

## REVIEW

[View Article Online](#)  
[View Journal](#) | [View Issue](#)

 CrossMark  
click for updates

Cite this: RSC Adv., 2015, 5, 39726

## Labelling of brassinosteroids by isotopes of hydrogen and carbon

 Mahadeo R. Patil,<sup>a</sup> Tomáš Elbert<sup>\*b</sup> and Rangappa S. Keri<sup>\*a</sup>

The brassinosteroids (BRs) are a class of native plant growth regulating substances with high biological activity even at very low concentration. These compounds have been rigorously explored and it has been found that they are not only growth regulators in plants but also promising antiviral agents. Recently, it has been reported that natural BRs exhibit relatively interesting anticancer activities. Up to now, the basic anticancer potential of BRs against several normal and human cancer cell lines has been determined. Natural BRs, at micromolar concentrations, impart cell growth-inhibitory responses in several human cancer cell lines without affecting the normal cells. To study the mechanism of action of BRs at the molecular level, the corresponding isotopically labelled compounds are essential. The latter BRs are essential for the investigation of biosynthesis, metabolism, transport and distribution in plants. This venture ultimately led us to explore the labeling of BRs by isotopes of hydrogen and carbon and the related technique to do this. The present review will shed light on the synthetic avenues in this field from the time of the discovery of labelled BRs up until their most recent advances.

 Received 7th March 2015  
 Accepted 27th April 2015

 DOI: 10.1039/c5ra04081g  
[www.rsc.org/advances](http://www.rsc.org/advances)

### 1. Introduction

Brassinosteroids (BRs) represent a class of naturally occurring phytohormones with various physiological activities and

ubiquitous distribution in the plant kingdom.<sup>1</sup> The vicinal diol grouping on ring A is typical of the BR-plant hormones discovered thirty years ago.<sup>2</sup> In Fig. 1, the formulae of two typical BRs-24-epibrassinolide **1** and castasterone **2** – are given. These compounds have been extensively studied and it has been found that they exhibit not only growth regulation functions in plants but also promising antiviral activity.<sup>3</sup>

Recently, molecular studies directed towards the essential role of BRs in plant growth and development<sup>4</sup> and their chemical synthesis, biological mode of action, and practical application in agriculture and horticulture<sup>5,6</sup> have greatly intensified.

<sup>a</sup>Centre for Nano and Material Sciences, Jain University, Jain Global Campus, Bangalore 562112, Karnataka, India. E-mail: keriphd@gmail.com; sk.rangappa@jainuniversity.ac.in

<sup>b</sup>Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Flemingovo nám. 2, 16610 Prague 6, Czech Republic. E-mail: elbert@uochb.cas.cz



*Mahadeo R. Patil received his MSc degree in Organic Chemistry in 2007 from S. R. T. M. University, Nanded, Maharashtra, India. Then he moved to NCL, Pune, India to work as a research assistant. Further, he spent 4 years at IOCB, Prague, Czech Republic to work on research project as an Assistant Scientist. There his research work focused on the synthesis of isotopically labelled brassinosteroids and their application. Presently he is a Doctoral student in CNMS, Bangalore, India working with Dr. Rangappa Keri and his research is mainly focused on synthesis and biological activity of novel heterocyclic compounds.*



*Tomaš Elbert earned his Ph.D. degree working on sugar chemistry at the Faculty of Natural Sciences, Charles University, Prague, in 1980. He started to work on radioisotopes in 1980 at the Institute for Research, Production and Application of Radioisotopes, Prague. After that he served as Assistant professor of Organic Chemistry at Faculty of Natural Sciences, Charles University. Presently, he is head of the Laboratory of Radioisotopes at the Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences. In 2011 he was awarded with the IIS-CED Award 2011 for his work in the Advisory Board of IIS-CED.*



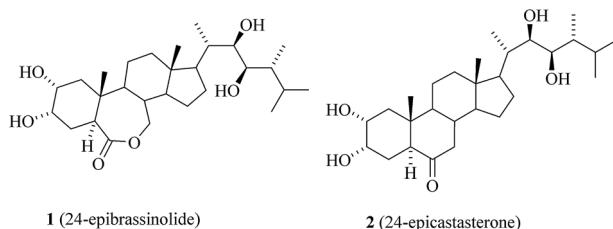


Fig. 1 Structures of 24-epibrassinolide (24-epiBL) and 24-epicastasterone (24-epiCS).

The essentialness of isotopically labelled BRs in the investigation of the biosynthesis, metabolism, transport and distribution of endogenous BRs in plants has been documented. In such studies, BRs labelled with isotopes of hydrogen are most frequently used to explain the biosynthesis, metabolism and mode of action at a molecular level.

Therefore, the numerous strategies for the synthesis of BRs labelled with deuterium ( $^2\text{H}$  or D) and tritium ( $^3\text{H}$  or T) in the side chain or in the ring system have been developed.<sup>7</sup> BRs are formed by a biosynthetic network of alternative pathways and sub-pathways. A number of feeding experiments using labelled BRs precursors as a substrate are necessary to elucidate these pathways. In these reactions, labelled BRs are being used as internal standards for qualitative analysis of endogenous BRs and they have been employed in biosynthetic experiments.<sup>8-11</sup> Some of the deuterium-labelled 24-methylenecholesterol and related C<sub>28</sub> steroids were used as both, internal standards in quantitative analyses and substrate, for metabolic studies in BRs deficient mutants of *Arabidopsis thaliana* and *Pisum sativum*. The biosynthetic sequence from campesterol<sup>12</sup> to campestanol in *Arabidopsis thaliana* was determined by identification of each intermediate followed by feeding experiments with deuterium-labelled intermediates.<sup>13,14</sup> The dwarf pea (*Pisum sativum*) mutants Ika and Ikb are BRs insensitive and deficient, respectively. The latter mutant was rescued to wild type by exogenous application of labelled brassinolide and its precursors. Feeding

experiments using deuterium labelled 24-methylenecholesterol indicated that an Ikb mutant is unable to isomerize and/or reduce the double bond.

Recently, brassinolide biosynthetic pathways have been elucidated by feeding deuterium-labelled intermediate to suspension cultures of *Catharanthus roseus*.<sup>15,16</sup> Identification of castasterone with one of the hydroxyl groups lacking and application of its labelled analogue in feeding experiments<sup>9</sup> demonstrated that the brassinolide biosynthesis proceeds *via* initial hydroxylation at C-22 followed by an introduction of the hydroxyl group at C-23. Deuterium labelled of secasterol, teasterone and typhasterol, upon administration to rye seedlings, were incorporated into secasterone and 2,3-diepisecasterone, indicating a biosynthetic route *via* teasterone/typhasterol to secasterol to 2,3-epoxybrassinosteroids, secasterone in seedlings of *Secale cereal*. Similarly, deuterated secasterone upon administration resulted in deuterated castasterone and 2-epicastasterone used in biosynthetic sub-pathways from typhasterol/teasterone *via* 2,3-epoxybrassinosteroids intermediate to castasterone (CS).<sup>17</sup> Some of the  $^{14}\text{C}$  labelled BRs, namely (22R,23R)- and (22S,23S)-[4- $^{14}\text{C}$ ]-24-epiBL (A and B in Scheme 11, respectively) were used to facilitate metabolic and distribution studies of (22R,23R)-24-epiBL and (22S,23S)-24-epiBL as well as to study their role in the growth of grain and vegetables as they are promising candidates for agriculture application.<sup>18</sup> Predominantly the  $^{14}\text{C}$ -labelled epiBL (Scheme 10) was used in the uptake and the transport study of exogenously applied epibrassinolide on seedlings of cucumber and wheat. When applied to roots,  $^{14}\text{C}$ -epiBL was readily taken up and swiftly transported throughout both the plant species. When  $^{14}\text{C}$ -epiBL was applied to the adaxial surface of a young cucumber leaf, it was readily taken up, however, transported very slowly compared with the previous case. In wheat leaves,  $^{14}\text{C}$ -epiBL was transported only in the apical direction from the treated spot after 3 days of treatment; however, it was not transported from the treated leaf to the other leaves or organs even after seven days. Recently, isotopically labelled [7,7- $^2\text{H}_2$ ] epibrassinolide was used for biosynthetic transformation studies.<sup>19</sup> This compound found useful for biochemical and physiological investigation in the plant.<sup>20</sup> Simultaneously, it was also discovered that natural BRs exhibit relatively interesting anticancer activities. So far, potential anticancer activities of 24-epiBL 1 and 24-epicastasterone 2 on several human cancer cell lines have been determined.<sup>19</sup> 24-Episecasterol was prepared and found cytotoxic against human breast carcinoma MCF-7 (Michigan Cancer Foundation) cells.<sup>21</sup> It was also demonstrated that non-plant cells, yeast WAT21, generates a steroid plant hormone castasterone. To understand how castasterone is generated in WAT21 cells, deuterium labelled 6-deoxo-[26,28- $^2\text{H}_6$ ]teasterone(TE), 6-deoxo-[26,28- $^2\text{H}_6$ ]typhasterol (TY) and 6-deoxo-[26,28- $^2\text{H}_6$ ](CS) were fed to WAT21 cells and their metabolites were isolated. [26,27- $^2\text{H}_6$ ] labelling of brassinolide, castasterone, typhasterol, and teasterone were reported by Takatsuto and Ikekawa in 1986.<sup>22</sup> Deuterium labelled 6-deoxo-BRs were identified as biosynthetic precursors of CS in WAT21 cells.<sup>23</sup> In this review, a variety of labelling



Dr. Rangappa Keri is currently working as an Assistant Professor at Jain University, Bangalore, Karnataka, India. Before joining Jain University, he did his post-doctoral work at IST, Universidade de Lisboa, Lisboa, Portugal and Kyung Hee University, Seoul, South Korea. His research interests concern Organic and Medicinal chemistry. Especially, development of new drugs including acetyl acetylcholinesterase (AChE) inhibitors, anti-cancer, neuroprotective agents and anti-virals by organic synthesis. Also, development of organometal and metaloorgano-catalyzed enantioselective methods for the synthesis of heterocyclic scaffolds.



procedures of BRs with stable and radioactive isotopes of hydrogen and carbon will be presented.

## 2. Synthesis of deuterium-labelled BRs

### 2.1. BRs with deuterium labels in the side chain

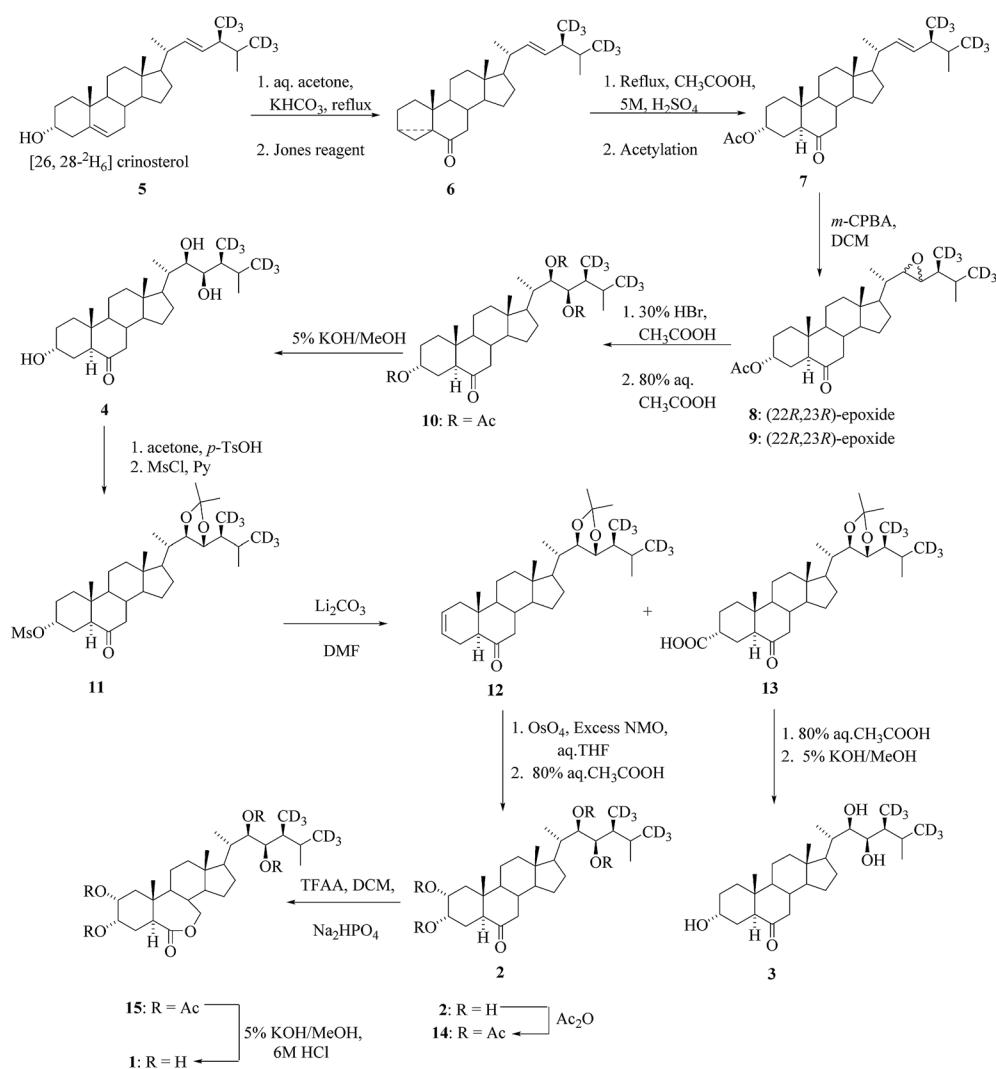
#### 2.1.1. $[26-^2\text{H}_3]$ , $[26,27-^2\text{H}_6]$ and $[26,28-^2\text{H}_6]$ labelled BRs.

