Biosci. Biotechnol. Biochem., **64** (9), 1915–1922, 2000

Synthesis of 2-*C*-Methyl-D-erythritol and 2-*C*-Methyl-L-threitol; Determination of the Absolute Configuration of 2-*C*-Methyl-1,2,3,4-butanetetrol Isolated from *Phlox sublata L*

Isao SAKAMOTO,[1] Kazuo ICHIMURA,[2] and Hiroshi OHRUI[1,†]

[1]*Divisionof Life Science, Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori Amamiyamachi, Aoba-ku, Sendai 981-8555, Japan*
[2]*NationalResearch Institute of Vegetables, Ornamental Plants and Tea, Ano, Mie 514-2392, Japan*

Received March 16, 2000; Accepted April 26, 2000

2-*C*-Methyl-D-erythritol (A) and 2-*C*-methyl-L-threitol (B) were respectively synthesized from D-glucose and D-galactose. The 2-*C*-methyl-1,2,3,4-butanetetrol compound (C) recently isolated from *Phlox sublata L* was confirmed to be A by comparing the CD and ^{1}H-NMR spoectra of its tri-*O*-benzoate with those of A and B.

Key words: 2-*C*-methyl-D-erythritol; non-mevalonate isoprenoid biosynthesis; *Phlox sublata L*; absolute configuration

2-*C*-Methyl-D-erythritol [**(A)**, (2*S*, 3*R*)-2-*C*-methyl-1,2,3,4-butanetetrol], a putative C_5 intermediate in the mevalonate-independent pathway for isoprenoid biosynthesis,[1)] has been isolated from *Convolvulus glomeratus*,[2)] *Liriodendron tulipiferqa L*,[3)] and *Ferula sinaica*.[4)] The recent isolation of 2-*C*-methyl-1,2,3,4-butanetetrol (**C**) from *Phlox sublata L* by Ichimura (K. Ichimura, unpublished results) prompted us to synthesize **A** and its diastereomer, 2-*C*-methyl-L-threitol [**B**, (2*R*, 3*R*)-2-*C*-methyl-1,2,3,4-butanetetrol] and to propose a method for the unequivocal determination of the absolute configurations of 2-*C*-methyl-1,2,3,4-butanetetrols. In this paper, we describe the stereoselective syntheses of **A** from D-glucose and of **B** from D-galactose, and a spectrometric method for determining the absolute configurations of 2-*C*-methyl-1,2,3,4-butanetetrols. Our synthetic strategy was designed to permit the introduction of stable or radioactiveisotopes in different steps, thus leading to a set of labelled compounds that would be necessary for mechanistic studies of the non-mevalonate isoprenoid biosynthetic pathway.

Results and Discussion

In 1976, Anthonsen *et al.* synthesized *racemic* 2-*C*-methyl-erythritol and determined the *erythro* stereochemistry for **A** by comparing their ^{1}H-NMR spectra.[2)] They also determined the D-configuration of **A** by comparing the CD spectrum of its molybdate(VI) complex with those of 2-*C-isobutyl*-D-erythritol and 2-*C-isobutyl*-D-threitol of known absolute configuration.[6)] Several studies on the synthesis of compounds related with **A** have been reported in the literatures.[7)] In the course of the preparation of this paper, a study on the synthesis of **A** from 1,2:5,6-di-*O*-isopropylidene-D-mannitol has appeared.[8)] However, the $[\alpha]_D$ value reported in that work is 50% smaller than the values previously reported.[2-4)] The inconsistency of the reported $[\alpha]_D$ value for **A** may have been due to the difficulty in purifying polyhydroxylated compounds. Since **C** has two asymmetric carbons, four optical isomers are theoretically possible for **C**. The combination of two analytical methods, one to discriminate *threo* and *erythro* stereochemistry and the other to discriminate D- and L-configuration, can determine the absolute configuration of **C**. In order to determine the absolute stereochemistry of **C**, we synthesized two diastereomers of **C**, **A** from D-glucose and **B** from D-galactose.

