SAPIENZA
UNIVERSITÀ DI ROMA

Synthesis, characterization and application of new amphiphilic compounds as precursors of functional materials

PhD in Chemical Sciences

XXXIV cycle

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Abstract

In the last two decades or so, bile acids and their synthetic derivatives have been shown to be useful building blocks in the preparation of particular nanostructures in a bottom-up approach. This work is part of that research field and focused on the preparation of new derivatives of bile acids and on the study of their properties that make them versatile in a wide range of research field, from medicinal chemistry and catalysis to surface chemistry and molecular electronics.

In particular, this research work dealt with the synthesis of new aromatic derivatives through click reactions, an innovative synthetic method never described in literature to link aromatic subunits to bile acids, in order to evaluate their aggregation properties and the possibility of exploiting this functionalization method on this type of substrate. This is what will be shown in Chapter 2.

In Chapter 3, bile acids were then tested also as useful surfactants essential for amino acids catalytic activity carried out in only water.

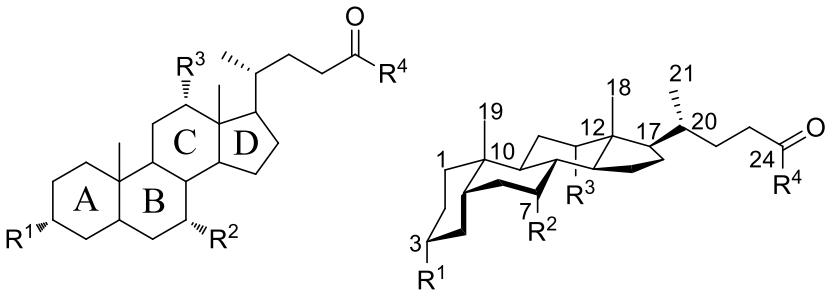
Furthermore, in Chapter 4, from the positive data of the aforementioned tests arose the idea of directly functionalizing these amphiphilic molecules with amino acid subunits, also evaluating if this could give a greater contribution by increasing yields, diastereoisomeric and enantiomeric excesses, given the intrinsic characteristics of molecules capable of inducing greater stereoselectivity.

Finally, ferrocenyl derivatives were synthesized, connecting the subunits in C-3 position or on steroid skeleton side chain in order to obtain molecules capable of functionalizing graphene surfaces, deposited on electrodes, in order to prevent their oxidation while still allowing the ET and more generally molecules useful in molecular electronics. This was described in final Chapter.

Chapter 1: Introduction to Bile Acids and their properties

1.1 Bile acids in biology and their unique use as surfactants

Bile acids are natural surfactants found in mammals (Figure 1.1). In humans they are produced in the liver starting from cholesterol and have a distinctive structure, characterized by a rigid and flat steroid skeleton, bearing a carbon chain ending with an acid function or a group of carboxylic derivation (as in the case of taurocholic acid or glycolic) and from hydroxyl groups which vary in number, stereochemistry and position.[1]



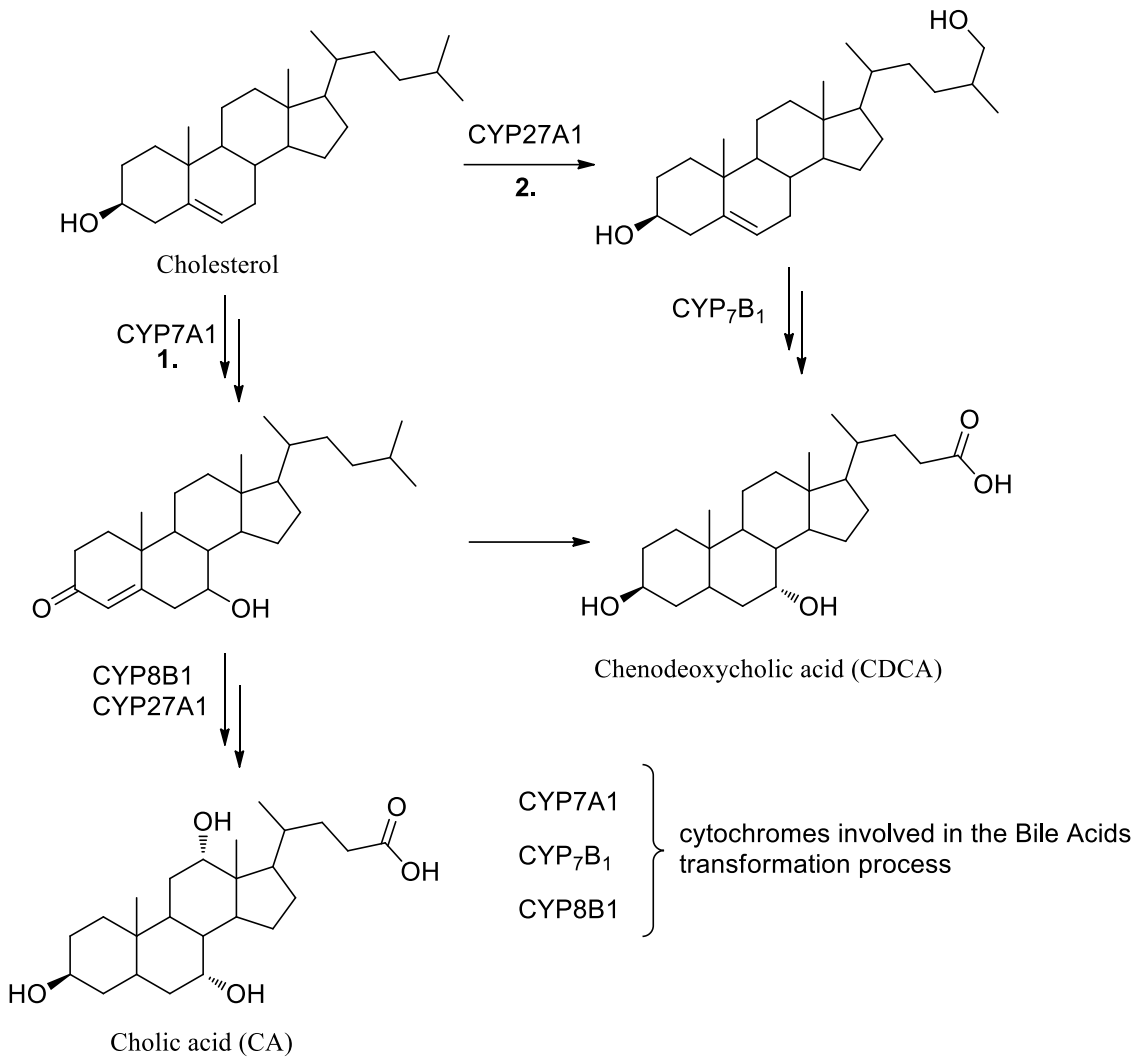
	R^1	R^2	R^3	R^4
1.1a cholanic acid	H	H	H	OH
1.1b cholic acid	OH	OH	OH	OH
1.1c chenodeoxycholic acid	OH	OH	H	OH
1.1d deoxycholic acid	OH	H	OH	OH
1.1e lithocholic acid	OH	H	H	OH
1.1f glycocholate	OH	OH	OH	$NH_2CH_2CO_2$-
1.1g taurocholate	OH	OH	OH	$NHCH_2CH_2SO_3$-

Figure 1.1. Some of bile acids produced in the liver.

The physiological evolution of cholesterol steroid nucleus involves several hydroxylations of the tetracyclic core and the loss an isopropyl group from the side chain, affording the so-called primary BAs, among which two of the most common are cholic acid (HC) **1.1b** and chenodeoxycholic acid (HCDC) **1.1c**. Further chemical modifications of primary BAs give the secondary BAs, such as deoxycholic (HDC) **1.1d** and lithocholic (HLC) **1.1e** acids.[2]

The conversion of cholesterol into bile acids is carried out through the complex action of a set of seventeen enzymes and represents the main cholesterol catabolism pathway. Although course and intracellular transport still remain not well defined and sequence-confirmed, four different synthetic pathways have been identified, each of which culminating in the synthesis

of one or more bile acids. As an example, is shown below the classical or "neutral" way, through which about 90% of all bile acids are produced, and the alternative or "acid" way (Scheme 1.1).[3,4]



Scheme 1.1. Two main bile acids biosynthetic pathway (1-"neutral way", 2-"acid way").

The most evolved mammalian BAs have a 5β-configuration with α-hydroxyl groups. Primary BAs under physiological conditions are normally conjugated with glycine and taurine (Figure 1.2), since the conjugation increases the solubility in water and plays an important physiological role in mammals enabling the digestion of cholesterol, fats, fatty acids and lipid-soluble vitamins and their absorption through the intestine. The BAs are then almost completely reabsorbed by the intestine and they recirculate to the liver via the portal vein in a process called enterohepatic circulation, of great importance in the cholesterol homeostasis.

The particular biological role of BAs finds a reflection both in their very specific molecular structure, which renders them facial amphiphiles, and in their self-assembly behaviour.[5,6]

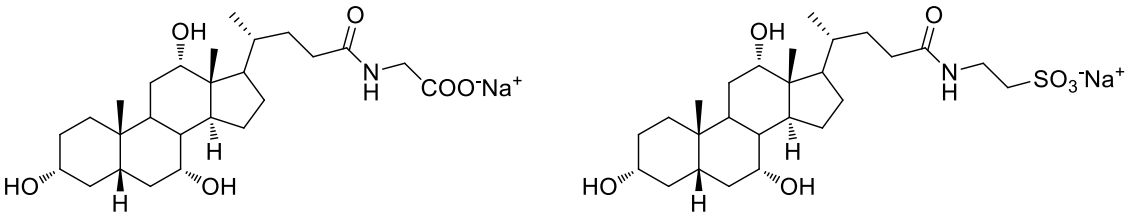


Figure 1.2. Molecular structure of the sodium glycocholate (left) and sodium taurocholate (right).

Their main physiological function is to promote digestion and absorption in the intestinal tract of lipids and some vitamins through the formation of micelles. They also have other functions, such as eliminating excess cholesterol and bilirubin, heavy metals and drug metabolites, regulating the bacterial flora of the intestinal tract and, to a lesser extent, regulating some receptors, enzymes and ion channels.[7]

Generally, lipophilic compounds, including molecules used in pharmacology, having low solubility in water, are well solubilized in bile salt micelles in the intestine, leading to an increase in absorption and bioavailability.

It has been shown that bile acids can act as drug absorption enhancers, increasing their solubility in case of excessive hydrophobicity and promoting their chemical and enzymatic stability.[8] Therefore, bile acids can improve the bioavailability of non-polar or highly hydrophilic drugs through the micellar solubility process and their interactions with biological membranes. They are also ideal elements to be exploited for synthetic purposes thanks to the stiffness of the steroid skeleton, the different position of the hydroxyl groups, the enantiomeric purity, the availability and the low cost. A further advantage of bile acids functionalization is also represented by their resistance to pH and enzymes of the gastrointestinal tract.[9]

Each bile acid exhibits a hydrophilic polar region (face α), where the hydroxyl groups are located, opposite to a non-polar hydrophobic region (face β), on which there are the C18 and C19 methyl groups (Figure 1.3).[10]

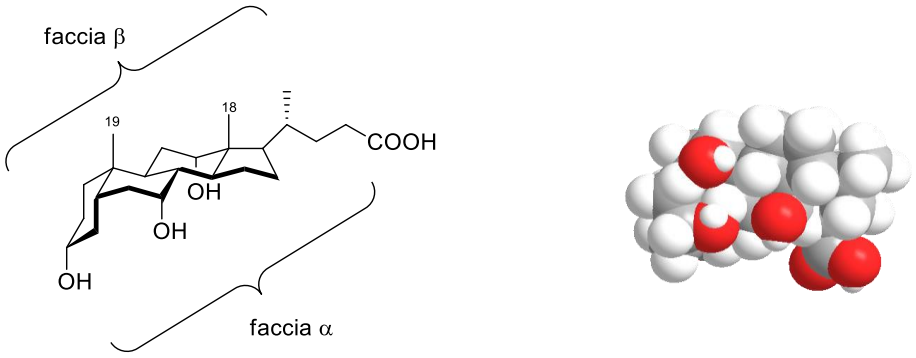


Figure 1.3. Cholic acid spatial representation.

All these structural characteristics also give bile acids peculiar amphipathic properties that give them the ability to act as physiological surfactants.[11]

The high structural rigidity and the lack of a clear separation between the hydrophobic part and the hydrophilic part allow the bile acids, exceeding a certain concentration value, to self-assemble in water in different aggregates in respect of traditional surfactants through electrostatic and hydrophobic interactions and hydrogen bonds.

The aggregation of BAs is the result of an intricate interplay between the hydrophobic effect and the intermolecular hydrogen bond formation. Indeed, hydrogen bonding provides a complementary mechanism for auto-association in addition to the unspecific hydrophobic effect, which is relatively weak and complex in BAs. The directional properties and the specific nature of the hydrogen bonds (which depends on the number, position and stereochemistry of hydroxyl groups) introduce an additional "rigidity" into the aggregates and limit the range of possible orientations. In addition, the carboxylic group provides a charge, being dissociated under physiological conditions. Thus, all these factors result in a very complex self-assembly behaviour.

1.2 Bile Acids as supramolecular aggregates

As anticipated, due to their molecular features, BAs auto-associate forming highly ordered architectures, in contrast to micelles formed by conventional amphiphiles (Figure 1.4). The size of such ordered aggregates, their dimension and shape strongly depend on the BA nature and on the experimental parameters, such as pH and electrolyte concentration.[12]

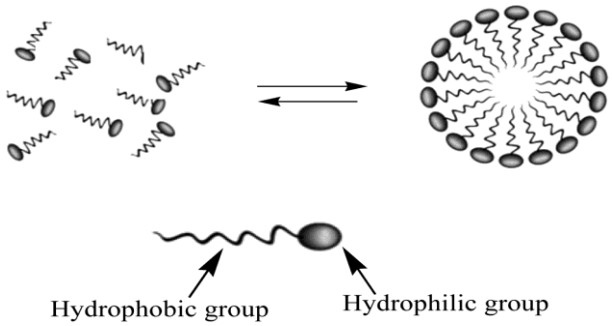


Figure 1.4. Classical aggregation behavior of conventional surfactants in aqueous solutions.

This dependence can be clearly illustrated by the different behaviour of cholic acid, deoxycholic acid and lithocholic acid in basic conditions. Indeed, while the latter compound forms tubules at alkaline pH,[13,14] cholic acid and deoxycholic (as well as their glycine and taurine conjugates) form, in the same conditions, globular[6,15,16] and rod-like[17,18] aggregates, respectively. In the last decades, several models have been proposed to describe their structures, the earliest of which considers the formation of primary and secondary micelles.[19] According to this model, at concentration values close to the critical aggregation concentration (cac), molecules self-organize directing their hydrophobic sides towards the interior and the hydrophilic one in contact with water, forming the primary micelle (Figure 1.5b), whose structure depends on the number and type of BA in it. At higher concentrations, hydrogen bonds between the micelles lead to elongated secondary micelles (Figure 1.5c), in which the hydrophobically stabilized primary units are held together by hydrogen bonding. The second model proposed suggests the formation of disk-like micelles (Figure 1.5d), with a variable diameter, in which all the molecules point the hydrophobic side to the inner side of the aggregate.[20] Instead, in the third one the self-organization is explained as driven by polar interactions: the molecules are organized in a helical arrangement (Figure 1.5e) whose interior is filled with water and all the hydrophobic surfaces are directed towards the outside. This model is based on the structure of the aggregate in the crystalline state, but it also well represents the structures in the liquid state.[21,22]

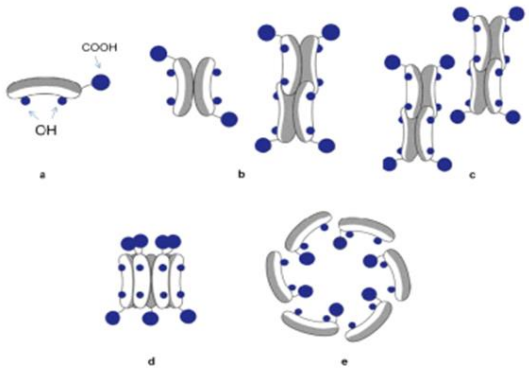


Figure 1.5. Schematic representation of a) a dihydroxy-BA and of different models for micellar structures: b) different primary micelles, c) secondary micelle, d) disk-like micelle and e) helical micelle.

In principle, several topologies of aggregation can be accessible to a BA, so there are some structural features that are crucial in pointing out the final structures of the aggregates. The most important is the number of hydroxyl groups on the tetracyclic system, since it determines the number of hydrogen bonds that can be involved in stabilizing the aggregates inevitably influencing their shape. Moreover, the curved and rigid skeleton limits the possible orientation of the molecules in the aggregates. As a matter of fact, from the comparative studies of the aggregation behaviour of cholic acid with respect to the one of deoxycholic acid and lithocholic acid, it became evident that the additional hydroxyl group in cholic one prevented both gel or viscous fluid formation, which was observed for other BAs, as in case of deoxycholic, that showed the self-organization in fibers as the pH was lowered to neutrality, with the consequent formation of a thixotropic gel.[15,25,26]

1.3 Bile acids and their derivatives: applications fields.

BAs are optimal substrates in the search for new compounds to be applied in many high-interest scientific fields spacing from supramolecular chemistry, material chemistry, and nanotechnology to medicinal chemistry and drug delivery.

As already evidenced, they are involved in the entheropathic circulation and correspondingly in multiple processes that require molecular recognition, they self-organize in different structures both in the solid state and in aqueous solutions and they are widespread in the majority of species of higher animals, thus being inexpensive and readily available starting materials. Consequently, in the last twenty years BAs and their derivatives have been

exploited in diverse areas of chemistry as molecular and ionic receptors, templates/chiral auxiliaries for asymmetric synthesis, host-guest interaction-based systems, protein or drug nanoparticle stabilizers, drug carriers, low molecular mass organo/hydrogelators, etc.[25-27]

All these reasons have significantly stimulated the developing of new methods to functionalize the BA skeleton, together with the preparation of a large number of new derivatives and this has been gaining an increasing interest as a very active research field.

The synthesis of BA derivatives takes advantage from the presence of various functional groups, which can be selectively modified, according to the huge number of stereoselective transformations and chemical modifications of BAs described in the literature so far. It is not possible to summarize completely all these methods here, but it is useful to cite the main transformations that BA functional groups can afford. To cut a long story short, the carboxylic group may be esterified, reduced, amidated or subjected to salt formation with metal ions, alkaloids or organic bases, whereas the hydroxyl groups can be regioselectively transformed as they show a differentiated reactivity: 3-OH is the only equatorial one, and thus the most reactive, being both the axial 7- and 12-OH sterically hindered.[28-33]

1.3.1 Bottom up systems

In the last years, a constantly growing interest has been shown in the fabrication of micro- and nanosystems for several applicative purposes, with the so-called bottom-up approach. In a bottom-up process, relatively simple building blocks interact with each other in a coordinated way to form large, more complex, stable and structurally well-defined aggregates linked by non-covalent interactions. In particular the molecular self-assembly represents one of the most important tools for a bottom-up synthesis.[34-36] Micro- and nano-structures formed by such approach find an incredible number of different applications, the most important of which are in the catalyst design, sensor and conducting devices tailoring.

A main goal along the way to nanomaterials is the synthesis of "smart molecules", that is systems which respond to external stimuli such as pH, temperature, light, electric field, chemicals and electrolyte concentration, affording dramatic variations in their aggregation properties, thus changing the nature of the nanomaterials formed. The development of such molecules has consequently a tremendous potential for applications in biotechnology, in drug delivery, sensors, bioseparations.[37-42]

With the aim of preparing new materials by self-assembly of relatively simple starting materials several biological molecules such as peptides, lipids, steroids have been

investigated.[37,38,43-46] Indeed, many of these biomolecules possess natural uncommon self-assembling properties, strongly affected by their chemical structure. Moreover, biomolecules usually show a wide variety of different functional groups in their structure, and this last feature can provide them with the ability to afford "intelligent" micro- and nanosystems.
In the last years, a number of BAs derivatives have been synthesized and investigated.

1.3.2 BAs as supramolecular systems

Their curved and rigid amphipathic backbone, enantiomeric purity, high levels of functionality and ease of functionalization render BAs particularly attractive scaffolds in supramolecular chemistry, in particular in the design of rigid pre-organized molecular systems to create clefts, cavities and other types of binding surfaces.[47]
The design of macrocycle synthetic receptors with molecular cavities is an important field in supramolecular chemistry. These host molecules in fact can serve as model compounds for more complex biological systems, for instance they can simulate the molecular recognition shown by enzymatic processes. The BA skeleton rigidity makes it suitable to the formation of a cavity, thus they were used in the preparation of macrocycles, such as cholaphanes (Figure1.6), which consist of two to four BA units joined by various spacer groups and have been shown to bind in their cavities carbohydrates and cations with a selective affinity, depending on the cavity size.[48-50]

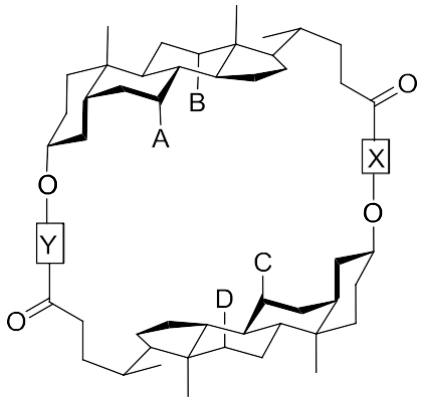


Figure 1.6. Structure of a generic head-to-tail cholaphane (A-D = binging/catalytic functionality, X,Y = spacer)

Examples of other cyclic structures involving BAs reported in the literature are cyclocholates,[51] BA-based molecular boxes,[52] crown ethers,[53] cyclophanes[54] and cryptands.[55]

These architectures provide frameworks for the construction of enantioselective hosts as well as chiral ligands or auxiliaries.

BA derivatives have also been exploited in the realization of acyclic structures, such as cleft-type compounds,[56] podand-type architectures ("cholapods") (Fig. 1.7.a)[57-59] and molecular tweezers (Fig. 1.7b)[60] as receptors for the recognition of anions, nucleobases, aminoacids or aromatic molecules or as transporters of anions across bilayer membrane. Moreover, the BA facial amphipathy has been exploited in preparing ionophores for the transport of ions through liposomal membranes[61] and in the development of so-called molecular umbrellas, that is, molecules potentially able to behave as carriers of hydrophilic compounds through phospholipid bilayers, by "shielding" their highly polar character when in contact with the hydrophobic core of a lipid bilayer.[62,63]

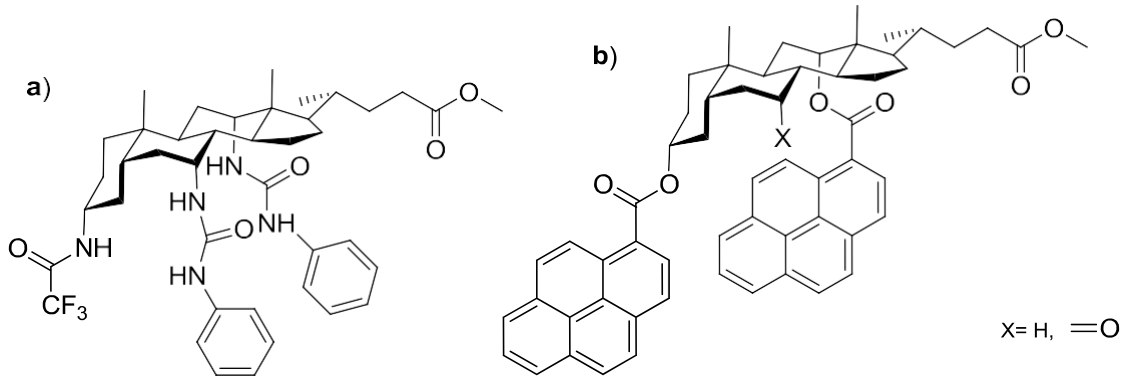


Figure 1.7. a) "Cholapod" derived from cholic acid, acts as anion receptor, molecules of this family are powerful anion receptors and carriers across the bilayer membranes, b) BA-based tweezers, receptors for a number of electron deficient aromatics.

1.3.3 BAs as gelators

In the recent years, gel-phase materials obtained from low molecular weight building blocks have been considered of particular interest because of the potential application in materials science, in the fields of tissue engineering, biomineralization, gels formation[64] and molecular electronics. The gelation of low molecular mass organic gelators (LMOGs) is mainly due to intermolecular interactions, namely hydrogen bonding, π-π stacking and Van der Waals interactions, leading to fibrous network formation.[64-66]

Several molecules have been studied as LMOGs, such as sugars, ureas, amino acids, steroids. Among these, BAs derivatives have shown efficient gelling properties. As previously

mentioned, some natural BAs self-organize to give supramolecular gels[23,24] and, given that the BA structure deeply affects the self-assembly behaviour, in the last years a number of derivatives have been reported showing that different derivatizations dramatically modify the aggregation process and lead in certain conditions to gelation. A majority of these BA derivatives have a general structure with an intact steroidal backbone and a variable side chain group. They have shown extraordinary gelling properties. By way of example, the HDC- and HLC-based hydroxypropylamides[67] (Figure 1.8a), the HC cholanamides[68] (Figure 1.8b) and α-amminoesters[69] (Figure 1.8c) have proved to be effective organogelators.

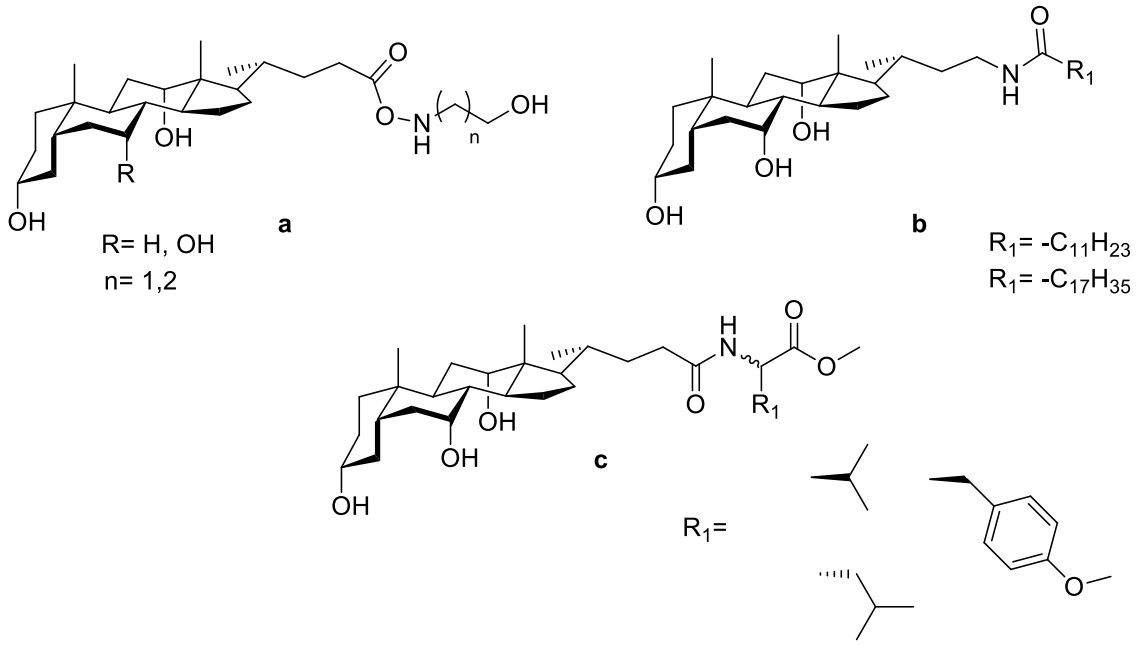


Figure 1.8. Molecular structure of a) deoxycholic and lithocholic acid based, b) and c) based organogelators.

BA derivatives have been known to lead also to gelation of water and electrolyte aqueous solutions under defined conditions[23,24] and hydrogels are of great importance because they are materials for biomedical applications, such as drug-delivery systems[70] or tissue engineering.[71] The deoxycholic acid cationic derivatives (Fig. 1.9a) and neutral hydroxylamides of BAs (Fig. 1.9b) are efficient gelators in aqueous media,[72] as well as the cholic acid trimer (Fig. 1.10).[73]

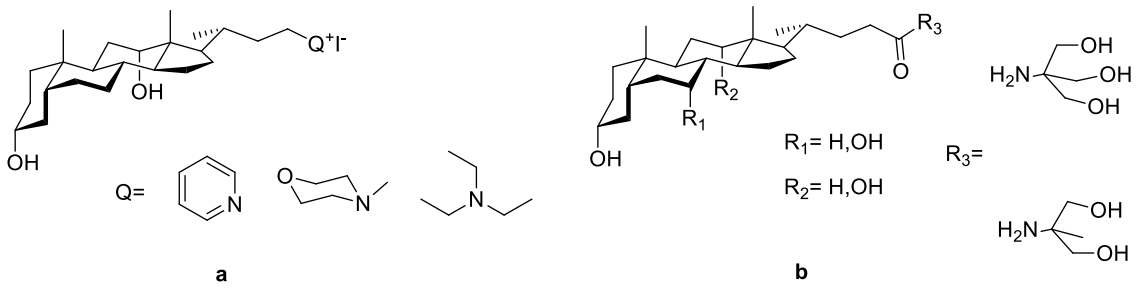


Figure 1.9. Molecular structures of some BA-based hydrogelators.

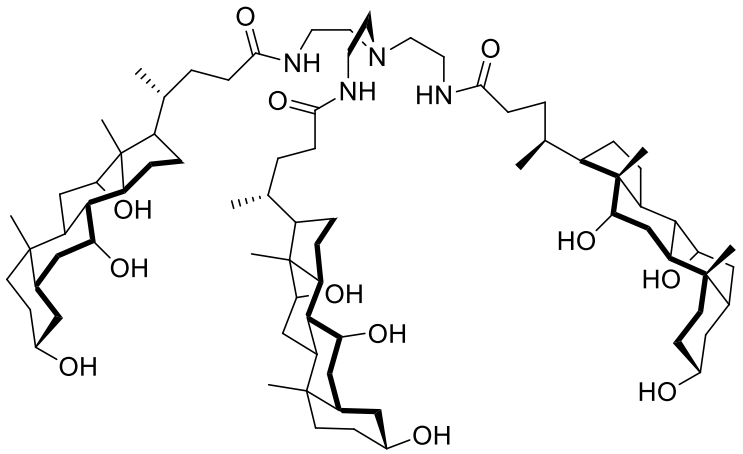


Figure 1.10. Cholic acid trimer.

1.3.4 Unusual structures of BAs and their uses as sensors

The derivatization of BAs has been shown to lead to supramolecular aggregates with uncommon morphologies not formed by natural bile salts. It has lately been demonstrated that the extension of the tetracyclic system by introducing a bulky hydrophobic group leads to new structures. The introduction of an adamantyl group in the position C3 of cholic acid (Fig.1.11) led to lamellar structures.[74] This kind of assemblies can find several application, notably as templates in the formation of metal nanowires, due to the well-known tendency of lamellar structures to form wires or nanotubes.[75,76]

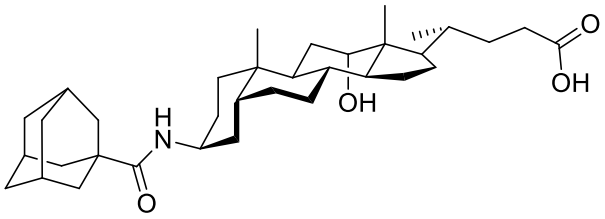


Figure 1.11. Molecular structure of the cholic acid adamantyl derivative.

Recently, this derivative and its analogues from the deoxycholic acid have been proved to be good drug nanoparticle stabilizers.[77]

Functionalization in C3 has also led to external stimuli-responsive assemblies, further confirming the potentialities of the use of BA derivatives in nanotechnology. A particularly interesting behaviour has been shown by the *t*-butylphenyl- (Fig. 1.12a) and naphthyl- (Fig. 1.12b) amide derivatives of cholic acid.

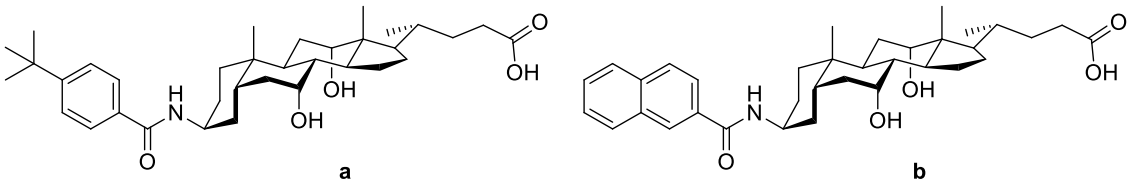


Figure 1.12. Molecular structures of the t-butylphenyl- (**a**) and naphthyl-(**b**) amide derivatives of cholic acid.

The first compound in aqueous solution forms vesicles which reversibly interconvert into tubules through a temperature-triggered process.[78,79] Moreover, the mixtures of the anionic form (Fig. 1.13a) and cationic derivative (Fig. 1.13b) of the *t*-butylphenylderivative show to self-assemble into tubules whose charge can be tuned by controlling the mixture stoichiometry.[80]

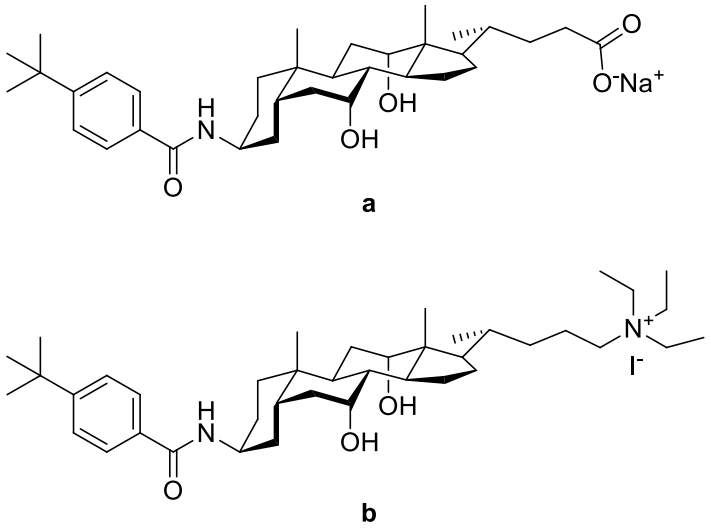


Figure 1.13. a) Cholic acid t-butylphenyl amide derivative in its carboxylate form and b) its cationic derivative.

Naphthyl derivative (Fig. 1.12b) is instead capable to aggregate into pH-sensitive tubules. By increasing the pH, the tubule walls open up leading to the disaggregation of the assemblies,[81] making this compound particularly attractive as potential drug carrier. The auto-association of BA derivatives has also been exploited in the realization of assembled systems in the crystalline state. Trisphenylurea cholapod (Figure 1.14), synthetized in the recent past and obtained by derivatizing all the three cholic acid hydroxyl groups, crystallizes from acetone/water or methyl acetate/water giving self-assembled nanoporous crystals, with chiral unidirectional channels large enough to accommodate a wide variety of molecules including dyes or substrates for catalysis (Figure 1.15).

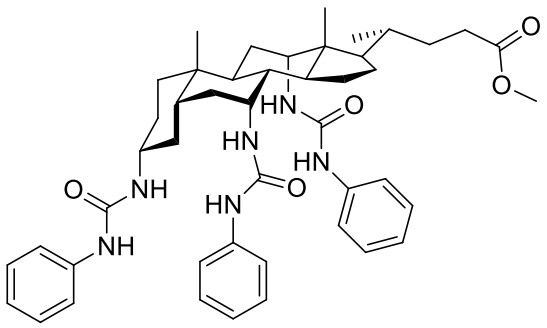


Figure 1.14. Tris-phenylurea cholapod obtained from cholic acid.

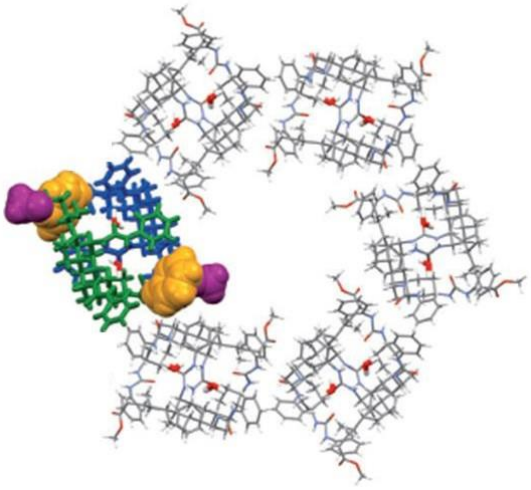


Figure 1.15. Packing of tris-phenylurea cholapod viewed along the crystallographic c axis, two molecules are highlined, one green and one blue, the methyl ester and the phenylurea at C3 are shown in the space-filling mode (in magenta and yellow, respectively).

A large number of derivatives have been prepared by varying the nature of the groups of the molecule, namely at the position C3 and at the end of the aliphatic side chain. In this way, it was possible to tune the diameter of the cavities and to engineer nanoporous organic alloys by mixing different isostructural derivatives and thus tuning the crystal properties.[82] These structures are of particular interest given that nanoporous crystals find application in catalysis, separation techniques, gas storage or sensors.

Sodium cholate and sodium deoxycholate were also used as reducing and capping agents (Figure 1.16) for the fabrication of Au nanoparticles (NPs) for applications in colorimetric DNA sensors.[83]

The method allowed for the fabrication of bile salt encapsulated Au NPs of controlled size and shape. It has been demonstrated that hydroxyl groups of the bile salts were involved in Au^{3+} reduction, while COO^- groups provided bonding to the NP surface. The colour change due to aggregation of the NPs was successfully used for the detection of Gemini viruses.[84]

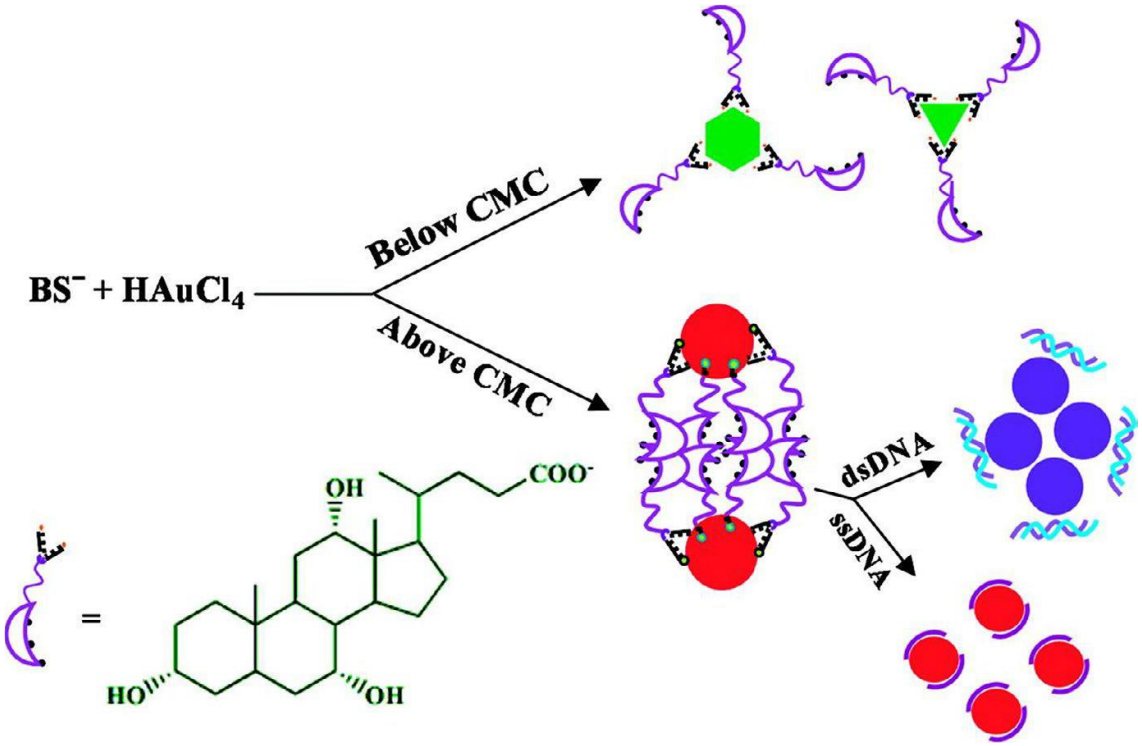


Figure 1.16. Schematic of the synthesis of bile salt (BS) capped Au nanoparticles and colorimetric sensing of DNA.

Cholic acid-capped Au nanoclusters have been successfully employed as fluorescent probes for creatinine detection.[84] The detection mechanism involved hydrogen bonding of cholic acid and creatinine. The sensors can be used for diagnosis and therapy of kidney disease.
Bile acids are also used in the surface modification of electrodes, and in particular the graphite ones, as these amphiphiles is a nanostructured material. By covering the surface they increase the sensitivity of the aforementioned electrodes. This characteristic has led to the development of molecules that were at the same time mediator of electrons and excellent dispersant of carbon nanotubes, thus favoring their deposition.
The introduction of a ferrocenyl subunit within the structure of a classic-type surfactant has already been described, as the ferrocene as a substituent was chemically stable in solution in a wide range of conditions, and its redox potential made it easy to change in its oxidation state. The oxidation of the ferrocene subunit to ferrocenium ion led to a change in the formal charge of this unit and therefore there was an important change in the polarity properties of the surfactant, and consequently in the aggregative properties.
In the literature it is indeed possible to find numerous examples of ferrocenyl-substituted surfactants. The reported derivatives are neutral, or cationic or anionic (Figure 1.17). The

interesting aspect was that depending on the structure of the surfactant and the position of the ferrocene within it, a wide range of different surfactant properties was observed.[85]

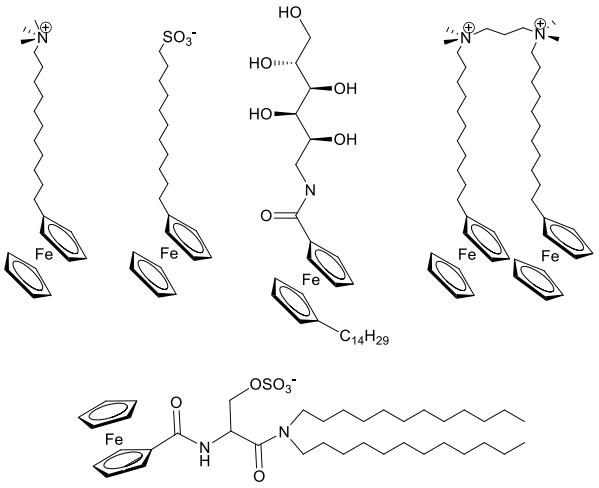


Figure 1.17. Some examples of neutral, cationic or anionic ferrocenyl-substituted classic-type surfactants.

1.4 References

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Chapter 2: Synthesis and characterization of new aromatic bile acids' derivatives through click chemistry

2.1 Introduction

Among the synthetic methods developed in the last decades, a significant popularity was gained by a group of reactions that is often referred to as "click chemistry". This synthetic methods, which fulfills many criteria, such as a stable linkage, minimal cross-reactivity with other functional groups, high yield reaction, no appreciable amounts of side products, and benign reaction conditions, showed its great potential for use in the synthesis of a very large number of interesting compounds.

The reactions belonging to this group are generally driven by a high thermodynamic force (> 20 kcal mol^{-}1), which gives rise to highly modular and stereospecific reactions with high yields.[1]

While a range of chemical reactions can in principle fulfill these criteria, successful examples often originate from five broad classes of reactions that appear to fit the framework of click chemistry exceptionally well: cycloaddition of unsaturated species (1,3-dipolar cycloaddition or [4+2]-cycloaddition (Diels–Alder)), nucleophilic substitution/ring-opening reactions, carbonyl reactions of the non-aldol type and addition to carbon–carbon multiple bonds.[1]

In particular, the first two types of reactions have recently played a crucial role in many fields of science, from material chemistry (nanomaterial preparations,[2-16] surface engineering,[17-25] multifunctional polymer design,[14,26-50] self-assembling molecules[51-58]) to medicinal chemistry[59], in which this modification method, which seems to be the cream of the crop towards C-N bond forming-reactions of five-membered heterocycles, has found his own important role, also due to its versatility, in the synthesis of a large number of bioconjugates showing anticancer activities (hybrids containing chalcone,[60-68] amide[69-80], coumarine,[81-87] azole[88-94] or polycyclic[95-101] analogues) (Figure 2.1).

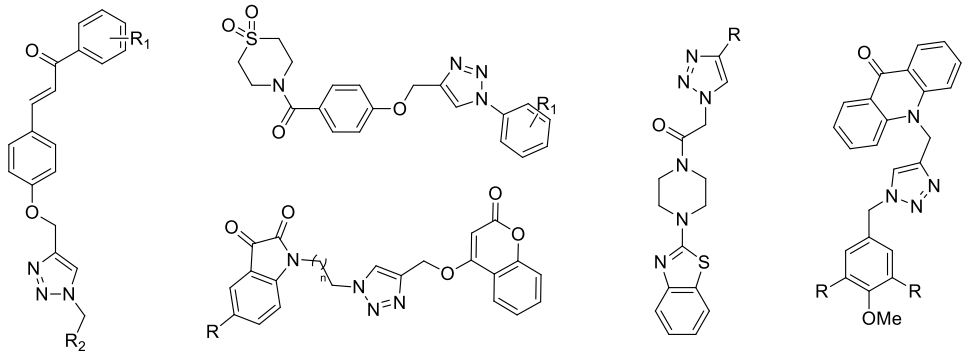
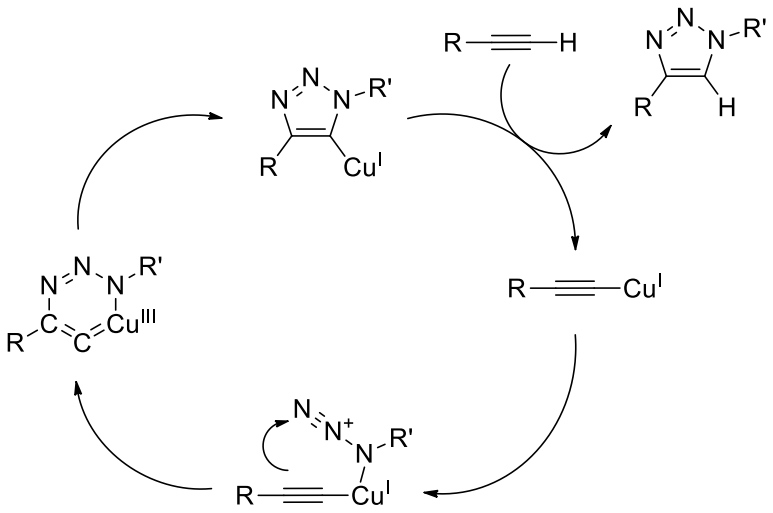


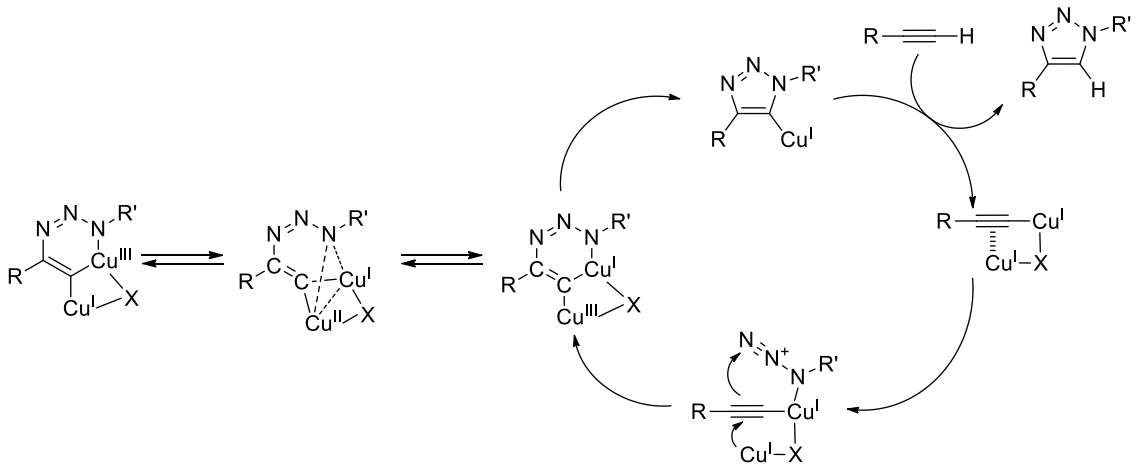
Figure 2.1. Chalcone, amide, coumarine, azole, polycyclic analogues.

The 1,3-dipolar cycloaddition of azides and alkynes, also known as Huisgen cycloaddition reaction,[102,103] involves dipolarophiles reacting with 1,3-dipolar compounds to form five-membered heterocycles including triazoles. The original Huisgen reaction was non-regioselective and required high temperature and pressure so it was largely ignored for a long time. In the last two decades, the use of catalytic amounts of Cu(I) was shown to improve the effectiveness of the reaction, leading to fast, highly efficient and regioselective azide–alkyne cycloadditions at room temperature in organic medium (Scheme 2.1).[104]



Scheme 2.1. First proposed mechanism for the CuAAC reaction.

In recent years, Sharpless and Fokin demonstrated that copper-catalyzed azide-alkyne cycloaddition (CuAAC) can be successfully performed in polar media such as t-butyl alcohol, ethanol or pure water (Scheme 2.2).[105]



Scheme 2.2. Current accepted CuAAC mechanism.

The widely use of the copper catalysed alkyne-azide cycloaddition reaction, the so called CuAAC, and its remarkable efficiency are demonstrated with the fact that it is the method of choice even for DNA click chemistry and, in general, for biological and nanotechnological applications or in drug research in the field of peptide-based drug discovery (Figure 2.2).[106,107,108]

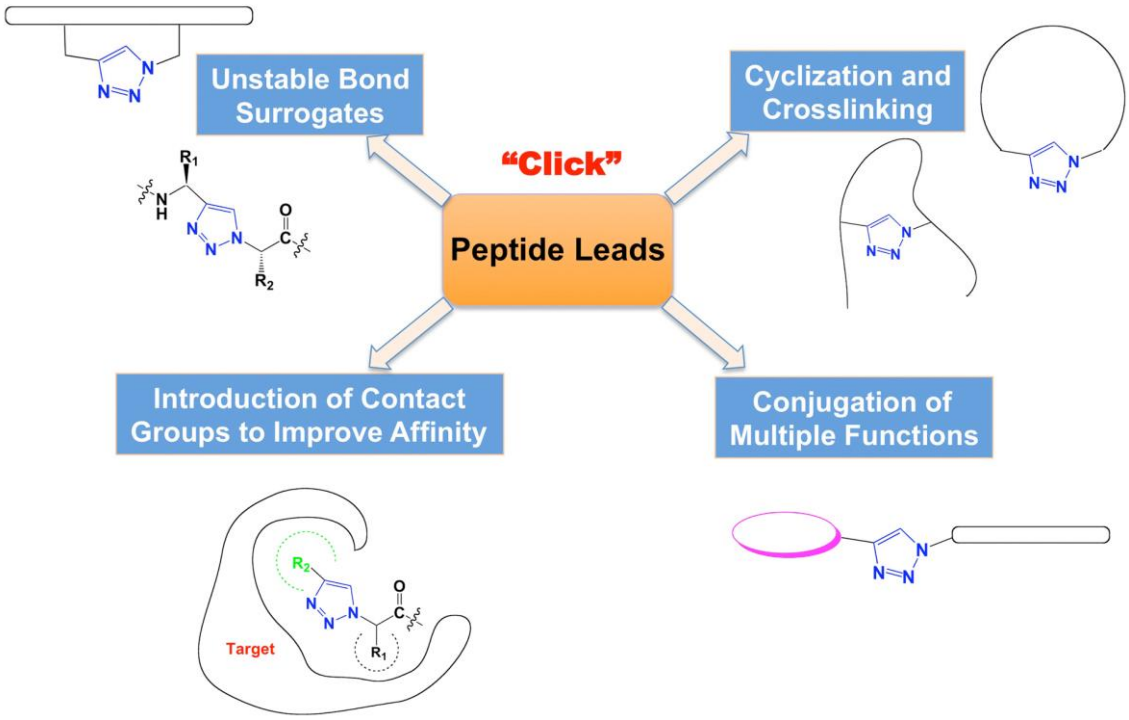


Figure 2.2. Click chemistry in peptide-based drug discovery.

Thanks to the large versatility and to the huge possibility in using the CuAAC method, further studies involving irradiation with microwaves and high-intensity ultrasound were also

recently conducted.[109]

The work described in this chapter stems from the well-known self-assembly behavior of bile acids' derivatives bearing aromatic substituents which are already described in literature[110,111] and it is aimed to the synthesis of new bile acid aromatic conjugates linked through a triazole group formed following click reaction (1,3-dipolar cycloaddition) and to their possible aggregation skill improvement. In future, these compounds could also be studied in order to evaluate their physical-chemistry properties.

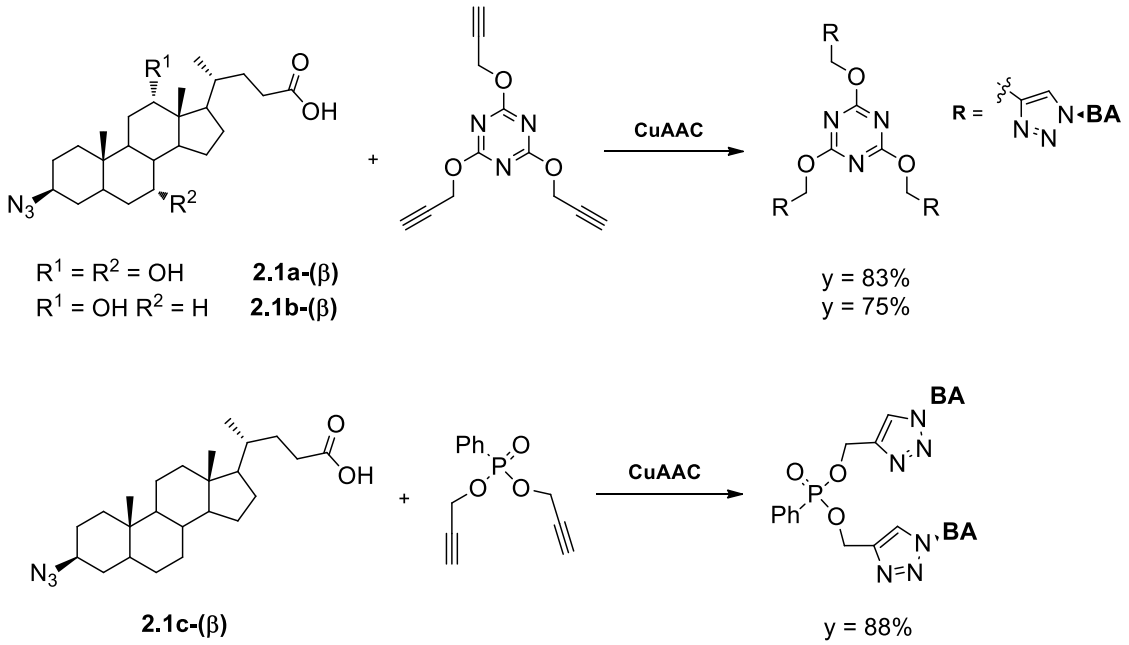
Starting from the product obtained using phenylacetylene as the model substrate, studies were also conducted to clarify the reason for the initial low yields by conducting the same reactions on other types of non-aromatic substrates, in search of the most ideal possible conditions for optimizing the quantity of product obtained.

2.2 Choice of target molecules

Since it was already shown that the expansion of the hydrophobic region of the BA skeleton by introducing a bulky non polar substituent at the position C3 significantly modify the BA self-assembly properties leading to uncommon supramolecular assemblies, we decided to follow a similar strategy introducing aromatic subunits into the position 3 of the tetracyclic system by Click Chemistry. This could, in principle, lead to a structure bearing aromatic hydrophobic groups, such as phenyl and 1,2,3-triazole moieites, capable to give intermolecular π-π stacking interactions crucial in self-assembly processes.[112]

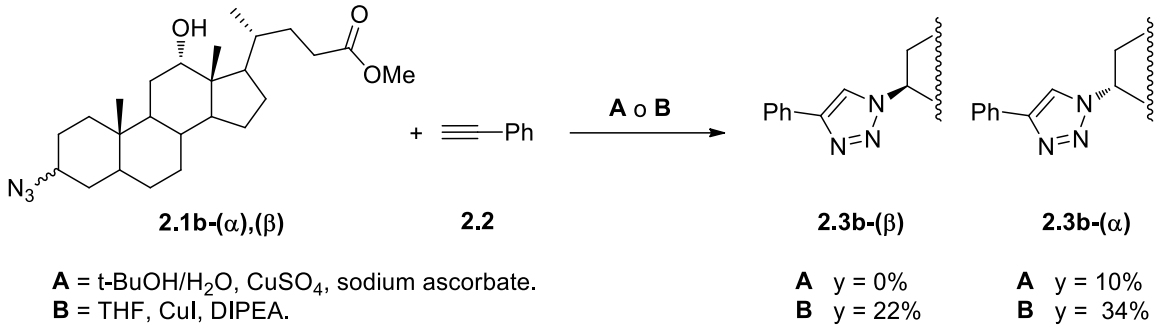
In literature there are examples of functionalization of bile acids by CuAAC reaction, but they mainly concerned the reaction carried out on the side chain.[113]

The few examples found involving the C-3 functionalization of 3-azido-substituted bile acids featured the use of non-aromatic terminal alkynes, as shown by some examples in Scheme 2.3.[114a-e]



Scheme 2.3. Examples of C-3 functionalization with non-aromatic terminal alkynes.

Since the attention of our group was focused on the introduction of aromatic subunits on the C-3 in order to improve the aggregative abilities of the BAs, the work started with the study of the reaction CuAAC on epimeric azides **2.1a-c** with phenylacetylene **2.2** used as model compound. During this study, we ran the reactions adopting the two most popular conditions reported in literature.[115,116] In the first one, a catalytic amount of Cu(II) sulfate was used as source of copper in the presence of sodium ascorbate as reducing agent, in the classic *t*-BuOH / H_2O mixture (conditions **A**). Alternatively, the reaction was conducted in organic solvent (THF), in association with the catalytic use of cuprous iodide and diisopropylethylamine (conditions **B**). However, the preliminary results obtained showed generally low yields, but the stereochemistry of C-3 seemed to play a role. Indeed, when the reaction involved the substrates with the azido group at C-3 in α configuration, the yields were slightly higher (Scheme 2.4).



Scheme 2.4. Low initial yields in preliminary studies.

Thus, we decided to continue this study on all the 3-azido bile acid esters derivatives from cholic, deoxycholic and litocholic acids.

To clarify the effect of the possible steric hindrance of the substituent on the triple bond, two model compounds were therefore chosen to react with the azide derivatives, phenylacetylene **2.2** and the acyclic 1-heptine **2.4**, (Figure 2.3).

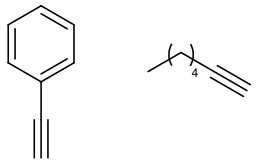


Figure 2.3. Phenylacetylene and 1-heptine involved in reaction with azide derivatives.

We also wanted to verify if the stereochemistry at C-3 in the azide substrates was crucial to obtain good yields.

The variation of a reaction condition, i.e. the use of a catalytic or stoichiometric amount of the copper used, was also studied.

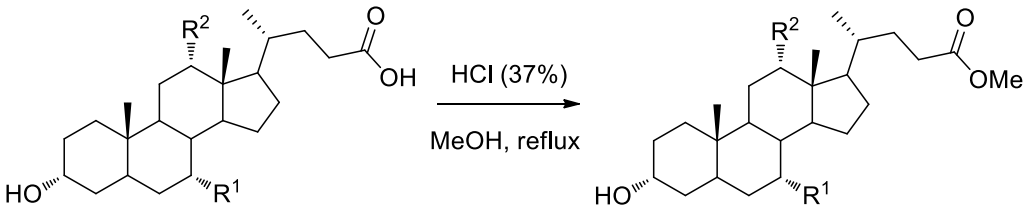
Once the optimal conditions for the click reaction among the compounds already mentioned have been found, the next step involved the reaction applied to more complex, variously substituted aromatic alkynes; a fact that would have confirmed the usability of the reaction and constituted an enrichment of the reaction scope, with products that could constitute excellent intermediates for further reactions.

2.3 Studies on reaction models

The experimental work started with the synthesis of epimeric azides **2.1a-c** deriving from **2.5a-c** acids. The synthetic procedures for the preparation of these azides are widely available in the literature[117,114b] and, although different for the α and β epimers, all involve the transformation of the hydroxyl group on C-3 (in a selective manner, thanks to its greater reactivity) in a good leaving group (mesylate) with configuration retention or inversion. Then mesylates are reacted with sodium azide in order to introduce the azide group with a neat inversion of configuration.

A preliminary stage of protection of the -COOH group as methyl ester, avoiding interference of the carboxyl in subsequent reactions, was necessary and it was performed using a Fischer esterification reaction.

The bile acids **2.5a-c** were then esterified using MeOH, also used as solvent, under acid catalysis conditions, given by the presence of HCl (37%) in trace quantities (Scheme 2.5).

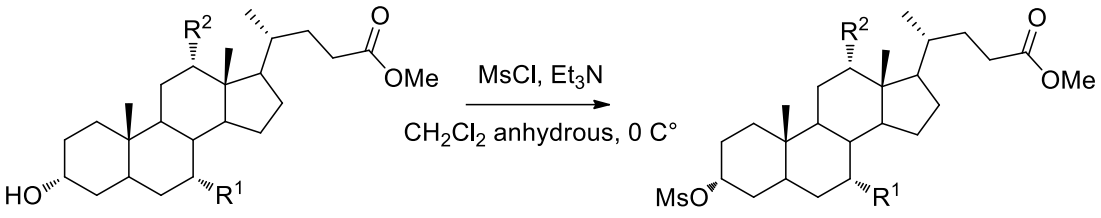


R1 = **R2** = OH	**2.5a**	**R1** = **R2** = OH	**2.6a**	y= 70%
R1 = H **R2** = OH	**2.5b**	**R1** = H **R2** = OH	**2.6b**	y= 92%
R1 = **R2** = H	**2.5c**	**R1** = **R2** = H	**2.6c**	y= 58%

Scheme 2.5. Bile acids esterification using Fischer conditions.

Then, the desired esters **2.6a-c** were purified by crystallization and obtained with excellent yields.

The next step was the transformation of the hydroxyl group on C-3 into its methanesulfonate ester in both possible configurations. The **2.7a-c** mesylates with retained C-3 configuration were obtained by treating the **2.6a-c** esters solubilized in DCM with methanesulfonyl chloride in the presence of triethylamine (Scheme 2.6).

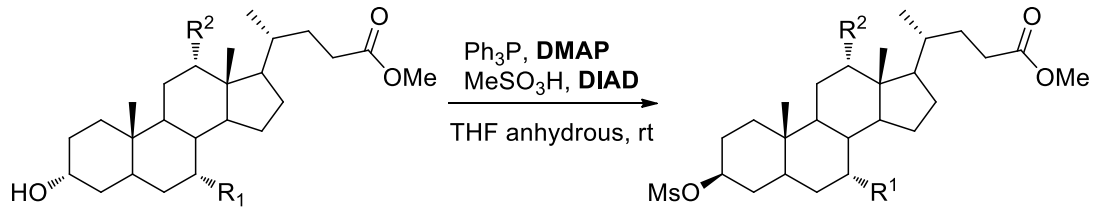


$R^1 = R^2 = OH$	**2.6a**	$R^1 = R^2 = OH$	**2.7a-(α)**
$R^1 = H$ $R^2 = OH$	**2.6b**	$R^1 = H$ $R^2 = OH$	**2.7b-(α)**
$R^1 = R^2 = H$	**2.6c**	$R^1 = R^2 = H$	**2.7c-(α)**

Scheme 2.6. C-3 hydroxyl group mesylation.

The reaction products were obtained with a suitable purity to be able to continue the synthetic work without needing any purification.

The compounds **2.7a-c**, obtained with an inverted C-3 configuration were also synthesized from **2.6a-c** esters, but through the reaction with methanesulfonic acid according to the Mitsunobu protocol,[118] shown in Scheme 2.7.



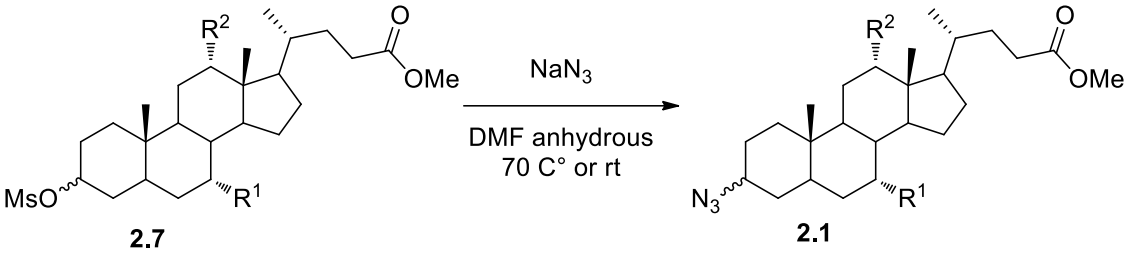
$R^1 = R^2 = OH$	**2.6a**	$R^1 = R^2 = OH$	**2.7a-(β)**
$R^1 = H$ $R^2 = OH$	**2.6b**	$R^1 = H$ $R^2 = OH$	**2.7b-(β)**
$R^1 = R^2 = H$	**2.6c**	$R^1 = R^2 = H$	**2.7c-(β)**

Scheme 2.7. Mesylation in Mitsunobu conditions.

The reaction was carried out by dissolving the esters in anhydrous THF in the presence of triphenylphosphine, 4-dimethylaminopyridine (DMAP), N,N'-diisopropylcarbodiimide (DIAD) and $MeSO_3H$. Again, also in this case, the raw material was directly used in the subsequent azidation procedure.

Compounds **2.7a-c(α)** and **2.7a-c(β)** were then employed in the substitution reaction of the methanesulfonate group with the azide function using NaN_3 and anhydrous DMF as reaction solvent. The **(β)-** mesylates showed very good reactivity at room temperature, while a satisfying conversion the **(α)-** stereoisomers required the temperature to be raised to 70 °C. The compounds **2.1a-c(α)** and **2.1a-c(β)** were then obtained, after chromatographic purification. The results are reported in Table 2.1 and the products yields, isolated by

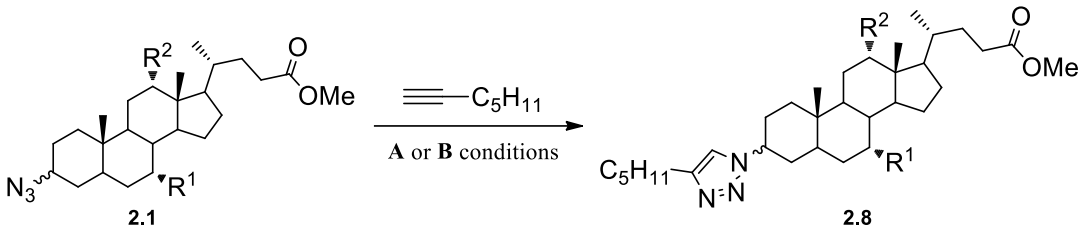
chromatography, were calculated on two stages starting from esters **2.6a-c**.



Mesylate	R^1	R^2	T(C°)	Product	Yields(%)
2.7a-(α)	OH	OH	70 C°	**2.1a-(β)**	54
2.7b-(α)	H	OH	70 C°	**2.1b-(β)**	57
2.7c-(α)	H	H	70 C°	**2.1c-(β)**	27
2.7a-(β)	OH	OH	rt	**2.1a-(α)**	76
2.7b-(β)	H	OH	rt	**2.1b-(α)**	80
2.7c-(β)	H	H	rt	**2.1c-(α)**	83

Table 2.1. Azidation reaction conditions and yields.

Once the epimeric azides **2.1** were obtained, it was possible to start the study of the CuAAC reaction with the two terminal alkynes chosen as model compounds, namely 1-heptine and phenylacetylene. As anticipated in the previous paragraph, the reaction was carried out in two different conditions, i.e. in a *t*-BuOH/H_2O mixture, using Cu (II) sulfate in the presence of a reducing agent (conditions A, Table 2.2), or in THF with Cu (I) iodide (conditions B, Table 2.2). The results obtained using the metal in catalytic and then stoichiometric quantities were compared during this study. Therefore, reactions started with the "click" between the azide compounds **2.1(α)** and **2.1(β)** and 1-heptine and the results are shown in Table 2.2.



A conditions : t-BuOH/H_2O, $CuSO_4$ $5H_2O$, sodium ascorbate, T = 60 C°
B conditions : THF anhydrous, CuI, DIPEA

Azide	R^1	R^2	Conditions	Product	y% **cat.**	y% **stoich.**
2.1a-(α)	OH	OH	**A**	**2.8a-(α)**	27	82
2.1a-(α)	OH	OH	**B**	**2.8a-(α)**	30	51
2.1b-(α)	H	OH	**A**	**2.8b-(α)**	33	73
2.1b-(α)	H	OH	**B**	**2.8b-(α)**	33	48
2.1c-(α)	H	H	**A**	**2.8c-(α)**	-	-
2.1c-(α)	H	H	**B**	**2.8c-(α)**	-	-
2.1a-(β)	OH	OH	**A**	**2.8a-(β)**	10	63
2.1a-(β)	OH	OH	**B**	**2.8a-(β)**	-	28
2.1b-(β)	H	OH	**A**	**2.8b-(β)**	23	65
2.1b-(β)	H	OH	**B**	**2.8b-(β)**	-	-
2.1c-(β)	H	H	**A**	**2.8c-(β)**	-	-
2.1c-(β)	H	H	**B**	**2.8c-(β)**	-	-

Table 2.2. A & **B** conditions for click reaction.

It is odd to note that the azides **2.1c** deriving from lithocholic acid were found to be unreactive, while those deriving from cholic and deoxycholic acid showed conversion in all the conditions used. The use of stoichiometric quantities of Cu led to a significant increase in yields in almost all cases. The explanation for this trend can be found in the reaction kinetics. A study of CuAAC reactions in the absence of ligands, such as TBTA (tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine), has shown that an excess of copper leads the reaction to be of zero order with respect to the metal, making the turnover of the catalyst unnecessary. Conversely, a catalytic amount of copper makes the reaction second-order with respect to copper, lowering the overall rate and yield of the reaction.[119]

Comparing the yields obtained in conditions A and B, generally, the reactions with $CuSO_4$ pentahydrate as catalyst showed better yields than the use of copper iodide. This is due to the fact that the iodide ion remains a good binder for Cu(I), and can interfere with the formation of the acetylide intermediate,[120] leading to slower reactions and lower yields.

Regarding the influence of the configuration at C-3, the yield data appear to be better for the

azides **2.1a-b**. These data confirm previous results obtained in the recent past.

So, in light of this, it has been hypothesized that the cause of this trend is due to the aggregation phenomena of compounds **2.1**, which form micelles in which the $-N_3$ group is, in the case of β azides, more cluttered than the other epimer; this assumption, however, has still to be confirmed (Figure 2.4).

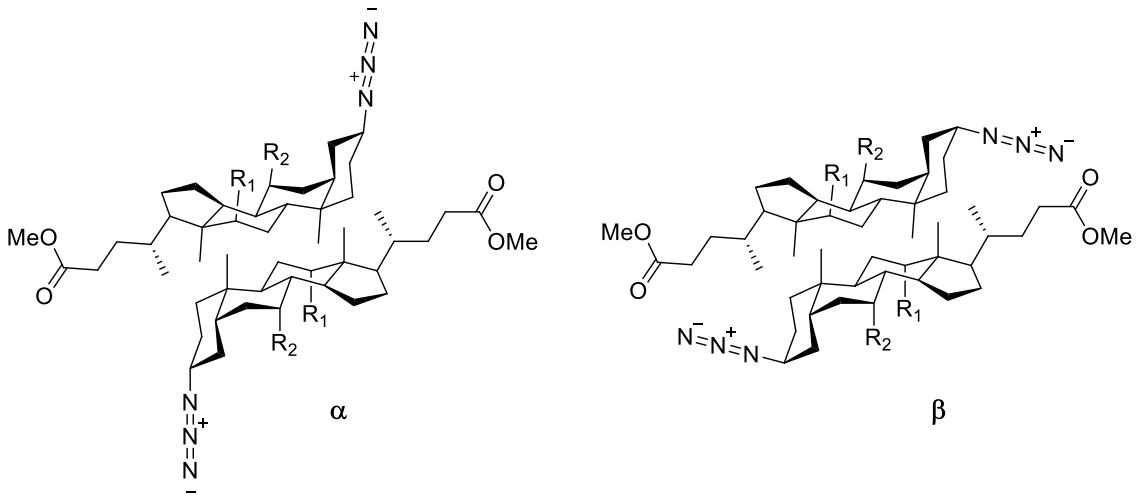
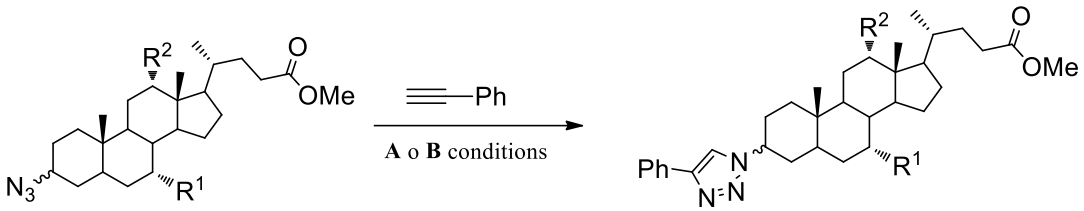


Figure 2.4. Possible aggregation phenomena between azide molecules.

We then switched to the use of an alkyne bearing an aromatic substituent, the phenylacetylene **2.2**. The click reactions were then performed between compounds **2.1a-c(α)** and **2.1a-c(β)** and the above-mentioned alkyne, under the same conditions adopted for the reactions with 1-heptine. The results are reported in Table 2.3.



A conditions : t-BuOH/H_2O, $CuSO_4$ $5H_2O$, sodium ascorbate, T= 60 C°
B conditions : THF anhydrous, CuI, DIPEA

Azide	R^1	R^2	Conditions	Product	y% **cat**	y% **stoich.**
2.1a-(α)	OH	OH	**A**	**2.3a-(α)**	-	50
2.1a-(α)	OH	OH	**B**	**2.3a-(α)**	42	74
2.1b-(α)	H	OH	**A**	**2.3b-(α)**	10	68
2.1b-(α)	H	OH	**B**	**2.3b-(α)**	34	76
2.1c-(α)	H	H	**A**	**2.3c-(α)**	20	36
2.1c-(α)	H	H	**B**	**2.3c-(α)**	41	58
2.1a-(β)	OH	OH	**A**	**2.3a-(β)**	-	82
2.1a-(β)	OH	OH	**B**	**2.3a-(β)**	14	35
2.1b-(β)	H	OH	**A**	**2.3b-(β)**	-	89
2.1b-(β)	H	OH	**B**	**2.3b-(β)**	22	24
2.1c-(β)	H	H	**A**	**2.3c-(β)**	-	-
2.1c-(β)	H	H	**B**	**2.3c-(β)**	10	20

Table 2.3. Click reaction results in **A** and **B** conditions.

In addition to the effects previously discussed, consistent also for the reactions with phenylacetylene, the presence of an aromatic group, which can conjugate and therefore stabilize the triazole ring of the final product, seems to significantly increase the yields of the CuAAC reactions, both for the stereoisomers α than for β, which show comparable yields.
As in the previous case, the reaction appears to present problems in yields when catalytic amounts of copper are used.
Therefore, in addition to conducting reactions (**B** conditions) with increasing quantities of copper to evaluate the increase in yields (Figure 2.5), methodological changes were also made regarding the timing and manner of adding copper sulphate and sodium ascorbate.

Cu^{++} (% mol)	Yields (%)
5	11
20	33
40	58
60	65
80	77
100	82

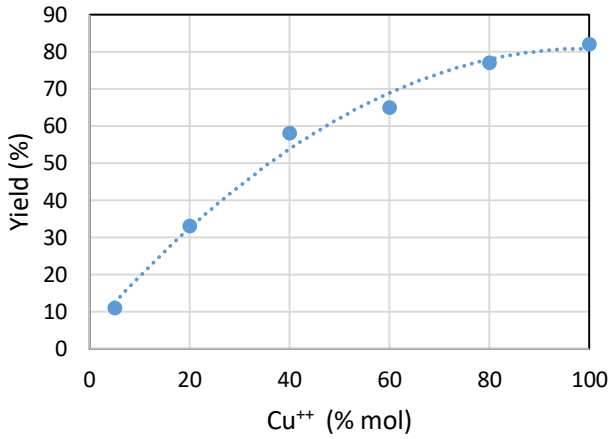


Figure 2.5. Yield of the click reaction as a function of the quantity of Cu^{++}.

Other tests have seen coordinating ions such as Mg^{2+} and Ca^{2+} introduced into the reaction system in order to evaluate their possible influence on the reaction yields. In these tests we saw that the Ca^{2+} ion, as CaCl, does not influence the conversion of the reaction in any way, while the Mg^{2+} ion, as $MgSO_4$, provides a slight increase in yields when present together with the reagents (Table 2.4).

Cu^{++} (% mol)	Mg^{++} (eq)	Ca^{++} (eq)	Yield (%)
5	/	/	11
5	0.5	/	19
5	1	/	35
5	/	0.5	12

5	/	1	10
20	/	/	33
20	0.5	/	44
20	1	/	51
20	/	0.5	30
20	/	1	28

Table 2.4. Test involving the use of Mg^{++} and Ca^{++}.

To find an ideal reaction condition that could also be suitable for subsequent reactions aimed at synthesizing further aromatic conjugates, tests were also carried out in other solvents or solvent mixtures: the reaction was then repeated using Cu^{2+} at 20% and carrying out the reaction in 100% THF with a yield of 48% and in a mixture THF/H_2O = 7:3 with a yield of 76%.

Finally, the best conditions, also considering the amount of copper, i.e. 5% (the minimum amount used), were found in a test in which the solutions of $CuSO_4$ and sodium ascorbate were prepared by dissolving the powders in two separate aliquots of 0.5 mL of H_2O and then together and suddenly added in the reaction system.

2.4 Synthesis of various aromatic conjugates

From the positive results obtained from the first optimization tests it was evident the possibility of conjugating aromatic rings with the steroid skeleton in a fairly simple way, even though it remained to be verified whether this reaction was equally efficient with alkynes with a more extensive aromaticity or with more complex aromatic alkynes.

Therefore, we initially dealt with synthesizing alkynes with greater conjugation and a greater steric hindrance such as naphthylacetylene and anthracenylacetylene (Figure 2.6).

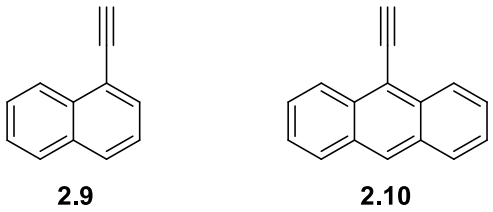
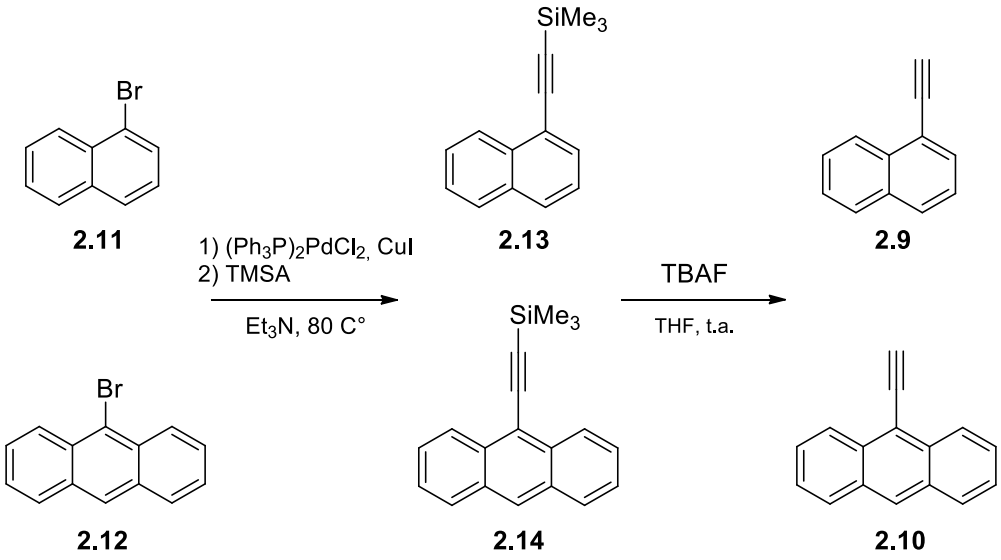


Figure 2.6. Naphtylacetylene and anthracenylacetylene with a more extensive aromaticity.

In this sense, starting from 1-bromonaphthalene and 9-bromoanthracene, through a Sonogashira reaction at 80°C and under argon atmosphere with TMSA in the presence of $(Ph_3P)_2PdCl_2$, CuI and using Et_3N as a solvent, we obtained the alkyne derivatives we were interested in with a yield of 86% and 74% respectively.

Subsequently the TMS group was easily removed with TBAF in anhydrous THF. Compounds **2.9** and **2.10** were then obtained after purification on a chromatographic column with a yield of 93% and 90% respectively (Scheme 2.8).



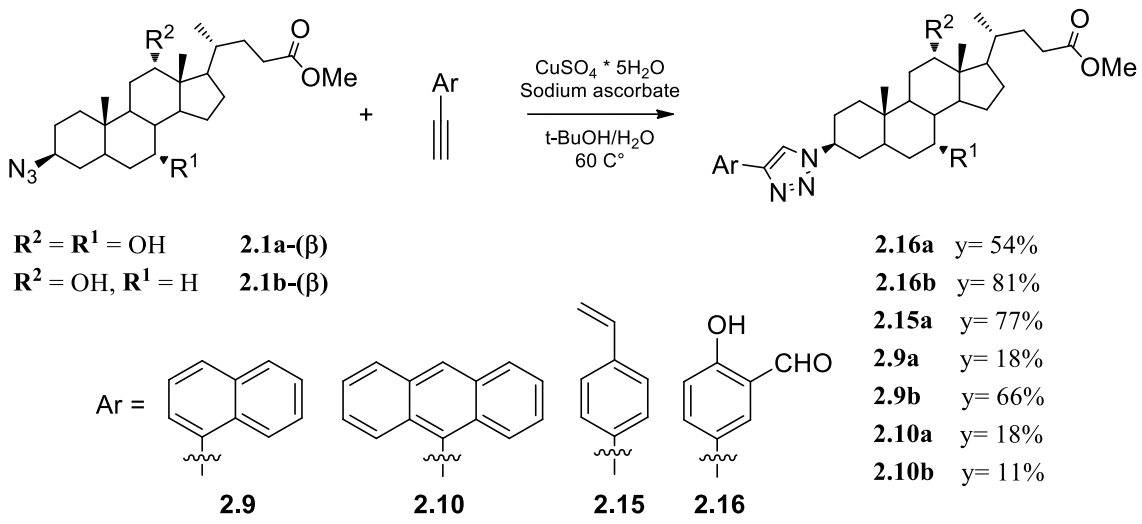
Scheme 2.8. Naphtylacetylene and anthracenylacetylene synthesis.

Furthermore, for more complex alkynes we have instead chosen bromoderivatives such as 4-bromostyrene and *p*-bromosalicylaldehyde.

For the first reagent the reaction conditions were the same with the difference that the temperature required for the Sonogashira reaction was slightly lower (50 °C), while for the latter MeOH was used as solvent in the desilylation reaction.

Once the necessary substrates were obtained, we proceeded with the click reactions.

Compounds **2.1a** and **2.1b** were chosen as azide substrates, having reported the highest yields in the studies made so far. The choice of the reaction conditions was therefore dictated by the collected data, which highlighted the optimal reaction conditions in aqueous environment and stoichiometric quantities of $CuSO_4$ dissolving it and sodium ascorbate in two separate aliquots of 0.5 mL of H_2O and then together and suddenly added in the reaction system. Compounds **2.9a,b**, **2.10a,b**, **2.15a** and **2.16a,b** were thus obtained with yields reported in Scheme 2.9, after purification on a chromatographic column.



Scheme 2.9. Click reaction on more complex alkynes.

Many aromatic derivatives were then synthesized proving the efficiency and the versatility of this method, also studying and optimizing Click reaction conditions in order to obtain higher yields in respect of the literature data, also aiming at the use of greener approaches than those in which THF and N,N-diisopropylethylamine are usually used.

2.5 Experimental section

All commercial compounds and solvents used were purchased from Sigma-Aldrich and used without further purification. All dry solvents were distilled following standard procedures before use. The ^{1}H and ^{13}C NMR spectra were performed with the Bruker 400 spectrometer, operating at 400 MHz for ^{1}H and at 100 MHz for ^{13}C using 5mm diameter tubes in $CDCl_3$, MeOD and DMSO-d6. The progress of the reactions was followed by thin layer chromatography (TLC) using Merck Kieselgel 60 F254 silica gel plates (0.25mm thick). The products were revealed on a plate using as indicators:

- 12% phosphomolybdic acid;
- Sulfuric acid (95:5) in H2O;
- UV lamp (λ = 254 nm).

Products isolation and purification by chromatography were carried out by chromatography column using Merck-Kieselgel 60 silica gel (0.063-0.20 mm, 70-230 mesh) as the stationary phase.

High resolution ESI mass spectra were carried out on a Q-TOF Micro spectrometer operating in a positive ion mode.

Synthesis of methyl esters 2.6 starting from the corresponding acids

The desired bile acid was dissolved in abundant MeOH in a one-necked flask fitted with bubble refrigerant. Subsequently, drop by drop, 0.1 mL of HCl (37%) were added and the mixture was refluxed for 1 hour. The reaction mixture was then brought to room temperature and the clean product is crystallized at -20 ° C overnight. The ester obtained was then vacuum filtered, washed with abundant and cold MeOH and dried. The corresponding esters were obtained with a **2.6a**: 70%; **2.6b**: 92%; **2.6c**: 58% yield.

Esters mesylation through the Mitsunobu reaction (configuration retention)

In a three-necked flask equipped with a calcium chloride valve and a refrigerating column and previously flamed under a flow of argon, 1 eq. of ester in anhydrous THF, 2 eq. of DMAP and 3 eq. of Ph_3P were dissolved. Then 2 eq. of $MeSO_3H$ and 3 eq. of DIAD were added drop by drop and the reaction was left under magnetic stirring for 24h. The reaction was controlled by TLC using Petroleum Ether:AcOEt = 7:3 as eluent mixture while over time the solution changes from white to yellow and releasing heat. Once the reaction is complete, the solvent is then evaporated at reduced pressure until an orange oily residue is

obtained, used directly for the next synthetic stage.

Mesylation of esters by nucleophilic substitution

In a 3-necked flask, equipped with bubble refrigerant, calcium chloride valve and dripping funnel, previously flamed, 1 eq. of methyl ester was dissolved in 4 mL of anhydrous CH_2Cl_2. 2 eq. of Et_3N were added at 0 °C and, drop by drop, also a solution composed of 1.1 eq. of MsCl in 3.5 mL of anhydrous CH_2Cl_2. The reaction mixture was left under stirring at room temperature and then controlled by TLC. Once the reaction was complete, the mixture was transferred to a separatory funnel, 15 mL of water was added and extracted with ethyl acetate (3 x 15 mL). The organic phase was washed with a saturated solution of NaCl and dried over Na_2SO_4. The drying agent was then removed by filtration and the solvent by evaporation at reduced pressure. In this way, an oily crude containing a degree of purity sufficient to be used entirely in the subsequent synthetic stage was obtained.

Synthesis of azide derivatives from the Mitsunobu products

The crude residue of mesylates **2.7** obtained from the Mitsunobu reaction was dissolved, in a 25 mL flask, in 5 mL of anhydrous DMF. 3 eq of NaN_3 were added to the solution. The reaction mixture was left under stirring, at room temperature and argon atmosphere for 48 hours and the trend was controlled by TLC. Once the reaction is complete, the mixture was transferred to a separatory funnel, 15 mL of water was added and extracted with ethyl acetate (3 x 15 mL). The organic phase was washed with a saturated solution of NaCl and dried over Na_2SO_4. The drying agent was then removed by filtration and the solvent by evaporation at reduced pressure. An oily residue was thus obtained which was, in the case of compounds **2.1a-(α)** and **2.1b-(α)**, purified by means of a chromatographic column (silica, petroleum ether/ethyl acetate = 6:4). In the case of the compound **2.1c-(α)**, the purification was performed with a series of washes in petroleum ether, which selectively solubilized the azide compound to the detriment of impurities. The desired compounds were obtained **2.1a-(α)**: 54%; **2.1b-(α)**: 57%; **2.1c-(α)**: 27%.

Synthesis of azide derivatives from the nucleophilic substitution

The crude residue of **2.7a-c(α)** mesylates was dissolved in anhydrous DMF in a one-necked flask fitted with a bubble cooler. 9 eq. of NaN_3 were added to the solution. The mixture was left under stirring in argon atmosphere at 80 °C for 24 hours. The progress of the reaction

was controlled by TLC. Once the reaction was complete, the mixture was transferred to a separatory funnel, 15 mL of water was added and extracted with ethyl acetate (3 x 15 mL). The organic phase was washed with a saturated solution of NaCl and dried over Na_2SO_4. The drying agent is then removed by filtration and the solvent by evaporation at reduced pressure. In this way, after purification on a chromatographic column, the desired compounds were obtained **2.1a-(β)**: 76%; **2.1b-(β)**: 80%; **2.1c-(β)**: 83%.

Synthesis of conjugates derivatives through click reaction in aqueous conditions

In a single-necked flask equipped with a bubble cooler, 1 eq. of azide and 1eq. of alkyne were transferred in a solution of t-BuOH/H_2O in a 7:3 ratio. Subsequently, 1.5 eq. of sodium ascorbate and 1 eq. of copper sulphate pentahydrate, working in stoichiometric conditions, were added to this mixture. In the procedure with catalytic quantities, 0.3 eq of copper sulphate and 0.5 eq. of sodium ascorbate were added. The reaction was brought to 60 °C and left under stirring for 24 hours. The progress of the reaction was controlled by TLC. Once the reaction was complete, the mixture was transferred to a separatory funnel, 15 mL of water was added and extracted with ethyl acetate (3 x 15 mL). The organic phase was washed with a saturated solution of NaCl and dried over Na_2SO_4. The drying agent was then removed by filtration and the solvent by evaporation at reduced pressure, obtaining the desired compounds after purification in a chromatographic column.

Synthesis of conjugates derivatives in THF conditions

In a single-necked flask 1 eq. of azide and 1 eq. of alkyne were dissolved in anhydrous THF. Subsequently, 1 eq. of CuI were added into the reaction flask if operating under stoichiometric conditions or 0.3 eq. of CuI if operating in catalytic conditions. The solution turned yellow and at this point 13 eq. of DIPEA were added. The reaction was left under magnetic stirring at room temperature for 16 hours. The progress of the reaction was controlled by TLC. Once the reaction is complete, the mixture was transferred to a separatory funnel, 15 mL of water was added and extracted with ethyl acetate (3 x 15 mL). The organic phase was washed with a saturated solution of NaCl and dried over Na_2SO_4. The drying agent was then removed by filtration and the solvent by evaporation at reduced pressure. An oily residue was thus obtained which was purified by means of a chromatographic column obtaining the desired compounds.

Sonogashira reaction procedure for 2.9(naftalene) and 2.10(antracene) derivative synthesis

In a Schlenk tube, previously flamed and placed under an inert atmosphere of argon, 1 eq. of bromoderivative, 0.03 eq. of $(Ph_3P)_2PdCl_2$ catalyst and 0.05 eq. of cuprous iodide were added in 15 mL of anhydrous Et_3N. The mixture was heated to 80 °C and subsequently 1.5 eq. of TMSA were added in the hermetically sealed tube. The reaction was then left hot under magnetic stirring for 4 hours. Reaction progress was controlled by TLC (petroleum ether/ethyl ether = 95:5). Once the reaction was complete, filtration was carried out using a Gooch funnel, in which a 3 cm layer of celite was deposited, washing the filtering layer with abundant AcOEt. At this point, the crude was purified in a chromatographic column. Compounds **2.9** and **2.10** were thus obtained with a yield of 86% and 74%.

Sonogashira reaction procedure for 4-(trimethylsilyl)ethinylstyrene derivative synthesis

In a Schlenk tube, equipped with a magnetic stir bar, protected from light and under an argon atmosphere, 10 mL of anhydrous Et_3N, 1 eq. of 4-bromostyrene, 2.3 eq. of trimethylsilylacetylene (TMSA) and 0.03 eq. of $(Ph_3P)_2PdCl_2$ were added. The reaction mixture was then brought to 40 °C and after 5 minutes 0.05 eq. of cuprous iodide were added. Instantly, a change in the solution to black was noticed. The mixture was then left under stirring, under a flow of Argon and at 75 °C for 48 hours. The mixture was monitored throughout the reaction time by TLC in pure hexane. Once the reaction was over, the triethylammonium precipitate was filtered, the solvent was evaporated under reduced pressure. A brown crude which was then purified by chromatographic column using pure hexane as eluent mixture was obtained.

The pure product appears as an oily residue with a yield of 81%.