Brassinolide (BL) and related C-28 BRs as castasterone (CS), typhasterol (TY), and teasterone (TE) occur in a wide variety of higher plants.<sup>24,25</sup> The synthesis of  $[26,28-^2\text{H}_6]$  brassinolide 1,  $[26,28-^2\text{H}_6]$ CS 2,  $[26,28-^2\text{H}_6]$ TY 3 and  $[26,28-^2\text{H}_6]$ TE 4 as an internal standards for GC-MS assays of BRs was reported in the literature.<sup>26,27</sup>  $[26,28-^2\text{H}_6]$ crinosterol<sup>28</sup> 5 served as a starting material for the synthesis of four deuterated BRs (1-4) (Scheme 1).

Alternatively, labelled BRs containing three or six deuterium atoms appended in the terminal methyl groups of the side chain (in a position ensuring lack of isotopic exchange) were prepared from stigmasterol or bisnororcholenic acid. There are

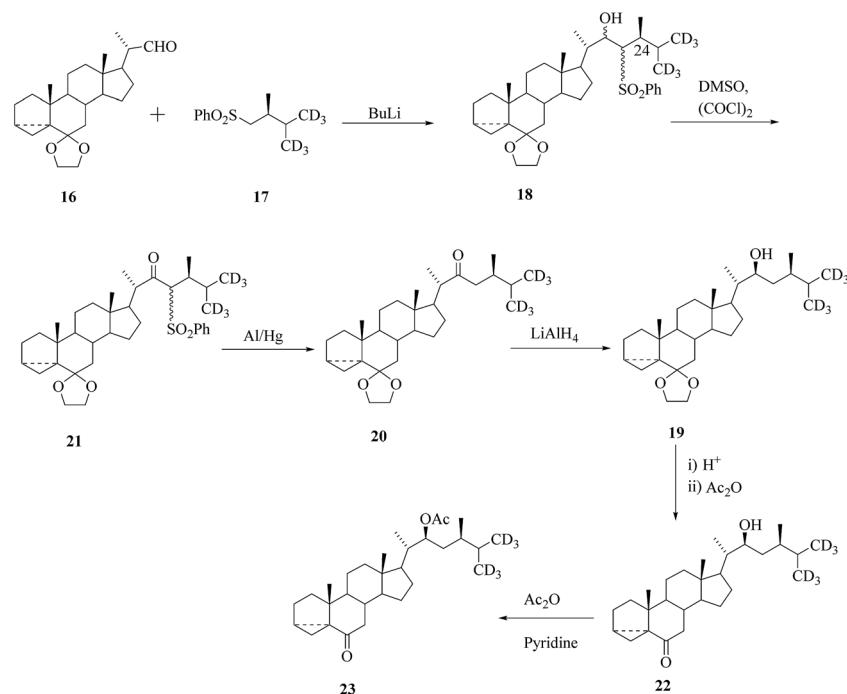
main strategies for the construction of the side chain containing an asymmetric centre at C-24 of the compound 18 and these are based on the coupling of 16-aldehyde with an appropriate chiral sulfone synthon 17. These methodologies were also used for the stereoselective construction of the side chain labelled BRs. Preparation of trideuterated fragment 22 in the side chain of BRs<sup>29,30</sup> through Claisen rearrangement for stereoselective construction of the steroid side chain. Construction of the side chain of hexa-deuterated BR was performed *via* coupling of sulfone 17 with aldehyde 16 (Scheme 2).

Introduction of functional groups at the cyclic part of the steroids could be done prior to the construction stages of the side chain based on various 22-aldehydes.<sup>31,32</sup> As a result, the aldehyde 25 was the best choice for the synthesis of labelled BR derivatives with functional groups tethered to the cyclic part and it was suitable for the preparation of many highly functionalized BRs. The aldehyde 25 was prepared either from stigmasterol 24 by traditional method<sup>33,34</sup> in 3 steps or from 23,24-bisnorcholelic acid<sup>35</sup> 26 (Scheme 3). An important part of the convergent synthesis was the preparation of the chiral intermediate 17, which

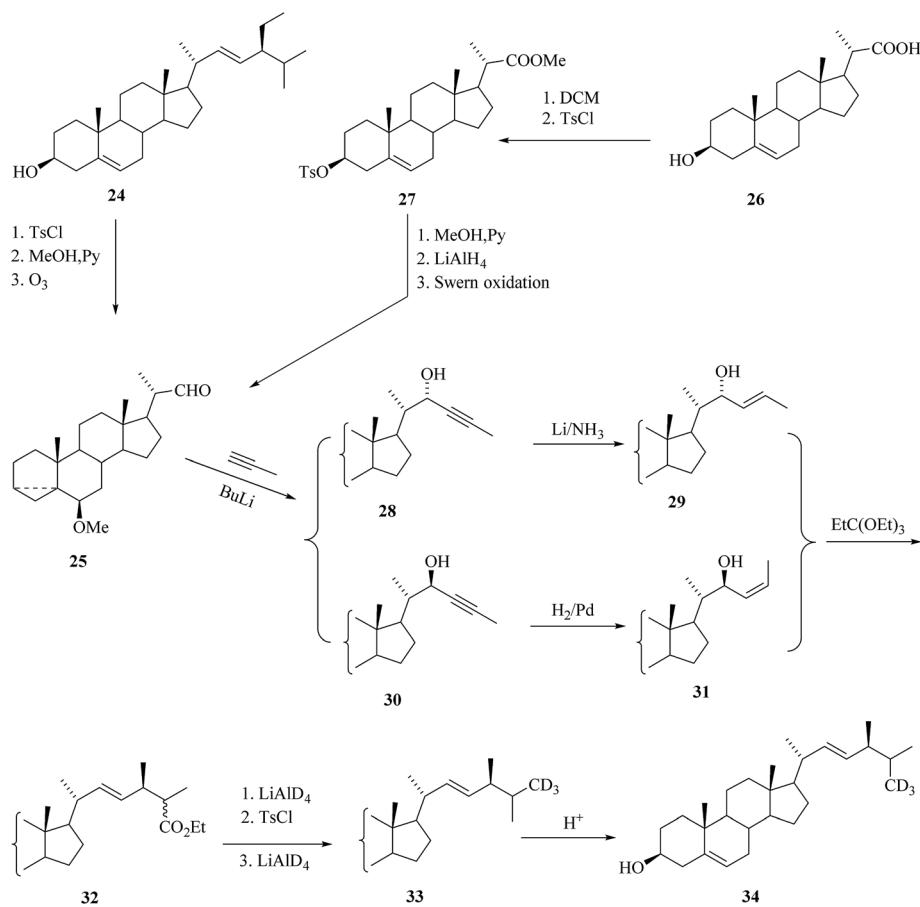


Scheme 1 Synthesis of  $[26,28-^2\text{H}_6]$ BL (1),  $[26,28-^2\text{H}_6]$ CS (2),  $[26,28-^2\text{H}_6]$ TY (3) and  $[26,28-^2\text{H}_6]$ TE (4).



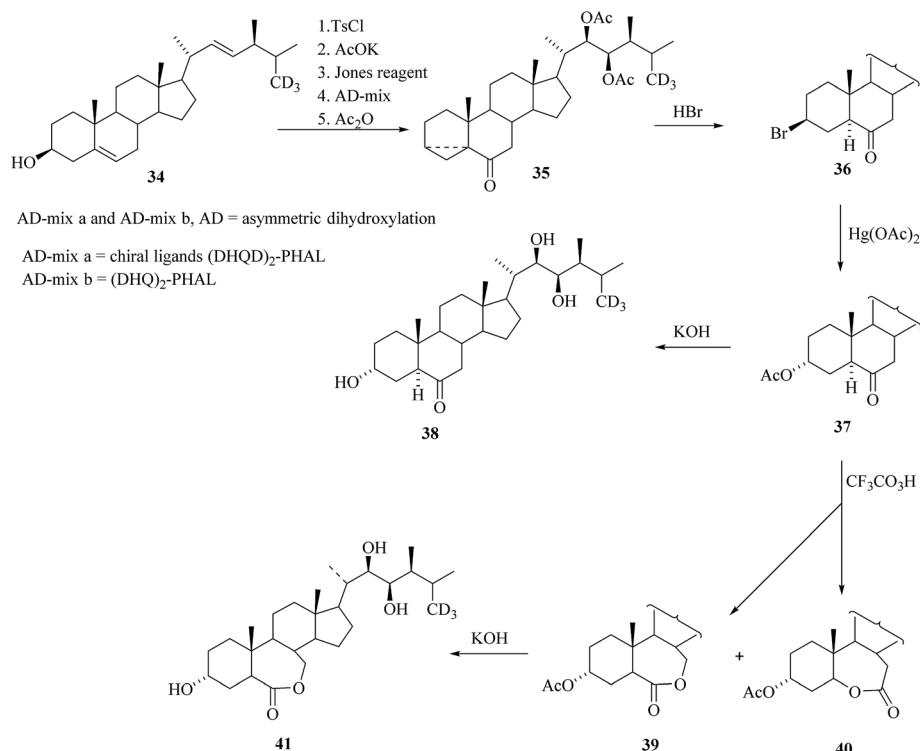


Scheme 2 Construction of the side chain of hexa-deuterated BRs.



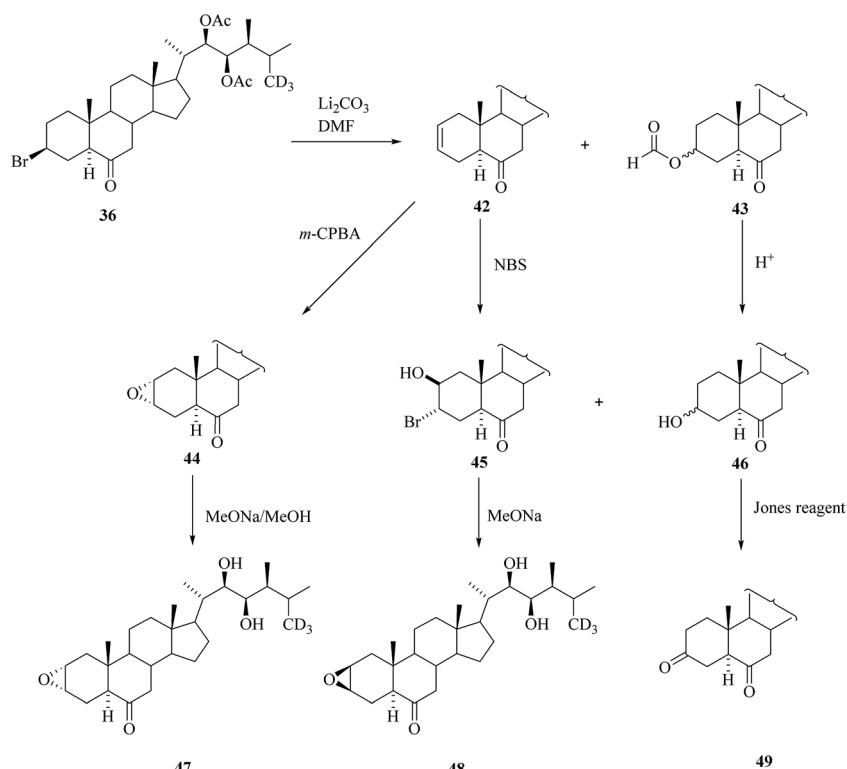
Scheme 3 Construction of the side chain of [26-2H3]BRs.



Scheme 4 Synthesis of  $[26-^2\text{H}_3]\text{TY}$  38 and related compounds.

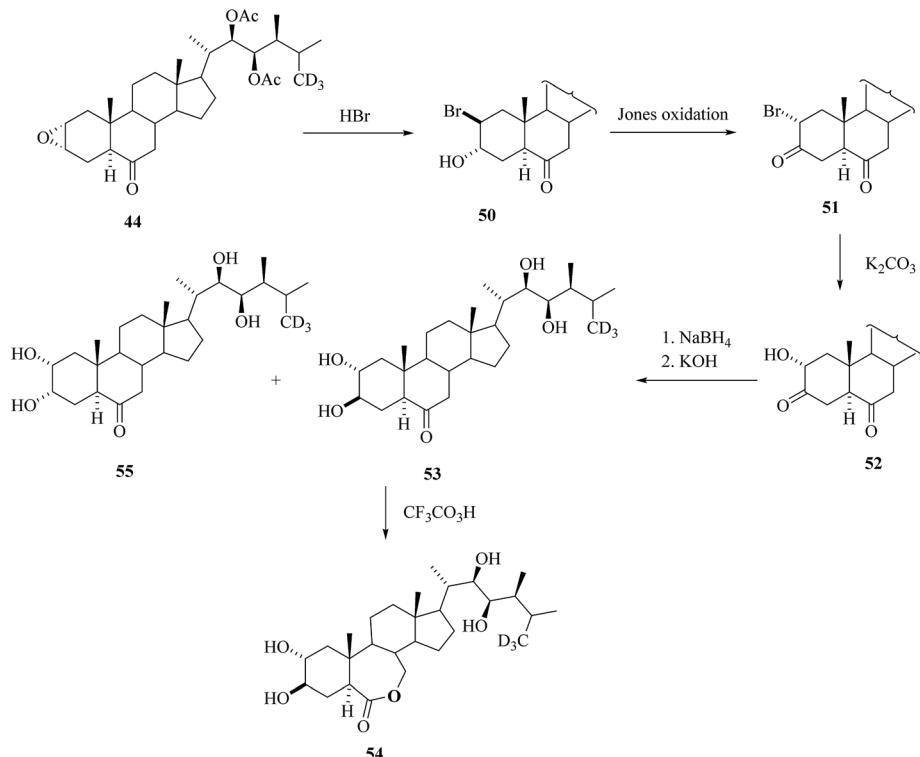
was accompanied through  $(2R)$ -3-hydroxy-2-methylpropanoate.<sup>35,36</sup> The synthesis of precursor of  $[26-^2\text{H}_3]$ -brassinosteroids 34 for biochemical studies from the starting material 25 is depicted

in Scheme 3. In the first convergent strategies based on the Claisen rearrangement of a single isomer of allylic alcohol is considered useful in this rearrangement to access certain



Scheme 5 Synthesis of deuterated BRs with an epoxy group in ring A.

