Facoltà di Scienze Matematiche Fisiche e Naturali

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Synthesis of antioxidant compounds and polyfunctionalized intermediates by transition metal-mediated reactions.

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1. Introduction

1.1 Free radicals and oxidative stress

Free radicals, "the price we pay for breathing" are essential for health and life in moderation and harmful to health and life in excess. Reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), nitric oxide ($NO^{\bullet}$) and peroxynitrite ($ONOO^-$) are products of oxygen metabolism in all aerobic organisms. ROS are generated as a result of energy production from mitochondria (from the electron transport chain), as part of an antimicrobial[1] or antiviral[2] response, as well as detoxification reactions carried out by the cytochrome P-450 system.[3,4] Environmental agents such as ultraviolet light, ionizing radiation, redox chemicals and cigarette smoke also readily generate ROS. The antioxidant defense system in most cells is composed of two components, the antioxidant enzymes component which includes enzymes such as superoxide dismutase, catalase and glutathione peroxidase, and the low molecular weight antioxidants component that includes vitamins A and E, ascorbate, glutathione and thioredoxin. These substances are the body's natural defense against endogenous generated ROS and other free radicals, as well as ROS generated by external environmental factors. Oxidative stress occurs when the production of ROS exceeds the body's natural antioxidant defense mechanisms, causing damage to biomolecules such as lipids, proteins and DNA. Oxidative DNA damage has been implied as a cause of cancer,[5,6] aging and neurodegenerative diseases such as Alzheimer's and Parkinson's,[7,8,9,10] cardiovascular diseases such as arteriosclerosis[11,12] and it is the

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[1] Weiss, S. J. and Lo Buglio, A. F., *Laboratory Investigation*, **1982**, *47*, 5-18.

[2] Griot, C., Burge, T., Vandevelde, M., and Peterhans, E., Acta Neuropathol., **1989**, *78*, 396-403.

[3] Scholz, W., Schutze, K., Kunz, W., and Schwarz, M., *Cancer Res.,* **1990**, *50*, 7015-22.

[4] Cederbaum, A. I., *Free Radical Biology & Medicine*, **1989**, *7*, 559-67.

[5] Huang, X., *Mutation Research*, **2003**, *533*, 153-171.

[6] Hajiliadis, N. D., *Netherlands: Kluwer Academic Press*, **1997** (Cytotoxic, mutagenic and carcinogenic potential of heavy metals related to human environment).

[7] Markesbery, W. R., Lovell, M. A., *Antioxidant and Redox Signalling*, **2006**, *8*, 2039-2045.

[8] Halliwell, B., *Drugs and Aging*, **2001**, *18*, 685-716.

[9] Markesbery, W. R., *Free Radical Biology and Medicine*, **1997**, *23*, 134-147.

[10] Markesbery, W. R., *Brain Pathology*, **1999**, *9*, 133-146.

[11] Vokurkova, M., Xu, S., *Future Cardiology*, **2007**, *3*, 53-63.

[12] Steinberg, D., *The Journal of Biological Chemistry*, **1997**, *272*, 20963-20966.

primary cause of cell death and tissue damage resulting from heart attack and stroke.[13,14] Therefore, prevention of oxidative stress caused by ROS and RNS has several implication for the prevention and treatment of diseases.

1.1.1 The role of transition metals in ROS generation: Fenton reactions

Formation of biological peroxides (H_2O_2) is a natural process in aerobic organisms.[15] Moreover, cell signaling mechanisms often involve ROS or RNS such as H_2O_2, $NO^{\bullet}$ and $O_2^{\bullet -}$ that can form more potent oxidants if not carefully regulated, leading to cellular damage and oxidative stress.[16,17,18] H_2O_2 is commonly reduced *in vivo* by either Fe^{2+} or Cu^{+} resulting in the formation of $^{\bullet}OH$ through Fenton-type reactions (Scheme 1).

$$Fe^{2+} \text{ or } Cu^{+} + H_2O + H^{+} \longrightarrow Fe^{3+} \text{ or } Cu^{2+} + {}^{\bullet}OH + H_2O \qquad (1)$$

$$2\, O_2^{\bullet -} + 2H^{+} \longrightarrow H_2O_2 + O_2 \qquad (2)$$

$$[2Fe^{2+}2Fe^{3+} - 4S] + O_2^{\bullet -} + 2H^{+} \longrightarrow [Fe^{2+}3Fe^{3+} - 4S] + H_2O_2 \qquad (3)$$

$$O_2^{\bullet -} + Fe^{3+} \text{ or } Cu^{2+} \longrightarrow O_2 + Fe^{2+} \text{ or } Cu^{+} \qquad (4)$$

$$O_2^{\bullet -} + H_2O_2 + H^{+} \longrightarrow O_2 + {}^{\bullet}OH + H_2O \qquad (5)$$

Scheme 1.1 Fenton and Haber-Weiss reactions.

Hydroxyl radical, the most active ROS known, has been supposed to extract a hydrogen atom from biological substrates at diffusion-limited rates.[19] Oxidative damage of DNA can occur at both the phosphate skeleton (strand breakage)[20] and nucleotide bases.[21] Iron-mediated DNA damage has been thought to be primary due to solvated iron that is not bound to proteins[22] (such as hemoglobin

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[13] Ide, T., Tsutsui, H., Hayashidani, S., Kang, D., Suematsu, N., Nakamura, K.-I. et al, *Circ. Res.*, **2001**, *88*, 529-535.

[14] Chevion, M., Berenshtein, E., Zhu, B.-Z., *Reactive oxygen species in biological system*, **1999**

[15] Gilbert, D. L., Coltn, C. A., **1993**, *Reactive Oxygen Species in Biological Systems* New York: Plenum Publisher.

[16] Droge, W., *Physiological Reviews,* **2002**, *82*, 47-95.

[17] Bredt, D. S., Snyder, S. H., *Annual Review of Biochemistry*, **1994**, *63*, 175-195.

[18] Suzuki, Y. J., Forman, H. J., Sevanian, A., *Free Radical Biology and Medicine*, **1997**,*22*, 269-285.

[19] Keyer, K., Gort, A. S., Imalay, J. A., *Journal of Bacteriology*, **1995**, *177*, 6782-6790.

[20] Imlay, J. A., Linn, S., *Science*, **1988**, *240*, 1302-1309.

[21] Lu, A.-L., Li, X., Gu, Y., Wright, P. M., Chang, D.-Y., *Cell Biochem. Biophis.*, **2001**, *35*, 141-170.

[22] Andrews, N. C., *Am. J. Physiol.*, **2004**, *287*, C1537-C1538.

or transferrin). Moreover, Fenton-type reaction also releases iron from proteins (reaction 3, Scheme 1), increasing the non-protein bound iron concentration.[23]

1.1.2 Antioxidant vs pro-oxidant activity

*"*Every antioxidant, including vitamin antioxidants, is in fact a redox (reduction-oxidation) agent, protecting against free radicals in some circumstances, promoting free radical generation in others. Excessive antioxidant action can adversely affect key physiological processes."[24]

ROS, as well as reactive nitrogen species (RNS) are key agents in the regulation of cell functions by acting as secondary messengers in intracellular signaling cascades (Alanko et al., 1999; Valko et al., 2007) Pro-oxidants are chemicals that induce oxidative stress, either through creating reactive oxygen species or inhibiting antioxidant systems. Some substances can act as either antioxidants, or pro-oxidants, depending on the specific set of conditions. Some of the conditions that are important include the concentration of the chemical and if oxygen or transition metals are present. While thermodynamically very favored, reduction of molecular oxygen or peroxide to superoxyde or hydroxy radical is fortunately spin forbidden. This greatly reduces the rates of these reactions, thus allowing aerobic life to exist.

1.2 Polyphenols

1.2.1 The term "polyphenols": strict definition and common usage

Plant-derived natural products were firstly referred to as "vegetables tannins" due to their usage in leather-making process. In the late '50, Theodore Withe stated that "tannin" should refer to plant polyphenolic materials having molecular masses between 500 and 3000 Da and sufficiently large number of phenolic groups to be capable of forming hydrogen-bonded cross-linked structures with collagen molecules (the act of tanning). Subsequently, in 1962, E. C. Bate-Smith and T. Swain gave a slight different interpretation of the term, defining polyphenols as "water-soluble phenolic compounds having molecular weights between 500 and 3000 Da and, beside giving the usual phenolic reaction, they have special properties such as the ability to precipitate alkaloids gelatins

[23] Biemond, P., Swaak, A. J. G., van Eijk, H. G., Koster, J. F., *Free Rad. Biol. Med.*, **1988**, *4*, 185-198.

[24] Food and Drug Administration, 1993

and other proteins from solution".[25] The latter definition was further expanded by E. Haslam, who suggested to refine it as follow: "the term polyphenols should be used as a descriptor for water-soluble plant phenolic compounds having molecular masses ranging from 500 to 3000-4000 Da and possessing 12 to 16 phenolic hydroxy groups on five to seven aromatic rings per 1000 Da of relative molecular mass. Furthermore, the compounds should undergo the usual phenolic reactions and have the ability to precipitate some alkaloids, gelatin and other proteins from solution".[26] Nowadays, the WBSSH (White Bate-Smith Swain Haslam) definition tends to evolve, since alternative meanings of the term "polyphenols" have emerged. In fact, scientists from industry and academia more and more often refer to simple plant monophenolic compounds as polyphenols. In this wider sense, small molecules such as hydroxytyrosol (3,4-dihydroxyphenylethanol), terpenoids or tyrosine-derived alkaloids are allowed to join the big family of polyphenols. For the purpose of this thesis, the term "polyphenols" will embrace all plant-derived phenolics.

1.2.2 Classification

N° of C atoms	Basic skeleton	Class	Examples
6	C_6	Simple phenols Benzoquinones	Catechol, Hydroquinone 2,6-dimethoxybenzoquinone
7	C_6-C_1	Phenolic acids	Gallic, Salicylic
8	C_6-C_2	Tyrosine derivatives Phenylacetic acids	Tyrosol p-hydroxyphenylacetic acid
9	C_6-C_3	Hydroxycinnamic acids Coumarines Chromones	Caffeic, Ferulic Aesculetin Eugenin
10	C_6-C_4	Naphtoquinones	Juglone
13	C_6-C_1-C_6	Xanthones	Mangiferin
14	C_6-C_2-C_6	Stilbenes Antraquinones	Resveratrol Emodin
15	C_6-C_3-C_6	Flavonoids Isoflavonoids	Quercetin, Apigenin Genistein

[25] Swain, T., Bate-Smith, E. C. *Comparative Biochemistry III*, **1962**, pp. 755-809. New York Academic Press.

[26] Haslam, E., Cai, Y. *Nat. Prod. Rep.*, **1994**, *11*, 41-66.

N° of C atoms	Basic skeleton	Class	Examples
18	$(C_6\text{-}C_3)_2$	Lignanes Neolignanes	Pinoresinol Euseridin
30	$(C_6\text{-}C_3\text{-}C_6)_2$	Biflavonoids	Amentoflavone

Table 1.1 The major classes of phenolic compounds in plants.

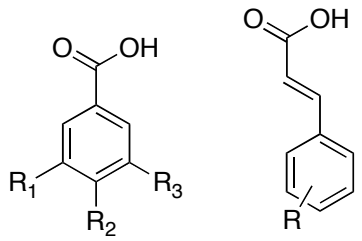

Figure 1.1 Structures of benzoic and cynnamic acids

Phenolic acids: Two classes can be distinguished: benzoic acid derivatives and cinnamic acid derivatives. The hydroxycinnamic acids are more common in edible plants than hydrobenzoic ones and consist of *p*-cumaric, ferulic, caffeic and sinapic acids. Caffeic acid, both free and esterified, is generally the most abundant phenolic acid in plant and represent from 75 to 100% of hydroxycinnamic acid composition of most fruits.[27]

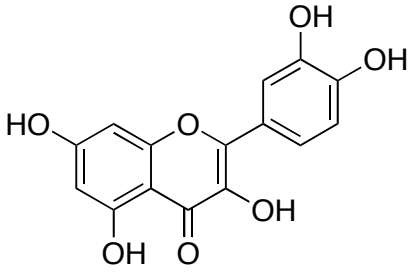

Figure 1.2 Structure of quercetin, a flavonol.

Flavonoids: Flavonoids are polyphenolic compounds that are ubiquitous in nature and are classified, depending on their chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. They can be found as hydroxylated, methoxylated and /or glycosylated derivatives. The linked sugar is often glucose or rhamnose; the number of sugar is commonly one, but it can be two or three and there are several position of substitution (C-7 position is the most preferred). The glycosylation greatly affects the bioavailability of flavonoids as well as the absorption.[28]

[27] Manach, C., Scalbert, A., Morand C., Rémésy C., Jimenez L., *Am. J. Clin. Nutr.*, **2004**, *79*, 727-747.

[28] Rice-Evans, C. A., Miller, N. J., Paganga, G., *Free Radic. Biol. Med.*, **1996**, *20*, 933-956

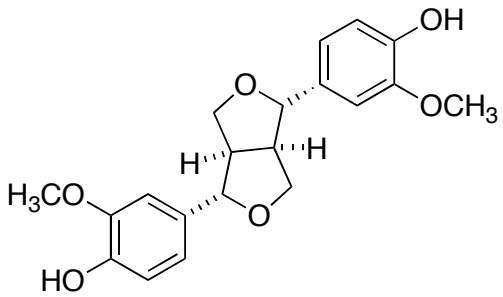

Figure 1.3 Structure of (+)-pinoresinol, a dimeric lignan

Lignans: Lignans are non-carbohydrate plant constituent characterized by 2 C_6-C_3 units linked together. They are constituents of lignin, a polymeric material found in plants as cell wall strengthener. Different classes of lignans are classified depending on the different linkage between the side chain carbon atoms of monomeric units.

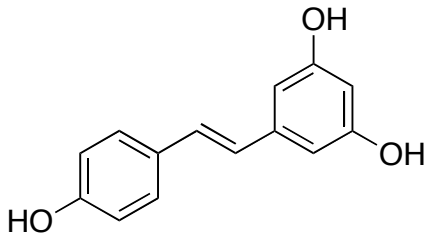

Figure 1.4 Structure of resveratrol

Stilbenes: Stilbenes are plant secondary metabolites, synthesized in response to pathogen attack. They are characterized by a 1,2-diphenylethylene backbone, with one or more substituent on the aromatic rings. Numerous structural variations have been identified, depending on the configuration of the carbon–carbon double bond, the number of hydroxyl functionalities, and the extent to which the phenol groups are substituted with sugars, methoxy, or other alkoxy groups. Resveratrol (3,5,4' -trihydroxy-*trans*-stilbene) is the most famous among the family, extensively studied since it was postulated to be involved in the health benefits associated with a moderate consumption of red wine.[29]

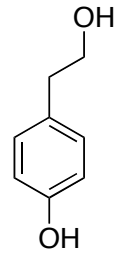

Figure 1.5 Structure of tyrosol

Phenethyl alcohols: They are characterized by a phenethyl structure derived from tyrosine metabolism (shikimic acid pathway). The aromatic ring can be mono- or poly-substituted by free or protected hydroxyl groups.

[29] Siemann, E. H., Creasy, L. L., *Am. J. Enol. Vitic.,* **1992**, *43*, 49-52.

1.2.3 Sources and biological activities

Polyhydroxylated compounds are widely distributed in the plant kingdom. They can be found in green[30,31] and black teas[32,33], coffee[34], fruits[35,36], vegetables[37,38], olive oil[39,40], red and white wines[41,42] and chocolate[43] (Fig. 1.6). People with diets rich in fruits and vegetables may consume one or more grams per day of these compounds. In most cases, foods contain complex mixtures of polyphenols, which are depending on the variety of plants. For many plants product, the polyphenols composition knowledge is often limited to one or a few varieties and data sometimes do not concern the edible part. Furthermore, numerous factors other than variety may affect the polyphenol content of plants; these factors include ripeness at the time of harvest, environmental factors (soil type, sun exposure, rainfall, culture type, etc.), processing and storage.

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[30] Sutherland, B. A., Rahman, R. M. A., Appleton, I., *J. Nutr. Biochem.,* **2006**, *17, 291-306.*

[31] Cabreara, C., Artacho, R., Gimenez, R., *J. Am. Coll. Nutr.*, **2006**, *25*, 79-99.

[32] Gardner, E. J., Ruxton, C. H. S., Leeds, A. R., *Eur. J. Clin. Nutr.*, **2007**, *61*, 3-18.

[33] Vinson, J. A., *Advances in Experimental Medicine and Biology*, **1998**, *439*, 151-164.

[34] Nardini, M., Cirillo E., Natella F., Scaccini, C., *J. of Agric. Food Chem.*, **2002**, *50*, 5735-5741.

[35] Vinson, J. A., Su, X., Zubik, L., Bose, P., *J. of Agric. Food Chem.*, **2001**, *49*, 5315-5321.

[36] Mertens-Talcott, S. U., Jilma-Stohlawetz, P., Rios, J., Hingorani, L., Derendorf, H., *J. of Agric. Food Chem.*, **2006**, *54*, 8956-8961.

[37] Vinson, J. A., Hao, Y., Su, X., Zubik, L., *J. of Agric. Food Chem.*, **1998**, *46*, 3630-3634.

[38] Oboh, G., Rocha, J. B. T., *Eur. Food Res. Technol.*, **2007**, *225*, 239-247.

[39] Gutiérrez, F., Arnaud, T., Garrido, A., *J. Sci. Food Agric.*, **2001**, *81*, 1463-1470.

[40] Visioli, F., Bellomo, G., Galli, C., *Biochem. Biophys. Res. Commun.*, **1998**, *247*, 60-64.

[41] Lodovici, M., Guglielmi, F., Casalini, C., Meoni, M., Cheynier, V., Dolara, P., *Eur. J. Nutr.*, **2001**, *40*, 74-77.

[42] Makris, D. P., Psarra, E., Kallithraka, S., Kefalas, P., *Food Res. Int.*, **2003**, *36*, 805-814.

[43] Vinson, J. A., Proch, J., Zubik, L., *J. of Agric. Food Chem.*, **1999**, *47*, 4821-4824.

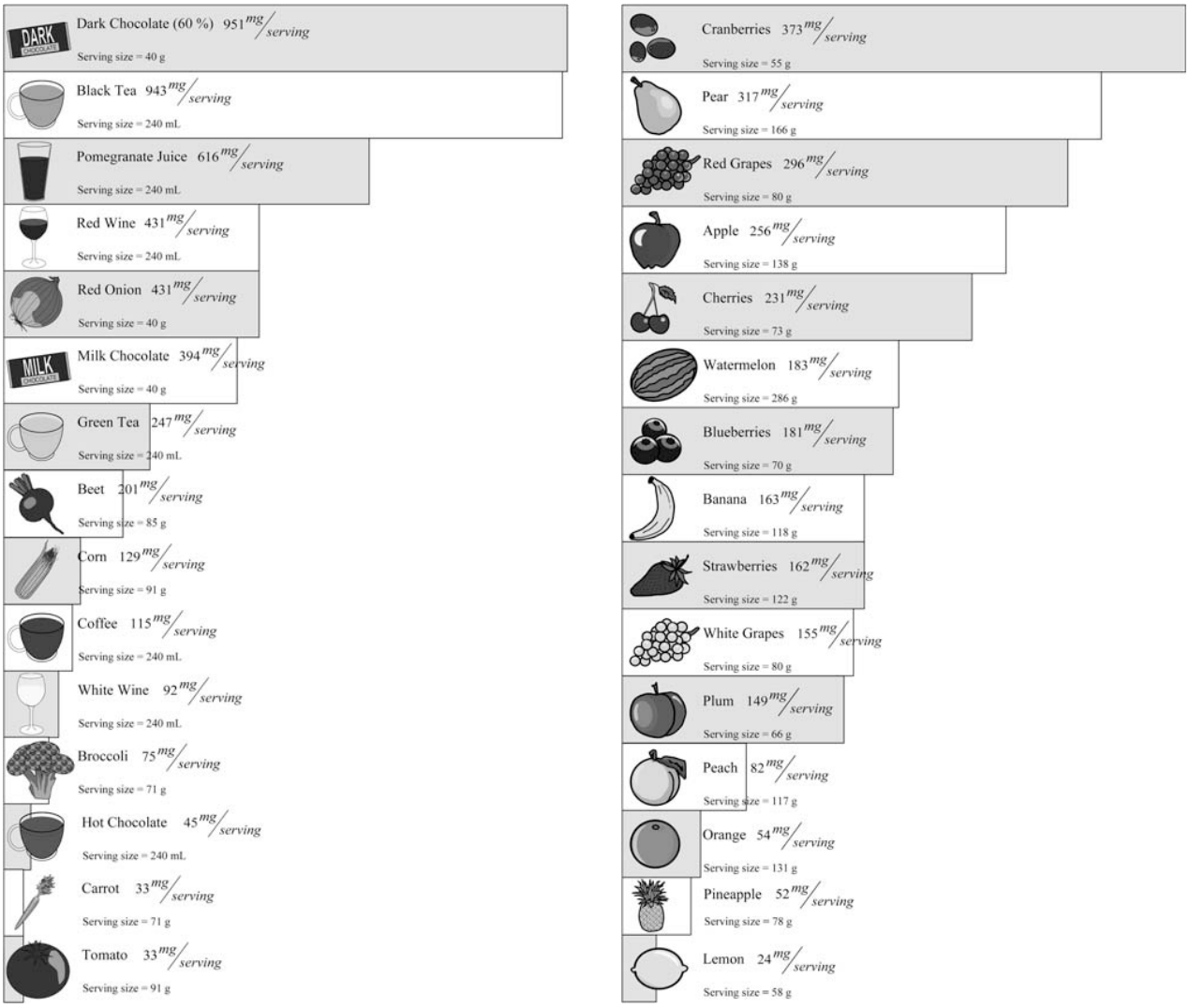

Figure 1.6 A chart showing the phenolic content of selected fruits, vegetables, beverages and chocolate in milligrams per serving.

Polyphenols exhibit a wide range of biological activities as a consequence of their antioxidant activity. They inhibit LDL oxidation *in vitro*[44] and protect DNA from oxidative damage.[45] It is well established that LDL oxidation is strongly correlated to cardiovascular diseases[46] such as myocardial infarction and stroke; thus any reduction of LDL oxidation could result in prevention of heart-associated diseases. Flavonoids show anti-inflammatory and anti-thrombotic properties[47,48] as well as anti-cancer activity. In particular, quercetin has shown anti-proliferative and antineoplastic

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[44] Frankel, E. N., Kanner, J., German, J. B., Parks, E., Kinsella, J. E., *Lancet*, **1993**, *341*, 454-457.

[45] Halliwell, B., *Nutr. Rev.,* **1999**, *57*, 104-113.

[46] Diaz, M. N., Frei, B., Vita, J. A., Keaney, J. F., *N. Engl. J. Med.*, **1997**, *337*, 408-416.

[47] Gerristen, M. E., Carley, W. W., Ranges, G. E., Shen, C. P., Phan, S. A., Ligon, G. F., Perry, C. A., *Am. J. Pathol.*, **1995**, *147*, 278-292.

[48] Muldoon, M. F., Kritchevsky, S. B., *Brit. Med. J.*, **1996**, *312*, 458-459.

activities *in vitro* against numerous cell lines.[49,50] It is thought that flavonoids anti-tumor activity depends on various mechanisms, including kinase inhibition and apoptosis by cell cycle arrest. All in all, several epidemiological studies show that a consumption of fresh fruits and vegetables plays a preventive role against chronic diseases (stroke,[51,52] heart disease,[53,54] cancer[55,56]). Unfortunately, evidence in human is still limited and controversial[57] since, at present, *in vivo* data are far from conclusive. It has to be remembered that the protective effect on human health, exerted by consumption of fresh fruits and vegetables, must be due to a variety of constituents (including vitamins, minerals and fibers) as well as polyphenols. All of them are supposed to act in a synergic manner.

1.2.4 Physicochemical properties and reactivity

A phenol functional group is an amphiphilic moiety made up by a planar aromatic ring (hydrophobic) and a polar hydroxy substituent (hydrophilic). The hydroxy moiety can act as a H-bond donor or as an acceptor (Scheme 2), while the aromatic ring can form hydrophobic van der Waals interactions with biomolecules such as proteins.[58] The $\pi \rightarrow \pi^*$ absorption maximum of polyphenols in water is red-shifted with respect to benzene itself due to the presence of additional hydroxy group and/or electron-withdrawing groups in *para* position. Hence, polyphenolic compounds provide protection against DNA-damaging solar radiation (UV-B light range, 270-320 nm).[59]

The presence of a single hydroxy group on the almost inert aromatic system drastically changes the reactivity. In fact, phenols can be considered as enols with a weak nucleophilic character that can be enhanced by deprotonation into phenolate anion ($pK_a \approx 8$-12, moderate but still exploitable acidity in biological system). Hence, polyphenols can react either as carbon or oxygen nucleophiles (Scheme 2). Phenols can also undergo oxidative process by hydrogen abstraction due to the relatively weak

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[49] Seeram, N. P., Adams, L. S., Hardy, M. L., Heber, D., *J. Agric. Food Sci.*, **2004**, *52*, 2512-2517.

[50] Ramos, S., Alia, M., Bravo, L., Goya, L., *J. Agric. Food Chem.*, **2005**, *53*, 1271-1280.

[51] Ness, A. R., Powles, J. W., *Int. J. Epidemiol.*, **1997**, *6*, 1-13.

[52] Peterson, J., Dwyer, J., *Nutr. Res.*, **1998**, *12*, 1995-2018.

[53] Frei, B. C., *Crit. Rev. Food Sci.*, **1995**, *35*, 83-97.

[54] Gei, K. F., *J. Nutr. Biochem.*, **1995**, *6*, 206-236.

[55] Ingram, D., Sanders, K., Kolybaba, M., Lopez, M., *Lancet*, **1997**, *983*, 990-994.

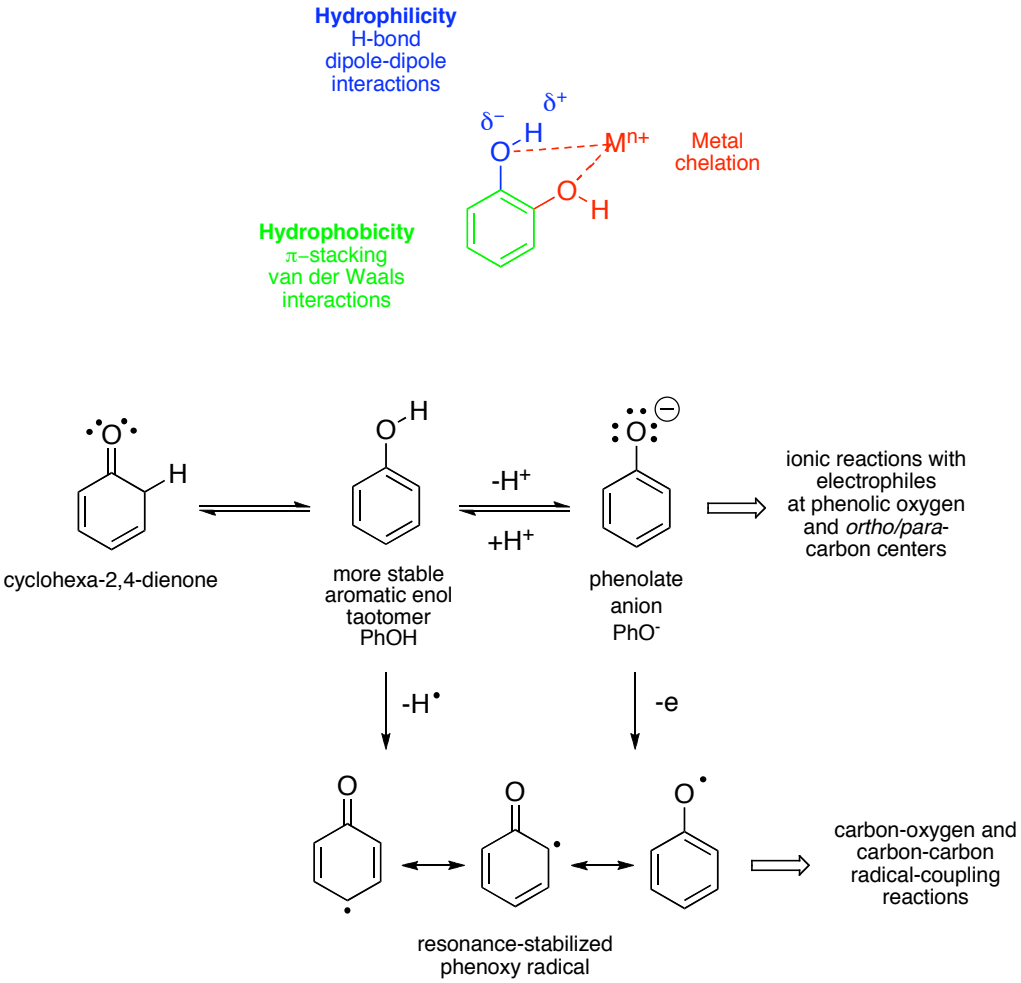
[56] Block, G., Patterson, B., Subar, A., *Nutr. Cancer*, **1992**, *17*, 1-29.

[57] Wang, W., Goodman, M. T., *Nutr. Res.*, **1999**, *19*, 191-202.

[58] Dangles, O., Dufour, C., *Recent advances on polyphenols research, Vol I*, Wiley-Blackwell, Oxford, **2008**, pp. 67-87.

[59] Harborne, J. B., Williams, C. A., *Phytochemistry*, **2000**, *55*, 481-504.

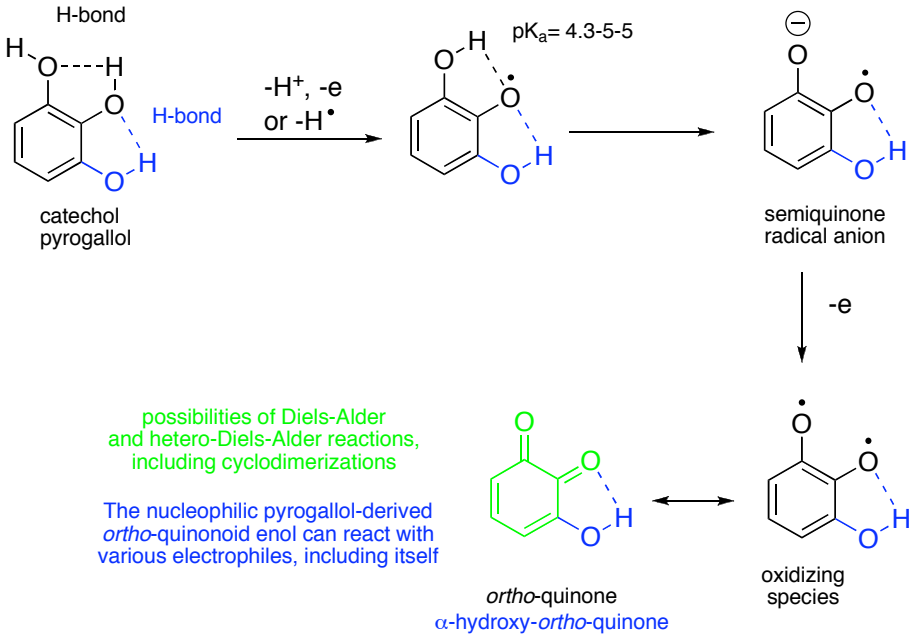
O-H bond dissociation enthalpy (BDE, 87-90 Kcal/mol in gas phase).[60] The stability of the phenoxy radical formed is drastically influenced by the presence of alkoxy and/or alkyl group in *orto* or *para* positions. Moreover, phenols can be converted into phenoxenium cation (PhO^+) in slightly acidic oxidation condition, often encountered in biological systems. Phenoxenium cation are extremely reactive intermediates towards nucleophilic aromatic substitution.[61]



Scheme 1.2 Physicochemical properties and reactivity of phenolics.

[60] Blanksby, S. J., Ellison, G. B., *Acc Chem. Res.*, **2003**, *36*, 255-263.

[61] Quideau, S., Pouységu, L., Deffieux, D., *Curr. Org. Chem.*, **2004**, *8*, 113-148.



Scheme 1.3 Oxidative dehydrogenation of catechol- and pyrogallol-type phenols.

1.3 Polyhydroxylated compounds: antioxidant activity

Eat five servings of fruits and vegetables per day! This is what is highly recommended to stay healthy. Many mechanisms have been proposed for polyphenols prevention of oxidative stress and ROS-RNS generation. Radical scavenging is the most widely published mechanism for polyphenols antioxidant activity. (Scheme 3) Nonetheless, it should be also kept in mind the presence of metal chelation and inhibition of key-enzymes in ROS generation.

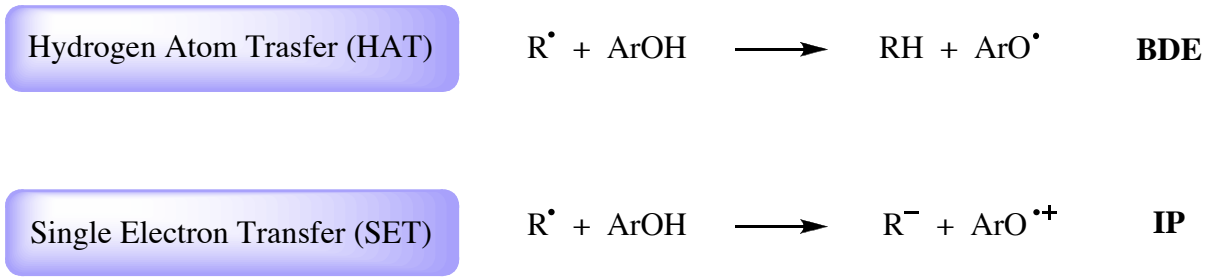
In this context, it is important to recall the possibility of any redox agent to exert pro-oxidant activity and promote free radicals generation in certain circumstances.

1.3.1 Radical scavenging pathways

Two main radical scavenging-based mechanisms have been proposed for polyphenols:[62] The first arises from the ability to donate a hydrogen atom to a free radical (R•) and break chain reactions, while the second occurs when a single electron is transferred. (Scheme 4) The efficiency of each process can be correlated to an appropriate parameter as shown above. Bond dissociation enthalpy (BDE) of the phenolic O-H bond is linked to HAT mechanism since the lower the BDE value, the

[62] Wright, J. S., Johnson, E. R., DiLabio, G. A., *J. Am. Chem. Soc.*, **2001**, *123*, 1173-1183.

easier the homolitic rupture of the O-H bond and the reaction with free radicals are. Similarly, ionization potential (IP) is another important tool in evaluating the phenols antioxidant activity SET-related; the lower the IP, the easier the electron transfer is.



Scheme 1.4 Hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms. BDE (bond dissociation enthalpy) and IP (ionization potential) are the two physicochemical parameters used to determine the efficacy of each process, respectively.

Among polyphenols, catecholic and pyrogallolic species (hydroxytyrosol, gallic acid, epicatechin) act well as H-atom donor due to the good stability of the resulting phenoxy radical conferred by intramolecular H-bond with the adjacent hydroxy group(s).[63] Lower IP values are calculated for molecules having an extended electronic delocalization due to resonance effects and structural planarity. Such polyphenols (resveratrol, kaempferol) act as antioxidant by donating a single electron to free radicals.[64]

1.3.2 Metal chelation

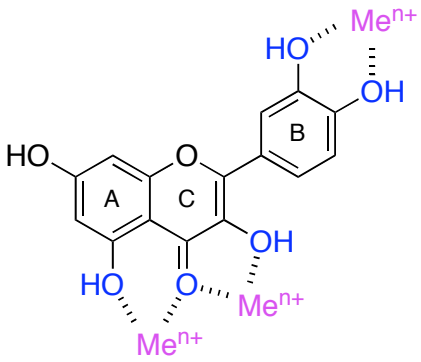


Figure 1.7 Binding sites for trace metals in quercetin.

As mentioned before, transition metals (mainly iron) are implied in ROS generation i*n vivo* through Fenton-type reactions (1 and 3, *par 1.1.1*). Understanding the biochemistry of iron has become a primary matter in literature due to its involvement in DNA damage, cell death and oxidative stress. It is well established that transition metal can be chelated by polyphenols, leading to stable complexed compounds.[65] The complex stability can be correlated to the acidity of the polyphenols, as far as the ionized forms of the latter are concerned. In fact, the anionic form of the polyphenols is often involved in metals chelation. For flavonoids, the 4'-OH in the ring

[63] Leopoldini, M., Marino, T., Russo, N., Toscano, M., *J. Phys. Chem.*, **2004**, *108*, 4916-4922.

[64] See ref. 63

[65] Brown, J. E., Khodr, H., Hider, R. C., Rice-Evans, C. A., *Biochem. J.*, **1998**, *330*, 1173-1178.

B is the most preferred deprotonation site, followed by the 7-OH in the ring A.[66] Nonetheless, when the deprotonated compound can stabilize the negative charge through conjugation, alternative sites for chelation arise. This is the case of quercetin, that form the lower energy adduct by involving the carboxylic oxygen on ring C and the C5 hydroxy group.[67] (Fig. 1.7)

1.3.3 ROS production-related enzymes inhibition

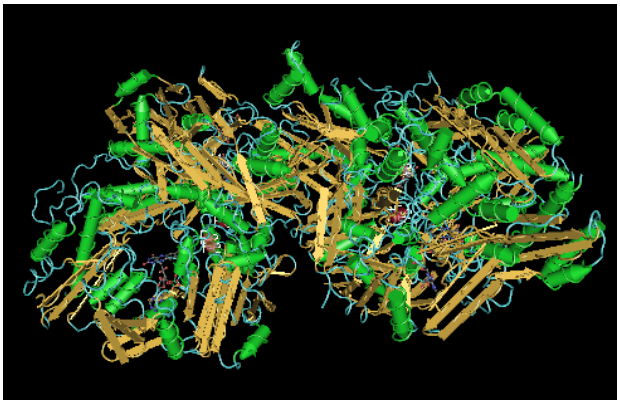


Figure 1.8 Crystal structure of bovine xanthine oxidase in complex with quercetine

Another way through which polyphenols could exert antioxidant activity is inhibition of enzymes responsible of ROS and RNS production. Flavonoids are capable to inhibit the enzymes involved in superoxide anion production, such as xanthine oxidase,[68] (Fig. 1.8) and protein kinase C.[69] Xanthine oxidase is implicated in oxidative damage because it reacts with molecular oxygen and releases superoxide. Quercetin and luteolin are potent inhibitors of xanthine oxidase.[70] Flavonoids inhibit nitric oxide synthase that generates nitric oxide, which can further react with free radicals to produce peroxynitrite species (RNS).[71] Moreover, flavonoids have been also shown to inhibit cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase and NADH oxidase, all responsible for ROS generation.[72]

1.3.4 Pro-oxidant activity and its exploitation on development of anticancer drugs

As each redox system, polyphenols can scavenge or produce free radicals. Their activity depends on many factors, including structural and chemical features, redox potential with respect to those of the

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[66] Leopoldini, M., Russo, N., Chiodo, S., Toscano, M., *J. Agric. Food Chem.*, **2006**, *54*, 6343–6351.

