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DOTTORATO IN SCIENZE CHIMICHE E FARMACEUTICHE

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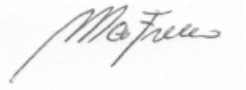

SYNTHETICAL STUDIES ON ARACHIDONIC ACID METABOLITES FOR DIAGNOSTIC PURPOSES

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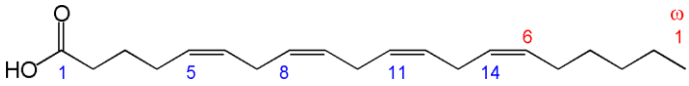
CONTENTS

INTRODUCTION

ARACHIDONIC ACID

Arachidonic acid ($\Delta^{5,8,11,14}$-eicosatetraenoic acid, AA) is a carboxylic acid with 20-carbon chain and four unsaturations, all with the Z-configuration (figure 1). It belongs to the ω-6 unsaturated fatty acids class, because the first double bond is located on the carbon 6 from the methyl terminus (ω end)[1].



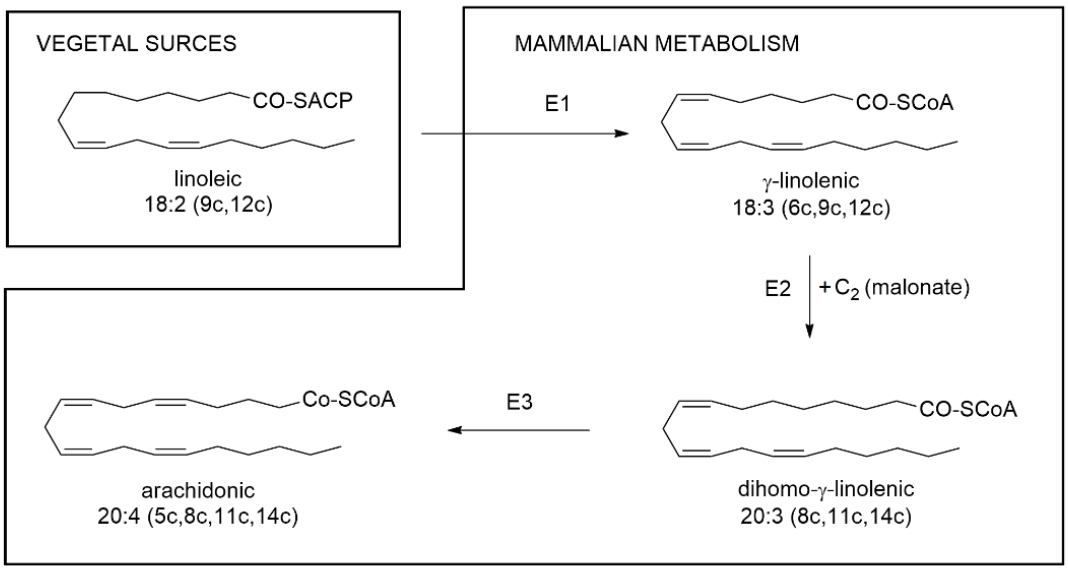
Arachidonic Acid

FIGURE 1

Arachidonic acid and esterified arachidonate are ubiquitous components of every mammalian cell. This twenty carbon fatty acid with four double bonds was first isolated and identified from mammalian tissues in 1907 by Percival Hartley. The name arachidonic acid was suggested in 1913 by J. Lewkowitsch because of its relationship with its saturated analogue, the arachidic acid (20:0). The position of the four double bonds was clear only in the 1940 thanks to the work of Smedley-Maclean, but to have experimental evidences of the *cis*-configuration of the double bonds must wait the 50's with the advent of the infrared spectroscopy. [2]

Arachidonic acid is one of the most abundant polyunsaturated fatty acids in mammalian cells. It is predominantly present esterified in glycerophospholipids in biological membranes and thanks to its double bonds helps to maintain the fluidity of the cell wall. It is not considered an essential fatty acid because mammalian organisms are able to synthesize AA from linoleic acid after enzymatic desaturation and elongation of the carbon chain (*figure 2*).

[1] Paul M Dewick; *Medicinal Natural Product a Biosynthetic Approach*, **2008**, 3rd edition
[2] Sarah A. Martin, Alan R. Brash, Robert C. Murphy, *J Lipid Res.*, **2016**, 57(7), 1126-1132



E1: Δ^6-desaturase; E2: C_{18} elongase; E3: Δ^5-desaturase

FIGURE 2

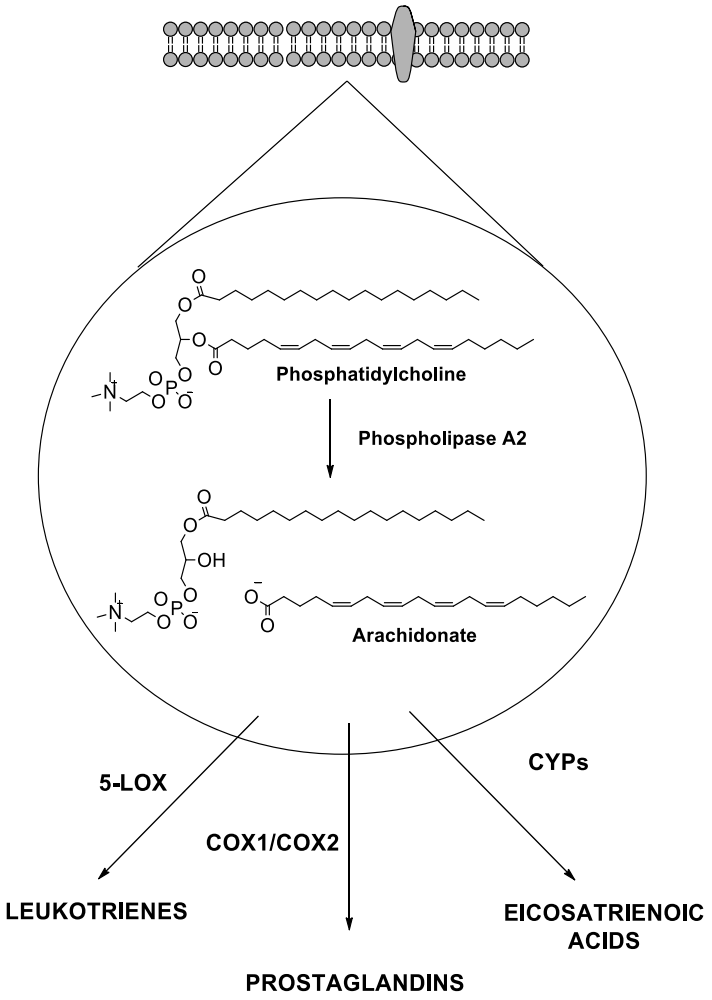


FIGURE 3

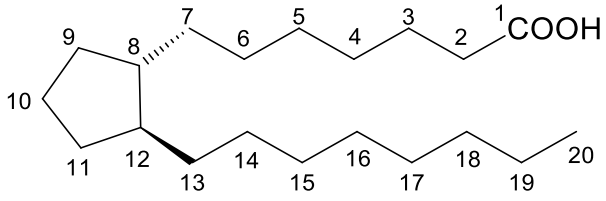
In addition, when it is present in its free form, it could be involved in the cellular signaling. In fact, the lipase A2, following appropriate external stimuli, is able to free arachidonic acid which in turn is then metabolized via enzymatic pathways including the cyclooxygenase (COX) and lipoxygenase (LOX) pathways to generate 2-series prostaglandins (PGs) and thromboxanes (Txs) or 4-series leukotrienes (LTs) and eicosatetraenoic acids (ETEs)[3] as shown in *figure 3*. These kinds of metabolites cover several roles in mammalian biology, they are involved in the inflammatory processes, in platelet aggregation, vasoconstriction and many other important processes.

[3] Brunton L.L.; Lazo J. S.; Parker K. L., Goodman & Gilman's The Pharmacological Basis of Therapeutics, 11th edition

PROSTAGLANDINS

As mentioned before, a family of metabolites of the arachidonic acid of grate interest are prostaglandins (PGs). Prostaglandins are a modified C-20 fatty acids. They were discovered in ovine seminal fluid by U. S. von Euler in 1935, he was working on the smooth muscle-contracting activity of seminal plasma and recognized the active material in a lipid-soluble acid, which he named prostaglandin, believing that substance was secreted by the prostatic gland[4]. Only in the early 60's, thanks to the works of Samuelsson and Bergström, the structure and the biosynthesis of some prostaglandins were elucidated[5]. Today is well known that prostaglandins are extensive diffuse in animal tissues, but they are produced only in hormone-like concentrations, and It has been demonstrated that they have multiple biological activities. Prostaglandins play a role in regulation of blood pressure, smooth muscle contraction, platelet aggregation, gastric and intestinal secretions and many other functions, but they play their major role in inflammatory and immune responses. Their potential for drug use is extremely high, but it has proved difficult to separate the various biological activities into individual agents[1].

The basic skeleton of a prostaglandin is analogue to prostanoic acid (*figure 4*), C-20 fatty acid cyclized into a cyclopentane ring, a C-7 side chain with the carboxylic group terminus (named α chain) and a C-8 side chain that bring the methyl end (named ω chain). The ω and the α chains presents a *trans*-configuration in respect to the cyclopentane, which is the thermodynamically favorited.



PROSTANOIC ACID

FIGURE 4

[4] Von Euler, U.S.; *Wien Klin Wochenschr;* **1935**; 14(33); 1182-1183.
[5] Bergström, S.; *J Am Oil Chem Soc*; **1965**; 42; 608-609

Prostaglandins could be classified in three different series in respect to which unsaturated fatty acid (UFAs) is the precursor. They are biosynthesized by the action of cyclooxygenases (COX1 and COX2) from three different UFAs, namely ***dihomo-γ-linolenic acid***, ***arachidonic acid***, and ***$\Delta^{5,8,11,14,17}$-eicosapentaenoic acid***, which yield prostaglandins of the 1-, 2-, and 3-series respectively. The numerals refer to the number of double-double bonds presents. Being arachidonic acid the most important fatty acid in most animals, PGs of the 2-series are the most abundant in mammalian tissues. Furthermore, prostaglandins are catalogued according to the structures of the 5-membered ring, to every structure correspond a letter from **A** to **J** *(figure 5)*.

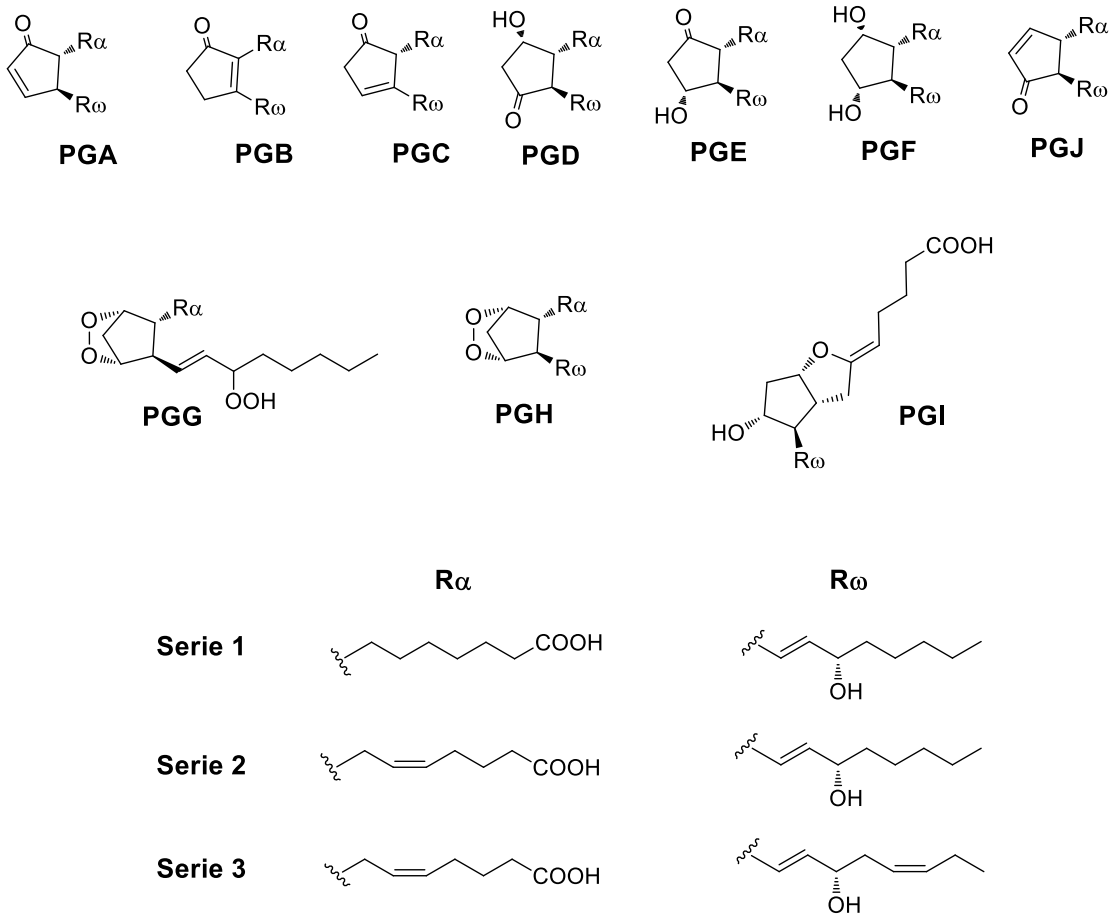


FIGURE 5

The oxidation of UFAs by COX afford first G-type prostaglandins (PGG) that is enzymatically transformed into H-type PGs (PGH). The action of other isomerases and synthases can transform the cyclic endoperoxide giving E, D, I and F-type prostaglandins, named parent

prostaglandins. Moreover, the non-enzymatically processes can produce a dehydration of PGD and PGE yielding the more stable cyclopentenonic prostaglandins like PGA and PGJ[3,6].

[6] Coleman, R. A.; Smith, W. L.; Narummiya, S. *Pharmacol. Re*V. **1994**, *46*, 205.

PROSTAGLANDINS SYNTHESIS

Thanks to the diversity of their biological activities and the potency of their derivatives, even if they are present at very low concentrations in mammalian biological fluids, prostaglandins induced a very intense activity both in chemistry and in biology since they were discovered[7]. By the mid-l960s, there was widespread belief that PGs would be useful therapeutic agents in a large number of diseases and efforts to synthesize the natural compounds and structurally modified analogs became intense. As synthetic targets, prostaglandins represent an exciting challenge, just think to the high number of stereogenic centers. For example, in the F-type prostaglandins have five stereocenters and four of them are set on the cyclopentane ring and one on the C-15 in allylic position. Furthermore, is necessary to find a route that can introduce a *cis* and a *trans* di substituted olefins at the same time.

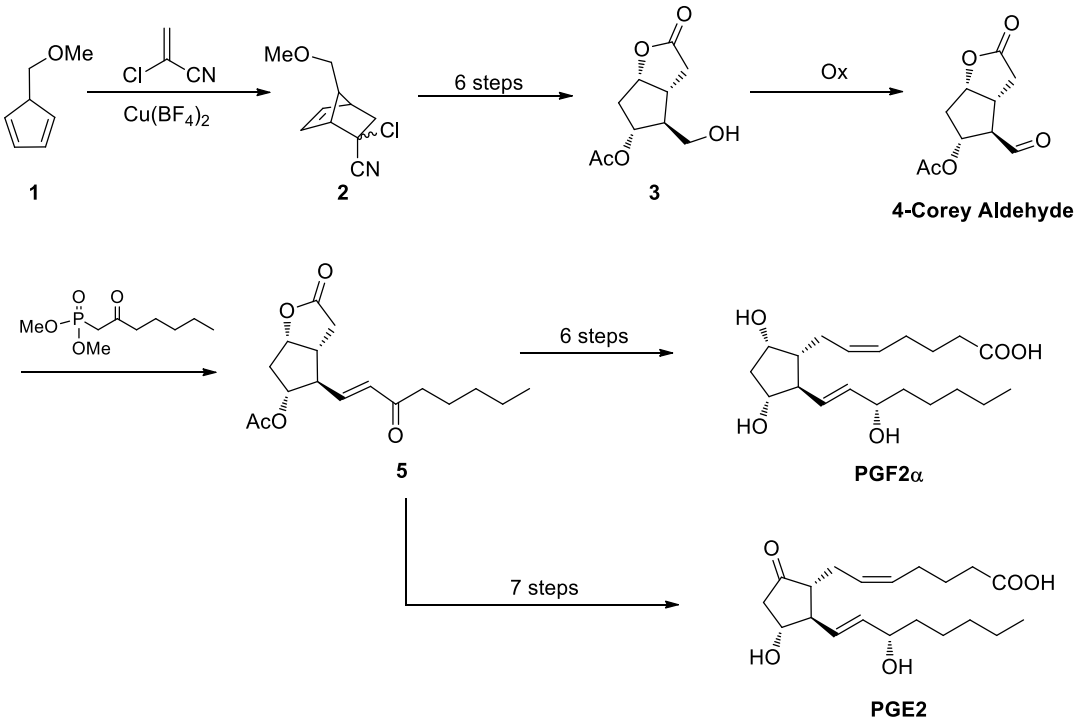
Over the years, several strategies were developed, and we can group them into three different approaches[8]:

1. The first approach involves, in a first time the synthesis of the cyclopentanic core with appropriate substituents in the right positions to add the ω and the α chains in succession in second time. In this approach the development of the Corey lactone was a milestone[7]. In a brilliant work published in 1969, E.J. Corey described for the first time the synthesis of prostaglandins F2α and E2 based on the use of lactone **4** as common building block. This useful intermediate was prepared in 7 steps via a copper catalyzed Diels-Alder reaction between cyclopentadiene **1** with 2-chloro-acrylonitrile (*scheme 1*)[9].

[7] (a) Corey, E. J. *Angew. Chem*., Int. Ed. Engl. **1991**, 30, 455. (b) *The Synthesis of Prostaglandins*; Mitra, A., Ed.; John Wiley and Sons: New York, **1977**.

[8] Chandrasekhar, J. S. Yadav, R. Grée*, Chemical Reviews* **2007**, *107*, 3286.

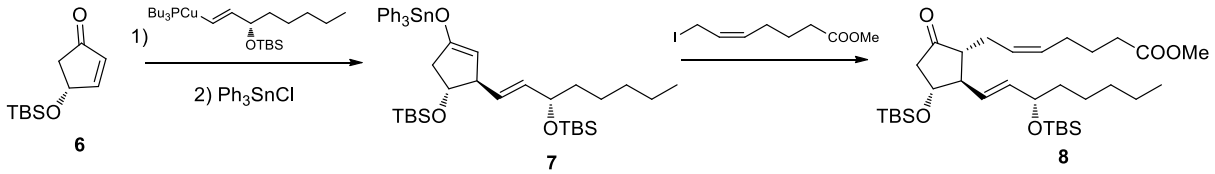
[9] a) Corey, E. J.; Weinshenker, N. M.; Schaaf, T. K.; Hube, W.; *J. Am. Chem. Soc.* **1969**, 91(20), 5675-5677. (b) E. J. Corey, T. K. Schaaf, W. Huber, U. Koelliker, N. M. Weinschenker, J. Am. Chem. Soc. **1970**, 92, 397. (c) Corey, E. J. Angew. Chem., Int. Ed. **2002**, 41, 1650.



SCHEME 1

2. The second approach is based on a two-component coupling; the cyclic core is synthetized starting from a structure that already includes one of the side-chains. The second side-chain is introduced later in the synthesis.

3. The third strategy is based on a one pot three-component coupling, and consists on a 1-4 addition of the ω chain on an appropriate cyclopentenone followed by the trapping of the enolate by the appropriate α side chain as electrophile. This refined strategy was porposed and developed by R. Noyori[10] (*scheme 2*).

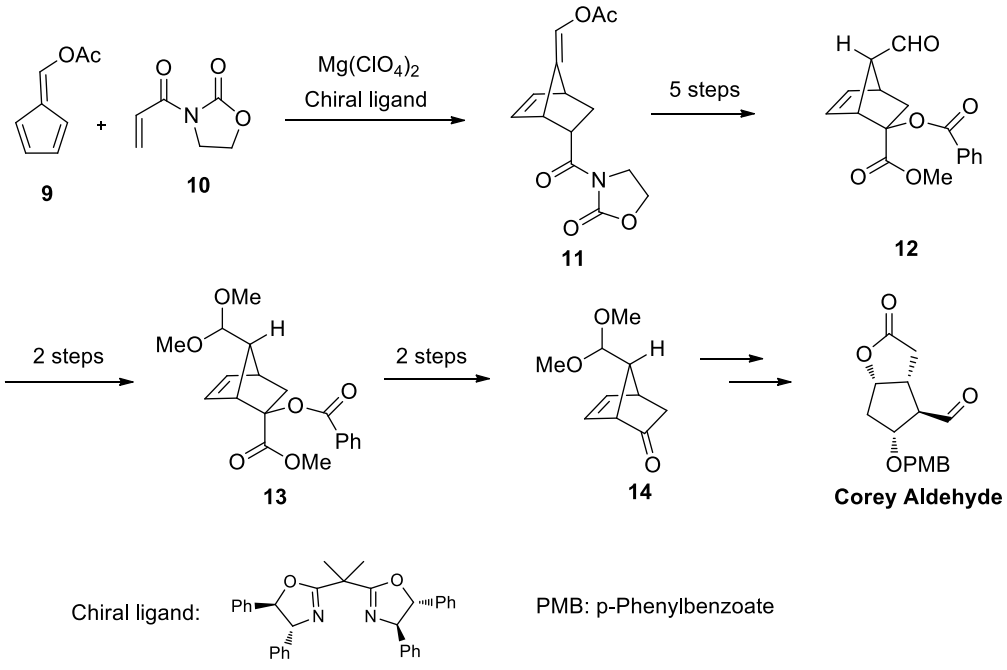


SCHEME 2

[10] Suzuki, M.; Yanagisawa, A.; Noyori, R. *J. Am. Chem. Soc.* **1988**, 110, 4718

Of these three approaches, what was more widely developed and used was undoubtedly the strategy invented by prof. E.J. Corey. Thanks to its high versatility the Corey aldehyde **4** has been used by several laboratories to prepare prostaglandins and their derivatives on any scale. In the original version of the 1969, the Diels-Alder reaction was performed in a stereospecific but not enantioselective fashion, and followed by an enantiomeric resolution in a later step. The development of this strategy has seen the publications several ways to get optically pure Corey aldehyde (**4**).

A recent example in this direction is the one published by G. Zanoni et all in 2014[11]. His synthesis is based on an asymmetric Diels-Alder reaction between Acetoxyfulvene and 3-Acryloyl-1,3-oxazolidin-2-one, and on the epimerization of the aldehyde **12** (*scheme 3*). The Corey aldehyde is afforded with the 95% *ee*.

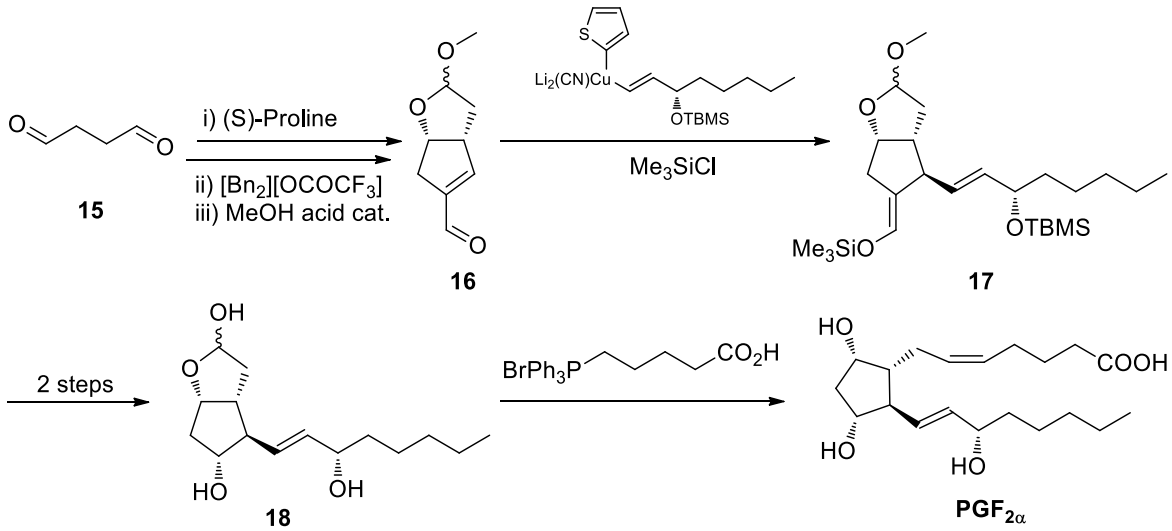


SCHEME 3

Another important achievement in the route opened by E.J. Corey is the strategy purposed by Aggarwal et all, in his work he performed the synthesisPGF2α and its derivative, Latanoprost and Bimatoprost, in 7 steps using a new building block, different in structure but similar in concept to which developed by Corey. This strategy is based on the

[11] Valli, M.; Chiesa, F.; Gandini, A.; Porta, A.; Vidari, G.; Zanoni, G. *J. Org. Chem*. **2014**, 79, 2632.

organocatalytic synthesis of the unsaturated aldehyde **16** in 99:1 e.r., followed by the conjugate addition of the ω chian (*scheme 4*)[12].



SCHEME 4

[12] a) Coulthard, G.; Erb, W.; Aggarwal, V. K. *Nature* **2012**, *489*, 278. b) Prévost, S.; Thai, K.; Schützenmeister, N.; Coulthard, G.; Erb, W.; Aggarwal, V.K. *Organic Letters* **2015**, *17*, 504.

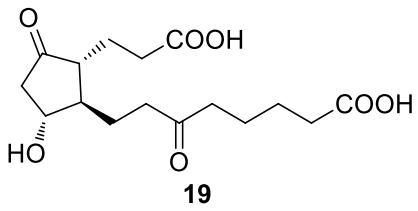
AIM OF THE THESIS

As mentioned before prostaglandins are implicated in several biochemical processes in mammalian. Their hormone-like behavior due to external stimuli, often related to particular pathologies or diseases like cancer or inflammatory processes, make prostaglandins interesting biomarkers. Unfortunately, because of their poor stability and reactivity the quantification of PGs in animals and humans is quite difficult. The measurement of prostaglandins in various biological fluids could be affected by artefactual or *ex vivo* generation of PGs derivatives. However, the evaluation of systemic production of prostaglandins is important to build up a diagnostic method. A solution to the problem is the evaluation of the more stable and polar products of prostaglandin metabolism directly in urine.

For these reasons with this work we propose the synthesis based on the Corey approach for the synthesis of two PGs urinary metabolites as standards for HPLC-MS analysis of biological samples.

The first is the fully stereodefined total synthesis of the major urinary metabolite of prostaglandin E_2 (*figure 6*).

The second is a synthetical study for the synthesis of the proposed urinary metabolite of the 15d-PGJ_2 labeled with deuterium on the ω side chain (*figure 6*). The structure of this molecule has not been determined jet, thus the aim of this work is also to lay the groundwork for the structural determination.



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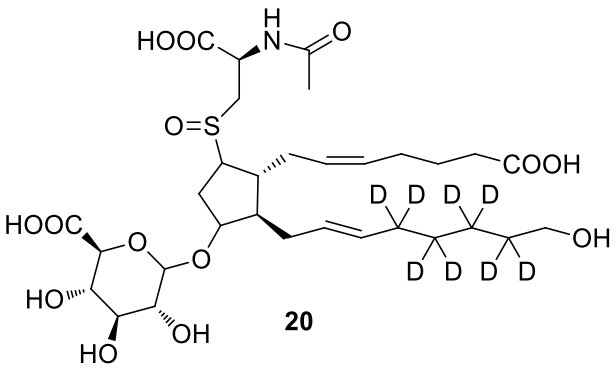
PGE_2 URINARY METABOLITE



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15d-PGJ_2 URINARY METABOLITE

FIGURE 6

SYNTHESIS OF THE PGE_2 URINARY METABOLITE

PROSTAGLANDIN E_2

PGE_2 exert a variety of physiological functions in mammalian cell. For example, PGE_2 modulates local vascular tone in various tissues, regulates sodium and water excretion by the kidney, and maintains normal gastric homeostasis. PGE_2 is involved also in inflammatory processes, in which plays a key role. Various studies have demonstrated that PGE_2 pathway is involved in several inflammatory diseases, like rheumatoid arthritis chronic periodontitis and cardiovascular diseases[13]. There are also evidences of implication of PGE_2 with cancer, in particular in cellular growth and malignant transformations. Was provided evidences of PGE_2 induce significant alteration in cells of colorectal carcinoma[14], non-small cell lung cancer (NSCLC)[15], head and neck cancer[16], breast cancer[17], and prostate cancer[18] increasing proliferation, migration, and invasiveness of tumor cells.

Among the various prostaglandins parents what has been given more attention is the PGE_2. PGE_2 is synthesized by the action of the isomerases PGE synthases on the PGH_2, a cycloendoperoxide produced by COX form arachidonic acid. In human cells are known three different type of PGE synthases: two are microsomal and one is cytosolic, of these three isomerases the microsomal PGE synthase 1 (mPGES-1) is expressed after pro-inflammatory stimuli, and it can be considered the principal source of PGE_2 in inflammation[19]. The effects of PGE_2 are due to its interaction with four distinct PGE_2 receptors (EP1, EP2, EP3, and EP4) that are G-protein-coupled cell surface membrane receptors. COX inhibitors, including traditional nonsteroidal anti-inflammatory drugs

[13] (a) Pihlstrom, B.L.; Michalowicz, B.S.; Johnson, N.W. *Lancet.* **2005**, 366(9499), 1809. (b) de Hair, M. J. H.; Leclerc, P.; Newsum, E. C.; Maijer, K. I.; van de Sande, M. G. H.; Ramwadhdoebe, T. H.; van Schaardenburg, D.; van Baarsen, L. G. M.; Korotkova, M.; Gerlag, D. M.; Tak, P.P.; Jakobsson, P.J. *PLoS One* **2015**, 10(7), e0133669/1. (c) Mapp, P.I. *Ann Rheum Dis* **1995**; 54(5), 398.

[14] Sheng, H.; Shao, J.; Washington, M. K.; DuBois, R. N. *J Biol Chem* **2001**, 276, 18075.

[15] Murphey, L. J.; Williams, M. K.; Sanchez, S. C.; Byrne, L. M.; Csiki, I.; Oates, J. A.; et al. *Anal Biochem* **2004**, 334, 266.

