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Ciclo XXIX

Development of new synthetic and analytical methods for the investigation of specific polyphenolic human metabolites

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GENERAL INTRODUCTION

Science is a privileged interpreter of Nature. Nature is always offering continuing transformation of the physical world and the scientific interpretation of these transformations in the wide domain of polyphenol natural products along with the desire of facing new horizons propelled the research during my three-years-long PhD studies. I tried to face this domain from a double perspective, the nutrition world and the organic synthesis world, as two curious observers are looking toward the same horizon. The intense transformation of polyphenol molecules in human body brings about many types of metabolites, whose structural complexity have stimulated modern organic chemistry toward the creation of new strategies and methods to access them by synthesis. In particular, the invention and exploitation of new methodologies to selectively drive chemical transformations of specific substrates is of paramount interest in modern synthesis.

On these grounds, the development of chemical strategies including the asymmetric vinylogous Mukaiyama aldol reaction, the intra- and intermolecular biaryl coupling, and conjugation reactions of phenol substrates allowed access to specific polyphenol metabolites, in particular γ-valerolactones and urolithins. The synthetic obtainment of these targets gave us the possibility to study their molecular structure and investigate their metabolic profiles, thus offering new "interpretations" of Nature.

INTRODUZIONE GENERALE

La scienza è una pregevole interprete della Natura. Quello che la Natura continua a offrire è una perpetua trasformazione. L'interpretazione di questa trasformazione all'interno del popolare mondo dei polifenoli col desiderio di affrontarne nuovi orizzonti ha generato il lavoro di ricerca che ho seguito durante i tre anni del mio dottorato, in cui due osservatori hanno cercato lo stesso orizzonte: il mondo della nutrizione e quello della chimica organica.

L'intensa trasformazione dei polifenoli nel corpo umano genera diversi tipi di metaboliti, le cui strutture hanno fornito l'occasione alle moderne metodologie chimiche di esprimere la loro potenzialità sintetica.

La chimica organica è appunto lo studio delle trasformazioni subite e innescate dalle entità molecolari. Come sfruttare e governare queste trasformazioni chimiche in funzione della natura del substrato e per l'ottenimento di determinati motivi strutturali è lo scopo dell'indagine metodologica. Su questa linea di ricerca l'indispensabile studio di nuove strategie, fra cui la reazione asimmetrica viniloga di Mukaiyama, gli accoppiamenti biarilici intra- ed intermolecolari e le reazioni di coniugazione di substrati fenolici hanno permesso l'accesso a specifici metaboliti polifenolici, in particolare i metaboliti valerolattonici e le urolitine. L'ottenimento sintetico di questo tipo di strutture ha fornito quindi la possibilità di analizzarne rigorosamente la struttura molecolare e di indagarne il profilo metabolico aprendo la strada a nuove "interpretazioni".

CHAPTER 1

Chiral valerolactone metabolites: when challenging synthetic targets meet relevant food science

1.1. Classifications of polyphenols: flavonoid and *non*-flavonoid compounds

The plant kingdom produces an overwhelming array of structurally diverse secondary metabolites, among which flavonoids and related phenolic and (poly)phenolic compounds constitute one of the most numerous and widely distributed group of natural products. While not essential for the successful growth and development of most plants, phenolic compounds can occur in high concentrations in some species and are referred to as secondary metabolites. *In planta* (poly)phenols have various functions, including protecting plants from herbivores and microbial infections, attractants for pollinators and seed dispersing animals, allelopathic agents, UV protectants, and signal molecules in the formation of nitrogen-fixing root nodules.[1] Fruits, vegetables, whole grains and other types of foods and beverages such as tea, chocolate and wine are rich sources of polyphenols.

To date, more than 8000 structures have been classified as members of the phytochemical class of (poly)phenols and among them over 4000 flavonoids have been identified.[2] Although polyphenols are chemically characterized as compounds with phenolic structural features, this group of natural products is highly diverse and contains several sub-groups of phenolic compounds.

The diversity and wide distribution of (poly)phenols in plants have led to different ways of categorizing these naturally occurring compounds.

Polyphenols have been classified by their source of origin, biological function or according to their chemical structures. Generally, the chemical structure determines their classification in two bigger classes, namely flavonoids and non-flavonoids.

As shown in Figure 1.1, flavonoids are polyphenolic compounds comprising 15 carbons with two aromatic rings (A ring ang B ring) connected by a three-carbon bridge (C6-C3-C6 compounds). Due to the hydroxylation pattern and variations in the chromane ring (ring C), flavonoids can be further divided into different sub-groups such as flavones, flavonols, flavan-3-ols, isoflavones, flavanones, and anthocyanidins. Other flavonoids that are minor dietary components are chalcones, dihydrochalcones, dihydroflavonols, flavan-3,4-diols, coumarins, and aurones. The basic flavonoid skeleton can have numerous substituents: hydroxyl groups are usually present at the 4', 5 and 7 position. Furthermore, the majority of flavonoids occur naturally as glycosides rather than aglycones.

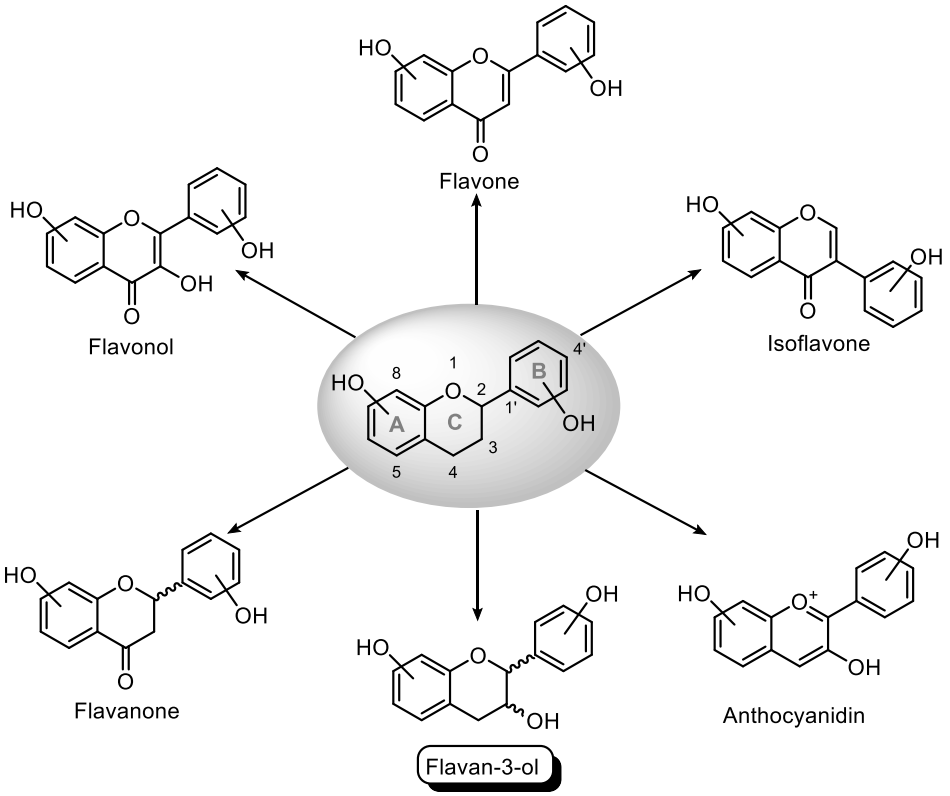


Figure 1.1. *Structure of the flavonoid skeleton and different flavonoid compounds*

Phenolic acids are non-flavonoid polyphenolic compounds which can be further divided into two main types, benzoic acid and cinnamic acid derivatives based on C1–C6 and C3–C6 backbones (Figure 1.2). Gallic acid is the most common phenolic acid, and occurs widely as complex sugar esters in gallotannins. Gallic acid is the base unit of gallotannins whereas gallic acid and hexahydroxydiphenic acid are both subunits of ellagitannins.

The C6–C3 hydroxycinnamates occur mainly as conjugates, for example, with tartaric acid or quinic acid, and collectively are referred to as chlorogenic acids.

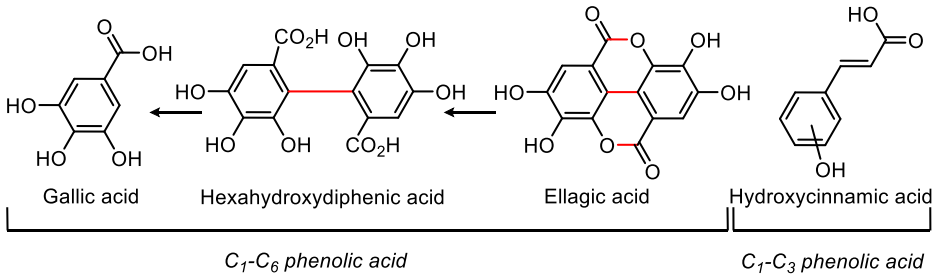


Figure 1.2. *Base-units of non-flavonoid phenolic compounds.*

1.1.1. A focus on Flavan-3-ol subclass

Flavan-3-ols are the most complex subclass of flavonoids, ranging from simple monomers to the oligomeric and polymeric proanthocyanidins, which are also known as nonhydrolyzable condensed tannins.[3] This class of compounds is characterized by the absence of the double bond between C2 and C3, and the absence of the C4 carbonyl in ring C. This characteristic and the hydroxylation on C3 allow flavanols to have two stereogenic centers at C2 and C3 of the monomeric flavan-3-ol (Figure 1.3), thereby producing four possible stereoisomers for each level of B-ring hydroxylation. The most common constitutional isomer features two hydroxyl groups in C3′ and C4′ positions of ring B, namely "catechin", the diastereoisomer with a C3′-C4′ *trans* configuration and "epicatechin", with the corresponding *cis* configuration. Each of these two diastereoisomers exist in two enantiomeric forms: *i.e.*, (+)-catechin, (−)-catechin, (+)-epicatechin and (−)-epicatechin.[3] (+)-Catechin and (−)-epicatechin are widespread in nature, while others such as (−)-epiafzelechin with only one hydroxyl group in C4′ position have a more limited distribution (Figure 1.3).[4] Catechin and epicatechin can form polymers, which are often referred to as proanthocyanidins because an acid-catalyzed cleavage of the polymeric chains produces anthocyanidins.[4] Oligomeric and polymeric

proanthocyanidins have an additional chiral center at C4. Depending on the interflavanic linkages, proanthocyanidins of type A or type B can be distinguished. Type B proanthocyanidins are formed by oxidative coupling between the C4 of the upper monomer and the C6 or C8 of the adjacent lower or extended unit to create oligomers or polymers. As summarized in Table 1.1, the B-type proantocyanidin dimers can be hetero-dimers between two units of epicatechin and catechin (procyanidin B1, B4, B7, B8) or homo-dimers constitued by the same unit of epicatechin or catechin (procyanidin B2, B3, B4, B5). Type A proanthocyanidins have an additional C2→*O*–C7 or C2→*O*–C5 ether linkage between C2 in the ring C of one monomer and C7 or C5 in the A-ring of the other monomer producing fused pentacyclic compounds (Figure 1.3).[5] Most commonly, type A proanthocyanidins are procyanidin A1 and A2, respectively a hetero-dimer and homodimer (Table 1.1). Proanthocyanidins are traditionally considered condensed tannins, because they are not susceptible to cleavage by hydrolysis.

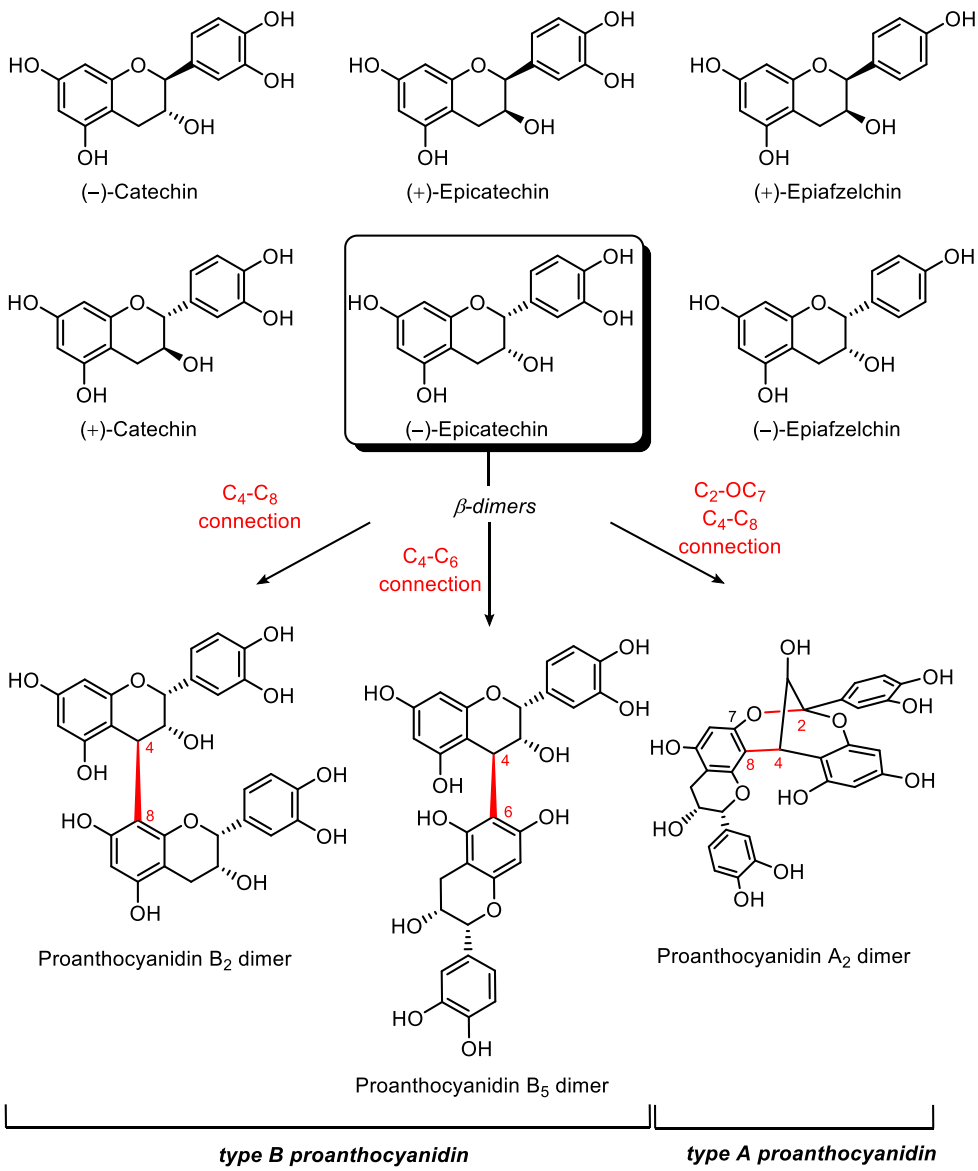


Figure 1.3. *Panel of the most common flavan-3-ol monomers and their proanthocyanidin dimers.*

Proanthocyanidins can occur as polymers of up to 50 units. Proanthocyanidins that consist exclusively of (epi)catechin units are called procyanidins, and are the most abundant type of proanthocyanidins in plants. Among this polymeric compounds are included the less common proanthocyanidins containing

(epi)afzelechin or (epi)gallocatechin subunit, named propelargonidins and prodelphinidins, respectively.[4,6] However, most food proanthocyanidins are hetero-oligomers with monomeric units that vary in the number and pattern of hydroxylation. Not all proanthocyanidins are made up exclusively of flavan-3-ol subunits. For instance, linkages with anthocyanins and flavonols may occur.[7,8]

Table 1.1. *Main proanthocyanidin dimers between (−)-epicatechin (EC) and (+) catechins (C).*

	proanthocyanidin			
Interflavonoid bond	**Hetero-dimer**		**Homo-dimer**	
	EC - C	**C - C**	**EC - EC**	**C - C**
B-Type (C_4→C_8)	Procyanidin B1 EC (4β→8) C	Procyanidin B4 C (4α→8) EC	Procyanidin B2 EC (4β→8) EC	Procyanidin B3 C (4α→8) C
B-Type (C_4→C_6)	Procyanidin B7 EC (4β→8) C	Procyanidin B8 C (4α→8) EC	Procyanidin B5 EC (4β→8) EC	Procyanidin B6 C (4α→8) C
A-Type($C_{4\beta}$→C_6, $C_{2\beta}$→O-C_7)	Procyanidin A1 EC - C	–	Procyanidin A2 EC - EC	–

Dark chocolate is a rich source of proanthocyanidins derived from the roasted seeds of cocoa (*Theobroma cacao*) and sensible amounts can also be found in red wine, skins of grapes and berries, such as cranberries (*Vaccinium macrocarpum*). Green tea (*Camellia sinensis*) represents an important source of flavan-3-ol monomers with the main components being (-)-epigallocatechin, (+)-gallocatechin, (−)-epigallocatechin-3-*O*-gallate, and (-)-epicatechin-3-*O*-gallate. The levels of these flavan-3-ols decline during fermentation of the green leaves to produce black tea, principally as a result of the action of polyphenol oxidase, and there is a concomitant accumulation of theaflavins and thearubigins.[9] Theaflavin, theaflavin-3-*O*-gallate, theaflavin-3'-*O*-gallate, and

theaflavin-3,3'-*O*-digallate are dimer-like structures that contribute to the quality of the black tea beverage. The brownish, water-soluble, high-molecular-weight thearubigins are the major phenolic fraction in black tea.

1.2. Polyphenols fate in human

The absorption, distribution, metabolism, and excretion of flavonoids and related phenolics after dietary intake have been the focus of increasing research efforts in recent years. The interest oriented to (poly)phenol metabolism, also attributed to their recently demonstrated health-promoting benefits,[10] led the scientific community to elucidate the main metabolic pathway/s which contribute to (poly)phenol biotransformation. In order to exert a health benefit, an active ingredient compound needs to withstand food processing, to be released from the food matrix after ingestion, being bioaccessible in the gastrointestinal tract, undergo metabolism and finally reach the target tissue of action.[10] It means that the bioactive compound needs to be bioavailable before it can have an effect. As all nutrients and non-nutrients, once introduced in the organism through the diet, (poly)phenols are subjected to an intense metabolism, able to convert the native compounds into the corresponding conjugated derivatives, as well as into smaller and deeply modified molecules, which in turn could be further conjugated. Although great strides have been made in the last decades, some steps of the (poly)phenol metabolism remain unclear and are interesting point of research. The complexity of this class of phytochemicals, concerning both the number and the variety of their structure, contributes to the difficult investigation of their comprehensive metabolic fate.

Most (poly)phenols found in foods exist as esters, glycosides or polymers: it means that they cannot be absorbed as such, but need some structural

modification prior absorption. After ingestion, (poly)phenol glycosides could be partially modified in the oral cavity by mastication and by the hydrolysing activity of saliva, although after passing through the stomach and the gastrointestinal lumen, several digestive fluids containing different enzymes continue to break down the food matrix.[11,12] The absorption of a minimal part of the ingested (poly)phenols occurs in the small intestine. The molecular size is a key factor for bioactive compound, since it considerably affects its absorption. For example, it is well known that high molecular weight compounds, such as the oligomeric proanthocyanidins, do not pass through the intestinal cells unless they are firstly broken down.[13] For what concerns flavonoids, the sugar moiety represents a key structural determinant for their absorption in humans.[14] The β-glucoside compounds are generally cleaved to release the aglycone as a result of the action of lactase phlorizin hydrolase (LPH), an enzyme located in the brush border of the small intestine epithelial cells, which shows specificity principally for flavonoid-O-β-D-glucosides.[14,15] The released aglycone may then enter the epithelial enterocytes by passive diffusion, due to its increased lipophilicity caused by loss of the glycosidic part, and to its proximity to the cellular membrane. Alternatively, the hydrolysis of the (poly)phenol glucoside may be mediated by a cytosolic β-glucosidase (CBG) within the epithelial cells.[16,17] The polar glucosides must enter the epithelial cells, possibly with the involvement of the active sodium-dependent glucose transporter 1 (SGLT1), to allow the CBG-catalyzed hydrolysis to occur. However, the involvement of this active transporter has to be clarified.[18] Once absorbed, (poly)phenols and related phenolics follow the common metabolic pathway of exogenous organic substances, like drugs and most xenobiotics.[11] In the enterocytes, aglycones undergo to Phase II metabolism, through the action of specific Phase II enzymes, resulting in conjugated forms with methyl groups (by the action of catechol-*O*-methyltransferases, COMT), sulfate moieties (by the

enzymatic activity of sulphotransferases, SULT), and/or glucuronyl groups (by uridine-5'-diphosphate glucuronosyl-transferases, UGT).[18] Some metabolites can efflux back from the enterocyte into the intestinal lumen by an adenosine triphosphate-binding cassette (ABC) family of transporters, which include multidrug resistance protein (MRP) and P-glycoprotein.[16-18] The ABC family of transporters contribute to efflux mechanisms, that can hamper the bioavailability of bioactive food compounds. Since flavonoids are well known substrates for the ABC transporters, the specific affinity with these transporters has been suggested as one of the main reasons for the poor bioavailability of these bioactive compounds.[19,20] MRP-3 and the glucose transporter GLUT2 have also been implicated in the efflux of metabolites from the basolateral membrane of the enterocytes.[21] The enterocyte-conjugated metabolites, as well as the flavonoids that escaped conjugation in the enterocytes, pass into hepatocytes via the hepatic portal vein. In the liver, further Phase II conjugation takes place, and from the liver, potentially bioactive metabolites can be excreted either into systemic circulation or, via enterohepatic recirculation, can be recycled back to the small intestine through bile excretion.[22] (Poly)phenol metabolites present in the systemic circulation are finally excreted into urine.[16,17,23] Due to the complex structure of (poly)phenols, a large portion of these phytochemicals is not absorbed in the small intestine and reaches the large intestine, where phenolic compounds are metabolized by the host microbiota. Some studies reported the analysis of ileal fluid collected from ileostomists after the ingestion of various (poly)phenol-rich foods. It has been demonstrated that even when dietary (poly)phenols are absorbed in the proximal gastro-intestinal tract, substantial quantities nonetheless pass from the small to the large intestine.[24] The high molecular weight compounds as well as glycosides, glucuronides, sulfates, amides, esters and lactones are cleaved by microbial enzyme activity to release the aglycones, which undergo ring fission,

leading to the production of smaller molecules, which in turn can be subjected to reduction, decarboxylation, demethylation and dehydroxylation reactions.[11,25]

The so formed low molecular weight colonic catabolites can be efficiently absorbed *in situ* and, once into the liver, can undergo phase II metabolism, before entering the circulation and being excreted in urine.[26-28]

1.2.1. The stereochemical effect of flavan-3-ol on their bioavailability

The main dietary sources of flavan-3-ols and proanthocyanidins are cocoa, tea, and red wine, and this is the reason why most of the available studies on bioavailability and metabolism of this class of phytochemicals are based on the consumption of these food items. The bioavailability of flavan-3-ols has been largely investigated and recently Ottaviani et al. reported that the different stereochemical asset of (epi)catechin influences the bioavailability and the metabolism of the molecule.[27] Ottaviani et al. investigated the bioavailability of different enantiomeric forms of flavan-3-ol monomers in a study in which adult human males consumed equal quantities of (−)-epicatechin, (−)-catechin, (+)-epicatechin, and (+)-catechin in a cocoa drink. Based on plasma concentrations and urinary excretion, the bioavailability of the stereoisomers was ranked as (−)-epicatechin > (+)-epicatechin = (+)-catechin > (−)-catechin. There were also differences in the metabolic fate of the catechin and epicatechin epimers, as reflected in the ratios of their 3C- and 4C-*O*-methylated metabolites. In addition, the levels of nonmethylated metabolites of (−)- and (+)-epicatechin in plasma and urine differed, demonstrating that flavan-3-ol stereochemistry also affects metabolic pathways other than *O*-methylation. The samples were analyzed as aglycones released by glucuronidase/sulfatase treatment, so it was not possible to determine in detail to what degree this impacted the

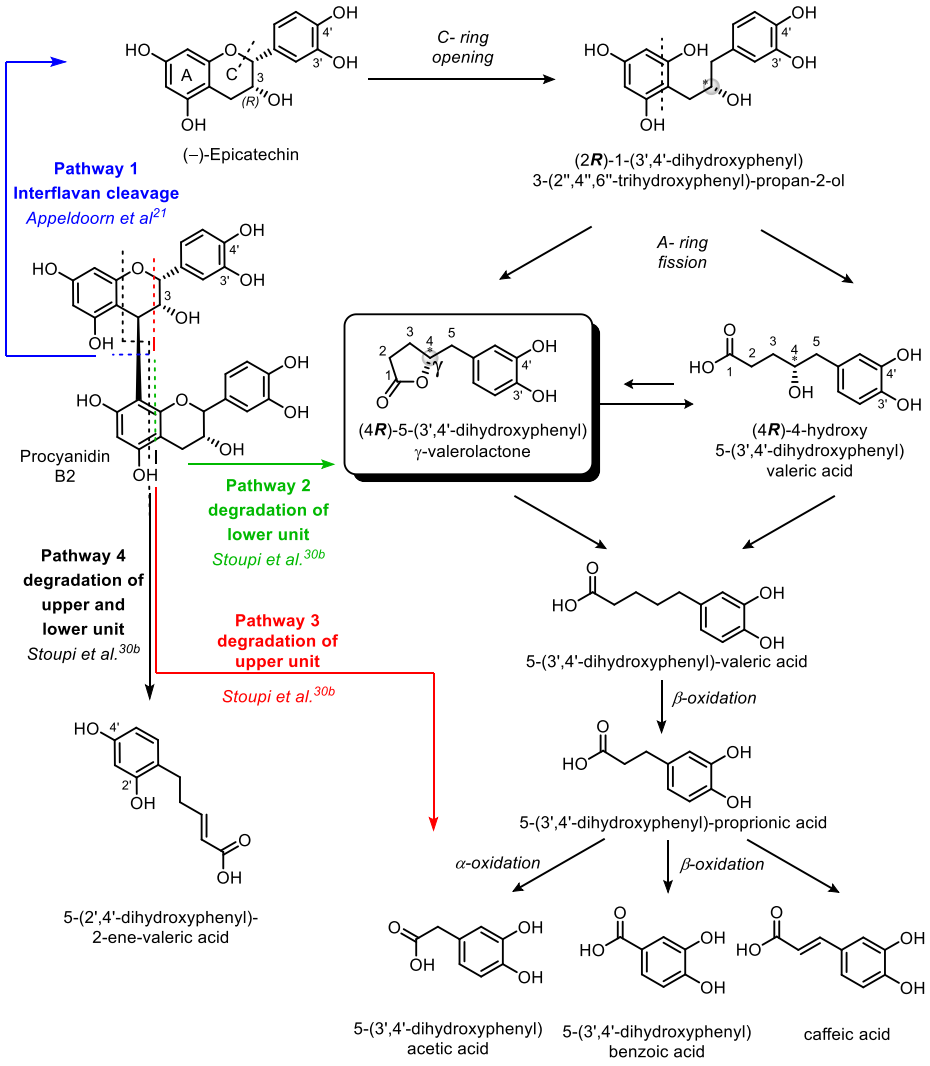
production of glucuronide and sulfate metabolites. As the individual flavan-3-ol stereoisomers in cocoa products and green tea used in feeding studies are usually not determined, this may explain the level of approximation related to the different (epi)catechin metabolic profiles reported in literature.

1.2.2. Microbiota catabolism of flavan-3-ols

In the case of flavan-3-ols, studies performed on ileostomy patients (i.e. patients whose colon has been removed surgically), revealed that approximately 70% of the ingested monomeric flavan-3-ols from green tea could pass from the small to the large intestine, with 33% corresponding to the intact parent compounds.[28] Recently, it has been reported that after oral administration of [^{14}C]procyanidin B2, 63% of the total radioactivity was excreted via urine, indicating that a large quantity of the parent compound is degraded by the gut microflora.[29] The recognition that the colon is a very active organ for the metabolism of flavan-3-ols, particularly proanthocyanidins, has led to a resurgence in the study of the biotransformation of these compounds and other polyphenols by the intestinal microbiota and their implication in the overall bioavailability and bioactivity of polyphenols.[4,25-29]

The complex catabolism of B-type proanthocyanidins involves interflavan cleavage to obtain a monomer unit (Pathway 1, Scheme 1.1), followed by C-ring opening, lactonization, decarboxylation, dehydroxylation, and oxidation reactions, among others.[25] Although numerous in vitro fermentation and in vivo studies have been carried out in recent years, the accumulated knowledge has only led to partial elucidation of the catabolic route of monomeric and B-type dimeric structures.[30-31] In the case of galloylated monomeric flavan-3-ols (ECG and EGCG), the microbial catabolism usually starts with the rapid cleavage of the gallic acid ester moiety by microbial esterases, releasing free

(epi)catechin.[31] The C-ring is subsequently opened, giving rise to diphenylpropan-2-ol, which is later converted into 5-(3',4'-dihydroxyphenyl)-γ-valerolactone (starting from epicatechins) or 5-(3',4',5'-trihydroxyphenyl)-γ-valerolactone (starting from epigallocatechins). The valerolactone ring is then hydrolyzed to 5-(3',4'-dihydroxyphenyl)-valeric acid and/or 4-hydroxy-5-(3',4'-dihydroxyphenyl)-valeric acid (Scheme 1.1).[32] The identification of this latter compound was firstly proposed by Khori et al.[31b] and recently confirmed by Llorach et al.[33] through the analysis of urine samples collected after cocoa consumption in humans, as well as by Stoupi et al.[30b] after *in vitro* fermentations carried out with human faeces in the presence of (−)-epicatechin and procyanidin B2.



Scheme 1.1. *Exemplified trasformations of procyanidin B2 by gut microbiota action.*

Although it was first proposed that 4-hydroxy-5-(hydroxyphenyl) valeric acids could arise from the degradation of diphenylpropan-2-ols, concurrently with hydroxyphenyl-γ-valerolactones, it has recently been suggested that they are formed instead from hydroxyphenyl-γ-valerolactones, and that an interconversion between both forms 4-hydroxy-5-(hydroxyphenyl)-valeric acids and 5-(hydroxyphenyl)-γ-valerolactones may exist.[33] Subsequent

biotransformations of these valeric acids give rise to hydroxyphenylpropionic and hydroxybenzoic acids by successive loss of carbon atoms from the side chain through β-oxidation (Scheme 1.1).[24e] The possible formation of 3,4-dihydroxyphenylacetic acid via α-oxidation of 3,4-dihydroxyphenylpropionic acid by microbial catabolism of monomeric flavan-3-ols, has been widely debated.[32] Another possible metabolic way was proposed by Stoupi et al[30b], where 5-(3',4'-dihydroxyphenyl)-γ-valerolactone could result from the direct degradation of lower unit of procyanidin B2 (Pathway 2, Scheme 1.1), while 3,4-dihydroxyphenylacetic acid could derive from the cleavage of the upper unit of dimeric procyanidins (Pathway 3, Scheme 1.1), without discarding other possible pathways proposed by Appeldoorn et al[21] (Pathway 1, Scheme 1.1). The possible depolymerization of dimeric structures into monomeric units has been recently confirmed to occur but to a lesser extent, representing less than 10% in the case of procyanidin B2.[30b] Other microbial metabolites arising exclusively from the catabolism of dimeric procyanidins have recently been identified, such as 5-(2',4'-dihydroxyphenyl)-2-ene-valeric acid, derivided by simultaneous degradation of upper and lower units of procyanidin B2 (Pathway 4, Scheme 1.1).[30b]

The microbial metabolism includes also dehydroxylation reactions, which can occur in different positions of the aromatic ring depending by the substrate. Once absorbed, the microbial metabolites from flavan-3-ols are mainly metabolized in the liver by phase II enzymes as conjugated derivatives that are subsequently eliminated in urine. At the same time, a portion of microbial metabolites (non-conjugated microbial metabolites) is eliminated in the faeces.

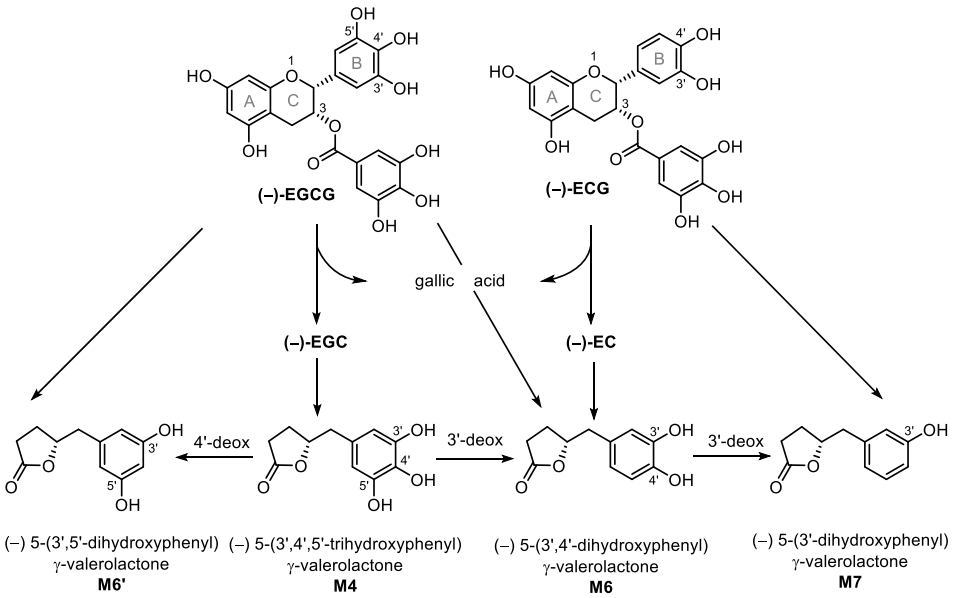
1.2.3. Focusing on γ-valerolactone metabolites: past identifications and open questions

In the study of flavan-3-ols biotrasformation and the search of their potential beneficial effects, γ-valerolactones represent a new and interesting topic of investigation both as metabolomic standards, as well as challenging synthetic targets. At first, since several metabolites are shared by the microbial catabolism of different flavonoids,[18] hydroxyphenyl-γ-valerolactones are considered specific microbial metabolites and so potential biomarkers of flavan-3-ol consumption in humans, as confirmed by studying the metabolomics pattern after intake of green tea,[34] cocoa products[35] and almond skins.[36] Furthermore, it has been estimated that a high percentage of colonic breakdown flavan-3-ols metabolites are represented by hydroxyphenyl-γ-valerolactone structures. Regrettably, their quantitative identification in human body and the exact recognition of a role as key-intermediates in different catabolic pathways have often being hampered or approximated by the absence of useful quantities of validated analytical standards.

To this day, the most studied food matrix with high content of polyphenols has been tea, for its widespread consumption and health benefits proven in animal experiments and in some human studies including the prevention of cancer and heart disease.[18] Tea is manufactured in three basic forms: green tea, oolong tea, and black tea, of which the former represents the richest source of polyphenols. There are four major catechins of which tea is rich: (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)-epicatechin (EC). Of all, EGCG is the most abundant and it may account for 50–80% of the total catechin content.

Many *in vitro* and *in vivo* experiments were developed to investigate the metabolic fate of tea catechins. To mimic events taking place in the large

intestine, the *in vitro* assays were conducted incubating the isolated tea catechin or the leaves of green tea with fecal slurry[30,37] in anaerobic conditions and monitoring their degradation to phenolic acid and aromatic catabolites.



Scheme 1.2. *Valerolactone metabolites derived from (−)-EGCG and (−)-ECG.*

In these studies, the incubation of (-)-epigallocatechin gallate (**EGCG**) produced (-)-epigallocatechin (**EGC**) through the hydrolysis of gallic acid and significative amounts of 5-(3',4',5'-trihydroxyphenyl)-γ-valerolactone (**M4**), 5-(3',5'-dihydroxyphenyl)-γ-valerolactone (**M6'**) and its regioisomer 5-(3',4'-dihydroxyphenyl)-γ-valerolactone (**M6**). These latter two metabolites are formed through dehydroxylation respectively in position 3' and 4' of aromatic ring of **M4** or directly of gallocatechins substrate. Instead, (-)-epicatechin gallate (**ECG**) and its hydrolyzed (-)-epicatechin (**EC**) were degraded into 5-(3',4'-dihydroxyphenyl)-γ-valerolactone (**M6**) and its 4'-deoxygenated 5-(3'-hydroxyphenyl)-γ-valerolactone (**M7**), while no trace of 5-(4'-hydroxyphenyl)-γ-valerolactone (regioisomer of **M7**) were formed (Scheme 1.2). These

transformation suggests that a dehydroxylation process can occur either in 3'-position or in 4'-position of the aromatic ring of **M4** and ECGC substrates (*not shown*), whereas ECG and **M6** are subjected to dehydroxylation preferably in position 3'.

One of the main problems related to this kind of *in vitro* experiments is related to the many key variables to be considered in their executions: in fact, factors such as experimental conditions, incubation time, detection method, and composition of the bacteria broth, influence significantly the outcome of the experiment. Furthermore, these experiments usually discount the action of Phase II enzymes limitating the analysis to unconjugated metabolites.

To complement the *in vitro* incubations, different *in vivo* studies were conducted, assessing the catabolites excreted in urine and in plasma after the ingestion of catechins or green tea and water by healthy subjects.[34] In this respect, Del Rio et al[38] carried out a study in which urines of 20 healthy volunteers were collected for 24 h after green tea intake and valerolactone metabolites were excreted in quantities equivalent to 36% of polyphenols intake. The "sample collection time" is an important factor to be considered in these kinds of investigation. In fact, Calani et al.[34c] proved that the excretion of colonic valerolactones continues far beyond 24 h after ingestion by monitoring the urinary excretion of (epi)catechin catabolites by 20 healthy volunteers for 48 h after ingestion of green tea. On a mole-for-mole basis, some volunteers exhibited a 100% recovery of flavan-3-ols in urine, whereas in other subjects, excretion was <30% of intake. While this conclusion is obviously an approximation because of the presence of several variables (such as different volunteers, flavan-3-ol intakes, and analytical methodologies), it does demonstrate that there is a very high urinary recovery of flavan-3-ols, principally in the form of colon-derived catabolites, mainly represented by γ-valerolactone metabolites.

Arguably, this suggests that colonic ring-fission catabolism could be a key factor in the bioactivity of green tea flavan-3-ols. Furthermore, these studies put in evidence the relevance of valerolactone breakdown products in the evaluation of flavan-3-ols bioavailability.

Summarily, up to 138 metabolites were detected after green tea consumption, including 48 valerolactone and valeric acid conjugates, among which **M4**, **M6'**, **M6**, **M7** valerolactones and the corresponding glucuronides of **M4** and **M6** derivatives were the most abundant.

The aforementioned studies showed some variability in the metabolic profiles: the reason for these discrepancies, especially the dominance of glucuronides in some studies and sulfates in others, could be due to the response of the mass spectrometers used by the different groups, that may vary to some extent, which cannot be determined in the absence of reference compounds. Thus, the different sensitivity of these instrumentations to sulfates versus glucuronides could be responsible for the observed variability of the results. To overcome this issue, it could be convenient to perform these studies on aglycones after enzymatic (sulfatase/glucuronidase) treatment; but even in this case some variability could be observed in different batches, due to variable efficiency of enzymatic hydrolysis.

Moreover, the detection of aglycones after decojugating processes implies the exclusion of methoxy derivatives, which constitute a relevant fraction of urinary metabolites.[34]

Another interesting factor to be considered is the chirality of these metabolites. The γ-valerolactones possess a stereogenic center at the C4 position, presumably derived from the C3 stereocenter of the catechin progenitor; thus they can potentially exist in two enantiomeric forms. This feature arose the question about the enantiopurity of the metabolite in the human body. After the incubation of EGCG with human intestinal bacteria,

Meselhy et al.[31a] isolated **M6** and **M7**, that showed a negative optical activity. Then Li et al.[34b] isolated **M4** and **M6** from urine samples and both the molecules exhibited negative optical activity confirming Meselhy's data. Although this result cannot give information about the absolute configuration of the metabolites or their degree of enantiopurity, this is an important factor to consider in the investigation of health benefits related to these specific metabolites.

1.3. Bioactive valerolactone metabolites

Lots of epidemiological and biological studies exist attributing health benefits against chronic deseases to polyphenol-rich foods as green tea, red wine and fruits, but very few reports deal with the health benefits associated to γ-valerolactones, principally due to their lack of commercial availability.[18] In this context, the developed biological models investigated predominantly the anti-proliferative, anti-inflammatory and anti-oxidant activity.[32]

Anti-proliferative activity. In 2005, Lambert et al.[39] discovered that racemic **M4** (obtained by synthesis) showed a bigger inibition effect against the growth of a series of immortalized and malignant human cell lines than its trimethoxylated derivative, with the exception of colon cancer cells (HCT-116), and immobilized human (INT407) and rat (IEC-6) intestinal cells, which were not sensitive to the growth-inhibitory effects of the compound. Furthermore, **M4** was also more effective in the inhibition of the growth of colon (HT-29) and oesophagus (KYSE150) cancer cells than racemic **M6** valerolactone and its mono- and di-methoxylated derivatives. However, the growth-inhibitory effects of this metabolite were lower than those of the EGCG precursor.

Anti-inflammatory effects. The inflammation process implies the activaction and control of a network of many intracellular signaling pathways, that increase the NO production, iNOS production and the activation of some matrix degradating enzymes (e. g. metalloproteases MMPs) associated to the tissue damage or remodelling. Based on these observations, it is not surprising that all these factors are regarded as key issues for the development of new anti-inflamamtory strategies.

Lambert et al.[39] reported that *rac*-**M4** showed inhibition of NO production in murine macrophage cells, while its trimethoxylated derivative did not show any activity. Moreover, Uhlenhut and Hogger[40] recently showed for the first time that *rac*-**M6** inhibited nitrite production and iNOS in a concentration-dependent fashion. However, the authors found these effects to require a much higher concentration of **M6** than those previously detected in human plasma samples. To understand this, they investigated a possible accumulation of 5-(3',4'-dihydroxyphenyl)-γ-valerolactone in cells and indeed observed high-capacity binding of this molecule to macrophages, monocytes, and endothelial cells involving a mechanism of facilitated transport into cells.

Grimm et al.[41] investigated the bioactivity of *rac*-**M6** and its 3'-*O*-methoxy-derivate against the action of some matrix metalloproteases (MMPs), a family of zinc-dependent proteolytic enzymes involved in some tessue components degradation, as collagene and elastin degradation. Both metabolites showed a strong inhibitory effect against the MMPs action through a direct binding with these ezymatic targets.

Antioxidant activity. The antioxidant activity of *rac*-**M6** and its 3'-*O*-methoxy-derivate has been tested against superoxide radicals, as well as by the ferric reducing antioxidant potential (FRAP).[41] In the radical scavenging test, *rac*-**M6** was more effective than (+)-catechin, ascorbic acid and trolox, whereas related

3'-*O*-methoxy-derivate did not exhibit antioxidant activity. In the reducing test, the order of values was: *rac*-**M6** > (+)-catechin > ascorbic acid > 5-(3-methoxy-4-hydroxyphenyl)-γ-valerolactone. In addition, *rac*-**M6** and its chemical analogue 3-(3',4'-dihydroxyphenyl)-δ-valerolactone showed very similar antioxidant capacity by measuring the ORAC (oxygen radical absorbance capacity).[42]

Although all these *in vitro* essays represent only a starting point for further studies, they contain several important limitations: first, all metabolites were tested as racemic mixtures, even though the "natural" isolated metabolite is optically active. Furthermore, only some methylated conjugated metabolites are considered, whilst data on the corresponding glucuronidated and sulfated forms are still missing.

1.4. Conclusion and perspective

The aim of this chapter was to provide a broad overview of the wide and complex world of polyphenols in the food science realm, outlining the increasing relevance that phenyl-γ-valerolactone metabolites ownes within it.
In the first section, the chemical classification of polyphenols was described focusing on the most popular class of flava-3-ols, which has become a major topic in human nutrition research. Indeed, many efforts have been made to build databases on the (poly)phenol content of foods and to understand the complex relationship between (poly)phenol intake and improvements in human health concerning effects on cognition, age-related cognitive decline, cancer, urinary infections and cardiovascular deseases. The following step carried on by the scientific comunity has been centered upon the understanding of the fate of polyphenol compounds in the human body to better comprehend and

rationalize the chemical nature of the active molecules present at physiological levels. The second section of this chapter was intended to map out the improvements achieved in the identification of most relevant polyphenol metabolites, among wich chiral γ-valerolactones represent potentially biomarkers of polyphenols intake. Despite these promising advances, at present, there is insufficient evidence to draw definitive conclusions on the efficacy of most (poly)phenol molecules, also due to the lack of suitable amounts of chiral non racemic metabolite standards.

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CHAPTER 2

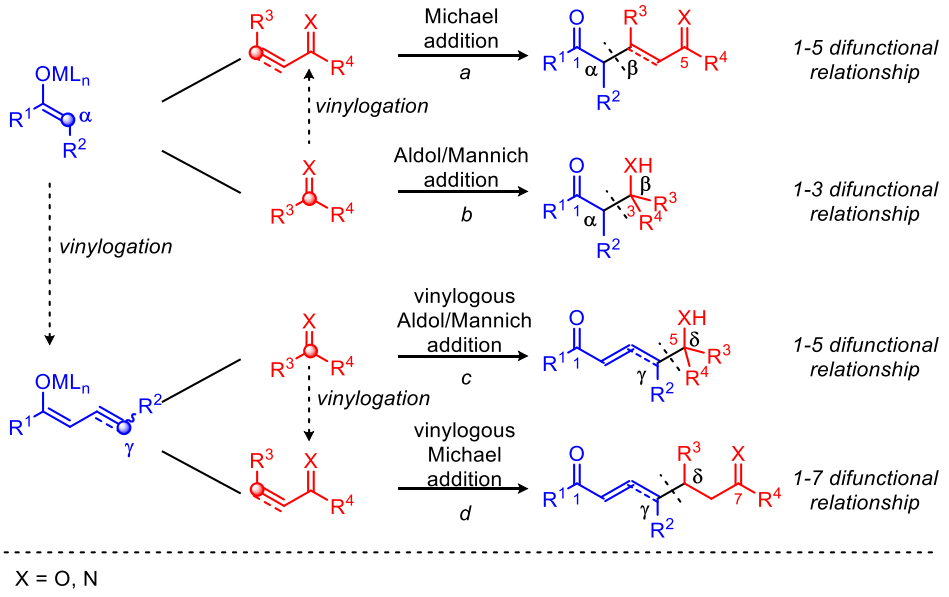
The catalytic, enantioselective, vinylogous aldol reaction (VMAR) with heterocyclic donor systems

2.1. Introduction: the pillars of organic synthesis

The wide panel of compounds that Nature masterfully synthesizes in living organism (the so called "natural products") has always been an inspiration source for the organic chemistry community that often draws from such limitless library of compounds a plethora of valuable synthetic and bioactive targets, useful scaffolds to be transformed, as well as precious chiral reagents. The specific chiral and structural features of biologically active compounds offer many synthetic challenges concerning the ways of new bonds construction and especially the tactics to obtain chiral non-racemic products. A useful strategy for synthesizing such molecules is to devise methods that provide opportunities for using enantioselective catalysis. In using this tactic, the desire for a particular target structure ultimately drives the development of catalytic methods. New enantioselective catalytic methods contribute to a greater fundamental understanding of how bonds can be constructed and lead to valuable synthetic technologies that are useful for a variety of applications.

In this context, the formation of new carbon-carbon bonds is one of the most important transformations for the three-dimensional construction of the molecular framework of many organic molecules by synthesis. In this respect, aldol-, Mannich-, and Michael-type addition reactions between carbon-centered nucleophiles (enolates) and suitably activated aldehydes, imines, and α,β-carbonyl acceptors respectively, are among the fundamental and most

studied transformations in organic synthesis. In fact, over the years, many efficient and stereoselective variants have been developed and applied to the successful total synthesis of increasingly complex bioactive scaffolds.



Scheme 2.1. *Vinylogous evolution of the Aldol, Mannich and Michael reaction and related increasing molecular diversity.*

The "principle of vinylogy"[1] explains how the influence of a functional group may be relayed to a distant point in the molecule when two reactive sites are connected by conjugated unsaturated linkages, such as double and triple bonds or aromatic moieties. This principle has been applied, over the years, to the majority of polar carbon-carbon bond-forming reactions of various repute, including the venerable Michael addition reaction, where the electrophilic site C=X (1,2-addition) is "usurped" by a remote conjugated RC=CR-C=X position (1,4-addition) (Scheme 2.1, eqs a, d). The aldol addition reaction and the related Mannich process, both fundamental pillars of organic synthesis (Scheme 2.1, eq b), have not escaped this fate, and both of their vinylogous

extensions have emerged as extremely valuable synthetic methodologies (Scheme 2.1, eq c).[2] The structural motives arising from reactions involving "vinylogated" donor and/or acceptor components are intrinsically more adorned than their "normal" counterparts, as they embody extended carbon skeletons, additional functionality, and increasing stereochemical complexity. Whereas the normal aldol addition provides access to 1,3-difunctional relationships (Scheme 2.1, eq b), the vinylogous extension of this reaction allows 1,5-difunctional subunits to be constructed (Scheme 2.1, eq c). This vinylogous modification of the aldol reaction is possible when γ-enolizable α,β-unsaturated carbonyl substrates are employed as "extended dienolates". This process leads to the formation of or δ-hydroxy-α,β-unsaturated carbonyl compounds in which up two stereocenters and one double bond can be created simultaneously. All of these attributes qualify the vinylogous aldol, the vinylogous Mannich, and the vinylogous Michael addition reactions as immensely useful, strategic maneuvers in the art of contemporary organic synthesis (Scheme 2.1).[2] Nevertheless, the higher molecular complexity is associated to more critical issues concerning the reaction outcome: *i*. production and control of (poly)enolate geometry, *ii*. channeling of the correct reactivity, *iii*. driving of the chemo-, regio- and stereoselectivity. These challenges are faced through different strategies, among which the search of suitable donors or acceptors with specific structural features and intrinsic vinylogous reactivity represent a fundamental task.

Among the most popular vinylogous nucleophiles, furan (**A**), thiophene- (**B**), pyrrole- (**C**), and indole (**D**) -based heterocyclic silyl dienolates represent such as a family of valuable d^4 donor reagents through which a varied repertoire of multifunctional butenolide-related frameworks, often found in architecturally complex natural products, can be readily obtained (Figure 2.1). Other interesting prototypical donors are their pro-nucleophilic precursors, such as

2(5*H)*- and 2(3*H)*-furanones (**A'**, **A''**), pyrrolinone (**C'**), 3-alkylidene oxindole (**D'**).[3]

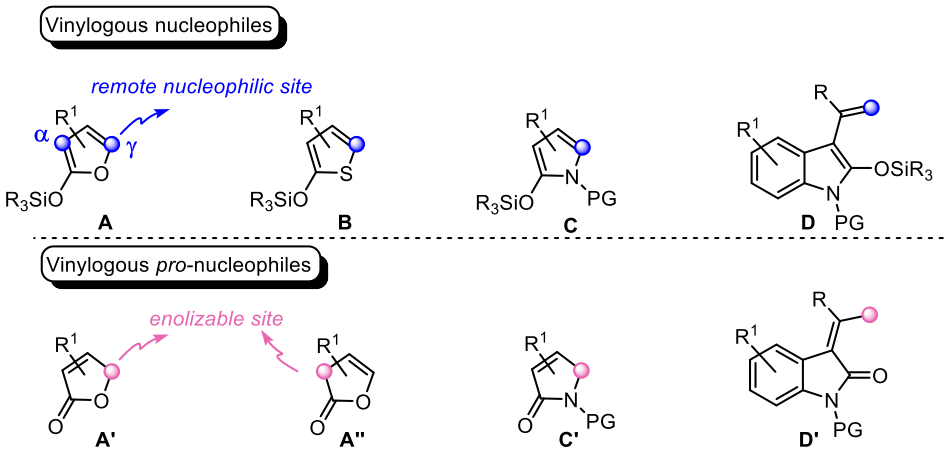
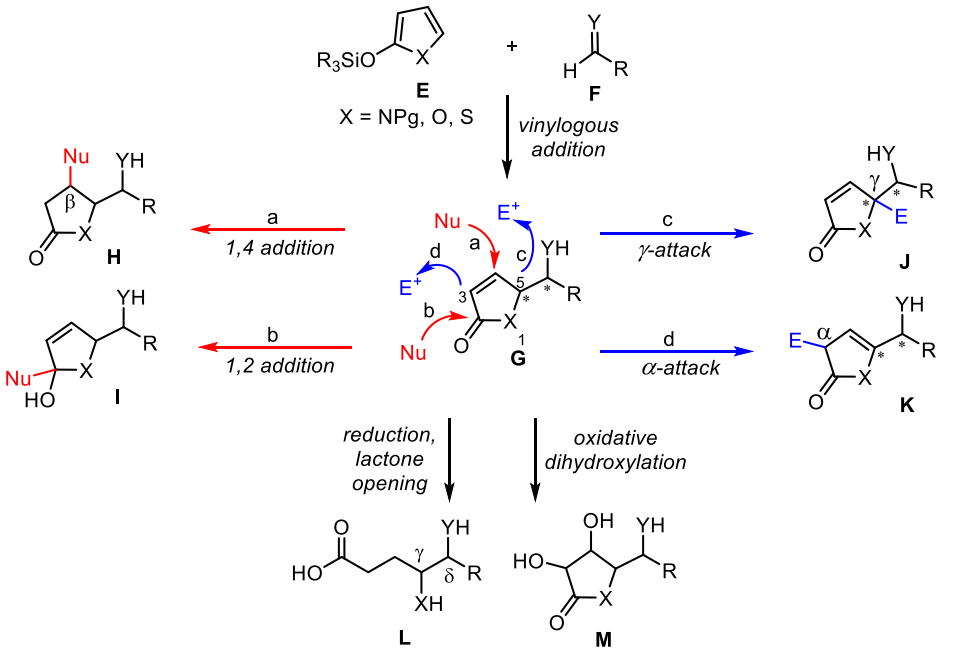


Figure 2.1. *Structures of popular nucleophilic and pro-nuclephilic vinylogous donor heterocycles.*

The additions of the aforementioned systems A-C to an electrophile of type **F** (Scheme 2.2) leads to the formation of the intermediary adducts of type **G**, which can be viewed as ideal template to forge densely functionalized molecular frames and heteroatom-containing target structures. In fact the butenolide-like substrate **G** in turn possess various reactive sites, that can be exploited in different fashion: the nucleophilic attack on C4 (1,4-addition) and C2 (1,2-addition) could furnish β-substituted saturated heterocycles of type **H** and hemiacetal structures of type **I** (Scheme 2, paths a, b), respectively. On the other hand, the reiterative γ-enolization of the system could unmask the nucleophilicity at C3 or C5, thereby assembling differently substituted heterocycles of type **J** and **K** (Scheme 2, paths c, d). At the same time, the manipulation of the double bond within **G** could provide the γ,δ-disubstituted open-chain segment **L** through reduction and ring opening or, alternately, the 1,2-diol scaffold **M** through oxidative dihydroxylation. Looking at this colorful

scenario (Scheme 2.2), the heterocyclic donor compound **E** clearly exhibits an improved functional complexity as compared to that obtained with the corresponding acyclic counterparts (Scheme 2.1).