Synthesis of A from D-glucose

1,2:5,6 - Di - *O* - cyclohexylidene - α - D - *ribo* - hexofuranos-3-ulose (**1**),[9)] was reacted with methyl magnesium bromide to give **2** in a 91% yield. The stereochemistry at the 3-position of **2** was confirmed by NOE experiments as shown in Fig. 1. The OH group of **2** was protected with a benzyl group to give

† To whom correspondence should be addressed. Fax: +81-22-717-8806; E-mail: ohrui@biochem.tohoku.ac.jp
Abbreviations: THF, tetrahydrofuran; DMSO, dimethyl sulfoxide; DMF, *N*,*N*-dimethylformamide

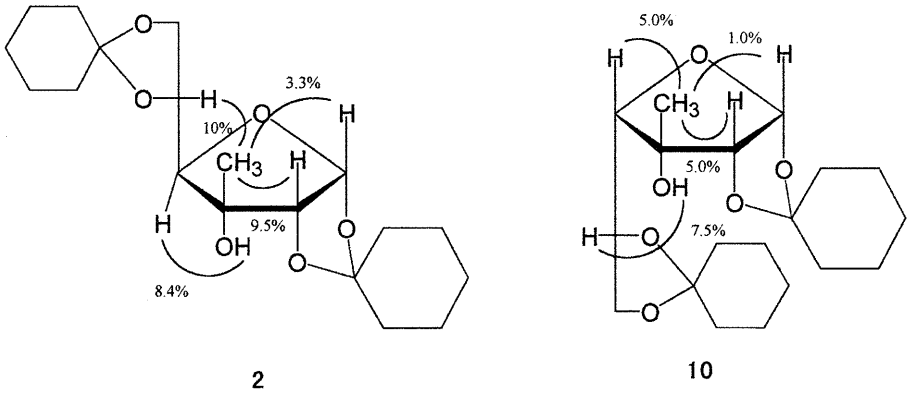
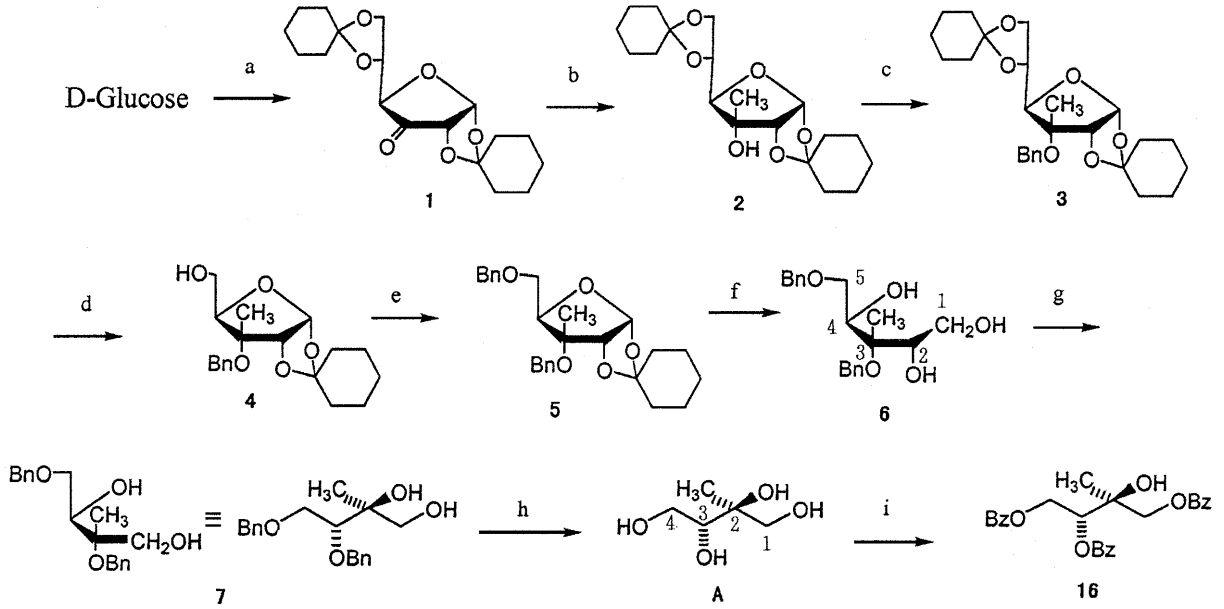


Fig. 1. NOE data for **2** and **10**.