[16] Camacho, M.; Leon, X.; Fernandez-Figueras, M. T.; Quer, M.; Vila, L., *Head Neck* **2008**, 30, 1175.

[17] Subbaramaiah, K.; Hudis, C.; Chang, S. H.; Hla, T.; Dannenberg, A. J., *J Biol Chem* **2008,** 283, 3433.

[18] Jain, S.; Chakraborty, G.; Raja, R.; Kale, S.; Kundu, G. C. 2008.
Cancer Res 68:7750.

[19] Kudo, I.; Murakami, M., *J Biochem Mol Biol* **2005**, 38, 633.

(NSAIDs) such as aspirin and selective COX-2 inhibitors, and mPGES-1 inhibitors[20], are utilized to block the synthesis and consequent deleterious effects of this molecule.

QUANTIFICATION OF PGE2 PRODUCTION IN VIVO

Previously we mentioned the importance of prostaglandin quantification, concerning *in vitro* experiments the solution to the problem is less problematic, several methods, including immunoassay and mass spectrometry, have been developed. However, the building up of a method capable to measure the systemic production of eicosanoids in humans or animals is more challenging. In fact, the quantification of PGs in biological fluids is often affected by artifacts or *ex vivo* generation of these molecules during the sampling. The measurement of free eicosanoids and their metabolites in urine is a non-invasive and representative method for the determination of their whole body production[21].

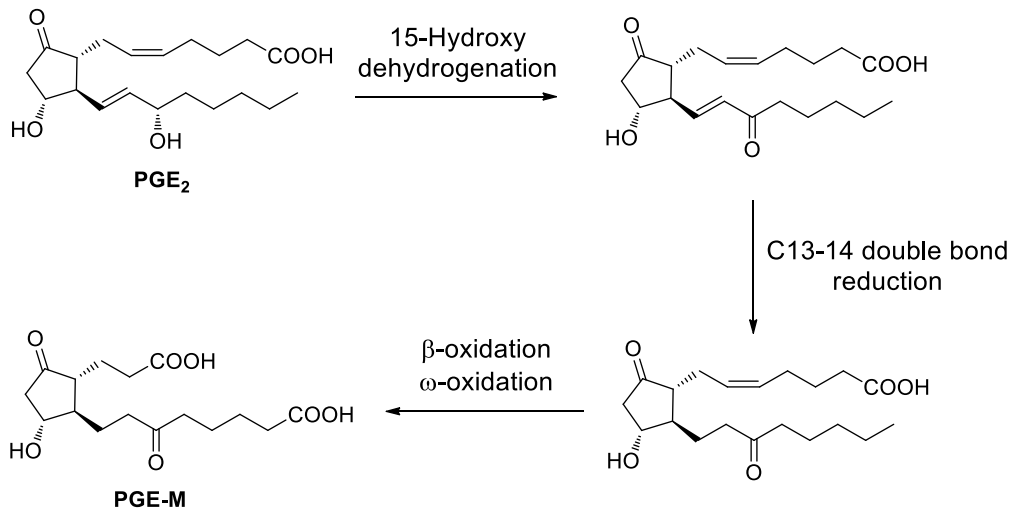
Because of its relationship with various type of diseases and pathologies, prostaglandin E_2 can be considered an interesting biomarker for diagnostic applications. Unfortunately, the quantification of PGE_2 in urine does not reflect the real systemic production, almost all free PGE_2 in urine is produced locally in the kidney[22]. Studies has demonstrated that the most representative index of the whole body production of PGE_2 is its urinary metabolite, (1,2,3,4-Tetranor)-9,15-dioxo-11-hydroxy-13,14-dihydroprostanedioic acid (PGE-M). The first facile and robust methodology for the quantification of PGE-M by liquid chromatography-mass spectrometry (LC-MS) was published by Murphey and colleagues in 2004[15]. The possibility to quantify accurately this metabolite allowed to discover the implication of PGE_2 with a number of diseases and related lifestyle factors. For examples was demonstrated that tobacco users present higher level of PGE-M than never tobacco users[23] and PGE-M levels are increased in patients with colorectal cancer[24], head and neck squamous cell carcinoma[25], and NSCLC [15]. Furthermore, in head and neck squamous cell

[20] Iyer, J. P.; Srivastava, P. K.; Dev, R.; Dastidar, S. G.; Ray, A. *Expert Opin Ther Targets* **2009**, 13, 849.
[21] Catella, F.; Nowak, J.; Fitzgerald, G. A. *Am J Med* **1986**, 81(2), 23
[22] Frolich, J. C.; Wilson, T. W.; Sweetman, B. J.; Smigel, M.; Nies, A. S.; Carr, K.; et al. *J Clin Invest* **1975**, 55, 763.
[23] Kekatpure, V.D; Naveen BS, Wang, H.; Zhou, X.K.; Kandasamy, C.; Sunny, S.P.; Suresh, A.; Milne, G.L .; et al. *Cancer Prev Res* **2016**; 9(6), 428.
[24] Johnson, J. C.; Schmidt, C. R.; Shrubsole, M. J.; Billheimer, D. D.; Joshi, P. R.; Morrow, J. D.; et al. *Clin Gastroenterol Hepatol* **2006**, 4, 1358
[25] Kekatpure, V. D.; Boyle, J. O.; Zhou, X. K.; Duffield-Lillico, A. J.; Gross, N. D.; Lee, N. Y.; et al. *Cancer Prev Res* **2009**, 2, 957

carcinoma, PGE-M was prognostic for disease progression[23]. PGE-M can be used also as biomarker to predict-select who will have a positive response in the treatment with COX2-inhibitors associated with chemotherapy and other treatments for recurrent NSCLC[26].

PGE2 URINARY METABOLITE DISCOVER AND PREVIOUS SYNTHESIS

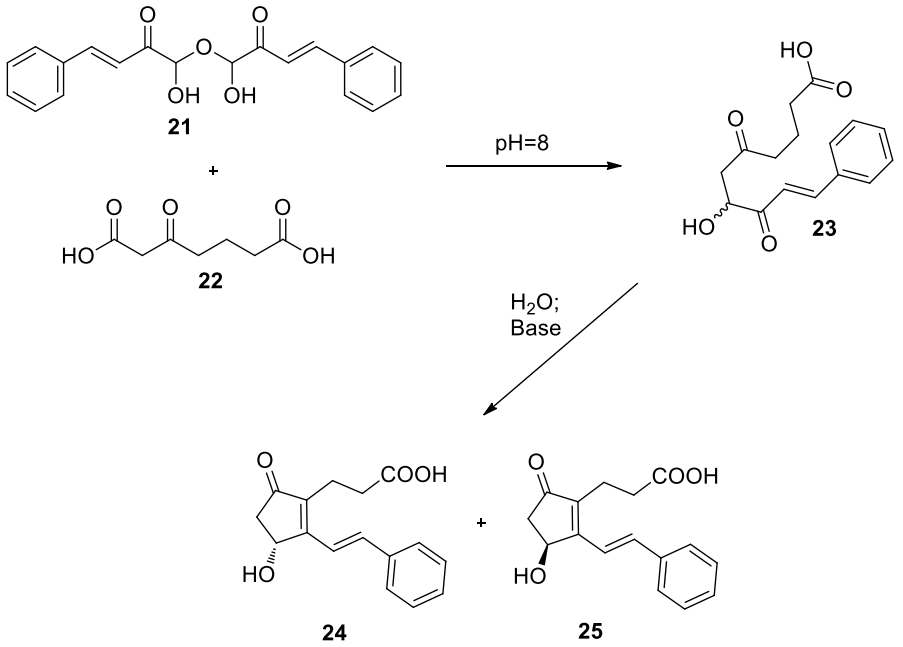
The structure of the major human urinary metabolite of PGE_2 was determined by Hamberg and Samuelsson in 1969[27]. After intravenous infusion of PGE_2 labeled with tritium in a man they recovered the radioactive fraction of the urine acidic extract, and analyzed it by gas-liquid partition chromatography coupled with mass spectrometry, thus identifying the major urinary metabolite of PGE_2 in human as (1,2,3,4-Tetranor)-9,15-dioxo-11-hydroxy-13,14-dihydroprostanedioic acid. Two years later, in 1971 the same authors published the results of a research in which was clarified the sequence of metabolic reactions that afford the PGE_2 urinary metabolite[28]. They demonstrate that the formation of PGE-M involves four sets of reaction, oxidation of the hydroxyl group on C-15, reduction of the *trans* double bond, two steps of β-oxidation and ω-oxidation. It is also possible that the first two reaction precede the others and moreover, is reasonable that the reduction of the double bond were activated by the conjugation (*scheme 5*).



SCHEME 5

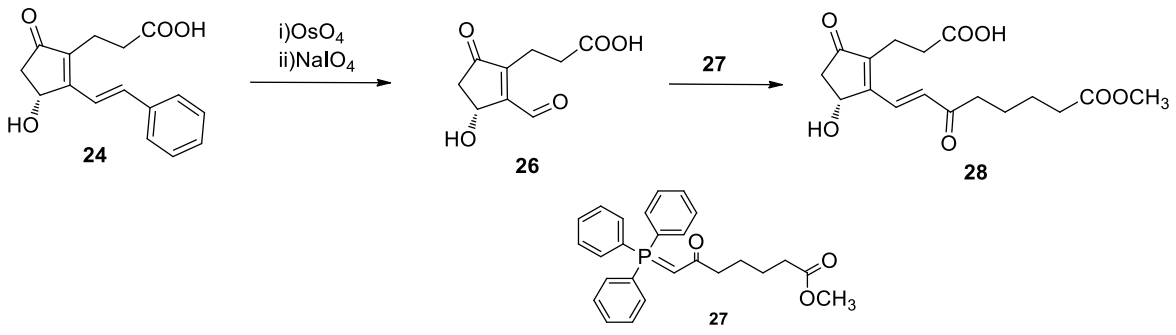
[26] (a) Reckamp, K.; Gitlitz, B.; Chen, L. C.; Patel, R.; Milne, G.; Syto, M.; et al. *Cancer* **2011**, 117, 809. (b) Csiki, I.; Morrow, J. D.; Sandler, A.; Shyr, Y.; Oates, J.; Williams, M. K.; et al. *Clin Cancer Res* **2005**, 11, 6634.
[27] Hamberg, M.; Samuelsson, B. *J. Am. Chem. Soc.* **1969**, 91(8), 2177.
[28] Hamberg, M.; Samuelsson, B. *J. Biol. Chem.* **1971**, 246, 6713.

The first total synthesis of the PGE-M was published by Boot and coworkers in 1974[29]. In this work the authors purposed a six steps synthesis in order to obtain optically pure metabolite in its natural form. The process is based on the resolution of the racemic carboxylic acid **25** and **26**, which was obtained by cyclization of the condensation product of the di-acid **22** with **21** (*scheme 6*).

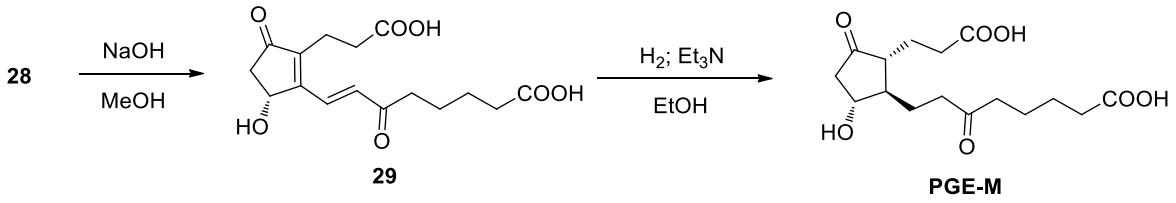


SCHEME 6

The oxidative cleavage of the styril group of III afforded the unstable aldehyde N which was immediately treated with the phosphorane **27**. The Wittig product **28** was treated with methanolic sodium hydroxide and subjected to hydrogenation in ethanol and triethylamine over 10% Pd/C as catalyst affording the PGE-M in its (-)-optically pure form (*scheme 7*).

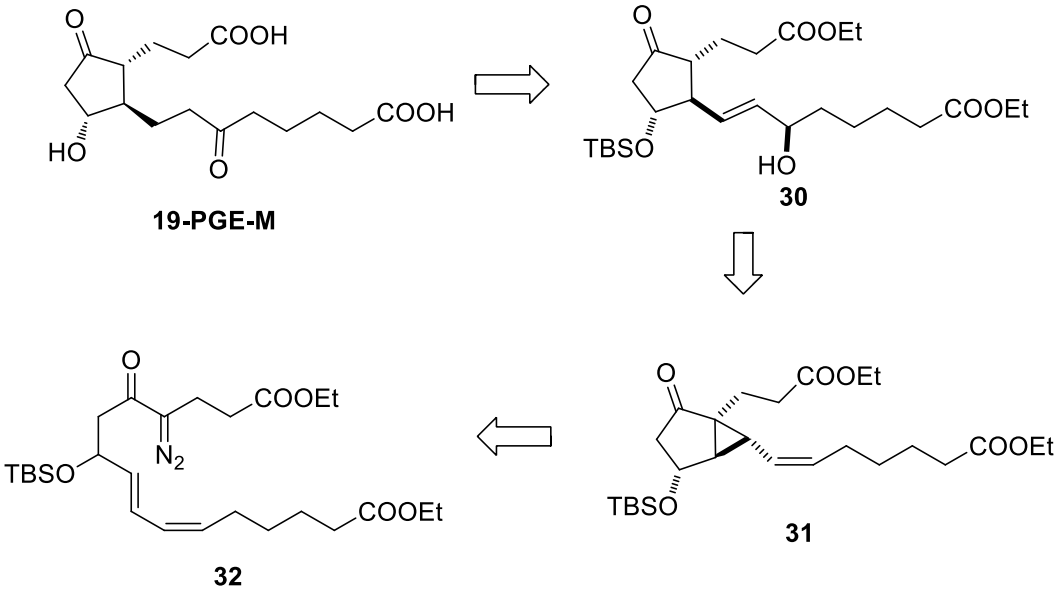


[29] Boot, J. R.; Foulis, M. J.; Gutteridge, N. J. A.; Smith, C. W. *Prostaglandins* **1974**, 8, 439.



SCHEME 7

In recent years Taber described a new strategy for the synthesis of PGE-M[30]. The approach was similar to which he used for the synthesis of other prostanoids[31] that is based on cyclization of the diazoketone **32** like showed in the retrosynthetical *scheme 8*.

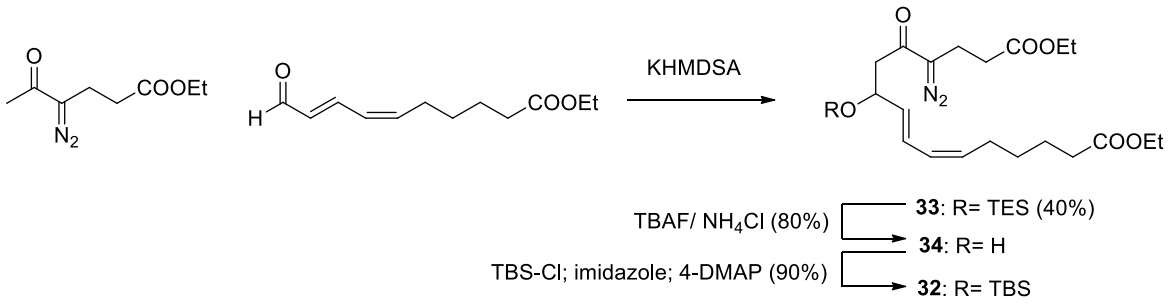


SCHEME 8

The diazoketone **32** was obtained in racemic mixture by aldol condensation between **33** and **34** followed by protecting group exchange in order to improve the resistance of the protecting group during the ring opening step (*scheme 9*). The cyclization step was performed using Rhodium octanoate as catalyst. The reaction provided a mixture of diastereoisomers **31** and **35** in a ratio of 65:35. They were not able to separate the mixture, in order to enable the purification of the mixture they submitted the diastereoisomers to

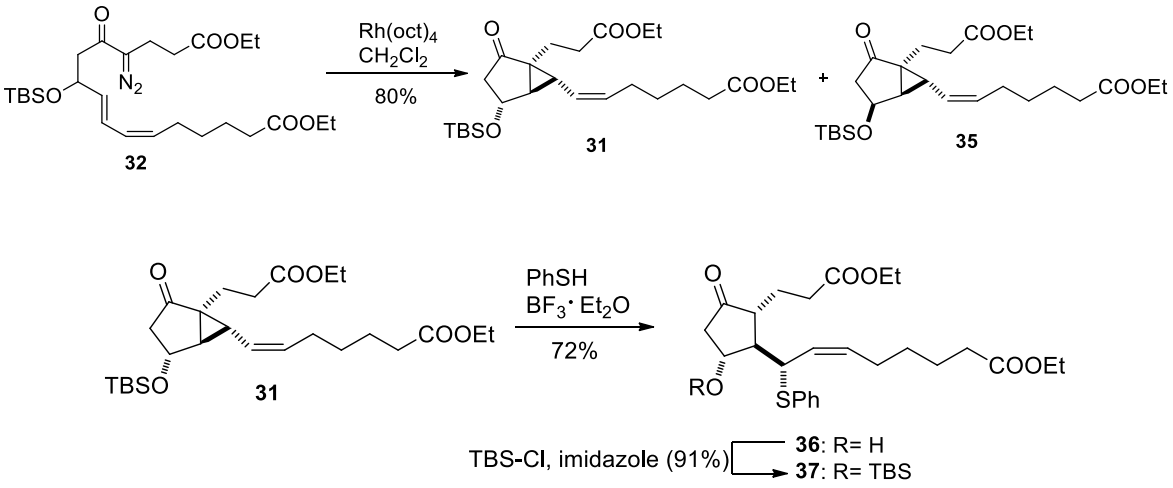
[30] (a)Taber, D. F.; Gu, P. *Tetrahedron* **2009**, 65, 5904. (b) Taber, D. F.; Teng, D. *J. Org. Chem.* **2002**, 67, 1607
[31] (a) Taber, D. F.; Hoerrner, R. S. *J. Org. Chem.* **1992**, 57, 441. (b) Taber, D. F.; Herr, R. J.; Gleave, D. M. *J. Org. Chem.* **1997**, 62, 194

reduction with diisobutylaluminium hydride (DIBALH), separation and then oxidation with Dess-Martin periodinane obtaining only **31** as a pair of enantiomers.



SCHEME 9

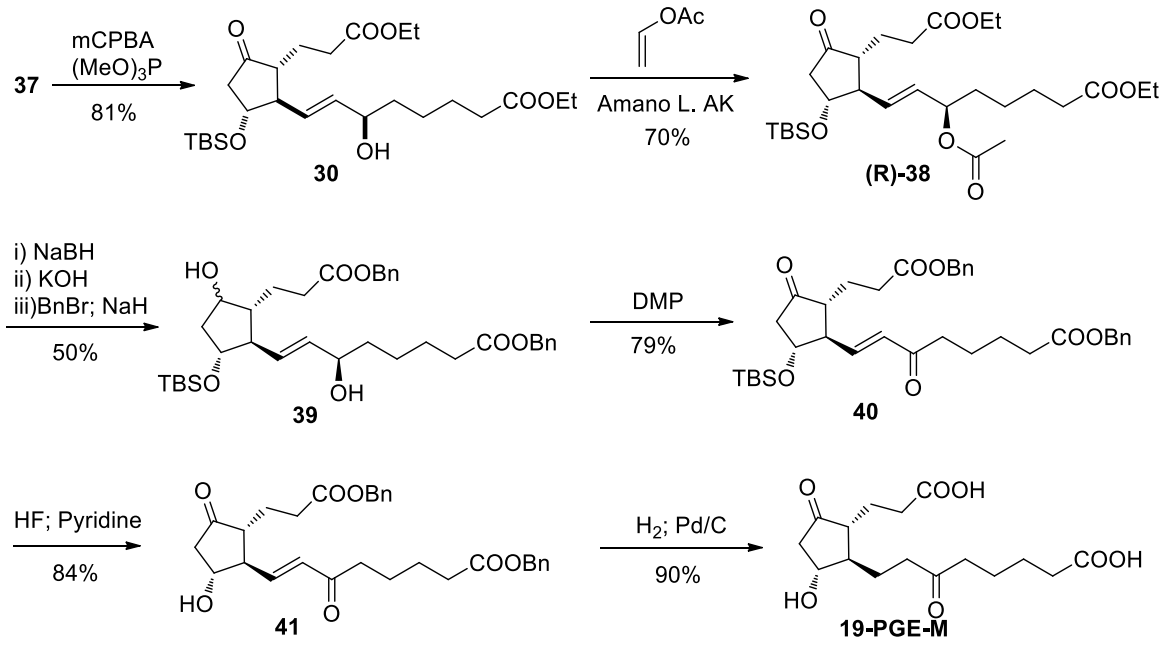
The bicyclic compound **31** was submitted to the ring opening by treatment with stoichiometric thiophenol and boron trifluoride dietylether complex at -10°C affording the thioether **36** with the right side-chain *trans* relative configuration. The reaction condition produced the loss of the *t*-butyldimethyl sylilether that was restored to preserve from oxidation in the next steps (*scheme 10*).



SCHEME 10

After oxidation and Mislow-Evans rearrangement the allyl alcohol **30** was recovered as racemic mixture. The right optically pure enantiomer was obtained as allyl acetate **(R)-38** after enzymatic resolution with R-selective Amano Lipase AK. The authors also highlighted the importance to have a Z-double bond on **37** and its precursor in order to recover the (R)-allyl alcohol with the same absolute configuration of the other stereogenic center as

natural prostaglandins. The enantiomerically pure acetate **(R)-38** was then reduced with sodium borohydride, submitted to basic hydrolysis and finally transformed in the bis benzyl ester **39**. The synthesis was finally concluded by oxidation pf **39**, deprotection of the silylesther **40** with hydrogen fluoride and hydrogenation of the enone **41** affording the optically pure **PGE-M** as free diacid (*scheme 11*).

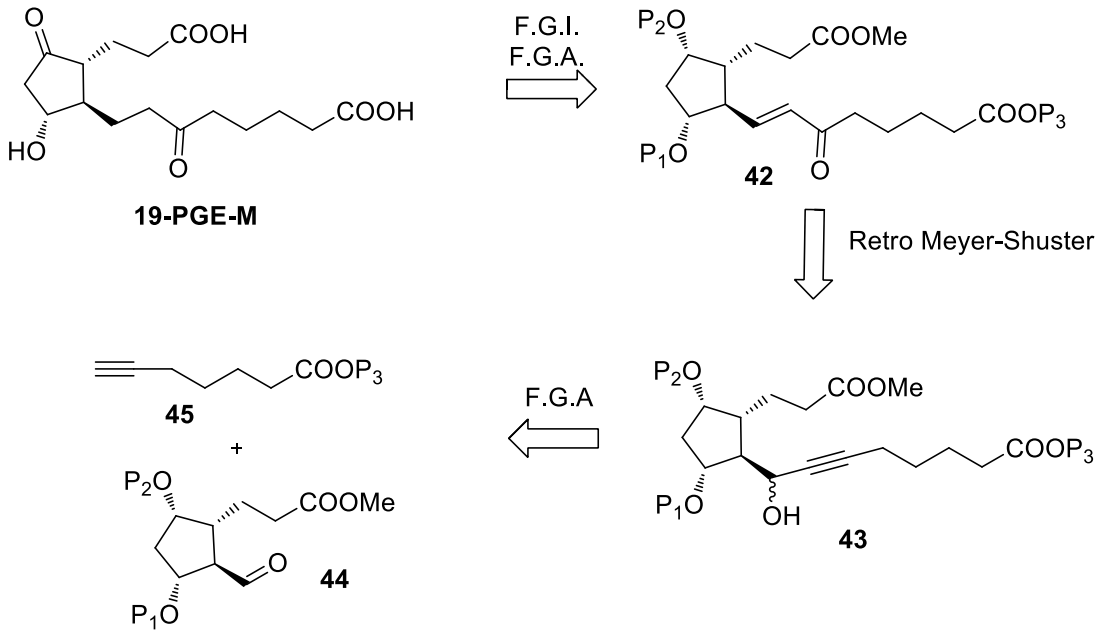


SCHEME 11

RETROSYNTHETIC ANALYSIS

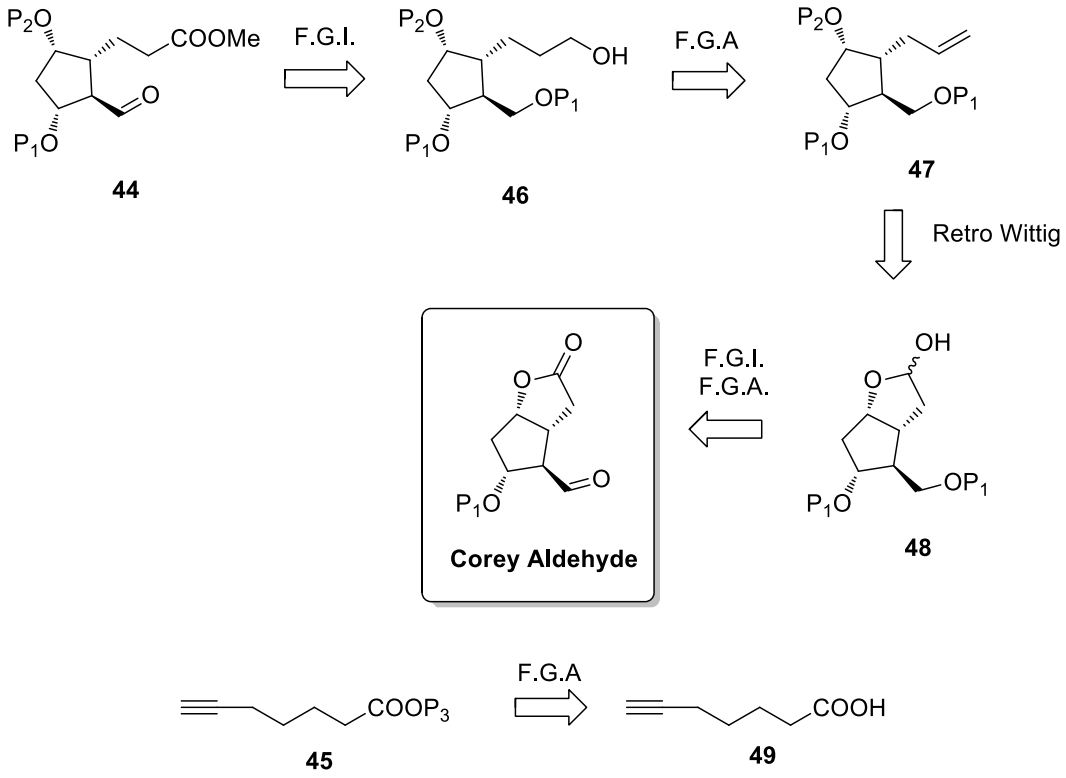
In this work we purpose a new and stereoselective synthetic strategy for the synthesis of the PGE_2 urinary metabolite. As showed before in the past were published various synthesis of this metabolite but they were all based on the resolution of a racemic mixture forming a diastereomeric salt or by enzymatic reactions.

The approach we used for the synthesis of this molecule is based on the identification of a route for the introduction of the side chains starting from a building block that already has the correct absolute configuration of stereogenic centers.



SCHEME 12

In which concern the introduction of the ω-side chain (the longest one with the carbonyl function) we identify two building blocks, the aldehyde **44** and the alkyne **45** as precursors of the cyclopentanic core and the side-chain relatively (*scheme 12*). In fact, we can formally lead the target molecule to the enone **42** applying the right functional group additions and interconversions (F.G.A.; F.G.I.), and employing a retro Meyer-Shuster rearrangement we can identify the propargylic alcohol **43**. Applying the appropriate disconnection of the bond between the alkyne and the hydroxyl function the compound **43** can be traced back to the building blocks **44** and **45.**



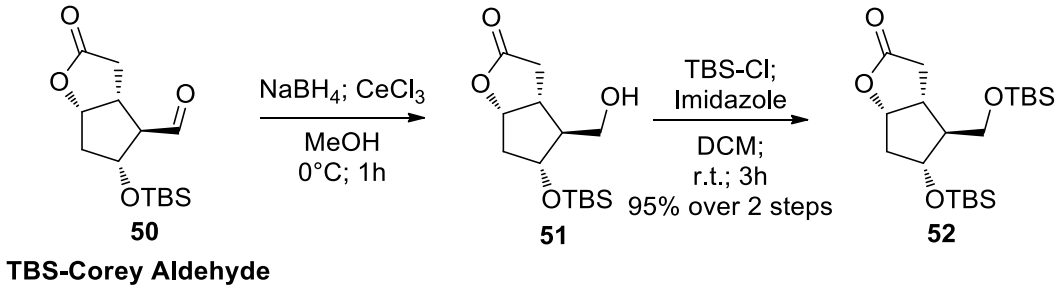
SCHEME 13

While the alkyne **45** can be easily reconducted to the commercially available free acid **49**, the aldehyde **44** can be obtained from the terminal alkene **47** passing by the free alcohol **46** applying the right functional group interconversion and addition. A retro Wittig olefination allows us to identify the lactol **48** as precursor of **47**. Through the application of the opportune functional group interconversions and addition **48** can be bring back to the commercially available Corey aldehyde (*scheme 13*).

SYNTHESIS OF THE PGE2 URINARY METABOLITE

The experience of our group in the synthesis of prostanoids has permitted to identify it the Corey Aldehyde, protected as *tert*-butyldimethylsylil ether on the secondary hydroxyl function, as ideal starting material. It has all the stereogenic centers with the same relative configuration of natural prostaglandins. This important building block can be purchased optically pure or can be synthesized with high enantiomeric excess with the methodology developed by our laboratory from acetoxyfulvene[11].

The first step of the synthesis involed the reduction of the aldehyde **50** in the Luche conditions with sodium borohydride and cerium chloride to give the hydroxy-lactone **51**. The alcohol thus obtained was submitted to protection as sylil-ether by treatment with *tert*-butyldiemthylsylil-chloride affording the bis-TBS derivative **52** in 95% yield over 2 steps (*scheme 14*).