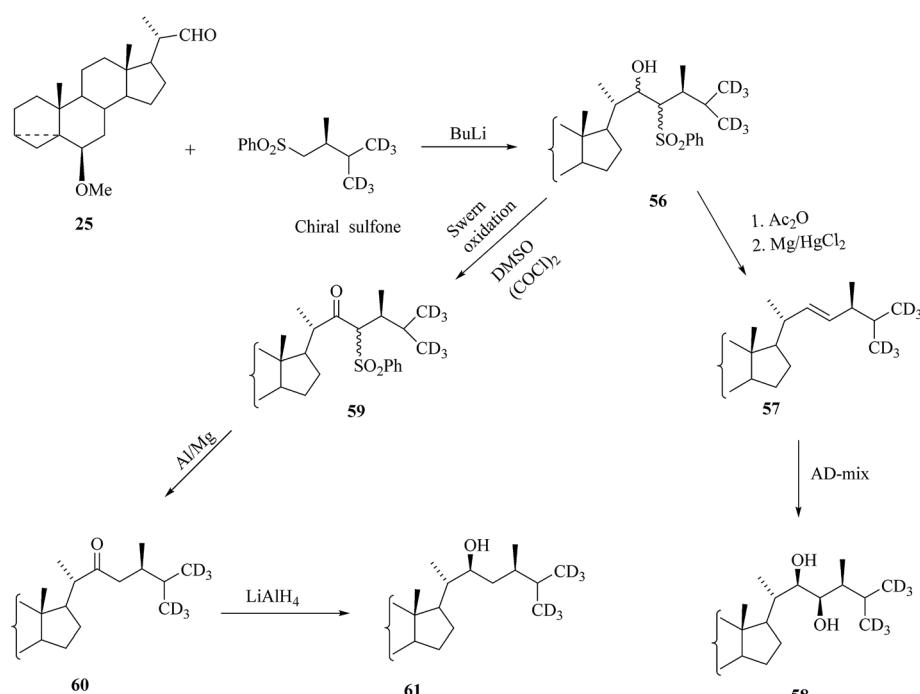


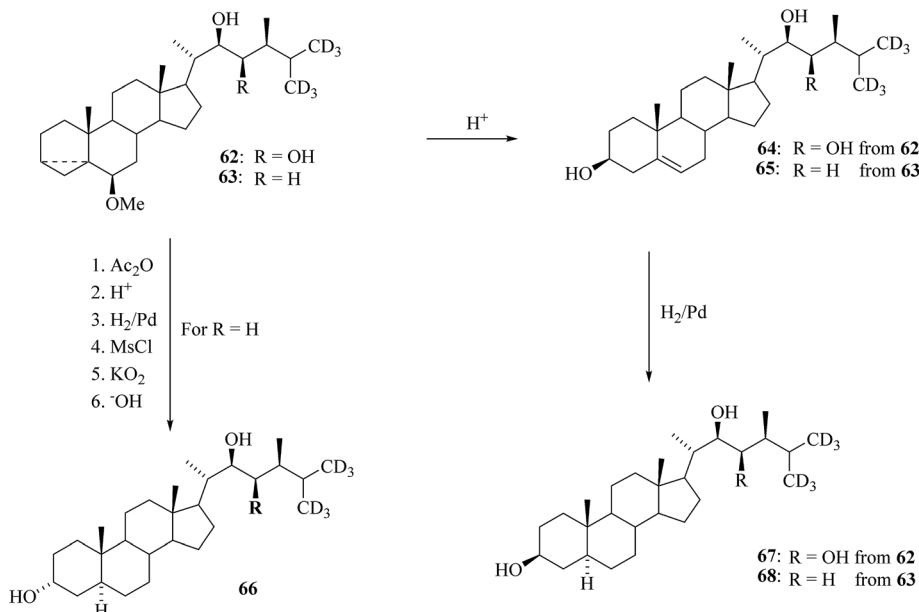


Scheme 6 Synthesis of deuterated tetra-hydroxyl BRs.

product.<sup>27,28,37,38</sup> A Claisen rearrangement has been widely used in the preparation of  $\Delta^{22}$ -steroids containing an alkyl substituent with predictable stereochemistry at C-24. Then the ester compounds 32 were prepared *via* isomeric acetylenic alcohols 28

and 30 and the allylic alcohol intermediates 29 and 31 (Scheme 3). Successive reduction of the ester 32 with LiAlD<sub>4</sub> followed by tosylation and deuteride reduction gave compound 33 containing three deuterium atoms in the terminal part of the side chain.<sup>30</sup>

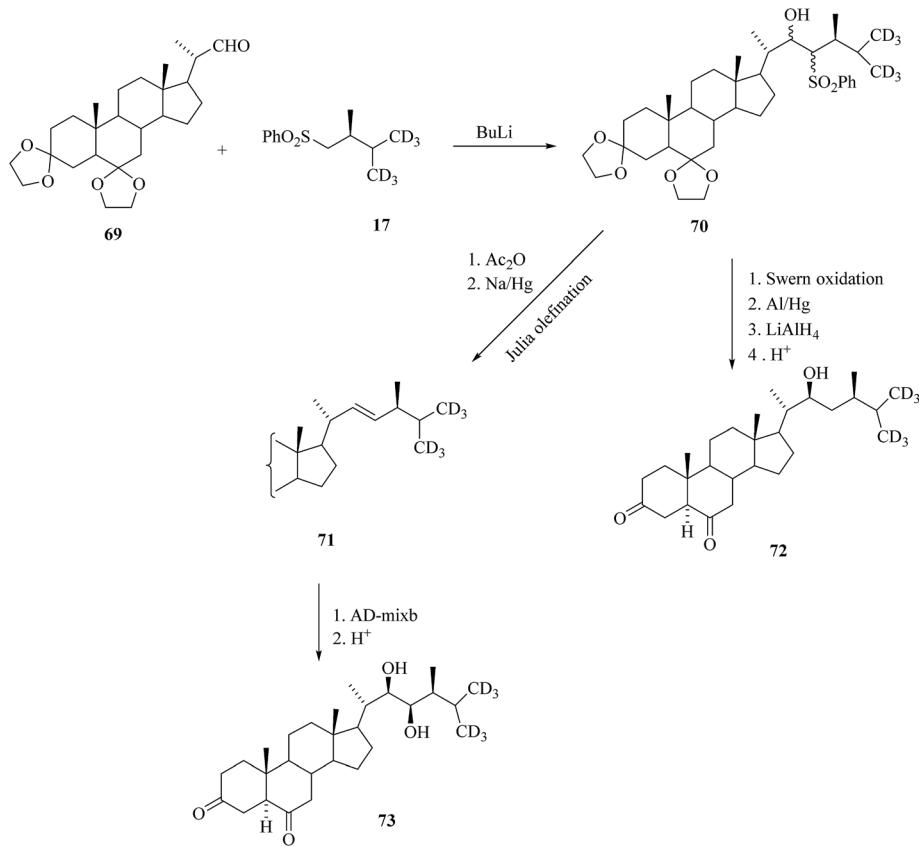
Scheme 7 Construction of the side chain of [26,27-<sup>2</sup>H<sub>6</sub>]BRs.

Scheme 8 Modification of rings A and B of [26,27-<sup>2</sup>H<sub>6</sub>]BRs.

Regeneration of the cyclic part of 33 by acid treatment to yield the deuterated crinosterol 34 (Scheme 3).

After formation of a 3-membered ring and oxidation on C-6 by standard sequence of reactions starting from the 34 and then the sharpless oxidation of side chain double bond using

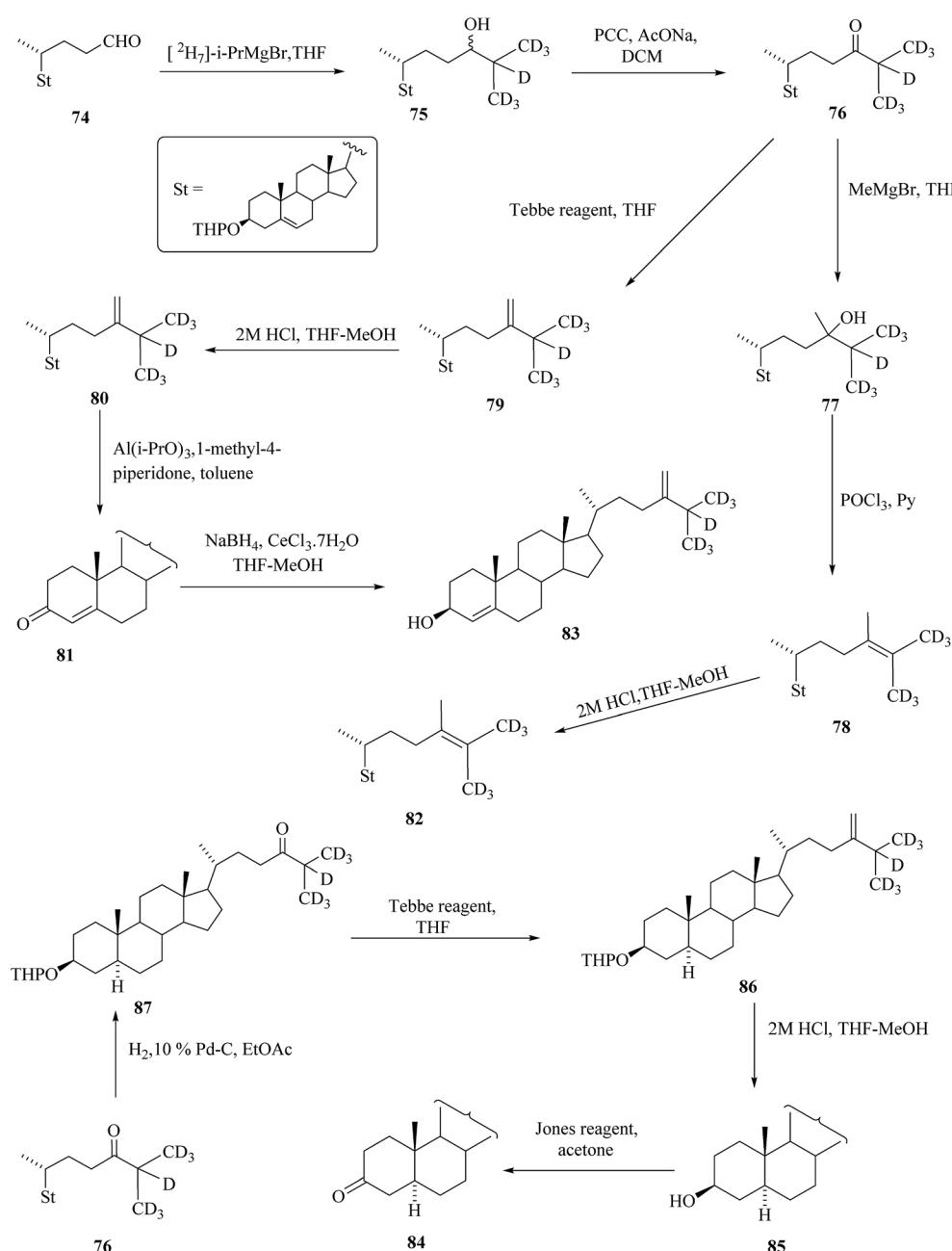
(DHQD)<sub>2</sub>-PHAL as a ligand and osmium tetroxide as catalyst led to expected alcohol and followed by acetylation to obtained the compound 35 and then to bromide 36. Nucleophilic substitution of the bromide in 36 offered 3 $\alpha$ -acetoxyketone 37 and after deacetylation [26-<sup>2</sup>H<sub>3</sub>]TY 38 was obtained. Baeyer-

Scheme 9 Synthesis of 3,6-diketo-[26,27-<sup>2</sup>H<sub>6</sub>] BRs.

Villiger oxidation of **37** yielded lactone **39** along with its regioisomer **40**. Deprotection of the hydroxyl groups furnished 2-dehydroxy-[26-<sup>2</sup>H<sub>3</sub>] brassinolide **41** (Scheme 4).

Bromide **36** found useful as an intermediate for the preparation of deuterated BRs with an epoxy group in ring A (Scheme 5). Dehydrobromination of **36** offered 6-ketone **42** as the main product of the reaction with formic ester **43** as a byproduct. Epoxidation of **42** ensued stereoselectively to yield 2 $\alpha$ ,3 $\alpha$ -epoxy-acetate **44** that was then deprotected to 2,3-[26-<sup>2</sup>H<sub>3</sub>]episecasterone **47**. Compound 2,3-[26-<sup>2</sup>H<sub>3</sub>] secasterone **48** was obtained *via* bromohydrin **45** from **42**. Formate **43** can be used for the preparation of 3,6-diketo BRs **49** *via* 3-dehydroteasterone **46** (Scheme 5).