[67] See ref. 66

[68] Hanasaki, Y., Ogawa, S., Fukui, S., *Free Rad. Biol. Med.*, **1994**, *16*, 845-850.

[69] Ursini, F., Maiorino, M., Morazzoni, P., Roveri, A., Pifferi, G., *Free Rad. Biol. Med.*, **1994**, *16*, 547-553.

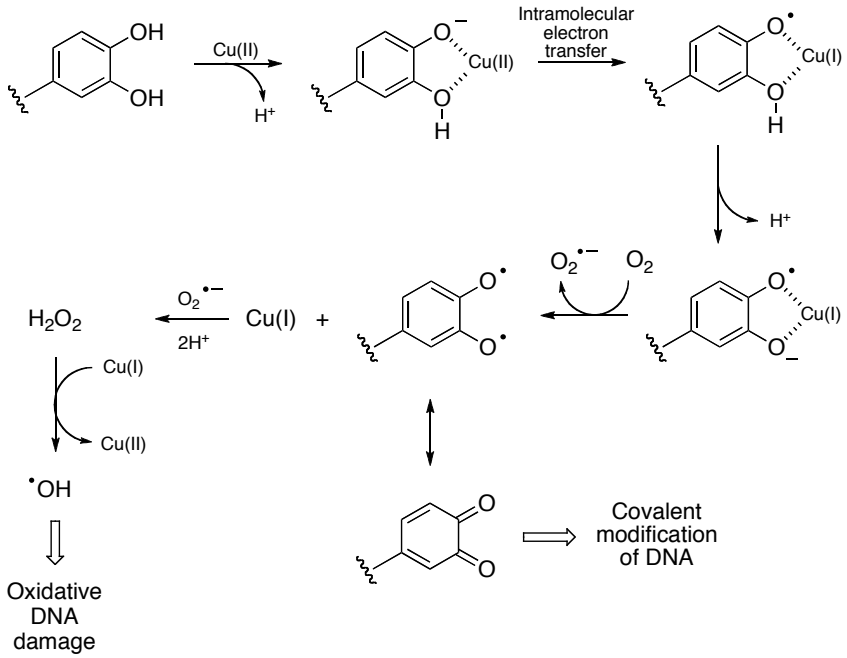
[70] Cos, P., Ying, L., Calomme, M., Hu, J. P., Cimanga, K., Van Poel, B., Pieters, L., Vlietinck, A. J., Vanden Berger, D., *J. Nat. Prod.*, **1998**, *61*, 71-76.

[71] Shutenko, Z., Henry, Y., Pinard, E., Seylaz, J., Potier, P., Berthet, F., Girard, P., Sercombe, R., *Biochem. Pharmacol.*, **1999**, *57*, 199-208.

[72] Brown, J. E., Khodr, H., Hider, R. C., Rice-Evans, C., *Biochem. J.*, **1998**, *330*, 1173-1178.

species with which they interact, the BDE and the IP values of the phenol unit and bioavailability.[73,74]

The pro-oxidant effect has been thought to have an active role in the chemopreventive effect of polyphenols against carcinogenesis.[75] As an example, Zheng and co-workers have recently studied the copper-mediate DNA breakage induced by resveratrol and its analogues.[76] A pro-oxidation mechanism for copper(II)-mediated DNA damage by catecholic or pyrogallolic plant polyphenols has been proposed (Scheme 5).



Scheme 1.5 Proposed pro-oxidation mechanism for Cu(II)-mediated DNA damage by catecholic or pyrogallolic plant polyphenols.

As shown in the scheme, the pro-oxidant property can be exert by reduction of metals (mainly copper, due to its lower standard reduction potential: Cu^{2+}/Cu →0.15 V vs. Fe^{3+}/Fe^{2+} →0.77 V) after chelation. Moreover, the electrophilic *ortho*-quinone thus generated can induce covalent DNA damage and protein and peptide covalent modifications.[77] (Scheme 5) In conclusion, in normal cells polyphenols could prevent cancer through HAT-based ROS scavenging, while in cancer cells,

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[73] Wollgast, J., Anklam, E., *Food Res. Int.*, **2000**, *33*, 449-459.

[74] Dreosti, I. E., *Nutrition*, **2000**, *16,* 692-694.

[75] Fan, G.-J., Jin, X.-L., Qian, Y.-P., Wang, Q., Yang, R.-T., Dai, F., Tang, J.-J., Shang, Y.-J., Cheng, L.-X., Yang, J., Zhou, B., *Chem. Eur. J.*, **2009**, *15*, 12889-12899.

[76] Zheng, L. F., Wei, Q.-Y., Cai, Y.-J., Fang, J.-G., Zhou, B., Yang, L., Liu, Z.-L., *Free Radical Biol. Med.*, **2006**, *41*, 1807-1816.

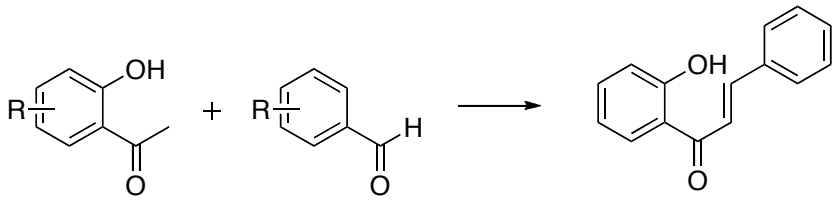
[77] Feldman, K. S., Sambandam, A., Bowers, K. E., Appel, H. M., *J. Org. Chem.*, **1999**, *64*, 5794-5803.

characterized by higher oxidative stress level,[78] they could further increase ROS level through intramolecular SET-based reduction of Cu(II), causing cytotoxic DNA breakage.

1.4 Conventional syntheses of polyhydroxylated compound

1.4.1 Flavonoids

Due to the increasing interest on flavonoids in biology and pharmacology, numerous total syntheses have been developed in the last decades. The methodology most widely used to prepare flavonoids involves the isomerization of appropriately substituted 2-hydroxy chalcones, in turn obtained by an aldol condensation reaction between a 2-hydroxyacetophenone and a benzaldehyde. (Scheme 6)



2-hydroxyacetophenone benzaldehyde 2-hydroxychalcone

Scheme 1.6 Aldol condensation between substituted acetophenones and benzaldehydes.

These cyclizations have been carried out under numerous conditions using acids,[79,80] bases,[81,82] silica gel,[83] heat,[84,85] light,[86,87] electrolysis,[88] Ni/Zn/K halides[89] and others.[90,91,92] (Scheme 7)

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[78] Pelicano, H.,Carney, D., Huang, P., *Drug Resist. Updates*, **2004**, *7*, 97-110.

[79] Reichel, L., Proksch, G., *Ann. Chem.* **1971**, *745*, 59-70.

[80] Nabaei-Bidhendi, G., Bannerjee, N. R., *J. Indian Chem. Soc.*, **1990**, *67*, 43-45.

[81] Keane, D. D., Marathe, K. G., O'Sullivan, W. I., Philbin, E. M., Simons, R. M., Teague, P. C., *J. Org. Chem.*, **1970**, *35*, 2286–2290.

[82] Climent, M. J., Garcia, H., Iborra, S., Miranda, M. A., Primo, J., *Hetrocycles*, **1989**, *29*, 115–117.

[83] Sangawan, N. K., Varma, B. S., Dhindsa, K. S., *Chem. Ind. (London)* **1984**, 271–272.

[84] Harris, T. M., Carney, R. L., *J. Am. Chem. Soc.*, **1967**, *89*, 6734–6741.

[85] Hoshino, Y., Takeno, N., *Bull. Chem. Soc. Jpn.*, **1986**, *59*, 2903–2904.

[86] Matsushima, R., Kageyama, H., *J. Chem. Soc., Perkin Trans. 2*, **1985**, 743–748.

[87] Pandey, G., Krishna, A., Kumaraswamy, G., *Tetrahedron Lett.* **1987**, *28*, 4615–4616.

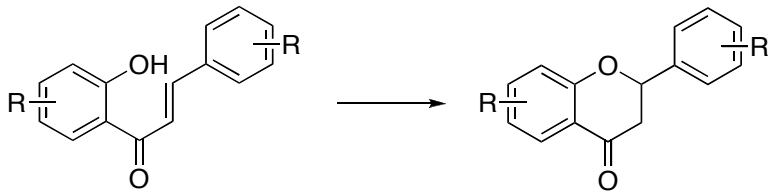
[88] Sanicanin, Z., Tabakovic, I., *Tetrahedron Lett.*, **1986**, *27*, 407–408.

[89] Ali, S. M., Iqbal, J., Ilyas, M., *J. Chem. Res. (S)*, **1984**, 236–240.

[90] Chen, H. Y., Dykstra, K. D., Birzin, E. T., Frisch, K., Chan, W., Yang, Y. T., Mosley, R. T., DiNinno, F., Rohrer, S. P., Schaeffer, J. M., Hammond, M. L., *J. Bioorg. Med. Chem. Lett.*, **2004**, *14*, 1417–1421.

[91] Choudary, B. M., Ranganath, K. V. S., Yadav, J., Kantam, M. L., *Tetrahedron Lett.*, **2005**, *46*, 1369–1371.

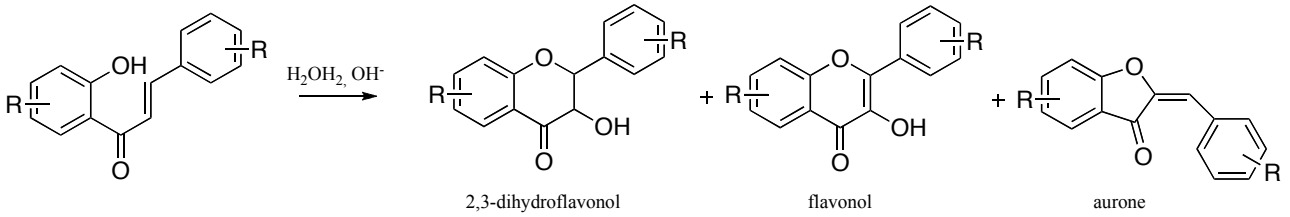
[92] Dauzonne, D., Monneret, C., *Synthesis*, **1997**, *11*, 1305–1308.



Scheme 1.7 2-hydroxychalcones cyclization.

Flavanones thus prepared can be converted into the corresponding flavones by dehydrogenation. The choice of the reactants depends on the nature and reactivity of the substituent on the aromatic rings.[93,94]

Flavonols and 2,3-dihydroflavonols can be synthesized from 2-hydroxychalcones exploiting the AFO (Algar-Flynn-Oyamada) protocol developed in 1934.[95] To avoid the formation of aurones-like related compound, milder reaction conditions and a strictly controlled approach should be used.[96] (Scheme 8)



Scheme 1.8 The AFO approach to the synthesis of flavan-3-ols and flavonols.

1.4.2 Stilbenes

The first laboratory preparation of resveratrol dates back to 1941.[97] Since then, organic synthesis has developed towards new and sophisticated methodologies due in large measure to the influx of recent, rapidly evolving organometallic catalysis. The Wittig reaction has proved to be quite versatile in the preparation of different substituted stilbenes.[98,99] Even though the reaction is not sensitive to atmospheric oxygen, allowing simple practical procedure, a mixture of *cis*- and *trans*-

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[93] Singh, O. V., Kapoor, R. P., *Tetrahedron Lett.*, **1990**, *31*, 1459-1462.

[94] Prasak, O., Pahuja, S., Moriarty, R. M., *Synth. Comm.*, **1990**, *20*, 1417-1422.

[95] Geissman, T. A., Fukushima, D. K., *J. Am. Chem. Soc.*, **1948**, *70*, 1686-1689.

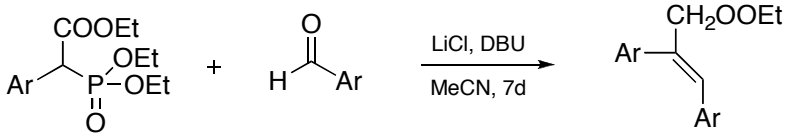
[96] Patonay, T., Toth, G., Adam, W., *Tetrahedron*, **1993**, *34*, 5055–5058.

[97] Späth, E., Kromp, K., *Ber. Dtsch. Chem. Ges.*, **1941**, *74*, 189-192.

[98] Wadsworth, W. S., Emmons, W. D., *J. Am. Chem. Soc.*, **1961**, *83*, 1733-1739.

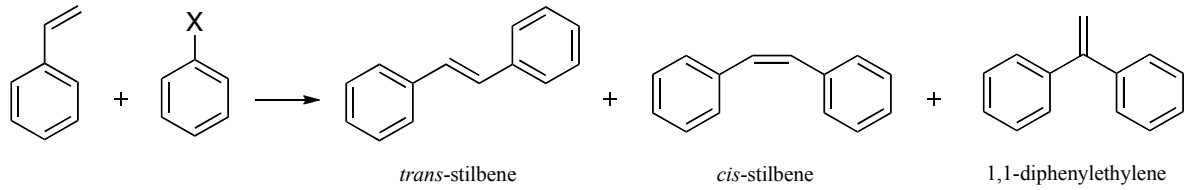
[99] Horner, L., Hoffmann, H. M. R., Wippel, H. G., *Chemische Berichte*, **1958**, *91*, 61-63.

stilbene is obtained. The Horner–Wadsworth–Emmons reaction employes stabilized phosphonate carbanions with aldehydes (or ketones) to form predominantly *E*-alkenes. By this way 2,2'-aryl-substituted cinnamic acid esters were synthesized.[100] (Scheme 9)



Scheme 1.9 Scheme of synthesis of 2,2-aryl-substituted cinnamic acid esters.

The palladium-catalyzed olefination of arylhalydes (Heck reaction) is another useful tool to synthesize stilbenoids. (Scheme 10) According to this approach, stilbenes may be obtained by coupling a styrene with an aryl derivative Ar–X.[101] The regioselectiviy of the reaction can be modulated by a relevant choice of the reaction conditions.[102]



Scheme 1.10 Heck reaction's products.

In addition to conventional Heck reaction, nucleophilic organoboron species can be coupled with arylhalydes or diazonium salts (Suzuki reaction) for the synthesis of stilbenes derivatives. Despite the reaction proceed without addition of any bases, the reaction times were extended.[103]

1.5 C-O bond formation through Cu-catalyzed process

Formally copper catalyzed couplings are analogous to palladium and nickel catalyzed reactions. Carbon-carbon and carbon-heteroatom bonds can be formed in such transformations alike. From the mechanistic point of view there is a significant difference between nickel, palladium and copper catalyzed processes however. While in the former cases the catalyst usually oscillates between the 0 and +2 oxidation states, in copper mediated transformations the common oxidation numbers are +1, +2 and +3. There are two distinguishable classes of copper catalyzed processes.

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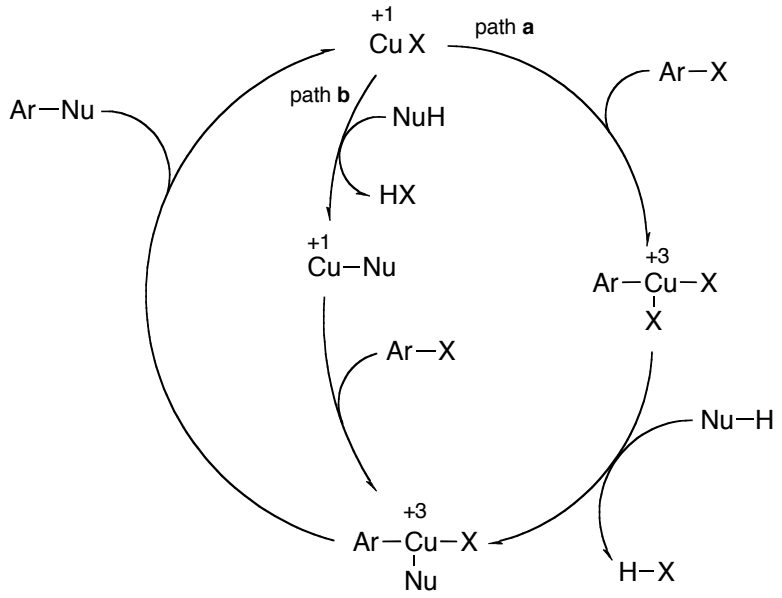
100 Ianni, A., Waldvogel, S. R., *Synthesis*, **2006**, *13*, 2103-2112.

101 See for example: Guiso, M., Marra, C., Farina, A., *Tetrahedron Lett.*, **2002**, *43*, 597-598.

102 Reetz, M. T., Lohmer, G., Lohmer, R., Westermann, E., *Ger. Offen.*, DE 19843012 (**2000**).

103 Andrus, M. B., Song, C., *Org. Lett.*, **2001**, *3*, 3761-3764.



Scheme 1.11 Cu(I)-catalyzed coupling reaction mechanism.

Reactions catalyzed by copper(I) salts usually couple a reagent capable of oxidative addition (e.g. an aryl halide) and another reagent that can attach itself to the copper via transmetalation/ coordination. Common examples include nitrogen, oxygen and carbon nucleophiles, such as alcohols, amines, azoles, cyanide and malonates. In these processes the sequential attachment of the coupling partners to the copper centre results in the formation of a copper(III) complex (path **a,** Scheme 11), which, on release of the product in reductive elimination, returns to the catalytically active +1 oxidation state. It is not always clear if the oxidative addition or the transmetalation step is the opening of the catalytic cycle (path **b**, Scheme 11) as most nucleophiles exhibit a strong affinity towards copper salts and form stable complexes.

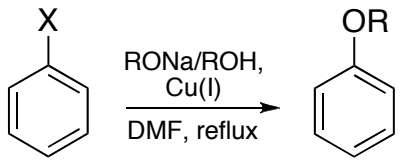
The other class of Cu-catalyzed reactions follow a different route, involving Cu(II) salts and arylboronic acids that undergo nucleophilic displacement. For the purpose of this thesis this class of reactions will not be discussed any further.

2. Aim of the work

2.1 Oxyfunctionalization of flavonoids

Flavonoids are molecules with antioxidant properties widely occurring in the plant kingdom. The specific properties of these molecules are due to the presence of aromatic moieties and of many oxygenated groups.[104] Methoxylated flavones exhibiting interesting biological activities have recently been isolated from plants.[105] Despite the biological importance evidenced for this class of natural compounds, an extensive investigation into the activities of most of them is limited because of their scarce availability. Moreover, the synthesis and/or structural modification of these polyphenolics is often complex, low yielding and expensive.[106,107,108] Because of the increasing interest in these molecules, methods for their synthesis and structural modification are the goals of several research groups.

Our interest in this field is to exploit our experience in the functionalization of aromatic compounds to prepare natural-occurring and new flavonoids that are potentially useful as drugs or food preservatives. Recently we reported a protocol for introducing oxygenated moieties into activated aromatic rings.[109] A selective bromination, followed by a methanolysis protocol, was performed on a series of natural compounds with the aim to improve their antioxidant properties.[110] (Scheme 2.1) Previously reported oxygenation methods of flavonoids, i.e., catalyzed hydrogen peroxide[111] or potassium persulfate[112] systems are directed to introduce hydroxyl groups in the skeleton and are usually low yielding.



Scheme 2.1 Copper(I)-catalyzed synthesis of aryl-alkyl ethers.

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[104] Fernandez-Bolanos, J., Felizon, B., Brenes, M., Guillen, A., Heredia, A., *J. Am. Oil Chem. Soc.*, **1998**, *75*, 1–7.

[105] Aroonrerk, N., Punjanon, T., Suksamrarn, S., Kongkun, S., *Arch. Pharm. Res.*, **2003**, *26*, 816–820.

[106] Matuyama, K., Tamanaka, K., Nishinaga, A., Inada, A., Nakaishi, T., *Tetrahedron Lett.*, **1989**, *30*, 4145–4148.

[107] Sanicanin, Z., Tabakovic, I., *Tetrahedron Lett.*, **1986**, *27*, 407–408.

[108] Stermitz, F. R., Adamovics, J. A., Geigert, J., *Tetrahedron*, **1975**, *31*, 1593–1595.

[109] Bovicelli, P., Antonioletti, R., Barontini, M., Borioni, G., Bernini, R., Mincione, E., *Tetrahedron Lett.*, **2005**, *46*, 1255–1257.

[110] Bovicelli, P., *J. Pharm. Pharmacol.*, **2007**, *59*, 1703–1710.

[111] Gao, H., Kawabata, J., *Biosci. Biotechnol. Biochem.*, **2004**, *68*, 1858–1864.

[112] Barron, D., Jolivert, S., Crouzet, J.-M., Mariotte, A.-M., *Tetrahedron Lett.*,**1992**, *33*, 7137–7140.

In particular the methanolysis protocol (R = Me, Scheme 2.1), developed in our laboratories, is actually considered of general value and can be used as routine synthetic step. Also sensitive compounds, such as flavones are usually submitted to the process with success.[113] Our protocol consists in mixing at room temperature NaOMe and CuBr in DMF, which is then added to the bromo-aromatic substrate in DMF at 120 °C and the methanolysis reaction occurs in few minutes. It is noteworthy that the bromo-aromatic substrates used do not need to be highly activated. Moreover, substrates with different substitution patterns on the aromatic ring underwent methanolysis even if sterically hindered and/or despite the presence of *ortho* hydroxy group. The process was used with success in the preparation of some rare flavones such as 3'-demethoxysudachitin,[114] compound present in several plant extracts often used in traditional medicines and known to possess a number of biological activities. At the present we are studying the scope and limitations of the method and the possibility to exploit it to synthesize a series of flavones.

Selective bromination and flavanones oxidation were the fundamental key steps towards the achievement of the actual substrate that underwent methanolysis. A preliminary screening on the efficiency and the regioselectivity of some bromination methodologies were made in order to optimize the bromo-derivate synthesis; a recently reported protocol[115] to oxidize α,β-unsatured ketones were successfully applied on flavanones in order to convert them into fully-conjugated flavones.

2.2 Reactivity and functionalization of tyrosol

Tyrosol, a commercially available simple phenol, is one of the most abundant component of the unsaponifiable fraction (1-2%) of virgin olive oil.[116] It can be found in olive mill waste water and thus it is a good candidate as starting material for large-scale syntheses. More potent antioxidants hyroxytyrosol and 3,5-dihydroxytyrosol have been recently synthesized by our research group starting from tyrosol.[117] Keeping our attention on this topic, synthetic strategies towards the synthesis of new and potentially good antioxidants were developed during this thesis. In particular,

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[113] Bovicelli, P., D'Angelo, V., Collalto, D., Verzina, A., D'Antona, N., Lambusta, D., *J. Pharm. Pharmacol.*, **2008**, *59*, 1697–1701.

[114] Bovicelli, P., D'Angelo, V., Collalto, D., Verzina, A., D'Antona, N., Lambusta, D., *J. Pharm. Pharmacol.*, **2007**, *12*, 1697-1701.

[115] Nicolau, K. C., Montagnon, T., Baran, P. S., *Angew. Chem. Int. Ed.*, **2002**, *41*, 993-996.

[116] Murkovic, M., Lechner, S., Pietzka, A., Bratacos, M., Katzogiannos E., *J. Biochem. Biophys. Methods*, **2004**, *61*, 155-160.

[117] See ref. 110

new tyrosol derivatives bearing 3,4,5-trihydroxy substitution pattern and 2,3-dihydrobenzofurans were synthesized. As a case in point the unique reactivity of phenethyl structure were investigated. As previously reported in literature, halo- and/or tosyl- derivates of phenethyl alcohol can undergo rearrangement to form phenonium ion after the loss of the leaving group. [118] Carboxymethylated derivatives were found to undergo the same rearrangement under relatively mild reaction conditions, making this methodology exploitable to form Nu-C bond, even using weak carbon nucleophiles.

2.3 Copper-catalyzed silver acetylide-azide cycloaddition

A side project has been conducted under the supervision of Dr. Spencer J. Williams at Melbourne University. The possibility of silver acetylides to undergo cycloaddition under CuAAC (Cu-catalyzed alkyne-azide cycloaddition) condition was investigated. For this purpose a wide range of silver acetylides were synthesized. Moreover, a mechanistic explanation about the course of the reaction was proposed exploiting mass spectroscopy experiments. Due to the complexity and the fullness of the subject, a separate chapter of this thesis, including a brief overview on Huisgen reaction and its recent developments, will be dedicated to this topic.

[118] Brusco, Y., Berroteran, N., Lorono, M., Cordova, T., Chuchani, G., *J. Phys. Org. Chem.*, **2009**, *22*, 1022-1029.

3. Results and discussion - Flavonoids

3.1 Covergent synthesis of mosloflavone, negletein and baicalein

The synthetic pathway that was employed to obtain mosloflavone (**11**), negletein (**13**) and baicalein (**14**) is summarized in Scheme 3.4. A semi-synthetic approach towards the synthesis of **11**, **13** and **14** was applied. Crysin (**6**), a cheap and commercially available flavone, was chosen as starting material.[119]

Initially the bromination step was performed with a oxone/NaBr system, which produces a highly reactive electrophilic species, which are able to brominate activated aromatic systems, such as in the case of hydroxytyrosol[120] and bioactive biphenols syntheses.[121] The method is not useful in the case of the A-ring of flavones, since its efficiency does not allow a discrimination between different highly activated sites, i.e., the C6 and C8 positions of crysin (**6**). For this reason other brominating agents have been explored. Tetrabutylamonium tribromide (TBATB) is reported in literature as an efficient generator of HBr[122] but it is also able to brominate double bonds, such as in the case of some chalcones.[123] This reagent showed to be very efficient in the selective bromination of crysin dimethyl ether (**1**) in the 6-position, as already reported for a similar iodination reaction.[124] (Scheme 3.1) On the contrary, if one or both the hydroxyl groups were left unprotected a mixture of bromo derivatives were obtained in any condition. In the case of 7-methylcrysin (**3**) the reaction led to a mixture, which tended towards the 6,8-dibromo derivative with an excess of the reagent. Under mild conditions it was possible to obtain a mixture of two monobromo derivatives (**4**:**5** 2:1 ratio), inseparable by common chromatographic techniques. In the case of crysin (**6**) the dibromo derivative was already present at low conversion and then 6,8-dibromo crysin (**7**) was the only product obtainable in good yields. *N*-Bromosuccinimide (NBS) had a different behavior with 5,7-

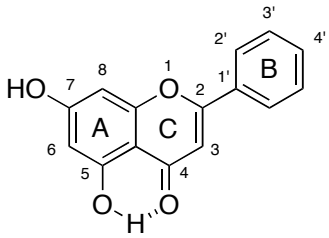


Figure 3.1 Crysin

[119] Righi, G., Antonioletti, R., Proietti Silvestri, I., D'Antona, N., Lambusta, D., Bovicelli, P., *Tetrahedron*, **2010**, *66*, 1294-1298.

[120] Bovicelli, P., Antonioletti, R., Mancini, S., Causio, S., Borioni, G., Annnendola, S., Barontini, M., *Synth. Commun.*, **2007**, *37*, 4245–4252.

[121] Bovicelli, P., Antonioletti, R., Onori, A., Delogu, G., Fabbri, D., Dettori, M. A., *Tetrahedron*, **2006**, *62*, 635–639.

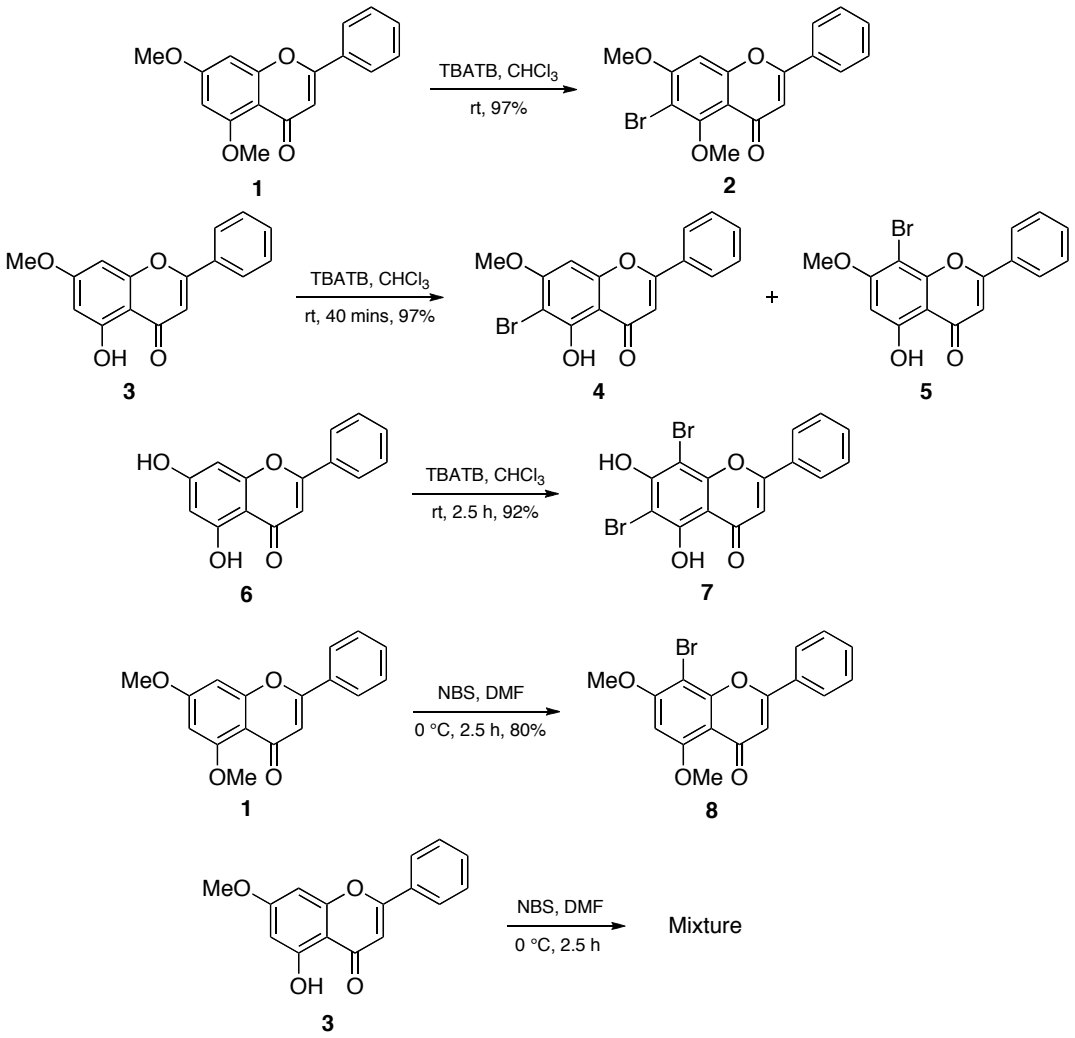
[122] Gopinath, R., Haque, S. J., Patel, B. K., *J. Org. Chem.*, **2002**, *67*, 5842–5845.

[123] Bose, G., Mondal, E., Khan, A. T., Bordoloi, M. J., *Tetrahedron Lett.*, **2001**, *42*, 8907– 8909.

[124] Quintin, J., Lewin, G., *Tetrahedron Lett.*, **2004**, *45*, 3635–3638.

dimethylcrysin (**1**) being able to brominate the C8-position, but it failed to selectively functionalize flavones with free hydroxyl groups. (Scheme 3.1)

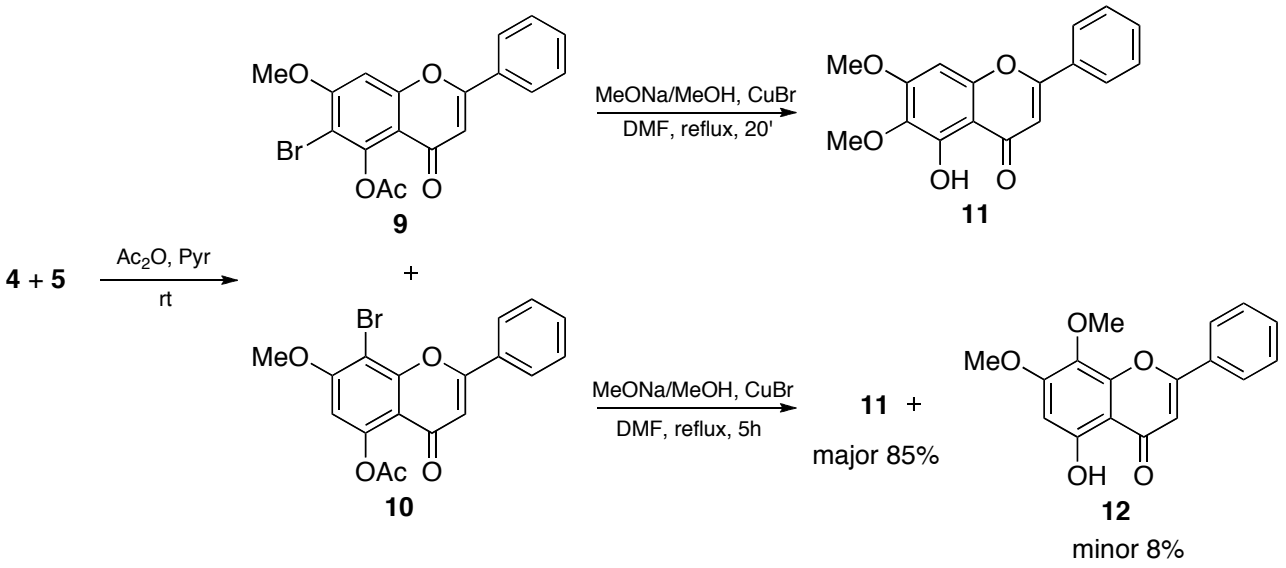
In order to modulate the reactivity and to distinguish the C6 and C8 positions, mono- and di-methyl ether derivatives of **6** were synthesized. Exploiting the different reactivities of C5 and C7 hydroxyl groups, a selective methylation at the oxygen atom on C7 can be conveniently achieved using dimethyl solphate as methylating reagent. The regioselectivity of the reaction is due to an intramolecular hydrogen bond between the hydrogen atom of C5-OH group on ring A and the oxygen on the ketone group on ring C that drastically lowers the C5-OH nucleophilicity (Figure 3.1). Enhancing the temperature at reflux temperature of acetone (the solvent of the reaction) counter the effect of C5-OH and C7-OH different reactivities and the dimethyl-derivate (**1**) is easily achieved.



Scheme 3.1 Selective bromination of flavones.

With the aim to exploit the bromination/methanolysis protocol in order to obtain flavones with a higher oxygenation degree, **2** and **8** have been reacted in methanolysis conditions. Surprisingly any

attempt at methanolysis of **2** and **8** failed to give any isolable product. In fact, most of the substrate remained unreacted (no dehalogenation product was isolated) even after 5 hours while no main product has been detected in the reaction mixture. This negative result seems consistent with a scarce reactivity of the substrate towards the formation of the reactive complex with Cu(I). This behavior was probably due to the sterical hinderance of two methoxyl groups. Then, with the aim of exploiting our protocol to obtain polyoxygenated flavones, the compounds obtained from bromination of **3** were purified as acetyl derivatives. (Scheme 3.2)



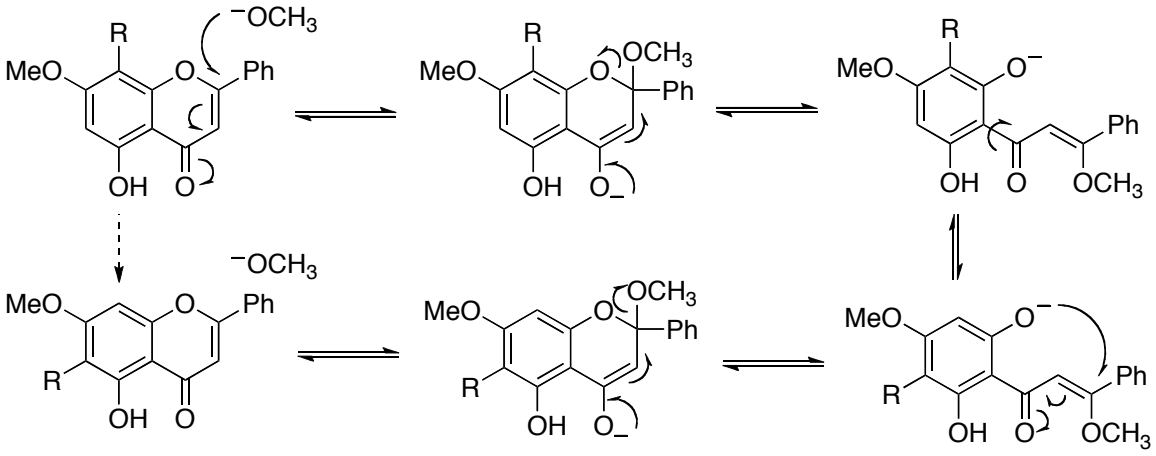
Scheme 3.2 Methanolysis of monobromo-derivatives

Compounds **9** and **10** were submitted to the usual methanolysis step and **11** was obtained as the main product from both the substrates (Scheme 3.2). In the case of **9** the reaction occurred in few minutes, while in the case of **10**, 5 hours were required to converge an initial complex mixture towards the main product. This behavior led us to hypothesize a process in which, after the preliminary deacetylation, a rearrangement occurred in which an open form of the ring C was involved. This particular rearrangement, known as Wessely-Moser rearrangement, has been already reported for flavones only in acid media[125,126] via an equilibrium of open/close forms leading to the more thermodynamically stable compound. A similar mechanism can be proposed in basic conditions. (Scheme 3.3) The methoxy group can cause the C ring opening reacting at the electrophilic C2 carbon on the double bond . The closure can take place both on the same oxygen (i.e., the C9-OH) or on the oxygen on C5 because of the rotation of C10-C4 bond. The latter give, as final product, the flavone with the substituent (R on Scheme 3.3) on C6. In this case **11** was the

[125] Shinomiya, K., Hano, Y., Nomura, T., *Heterocycles*, **2000**, *53*, 877–886.