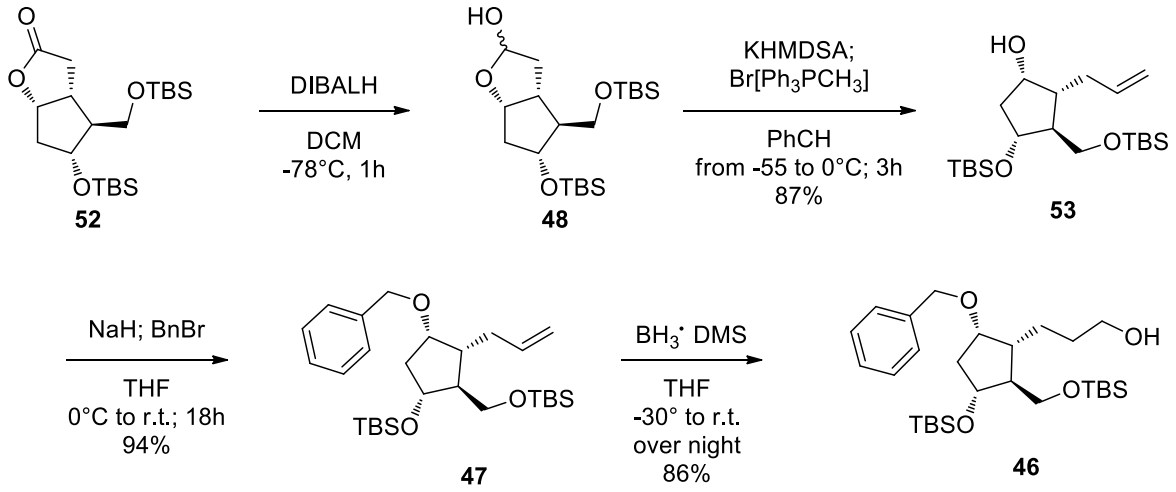


SCHEME 14

The use of the same protecting group for both hydroxyl functions, might seem a not ideal choice. However, the synthesis made using an orthogonal protecting group to TBS, as the PMB, has brought no improvements in terms of yield both in protection and in deprotection reaction. Moreover, the presence of a p-metoxybenzyl ether interferes with subsequent steps of oxidation lowering the yield. For these and also for economic reasons, the choice of the protecting group fell on TBS.

The introduction of the α-side chain was easily accomplished via homologation of the upper side chain with one extra carbon. The additional carbon was introduced by Wittig olefination with methyltriphenylphosfonium bromide on the hemiacetal **48**, previously obtained by treatment of **52** with diisobutylaluminium hydride (*scheme 15*). This the reaction sequence of lactone reduction-Wittig olefination, purposed by Corey since 1969[9],

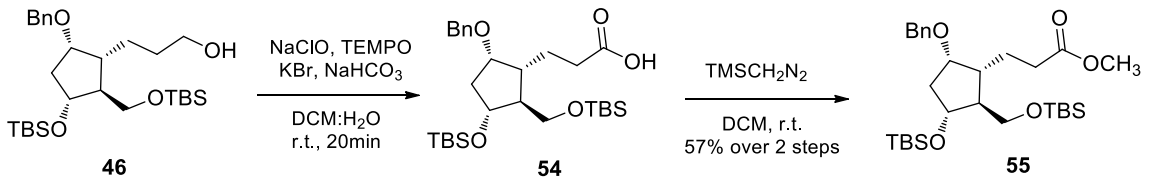
has been widely used by our research group for the synthesis of other prostaglandins and prostanoids[32].



SCHEME 15

The Wittig olefination is followed by protection of secondary alcohol **53** as benzyl ether by treatment with sodium hydride and benzyl bromide affording **47**.

The formation of the terminal olefin paves the way for the introduction of the carboxylic moiety. As showed in *scheme 15* the hydroboration reaction on **47** with borane dimethylsulfide complex (BH_3·DMS) allowed to obtain the primary alcohol **46** in good yield. The compound thus obtained is then subjected to oxidation reaction in order to form carboxylic acid derivative **54**. In a first trial the oxidation of **46** has been performed directly in one step by treatment with sodium hypochlorite and 2,2,6,6-Tetramethylpiperidine 1-oxyl (TEMPO) in the condition developed by Anelli[33] (*scheme 16*).

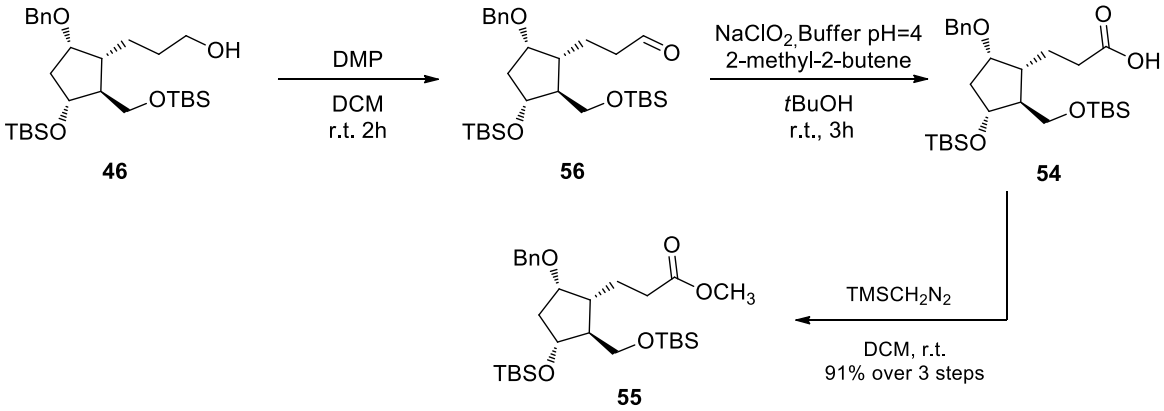


SCHEME 16

[32] (a) Zanoni, G.; Castronovo, F.; Perani, E.; Vidari, G. *J. Org. Chem.* **2003**, 68(17), 6803. (b) Zanoni, G.; Porta, A.; Castronovo, F.; Vidari, G. *J. Org. Chem.* **2003,** 68(15), 6005. (c) Porta, A.; Vidari, G.; Zanoni, G. *J. Org. Chem.* **2005**, 70(12), 4876. (d) Zanoni, G.; Brunoldi, E. M.; Porta, A.; Vidari, G. *J. Org. Chem.* **2007**, 72(25), 9698.

[33] Anelli, P. L.; Biffi, C.; Montanari, F.; Quici, S. *J. Org. Chem.*, 1987, 52 (12), 2559

The reaction was very fast, it takes about only 20 min to completely consume the starting material, but the yield, based on the recovered methyl ester, obtained by treatment of **54** with trimethylsylildiazomethane, was quite modest. Thus, we decided to carry on the oxidation in a step by step way. This new protocol involves first the transformation of alcohol **46** into its relative aldehyde by treatment with Dess-Martin periondinane and then a further oxidation under Pinnick conditions to give the desired carboxylic acid **54**. The steps involved and the reaction condition are showed in the *scheme 17*.

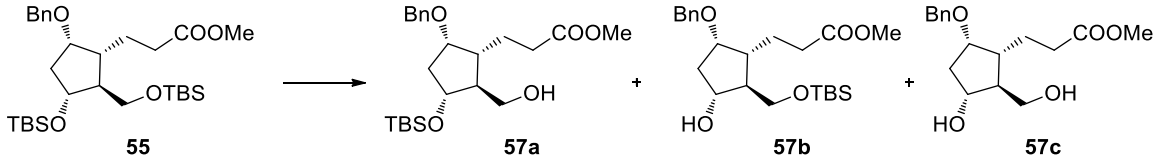


SCHEME 17

The second methodology afford the methyl ester **55** with significant improvement in term of yield, 33% more with respect to the direct oxidation.

The further step is very challenging; it involves selective removal of the primary *tert*-butyldimethylsylil ether. Several tests were carried out in order to find the best reaction conditions. We screened before some deprotection protocols with fluorides and subsequently we tested solvolytic conditions by acid catalysis with different lipophilicity of solvent mixture.

The tests performed and reaction conditions used are listed in the following table (*table 1*). The first five entry involves the use of fluorides in different pH condition and solvent polarity. The reactions are very fast; they take only three or four hours with total conversion of the starting material. However, from the results obtained in these conditions it denotes an almost total lack of selectivity towards the primary silyl ether with respect to the secondary. Probably the high affinity of fluorides towards the silicon does not leave the possibility of exploiting the difference between lability of the two silyl ethers also by modulating the pH and polarity of the solvent.



Entry	Solvent	Reagent	Temp.	Time	57a[I]	57b[I]	57c[I]	Conv.
1	MeCN/MTBE 1:1	HF[II] (6 equiv.)	0°C	3 h	13%	12%	72%	100%
2	THF	HF/Py[III]	r.t.	3 h	11%	12%	65%	100%
3	THF	TBAF	r.t.	3 h	18%	10%	70%	100%
4	MeCN	NaF/HF[IV]	0°C	4 h	30%	25%	40%	100%
5	MeOH	NH_4F (4 equiv.)	r.t.	4 h	35%	28%	32%	100%
6	THF/H_2O 1:1	A_cOH (0.5 equiv.)	r.t.	52 h	0%	0%	0%	0%
7	MeOH/DCM 1:1	PPTS (0.5 equiv.)	r.t.	2 h	10%	0%	80%	80%
8	EtOH/DCM 1:1	PPTS (0.5 equiv.)	r.t.	50 h	70%	0%	10%	90%
9	iPrOH/DCM 1:1	PPTS (0.5 equiv.)	r.t.	120 h	70%	0%	5%	10%

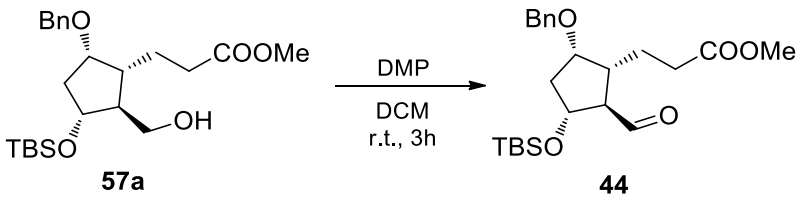
[I] The percentage yields are based on the recover starting material.
[II] The reagent has been added in small portions as a titration.
[III] 15equiv. of HF and 10equiv. of Pyridine.
[IV] As buffer solution pH= 4.5

TABLE 1

With the entry 6 we tested the removal of the primary TBS by acid catalysis in hydrolytic conditions. In this case after 52 hours no reaction did not occur and the starting material was entirely recovered. Thus, in the next three entries we performed the deprotection in anhydrous solvolytic condition in a mixture of dichloromethane/alcohol with pyridimium p-toluensulfonate as acidic catalyst. The alcohols screened are methanol, ethanol and isopropanol. In all the entries is evident that these reaction conditions ensure the correct selectivity (no trace of **57b** was recovered), but the reaction is faster the more acid is the solvent. In fact, with methanol the reaction is very fast (2 hours) and it leads to immediate deprotection also of the secondary hydroxyl function. Conversely, isopropanol affords a

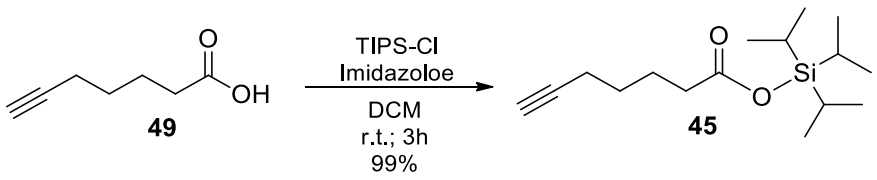
high selectivity even if with a very low conversion percentage after five days. The best results are obtained with a solvent mixture of ethanol and dichloromethane 1:1 (entry 8, *table 1*) which affords the desired product **57a** with the 70% yield and the 90% of conversion.

The thus obtained alcohol **57a** is subjected to treatment with Dess-Martin periodinane to give the relative aldehyde **44** (*scheme 18*).



SCHEME 18

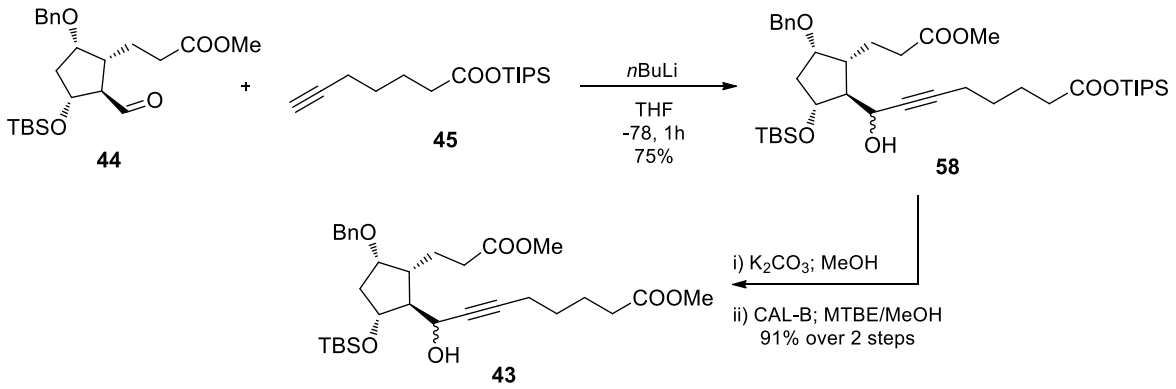
This unstable aldehyde was used immediately for the synthesis of the propargylic alcohol **43**. This steps involves the use of the ester **45**[34] that can be prepared in an easy way from commercially available heptynoic acid **49** by treatment with triisopropylsylil chloride like showed in *scheme 19*.



SCHEME 19

This unusual protecting group for carboxylic acids is important, in our case, thanks to the steric hindrance to avoid nucleophilic addition to the carboxyl function during the treatment with n-butyl lithium in the following step. The nucleophilic addition of the alkyne **45** on the aldehyde **44** afforded the propargylic alcohol **58** in good yield. Transesterification of TIPS ester in presence of the free propargylic acohol is accomplished in a biocatalytic approach with *candida antarctica* lipase B (CAL-B) in the presence of methanol (*scheme 20*).

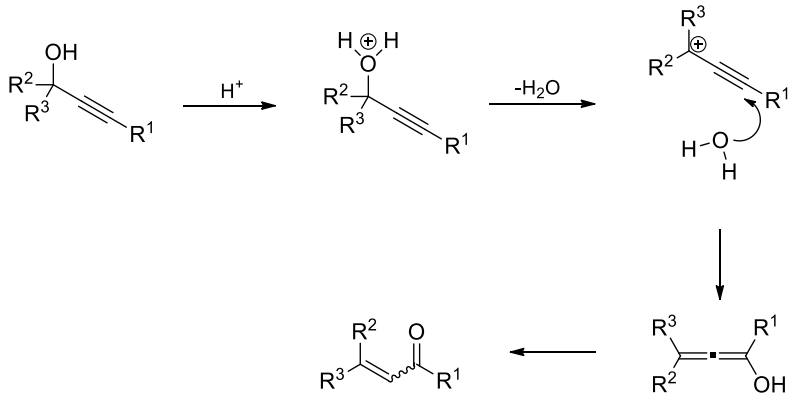
[34] Porta, A.; Chiesa, F.; Quaroni, M.; Persico, M.; Moratti, R.; Zanoni, G.; Vidari, G. *Eur. J. Org. Chem.* **2014**, 2014(10), 2111.



SCHEME 20

The compound **43** may now be subjected to Meyer-Schuster rearrangement.

For Meyer-Schuster rearrangement we consider the acid promoted isomerization of a propargylic alcohol into α,β-unsaturated carbonyl compound[35] (*scheme 21*).



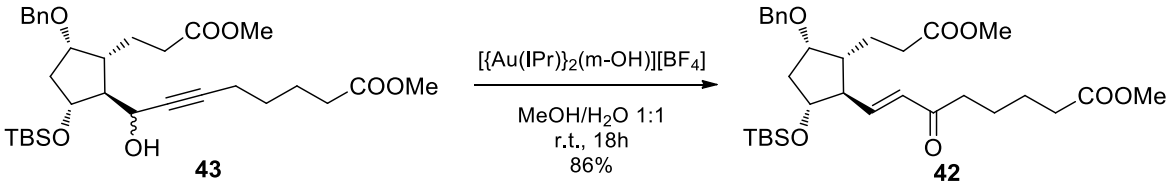
SCHEME 21

In recent year new approaches based on the use of transition-metal catalysts have been discovered, enabling this reaction under mild conditions with much more efficiency and selectivity than classical methods[36]. Among the catalysts that have helped make the Meyer-Schuster rearrangement of an important tool in organic synthesis, there are certainly the gold (I) N-heterocyclic carbenes complexes (NHC). This type of catalysis, developed by prof.

[35] (a) Cadierno, V.; Crochet, P.; Garcı´a-Garrido, S. E.; Gimeno, J. *Dalton Trans*. **2010**, 39, 4015. (b) Meyer, K. H.; Schuster, K. *Chem. Ber.* **1922**, 55, 819.

[36] (a) Engel, D. A.; Dudley, G. B. *Org. Biomol. Chem.* **2009**, 7, 4149. (b) Stefanoni, M.; Luparia, M.; Porta, A.; Zanoni, G.; Vidari, G. A. *Chem. Eur. J.* **2009**, 15, 3940.

Nolan[37], was studied and applied to total synthesis by our research group[38]. In particular, Meyer-Schuster reaction was used for the introduction of the ω-chain in prostaglandins and their analogues synthesis such as $PGF_{2\alpha}$ and Latanoprost[39]. Thanks to these skills developed over the years, we have thought to use this reaction for the introduction of the carbonyl function on the ω side chain in an atom economical fashion. Starting from propargylic alcohol **43** we obtained enone **42** in good yield catalyzing the reaction with $[\{Au(IPr)\}_2(m\text{-}OH)][BF_4]$ (IPr =1,3-bis(2,6-diisopropylphenyl)imidazol-2-ylidene) in a mixture of methanol and water (*scheme 22*).



IPr = 1,3-bis(2,6-diisopropylphenyl)imidazol-2-ylidene

SCHEME 22

The dinuclear structure of this complex give to the catalyst stability and resilience to decomposition, and avoid the use of the air-, light-, and moisture-sensitive silver salts of type AgX (X = BF_4, SbF_6, OTf, PF_6) to abstract chloride from the usually employed [(L)AuCl] (L=ligand) complexes[39b]. The X-ray structure of the catalyst is shown in *figure 7*[40]. The mechanism of action of $[\{Au(IPr)\}_2(m\text{-}OH)][BF_4]$ has not been investigated yet, but is reasonable that it is similar to which purposed by Nolan for the mono nuclear complex[37] (scheme 23).

[37]Ramon, R. S.; Marion, N.; Nolan, S. P. *Tetrahedron* **2009**, 65(9), 1767.

[38] (a) Merlini, V.; Gaillard, S.; Porta, A.; Zanoni, G.; Vidari, G.; Nolan, S. P. *Tetrahedron Lett.* **2011**, 52(10), 1124. (b) Bugoni, S.; Boccato, D.; Porta, A.; Zanoni, G.; Vidari, G. *Chem. Eur. J.* **2015**, 21(2), 791. (c) Beretta, R.; Giambelli Gallotti, M.; Penne, U.; Porta, A.; Gil Romero, J. F.; Zanoni, G.; Vidari, G. *J. Org. Chem.* **2015**, 80(3), 1601.

[39] (a) Zanoni, G.; D'Alfonso, A.; Porta, A.; Feliciani, L.; Nolan, S. P.; Vidari, G. *Tetrahedron* **2010**, 66(38), 7472. (b) Ramon, R. S.; Gaillard, S.; Slawin, A. M. Z.; Porta, A.; D'Alfonso, A.; Zanoni, G.; Nolan, S. P. *Organometallics* **2010**, 29(16), 3665

[40] Gaillard, S.; Bosson, J.; Ramón, R.S.; Nun, P.; Slawin, A.M.Z.; Nolan, S.P. *Chem. Eur. J.* **2010,** 16 (2010), 13729.

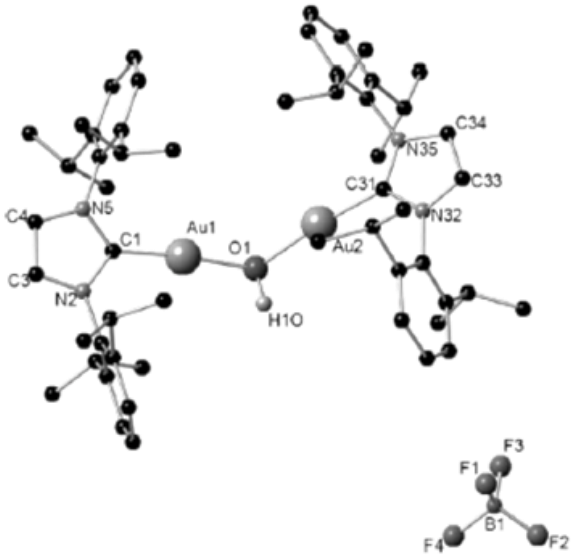
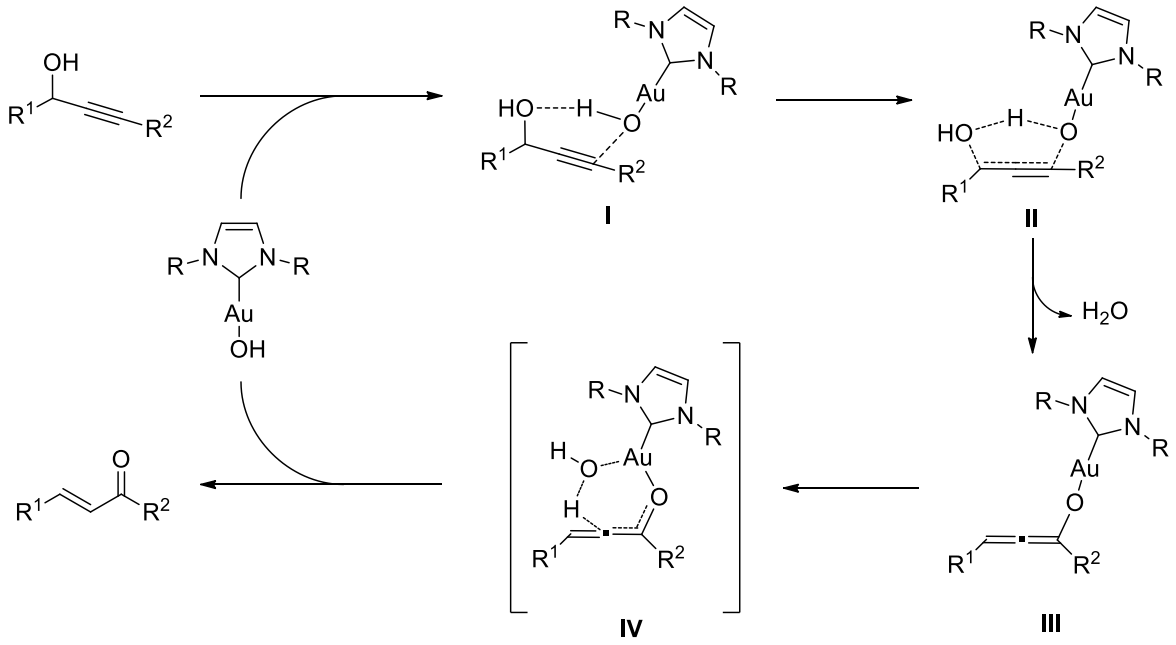


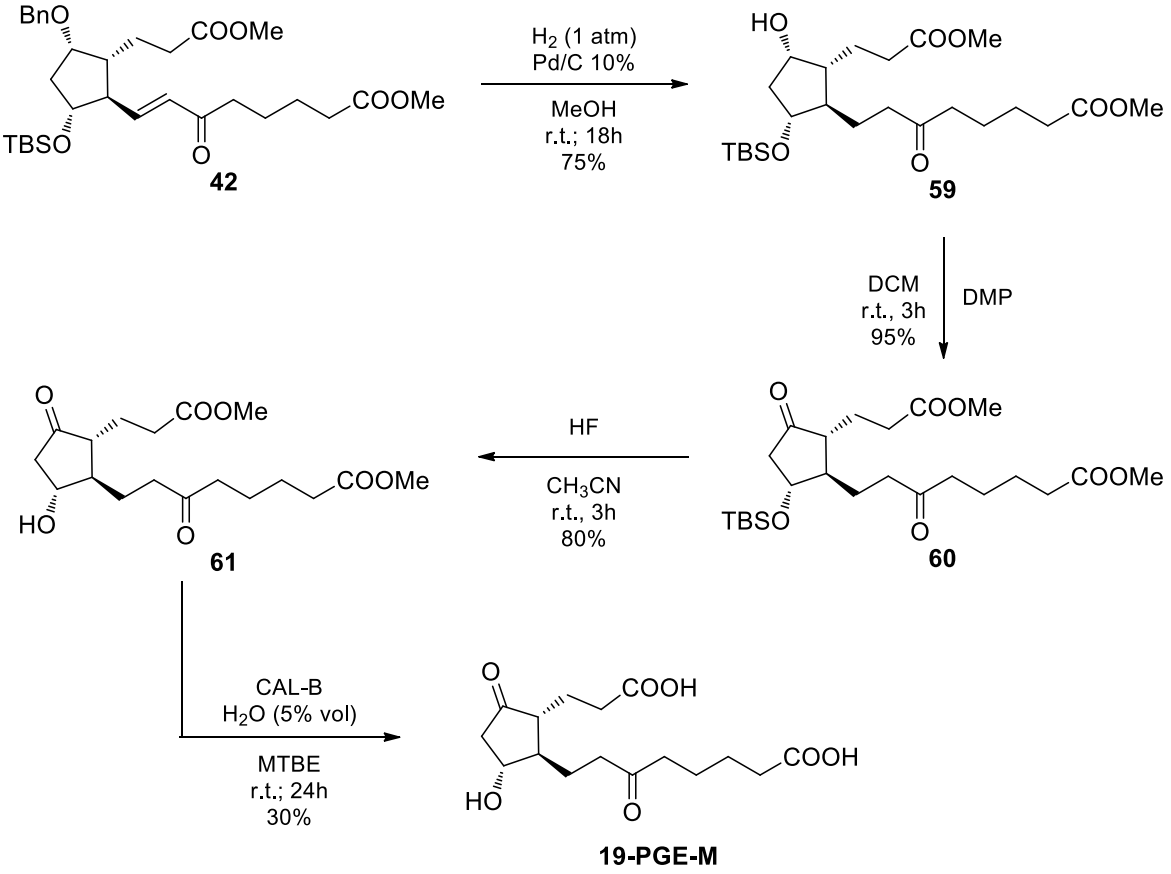
FIGURE 7[40]



SCHEME 23

The next steps of the synthesis of PGE-M involved the hydrogenation of the enone **42** that allowed the reduction of the double bond on the side chain together with the cleavage of the benzyl group leading to **59** as free alcohol. The subsequent oxidation of **59** with Dess-Martin periodinane afforded the diketone **60** that was subjected to the removal of the sylil

ether with HF. The hydrolysis of the diester **61** to give the desired prostaglandin E2 urinary metabolite was performed in an enzymatic fashion with Candida Antarctica lipase B in presence of water. The yield of this last step was not as high as expected, probably the upper side chain is too short to interact well with the active site of the enzyme.



SCHEME 24

CONLUSIONS

With this work, we developed the first example of stereoselective synthesis of the prostaglandin E_2 urinary metabolite based on a three-component approach that employ the Corey Aldehyde as principal building block. Our strategy brings an improvement over others examples previously reported in literature based on resolution of racemic mixture.

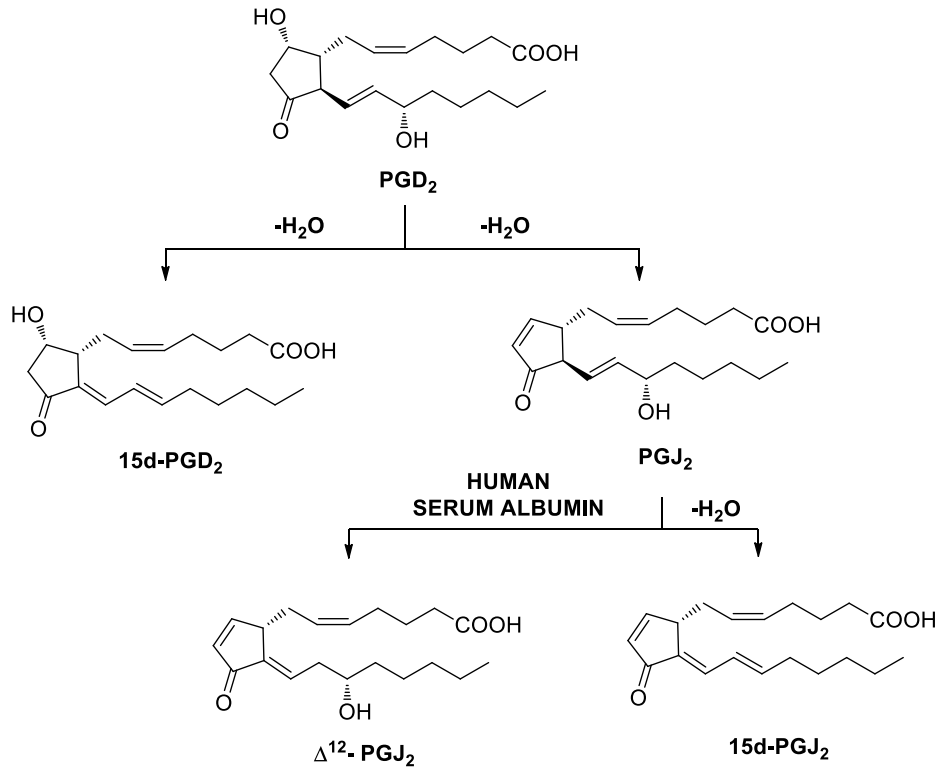
A particularly interesting aspect of our synthesis is the way in which the ω-chain has been introduced. The key step of the route is the gold (I) catalyzed Meyer-Schuster rearrangement of the propargylic alcohol **43** which allowed the introduction of the carbonyl function in the right position on the chain.

Moreover, we have improved a methodology already known in the literature[41] for the introduction of the α-chain thanks to a mild two steps oxidation of the alcohol **46.**

[41] Lin, C.H. *J. Org. Chem.* **1976**, 41(25), 4045

15-DEOXI-$\Delta^{12,14}$-PROSTAGLANDIN J_2

15-deoxy-$\Delta^{12,14}$-prostaglandin J2 (15d-PGJ_2) is a cyclopentenone-type prostaglandin with a wide spectrum of physiological activities. Like all the other prostaglandins of the J_2 series, 15d-PGJ_2 is a product of nonenzymatically dehydration of prostaglandin D_2 (PGD_2). Shibata in 2002 studied the metabolism of PGD_2 demonstrating that PGJ_2 is directly converted into 15d-PGJ_2 in a nonenzymatically fashion rather than in a two steps way passing by Δ^{12}-PGJ_2 (*scheme 25*)[42].