The epoxide **44** was used as key intermediate for the synthesis of [26-<sup>2</sup>H<sub>3</sub>] BRs **53–55** as depicted in Scheme 6.<sup>30</sup> *trans*-Dixial ring opening of epoxide **44** with the action of HBr led to bromohydrin **50**. Jones oxidation ensued with the formation of a ketonic group at C-3 and inversion of configuration at C-2 (bromoketone **51**). After nucleophilic substitution of bromine by a hydroxyl group, the hydroxyl ketone **52** was isolated. The regiospecific and stereoselective reduction of **52** followed by saponification to remove acetate groups in the side chain furnished a mixture of 3-epi[26-<sup>2</sup>H<sub>3</sub>]CS **53** and [26-<sup>2</sup>H<sub>3</sub>]CS **55**. Bayer–Villiger oxidation of **53** with trifluoroperoxoacetic acid offered 3-[26-<sup>2</sup>H<sub>3</sub>]epiBL **54**.



Scheme 10 Phytosterol and their deuterium-labelled analogues.

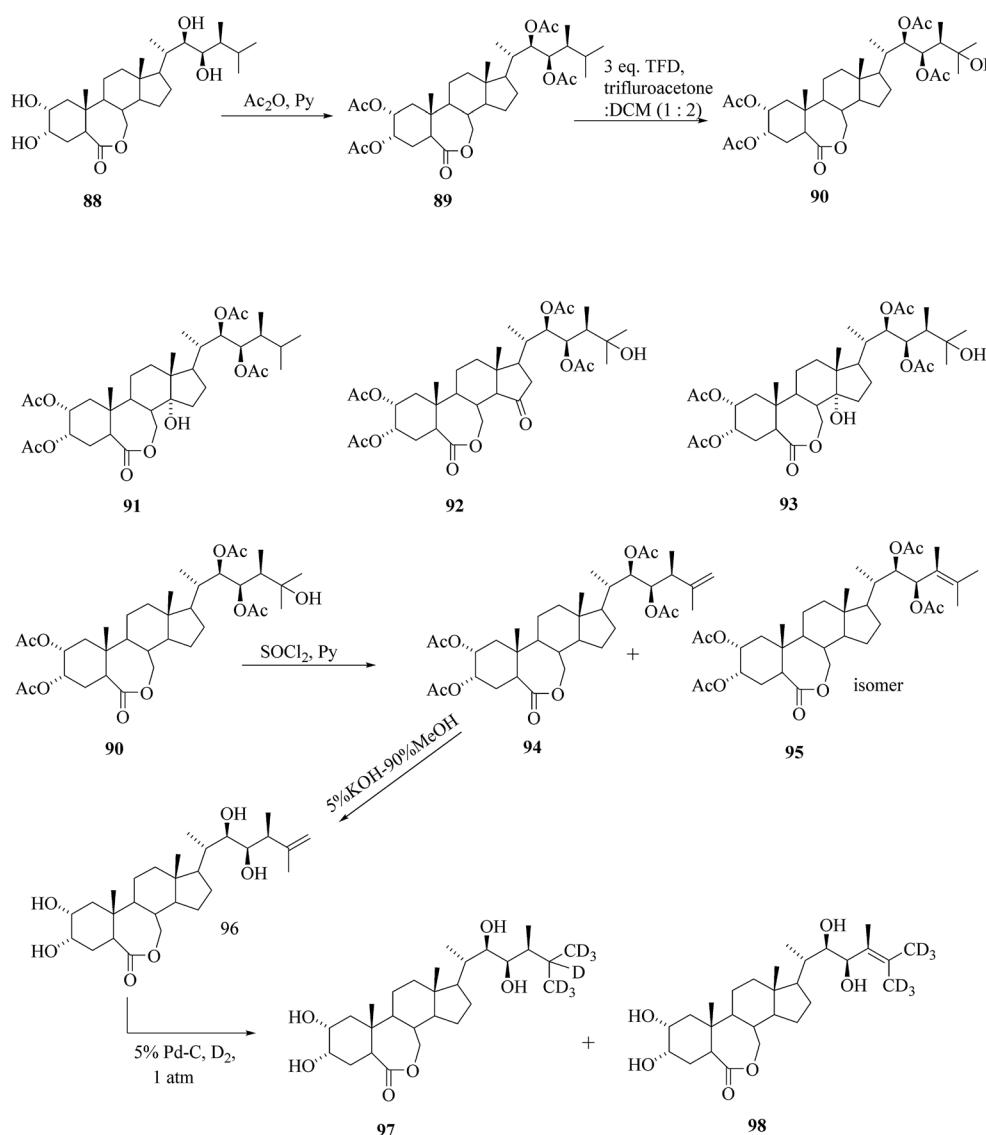


An important convergent approach using the side chain fragment was used for the preparation of the labelled BRs containing six deuterium atoms at C-26 and C-27.<sup>35,36</sup> Coupling of the appropriate aldehyde with lithium salt of chiral sulfone led to compounds **56** (Scheme 7).

Then alcohol **56** is functionalized by modified Julia olefination protocol *via* acetylation followed by reduction elimination with magnesium amalgam to furnish the olefin **57**. Introduction of a (22*R*,23*R*)-diol function was attained by using asymmetric dihydroxylation (AD) as discussed in the earlier case for [26-<sup>2</sup>H<sub>3</sub>] BRs. Synthesis of a derivative **61** demonstrated the methodology applied in the preparation of BRs containing one hydroxyl group in the side chain. Swern oxidation of hydroxyl sulfones **56** directed to the ketosulfones **59** and then desulfurization with aluminium amalgam was smoothly resulted in the formation of the ketones **60**. The further reduction of **60** with lithium aluminium hydride to offered desired (22*S*)-alcohols **61** as the main product (Scheme 7). Mono- and dihydroxy

derivatives **62** and **63**, respectively allowed easy access to a variety of corresponding [26,27-<sup>2</sup>H<sub>6</sub>]BRs as shown in Scheme 8.  $\Delta^5$ -3 $\beta$  Alcohols **64** and **65** were synthesized from **62** and **63** in a dioxane–water solution olfin presence of toluenesulfonic acid at elevated temperature. Hydrogenation of double bond over palladium bequeathed 6-deoxoesterone **67** and 6-deoxocathasterone **68**. The preparation of 3 $\alpha$ -alcohol **66** involved inversion configuration at C-3 *via* nucleophilic substitution of intermediate mesylate with potassium superoxide.

An attempt to prepare labelled brassinolide biosynthetic precursors having 3,6-diketone moiety was first reported with by-products similar to **42** obtained from dehydrobromination of 3 $\beta$ -bromides (see also Scheme 5). It is proved incompatible with the removal of the acetate protecting groups in the side chain and the problem was solved by using aldehyde **69** as a key intermediate (Scheme 9). Essentially, the same protocol as that described in Scheme 7 was used for the preparation of 3-dehydro-[26,27-<sup>2</sup>H<sub>6</sub>]cathasterone **72** and 3-dehydro-[26,27-<sup>2</sup>H<sub>6</sub>] TE **73**.<sup>35</sup>

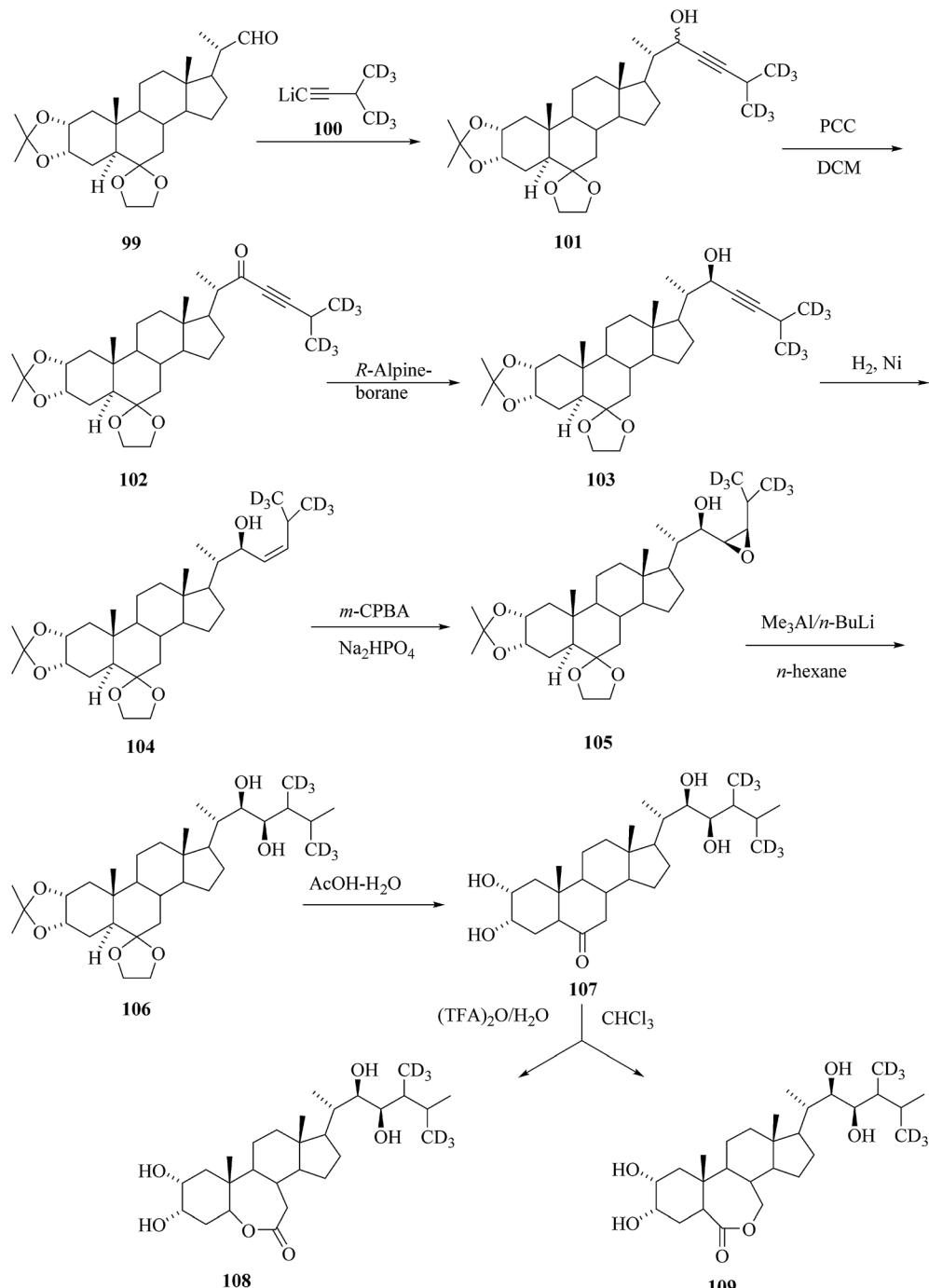


Scheme 11 Synthesis of [25,26,27-<sup>2</sup>H<sub>7</sub>]IBL.



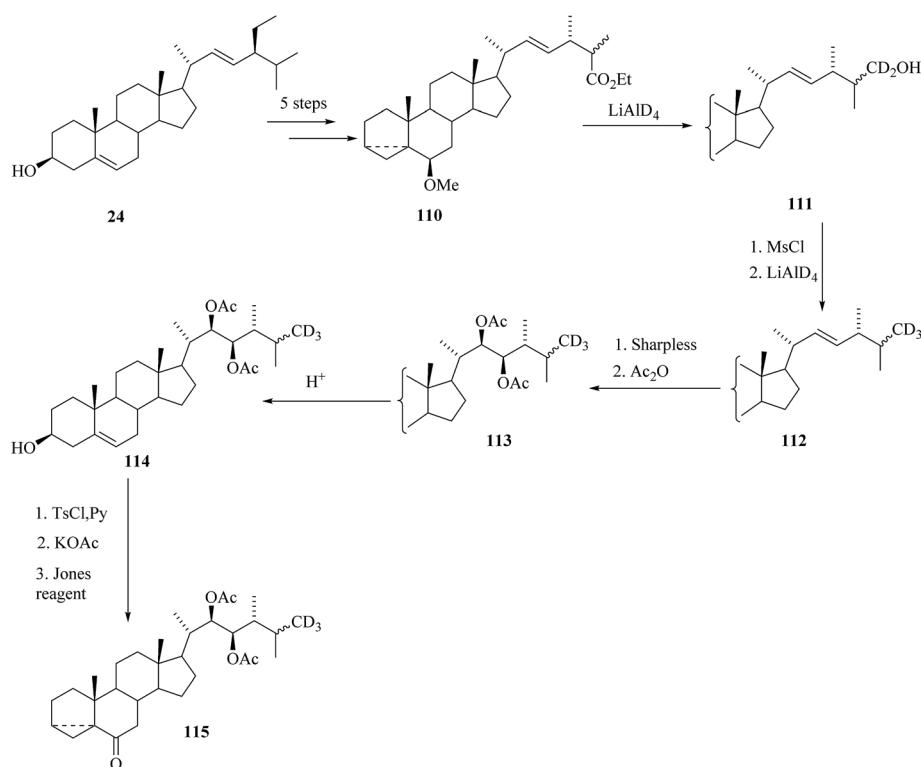
**2.1.2. Synthesis of deuterio-labelled 24-methylenecholesterol and related steroids.** Anastasia *et al.* reported the synthesis of BRs with one deuterium atom,<sup>39</sup> however, usually four and more deuterium atoms in the molecule are required for the MS standards. Contemporarily, Takatsuto *et al.* reported labelling of the side chain of targeted steroid by more than one deuterium atom. It is described in the work that the synthesis of deuterium labelled C<sub>28</sub> steroids **80** to **86** from the known 3 $\beta$ -tetrahydropyranoyloxychole-5-en-24-al **74** (Scheme 10).<sup>40</sup>

**2.1.3. Synthesis of [25,26,27-<sup>2</sup>H<sub>7</sub>]BL from parent brassinolide.** Several routes for the synthesis of labelled BRs have been reported so far,<sup>41</sup> however, all are quite lengthy multi step reactions which are neither eco-friendly nor cost-effective. The easy way to synthesize the side chain labelled BRs from parent BRs includes three basic steps; C-25 hydroxylation, dehydration to create double bond between C25-C26 and deuterioxygenation or tritiation of the double bond. Based on these basic steps, the preparation of [25,26,27-<sup>2</sup>H<sub>7</sub>]BL **97** from brassinolide **88** as a starting



Scheme 12 Synthesis of [26,28-<sup>2</sup>H<sub>6</sub>]CS and [26,28-<sup>2</sup>H<sub>6</sub>]BL.