[126] Lareget, R., Lockhart, B., Renard, P., Largeron, M., *Biorg. Med. Chem. Lett.*, **2000**, *10*, 835–838.

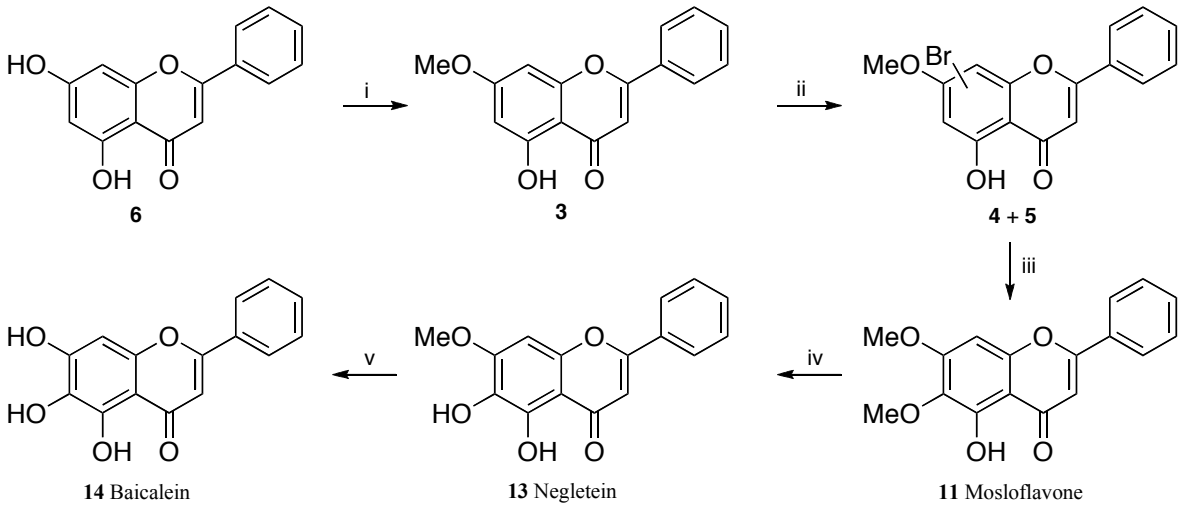
preferred product. The exact structure of compounds **11** and **12** has been assigned after a methylation of the C5 hydroxyl group.



Scheme 3.3 Proposed mechanism for the Wessely-Moser type rearrangement in basic media

For the purpose of this synthesis the mixture of **4** and **5** was reacted with MeONa and CuBr without further purification, since both the bromoderivates gave compound **11** as preferred product.

Mosloflavone **11** is a natural occurring flavone extracted from *Desmos Chinensis*.[127] By progressive selective demethylation steps, two more natural products were produced: negletein (**13**), component of *Centaurea clementei*,[128] and baicalein (**14**), component of *Scutellaria baicalensis*.[129] (Scheme 3.4) Despite a longer reaction time with respect to BBr_3 system, the HBr/AcOH methodology was chosen due to the easier work up and the better product recovery.



14 Baicalein **13** Negletein **11** Mosloflavone

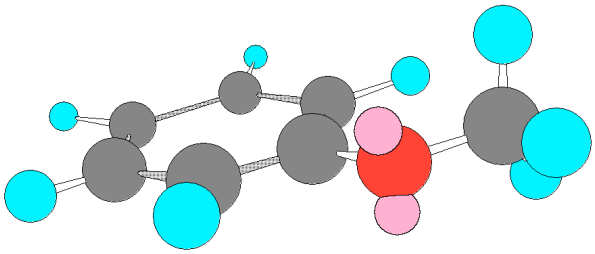
[127] Van Kiem, P., Van Minh, C., Huong, H. T., Lee, J. J., Lee, I. S., Kim, Y. H., *Arch. Pharmacal. Res.*, **2005**, *28*, 1345-1349.

[128] Gonzalez Collado, I., Macias, F. A., Massanet, G. M., Rodriguez Luis, F., *J., Nat. Prod.*, **1985**, *48*, 819–822.

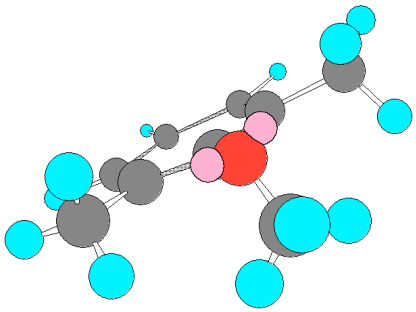
[129] Li-Weber, M., *Cancer Treat. Rev.*, **2009**, *35*, 57–68.

Scheme 3.4 Convergent synthesis of **11**, **13** and **14**. i) $(CH_3O)_2SO_2$, K_2CO_3, acetone, rt; ii) TBATB, $CHCl_3$, rt; iii) MeONa/MeOH, CuBr, DMF, reflux; iv) HBr, AcOH, reflux, 3h; v) HBr, AcOH, reflux, 18h.

Compound **13** was obtained despite in literature a similar reaction affording a different regioisomer was reported.[130] In the ^{13}C NMR spectrum the methoxy group of **13** has a chemical shift of 56.3 ppm, value compatible with a methoxyl having at least one *ortho* position free of functional groups. In fact, a methoxy substituent on an aromatic system with no sterical hinderance can lie in the plane of the ring. In this conformation there is the maximum overlap between the lone pair of the oxygen and the π-orbitals of the aromatic ring. The methoxyl carbon is then shielded by the conjugated electrons and chemical shifts occur between 55.0 and 56.5 ppm.



Methoxy group having free *ortho* positions: oxygen lone pairs (pink) can conjugate with π-orbitals of the aromatic ring.



Methoxy group with no free *ortho* positions: minimum overlapping between oxygen lone pairs and π-orbitals of the aromatic ring.

Figure 3.2 3D graphical representation of *ortho* substituents influence on MeO- chemical shift.

When the methoxyl group has both the *ortho* positions occupied by bulky substituents, this conformation is disfavoured, the oxygen is not fully conjugated with the aromatic ring and the methoxy carbon is deshielded to 59.5–63.6 ppm.[131] The ^{13}C NMR shifts were also used to determine the exact structure of compounds **11** and **12**. After a preventive methylation of the hydroxyl group in C5, compound **11** has two carbon signals in the region of 60 ppm and only one at

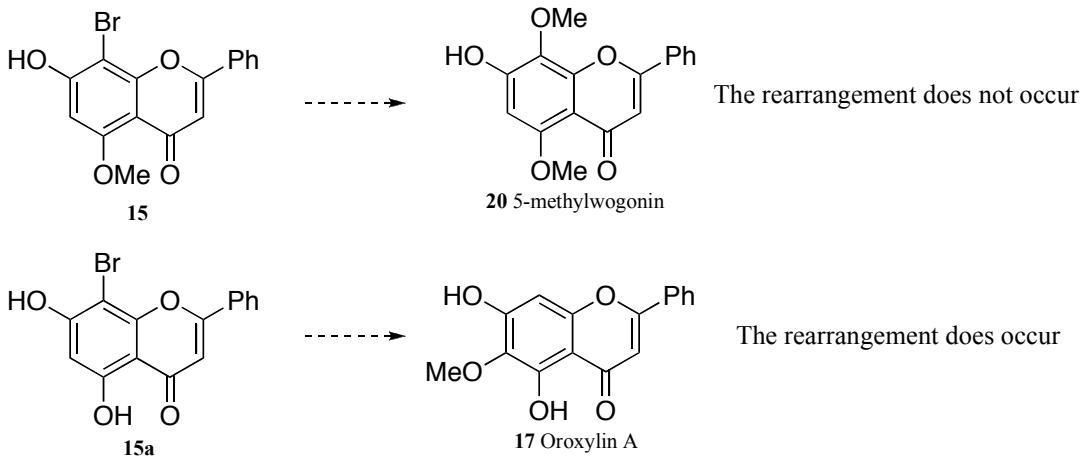
[130] Huang, W.-H., Chien, P.-Y., Yang, C.-H., Lee, A.-R., *Chem. Pharm. Bull.*, **2003**, *51*, 339–340.

[131] Panichpol, K., Waterman, P. G., *Phytochemistry*, **1978**, *17*, 1363–1367.

higher fields (56 ppm). On the contrary, compound **12** presents one signal at 56 ppm and two at 60 ppm; indeed, the right structure for each compound is the one depicted above. (Scheme 3.2)

3.2 Divergent synthesis of wogonin and oroxilin A

Exploiting the Wessely-Moser rearrangement two more natural flavone, wogonin (**16**) and oroxilin A (**17**), have been synthesized. Wogonin is an uncommon flavone extracted from *Scutellaria baicalensis*.[132] Recently it has been demonstrated that wogonin exerts anxiolytic effect as benzodiazepine receptor ligand without exhibiting sedative and myorelaxant side-effects.[133] Wogonin and oroxylin A have both the common substitution pattern of flavonoids, i.e. the C5 and C7 hydroxyl groups. They only differ in the methoxy substituent position that varies from C6 (oroxylin A) to C8 (wogonin). Keeping in mind the Wessely-Moser type rearrangement, it is easy to understand that both the compounds can be synthesized starting from the same precursor and exploiting or not the rearrangement during the methanolysis step. (Scheme 3.5)



Scheme 3.5 Exploitation of Wessely-Moser rearrangemet in oroxylin A and wogonin synthesis.

The Wessely-Moser rearrangement can be avoided protecting the C5 hydroxyl group with a base-resistant protecting group. (Scheme 3.3) Nevertheless, the regioselectivity of the bromination step is modulated by substituents on C5 and C7 oxygen atom, thus a wise choice of C5 and C7 protecting groups is needed. The inexpensive flavone crysin was chosen as starting material as in the previous case. A benzylation of C7-OH followed by a methylation of C5-OH led to compound **18** in one-pot

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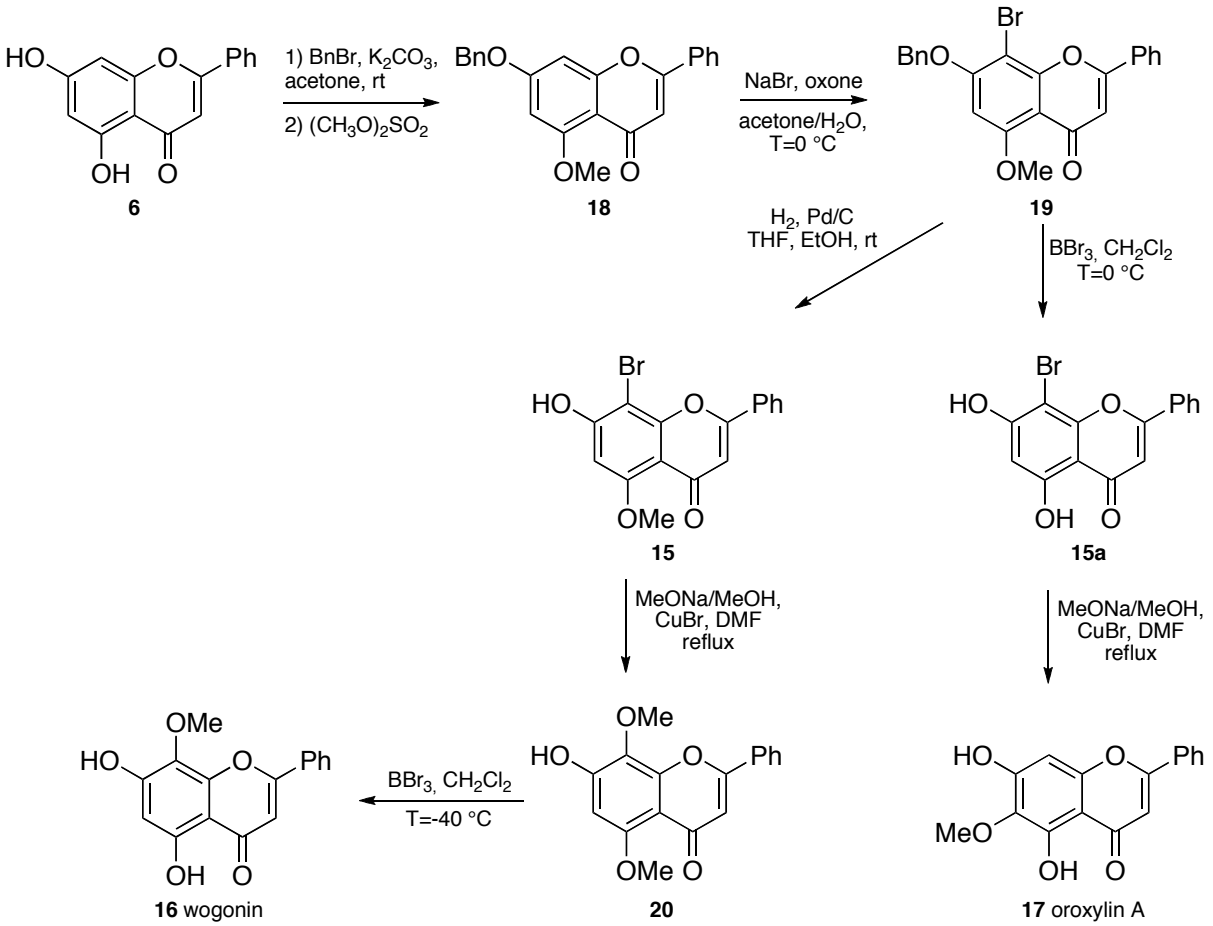
[132] Hui, K. M., Huen, M. S. Y., Wang, H. Y., Zheng, H., Sigel, E., Baur, R., Ren, H., Li, Z. W., Wong, J T F., Xue, H., *Biochem. Pharmacol*., **2002**, *64*, 1415-1424.

[133] See ref. 132

two steps process. **18** was then treated with TBATB at rt. Since no reaction occurred, the reaction mixture was heated up to 60 °C but a complex mixture of product was obtained.



Scheme 3.6 Divergent synthesis of oroxylin A and wogonin.

When NaBr-oxone system was tested on substrate **18** a perfect regioselectivity of the reaction was detected at T=0 °C since **18** was completely converted into the 8-bromo derivate **19**. (Scheme 3.6) The structure was assigned by ^{13}C NMR spectroscopy. Compound **19** can be conveniently deprotected on C7-OH and/or C5-OH in order to obtain a bromo-derivate with (**15a**) or without (**15**) a free hydroxyl group on C5. This is the key step in which the synthesis of wogonin and oroxylin A diverges. (Scheme 3.6) A methanolysis reaction on **15a** directly led to oroxylin A (**17**) since the substrate underwent a complete rearrangement after 5 hours. The same reaction performed on **15** led to 7-hydroxy-5,8-dimethoxyflavone (**20**) in few minutes. **20** was then selectively demethylated exploiting the higher reactivity of C5-OMe with respect to the others present in the molecule. **16** was then achieved in satisfactory yield.

3.3 Convergent synthesis of scutellarein

Recently scutellarein, a flavone extracted from *Scutellaria*, became noteworthy due to its interesting biological activities reported by different research group like cytotoxic activity on human leukaemia cells,[134] inhibitory activity towards 17β-HSD[135] and human salivary α-amylase.[136] It is also known to be an antagonist of tromboxane A_2 receptor,[137] leading to the formulation of new drugs involved in the thrombotic diseases. To the best of our knowledge, no synthesis of scutellarein was reported until now for preparative purposes.[138]

The approach to the synthesis of scutellarein consisted in finding out a new and easily reproducible way to prepare it starting from inexpensive compounds. Referring to the previous work, in which crysin was converted to baicalein using a bromination/methanolysis protocol,[139] the dimethylated apigenin (5-hydroxy-7,4'-dimethoxyflavone, **23**) was chosen as starting material. Being apigenin very expensive and scarcely available in nature, our first goal was to convert naringenin, the flavanone precursor, into the above corresponding flavone. It is well known in literature that flavones have enhanced biological activities than flavanones.[140,141,142,143] The oxidation step was firstly carried out using I_2 (0.3-0.5 eq.) in DMSO in acidic media. (Scheme 3.7) Unfortunately a low conversion of the substrates **21** was observed. Trying to force the reaction adding an excess (1.2 eq.) of iodine improved the conversion but got worse the chemoselectivity of the reaction since iodination at the aromatic ring A was competitively achieved.

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[134] Plochmann, K., Korte, G., Koutsilieri, E., Richling, E., Riederer, P., Rethwilm, A., Schreier, P., Scheller, C., *Arch. Biochem. Biophys.*, **2007**, *460*, 1-9.

[135] Brozic, P., Kocbek, P., Sova, M., Kristl, J., Martens, S., Adamski, J., Gobec, S., Rizner, T. L., *Mol. Cell. Endocrinol.*, **2009**, *301*, 229-234.

[136] Lo Piparo, E., Scheib, H., Frei, N., Williamson, G., Grigorov, M., Chou, C. J., *J. Med. Chem.*, **2008**, *51*, 3555-3561.

[137] Navarro-Nuñez, L., Castillo, J., Lozano, M. L., Martinez, C., Benavente-Garcia, O., Vicente, V., Rivera, J., *J. Agric. Food Chem.*, **2009**, *57*, 1589-1594.

[138] Gao, H., Kawabata, J., *Biosci. Biotechnol. Biochem.,* **2004***, 68*, 1858-1864.

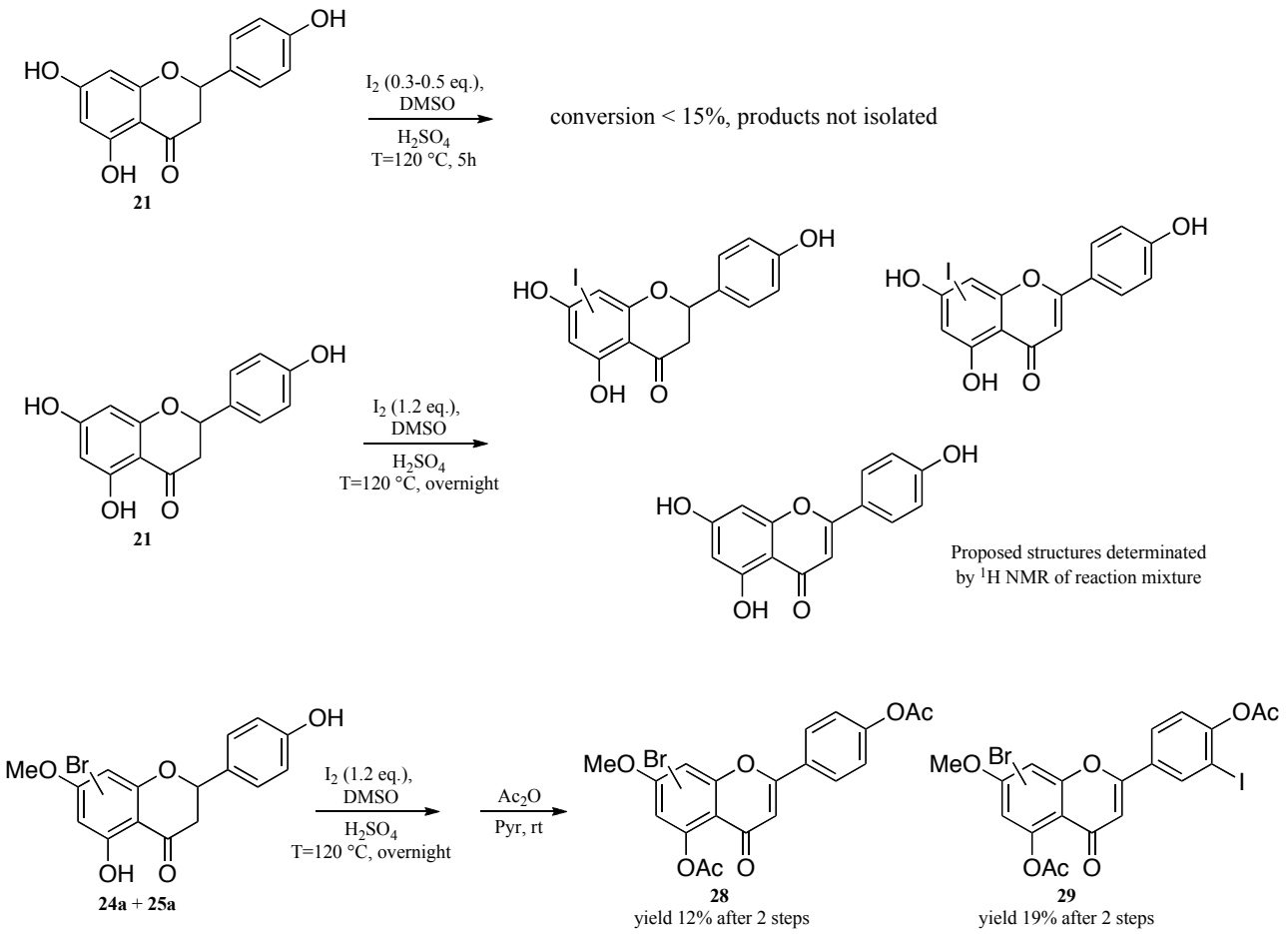
[139] See ref. 120

[140] Ares, J. J., Outt, P. E., Randall, J. L., Johnston, J. N., Murray, P. D., O'Brien, L. M., Weisshaar, P. S., Ems, B. L., *Bioorg. Med. Chem. Lett.*, **1996**, *6*, 995-998.

[141] Park, Y. H., *J. Ocul. Pharmacol. Ther.*, **2004**, *20*, 189-200.

[142] Farkas, O., Jakus, J., Heberger, K., *Molecules*, **2004**, *9*, 1079-1088.

[143] Tapas, A. R., Sakarkar, D. M., Kakde, R. B., *Trop. J. Pharm. Res.,* **2008**, *7*, 1089-1099.



Scheme 3.7 Flavanone/flavone oxidation with I_2/DMSO protocol

Referring to a previous work reported by Bovicelli and co-workers[144] in which I_2/DMSO oxidation of bromoflavanones was achieved in good yield, 6-bromo-5,4'-dihydroxy-7-methoxyflavanone (**25a**) and 8-bromo-5,4'-dihydroxy-7-methoxyflavanone (**24a**) were synthesized according to the the synthetic pathway described above. (see Scheme 3.4, steps 1 and 2) The I_2/DMSO oxidation protocol was then applied to **24a** and **25a** with the aim to obtain the corresponding flavones **24** and **25**. As in the previous case, a complex mixture, inseparable by common chromatographic techniques, was obtained. After a preliminary acetylation of the free hydroxyl groups, two main products (**28** and **29**) were isolated. **28** was the expected flavone while **29** turned out to be the 3'-iodoflavone. Hence, beside the C2-C3 double bond formation, an electrophilic aromatic substitution at ring B happened. (Scheme 3.7) Since the reaction failed to be explicable for synthetic purposes, other methodologies were examined.

[144] Bovicelli, P., D'Angelo, V., Collalto, D., Verzina A., D'Antona N., Lambusta, D., *J. Pharm. Pharmacol.*, **2007**, *59*, 1697-1701.

Recently, Nicolau and co-workers [145] reported a new and efficient method to oxidize C_α-C_β bond of aldehydes and ketones. They introduced a new class of oxidants, the complexes of 2-iodoxybenzoic acid (IBX) with *N*-oxides, which often exhibit superior reactivity than that of the parent reagent. (Figure 3.3) Heteroatom oxide ligands when linked to IBX provide a modified electronic environment around the iodine center, which enhances the propensity of these reagents to serve as electron trap. The goodness and efficiency of this class of compounds can be attributed to the low temperature employed that allow this reaction to be accomplished also on labile substrates. Among the wide range of *N*-oxides examined as potential ligands, *N*-methylmorpholine-*N*-oxide (NMO) was proposed as one of the best due to the good reaction rate and general and mild reaction conditions. Hence, the oxidation step was carried out with the complex NMO•IBX in DMSO at room temperature. The reactive complex is readily prepared by mixing equivalent amount of the two reagent at room temperature in DMSO. For this oxidative protocol, Nicolaou et al. proposed a single electron transfer (SET) mechanism, as described in Scheme 3.8.

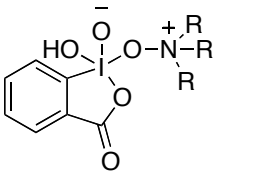
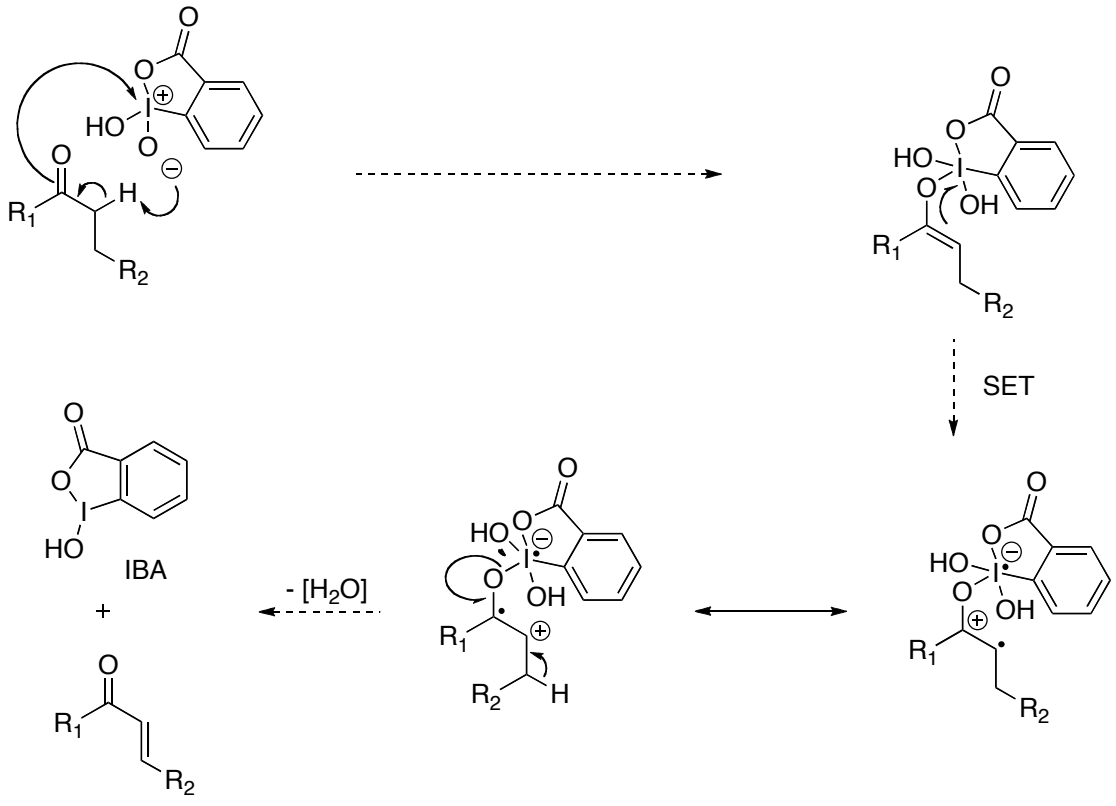


Figure 3.3 IBX• N-oxides complex



Scheme 3.8 Postulated mechanism for the IBX-mediated dehydrogenation of ketones and aldehydes to α,β-unsatured carbonyl compounds.

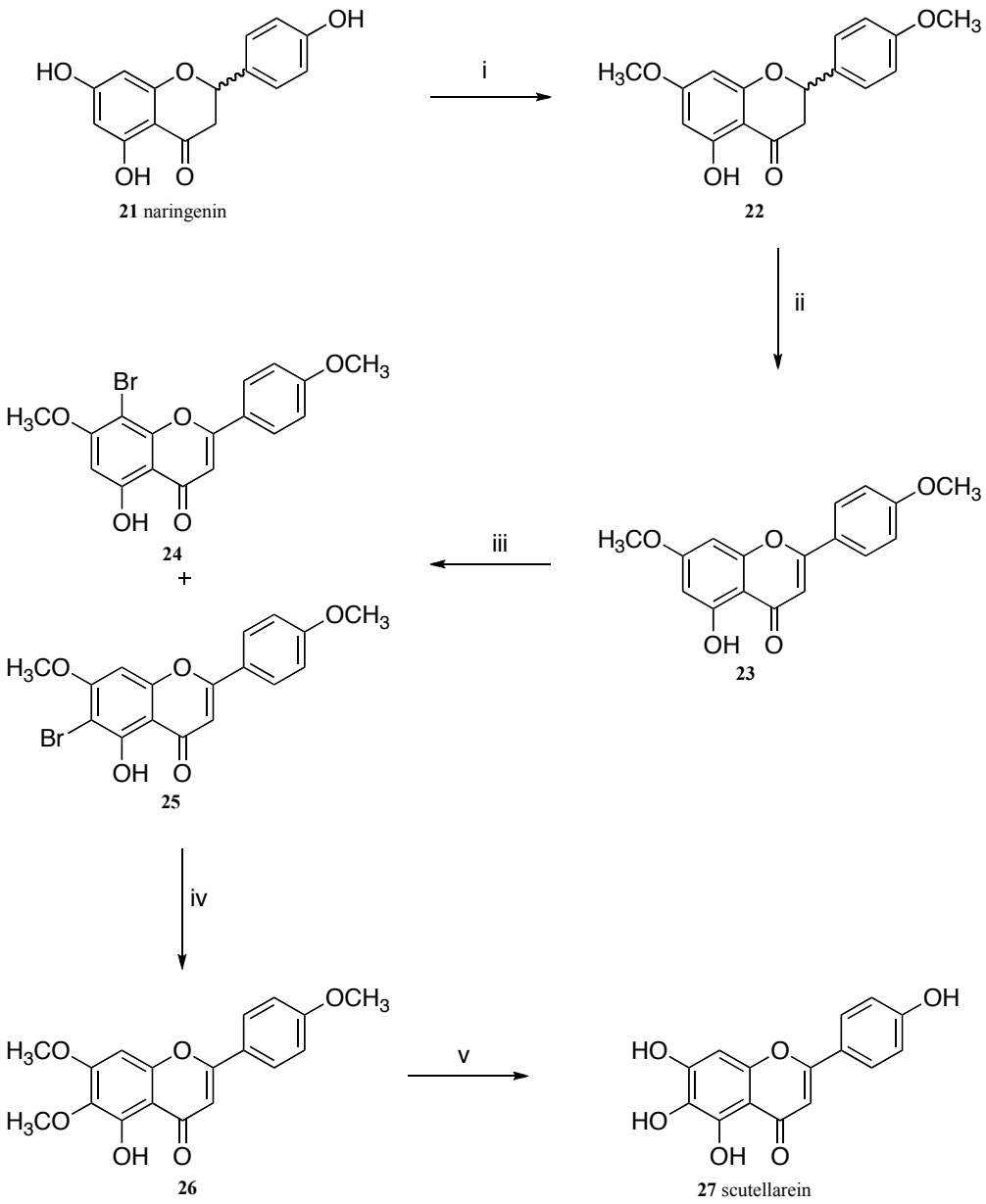
[145] Nicolau, K. C., Montagnon, T., Baran, P. S., *Angew. Chem. Int. Ed.*, **2002**, *41*, 993-996.

In this particular case, to convert naringenin into apigenin, (Scheme 3.9) a previous methylation of hydroxyl groups[146] was necessary to avoid chemoselective issues arising from the presence of free hydroxyl groups since it was recently reported by Pettus and co-workers that IBX can easily oxidize phenolsl to *ortho*-quinones.[147] Despite what has been reported for similar substrates,[148] a complete protection of all three hydroxyl groups is useless since a free hydroxyl group on C5 does not affect the oxidation reaction. The following bromination step led to a mixture of regioisomer, i.e. 6-bromo-5-hydroxy-7-methoxyflavone **25** and 8-bromo-5-hydroxy-7-methoxyflavone **24**, in accordance with the previous work. Tetrabutylamonium tribromide (TBATB) demonstrated to have a perfect regioselectivity towards the A ring since no bromination occurred on B ring.

[146] Jurd, L., *J. Org. Chem.,* **1962**, *27*, 1294-1297.

[147] Selenski, C., Pettus, T. R., *Tetrahedron*, **2006**, *62*, 5298-5307.

[148] Barontini, M., Bernini, R., Crisante, F., Fabrizi, G., *Tetrahedron*, **2010**, *66*, 6047-6053.



Scheme 3.9 Convergent synthesis of scutellarein. i) Acetone, K_2CO_3, $(CH_3O)_2SO_2$ ii) NMO, IBX, DMSO iii) TBATB, $CHCl_3$ iv) CuBr, MeONa, DMF v) HBr, AcOH. Overall yield 62% from naringenin.

The mixture of monobromoflavones was directly converted to 5-hydroxy-6,7,4'-trimethoxyflavone (**26**) with copper bromide and sodium methoxyde in DMF. A Wessely-Moser rearrangement occurs (see for example Scheme 3.3) in basic media during the methanolysis step as reported for crysin.[149] The final selective demethylation steps led to scutellarein (**27**) passing through the methyl derivatives. The proposed synthesis occurred in 62% overall yield,[150] allowing to prepare the target compound for drug uses and for extensive biological studies.

[149] See ref. 120.

[150] Righi, G., Proietti Silvestri, I., Barontini, M., Crisante, F., Di Manno, A., Pelagalli, R., Bovicelli, P., *Natural Product Research*, DOI:

3.4 Investigation on IBX behavior on flavonoids

In recent years, the chemistry of hypervalent iodine compounds has experienced a drastic growth[151,152] and a broad variety of hypervalent iodine reagents have been prepared for new, mild and useful synthetic transformation. Large atomic size and low ionization potential compared to other halogens make iodine a suitable candidate to form polycoordinate compounds. Multivalent iodine reagents are now used extensively in organic synthesis as a mild, safe, economic and environmentally-friendly alternative to heavy-metal reagents such as lead(IV), thallium(III), mercury(II), chromium(VI), etc.

2-iodoxybenzoic acid (IBX) was firstly prepared over 100 years ago by Hartmann and Meyer.[153] Since then, its synthesis has been improved and it has found use in a wide panel of organic reactions,[154] such as the oxidative demethylation of *ortho*-methoxyphenols[155] or the aromatic hydroxylation of phenols.[156,157] Due to an extended linkage of intermolecular secondary I···O bonding interactions,[158] IBX has a polymeric structure that renders it essentially insoluble in most organic solvents (exception made for DMSO). Nevertheless, the reaction solvents used are numerous (EtOAc, MeOH, DMF or *tert*-BuOH, to name a few) and they belong to different class of compounds. With the aim to keep exploring new and high yielding synthetic strategy of potential antioxidant polyphenols, IBX seemed to be a efficient and reliable tool to increase the antioxidant potential of simple phenols converting them into the corresponding catechols.

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[151] Wirth, T., *Angew. Chem., Int. Ed.*, **2005**, *44*, 3656-3665.

[152] Hirofumi, T., Yasuyuki, K., *Adv. Synth. Catal.*, **2004**, *346*, 111-124.

[153] Hartman, C., Mayer, V., *Chem. Ber.*, **1893**, *26*, 1727-1732.

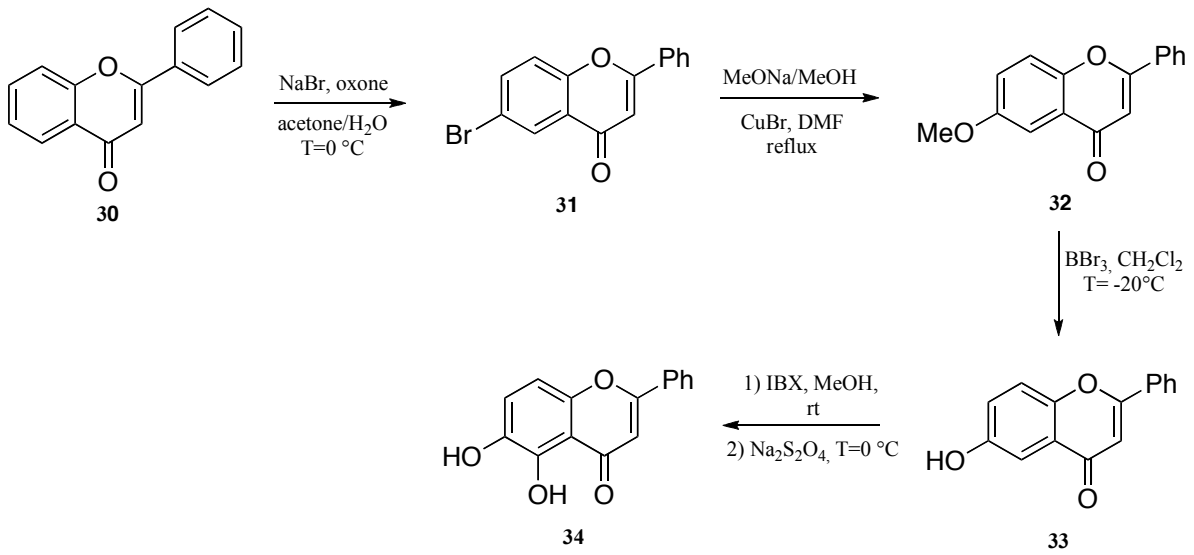
[154] For an overview, see: (a) Wirth, T., *Angew. Chem., Int. Ed.* **2001**, *40*, 2812- 2814; for recent developments, see: (b) Nicolaou, K. C., Baran, P. S., Zhong, Y.-L., Barluenga, S., Hunt, W. K., Kranich, R., Vega, J. A., *J. Am. Chem. Soc.*, **2002**, *124*, 2233-2244; (c) see ref. 27.