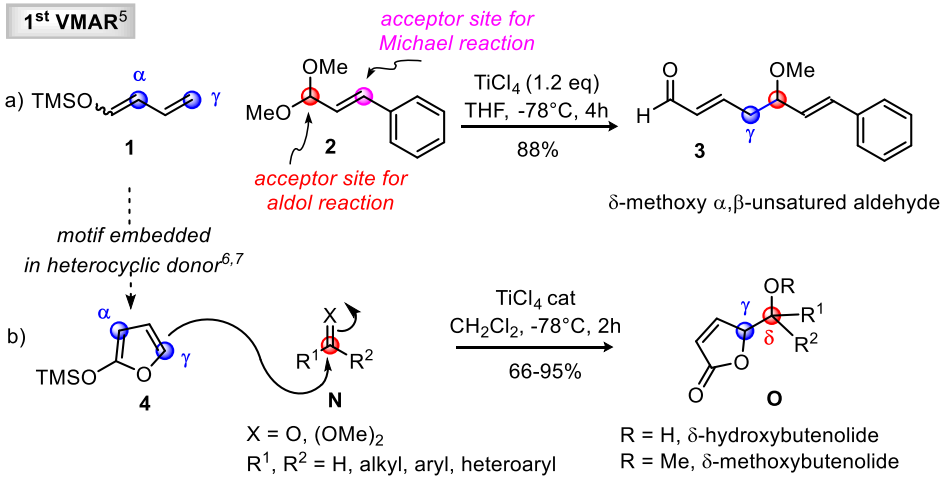


Scheme 2.2. *Multiple choices for the elaboration of butenolide-like product* ***G****.*

In this chapter, the focus will be placed on the evolution and recent advances of the synthetically useful catalytic, enantioselective, vinylogous aldol reaction of heterocycle-based dienoxysilane donors, highlighting those protocols that allow access to chiral non-racemic substances with high efficiency and stereoselectivity. In particular, strong emphasis will be devoted to the VMAR between silyloxydiene heterocycles and suitable aldehydes, promoted by Denmark's bisphosphoramide-$SiCl_4$ chiral complex, a key reaction that we exploited and optimized for the enantioselective total synthesis of chiral, enantiopure γ-valerolactone metabolites (see Chapter 3).

2.2. The origin of the vinylogous Mukaiyama aldol reactions (VMAR)

In 1973 Mukaiyama published a new protocol for an aldol-type reaction using trimethylsilyl enol ethers with ketones or aldehydes in the presence of titanium tetrachloride (*not shown*).[4] Two years later, in an application of Fuson's principle of vinylogy, Mukaiyama and Ishida published the first vinylogous Mukaiyama aldol reaction (VMAR) using crotonaldehyde derived silyl dienol ether **1** and cinnamaldehyde dimethyl acetal **2** with $TiCl_4$ as the Lewis acid in analogy to their pivotal 1973 paper (Scheme 2.3, eq a).[5]



Scheme 2.3. *First vinylogous Mukaiyama Aldol Reaction (VMAR) and its first application using heterocyclic donor systems.*

Further advancements of this indirect strategies didn't escape from the so called "butenolide methodology": in the pioneering work by Yoshii[6] and Asaoka[7] in the late 1970s, the first Lewis acid-promoted vinylogous aldol-type functionalization of 2-(trimethylsiloxy)furan **4** with carbonyl acceptors such as aldehydes, ketones, ortho esters, and acetals of type **N** was introduced (Scheme 2.3, eq b). The fact that the silicon metal leads the system reactivity to

the remote site unfolding the nucleophilicity at the γ-position, in contrast to the corresponding metal dienolates, can be rationalized by different orbital coefficients and/or electrophilic susceptibility. Analysis of these values shows that for metal dienolates, both the orbital coefficients and electrophilic susceptibility are larger at the α-position, whereas for vinylogous silyl ketene acetals, the γ-position displays larger values (Figure 2.2).

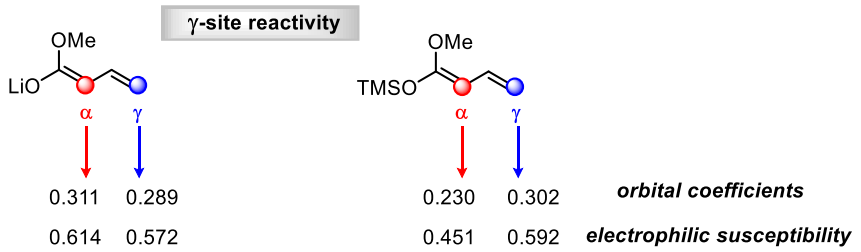


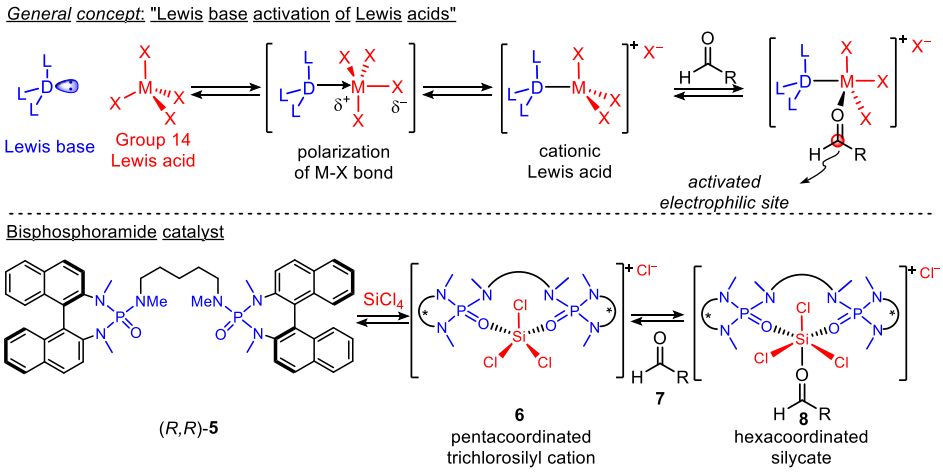
Figure 2.2. *Rationalization of the vinylogous reactivity of vinylogous silyl ketene acetals by comparison of orbital coefficients and electrophilic susceptibility with metal dienolates.*

2.3. Denmark's bisphosphoramide-$SiCl_4$ chiral complex in enantioselective VMAR

Despite the important advancements described in the previous section, the stereoselectivity issue remained open. Various efforts were made to obtain chiral non-racemic compounds using chiral reagents, chiral catalyst and kinetic resolution methods. Among these strategies, the silyloxy diene heterocyclic chemistry found a growing and fruitful field with the advent of the asymmetric (organo)catalysis, in which a chiral "small" molecule is engaged both in the acceleration of the organic transformation and in the transmission of the chiral information to the final product.

Among the many chiral catalysts utilized for VMAR,[8] Prof. S. Denmark and his team proved that the bisphosphoramide catalyst **5** in complex with silicon

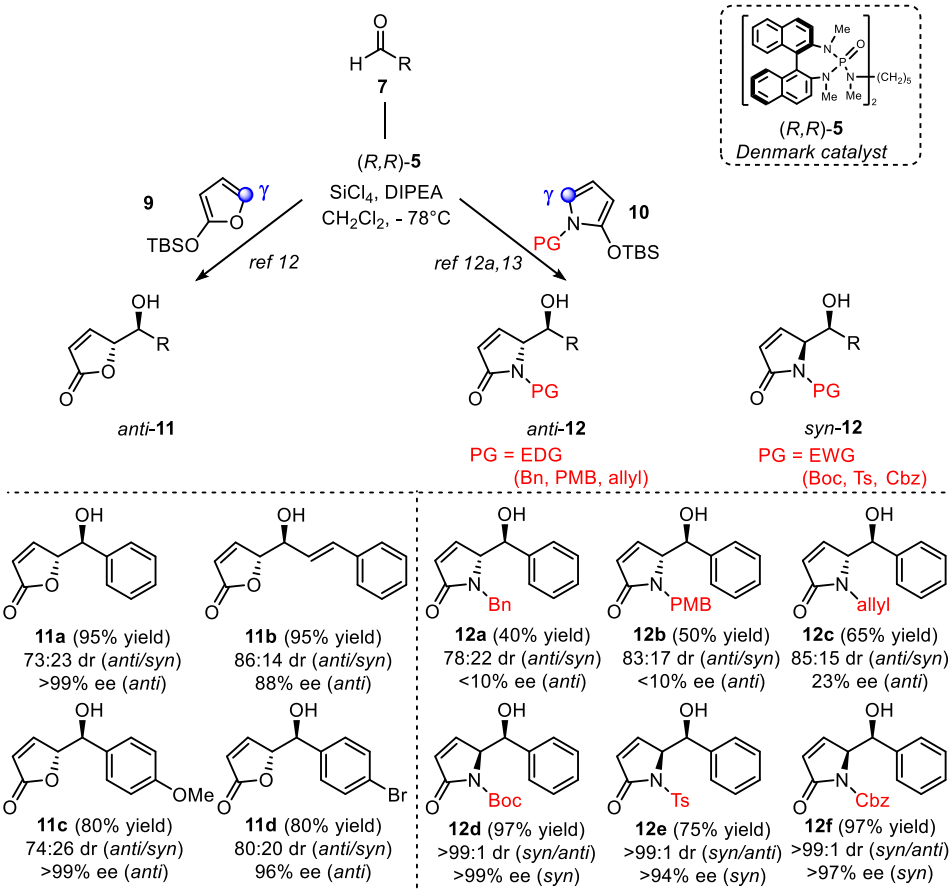
tetrachloride ($SiCl_4$) may well serve the purpose in this environment, ensuring high levels of diastereo- and enantioselectivity.[9] The action of this catalytic system is based on the general concept of "Lewis base activation of Lewis acids", according to which the Lewis base-Lewis acid interaction causes an electronic redistribution within the silicon complex system such that the activating ability of the Lewis acid toward the acceptor of the reaction is enhanced (Scheme 2.4, above).[10]



Scheme 2.4. *General Lewis acid-base complexation (above) and its application for bisphosphoramide catalyst* ***5*** *(below).*

In detail, the mechanistic pathway rationalized by Denmark et al. for VMAR invokes the formation of pentacoordinate siliconium ion **6** through the interaction of the two phosphoramide moieties of the axially chiral compound **5** (strong, neutral Lewis base) to the weak Lewis acid $SiCl_4$ (Scheme 2.4, below). In line with Gutmann's analysis,[11] this binding leads to polarization of the silicon–chlorine bond and the displacement of a chloride to form the active chiral trichlorosilyl cation **6**. This species can then bind to the aldehyde **7** to form hexacoordinated silycate **8**, where the activated aldehyde acceptor is

more susceptible to the attack from the vinylogous enolate, and the intermolecular carbon–carbon bond formation step can proceed.



Scheme 2.5. *Asymmetric VMAR with pyrrole and furan based dienoxy silanes.*

Inspired by this appealing and promising catalytic system, Curti et al.[12,13] exploited the bisphosphoramide-$SiCl_4$ complex in the vinylogous aldol reaction of furan- and pyrrole-based nucleophiles **9** and **10** (Scheme 2.5). This methodology allowed entry to a variety of δ-hydroxylated γ-butenolide-type frameworks with high efficiency and valuable margins of regio-, diastereo-, and enantioselectivity. Noteworthy, the nature of heteroatom in the silyloxy diene scaffolds heavily impacted the diastereoselectivity of the reaction, favoring the

formation of *anti*-configured adducts (**11a-d**, **12a-c**, Scheme 2.5) using either silyloxy furan **9** or pyrroles **10** carrying electron-donating *N*-substituents (Bn, PMB, allyl). Using instead pyrroles **10** having electron-withdrawing *N*-substituents (Boc, Ts, Cbz) gave rise to *syn*-disposed adducts (**12d-f**, Scheme 2.5), preferentially, with complete reversal of diastereoselectivity,.

As depicted in Figure 2.3, this peculiar behavior can be explained through a supplementary interaction between *N*-electron-withdrawing group and the hypervalent silicon atom of the chiral catalyst, which exposes the prochiral *re*-face of pyrrole in its γ-site and affording *syn*-disposed adducts. On the contrary, lacking coordination at silicon, steric effects prevail, thereby exposing the prochiral *si*-face of the pyrrole or furan nucleophiles in the transition state, ultimately generating *anti*-disposed structures. In line with Denmark experimentation and theoretical studies,[9] the chiral bisphosphoramide catalyst is prone to shield the *si*-face of the aldehyde carbonyl, while better involving the *re*-face of the acceptor in the transition state.

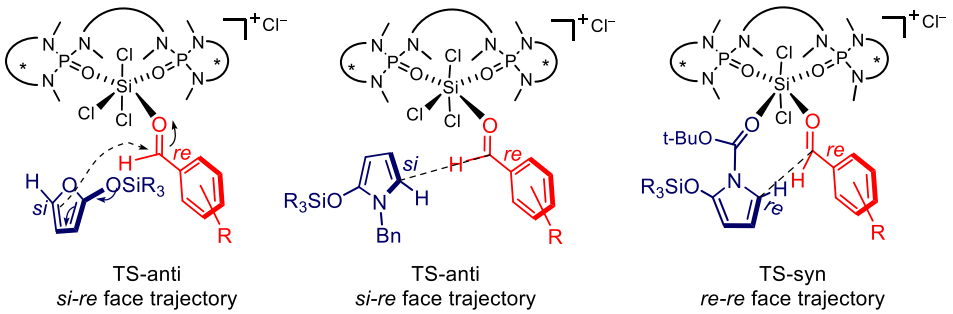


Figure 2.3. *Proposed transition state models for the heterocyclic VMARs.*

Following the evolution of this chemistry, the further exploitation of Denmark system allowed exploring new horizons of the vinylogous Mukaiyama aldol reaction with silyloxyindole matrices[14] and hypervinylogous substrates[15] generating molecular motifs with increasing complexity.

2.4. Summary and conclusion

The aim of this chapter was to emphasize the merits of the enantioselective vinylogous Mukaiyama aldol reaction (VMAR) as one of the most useful transformations in synthetic organic chemistry to forge "Nature-inspired" molecular entities.

In the first section, a general introduction concerning the importance of new C-C bond-forming strategies, the principle of vinylogy and the design of new structural building blocks with vinylogous heterocyclic donor systems is outlined. The birth of the Mukaiyama aldol reaction in its racemic format as precious protocol offering a chance to investigate new horizons for vinylogous transformations is presented in the second section. The primary objective of the third section is to provide conceptual foundation for new opportunities in asymmetric endeavours presented by recognizing the unique characteristics of Lewis acid-assisted/Lewis base catalysis. The final section illustrates a specific application of this strategy: the catalytic, asymmetric vinylogous Mukaiyama aldol reactions of pyrrole- and furan-based dienoxy silanes.

This brief description may give an idea of how a specific chemical methodology study arises from wide and interdisciplinary disciplines, where various basic and pioneering discoveries merge together to achieve scientific discovery and advancement. The next step will be exploring potential useful applicability of the developed methodology.

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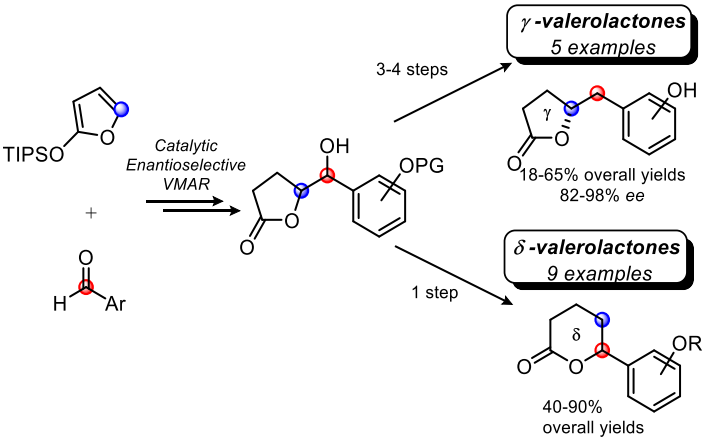
Chemodivergent Approach to γ-Valerolactone Flavan-3-ol Metabolites and δ-Lactone Analogues*

Abstract. The chemodivergent synthesis of various hydroxyphenyl γ-valerolactones and δ-valerolactones was achieved starting from 2-silyloxyfuran and alkoxy-substituted benzaldehydes as common precursors. Key synthesis steps included an enantioselective vinylogous Mukaiyama aldol reaction and a Barton-McCombie deoxygenation (for γ-lactones) or a one-pot reductive ring expansion (for δ-lactones). Five enantioenriched γ-valerolactone targets were obtained in 5-6 steps, 18-63% overall yields and 82-98% *ee*, paving the way for the straightforward entry to this class of biologically effective and poorly available flavan-3-ol metabolites. On the other hand, TMSCl/NaI-based conditions were found for the efficient one-pot deoxygenation/ hydrolactonization of phenolic butanolides to racemic δ-lactones.



*C. Curti, N.Brindani, L. Battistini, A. Sartori, G. Pelosi, P. Mena, F. Brighenti, F. Zanardi, D. Del Rio, *Adv. Synth. Catal.* **2015**, 357, 4082–4092. doi:10.1002/adsc.201500705

3.1. Introduction

In the past few decades, the search for novel and efficient methodologies addressed at the stereoselective synthesis of diverse, highly functionalized small molecules has been a preeminent goal of many skilled organic chemists. Such an effort resulted in a wide array of new asymmetric transformations to be added in the toolbox of organic reactions already at hand.[1] In this context, the preparation of chiral, small-sized lactones such as γ- and δ-valerolactones (namely 5-methyldihydrofuran-2(3*H*)-one **A**, and tetrahydro-2*H*-pyran-2-one **B**, Figure 3.1) has attracted considerable attention, as they represent the central core of a vast set of natural and non-natural molecules.

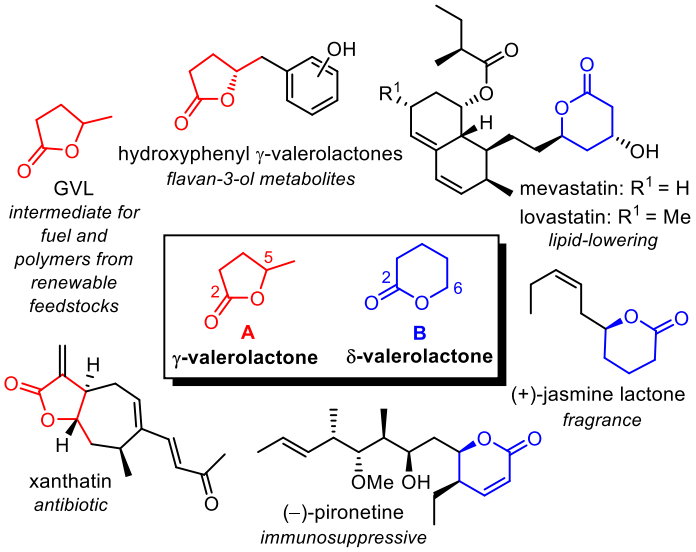


Figure 3.1. *Representative natural and non-natural compounds embedding the γ- and δ-valerolactone module.*

Their application embrace many research areas including drug discovery and bio-medicine,[2] fuel- and polymer-related chemistry,[3] and food science and technology. In the latter area, in particular, these scaffolds are studied as important flavour and aroma constituents,[4] as well as metabolites derived by

human gut microbiota transformation of flavan-3-ols (Figure 3.1). In particular, the γ-valerolactone motif is embedded in the (poly)hydroxyphenyl-γ-valerolactone microbial metabolites. As widely described in Chapter 1, the scientific evidence accumulated during the last decade, though, indicates that the beneficial effects of flavan-3-ols in the human organism are mainly attributed not to the parent set of compounds present *in planta*, but rather to the corresponding enteric metabolites, and in particular to those derived from their microbial catabolism occurring in the colon, mainly represented by hydroxyphenyl γ-valerolactone derivatives of type **1**-**5** (Figure 3.2).[5-7] These chiral metabolites feature a butyrolactone core bearing a hydroxylated benzyl moiety at its 5*R*-configured stereocenter. The different hydroxy substitution pattern on the phenyl ring differentiates each metabolite, so that 4-hydroxy- (**1**), 3-hydroxy- (**2**), 3,4-dihydroxy- (**3**), 3,5-dihydroxy- (**4**), and 3,4,5-trihydroxy- (**5**) derivatives have been isolated and identified in different metabolomic studies as effective flavan-3-ol metabolites (Figure 3.2).[7]

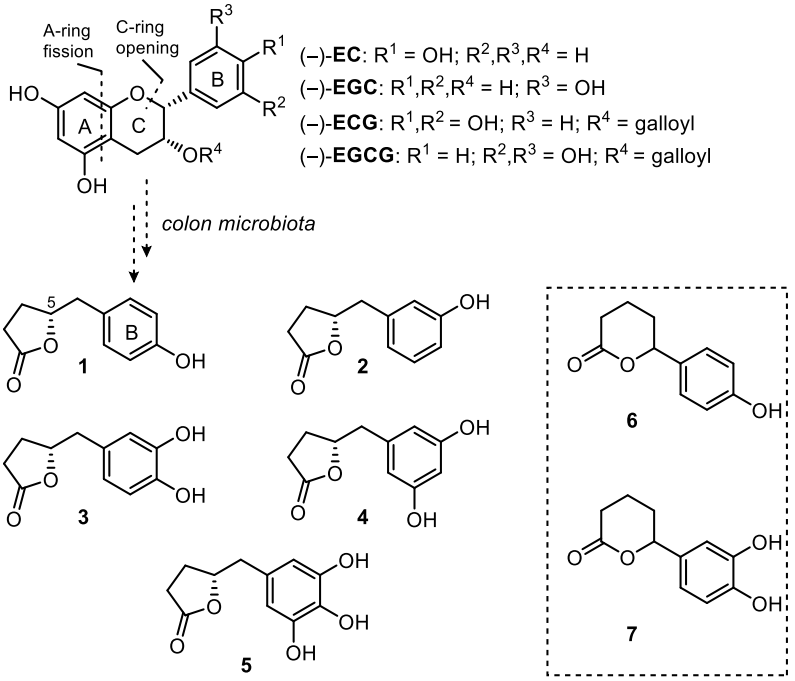
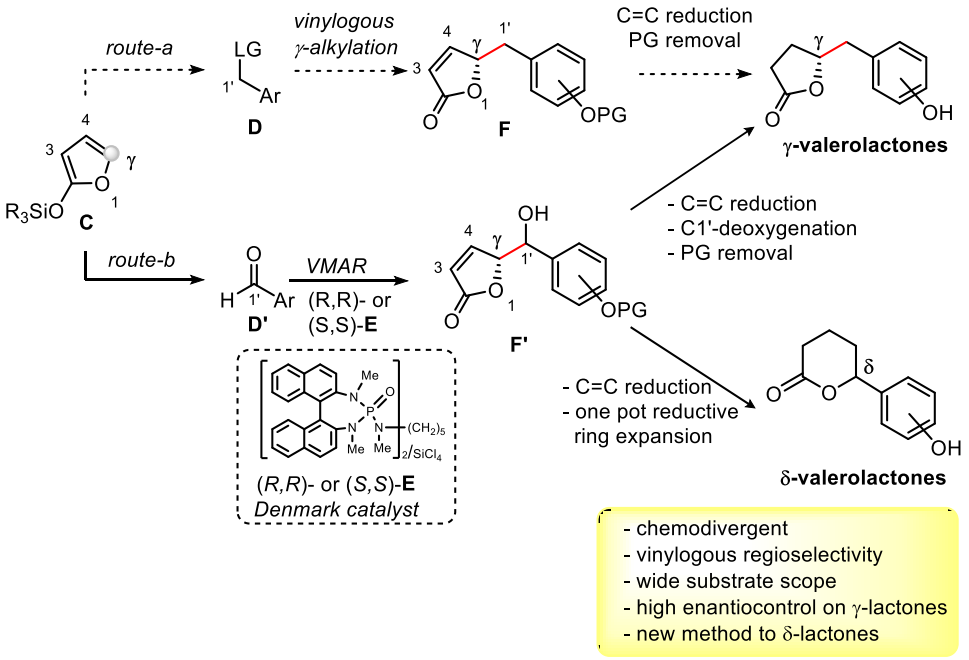


Figure 3.2. *Structures of natural catechins (top). Flavan-3-ol-derived γ-valerolactone metabolites* ***1-5*** *targeted in this work (bottom). In the hatched box, non-natural δ-valerolactones accessed in this work.*

Despite the interesting pharmacological profile exhibited by these phenolic lactones, their extremely scarce availability from either biological samples or commercial suppliers *de facto* precluded their use as both analytical/metabolomic standards and bioactive pharmacological probes. To address this issue, chemical synthesis represents an attractive and viable option. A few synthetic ventures to γ-valerolactones were reported in recent years,[8] by the Lambert and Nakajima groups. The first one accessed compounds **3** and **5** in a racemic format in 7-8 steps and 5-10% overall yields; the latter applied a chironic approach to the diastereoselective synthesis of **2**-**5** in 30-37% overall yields over 7-8 global steps. Even with these notable precedents, the implementation of short, efficient and enantioselective approaches to these scaffolds remains urgent and still topical.



Scheme 3.1. Two possible "butenolide routes" using asymmetric vinylogous alkylation (*route-a*) and asymmetric VMAR (*route-b*) and chemodivergent strategy to targeted hydroxyphenyl γ- and δ-valerolactones.

Looking at the chemical features of the five final targets (**1-5**), we envisaged two possible synthetic routes, which could potentially exploit the intrinsic vinylogous reactivity of furan-based sililoxy diene **C**. In *route-a,* the vinylogous aldol reaction between **C** and a suitable electrophile **D** with a good leaving group would afford butenolide **F**, that after sequential double C-C bond reduction and deprotection steps achieves the desired final γ-valerolactone. Instead, *route-b* takes inspiration from a previous work by Curti et al,[9] that reported the catalytic, enantioselective version of the vinylogous Mukaiyama aldol reaction (VMAR)[10] between furan- or pyrrole-based silyloxydienes and a series of aromatic and heteroaromatic aldehydes (Scheme 2.5, Chapter 2). In that instance, the Denmark's chiral bisphosphoramide (*R,R*)-**E**/silicon tetrachloride system[11] performed as an excellent Lewis base-Lewis acid catalyst

pair, providing a variety of δ-hydroxylated butenolide frameworks **F'** (Scheme 3.1) and their nitrogen counterparts with high efficiency and valuable margins of regio-, diastereo-, and enantioselectivities.[10b] According to *route-b*, scaffolds **F'** could easily consign the γ-valerolatone skeleton through double C-C bond reduction followed by a key deoxygenation step at the C1'-carbinol. Eager to exploit the success of the previously reported methodology to a useful synthetic target, we followed this second line. This chapter describes the successful implementation of this plan (*route*-b), as exemplified by the synthesis of five highly enantioenriched valerolactone products **1**-**5** (Figure 3.2) merging the merits of asymmetric catalysis with the exquisite synthetic versatility of vinylogous transformations. Furthermore, while conducting these synthetic studies, it was found that the TMSCl/NaI couple promoted an unprecedented reductive ring-expansion reaction starting from the reduced butanolide from **F'**, to furnish several δ-valerolactone analogues of type **6** and **7** as racemates. The results of this endeavor and insights toward understanding the mechanism and substrate viability of the latter reaction are also outlined.

3.2. Results and Discussion

3.2.1. Synthesis of γ-Valerolactones

As suggested by our plan, entering the γ-valerolactone targets imposed adaptation of the enantioselective VMAR to the requisite substrates, namely furan **C** and phenolic aldehyde **D'** (Scheme 3.1). Though supported by the successful precedents by Curti et al,[9] this new endeavor was not for granted, as some crucial points had to be faced. *First*, it was anticipated that both the substitution pattern and the nature of the protecting groups on phenolic aldehydes would affect the efficiency and stereocontrol of VMAR, due to

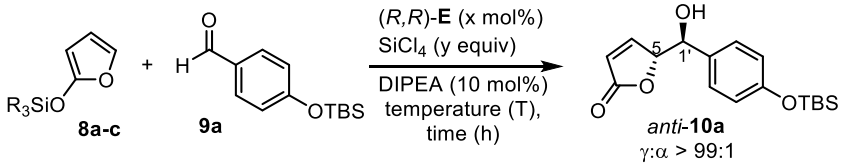
electronic and steric bias in the VMAR transition state. *Second*, the notable vinylogous regioselectivity (γ over α) experienced in the past VMARs had to be respected even in this case, since only the γ-attack would directly install the projected new C5-C1' bond within **F'**. *Third*, the high enantiomeric purity of the targets (*R*-C5 in natural products) directly correlates to the enantiofacial discrimination during VMAR, since VMAR is responsible for the installation of the C5 stereocenter (and that of C1', which is lost) and the synthesis is stereoconservative. *Last*, the previously reported VMAR predicted a preferential *anti*-diastereoselectivity, with minor *syn* isomers being epimeric at C5.[10b] The diastereofacial control during the present VMAR could indeed be quite different, with possible unexpected results.

Starting from the previously developed conditions and using 4-(*tert*-butyldimethylsilyloxy)benzaldehyde (**9a**) as the model acceptor, critical experimental parameters were briefly surveyed, including the nature of the silicon substituent of the nucleophile, catalyst loading, molar ratios between reagents, and reaction temperature (Table 3.1).

At first, we considered the influence of the steric hindrance related of the donor counterpart by examining the silyl component in the furan from TMS to TBS and TIPS (Table 1, entries 1-3). Upon this screening, triisopropylsilyloxy furan (**8c**) turned out to give the best results as the vinylogous nucleophile, in terms of both reaction efficiency, and diastereo/enantiocontrol. Thus, we evaluated the reaction conditions related to the loading of $SiCl_4$ and bisphosphoramide, the time and the reaction temperature. Finally, we found that exposure of furan **8** to silyloxy benzaldehyde **9a** in the presence of a combination of freshly distilled $SiCl_4$ (1.5 equiv), commercial bisphosphoramide (*R,R*)-**I** (3 mol%) and diisopropylethyl amine (DIPEA, 10 mol%) in CH_2Cl_2 at $-78°C$ gave, after 12 h, the desired δ-hydroxylated butenolide **10a** with best yield and diastereo/enantioselection levels (Table 3.1, entry 8). Compound **10a** was

obtained as a 3:1 *anti/syn* diastereomeric mixture in a 97% combined isolated yield, with exclusive γ-regioselectivity (>99:1 γ vs α) and excellent enantiocontrol (97% *ee*) for the major (5*R*,1'*S*)-configured isomer.

Table 3.1. *Screening results for VMAR of furan based silyloxy diene* ***8*** *and benzaldehyde* ***9a****.*



entry	R_3Si	(*R,R*)-**E** (x mol%)	$SiCl_4$ (y mol%)	T (°C)	Time (h)	Yield[b] %	*dr*[c] (*anti/syn*)	*ee*[d](*anti*) (%)
1	TMS (**8a**)	3	1.1	-78	12	82	65:35	96
2	TBS (**8b**)	3	1.1	-78	12	80	68:32	96
3	TIPS (**8c**)	3	1.1	-78	12	78	75:25	97
4	TIPS	3	1.5	-78	12	85	75:25	97
5	TIPS	3	1.5	-50	8	81	68:32	92
6	TIPS	1	1.5	-78	16	55	65:35	95
7[e]	TIPS	3	1.5	-78	16	90	75:25	95
8[f]	**TIPS**	**3**	**1.5**	**-78**	**12**	**97**	**75:25**	**97**

[a] Unless otherwise noted, all reactions were carried out in the presence of furan **8**/aldehyde **9a** (2/1 mol. ratio), (*R,R*)-**I** (x mol%), $SiCl_4$ (y equiv) DIPEA (10 mol%), with a substrate concentration of 0.1M (0.4 mmol scale) in CH_2Cl_2 at the indicated time and temperature.

[b] Refers to isolated combined yields.

[c] Determined by ^1H NMR analysis of the crude.

[d] Determined by chiral HPLC analysis. [e] molar ratio **8/9a** = 1:1. [f] molar ratio **8/9a** = 1:1.5.

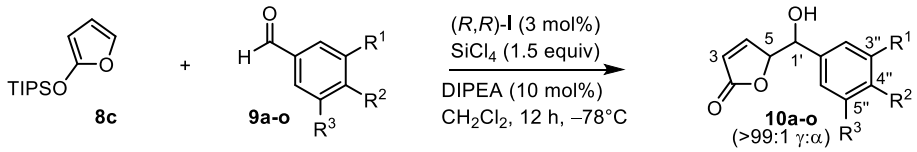
With these results established, we next explored the generality of this catalytic, enantioselective VMAR focusing on diversely protected mono-, di-,

and tri-alkoxy benzaldehydes **9b-o**, whose substitution pattern on the phenyl ring directly correlated to the targeted γ-valerolactones. All reactions performed with good to excellent efficiency with typical combined yields of products **10** ranging from 80% to 98%, with the exception of trialkoxy-substituted compound **10n** which was obtained in a 65% yield (Table 3.2).

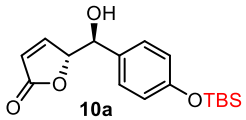
Conversely, the number, nature and position of the alkoxy groups of the aldehyde phenyl ring highly affected the stereochemical output of the VMAR reaction. As a rule, good *anti*-selectivity was observed for most compounds, with the exception of di- and tri-*tert*-butyldimethylsilyloxy derivatives **10g**, **10j**, and **10m** for which either inverted *syn*-selectivity (**10g**, **10m**) or no selectivity at all (**10j**) were attained. High enantiocontrol was also observed in most cases, with enantiomeric excesses ranging from 82% to 99% (Table 3.2). As exceptions, compounds **10f**, **10j**, **10m**, and **10n** were isolated with <70% *ee* or as racemic mixtures, probably due to scarce enantiofacial discrimination of the chiral catalyst or inherent high reactivity of the aldehyde substrates favoring the competitive achiral $SiCl_4$-promoted VMAR background. Notably, while the majority of butenolide products **10** possessed the expected *S*-C1' absolute configuration (resulting from an attack on the *Re*-face of the aldehyde), a striking and unprecedented enantiofacial inversion was experienced for polymethoxy- and polybenxyloxy-substituted products **10k**, **10l**, **10n**, and **10o**, for which a *R*-C1' configuration was assigned (*Si*-face attack).[12]

The C5 absolute configuration of VMAR products **10d**, **10h**, *ent*-**10k**, and *ent*-**10o** were confirmed by chemical correlation to the respective target products **2**-**5**, which are known compounds (*vide infra*). With a well-stocked set of differently-protected enantioenriched butenolides **10a-o** at hand, compounds **10b**, **10d**, **10h**, *ent*-**10k**,[14] and *ent*-**10o**[14] were selected to move forward to the corresponding targeted *R*-configured lactones **1**-**5**.

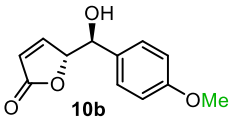
Table 3.2. *Substrate scope of the catalytic, enantioselective VMAR between furan* ***8*** *and alkoxyl benzaldehyde* ***9****.*[a-d)]



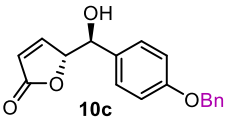
4''-alkoxy



10a
97% Yield
75:25 *dr* (*anti*/*syn*)
97% *ee* (*anti*)

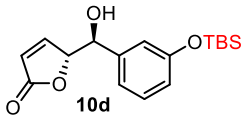


10b
90% Yield
85:15 *dr* (*anti*/*syn*)
99% *ee* (*anti*)

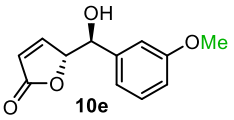


10c
85% Yield
72:28 *dr* (*anti*/*syn*)
99% *ee* (*anti*)

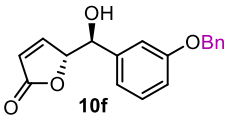
3''-alkoxy



10d
98% Yield
72:28 *dr* (*anti*/*syn*)
90% *ee* (*anti*)

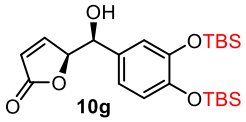


10e
94% Yield
74:26 *dr* (*anti*/*syn*)
82% *ee* (*anti*)

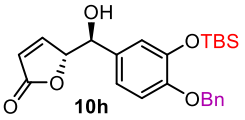


10f
96% Yield
64:36 *dr* (*anti*/*syn*)
20% *ee* (*anti*)

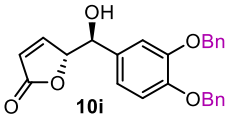
3'',4''-dialkoxy



10g
85% Yield
17:83 *dr* (*anti*/*syn*)
95% *ee* (*syn*)

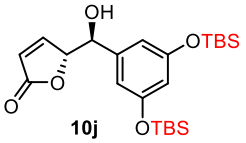


10h
95% Yield
75:25 *dr* (*anti*/*syn*)
99% *ee* (*anti*)

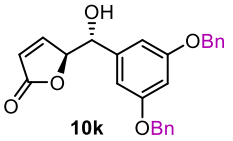


10i
80% Yield
69:31 *dr* (*anti*/*syn*)
93% *ee* (*anti*)

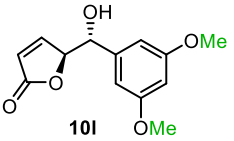
3'',5''-dialkoxy



10j
80% Yield
56:44 *dr* (*anti*/*syn*)
racemic (*anti*)

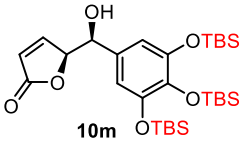


10k
98% Yield
82:18 *dr* (*anti*/*syn*)
94% *ee* (*anti*)

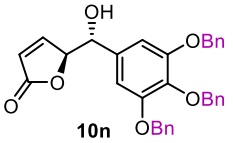


10l
93% Yield
80:20 *dr* (*anti*/*syn*)
89% *ee* (*anti*)

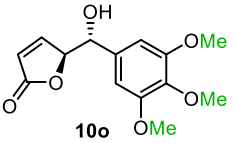
3'',4'',5''-trialkoxy



10m
80% Yield
9:91 *dr* (*anti*/*syn*)
60% *ee* (*syn*)[e)]



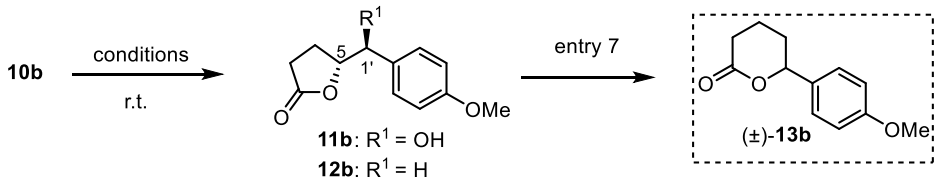
10n
65% Yield
77:23 *dr* (*anti*/*syn*)
69% *ee* (*anti*)



10o
84% Yield
70:30 *dr* (*anti*/*syn*)
82% *ee* (*anti*)[e)]

Initially, attempts were made to perform the C3-C4 double bond reduction and the C1'-carbinol deoxygenation according to a *one pot* procedure. To this end, several reducing agents and different reaction conditions were scrutinized on model butenolide **10b**, and formation of butanolide **11b** versus doubly-reduced valerolactone **12b** was registered (Table 3.3). Unfortunately, under the scrutinized reaction conditions, we were unable to detect any useful quantities of valerolactone **12b**, and only saturated butyrrolactone **11b** could be isolated efficiently. Of note, nickel boride-promoted olefin reduction (Table 3.3, entries 5 and 6,) resulted in a more efficient reaction with respect to common catalytic hydrogenation options, yielding butanolide **11b** in 95% yield.

Table 3.3. *Transformation of butenolide **10b** toward γ-valerolactone **12b**: reduction attempts.*



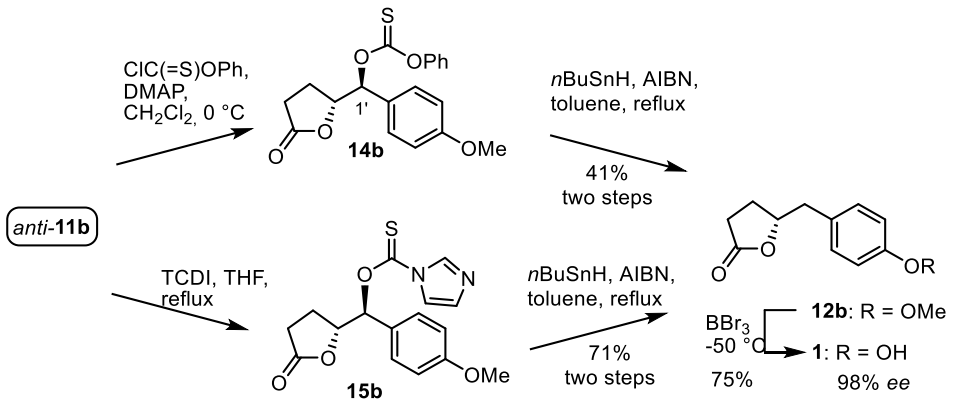
Entry	Conditions	Solvent	Time (h)	yield (%)[a]		
				11b	**12b**	**13b**
1	H_2, Pd/C (10%)	EtOAc	12	89	-	-
2[b]	H_2, Pd/C (10%)	MeOH	24	85	-	-
3	H_2, Raney	MeOH	24	80	-	-
4	H_2 (3atm), Raney	MeOH	48	60	<5	-
5	$NiCl_2$, $NaBH_4$	MeOH	24	95	-	-
6[b]	$NiCl_2$, $NaBH_4$	MeOH	24	95	-	-
7[c) d)]	TMSCl, NaI	CH_3CN	3	-	-	50

[a] Isolated yield, after column chromatography. [b] Reaction carried out at 40 °C, [c] Reaction performed on butanolide **11b** using a 1:1 mixture (6 equiv) of TMSCl and NaI, in CH_3CN at room temperature. [d] Enantiomeric excess *ee* of **13b** <5%.

We then tried several multi-step options, in which the deoxygenation step was performed on the reduced butanolide scaffold, upon functionalization of the C1'-carbynol. Quite surprisingly, treating butanolide **11b** with excess of a 1:1 mixture of TMSCl/NaI in CH_3CN - a common reagent used to reduce secondary benzylic alcohols under mild reaction conditions[15] a reductive ring expansion occurred, furnishing γ-valerolactone **13b** in an appreciable 50% yield, albeit with complete loss of optical activity (<5% *ee*, Table 3.3, entry 7). This unexpected result did not pass unnoticed, and development of parallel chemistry toward six-membered lactones was programmed (see next paragraph).

We then moved to investigate the viability of a Barton McCombie-type two-step approach, which would provide the projected lactone **12b** starting from butanolide **11b** via radical C1'-deoxygenation (Scheme 3.2).[16a] Thus, treatment of butanolide **11b** with *O*-phenyl chlorothionoformate and DMAP at 0 °C afforded xantate **14b** (Scheme 3.2, top), which was reduced to the corresponding γ-valerolactone **12b** (*n*Bu_3SnH, AIBN) in a 44% yield for the two steps. Gratifyingly, we could improve the yield of this transformation by using a thiocarbamate intermediate instead of the xantate.[16b] As depicted in Scheme 3.2 (bottom), treatment of **11b** with thionocarbonyl-1,1-diimidazole (TCDI) in THF at reflux for 7 h afforded thiocarbamate **15b** that, by using the abovementioned deoxygenation procedure, yielded γ-valerolactone **12b** in an improved 71% yield for the two steps. Finally, deprotection of the *para*-methoxy group with BBr_3 in anhydrous CH_2Cl_2 at −50 °C for 24 h smoothly afforded the targeted (*R*)-5-(4-hydroxyphenyl)-γ-valerolactone (**1**) in a 75% yield, corresponding to a nice 39% overall yield for the five steps from furan **8**. To the best of our knowledge, this is the first asymmetric entry to valerolactone (−)-**1**, whose 98% *ee* enantiopurity (as assessed via chiral HPLC) was in fine agreement with that of its precursor intermediate **10b** (compound **1**: $[\alpha]_D^{20}$

−22.0 (*c* 1.6, MeOH); lit.[17] $[\alpha]_D^{20}$ 0.0 (*c* 0.5, MeOH) for a naturally derived sample).



Scheme 3.2. *Barton McCombie-type two-step C1'-deoxygenation of butanolide* ***11b****. AIBN = 2,2-azobisisobutyronitrile; TCDI = thionocarbonyl-1,1-diimidazole.*

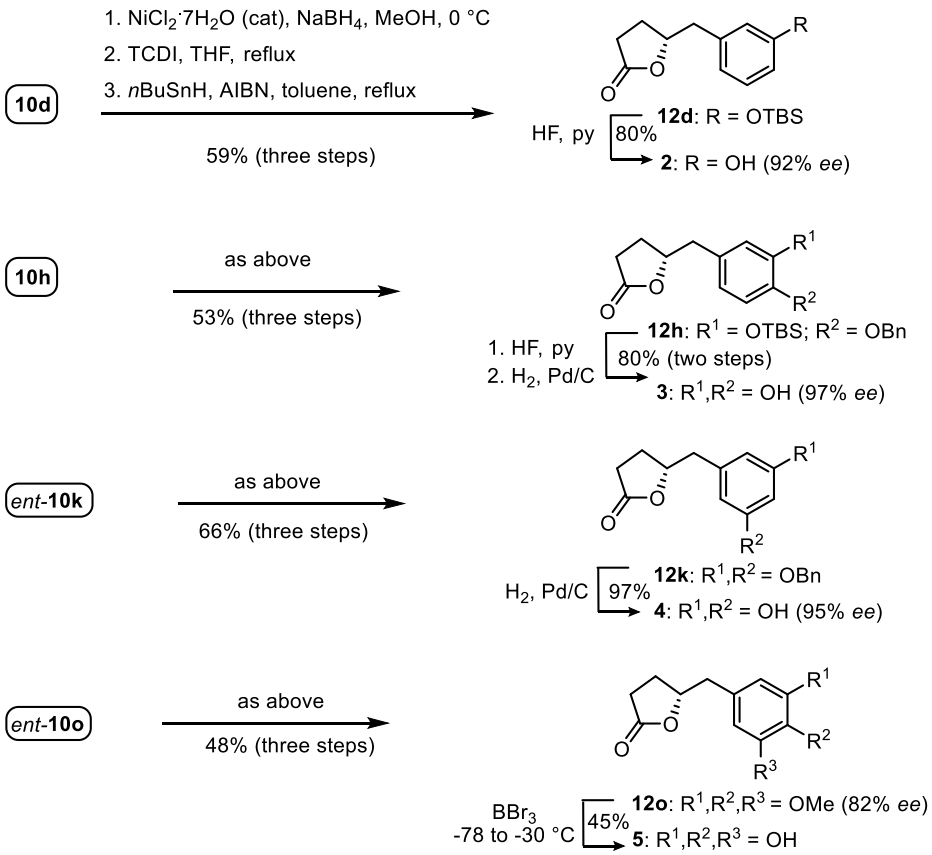
With the preparation of γ-valerolactone **1** successfully achieved, we proceeded to the synthesis of parent compounds **2**-**5**. As described in Scheme 3.3, by following the optimized synthetic procedures used for the preparation of **1**, *i.e.* double-bond reduction, thiocarbamate formation, and radical C1'-deoxygenation, butenolides **10d**, **10h**, *ent*-**10k**, and *ent*-**10o** were advanced to the corresponding protected valerolactones **12** in 68% to 81% yields for the three steps. Finally, cleavage of the protecting groups by standard procedures (e.g. HF·pyridine for the silyl group; H_2, Pd/C for benzyl, and BBr_3 from −78 °C to −30 °C for the methoxy group) afforded valerolactones **2**-**5** quite efficiently, thus validating both efficiency and versatility of the whole plan.

Valerolactone **2** was isolated in a 46% overall yield as white crystals; its levorotatory optical rotation sign $[\alpha]_D^{20}$ −18.0 (*c* 0.5, MeOH) was in accordance with that observed for previously reported samples (lit.[18] $[\alpha]_D^{20}$ −11.6 (*c* 0.1, MeOH) for a naturally derived sample; lit.[8c] $[\alpha]_D^{20}$ −29.2 (*c* 1.0, MeOH) for a synthesis-derived sample), thus confirming its 5*R* absolute configuration.

Dihydroxylated valerolactone **3**, a white crystal (global yield 41%), showed a negative optical rotation value ($[\alpha]_D^{20}$ −12.2 (*c* 1.0, MeOH)) almost coincident to those reported in literature (e.g. lit.[19] $[\alpha]_D^{30}$ −12.0 (*c* 0.1, MeOH) for a natural sample; lit.[8c] $[\alpha]_D^{20}$ −16.7 (*c* 0.7, MeOH) for a synthesis-derived sample).[8a,18]

Target valerolactone **4** was isolated in 63% overall yield as an amorphous solid exhibiting an optical activity ($[\alpha]_D^{20}$ −11.8 (*c* 0.4, MeOH)) quite similar to that observed for a naturally occurring sample (lit.[20] $[\alpha]_D^{20}$ −12.9 (*c* 0.4, MeOH)), albeit with discrepancies from a synthesis-derived product (lit.[8c] $[\alpha]_D^{20}$ −20.6 (*c* 0.97, MeOH). Finally, crystalline trihydroxylated valerolactone **5** was isolated in a 18% global yield and showed a negative optical rotation ($[\alpha]_D^{20}$ −8.0 (*c* 0.2, MeOH) almost in agreement with literature data (lit.[8c] $[\alpha]_D^{20}$ −12.6 (*c* 0.99, MeOH)).

Also, the enantiomeric excesses of target compounds **2**-**5** nicely matched those of the respective butenolide precursors, confirming that the synthetic strategy is stereoconservative.



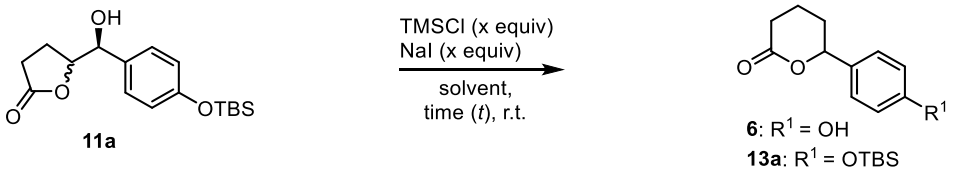
Scheme 3.3. *Completion of the synthesis of γ-valerolactones* ***2****-****5****. AIBN = 2,2-azobisisobutyronitrile; TCDI = thionocarbonyl-1,1-diimidazole.*

3.2.2. Unexpected Reductive Ring Expansion: Synthesis of δ-Valerolactones

As described before, while surveying a viable and efficient reduction-deoxygenation sequence for the synthesis of γ-valerolactone **12b**, we observed the unexpected generation of the expanded δ-valerolactone (±)-**13b** when butanolide **11b** was exposed to excess TMSCl/NaI (6 mol equiv of a 1:1 mixture) in acetonitrile (Table 3.3, entry 7). Though this reaction was only moderately efficient (50% isolated yield) with negligible stereocontrol (<5% *ee*), it served as an inspiration for further investigations into the scope and limitations of this

quite interesting and unprecedented reductive γ-lactone to δ-lactone expansion.[21]

Table 3.4. *Reductive γ-lactone to δ-lactone expansion: optimization of reaction parameters.*



entry	**11a**	x (equiv)	solvent	*t* (h)	yield (%)[a] **6** (**13a**)[b]
1	*anti*	6	CH_3CN	3	70 (0)
2[c]	*anti*	6	hexane	3	50 (5)
3	*anti*	6	CH_2Cl_2	3	50 (25)
4[d]	*anti*	6	CH_3CN	24	55 (0)
5[e]	*anti*	6	CH_3CN	1	60 (0)
6	*syn*	6	CH_3CN	3	70 (0)
7[f]	***anti/syn* (2:1)**	**6**	**CH_3CN**	**3**	**76 (0)**
8	*anti/syn* (2:1)	4	CH_3CN	3	40 (5)
9	*anti/syn* (2:1)	1	CH_3CN	24	<10 (10)
10	*anti/syn* (2:1)	10	CH_3CN	3	61 (0)
11[g]	***anti/syn* (2:1)**	**4**	**CH_2Cl_2**	**1**	**0 (75)**

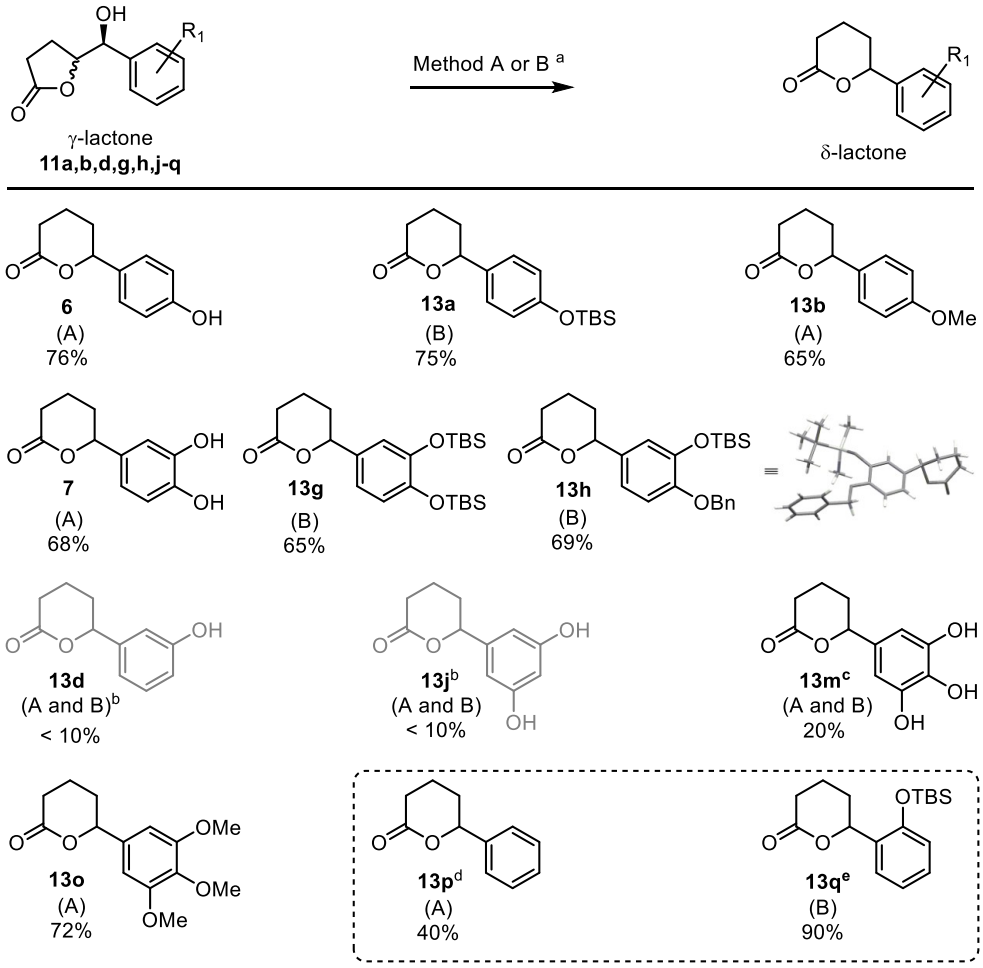
[a] Isolated yield of **6** after chromatographic purification; isolated yield of protected lactone **13a** in parenthesis.
[b] The enantiomeric excess (*ee*%) of **6** and **13a** resulted <5% in all entries, as determined by chiral HPLC analysis.
[c] A mixture of TMSCl/NaI/CH_3CN (1:1:1) was used in the indicated solvent (see ref. [15a]). [d] Reaction carried out at 25 °C. [e] Reaction carried out at 45 °C.
[f] Method A.
[g] Method B.