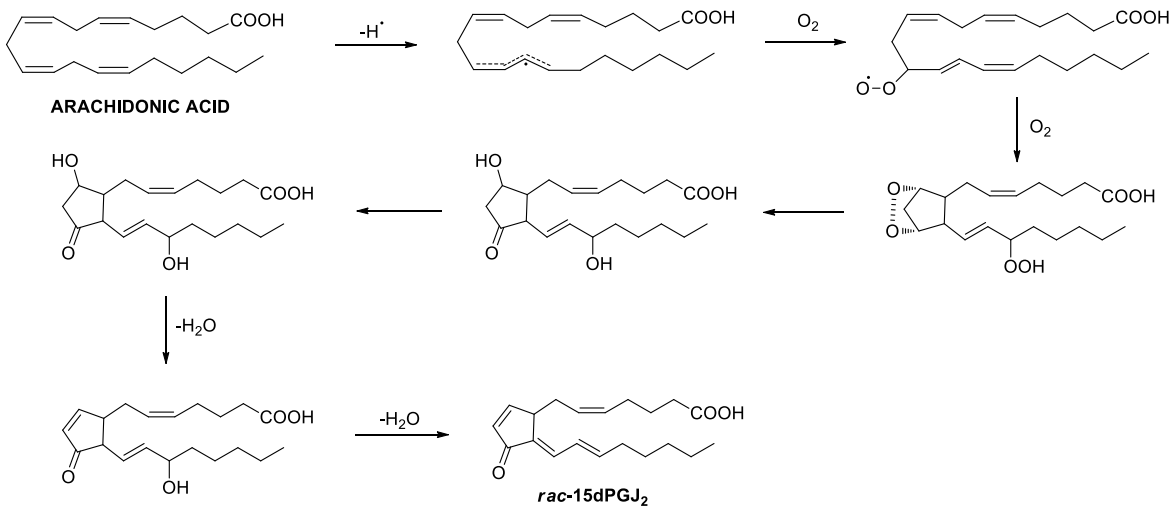
SCHEME 25

The polyunsaturated carbonyl moiety makes the 15d-PGJ_2 very reactive against nucleophiles especially thiols such as glutathione (GSH) and cysteine residues. The electrophilicity of 15d-PGJ_2 makes this compound biologically active.

[42] Shibata, T; Kondo, M; Osawa, T; Shibata, N; Kobayashi, M; Uchida, K. *J. Biol. Chem.* **2002**, 277, 10459.

15d-PGJ_2 is known as a potent ligand for the peroxisome proliferator-activated receptor γ (PPARγ) through Michael addition of cysteine-258 in the ligand-binding domain[43]. PPARγ is a member of the nuclear receptor superfamily and a ligand-activated transcription factor that regulate the glucose homeostasis, adipocyte differentiation and lipid metabolism[44]. In addition 15d-PGJ_2 exert potent anti-inflammatory activity repressing the NF-κB transcription factor with a PPARγ dependent mechanism [45] . Moreover, has been demonstrated that 15d-PGJ_2 inhibit the inflammatory response blocking the gene transcription covalently binding the critical cysteine residues in I*κ*B kinase and the DNA binding domain of NF-*κ*B[46].

In 2013 Liu and coworkers found that after ischemic attack the production of cyclopentenone D_2 and J_2 series prostaglandins is increased in rat brain. In particular, 15d-PGJ_2 is the most abundant cyclopentenone prostaglandin in post-stroke tissues[47].



SCHEME 26

15d-PGJ_2 can be considered a product of lipid peroxidation of arachidonic acid. A wide series of 15d-PGJ_2-like compounds is formed in vivo under oxidative stress conditions[48].

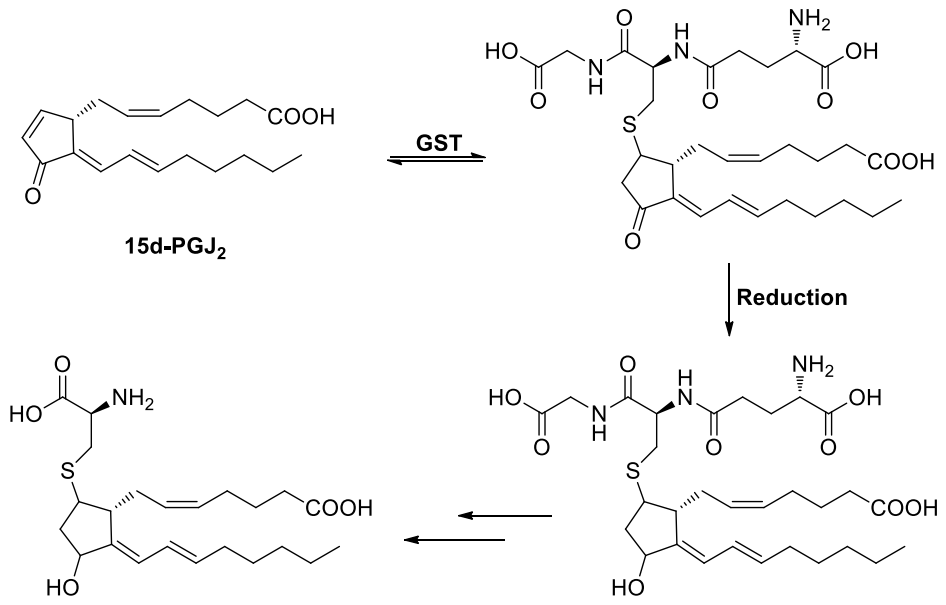
[43] Forman, B.M.; Tontonoz, P.; Chen, J.; Brun, R.P.; Spiegelman, B.M.; Evans, R.M. *Cell.* **1995**, 83, 803.
[44] Kliewer, S.A.; Lenhard, J.M.; Willson, T.M.; Patel, I.; Morris, D.C.; Lehmann J.M. *Cell.* **1995**, 83, 813.
[45] Straus, D.S.; Glass, C. *Med. Res. Rev.* **2001**, 21, 3,185.
[46] Straus, D. S.; Pascual, G.; Li, M., Welch, J. S.; Ricote, M.; Hsiang, C. H.; Sengchanthalangsy, L. L.; Ghosh, G.; Glass, C. K. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 4844.
[47] Liu, H.; Li, W.; Ahmad, M.; Rose, M. E.; Miller, T.E.; Yu, M.; Chen, J.; Pascoe, J.L.; Poloyac, S.M.; Hickey, R.W.; Graham, S. H. *Neurotox Res.* **2013**, 24, 191.
[48] Hardy, K. D.; Cox, B. E.; Milne, G. L.; Yin, H.; Roberts, L. J. *J. Lip. Res.* **2011**, 52, 113.

The abstraction of a bisallylic hydrogen from the carbon 13 of arachidonic acid and the addition of a molecule of oxygen to form a peroxyl radical, the radical undergoes 5- *exo* cyclization, and a second molecule of oxygen is added to the backbone of the compound to form PGG_2 -like compounds. This unstable intermediate can undergo spontaneous rearrangement affording the 15d-PGJ_2 as racemic mixture of all the possible stereoisomer (*scheme 26*).

15-DEOXI-$\Delta^{12,14}$-PROSTAGLANDIN J_2 METABOLISM

The electrophilicity of 15d-PGJ_2 render this molecule biologically active, but its high reactivity makes difficult to determine the extent the extent to which it is formed in cells and in vivo in humans and animals. In 2003 Bell-Parikh and colleagues measured the concentration of free 15d-PGJ_2 in urine, finding it in a very small amount not representative of the systemic production[49]. This finding can be explained with the high reactivity of the 15d-PGJ_2.

Despite the high interest in the biological activity, to date, the whole metabolic pathway is not clear yet.

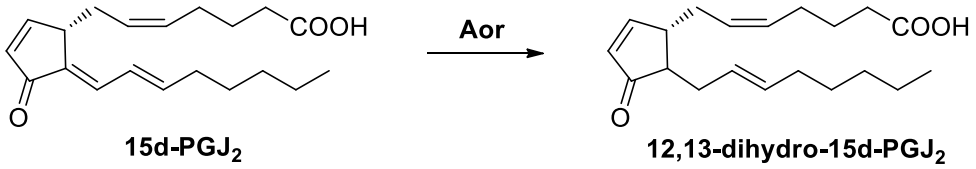


SCHEME 27

[49]Bell-Parikh, L. C.; Ide, T.; Lawson, J. A.; McNamara, P.; Reilly, M.; FitzGerald, G. A. *J. Clin. InVest.* **2003**, *112*, 945.

Prof. Morrow in collaboration with our research group find that 15d-PGJ_2 is metabolized by conjugation with glutathione (GSH) by human hepatocytes HepG2, which contains glutathione transferases (GSTs)[50]. The glutathione derivatives are subsequently converted into cysteine conjugates by hydrolysis of glycine and glutamic acid residues and reduction of the carbonyl on the carbon 11 into hydroxyl group (*scheme 27*).

Another study by Yu demonstrated that 15d-PGJ_2 can be metabolized by alkenal/one oxidoreductase (Aor) in rat[51]. Aor hydrogenates the double bond on carbon 12 reducing the reactivity of the metabolite (*scheme 28*).



15d-PGJ_2 **12,13-dihydro-15d-PGJ_2**

SCHEME 28

Prof. Morrow showed that, in experimental animals, the infusion of 15-A_{2t}IsoP results in the excretion of the corresponding polar and water-soluble *N*-acetyl cysteine sulfoxide conjugate[52]. Thus, it is possible to affirm, according with Morrow, that "In addition to conjugation with GSH, it is also reasonable to consider that 15-d-PGJ_2 may undergo metabolism in vivo to other polar metabolites including glucuronide conjugates or via β or ω-oxidation as is observed for other PGs"[49]. It is then probably that the structure of the principal urinary metabolite of 15-d-PGJ_2 is similar to which proposed in *figure 6*.

[50] Brunoldi, E. M.; Zanoni, G.; Vidari, G.; Sasi, S.; Freeman, M. L.; Milne, G. L.; Morrow, J.D. *Chem Res Toxicol* **2007**, 20, 1528.

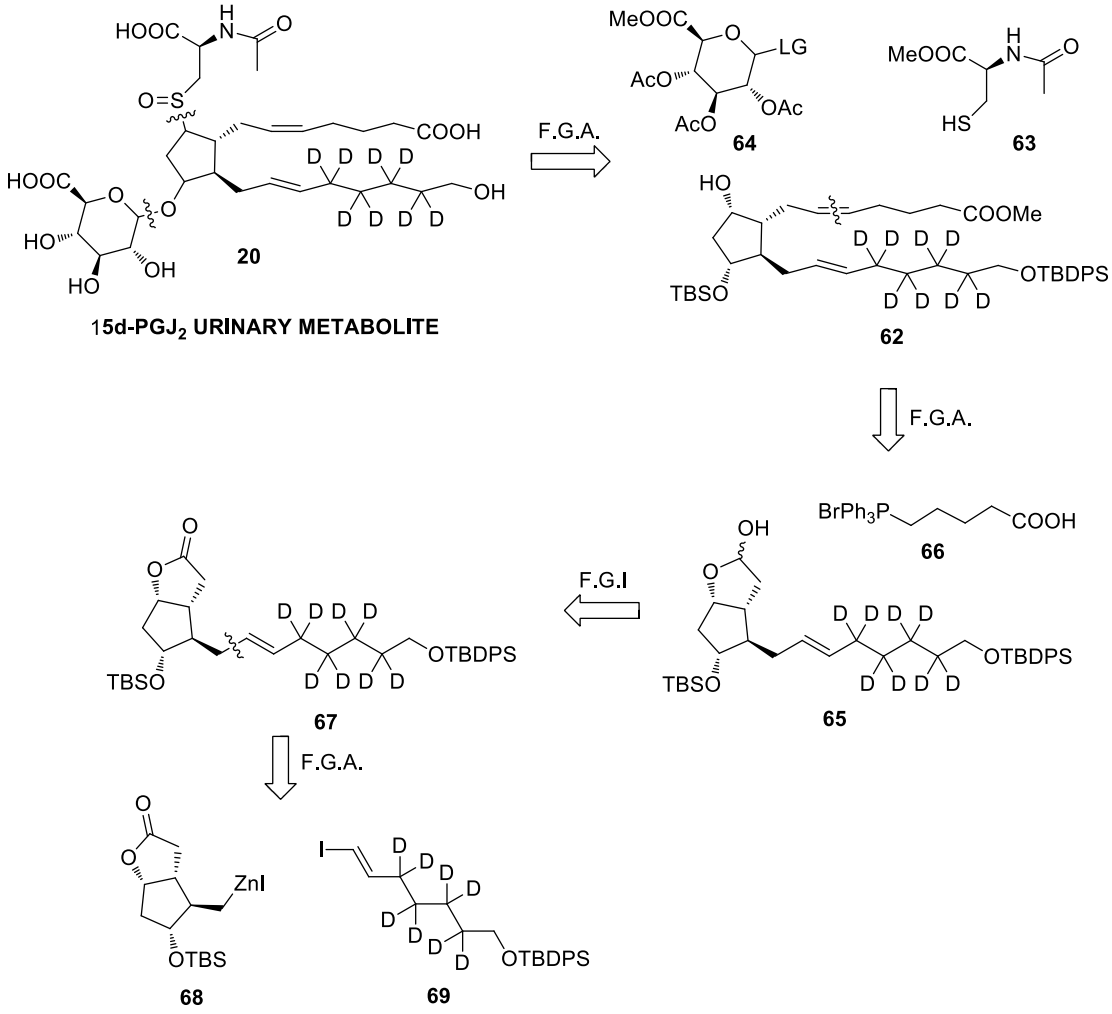
[51] Yu, X.; Egner, P.A.; Wakabayashi, J.; Wakabayashi, N.; Yamamoto, M.; Kensler, T.W. *J. Biol. Chem.* **2006**, 281(36), 26245.

[52] Milne, M.L.; Ling Gao, L.; Porta, A.; Zanoni, G.; Vidari, G.; Morrow, J.D. *J. Biol. Chem.* **2005**, 280(26), 25178.

RETROSYNTHETICAL ANALYSIS

For the synthesis of the 15dPGJ$_2$-UM we thought to a five components approach based on the use of the Corey Aldehyde that provide the right configuration of the stereogenic centers. The unusual position of the double bond on the ω-chain, on the carbon 14 instead of carbon 13 as usual in prostaglandins, suggest to employ a Negishi cross coupling for the introduction of the ω-chain.

The five principal building blocks can be easily identified applying the right disconnection on the target molecule **20** like showed in the *scheme 29*.

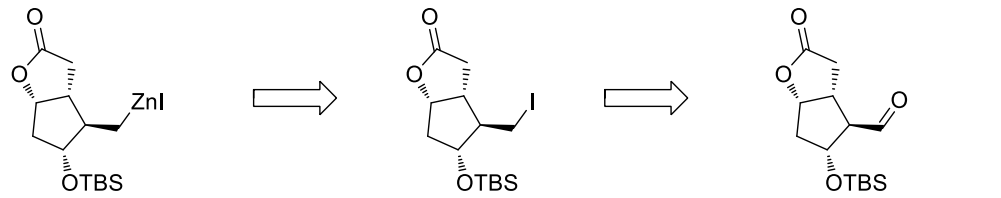


SCHEME 29

Disconnecting the O-glycosylic bond and that between the cyclopentanic ring and the sulfoxide we can identify the synthons **62**, **63**, **64** of the prostaglandin skeleton, the cysteine moiety and the glycosylic portion respectively. The synthon **62** can be bring back to the

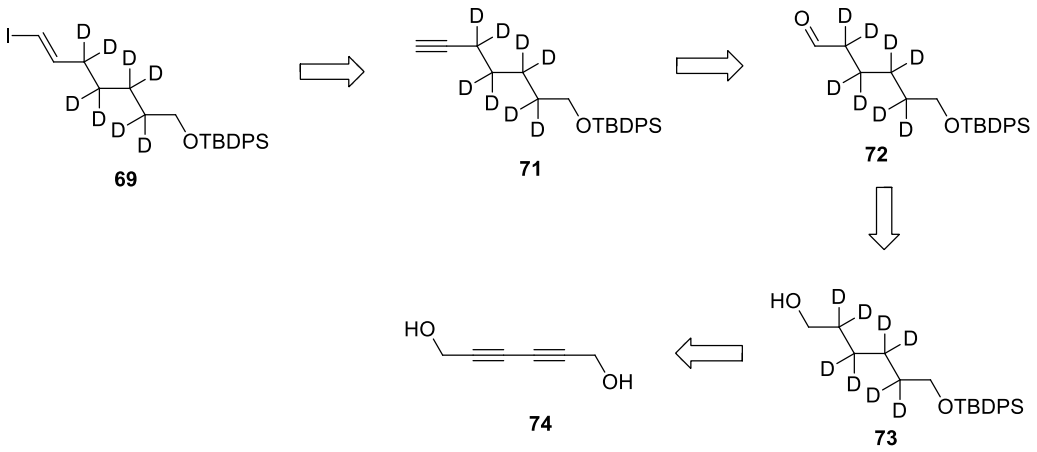
sulfonium salt **66** and the lactol **65** alpplying a retro Wittig olefination. A retro reduction on the lactol **66** afford the lactone **67**. Then a retro Negishi coupling bring back to the zinc derivative **68**, principal building block for the cyclopentanic core, and the vinyl iodide **69** as precursor of the ω side chain.

A series of functional group interconversion on the lactone **68** leads to the famous **50-TBS-Corey Aldehyde** (*scheme 30*).



SCHEME 30

The vinyl iodide **69** with a functional group interconversion is brought back to the terminal alkyne **71** from which a retro Ohira-Bestmann homologation allowed us to identify the synthon **72**. Finally a functional group interconversion and a functional group addition bring back the aldehyde **72** to the diol **74** (*scheme 31*).



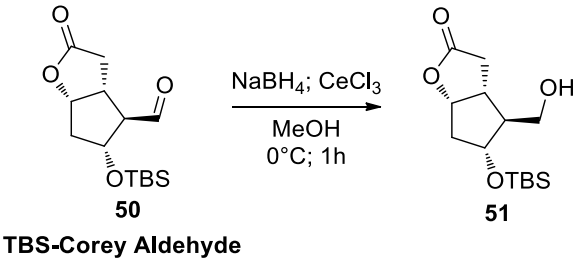
SCHEME 31

INTRODUCTION OF THE ω-CHAIN

NEGISHI COUPLING APPROACH

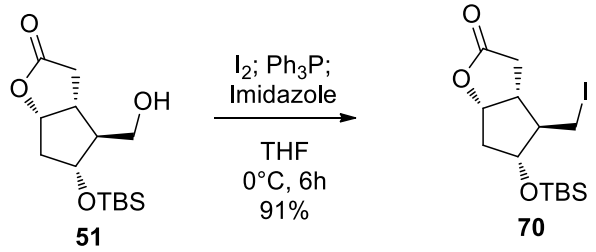
The main feature of this approach is the way in which we have tried to introduce the ω-chain; firstly we tried an organometallic approach based on Negishi coupling. For do this it is necessary to synthesize iodine-derivative of Corey lactone starting from the readily commercial available aldehyde **50**.

The first step of the synthesis, analogously to the synthesis previously explained of the PGE-M, involves the reduction of TBS-Corey Aldehyde with sodium borohydride under Luche condition (*scheme 32*).



SCHEME 32

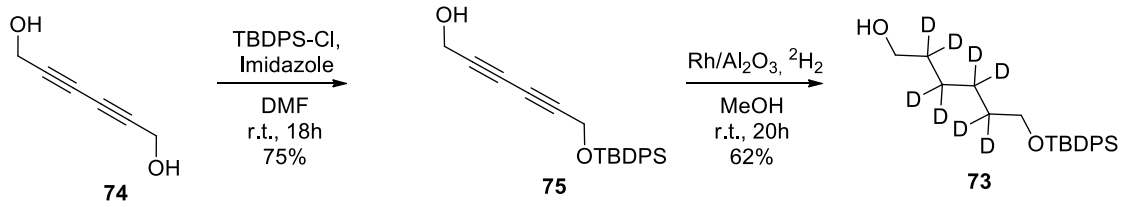
Subsequently alcohol **51** was transformed into iodide derivative **70** by treatment with iodine using Mitsunobu-like condition (*scheme 33*), the reaction gave desired iodide in highly satisfactory yield and with high purity.



SCHEME 33

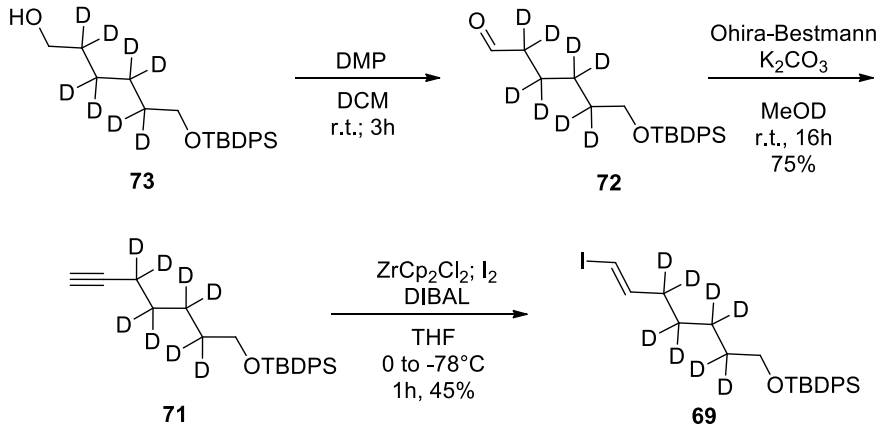
The side chain building block **69** is prepared starting from 2,4-hexanediyne-1,6-diol **74**. The diol is transformed selectively and in good yield in the mono *tert*-butyldiphenylsylil-

derivative (TBDPS) **75**. subsequently compound **75** has been subjected to reduction of the two triple bonds under deuterium atmosphere in the presence of rhodium on activated alumina as catalyst (*scheme 34*).



SCHEME 34

The alcohol **73** is then oxidized to aldehyde **72** with Dess-Martin periodinane enabling the homologation of the chain transforming the C-O double bond into a C-C triple bond with Ohira-Bestmann reagent[53] (*scheme 35*). This reaction, a modification of the Seyferth-Gilbert homologation, uses a dimethyl (1-diazo-2-oxopropyl) phosphonate (*figure 8*). In the *scheme 36* is reported the mechanism of action of the Ohira-Bestmann homologation[54]. Is important in this step to use deuterated methanol as solvent in order to prevent the loss two deuterium atoms in the α position with respect to the aldehyde.

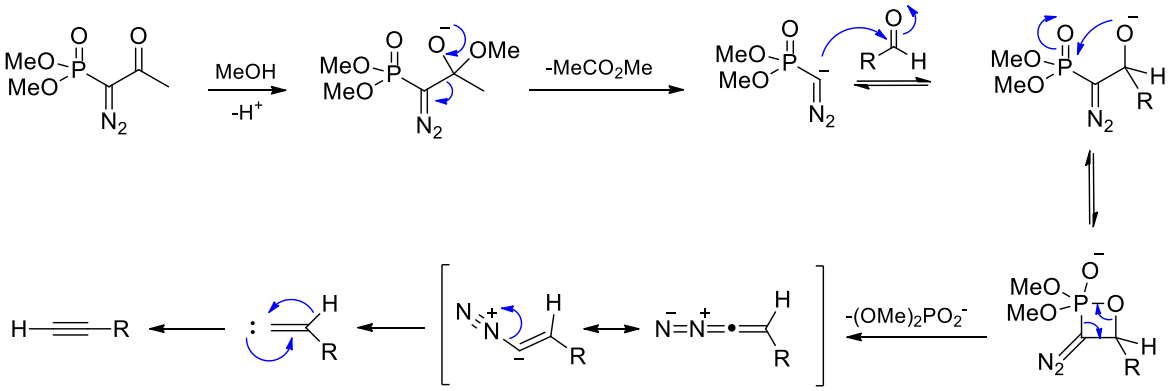


SCHEME 35

[53] (a) Ohira, S. *Synth. Commun*. **1989**, 19, 561. (b) Müller, S.; Liepold, B.; Roth, G.J.; Bestmann, H.J.; *Synlett* **1996**, 521. (c) Roth, G.J.; Liepold, B.; Müller, S.G.; Bestmann, H.J. *Synthesis* **2004**, 59.
[54] Gilbert, J. C.; Weerasooriya, U. *J. Org. Chem.*, **1982**, *47*, 1837-1845.

Ohira-Bestmann Reagent Schwartz reagent

FIGURE 8

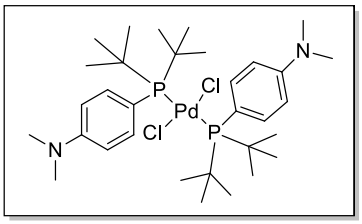


SCHEME 36

The terminal alkyne **71** is then transformed into *E*-vinyl iodide **69** by treatment with Schwartz's reagent (*figure 8*) and iodine. The zirconocene chloride hydride was generated *in situ* replacing with DIBAL a chloride ligand of zirconocene dichloride with a hydride[55].

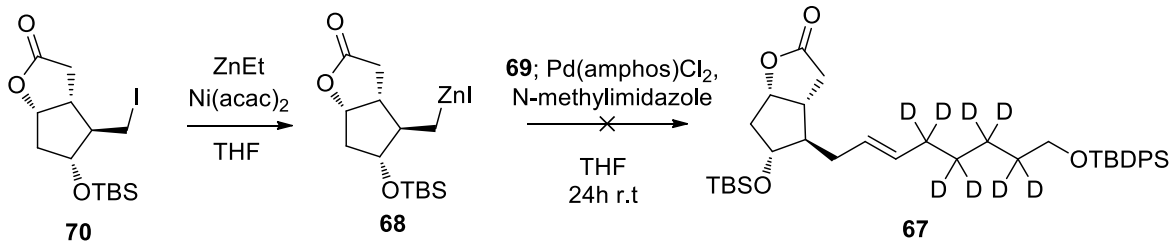
The sp^2-sp^3 cross-coupling reported by Negishi[56] decades ago, needs the synthesis of the organozinc intermediate **68**. The Zn oxidative insertion in the C-I bond and the cross-coupling were carried out in a one pot/two steps fashion. The formation of the organozinc derivative was prepared following the Knochel protocol[57a], procedure used in the past by our research group on a similar lactone[57b]. It involves the treatment of Iodide **70** with diethylzic in the presence of a catalytic amount of $Ni(acac)_2$. The subsequent palladium catalyzed Negishi coupling was performed mixing the organozinc previously prepared with *E*-vinyl iodide **69** and $PdCl_2(Amphos)_2$ as catalyst (*figure 9)*. N-methylimidazole were used to provide the retention of configuration of the resulting olefin in according with Lipshutz protocol[58].

[55] Wang, G; Mohan, S.; Negishi, E.-I. *Proc. Natl. Acad. Sci. USA* **2011**, 108(28), 11344.
[56] Negishi, E.-I.; King, A.O.; Okukado, N. *J. Org. Chem.* **1977**, 42, 182.
[57] (a) Vettel, S.; Vaupel, A.; Knochel, P. *J. Org. Chem.* **1996**, 61, 7473. (b) Zanoni, G.; Porta, A.; Brunoldi, E.; Vidari, G. *J. Org. Chem.* **2006**, 71 (22), 8459.
[58] Krasovskiy, A.; Lipshutz B.H. *Org. Lett.* **2011**, 13(15), 3822.



Pd(amphos)Cl_2

FIGURE 9

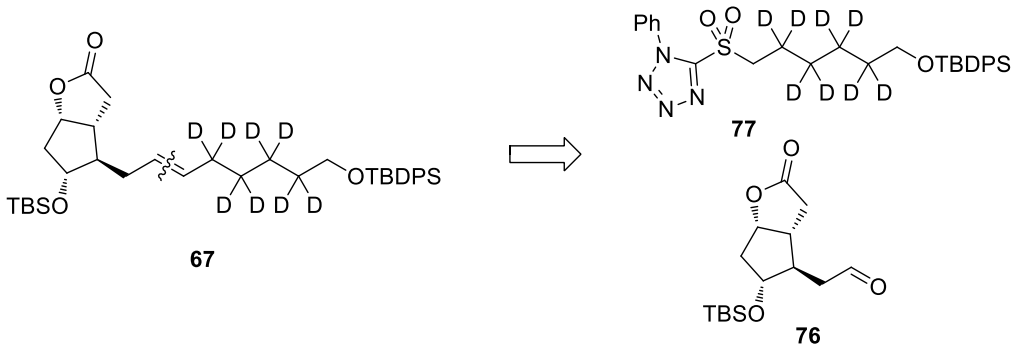


70 **68** **67**

SCHEME 37

Unfortunately, after several attempts we were never able to isolate the desired product (*scheme 37*). Furthermore, even after several trials we have never been able to determine if the problem was due to the formation of organozinc or to the cross-coupling reaction. For these reasons we decided to change our approach to the syntehsis.

JULIA-KOCIENSKI APPROACH



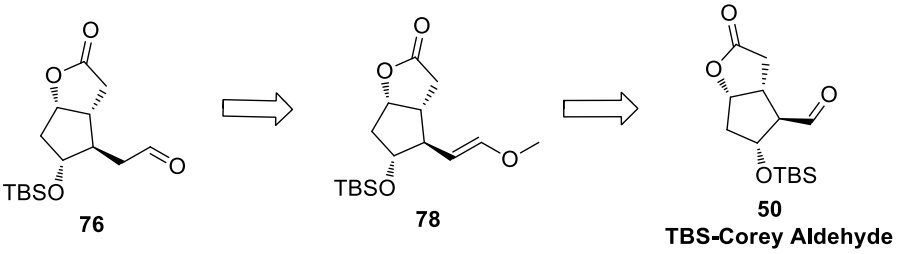
67 **77** **76**

SCHEME 38

Carefully observing the synthon **67** we thought to modify the synthetic approach by disconnecting directly the double bond, rather than the bond between the CH_2- and the olefinic system of the side chain. A retro Julia-Kocienski allows us to identify the two

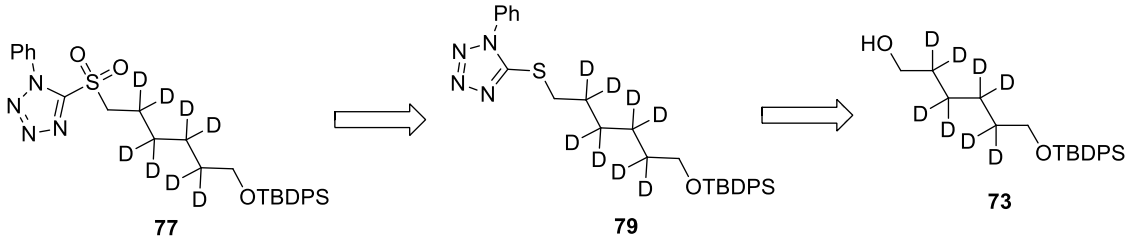
synthons necessary for the introduction of the ω chain: the aldehyde **76** and the sulfone **77** (*scheme 38*).