[155] Ozanne, A., Pouysegu, L., Depernet, D., Francois, B., Quideau, S., *Org. Lett.*, **2003**, *5*, 2903-2906.

[156] Magdziak, D., Rodriguez, A. A., Van De Water, R. W., Pettus, T. R. R., *Org. Lett.*, **2002**, *4*, 285-288.

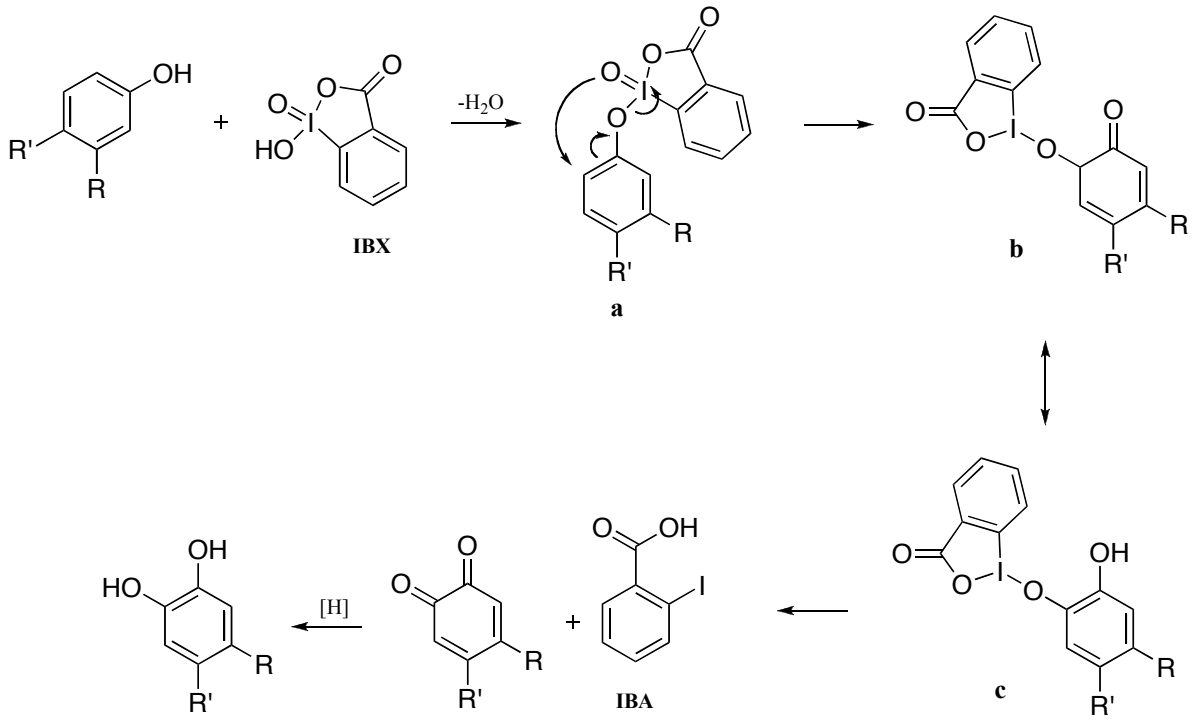
[157] Huang, Y., Zhang, J., Pettus, T. R. R., *Org. Lett.*, **2005**, *7*, 5841-5844.

[158] Stevenson, P. J., Treacy, A. B., Nieuwenhuyzen, M. J., *Chem. Soc., Perkin Trans, 2*, **1997**, 589-591.



Scheme 3.10 Synthesis of 5,6-dihydroxyflavone

A simple mono-functionalized flavone was examined firstly. For this purpose, 6-bromoflavone (**31**) was synthesized as reported by Bovicelli and co-workers[159] starting from the commercially available flavone **30**. **31** was converted into **33** in two steps applying the usual methanolysis/ demethylation protocol. **33** was treated with IBX in MeOH at room temperature and subsequently with a reductant to obtain 5,6-hydroxyflavone (**34**) with a perfect regioselectivity. (Scheme 3.10) The process most likely follows the proposed pathway shown in Scheme 3.11.



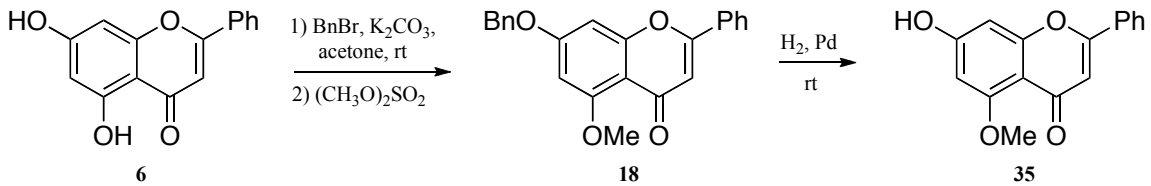
Scheme 3.11 Proposed mechanism for IBX-mediated conversion of phenols into *o*-quinones

[159] Bovicelli, P., Bernini, R., Antonioletti, R., Mincione, E., *Tetrahedron Lett.*, **2002**, *43*, 5563-5567.

The substrate combines with IBX to extrude H_2O, producing the I(V) intermediate **a**, which seems to intramolecularly deliver the oxygen to the most nucleophilic and least congested *ortho*-site on the starting phenol. During this delivery process, the I(V) atom in intermediate **a** is concurrently reduced to I(III), giving the intermediate **b**, which in turn undergoes tautomerization to produce the intermediate **c**. Intermediate **c** then oxidatively collapses with contemporary reduction of the iodine atom to produce *o*-quinone and 2-iodobenzoic acid (IBA). This highly regioselective oxidation of phenols to *o*-quinones with IBX is remarkable, because it represents a double oxidation: a hydroxy residue is regioselectively installed and the resulting catechol intermediate is oxidized. (Scheme 3.11) In this particular case, 6-hydroxyflavone (**33**) undergoes oxidation on C5 despite C7 position is less hindered and equally activated. Probably, the great stability arising from the formation of an internal H-bond between C5-OH and the carbonil oxygen on ring C force the reaction towards position C5.

Baicalein (**14**), negletein (**13**), mosloflavone (**11**) and 5,6-dihydroxyflavone (**34**) were tested for their antioxidant activity in collaboration with prof. Incerpi research group of "Roma 3" University. It is noteworthy to highlight that flavone **34** has a naturally occurring uncommon substitution pattern, lacking of C7 hydroxyl group. Interestingly, 5,6-dihydroxyflavone showed a superior antioxidant activity with respect to negletein, that presents the same catecholic substitution (unpublished results). Ongoing work is focused on rationalizing and elucidating structure-activity relationship (SAR) results in order to better project and synthesize new powerful antioxidant.

IBX-mediated oxidation of di-substituited flavones was then considered. Crysin (**6**) and its mono-methylated derivatives **3** and **35** have been chosen as substrates. **35** has been synthesized from **18**, selectively deprotecting C7 hydroxyl group. (Scheme 3.12)



6 **18** **35**

Scheme 3.12 Synthesis of 5,6-dihydroxyflavone

Results are summarized in table 3.1. As shown in entries 1 and 2, no reaction was observed for crysin when treated with stochiometric amount of IBX in MeOH (both at T=0 °C or at rt) or in DMF. In order to avoid issues arising from the presence of two free hydroxyl groups, 7-methylcrysin was tested under the same reaction conditions. (entry 3, table 3.1) No improvements

were detected, hence MeOH was replaced by $CHCl_3$ according to Pezzella and co-workers[160] that reported chloroform to be suitable in this kind of reaction. (entry 4, table 3.1) Again the reaction gave no conversion. The substrate was then replaced by 5-methylcrysin. (entries from 6 to 11, table 3.1) Probably, the unreactivness of **3** could be imputable to the extremely low reactivity of C5-OH due to the internal H-bond. Reaction of 5-methylcrysin (**35**) with 1.1 eq. of IBX in MeOH at rt led to a mixture of products and a 70% of recovered substrate. The same result was obtained increasing the IBX amount to 2.0 equivalents.

Entry	Substrate	Solvent	Temperature	Reagent	Product(s)
1	Crysin	MeOH	0 °C	IBX (1.1 eq.)	no reaction
2	Crysin	MeOH	rt	IBX (1.1 eq.)	no reaction
3	Crysin	DMF	rt	IBX (1.1 eq.)	no reaction
4	7-methylcrysin	MeOH	rt	IBX (1.1 eq.)	no reaction
5	7-methylcrysin	$CHCl_3$	rt	IBX (1.1 eq.)	no reaction
6	5-methylcrysin	MeOH	rt	IBX (1.1 eq.)	30% conversion
7	5-methylcrysin	MeOH	rt	IBX (2.0 eq.)	30% conversion
8	5-methylcrysin	$CHCl_3$	rt → 50 °C	IBX (2.0 eq.)	no reaction
9	5-methylcrysin	DMSO	rt	IBX (2.0 eq.)	no reaction
10	5-methylcrysin	DMSO	rt → 80 °C	IBX (2.0 eq.)	20% conversion
11	5-methylcrysin	t-BuOH	reflux	IBX (2.0 eq.)	no reaction

Table 3.1 Oxidation of 5,7-difunctionalized flavones with IBX

Different solvents were tested at different temperature (entries from 8 to 11, table 3.1) but no significant improvements have been obtained. Furthermore the extremely low conversion achieved in each case and the contrasting results obtained discouraged any further investigations.

Only one report is known in literature in which IBX is successfully used in a catalytic amount. Thottumakara and co-workers[161] reported that primary and secondary alcohols can be oxidized

[160] Pezzella, A., Lista, L., Napolitano, A., D'Ischia, M., *Tetrahedron Lett.*, **2005**, *46*, 3541-3544.

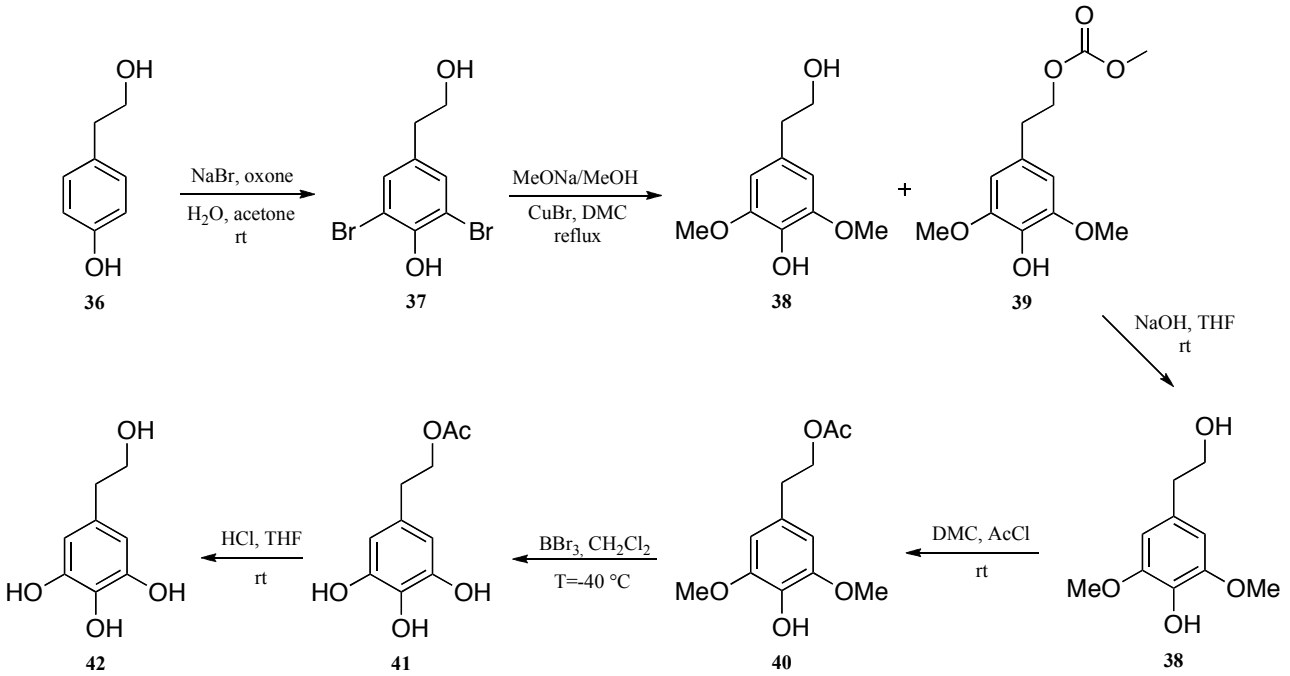
[161] Thottumkara, A. P., Bowsher, M. S., Thottumkara, K. V., *Org. Lett.*, **2005**, *7*, 2933-2936.

using a catalytic amount of *ortho*-iodobenzoic acid (IBA, the IBX precursor) and oxone as co-oxidant in MeCN/H_2O at 70 °C. The simple idea behind this innovative methodology arise from the possibility to utilize the inexpensive oxone in stochiometric amount instead of the potentially explosive IBX. As far as our knowledge, no catalytic procedure has been reported in literature for IBX-mediated oxidation of phenols. 6-hydroxyflavone (**33**) was chosen as substrate due to its facile conversion in non-catalytic conditions. A first attempt was carried out using 0.1 eq. of IBA and stochiometric amount of oxone in a 1:1 mixture of acetone and water at rt. Since no conversion of the substrate was detected, the mixture was heated to 70 °C, the temperature at which the oxidation of IBA to IBX occurs. No improvement in substrate conversion has been achieved and the catalytic use of IBX has not been investigated any further.

4. Results and discussion - Tyrosol

4.1 Synthesis of gallic acid analogs

In this work, an efficient methodology to synthesize 3,4,5−trihydroxyphenethyl alcohol and its methylated derivate starting from tyrosol, a molecule found in olive oil production waste,[17] is described. The pyrogallol moiety was achieved using a bromination/methanolysis protocol recently developed by our research group. The complete synthetic pathway is depicted in scheme 4.1.



Scheme 4.1 Synthesis of 3,5-dihydroxytyrosol

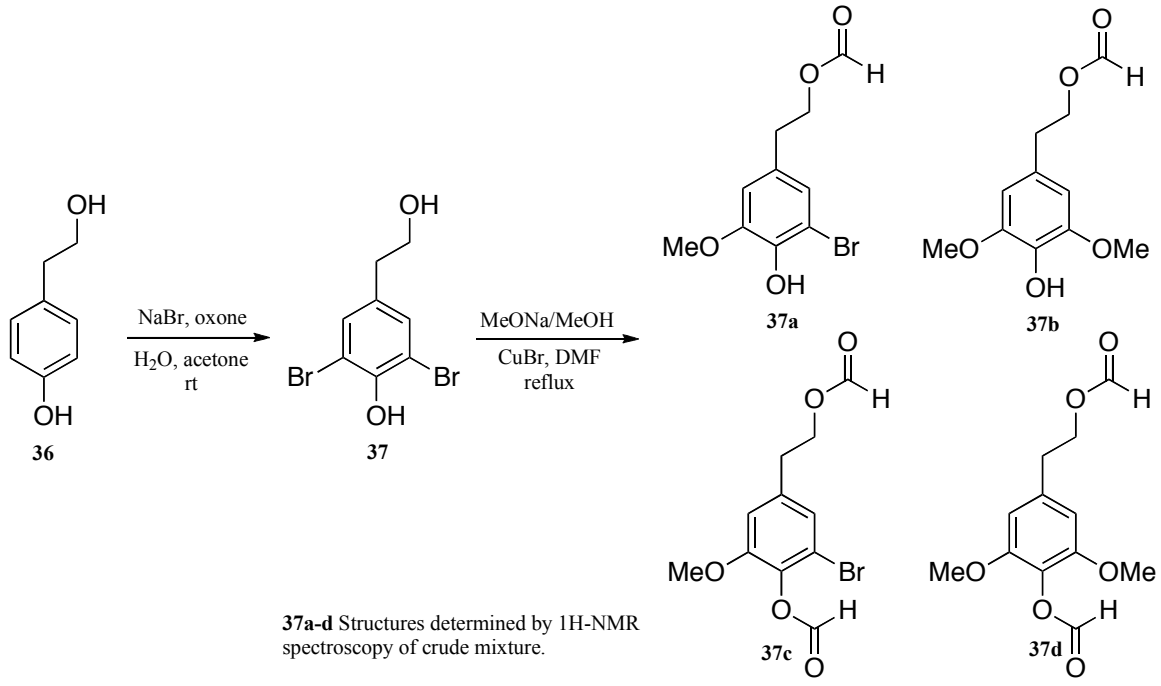
The bromination step was carried out with NaBr/oxone system modifying the previous reported procedure. (See scheme 3.6) In this case, acetone was successfully replaced by dimethyl carbonate (DMC). DMC is a versatile and environmentally innocuous material for chemical process.[162] It has been successfully used to introduce a methyl group at the α-position of arylacetonitrile and methyl aryl acetate.[163] It has also been used for selective monomethylation of primary aromatic amines.[164] DMC is a nontoxic, environmentally safe reagent that can be used as a "green substitute" for toxic reagent used in conventional methylation and carboxymethylation reactions. In our case, despite a low solubility in water (12% w/w), excellent results in conversion and yield were obtained.

[162] Mauri, M. M., Romanno, U., Rivetti, F., *Ing. Chim. Ital.*, **1985**, *21*, 6-12.

[163] Tundo, P., Selva, M., Bomben, A., *Org. Synth.*, **1998**, *76*, 167-177.

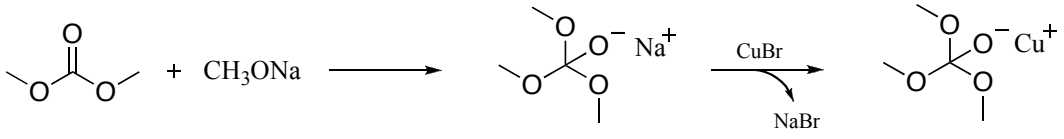
[164] Selva, M., Bomben, M., Tundo, P., *J. Chem. Soc. Perkin Trans. 1,* **1997**, *7*, 1041-1046.

(Scheme 4.1) The dibromo-derivate (**37**) was then treated with CuBr and MeONa in DMF in order to obtain the expected dimethoxy-derivate (**38**). Unfortunately the reaction led to a complex mixture of products. Due to a longer time of reaction with respect to the previous reported methanolysis reactions, both the free hydroxyl groups on the substrate underwent partial formylation. In addition, the methanolysis reaction did not reach the completion and mono-methoxylated products were isolated. (Scheme 4.2)



Scheme 4.2 Proposed products of methanolysis reaction in DMF

The replacement of DMF by DMC avoided the formation of a large number of side-products, even if a 1:1 mixture of 3,5-dimethoxytyrosol (**38**) and 3,5-dimethoxytyrosol carboxymethylester (**39**) was obtained. It is worthy of note that a significant enhancement of reactivity was detected with respect to the reaction in which DMF was used as the solvent. In fact, the refluxing temperature of DMC is 90 °C, considerably lower than DMF. Despite that, the reaction was immediate and the conversion was complete. The improved reactivity is probably due to the involvement of DMC in the formation of a reactive complex.[165] (Scheme 4.3)

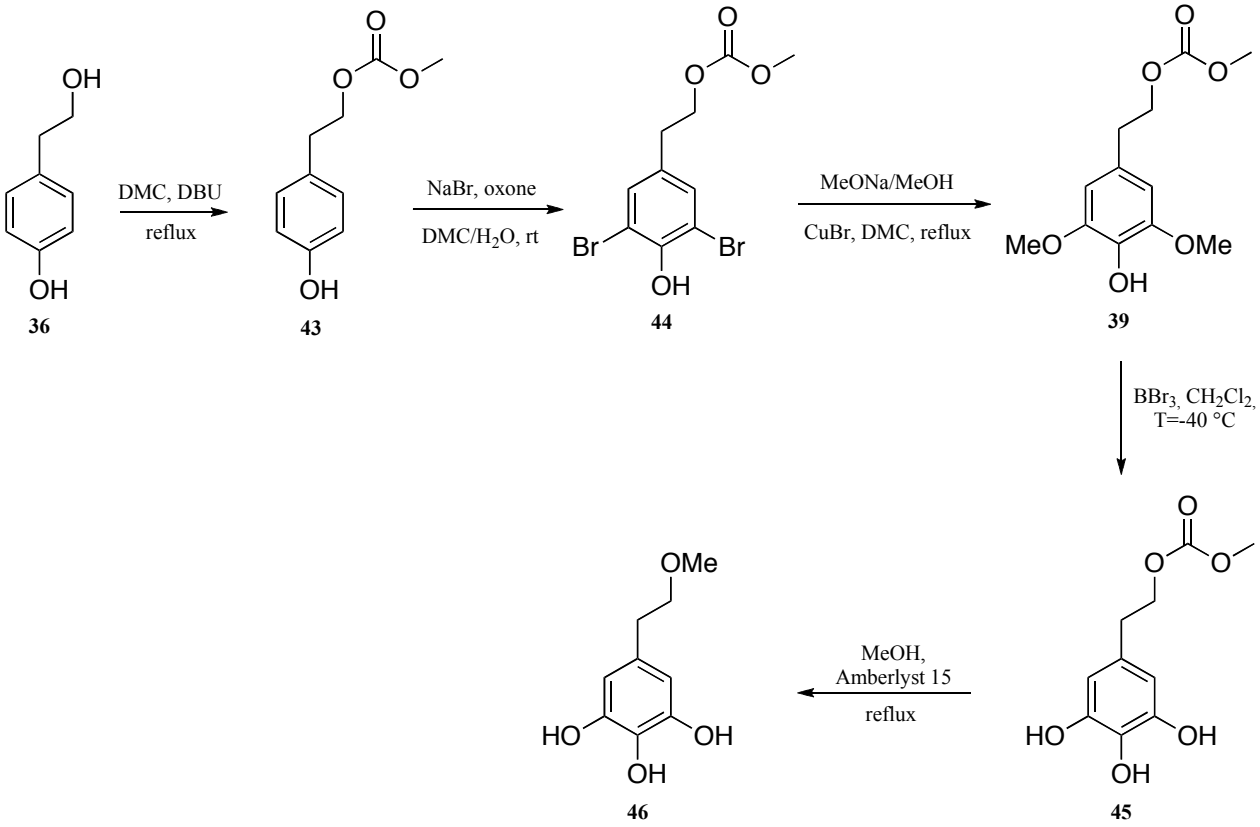


Scheme 4.3 Proposed mechanism for the involvement of DMC the formation of a reactive complex

[165] Capdevielle, P., Maumy, M., *Tetrahedron Lett.*, **1993**, *34*, 1007-1010.

The crude mixture **38** + **39** was hydrolyzed in basic conditions, since carboxymethylester group is acid-resistant. A selective acetylation reaction followed and the desired compound **40** was obtained. (Scheme 4.1) Acetyl group on the side chain was essential due to the following demethylation step. Finally the methoxy groups were deprotected with BBr_3 at low temperature to avoid the competitive bromination of the side chain. The last step was the hydrolysis of the acetyl group in acidic media to obtain the target compound **42**. (Scheme 4.1). The choice of an acid hydrolysis arise from the fact that the final product is water-soluble, hence a non-aqueous work-up was necessary. Hydrolysis in a mixture of HCl and THF allowed to simply remove the solvent under reduced pressure, since no solid by-products are formed during the reaction.

With the purpose of diminishing the polarity of **42** and enhancing its bioavailability, the methylether derivate **46** has been synthesized through a similar synthetic pathway. (Scheme 4.4) The choice of the new protecting group lies with convenient characteristics of DMC reactivity. Preliminary investigation on carboxymethylate phenethyl system showed the ability of carboxymethyl group to rearrange in acidic media into methyl ether. (See paragraph 4.2) Moreover, the carboxymethyl group simplifies the synthetic pathway since it is consistent with methanolysis conditions.



Scheme 4.4 Synthesis of 3,5-dihydroxytyrosol methylether

The first step was a selective protection of the more nucleophilic primary alcohol moiety on the side chain, already reported in literature.[166] DMC was used as solvent/reactant. The pyrogallol moiety was then built as in the previous case, exploiting the bromination/methanolysis protocol. (Scheme 4.4). Since compound **38** was protected as carboxymethylester, the methanolysis reaction led to the dimethoxy-derivate **39** as the only product. The deprotection of **39** with BBr_3 led to the carboxymethylated compound bearing a free pyrogallol moiety. The reaction was carried out at -40 °C to prevent the replacement of the oxygenated moiety on the side chain by bromine. **46** was obtained refluxing **45** in methanol and amberlyst 15. The methyl ether formation is supposed to happen through a rearrangement of carboxymethylester group in which the phenonium ion is involved.

The pirogallol compound **42** and the methylated derivative **46** were evaluated for their toxicity to better understand the potential role of the pyrogallol moiety on drugs development. In collaboration with the department of agrobiology and agrochemistry of Tuscia University, genotoxicity, citotoxicity and antioxidant activity tests have been performed on both the molecules. Based on the data analysed it can be affirmed that none of the two molecules show cytotoxicity or genotoxicity at doses lower than 200 μM on ovary hamster cells (CHO). To investigate whether compound **42** and **46** confer protection against H_2O_2 induced chromosome aberration in CHO, cell viability after 200 μM H_2O_2 incubation for 2h with or without **42** and **46** pre-treatment for 15 minutes has been detected. The results showed that pre-treatment prevented H_2O_2 damage in both cases and compound **46** demonstrated to have higher scavenger activity than compound **42**. Since both of them bear a pyrogallol moiety, the higher scavenger activity of **46** is probably due to its enhanced lipophilicity, resulting in an easier cell-membrane penetration.

4.2 Phenonium ion

The phenoium ion was described for the first time by Cram[167,168,169] in the 40's as intermediate in the solvolysis reactions of phenetyl chlorides and tosylates. In the 70's Olah[170,171] and co-workers characterized some stable phenonium ions prepared from their parent chlorides in SbF_5/SO_2ClF solution via NMR spectroscopy. The life time of these cations strictly depends by the ring

[166] Bernini, R., Mincione, E., Barontini, M., Crisante, F., *J. Agric. Food Chem.*, **2008**, *56*, 8897-8904.

[167] Cram, D. J., *J. Am. Chem. Soc*., **1949**, *71*, 3863-3870.

[168] Cram, D. J., Davis, R., *J. Am. Chem. Soc.*, **1949**, *71*, 3875-3883.

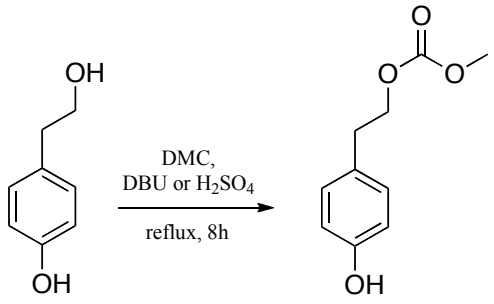
[169] Cram, D. J. *J. Am. Chem. Soc.*, **1964**, *86*, 3767-3772.

[170] Olah, G. A., Porter, R. D., *J. Am. Chem. Soc*., **1971,** *93*, 6877-6887.

[171] Olah, G. A., Porter, R. D., *J. Am. Chem. Soc*., **1970,** *92*, 7627-7629.

substitution.[172] Since Cram's and Olah's pioneering studies, the phenonium ion received considerable interest but nowadays it is not used for synthetic purposes. In this work a new pathway to form phenonium ions, which could open ways for its exploit in synthetic strategies, is described.

In recent years we have been focused on developing methods for selective protection of the alcohol moiety with respect to the phenol one. Our aim was the protection of the alcoholic moiety of tyrosol for further transformation. (Scheme 4.5)



36 **43**

Scheme 4.5 Selective protection of alcohol moiety.

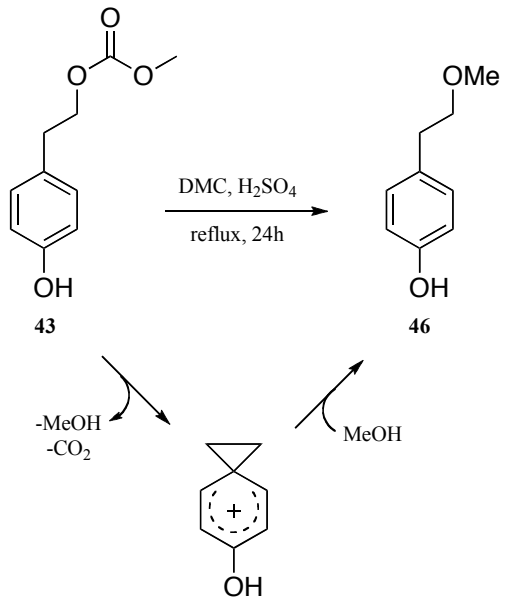
The objective was reached using dimethylcarbonate in basic (DBU) or acid (H_2SO_4) catalysts.[173] 4-hydroxyphenethyl carboxymethylester (**43**) was obtained in excellent yields. The method is eco-sustainable too.[174] (Scheme 4.5)

In the course of our studies we realized that the reaction of tyrosol carboxymethylester (**43**) with an excess of H_2SO_4 led to 4-hydroxyphenetyl methyl ether (**43a**) in almost quantitative yield. The course of the reaction involves reasonably a phenonium ion produced by decomposition of the carboxymethylester group into CO_2 and methanol, the latter one acting as nucleophile for the completion of the process. (Scheme 4.6)

[172] Mustanir, S. T., Shuhei, I., Masaaki M., *ARKIVOC*, **2008**, (*x*), 135-150

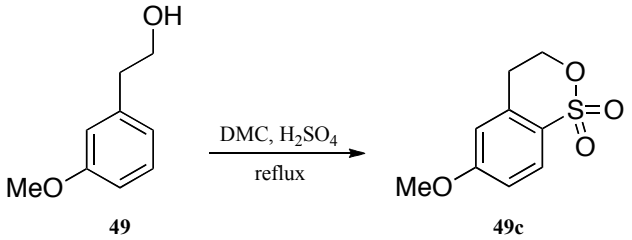
[173] See ref. 164

[174] Tundo, P., Selva, M., *Acc. Chem. Res*., **2002**, *35*, 706–716.



Scheme 4.6 Proposed mechanism for methyl ether formation

It has to be noticed that when 3-methoxyphenethyl alcohol (**49**) underwent the same reaction, electrophilic aromatic substitution happened and the following ring closure led to compound **49c** as the only product. (Scheme 4.7)

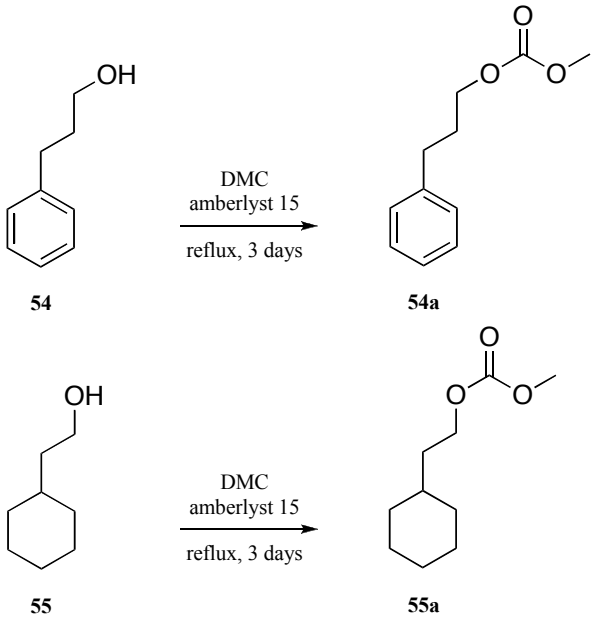


Scheme 4.7 Cyclic sulphonate from 3-methoxyphenethyl alcohol **49**

Moreover, in every case a large excess of H_2SO_4 was necessary to completely consume the substrate because of the contemporary formation of dimethyl sulfate as by-product. To avoid these side reactions we successfully used amberlyst 15 instead of H_2SO_4. An additional advantage in using heterogeneous reaction conditions was found in the work-up procedure, which resulted in a simple filtration and solvent evaporation, with a total recovery of the methylated product. In the new procedure a domino reaction can be promoted from **36**, which refluxed in DMC in the presence of amberlyst 15 led in 36 hours directly to **46** without isolate the carboxymethylester **43**. (Scheme 4.9) The reported procedure is, to our knowledge, the first example of selective etherification of an

alcoholic moiety with respect to a phenyl one. The best synthesis of compound **46** reported in literature is a three steps procedure.[175]

Some more experiments have been made to evidence the phemonium ion as the intermediate in the process. First of all, to confirm the hypothesis of an effective involvement of phenonium ion, two similar substrates (**54** and **55**) unable to form phenonium ion have been reacted in the same conditions. (Scheme 4.8)

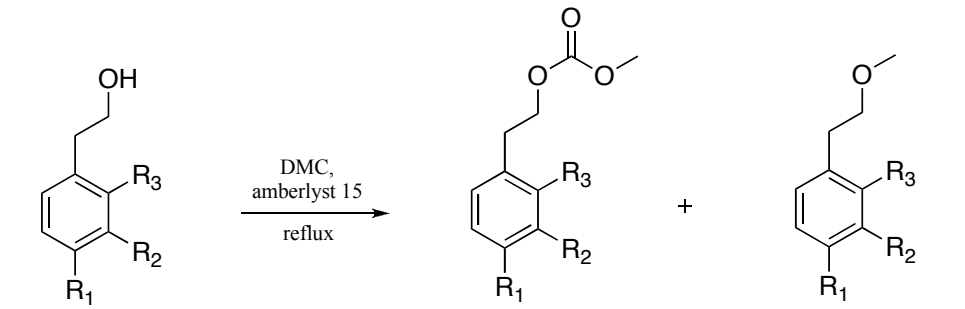


Scheme 4.8 Evidence for the involvement of phenonium ion in phenethyl methyl carbonate.

As shown in the scheme 4.8, even reacting the substrates for three days and forcing the reaction conditions, the only products afforded are the carboxymethylester-derivates **54a** and **55a**, hence the phenethyl structure is essential for the reaction to proceed.

As reported in literature, phenonium ion formation is strictly dependent on aromatic ring substitution. In order to investigate the influence of different substituents on the aromatic ring, seven more substrates were tested under the same reaction conditions. (Scheme 4.9) The reaction was quenched after 36 hours in order to obtain a qualitative measure of the activation of each substituent. The formation of methylether- and carboxymethylester-derivative was determined by ^{1}H NMR spectroscopy of the crude mixture through integration of relevant peaks. Data are summarized in table 4.1.

[175] Madrona, A., Pereira-Caro, G., Mateos, R., Rodríguez, G., Trujillo, M., Fernández-Bolaños, J., Espartero J. L., *Molecules*, **2009**, *5*, 1762-1772.



36 R_1=OH, R_2=R_3=H
47 R_1=R_2=R_3=H
48 R_1=OMe, R_2=R_3=H
49 R_2=OMe, R_1=R_3=H
50 R_3=OMe, R_2=R_3=H
51 R_1=R_2=OH, R_3=H
52 R_1=NO_2, R_2=R_3=H
53 R_2=CF_3, R_1=R_3=H

43 R_1=OH, R_2=R_3=H
47a R_1=R_2=R_3=H
48a R_1=OMe, R_2=R_3=H
49a R_2=OMe, R_1=R_3=H
50a R_3=OMe, R_2=R_3=H
51a R_1=R_2=OH, R_3=H
52a R_1=NO_2, R_2=R_3=H
53a R_2=CF_3, R_1=R_3=H

46 R_1=OH, R_2=R_3=H
47b R_1=R_2=R_3=H
48b R_1=OMe, R_2=R_3=H
49b R_2=OMe, R_1=R_3=H
50b R_3=OMe, R_2=R_3=H
51b R_1=R_2=OH, R_3=H
52b R_1=NO_2, R_2=R_3=H
53b R_2=CF_3, R_1=R_3=H

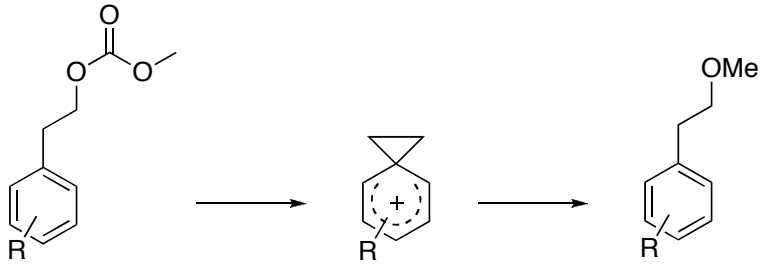
Scheme 4.9 Scope of the reaction. The reaction was stopped after 36 hours.

Entry	Substrate	Yield A [a]	Yield B [a]
1	**36** R_1=OH, R_2=R_3=H	-	> 95%
2	**47** R_1=R_2=R_3=H	83%	17%
3	**48** R_1=OMe, R_2=R_3=H	-	> 95%
4	**49** R_2=OMe, R_1=R_3=H	76%	24%
5	**50** R_3=OMe, R_1=R_2=H	-	> 95%
6	**51** R_1=R_2=OH, R_3=H	-	> 95%
7	**52** R_1=NO_2, R_2=R_3=H	88%	12%
8	**53** R_2=CF_3, R_1=R_3=H	86%	14%

Table 4.1 Yields of carboxymethylester- and methylether-derivatives.
[a] A and B refer to carboxymethylester- and methylether-derivatives respectively.

The data reported in table 4.1 shows that the reaction is promoted by ERGs in *ortho* or *para* positions, while is disfavoured by EWGs, as expected according to the mechanism proposed.
In collaboration with Dr. Spezia from French CNRS, relative energies of five representative systems were analyzed. We have considered three systems to understand the differences in reactivity, the carboxymethylester (system **1-H**), the *o*-, *m*- and *p*-MeO-carboxymethylester (systems **1-o-OMet**, **1-m-OMet** and **1-p-OMet**) and the *p*-NO_2-carboxymethylester (system **1-NO_2**). They were chosen since they are representative of the electron donating and electron withdrawing groups for which different reactivity was observed. For all the systems we have optimized the structure with the

B3LYP/6-311G(d,p)/PCM method. From the resulting minima we have investigated energetics of the reaction corresponding to the formation of the intermediate phenonium ion where the proton is donated by H_2SO_4, thus releasing CO_2 and MeOH, and then the final product. Results are reported in table 4.2 where for each reaction the reference relative energy was set for system **1-**.