The combination of TMSCl with NaI, usually in CH_3CN as the solvent, is a useful reagent for the in situ formation of iodotrimethylsilane (TMSI), which has been widely exploited to promote several transformations including the reduction of secondary benzylic alcohols,[15] the cleavage of esters and lactones,[22] and the hydroamination and hydroetherification of olefins.[23]

We commenced our studies by screening different reaction conditions in the attempt to optimize both yield and enantioselectivity. Initially, we chose silyloxy butanolide *anti*-**11a** (readily available in high yield by reduction of the parent butenolide *anti*-**10a**) as the model substrate, envisaging the possibility to obtain the deprotected δ-lactone **6** in a single step, due to the liability of the silyl group in an acidic environment. Indeed, using the same reagents and reaction conditions (TMSCl/NaI, CH_3CN, room temperature) previously employed for the conversion of **11b** to **13b** (Table 3.4), butanolide *anti*-**11a** was readily transformed to the deprotected lactone **6** with a good 70% isolated yield, but again as an almost racemic mixture (<5% *ee*) (Table 3.4, entry 1). Solvent and temperature didn't affect much the efficiency and the enantioselectivity of the process (entries 2-5) even though, using CH_2Cl_2 as the solvent, a 2:1 mixture of silylated and deprotected lactones **13a** and **6** could be isolated (entry 3). The reaction resulted also poorly affected by the configuration of the starting butanolide; thus, either pure *syn*-**11a** or a 2:1 (*anti:syn*) mixture of **11a** reacted similarly in CH_3CN, affording **6** as the sole product in comparable yields (70% and 76% yields, respectively, entries 6 and 7). On the other hand, the reaction efficiency resulted highly dependent on the amount of reagents employed: in fact, diminishing the quantity of the TMSCl/NaI couple to 4 mol equiv or 1 mol equiv, resulted in consistent drop of the overall yield (40% and <10%, respectively, entries 8 and 9). In any case, only racemic products were obtained. We concluded that the best reaction conditions for the conversion of **11a** to **6** consisted in the use of excess

TMSCl/NaI as a 1:1 mixture (6 mol equiv) in CH_3CN at room temperature, even starting from an *anti/syn* mixture of butanolide **11a** (76% yield, entry 7).

Table 3.5. *Reductive γ-lactone to δ-lactone expansion: scope and limitations.*



a)Method A: see entry 7 of Table 3.3. Method B: see entry 11 of Table 3.3.
b)Only deprotected starting material could be recovered.
c)Conversion before chromatography; due to high product instability, lactone **13m** couldn't be isolated.
d)24 h reaction time.
e)30 min reaction time.

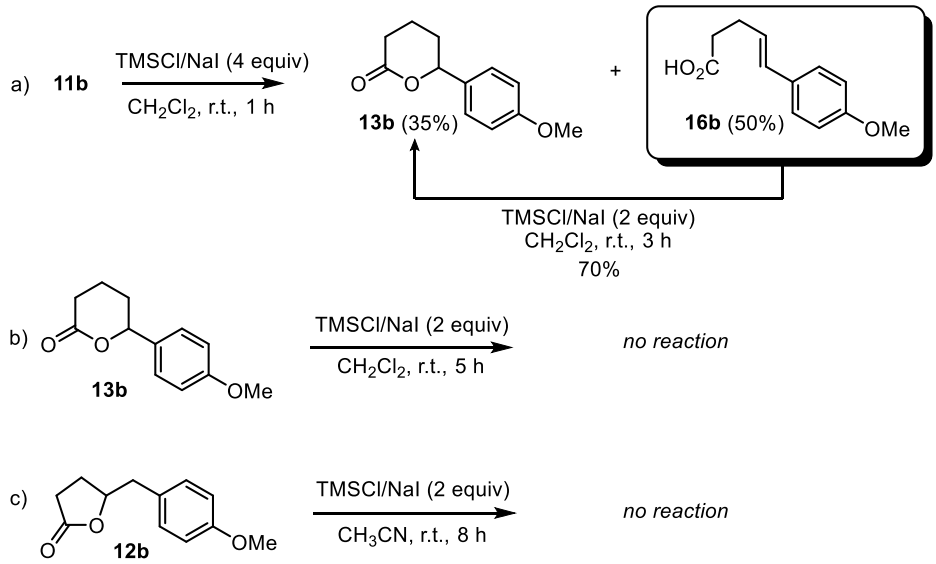
Interestingly, running the reaction in CH_2Cl_2 with no trace of CH_3CN and using 4 mol equiv of TMSCl/NaI (1:1) proved viable, yielding silylated δ-lactone **13a** as the sole product, in a remarkable 75% yield after 1h reaction time (Table 3.4, entry 11). Thus, starting from the silylated butanolide **11a**, we ended up with two protocols enabling the one pot formation of deprotected δ-valerolactone (method A), or protected δ-valerolactone (method B) as complementary options.

Next, we sought to examine the reaction scope with several γ-lactone scaffolds of type **11** (Table 3.5). As for monosubstituted butanolides, in addition to **11a**, the reaction performed well also on the *p*-methoxy-derivative **11b**, furnishing protected δ-valerolactone **13b** in a good 65% yield using method A. Similar results were obtained with bis-silyloxybutanolide **11g,** which gave either deprotected lactone **7** (method A) or the protected counterpart **13g** (method B) as the sole products in 68% and 65% yields, respectively. Interestingly, method B was also successfully applied to orthogonally protected γ-lactone **11h**, affording crystalline δ-lactone **13h** (69% yield, Table 3.5), whose structure was further certified by single crystal X-ray analysis.[24]

On the other hand, butanolides **11d** and **11j**, featuring *meta*-positioned mono-OTBS and bis-OTBS substituents respectively, failed to give the related δ-lactones **13d** and **13j**, and only deprotected starting materials could be isolated at the end of the reaction.

Finally, trisilyloxy-butanolide **11m** could be converted to the corresponding δ-lactone **13m** but we were unable to isolate it in an appreciable yield due to its high instability during the work up procedures. Trimethoxy-derivative **11o**, instead, performed well with method A, producing protected lactone **13o** in an appreciable 72% isolated yield. As control experiments, unsubstituted benzaldehyde-derived butanolide **11p** and *ortho*-substituted substrate **11q** were considered. The former proved to be reluctant to react under the

conditions of method A, and afforded lactone **13p** with a modest 40% isolated yield after 24 h. The latter, instead, reacted very fast according to method B, giving protected δ-valerolactone **13q** very efficiently (90% yield) after 30 minutes at room temperature. These observations led us to conclude that the reaction viability strictly depended on the substitution pattern of the starting butanolide **11**. In particular, *ortho*- or *para*-positioned alkoxy substituents seem to accelerate the reaction, while missing alkoxy substituents in these positions partially or completely inhibited the reaction, as in the case of butanolides **11d**, **11j**, and **11p**.



Scheme 3.4. *Control experiments for the reductive ring expansion reaction.*

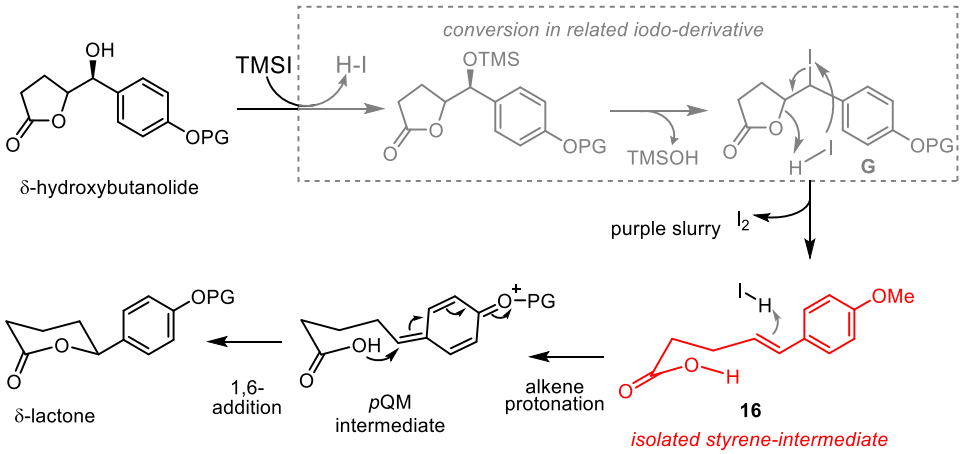
To rationalize the observed reaction outcome, and propose a plausible mechanism, a series of further control reactions were performed, as outlined in Scheme 3.4. Treatment of γ-lactone **11b** with TMSCl/NaI in CH_2Cl_2 for 1 h (method B) returned the expected expanded lactam **13b** (35% yield) along with considerable quantities of pentenoic acid **16b** (50% yield) (Scheme 3.4, eq. a). To verify whether open-chain carboxylic acid **16b** is a key intermediate during

the ring expansion or simply a degradation product, it was further treated with TMSCl/NaI (2 equiv) under the optimized conditions (3 h reaction time) giving lactone **13b** in a rewarding 70% yield (eq a). Conversely, neither the target **13b** or deoxygenated γ-lactone **12b** were able to convert to **16b** under the same conditions (eqs. b and c), supporting the notion that styrene **16b** is indeed a key reaction intermediate.

Thus, as shown in Scheme 3.5, we hypothesize that saturated δ-hydroxy-butanolide **11** is initially converted to its iodo-derivative, via a well-accepted reaction pathway that involves silylation of the secondary benzylic alcohol and subsequent nucleophilic displacement of the TMSO group by iodine anion.[15] At this stage, a concerted C1'-reduction/lactone opening sequence on **G**, promoted by the in situ generated hydrogen iodide, produces styrene intermediate **19** and molecular iodine, as testified by sudden turning of the whitish slurry to intense purple color.

Then, an intramolecular hydrolactonization reaction occurs,[25] in which the styrene double bond is first protonated generating a secondary benzylic carbocation that easily undergoes 6-*exo*-*trig* ring closure by the carboxylic group. This mechanistic proposal may explain why the *para*-alkoxy (or *ortho*-alkoxy) substitution was necessary for a fast and efficient ring expansion reaction. In fact, the presence of such substituents may favor the alkene protonation (possibly through the previously generated HI), with the transient formation of a *para*-quinone methide *p*QM (or *ortho*-quinone methide) intermediate, that may undergo a subsequent intramolecular 1,6-nucleophilic addition by the carboxylic function to afford the δ-valerolactone product. In line with this mechanistic hypothesis, the chiral lactone product emerges as a racemate since the stereocenters of the starting lactone are lost during the reaction path. To the best of our knowledge, this is the first example of a

tandem deoxygenation/hydrolactonization reaction involving five to six membered ring expansion.



Scheme 3.5. *Proposed mechanism for the "one pot" reductive ring expansion reaction via tandem deoxygenation/hydrolactonization sequence.*

3.3. Stereochemical assessment

The relative and absolute configuration of major *anti*-butenolides **10a-f, 10h,** and **10i**, were assigned as (5*R*,1'*S*) by analogy with the corresponding *p*-bromophenyl analogue (5*R*,1'*S*)-**17** obtained via a similar (*R,R*)-**E** promoted VMAR reaction, and whose structure was certified by X-ray analysis, as reported in previous paper (Figure 3.2).[10b, 13]

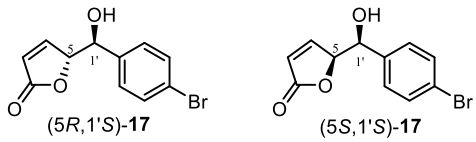


Figure 3.2. *Chemical Structure of reference compounds (5R,1'S)-**17** and (5S,1'S)-**17** whose absolute configuration was determined in a previous work by X-ray analysis (see ref. 10b)*

Furthermore, (5*R*) absolute configuration of VMAR products **10b**, **10d**, and **10h**, were confirmed by chemical correlation to the corresponding products (−)-**1**, (−)-**2**, and (−)-**3** which are known compounds.

Similarly, relative and absolute configuration of major *syn*-butenolides **10g**, and **10m**, were assigned as (5*S*,1′*S*) by analogy with the corresponding *p*-bromophenyl analogue (5*S*,1′*S*)-**17** obtained via a similar (*R,R*)-**E** promoted VMAR reaction, and whose structure was certified by X-ray analysis, as reported in our previous paper.[9b]

In addition, C5-C1′ *anti* or *syn* relative configuration were confirmed by comparison of the diagnostic H1′-NMR chemical shift data and $^{3}J_{5,1'}$ values as described in Table 3.6 and Chart 3.1. Accordingly, a chemical shift of the H1′ proton between 4.9 and 5.1 ppm with a $^{3}J_{5,1'}$ between 2.8 and 4.6 relate to an *anti*-configured butenolide (Table 3.6, left); while, a more shielded signal between 4.5 and 4.7 ppm with a larger $^{3}J_{5,1'}$ between 6.8 and 7.3 account for a *syn*-configured butenolide (Table 3.6, right).

Relative configuration of major adducts **10k**, **10l**, **10n**, and **10o** were assigned as 5,1′-*anti* by analogy to compound *anti*-**17** as described in Table 3.6, and Chart 3.1. Moreover, C5-absolute configuration of compounds *ent*-**10k** and *ent*-**10o** were confirmed to be *R* by chemical correlation to the corresponding valerolactone products (-)-**4** and (-)-**5** which are known compounds (see text). Consequently, the reported (5*S*,1′*R*)-configuration of **10k** and **10o** was established, and by analogy **10l** and **10n**.

Table 3.6. *^{1}H NMR (H1') and $^{3}J_{5,1'}$ data comparison between known butenolides anti-**17** (left) and syn-**17** (right) and titled compounds **10a-o**.*[a]

anti			***syn***	
H1'-NMR (ppm)	**$^{3}J_{5,1'}$ (Hz)**	**Compounds**	**$^{3}J_{5,1'}$ (Hz)**	**H1'-NMR (ppm)**
5.07	4.4	**17**[10b]	6.8	4.75
5.00	4.6	**10a**	7.1	4.63
4.90	4.6	**10b**	7.0	4.58
5.02	4.1	**10c**	7.2	4.65
5.09	4.0	**10d**	7.1	4.65
5.11	3.9	**10e**	7.1	4.70
5.10	4.0	**10f**	7.1	4.68
4.99	4.4	**10g**	7.2	4.57
4.96	3.9	**10h**	7.3	4.59
5.06	3.7	**10i**	7.0	4.57
5.05	3.8	**10j**	7.3	4.54
5.00	4.2	**10k**	7.2	4.60
5.07	4.1	**10l**	7.0	4.63
5.10	3.0	**10m**	7.3	4.49
4.97	4.1	**10n**	7.3	4.52
5.01	2.8	**10o**	7.1	4.65

[a] All reported data for purified major adducts were determined by ^{1}H NMR (300/400 MHz, $CDCl_3$) analysis; while for minor adducts, data were extrapolated from the ^{1}H NMR (300/400 MHz, $CDCl_3$) analysis of the crude.

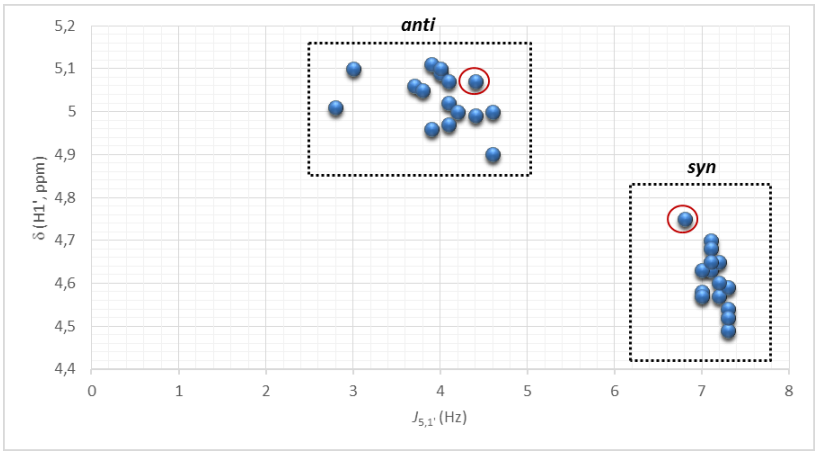


Chart 3.1. *Graphical representation of δ(ppm)/$J_{5,1'}$(Hz) data comparison reported in Table 6. Red-circled data refer to anti-**17** and syn-**17** values.*

3.4. Conclusion

We planned and developed a rapid approach for the synthesis of highly enantioenriched hydroxyphenyl γ-valerolactones, which are biologically effective and scarcely available metabolites of plant-derived flavan-3-ols. We also fortuitously discovered and developed a concise route for the synthesis of racemic hydroxyphenyl δ-valerolactones, the unnatural expanded isomeric counterparts of γ-lactones. After a common key initial step - an enantioselective and catalytic vinylogous Mukaiyama aldol reaction between 2-triisopropylsilyloxufuran and alkoxy-substituted benzaldehydes – the two paths diverge: a stereoconservative reduction/deoxygenation sequence gives the five-membered rings, while a one-pot deoxygenation/hydrolactonization reaction produce the six membered rings. Good yields were generally observed, and good to excellent diastereo- and enantioselectivities were attained for δ-lactones. We believe that this work nicely testifies how a basic reaction (i.e. the VMAR) can be advanced toward the synthesis of bioactive natural products and the discovery of unprecedented reaction itineraries.

3.5. Experimental section

Typical Procedure for the VMAR between 8 and alkoxy-benzaldehydes 9; Representative Procedure 1

Diisopropylethylamine (DIPEA, 7.3 µL, 0.04 mmol) was added via syringe to a solution of bisphosphoramide **I** (10.5 mg, 0.01 mmol) in anhydrous CH_2Cl_2 (2.0 mL), under argon atmosphere at room temperature. To this solution, aldehyde **9** (0.6 mmol) dissolved in anhydrous CH_2Cl_2 (0.8 mL), was added in one portion, and the reaction mixture was cooled to –78 °C under stirring. After 15 min, a solution of freshly distilled $SiCl_4$ (71 µL, 0.6 mmol) in anhydrous CH_2Cl_2 (0.7 mL) was added to the reaction mixture, followed by the dropwise addition of furan **8** (100 mg, 0.4 mmol, 1.0 equiv) dissolved in 0.5 mL of anhydrous CH_2Cl_2. The resulting mixture was stirred at –78 °C for 12 h, whereupon chilled CH_2Cl_2 (5.0 mL) was added and the cold reaction mixture was poured into a rapidly stirring solution of 1:1 sat. aq $NaHCO_3$/brine (25 mL) at 0 °C. This biphasic mixture was stirred vigorously for 2 h after which the organic layer was extracted with EtOAc (3×20 mL). The combined organic extracts were dried over Na_2SO_4, filtered and the filtrate was concentrated in vacuo. The residue was purified by silica gel flash chromatography to afford the desired adduct **10**. Catalyst **I** could be recovered (~90%) by eluting the flash column with 90:10 (EtOAc/MeOH-NH_3) mixture.

(5*R*,1'*S*)-10a: R_f = 0.30 (70/30 petroleum ether/EtOAc); $[\alpha]_D^{20}$ = +138.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 (dd, *J* = 5.8, 1.5 Hz, 1H, H4), 7.25 (m, 2H, Ar), 6.87 (m, 2H, Ar), 6.18 (dd, *J* = 5.8, 2.0 Hz, 1H, H3), 5.15 (ddd, *J* = 4.6, 1.8, 1.8 Hz, 1H, H5), 5.00 (d, *J* = 4.6 Hz, 1H, H1'), 1.95 (bs, OH), 0.99 (s, 9H, *tert*-Bu, TBS), 0.21 (s, 6H, CH_3, TBS); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.9 (Cq, C2), 156.2

(CH, C4), 153.1 (Cq, Ar), 131.0 (Cq, Ar), 127.5 (2C, CH, Ar), 123.4 (CH, C3), 120.6 (2C, CH, Ar), 86.6 (CH, C5), 73.3 (CH, C1'), 25.9 (3C, CH_3, *tert*-Bu, TBS), 18.4 (Cq, *tert*-Bu, TBS), −4.2 (2C, CH_3, TBS); HR-MS (ESI): *m/z* = 321.1502, calcd. for $[C_{17}H_{24}O_4Si + H]^+$: 321.1522. ee%: Chiral HPLC (Chiralcel OD-H, 95/5 Hexane/Ethanol, 1.0 mL/min, 254 nm): *R*t 11.12 min (minor), 12.17 min (major) (97% ee).

(5*R*,1'*S*)-10c: R_f = 0.29 (65/35 petroleum ether/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35-7.47 (m, 6H, CH_2Ph, H4), 7.34 (m, 2H, Ar), 7.02 (m, 2H, Ar), 6.19 (dd, *J* = 5.8, 1.9 Hz, 1H, H3), 5.15 (ddd, *J* = 4.5, 1.7, 1.7 Hz, 1H, H5), 5.10 (s, 2H, CH_2Ph), 5.02 (dd, *J* = 4.1, 4.1 Hz, 1H, H1'), 2.67 (d, *J* = 3.9 Hz, OH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.2 (Cq, C2), 159.1 (Cq, Ar), 153.2 (CH, C4), 136.9 (Cq, Ph), 130.9 (Cq), 128.8 (2C, CH, CH_2Ph), 128.3 (CH, CH_2Ph), 127.7 (2C, CH, CH_2Ph), 127.5 (2C, CH, Ar), 123.3 (CH, C3), 115.3 (2C, CH, Ar), 86.7 (CH, C5), 73.1 (CH, C1'), 70.3 (CH_2, CH_2Ph). HR-MS (ESI) m/z = 297.1132 $[M + H]^+$Calcd.: m/z 297.1126 $[C_{18}H_{16}O_4 + H]^+$. Opt. Rot. $[\alpha]_D^{20}$ +160.5 (*c* 1.0 g/100mL, $CHCl_3$); ee%: Chiral HPLC (Welk O1, 90/10 Hexane/Ethanol, 1.0 mL/min, 254 nm): *R*t 25.28 min (major), 27.81 min (minor) (99% ee).

(5*R*,1'*S*)-10d: R_f = 0.33 (70/30 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ +113.0 (c 1.1 g/100mL, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30 (dd, *J* = 5.8, 1.5 Hz, 1H, H4), 7.28 (m, 1H, Ar), 6.98 (bd, *J* = 7.6 Hz, 1H, Ar), 6.90 (m, 1H, Ar), 6.82 (dd, *J* = 8.0, 2.3 Hz, 1H, Ar), 6.18 (dd, *J* = 5.8, 1.8 Hz, 1H, H3), 5.17 (ddd, *J* = 4.1, 1.8, 1.8 Hz, 1H, H5), 5.09 (dd, *J* = 4.0, 4.0 Hz, 1H, H1'), 2.90 (d, *J* = 4.0 Hz, 1H, OH), 0.99 (s, 9H, *tert*-Bu, TBS), 0.22 (s, 6H, CH_3, TBS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.3 (Cq, C2), 156.2 (CH C4), 153.0 (Cq, Ar), 140.0 (CH, Ar), 130.0 (Cq, Ar), 123.4 (CH, C3), 120.2 (CH, Ar), 119.0 (CH, Ar), 117.9 (CH, Ar), 86.8 (CH, C5), 72.8 (CH, C1´), 25.8 (3C, *tert*-Bu, TBS), 18.38 (Cq, *tert*-Bu, TBS), −4.21 (2C, CH3, TBS).

HR-MS (ESI) = m/z 321.1535 $[M + H]^+$Calcd.: m/z 321.1522 $[C_{17}H_{24}O_4Si + H]^+$; ee%: Chiral HPLC (Chiralcel OD-H, 90/10 Hexane/Ethanol, 0.6 mL/min, 254 nm): *R*t 12.27 min (major), 13.88 min (minor) (90% ee).

(5*R*,1'*S*)-10e: R_f = 0.25 (60/40 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ +125.4 (c 1.0 g/100mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36 (m, 2H, H4, Ar), 6.98 (m, 2H, Ar), 6.90 (dd, *J* = 8.9, 1.8 Hz, 1H, Ar), 6.18 (dd, *J* = 5.6, 1.2 Hz, 1H, H3), 5.20 (m, 1H, H5), 5.11 (d, *J* = 3.9 Hz, 1H, H1'), 3.84 (s, 3H, OMe), 2.90 (bs, 1H, OH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.2 (Cq, C2), 160.1 (Cq, Ar), 153.0 (CH, C4), 140.1 (Cq, Ar), 130.0 (CH, Ar), 123.4 (CH, C3), 118.4 (CH, Ar), 114.1 (CH, Ar), 111.8 (CH, Ar), 86.7 (CH, C5), 73.0 (CH, C1'), 55.5 (CH_3, OMe). HR MS (ESI) m/z 221.0843 $[C_{12}H_{12}O_4 + H]^+$; Calcd.: m/z 221.0813. ee%: Chiral HPLC (WELK-O1, 85/15 Hexane/Ethanol, 1.0 mL/min, 254 nm): *R*t 11.18 min (major), 12.30 min (minor) (82% ee).

(5*R*,1'*S*)-10f: R_f = 0.31 (65/35 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ +28.6 (c 1.0 g/100mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35-7.47 (m, 6H, Ar), 7.25 (dd, *J* = 5.8, 1.5 Hz, 1H, H4), 7.05 (dd, *J* = 2.0, 2.0 Hz, 1H, Ar), 6.99 (m, 2H, Ar), 6.16 (dd, *J* = 5.8, 2.0 Hz, 1H, H3), 5.16 (ddd, *J* = 4.1, 1.8, 1.8 Hz, 1H, H5), 5.11 (s, 2H, $\underline{CH_2}$Ph), 5.10 (dd, *J* = 4.0, 4.0 Hz, 1H, H1´), 2.86 (bd, *J* = 4.0 Hz, 1H, OH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.2 (Cq, C2), 159.2 (Cq, Ar), 152.9 (CH, C4), 140.0 (Cq, Ar), 136.9 (Cq, Ph), 130.1 (CH, Ar) 128.8 (2C, CH, $CH_2\underline{Ph}$), 128.2 (CH, Ph), 127.7 (2C, $CH_2\underline{Ph}$), 123.4 (CH, C3), 118.7 (CH, Ar), 115.1 (CH, Ar), 112.7 (CH, Ar), 86.7 (CH, C5), 72.9 (CH, C1'), 70.2 (CH_2, $\underline{CH_2}$Ph). HR-MS (ESI): m/z = 297.1142 $[M + H]^+$; ee%: Chiral HPLC (WELK-O1, 90/10 Hexane/Ethanol, 1.0 ml/min, 254 nm): *R*t 17.84 min (major), 19.94 min (minor) (20 %ee).

(5*S*,1'*S*)-10g: R_f = 0.37 (70/30 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ $[\alpha]_D^{20}$ = − 68.9 (c 0.5 g/100mL, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.14 (dd, *J* = 5.8, 1.6 Hz, 1H, H4), 6.79-6.86 (m, 3H, Ar), 6.12 (dd, *J* = 5.8, 1.9 Hz, 1H, H3), 5.11 (ddd, *J* = 7.3, 1.8, 1.8 Hz, 1H, H5), 4.57 (d, *J* = 7.2 Hz, 1H, H1'), 2.66 (bs, OH), 1.00 (s, 9H, *tert*-Bu, TBS), 0.99 (s, 9H, *tert*-Bu, TBS), 0.17 (s, 12H, CH_3, TBS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.7 (Cq, C2), 153.4 (CH, C4), 147.7 (Cq, Ar), 147.3 (Cq, Ar), 131.0 (Cq, Ar), 123.0 (CH, C3), 121.4 (CH, Ar), 120.0 (CH, Ar), 119.7 (CH, Ar), 87.3 (CH, C5), 75.6 (CH, C1'), 26.1 (6C, CH_3, *tert*-Bu, TBS), 18.6 (2C, Cq, *tert*-Bu, TBS), −3.89 (4C, CH_3, TBS). HR-MS (ESI): m/z 451.2346 $[M + H]^+$; Calcd.: 451.2336 $[C_{23}H_{38}O_5Si_2 + H]^+$; ee%: Chiral HPLC (Chiralcel OD-H, 95/5 Hexane/Ethanol, 1.0 mL/min, 254 nm): *R*t 6.40 min (minor), 6.86 min (major) (95% ee).

(5*R*,1'*S*)-10h: R_f = 0.43 (65/35 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ +100 (c 0.3 g/100mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33-7.44 (m, 6H, Ph, H4), 6.91 (m, 3H, Ar), 6.17 (dd, *J* = 5.8, 1.8 Hz, 1H, H3), 5.13 (ddd, *J* = 4.4, 2.2, 2.2 Hz, 1H, H5), 5.06 (s, 2H, $\underline{CH_2}$Ph), 4.96 (dd, *J* = 3.9, 3.9 Hz, 1H, H1'), 2.43 (d, *J* = 3.7 Hz, 1H, OH), 0.94 (s, 9H, *tert*-Bu, TBS), 0.09 (s, 6H, CH_3, TBS). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.1 (Cq, C2), 153.0 (CH, C4), 150.6 (Cq, Ar), 145.8 (Cq, Ar), 136.9 (Cq, Ph), 131.3 (Cq, Ar), 128.7 (2C, CH, Ph), 128.2 (CH, Ph), 128.0 (2C, CH, Ph), 123.4 (CH, C3), 119.5 (CH, Ar), 119.0 (CH, Ar), 114.1 (CH, Ar), 86.7 (CH, C5), 72.9 (CH, C5), 71.0 (CH_2, $\underline{CH_2}$Ph), 25.9 (3C, *tert*-Bu, TBS), 18.6 (Cq, *tert*-Bu, TBS), −4.4 (2C, CH_3, TBS). HR-MS (ESI): m/z 427.1961 $[M + H]^+$Calcd.: m/z 427.1940 $[C_{24}H_{30}O_5Si + H]^+$; ee%: Chiral HPLC (WELK-O1, 90/10 Hexane/Ethanol, 1.0 ml/min, 254 nm): *R*t 13.56 min (major), 15.46 min (minor) (99% ee).

(5*R*,1'*S*)-10i: Rf = 0.27 (65/35 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ +91.0 (c 1.0 g/100mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47 (m, 4H, Ph),

7.33-7.42 (m, 6H, Ph), 7.09 (dd, *J* = 5.8, 1.4 Hz, 1H, H4), 6.96 (d, *J* = 1.8 Hz, 1H, H2''), 6.85 (d, *J* = 8.2 Hz, 1H, H5''), 6.89 (dd, *J* = 8.3, 1.8 Hz, 1H, H6''), 6.12 (dd, *J* = 5.8, 1.8 Hz, 1H, H3), 5.25 (d, *J* = 12.3 Hz, 1H, $\underline{CH_2}$Ph), 5.20 (s, 2H, $\underline{CH_2}$Ph), 5.20 (d, *J* = 12.3 Hz, 1H, $\underline{CH_2}$Ph), 5.06 (ddd, *J* = 3.5, 1.8, 1.8 Hz, 1H, H5), 5.06 (dd, *J* = 3.7, 3.7 Hz, 1H, H1'), 2.90 (d, *J* = 3.6 Hz, 1H, OH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.9 (Cq, C2), 152.8 (CH, C4), 149.3 (Cq, Ar), 149.1 (Cq, Ar), 137.2 (Cq, Ph), 137.2 (Cq, Ph), 131.2 (Cq, Ar), 128.8 (4C, CH, Ph), 128.1 (CH, Ph), 128.1 (CH, Ph), 127.6 (2C, CH Ph), 127.5 (2C, CH Ph), 123.3 (CH, C2), 119.4 (CH, C6''), 115.0 (CH, C5''), 113.2 (CH, C2''), 86.5 (CH, C5), 73.0 (CH, C1'), 71.5 (CH_2, $\underline{CH_2}$Ph), 71.4 (CH_2, $\underline{CH_2}$Ph). HR-MS (ESI): m/z = 402.1485 $[M + H]^+$; ee%: Chiral HPLC (Chiralcel OD-H, 88/12 Hexane/Ethanol, 1 ml/min, 254 nm): *R*t 31.10 min (minor), 36.84 min (major) (93% ee).

(5*R*,1'*S*)-10j: R_f = 0.39 (80/20 petroleum ether/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30 (dd, *J* = 5.8, 1.3 Hz, 1H, H4), 6.51 (d, *J* = 2.0 Hz, 2H, H2'', H6''), 6.32 (t, *J* = 2.1 Hz, 1H, H4''), 6.20 (dd, *J* = 5.8, 1.8 Hz, 1H, H3), 5.16 (ddd, *J* = 3.9, 2.0, 2.0 Hz, 1H, H5), 5.05 (dd, *J* = 3.8, 3.8 Hz, 1H, H1'), 2.50 (dd, *J* = 3.3, 3,3 Hz, 1H, OH), 0.99 (s, 18H, *tert*-Bu, TBS), 0.22 (s, 12H, Me, TBS). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.0 (Cq, C2), 157.1 (2C, Cq, C3'', C5''), 152.7 (CH, C4), 140.4 (Cq, C1''), 123.5 (CH, C3), 112.2 (CH, C4''), 111.2 (2C, CH, C2'', C6''), 86.6 (CH, C5), 72.7 (CH, C1'), 25.9 (6C, CH_3, *tert*-Bu, TBS), 18.4 (2C, Cq, *tert*-Bu, TBS), −4.2 (4C, CH_3, TBS). HR-MS-(ESI): m/z 451.2361 $[M + H]^+$; Calcd.: m/z 451.2336 $[C_{23}H_{38}O_5Si_2 + H]^+$.

(5*S*,1'*R*)-10k: R_f = 0.27 (65/35 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ −108.4 (*c* 1.0 g/100mL, $CHCl_3$), Opt. Rot.(for *ent*-**11k**) $[\alpha]_D^{20}$ +109.0 (*c* 1.0 g/100mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33-7.46 (m, 10H, $CH_2\underline{Ph}$), 7.19 (dd, *J* = 5.8, 1. Hz, 1H, H4), 6.63 (m, 3H, Ar), 6.15 (dd, *J* = 5.8, 1.9 Hz, 1H, H3),

5.13 (ddd, *J* = 4.2, 1.9, 1.9 Hz, 1H, H5), 5.11 (m, 4H, $\underline{CH_2}Ph$), 5.06 (dd, *J* = 4.2 Hz, 1H, H1'), 2.58 (bs, 1H, OH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.0 (Cq, C2), 160.4 (2C, Cq, Ar), 152.8 (CH, C4), 140.7 (Cq, Ar), 136.8 (2C, Cq, $CH_2\underline{Ph}$), 128.8 (4C, CH, $CH_2\underline{Ph}$) 128.3 (2C, CH, $CH_2\underline{Ph}$), 127.7 (4C, CH, $CH_2\underline{Ph}$), 123.4 (CH, C3), 105.4 (2C, CH, Ar), 102.3 (CH, Ar), 86.5 (CH, C5), 73.0 (CH, C1'), 70.4 (2C, CH_2, $\underline{CH_2}Ph$). HR-MS-(ESI) m/z = 402.1482 $[M + H]^+$, Calcd.: m/z 402.1467 $[C_{25}H_{22}O_5 + H]^+$; ee%: Chiral HPLC (WELK-O1, 90/10 Hexane/Ethanol, 1.0 ml/min, 254 nm): *R*t 26.70 min (minor), 31.36 min (major) (94% ee).

(5*S*,1'*R*)-10l: R_f = 0.52 (40/60 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ −101.5 (*c* 1.0 g/100mL, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.35 (dd, *J* = 5.8, 1.3 Hz, 1H, H4), 6.56 (d, *J* = 2.1 Hz, 2H, H2'', H6''), 6.44 (dd, *J* = 2.2, 2.2 Hz, 1H, H4''), 6.19 (dd, *J* = 5.8, 1.8 Hz, 1H, H3), 5.20 (ddd, *J* = 4.1, 2.0, 2.0 Hz, 1H, H5), 5.07 (d, *J* = 4.1 Hz, 1H, H1'), 3.82 (s, 6H, OMe), 2.84 (bs, 1H, OH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.1 (Cq, C2), 161.3 (2C, C3'', C5''), 152.9 (CH, C4), 140.9 (Cq, Ar), 123.4 (CH, C3), 104.1 (2C, C2'', C6''), 100.4 (CH, C4''), 86.6 (CH, C5), 72.3 (CH, C1'), 55.6 (2C, CH_3, OMe). HR-MS-(ESI) = m/z 251.0919 $[C_{13}H_{14}O_5 + H]^+$; ee%: Chiral HPLC (WELK-O1, 85/15 Hexane/Ethanol, 1.0 ml/min, 254 nm): *R*t 15.36 min (minor), 16.98 min (major) (89% ee).

(5*S*,1'*S*)-10m: R_f = 0.23 (90/10 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ −26.5 (*c* 1.0 g/100mL, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.10 (dd, *J* = 5.8, 1.5 Hz, 1H, H4), 6.51 (s, 2H, C2'', C6''), 6.13 (dd, *J* = 5.8, 2.0 Hz, 1H, H3), 5.08 (ddd, *J* = 7.3, 1.8, 1.8 Hz, 1H, H5), 4.49 (d, *J* = 7.3 Hz, 1H, H1'), 2.88 (bs, OH), 0.98 (s, 9H, *tert*-Bu, TBS), 0.93 (s, 18H, *tert*-Bu, TBS), 0.21 (s, 6H, Me, TBS), 0.20 (s, 6H, Me, TBS), 0.11 (s, 6H, Me, TBS).^{13}C NMR (100 MHz, $CDCl_3$): δ 172.6 (Cq, C2), 153.2 (CH, C4), 149.1 (2C, Cq, C3'', C5''), 139.0 (Cq, Ar), 129.9 (Cq, Ar), 123.0 (CH, C3), 112.8 (2C, CH, C2'', C6''), 87.3 (CH, C5), 75.6 (CH, C1'), 26.3 (9C, CH_3, *tert*-Bu,

TBS), 19.0 (2C, Cq, *tert*-Bu, TBS), 18.6 (Cq, *tert*-Bu, TBS), −3.4 (4C, CH_3, TBS), −3.7 (2C, CH_3, TBS). HR-MS-(ESI) m/z = 581.3168 $[M + H]^+$; Calcd.: m/z 581.3149 $[C_{29}H_{52}O_6Si_3 + H]^+$; ee%: For compound **10m**, for which no conditions for direct measurement of the enantiomeric purity via chiral HPLC analysis were found, ee% value was calculated indirectly via chiral HPLC analysis of the corresponding saturated butanolide (5*S*,1′*S*)-**11m**: Chiral HPLC (CHIRALCEL OD-H, 97/3 Hexane/Ethanol, 0.7 ml/min, 254 nm): *R*t 8.77 min (major), 11.45 min (minor) (73%).

(5*S*,1′*R*)-10n: R_f = 0.50 (60/40 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ −43.8 (*c* 0.5 g/100mL, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.36-7.47 (m, 12H, CH_2Ph), 7.29 (m, 3H, CH_2Ph), 6.98 (dd, *J* = 5.8, 1.4 Hz, 1H, H4), 6.63 (s, 2H, C2′′, C6′′), 6.09 (dd, *J* = 5.8, 1.8 Hz, 1H, H3), 5.15 (s, 2H, CH_2Ph), 5.14 (s, 2H, CH_2Ph), 5.10 (s, 2H, CH_2Ph), 5.03 (ddd, *J* = 4.1, 1.9, 1.9 Hz, 1H, H5), 4.97 (dd, *J* = 4.1, 3.7 Hz, 1H, H1′), 2.40 (d, *J* = 3.7 Hz, 1H, OH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.0 (Cq, C2), 153.1 (2C, Cq), 152.7 (CH, C4), 138.5 (Cq), 137.8 (Cq), 137.1 (2C, Cq), 133.7 (Cq), 128.8 (6C, CH, Ph), 128.4 (2C, CH, Ph), 128.2 (2C, CH, Ph), 127.7 (5C, CH, Ph), 123.3 (CH, C3), 105.9 (2C, CH, Ar), 86.4 (CH, C5), 75.4 (CH_2, CH_2Ph), 73.0 (CH, C1′), 71.4 (2C, CH_2, CH_2Ph). HR-MS (ESI) m/z = 509.1991 $[M + H]^+$; Calcd.: m/z 509.1964 $[C_{32}H_{28}O_6 + H]^+$; ee%: Chiral HPLC (CHIRALCEL OD-H, 90/10 Hexane/Ethanol, 1.0 ml/min, 254 nm): *R*t 46.64 min (major), 56.24 min (minor) (69%).

(5*S*,1′*R*)-10o: R_f = 0.38 (25/75 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ −89.6 (*c* 1.0 g/100mL, $CHCl_3$), Opt. Rot. (for *ent*-**10o**) $[\alpha]_D^{20}$ +90.0 (*c* 1.0 g/100mL, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40 (dd, *J* = 5.8, 1.5 Hz, 1H, H4), 6.62 (s, 2H, H2′′, H6′′), 6.20 (dd, *J* = 5.8, 2.0 Hz, 1H, H3), 5.19 (ddd, *J* = 4.5, 1.9, 1.9 Hz, 1H, H5), 5.01 (dd, *J* = 2.8, 2.8 Hz, 1H, H1′), 3.88 (s, 6H, OMe), 3.85 (s, 3H,

OMe), 3.00 (d, J = 3.0 Hz, 1H, OH). ^{13}C NMR (100 MHz): δ 173.2 (Cq, C2), 153.7 (CH, C4), 153.2 (2C, Cq, C3'', C5''), 137.9 (Cq, Ar), 134.3 (Cq, Ar), 123.3 (CH, C3), 103.1 (2C, CH, C2'', C6''), 86.7 (CH, C5), 73.3 (CH, C1'), 61.0 (CH_3, OMe), 56.4 (2C, CH_3, OMe). HR-MS (ESI) m/z = 281.1053 $[M + H]^+$; Calcd.: m/z 281.1025 $[C_{14}H_{16}O_6 + H]^+$; ee%: For compound **10o**, for which no conditions for direct measurement of the enantiomeric purity via chiral HPLC analysis were found, ee% was calculated indirectly via chiral HPLC analysis of the corresponding saturated butanolide (5*S,*1'*R*)-**11o**: Chiral HPLC (Chiralcel OD-H, 90/10 Hexane/Ethanol, 1.0 mL/min, 254 nm): *R*t 22.65 min (major), 26.83 min (minor) (82% ee).

Typical Procedure for the butenolide reduction (Table 3, entry 5)

Representative Procedure 2

Preparation of (+)-(*R*)-5-[(*S*)-hydroxy(4-(methoxyphenyl)methyl]dihydrofuran-2(5H)-one (**11b**)**:** A solution of a 85:15 (*anti/syn*) mixture of butenolide **10b**[10b] (82 mg, 0.37 mmol, 1.0 equiv) in 4 mL of absolute MeOH, was cooled to 0 °C and treated with $NiCl_2$•$6H_2O$ (21.4 mg, 0.09 mmol). The resulting mixture was stirred at the same temperature for 5 min before the addition of $NaBH_4$ (15.5 mg, 0.41 mmol). After 30 min, the reaction mixture was quenched with saturated aqueous NH_4Cl solution and extracted with CH_2Cl_2 (3×5 mL). The combined extracts were dried ($MgSO_4$) and concentrated under vacuum. Flash chromatographic purification (elution by gradient from 60:40 to 45:55 Petroleum Ether/EtOAc) afforded 66.1 mg of saturated lactone (5*R*,1'*S*)-**11b** (major isomer) as an amorphous solid, and 11 mg of lactone (5S,1'S)-**11b** (minor isomer) as a colourless resin (95% isolated combined yield).

(5*R*,1'*S*)-11b: R_f 0.38 (40/60 petroleum ether/EtOAc); $[\alpha]_D^{20}$ = +58.3 (c 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31 (m, 2H, Ar), 6.91 (m, 2H, Ar), 5.04 (dd, *J* = 3.3, 3.3 Hz, 1H, H1'), 4.67 (ddd, *J* = 7.6, 6.6, 3.2 Hz, 1H, H5), 3.82 (s, 3H, OMe), 2.88 (bs, OH), 2.53 (ddd, *J* = 17.7, 10.2, 6.2 Hz, 1H, H3a), 2.43 (ddd, *J* = 17.7, 9.6, 7.4 Hz, 1H, H3b), 2.26 (dddd, *J* = 13.5, 10.1, 7.1, 7.1 Hz, 1H, H4a), 1.96 (dddd, *J* = 13.5, 9.9, 7.5, 6.5 Hz, 1H, H4b); ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.0 (Cq, C2), 159.5 (Cq, Ar), 130.8 (Cq, Ar), 127.5 (2C, CH, Ar), 114.1 (2C, CH, Ar), 83.6 (CH, C5), 73.3 (CH, C1'), 55.5 (CH3, OMe), 28.7 (CH_2, C3), 21.0 (CH_2, C4); HR-MS (ESI): *m/z* = 223.0994, calcd. for $[C_{12}H_{14}O_4 + H]^+$: 223.0970.

(5*R*,1'*S*)-11d: R_f = 0.36 (70/30 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ +32.8 (*c* 1.0 g/100mL, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23 (dd, *J* = 7.8, 7.8 Hz, 1H, Ar), 6.97 (bd, *J* = 7.6 Hz, 1H, Ar), 6.91 (bs, Ar), 6.78 (dd, *J* = 8.0, 1.8 Hz, 1H, Ar), 5.10 (dd, *J* = 3.3, 3.3 Hz, 1H, H1'), 4.68 (ddd, *J* = 7.8, 6.2, 2.9 Hz, 1H, H5), 3.10 (d, *J* = 3.9 Hz, 1H, OH), 2.63 (ddd, *J* = 17.7, 10.2, 6.1 Hz, 1H, H3a), 2.44 (ddd, *J* = 17.7, 10.0, 7.3 Hz, 1H, H3b), 2.28 (dddd, *J* = 13.0, 10.2, 7.0, 6.2 Hz, 1H, H4a), 1.97 (dddd, *J* = 13.2, 10.1, 7.8, 6.2 Hz, 1H, H4b), 1.00 (s, 9H, *tert*-Bu, TBS), 0.21 (s, 6H, Me, TBS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.3 (Cq, C2), 156.1 (Cq, Ar), 140.3 (CH, Ar), 129.7 (Cq, Ar), 119.7 (CH, Ar), 119.1 (CH, Ar), 118.0 (CH, Ar), 83.7 (CH, C5), 73.1 (CH, C1'), 28.8 (CH_2, C3), 25.8 (3C, CH_3, *tert*-Bu, TBS), 20.6 (CH_2, C4), 18.4 (Cq, tert-Bu, TBS), −4.2 (2C, CH_3, TBS). HR-MS-(ESI) m/z 323.1692 $[M + H]^+$; Calcd.: m/z 323.1678 $[C_{17}H_{26}O_4Si + H]^+$.

(5*R*,1'*S*)-11h: R_f = 0.32 (70/30 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ +28.5 (*c* 1.0 g/100mL, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34-7.46 (m, 5H, Ph), 6.91 (m, 3H, Ar), 5.06 (s, 2H, CH_2Ph), 5.03 (d, *J* = 3.1 Hz, 1H, H1'), 4.66 (ddd, *J* = 7.7, 6.5, 3.2 Hz, 1H, H5), 2.56 (ddd, *J* = 17.7, 10.0, 6.0 Hz, 1H, H3a), 2.45 (ddd, *J* = 17.7, 9.8, 7.6 Hz, 1H, H3b), 2.27 (dddd, *J* = 12.9, 10.2, 7.4, 6.5 Hz, 1H,

H4a), 1.98 (dddd, *J* = 13.5, 9.6, 7.6, 6.1 Hz, 1H, H4b), 0.96 (s, 9H, *tert*-Bu, TBS), 0.10 (s, 6H, Me, TBS). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.8 (Cq, C2), 150.2 (Cq, Ar), 145.7 (Cq, Ar), 137.0 (Cq, Ph), 131.6 (Cq, Ar), 128.6 (2C, CH, Ph), 128.2 (CH, Ph), 128.0 (2C, CH, Ph), 119.5 (CH, Ar), 119.1 (CH, Ar), 114.1 (CH, Ar), 83.5 (CH, C5), 73.2 (CH, C1'), 71.0 (CH_2, $\underline{CH_2}$Ph), 28.8 (CH_2, C3), 25.9 (3C, CH_3, *tert*-Bu, TBS), 20.9 (CH_2, C4), 18.6 (Cq, *tert*-Bu, TBS), −4.36 (CH_3, TBS), −4.38 (CH_3, TBS). HR-MS-(ESI) m/z = 429.2021 $[M + H]^+$; Calcd.: m/z 429.2097 $[C_{24}H_{32}O_5Si + H]^+$.

(5*R*,1'*S*)-11k: R_f = 0.44 (55/45 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ +27.8 (*c* 1.0 g/100mL, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32-7.46 (m, 10H, Ph), 6.64 (bd, *J* = 2.2 Hz, 2H, H2'', H6''), 6.59 (t, *J* = 2.2 Hz, 1H, H4''), 5.06 (m, 5H, $\underline{CH_2}$Ph, H1'), 4.66 (ddd, *J* = 7.7, 6.4, 3.0 Hz, 1H, H5), 2.56 (ddd, *J* = 17.8, 10.2, 6.0 Hz, 1H, H3a), 2.44 (ddd, *J* = 17.7, 9.9, 7.6 Hz, 1H, H3b), 2.21 (dddd, *J* = 13.5, 9.9, 7.7, 6.0 Hz, 1H, H4a), 1.90 (dddd, *J* = 13.5, 9.9, 7.7, 6.0 Hz, 1H, H4b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.9 (Cq, C2), 160.1 (2C, Cq, C3'', C5''), 140.9 (Cq, C1''), 136.6 (2C, Cq, Ph), 128.6 (4C, CH, Ph), 128.1 (2C, CH, Ph), 128.0 (4C, CH, Ph), 105.2 (2C, CH, C2'', C6''), 101.6 (CH, C4''), 83.1 (CH, C5), 73.3 (CH, C1'), 70.1 (2C, CH_2, $\underline{CH_2}$Ph), 28.6 (CH_2, C3), 20.6 (CH_2, C4). HR-MS (ESI) m/z = 405.1711 $[M + H]^+$; Calcd.: m/z 405.1702 $[C_{25}H_{24}O_5 + H]^+$.

(5*R*,1'*S*)-11o: R_f = 0.41 (25/75 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ +24.1 (*c* 0.6 g/100mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.60 (s, 2H, H2'', H6''), 5.01 (d, *J* = 1.9 Hz, 1H, H1'), 4.68 (ddd, *J* = 7.7, 6.3, 3.2 Hz, 1H, H5), 2.81 (bs, OH), 3.86 (s, 6H, OMe), 3.83 (s, 3H, OMe), 2.56 (dddd, *J* = 17.8, 10.1, 6.1, 1.7 Hz, 1H, H3a), 2.45 (ddd, *J* = 17.7, 10.0, 7.4 Hz, 1H, H3b), 2.26 (dddd, *J* = 13.3, 10.2, 7.3, 6.3 Hz, 1H, H4a), 1.98 (dddd, *J* = 13.6, 10.1, 7.7, 6.0 Hz, 1H, H4b). ^{13}C NMR (100 MHz): δ 177.9 (Cq, C2), 153.6 (2C, Cq, C3'', C5''), 137.8 (Cq, Ar), 134.4 (Cq, Ar), 103.2 (2C, CH, C2'', C6''), 83.4 (CH, C5), 73.7 (CH, C1'), 61.0 (CH_3, OMe),

56.4 (2C, CH_3, OMe), 28.8 (CH_2, C3), 21.1 (CH_2, C4). HR-MS (ESI) m/z = 283.1195 $[M + H]^+$, Calcd.: m/z 283.1181 $[C_{14}H_{18}O_6 + H]^+$; ee%: Chiral HPLC (WELK-O1, 80/20 Hexane/Ethanol-0.1%TFA, 0.9 ml/min, 254 nm): *R*t 33.24 min (major), 36.06 min (minor) (91.0:9.0 er).

Typical Procedure for Barton-McCombie deoxygenatiion

Representative Procedure 3

Preparation of (*R*)-5-(4-methoxybenzyl)dihydrofuran-2(3*H*)-one (–)-**12b.** According to a known procedure,[16b] thionocarbonyldiimidazole (TCDI) (107 mg, 0.6 mmol) was added to a solution of butanolide **11b** (66 mg, 0.3 mmol, 1.0 equiv) in anhydrous THF (5 mL). The mixture was heated at reflux temperature for 7 h and then cooled, and the solvent was evaporated to yield thiocarbamate intermediate **15b** (74 mg) as a crude that was used as such, without further purifications.

A solution of crude thiocarbamate **15b** (74 mg, 0.22 mmol) in toluene (7 mL) containing AIBN (0.2 M solution in toluene, 1.1 mL, 0.22 mmol) was added by a syringe, within 20 min, to a refluxing mixture of tri-*n*-butyltinhydride (237 μL, 0.88 mmol) in toluene (15 mL). The mixture was heated under reflux for 1 h and then the solvent was evaporated, and the residue was chromatographed on silica gel (75/25 Petroleum Ether/EtOAc) to give pure (–)-**12b** (43 mg, 71% two steps) as a colourless resin.

(–)-12b: R_f 0.50 (70/30 petroleum ether/EtOAc); $[\alpha]_D^{20}$ = −8.7 (c 0.9, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.16 (m, 2H, Ar), 6.87 (m, 2H, Ar), 4.71 (dddd, *J* = 6.7, 6.7, 6.7, 6.7 Hz, 1H, H5), 3.80 (s, 3H, OMe), 3.01 (dd, *J* = 14.1, 5.9 Hz, 1H, H1'a), 2.89 (dd, *J* = 14.1, 6.2 Hz, 1H, H1'b), 2.47 (ddd, *J* = 17.8, 9.3, 9.3 Hz, 1H, H3a), 2.36 (ddd, *J* = 17.7, 9.2, 4.7 Hz, 1H, H3b), 2.23 (dddd, *J* = 12.6, 9.7, 6.7, 4.9 Hz,

1H, H4a), 1.95 (dddd, *J* = 12.8, 9.2, 9.2, 7.7 Hz, 1H, H4b); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.3 (Cq, C2), 158.8 (Cq, Ar), 130.7 (2C, CH, Ar), 128.0 (Cq, Ar), 114.2 (2C, CH, Ar), 81.1 (CH, C5), 55.5 (CH_3, OMe), 40.6 (CH_2, C1') 28.8 (CH_2, C3), 27.1 (CH_2, C4); HR-MS (ESI): *m/z* = 207.1030, calcd. for $[C_{12}H_{14}O_3 + H]^+$: 207.1021.

