Synthesis of hexadeuterated 23-dehydroxybrassinosteroids

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Abstract

Two hexadeuterated brassinosteroids (BS) (26,27-2H6-23-dehydroxycastasterone and 26,27-2H6-cathasterone) containing a hydroxy group at C22 instead of the 22R,23R-diol function characteristic for most compounds of this class were prepared for biochemical studies. The corresponding non-deuterated compounds are considered intermediates in brassinolide biosynthesis. The carbon skeleton of the side chain with proper stereochemistry at C24 was prepared from commercially available (2R)-3-hydroxy-2-methylpropanoate. This low molecular fragment was coupled to the tetracyclic steroidal fragment through the reaction of the appropriate sulfone with C22 aldehyde. Formation of the necessary configuration of the 22-hydroxy group was achieved by hydride reduction of the corresponding ketone. Deuterium atoms at C26 and C27 originated from [2H3]methyl iodide used for alkylation of the intermediate sulfone.

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1. Introduction

The presence of many functional groups in the brassinolide molecule implies that its biosynthesis is a multistep process. Detailed knowledge of the biosynthetic pathway is very important for a better understanding of the physiological processes and subtle mechanisms of the action of brassinosteroids (BS) in plants. Most BS known to date contain a 22R,23R-diol function, which is essential for their biological activity [1,2]. Identification of cathasterone lacking one of the hydroxy groups and application of its labeled analog in feeding experiments [3] showed that brassinolide biosynthesis proceeds via initial hydroxylation at C22, followed by introduction of the hydroxy group at C23 (Scheme 1). This finding has been confirmed by identification of the CPD gene as a microsomal cytochrome P450-dependent 23α-hydroxylase [4]. However, in addition to the established biosynthetic sequences, hitherto unknown alternative subpathways or species-specific biosynthetic routes may occur. It is known, for example, that even within one biological species, different biosynthetic routes to brassinolide exist. Thus, in Catharanthus roseus, the so-called ‘early C6-oxidation pathway’ and the ‘late C6-oxidation pathway’ operate independently [5,6]. In this respect, the main objective of the present work was the elaboration of a synthetic approach to and preparation of deuterated 23-dehydroxybrassinosteroids. Such compounds containing six deuterium atoms in the side chain are essential for biosynthetic studies of relevant brassinolide precursors [7].

2. Experimental

2.1. General

Melting points were recorded on a Boetius micromelting point apparatus and are uncorrected. IR spectra were recorded on an UR-20 spectrophotometer. 1H and 13C NMR spectra were recorded on a Bruker AC-200 (200 MHz for 1H, 50 MHz for 13C) spectrometer using TMS as an internal standard in CDCl3. Accurate mass measurements were carried out on a Micromass MassSpec mass spectrometer operating in the 70 eV EI mode. Samples were introduced by direct probe for accurate mass measurement by peak matching. Chemicals were purchased from Aldrich, Fluka, and Steraloids Chemical Companies and were used as received. [2H3]Methyl iodide (99.5%) was supplied by Deutero GmbH. Reactions were monitored by TLC using...
aluminum or plastic sheets precoated with silica gel 60 F254 (Merck Art. 7734). Column chromatography was carried out on Kieselgel 60 (Merck Art. 5715).

2.2. 2-[(2R)-2-Methyl-3-(4-methylphenylsulfonyloxy)propyloxy]tetrahydro-2H-pyran (5)

HCl (36%, 2.5 ml) was added to a solution of (2R)-3-hydroxy-2-methylpropanoate (2) (10 ml, 90 mmol) in dihydro-pyrrane (25 ml). After incubation at ambient temperature for 24 h, pyridine (25 ml) was added. The solvent was partially pyrane (25 ml). After incubation at ambient temperature for 2 h, diluted with water (400 ml), and extracted with petroleum ether. The organic layer was dried over Na2 SO 4 and evaporated to give crude (2R)-2-methyl-3-tetrahydro-2H-pyryloxypropanoate, which was dissolved in Et2 O (200 ml). LiAlH 4 (13.5 g, 0.35 mol) was added portionwise over 1 h, and the reaction mixture was stirred for an additional 1 h and filtered through a plug of SiO 2 with the aid of EtOAc.