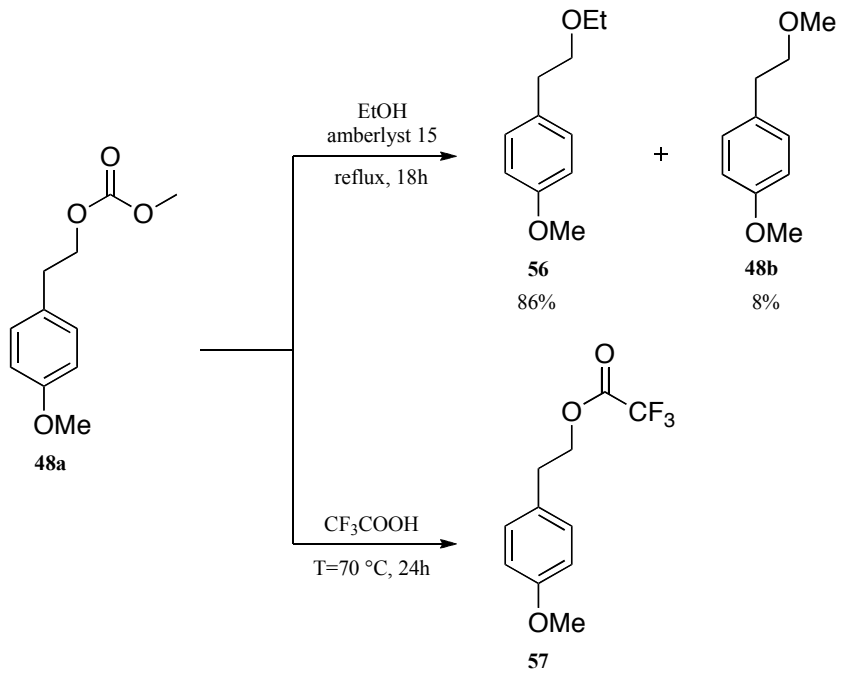
1- **phenonium-** **2-**

R	1-	Phenonium-	2-
H	0.	+0.40	-15.06
o-OMe	0.	-6.29	-15.01
m-OMe	0.	+2.19	-15.06
p-OMe	0.	-7.35	-15.22
p-NO_2	0.	+12.00	-15.54

Table 4.2 DFT results for five representative carboxymethylester derivates

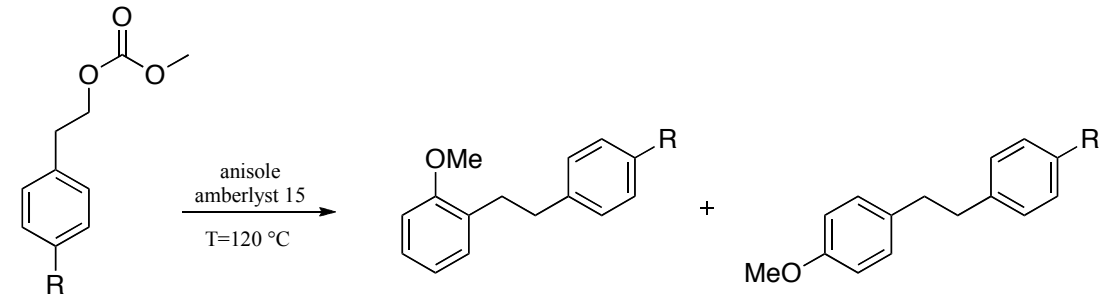
As clearly shown from DFT results, the phenonium ion is stabilized in the case of an electron donating group in position *ortho* or *para*, while it is not favored in the case of the same electron donating group in position *meta* or an electron withdrawing group. On the other hand the final product has roughly the same energy whatever is the substituent group on the ring. This confirms the reaction pathway suggested by experiments, and the formation of the phenonium ion is the determining step allowing (or not) the reaction to proceed further.

The possibility to produce phenonium ions in a so easy way, open perspectives for synthetic purposes. Performing the reaction in the presence of different nucleophiles, mixture of ethers were obtained, but when the nucleophile was also the solvent, a solvolysis occurred. As an example the reaction in ethanol led mainly to ethyl ether and in trifluoroacetic acid the corresponding trifluoroacetate was the only product (Scheme 4.10).



Scheme 4.10 Solvolysis of carboxymethylester **48a**

In the latter case methanol produced by decomposition of carboxymethylester group is neutralized by trifluoroacetic acid and cannot react with phenonium ion. Despite in literature the phenonium ion is usually made by solvolysis,[176,177] in our case the ion is produced by decomposition of carboxymethylester and can be potentially exploited to react with a number of nucleophiles. A first example is the carbon-carbon bond formation using anisol as the nucleophile (Scheme 4.11).



43 R=OH
48 R=OMe

58a R=OH
59a R=OMe

58b R=OH
59b R=OMe

Scheme 4.11 C-C bond formation through phenonium ion

In this case the interesting class of stylbenoids is afforded, and stylbenes can be obtained by further transformations. Compounds **58a** and **58b** were characterized as acetyl derivatives (**58c** and **58d** respectively) In summary a new and efficient method for formation of phenonium ion is reported

[176] Fujio, M., Nakamoto, Y. K., Yatsugi, K., Goto, N., Kim, S. H., Tsuji, Y., Rappoport, Z., Tsuno, Y., *Bulletin of the Chemical Society of Japan*, **1995**, *68*, 2603-2617.

[177] Brusco, Y., Berroteran, N., Lorono, M., Còrdova, T., Chuchani, G., *J. Phys. Org. Chem.*, **2009**, *22,* 1022-1029.

and some possible applications in synthesis are described. Worthy of note is the possibility to induce the carbon-carbon bond formation, with which the important class of stylbenes can be approached.

4.3 Synthesis of functionalized 2,3-dihydrobenzofurans and chromans

Among them, benzofuran derivatives are a major group of biologically active heterocycles, which are usually important constituents of plant extracts used in medicinal chemistry for their various biological activities. Benzofuran scaffold (Figure 4.1) displays potent biological properties including antifungal activity[178,179] as well as antibacterial[180] and angiogenesis inhibitory properties.[181] A benzofuran derivative, a novel myristoyltransferase inhibitor, has been reported as antifungal agent.[182] *N*-Myristoyltransferase has been proven to be essential for the viability of fungi, including medically important pathogenic fungi, *Candida albicans*[183] and *Cryptococcus neoformans*[184,185] making it a possible target for the development of antifungal agents with a novel mode of action. Due to their diverse activities, much attention has been paid to synthetic strategies to access these systems, and a number of methods have been developed.[186,187,188] Most of these methods exploit an intramolecular closure of a properly substituited ketone moiety to afford the fused furan ring. The closure can be achieved

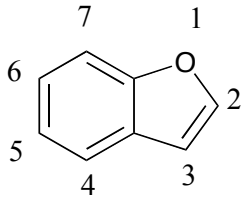


Figure 4.1 Benzofuran scaffold

[178] Masubuchi, M., Kawasaki, K., Ebiike, H., Ikeda, Y., Tsujii, S., Sogabe, S., Fujii, T., Sakata, K., Shiratori, Y., Aoki, Y., Ohtsuka, T., Shimma, N., *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 1833-1837.

[179] Masubuchi, M., Ebiike, H., Kawasaki, K., Sogabe, S., Morikami, K., Shiratori, Y., Tsujii, S., Fujii, T., Sakata, K., Hayase, M., Shindoh, H., Aoki, Y., Ohtsuka, T., Shimma, N., *Bioorg. Med. Chem.*, **2003**, *11*, 4463-4478.

[180] Abdel-Aziz, H. A., Mekawey, A. A. I., Dawood, K. M., *Eur. J. Med. Chem.*, **2009**, *44*, 3637-3644.

[181] Chen, Y., Chen, S., Lu, X., Cheng, H., Ou, Y., Cheng, H., Zhou, G.-C., *Bioorg. Med. Chem. Lett.*, **2009**, *19*, 1851-1854.

[182] See ref. 179 and 180

[183] Weinberg, R. A., McWherter, C. A., Freeman, S. K., Wood, D. C., Gordon, J. I., Lee, S. C., *Mol. Microbiol.*, **1995**, *16*, 241-250.

[184] Lodge, J. K., Jackson-Machelski, E., Toffaletti, D. L., Perfect, J. R., Gordon, J. I., *Proc. Natl. Acad. Sci. U.S.A.*, **1994**, *91*, 12008-12012.

[185] Lodge, J. K., Jackson-Machelski, E., Higgins, M., McWherter, C. A., Sikorski, J. A., Devadas, B., Gordon, J. I., *J. Biol. Chem.*, **1998**, *273*, 12482-12491.

[186] Nakamura, I., Mizushima, Y., Yamagishi, U., Yamamoto, Y., *Tetrahedron*, **2007**, *63*, 8670-8676.

[187] Sakai, N., Uchida, N., Konakahara, T., *Tetrahedron Lett.*, **2008**, *49*, 3437-3440.

[188] Zhang, J.W., Zhang, Y., Zhang, Y.S., Herndon, J.W., *Tetrahedron*, **2003**, *59*, 5609-5616.

using ruthenium,[189] palladium[190] or other transition metals[191,192] as catalysts that can be assisted (or not) by ligands.[193]

The central role of heterocycles in life sciences and natural product chemistry provides a constant drive for the development of even more efficient methods for their preparation. Among them, benzopyran and 3,4-dihydrobenzopyran nuclei are present in many biologically active compounds, such as α-tocopherol or vitamin E,[194,195] levcromakalim,[196] cannabichromene[197] and ubichromenol or cordiachromene.[198]

Previuos reports by Buckwald and co-workers in the last two decades describe a synthesis of heterocycles *via* Pd-catalyzed C-O bond forming reaction.[199] The protocol was then improved employing di-*tert*-butylphosphinobiaryl ligands.[200] The reaction proceeds under mild conditons using weak bases as K_3PO_4 or Cs_2CO_3 and enantioenriched alcohol were successfully tested as well. The reaction was used in the synthesis of MKC-242, an antidepressant currently in clinical trial. Although the economic attractiveness of copper has led to remarkable progress in the development of copper-catalyzed coupling reactions,[201] palladium-cayalyzed coupling reactions own the major role in organic synthesis.

Herein, a ligand-free copper(I) catalyst system that allows efficient synthesis of functionalized 2,3-dihydrobenzofurans and chromans through intramolecular cyclization is described. The use of copper(I) salts brings unquestionable advantages in terms of expensiveness and practicability. Moreover no bulky or difficult-to-handle ligands were needed to drive the reaction. The synthetic pathway is illustrated in scheme 4.12. The catechol moiety has been build by oxidation of the aromatic ring with IBX that afforded **61** in excellent yield. The following protection of both the hydroxyl groups was necessary to avoid issues arising from the high solubility of the catechol

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[189] Varela-Fernández, A., González-Rodríguez, C., Varela, J. A., Castedo, L., Saá, C., *Org. Lett.*, **2009**, *11*, 5350-5353.

[190] Wang, J.-R., Manabe, K., *J. Org. Chem.*, **2010**, *75*, 5340-5342.

[191] Lu, B., Wang, B., Zhang, Y., Ma, D., *J. Org. Chem.*, **2007**, *72*, 5337-5341.

[192] Nakamura, I., Mizushima, Y., Yamamoto, Y., *J. Am. Chem. Soc.*, **2005**, *127*, 15022-15023.

[193] Anderson, K. W., Ikawa, T., Tundel, R. E., Buchwald, S. L., *J. Am. Chem. Soc.*, **2006**, *128*, 10694-10695.

[194] Ames, S. R., Ludwig, M. I., Nelan, D. R., Robeson, C. D., *Biochemistry*, **1963**, *2*, 188-190.

[195] Machin, L. J., Gabriel, E., Brin, M., *J. Nutr.*, **1982**, *112*, 1437-1440.

[196] Ashood,V. A., Buckingham, R. E., Cassidy, F., Evans, J. M., Faruk, E. A., Hamilton, T. C., Nash, D. T., Stemp, G., Wihcoks, K., *J. Med. Chem.*, **1986**, *29*, 2194-2201.

[197] Holley, J. H., Hadley, K. W., Turner, C. E., *J. Pharm. Sci.*, **1975**, *64*, 892-895.

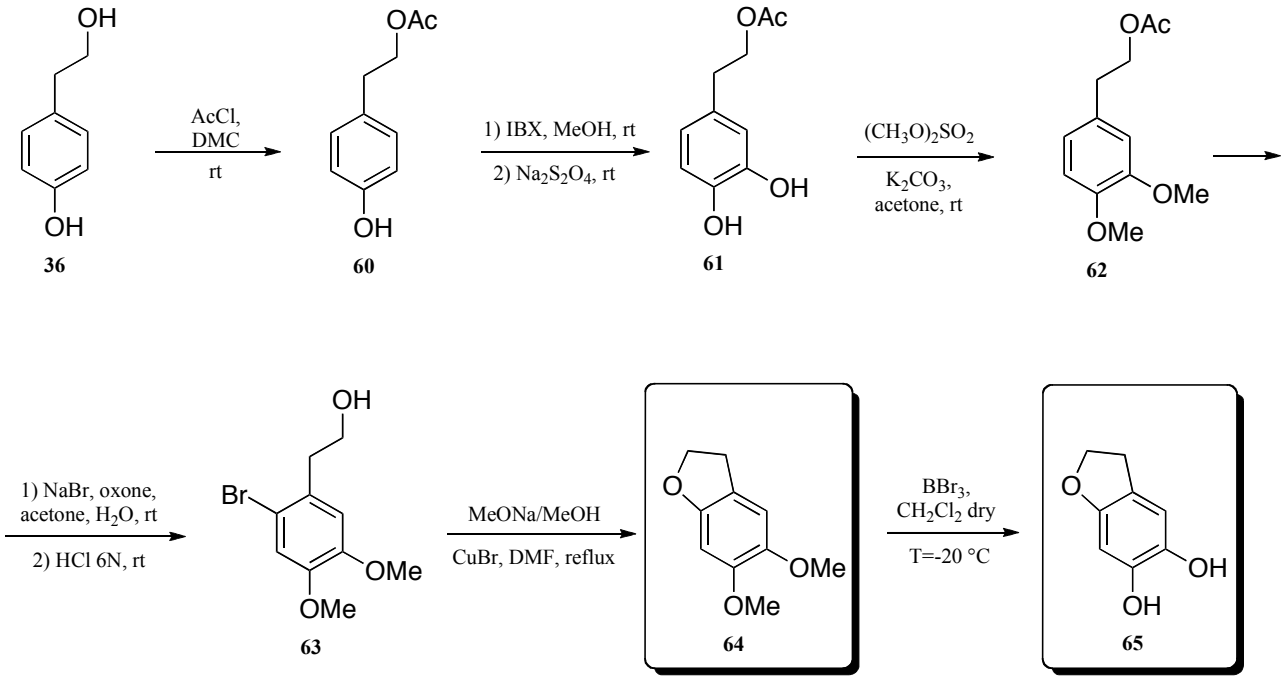
[198] Mc Hale, D., Green, J., *Chem. & Ind*., **1962**, 1867.

[199] Palucki, M., Wolfe, J. P., Buckwald, S. L., *J. Am. Chem. Soc.*, **1996**, *118*, 10333-10334.

[200] Kuwabe, S.-I., Torraca, K. E., Buchwald, S. L., *J. Am. Chem. Soc.*, **2001**, *123*, 12202-12206.

[201] For review see: Ley, S. V., Thomas, A. W., *Angew. Chem. Int. Ed.*, **2003**, *42*, 5400-5449; Monnier, F., Taillefer, M., *Angew. Chem. Int. Ed*., **2008**, *47*, 3096-3099; Ma, D., Cai, Q., *Acc. Chem. Res.*, **2008**, *41*, 1450-1460.

compound in aqueous phase. The next bromination reaction with NaBr/oxone afforded the 6-bromoderivate and the following acidic work-up led to the deprotected derivate **63**. Initially, the cyclization was carried out according to Mangas-Sànchez et al.[202] that reported good yields on non-functionalized arylhalides. Unfortunately the conversion was significantly poor for compound **63** hence a new approach was needed. With respect to the previous method, toluene was replaced with DMF and sodium hydride with a solution of sodium methoxyde in methanol. Using this reaction conditions the closure took place in less than 30 minutes with a complete conversion of the substrate. It is noteworthy to highlight that no intermolecular Ullmann reaction occurred despite sodium methoxyde was used as a base, indicating that the intramolecular cyclization is much more favorable than the intermolecular reaction.



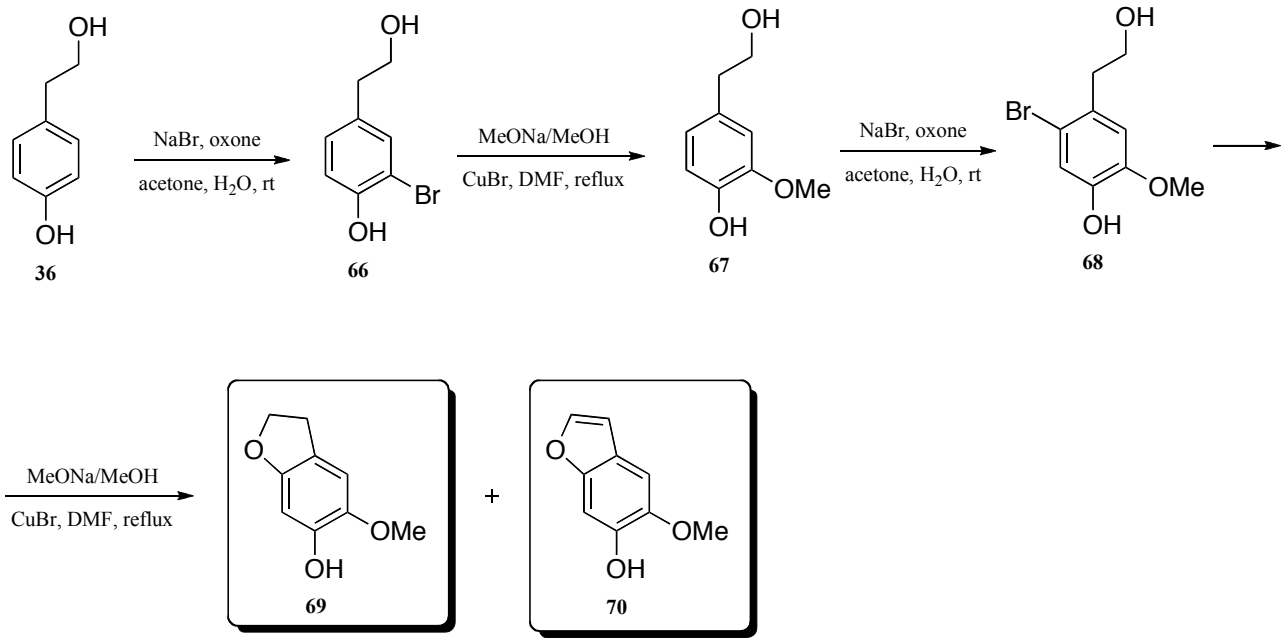
Scheme 4.12 Synthesis of 5,6-dimethoxy-2,3-dihydrobenzofuran and 5,6-dihydroxy-2,3-dihydrobenzofuran

The final demethylation step led to 5,6-dihydroxy-2,3-dihydrobenzofuran **65**. The Cu-catalyzed ring-closing reaction was also investigated in different conditions. As already reported in paragraph 4.1, DMF was replaced with DMC. The new solvent brought unquestioned advantages on reactiveness. In fact, the reaction was over in few minutes at 80 °C (120 °C in DMF) and the amount of copper utilized was significantly diminished (see experimental for detailed informations). Moreover, the use of DMC is more attractive than DMF in view of its lower cost, lower toxicity and ease of recovery of the product.

[202] Mangas-Sànchez, J., Busto, E., Gotor-Fernandez, V., Gotor, V., *Org. Lett.*, **2010**, *12*, 3498-3501.

This is the first synthesis reported for compound **64** and **65**. Theoretical studies on predicted IP and BDE values for compound **65** have been made by Mohajeri and co-workers.[203] The authors compare a series of potential antioxidants on the base of heterocyclic ring size and aryl substitution. They showed that 5-membered heterocyclic ring reduces IP and BDE values with respect to 6-membered parent compounds. Moreover they confirmed that a catechol moiety further decreases BDE and IP values for O-H bond with respect to phenol derivatives.

With the aim to differently functionalize the benzofuranoid scaffold, a different synthetic pathway was developed. 6-hydroxy-5-methoxy-2,3-dihydrobenzofuran **69** was chosen as the target molecule, characterized by a methoxy group adiacent to a hydroxyl group. In fact, this substitution on the aromatic ring is often a recurring theme on biologically active natural products.



Scheme 4.13 Synthesis of 6-hydroxy-5-methoxy-2,3-dihydrobenzofuran and 6-hydroxy-5-methoxybenzofuran

The synthetic pathway followed the previous one. Products **69** and **70** were achieved in a 2:1 ratio respectively. It is still under investigation why the benzofuran derivative is produced only when a hydroxyl group is left unprotected.

In conclusion, an efficient methodology for the synthesis 2,3-dihydrobenzofurans derivatives via an intramolecular cyclization of a aryl bromide and a primary alcohol under the catalysis of Cu(I) bromide is reported. To the best of our knowledge, this is the first example of intramolecular Cu-catalyzed C-O bond forming reaction carried out under non anhydrous condition. In addition, DMC was found to be an excellent solvent, allowing the reaction to occur in milder conditions.

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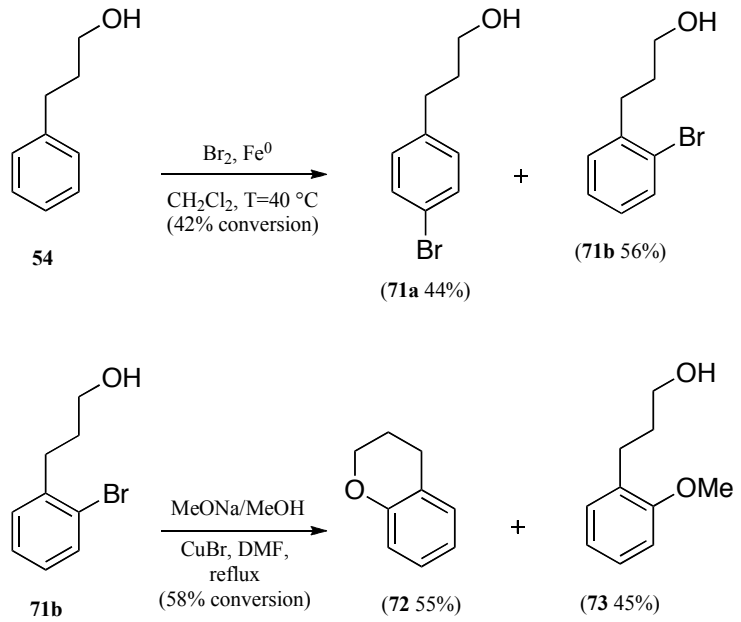
[203] Mohajeri, A., Asemani, S. S., *J. Mol. Struc.*, **2009**, *930*, 15-20.

The scope of the Cu-catalyzed ring-closing reaction was further investigated. Phenylpropyl alcohol (**54**) was chosen as substrate in order to obtain a six-membered heterocyclic ring. (Chroman scaffold)



Scheme 4.14 Synthesis of benzopyran.

The *ortho*-bromo derivate was achieved via a bromination step with Br_2 and Fe(0). (Scheme 4.14) The resulting mixture was separated through flash column chromatography. In this case the intermolecular methanolysis reaction is competitive with ring closure reaction and affords roughly 50% of methoxylated derivative **73**. Ongoing studies will evidence whether ring size or unactivation of the substrate are responsible for this different result.

5. Conclusions

5.1 Synthesis of biologically active flavones

A series of naturally occurring flavones have been synthesized starting from easily available compounds.[204] Selective bromination of substrates carried on with different bromination systems was the key step that led to each target molecules. The following Cu-catalyzed C-O bond forming reaction has been modified according to the different reactivity of each substrates. Moreover, evidences for a Wessely-Moser type rearrangement in basic media has been provided for the first time. Specific synthetic strategies have been developed and the Wessely-Moser rearrangement has been exploited in order to obtain the desired substitution pattern on the aromatic A ring of each flavones.

Mosloflavone, negletein and baicalein were tested for Fe chelating activity in collaboration with Dr. Mladenka and co-workers.[205] Ongoing works in collaboration with Prof. Incerpi are focused on assessing the antioxidant activities of above mentioned flavones. Unpublished results confirmed that the presence of a galloyl moiety is the best for flavonoid-like antioxidant such as baicalein, that showed very good antioxidant activity at very low concentration (picomolar range).

5.2 Synthesis of phenethyl alcohols bearing galloyl moiety

New syntheses for gallic acid analogs starting from tyrosol have been developed. Selective protections of primary alcohol with regard to phenol have been achieved. The synthetic pathway exploits a one pot Cu-catalyzed double C-O bond forming reaction on aryl dibromide. This step was efficiently achieved despite the presence of an *ortho* oxygenated moiety. In fact, despite what already reported in literature,[206] the reaction led to the expected product (contemporary double C-O bond formation on an aryl dibromide) with no formation of dehalogenated side-products. The reaction conditions were improved replacing DMF with DMC. DMC showed to be an excellent

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[204] Righi, G., Antonioletti, R., Proietti Silvestri, I., Lambusta, D., D'Antona, N., Bovicelli, P., *Tetrahedron*, **2010**, *66*, 1294-1298.

[205] Mladenka, P., Makakova, K., Filipsky, T., Zatloukalova, L., Jahodar, L., Bovicelli, P., Proietti Silvestri I., Hrdina, R., Saso, L., *J. Inorg. Biochem.*, **2011**, *105*, 693-701.

[206] Lindley, J., *Tetrahedron*, **1984**, *40*, 1433-1456.

solvent in Cu-catalyzed C-O coupling reaction since the substrates underwent methanolysis under mild conditions as already reported for less substituted substrates.[207]

Cytotoxicity and genotoxicity were evaluated for compounds **42** and **46** in collaboration with Dr. Pepe. Moreover antioxidant activity of both the compounds was tested. The results obtained showed that a methyl ether moiety on the side chain improved the antioxidant activity and this is probably due to the enhanced lipophilicity of the molecule resulting in a easier cell membrane permeability.[208]

5.3 Novel synthesis of stilbenoids via C-C coupling reaction

This work demonstrates that phenethyl methyl carbonates synthesized form tyrosol and similar substrates can form phenonium ion in relative mild conditions. Phenonium ion is produced by loss of CO_2 and methanol in acidic media at 90 °C also by unactivated aryl systems. The possibility to form phenonium ion in a so easy way open perspectives for synthetic purpose. For instance, common organic solvents (EtOH, CF_3COOH) can be used as nucleophiles to form the corresponding ether or ester. In this case a formal selective protection of a primary alcohol with respect to phenol is achieved. Worth of note is the possibility to use weak carbon nucleophiles as anisole to induce C-C bond formation. By this way the interesting class of stilbenoids can be approached.

Theoretical studies in collaboration with Dr. Spezia confirmed the proposed mechanism, showing that phenethyl methyl carbonates can be exploited as useful tools in organic synthesis.

5.4 Synthesis of 2,3-dihydrobenzofurans

First syntheses of 2,3-dihydro-5,6-dimethoxybenzofuran, 2,3-dihydro-5,6-dihydroxybenzofuran and 2,3-dihydro-5-methoxy-6-hydroxybenzofuran starting from tyrosol have been accomplished *via* a Cu-catalyzed ring-closing reaction. Reaction conditions were optimized in order to generalize the scope of the reaction, including the formation of six-membered ring starting from a similar substrate. With respect to recently published metal catalyzed ring-closing reactions it is noteworthy that non anhydrous and ligand-free conditions have been applied. Both DMF and DMC were used

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[207] Capdevielle, P., Maumy, M., *Tetrahedron Lett.*, **1993**, *34*, 1007-1010.

[208] Barontini, M., Proietti Silvestri, I., Nardi, V., Pepe, G., Crisante, F., Bovicelli, P., Righi, G., *Eur. J. Med. Chem.*, *submitted*

as solvent, the latter showing better performances in terms of rate of the reaction and temperature at which the reaction is carried out.

Ongoing works are focused on synthesizing the corresponding benzofurans through an oxidation step with NBS and AIBN.

6. Experimental

6.1 General

Unless otherwise stated, reactions were carried out under standard atmosphere. Yields refer to isolated and spectroscopically homogeneous compounds. NMR spectra were recorded on a VARIAN Mercury 3000 instrument (^{1}H, 300 MHz; ^{13}C, 75 MHz). Chemical shifts were calculated from the residual solvent signals of δ_H 2.04 ppm and δ_C 206.0 ppm in acetone-d_6, δ_H 7.26 ppm and δ_C 77.0 ppm in chloroform-d, δ_H 2.49 ppm and δ_C 39.5 in dimethyl sulfoxide-d_6. Melting points were measured on a Mettler FP80 instrument and were uncorrected. HRMS were performed on a Q-TOF MICRO spectrometer (Micromass, now Waters, Manchester, UK) equipped with an ESI source. IR spectra were recorded on a IR-470 Shimadzu spectrophotometer. All chromatographic purifications were performed on silica gel (100–200 mesh from E. Merck, Germany). Thin layer chromatography (TLC) was performed on precoated silica gel 60 F_{254} aluminium sheets (Merck Italia) using mixtures of hexane-ethyl acetate unless otherwise stated. Detection was effected by either charring in a mixture of phosphomolybdic acid in EtOH and/or by visualization in UV light. Flash chromatography was performed using forced flow with Fisher silica gel (60 mesh). Solvents were evaporated under reduced pressure using a rotary evaporator. Organic solvents and reagents used for the chemical synthesis and for chromatography acquired from Sigma Aldrich Italia were of analytical grade and were used without further purification unless otherwise stated.

TBATB was synthesized as reported in literature.[209]

IBX was synthesized as reported in literature.[210]

NBS was recrystalized from water.

6.2 General procedures

General procedure for complete methylation of flavonoids

To a solution of substrate (4.00 mmol) in acetone (30 mL), K_2CO_3 (12.00 mmol) and $(CH_3O)_2SO_2$ (16.00 mmol) were added. The mixture was stirred for 6 hours at 60 °C and monitored by TLC. After completion of the reaction the mixture was quenched with NH_4OH (5 mL of 10% sol in water) and acetone was removed under vacuum. The residue was dissolved in a small amount of

[209] Bora, U., Chaudhuri, M. K., Dey, D., Dhar, S. S., *Pure Appl. Chem.*, **2001**, *73*, 93-102.

[210] Frigerio, M., Santagostino, M., Sputore, S., *J. Org. Chem.*, **1999**, *64*, 4537-4538.

ethyl acetate and HCl 2M was added until the mixture was acidic. The mixture was extracted with ethyl acetate (3 x 30mL) and the combined organic layers were washed with brine (3 x 30mL) and dried over anhydrous sodium sulfate. The solvent was removed to obtain the crude product.

General procedure for selective methylation of flavonoids

To a solution of substrate (4.00 mmol) in acetone (30 mL), K_2CO_3 (12.00 mmol) and $(CH_3O)_2SO_2$ (16.00 mmol) were added. The mixture was stirred for 1.5 hours at rt and monitored by TLC. After completion of the reaction the mixture was quenched with NH_4OH (5 mL of 10% sol in water) and acetone was removed under vacuum. The residue was dissolved in a small amount of ethyl acetate and HCl 2M was added until the mixture was acidic. The mixture was extracted with ethyl acetate (3 x 30mL) and the combined organic layers were washed with brine (3 x 30mL) and dried over anhydrous sodium sulfate. The solvent was removed to obtain the crude product.

General procedure for acetylation with Ac_2O/pyr

To the substrate (2.00 mmol) a 1:1 mixture of pyridine and acetic anhydride (2 mL) was added. The reaction mixture was stirred overnight. The mixture was poured in a cold 2M solution HCl in water (20 mL) and extracted with ethyl acetate (3 x 5 mL). The organic layers were sequentially washed with a 2M solution of HCl in water (2 x 5mL), a saturated solution of $NaHCO_3$ (2 x 5mL) and brine (5 mL) and dried over anhydrous sodium sulfate. Evaporation of the solvent under reduced pressure gave the crude product.

General procedure for acetylation with Ac_2O/AcOH

To the substrate (2.00 mmol) a 1:1 mixture of acetic acid and acetic anhydride (2 mL) was added. The reaction mixture was stirred overnight. The mixture was diluited with ethyl acetate (5 mL). The organic layers were washed with a saturated solution of $NaHCO_3$ (3 x 5mL) and with brine (5 mL) and dried over anhydrous sodium sulfate. The solvent was removed to obtain the crude product.

General procedure for acetylation with DMC/AcCl

AcCl (1.1 mmol) was added to a solution of the substrate (1.0 mmol) in DMC (30 mL) at rt. The reaction was stirred at rt overnight. The reaction was quenched with water (20 mL) and the mixture was extracted with EtOAC (3 x 20 mL). The combined organic layer was washed with brine (3 x 20 mL) and dried over anhydrous sodium sulfate. The solvent was removed to obtain the crude product.

General procedure for benzylation

To a solution of the substrate (2.00 mmol) in acetone (15 mL) potassium carbonate (6.00 mmol) and benzyl bromide (2.00 mmol) were added. The suspension was stirred at rt until the reaction was over, then acetone was removed under reduced pressure. The residue was diluited with ethyl acetate (15 mL) and water (15 mL) and neutralized with a 6M solution of HCl. The aqueous layer was extracted with ethyl acetate (2 x 10 mL) and the combined organic layer was washed with brine (3 x 10 mL) and dried over anhydrous sodium sulfate. The solvent was removed to obtain the crude product.

General procedure for reductive debenzylation

In a two necks round bottom flask equipped with a 2 liters hydrogen insufflating device, the substrates (1.0 mmol) was dissolved in a mixture 1:1 of EtOH and THF (50 mL). $Pd(OH)_2$ 20% (0.05 mmol) was added and the reaction was stirred for 18 hours. The reaction mixture was filtered through celite and washed with EtOH (20 mL). The organic solvent was evaporated under reduced pressure to give the crude product.

General procedure for bromination with TBATB

To a solution of the substrate (2.00 mmol) in chloroform (7 mL), TBATB (4.00 mmol) was added in one portion. After 2.5 hours under stirring at rt the mixture was diluted with water (20 mL) and extracted with ethyl acetate (3 x 20 mL). The combined organic layers were washed with brine (3 x 15 mL) and dried over anhydrous sodium sulfate. The solvent was removed to obtain the crude product.

General procedure for bromination with NaBr/oxone/acetone

To a solution of the substrate (2.00 mmol) in acetone (20 mL) NaBr (2.00 mmol) was added, then a solution of oxone (1.00 mmol) in water (20 mL) was added dropwise at 0 °C. The reaction mixture was stirred until completion, then acetone was removed under vacuum. The resulting aqueous layer was extracted with ethyl acetate (3 x 15 mL) and the combined organic layer was washed with brine (3 x 15 mL) and dried over anhydrous sodium sulfate. The solvent was removed to obtain the crude product.

General procedure for bromination with NaBr/oxone/DMC

To a solution of the substrate (2.00 mmol) in DMC (20 mL) NaBr (2.00 mmol) was added, then a solution of oxone (1.00 mmol) in water (20 mL) was added dropwise at 0 °C. The reaction mixture was stirred until completion, then the mixture was extracted with ethyl acetate (3 x 15 mL) and the combined organic layer was washed with brine (3 x 15 mL) and dried over anhydrous sodium sulfate. The solvent was removed to obtain the crude product.

General procedure for bromination of flavonoids with NBS

To a solution of the substrate (1.00 mmol) in DMF (5 mL) NBS (1.00 mmol) was added in one portion. The reaction mixture was stirred at 0 °C for 2.5 hours or until the complete consuption of the substrate, then was poured in a 2 M solution of HCl (5 mL) at 0 °C. The mixture was extracted with CH_2Cl_2 (3 x 10 mL) and the combined organic layer was washed with brine (3 x 10 mL) and dried over anhydrous sodium sulfate. The solvent was removed to obtain the crude product.

General procedure for demethylation with BBr_3

The substrate (1.00 mmol) was dissolved in dry CH_2Cl_2 (5 mL) at -40 °C. BBr_3 (3.00 mmol, 0.28 mL) was added dropwise to the solution and the reaction was monitored by TLC. After the total consumption of the substrate MeOH (1 mL) was added and the reaction mixture was poured into water (10 mL). The aqueous layer was extract with AcOEt (3 x 7 mL) and the combined organic layer was washed with brine (3 x 10 mL) and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to obtain the crude product.

General procedure for demethylation with HBr/AcOH

A solution of the substrate (1.00 mmol) in acetic acid (15.0 mL) and hydrobromidic acid (7.5 mL, 47% in water) was refluxed for 3 hours or until the completion of the reaction, then the solution was cooled to rt and poured into ice. The resulting precipitate was filtered and washed with cold water and dried in oven (60 °C) overnight.

General procedure for deacetylation with NaOH/THF

The substrate (2.00 mmol) was dissolved in a 1:1 mixture of THF and NaOH 2M (10 mL). The reaction was left stirred overnight, then ethyl acetate (15 mL) was added and the aqueous layer was extracted with ethyl acetate (3 x 10 mL). The combined organic layer was washed with brine (3 x 10 mL) and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to afford the crude product.

General procedure for deacetylation with HCl/THF

A solution of the substrate (0.5 mmol) in a 1:1 mixture of THF and HCl 6 M (5 mL) was stirred at rt. After 1 hour all the substrate was consumed. The solvent was evaporated under reduced pressure to afford the product.