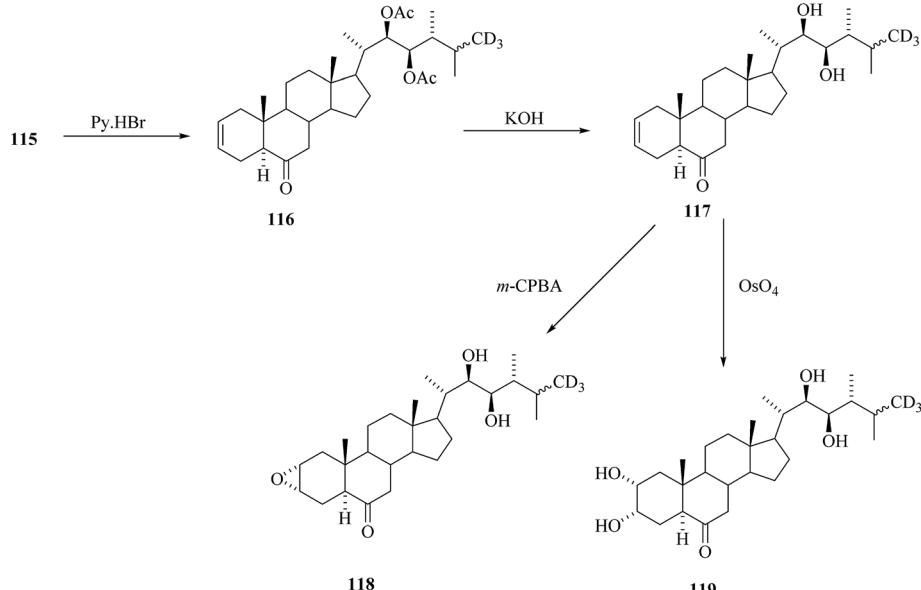


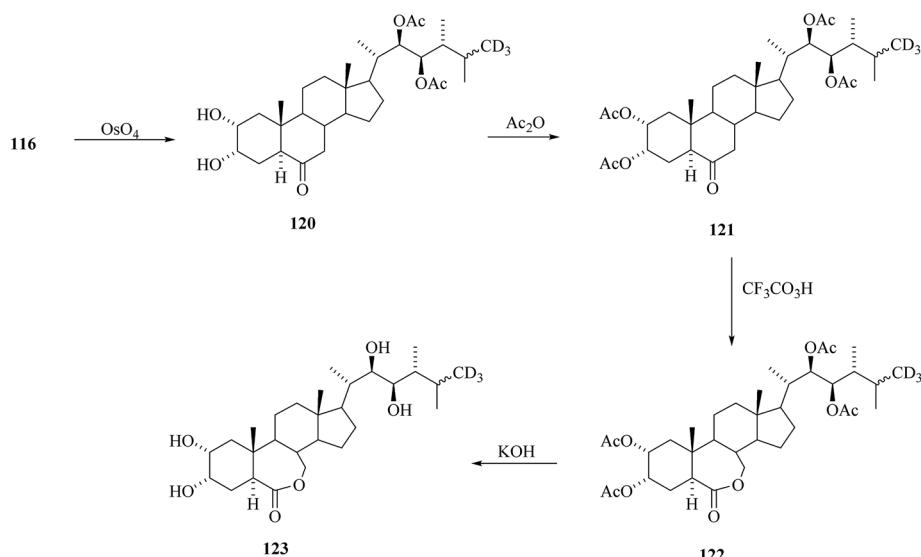


Scheme 13 Synthesis of cycloketone 115 from stigmasterol 24.

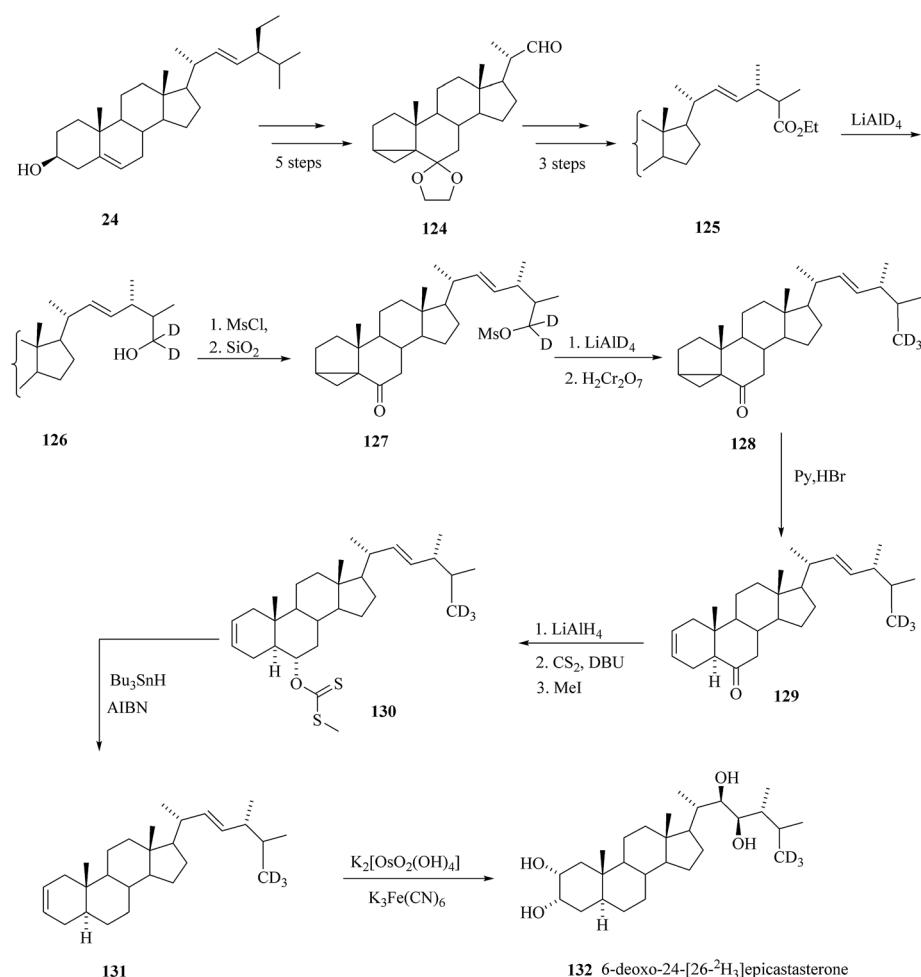
material was achieved<sup>42</sup> (Scheme 11). 25-Oxy-functionalization of tetra-*O*-acetyl BL 89 with TFD offered predominantly 25-hydroxy compound 90 while 14-hydroxy, 25-hydroxy-15-oxo- and 14,25-dihydroxy derivatives 91, 92, 93, respectively were identified as byproducts. Dehydration of 90 headed to the formation of a mixture of the compounds 94 and 95 (which were unseparable) and then the mixture of

isomers were subjected to deprotection of tetra-*O*-acetyl groups. Subsequently the mixture of isomers were treated with KOH in aqueous MeOH at room temperature until the lactone ring completely opened to the carboxylate and then at refluxing temperature. After recyclization of lactone ring and desalting the crystalline compound 96 was obtained. Reduction of the double bond of  $\Delta^{25(26)}$  BL 96 using

Scheme 14 Synthesis of 24-[26-<sup>2</sup>H<sub>3</sub>] epiCS 119.

Scheme 15 Synthesis of deuterated 24-[26- $^2\text{H}_3$ ] epiBL 123.

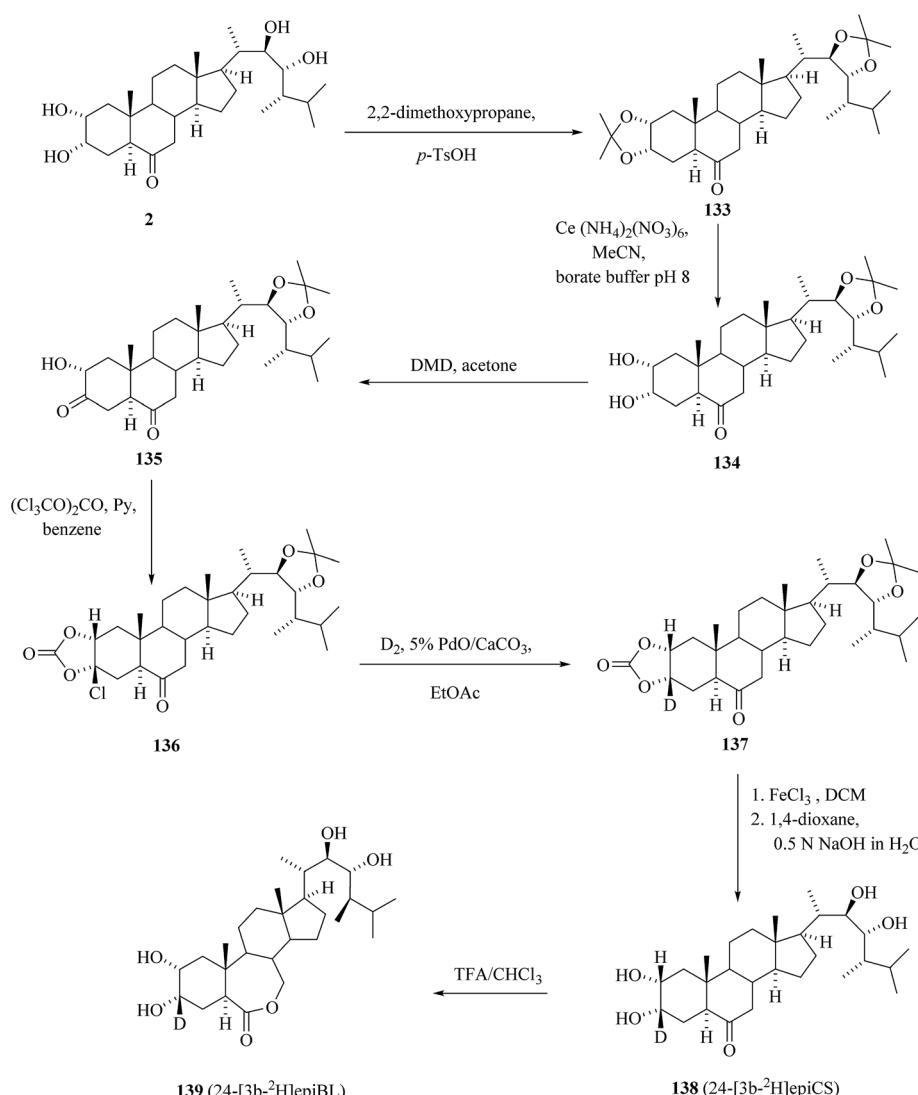
deuterium gas and 5% Pd/C as catalyst was preceded with extensive isotopic exchange, which resulted in high deuterium incorporation leading to the formation of [25,26,27- $^2\text{H}_7$ ] BL 97 with 60% isotopic purity. The partial migration of double bond provided the minor byproduct  $\Delta^{24(25)}$  [26,27- $^2\text{H}_6$ ]BL 98 with 68% isotopic purity.

Scheme 16 Synthesis of 6-deoxo-24-[26- $^2\text{H}_3$ ] epiCS 132.

**2.1.4. Alternative way to  $[26,28-^2\text{H}_6]$ brassinolide and  $[26,28-^2\text{H}_6]$ castasterone.** Kolbe *et al.*<sup>43</sup> reported the synthesis of deuterium labelled BRs  $[26,28-^2\text{H}_6]$ CS **107** and  $[26,28-^2\text{H}_6]$ BL **108** starting from 6,6-ethylenedioxy-20-formyl- $2\alpha,3\alpha$ -isopropylidenedioxy- $5\alpha$ -pregnane **99** and 3-[methyl- $^2\text{H}_3$ ]methyl-[4,4,4- $^2\text{H}_3$ ]but-1- $\text{yne}$  **100**, respectively (Scheme 12). The acetylenic alcohol **101** was oxidized by pyridinium chlorochromate and stereoselective reduction of ketone **102** yielded stereospecifically the acetylenic alcohol **103**. Partial hydrogenation on Raney® Ni gave an allylic alcohol **104**. The double bond was epoxidized by 3-chloroperbenzoic acid and then epoxide **105** was treated by trimethylaluminium/n-butyllithium reagent. Opening of the epoxide ring in **105** leads to the unusual methyl group migration from C-26 to C-25 took place during methylation and then subjected to hydrolysis to furnish  $[26,28-^2\text{H}_6]$ CS **107** was obtained instead of  $[26,27-^2\text{H}_6]$ -labelled isomer. Baeyer–Villiger oxidation of  $[26,28-^2\text{H}_6]$ castasterone **107** gave mixture of regioisomers **109** and **108** in which  $[26,28-^2\text{H}_6]$ BL **109** prevailed (**109 : 108 = 6 : 1**).