2.3. 2-[(2R)-2-Methyl-3-phenylsulfonyloxypropyloxy]tetrahydro-2H-pyran (6)

Thiophenol (30.6 ml, 0.3 mol) was added to a solution of sodium methoxide prepared from sodium (18 g, 0.78 mol) and MeOH (300 ml). A solution of the tosylate 5 (19.0 g, 58 mmol) in MeOH (150 ml) was then added, and the mixture was left to stand at ambient temperature for 14 h, diluted with water, and extracted with petroleum ether. The organic layer was dried over Na2SO4 and evaporated to give crude (2R)-2-methyl-1-phenylsulfanyl-3-tetrahydro-2H-pyryloxypropane, which was dissolved in CHCl3 (400 ml), and m-chloroperbenzoic acid (57 g, 0.33 mol) was added. The mixture was stirred at room temperature for 3 h, washed with 25% aqueous NaOH, water, dried over Na2SO4, and evaporated. The residue was chromatographed on SiO2 with petroleum ether–EtOAc (20 : 1) to give the tosylate 5 (19.0 g, 64%) as an oil.

2.4. 2-[(2R)-2-Methyl-3-[(2H)butyloxy]tetrahydro-2H-pyran (7)

A solution of BuLi (2.5 M in hexane, 40 ml, 0.1 mol) was added at –30 °C to a solution of sulfone 6 (10.0 g, 30.5 mmol) in THF (250 ml). The mixture was stirred for 15 min, after which a solution of CD3 I (3.4 ml, 55 mmol) in THF (10 ml) was added, and the temperature was gradually increased to 10 °C over 30 min. The mixture was recooled to –30 °C, and a solution of BuLi (2.5 M in hexane, 20 ml, 50 mmol) was added. The mixture was maintained at this temperature for 15 min, and a second portion of CD3 I (3.4 ml, 55 mmol) in THF (10 ml) was introduced. After 15 min, the mixture was allowed to warm to room temperature, after which it was diluted with water and extracted with EtOAc. The organic layer was dried over Na2SO4, evaporated, and the residue was chromatographed on SiO2 with petroleum ether–EtOAc (20 : 1) to give the deuterated derivative 7 (5.5 g, 50%) as an oil. IR (cm−1): 2950, 2875, 2245, 1450, 1390, 1360, 1305, 1155, 1085, 1040, 1030, 1010, 980. 1H NMR δ: 1.12 (dd, 3H, J = 8.5, 2.5 Hz, 2-Me), 2.24–2.46 (m, 1H, C2 –H), 2.86–3.16 (m, 2H, CH2 –S), 3.30–3.84 (m, 4H, CH2 –O), 4.42–4.56 (m, 2H, O–CH–O), 7.50–7.98 (m, 5H, Ph). 13C NMR δ: 17.25, 19.36, 19.46, 25.35, 29.34, 29.62, 29.65, 30.45, 59.17, 59.47, 62.18, 62.31, 70.43, 71.17, 127.86, 129.25, 133.54, 140.05. HRMS calc. for C15 H20 O3 S: 280.113317. Found: 280.113152. EI-MS m/z (%): 85 (100), 101 (45), 143 (40), 197 (52), 215 (14), 240 (0.5), 280 (0.3) [M – H2O]++.

2.5. (2R)-2-Methyl-3-[4-3H]methyl-3-phenylsulfonyl-4-[4-14C]butyl phenyl sulfide (I)

A mixture of 7 (5.5 g, 16.5 mmol) and HCl (36%, 1 ml) in MeOH (250 ml) was stirred at room temperature for 40 min. Pyridine (1 ml) was added, and the solvent was evaporated under reduced pressure. The residue was chromatographed on SiO2 with petroleum ether–EtOAc (15:1) to give the starting tosylate 9 (8.0 g, 15% recovery) and the sulfide 10 (3.5 g, 68%) as an oil. IR (cm−1): 2990, 2940, 2885, 2220, 2080, 1600, 1490, 1450, 1360, 1105, 1030. 1H NMR δ: 0.96 (d, 3H, J = 6.7 Hz), 1.02 (d, 3H, J = 8.5 Hz), 1.52–1.72 (m, 1H, C2–H), 2.26–2.46 (m, 1H, C2–H), 2.72 (dd, 1H, J = 13, 8.5 Hz, C1–H), 3.02 (dd, 1H, J = 13, 5Hz, C1–H), 3.90 (s, 1H, O–CH–O), 7.42–7.86 (m, 5H, Ph). 13C NMR δ: 13.12, 13.60, 37.19, 37.45, 65.86, 128.50, 128.74, 128.90, 130.14, 130.24, 133.51, 136.07. HRMS calc. for C13H20D5O2S: 340.14378. Found: 340.14364. 1H-MS m/z (%): 77 (9), 89 (16), 122 (100), 180 (5), 199 (17) [M−PhSO2]− 240 (21) [M]+.