(–)-12d: TLC: R_f = 0.31 (75/25 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ −4.3 (*c* 1.0 g/100mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.17 (dd, *J* = 7.8, 7.8 Hz, 1H, Ar), 6.82 (bd, *J* = 7.6 Hz, 1H, Ar), 6.74 (dd, *J* = 8.1, 1.7 Hz, 1H, Ar), 6.70 (dd, *J* = 2.0, 2.0 Hz, 1H, Ar), 4.71 (dddd, *J* = 6.7, 6.7, 6.7, 6.7 Hz, 1H, H5), 3.05 (dd, *J* = 13.9, 5.9 Hz, 1H, H1'a), 2.86 (dd, *J* = 13.9, 6.6 Hz, 1H, H1'b), 2.48 (ddd, *J* = 18.6, 9.8, 9.8 Hz, 1H, H3a), 2.40 (ddd, *J* = 17.7, 9.3, 4.8 Hz, 1H, H3b), 2.24 (dddd, *J* = 12.8, 9.5, 6.7, 4.8 Hz, 1H, H4a), 1.95 (dddd, *J* = 12.9, 9.3, 9.3, 7.6 Hz, 1H, H4b), 0.98 (s, 9H, *tert*-Bu, TBS), 0.19 (s, 6H, Me, TBS).^{13}C NMR (75 MHz, $CDCl_3$): δ 177.2 (Cq, C2), 156.0 (Cq, Ar), 137.5 (CH, Ar), 129.7 (Cq, Ar), 122.6 (CH, Ar), 121.4 (CH, Ar), 118.8 (CH, Ar), 80.9 (CH, C5), 41.3 (CH_2, C1') 28.8 (CH_2, C3), 27.3 (CH_2, C4), 25.8 (3C, CH_3, *tert*-Bu, TBS), 18.4 (Cq, *tert*-Bu, TBS), −4.2 (2C, CH_3, TBS). HR-MS-(ESI) m/z 307.1738 $[M + H]^+$, Calcd.: m/z 307.1729 $[C_{17}H_{26}O_3Si + H]^+$.

(–)-12h: R_f = 0.53 (70/30 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ −4.8 (*c* 1.0 g/100mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32-7.45 (m, 5H, Ph), 6.85 (m, 1H, Ar), 6.74 (m, 2H, Ar) 5.03 (s, 2H, $\underline{CH_2}$Ph), 4.68 (dddd, *J* = 6.6, 6.6, 6.6, 6.6 Hz, 1H, H5), 2.97 (dd, *J* = 14.0, 5.7 Hz, 1H, H1'a), 2.81 (dd, *J* = 14.0, 6.4 Hz, 1H, H1'b), 2.45 (ddd, *J* = 17.9, 9.3, 9.3 Hz, 1H, H3a), 2.38 (ddd, *J* = 17.7, 9.3, 4.8 Hz, 1H, H3b), 2.24 (dddd, *J* = 12.8, 9.5, 6.7, 4.8 Hz, 1H, H4a), 1.93 (dddd, *J* = 12.8, 9.2, 9.2, 7.4 Hz, 1H, H4b), 0.97 (s, 9H, *tert*-Bu, TBS), 0.12 (s, 3H, CH_3, TBS), 0.11 (s, 3H, CH_3, TBS). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.2 (Cq, C2), 149.6 (Cq, Ar), 145.6 (Cq, Ar), 137.2 (Cq, Ph), 129.0 (Cq, Ar), 128.6 (2C, CH, Ph), 128.1 (CH, Ph),

128.0 (2C, CH, Ph), 122.8 (CH, Ar), 122.5 (CH, Ar), 114.5 (CH, Ar), 81.0 (CH, C5), 71.1 (CH_2, $\underline{CH_2}$Ph), 40.7 (CH_2, C1'), 28.8 (CH_2, C3), 27.1 (CH_2, C4), 25.9 (3C, CH_3, *tert*-Bu, TBS), 18.58 (Cq, *tert*-Bu, TBS), −4.36 (2C, CH_3, TBS). HRMS(ESI) m/z = 413.2162 $[M + H]^+$ Calcd.: m/z 413.2148 $[C_{24}H_{32}O_4Si + H^+]$.

(–)-12k: R_f = 0.44 (70/30 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ −10.2 (*c* 1.0 g/100mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28-7.45 (m, 10H, Ph), 6.55 (bs, 2H, H2'', H6''), 6.49 (bs, 1H, H4''), 5.04 (s, 4H, $\underline{CH_2}$Ph), 4.72 (dddd, *J* = 6.6, 6.6, 6.6, 6.6 Hz, 1H, H5), 3.03 (dd, *J* = 14.0, 5.9 Hz, 1H, H1'a), 2.86 (dd, *J* = 13.9, 6.4 Hz, 1H, H1'b), 2.47 (ddd, *J* = 17.9, 9.2, 9.2 Hz, 1H, H3a), 2.38 (ddd, *J* = 17.8, 9.4, 4.9 Hz, 1H, H3b), 2.22 (dddd, *J* = 12.3, 9.9, 6.7, 5.4 Hz, 1H, H4a), 1.92 (dddd, *J* = 13.0, 9.2, 9.2 Hz, 1H, H4b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.9 (Cq, C2), 160.2 (2C, Cq, C3'', C5''), 138.3 (Cq, C1''), 136.9 (2C, Cq, Ph), 128.7 (4C, CH, Ph), 128.2 (2C, CH, Ph), 127.7 (4C, CH, Ph), 108.9 (2C, CH, C2'', C6''), 100.8 (CH, C4''), 80.7 (CH, C5), 70.3 (2C, CH_2, $\underline{CH_2}$Ph), 41.7 (CH_2, C1') 28.8 (CH_2, C3), 27.2 (CH_2, C4); HR-MS (ESI) m/z = 389.1768 $[M + H]^+$ Calcd.: m/z 389.1752 $[C_{25}H_{24}O_4 + H]^+$.

(–)-12o: R_f = 0.47 (25/75 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ −12.6 (*c* 1.0 g/100mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.60 (s, 2H, H2'', H6''), 4.75 (dddd, *J* = 6.6, 6.6, 6.6, 6.6 Hz, 1H, H5), 3.85 (s, 6H, OMe), 3.83 (s, 3H, OMe), 2.97 (dd, *J* = 14.1, 6.0 Hz, 1H, H1'a), 2.90 (dd, *J* = 14.1, 5.7 Hz, 1H, H1'b), 2.48 (ddd, *J* = 17.8, 9.1, 9.1 Hz, 1H, H3a), 2.37 (ddd, *J* = 17.7, 9.4, 4.7 Hz, 1H, H3b), 2.28 (dddd, *J* = 12.8, 9.8, 6.8, 4.7 Hz, 1H, H4a), 1.96 (dddd, *J* = 12.8, 9.3, 9.3, 7.5 Hz, 1H, H4b);^{13}C NMR (100 MHz, $CDCl_3$): δ 177.2 (Cq, C2), 153.5 (2C, Cq, C3'', C5''), 137.2 (Cq, Ar), 131.8 (Cq, Ar), 106.6 (2C, CH, C2'', C6''), 80.9 (CH, C5), 61.0 (CH_3, OMe), 56.3 (2C, CH_3, OMe), 41.8 (CH_2, C1'), 28.8 (CH_2, C3), 21.1 (CH_2, C4). HRMS(ESI) m/z = 267.1220 $[M + H]^+$ Calcd.: m/z 267.1232 $[C_{14}H_{18}O_5 + H]^+$.

Preparation of final γ-valerolactone targets:

(*R*)-5-(4-hydroxybenzyl)dihydrofuran-2(3*H*)-one (−)-**1** **(Representative Procedure 4)** To a solution of protected γ-valerolactone (−)-**12b** (43 mg, 0.2 mmol, 1.0 equiv) in anhydrous CH_2Cl_2 (5 mL), at −78 °C, BBr_3 (1.0 M in CH_2Cl_2, 420 μL, 0.4 mmol) was slowly added and the resulting solution was kept under vigorous stirring at the same temperature for 3h. The solution was than warmed to −30 °C, and after 3h the reaction was quenched with the addition of a small quantity of solid $NaHCO_3$ (20 mg), followed by a saturated aq. solution of $NaHCO_3$ (10mL). The temperature was warmed to 25 °C and the resulting slurry was extracted with EtOAc (3×15 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated under vacuum. Flash chromatographic purification (50/50 Petroleum Ether:EtOAc) of the crude afforded targeted γ-valerolactone (−)-**1** (30 mg, 75%) as white crystals. R_f 0.30 (65/35 petroleum ether/EtOAc); mp 121-124 °C; $[\alpha]_D^{20}$ = −22.0 (c 1.6, MeOH); ^{1}H NMR (400 MHz, MeOD): δ 6.99 (m, 2H, Ar), 6.65 (m, 2H, Ar), 4.66 (dddd, *J* = 6.2, 6.2, 6.2, 6.2 Hz, 1H, H5), 2.85 (dd, *J* = 14.1, 5.8 Hz, 1H, H1'a), 2.8 (dd, *J* = 14.1, 6.0 Hz, 1H, H1'b), 2.40 (ddd, *J* = 17.7, 9.6, 8.7 Hz, 1H, H3a), 2.25 (ddd, *J* = 17.7, 9.5, 4.7 Hz, 1H, H3b), 2.16 (dddd, *J* = 12.7, 9.8, 6.9, 4.8 Hz, 1H, H4a), 1.87 (dddd, *J* = 12.8, 9.1, 9.1, 7.4 Hz, 1H, H4b); ^{13}C NMR (100 MHz, MeOD): δ 180.4 (Cq, C2), 157.5 (Cq, Ar), 131.7 (2C, CH, Ar), 128.5 (Cq, Ar), 116.4 (2C, CH, Ar), 83.4 (CH, C5), 41.4 (CH2, C1'), 29.6 (CH_2, C3), 28.0 (CH_2, C4); HR-MS (ESI): *m/z* = 191.0724, calcd. for $[C_{11}H_{12}O_3 - H]^-$: 191.0708; Chiral HPLC [WELK-O1, 80/20 Hexane/Ethanol (0.1%TFA), 1.0 ml/min, 254 nm]: (−)-**1**, *R*t 8.67 min (99%); *R*t 7.93 min (1%).

(*R*)-5-(3-hydroxybenzyl)dihydrofuran-2(3*H*)-one (−)-**2** **(Representative Procedure 5)**: To a solution of protected γ-valerolactone **12d** (72 mg, 0.23 mmol, 1.0 equiv) in THF (3 mL) at room temperature, was slowly added HF

(~70% in pyrydine, 90 μL, 2.3 mmol) and the resulting white slurry was kept under vigorous stirring at the same temperature for 48h. The reaction was then neutralized with a saturated aq. solution of $NaHCO_3$ (5 mL) and then extracted with EtOAc (3 × 5 ml). The combined organic extracts were dried ($MgSO_4$) and concentrated under vacuum. Flash chromatographic purification (55:45 Petroleum Ether/EtOAc) of the crude afforded 36 mg of targeted γ-valerolactone (−)-**2** (80%) as a colourless resin. R_f 0.33 (65/35 petroleum ether/EtOAc); $[\alpha]_D^{20}$ = −18.0 (c 0.5, MeOH); ^{1}H NMR (400 MHz, MeOD): δ 7.04 (dd, *J* = 7.8, 7.8 Hz, 1H, Ar), 6.64 (m, 2H, Ar), 6.61 (ddd, *J* = 8.0, 2.5, 0.9 Hz, 1H, Ar), 4.68 (dddd, *J* = 6.7, 6.7, 6.7, 6.7 Hz, 1H, H5), 2.88 (dd, *J* = 14.0, 6.5 Hz, 1H, H1'a), 2.80 (dd, *J* = 14.0, 6.0 Hz, 1H, H1'b), 2.43 (ddd, *J* = 17.8, 9.7, 9.1 Hz, 1H, H3a), 2.31 (ddd, *J* = 17.7, 9.5, 4.7 Hz, 1H, H3b), 2.18 (dddd, *J* = 12.9, 9.7, 6.8, 4.6 Hz, 1H, H4a), 1.88 (dddd, *J* = 12.9, 9.3, 9.3, 7.5 Hz, 1H, H4b); ^{13}C NMR (100 MHz, MeOD): δ 180.3 (Cq, C2), 158.7 (Cq, Ar), 139.4 (CH, Ar), 130.7 (CH, Ar), 121.8 (Cq, Ar), 117.5 (CH, Ar), 114.8 (CH, Ar), 83.1 (CH, C5), 42.2 (CH_2, C1'), 29.6 (CH_2, C3), 28.2 (CH_2, C4); HR-MS (ESI): *m/z* = 191.0718, calcd. for $[C_{11}H_{12}O_3 - H]^-$: 191.0708; Chiral HPLC [WELK-O1, 80/20 Hexane/Ethanol (0.1%TFA), 1.0 ml/min, 254 nm]: (−)-**2**, *R*t 10.27 min (96%); (+)-**2**, *R*t 9.48 min (4%).

Preparation of (*R*)-5-(3,4-dihydroxyphenyl)dihydrofuran-2(3*H*)-one (−)-**3**: γ-Valerolactone **12h** was prepared in two steps according to Representative Procedure 5 using: valerolactone 12h (72 mg, 0.17 mmol, 1.0 equiv), and HF (~70% in pyrydine, 320 μL, 1.7 mmol) in THF (3 mL) at room temperature. After 48h the reaction was neutralized with a saturated aq. solution of $NaHCO_3$ (5 mL) and then extracted with EtOAc (3×5 ml). The combined organic extracts were dried ($MgSO_4$) and concentrated under vacuum. Flash chromatographic purification (Petroleum Ether:EtOAc 55:45) of the crude afforded 44 mg of related desilylated valerolactone (85%) as an amorphous solid. R_f = 0.19 (70/30

petroleum ether/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33-7.40 (m, 5H, Ph), 6.84 (d, *J* = 8.2 Hz, 1H, Ar), 6.79 (d, *J* = 2.0 Hz, 1H, Ar), 6.67 (dd, *J* = 8.2, 2.0 Hz, 1H, Ar), 5.64 (bs, OH), 5.07 (s, 2H, $\underline{CH_2}$Ph), 4.67 (dddd, *J* = 6.6, 6.6, 6.6, 6.6 Hz, 1H, H5), 2.96 (dd, *J* = 14.0, 6.0 Hz, 1H, H1'a), 2.80 (dd, *J* = 14.0, 6.4 Hz, 1H, H1'b), 2.43 (ddd, *J* = 17.9, 9.3, 9.3 Hz, 1H, H5a), 2.39 (ddd, *J* = 17.7, 9.3, 4.9 Hz, 1H, H5b), 2.24 (dddd, *J* = 12.8, 9.5, 6.7, 4.8 Hz, 1H, H4a), 1.91 (dddd, *J* = 12.8, 9.2, 9.2, 7.5 Hz, 1H, H4b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.3 (Cq, C2), 146.1 (Cq, Ar), 145.0 (Cq, Ar), 136.5 (Cq, Ph), 129.6 (Cq, Ar), 129.0 (2C, CH, Ph), 128.7 (CH, Ph), 128.0 (2C, CH, Ph), 121.2 (CH, Ar), 116.0 (CH, Ar), 112.6 (CH, Ar), 81.1 (CH, C5), 71.5 (CH_2, $\underline{CH_2}$Ph), 40.9 (CH_2, C1'), 28.9 (CH_2, C2), 27.3 (CH_2, C3); HR-MS-(ESI) m/z 299.1297 $[M + H]^+$Calcd.: m/z 299.1283 $[C_{18}H_{18}O_4 + H]^+$; Opt. Rot. $[\alpha]_D^{20}$ −5.6 (*c* 0.36 g/100mL, $CHCl_3$).

This intermediate was then subjected to standard debenzylation procedure (**Representative Procedure 6**) To a solution of mono-protected valerolactone (44 mg, 0.15 mmol, 1.0 equiv) in a degassed EtOAc (15 mL), was added Pd (10% on carbon, 20 mg). To this black suspension H_2 was flushed and kept sealed under pressure for 3h. After this period the H_2 was removed under vacuum, the resulting suspension was filtered in EtOAc, and the residue concentrated. Flash chromatographic purification (Petroleum Ether/EtOAc-2% glacial AcOH 60:40) of the crude afforded 29 mg of pure □-valerolactone **3** (95%) as white crystals.

(–)-3: White crystals; mp. 141-143 °C; R_f 0.29 (30/70 petroleum ether/EtOAc); $[\alpha]_D^{20}$ = −12.2 (c 1.0, MeOH); ^{1}H NMR (400 MHz, MeOD): δ 6.71 (m, 2H, Ar), 6.58 (dd, *J* = 8.0, 2.1 Hz, 1H, Ar), 4.74 (dddd, *J* = 6.3, 6.3, 6.3, 6.3 Hz, 1H, H5), 2.91 (dd, *J* = 14.0, 6.1 Hz, 1H, H1'a), 2.81 (dd, *J* = 14.0, 6.1 Hz, 1H, H5b), 2.50 (ddd, *J* = 18.5, 9.7, 8.9 Hz, 1H, H3a), 2.35 (ddd, *J* = 17.6, 9.3, 4.8 Hz, 1H, H3b), 2.24 (dddd, *J* = 12.8, 9.8, 6.8, 4.9 Hz, 1H, H4a), 1.97 (dddd, *J* = 12.8, 9.4, 9.4, 7.3 Hz, 1H, H4b); ^{13}C NMR (75 MHz, MeOD): δ 180.5 (Cq, C2), 146.4 (Cq, Ar), 145.4 (Cq,Ar), 129.2 (Cq,Ar), 122.0 (CH, Ar), 117.8 (CH, Ar), 116.5 (CH, Ar), 83.4 (CH,

C5), 41.6 (CH_2, C1') 29.6 (CH_2, C3), 28.0 (CH_2, C4); HR-MS (ESI): *m/z* = 207.0673, calcd. for $[C_{11}H_{13}O_4 - H]^-$: 207.0657; Chiral HPLC [Chiralcel OD-H, 90/10 Hexane/Ethanol-0.1%TFA, 1.0 ml/min, 254 nm]: (−)-**3**, *R*t 28.17 min (98.6%); (+)-**3**, *R*t 25.99 min (1.4%).

(−)-(*R*)-5-(3,5-dihydroxybenzyl)dihydrofuran-2(3*H*)-one **(–)-4**: Targeted γ-valerolactone (−)-**4** was prepared according to Representative Procedure 6, starting from protected lactone **12k** (103 mg, 0.27 mmol, 1.0 equiv) in a degassed EtOAc (20 mL), and using Pd (10% on carbon, 20 mg) as the catalyst. To the vigorously stirred black suspension was flushed H_2, and the reaction was kept sealed under pressure of H_2 for 3h. After this period the H_2 was removed under vacuum, the resulting suspension was filtered in EtOAc, and the residue concentrated. Flash chromatographic purification (Petroleum Ether:EtOAc from 35:65) of the crude afforded 54.5 mg of pure (−)-valerolactone **4** (97%) as amorphous solid. R_f 0.26 (50/50 petroleum ether/EtOAc); $[\alpha]_D^{20}$ = −11.8 (c 0.44, MeOH); ^{1}H NMR (400 MHz, MeOD): δ 6.22 (bd, *J* = 2.2 Hz, 2H, H2'', H6''), 6.16 (d, *J* = 2.2 Hz, 1H, H4''), 4.76 (dddd, *J* = 6.7, 6.7, 6.7, 6.7 Hz, 1H, H5), 2.90 (dd, *J* = 13.9, 6.3 Hz, 1H, H1'a), 2.80 (dd, *J* = 13.9, 6.1 Hz, 1H, H1'b), 2.53 (ddd, *J* = 17.8, 9.6, 9.1 Hz, 1H, H3a), 2.41 (ddd, *J* = 17.7, 9.4, 4.7 Hz, 1H, H3b), 2.28 (dddd, *J* = 12.7, 9.7, 6.8, 4.7 Hz, 1H, H4a), 1.98 (dddd, *J* = 12.8, 9.2, 9.2, 7.5 Hz, 1H, H4b); ^{13}C NMR (75 MHz, MeOD): δ 180.4 (Cq, C2), 159.8 (2C, Cq, C3'', C5''), 140.0 (Cq, C1''), 109.1 (2C, CH, C2'', C6''), 102.2 (CH, C4''), 83.1 (CH, C5), 42.3 (CH_2, C1') 28.6 (CH_2, C3), 28.2 (CH2, C4); HR-MS (ESI): *m/z* = 207.0639, calcd. for $[C_{11}H_{12}O_4 - H]^-$: 207.0657; Chiral HPLC [Chiralcel OD-H, 95/5 Hexane/Ethanol-0.1%TFA, 1.1 ml/min, 254 nm]: (−)-**4**, *R*t 103.29 min (97.8%); (+)-**4**, *R*t 99.21 min (2.2%).

(*R*)-5-(3,4,5-trimethoxybenzyl)dihydrofuran-2(3*H*)-one **(–)-5**: was prepared according to Representative Procedure 4, using lactone **12o** (42 mg, 0.16 mmol,

1.0 equiv) in anhydrous CH_2Cl_2 (10 mL), and using BBr_3 (1.0 M in CH_2Cl_2, 940 μL, 0.94 mmol) as the catalyst. The solution was stirred at −78 °C for 16h and then warmed to −30 °C. After other 6h the reaction was quenched with the addition of a small quantity of $NaHCO_3$ (20 mg), followed by a saturated aq. solution of $NaHCO_3$ (10mL). The temperature was warmed to 25 °C and the resulting slurry was extracted with EtOAc (3 × 15 ml). The combined organic extracts were dried ($MgSO_4$) and concentrated under vacuum. Flash chromatographic purification (Toluene:EtOAc 50:50) of the crude afforded 15.8 mg of targeted γ-valerolactone **(–)-5** (45%) as white powder. R_f 0.27 (10/90 petroleum ether/EtOAc-2% AcOH); $[\alpha]_D^{20}$ = −8.0 (c 0.2, MeOH); ^{1}H NMR (400 MHz, MeOD): δ 6.26 (s, 2H, H2'', H6''), 4.73 (dddd, *J* = 6.9, 6.9, 6.9, 6.9 Hz, 1H, H5), 2.84 (dd, *J* = 14.0, 6.1 Hz, 1H, H1'a), 2.74 (dd, *J* = 14.0, 6.2 Hz, 1H, H1'b), 2.50 (ddd, *J* = 17.7, 9.7, 8.8 Hz, 1H, H3a), 2.36 (ddd, *J* = 17.7, 9.4, 4.8 Hz, 1H, H3b), 2.26 (dddd, *J* = 12.6, 9.7, 6.8, 4.8 Hz, 1H, H4a), 1.98 (dddd, *J* = 12.7, 9.2, 9.2, 7.3 Hz, 1H, H4b). ^{13}C NMR (100 MHz, MeOD): δ 180.4 (Cq, C2), 147.2 (2C, Cq, C3'', C5''), 133.1 (Cq, Ar), 128.5 (Cq, Ar), 109.6 (2C, CH, C2'', C6''), 83.4 (CH, C5), 41.8 (CH_2, C1'), 29.6 (CH_2, C3), 28.0 (CH_2, C4). HR-MS (ESI): *m/z* = 223.0619, calcd. for $[C_{11}H_{12}O_5 - H]^-$: 223.0606.

Typical Procedure for the Reductive Ring Expansion Reaction of Table 3.4.

(Method A: entry 3, Table 3.4): Representative Procedure 7

To a solution of NaI (180 mg, 1.2 mmol) in CH_3CN (1.5 mL) at 0 °C, under Argon atmosphere, was slowly added chlorotrimethylsilane (TMSCl, 152 μL, 1.2 mmol) and the resulting whitish suspension was kept under vigorous stirring at room temperature. After 5 minutes, a solution of an *anti/syn* mixture of butanolide **11** (0.2 mmol, 1.0 equiv) in CH_3CN (0.5 mL) was slowly added turning the

reaction slurry from white to purple. After 3h, the reaction was quenched with a saturated aqueous solution of $Na_2S_2O_3$ (2.5 mL) and brine (5 mL) and extracted with EtOAc (3×10 mL). The combined extracts were dried ($MgSO_4$), filtered, and the combined organic extracts concentrated under vacuum. Flash chromatographic purification of the crude afforded the pure δ-valerolactones reported in Table 3.5.

(±)-6: Amorphous solid; R_f 0.25 (50/50 petroleum ether/EtOAc); ^{1}H NMR (400 MHz, MeOD): δ 7.22 (m, 2H, Ar), 6.81 (m, 2H, Ar), 5.34 (dd, *J* = 10.8, 3.2 Hz, 1H, H6), 2.71 (dddd, *J* = 17.7, 6.6, 6.6, 1.3 Hz, 1H, H3a), 2.56 (ddd, *J* = 17.7, 7.4, 7.4 Hz, 1H, H3b), 1.86-2.11 (m, 4H, H4, H5). ^{13}C NMR (100 MHz, MeOD): δ 175.2 (Cq, C2), 158.9 (Cq, Ar), 132.3 (Cq, Ar), 128.7 (2C, CH, Ar), 116.4 (2C, CH, Ar), 83.9 (CH, C6), 31.4 (CH_2, C5), 30.2 (CH_2, C3), 19.7 (CH_2, C4). HR-MS (ESI): *m/z* = 191.0723, calcd. for $[C_{11}H_{12}O_3-H]^-$: 191.0708.

(±)-7: R_f 0.25 (30/70 petroleum ether/EtOAc); ^{1}H NMR (300 MHz, MeOD): δ 6.69 (m, 3H, Ar), 5.27 (dd, *J* = 10.6, 3.1 Hz, 1H, H6), 2.69 (ddd, *J* = 17.9, 6.0, 6.0 Hz, 1H, H3a), 2.53 (ddd, *J* = 17.4, 7.7, 7.7 Hz, 1H, H3b), 1.84-2.11 (m, 4H, H4, H5). ^{13}C NMR (100 MHz, MeOD): δ 175.2 (Cq, C2), 146.7 (Cq, Ar), 146.6 (Cq, Ar), 133.0 (Cq, Ar), 118.9 (CH, Ar), 116.3 (CH, Ar), 114.5 (CH, Ar), 83.9 (CH, C6), 31.4 (CH_2, C5), 30.2 (CH_2, C3), 19.6 (CH_2, C4). HR-MS (ESI): *m/z* = 207.0671, calcd. for $[C_{11}H_{12}O_4 - H]^-$: 207.0657.

(±)-13b: R_f = 0.31 (55/45 petroleum ether/ethyl acetate)**;** ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.27 (m, 2H, Ar), 6.90 (m, 2H, Ar), 5.30 (dd, *J* = 10.6, 3.0 Hz, 1H, H6), 3.81 (s, 3H, OMe), 2.70 (ddd, *J* = 17.7, 6.6, 6.6 Hz, 1H, H3a), 2.56 (ddd, *J* = 17.7, 8.0, 8.0 Hz, 1H, H3b), 2.13 (dddd, *J* = 12.9, 4.1, 4.1, 4.1 Hz, 1H, H5a), 1.98 (m, 2H, H4), 1.87 (dddd, *J* = 13.7, 10.7, 8.6, 7.3 Hz, 1H, H5b); ^{13}C NMR (100 MHz,

$CDCl_3$): δ 171.7 (Cq, C2), 159.8 (Cq, Ar), 132.0 (Cq, Ar), 127.4 (2C, CH, Ar), 114.1 (2C, CH, Ar), 81.7 (CH, C6), 55.5 (CH_3, OMe), 30.5 (CH_2, C5), 29.6 (CH_2, C3), 18.8 (CH_2, C4)**;** HRMS(ESI) m/z 207.1034 $[M + H]^+$; Calcd.: m/z 207.1021 $[C_{12}H_{14}O_3 + H]^+$.

(±)-**13o:** colourless resin; R_f = 0.30 (30/70 petroleum ether/ethyl acetate); ^1H NMR (400 MHz, $CDCl_3$): δ 6.55 (s, 2H, H2', H6'), 5.27 (dd, *J* = 10.8, 3.3 Hz, 1H, H6), 3.86 (s, 6H, OMe), 3.84 (s, 3H, OMe), 2.71 (ddd, *J* = 17.8, 6.4, 6.4 Hz, 1H, H3a), 2.58 (ddd, *J* = 17.6, 7.8, 7.8 Hz, 1H, H3b), 2.16 (dddd, *J* = 13.3, 4.3, 4.3, 4.3 Hz, 1H, H5a), 1.99 (m, 2H, H4), 1.86 (ddd, *J* = 13.8, 10.7, 7.8, 7.8 Hz, 1H, H5b); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5 (Cq, C2), 153.6 (2C, Cq, C3', C5'), 138.0 (Cq, Ar), 135.5 (Cq, Ar), 103.0 (2C, CH, C2', C6'), 81.8 (CH, C6), 61.0 (CH_3, OMe), 56.4 (2C, CH_3, OMe), 30.8 (CH_2, C5), 29.6 (CH_2, C3), 18.8 (CH_2, C4); HR-MS (ESI) m/z = 267.1221 $[M + H]^+$Calcd.: m/z 267.1232 $[C_{14}H_{18}O_5 + H]^+$.

(±)-13p: colourless resin; R_f = 0.46 (50/50 petroleum ether/ethyl acetate); ^1H NMR (400 MHz, $CDCl_3$): δ 7.28-7.42 (m, 5H, Ph), 5.38 (dd, *J* = 10.5, 3.5 Hz, 1H, H6), 2.73 (dddd, *J* = 17.8, 6.5, 6.5, 1.0 Hz, 1H, H3a), 2.60 (ddd, *J* = 17.7, 7.7, 7.7 Hz, 1H, H3b), 2.20 (dddd, *J* = 13.6, 4.4, 4.4, 4.4 Hz, 1H, H5a), 2.00 (m, 2H, H4), 1.89 (ddd, *J* = 13.5, 10.3, 8.8, 6.9 Hz, 1H, H5b); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5 (Cq, C2), 139.9 (Cq, Ph), 128.8 (2C, CH, Ph), 128.4 (CH, Ph), 125.9 (2C, CH, Ph), 81.8 (CH, C6), 30.7 (CH_2, C5), 29.7 (CH_2, C3), 18.8 (CH_2, C4); HRMS(ESI) m/z = 177.0926 $[M + H]^+$; Calcd.: m/z 177.0915 $[C_{11}H_{12}O_2 + H]^+$

(Method B: entry 11, Table 3.4): Representative Procedure 8

To a solution of NaI (120 mg, 0.8 mmol) in anhydrous CH_2Cl_2 (1.5 mL) at 0 °C, under Argon atmosphere, was slowly added chlorotrimethylsilane (TMSCl, 102 μL, 0.8 mmol) and the resulting whitish suspension was kept under vigorous

stirring at room temperature. After 5 minutes, a solution of a 2:1 (*anti/syn*)-mixture of butanolide **11** (65 mg, 0.2 mmol, 1.0 equiv) in anhydrous CH_2Cl_2 (0.5 mL) was slowly added turning the reaction slurry from white to purple. After 1h, the reaction was quenched with a saturated aqueous solution of $Na_2S_2O_3$ (2.5 mL) and brine (5 mL) and extracted with EtOAc (3×10 mL). The combined extracts were dried ($MgSO_4$), filtered, and the combined organic extracts concentrated under vacuum. Flash chromatographic purification (Petroleum Ether:EtOAc 60:40) of the crude afforded 46 mg of protected δ-valerolactone reported in Table 3.5.

(±)-13a: colourless resin; R_f = 0.24 (80/20 petroleum ether/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.24 (m, 2H, Ar), 6.85 (m, 2H, Ar), 5.30 (dd, *J* = 10.7, 3.4 Hz, 1H, H6), 2.72 (ddd, *J* = 18.1, 6.3, 6.3 Hz, 1H, H3a), 2.58 (ddd, *J* = 17.6, 7.9, 7.9 Hz, 1H, H3b), 2.15 (dddd, *J* = 13.0, 4.0, 4.0, 4.0 Hz, 1H, H5a), 2.00 (m, 2H, H4), 1.88 (dddd, *J* = 13.8, 10.5, 9.1, 6.8 Hz, 1H, H5b), 1.00 (s, 9H, *tert*-Bu, TBS), 0.21 (s, 6H, CH_3, TBS); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.7 (Cq, C2), 155.9 (Cq, Ar), 132.6 (Cq, Ar), 127.4 (2C, CH, Ar), 120.3 (2C, CH, Ar), 81.8 (CH, C6), 30.5 (CH_2, C5), 29.6 (CH_2, C3), 25.9 (3C, CH_3, *tert*-Bu, TBS), 18.9 (CH_2, C4), 18.4 (Cq, *tert*-Bu, TBS), −4.2 (2C, CH_3, TBS); HR-MS (ESI) m/z = 307.1739 $[M + H]^+$; Calcd.: m/z 307.1729 $[C_{17}H_{26}O_3Si + H]^+$.

(±)-**13g**: R_f = 0.51 (75/25 petroleum ether/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.82 (m, 3H, Ar), 5.25 (dd, *J* = 10.4, 3.3 Hz, 1H, H6), 2.70 (ddd, *J* = 17.8, 6.7, 6.7 Hz, 1H, H3a), 2.56 (ddd, *J* = 17.7, 7.8, 7.8 Hz, 1H, H3b), 2.13 (dddd, *J* = 13.0, 4.2, 4.2, 4.2 Hz, 1H, H5a), 1.98 (m, 2H, H4), 1.86 (dddd, *J* = 13.7, 9.9, 8.8, 6.7 Hz, 1H, H5b), 1.00 (s, 18H, *tert*-Bu, TBS), 0.21 (s, 12H, CH_3, TBS); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.7 (Cq, C2), 147.2 (Cq, Ar), 147.0 (Cq, Ar), 132.9 (Cq, Ar), 121.2 (CH, Ar), 119.2 (2C, CH, Ar), 81.6 (CH, C6), 30.4 (CH_2, C5), 29.9 (CH_2, C3),

26.1 (6C, CH_3, *tert*-Bu, TBS), 18.6 (CH_2, C4), 18.6 (2C, Cq, *tert*-Bu, TBS), −3.9 (4C, CH_3, TBS).HR-MS m/z = 409.2795 $[M + H]^+$ (ESI) Calcd.: m/z 409.2774 $[C_{23}H_{40}O_4Si_2 + H]^+$.

(±)-**13h:** yellow crystals m. p.: 97-98 °C; R_f = 0.47 (70/30 petroleum ether/ethyl acetate)**;** ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34-7.45 (m, 5H, CH_2Ph), 6.89 (m, 3H, Ar), 5.26 (dd, *J* = 10.5, 3.4 Hz, 1H, H6), 5.07 (s, 2H, CH_2Ph), 2.71 (ddd, *J* = 17.9, 6.3, 6.3 Hz, 1H, H3a), 2.58 (ddd, *J* = 17.7, 7.7, 7.7 Hz, 1H, H3b), 2.13 (dddd, *J* = 13.9, 3.9, 3.9, 3.9 Hz, 1H, H5a), 1.98 (m, 2H, H4), 1.87 (dddd, *J* = 13.8, 10.3, 8.9, 6.8 Hz, 1H, H5b), 0.98 (s, 9H, *tert*-Bu, TBS), 0.12 (s, 6H, CH_3, TBS). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.7 (Cq, C2), 150.4 (Cq, Ar), 145.6 (Cq, Ar), 137.1 (Cq, Ph), 132.9 (Cq, Ar), 128.6 (2C, CH, CH_2Ph), 128.2 (CH, CH_2Ph), 128.0 (2C, CH, CH_2Ph), 119.4 (CH, Ar), 119.1 (CH, Ar), 114.0 (CH, Ar), 81.5 (CH, C6), 71.1 (CH_2, CH_2Ph), 30.4 (CH_2, C5), 29.6 (CH_2, C3), 25.9 (3C, CH_3, *tert*-Bu, TBS), 18.7 (CH_2, C4), 18.6 (Cq, *tert*-Bu, TBS), −4.3 (2C, CH_3, TBS). HR-MS (ESI) m/z = 413.2125 $[M + H]^+$, Calcd.: m/z 413.2148 $[C_{24}H_{32}O_4Si + H]^+$

(±)-13q: colourless resin; R_f = 0.26 (80/20 petroleum ether/ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40 (dd, *J* = 7.6, 1.6 Hz, 1H, Ar), 7.21 (ddd, *J* = 7.7, 7.7, 1.8 Hz, 1H, Ar), 7.01 (ddd, *J* = 7.5, 7.5, 0.9 Hz, 1H, Ar), 6.83 (dd, *J* = 8.1, 1.0 Hz, 1H, Ar), 5.70 (dd, *J* = 10.2, 3.2 Hz, 1H, H6), 2.72 (ddd, *J* = 17.7, 6.3, 6.3 Hz, 1H, H3a), 2.62 (ddd, *J* = 17.8, 7.4, 7.4 Hz, 1H, H3b), 2.22 (dddd, *J* = 13.1, 4.0, 4.0, 4.0 Hz, 1H, H5a), 1.97 (m, 2H, H4), 1.77 (dddd, *J* = 14.0, 10.2, 9.2, 6.8 Hz, 1H, H5b), 1.03 (s, 9H, *tert*-Bu, TBS), 0.30 (s, 3H, CH_3, TBS), 0.27 (s, 3H, CH_3, TBS). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.0 (Cq, C2), 152.0 (Cq, Ar), 130.6 (CH, Ar), 129.0 (CH, Ar), 126.9 (CH, Ar), 121.6 (CH, Ar), 118.3 (CH, Ar), 77.3 (CH, C6), 29.9 (CH_2, C3), 29.4 (CH_2, C5), 25.9 (3C, CH_3, *tert*-Bu, TBS), 18.8 (CH_2, C4), 18.4 (Cq, *tert*-Bu, TBS),

−3.8 (CH_3, TBS), −4.1 (CH_3, TBS). HR-MS (ESI) m/z 307.1743 $[M + H]^+$; Calcd.: m/z 307.1729 $[C_{17}H_{26}O_3Si + H]^+$.

Synthesis of styrene intermediate: (*E*)-5-(4-methoxyphenyl)pent-4-enoic acid **(16b)**

Compound **16b** was obtained following Representative Procedure 8, starting from a 2:1 (*anti/syn*)-mixture of butanolide **11b** (45 mg, 0.2 mmol, 1.0 equiv), and using NaI (120 mg, 0.8 mmol), and (TMSCl, 102 μL, 0.8 mmol) in CH_2Cl_2 (2 mL) under Argon atmosphere. After 1h, the reaction was quenched with a saturated aqueous solution of $Na_2S_2O_3$ (2.5 mL) and brine (5 mL) and extracted with EtOAc (3×10 mL). The combined extracts were dried ($MgSO_4$), filtered, and the combined organic extracts concentrated under vacuum. Flash chromatographic purification (Petroleum Ether:EtOAc 20:80) of the crude afforded 20.6 mg of protected styrene **16b** (50%) as colourless resin, and 14.4 mg (35%) of □-valerolactone **13b** as a pale yellow resin.

16b: R_f = 0.28 (25/75 petroleum ether/ethyl acetate); ^{1}H NMR (400 MHz, MeOD): δ 7.29 (m, 2H, Ar), 6.85 (m, *2*H, Ar), 6.40 (d, *J* = 15.8 Hz, 1H, H5), 6.12 (ddd, *J* = 15.8, 6.5, 6.5 Hz, 1H, H4), 3.79 (s, CH_3, OMe), 2.39-2.52 (m, 4H, H2, H3); ^{13}C NMR (100 MHz, MeOD): δ 177.1 (Cq, C2), 160.6.0 (Cq, Ar), 131.8 (Cq, Ar), 130.3 (CH, C5), 128.3 (2C, CH, Ar), 127.4 (CH, C4), 115.0 (2C, CH, Ar), 55.8 (CH_3, OMe), 35.1 (CH_2), 29.5 (CH_2); HR MS (ESI) m/z = 205.0877 $[M - H]^-$, Calcd.: m/z 205.0864 $[C_{12}H_{23}O_3 - H]^-$.

3.6. References

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CHAPTER 4

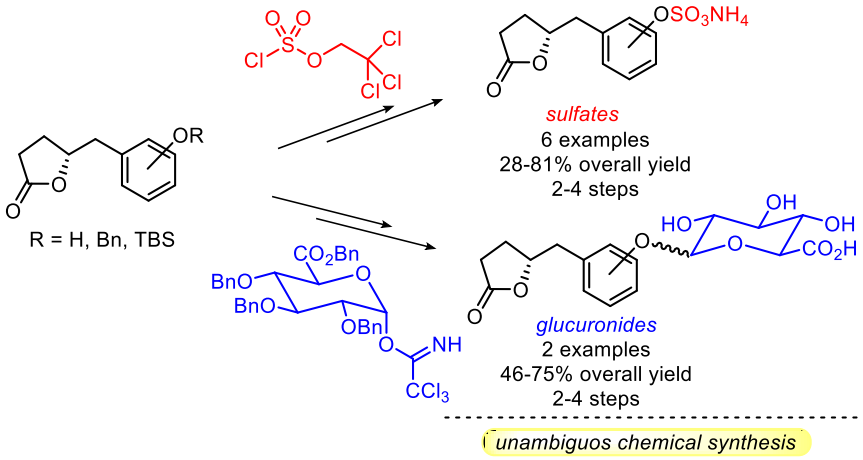
Synthetic and analytical strategies for the quantification of phenyl-γ-valerolactone conjugated metabolites in human urine[†]

Scope: The contribution of the gut microbiota to the metabolism of catechins and proanthocyanidins remains still unclear. Although phenyl-γ-valerolactones have been pointed out as the most representative metabolites of these flavan-3-ols, their accurate quantification has not been addressed because of a lack of appropriate bioanalytical standards. This work aimed at synthesizing a set of sulphate- and glucuronide-conjugated phenyl-γ-valerolactones and at developing an analytical UHPLC-ESI-MS/MS method for their quantification in urine samples.

Methods and results: Eight glucuronide and sulphate conjugates of hydroxyphenyl-γ-valerolactones were synthesized for the first time. They were used as analytical standards, together with 5 phenyl-γ-valerolactone aglycones, for the development of a high-throughput, validated method. Chromatographic and MS conditions were optimized. The method validation showed acceptable linearity, intra-day and inter-day repeatability, and accuracy, with the analytical range, limit of detection (LOD), and lower limit of quantification (LLOQ) varying notably among compounds. The method was used to calculate the excretion of phenyl-γ-valerolactones in healthy subject consuming green tea, providing novel information on the real concentrations of phenyl-γ-valerolactones in urine.

[†] *Authors contributions:* Nicoletta Brindani, Pedro Mena*, Luca Calani, Iris Benzie, Sui-Wai Choi, Furio Brighenti, Franca Zanardi, Claudio Curti, Daniele Del Rio.
(manuscript ready for submission)

Conclusion: This work opens the door to better studying the bioavailability of flavan-3-ols and the real exposition to flavan-3-ol sources, as well as to define the bioactivity of these colonic metabolites in cell assays.



4.1. Introduction

As detailed in the Chapter 1, increased understanding of metabolic processes involving conjugation reaction as sulfation and glucuronidation reactions has led to growing appreciation of the role and significance of Phase II metabolites. Besides their canonical role as final, easily eliminable forms of drugs and xenobiotics , often performing an important detoxification role in the living bodies, glucuronide and sulfate derivatives may have significant biological activity in their own right.

Even γ-valerolactone aglycones produced in the gut from flava-3-ols don't escape to these modifications (*see* Chapter 1).

Several studies, mostly through LC-MS or NMR analytical techniques, have confirmed 5-(hydroxyphenyl)-*γ*-valerolactone structures as the main products

derived from flavan-3-ols metabolism[1-5] and a variety of methods have been developed aiming at targeting flavan-3-ol metabolites in biological fluid.[6-11] However, without appropriate reference standards, most of the analyses remain only qualitative or semi-quantitative,[12] as the complex composition of biological samples, the possible regioisomeric forms, and the likely low MS ionization of some derivatives, among other factors, may hamper the unambiguous identification and absolute quantification of these metabolites.

Actually, the lack of reliable reference compounds of hydroxyphenyl-γ-valerolactones has restricted their use in both analytical methods and *in vitro* bioactivity assays, so far.[13] Nevertheless, progresses in asymmetric synthesis carried out by our research group (*see* Chapter 3) have overcome this situation and now the enantioselective synthesis of hydroxyphenyl-γ-valerolactone aglycones has been reported (compounds **1**-**5**, Figure 4.1).[14] In the framework of the "joint venture" between chemical synthesis and analytical techniques, the development of a validated analytical method for the quali-quantitative determination of these metabolites in biological fluids is really needed.

In this paper, the synthesis of 8 *O*-glucuronide and *O*-sulfate conjugated metabolites (compounds **6-12**, Figure 4.1) starting either from chiral aglycones or from suitable protected valerolactone precursors, is reported for the first time. Furthermore, using the synthesized compounds as chemically unambiguous authentic standards, an analytical UHPLC-ESI-MS/MS method to quantify phenyl-γ-valerolactone metabolites in urinary samples has been successfully developed. The viability of this method was tested by evaluating the urinary excretion of phenyl-γ-valerolactones after consumption of green tea, one of the most popular beverages worldwide and one of the major dietary sources of flavan-3-ols, in a population of 16 volunteers.

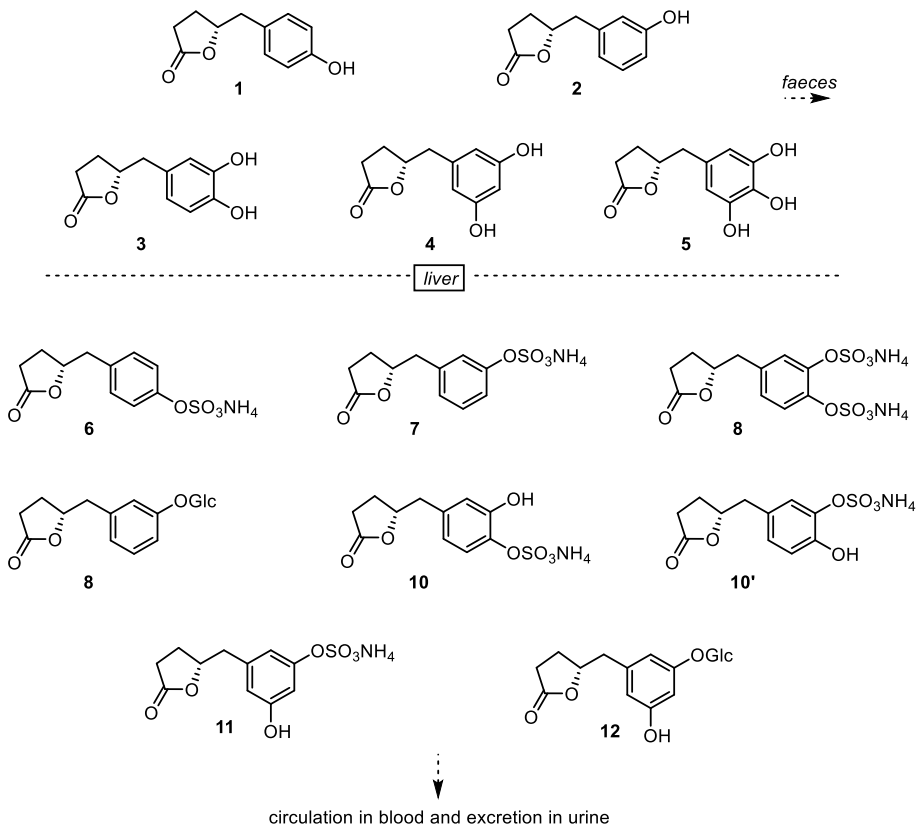


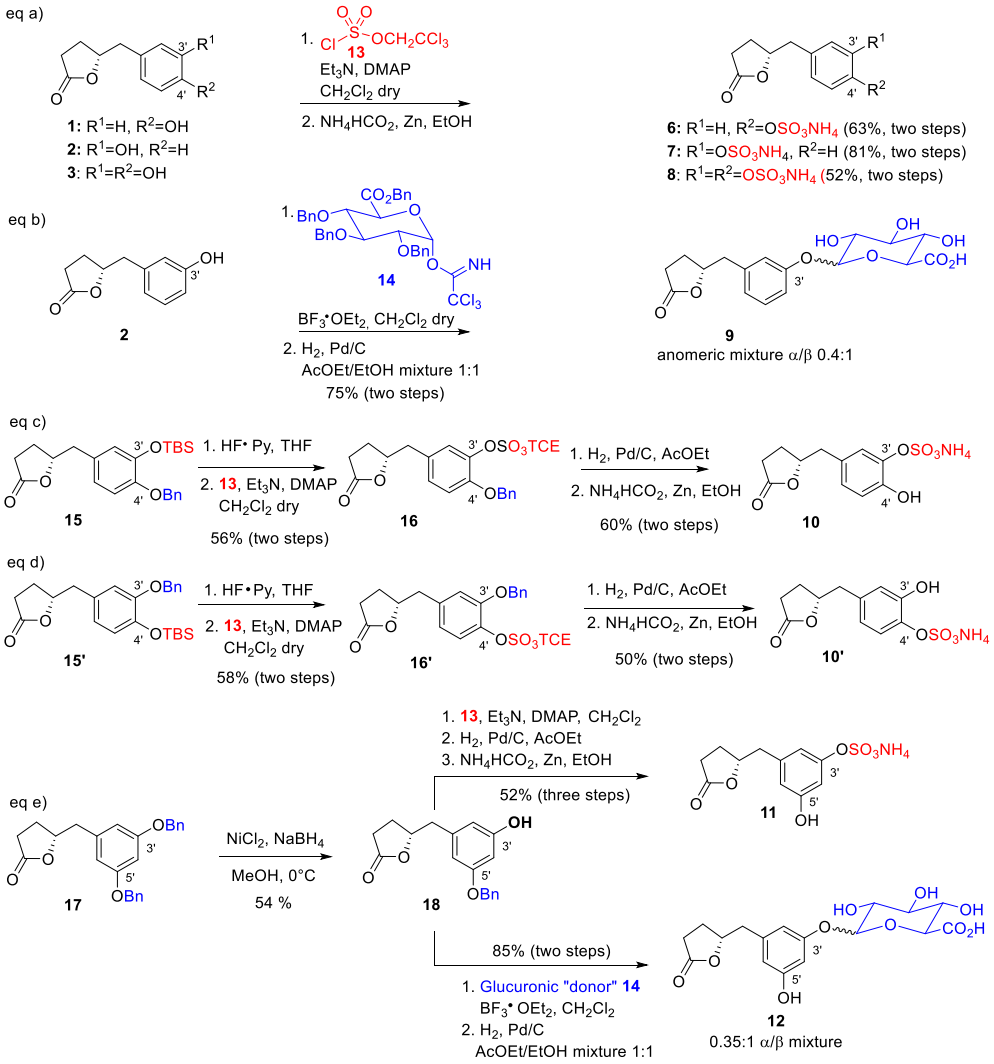
Figure 4.1. *Panel of thirteen synthesized valerolactone metabolites (aglicones and conjugates)* ***1****-****12****. (Glc = glucuronyl).*

4.2. Results and discussion

4.2.1. Synthesis of conjugated phenyl-γ-valerolactones

Considering previous studies accounting for the transformation into conjugated phenyl-γ-valerolactones of flavan-3-ols by gut microbiota and human Phase II enzymatic pools,[1,2,5] different glucuronide and sulphate conjugates were synthesized. *O*-Sulfated isomers **6** and **7** were synthesized using 2,2,2-trichloroethyl chlorosulphate (TCECS, **13**) as sulfate "donor" in presence of Et_3N, DMAP in CH_2Cl_2, starting respectively from the corresponding

5-(hydroxyphenyl)-valerolactone **1** and **2** (Scheme 4.1, eq a). The next removal of TCE moiety with Zn dust and ammonium formate afforded the target products as ammonium salts, with 63% and 81% of yield, respectively, after two steps. Following the aforementioned synthetic way, the di-*O*-sulphated metabolite **8** was prepared starting from the related aglycone **3** with a 52% overall yield (Scheme 4.1, eq a).



Scheme 4.1. *Synthetic routes towards phenyl-γ-valerolactone conjugates.*

The phenolic group of aglycone (*R*)-**2** was also conjugated with benzyl glucuronate "donor" **14** to give the protected glucuronidated adduct in 78% yield as an anomeric 0.4:1 α:β mixture. Finally, total debenzylation with H_2 Pd/C in EtOH/AcOEt (1:1) mixture afforded the desired metabolite **9** in quantitative yield (Scheme 4.1, eq b).

As depicted in Scheme 4.1 (eqs c, d), the two monosulfated regioisomers **10** and **10'**, both coming from γ-valerolactone **3**, were synthesized starting from orthogonally protected precursors **15** and **15'**, respectively. For the synthesis of 3'-*O*-sulfated metabolite **10**, precursor **15** was desilylated by HF ▪Py unmasking the phenolic group at the 3' position, which was then sulfated with compound **13** with a good 70% yield (Scheme 4.1, eq c). At this point, removal of the benzyl group with H_2, Pd/C in AcOEt and the cleavage of TCE group with Zn dust, ammonium formate afforded metabolite **10'** with a good 60% yield after two steps. The same treatment of desilylation, sulphation, benzyl and TCE cleavages converted the regioisomer precursor **15'** into the target molecule **10'** in a nice 38% overall yield (Scheme 4.1, eq d).

Due to the chemical equivalence of the two phenolic groups in 3' and 5' position of valerolactone **4**, the synthesis of mono-conjugated metabolites **11** and **12** did not require the orthogonal protection strategy. As shown in Scheme 4.2 (eq e), the dibenzylated valerolactone scaffold **17** was subjected to a mild deprotection with $NiCl_2$, $NaBH_4$ in MeOH, giving the mono-protected compound **18** with an acceptable 52% of yield. Intermediate **18** represented a divergent point toward the final products **11** and **12**. In fact, the sulfation and sequential reductive cleavage of benzyl and TCE moieties afforded the final target **12** with 52% yield after three steps (Scheme 4.1, eq e). On the other hand, coupling **18** with the trichloroacetimidate **14** gave the protected glucuronide as anomeric 0.35:1- α/β mixture with a very good 85% yield. Treatment of this polybenzylated mixture with H_2, Pd/C afforded in only one step the total

cleavage of all benzyl groups yielding the targeted metabolite **12** in quantitative yield.

Despite the synthesis of some phenyl-γ-valerolactone scaffolds has already been reported,[13-15] this is the first time, to the best of our knowledge, that the synthesis of authentic bioanalytical standards of sulfate- and glucuronide-conjugated phenyl-γ-valerolactones is reported.

4.2.2. Development and optimization of the UHPLC-ESI-MS/MS method

One of the aims of this work was to develop a quick method to quantify phenyl-γ-valerolactones in human urine. Six UHPLC columns (Knauer BlueOrchid C18, Restek Ultra AQ C18, Waters Acquity UPLC HSS T3, Phenomenex Kinetex PFP, Phenomenex Kinetex EVO C18 2.6 µm, and Phenomenex Kinetex EVO C18 1.7 µm) often used for the separation of phenolic metabolites were utilized. Column length was a critical characteristic to allow the separation of isomers **6** and **7**, for which long columns were required (100 mm). Both Kinetex EVO C18 and the Acquity HSS T3 columns provided the best peak resolutions at their optimal flow rates, but the EVO C18 2.6 µm was preferred since lower operating pressures were achieved due to its higher particle size (2.6 µm in comparison with 1.8 µm of the Acquity and 1.7 µm of the other Kinetex EVO C18). Despite the Restek Ultra AQ C18 was similar to the EVO C18 2.6 µm in terms of length and particle size, peak shape for sulphated derivatives was poor in the former after repeated analyses. Flow rates under 0.4 mL/min resulted in poor peak resolution. Regarding mobile phase solvents, acetonitrile but not methanol improved peak shape for sulphated conjugates (approximately 35%). All phenyl-γ-valerolactones eluted within 12 minutes and all compounds, including isomers, were well separated under the above described chromatographic conditions. However, this method did not succeed to separate

co-eluting isomers **10** and **10'**, and analysis times longer than 30 minutes were required to achieve an acceptable separation. This fact had been previously observed by other authors using longer gradients.[5]

The MS/MS related parameters were optimized for each individual compound separately, by performing direct infusion experiments (Table 4.1). A greater sensitivity was reached in negative ionization condition for all the compounds. In general, sulphated compounds responded better to ES ionization conditions with respect to their glucuronidated counterparts and to free forms of phenyl-γ-valerolactones. As it had been previously reported,[3] the deprotonated aglycone ions of phenyl-γ-valerolactone conjugates were always the predominant peaks in the fragment ion MS spectra. Two selective SRM transitions were used for each metabolite, making a robust qualitative and quantitative information easily achievable.[16]

A particular behaviour in terms of peak resolution and ionization was observed for 5-phenyl-γ-valerolactone-3',4'-di-*O*-sulfate (**8**). This compound showed an asymmetric peak shape, characterised by a severe peak tail, and also showed a limited ionization, characterized by the co-presence of four different molecular ions: the doubly-charged molecular ion ($[M-2H]^{2-}$) at *m/z* 183 (100% of relative abundance), two in-source fragments of one and two sulphate moieties yielding molecular ions at *m/z* 287 and 207 (30% of relative abundance for both ions), and the single molecular ion ($[M-H]^{-}$) at *m/z* 367 (10% of relative abundance). Unfortunately, it was not possible to improve its chromatographic and ionization features, despite multiple efforts.