**2.1.5. Synthesis of  $[26-^2\text{H}_3]$ -epibrassinolide and its precursors.** A further study towards the synthesis of labelled BRs containing three deuterium atoms in the terminal part of the side chain starting from commercially available stigmasterol **24** is reported by Khrapach *et al.*<sup>32</sup> 3,5-cyclo ester **110** was prepared from stigmasterol **24** in 5 steps. Labelling with deuterium was done in two steps. Reduction of 3,5-cyclo ester **110** with lithium aluminium deuteride gave alcohol **111** (Scheme 13) and then mesylation of the alcohol **111** followed by reduction with  $\text{LiAlD}_4$  was introduced the third deuterium to the side chain end methyl group and thus  $\Delta^{22}$ -derivative **112** was obtained. Asymmetric dihydroxylation of the double bond in **112** followed by acetylation yielded (22*R*,23*R*)-diacetate **113**. A methoxy group at C-6 was replaced by an oxo group in four steps to give cycloketone **115** that was used for further construction of cyclic part of brassinosteroids (Scheme 13).

Treatment of compound **115** in boiling dimethyl acetamide in the presence of Py. HBr directed to the preparation of



Scheme 17 Synthesis of 24-[3 $\beta$ - $^2\text{H}$ ]epiCS and 24-[3 $\beta$ - $^2\text{H}$ ]epiBL.



compound **116** containing 2,3 double bond in the ring A (Scheme 14).

Removal of acetyl masking groups revealed the presence of 24-[26- $^2$ H<sub>3</sub>]episecasterol **117**. Recently, 24-episecasterol was found cytotoxic against MCF-7 cells.<sup>44</sup> Epoxidation of the olefin **117** gave deuterated epoxide **118** and dihydroxylation of olefin **117** gave 24-[26- $^2$ H<sub>3</sub>]epiCS **119** (Scheme 14). 24-[26- $^2$ H<sub>3</sub>]epiBL **123** was eventually prepared from protected 24-[26- $^2$ H<sub>3</sub>]episecasterol **116** (Scheme 15). The diacetate of [26- $^2$ H<sub>3</sub>] epics **120** obtained by dihydroxylation of **116** was acetylated and converted by Baeyer–Villiger oxidation to tetracetate of 24-[26- $^2$ H<sub>3</sub>]epiBL **121**. After alkaline hydrolysis of acetate groups and acidic workup to restore the lactone ring in 24-[26- $^2$ H<sub>3</sub>] epibrassinolide **123**. It is worth mentioning here that this paper is reporting the protocols enabling the direct Baeyer–Villiger oxidation of 24-epicastasterone to 24-epibrassinolide with more than satisfactory yields and without need of the protection of hydroxy groups.<sup>45,46</sup>

**2.1.6. Synthesis of 6-deoxo-24-[26- $^2$ H<sub>3</sub>]epiCS.** The position and reaction sequence for introducing the label was selected based on the previous experience with the synthesis of deuterated BRs (Scheme 16).

Stigmasterol **24** was a starting compound that was converted in five steps to 22-aldehyde **124**. Claisen rearrangement was the key step in conversion of 22-aldehyde **124** to the ester **125**. Using the reduction with LiAlD<sub>4</sub>-mesylation-reduction with LiAlD<sub>4</sub> sequence as described above the ester group of **125** was converted to [ $^2$ H<sub>3</sub>] methyl group in **128**. Rearrangement of 3,5-cyclo derivative **128** to  $\Delta^2$  derivative **129** was achieved by boiling with pyridinium hydrobromide. Deoxygenation of at C-6 was done *via* methyl xanthate **130** and for final *cis* hydroxylation potassium osmate was used. Thus, the 6-deoxo-24-[26- $^2$ H<sub>3</sub>]epiCS **132** was prepared<sup>47</sup> in overall yield 18% calculated on ester **125**.

## 2.2. BRs with deuterium label in ring A

### 2.2.1. Stereospecific labelling of brassinosteroids with hydrogen isotope.

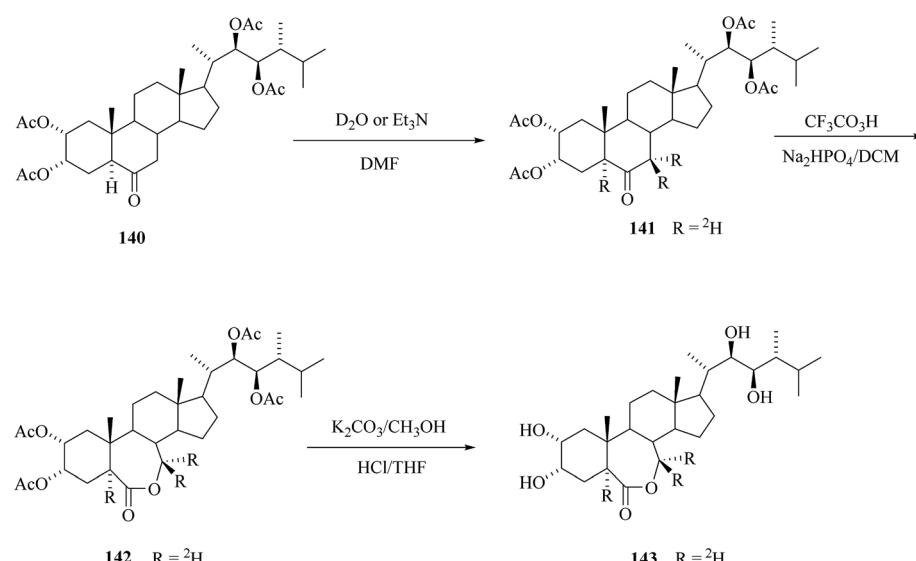
As the deuterium labelled BRs must have at

least 4 deuterium atoms in metabolically stable positions to be useful as MS internal standards the deuterium labelling in ring A is not very attractive. On the other hand ring A is good target for labelling with tritium especially if the aim is to prepare suitable precursor for tritiation from the non-labelled target BR. It is the rule to model the tritiation reactions first with deuterium and therefore we developed recently general stereospecific method of introduction of deuterium to ring A of BRs having 2 $\alpha$ ,3 $\alpha$ -dihydroxy group.

In quest for the suitable precursor for tritiation we discovered a stereospecific reaction of  $\alpha$ -hydroxy ketone **135**, prepared in three steps from 24-epicastasterone **2** in 47% yield, with triphosgene<sup>48</sup> giving in 99% yield 3 $\beta$ -chloro-2 $\alpha$ ,3 $\alpha$ -(carbonyldioxy) derivative **136** (Scheme 17).<sup>45</sup> While catalytic reductive dechlorination is frequently used for the introduction of hydrogen isotopes on aromatic rings<sup>49</sup> to our best knowledge there are no literature data available for either aliphatic or alicyclic substrates. Catalytic dehalogenation of chlorocarbonate **136** with deuterium gas on 5% PdO/CaCO<sub>3</sub> in the presence of triethylamine in ethyl acetate afforded the 2 $\alpha$ ,3 $\alpha$ -(carbonyldioxy) [3 $\beta$ - $^2$ H]derivative **137** in 65% yield and 80% isotopic enrichment. Removal of protecting groups in two-step one pot sequence gave 24-[3 $\beta$ - $^2$ H] epicastasterone **138** in 91% yield on **137**. Baeyer–Villiger oxidation of [3 $\beta$ - $^2$ H]epiCs **138** with trifluoroperoxyacetic acid gave 24-[3 $\beta$ - $^2$ H]epiBL **139** in a 65% yield with no loss of deuterium label.

## 2.3. BRs with deuterium label in ring B

**2.3.1. Synthesis of 24-[5,7,7- $^2$ H<sub>3</sub>]epiBL.** For BRs containing keto group the base catalyzed exchange of  $\alpha$ -hydrogens with  $^2$ H<sub>2</sub>O can be exploited for deuterium introduction. Kolbe *et al.*<sup>20</sup> reported the preparation of 24-[5,7,7- $^2$ H<sub>3</sub>]epiBL starting from tetracetate of 24-epiCS **140** (Scheme 18) or alternatively from 2,3,22,23-bis-isopropylidenedioxy-24-epiCS **133** (see Scheme 17). The exchange with  $^2$ H<sub>2</sub>O in DMF was catalyzed by triethylamine. Bayer–Villiger oxidation with CF<sub>3</sub>CO<sub>3</sub>H of the obtained



Scheme 18 Synthesis of 24-[5,7,7- $^2$ H<sub>3</sub>] epiBL.



labelled 24-epiCS derivative **141** and its final deprotection gave 24-[5,7,7-<sup>2</sup>H<sub>3</sub>]epiBL **143**. This procedure has the advantage of specific introduction of labelling at a late stage, whereas three deuterium atoms were introduced in a stable position at C-5 and C-7. A procedure reported by Allevi *et al.*<sup>50</sup> is less convenient because it uses high excess of labelled water and methanol and there is the need of separation of side chain epimers.

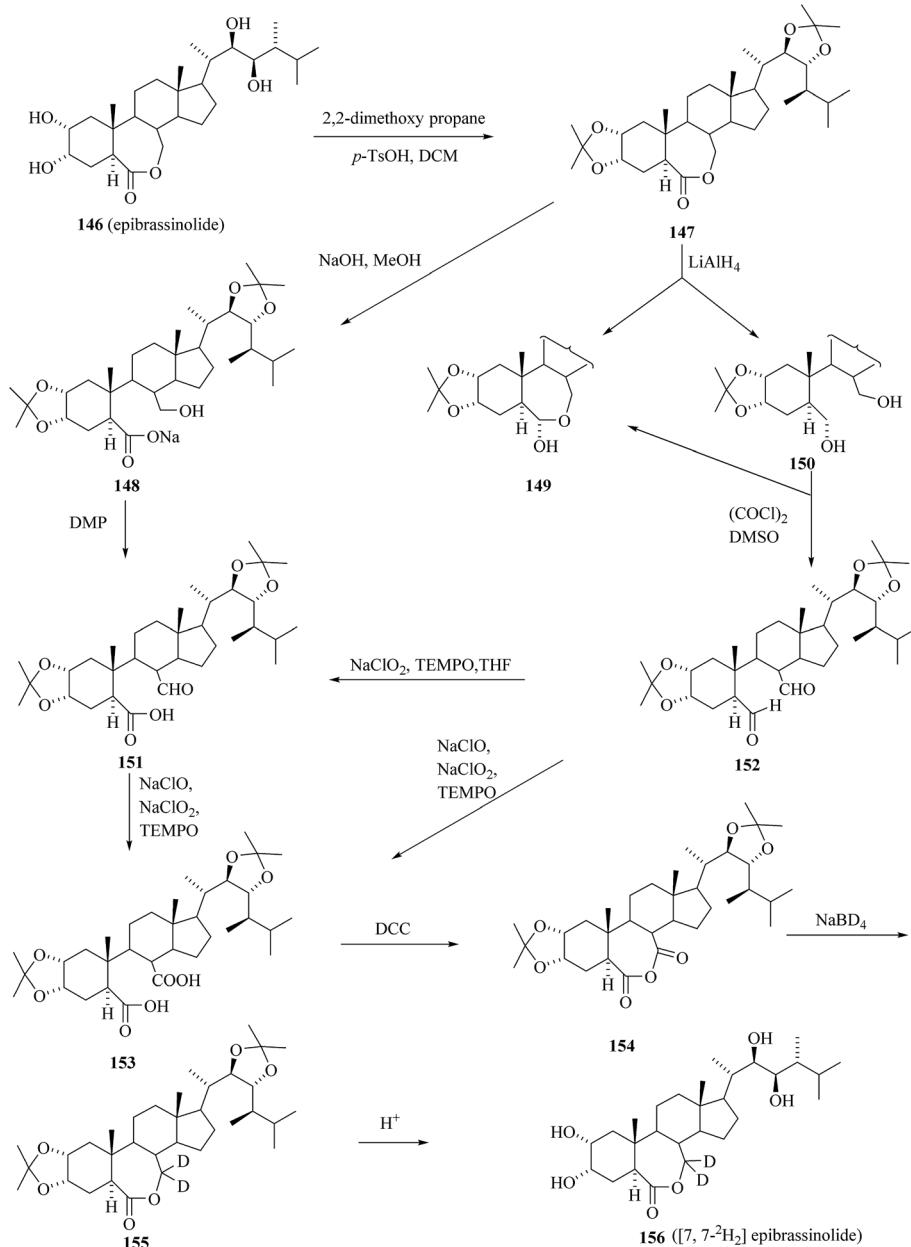
**2.3.2. Synthesis of 24-[7,7-<sup>2</sup>H<sub>2</sub>]epiBL.** The intended transformation of lactone group in epibrassinolide **146** was carried out after protecting both diol functions by isopropylidenation. The key transformation is the preparation of 6,7-seco diacid **153** from diacetone **147** in three step and then formation of the cyclic anhydride **154** from 6,7-seco diacid **153** (Scheme 19). Regioselective reduction of anhydride **154** with NaBD<sub>4</sub> gave

deuterated diacetone **155**. Acidic hydrolysis of acetonide groups on **155** provided [7,7-<sup>2</sup>H<sub>2</sub>]epiBL **156** with 82% isotopic enrichment.<sup>51</sup>