Scheme 1. Synthesis of 2-*C*-Methyl-D-erythritol (A) from D-Glucose.

Reagents & conditions: a) (1) cyclohexanone, H_2SO_4, 50%, (2) P_2O_5, DMSO, DMF, 50°C, 70%; b) CH_3MgCl, THF, rt, 91%; c) BnCl, KOH, reflux, 85%; d) (1) 70% AcOH, 70°C, 83%; (2) $NaIO_4$, EtOH, H_2O, then $NaBH_4$, 93%; e) BnCl, KOH, reflux, 87%; f) (1) 30% trifluoroacetic acid, reflux, 1 h, 80%; (2) $NaBH_4$, EtOH, 24 h, 70%; g) $NaIO_4$, MeOH, H_2O, then $NaBH_4$, 93%; h) H_2, 10% Pd C, MeOH, 24 h, 85%; i) BzCl, pyridine, 75%.

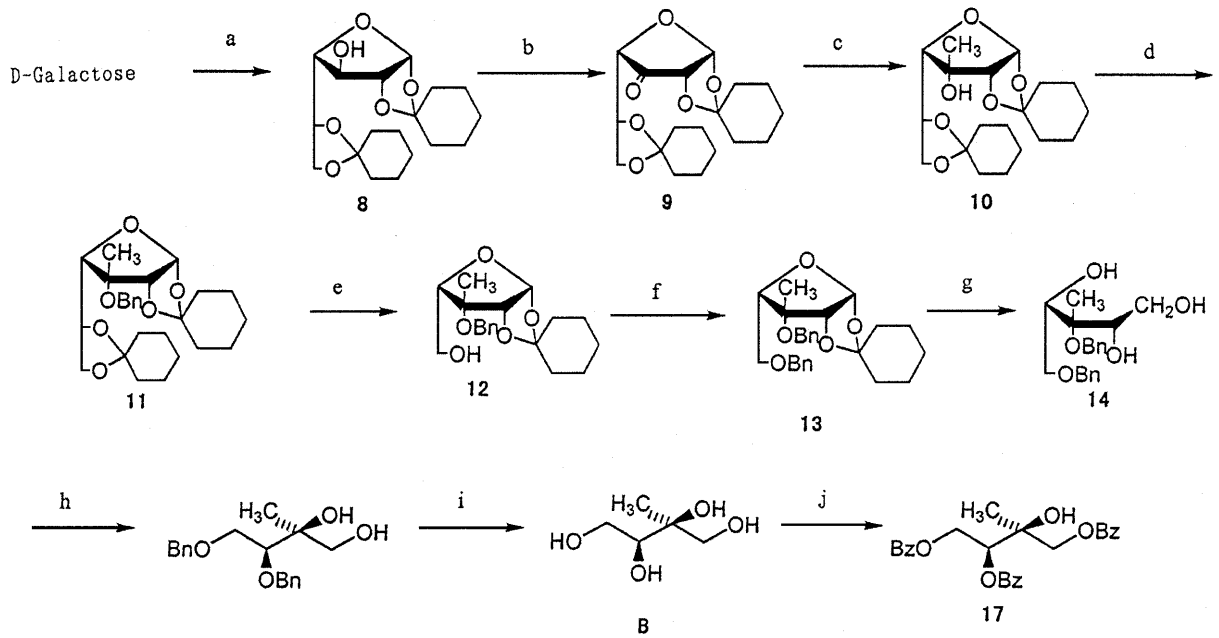
3, which prevented the migration of the cyclohexylidene group from the 1,2- to 2,3-position under acidic conditions. Selective hydrolysis of the 5,6-*O*-cyclohexylidene group of **3** in 30% acetic acid and subsequent IO_4^- oxidation and $NaBH_4$ reduction afforded pentose **4** in a 76% yield. The OH group of **4** was protected with a benzyl group to give **5**. Removal of the 1,2-*O*-cyclohexylidene group of **5** and subsequent $NaBH_4$ reduction of the resulting hemiacetal yielded 3-*C*-methyl-D-ribitol derivative **6** in a 56% yield. IO_4^- oxidation of **6** and then $NaBH_4$ reduction of the resulting tetrose gave 3,4-di-*O*-benzyl-3-*C*-methyl-D-erythritol (**7**) which was de-*O*-benzylated to afford **A** (15% from **1**; Scheme 1).