Despite the feasibility of analysing different classes of flavan-3-ol metabolites, this method was exclusively developed for phenyl-γ-valerolactones because the lack of authentic standards has hindered accurate calibration and absolute quantification of these phenolic metabolites so far.[12] The method allowed the simultaneous resolution and quantification of 12 authentic standards of phenyl-

γ-valerolactones within 12 minutes. The analysis time was short if compared to other methods that detected phenyl-γ-valerolactones as well as other flavan-3-ol metabolites in 26-70 min,[1,3-6,17,18] and in line with other UHPLC methods resolving a high number of phenolic metabolites in 10-12 min.[8-11]

4.2.3. Method validation

Selectivity

To determine whether endogenous peaks from human urine or other sample components co-eluted with the analytes of interest, selectivity was evaluated in diluted blank matrix spiked or not with phenyl-γ-valerolactones. In all cases, no interference signals from the matrix at the specific SRM transitions were observed. The concomitant presence of 5-(3'-hydroxyphenyl)-γ-valerolactone (**2**) and 5-phenyl-γ-valerolactone-3'-*O*-sulfate (**7**) in the sample caused a loss of selectivity for the former due to the in-source fragmentation of the latter. For all the other analysed compounds, the method was characterized by a high selectivity.

Linearity, limit of detection and limits of quantification

Calibration curves were established using diluted blank urine for matrix-match calibration. Different concentrations levels, covering the expected range for each compound and ranging from its LLOQ to its UPLOQ were used. Calibration curves were forced to pass through the origin and the regression line best fitting data (linear or quadratic) was used. Most of the compounds were fitted linearly, but compounds **5**, **10**, and **10'** fitted quadratic calibration curves (Table 4.2). All the compounds showed R^2 higher than 0.987 (Table 4.2).

Concentration ranges, LODs, LLOQs, and UPLOQs varied largely among the different analytes (Table 4.2), with most of the compounds displaying analytical

ranges along 3-5 orders of magnitude, with the exception of unconjugated mono- and trihydroxy-phenyl-γ-valerolactones (**1**, **2**, and **5**) and 5-phenyl-γ-valerolactone-3',4'-di-*O*-sulfate (**8**). LOD values varied from 0.2 to 1,113 nM and the median LOD was 6.2 nM. With respect to the LLOQ, it ranged from 0.6 to 2,227 nM and the median LLOQ was 12.4 nM. UPOQ varied between 66,667 and 133,333 nM, with median values for 1,000,000 nM. LODs of compounds **1**, **2**, **4**, **5**, and **8** were above 20 nM, mostly because of their poor ionization. On the contrary, mono-sulfated hydroxy- and dihydroxy-phenyl-γ-valerolactones (compounds **6**, **7**, **10** and **10'**) had LODs and LLOQs below 1.5 and 10 nM, respectively. These LOD and LLOQ values, in the low nM range, were in agreement or even lower than those reported for 5-(3',4'-dihydroxyphenyl)-γ-valerolactone (**3**), 5-(3'-hydroxyphenyl)-γ-valerolactone-4'-*O*-sulfate (**10**), and other phenolic metabolites.[6,10,11,19,20]

Precision and accuracy

The intra-day and inter-day precision, calculated as the respective relative standard deviation (% RSD), was determined at three concentrations (L1-L3) (Table 4.2). The intra-day precision was lower than 15% for all the compounds at L2 and L3, while it was within 20% for most of the compounds at the LLOQ (L1). Average intra-day precision values (%) were 10.6 ± 6.7, 7.7 ± 4.3, and 2.2 ± 3.0 for L1, L2, and L3, respectively. The values of the inter-day precision were lower than 20% at L1 and fell within 15% at L2 and L3 for most of the compounds. Average values for inter-day precision (%) were 12.1 ± 6.6, 8.6 ± 3.7, and 6.1 ± 2.8 for L1, L2, and L3, respectively. The accuracy was excellent for most of the compounds, with values ranging from 86.6% for 5-(3',4',5'-trihydroxyphenyl)-γ-valerolactone to 121.9% for 5-(4'-hydroxyphenyl)-γ-valerolactone-3'-*O*-sulfate (Table 4.2). Average accuracy (%) was 101.4 ± 10.9.

Overall, the method met the acceptance criteria of FDA for intra- and inter-day precision, and accuracy.[21]

4.2.4. Method application: urinary excretion of phenyl-γ-valerolactones after consumption of green tea

Of the 13 metabolites targeted within the present UHPLC-ESI-MS/MS method, 10 compounds were identified and quantified in urine samples of subjects consuming green tea and following an unrestricted diet. In this set of analyses, 5-(3′-hydroxyphenyl)-γ-valerolactone (**2**) and 5-phenyl-γ-valerolactone-3′,4′-di-*O*-sulfate (**8**) were not detected, and their absence could be related to their intrinsic poor selectivity and resolution, respectively. Nevertheless, their absence in the samples could not be completely ruled out. On the other hand, it was impossible to distinguish between 5-(3′-hydroxyphenyl)-γ-valerolactone-4′-*O*-sulfate (**10**) and 5-(4′-hydroxyphenyl)-γ-valerolactone-3′-*O*-sulfate (**10′**) due to their chromatographic behaviour. These isomers were quantified using **10′** as reference compound.

There were no statistically significant differences in the excretion of most of the phenyl-γ-valerolactones between the water control and the green tea supplementation periods (p>0.05). This fact could be linked to the limited contribution of normal dosages of green tea to the total pool of circulating phenyl-γ-valerolactones under free-living conditions (with no dietary restrictions). However, green tea supplementation guaranteed the presence of phenolic scaffolds allowing the formation of 5-(3′,4′,5′-trihydroxyphenyl)-phenyl-γ-valerolactone (**5**), since it is mainly produced by the colonic catabolism of (–)-epigallocatechin (EGC) and (–)-epigallocatechin-3-gallate (EGCG),[1] contained in green tea but not in other flava-3-ol rich sources, like cocoa or red wine. Maximum urinary concentrations varied between 515

nM for 5-phenyl-γ-valerolactone-4'-*O*-sulphate (**6**) and 132,111 nM for 5-(hydroxyphenyl)-γ-valerolactone-*O*-sulphate isomers (**10**/**10'**). In terms of absolute excretion, maximum values ranged from 84 nmol/mmol creatinine for metabolite **6** to 15,697 nmol/mmol creatinine for isomers **10**/**10'** (*not shown*). The most abundant compounds were **4** and **10**/**10'**, although the relative contribution of each phenyl-γ-valerolactone to the total urinary excretion varied notably among subjects. With respect to minimum urinary concentrations, it should be noted that some phenyl-γ-valerolactones were not produced/excreted by some volunteers, and this can be related to the large inter-individual variability existing in the production of these colonic metabolites.[5,13,17,22-25]

The urinary concentrations recorded for some phenyl-γ-valerolactones, in particular **4** and **10**/**10'**, were quite high (reaching 132 μM). Comparison with other works is avoided, since most of them quantified phenyl-γ-valerolactones without using synthesised exact standards, or because the analysed samples were hydrolysed by using sulphatase and β-glucuronidase enzymes before analysis.[4,17,18] In this sense, these data and the accurate quantification of phenyl-γ-valerolactones with their respective reference compounds may lead to the redefinition of the recovery and bioavailability of flavan-3-ols.

4.3. Conclusions

This work described for the first time the synthetic procedure for 8 sulfate- and glucuronide-conjugated phenyl-γ-valerolactones. A quick, selective, sensitive, and reproducible validated UHPLC-ESI-MS/MS method allowing the quantification of up to 13 phenyl-γ-valerolactones in human urine was also developed. Moreover, the analytical challenges faced when dealing with some of these molecules were reported to save researchers in the field from further

future unsuccessful attempts. The analytical method allowed, for the first time, the accurate quantification of 10 phenyl-γ-valerolactones in urine samples of subjects consuming green tea, by using exact reference compounds. Additional efforts are needed to extrapolate the application of this analytical method to other biological samples, such as plasma and faeces, likely by using clean-up steps. However, this point requires further investigations since optimization of solid-phase extraction may be of critical importance. In addition, thanks to the information on the ionization properties of the synthesized compounds, the method could be extended to other phenyl-γ-valerolactones for which their pure forms are still lacking.

The availability of phenyl-γ-valerolactone conjugates as authentic bioanalytical standards will also allow the use of these key flavan-3-ol metabolites in cells assays, in order to shed light on their *in vitro* putative bioactivity. Moreover, the quantification of phenyl-γ-valerolactones in urine samples by comparison with authentic synthesized standards will open the door to better studying the bioavailability of flavan-3-ols and the real exposure of populations to flavan-3-ol sources. Overall, the present work can provide valuable insights in the future study of the fate of flavan-3-ols and phenyl-γ-valerolactones in the human body, as well as help in the understanding of their potential role in the prevention of chronic diseases.

Table 4.1. *Retention times and optimized SRM conditions for identification and quantification of phenyl-γ-valerolactones*

No.	Compound	RT (min)	Parent ion (m/z)	S-lens	Quantifier		Qualifier	
					Product ion (m/z)	CE (V)	Product ion (m/z)	CE (V)
1	5-(4′-hydroxyphenyl)-γ-valerolactone	5.26	191	70	147	13	106	31
2	5-(3′-hydroxyphenyl)-γ-valerolactone	4.81	191	70	147	20	106	31
3	5-(3′,4′-dihydroxyphenyl)-γ-valerolactone	3.94	207	75	163	20	122	25
4	5-(3′,5′-dihydroxyphenyl)-γ-valerolactone	3.54	207	75	163	18	123	20
5	5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone	2.19	223	78	179	21	138	26
6	5-phenyl-γ-valerolactone-4′-O-sulphate	4.53	271	93	191	23	147	35
7	5-phenyl-γ-valerolactone-3′-O-sulphate	4.71	271	92	191	23	106	48
8	5-phenyl-γ-valerolactone-3′,4′-di-O-sulphate	4.97	367	52	287	12	207	33
9	5-phenyl-γ-valerolactone-3′-O-glucuronide	4.03	367	93	191	25	147	43
10	5-(3′-hydroxyphenyl)-γ-valerolactone-4′-O-sulphate	4.46	287	96	207	23	163	34
10′	5-(4′-hydroxyphenyl)-γ-valerolactone-3′-O-sulphate	4.42	287	96	207	23	163	35
11	5-(5′-hydroxyphenyl)-γ-valerolactone-3′-O-sulphate	3.83	287	96	207	23	163	35
12	5-(5′-hydroxyphenyl)-γ-valerolactone-3′-O-glucuronide	2.11	383	87	207	24	163	40

CE: collision energy

Table 4.2. *Parameters for quantification of phenyl-γ-valerolactones in human urine samples by HPLC-ESI-MS/MS*

AC	Calibration curve	R^2	LOD (nM)	LLOQ (nM)	ULOQ (nM)	Precision intra-day (% RSD)			Precision inter-day (% RSD)			Accuracy (%)
						L1	L2	L3	L1	L2	L3	
1	y = 2982x	0.989	556	1113	100000	5.2	3.9	4.2	6.9	7.5	4.3	112.6
2	y = 891x	0.989	1113	2227	100000	8.6	6.3	3.0	7.7	8.6	6.9	99.8
3	y = 93122x	0.991	6.2	12.4	100000	16.8	0.1	1.8	16.1	1.8	3.4	102.6
4	y = 6245x	0.996	61.6	123	99852	18.9	5.1	0.1	21.7	7.8	3.6	96.5
5	$y = 39.4x^2 + 76.9x$	0.998	1112	2223	66695	23.6	12.5	8.8	17.2	15.2	5.3	86.6
6	y = 94304x	0.990	1.2	2.5	133333	2.8	10.2	1.0	3.3	7.6	6.8	106.7
7	y = 341669x	0.996	1.2	6.2	100000	6.6	9.3	0.2	10.8	4.4	7.0	117.3
8	y = 131.85x	0.993	1110	2220	100000	6.0	2.1	1.3	16.2	13.8	11.1	94.3
9	y = 16626x	0.993	2.5	12.4	66722	8.2	13.0	0.0	17.6	12.8	7.8	87.9
10	$y = -2231x^2 + 396261x$	0.995	0.2	0.6	100000	12.9	7.8	7.9	13.6	5.8	6.5	94.7
10'	$y = -1856x^2 + 428010x$	0.988	0.6	1.2	100000	4.9	5.6	0.1	1.0	8.5	6.8	121.9
11	y = 136366x	0.989	6.2	12.3	100000	4.9	10.0	0.2	5.5	8.9	9.7	104.6
12	y = 11046x	0.993	12.3	24.7	66667	17.8	14.3	0.1	19.5	9.7	0.3	92.8

AC = analyzed compound

4.4. Experimental section

4.4.1. Urine collection and processing

Urine samples for method validation were obtained from subjects consuming green tea. In particular, sixteen healthy adults aged between 35 to 50 were recruited. Subjects were non-smokers with no previous history of chronic diseases, did not take regularly (daily) green tea or vitamin/herbal supplements, and had not special dietary preferences, e.g. vegetarianism. They were not under long-term medication, had not been hospitalised in the previous 12 months, and had not received medical care in the past three months. Subjects with Body Mass Index (BMI) higher than 27 kg/m^2 were excluded. Written consent was obtained and all procedures complied with the Declaration of Helsinki. The study was approved by the Human Subjects Ethics Sub-committee of the Hong Kong Polytechnic University.

Subjects were assigned to have either tea or water first on a randomised, single-blinded basis. On day 1 of each subject's participation, baseline urine samples for their 7-day treatment (supplementation study) were collected into containers without any preservative and stored frozen (−80 °C) until used. From day 1, all subjects were required to drink either 200 mL of 1% w/v green tea (pre-rain Loong-cheng tea leaves, kindly provided by Ying Kee Tea House, HKSAR) or hot water twice a day (preferably, in the morning and at night) for seven consecutive days (tea bags of green tea were supplied), and they would return to the laboratory on day 8, when urine samples were collected as previously reported. Subjects then went through a 4-week washout period, after which the procedures of 7 days' supplementation were repeated, with each subject crossed-over onto the other treatment. Urine samples were collected again. Compliance was assessed by counting up the number of tea

bags returned from green tea supplementation group and by inquiry to both groups. A compliance >80% was regarded as satisfactory.

Urine samples were defrosted, vortexed, diluted in 0.1% formic acid in water (1/4, v/v), centrifuged at 18000 *g* for 5 min, and filtered through 0.22 µm nylon filters prior to the analysis by UHPLC-ESI-MS/MS.

4.4.2. UHPLC-ESI-QqQ-MS/MS

All synthesized standards and samples were analysed by UHPLC DIONEX Ultimate 3000 equipped with a TSQ Vantage triple quadrupole mass spectrometer (Thermo Fisher Scientific Inc., San Jose, CA, USA) fitted with a heated-electrospray ionization source (H-ESI-II; Thermo Fisher Scientific Inc.).

Separations were performed with a Kinetex EVO C18 (100 × 2.1 mm), 2.6 µm particle size (Phenomenex). For UHPLC, mobile phase A was 0.2% formic acid in water and mobile phase B was acetonitrile containing 0.2% formic acid. The gradient started with 5%B, keeping isocratic conditions for 0.5 min, reaching 95%B at 7 min, followed by 1 minute at 95% B and then 4 min at the start conditions to re-equilibrate the column. The flow rate was set at 0.4 mL/min, the injection volume was 5 µL, and the column was thermostated at 40°C.

The MS worked in negative ionization mode with capillary temperature at 270 °C, while the source at 300 °C. The sheath gas flow was 60 units, while auxiliary gas pressure was set to 10 units. The source voltage was 3 kV. Ultra high-purity argon gas was used for collision-induced dissociation (CID). Each synthesized compound was directly infused into the ESI source (5 µg/mL at a flow rate of 10 µL/min) in combined mode with a background mode of 70/30 v/v of phase A/phase B at 0.3 mL/min. Characteristic MS conditions (S-lens RF amplitude voltage and collision energy) were optimized for each phenyl-γ-valerolactone. The applied method consisted in the selective determination of each target precursor ion by the acquisition of characteristic product ions in the “selected

reaction monitoring" (SRM) mode. Two molecular transitions were used to qualify and quantify phenyl-γ-valerolactone conjugates. Data processing was performed using Xcalibur software from Thermo Scientific.

4.4.3. Method validation

The method was validated for selectivity, calibration curve, range, limit of detection (LOD), lower limit of quantification (LLOQ), upper limit of quantification (ULOQ), intra-day and inter-day precision, and accuracy. Method validation was carried out on diluted blank urine samples spiked with the synthesized phenyl-γ-valerolactones and according to Food and Drug Administration (FDA) guidelines.[21] Blank urine samples were kindly provided by three healthy volunteers following a phenolic-free diet for 72 h.

Compounds were individually dissolved in dimethyl sulfoxide at 10 mM and individual stock solutions were diluted and pooled to obtain a standard solution at 200 µM in 1 mL 0.1% formic acid in acetonitrile. Working dilutions of phenyl-γ-valerolactones from the standard pool solution were prepared in 0.1% formic acid in water/blank urine (4/1, v/v), with concentrations ranging from 0.1 nM to 133 µM. Compound **10** was prepared individually following the same procedure. A minimum of 14 concentration levels were used.

Selectivity was assessed by analysing diluted blank urine samples spiked or not with phenyl-γ-valerolactones at the LLOQ. The evaluation of the range of calibration curves was based on data fitting to linear or quadratic regressions, prioritizing linear fitting. Acceptable fitting was estimated by using the coefficient of determination (R^2). The LOD and LLOQ for each compound were determined as the concentration in which the quantifier transition showed a signal-to-noise (S/N) ratio ≥3 and ≥10, respectively. The intra-day precision (repeatability) and inter-day precision (semi-reproducibility) of the method, reported as the relative standard deviation (% RSD), was evaluated at the LLOQ

of each compound (L1) and at two higher concentration levels (5xLOQ, L2, and 10xLOQ, L3). Each solution was injected randomly three times per day in three different days. The acceptance criteria was RSD <20% for L1 and <15% for both L2 and L3. Accuracy was calculated in terms of recovery rate for the L2 concentration level of each compound, as the ratio between the mean recorded concentration and the spiked concentration, multiplied by 100.

4.4.4. Data and statistical analysis

All analyses were performed in triplicate for method validation. Data are reported as mean ± standard deviation (SD). Statistical analysis was carried out using the IBM SPSS Statistics 23.0 software package (IBM, Chicago, IL, USA). Non-parametric Kruskal-Wallis test was performed and, when significant (p<0.05), the Mann-Witney *U* test was applied to define specific differences in the urinary excretion of phenyl-γ-valerolactones.

4.4.5. Chemicals

Dichloromethane (HPLC grade), was dried by distillation on CaH_2 according to standard procedures. THF dry and Et_2O dry were distilled on Na/Benzophenone. Solvents for chromatography and filtration including hexane, ethyl acetate, dichloromethane, anhydrous ethanol, methanol, DMF, toluene and 2-propanol were ACS or HPLC grade and were used as received. Petroleum ether for flash chromatography was ACS grade (bp≥90% 40-60 °C) and was used as such without further purifications. Ammonia-methanol mixture was prepared by bubbling liquid ammonia in methanol at 0 °C for 30 min.

Unless otherwise noted, all reactions were performed in oven-dried or flame-dried glassware under an atmosphere of nitrogen or argon. Air-sensitive

reagents and solutions were transferred via syringe or cannula and were introduced to the apparatus through rubber septa. Reagents were supplied from commercial sources without further purification. Valerolactone aglycones **1-5** and valerolactone precursors **15**, **15'** and **17** were prepared in-house using the synthetic strategy outlined previously by Curti et al.[14] Denmark's chiral bis-phosphoramides (R,R) (352310-87-3), and (S,S) (873306-78-6) were commercially available, and were used as such, without further purifications. 2,2,2-trichloroethyl chlorosulfate **13** (TCECS),[26] benzyl 2,3,4-tri-*O*-benzyl-1-*O*-(trichloroacetimidoyl)-α-D-glucuronate **14**,[27] triisopropylsilyloxy-furan (TIPSOF),[28] 3-(benzyloxy)-4-hydroxy-benzaldehyde[29] were prepared according to reported procedures.

NMR spectra were recorded at 300 MHz or 400 MHz (^{1}H) and 75 MHz or 100 MHz (^{13}C). Spectra were referenced to tetramethylsilane (0.0 ppm, 1H; 0.0 ppm, 13C, in $CDCl_3$). Chemical shifts (δ) are reported in parts per million (ppm), and multiplicities are indicated as s (singlet), d (doublet), t (triplet), q (quartet), sext (sextet), sept (septet), dd (double doublet), m (multiplet), and b (broad). Coupling constants, *J*, are reported in Hertz. ^{1}H and ^{13}C NMR assignments are corroborated by 1D and 2D experiments (gCOSY, gHSQC, DEPT). Optical rotation data ($[\alpha]_D^{20}$) were obtained on a digital Perkin Elmer polarimeter at 589 nm (NaD) and 20 °C using a 100 mm cell with a 1 mL capacity and are given in units of 10^{-1} deg cm^2 g^{-1}.

<u>Synthesis of compound 6</u>: Representative Procedure for the synthesis of *O*-Sulfate γ-valerolactones

***Representative Procedure 1* for the preparation of the 2,2,2-Trichloroprotected sulfates.**

(*R*)-5-phenyl-γ-valerolactone 4'-*O*-trichloroethylsulfate. According to a known procedure[26] to a solution of **1** (10.0 mg, 0.05 mmol) in DCM dry (3 mL), Et_3N (8.4 µL, 0.06 mmol, 1.2 equiv), DMAP (6.1 mg, 0.05 mmol, 1 equiv) and 2,2,2-trichloroethyl chlorosulfate TCECS (74.4 mg, 0.3 mmol, 6 equiv) were sequentially added. The solution was stirred at room temperature for 16 h. The resulting white suspension was diluted with EtOAc (6 mL) and washed with H_2O (6 mL), 1 N HCl (6 mL), and brine (6mL). The organic layer was dried (Na_2SO_4), and concentrated under vacuum. The residue was chromatographed on silica gel (elution by gradient from 75:25 to 65:35 Petroleum Ether/EtOAc) to give the corresponding pure protected sulfate intermediate (15.1 mg, 75%) as a pale yellow resin. TLC: R_f = 0.33 (40/60 petroleum ether/ethyl acetate). Opt. Rot. $[\alpha]_D^{20}$ = − 21.0 (c 1.0 g/100mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.01 (s, 4H, Ar), 4.91 (s, 2H, $\underline{CH_2}CCl_3$), 4.76 (dddd, *J* = 7.9, 6.8, 6.8, 5.6 Hz, 1H, H4), 3.10 (dd, *J* = 14.3, 6.8 Hz, 1H, H5a), 3.02 (dd, *J* = 14.3, 5.4 Hz, 1H, H5b), 2.51 (ddd, *J* = 17.8, 9.5, 9.5 Hz, 1H, H2a), 2.43 (ddd, *J* = 17.8, 9.3, 4.3 Hz, 1H, H2b), 2.33 (dddd, *J* = 12.9, 9.6, 6.6, 4.3 Hz, 1H, H3a), 1.92 (dddd, *J* = 12.9, 9.4, 9.4, 8.0 Hz, 1H, H3b).^{13}C NMR (100 MHz, $CDCl_3$): δ 176.9 (Cq, C1), 149.3 (Cq Ar), 136.4 (Cq Ar), 131.3 (2C, CH Ar), 121.5 (2C, CH Ar), 92.5 (Cq, $CH_2\underline{CCl_3}$), 80.6 (CH_2, $\underline{CH_2}CCl_3$), 80.5 (CH, C4), 40.9 (CH_2, C5), 28.8 (CH_2, C2), 27.5 (CH_2, C3). MS (ESI, 50eV): Calcd.: $[M+Na^+]$: 424.9 Found: $[M+Na^+]$: 425.1.

***Representative Procedure 2* for the Removal of 2,2,2-Trichloroethyl Group**

(*R*)-5-phenyl-γ-valerolactone 4' *O*-Sulfate (6). According to a known procedure,[26] to a solution of protected intermediate valerolactone (15.1 mg, 0.04 mmol) in absolute EtOH (2 mL) ammonium formate (15.1 mg, 0.24 mmol, 6 equiv) and Zn dust (5.2 mg, 0.08 mmol, 2 equiv) were sequentially added The solution was stirred until all reagents were consumed, as determined by TLC (2 h). The reaction was filtered through Celite and the supernatant was

concentrated in vacuum. The residue was subjected to flash chromatography (EtOAc/MeOH 80/20) to give the desired sulphated valerolactone **6** as an amorphous solid (9.7 mg, 84%). TLC: R_f = 0.19 (90/10 ethyl acetate/MeOH); Opt. Rot. $[\alpha]_D^{20}$ −19.0 (c 0.7 g/100mL,CH_3OH); ^{1}H NMR (400 MHz, MeOD): δ 7.22 (m, 4H, Ar), 4.71 (dddd, *J* = 6.8, 6.8, 6.8, 6.8 Hz, 1H, H4), 3.03 (dd, *J* = 14.0, 6.5 Hz, 1H, H5a), 2.90 (dd, *J* = 14.0, 6.0 Hz, 1H, H5b), 2.51 (ddd, *J* = 17.7, 9.4, 9.4 Hz, 1H, H2a), 2.40 (ddd, *J* = 17.7, 9.4, 4.5 Hz, 1H, H2b), 2.27 (dddd, *J* = 12.8, 9.7, 6.7, 4.5 Hz, 1H, H3a), 1.97 (dddd, *J* = 12.8, 9.3, 9.3, 7.5 Hz, 1H, H3b). ^{13}C NMR (100 MHz, MeOD): 180.3 (Cq, C1), 153.0 (Cq, Ar), 134.5 (Cq, Ar), 131.4 (2C, CH, Ar), 122.7 (2C, CH, Ar), 83.2 (CH_2, C4), 41.6 (CH, C5), 29.7 (CH_2, C2), 28.2 (CH_2, C3).

Synthesis of compound 7:

(*R*)-5-phenyl-γ-valerolactone-3'-*O*-trichloroethylsulfate. The protected sulfate was prepared according to the Representative Procedure 1 using: *γ*-valerolactone **2** (28.8 mg, 0.15 mmol, 1 equiv), Et_3N (25 µL, 0.18 mmol), DMAP (18 mg, 0.15 mmol), TCECS (223.1 mg, 0.9 mmol) in DCM dry (6 mL) at room temperature for 16h. Flash chromatographic purification (elution by gradient from 75:25 to 65:35 Petroleum Ether/EtOAc) afforded a protected sulfate intermediate (51.1 mg, 84% yield) as a pale yellow resin. TLC: R_f = 0.33 (40/60 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ −15.2 (c 0.8 g/100mL,$CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40 (dd, *J* = 8.1, 8.1 Hz, 1H, Ar), 7.24-7.28 (m, 3H, Ar), 4.84 (s, 2H, $\underline{CH_2}CCl_3$), 4.71 (dddd, *J* = 7.9, 6.7, 6.7, 5.5 Hz, 1H, H4), 3.07 (dd, *J* = 14.4, 6.9 Hz, 1H, H5a), 2.99 (dd, *J* = 14.3, 5.4 Hz, 1H, H5b), 2.51 (ddd, *J* = 17.8, 9.5, 9.5 Hz, 1H, H2a), 2.44 (ddd, *J* = 17.8, 9.3, 4.4 Hz, 1H, H2b), 2.32 (dddd, *J* = 12.8, 9.5, 6.6, 4.4 Hz, 1H, H3a), 1.95 (dddd, *J* = 12.8, 9.4, 9.4, 7.9 Hz, 1H, H3b). ^{13}C NMR (100 MHz, $CDCl_3$): 176.7 (Cq, C1), 150.3 (Cq, Ar), 139.1 (Cq, Ar), 130.5 (CH, Ar), 129.2 (CH, Ar), 122.2 (CH, Ar), 119.8 (CH, Ar), 92.5 (Cq, $CH_2\underline{CCl_3}$), 80.6

(CH_2, $\underline{CH_2}CCl_3$), 80.3 (CH, C4), 41.2 (CH_2, C5), 28.8 (CH_2, C2), 27.5 (CH_2, C3); MS (ESI, 50eV): Calcd.: $[M+Na^+]$: 424.9, Found: $[M+Na^+]$: 425.1

(*R*)-5-phenyl-γ-valerolactone-3'-*O*-Sulfate (7): Sulfate **7** was prepared according to the Representative Procedure 2 using: 3'-*O*-trichloroethylsulfate of (*R*)-5-(3'-hydroxyphenyl)-γ-valerolactone (51.1 mg, 0.13 mmol, 1 equiv), ammonium formate (47.9 mg, 0.76 mmol), Zn dust (16.6 mg, 0.25 mmol) in absolute EtOH (6.5 mL) for 45 minutes. Flash chromatographic purification (elution by gradient from 95:5 to 85:15 EtOAc/MeOH) afforded pure sulfate valerolactone 7 (36.5 mg, 97% yield) as a colourless resin (R_f = 0.20 (90/10 ethyl acetate/MeOH); Opt. Rot. $[\alpha]_D^{20}$ −15.7 (c 0.6 g/100mL,MeOH); ^{1}H NMR (400 MHz, MeOD): δ 7.29 (dd, *J* = 7.8, 7.8 Hz, 1H, Ar), 7.24 (dd, *J* = 1.8, 1.8 Hz, 1H, Ar), 7.21 (ddd, *J* = 8.0, 2.1, 1.2 Hz, 1H, Ar), 7.10 (ddd, *J* = 7.5, 1.3, 1.3 Hz, 1H, Ar), 4.82 (dddd, *J* = 6.8, 6.8, 6.8, 6.8 Hz, 1H, H4), 3.06 (dd, *J* = 14.0, 6.6 Hz, 1H, H5a), 2.97 (dd, *J* = 14.0, 6.0 Hz, 1H, H5b), 2.54 (ddd, *J* = 17.8, 9.4, 9.4 Hz, 1H, H2a), 2.43 (ddd, *J* = 17.8, 9.4, 4.6 Hz, 1H, H2b), 2.30 (dddd, *J* = 12.7, 9.7, 6.7, 4.5 Hz, 1H, H3a), 2.00 (dddd, *J* = 12.7, 9.2, 9.2, 7.6 Hz, 1H, H3b). ^{13}C NMR (100 MHz, MeOD): 180.3 (Cq, C1), 154.2 (Cq, Ar), 139.3 (Cq, Ar), 130.3 (CH, Ar), 127.2 (CH, Ar), 123.7 (CH, Ar), 123.0 (CH, Ar), 83.0 (CH, C4), 42.0 (CH_2, C5), 29.6 (CH_2, C2), 28.2 (CH_2, C3).

Synthesis of compound 8:

(*R*)-5-phenyl-γ-valerolactone-3',4'-di*O*-trichloroethylsulfate. The protected sulfate intermediate was prepared according to Representative Procedure 1 using: *γ*-valerolactone **3** (14.5 mg, 0.07 mmol, 1 equiv), Et_3N (24 µL, 0.16 mmol), DMAP (17 mg, 0.14 mmol), TCECS (208 mg, 0.84 mmol) in DCM dry (6 mL) at room temperature for 48h. Flash chromatographic purification (elution by

gradient: from 60/40 to 55/45 Petroleum Ether/EtOAc) afforded protected sulfate as a pale yellow resin (26.5 mg, 60% yield). TLC: R_f = 0.51 (55/45 petroleum ether/ethyl acetate). Opt. Rot. $[\alpha]_D^{20}$ −24.0 (c 1.0 g/100mL,$CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (d, *J* = 8.5, H5'), 7.46 (bs, H2'), 7.31 (bd, *J* = 8.5 Hz, H6'), 4.94 (s, 2H, CH_2, $\underline{CH_2}CCl_3$), 4.92 (s, 2H, CH_2, $\underline{CH_2}CCl_3$), 4.68 (dddd, *J* = 6.7, 6.7, 6.7, 6.7 Hz, 1H, H4), 3.02 (m, 2H, H5), 2.42-2.58 (m, 2H, H2), 2.31-2.39 (m, 1H, H3a), 1.88-1.98 (m, H3b). ^{13}C NMR (100 MHz, $CDCl_3$): 176.4 (Cq, C1), 141.1 (Cq, Ar), 140.1 (Cq, Ar), 138.5 (Cq, Ar), 130.2 (CH, Ar), 124.3 (CH, Ar), 123.5 (CH, Ar), 92.3 (2C, Cq, $CH_2\underline{CCl_3}$), 81.1 (CH_2, $\underline{CH_2}CCl_3$), 81.0 (CH_2, $\underline{CH_2}CCl_3$), 79.9 (CH_2, C4), 41.0 (CH_2, C5), 28.8 (CH_2, C2), 27.7 (CH_2, C3). MS (ESI, 50eV): Calcd.: [M+Na^+]: 650.8, Found: [M+Na^+]: 650.9

(*R*)-5-phenyl-γ-valerolactone-3',4'-di-*O*-sulfate (8): Sulfate **8** was prepared according to the Representative Procedure 2 using: 3', 4'-*O*-ditrichloroethylsulfate of (*R*)-5-(3',4'-dihydroxyphenyl)-γ-valerolactone (24.5 mg, 0.04 mmol, 1 equiv), ammonium formate (30.2 mg, 0.48 mmol), Zn dust (10.5 mg, 0.16 mmol) in absolute EtOH (8 mL) for 16 h. Flash chromatographic purification (80:20 EtOAc/MeOH) afforded sulfate valerolactone **8** (14.0 mg, 87% yield). White amorphous solid; R_f = 0.21 (80/20 AcOEt/MeOH); Opt. Rot. $[\alpha]_D^{20}$ −12.1 (c 0.9 g/100mL, MeOH); ^{1}H NMR (300 MHz, MeOD): δ 7.50 (d, *J* = 8.4 Hz, 1H, H5'), 7.48 (d, *J* = 1.9, 1H, H2'), 7.05 (dd, *J* = 8.4, 2.2, 1H, H6'), 4.79 (dddd, *J* = 6.6, 6.6, 6.6, 6.6 Hz, 1H, H4), 3.02 (dd, *J* = 14.1, 6.5 Hz, 1H, H5a), 2.93 (dd, *J* = 14.1, 6.1 Hz, 1H, H5b), 2.54 (ddd, *J* = 17.8, 9.4, 9.4 Hz, 1H, H2a), 2.43 (ddd, *J* = 17.8, 9.2, 4.5 Hz, 1H, H2b), 2.29 (dddd, *J* = 12.7, 9.5, 6.6, 4.5 Hz, 1H, H3a), 1.99 (dddd, *J* = 12.7, 9.3, 9.3, 7.6 Hz, 1H, H3b). ^{13}C NMR (100 MHz, MeOD): 180.3 (Cq, C1), 145.4 (Cq, Ar), 144.3 (Cq, Ar), 135.1 (Cq, Ar), 127.3 (CH, Ar), 124.8 (CH, Ar), 123.7 (CH, Ar), 83.0 (CH, C4), 41.6 (CH_2, C5), 28.7 (CH_2, C2), 28.2 (CH_2, C3).

Synthesis of compound 10:

(*R*)-5-(4'-benzyloxyphenyl)-γ-valerolactone-3'-*O*-trichloroethylsulfate (16): Protected sulfate **16** was prepared according to the Representative Procedure 1 using: compound (*R*)-5-[(4-benzyloxy)-3-hydroxyphenyl]dihydrofuran-2(3*H*)-one (previously synthesized by Curti et al)[3] (26.5 mg, 0.09 mmol, 1 equiv), Et_3N (15 µL, 0.11 mmol), DMAP (11 mg, 0.09 mmol), TCECS (132 mg, 0.53 mmol) in DCM dry (5 mL) at room temperature for 16 h. Flash chromatographic purification (elution by gradient: from 60/40 to 55/45 Petroleum Ether/EtOAc) afforded protected sulfate **16** as a pale yellow resin (32.1 mg, 70 %). TLC: R_f = 0.51 (45/15/40 petroleum ether/DCM/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ −10.8 (c 0.4 g/100mL,$CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): 7.33-7.45 (m, 5H, Ph), 7.28 (d, *J* = 2.1 Hz, 1H, H2'), 7.17 (dd, *J* = 8.5, 2.1 Hz, 1H, H6'), 7.01 (d, *J* = 8.5 Hz, 1H, H5'), 5.09 (s, 2H, $\underline{CH_2}$Ph), 4.72 (s, 2H, $\underline{CH_2}CCl_3$), 4.68 (dddd, *J* = 6.5, 6.5, 6.5, 6.5 Hz, 1H, H4), 2.97 (dd, *J* = 14.3, 6.4 Hz, 1H, H5a), 2.91 (dd, *J* = 14.3, 5.6 Hz, 1H, H5b), 2.45 (ddd, *J* = 17.7, 9.5, 9.5 Hz, 1H, H2a), 2.39 (ddd, *J* = 17.7, 9.2, 4.4 Hz, 1H, H2b), 2.29 (dddd, J = 12.9, 9.5, 6.7, 4.4 Hz, 1H, H3a), 1.94 (dddd, *J* = 12.9, 9.4, 9.4, 7.9 Hz, 1H, H3b). ^{13}C NMR (75 MHz, $CDCl_3$): 176.9 (Cq, C1), 149.5 (Cq, Ar), 138.8 (Cq, Ar), 135.7 (Cq, Ar), 130.1 (CH, Ar), 129.6 (CH, Ar), 129.1 (2C, CH, Ar), 129.0 (Cq), 128.2 (2C, CH, Ar), 124.6 (CH, Ar), 121.2 (CH, Ar), 114.6 (CH, Ar), 112.5 (CH, Ar), 92.6 (Cq, $CH_2\underline{CCl_3}$), 80.5 (2C, $\underline{CH_2}CCl_3$, C4), 40.5 (CH, C5), 28.8 (CH_2, C2), 27.3 (CH_2, C3); MS (ESI, 50eV): Calcd.: [M+Na+]: 531.0, Found: [M+Na+]: 530.9

(*R*)-5-(4'-hydroxyphenyl)-γ-valerolactone-3'-*O*-Sulfate (10): To a solution of compound **16** (17.9 mg, 0.035 mmol, 1.0 equiv) in a degassed EtOAc (7 mL), Pd (10% on carbon, 10 mg) was added. To this black suspension, H_2 was flushed and kept sealed under pressure for 1 h. After this period the H_2 was removed under vacuum the resulting suspension was filtered in EtOAc, and the residue

concentrated to yield the desired debenzylated product (10.8 mg) as a crude that was used as such, without further purifications. The crude (10.8 mg, 0.03 mmol) was then subjected to TCE cleavage according to the Representative Procedure 2 using ammonium formate (11.4 mg, 0.18 mmol), Zn dust (3.9 mg, 0.06 mmol) in absolute EtOH (2 mL) for 45 minutes. Flash chromatographic purification (85:15 EtOAc/MeOH) afforded sulfate valerolactone **10** as an amorphous solid (6.4 mg, 60% two steps). R_f = 0.16 (90/10 AcOEt/MeOH); Opt. Rot. $[\alpha]_D^{20}$ −7.8 (c 0.4 g/100mL, MeOH); ^{1}H NMR (400 MHz, MeOD): δ 7.20 (d, *J* = 2.1 Hz, 1H, H2'), 6.93 (dd, *J* = 8.3, 2.1 Hz, 1H, H6'), 6.83 (d, *J* = 8.3 Hz, 1H, H5'), 4.75 (dddd, *J* = 6.9, 6.9, 6.9, 6.9 Hz, 1H, H4), 2.97 (dd, *J* = 14.0, 6.1 Hz, 1H, H5a), 2.84 (dd, *J* = 14.0, 6.5 Hz, 1H, H5b), 2.50 (ddd, *J* = 17.8, 9.2, 9.2 Hz, 1H, H2a), 2.38 (ddd, *J* = 17.7, 9.4, 4.7 Hz, 1H, H2b), 2.26 (dddd, *J* = 12.8, 9.7, 6.8, 4.7 Hz, 1H, H3a), 1.98 (dddd, *J* = 12.8, 9.2, 9.2, 7.4 Hz, 1H, H3b). ^{13}C NMR (100 MHz, MeOD): 180.3 (Cq, C1), 149.6 (Cq, Ar), 141.4 (Cq, Ar), 129.3 (Cq, Ar), 128.4 (CH, C6'), 125.3 (CH, C2'), 118.5 (CH, C5'), 83.0 (CH, C4), 41.3 (CH_2, C5), 29.6 (CH_2, C2), 28.1 (CH_2, C3).

Synthesis of compound 10':

3-benzyloxy-4-(*tert*-butyldimethylsilyloxy)benzaldehyde. Et_3N (118µL, 0.85 mmol, 1.5 equiv), DMAP (7 mg, 0.06 mmol, 0.1 equiv), TBSCl (128 mg, 0.85 mmol, 1.5 equiv) were added to a solution of 3-(benzyloxy)-4-hydroxybenzaldehyde[9] (129 mg, 0.56 mmol, 1 equiv) in CH_2Cl_2 dry (6 mL). The solution was stirred at room temperature for 2 h. The resulting white suspension was diluted with CH_2Cl_2 (6 mL) and washed with H_2O (3 mL), 1 N HCl (2 mL), and brine (2mL). The organic layer was dried (Na_2SO_4), and concentrated under vacuum. The residue was chromatographed on silica gel (90:10 Petroleum Ether/EtOAc) to give pure product (184.7 mg, 95%) as a pale

yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): 9.86 (s, CHO), 7.52 (d, J = 1.9 Hz, 1H, H2'), 7.34-7.49 (m, 5H, CH_2Ph), 7.42 (dd, J = 8.1, 1.8 Hz, 1H, H6'), 7.01 (d, *J* = 8.0 Hz, 1H, H5'), 5.12 (s, 2H, CH_2Ph), 0.99 (s, 9H, *tert*-Bu, TBS), 0.16 (s, 6H, CH_3, TBS). ^{13}C NMR (100 MHz, $CDCl_3$): δ 191.2 (CHO), 151.8 (Cq, Ar), 151.1 (Cq, Ar), 136.3 (Cq, Ar), 131.1 (Cq, Ar), 128.7 (2C, CH, Ar), 128.4 (CH, Ar), 128.2 (2C, CH, Ar), 126.5 (CH, Ar), 121.0 (CH, Ar), 112.0 (CH, Ar), 70.9 (CH_2, CH_2Ph), 25.7 (3C, *tert*-Bu, TBS), 18.6 (Cq, *tert*-Bu, TBS), −4.4 (2C, CH_3, TBS); MS (ESI, 50eV): Calcd.: [M+Na+]: 365.3, Found: [M+Na+]: 365.1.

(+)-(*R*)-5-[(*S*)-hydroxy(3-benzyl-4-(*tert*-buthyldimethylsilyl)oxy)phenyl) methyl]furan-2(5*H*)-one. According to a known procedure,[14] diisopropylethylamine (DIPEA, 17 µL, 0.1 mmol) was added via syringe to a solution of bisphosphoramide Denmark-(*R*,*R*) (25.0 mg, 0.03 mmol) in anhydrous CH_2Cl_2 (5.0 mL), under argon atmosphere at room temperature. To this solution, 3-benzyloxy-4-(*tert*-butyldimethylsilyloxy)benzaldehyde. (514.0 mg, 1.5 mmol), dissolved in anhydrous CH_2Cl_2 (2.0 mL), was added in one portion, and the reaction mixture was cooled to –78 °C under stirring. After 15 min, a solution of freshly distilled $SiCl_4$ (172.2 µL, 1.5 mmol) in anhydrous CH_2Cl_2 (1.8 mL) was added dropwise to the reaction mixture, followed by the dropwise addition of TIPSOF (240.4 mg, 1.0 mmol, 1.0 equiv) dissolved in 1.2 mL of anhydrous CH_2Cl_2. The resulting mixture was stirred at –78 °C for 12 h, whereupon chilled CH_2Cl_2 (12.0 mL) was added and the cold reaction mixture was poured into a rapidly stirring solution of 1:1 sat. aq $NaHCO_3$/brine (60 mL) at 0 °C. This biphasic mixture was stirred vigorously for 2 h after which the organic layer was extracted with EtOAc (3 × 60 mL). The combined organic extracts were dried over Na_2SO_4, filtered and the filtrate was concentrated in vacuo. The residue was purified by silica gel flash chromatography (60/40 Petroleum Ether:EtOAc) to yield product the desired product (416 mg, 97%

combined yield) as an inseparable *anti*/*syn* mixture of isomers. The dr (*anti/syn*) of the reaction was determined to be 65:35 by ^{1}H NMR analysis of the crude reaction mixture. Catalyst Denmark-(*R,R*) could be recovered (90%) by eluting the flash column with 90:10 (EtOAc/MeOH-NH_3) mixture. For a useful characterization of compound anti-butenolide product, a portion of the resulting mixture was further purified by silica gel flash chromatography (elution by gradient from 70:30 to 65:35 Petroleum Ether/EtOAc) yielding pure *anti*-butenolide (172 mg) as an amorphous solid. Major isomer: TLC: R_f = 0.45 (65/35 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ +115 (c 0.5 g/100mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32-7.46 (m, 5H, Ph), 7.18 (dd, *J* = 5.8, 1.5 Hz, 1H, H3), 6.95 (d, *J* = 1.9 Hz, H2'), 6.90 (d, *J* = 8.1 Hz, 1H, H5'), 6.85 (dd, *J* = 8.1, 2.0 Hz, 1H, H6'), 6.14 (dd, *J* = 5.8, 2.0 Hz, 1H, H2), 5.06-5.13 (m, 3H, CH_2Ph, H4), 5.01 (d, *J* = 4.3 Hz, 1H, H5), 2.06 (s, 1H, OH), 0.99 (s, 9H, *tert*-Bu, TBS), 0.14 (s, 6H, CH_3, TBS). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.1 (Cq, C1), 153.0 (CH, C3), 150.5 (Cq, Ar), 145.8 (Cq, Ar), 136.9 (Cq, Ph), 131.6 (Cq, Ar), 128.7 (2C, CH, Ph), 128.2 (CH, Ph), 128.1 (2C, CH, Ph), 123.4 (CH, C2), 121.3 (CH, C5'), 119.1 (CH, C6'), 112.2 (CH, C2'), 86.7 (CH, C4), 73.0 (CH, C5), 71.0 (CH_2, CH_2Ph), 25.9 (3C, *tert*-Bu, TBS), 18.6 (Cq, *tert*-Bu, TBS), −4.3 (CH_3, TBS), −4.4 (CH_3, TBS); MS (ESI, 50eV): Calcd.: [M+Na^+]: 449.3, Found: [M+Na^+]: 449.2; Chiral HPLC (WELK-O1, 90/10 Hexane/Ethanol, 1.0 ml/min, 254 nm): *R*t 10.46 min (major), 11.65 min (minor) (98% ee).

(+)-(*R*)-5-[(*S*)-(3-benzyloxy)-4-(*tert*-butyldimethylsilyloxyphenyl)methyl] dihydrofuran-2(5*H*)-one. A solution of a 65:35 (*anti/syn*) mixture of 5-[(*S*)-hydroxy(3-benzyl-4-(*tert*-buthyldimethylsilyl)oxy)phenyl) methyl]furan-2(5*H*)-one (384 mg, 0.90 mmol, 1.0 equiv) in 10 mL of absolute MeOH, was cooled to 0 °C and treated with $NiCl_2 \cdot 6H_2O$ (53.5.0 mg, 0.22 mmol). The resulting mixture was stirred at the same temperature for 5 min before the addition of $NaBH_4$

(33.9 mg, 0.90 mmol). After 30 min, the reaction mixture was quenched with saturated aqueous NH_4Cl solution and extracted with CH_2Cl_2 (3×5 mL). The combined extracts were dried ($MgSO_4$) and concentrated under vacuum. Flash chromatographic purification (65:35 Petroleum Ether/EtOAc) afforded 240.0 mg of *anti* saturated lactone (major isomer) as an amorphous solid, and 128.2 mg of *syn*-lactone (minor isomer) as a colourless resin (95% isolated combined yield). TLC: R_f = 0.40 (65/35 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ +32.4 (*c* 0.8 g/100mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31-7.46 (m, 5H, Ph), 6.97 (d, *J* = 1.5 Hz, 1H, H2'), 6.88 (d, *J* = 8.1 Hz, 1H, H5'), 6.83 (dd, *J* = 8.2, 1.7 Hz, H6'), 5.10 (d, *J* = 12.3 Hz, 1H, $\underline{CH_2}$Ph), 5.07 (d, *J* = 12.5 Hz, 1H, $\underline{CH_2}$Ph), 5.02 (d, *J* = 3.0 Hz, 1H, H5), 4.64 (ddd, *J* = 7.7, 6.7, 3.1 Hz, 1H, H4), 2.48 (ddd, *J* = 17.9, 10.0, 6.2 Hz, 1H, H2a), 2.40 (ddd, *J* = 17.7, 9.7, 7.9 Hz, 1H, H2b), 2.17 (dddd, *J* = 13.7, 9.9, 7.5, 7.5 Hz, 1H, H3a), 1.88 (dddd, *J* = 13.5, 9.8, 7.5, 6.6 Hz, 1H, H4b), 0.98 (s, 9H, *tert*-Bu, TBS), 0.13 (s, 6H, Me, TBS). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.9 (Cq, C1), 150.4 (Cq, Ar), 145.5 (Cq, Ar), 137.0 (Cq, Ph), 131.8 (Cq, Ar), 128.6 (2C, CH, Ph), 128.1 (CH, Ph), 128.1 (2C, CH, Ph), 121.2 (CH, C5'), 119.1 (CH, C6'), 112.3 (CH, C2'), 83.5 (CH, C4), 73.4 (CH, C5), 71.0 (CH_2, $\underline{CH_2}$Ph), 28.7 (CH_2, C2), 25.9 (3C, CH_3, *tert*-Bu, TBS), 20.9 (CH_2, C3), 18.5 (Cq, *tert*-Bu, TBS), −4.4 (CH_3, TBS), −4.4 (CH_3, TBS); MS (ESI, 50eV): Calcd.: [M+Na^+]: 451.2, Found: [M+Na^+]: 451.3.

(–)-(*R*)-5-[(3-benzyloxy)-4-(*tert*-butyldimethylsilyloxy)phenyl] dihydrofuran-2(3*H*)-one (15'). Thionocarbonyldiimidazole (TCDI) (300 mg, 1.68 mmol) was added to a solution of butanolide (*R*)-5-[(*S*)-(3-benzyloxy)-4-(*tert*-butyldimethylsilyloxyphenyl)methyl] dihydrofuran-2(5*H*)-one (240 mg, 0.56 mmol, 1.0 equiv) in anhydrous THF (10 mL). The mixture was heated at reflux temperature for 8 h and then cooled, and the solvent was evaporated. The reagent in excess was removed by flash chromatography (50/50 Petroleum

Ether/EtOAc) to yield desired thiocarbamate intermediate(235 mg). AIBN (0.2 M solution in toluene, 2.2 mL) was added to a solution of the thiocarbamate (235 mg, 0.43 mmol) and tri-*n*-butyltinhydride (470 μL, 1.74 mmol) in toluene (8 mL). The mixture was heated under reflux for 1 h and then the solvent was evaporated, and the residue was chromatographed on silica gel (elution by gradient from 80/20 to 70/30 Petroleum Ether:EtOAc) to give pure (−)-**15'** (170 mg, 73% two steps) as a colorless resin. TLC: R_f = 0.48 (65/35 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ −4.9 (*c* 0.5 g/100mL, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31-7.44 (m, 5H, Ph), 6.80 (d, *J* = 8.0 Hz, 1H, H5'), 6.76 (d, *J* = 2.0 Hz, 1H, H2'), 6.68 (dd, *J* = 8.0, 2.0 Hz, H6'), 5.00 (s, 2H, CH_2Ph), 5.02 (d, *J* = 3.0 Hz, 1H, H5), 4.68 (dddd, *J* = 6.4, 6.4, 6.4, 6.4 Hz, 1H, H4), 2.95 (dd, *J* = 14.1, 5.7 Hz, 1H, H5a), 2.83 (dd, *J* = 14.1, 6.2 Hz, 1H, H5b), 2.41 (ddd, *J* = 17.7, 9.7, 8.9 Hz, 1H, H2a), 2.26 (ddd, *J* = 17.7, 9.4, 5.0 Hz, 1H, H2b), 2.16 (dddd, *J* = 12.7, 9.7, 6.9, 5.0 Hz, 1H, H3a), 1.87 (dddd, *J* = 12.9, 9.2, 9.2, 7.3 Hz, 1H, H4b), 0.96 (s, 9H, *tert*-Bu, TBS), 0.10 (s, 6H, Me, TBS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.4 (Cq, C1), 150.2 (Cq, Ar), 144.6 (Cq, Ar), 137.1 (Cq, Ph), 129.2 (Cq, Ar), 128.5 (2C, CH, Ph), 128.0 (3C, CH, Ph), 122.4 (CH, C6'), 121.3 (CH, C5'), 115.7 (CH, C2'), 81.0 (CH, C4), 71.3 (CH_2, $\underline{CH_2}$Ph), 41.0 (CH_2, C5), 28.8 (CH_2, C2), 27.0 (CH_2, C3), 25.9 (3C, CH_3, *tert*-Bu, TBS), 18.5 (Cq, *tert*-Bu, TBS), −4.4 (2C, CH_3, TBS). MS (ESI, 50eV): Calcd.: [M+Na^+]: 435.3, Found: [M+Na^+]: 435.1.