General procedure for methanolysis in DMF

To a suspension of CuBr (0.73 mmol) in DMF (2.2 mL) a 25% solution of sodium methoxide in methanol (6.63mL, 29.15 mmol) was added at rt and left under stirring until a bright blue colour appeared (about 1h). The mixture was added to a solution of the substrate (0.91 mmol) in DMF (3 mL) at 120 °C in 2 mL portions. The mixture was left stirring for 40 min, then cooled to rt, quenched with a cold 2M solution of HCl in water (13 mL) and extracted with ethyl acetate (3 x 20 mL). The extracts were washed with brine (3 x 10 mL), dried over anhydrous sodium sulfate and the solvent evaporated under reduced pressure. When a Wessely-Moser rearrangement occurred, the reaction was refluxed for 4.5 hours.

General procedure for methanolysis in DMC

To a suspension of CuBr (0.73 mmol) in DMC (2.2 mL) a 25% solution of sodium methoxide in methanol (6.63mL, 29.15 mmol) was added at rt and left under stirring until a bright blue colour appeared (about 1h). The mixture was added to a solution of the substrate (0.91 mmol) in DMC (3 mL) at reflux in 2 mL portions. The mixture was left stirring for 40 min, then cooled to rt, quenched with a cold 2M solution of HCl in water (13 mL) and extracted with ethyl acetate (3 x 20 mL). The extracts were washed with brine (3 x 10 mL), dried over anhydrous sodium sulfate and the solvent evaporated under reduced pressure.

General procedure for oxidation with I_2/DMSO

To a solution of the substrate (2.0 mmol) in DMSO (5 mL) iodine (0.2 mmol) and sulphuric acid 96% (1 drop) were added. The reaction mixture was heated to 100 °C and stirred for 24 hours. After the complete consumption of the substrate, the reaction was quenched with $Na_2S_2O_3$

General procedure for oxidation of phenols with IBX

IBX (2.0 mmol) was added to a mixture of the substrate (1.0 mmol) in MeOH (3 mL) at rt. The reaction mixture was stirred at rt until the substrate was over. The reaction was quenched with a

solution of $Na_2S_sO_4$ (3.0 mmol) in water (5 mL). The reaction mixture was extracted with EtOAc (3 x 5 mL) and the combined organic layer was washed with brine (3 x 5 mL) and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure.

General procedure for oxidation with IBX/NMO

N-methylmorpholine-*N*-oxide (123 mg, 1.09 mmol) was added to a solution of 2-iodoxybenzoic acid (222 mg, 1.09 mmol) in DMSO (0.8 mL). When a clear solution was obtained (about 15 min), the substrate (0.33 mmol) was added and the reaction mixture was stirred for 48 h at rt. The mixture was then diluted whit ethyl acetate (5 mL) and the organic layer was sequentially washed with a saturated solution of $NaHCO_3$ (2 x 3 mL) and brine (3 mL) and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure.

General procedure for carboxymethylation of phenethyl alcohols in H_2SO_4

H_2SO_4 (0.1 mL) was added to a solution of the substrate (1.0 mmol) in DMC (5 mL). The reaction mixture was stirred at reflux for 2 days adding H_2SO_4 (0.1 mL) every 12 hours, then was filtered through celite and washed with MeOH (15 mL). A solution of NaOH 2 M (10 mL) was added and the biphasic solution was left stirred for 2 hours. The organic layer was washed with brine (3 x 5 mL) and the solvent was removed under vacuum as an azeotropic mixture (DMC:MeOH 1:3). The residual product was characterized without further purification.

General procedure for carboxymethylation of phenethyl alcohols in Amberlyst 15

Amberlyst 15 (1.0 mmol) was added in five portions of 0.2 mmol to a solution of the alcohol (1.0 mmol) in DMC (5 mL). The reaction mixture was stirred at reflux for 2 days. The reaction mixture was filtered and washed with MeOH and the solvent was evaporated as an azeotropic mixture with MeOH (DMC:MeOH 1:3) to obtain the product.

General procedure for C-C coupling

Amberlyst 15 (1.0 mmol) was added to a solution of the appropriate substrate (1.0 mmol) in anisole (3 mL). The reaction was left stirred for 2 days at 120 °C and then the solvent was concentrated under vacuum. Purification through silica gel (eluent: hexane/EtOAc 95/5) afford two regioisomers.

Synthesis of tyrosol ethyl ether

Amberlyst 15 (1.0 mmol) was added to a solution of carboxymethylated tyrosol (1.0 mmol, 196 mg) in EtOH (5 mL). The reaction was stirred at reflux for 36 hours and then filtrated through celite. The organic solvent was evaporated under vacuum. The crude product was purified through silica gel (eluent: hexane/EtOAc 7/3).

Synthesis of tyrosol trifluoroacetate

Trifluoroacetic acid (5 mL) was added to carboxymethylated tyrosol (1.0 mmol, 196 mg). The mixture was stirred at reflux for 48 hours and then the solvent was evaporated under vacuum.

6.3 Analytical and spectroscopical characterization of compounds

5,7-dimethylcrysin (1)

97% yield. Yellow powder. mp 201-202 °C (lit. 201.7°C). ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.87 (3H, s, CH_3O), 3.90 (3H, s, CH_3O), 6.37 (1H, d, *J*=2.2 Hz, Ar-H), 6.56 (1H, d, *J*=2.2 Hz, Ar-H), 6.67 (1H, s, C3-H), 7.49 (3H, m, Ar-H), 7.86 (2H, m, Ar-H); analytical were in agreement with those reported in literature.[211,212]

6-bromo-5,7-dimethylcrysin (2)

97% yield. Gummy liqiud. IR $\nu_{(max)}$ ($CHCl_3$) 1640, 1350, 1165 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ= 3.95 (3H, s, CH_3O), 3.97 (3H, s, CH_3O), 6.41 (1H, s, C8-H), 6.73 (1H, s, C3-H), 7.45 (3H, m, Ar-H), 7.90 (2H, m, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ= 56.7, 58.9, 92.4, 96.7, 107.3, 108.9, 126.0, 128.8, 177.0, 130.5, 131.5, 154.9, 160.2, 160.5, 161.0, 177.0; HRMS: *m/z*=382.9885. [M +Na]$^{+}$, calcd. 382.9895 for $C_{17}H_{13}BrO_4Na$.

7-methylcrysin (3)

96% yield. Light yellow needles. mp 166-168 °C (lit. 166.6 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.88 (3H, s, CH_3O), 6.38 (1H, d, *J*=2.0 Hz, Ar-H), 6.50 (1H, d, *J*=2.0 Hz, Ar-H), 6.64 (1H, s, C3-H), 7.53-7.55 (3H, m, Ar-H), 7.88-7.91 (2H, m, Ar-H); analytical were in agreement with those reported in literature.[213]

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[213] See ref. 213

6-bromo-5-hydroxy-7-methoxyflavone (4) and 8-bromo-5-hydroxy-7-methoxyflavone (5)

4 and **5** were characterized as acetyl derivatives (**9** and **10** respectively) after chromatography on silica gel (hexane/ethyl acetate 8:2).

9: 65% yield from **3**. R_f 0.36. IR $\nu_{(max)}$ ($CHCl_3$) 1715, 1650, 1350, 1165 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ=2.49 (3H, s, CH_3COO), 4.01 (3H, s, CH_3O), 6.61 (1H, s), 6.91 (1H, s), 7.48-7.54 (3H, m, Ar-H), 7.81 (2H, m, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=20.9, 57.0, 98.0, 105.7, 108.5, 112.2, 126.1, 129.0, 131.2, 131.6, 148.0, 157.5, 160.0, 162.0, 168.3, 175.6; HRMS: *m/z*=410.9839. $[M+Na]^+$, calcd. 410.9844 for $C_{18}H_{13}BrO_5Na$.

10: 32% yield from **3**. R_f 0.33. IR $\nu_{(max)}$ ($CHCl_3$) 1715, 1645, 1350, 1160 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ=2.45 (3H, s, CH_3COO), 4.01 (3H, s, CH_3O), 6.66 (1H, s), 6.68 (1H, s), 7.51-7.55 (3H, m, Ar-H), 7.98 (2H, m, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=21.1, 57.0, 97.5, 104.3, 107.7, 111.9, 126.4, 129.1, 131.0, 131.8, 149.9, 154.7, 160.0, 162.2, 169.4, 176.3; HRMS: *m/z*=410.9848. $[M+Na]^+$, calcd. 410.9844 for $C_{18}H_{13}BrO_5Na$.

6,8-dibromocrysin (7)

92% yield. Pale yellow oil. ^1H NMR (300 MHz, acetone-d_6): δ=7.03 (1H, s, C3-H), 7.59-7.62 (3H, m, Ar-H), 8.09-8.12 (2H, m, Ar-H), 13.71 (1H, s, C5-OH); analytical data were in agreement with literature.[214]

8-bromo-5,7-dimethoxyflavone (8)

80% yield. Yellow oil. ^1H NMR (300 MHz, acetone-d_6): δ=3.96 (3H, s, CH_3O), 3.98 (3H, s, CH_3O), 6.41 (1H, s), 6.71 (1H, s), 7.47 (3H, m, Ar-H), 7.96 (2H, m, Ar-H); ^{13}C NMR (75 MHz, acetone-d_6): δ=56.4, 56.5, 90.9, 92.2, 108.1, 109.6, 126.1, 128.9, 131.0, 131.4, 160.2, 160.3, 160.8, 162.7, 177.4; HRMS: *m/z*=382.9853. $[M]^+$, calcd. 382.9895 for $C_{17}H_{13}BrO_4$.

5-hydroxy-6,7-dimethoxyflavone (mosloflavone, 11)

87% yield after chromatography (hexane:ethyl acetate 8:2). Yellow powder. mp 159-160 °C (lit. 159-160). ^1H NMR (300 MHz, $CDCl_3$): δ=3.91 (3H, s, CH_3O), 3.95 (3H, s, CH_3O), 6.54 (1H, s), 6.64 (1H, s), 7.53-7.55 (3H, m, Ar-H), 7.84-7.87 (2H, m, Ar-H), 12.67 (1H, s, C5-OH); analytical were in agreement with those reported in literature.[215]

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5-hydroxy-7,8-dimethoxyflavone (12)

80% yield after chromatography (hexane:ethyl acetate 8:2). Gummy liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.93 (3H, s, CH_3O), 3.94 (3H, s, CH_3O), 6.42 (1H, s), 6.66 (1H, s), 7.52-7.56 (3H, m, Ar-H), 7.91-7.94 (2H, m, Ar-H), 12.54 (1H, s broad, C5-OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=56.3, 61.6, 95.9, 105.0, 105.3, 126.3, 129.1, 131.4 131.9, 149.5, 147.6 158.7, 163.9 182.7. Other data agreed with those reported in literature.[216]

5,6-dihydroxy-7-methoxyflavone (negletein, 13)

96% yield. Yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ=4.01 (3H, s, CH_3O), 6.62 (1H, s), 6.69 (1H, s), 7.52-7.56 (3H, m, Ar-H), 7.88-7.91 (2H, m, Ar-H), 12.51 (1H, s broad, C5-OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=56.6, 90.6, 105.6, 105.8, 126.4, 126.5, 129.2, 129.8, 131.9, 145.8, 150.8, 153.1, 164.3, 182.7. Other data agreed with those reported in literature.[217]

5,6,7-trihydroxyflavone (baicalein, 14)

85% yield. Yellow powder. Data were identical to that of an original sample from Sigma-Aldrich Co.

8-bromo-7-hydroxy-5-methoxyflavone (15)

92% yield. Light yellow needles. mp 245.9-247.3 °C ^{1}H NMR (300 MHz, acetone-d_6): δ=3.94 (3H, s, CH_3O), 6.46 (1H, s), 6.87 (1H, s), 7.60-7.64 (3H, m, Ar-H), 8.03-8.07 (2H, m, Ar-H), 10.57 (1H, s, C5-OH); ^{13}C NMR (75 MHz, acetone-d_6): δ=56.7, 91.8, 92.0, 107.8, 109.1, 126.8, 128.8, 130.1, 131.0, 157.4, 160.9, 162.2, 164.0, 178.4; HRMS: *m/z*=368.9739. $[M+Na]^+$, calcd. 368.9733 for $C_{16}H_{11}BrO_4Na$.

8-bromo-5,7-dihydroxyflavone (15a)

87% yield. Yellow gummy liquid. ^{1}H NMR (300 MHz, acetone-d_6): δ=6.53 (1H, s), 6.93 (1H, s), 7.63-7.67 (3H, m, Ar-H), 8.17-8.22 (2H, m, Ar-H), 10.40 (1H, s, C5-OH); ^{13}C NMR (75 MHz, acetone-d_6): δ=91.0, 91.8, 108.5, 109.3, 126.4, 129.8, 130.5, 130.9, 156.8, 160.1, 162.5, 163.7, 182.4; HRMS: *m/z*=354.9588. $[M+Na]^+$, calcd. 354.9576 for $C_{15}H_9BrO_4Na$.

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5,7-dihydroxy-8-methxoyflavone (wogonin, 16)

91% yield. Bright yellow crystals. mp 203-204 °C (lit.[218] 203). ^{1}H NMR (300 MHz, DMSO-d_6): δ=3.82 (3H, s, OCH_3), 6.30 (1H, s), 7.01 (1H, s), 7.62-7.65 (3H, m, Ar-H), 8.08-8.12 (2H, m, Ar-H), 12.54 (1H, s, C5-OH); Other data agreed with those reported in literature.[219]

5,7-dihydroxy-6-methoxyflavone (oroxilin A, 17)

83% yield. Yellow powder. mp 198-199 °C (lit.[220] 195-197). ^{1}H NMR (300 MHz, $CDCl_3$): δ=4.02 (3H, s, CH_3O), 6.44 (1H, s), 6.70 (1H, s), 7.53-7.57 (3H, m, Ar-H), 7.90-7.93 (2H, m, Ar-H), 12.44 (1H, s, C5-OH); HRMS: *m/z*=307.0564. $[M+Na]^+$, calcd. 307.0577 for $C_{16}H_{12}O_5Na$. Other data agreed with those reported in literature.[221]

7-benzyloxy-5-methoxyflavone (18)

95% yield. White powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.95 (3H, s, CH_3O), 5.17 (2H, s, CH_2Ph), 6.48 (1H, d, *J*=2.1 Hz, Ar-H), 6.67 (1H, d, *J*=2.1 Hz, Ar-H), 6.74 (1H, s, C3-H) 7.38-7.54 (8H, m, Ar-H), 7.86-7.89 (2H, m, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=55.9, 70.7, 92.8, 94.5, 104.5, 105.1, 126.1, 126.2, 126.8, 126.9, 128.1, 128.4, 131.3, 135.9, 159.2, 160.6, 162.0, 162.8, 177.5; HRMS: *m/z*=381.1103. $[M+Na]^+$, calcd. 381.1097 for $C_{23}H_{18}O_4Na$.

7-benzyloxy-8-bromo-5-methoxyflavone (19)

95% yield. Yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.89 (3H, s, CH_3O), 5.22 (2H, s, CH_2Ph), 6.41 (1H, s), 6.63 (1H, s), 7.32-7.47 (8H, m, Ar-H), 7.90-7.93 (2H, m, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=56.5, 71.1, 93.8, 93.9, 108.3, 125.9, 127.0, 128.3, 128.7, 129.0, 129.1, 131.0, 131.4, 135.5, 155.2, 159.3, 160.1, 160.7, 177.2; HRMS: *m/z*=459.0193. $[M+Na]^+$, calcd. 459.0202 for $C_{23}H_{17}BrO_4Na$.

7-hydroxy-5,8-dimethoxyflavone (20)

88% yield after chromatography (eluent: hexane/ethyl acetate 7/3). ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.95 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 6.41 (1H, s), 7.03 (1H, s), 7.38-7.51 (3H, m, Ar-H), 7.89-7.92 (2H, m, Ar-H); other data were in agreement with those reported in literature.[222]

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5-hydroxy-7,4'-dimethoxyflavanone (22)

90% yield after chromatography (eluent: hexane/ethyl acetate 7/3). Yellow needles. mp 113.8-114.5 °C (lit.[223] 114-115). ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.77 (1H, dd, *J*=3 Hz, *J*=17.1 Hz, C3-H), 3.10 (1H, dd, *J*=12.9 Hz, *J*=17.1 Hz, C3-H), 3.80 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 5.35 (1H, dd, *J*=12.9 Hz, *J*= 3 Hz, C2-H), 6.03 (1H, d, *J*=2.4 Hz, CH), 6.06 (1H, d, *J*=2.4 Hz, CH), 6.95 (2H, d, *J*= 8.7 Hz, CH), 7.38 (2H, d, *J*= 8.7 Hz, CH), 12.04 (1H, s, C5-OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=43.1 (CH_2), 55.3 (OCH_3), 55.6 (OCH_3), 79.0, 94.2, 95.0, 103.1, 114.2, 127.7, 130.4, 160.0, 162.9, 164.1, 167.9, 195.9 (C=O). HRMS: *m/z*= 323.0873. $[M+Na]^+$, calcd. 323.0890 for $C_{17}H_{16}O_5Na$.

5-hydroxy-7,4'-dimethoxyflavone (23)

92% yield after chromatography (eluent: hexane/ethyl acetate 8/2). Yellow needles. mp 169.1-172.2 °C, (lit.[224] 167-170). ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.85 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 6.32 (1H, d, *J*=2.4 Hz, CH), 6.43 (1H, d, *J*=2.4 Hz, CH), 6.52 (1H, s, C3-H), 6.97 (2H, d, *J*=9 Hz, CH), 7.79 (2H, d, *J*=9 Hz, CH), 12.78 (1H, s, C5-OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=55.7 (OCH_3), 55.9 (OCH_3), 92.8, 98.2, 104.5, 105.7, 114.7, 128.2, 157.9, 162.4, 162.8, 164.2, 165.6, 182.6 (C=O). HRMS: *m/z*= 321.0758. $[M+Na]^+$, calcd. 321.0733 for $C_{17}H_{14}O_5Na$.

8-bromo-5hydroxy-7,4'-dimethoxyflavone (24) and 6-bromo-5hydroxy-7,4'-dimethoxyflavone (25)

The mixture was used in the following step without any further purification. Yield 94%.

Compounds **24** and **25** were characterized after column chromatography on silica gel (eluent: hexane/ethyl acetate 8/2).

24: Yellow powder. mp 248.3-249.7 °C, (lit.[225] 249-250). ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.90 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 6.43 (1H, s), 6.61 (1H, s), 7.00 (2H, d, *J*=6.6 Hz, CH), 7.93 (2H, d, *J*=6.6 Hz, CH), 12.14 (1H, s, C5-OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=55.5 (OCH_3), 56.7 (OCH_3), 88.0, 94.2, 103.5, 103.6, 114.7, 122.9, 123.1, 128.0, 128.4, 156.5, 161.3, 161.6, 162.8, 164.2, 182.2 (C=O); HRMS: *m/z*= 398.9882. $[M+Na]^+$, calcd. 398.9839 for $C_{17}H_{13}BrO_5Na$.

25: Yellow powder. mp 248.1-249.5 °C (recryst. from acetone). ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.87 (3H, s, OCH_3), 4.00 (3H, s, OCH_3), 6.55 (1H, s), 6.61 (1H, s), 7.02 (2H, d, *J*=6.6 Hz, CH),

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7.82 (2H, d, *J*=6.6 Hz, CH), 12.36 (1H, s, C5-OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=55.5 (OCH_3), 56.8 (OCH_3), 90.7, 95.9, 104.2, 105.8, 114.5, 123.0, 123.2, 128.3, 128.4, 153.2, 158.2, 161.5, 162.7, 164.1, 181.7 (C=O); HRMS: *m/z*= 398.9868. $[M+Na]^+$, calcd. 398.9839 for $C_{17}H_{13}BrO_5Na$.

5-hydroxy-6,7,4'-trimethoxyflavone (26)

82% yield. Pale yellow needles. mp 180.3-181.1 °C, (lit.[226] 180). ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.87 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 6.52 (1H, s), 6.58 (1H, s), 6.98 (2H, d, *J*=8.1 Hz, CH), 7.81 (2H, d, *J*=8.1 Hz, CH), 12.32 (1H, s, C5-OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=55.5 (OCH_3), 56.3 (OCH_3), 60.8 (OCH_3), 90.6, 104.2, 106.2, 114.5, 123.6, 128.0, 132.7, 153.1, 153.2, 158.7, 162.6, 164.0, 182.7 (C=O). HRMS: *m/z*= 351.0798. $[M+Na]^+$, calcd. 351.0839 for $C_{18}H_{16}O_6Na$.

5,6,7,4'-tetrahydroxyflavone (scutellarein, 27)

97% yield. Yellow powder. Analytical data agreed with those reported in literature.[227]

6-bromoflavone (31)

91% yield. Pale yellow solid. mp 192-194 °C (lit.[228] 191). ^{1}H NMR (300 MHz, acetone-d_6): δ=8.34 (1H, d, *J*=2.4 Hz, C5-H), 7.92 (2H, dd, *J*=4.7 and 2.1, Ar-H), 7.79 (1H, dd, *J*=4.7 and 2.4, Ar-H), 7.42-7.59 (4H, m, Ar-H), 6.84 (s, 1H, C3-H); other data were in agreement with those reported in literature.[229]

6-methoxyflavone (32)

89% yield. Pale yellow powder. Data were identical to that of an original sample from Sigma-Aldrich Co.

6-hydroxyflavone (33)

91% yield. Pale yellow powder. Data were identical to that of an original sample from Sigma-Aldrich Co.

5,6-dihydroxyflavone (34)

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96% yield. Yellow powder. mp 184.9-186.4 °C (lit.[230] 189) ^{1}H NMR (300 MHz, acetone-d_6): δ=6.85 (1H, s, C3-H), 7.09 (1H, d, *J*=9.0 Hz, Ar-H), 7.31 (1H, d, *J*=9.0 Hz, Ar-H), 7.60-7.66 (3H, m, Ar-H), 8.07-8.12 (2H, m, Ar-H), 12.65 (1H, s, C5-OH); other data were in agreement with those reported in literature.[231]

7-hydroxy-5-methoxyflavone (35)

93% yield. Withe powder. mp 240.9-242.3 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ=3.80 (3H, s, OCH_3), 6.39 (1H, d, *J*=2.1 Hz, Ar-H), 6.56 (1H, d, *J*=2.1 Hz, Ar-H), 6.71 (1H, s, C3-H), 7.54-7.57 (3H, m, Ar-H), 7.98-8.02 (2H, m, Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6): δ=55.8, 95.2, 96.6, 108.0, 115.6, 125.8, 129.0, 131.0, 131.2, 159.1, 159.3, 160.6, 162.7, 187.1; HRMS: *m/z*= 291.0639. $[M+Na]^+$, calcd. 291.0628 for $C_{16}H_{12}O_4Na$.

3,5-dibromotyrosol (37)

92% yield. Pale yellow solid. mp 91-92 °C (lit.[232] 93) ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.74 (2H, t, *J*=6.6 Hz, CH_2-Ar), 3.80 (2H, t, *J*=6.6 Hz, CH_2-OH), 6.00 (1H, broad s, OH), 7.31 (2H, s, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=37.7, 63.3, 110.0, 132.6, 133.4, 148.2; other data were in agreement with those reported in literature.[233]

3,5-dimethoxytyrosol (38)

46% yield. Oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.76 (2H, t, *J*=6.6 Hz, CH_2-Ar), 3.80 (2H, t, *J*=6.6 Hz, -CH_2OH), 3.83 (s, 6H, -OCH_3), 6.42 (s, 2H, Ph-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ= 39.2, 56.2, 63.7, 105.4, 129.3, 133.2, 147.0; HRMS: *m/z*= 198.0906. $[M]^+$, calcd. 198.0892 for $C_{10}H_{14}O_4$.

4-hydroxy-3,5-dimethoxyphenethyl methyl carbonate (39)

87% yield. Oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.88 (2H, t, *J*=7.2 Hz, Ph-CH_2-), 3.75 (3H, s, OCO_2CH_3), 3.84 (6H, s, OCH_3), 4.29 (2H, t, *J*=7.2 Hz, -$CH_2OCO_2CH_3$), 6.43 (2H, s, Ph-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=35.2, 54.6, 56.2, 68.4, 105.5, 128.1, 133.5, 147.0, 155.6. HRMS: *m/z*= 256.0958. $[M]^+$, calcd. 256.0947 for $C_{12}H_{16}O_6$.

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[233] See ref. 233

4-hydroxy-3,5-dimethoxyphenethyl ethanoate (40)

93% yield. Oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.02 (3H, s, -$OCOCH_3$), 2.83 (2H, t, *J*=6.9 Hz, Ph-CH_2-), 3.83 (6H, s, -OCH_3), 4.22 (2H, t, *J*=6.9 Hz, -CH_2OCO-), 6.40 (2H, s, Ph-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=21.0, 35.2, 56.3, 65.1, 105.5, 128.7, 133.4, 147.0, 171.0; HRMS: *m/z*= 240.1012. $[M]^+$, calcd. 240.0998 for $C_{12}H_{16}O_5$.

3,4,5-trihydroxyphenethyl ethanoate (41)

84% yield. Oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.98 (3H, s, -$OCOCH_3$), 2.70 (2H, t, *J*=7.0 Hz, Ph-CH_2-), 4.15 (2H, t, *J*=7.0 Hz, -CH_2OCO-), 6.17 (1H, s, OH), 6.28 (2H, s, Ph-H), 6.76 (2H, s, -OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=34.4, 65.0, 108.2, 117.6, 130.2, 130.9, 145.5, 171.0; HRMS: *m/z*= 212.0669. $[M]^+$, calcd. 212.0685 for $C_{10}H_{12}O_5$.

3,4,5-trihydroxyphenethyl alcohol (42)

91% yield. Oil. ^{1}H NMR (300 MHz, D_2O): δ=2.45 (2H, t, *J*=6.6 Hz, Ph-CH_2-), 3.54 (2H, t, *J*=6.6 Hz, -CH_2OH), 6.20 (2H, s, Ph-H); ^{13}C NMR (75 MHz, D_2O): δ=37.3, 62.7, 109.0, 131.7, 131.8, 145.4; HRMS: *m/z*= 170.0534. $[M]^+$, calcd. 170.0579 for $C_8H_{10}O_4$.

4-hydroxyphenethyl methyl carbonate (43)

98% yield. Oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.88 (2H, t, *J*=7.1 Hz, Ph-CH_2), 3.75 (3H, s, -OCO_2CH_3), 4.28 (2H, t, *J*=7.1 Hz, C*H*$_2OCO_2CH_3$), 5.37 (1H, s, -OH), 6.75 (2H, d, *J*=8.6 Hz, Ph-H), 7.06 (2H, d, *J*=8.5 Hz, Ph-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=34.2, 54.8, 68.7, 115.4, 129.1, 130.0, 154.4, 155.8; HRMS: *m/z*= 196.0711. $[M]^+$, calcd. 196.0736 for $C_{10}H_{12}O_4$.

4-hydroxyphenethyl methyl ether (43a)

95% yield. Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.82 (2H, t, *J*=6.9 Hz, Ph-CH_2-), 3.36 (3H, s, OCH_3), 3.58 (2H, t, *J*=6.9 Hz, -C*H*$_2OCO_2CH_3$), 6.75 (2H, d, *J*=8.7 Hz, Ar-H), 7.08 (2H, d, *J*=8.7 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=35.4, 58.7, 74.1, 115.4, 115.6, 130.1, 130.2; HRMS: *m/z*= 152.0844. $[M]^+$, calcd. 152.0837 for $C_9H_{12}O_2$.

3,5-dibromo-4-hydroxyphenethyl methyl carbonate (44)

84% yield. Oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.86 (2H, t, *J*=6.8 Hz, Ph-CH_2-), 3.77 (3H, s, OCO_2CH_3), 4.28 (2H, t, *J*=6.8 Hz, -$CH_2OCO_2CH_3$), 7.31 (2H, s, Ph-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=33.3, 54.6, 67.5, 109.7, 131.7, 132.1, 148.1, 155.3; HRMS: *m/z*= 351.8975. $[M]^+$, calcd. 351.8946 for $C_{10}H_{10}Br_2O_4$.

3,4,5-trihydroxyphenethyl methyl carbonate (45)

90% yield. Oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.74 (2H, t, *J*=7.2 Hz, Ph-CH_2-), 3.74 (3H, s, OCO_2CH_3), 4.23 (2H, t, *J*=7.2 Hz, -C*H*$_2$$OCO_2CH_3$), 6.31 (2H, s, Ph-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=34.6, 54.9, 68.6, 108.6, 129.5, 130.4, 144.3, 161.0; HRMS: *m/z*= 228.0606. $[M]^+$, calcd. 228.0634 for $C_{10}H_{12}O_6$.

3,4,5-trihydroxyphenethyl methyl ether (46)

74% yield. Oil. ^{1}H NMR (300 MHz, D_2O): δ=2.59 (2H, t, *J*=6.6 Hz, Ph-CH_2-), 3.54 (3H, s, -OCH_3), 3.56 (2H, t, *J*=6.6 Hz, -CH_2OCH_3), 6.23 (2H, s, Ph-H); ^{13}C NMR (75 MHz, D_2O): δ=33.8, 58.4, 74.5, 109.1, 128.9, 135.6, 145.8; HRMS: *m/z*= 184.0752. $[M]^+$, calcd. 184.0736 for $C_9H_{12}O_4$.

phenethyl methyl carbonate (47a)

83% yield. Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.98 (2H, t, *J* = 7.2 Hz, Ph-*CH*$_2$-), 3.77 (3H, s, COO-*CH*$_3$), 4.35 (2H, t, *J*=7.2 Hz, Ph-*CH*$_2$-), 7.29 (5H, m, Ph-*H*); ^{13}C NMR (75 MHz, $CDCl_3$): δ=35.3, 54.8, 68.5, 126.8, 128.7, 129.0, 137.4, 155.8; HRMS: *m/z*= 184.0752. $[M]^+$, calcd. 184.0736 for $C_9H_{12}O_4$.

phenethyl methyl ether (47b)

17% yield. Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.92 (2H, t, *J*=7.2 Hz, Ph-CH_2-), 3.39 (3h, s, OCH_3), 3.64 (2H, t, *J*=7.2 Hz, C*H*$_2$-OCH_3), 7.22-7.35 (5H, m, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=36.3, 58.7, 73.7, 126.3, 128.5, 128.9, 139.1; other data agreed with those reported in literature.[234]

1-methoxy-4-(2-methoxyethyl)benzene (48b)

>95% yield. Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.88 (2H, t, *J*=7.2 Hz, Ph-CH_2-), 3.40 (3H, s, OCH_3), 3.62 (2H, t, *J*=7.2 Hz, C*H*$_2$-OCH_3), 3.80 (3H, s, Ph-OCH_3), 6.89 (2H, d, *J*=8.4 Hz, Ph-H), 7.19 (2H, d, *J*=8.4 Hz, Ph-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=35.8, 55.4, 57.9, 73.4, 113.5, 129.5, 131.4, 158.7. Other data agreed with those reported in literature.[235]

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[235] Vechorkin, O., Proust, V., Hu, X., *Journal of the American Chemical Society*, **2009** , *28*, 9756-9766.

3-methoxyphenethyl methyl carbonate (49a)

76% yield. Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.96 (2H, t, *J*=7.2 Hz, Ph-CH_2-), 3.77, (3H, s, COO-*CH_3*), 3.80 (3H, s, OCH_3), 4.34 (2H, t, *J*=7.2 Hz, CH_2-O), 6.77-6.83 (3H, m, Ar-H), 7.20-7.25 (1H, m, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=35.2, 54.7, 55.1, 68.3, 112.0, 114.7, 121.2, 129.6, 138.8, 155.7, 159.8; HRMS: *m/z*= 233.0796. $[M+Na]^+$, calcd. 233.0784 for $C_{11}H_{14}O_4Na$.

3-methoxyphenethyl methyl ether (49b)

24% yield. Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.87 (2H, t, *J*=7.2 Hz, Ph-CH_2-), 3.36 (3H, s, OCH_3), 3.60 (2H, t, *J*=7.2 Hz, CH_2-O), 3.80 (3H, s, Ph-OCH_3), 6.74-6.83 (3H, m, Ar-H), 7.18-7.25 (1H, m, Ar-H). Other data agreed with those reported in literature.[236]

2-methoxyphenethyl methyl ether (50b)

>95% yield. Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.91 (2H, t, *J*=7.2 Hz, Ph-CH_2-), 3.36 (3H, s, OCH_3), 3.58 (2H, t, *J*=7.2 Hz, CH_2-O), 3.82 (3H, s, Ph-O*CH_3*), 6.88 (2H, m, Ph-H), 7.19 (2H, m, Ph-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=30.6, 55.3, 58.4, 72.3, 110.3, 120.4, 127.5, 130,5; other data were in agreement with those reported in literature.[237]

3,4-dihydroxyphenethyl methyl ether (51b)

>95% yield. White solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.88 (2H, t, *J*=7.2 Hz, Ph-CH_2-), 3.35 (3H, s, OCH_3), 3.61 (2H, t, *J*=7.2 Hz, CH_2-O), 6.70-6.78 (3H, m, Ar-H); other data were in agreement with those reported in literature.[238]

methyl 4-nitrophenethyl carbonate (52a)

88% yield. Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.05 (2H, t, *J*=6.6 Hz, Ph-CH_2-), 3.71 (3H, s, C=OO-*CH_3*), 4.35 (2H, t, *J*=6.6 Hz, CH_2-O), 7.36 (2H, d, *J*=9.0 Hz, Ar-H), 8.10 (2H, d, *J*=9.0 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=35.1, 55.0, 67.4, 123.9, 130.0, 145.5, 147.2, 155.7; HRMS: *m/z*=248.0533. $[M+Na]^+$, calcd. 248.0529 for $C_{10}H_{11}O_5NNa$.

1-(2-methoxyethyl)-4-nitrobenzene (52b)

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[238] Pereira-Caro, G., Bravo, L., Mateos, R., Madrona, A., Espartero, J. L., *J. Agric. Food Chem.*, **2010**, *58*, 789-806.

12% yield. White crystals. mp 64.8-66.1 °C (lit.[239] 64 °C) ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.97 (2H, t, *J*=6.6 Hz, Ph-*CH*$_2$-), 3.33 (3H, s, OCH_3), 3.63 (2H, t, *J*=6.6 Hz, C*H*$_2$-OCH_3), 7.38 (2H, d, *J*=8.7 Hz, Ar-H), 8.13 (2H, d, *J*=8.7 Hz, Ar-H). Other data agreed with those reported in literature.[240]

methyl 3-(trifluoromethyl)phenethyl carbonate (53a)

86% yield. Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.04 (2H, t, *J*=6.9 Hz, Ph-*CH*$_2$-), 3.76 (3H, s, C=OO-*CH*$_3$), 4.36 (2H, t, *J*=6.9 Hz, CH_2-O), 7.46 (m, 4H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=35.1, 54.9, 67.9, 123.8, 125.8, 126.1, 129.2, 132.4, 132.5, 138.4, 155.8;

1-(2-methoxyethyl)-3-(trifluoromethyl)benzene (53b)

14% yield. Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.90 (2H, t, *J*=6.9 Hz, Ph-*CH*$_2$-), 3.34 (3H, s, OCH_3), 3.86 (2H, t, *J*=6.9 Hz, C*H*$_2$-OCH_3), 7.44 (m, 4H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=35.5, 58.8, 68.5, 123.1, 125.9, 126.1, 128.4, 132.1, 132.8, 139.4;

methyl 3-phenylpropyl carbonate (54a)

>95% yield. Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.04 (2H, m), 2.73 (2H, t, *J*=8.1 Hz, Ar-CH_2), 3.81 (3H, s, OCH_3), 4.23 (2H, t, *J*=8.1 Hz, CH_2-O), 7.24 (5H, m, Ar-H). Other data agreed with those reported in literature.[241]

2-cyclohexylethyl methyl carbonate (55a)

>95% yield. Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.88-1.71 (13H, m), 3.75 (3H, s, OCH_3), 4.15 (2H, t, *J*=6.6, CH_2-O); ^{13}C NMR (75 MHz, $CDCl_3$): δ=26.2, 26.5, 33.2, 33.5, 34.3, 36.1, 54.6, 66.4, 70.9, 156.0;

1-(2-ethoxyethyl)-4-methoxybenzene (56)

86% yield after column chromatography. (Eluent: hexane/EtOAC 8/2) Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.20 (3H, t, *J*=7.2 Hz, OCH_2-*CH*$_3$), 2.84 (2H, t, *J*=7.2 Hz, Ph-CH_2), 3.50 (2H, q, *J*=7.2 Hz, O*CH*$_2$-CH_3), 3.59 (2H, t, *J*=7.2 Hz, CH_2-O), 3.79 (3H, s, Ph-OCH_3), 6.83 (2H, d, *J*=8.4,

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Ph-*H*), 7.14 (2H, d, *J*=8.4, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=15.4, 35.7, 55.4, 66.4, 72.0, 114.0, 130.0, 131.3, 158.3;

4-methoxyphenethyl 2,2,2-trifluoroethanoate (57)

>95% yield. Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.99 (2H, t, *J*=6.9 Hz, Ph-CH_2-), 3.80 (3H, s, Ph-OCH_3), 4.51 (2H, t, *J*=6.9 Hz, CH_2-O), 4.97 (1H, s broad, OH), 6.88 (2H, d, *J*=8.7, Ar-H), 7.15 (2H, d, *J*=8.7, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=34.0, 55.4, 68.8, 114.4, 114.7 (CF_3), 128.4, 130.1, 158.2 (C=O), 158.9; HRMS: *m/z*=248.0675. $[M]^+$ calcd. 248.0660 for $C_{11}H_{11}F_3O_3$

4-(2-methoxyphenethyl)phenyl ethanoate (58c)

34% yield from **43**. Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.31 (3H, s, C=OCH_3), 2.90 (4H, m, Ar-*CH_2*-*CH_2*-Ar), 3.83 (3H, s, OCH_3), 6.89 (2H, m, Ar-H), 7.00 (2H, d, *J*=8.4 Hz, Ar-H), 7.12 (1H, dd, *J*=7.2 Hz, *J*=1.5 Hz, Ar-H), 7.20 (1H, dd, *J*=7.2 Hz, *J*=1.8 Hz, Ar-H), 7.22 (2H, d, *J*=8.4 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=21.1, 32.4, 35.7, 55.3, 110.4, 120.4, 121.2, 127.3, 129.4, 129.9, 130.1, 140.0, 148.8, 157.5, 169.6;

4-(4-methoxyphenethyl)phenyl ethanoate (58d)

45% yield from **43**. White solid. mp …. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.30 (3H, s, C=OCH_3), 2.87 (4H, m, Ar-*CH_2*-*CH_2*-Ar), 3.80 (3H, s, OCH_3), 6.84 (2H, d, *J*=8.7 Hz, Ar-H), 7.00 (2H, d, *J*=8.4 Hz, Ar-H), 7.10 (2H, d, *J*=8.4 Hz, Ar-H), 7.18 (2H, d, *J*=8.7 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=21.1, 36.9, 37.5, 55.2, 113.8, 121.3, 129.4, 129.4, 133.6, 139.4, 148.9, 158.0, 169.5;

2,4'-dimethoxy-dibenzyl (59a)

28% yield after column chromatography (eluent: hexane/EtOAC 7/3). Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.82-2.88 (4H, m, Ar-*CH_2*-*CH_2*-Ar), 3.80 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 6.82-6.91 (4H, m, Ar-H), 7.09-7.21 (4H, m, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=32.7, 35.4, 55.2, 55.3, 110.3, 113.7, 120.4, 127.2, 129.4, 129.9, 130.4, 134.6, 157.6, 157.8;

1,2-bis(4-methoxyphenyl)ethane (59b)

47% yield after column chromatography (eluent: hexane/EtOAC 7/3). White solid. mp 118.2-120.0 °C (lit.[242] 123-125); ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.83 (s, 4 H, Ph-*CH_2*-*CH_2*-Ph), 3.79 (s, 6 H,

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Ph-O*CH*$_3$), 6.82 (d, *J*=8.7 Hz, 2H, Ph-*H*), 7.09 (d, *J* = 8.7 Hz, 2H, Ph-*H*); ^{13}C NMR (75 MHz, $CDCl_3$): δ=37.4, 55.4, 113.8, 129.5, 134.1, 157.9; other data were in agreement with those reported in literature.[243]

2-(4-hydroxyphenyl)-ethyl acetate (60)

Yield >95%. White solid. mp 55.4-56.8 °C (lit.[244] 57-58); ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.09 (3H, s, C=OCH_3), 2.90 (2H, t, *J*=7.2 Hz, Ar-CH_2), 4.24 (2H, t, *J*=7.2 Hz, CH_2-O), 6.75 (2H, d, *J*=8.5 Hz, Ar-H), 7.04 (2H, d, *J*=8.5 Hz, Ar-H); other data agreed with those reported in literature.[245]

2-(3,4-dihydroxyphenyl)-ethyl acetate (61)

White solid. mp 80.4-81.9 °C (lit.[246] 81-83); ^{1}H NMR (300 MHz, DMSO-d_6): δ=1.98 (3H, s, C=OCH_3), 2.68 (2H, t, *J*=7.1 Hz, Ar-CH_2), 4.10 (2H, t, *J*=7.1 Hz, CH_2-O), 6.45 (1H, dd, *J*=2.1 Hz, *J*=8.0 Hz, Ar-H), 6.61 (1H, d, *J*=2.1 Hz, Ar-H), 6.65 (1H, d, *J*=8.0 Hz, Ar-H); other data agreed with those reported in literature.[247]

3,4-dimethoxyphenethyl ethanoate (62)

97% yield. Pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.98 (3H, s, OCOCH_3), 2.82 (2H, t, *J*=6.9 Hz, CH_2-Ph), 3.79 (3H, s, Ph-OCH_3), 3.81 (3H, s, Ph-OCH_3), 4.20 (2H, t, *J*=6.9 Hz, CH_2-O), 6.69-6.77 (3H, m, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=20.8, 34.6, 55.8, 55.9, 64.9, 111.4, 112.3, 120.8, 130.3, 147.8, 148.9, 170.7; HRMS: *m/z*= 224.1055. $[M]^+$, calcd. 224.1049 for $C_{12}H_{16}O_4$.