2.8. (2S)-2-Methyl-3-[4-3H]methyl-4-[4-14C]butyl phenyl sulfide (II)

Mg (2 g, 82 mmol) was added to a vigorously stirred solution of 10 (3.3 g, 9.7 mmol) in MeOH (200 ml). The mixture was stirred for 2 h, the resultant precipitate was filtered, and the filtrate was neutralized with 3N HCl. The acidified aqueous layer was extracted with petroleum ether, and the combined organic extracts were dried over Na2SO4, evaporated, and chromatographed on SiO2 with petroleum ether–EtOAc (20:1) to give the sulhide 11 (7.7 g, 88%) as an oil. IR (cm−1): 2970, 2940, 2885, 2220, 2080, 1600, 1490, 1450, 1360, 1105, 1030. 1H NMR δ: 0.96 (d, 3H, J = 7 Hz, 2-Me), 1.52–1.72 (m, 1H, C2–H), 2.72 (dd, 1H, J = 12.5, 8.5 Hz, C1–H), 3.02 (dd, 1H, J = 13, 5Hz, C1–H), 3.78–3.92 (m, 3H, Ph). 13C NMR δ: 15.12, 18.99, 30.95, 38.31, 38.88, 40.39, 125.49, 125.67, 128.74, 128.97, 137.52. HRMS calc. for C13H20D5S: 200.15083. Found: 200.14987.

2.9. (2S)-2-Methyl-3-[4-3H]methyl-4-[4-14C]butyl phenyl sulfone (II)

MCPBA (5.8 g, 34 mmol) was added to an ice-bath cooled solution of sulfide 11 (7.7 g, 8.5 mmol) in CHCl3 (100 ml). The mixture was stirred for 3 h at room temperature and then, washed with NaHCO3 (25% solution) and water. The organic layer was extracted with petroleum ether. The combined organic phases were washed with saturated NaHCO3, dried over Na2SO4, and evaporated under reduced pressure. The residue was chromatographed on SiO2 with petroleum ether–EtOAc (15:1) to give the sulfone 12 (8.0 g, 61%) as an oil. 1H NMR δ: 1.02 (d, 3H, J = 7Hz, 2-Me), 1.90–2.10 (m, 1H, C2–H), 2.88 (dd, 1H, J = 14, 8.5 Hz, C1–H), 3.10 (dd, 1H, J = 14, 3.5 Hz, C1–H), 7.45–7.96 (m, 5H, Ph). 13C NMR δ: 13.91, 14.05, 19.40, 25.32, 30.56, 30.59, 36.40, 36.65, 62.12, 62.20, 65.26, 60.62, 69.94, 98.98, 109.07, 126.82, 130.30, 133.66, 136.26, 137.52. HRMS calc. for C13H20D5O2S+: 232.140412. Found: 232.140400. 1H-MS m/z (%): 77 (67), 90 (100), 125 (13), 143 (97), 156 (7), 184 (5), 232 (3) [M]+.
A solution of BuLi in hexane (2.5 M, 5 ml, 12.5 mmol) was added to a stirred solution of sulfone 12 (0.9 g, 3.9 mmol) in THF (15 ml) under an argon atmosphere at −80 °C. After stirring at −70 °C for 1 h, the mixture was allowed to warm to room temperature. 

NH4Cl (2 g) was added, and the mixture was diluted with water and extracted with EtOAc. The organic layer was dried over Na2SO4 and evaporated to give 15 (350 mg, 65%) as an oil. IR (cm−1): 2270, 2265, 2220, 1720, 1470, 1385, 1170, 1110, 1080. 1H NMR δ: 0.73 (s, 3H, 18-Me), 1.02 (s, 3H, 19-Me), 3.68-4.08 (m, 4H, –CH2–O–).