(*R*)-5-[(3-benzyloxy)-4-hydroxyphenyl]dihydrofuran-2(3*H*)-one. To a solution of protected γ-valerolactone **15'** (150 mg, 0.36 mmol, 1.0 equiv) in THF (6 mL) at room temperature, HF (~70% in pyrydine, 95 μL, 3.6 mmol) was slowly added and the resulting white slurry was kept under vigorous stirring at the same temperature for 48 h. The reaction was then neutralized with a saturated aq. solution of $NaHCO_3$ (5 mL) and then extracted with EtOAc (3 × 5 ml). The combined organic extracts were dried ($MgSO_4$) and concentrated under

vacuum. Flash chromatographic purification (60:40 Petroleum Ether/EtOAc) of the crude afforded 81 mg of the mono-protected intermediate (75%) as a colourless resin. TLC: R_f = 0.42 (55/45 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ −4.0 (*c* 0.5 g/100mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29-7.46 (m, 5H, Ph), 6.90 (d, *J* = 8.0 Hz, 1H, H5'), 6.84 (d, *J* = 1.9 Hz, 1H, H2'), 6.75 (dd, *J* = 8.1, 1.9 Hz, H6'), 5.14 (d, *J* = 11.5, 1H, $\underline{CH_2}$Ph), 5.11 (d, *J* = 11.6, 1H, $\underline{CH_2}$Ph), 4.68 (dddd, *J* = 6.0, 6.0, 6.0, 6.0 Hz, 1H, H4), 2.97 (dd, J = 14.2, 5.8 Hz, 1H, H5a), 2.89 (dd, J = 14.2, 6.0 Hz, 1H, H5b), 2.45 (ddd, *J* = 17.8, 9.4, 9.4 Hz, 1H, H2a), 2.32 (ddd, *J* = 17.8, 9.5, 4.9 Hz, 1H, H2b), 2.22 (dddd, *J* = 12.7, 9.7, 6.8, 4.9 Hz, 1H, H3a), 1.92 (dddd, *J* = 12.8, 9.0, 9.0, 7.2 Hz, 1H, H4b).^{13}C NMR (100 MHz, $CDCl_3$): δ 177.4 (Cq, C1), 145.9 (Cq, Ar), 145.1 (Cq, Ar), 136.4 (Cq, Ph), 128.9 (2C, CH, Ph), 128.6 (CH, Ph), 128.1 (2C, CH, Ph), 127.7 (Cq, Ar), 122.8 (CH, C6'), 114.9 (CH, C5'), 113.8 (CH, C2'), 81.1 (CH, C4), 71.0 (CH_2, $\underline{CH_2}$Ph), 41.0 (CH_2, C5), 28.8 (CH_2, C2), 27.0 (CH_2, C3). MS (ESI, 50eV): Calcd.: [M+Na^+]: 321.11, Found: [M+Na^+]: 321.10.

(*R*)-5-(3'-benzyloxyphenyl)-γ-valerolactone-4'-*O*-trichlorosulfate (16'). Protected sulfate **16'** was prepared according to the Representative Procedure 1 using: (*R*)-5-[(3-benzyloxy)-4-hydroxyphenyl]dihydrofuran-2(3*H*)-one (46.0 mg, 0.15 mmol, 1 equiv), Et_3N (26 µL, 0.18 mmol), DMAP (18.8 mg, 0.15 mmol), TCECS **13** (229 mg, 0.92 mmol) in DCM dry (7 mL) at room temperature for 16 h. Flash chromatographic purification (elution by gradient: from 60/40 to 55/45 Petroleum Ether/EtOAc) afforded protected sulfate **15**' as a pale yellow resin (61.0 mg, 77 %). TLC: R_f = 0.28 (55/45 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ −19.1 (*c* 1.0 g/100mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35-7.49 (m, 6H, $CH_2\underline{Ph}$, H5'), 7.00 (d, *J* = 1.9 Hz, 1H, H2'), 6.89 (dd, *J* = 8.3, 1.9 Hz, H6'), 5.14 (d, *J* = 11.4, 1H, $\underline{CH_2}$Ph), 5.12 (d, *J* = 11.6, 1H, $\underline{CH_2}$Ph), 4.70-4.77 (m, 3H, H4, $\underline{CH_2}CCl_3$), 3.03 (dd, *J* = 14.3, 6.5 Hz, 1H, H5a), 2.98 (dd, *J* = 14.4, 5.5 Hz, 1H, H5b),

2.51 (ddd, *J* = 17.9, 9.6, 9.6 Hz, 1H, H2a), 2.40 (ddd, *J* = 17.8, 9.4, 4.4 Hz, 1H, H2b), 2.30 (dddd, *J* = 12.8, 9.7, 6.7, 4.4 Hz, 1H, H3a), 1.92 (dddd, *J* = 12.8, 9.3, 9.3, 7.8 Hz, 1H, H4b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.9 (Cq, C1), 150.4 (Cq, Ar), 138.0 (Cq, Ar), 137.4 (Cq, Ph), 135.5 (Cq Ar), 129.1 (2C, CH, Ph), 128.8 (CH, Ph), 128.2 (2C, CH, Ph), 123.7 (CH, C5'), 122.3 (CH, C6'), 115.7 (CH, C2'), 92.6 (Cq, TCE), 80.4 (2C, $\underline{CH_2}CCl_3$, C4), 71.0 (CH_2, $\underline{CH_2}Ph$), 41.4 (CH_2, C5), 28.8 (CH_2, C2), 27.4 (CH_2, C3). MS (ESI, 50eV): Calcd.: [M+Na^+]: 531.0, Found: [M+Na^+]: 530.9

(*R*)-5-(3'-hydroxyphenyl)-γ-valerolactone-4'-*O*-Sulfate (10'). To a solution of compound **16'** (55.3 mg, 0.11 mmol, 1.0 equiv) in a degassed EtOAc (15 mL), Pd (10% on carbon, 30 mg) was added. To this black suspension, H_2 was flushed and kept sealed under pressure for 1 h. After this period, the H_2 was removed under vacuum, the resulting suspension was filtered in EtOAc, and the residue concentrated to yield the desired debenzylated intermediate (31.9 mg) as a crude that was used as such, without further purifications. The crude (31.9 mg, 0.08 mmol) was then subjected to TCE cleavage according to the Representative Procedure 2 using ammonium formate (32 mg, 0.46 mmol), Zn dust (29.8 mg, 0.17 mmol) in absolute EtOH (6 mL) for 45 minutes. Flash chromatographic purification (85:15 EtOAc/MeOH) afforded sulfate valerolactone **9'** as an amorphous solid (16.5 mg, 50% two steps). White amorphous solid; R_f = 0.15 (90/10 AcOEt/MeOH); Opt. Rot. $[\alpha]_D^{20}$ −15.9 (*c* 1.0 g/100mL, CH_3OH); ^{1}H NMR (400 MHz, MeOD): δ 7.21 (d, *J* = 8.2 Hz, H5'), 6.82 (d, *J* = 2.1 Hz, 1H, H2'), 6.71 (dd, *J* = 8.2, 2.1 Hz, H6'), 4.75 (dddd, *J* = 6.6, 6.6, 6.6, 6.6 Hz, 1H, H4), 2.96 (dd, *J* = 14.0, 6.4 Hz, 1H, H5a), 2.86 (dd, *J* = 14.0, 6.0 Hz, 1H, H5b), 2.52 (ddd, *J* = 17.8, 9.4, 9.4 Hz, 1H, H2a), 2.41 (ddd, *J* = 17.7, 9.4, 4.5 Hz, 1H, H2b), 2.26 (dddd, *J* = 12.8, 9.7, 6.7, 4.5 Hz, 1H, H3a), 1.96 (dddd, *J* = 12.8, 9.3, 9.3, 7.7 Hz, 1H, H3b). ^{13}C NMR (100 MHz, MeOD): δ 180.3 (Cq, C1),

150.6 (Cq, Ar), 140.2 (Cq, Ar), 136.1 (Cq, Ar), 124.2 (CH, C5'), 122.0 (CH, C6'), 119.5 (CH, C2'), 83.1 (CH, C4), 41.7 (CH_2, C5), 29.7 (CH_2, C2), 28.2 (CH_2, C3).

Synthesis of compound 11:

(-)-(*R*)-5-[(5-benzyloxy)-3-hydroxyphenyl]dihydrofuran-2(3*H*)-one (18). A solution of protected valerolactone **17** (50 mg, 0.13 mmol, 1.0 equiv) in 5 mL of absolute MeOH, was cooled to 0 °C and treated with $NiCl_2 \cdot 6H_2O$ (61.3 mg, 0.26 mmol). The resulting mixture was stirred at the same temperature for 5 min before the addition of $NaBH_4$ (30 mg, 0.77 mmol). After 30 min, the reaction mixture was quenched with saturated aqueous NH_4Cl solution and extracted with CH_2Cl_2 (3×5 mL). The combined extracts were dried ($MgSO_4$) and concentrated under vacuum. Flash chromatographic purification (elution by gradient from 70:30 to 60:40 Petroleum Ether/EtOAc) afforded monoprotected valerolactone **18** (21 mg) as an amorphous solid (54% yield). TLC: R_f = 0.51 (50/50 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ −10.5 (*c* 1.0 g/100mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29-7.41 (m, 5H, $CH_2\underline{Ph}$), 6.40 (s, 2H, H2', H6'), 6.30 (s, 1H, H4'), 6.04 (bs, OH), 5.00 (s, 2H, $\underline{CH_2}Ph$), 4.71 (dddd, *J* = 6.6, 6.6, 6.6, 6.6 Hz, 1H, H4), 2.93 (dd, *J* = 14.0, 5.9 Hz, 1H, H5a), 2.86 (dd, *J* = 14.0, 6.0 Hz, 1H, H5b), 2.45 (ddd, *J* = 18.0, 9.3, 9.3 Hz, 1H, H2a), 2.35 (ddd, *J* = 17.6, 9.3, 4.8 Hz, 1H, H2b), 2.18-2.26 (m, 1H, H3a), 1.86-1.96 (m, 1H, H3b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.0 (Cq, C1), 160.3 (Cq), 157.4 (Cq), 138.4 (Cq), 137.0 (Cq, Ph), 128.8 (2C, CH, Ph), 128.2 (CH, Ph), 127.7 (2C, CH, Ph), 109.5 (CH, Ar), 108.8 (CH, Ar), 101.2 (CH, Ar), 81.1 (CH, C4), 70.3 (CH_2, $\underline{CH_2}Ph$), 41.4 (CH_2, C5) 28.9 (CH_2, C2), 27.1 (CH_2, C3). MS (ESI, 50eV): Calcd.: $[M+Na^+]$: 321.1; Found: $[M+Na^+]$: 321.1.

(*R*)-5-(5′-benzyloxyphenyl)-γ-valerolactone-3′-*O*-trichlorosulfate. Protected sulfate was prepared according to the Representative Procedure 1 using: compound **18** (20.4 mg, 0.06 mmol, 1 equiv), Et_3N (12 µL, 0.08 mmol), DMAP (8.5 mg, 0.06 mmol), TCECS (90 mg, 0.36 mmol) in DCM dry (7 mL) at room temperature for 16 h. Flash chromatographic purification (elution by gradient: from 70/30 to 60/40 Petroleum Ether/EtOAc) afforded desired protected sulfate as a pale yellow resin (24.1 mg, 74%). TLC: R_f = 0.50 (70/30 petroleum ether/ethyl acetate); Opt. Rot. $[\alpha]_D^{20}$ −20.1 (*c* 1.0 g/100mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33-7.41 (m, 5H, CH_2Ph), 6.87 (m, 3H, Ar), 5.06 (s, 2H, CH_2Ph), 4.81 (s, 2H, CH_2CCl_3), 4.69 (dddd, *J* = 6.6, 6.6, 6.6, 6.6 Hz, 1H, H4), 3.01 (dd, *J* = 14.2, 6.7 Hz, 1H, H5a), 2.93 (dd, *J* = 14.3, 5.5 Hz, 1H, H5b), 2.50 (ddd, *J* = 17.9, 9.5, 9.5 Hz, 1H, H2a), 2.42 (ddd, *J* = 17.8, 9.3, 4.4 Hz, 1H, H2b), 2.24-2.33 (m, 1H, H3a), 1.87-1.96 (m, 1H, H3b). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.7 (Cq, C1), 160.1 (Cq, Ar), 150.9 (Cq, Ar), 139.6 (Cq, Ar), 136.1 (Cq, Ph), 128.9 (2C, CH, Ph), 128.5 (CH, Ph), 127.7 (2C, CH, Ph), 116.0 (CH, Ar), 114.4 (CH, Ar), 106.7 (CH, Ar), 92.6 (Cq, CH_2CCl_3), 80.6 (CH_2, CH_2CCl_3), 80.2 (CH, C4), 70.7 (CH_2, CH_2Ph), 41.5 (CH_2, C5) 28.8 (CH_2, C2), 27.5 (CH_2, C3). MS (ESI, 50eV): Calcd.: $[M+Na^+]$: 531.0, Found: $[M+Na^+]$: 531.0.

(*R*)-5-(5′-hydroxyphenyl)-γ-valerolactone-3′ *O*-Sulfate (11). To a solution of compound of 5-(5′-benzyloxy)-γ-valerolactone-3′-*O*-trichlorosulfate (24.5 mg, 0.05 mmol, 1.0 equiv) in a degassed EtOAc (8 mL), Pd (10% on carbon, 15 mg) was added. To this black suspension, H_2 was flushed and kept sealed under pressure for 1 h. After this period the H_2 was removed under vacuum, the resulting suspension was filtered in EtOAc, and the residue concentrated to yield the desired debenzylated intermediate (14 mg) as a crude that was used as such, without further purifications. The crude (14 mg, 0.03 mmol) was then subjected to TCE cleavage according Representative Procedure 2 using

ammonium formate (13 mg, 0.20 mmol), Zn dust (3.9 mg, 0.06 mmol) in absolute EtOH (3 mL) for 45 minutes. Flash chromatographic purification (85:15 EtOAc/MeOH) afforded sulfate valerolactone **11** as a pale yellow resin (10.6 mg, 70% two steps). Pale yellow resin; R_f = 0.23 (85/15 AcOEt/MeOH); Opt. Rot. $[\alpha]_D^{20}$ −13.4 (*c* 0.5 g/100mL, MeOH); ^{1}H NMR (400 MHz, MeOD): δ 6.72 (dd, *J* = 1.8, 1.8 Hz, 1H, H2'), 6.70 (dd, *J* = 2.1, 2.1 Hz, 1H, H4'), 6.54 (dd, *J* = 1.8, 1.8 Hz, 1H, H6'), 4.79 (dddd, *J* = 6.8, 6.8, 6.8, 6.8 Hz, 1H, H4), 2.98 (dd, *J* = 13.9, 6.4 Hz, 1H, H5a), 2.86 (dd, *J* = 13.9, 6.2 Hz, 1H, H5b), 2.54 (ddd, *J* = 18.0, 9.4, 9.4 Hz, 1H, H2a), 2.44 (ddd, *J* = 17.8, 9.4, 4.6 Hz, 1H, H2b), 2.29 (dddd, *J* = 12.7, 9.7, 6.7, 4.6 Hz, 1H, H3a), 2.00 (m, dddd, *J* = 12.8, 9.2, 9.2, 7.6 Hz, 1H, H3b). ^{13}C NMR (100 MHz, MeOD): δ 180.3 (Cq, C1), 159.4 (Cq, Ar), 155.0 (Cq, Ar), 139.7 (Cq, Ar), 114.7 (CH, C2'), 114.2 (CH, C6'), 108.3 (CH, C4'), 83.0 (CH, C4), 42.2 (CH_2, C5), 29.6 (CH_2, C2), 28.2 (CH_2, C3).

<u>Synthesis of compound 9.</u> Representative Procedure for the synthesis of *O*-Glucuronide γ-valerolactones.

<u>Representative procedure 3</u> for glucuronidation with trichloroacetimidate 14.

5-phenyl-γ-valerolactone-3'-*O*-(benzyl-2,3,4-tri-*O*-benzyl-D-glucopyranosyluronate). According to a known procedure,[30] a solution of valerolactone **2** (10.4 mg, 0.05 mmol, 1 equiv) in CH_2Cl_2 (1 mL) was added dropwise to a solution of α-D-glucuronide trichloroacetimidate **14** (69.9 mg, 0.1 mmol, 2 equiv) in CH_2Cl_2 (3 mL). The mixture was cooled to 0 °C, follow up by the addition of $BF_3{\cdot}OEt_2$ (1.5 µL, 0.01 mmol, 0.2 equiv). After 2 h, Et_3N was added and the solution was concentrated under vacuum. The residue was purified by flash chromatography on silica gel (from 85:15 to 80:20 Petroleum ether/EtOAc) to afford the corresponding protected glucuronide (28.8 mg, 79%) as a mixture of α : β anomers in 0.40:1.0 ratio. The anomeric ratio was

determined by ^{1}H NMR (400 MHz). A little amount of β-anomer glucuronide (4.6 mg) was purified by semipreparative HPLC (CN-10µM, 250 x 10 mm, hexane/anhydrous EtOH 90:10, flow rate 4 mL/min, detection at 254 nm, Rt β = 33.47 minutes). β-anomer: TLC: R_f = 0.57 (60/40 petroleum ether/ethyl acetate). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28-7.34 (m, 19H, Ar), 7.16 (m, 2H, Ar), 6.97 (m, 2H, Ar), 6.90 (bs, 1H, Ar), 5.21 (d, *J* = 12.2 Hz, 1H, $CO_2\underline{CH_2}Ph$), 5.16 (d, *J* = 12.2 Hz, 1H, $CO_2\underline{CH_2}Ph$), 5.05 (d, *J* = 6.8 Hz, 1H, H1''), 5.04 (d, *J* = 11.1 Hz, 1H, $\underline{CH_2}Ph$), 4.94 (d, *J* = 10.9 Hz, 1H, $\underline{CH_2}Ph$), 4.84 (d, *J* = 10.9 Hz, 1H, $\underline{CH_2}Ph$), 4.83 (d, *J* = 11.0 Hz, 1H, $\underline{CH_2}Ph$), 4.78 (d, *J* = 10.6 Hz, 1H, $\underline{CH_2}Ph$), 6.68 (dddd, *J* = 6.8, 6.8, 6.8, 6.8 Hz, 1H, H4), 4.55 (d, *J* = 10.8 Hz, 1H, $\underline{CH_2}Ph$), 4.09 (d, *J* = 9.6 Hz, 1H, H5''), 3.97 (dd, *J* = 9.0, 9.0 Hz, 1H, H4''), 3.80 (dd, *J* = 9.0, 9.0 Hz, 1H, H3''), 3.77 (dd, *J* = 9.0, 9.0 Hz, 1H, H2''), 3.05 (dd, *J* = 13.8, 6.0 Hz, 1H, H5a), 2.85 (dd, *J* = 13.8, 6.4 Hz, 1H, H5b), 2.47 (ddd, *J* = 17.8, 9.2, 9.2 Hz, 1H, H2a), 2.37 (m, 1H, H2b), 2.21 (m, 1H, H3a), 1.90 (m, 1H, H3b). ^{13}C NMR (100 MHz): δ 177.4 (Cq, C1), 168.5 (Cq, CO_2Bn), 157.8 (Cq, Ar), 138.7 (Cq, Ar), 138.5 (Cq, Ar), 138.3 (Cq, Ar), 138.2 (Cq, Ar), 135.5 (Cq, Ar), 130.4 (CH, Ar), 129.1 (2C, CH, Ar), 129.0 (CH, Ar), 128.9 (5C, CH, Ar), 128.8 (2C, CH, Ar), 128.7 (3C, CH, Ar), 128.4 (3C, CH, Ar), 128.3 (2C, CH, Ar), 128.2 (CH, Ar), 128.1 (CH, Ar), 124.8 (CH, Ar), 118.7 (CH, Ar), 116.0 (CH, Ar), 102.5 (CH, C1''), 84.2 (CH_2, $CO_2\underline{CH_2}Ph$), 82.1 (CH, C4), 81.0 (CH), 79.5 (CH), 76.3 (CH_2), 75.6 (CH_2), 75.5 (CH), 75.2 (CH), 67.9 (CH_2), 41.7 (CH_2, C5), 29.2 (CH_2, C2), 27.7 (CH_2, C3).

5-phenyl-γ-valerolactone-3'-*O*-glucuronide (9).

To a solution of the previously obtained anomeric mixture of benzylated glucoronide (21.9 mg, 0.03 mmol, 1.0 equiv) in a degassed EtOAc/EtOH 1:1 (15 mL), was added Pd (10% on carbon, 10 mg). To this black suspension H_2 was flushed and kept sealed under pressure for 6h. After this period the H_2 was removed under vacuum, the resulting suspension was filtered in EtOAc/EtOH

mixture, and the residue concentrated to yield targeted compound **9** (10.5 mg,95%) as white amorphous solid. TLC: Rf = 0.15 (80/20 AcOEt/MeOH, 2% AcOH). ^{1}H NMR (400 MHz, MeOD): δ 7.23-7.39 (m, 1.4 H, Hα Ar, Hβ Ar), 6.95-7.09 (m, 4.2 H, 3Hα Ar, 3Hβ Ar), 5.56 (d, *J* = 3.6 Hz, 0.4 H, H1''α), 4.98 (d, *J* = 7.7 Hz, 1H, H1''β), 4.82 (m, 1.4 H, H4α, H4β), 4.15 (d, *J* = 9.9 Hz, 0.4H, H5''α), 4.01 (d, *J* = 9.7 Hz, 1H, H5''β), 3.88 (dd, *J* = 9.2, 9.2 Hz, 0.4H, H3''α), 3.59-3.66 (m, 1.8H, H2α, H4α, H4β), 3.51 (m, 2H, H2''β, H3''β), 3.04 (dd, *J* = 14.0, 7.0 Hz, 0.4H, H5aα), 3.03 (dd, *J* = 13.8, 6.4 Hz, 1H, H5aβ), 2.96 (dd, *J* = 13.9, 5.8 Hz, 1H, H5bβ), 2.95 (dd, *J* = 13.9, 5.6 Hz, 0.4H, H5bα), 2.34-2.58 (m, 2.8H, H2α, H2β), 2.24-2-32 (m, 1.4H, H3aα, H3aβ), 1.94-2.04 (m, 1.4H, H3bα, H3bβ). ^{13}C NMR (100 MHz, MeOD): δ 180.3 (2C, Cq, C1α, C1β), 159.4 (2C, Cq α,β), 158.7 (2C, Cq α,β), 139.7 (Cq, Ar α), 139.4 (Cq, Ar β), 130.7 (CH, Ar α), 130.6 (CH, Ar β), 125.0 (CH, Ar β), 124.9 (CH, Ar α), 119.3 (2C, CH, Ar α, β), 116.7 (CH, Ar α), 116.6 (CH, Ar β), 102.6 (CH, C1''β), 99.3 (CH, C1''α), 83.1 (CH, C4α), 82.9 (CH, C4β), 77.8 (CH, Glc-β) 76.3 (CH, Glc-α), 74.8 (2C, CH, Glc-α, Glc-β), 74.8 (CH, Glc-α), 73.9 (CH, Glc-α), 73.9 (CH, Glc-β), 73.1 (CH, Glc-β), 42.2 (CH_2, C5α), 42.1 (CH_2, C5β), 29.6 (2C, CH_2, C2α, C2β), 28.3 (CH_2, C3α), 28.1 (CH_2, C3β). MS (ESI, 50eV): Calcd.: [M−H$^+$]: 367.1Found: [M−H$^+$]: 367.2

Synthesis of compound 12:

5-(5'-benzyloxyphenyl)-γ-valerolactone-3'-*O*-(benzyl-2,3,4-tri-*O*-benzyl-D-glucopyranosyluronate).

The protected glucuronide was prepared according the Representative Procedure 3 using: monoprotected valerolactone **18** (21mg, 0.07 mmol), tetrabenzylated glucuronic "donor" **14** (87.4 mg, 0.13 mmol), BF_3▪OEt_2 (1.8 µL, 0.014 mmol) in CH_2Cl_2 (3 mL). Flash chromatographic purification (elution by gradient from 85:15 to 75:25 Petroleum Ether/AcOEt) afforded desired protected glucuronide valerolactone as 1:0.35 mixture β:α (49.5 mg, 85% yield)

as a white amorphous solid. TLC: Rf = 0.42 (70/30 petroleum ether/ethyl acetate), ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.14-7.34 (m, 34H, CH_2Ph α, β), 6.65 (dd, *J* = 2.1, 2.1 Hz, 0.35H, Ar α), 6.60 (m, 2.30 H, 2H Ar β, 1H Ar α), 6.55 (dd, *J* = 1.7, 1.7 Hz, 0.35H, H Ar α), 6.52 (dd, *J* = 1.6, 1.6 Hz, 1H, H Ar β), 5.22 (d, *J* = 12.3 Hz, 1H, CH_2Ph β), 5.19 (d, *J* = 12.0 Hz, 0.35H, CH_2Ph α), 5.17 (d, *J* = 12.8 Hz, 1H, CH_2Ph β), 5.13 (d, *J* = 12.5 Hz, 0.35H, CH_2Ph α), 4.98-5.05 (m, 5H, CH_2Ph α,β, H1''β), 4.93 (d, *J* = 10.9 Hz, 1H, CH_2Ph β), 4.89 (d, *J* = 10.9 Hz, 0.35H, CH_2Ph α), 4.77-4.84 (m, 3.7H, CH_2Ph α, β), 4.69 (d, *J* = 11.9 Hz, 0.40H, CH_2Ph α), 4.66 (dddd, *J* = 6.7, 6.7, 6.7, 6.7 Hz, 1.35H, H4α, H4β), 4.55 (d, *J* = 10.7, 1H, CH_2Ph β), 4.49 (d, *J* = 10.7, 0.35H, CH_2Ph α), 4.40 (d, *J* = 10.0 Hz, 0.35H, H5''α) 4.20 (dd, *J* = 9.3, 9.3 Hz, 0.38H, H3''α), 4.09 (d, *J* = 9.6 Hz, 1H, H5''β), 3.97 (dd, *J* = 9.4, 9.4 Hz, 1H, H4''β), 3.73-3.80 (m, 2.34 Hz, H2''α, H2''β, H3''β), 3.01 (dd, *J* = 13.9, 5.9 Hz, 1.36H, H5aα, H5aβ), 2.79 (dd, *J* = 14.0, 6.7 Hz, 1.36H, H5bα, H5bβ), 2.34-2.50 (m 2.74H, H2α, H2β), 2.14-2.24 (m, 1.35H, H3aα, H3aβ), 1.82-1.94 (m, 1.37H, H3bα, H3bβ). MS (ESI, 50eV): Calcd.: $[M+Na^+]$: 857.3, Found: $[M+Na^+]$: 857.4

5-(5'-hydroxyphenyl)-γ-valerolactone-3'-*O*-glucuronide (12).

Valerolactone **12** with the classical procedure of catalytic hydrogenation using previously synthesized anomeric miture of polybenzylated glucuronide (30 mg, 0.036 mmol),degassed EtOAc/EtOH 1:1 (20 mL), Pd (10% on carbon, 10 mg), H_2 for 5 hours. After this period the H_2 was removed under vacuum, the resulting suspension was filtered in EtOH, and the residue concentrated to afford valerolactone **12** (14 mg, quantitative yield) as white amorphous solid. TLC: Rf = 0.10 (80/20 ethyl acetate/MeOH-2% AcOH), ^{1}H NMR (400 MHz, MeOD): δ 6.60 (dd, *J* = 1.8, 1.8 Hz, 0.36H, Ar α), 6.53 (m, 1.30 H, H Ar β, H Ar α), 6.48 (dd, *J* = 2.2, 2.2 Hz, 1H, H Ar β), 6.41 (m, 1.31H, H Ar β), 5.50 (d, *J* = 3.6 Hz, 0.35H, H1'' α), 4.90 (d, *J* = 7.6 Hz, 1H, H1'' β), 4.79 (dddd, *J* = 6.9, 6.9, 6.9, 6.9 Hz, 1.5H, H4α, H4β), 4.06 (d, *J* = 10.0 Hz, 0.37H, H5''α), 3.90 (d, *J* = 9.6 Hz, 1H, H5''β), 3.87 (dd,

J = 9.2, 9.2 Hz, 0.36H, H3''α), 3.57-3.61 (m, 1.77H, H4''β, H4''α, H2''α), 3.46-3.53 (m, 2H, H3''β, H2''β), 2.97 (dd, *J* = 14.3, 6.6 Hz, 0.36H, H5aα), 2.95 (dd, *J* = 14.0, 6.2 Hz, 1H, H5aβ), 2.86 (dd, *J* = 14.1, 6.2 Hz, 1H, H5bβ), 2.84 (dd, *J* = 14.0, 6.3 Hz, 0.38H, H5bα), 2.34-2.56 (m 2.75H, H2α, H2β), 2.23-2.32 (m, 1.38H, H3aα, H3aβ), 1.94-2.04 (m, 1.37H, H3bα, H3bβ). ^{13}C NMR (100 MHz, MeOD): δ 180.3 (2C, Cq, C1α, C1β), 160.3 (2C, Cq α, Cq β), 159.8 (Cq α), 159.7 (Cq β), 159.6 (2C, Cq α, Cq β), 140.3 (Cq α), 140.0 (Cq β), 112.2 (CH Ar, β), 112.1 (CH Ar, α), 110.6 (CH Ar, α), 110.4 (CH Ar, β), 104.0 (CH Ar, α), 103.9 (CH Ar, β), 102.5 (CH, C1''β), 99.2 (CH, C1''α), 83.1 (CH, C4α), 82.9 (CH, C4β), 77.7 (CH, Glc-β), 76.6 (CH, Glc-α), 74.8 (2C, CH, Glc-β), 74.7 (CH, Glc-α), 73.8 (CH, Glc-α), 73.4 (CH, Glc-β), 73.1 (CH, Glc-α), 42.3 (CH_2, C5α), 42.2 (CH_2, C5β), 30.9 (CH_2, C2α), 29.6 (CH_2, C2β), 28.3 (CH_2, C3α), 28.1 (CH_2, C3β). MS (ESI, 50eV): Calcd.: [M−H^+]: 383.1, Found: [M−H^+]: 383.2.

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CHAPTER 5

5-(3',4'-dihydroxyphenyl)-γ-valerolactone and its sulfate conjugates: representative circulating metabolites of flavan-3-ols, exhibiting anti-adhesive activity against uropathogenic *Escherichia coli* in bladder epithelial cells[‡]

Abstract: Cranberries as well as other foodstuffs rich in proanthocyanidins and monomeric flavan-3-ols have shown preventive effects against urinary tract infections (UTI). Despite dihydroxyphenyl-γ-valerolactone derivatives are among the main metabolites occurring in urine after the microbial metabolism of procyanidins and catechins, they have never been tested for their inhibitory effects on the adherence of uropathogenic *Escherichia coli* (UPEC) to uroepithelial cells. This paper studied the inhibition of the adherence of UPEC ATCC® 53503™ to T24 epithelial bladder cells by differently sulfated dihydroxyphenyl-γ-valerolactones at physiological concentrations. Moreover, the transformations of these molecules in cell media were evaluated by UHPLC-MS^n. All dihydroxyphenyl-γ-valerolactone derivatives showed anti-adhesive activity at 100 µM, while 5-(3'-hydroxyphenyl)-γ-valerolactone-4'-*O*-sulfate also showed uroprotective effects at 50 µM. Some compounds underwent extensive metabolism during cell incubation, mainly deconjugation of sulfate moieties and opening of the lactone ring. These results shed light on the potential active role of flavan-3-ol metabolites behind the prophylactic effect of cranberries against UTI.

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5.1. Introduction

Uropathogenic *Escherichia coli* (UPEC) is the infective agent for 70–90 % of urinary tract infections (UTI), the most common type of nosocomial infection worldwide.[1] The development of antibiotic resistance and the morbidity associated with UTI have impelled new prophylaxis or therapies by antiadhesive compounds.[2] Cranberry intake has usually shown prophylactic effects against UTI, and its components or metabolites seem to operate in the phase of bacterial adherence to the uroepithelial cells, preventing UTI colonization and progression.[3] Lately, green tea intake has been also reported to have antimicrobial activity against UPEC, and synergistic effect with antibiotics.[4]

Monomeric flavan-3-ols (+)-catechin, (-)-epicatechin, (-)-epicatechin-3-*O*-gallate, and (-)-epigallocatechin-3-*O*-gallate as well as their oligomeric condensation products, proanthocyanidins (PACs), have been pointed out as the potential responsible agents for the preventive effects of cranberries on UTI.[5,6] These compounds have also exhibited several health-promoting activities, including antioxidant, antimicrobial, antiproliferative, cardiopreventive, anti-viral, and neuro-protective activities.[7,8] However, while a slight proportion of these dietary bioactive compounds are absorbed intact, substantial amounts of low molecular weight catabolites are absorbed after biotransformation by the colon microflora.[9] Approximately two-thirds of the ingested flavan-3-ols are converted to phenolic and aromatic acids of microbial origin that enter the bloodstream and are excreted in urine.[7,9] In the microbial catabolic degradation of flavan-3-ols, phenylvalerolactone and phenylvaleric acid derivatives are initially formed, to be then further degraded into phenylpropionic, phenylacetic, cinnamic and benzoic acids, and other end products.[10,11] Among the main colonic breakdown products of flavan-3-ols, a particular role seems to be played by 5-(3′,4′,5′-trihydroxyphenyl)-γ-

valerolactone and 5-(3',4'-dihydroxy)-γ-valerolactone.[7,12] Although the metabolic potential of the microbiota towards the formation of hydroxyphenyl-γ-valerolactone seems to be greatly variable among individuals, and despite the catabolic pathways involved in flavan-3-ol degradation have not been fully elucidated, it has been described that absorbed metabolites are conjugated to glucuronidated and sulfated forms.[7,11,13] Among these conjugated catabolites, hydroxyphenyl-γ-valerolactone sulfates have been reported to be the predominant phase II metabolites excreted after the intake of flavan-3-ol-rich foodstuffs.[7,13]

In vitro studies dealing with the bioactivity of phenolic compounds have usually applied molecules never appearing *in vivo*, as tested in their unmetabolised forms, or concentrations far from those achievable in circulation in the context of a normal diet. To test the right molecules at the right concentrations in cell-based biological experiments, physiological metabolites should be available. So far, the lack of authentic standards of polyhydroxyphenyl-γ-valerolactone has limited their use in biological testing.[11] However, recent advances in the asymmetric synthesis of these molecules has overcome these limitations and made their use in cell assays easily achievable.[14]

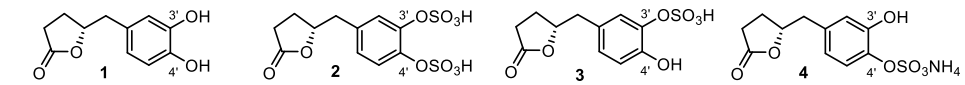

Figure 5.1. *Molecular structures of tested compounds.*

Recently, we demonstrated for the first time the *in vitro* antiadhesive activity against UPEC of some low-molecular-weight phenolic metabolites (i.e., simple phenols and phenolic acids), although it was not possible to establish a consistent structure-activity relationship from these data.[15] In the present

chapter we report that the previously synthesized γ-valerolactones and conjugated forms (sulfated) (*see* Chapters 3, 4), which coincide with cranberry-derived microbial metabolites, were tested for their capacity to inhibit the adherence of uropathogenic *Escherichia coli* (UPEC) ATCC®53503™ to T24 epithelial bladder cells (Figure 5.1). This is, to the best of our knowledge, the first time that the main conjugated metabolites derived from the microbial degradation products of monomeric flavan-3-ols and PACs are tested.

5.2. Results and discussion

5.2.1. Anti-adhesive effects of dihydroxyphenyl-γ-valerolactone derivatives on the adherence of *E. coli* to bladder epithelial cells

Inhibition of adherence of UPEC ATCC®53503™ to T24 bladder epithelial cells was assessed at different, physiologically realistic concentrations (10, 50, and 100 μM) of 5-(3',4'-dihydroxyphenyl)-γ-valerolactone derivatives differently conjugated with sulfate moieties (Table 5.1). These concentrations did not result in any cytotoxicity (data not shown). The percentage of adherence of UPEC ATCC®53503™ to T24 epithelial cells was 9.76 ± 1.33. The four flavan-3-ol metabolites tested, [5-(3',4'-dihydroxyphenyl)-γ-valerolactone (**1**), 5-phenyl-γ-valerolactone-3',4'-disulfate (**2**), 5-(4´-hydroxyphenyl)-γ-valerolactone-3'-*O*-sulfate (**3**), and 5-(3'-hydroxyphenyl)-γ-valerolactone-4'-*O*-sulfate (**4**)] were found to inhibit the adherence of UPEC depending on the concentration used (Table 5.1). All the phenyl-γ-valerolactone derivatives tested were able to significantly inhibit the adherence of UPEC to uroepithelial cells at concentrations of 100 μM, but 5-(3'-hydroxyphenyl)-γ-valerolactone-4'-*O*-sulfate (**4**) also inhibited significantly the adherence of UPEC to T24 cells when

incubated at 50 μM, while its monosulfate isomer conjugated in 3′ did not show any statistically significant inhibitory effect.

Table 5.1. Inhibition (%) of the adherence of *E. coli* ATCC® 53503™ to ATCC® HTB4™ cells by dihydroxyphenyl-γ-valerolactones.

Compound	Concentration (μM)		
	10	50	100
5-(3′,4′-dihydroxyphenyl)-γ-valerolactone	6.79±3.92	9.95±8.28	19.4±10.3*
5-phenyl-γ-valerolactone-3′,4′-disulfate	0.22±0.71	14.7±1.5	30.3±3.6**
5-(4′-hydroxyphenyl)-γ-valerolactone-3′-*O*-sulfate	11.9±1.7	10.2±3.9	22.2±5.9**
5-(3′-hydroxyphenyl)-γ-valerolactone-4′-*O*-sulfate	10.1±3.1	16.1±6.1*	24.2±3.1**

* Mean significantly different from zero (p<0.05) using one-sample t-test. ** Mean significantly different from zero (p<0.01) using one-sample t-test.

5.2.2. Metabolism of γ-valerolactone derivatives in bladder epithelial cells

The stability of 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone and its sulfate derivatives, together with the presence of newly-formed metabolites were assessed in cell cultures at the end of each experiment (after 1 h of incubation with UPEC plus 1 h of co-incubation with UPEC and T24 cells). To investigate the role of UPEC and bladder epithelial cells in the peripheral metabolism of these flavan-3-ol metabolites, cell media non-incubated with γ-valerolactones and UPEC-free T24 cells (non-inoculated) were used as controls. Different metabolic reactions, including deconjugation, further conjugation with methyl, glucuronide, sulfate, and glutathione moieties, (de)hydroxylation, and formation of phenyl-valeric acids, were monitored by UHPLC-MS/MS analysis. All the compounds were stable under cell culture conditions. However, while 5-

(3’,4’-dihydroxyphenyl)-γ-valerolactone did not undergo any metabolic transformation, its sulfate derivatives were instead modified (Figure 5.2). These transformations were limited to deconjugation of sulfate moieties and/or opening of the lactone ring. Three newly-formed metabolites, all phenyl-valeric acid derivatives likely formed by lactone ring opening, were identified according to their retention times and mass spectra (Table 5.2). *E.coli* did not contribute to the modifications exerted by bladder epithelial cells, as there were no statistically significant differences between the levels of flavan-3-ol metabolites in presence or absence of the UPEC inoculum (p>0.05 for all the γ-valerolactones tested).

A weak desulfation (~0.5%) of 5-phenyl-γ-valerolactone-3’,4’-di-*O*-sulfate was observed, both at 3’ and 4’ positions (Figure 5.2). The loss of the 4’ sulfate moiety was slightly higher than in 3’ (p=0.031), and 5-(3’,4’-dihydroxyphenyl)-γ-valerolactone was not detected, suggesting the absence of a complete desulfation process. Cell media incubated with 5-phenyl-γ-valerolactone-3’,4’-di*O*-sulfate also contained traces of 5-phenyl-valeric acid-3’,4’-di*O*-sulfate. Interestingly, mono-sulfated isomers of 5-(3’,4’-dihydroxyphenyl)-γ-valerolactone were differently metabolised (Figure 5.2): 5-(4’-hydroxyphenyl)-γ-valerolactone-3’-*O*-sulfate suffered minor desulfation (~2%) but not lactone ring opening, while its isomer 5-(3’-hydroxyphenyl)-γ-valerolactone-4’-*O*-sulfate underwent both lactone opening and dehydroxylation (yielding monohydroxyphenyl-valeric acids), as well as loss of the sulfate moiety. Nevertheless, the presence of monohydroxyphenyl-valeric acids (both free and sulfated) was detected only at trace levels.

Table 5.2. *Flavan-3-ol metabolites detected in bladder epithelial cells and bladder epithelial cells inoculated with uropathogenic E. coli.*

Metabolite	Retention time (min)	$[M-H]^-$ (*m/z*)	MS^2 ion fragments (*m/z*)	MS^3 ion fragments (*m/z*)
5-phenyl-γ-valerolactone 3′,4′-diO-sulfate *	1.65	367	287, 299	207
5-(hydroxyphenyl)-valeric acid *	2.42	193	149, 157	
5-(phenyl)-valeric acid-3′,4′-di*O*-sulfate	2.65	369	289	
5-(sulfate-phenyl)-valeric acid	3.08	273	193	149
5-(4′-hydroxyphenyl)-γ-valerolactone-3′-*O*-sulfate *	3.85	287	207	163, 122
5-(3′-hydroxyphenyl)-γ-valerolactone-4′-*O*-sulfate *	4.00	287	207	163, 122
5-(3′,4′-dihydroxyphenyl)-γ-valerolactone *	4.31	207	163, 122	

* Compound identified by comparing retention times and MS data with those of reference compounds

5.3. Discussion

Cranberry consumption has been indicated to be effective in decreasing the occurrence and severity of UTI in women.[8,17] This fact has been attributed to the ability of cranberry A-type PACs to inhibit the adherence of UPEC to the bladder epithelium.[3,8] However, evidence of the limited absorption of PACs and their extensive metabolism by gut microbiota raise serious concerns about the physiological relevance of previous experiments,[7,9-11]and points to single flavonoids, phenolic acids, and phenyl-γ-valerolactones as the biologically

plausible candidates to exert anti-adhesive activity within the bladder. A methodology optimized by our research group to assess the adherence of UPEC ATCC®53503™ to T24 epithelial cells proved the inhibition of adherence of UPEC to bladder cells by a series of microbial-derived phenolic catabolites (catechol, benzoic acid, protocatechuic acid, vanillic acid, phenylacetic acid and 3,4-dihydroxyphenylacetic acid).[15] The present work has demonstrated, for the first time, the anti-adhesive effects of phenyl-γ-valerolactones, which are by far more representative catabolic products of cranberry (and possibly other sources) PACs. Actually, dihydroxyphenyl-γ-valerolactone derivatives have been identified to be one of the main circulating metabolites after consumption of flavan-3-ol-rich foodstuffs, reaching high μM urinary concentrations.[18-20] Their anti-adhesive activity at physiologically realistic concentrations (Table 5.1) adds a paramount step to the process of understanding what lies beneath the UTI preventive properties of cranberry-based products and other flavan-3-ol and PACs rich sources. However, the mechanisms of action through which these compounds exert their anti-adhesive actions, as well as their potential interactions with other phenolic metabolites inhibiting the adhesion of UPEC to bladder epithelium, remain still to be elucidated.

Cell metabolism is a point to be taken into account while assessing the bioactivity of phenolic derivatives, since the biological effects of the tested parent molecules may be modified by the ability of some cell lines to take up and metabolize phenolic compounds. Although gastrointestinal tract cells and hepatocytes are key in the absorption and metabolism of phenolic compounds,[7,8] other cell types not directly linked to the digestive system may also metabolize circulating molecular scaffolds and, thus, change their biological activity. Therefore, the possible transformations of phenyl-γ-valerolactones in bladder epithelial cells infected or not with uropathogenic *E.*

coli were evaluated to fully clarify the real metabolites behind the effects exhibited by these important flavan-3-ol colonic metabolites.

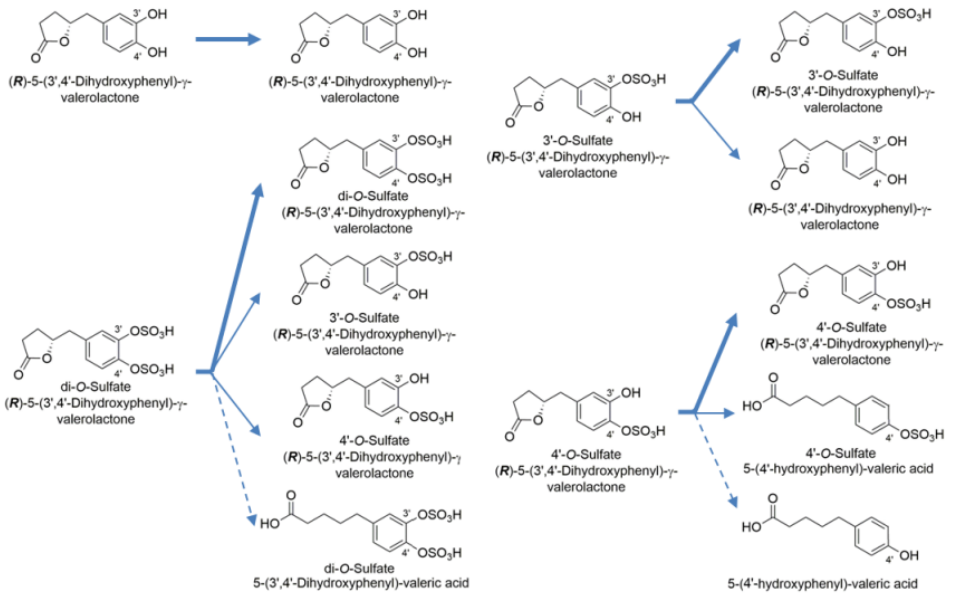

Figure 5.2. *Metabolic reactions occurring in bladder epithelial cells and bladder epithelial cells inoculated with uropathogenic E. coli in the presence of dihydroxyphenyl-γ-valerolactone derivatives. Bold arrows indicate major pathways, which correspond to incubated metabolites; normal arrows, newly-formed metabolites and dotted arrows, newly-formed metabolites detected at trace levels.*

Bladder epithelial cells were not able to metabolise 5-(3',4'-dihydroxyphenyl)-γ-valerolactone, in contrast to blood cells, which have been reported to form a broad array of metabolites, mainly glutathione conjugates.[21] Neither methylation nor sulphation, previously described as relevant peripheral transformations of catechins and ellagitannin catabolites,[8,16,22] were observed here. In this case, sulfate conjugates of dihydroxyphenyl-γ-valerolactones underwent an extensive metabolism by bladder epithelial cells, moving far beyond the classical reactions taking place at gut epithelium and/or liver level. Desulphation was the main metabolic reaction exerted by bladder epithelial cells, although the rate of deconjugation was quite low (0.5-2%).

Hydroxyphenyl-valeric acids, which had previously been reported to be metabolites derived from the catabolism of flavan-3-olswere also formed. [11,12,20] The presence of monohydroxyphenyl-valeric acids in the cell media incubated with 5-(3'-hydroxyphenyl)-γ-valerolactone-4'-*O*-sulfate, which were not detected in the media incubated with 5-(4'-hydroxyphenyl)-γ-valerolactone-3'-*O*-sulfate, might be responsible for the small differences observed in the bioactivity of these two monosulfated dihydroxyphenyl-γ-valerolactones. The formation of phenyl-valeric acids could be attributed, at least partially, to the interconversion between the lactone form and the open form, which might also take place easily during handling due to pH conditions.[23] On the other hand, despite the high ability of *E. coli* to metabolize phenolic structures,[24] UPEC did not seem to contribute to the transformations carried out by bladder epithelial cells; however, differences after longer incubation times cannot be ruled out.

5.4. Conclusions

The inhibition of UPEC adherence to bladder epithelial cells exerted by sulfated phenyl-γ-valerolactones, possibly the most relevant metabolites derived from the microbial and then hepatic transformation of PACs and monomeric flavan-3-ols, has been reported in this work for the first time. The prophylactic effects against UTI of cranberry products and other foods or supplements rich in flavan-3-ols might be reasonably mediated by these molecules. Further experiments are required to fully determine the contribution of these molecules to the anti-adhesive activity of the pool of urinary phenolic metabolites present in concomitance after cranberry consumption. Similarly, efforts should be addressed to understanding how inter-individual variations in the production of phenyl-γ-valerolactones may influence their impact on the prevention of UTI.

5.5. Experimental section

5.5.1. Chemicals

Compounds were dissolved at a concentration of 1,000 µM in Dulbecco's phosphate-buffered saline (DPBS, Lonza Walkersville, Inc., USA) containing 0.01% DMSO. Stock solutions were filtered through 0.22 µm pore-size filters. Assay solutions (200, 100 and 20 µM) were prepared via serial dilutions in sterilized DPBS.

The solvents used for LC-MS were supplied by Carlo Erba Reagents (Milan, Italy). Ultrapure water from MilliQ system (Millipore, Bedford, MA, USA) was used throughout the experiment. 5-(3'-hydroxyphenyl)-valeric acid was purchased from Alfa Aesar (Ward Hill, MA, USA).

The tested compounds **1-4** (Figure 5.1) were synthesized as described in Chapter 3, 4.

5.5.2. Bladder epithelial cells and cytotoxicity assays

Bladder T24 epithelial cells (ATCC® HTB4™) were grown and maintained in McCoy's 5A medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% (v/v) fetal bovine serum, at 37 ºC, in an atmosphere of 5% CO_2 /95 % air, and at constant humidity. Cells were seeded in 96- (cytotoxicity assays) or 24-well (adherence assays) tissue plates and grown approximately for 24 h to enable cell attachment and to obtain a cell monolayer.

Cytotoxicity of all tested γ-valerolactone derivatives against T24 cells was performed using the colorimetric 3-[4,5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide (MTT) assay, as previously described (González de Llano et al., 2015).

5.5.3. UPEC adherence assays

The UPEC strain (ATCC® 53503™) that express P fimbriae (Brinton & Fusco, 1988) was grown in TSB (Tryptic Soy Broth, Scharlau Chemie S.A., Barcelona, España) at 37 ºC. UPEC inocula were prepared from overnight cultures harvested by centrifugation (10,000 g, 10 min, 4 ºC) and re-suspended in DPBS to achieve an inoculum of 10^8 CFU/mL.

The UPEC inoculum was incubated with the same volume of γ-valerolactone solution for 1 h, at 37 ºC, in agitation (180 rpm). Then, confluent T24 cell monolayers ($5x10^5$cells/well) were washed with DPBS solution to eliminate antibiotic and overlaid with 0.5 mL of the UPEC bacteria pre-incubated with each γ-valerolactone solution or with PBS (control). Plates were incubated for 1 h at 37 ºC under 5% CO_2 atmosphere. After incubation, an aliquot from each well was removed and frozen for further UHPLC-MS/MS analysis. Wells were softly washed with DPBS solution to remove unbound bacteria. Cells and adhered bacteria were then detached by trypsinization and sonicated in an ultrasonic sonication bath (3 pulses, 10 seconds on, 3 seconds off) at 40 kHz to recover bacteria associated with cells. Bacterial counts (UFC/mL) were carried out by serial dilution plate method in TSA (Tryptic Soy Agar, Scharlau Chemie S.A., Barcelona, España) plates after incubation at 37 ºC for 24 h. Assays were carried out in triplicate and experiments were repeated at least twice. The adherence percentage (%) was calculated as the number of adhered bacteria (CFU/mL) relative to the total number of bacteria added initially x 100. The percentage of inhibition by γ-valerolactones was calculated as [1- (% $Adherence_{sample}$/% $Adherence_{control}$)] x100.

5.5.4. UHPLC-MS/MS analysis of cell media

Cell media supernatants were collected and analysed by UHPLC-MS^n to determine the stability and metabolism of hydroxyphenyl-γ-valerolactones in cell culture. Cell media were extracted according to Sala et al.[16] Samples were analysed using an Accela UHPLC 1250 equipped with a linear ion trap-mass spectrometer (LTQ XL, Thermo Fisher Scientific Inc., San Jose, CA, USA) fitted with a heated-electrospray ionization probe (H-ESI-II; Thermo Fisher Scientific Inc.). Separations were performed using a Waters Acquity UPLC HSS T3column (2.1 mm × 100 mm, 1.8 µm particle size, Waters, Milford, MA, USA), with an injection volume of 5 µL, column oven temperature of 40 °C and elution flow rate of 0.5 mL/min. The initial gradient was 95% of 0.1% aqueous formic acid and 5% acetonitrile (in 0.1% formic acid), reaching 65% acetonitrile at 6.5 min.

The MS conditions included: negative ionization mode, capillary temperature of 275 °C and source heater temperature of 300 °C, sheath gas flow of 60 units, auxiliary gas of 5 units, source voltage of 4 kV and capillary voltage and tube lens of -33 and -98 V, respectively. The applied MS method consisted in the selective determination of each target precursor ion by the acquisition of characteristic product ions in full scan mode, with collision induced dissociation (CID) equal to 30 (arbitrary units). Pure helium gas was used for CID. Data processing was performed using Xcalibur software from Thermo Scientific. Quantification was carried out with calibration curves of standards, when available.

5.5.5. Statistical analysis

SPSS 21.0 for Windows (SPSS Science, Chicago, IL, USA) was used to perform *t*-tests. Statistical significance was declared at $p<0.05$ (two-sided).

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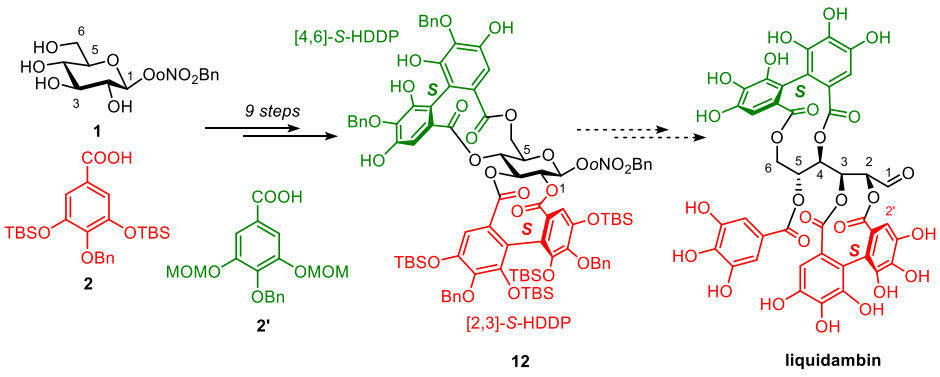
CHAPTER 6

Biomimetic total synthesis of members of the C-glucosidic subclass of ellagitannins

This work was made in the period November 2015-March 2016 at the Institut Européen de Chimie et Biologie (IECB), Bordeaux, France under the supervision of Prof. Stéphane Quideau.

Abstract. This chapter describes the advancements toward the biomimetic total synthesis of liquidambin, an open-chain ellagitannin compound characterized by two [4,6:2,3]-*bis*-(***S***)-HDDP units. The success of the designed strategy was proven by synthesizing the precious advanced intermediate **12** starting from *D*-glucose. Key maneuvers were two consecutive *bis*-esterifications with suitable protected galloyl units and two atroposelective biarylic couplings performed through a specific strategic sequence of protection/deprotection operations. In particular, the formation of both (***S***)-HHDP bridges were performed by an intramolecular oxidative aryl-aryl coupling promoted by $CuCl_2$-*n*$BuNH_2$ with perfect control of the axial chirality, disclosing a different intrinsic reactivity of the phenolic moieties depending on their specific disposition on the sugar core.