The aldehyde **76** can be easily prepared from TBS-Corey aldehyde applying first a functional group interconversion and then a retro Wittig olefination (*scheme 39*).



SCHEME 39

The sulfone instead can be traced back to the **79** sulfide through a retro oxidation. A retro Mitsunobu on the sulfide brings us back to the already known alcohol 55 (*scheme 40*).

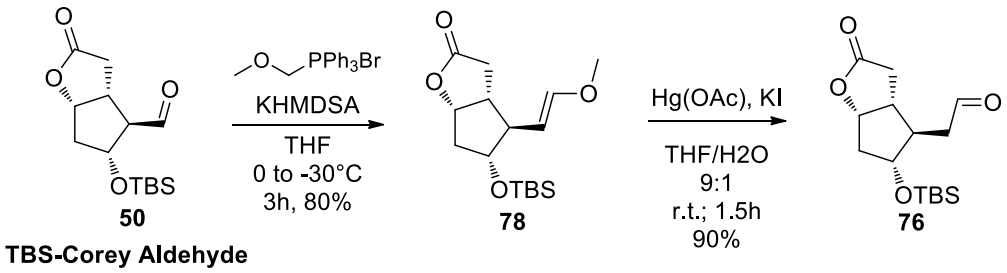


SCHEME 40

The introduction of the side chain forming the double bond requires homologation (with an additional carbon atom) of the branch that brings the aldehyde function and the use of an olefination reaction that leads to the selective formation of a double bond with E geometry.

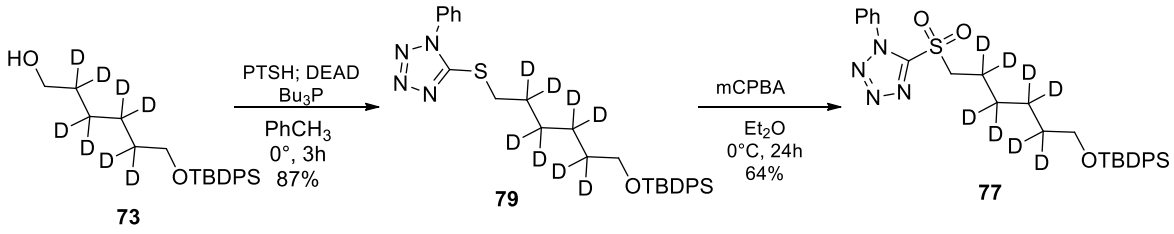
The homologation of the Corey Aldehyde was carried out by the introduction of an additional carbon with a Wittig olefination using methoxymethyltriphenilphosphonium bromide forming the enolether WW. The hydrolysis catalyzed by mercury (II) acetate in aqueous THF give the desired aldehyde **76** (*scheme 41*)[59].

[59] (a) Derrick L. J. Clive, D. L. J.; Cheng, H.; Gangopadhyay, P.; Huang, X.; Prabhudas, B. *Tetrahedron* **2004**, 60, 4205. (b) Larock, R. C.; Hsu, M. H.; Narayanan, K; *Tetrahedron* **1987**, 43(13), 2891.



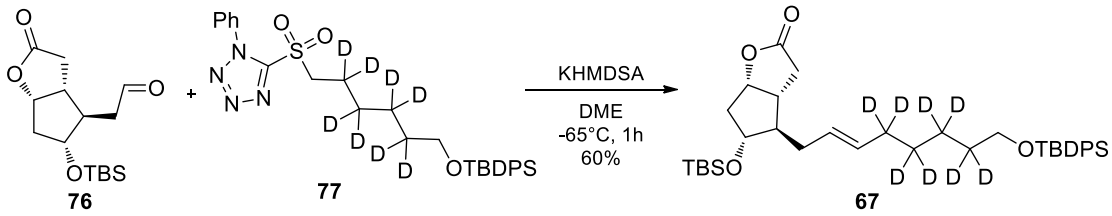
SCHEME 41

The deuterated chain was prepared from the intermediate **73**, which was converted into sulfide **79** using a classical Mitsunobu reaction[60] with 1-phenyl-1H-tetrazole-5-thiol (PTSH) as a soft nucleophyle. The resulting thioether **79** was then oxidized to sulfone **77** m-chloroperbenzoic acid (*scheme 42*).



SCHEME 42

Finally, the Julia olefination performed using a sterically hindered base with potassium as counter ion and 1,2-dimethoxyethane as a solvent provides the desired product with only stereochemistry E around double bond.

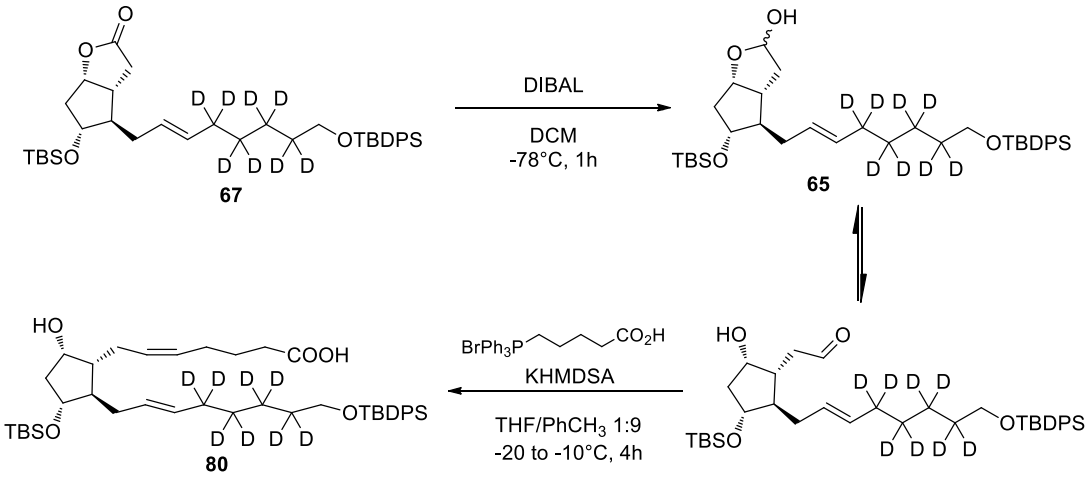


SCHEME 43

[60] Matteo Valli, M.; Bruno, P.; Sbarbada, D.; Porta, A.; Vidari, G.; Zanoni, G. *J. Org. Chem*. **2013**, 78, 5556.

INTRODUCTION OF THE α-CHAIN

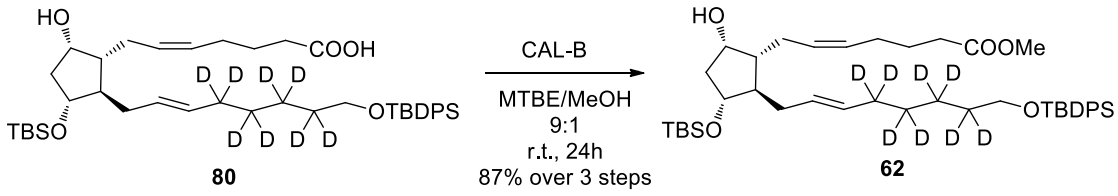
The installation of α chain was then performed followed the classic approach[9]. The reduction of the lactone **67** with DIBAL-H gave lactol **65**, which upon Wittig condensation with 4-carboxybutyl)-triphenylphosphonium bromide delivered carboxylic acid **80** as a single Z stereoisomer (*Scheme 44*).



SCHEME 44

The wittig olefination was carried out treating lactol **65** with the ylide generated from the phosphonium salt and KHMDS (4.8 equiv) in THF/toluene (1:9), from -20°C to -10°C for 4h. These reaction conditions were fine tuned in the past by our laboratory for the synthesis of PGs of the F and E type in order to avoid the 1,5-TBS shift[39].

Exposure of carboxylic acid **80** to Candida Antarctica Lipase B in MTBE/MeOH 9:1 afforded methyl ester **62** in high yield (*scheme 45)*.

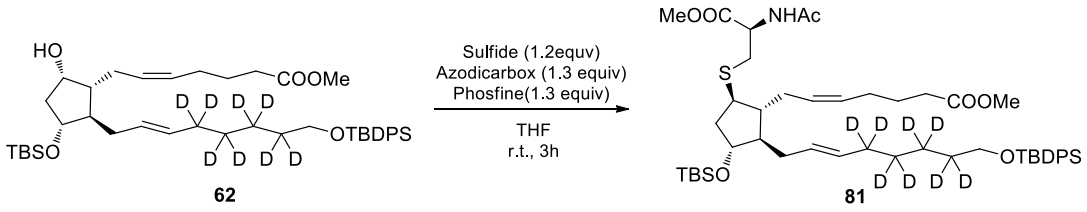


SCHEME 45

INTRODUCTION OF THE N-ACETYLCYSTEINE MOIETY

CYSTEINE ADDITION VIA MITSUNOBU REACTION

The easiest way for the addition of the N-acetylcystein methyl ester minimizing the number of steps is a nucleophilic substitution on the hydroxyl group of **62**. However, the structure of this intermediate does not allow to exploit the classical methodology, transforming the hydroxyl function into good leaving group and then replace it with a nucleophilic attack by the thiol or thiolate of cysteine substructure. The elimination reaction, in competition with the nucleophilic substitution, would lead to the formation of a stable trisubstituted olefin, favored compared to the substitution product. For this reason we have focused our attention on the Mitsunobu reaction, which due to its mechanism of action and to mild reactions conditions, reduces the risk of elimination as a side reactions. The tests performed and reaction conditions used are listed in the following table (*table 2*).



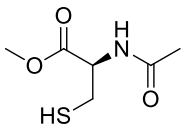
Entry	Sulfide	Diazo-Comp.	Phosphine	Yield (%)
1[a]	N-acetyl-L-Cystine methyl ester	\\	nBu_3P	0
2[b]	N-acetyl-L-Cysteine methyl ester	DEAD	Ph_3P	0
3[b]	N-acetyl-L-Cysteine methyl ester	DEAD	nBu_3P	0
4[b]	N-acetyl-L-Cysteine methyl ester	DIAD	Ph_3P	0
5[b]	N-acetyl-L-Cysteine methyl ester	DIAD	nBu_3P	0

a) N-acetyl-L-Cystine dimethyl ester 3 equiv.; nBu_3P 3 equiv.
b) Nacetyl-L-cysteine methyl ester 1.2 equiv.; Phosphine 1.3 equiv.; Azadicarboxylate 1.3 equiv.

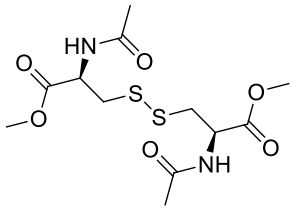
Table 2

In the first entry we performed the reaction using the N-acetyl-L-cystine methyl ester (dimer of N-acetyl-L-cysteine), in the presence of n-tributyl phosphine. In the others four entries were screened all the possible combination between the two azodicarboxylate,

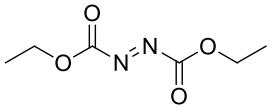
diethyl azodicarboxylate (DEAD) and diisopropyl azodicarboxylate (DIAD), and the two phosphine, triphenylphosphine and tributylphosphine, employing N-acetyl-L-cysteine methyl ester as sulfide surce. The structures of the two sulfide the azodicarboxylate used for the trials are showed in *figure 10*. The two sulfide were prepared starting from commercial available N-acetyl-L-cysteine and L-Cystine dimethyl ester dihydrochloride following the procedure published by Matile[48a] and Serafinowski[61b].



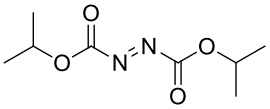
N-acetyl-L-Cysteine methyl ester



N-acetyl-L-Cystine di methyl ester



Diethyl azodicarboxylate (DEAD)

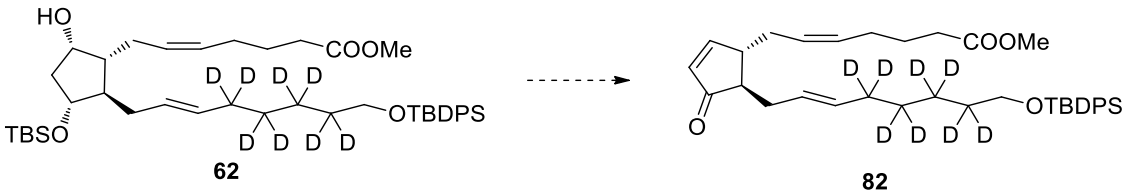


Diisopropyl azodicarboxylate (DIAD)

FIGURE 10

Unfortunately, the tests carried out have not brought the desired results. Indeed, in none of the tests trace of the desired product was isolated. It is reasonable that the steric hindrance due to the α-side chain forbid the substitution.

The negative results obtained made it necessary to change the synthetic approach. It has therefore decided to use a more safety Thia-Michael reaction, typical reaction of α,β-unsaturated carbonyl substartes. For do this we have faced a very challenging conversion of the cyclopentanol **62** into cyclopentenone **82** (*figure 11*).



62 **82**

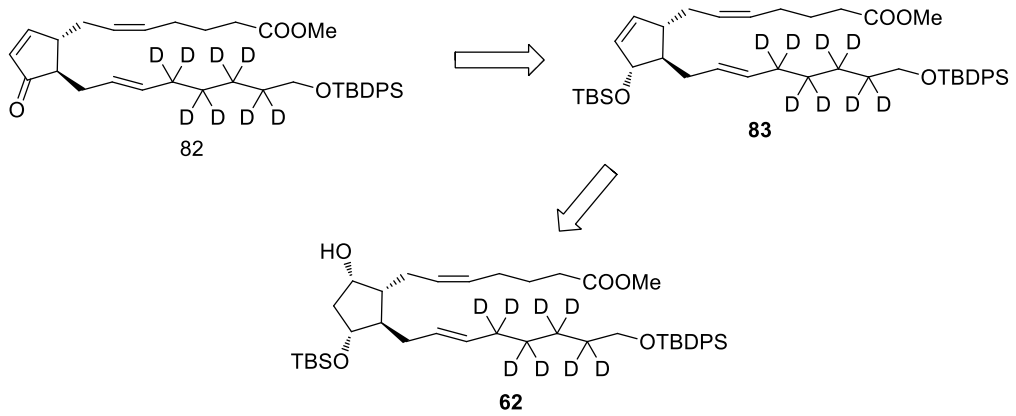
FIGURE 11

[61] a) Bang, E.; Gasparini, G.; Molinard, G.; Roux, A.; Sakai, N.; Matile, S. *J. Am. Chem. Soc.* **2013**, 135(6), 2088. b) Serafinowski, P.; Dorland, E.; Harrap, K.R. *J. Med. Chem*. **1992**, 35, 4575.

CYCLOPENTENONE SYNTHESIS

DIRECT DEHYDRATION

The first approach we purposed traces the cyclopentenone **82** back to cyclopentanol **62** passing by allylsylil ether **83**, like showed in the following brief retrosynthetical *scheme 46*.



SCHEME 46

This strategy involves a tricky elimination step. Indeed, a classic S_{n2} reaction can not be performed, in accordance with the Zaitsev rule would be formed primarily the more substituted olefin, that is the double bond between carbons 8 and 9 and not from 9 to 10 as in the desired compound (*figure 12*).

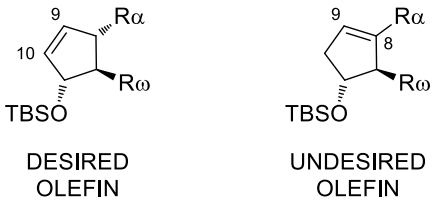
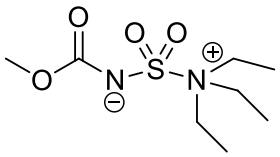


FIGURE 12

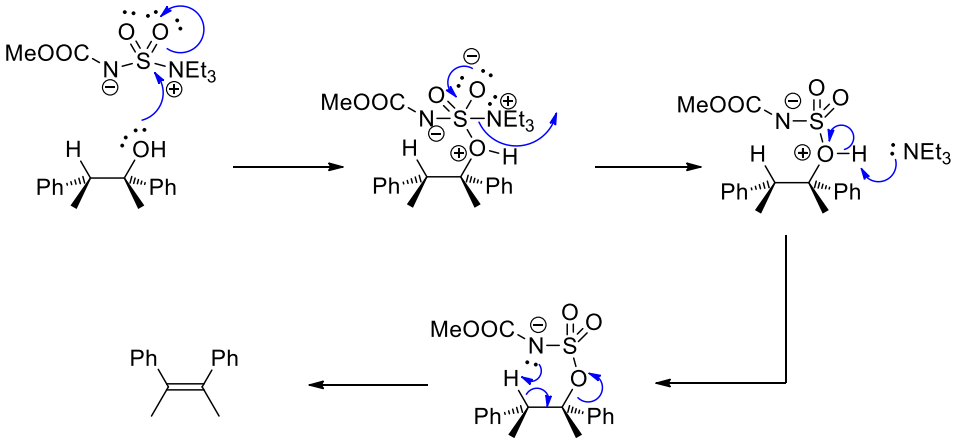
An accurate search in literature has allowed us to identify the Burgess reagent (*figure 13*) as a suitable method able to perform dehydration of the compound **62** with removal of the β *syn*-proton with respect to the hydroxyl group. The structure and the mechanism of action of the Burgess inner salt is showed in *scheme 47* [62].

[62] Burgess, E. M.; Penton, H. R. Jr., Taylor, E. A. *J. Org. Chem*. **1973**, 38, 26.



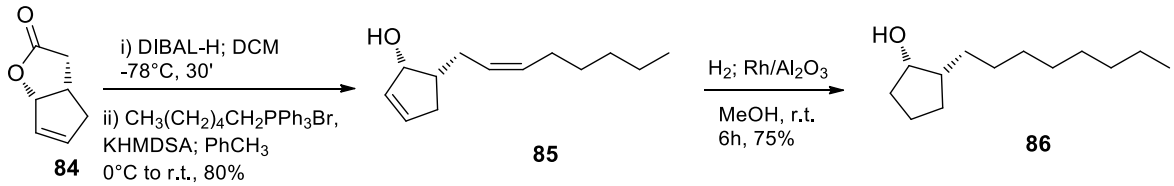
Burgess reagent

FIGURE 13

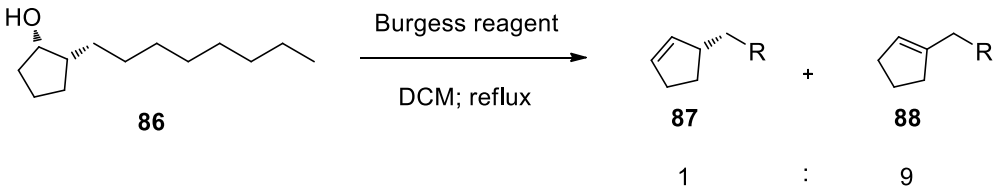


SCHEME 47

The reaction was first tested on a model molecule easily synthesized from Curran lactone RR in three steps (reduction to lactol, Wittig olefination, hydrogenation). The reaction conditions are showed in the *scheme 48*. After synthetizing the model molecule we immediately tested the dehydration reaction refluxing the cyclopentenone with Burgess inner salt in dichloromethane. The GC-MS and NMR analysis of the recovered product showed that the reaction afforded a mixture of olefins in 9:1 ratio in favor to the undesired one (*scheme 49*). It is reasonable that the triethylamine which is liberated during the reaction promotes the elimination according to the classic mechanism.



SCHEME 48

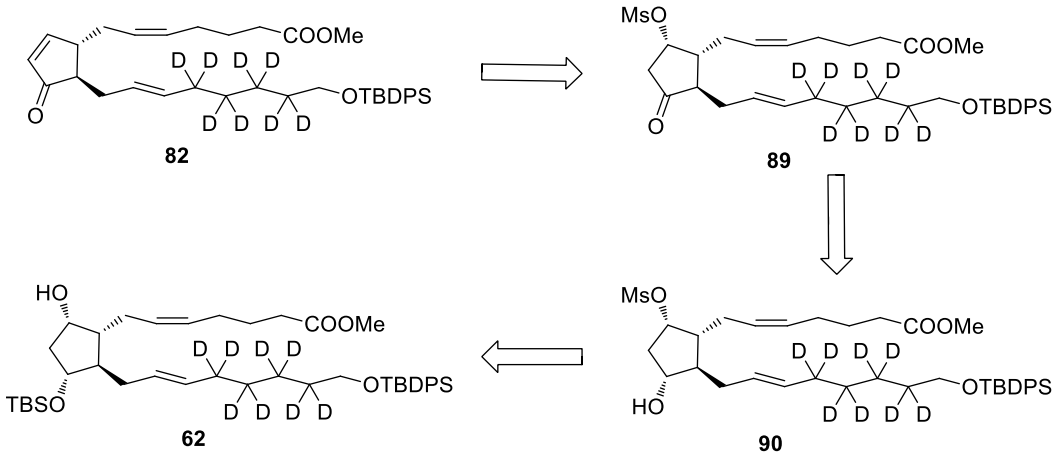


SCHEME 49

Also in this case the results have not been encouraging and it was necessary to modify the synthesis again.

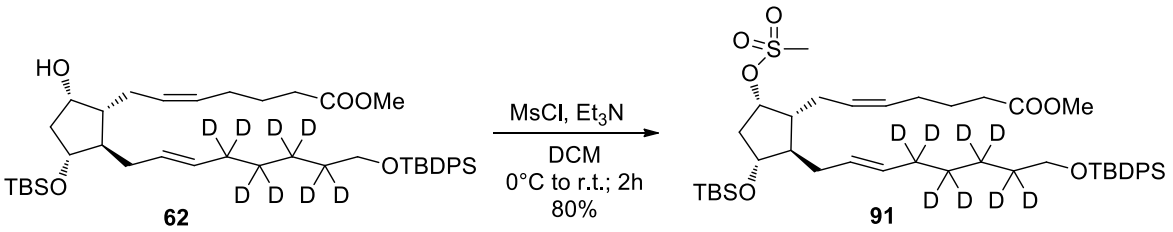
METHANESULPHONYL DERIVATIVE

In this new route for the synthesis of the cyclopentenonic system (*scheme 50*) we have thought to use, as driving force of the elimination, the effect of conjugation with the carbonyl in position 11 of the compound **89**. This intermediate can be obtained from compound **90** which can be easily brought back to cyclopentanol **62**. This strategy extends the synthesis and involves quite difficult passages, but it ensures the formation of the right cyclopentenone ring.



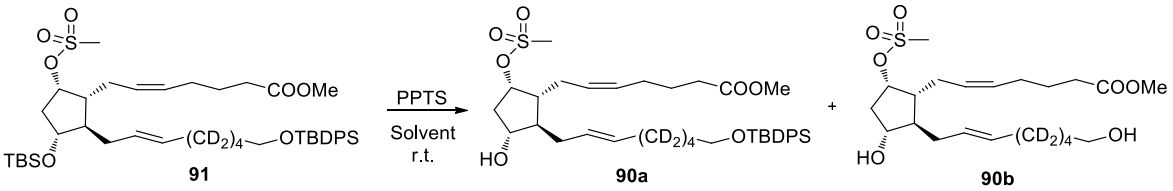
SCHEME 50

As firs step, the hydroxyl group of cyclopentanol **62** has been transformed into good leaving group by converting it into mesilate **91** (*scheme 51*). This delicate functional group works also as protecting group for hydroxyl function on the carbon 9 in order to prevent its oxidation in subsequent steps.



SCHEME 51

The thus obtained mesilate TT was subjected to a selective removal of the secondary *tert*-butyldimethylsilyl group in the presence of primary tert-butyldiphenylsilyl ether.

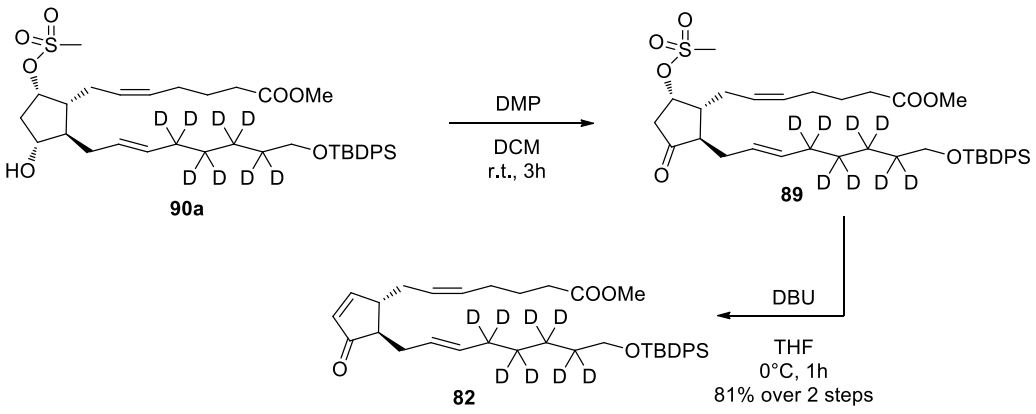


Entry	Solvent	PPTS mol%	Time	90a	90b	Conv.
1	DCM/MeOH 1:1	30	18h	33%	28%	100%
2	DCM/EtOH/MeOH 1:1:1	30	36h	33%	32%	100%
3	DCM/iPrOH/MeOH 1:1:1	30	36h	36%	34%	100%
4	DCM/EtOH 1:1	30	24h	38%	38%	100%
5	iPrOH	50	72h	88%	trace	15%
6	iPrOH/EtOH 2:1	30	36h	60%	10%	70%

TABLE 3

A similar reaction has been reported for the synthesis of the PGE_2 urinary metabolite in the previous chapter (*scheme 18*); in that case we performed the removal of a primary TBS instead of the secondary one, in this case the reaction is more tricky because we want to remove a secondary, but more labile silyl ether, in presence of a primary, but less labile protecting group. During the synthesis of PGE-M we found that the best results in term of selectivity were achieved with acidic catalysis (see *table 1*), thus also in this case we used

the same type of catalysis. The tests performed and reaction conditions used are listed in table (*table 3*). We made a solvent screening in order to find the best reaction condition. In the first three entries we used methanol as co-solvent and we tried to modulate the hardness of the solvent mixture mixing it with ethanol or isopropanol. The conversion of the starting material was complete but with a low selectivity, furthermore a partial decomposition of the substrate or of the products has been noted. In entry 4 the mixture DCM/EtOH gave about the same results of the three previous tests with a partial decrease of decomposition. In entry 5 we used pure absolute isopropanol as solvent, in these conditions the reaction was strongly slowed down (15% of conversion after 72 hours), but the selectivity was high and no decomposition does occur. Building on previous results, with the entry 6 we tried to improve conversion and reduce the reaction times. We then add 33% of absolute ethanol to isopropanol in order to increase the acidity of the solvent mixture making the catalysis more energetic. This test presents a good selectivity, an improved global yield (42%) and a good conversion even if not complete. The reaction conditions showed in entry 6 are those used for the development of the next steps in total synthesis of target molecule synthesis.

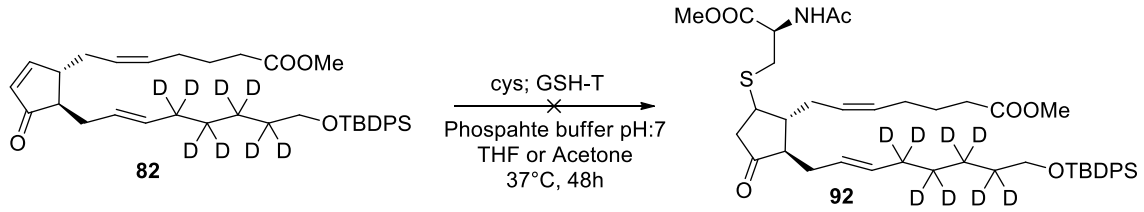


SCHEME 52

The oxidation with DMP of cyclopentanol **90** and the elimination of the mesilate by treatment of ketone **89** with DBU in dry tetrahydrofuran gave, as expected, the desired cyclopentenone in good yield (*scheme 52*).

CYSTEINE ADDITION VIA MICHAEL REACTION

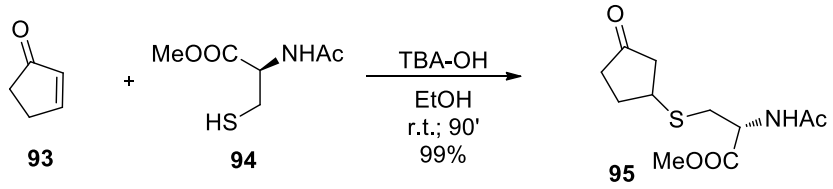
The synthesis of alpha-beta unsaturated carbonyl **82** has finally allowed us to study the addition of the cysteine moiety through Thia-Michael reaction. Initially we thought to use a biomimetic strategy using an enzymatic reaction catalyzed by glutathione transferase (*scheme 53*), in order to pay attention to the green and atom economical aspects.



SCHEME 53

The α,β-unsaturated carbonyl was incubated with the enzyme and N-acetyl-L-cysteine methyl ester in Phosphate buffer pH=7 for several hours. In order to increase the solubility of the substrate we used THF or acetone as co-solvents. However, after 48h under stirring at 37°C was not noticed product formation; probably the lipophilic substrate is not able to reach the active site of the enzyme in a prevalently aqueous medium.

We decided to catalyze, as an alternative to the glutathione transferase, the Michael reaction with tetrabutylammonium hydroxide.