LiAlH4 (200 mg, 5.3 mmol) was added to a stirred solution of ketone 15 (350 mg, 0.76 mmol) in THF. Stirring was maintained for 30 min, and then, excess LiAlH4 was destroyed by careful addition of water (0.8 ml) and NaOH solution (15%, 0.2 ml). The precipitate was filtered, and the filtrate was dried over Na2SO4 and evaporated to give (22S,24R)-[26,27-2H6]-22-hydroxy-24-methyl-6,6-ethylenedioxy-3α,5-cyclo-5α-cholestane-6-one (16) (330 mg, 94%) as an oil, which was used without further purification for the next step.

Alcohol 16 (270 mg, 0.58 mmol) was dissolved in acetonitrile (137 ml), and a solution of TsOH (80 ml, 0.58 mol) was added dropwise, and the cooling bath was removed. After warming to room temperature, the mixture was diluted with water and extracted with EtOAc. The organic layer was chromato- graphed on SiO2 with petroleum ether–EtOAc (20:1) to give the oxo sul- fonate 210 (70%, 70%) as an oil: IR (cm−1): 2920, 2880, 2225, 1720, 1690, 1660, 1455, 1390, 1310, 1150, 1080. 1H NMR δ: 0.72 (s, 3H, 18-Me), 1.00 (s, 3H, 19-Me), 3.52–4.10 (m, 4H, –CH2–O–). 13C NMR δ: 0.46, 34.21, 34.86, 35.35, 37.62, 39.25, 39.65, 40.17, 41.12, 42.61, 44.70, 45.59, 46.00, 46.33, 46.73, 47.36, 51.86, 56.85, 63.72, 64.66, 64.68, 70.93, 71.06, 109.67, 127.68, 128.09, 128.97, 133.08, 143.90, 209.53. HRMS calc. for CsH211D18O5: 420.39128. Found: 420.388802.

El-MS m/z (%): 121 (32), 136 (41), 161 (29), 229 (12), 285 (27), 300 (100), 329 (9), 402 (8) [M − H2O]+, 420 (50) [M]+.

A mixture of alcohol 17 (140 mg, 0.33 mmol), Ac2O (0.5 ml) and pyridine (1 ml) was stirred at room temperature for 5 h, after which it was diluted with water, and extracted with EtOAc. The organic layer was dried over Na2SO4 and evaporated. The residue was chromatographed on SiO2 with petroleum ether–EtOAc (20:1) to give the acetate 18 (154 mg, 100%) as an oil. 1H NMR δ: 0.70 (s, 3H, 18-Me), 0.82 (d, 3H, J = 6.5 Hz, 21- or 28-Me), 0.95 (d, 3H, J = 6.5 Hz, 28- or 21-Me), 1.02 (s, 3H, 19-Me), 1.49–5.08 (m, 1H, C22–H). 13C NMR δ: 11.59, 11.83, 12.60, 15.49, 19.64, 21.23, 22.87, 23.99, 25.88, 28.07, 31.64, 33.47, 34.81, 34.99, 35.21, 35.73, 38.20, 39.69, 42.62, 44.69, 46.05, 46.26, 46.71, 52.48, 56.86, 74.84, 170.69, 209.44.

21. (22S,24R)-[26,27-2H6]H22-Acetoxy-24-methyl-3α, 5-cyclo-5α-cholestane-6-one (18)
HBr (48%, 0.24 ml) was added to a solution of 18 (75 mg, 0.16 mmol) in AcOH (2 ml). The mixture was stirred for 30 min, diluted with water, and extracted with CHCl3. The organic layer was washed consecutively with water, saturated NaHCO3, and then, it was dried over Na2SO4 and evaporated. The residue was chromatographed on SiO2 with petroleum ether–EtOAc (20:1) to give the bromide 19 (88 mg, 100%) as an oil. 1H NMR δ: 0.66 (s, 3H, 18-Me), 0.80 (s, 3H, 19-Me), 2.04 (s, 3H, OAc), 3.94 (m, 1H, C3–H), 5.00–5.12 (m, 1H, C22–H). 13C NMR δ: 11.80, 12.56, 13.08, 15.50, 21.30, 23.89, 27.95, 31.63, 32.34, 33.40, 35.00, 35.78, 37.80, 38.22, 39.19, 39.38, 40.64, 42.89, 46.50, 50.47, 52.53, 53.84, 56.58, 58.96, 74.82, 170.22, 209.50. HRMS calc. for C21H29BrD7O: 484.300992. Found: 484.301857. Calc. for C21H29BrO7: 482.303038. Found: 482.303668. ElMS m/z (%): 95 (64), 103 (63), 149 (28), 270 (19), 336 (16), 350 (100), 352 (98), 377 (37), 397 (67), 380 (75), 382 (58), 462 (15), 482 (35), 484 (42), 542 (1) [M]+, 544 (1) [M]+.