6.1. Introduction

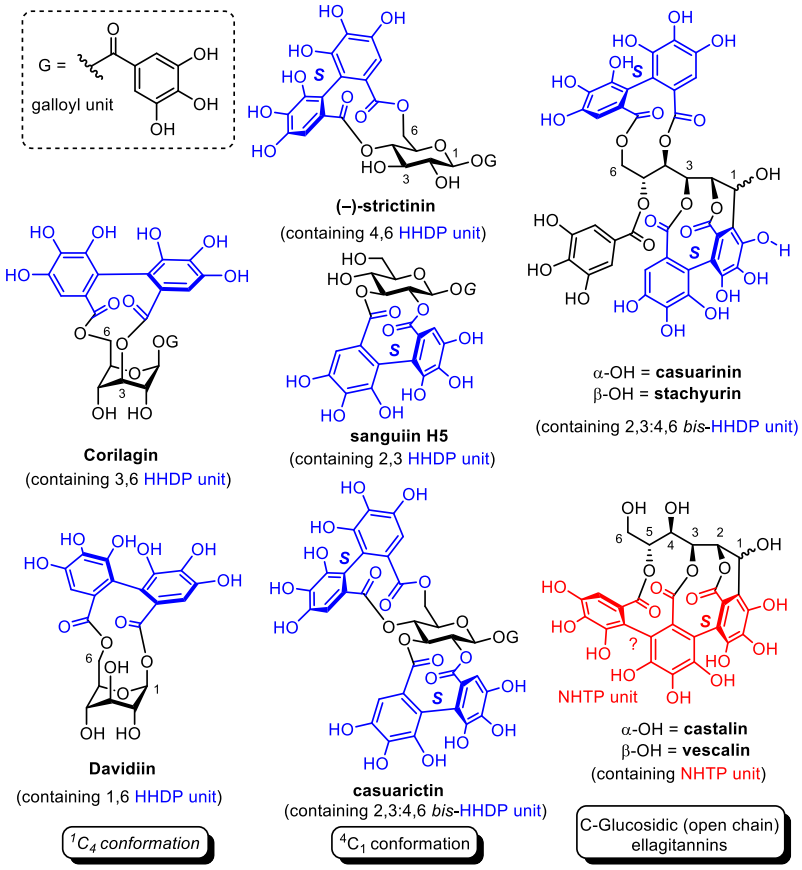


Figure 6.1. *Example of different types of ellagitannins.*

Ellagitannins constitute one of the major classes of polyphenolic natural products and are derived from the secondary metabolism of dicotyledonous plant species of the *Angiospermae*.[1] Research interest in these plant polyphenols initially emerged from the discovery of their occurrence in numerous herbal remedies used in traditional Asian medicine and the remarkable biological activities related to their antioxidant, antiviral, and host-mediated anti-tumor properties; an interest that was further fed by the

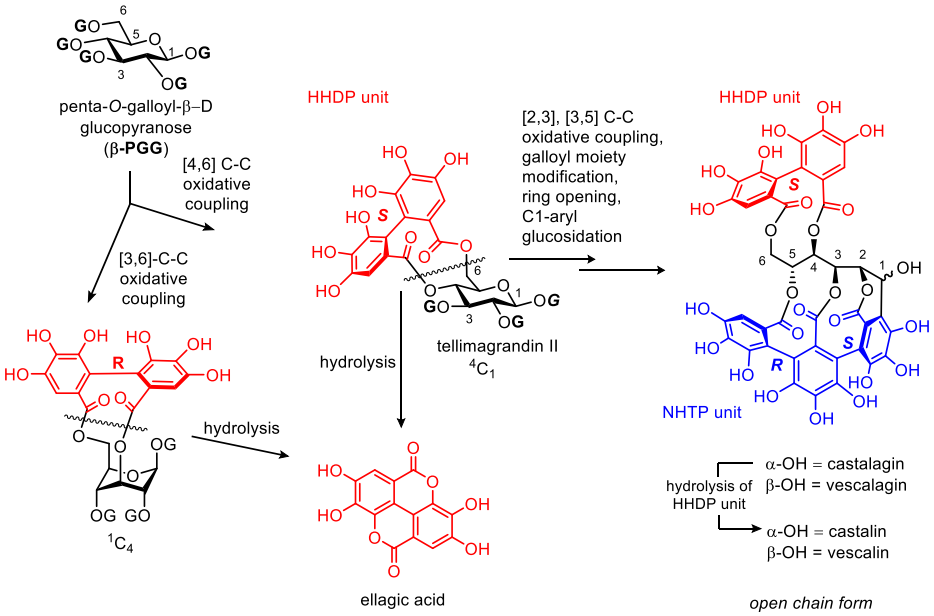
determination of their unusual molecular structures.[1] In particular, several reports of Quideau's group showed the interesting targeting ability of some wine ellagitannins toward the human topoisomerase II enzyme, a nuclear enzyme involved in DNA processes such as replication, transcription, chromosome condensation and segregation, suggesting a potential anti-proliferative activity and potential use of these molecules as new anti-cancer drugs.[2] Furthermore, the same group discovered that vescalagin (Scheme 6.1) is capable of inhibiting the activity of certain cells (endothelial and smooth muscle cells) by dismantling their actin cytoskeleton, opening the way toward a potential novel therapy against osteoporosis.[3]

Since the half nineties of the last century, the major contributions for the structural determination of ellagitannins look back to the seminal works by German chemists Schmidt and Mayer and the Japaneese groups of Okuda, Yoshida, Nishioka and Kouno; these scientists isolated and fully characterized nearly 1000 members of ellagitannins from various plant sources, rendering this sub-class of polyphenolic plant metabolites the largest group of known tannin molecules.[4]

From a structural point of view, the ellagitannins represent complex naturally occurring archetypes of polyphenolic clusters that adopt either disc-like or ball-like shapes. Despite their great structural variety, the ellagitannins' scaffold could be traced back to a common glucosydic core embedding several galloyl moieties. The structural variation among ellagitannins mainly depends on (1) the open- or close-forms of the sugar core, (2) the extent of galloylation, (3) the site and number of intramolecular C-C biaryl connections, (4) the oligomerization degree via oxidative C-O coupling (Figure 6.1).

As depicted in Scheme 6.1, the structural variety of ellagitannins arises from their biosynthetic route *in planta*, which starts from a common penta-*O*-galloyl-α-D-glucopyranosidic (β-PGG) precursor, which generates the so-called

hexahydroxydiphenoyl (HHDP) moiety through an intramolecolar oxidative C-C coupling of appropriately juxtaposed galloyl groups, as proposed by Schimdt and Mayers.[5] The HHDP biaryl unit is the structural determinant that defines hydrolyzable tannins as ellagitannins. In fact, hydrolytic release of HHDP units from ellagitannins gives rise to their facile and unavoidable conversion into the ellagic acid bis-lactone (Scheme 6.1), from which these natural products are named. This transformation constitutes the basis of current dogma in ellagitannin chemistry: for example, a first C-C biarylic coupling between the phenolic galloyl groups in position [4-6] affords tellimagrandin II, characterized by a glucose core constrained in a $^4C_1$ conformation. Further transformation consisting of intramolecolar biaryl coupling, galloylation degree modification, and ring opening may sequentially occur,affording more complex structures including the final derivatives vescalagin and castalagin. These natural products are the most attractive examples of open-form ellagitannins, due to their peculiar putative biological activity and intriguing structural features.[4] They contain the HHDP base motif, a nonahydroxyterphenoyl moiety (NHTP) and a peculiar C1-arylglucosidic connection giving the possibility to have α (castalagin) and β (vescalagin) anomeric forms, that could be transformed into vescalin and castalin after the HHDP unit realese. Another possibility involves the [1,6]-, [2,4]-, and/or [3,6]-C-C oxidative coupling of β-PGG precursor generating a series of less-stable $^1C_4$ glucopyranosic ellagitannin conformers (Scheme 6.1).



Scheme 6.1. *Putative biosynthetic routes of ellagitannins (G = galloyl moiety).*

6.1.1. Stereochemical consideration

The HHDP and NHTP units possess an axial chirality (atropoisomerism) giving the possible production of different stereoisomeric forms.

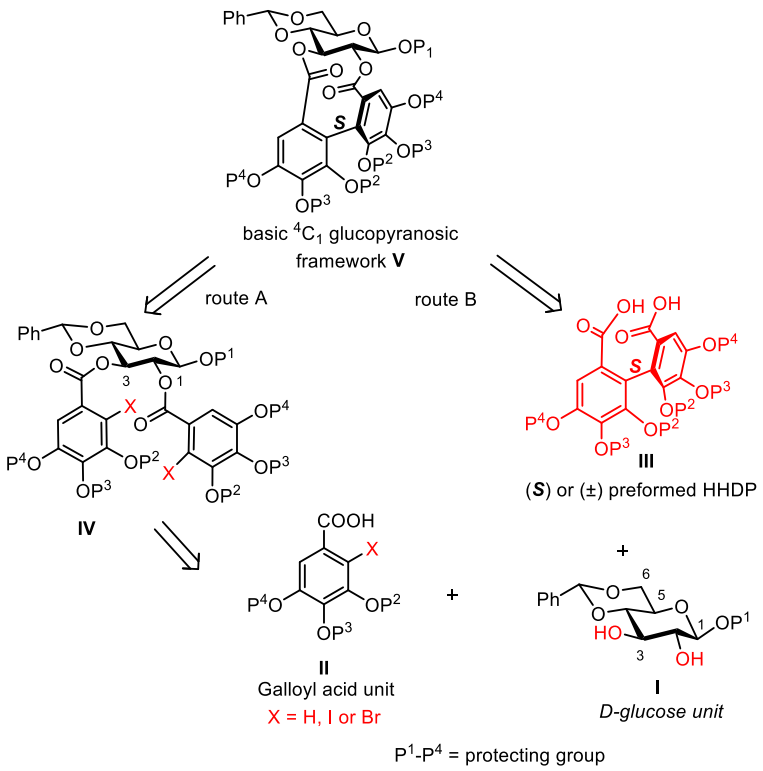
In the past, the stereochemistry of HHDP was intensely investigated by Haslam's, Nonaka's and Shimidt's groups,[4-6] leading to the conclusion that these chiral biaryl units almost exclusively adopt the *S*-configuration in $^4C_1$ ellagitannin structures, whereas both *R*- and *S*-configurations are observed in $^1C_4$ ellagitannins. The diastereoselectivity in the biosynthetic pathway is simply dictated by the geometrical constraints imposed by the glucopyranose ring, as proposed by Schimdt[5b] and Haslam[6a,b] and later refined by molecular mechanics-based analysis of substrate conformations.[4c,7,8]

To this day, the stereochemical attribution of NHTP unit is more uncertain. The original stereostructure of the triphenoyl moiety in vescalagin and castalagin and related HHDP-hydrolyzed vescalin and castalin was proposed to be [*S,S*],

based on electronic circular dichroism (ECD) values compared to well-known simpler analogues.[9] Later, computational studies by Vivas et al.[10] and Tanaka et al.[11] showed that the structures with the triphenoyl moieties in the (*S,R*) configuration represent the most stable form of these compounds. Furthermore, the calculated Cotton effect of (*S,R*)-vescalin and (*S,R*)-castalin were almost in agreement with the experimental ECD spectrum of related methylated derivates rendering the previous ECD-based studies inappropriate.[11]

6.1.2. Synthetic challenges

In this scenario, the chemical synthesis of some still untargeted ellagitannins offers an appealing challenge to completely understand their biosynthesis, biological activity and structural features, in particular for what concerns the stereochemistry of the NHTP unit. The biosynthetic route represents an inspiration source offering at the same time different challenges for organic synthesis: chemoselectivity, regioselectivity, and stereoselectivity, especially the atroposelectivity related to the galloyl coupling. Thus, planning of a truly appealing synthetic strategy has to face efficiency and brevity of the execution, and especially all these selectivity issues.



Scheme 6.2. *Principal strategies for HHDP-$^4C_1$ glucopyranonosic ellagitannin framework.*

In the past, organic chemists targeted some $^4C_1$-glucopyranosic ellagitannins mainly exploiting two strategies (i.e., routes A and B) as depicted in Scheme 6.2.[4a,b] Route A is based on a (biomimetic) biaryl coupling of the galloyl residues of an intermediate of type **IV**, which results from an esterification of a suitably protected/activated gallic acid **II** with a *D*-glucose-derived diol such as **I**. The alternative route B relies on a double esterification of a *D*-glucose diol derivative **I** with a preformed, and suitably protected, hexahydroxydiphenoic acid of type **III**, used either in its enantiopure form or in its racemic version (preformed HHDP approach).

The development of a stereoselective chemistry of polyhydroxylated molecules entails positive and negative strategic role played by the protecting groups modulating the key intramolecular biaryl coupling. Indeed, the kind and the

well-defined arrangement of protective groups have a tremendous impact on both the reactivity and the regioselectivity of the intramolecular key biaryl coupling of the polyphenolic structure. Obviously, the choice of the protecting groups has also to take into consideration the capability of easy manipulation of intermediates and final products. In fact, the well documented difficulties associated with purification and isolation of polydroxylated natural products[12] highlight the necessity of preparing pure synthetic precursors which, upon simple deprotection, will deliver impurity-free ellagitannin products. Thus, protecting groups not only must modulate the reactivity of the phenolic rings but they also provide "handles" compatible with delicate chromatographic purification at the end of the synthesis.

6.2. Synthetic planning of open-chain ellagitannins

Based on previously reported syntheses of ellagitannins,[4] we designed and implemented a bio-inspired divergent and stereoselective synthetic route toward the still untargeted open-chain aldehyde liquidambin and the more complex vescalagin and castalagin. Liquidambin was first isolated in 1987 by Okuda et al.[13] and its synthesis could help understanding the biochemical events that mediate the step from the glucopyranosic ellagitannin class to the open-chain C-glucosidic counterparts, that still remains a matter of speculation. In this context, it is reasonable to envisage that the unveiled electrophilic aldehyde function of liquindambin could be exposed to an intramolecular aldol-type attack by the phenolic [2,3]-HHDP unit to forge the characteristic C-glucosidic bond of a series of natural ellagitannins including the more complex vescalagin and castalagin. Moreover, this offers a new chemical field of interest, concerning the role and reactivity of the aldehyde function of these

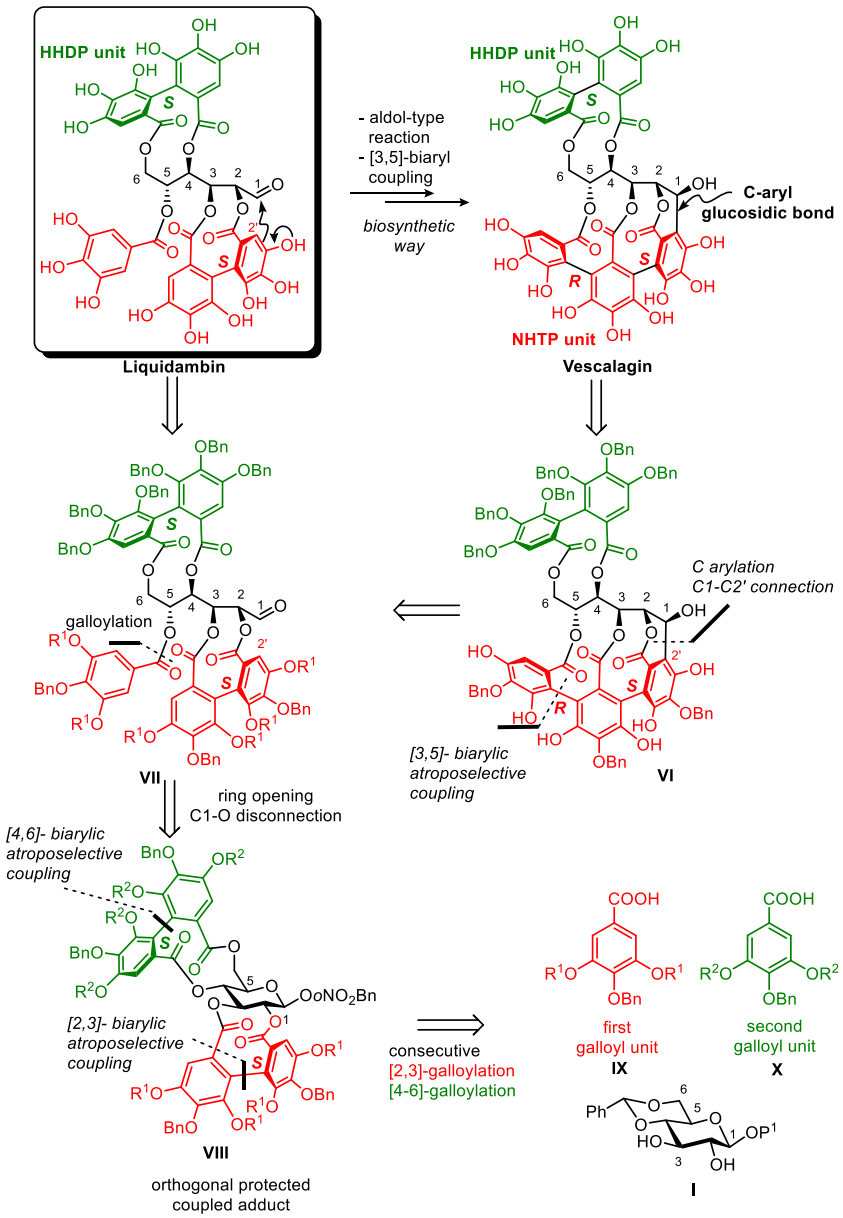
kinds of polyphenolic architectures, in addition to the most studied biaryl couplings.

As conceptualized in Scheme 6.3, our retrosynthetic plan identified polybenzylated compound **VI** as the key immediate precursor of vescalagin; **VI** may be accessed by an intramolecular C-arylation reaction and a [3,5]-biarylic atroposelective coupling of open intermediate **VII** which represents the point of divergence in the synthesis of both liquidambin and vescalagin. The choice of benzyl functionalities as protecting groups of advanced intermediate **VI** appeared to be the best option, since their cleavage procedure (palladium-catalyzed hydrogenation) are usually carried out under mild conditions and fast and easy experimental techniques (i.e. filtration) that seemed to best warrant purification of the final product avoiding difficulties associated with isolation and purification of perhydroxylated natural products.

In the synthetic direction, the sequential esterification of protected glucose **I** with suitably protected galloyl units **IX** and **X**, followed by the [2,3] and [4,6] intramolecular biaryl coupling provide the ortoghonally protected tetragalloyl intermediate **VIII**, that can be converted into building-block **VII** through opening of the sugar core. It is important to underline two considerations: i) the use of symmetrically-protected galloyl units **IX** and **X** to avoid regioselectivity issues, and ii) the engagement of two differently protected galloyl units to control the reactivity of polyphenol substrates during the double biaryl coupling (*vide infra*).

It is worth noting that this approach is inspired by those transformations performed by Nature in its biosynthetic pathway; obviously Nature faces the difficulties associated with these reactions by using specific enzymes that stereoselectively and regioselectively drive the intramolecular biaryl coupling (phenol oxidase) and the following ring-opening (enzymatic 5-*O*-galloylation

reaction) with high efficiency,). On the other hand, the chemical synthesis hasn't got this advantage!



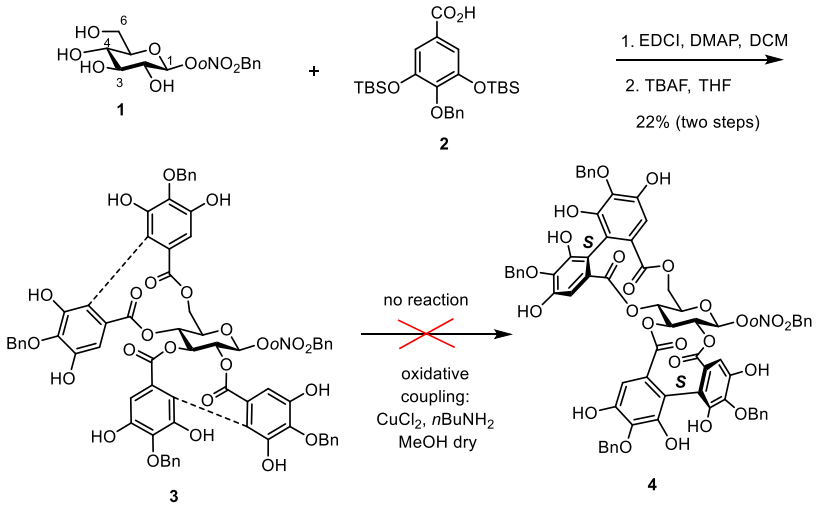
Scheme 6.3. *Retrosynthetic plan of open-chain ellagitannins liquidambin and vescalagin.*

As outlined before, from a synthetic point of view, there are several key crucial aspects to consider to efficiently shape the ellagitannins' architecture: *i.* the choice of suitable protecting groups with the corresponding sequence of protection and deprotection reactions; *ii.* the atroposelective intramolecular coupling; *iii.* the opening of the sugar moiety, and *iv.* the installation of the C-aryl glucosidic bond.

6.3. Results and Discussion

From a structural point of view, liquidambin is characterized by an "exposed" aldehyde group of the open-sugar and five galloyl moieties, four of which coupled two by two through an (***S***,***S***) atropoisomeric biaryl bond. At the beginning (Scheme 6.4), we tried to install these two atropoisomeric bonds starting from tetragalloyl compound **3**, in turn obtained by tetraesterification of *D*-glucose derivative **1** with protected gallic acid **2**, followed by total deprotection of the phenolic silyl groups. Unfortunately, the desired simultaneous intramolecular [4,6] and [2,3]-biaryl couplings using copper catalysis didn't occur and no desired product **4** could be detected, probably due to the concomitant undesired polymerization of compound **3**.

In order to avoid this side reaction, we decided to perform the two biaryl coupling sequentially through the use of the orthogonally protected precursor **10** (Scheme 6.5). Thus, we started masking the 4,6-dihydroxy functionalities of sugar **1** with a benzylidene moiety with $ZnCl_2$ and benzaldeyhyde. The regioselectivity of this protection was driven by the enhanced nucleophilicity of primary alcohol at the C6 position. Then, selective esterification of the remaining C2-C3 diol with suitably protected gallic acid **2** under modified Steglich's conditions[14] afforded fully protected sugar **5** in a 66% yield for the two steps.



Scheme 6.4. *Attempt of simultaneus [2,3]-[4,6] biarylic coupling.*

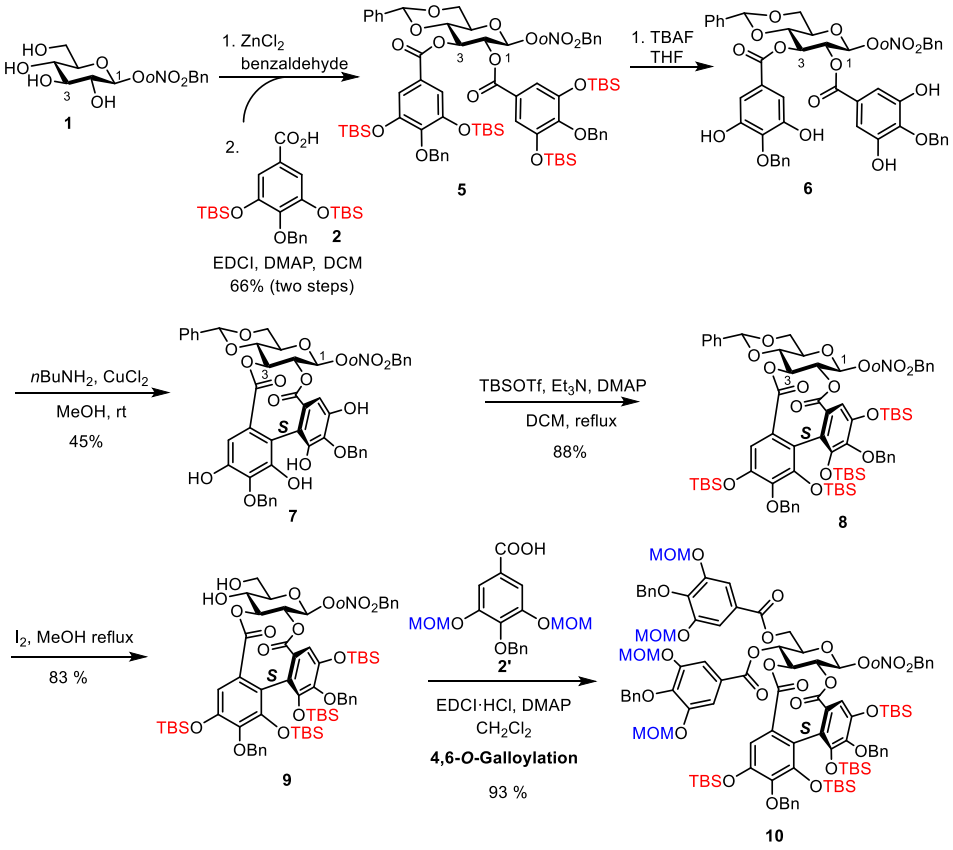
Desilylation of compound **5** was then mandatory in order to set up the substrate for the next key intramolecular oxidative coupling. This was mediated by a copper-amine complex ($CuCl_2$-*n*$BuNH_2$), as previously successfully applied by Yamada's group for the synthesis of (-)-corilagin[14] and (-)-strictinin.[15] Here, the coupling was successful, affording the desired (***S***)-configured derivative **7** as the sole isomer, with an acceptable yield of 45%. The (***S***)-stereochemistry of the newly formed biaryl bond was induced by the pre-existent chirality of the sugar core. For the next manipulations, the phenolic groups of compound **7** were silylated again affording protected adduct **8** in a 88% yield. At this point, inspired by a previously work of Feldman,[16] the selective cleavage of the benzylidene moiety with I_2 unmasked the [4,6] alcoholic groups of the intermediate **8**, furnishing diol **9** with a very good 83% yield, which could be now esterified with the second bulk of gallic acid **2'**, where the two phenolic functionalities in *meta*-position were protected as methoxymethyl (MOM) groups. The choice of the MOM group was justified by its easy way of installation, its stability under the strongly basic and weakly acidic conditions

necessary for removal of the other protecting groups such as silyl, alkoxy, acyl, or benzyl functions, and at the same time it results more easily removable than a standard methyl group. Finally, the 4,6-*O*-galloylation of substrate **9** allowed the access to the orthogonally protected intermediate **10** with an excellent 93% of yield.

With intermediate **10** in hand, we then moved on, trying the selective deprotection of MOM groups in the presence of TBS groups: a step that proved to be more troublesome than expected. At the beginning, we tried this deprotection maneuver using the same conditions of benzylidene cleavage (I_2 in MeOH), but MOM groups resist to these conditions; when harsher reaction conditions were used (higher temperatures, prolonged reaction time or higher loading of iodine), substrate **10** underwent partial desilylation before removal of MOM groups, thus considerably lowering the efficiency of the overall process.

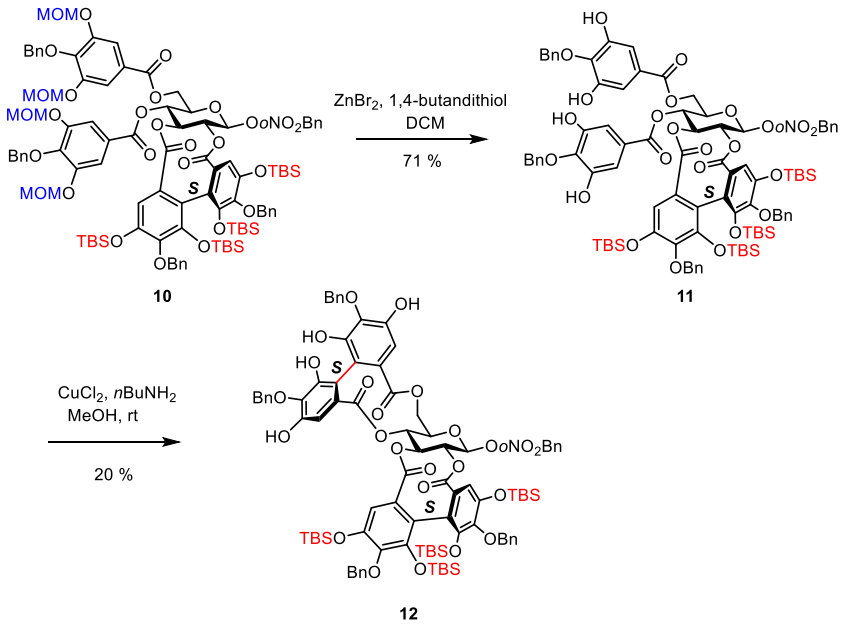
After this evidences, we succeeded in removing selectively the MOM groups under mild treatment with $ZnBr_2$ and 1,4-butandithiol in CH_2Cl_2 at room temperature, yielding the desired intermediate **11** in a goof 71% yield (**Scheme 6.6**).[17]

We next moved to the second atroposelective intramolecular [4,6] biaryl oxidative coupling: using the previously optimized conditions with $CuCl_2$ and *n*-butylamine (Scheme 6.6). In the event, we isolated the advanced intermediate **12** in 20%yield. Despite the scarce efficiency of this last coupling (that requires future optimization), we noticed that the [4-6] coupling proceeds faster than the [2-3]-coupling, probably due to conformational bias.



Scheme 6.5*. Synthetic route toward orthogonally protected intermediate* ***10****.*

We expect that the judicious modulation of the reaction conditions including reaction time, copper catalyst loading, and reactants concentration could help the successful outcome of this transformation, overcoming possible competitive intermolecular reactions, as similarly observed by Yamada during his total synthesis of (-)-strictinin.[15]



***Scheme 6.6**. Selective cleavage of MOM groups and second [4,6]-biaryl coupling.*

6.4. Conclusion and perspectives

In conclusion, in the scenario of the programmed total synthesis of ellagitannins we reached an important point along the synthesis of liquidambin and vescalagin, by providing access to advanced intermediate **12**, which features the presence of synthetically challenging [4,6] and [2,3] biaryl bonds and orthogonally protected phenol rings. Simple unmasking of the aldehyde function within **12** would then directly provide open-chain liquidambin.

The proposed synthetic path may pave the way to a new stereo- and regioselective approach to the total synthesis of ellagitannins while providing opportunities in the development of new chemical methodologies in the field of sugar-opening strategies, aldol-type reactions of phenols, and overall understanding of the non-trivial polyphenol chemistry.

6.5. Experimental Section

General experimental method

All moisture and oxygen sensitive reactions were carried out in flame-dried glassware under inert atmosphere with dry solvents. Dichloromethane (CH_2Cl_2) was purified immediately before use by filtration through activated alumina columns under nitrogen. Methanol (MeOH) was dried from molecular sieves (3 Å) under nitrogen prior to use. Acetone, ethyl acetate (EtOAc), petroleum ether (PET), cyclohexane and diethyl ether (Et_2O) were used as received. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Evaporations were conducted under reduced pressure at temperatures less than 45 °C. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm E. Merck silica plates (60 F254), using UV light for visualization and a potassium permanganate solution and heat as the developing agent. Column chromatography was carried out under positive pressure using 40-63 µm silica gel (Merck) and the indicated solvents. NMR spectra of samples in the indicated solvents were recorded on Bruker Avance 300 and were calibrated using residual solvent as an internal reference. The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, m= multiplet and br = broad singlet. Carbon multiplicities were determined by DEPT135 experiments. Diagnostic correlations were obtained by two-dimensional COSY, HSQC and HMBC experiments.

Starting materials and other materials

The synthesis of compound **1**[18] from commercially available 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl bromide, **2**[18], **2'**[14] from methyl gallate and **6**[14] from **1** were made as previously described.

Compound 3. According to the procedure described in the literature,[18] to a solution of **1** (128 mg, 0.406 mmol, 1 equiv) and **2** (952.5 mg, 1.95 mmol, 4.8 equiv) in CH_2Cl_2 (12 ml), DMAP (198.3, 1.62 mmol, 4 eq decidi equiv o eq in tutto il testo) and EDC-HCl (373.6 mg, 1.95 mmol, 4.8 eq) were added at room temperature. The solution was purged with nitrogen and stirred at room temperature for 18 h. A 1M aqueous solution of H_3PO_4 (10 ml) was then added to quench the reaction and the mixture was extracted with CH_2Cl_2 (3 x 10 ml). The organic layer was then washed with brine (2 x 10 ml), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified by column chromatography with dietyl ether:petroleum ether (1:99 →20:80-1% Et_3N) to furnish **3** (280 mg, 3.12 mmol, **31 %**) as a white foam. NMR^{1}H (300 MHz, $CDCl_3$): δ (ppm): 0.06 (s, 12H, CH_3, TBS), 0.09 (s, 6H, CH_3, TBS), 0.10 (s, 6H, CH_3, TBS), 0.12 (s, 6H, CH_3, TBS), 0.13 (s, 6H, CH_3, TBS), 0.17 (s, 6H, CH_3, TBS), 0.18 (s, 6H, CH_3, TBS), 0.87 (s, 18H, *tert*-Bu, TBS), 0.91 (s, 18H, *tert*-Bu, TBS), 0.92 (s, 18H, *tert*-Bu, TBS), 0.94 (s, 18H, *tert*-Bu, TBS), 4.13 (ddd, *J* = 9.7, 5.6, 1.8 Hz, 1H, H5), 4.32 (dd, *J* = 12.4, 5.7 Hz, H6a), 4.62 (dd, *J* = 12.3, 2.0 Hz, 1H, H6b), 4.94-5.09 (m, 10H, CH_2, H1), 5.28 (d, *J*= 15.4 Hz, 1H, CH_2), 5.52 (dd, *J* = 9.9, 3.4 Hz, 1H, H2), 5.55 (dd like t, *J* = 9.9, 9.9 Hz, 1H, H4), 5.83 (dd like t, *J* = 9.7, 9.7 Hz, 1H, H3), 7.03 (s, 2H, Ar galloyl), 7.12 (s, 4H, Ar galloyl), 7.25 (s, 2H, Ar galloyl), 7.28/7.48 (m, 22H, Ar), 7.72 (dd, *J* = 7.8, 0.8 Hz, 1H, Ar), 8.01 (dd, *J* = 1.3, 8.1 Hz, 1H, Ar). NMR^{13}C (75 MHz, $CDCl_3$): δ (ppm) 165.9 (Cq, C=O), 165.0 (Cq, C=O), 164.7 (2C, C=O), 150.0/149.7 (8C, Cq Ar), 147.1 (Cq Ar), 146.9

(Cq Ar), 146.8 (2C, Cq Ar), 146.7 (Cq Ar), 138.0 (Cq Ar), 137.9 (Cq Ar), 137.8 (2C, Cq Ar), 134.2 (CH, Ar), 134.0 (CH, Ar), 128.9 (CH, Ar), 128.3 (CH, Ar), 128.1 (CH, Ar), 128.0 (CH, Ar), 127.9 (CH, Ar), 127.8 (CH, Ar), 127.7 (CH, Ar), 124,7 (2C, Cq, Ar), 124.3 (Cq, Ar), 124.0 (Cq, Ar), 123.8 (Cq, Ar), 116.5/116.4 (4C, CH galloyl), 116.3 (2C, CH galloyl), 116.2 (2C, CH, galloyl), 101.5 (C1), 74.3 (CH_2), 74.30 (CH_2), 73.5 (CH, C5), 72.6 (CH, C3), 72.0 (CH, C2), 69.4 (CH, C4), 68.6 (CH_2), 62.9 (CH_2, C6), 26.0 (12C, CH_3, *tert*-Bu, TBS) 25.9 (12C, CH_3, *tert*-Bu, TBS), 18.6 (2C, Cq, *tert*-Bu, TBS), 18.5 (4C, Cq, *tert*-Bu, TBS), 18.4 (2C, Cq, *tert*-Bu, TBS), −4.3 (8C, CH_3, TBS), –4.2 (8C, CH_3, TBS).

Compound 4. To a solution of **3** (270 mg, 0.12 mmol, 1 eq) in dry THF (10 ml), a 1M solution of TBAF in THF (1.47 ml, 1.47 mmol, 12 eq) was added. The solution was stirred under nitrogen at room temperature for 2 hours. The reaction mixture was then diluted with 1M aqueous solution of H_3PO_4 (10 ml). The product was extracted with AcOEt and the organic layer was washed with brine (3 ml), dried over Na_2SO_4 and concentrated *in vacuo*. Crude material was purified by column chromatography (ethyl acetate : petroleum ether 60:40 → 80:20) to afford **4** as a pale orange foam (110 mg, 0.085 mmol, **71 %**). ^{1}H NMR (300 MHz, acetone-d_6): δ 4.40-4.49 (m, 2H, H5, H6a), 4.58 (m, 1H, H6b), 5.10 (s, 2H, $\underline{CH_2}$Ph), 5.13 (s, 2H, $\underline{CH_2}$Ph), 5.17 (s, 2H, $\underline{CH_2}$Ph), 5.20 (d, *J* = 13.7 Hz, 1H, $\underline{CH_2}o$$NO_2$Ph), 5.21 (s, 2H, $\underline{CH_2}$Ph), 5.28 (d, *J* = 7.7 Hz, 1H, H1), 5.32 (d, *J* = 14.4 Hz, 1H, $\underline{CH_2}o$$NO_2$Ph), 5.51 (dd, *J* = 9.8, 8.0 Hz, 1H, H2), 5.64 (dd like t, *J* = 9.6, 9.6 Hz, 1H, H4), 5.90 (dd like t, *J* = 9.7, 9.7 Hz, 1H, H3), 6.96 (s, 2H, Ar galloyl), 7.02 (s, 2H, Ar galloyl), 7.09 (s, 2H, Ar galloyl), 7.17 (s, 2H, Ar galloyl), 7.27-7.55 (m, 22H, Ar), 7.75 (m, 1H, Ar), 8.06 (m, 1H, Ar), 8.36-8.39 (bs, 8H). ^{13}C NMR (75 MHz, acetone-d_6): δ 166.1 (Cq, C=O), 165.8 (Cq, C=O), 165.4 (2C, Cq, C=O), 151.4 (4C, Cq, Ar), 151.3 (4C, Cq, Ar), 148.0 (Cq, Ar), 139.6 (Cq, Ar), 139.5 (Cq, Ar), 139.4 (Cq, Ar), 139.2 (Cq, Ar), 138.7 (Cq, Ar), 138.6 (Cq, Ar), 138.6 (Cq, Ar), 138.5 (Cq,

Ar), 135.0 (Cq, Ar), 134.6 (CH, Ar), 129.3 (3C, CH, Ar), 129.3 (3C, CH, Ar), 129.2 (2C, CH, Ar), 129.2 (CH, Ar), 129.0 (2C, CH, Ar), 128.9 (2C, CH, Ar), 128.8 (CH, Ar), 128.8 (CH, Ar), 128.7 (CH, Ar), 128.7 (CH, Ar), 126.1 (Cq, Ar), 125.6 (Cq, Ar), 125.4 (CH, Ar), 125.3 (Cq, Ar), 125.3 (Cq, Ar), 110.3 (4C, CH, Ar), 110.2 (4C, CH, Ar), 101.5 (CH, C1), 74.7 (CH_2, $\underline{CH_2}$Ph), 74.6 (CH_2, $\underline{CH_2}$Ph), 74.6 (CH_2, $\underline{CH_2}$Ph), 74.6 (CH_2, $\underline{CH_2}$Ph), 73.7 (CH, C3), 72.9 (CH, C5), 70.0 (CH, C4), 68.5 (CH_2, $\underline{CH_2}$*o*NO_2Ph), 63.1 (CH_2, C6).

Compound 7. To a stirred solution of $CuCl_2$ (56 mg, 0.42 mmol, 5 eq) in dry methanol (1.5 ml), *n*-butylamine (165µl, 1.7 mmol, 20 eq) was added at room temperature, and the mixture was stirred for 45 minutes. To this blue solution, a solution of **7** (75 mg, 0.08 mmol, 1 eq) in dry methanol (1 ml) was added and the mixture was stirred for 30 minutes. The reaction mixture was then poured into a 1:1 mixture of saturated aqueous solution of NH_4Cl: ethyl acetate (35 ml) and stirred at room temperature for 15 min. The mixture was next extracted with AcOEt. The combined organic layers were washed with brine (10 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by column chromatography with a mixture of acetone:CH_2Cl_2 (0:100 → 5:95) to give (*S*)-**8** as a brown powder (43 mg, 0.05 mmol, 45 %). NMR ^{1}H (300 MHz, acetone-*d6*): δ 3.82 (ddd like dt, *J* = 9.6, 4.6, 4.6 Hz, 1H, H5), 3.94 (dd like t, *J* = 9.8, 9.8 Hz, 1H, H6a), 4.05 (dd like t, *J* = 9.2, 9.2 Hz, 1H, H4), 4.40 (dd, *J* = 10.1, 4.7 Hz, 1H, H6b) 5.00 (dd like t, 1H, *J* = 8.4, 8.4 Hz, H2), 5.12-5.26 (m, 7H, H1, CH_2 *o*NO_2Bn, CH_2 Bn), 5.35 (dd like t, *J* = 8.2, 8.2 Hz, 1H, H3), 5.76 (s, 1H, CH benzylidene), 6.58 (s, 1H, Ar), 6.73 (s, 1H ,Ar), 7.28-7.54 (m, 15H, Ar), 7.63 (ddd like td, *J* = 7.4, 7.4, 1.8 Hz, 1H, Ar), 7.80-7.89 (m, 2H), 8.11 (dd, 1H, *J* = 8.1, 0.9Hz, Ar), 8.40 (s, 2H, OH). NMR ^{13}C (75 MHz, acetone-*d6*): δ (ppm) 168.9 (Cq, C=O), 168.4 (Cq, C=O), 150.5 (Cq Ar), 150.5 (Cq Ar), 150.3 (Cq Ar) 150.2 (Cq Ar), 149.2 (Cq Ar), 138.7 (Cq, Ar), 138.6 (Cq, Ar), 138.5 (Cq, Ar), 134.6 (Cq,),

134.3 (CH, Ar), 131.1 (CH, Ar), 130.9 (CH, Ar), 129.9 (CH, Ar), 129.8 (CH, Ar), 129.7 (CH, Ar), 129.3 (CH, Ar), 129.3 (CH, Ar), 129.0 (CH, Ar), 128.9 (CH, Ar), 127.3 (CH, Ar), 125.5 (CH, Ar), 114.2 (CH, Ar), 113.9 (CH, Ar), 107.1 (CH, Ar), 107.0 (CH, Ar), 102.3 (CH, Benzylidene), 100.8 (CH, C1), 78.2 (CH, C4), 76.9 (CH, C2), 76.4 (CH, C3), 74.8 (CH_2, Bn), 69.0 (CH_2, C6), 68.6 (CH_2, *o*NO_2Bn), 67.8 (CH, C5). MS (ESI): m/z = 1735.4 $[M-H^+]^-$.

Compound 8. To a solution of **7** (1900 mg, 2.14 mmol, 1 eq) in dry CH_2Cl_2 (3 ml) triethylamine (6 ml, 42.9 mmol, 20 eq), TBSOTf (2.87 ml, 12.87 mmol, 6 eq) and DMAP (262 mg, 2.14 mmol, 1 eq) were sequentially added under argon atmosphere. The reaction mixture was stirred to reflux for 7 h. The solution was then diluted with CH_2Cl_2 (25.5 mL) and washed successively with a saturated solution of $NaHCO_3$ (15 mL), brine (15 mL), dried over $MgSO_4$ and concentrated under reduced pressure to afford the crude mixture as an orange oil. The crude material was purified by column chromatography (NEt_3:CH_2Cl_2:cyclohexane 1:10:90 → 1:40:60) to give (*S*)-**8** as an yellow foam (2526 mg, 1.88 mmol, 88 %). NMR ^{1}H (300 MHz, acetone-*d6*): δ (ppm): −0.30 (s, 3H, CH_3, TBS), −0.28 (s, 3H, CH_3, TBS), 0.07 (s, 3H, CH_3, TBS), 0.08 (s, 3H, CH_3, TBS), 0.23 (s, 6H, CH_3, TBS), 0.25 (s, 3H, CH_3, TBS), 0.28 (s, 3H, CH_3, TBS), 0.79 (s, 9H, *tert*-Bu, TBS), 0.81 (s, 9H, *tert*-Bu, TBS), 0.94 (s, 9H, *tert*-Bu, TBS), 0.96 (s, 9H, *tert*-Bu, TBS), 3.87 (ddd like dt, *J* = 9.6, 4.7, 4.7 Hz, 1H, H5), 3.97 (dd like t, *J* = 10.1, 10.1 Hz, 1H, H6a), 4.04 (dd like t, *J* = 9.2, 9.2 Hz, 1H, H4), 4.42 (dd, J = 10.1, 4.7Hz, 1H, H6b), 4.98 (dd like t, *J* = 7.3 Hz, 1H, H2), 5.06-5.25 (m, 7H, H1, CH_2 *o*NO2Bn, CH_2 Bn), 5.35 (dd like t, *J* = 9.2, 9.2 Hz, 1H, H3), 5.76 (s, 1H, benzylidene), 6.76 (s, 1H, Ar), 6.94 (s, 1H,Ar), 7.28-7.52 (m, 15H, Ar), 7.63 (ddd like td, J = 8.2, 8.2, 1.8Hz, 1H, Ar), 7.75-7.87 (m, 2H, Ar), 8.06 (dd, *J* = 8.2, 1.0 Hz, 1H). NMR ^{13}C (75 MHz, acetone-*d6*): δ (ppm) 168.9 (Cq, C=O), 168.3 (Cq, C=O), 150.0 (Cq, Ar), 149.9 (Cq, Ar), 149.2 (Cq, Ar), 144.8/144.7 (Cq, Ar), 138.9 (Cq,

Ar), 138.6 (Cq, Ar), 134.3 (Cq, Ar), 133.6 (CH, Ar), 131.1 (CH, Ar), 131.1 (CH, Ar), 130.4 (CH, Ar), 130.0 (CH, Ar), 129.8 (CH, Ar), 128.9 (CH, Ar), 128.9 (CH, Ar), 128.2 (CH, Ar), 128.2 (CH, Ar), 127.5 (CH, Ar), 127.4 (CH, Ar), 127.1 (CH, Ar), 125.4 (CH, Ar), 122.5 (CH, Ar), 122.4 (CH, Ar), 113.4 (CH, Ar galloyl unit), 113.2 (CH, Ar galloyl unit), 102.2 (CH, benzylidene), 101.1 (C1), 78.3 (CH, C4), 77.3 (CH, C3), 76.8 (CH, C2), 74.7 (CH_2), 74.6 (CH_2), 69.2 (CH_2, C6), 69.0 (CH_2), 67.7 (CH, C5), 26.4 (6C, CH_3, *tert*-Bu, TBS), 26.3 (6C, CH_3, *tert*-Bu, TBS), 19.1 (Cq, *tert*-Bu, TBS), 19.0 (2C, *tert*-Bu, TBS), 18.8 (Cq, *tert*-Bu, TBS), -3.6 (CH_3, TBS), -3.7 (CH_3, TBS), -3.9 (CH_3, TBS), -4.0 (CH_3, TBS), -4.2 (CH_3, TBS), -4.2 (CH_3, TBS), -4.5 (CH_3, TBS), -4.6 (CH_3, TBS). MS (ESI): m/z = 1364.56 $[M+Na]^+$

Compound 9. To a solution of **8** (350 mg, 0.26 mmol, 1 eq) in CH_2Cl_2 (3 ml) and MeOH (3 ml), I_2 (80 mg, 0.313 mmol, 1.2eq) was added under argon. The mixture was heated at reflux (60 °C) under argon for 17 h. The solution was cooled, diluted with EtOAc (7 ml), washed with saturated $Na_2S_20_3$ solution and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified by column chromatography with cyclohexane:EtOAc (80:20 → 70:30) to furnish **9** (274 mg, 0.22 mmol, 83 %) as a white foam. NMR ^{1}H (300 MHz, acetone-d_6): δ (ppm) : -0.29 (s, 3H, CH_3, TBS), -0.28 (s, 3H, CH_3, TBS), 0.08 (s, 3H, CH_3, TBS), 0.08 (s, 3H, CH_3, TBS), 0.22 (s, 3H, CH_3, TBS), 0.24 (s, 3H, CH_3, TBS), 0.25 (s, 3H, CH_3, TBS), 0.29 (s, 3H, CH_3, TBS), 0.80 (s, 9H, *tert*-Bu, TBS), 0.81 (s, 9H, *tert*-Bu, TBS), 0.93 (s, 9H, *tert*-Bu, TBS), 0.95 (s, 9H, *tert*-Bu, TBS), 3.59-3.65 (m, 1H, H5), 3.77-3.96 (m, 3H, H6, H4), 4.85 (dd, 1H, *J* = 9.3, 8.1 Hz, H2), 4.99 (d, *J* = 8.1 Hz, 1H, H1), 5.05-5.18 (m, 6H, CH_2, $\underline{CH_2}$Ph, $\underline{CH_2}o$$NO_2$Ph, H3), 5.25 (d, *J* = 13.8 Hz, 1H, CH_2), 6.87 (s, 1H, Ar galloyl), 6.90 (s, 1H, Ar galloyl), 7.29-7.42 (m, 6H, Ph), 7.50-7.52 (m, 4H, Ph), 7.62 (ddd, *J* = 8.2, 7.4, 1.6 Hz, 1H, Ar), 7.77 (td, *J* = 7.2, 1.2 Hz, 1H, Ar), 7.83 (dd, *J* = 7.7, 1.4 Hz, 1H, Ar), 8.05 (dd, *J* = 8.0, 1.0 Hz, 1H, Ar). NMR ^{13}C (75 MHz, acetone-d_6): δ

(ppm) 169.2 (Cq, C=O), 168.4 (Cq, C=O), 149.9 (2C, Cq Ar), 149.8 (2C, Cq Ar), 149.2 (Cq Ar), 144.6 (2C, Cq Ar), 138.9 (2C, Cq Ar), 134.3 (CH Ar), 133.9 (Cq Ar), 131.5 (CH Ar), 131.3 (CH Ar), 130.3 (CH Ar), 129.8 (CH Ar), 128.9 (4C, CH Ar), 128.1 (CH Ar), 127.4 (CH Ar), 127.4 (2C, CH Ar), 125.3 (CH Ar), 122.5 (Cq Ar), 122.4 (Cq Ar), 113.4 (CH, Ar galloyl), 113.2 (CH, Ar galloyl), 100.3 (CH, C1), 81.0 (CH, C3), 78.0 (CH, C5), 76.7 (CH, C2), 74.6 (CH_2), 74.6 (CH_2), 68.6 (CH_2), 68.2 (CH, C4), 62.0 (CH_2, C6), 26.4 (6C, CH_3, *tert*-Bu, TBS),26.2 (6C, CH_3, *tert*-Bu, TBS), 19.0 (Cq, *tert*-Bu, TBS), 18.9 (2C, *tert*-Bu, TBS), 18.8 (Cq, *tert*-Bu, TBS), -3.6 (C_{10}), -3.7 (CH_3, TBS), -3.9 (CH_3, TBS), -4.0 (CH_3, TBS), -4.1 (CH_3, TBS), -4.2 (CH_3, TBS), -4.5 (CH_3, TBS), -4.6 (CH_3, TBS). MS (ESI): m/z = 1276.50 $[M+Na]^+$

Compound 10 To a solution of **9** (160.0 mg, 0.13 mmol, 1 eq) and **2'** (133 mg, 0.38 mmol, 3 eq) in CH_2Cl_2 (5 ml), DMAP (109.0 g, 0.89 mmol, 7 eq) and EDC-HCl (122.2 mg, 0.64 mmol, 5 eq) were sequentially added. The solution was purged with nitrogen and stirred at room temperature for 1 8h. A 1M aqueous solution of H_3PO_4 (5 ml) was then added to quench the reaction and the mixture was extracted with CH_2Cl_2 (3 x 10 ml). The organic layer was washed with brine (2 x 10 ml), dried over Na_2SO_4 filtered and concentrated under reduced pressure. The resulting residue was purified by column chromatography (elution by gradient from 60:40 to 55:45 petroleum ether/diethyl ether-1% Et_3N) to furnish **10** (230 mg, 0.12 mmol, **93 %**) as a white foam. NMR ^1H (300 MHz, acetone-d_6): δ (ppm): -0.34 (s, 3H, CH_3, TBS), -0.29 (s, 3H, CH_3, TBS), 0.06 (s, 6H, CH_3, TBS), 0.23 (s, 3H, CH_3, TBS), 0.24 (s, 3H, CH_3, TBS), 0.26 (s, 3H, CH_3, TBS), 0.31 (s, 3H, CH_3, TBS), 0.77 (s, 9H, *tert*-Bu, TBS), 0.79 (s, 9H, *tert*-Bu, TBS), 0.84 (s, 18H, *tert*-Bu, TBS), 3.46 (s, 6H, CH_3 MOM), 3.48 (s, 6H, CH_3, MOM), 4.43-3.50 (m, 2H, H5, H6a), 4.83 (m, 1H, H6b), 5.04-5.16 (m, 9H, H2, $\underline{CH_2}o NO_2Ph$, $\underline{CH_2}Ph$), 5.24-5.31 (m, 11H, $\underline{CH_2}Ph$, CH_2 MOM, H1), 5.48 (dd like t, *J* = 9.4, 9.4 Hz, 1H, H3), 5.63 (dd like t, *J* = 9.7, 9.7 Hz, 1H, H4), 6.6

(s, 1H, Ar galloyl), 6.95 (s, 1H, Ar galloyl), 7.28-7.42 (m, 11H, Ar), 7.60-7.65 (m, 14H, Ar), 7.76 (ddd, *J* = 1.3, 7.6, 7.6 Hz, 1H, Ar), 7.84 (dd, *J* = 1.2, 7.7 Hz, 1H,Ar), 8.05 (dd, *J* = 1.2, 8.1 Hz, 1H, Ar). NMR^{13}C (75 MHz, acetone-d_6): δ (ppm) 168.8 (Cq, C=O), 168.3 (Cq, C=O), 165.8 (Cq, C=O), 165.2 (Cq, C=O), 152.1 (2C, Cq Ar), 152.0 (2C, Cq Ar), 150.1 (2C, Cq Ar), 150.0 (2C, Cq Ar), 149.2 (Cq Ar), 144.9 (Cq Ar), 144.8 (Cq Ar), 144.5 (Cq Ar), 144.4 (Cq Ar), 139.0 (Cq Ar), 138.9 (Cq Ar), 138.8 (Cq Ar), 138.7 (Cq Ar), 134.3 (CH Ar), 133.5 (CH Ar), 131.0 (CH Ar), 130.7 (2C, CH Ar), 129.9 (Cq, Ar), 129.0 (CH Ar), 129.0 (CH Ar), 128.9 (CH Ar), 128.7 (CH Ar), 128.2 (CH Ar), 127.5 (CH Ar), 125.9 (Cq Ar), 125.4 (CH, Ar), 125.2 (Cq Ar), 122.3 (Cq Ar), 121.8 (Cq Ar), 113.5 (CH, Ar galloyl), 113.0 (2C, CH, Ar galloyl), 112.8 (2C, CH, Ar galloyl), 112.6 (CH, Ar galloyl), 100.4 (C1), 96.2 (4C, CH_2, MOM), 77.6 (CH, C3), 76.4 (CH, C2), 75.6 (2C, CH_2), 74.7 (CH_2), 74.6 (CH_2), 73.0 (CH, C5), 69.3 (CH, C4), 69.1 (CH_2), 63.7 (CH_2, C6), 56.6 (4C, CH_3, MOM), 26.4 (9C, CH_3, *tert*-Bu, TBS), 26.2 (3C, CH_3, *tert*-Bu, TBS), 19.1 (Cq, *tert*-Bu, TBS), 19.0 (Cq, *tert*-Bu, TBS), 18.8 (2C, Cq, *tert*-Bu, TBS), -3.5 (2C, CH_3, TBS), -3.7 (2C, CH_3, TBS), -3.9 (2C, CH_3, TBS), -4.0 (2C, CH3, TBS), -4.1 (2C, CH_3, TBS), -4.2 (2C, CH_3, TBS), -4.5 (2C, CH_3, TBS)**.** MS (ESI): m/z = 1937.53 $[M+Na]^+$