*Synthesis of **B** from D-galactose*

1,2:5,6-Di-*O*-cyclohexylidene-α-D-galactofuranose (**8**) that has recently been developed in our laboratory[10] was oxidized with DMSO-P_2O_5[8] to afford 3-ketone **9** in a 61% yield. The conversion of **9** to **B** was performed by a procedure essentially the same as that described for the conversion of **1** to **A** as shown in Scheme 2.

*Determination the absolute configuration of **C** by comparing the CD and ^{1}H-NMR spectra of its tri-**O**-benzoate with those of the tri-**O**-benzoates of **A** and **B***

The sign and magnitude for the optical rotation of optically active compounds are very important physical properties to identify them. Although the reported $[\alpha]_D$ values for **A** were inconsistent,[2-4,7] their signs were all positive, $[\alpha]_D$ of our **A** being +20.9° in H_2O (+15.7° in MeOH) and that of our **B** being −16.1°



Scheme 2. Synthesis of 2-*C*-Methyl-L-threitol (B) from D-Galactose.
Reagents & conditions: a) cyclohexanone, DMF, PPTS, 66%; b) P_2O_5, DMSO, 50°C, 61%; c) CH_3MgCl, THF, rt, 99%; d) BnCl, KOH, reflux, 81%; e) (1) 70% AcOH, 70°C, 82%; (2) $NaIO_4$, EtOH, H_2O, then $NaBH_4$, 86%; f) BnCl, KOH, reflux, 83%; g) (1) 30% trifluoroacetic acid, rt, 24 h, 85%; (2) $NaBH_4$, EtOH, 24 h, 86%; h) $NaIO_4$, MeOH, H_2O, then $NaBH_4$, 78%; i) H_2, 10% Pd C, MeOH, 24 h, 86%; j) BzCl, pyridine, 70%.

in H_2O ($-11.7°$ in MeOH). The signs are opposite between the D- and L-configurations regardless of their *erythro* or *threo* stereochemistry. Thus, the sign can be diagnostic for the determination of D, L of 2-*C*-methyl-1,2,3,4-butanetetrols. However, the difference in magnitude between the *erythro* and *threo* isomers is small and this small difference can not be used for the determination of their *erythro* or *threo* configuration because the reported $[\alpha]_D$ values were inconsistent. Therefore, we decided to determine the absolute configuration of **C** by a much reliable method. Compound **A** and **B** were benzoylated to their tri-*O*-benzoates **16** and **17**, which can be easily purified, and their CD spectra were measured (Fig. 2a). These CD spectra appeared as exiton-type to show that the terminal vicinal diol system favoured a *gauche-trans* conformation (Fig. 3) and therefore their absolute configurations. These results are consistent with those for the previous terminal diols[10] showing that the CD method can be used for determining the D- or L-configuration of 2-*C*-methyl-1,2,3,4-butanetetrols in spite of the additional 1,3-di-*O*-benzoate system.[10] The ^{1}H-NMR spectra for **16** and **17** are shown in Fig. 2b. The signals of both the C-1 and C-4 methylene protons are quite different between them (the difference in chemical shift of the C-1 methylene protons of **16** is smaller than that of **17**, while that of the C-4 methylene protons of **16** is larger than that of **17**). Thus, the ^{1}H-NMR spectra can be used for the unequivocal discrimination of their *erythro* and *threo* stereochemistry. Accordingly, the absolute configurations of 2-*C*-methyl-1,2,3,4-butanetetrols can be unambiguously determined by comparing both the CD and ^{1}H-NMR spectra of the tri-*O*-benzoate with those of the tri-*O*-benzoates of **A** and **B**. Both the CD and ^{1}H-NMR spectra of the tri-*O*-benzoate of **C** were identical with those of the tri-*O*-benzoate of **A**. Thus, **C** was in fact confirmed to be **A**.