A mixture of bromide 19 (88 mg, 0.16 mmol), Li2CO3 (300 mg) and DMF (3 ml) was refluxed for 1 h, allowed to cool to ambient temperature, diluted with water, and extracted with CHCl3. The organic layer was dried over Na2SO4 and evaporated. The residue was chromatographed on SiO2 with petroleum ether–EtOAc (20:1 → 12:1) to give: (a) 22-acetoxy-24-methyl-5a-cholest-2-en-6-one (20) (40 mg, 53%) as an oil. 1H NMR δ: 0.68 (s, 3H, 18-Me), 0.72 (s, 3H, 19-Me), 0.84 (d, 3H, J = 6.5 Hz, 21- or 28-Me), 0.96 (d, 3H, J = 6.5 Hz, 28- or 21-Me), 2.05 (s, 3H, OAc), 5.00–5.12 (m, 1H, C22–H), 5.64 (m, 2H, C2– and C1–H). 13C NMR δ: 11.75, 12.58, 13.52, 15.51, 21.14, 21.30, 21.71, 23.90, 27.96, 31.66, 35.00, 35.75, 37.74, 38.19, 39.48, 39.40, 42.72, 46.92, 52.53, 53.42, 58.83, 65.63, 68.44, 124.48, 125.01, 170.77, 211.90. HRMS calc. for C23H31D7O2: 462.398006. Found: 462.397206. ElMS m/z (%): 93 (21), 107 (19), 121 (22), 279 (5), 243 (5), 270 (17), 297 (16), 300 (18), 402 (12), 434 (17), 447 (68) [M + CH3]+, 462 (100) [M]+; (b) 22-acetoxy-24-methyl-5a-cyclo-5a-cholest-6-one (18) (20 mg, 27%) as an oil.

A mixture of bromide 19 (88 mg, 0.16 mmol), Li2CO3 (300 mg) and DMF (3 ml) was refluxed for 1 h, allowed to cool to ambient temperature, diluted with water, and extracted with CHCl3. The organic layer was washed with saturated NaHCO3, dried over Na2SO4, and evaporated. The residue was chromatographed on SiO2 with petroleum ether–EtOAc (20:1 → 10:1) to give the diacetate 22 (45 mg, 68%) as an oil. 1H NMR δ: 0.70 (s, 3H, 18-Me), 0.78 (s, 3H, 19-Me), 0.84 (d, 3H, J = 6.5 Hz, 21- or 28-Me), 0.98 (d, 3H, J = 6.5 Hz, 28- or 21-Me), 2.02 (s, 6H, AcO), 4.66 (m, 1H, C3–H), 5.98–5.12 (m, 1H, C22–H). 13C NMR δ: 11.83, 12.57, 13.04, 15.49, 20.32, 21.47, 23.90, 26.10, 26.83, 27.95, 31.65, 34.98, 35.71, 36.42, 37.93, 38.15, 39.40, 40.41, 42.89, 46.56, 52.50, 53.81, 54.67, 56.53, 72.82, 74.84, 170.62, 170.77, 210.26. HRMS calc. for C23H31D7O2: 462.398006. Found: 462.397766. ElMS m/z (%): 95 (43), 103 (38), 107 (26), 121 (23), 149 (15), 177 (11), 229 (11), 271 (14), 300 (47), 350 (100), 357 (72), 402 (15), 447 (10), 462 (78) [M + AcOH]+, 522 (1.5) [M]+.

Diacetate 23 (43 mg, 0.082 mmol) was treated with a solution of KOH in MeOH (2%, 8 ml) at room temperature for 50 min. AcOH was added to neutralize the mixture, and the solvent was evaporated under reduced pressure. The residue was chromatographed on SiO2 with petroleum ether–EtOAc (10:1 → 1:1) to give monooacetate 24 (37 mg, 94%) as an oil. 1H NMR δ: 0.66 (s, 3H, 18-Me), 0.76 (s, 3H, 19-Me), 0.84 (d, 3H, J = 6.5 Hz, 21- or 28-Me), 0.96 (d, 3H, J = 6.5 Hz, 2
28- or 21-Me), 2.04 (s, 3H, –CH3–CO–), 3.60 (m, 1H, 25-α-OH), 2.52 (s, 3H, –CH3–CO–), 3.78 (m, 1H, 24-Me).