Sonogashira reaction procedure for 2-hydroxy-5-((trimethylsilyl)-ethynyl)benzaldehyde derivative synthesis

In a Schlenk tube, previously flamed and placed under an inert atmosphere of argon, 1 eq. of 5-bromosalicylaldehyde, 0.03 eq. of Pd catalyst and 0.05 equivalents of cuprous iodide in 15 mL of anhydrous Et_3N were added. The mixture was heated to 80 °C and subsequently 1.5 equivalents of TMSA were added in the hermetically sealed tube. The reaction was then

left under magnetic stirring for 4 hours. Reaction progress was controlled by TLC (petroleum ether/ethyl ether = 95:5). Once the reaction was complete, filtration was carried out using a Gooch funnel, in which a 3 cm layer of celite was deposited, washing the filtering layer with abundant AcOEt. At this point, the crude was purified by chromatographic column. Final product was thus obtained with a yield of 87%.

2.6 Characterization data

Cholic acid methyl ester

^{1}H-NMR (300 MHz, $CDCl_3$) δ(ppm): 0.66 (s, 3H); 0.87 (s, 3H); 0.96 (d, 3H, 3J= 6,0 Hz); 1.04-2.41 (mc, 24 H, steroidic backbone and side chain); 3.06(s, 3H); 3.46 (m, 1H); 3.65 (s, 3H); 3.83 (m, 1H); 3.94 (m, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ(ppm): 12.56, 17.40, 22.54, 23.30, 26.45, 27.59, 28.26, 30.50, 30.99, 34.74, 34.84, 35.36, 39.59, 41.56, 41.74, 46.52, 47.09, 51.58, 68.54, 71.98, 73.14, 174.90. MS (ESI): m/z (%) 422.3038 $[M+H]^+$.

Deoxycholic acid methyl ester

^{1}H-NMR (300 MHz, $CDCl_3$) δ(ppm): 0.66 (s, 3H), 0.89 (s, 3H), 0.94 (d, 3H, 3J= 6,0 Hz), 0.99-2.10 (mc, 26 H, steroidic back bone, side chain and 2 OH), 2.12-2.42 (m, 2H), 3.58 (m, 1H), 3.65 (s, 3H), 3.96 (bs, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ(ppm): 12.69, 17.24, 23.09, 23.62, 26.09, 27.09, 27.44, 28.57, 30.34, 30.86, 31.07, 33.56, 34.08, 35.13, 35.19, 35.97, 36.34, 42.02, 46.44, 47.22, 48.19, 51.48, 71.70, 73.10, 174.74. MS (ESI): m/z (%) 406.3087 $[M+H]^+$.

(4R)-methyl 4-((3R,7R,10S,12S,13R)-7,12-dihydroxy-10,13-dimethyl-3-((methylsulfonyl)oxy)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 0.66 (s, 3H), 0.88 (s, 3H), 0.95 (d, 3H, 3J= 6,0 Hz), 1.01-2.46 (mc, 26H, steroidic backbone, side chain and 2 OH), 2.97 (s, 3H), 3.64 (s, 3H), 3.84 (m, 1H), 3.96 (m, 1H), 4.48 (m, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 12.48, 17.31, 22.26, 23.13, 26.48, 27.45, 27.91, 28.17, 30.81, 31.03, 34.20, 34.46, 34.78, 35.21, 36.02, 38.84, 39.38, 41.40, 41.80, 46.49, 47.16, 51.54, 68.11, 72.86, 82.90, 174.80. MS (ESI): m/z (%) 500.2813 $[M+H]^+$.

(4R)-methyl 4-((3R,10S,12S,13R)-12-hydroxy-10,13-dimethyl-3-((methylsulfonyl)oxy)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 0.65 (s, 3H), 0.89 (s, 3H), 0.95 (d, 3H, 3J=6,04 Hz), 1.01-2.41 (mc, 27 H, steroidic backbone, side chain and 1 OH), 2.99 (s, 3H), 3.64 (s, 3H), 3.97 (m, 1H), 4.62 (m, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 12.71, 17.30, 22.90, 23.13, 25.90, 26.77, 27.40, 27.68, 28.61, 30.84, 31.04, 33.25, 33.55, 33.88, 34.81, 35.03, 35.88, 38.87, 42.07, 46.45, 47.30, 48.13, 51.51, 72.93, 82.72, 174.67. MS (ESI): m/z (%) 484.2863 $[M+H]^+$.

(4R)-methyl 4-((3R,7R,10S,12S,13R)-3-azido-7,12-dihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

^{1}H-NMR (300 MHz, $CDCl_3$) δ(ppm): 0.67 (s, 3H), 0.91 (s, 3H), 0.97 (d, 3H, ^{3}J= 6,0 Hz), 1.09-2.56 (mc, 26 H, steroidic backbone, side chain and 2 OH), 3.15 (m, 1H), 3.65 (s, 3H), 3.85 (m, 1H), 3.96 (m, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ(ppm): 12.42, 17.27, 21.81, 22.51, 23.15, 26.80, 27.45, 28.20, 30.82, 34.57, 35.25, 39.61, 41.86, 46.52, 47.20, 51.40, 61.32, 68.21, 70.02, 70.29, 72.11, 72.98, 76.56, 77.41, 174.70. MS (ESI): m/z (%) 447.3102 $[M+H]^+$.

(4R)-methyl 4-((3R,10S,12S,13R)-3-azido-12-hydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 0.68 (s, 3H), 0.93 (s, 3H), 0.98 (d, 3H, 3J= 6,4 Hz), 1.07-1.95 (mc, 25 H, steroidic backbone, side chain and 1 OH), 2.12-2.42 (m, 2H), 3.34 (m, 1H), 3.67 (s, 3H), 3.99 (bs, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 12.73, 17.32, 23.23, 23.59, 26.02, 26.68, 27.02, 27.41, 28.69, 30.87, 31.04, 32.44, 33.67, 34.18, 35.05, 35.39, 35.98, 43.35, 46.48, 47.33, 48.15, 51.50, 61.24, 73.03, 174.67. MS (ESI): m/z (%) 431.3159 $[M+H]^+$.

(4R)-methyl 4-((3R,10S,13R)-3-azido-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 0.64 (s, 3H), 0.91 (m, 6H), 0.96-2.39 (mc, 28 H, steroidic backbone and side chain), 3.66 (s, 3H), 3.85. ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 12.01, 18.24, 20.80, 23.54, 24.20, 26.37, 26.92, 27.35, 27.16, 28.18, 30.99, 31.05, 32.62, 34.71, 35.36, 35.51, 35.81, 40.16, 40.23, 42.22, 42.71, 55.94, 56.45, 61.24, 174.74. MS (ESI): m/z (%) 415.3203 $[M+H]^+$.

(4R)-methyl 4-((3S,7R,10S,12S,13R)-3-azido-7,12-dihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

^{1}H-NMR (300 MHz, $CDCl_3$) δ(ppm): 0.67 (s, 3H), 0.91 (s, 3H), 0.97 (d, 3H, 3J= 6,0 Hz), 1.09-2.56 (mc, 26 H, steroidic backbone, side chain and 2 OH), 3.65 (s, 3H), 3.84 (m, 1H), 3.88 (s, 1H), 3.96 (m, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ(ppm): 12.48, 17.31, 22.82, 23.18, 24.54, 26.12, 27.46, 28.44, 30.48, 31.83, 31.07, 33.01, 34.15, 35.08, 35.19, 36.75, 39.40, 41.87, 46.54, 47.25, 51.49, 58.71, 68.41, 72.98, 174.69. MS (ESI): m/z (%) 447.3099 $[M+H]^+$.

(4R)-methyl 4-((3S,10S,12S,13R)-3-azido-12-hydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

^{1}H-NMR (300 MHz, $CDCl_3$) δ(ppm): 0.65 (s, 3H), 0.94 (d, 3H, 3J=5,72 Hz), 0.88 (s, 3H), 0.98-2.40 (mc, 27 H, steroidic backbone, side chain and 1 OH), 3.58 (m, 1H), 3.64 (s, 3H), 3.95 (m, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ(ppm): 12.69, 17.24, 23.11, 23.64, 26.10, 27.11, 27.46, 28.61, 30.87, 31.08, 33.58, 34.09, 35.16, 35.21, 35.99, 36.37, 42.04, 46.45, 47.22, 48.19, 51.47, 58.86, 73.07, 174.71. MS (ESI): m/z (%) 431.3153 $[M+H]^+$.

(4R)-methyl 4-((3S,10S,13R)-3-azido-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

^{1}H-NMR (300 MHz, $CDCl_3$) δ(ppm): 0.63 (s, 3H), 0.89 (d, 3H, 3J=6,31), 0.93 (s, 3H), 1.02-2.39 (mc, 28 H, steroidic backbone and side chain), 3.65 (s, 3H), 3.94 (m, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ(ppm): 12.03, 18.25, 20.98, 23.76, 24.15, 24.68, 26.26, 26.51, 28.16, 30.17, 30.65, 30.98, 31.03, 34.94, 35.34, 35.62, 37.29, 40.11, 40.15, 42.72, 51.46, 55.97, 56.56, 58.76, 174.72. MS (ESI): m/z (%) 415.3202 $[M+H]^+$.

(4R)-methyl 4-((3R,7R,10S,12S,13R)-7,12-dihydroxy-10,13-dimethyl-3-(4-pentyl-1H-1,2,3-triazol-1-yl)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

^{1}H-NMR (300 MHz, $CDCl_3$) δ(ppm): 0.67 (s, 3H), 0.90 (d, 1H), 0.91 (s, 3H), 0.97 (d, 3H, 3J= 6,0 Hz), 1.09-2.56 (mc, 34 H, steroidic backbone, side chian and 2 OH), 3.65 (s, 3H), 3.84 (m, 1H), 3.88 (s, 1H), 5.20 (m, 1H), 7.55 (s, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ(ppm): 12.48, 17.31, 22.82, 23.18, 24.54, 26.12, 27.46, 28.44, 30.48, 31.83, 31.07, 33.01, 34.15, 35.08, 35.19, 36.75, 39.40, 41.87, 46.54, 47.25, 51.49, 61.07, 68.41, 72.98, 174.69. MS (ESI): m/z (%) 543.4039 $[M+H]^+$.

(4R)-methyl 4-((3R,10S,12S,13R)-12-hydroxy-10,13-dimethyl-3-(4-pentyl-1H-1,2,3-triazol-1-yl)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

^{1}H-NMR (300 MHz, $CDCl_3$) δ(ppm): 0.67 (s, 3H), 0.90 (d, 3H), 0.91 (s, 3H), 0.97 (d, 3H, ^{3}J= 6,0 Hz), 1.09-2.56 (mc, 35 H, steroidic backbone, side chain, alkyne and 1 OH), 3.65 (s, 3H), 3.88 (s, 1H), 5.20 (m, 1H), 7.55 (s, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ(ppm): 12.48, 17.31, 22.82, 23.18, 24.54, 26.12, 27.32, 27.46, 28.44, 30.48, 31.83, 31.07, 33.01, 34.15, 35.08, 35.19, 36.75, 39.40, 41.87, 46.54, 47.25, 51.49, 61.07, 72.98, 174.69. MS (ESI): m/z (%) 527.4089 $[M+H]^+$.

(4R)-methyl 4-((3S,7R,10S,12S,13R)-7,12-dihydroxy-10,13-dimethyl-3-(4-pentyl-1H-1,2,3-triazol-1-yl)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

^{1}H-NMR (300 MHz, $CDCl_3$) δ(ppm): 0.67 (s, 3H), 0.90 (d, 3H), 0.91 (s, 3H), 0.97 (d, 3H, 3J= 6,0 Hz), 1.09-2.56 (mc, 34H, steroidic backbone, side chain, heptine and 2 OH), 3.65 (s, 3H), 3.84 (m, 1H), 3.88 (s, 1H), 5.58 (m, 1H), 7.55 (s, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ(ppm): 12.48, 17.31, 22.82, 23.18, 24.54, 26.12, 27.46, 28.44, 30.48, 31.83, 31.07, 33.01, 34.15, 35.08, 35.19, 36.75, 39.40, 41.87, 46.54, 47.25, 51.49, 58.07, 68.41, 72.98, 174.69. MS (ESI): m/z (%) 543.4039 $[M+H]^+$.

(4R)-methyl 4-((3S,10S,12S,13R)-12-hydroxy-10,13-dimethyl-3-(4-pentyl-1H-1,2,3-triazol-1-yl)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

^{1}H-NMR (300 MHz, $CDCl_3$) δ(ppm): 0.67 (s, 3H), 0.90 (d, 3H), 0.91 (s, 3H), 0.97 (d, 3H, ^{3}J= 6.0 Hz), 1.09-2.56 (mc, 35 H, steroidic backbone, side chain and 1 OH), 3.65 (s, 3H), 33.88 (s, 1H), 5.58 (m, 1H), 7.55 (s, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ(ppm): 12.48, 17.31, 22.82, 23.18, 24.54, 26.12, 26.81, 27.46, 28.44, 30.48, 31.83, 31.07, 33.01, 34.15, 35.08, 35.19, 36.75, 39.40, 41.87, 46.54, 47.25, 51.49, 58.07, 72.98, 174.69. MS (ESI): m/z (%) 527.4089 $[M+H]^+$.

(4R)-methyl 4-((3R,7R,10S,12S,13R)-7,12-dihydroxy-10,13-dimethyl-3-(4-phenyl-1H-1,2,3-triazol-1-yl)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

^{1}H-NMR (300 MHz, $CDCl_3$) δ(ppm): 0.67 (s, 3H), 0.89 (s, 3H), 0.97 (d, 3H, 3J= 6,2 Hz), 1.22-2.81 (mc, 26 H, steroidic backbone, side chain and 2 OH), 3.65 (s, 3H), 3.88 (m, 1H), 4.05 (s, 1H), 5.29 (s, 1H), 7.33-7.79 (5H, aromatic), 7.86 (m, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ(ppm): 12.49, 14.07, 17.28, 22.54, 23.14, 23.85, 26.73, 27.49, 28.33, 29.49, 29.68, 30.78, 31.06, 31.91, 34.31, 34.73, 35.43, 36.21, 39.35, 40.90, 41.98, 46.57, 47.26, 51.47, 61.07, 68.18,

73.12, 117.62, 125.67, 127.98, 128.71, 130.63, 174.78. MS (ESI): m/z (%) 549.3569 $[M+H]^+$.

(4R)-methyl 4-((3R,10S,12S,13R)-12-hydroxy-10,13-dimethyl-3-(4-phenyl-1H-1,2,3-triazol-1-yl)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

1H-NMR (400 MHz, $CDCl_3$) δ(ppm): 0.71 (s, 3H), 0.96 (d, 3H, 3J= 6,4 HZ), 1.01 (s, 3H), 1.08-2.41 (mc, 28 H, steroidic backbone, side chain and 1 OH), 3.67 (s, 3H), 4.06 (m, 1H), 7.31-7.44 (mc, 5H, aromatic), 7.83 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 12.75, 17.31, 23.23, 23.66, 26.03, 26.92, 27.47, 28.09, 28.81, 30.90, 31.12, 33.78, 33.98, 34.32, 35.15, 35.70, 36.03, 42.72, 46.53, 47.35, 48.11, 51.50, 61.01, 73.01, 117.55, 125.68, 127.98, 128.77, 130.80, 147.23, 174.78. MS (ESI): m/z (%) 533.3620 $[M+H]^+$.

(4R)-methyl 4-((3R,10S,13R)-10,13-dimethyl-3-(4-phenyl-1H-1,2,3-triazol-1-yl)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 0.69 (s, 3H), 0.94 (d, 3H, 3J= 6,4 HZ), 1.04 (s, 3H), 1.06-2.41 (mc, 29 H, steroidic backbone and side chain), 3.67 (s, 3H), 7.32-7.45 (mc, 5H, aromatic), 7.86 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 12.06, 18.28, 20.90, 23.50, 24.17, 26.32, 26.99, 28.17, 30.99, 31.06, 34.09, 34.80, 35.36, 35.83, 35.86, 40.01, 40.66, 42.74, 51.49, 55.97, 56.34, 61.17, 117.57, 125.71, 128.03, 128.79, 130.73, 147.17, 174.74. MS (ESI): m/z (%) 517.3670 $[M+H]^+$.

(4R)-methyl 4-((3S,7R,10S,12S,13R)-7,12-dihydroxy-10,13-dimethyl-3-(4-phenyl-1H-1,2,3-triazol-1-yl)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

^{1}H-NMR (300 MHz, $CDCl_3$) δ(ppm): 0.72 (s, 3H), 0.91 (s, 3H), 1.01 (d, 3H, 3J= 6,4 Hz), 1.15-2.86 (mc, 26 H, steroidic backbone and side chain), 3.69 (s, 3H), 3.93 (bs, 1H), 4.00 (bs, 1H), 7.32-7.45 (5H, aromatic), 7.88 (m, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ(ppm): 12.55, 17.37, 22.83, 23.21, 23.39, 24.94, 26.70, 27.50, 28.50, 29.69, 30.57, 30.89, 31.09, 32.58, 33.96, 34.94, 35.19, 36.83, 39.48, 41.94, 46.58, 47.31, 51.44, 57.00, 68.31, 72.97, 125.70, 128.07, 128.82, 130.63, 174.73. MS (ESI): m/z (%) 549.3570 $[M+H]^+$.

(4R)-methyl 4-((3S,10S,12S,13R)-12-hydroxy-10,13-dimethyl-3-(4-phenyl-1H-1,2,3-triazol-1-yl)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

^{1}H-NMR (300 MHz, $CDCl_3$) δ(ppm): 0.71 (s, 3H), 0.93 (s, 3H), 1.00 (d, 3H, 3J= 6,4 Hz), 1.08-2.50 (mc, 28 H, steroidic backbone and side chain), 3,69 (s, 3H), 4.04 (s, 1H), 7.32-7.46

(5H, aromatic), 7.87 (m, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ(ppm): 12.76, 17.38, 23.53, 23.58, 24.90, 25.84, 26.40, 27.45, 28.77, 29.89, 30.56, 30.89, 31.07, 33.63, 34.36, 35.06, 35.82, 37.30, 46.55, 47.44, 48.35, 51.53, 56.81, 73.16, 118.82, 125.69, 127.99, 128.80, 130.82, 147.16, 174.70. MS (ESI): m/z (%) 533.3617 $[M+H]^+$.

(4R)-methyl 4-((3S,10S,13R)-10,13-dimethyl-3-(4-phenyl-1H-1,2,3-triazol-1-yl)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 0.69 (s, 3H), 0.94 (d, 3H, 3J= 6,4 Hz), 1.04 (s, 3H), 1.06-2.41 (mc, 29 H, steroidic backbone and side chain), 3.67 (s, 3H), 7.32-7.45 (mc, 5H, aromatic), 7.86 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 12.06, 18.29, 21.03, 23.27, 23.79, 26.15, 26.48, 27.37, 27.93, 28.19, 28.42, 28.97, 29.70, 29.96, 30.50, 30.79, 30.95, 31.01, 34.85, 35.38, 37.33, 40.54, 42.78, 51.50, 56.03, 56.61, 125.74, 128.13, 128.84, 130.63, 174.74. MS (ESI): m/z (%) 517.3671 $[M+H]^+$.

2-hydroxy-5-((trimethylsilyl)ethynyl)benzaldehyde

^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 0.23 (s, 9H), 6.92 (d, 1H, J=8.65), 7.59 (dd, 1H, J=2.01, 8.63 Hz), 7.69 (d, 1H, J=2.0 Hz), 9.84 (s, 1H), 11.09 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ(ppm): -0.09, 93.79, 103.13, 115.09, 117.93, 120.31, 137.33, 140.11, 161.49, 195.98. MS (ESI): m/z (%) 218.0766 $[M+H]^+$.

5-ethynyl-2-hydroxybenzaldehyde

^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 3.04 (s, 1H), 6.95 (d, 1H, 3J=8.63), 7.62 (d, 1H, 3J=8.63 Hz), 7.72 (s, 1H), 9.86 (s, 1H), 11.19 (s, 1H, -OH). ^{13}C NMR (100 MHz, $CDCl_3$) δ(ppm): 77.43, 81.86, 113.97, 118.11, 120.38, 137.48, 140.16, 161.81, 195.91. MS (ESI): m/z (%) 146.0372 $[M+H]^+$.

(4R)-methyl 4-((3S,7R,10S,12S,13R)-3-(4-(3-formyl-4-hydroxyphenyl)-1H-1,2,3-triazol-1-yl)-7,12-dihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 0.70 (s, 3H), 0.91 (s, 3H), 0.98 (d, 3H, J= 6,0 Hz), 1.03-2.42 (mc, 27 H, steroidic backbone and side chain), 3.67 (s, 3H), 3.86 (bs, 1H), 4.00 (bs, 1H), 4.96 (m, 1H), 6.97 (d, 1H, J= 8,8 Hz), 7.63 (d, 1H, J= 8,8 Hz), 7.73 (bs, 1H), 9.87 (m, 1H), 11.03 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 12.48, 17.31, 21.71, 21.95, 22.57, 23.19,

27.50, 28.20, 30.82, 31.04, 34.55, 34.77, 35.28, 35.37, 35.47, 39.41, 41.84, 41.89, 46.54, 47.20, 51.53, 61.33, 68.24, 73.01, 118.12, 137.51, 140.19, 161.72, 174.83, 195.98. MS (ESI): m/z (%) 593.3468 $[M+H]^+$.

(4R)-methyl 4-((3S,10S,12S,13R)-3-(4-(3-formyl-4-hydroxyphenyl)-1H-1,2,3-triazol-1-yl)-12-hydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 0.70 (s, 3H), 0.91 (s, 3H), 0.98 (d, 3H, J= 6,0 Hz), 1.03-2.42 (mc, 29 H, steroidic backbone and side chain), 3.67 (s, 3H), 4.00 (bs, 1H), 4.96 (m, 1H), 6.97 (d, 1H, J= 8,8 Hz), 7.63 (d, 1H, J= 8,8 Hz), 7.73 (bs, 1H), 9.87 (m, 1H), 11.03 (s, 1H, -OH). ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 12.48, 17.31, 21.71, 21.95, 22.57, 23.19, 27.50, 28.20, 30.82, 31.04, 34.55, 34.77, 35.28, 35.37, 35.47, 39.41, 41.84, 41.89, 46.54, 47.20, 51.53, 61.33, 73.01, 118.12, 137.51, 140.19, 161.72, 174.83, 195.98. MS (ESI): m/z (%) 577.3519 $[M+H]^+$.

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Chapter 3: Effective and Green Promotion of Proline-Catalyzed Aldol Reaction in Water by Bile Acid Salt Surfactants

3.1 Introduction

Green Chemistry is a relatively new emerging field that has the goal to work at the molecular level to achieve sustainability. This field has received widespread interest in the past decade due to its ability to harness chemical innovation to meet environmental and economic goals simultaneously. It is defined as the "design of chemical products and processes to reduce or eliminate the use and generation of hazardous substances."[1,2]

Among the criteria that a green chemical process should fulfill, the use of a non-toxic solvent (or no solvent at all) is of primary importance, to minimize both reaction waste generation and environmental impact.

Such criteria would be largely fulfilled if the major component of the reaction mixture, i.e. the solvent, is water which is a suitable solvent in various reactions.

Another important principle in tailoring a green process is the choice of catalytic reactions in place of stoichiometric ones. Comparing the various kind of catalysts available, although metal catalyzed reactions[3] have wider substrate scope, they are associated with some drawbacks, such as high cost involved in the preparation of catalysts, and the toxicity of metals, which sometimes are difficult to separate from reaction products.

A good alternative is organocatalysis, which is the use of small organic molecules as catalysts. Compared to metals, these organic catalysts show to be more stable, cheap, non-toxic, readily available, and environmentally friendly. Furthermore, organocatalytic reactions are less or completely not sensitive to the presence of water or air in comparison to metal catalyzed reactions. Moreover, in contrast to biocatalysts such as enzymes, which are highly substrate specific and cannot tolerate even a minor change in the structure of the reactants, they provide broad substrate scope.[4] Again, small organic molecules are known to give high catalytic activity and stereospecificity just like enzymes and this is another reason of their use in place of biocatalysts. These relatively small organic molecules activate substrates either through strong interactions such as covalent bonding or weak interactions such as van der Waals forces or hydrogen bonding.[5,6,7] These interactions result in considerable acceleration

of the reaction rate.[8,9,10]

The notion of "green chemistry" has also encouraged synthetic organic chemists to include water as a solvent. Moreover, incredible selectivity and activity can be achieved through the addition of amphiphiles with a defined structure. Temperature and concentration can affect the surfactants self-assembly influencing the supramolecular assemblies. Reactions in presence of amphiphiles are typically conducted thanks to the formation of spherical aggregates in the nanometer range, that is, micelles.[11,12] There is a strong polarity gradient between the two surfaces possessing different affinity with the aqueous medium, and this is the reason why both nonpolar and polar reagents can be solubilized.

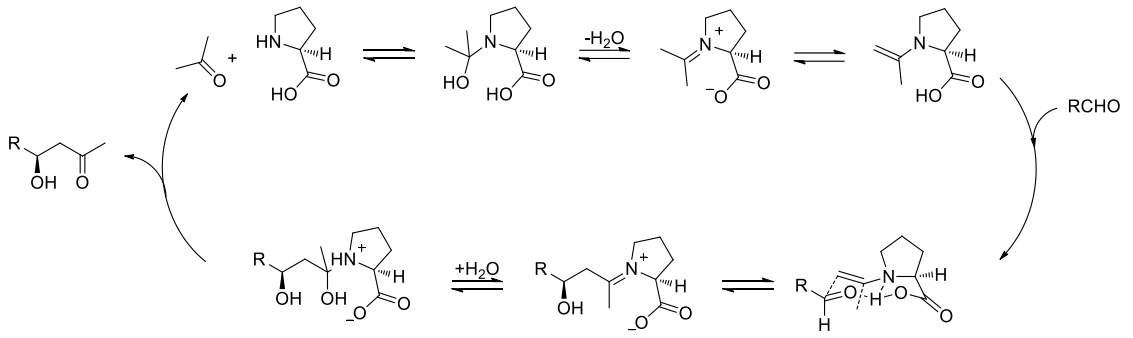
3.1.1 BAs as surfactant forming micelles

Bile salts (BSs) are the most important biologically derived detergent-like molecules. They possess two different regions characterized by two opposite polarity: the α-face is the hydrophilic region and the β-face is the hydrophobic one. Unlike conventional surfactants, BSs do not show a well-defined critical micellar concentration (cmc). Indeed, upon increase of BS concentration the transition from monomeric to micellar solution occurs stepwise in a broad region of concentrations. This process provides micelles of 2-15 BS molecules, corresponding to much lower aggregation numbers than those of conventional surfactants. The aggregation tendency strongly depends on the BS nature and it is generally greater the larger their hydrophobicity, thus increasing for example by passing from cholates to deoxycholates families. However, specific effects related to the position and to the orientation of the hydroxyl groups are also observed to significantly affect the BSs self-assembly.

In these micellar systems, as will be explained in the following paragraphs, it is possible to carry out the reactions. In the literature there are examples of reactions in micelles formed by bile acids dating back to 1974 and 1978.[58a]

3.1.2 Proline as organocatalysts in surfactants forming micelles

There are many examples of the use of small molecules as organocatalysts in literature and proline is one of them. The initial events that triggered the development of organocatalysis using this catalyst were the first works by List, Lerner and Barbas who demonstrated that proline was an excellent catalyst for intermolecular asymmetric aldol reactions (Scheme 3.1).[13,14]



Scheme 3.1. Proposed mechanism for L-Pro catalyzed Aldol reaction.

From that point, proline and its derivatives are used more and more. Despite this, there are almost no works in which proline is used in water as solvent in aldol reactions without loss of stereoselectivity and high yields. Furthermore, there are very few works in which proline is used in aqueous media in the presence of surfactants that form micelles.[15] Most commonly, proline-catalyzed processes are carried out in vigorously agitated biphasic systems, with the reaction occurring at the interphase.[16]

Recently, the need to turn towards a more eco-sustainable chemistry has prompted the chemical industry to develop suitable conditions for typical reactions of organic catalysis to take place in an aqueous environment.

Initially water, despite having peculiar characteristics such as high surface tension, high polarity and the ability to form hydrogen bonds, was not considered a suitable solvent for organocatalytic reactions, for various reasons, among which the insolubility of the great part of the organic molecules in it. In addition to this, water can in principle interfere with the transition state formed between the organocatalyst and substrate molecules, modifying the pattern of weak interactions that are created between them, thus deteriorating the catalytic activity and the stereocontrol. Therefore, reactions in water were assumed to lead to slow reaction rates and lower yields of the desired products. In this regard, it was observed that aldol reactions carried out in favorable conditions, therefore in aprotic polar solvents, in the presence of a quantity of water greater than 4%, lead to a substantial decrease in yields and enantioselectivity.

The solution to the problems discussed so far on the use of water as a solvent for organocatalytic reactions came from the addition of surfactants to the reaction mixtures.

The use of organic solvents therefore becomes unnecessary since in water the self-assembled micelles provide a lipophilic environment within them that acts as a suitable reaction environment. Furthermore, a quantity equal to 1-2% by weight of surfactant in water is

generally sufficient to easily exceed the CMC required, and therefore to have the formation of micelles a catalytic quantity of the amphiphile is sufficient.

And since the organocatalytic reactions that must be carried out in water use, as mentioned, a small molecule as a catalyst, an approach that is widely described is the synthesis of surfactants which in their structure not only have lipophilic and hydrophilic portions, but also suitable functional groups to bind the catalyst.

An example is PQS-Proline, which in water provides micelles whose diameter of 79 nm was determined via DLS (Figure 3.1).

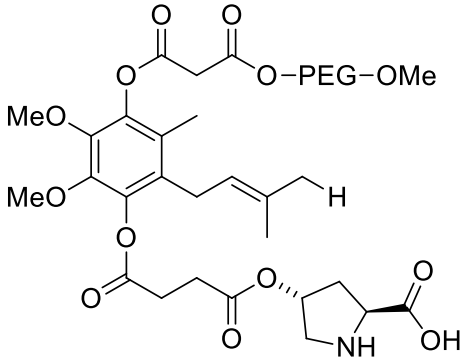


Figure 3.1. Organocatalyst PQS-Proline.

These micelles have proved to be ideal reactors within which homogeneous organic catalysis can occur, similarly to the case of aldol condensation catalyzed by the single proline molecule.

3.1.3 Reactions in presence of amphiphiles: micellar systems and microemulsions

Sometimes, as in everyday things, there are incompatibilities between reacting species. There are also ways to solve these problems. A solution could be find a solvent or a solvent combination capable to dissolve both the hydrophilic and lipophilic species.

A wide number of aprotic solvent is very useful to overcome the solubilization problem but these are usually unsuitable for large scale work due to high cost, toxicity and high boiling temperature non very fit for vacuum evaporation.

Another solution could be carrying out the reactions in a mixture of two immiscible solvent under vigorous stirring, and in presence of a phase-transfer reagent, in particular quaternary ammonium compounds, which may increase the contact surface between the phases.

A simpler approach can be the use of microemulsions or a related type of organized solution such as micellar systems. The former are excellent "solvent" both for hydrophobic organic

compounds and for inorganic salts or entities wich not solubilize in organic media and being macroscopically homogeneous but microscopically dispersed, they can be placed between the solvent-based one-phase systems and the true two-phase systems. Furthermore microemulsions have found many technical applications due to their capabilities to solubilize a very large spectrum of substances in one single formulation; not only this can be a way to overcome compatibility problems, but the ability of microemulsions to compartimentalize and concentrate reactants can also lead to rate enhancement[17-19] or inhibition compared to one-phase system reaction rates (this phenomenon was recognized relatively early on and was investigated extensively, as reflected in early review articles and a monograph published in 1975)[20a,b] and it can be exploited to induce regioselectivity.[21-34]

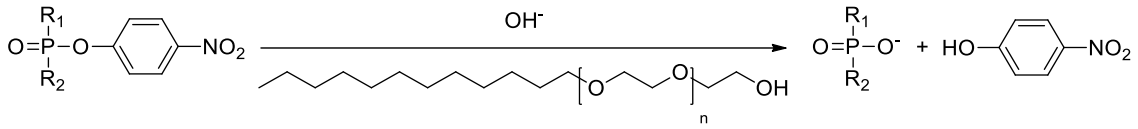
Microemulsions and micellar solution have much in common. They are both thermodynamically stable, which means that they will remain one-phase systems for ever (provided the surfactants stay intact). They form spontaneously when the components are mixed. They are transparent, usually low viscous, and they are both highly dynamic systems. Microemulsions can be regarded as micellar solutions that have solubilized oil into the apolar surfactant tail region.[35,36] Emulsions are very different. They are not thermodynamically stable and considerable mechanical energy is normally needed to create them. All emulsions will eventually separate into an oil and a water phase. Some emulsions, for instance in the food area, are said to be "stable" but that does not mean that they are thermodynamically stable. It just means that the time until phase separation occurs is long. Emulsions are not dynamic systems, rather they are static. The interface in an emulsion does not constantly disintegrate and reform as it does in a microemulsion and in a micellar system. The dimensions in an emulsion is also much larger, typically in the micrometer range, which means that the oil-water interface in an emulsion is orders of magnitude smaller than in a microemulsion.

Micelles are especially simple spherical supramolecules, which are formed by amphiphiles in water or media similar to water. A micellar system appears to be homogeneous since these aggregates are of colloidal size; however, in reality the absorbed reactants are in a microheterogeneous two-phase system.[37]

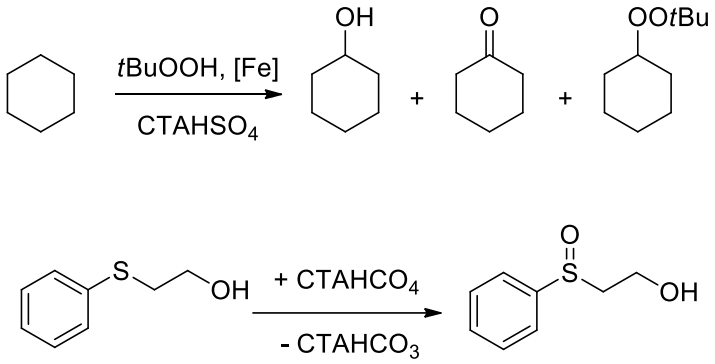
More-recent examples of organic reactions conducted under micellar conditions include catalytic reactions, reactions from inorganic chemistry which take place in micelles,[38] photochemical reactions in organized systems,[39] systematically constructed "artificial enzymes",[40] and the very extensive area of surfactant-influenced polymerization.[41]

Then, micellar systems play their role in solvolyses of esters and related nucleophilic reactions

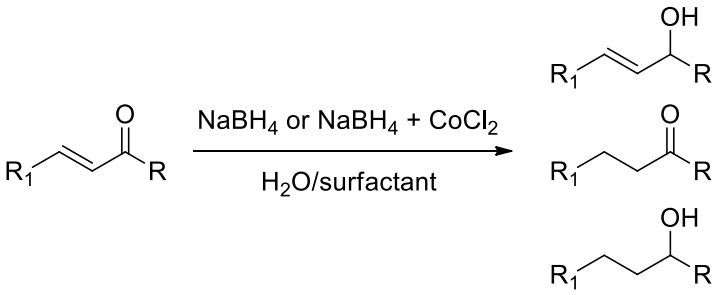
(Scheme 3.2),[42-48] oxidations (Scheme 3.3),[49-57] reductions (Scheme 3.4)[58-63] and also in C-C coupling reactions (Scheme 3.5).[64-77]



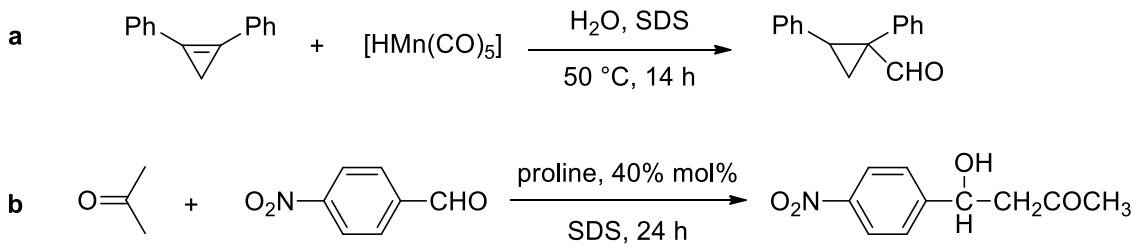
Scheme 3.2. Reaction of 1-chloro-2,4-dinitrobenzene and nitrophenyl esters of phosphoric acid in a basic non-ionic micellar medium.



Scheme 3.3. Cyclohexane oxidation and sulfoxidation.



Scheme 3.4. Reduction of a,b-unsaturated ketones with sodium borohydride in a micellar medium.



Scheme 3.5. a) Stoichiometric hydroformylation with manganese pentacarbonyl hydride and b) micellar aldol reaction catalyzed by L-proline.

There is also a connection with enzymatic catalysis because hydrophilic and hydrophobic regions exist in both supramolecular and macromolecular structures.[78]

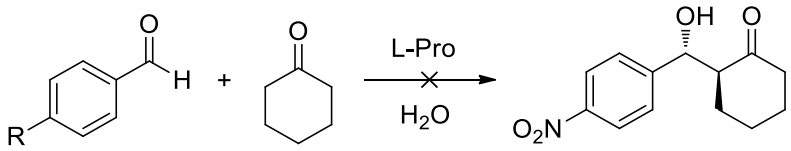
3.2 Results and discussion

Since the previous experience our group have been gaining on chemistry of bile acid salts, we decided to evaluate the promoting role of an easily available non-classical surfactant, that is, sodium deoxycholate (NaDC), in aldol reactions carried out in pure water. Indeed, the amphiphilic properties of NaDC,[79] based on the differently polar faces of its rigid polycyclic structure, could afford the formation of non-classical chiral aggregates able to provide a suitable lipophilic environment in water for the organic compounds to merge and react. In this Chapter the results are reported.

3.2.1 Effective promotion of L-Pro-catalyzed Aldol reaction in water by NaDC

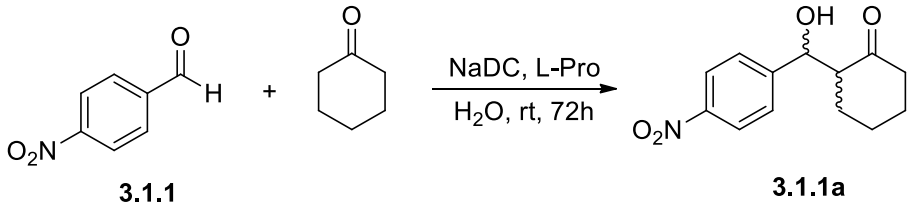
The preliminary reactions were carried on a model system based on cyclohexanone and *p*-nitrobenzaldehyde. As regards the proline load, aldehyde/ketone/proline millimolar ratio (1:5:0.1) was used (0.1 mmol of aldehyde in 1 mL of water). Such molar conditions were already described for similar reaction run with proline-derived amphiphilic catalysts in water.[80]

First, we decided to carry out control experiments run on the organic substrates and proline in water, but without sodium deoxycholate, showing no significant reagent conversion in a distinctly biphasic mixture, even with activated aromatic aldehydes such as p-NO_2-benzaldehyde (Scheme 3.6).



Scheme 3.6. L-Pro catalyzed aldol reaction showing no reagent conversion.

NaDC was then added at room temperature (10 mol% with respect to aldehyde), turning the reaction mixture from bulk dispersion of organic phase in water into an almost neutral pH (7.9) finely dispersed opalescent system. Evidence of formation of aldol products was observed since the early reaction times, but total substrate conversion required three days to be complete (Scheme 3.7). Both the yields and the reaction time showed not to be affected by a molar increase of NaDC (Table 3.1), yielding predominantly to the *anti* aldol product with high diastereo- and enantioselectivity (up to 78:28 dr, up to 87% ee). The anti-diastereoselectivity is in accordance with literature data on proline-catalysed aldol reactions carried out in classical organic solvents.



Scheme 3.7. Effective promotion of aldol reaction in presence of NaDC.

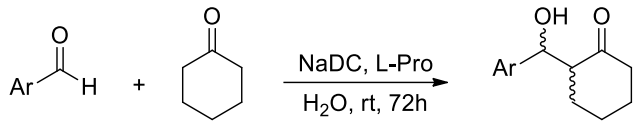
NaDC load [mol%]	Yield[a] [%]	dr[b] (anti:syn)	*ee* anti[c] [%]
1	46	70:30	84
5	73	68:32	82
10	80	72:28	87
20	83	72:28	85
30	82	71:29	87

[a]Calculated on isolated product after flash column chromatography.;
[b]determined by NMR spectra and confirmed *via* HPLC on a chiral stationary phase;
[c]Determined *via* HPLC analysis on a chiral stationary phase.

Table 3.1. Molar increase of NaDC load does not affect neither yields nor reaction time.

Furthermore, a control reaction of organic substrates was carried out in water in the same pH conditions without NaDC. In such conditions, no conversion was observed within days, clearly assessing the positive role of surfactant in the reaction.

The reaction was then carried out in the same conditions on several aromatic aldehydes, and the results are reported in Table 2.



3.X **3.Xa**

Entry	Ar	Yield[a] [%]	dr[b] (anti:syn)	*ee* anti[c] [%]
1	Ph	47	90:10	23
2	3-NO_2-C_6H_4	70	90:10	98
3	2-NO_2-C_6H_4	67	≥99:1	99
4	4-CN-C_6H_4	82	68:32	24
5	2-F-C_6H_4	99	78:22	39
6	4-Br-C_6H_4	95	78:22	26
7	3-Br-C_6H_4	94	69:31	20
8	2-Br-C_6H_4	97	48:52	8
9	3-Cl-C_6H_4	99	78:22	21
10	2-Cl-C_6H_4	98	97:3	6
11	4-OMe-C_6H_4	17	≥99:1	9
12	3-OMe-C_6H_4	77	82:18	25
13	3-OH-C_6H_4	40	90:10	16
14	1-naphthyl	38	90:10	31

[a]Calculated on isolated product after flash column chromatography;

[b]determined by NMR spectra and confirmed *via* HPLC on a chiral stationary phase;

[c]determined *via* HPLC analysis on a chiral stationary phase.

Table 3.2. Aldol reaction on several aromatic aldehydes in presence of NaDC expanding the reaction scope.

As can be seen from the Table 3.2 the reaction occurred in high yield; also good diastereomeric ratios were found by analysing NMR spectra. Although it is the first test in which the reaction takes place in a mainly aqueous system thanks to the presence of DC as a surfactant, in some cases (entry 2, 3, 4) even high enantiomeric excesses have been found.

A first interpretation of these results suggests this might be connected in some way to the pronounced dipole moment of nitro group, which closely interacts with the polar chiral surfactants concave face.

3.2.2 L-Proline interaction with sodium deoxycholate

Moreover, we can speculate on L-Proline interaction with sodium deoxycholate.

Carrying two hydroxyl groups on the steroidal skeleton and a carboxylic residue as a polar head, NaDC is considered a "facial" amphiphile.[81] It's already known that such molecules are capable of intriguing self-assembly behaviour[82] when structural[83] or physico-chemical[84,85] parameters are varied. In water solvent, pH values near acidity and concentration above cmc (critical micelle concentration), NaDC aggregates in micelles with very low aggregation numbers (Nagg < 10).[86] When pH value is increased near neutrality, aggregation number greatly rises up to several hundreds of units per micelle (Nagg ~ 500), until elongated structures such as fibers are formed, and solution undergoes abrupt gelation.[87]

On the other hand, the polar amino acid proline presents two moieties, $=NH_2^+$ (pKa 10.60) and $-COO^-$ (pKa 1.20), that make proline a zwitterionic amino acid when pH values reach neutrality.

The interaction between proline and NaDC was herein tested through dynamic light scattering (DLS) to assess signs of self-assembly behaviour when these two molecules are mixed in water at pH near to neutrality and equimolar ratio.

The DLS results, consisting of autocorrelation function and size distribution are shown in Figure 3.2a. The correlation function indicates a monodisperse population of colloidal aggregates of considerable size.

A more precise indication of sizes distribution is reported in Figure 3.2b. The peak of size distribution as a function of the scattering intensity is centred around 170 nm, indicating that remarkable spheroidal aggregates are formed when an equimolar amount of proline is mixed with NaDC at pH 7,9.

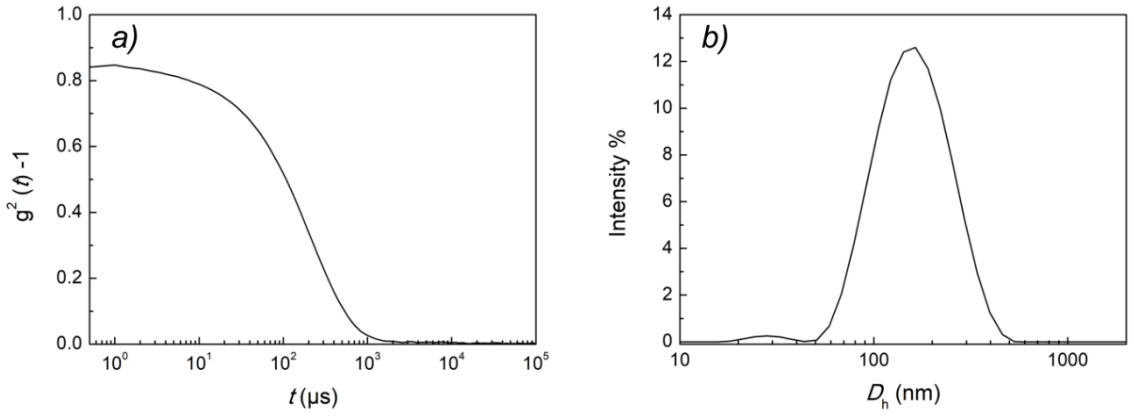


Figure 3.2. DLS measurement of L-Pro:NaDC 1:1, 34 mM water solution at pH 7.8 and 20°C. a) Autocorrelation function. b) Corresponding scattered intensity peak as a function of particle hydrodynamic diameter distribution.

As reported in literature, NaDC water solutions at concentrations above 5 mM and neutral pH tend to undergo sol-gel transition due to fibrils elongation and entanglement. This effect was not observed in this case as the NaDC/L-Proline solution remained transparent and not rheologically affected, indicating that L-proline heavily impacts the NaDC self-assembly behaviour intercalating strongly into its bilayer, inhibiting the micellar elongation and constraining the self-assembly to the bilayer stage. The interaction, probably due to charge interplay and hydrogen bond stabilization between the two species, results firstly in L-proline intercalation within the NaDC packing units, then in isotropic bilayer/vescicular growth,[88] behaviour not observed in L-proline and NaDC separated water solutions at the same conditions which show a gel on one side and a solution on the other one.

However, at reaction conditions, when organic reagents are mixed with water phase, an emulsion is formed and probably stabilized by the NaDC surfactant. This stabilization effect, likely the result of such L-proline/NaDC charge interplay, has two advantages: i) stabilizes the emulsion, delaying Ostwald ripening[89-91] of the unstable dispersed phase, ii) maintains L-proline packed in a fixed position, enhancing its ability to process the reagents in a more efficient way consequently increasing the reaction yield.

3.3 Experimental section

3.3.1 General Informations

Unless otherwise noted, all commercially available compounds were used without further purification. Trends of reactions was monitored by thin layer chromatography (TLC) Merck Kieselgel 60 F254 (0.25mm thickness). Visualization of the developed TLC plates was performed with UV irradiation (254 nm) or by staining with phosphomolybdic acid 12% solution. ^{1}H and ^{13}C spectra were recorded at room temperature on Bruker Avance 400. Analytical HPLC was performed on a Varian 9002 HPLC instrument equipped with a refractive index detector (Schambeck, RI2000), using chiral stationary phases (Chiralpak IA, IB and IC).

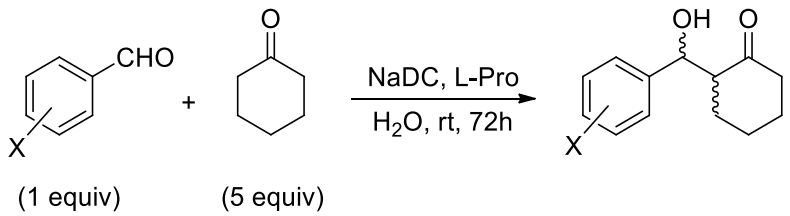
High-resolution ESI mass spectra were carried out on a Q-TOF Micro spectrometer operating in a positive ion mode.

Dynamic Light Scattering measurements.

The measurements were performed using a Malvern Zetasizer Nano ZS equipped with a He-Ne laser (λ = 633 nm), working in backscattering mode (θ = 173°), and with an automated attenuator. The reported results are stated as the average of three consecutive measurements at the controlled temperature of 20 °C. Prior to the measurement, the 34 mM water solution of L-Pro:NaDC 1:1 mixture was filtered using a Millipore ø= 0.22 μm filter, transferred in a PMMA cuvette and finally placed in the instrument.

3.3.2 General procedure and analytical data

General procedure

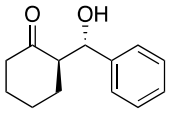


Sodium deoxycholate (0.1 eq, 0.03 mmol), aldehyde (1 eq, 0.30 mmol), cyclohexanone (5 eq, 1.50 mmol) and L-Proline (1 eq, 0.3 mmol) were added into a one-necked bottom flask at rt. Water (1 mL) was added, and the resulting solution was allowed to stir at rt for 72 h. The mixture was stirred at room temperature until complete conversion of the substrates were achieved by TLC monitoring. Once the reaction is complete the reaction mixture was diluted

with H_2O (2 mL) and extracted with AcOEt (3 mL x 3). The volatiles were then removed in vacuo to afford the crude product which was subsequently purified by flash chromatography on silica gel (eluting with 30% EtOAc/Petroleum ether) to afford the aldol products.
Unless otherwise stated the derivatives have been previously synthesised and all analytical data are consistent with literature values.

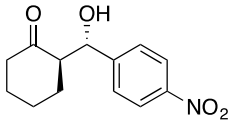
NMR Spectra

(R)-2-((S)-hydroxy(phenyl)methyl)cyclohexan-1-one (3.1a)



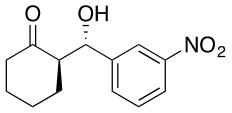
The product **3.1a** was isolated after flash chromatography on silica gel (P/EtOAc 7:3) as colourless oil (29 mg, 47% yield). **Molecular formula**: $C_{14}H_{16}O_2$. **Molecular mass**: 204.26 g mol^{-1}. **HPLC**: Chiralpak IB, Hexane/iPrOH 9:1, 0.5 mL/min, *anti* diastereomer (major) : τ_{major} = 14.8 min τ_{minor} = 17.3 min; *syn* diastereomer (minor) : τ_{major} = 12.9 min τ_{minor} = 12.4 min. **dr:** *anti*/*syn* 90:10 (from NMR analysis); *anti*/*syn* 91:9 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.31 (m, 5H, CH_{ar}), 4.78 (d, *J* = 8.8 Hz, 1H, C*H*Ph), 3.58 (br, 1H, O*H*), 2.62 (m, 1H), 2.48 (m, 1H), 2.36 (m, 1H), 2.08 (m, 1H), 1.77 (m, 1H), 1.66 (m, 1H), 1.56 (m, 2H), 1.31 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 215.6, 141.0, 128.4 (2C), 127.9, 127.0 (2C), 74.8, 57.4, 42.7, 30.9, 27.8, 24.7 ppm; **HRMS (ESI)**: Calcd for $C_{14}H_{17}O_2^+$ ($[M+H]^+$) 205.1223. Found 205.1215.

(R)-2-((S)-hydroxy(4-nitrophenyl)methyl)cyclohexan-1-one (3.1.1a)



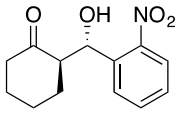
The product **3.1.1a** was isolated after flash chromatography on silica gel (P/EtOAc 7:3) as yellowish solid (60 mg, 80% yield). **Molecular formula**: $C_{13}H_{15}NO_4$. **Molecular mass**: 249.27 g mol^{-1}. **HPLC**: Chiralpak IB, Hexane/iPrOH 95:5, 1.0 mL/min, *anti* diastereomer (major) : τ_{major} = 24.0 min τ_{minor} = 31.6 min; *syn* diastereomer (minor) : τ_{major} = 19.8 min τ_{minor} = 22.4 min. **dr:** *anti*/*syn* 72:28 (from NMR analysis); *anti*/*syn* 66:34 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.20 (m, 2H, C*H*$_{ar}$), 7.50 (m, 2H, C*H*$_{ar}$), 4.89 (d, *J* = 8.4 Hz, 1H, C*H*Ph), 4.08 (br, 1H, O*H*), 2.59 (m, 1H), 2.49 (m, 1H), 2.37 (m, 1H), 2.11 (m, 1H), 1.69 (m, 2H), 1.54 (m, 2H), 1.38 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 214.8, 149.1, 148.4, 127.9 (2C), 123.6 (2C), 74.0, 57.2, 42.7, 30.8, 27.6, 24.7 ppm; **HRMS (ESI)**: Calcd for $C_{13}H_{16}NO_4^+$ ($[M+H]^+$) 250.1074. Found 250.1065.

(R)-2-((S)-hydroxy(3-nitrophenyl)methyl)cyclohexan-1-one (3.2a)



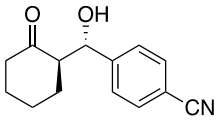
The product **3.2a** was isolated after flash chromatography on silica gel (P/EtOAc 7:3) as yellowish oil (52 mg, 70% yield). **Molecular formula**: $C_{13}H_{15}NO_4$. **Molecular mass**: 249.27 g mol^{-1}. **HPLC**: Chiralpak IA, Hexane/iPrOH 9:1, 0.6 mL/min, *anti* diastereomer (major) : τ_{major} = 32.3 min τ_{minor} = 39.5 min; *syn* diastereomer (minor) : τ_{major} = 25.0 min τ_{minor} = 28.3 min. **dr:** *anti*/*syn* 90:10 (from NMR analysis); *anti*/*syn* 87:13 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.15 (m, 2H, C*H*$_{ar}$), 7.65 (m, 1H, C*H*$_{ar}$), 7.51 (m, 1H, CH$_{ar}$), 4.88 (d, *J* = 8.4 Hz, 1H, C*H*Ph), 4.13 (br, 1H, O*H*), 2.61 (m, 1H), 2.48 (m, 1H), 2.38 (m, 1H), 2.10 (m, 1H), 1.81 (m, 1H), 1.67 (m, 2H), 1.56 (m, 2H), 1.38 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 214.9, 148.3, 143.3, 133.2, 129.3, 122.8, 122.0, 74.0, 57.1, 42.6, 30.7, 27.6, 24.6 ppm; **HRMS (ESI)**: Calcd for $C_{13}H_{16}NO_4^+$ ([M+H]$^+$) 250.1074. Found 250.1069.

(R)-2-((S)-hydroxy(2-nitrophenyl)methyl)cyclohexan-1-one (3.3a)



The product **3.3a** was isolated after flash chromatography on silica gel (P/EtOAc 7:3) as brownish solid (50 mg, 67% yield). **Molecular formula**: $C_{13}H_{15}NO_4$. **Molecular mass**: 249.27 g mol^{-1}. **HPLC**: Chiralpak IA, Hexane/iPrOH 9:1, 1 mL/min, *anti* diastereomer (major) : τ_{major} = 19.3 min τ_{minor} = 20.0 min; *syn* diastereomer (minor) : τ_{major} = nd τ_{minor} = nd. **dr:** *anti*/*syn* ≥99:1 (from NMR analysis); *anti*/*syn* ≥99:1 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.83 (dd, *J* = 1.3, 8.2), 1H, C*H*$_{ar}$), 7.75 (dd, *J* = 1.3, 7.9) Hz, 1H, C*H*$_{ar}$), 7.62 (td, *J* = 1.3, 7.8 Hz, 1H, C*H*$_{ar}$), 7.42 (m, 1H, C*H*$_{ar}$), 5.43 (d, *J* = 7.1 Hz, 1H, C*H*Ph), 4.16 (br, 1H, O*H*), 2.75 (m, 1H), 2.44 (m, 1H), 2.33 (m, 2H), 2.08 (m, 1H), 1.83 (m, 1H), 1.73 (m, 2H), 1.57 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 215.0, 136.6, 133.1, 129.0, 128.4, 124.1, 69.7, 57.3, 42.8, 31.1, 27.8, 25.0 ppm; **HRMS (ESI)**: Calcd for $C_{13}H_{16}NO_4^+$ ([M+H]$^+$) 250.1074. Found 250.1066.

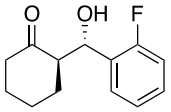
4-((S)-hydroxy((R)-2-oxocyclohexyl)methyl)benzonitrile (3.4a)



The product **3.4a** was isolated after flash chromatography on silica gel (P/EtOAc 7:3) as yellowish oil (56 mg, 82% yield). **Molecular formula**: $C_{14}H_{15}NO_2$. **Molecular mass**: 229.28 g mol^{-1}. **HPLC**:

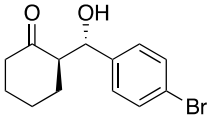
Chiralpak IA, Hexane/iPrOH 9:1, 1 mL/min, *anti* diastereomer (major) : τ_{major} = 36.9 min τ_{minor} = 25.5 min; *syn* diastereomer (minor) : τ_{major} = 18.1 min τ_{minor} = 21.7 min. **dr:** *anti*/*syn* 68:32 (from NMR analysis); *anti*/*syn* 67:33 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.59 (m, 2H, C*H*$_{ar}$), 7.41 (m, 2H, C*H*$_{ar}$), 4.81 (d, *J* = 8.3 Hz, 1H, C*H*Ph), 4.07 (br, 1H, O*H*), 2.55 (m, 1H), 2.43 (m, 1H), 2.32 (m, 1H), 2.06 (m, 1H), 1.79 (m, 1H), 1.66 (m, 1H), 1.50 (m, 2H), 1.31 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 214.8, 146.5, 132.1 (2C), 127.8 (2C), 118.7, 111.6, 74.1, 57.1, 42.6, 30.7, 27.6, 24.6 ppm; **HRMS (ESI)**: Calcd for $C_{14}H_{16}NO_2^+$ ([M+H]$^+$) 230.1176. Found 230.1171.

(R)-2-((S)-(2-fluorophenyl)(hydroxy)methyl)cyclohexan-1-one (3.5a)



The product **3.5a** was isolated after flash chromatography on silica gel (P/EtOAc 7:3) as greenish oil (66 mg, 99% yield). **Molecular formula**: $C_{13}H_{15}FO_2$. **Molecular mass**: 222.26 g mol^{-1}. **HPLC**: Chiralpak IC, Hexane/iPrOH 95:5, 1.0 mL/min, *anti* diastereomer (major) : τ_{major} = 26.4 min τ_{minor} = 33.3 min; *syn* diastereomer (minor) : τ_{major} = 14.7 min τ_{minor} = 13.0 min. **dr:** *anti*/*syn* 78:22 (from NMR analysis); *anti*/*syn* 91:9 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.40 (m, 1H, C*H*$_{ar}$), 7.13 (m, 1H, C*H*$_{ar}$), 7.05 (m, 1H, C*H*$_{ar}$), 6.89 (m, 1H, C*H*$_{ar}$), 5.08 (d, *J* = 8.8 Hz, 1H, C*H*Ph), 4.00 (br, 1H, O*H*), 2.57 (m, 1H), 2.35 (m, 1H), 1.95 (m, 1H), 1.67 (m, 1H), 1.48 (m, 4H), 1.31 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 214.9, 161.2, 129.1, 128.3, 124.3, 123.8, 114.9, 67.7, 57.0, 42.4, 41.8, 26.9, 24.9 ppm; **HRMS (ESI)**: Calcd for $C_{13}H_{16}FO_2^+$ ([M+H]$^+$) 223.1129. Found 223.1119.

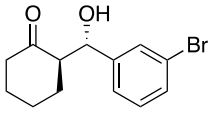
(R)-2-((S)-(4-bromophenyl)(hydroxy)methyl)cyclohexan-1-one (3.6a)



The product **3.6a** was isolated after flash chromatography on silica gel (P/EtOAc 7:3) as yellow oil (81 mg, 95% yield). **Molecular formula**: $C_{13}H_{14}BrO_2$. **Molecular mass**: 283.17 g mol^{-1}. **HPLC**: Chiralpak IC, Hexane/iPrOH 95:5, 1 mL/min, *anti* diastereomer (major) : τ_{major} = 31.4 min τ_{minor} = 40.0 min; *syn* diastereomer (minor) : τ_{major} = 27.4 min τ_{minor} = 29.0 min. **dr:** *anti*/*syn* 78:22 (from NMR analysis); *anti*/*syn* 88:12 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.43 (m, 2H, C*H*$_{ar}$), 7.17 (m, 2H, C*H*$_{ar}$), 4.73 (d, *J* = 8.7 Hz, 1H, C*H*Ph), 2.53 (m, 1H), 2.44 (m, 1H), 2.05 (m,

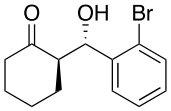
1H), 1.77 (m, 1H), 1.57 (m, 4H), 1.40 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 215.2, 140.1, 131.4 (2C), 128.7 (2C), 121.7, 74.1, 57.3, 42.6, 30.7, 27.7, 24.7 ppm; HRMS (ESI): Calcd for $C_{13}H_{15}BrO_2Na^+$ ($[M+Na]^+$) 305.0148. Found 305.0152.

(R)-2-((S)-(3-bromophenyl)(hydroxy)methyl)cyclohexan-1-one (3.7a)



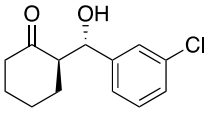
The product **3.7a** was isolated after flash chromatography on silica gel (P/EtOAc 7:3) as colorless oil (94 mg, 94% yield). **Molecular formula**: $C_{13}H_{15}BrO_2$. **Molecular mass**: 283.17 g mol^{-1}. **HPLC**: Chiralpak IB, Hexane/iPrOH 95:5, 1 mL/min, *anti* diastereomer (major) : τ_{major} = 12.4 min τ_{minor} = 10.7 min; *syn* diastereomer (minor) : τ_{major} = 9.1 min τ_{minor} = 8.7 min. **dr:** *anti*/*syn* 69:31 (from NMR analysis); *anti*/*syn* 64:36 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.41 (m, 1H, C*H*$_{ar}$), 7.32 (m, 1H, C*H*$_{ar}$), 7.14 (m, 2H, C*H*$_{ar}$), 4.67 (d, *J* = 8.6 Hz, 1H, C*H*Ph), 3.61 (br, *1*H, O*H*), 2.50 (m, 1H), 2.36 (m, 1H), 1.98 (m, 1H), 1.70 (m, 1H), 1.57 (m, 1H), 1.50 (m, 2H), 1.43 (m, 1H) ppm. **^{13}C NMR** (151 MHz, $CDCl_3$) δ = 214.8, 143.6, 130.8, 130.0, 129.8, 125.8, 122.4, 73.9, 57.2, 41.9, 30.7, 27.7, 24.6 ppm; **HRMS (ESI)**: Calcd for $C_{13}H_{16}BrO_2^+$ ($[M+H]^+$) 283.0328. Found 283.0335.

(R)-2-((S)-(2-bromophenyl)(hydroxy)methyl)cyclohexan-1-one (3.8a)



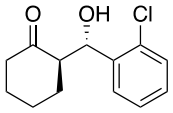
The product **3.8a** was isolated after flash chromatography on silica gel (P/EtOAc 6:4) as yellow oil (97 mg, 97% yield). **Molecular formula**: $C_{13}H_{15}BrO_2$. **Molecular mass**: 283.17 g mol^{-1}. **HPLC**: Chiralpak IC, Hexane/iPrOH 9:1, 1.0 mL/min, *anti* diastereomer (major) : τ_{major} = 15.6 min τ_{minor} = 19.2 min; *syn* diastereomer (minor) : τ_{major} = 8.7 min τ_{minor} = 9.6 min. **dr:** *anti*/*syn* 48:52 (from NMR analysis); *anti*/*syn* 42:58 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.39 (m, 1H, CH_{ar}), 7.34 (m, 1H, CH_{ar}), 7.17 (m, 1H, CH_{ar}), 6.97 (m, 1H, CH_{ar}), 5.16 (d, *J* = 8.1 Hz, 1H, C*H*Ph), 2.53 (m, 1H), 2.30 (m, 1H), 1.90 (m, 1H), 1.77 (m, 1H), 1.49 (m, 2H), 1.41 (m, 1H), 1.36 (m, 1H), 1.27 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 211.8, 140.8, 132.3, 128.9, 128.4, 127.7, 127.0, 72.5, 57.6, 42.5, 30.4, 27.7, 25.5 ppm; **HRMS (ESI)**: Calcd for $C_{13}H_{15}BrO_2Na^+$ ($[M+Na]^+$) 305.0148. Found 305.0155.

(R)-2-((S)-(3-chlorophenyl)(hydroxy)methyl)cyclohexan-1-one (3.9a)



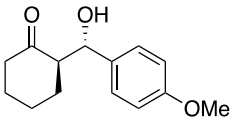
The product **3.9a** was isolated after flash chromatography on silica gel (P/EtOAc 7:3) as yellow oil (71 mg, 99% yield). **Molecular formula**: $C_{13}H_{15}ClO_2$. **Molecular mass**: 238.71 g mol^{-1}. **HPLC**: Chiralpak IB, Hexane/iPrOH 95:5, 1 mL/min, *anti* diastereomer (major) : τ_{major} = 10.2 min τ_{minor} = 11.9 min; *syn* diastereomer (minor) : τ_{major} = 8.7 min τ_{minor} = 8.3 min. **dr:** *anti*/*syn* 78:22 (from NMR analysis); *anti*/*syn* 62:38 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.25 (m, 1H, C*H*$_{ar}$), 7.16 (m, *2*H, C*H*$_{ar}$), 7.09 (m, 1H, C*H*$_{ar}$), 4.68 (d, *J* = 8.6 Hz, 1H, C*H*Ph), 3.75 (br, 1H, OH), 2.49 (m, 1H), 2.36 (m, 1H), 2.29 (m, 1H), 1.97 (m, 1H), 1.68 (m, 1H), 1.48 (m, 3H), 1.20 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 214.8, 143.3, 134.1, 129.5, 127.8, 127.1, 125.3, 73.9, 57.2, 42.5, 30.6, 27.0, 24.5 ppm; **HRMS (ESI)**: Calcd for $C_{13}H_{16}ClO_2^+$ ([M+H]$^+$) 239.0833. Found 239.0826.

(R)-2-((S)-(2-chlorophenyl)(hydroxy)methyl)cyclohexan-1-one (3.10a)



The product **3.10a** was isolated after flash chromatography on silica gel (P/EtOAc 7:3) as yellowish oil (70 mg, 98% yield). **Molecular formula**: $C_{13}H_{15}ClO_2$. **Molecular mass**: 238.71 g mol^{-1}. **HPLC**: Chiralpak IB, Hexane/iPrOH 95:5, 1 mL/min, *anti* diastereomer (major) : τ_{major} = 8.9 min τ_{minor} = 9.8 min; *syn* diastereomer (minor) : τ_{major} = nd τ_{minor} = nd. **dr:** *anti*/*syn* ≥99:1 (from NMR analysis); *anti*/*syn* ≥99:1 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.47 (m, 1H, C*H*$_{ar}$), 7.23 (m, 2H, C*H*$_{ar}$), 7.12 (m, 1H, C*H*$_{ar}$), 5.28 (d, *J* = 8.2 Hz, 1H, C*H*Ph), 3.90 (br, 1H, OH), 2.60 (m, 1H), 2.38 (m, 1H), 2.29 (m, 1H), 1.99 (m, 1H), 1.71 (m, 1H), 1.56 (m, 3H), 1.35 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 215.0, 139.2, 132.8, 129.1, 128.7, 128.3, 127.2, 70.2, 57.6, 42.6, 30.3, 27.0, 24.8 ppm; **HRMS (ESI)**: Calcd for $C_{13}H_{16}ClO_2^+$ ([M+H]$^+$) 239.0833. Found 239.0837.

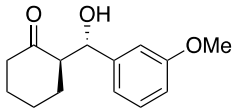
(R)-2-((S)-hydroxy(4-methoxyphenyl)methyl)cyclohexan-1-one (3.11a)



The product **3.11a** was isolated after flash chromatography on silica gel (P/EtOAc 6:4) as orange oil (12 mg, 17% yield). **Molecular formula**: $C_{14}H_{18}O_3$. **Molecular mass**: 234.30 g mol^{-1}. **HPLC**: Chiralpak IA, Hexane/iPrOH 95:5, 1 mL/min, *anti* diastereomer (major) : τ_{major} = 33.4 min τ_{minor} =

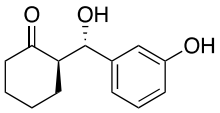
32.0 min; *syn* diastereomer (minor) : τ_{major} = nd τ_{minor} = nd. **dr:** *anti*/*syn* ≥99:1 (from NMR analysis); *anti*/*syn* ≥99:1 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.24 (m, 2H, C*H*$_{ar}$), 6.87 (m, 2H, C*H*$_{ar}$), 4.74 (d, *J* = 8.9 Hz, 1H, C*H*Ph), 3.8 (s, 1H, OCH_3), 2.59 (m, 1H), 2.47 (m, 1H), 2.35 (m, 1H), 2.08 (m, 1H), 1.78 (m, 1H), 1.66 (m, 1H), 1.58 (m, 2H), 1.51 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 215.7, 159.3, 133.2, 128.2 (2C), 113.8 (2C), 74.3, 57.5, 55.3, 42.7, 30.8, 27.8, 24.7 ppm; **HRMS (ESI)**: Calcd for $C_{14}H_{18}O_3Na^+$ ([M+Na]$^+$) 257.1148. Found 257.1154.

(R)-2-((S)-hydroxy(3-methoxyphenyl)methyl)cyclohexan-1-one (3.12a)



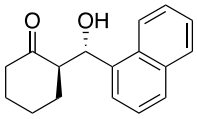
The product **3.12a** was isolated after flash chromatography on silica gel (P/EtOAc 6:4) as yellowish oil (54 mg, 77% yield). **Molecular formula**: $C_{14}H_{18}O_3$. **Molecular mass**: 234.30 g mol^{-1}. **HPLC**: Chiralpak IA, Hexane/iPrOH 95:5, 1 mL/min, *anti* diastereomer (major) : τ_{major} = 30.2 min τ_{minor} = 28.8 min; *syn* diastereomer (minor) : τ_{major} = 16.1 min τ_{minor} = 18.8 min. **dr:** *anti*/*syn* 82:18 (from NMR analysis); *anti*/*syn* 71:29 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.23 (m, 1H, C*H*$_{ar}$), 6.88 (m, 2H, C*H*$_{ar}$), 6.82 (m, 1H, C*H*$_{ar}$), 4.75 (d, *J* = 8.8 Hz, 1H, C*H*Ph), 3.80 (s, *3*H, OCH_3), 3.61 (br, 1H, OH), 2.59 (m, 1H), 2.46 (m, 1H), 2.34 (m, 1H), 2.06 (m, 1H), 1.77 (m, 1H), 1.57 (m, 3H), 1.28 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 215.5, 159.7, 142.6, 129.3, 119.5, 113.4, 112.4, 74.7, 57.4, 55.2, 42.6, 30.8, 27.8, 24.7 ppm; **HRMS (ESI)**: Calcd for $C_{14}H_{18}O_3Na^+$ ([M+Na]$^+$) 257.1148. Found 257.1156.

(R)-2-((S)-hydroxy(3-hydroxyphenyl)methyl)cyclohexan-1-one (3.13a)



The product **3.13a** was isolated after flash chromatography on silica gel (P/EtOAc 6:4) as brownish oil (26 mg, 40% yield). **Molecular formula**: $C_{13}H_{16}O_3$. **Molecular mass**: 220.27 g mol^{-1}. **HPLC**: Chiralpak IC, Hexane/iPrOH 9:1, 1.0 mL/min, *anti* diastereomer (major) : τ_{major} = 59.8 min τ_{minor} = 55.7 min; *syn* diastereomer (minor) : τ_{major} = 43.8 min τ_{minor} = 40.1 min. **dr:** *anti*/*syn* 90:10 (from NMR analysis); *anti*/*syn* 93:7 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.14 (m, 1H, C*H*$_{ar}$), 6.79 (m, 2H, C*H*$_{ar}$), 6.71 (m, 2H, C*H*$_{ar}$), 4.74 (d, J = 8.8 Hz, 1H, C*H*Ph), 2.59 (m, 1H), 2.46 (m, 1H), 2.35 (m, 1H), 2.05 (m, 1H), 1.75 (m, 1H), 1.58 (m, 3H), 1.25 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 216.2, 156.2, 142.1, 129.5, 119.3, 115.4, 113.9, 74.8, 57.0, 42.6, 30.8, 27.8, 24.5 ppm; **HRMS (ESI)**: Calcd for $C_{13}H_{17}O_3^+$ ([M+H]$^+$) 221.1172. Found 221.1166.

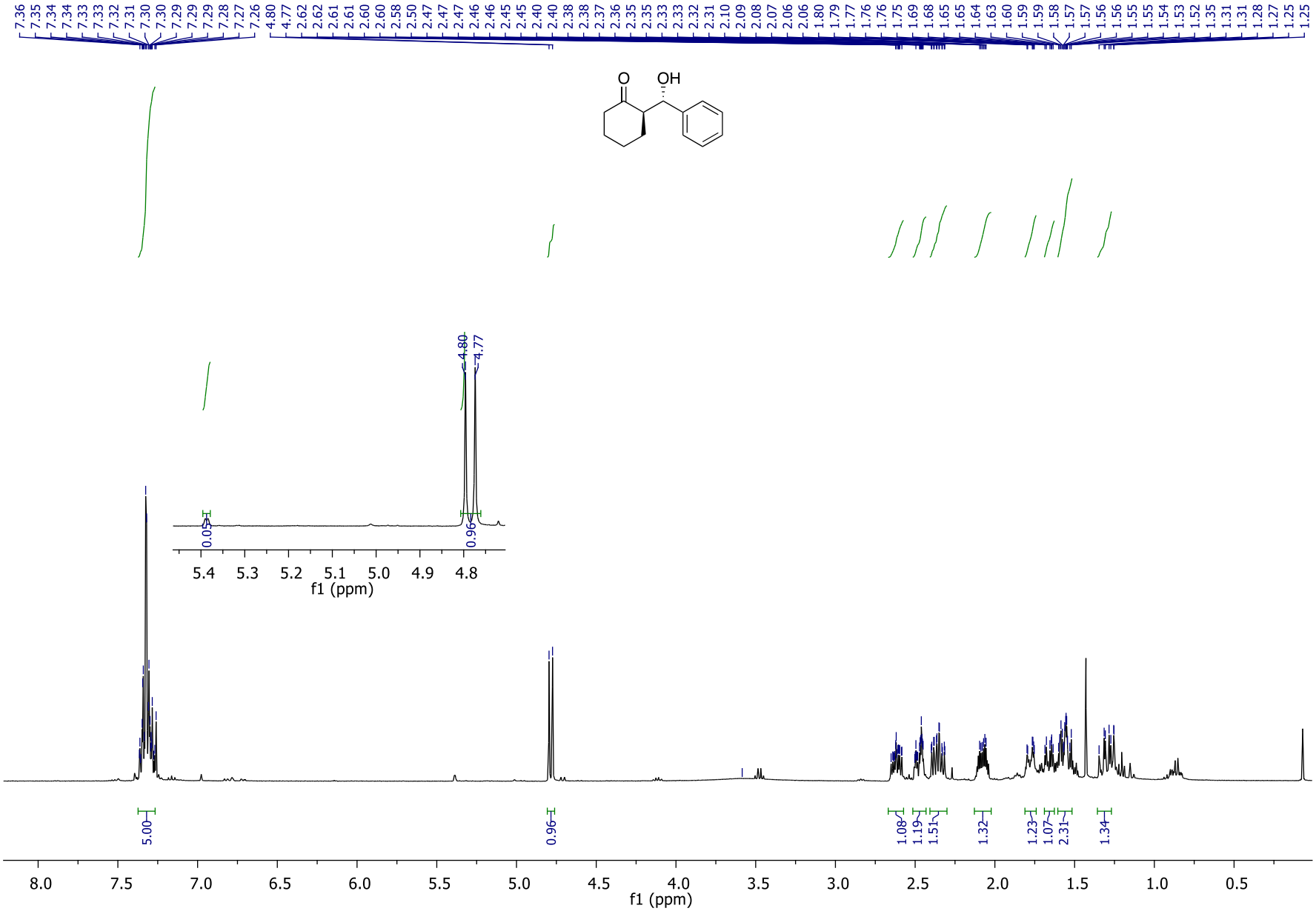
(R)-2-((S)-hydroxy(naphthalen-1-yl)methyl)cyclohexan-1-one (3.14a)

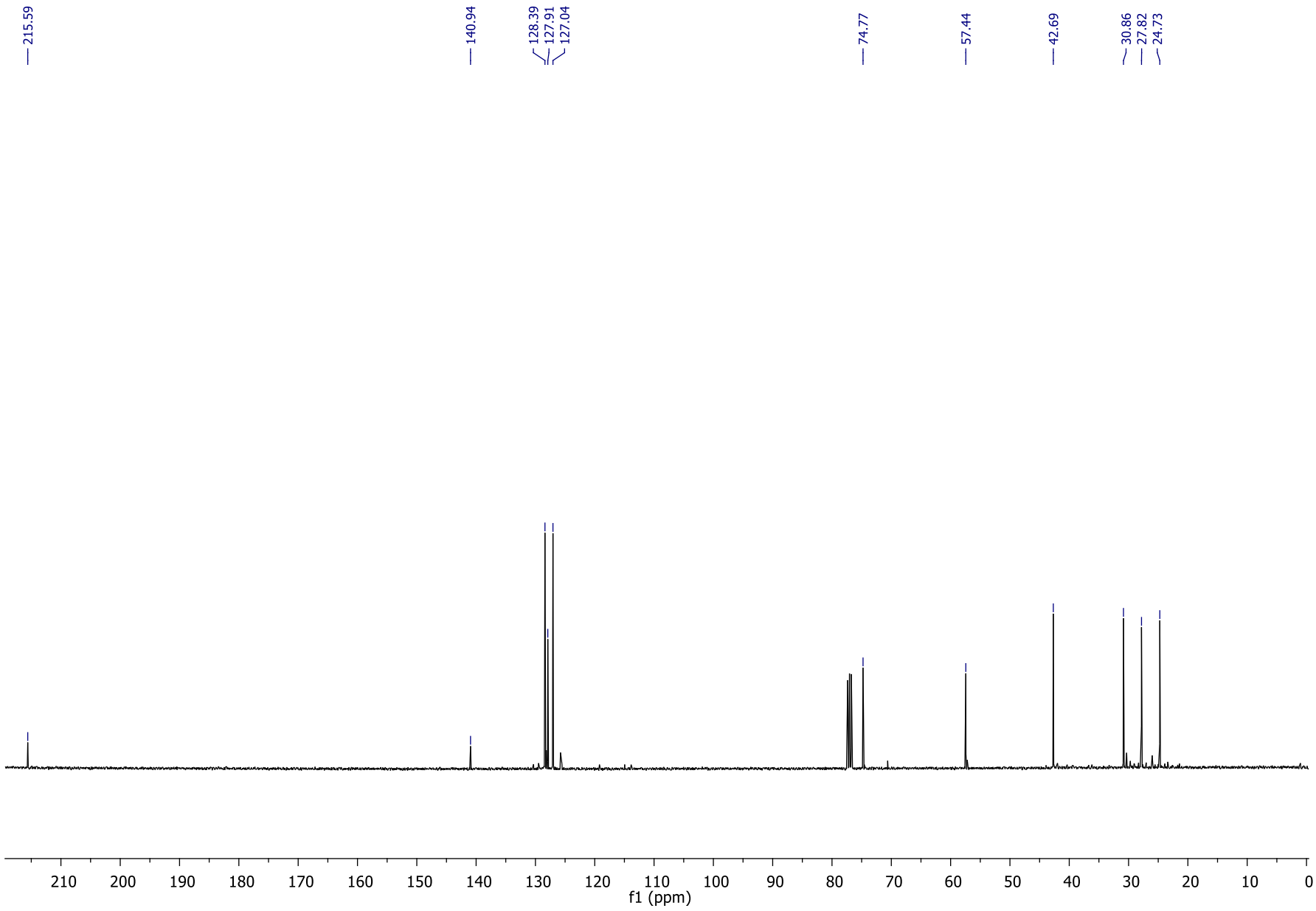


The product **3.14a** was isolated after flash chromatography on silica gel (P/EtOAc 7:3) as brown oil (29 mg, 38% yield). **Molecular formula**: $C_{17}H_{18}O_2$. **Molecular mass**: 254.33 g mol^{-1}. **HPLC**: Chiralpak IA, Hexane/iPrOH 95:5, 1 mL/min, *anti* diastereomer (major) : τ_{major} = 24.6 min τ_{minor} = 29.2 min; *syn* diastereomer (minor) : τ_{major} = 14.0 min τ_{minor} = 12.0 min. **dr:** *anti*/*syn* 90:10 (from NMR analysis); *anti*/*syn* 80:20 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.25 (m, 1H, CH_{ar}), 7.85 (m, 1H, CH_{ar}), 7.79 (m, 1H, CH_{ar}), 7.54 (m, 1H, CH_{ar}), 7.47 (m, 3H, CH_{ar}), 5.57 (d, J = 8.8 Hz, 1H, C*H*Ph), 2.67 (m, 1H), 2.5 – 1.3 (m, 9H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 216.4, 129.6, 128.9, 128.4, 128.1, 126.0, 125.5, 125.34, 125.31, 125.2, 123.9, 72.0, 57.4, 42.0, 31.4, 27.0, 24.9 ppm; **HRMS (ESI)**: Calcd for $C_{17}H_{19}O_2^+$ ($[M+H]^+$) 255.1380. Found 255.1375.