2-bromo-4,5-dimethoxyphenethyl ethanoate (63)

91% yield. Pale yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.98 (3H, s, OCOCH_3), 2.94 (2H, t, *J*=7.2 Hz, CH_2-Ph), 3.78 (3H, s, Ph-OCH_3), 3.79 (3H, s, Ph-OCH_3), 4.20 (2H, t, *J*=6.9 Hz, CH_2-O), 6.69 (1H, s, Ar-H), 6.94 (1H, s, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=20.9, 34.9, 56.0, 56.1, 63.5, 113.7, 114.4, 115.6, 123.4, 129.0, 148.4, 170.9; HRMS: *m/z*= 302.0167. $[M]^+$, calcd. 302.0154 for $C_{12}H_{15}BrO_4$.

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5,6-dimethoxy-2,3-dihydrobenzofuran (64)

88% yield. White needles. ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.12 (2H, t, *J*=8.7 Hz, CH_2-Ph), 3.80 (3H, s, Ph-OCH_3), 3.81 (3H, s, Ph-OCH_3), 4.52 (2H, t, *J*=8.7 Hz, CH_2-O), 6.43 (1H, s, Ar-H), 6.75 (1H, s, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=30.1, 56.2, 57.1, 71.7, 95.2, 109.7, 116.8, 143.4, 149.5, 154.4; HRMS: *m/z*= 180.0781 $[M]^+$, calcd. 180.0786 for $C_{10}H_{12}O_3$.

5,6-dihydroxy-2,3-dihydrobenzofuran (65)[248]

81% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.06 (2H, t, *J*=7.2 Hz, Ar-CH_2), 3.55 (2H, t, *J*=7.2 Hz, CH_2-O), 6.39 (1H, s, Ar-H), 6.64 (1H, s, Ar-H);

3-bromotyrosol (66)

92% yield. Pale yellow oil. ^{1}H NMR (300 MHz, acetone-d_6): δ=2.78 (2H, t, *J*=6.4 Hz, Ar-CH_2), 3.82 (2H, t, *J*=6.4 Hz, CH_2-OH), 6.94 (1H, d, *J*=8.3 Hz, Ar-H), 7.08 (1H, dd, *J*=1.7 Hz, *J*=8.3 Hz, Ar-H), 7.34 (1H, d, *J*=1.7 Hz, Ar-H); other data were in agreement with those reported in literature.[249]

3-methoxytyrosol (67)

83% yield. Gummy liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.80 (2H, t, *J*=6.3 Hz, CH_2-Ph), 3.84 (2H, t, *J*=6.3 Hz, CH_2-O), 3.88 (3H, s, OCH_3), 6.70-6.73 (2H, m, Ar-H), 6.86 (1H, d, *J*=8.1 Hz, Ar-H); other data were in agreement with those reported in literature.[250]

5-bromo-3-methoxytyrosol (68)

94% yield. Yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.93 (2H, t, *J*=7.8 Hz, CH_2-Ph), 3.84 (2H, t, *J*=7.8 Hz, CH_2-O), 3.86 (3H, s, OCH_3), 6.75 (1H, s, Ar-H), 7.09 (1H, s, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=35.1, 56.3, 63.7, 113.9, 114.6, 115.8, 123.6, 129.2, 148.6;

6-hydroxy-5-methoxy-2,3-dihydrobenzofuran (69)[251]

63% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.11 (2H, t, *J*=8.7 Hz, CH_2-Ph), 3.91 (3H, s, Ph-OCH_3), 4.52 (2H, t, *J*=8.7 Hz, CH_2-O), 6.99 (1H, s, Ar-H), 7.10 (1H, s, Ar-H);

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[248] This compound was not fully characterized due to the fact that the work is yet to be finished.

[249] Bovicelli, P., Antonioletti, R., Mancini, S., Causio, S., Borioni, G., Ammendola, S., Barontini, M., *Synth. Comm.*, **2007**, *37*, 4245-4252.

[250] Christophoridou, S., Dais, P., Tseng, L.-H., Spraul, M., *J. Agric. Food Chem.*, **2005**, *53*, 4667-4679.

[251] This compound was not fully characterized due to the fact that the work is yet to be finished.

6-hydroxy-5-methoxybenzofuran (70)[252]

30% yield. ^{1}H NMR (300 MHz, CDCl3): δ=3.81 (3H, s, Ph-OCH$_3$), 6.45 (1H, s, Ar-H), 6.65 (1H, d, *J*=2.1 Hz, C3-H), 6.74 (1H, s, Ar-H), 7.50 (1H, d, *J*=2.1 Hz, C2-H);

4-bromophenylpropyl alcohol (71a)

19% yield after column chromatography. (Eluent: hexane/EtOAc 85/15). ^{1}H NMR (300 MHz, CDCl3): δ=1.81-1.91 (2H, m, Ar-CH$_2$-*CH$_2$*), 2.67 (2H, t, *J*=7.8 Hz, Ar-CH$_2$), 3.66 (2H, t, *J*=7.8 Hz, CH$_2$-OH), 7.07 (2H, d, *J*=8.1 Hz, Ar-H), 7.40 (2H, d, *J*=8.1 Hz, Ar-H); other data were in agreement with those reported in literature.[253]

2-bromophenylpropyl alcohol (71b)

23% yield after column chromatography. (Eluent: hexane/EtOAc 85/15). ^{1}H NMR (300 MHz, CDCl$_3$): δ=1.87-1.94 (2H, m, Ar-CH$_2$-*CH$_2$*), 2.84 (2H, t, *J*=6.3 Hz, Ar-CH$_2$), 3.70 (2H, t, *J*=6.3 Hz, CH$_2$-OH), 7.03-7.08 (1H, m, Ar-H), 7.23-7.26 (2H, m, Ar-H), 7.46-7.57 (1H, m, Ar-H); other data were in agreement with those reported in literature.[254]

chroman (72)

32% yield from **71b** after column chromatography. (Eluent: hexane/EtOAc 75/25). Pale yellow powder. Data were identical to that of an original sample from Sigma-Aldrich Co.

2-methoxyphenylpropyl alcohol (73)

26% yield from 71b after column chromatography. (Eluent: hexane/EtOAc 75/25). Light yellow oil. ^{1}H NMR (300 MHz, CDCl3): δ=1.83-1.89 (2H, m, Ar-CH$_2$-*CH$_2$*), 2.73 (2H, t, *J*=6.3 Hz, Ar-CH$_2$), 3.60 (2H, t, *J*=6.3 Hz, CH$_2$-OH), 3.84 (3H, s, OCH$_3$), 6.85-6.93 (1H, m, Ar-H), 7.14-7.25 (2H, m, Ar-H), 7.53 (1H, d, *J*=8.1 Hz, Ar-H); other data were in agreement with those reported in literature.[255]

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[252] This compound was not fully characterized due to the fact that the work is yet to be finished.

[253] Molander, G. A., Argintaru, O. A., Aron, I. Dreher, S. D., *Org. Lett.,* **2010***, 12*, 5783-5785.

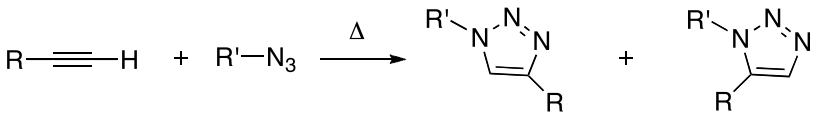
[254] Tsuji, H., Yamagata, K.-I., Itoh, Y., Endo, K., Nakamura, M., Nakamura, E., *Angew. Chem. Int. Ed*., **2007**, *46*, 8206-8208.

[255] Liang, H., Ciufolini, M. A., *Org. Lett*., **2010,** *16***,** 13262-13270.

7. Cu^{I}-catalyzed silver acetylide-azide cycloaddition

7.1 Introduction

Huisgen 1,3-dipolar cycloadditions are pericyclic reactions that afford heterocyclic compounds from acyclic unsaturated reactants.[256] Perhaps the most widely known member of this family is the cycloaddition of terminal alkynes and azides to give triazoles.[257] The Huisgen (2 + 3)[258] thermal cycloaddition of alkynes and azides requires high reaction temperatures and lacks regioselectivity, consequently this reaction has seen only limited use.



Scheme 7.1 Huisgen 1,3-dipolar cycloaddition

A condition for such a reaction to take place is a certain similarity of the interacting HOMO and LUMO orbitals, depending on the relative orbital energies of both the dipolarophile and the dipole. EWGs on the dipolarophile normally favour an interaction of the LUMO of the dipolarophile with the HOMO of the dipole that leads to the formation of the new bonds, whereas ERGs on the dipolarophile normally favour the inverse of this interaction.

The Cu^{I}-catalyzed azide-alkyne cycloaddition (CuAAC) is a variant of the Huisgen azide-alkyne cycloaddition,[259,260] independently reported in 2002 by the Meldal and Sharpless groups.[261] The use of copper(I) gives perfect regioselectivity and enhances the rate of the reaction. These characteristics, together with experimental simplicity, make the CuAAC reaction a useful workhorse in synthetic and medicinal chemistry,[262] material science[263] and polymer chemistry.[264]

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[256] Huisgen, R., in *1,3-Dipolar cycloaddition Chemistry* (Ed.: A. Padwa), Wiley, New York, **1984**, pp. 1-76

[257] Huisgen, R., *Pure Appl. Chem.*, **1989**, *61*, 613-628.

[258] 2 π-electrons of the dipolarophile and 4 electrons of the dipolar compound participate in a concerted, pericyclic shift and the reaction is therefore a $[2_s+4_s]$ cycloaddition. Many authors still use "[2+3] cycloaddition", which counts the number of involved atoms but does not follow IUPAC recommendations.

[259] Tornøe, C. W., Christensen, C., Meldal, M., *J. Org. Chem.*, **2002**, *67*, 3057-3064.

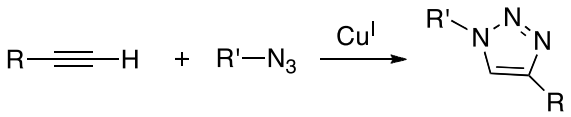
[260] Rostovtsev, V. V., Green, L. G., Fokin, V. V., Sharpless, K. B., *Angew. Chem. Int. Ed.*, **2002**, *41*, 2596-2599.

[261] Meldal M., Tornøe, C. W., *Chem Rev.*, **2008**, *108*, 2952-3015.

[262] Kolb, H. C., Sharpless, K. B., *Drug Discovery Today*, **2003**, *8*, 1128-1137.

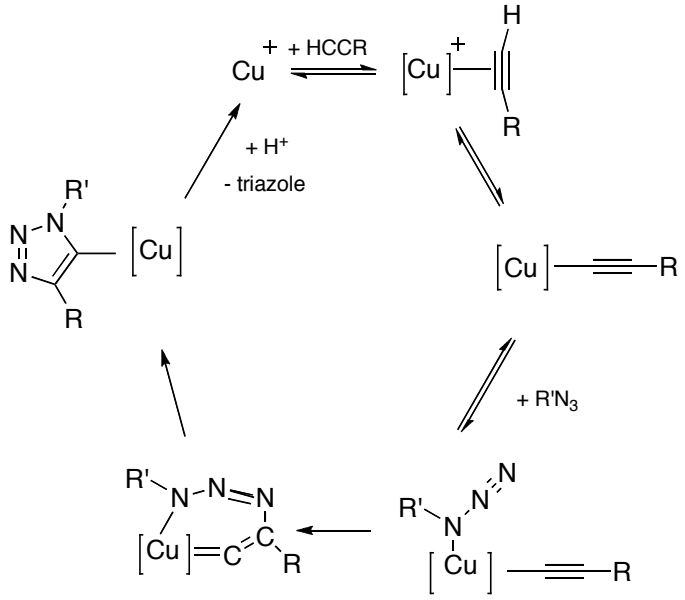
[263] Hawker, C. J., Fokin, V. V., Finn, M. G., Sharpless, K. B., *Aust. J. Chem.*, **2007**, *60*, 381-383.

[264] Evans, R. A., *Aust. J. Chem.*, **2007**, *60*, 384-395.



Scheme 7.2 CuAAC

The proposed mechanism begins with the formation of copper(I) acetylide. Extensive density functional theory calculation[265] offer compelling evidence which strongly disfavours the direct (2+3) concerted cycloaddiction and points to a stepwise annealing sequence that pass through a six membered intermediate containing copper.

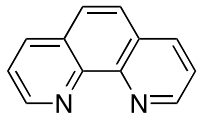


Scheme 7.3 Proposed CuAAC mechanism

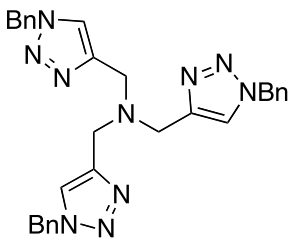
One of the preferred methods is the use of a Cu^{II} source and a reducing agent in large excess, such as $CuSO_4$ and sodium ascorbate. The presence of the reducing agent reduces the sensitivity of the reaction to oxygen allowing it to be performed in open-air conditions. However, potential oxidative side reaction can occur and cause the degradation of the copper source. For this reason, polydentate ligands are often used to maintain the necessary concentration of catalytically-active copper(I). Many ligands also provide a rate enhancement to the reaction.[266] Examples of such ligands are TBTA (*tris*-(benzyltriazolylmethyl)amine), 1,10-phenanthroline and $(BimH)_3$.

[265] Doyle, M. P., et al., in *Modern Catalytic Methods for Organic Synthesis with Diazo Compounds* Wiley (New York), **1997**, 163-248.

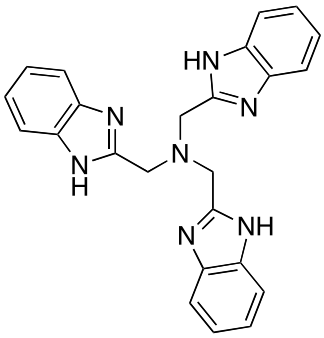
[266] V. O. Rodionov, S. I. Presolski, D. D. Diaz, V. V. Fokin, M. G. Finn, *J. Am. Chem. Soc.*, **2007**, *129*, 12705-12712.



1,10-phenanthroline



TBTA



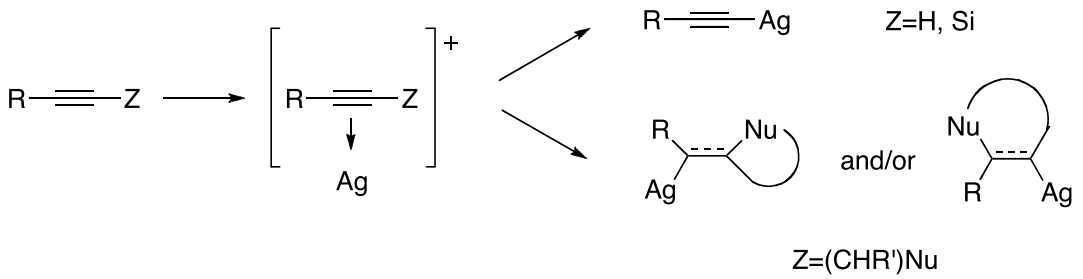
$(BimH)_3$

Figure 7.1 Structures of Cu(I) ligands

The efficiency and selectivity of the CuAAC for the exclusive formation of 1,4-triazoles are a consequence of the reactivity of the *in situ* generated copper(I) acetylides. The reaction of the copper acetylide with an azide yields a copper(I) triazolide, which undergoes protonation/ demetallation to afford the triazole.

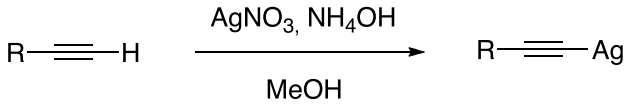
The unique reactivity of copper(I) compounds has long been appreciated. Cu^{I}, but not Cu^{II}, forms a variety of compound with Cu-C bonds. Alkyl copper(I) compounds may be obtained by interaction of Cu^{I} halides with organolithium or Grignard compounds. Alkyl copper(I) compounds usually decompose readily, but they can be stabilized by phosphine ligands. Cu^{I} amine solutions react with terminal acetylenes to give yellow or red precipitates termed ladder polymers, that are believed to have RCC-Cu units π-bonded to another Cu atom. Cu^{I} acetylides are useful intermediates for the synthesis of a variety of organic acetylenic compounds and heterocycles, by reaction with aryl and other halides, e.g. Sonogashira, Castro-Stephens, and Cadiot-Chodkiewicz couplings.

Copper is a member of group 11 of the periodic table, and shares many properties with silver and gold. The d^{10} electronic configurations of Cu^{I}, Ag^{I} and Au^{I} favours interaction with unsaturated systems that possess low-energy vacant orbitals, especially alkynes, allenes and alkenes. From an organic chemistry point of view, this alkynophilicity has dramatic consequence on the behaviour of such π-systems. Upon coordination to Ag^{I} or Au^{I}, π-systems becomes susceptible to nucleophilic addition, and if heteronucleophile is present within the same molecule the formation of heterocycles can occur. If the substrate is a terminal alkyne, different reactivity may manifest as the initial π-complex may convert to the corresponding Ag^{I} or Au^{I} acetylide.



Scheme 7.4 Different behaviour of internal and terminal alkynes

Gold and silver acetylides are among the oldest organometallics known. Silver acetylides are especially straightforward to prepare: addition of the acetylene to aqueous ammoniacal silver nitrate solution affords the silver acetylide as a precipitate, which can be collected by filtration and stored in absence of light for months without significant decomposition.



Scheme 7.5 Classical synthesis of silver acetylides

In comparison to other metal acetylides (e.g. Na, Cu, Li), the use of silver acetylides in synthesis has been limited perhaps as a consequence of their relatively low reactivity and/or poor solubility. Despite their long history and ready preparation, they have seen little use in organic chemistry, being predominantly used as nucleophiles in addition/elimination reactions or substitution reactions.[267] Very recently it has been shown that silver acetylides can undergo transmetalation to palladium and participate in copper-free Sonogashira reactions.[268]

Gold(I) acetylides can be prepared by reaction of gold halides with acetylide anions or by depolymerising ligand disruption of polymeric gold(I) acetylides. The resulting compounds are frequently stable in air. Like silver acetylides, gold acetylides are also unreactive with organic azides. Upon treatment with copper(I), gold acetylides undergo cycloaddition with azides to form gold triazolides,[269] which can be isolated. This reaction is limited to terminal alkynes and the products are frequently stable in air.

Recently it has been demonstrated that also dialkyl aluminium acetylides can react with organic azides to afford 1,4-disubstituited-5-aluminium-1,2,3-triazoles under copper(I) catalysis.[270] The reaction has a perfect regioselectivity. This selectivity is noteworthy, as the copper-catalized

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[267] Halbes-Letinois, U., Weibel, J. M., Pale P., *Chem. Soc. Rev.*, **2007**, *36*, 759-769.

[268] Dillinger, S., Bertus, P., Pale, P., *Org. Lett.,* **2001**, *3*, 1661-1664.

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[270] Zhou, Y., Lecourt T., Micouin, L., *Angew. Chem. Int. Ed.*, **2010**, *49*, 2607-2610.

cycloaddition of internal alkynes and azides is known to be poorly regioselective. The aluminium derivatives can further react to afford a wide range of different organic compounds. These transformations clearly show that, despite its stability during the triazole formation, the carbon-aluminium bond is still reactive enough to be engaged in further synthetic transformation.

Another notable exception is the copper(I)-catalized cycloaddition reaction of 1-iodoalkynes[271] and 1-bromoalkynes[272] with organic azides affording 1,4,5-trisubstituted triazoles in which the alkyne substituent is found in the 5 position of the resulting 1,2,3-triazole.

Internal alkynes (RCCR') can react under the CuAAC conditions as well.[273] Nolan and co-workers demonstrated that using NHC (*N*-heterocyclic carbenes) as ligands in the copper(I) catalyzed cycloaddition allows internal alkynes to perform the reaction.[274]

Copper acetylides are postulated intermediates in several organic reactions, for example the Sonogashira coupling and Cadiot-Chodkiewicz couplings. They are often heat and shock sensitive and highly explosive, so it will be very useful to investigate the possibility to use silver acetylides as precursor of copper acetylides. Transmetalation of copper acetylides with palladium occurs in the proposed mechanism of Sonogashira reaction. The CuAAC reaction is complemented by the ruthenium(I)-catalyzed azide-alkyne cycloaddition (RuAAC) reaction, which provides 1,5-disubstituted and 1,4,5-trisubstituted triazoles.[275,276] A metal-free protocol employing catalytic tetraalkylammonium hydroxide in DMSO also affords 1,5-triazoles exclusively.[277]

Whereas the cycloaddition chemistry of acetylides of the group 11 metals copper and gold with azides have been well studied, the corresponding reactions of the intervening element silver have been largely overlooked.[278] Stimulated by the similar electronic configurations of the congeners copper(I), silver(I) and gold(I) (s^0d^{10}) we investigated the reactivity of silver acetylides in azide/ alkyne cycloaddition reactions with and without copper(I) catalyst. The CuAAC reaction was chosen as model reaction due to its practicability and special contemporary interest. Different

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[271] Hein, J. E., Tripp, J. C., Krasnova, L. B., Sharpless, K. B., Fokin, V. V., *Angew. Chem. Int. Ed.*, **2009**, *48*, 8018-8021.

[272] Kuijpers, B. H. M., Dijkmans, G. C. T., Groothuys, S., Quaedflieg, P. J. L., Blaauw, M. R. H., van Delft F. L., Rutjes, F. P., *Synlett*, **2005**, *20*, 3059-3062.

[273] Candelon, N., Lastecoueres, D., Diallo, A. K., Aranzaes, J. R., Astruc D., Vincent, J. M., *Chem. Commun.*, **2008**, *6*, 741-743.

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[276] Zhang, L., Chen, X., Xue, P., Sun, H. H., Williams, I. D., Sharpless, K. B., Fokin V. V., Jia, G., *J. Am. Chem. Soc.*, **2005**, *127*, 15998-15999.

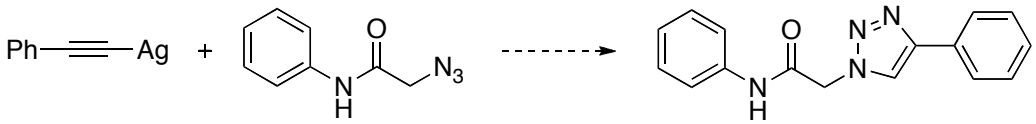
[277] Kwok, S. W., Fotsing, J. R., Fraser, R. J., Rodionov, V. O., Fokin, V. V., *Org. Lett.*, **2010**, *12*, 4217-4219.

[278] Aucagne, V., Leigh, D. A., *Org. Lett.*, **2006**, *8*, 4505-4507.

organic azides and silver acetylides were synthesized to exploit the cycloaddition reaction with a wide range of substrate.

7.2 Results and discussion

As highlighted in the Introduction, silver(I) and copper(I) share many similarities arising from their electronic configuration. Since copper acetylides are known to be intermediates in the azide-alkyne cycloaddition, we initially investigated whether silver acetylides can react with organic azides. We investigated the reaction of a stoichiometric quantity of silver phenylacetylide **1** with a model azide (**2**) in different solvents without any catalyst.



1 **2** **3**

Entry	Acetylide equivalents	Azide equivalents	Solvent	Time and temperature	Yield
1	1	1	Pyridine	rt, 24 h	No reaction
2	1	1	NMM	rt, 24 h	No reaction
3	1	1	NMM	Reflux, 24 h	1,4 product 2.4% 1,5 product 2.7% substrate 94.8%

Table 7.1 Solvent and temperature

Silver acetylides are polymeric species with limited solubility[279] and we investigated the reaction in both *N*-methylmorpholine, which has seen use as a solvent in nucleophilic substitution reactions of silver acetylides,[280,281] and pyridine.[282] The latter solvent dissolves the Ag-acetylide most likely as a consequence of Ag-N(pyridine) coordinate bond formation breaking up aggregates.

[279] Létinois-Halbes, U., Pale P., Berger, S., *J. Org. Chem.*, **2005**, *70*, 9185-9190.

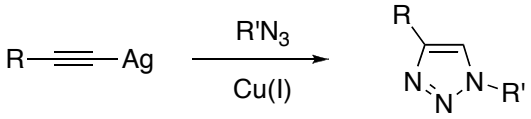
[280] Pouwer, R. H., Williams, C. M., Raine, A. L., Harper, J. B., *Org. Lett.*, **2005**, *7*, 1323-1325.

[281] Pouwer, R. H., Harper, J. B., Vyakaranam, K., Michl, J., Williams, C. M., Jessen, C. H., Bernhardt, P. V., *Eur. J. Org. Chem.*, **2007**, *2*, 241-248.

[282] Davis R. B., Scheiber, D. H., *J. Am. Chem. Soc.*, **1956**, *78*, 1675- 1678.

The choice of solvents was limited by the poor solubility of silver phenylacetylide, which requires a strongly coordinating aprotic solvent. Silver phenylacetylide is soluble in pyridine but no reaction was observed at room temperature (entry 1, Table 7.1). Silver phenylacetylide is poorly soluble in *N*-methylmorpholine, but this solvent has been reported to be effective in promoting the nucleophilic substitution of bridgehead adamantly iodides by phenyl acetylide. No reaction occurred at room temperature in *N*-methylmorpholine (entry 2, Table 7.1), and upon heating at reflux a small amount of regioisomeric triazoles were obtained (entry 3, Table 7.1). The formation of these triazoles is attributed to the partial hydrolysis of the silver acetylide followed by a thermal (2 + 3) Huisgen reaction. The formation of a mixture of regioisomers is diagnostic for the classic Hüisgen (2 + 3) cycloaddition reaction.

The use of copper(I) as catalyst was studied next. Both preformed copper(I) in *Tetrakis*(acetonitrile)copper(I) hexafluorophosphate $[Cu(MeCN)_4]PF_6$ and *in situ* generated copper(I) from $Cu^{II}SO_4$/sodium ascorbate were used. While $Cu(MeCN)_4PF_6$ was soluble in pyridine, a solution of pyridine/water 10:1 was necessary to dissolve the sodium ascorbate. A separate study carried out under the same reaction condition showed that sodium ascorbate did not affect silver acetylides. In both cases the formation of a 1,4-triazole was observed (entries 1 and 2, Table 7.2), but neither reaction went to completion after 24 h. The use of $Cu(MeCN)_4PF_6$ was superior to the use of $CuSO_4$/sodium ascorbate. Increasing the amount of silver acetylide to 1.5 equivalents, afforded no improvements in either the yield or in the substrate conversion (entry 3, Table 7.2).



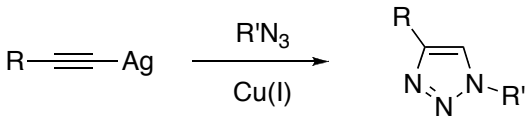
Entry	Acetylide equivalents	Azide equivalents	Copper source and equivalents	Solvent	Time and conditions	Yield
1	1	1	$Cu(MeCN)_4PF_6$ 5% mol	Pyridine	rt, 24 h	Product 66% Yield 63%
2	1	1	$CuSO_4$ 5% mol/ Na ascorbate 10% mol	Pyridine/H_2O 10:1	rt, 24 h	Product 50% Yield 27%
3	1.5	1	$Cu(MeCN)_4PF_6$ 5% mol	Pyridine	rt, 24 h	Product 70% Yield 54%

4	1	1	$Cu(MeCN)_4PF_6$ 5% mol	Pyridine	rt, 24 h, under N_2	Product 100% Yield 75%

Table 7.2 Optimization of the reaction conditions

Miller and co-workers reported on the dramatic effectiveness of solvent sparging with nitrogen to reduce the dissolved oxygen content. It was anticipated that a low concentration of oxygen in pyridine should prevent the oxidation of Cu^{I} to Cu^{II}. Sparging of the pyridine solvent with nitrogen for 10 min before the addition of reactants followed by running the reaction under a nitrogen atmosphere resulted in a complete consumption of the azide substrate and isolation of the 1,4-triazole as the sole product (entry 4, Table 7.2). Exclusive formation of the 1,4-triazole was confirmed by analytical data[283] and comparison with authentic material prepared by CuAAC reaction.[284]

With the aim to understand the efficiency of the Cu^{I} catalysis, the reaction was repeated using different concentrations of $Cu(MeCN)_4PF_6$. While 75% yield was obtained using 5 mol% $Cu(MeCN)_4PF_6$, the yield was reduced to 57% with 1 mol% and only 14% with 0.2 mol% (entries 1, 2 and 3, Table 7.3).



Entry	Acetylide equivalents	Azide equivalents	Copper source and equivalents	Solvent	Time and conditions	Yield
1	1	1	$Cu(MeCN)_4PF_6$ 5 mol%	Pyridine	rt, 24 h, under N_2	Product 100% Yield 75%
2	1	1	$Cu(MeCN)_4PF_6$ 1 mol%	Pyridine	rt, 24 h, under N_2	Product 78% Yield 57%
3	1	1	$Cu(MeCN)_4PF_6$ 0.2 mol %	Pyridine	rt, 24 h, under N_2	Product 21% Yield 14%

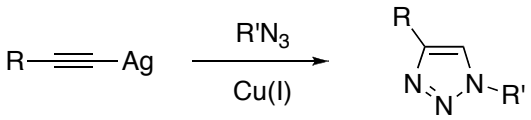
Table 7.3 Study of catalytic activity

[283] Ananthanarayanan, C., Ramakrishnan, V. T., *Indian J. Chem., Sect B*, **1989**, *28*, 228-230.

[284] Donnelly, P. S., Zanatta, S. D., Zammit, S. C., White J. M., Williams, S. J., *Chem. Commun.*, **2008**, *7*, 2459-2461.

We next examined whether ligands which have been shown to be effective for the CuAAC reaction would influence the efficiency of this process. Polydentate ligands are often used to maintain the necessary concentration of catalytically active Cu^{I}, and protect it from side reactions such as disproportionation. In some cases they have also been observed to provide a rate enhancement of the reaction. Ligands were added to be equimolar with the copper source. The electron rich 1,10-phenanthroline[285] and $(BimH)_3$ (*tris*(2-benzimidazolylmethyl)amine)[286,287] ligands (Table 7.4) in conjunction with CuI are reported to accelerate the rate of CuAAC reactions, although in both cases the cycloaddition reactions are sensitive to oxygen. On the other hand the *tris*-triazole ligand TBTA (*tris*(1-benzyl-1*H*-1,2,3-triazol-4-ylmethyl)amine) (Table 7.4) has emerged as a popular auxiliary ligand that enhances reaction yields through stabilizing the copper(I) oxidation state,[288,289] thereby improving the turnover number of the catalytic copper centers, although no rate enhancement is observed.[290]

Use of the copper(I)-stabilizing ligand TBTA did not provide any significant improvements to the yield (entries 1 and 2, Table 7.4). On the other hand the accelerating ligands $(BimH)_3$ and 1,10-phenanthroline gave significant improvements at 1 mol% copper concentrations (entries 3 and 5, Table 7.4), but were unable to drive reactions at 0.2 mol% copper to completion.



Entry	Solvent	Copper source and equivalents	Ligand	Time and conditions	Yield
1	Pyridine	$Cu(MeCN)_4PF_6$ 1 mol%	TBTA	rt, 24 h, under N_2	Product 66% Yield 57%
2	Pyridine	$Cu(MeCN)_4PF_6$ 0.2 mol %	TBTA	rt, 24 h, under N_2	Product 21% Yield 19%
3	Pyridine	$Cu(MeCN)_4PF_6$ 1 mol%	1,10-phenathroline	rt, 24 h, under N_2	Product 100% Yield 78%
4	Pyridine	$Cu(MeCN)_4PF_6$ 0.2 mol %	1,10-phenathroline	rt, 24 h, under N_2	Product 95% Yield 66%
5	Pyridine	$Cu(MeCN)_4PF_6$ 1 mol %	$(BimH)_3$	rt, 24 h, under N_2	Product 100% Yield 69%
6	Pyridine	$Cu(MeCN)_4PF_6$ 0.2 mol %	$(BimH)_3$	rt, 24 h, under N_2	Product 85% Yield 66%

Table 7.4 Role of ligands

[285] Lewis, W. G., Magallon, F. G., Fokin V. V., Finn, M. G., *J. Am. Chem. Soc.*, **2004**, *126*, 9152-9153.

[286] See ref. 266

[287] Rodionov, V. O., Presolski, S. I., Gardinier, S., Lim, Y. H., Finn, M. G., *J. Am. Chem. Soc.*, **2007**, *129*, 12696-12704.

[288] See ref. 284

[289] Chan, T. R., Hilgraf, R., Sharpless K. B., Fokin, V. V., *Org. Lett.*, **2004**, *6*, 2853-2855.

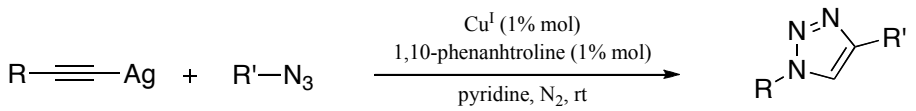
[290] See ref. 266

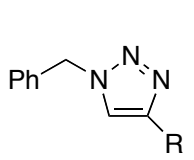
The preferred conditions for the copper(I)-catalyzed silver acetylide azide cycloaddition (CuAgAAC) reaction were using 1 mol% $[Cu(MeCN)_4]PF_6$ and 1,10-phenanthroline, under nitrogen at room temperature in pyridine (entry 3, table 7.4). The scope of the CuAgAAC reaction was examined with the results outlined in Figure 7.2. In general, the 1,4-disubstituted triazoles **3-17** were obtained in good yields (69-96%). Satisfactory yields were obtained with both aryl and alkylacetylenes. The presence of an *ortho* methoxy group did not appreciably affect the yield of **8** and **13**. The synthesis of the *bis*-triazoles **16** and **17** required more forcing conditions to effect formation of two triazole groups, with the silver acetylide increased to 3 equivalents with respect to the azide and 15 mol% $[Cu(MeCN)_4]PF_6$ and 6 mol% 1,10-phenanthroline. A benefit of the CuAgAAC reaction is its ability to utilize silver acetylides of low molecular weight alkynes such as propyne and 1-butyne, which are generally under-represented as substrates for the CuAAC reaction leading to sparse reports of 4-methyl and 4-ethyl-substituted 1,4-triazoles.[291,292,293]

[291] Schramm, H., Saak, W, Hoenke C., Christoffers, J., *Eur. J. Org. Chem.*, **2010**, *9*, 1745-1753.