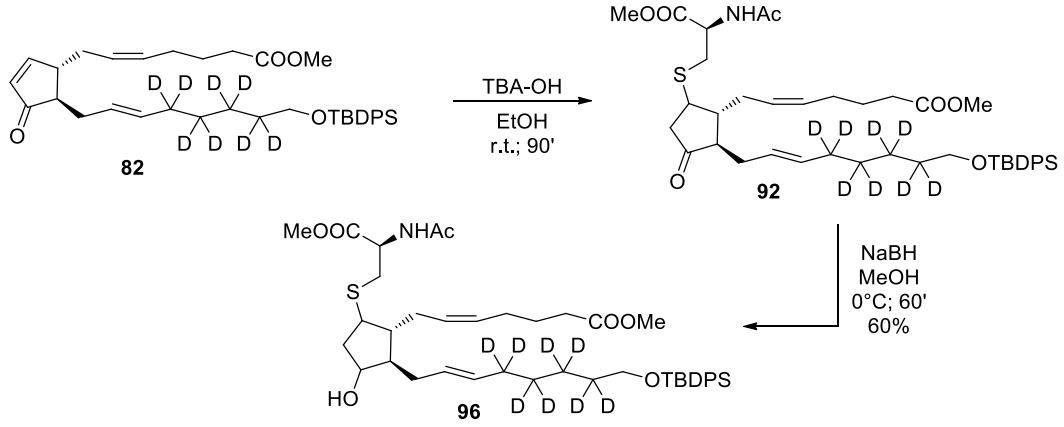


SCHEME 54

This procedure was developed by Nicponski[63] where it uses the same type of thiol and the simple cyclopentenone as Michael acceptor (*Scheme 54*). The reaction carried on **82** did not show significant differeces from that published by Nicponski. Is important to respect the reaction times in order to prevent transesterification; the use of methanol as solvent

[63] Nicponski, D. R.; Marchi, J. M. *Synthesis* **2014**, 46 (13), 1725.

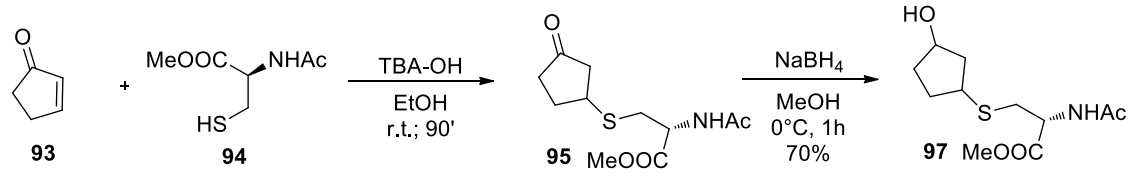
negatively affected the overall yield. The Michael adduct was subsequently reduced with $NaBH_4$ in methanol (*scheme 55*).



SCHEME 55

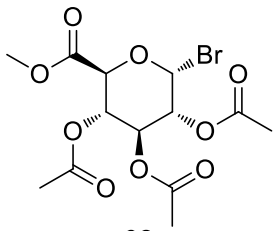
STUDY ON GLYCOSYLATION

Thioester thus obtained it is then ready to be subjected to glycosylation reaction with a suitable derivative of glucuronic acid. However, not being known in the literature examples of glycosylation on the basic skeleton of prostaglandin, we preferred to study the reaction with the help of a model molecule. This model was synthesized using the same reaction sequence used for the synthesis of thioether **96** (*scheme 56*)**.**



SCHEME 56

After a careful search in literature, the first glycosyl-donor we tested was the Acetobromo-α-D-glucuronic acid methyl ester **98** (*figure 14*). This feature was motivated by the fact that this bromide can be easily synthesized in few steps and the acetates can be hydrolyzed simultaneously with the methyl esters, thus reducing the number of synthetic steps.



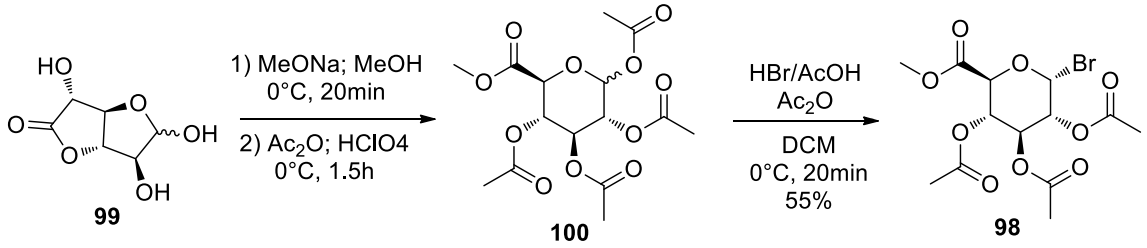
98

Acetobromo-α-D-glucuronic acid methyl ester

FIGURE 14

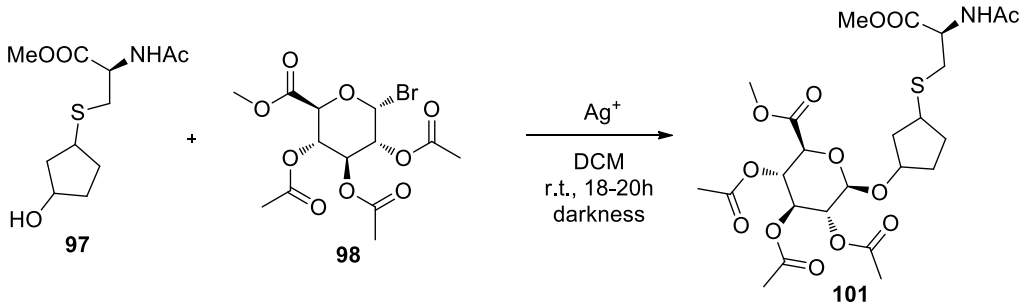
This building block can be easily obtained in three steps synthesis starting from D-Glucurono-6,3-lactone **99**[64]. The lactone was treated with sodium methoxide, acetylated with acetic anhydride and then brominated by treatment with hydrogen bromide in acetic acid (*scheme 57*).

[64] a) Jongkees, S. A. K.; Withers, S. G. *J. Am. Chem. Soc.* **2011**, 133 (48), 19334. b) Bollenback, G. N.; Long, J. W.; Benjamin, D. G.; Lindquist, J. A. J. Am. Chem. Soc. **1955**, 77 (12), 3310.



SCHEME 57

Various tests were carried out using the bromide **98** as a glycosyl donor under Koenigs-Knorr-like reaction conditions[65]. The tests carried out and the reaction conditions are listed in *Table 4*.



Entry	Catalyst	Catalyst loading	Additives	MS* (4Å)	Yield
1	Ag_2CO_3	2.0 equiv.	//	//	0%
2	Ag_2O	2.2 equiv.	//	//	0%
3	AgOTf	2,5 equiv.	//	//	0%
4	AgOTf	2.5 equiv.	2,6-lutidine	//	0%
5	AgOTf	2.5 equiv.	2,6-lutidine	150 mg	0%

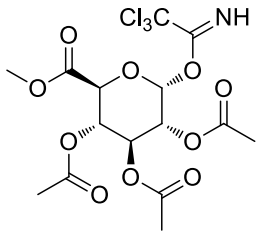
All the reactions were performed in the dark
*MS=molecular sieves

TABLE 4

None of the tests has shown formation of the desired glycosylated product, it is then thought to modify the approach by changing the type of activation of the anomeric carbon.

[65] a) Moench, B.; Gebert, A.; Emmerling, F.; Becker, R.; Nehls, I. *Carbohydr. Res.* **2012**, 352, 186. b) Khmelnitsky, Y. L.; Mozhaev, V. V.; Cotterill, I. C.; Michels, P. C.; Boudjabi, S.; Khlebnikov, V.; Madhava, R.; Wagner, G. S.; Hansen, H. C. *Eur. J. Med. Chem.* **2013**, 64, 121. c) Harnor, S. J.; Rennison, T.; Galler, M.; Cano, C.; Griffin, R. J.; Newell, D. R.; Golding, Bernard T. *Med. Chem. Comm.* **2014**, 5(7), 984.

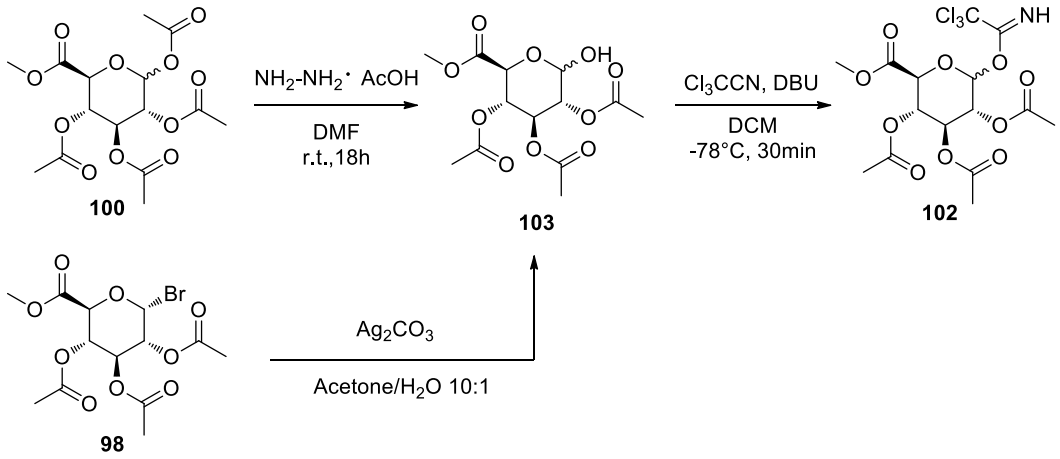
We decided to employ the trichloroacetimidate derivative **102** (*figure 15*). This more classic approach reduces the risk of light degradation of the catalyst.



102
Methyl triacetylglucuronate trichloroacetimidate

FIGURE 15

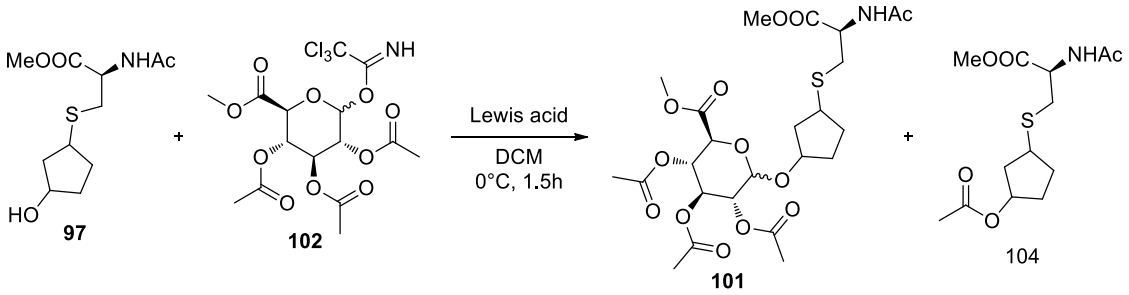
The trichloroacetimidate was synthesized by selective O-deacetylation of methyl 1,2,3,4-tetra-*O*-acetyl-α/β-D-glycopyranuronate **100**[51a] or by hydrolysis of the bromide **98** [66] followed by treatment of emiacetal **103** with 1,8-diazabicyclo[5,4,0]-undec-7-ene (DBU) and trichloroacetonitrile (*scheme 58*).



SCHEME 58

This glycosyl donor was then employed in glycosylation trials with thioether **97** (*table 5*). Three different type of catalysts were tested: $BF_3{\cdot}Et_2O$[67], trimethylsilyl triflate[68] and gold(I) chloride[69]. Also this time no traces of desired product has been noted.

[66] Pilgrim, W.; Murphy, P. V. *J. Org. Chem.* **2010**, 75(20), 6747.
[67] Ferguson, J. R.; Harding, J.R.; Killick, D. A.; Lumbard, K. W.; Scheinmann, F.; Stachulski A. V. *J. Chem. Soc., Perkin Trans. 1* **2001**, 3037.
[68] Zhao, W.; Kong, F. *Bioorg. Med. Chem.* **2005**, 13, 121.
[69] Peng, P.; R. Schmidt R. J. Am. Chem. Soc. **2015**, 137, 12653.

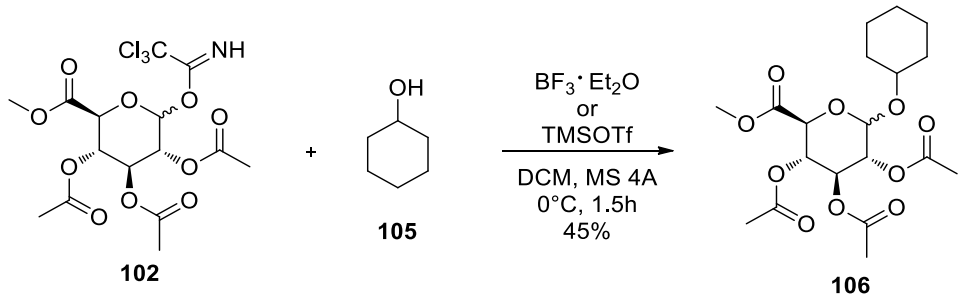


Entry	Catalyst	Cat. Loading	Time	Temp.	101	104
1	$BF_3{\cdot}Et_2O$	1.1 equiv.	1.5 h	0°C	0%	30%
2	TMSOTf	0.05 equiv.	2 h	0°C	0%	30%
3	AuCl	0.1 equiv.	72 h	-60°C	0%	0%

All the tests were performed using the 100%w/w of 4Å molecular sieves

TABLE 5

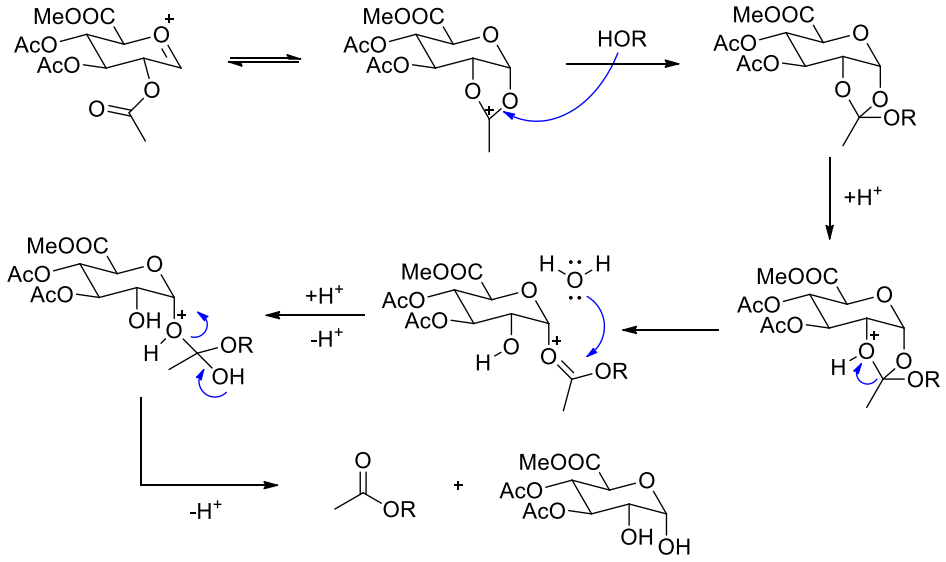
In the first two tests we noted the formation of the acetylated thioester GG as major reaction product (30% yield). The formation of acyl transfer side product in glycosylation is reported in several cases in literature[70], however it seems difficult to identify the cause of this side reaction, because the published results show substantial differences from case to case, although this problem seems to occur more when the glycosyl acceptor is not very reactive[57d]. And this seems to be the case here: the reaction of glycosylation performed replacing the thioether **97** with the cyclohexanol **105** led to the formation of the correct product with a 45% yield (*scheme 59*).



SCHEME 59

[70] a) Harding, J.R.; King, C.D.; Perrie, J.A.; Sinnottb, D.; Stachulski, A.V. *Org. Biomol. Chem.* **2005**, 3, 1501. b) Belot, F.; Jacquinet, J. C. *Carbohydr. Res.* **1996**, 290, 79. c) Urban, F. J.; Moore, B. S.; Breitenbach, R. *Tetrahedron Lett.* **1990**, 31, 4421–4424. d) Janusz Madaj, J.; Trynda, A.; Jankowska, M.; Wis´niewski, A. *Carbohydr. Res.* **2002**, 337, 1495.

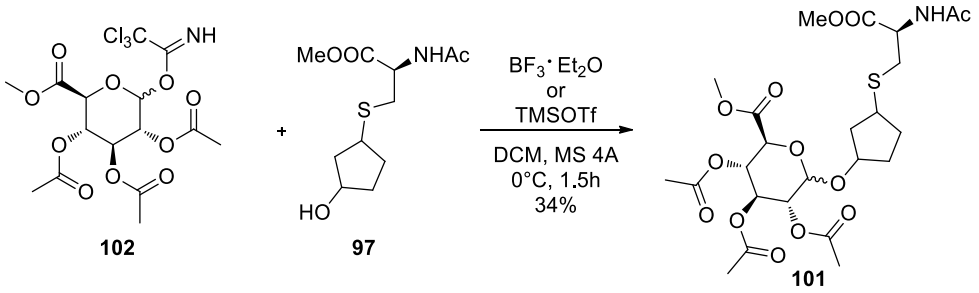
On the other hand is reasonable to think that to favor the acyl group migration is the moisture present in the reaction ambient.



SCHEME 60

It is known that the presence of ester groups next to the anomeric position stabilize the oxacarbenium ion which is generated by treating the trichloroacetimidate with a Lewis acid. The direct attack to the carbonyl carbon is responsible for the formation of the orthoester[71] which can be hydrolyzed in acidic conditions in presence of water (*scheme 60*). In complete agreement with this theory the tests of glycosylation were carried out again under the same reaction conditions of entry 1 and 2 of *table 5*, but paying more attention to keep anhydrous the medium (*scheme 61*). The molecular sieves were freshly activated, the glycosyl donor and acceptor were dried under azeotropic condition from toluene and left under high vacuum overnight.

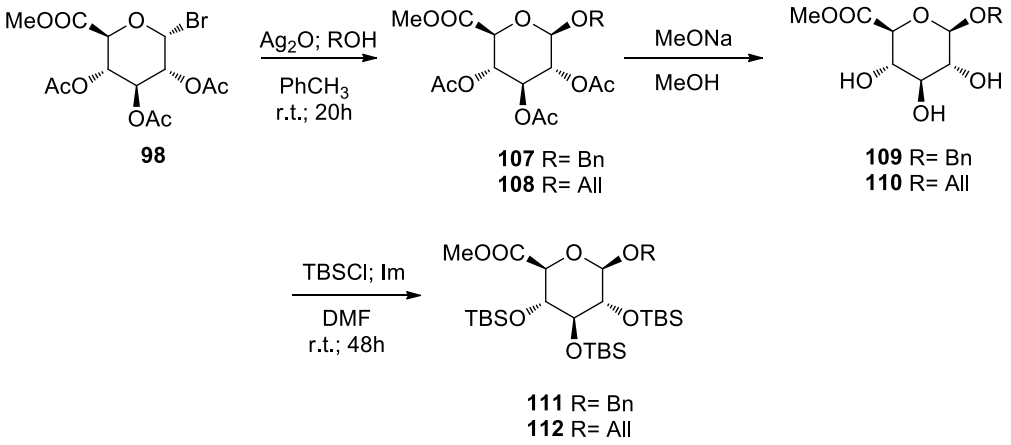
[71] Mydock, L. K.; Demchenko, A. V. *Org. Biomol. Chem.* **2010**, 8, 497.



SCHEME 61

The reaction performed under high dryness afforded the desired compound in a 34% yield both with boron trifluoride and trimethylsilyl triflate as catalyst and in both the cases no traces of transacetylated product has been shown.

However, in order to improve the yield of the glycosylation we embarked a new synthesis of a glucuronate trichloracetimidate with the three hydroxyl groups protected as *tert*-butyldimethylsilyl derivatives.

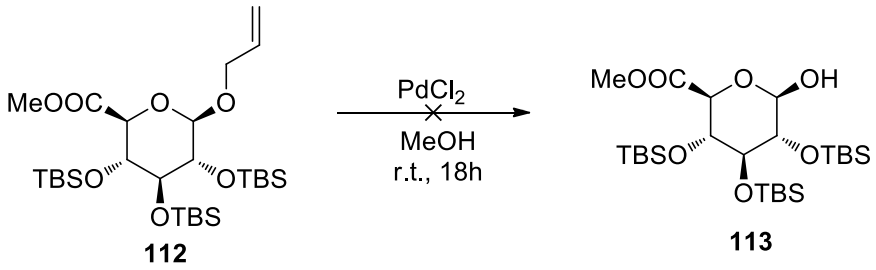


SCHEME 62

This type of protection of the hydroxyl groups prevents the formation of the orthoester and consequently should reduce the formation of side products; moreover, TBS-ether can be removed simultaneously with the terminal silyl ether on the ω-side chain during the synthesis of the target molecule. To use this strategy is necessary to protect the anomeric hydroxyl in orthogonal manner. We then decided to try to derivatization the ester glucuronide as benzyl and allyl acetal. As showed in *scheme 62*, is possible to obtain the

allyl or the benzyl TBS-methyl glucuronate starting from the bromide **98** according to a procedure known in the literature[72].

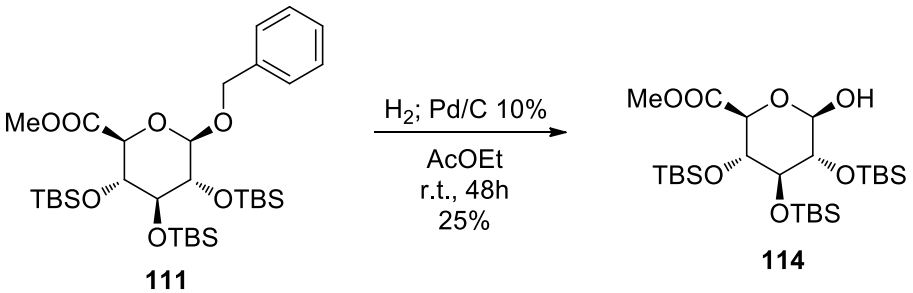
Unfortunately, in both cases the acetal cleavage procedure has not brought the desired results. Indeed, the removal of the allylic portion on compound **112** with palladium dichloride led to substrate degradation. The reaction conditions are shown in *scheme 63*.



112 **113**

SCHEME 63

The hydrogenation reaction of the compound benzoate **111** instead, has provided low overall yields and a low conversion percentage after 48h (30%) (*scheme 64*).



111 **114**

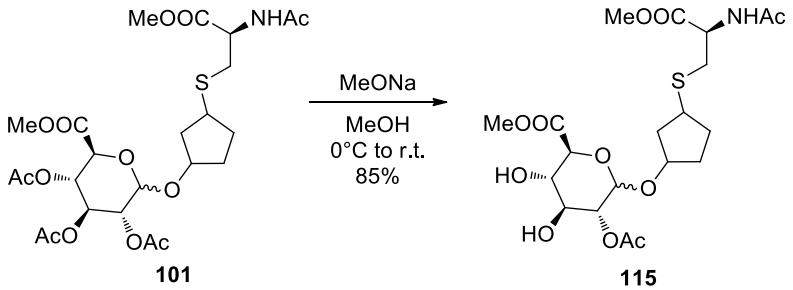
SCHEME 64

Because of these problems we have seen fit to abandon this path because the number of synthetic steps and low yields would not lead any advantage to the synthesis of the target molecule.

At this stage we would have to study the glycosylation reaction, under the conditions reported in the *scheme 61*, on the compound **96**, unfortunately for reasons of time this it has not been possible.

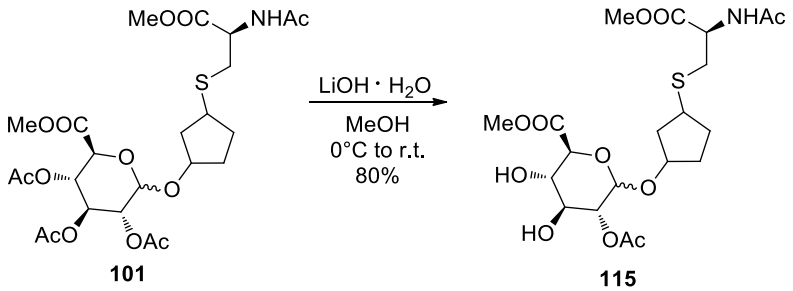
[72] a) Kirschning, A.; Ries, M.; Domann, S.; Martin, W.; Albrecht, W.; Arnold, P.; Laufer, S. *Biorg. Med. Chem. Lett.* **1997**, 7(7), 903. b) Silverberg, L.J.; Dillon, J.L.; Vemishetti, P.; Sleezer, P.D.; Discordia, R.P.; Hartung, K.B.; Gao, Q. *Org. Proc. Res. Dev.* **2000**, 4(1), 34.

But we made some tests of removal of the acetate groups from compound **101**. The first test performed concerns the solvolysis of the acetates of **101** by treatment with sodium methoxide in methanol (*scheme 65*). In these conditions, even after several hours under stirring at room temperature the reaction afforded only the monoacetate product **115**. Results were confirmed by NMR and mass spectrometry analysis; we do not know precisely what is the acetyl group which is not removed, but it is reasonable that it could be the acetate on the vicinal position to the anomeric carbon because it is the more sterically hindered.



SCHEME 65

It was also performed a test on the same substrate hydrolysis with lithium hydroxide hydrate both in a catalytic (0.1 equiv.) and stoichiometric (1.5 equiv.) amount (*scheme 66*). These more energetic conditions should have led to the removal of all the acetates and also the methyl esters. However, the reaction has not shown differences compared to the test with methoxide.



SCHEME 66

CONCLUSIONS

With this work, we performed a synthetical study on the proposed principal urinary metabolite of 15-deoxi-prostaglan J_2 to optimize all the crucial synthetic steps.

The study on the introduction of the ω chain led to the identification of an approach based on a Julia-Kocienski olefination between the sulfone **77** and the aldehyde **76**. this strategy allowed to obtain the total stereoselectivity on the formation of the E double bond.

The best condition for the introduction of the cysteine moiety were found using a Thia-Michael reaction catalyzed by tetrabutylammonium hydroxide. To do this we developed a methodology able to convert the cyclopentanol **62** into the right cyclopentanone **82**.

Finally we studied the glycosylation reaction on a model molecule in order to find the best condition for the introduction of the glucuronic acid as O-glycoside. Unfortunately for reason of time we did not try the reaction on the right intermediate **96** and so we were unable to conclude the synthesis of the target molecule.

We hope conclude the synthesis in the future in order to determine the real structure of this important metabolite.

GLOSSARY

AA	Arachidonic acid
AcOEt	Ethyl acetate
AcOH	Acetic acid
All	Allyl
Bn	Benzyl
BRINE	aqueous saturated solution of NaCl.
t-BuOH	*tert*-butanol
CAL-B	*candida antarctica* lipase B
DBU	1,5-diazabiciclo[5.4.0]undec-5-ene
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	Dietyl azodicarboxylate
DIAD	Diisopropyl azodicarboxylate
DME	Dimethoxyethane
DMF	Dimetylformamide
DMP	Dess-Martin Periodinane
DMAP	4-Dimenthyl-amino-pyridine
EtOH	Ethanol
Im	Imidazole
iPrOH	*iso*-Propanol
KHMDS	Potassium hexamethyldisilylamide
mCPBA	4-Chlorobenzoic acid
MeOH	Methanol
MTBE	Methyl *tert*-butyl ether
PG	Prostaglandin
PGE_2	Prostaglandin E_2
PGE-M	Prostaglandin E_2 urinary metabolite
15d-PGJ_2	15-deoxi-$\Delta^{12,14}$-prostaglandin J_2
15d-PGJ_2-M	15-deoxi-$\Delta^{12,14}$-prostaglandin J_2 urinary metabolite
PMB	4-Methoxy-benzyl

PPTS	Pyridinium-*p*-toluen Sulfonate
PTSA	*p*-Toluensulfonic acid
PTSH	1-Phenyl-1H-tetrazol-5-thiol
Py	Pyridine
TBAF	Tetrabutylammonium fluoride
TBA-OH	Tetrabutylammonium hydroxide
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
TEMPO	2,2,6,6-Tetramethyl-1-piperidinyloxy
THF	Tetrahydrofurane
TIPS	Triisopropylsilyl
TMSOTf	Trimethylsilyl triflate

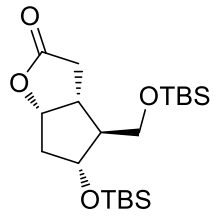
EXPERIMENTAL SECTION

GENERAL PROCEDURES

All solvents were of commercial quality and were purified by distillation over the drying agents indicated: THF (Na/benzophenone), CH_2Cl_2 and hexane (CaH_2), toluene (Na/K). All other reagents were used as supplied. All moisture-sensitive reactions were carried out under a positive static atmosphere of Ar in flame-dried glassware. Syringes and needles for the transfer of reagents were dried at 140 °C and allowed to cool in a desiccator over P_2O_5 before use. Routine monitoring of reactions was performed using silica gel 60 (0.25 mm) aluminum-supported TLC plates. Compounds were visualized by UV irradiation at a wavelength of 254 nm or stained by exposure to a 0.5% solution of vanillin in H_2SO_4/EtOH, followed by charring. Flash column chromatography (FCC) was performed on silica gel (40−63 μm). Yields are reported for isolated compounds with >96% purity established by NMR unless otherwise indicated. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, in the solvents indicated; chemical shifts (δ) are given in ppm relative to TMS, and coupling constants (J) are in hertz. The solvent signals were used as references, and the chemical shifts were converted to the MS scale ($CDCl_3$: δC 77.00; residual $CHCl_3$ in $CDCl_3$: δH 7.26; CD_2Cl_2: δC 53.8; residual CH_2Cl_2 in ; CD_2Cl_2: δH 5.32 ppm). COSY, DEPT, and NOESY spectra were recorded using a standard pulse program library. The number of H-atoms attached to each C-atom (s = 0H, d = 1H, t = 2H, q = 3H) was determined by DEPT experiments. Optical rotations were recorded on a digital polarimeter at 589 nm, with concentration (c) in g/100 mL. Mass spectrometry was performed by LTQ using heated electrospray ionization (ESI).

Synthesis of 52

(3aR,4S,5R,6aS)-5-((*tert*-butyldimethylsilyl)oxy)-4-(((*tert*-butyldimethylsilyl)-oxy)methyl)-hexahydro-2H-cyclopenta[b]furan-2-one



Chemical Formula: $C_{20}H_{40}O_4Si_2$
Molecular Weight: 400,70

To a stirred solution, cooled to -20°C, of $NaBH_4$ (38mg, 1mmol) in dry methanol (4mL) was added a catalytic amount of $CeCl_3$ (8mg, 0.03 mmol). The aldehyde **50**, dissolved into 2 mL od methanol, was added to the mixture. After 1h under stirring the reaction was quenched by addition of acetone (3mL) and acetic acid (120µL). The methanol was evaporated and the residue was taken up with DCM (10mL) and saturated aqueous solution of NH_4Cl (10mL). The layers were separated and the aqueous layer was extracted with DCM (5x10mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure affording the crude alcohol **51**.