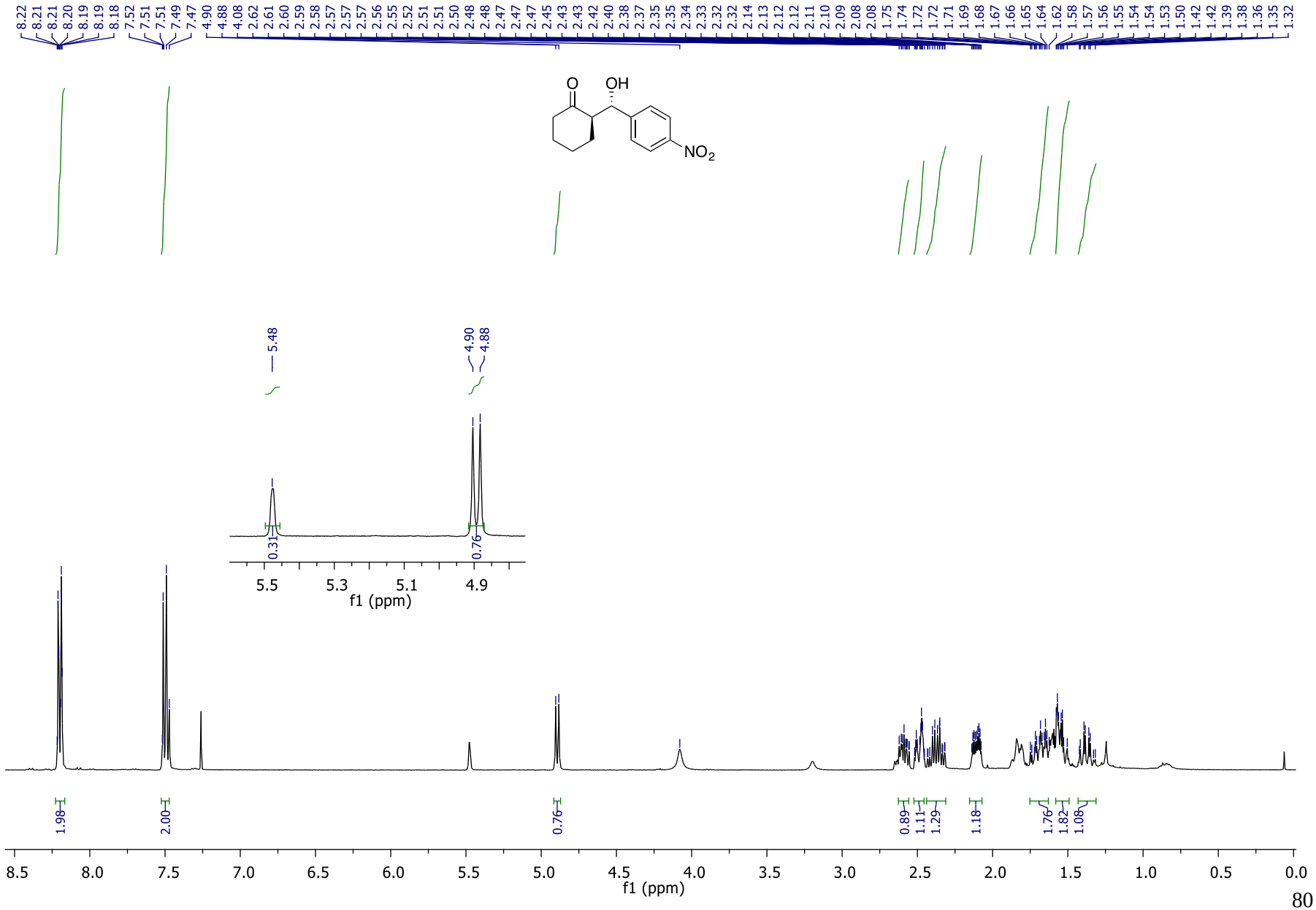
Two diastereomers: anti > syn

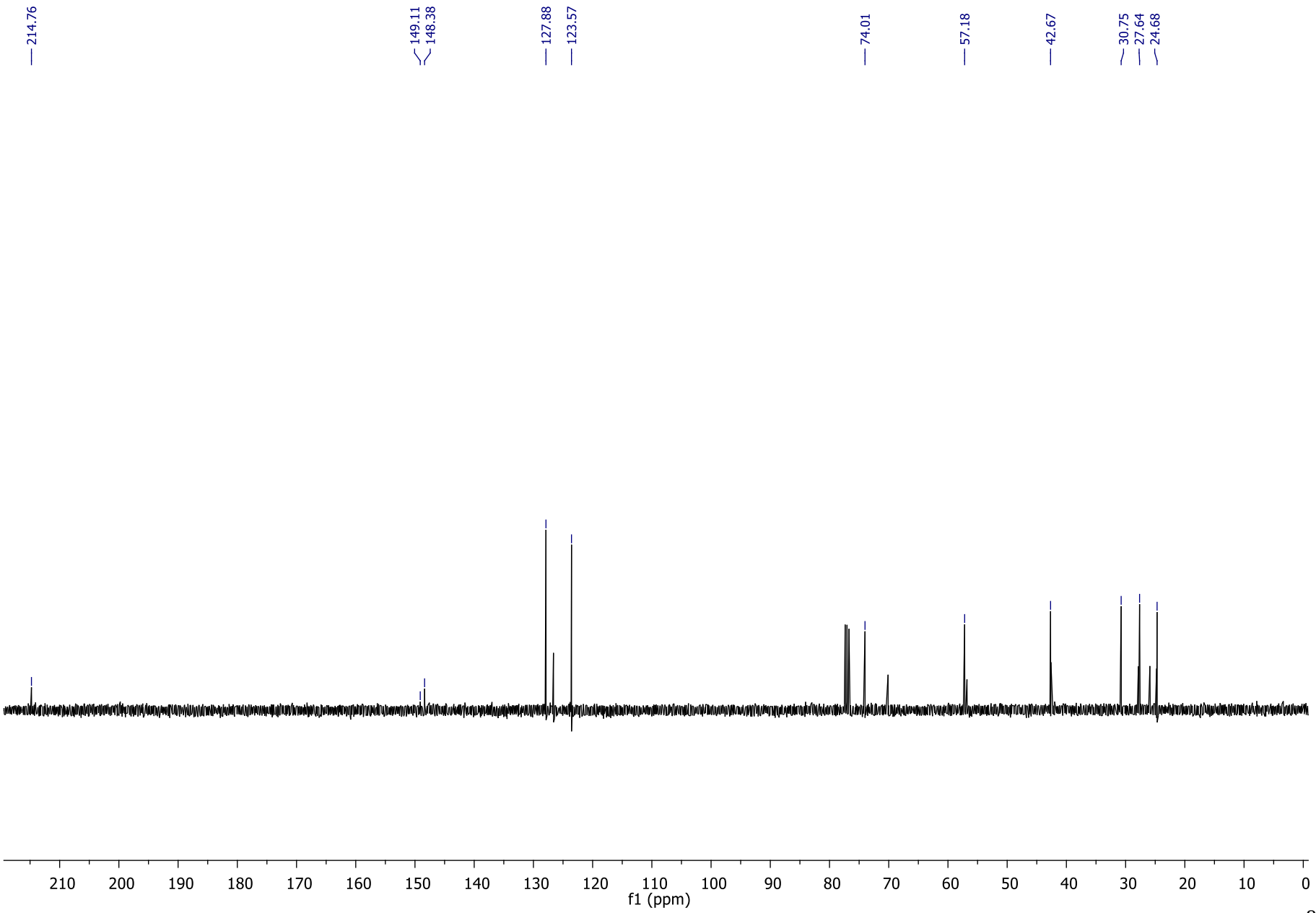
(R)-2-((S)-hydroxy(phenyl)methyl)cyclohexan-1-one (3.1a)



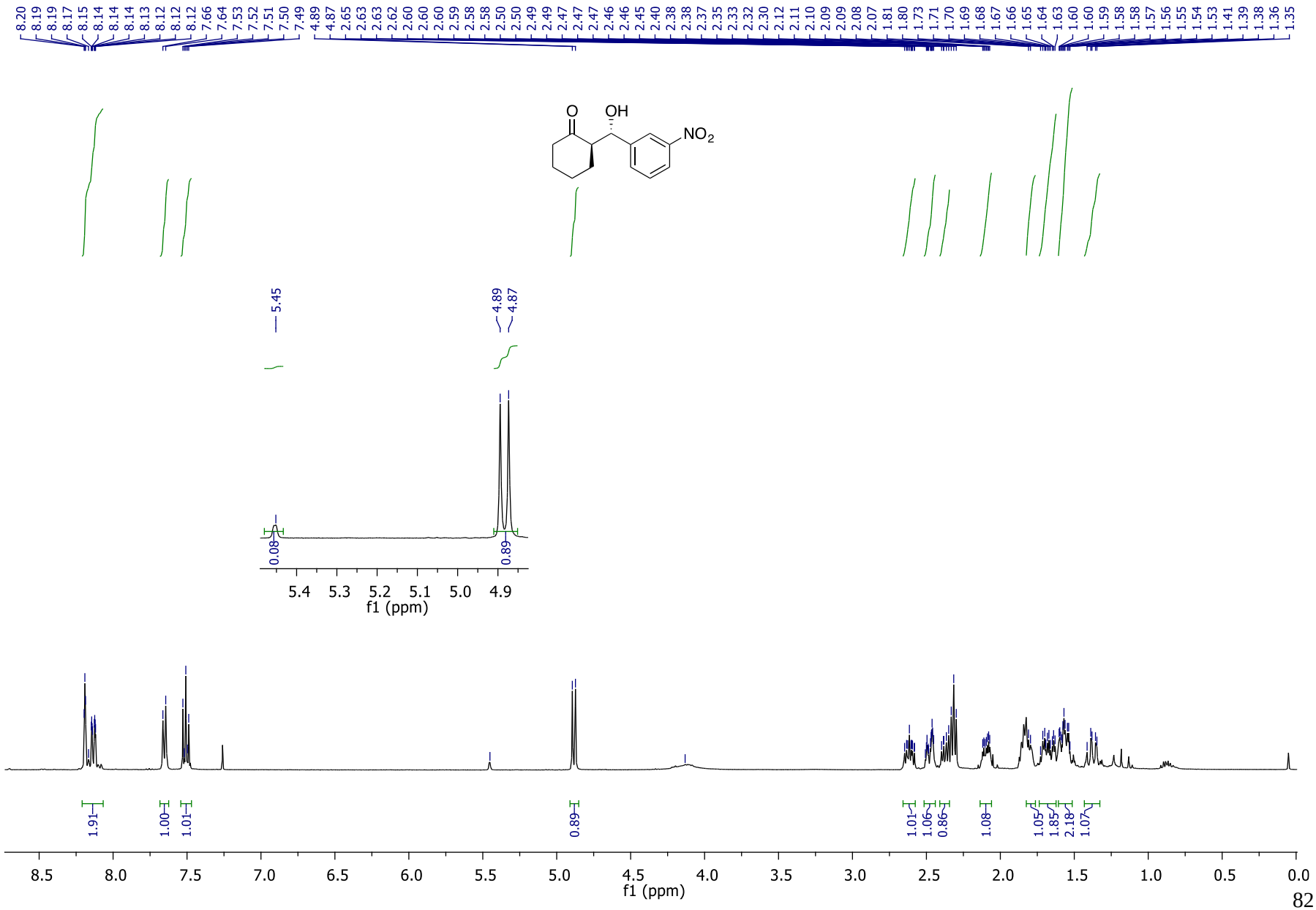

215.59
140.94
128.39
127.91
127.04
74.77
57.44
42.69
30.86
27.82
24.73
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

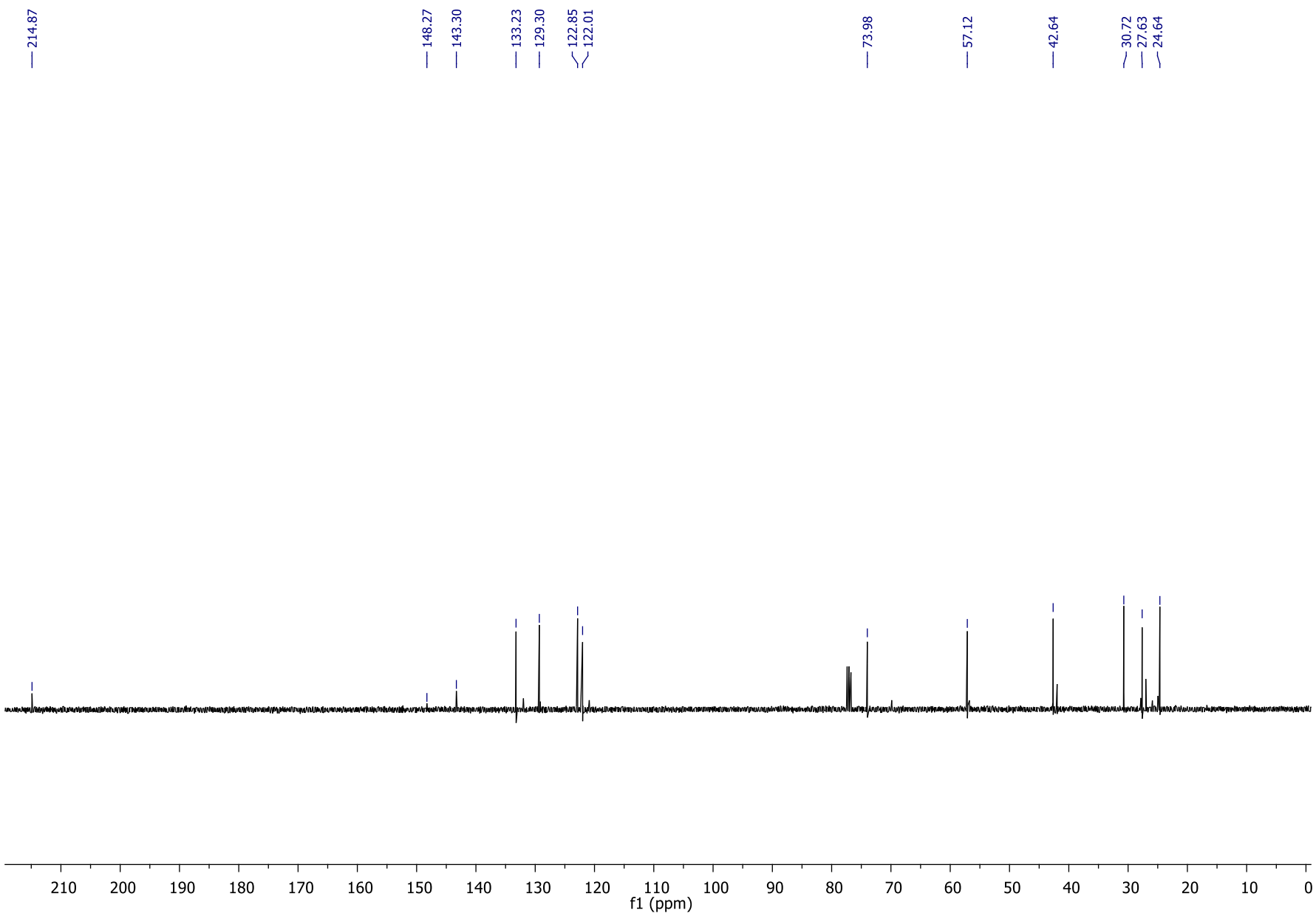
(R)-2-((S)-hydroxy(4-nitrophenyl)methyl)cyclohexan-1-one (3.1.1a)



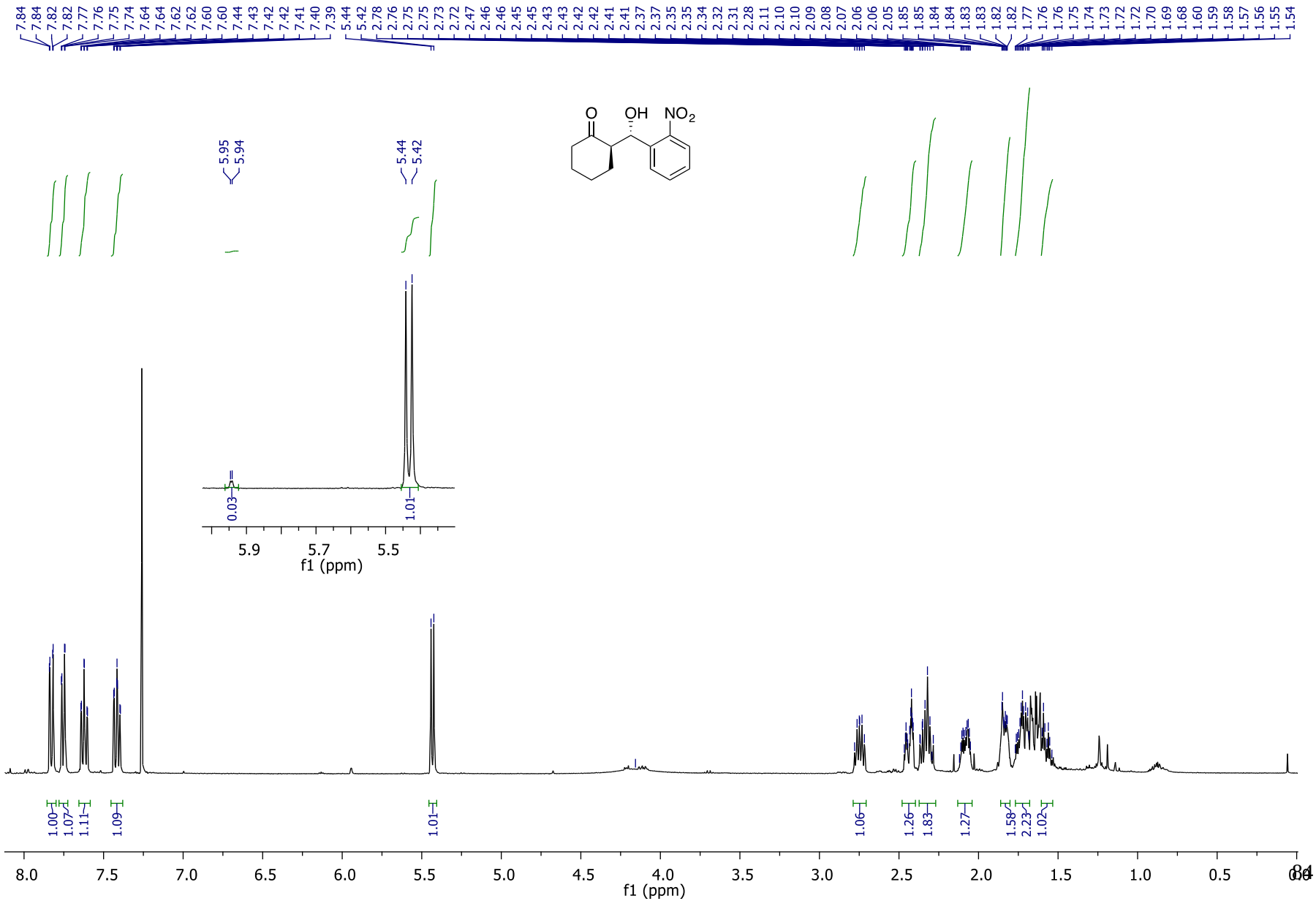

214.76
149.11
148.38
127.88
123.57
74.01
57.18
42.67
30.75
27.64
24.68
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

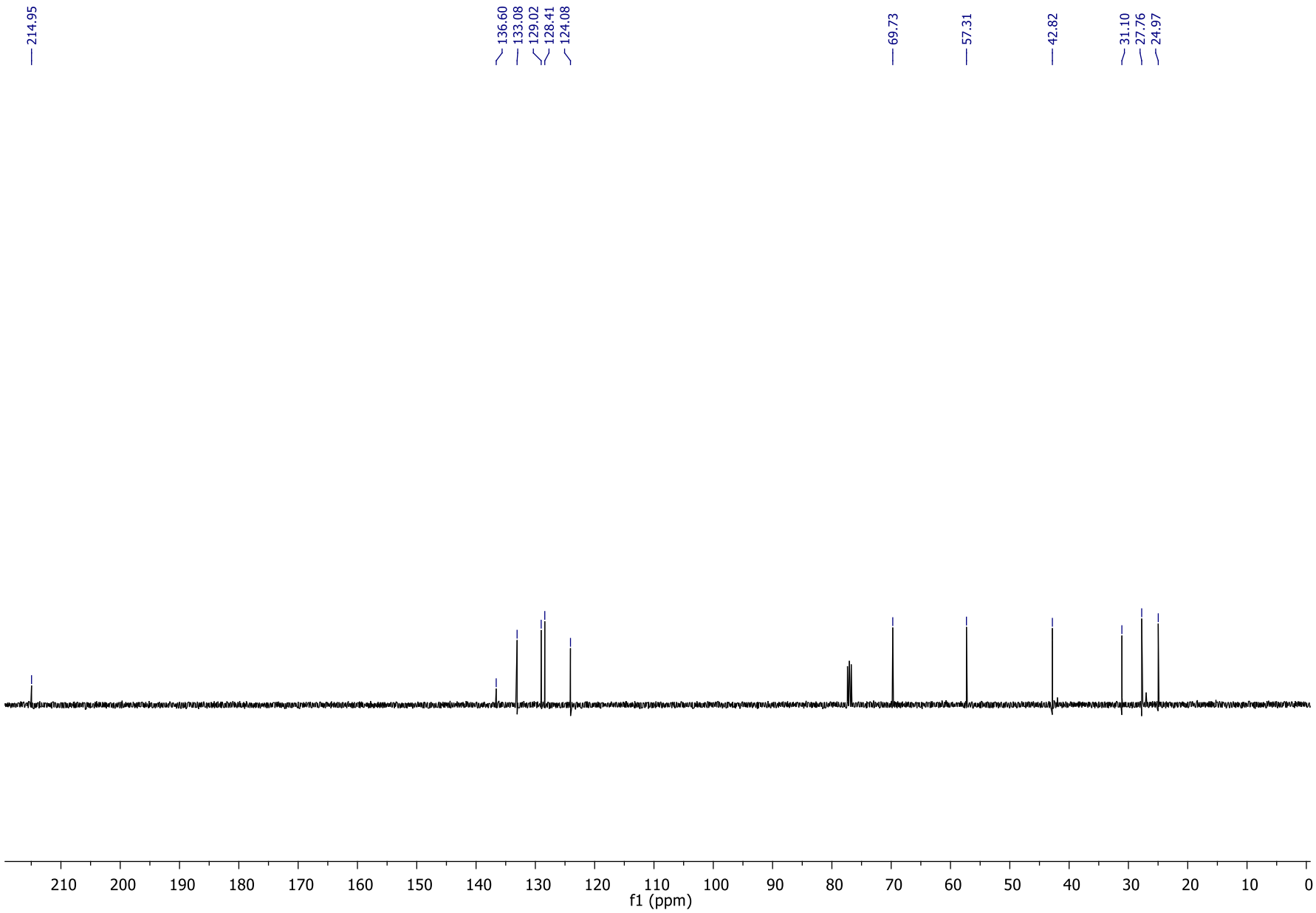
(R)-2-((S)-hydroxy(3-nitrophenyl)methyl)cyclohexan-1-one (3.2a)



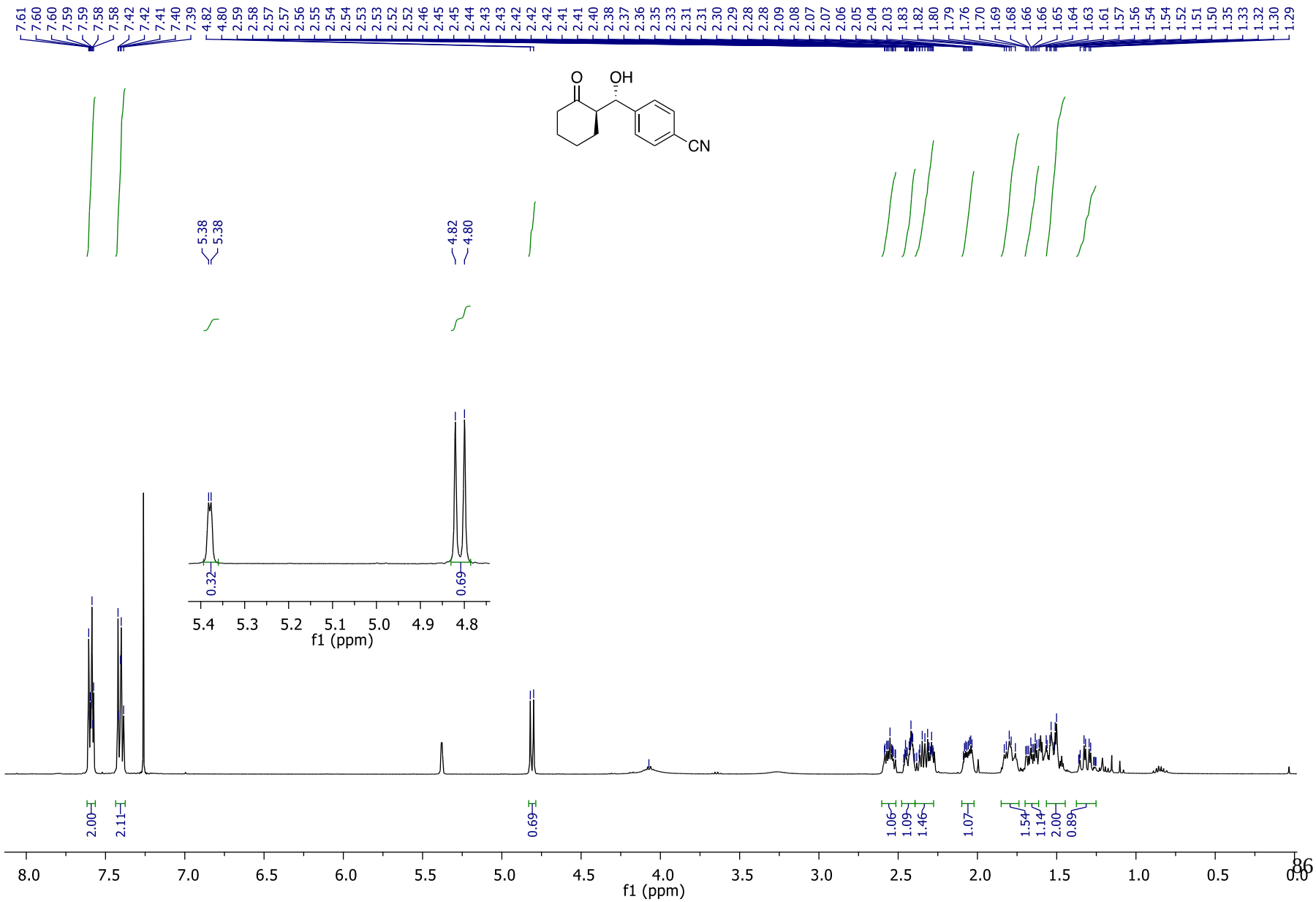

214.87
148.27
143.30
133.23
129.30
122.85
122.01
73.98
57.12
42.64
30.72
27.63
24.64
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

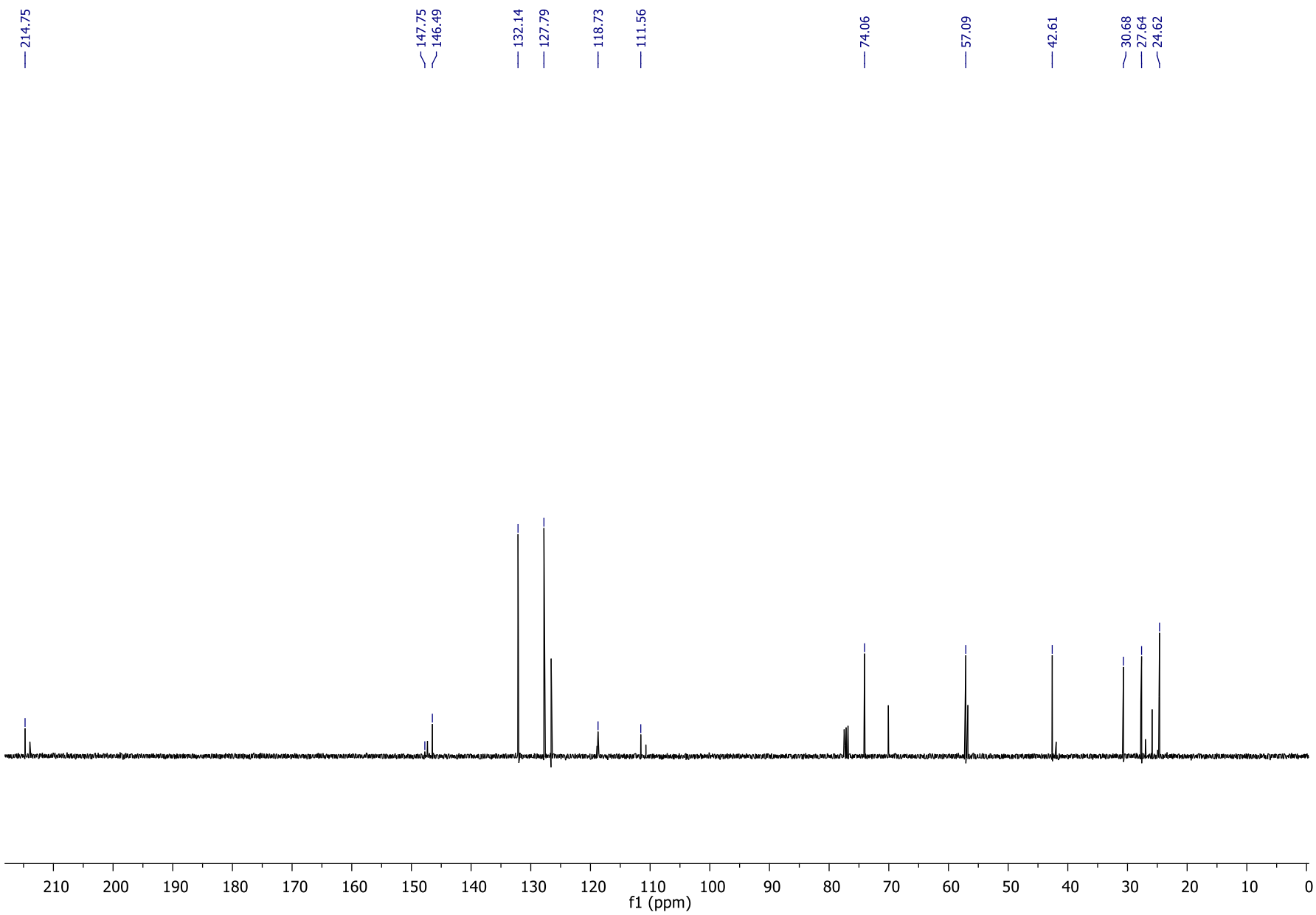
(R)-2-((S)-hydroxy(2-nitrophenyl)methyl)cyclohexan-1-one (3.3a)



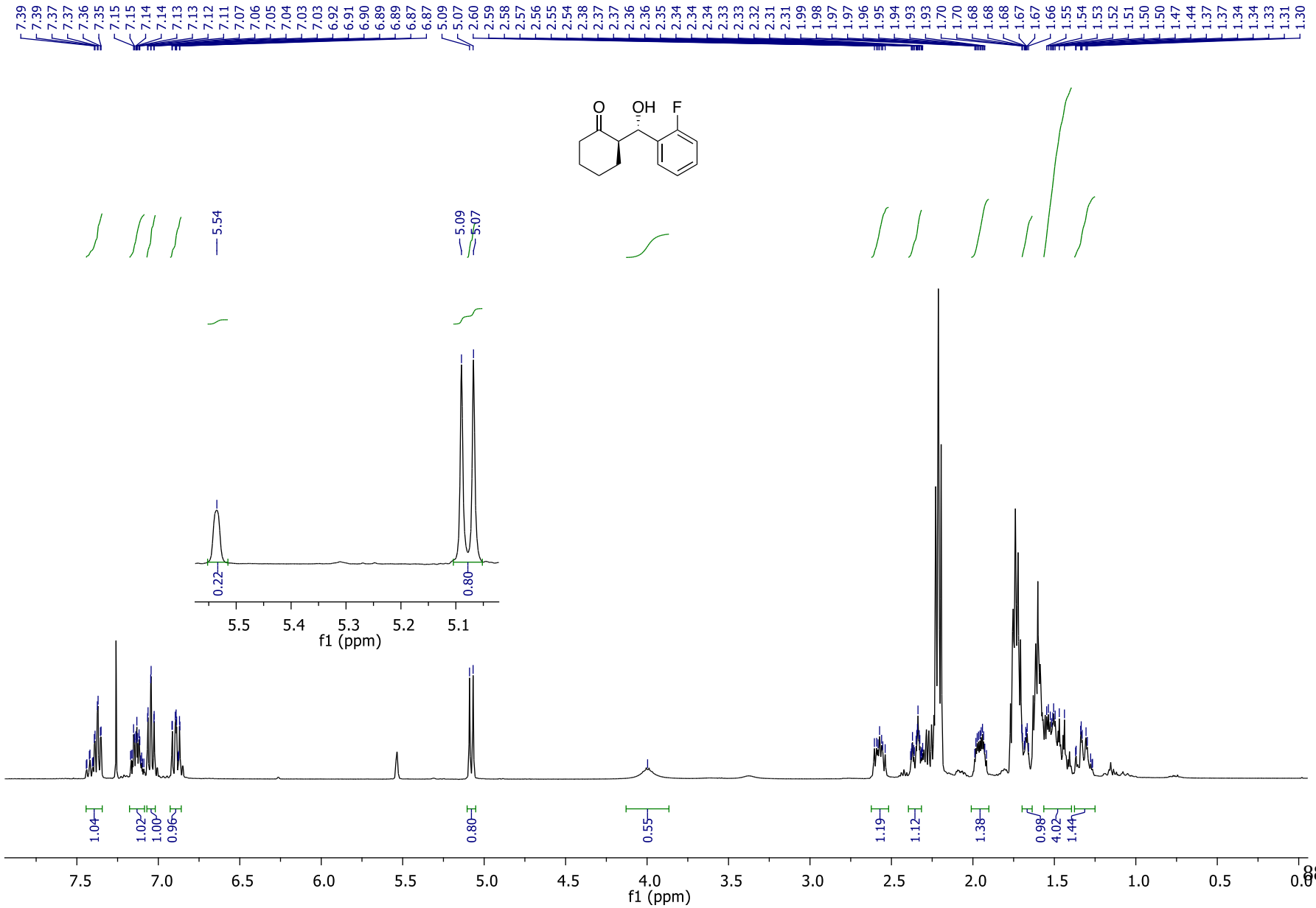

214.95
136.60
133.08
129.02
128.41
124.08
69.73
57.31
42.82
31.10
27.76
24.97
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

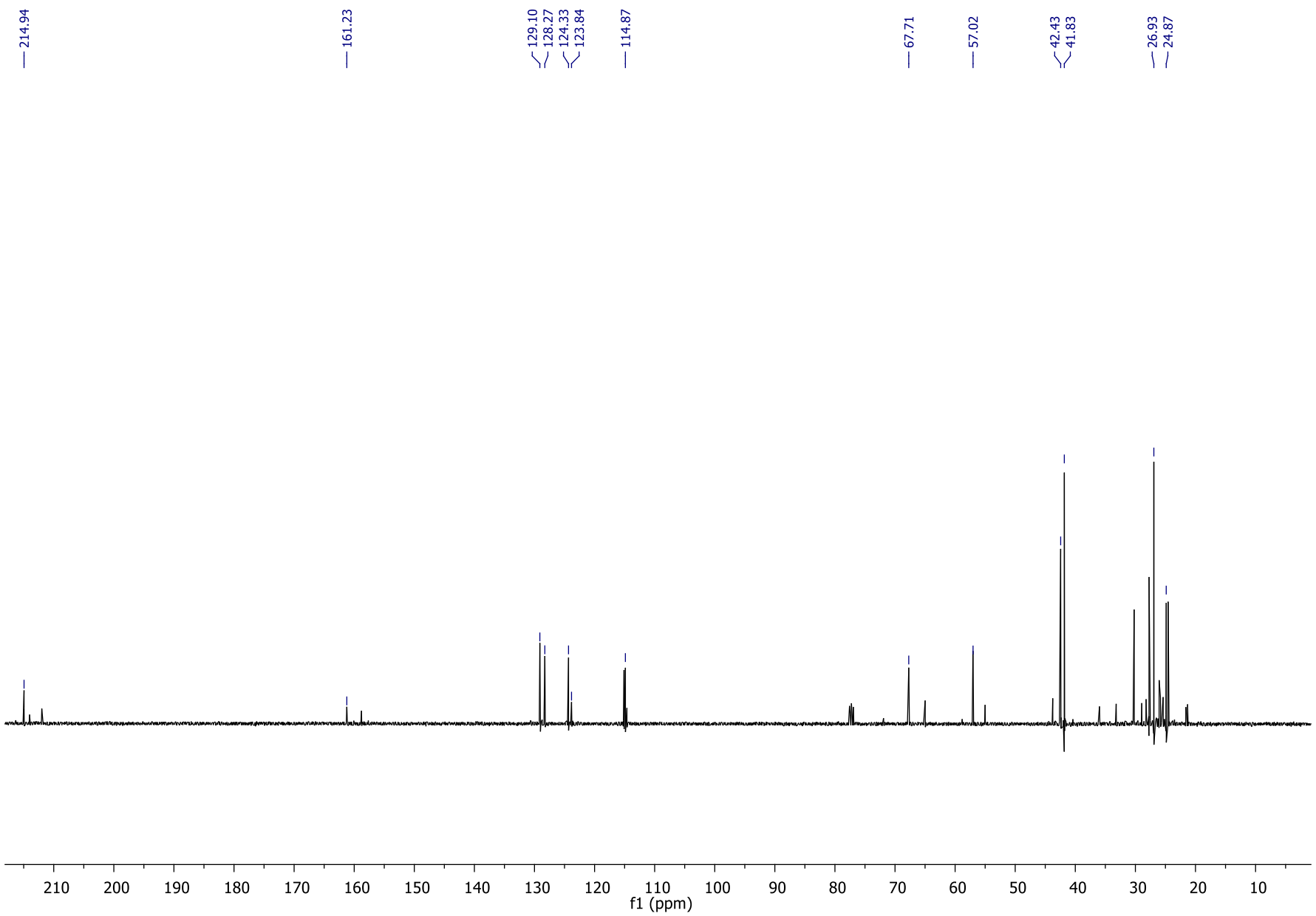
4-((S)-hydroxy((R)-2-oxocyclohexyl)methyl)benzonitrile (3.4a)



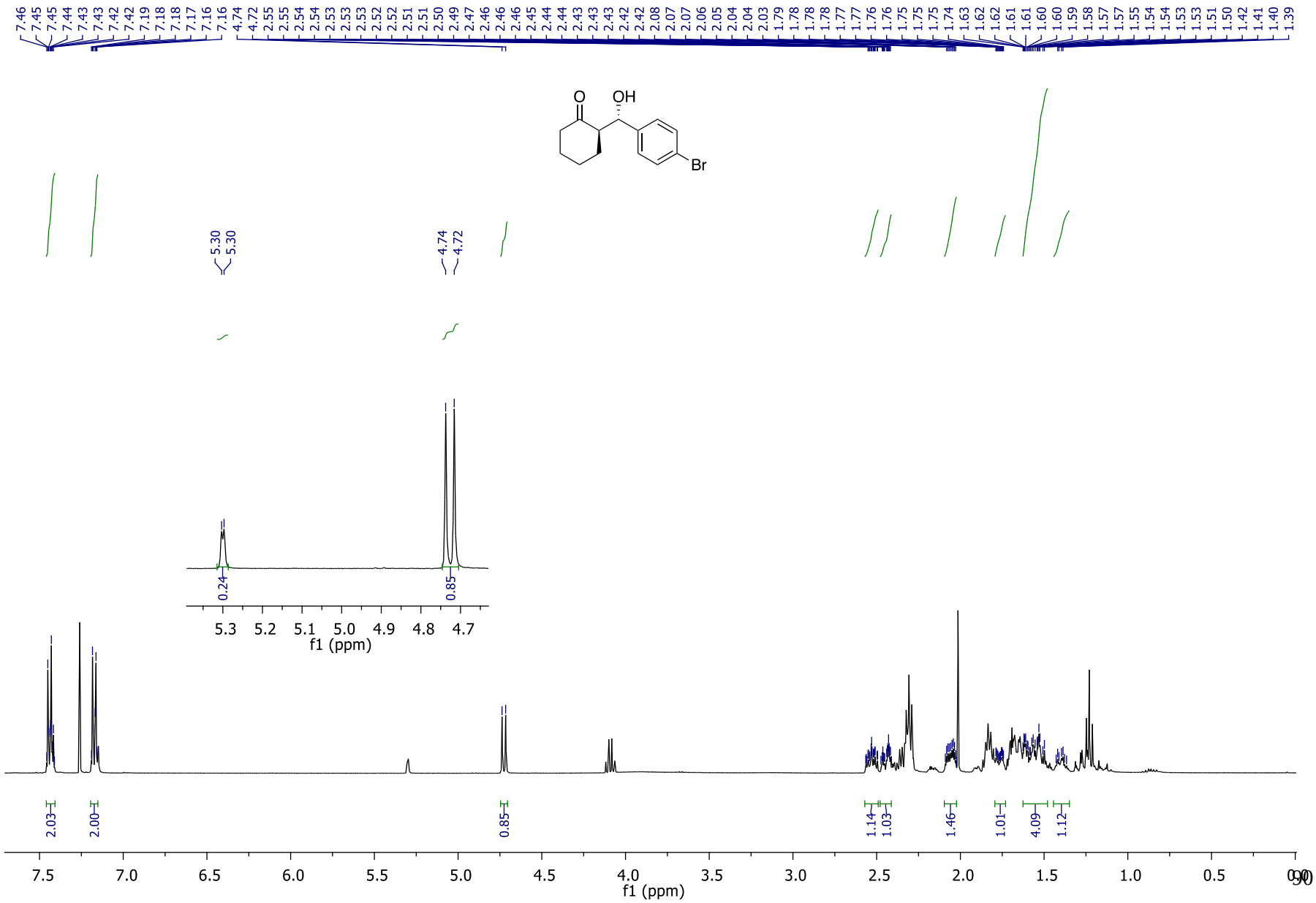

214.75
147.75
146.49
132.14
127.79
118.73
111.56
74.06
57.09
42.61
30.68
27.64
24.62
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

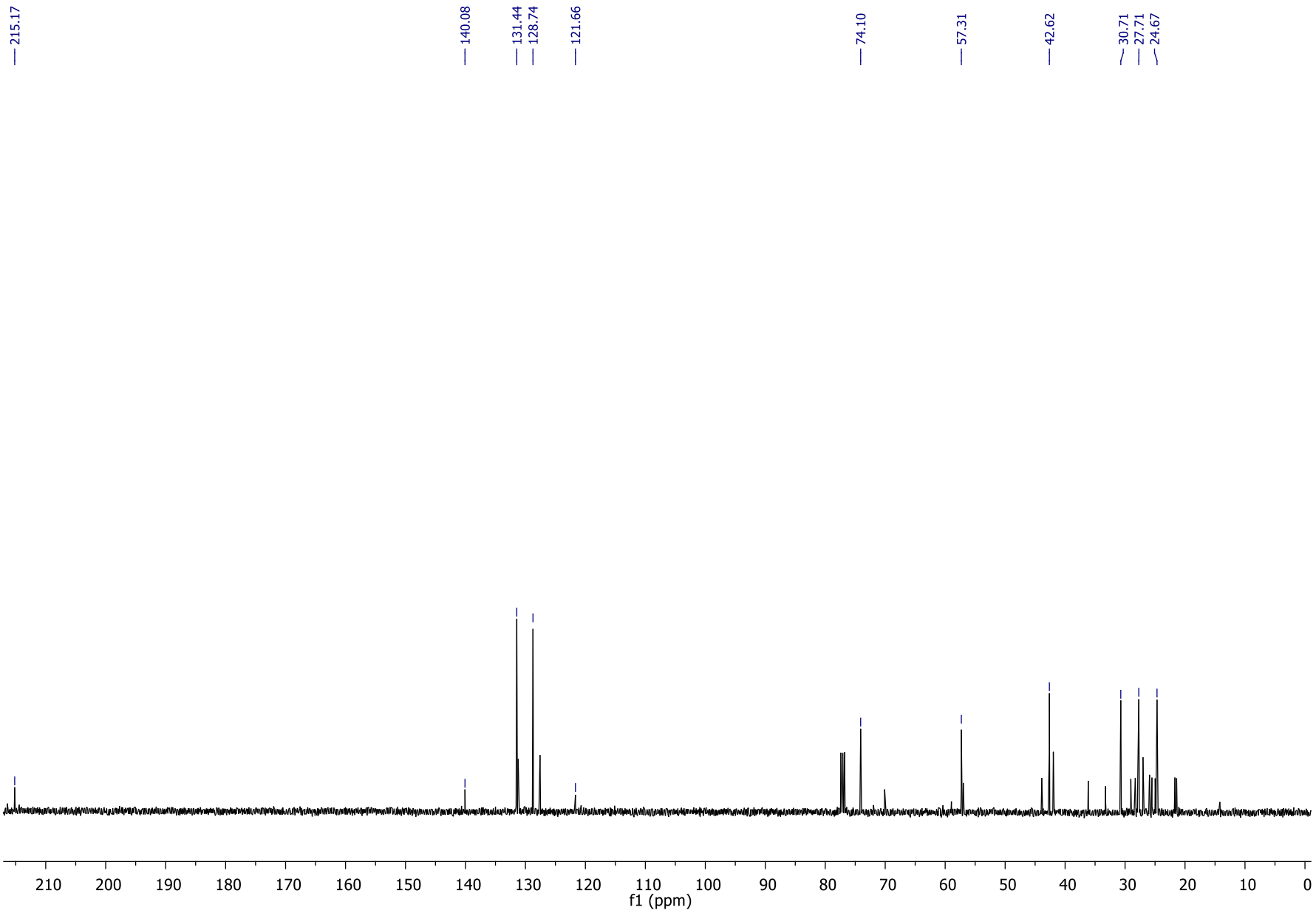
(R)-2-((S)-(2-fluorophenyl)(hydroxy)methyl)cyclohexan-1-one (3.5a)



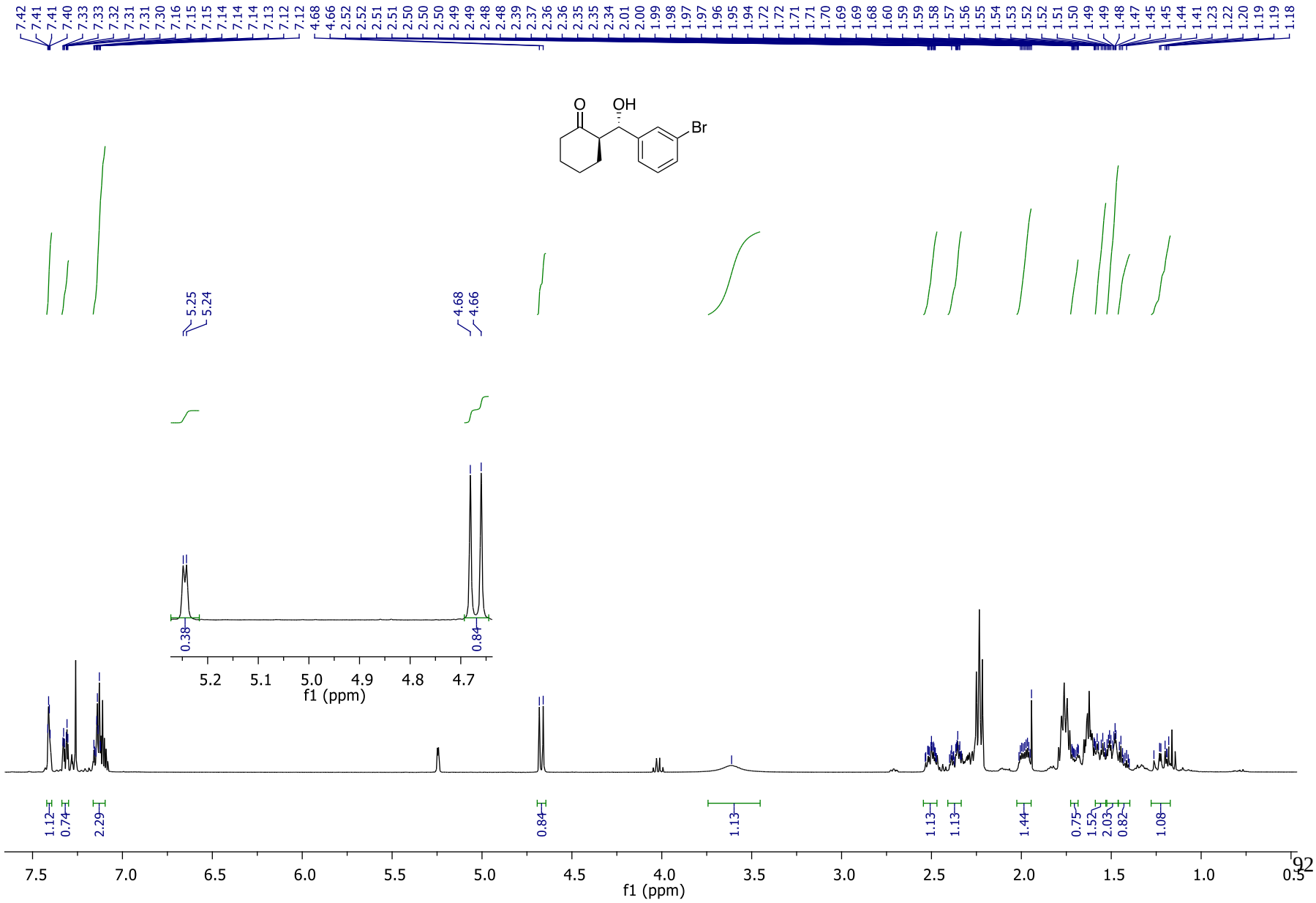

214.94
161.23
129.10
128.27
124.33
123.84
114.87
67.71
57.02
42.43
41.83
26.93
24.87
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

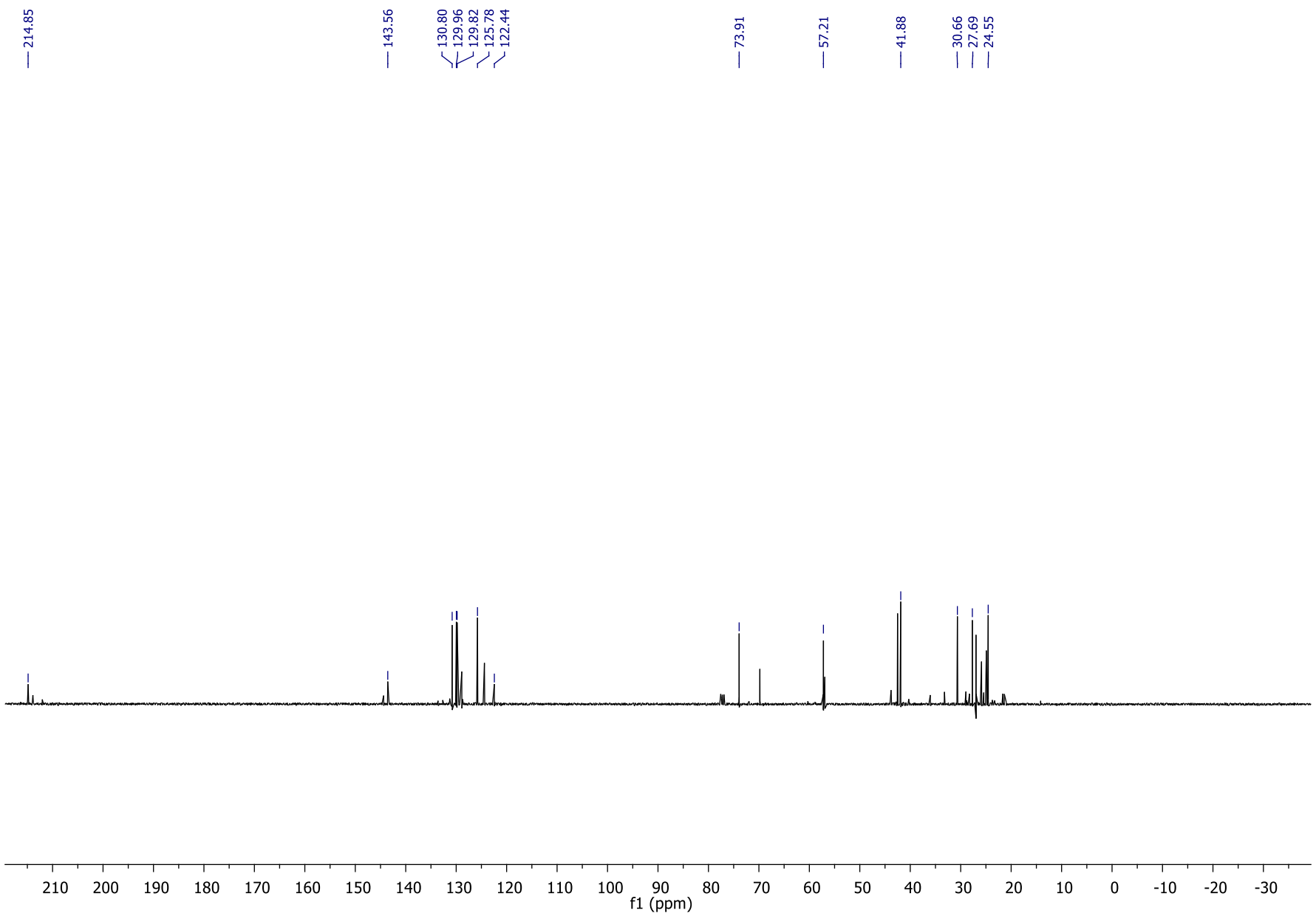
(R)-2-((S)-(4-bromophenyl)(hydroxy)methyl)cyclohexan-1-one (3.6a)



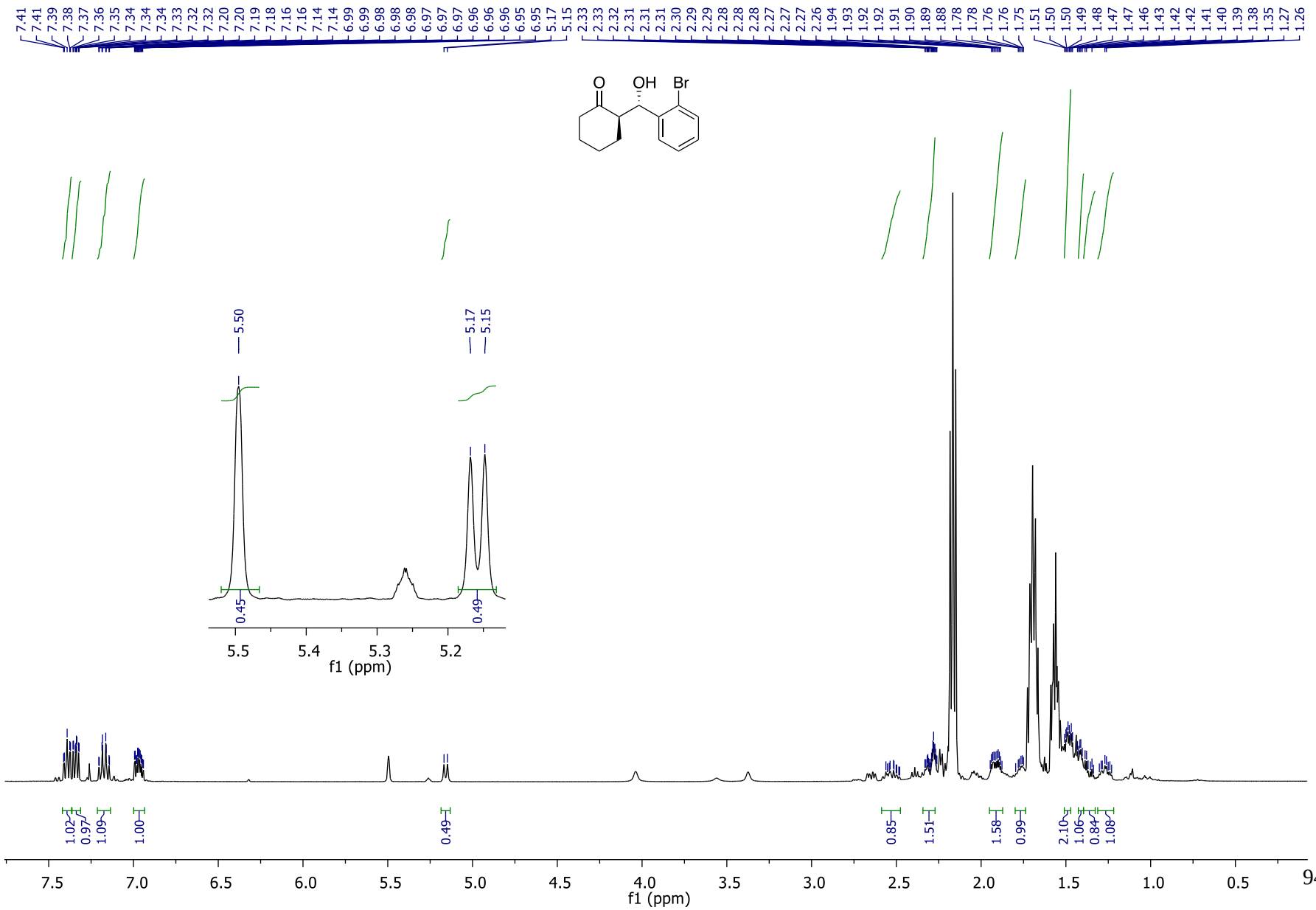

215.17
140.08
131.44
128.74
121.66
74.10
57.31
42.62
30.71
27.71
24.67
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

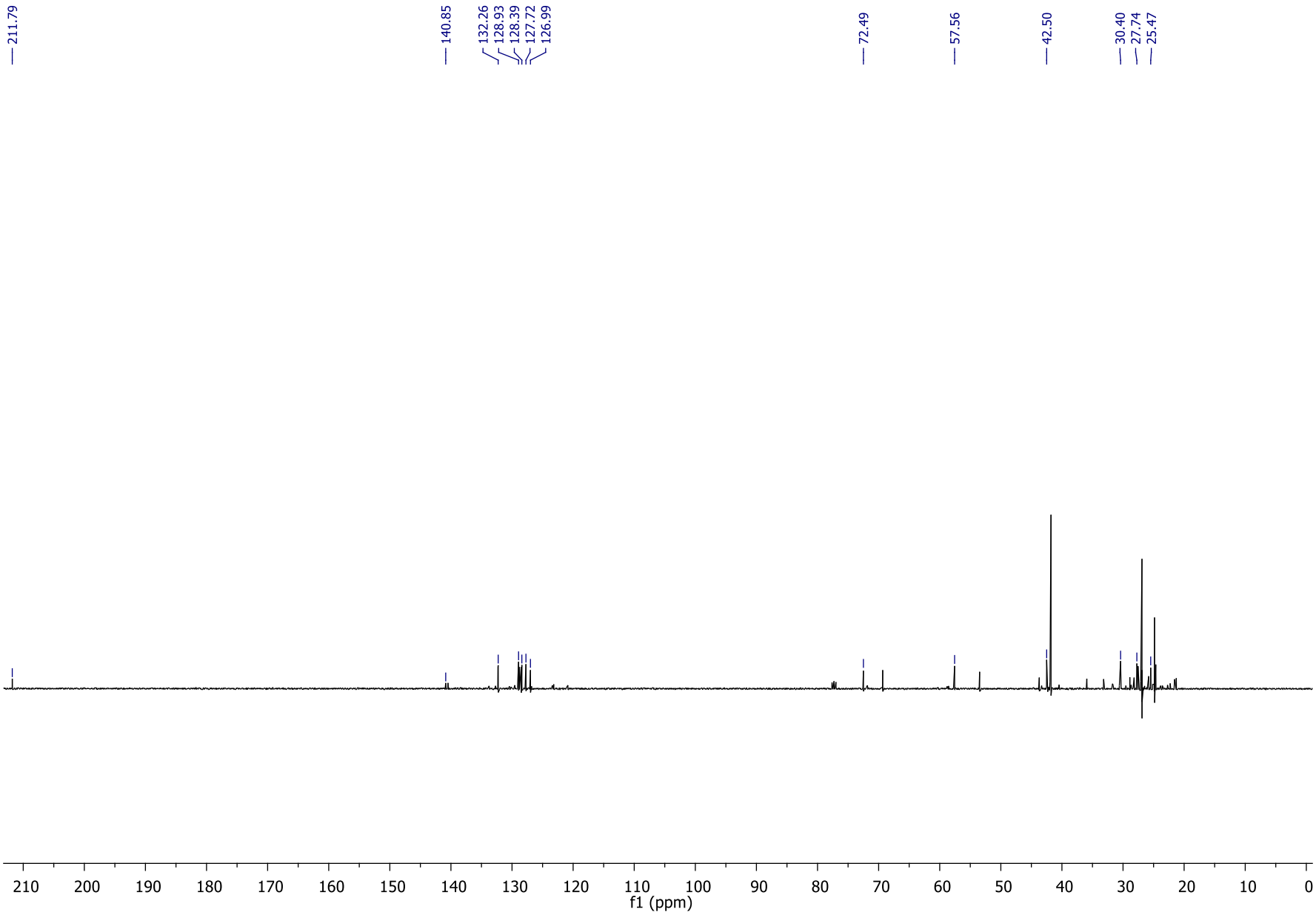
(R)-2-((S)-(3-bromophenyl)(hydroxy)methyl)cyclohexan-1-one (3.7a)



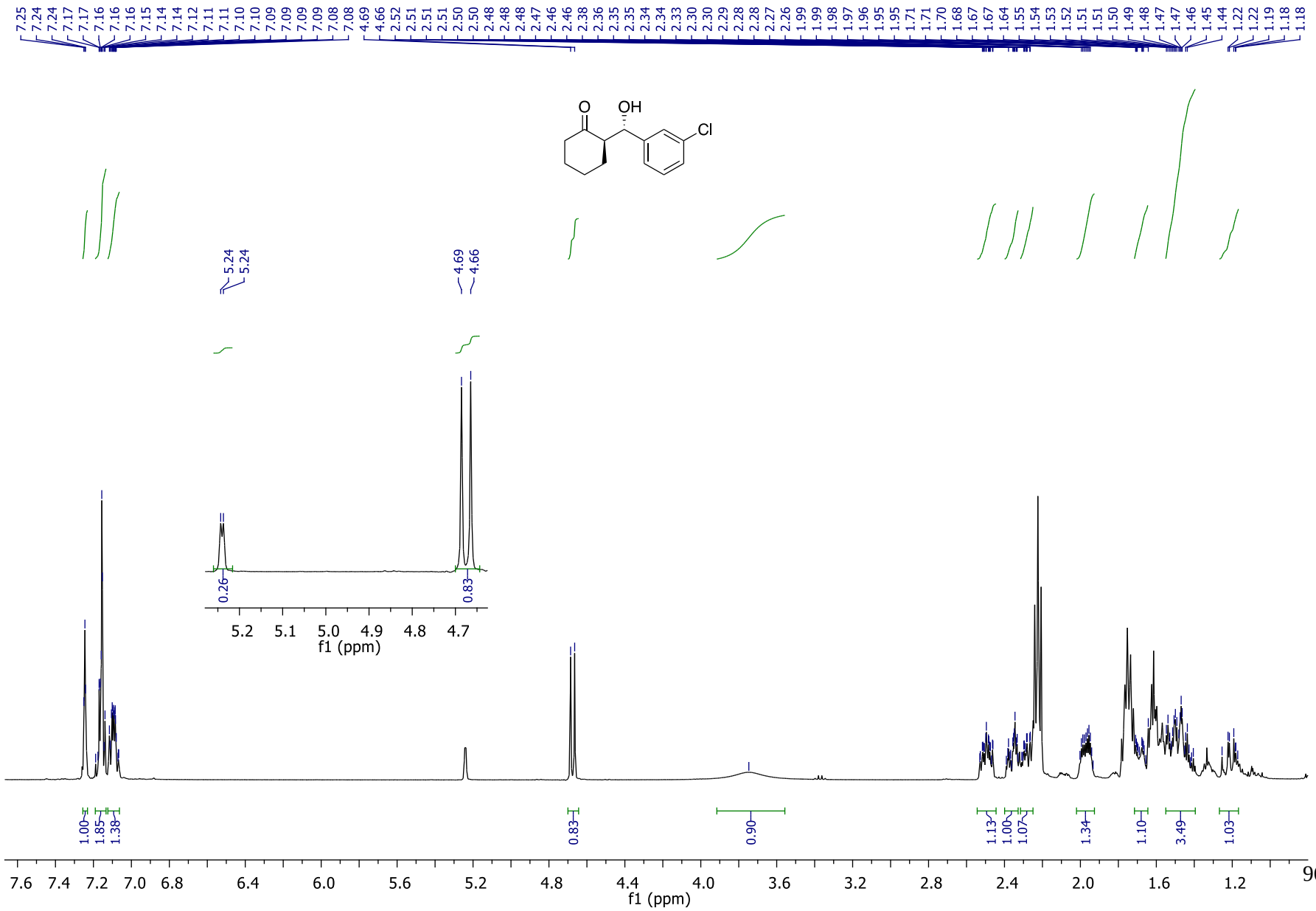

214.85
143.56
130.80
129.96
129.82
125.78
122.44
73.91
57.21
41.88
30.66
27.69
24.55
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30
f1 (ppm)

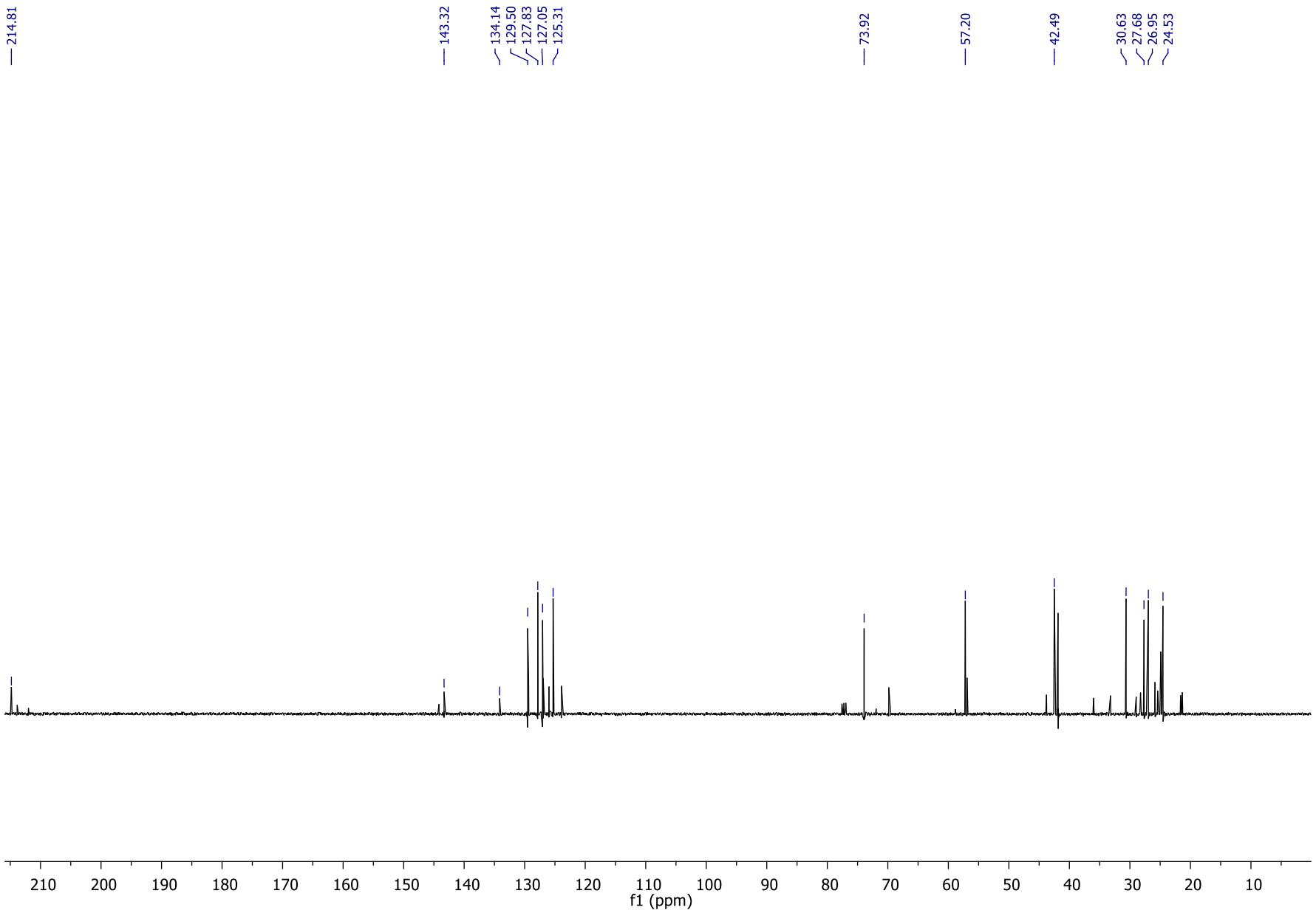
(R)-2-((S)-(2-bromophenyl)(hydroxy)methyl)cyclohexan-1-one (3.8a)



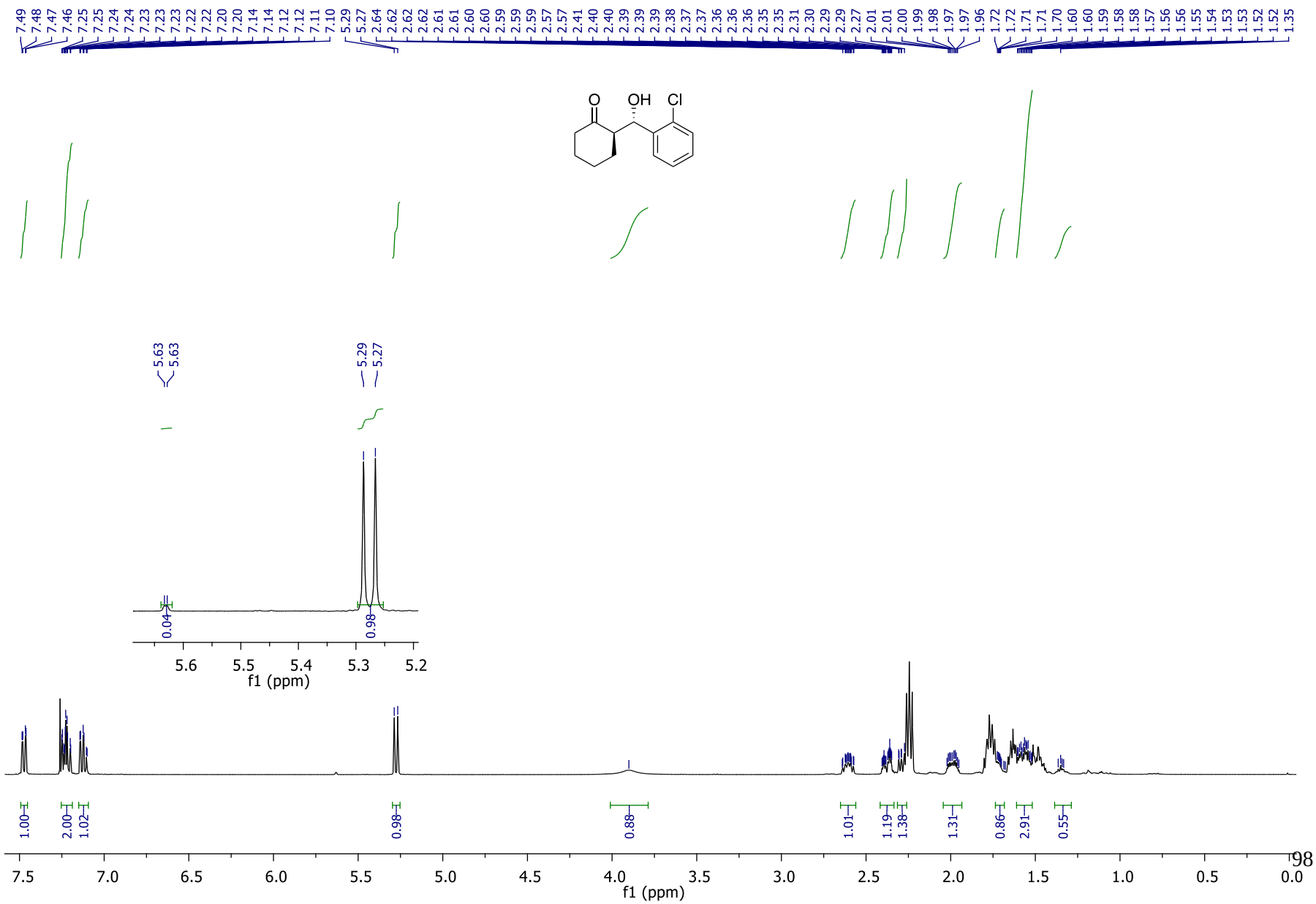

211.79
140.85
132.26
128.93
128.39
127.72
126.99
72.49
57.56
42.50
30.40
27.74
25.47
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

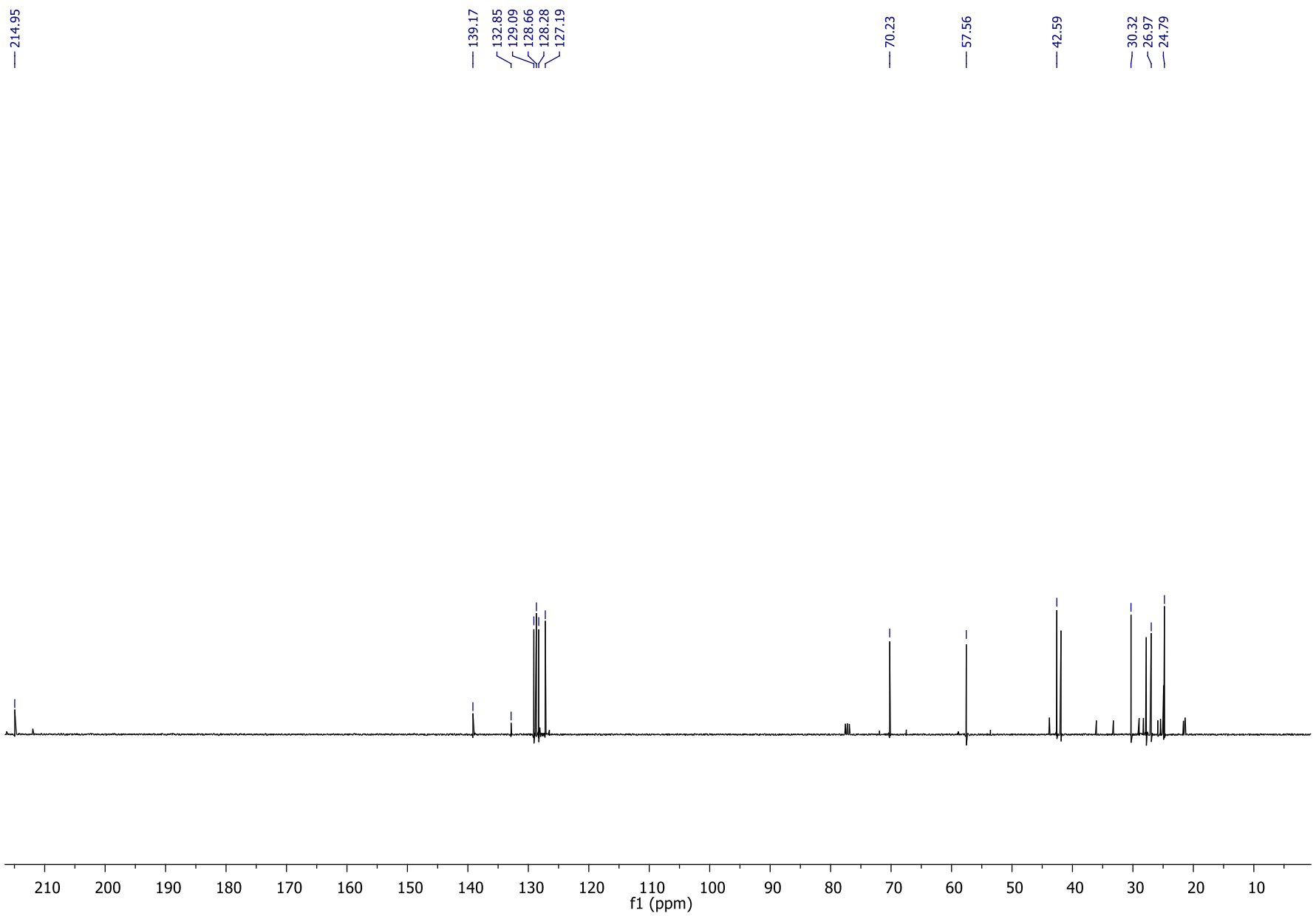
(R)-2-((S)-(3-chlorophenyl)(hydroxy)methyl)cyclohexan-1-one (3.9a)



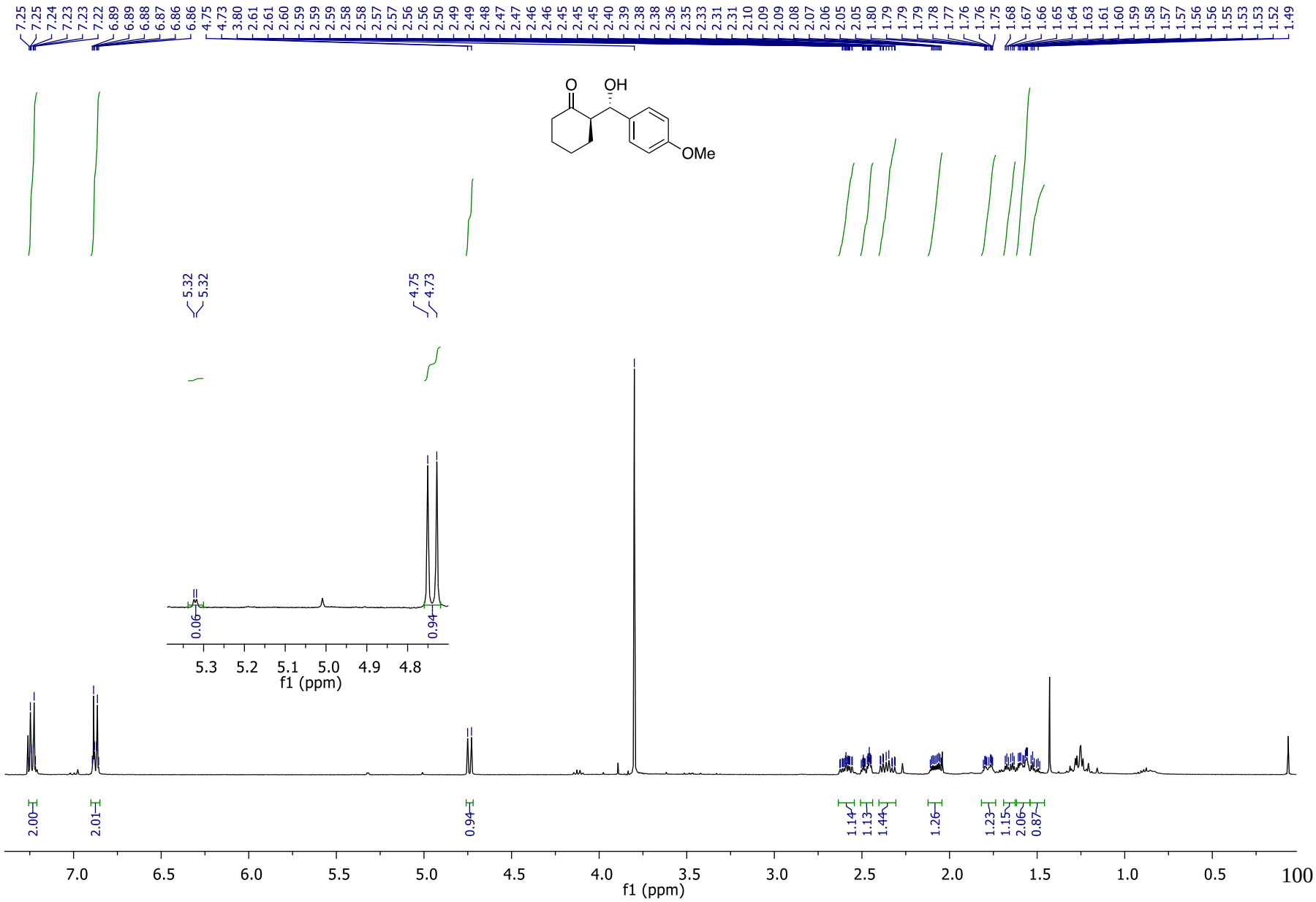

214.81
143.32
134.14
129.50
127.83
127.05
125.31
73.92
57.20
42.49
30.63
27.68
26.95
24.53
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

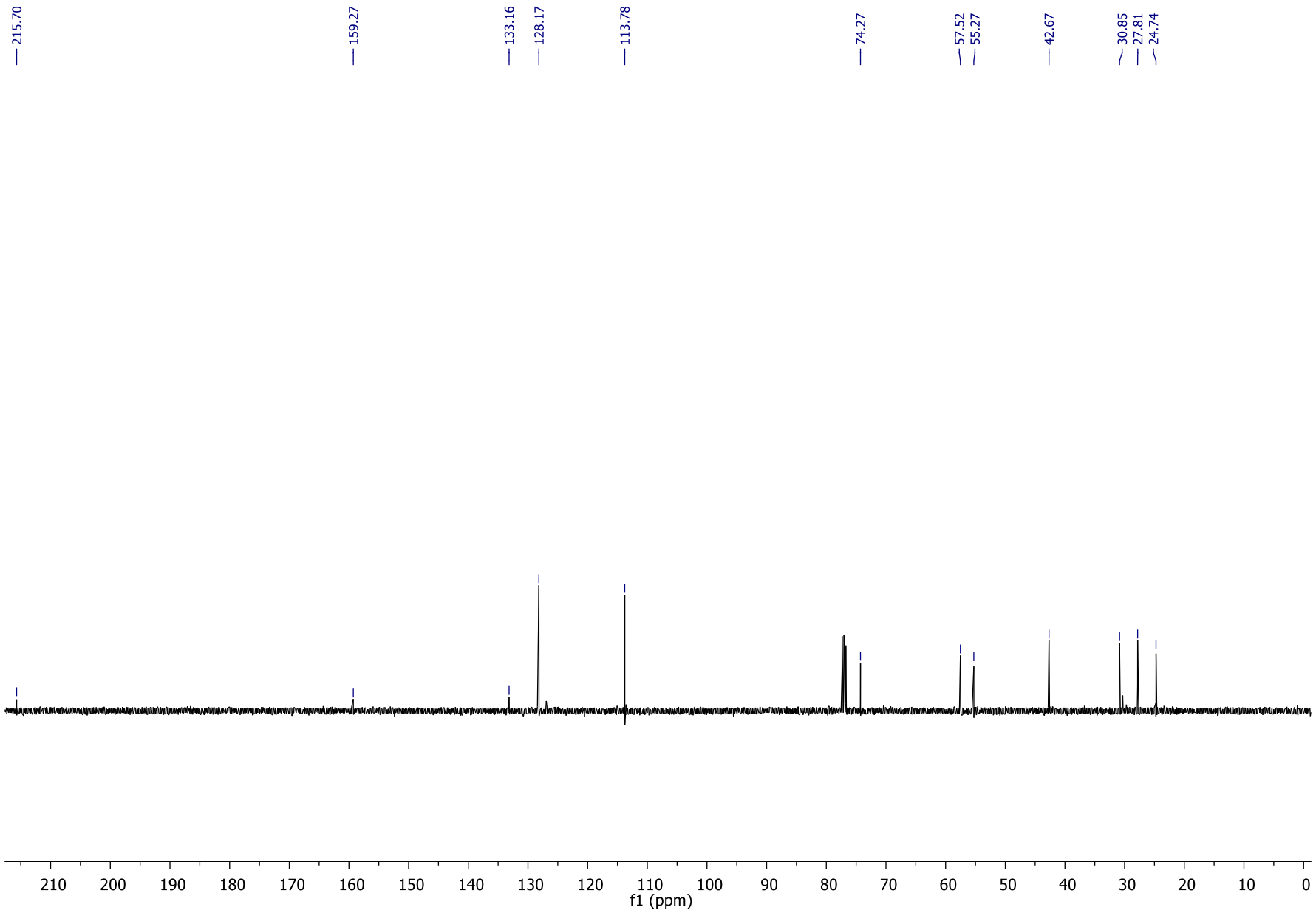
(R)-2-((S)-(2-chlorophenyl)(hydroxy)methyl)cyclohexan-1-one (3.10a)



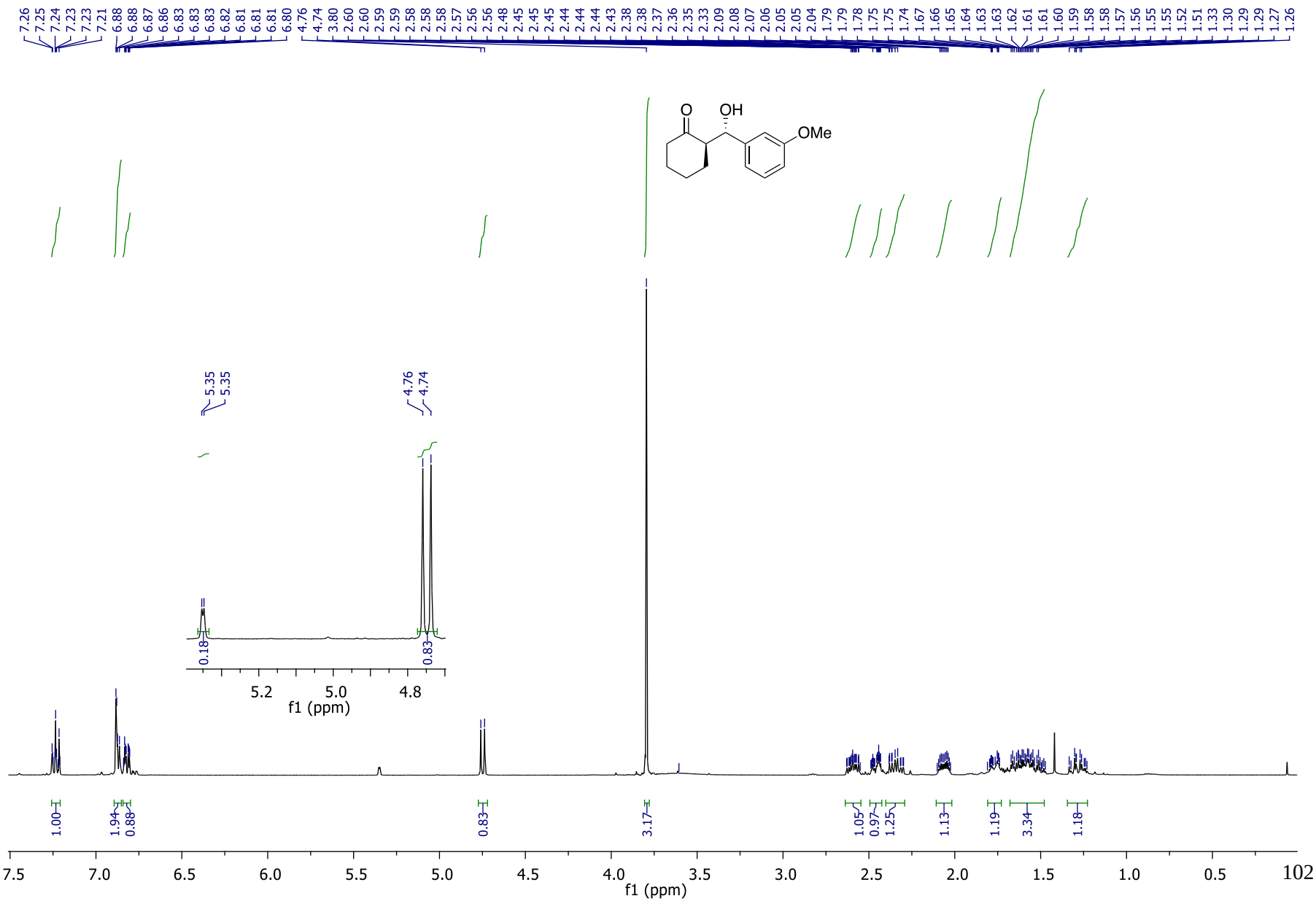

214.95
139.17
132.85
129.09
128.66
128.28
127.19
70.23
57.56
42.59
30.32
26.97
24.79
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

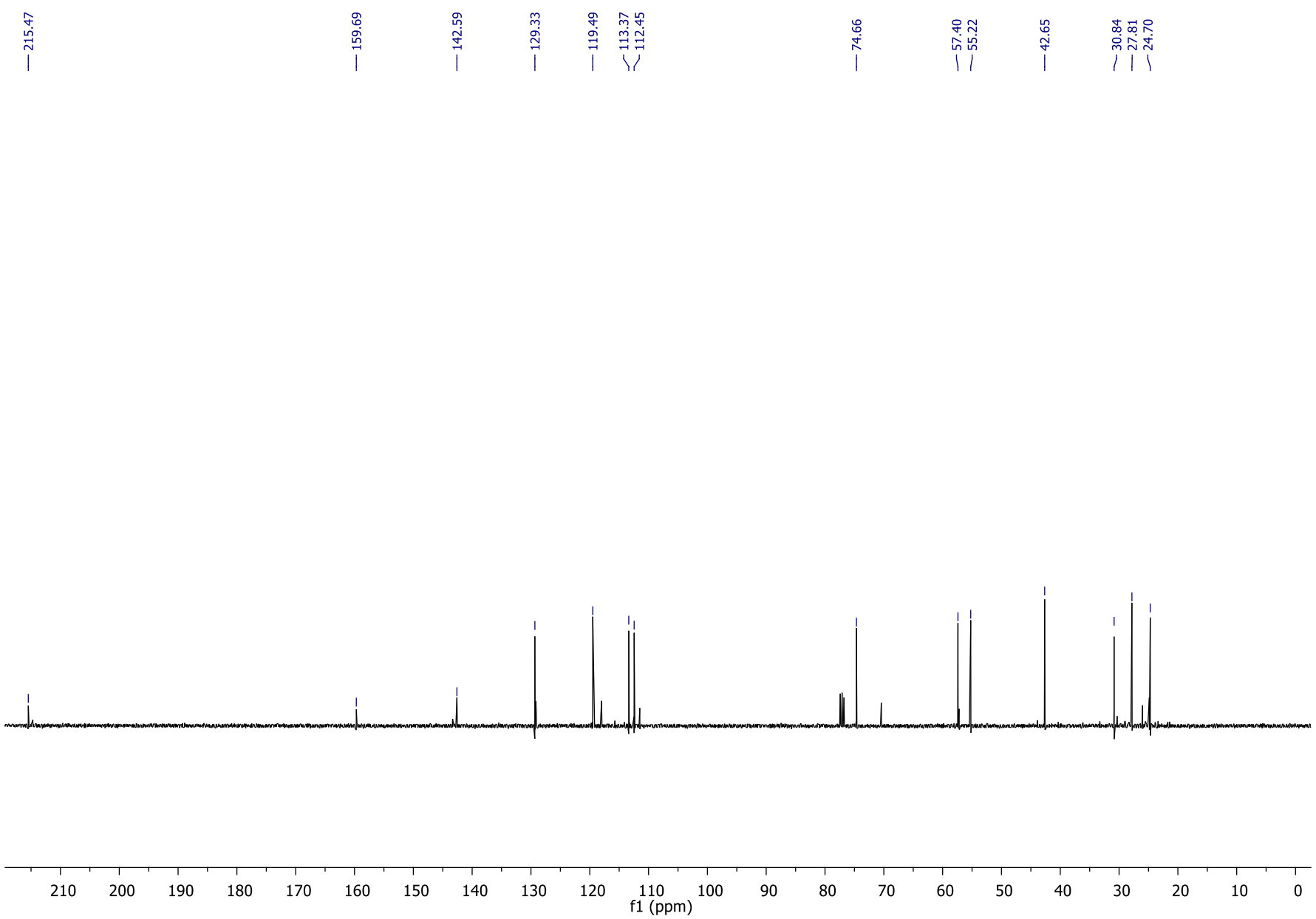
(R)-2-((S)-hydroxy(4-methoxyphenyl)methyl)cyclohexan-1-one (3.11a)



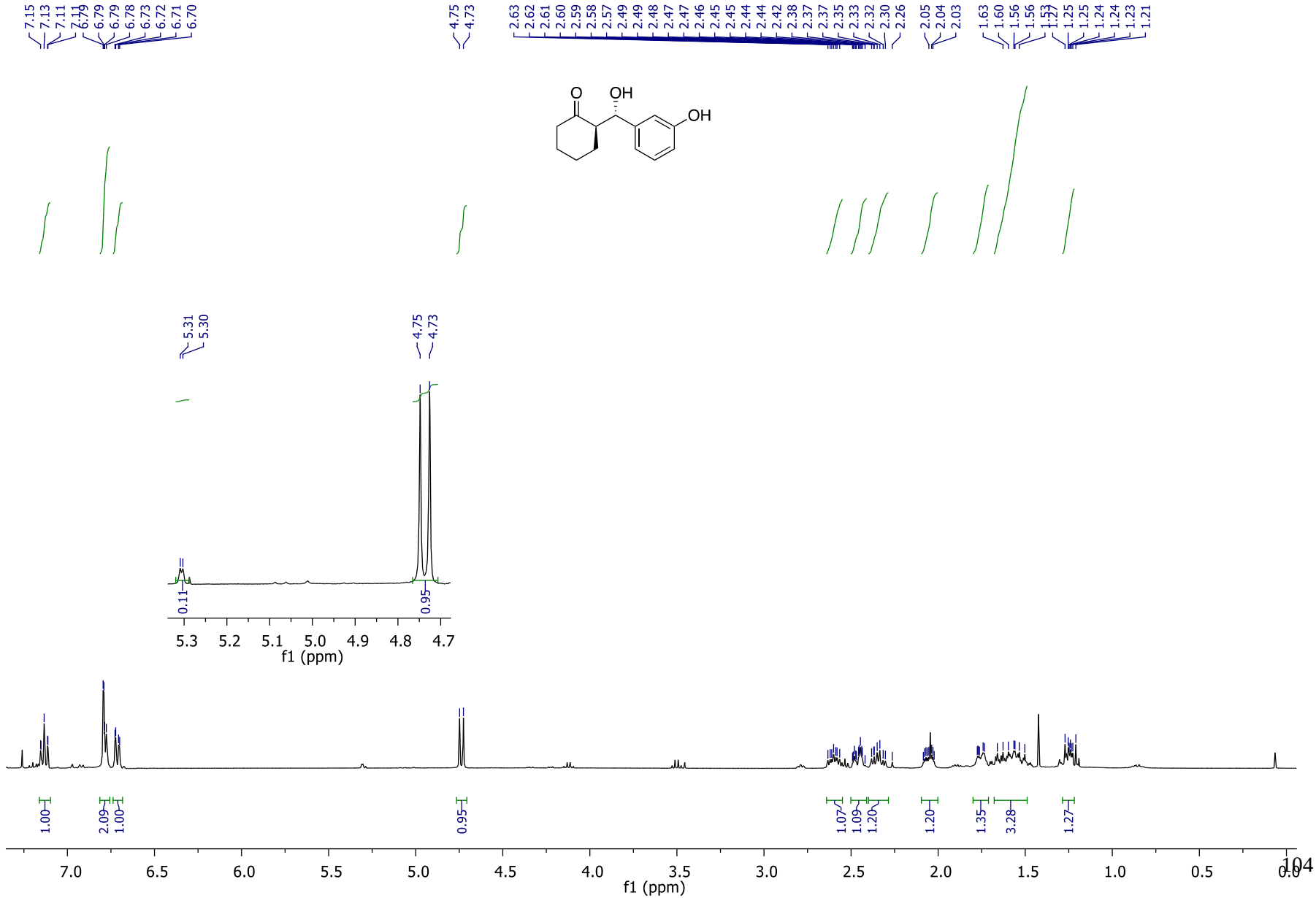

215.70
159.27
133.16
128.17
113.78
74.27
57.52
55.27
42.67
30.85
27.81
24.74
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

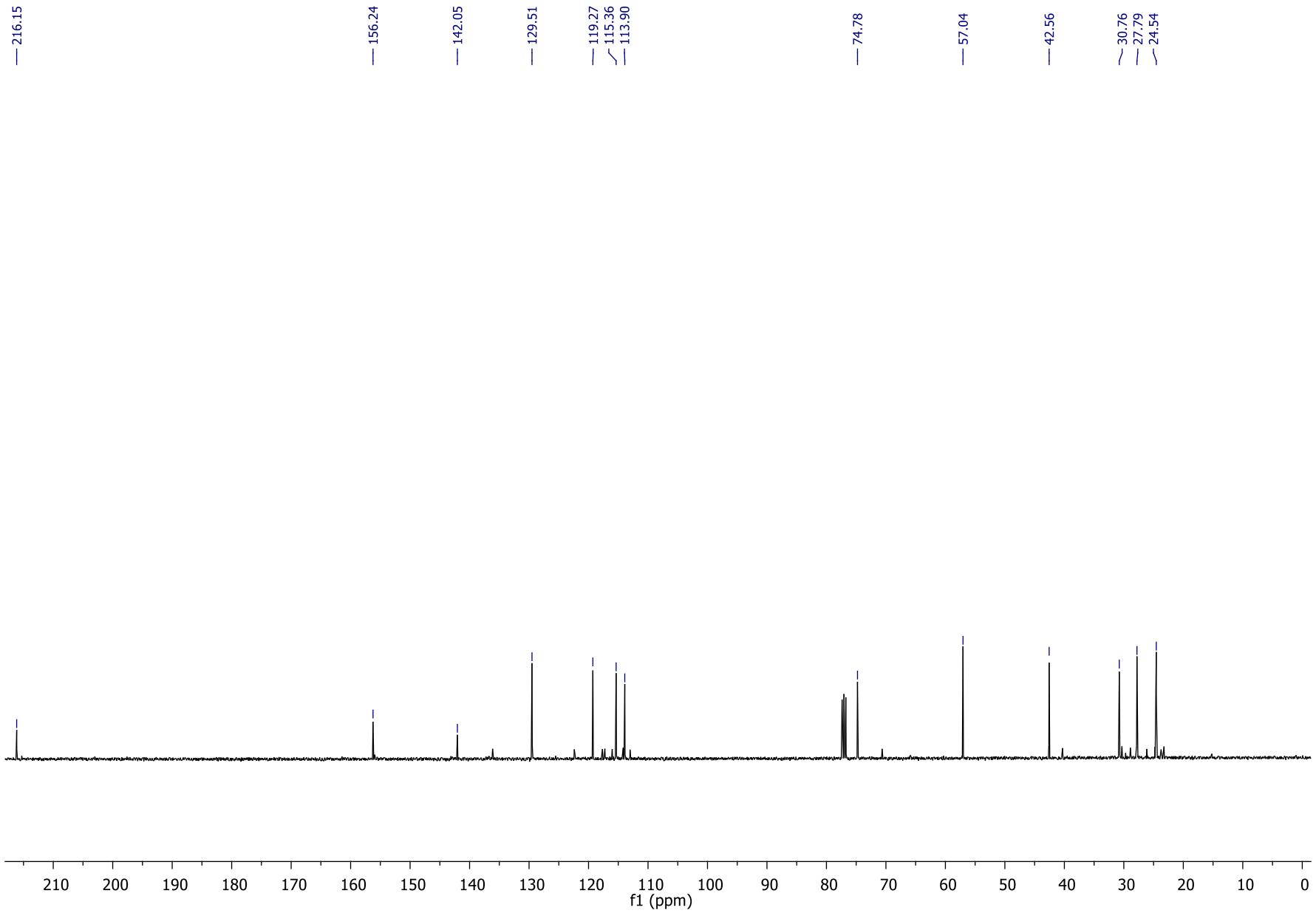
(R)-2-((S)-hydroxy(3-methoxyphenyl)methyl)cyclohexan-1-one (3.12a)



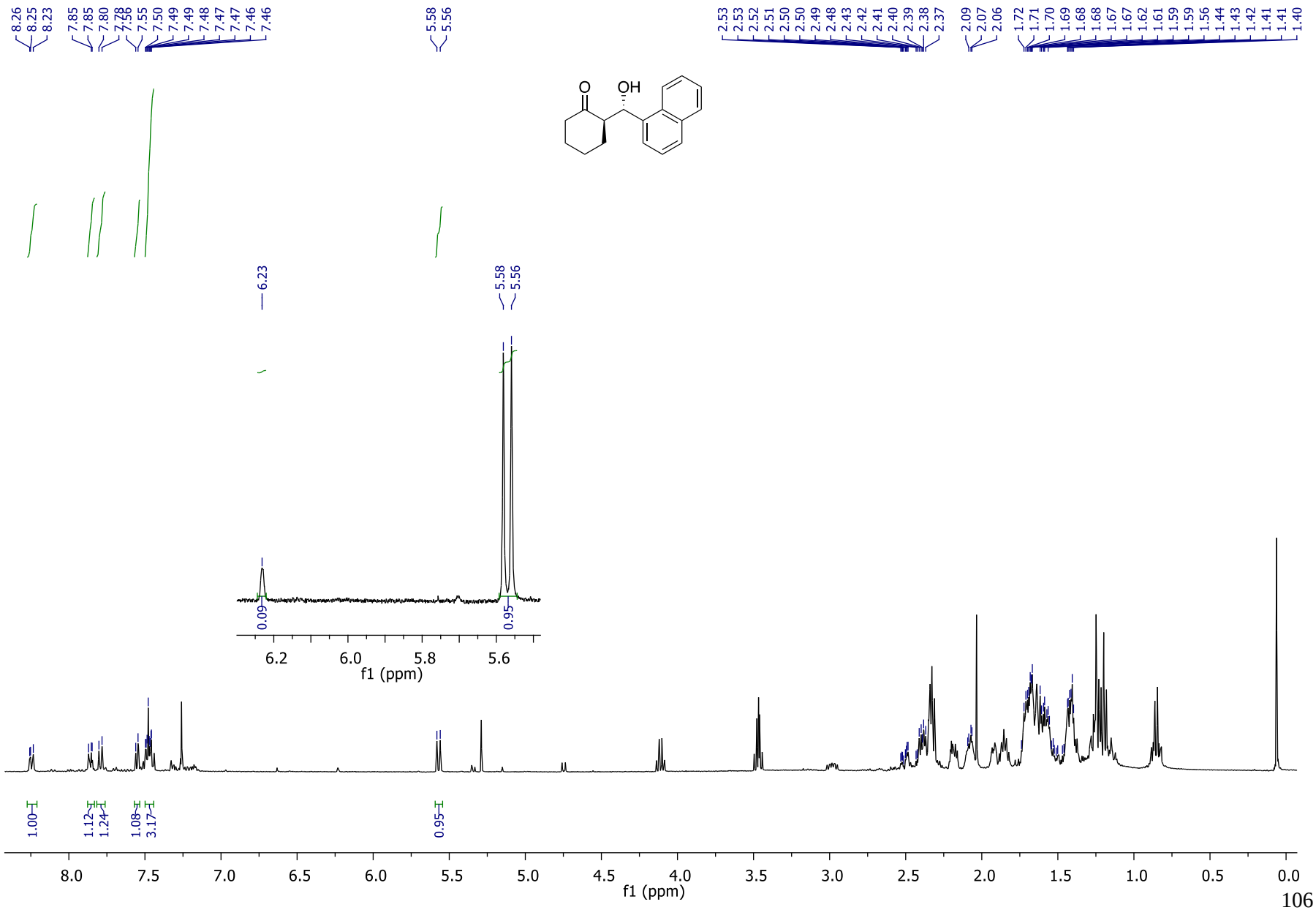

215.47
159.69
142.59
129.33
119.49
113.37
112.45
74.66
57.40
55.22
42.65
30.84
27.81
24.70
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