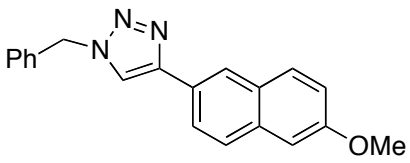
[292] Dörfler, M., Tschammer, N., Hamperl, K., Hübner H., Gmeiner, P., *J. Med. Chem.*, **2008**, *51*, 6829-6838.

[293] Cosyn, L., Palaniappan, K. K., Kim, S. K., Duong, H. T., Gao, Z. G., Jacobson K. A., Van Calenbergh, S., *J. Med. Chem.*, **2006**, *49*, 7373-7383.

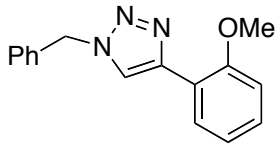




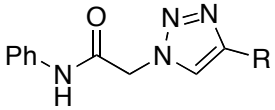
4 R=Ph (70%)
5 R=n-C_4H_9 (73%)
6 R=Et (71%)



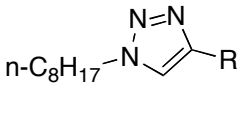
7 (73%)



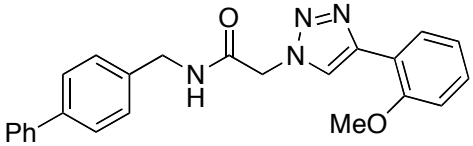
8 (73%)



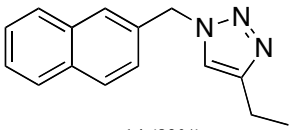
3 R=Ph (81%)
9 R=n-C_4H_9 (88%)
10 R=Et (91%)



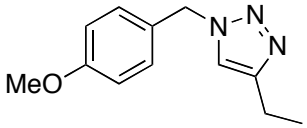
11 R=Ph (96%)
12 R=Et (69%)



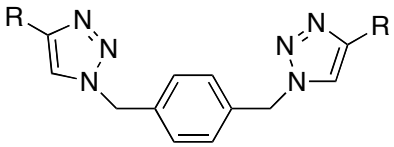
13 (90%)



14 (83%)



15 (84%)



16 R=Ph (69%)[a]
17 R=n-C_4H_9 (75%)[a]

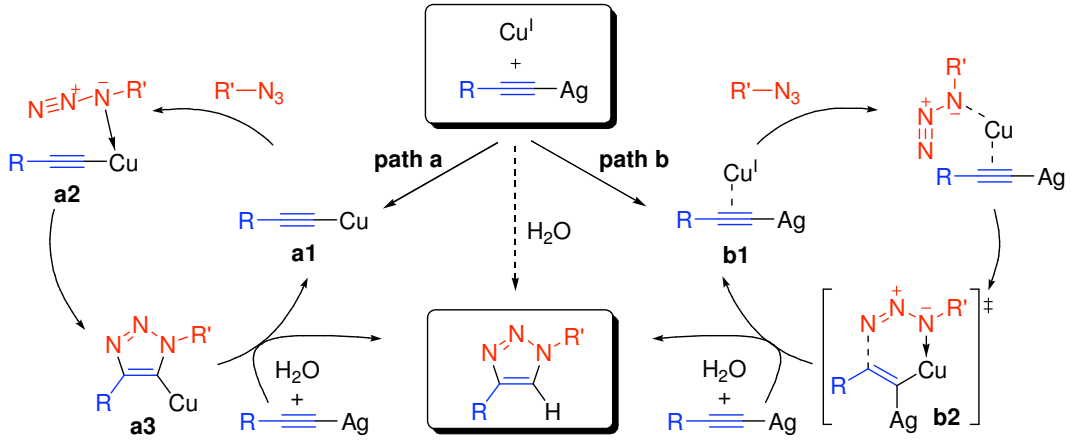
Figure 7.2 Scope of the copper(I)-catalyzed silver acetylide azide cycloaddition. Conditions: silver acetylide (1 eq), azide (1 eq), $[Cu(MeCN)_4]PF_6$ (1 mol%), 1,10-phenanthroline (1 mol%), pyridine, rt, 24 h. [a] Silver acetylide (3 eq), azide (1 eq), $[Cu(MeCN)_4]PF_6$ (5 mol%), 1,10-phenanthroline (2 mol%)

7.3 Mechanistic studies

Two possible mechanistic pathways for the CuAgAAC reaction can be propposed based on the analogous processes for CuAAC reactions of terminal acetylenes and non-terminal acetylenes. One pathway (Scheme 7.6, **path a**) involves the transmetalation of the silver acetylide to a copper acetylide intermediate **a1**,[294] which then undergoes reaction with an azide to afford a 6-membered

[294] Buckley, B. R., Dann, S. E., Harris, D. P., Heaney, H., Stubbs, E. C., *Chem. Commun.*, **2010**, *46*, 2274-2276.

metallocycle **a2**. This metallocycle contracts to form a 5-cuprotriazolide **a3**[295] which is protonated to afford the 1,4-triazole. The second pathway (Scheme 7.6, **path b**) invokes the formation of a π-complex with the alkyne **b1**, which undergoes cycloaddition to **b2**, followed by expulsion of copper(I).



Scheme 7.6 Proposed mechanisms for CuAgAAC reaction

In order to test the potential for silver acetylide to undergo transmetallation, we studied a member of a family of previously described discrete molecular species $[(RCC)_{12}Ag_{14}X]^+$ (X=F, Cl, Br).[296] These consist of a cage compound with 14 silver atom arranged in a rhombic dodecahedron surrounding a central halide atom.[297] The silver atoms are held together by a combination of bridging alkynyl groups and argentophilic Ag···Ag interactions. As molecular species they comprise a useful model for the less tractable polymeric silver acetylides, as they are likely to posses similarities in their bonding patterns. $[(C_3H_7CC)_{12}Ag_{14}Cl]^+$ can be generated by reformation of polymeric silver pentynide and conveniently studied by electrospray ionization mass spectroscopy (ESI-MS).[298] Treatment of $[(C_3H_7CC)_{12}Ag_{14}Cl]^+$ with increasing concentration of $[Cu(MeCN)_4]PF_6$ in acetonitrile/water afforded a series of copper(I) exchanged clusters $[(C_3H_7CC)_{12}Ag_nCu_mCl]^+$ (n=8-13, m=1-6, n+m=14), in which a maximum of 6 silver(I) centres are exchanged to afford $[(C_3H_7CC)_{12}Ag_8Cu_6Cl]^+$ (Figure 7.3). Varying the time of incubation did not alter the degree of incubation, suggesting that the exchange process is rapid. We conclude that partial Cu^I/Ag^I

[295] Nolte, C., Mayer, P., Straub, B. F., *Angew. Chem. Int. Ed.*, **2007**, *46*, 2101-2103.

[296] Rais, D., Mingos, D. M. P., Vilar, R., White, A. J. P., Williams, D. J., *J. Organometallic Chem.*, **2002**, *652*, 87-93.

[297] For other examples of alkynyl Ag(I)/Cu(I) clusters see: Abu-Salah, O. M., Hussain M. S., Schlemper, E. O., *J. Chem. Soc., Chem. Commun.*, **1988**, 1212-1213; Yin, Q., Zhang, L. Y., Shi, L. X., Mao, Z. W., Chen, Z. N., *Inorg. Chem.*, **2004**, *43*, 3484-3491; Rais, D., Yau, J., Mingos, M. P., Vilar, R., White, A. J. P., Williams, D. J., *Angew. Chem. Int. Ed.*, **2001**, *40*, 3464-3467.

[298] Wang, F. Q., Khairallah, G. N., Koutsantonis, G. A., Williams, C. M., Callahan D. L., O'Hair, R. A., *Phys. Chem. Chem. Phys.*, **2009**, *11*, 4132-4135.

exchange is a facile process, and that it may also occur in polymeric silver acetylides or silver acetylides dissolved in pyridine.

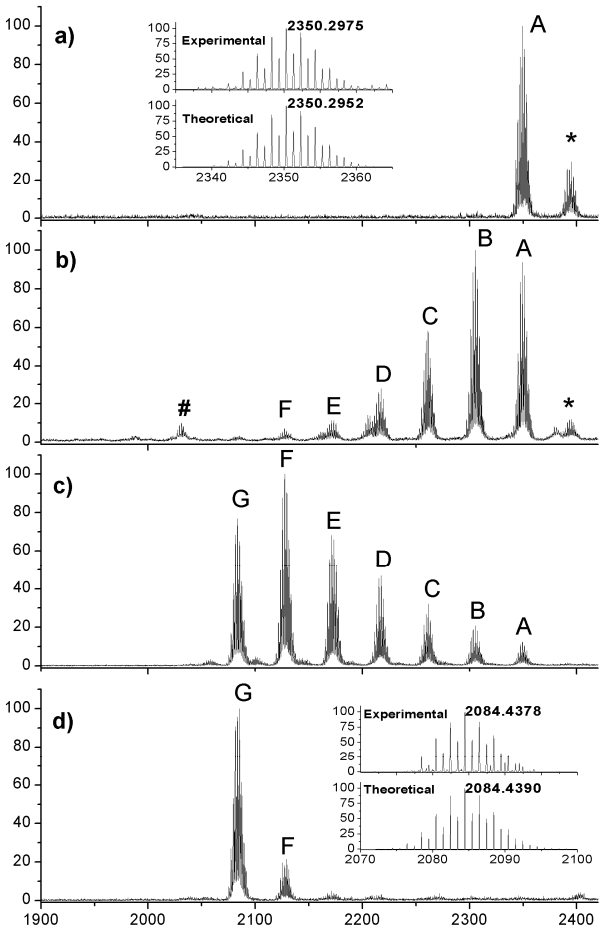


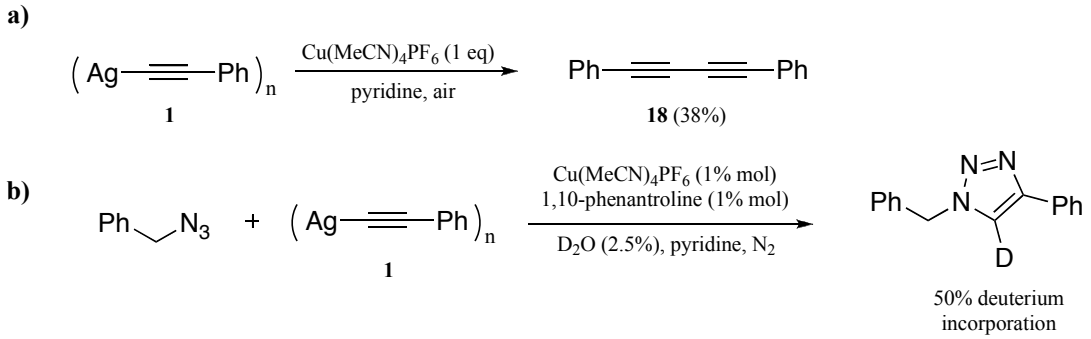
Figure 7.3 ESI-MS spectra of solutions of $AgCCC_3H_7$:$Cu(MeCN)_4PF_6$ in $MeCN/H_2O$. Concentration ratio (a) 1:0; (b) 1:0.2; (c) 1:0.5; (d) 1:1. Peaks observed correspond to the general formula $[Ag_nCu_mCl(CCC_3H_7)_{12}]^+$ (n=8-14, m=0-6, n +m=14). **A**: n=14, m=0; **B**: n=13, m=1; **C**: n=12, m=2; **D**: n=11, m=3; **E**: n=10, m=4; **F**: n=9, m=5; **G**: n=8, m=6. *=$[(AgCCC_3H_7)_{13}Ag]^+$, #= $[(AgCCC_3H_7)_{10}Ag]^+$. The inset in (a) and (d) correspond to experimental and theoretical isotope distributions and mass of peaks **A** and **G**, respectively.

To gain evidence that copper acetylides formed by transmetallation of silver acetylides are reaction intermediates, the Glaser coupling of silver acetylides under Cu(I) catalysis was investigated. (Schem 5.7a) The Glaser reaction is a classical method to prepare 1,3-diynes through the oxidative homocoupling of terminal alkynes.[299,300] Treatment of silver phenylacetylide **1** with stoichiometric $[Cu(MeCN)_4]PF_6$ in pyridine at 80 °C in the presence of O_2 afforded 1,4-diphenylbuta-1,3-diyne **18** in an unoptimized 38% yield. In the absence of copper, only traces of the diyne could be observed. Together with the ESI-MS study of Cu^I/Ag^I exchange in the molecular Mingos cluster we conclude

[299] Glaser, C., *Ber. Dtsch. Chem. Ges.*, **1869**, *2*, 422-424.

[300] Siemsen, P., Livingston, R. C., Diederich, F., *Angew. Chem. Int. Ed.*, **2000**, *39*, 2632-2657.

that transmetallation of silver acetylides to copper acetylides can occur readily, although the precise nature of the silver alkynyls formed when silver acetylides are dissolved in pyridine is not known.[301]



Scheme 7.7 Mechanistic investigations into the CuAgAAC reaction. (a) Copper-promoted Glaser reaction of silver phenylacetylide. (b) C5- deuterium incorporation from D_2O.

We next studied the origin of the proton at C5 of the triazole. Inclusion of D_2O into a standard reaction of silver phenylacetylide **1** and benzyl azide in dry pyridine resulted in 50% incorporation of deuterium as determined by ^1H NMR and mass spectrometric analysis (Scheme 7.7b). As the CuAgAAC reaction we have developed utilizes non-dried pyridine, the C5-triazole proton originates from the solvent.

7.4 Conclusion

This work demonstrates a new copper(I)-catalyzed cycloaddition reaction of silver acetylides with azides. The key to this reaction is the judicious choice of pyridine as solvent, which possesses powerful solvating properties for a wide range of silver acetylides. Mechanistic evidence supports a pathway proceeding by way of transmetalation to copper acetylides, consistent with **path a** (Scheme 7.6). This work represents only the second case for which transmetalation of silver acetylides has been observed, extending the seminal work showing that silver acetylides can enter the palladium catalytic cycle of cross-coupling reactions[302] and demonstrating that silver acetylides can participate in other Cu(I)-catalyzed processes such as the Glaser reaction. The new catalytic CuAgAAC reaction provides a practical synthesis of 1,4-triazoles derived from low molecular weight alkynes by their conversion to readily handled polymeric silver acetylides, and is

[301] For structural studies of pyridyl gold(I) alkynyls see: Kilpin, K. J., Horvath, R., Jameson, G. B., Telfer, S. G., Gordon, K. C., Crowley, J. D., *Organometallics*, **2010**, *29*, 6186-6195.

[302] Dillinger, S., Bertus P., Pale, P., *Org. Lett.*, **2001**, *3*, 1661-1664.

complementary to other recently reported processes using copper(I)-catalyzed cycloaddition of TMS-alkynes[303] and of calcium carbide.[304]

7.5 Experimental

Petroleum spirits refers to a mixed fraction boiling at 40–60 ºC. Thin layer chromatography (t.l.c) was performed with Merck Silica Gel 60 F254, using mixtures of petroleum spirits-ethyl acetate unless otherwise stated. Detection was effected by either charring in a mixture of 5% sulfuric acid-MeOH and/or by visualization in UV light. Melting points were obtained on a Reichert-Jung hot-stage apparatus and are corrected. NMR spectra were obtained on Varian Inova 400 or 500 instruments (Melbourne, Australia). Flash chromatography was performed according to the method of Still *et al*. with Merck Silica Gel 60, using adjusted mixtures of ethyl acetate-petroleum spirits unless otherwise stated. Solvents were evaporated under reduced pressure using a rotary evaporator. High resolution mass spectrometry was performed on a Finnigan hybrid LTQ-FT mass spectrometer (Thermo Electron Corp.). Azides were prepared as described in the literature.[305,306]

7.5.1 General procedures for the preparation of silver acetylides

Silver acetylides were prepared according to the procedures of Davies and Scheiber[307] or Viterisi et al.[308]

(a) A solution of $AgNO_3$ (5 mmol) in a mixture 1:1 of EtOH and 15 M aqueous NH_3 (10 mL) was added to a stirred solution of the alkyne (5 mmol) in EtOH (5 mL) at rt. The resulting precipitate was collected, washed with EtOH, ether and petroleum spirits and dried in the dark.

(b) A solution of $AgNO_3$ (1 eq) in a mixture of CH_3CN and Et_3N (2:1, 10 mL) was added to a stirred solution of the alkyne (1 eq) in CH_3CN (5 mL) at rt. The resulting precipitate was collected, washed with CH_3CN and dried in the dark.

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[303] Cuevas, F., Oliva, A. I., Pericàs, M. A., *Synlett*, **2010**, *12*, 1873-1877.

[304] Gonda, Z., Lorincz, K., Novák, Z., *Tetrahedron Lett.*, **2010**, *51*, 6275-6277.

[305] van der Peet, P., Gannon, C. T., Walker, I., Dinev, Z., Angelin, M., Tam, S., Ralton, J. E., McConville M. J., Williams, S. J., *ChemBioChem*, **2006**, *7*, 1384-1391.

[306] Campbell-Verduyn, L. S., Mirfeizi, L., Dierckx, R. A., Elsinga, P. H., Feringa, B. L., *Chem. Commun.*, **2009**, *6*, 2139-2141.

[307] See ref. 306

[308] Viterisi, A., Orsini, A., Weibel, J.-M., Pale, P., *Tetrahedron Lett.*, **2006**, *47*, 2779-2781.

(c) TMS-alkyne (1 eq) was added to a solution of silver nitrate (1 eq) in ethanol (10 mL) at rt. The resulting precipitate was filtered, washed with ethanol and dried in the dark.

Synthesis of chloroacetanilide

Chloroacetyl chloride (1.2 equiv) was added to a stirred solution of aniline (1 equiv) in dichloromethane (15-20 mL) in the presence of pyridine (2 equiv) at 0 °C over 30 min. The reaction was stirred for a further 60 min at room temperature. Water (1 mL) was added and the mixture was stirred vigorously for 5 min, then was sequentially washed with water, aq. 1 M HCl and sat. aq. $NaHCO_3$. The organic phase was dried ($MgSO_4$) and the solvent evaporated under reduced pressure to give the crude bromide (4.44 g), which was used directly in the next step.

Synthesis of azidoacetanilide (2)

A mixture of chloroacetanilide (1 equiv) and NaN_3 (1.5 equiv) in DMSO (10 ml) was stirred for 2.5 h at rt. The reaction mixture was diluted with ice/water (20 mL). The mixture was extracted with dichloromethane (3 × 10 mL) and the combined organic layer washed with water (2 × 20 mL) and brine (20 ml). the organic phase was dried ($MgSO_4$), filtered and the solvent removed under reduced pressure and the residue recrystallized to afford the azide as brown needles (2.58 g, 68% over two steps); m.p. 83-84 °C (EtOAc/pet. Spirits; lit.[ref] 83 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.12 (s, 2H, CH_2), 7.13-7.54 (m, 5H, Ph), 8.07 (bs, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 54.04 (CH_2), 121.79, 126.13, 130.19, 137.83 (Ph), 165.66 (C=O); IR ν (cm^{-1}) 1672 str. (C=O), 2102 str. (N_3).

7.5.2 General procedure for Cu(I)-catalyzed silver acetylide and azide cycloaddition

Silver acetylide (1 eq.) was added to a solution of azide (1 eq.) in pyridine (4 mL). The reaction mixture was sparged with N_2 for 10 min, then $Cu(MeCN)_4PF_6$ (1% mol) and 1,10-phenanthroline (1% mol) were added. The reaction mixture was stirred for 24 h at room temperature in the dark under N_2, then was diluted with dichloromethane (15 mL) and aq. 15 M NH_3 solution (15 mL). The mixture was filtered through Celite and the filtrate was sequentially washed with aq. 15 M NH_3 aq (15 mL) and water (2 × 15 mL). The organic phase was dried ($MgSO_4$) and the solvent was evaporated under reduced pressure to afford the product.

***N*-Phenyl-2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)acetamide (3)**

White needles from DMSO/H_2O, mp 244.5-246.5 °C (lit.[309] 243-245 °C). ^{1}H NMR (500 MHz, d_6-DMSO): δ=5.38 (2H, s, CH_2), 7.09 (1H, t, *J* 7.5 Hz), 7.33 (3H, t, *J* 8.0 Hz), 7.45 (2H, t, *J* 8.0 Hz), 7.59 (2H, d, *J* 7.5 Hz), 7.88 (2H, d, *J* 8.0 Hz), 8.59 (1H, s, triazole-H), 10.49 (1H, s, NH); ^{13}C NMR (100.5 MHz, d_6-DMSO): δ=52.4 (1C, CH_2), 119.2, 123.0, 123.8, 125.1, 127.8, 128.9, 130.2, 138.4, 146.2 (14C, Ar), 164.1 (1C, C=O).

1-Benzyl-4-phenyl-1*H*-1,2,3-triazole (4)

White crystals from DMSO/H_2O, mp 120-122.5 °C (lit.[310] 122-124 °C). ^{1}H NMR (500 MHz, d_6-DMSO): δ=5.64 (2H, s, CH2), 7.37 (8H, m, Ar-H), 7.84 (2H, dd, *J* 1.5, 7.5 Hz, Ar-H), 8.63 (1H, s, triazole-H); ^{13}C NMR (100.5 MHz, d_6-DMSO): δ=53.0 (1C, CH_2), 121.5, 125.1, 127.9, 128.1, 128.4, 128.8, 128.9, 130.6, 136.0, 146.6 (14C, Ar).

1-Benzyl-4-butyl-1*H*-1,2,3-triazole (5)

White crystals from DMSO/H_2O, mp 60-62 °C, (lit.[311] 62-63 °C). ^{1}H NMR (500 MHz, d_6-DMSO): δ=0.87 (3H, t, *J* 7.5 Hz, CH_3) 1.30 (2H, m, CH_2), 1.55 (2H, m, CH_2), 2.59 (2H, t, *J* 7.5 Hz, CH_2C=C), 5.52 (2H, s, CH_2Ph), 7.26-7.38 (5H, m, Ar-H), 7.87 (1H, s, triazole-H); ^{13}C NMR (100.5 MHz, d_6-DMSO): δ=13.7, 21.7, 24.6, 31.1, 52.6 (5C, 4 × CH_2,1 × CH_3), 121.9, 127.8, 128.0, 128.7, 136.3, 147.2 (8C, Ar).

4-Ethyl-1-benzyl-1*H*-1,2,3-triazole (6)

White crystals, mp 53-55°C. 1H NMR (500 MHz, d_6-DMSO): δ=1.17 (3H, t, *J* 7.5 Hz, CH_3), 2.62 (2H, q, *J* 7.5 Hz, C**H**$_2$$CH_3$), 5.55 (2H, s, C**H**$_2$Ph), 7.32 (5H, m, Ph), 7.88 (1H, s, triazole-H); ^{13}C NMR (100.5 MHz, d_6-DMSO): δ=13.6, 18.5, 52.7 (3C, 2 × CH_2, 1 × CH_3), 121.5, 127.8, 127.9, 128.7, 136.3, 148.7 (8C, Ar); HR-ESIMS: *m/z*=188.11793 [M+H]+, calcd. 188.11822 for $C_{11}H_{14}N_3$.

1-Benzyl-4-(2-methoxynaphthalen-7-yl)-1*H*-1,2,3-triazole (7)

Colourless crystals, mp 215.5-218 °C. 1H NMR (500 MHz, d_6-DMSO): δ=3.87 (3H, s, CH_3), 5.66 (2H, s, CH_2), 7.17 (1H, dd, *J* 2.5, 9.0 Hz, Ar-H), 7.32-7.41 (6H, m, Ar-H), 7.85-7.87 (2H, m, Ar-H), 7.93 (1H, dd, *J* 2.5, 9.0 Hz, Ar-H), 8.31 (1H, d, *J* 2.5 Hz, Ar-H), 8.67 (1H, s, triazole-H); ^{13}C NMR (100.5 MHz, d_6-DMSO): δ=53.0, 55.1 (CH_2, CH_3), 106.1, 118.8, 121.2, 123.3, 124.0, 125.8, 127.1,

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[309] See ref. 306

[310] Chtchigrovsky, M., A. Primo, P. Gonzalez, K. Molvinger, M. Robitzer, F. Quignard and F. Taran, *Angew. Chem. Int. Ed.*, 2009, **48**, 5916-5920.

[311] Cuevas, F., Oliva, A. I., Pericàs, M. A., *Synlett*, **2010**, *12*, 1873-1877.

127.8, 128.0, 128.4, 128.6, 129.3, 133.8, 135.8, 146.7, 157.4 (Ar); HR-ESIMS: *m/z*=316.14438 [M +H]+, calcd. 316.14444 for $C_{20}H_{17}N_3O$.

1-Benzyl-4-(2-methoxyphenyl)-1*H*-1,2,3-triazole (8)

White crystals from DMSO/H_2O, mp 167.5-169.5 °C. ^{1}H NMR (500 MHz, d_6-DMSO): δ=3.88 (3H, s, CH_3), 5.66 (2H, s, CH_2), 7.04 (1H, t, *J* 7.5 Hz, Ar), 7.15 (1H, dd, *J* 1.0, 8.0 Hz, Ar), 7.30-7.38 (6H, m, Ar), 8.15 (1H, dd, *J* 2.0, 8.0 Hz, Ar), 8.46 (1H, s, triazole-H); ^{13}C NMR (100.5 MHz, d_6-DMSO): δ=52.7 (1C, CH_2), 55.5 (1C, OMe), 111.5, 119.0, 120.6, 124.1, 126.5, 127.8, 128.1, 128.8, 129.0, 136.3, 142.0, 155.3 (14C, Ar); HR-ESIMS: *m/z*=266.12872 [M+H]+, calcd. 266.12879 for $C_{16}H_{16}N_3O$.

2-(4-Butyl-1*H*-1,2,3-triazol-1-yl)-*N*-phenylacetamide (9)

White crystals from DMSO/H_2O, mp 167.5-169.5 °C. ^{1}H NMR (500 MHz, d_6-DMSO): δ=0.81 (3H, t, *J* 7.5 Hz, CH_3), 1.23 (2H, m, CH_2), 1.51 (2H, m, CH_2), 2.58 (2H, t, *J* 7.5 Hz, CH_2), 5.18 (2H, s, CH_2CO), 7.07 (1H, t, *J* 7.5 Hz, Ar-H), 7.29 (2H, t, *J* 7.5 Hz, Ar-H), 7.44 (2H, d, *J* 8.5 Hz, Ar-H), 7.76 (1H, s, triazole-H), 10.41 (1H, s, NH); ^{13}C NMR (100.5 MHz, d_6-DMSO): δ=14.1, 22.1, 25.6, 31.6, 52.5 (5C, 4 × CH_2, 1 × CH_3), 119.6, 123.9, 124.2, 129.3, 138.8, 147.1 (8C, Ar), 164.8 (1C, C=O); HR-ESIMS: *m/z*=259.15527 [M+H]+, calcd. 259.15534 for $C_{14}H_{19}N_4O$.

2-(4-Ethyl-1*H*-1,2,3-triazol-1-yl)-*N*-phenylacetamide (10)

White crystals, mp 158-160 °C. ^{1}H NMR (500 MHz, d_6-DMSO): δ=1.21 (3H, t, *J* 7.5 Hz, CH_3), 2.65 (2H, q, *J* 7.5 Hz, C**H**$_2$CH$_3$), 5.26 (2H, s, CH_2CO), 7.08 (1H, t, *J* 7.5 Hz, Ar), 7.33 (2H, t, *J* 7.5 Hz, Ar), 7.58 (2H, d, *J* 7.5 Hz, Ar), 7.86 (1H, s, triazole-H), 10.4 (1H, s, NH); ^{13}C NMR (100.5 MHz, d_6-DMSO): δ=13.7 (CH_3), 18.4, 52.1 (CH_2), 119.2, 123.0, 123.7, 128.9, 138.4, 148.1 (Ar), 164.3 (C=O); HR-ESIMS: *m/z*=231.12399 [M+H]+, calcd. 231.12404 for $C_{12}H_{15}N_4O$.

4-Phenyl-1-octyl-1*H*-1,2,3-triazole (11)

White crystals, mp 76-78 °C (lit.[312] 74-75 °C). ^{1}H NMR (500 MHz, d_6-DMSO): δ=0.83 (3H, t, *J* 7 Hz, CH3), 1.22-1.28 (10H, m, 5 × CH_2), 1.84 (2H, t, *J* 7 Hz, C**H**$_2$CH$_2$N), 4.37 (2H, t, *J* 7 Hz, C**H**$_2$-N), 7.31 (1H, t, *J* 8 Hz, Ar), 7.43 (2H, t, *J* 8 Hz, Ar), 7.83 (2H, d, J 8.5 Hz, Ar), 8.57 (1H, s, triazole-H); ^{13}C NMR (100.5 MHz, d_6-DMSO): δ=13.9 (CH_3), 22.0, 25.8, 28.3, 28.5, 29.6, 31.1, 49.5 (8C, 8 × CH_2), 121.2, 125.1, 127.7, 128.8, 130.9, 146.2 (8 C, Ar).

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[312] Campbell-Verduyn, L. S., Mirfeizi, L., Dierckx, R. A., Elsinga, P. H., Feringa, B. L., *Chem. Commun.*, **2009**, *6*, 2139-2141.

4-Ethyl-1-octyl-1*H*-1,2,3-triazole (12)

Yellow solid, mp 37.5-39 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ=0.83 (3H, t, *J* 7.0 Hz, $(CH_2)_7C\mathbf{H}_3$), 1.19-1.26 (13H, m, 5 × CH_2, 1 × CH_3), 2.70 (2H, q, Ar$C\mathbf{H}_2CH_3$), 4.25 (2H, t, *J* 7.5 Hz, CH_2N), 7.23 (1H, s, triazole-H); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ=13.6, 13.9, 18.9, 22.5, 26.4, 28.3, 28.9, 30.2, 31.6, 50.1 (10C, 10 × CH_2), 119.9, 149.6 (2C, Ar); HR-ESIMS: *m/z*=210.19646 [M+H]+, calcd. 210.19647 for $C_{12}H_{23}N_3$.

2-(4-(2-Methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)-(*N*-(4-biphenyl))acetamide (13)

Colourless crystals, mp 182-183 °C. ^{1}H NMR (500 MHz, d_6-DMSO): δ=3.91 (3H, s, CH_3), 5.24 (2H, s, CH_2), 7.04 (1H, t, *J* 8.0 Hz, Ar-H), 7.05 (1H, d, *J* 8.0 Hz, Ar-H), 7.31-7.38 (4H, m, Ar-H), 7.39-7.47 (2H, m, Ar-H), 7.62-7.65 (4H, m, Ar-H), 8.16 (1H, dd, *J* 1.5, 7.5 Hz, Ar-H), 8.38 (1H, s, triazole-H); ^{13}C NMR (100.5 MHz, d_6-DMSO): δ=42.1, 51.6, 55.5 (3C, 2 × CH_2, 1 × CH_3), 111.5, 119.1, 120.6, 125.5, 126.5, 126.6, 126.7, 127.4, 128.0, 128.8, 128.9, 138.0, 139.0, 139.9, 141.5, 155.3 (20C, Ar), 165.6 (1C, C=O); HR-ESIMS: *m/z*=399.18148 [M+H]+, calcd. 399.18155 for $C_{24}H_{23}N_4O_2$.

4-Ethyl-1-((naphthalen-6-yl)methyl)-1*H*-1,2,3-triazole (14)

White solid, mp 73.5-76 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ=1.23 (3H, t, *J* 7.5 Hz, $(CH_2)_7C\mathbf{H}_3$), 2.72 (2H, q, $C\mathbf{H}_2CH_3$), 5.62 (2H, s, CH_2N), 7.21 (1H, s, triazole-H), 7.34 (1H, dd, *J* 1.5, 8.5 Hz, H7), 7.48-7.51 (2H, m, Ar), 7.18 (1H, d, *J* 1.5 Hz, H5), 7.80-7.83 (3H, m, Ar); ^{13}C NMR (100.5 MHz, CDCl3): δ=13.5, 19.0 (2C, CH_2CH_3), 54.1 (1C, CH_2N), 120.1, 125.3, 126.5, 126.6, 127.2, 127.7, 127.8, 129.0, 132.3, 133.0, 133.1, 150.2 (12C, Ar); HR-ESIMS: *m/z*=238.13385 [M+H]+, calcd. 238.13387 for $C_{15}H_{16}N_3$.

1-(4-Methoxybenzyl)-4-ethyl-1*H*-1,2,3-triazole (15)

White solid, mp 65.1-66.3 ºC. ^{1}H NMR (500 MHz, $CDCl_3$): δ=1.23 (3H, t, *J* 7.5 Hz, $CH_2C\mathbf{H}_3$), 2.72 (2H, q, $C\mathbf{H}_2CH_3$), 3.80 (3H, s, OMe), 5.41 (2H, s, CH_2N), 6.88 (2H, d, *J* 9.0 Hz, Ar), 7.15 (1H, s, triazole-H), 7.21 (2H, d, *J* 9.0 Hz, Ar); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ=13.6, 19.0 (2C, CH_2CH_3), 53.6, 55.3 (2C, CH_2N, OMe), 114.4, 119.8, 126.9, 129.6, 148.8, 159.8 (8C, Ar); HR-ESIMS: *m/z*=218.12877 [M+H]+, calcd. 218.12879 for $C_{12}H_{16}N_3O$.

1,4-*bis*((4-Phenyl-1*H*-1,2,3-triazol-1-yl)methyl)benzene (16)

White crystals from EtOAc/pet. spirit, mp 207-209 °C. ^{1}H NMR (400 MHz, d_6-DMSO): δ=5.64 (2H, s, CH_2), 7.37 (4H, s, C_6H_4), 7.34-7.30 (2H, m, Ph), 7.44-7.41 (4H, m, Ph), 7.84-7.81 (4H, m, Ph), 8.62 (2H, s, triazole-H); ^{13}C NMR (100 MHz, d_6-DMSO): δ=52.5 (CH_2), 99.7, 121.3, 125.0, 127.7, 128.2, 128.6, 130.5, 135.8, 146.5 (Ar); HR-ESIMS: *m/z*=393.18207 [M+H]+, calcd. 393.18222 for $C_{24}H_{21}N_6$.

1,4-*bis*((4-Butyl-1*H*-1,2,3-triazol-1-yl)methyl)benzene (17)

White crystals, mp 168-171 °C. ^{1}H NMR (500 MHz, d_6-DMSO): δ=0.86 (6H, t, *J* 7.5 Hz, 2 × CH_3), 1.28 (4H, m, C**H**$_2$$CH_2$), 1.53 (4H, m, CH_2CH_3), 2.57 (4H, t, *J* 7.5 Hz, CH_2C=C), 5.51 (2H, s, C**H**$_2$Ar), 7.19 (1H, s, triazole-H), 7.23 (4H, s, C_6H_4); ^{13}C NMR (100.5 MHz, d_6-DMSO): δ=13.7, 21.7, 24.6, 31.1 (8C, 6 × CH_2, 2 × CH_3), 52.3 (2C, CH_2Ph), 121.9, 128.2, 136.1, 147.3 (10C, Ar); HR-ESIMS: *m/z*=353.24484 [M+H]+, calcd. 352.24482 for $C_{20}H_{29}N_6$.

Preparation of 1,4-diphenylbuta-1,3-diyne (18)

[Cu(MeCN)$_4$]PF_6 (385 mg, 1.03 mmol) was added to a solution of silver phenylacetylide (214 mg, 1.02 mmol) in pyridine (4.00 mL). The reaction mixture was stirred at 80 °C exposed to air for 18 h. Conc. aq. ammonia (20.0 ml) was added to the mixture followed by stirring for 5 min. The reaction mixture was filtered through filter aid. The aqueous phase was extracted with dichloromethane (3 × 15 ml), washed with conc. aq. ammonia and water, dried ($MgSO_4$) and solvent removed under reduced pressure. Recrystallization (EtOAc/pet. spirit) afforded 1,4-diphenylbuta-1,3-diyne (102 mg, 38%), mp 86-88 °C, lit.[313] 86-87 °C. ^{1}H NMR (500 MHz, d_6-DMSO) δ 7.33-7.38 (6H, m, Ph), 7.52-7.55 (4H, m, Ph); ^{13}C NMR (125 MHz, d_6-DMSO) δ 73.9, 81.6 (2C, C≡C), 121.8, 128.4, 129.2, 132.5 (6C, Ph).

[313] Wex, B., Kaafarani, B. R., Oliver, A. G., Krause Bauer, J. A., Neckers, D. C., *J. Org. Chem.*, **2003**, *68*, 8258-8260.

8. Abbreviations

AFO Algar-Flynn-Oyamada
BDE Bond dissociation enthalpy
CuAAC Copper-catalyzed azide-alkyne cycloaddition
CuAgAAC Copper-catalyzed silver acetylides-azide cycloaddition
DBU 1,8-diazabicyclo[5.4.0]undec-7-ene
DFT Density-functional-theory
DMC Dimethyl carbonate
DMF Dimethylformamide
DMSO Dimethyl sulfoxide
DNA Deoxyribonucleic acid
ERG Electron-releasing group
ESI-MS electrospray ionization mass spectrometry
EWG Electron-withdrawing group
HAT Hydrogen atom transfer
HOMO Highest occupied molecular orbital
IBA 2-iodobenzoic acid
IBX 2-iodoxybenzoic acid
IP Ionization potential
IR Infrared
LDL Low density lipoprotein
LUMO Lowest unoccupied molecular orbital
NADH Nicotinamide adenine dinucleotide
NBO Natural bond orbital
NBS *N*-bromosucinimide
NHC *N*-heterocyclic carbenes
NMM *N*-methylmorpholine
NMO *N*-methylmorpholine-*N*-oxide
NMR Nuclear magnetic resonance
RNS Reactive nitrogen species
ROS Reactive oxygen species
SAR Structure-activity relationship
SET Single electron tranfer
TBAB Tetrabuthylamonium bromide
TBATB Tetrabuthylamonium tribromide
TBTA *tris*-(benzyltriazolylmethyl)amine
THF Tetrahydrofuran