To a solution of crude **51** in DCM (10mL) were added in the order imidazole (204mg, 3mmol) and *tert*-butyldimethylsilyl chloride (175mg, 1.15mmol). The reaction was stirred at room temperature for 5h and then was quenched by addition of saturated aqueous solution of $NaHCO_3$. The layers were separated and the aqueous layer was extracted with DCM (3x10mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography SiO_2 (hexane/AcOEt 9:1) affording 380mg of pure lactone **52** (95% yield).

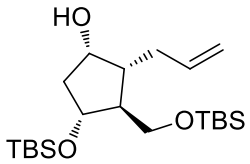
^{1}H NMR (300 MHz, $CDCl_3$): 4.84 (dt, 1H, J=6.9, 4.4 Hz), 4.10 (q, 1H, J=5.4 Hz), 3.37 (d, 2H, J=4.9 Hz), 2.76 (m, 2H), 2.54 (d, 1H, J=17.6Hz), 2.08 (t, 2H, J=5.5 Hz), 2.01 (m, 1H, J=5.8, 5.6, 5.4 Hz), 1.05 (s, 18H), 0.09 (s, 6H), 0.05 (s, 6H).

^{13}C NMR (75 MHz, $CDCl_3$): 177.1 (s), 83.8 (d), 74.7 (d), 63.7 (t), 56.5 (d), 40.8 (t), 38.8 (d), 35.5 (t), 25.9 (q), 25,6 (q), 18.2 (s), 17.7 (s), -4.8 (q), -5.0 (q), -5.6 (q)

H-ESI MS (m/z): 423.6 [M^++Na]

Synthesis of 53

(1S,2R,3S,4R)-2-allyl-4-((tert-butyldimethylsilyl)oxy)-3-(((tert-butyldimethyl-silyl)oxy)-methyl)-cyclopentanol



Chemical Formula: $C_{21}H_{44}O_3Si_2$
Molecular Weight: 400,74

DIBAL-H (1M in hexanes, 288 µL, 0.288 mmol) was added dropwise to a stirred solution of lactone **52** (100mg, 0.250 mmol) in dry DCM (5mL) precooled to – 78°C under an argon atmosphere. Stirring was continued for 30 min and then the excess reducing agent was destroyed by addition of a saturated solution of NH_4Cl (2mL) at –78°C. The mixture was gradually warmed to room temperature and diluted with DCM (3mL), followed by addition of a saturated solution of sodium and potassium tartrate (6 mL). The two layers were separated and the aqueous layer was extracted with DCM (3x5mL); the combined organic layers were dried on $MgSO_4$, filtered, and evaporated in vacuo to give the crude expected lactol **48**.

KHMDSA 0.5M in $PhCH_3$ (1.7 mL, 0.85 mmol) was added dropwise to a suspension of methyltriphenylphosphonium bromide (330 mg, 0.85 mmol) in dry toluene (6 mL). The mixture was stirred for 45 min. at room temperature. The suspension was cooled at -55°C and a solution of lactol **48** in toluene (1.5mL) was added *via cannula*. The mixture was allowed to warm to 0 ° C and was stirred until completion of the reaction. A saturated aqueous solution of NH_4Cl was added and the two layers were separated. The aqueous layer was extracted three times with 15ml of DCM. The combined organic layers were dried on Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel (hexane/AcOEt 95:5) affording 87mg of pure **53** (87% over 2steps).

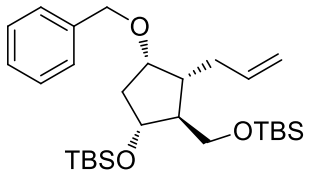
^{1}H NMR (300 MHz, $CDCl_3$): 5.89 (m, 1H), 5.10 (d, 1H, J=16.7 Hz), 5.05 (d, 1H, J=10.0 Hz), 4.23 (d, 1H, J=4.2 Hz) 4.11 (bs, 1H), 3.63 (dd, 1H, J=10. 1Hz, 4.2 Hz), 3.35 (dd, 1H, J=10.0 Hz, 6.65 Hz) 3.02 (bs, 1H), 2.48 (m, 1H), 2.20 (m, 1H), 1.78 (m, 3H), 1.56 (m, 1H), 0.89 (s, 18H), 0.09 (s, 6H), 0.05 (s, 6H).

^{13}C NMR (75 MHz, $CDCl_3$): 137.9 (d), 11.2 (t), 77.25 (d), 75.4 (d), 63.7 (t), 55.3 (d), 46.56 (d), 42.4 (t), 34.3 (t), 25.8 (q), 25,6 (q), 18.2 (s), 17.7 (s), -4.8 (q), -5.0 (q), -5.6 (q), -5.6 (s).

H-ESI MS (m/z): 423.6 [M^{+}+Na]

Synthesis of 47

(((1S,2R,3S,5R)-2-allyl-3-(benzyloxy)-5-((tert-butyldimethylsilyl)oxy)cyclopentyl)methoxy)-(tert-butyl)dimethylsilane



Chemical Formula: $C_{28}H_{50}O_3Si_2$
Molecular Weight: 490,87

53 (82mg, 0.20 mmol) was dissolved in dry THF (2 mL) and the mixture cooled to 0°C. NaH (60% in oil, 10mg, 0.25mmol) was added in one portion. The suspension was stirred at 0°C for 45 min. Benzyl bromide (49µL, 0.40 mmol) was then added to the mixture dropwise. The temperature was allowed to reach r.t. and stirring is continued for further 18h. then reaction was quenched by addition of saturated solution of NH_4Cl (5mL) and Et_2O (5mL). The layers were separated and the aqueous layer was extracted with Et_2O (3x5mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by The residue was purified on silica gel column chromatography (hexane/MTBE 98:2) affording pure **47** (92 mg, 94% yield).

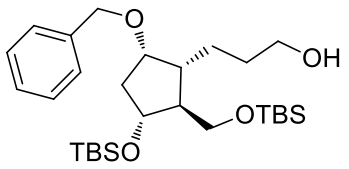
^{1}H NMR (300 MHz, $CDCl_3$): 7.32 (m, 5H), 5.84, (m, 1H), 5.05 (d, 1H, J=17.1 Hz), 4.95 (d, 1H, J=10.1 Hz), 4.57 (d, 1H, J= 11.9Hz), 4.37 (d, 1H, J= 11.9 Hz), 4.11 (m, 1H), 3.84 (m, 1H), 3.67 (dd, 2H, J=30.0 Hz, 10.0 Hz), 2.48 (m, 1H), 2.16 (m, 2H), 1.76 (m, 3H), 0.91 (s, 18H), 0.06 (s, 12H).

^{13}C NMR (75 MHz, $CDCl_3$): 139.0 (s), 138.0 (d),128.1 (d), 127.4 (d), 127.1 (d), 115.0 (t), 78.4 (d), 71.9 (d), 70.3 (t), 60.0 (t), 52.9 (d), 42.3 (d), 40.0 (t), 31.85 (t), 25.8 (q), 18.1 (s), 17.9 (s), -4.6 (q), -4.9 (q), -5.6 (q), -5.7 (s).

H-ESI MS (m/z): 513.2 [M^++Na]

Synthesis of 46

3-((1R,2S,3R,5S)-5-(benzyloxy)-3-((tert-butyldimethylsilyl)oxy)-2-(((tert-butyldimetylsilyl) oxy)methyl)cyclopentyl)propan-1-ol



Chemical Formula: $C_{28}H_{52}O_4Si_2$
Molecular Weight: 508,88

47 (76mg, 0.12mmol) was dissolved in 1.8mL of dry THF under static Ar atmosphere. The solution was cooled to -30°C and BH_3 dimethylsulfide complex (2M, 89µL, 0.18mmol) was added dropwise. The mixture was allowed to worm to r.t. and the stirring was continued overnight. The reaction was quenched by addition of K_2CO_3 (207mg, 1.5mmol) and H_2O_2 (30%w/w, 153µL, 1.5mmol). The mixture was stirred for additional 2h. Brine (2mL), H_2O (2mL) and Et_2O (5mL) were added. The layers were separated and the aqueous layer was extracted with Et_2O (3x5mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by The residue was purified on silica gel column chromatography (hexane/AcOEt 9:1) affording pure **46** (67 mg, 86% yield).

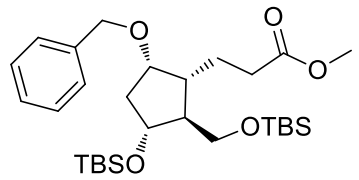
^{1}H NMR (300 MHz, $CDCl_3$): 7.35 (m, 5H), 4.60 (d, 1H, J= 11.9Hz), 4.37 (d, 1H, J= 11.9 Hz), 4.11 (m, 1H), 3.84 (m, 1H), 3.70 (m, 1H), 3.62 (m, 3H), 2.1 (m, 1H), 1.62 (m, 7H), 0.91 (s, 18H), 0.06 (s, 12H).

^{13}C NMR (75 MHz, $CDCl_3$): 138.9 (s), 128.1 (d), 127.5 (d), 127.2 (d), 78.1 (d), 72.0 (d), 70.1 (t), 63.2 (t), 60.0 (t), 53.3 (d), 42.2 (d), 39.9 (t), 31.0 (t), 25.8 (q), 22.9 (t), 18.1 (s), 17.9 (s), -4.6 (q), -4.9 (q), -5.6 (q), -5.7 (s).

H-ESI MS (m/z): 531.4 [M^++Na]

Synthesis of 55

Methyl 3-((1R,2S,3R,5S)-5-(benzyloxy)-3-((tert-butyldimethylsilyl)oxy)-2-(((tert-butyldimethylsilyl)oxy)methyl)cyclopentyl)propanoate



Chemical Formula: $C_{29}H_{52}O_5Si_2$
Molecular Weight: 536,89

Direct oxidation

To a vigorously stirred and ice water-cooled biphasic mixture of DCM- H_2O 12:1 (1.1 mL) containing **46** (90mg, 0.18mmol), TEMPO (4.4mg, 0.02mmol), KBr (2mg, 0.02mmol) and Aliquat 336 (4µL, 0.01mmol) was added NaClO buffered to pH 9.5 with sodium bicarbonate in small portion until complete conversion of **46**. The reaction was monitored by TLC (hexane/AcOEt 9:1). The reaction was quenched by addition of 10mL of saturated aqueous solution of H_2KPO_4 and 10mL Et_2O. The layers were separated and the aqueous layer was extracted with Et_2O (3x10mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude was dissolved in Et_2O (2mL) and an excess of trimethylsilyldiazomethane (2M in Et_2O, 270µL, 0.54 mmol) was added. The acid was completely consumed in few minutes. The solvent was then removed under vacuum and the residue was purified by SiO_2 column chromatography (hexane/AcOEt 98:2) affording 54 mg of the desired methyl ester (57% yield).

Two step oxidation

To a solution of **46** (190mg, 0.37 mmol) in DCM (4 mL) was added Dess-Martin periodinane (173mg, 0.41mmol). The reaction mixture was stirred at r.t. for 2h. Saturated aqueous solution of $NaHCO_3$ (2mL), $Na_2S_2O_3$ (2mL), water (2mL) and DCM (2mL) were added. The layers were separated and the aqueous layer was extracted with DCM (3x5mL). The

combined organic layers were washed with BRINE (5mL) and dried over Na_2SO_4, filtered and concentrated under reduced pressure affording crude **56**.

To a solution of crude **56**, 2-methyl-2-butene (300 µL, 2.8 mmol) and NaH_2PO_4 (3mL of a saturated aqueous solution) in *t*-BuOH (7mL) was added sodium chlorite (67mg ,0.56) in H_2O (3mL) and the resulting mixture was stirred at room temperature. After 2 h, H_2O (10mL), brine (5mL) and DCM (20mL) were added. layers were separated and the aqueous layer was extracted with DCM (3x15mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude was dissolved in Et_2O (5mL) and an excess of trimethylsilyldiazomethane (2M in Et_2O, 555 µL, 1.11 mmol) was added. The acid was completely consumed in few minutes. The solvent was then removed under vacuum and the residue was purified by SiO_2 column chromatography (hexane/AcOEt 9:1) affording 180 mg of the desired methyl ester (91% yield over three steps).

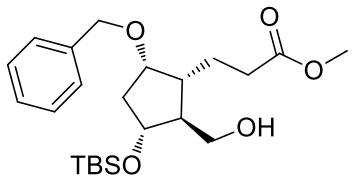
^{1}H NMR (300 MHz, $CDCl_3$): 7.35 (m, 5H), 4.63 (d, 1H, J= 11.9Hz), 4.36 (d, 1H, J= 11.9 Hz), 4.10 (m, 1H), 3.77 (m, 1H), 3.67 (m, 5H), 2.31 (m, 2H), 2.10 (m, 1H), 1.98 (m, 1H), 1,77 (m, 5H), 0.91 (s, 18H), 0.06 (s, 12H).

^{13}C NMR (75 MHz, $CDCl_3$): 174.2 (s), 138.9 (s), 128.1 (d), 127.4 (d), 127.2 (d), 77.8 (d), 72.1 (d), 70.0 (t), 60.1 (t), 53.3 (d), 51.3 (q), 41.8 (d), 39.8 (t), 32.3 (t), 25.8 (q), 22.8 (t), 18.1 (s), 17.9 (s), -4.6 (q), -4.9 (q), -5.6 (q), -5.7 (s).

H-ESI MS (m/z): 537.2 [M^++H]; 560.2 [M^++Na]

Synthesis of 57a

methyl 3-((1R,2S,3R,5S)-5-(benzyloxy)-3-((tert-butyldimethylsilyl)oxy)-2-(hydroxymethyl)-cyclopentyl)propanoate



Chemical Formula: $C_{23}H_{38}O_5Si$
Molecular Weight: 422,63

55 (50 mg, 0.09 mmol) was dissolved in a mixture of DCM/EtOH 1:1 (1mL) and PPTS was added (10 mg, 0.04mmol). The reaction was stirred for 50h at room temperature. The reaction was quenched by addition of phosphate buffer (2mL, pH=7) and DCM (3mL). The layers were separated and the aqueous layer was extracted with DCM (3x3mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified on silica gel column chromatography (hexane/AcOEt 9:1) affording the desired compound **57a** as a colorless oil (73 mg, 70% based on the converted starting material). 5mg of starting material were recovered (90% of conversion).

^{1}H NMR (300 MHz, $CDCl_3$): 7.35 (m, 5H), 4.63 (d, 1H, J= 11.9Hz), 4.36 (d, 1H, J= 11.9 Hz), 4.10 (m, 1H), 3.77 (m, 3H), 3.67 (s, 3H), 2.27 (m, 4H), 1.98 (m, 2H), 1,73 (m, 3H), 0.91 (s, 9H), 0.09 (s, 6H).

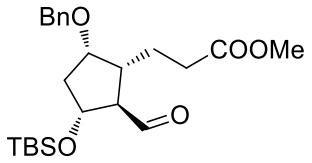
^{13}C NMR (75 MHz, $CDCl_3$): 174.5 (s), 138.6 (s), 128.1 (d), 127.4 (d), 127.3 (d), 77.0 (d), 74.1 (d), 70.1 (t), 62.1 (t), 52.4 (d), 51.5 (q), 41.7 (d), 39.9 (t), 31.9(t), 25.7 (q), 22.0 (t), 17.8 (s), -4.4 (q), -5.0 (q).

H-ESI MS (m/z): 423.3 [M^++H]

$[\alpha]^D_{20}$= +41.09 (c=0.55, CH_2Cl_2)

Synthesis of 44

methyl 3-((1R,2R,3R,5S)-5-(benzyloxy)-3-((tert-butyldimethylsilyl)oxy)-2-formylcyclopent-yl)propanoate



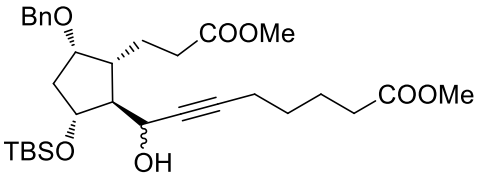
Chemical Formula: $C_{23}H_{36}O_5Si$
Molecular Weight: 420,61

To a solution of **57a** (170mg, 0.40 mmol) in DCM (5 mL) was added Dess-Martin periodinane (197mg, 0.46mmol). The reaction mixture was stirred at r.t. for 3h. Saturated aqueous solutions of $NaHCO_3$ (10mL), $Na_2S_2O_3$ (10mL) and hexane/Et_2O 1:1 (50mL) were added. The layers were separated and the aqueous layer was extracted with hexane/AcOEt 1:1 (3x15mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was taken up with hexane/AcOEt 1:1 and filtered on a pad of SiO_2. The solvent was removed under reduced pressure and the residue containing crude **44** was used in the next step without further purifications.

H-ESI MS (m/z): 443 $[M+Na]^+$

Synthesis of 43

methyl 8-((1S,2R,3S,5R)-3-(benzyloxy)-5-((tert-butyldimethylsilyl)oxy)-2-(3-methoxy-3-oxopropyl)cyclopentyl)-8-hydroxyoct-6-ynoate



Chemical Formula: $C_{31}H_{48}O_7Si$
Molecular Weight: 560,79

n-BuLi (1.6 M in hexane, 275 µL) was added to a solution of **45**[34] (130 mg, 0.46 mmol) in dry THF (2 mL) at -78 °C. After stirring at -78°C for 30 min aldehyde **44** in dry THF (1 mL) was added. After 30 min the reaction was quenched with aq. solution of NH_4Cl (4 mL) and the solution was diluted with Et_2O (5 mL). The layers were separated and the aqueous phase was extracted with Et_2O (3x5 mL). The combined organic phases were dried on Na_2SO_4, filtered, and concentrated under reduced pressure. The residue containing **58** was dissolved in absolute methanol (4 mL) and K_2CO_3 (3 mg, 0.02mmol) was added. The mixture was stirred at room temperature until complete conversion of starting material (TLC hexane/AcOEt 7:3). The reaction was quenched by addition of 90µL of AcOH. The methanol was evaporated and the residue was taken up with aq. NH_4Cl (5 mL) and DCM (5 mL). The layers were separated and the aqueous layer was extracted with DCM (3x5 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was dissolved in a mixture of MTBE/MeOH (4 mL) 9:1 and 100 mg of CAL-B supported on polystyrene were added. The heterogeneous mixture was gently stirred at 30°C for 20h. after this time the mixture was filtered and the solvent was removed under reduced pressure. The crude was purified by column chromatography on silica gel hexane/AcOEt 8:2 affording the desired methyl ester **43** pure (163 mg, 73% over 4 steps).

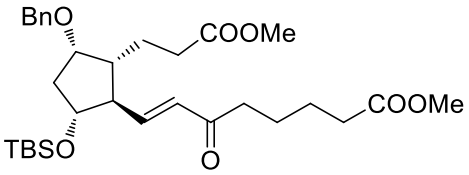
^{1}H NMR (300 MHz, $CDCl_3$): 7.35 (m, 5H), 4.48 (m, 3H), 3.85 (m, 2H), 3.68 (s, 3H), 3.66 (s, 3H), 2.27 (m, 10H), 1.77 (m, 5H), 1,56 (m, 2H), 0.91 (s, 9H), 0.09 (m, 6H).

^{13}C NMR (75 MHz, $CDCl_3$): 174.7(s), 173.7 (s), 138.6 (s), 128.2 (d), 127.3 (d), 127.3 (d), 85.7 (s),79.8 (s), 77.7 (d), 76.7 (d), 73.1 (d), 70.4 (t), 64.1 (d), 57.42 (q), 54.3 (d), 51.4 (q), 41.8 (d), 39.3 (t), 33.37 (t), 32.1 (t), 27.88 (t), 25.7 (q), 24.1 (t), 22.4 (t), 18.3 (t), 17.8 (s), -4.5 (q), -5.0 (q).

H-ESI MS (m/z): 583 $[M+Na]^+$

Synthesis of 42

(E)-methyl 8-((1R,2R,3S,5R)-3-(benzyloxy)-5-((tert-butyldimethylsilyl)oxy)-2-(3-methoxy-3-oxopropyl)cyclopentyl)-6-oxooct-7-enoate



Chemical Formula: $C_{31}H_{48}O_7Si$
Molecular Weight: 560,79

43 (84 mg, 0.15 mmol) was dissolved in MeOH (1 mL), then [{Au(IPr)}$_2$(m-OH)][BF_4] (3.84 mg, 0.003 mmol) was added followed by distilled H_2O (100 µL). The solution was stirred at room temperature until full conversion of **43** was indicated by TLC (hexane/acetate 8:2). Volatile compounds were removed under reduced pressure and the residue was purified by column chromatography on silica gel (hexane/AcOEt 8:2) affording the enone **42** (72 mg, 86%) as pale yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$): 7.35 (m, 5H), 6.64 (dd, 1H, J=15.6 Hz, 5.4 Hz), 6.19 (d, 1H, J= 15.6), 4.61 (d, 1H, 11.9 Hz), 4.33 (d, 1H, J=11.9), 3.95 (q, 1H, J=6.21 Hz), 3.76 (m, 1H), 3.66 (s, 3H), 3.66 (s, 3H), 2.56 (m, 3H), 2.30 (m, 5H), 1.90 (m, 1H), 1.77 (m, 1H), 1.67 (m, 6H), 0.87 (s, 9H), 0.01 (s, 3H), -0.01 (s, 1H).

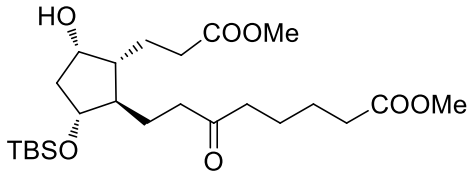
^{13}C NMR (75 MHz, $CDCl_3$): 199.2 (s), 173.8 (s), 147.6 (d), 138.4 (s), 131.7 (d), 128.2 (d), 127.5 (d), 127.4 (d), 77.0 (d), 76.9 (d), 70.12 (t), 55.26 (d), 51.33 (q), 46.7 (d), 40.57 (t), 39.91 (t), 33.73 (t), 31.9 (t), 25.6 (q), 24.4 (t), 23.4 (t), 22.5 (t), 17.9 (s), -4.7 (q).

H-ESI MS (m/z): 583 $[M+Na]^+$

$[\alpha]^{D}_{20}$= +29.4 (c=0.84, CH_2Cl_2)

Synthesis of 59

methyl 8-((1R,2R,3S,5R)-5-((tert-butyldimethylsilyl)oxy)-3-hydroxy-2-(3-methoxy-3-oxopropyl)cyclopentyl)-6-oxooctanoate



Chemical Formula: $C_{24}H_{44}O_7Si$
Molecular Weight: 472,69

To a solution of **42** (61 mg, 0.11 mmol) in MeOH (4 mL) was added Pd on carbon (10% w/w, 12 mg, 0.01 mmol). The heterogenous mixture was stirred under a positive atmosphere of hydrogen until complete conversion of the starting material (TLC hexane/AcOEt 7:3). The mixture was then filtrated to remove the catalyst and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (hexane/AcOEt 7:3) affording the pure **59** (39 mg, 75%) as a colorless oil.

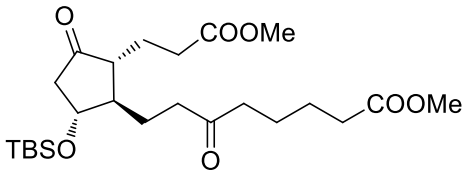
^{1}H NMR (300 MHz, $CDCl_3$): 4.15 (m, 1H), 3.94 (m, 1H), 3.66 (s, 6H), 3.03 (d, 1H, J=10.4 Hz), 2.46 (m, 8H), 1.85 (m, 4H), 1.62 (m, 7H), 1.43 (m, 1H), 0.87 (s, 9H), 0.08 (s, 6H).

^{13}C NMR (75 MHz, $CDCl_3$): 209.8 (s), 174.3 (s), 173.7 (s), 79.9 (d), 74.4 (d), 52.8 (d), 51.3 (q), 50.5 (d), 42.2 (t), 41.9 (t), 40.9 (t), 33.7 (t), 32.5 (t), 27.9 (t), 25.6 (q), 25.2 (t), 24.3 (t), 23.0 (t), 17.6 (s), -4.9 (q).

$[\alpha]^{D}_{20}$= +16.66 (c=0.24, CH_2Cl_2)

Synthesis of 60

methyl 8-((1R,2R,5R)-5-((tert-butyldimethylsilyl)oxy)-2-(3-methoxy-3-oxopropyl)-3-oxocyclopentyl)-6-oxooctanoate



Chemical Formula: $C_{24}H_{42}O_7Si$
Molecular Weight: 470,67

To a solution of **59** (20 mg, 0.04 mmol) in DCM (2 mL) was added Dess-Martin periodinane (20mg, 0.05mmol). The reaction mixture was stirred at r.t. for 3h. Saturated aqueous solutions of $NaHCO_3$ (2mL), $Na_2S_2O_3$ (2mL) and DCM (2 mL) were added. The layers were separated and the aqueous layer was extracted with DCM (3x3mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on SiO_2. The elution with hexane/AcOEt 8:2 gave 19 mg of pure ketone **60** (95% yield).

^{1}H NMR (300 MHz, $CDCl_3$): 4.15 (m, 1H), 3.67 (s, 6H), 2.63-2.26 (m, 8H), 2.07 (m, 1H), 2.00-1.53 (m, 11H), 0.92 (s, 9H), 0.12 (s, 3H), 0.09 (s, 3H).

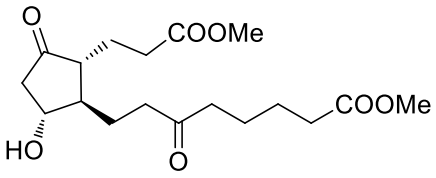
^{13}C NMR (75 MHz, $CDCl_3$): 217.0 (s), 210.3 (s), 174.2 (s), 74.5 (d), 52.8 (d), 52.1 (q), 52.1 (q), 49.8 (d), 48.2 (t), 43.0 (t), 41.0 (t), 34.5 (t), 32.0 (t), 26.8 (t), 26.3 (q),25.3 (t), 25.2 (t), 23.9 (t), 18.5 (s), -4.0 (q), -4.4 (q).

H-ESI MS (m/z): 471 $[M+H]^+$; 493 $[M+Na]^+$

$[\alpha]^D_{20}$= -28.36° (c=0.55, CH_2Cl_2)

Synthesis of 61

Methyl 8-((1R,2R,5R)-5-hydroxy-2-(3-methoxy-3-oxopropyl)-3-oxocyclopentyl)-6-oxooctanoate



Chemical Formula: $C_{18}H_{28}O_7$
Molecular Weight: 356,41

Aqueous HF (48%, 126 µL, 3.5 mmol) was added to a stirred solution of ketone **60** (48 mg, 0.1 mmol) in MeCN (8 mL) in a PE test tube. After 18 h phosphate buffer (pH 6.8, 10 mL) was added. The layers were separated and the aqueous layer was extracted with AcOEt (6x6 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel. Elution with hexane/AcOEt 1:1 gave pure **61** (29 mg, 80%) as a colorless oil.

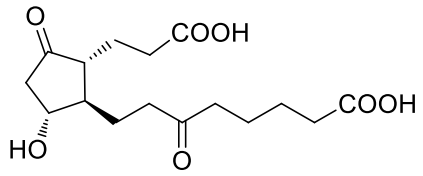
^{1}H NMR (300 MHz, CD_3CN): 4.05 (m, 1H), 3.66 (s, 6H), 3.21 (d, 1H, J=4.4 Hz), 2.46 (m, 8H), 2.12 (m, 2H), 2.01-1.50 (m, 10H)

^{3}C NMR (75 MHz, CD_3CN): 216.4 (s), 210.4 (s), 173.6 (s), 173.5 (s), 71.9 (d), 52.2 (d), 51.0 (q), 50.9 (q), 48.2 (d), 46.6 (t), 41.7 (t), 39.9 (t), 39.4 (t), 33.3 (t), 30.9 (t), 25.5 (t), 24.2 (t), 24.0 (t), 22.9 (t).

H-ESI MS (m/z): 379 $[M+Na]^+$

Synthesis of 19

8-((1R,2R,5R)-2-(2-carboxyethyl)-5-hydroxy-3-oxocyclopentyl)-6-oxooctanoic acid (PGE-M)



Chemical Formula: $C_{16}H_{24}O_7$
Molecular Weight: 328,36

The bis-methyl ester **61** (24 mg, 0.07 mmol) was dissolved in HPLC-grade MTBE (1.5 mL), and HPLC-grade H_2O (130 µL, 7.2 mmol) was added. Solid-supported CAL-B (2 mg) was added to the mixture, and the suspension was gently stirred at room temperature for 18 h. The enzyme was removed by filtration through a sintered glass funnel, and the solid was carefully washed with MeCN/MTBE (1:1; 4x2 mL). The filtrates were combined, and the solvents were evaporated under vacuum (CAUTION: without heating). The residue was purified by RP-18 column chromatography (MeOH/H_2O 8:2) to give pure PGE-M (7 mg, 30%) as a pale yellow oil.

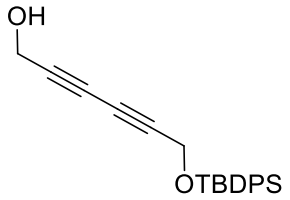
The spectroscopic data match those reported in literature[30a].

H-ESI MS (m/z): 327 $[M\text{-}H^+]^-$

$[\alpha]^D_{20}$= -11.2 (c=0.20, MeOH) reported in literature[30a]: $[\alpha]^D_{20}$= -9.1 (c=0.10, MeOH)

Synthesis of 75

6-((*tert*-butyldiphenylsilyl)oxy)hexa-2,4-diyn-1-ol



Chemical Formula: $C_{22}H_{24}O_2Si$
Molecular Weight: 348,51

To a stirred solution of 2,4-hexadiyne-1,6-diol (250mg, 2.27mmol) and imidazole (205.9 mg, 0.75 eq) in DMF (5.7 mL) was added dropwise a solution of *t*-butyldiphenylsilyl chloride (295.3 mg, 0,5 eq)in DMF (1.1mL) at 0 °C. After stirring at room temperature for 18h the mixture was worked up with Brine (15mL) and MTBE (4x10 ml). The combined extracts were dried with anhydrous sodium sulfate and the solvent was removed under vacuum. The crude was purified by silica gel flash column chromatography (hex/AcOEt 8:2) to yield the desired product (298 mg, 75% yield) as a pale yellow oil.