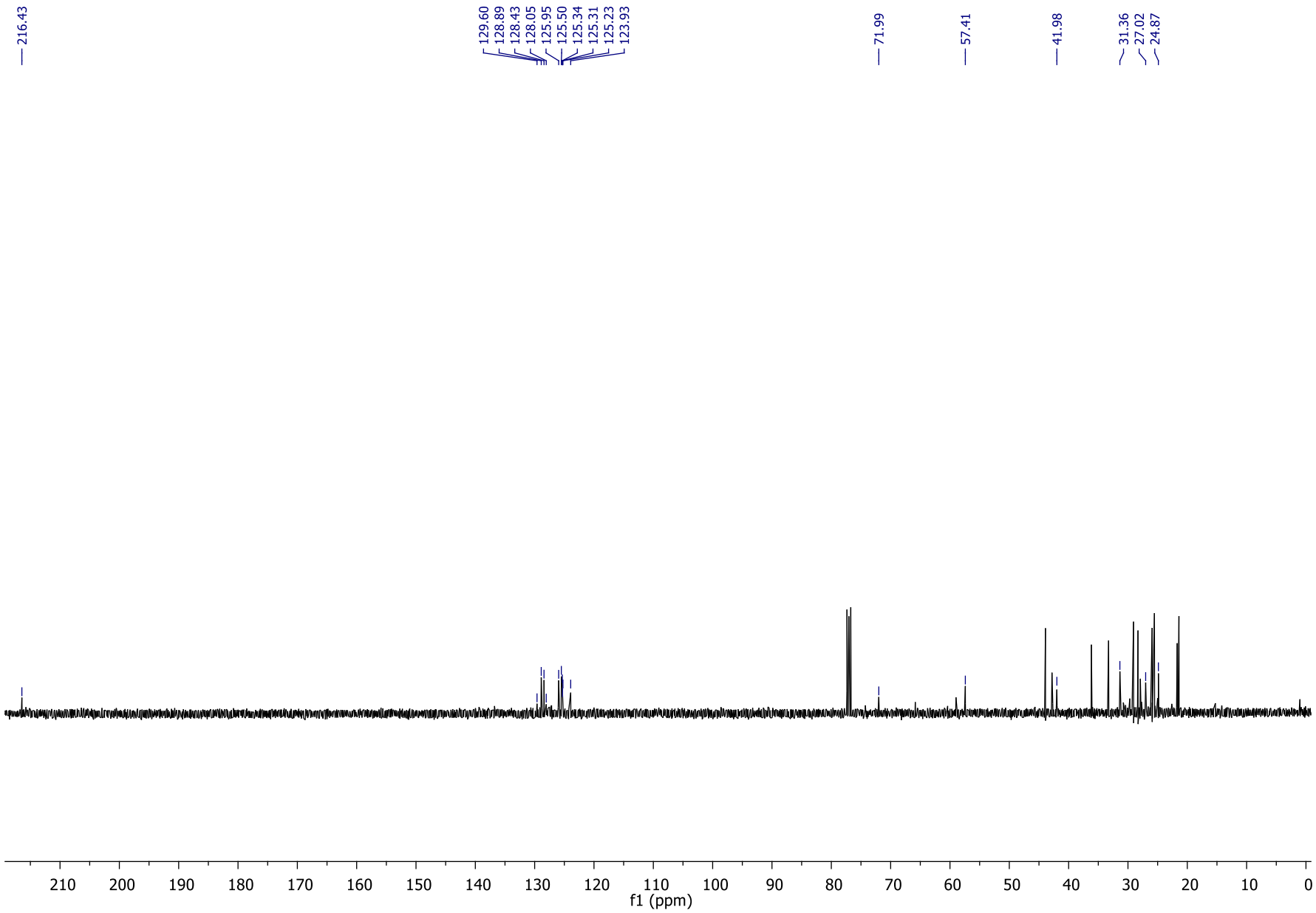
(R)-2-((S)-hydroxy(3-hydroxyphenyl)methyl)cyclohexan-1-one (3.13a)



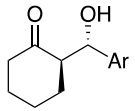

216.15
156.24
142.05
129.51
119.27
115.36
113.90
74.78
57.04
42.56
30.76
27.79
24.54
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

(R)-2-((S)-hydroxy(naphthalen-1-yl)methyl)cyclohexan-1-one (3.14a)



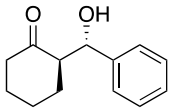

216.43
129.60
128.89
128.43
128.05
125.95
125.50
125.34
125.31
125.23
123.93
71.99
57.41
41.98
31.36
27.02
24.87
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

HPLC Data



Ar	Yield [%]	HPLC conditions						dr (*anti*:*syn*) from NMR	dr (*anti*:*syn*) from HPLC	*ee* anti [%]	*ee* syn [%]
		Column	Eluent	RT (major ent) *anti* [min]	RT (minor ent) *anti* [min]	RT (minor ent) *syn* [min]	RT (minor ent.) *syn* [min]				
Ph	47	IB	Hexane/iPrOH 9:1 (0.5 ml/min)	14.8	17.3	12.9	12.4	90:10	91:9	23	23
4-NO_2-C_6H_4	80	IB	Hexane/iPrOH 95:5 (1 ml/min)	24.0	31.6	19.8	22.4	72:28	66:34	87	29
3-NO_2-C_6H_4	70	IB	Hexane/iPrOH 95:5 (1 ml/min)	22.1	30.2	18.6	20.0	90:10	86:14	98	56
2-NO_2-C_6H_4	67	IA	Hexane/iPrOH 9:1 (1 ml/min)	19.3	20.0	nd	nd	≥99:1	≥99:1	99	nd
4-CN-C_6H_4	82	IB	Hexane/iPrOH 95:5 (1 ml/min)	27.2	35.2	22.1	25.4	68:32	67:33	24	5
2-F-C_6H_4	99	IC	Hexane/iPrOH 95:5 (1 ml/min)	26.4	33.3	14.7	13.0	78:22	91:9	39	1
4-Br-C_6H_4	95	IC	Hexane/iPrOH 95:5 (1 ml/min)	31.4	40.0	27.4	29.0	78:22	88:12	26	33
3-Br-C_6H_4	94	IB	Hexane/iPrOH 95:5 (1 ml/min)	12.4	10.7	9.1	8.7	69:31	64:36	20	22
2-Br-C_6H_4	97	IC	Hexane/iPrOH 9:1 (1 ml/min)	15.6	19.2	8.7	9.6	48:52	42:58	8	65
3-Cl-C_6H_4	99	IB	Hexane/iPrOH 95:5 (1 ml/min)	10.2	11.9	8.7	8.3	78:22	62:38	21	26
2-Cl-C_6H_4	98	IB	Hexane/iPrOH 95:5 (1 ml/min)	8.9	9.8	nd	nd	97:3	≥99:1	6	nd
4-OMe-C_6H_4	17	IA	Hexane/iPrOH 95:5 (1 ml/min)	33.4	32.0	nd	nd	≥99:1	≥99:1	9	nd
3-OMe-C_6H_4	77	IA	Hexane/iPrOH 95:5 (1 ml/min)	30.2	28.8	16.1	18.8	82:18	71:29	25	17
3-OH-C_6H_4	40	IC	Hexane/iPrOH 9:1 (1 ml/min)	59.8	55.7	43.8	40.1	90:10	93:7	16	28
1-naphthyl	38	IA	Hexane/iPrOH 95:5 (1 ml/min)	24.6	29.2	14.0	12.0	90:10	80:20	31	26

(R)-2-((S)-hydroxy(phenyl)methyl)cyclohexan-1-one (3.1a)



30/11/2020 16:22 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR138rac.PRM Page 1 of 2

Clarity - Chromatography SW
DataApex 2006
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Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR138rac.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 30/11/2020 15:38:35
Acquired Date : 30/11/2020 15:38:34
By : Fabrizio

Printed Version Info:
Printed Version : 30/11/2020 16:21:56
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 30/11/2020 16:22:20
By : Fabrizio

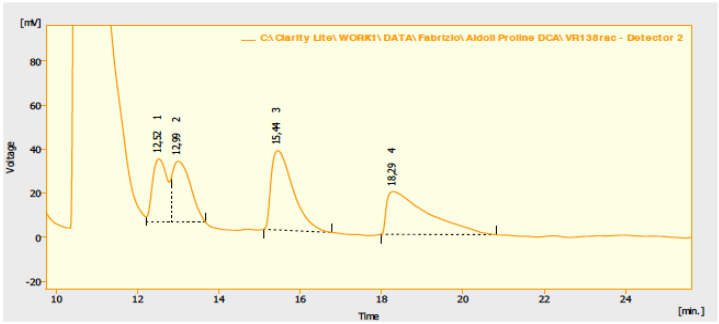
Sample Info:
Sample ID : VR138rac
Sample : Ph aldol racemate
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 30/11/2020 16:21

Column : IB
Mobile Phase : H/IPrOH 9:1
Flow Rate : 0.5 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 6.28 min and 7.27, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



30/11/2020 16:22 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR138rac.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR138rac - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	12,524	729,913	28,591	17,0	25,6	0,48	
2	12,992	844,460	27,576	19,7	24,7	0,52	
3	15,444	1351,312	35,997	31,5	32,3	0,58	
4	18,292	1369,208	19,425	31,9	17,4	1,02	
	Total	4294,893	111,590	100,0	100,0		

30/11/2020 16:24 Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\CAMPIONI FINO 30 NOV\VR138.PRM Page 1 of 2

Clarity - Chromatography SW
DataApex 2006
www.dataapex.com

Chromatogram Info:
File Name : C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\CAMPIONI FINO 30 NOV\VR138.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 17/11/2020 09:32:59
Acquired Date : 17/11/2020 09:35:20
By : Fabrizio

Printed Version Info:
Printed Version : 30/11/2020 16:24:25
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 30/11/2020 16:24:40
By : Fabrizio

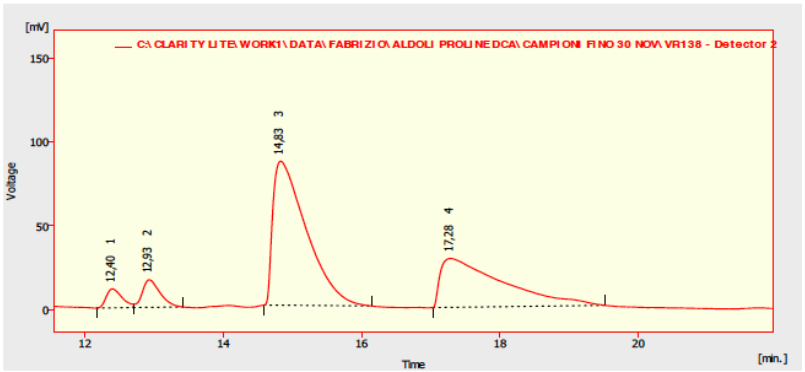
Sample Info:
Sample ID : VR138
Sample : Ph aldol
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 30/11/2020 16:24

Column : IB
Mobile Phase : H/IPrOH 9:1
Flow Rate : 0.5 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 6.28 min and 7.27, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



30/11/2020 16:24 Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\CAMPIONI FINO 30 NOV\VR138.PRM Page 2 of 2

Result Table (Uncal - C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\CAMPIONI FINO 30 NOV\VR138 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	12,396	179,475	11,337	3,5	7,9	0,26	
2	12,928	292,800	16,466	5,6	11,5	0,28	
3	14,828	2898,402	85,893	55,7	60,1	0,52	
4	17,284	1830,189	29,184	35,2	20,4	0,97	
	Total	5200,866	142,880	100,0	100,0		

(R)-2-((S)-hydroxy(4-nitrophenyl)methyl)cyclohexan-1-one (3.1.1a)

Clarity - Chromatography SW
DataApex 2006
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Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR116rac.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 11/01/2021 11:10:17
Acquired Date : 11/01/2021 11:10:17
By : Fabrizio

Printed Version Info:
Printed Version : 11/01/2021 11:32:20
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 11/01/2021 11:32:49
By : Fabrizio

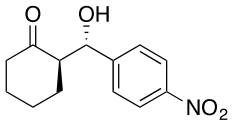
Sample Info:
Sample ID : VR116rac
Sample : 4-NO2 aldol rac
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

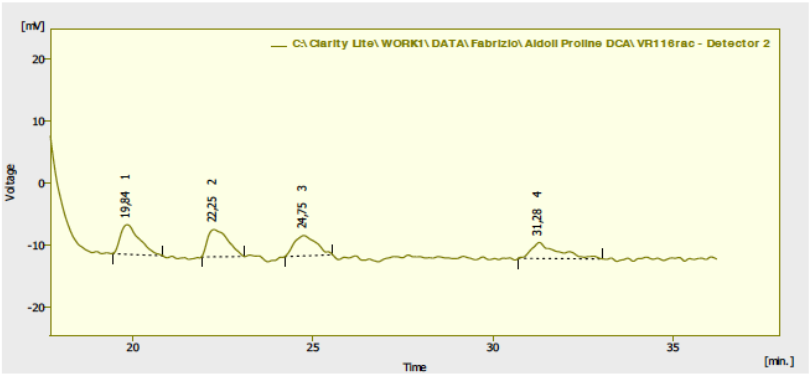
Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 11/01/2021 11:32

Column : IB
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0





Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR116rac - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	19,844	175,242	4,762	28,0	31,7	0,58	
2	22,252	174,628	4,409	27,9	29,3	0,66	
3	24,748	141,350	3,282	22,6	21,8	0,76	
4	31,284	134,738	2,584	21,5	17,2	0,67	
	Total	625,957	15,038	100,0	100,0		

Clarity - Chromatography SW
DataApex 2006
www.dataapex.com

Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR116.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 11/01/2021 10:39:21
Acquired Date : 11/01/2021 10:39:21
By : Fabrizio

Printed Version Info:
Printed Version : 11/01/2021 11:32:18
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 11/01/2021 11:32:57
By : Fabrizio

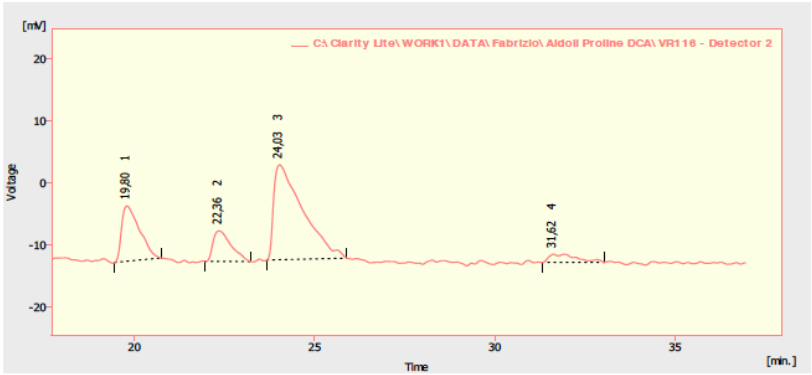
Sample Info:
Sample ID : VR116
Sample : 4-NO2 aldol
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 11/01/2021 11:32

Column : IB
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR116 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	19,800	307,441	8,912	21,8	29,3	0,57	
2	22,356	171,478	4,913	12,1	16,1	0,54	
3	24,032	862,004	15,321	61,1	50,3	0,86	
4	31,620	70,998	1,318	5,0	4,3	0,86	
	Total	1411,921	30,463	100,0	100,0		

(R)-2-((S)-hydroxy(3-nitrophenyl)methyl)cyclohexan-1-one (3.2a)

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Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\Report\3-NO2rac.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 11/01/2021 16:11:55
Acquired Date : 11/01/2021 12:53:57
By : Fabrizio

Printed Version Info:
Printed Version : 11/01/2021 13:09:18
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 11/01/2021 16:12:42
By : Fabrizio

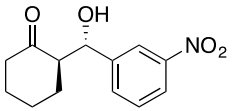
Sample Info:
Sample ID : VR124rac
Sample : 3-NO2 aldol rac
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

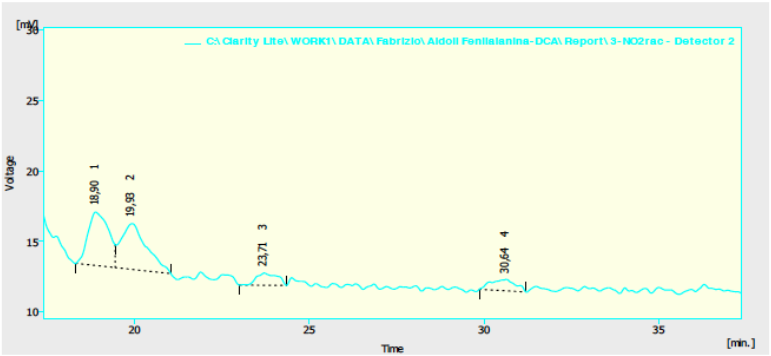
Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 11/01/2021 16:11

Column : IB
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0





Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\Report\3-NO2rac - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	18,900	156,317	3,793	39,1	43,4	0,73	
2	19,932	166,773	3,253	41,8	37,3	0,86	
3	23,712	36,155	0,870	9,1	10,0	0,79	
4	30,636	40,146	0,816	10,1	9,3	0,87	
	Total	399,391	8,732	100,0	100,0		

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Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR124.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 11/01/2021 12:19:33
Acquired Date : 11/01/2021 12:25:02
By : Fabrizio

Printed Version Info:
Printed Version : 11/01/2021 13:08:53
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 11/01/2021 16:12:22
By : Fabrizio

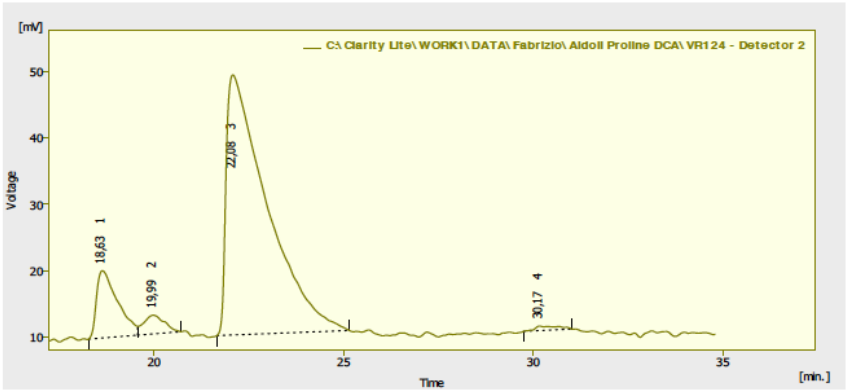
Sample Info:
Sample ID : VR124
Sample : 3-NO2 aldol
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 11/01/2021 13:08

Column : IB
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

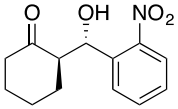
Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR124 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	18,632	391,214	10,115	11,4	19,2	0,63	
2	19,988	109,084	2,821	3,2	5,3	0,69	
3	22,080	2914,926	39,179	84,7	74,2	1,10	
4	30,172	27,078	0,670	0,8	1,3	0,92	
	Total	3442,301	52,786	100,0	100,0		

(R)-2-((S)-hydroxy(2-nitrophenyl)methyl)cyclohexan-1-one (3.3a)



26/01/2021 15Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE NON LEGATA DCA\IA_9_1_1MLMIN\VR125RAC.PRM Page 1 of 2

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Chromatogram Info:

File Name	: C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE NON LEGATA DCA\IA_9_1_1MLMIN\VR125RAC.PRM	File Created	: 16/12/2020 17:22:56
Origin	: Acquired	Acquired Date	: 16/12/2020 17:41:21
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

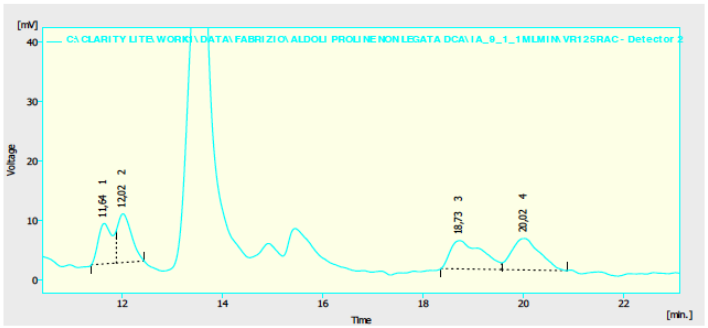
Printed Version	: 26/01/2021 15:19:00	Printed Date	: 26/01/2021 15:19:17
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

Sample Info:

Sample ID	: VR125rac	Amount	: 0
Sample	: 2-NO2 aldol racemate	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 26/01/2021 15:19
Column	: IA	Detection	: RI
Mobile Phase	: H/IPrOH 9:1	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)		
Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



26/01/2021 15Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE NON LEGATA DCA\IA_9_1_1MLMIN\VR125RAC.PRM Page 2 of 2

Result Table (Uncal - C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE NON LEGATA DCA\IA_9_1_1MLMIN\VR125RAC - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	11,644	133,468	6,765	18,9	27,1	0,38	
2	12,020	153,933	8,173	21,9	32,7	0,32	
3	18,728	210,696	4,746	29,9	19,0	0,79	
4	20,020	206,364	5,277	29,3	21,1	0,64	
	Total	704,461	24,961	100,0	100,0		

16/12/2020 18:11 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR125.PRM Page 1 of 2

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Chromatogram Info:

File Name	: C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR125.PRM	File Created	: 16/12/2020 18:05:08
Origin	: Acquired	Acquired Date	: 16/12/2020 18:11:10
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

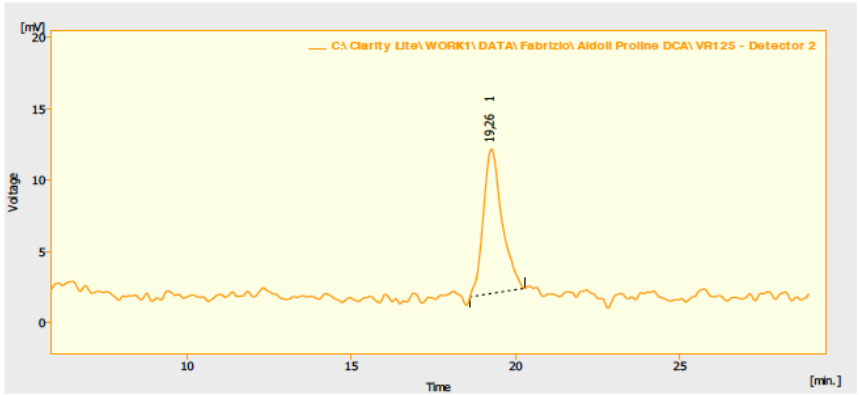
Printed Version	: 16/12/2020 18:11:30	Printed Date	: 16/12/2020 18:11:40
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

Sample Info:

Sample ID	: VR125	Amount	: 0
Sample	: 2-NO2 aldol	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 16/12/2020 18:11
Column	: IA	Detection	: RI
Mobile Phase	: H/IPrOH 9:1	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)		
Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



16/12/2020 18:11 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR125.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR125 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	19,264	402,155	10,121	100,0	100,0	0,59	
	Total	402,155	10,121	100,0	100,0		

4-((S)-hydroxy((R)-2-oxocyclohexyl)methyl)benzonitrile (3.4a)

12/01/2021 09:26 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\VR126rac.PRM Page 1 of 2

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Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\VR126rac.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 12/01/2021 09:08:34
Acquired Date : 12/01/2021 09:08:34
By : Fabrizio

Printed Version Info:
Printed Version : 12/01/2021 09:25:49
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 12/01/2021 09:26:03
By : Fabrizio

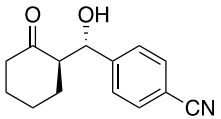
Sample Info:
Sample ID : VR126rac
Sample : 4-CN aldol rac
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

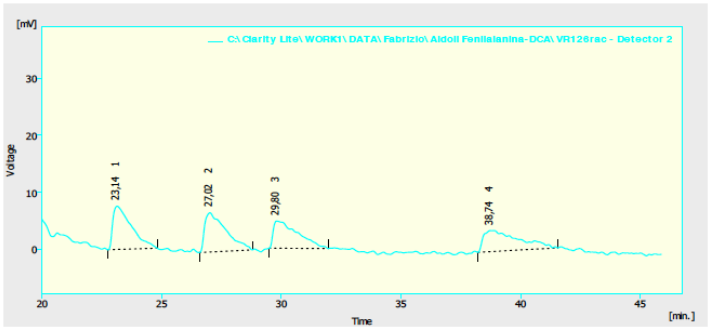
Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 12/01/2021 09:25

Column : IB
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0





12/01/2021 09:26 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\VR126rac.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\VR126rac - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	23,136	408,808	7,584	26,4	33,1	0,86	
2	27,024	424,729	6,859	27,4	30,0	1,02	
3	29,804	341,880	4,757	22,1	20,8	1,17	
4	38,740	372,245	3,691	24,1	16,1	1,76	
	Total	1547,661	22,890	100,0	100,0		

11/01/2021 18:09 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\VR126.PRM Page 1 of 2

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Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\VR126.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 11/01/2021 17:33:54
Acquired Date : 11/01/2021 17:33:54
By : Fabrizio

Printed Version Info:
Printed Version : 11/01/2021 18:08:49
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 11/01/2021 18:09:02
By : Fabrizio

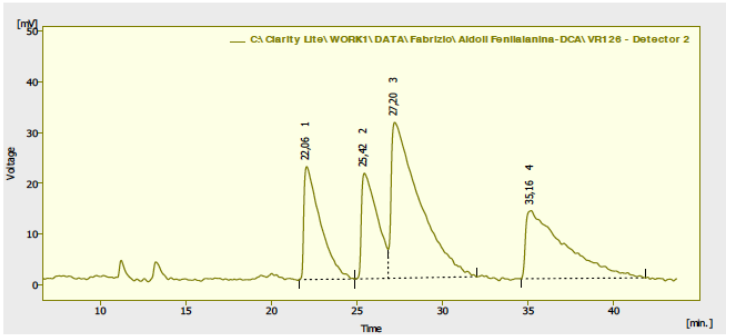
Sample Info:
Sample ID : VR126
Sample : 4-CN aldol
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 11/01/2021 18:08

Column : IB
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0

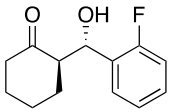


11/01/2021 18:09 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\VR126.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\VR126 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	22,060	1492,403	22,232	17,5	25,5	1,00	
2	25,424	1340,608	20,801	15,8	23,9	1,10	
3	27,196	3527,308	30,707	41,5	35,2	1,67	
4	35,164	2145,408	13,416	25,2	15,4	2,38	
	Total	8505,727	87,156	100,0	100,0		

(R)-2-((S)-(2-fluorophenyl)(hydroxy)methyl)cyclohexan-1-one (3.5a)



03/12/2020 14:13 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR128rac.PRM Page 1 of 2

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Chromatogram Info:

File Name	: C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR128rac.PRM	File Created	: 03/12/2020 12:13:45
Origin	: Acquired	Acquired Date	: 03/12/2020 13:10:23
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

Printed Version	: 03/12/2020 14:13:44	Printed Date	: 03/12/2020 14:13:51
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

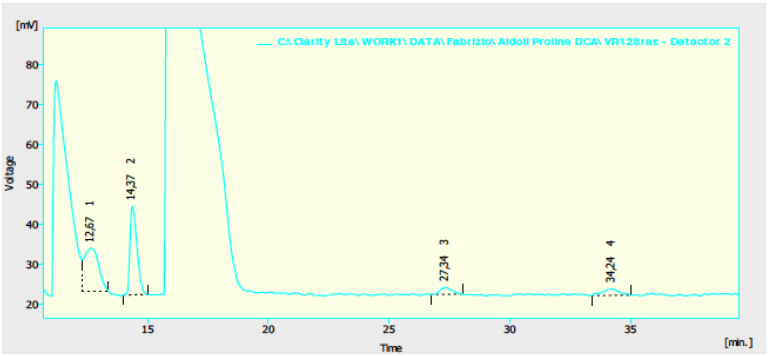
Sample Info:

Sample ID	: VR128rac	Amount	: 0
Sample	: 2-F aldol racemate	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 03/12/2020 14:13
Column	: IC	Detection	: RI
Mobile Phase	: H/IPrOH 95:5	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)		

Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



03/12/2020 14:13 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR128rac.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR128rac - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	12,668	458,840	10,784	44,0	29,7	0,74	
2	14,368	446,910	22,154	42,9	61,0	0,32	
3	27,344	58,318	1,750	5,6	4,8	0,56	
4	34,244	78,243	1,639	7,5	4,5	0,70	
	Total	1042,311	36,327	100,0	100,0		

03/12/2020 14:14 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR128.PRM Page 1 of 2

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Chromatogram Info:

File Name	: C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR128.PRM	File Created	: 03/12/2020 13:42:12
Origin	: Acquired	Acquired Date	: 03/12/2020 13:42:11
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

Printed Version	: 03/12/2020 14:14:18	Printed Date	: 03/12/2020 14:14:44
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

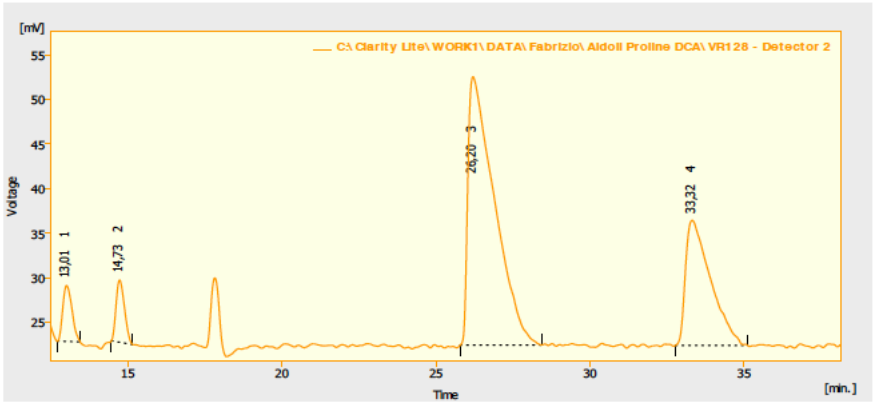
Sample Info:

Sample ID	: VR128	Amount	: 0
Sample	: 2-F aldol	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 03/12/2020 14:14
Column	: IC	Detection	: RI
Mobile Phase	: H/IPrOH 95:5	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)		

Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



03/12/2020 14:14 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR128.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR128 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	13,012	129,634	6,272	4,5	10,9	0,34	
2	14,732	132,389	6,972	4,6	12,2	0,31	
3	26,204	1798,267	30,059	63,1	52,4	0,95	
4	33,320	791,510	14,016	27,8	24,5	0,88	
	Total	2851,800	57,320	100,0	100,0		

(R)-2-((S)-(4-bromophenyl)(hydroxy)methyl)cyclohexan-1-one (3.6a)

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Chromatogram Info:

File Name	: C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IC_95_5_1MLMIN\VR131RAC.PRM	File Created	: 07/12/2020 10:44:47
Origin	: Acquired	Acquired Date	: 07/12/2020 11:30:06
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

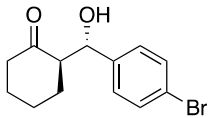
Printed Version Info:

Printed Version	: 07/12/2020 13:06:35	Printed Date	: 07/12/2020 13:06:41
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

Sample Info:

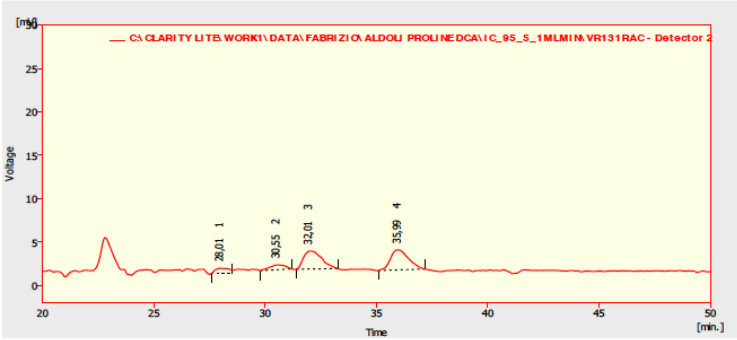
Sample ID	: VR131rac	Amount	: 0
Sample	: 4-Br aldol racemate	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 07/12/2020 13:06
Column	: IC	Detection	: RI
Mobile Phase	: H/IPrOH 95:5	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)		



Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



Result Table (Uncal - C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IC_95_5_1MLMIN\VR131RAC - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	28,012	26,857	0,631	8,9	11,3	0,80	
2	30,552	26,897	0,565	8,9	10,1	0,80	
3	32,012	117,629	2,080	39,0	37,2	0,94	
4	35,988	130,585	2,311	43,2	41,4	0,90	
	Total	301,967	5,586	100,0	100,0		

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Chromatogram Info:

File Name	: C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR131.PRM	File Created	: 07/12/2020 11:53:50
Origin	: Acquired	Acquired Date	: 07/12/2020 11:53:50
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

Printed Version	: 07/12/2020 12:25:20	Printed Date	: 07/12/2020 12:25:28
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

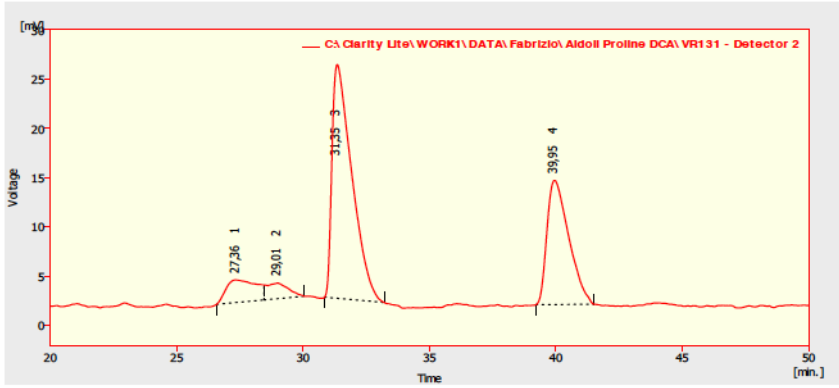
Sample Info:

Sample ID	: VR131	Amount	: 0
Sample	: 4-Br aldol	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 07/12/2020 12:25
Column	: IC	Detection	: RI
Mobile Phase	: H/IPrOH 95:5	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)		

Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

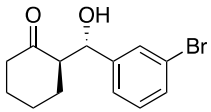
Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR131 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	27,364	188,262	2,285	8,1	5,7	1,56	
2	29,012	96,326	1,581	4,1	3,9	1,08	
3	31,352	1296,155	23,706	55,5	59,0	0,86	
4	39,952	754,748	12,589	32,3	31,3	0,95	
	Total	2335,491	40,163	100,0	100,0		

(R)-2-((S)-(3-bromophenyl)(hydroxy)methyl)cyclohexan-1-one (3.7a)



22/12/2020 18:25 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR130rac.PRM Page 1 of 2

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Chromatogram Info:

File Name	: C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR130rac.PRM	File Created	: 22/12/2020 18:11:40
Origin	: Acquired	Acquired Date	: 22/12/2020 18:11:40
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

Printed Version	: 22/12/2020 18:25:07	Printed Date	: 22/12/2020 18:25:15
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

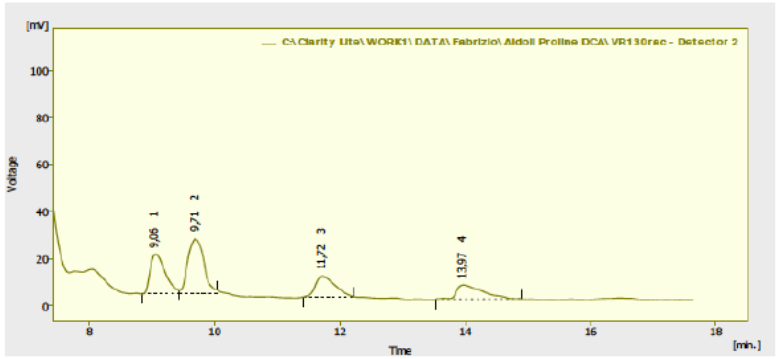
Sample Info:

Sample ID	: VR130rac	Amount	: 0
Sample	: 3-Br aldol	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 22/12/2020 18:25
Column	: IB	Detection	: RI
Mobile Phase	: H/IPrOH 95:5	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)		

Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



22/12/2020 18:25 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR130rac.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR130rac - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	9,056	284,254	16,943	29,3	30,6	0,27	
2	9,712	318,450	23,767	32,9	42,9	0,23	
3	11,724	188,375	8,725	19,4	15,7	0,35	
4	13,972	177,440	6,017	18,3	10,9	0,46	
	Total	968,519	55,452	100,0	100,0		

22/12/2020 18:04 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR130.PRM Page 1 of 2

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Chromatogram Info:

File Name	: C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR130.PRM	File Created	: 22/12/2020 17:56:33
Origin	: Acquired	Acquired Date	: 22/12/2020 17:56:33
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

Printed Version	: 22/12/2020 18:04:15	Printed Date	: 22/12/2020 18:04:24
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

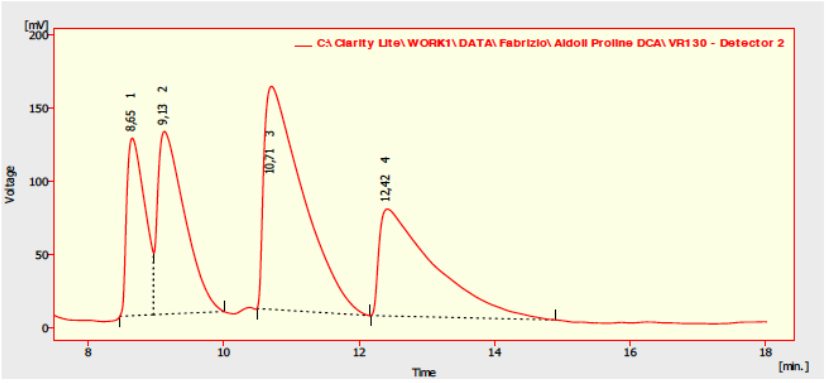
Sample Info:

Sample ID	: VR130	Amount	: 0
Sample	: 3-Br aldol	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 22/12/2020 18:04
Column	: IB	Detection	: RI
Mobile Phase	: H/IPrOH 95:5	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)		

Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				

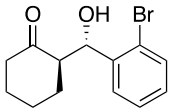


22/12/2020 18:04 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR130.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR130 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	8,652	2241,065	121,060	14,0	25,7	0,34	
2	9,128	3488,761	124,610	21,9	26,5	0,44	
3	10,708	6111,499	152,086	38,3	32,3	0,62	
4	12,416	4111,907	72,860	25,8	15,5	0,79	
	Total	15953,233	470,616	100,0	100,0		

(R)-2-((S)-(2-bromophenyl)(hydroxy)methyl)cyclohexan-1-one (3.8a)



15/12/2020 15:17 Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IC_9_1_1MLMIN\VR129RAC.PRM Page 1 of 2

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Chromatogram Info:

File Name	: C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IC_9_1_1MLMIN\VR129RAC.PRM	File Created	: 10/12/2020 13:21:38
Origin	: Acquired	Acquired Date	: 10/12/2020 13:33:21
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

Printed Version	: 15/12/2020 15:17:38	Printed Date	: 15/12/2020 15:17:47
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

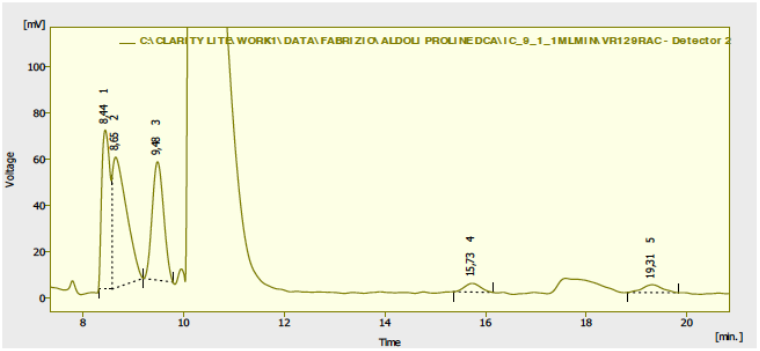
Sample Info:

Sample ID	: VR129rac	Amount	: 0
Sample	: 2-Br aldol racemate	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 15/12/2020 15:17
Column	: IC	Detection	: RI
Mobile Phase	: H/IPrOH 9:1	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)		

Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



15/12/2020 15:17 Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IC_9_1_1MLMIN\VR129RAC.PRM Page 2 of 2

Result Table (Uncal - C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IC_9_1_1MLMIN\VR129RAC - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	8,444	801,924	68,662	27,9	37,5	0,22	
2	8,648	1085,303	56,434	37,7	30,8	0,33	
3	9,480	811,311	51,101	28,2	27,9	0,26	
4	15,728	83,392	3,744	2,9	2,0	0,36	
5	19,308	93,353	3,387	3,2	1,8	0,41	
	Total	2875,283	183,329	100,0	100,0		

15/12/2020 15:19 Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IC_9_1_1MLMIN\VR129.PRM Page 1 of 2

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Chromatogram Info:

File Name	: C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IC_9_1_1MLMIN\VR129.PRM	File Created	: 10/12/2020 13:39:00
Origin	: Acquired	Acquired Date	: 10/12/2020 14:11:15
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

Printed Version	: 15/12/2020 15:18:28	Printed Date	: 15/12/2020 15:19:04
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

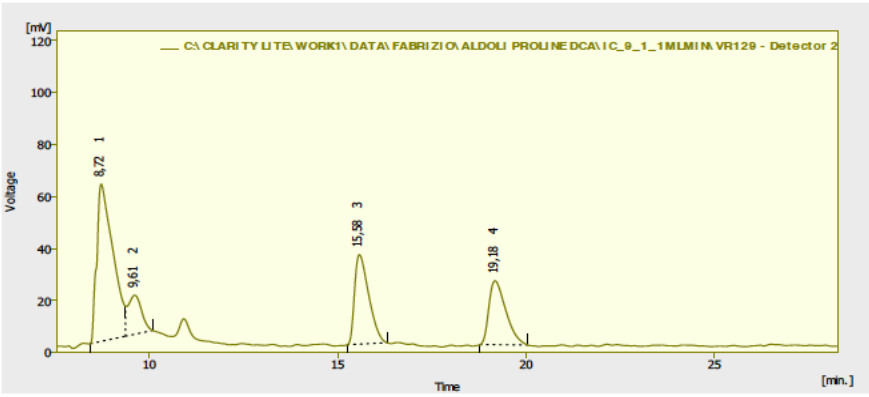
Sample Info:

Sample ID	: VR129	Amount	: 0
Sample	: 2-Br aldol	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 15/12/2020 15:18
Column	: IC	Detection	: RI
Mobile Phase	: H/IPrOH 9:1	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)		

Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



15/12/2020 15:19 Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IC_9_1_1MLMIN\VR129.PRM Page 2 of 2

Result Table (Uncal - C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IC_9_1_1MLMIN\VR129 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	8,724	1807,370	60,467	46,8	44,9	0,48	
2	9,612	385,150	15,028	10,0	11,2	0,46	
3	15,576	901,829	34,442	23,3	25,6	0,42	
4	19,176	769,180	24,603	19,9	18,3	0,49	
	Total	3863,529	134,540	100,0	100,0		

(R)-2-((S)-(3-chlorophenyl)(hydroxy)methyl)cyclohexan-1-one (3.9a)

22/12/2020 17:41 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR133rac2.PRM Page 1 of 2

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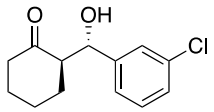
Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR133rac2.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 22/12/2020 17:34:27
Acquired Date : 22/12/2020 17:34:27
By : Fabrizio

Printed Version Info:
Printed Version : 22/12/2020 17:40:49
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 22/12/2020 17:41:02
By : Fabrizio

Sample Info:
Sample ID : VR133rac2
Sample : 3-Cl aldol rac
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

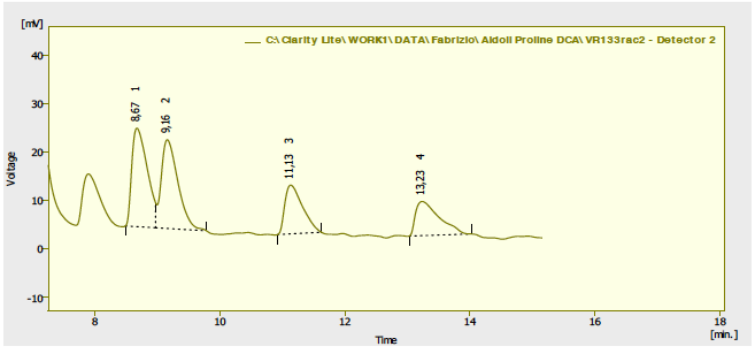
Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 22/12/2020 17:40

Column : IB
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :



Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



22/12/2020 17:41 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR133rac2.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR133rac2 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	8,672	349,056	20,340	32,4	36,5	0,29	
2	9,160	345,493	18,319	32,0	32,8	0,29	
3	11,132	198,411	10,084	18,4	18,1	0,32	
4	13,232	185,879	7,058	17,2	12,6	0,42	
	Total	1078,839	55,800	100,0	100,0		

22/12/2020 17:25 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR133.PRM Page 1 of 2

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Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR133.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 22/12/2020 16:31:31
Acquired Date : 22/12/2020 17:05:09
By : Fabrizio

Printed Version Info:
Printed Version : 22/12/2020 17:25:39
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 22/12/2020 17:25:50
By : Fabrizio

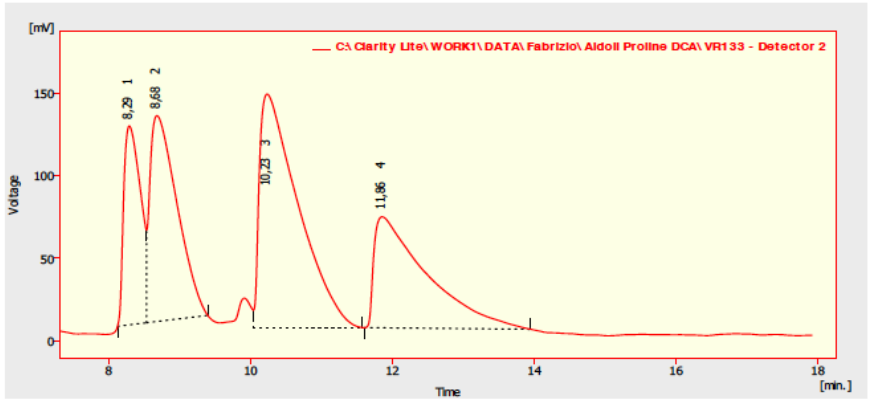
Sample Info:
Sample ID : VR133
Sample : 3-Cl aldol
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 22/12/2020 17:25

Column : IB
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0

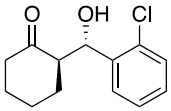


22/12/2020 17:25 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR133.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR133 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	8,288	1976,566	120,456	14,0	26,5	0,32	
2	8,680	3345,059	124,696	23,7	27,5	0,45	
3	10,232	5346,210	141,394	37,8	31,2	0,59	
4	11,856	3468,883	67,280	24,5	14,8	0,74	
	Total	14136,717	453,825	100,0	100,0		

(R)-2-((S)-(2-chlorophenyl)(hydroxy)methyl)cyclohexan-1-one (3.10a)



22/12/2020 16:04 Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IB_95_5_1MLMIN\VR132RAC.PRM Page 1 of 2

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Chromatogram Info:

File Name	: C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IB_95_5_1MLMIN\VR132RAC.PRM	File Created	: 22/12/2020 13:58:43
Origin	: Acquired	Acquired Date	: 22/12/2020 14:11:33
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

Printed Version	: 22/12/2020 16:04:26	Printed Date	: 22/12/2020 16:04:34
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

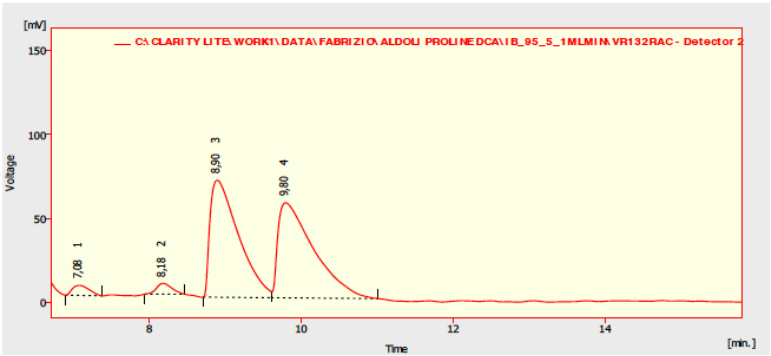
Sample Info:

Sample ID	: VR132rac	Amount	: 0
Sample	: 2-Cl aldol rac	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 22/12/2020 16:04
Column	: IB	Detection	: RI
Mobile Phase	: H/IPrOH 95:5	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)		

Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



22/12/2020 16:04 Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IB_95_5_1MLMIN\VR132RAC.PRM Page 2 of 2

Result Table (Uncal - C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IB_95_5_1MLMIN\VR132RAC - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	7,080	90,884	5,940	2,4	4,3	0,26	
2	8,184	91,457	6,472	2,4	4,7	0,22	
3	8,896	1814,767	69,717	47,1	50,2	0,41	
4	9,796	1855,586	56,643	48,2	40,8	0,50	
	Total	3852,694	138,771	100,0	100,0		

22/12/2020 16:03 Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IB_95_5_1MLMIN\VR132.PRM Page 1 of 2

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Chromatogram Info:

File Name	: C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IB_95_5_1MLMIN\VR132.PRM	File Created	: 22/12/2020 14:43:03
Origin	: Acquired	Acquired Date	: 22/12/2020 14:43:03
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

Printed Version	: 22/12/2020 16:03:40	Printed Date	: 22/12/2020 16:03:48
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

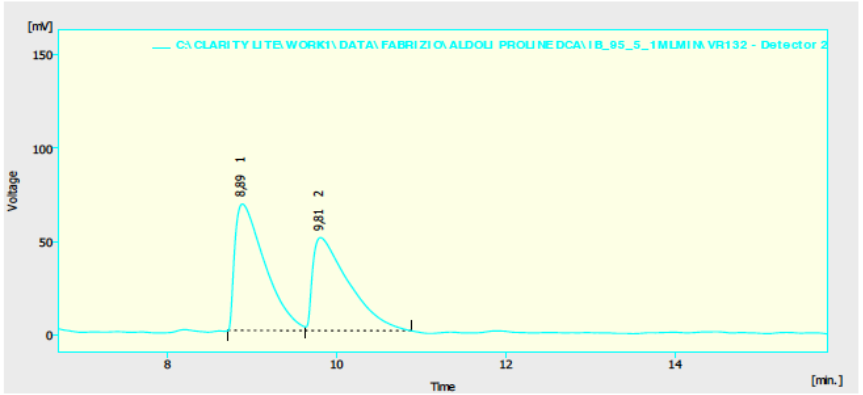
Sample Info:

Sample ID	: VR132	Amount	: 0
Sample	: 2-Cl aldol	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 22/12/2020 16:03
Column	: IB	Detection	: RI
Mobile Phase	: H/IPrOH 95:5	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)		

Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



22/12/2020 16:03 Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IB_95_5_1MLMIN\VR132.PRM Page 2 of 2

Result Table (Uncal - C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IB_95_5_1MLMIN\VR132 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	8,888	1674,715	67,699	53,1	57,6	0,39	
2	9,812	1478,096	49,788	46,9	42,4	0,46	
	Total	3152,811	117,487	100,0	100,0		

(R)-2-((S)-hydroxy(4-methoxyphenyl)methyl)cyclohexan-1-one (3.11a)

21/12/2020 14:29 Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\VR135RAC.PRM Page 1 of 2

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Chromatogram Info:

File Name	: C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\VR135RAC.PRM	File Created	: 18/12/2020 19:03:44
Origin	: Acquired	Acquired Date	: 18/12/2020 19:49:33
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

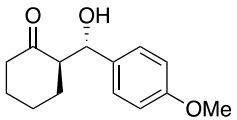
Printed Version Info:

Printed Version	: 21/12/2020 14:29:31	Printed Date	: 21/12/2020 14:29:37
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

Sample Info:

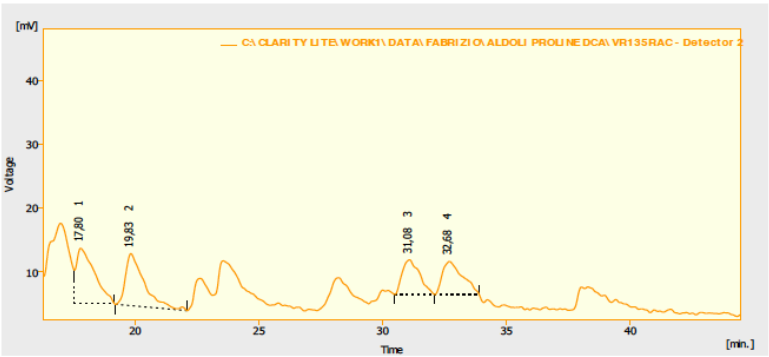
Sample ID	: VR135rac	Amount	: 0
Sample	: 4-OMe aldol rac	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 21/12/2020 14:29
Column	: IA	Detection	: RI
Mobile Phase	: H/IPrOH 95:5	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)		



Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



21/12/2020 14:29 Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\VR135RAC.PRM Page 2 of 2

Result Table (Uncal - C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\VR135RAC - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	17,796	452,077	8,683	31,5	31,6	0,90	
2	19,828	426,999	8,149	29,7	29,7	0,75	
3	31,080	264,518	5,440	18,4	19,8	0,83	
4	32,684	292,979	5,177	20,4	18,9	0,94	
	Total	1436,573	27,448	100,0	100,0		

21/12/2020 14:28 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR135_3.PRM Page 1 of 2

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Chromatogram Info:

File Name	: C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR135_3.PRM	File Created	: 21/12/2020 13:39:10
Origin	: Acquired	Acquired Date	: 21/12/2020 13:39:10
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

Printed Version	: 21/12/2020 14:28:11	Printed Date	: 21/12/2020 14:28:19
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

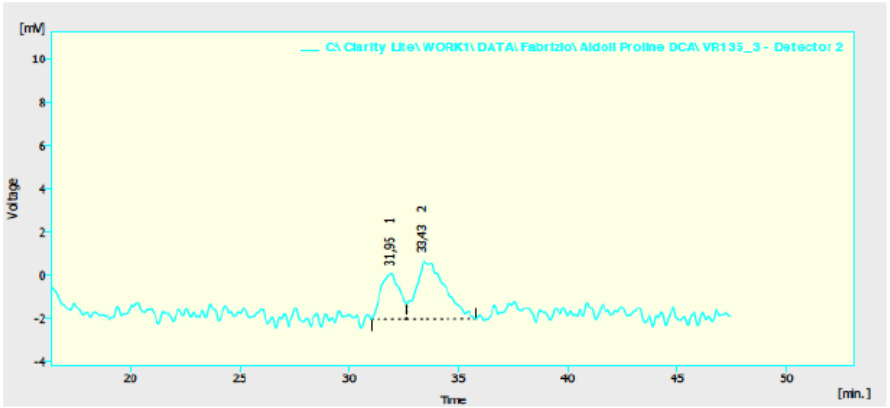
Sample Info:

Sample ID	: VR135_3	Amount	: 0
Sample	: 4-OMe aldol	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 21/12/2020 14:28
Column	: IA	Detection	: RI
Mobile Phase	: H/IPrOH 95:5	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)		

Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



21/12/2020 14:28 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR135_3.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR135_3 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	31,948	126,868	2,107	34,1	43,8	1,08	
2	33,428	245,294	2,699	65,9	56,2	1,42	
	Total	372,162	4,806	100,0	100,0		

(R)-2-((S)-hydroxy(3-methoxyphenyl)methyl)cyclohexan-1-one (3.12a)

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Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR134_rac.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 02/12/2020 15:48:36
Acquired Date : 02/12/2020 15:58:35
By : Fabrizio

Printed Version Info:
Printed Version : 02/12/2020 15:59:00
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 02/12/2020 15:59:06
By : Fabrizio

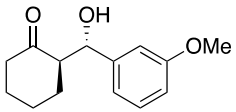
Sample Info:
Sample ID : VR134_rac
Sample : 3-OMe aldol rac
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

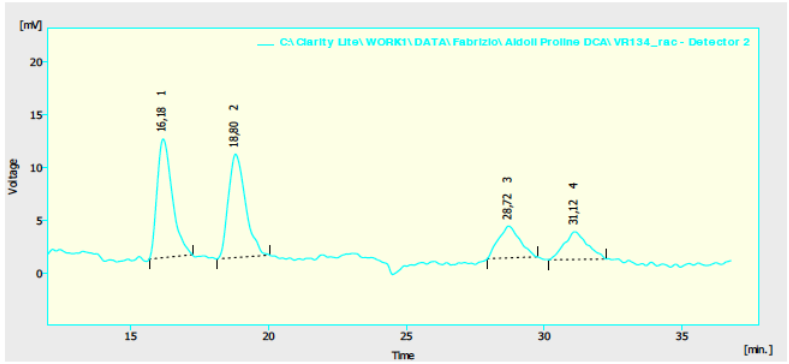
Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 02/12/2020 15:59

Column : IA
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0





Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR134_rac - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	16,180	431,094	11,239	36,8	42,1	0,60	
2	18,804	423,816	9,794	36,2	36,7	0,68	
3	28,720	162,194	3,015	13,8	11,3	0,90	
4	31,116	154,627	2,630	13,2	9,9	0,98	
	Total	1171,731	26,679	100,0	100,0		

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Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR134.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 02/12/2020 16:38:27
Acquired Date : 02/12/2020 16:38:27
By : Fabrizio

Printed Version Info:
Printed Version : 02/12/2020 16:39:43
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 02/12/2020 16:39:50
By : Fabrizio

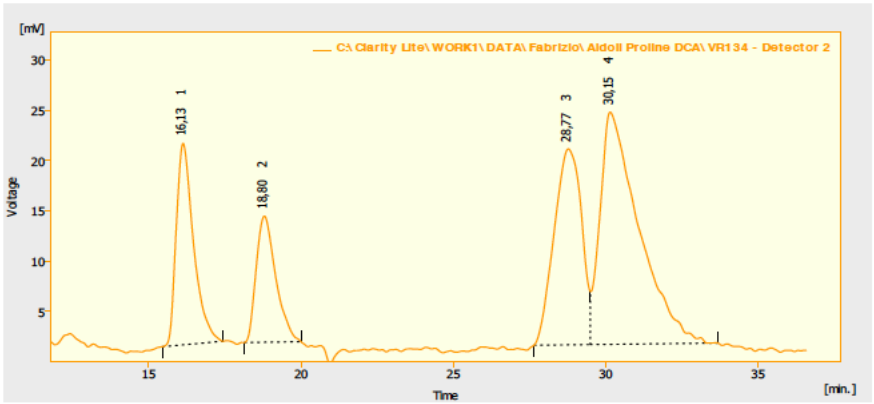
Sample Info:
Sample ID : VR134
Sample : 3-OMe aldol
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 02/12/2020 16:39

Column : IA
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

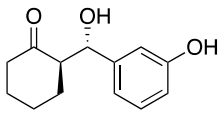
Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR134 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	16,132	791,151	20,035	17,1	26,7	0,60	
2	18,800	560,770	12,534	12,1	16,7	0,70	
3	28,772	1221,440	19,473	26,4	25,9	1,04	
4	30,152	2050,312	23,071	44,3	30,7	1,32	
	Total	4623,673	75,113	100,0	100,0		

(R)-2-((S)-hydroxy(3-hydroxyphenyl)methyl)cyclohexan-1-one (3.13a)



03/12/2020 18:50 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR137rac_2.PRM Page 1 of 2

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Chromatogram Info:

File Name	: C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR137rac_2.PRM	File Created	: 03/12/2020 18:08:20
Origin	: Acquired	Acquired Date	: 03/12/2020 18:08:20
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

Printed Version	: 03/12/2020 18:49:56	Printed Date	: 03/12/2020 18:50:09
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

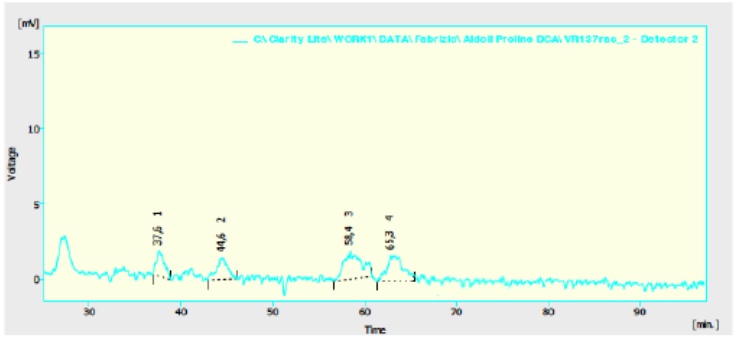
Sample Info:

Sample ID	: VR137rac_2	Amount	: 0
Sample	: 3-OH aldol racemate	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 03/12/2020 18:49
Column	: IC	Detection	: RI
Mobile Phase	: H/IPrOH 9:1	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)		

Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



03/12/2020 18:50 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR137rac_2.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR137rac_2 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	37,616	103,258	1,702	15,2	25,4	0,91	
2	44,568	120,575	1,429	17,7	21,3	1,30	
3	58,448	239,168	1,849	35,1	27,6	2,16	
4	65,288	217,902	1,726	32,0	25,7	1,60	
	Total	680,903	6,706	100,0	100,0		

03/12/2020 17:00 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR137.PRM Page 1 of 2

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Chromatogram Info:

File Name	: C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR137.PRM	File Created	: 03/12/2020 16:09:01
Origin	: Acquired	Acquired Date	: 03/12/2020 16:09:01
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

Printed Version	: 03/12/2020 17:00:31	Printed Date	: 03/12/2020 17:00:40
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

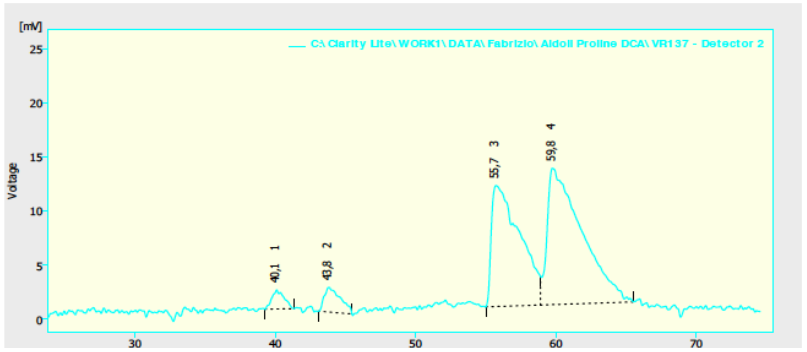
Sample Info:

Sample ID	: VR137	Amount	: 0
Sample	: 3-OH aldol	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 03/12/2020 17:00
Column	: IC	Detection	: RI
Mobile Phase	: H/IPrOH 9:1	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)		

Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



03/12/2020 17:00 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR137.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR137 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	40,112	106,266	1,773	2,6	6,4	1,00	
2	43,820	186,111	2,281	4,6	8,2	1,38	
3	55,728	1580,781	11,207	39,0	40,2	2,56	
4	59,772	2176,467	12,634	53,7	45,3	2,69	
	Total	4049,625	27,895	100,0	100,0		

(R)-2-((S)-hydroxy(naphthalen-1-yl)methyl)cyclohexan-1-one (3.14a)

Clarity - Chromatography SW
DataApex 2006
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Chromatogram Info:

File Name	: C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR138rac.PRM	File Created	: 30/11/2020 15:38:35
Origin	: Acquired	Acquired Date	: 30/11/2020 15:38:34
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

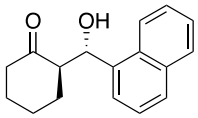
Printed Version Info:

Printed Version	: 30/11/2020 16:21:56	Printed Date	: 30/11/2020 16:22:20
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

Sample Info:

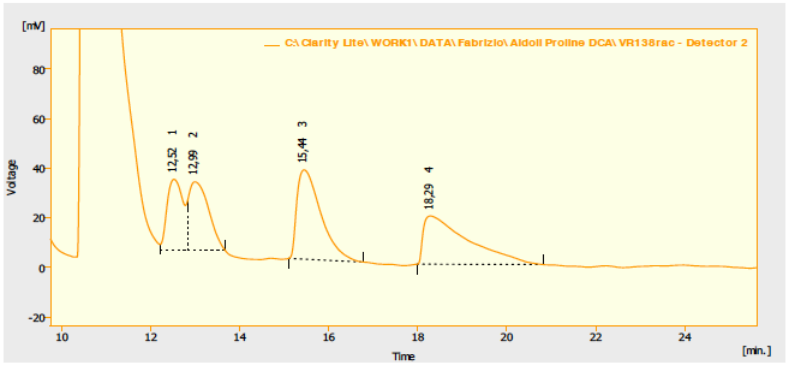
Sample ID	: VR138rac	Amount	: 0
Sample	: Ph aldol racemate	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 30/11/2020 16:21
Column	: IB	Detection	: RI
Mobile Phase	: H/IPrOH 9:1	Temperature	:
Flow Rate	: 0.5 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 6.28 min and 7.27, checked with blank injection)		



Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR138rac - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	12,524	729,913	28,591	17,0	25,6	0,48	
2	12,992	844,460	27,576	19,7	24,7	0,52	
3	15,444	1351,312	35,997	31,5	32,3	0,58	
4	18,292	1369,208	19,425	31,9	17,4	1,02	
	Total	4294,893	111,590	100,0	100,0		

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Chromatogram Info:

File Name	: C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\CAMPIONI FINO 30 NOV\VR138.PRM	File Created	: 17/11/2020 09:32:59
Origin	: Acquired	Acquired Date	: 17/11/2020 09:35:20
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

Printed Version	: 30/11/2020 16:24:25	Printed Date	: 30/11/2020 16:24:40
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

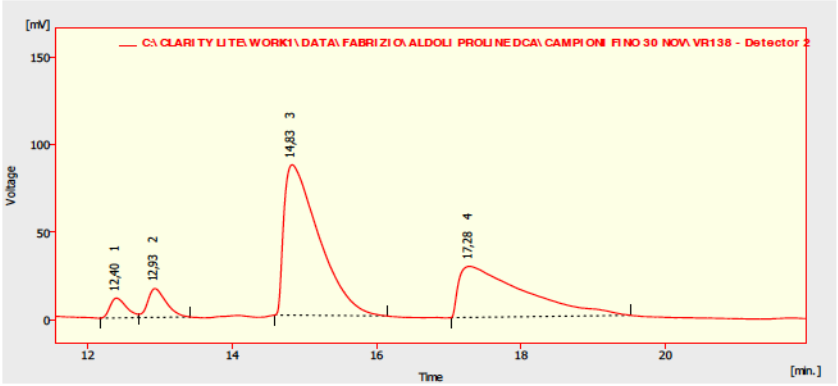
Sample Info:

Sample ID	: VR138	Amount	: 0
Sample	: Ph aldol	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 30/11/2020 16:24
Column	: IB	Detection	: RI
Mobile Phase	: H/IPrOH 9:1	Temperature	:
Flow Rate	: 0.5 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 6.28 min and 7.27, checked with blank injection)		

Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



Result Table (Uncal - C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\CAMPIONI FINO 30 NOV\VR138 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	12,396	179,475	11,337	3,5	7,9	0,26	
2	12,928	292,800	16,466	5,6	11,5	0,28	
3	14,828	2898,402	85,893	55,7	60,1	0,52	
4	17,284	1830,189	29,184	35,2	20,4	0,97	
	Total	5200,866	142,880	100,0	100,0		

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Chapter 4: Amino acids-BAs Conjugates promoted Aldol reactions in water

4.1 Introduction

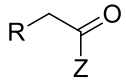
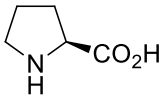
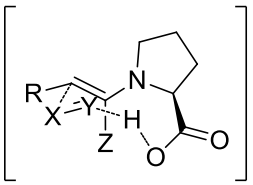
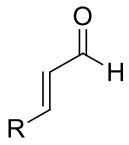
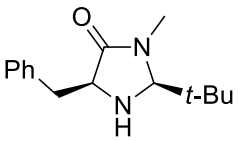
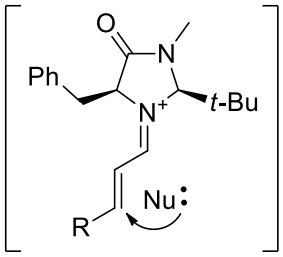
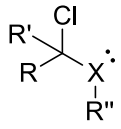
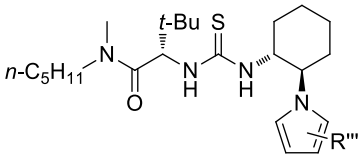
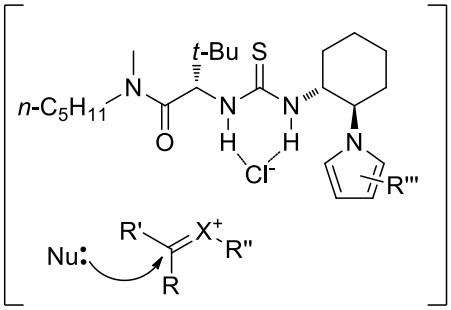
A very important field of organic chemistry, as already discussed in Chapter 3, is organic catalysis, able to give a great help to the selective synthesis of compounds with one or more chiral centers, until recently only achievable by complex architectures like those of enzymes. For a long time, it was thought that, if the enzymatic pathway fails, a catalyst containing a transition metal combined with chiral ligands could be the right solution because it is capable of efficiently promoting reactions that otherwise could not occur. This belief has been dispelled in recent years, given that there has been a huge development in biomolecular chemistry, which aims to perfect enzymes so as not to resort to organometallic catalysts in view of a more eco-sustainable chemistry.

In addition to the methods just mentioned, in the last decade, another approach that attempted to mimic enzymatic activity and that did not require the use of transition metals, namely organocatalysis has been developed.[1,2]

Although organocatalyzed reactions had been taking place for many years, we had to wait until 2000 to have a commonly accepted definition of this branch of modern chemistry, which reached its peak today with the recent awarding of the Nobel Prize for studies in this field to Benjamin List and David MacMillan.

David W. C. MacMillan coined and defined the term organocatalysis as "the use of low molecular weight organic molecules as catalysts in organic reactions". In fact, organocatalysis is an approach that uses small organic molecules (MW<1000) as chiral catalysts. The main advantage of these catalysts is the possibility to carry out reactions in completely "user-friendly" conditions, without resorting to particularly complex procedures: generally no inert atmosphere, nor particular temperatures and pressures, or particular reaction equipment is required. Furthermore, these substances are easily available, characterized by low cost and low toxicity since they are mainly biological compounds such as sugars, peptides and amino acids, or derivatives of these compounds.[3]

Organocatalysts perform their function through different types of catalysis depending on the structure of the organocatalyst itself and on the interactions it establishes with the substrate. In general terms, the catalysis carried out by organic catalysts can be divided into two main categories: a covalent type catalysis and a non-covalent type one. Organocatalysts containing secondary amino functionalities perform their function by means of a covalent catalysis by passing through the formation of a C-N bond between catalyst and substrate. Instead, thiourea-based organocatalysts exploit ionic interactions or hydrogen bonds to catalyze asymmetric organic reactions (Table 4.1).[4]

Substrate	Catalyst	Substrate activation
 + $X{=}CZ_2$ R = $-(CH_2)_n-$, $-(CH_2)_nCH_3$ X = C, N, O, S Z = alkyl, H		
		
 X = O, NR R, R', R'', R''' = alkyl, aryl		

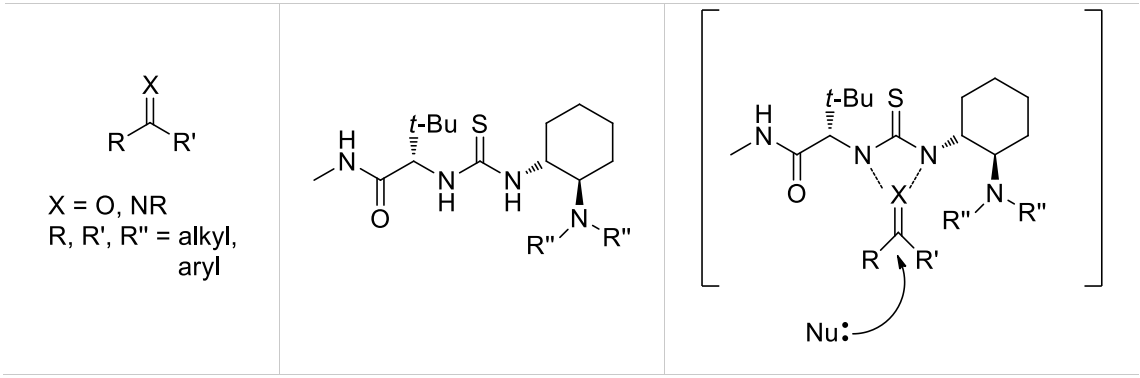
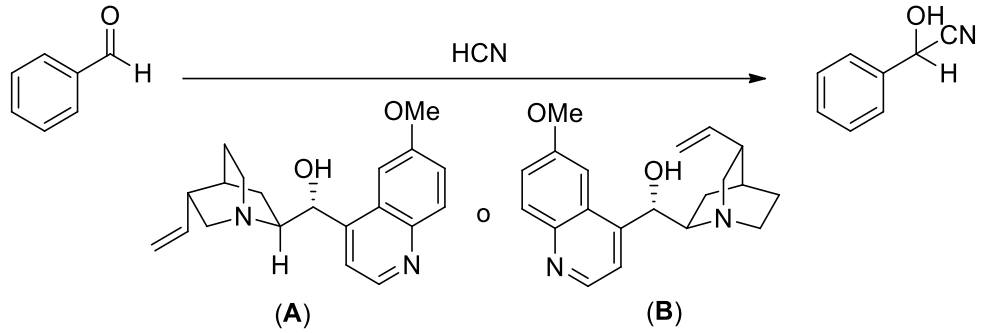


Table 4.1. Schematic representation of diverse types of organocatalysts substrates activation.

All these characteristics, especially the absence of transition metals, make organocatalysts an excellent tool to be used in green chemistry[5] but, despite their high versatility, they still find limited use in the field of industrial synthesis[6] and nanotechnologies.[7]

The first organocatalyzed asymmetric syntheses date back to lots decades ago. In fact, the first of these was reported in the literature in 1912 by Bredig and Fiske[8] who tried to carry out an addition reaction of hydrogen cyanide (HCN) to benzaldehyde using the alkaloids quinine or quinidine as catalysts, providing a optically active product, obtainable in both configurations (Scheme 4.1).



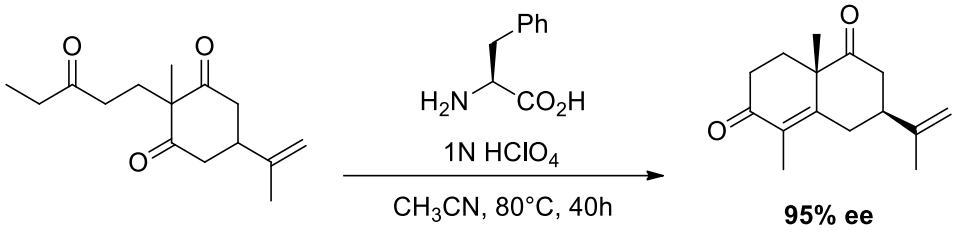
Scheme 4.1. The alkaloids (-)-quinine (**A**) or (+)-quinidine (**B**) used by Bredig and Fiske in the addition of HCN to aldehydes.

Although the reaction occurred, the enantiomeric excess did not exceed 10%, a very insufficient value for the preparatory purposes.

However, given the numerous advantages that this new approach to asymmetric catalysis offered, it is not surprising that research groups from around the world were quickly attracted on the field of asymmetric organocatalysis.

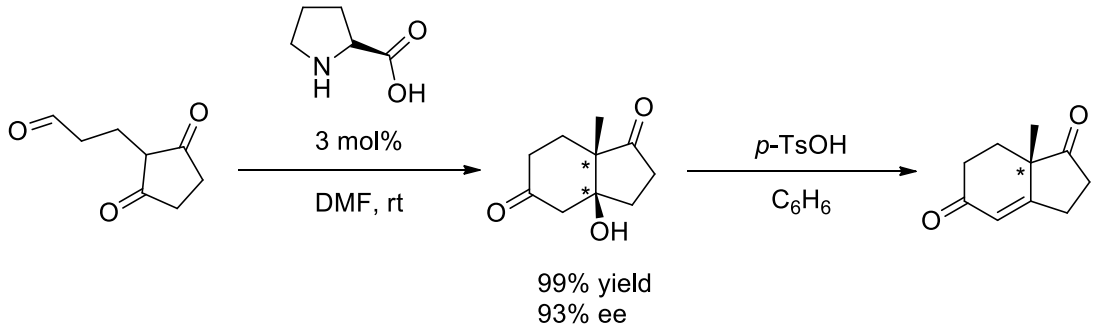
Initially, aspiring to imitate enzymatic efficiency, scientists' attention turned to amino acids, transforming them into countless auxiliaries, catalysts and ligands; in most examples, amino acids are used as a source of chirality and both amino and acid groups are often functionalized to perform this action.[9]

The use of primary amino acids as organic catalysts is widely described in the literature, phenylalanine, for example, is applied in numerous Aldol condensation reactions, including intramolecular ones (Scheme 4.2), leading to products with high enantioselectivity.[10]



Scheme 4.2. Intramolecular Aldol reaction example.

Inter- and intra-molecular aldol condensations, fundamental in organic chemistry as they lead to the formation of new C-C bonds, were among the first reactions for which organocatalytic versions were developed. In fact, among the first enantioselective organocatalytic transformations of interest we can find the Hajos-Parrish-Eder-Sauer-Wiechert reaction of 1971 (as well as the first intramolecular asymmetric aldol reaction invented by chemists) which consisted in the preparation of the precursor of the Wieland-Miescher ketone catalyzed by L-proline (Scheme 4.3).[11]



Scheme 4.3. Hajos-Parrish-Eder-Sauer-Wiechert.

4.1.2 α-Amino acids as potential and powerfull organocatalysts

As already described, α-amino acids (Figure 4.1) were largely used as efficient organocatalysts in a wide spectrum of organic reactions.

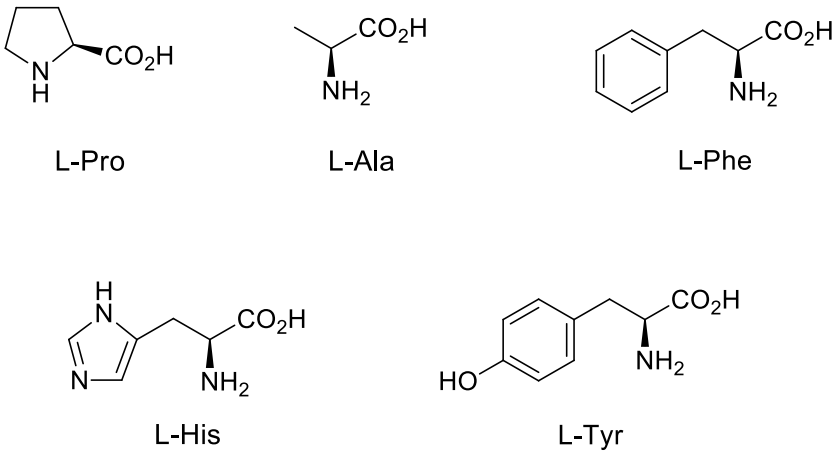
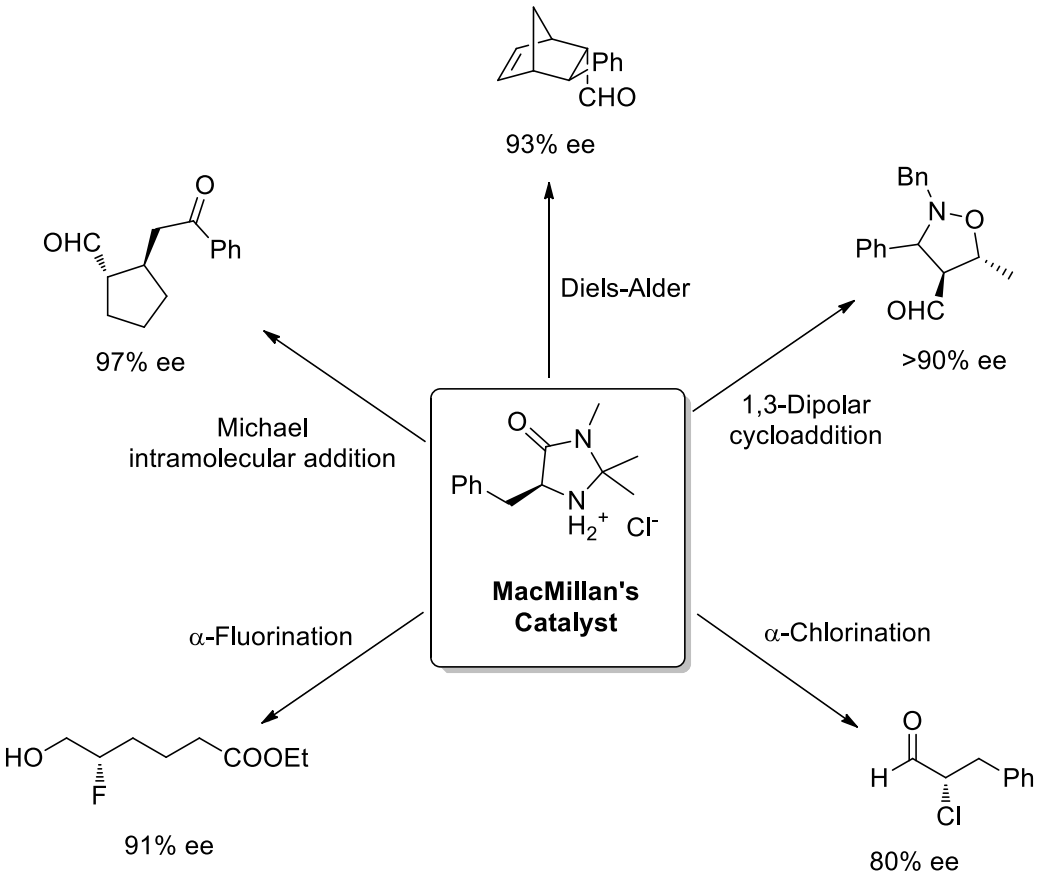


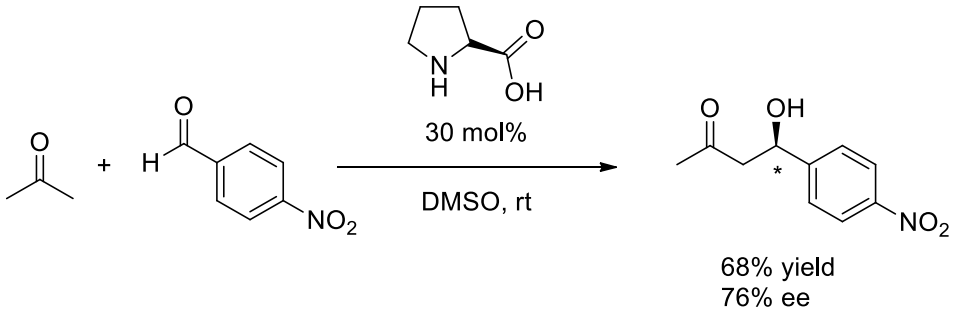
Figure 4.1. Some of the α-amino acids used as organocatalyst.

Although the direct use of chiral α-amino acids in the organocatalysis has been seen as the favorite approach due to their commercial availability at negligible costs, methods have been developed over time in order to modify, from synthetic point of view of their structure, to modify their catalytic and polarity properties, but also the electronic and/or steric properties of the amino and carboxylic groups, which are necessary, as will be seen later, for catalysis.[12] A successful example of the latter approach is MacMillan's imidazolidinone catalyst, obtained from L-phenylalanine and its derivatives, which have been successfully employed as efficient catalysts for a variety of organic reactions, including Diels-Alder's cycloadditions,[13] 1,3-dipolar cycloadditions,[14] α-chlorinations,[15] α-fluorinations[16] and intramolecular Michael additions[17] (Scheme 4.4).



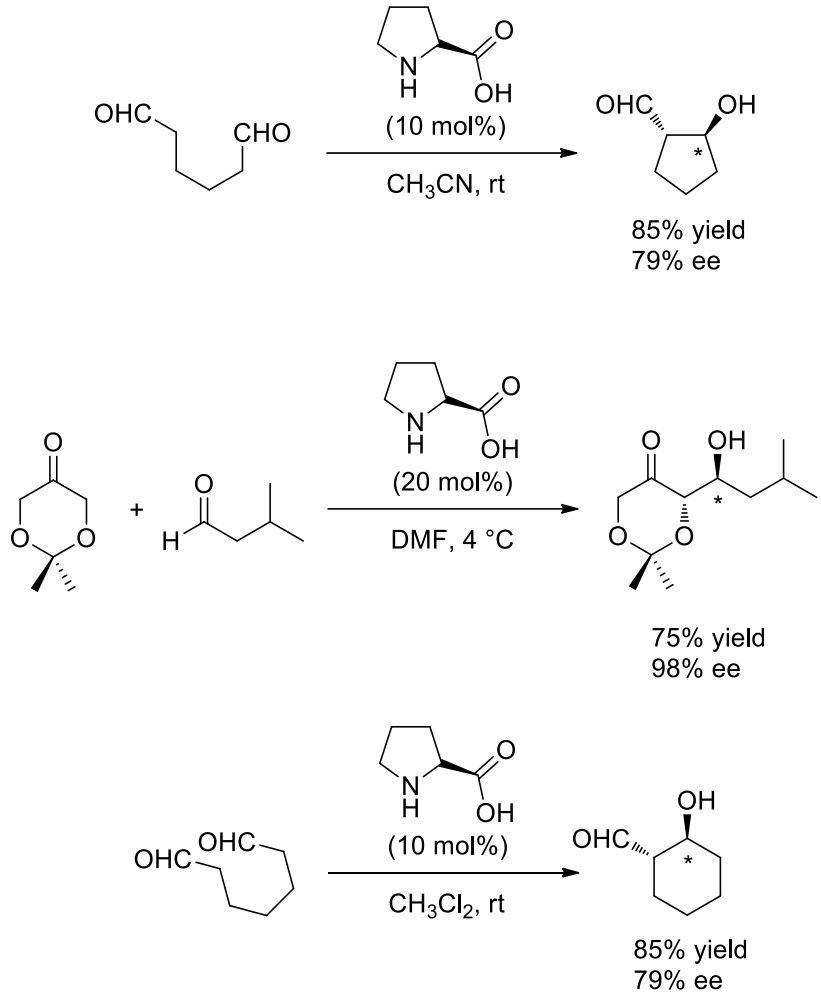
Scheme 4.4. McMillan catalyst promoted reactions.

The first evidence of the use of chiral α-amino acids to catalyze an asymmetric intermolecular aldol reaction was the work of List, Lerner and Barbas in 2000 in which L-proline was used (Scheme 4.5), following the pioneering 1971 study by Hajos and Parrish.[17]



Scheme 4.5. L-Pro catalyzed asymmetric intermolecular Aldol reaction.

In the last two decades, a considerable number of applications of L-proline to inter- and intra-molecular aldol condensations have been considered (Scheme 4.6).[19]



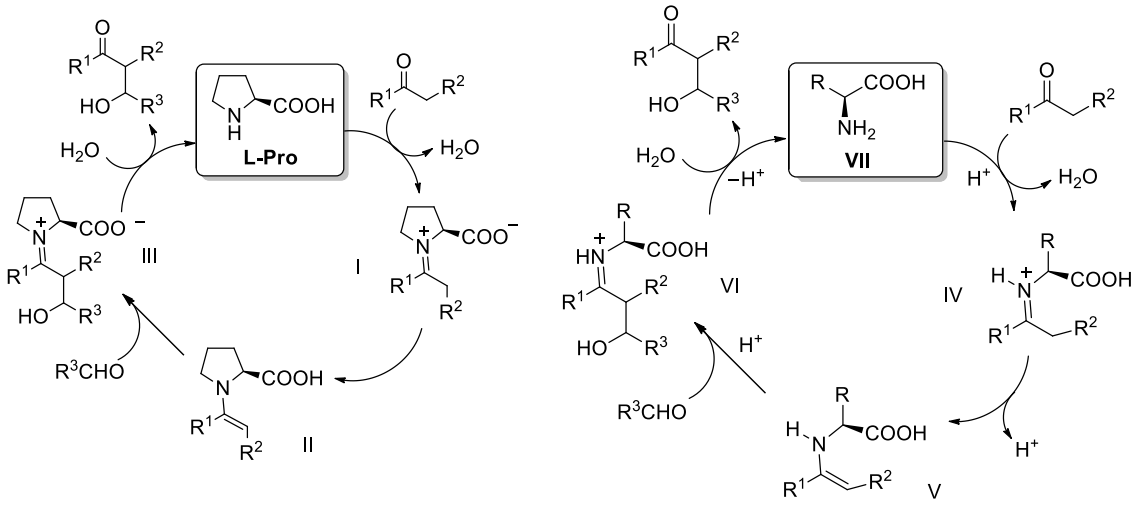
Scheme 4.6. Intra- and inter-molecular catalyzed Aldol reaction by L-Pro.

Until the early 2000s, proline remained the only widely studied α-amino acid as an organocatalyst of the aldol reaction and, in general, of other organic reactions. However, also other chiral α-amino acids have in principle the same catalytic ability, as they have an amino group and therefore are potentially capable of carrying out a *via* enamine catalysis.

However, it has been observed that α-amino acids with the primary amino function, including phenylalanine, valine, histidine and tyrosine, although they were efficient in catalyzing intramolecular aldol condensations, particularly when used together with Brønsted acid species such as perchloric acid or camphorsulfonic acid (CSA), they appeared to have little activity as catalysts of intermolecular aldol reactions.[20]

One reason for this ineffectiveness could consist in the fact that, unlike proline, an amino acid with a primary amino group reacting with a carbonyl compound, mainly gives the iminium ion IV, and not the enamine form V, more stabilized by hyperconjugation, which is postulated as a fundamental intermediate in the catalytic cycle. Therefore, for the primary

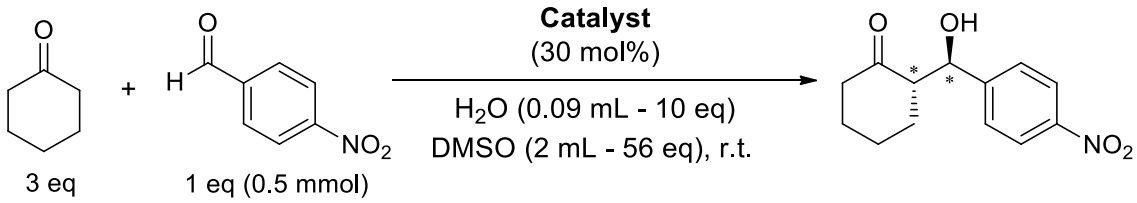
amino acids to give a catalytic mechanism *via* enamine it is essential that they proceed to the tautomerization of the iminium ion IV into the enamine form V (Scheme 4.7).



Scheme 4.7. *Via* enamine intermolecular Aldol reactions. Comparison between L-Pro and primary amino acid catalytic cycle.

In addition to proline, the work published by Cordova et al in 2006 is very important because it reports that primary amino acids such as phenylalanine, alanine, valine, and isoleucine can be realistically used as efficient organocatalysts in intermolecular aldol reactions, if such reactions are carried out in organic solvent containing a small amount of water.[21]

In particular, in the aldol reaction between cyclohexanone and *p*-nitrobenzaldehyde in the above conditions (Scheme 4.8) the *anti* aldol product is selectively obtained with high yields and with an enantiomeric excess greater than 99% (Table 4.2).



Schema 4.8. Small amount of water containing Aldol reaction catalyzed by primary amino acids in organic solvent.

Catalyst	Time (hours)	Yield (%)	d.r. (*anti*/*syn*)	e.e. (%)
(S)-Ala	72	95	15:1	92
(S)-Val	72	98	37:1	>99
(S)-Phe	48	70	3:1	73

Table 4.2. Aldol catalyzed reaction results.

Generally, however, the α-amino acids catalyzed aldol reactions reach a high enantioselectivity in aprotic polar organic solvents such as dimethylsulfoxide (DMSO) and N, N-dimethylformamide (DMF); however, the use of these solvents makes the workup and recycling of the catalyst more complex.[22]

Recently, the need to turn towards a more eco-sustainable chemistry has prompted the chemical industry to develop suitable conditions for typical reactions of organic catalysis to take place in an aqueous environment.

Initially, water, despite having peculiar characteristics such as high surface tension, high polarity and the ability to form hydrogen bonds, was not considered a suitable solvent for organocatalytic reactions, for various reasons, among which the insolubility of the great part of the organic molecules in it. In addition to this, water can in principle interfere with the transition state formed between the organocatalyst and substrate molecules, modifying the pattern of weak interactions that are created between them, thus deteriorating the catalytic activity and stereo-control.[23]

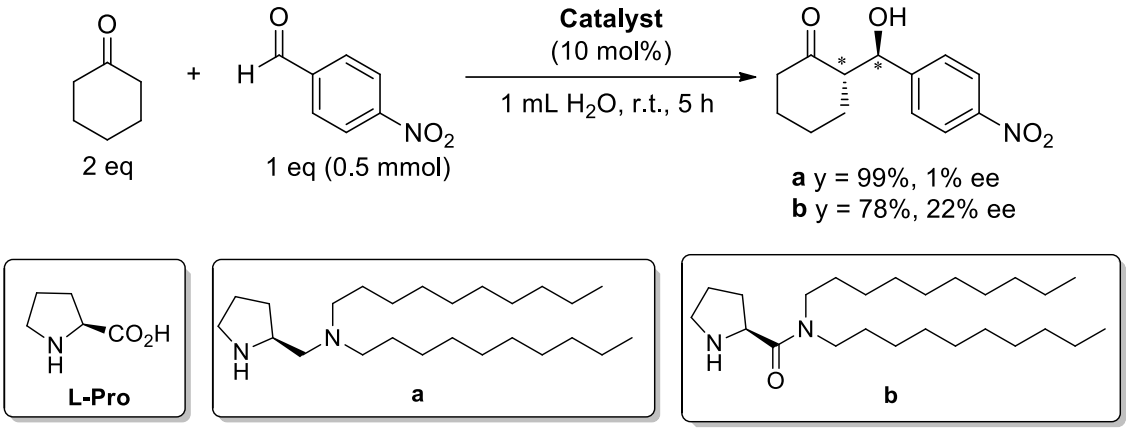
Therefore, reactions in water were assumed to lead to slow reaction rates and lower yields of the desired products. In this regard, it was observed that the Aldol reactions carried out in favorable conditions, therefore in aprotic polar solvents, in the presence of a quantity of water greater than 4%, lead to a substantial decrease in yields and enantioselectivity.[24]

The solution to the problems discussed so far on the use of water as a solvent for organocatalytic reactions came from the addition of surfactants to the reaction mixtures, topic already discussed in the previous Chapter.