### 3. Synthesis of tritium-labelled BRs

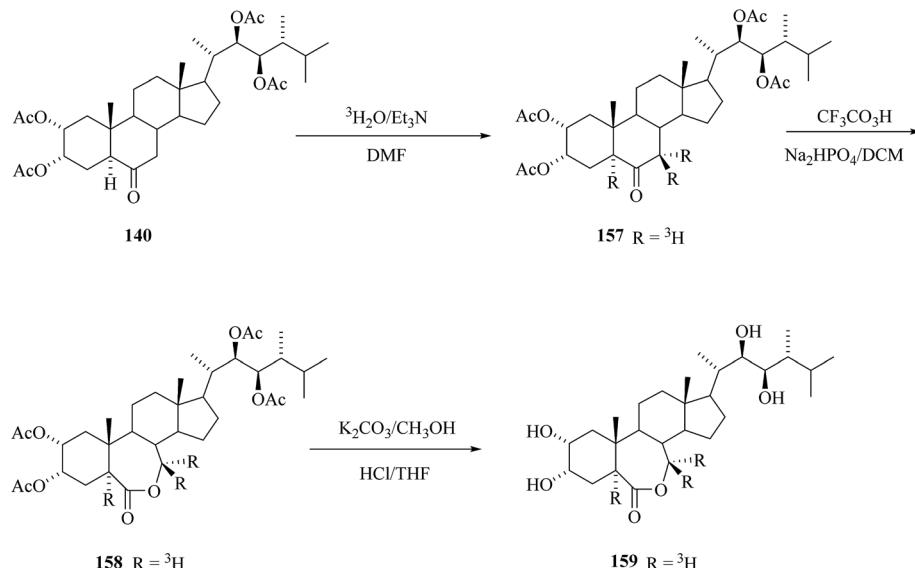
#### 3.1. Synthesis of 24-[5,7,7-<sup>3</sup>H<sub>3</sub>]epiBL

By the same method as described above for the preparation of corresponding deuterium labelled 24-epiBL **143** tritium was introduced to tetracetate of 24-epiCS **140** by base catalyzed exchange reaction with tritiated water having specific activity 1.1 Ci mL<sup>-1</sup>. After Bayer–Villiger oxidation of **157** and removal of acetyl protecting groups 24-[5,7,7-<sup>3</sup>H<sub>3</sub>] epiBL with specific activity (S.A.) 6 mCi mmol<sup>-1</sup>.<sup>20</sup> The same sequence was



Scheme 19 Synthesis of 24-[7,7-<sup>2</sup>H<sub>2</sub>]epiBL.

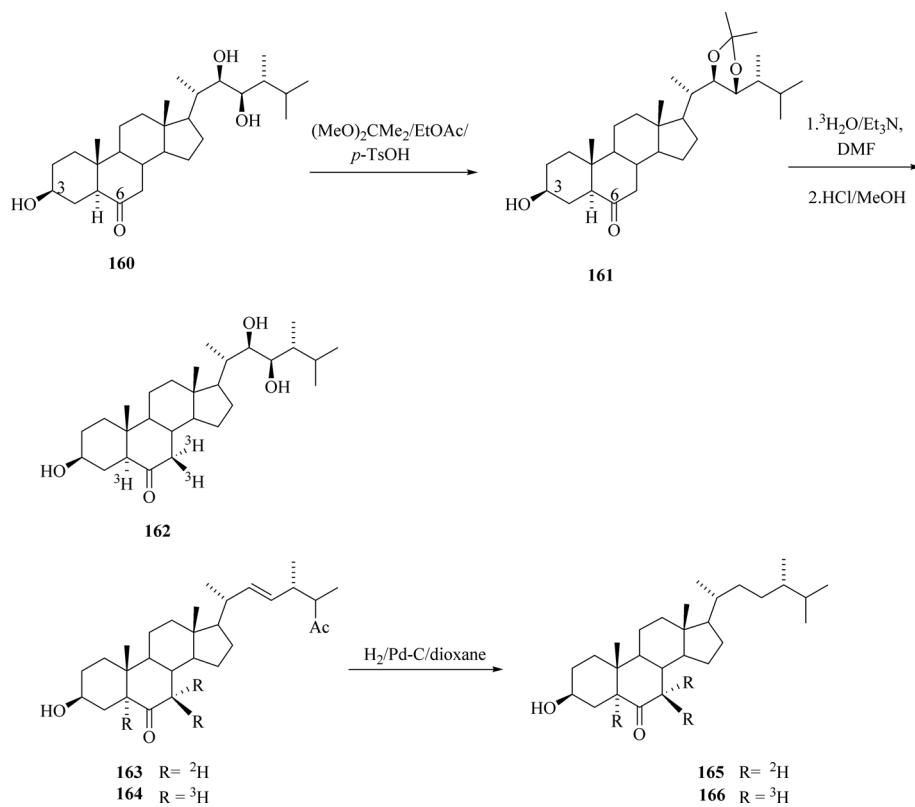


Scheme 20 Synthesis of 24-[5,7,7-<sup>3</sup>H]epiBL.

performed also with 2,3,22,23-bis-isopropylidenedioxy-24-epiCS **133** as starting compound. The advantage of the use of isopropylidene protecting groups is the more simple deprotection step – the lactone ring is not opened under acidic hydrolysis conditions as the opposite is true for basic hydrolysis needed for the acetate group cleavage (Scheme 20).

### 3.2. Synthesis of 24-[5,7,7-<sup>3</sup>H]epiTE, 6-oxo-24 $\beta$ -methyl-22-dehydro[5,7,7-<sup>3</sup>H]cholestanol and 6-oxo-24-[5,7,7-<sup>3</sup>H]epicampestanol

All title compounds were prepared by base catalysed exchange of  $\alpha$ -keto hydrogens with tritiated water<sup>52</sup> with S.A. = 0.8 Ci mmol<sup>-1</sup> (Scheme 21). 24-[5,7,7-<sup>3</sup>H]epiteasterone **162** was prepared with S.A. = 1.5 mCi mmol<sup>-1</sup> 6-oxo-24 $\beta$ -methyl-22-



Scheme 21 Labelling of biogenetic BPs precursors.

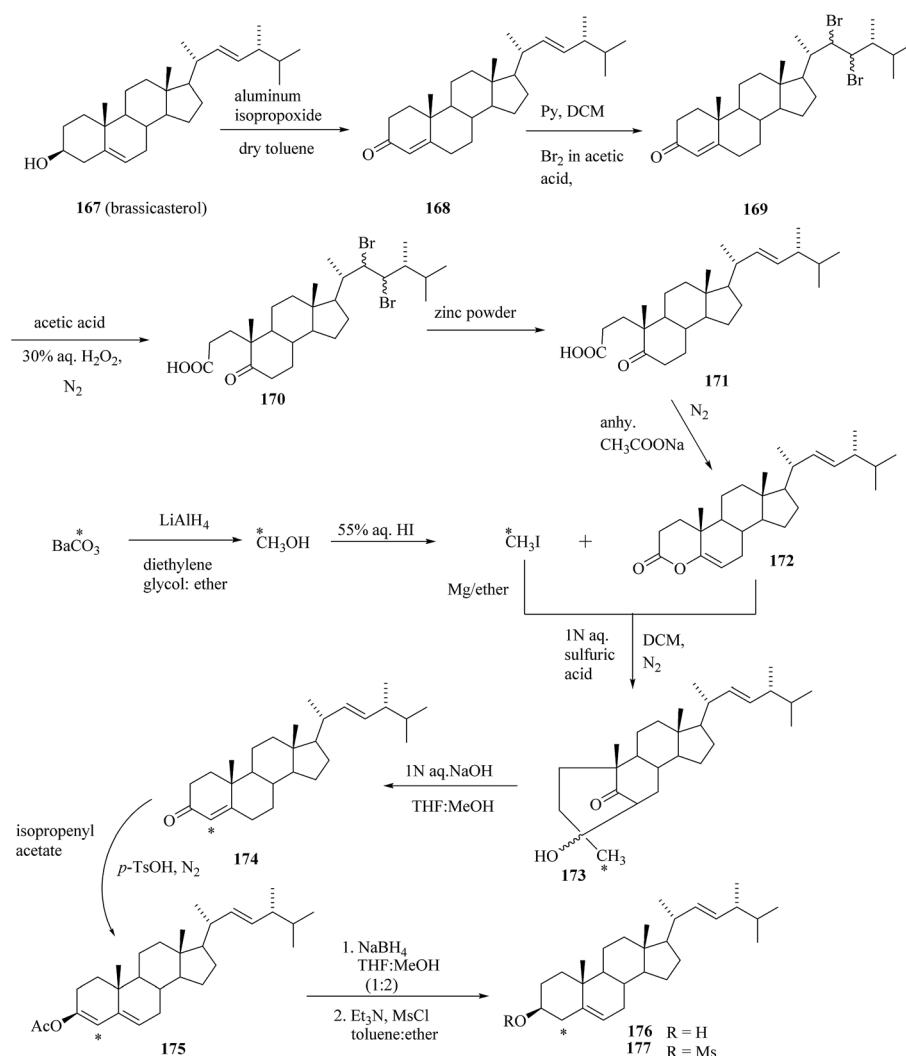
dehydro[5,7,7,-<sup>3</sup>H] cholestanol **164** was labelled without protection of hydroxyl groups and its S.A. was not indicated. Cholestanol derivative **164** was further converted *via* hydrogenation of the double bond in the side chain catalysed by 10% Pd/C and then 6-oxo-24-[5,7,7-<sup>3</sup>H]epicampestanol purified by column chromatography had S.A. = 3.5 mCi mmol<sup>-1</sup>.

## 4. Synthesis of <sup>14</sup>C-labelled BRs

### 4.1. Synthesis of (22R,23R)-and (22S,23S)-24-[4-<sup>14</sup>C] epiBL

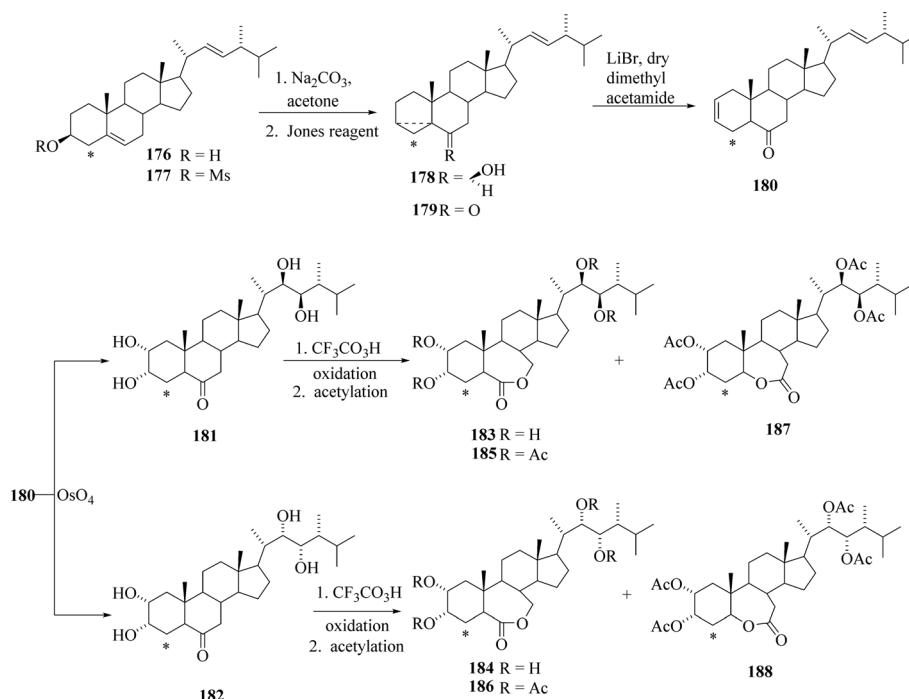
There is only one report available in the literature on <sup>14</sup>C-labelled brassinosteroids so far. Seo *et al.*<sup>53</sup> have reported the synthesis of [4-<sup>14</sup>C]-labelled epiBL **183** and **184**. The C-4 position in epiBL **183** was selected for <sup>14</sup>C labelling because of its stability to metabolic loss and easy way to do the preparation. According to the established method reported in the literature for incorporation of <sup>14</sup>C into the C-4 position of steroids, the enol lactone **172** was synthesized from the starting material brassicasterol **167** in five steps (Scheme 22). This lactone **172** was then treated with [<sup>14</sup>C] methyl iodide (prepared by known

method from barium [<sup>14</sup>C]carbonate *via* [<sup>14</sup>C]methanol) to give bridged ketone **173**. Alkaline treatment of **173** in MeOH gave [4-<sup>14</sup>C]brassicasterone **174**. Acetylation of **174** with isopropenyl acetate under acid catalysis gave the enol acetate **175** that was reduced with sodium borohydride in methanol to give [4-<sup>14</sup>C]-brassicastero **176** as the major product 3,5-cyclo-6-ol **178** was obtained in 91.7% yield by mesylation to give **177** followed by treatment with sodium carbonate in acetone (Scheme 23). Jones oxidation of **178** gave 3,5-cyclo-6-one **179**, which was treated with lithium bromide and camphor sulfonic acid in dimethyl acetamide to rearrange to 2,22-diene-6-one **180** in quantitative yield. Oxidation of **180** with osmium tetroxide gave a stereoisomeric mixture of 2,3,22,23-tetraols **181** and **182** which is separated through the repeated chromatography and recrystallization. Bayer-Villiger oxidation with TFA in dichloromethane gave the (22R,23R)-7-oxa-lactone **183** contaminated with a small amount of its 6-oxa isomer. (22S,23S)-Tetraol also gave the (22S,23S)-7-oxa-lactone **184** contaminated with its 6-oxa isomer. The final products (22R,23R)-24-[4-<sup>14</sup>C]epiBL **183** and (22S,23S)-24-[4-<sup>14</sup>C]-epiBL **183** were obtained in 3.20% and 4.46% radiochemical



Scheme 22 Synthetic pathway to (22R,23R)- and (22S,23S)-24-[4-<sup>14</sup>C] epiBL I.