If isotope-labelled methylmagnesium bromide, $NaBH_4$ or $LiAlH_4$ were to be used in our synthesis of **A**, regioselectively isotope-labelled **A**, which would be useful for studying the non-mevalonate biosynthesis of isoprenoids, could be prepared.

Experimental

General methods. Melting point (mp) data were recorded with Shibata melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded with a Varian UNITY plus-500 spectrometer at 21–23°C in $CDCl_3$ using Me_4Si as an internal standard, and mass spectra were recorded with a Hitachi M-80B spectrometer at 70 eV. Specific rotation values were measured at 22°C with a JASCO DIP-360 instrument at 589 nm. Merck silica gel Art. 9385 was used for column chromatography, and Merck silica gel Art. 5554 for analytical thin-layer chromatography.

1,2:5,6-Di-O-cyclohexylidene-3-C-methyl-α-D-allofuranose (2). To a solution of **1** (1014 mg, 3 mmol) in dry THF (40 ml) under Ar was added dropwise

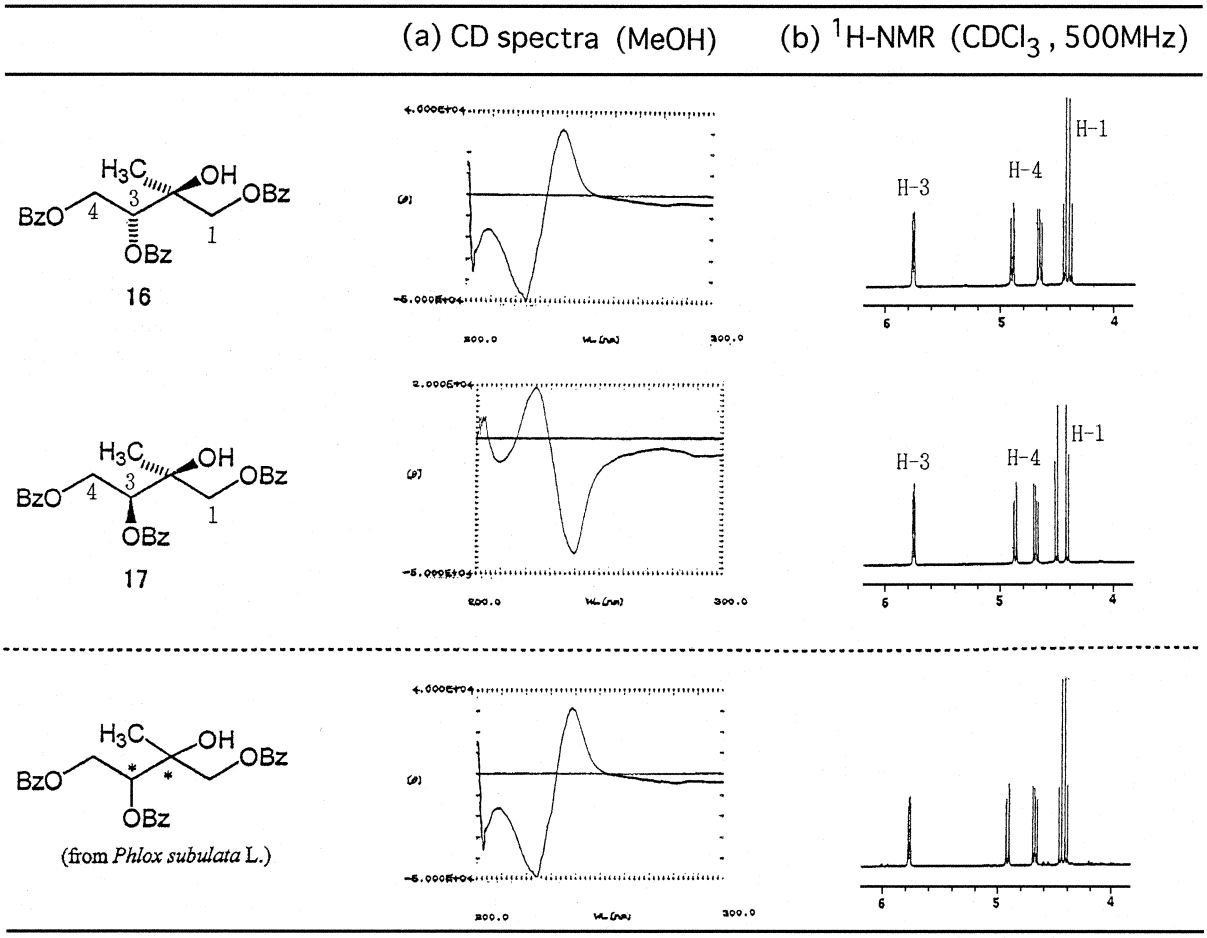


Fig. 2. CD Spectra and ^{1}H-NMR Spectra for **16**, **17** and 1,3,4-tri-*O*-Benzoate of **C**.