4.2 Choice of the amino acid-BA derivatives

The Aldol reactions reported in the literature, carried out in water and using proline as an organocatalyst, do not lead to the formation of any Aldol product.

However, it is possible to use water as an exclusive reaction medium obtaining, at the same time, good yields of the Aldol product and sometimes even a well-determined stereoselectivity. This was experimentally demonstrated in the work of Mase et al. in 2006, in fact, carrying out the Aldol reaction between cyclohexanone and *p*-nitrobenzaldehyde exclusively in water using as organocatalysts of proline derivatives containing hydrophobic alkyl chains (Scheme 4.9) there is an increase in the yields of the Aldol product and a very specific *anti*/*syn* diastereoselection (Table 4.3).[25]



Scheme 4.9. Asymmetric Aldol reactions catalyzed by organic surfactants in water.

Catalyst	Time (h)	Yield (%)	d.r. (*anti*:*syn*)	e.e. (%)
L-Pro	96	0	-	-
1a	5	99	94:6	1
1b	5	78	84:16	22

Table 4.3. Aldol reaction results using surfactant-catalyst derivatives.

Furthermore, cholic and deoxycholic acid derivatives had been prepared and designed in order to give pH sensitive aggregative behaviors. For this purpose, amino acid units were linked to the C-3 of the polycyclic steroid skeleton of excellent natural surfactants such as bile acids (as previously discussed). In particular, derivatives **4.1** and **4.2** in which the

aforementioned acids were linked through their C-3 to phenylalanine through an amide function as a linker were prepared (Figure 4.2).

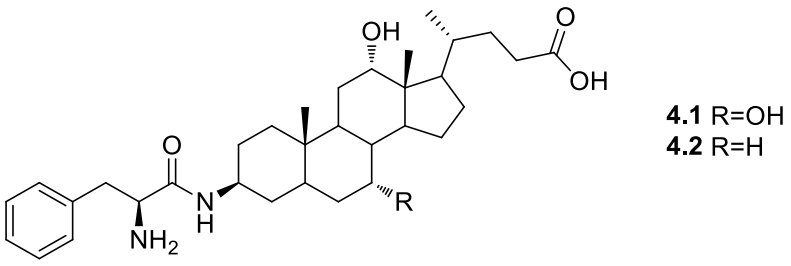


Figure 4.2. Cholic and deoxycholic acid derivatives.

These compounds showed the formation of diversified supramolecular aggregates in water depending on the pH of the aqueous medium.[26]

This ability to form spherical micelles among the possible architectures inspired also the synthesis of the new C-3 epimers of the prolin derivative of deoxycholic acid **4.3-(α)** and **4.3-(β)** (Figure 4.3).

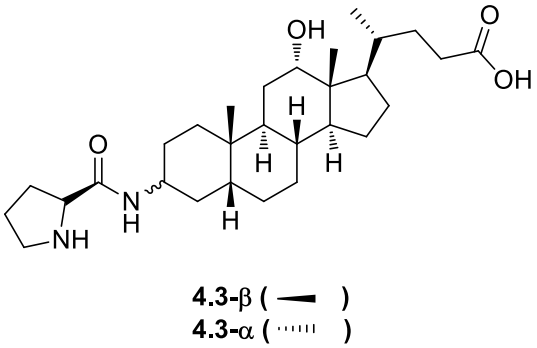


Figure 4.3. New C-3 L-Pro-BA derivatives.

The synthesis of both C-3 epimers would also have elucidated whether or not the different configuration plays a crucial role in the formation of aggregates, therefore also evaluating the ability to work as catalysts.

In fact, subsequently to the derivatives synthesis, the work would continue verifying their effectiveness as modified amino acids organocatalysts in simple Aldol condensation reactions in aqueous environment involving aromatic aldehydes, which are not able to form enolates, with symmetrical structure ketones, such as cyclohexanone.

Moreover, the synthesis of derivatives, as will be shown, would have involved the preparation of amino ester intermediates **4.4a** and **4.4b** whose hydrolysis reaction would have led to carboxylic acids **4.5a** and **4.5b** (Figure 4.4).

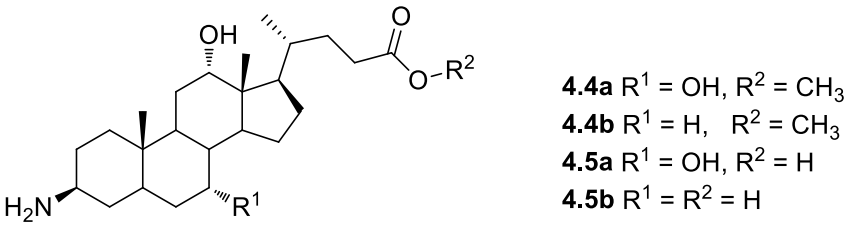
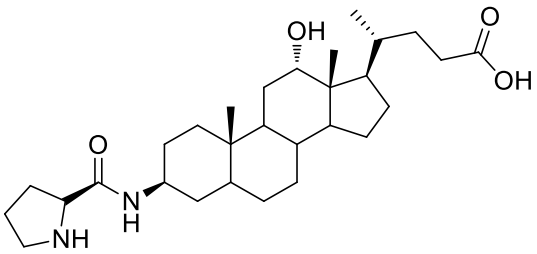


Figure 4.4. Amino ester intermediates.

These compounds showed the structural characteristics that could have made them effective organocatalysts, that is, a primary amino group linked to a structure with considerable rigidity. For this reasons they have also been verified as organocatalysts.

4.3 Synthesis of chosen amino acid-BAs derivatives

The first step of this work was the synthesis of **4.3-(β)** shown in Figure 4.5.

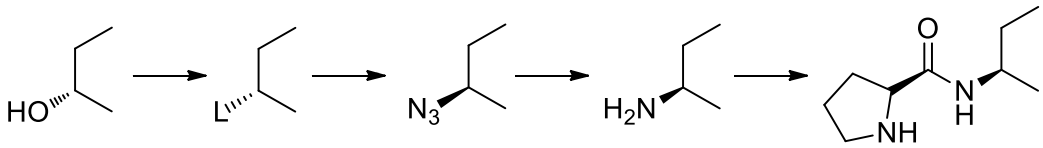


4.3-(β)

Figure 4.5. First target molecule synthesis.

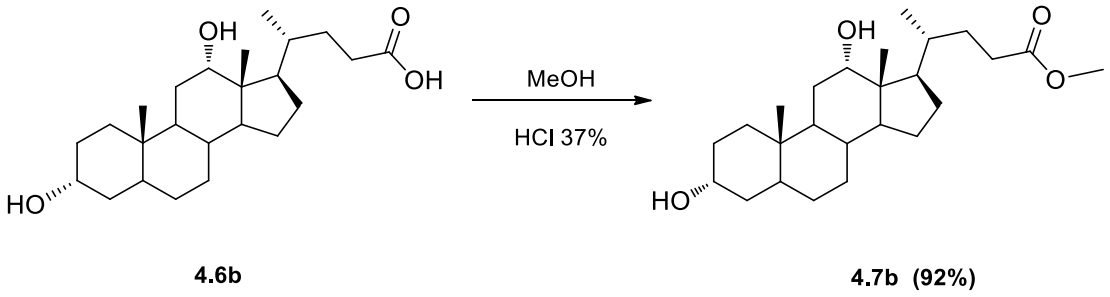
The general synthetic strategy chosen for the crucial amine intermediate and for the final amide derivative was that described in the literature, shown in Scheme 4.10.[27]

It uses the C-3 hydroxyl conversion into a good leaving group with configuration retention, then substitution with the azide group with inversion of configuration at C-3, and reduction of the azide function to amine. In the final stage, the amine undergoes condensation with the proline carboxylic function suitably protected.



Scheme 4.10. Chosen synthetic strategy for **4.3-(β)** synthesis.

However, before proceeding with the aforecited reactions, it was necessary to protect the carboxylic group of deoxycholic acid **4.6b** as methyl ester **4.7b**, according to the Fischer protocol already described, using methanol under acid catalytic conditions for HCl. In this way, the desired ester **4.7b** was obtained, following cold precipitation and vacuum filtration, with a yield of 92% (Scheme 4.11).

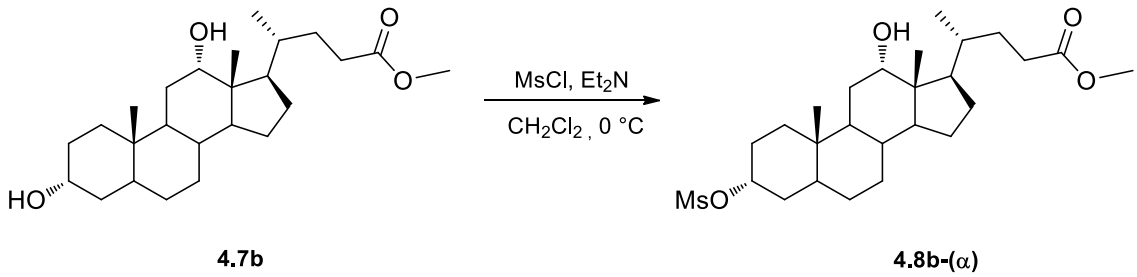


4.6b **4.7b (92%)**

Scheme 4.11. Fischer esterification.

At this point, it was possible to manipulate the hydroxyl function in C-3, known for its greater reactivity compared to the analogous functionality in position C-12,[28] through its conversion into the corresponding **4.8b-(α)** methanesulfonate ester (mesylate), which is very reactive in bimolecular nucleophilic substitution reactions.

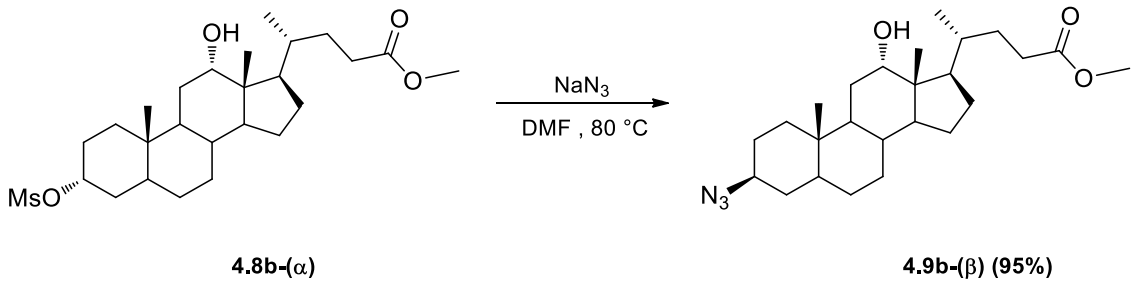
The mesylate synthesis reaction was then carried out by treating compound **4.7b** in anhydrous CH_2Cl_2 with methanesulfonyl chloride (MsCl) in the presence of triethylamine (Et_3N) (Scheme 4.12).



4.7b **4.8b-(α)**

Scheme 4.12. Mesylate synthesis.

This product was not isolated, to avoid any decomposition in chromatographic column and was then replaced with an azide group, with consequent configuration inversion, through a bimolecular nucleophilic substitution, carried out in N,N-dimethylformamide (DMF) by adding sodium azide (NaN_3) at a temperature of 80 °C (Scheme 4.12).



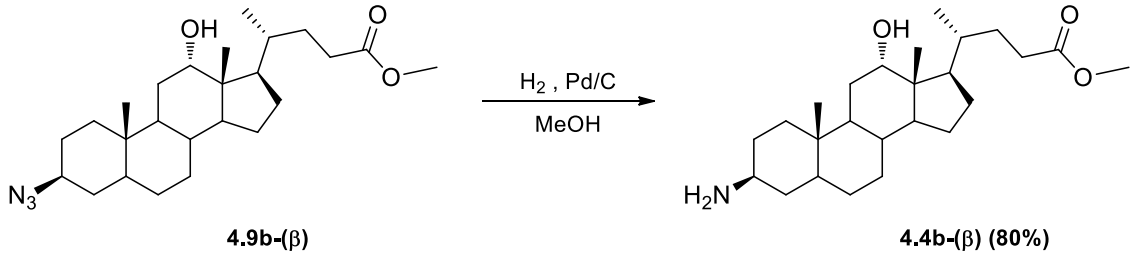
4.8b-(α) **4.9b-(β) (95%)**

Scheme 4.12. Azido-derivative **4.9b-(β)** synthesis.

The crude obtained was purified by chromatography obtaining the desired azide **4.9b-(β)** with a total 95% yield, calculated starting from methyl deoxycholate **4.7b**.

The azide group of **4.9b-(β)** at this point could be easily transformed into the primary amino group that we wanted to insert in position C-3, through a simple reduction reaction, by catalytic hydrogenation in heterogeneous phase.

The compound **4.9b-(β)** was then dissolved in MeOH in the presence of a catalytic quantity of Pd at 10% by weight on carbon, under magnetic stirring and in an hydrogen atmosphere, as illustrated in Scheme 4.13.



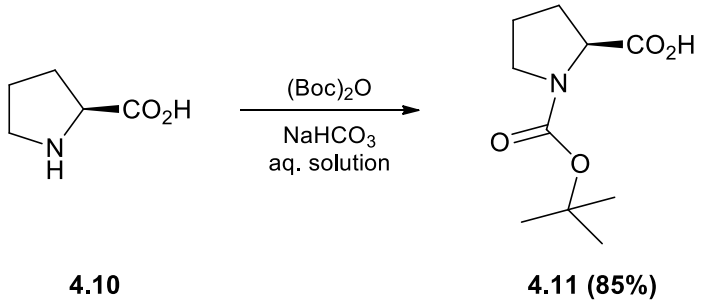
Scheme 4.13. Azido-derivative reduction.

The amino-derivative **4.4b-(β)** was obtain with an 80% yield after purification on chromatographic column.

At this point, the synthetic sequence involved the condensation of the carboxylic group of the protected N-Boc proline **4.11** with the primary amino function of the compound **4.4b-(β)**.

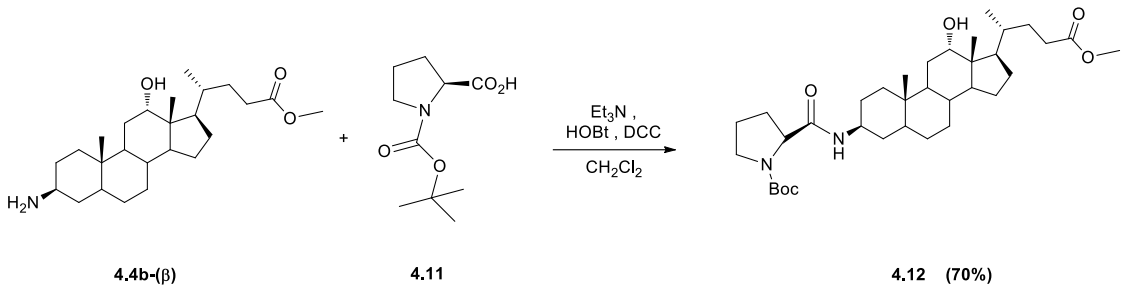
Compound **4.11** was therefore preliminarily prepared, protecting the proline **4.10** nitrogen with di-*tert*-butyldicarbonate.

Relying on protocols widely used in the literature,[29] the reaction was carried out in aqueous conditions, leading to compound **4.11** with a yield of 85% (Scheme 4.14).



Scheme 4.14. N-Boc protected

The subsequent condensation reaction between compound **4.11** and amine **4.4b-(β)** was carried out in dichloromethane, in the presence of triethylamine (Et_3N), hydroxybenzotriazole (HOBt) and dicyclohexylcarbodiimide (DCC) (Scheme 4.15).

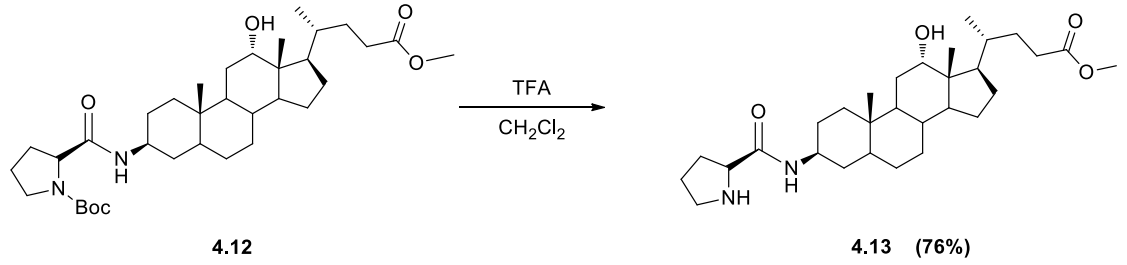


4.4b-(β) 4.11 4.12 (70%)

Scheme 4.15. Condensation reaction between **4.11** and **4.4b-(β)**.

The crude was purified on a chromatographic column, obtaining the desired product **4.11** with a yield of 70%.

The next step was the removal of the *t*-butoxycarbonyl protecting group in a reaction with trifluoroacetic acid (TFA) in dichloromethane (Scheme 4.16).

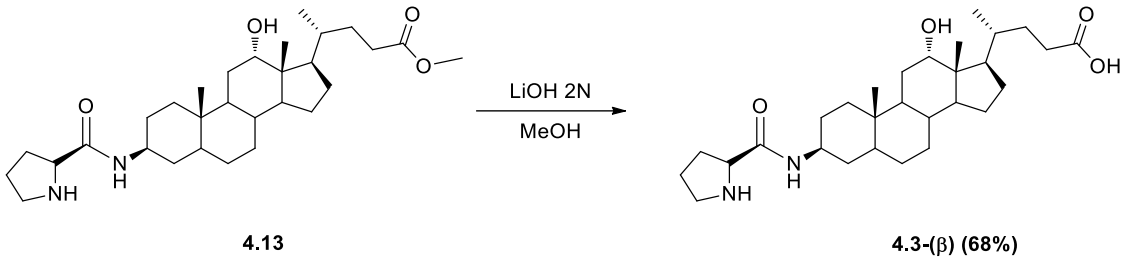


4.12 4.13 (76%)

Scheme 4.16. Boc protecting group removal.

The crude, after extraction, was purified on a chromatographic column, which gave the expected product with a yield of 76%.

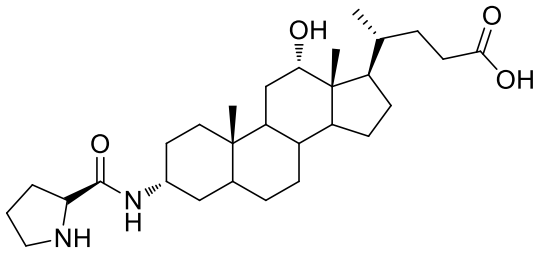
The synthesis of the desired catalyst **4.3-(β)** was then achieved carrying out the hydrolysis of the compound **4.13** with an aqueous solution of LiOH 2N in methanol, conditions which allowed the selective hydrolysis of the ester group only (Scheme 4.17). The compound **4.3-(β)** was then obtained, after purification, with 68%.



4.13 4.3-(β) (68%)

Scheme 4.17. Synthesis of first organocatalyst **4.3-(β)**.

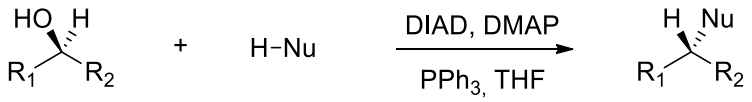
The work continued with the synthesis of catalyst **4.3-(α)** with an inverted configuration (Figure 4.6).



4.3-(α)

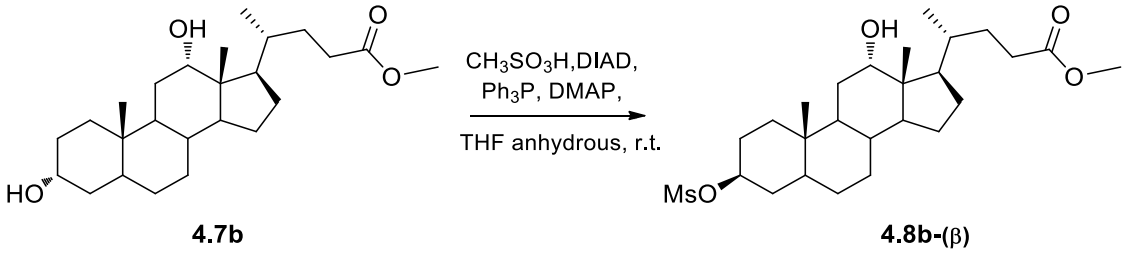
Figure 4.6. Catalyst **4.3-(α)** with inverted configuration.

To achieve this goal it was used the Mitsunobu reaction, which general scheme is here reported (Scheme 4.18).[30]



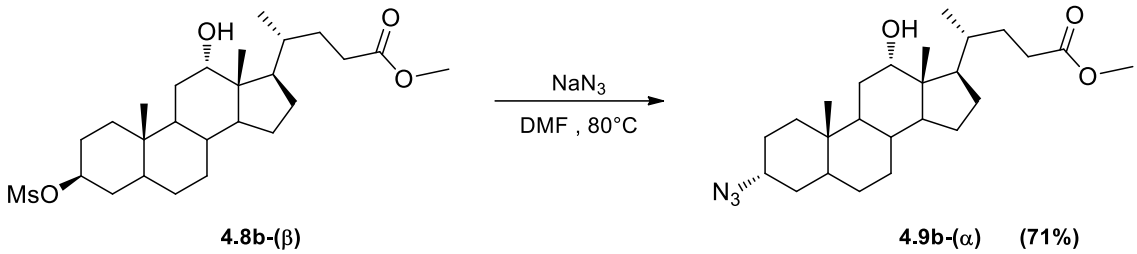
Scheme 4.18. Examples of Mistunobu reaction.

This reaction show a "formal" substitution with configuration inversion of a hydroxyl group with a nucleophile that has protons with a certain acidity, in the presence of triphenylphosphine (Ph_3P), diisopropylazodicarboxylate (DIAD) and N,N-dimethylaminopyridine (DMPA). The reaction just described was then carried out under the conditions reported in the literature, on methyl deoxycholate **4.7b**, using methanesulfonic acid as the nucleophile, in THF at room temperature for 48 hours (Scheme 4.19).



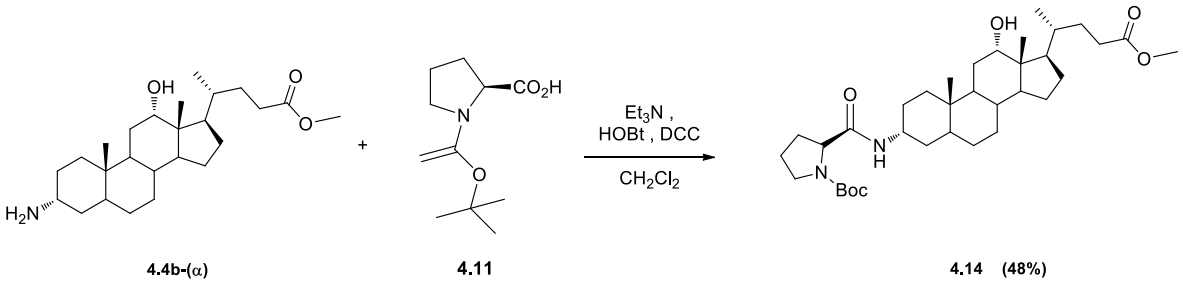
Scheme 4.19. Mesylation with configuration retention.

Unfortunately, the **4.8b-(β)** mesyl derivative thus obtained was not sufficiently stable to be purified on a chromatographic column, as this would have led to its decomposition on silica. The next stage involved the introduction of the azide group in position 3, through a nucleophilic S_N2 substitution, which involved a configuration inversion. The azidation reaction was carried out with sodium azide in DMF at room temperature (Scheme 4.20).



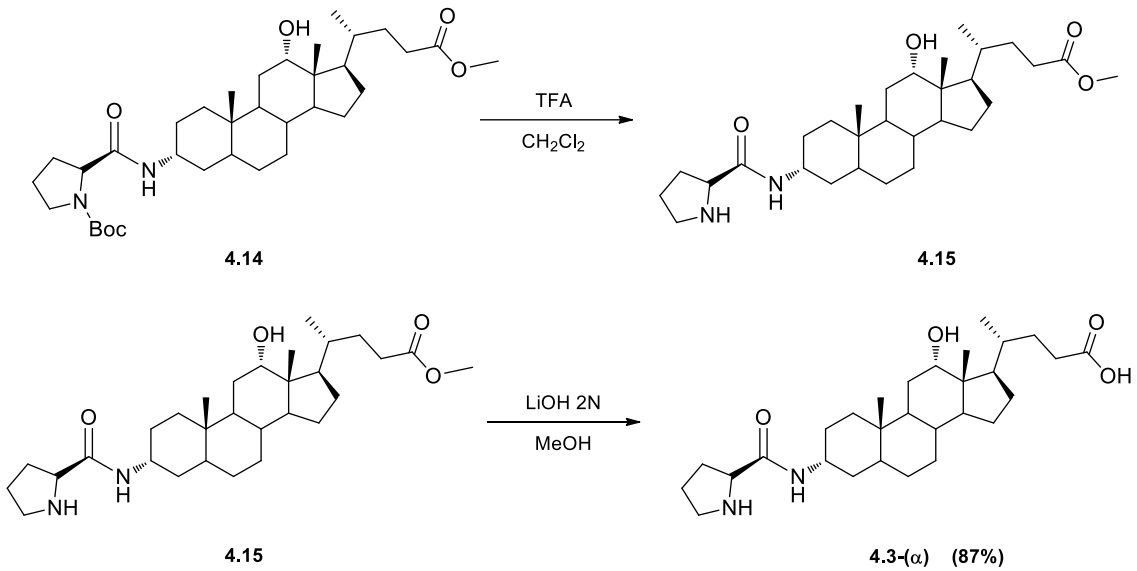
Scheme 4.20. Azidation of compound **4.8b-(β)**.

The crude was purified and the corresponding azide **4.9b-(α)** was obtained with a 71% yield. The azide group of **4.9b-(α)** was easily transformed into the primary amino group with the same method used in the preparation of **4.9b-(β)** obtaining **4.4b-(α)** with a yield of 40%. After the condensation in the same conditions, as the previous reduction reaction, between **4.4b-(α)** and N-Boc proline **4.11**, **4.14** was obtained in 48% yield (Scheme 4.21).



Scheme 4.21. Condensation reaction between **4.4b-(α)** and N-Boc proline.

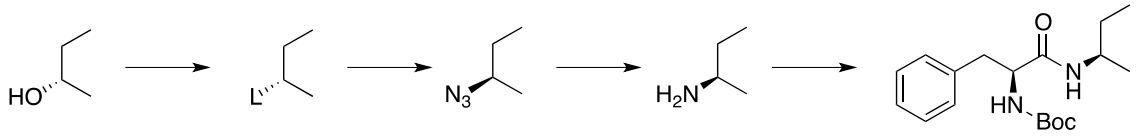
The Boc protecting group and the ester function were cleaved also with the procedure used before as shown in the Scheme 4.22 and the second organocatalyst **4.3-(α)** was obtained.



4.14 **4.15** **4.15** **4.3-(α) (87%)**

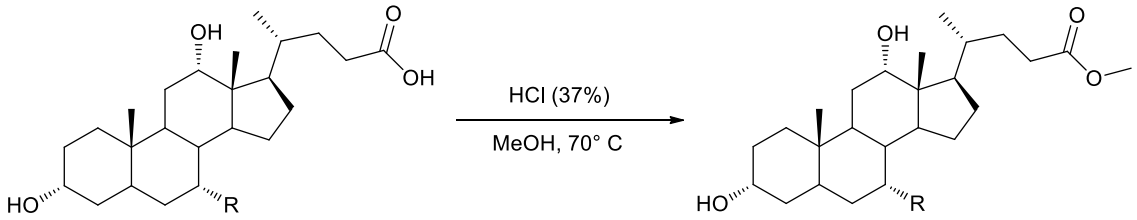
Scheme 4.22. Cleavage of Boc protecting group and ester portion.

The work continued with the synthesis of phenylalanine derivatives **4.1** and **4.2** (Figure 4.2) according to already reported procedure cited before and the final condensation with the amino acid (Scheme 4.23).



Scheme 4.23. Followed procedure for the synthesis of phenylalanine derivatives.

First of all, as in the synthesis of the previous derivatives, esters were obtained from compound **4.6a** and **4.6b** (Scheme 4.24).

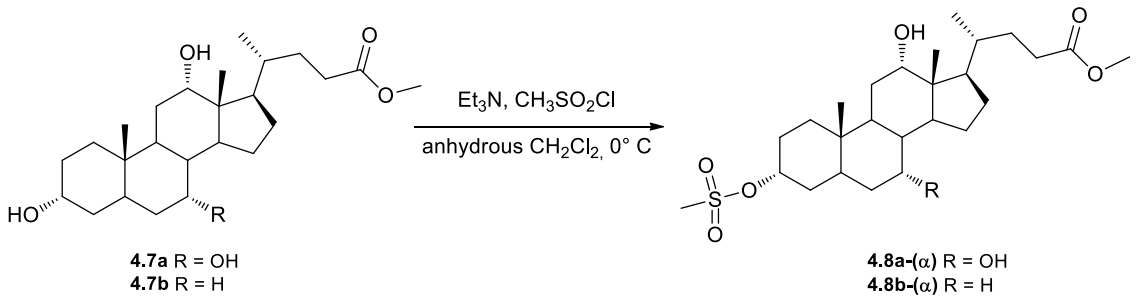


4.6a R = OH
4.6b R = H

4.7a R = OH (92%)
4.7b R = H (90%)

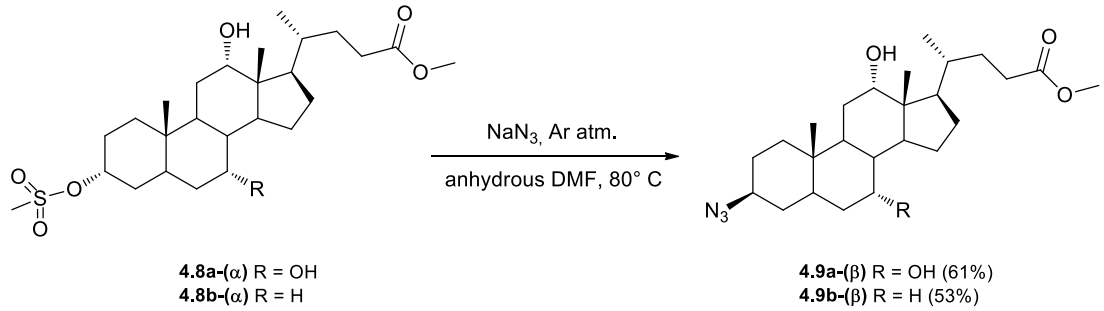
Scheme 4.24. Esters **4.7a,b** preparation.

Once again, the C-3 hydroxyl function of both compounds was mesylated with configuration retention (Scheme 4.25).



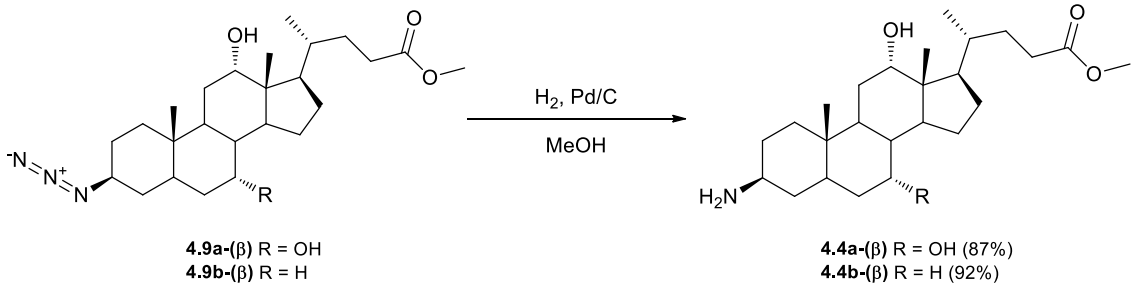
Scheme 4.25. Mesylation with configuration retention.

The mesyl-derivatives were then azidated as described before in order to obtain azido-derivatives **4.9a,b-(β)** with 61% and 53% yields as shown in Scheme 4.26.



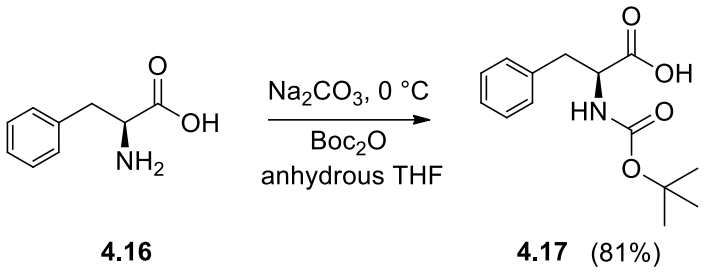
Scheme 4.26. Azido-derivative **4.9a,b-(β)** synthesis.

The C-3 azido-group was then reduced in MeOH in the presence of Pd/C and H_2 atmosphere (Scheme 4.27).



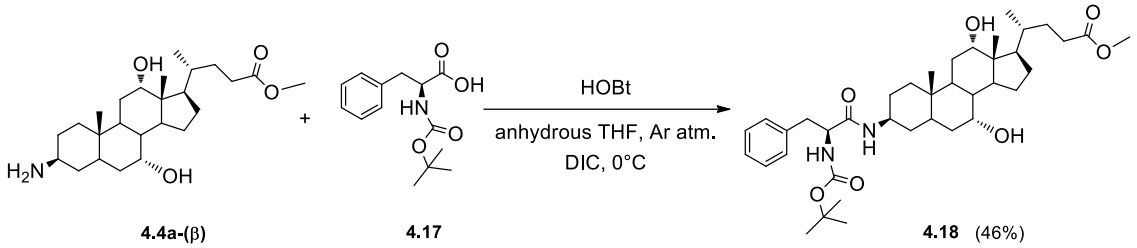
Scheme 4.27. C-3 Azido-group reduction.

Before the condensation reaction between the amino acid and the amino-derivatives, L-phenylalanine was N-Boc protected with the procedure already described (Scheme 4.28).

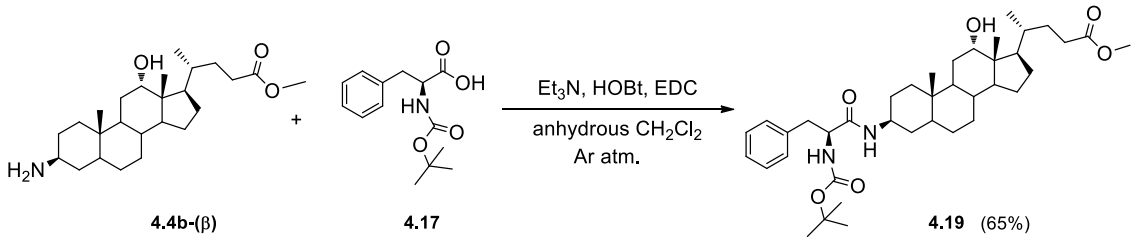


Scheme 4.28. N-Boc protection of L-Phe.

This time, the condensation reactions between N-Boc protected amino acid **4.17** and amino-derivatives **4.4a,b-(β)** were performed with DIC (N,N'-diisopropylcarbodiimide) and EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) as shown in Scheme 4.29 and Scheme 4.30.

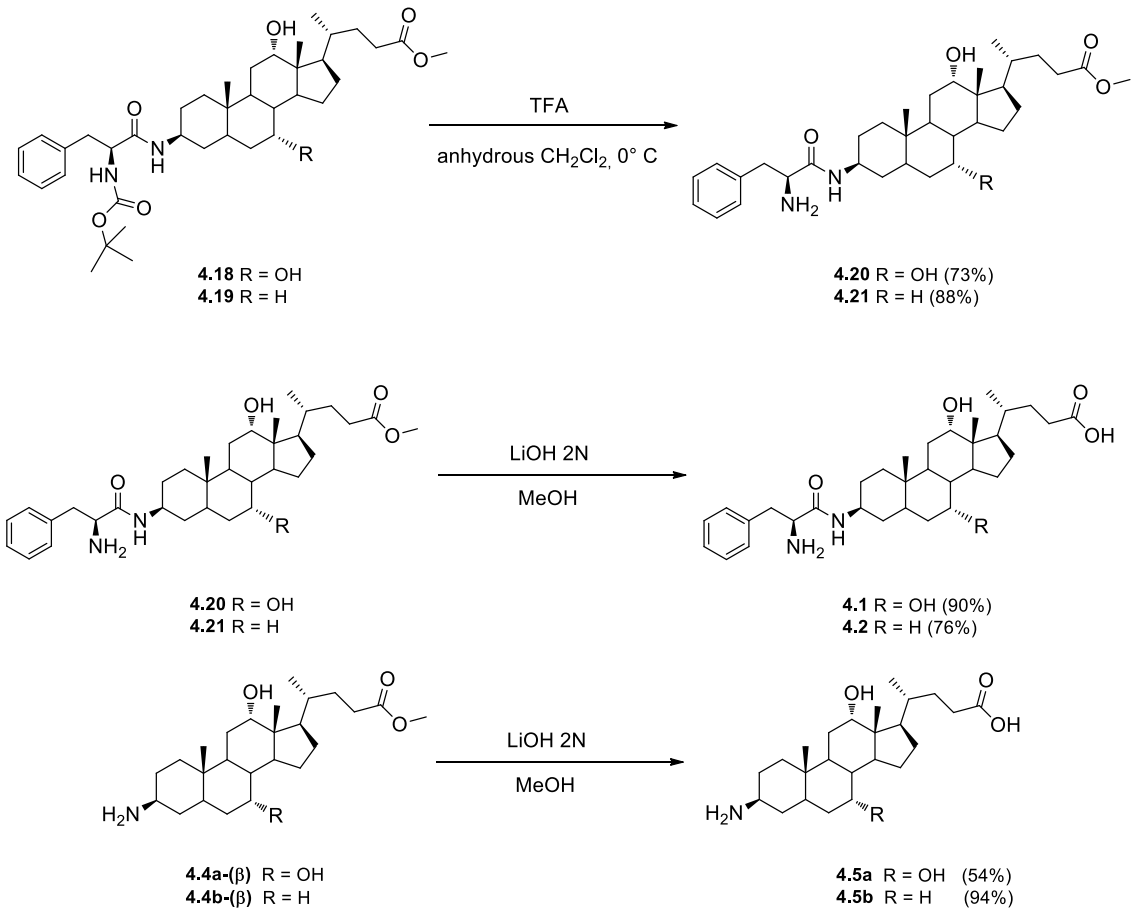


Scheme 4.29. Condensation reaction between **4.4a-(β)** and **4.17**.



Scheme 4.30. Condensation reaction between **4.4b-(β)** and **4.17**.

Tert-butoxycarbonyl group and ester portions was then removed from **4.18**, **4.19** and **4.4 b-(β)** using TFA first and then with LiOH 2N, with the methods already used for previous organocatalysts (Scheme 4.31).



Scheme 4.31. Deprotection of amino and acidic functions affording organocatalysts **4.1**, **4.2** and **4.4a,b-(β)**.

Organocatalysts **4.1**, **4.2**, **4.3** and **4.4a,b-(β)** (Figure 4.7) were ready to be tested in simple Aldol reactions involving cyclohexanone **4.22** and variously substituted aromatic aldehydes.

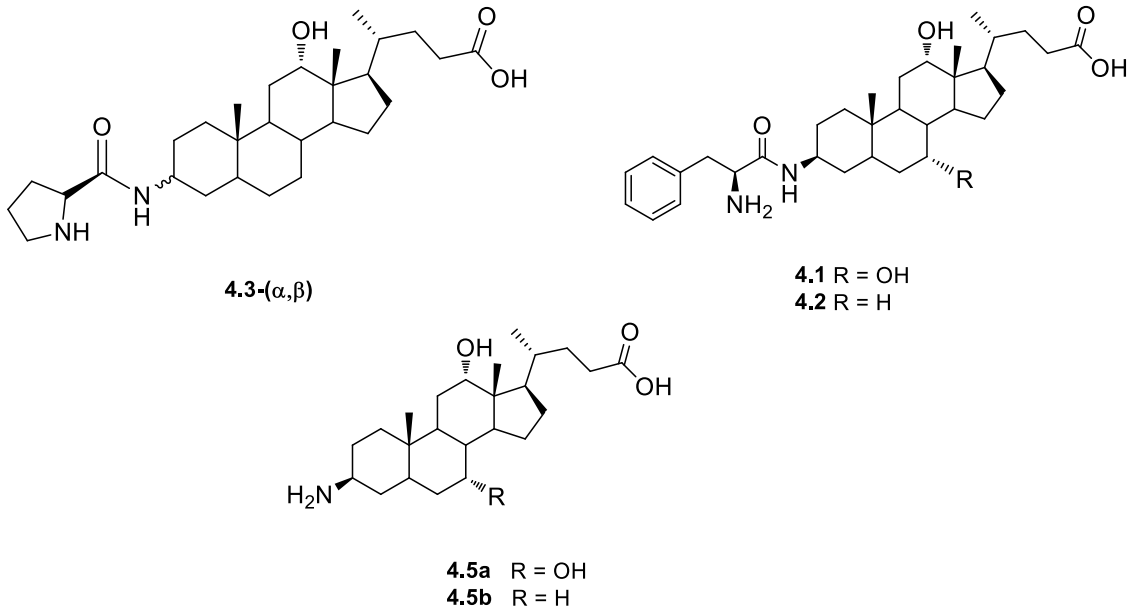


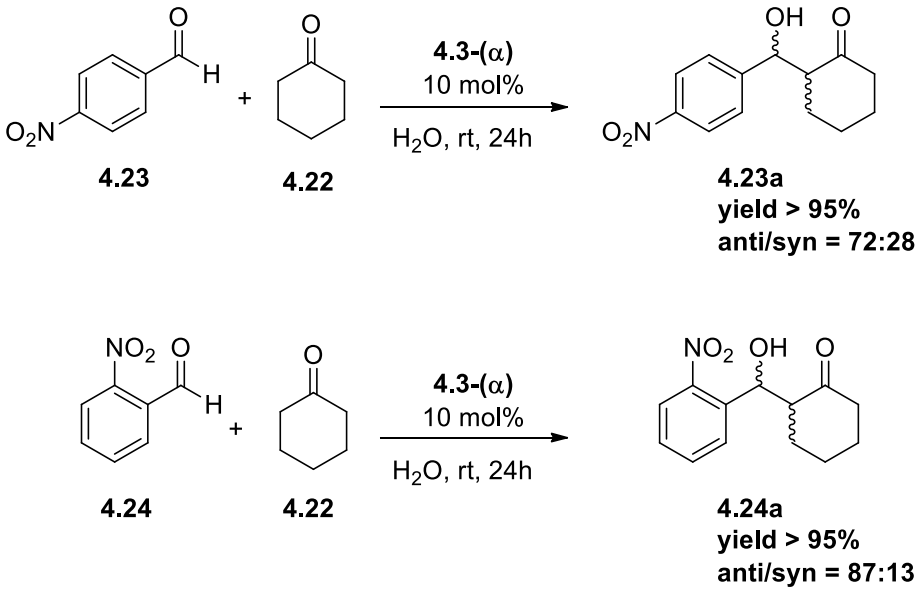
Figure 4.7. Amino acid based organocatalysts synthesized.

4.4 Synthesized organocatalysts tests in Aldol reactions

In order to evaluate the catalytic activity of synthetized organocatalysts they were tested in Aldol reactions, in 100% water in reactions with cyclohexanone and variously substituted aldehydes. Since, as will be shown, using more than 10 mol% of catalyst did not lead to significant increases in yields, this quantity was chosen as a model in the reactions. All aldols were observed by NMR spectroscopy and all yields are intended after purification on a chromatographic column

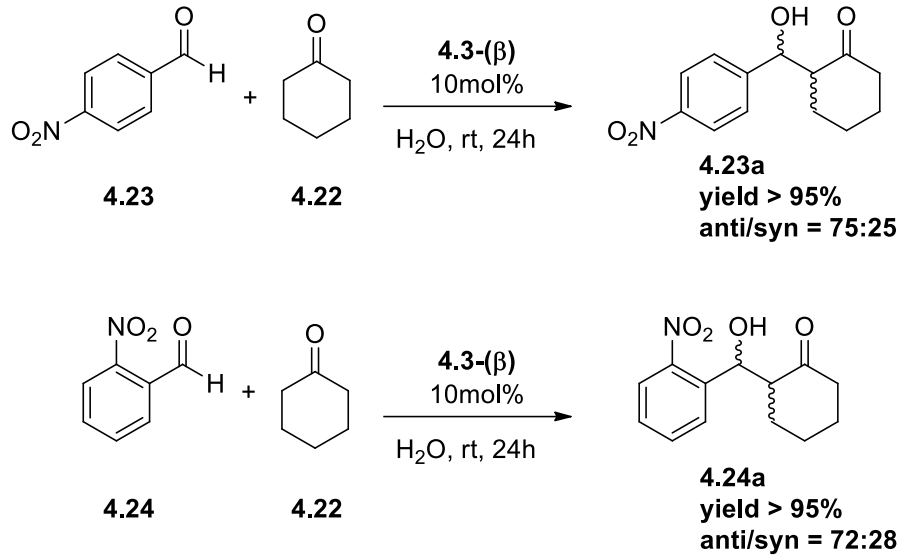
4.4.1 L-Pro derivatives tests

The first catalyst tested in two model Aldol reactions was **4.3-(α)**. It shows a good efficiency and the reactions gave good yields and diastereoisomeric ratios as shown in Scheme 4.32.



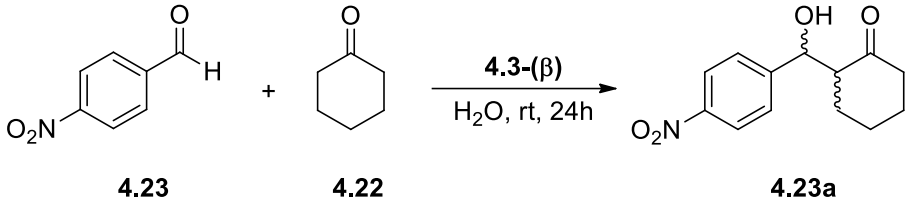
Scheme 4.32. First reactions carried out with organocatalyst **4.3-(α)**.

Then, since tests with catalyst **4.3-(β)** showed almost same result in terms of yields and dr (Scheme 4.33), the study on this L-Pro derivative continued with the β one.



Scheme 4.33. First tests with **4.3-(β)** organocatalyst.

Both the yields and the reaction time showed not to be affected by a molar increase of **4.3-(β)** (Table 4.4), yielding predominantly to the anti aldol product with high diastereo- and enantioselectivity. The anti-diastereoselectivity is in accordance with literature data on proline-catalysed aldol reactions carried out in classical organic solvents.



Scheme 4.34. Effective promotion of aldol reaction in presence of **4.3-(β)**.

Catalyst load [mol%]	Yield[a] [%]	dr[b] (anti:syn)	*ee* anti[c] [%]
1	49	75:25	83
5	65	69:31	87
10	87	77:23	84
20	86	74:26	82

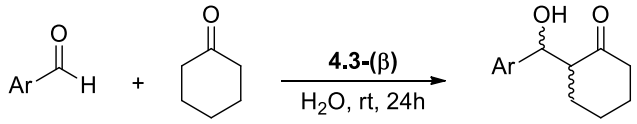
30	89	71:29	86

[a]Calculated on isolated product after flash column chromatography.;
[b]determined by NMR spectra and confirmed *via* HPLC on a chiral stationary phase;
[c]Determined *via* HPLC analysis on a chiral stationary phase.

Table 4.4. Molar increase of catalyst load does not affect neither yields nor reaction time or dr and ee.

Furthermore, a control reaction of organic substrates was carried out in water in the same pH conditions without catalyst. In such conditions, no conversion was observed within days, clearly assessing the positive role of surfactant in the reaction.

The reaction was then carried out in the same conditions (10 mol% catalyst) on several aromatic aldehydes, and the results are reported in Table 4.5.



4.X **4.22** **4.Xa**

Entry (X =)	Ar	Yield[a] [%]	dr[b] (anti:syn)	*ee* anti[c] [%]
23	4-NO_2-C_6H_4	99	77:23	19
24	2-NO_2-C_6H_4	70	78:22	14
25	3-NO_2-C_6H_4	67	80:20	18
26	4-CN-C_6H_4	82	73:27	33
27	2-F-C_6H_4	99	95:5	38
28	4-Br-C_6H_4	95	82:18	15
29	3-Br-C_6H_4	94	84:16	25
30	2-Br-C_6H_4	97	97:3	23
31	3-Cl-C_6H_4	99	74:26	27
32	2-Cl-C_6H_4	98	88:12	20
33	4-OMe-C_6H_4	17	87:13	55
34	3-OMe-C_6H_4	77	69:31	30
35	3-OH-C_6H_4	40	97:3	29
36	1-naphthyl	38	82:18	1

[a]Calculated on isolated product after flash column chromatography;
[b]determined by NMR spectra and confirmed *via* HPLC on a chiral stationary phase;
[c]determined *via* HPLC analysis on a chiral stationary phase.

Table 4.5. Aldol reaction on several aromatic aldehydes in presence of **4.3-(β)** expanding the reaction scope.

As can be seen from the Table 4.5 the reaction occurred in high yield and good diastereomeric ratios were found by analysing NMR spectra.

4.4.2 Experimental section for L-Pro derivatives

General Informations

Unless otherwise noted, all commercially available compounds were used without further purification. Trends of reactions was monitored by thin layer chromatography (TLC) Merck Kieselgel 60 F254 (0.25mm thickness). Visualization of the developed TLC plates was performed with UV irradiation (254 nm) or by staining with phosphomolybdic acid 12% solution. ^{1}H and ^{13}C spectra were recorded at room temperature on Bruker Avance 400. Analytical HPLC was performed on a Varian 9002 HPLC instrument equipped with a refractive index detector (Schambeck, RI2000), using chiral stationary phases (Chiralpak IA, IB and IC).

High-resolution ESI mass spectra were carried out on a Q-TOF Micro spectrometer operating in a positive ion mode.

All compounds already described were not reported again.

Catalyst synthesis

In a three-necked flask, equipped with bubble refrigerant, under inert atmosphere, 0.29 mmol of amine and 1 eq of N-BOC Proline were in CH_2Cl_2 were added in this order. Then, at 0 °C, 0.041 mL (1 eq) of Et_3N, 40 mg (1 eq) of benzohydroxytriazole and 61 mg (1eq) of DCC were added. The reaction was left for 30 minutes at 0 °C and subsequently brought to room temperature for 48 hours.

At the end of the reaction, the raw product was filtered to remove the dicyclohexylurea and purified on a chromatographic column with an eluent mixture of petroleum ether/ethyl acetate (4:6).

Then in a three-necked flask, equipped with a bubble cooler, a dropping funnel and placed under an inert atmosphere, 85 mg (0.14 mmol) of the previous compound were dissolved in 5 ml of CH_2Cl_2. 0.097 mL (9 eq) of TFA at 0 °C was then added dropwise. The reaction was checked periodically until completion. At the end of the reaction, the crude was processed with Na_2HCO_3 (2 x 10mL), brine (2 x 10mL), and H_2O (2 x 10mL); then dried over sodium sulphate and the solvent removed under vacuum. The reaction crude is purified by means of a chromatographic column (packed with conditioned silica thanks to methanol) in gradient, using ethyl acetate to elute the impurities and CH_2Cl_2 / MeOH (9:1) to elute the product.

To finally obtain the catalyst, 40 mg (0.079 mmol) of the previous compound were dissolved in 1 mL of MeOH in a one-necked Liebig flask at 50 °C. 1 mL of 2N LiOH was slowly added

and the reaction was checked periodically every 5 minutes. The reaction was complete within 30-40 minutes, after which the methanol was evaporated under reduced pressure (50-60 °C) and the remaining aqueous phase was transferred to a separatory funnel and extracted with Et_2O (2 x 5mL). The aqueous phases were then acidified with 2N hydrochloric acid and extracted with ethyl acetate (4 x 5mL). The organic phases were combined and washed with water until neutral.

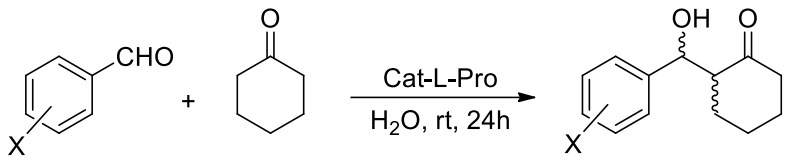
The raw reaction product was purified on a chromatographic column with an eluent mixture $CHCl_3$/MeOH (95:5).

NMR Spectra

^{1}H-NMR (400 MHz, DMSO) **(α)** δ(ppm): 0,67 (s, 3H), 0,93 (3, 3H), 0,95 (d, 3H, J=6,05 Hz), 0,98-2,50 (cm, 27H, CH and CH_2 steroidic backbone and side chain), 1,80-2,30 (cm, 4H), 3,40 (m, 1H), 3,50 (m, 2H), 3,97 (m, 1H), 4,20 (m, 1H). HRMS (ESI): Calcd for $C_{29}H_{49}N_2O_4^+$ ($[M+H]^+$) 489.3692. Found 489.3695.

^{1}H-NMR (400 MHz, DMSO) **(β)** δ(ppm): 0,67 (s, 3H, CH3-18); 0,93 (3, 3H, CH3-19); 0,95 (d, 3H, J=6,05 Hz CH3-21); 0,98-2,50 (mc, 27H, CH e CH2 steroidic backbone and side chain); 1,80-2,30 (mc, 4H; CH2-27 e CH2-26); 3,40 (m, 1H, CH-3); 3,50 (m, 2H, CH2-28); 3,97 (m, 1H, CH-12); 4,20 (m, 1H, CH-35). HRMS (ESI): Calcd for $C_{29}H_{49}N_2O_4^+$ ($[M+H]^+$) 489.3692. Found 489.3697.

General procedure for Aldol reaction



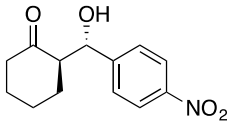
(1 equiv) **4.23-36** (5 equiv) **4.22** **4.23-36a**

Catalyst (0.1 eq, 0.03 mmol), aldehyde (1 eq, 0.30 mmol), cyclohexanone (5 eq, 1.50 mmol) were added into a one-necked bottom flask at rt. Water (1 mL) was added, and the resulting solution was allowed to stir at rt for 24 h. The mixture was stirred at room temperature until complete conversion of the substrates were achieved by TLC monitoring. Once the reaction is complete the reaction mixture was extracted with AcOEt (3 mL x 3) and the catalyst was recovered in the aqueous phase. The volatiles were then removed in vacuo to afford the crude product which was subsequently purified by flash chromatography on silica gel (eluting with 30% EtOAc/Petroleum ether) to afford the aldol products.

Unless otherwise stated the derivatives have been previously synthesised and all analytical data are consistent with literature values.

NMR Spectra

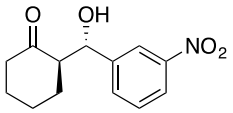
(R)-2-((S)-hydroxy(4-nitrophenyl)methyl)cyclohexan-1-one **(4.23a)**



The product **4.23a** was isolated after flash chromatography on silica gel (P/EtOAc 7:3) as yellowish solid (74 mg, 99% yield). **Molecular formula**: $C_{13}H_{15}NO_4$. **Molecular mass**: 249.27 g mol^{-1}. **HPLC**: Chiralpak IB, Hexane/iPrOH 95:5, 1.0 mL/min, *anti* diastereomer (major) : τ_{major} = 25.7 min τ_{minor} = 31.8 min; *syn* diastereomer (minor) : τ_{major} = 21.2 min τ_{minor} = 23.4 min. **dr:** *anti*/*syn* 77:23 (from NMR analysis); *anti*/*syn* 54:46 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.18 (m, 2H, C*H*$_{ar}$), 7.48 (m, 2H, C*H*$_{ar}$), 4.88 (d, J = 8.4 Hz, 1H, C*H*Ph), 2.59 (m, 1H), 2.46 (m, 1H), 2.37 (m, 1H), 2.08 (m, 1H), 1.65 (m, 2H), 1.53 (m, 2H), 1.30 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 214.7, 148.5, 147.5, 127.9 (2C),

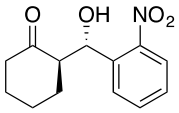
123.5 (2C), 73.9, 57.2, 42.6, 30.7, 27.6, 24.6 ppm; HRMS (ESI): Calcd for $C_{13}H_{16}NO_4^+$ ($[M+H]^+$) 250.1074. Found 250.1078.

(R)-2-((S)-hydroxy(3-nitrophenyl)methyl)cyclohexan-1-one **(4.25a)**



The product **4.25a** was isolated after flash chromatography on silica gel (P/EtOAc 7:3) as yellowish oil (69 mg, 67% yield). **Molecular formula**: $C_{13}H_{15}NO_4$. **Molecular mass**: 249.27 g mol^{-1}. **HPLC**: Chiralpak IB, Hexane/iPrOH 95:5, 1 mL/min, *anti* diastereomer (major) : τ_{major} = 22.1 min τ_{minor} = 30.2 min; *syn* diastereomer (minor) : τ_{major} = 18.6 min τ_{minor} = 20.0 min. **dr:** *anti*/*syn* 80:20 (from NMR analysis); *anti*/*syn* 51:49 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.15 (m, 2H, CH_{ar}), 7.66 (m, 1H, CH_{ar}), 7.51 (m, 1H, CH_{ar}), 4.89 (d, J = 8.5 Hz, 1H, C*H*Ph), 3.48 (br, 1H, O*H*), 2.62 (m, 1H), 2.48 (m, 1H), 2.36 (m, 1H), 2.10 (m, 1H), 1.83 (m, 1H), 1.61 (m, 2H), 1.56 (m, 2H), 1.37 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 214.9, 148.3, 143.3, 133.2, 129.3, 122.8, 122.0, 73.9, 57.1, 42.6, 30.7, 27.6, 24.6 ppm; HRMS (ESI): Calcd for $C_{13}H_{16}NO_4^+$ ($[M+H]^+$) 250.1074. Found 250.1075.

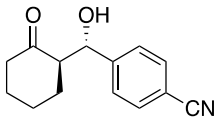
(R)-2-((S)-hydroxy(2-nitrophenyl)methyl)cyclohexan-1-one **(4.24a)**



The product **4.24a** was isolated after flash chromatography on silica gel (P/EtOAc 7:3) as brownish solid (72 mg, 70% yield). **Molecular formula**: $C_{13}H_{15}NO_4$. **Molecular mass**: 249.27 g mol^{-1}. **HPLC**: Chiralpak IA, Hexane/iPrOH 9:1, 1 mL/min, *anti* diastereomer (major) : τ_{major} = 19.3 min τ_{minor} = 23.7 min; *syn* diastereomer (minor) : τ_{major} =12.5 τ_{minor} = 11.5. **dr:** *anti*/*syn* 78:22 (from NMR analysis); *anti*/*syn* 70:30 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.83 (dd, J = 1.4, 8.2), 1H, CH_{ar}), 7.75 (dd, J = 1.3, 7.9) Hz, 1H, CH_{ar}), 7.63 (m, 1H, CH_{ar}), 7.41 (m, 1H, CH_{ar}), 5.43 (d, J = 7.1 Hz, 1H, C*H*Ph), 3.37 (br, 1H, O*H*), 2.75 (m, 1H), 2.42 (m, 1H), 2.32 (m, 2H), 2.08 (m, 1H), 1.84 (m, 1H), 1.61 (m, 4H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 214.8, 148.7, 136.6, 133.0, 129.0, 128.4, 124.0, 69.6, 57.3, 42.8,

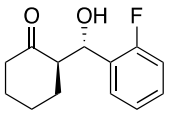
31.0, 27.7, 24.9 ppm; HRMS (ESI): Calcd for $C_{13}H_{16}NO_4^+$ ([M+H]$^+$) 250.1074. Found 250.1076.

4-((S)-hydroxy((R)-2-oxocyclohexyl)methyl)benzonitrile **(4.26a)**



The product **4.26a** was isolated after flash chromatography on silica gel (P/EtOAc 7:3) as yellowish oil (55 mg, 80% yield). **Molecular formula**: $C_{14}H_{15}NO_2$. **Molecular mass**: 229.28 g mol^{-1}. **HPLC**: Chiralpak IB, Hexane/iPrOH 95:5, 1 mL/min, *anti* diastereomer (major) : τ_{major} = 26.4 min τ_{minor} = 33.4 min; *syn* diastereomer (minor) : τ_{major} = 21.2 min τ_{minor} = 24.7 min. **dr:** *anti*/*syn* 73:27 (from NMR analysis); *anti*/*syn* 53:47 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.61 (m, 2H, C*H*$_{ar}$), 7.16 (m, 2H, C*H*$_{ar}$), 4.82 (d, *J* = 8.4 Hz, 1H, C*H*Ph), 3.79 (br, 1H, O*H*), 2.56 (m, 1H), 2.45 (m, 1H), 2.33 (m, 1H), 2.08 (m, 1H), 1.81 (m, 1H), 1.66 (m, 1H), 1.52 (m, 2H), 1.33 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 214.8, 146.4, 132.1 (2C), 127.8 (2C), 118.7, 111.6, 74.1, 57.1, 42.6, 30.7, 27.6, 24.6 ppm; HRMS (ESI): Calcd for $C_{14}H_{16}NO_2^+$ ([M+H]$^+$) 230.1176. Found 230.1178.

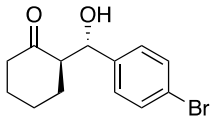
(R)-2-((S)-(2-fluorophenyl)(hydroxy)methyl)cyclohexan-1-one **(4.27a)**



The product **4.27a** was isolated after flash chromatography on silica gel (P/EtOAc 7:3) as greenish oil (66 mg, 99% yield). **Molecular formula**: $C_{13}H_{15}FO_2$. **Molecular mass**: 222.26 g mol^{-1}. **HPLC**: Chiralpak IC, Hexane/iPrOH 95:5, 1.0 mL/min, *anti* diastereomer (major) : τ_{major} = 26.4 min τ_{minor} = 33.3 min; *syn* diastereomer (minor) : τ_{major} = 14.7 min τ_{minor} = 12.6 min. **dr:** *anti*/*syn* 95:5 (from NMR analysis); *anti*/*syn* 99:1 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.44 (m, 1H, C*H*$_{ar}$), 7.22 (m, 1H, C*H*$_{ar}$), 7.12 (m, 1H, C*H*$_{ar}$), 6.97 (m, 1H, C*H*$_{ar}$), 5.15 (d, *J* = 8.7 Hz, 1H, C*H*Ph), 3.82 (br, 1H, O*H*), 2.64 (m, 1H), 2.43 (m, 1H), 2.04 (m, 1H), 1.80 (m, 1H), 1.56 (m, 4H), 1.40 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$)

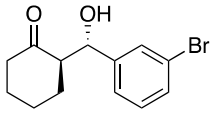
δ = 215.2, 161.3, 129.2, 128.3, 128.2, 124.4, 115.0, 68.0, 57.1, 42.6, 30.3, 27.8, 24.7 ppm; HRMS (ESI): Calcd for $C_{13}H_{16}FO_2^+$ ($[M+H]^+$) 223.1129. Found 223.1132.

(R)-2-((S)-(4-bromophenyl)(hydroxy)methyl)cyclohexan-1-one **(4.28a)**



The product **4.28a** was isolated after flash chromatography on silica gel (P/EtOAc 7:3) as yellow oil (84 mg, 95% yield). **Molecular formula**: $C_{13}H_{15}BrO_2$. **Molecular mass**: 283.17 g mol^{-1}. **HPLC**: Chiralpak IC, Hexane/iPrOH 95:5, 1 mL/min, *anti* diastereomer (major) : τ_{major} = 31.4 min τ_{minor} = 40.0 min; *syn* diastereomer (minor) : τ_{major} = 27.4 min τ_{minor} = 29.0 min. **dr:** *anti*/*syn* 82:18 (from NMR analysis); *anti*/*syn* 98:2 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.46 (m, 2H, C*H*$_{ar}$), 7.19 (m, 2H, C*H*$_{ar}$), 4.74 (d, *J* = 8.7 Hz, 1H, C*H*Ph), 3.98 (br, 1H, O*H*), 2.54 (m, 1H), 2.46 (m, 1H), 2.08 (m, 1H), 1.78 (m, 1H), 1.69 (m, 2H), 1.58 (m, 4H), 1.42 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 215.2, 140.0, 131.5 (2C), 128.8 (2C), 121.7, 74.2, 57.3, 42.7, 30.7, 27.7, 24.7 ppm; HRMS (ESI): Calcd for $C_{13}H_{15}BrO_2Na^+$ ($[M+Na]^+$) 305.0148. Found 305.0151.

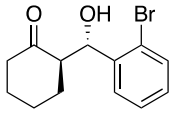
(R)-2-((S)-(3-bromophenyl)(hydroxy)methyl)cyclohexan-1-one **(4.29a)**



The product **4.29a** was isolated after flash chromatography on silica gel (P/EtOAc 7:3) as colorless oil (83 mg, 94% yield). **Molecular formula**: $C_{13}H_{15}BrO_2$. **Molecular mass**: 283.17 g mol^{-1}. **HPLC**: Chiralpak IB, Hexane/iPrOH 95:5, 1 mL/min, *anti* diastereomer (major) : τ_{major} = 9.9 min τ_{minor} = 11.5 min; *syn* diastereomer (minor) : τ_{major} = 8.3 min τ_{minor} = 8.8 min. **dr:** *anti*/*syn* 84:16 (from NMR analysis); *anti*/*syn* 83:17 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.43 (m, 1H, C*H*$_{ar}$), 7.34 (m, 1H, C*H*$_{ar}$), 7.15 (m, 2H, C*H*$_{ar}$), 4.68 (d, *J* = 8.6 Hz, 1H, C*H*Ph), 2.51 (m, 1H), 2.38 (m, 1H), 2.28 (m, 1H), 2.00 (m, 1H), 1.55 (m, 4H), 1.25 (m, 1H) ppm. **^{13}C NMR** (151 MHz, $CDCl_3$) δ = 214.9, 143.6, 130.8, 130.0, 129.9, 125.8,

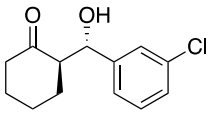
122.5, 74.0, 57.2, 42.6, 30.7, 27.7, 24.6 ppm; HRMS (ESI): Calcd for $C_{13}H_{16}BrO_2^+$ ($[M+H]^+$) 283.0328. Found 283.0331.

(R)-2-((S)-(2-bromophenyl)(hydroxy)methyl)cyclohexan-1-one **(4.30a)**



The product **4.30a** was isolated after flash chromatography on silica gel (P/EtOAc 6:4) as yellow oil (84 mg, 97% yield). **Molecular formula**: $C_{13}H_{15}BrO_2$. **Molecular mass**: 283.17 g mol^{-1}. **HPLC**: Chiralpak IC, Hexane/iPrOH 9:1, 1.0 mL/min, *anti* diastereomer (major) : τ_{major} = 15.6 min τ_{minor} = 19.2 min; *syn* diastereomer (minor) : τ_{major} = 8.7 min τ_{minor} = 9.6 min. **dr:** *anti*/*syn* 97:3 (from NMR analysis); *anti*/*syn* 94:6 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.50 (m, 2H, CH_{ar}), 7.32 (m, 1H, CH_{ar}), 7.11 (m, 1H, CH_{ar}), 5.29 (d, J = 8.1 Hz, 1H, C*H*Ph), 2.67 (m, 1H), 2.44 (m, 1H), 2.23 (m, 1H), 2.06 (m, 1H), 1.80 (m, 1H), 1.57 (m, 4H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 215.1, 140.8, 132.5, 129.1, 128.5, 127.9, 123.4, 72.8, 57.7, 42.7, 30.6, 27.8, 24.9 ppm; HRMS (ESI): Calcd for $C_{13}H_{15}BrO_2Na^+$ ($[M+Na]^+$) 305.0148. Found 305.0152.

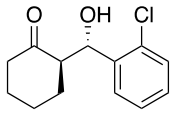
(R)-2-((S)-(3-chlorophenyl)(hydroxy)methyl)cyclohexan-1-one **(4.31a)**



The product **4.31a** was isolated after flash chromatography on silica gel (P/EtOAc 7:3) as yellow oil (65 mg, 99% yield). **Molecular formula**: $C_{13}H_{15}ClO_2$. **Molecular mass**: 238.71 g mol^{-1}. **HPLC**: Chiralpak IB, Hexane/iPrOH 95:5, 1 mL/min, *anti* diastereomer (major) : τ_{major} = 9.6 min τ_{minor} = 11.2 min; *syn* diastereomer (minor) : τ_{major} = 8.4 min τ_{minor} = 8.1 min. **dr:** *anti*/*syn* 74:26 (from NMR analysis); *anti*/*syn* 76:24 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.18 (m, 1H, CH_{ar}), 7.09 (m, *2*H, CH_{ar}), 7.03 (m, 1H, CH_{ar}), 4.63 (d, J = 8.5 Hz, 1H, C*H*Ph), 3.41 (br, 1H, OH), 2.44 (m, 1H), 2.28 (m, 1H), 2.22 (m, 1H), 1.90 (m, 1H), 1.55 (m, 3H), 1.42 (m, 1H), 1.15 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 214.5,

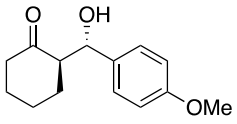
143.4, 134.0, 129.4, 127.7, 127.0, 125.3, 73.7, 57.1, 41.8, 30.5, 26.9, 24.4 ppm; HRMS (ESI): Calcd for $C_{13}H_{16}ClO_2^+$ ([M+H]$^+$) 239.0833. Found 239.0835.

(R)-2-((S)-(2-chlorophenyl)(hydroxy)methyl)cyclohexan-1-one **(4.32a)**



The product **4.32a** was isolated after flash chromatography on silica gel (P/EtOAc 7:3) as yellowish oil (62 mg, 98% yield). **Molecular formula**: $C_{13}H_{15}ClO_2$. **Molecular mass**: 238.71 g mol^{-1}. **HPLC**: Chiralpak IB, Hexane/iPrOH 95:5, 1 mL/min, *anti* diastereomer (major) : τ_{major} = 8.9 min τ_{minor} = 9.8 min; *syn* diastereomer (minor) : τ_{major} = nd τ_{minor} = nd. **dr:** *anti*/*syn* 88:12 (from NMR analysis); *anti*/*syn* ≥99:1 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.53 (m, 1H, C*H*$_{ar}$), 7.29 (m, 2H, C*H*$_{ar}$), 7.18 (m, 1H, C*H*$_{ar}$), 5.33 (d, *J* = 8.2 Hz, 1H, C*H*Ph), 3.93 (br, 1H, OH), 2.66 (m, 1H), 2.43 (m, 1H), 2.32 (m, 2H), 2.06 (m, 1H), 1.81 (m, 1H), 1.54 (m, 2H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 215.2, 139.1, 132.9, 128.7, 128.5, 127.2, 126.6, 70.4, 57.6, 42.7, 30.4, 27.8, 24.9 ppm; HRMS (ESI): Calcd for $C_{13}H_{16}ClO_2^+$ ([M+H]$^+$) 239.0833. Found 239.0834.

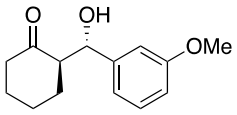
(R)-2-((S)-hydroxy(4-methoxyphenyl)methyl)cyclohexan-1-one **(4.33a)**



The product **4.33a** was isolated after flash chromatography on silica gel (P/EtOAc 6:4) as orange oil (35 mg, 17% yield). **Molecular formula**: $C_{14}H_{18}O_3$. **Molecular mass**: 234.30 g mol^{-1}. **HPLC**: Chiralpak IA, Hexane/iPrOH 95:5, 1 mL/min, *anti* diastereomer (major) : τ_{major} = 32.0 min τ_{minor} = 30.9 min; *syn* diastereomer (minor) : τ_{major} = 17.1 τ_{minor} = 20.0. **dr:** *anti*/*syn* 87:13 (from NMR analysis); *anti*/*syn* 94:6 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.23 (m, 2H, C*H*$_{ar}$), 6.87 (m, 2H, CH$_{ar}$), 4.74 (d, *J* = 8.9 Hz, 1H, C*H*Ph), 3.79 (s, 3H, OCH_3), 2.59 (m, 1H), 2.47 (m, 1H), 2.35 (m, 1H), 2.08 (m, 1H), 1.77 (m, 1H), 1.65 (m, 1H), 1.58 (m, 2H), 1.51 (m, 1H), 1.26 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$)

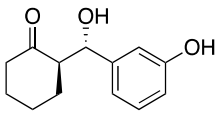
δ = 215.7, 159.3, 133.2, 128.2 (2C), 113.8 (2C), 74.3, 57.5, 55.3, 42.7, 30.8, 27.8, 24.7 ppm; HRMS (ESI): Calcd for $C_{14}H_{18}O_3Na^+$ ([M+Na]$^+$) 257.1148. Found 257.1151.

(R)-2-((S)-hydroxy(3-methoxyphenyl)methyl)cyclohexan-1-one **(4.34a)**



The product **4.34a** was isolated after flash chromatography on silica gel (P/EtOAc 6:4) as yellowish oil (66 mg, 77% yield). **Molecular formula**: $C_{14}H_{18}O_3$. **Molecular mass**: 234.30 g mol^{-1}. **HPLC**: Chiralpak IA, Hexane/iPrOH 95:5, 1 mL/min, *anti* diastereomer (major) : τ_{major} = 33.4 min τ_{minor} = 32.0 min; *syn* diastereomer (minor) : τ_{major} = 17.3 min τ_{minor} = 20.0 min. **dr:** *anti*/*syn* 69:31 (from NMR analysis); *anti*/*syn* 78:22 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.25 (m, 1H, C*H*$_{ar}$), 6.83 (m, 3H, C*H*$_{ar}$), 4.74 (d, *J* = 8.8 Hz, 1H, C*H*Ph), 3.78 (s, 3H, OCH_3), 2.57 (m, 1H), 2.44 (m, 1H), 2.33 (m, 1H), 2.03 (m, 1H), 1.88 (m, 1H), 1.62 (m, 3H), 1.25 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 215.5, 159.7, 142.6, 129.3, 119.5, 113.3, 112.4, 74.6, 57.4, 55.2, 42.6, 30.8, 27.8, 24.6 ppm; HRMS (ESI): Calcd for $C_{14}H_{18}O_3Na^+$ ([M+Na]$^+$) 257.1148. Found 257.1150.

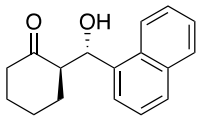
(R)-2-((S)-hydroxy(3-hydroxyphenyl)methyl)cyclohexan-1-one **(4.35a)**



The product **4.35a** was isolated after flash chromatography on silica gel (P/EtOAc 6:4) as brownish oil (25 mg, 40% yield). **Molecular formula**: $C_{13}H_{16}O_3$. **Molecular mass**: 220.27 g mol^{-1}. **HPLC**: Chiralpak IC, Hexane/iPrOH 9:1, 1.0 mL/min, *anti* diastereomer (major) : τ_{major} = 65.3 min τ_{minor} = 62.0 min; *syn* diastereomer (minor) : τ_{major} = 44.4 min τ_{minor} = 48.4 min. **dr:** *anti*/*syn* 97:3 (from NMR analysis); *anti*/*syn* 94:6 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.15 (m, 1H, C*H*$_{ar}$), 6.80 (m, 2H, CH$_{ar}$), 6.72 (m, 2H, CH$_{ar}$), 4.74 (d, J = 8.8 Hz, 1H, C*H*Ph), 2.60 (m, 1H), 2.47 (m, 1H), 2.35 (m, 1H), 2.08 (m, 1H), 1.77 (m, 1H), 1.67 (m, 1H), 1.55 (m, 2H), 1.30 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 216.0,

156.1, 142.2, 129.5, 119.4, 115.2, 113.8, 74.8, 57.1, 42.6, 30.8, 27.8, 24.6 ppm; HRMS (ESI): Calcd for $C_{13}H_{17}O_3^+$ ($[M+H]^+$) 221.1172. Found 221.1174.

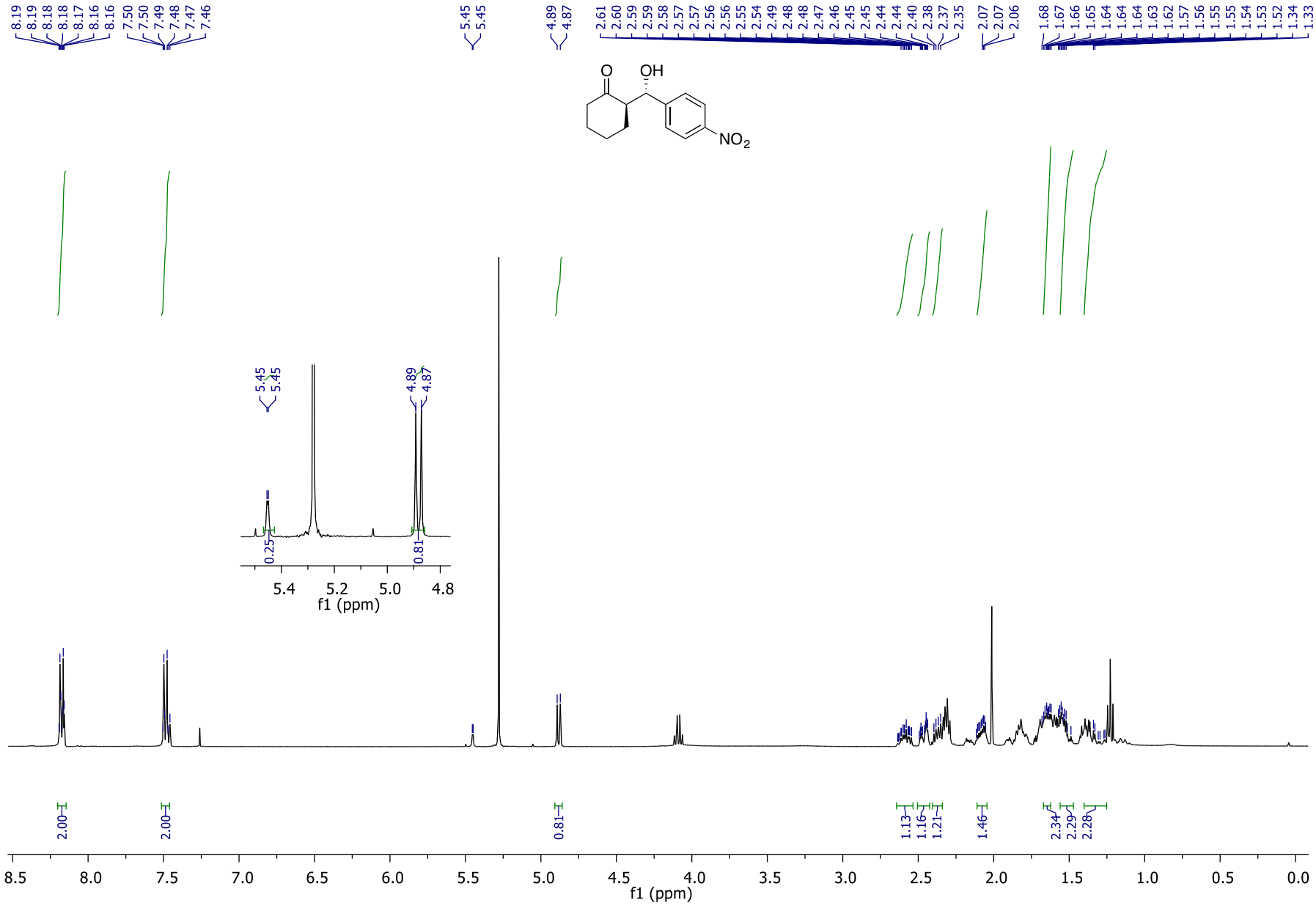
(R)-2-((S)-hydroxy(naphthalen-1-yl)methyl)cyclohexan-1-one **(4.36a)**

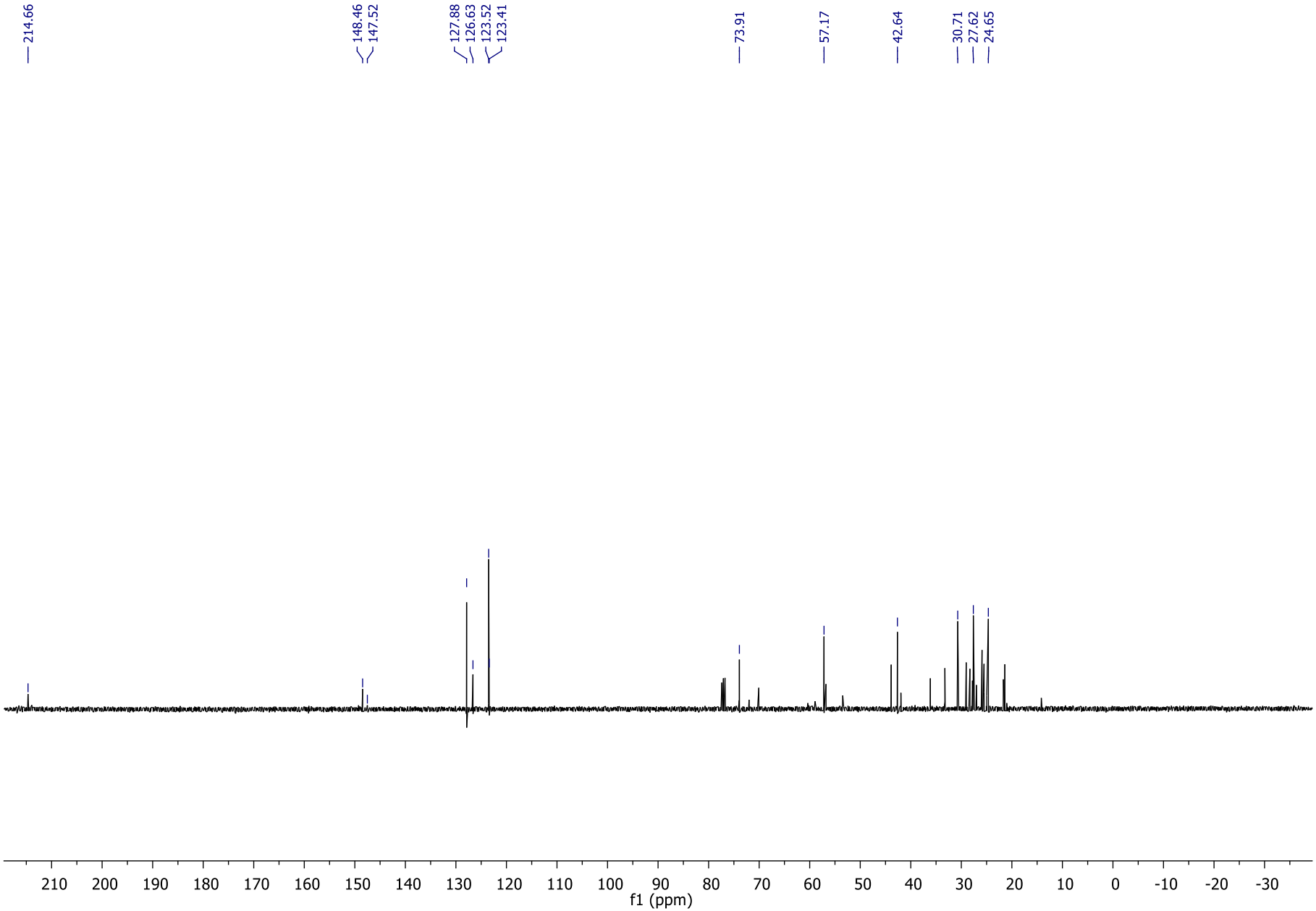


The product **4.36a** was isolated after flash chromatography on silica gel (P/EtOAc 7:3) as brown oil (31 mg, 38% yield). **Molecular formula**: $C_{17}H_{18}O_2$. **Molecular mass**: 254.33 g mol^{-1}. **HPLC**: Chiralpak IA, Hexane/iPrOH 95:5, 1 mL/min, *anti* diastereomer (major) : τ_{major} = 26.5 min τ_{minor} = 32.5 min; *syn* diastereomer (minor) : τ_{major} = 14.9 min τ_{minor} = 12.7 min. **dr:** *anti*/*syn* 82:18 (from NMR analysis); *anti*/*syn* 64:36 (from HPLC analysis). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 8.25 (m, 1H, CH_{ar}), 7.87 (m, 1H, CH_{ar}), 7.78 (m, 1H, CH_{ar}), 7.56 (m, 1H, CH_{ar}), 7.48 (m, 3H, CH_{ar}), 5.58 (d, J = 8.8 Hz, 1H, C*H*Ph), 2.99 (m, 1H), 2.51 (m, 1H), 2.40 (m, 1H), 2.07 (m, 1H), 1.70 (m, 4H), 1.36 (m, 1H) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 215.8, 136.8, 133.9, 131.4, 128.9, 128.4, 126.0, 125.5, 125.4, 123.9, 122.4, 72.1, 57.4, 42.8, 31.4, 27.9, 24.9 ppm; HRMS (ESI): Calcd for $C_{17}H_{19}O_2^+$ ($[M+H]^+$) 255.1380. Found 255.1383.