Scheme 23 Synthetic pathway to (22R,23R)- and (22S,23S)-24-[4-<sup>14</sup>C] epiBL II.

yield (based on  $\text{Ba}^{14}\text{CO}_3$ ), respectively. Specific radioactivity of **183** and **183** was 56.8 mCi  $\text{mmol}^{-1}$ .

## 5. Summary

The number of published synthesis of BRs labelled either by stable isotopes or by radioisotopes reflects their importance for the biochemical studies of this interesting group of plant growth regulators. The main effort was devoted to the synthesis of multideuterated BRs in side chain as internal standards for MS. For labelling with tritium the methods developed for multi-deuterated BRs are not useful. The methods using exchange with tritiated water were described. However, of in this way prepared BRs have specific activities only in order of several mCi  $\text{mmol}^{-1}$ . What remains to be done is to prepare the BRs ligands with the specific activities of order tens of Ci  $\text{mmol}^{-1}$  to enable the search for BRs receptors. Only one example of the synthesis of <sup>14</sup>C-labelled BR reflects the shortage and fast increase of price of  $\text{Ba}^{14}\text{CO}_3$  in recent years. Notwithstanding, some BRs are considered as potential drugs and if they will pass the preclinical sieve, there will be certainly need for their labelling with <sup>14</sup>C.

## List of abbreviations

Ac <sub>2</sub> O	Acetic anhydride	D	Deuterium
AIBN	Azobisisobutyronitrile	DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
Aq	Aqueous	DCC	Dicyclohexylcarbodiimide
BL	Brassinolide	DCM	Dichloromethane
BR	Brassinosteroid	(DHQ) <sub>2</sub> -PHAL	Dihydroquinine 1,4-phthalazinediyl diether
BuLi	Butyllithium	(DHQD) <sub>2</sub> -PHAL	Dihydroquinidine 1,4-phthalazinediyl diether
CS	Castasterone	DMD	Dimethylidioxirane
		DMF	Dimethylformamide
		DMP	4-Dimethylamino Pyridine
		DMSO	Dimethyl sulfoxide
		epiBL	epibrassinolide
		Et <sub>3</sub> N	Triethyl amine
		EtOAc	Ethyl acetate
		Hg(OAc) <sub>2</sub>	Mercury(II) acetate
		LiAlD <sub>4</sub>	Lithium Aluminum Deuteride
		m-CPBA	meta-Chloroperoxybenzoic acid
		MCF	Michigan cancer foundation
		MeOH	Methanol
		MsCl	Methanesulfonyl chloride
		Na/Hg	Sodium amalgam
		NBS	N-Bromosuccinic imide
		PCC	Pyridinium chlorochromate
		p-TsOH	p-Toluenesulfonic acid
		Py	Pyridine
		T	Tritium
		TE	Teasterone
		TEMPO	2,2,6,6-Tetramethylpiperidinyl-1-oxyl
		TFD	Trifluoromethylidioxirane
		THF	Tetrahydrofuran
		TsCl	4-Toluenesulfonyl Chloride
		TY	Typhasterol



## Acknowledgements

This work was realized at IOCB, Prague, Czech Republic and supported by Academy of Sciences of the Czech Republic (RVO 61388963), by Grant IAA400550801 of the Grant Agency of the Academy of Sciences of the Czech Republic and by CNMS, Jain University, Bangalore. The authors wish to express thanks to all for financial support.

## References

- 1 S. Fujioka and A. Sakurai, *Nat. Prod. Rep.*, 1997, **14**, 1–10.
- 2 A. Bajguz and A. Tretyn, *Phytochemistry*, 2003, **62**, 1027–1046.
- 3 V. Castilla, J. Ramirez and C. E. Coto, *Curr. Med. Chem.*, 2010, **17**, 1858–1873.
- 4 S. D. Clouse, *Plant J.*, 1996, **10**, 1–8.
- 5 V. A. Khripach, V. N. Zhabinskii and A. de Groot, *Brassinosteroids: A New Class of Plant Hormones*, Academic Press, San Diego, 1999.
- 6 *Brassinosteroids: Steroidal Plant Hormones*, ed. A. Sakurai, T. Yokota and S. D. Clouse, Springer, Tokyo, 1999.
- 7 V. Marquardt and G. Adam, in *Chemistry of Plant Protection*, ed. W. Ebing, Springer, Berlin, 1991, vol. 7, pp. 103–139 and the references cited therein.
- 8 H. Suzuki, T. Inoue, S. Fujioka, T. Saito, S. Takatsuto, T. Yokota, N. Murofushi, T. Yanagisawa and A. Sakurai, *Photochemistry*, 1995, **40**, 1391–1397.
- 9 S. Fujioka, T. Inoue, S. Takatsuto, T. Yanagisawa, T. Yokota and A. Sakurai, *Biosci., Biotechnol., Biochem.*, 1995, **59**, 1543–1547.
- 10 S. Takatsuto, C. Gotoh, T. Noguchi, T. Nomura, S. Fujioka and T. Yokota, *J. Chem. Res., Synop.*, 1998, 206–207.
- 11 H. Suzuki, S. Fujioka, S. Takatsuto, T. Yokota, N. Murofushi and A. Sakurai, *J. Plant Growth Regul.*, 1994, **13**, 21–26.
- 12 A. Sakurai and S. Fujioka, *Biosci., Biotechnol., Biochem.*, 1997, **61**, 757–762.
- 13 S. Fujioka, J. Li, Y.-H. Choi, H. Seto, S. Takatsuto, T. Noguchi, T. Watanabe, H. Kuriyama, T. Yokota, J. Chory and A. Sakurai, *Plant Cell*, 1997, **9**, 1951–1962.
- 14 T. Noguchi, S. Fujioka, S. Takatsuto, S. Yoshida, A. Sakurai, J. Li and J. Chory, Abstract Papers of 32nd Annual Meeting of The Japanese Society for Chemical Regulation of Plants, Tokyo, 1997, p. 93.
- 15 S. Fujioka and A. Sakurai, *Physiol. Plant.*, 1997, **100**, 710–715.
- 16 T. Yokota, *Trends Plant Sci.*, 1997, **2**, 137–143.
- 17 A. Antonchick, A. Svatoš, B. Schneider, O. V. Konstantinova, V. N. Zhabinskii and V. A. Khripach, *Phytochemistry*, 2005, **66**, 65–72.
- 18 S. Takatsuto and N. Ikekawa, *Chem. Pharm. Bull.*, 1984, **32**, 2001–2004.
- 19 (a) J. Malíková, J. Swaczynová, Z. Kolář and M. Strnad, *Phytochemistry*, 2008, **69**, 418–426; (b) J. Steigerová, J. Oklešková, M. Levková, L. Rárová, Z. Kovář and M. Strnad, *Chem.-Biol. Interact.*, 2010, **188**, 487–496.
- 20 A. Kolbe, V. Marquardt and G. Adam, *J. Labelled Compd. Radiopharm.*, 1992, **31**, 801–805.
- 21 V. A. Khripach, V. N. Zhabinskii, O. V. Gulyakevich, O. V. Konstantinova, A. Y. Misharin, A. R. Mekhtiev, V. P. Timofeev and Y. V. Tkachev, *Russ. J. Bioorg. Chem.*, 2010, **36**, 746–754.
- 22 S. Takatsuto and N. Ikekawa, *Chem. Pharm. Bull.*, 1986, **34**, 4045–4049.
- 23 S. C. Lee, S. Joo, C. Park, S. Son, J. Youn, M. Kim, S. Jeong and S. Kim, *Bull. Korean Chem. Soc.*, 2011, **32**, 332–334.
- 24 G. Adam, A. Porzel, J. Schmidt, B. Schneider and B. Voigt, in *Studies in Natural Products Chemistry*, ed. Atta-ur-Rahman, Elsevier, Amsterdam, 1996, vol. 18, pp. 495–549.
- 25 M. D. Grove, G. F. Spencer, W. K. Rohwedder, N. Mandava, J. F. Worley, J. D. Warthen, G. L. Steffens, J. L. Flippin-Anderson and J. C. Cook, *Nature*, 1979, **225**, 1065–1066.
- 26 S. Takatsuto and N. Ikekawa, *Chem. Pharm. Bull.*, 1986, **34**, 1415–1418.
- 27 S. Takatsuto and N. Ikekawa, *Chem. Pharm. Bull.*, 1986, **34**, 4045–4049.
- 28 S. Takatsuto and N. Ikekawa, *J. Chem. Soc., Perkin Trans. 1*, 1986, 591–593.
- 29 V. A. Khripach, V. N. Zhabinskii, O. V. Konstantinova and N. B. Khripach, *Tetrahedron Lett.*, 2000, **41**, 5765–5767.
- 30 V. A. Khripach, V. N. Zhabinskii, O. V. Konstantinova, N. B. Khripach, A. P. Antonchick and B. Schneider, *Steroids*, 2002, **67**, 587–595.
- 31 K. Mori, M. Sakakibara, Y. Ichikawa, H. Ueda, K. Okada, T. Umemura, G. Yabuta, S. Kuwahara, M. Kondo, M. Minobe and A. Sogabe, *Tetrahedron*, 1982, **38**, 2099–2109.
- 32 V. A. Khripach, V. N. Zhabinskii, V. K. Olkhovick and F. A. Lakhvich, *Zh. Org. Khim.*, 1990, **26**, 1966–1976.
- 33 J. A. Steele and E. Mosettig, *J. Org. Chem.*, 1963, **28**, 571–572.
- 34 J. J. Pappas, W. P. Keaveney, E. Gancher and M. Berger, *Tetrahedron Lett.*, 1966, **7**, 4273–4278.
- 35 A. P. Antonchick, B. Schneider, V. N. Zhabinskii and V. A. Khripach, *Steroids*, 2004, **69**, 617–628.
- 36 V. A. Khripach, V. N. Zhabinskii, A. P. Antonchick, O. V. Konstantinova and B. Schneider, *Steroids*, 2002, **67**, 1101–1108.
- 37 M. Anastasia, P. Allevi, P. Ciuffreda and A. Fiechchi, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2365–2367.
- 38 M. Anastasia, P. Ciuffreda, M. Delpuppo and A. Fiechchi, *J. Chem. Soc., Perkin Trans. 1*, 1983, 379–382.
- 39 M. Anastasia, P. Allevi, P. Ciuffreda, A. Fiechchi, P. Gariboldi and A. Scala, *J. Chem. Soc., Perkin Trans. 1*, 1985, 595–599.
- 40 M. Koizumi, M. Ishiguro, M. Yasuda and N. Ikekawa, *J. Chem. Soc., Perkin Trans. 1*, 1983, 1401–1410.
- 41 V. Marquardt and G. Adam, *Chemistry of plant protection*, ed. W. Ebing, Springer, Berlin, 1991, vol. 7, p. 103.
- 42 H. Seto, S. Fujioka, H. Koshino, S. Yoshida, T. Watanabe and S. Takatsuto, *Tetrahedron Lett.*, 1998, **39**, 7525–7528.
- 43 A. Kolbe, A. Porzel, J. Schmidt and G. Adam, *J. Labelled Compd. Radiopharm.*, 2003, **46**, 231–242.
- 44 V. A. Khripach, V. N. Zhabinskii, O. V. Gulyakevich, O. V. Konstantinova, A. Y. Misharin, A. R. Mekhtiev, V. P. Timofeev and Y. V. Tkachev, *Russ. J. Bioorg. Chem.*, 2010, **36**, 746–754.



45 A. Marek, M. R. Patil, B. Klepetářová, L. Kohout and T. Elbert, *Tetrahedron Lett.*, 2012, **53**, 2048–2050.

46 L. Kohout, *Collect. Czech. Chem. Commun.*, 1994, **59**, 457–460.

47 V. A. Khripach, V. N. Zhabinskii and O. V. Gulyakevich, *Chem. Nat. Compd.*, 2012, **48**, 601–605.

48 H. Eckert and B. Forster, *Angew. Chem., Int. Ed. Engl.*, 1987, **26**, 894–895.

49 R. Voges, J. R. Heys and T. Moenius, *Preparation of compounds labelled with tritium and Carbon-14*, John Wiley & Sons, Chichester, 2009, pp. 133–144.

50 P. Allevi, M. Anastasia, R. Cerana, P. Ciuffreda and P. Lado, *Phytochemistry*, 1988, **27**, 1309–1313.

51 V. A. Khripach, N. B. Khripach, V. N. Zhabinskii, Y. Y. Zhiburtovich, B. Schneider and A. De Groot, *J. Labelled Compd. Radiopharm.*, 2007, **50**, 1153–1158.

52 A. Kolbe, B. Schneider, B. Voigt and G. Adam, *J. Labelled Compd. Radiopharm.*, 1998, **41**, 131–137.

53 S. Seo, T. Nagasaki, Y. Katsuyama, F. Matsubara, T. Sakata, M. Yoshioka and Y. Makisumi, *J. Labelled Compd. Radiopharm.*, 1989, **27**, 1383–1393.