Compound 11 To a stirred solution of **10** (191.5 mg, 0.10 mmol, 1 equiv) in CH_2Cl_2 (2 ml), $ZnBr_2$ (90 mg, 0.40 mmol, 4 equiv) and 1,4-butanedithiol (35 µL, 0.3 mmol, 3 equiv) were sequentially added. After stirring for 7 h at room temperature, the resulting mixture was diluted with CH_2Cl_2 (10 mL). Saturated $NaHCO_3$ (3 ml) was added slowly at 0 °C and the mixture was filtered through Celite. The aqueous layer was separated and further extracted with CH_2Cl_2 (3 x 5 mL). The combined organic layers were washed with brine (3 ml), dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash column chromatography (7:2.8:0.2 DCM/Cyclohexane/MeOH) to afford the product **11** as a white foam (125 mg, 0.071 mmol, 71% yield). NMR ^{1}H (300

MHz, acetone-d_6): δ (ppm) : -0.36 (s, 3H, CH_3, TBS), -0.28 (s, 3H, CH_3, TBS), 0.06 (s, 6H, CH_3, TBS), 0.23 (s, 3H, CH_3, TBS), 0.24 (s, 3H, CH_3, TBS), 0.26 (s, 3H, CH_3, TBS), 0.30 (s, 3H, CH_3, TBS), 0.77 (s, 9H, *tert*-Bu, TBS), 0.80 (s, 9H, *tert*-Bu, TBS), 0.93 (s, 9H, *tert*-Bu, TBS), 0.94 (s, 9H, *tert*-Bu, TBS), 4.43-4.6 (m, 2H, H5, H6a), 5.03-5.25 (m, 13H, H6b, $\underline{CH_2}o NO_2Ph$, $\underline{CH_2}Ph$, H2, H1), 5.45 (dd like t, *J* = 9.7, 9.7 Hz, H4), 5.59 (dd like t, *J* = 9.7, 9.7 *Hz*, H3), 6.60 (s, 1H, Ar galloyl), 6.96 (s, 1H, Ar galloyl), 7.17 (s, 2H, Ar galloyl), 7.17 (s, 2H, Ar galloyl), 7.42-7.54 (m, 20H, Ar), 7.62 (ddd like td, *J* = 7.8, 7.8, 1.5 Hz, 1H, Ar), 7.75 (ddd like td, *J* = 7.7, 7.7, 1.4 Hz, 1H, Ar), 7.83 (dd, *J* =7.9, 1.3 Hz, 1H, Ar), 8.05 (dd, *J* =8.0, 1.2Hz, 1H, Ar). $NMR^{13}C$ (75 MHz, acetone-d_6): δ (ppm) 168.7 (Cq, C=O), 168.3 (Cq, C=O), 166.2 (Cq, C=O), 165.1 (Cq, C=O), 151.4 (2C, Cq Ar), 150.0 (2C, Cq Ar), 149.9 (2C, Cq Ar), 149.2 (Cq Ar), 144.8 (Cq Ar), 144.5 (Cq Ar), 139.6 (Cq Ar), 139.2 (Cq Ar), 139.0 (Cq Ar), 138.9 (Cq Ar), 138.6 (Cq Ar), 138.5 (Cq Ar), 134.3 (CH Ar), 133.4 (CH Ar), 130.9 (CH Ar), 130.6 (CH Ar), 129.9 (Cq Ar), 129.3 (CH Ar), 129.2 (CH Ar), 129.0 (CH Ar), 128.9 (CH Ar), 128.7 (CH Ar), 128.2 (CH Ar), 127.5 (CH Ar), 127.4 (CH, Ar), 126.0 (Cq Ar), 125.4 (CH Ar), 125.2 (Cq Ar), 122.3(Cq Ar), 121.8 (Cq Ar), 113.5 (CH, Ar galloyl), 112.6 (CH, Ar galloyl), 110.3 (2C, CH, Ar galloyl), 110.1 (2C, CH, Ar galloyl), 100.4 (CH, C1), 77.6 (CH, C3), 76.5 (C2), 74.7 (2C, CH_2), 74.6 (2C, CH_2), 73.0 (CH, C5), 69.1 (CH_2), 68.4 (CH, C4), 63.3 (CH_2, C6), 26.4 (3C, CH_3, *tert*-Bu, TBS), 26.3 (6C, CH_3, *tert*-Bu, TBS), 26.2 (3C, CH_3, *tert*-Bu, TBS), 19.1 (Cq, *tert*-Bu, TBS), 18.9 (Cq, *tert*-Bu, TBS), 18.8 (Cq, *tert*-Bu, TBS), 18.7 (Cq, *tert*-Bu, TBS), -3.4 (CH_3, TBS), -3.7 (CH_3, TBS), -3.9 (CH3, TBS), −4.0 (CH3, TBS), −4.0 (CH3, TBS), −4.2 (CH3, TBS), −4.6 (CH3, TBS), −4.6 (CH3, TBS). MS (ESI): m/z = 1761.4 $[M+Na]^{+;}$ MS (ESI): m/z = 1735.4 $[M-H^+]^-$

Compound 12. To a stirred solution of $CuCl_2$ (68 mg, 0.51 mmol, 5 eq) in dry methanol (2 ml), *n*-butylamine (300 µl, 3.0 mmol, 30 eq) was added at room temperature, and the mixture was stirred for 45 minutes. To this blue solution,

a solution of **11** (176 mg, 0.10 mmol, 1 eq) in dry methanol (2 ml) was added and the mixture was stirred for 10 minutes. The reaction mixture was then poured into a 1:1 mixture of saturated aqueous solution of NH_4Cl: ethyl acetate (35 ml) and stirred at room temperature for 15 min. The mixture was next extracted with AcOEt. The combined organic layers were washed with brine (10 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by column chromatography (elution by gradient from 90:10 DCM/cyclohexane to 90:8:2 DCM/cyclohexane/acetone) to give **12** as a brown foam (34 mg, 0.02 mmol, **20 %**). NMR ^{1}H (300 MHz, acetone-d_6): δ (ppm) -0.30 (s, 3H, CH_3, TBS), -0.29 (s, 3H, CH_3, TBS), 0.05 (s, 3H, CH_3, TBS), 0.09 (s, 3H, CH_3, TBS), 0.12 (s, 3H, CH_3, TBS), 0.18 (s, 3H, CH_3, TBS), 0.24 (s, 3H, CH_3, TBS), 0.27 (s, 3H, CH_3, TBS), 0.79 (s, 18H, *tert*-Bu, TBS), 0.84 (s, 9H, *tert*-Bu, TBS), 0.95 (s, 9H, *tert*-Bu, TBS), 3.99 (d, *J* = 13.0 Hz, 1H), 4.38 (dd, *J* = 9.9; 5.9 *Hz*, 1H), 4.99-5.37 (m, 15H), 6.56 (s, 1H, Ar galloyl), 6.63 (s, 1H, Ar galloyl), 6.68 (s. 1H, Ar galloyl), 6.96 (s, 1H, Ar galloyl), 7.26-7.66 (m, 21H, Ar), 7.79 (ddd like td, *J* = 1.1, 7.7, 7.7 Hz, 1H, Ar), 7.85 (dd, *J* = 1.3, 7.7 Hz, 1H, Ar), 8.06 (dd, *J* = 0.9, 8.0 Hz, 1H, Ar), 8.39 (bs, OH), 8.43 (bs, OH). MS (ESI): m/z = 1759.6 $[M+Na]^+$; MS (ESI): m/z = 1735.3 $[M-H^+]^-$

6.6. References

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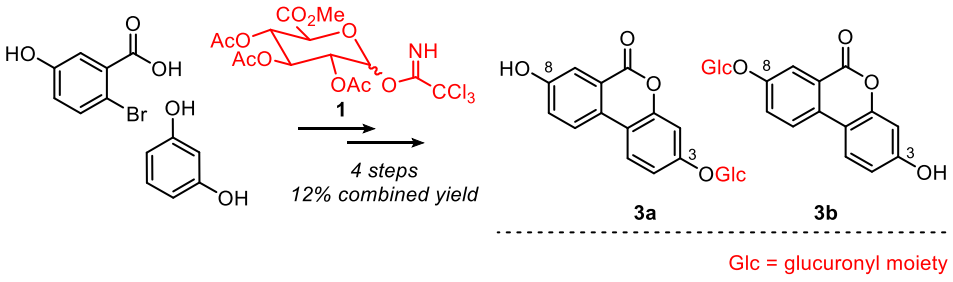
Synthesis of regioisomeric variants of mono glucuronide urolithin A

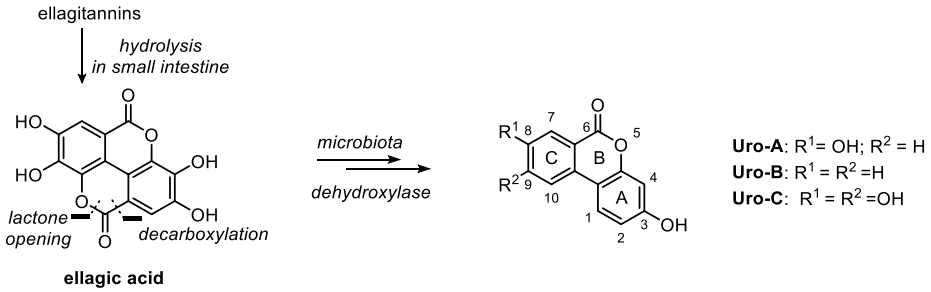
Abstract: A simple strategy to chemically synthesize 3-*O*-β-D-glucuronide and 8-*O*-β-D-glucuronide of urolithin-A starting from 2-bromo-5-hydroxybenzoic acid and resorcinol is described. With a common synthetic route encompassing four steps, we were able to obtain both the isolated regioisomers **3a** and **3b** in 12% combined yield. The key steps include the exploitation of Ullmann reaction for construction of the dibenzopyranone core of urolithin A and the conjugation of phenolic group with the activated glucuronic acid "donor" **1**, that ensures the complete β-stereoselectivity of glucuronidation.



7.1. Introduction

Ellagitannins (ET) are non-flavonoid polyphenols found in raspberries, pomegranates, oak-aged red wine, tea, and other fruits, as well as in plant-based medicines, and they have shown various interesting biological activities ranging from anti-angiogenic[1] to anti-atherogenic properties (*see* also Chapter 6).[2] These macromolecules show very low bioavaibility due to an extensive microbial metabolism in human body producing mainly polyphenolic compounds known as urolithins (Scheme 7.1), that seem to be the true responsible for the *in vivo* health effects.[3] The biological properties attributed to urolithins based on studies carried out *in vitro* are numerous and diverse: antimalarial activity,[4] anticarcinogenic activity related to topoisomerase inhibition,[5] antimicrobial activity through the inhibition of quorum sensing,[6] estrogenic modulation,[7] among others.[8]

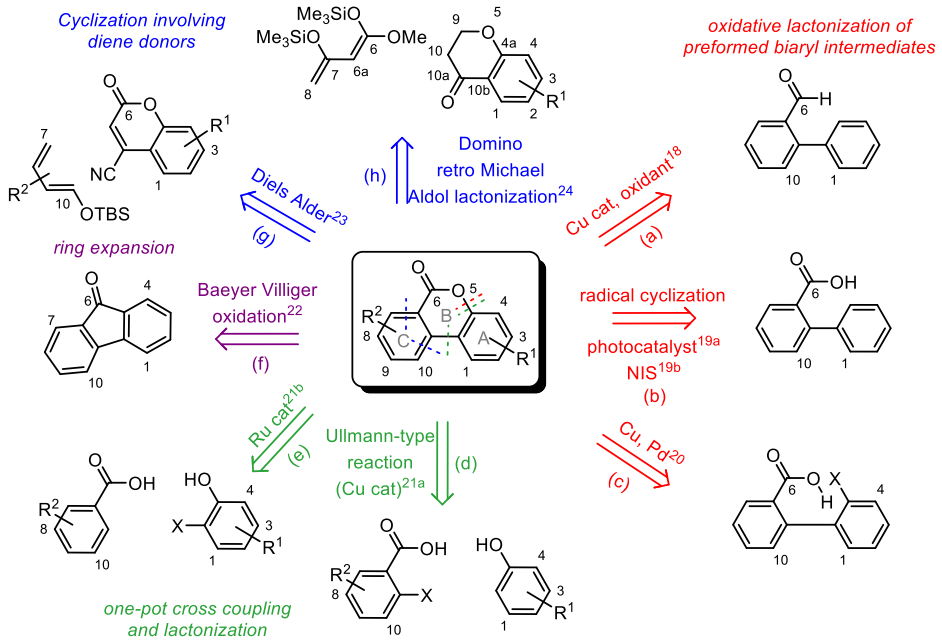
The biogenetic pathway accessing the urolithin skeleton starts from ellagic acid (previously released from ellagitannins) through the opening and decarboxylation of one lactone moiety by the action of a lactonase/decarboxylase enzyme. The sequential removal of several phenolic OH groups (dehydroxylase activities) yields the selected urolithin that is firstly absorbed mainly in the large intestine and then metabolized by Phase II enzymes in the liver affording the corresponding methylated, sulfated and glucuronidated conjugates. Finally, these conjugated urolithins reach the kidneys and are excreted via the urine.



Scheme 7.1. *Putative metabolic pathway for urolithins production in human body.*

From a structural point of view, urolithins are dibenzo[b,d]pyran-6-one derivatives, that can be considered a combination of coumarin (ring A,B) and isocoumarin (ring B,C). The different hydroxyl-substitution pattern on the phenyl rings differentiates a wide range of metabolic derivatives, among which the most produced are 3-hydroxy (**Uro-B**), 3,8-dihydroxy (**Uro-A**), 3,8,9-trihydroxy (**Uro-C**)-6*H*-dibenzo[b,d]pyran-6-one, known as urolithin A, urolithin B and urolithin C, respectively (Scheme 7.1). The main metabolites detected in human plasma after the intake of ET-containing foods are the mono-glucuronides of Uro-A, Uro-B and Uro-C, with total concentrations of these metabolites ranging from ~0.2 μM up to~18.6 μM.[9,10]

However, there are some uncertainties in relation to the dietary intake assessment methods currently used in the epidemiological studies. In fact, reliable biomarkers for the intake of dietary polyphenols are needed to get better insight into their health effects.[11] For this purpose, chemical synthesis can provide those ellagitannin metabolic standards useful to investigate their biological activity, metabolic synthetic pathways and identification of specific biomarker compounds of ellagitannins food intake.[8]



Scheme 7.2. *Synthetic strategies for the construction of the dibenzopyranone core (NIS = N-iodosuccimide).*

The dibenzopyranone core has been the focus of many synthetic strategies, being a structural motif recurring in a wide range of heterocyclic natural and bioactive compounds with bactericidal and antitumor properties such as the gilvocarcins,[12] the ravidomycins,[13] and arnottin I,[14] as well as urolithin itself. Furthermore, dibenzopyranone scaffolds have been used as intermediates for the synthesis of many pharmaceutically interesting compounds, such as progesterone,[15] androgen receptor ligands,[16] and endothelial cell proliferation inhibitors.[17]

As depicted in Scheme 7.2, the several synthetic methods developed so far can be broadly classified according to the key step(s) of the synthesis: (1) lactonization of preformed biaryl intermediates such as 2-aryl-benzaldehyde (path a),[18] 2-aryl-benzoic acid (path b)[19] and 2-halobiarylcarboxylic acids (path c);[20] (2) simultaneous biaryl coupling and lactonization using carboxylic acid and

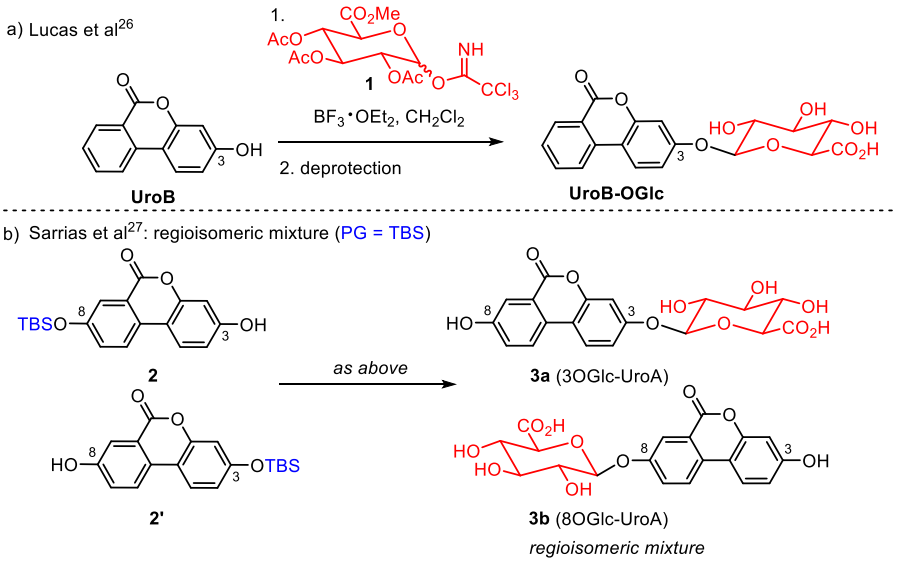
phenolic matrices (paths d and e);[21] (3) rearrangement of polycyclic compounds, that include the Baeyer Villiger oxidation of fluorenone (path f);[22] (4) cyclization to form the C ring (or B and C rings), with or without subsequent aromatization. Two examples of this latter approach include the Diels-Alder cycloaddition of 4-cyanocoumarins with 1-silyloxydienes (path g)[23] and domino *retro*-Michael–aldol–lactonization reactions of 1,3-bis-(trimethylsilyloxy)-1,3-butadienes with activated 2,3-dihydropyrans (path h).[24]

Another area of research that attracted the attention of organic chemists is the development of chemo-selective glucuronidation procedures, since this kind of glycosidic moiety is involved in the metabolism of many drugs and endogenous compounds and can confer additional biological properties to a substrate. In glucuronidation reactions, electrophilic glucuronic acid-equivalents (e.g. trichloroacetimidates, *vide infra*) are attached to the phenol group of an aglycone at the anomeric carbon atom forming *O*-glycosidic bonds. Although sharing all common aspects with general glycosidations, the synthesis of glucuronidated compounds is particularly challenging, since it requires higher activation for a given aglycone, due to the presence of the C-5 carboxylic group in the glucuronide reactant, that decreases the reactivity of the anomeric position.[25] Enzymatic synthesis has been developed, however this requires the use of either expensive glucuronyl transferases or liver microsomes and tedious purification procedures of the crude mixtures. In this context, chemical synthesis represents a viable option, and several strategies have been developed to fulfill a feasible, efficient and chemo-selective conjugation of different polyphenol-related molecules.[25] Concerning the use of different kinds of glucuronic acid "donors", some aspects have to be considered: *i*. the stability of the glucuronic acid; *ii*. the use of suitable OH-protecting groups on the sugar core whose installation/removal procedures must be compatible with the integrity of aglycone functionalities and stereochemistry; and *iii*. the type of the

C-2 appendage on the sugar, that dictates the α,β anomeric diastereoselection of the conjugation reaction (anomeric effect).[25]

In 2009 Lucas et al.[26] reported the diastereoselective synthesis of urolithin B β-glucuronide using the trichloroacetimidate (TCA) form of methyl 2,3,4-tri-*O*-acetyl-α-D-glucuronate **1** (Scheme 7.3, eq. a). The reaction, carried out in CH_2Cl_2, was promoted by $BF_3{\cdot}OEt_2$ (0.25 equivalents) yielding the desired product in 93% yield and complete β diastereoselectivity.

In 2013 González-Sarrías et al.[27] obtained in a similar way an inseparable regioisomeric mixture of 3- and 8-monoglucuronidated urolithin A **3a** and **3b**, starting from mono-silylated mixture of precursors **2a** and **2b** (Scheme 7.3, eq. b). In addition, although in the case of urolithin A there are two possible non-equivalent phenolic OH in 3 and 8 that may be conjugated, it has been suggested that the former would be the preferred conjugating position in the human metabolic pathway, but supporting evidences about this are still lacking.[28] In order to investigate the real Phase II metabolic pathway of urolithin A and to unveil the potential biological activity of the corresponding metabolites, we designed a practical and versatile synthetic plan aimed at the chemo- and diastereoselective synthesis of isolated urolithin A monoglucuronides **3a** and **3b**, in useful quantities to be tested (*see next section*).

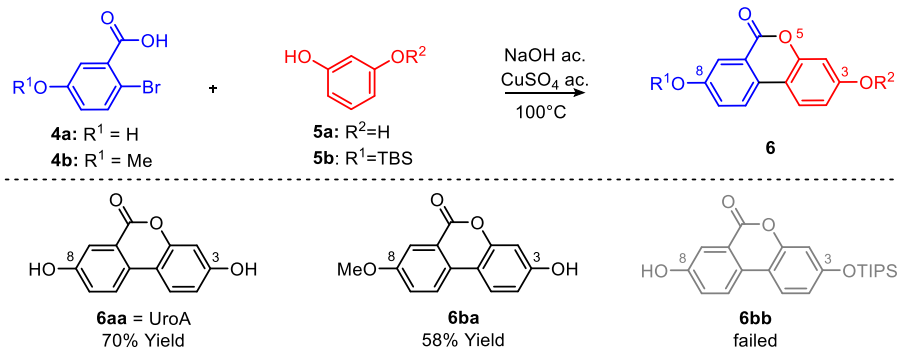


Scheme 7.3*. Previous reports of glucuronidation of urolithins.*

7.2. Results and discussion

For the construction of the chromen-6-one skeleton **6** (Scheme 7.4) we chose to exploit the well-known copper-catalyzed Ullman reaction between 2-bromo-5-hydroxy-benzoic acids of type **4** and resorcinols of type **5**,[21a] because it provides the desired product in only one step with the substitution pattern of urolithin A starting from readily available starting materials. We performed a preliminary screening of reactants using both free- and suitably protected starting materials **4** and **5**. The screened protecting groups are reported in Scheme 7.4 together with the corresponding outcomes. The aim was to explore several options to test the efficacy of the reaction alternating the protection on benzoic acid **4** or on resorcinol **5**: in this way we looked forward to obtain a useful mono-protected intermediate **6** suitable for the subsequent regioselective glucuronidation of the substrate. The direct reaction between unprotected 2-bromo-5-hydroxy-benzoic acid **4a** and resorcinol **5a** produced

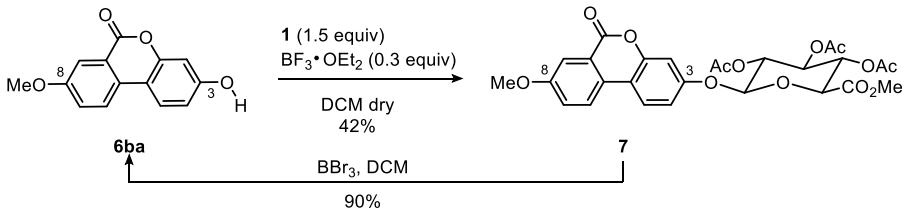
urolithin A **6aa** with a good 70% yield. The yield of the Ullmann coupling resulted viable although less efficient (58 %) when the commercial available 2-bromo-5-methoxy-benzoic acid **4b** was used. The protection of one phenolic group of resorcinol with, for example, the triisopropilsilyl group, proved convenient in terms of easiness of protection/deprotection procedures but resulted detrimental in terms of reactivity of the substrates and efficiency of the overall reaction (probably due by the steric bulk of the protecting group) giving total recovery of the starting materials. Of note, at this point of the work, we had to rule out the use of the acetate as protecting group of choice of the phenolic OH of reactants, for its incompatibility with the reaction conditions of the coupling.



***Scheme 7.4**. Ulmann reaction between differently protected starting materials **4** and **5**.*

After the brief investigation described so far we decided to proceed with the coupling of mono-methylated urolithin A **6ba** with the polyacetylated glucuronic acid trichloroacetimidate donor **1** using boron trifluoride diethyl etherate as promoter; we obtained the β-glucuronide **7** with an acceptable yield of 42% as a single isomer (Scheme 7.5). The Intermediate **7** was then treated with boron tribromide (BBr_3) in dichloromethane, but instead of observing the desired demethylation at the C8, unconjugated urolithin A **6ba**

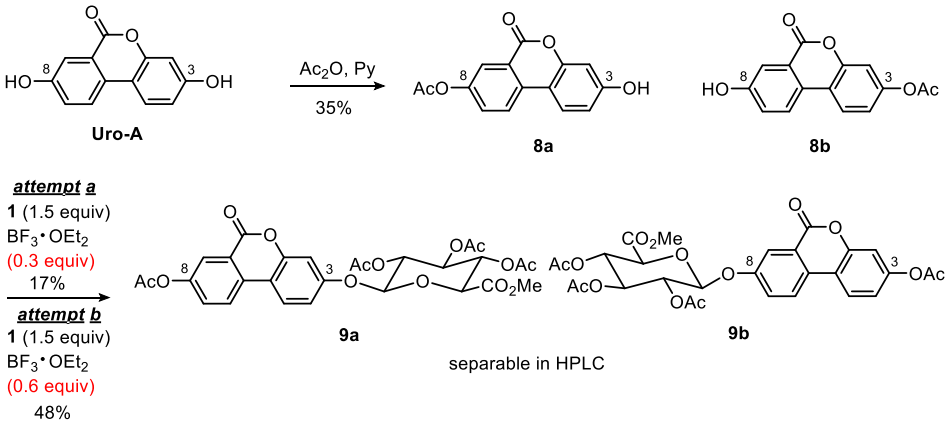
was recovered almost quantitatively with a yield of 90% proving that the cleavage of the methoxy group was incompatible with the glucuronic moiety of the substrate.



Scheme 7.5. *Glucuronidation of compound* ***6ba*** *and treatment of conjugated product* ***7*** *with* BBr_3.

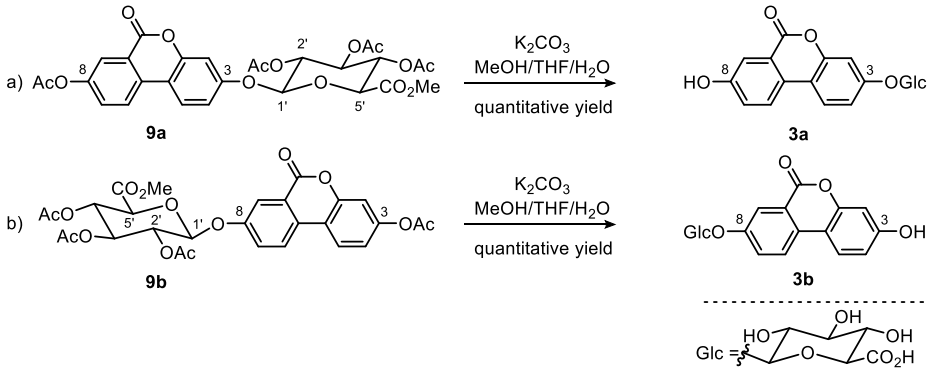
At this point we tried to perform the coupling under the same reaction conditions directly on the unprotected urolithin A **6aa** (not shown), exploiting the different reactivity of the two phenolic groups in 3 and 8, but the reaction failed probably due to the low solubility of urolithin A in the organic solvents of choice (DMF or DCM).

We thus revalued our initial synthetic strategy based on the functionalization of monoprotected urolithin A. Considering the mono-acetyl urolithin A, the potentially most advantageous substrate to be glucuronidated (since it contains the same acetyl group as in the glucuronic acid reagent **1,** additional deprotection steps in the achievement of the final target would be avoided), we decided to monoacetylate urolithin A directly rather than trying to obtain it by the Ulmann protocol using the corresponding protected starting material **4** and **5**.



Sheme 7.6. *Acetylation of urolithin A and glucuronidation of the mixture of mono-protected adducts* ***8****.*

The direct acetylation of urolithin A with acetic anhydride in pyridine afforded a 58:42 regioisomeric mixture of monoacetylated **8a** and **8b** with a 35% combined yield. The low yield of this step is due to the formation of the undesired diacetylated product with a remarkable (but undesired) 70% yield. Regrettably, the following coupling of monoacetylated compounds **8** with **1** resulted difficult, affording the related mixture of glucuronide products **9** with a scarce 17% combined yield, probably due to a competitive detrimental coordination of the boron trifluoride between vicinal acetyl groups in the substrate **8**. To overcome this competition, we performed the conjugation of **8** with **1** using higher activator loading (from 30 mol% to 60 mol%) and this attempt nicely increased the efficiency of the transformation to 48% yield with complete β-diastereoselctivity (Scheme 7.6, attempt b).



Scheme 7.7. *Synthesis of final targets* ***3a*** *and* ***3b*** *from isolated precursor* ***9a*** *and* ***9b****.*

Finally, each regioisomer **9a** and **9b** was separated through HPLC and then fully deprotected under mild conditions in the presence of potassium carbonate in a MeOH/THF/H_2O solvent mixture obtaining the isolated **3a** and **3b** in quantitative yield (Scheme 7.7).

7.3. Preliminary structural determination

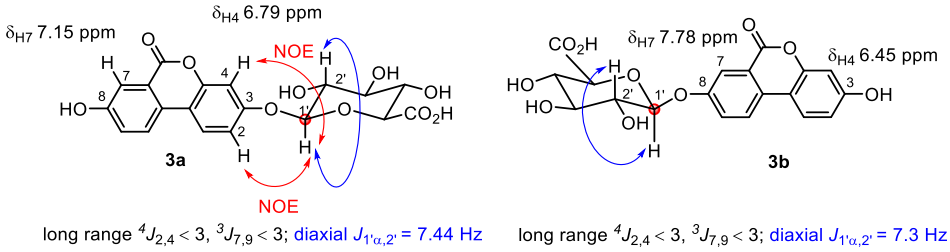


Figure 7.1. *Diagnostic NOE contacts and key inter-proton experimental coupling constants (J, Hz) for the structural elucidation of final targets* ***3a*** *and* ***3b*** *(*^{1}*H NMR, 400 MHz,* D_2O*).*

The final targets **3a** and **3b** were purified by reverse phase HPLC and in both cases the chromatogram showed the presence of two major chromatogram peaks, having the same MS and both ascribable to β anomers of the final targets (*vide infra*). Preliminary confirmation of the stereostructure of

compounds **3** as well as their conformational behavior in solution was made on the basis of 1D ^{1}H NMR and 2D experiments (D_2O).

The confirmation of the position of the glucuronic moiety for compound **3a** was proven through diagnostic NOE contacts (selective 1D NOE experiments) between H1' of the sugar and H4 and H2 protons of the aromatic moiety (Figure 7.1). The assignment of H4, in turn, was based on observation of inter-proton coupling constant (long range $^{4}J_{4,5} < 3$ Hz) and the shielded chemical shift (δ = 6.79 ppm), which were compared to the values of H7 in *ortho* position to the carbonyl function (δ = 7.15 ppm, long range $^{4}J_{7,9} < 3$) and its hetero-correlation with the carbon resonance at 104.4 ppm in the ^{13}C NMR (typical chemical shift of the C4 of resorcinol moiety).

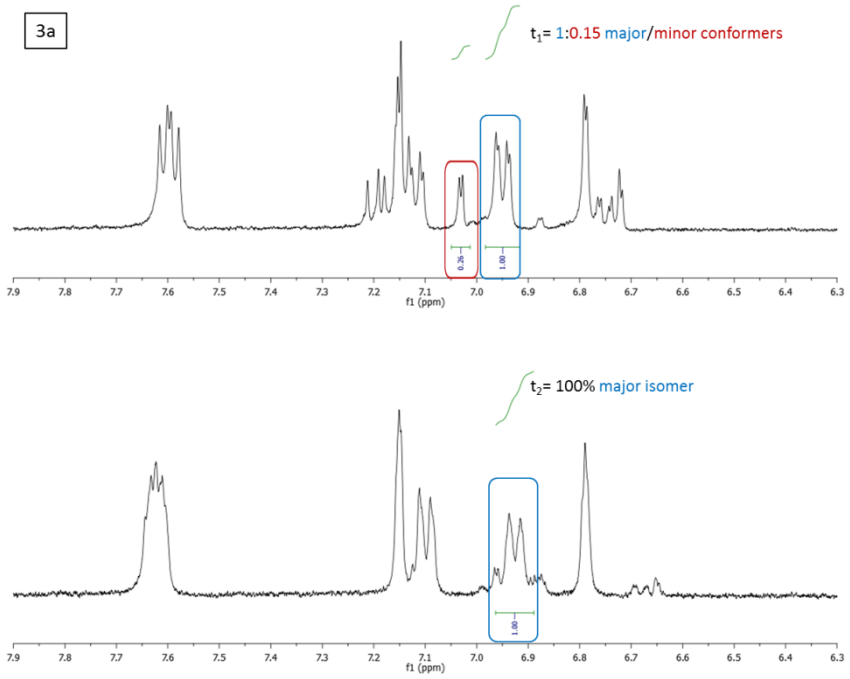


***Figure 7.2**. Time-dependent equilibrium between two conformational species of compound **3a**.*

For each regioisomer, the NMR spectra of each isolated chromatogram peak showed the coexistence of two species in equilibrium that we attributed to different conformers for several reasons. *First of all,* each conformer possesses a large vicinal H1'-H2' coupling ($J_{1',2'}$ > 7 Hz) of the glucuronide moiety, which is indicative of an axial orientation of both H1' and H2' protons in pyranose sugar, thus confirming the existence of monoglucuronide urolithin A exclusively as β-anomer. We reasoned that a role of hydrogen-bond network between different alcoholic, carboxylic and phenolic groups could be invoked for the stabilization of the two conformers, accounting for the observed behaviour. Furthermore, repeated NMR experiments taken at different time (ca. every 12 h) in D_2O (15 mM) at 25 °C for compound **3a** (Figure 7.2) and **3b** (Figure 7.3), showed a variation of the ratio between the two conformational species, demonstrating a dynamic equilibrium between the two species hardly ascribable to α to β anomeric switch or, worse, to 3 to 8 glucuronide scrambling (the latter also ruled out by the comparison of NMR spectra of **3a** with **3b**) (Figures 7.2 and 7.3). Further studies are on course to better clarify without doubt the structural nature and the spectroscopic behaviour of these intriguing target molecules

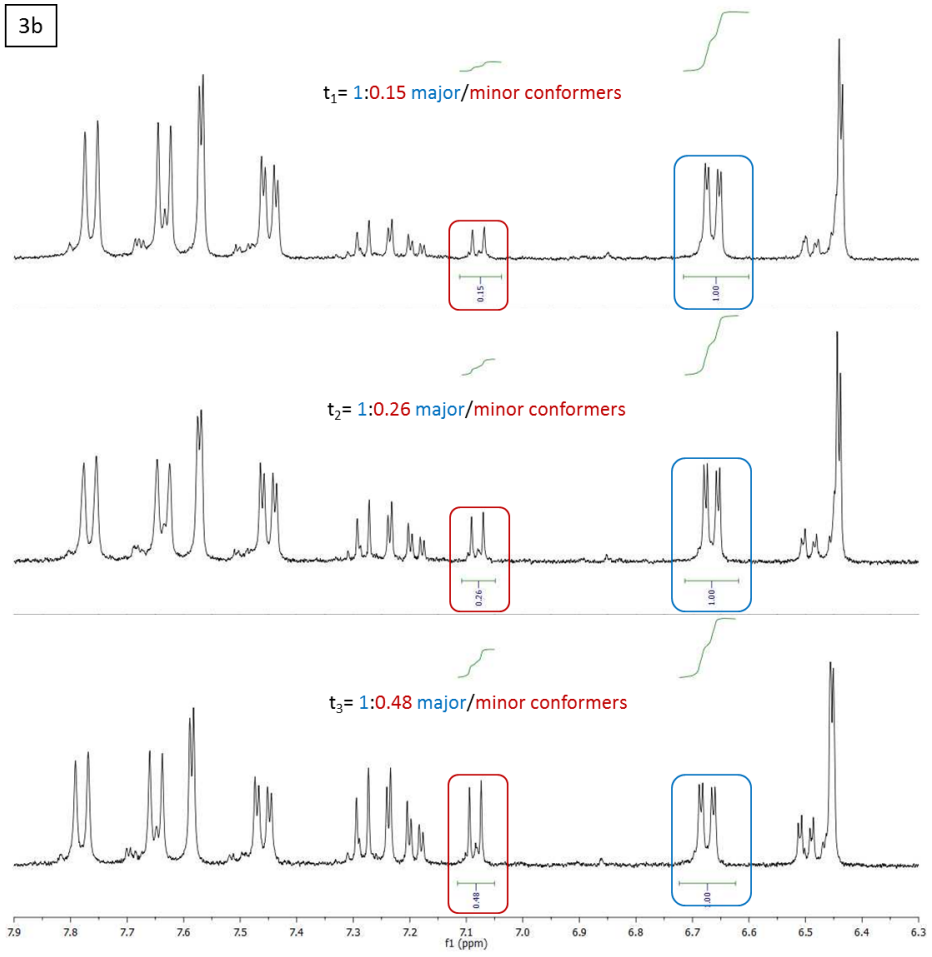


***Figure 7.3**. Time-dependent equilibrium between two conformational species of compound **3b**.*

7.4. Conclusion and perspectives

In conclusion, we accomplished a simple and versatile synthetic plan, getting access for the first time to the isolated regioisomeric monoglucuronide forms of urolithin A **3a** and **3b** with a final, still optimizable 12% yield and with almost complete β-diastereoselectivity. Moreover, a peculiar and unprecedented observed conformational behavior was ascertained and analyzed through NMR experiments.

The synthesized metabolites are of great interest, since their very low biological availability has precluded their use as analytical/metabolomics standards or bio/pharmacological candidates. In fact, these phenolic glucuronides will help to elucidate their actual contribution to the reported biological functions *in vivo* and will also find use in the more accurate determination of the metabolic and pharmacokinetic profiles of urolithins.

7.5. Experimental section

Unless otherwise noted, all reactions were performed in oven-dried or flame-dried glassware under an atmosphere of nitrogen or argon. Air-sensitive reagents and solutions were transferred via syringe or cannula and were introduced to the apparatus through rubber septa. Dichloromethane (HPLC grade) was dried by distillation on CaH_2 according to standard procedures. THF dry was distilled on Na/Benzophenone. Solvents for chromatography and filtration including hexane, ethyl acetate, dichloromethane, petroleum ether, anhydrous ethanol, methanol, dimethylformamide, toluene and 2-propanol were ACS or HPLC grade and used as received. Ammonia-methanol mixture was prepared by bubbling liquid ammonia in methanol at 0 °C for 30 min.

Analytical thin layer chromatography (TLC) was performed on silica gel 60 F254 pre-coated plates with visualization under short-wavelength UV light and by dipping the plates with molybdate reagent (aqueous H_2SO_4 solution of ceric sulphate/ammonium molybdate) followed by heating. Flash column chromatography was performed using 40-63 µm silica gel using the indicated solvent mixtures.

HPLC analyses were carried out using LiChroCART® 250-4 LiChrospher® 100 CN (5µm, 4.6x25) for analytical analysis; Luna CN 100A (10mm, 250x10), Superlco C18 column 250 × 10 mm, 10 µm for semi-preparative HPLC separations. Rotation data were obtained on a digital polarimeter at ambient temperature using a 100 mm cell with a 1 mL capacity and are given in units of 10^{-1} deg cm^2 g^{-1}.

NMR spectra were recorded at 300 MHz or 400 MHz (^1H) and 75 MHz or 100 MHz (^{13}C). Spectra were referenced to tetramethylsilane (0.0 ppm, ^1H; 0.0 ppm, ^{13}C, in $CDCl_3$). Chemical shifts (δ) are reported in parts per million (ppm), and multiplicities are indicated as s (singlet), d (doublet), t (triplet), q (quartet), sext

(sextet), sept (septet), dd (double doublet), m (multiplet), and b (broad). Coupling constants, *J*, are reported in Hertz. ^{1}H and ^{13}C NMR assignments are corroborated by 1D and 2D experiments (gCOSY, gHSQC, DEPT, and NOESY sequences).

ESI-mass spectra were recorded on API 150EX apparatus and are reported in the form of (*m/z*).

3-*O*-acetyl-8-hydroxy-(6*H*-(dibenzo[b,d]pyran-6-one) and 8-*O*-acetyl-3-hydroxy-(6*H*-(dibenzo[b,d]pyran-6-one) (**8a, 8b**) To a solution of urolithin A (160 mg, 0.7011 mmol, 1 equiv) in pyridine (7 mL), acetic anhydride (22.5 µL, 0.2208 mmol, 1.2 equiv) was added. The reaction mixture was stirred at room temperature for 30 hours, and then the solvent was removed under vacuum. Flash chromatographic purification (elution by gradient: from 91/4/4 to 80/10/10 Toluene/Acetone/MeOH) afforded product **8** (66.3 mg, 35% combined yield) as an inseparable 1:0.73 regioisomeric mixture **8a/8b.** ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99 (d, *J* = 2.3 Hz, H7a), 7.98 (d, *J* = 9.0 Hz, 1H, H10a), 7.95 (d, *J* = 8.6 Hz, 0.75H, H10b), 7.93 (d, *J* = 8.7 Hz, 0.80H, H1a), 7.83 (d, *J* = 8.8 Hz, 1H, H1b), 7.65 (d, *J* = 2.6, 1H, H7b), 7.50 (dd, *J* = 8.7, 2.6 Hz, 1H, H9a), 7.32 (dd, *J* = 8.7, 2.6 Hz, 1H, H9b), 7.10 (d, *J* = 2.3 Hz, 1H, H4b), 7.06 (dd, *J* = 8.7, 2.3 Hz, 1H, H2b), 6.82 (dd, *J* = 8.7, 2.4 Hz, 1H, H2a), 6.78 (dd, *J* = 2.4 Hz, H4a). TLC: R_f = 0.36 (90/5/5 Toluene/Acetone/MeOH).

3-*O*-acetyl-8-*O*-(methyl-2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-(6*H*-(dibenzo[b,d]pyran-6-one) (9a) and **8-*O*-(methyl-2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-3-hydroxy-(6*H*-(dibenzo[b,d]pyran-6-one) (9b)** A solution of the mono-acetylated isomeric mixture urolithin A **8** (50.0 mg, 0.18 mmol, 1.0 equiv) in CH_2Cl_2 (5 mL) was added dropwise to a solution of α-D-Glucuronide Trichloroacetimidate **1** (125.0 mg, 0.261 mmol, 1.5 equiv) in CH_2Cl_2 (3 mL). The mixture was cooled to 0 °C, and $BF_3{\cdot}OEt_2$ (14 µL, 0.11 mmol, 0.6 equiv) was added. After 4 h, Et_3N was added and the solution was concentrated under vacuum. The residue was purified by flash chromatography on silica gel (elution by gradient from 90:5:5 to 80:10:10 Toluene/Acetone/MeOH) to afford related protected glucuronide **9** (42.1 mg, 40%) as amorphous white solid. Little amounts of glucuronides **9a** (10.0 mg) and **9b** (10.3 mg) were purified by

semipreparative HPLC (CN-10µM, 250 x 10 mm, hexane/anhydrous EtOH 75:25, flow rate 3.5 mL/min, detection at 254 nm, Rt **9a** =27.84, Rt **9b** =24.44.

Data for **9a**: TLC: R_f = 0.40 (90/5/5 Toluene/Acetone/MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (d, *J* = 2.3 Hz, 1H, H7), 8.02 (d, *J* = 8.7 Hz, 1H, H10), 7.91 (d, *J* = 9.5 Hz, 1H, H1), 7.55 (dd, *J* = 8.7, 2.4 Hz, 1H, H9), 7.01 (m, 2H, H2, H4), 5.30-5.39 (m, 3H, H2', H3', H4'), 5.25 (d, *J* = 7.2 Hz, 1H, H1'), 4.26 (d, *J* = 8.9, 1H, H5'), 3.74 (s, 3H, CO_2CH_3 Glc), 2.36 (s, 3H, C8CO_2CH_3), 2.10 (s, 3H, OAc Glc), 2.06 (s, 6H, OAc Glc). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2 (Cq, C=O), 169.6 (Cq, C=O), 169.4 (Cq, C=O), 169.2 (Cq, C=O), 166.9 (Cq, C=O), 160.5 (Cq, C=O), 158.2 (Cq, Ar), 152.1 (Cq, Ar), 150.6 (Cq, Ar), 132.5 (Cq, Ar), 129.4 (CH, C9), 124.2 (CH, C1), 123.2 (CH, Ar), 123.1 (CH, Ar), 121.7 (Cq, Ar), 114.6 (CH, C2), 113.2 (Cq, Ar), 105.3 (CH, C4), 98.7 (CH, C1'), 72.9 (CH, C5'), 71.9 (CH, Glc), 71.1 (CH, Glc), 69.1 (CH, Glc), 53.3 (CH_3, CO_2Me), 21.2 (CH_3, C8OAc), 20.8 (2C, CH_3, OAc Glc), 20.7 (CH_3, OAc Glc).MS (ESI, 50eV): Calcd.: [M−H$^+$]: 586. Found: [M−H$^+$]: 287.3; Opt. Rot. $[\alpha]_D^{20}$ −28.2 (*c* 0.51 g/100mL, $CHCl_3$)

Data for **9b**: TLC: R_f = 0.40 (90/5/5 Toluene/Acetone/MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02 (d, *J* = 8.8 Hz, 1H, H10), 7.98 (d, *J* = 8.8 Hz, 1H, H1), 7.94 (d, *J* = 2.7 Hz, 1H, H7), 7.49 (dd, *J* = 8.8, 2.7 Hz, 1H, H9), 7.16 (d, *J* = 2.2, 1H, H4), 7.11 (dd, J = 8.8, 2.2 Hz, 1H, H2) 5.32-5.39 (m, 4H, H1', H2', H3', H4'), 4.29 (d, *J* = 8.9, 1H, H5'), 3.73 (s, 3H, $CO_2\underline{CH_3}$ Glc), 2.35 (s, 3H, C3$CO_2\underline{CH_3}$), 2.09 (s, 3H, OAc Glc), 2.07 (s, 6H, OAc Glc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2 (Cq, C=O), 169.6 (Cq, C=O), 169.4 (Cq, C=O), 169.1 (Cq, C=O), 166.9 (Cq, C=O), 160.6 (Cq, C=O), 156.8 (Cq, Ar), 151.8 (Cq, Ar), 151.3 (Cq, Ar), 130.1 (Cq, Ar), 125.9 (CH, C9), 123.9 (CH, Ar), 123.5 (CH, Ar), 122.2 (Cq, Ar), 118.7 (Cq, C2), 116.1 (CH, C7), 115.7 (Cq, Ar), 111.3 (CH, C4), 98.6 (CH, C1'), 72.8 (CH, C5'), 71.9 (CH, Glc), 71.2 (CH, Glc), 69.1 (CH, Glc), 53.2 (CH_3, $CO_2\underline{Me}$), 21.3 (CH_3, C3OAc), 20.8 (2C, CH_3, OAc Glc), 20.7 (CH_3, OAc Glc). Opt. Rot. $[\alpha]_D^{20}$ −25.4 (*c* 0.50 g/100mL, $CHCl_3$)

3-*O*-(β-D-glucopyranosyluronic acid)-8-hydroxy-(6*H*-(dibenzo[b,d]pyran-6-one) (3O-Glc-UroA). The glucuronidated precursor **9a** (10 mg, 0.017 mmol) and K_2CO_3 (5.9 mg, 0.043 mmol) were dissolved in MeOH/ THF/H_2O 5:2:1(1.6 mL). The reaction mixture was stirred at room temperature for 18 h and then the solvent was removed under vacuo. The crude was purified by RP-C18 (H_2O:CH_3CN, from 90:10 to 40:60). Fractions containing the desired product were concentrated affording 3-OGlc-UroA **3a** as a conformational equilibrium between two species in a 1:0.26 ratio (6.8 mg, quantitative yield) as a yellow amorphous solid. ^{1}H NMR (400 MHz, D_2O): δ 7.60 (d, *J* = 8.7 Hz, 1H, Ar major conformer), 7.59 (d, *J* = 8.7 Hz, 1H, Ar major conformer), 7.18 (d, *J* = 8.4 Hz, 0.25H, Ar minor conformer) 7.17 (d, *J* = 8.8 Hz, 0.25H, Ar minor conformer), 7.15 (d, *J* = 2,5 Hz, 1,3H, Ar major conformer and minor conformers), 7.12 (dd, *J* = 8.6, 2.6 Hz, 1H, Ar major conformer), 7.03 (d, *J* = 2.5 Hz, 0.25H, minor conformer), 6.95 (dd, *J* = 8.6, 2.2 Hz, 1.25 H, Ar major conformer and minor conformers), 6.79 (d, *J* = 2.2 Hz, 1H, Ar major conformer), 6.75 (dd, *J* = 8.7, 2.5 Hz, Ar minor conformer), 5.18 (d, *J* = 7.2 Hz, 1H, H1' major conformer), 5.14 (d, *J* = 7.3 Hz, 1H, H1' minor conformer) 4.00 (d, J = 9.6 Hz, H5’ major conformer), 3.93 (d, J = 8.9 Hz, H5’ minor conformer) 3.62-3.76 (m, 4H, H2', H3', H4' major and minor conformer). ^{13}C NMR (100 MHz, $CDCl_3$) of major conformer: δ 181.7 (Cq, C=O), 175.6 (Cq, C=O), 163.4 (Cq, Ar), 163.0 (Cq, Ar), 162.7 (Cq, Ar), 132.5 (Cq, Ar), 131.7 (CH, Ar), 123.4 (CH, Ar), 123.0 (CH, Ar), 120.9 (Cq, Ar), 119.9 (Cq, Ar), 115.0 (CH, Ar), 113.9 (CH, Ar), 104.4 (CH, Ar), 100.1 (CH, C1'), 76.5 (CH, C5'), 75.5 (CH, Glc), 73.0 (CH, Glc), 72.0 (CH, Glc).

8-*O*-(β-D-glucopyranosyluronic acid)-3-hydroxy-(6*H*-(dibenzo[b,d]pyran-6-one) (8O-Glc-UroA). The glucuronidated precursor **9b** (10.3 mg, 0.017 mmol) and K_2CO_3 (6 mg, 0.043 mmol) were dissolved in MeOH/ THF/H_2O 5:2:1(1.6 mL). The reaction mixture was stirred at room temperature for 18 h and then the

solvent was removed under vacuo. The crude was purified by RP-C18 (H_2O:CH_3CN, from 90:10 to 40:60). Fractions containing the desired product were concentrated affording 8-OGlc-UroA **3b** as a conformational equilibrium between two species in a 1:0.18 ratio (6.8 mg, quantitative yield) as a yellow amorphous solid. ^{1}H NMR (400 MHz, D_2O): δ 7.78 (d, *J* = 9.1 Hz, 1H, Ar major conformer), 7.65 (d, *J* = 8.9 Hz, 1H, Ar major conformer), 7.58 (d, *J* = 2.6 Hz, 1H, Ar major conformer) 7.45 (dd, *J* = 8.9, 2.7 Hz, 1H, Ar major conformer), 7.28 (d, *J* = 8,4 Hz, 0.18H, Ar minor conformer), 7.23 (d, *J* = 2.6 Hz, 0.18H, Ar minor conformer), 7.19 (dd, *J* = 8.4, 2.7 Hz, 0.18H, minor conformer), 7.08 (d, *J* = 8.5 Hz, 0.18 H, Ar minor conformer), 6.49 (dd, *J* = 8.4, 2.3 Hz, 1H, Ar minor conformer), 6.45 (d, *J* = 2.2 Hz, 1.2H, Ar major and minor conformer), 5.21 (d, *J* = 7.3 Hz, 1H, H1' major conformer), 5.19 (d, *J* = 8.5 Hz, 1H, H1' minor conformer) 4.01 (d, *J* = 9.4 Hz, H5’ major conformer), 3.94 (d, *J* = 9.3 Hz, H5’ minor conformer) 3.60-3.79 (m, 3.7 H, H2', H3', H4' major and minor conformer). ^{13}C NMR (100 MHz, $CDCl_3$) of major conformer: δ 181.7 (Cq, C=O), 175.6 (Cq, C=O), 164.2 (Cq, Ar), 163.9 (Cq, Ar), 161.0 (Cq, Ar), 131.1 (Cq, Ar), 125.6 (CH, Ar), 123.8 (CH, Ar), 123.1 (CH, Ar), 118.6 (Cq, Ar), 116.6 (Cq, Ar), 115.6 (CH, Ar), 114.9 (CH, Ar), 104.4 (CH, Ar), 100.2 (CH, C1'), 76.5 (CH, C5'), 75.5 (CH, Glc), 73.0 (CH, Glc), 72.0 (CH, Glc).

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FINAL REMARKS

This PhD work focused on the application of modern strategies and methodologies to the synthesis of relevant polyphenolic compounds, with the final goal of better understanding the transformations operated by Nature in the complex world of polyphenols.

The aim of Chapter 1 was to outline an overall vision of the complex world of polyphenols concerning the structural diversity, the chemical classification and their fate in the human body. Among the several molecules produced by the interaction of dietary polyphenols with the intestinal microbiota, chiral γ-valerolactones and urolithins emerge as the two main scaffolds derived from flavan-3-ols and ellagitannins, respectively. In vitro studies gave preliminary interesting clues about their bioactivity, but unavailability of useful quantities of these metabolites set limits for their rigorous investigation. The chemical synthesis offers the possibility to overcome these limits, by providing appreciable amounts of pure products.

Chapter 2 is a brief guide for the reader introducing the vinylogous principle and its application in the catalytic enantioselective Mukaiyama aldol reaction with heterocyclic donor systems; it represents one attractive methodology for the selective construction of useful building blocks.

The worlds of polyphenol metabolites and organic synthesis methodology meet each other in Chapter 3, where the asymmetric vinylogous Mukaiyama aldol reaction was applied in an innovative synthesis of enantioenriched γ-valerolactone scaffolds as aglyconic forms. Furthermore, during this itinerary, we accidentally discovered and developed a concise route for the synthesis of

racemic hydroxyphenyl δ-valerolactones, the unnatural expanded isomeric counterparts of γ-lactones.

Chapter 4 describes the ongoing efforts to obtain a wide panel of conjugated variants of γ-valerolatone metabolites through synthesis, by applying the modern sulfation and glucuronidation methods to aglyconic, suitable protected precursors. This part completes the project for the deep investigation of this specific class of metabolites, certifying their relevance in food science.

The biological results concerning the inhibition of uropathogenic *Escherichia coli* UPEC adherence to bladder epithelial cells exerted by some of the synthesized γ-valerolactone sulfates were reported in Chapter 5, suggesting that a real health benefit could be mediated by this type of molecules.

The research project at Institut Européen de Chimie et Biologie (IECB) of Bordeaux was reported in Chapter 6, where the other big class of polyphenols was considered: the ellagitannins. Herein the advancements toward the stereo- and regioselective total synthesis of liquidambin and vescalagin, two open-chain ellagitannins, were disclosed. Indeed, these synthetic targets provide an attractive field of action to the well-studied biaryl coupling methodology with phenol substrates.

The biaryl coupling and glucuronidation reactions found a further applicability in the regio- and diastereoselective synthesis of mono-glucuronide variants of urolithin A, detailed in Chapter 7.

In conclusion, this research program, conducted in equilibrium between the two fields of nutrition and organic chemistry, emphasizes how methodological investigations for the selective obtainment of relevant molecular targets may nicely flank and complement food science-centered metabolomic studies.

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