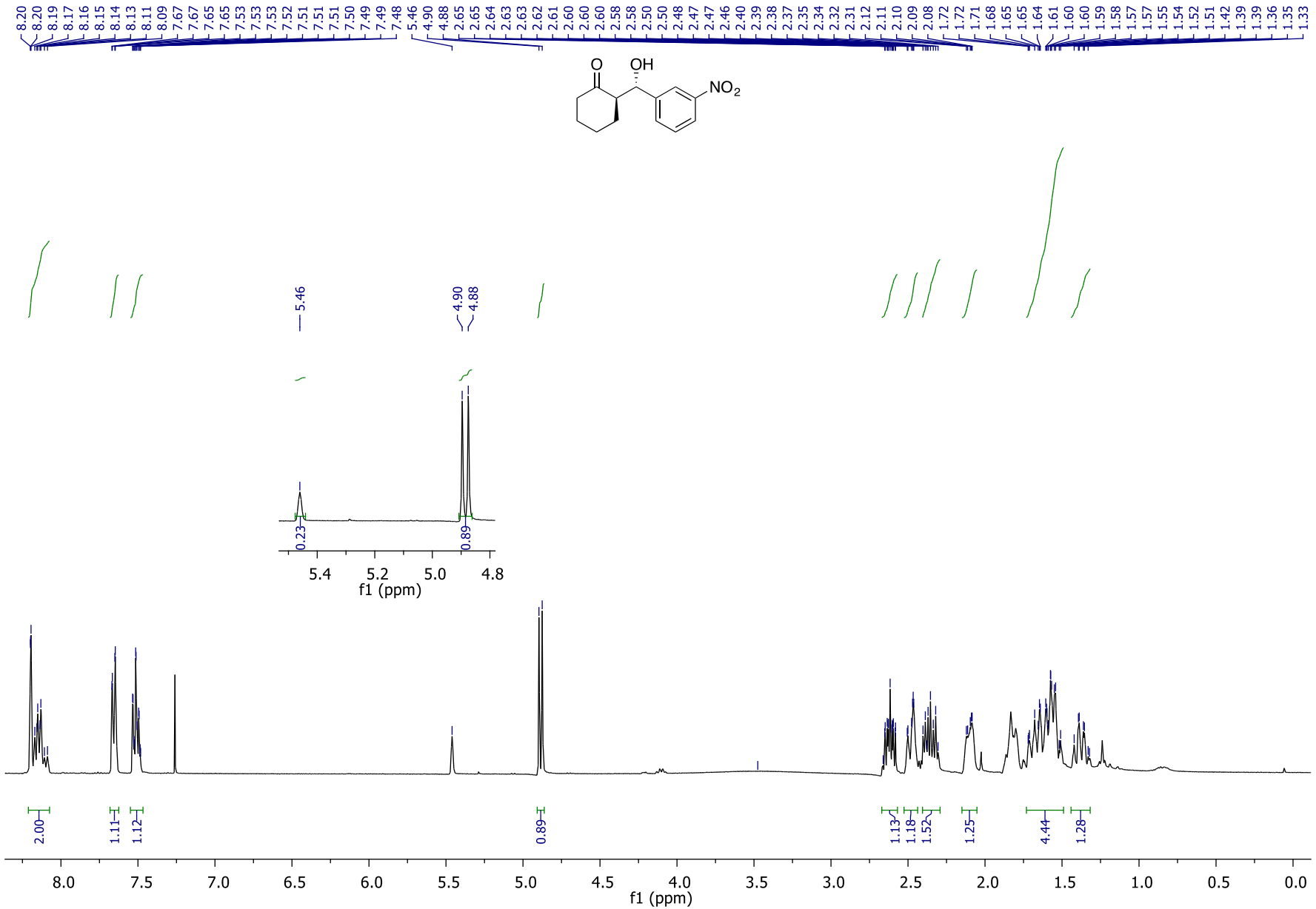
NMR Spectra (two diastereomers: *anti*>*syn*)

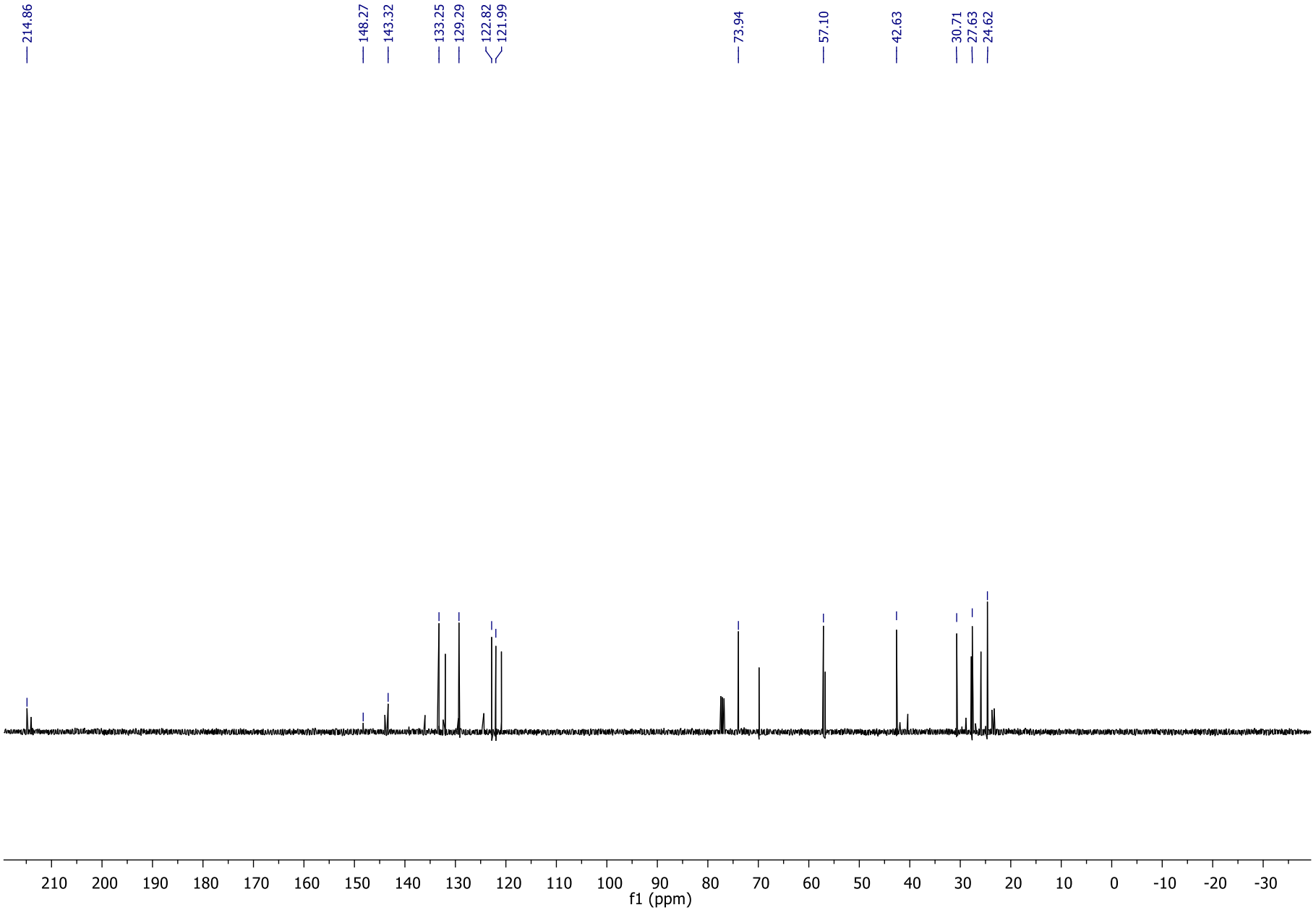
(R)-2-((S)-hydroxy(4-nitrophenyl)methyl)cyclohexan-1-one (4.23a)



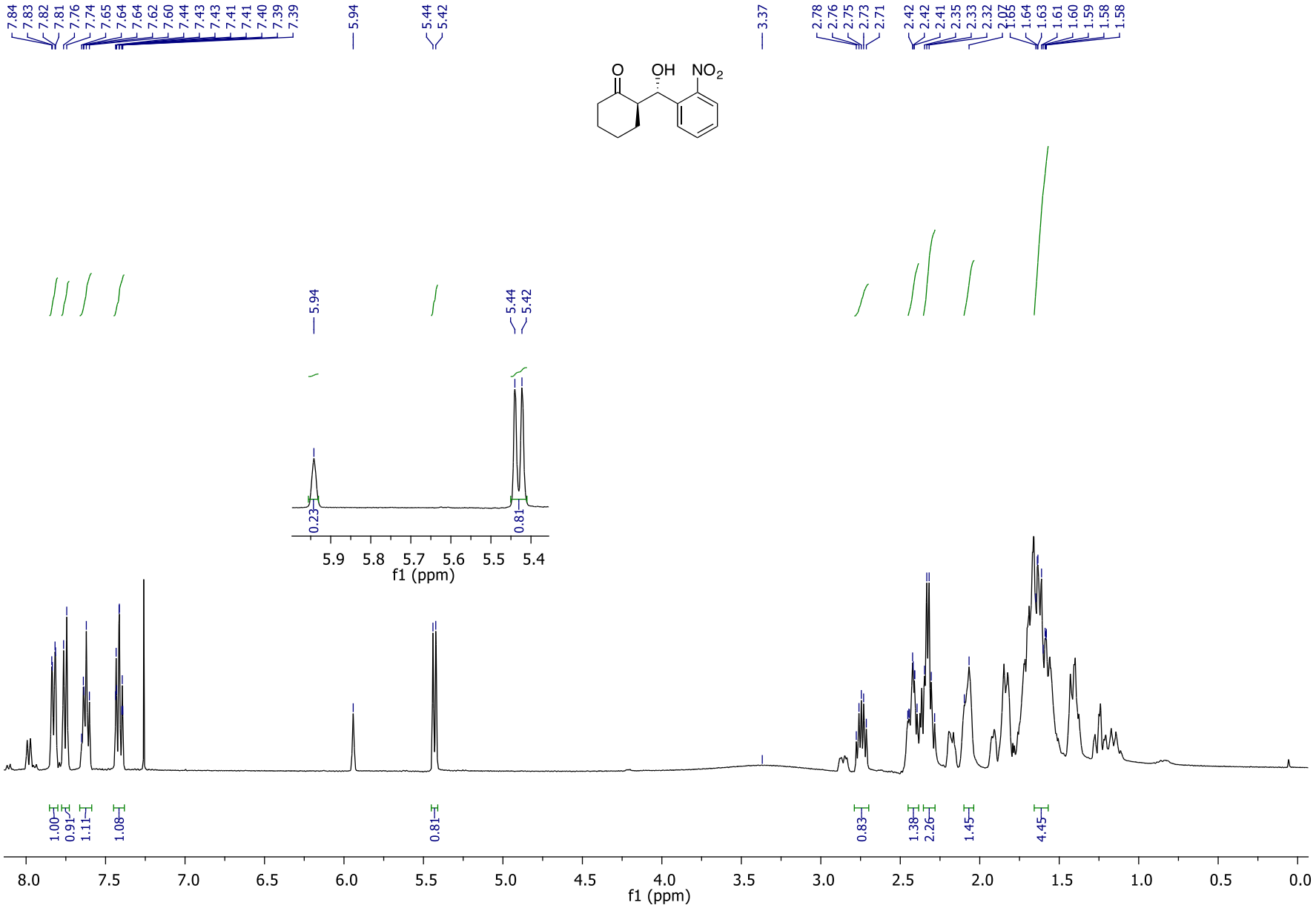

214.66
148.46
147.52
127.88
126.63
123.52
123.41
73.91
57.17
42.64
30.71
27.62
24.65
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30
f1 (ppm)

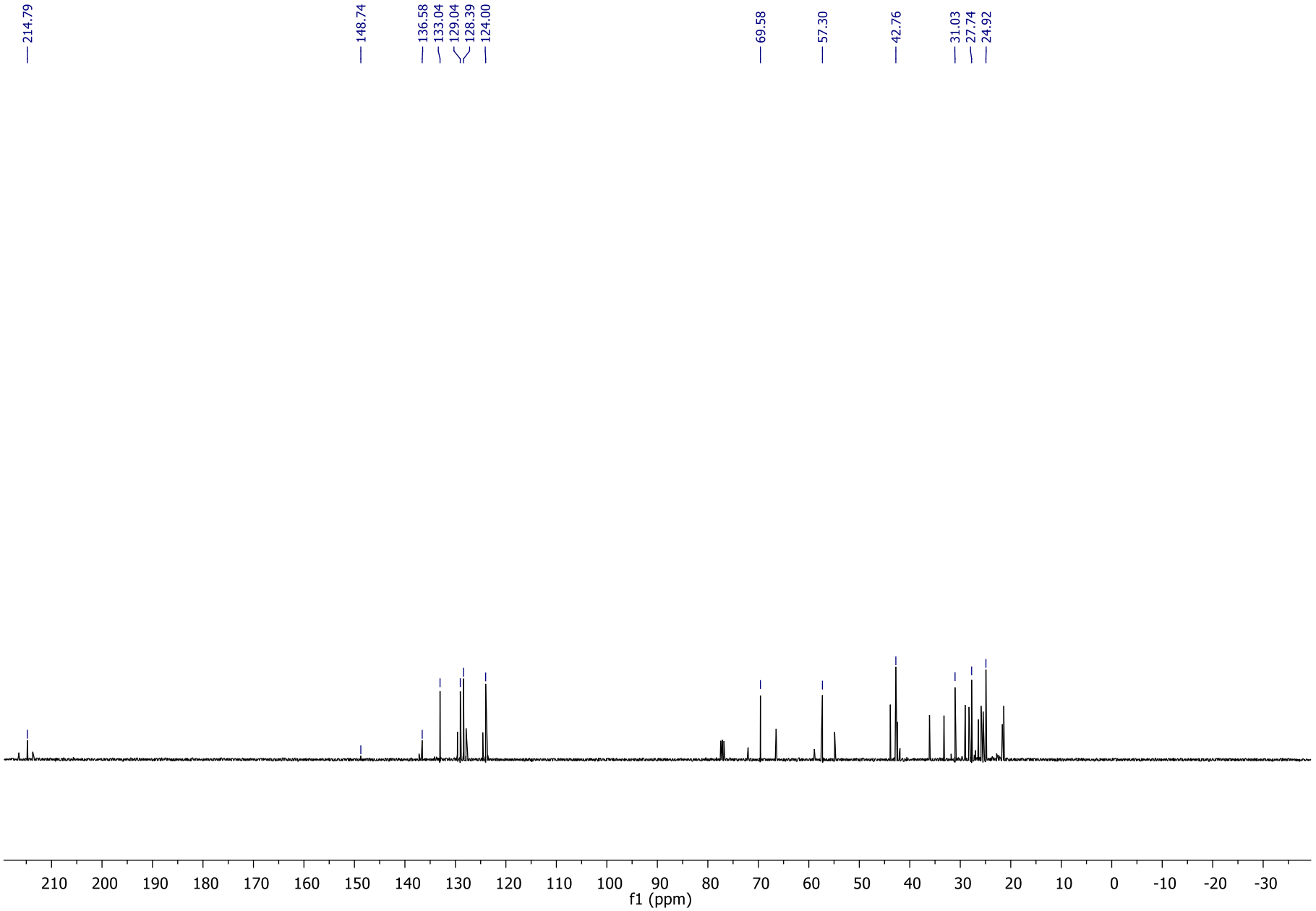
(R)-2-((S)-hydroxy(3-nitrophenyl)methyl)cyclohexan-1-one (4.25a)



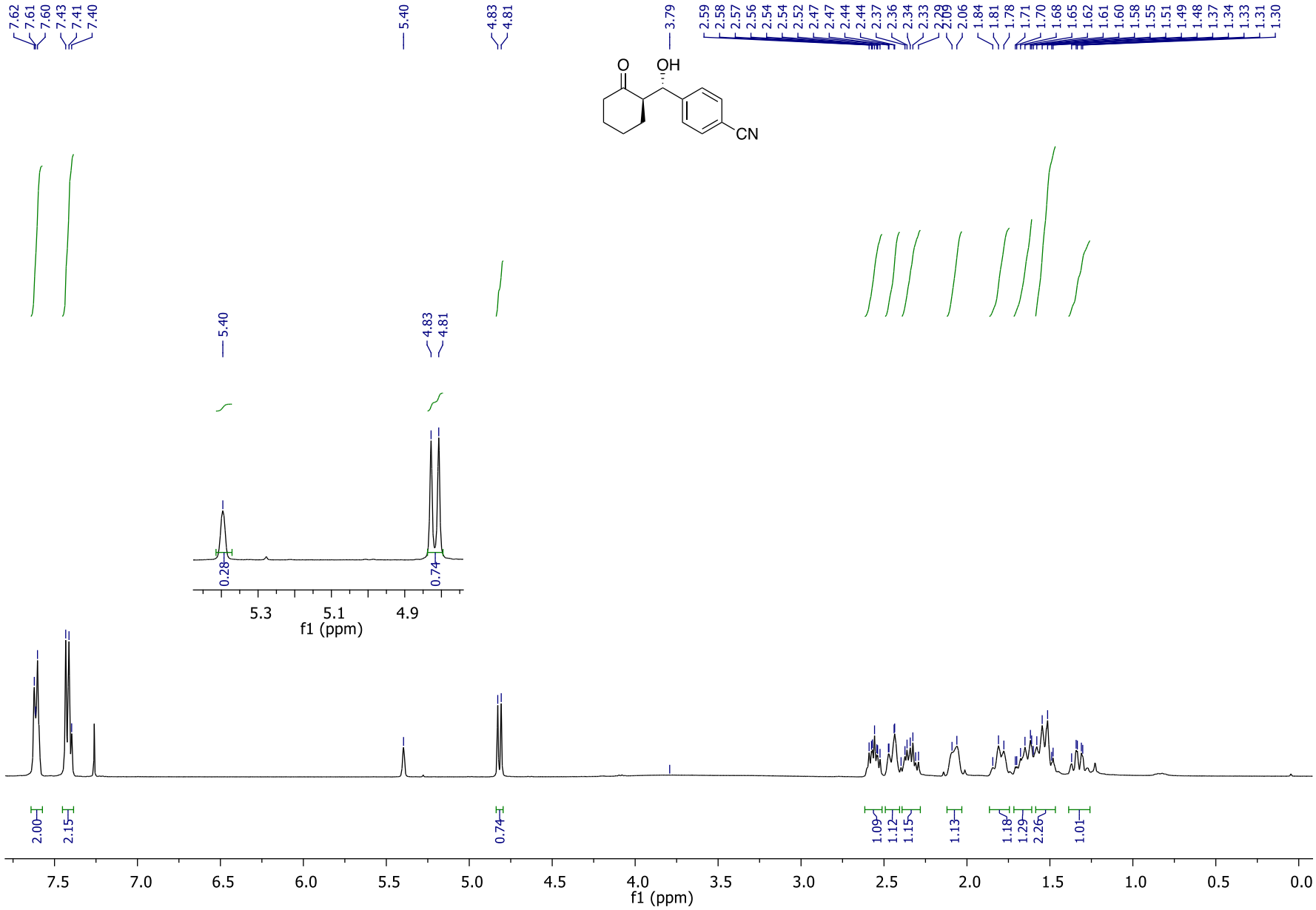

214.86
148.27
143.32
133.25
129.29
122.82
121.99
73.94
57.10
42.63
30.71
27.63
24.62
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30
f1 (ppm)

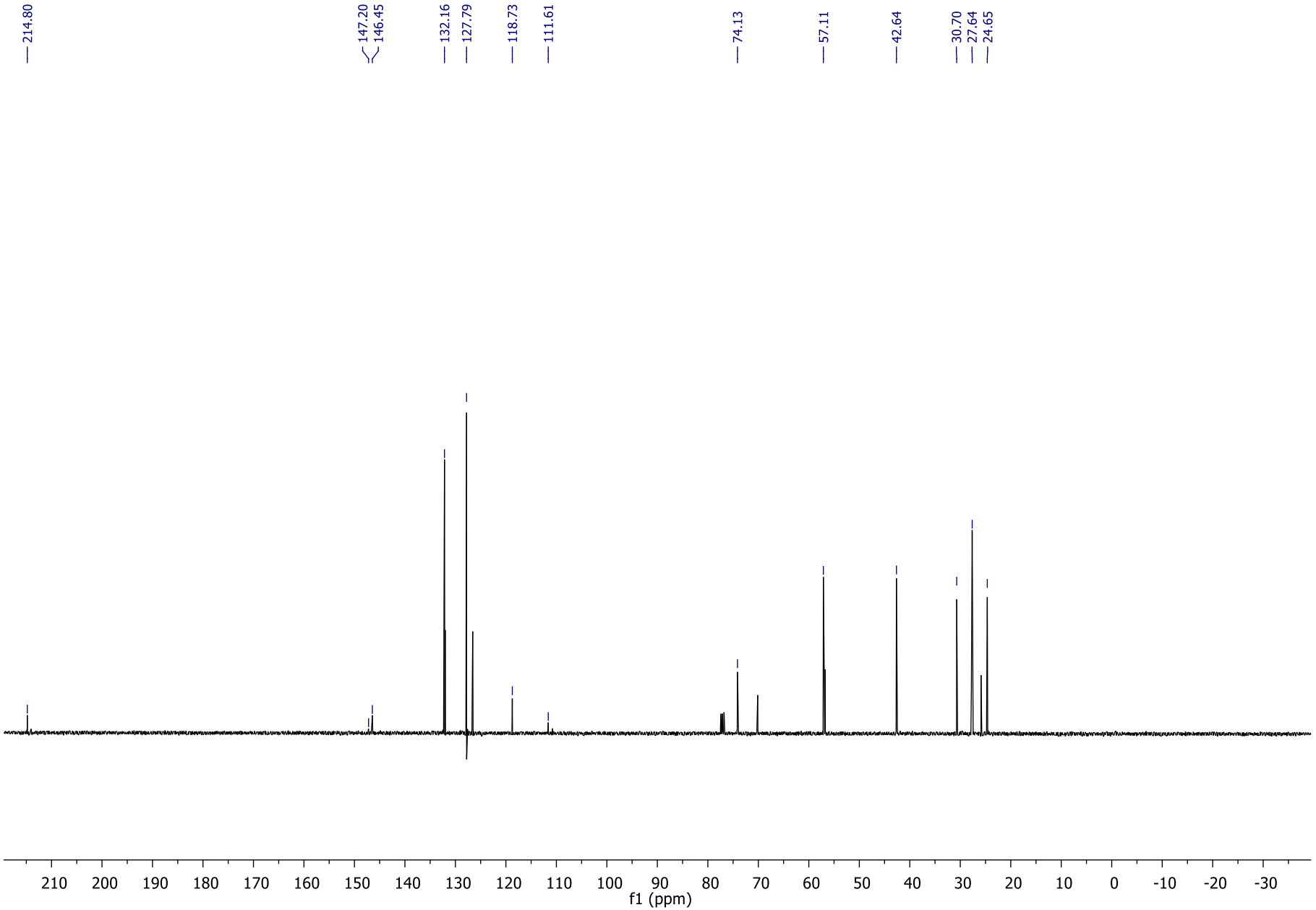
(R)-2-((S)-hydroxy(2-nitrophenyl)methyl)cyclohexan-1-one (4.24a)



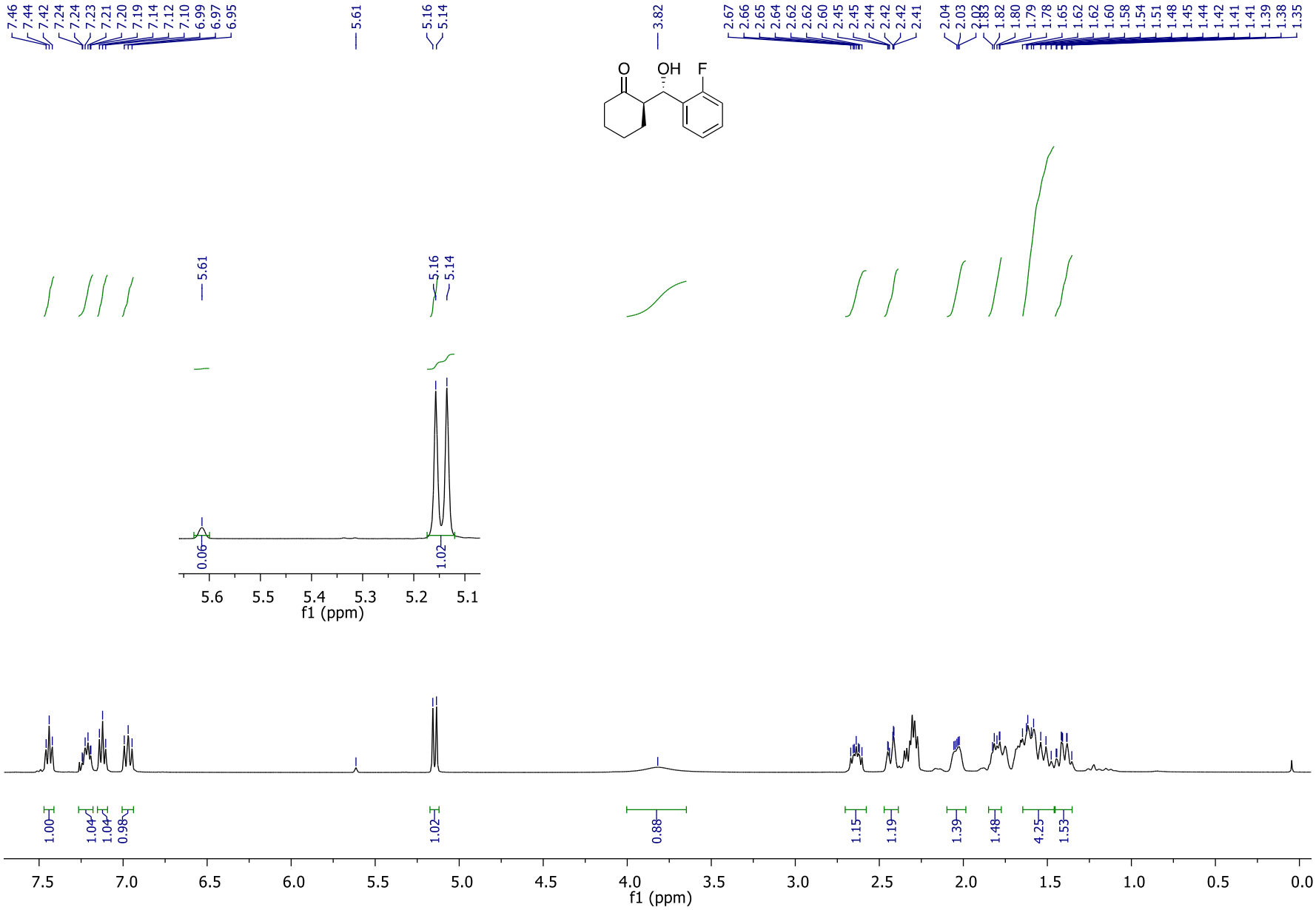

214.79
148.74
136.58
133.04
129.04
128.39
124.00
69.58
57.30
42.76
31.03
27.74
24.92
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30
f1 (ppm)

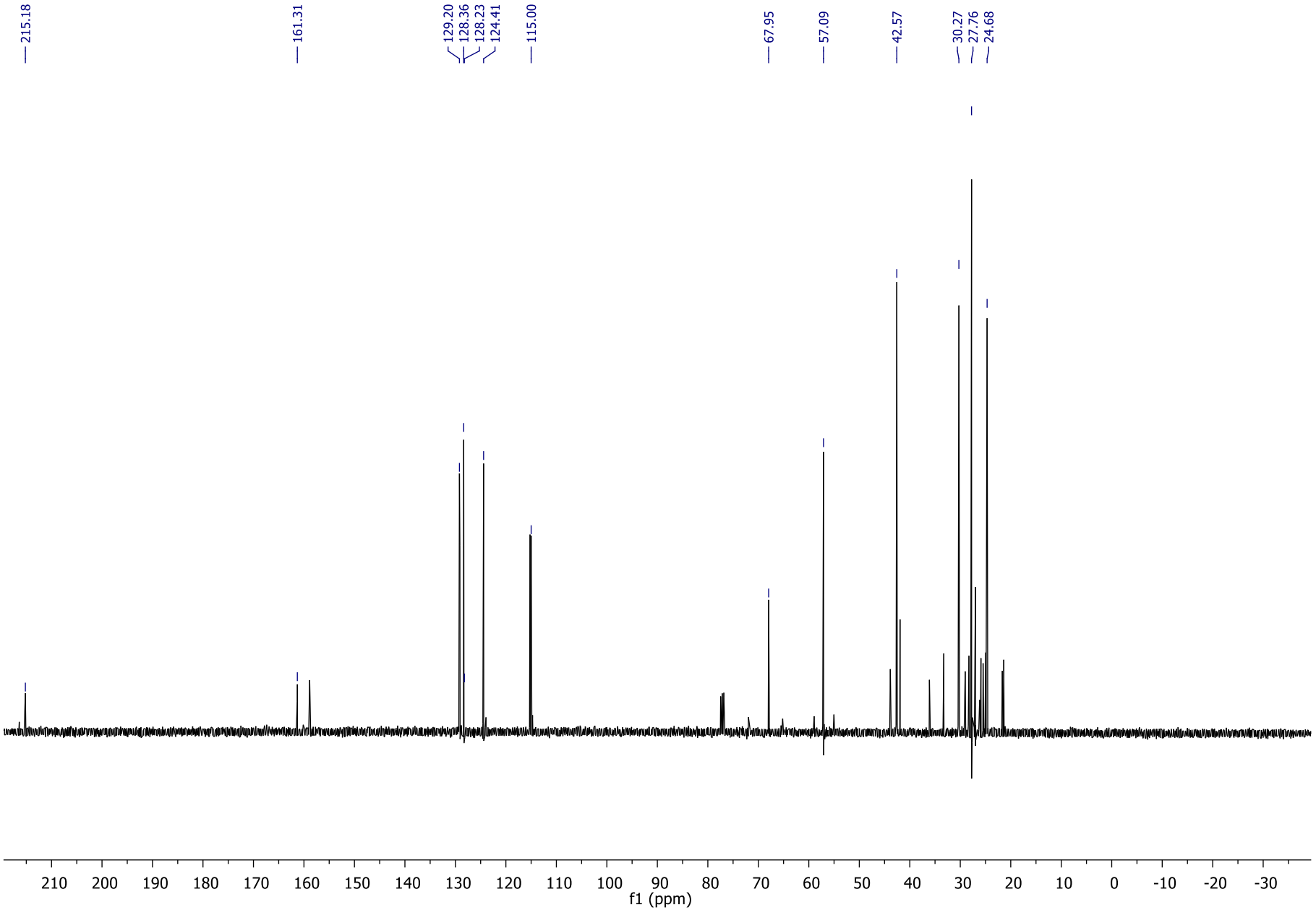
4-((S)-hydroxy((R)-2-oxocyclohexyl)methyl)benzonitrile (4.26a)



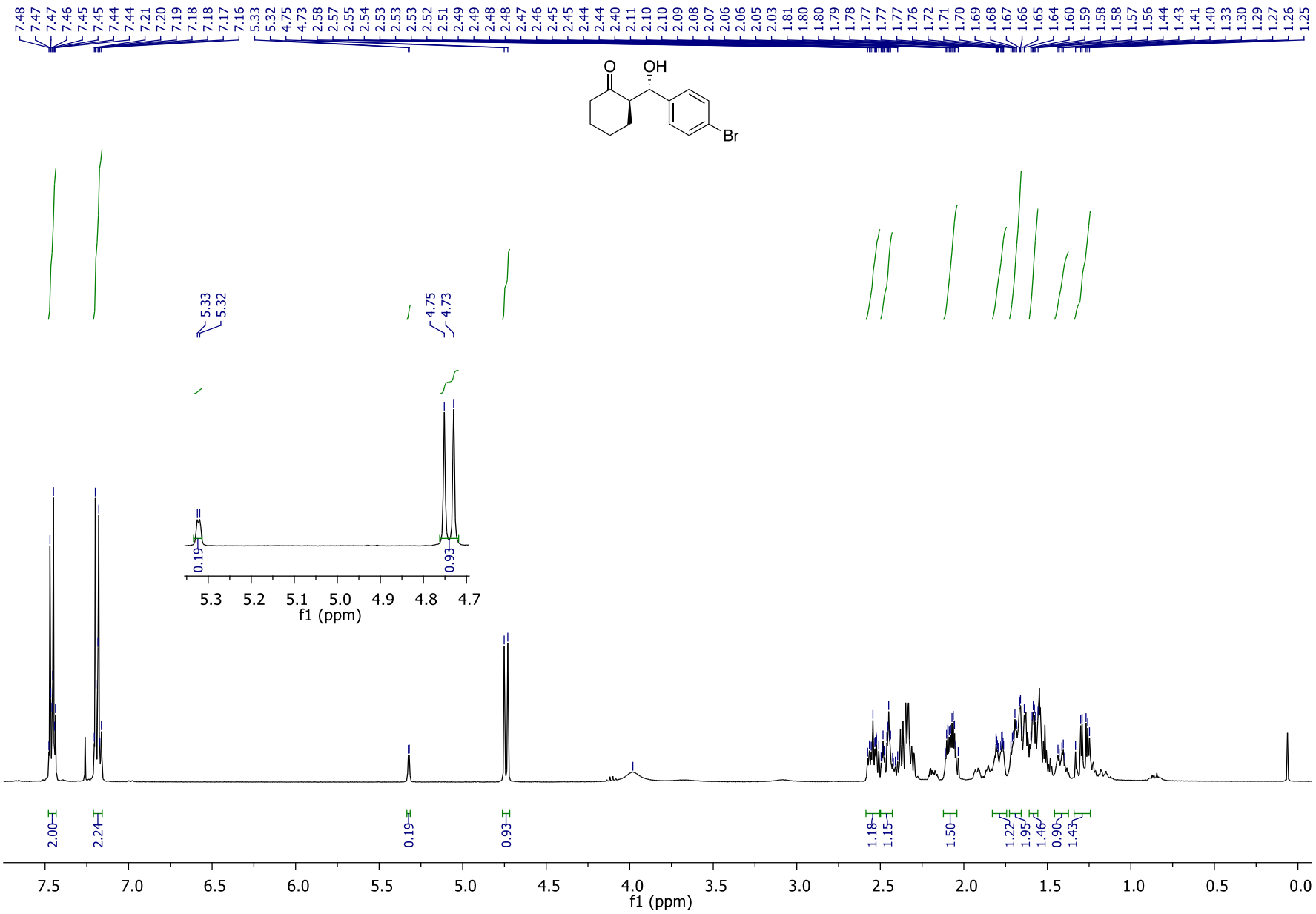

214.80
147.20
146.45
132.16
127.79
118.73
111.61
74.13
57.11
42.64
30.70
27.64
24.65
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30
f1 (ppm)

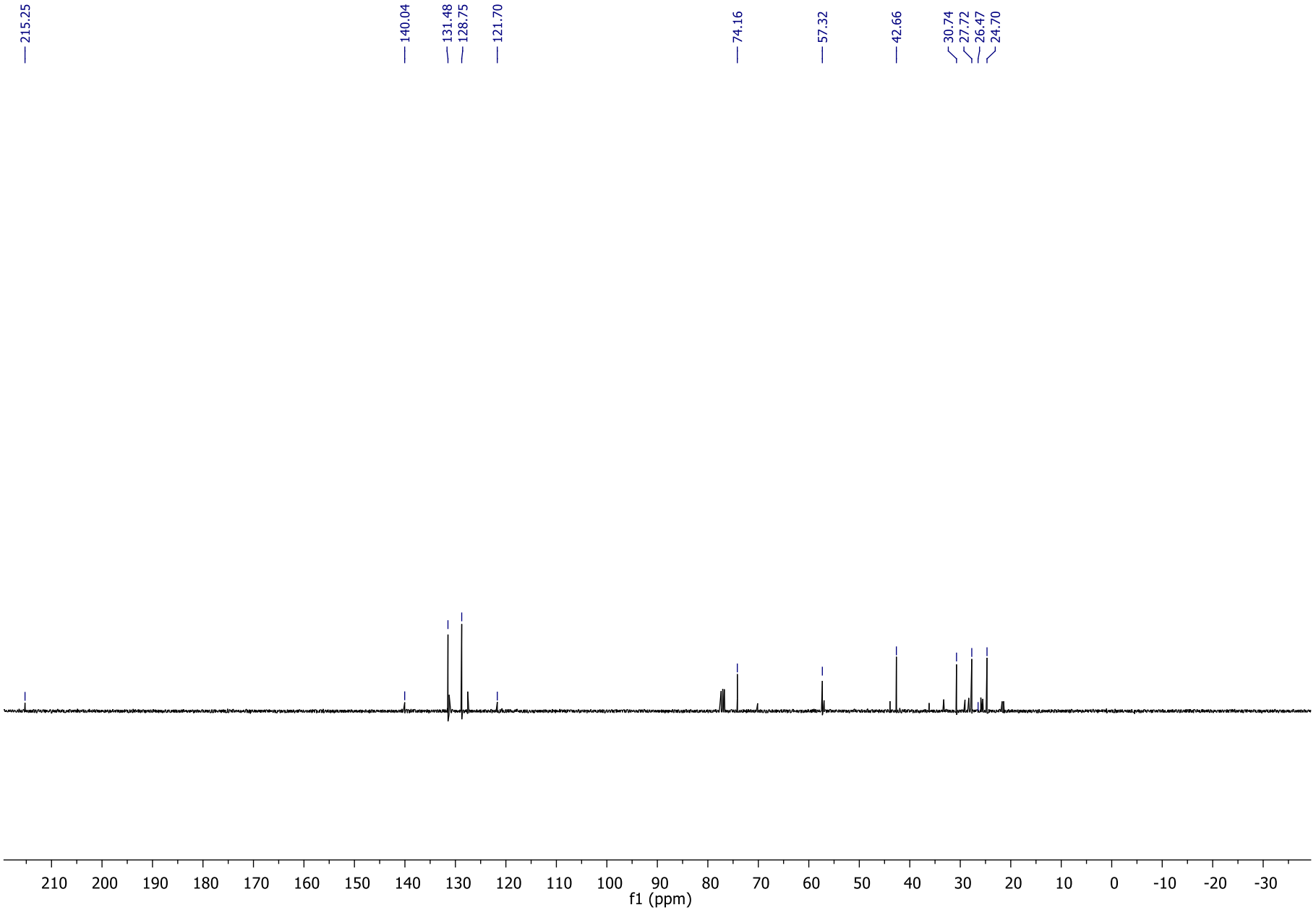
(R)-2-((S)-(2-fluorophenyl)(hydroxy)methyl)cyclohexan-1-one (4.27a)



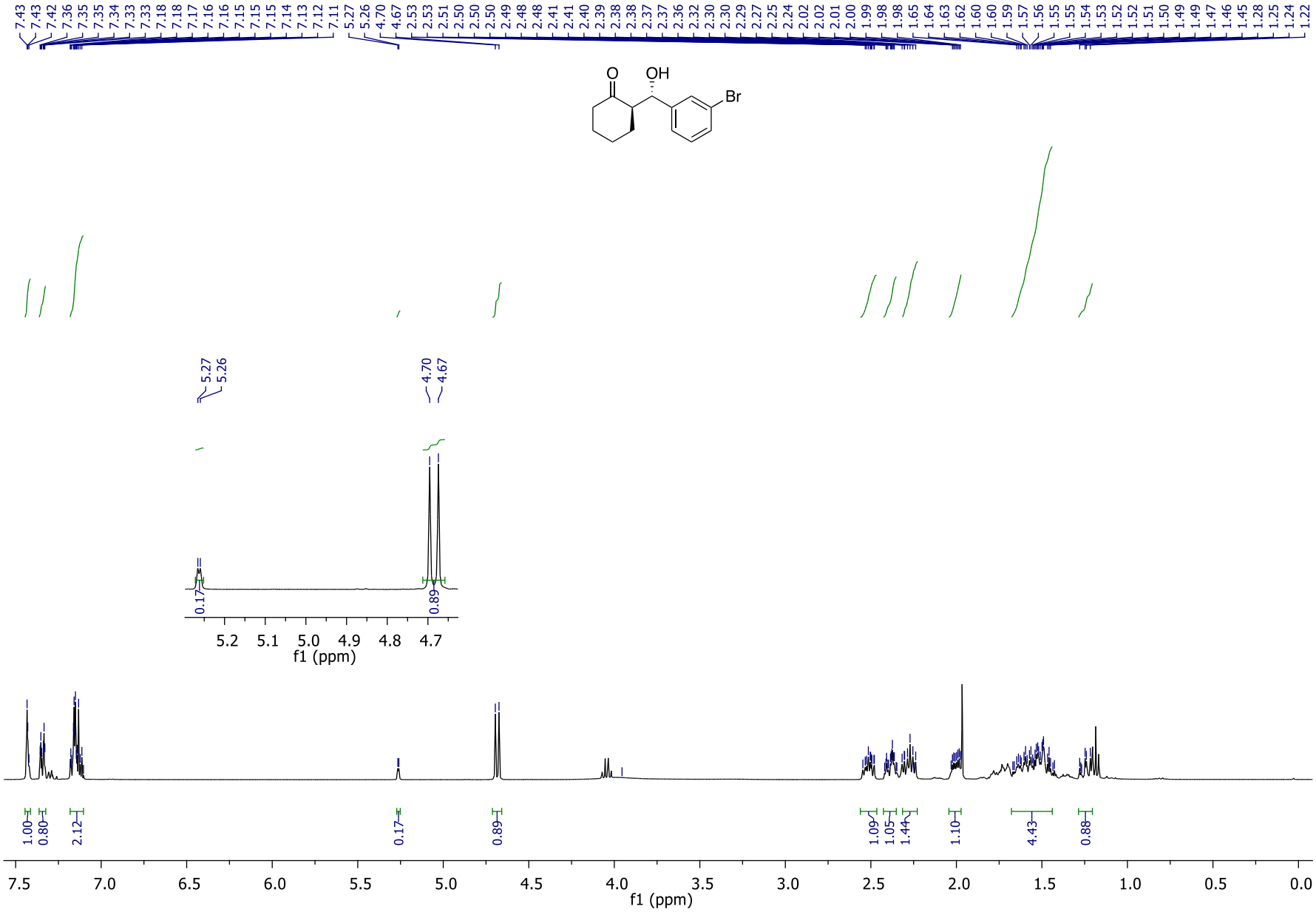

215.18
161.31
129.20
128.36
128.23
124.41
115.00
67.95
57.09
42.57
30.27
27.76
24.68
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30
f1 (ppm)

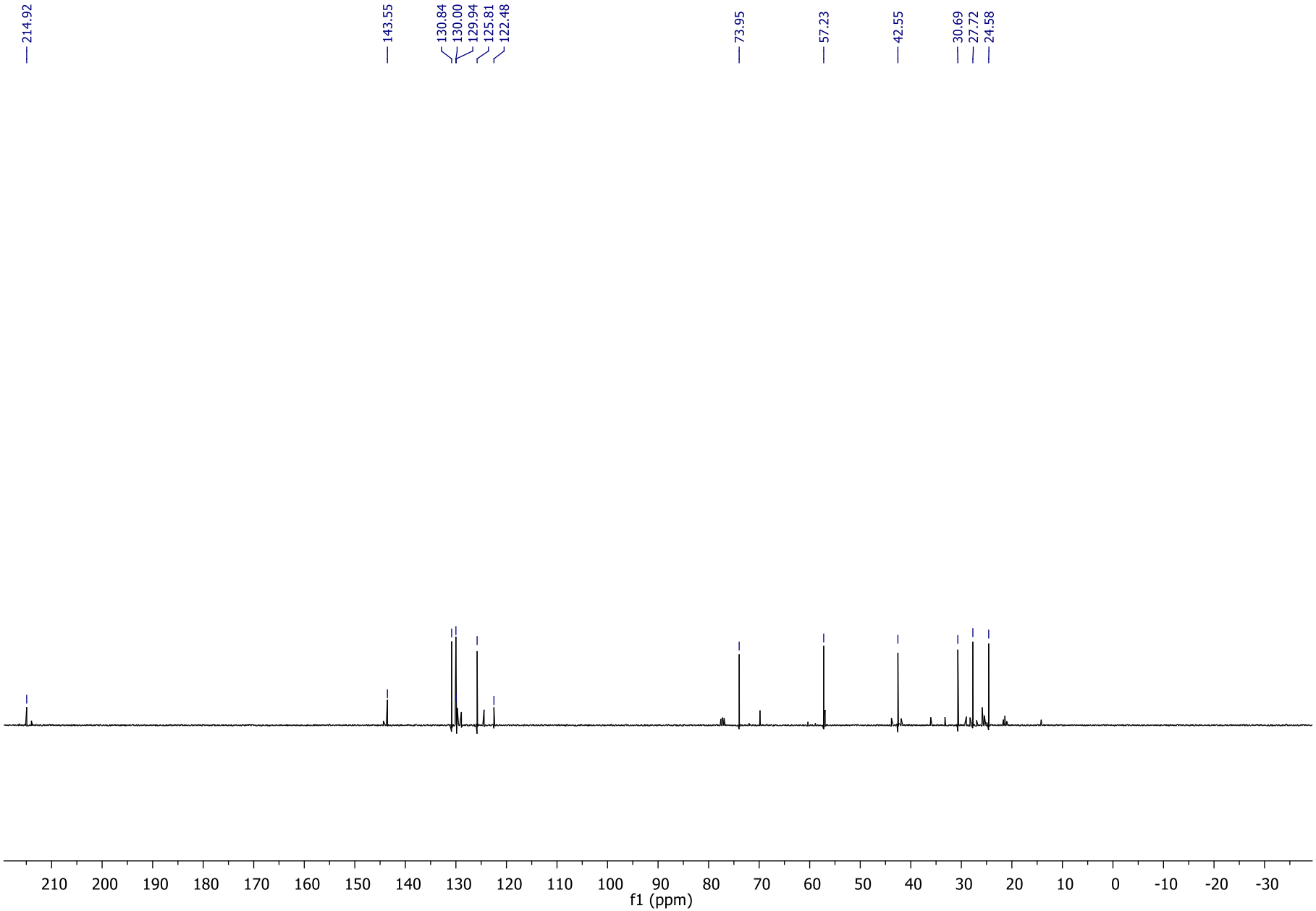
(R)-2-((S)-(4-bromophenyl)(hydroxy)methyl)cyclohexan-1-one (4.28a)



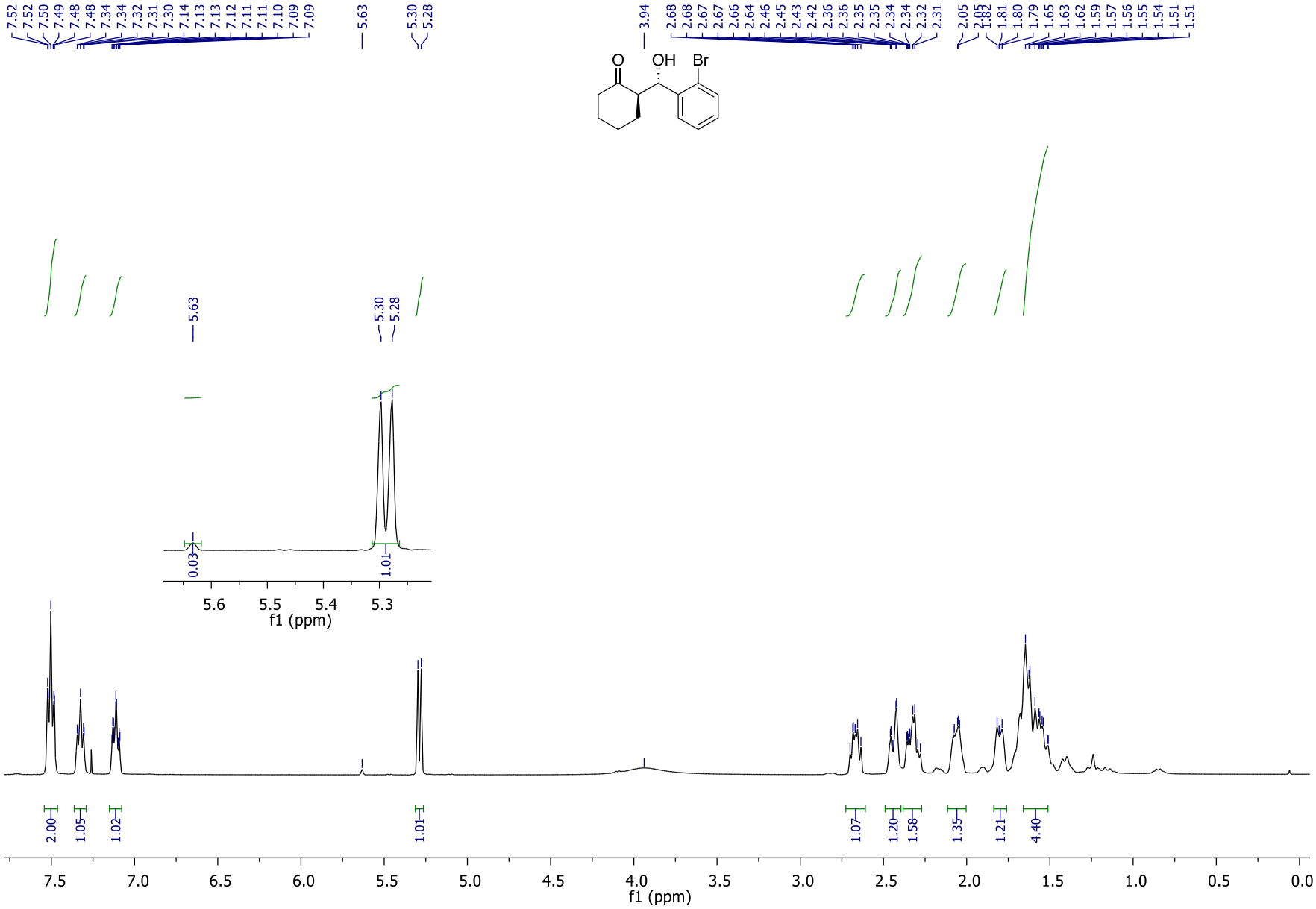

215.25
140.04
131.48
128.75
121.70
74.16
57.32
42.66
30.74
27.72
26.47
24.70
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30
f1 (ppm)

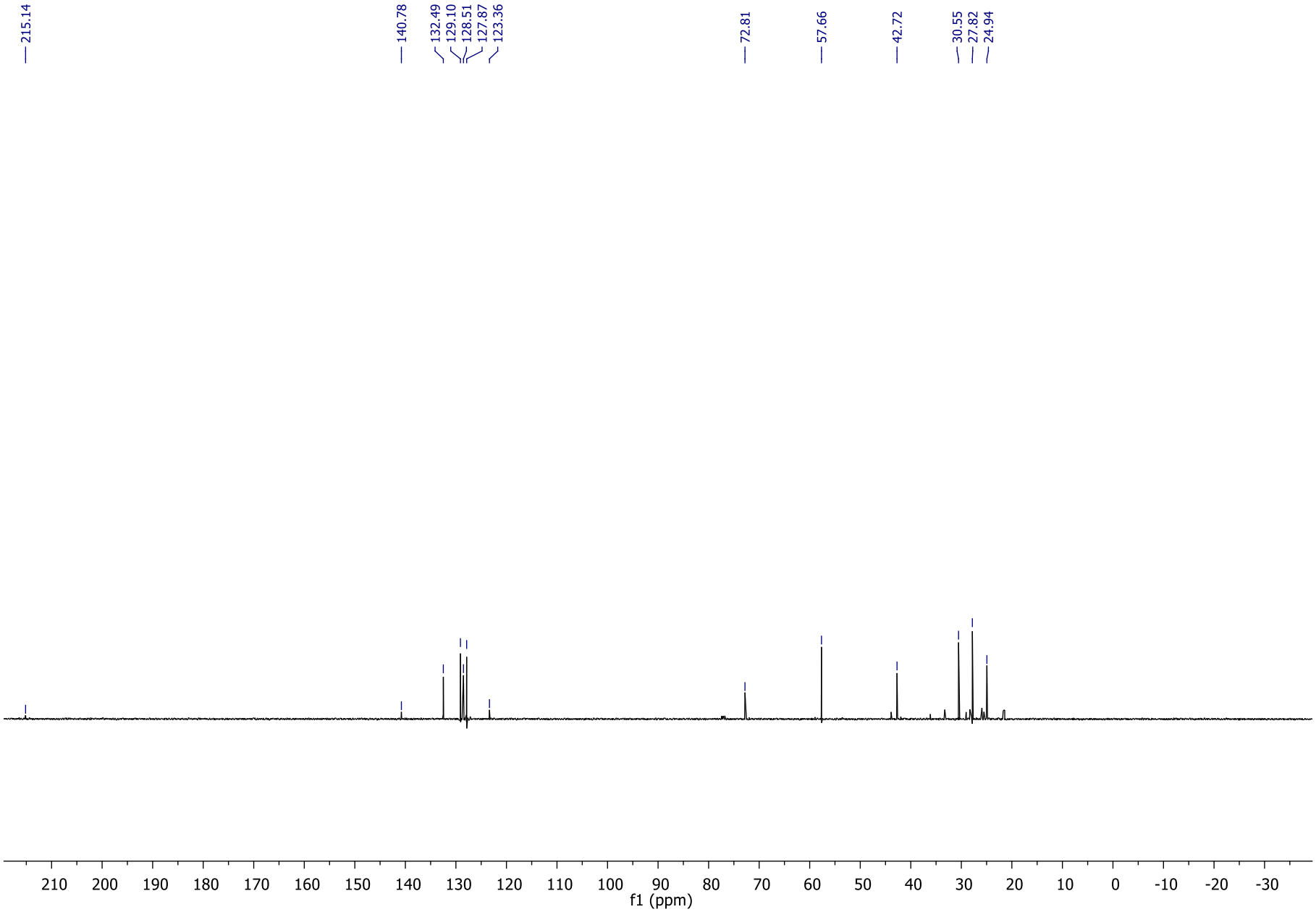
(R)-2-((S)-(3-bromophenyl)(hydroxy)methyl)cyclohexan-1-one (4.29a)



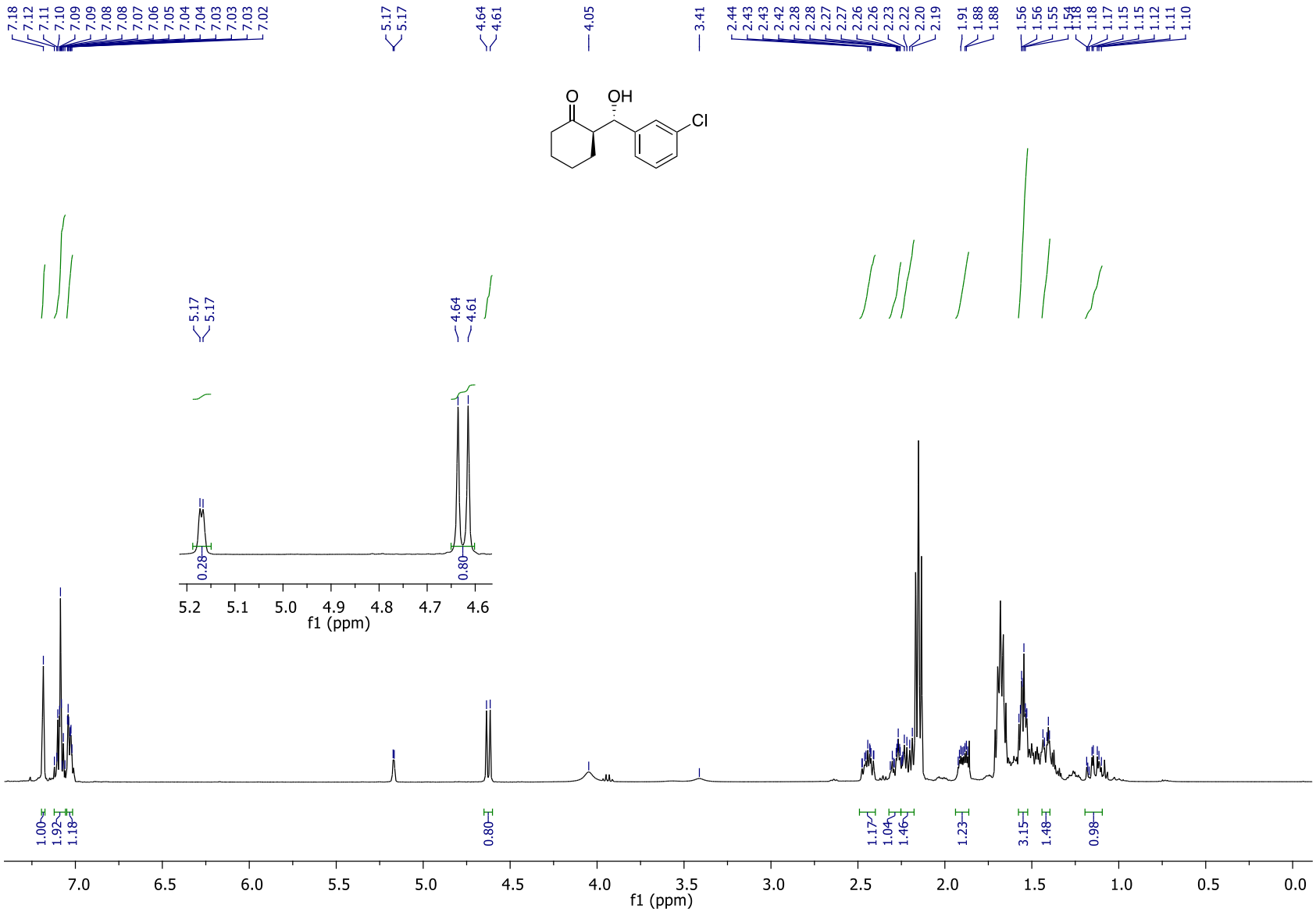

214.92
143.55
130.84
130.00
129.94
125.81
122.48
73.95
57.23
42.55
30.69
27.72
24.58
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30
f1 (ppm)

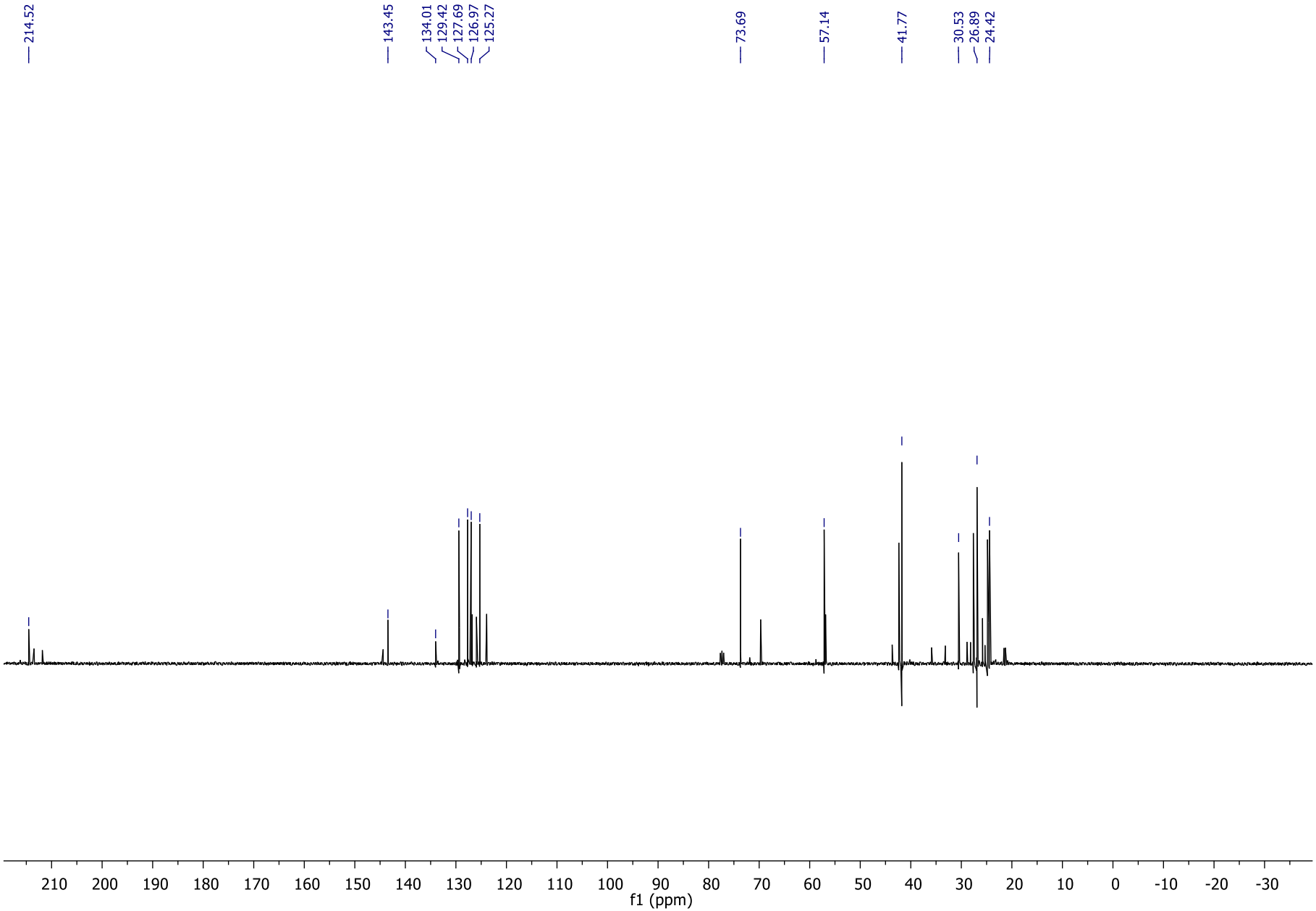
(R)-2-((S)-(2-bromophenyl)(hydroxy)methyl)cyclohexan-1-one (4.30a)



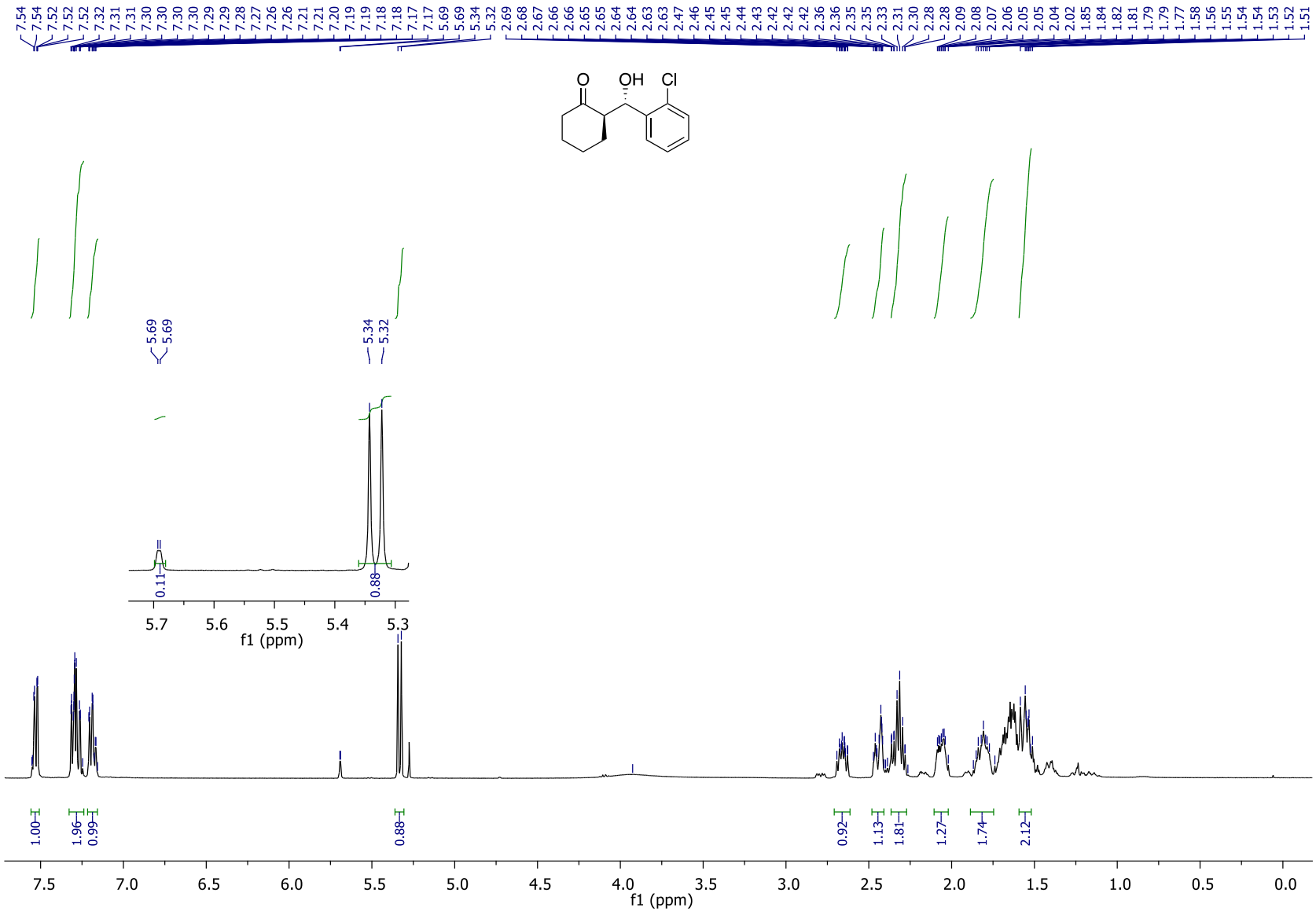

215.14
140.78
132.49
129.10
128.51
127.87
123.36
72.81
57.66
42.72
30.55
27.82
24.94
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30
f1 (ppm)

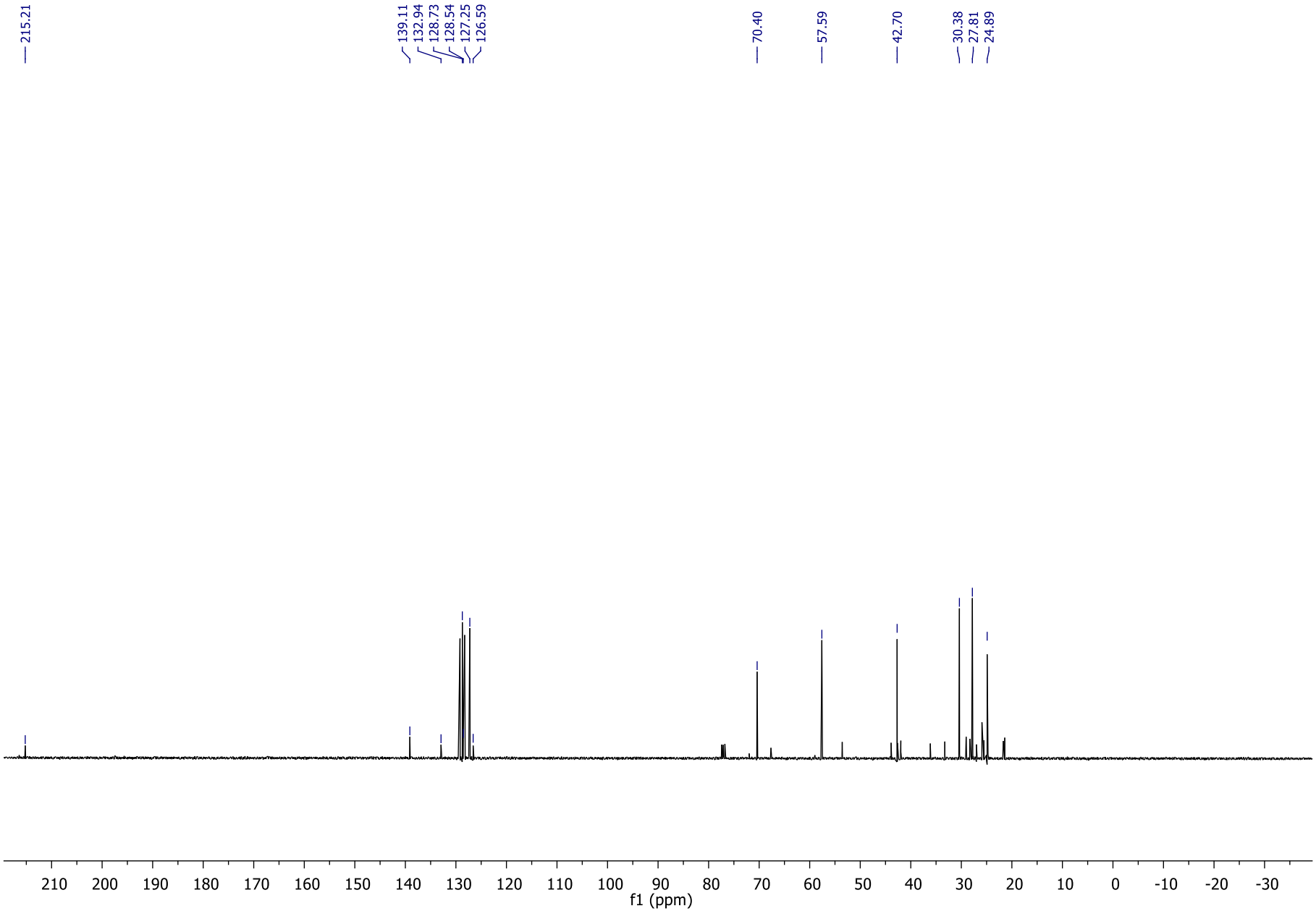
(R)-2-((S)-(3-chlorophenyl)(hydroxy)methyl)cyclohexan-1-one (4.31a)



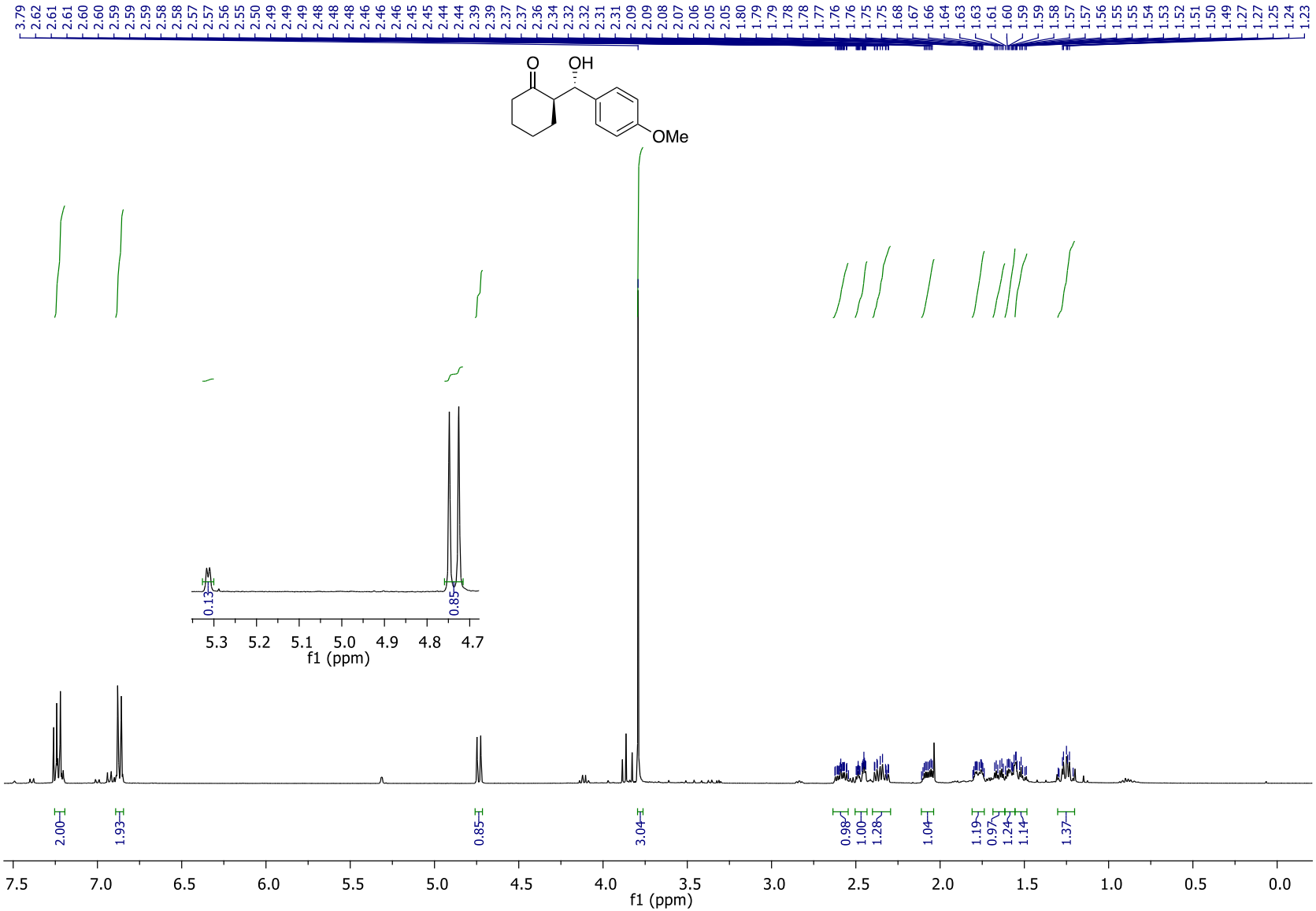

214.52
143.45
134.01
129.42
127.69
126.97
125.27
73.69
57.14
41.77
30.53
26.89
24.42
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30
f1 (ppm)

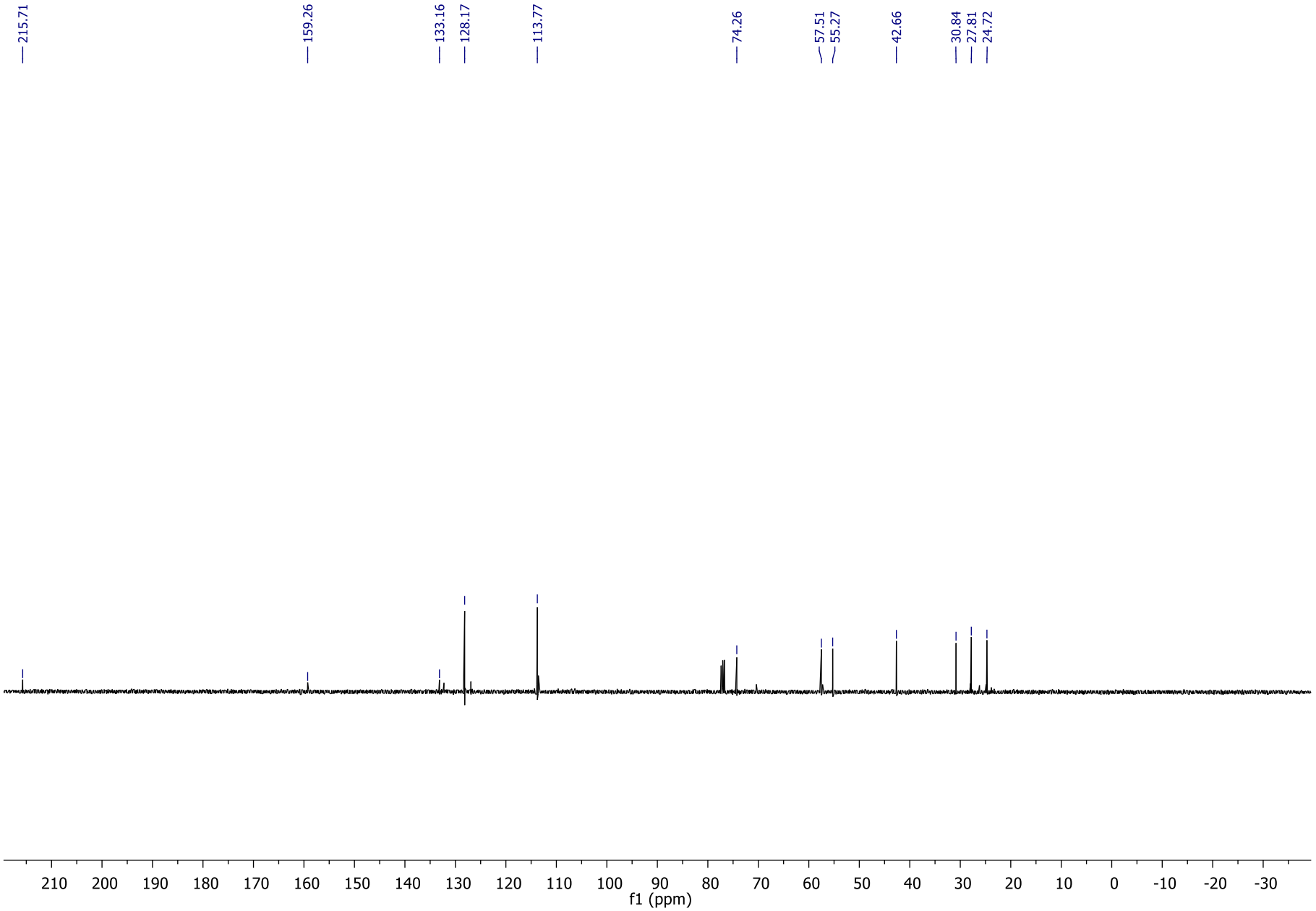
(R)-2-((S)-(2-chlorophenyl)(hydroxy)methyl)cyclohexan-1-one (4.32a)



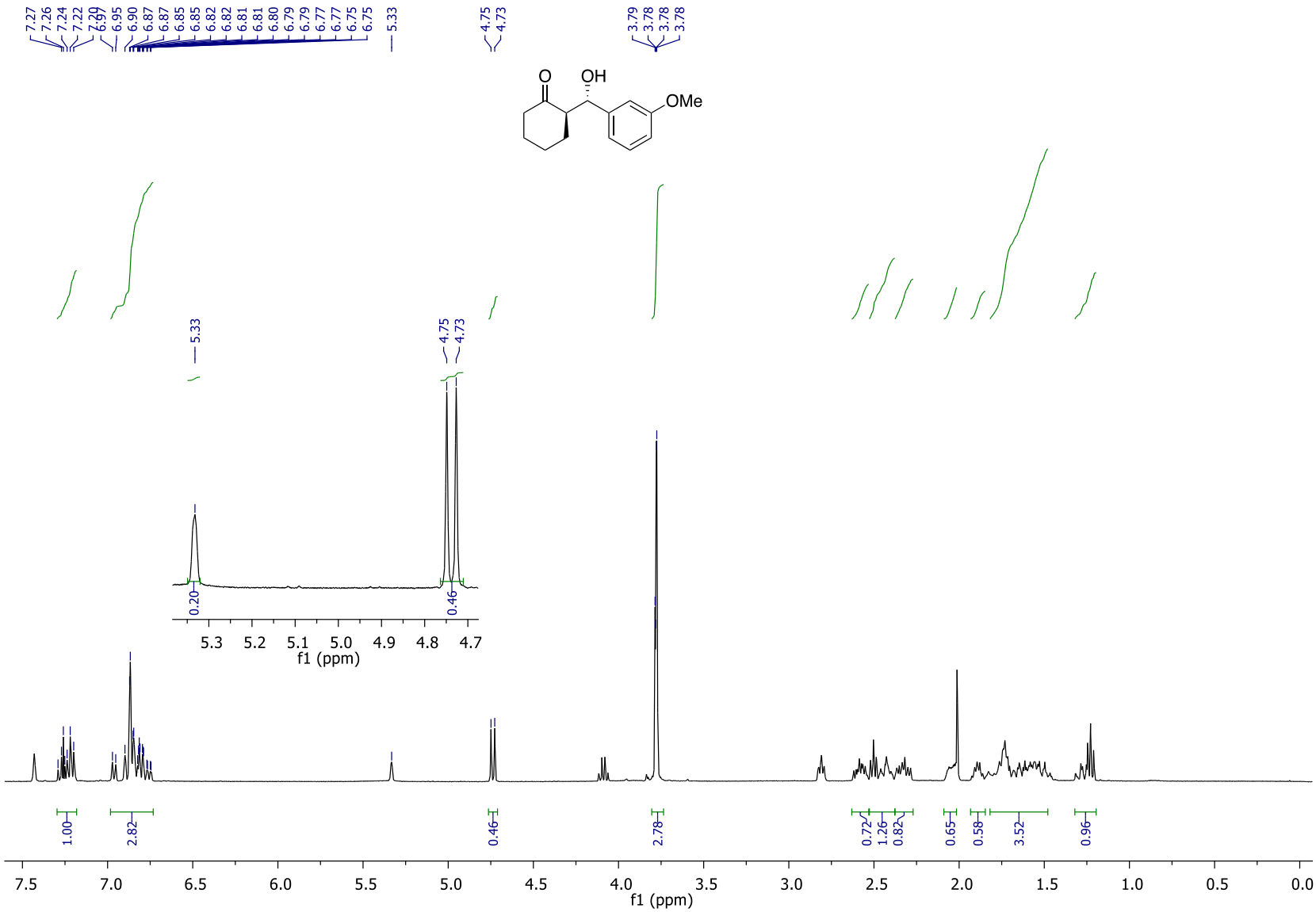

215.21
139.11
132.94
128.73
128.54
127.25
126.59
70.40
57.59
42.70
30.38
27.81
24.89
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30
f1 (ppm)

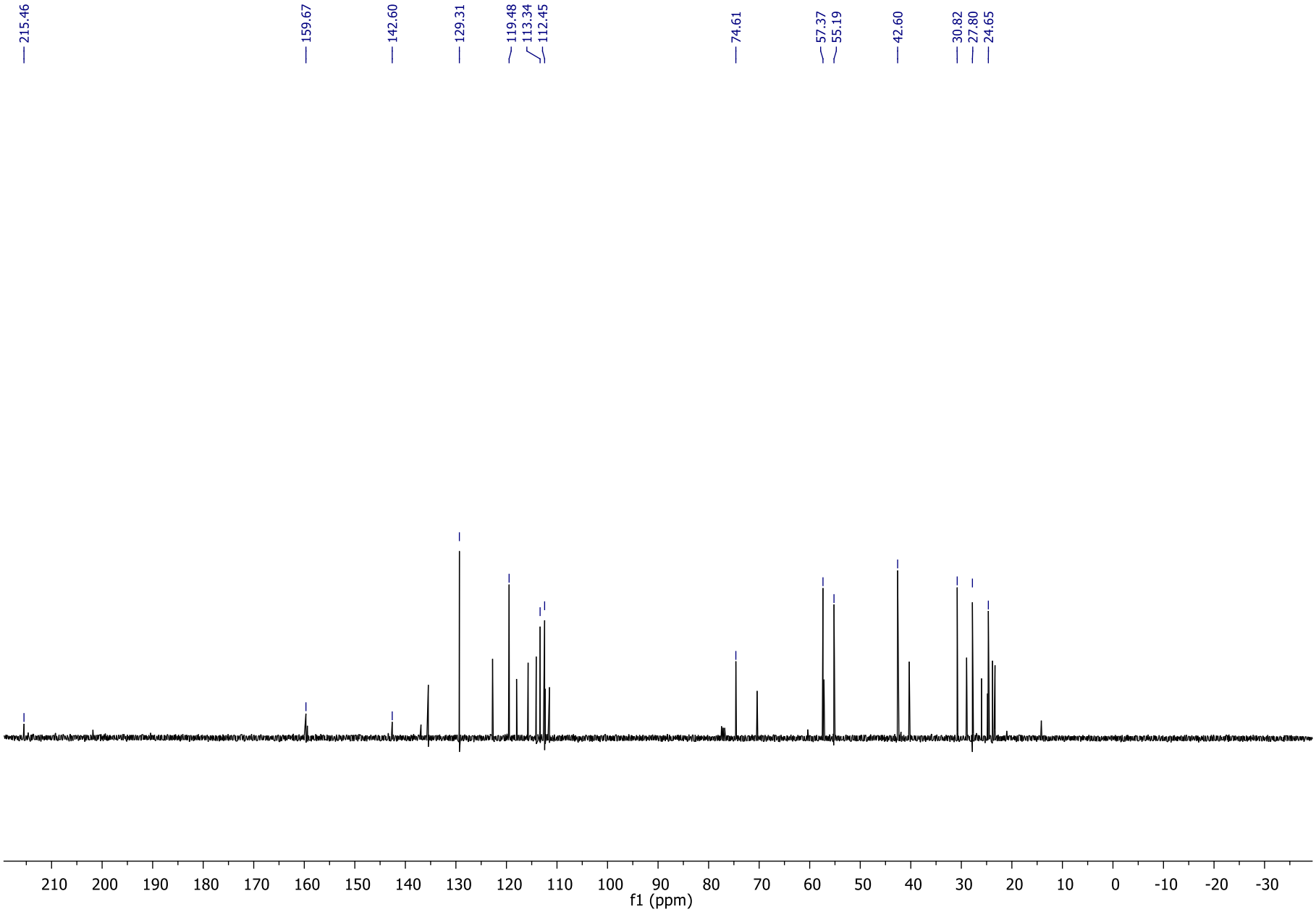
(R)-2-((S)-hydroxy(4-methoxyphenyl)methyl)cyclohexan-1-one (4.33a)



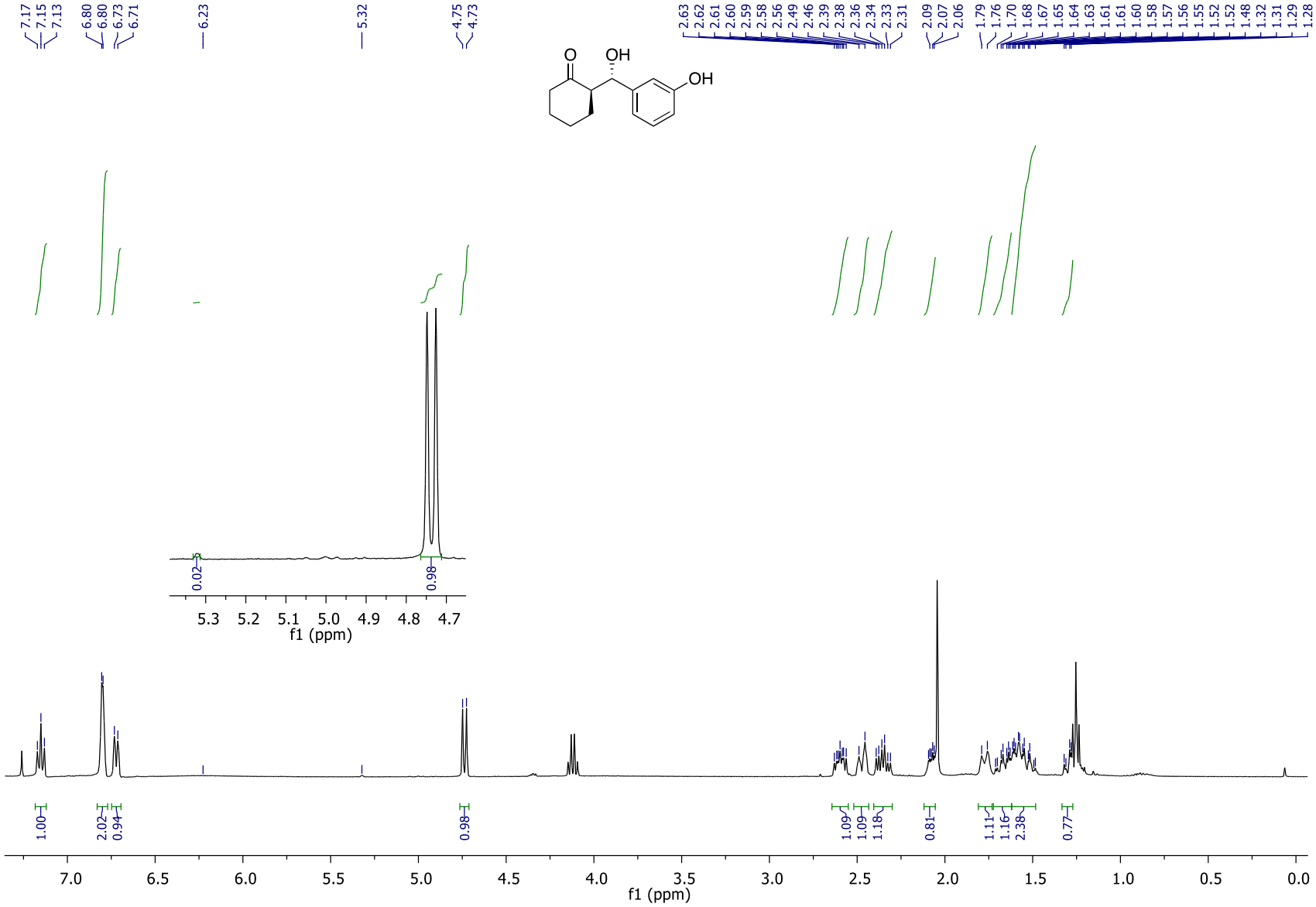

215.71
159.26
133.16
128.17
113.77
74.26
57.51
55.27
42.66
30.84
27.81
24.72
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30
f1 (ppm)

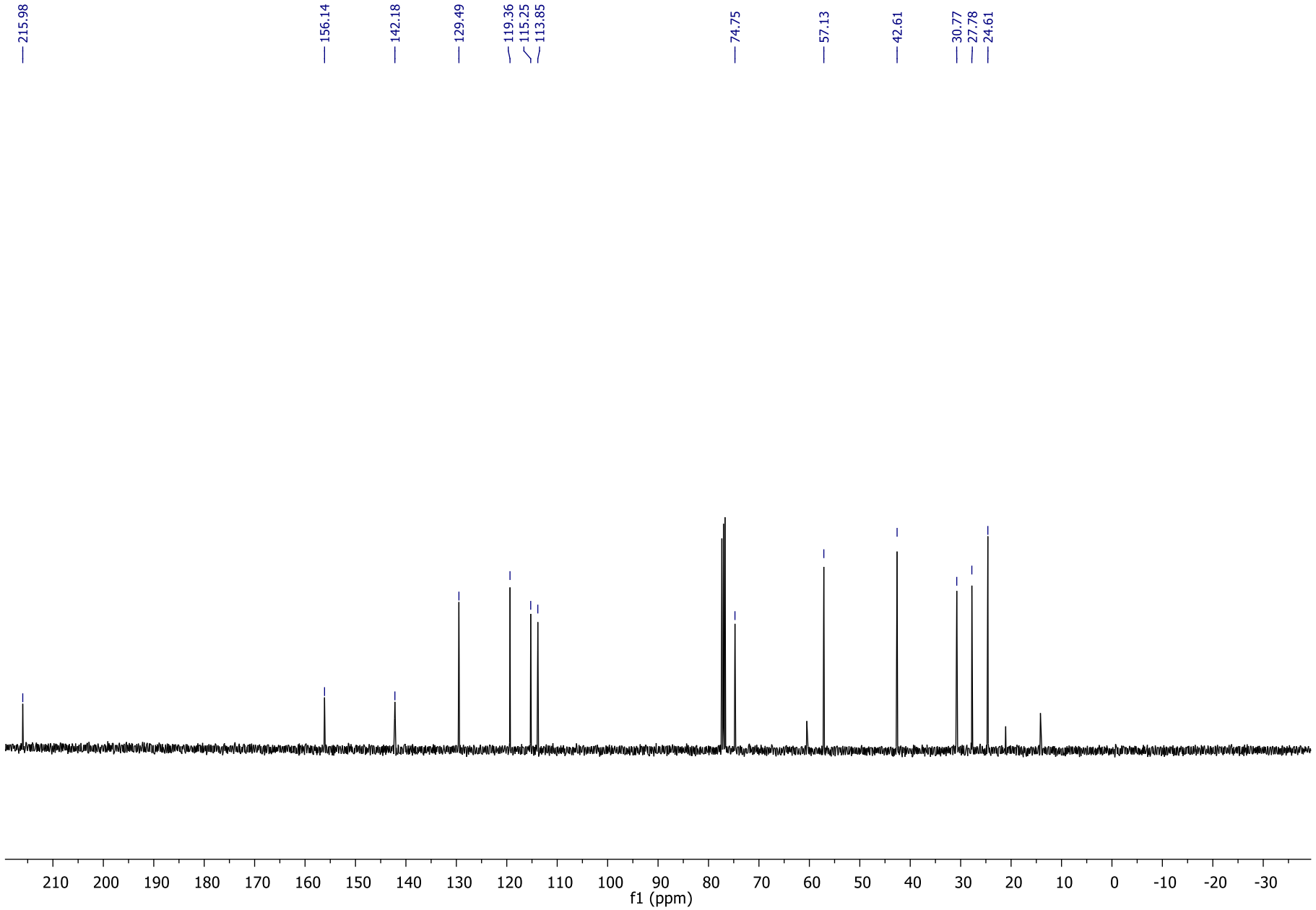
(R)-2-((S)-hydroxy(3-methoxyphenyl)methyl)cyclohexan-1-one (4.34a)



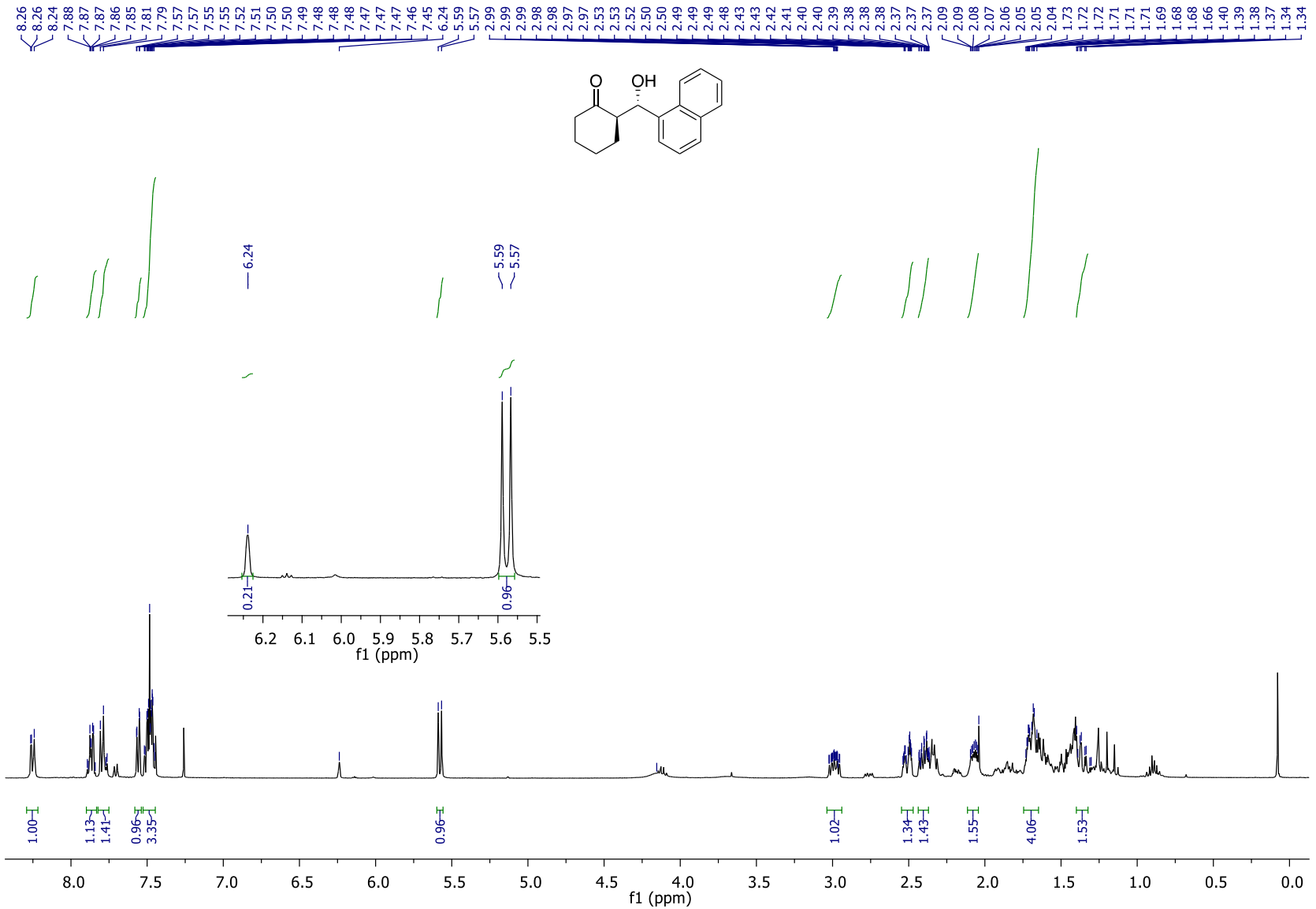

215.46
159.67
142.60
129.31
119.48
113.34
112.45
74.61
57.37
55.19
42.60
30.82
27.80
24.65
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30
f1 (ppm)

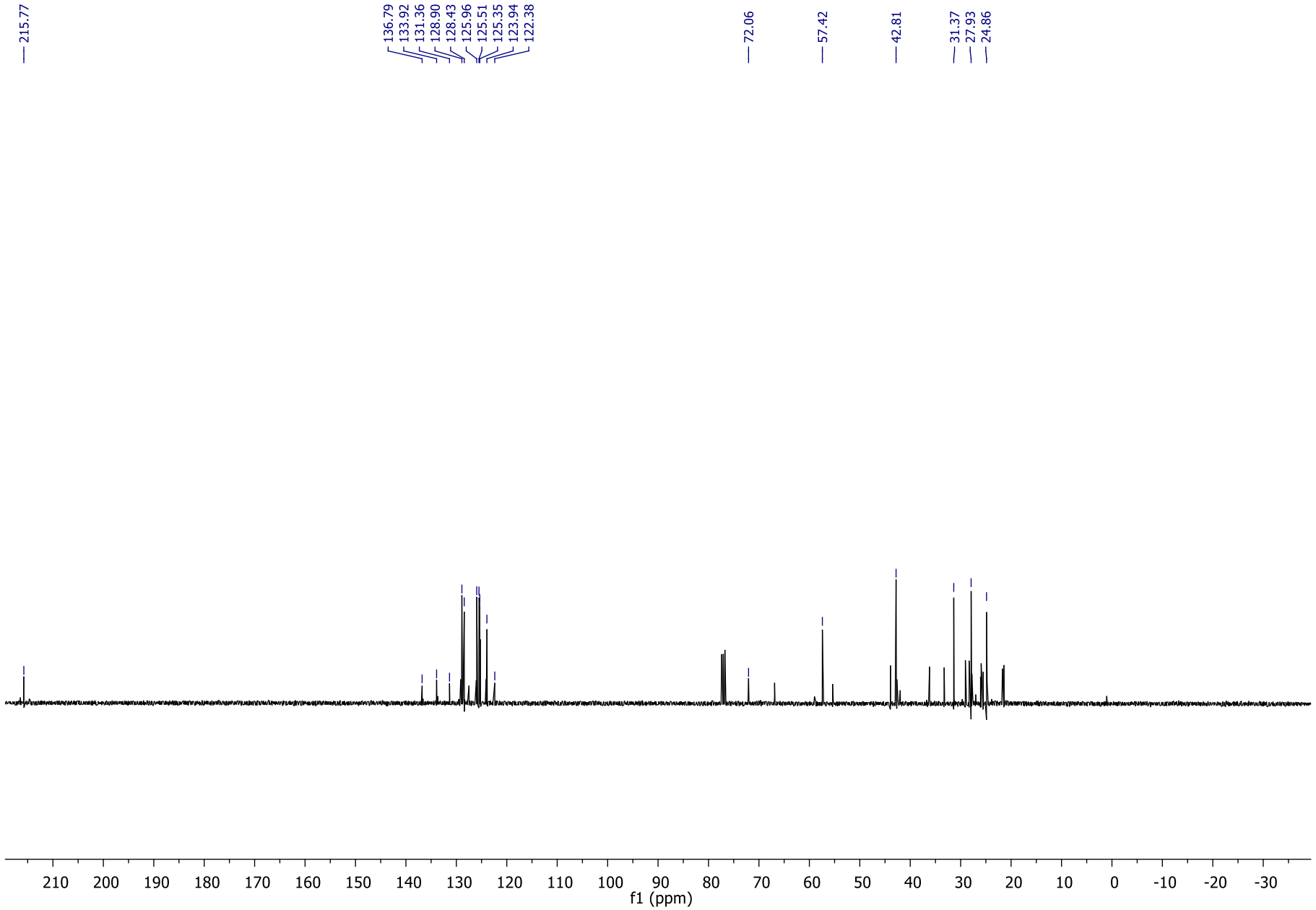
(R)-2-((S)-hydroxy(3-hydroxyphenyl)methyl)cyclohexan-1-one (4.35a)



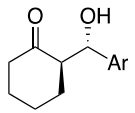

215.98
156.14
142.18
129.49
119.36
115.25
113.85
74.75
57.13
42.61
30.77
27.78
24.61
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30
f1 (ppm)

(R)-2-((S)-hydroxy(naphthalen-1-yl)methyl)cyclohexan-1-one (4.36a)




215.77
136.79
133.92
131.36
128.90
128.43
125.96
125.51
125.35
123.94
122.38
72.06
57.42
42.81
31.37
27.93
24.86
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20 -30
f1 (ppm)