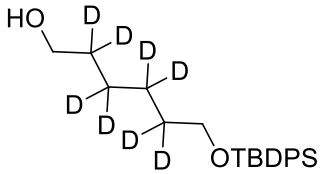
^{1}HNMR (300MHz, $CDCl_3$): δ 7.75 (dt, 4H, J=1.7, 7.6);7.47 (m, 6H); 4.45 (s, 2H); 4.30 (s, 2H); 2.10 (bs; 1H); 1.15 (s, 9H).

^{13}CNMR (75MHz, $CDCl_3$): δ 135.5 (d); 132.6 (q); 129.7 (d); 127.7 (d); 70.0 (s), 68.9 (s); 52.9 (t); 51.35 (t), 26.6 (q); 19.1 (s).

H-ESI MS (m/z): 371 $[M+Na]^+$

Synthesis of 73

D_8-6-((*tert*-butyldiphenylsilyl)oxy)-hexan-1-ol



Chemical Formula: $C_{22}H_{24}D_8O_2Si$
Molecular Weight: 364,62

To a magnetically stirred solution of **75** (928 mg, 0.861mmol) in methanol (9 mL) was added Rh on activated alumina 10% w/w (15 mg). The reaction mixture was stirred under deuterium atmosphere for 16h. The cathalyst was removed by filtration on a pad of celite and the filtrate was concentrated under vacuum. The crude was purified by flash chromatography on silica gel (DCM/AcOEt 98:2). 600 mg of the desired compound **73** were obtained (62% yield).

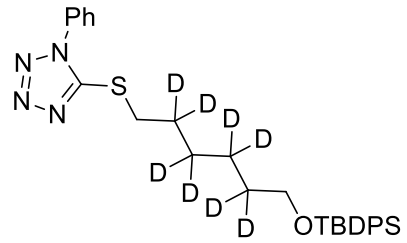
^{1}HNMR (300MHz, $CDCl_3$): δ 7.70 (m, 4H); 7.30 (m, 6H); 3.72 (s, 2H); 3.65 (s, 2H); 1.15 (s, 9H).

^{13}CNMR (75MHz, $CDCl_3$): δ 135.5 (d); 134.0 (s); 129.4 (d); 127.5 (d); 63.6(d); 62.8 (d), 26.8 (q); 19.1 (s).

H-ESI MS (m/z): 387 $[M+Na]^+$

Synthesis of 79

D_8-5-((6-((*tert*-butyldiphenylsilyl)oxy)hexyl)thio)-1-phenyl-1H-tetrazole



Chemical Formula: $C_{29}H_{28}D_8N_4OSSi$
Molecular Weight: 524,82

To a magnetically stirred solution of **73** (600 mg, 1.645 mmol) in dry toluene (11 mL) under Ar at rt were added Bu_3P (527 µL, 2.138 mmol), PTSH (352 mg, 1.974 mmol), and DEAD 40% in toluene (980 µL, 2.138 mmol). The reaction was completed after 3 h and was concentrated under vacuum. The residue was purified by column chromatography on silica gel using DCM/EtOAc 98:2 as eluent to afford the sulfide **79** as colorless oil (751mg, 87%).

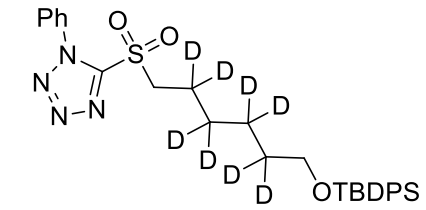
^{1}HNMR (300MHz, $CDCl_3$): δ 7.70 (m, 4H); 7.60 (m, 5H); 7.35 (m, 6H); 3.68 (s, 2H); 3.35 (s, 2H); 1.08 (s, 9H).

^{13}CNMR (75MHz, $CDCl_3$): δ 154.4 (s); 135.5 (d); 134.0 (s); 133.7 (s); 129.9 (d), 129.7 (d); 129.4 (d); 127.5 (d); 123.7 (d); 63.5(t); 33.0 (t), 26.8 (q); 19.1 (s).

H-ESI MS (m/z): 525 $[M+H]^+$

Synthesis of 77

D_8-5-((6-((*tert*-butyldiphenylsilyl)oxy)hexyl)sulfonyl)-1-phenyl-1H-tetrazole



Chemical Formula: $C_{29}H_{28}D_8N_4O_3SSi$
Molecular Weight: 556,82

To a magnetically stirred solution of **79** (708 mg, 1.35 mmol) in dry Et_2O (14mL) at 0°C was added mCPBA (512mg, 2.97mmol). The mixture was stirred at room temperature for 24h. the reaction was monitored by TLC (hex/AcOEt 8:2). The reaction was quenched by addition of saturated aq. $NaHCO_3$ (15mL). The aqueous layer was extracted with Et_2O (3x1 0mL), the combined organic layers were washed with brine, dried on Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by chromatography on silica gel using hex/AcOEt as eluent in gradient from 95:5 to 8:2 to afford the desired product **77** as colorless oil (474mg, 64% yield).

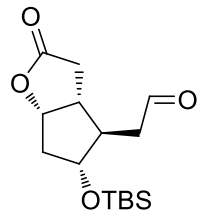
^{1}HNMR (300MHz, $CDCl_3$): δ 7.70 (m, 9H); 7.4 (m, 6H); 3.71 (s, 2H); 3.68 (s, 2H); 1.07 (s, 9H).

^{13}CNMR (75MHz, $CDCl_3$): δ 153.6 (s); 135.5 (d); 133.8 (s); 132.9 (s); 131.4 (d), 129.6 (d); 129.5 (d); 127.5 (d); 125.0 (d); 63.2 (t); 55.7 (t), 26.8 (q); 19.1 (s).

H-ESI MS (m/z): 557 $[M+H]^+$

Synthesis of 76

2-((3aR,4R,5R,6aS)-5-((*tert*-butyldimethylsilyl)oxy)-2-oxohexahydro-2H cyclopenta[b]furan -4-yl)acetaldehyde



Chemical Formula: $C_{15}H_{26}O_4Si$
Molecular Weight: 298,45

(methoxymethyl)triphenylphosphonium chloride (1208 mg, 3.52 mmol) was dissolved in dry PhMe (30 mL) under Ar, and KHMDS (6.8 mL of 0.5 M solution in PhMe, 3.40 mmol) was added dropwise at 0 °C. The suspension was stirred for 45 min at 0 °C, and then the stirring was stopped in order to separate the liquid layer from the solid residue. The red liquid layer was transferred to another dry round-bottom flask under Ar and was cooled to −78 °C. In another dry two-neck round-bottom flask the TBS-Corey Aldeyde **50** (400 mg, 1.41 mmol) was dissolved in dry PhMe (5 mL) under Ar. The solution of the aldehyde was added to the solution of the ylide dropwise, and the solution was allowed to reach rt. The reaction was monitored by TLC (hexane/EtOAc 8/2), and when it was complete, the reaction was stirred at r.t. for 15 min and was quenched with saturated aq. NH_4Cl (60 mL) and Et2O (50 mL). The layers were separated, and the aqueous layer was extracted with Et_2O (3 × 50 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under vacuum. The residue was partially purified by filtration on silica gel using hexane/AcOEt 8:2 as eluent to afford 380 mg of crude **78**.

The enolether **78** was dissolved in 20 mL of THF in a round bottom flask. To the solution was added 2mL of water and $Hg(OAc)_2$ (1.01 g, 3.16 mmol), the mixture after few seconds become deep yellow. After 1.5h the reaction was completed. The reaction was quenched by the addition of a solution of aq. KI 7% w/w (15 mL). The suspension turns from yellow to dark red and was filtered on a pad of celite. The filtrate was extracted with Et_2O (3x15mL). The combined organic layers were washed with aq. KI, $Na_2S_2O_3$, water and brine,

then dried on Na_2SO_4 and concentrated under vacuum. The residue was purified by column chromatography on silica gel using hexane/AcOEt 7:3 as eluent o afford the desired aldehyde **76** (300 mg, 71% yield over two steps).

^{1}HNMR (300MHz, CD_3CN): δ 9.70 (t, 1H, J= 1.4 Hz); 4.96 (td, 1H, J= 7 Hz, 2.3 Hz); 4.01 (q, 1H, J= 4.6 Hz); 2.79 (dd, 1H, J= 17.5 Hz, 10.2 Hz); 2.40 (m, 7H); 1.89-1.70 (2m, 1H); 0.88 (s, 9H); 0.08 (s, 6H).

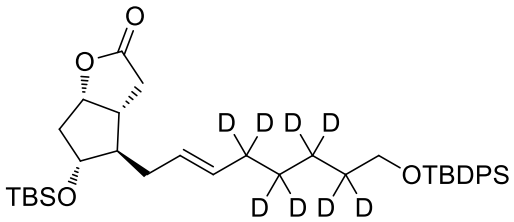
^{13}CNMR (75MHz, CD_3CN): δ 201.7 (d); 177.1(s); 83.6 (d); 77.6 (d), 48.6 (d); 46.2 (t), 42.2 (d); 40.1 (t); 35.3 (t); 25.1 (q); 17.5 (s); -5.6 (q); -5.8 (q).

H-ESI MS (m/z): 321 $[M+Na]^+$

$[\alpha]^D_{20}$= -16.7 (c=0.91, CH_2Cl_2)

Synthesis of 67

D_8-(3aR,4R,5R,6aS)-5-((*ter*t-butyldimethylsilyl)oxy)-4-((E)-8-((*tert*-butyldiphenylsilyl)-oxy)oct-2-en-1-yl)hexahydro-2H-cyclopenta[b]furan-2-one



Chemical Formula: $C_{37}H_{48}D_8O_4Si_2$
Molecular Weight: 629,06

Sulfone **77** (330 mg, 0.603 mmol) was dissolved in dry DME (5 mL) under Ar. The solution was cooled to −65 °C and KHMDS (1450µL, solution 0.5 M in toluene, 0.724 mmol) added dropwise. After 40 min, a solution of 7 (234 mg, 0.784 mmol) in dry DME (1 mL) under Ar was added dropwise to the reaction. The reaction mixture was stirred at −65 °C for 1 h and then allowed to reach room temperature. The reaction was quenched with saturated aq NH_4Cl (5 mL), H_2O (5 mL), and Et_2O (10 mL). The layers were separated, and the aqueous layer was extracted with Et_2O (3 × 10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified by column chromatography on silica gel using hexane/AcOEt 9:1 to afford the pure product **67** (227mg, yield = 60%).

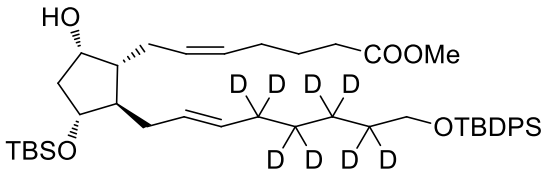
^{1}HNMR (300MHz, CD_3CN): δ 7.7(m, 4H); 7.4 (m, 6H); 5.4 (m, 2H); 4.96 (td, 1H, J= 7 Hz, 2.3 Hz); 3.95 (q, 1H, J= 3.9 Hz); 3.66 (s, 2H); 2.74 (m, 1H); 2.54 (m, 2H); 2.25-1.77 (m, 5H); 1.62 (bs, 1H); 1.16 (s, 9H); 0.98 (s, 9H); 0.10 (s, 6H).

^{13}CNMR (75MHz, CD_3CN): δ 177.4 (s); 135.4(d); 134.0 (s); 132.9 (d); 129.4 (d);127.5 (d); 83.6 (d); 63.6 (t); 54.6 (d); 41.8 (d); 40.4 (t); 36.0 (t); 35.7 (t); 26.7 (q); 25.6 (q); 19.1 (s); 17.8 (s); -4.8 (q); -5.1 (q).

H-ESI MS (m/z): 630 $[M+H]^+$ $[\alpha]^D_{20}$= -0.12 (c=0,91 CH_2Cl_2)

Synthesis of 62

(Z)-methyl 7-((1R,2R,3R,5S)-3-((*tert*-butyldimethylsilyl)oxy)-2-((E)-8-((*tert*-butyldiphenyl-silyl)oxy)oct-2-en-1-yl)-5-hydroxycyclopentyl)hept-5-enoate



Chemical Formula: $C_{43}H_{60}D_8O_5Si_2$
Molecular Weight: 729,22

DIBAL-H (1M in hexanes, 235 µL, 0.235 mmol) was added dropwise to a stirred solution of lactone **67** (227 mg, 0.361 mmol) in dry DCM (4 mL) precooled to – 78°C under an argon atmosphere. Stirring was continued for 30 min and then the excess reducing agent was destroyed by addition of a saturated solution of NH_4Cl at –78°C. The mixture was gradually warmed to room temperature and diluted with DCM, followed by addition of a saturated solution of sodium and potassium tartrate (6 mL). The two phases were separated and the aqueous one was extracted with DCM (3x4mL); the combined organic layers were dried on $MgSO_4$, filtered, and evaporated in vacuo to give the expected lactol **65**, that was employed in the following step without further purification.

KHMDSA 0.5M in $PhCH_3$ (6.4 mL, 3.21 mmol) was added dropwise to a suspension of (4-carboxybutyl)-triphenylphosphonium bromide (719.6 mg, 1.61 mmol) in dry THF (2 mL) under an argon atmosphere at 0°C. After having been stirred for 30 min at room temperature, the solution became orange. The solution was then cooled to -40°C and $PhCH_3$ (7mL) was added. A $PhCH_3$ (2.5 mL) solution of lactol **65** (0.361 mmol) was added dropwise via cannula. Stirring was continued for 3h at -30°C and then the solution was allowed to warm to 0°C. after an additional hour of stirring the reaction was quenched by adding a saturated solution of NH_4Cl (15mL) and acetic acid (200µL, 1.1 equiv. with respect to KHMDSA); Et_2O (10 mL) was added to the mixture and the organic layer was separated whereas the aqueous phase was extracted with Et_2O (3x10ml). The organic phases were combined, dried on $MgSO_4$, filtered, and concentrated under reduced pressure. crude acid

80 was used directly in the next step after a quick filtration on a pad of silica gel with AcOEt as eluent.

The residue was dissolved with a mixture of MTBE/MeOH 9:1 (4 mL) and lipase B from candida antarctica supported on polystyrene resin was added (95 mg). The heterogeneous mixture was gently stirred for 24h. the mixture was filtered to recover the enzyme and the solvent evaporated under vacuum. The crude was purified by flash column chromatography on silica gel with hexane/AcOEt 9:1 as eluent affording the pure methyl ester **62** (197 mg, 75% yield).

^{1}HNMR (300MHz, $CDCl_3$): δ 7.70(m, 4H); 7.40 (m, 6H); 5.38 (m, 4H); 4.15 (t, 1H, J= 3.6Hz); 4.05(d, 1H, J= 4.4 Hz); 3.68 (s, 3H); 3.67 (s, 2H); 2.48-2.10 (m, 8H); 1.85 (m, 2H); 1.75 (m, 4H); 1.06 (s, 9H); 0.89 (s, 9H); 0.08 (s, 6H).

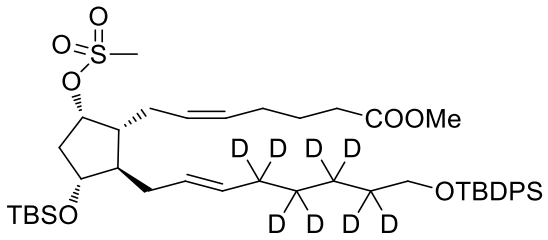
^{13}CNMR (75MHz, $CDCl_3$): δ 174.1 (s); 135.4(d); 134.0 (s); 132.3(d); 129.8 (d); 128.8 (d); 128.1 (d); 127.5 (d); 78.8 (d); 75.5 (d); 63.7 (t); 52.9 (d); 51.3 (q); 51.2 (d); 41.9 (t); 36.9 (t); 33.4 (t); 27.7 (t); 26.8 (q); 26.5 (t); 25.6 (q); 24.8 (t); 19.1 (s); 17.7 (s); -4.8 (q); -4.9 (q).

H-ESI MS (m/z): 751 $[M+Na]^+$

$[\alpha]^{D}_{20}$= +0.071 (c=0.95, CH_2Cl_2)

Synthesis of 91

D_8-(Z)-methyl 7-((1R,2R,3R,5S)-3-((*tert*-butyldimethylsilyl)oxy)-2-((E)-8-((*tert*-butyldiphenylsilyl)oxy)oct-2-en-1-yl)-5-((methylsulfonyl)oxy)cyclopentyl)hept-5-enoate



Chemical Formula: $C_{44}H_{62}D_8O_7SSi_2$
Molecular Weight: 807,31

A solution of methyl ester **62** (229 mg, 0.314mmol) in dry dichloromethane (3 mL) under Ar atmosphere was cooled to 0°C. Freshly distilled Et_3N (65 µL, 0.471 mmol) and subsequently methansulfonyl chloride were added dropwise. After 30 min the reaction was quenched by addition of phosphate buffer (5mL, pH=7) and DCM (3mL). The layers were separated and the aqueous layer was extracted with DCM (3x3mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified on silica gel column chromatography (hexane/AcOEt 9:1) affording pure mesilate **91** (203 mg, 80% yield).

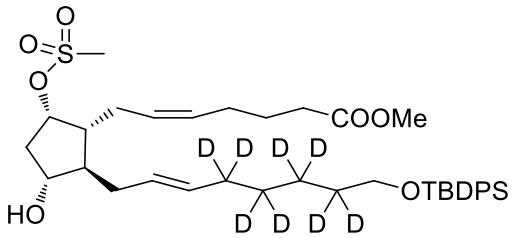
^{1}HNMR (300MHz, $CDCl_3$): δ 7.70(m, 4H); 7.40 (m, 6H); 5.38 (m, 4H); 4.95 (m, 1H); 3.95 (m, 1H); 3.68 (s, 2H); 3.66 (s, 3H); 3.00 (s, 3H); 2.45-1.80 (m, 10H); 1.66 (m, 4H); 1.08 (s, 9H); 0.90 (s, 9H); 0.08 (s, 6H).

^{13}CNMR (75MHz, $CDCl_3$): δ 174.1 (s); 136.3 (d); 135.0 (s); 133.9 (d); 131.0(d); 130.3 (d); 128.9 (d); 128.3 (d); 127.3 (d); 84.18 (d); 75.8 (d); 64.7 (t); 52.0 (d); 51.8 (d); 47.4 (q); 42.5 (t); 39.4 (q); 34.2 (t); 33.9 (t); 27.5 (d); 27.4 (q); 26.4 (q); 26.2 (t); 25.5 (t); 19.8 (s); 18.6 (s); -3.9 (q); -4.3 (q).

H-ESI MS (m/z): 829 $[M+Na]^+$ $[\alpha]^D_{20}$= +0.227 (c= 1.1, CH_2Cl_2)

Synthesis of 90a

D_8-(Z)-methyl 7-((1R,2R,3R,5S)-2-((E)-8-((*tert*-butyldiphenylsilyl)oxy)oct-2-en-1-yl)-3-hydroxy-5-((methylsulfonyl)oxy)cyclopentyl)hept-5-enoate



Chemical Formula: $C_{38}H_{48}D_8O_7SSi$
Molecular Weight: 693,05

The mesilate **91** (203 mg, 0.251mmol) was dissolved in absolute iPrOH (5mL) and PPTS was added (20mg, 0.075mmol). The reaction was stirred for 36h at room temperature. The reaction was quenched by addition of phosphate buffer (5mL, pH=7) and DCM (3mL). The layers were separated and the aqueous layer was extracted with DCM (3x3mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified on silica gel column chromatography (hexane/AcOEt 8:2) affording the desired compound **90a** as a colorless oil (73 mg, 60% based on the converted starting material).

^{1}HNMR (300MHz, CD_2Cl_2): δ 7.70(m, 4H); 7.44 (m, 6H); 5.49 (m, 4H); 5.02 (m, 1H); 3.99 (m, 1H); 3.69(m, 2H); 3.66 (s, 3H); 3.00 (s, 3H); 2.40-1.50 (m, 15H); 1.08 (s, 9H).

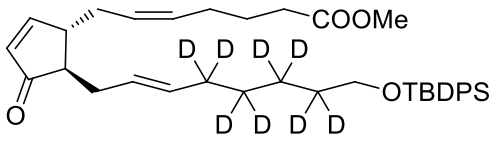
^{13}CNMR (75MHz, $CDCl_3$): δ 174.6 (s); 136.4 (d); 136.1 (s); 134.1 (d); 131.2 (d); 130.4 (d); 128.8 (d); 128.4 (d); 128.1 (d); 85.3 (d); 76.9 (d); 64.8 (t); 52.7 (d); 52.2 (d); 49.4 (q); 42.0 (t); 39.6 (q); 35.7 (t); 34.2 (t); 27.6 (t); 27.5 (q); 26.4 (t); 25.6 (t); 19.4 (s).

H-ESI MS (m/z): 715 $[M+Na]^+$

$[\alpha]^D_{20}$= +0.352 (c= 0.5, CH_2Cl_2)

Synthesis of 82

D_8-(Z)-methyl 7-((1S,5R)-5-((E)-8-((*tert*-butyldiphenylsilyl)oxy)oct-2-en-1-yl)-4-oxocyclopent-2-en-1-yl)hept-5-enoate



Chemical Formula: $C_{37}H_{42}D_8O_4Si$
Molecular Weight: 594,93

To a solution of **90a** (33mg, 0.048 mmol) in DCM (1 mL) was added Dess-Martin periodinane (24mg, 0.058mmol). The reaction mixture was stirred at r.t. for 3h. Saturated aqueous solution of $NaHCO_3$ (1mL), $Na_2S_2O_3$ (1mL), water (1mL) and DCM (3mL) were added. The layers were separated and the aqueous layer was extracted with DCM (3x1mL). The combined organic layers were washed with BRINE and dried over Na_2SO_4, filtered and concentrated under reduced pressure.

The crude containing **89** was dissolved in anhydrous THF (1 mL) under inert atmosphere of Argon. The mixture was cooled to 0°C and DBU (7 µL, 0.048mmol) were added dropwise. After 1h under magnetic stirring at 0°C the reaction was completed. Water (2mL) and DCM (3mL) were added. The layers were separated and the aqueous layer was extracted with DCM (3x1mL). The combined organic layers were washed with BRINE and dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified on silica gel column chromatography (hexane/AcOEt 9:1) affording the desired cyclopentenone **82** as a colorless oil (23 mg, 81% over two steps).

^{1}HNMR (300MHz, CD_2Cl_2): δ 7.70(dd, 4H, J= 7.5Hz, 2.5 Hz); 7.55 (dd, 1H, J= 5.7Hz, 2.3 Hz); 7.42 (m, 6H); 6.14 (dd, 1H, J= 5.7Hz, 1.9 Hz); 5.45 (m, 4H); 3.70 (s, 3H); 3.65 (s, 2H); 2.75 (m, 1H); 2.50(m, 1H); 2.30 (m, 6H); 2.15 (m, 2H); 1.76 (m, 2H); 1.06 (s, 9H).

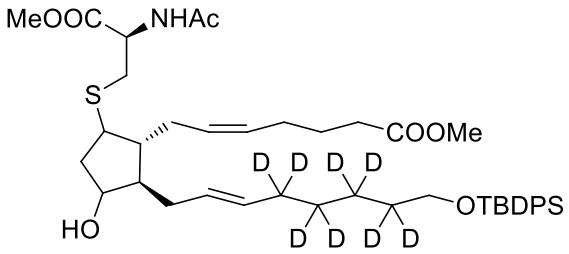
^{13}CNMR (75MHz, $CDCl_3$): δ 211.3 (s); 173.8 (s); 166.6 (d); 135.4 (d); 134.0 (s); 133.2 (d); 131.1 (d); 129.4 (d); 127.5 (d); 126.7 (d); 126.3 (d); 63.7 (t); 51.4 (q); 50.8 (d); 46.5 (d); 42.0 (t); 33.5 (t); 33.3 (t); 31.3 (t); 26.8 (q); 26.5 (t); 24.6 (t); 19.1 (s).

H-ESI MS (m/z): 617 $[M+Na]^+$

$[\alpha]^{D}_{20}$= +0.183 (c= 0.5, CH_2Cl_2)

Synthesis of 96

D_8-(Z)-methyl 7-((1R,2R)-5-(((R)-2-acetamido-3-methoxy-3-oxopropyl)thio)-2-((E)-8-((*tert*-butyldiphenylsilyl)oxy)oct-2-en-1-yl)-3-hydroxycyclopentyl)hept-5-enoate



Chemical Formula: $C_{43}H_{55}D_8NO_7SSi$
Molecular Weight: 774,16

To a clear solution of **82** (24mg, 0.04mmol) in ethanol (1 mL) 1 equivalent of N-acetyl-cysteine methyl ester **94** (7 mg) was added, and the mixture was stirred until homogenous. After this time, TBA-OH (40 wt% in H_2O, 1 mol%, 0.264 µL) was added in one portion, and the mixture was stirred for 90 min. The mixture was partionated between H_2O (4mL) and Et_2O (4mL). The aqueous layer was extracted with Et_2O (4x3mL). The combined organic layers were washed with BRINE and dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude containing **92** was dissolved with absolute methanol (0.5mL) and the solution was cooled to 0°C. $NaBH_4$ was then added to the mixture. After 1h under stirring the reaction was quenched by adding some drops of acetone, the mixture was stirred for additional 15 min. The solvent was removed under vacuum; the residue was taken up with AcOEt (5mL) and filtered on a thin pad of celite. The AcOEt was removed under reduced pressure. The residue purified by a silica gel column chromatography (hexane/AcOEt 4:6) to give 18mg of the desired compound **96** with the 60% yield over two steps.

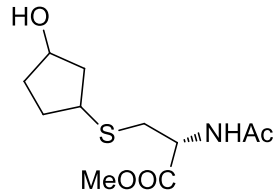
^{1}HNMR (300MHz, CD_3OD): δ 7.67(m, 4H); 7.43(m, 6H); 5.44 (m, 4H); 4.60 (m, 1H); 4.13 (m, 1H); 3.74(s, 3H); 3.66 (s, 5H); 3.03 (dd, 1H, J= 12.1 Hz, 5.2Hz); 2.79 (m, 2H); 2.36-2.00 (m 8H); 2.00 (s, 3H); 1.76-1.50 (m, 6H); 1.05 (s, 9H).

^{13}CNMR (75MHz, $CDCl_3$): δ 176.0 (s); 173.5 (s); 173.1 (s); 137.0 (d); 135.4 (s); 132.8 (d); 131.7 (d); 130.9 (d); 129.1 (d); 73.7 (d); 65.2 (t); 54.2 (s); 53.2 (s); 52.3 (q); 50.8 (q); 47.3 (s); 43.7 (t); 34.6 (t); 34.2 (t); 32.5 (t); 30.3 (t); 28.1 (t); 27.7 (q); 26.3 (t); 22.6 (q); 19.4 (s).

H-ESI MS (m/z): 796 $[M+Na]^+$

Synthesis of 97

(2R)-methyl 2-acetamido-3-((3-hydroxycyclopentyl)thio)propanoate



Chemical Formula: $C_{11}H_{19}NO_4S$
Molecular Weight: 261,34

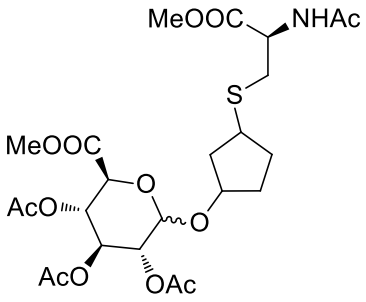
To a clear solution of cyclopent-2-en-1-one **93** (236 µL, 2.82mmol) in ethanol (28 mL) 1 equivalent of N-acetyl-cysteine methyl ester **94** (500 g) was added, and the mixture was stirred until homogenous. After this time, TBA-OH (40 wt% in H_2O, 1 mol%, 19 µL) was added in one portion, and the mixture was stirred for 90 min. The mixture was partionated between H_2O (mL) and Et_2O (4mL). The aqueous layer was extracted with Et_2O (4x3mL). The combined organic layers were washed with BRINE and dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude containing **95** was dissolved with absolute methanol (26 mL) and the solution was cooled to 0°C. $NaBH_4$ (117mg, 3.1 mmol) was then added to the mixture. After 1h under stirring the reaction was quenched by adding acetone, the mixture was stirred for additional 15 min. The solvent was removed under vacuum, the residue was taken up with AcOEt (30mL) and filtered on a pad of celite. The AcOEt was removed under reduced pressure. The residue purified by a silica gel column chromatography (DCM/MeOH98:2) to give 515 mg of the desired compound **97** with the 70% yield over two steps.

^{1}H NMR (200 MHz, $CDCl_3$): 6.51 (m, 1H), 4.81 (m, 1H), 4.32 (m, 1H), 3.76 (s, 3H), 3.4 (m, 1H), 3.01 (m, 2H),2.53 (bs, 1H), 2.26 (m, 1H), 2.02 (s, 3H), 1.75 (m, 6H).

H-ESI MS (m/z): 262 $[M+H]^+$; 284 $[M+Na]^+$.

Synthesis of 101

(3R,4S,5S,6S)-2-((3-(((R)-2-acetamido-3-methoxy-3-oxopropyl)thio)cyclopentyl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate



A solution of **97** (263mg, 0.536 mmol) in dry DCM (2.5 mL) was stirred under static atmosphere of Ar at room temperature with freshly activated 4Å molecular sieves for 1h. A solution of tricholroacetimidate **102** (170mg, 0.356 mmol) in dry DCM (1mL) was added, and after stirring for a further 1h the mixture was cooled to 0°C. $BF_3{\cdot}OEt_2$ (45µL, 0.356mmol) was added to mixture dropwise. After a 1.5h the reaction was quenched by addition of saturated aqueous $NaHCO_3$ (3mL) and AcOEt (3mL). The layers were separated and the aqueous layer was extracted with AcOEt (3x3mL). The combined organic layers were washed with BRINE and dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified on silica gel column chromatography (hexane/AcOEt 8:2) affording the desired glycosylated compound 101 (70 mg, 34% yield).
Alternatively, TMSOTf (0.5 equiv.) was added instead of $BF_3{\cdot}OEt_2$ in the above procedure.

^{1}H NMR (200 MHz, $CDCl_3$): 6.20 (m, 1H), 5.83 (d, J=5 Hz), 5.23 (m, 2H), 4.75 (m, 1H), 4.32 (m, 2H), 3.76 (s, 6H), 3.25 (m, 1H), 3.01 (m, 2H), 2.06 (m, 12H), 1.75 (m, 7H).

H-ESI MS (m/z): 578.3$[M+H]^+$; 600.2 $[M+Na]^+$