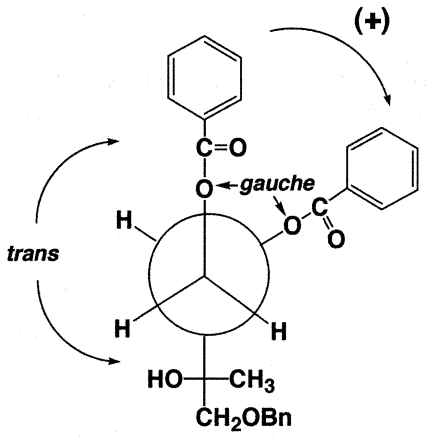


Fig. 3. Preferred *Gauche-trans* Conformation of the tri-*O*-Benzoate of **A**.

CH_3MgCl (10 ml, a 3.0 M solution in THF), and the mixture was stirred at room temperature for 30 min. An ammonium chloride solution (25% in water) was then added to the mixture while cooling in an ice-water bath. The mixture was extracted with ethyl acetate (20 ml × 5). The resulting extract was washed with water, dried over $MgSO_4$ and evaporated under reduced pressure to give crude crystals which were recrystallized from cyclohexane to give **2** (996 mg, 91%) as colourless crystals, mp 120–122°C, $[\alpha]_D$ + 16.6° (c 1.00, $CHCl_3$); NMR δ_H: 5.71 (1H, d, J = 3.6 Hz, H-1), 4.16 (1H, d, J = 3.66 Hz, H-2), 4.10 (1H, m, H-5), 3.91 (2H, m, H-6, 6′), 3.76 (1H, m, H-4), 2.74 (1H, OH), 1.81–1.30 (23H, m, cyclohexylidene, methyl). *Anal.* Found: C, 64.48; H, 8.40%. Calcd. for $C_{19}H_{30}O_6$: C, 64.38; H, 8.53%.

3-O-Benzyl-1,2:5,6-di-O-cyclohexylidene-3-C-methyl-α-D-allofuranose (3). A mixture of **2** (1062 mg, 3 mmol), powdered KOH (10 g) and benzyl chloride (10 ml) was refluxed while vigorously stirring for 5 h, and then cooled to room temperature. Water (20 ml) was added to the mixture, and the solution was extracted with ethyl acetate (10 ml × 3). The extract was washed with water, dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced presure to give a syrup. This syrup was submitted to silica gel column chromatography, eluting with a mixture of hexane and ethyl acetate (3:1, v/v) to give **3** (129 mg, 93%), $[\alpha]_D$ + 39.7° (c 1.21, $CHCl_3$); NMR δ_H: 7.44–7.24 (5H, m, Ph), 5.73 (1H, d, H-1, J = 3.6 Hz), 4.69 (1H, Ph-CH_2-O), 4.33 (1H, d, J = 3.66 Hz,

H-2), 4.32 (1H, Ph-CH_2-O), 4.14 (2H, m, H-4, 5), 4.05 (2H, m, H-6), 3.94 (1H, m, H-6′), 1.88–128 (23H, m, cyclohexylidene, methyl). *Anal.* Found: C, 70.35; H, 8.23%. Calcd. for $C_{26}H_{36}O_6$: C, 70.24; H, 8.16%.