HPLC DATA



Ar	Yield [%]	HPLC conditions						dr (*anti*:*syn*) from NMR	dr (*anti*:*syn*) from HPLC	*ee* anti [%]	*ee* syn [%]
		Column	Eluent	RT (major ent) *anti* [min]	RT (minor ent) *anti* [min]	RT (minor ent) *syn* [min]	RT (minor ent.) *syn* [min]				
4-NO_2-C_6H_4		IB	Hexane/iPrOH 95:5 (1 ml/min)	25.7	31.8	21.2	23.4	77:23	54:46	19	14
3-NO_2-C_6H_4		IB	Hexane/iPrOH 95:5 (1 ml/min)	22.1	30.2	18.6	20.0	80:20	51:49	14	1
2-NO_2-C_6H_4		IA	Hexane/iPrOH 9:1 (1 ml/min)	19.3	23.7	12.5	11.5	78:22	70:30	18	16
4-CN-C_6H_4		IB	Hexane/iPrOH 95:5 (1 ml/min)	26.4	33.4	21.2	24.7	73:27	53:47	33	22
2-F-C_6H_4		IC	Hexane/iPrOH 95:5 (1 ml/min)	26.4	33.3	14.7	12.6	95:5	99:1	38	0
4-Br-C_6H_4		IC	Hexane/iPrOH 95:5 (1 ml/min)	31.4	40.0	27.4	29.0	82:18	98:2	15	(20)
3-Br-C_6H_4		IB	Hexane/iPrOH 95:5 (1 ml/min)	9.9	11.5	8.3	8.8	84:16	83:17	25	3
2-Br-C_6H_4		IC	Hexane/iPrOH 9:1 (1 ml/min)	15.6	19.2	8.7	9.6	97:3	94:6	23	28
3-Cl-C_6H_4		IB	Hexane/iPrOH 95:5 (1 ml/min)	9.6	11.2	8.4	8.1	74:26	76:24	27	23
2-Cl-C_6H_4		IB	Hexane/iPrOH 95:5 (1 ml/min)	8.9	9.8	nd	nd	88:12	>99:1	20	nd
4-OMe-C_6H_4		IA	Hexane/iPrOH 95:5 (1 ml/min)	32.0	30.9	17.1	20	87:13	94:6	55	(24)
3-OMe-C_6H_4		IA	Hexane/iPrOH 95:5 (1 ml/min)	33.4	32.0	17.3	20.0	69:31	78:22	30	21
3-OH-C_6H_4		IC	Hexane/iPrOH 9:1 (1 ml/min)	65.3	62.0	44.4	48.4	97:3	94:6	29	13
1-naphthyl		IA	Hexane/iPrOH 95:5 (1 ml/min)	26.5	32.5	14.9	12.7	82:18	64:36	1	(40)

(R)-2-((S)-hydroxy(4-nitrophenyl)methyl)cyclohexan-1-one (4.23a)

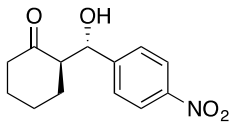
11/01/2021 11:32 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR116rac.PRM Page 1 of 2

Clarity - Chromatography SW
DataApex 2006
www.dataapex.com

Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR116rac.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 11/01/2021 11:10:17
Acquired Date : 11/01/2021 11:10:17
By : Fabrizio

Printed Version Info:
Printed Version : 11/01/2021 11:32:20
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 11/01/2021 11:32:49
By : Fabrizio

Sample Info:
Sample ID : VR116rac
Sample : 4-NO2 aldol rac
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

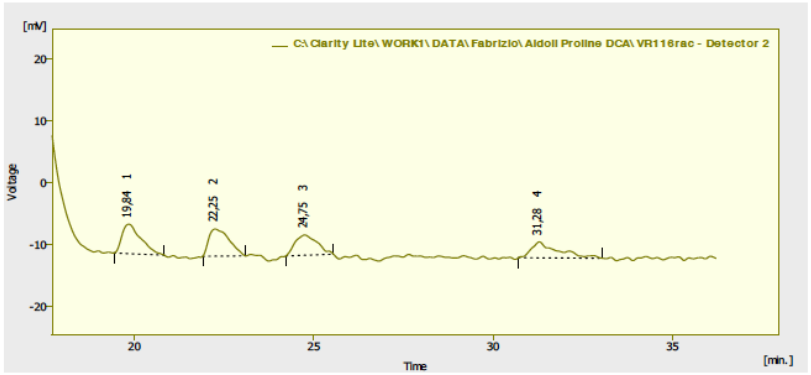


Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 11/01/2021 11:32

Column : IB
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



11/01/2021 11:32 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR116rac.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR116rac - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	19,844	175,242	4,762	28,0	31,7	0,58	
2	22,252	174,628	4,409	27,9	29,3	0,66	
3	24,748	141,350	3,282	22,6	21,8	0,76	
4	31,284	134,738	2,584	21,5	17,2	0,67	
	Total	625,957	15,038	100,0	100,0		

26/01/2021 11:13 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR103.PRM Page 1 of 2

Clarity - Chromatography SW
DataApex 2006
www.dataapex.com

Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR103.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 26/01/2021 11:06:40
Acquired Date : 26/01/2021 11:06:40
By : Fabrizio

Printed Version Info:
Printed Version : 26/01/2021 11:13:38
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 26/01/2021 11:13:45
By : Fabrizio

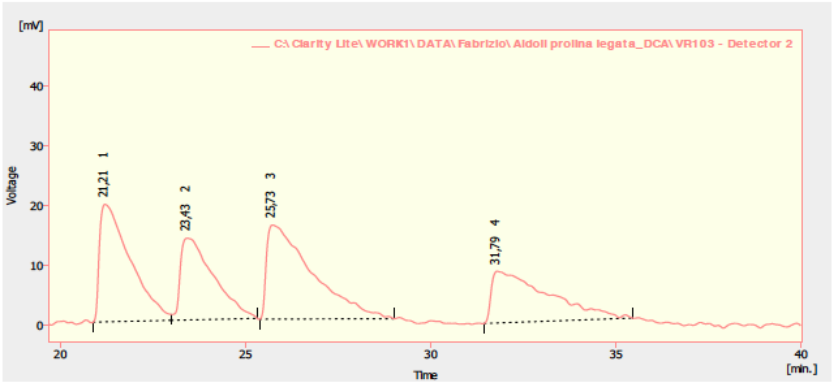
Sample Info:
Sample ID : VR103
Sample : 4-NO2 aldol
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 26/01/2021 11:13

Column : IB
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



26/01/2021 11:13 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR103.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR103 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	21,208	1136,862	19,691	26,7	34,0	0,91	
2	23,432	857,021	13,702	20,1	23,7	0,99	
3	25,732	1349,485	15,787	31,7	27,3	1,22	
4	31,788	918,684	8,676	21,6	15,0	1,90	
	Total	4262,053	57,856	100,0	100,0		

(R)-2-((S)-hydroxy(3-nitrophenyl)methyl)cyclohexan-1-one (4.25a)

11/01/2021 16:12 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\Report\3-NO2rac.PRM Page 1 of 2

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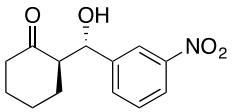
Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\Report\3-NO2rac.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 11/01/2021 16:11:55
Acquired Date : 11/01/2021 12:53:57
By : Fabrizio

Printed Version Info:
Printed Version : 11/01/2021 13:09:18
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 11/01/2021 16:12:42
By : Fabrizio

Sample Info:
Sample ID : VR124rac
Sample : 3-NO2 aldol rac
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

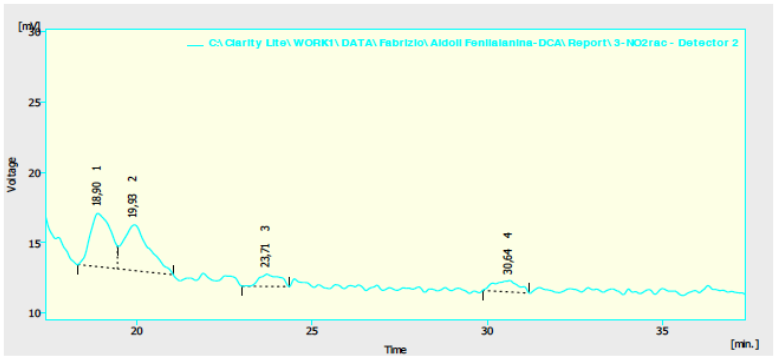
Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 11/01/2021 16:11

Column : IB
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :



Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



11/01/2021 16:12 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\Report\3-NO2rac.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\Report\3-NO2rac - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	18,900	156,317	3,793	39,1	43,4	0,73	
2	19,932	166,773	3,253	41,8	37,3	0,86	
3	23,712	36,155	0,870	9,1	10,0	0,79	
4	30,636	40,146	0,816	10,1	9,3	0,87	
	Total	399,391	8,732	100,0	100,0		

26/01/2021 12:36 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR102.PRM Page 1 of 2

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Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR102.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 26/01/2021 12:35:08
Acquired Date : 26/01/2021 12:35:08
By : Fabrizio

Printed Version Info:
Printed Version : 26/01/2021 12:36:12
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 26/01/2021 12:36:17
By : Fabrizio

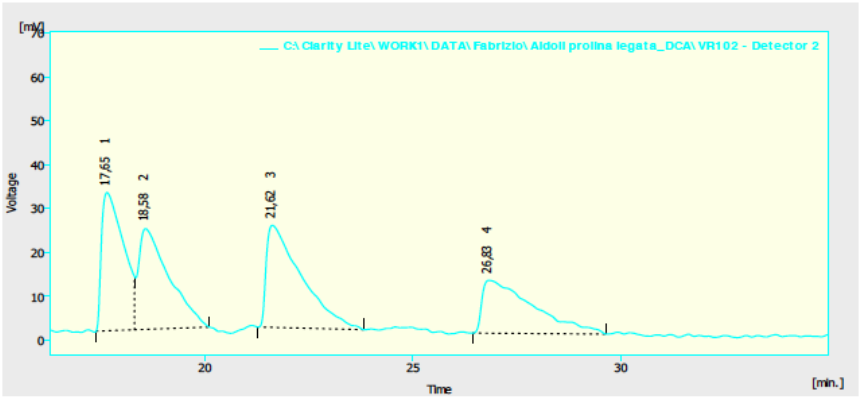
Sample Info:
Sample ID : VR102
Sample : 3-NO2 aldol
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 26/01/2021 12:36

Column : IB
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



26/01/2021 12:36 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR102.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR102 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	17,648	1159,980	31,537	24,5	35,1	0,68	
2	18,576	1182,437	22,964	24,9	25,6	0,84	
3	21,624	1365,170	23,265	28,8	25,9	0,88	
4	26,828	1033,876	12,058	21,8	13,4	1,29	
	Total	4741,462	89,824	100,0	100,0		

(R)-2-((S)-hydroxy(2-nitrophenyl)methyl)cyclohexan-1-one (4.24a)

26/01/2021 15:Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE NON LEGATA DCA\IA_9_1_1MLMIN\VR125RAC.PRM Page 1 of 2

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Chromatogram Info:
File Name : C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE NON LEGATA DCA\IA_9_1_1MLMIN\VR125RAC.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 16/12/2020 17:22:56
Acquired Date : 16/12/2020 17:41:21
By : Fabrizio

Printed Version Info:
Printed Version : 26/01/2021 15:19:00
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 26/01/2021 15:19:17
By : Fabrizio

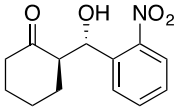
Sample Info:
Sample ID : VR125rac
Sample : 2-NO2 aldol racemate
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

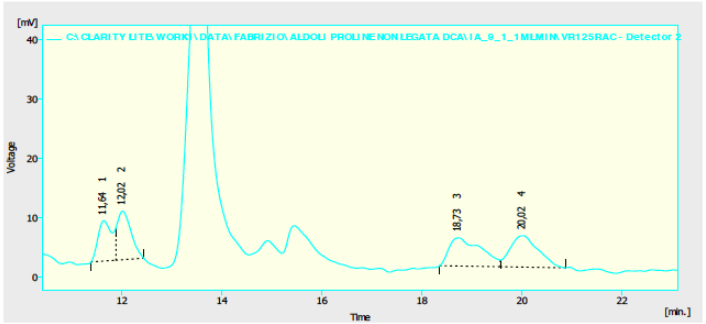
Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 26/01/2021 15:19

Column : IA
Mobile Phase : H/IPrOH 9:1
Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0





26/01/2021 15:Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE NON LEGATA DCA\IA_9_1_1MLMIN\VR125RAC.PRM Page 2 of 2

Result Table (Uncal - C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE NON LEGATA DCA\IA_9_1_1MLMIN\VR125RAC - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	11,644	133,468	6,765	18,9	27,1	0,38	
2	12,020	153,933	8,173	21,9	32,7	0,32	
3	18,728	210,696	4,746	29,9	19,0	0,79	
4	20,020	206,364	5,277	29,3	21,1	0,64	
	Total	704,461	24,961	100,0	100,0		

25/01/2021 15:43 Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINA LEGATA_DCA\IA_9-1_1MLMIN\VR101.PRM Page 1 of 2

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Chromatogram Info:
File Name : C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINA LEGATA_DCA\IA_9-1_1MLMIN\VR101.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 25/01/2021 10:14:25
Acquired Date : 25/01/2021 10:26:39
By : Fabrizio

Printed Version Info:
Printed Version : 25/01/2021 15:43:35
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 25/01/2021 15:43:47
By : Fabrizio

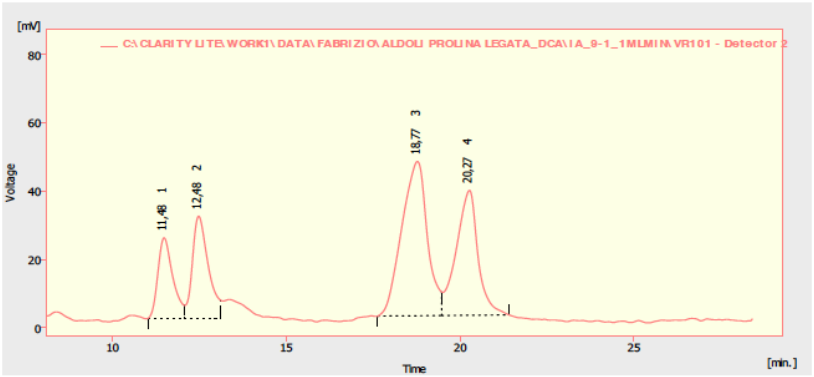
Sample Info:
Sample ID : VR101
Sample : 2-NO2 aldol
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 25/01/2021 15:43

Column : IA
Mobile Phase : H/IPrOH 9:1
Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



25/01/2021 15:43 Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINA LEGATA_DCA\IA_9-1_1MLMIN\VR101.PRM Page 2 of 2

Result Table (Uncal - C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINA LEGATA_DCA\IA_9-1_1MLMIN\VR101 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	11,480	699,165	23,550	12,5	17,4	0,46	
2	12,476	957,784	29,896	17,2	22,1	0,48	
3	18,772	2315,278	45,192	41,5	33,4	0,80	
4	20,268	1603,636	36,609	28,8	27,1	0,64	
	Total	5575,862	135,246	100,0	100,0		

4-((S)-hydroxy((R)-2-oxocyclohexyl)methyl)benzonitrile (4.26a)

12/01/2021 09:26 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\VR126rac.PRM Page 1 of 2

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Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\VR126rac.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 12/01/2021 09:08:34
Acquired Date : 12/01/2021 09:08:34
By : Fabrizio

Printed Version Info:
Printed Version : 12/01/2021 09:25:49
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 12/01/2021 09:26:03
By : Fabrizio

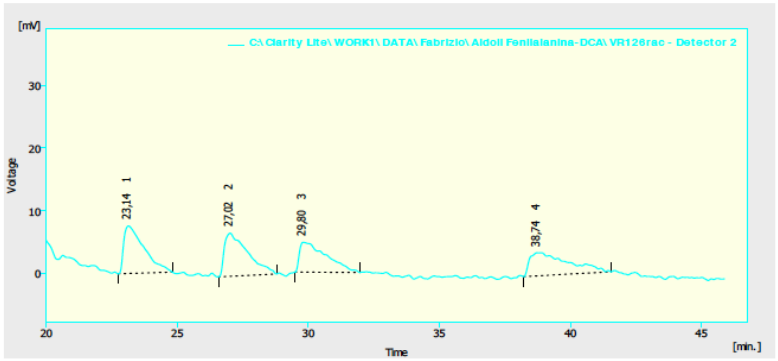
Sample Info:
Sample ID : VR126rac
Sample : 4-CN aldol rac
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 12/01/2021 09:25

Column : IB
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



12/01/2021 09:26 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\VR126rac.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\VR126rac - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	23,136	408,808	7,584	26,4	33,1	0,86	
2	27,024	424,729	6,859	27,4	30,0	1,02	
3	29,804	341,880	4,757	22,1	20,8	1,17	
4	38,740	372,245	3,691	24,1	16,1	1,76	
	Total	1547,661	22,890	100,0	100,0		

26/01/2021 12:01 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR106.PRM Page 1 of 2

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Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR106.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 26/01/2021 11:56:03
Acquired Date : 26/01/2021 11:56:03
By : Fabrizio

Printed Version Info:
Printed Version : 26/01/2021 12:01:06
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 26/01/2021 12:01:12
By : Fabrizio

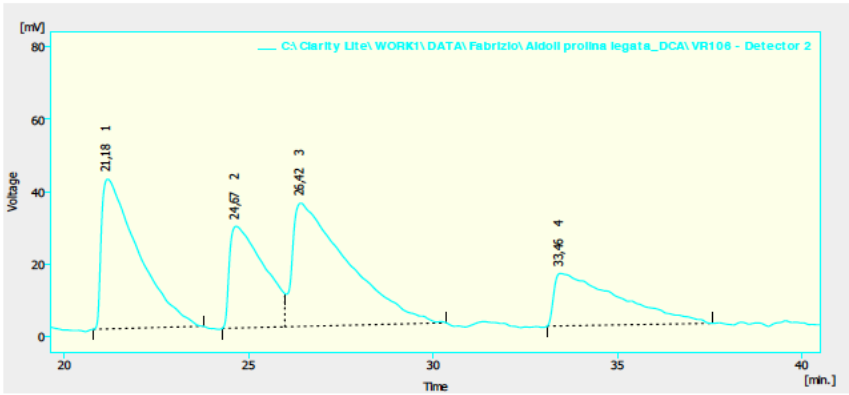
Sample Info:
Sample ID : VR106
Sample : 4-CN aldol
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 26/01/2021 12:01

Column : IB
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



26/01/2021 12:01 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR106.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR106 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	21,180	2879,518	41,440	28,6	35,1	1,06	
2	24,672	1839,252	28,111	18,3	23,8	1,20	
3	26,424	3578,328	34,086	35,5	28,8	1,58	
4	33,464	1779,725	14,527	17,7	12,3	1,94	
	Total	10076,823	118,165	100,0	100,0		

(R)-2-((S)-(2-fluorophenyl)(hydroxy)methyl)cyclohexan-1-one (4.27a)

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Chromatogram Info:

File Name	: C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR128rac.PRM	File Created	: 03/12/2020 12:13:45
Origin	: Acquired	Acquired Date	: 03/12/2020 13:10:23
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

Printed Version	: 03/12/2020 14:13:44	Printed Date	: 03/12/2020 14:13:51
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

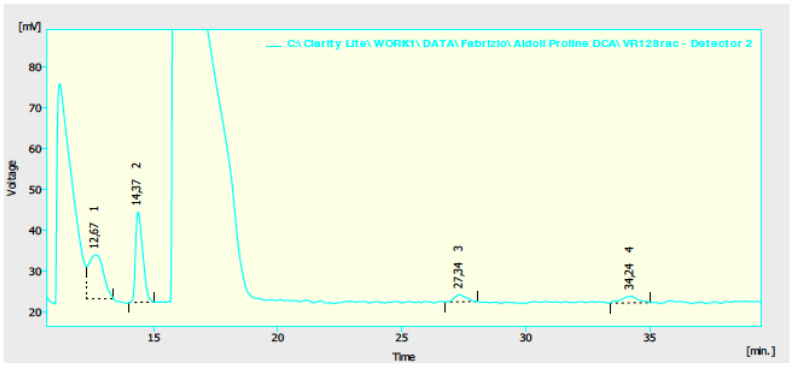
Sample Info:

Sample ID	: VR128rac	Amount	: 0
Sample	: 2-F aldol racemate	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 03/12/2020 14:13
Column	: IC	Detection	: RI
Mobile Phase	: H/IPrOH 95:5	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)		

Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR128rac - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	12,668	458,840	10,784	44,0	29,7	0,74	
2	14,368	446,910	22,154	42,9	61,0	0,32	
3	27,344	58,318	1,750	5,6	4,8	0,56	
4	34,244	78,243	1,639	7,5	4,5	0,70	
	Total	1042,311	36,327	100,0	100,0		

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Chromatogram Info:

File Name	: C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR108.PRM	File Created	: 25/01/2021 16:39:14
Origin	: Acquired	Acquired Date	: 25/01/2021 16:39:14
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

Printed Version	: 25/01/2021 16:41:48	Printed Date	: 25/01/2021 16:41:55
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

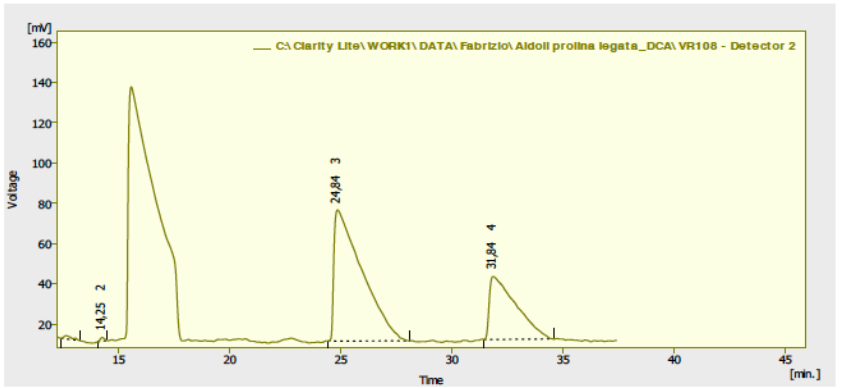
Sample Info:

Sample ID	: VR108	Amount	: 0
Sample	: 2-F aldol	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 25/01/2021 16:41
Column	: IC	Detection	: RI
Mobile Phase	: H/IPrOH 95:5	Temperature	:
Flow Rate	: 1 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)		

Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR108 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	12,640	43,647	1,669	0,5	1,7	0,34	
2	14,252	23,900	1,879	0,3	1,9	0,22	
3	24,844	5713,802	65,068	68,2	65,1	1,40	
4	31,844	2596,724	31,271	31,0	31,3	1,32	
	Total	8378,073	99,887	100,0	100,0		

(R)-2-((S)-(4-bromophenyl)(hydroxy)methyl)cyclohexan-1-one (4.28a)

07/12/2020 13:06 Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IC_95_5_1MLMIN\VR131RAC.PRM Page 1 of 2

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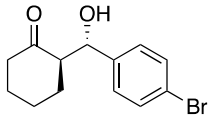
Chromatogram Info:
File Name : C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IC_95_5_1MLMIN\VR131RAC.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 07/12/2020 10:44:47
Acquired Date : 07/12/2020 11:30:06
By : Fabrizio

Printed Version Info:
Printed Version : 07/12/2020 13:06:35
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 07/12/2020 13:06:41
By : Fabrizio

Sample Info:
Sample ID : VR131rac
Sample : 4-Br aldol racemate
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

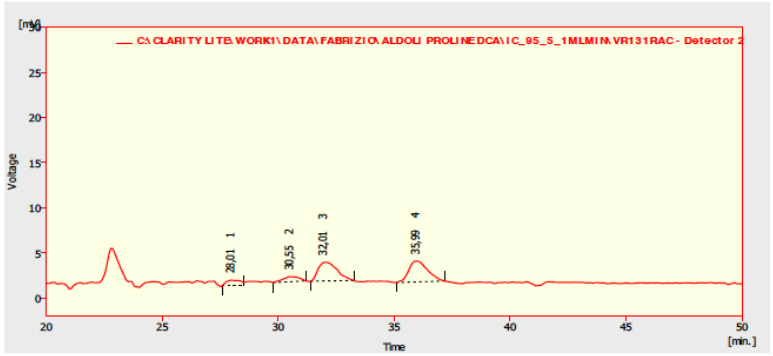
Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 07/12/2020 13:06

Column : IC
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :



Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



07/12/2020 13:06 Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IC_95_5_1MLMIN\VR131RAC.PRM Page 2 of 2

Result Table (Uncal - C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IC_95_5_1MLMIN\VR131RAC - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	28,012	26,857	0,631	8,9	11,3	0,80	
2	30,552	26,897	0,565	8,9	10,1	0,80	
3	32,012	117,629	2,080	39,0	37,2	0,94	
4	35,988	130,585	2,311	43,2	41,4	0,90	
	Total	301,967	5,586	100,0	100,0		

25/01/2021 17:34 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR110.PRM Page 1 of 2

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Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR110.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 25/01/2021 17:30:41
Acquired Date : 25/01/2021 17:30:41
By : Fabrizio

Printed Version Info:
Printed Version : 25/01/2021 17:34:23
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 25/01/2021 17:34:28
By : Fabrizio

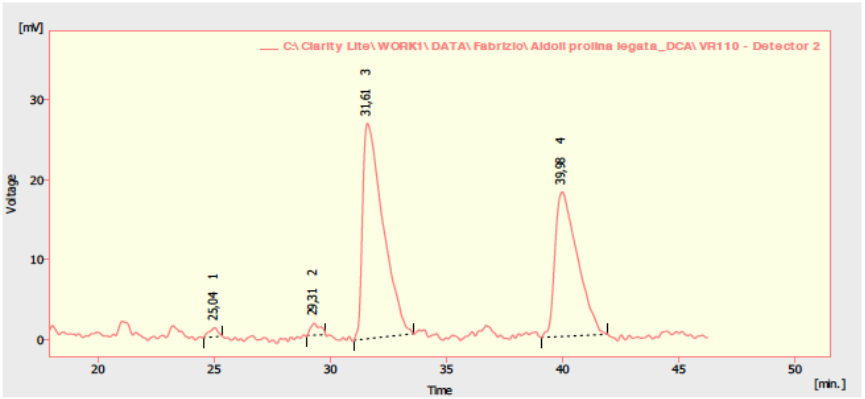
Sample Info:
Sample ID : VR110
Sample : 4-Br aldol
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 25/01/2021 17:34

Column : IC
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



25/01/2021 17:34 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR110.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR110 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	25,036	29,986	1,132	1,0	2,4	0,44	
2	29,312	43,197	1,502	1,5	3,1	0,60	
3	31,608	1649,874	26,976	56,1	56,5	0,96	
4	39,984	1217,953	18,095	41,4	37,9	1,08	
	Total	2941,010	47,704	100,0	100,0		

(R)-2-((S)-(3-bromophenyl)(hydroxy)methyl)cyclohexan-1-one (4.29a)

22/12/2020 18:25 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR130rac.PRM Page 1 of 2

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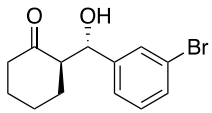


Chromatogram Info:

File Name	: C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR130rac.PRM	File Created	: 22/12/2020 18:11:40
Origin	: Acquired	Acquired Date	: 22/12/2020 18:11:40
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

Printed Version	: 22/12/2020 18:25:07	Printed Date	: 22/12/2020 18:25:15
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

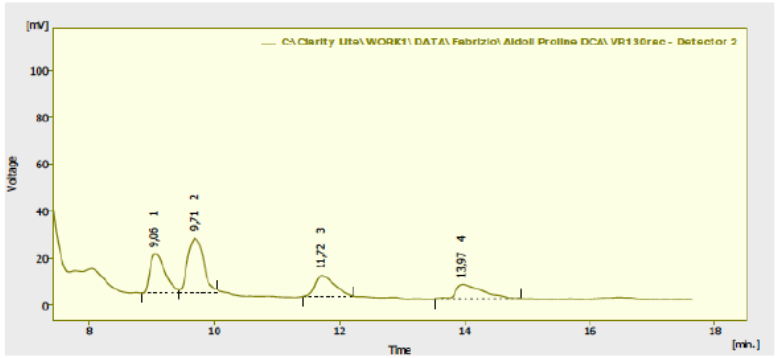
Sample Info:

Sample ID	: VR130rac	Amount	: 0
Sample	: 3-Br aldol	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 22/12/2020 18:25
Column	: IB	Detection	: RI
Mobile Phase	: H/IPrOH 95:5	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)		

Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



22/12/2020 18:25 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR130rac.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR130rac - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	9,056	284,254	16,943	29,3	30,6	0,27	
2	9,712	318,450	23,767	32,9	42,9	0,23	
3	11,724	188,375	8,725	19,4	15,7	0,35	
4	13,972	177,440	6,017	18,3	10,9	0,46	
	Total	968,519	55,452	100,0	100,0		

25/01/2021 18:09 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR121.PRM Page 1 of 2

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Chromatogram Info:

File Name	: C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR121.PRM	File Created	: 25/01/2021 17:59:55
Origin	: Acquired	Acquired Date	: 25/01/2021 18:06:41
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

Printed Version	: 25/01/2021 18:09:36	Printed Date	: 25/01/2021 18:09:46
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

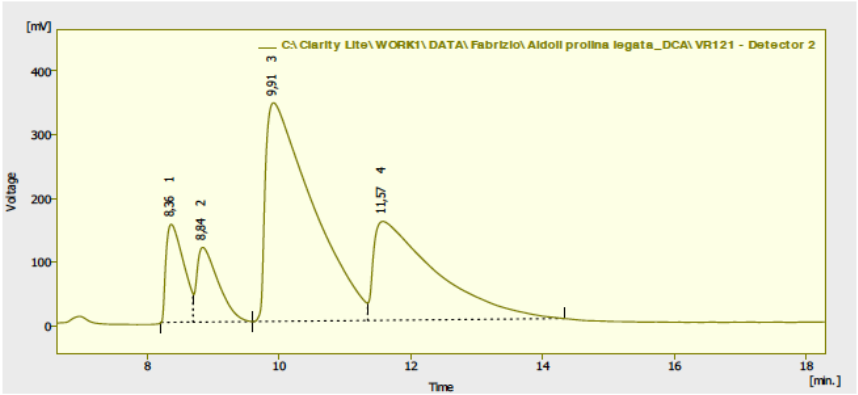
Sample Info:

Sample ID	: VR121	Amount	: 0
Sample	: 3-Br aldol	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 25/01/2021 18:09
Column	: IB	Detection	: RI
Mobile Phase	: H/IPrOH 95:5	Temperature	:
Flow Rate	: 1 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)		

Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



25/01/2021 18:09 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR121.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR121 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	8,360	2845,391	153,572	8,7	20,0	0,33	
2	8,840	2673,773	117,021	8,2	15,2	0,36	
3	9,912	17088,830	342,693	52,1	44,6	0,79	
4	11,572	10183,872	155,013	31,1	20,2	0,96	
	Total	32791,866	768,299	100,0	100,0		

(R)-2-((S)-(2-bromophenyl)(hydroxy)methyl)cyclohexan-1-one (4.30a)

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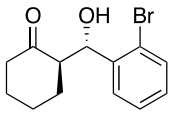
Chromatogram Info:
File Name : C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IC_9_1_1MLMIN\VR129RAC.PRM
File Created : 10/12/2020 13:21:38
Origin : Acquired
Acquired Date : 10/12/2020 13:33:21
Project : C:\Clarity Lite\Projects\Work1.PRJ
By : Fabrizio

Printed Version Info:
Printed Version : 15/12/2020 15:17:38
Printed Date : 15/12/2020 15:17:47
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
By : Fabrizio
Calibration File : None

Sample Info:
Sample ID : VR129rac
Amount : 0
Sample : 2-Br aldol racemate
ISTD Amount : 0
Inj. Volume [mL] : 0,1
Dilution : 1

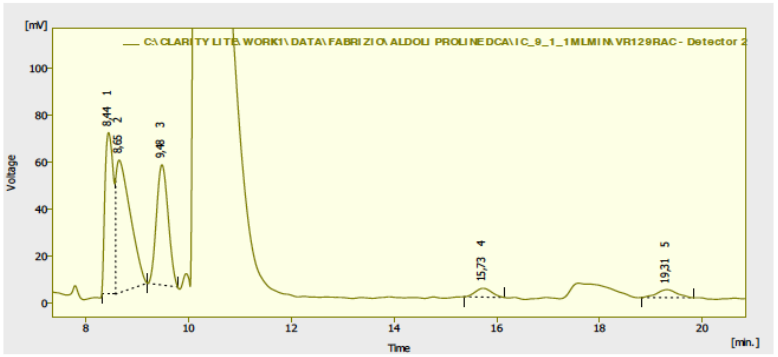


Method : Default1
By : DataApex Ltd.
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
Modified : 15/12/2020 15:17

Column : IC
Detection : RI
Mobile Phase : H/IPrOH 9:1
Temperature :
Flow Rate : 1.0 mL/min
Pressure :
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)

Autostop : None
External Start : Start - Restart, Down
Detector 1 : Detector 2
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Calibration File : None
Calculation : Uncal
Scale Factor : Not Used
Units After Scaling : Not Used
Uncal. Response : 0
Result Table Reports : All Peaks
Hide ISTD Peak : Enabled
GPC Calibration File :



Result Table (Uncal - C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IC_9_1_1MLMIN\VR129RAC - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	8,444	801,924	68,662	27,9	37,5	0,22	
2	8,648	1085,303	56,434	37,7	30,8	0,33	
3	9,480	811,311	51,101	28,2	27,9	0,26	
4	15,728	83,392	3,744	2,9	2,0	0,36	
5	19,308	93,353	3,387	3,2	1,8	0,41	
	Total	2875,283	183,329	100,0	100,0		

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Chromatogram Info:
File Name : C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINA LEGATA_DCA\IC_9-1_1MLMIN\VR109.PRM
File Created : 25/01/2021 12:26:18
Origin : Acquired
Acquired Date : 25/01/2021 12:27:11
Project : C:\Clarity Lite\Projects\Work1.PRJ
By : Fabrizio

Printed Version Info:
Printed Version : 25/01/2021 15:42:12
Printed Date : 25/01/2021 15:42:19
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
By : Fabrizio
Calibration File : None

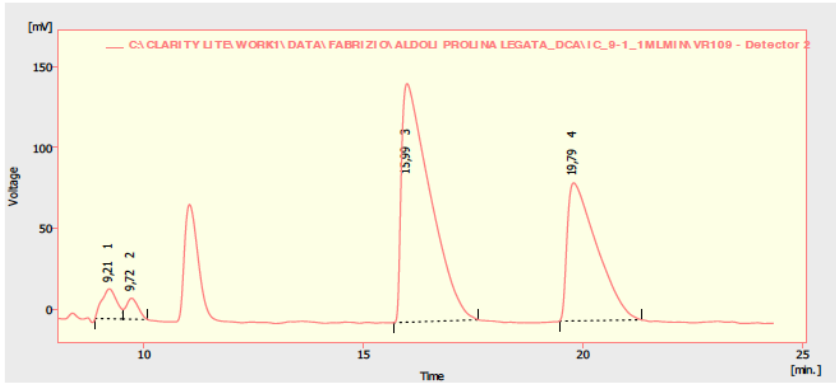
Sample Info:
Sample ID : VR109
Amount : 0
Sample : 2-Br aldol
ISTD Amount : 0
Inj. Volume [mL] : 0,1
Dilution : 1

Method : Default1
By : DataApex Ltd.
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
Modified : 25/01/2021 15:42

Column : IC
Detection : RI
Mobile Phase : H/IPrOH 9:1
Temperature :
Flow Rate : 1 mL/min
Pressure :
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)

Autostop : None
External Start : Start - Restart, Down
Detector 1 : Detector 2
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Calibration File : None
Calculation : Uncal
Scale Factor : Not Used
Units After Scaling : Not Used
Uncal. Response : 0
Result Table Reports : All Peaks
Hide ISTD Peak : Enabled
GPC Calibration File :



Result Table (Uncal - C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINA LEGATA_DCA\IC_9-1_1MLMIN\VR109 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	9,212	441,680	18,461	3,9	7,0	0,42	
2	9,716	248,325	12,920	2,2	4,9	0,34	
3	15,992	6677,618	147,143	58,8	55,8	0,72	
4	19,788	3997,160	85,103	35,2	32,3	0,75	
	Total	11364,784	263,626	100,0	100,0		

(R)-2-((S)-(3-chlorophenyl)(hydroxy)methyl)cyclohexan-1-one (4.31a)

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Chromatogram Info:

File Name	: C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR133rac2.PRM	File Created	: 22/12/2020 17:34:27
Origin	: Acquired	Acquired Date	: 22/12/2020 17:34:27
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

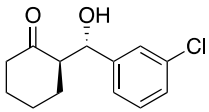
Printed Version Info:

Printed Version	: 22/12/2020 17:40:49	Printed Date	: 22/12/2020 17:41:02
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

Sample Info:

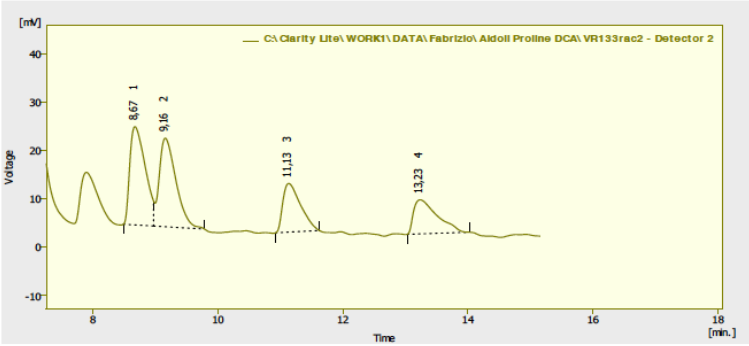
Sample ID	: VR133rac2	Amount	: 0
Sample	: 3-Cl aldol rac	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 22/12/2020 17:40
Column	: IB	Detection	: RI
Mobile Phase	: H/IPrOH 95:5	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)		



Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR133rac2 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	8,672	349,056	20,340	32,4	36,5	0,29	
2	9,160	345,493	18,319	32,0	32,8	0,29	
3	11,132	198,411	10,084	18,4	18,1	0,32	
4	13,232	185,879	7,058	17,2	12,6	0,42	
	Total	1078,839	55,800	100,0	100,0		

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Chromatogram Info:

File Name	: C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR119.PRM	File Created	: 26/01/2021 09:13:17
Origin	: Acquired	Acquired Date	: 26/01/2021 09:13:17
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

Printed Version	: 26/01/2021 09:14:40	Printed Date	: 26/01/2021 09:14:51
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

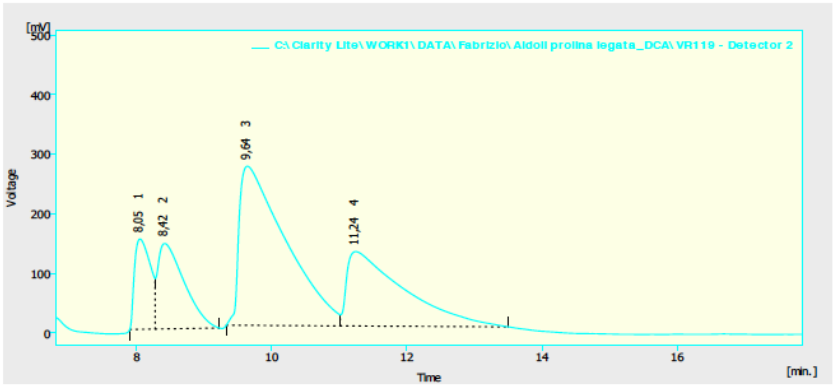
Sample Info:

Sample ID	: VR119	Amount	: 0
Sample	: 3-Cl aldol	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 26/01/2021 09:14
Column	: IB	Detection	: RI
Mobile Phase	: H/IPrOH 95:5	Temperature	:
Flow Rate	: 1 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)		

Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR119 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	8,052	2472,652	151,608	9,3	22,1	0,32	
2	8,416	3922,941	143,355	14,8	20,9	0,46	
3	9,640	12792,921	267,168	48,2	38,9	0,76	
4	11,244	7337,574	124,837	27,7	18,2	0,87	
	Total	26526,088	686,968	100,0	100,0		

(R)-2-((S)-(2-chlorophenyl)(hydroxy)methyl)cyclohexan-1-one (4.32a)

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Chromatogram Info:

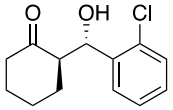
File Name	: C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IB_95_5_1MLMIN\VR132RAC.PRM	File Created	: 22/12/2020 13:58:43
Origin	: Acquired	Acquired Date	: 22/12/2020 14:11:33
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

Printed Version	: 22/12/2020 16:04:26	Printed Date	: 22/12/2020 16:04:34
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

Sample Info:

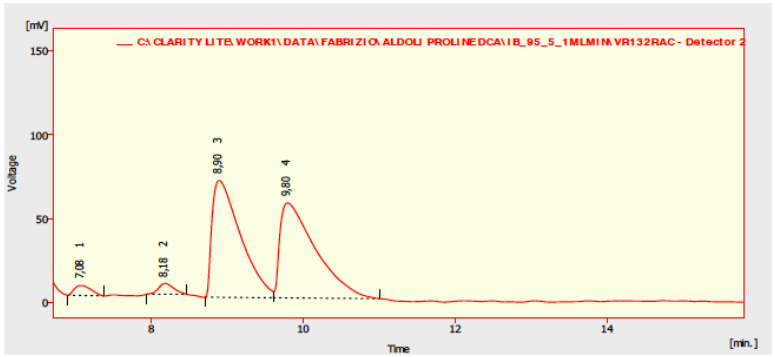
Sample ID	: VR132rac	Amount	: 0
Sample	: 2-Cl aldol rac	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1



Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 22/12/2020 16:04
Column	: IB	Detection	: RI
Mobile Phase	: H/iPrOH 95:5	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)		

Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



Result Table (Uncal - C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\IB_95_5_1MLMIN\VR132RAC - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	7,080	90,884	5,940	2,4	4,3	0,26	
2	8,184	91,457	6,472	2,4	4,7	0,22	
3	8,896	1814,767	69,717	47,1	50,2	0,41	
4	9,796	1855,586	56,643	48,2	40,8	0,50	
	Total	3852,694	138,771	100,0	100,0		

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Chromatogram Info:

File Name	: C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR113.PRM	File Created	: 25/01/2021 18:25:04
Origin	: Acquired	Acquired Date	: 25/01/2021 18:25:04
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

Printed Version	: 25/01/2021 18:25:32	Printed Date	: 25/01/2021 18:26:00
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

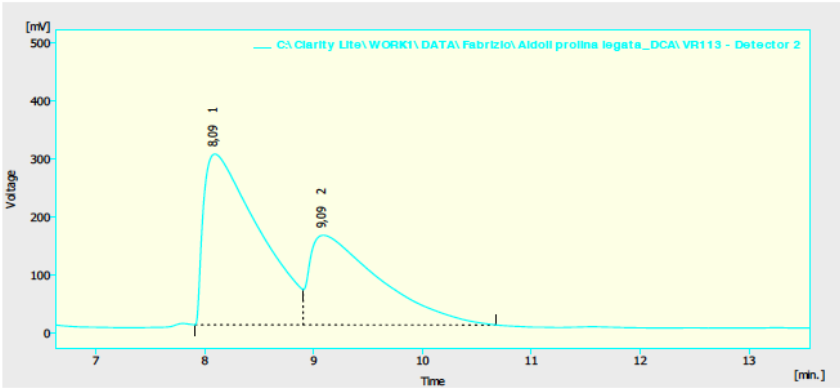
Sample Info:

Sample ID	: VR113	Amount	: 0
Sample	: 2-Cl aldol	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 25/01/2021 18:25
Column	: IB	Detection	: RI
Mobile Phase	: H/iPrOH 95:5	Temperature	:
Flow Rate	: 1 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)		

Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR113 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	8,092	10392,330	293,520	60,2	65,6	0,59	
2	9,088	6883,396	154,003	39,8	34,4	0,68	
	Total	17275,726	447,523	100,0	100,0		

(R)-2-((S)-hydroxy(4-methoxyphenyl)methyl)cyclohexan-1-one (4.33a)

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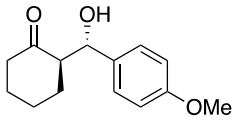
Chromatogram Info:
File Name : C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\VR135RAC.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 18/12/2020 19:03:44
Acquired Date : 18/12/2020 19:49:33
By : Fabrizio

Printed Version Info:
Printed Version : 21/12/2020 14:29:31
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 21/12/2020 14:29:37
By : Fabrizio

Sample Info:
Sample ID : VR135rac
Sample : 4-OMe aldol rac
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

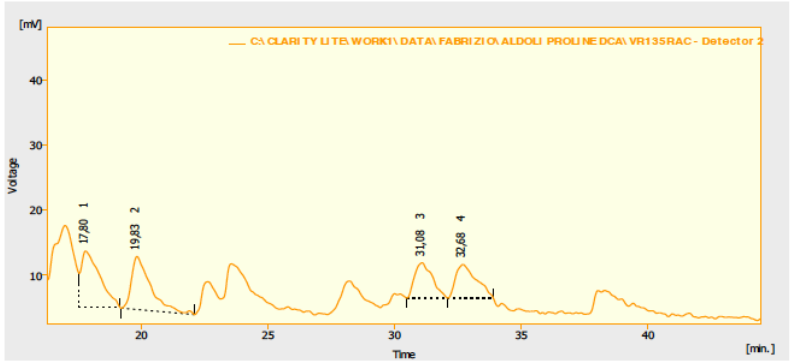
Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 21/12/2020 14:29

Column : IA
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :



Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



Result Table (Uncal - C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE DCA\VR135RAC - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	17,796	452,077	8,683	31,5	31,6	0,90	
2	19,828	426,999	8,149	29,7	29,7	0,75	
3	31,080	264,518	5,440	18,4	19,8	0,83	
4	32,684	292,979	5,177	20,4	18,9	0,94	
	Total	1436,573	27,448	100,0	100,0		

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Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR118.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 27/01/2021 11:23:32
Acquired Date : 27/01/2021 11:23:32
By : Fabrizio

Printed Version Info:
Printed Version : 27/01/2021 11:27:50
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 27/01/2021 11:28:00
By : Fabrizio

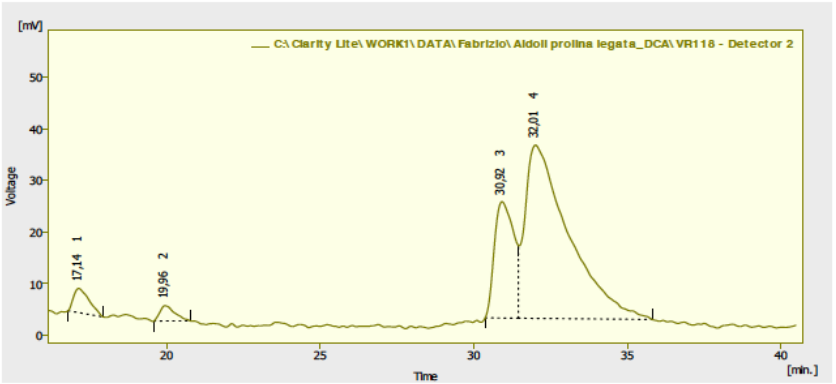
Sample Info:
Sample ID : VR118
Sample : 4-OMe aldol
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 27/01/2021 11:27

Column : IA
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR118 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	17,140	167,233	4,689	3,6	7,4	0,55	
2	19,956	99,808	2,961	2,2	4,6	0,53	
3	30,920	985,107	22,542	21,4	35,3	0,82	
4	32,012	3344,353	33,581	72,8	52,7	1,43	
	Total	4596,501	63,773	100,0	100,0		

(R)-2-((S)-hydroxy(3-methoxyphenyl)methyl)cyclohexan-1-one (4.34a)

02/12/2020 15:59 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR134_rac.PRM Page 1 of 2

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Chromatogram Info:

File Name	: C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR134_rac.PRM	File Created	: 02/12/2020 15:48:36
Origin	: Acquired	Acquired Date	: 02/12/2020 15:58:35
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

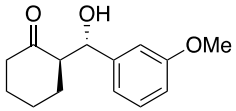
Printed Version Info:

Printed Version	: 02/12/2020 15:59:00	Printed Date	: 02/12/2020 15:59:06
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

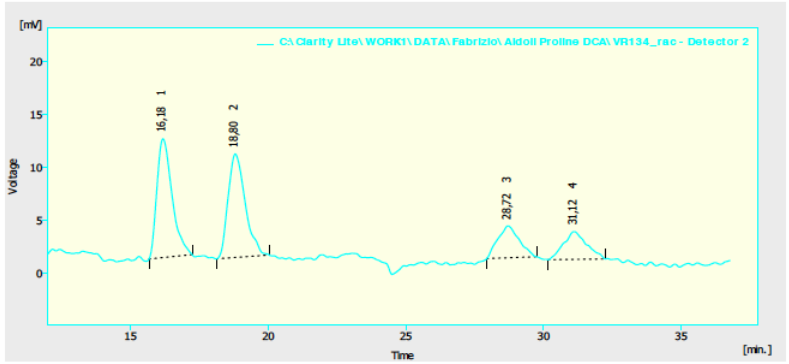
Sample Info:

Sample ID	: VR134_rac	Amount	: 0
Sample	: 3-OMe aldol rac	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 02/12/2020 15:59
Column	: IA	Detection	: RI
Mobile Phase	: H/IPrOH 95:5	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)		



Autostop	: None			External Start	: Start - Restart, Down
Detector 1	: Detector 2			Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.
Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



02/12/2020 15:59 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR134_rac.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR134_rac - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	16,180	431,094	11,239	36,8	42,1	0,60	
2	18,804	423,816	9,794	36,2	36,7	0,68	
3	28,720	162,194	3,015	13,8	11,3	0,90	
4	31,116	154,627	2,630	13,2	9,9	0,98	
	Total	1171,731	26,679	100,0	100,0		

27/01/2021 12:13 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR114_dil2.PRM Page 1 of 2

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Chromatogram Info:

File Name	: C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR114_dil2.PRM	File Created	: 27/01/2021 12:11:41
Origin	: Acquired	Acquired Date	: 27/01/2021 12:11:41
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

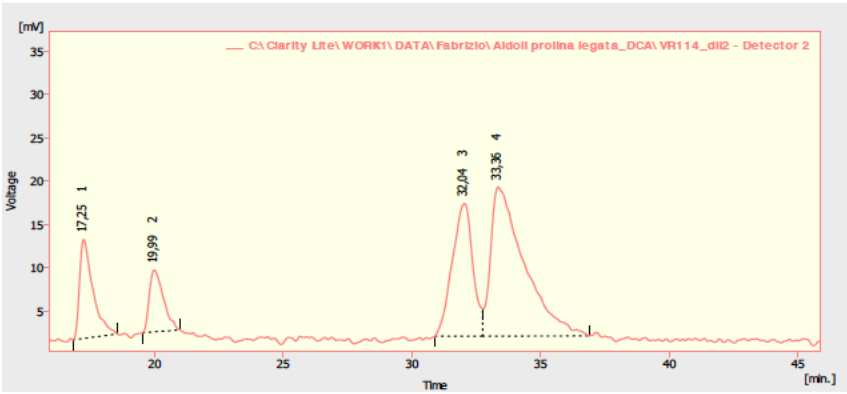
Printed Version	: 27/01/2021 12:13:40	Printed Date	: 27/01/2021 12:13:48
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

Sample Info:

Sample ID	: VR114_dil2	Amount	: 0
Sample	: 3-OMe aldol	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 27/01/2021 12:13
Column	: IA	Detection	: RI
Mobile Phase	: H/IPrOH 95:5	Temperature	:
Flow Rate	: 1 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)		

Autostop	: None			External Start	: Start - Restart, Down
Detector 1	: Detector 2			Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.
Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



27/01/2021 12:13 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR114_dil2.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR114_dil2 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	17,248	412,909	11,419	13,3	22,4	0,54	
2	19,992	270,845	7,135	8,7	14,0	0,61	
3	32,044	850,411	15,312	27,4	30,0	0,88	
4	33,360	1569,718	17,215	50,6	33,7	1,31	
	Total	3103,882	51,081	100,0	100,0		

(R)-2-((S)-hydroxy(3-hydroxyphenyl)methyl)cyclohexan-1-one (4.35a)

03/12/2020 18:50 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR137rac_2.PRM Page 1 of 2

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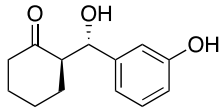
Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR137rac_2.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 03/12/2020 18:08:20
Acquired Date : 03/12/2020 18:08:20
By : Fabrizio

Printed Version Info:
Printed Version : 03/12/2020 18:49:56
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 03/12/2020 18:50:09
By : Fabrizio

Sample Info:
Sample ID : VR137rac_2
Sample : 3-OH aldol racemate
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

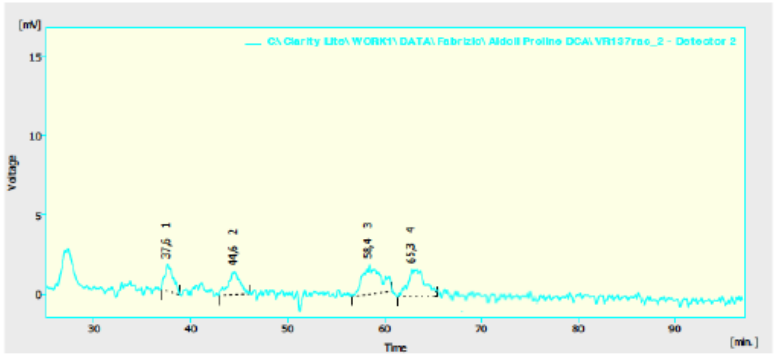


Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 03/12/2020 18:49

Column : IC
Mobile Phase : H/IPrOH 9:1
Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



03/12/2020 18:50 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR137rac_2.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR137rac_2 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	37,616	103,258	1,702	15,2	25,4	0,91	
2	44,568	120,575	1,429	17,7	21,3	1,30	
3	58,448	239,168	1,849	35,1	27,6	2,16	
4	65,288	217,902	1,726	32,0	25,7	1,60	
	Total	680,903	6,706	100,0	100,0		

25/01/2021 15:41 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR122.PRM Page 1 of 2

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Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR122.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 25/01/2021 13:45:37
Acquired Date : 25/01/2021 13:45:37
By : Fabrizio

Printed Version Info:
Printed Version : 25/01/2021 15:40:57
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 25/01/2021 15:41:03
By : Fabrizio

Sample Info:
Sample ID : VR122
Sample : 3-OH aldol
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 25/01/2021 15:40

Column : IC
Mobile Phase : H/IPrOH 9:1
Flow Rate : 1 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
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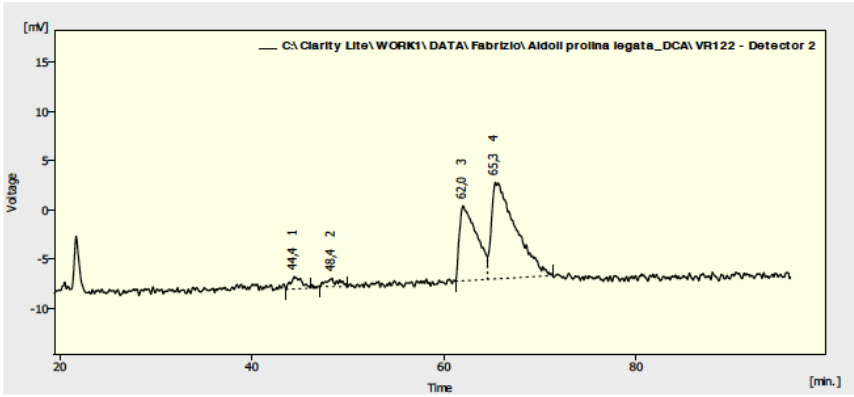

25/01/2021 15:41 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli prolina legata_DCA\VR122.PRM Page 2 of 2

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1	44,416	99,426	1,274	3,5	6,5	1,16	
2	48,352	76,042	0,865	2,7	4,4	0,70	
3	62,000	958,005	7,633	33,5	38,9	2,30	
4	65,344	1729,557	9,825	60,4	50,1	2,75	
	Total	2863,029	19,598	100,0	100,0		

(R)-2-((S)-hydroxy(naphthalen-1-yl)methyl)cyclohexan-1-one (4.36a)

30/11/2020 16:22 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR138rac.PRM Page 1 of 2

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By : Fabrizio

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Calibration File : None
Printed Date : 30/11/2020 16:22:20
By : Fabrizio

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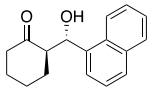

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By : DataApex Ltd.
Modified : 30/11/2020 16:21

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Detection : RI
Temperature :
Pressure :

Autostop : None
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External Start : Start - Restart, Down
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Scale Factor : Not Used
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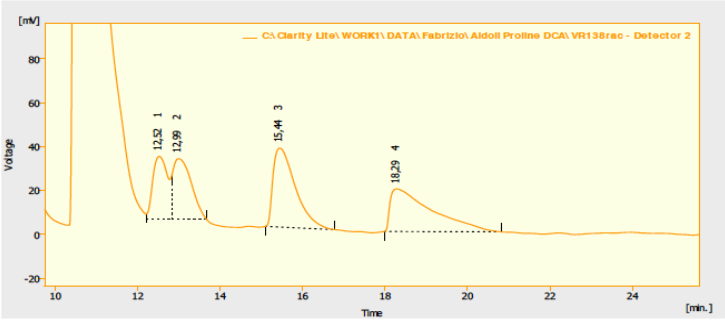

27/01/2021 15:18 Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINA LEGATA_DCA\VR107_2.PRM Page 1 of 2

Clarity - Chromatography SW
DataApex 2006
www.dataapex.com

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By : Fabrizio

Printed Version Info:
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Report Style : C:\Clarity Lite\Common\Chromatogram.sty
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Printed Date : 27/01/2021 15:18:14
By : Fabrizio

Sample Info:
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Amount : 0
ISTD Amount : 0
Dilution : 1

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Description : Default method for Instrument 1
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By : DataApex Ltd.
Modified : 27/01/2021 15:18

Column : IA
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
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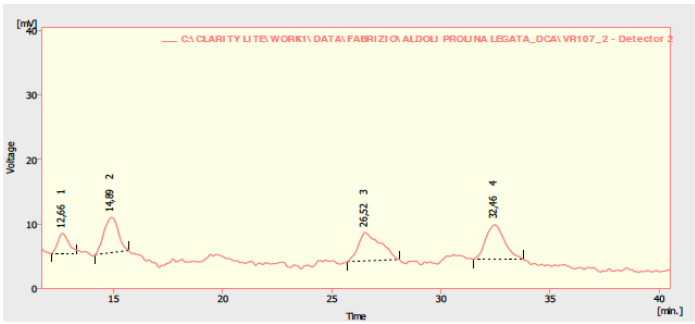

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Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR138rac - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	12,524	729,913	28,591	17,0	25,6	0,48	
2	12,992	844,460	27,576	19,7	24,7	0,52	
3	15,444	1351,312	35,997	31,5	32,3	0,58	
4	18,292	1369,208	19,425	31,9	17,4	1,02	
	Total	4294,893	111,590	100,0	100,0		

27/01/2021 15:18 Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINA LEGATA_DCA\VR107_2.PRM Page 2 of 2

Result Table (Uncal - C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINA LEGATA_DCA\VR107_2 - Detector 2)

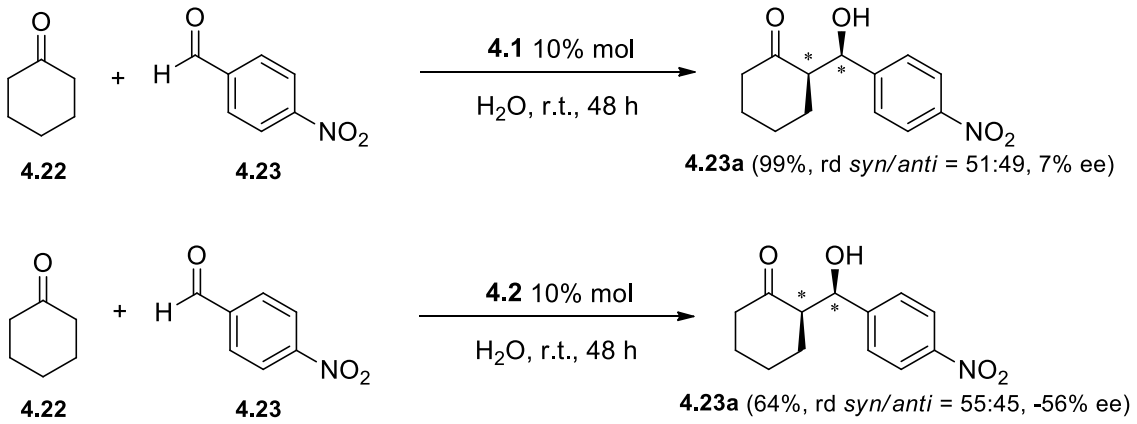
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	12,660	107,274	3,102	10,9	16,9	0,54	
2	14,888	248,562	5,471	25,2	29,8	0,74	
3	26,524	311,938	4,403	31,6	24,0	1,28	
4	32,460	319,108	5,366	32,3	29,3	0,94	
	Total	986,882	18,341	100,0	100,0		

4.4.3 L-Phe derivatives tests

Tests continued using **4.1** and **4.2** as organocatalysts in Aldol reactions, in water, between a model ketone, cyclohexanone **4.22**, and some variously substituted aromatic benzaldehydes under the same conditions as the model reaction already carried out with the proline catalyst. In this way, a first direct comparison between the results obtained with the two types of catalysts functionalized with two different amino acids would have been possible.

To be sure that the amphiphilic nature of the synthesized catalysts was important in determining their catalytic ability, a preliminary test was made using the same conditions described above and as catalyst L-phenylalanine amino acid. The reaction was monitored by thin layer chromatography (TLC), but even after more than 48 hours, no substrate conversion was observed.

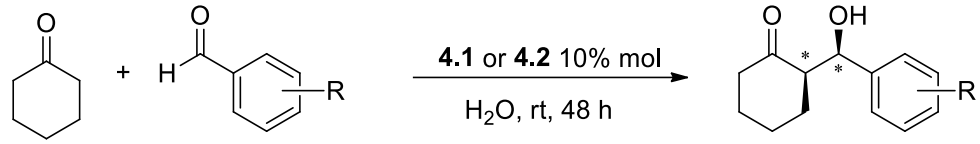
The first reactions carried out with the organocatalysts **4.1** and **4.2**, involved cyclohexanone **4.22** and *p*-nitrobenzaldehyde **4.23** (Scheme 4.35).



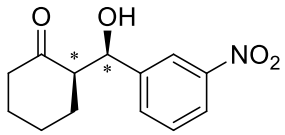
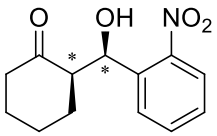
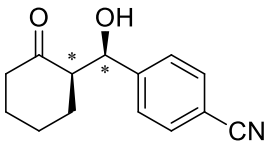
Scheme 4.35. Preliminary reactions carried out with catalysts **4.1** and **4.2**.

According to NMR spectra, the dr resulted not to be very high, and the total conversion of aldehydes into Aldol products took 48 hours. All the yields were calculated after purification on a chromatographic column and ee was calculated from chiral HPLC results.

Furthermore, in order to extend the verification of the catalytic activity to other aldehydes, the reactions was repeated using **4.24**, **4.25** and **4.26** aldehydes (Table 4.6).



5 **4.24-26** **4.24-26a**

Product	Catalyst	Yield (%)	dr (*syn*/*anti*)[a]	ee (%)[b]
 **4.25a**	**4.1**	84	56:44	-31
	4.2	54	70:30	-75
 **4.24a**	**4.1**	72	57:43	-2
	4.2	58	55:45	-6
 **4.26a**	**4.1**	59	56:44	10
	4.2	70	67:33	61

a Calculated on reaction crude
b Calculated on *syn* aldol

Table 4.6. Extending reaction scope results.

The results showed that catalyst **4.1** had a slightly greater efficiency and this could be pointed to its major dispersant ability, due to its greater polarity.

Furthermore, *syn* product was obtained in greater quantities and this resulted to be an unusual result compared to literature data concerning similar aldol reactions and compared to previous results obtained with the previous L-Pro-based catalyst.

4.4.4 Experimental section for L-Phe derivatives

General Informations

Unless otherwise noted, all commercially available compounds were used without further purification. Trends of reactions was monitored by thin layer chromatography (TLC) Merck Kieselgel 60 F254 (0.25mm thickness). Visualization of the developed TLC plates was performed with UV irradiation (254 nm) or by staining with phosphomolybdic acid 12% solution. ^{1}H and ^{13}C spectra were recorded at room temperature on Bruker Avance 400. Analytical HPLC was performed on a Varian 9002 HPLC instrument equipped with a refractive index detector (Schambeck, RI2000), using chiral stationary phases (Chiralpak IA, IB and IC).

High-resolution ESI mass spectra were carried out on a Q-TOF Micro spectrometer operating in a positive ion mode.

All compounds already described were not reported again.

Catalyst synthesis

Procedure for 4.1

In a 25 mL three-necked flask, previously flamed and placed under an Argon atmosphere, equipped with a $CaCl_2$ valve, were dissolved in this order: 0.15 g (1 eq, 0.000356 mol) of amino-derivative, 0.113 g (1.2 eq, 0.000427 mol) of L-Phe-N-Boc, 0.043 g (0.9 eq, 0.00032 mol) of HOBt in 5 mL of anhydrous THF. Subsequently, keeping the mixture at 0 °C in an ice bath, 0.039 mL (0.7 eq, 0.000249 mol) of DIC were added and the reaction mixture was stirred under an Argon atmosphere until the reaction was over, monitoring the development of the reaction by TLC. At the end of the reaction, the white suspension was filtered to remove the diisopropylurea and the filtrate was concentrated under reduced pressure. The resulting oily residue was re-taken up with EtOAc (7 mL) and the organic phase was transferred to a separatory funnel and washed with a 2N aqueous solution of citric acid (8 mL x 2) and then with a saturated aqueous solution of sodium bicarbonate (8 mL x 2) and with a saturated aqueous solution of sodium chloride (8 mL x 2). Finally, the organic solvent was dried over anhydrous sodium sulphate and filtered on a funnel with cotton wool in order to remove the drying agent. The EtOAc was evaporated at reduced pressure and the crude obtained (0.186 g) was purified on a chromatographic column using ethyl acetate/petroleum ether (3:7) mixture as eluent.

Then, in a 25 mL three-necked flask, previously flamed, 0.110 g (1 eq, 0.000164 mol) of amido-compound were dissolved in about 3 mL of anhydrous CH_2Cl_2. Subsequently, keeping the mixture at 0 °C in an ice bath, about 0.3 mL of TFA were added drop by drop. The mixture was kept under stirring and at room temperature for about 4 hours and the progress of the reaction was monitored by TLC. When the reaction was over the mixture was transferred into a separatory funnel and the organic phase was washed with a saturated aqueous solution of sodium bicarbonate (4 mL x 3), with water (3 mL x 2) and with an aqueous sodium chloride saturated solution (3 mL x 2). Finally, the organic phases were dried on anhydrous sodium sulphate and filtered on a funnel with cotton wool in order to remove the drying agent. The CH_2Cl_2 was evaporated under reduced pressure.

At this point, in a 10 mL neck flask, 0.07 g (1 eq, 0.00012 mol) of ester compound were dissolved in approximately 1 mL of MeOH. Then, approximately 1 mL of 2N LiOH was added. The mixture was kept under stirring and at room temperature for about 24 hours. The mixture was concentrated under reduced pressure up to half the volume and then acidified with 1N HCl until complete precipitation of the product (pH = 6.7). The precipitate was isolated by vacuum filtration on Hirsch and dried in an oven (30 °C) for about 24 hours.

Procedure for 4.2

The followed procedure in order to obtain compound **4.2** was the same as the previous one with a difference in the condensation reaction.

In a 25 mL three-necked flask, previously flamed and equipped with a $CaCl_2$ valve, 0.1 g (1 eq, 0.000247 mol) of amino-derivative, 0.066 g (1 eq, 0.000247 mol) of L-Phe-NBoc, 0.05 g (1.5 eq, 0.0003705 mol) of HOBt and 0.07 g (1.5 eq, 0.0003705 mol) of EDC were dissolved in approximately 4 mL of anhydrous CH_2Cl_2. Then 0.057 mL (1.65 eq, 0.000408 mol) of Et_3N were added and the reaction mixture was stirred under an Argon atmosphere until the reaction was over, monitoring the course of reaction by TLC. The mixture was transferred to a separatory funnel (recovering it with about 8 mL of CH_2Cl_2) and the organic phase was washed with an aqueous solution of 2N citric acid (8 mL x 2), then with a saturated aqueous solution of bicarbonate of sodium (8 mL x 2) and with a saturated aqueous solution of sodium chloride (8 mL x 2). Finally, it was dried over anhydrous sodium sulphate and filtered on a funnel with cotton wool in order to remove the drying agent. The CH_2Cl_2 was

evaporated under reduced pressure and the crude obtained was purified on a chromatographic column using the mixture diethyl ether/petroleum ether (7:3) as eluent.

NMR Spectra

Compound 4.1

^{1}H NMR (MeOH-d_4) δ: 0.73 (s, 3H), 0.86 - 0.96 (m, 3H), 1.04 (d, 3H, ^{3}J = 5.93 Hz), 1.06 – 2.40 (cm, 24H, CH_2 e CH steroidic backbone and side chain), 2.95 – 3.13 (m, 2H), 3.81 (bs, 1H), 3.77 – 4.01 (m, 2H), 4.33 - 4.52 (m, 1H), 7.28 (m, 5H). **^{13}C NMR** (MeOH-d_4) δ: 12.5, 17.4, 21.0, 21.2, 23.0, 24.1, 28.0, 28.2, 29.4, 30.2, 31.6, 32.9, 33.4, 35.4, 36.7, 38.0, 40.6, 41.9, 47.0, 51.3, 52.5, 53.9, 65.7, 68.6, 73.2, 73.5, 128.2, 129.4, 129.5, 129.7, 130.1, 130.3, 165.5, 175.7. HRMS (ESI): Calcd for $C_{33}H_{51}N_2O_5^+$ ($[M+H]^+$) 555.3798. Found 555.3800.

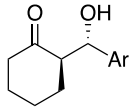
Compound 4.2

^{1}H NMR (DMSO-d_6) δ: 0.59 (s, 3H), 0.84 (s, 3H), 0.91 (d, 3H, ^{3}J = 6.42 Hz), 0.94 – 1.92 (cm, 25H, CH_2 e CH steroidic backbone and side chain), 2.02 - 2.29 (m, 1H), 2.70 - 2.91 (m, 1H), 3.77 (m, 1H), 3.89 (m, 1H), 4.14 (br, 1H), 7.15 - 7.28 (m, 5H), 7.74 (m, 1H). **^{13}C NMR** (DMSO-d_6) δ: 12.9, 17.4, 23.8, 23.9, 24.7, 26.1, 26.9, 27.6, 29.2, 30.4, 30.9, 31.2, 31.3, 32.6, 34.5, 35.4, 35.8, 36.9, 44.7, 46.5, 46.7, 47.9, 55.5, 71.6, 126.7, 128.5, 129.8, 138.2, 167.1, 171.8, 175.4. HRMS (ESI): Calcd for $C_{33}H_{51}N_2O_4^+$ ($[M+H]^+$) 539.3849. Found 539.3853.

Aldol NMR spectra are almost the same as the ones reported in the literature, except in the signals of the aldol characteristic proton on which the rd was calculated.

HPLC results

L-Phenylalanine-sodium cholate or phenylalanine-sodium deoxycholate in water



Ar	Yield [%]	HPLC conditions						dr (*anti*:*syn*) from NMR	dr (*anti*:*syn*) from HPLC	*ee* anti [%]	*ee* syn [%]
		Column	Eluent	RT (major ent) *anti* [min]	RT (minor ent) *anti* [min]	RT (major ent) *syn* [min]	RT (minor ent.) *syn* [min]				
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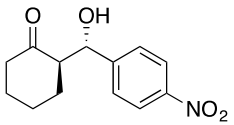
4-NO_2-C_6H_4		IB	Hexane/iPrOH 95:5 (1 ml/min)	26.8	33.6	23.6	22.0		41:59	18	-56
3-NO_2-C_6H_4		IB	Hexane/iPrOH 95:5 (1 ml/min)	25.0	20.7	17.7	17.1	44:56	50:50	-10	-31
3-NO_2-C_6H_4		IB	Hexane/iPrOH 95:5 (1 ml/min)	22.3	27.8	18.4	18.0		37:63	7	-75
2-NO_2-C_6H_4		IA	Hexane/iPrOH 9:1 (1 ml/min)	19.4	18.0	11.9	11.3	43:57	46:54	-25	-2
2-NO_2-C_6H_4		IA	Hexane/iPrOH 9:1 (1 ml/min)	19.0	20.4	12.0	11.7	35:65	53:47	31	(-)6
4-CN-C_6H_4		IB	Hexane/iPrOH 95:5 (1 ml/min)	32.1	26.2	23.9	20.7	44:56	42:58	-1	10
4-CN-C_6H_4		IB	Hexane/iPrOH 95:5 (1 ml/min)	38.0	29.3	25.7	23.0		35:65	-2	61

(R)-2-((S)-hydroxy(4-nitrophenyl)methyl)cyclohexan-1-one (4.23a)



11/01/2021 11:32 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR116rac.PRM Page 1 of 2

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DataApex 2006
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Flow Rate : 1.0 mL/min
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Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
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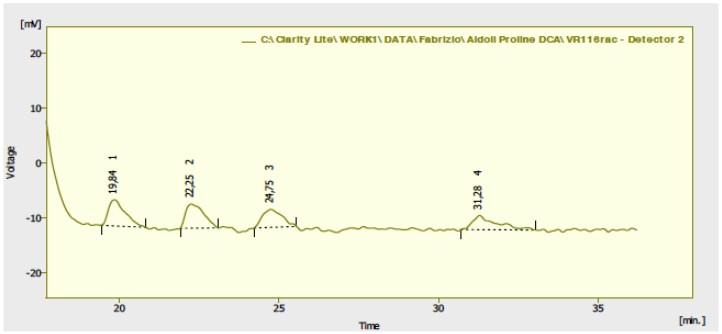

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Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Proline DCA\VR116rac - Detector 2)

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2	22,252	174,628	4,409	27,9	29,3	0,66	
3	24,748	141,350	3,282	22,6	21,8	0,76	
4	31,284	134,738	2,584	21,5	17,2	0,67	
	Total	625,957	15,038	100,0	100,0		

03/03/2021 11:2Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI FENILALANINA-COLATO\IB_95-5_1MLMIN\FP_4NO2.PRM Page 1 of 2

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By : Fabrizio

Sample Info:
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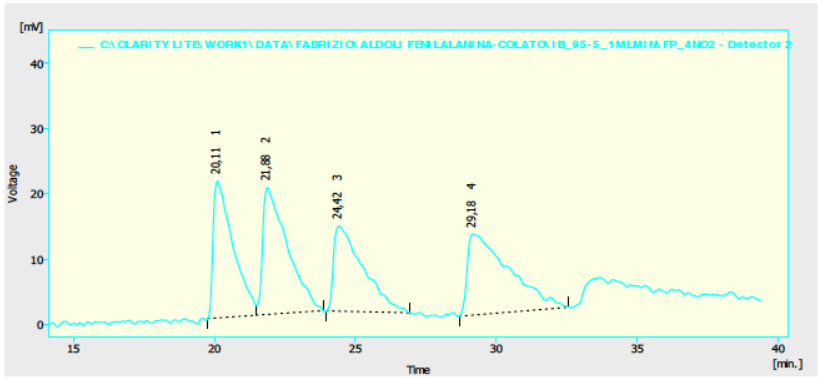
Flow Rate : 1 mL/min
Note :
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



03/03/2021 11:2Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI FENILALANINA-COLATO\IB_95-5_1MLMIN\FP_4NO2.PRM Page 2 of 2

Result Table (Uncal - C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI FENILALANINA-COLATO\IB_95-5_1MLMIN\FP_4NO2 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	20,112	1031,408	20,935	23,3	31,8	0,76	
2	21,880	1183,418	19,399	26,7	29,5	0,94	
3	24,424	947,380	13,044	21,4	19,8	1,12	
4	29,180	1271,631	12,386	28,7	18,8	1,70	
	Total	4433,837	65,763	100,0	100,0		

(R)-2-((S)-hydroxy(3-nitrophenyl)methyl)cyclohexan-1-one (4.25a)

11/01/2021 16:12 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\Report\3-NO2rac.PRM Page 1 of 2

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Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\Report\3-NO2rac.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 11/01/2021 16:11:55
Acquired Date : 11/01/2021 12:53:57
By : Fabrizio

Printed Version Info:
Printed Version : 11/01/2021 13:09:18
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 11/01/2021 16:12:42
By : Fabrizio

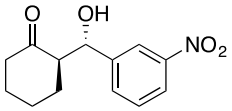
Sample Info:
Sample ID : VR124rac
Sample : 3-NO2 aldol rac
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

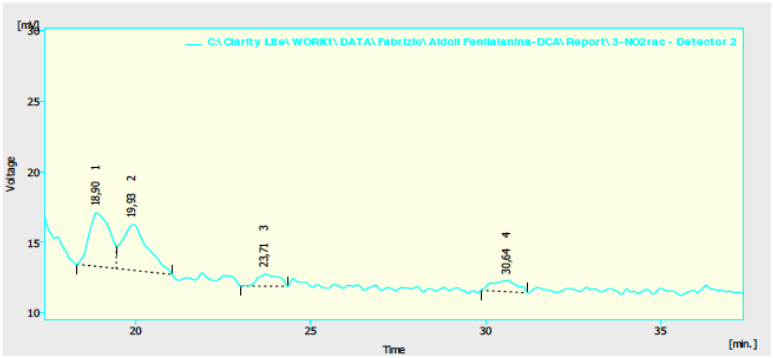
Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 11/01/2021 16:11

Column : IB
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0





11/01/2021 16:12 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\Report\3-NO2rac.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\Report\3-NO2rac - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	18,900	156,317	3,793	39,1	43,4	0,73	
2	19,932	166,773	3,253	41,8	37,3	0,86	
3	23,712	36,155	0,870	9,1	10,0	0,79	
4	30,636	40,146	0,816	10,1	9,3	0,87	
	Total	399,391	8,732	100,0	100,0		

03/03/2021 11:2Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI FENILALANINA-COLATO\IB_95-5_1MLMIN\FP_3NO2.PRM Page 1 of 2

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Chromatogram Info:
File Name : C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI FENILALANINA-COLATO\IB_95-5_1MLMIN\FP_3NO2.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 03/03/2021 11:08:44
Acquired Date : 03/03/2021 11:08:44
By : Fabrizio

Printed Version Info:
Printed Version : 03/03/2021 11:24:50
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 03/03/2021 11:24:55
By : Fabrizio

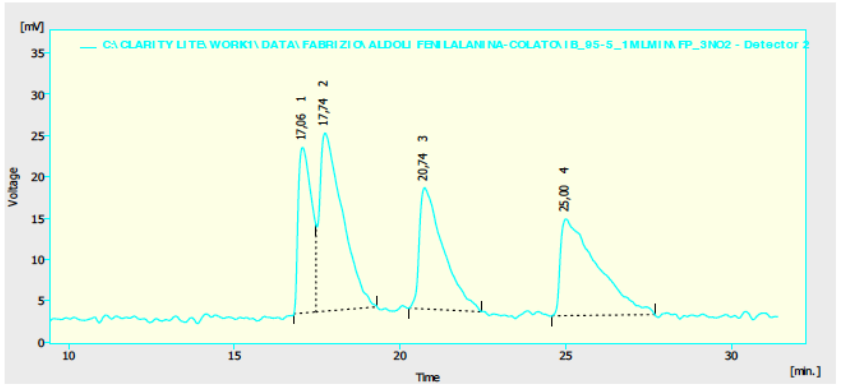
Sample Info:
Sample ID : FP_3NO2
Sample : Aldol 3NO2 fenal-col
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 03/03/2021 11:24

Column : IB
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1 mL/min
Note :
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0

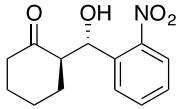


03/03/2021 11:2Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI FENILALANINA-COLATO\IB_95-5_1MLMIN\FP_3NO2.PRM Page 2 of 2

Result Table (Uncal - C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI FENILALANINA-COLATO\IB_95-5_1MLMIN\FP_3NO2 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	17,064	560,212	20,044	17,1	29,5	0,56	
2	17,740	1059,029	21,581	32,4	31,7	0,80	
3	20,744	740,164	14,706	22,6	21,6	0,74	
4	25,004	910,087	11,700	27,8	17,2	1,17	
	Total	3269,492	68,031	100,0	100,0		

(R)-2-((S)-hydroxy(2-nitrophenyl)methyl)cyclohexan-1-one (4.24a)



26/01/2021 15:Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE NON LEGATA DCA\IA_9_1_1MLMIN\VR125RAC.PRM Page 1 of 2

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Chromatogram Info:

File Name	: C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE NON LEGATA DCA\IA_9_1_1MLMIN\VR125RAC.PRM	File Created	: 16/12/2020 17:22:56
Origin	: Acquired	Acquired Date	: 16/12/2020 17:41:21
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

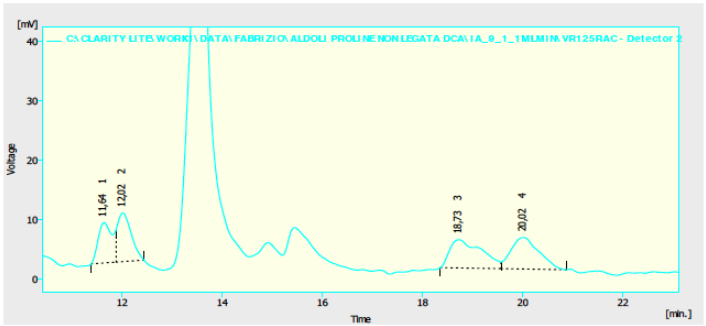
Printed Version	: 26/01/2021 15:19:00	Printed Date	: 26/01/2021 15:19:17
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

Sample Info:

Sample ID	: VR125rac	Amount	: 0
Sample	: 2-NO2 aldol racemate	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 26/01/2021 15:19
Column	: IA	Detection	: RI
Mobile Phase	: H/IPrOH 9:1	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)		
Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



26/01/2021 15:Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE NON LEGATA DCA\IA_9_1_1MLMIN\VR125RAC.PRM Page 2 of 2

Result Table (Uncal - C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE NON LEGATA DCA\IA_9_1_1MLMIN\VR125RAC - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	11,644	133,468	6,765	18,9	27,1	0,38	
2	12,020	153,933	8,173	21,9	32,7	0,32	
3	18,728	210,696	4,746	29,9	19,0	0,79	
4	20,020	206,364	5,277	29,3	21,1	0,64	
	Total	704,461	24,961	100,0	100,0		

03/03/2021 11:24Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI FENILALANINA-COLATO\IA_9-1_1MLMIN\FP_2NO2.PRM Page 1 of 2

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Chromatogram Info:

File Name	: C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI FENILALANINA-COLATO\IA_9-1_1MLMIN\FP_2NO2.PRM	File Created	: 03/03/2021 09:05:12
Origin	: Acquired	Acquired Date	: 03/03/2021 09:10:54
Project	: C:\Clarity Lite\Projects\Work1.PRJ	By	: Fabrizio

Printed Version Info:

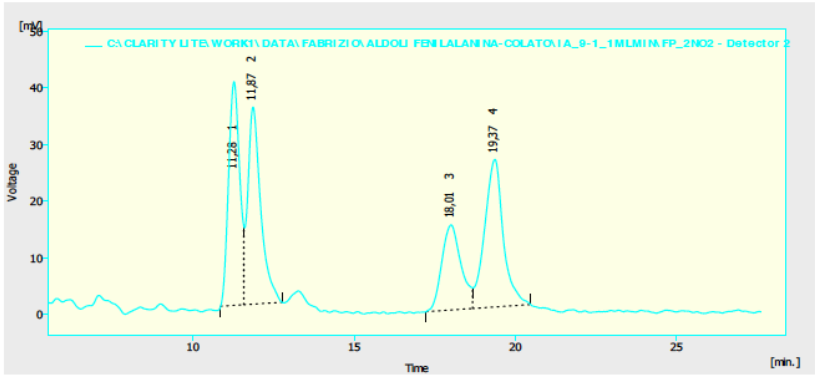
Printed Version	: 03/03/2021 11:23:40	Printed Date	: 03/03/2021 11:24:03
Report Style	: C:\Clarity Lite\Common\Chromatogram.sty	By	: Fabrizio
Calibration File	: None		

Sample Info:

Sample ID	: FP_2NO2	Amount	: 0
Sample	: Aldol 2NO2 fenal-col	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 03/03/2021 11:23
Column	: IA	Detection	: RI
Mobile Phase	: H/IPrOH 90:10	Temperature	:
Flow Rate	: 1 mL/min	Pressure	:
Note	:		
Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



03/03/2021 11:24Chromatogram C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI FENILALANINA-COLATO\IA_9-1_1MLMIN\FP_2NO2.PRM Page 2 of 2

Result Table (Uncal - C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI FENILALANINA-COLATO\IA_9-1_1MLMIN\FP_2NO2 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	11,276	941,697	39,483	26,1	34,2	0,40	
2	11,868	988,705	34,774	27,4	30,1	0,42	
3	18,008	627,000	15,045	17,4	13,0	0,65	
4	19,368	1053,496	26,049	29,2	22,6	0,59	
	Total	3610,898	115,350	100,0	100,0		

4-((S)-hydroxy((R)-2-oxocyclohexyl)methyl)benzonitrile (4.26a)

12/01/2021 09:26 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\VR126rac.PRM Page 1 of 2

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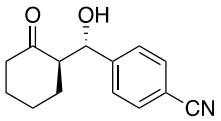
Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\VR126rac.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 12/01/2021 09:08:34
Acquired Date : 12/01/2021 09:08:34
By : Fabrizio

Printed Version Info:
Printed Version : 12/01/2021 09:25:49
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 12/01/2021 09:26:03
By : Fabrizio

Sample Info:
Sample ID : VR126rac
Sample : 4-CN aldol rac
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

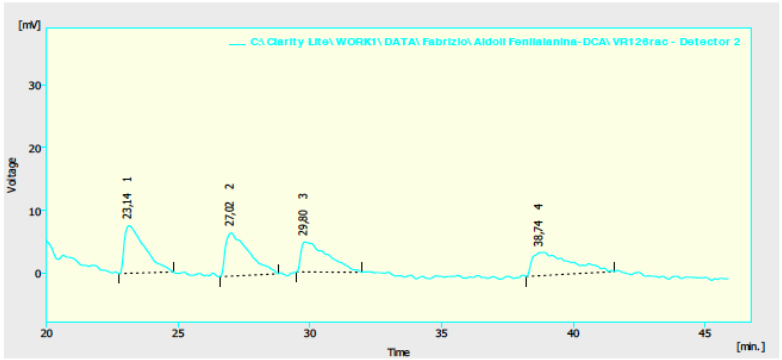


Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 12/01/2021 09:25

Column : IB
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



12/01/2021 09:26 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\VR126rac.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\VR126rac - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	23,136	408,808	7,584	26,4	33,1	0,86	
2	27,024	424,729	6,859	27,4	30,0	1,02	
3	29,804	341,880	4,757	22,1	20,8	1,17	
4	38,740	372,245	3,691	24,1	16,1	1,76	
	Total	1547,661	22,890	100,0	100,0		

03/03/2021 12:03 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli fenilalanina-colato\FP_4CN.PRM Page 1 of 2

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Chromatogram Info:
File Name : C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli fenilalanina-colato\FP_4CN.PRM
Origin : Acquired
Project : C:\Clarity Lite\Projects\Work1.PRJ
File Created : 03/03/2021 11:48:09
Acquired Date : 03/03/2021 11:48:09
By : Fabrizio

Printed Version Info:
Printed Version : 03/03/2021 12:02:58
Report Style : C:\Clarity Lite\Common\Chromatogram.sty
Calibration File : None
Printed Date : 03/03/2021 12:03:04
By : Fabrizio

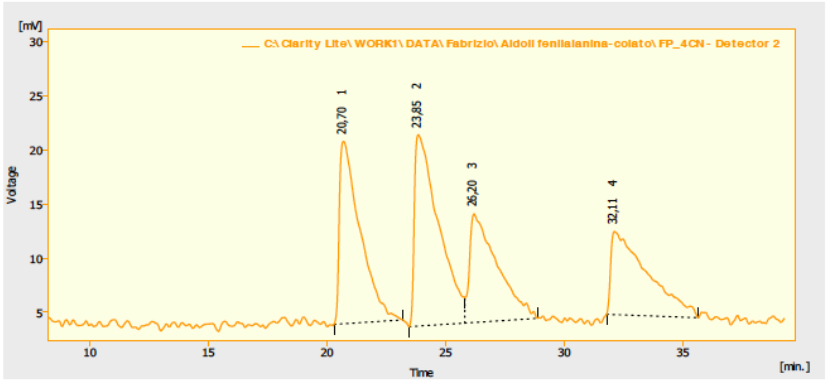
Sample Info:
Sample ID : FP_4CN
Sample : Aldol 4CN fenal-col
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

Method : Default1
Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 03/03/2021 12:02

Column : IB
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1 mL/min
Note :
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



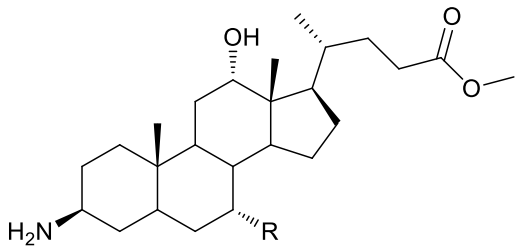
03/03/2021 12:03 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli fenilalanina-colato\FP_4CN.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli fenilalanina-colato\FP_4CN - Detector 2)

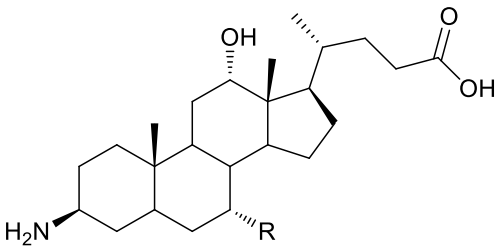
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	20,704	1024,011	16,858	26,1	32,2	0,93	
2	23,848	1248,484	17,684	31,9	33,8	1,12	
3	26,200	818,744	10,047	20,9	19,2	1,25	
4	32,112	826,813	7,685	21,1	14,7	1,57	
	Total	3918,052	52,274	100,0	100,0		

4.4.5 Amino-derivatives tests

Amino-derivatives **4.4a,b** and **4.5a,b** (Figure 4.8) were also tested as organocatalyst in order to verify both their catalytic efficiency, as they contain a primary amino group suitable for this porpose, and whether the ester function somehow could influence the reaction promotion process.



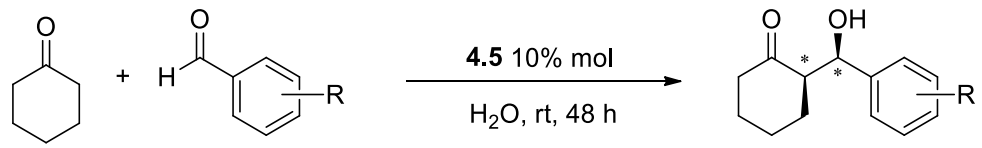
4.4a R = OH
4.4b R = H



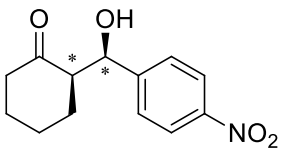
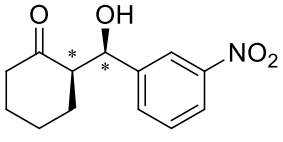
4.5a R = OH
4.5b R = H

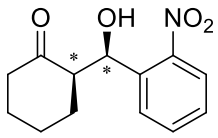
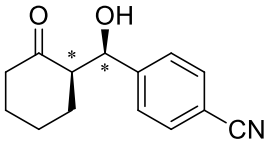
Figure 4.8. Amino-derivatives tested as catalysts.

Therefore, tests were carried out on aldol reactions in water similar to those carried out with previously synthesized compounds, using the same aromatic benzaldehydes, in which compounds **4.4a,b** and **4.5a,b** were the organocatalysts. The results are reported in Table 4.7.



5 **4.23-26** **4.23-26a**

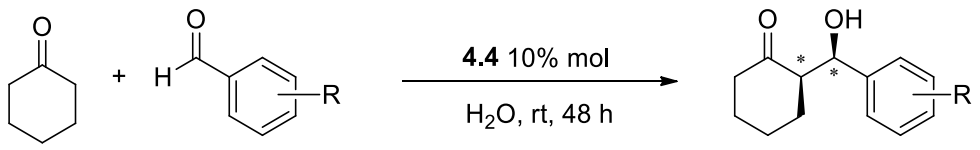
Product	Catalyst	Yield (%)	dr (*syn*/*anti*)[a]	ee (%)[b]
 **4.23a**	**4.5a**	28	-	-6
	4.5b	80	85:15	0
 **4.25a**	**4.5a**	20	-	-4
	4.5b	85	83:17	-24

 4.24a	**4.5a**	-	-	
	4.5b	72	68:32	0
 4.26a	**4.5a**	-	-	
	4.5b	80	72:28	-6

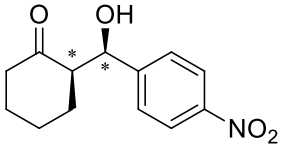
a Calculated on reaction crude
b Calculated on *syn* aldol

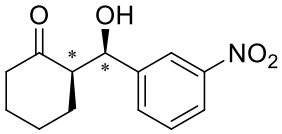
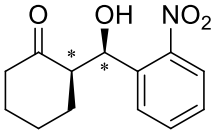
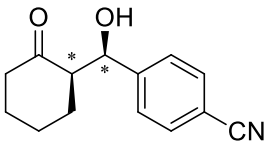
Table 4.7. Tests with **4.5** catalysts.