3. Results and discussion

Preparation of BS analogs containing a hydroxy group only at C22 in the side chain instead of the 22R:23R-diol function has been described earlier. These experiments were aimed at the synthesis of more simple derivatives than natural BS for practical purposes [8], for structure–activity relationship [9–11] and biosynthetic [12,13] studies, and to develop synthetic methodologies for steroidal side chain synthesis [14]. However, control over the substituent at C24 was exercised [15] only in one study. It is known that most natural BS contain a 24α-methyl group that is essential for biological activity. Synthesis of compounds with a 22α-hydroxy group and carbon side chain characteristic of brassinolide has not been reported before.

In this paper, we describe preparation of (26,27-2H6)23-dehydroxybrassinosteroids as depicted in the retrosynthetic Scheme 2.

Scheme 2. Synthesis of related (26,27-2H6) sterols has been reported [16,17], but none of these methods provided a facile entry into 2H-labeled BS. The standard approach to 22α-alcohols is reaction of organometallic reagents with 22C-aldehydes [18,19]. However, it suffers from the formation of a considerable amount (up to 40%) of isomeric 22β-alcohol. In this respect, better results were anticipated by the hydride reduction of 22-ketones [18]. Formulation of the desired stereochemistry at C24 required the use of a chiral synthetic building block. Our approach made use of (2R:3-hydroxy-2-methylpropanoate) [20].

Synthesis of the C23–C28 fragment of the side chain was performed as depicted in Scheme 3. The hydroxy group in 2 was protected as a tetrahydroxypropyl ether, and via a number of steps, the carbomethoxy group was transformed into a methylphenylsulfonyl moiety. Introduction of deuterium was achieved by twice repeated treatment of the sulfone 6 with butyl lithium and [2H3]methyl iodide. At this stage, the necessary carbon skeleton was prepared, and efforts were directed toward formation of functionality, which was required for the coupling of the low-molecular fragment to the steroidal aldehyde.

Sulfones are known to be of great synthetic utility in carbon–carbon bond forming reactions in the construction of steroidal side chains [19], including those characteristic of BS [20–22]. The same procedure was used for introduction of another phenylsulfonyl group. However, prior to the oxidation of the sulfide 11, the phenylsulfonyl group at C25 had to be removed. This was achieved by treatment of 10 with magnesium in methanol.

Construction of the necessary side chain was performed as depicted in Scheme 4. Addition of the lithium salt of sulfone 12 to the aldehyde 4, prepared in five steps from stigmasterol [23,24], gave a mixture of hydroxy sulfones 13. It has been shown [25] that a similar reaction produced a mixture of all possible isomers at C22 and C23. Taking into account that the total amount of 22α-alcohols was rather substantial, removal of phenylsulfonyl group at this stage seemed to be reasonable. However, all attempts of desulfurization of 13 failed, and the only isolated product was the Δ22-derivative, corresponding to a Julia olefination. Conversely, a similar
reaction is well known for keto sulfones [26, 27]. The initial attempt to oxidize 13 with CrO₃ in pyridine into the corresponding ketone gave poor results; the reaction proceeded slowly (2–3 days), and the yield of 14 was low. The problem was solved using Swern oxidation. Desulfurization of 14 with aluminum amalgam gave the desired 22-ketone 15 without epimerization of the adjacent chiral center. Hydride reduction of the ketone 15 led to the 22α-alcohol 16. This gave the required side chain, and for further transformations in the cyclic skeleton, the hydroxy group was protected as the acetate.

Construction of the required cyclic moiety was accomplished according to previously reported procedures [28] for the synthesis of [26-2H₃]BS (Scheme 5). Hydrobromic acid assisted cyclopropane ring opening in 18 followed by dehydrobromination of 19 gave the Δ₂-olefin 20, which was
Upon treatment with base, compound 22-23-dehydroxy analog of castasterone further subjected to hydroxylation to give the 21-23-ric acid gave the diacetate.

In conclusion, a new method for preparation of castasterone side chain have been developed. Two new brassinolide biosynthetic intermediates (as hexadeuterated derivatives 22 and 25) have been synthesized. The obtained compounds are considered as effective tools for elucidation of alternative subpathways in brassinolide biosynthesis.

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References