3-O-Benzyl-1,2-O-cyclohexylidene-3-C-methyl-α-D-ribofuranose (4). A solution of **3** (888 mg, 2 mmol) in 70% aq. acetic acid (25 ml) was kept at 70°C for 1 h and then cooled to room temperature. After being neutralized with 4 N NaOH, it was extracted with $CHCl_3$ (20 ml × 3). The resulting extract was washed with water, dried over $MgSO_4$ and filtered. The filtrate was evaporated to give a syrup which was purified by silicagel column chromatography with a mixture of hexane and ethylacetate (1:1, v/v) to give crystalline 3-*O*-benzyl-1,2-*O*-hexylidene-3-*C*-methyl-α-D-allofuranose (618 mg, 85%), mp 128–129°C, $[\alpha]_D$ + 10.9° (c 1.1, $CHCl_3$); NMR δ_H: 7.38–7.28 (5H, m, Ph), 5.86 (1H, d, J = 3.9 Hz, H-1), 4.63 (2H, Ph-CH_2-O), 4.43 (1H, d, J = 3.9 Hz, H-2), 4.34 (1H, m, H-5), 3.85 (1H, d, H-4), 3.73 (1H, m, H-6), 3.63 (1H, m, H-6′), 3.01 (1H, OH), 2.18 (1H, OH), 1.70–1.36 (13H, m, cyclohexylidene, methyl). *Anal.* Found: C, 66.01; H, 7.71%. Calcd. for $C_{20}H_{28}O_6$: C, 65.91; H, 7.74%. To a stirred solution of this allofuranose (728 mg, 2 mmol) in ethanol (40 ml) were added dropwise water (10 ml) and slowly a solution of $NaIO_4$ (786 mg in 60 ml of H_2O). After the mixture had been stirred at room temperature for 2 h, $NaBH_4$ (152 mg) was added to it. The mixture was stirred for 2 h more and evaporated under reduced pressure. The residue was partitioned between ethyl acetate (20 ml) and water (20 ml). The aqueous layer was extracted with ethyl acetate (10 ml × 2). The combined ethyl acetate layers were washed with water, dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure to give crude crystals which were recrystallized from cyclohexane to yield **4** (200 mg, 93%), mp 132–134°C, $[\alpha]_D$ + 41.5° (c 0.56, $CHCl_3$); NMR δ_H: 7.43–7.27 (5H, m, Ph), 5.80 (1H, d, J = 3.66 Hz, H-1), 4.61 (4H, Ph-CH_2-O), 4.36 (1H, d, J = 3.6 Hz, H-2), 4.21 (1H, m, H-4), 3.840–3.72 (2H, m, H-5, 5′), 1.90–1.41 (11H, m, cyclohexylidene, OH), 1.25 (3H, s, methyl). *Anal.* Found: C, 68.33; H, 7.83%. Calcd. for $C_{19}H_{26}O_5$: C, 68.24; H, 7.84%.

3,5-Di-O-benzyl-3-C-methyl-D-ribofuranose (5). A mixture of **4** (820 mg, 2 mmol) and powdered KOH (5 g) in benzyl chloride (10 ml) was refluxed for 5 h. After being cooled, the mixture was diluted with water (10 ml) and extracted with ether (10 ml × 2). The extract was washed with water, dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure to give a syrup. This syrup was purified by silica gel column chromatography, eluting with a mixture of hexane and ethyl acetate (10:1, v/v), to give **6** (788 mg, 93%) as a syrup, $[\alpha]_D$ + 39.2° (c 0.19, $CHCl_3$); NMR δ_H: 7.42–7.24 (10H, m, Ph), 5.80 (1H, d, J = 3.6 Hz, H-1), 4.62–4.50 (4H, $PhCH_2O$), 4.69 (1H, dd, H5), 3.56 (10H, m, cyclohexylidene), 1.18 (3H, s, methyl). *Anal.* Found: C, 73.45; H, 7.65%. Calcd. For $C_{26}H_{32}O_5$: C, 73.56; H, 7.60%.