As can be seen from these results, the catalyst **4.5b** allows to reach significantly higher yields than those of the catalyst **4.5a**. This is probably due to the fact that the increase in the polarity of the concave face of the bile acid could compromise and inhibit the formation of the iminium ion, a fundamental intermediate in *via* enamine catalysis, for solvation reasons.
Furthermore, also in this case the *syn* distereosimer was clearly formed in major quantities compared to the *anti* one.
Finally, tests of aldol reactions in water similar to those described above were carried out, using the compounds **4.4a** and **4.4b** as organocatalysts to evaluate a possible effect of the ester portion on the catalytic activity.
The results are reported in Table 4.8 in relation to aldols **4.23-26a**.



5 4.23-26 4.23-26a

Product	Catalyst	Yield (%)	dr (*syn*/*anti*)[a]	ee (%)[b]
 4.23a	**4.4a**	35	-	2
	4.4b	96	61:39	-4

 4.25a	**4.4a**	29	-	-27
	4.4b	83	65:35	-2
 4.24a	**4.4a**	6	-	-28
	4.4b	48	70:30	-28
 4.26a	**4.4a**	13	-	10
	4.4b	37	59:41	-6

a Calculated on reaction crude
b Calculated on *syn* aldol

Table 4.8. Results using catalysts **4.4**.

The ester nature of the catalyst **4.4** seemed not lead to a decrease in the catalytic activity, in fact, the yields obtained were good. Again, the catalyst **4.4b** proved to be much more effective in converting substrates than the catalyst **4.4a**.

4.4.5 Experimental section for amino-derivatives

General Informations

Unless otherwise noted, all commercially available compounds were used without further purification. Trends of reactions was monitored by thin layer chromatography (TLC) Merck Kieselgel 60 F254 (0.25mm thickness). Visualization of the developed TLC plates was performed with UV irradiation (254 nm) or by staining with phosphomolybdic acid 12% solution. ^{1}H and ^{13}C spectra were recorded at room temperature on Bruker Avance 400. Analytical HPLC was performed on a Varian 9002 HPLC instrument equipped with a refractive index detector (Schambeck, RI2000), using chiral stationary phases (Chiralpak IA, IB and IC).

High-resolution ESI mass spectra were carried out on a Q-TOF Micro spectrometer operating in a positive ion mode.

All compounds already described were not reported again.

NMR Spectra

Compound 4.4a

^{1}H NMR ($CDCl_3$) δ: 0.68 (s, 3H), 0.92 (s, 3H), 0.98 (d, 3H, ^{3}J = 6.21 Hz), 1.02 – 2.66 (cm, 24H, CH_2 e CH steroidic backbone and side chain), 2.75 (m, 1H), 3.66 (s, 3H), 3.84 (m, 1H), 3.97 (m, 1H). **^{13}C NMR** ($CDCl_3$) δ: 12.5, 17.4, 22.8, 23.3, 23.9, 26.1, 27.5, 28.5, 30.3, 30.9, 31.2, 33.5, 33.8, 34.8, 35.3, 35.4, 36.3, 39.4, 41.8, 46.5, 47.2, 51.5, 54.8, 68.4, 73.1, 174.7. HRMS (ESI): Calcd for $C_{25}H_{44}NO_4^+$ ($[M+H]^+$) 422.3270. Found 422.3273.

Compound 4.4b

^{1}H NMR ($CDCl_3$) δ: 0.66 (s, 3H), 0.94 - 0.96 (m, 6H), 0.99 – 2.45 (cm, 25H, CH_2 e CH steroidic backbone and side chain), 3.31 (bs, 1H), 3.64 (s, 3H), 3.97 (m, 1H). **^{13}C NMR** ($CDCl_3$) δ: 12.6, 17.2, 23.4, 23.6, 26.0, 26.6, 26.8, 27.4, 28.9, 29.5, 30.8, 31.0, 32.6, 32.9, 33.1, 34.6, 34.7, 35.0, 35.7, 36.2, 46.4, 48.3, 51.5, 73.1, 174.5. HRMS (ESI): Calcd for $C_{25}H_{44}NO_5^+$ ([M+H]+) 406.3321. Found 406.3323.

Compound 4.5a

^{1}H NMR (MeOH-d_4) δ: 0.72 (s, 3H), 0.94 - 1.04 (m, 6H), 1.08 – 2.42 (cm, 24H, CH_2 e CH steroidic backbone and side chain), 2.67 (m, 1H), 3.81 (m, 1H), 3.97 (m, 1H). **^{13}C NMR** (MeOH-d_4) δ: 12.6, 17.2, 22.5, 23.8, 27.2, 28.3, 29.2, 30.2, 30.8, 31.4, 31.9, 32.0, 34.5, 35.7, 35.8, 37.0, 40.5, 42.6, 47.1, 47.6, 57.5, 68.3, 73.4, 178.2. HRMS (ESI): Calcd for $C_{24}H_{42}NO_4^+$ ([M+H]+) 408.3114. Found 408.3118.

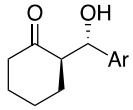
Compound 4.5b

^{1}H NMR (MeOH-d_4) δ: 0.72 (s, 3H), 1.00 - 1.04 (m, 6H), 0.99 (bs, 3H), 1.06 – 2.34 (cm, 25H, CH_2 e CH steroidic backbone and side chain), 2.67 (s, 1H), 3.98 (m, 1H). **^{13}C NMR** (MeOH-d_4) δ: 12.8, 17.4, 23.2, 23.8, 24.4, 26.5, 27.0, 28.3, 29.4, 29.6, 30.0, 31.4, 33.4, 33.7, 35.2, 35.5, 36.7, 36.8, 37.1, 47.2, 49.4, 57.4, 73.6, 182.7. HRMS (ESI): Calcd for $C_{24}H_{42}NO_3^+$ ([M+H]+) 392.3165. Found 392.3167.

Aldol NMR spectra are almost the same as the ones reported in the literature, except in the signals of the aldol characteristic proton on which the rd was calculated.

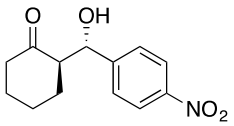
HPLC results

3-amino ester (deoxycholic acid derivative) or 3-amino ester (cholic acid derivative) in water



Ar	Yield [%]	HPLC conditions						dr (*anti*:*syn*) from NMR	dr (*anti*:*syn*) from HPLC	*ee* anti [%]	*ee* syn [%]
		Column	Eluent	RT (major ent) *anti* [min]	RT (minor ent) *anti* [min]	RT (major ent) *syn* [min]	RT (minor ent.) *syn* [min]				
4-NO_2-C_6H_4		IB	Hexane/iPrOH 95:5 (1 ml/min)	25.3	30.1	20.3	22.3		35:65	-10	-4
4-NO_2-C_6H_4		IB	Hexane/iPrOH 95:5 (1 ml/min)	27.2	33.8	23.7	21.6		30:70	-3	2
3-NO_2-C_6H_4		IB	Hexane/iPrOH 95:5 (1 ml/min)	21.1	25.9	17.9	16.9		35:65	-6	-2
3-NO_2-C_6H_4		IB	Hexane/iPrOH 95:5 (1 ml/min)	29.0	23.0	19.1	18.4		18:82	10	-27
2-NO_2-C_6H_4		IA	Hexane/iPrOH 9:1 (1 ml/min)	18.2	17.1	11.1	10.8		34:66	10	-28
2-NO_2-C_6H_4		IA	Hexane/iPrOH 9:1 (1 ml/min)	17.9	19.1	11.4	11.3		48:52	-12	-28
4-CN-C_6H_4		IB	Hexane/iPrOH 95:5 (1 ml/min)	27.4	34.6	20.3	24.2		26:74	8	-6
4-CN-C_6H_4		IB	Hexane/iPrOH 95:5 (1 ml/min)	38.3	29.7	22.9	26.6		45:55	-10	10

(R)-2-((S)-hydroxy(4-nitrophenyl)methyl)cyclohexan-1-one (4.23a)



11/01/2021 11:32 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoll Proline DCA\VR116rac.PRM Page 1 of 2

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By : Fabrizio

Sample Info:
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Sample : 4-NO2 aldol rac
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

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By : DataApex Ltd.
Modified : 11/01/2021 11:32

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Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
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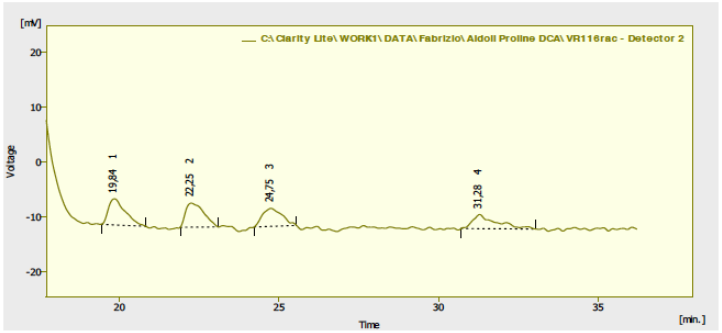
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



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	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	19,844	175,242	4,762	28,0	31,7	0,58	
2	22,252	174,628	4,409	27,9	29,3	0,66	
3	24,748	141,350	3,282	22,6	21,8	0,76	
4	31,284	134,738	2,584	21,5	17,2	0,67	
	Total	625,957	15,038	100,0	100,0		

19/04/2021 19:11 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\3-ammino estere colico\FP_4-NO2.PRM Page 1 of 2

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By : Fabrizio

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Calibration File : None
Printed Date : 19/04/2021 19:11:55
By : Fabrizio

Sample Info:
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Sample : 4-NO2 aldol
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

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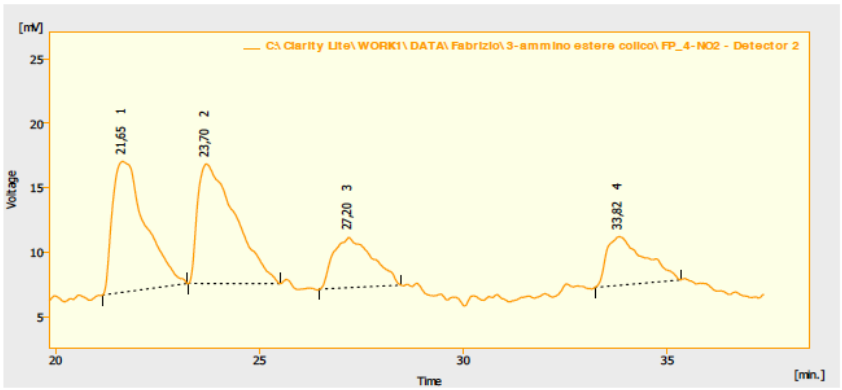
By : DataApex Ltd.
Modified : 19/04/2021 19:11

Column : IB
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1 mL/min
Note :
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



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1	21,652	570,711	10,156	34,0	37,5	0,87	
2	23,700	598,913	9,266	35,7	34,2	1,08	
3	27,196	261,010	3,903	15,6	14,4	1,22	
4	33,820	245,484	3,790	14,6	14,0	1,34	
	Total	1676,118	27,115	100,0	100,0		

(R)-2-((S)-hydroxy(3-nitrophenyl)methyl)cyclohexan-1-one (4.25a)

11/01/2021 16:12 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\Report\3-NO2rac.PRM Page 1 of 2

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By : Fabrizio

Sample Info:
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Sample : 3-NO2 aldol rac
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

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Description : Default method for Instrument 1
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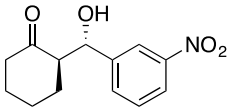
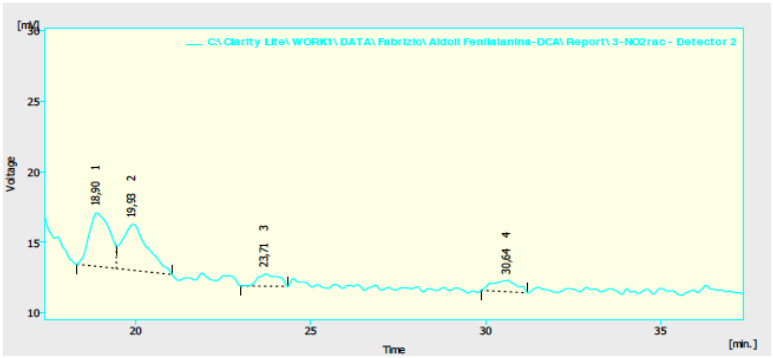
Modified : 11/01/2021 16:11

Column : IB
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0





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Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\Report\3-NO2rac - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	18,900	156,317	3,793	39,1	43,4	0,73	
2	19,932	166,773	3,253	41,8	37,3	0,86	
3	23,712	36,155	0,870	9,1	10,0	0,79	
4	30,636	40,146	0,816	10,1	9,3	0,87	
	Total	399,391	8,732	100,0	100,0		

19/04/2021 19:46 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\3-ammino estere colico\FP_3-NO2.PRM Page 1 of 2

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By : Fabrizio

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Printed Date : 19/04/2021 19:46:35
By : Fabrizio

Sample Info:
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Sample : 3-NO2 aldol
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

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Description : Default method for Instrument 1
Created : 31/08/2006 15:43
By : DataApex Ltd.
Modified : 19/04/2021 19:46

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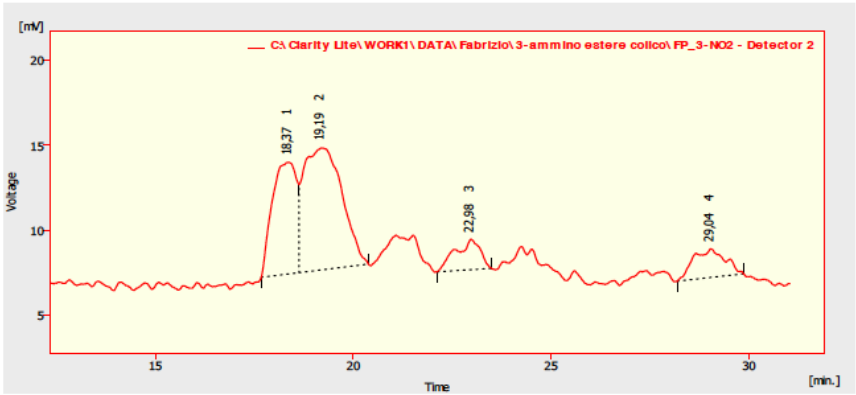
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1 mL/min
Note :
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



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Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\3-ammino estere colico\FP_3-NO2 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	18,372	281,101	6,588	29,8	38,1	0,75	
2	19,192	488,160	7,192	51,8	41,6	1,18	
3	22,976	78,354	1,802	8,3	10,4	0,85	
4	29,036	94,557	1,692	10,0	9,8	1,09	
	Total	942,172	17,275	100,0	100,0		

(R)-2-((S)-hydroxy(2-nitrophenyl)methyl)cyclohexan-1-one (4.24a)

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DataApex 2006
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Calibration File	: None		

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Sample	: 2-NO2 aldol racemate	ISTD Amount	: 0
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Description	: Default method for Instrument 1		
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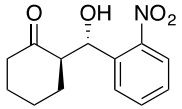
Column	: IA	Detection	: RI
Mobile Phase	: H/IPrOH 9:1	Temperature	:
Flow Rate	: 1.0 mL/min	Pressure	:
Note	: Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.2 and 3.84 min, checked with blank injection)		



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Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

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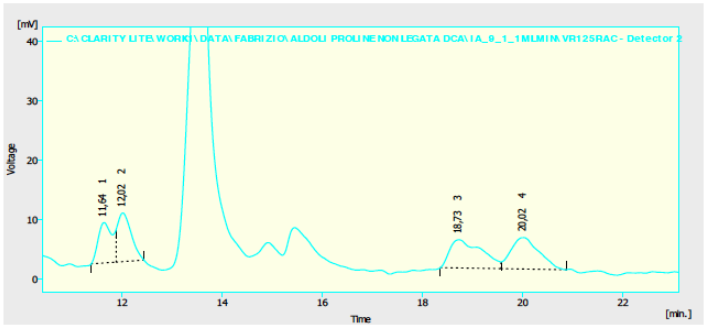
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



Result Table (Uncal - C:\CLARITY LITE\WORK1\DATA\FABRIZIO\ALDOLI PROLINE NON LEGATA DCA\IA_9_1_1MLMIN\VR125RAC - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	11,644	133,468	6,765	18,9	27,1	0,38	
2	12,020	153,933	8,173	21,9	32,7	0,32	
3	18,728	210,696	4,746	29,9	19,0	0,79	
4	20,020	206,364	5,277	29,3	21,1	0,64	
	Total	704,461	24,961	100,0	100,0		

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Chromatogram Info:

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Printed Version Info:

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Calibration File	: None		

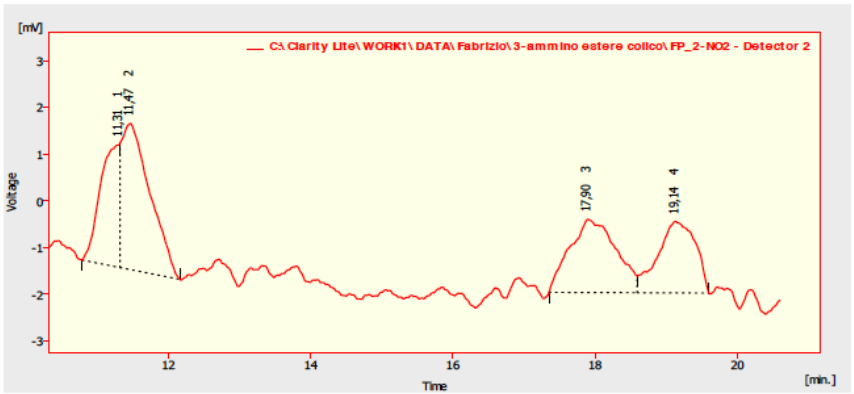
Sample Info:

Sample ID	: FP_2-NO2	Amount	: 0
Sample	: 2-NO2 aldol	ISTD Amount	: 0
Inj. Volume [mL]	: 0,1	Dilution	: 1

Method	: Default1	By	: DataApex Ltd.
Description	: Default method for Instrument 1		
Created	: 31/08/2006 15:43	Modified	: 19/04/2021 20:15
Column	: IA	Detection	: RI
Mobile Phase	: H/IPrOH 9:1	Temperature	:
Flow Rate	: 1 mL/min	Pressure	:
Note	:		

Autostop	: None	External Start	: Start - Restart, Down
Detector 1	: Detector 2	Range 1	: Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base	: Not Used	Calibration File	: None	Calculation	: Uncal
Scale Factor	: Not Used	Units After Scaling	: Not Used	Uncal. Response	: 0
Result Table Reports	: All Peaks	Hide ISTD Peak	: Enabled		
GPC Calibration File	:				



Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\3-ammino estere colico\FP_2-NO2 - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	11,312	48,570	2,641	18,5	29,8	0,33	
2	11,468	86,882	3,129	33,0	35,3	0,43	
3	17,896	71,126	1,568	27,0	17,7	0,79	
4	19,136	56,583	1,530	21,5	17,3	0,62	
	Total	263,162	8,867	100,0	100,0		

4-((S)-hydroxy((R)-2-oxocyclohexyl)methyl)benzonitrile (4.26a)

12/01/2021 09:26 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\VR126rac.PRM Page 1 of 2

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Chromatogram Info:
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By : Fabrizio

Printed Version Info:
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Printed Date : 12/01/2021 09:26:03
By : Fabrizio

Sample Info:
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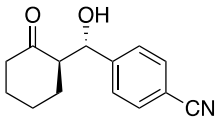
Dilution : 1



Method : Default1
Description : Default method for Instrument 1
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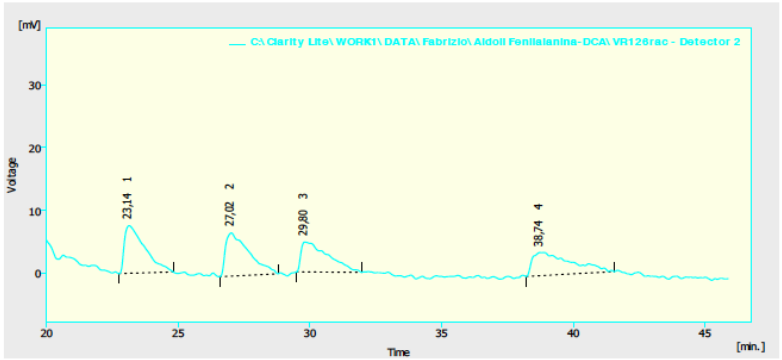
By : DataApex Ltd.
Modified : 12/01/2021 09:25

Column : IB
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1.0 mL/min
Note : Sample dissolved in Hexane/IPrOH 95:5 + DCM (solvent peak at 3.8 min, checked with blank injection)
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



12/01/2021 09:26 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\VR126rac.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\Aldoli Fenilalanina-DCA\VR126rac - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	23,136	408,808	7,584	26,4	33,1	0,86	
2	27,024	424,729	6,859	27,4	30,0	1,02	
3	29,804	341,880	4,757	22,1	20,8	1,17	
4	38,740	372,245	3,691	24,1	16,1	1,76	
	Total	1547,661	22,890	100,0	100,0		

19/04/2021 18:33 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\3-ammino estere colico\FP_4-CN.PRM Page 1 of 2

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Chromatogram Info:
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File Created : 19/04/2021 18:28:40
Acquired Date : 19/04/2021 18:28:40
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Calibration File : None
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By : Fabrizio

Sample Info:
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Sample : 4-CN aldol
Inj. Volume [mL] : 0,1
Amount : 0
ISTD Amount : 0
Dilution : 1

Method : Default1
Description : Default method for Instrument 1
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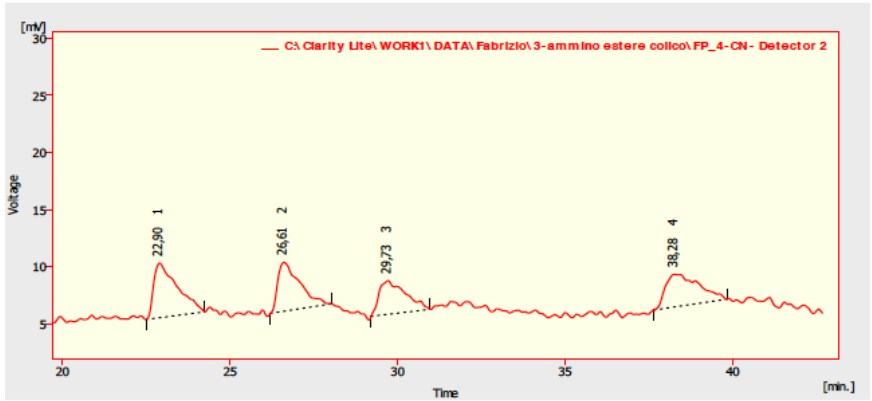
By : DataApex Ltd.
Modified : 19/04/2021 18:33

Column : IB
Mobile Phase : H/IPrOH 95:5
Flow Rate : 1 mL/min
Note :
Detection : RI
Temperature :
Pressure :

Autostop : None
Detector 1 : Detector 2
External Start : Start - Restart, Down
Range 1 : Bipolar, 1250 mV, 12,5 Samp. per Sec.

Base : Not Used
Scale Factor : Not Used
Result Table Reports : All Peaks
GPC Calibration File :
Calibration File : None
Units After Scaling : Not Used
Hide ISTD Peak : Enabled
Calculation : Uncal
Uncal. Response : 0



19/04/2021 18:33 Chromatogram C:\Clarity Lite\WORK1\DATA\Fabrizio\3-ammino estere colico\FP_4-CN.PRM Page 2 of 2

Result Table (Uncal - C:\Clarity Lite\WORK1\DATA\Fabrizio\3-ammino estere colico\FP_4-CN - Detector 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	22,900	233,113	4,766	29,7	32,1	0,82	
2	26,608	194,088	4,274	24,8	28,8	0,80	
3	29,728	161,297	2,939	20,6	19,8	0,99	
4	38,276	195,261	2,852	24,9	19,2	1,30	
	Total	783,758	14,831	100,0	100,0		

4.4.6 Other spectroscopic data

Spectroscopic data of compounds, which are not present in literature, are herein reported.

(R)-*tert*-butyl 2-(((3S,5R,8R,9S,10R,12S,13R,14S,17R)-12-hydroxy-17-((R)-5-methoxy-5-oxopentan-2-yl)-5,8,10,13-tetramethylhexadecahydro-1H-cyclopenta[a]phenanthren-3-yl)carbamoyl)pyrrolidine-1-carboxylate (4.12)

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 0.67 (s, 3H), 0.93 (s, 3H), 0.95 (d, 3H, *J*=6.05 Hz), 0.98-2.50 (cm, 27H, CH and CH_2 steroidic backbone and side chain), 1.47 (s, 9H), 2.20-2.48 (cm, 4H), 3.38-3.49 (m, 2H), 3.46 (m, 2H), 3.65 (s, 3H), 4.08 (m, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ(ppm): 12.7, 17.3, 23.1, 23.6, 23.6, 26.1, 27.1, 27.5, 28.4 (3C), 28.9, 30.6, 30.9, 31.0, 33.6, 34.1, 35.2, 35.2, 36.0, 36.4, 42.0, 46.5, 47.1, 47.3, 48.2, 48.5, 51.8, 73.1, 174.7. HRMS (ESI): Calcd for $C_{35}H_{59}N_2O_6^+$ ($[M+H]^+$) 603.4373. Found 603.4375.

(4R)-methyl 4-((3S,10S,12S,13R,17R)-12-hydroxy-10,13-dimethyl-3-((R)-pyrrolidine-2-carboxamido)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (4.13)

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 0.67 (s, 3H), 0.93 (3, 3H), 0.95 (d, 3H, J=6,05 Hz), 0.98-2.50 (cm, 27H, CH and CH_2 steroidic backbone and side chain), 1.80-2.30 (cm, 4H), 3.47 (m, 2H), 3.65 (s, 3H), 3.72 (m, 1H), 3.96 (m, 1H), 4.11 (m, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ(ppm): 12.7, 17.3, 23.1, 23.6, 23.6, 26.1, 27.1, 27.5, 28.4 (3C), 28.9, 30.6, 30.9, 31.0, 33.6, 34.1, 35.2, 35.2, 36.0, 36.4, 42.0, 46.5, 47.1, 47.3, 48.2, 48.3, 51.5, 73.7, 174.7. HRMS (ESI): Calcd for $C_{30}H_{51}N_2O_4^+$ ($[M+H]^+$) 503.3849. Found 503.3851.

(2R)-*tert*-butyl 2-(((3R,9S,10S,12S,13R,14S,17R)-12-hydroxy-17-((R)-5-methoxy-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-3-yl)carbamoyl)pyrrolidine-1-carboxylate (4.14)

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 0.67 (s, 3H), 0.93 (s, 3H), 0.95 (d, 3H, *J*=6.05 Hz), 0.98-2.50 (cm, 27H, CH and CH_2 steroidic backbone and side chain), 1.43 (s, 9H), 1.80-2.30 (cm, 4H), 3.40 (m, 2H), 3.50 (m, 2H), 3.65 (s, 3H), 3.97 (m, 1H), 4.20 (m, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ(ppm): 12.7, 17.3, 23.1, 23.6 (2C), 26.1, 27.1, 27.5, 28.4 (3C), 28.9, 30.6, 30.9, 31.0, 33.6, 34.1, 35.2, 35.2, 36.0, 36.4, 42.0, 46.5, 47.1, 47.3, 48.2, 48.3, 51.5, 73.7, 174.7. HRMS (ESI): Calcd for $C_{35}H_{59}N_2O_6^+$ ($[M+H]^+$) 603.4373. Found 603.4376.

(4R)-methyl 4-((3R,10S,12S,13R,17R)-12-hydroxy-10,13-dimethyl-3-((R)-pyrrolidine-2-carboxamido)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (4.15)

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 0.67 (s, 3H), 0.93 (3, 3H), 0.95 (d, 3H, J=6,05 Hz), 0.98-2.50 (cm, 27H, CH and CH_2 steroidic backbone and side chain), 1.80-2.30 (cm, 4H), 3.40 (m, 2H), 3.50 (s, 3H), 3.65 (m, 1H),3.97 (m, 1H), 4.20 (m, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ(ppm): 12.7, 17.3, 23.1, 23.6, 23.6, 26.1, 27.1, 27.5, 28.4 (3C), 28.9, 30.6, 30.9, 31.0, 33.6, 34.1, 35.2, 35.2, 36.0, 36.4, 42.0, 46.5, 47.1, 47.3, 48.2, 48.3, 51.5, 73.7, 174.7. HRMS (ESI): Calcd for $C_{30}H_{51}N_2O_4^+$ ($[M+H]^+$) 503.3849. Found 503.3853.

(4R)-methyl 4-((3S,7R,10S,12S,13R,17R)-3- ((S)-2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)-7,12-dihydroxy-10,13-dimethylhesadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (4.18)

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 0.68 (s, 3H), 0.88 (s, 3H), 0.99 (d, 3H, *J*=5.97 Hz), 1.41 (s, 9H), 1.06-2.45 (cm, 24H, CH and CH_2 steroidic backbone and side chain), 2.18-2.43 (m, 2H), 3.65 (s, 3H), 3.88 (m, 1H), 3.98 (m, 1H), 4.74 (m, 1H), 4.84 (m, 1H), 5.73 (m, 1H), 7.14-7.31 (m, 6H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ(ppm): 12.56, 17.43, 21.26, 23.65, 27.43, 28.35, 30.20, 30.93, 31.05, 33.78, 33.97, 34.00, 34.67, 35.17, 36.92, 39.36, 40.38, 41.17, 46.29, 47.03, 49.85, 51.50, 51.92, 52.35, 67.99, 68.45, 72.91, 79.50, 126.81, 128.40, 128.46, 128.48, 129.58, 136.55, 154.97, 166.23, 174.76. HRMS (ESI): Calcd for $C_{39}H_{61}N_2O_7^+$ ($[M+H]^+$) 669.4474. Found 669.4477.

(4R)-methyl 4-((3S,10S,12S,13R,17R)-3- ((S)-2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)-12-dihydroxy-10,13-dimethylhesadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (4.19)

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 0.65 (s, 3H), 0.81 (s, 3H), 0.95 (d, 3H, *J*=5.57 Hz), 1.42 (s, 9H), 0.96-1.96 (cm, 25H, CH and CH_2 steroidic backbone and side chain), 2.85-3.15 (m, 2H), 3.64 (s, 3H), 3.94 (m, 1H), 4.03 (bs, 1H), 4.24 (bs, 1H), 5.33 (bs, 1H), 5.80 (m, 1H), 7.13-7.32 (m, 5H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ(ppm): 12.72, 17.32, 23.59, 24.61, 25.82, 26.60, 27.40, 28.33, 28.81, 29.69, 30.26, 30.32, 30.90, 30.96, 31.02, 32.84, 34.38, 34.96, 35.05, 35.74, 37.41, 39.17, 45.13, 46.50, 47.23, 48.25, 51.49, 73.09, 125.47, 126.89, 128.74, 129.12, 129.18, 137.14, 155.27, 169.88, 174.69. HRMS (ESI): Calcd for $C_{39}H_{61}N_2O_6^+$ ($[M+H]^+$) 653.4525. Found 653.4527

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Chapter 5: Synthesis of BAs ferrocenyl derivatives for sensors and molecular electronic applications

5.1 BAs and carbon nanotubes interactions

A research field that has recently developed considers the covalent and non-covalent interactions between bile acids and carbon nanotubes (CNTs).

Carbon nanotubes are one of the allotropic forms of carbon and can be described by one or more planes of graphene wrapped together around themselves.[1]

The latter give rise to a cylindrical structure with a diameter of nanometers,[2,3] and a length ranging from hundreds of nanometers to micrometers. They can also be closed at the ends by hemispherical caps of the fullerenic type,[4,5] therefore formed by pentagonal and hexagonal rings which, by imparting the right curvature, allow the closure of the graphitic cylinder. The central part of the tube, on the other hand, is mainly formed by carbon atoms with sp^2 hybridization organized in hexagonal rings, although as the diameter of the cylinder decreases and therefore the curvature of the graphene sheet increases, there is an increase in the presence of sp^3 hybridization carbon atoms (Figure 5.1).

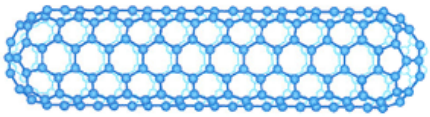


Figure 5.1. Carbon nanotube.

CNTs can generally be divided into two subsets: (i) Single Walled Carbon NanoTube (SWCNT), consisting of a single graphitic sheet and (ii) Multi Walled Carbon NanoTube (MWCNT), 16 consisting of several sheets wrapped one over the other in concentric manner (Figure 5.2).[6]

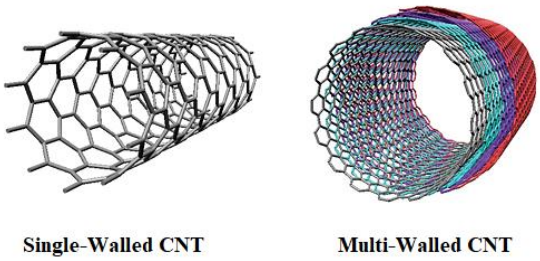


Figure 5.2. Single Walled nanotube or SWCNT (left), Multi Walled nanotube or MWCNT (right).

In these materials, very interesting characteristics have been found, such as high mechanical strength, good elasticity and excellent thermal and electrical conductivity, which allows them to behave as metal conductors or semiconductors.[7] Their conductivity properties make CNTs suitable for electrochemical applications. It has been noted that the deposition on the CNT electrode leads to a substantial increase in the electroactive surface area of the electrode itself and consequently, to an increase in the sensitivity of the measurements and in the speed of electronic transfer between analyte and detector. It was therefore natural to use them in sensors field.

Chemical sensors, in general, are devices capable of giving a quantitative response to the presence of a specific analyte. To do this, it is necessary that there is (i) a receptor, able to recognize the molecule under examination, and to undergo some modification of its chemical-physical properties after interaction with it; (ii) a transducer, which converts the information of the receptor modification into an electrical signal (Figure 5.3).

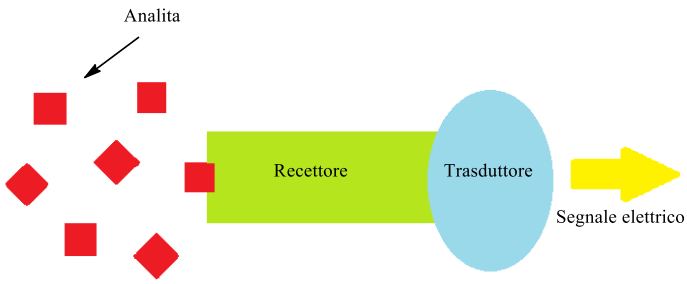


Figure 5.3. Schematic representation of a generic sensor.

Depending on the type of receptor and transducer used, we will obtain different types of sensors.

In electrochemical sensors field, the transducer consists of a chemically modified electrode on the surface able to host the receptor, which interacts with the analyte. If the receptor is a

biological compound then we are talking about electrochemical biosensors. These are divided into potentiometric, amperometric, conductometric and impedimetric, depending on the type of detector and the nature of the measured signal.

For our reserch purpose amperometric biosensors, through which the current variation produced by the interactions between the analyte and the recognition system is measured, are very insteresting. These particular biosensors are divided into first, second and third generation.

In the case of first-generation biosensors, they work indirectly, i.e. the biomolecule reacts with the substrate producing a secondary product that will be able to interact with the electrode. However, these biosensors have two major limitations, one is the formation of the secondary product may not be quantitative leading to a decrease in sensitivity, the second is due to the fact that other chemical species which participate in the electrode process may be present. An example of a first generation sensor is the glucose biosensor, in which glucose is transformed into gluconic acid by oxygen with the concomitant production of hydrogen peroxide, then detected through a process of oxidation, on an electrode of platinum (Figure 5.6). In this case the presence of ascorbic acid and uric acid, which can in turn be oxidized, exemplifies one of the aforementioned limits.[8]

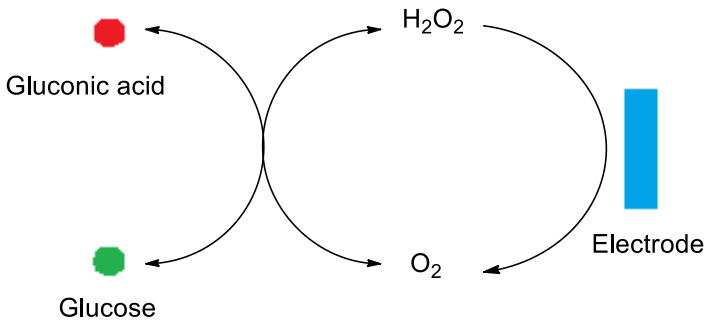


Figure 5.6. Example diagram of first generation electrochemical amperometric biosensor.

On the other hand, second generation biosensors use a redox mediator, able to reduce and to oxidize, which allows the transfer of the electronic charge from the biomolecule to the electrode surface, therefore they also work indirectly (Figure 5.7).[9] Obviously, the redox mediator must be stable in both forms, oxidized and reduced, the reaction must be fast and reversible and the redox potential must be low. The most widely used mediators are transition metal cations and their complexes such as ferrocene or even Prussian blue.[10]

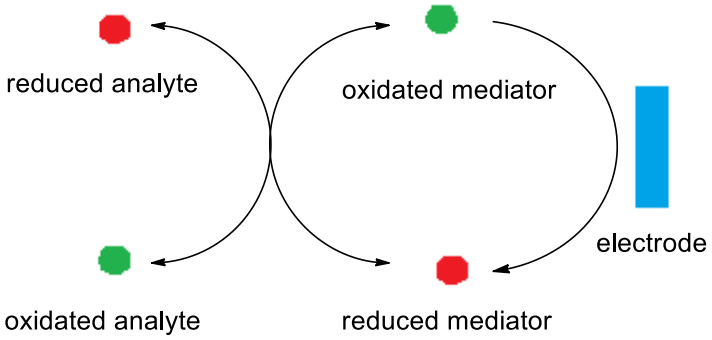


Figure 5.7. Second generation amperometric electrochemical biosensor.

Third generation biosensors work through a direct electronic transfer which takes place directly between the redox center of the biomolecule and the electrode surface (Figure 5.8).[11] These biosensors are mainly limited to proteins or enzymes in which the redox center is external and to electrodes functionalized with materials capable of minimizing the distance between biomolecule and electrode as much as possible.

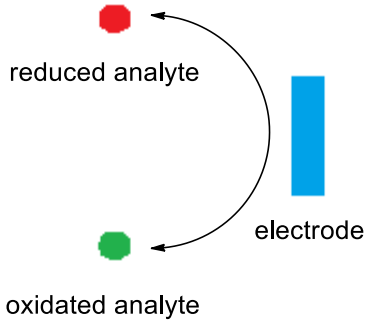


Figure 5.8. Example diagram of third generation electrochemical biosensors.

A major limitation in the use of CNTs as chemical and biological sensors in aqueous environments,[12-13] however, lies in their low dispersion capacity in both aqueous and organic solutions.[14] Faced with this, an attempt was made to increase its dispersion through the use of surfactants.

The surfactant-assisted dispersion process of CNTs is a process that requires an input of external energy (ultrasound), useful for separating the nanotubes and therefore for breaking the strong Van der Waals interactions between them. The carbon nanotubes walls, once separated, will be available as absorption sites for the surfactant molecules.

Based on these observations, bile salts have been observed to be effective dispersing agents, as their slightly bent rigid skeleton seems to adapt very effectively to the curvature of the CNT surface, preventing their re-aggregation.[15]

In fact, the cholate ions will wrap around the nanotube, orienting themselves perpendicularly to the cylinder axis, placing the hydrophilic face outwards and the hydrophobic face in contact with the surface of the CNTs (Figure 5.9).[16]

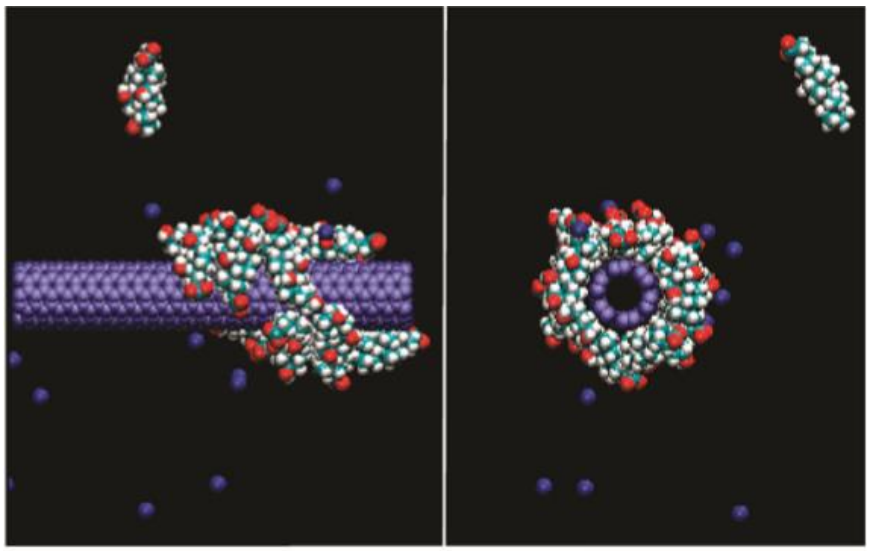


Figure 5.9. Absorption simulation of the sodium cholate (at a concentration of 75mM) on a SWCNT surface.

In addition to offering dispersing properties, bile acids could be an efficient way for the electrodes surface modification, being an element of the set of nano-structured materials, in order to increase their sensitivity.

This requires the development of a molecule, derivative of bile acid, which is at the same time a mediator of ET and an excellent dispersant of carbon nanotubes, favoring their deposition.

5.1.1 Ferrocenyl-surfactant derivatives

The introduction of a ferrocenyl subunit into the structure of a classical type surfactant has already been described, as the ferrocene is chemically stable in solution in a wide range of conditions, and its redox potential made it easy to change in its oxidation state. The oxidation of the ferrocene unit contained in the ferrocenium ion surfactant lead to a change in the formal charge, so there is an important change in the polarity properties of the surfactant, and consequently in the aggregative properties.

Numerous examples of ferrocenyl-substituted surfactants are reported in the literature. The reported derivatives were neutral, or cationic or anionic (some examples are shown in Figure

5.10). The interesting aspect was that depending on the structure of the surfactant and the position of the ferrocene within it, a wide range of different surfactant properties was observed.[17]

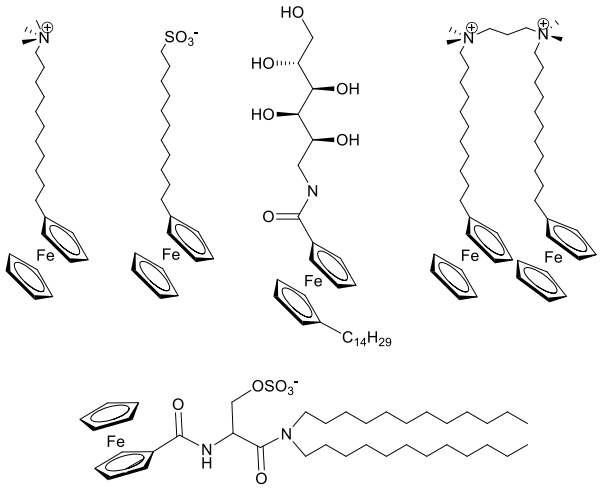


Figure 5.10. Already reported ferrocenyl-substituted surfactants examples.

5.2 Choice of the target molecules

Since no examples of the introduction of ferrocenyl units in non-classical surfactants such as bile acids were found.

With this in mind, given the premises made in the previous paragraphs, we decided to prepare cholic acid derivatives **5.1** and **5.2** having a ferrocenyl subunit in their skeleton, able to act as a redox mediator (Figure 5.11).

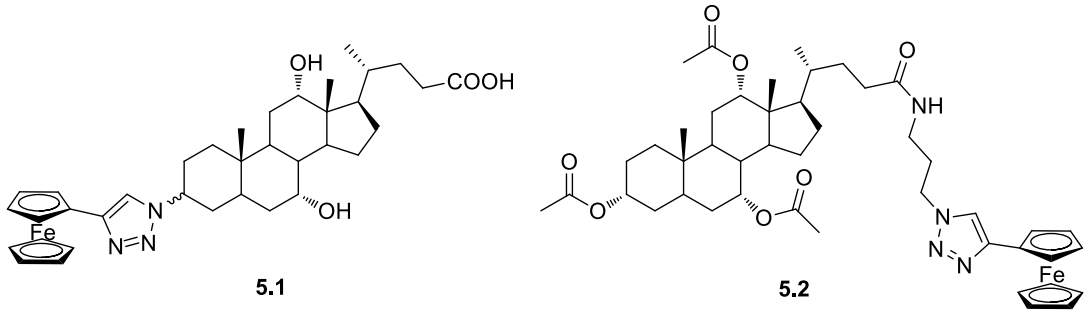


Figure 5.11. Target ferrocenyl derivatives chosen.

In compounds **5.1**, the ferrocenyl unit would have been linked to the C-3 of the steroid skeleton in both the relative configurations α and β. In particular, in derivative **5.1**, the choice of the triazole ring as linker was made in order to possibly increase the interactions with the nanotubes. Furthermore, as previously described in Chapter 2, in the literature there are no examples on the application of the Click reaction to bind purely aromatic subunits to C-3 of steroid compounds. Therefore, conducting this type of reaction would have somehow also provided data on the trend of the Click reaction involcing these substrates.

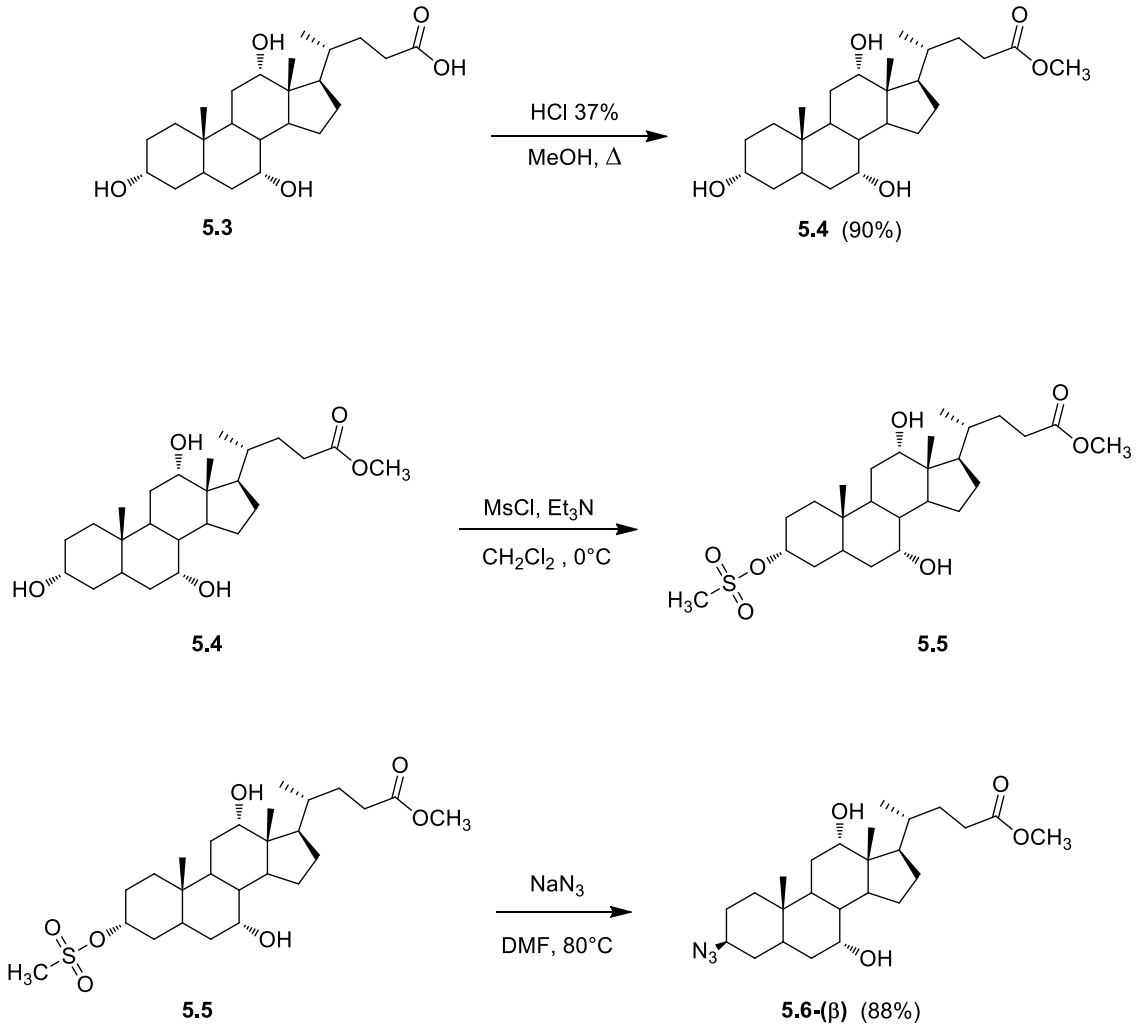
Furthermore, we hoped that the triazole ring of compounds **5.1** and **5.2**, together with the metallocene, could have facilitated the aggregation of derivatives through intermolecular π-π interactions.

In case of compound **5.2**, the introduction of ferrocene in the terminal position of this chain provided a new polar head, if the ferrocene had been oxidized to ferrocenium ion. The choice of acetyl groups as protectors of the hydroxyl functions was given by the fact that these functions could be selectively removed from each other, thus opening the way to a further targeted synthetic manipulation of the derivative.

These compounds could have been useful also in the dispersion of nanotubes and, later, it would have been interesting to study if they remained adhered to the nanotubes, once deposited on the electrode surface.

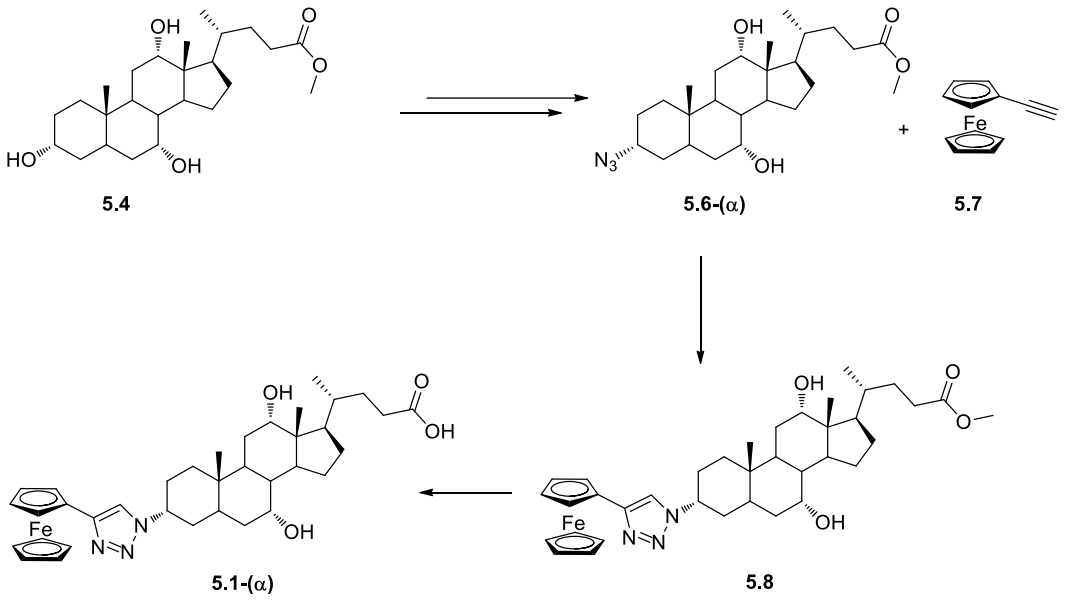
5.3 Synthesis of BA/ferrocene derivatives

β-C-3 azidoderivative was obtained with the same procedures decribed in the previous Chapters as shown in Scheme 5.1.



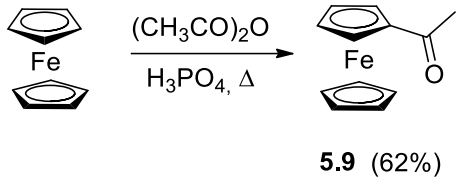
Scheme 5.1. Synthetic pathway followed for the synthesis of β-azidoderivative.

The substrate we had to prepare has a relative β-configuration on C-3 and a ferrocenyl subunit linked to the steroid skeleton through a triazole function. The followed synthesis pathway involved the preparation of epimer azide intermediate **5.6-(α)** from cholic acid, which was reacted in a copper catalyzed cycloaddition reaction with ethinylferrocene **5.7** (Scheme 5.2).



Scheme 5.2. Synthetic pathway chosen for compound **5.1-(α)**.

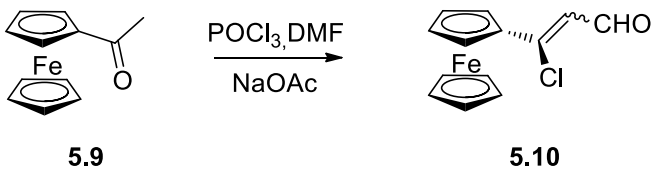
Ethinylferrocene **5.7** that we chose followed a literature[19] procedure and began with the Friedel-Crafts acetylation of ferrocene, commercially available, using acetic anhydride as a reagent and orthophosphoric acid as a catalyst, as illustrated in Scheme 5.3.



Scheme 5.3. Ferrocene acetylation in a classical Friedel-Crafts reaction.

The reaction crude was then purified on a chromatographic column, obtaining the desired product with a yield of 62%.

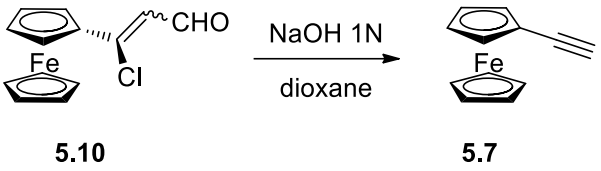
The synthesis continued obtaining 2-formyl-1-chlorovinylferrocene **5.10** through a Vilsmeier-Haack reaction with phosphorus oxychloride and dimethylformamide (Scheme 5.4).



Scheme 5.4. Vilsmeier-Haack reaction on compound **5.10**.

The synthetic stage that led to ethinylferrocene **5.7** involved an elimination reaction on

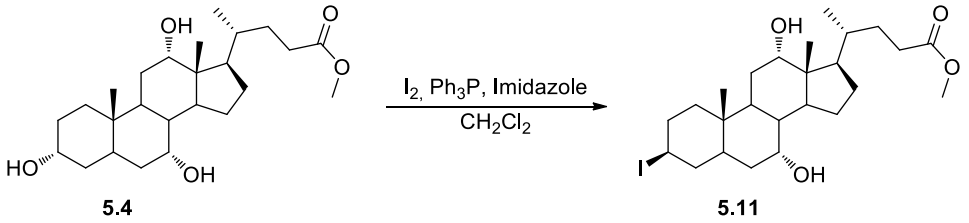
compound **5.10** according to the Rosemblum procedure[20] for treatment with a 1N solution of NaOH in anhydrous dioxane (Scheme 5.5).



5.10 **5.7**

Scheme 5.5. Obtaining ethinylferrocene through Rosemblum procedure.

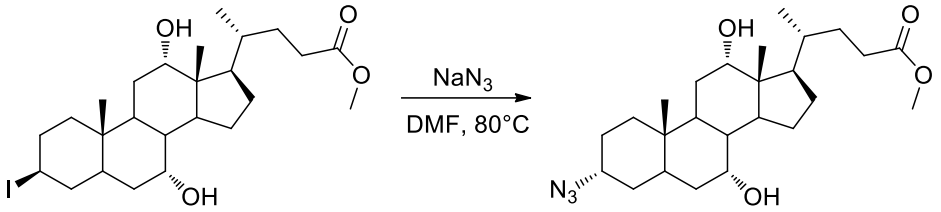
The azide derivative **5.6-(α)** synthesis with configuration retention was carried out by passing through the formation of 3-(β)-iodocholate **5.11** according to a Mitsunobu reaction variant[21] with triphenylphosphine, imidazole and molecular iodine, as represented in Scheme 5.6.



5.4 **5.11**

Scheme 5.6. Mitsunobu reaction on methyl cholate.

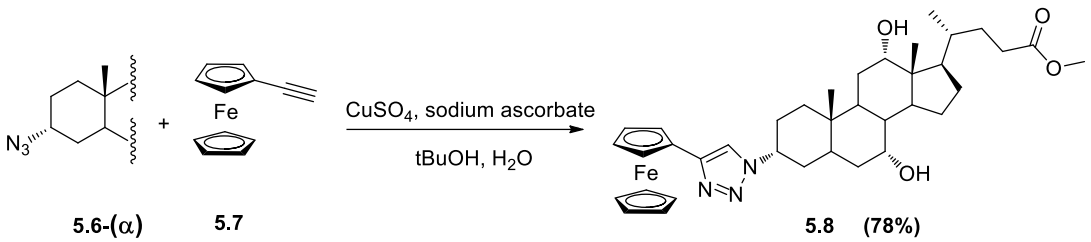
This compound was not purified by chromatographic column since the crude was used for the subsequent *SN2* nucleophilic substitution reaction using sodium azide in DMF, to obtain compound **5.6-(α)** with 76% yield (Scheme 5.7).



5.11 **5.6-(α) (76%)**

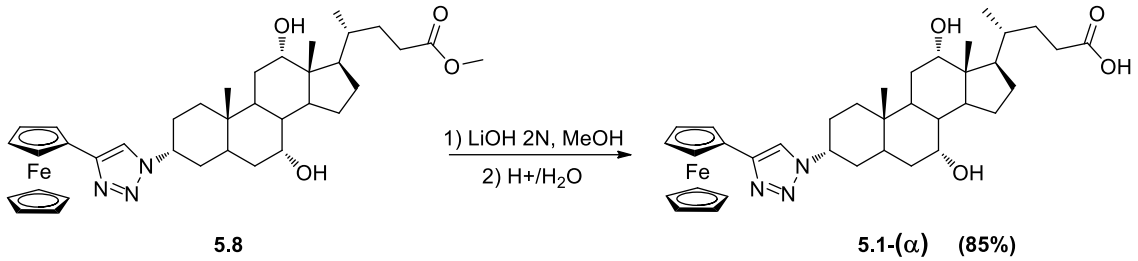
Scheme 5.7. Nucleophilic substitution with NaN_3 obtaining azido-derivative **5.6-(α)**.

Once the **5.6-(α)** azido-derivative was obtained, this was reacted with the previously synthesized ethinylferrocene **5.7**, forming the **5.8** compound by a click reaction, carried out in H2O/t-BuOH and in the presence of a slight excess of sodium ascorbate and CuSO4 (Scheme 5.8).



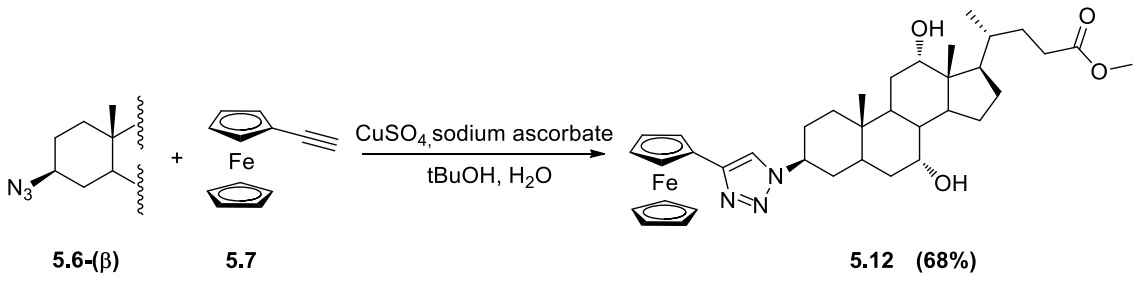
Scheme 5.8. Click reaction between azido-derivative **5.6-(α)** and ethinylferrocene **5.7**.

The compound, purified on a chromatographic column, was obtained with a yield of 78%. Finally, the cleavage of the ester portion was carried out with a LiOH 2N solution in methanol (Scheme 5.9) with a 85% yield after purification.



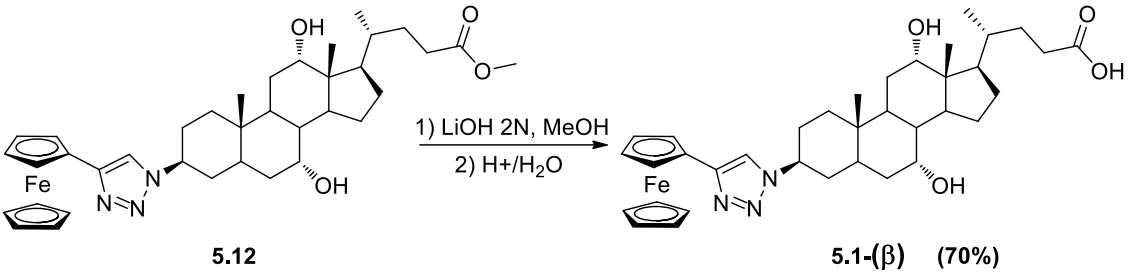
Scheme 5.9. Ester portion cleavage in alkaline conditions.

In order to synthesize **5.1-(β)** click reaction was carried out on compound **5.6-(β)** in the same conditions of α epimer with a 68% yield as shown in Scheme 5.10.



Scheme 5.10. Click reaction on β epimer.

Once purified, compound **5.12** was hydrolyzed in a LiOH 2N solution in methanol (Scheme 5.11).

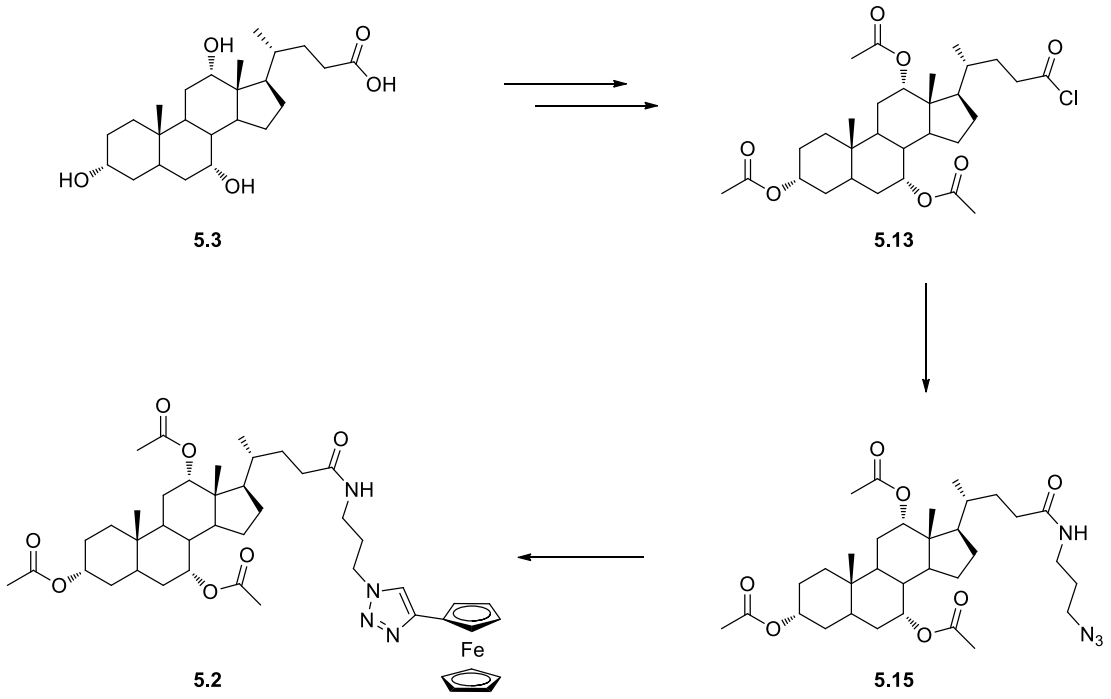


5.12 5.1-(β) (70%)

Scheme 5.11. Methyl ester cleavage of compound **5.21**.

Finally, to obtain compound **5.2** we had to functionalize the C-17 position on the side chain in order to carry out the click reaction with ethinylferrocene **5.7** and to observe any changes in the aggregative behavior.

First of all, the synthesis of **5.2**, illustrated in Scheme 5.12, started with the protection of the cholic acid hydroxyl groups and formation of the corresponding acyl chloride **5.13**. Subsequently it was reacted with 3-azidopropylamine **5.14** to give the azide derivative **5.15**, precursor of the final product **5.2**.



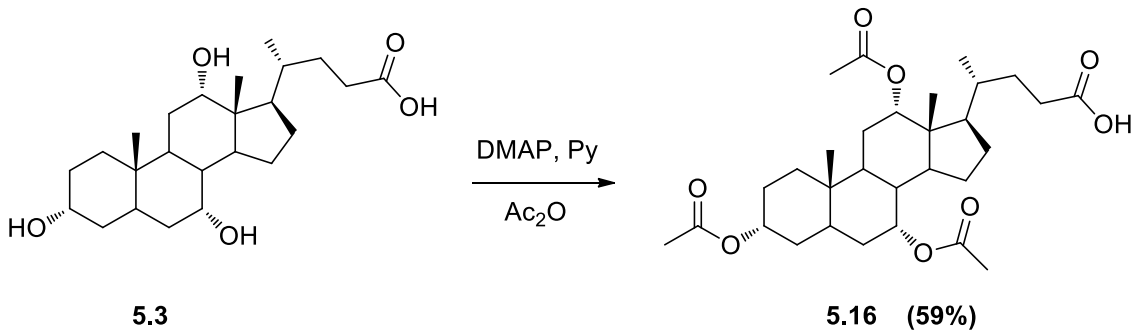
5.3 5.13 5.2 5.15

Scheme 5.11. Synthetic pathway for compound **5.2**.

The first objective of this synthetic strategy was the synthesis of compound **5.13**, which would be used for the subsequent amidation reaction.

Chlorination on C-17 required, as a preliminary step, the acetylation of the hydroxyl groups

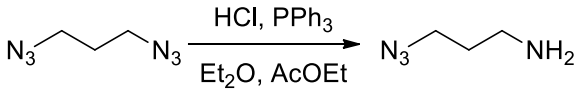
on the cholic acid steroid skeleton **5.3** to give compound **5.16** with 59% yield, which was obtained starting from cholic acid reaction with DMAP, pyridine and acetic anhydride (Scheme 5.12).



5.3 **5.16 (59%)**

Scheme 5.12. Peracetylation of compound **5.3**.

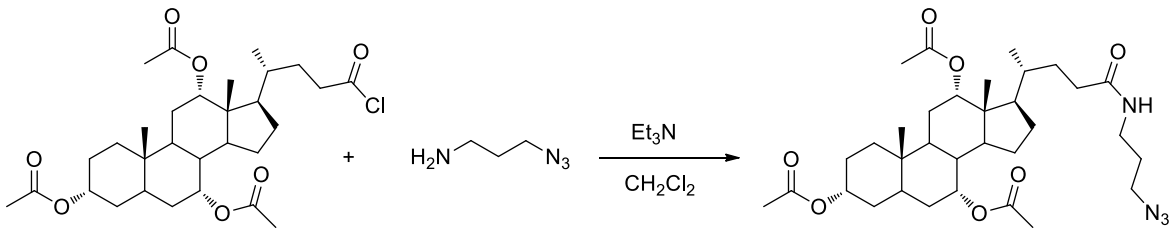
Once compound **5.16** was obtained, it was converted to the corresponding acyl chloride **5.13**, in a reaction with oxalyl chloride and DMF in anhydrous CH_2Cl_2. This compound was not purified and the raw product was used in reaction with 3-azidopropylamine **5.14**, obtained partially reducing the diazide **5.17** in diethyl ether with HCl 5% solution and PPh_3 (Scheme 5.13).



5.17 **5.14 (56%)**

Scheme 5.13. Partial reduction of 1,3-diazidopropane.

The 3-azidopropylamine **5.14** and the acyl chloride **5.13** were reacted in anhydrous DCM in presence of triethylamine (Scheme 5.14).

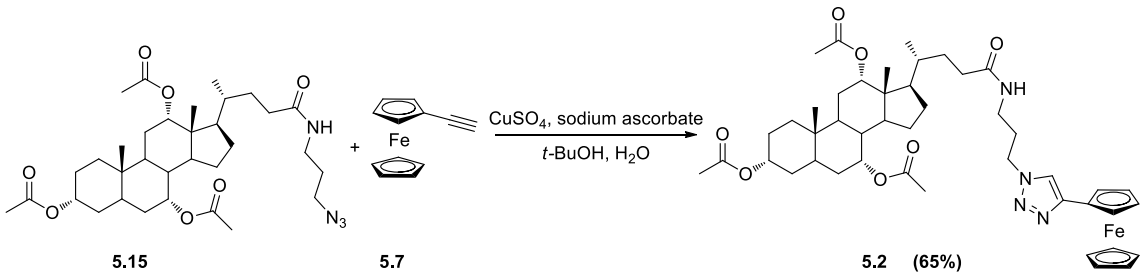


5.13 **5.14** **5.15**

Scheme 5.14. Nucleophilic acyl substitution.

Surprisingly, the amide **5.15** was obtained with a low yield (20%).

Finally, the synthetic work ended with the Click reaction, in *t*-BuOH/H_2O, with $CuSO_4$ and sodium ascorbate, between azide **5.15** and ethinylferrocene **5.7** (Scheme 5.15).



Scheme 5.15. Final Click reaction in order to obtain derivative **5.4**.

Compound **5.2** was obtained with a 65% yield after chromatographic purification.

5.4 Experimental section

All commercially available reagents used were supplied by Sigma-Aldrich and used without further purification.

All ^{1}H-NMR and ^{13}C-NMR spectra were performed with a Bruker Avance 400 spectrometer, 400 MHz for ^{1}H-NMR and at 100MHz for ^{13}C-NMR using 5mm diameter tubes in $CDCl_3$, DMSO and MeOD.

High-resolution ESI mass spectra were carried out on a Q-TOF Micro spectrometer operating in a positive ion mode.

The progress of the reactions was followed by thin layer chromatography (TLC) using Merck Kieselgel 60 F254 silica gel plates (0.25mm thick).

The products were revealed on a slide using as indicators:

• 12% phosphomolybdic acid.

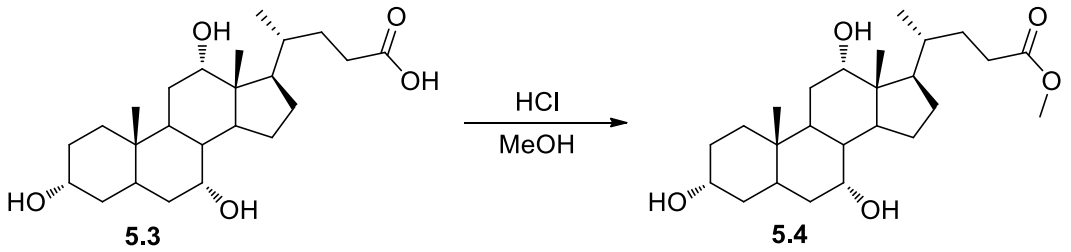
• UV lamp (λ = 254 nm)

• 95% sulfuric acid.

The isolation and purification of the products were carried out by column chromatography using Merck-Kieselgel 60 silica gel (0.063-0.20 mm, 70-230 mesh) as the stationary phase.

The anhydrous solvents used were: tetrahydrofuran (distilled on sodium), dichloromethane (distilled on phosphorus pentoxide), 1,4-dioxane (distilled on sodium) and N,N-dimethylfromamide (commercially available as anhydrous).

Synthesis of cholic acid methyl ester 5.4



5.3 **5.4**

The synthetic procedure is the same as described in Chapter 2 (as product **2.6a**)

Synthesis of mesyl derivative 5.5 starting from methyl cholate 5.4

The synthetic procedure is the same as described in Chapter 2 (as product **2.7-(α)**)

Synthesis of azido derivative 5.6-(β) from mesyl derivative 5.5

The synthetic procedure is the same as described in Chapter 2 (as product **2.5-(β)**)

Acetylferrocene (5.9) synthesis from ferrocene

1.0 g (5.4 mmol) of ferrocene, 3.3 mL (3.35 mmol) of acetic anhydride and 0.7 mL (13.0 mmol) of 85% orthophosphoric acid were added in this order in a 100 mL three-necked flamed flask equipped with bubble refrigerant, magnetic stir bar and CaCl2 valve and the mixture was stirred at 85 °C for forty-five minutes. At the end, the mixture was cooled in an ice bath, diluted with distilled water (7 mL) and neutralized with a 10% sodium hydroxide solution.

The precipitate was filtered on Hirsch, washed with distilled water and the coloured solution was transferred to a 100 mL separating funnel and treated with ethyl acetate (4x10 mL) (during processing, a brown pitch may form inside the flask reaction which is easily washed with ethyl ether).

The organic phases are combined and purified on a chromatographic column (eluent mixture of petroleum ether/ethyl ether 1:1) obtaining 0.505 g (2.21 mmol) of final product corresponding to a yield of 62%.

2-formyl-1-chlorovinylferrocene 5.10 preparation starting from acetylferrocene 5.9

0.1 g (0.438 mmol) of acetylferrocene dissolved in 0.2 mL (2.6 mmol) of anhydrous DMF were placed in a 50 mL three-necked flask equipped with bubble cooler, dropping funnel and magnetic stir bar at 0 °C under an argon atmosphere. In a schlenk tube, 0.2 mL (2.14 mmol) of phosphorus oxychloride in 0.2 mL (2.6 mmol) of anhydrous DMF were added under inert atmosphere and in an ice bath and the red complex thus obtained was transferred into the dropping funnel and added drop by drop for thirty minutes, again at 0 °C. The mixture was left under stirring for two hours until a change from brown to blue is observed. At the end the mixture was treated with 0.4 mL of ethyl ether, 0.5 g (3.67 mmol) of sodium acetate trihydrate, followed by 0.22 mL of distilled water. The ice bath was removed as soon as the double phase is formed, the organic one of red color and the aqueous one of blue color.

After three hours, the solution was transferred into a separatory funnel, 10 mL of distilled

water, 10 mL of ethyl ether were added and the organic phase was extracted. Subsequently, the aqueous phase was extracted with ethyl ether (4x1 mL) and the combined organic phases were washed with a saturated solution of $NaHCO_3$ (10 mL) and distilled water (4x1 mL), finally dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. 0.070 g (0.256 mmol) of an unpurified, purple colored crystalline compound was obtained.

Ethinylferrocene 5.7 synthesis

In a 50 mL three-necked flask equipped with bubble refrigerant, 100 mg (0.366 mmol) of 2-formyl-1-chlorovinylferrocene **5.10** were added in 1.2 mL (14 mmol) of anhydrous 1,4-dioxane and the whole mixture was refluxed under inert atmosphere. After five minutes of reflux, 1.14 mL of a boiling 1N sodium hydroxide solution were added and the mixture was kept under stirring and refluxing for about thirty minutes. The silicone bath was removed, the flask wass cooled to room temperature and the mixture neutralized with 1N HCl in the presence of an ice bath.

After neutralization, the compound was transferred to a separatory funnel, 5 mL of distilled water, 10 mL of petroleum ether were added and the organic phase was extracted; the aqueous phase was repeatedly washed with petroleum ether (4x10 mL) and the combined organic phases with a saturated solution of $NaHCO_3$ (10 mL) and distilled water (3x10 mL). The organic phase was dried with anhydrous sodium sulphate and evaporated at low pressure.

The obtained orange oily phase was purified on a chromatographic column (eluent mixture: pure petroleum ether) obtaining 44 mg (0.209 mmol) of ethinylferrocene corresponding to a yield of 57%.

Click reaction for the synthesis of compounds 5.8, 5.12 and 5.2

In a 25 mL flask, 1 eq of relative azide and 1 eq. of ethinyl ferrocene were added in 10 mL of a 7:3 mixture of *t*-BuOH/H_2O. Then 3 eq. of sodium ascorbate and finally 1 eq. of $CuSO_4$ were added.

The reaction was left at 60 °C and controlled by TLC. Since the reagents are still present after 12h, a further addition of sodium ascorbate and $CuSO_4$ was made.

At the end of the reaction, the mixture was transferred to a separatory funnel adding water 2 mL and extracted with ethyl acetate (5 mL x 3). The organic phases were dried over sodium

sulphate, filtered and dried under vacuum. The reaction crude was purified on a chromatographic column with an eluent mixture of petroleum ether/ethyl acetate (3:7).

Iododerivative 5.11 preparation starting from the methyl cholate 5.4

0.200 g (1 eq., 0.47 mmol) of methyl cholate in CH_2Cl_2 were added to a three-necked round bottom flask, equipped with bubble refrigerant and a $CaCl_2$ valve. Subsequently, 0.186 g (1.5 eq., 0.71 mmol) of triphenylphosphine and 0.096 g (3.0 eq, 1.42 mmol) of imidazole were added. The solution was left under stirring for five minutes, after which 0.18 g (1.5 eq., 0.71 mmol) of molecular iodine was added little by little, until the solution turned from transparent to intense red.

The mixture was left at room temperature for thirty minutes, after which it was transferred to a separatory funnel and separated with distilled water containing a few drops of 30% H_2O_2 (15 mL). The aqueous phase was then washed with ethyl acetate (15 mL x 3). The organic phases were then combined and extracted with $Na_2S_2O_5$ (15 mL x 3), until the solution changes from a brownish to a bright yellow color. At this point, the organic phase was dried over sodium sulphate, filtered and dried to obtain a crude weight of 0.406 g (0.762 mmol).

The obtained compound was used in the subsequent reaction.

Synthesis of azidoderivative 5.6-(α) from iododerivative 5.11

0.406 g (1 eq., 0.762 mmol) of methyl cholate and 0.149 g (3 eq., 2.29 mmol) of sodium azide were added in 5 mL of DMF in a three-necked flask, equipped with a liebig, under inert atmosphere. The reaction was left under stirring at room temperature for 24 hours.

Subsequently, the reaction mixture was transferred to a separatory funnel in which were added 20 mL of distilled water and extracted with ethyl ether (4 mL) and ethyl acetate (1 mL). The organic phases were collected and dried over sodium sulphate, filtered and brought to dryness.

The crude product thus obtained was purified on a chromatographic column with an eluent mixture of petroleum ether:ethyl acetate (8:2).

Compound **5.6-(α)** with a weight of 0.161 g (0.36 mmol) was obtained with a 77% yield.

Cholic acid peracetylation

In a single-necked round-bottomed flask, 1.5 g (3.67 mmol) of cholic acid and 205 mg (1.83 mmol) of DMAP were dissolved in 5 mL of pyridine. Then 1.73 mL of Ac2O were added and the reaction was left under stirring at room temperature for 12 h. Once the reaction was over it was quenched with acid water at pH=2. The aqueous phase was extracted 3 times with CH_2Cl_2 then the organic phase was extracted 3 times with acid water, dried on Na_2SO4 and the solvent is evaporated at reduced pressure. The crude is purified on a chromatographic column with an eluent mixture of petroleum ether/ethyl acetate (6:4). 1.162 g (2.17 mmol) of peracetylate were obtained with a yield of 59%.

Preparation of 5.13 from peracetylated 5.16

In a 25 mL flask, previously flamed, 320 mg (0.598 mmol) of peracetylate were placed in 5 mL of CH_2Cl_2, the flask was then placed in an ice bath and 0.2 mL (2.394 mmol) of oxalyl chloride was added. Finally, when the effervescences were finished, 3 drops of DMF were added. The reaction was left at room temperature for 4h. When it was over, the crude was brought to the rotavapor and used for the subsequent reaction as raw product.

Diazidation of 1,3-dibromopropane to obtain 1,3-diazidopropane 5.17

1.26 g (17.325 mmol) of NaN_3 and 0.5 mL (4.90 mmol) of 1.3 dibromopropane were added in 4 mL of DMF in a one-necked round-bottomed flask equipped with bubble refrigerant and previously flamed.

The reaction was left at 50 °C for one night and when it was over, 20 mL of ethyl ether were added and extracted with H_2O (5 mL x 3 times) and with Brine (5 mL x 10 times).

The organic phases were dried over sodium sulphate, filtered and dried by distilling in the rotavapor at atmospheric pressure.

610 mg (4.83 mmol) of crude product were obtained.

Monoreduction of compound 5.17

610 mg (4.84 mmol) of diazidopropane, 3 mL of AcOEt, 3 mL of Et2O and 5 mL of aqueous solution at 5% of HCl were added in a one-necked round-bottomed flask equipped with bubble cooler and previously flamed. Then 1.2 g of PPh_3 (4.74 mmol) were added little by little by immersing the flask in an ice bath. The reaction was left at room temperature for 16

h.

When the reaction was over, the mixture was placed in a separatory funnel and the organic phase was discharged. The aqueous phase was washed twice with CH_2Cl_2 (5 mL x 3 times), basified (pH>12) and finally extracted with CH2Cl2 (5 mL x 4 times).

The extracted organic phases were dried over sodium sulphate, filtered and dried by distilling them in a rotavapor at atmospheric pressure.

278 mg (2.77 mmol) of product were obtained with a yield of 57%.

Condensation between compounds 5.13 and 5.14

330 mg (0.596 mmol) of raw **5.13** were directly reacted with 119 mg (1.19 mmol) of amine **5.14** and 0.16 mL (1.19 mmol) of Et_3N while holding the round-bottomed flask in an ice bath. The mixture was left for 14 h at room temperature.

Once the reaction was over, CH_2Cl_2 was added and washed with a saturated solution of $NaHCO_3$. The aqueous phase was extracted with CH_2Cl_2 (5 mL x 3 times). The organic phases were then dried over $NaSO_4$ and the solvent was removed under reduced pressure. The reaction crude was purified on a chromatographic column with an eluent mixture of petroleum ether/ethyl acetate (6:4) obtaining 68 mg (0.11 mmol) of compound 20 with a yield of 18%.

5.5 Characterization data

Characterization data of some compounds have already been described in the previous Chapters and were not repeated in this section.

Acetylferrocene

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 2.38 (s, 3H), 4.19 (m, 5H), 4.49 (m, 2H), 4.76 (m, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 27.1, 69.8, 70.0, 72.5, 79.4, 202.3. MS (ESI): m/z (%) 229.0319 $[M+H]^+$.

(1-chloro-2-formylvinyl)ferrocene

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 4.22 (m, 5H), 4.55 (m, 2H), 4.75 (m, 2H), 6.38 (d, 1H, *J*=7.1 Hz), 10.07 (d, 1H, *J*=7.1). ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 69.8, 70.0, 72.2, 80.0, 120.3, 155.1, 190.7. MS (ESI): m/z (%) 274.9929 $[M+H]^+$.

Ethynylferrocene

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 2.73 (s, 1H), 4.20 (m, 2H), 4.23 (m, 5H), 4.47 (m, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 63.9, 68.7, 70.0, 71.7, 75.5, 82.6. MS (ESI): m/z (%) 211.0213 $[M+H]^+$.

(4R)-4-((3S,7R,10S,12S,13R,17R)-7,12-dihydroxy-3-((ferrocenecarbonyl)amino)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid methyl ester

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 0.58 (s, 3H), 0.89 (m, 6H), 1.15-2.56 (m, 26 H, steroidic backbone and side chain), 3.56 (s, 3H), 3.63 (m, 1H), 3.77 (m, 1H), 3.94 (m, 1H), 4.04 (m, 5H), 4.29 (m, 2H), 4.89 (m, 2H), 7.01 (d, 1H, *J*=5.4 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 12.5, 17.3, 22.9, 23.2, 24.5, 26.1, 27.4, 28.5, 30.5, 30.8, 31.1, 33.0, 34.1, 35.1, 35.2, 36.8, 39.4, 41.9, 46.5, 47.2, 51.6, 66.8, 68.9, 69.7, 70.2, 71.5, 77.4, 79.6, 168.7, 174.6. MS (ESI): m/z (%) 634.3198 $[M+H]^+$.

(4R)-4-((3S,7R,10S,12S,13R,17R)-7,12-dihydroxy-3-((ferrocenecarbonyl)amino)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 0.58 (s, 3H), 0.89 (m, 6H), 1.15-2.56 (m, 26 H, steroidic backbone and side chain), 3.63 (m, 1H), 3.77 (m, 1H), 3.94 (m, 1H), 4.11 (m, 5H), 4.31 (m, 2H), 4.89 (m, 2H), 7.01 (d, 1H, *J*=5.4 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 12.5, 17.3, 22.9, 23.2, 24.5, 26.1, 27.4, 28.5, 30.5, 30.8, 31.1, 33.0, 34.1, 35.1, 35.2, 36.8, 39.4, 41.9, 46.5, 47.2, 66.8, 68.9, 69.7, 70.2, 71.5, 77.4, 168.8, 175.3. MS (ESI): m/z (%) 620.3040 $[M+H]^+$.

(4R)-methyl 4-((3S,7R,10S,12S,13R,17R)-7,12-dihydroxy-3-iodo-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 0.67 (s, 3H), 0.91 (s, 3H), 0.96 (d, 3H, J=6.2 Hz), 1.15-2.56 (m, 26 H, steroidic backbone and side chain), 3.65 (s, 3H), 3.85 (m, 1H), 3.95 (m, 1H), 4.93 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 12.5, 17.3, 22.9, 23.2, 24.5, 26.1, 27.4, 28.5, 30.5, 30.8, 31.1, 33.0, 34.1, 35.1, 35.2, 36.8, 39.4, 41.9, 46.5, 47.2, 51.5, 68.4, 71.9, 73.0, 174.7. MS (ESI): m/z (%) 533.2831 $[M+H]^+$.

methyl 4-((3R)-7,12-dihydroxy-10,13-dimethyl-3-(4-ferrocenyl-1H-1,2,3-triazol-1-yl)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

^{1}H-NMR (400 MHz, DMSO) δ(ppm): 0.58 (s, 3H), 0.89 (m, 6H), 1.15-2.56 (m, 26 H, steroidic backbone and side chain), 3.14 (m, 1H), 3.63 (m, 1H), 3.65 (s, 3H), 3.77 (m, 1H), 4.11 (m, 5H), 4.31 (m, 2H), 4.89 (m, 2H), 7.95 (s, 1H). ^{13}C-NMR (100 MHz, DMSO) δ(ppm): 12.5, 17.3, 22.9, 23.2, 24.5, 26.1, 27.4, 28.5, 30.5, 30.8, 31.1, 33.0, 34.1, 35.1, 35.2, 36.8, 39.4, 41.9, 46.5, 47.2, 51.0, 31.3, 56.4, 65.6, 71.2, 71.4, 73.3, 74.4, 123.9, 145.9, 178.9. MS (ESI): m/z (%) 658.3304 $[M+H]^+$.

4-((3R)-7,12-dihydroxy-10,13-dimethyl-3-(4-ferrocenyl-1H-1,2,3-triazol-1-yl)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid

^{1}H-NMR (400 MHz, MeOD) δ(ppm): 0.74 (s, 3H), 1.2 (m, 6H), 1.15-2.56 (cm, 26 H, steroidic backbone and side chain), 3.14 (m, 1H), 3.69 (m, 1H), 3.83 (m, 1H), 4.04 (m, 5H), 4.31 (m, 2H), 4.71 (m, 2H), 7.95 (s, 1H). ^{13}C-NMR (100 MHz, MeOD) δ(ppm): 12.5, 17.3, 22.9, 23.2, 24.5, 26.1, 27.4, 28.5, 30.5, 30.8, 31.1, 33.0, 34.1, 35.1, 35.2, 36.8, 39.4, 41.9, 46.5, 47.2, 61.3,

67.5, 68.3, 69.2, 69.9, 72.6, 116.9, 117.6, 145.9. MS (ESI): m/z (%) 644.3149 $[M+H]^+$.

methyl 4-((3R)-7,12-dihydroxy-10,13-dimethyl-3-(4-phenyl-1H-1,2,3-triazol-1-yl)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate

^{1}H-NMR (400 MHz, DMSO) δ(ppm): 0.58 (s, 3H), 0.90 (m, 6H), 1.07-2.61 (cm, 26 H, steroidic backbone and side chain), 2.93 (m, 1H), 3.56 (s, 3H), 3.64 (m, 1H), 3.79 (m, 1H), 3.99 (m, 5H), 4.27 (m, 2H), 4.73 (m, 2H), 8.21 (s, 1H). ^{13}C-NMR (100 MHz, DMSO) δ(ppm): 12.8, 17.2, 22.9, 23.2, 24.5, 26.1, 27.4, 28.5, 30.5, 30.8, 31.1, 33.0, 34.1, 35.1, 35.2, 36.8, 39.4, 41.9, 46.5, 47.2, 51.0, 51.3, 56.1, 66.6, 68.6, 69.6, 71.4, 76.6, 120.2, 145.2, 174.2. MS (ESI): m/z (%) 658.3304 $[M+H]^+$.

4-((3R)-7,12-dihydroxy-10,13-dimethyl-3-(4-phenyl-1H-1,2,3-triazol-1-yl)hexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid

^{1}H-NMR (400 MHz, MeOD) δ(ppm): 0.72 (s, 3H), 0.90 (m, 3H), 1.2 (m, 3H), 1.11-2.36 (cm, 26 H, steroidic backbone and side chain), 3.03 (m, 1H), 3.83 (m, 1H), 3.98 (m, 1H), 4.05 (m, 5H), 4.33 (m, 2H), 4.78 (m, 2H), 8.03 (s, 1H). ^{13}C-NMR (100 MHz, MeOD) δ(ppm): 11.6, 16.2, 22.2, 22.8, 24.3, 27.4, 28.5, 30.5, 30.8, 31.1, 33.0, 34.1, 35.2, 36.8, 39.4, 41.9, 46.5, 47.2, 66.3, 67.5, 68.5, 69.2, 72.5, 119.1, 145.9, 176.8. MS (ESI): m/z (%) 644.3148 $[M+H]^+$.

1,3-diazidopropane

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 1.81 (q, 2H, J = 6.4 Hz), 3.40 (t, 4H, J = 6.5 Hz). ^{13}C-NMR (100 MHz, DMSO) δ(ppm): 28.2, 48.3. MS (ESI): m/z (%) 127.0729 $[M+H]^+$.

3-azidopropan-1-amine

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 1.47 (bs, 2H), 1.74 (q, 2H, J = 6.8 Hz), 2.80 (t, 2H, J = 6.8 Hz), 3.37 (t, 2H, J = 6.8 Hz). ^{13}C-NMR (100 MHz, DMSO) δ(ppm): 32.3, 39.3, 49.0. MS (ESI): m/z (%) 101.0829 $[M+H]^+$.

(4R)-4-((3R,7R,10S,12S,13R,17R)-3,7,12-triacetoxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 0.74 (s, 3H), 0.83 (m, 3H), 0.93 (s, 3H), 1.00-2.41 (cm, 34 H, steroidic backbone and side chain), 2.06 (s, 3H), 2.10 (s, 3H), 2.15 (s, 3H), 4.59 (m, 1H), 4.92 (s, 3H), 5.10 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 12.2, 17.4, 21.4 (2C), 21.6, 22.5, 22.8, 25.5, 26.8, 27.1, 28.8, 30.5, 30.6, 31.2, 34.3, 34.5, 34.6, 34.7, 37.7, 40.9, 43.4, 45.0, 47.3, 70.7, 74.1, 75.4, 170.4, 170.5 (2C), 179.0. MS (ESI): m/z (%) 535.3268 $[M+H]^+$.

(3R,7R,10S,12S,13R,17R)-17-((R)-5-((3-azidopropyl)amino)-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthrene-3,7,12-triyl triacetate

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 0.77 (s, 3H), 0.88 (m, 3H), 0.94 (s, 3H), 1.00-2.36 (cm, 34 H, steroidic backbone and side chain), 2.07 (s, 3H), 2.11 (s, 3H), 2.17 (s, 3H), 3.35 (m, 2H), 3.63 (m, 2H), 4.60 (m, 1H), 4.94 (m, 1H), 5.11 (m, 1H), 5.61 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 12.3, 18.8, 21.4 (2C), 21.6, 22.5, 22.8, 25.6, 26.7, 26.9, 27.3, 28.8, 31.2, 34.3, 34.6, 34.7, 34.9, 35.1, 37.7, 39.9, 40.9, 43.4, 45.1, 47.7, 48.9, 70.6, 74.0, 75.2, 161.8, 170.3, 170.4, 170.5. MS (ESI): m/z (%) 617.3917 $[M+H]^+$.

(3R,7R,10S,12S,13R,17R)-10,13-dimethyl-17-((R)-5-oxo-5-((3-(4-ferrocenyl-1H-1,2,3-triazol-1-yl)propyl)amino)pentan-2-yl)hexadecahydro-1H-cyclopenta[a]phenanthrene-3,7,12-triyl triacetate

^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 0.71 (s, 3H), 0.80 (m, 3H), 0.90 (s, 3H), 1.21-2.30 (cm, 34 H, steroidic backbone and side chain), 1.97 (s, 3H), 1.99 (s, 3H), 2.05 (s, 3H), 3.46 (m, 3H), 4.03 (m, 5H), 4.29 (m, 2H), 4.45 (m, 2H), 4.69 (m, 2H), 4.79 (m, 1H), 4.97 (s, 1H), 6.00 (s, 1H), 8.13 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 12.4, 18.5, 21.5, 21.6, 21.8, 22.6, 28.7, 35.1, 37.3, 43.4, 45.1, 47.6, 55.3, 66.7, 68.6, 73.8, 74.9, 75.3, 76.4, 121.1, 127.6, 129.9, 145.6, 162.6, 170.1, 170.2, 170.3. MS (ESI): m/z (%) 827.4049 $[M+H]^+$.

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