3,5-Di-O-benzyl-3-O-methyl-D-ribitol (6). A solution of **5** (848 mg, 2 mmol) in aq. 30% trifluoroacetic acid (50 ml) was refluxed for 1 h. After being cooled, the solution was neutralized with 4 N NaOH. To the solution were added ethanol (30 ml) and $NaBH_4$ (140 mg), and the mixture was stirred overnight. To the mixture was added acetone (5 ml), before evaporating under reduced pressure. The resulting residue was partitioned between $CHCl_3$ (20 ml) and water (20 ml). The aqueous layer was extracted with $CHCl_3$ (10 ml × 2), and the combined $CHCl_3$ layers were washed with water, dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to afford a syrup. This syrup was purified by silica gel column chromatography, eluting with a mixture of hexane and ethyl acetate (1:1, v/v), to give syrupy **6** (486 mg, 70%), $[\alpha]_D$ + 15.6° (c 1.0, $CHCl_3$); NMR δ_H: 7.36–7.23 (10H, m, Ph), 4.56 (2H, Ph-CH_2-O), 4.51 (2H, Ph-CH_2-O), 4.08 (1H, CH), 3.87–3.71 (4H, m, CH_2 × 2), 3.61 (1H, CH), 3.38 (2H, OH × 2), 3.15 (1H, OH), 1.45 (3H, s, methyl). *Anal.* Found: C, 69.15; H, 7.55%. Calcd for $C_{20}H_{24}O_5$: C, 69.34; H, 7.56%.

2,4-Di-O-benzyl-2-C-methyl-D-erythritol (7). To a solution of **6** (694 mg, 2 mmol) in MeOH (50 ml) were added dropwise H_2O (50 ml) and then $NaIO_4$ (860 mg in 50 ml of H_2O). The mixture was stirred for 2 h at room temperature and then $NaBH_4$ (240 mg) was added to it. The mixture was stirred for a further 2 h at room temperature and then evaporated under reduced pressure. The resulting residue was extracted with ethyl acetate (30 ml), and the extract was washed with water, dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to give crude **7** as a syrup. This syrup was purified by silica gel column chromatography, eluting with a mixture of hexane and ethyl acetate (1:1, v/v), to give syrupy **7** (600 mg, 95%), $[\alpha]_D$ + 19.1° (c 1.0, $CHCl_3$); NMR δ_H: 7.38–7.24 (10H, m, Ph), 4.59–4.49 (4H, Ph-CH_2-O), 4.03–3.97 (1H, m, H-3), 3.78 (1H, m, H-4), 3.72 (2H, d, J = 12 Hz, H-1), 3.60 (1H, m, H-4), 2.82 (1H, OH), 2.57 (1H, OH), 1.24 (3H, s, methyl). *Anal.* Found: C, 72.01; H, 7.55%. Calcd. For $C_{19}H_{24}O_4$: C, 72.13; H, 7.65%.

2-C-methyl-D-erythritol (A). A solution of **7** (316 mg, 1 mmol) in MeOH (10 ml) was stirred in the presence of 10% Pd-C (20 mg) under H_2 for 24 h at room temperature. After the catalyst had been

filtered off, the solution was concentrated to give crude **A** as a syrup. This syrup was purified by silica gel column chromatography, eluting with amixture of $CHCl_3$ and MeOH (3:1, v/v), to give an analytically pure sample of **A** (234 mg, 86%) as a syrup, $[\alpha]_D$ +20.9° (c 0.5, H_2O), +15.7° (c 1.0, MeOH); NMR δ_H: 3.86 (1H, m, H-4), 3.69–3.66 (1H, m, H-3), 3.63–3.56 (2H, m, H-1, H-4′), 3.48 (1H, d, J = 12 Hz, H-1′), 1.13 (3H, s, methyl). *Anal.* Found: C, 44.32; H, 8.76%. Calcd. for $C_5H_{12}O_4$: C, 44.11; H, 8.88%.

1,2,4-Tri-O-benzoyl-2-C-methyl-D-erythritol (16). To an ice-cooled solution of **A** (32 mg, 0.01 mmol) in pyridine (15 ml) was added benzoyl chloride (450 μl), and the mixture was stirred overnight at room temperature. To the mixture was added MeOH (15 ml), before stirring for 1 h at room temperature and then evaporating under reduced pressure. The resulting residue was dissolved in $CHCl_3$ (20 ml). The $CHCl_3$ solution was successively washed with water, aq. 3% H_2SO_4, sat. $NaHCO_3$, and water, dried over $MgSO_4$ and filtered. The filtrate was concentrated under-reduced pressure to give a syrup. This syrup was purified by silica gel column chromatography, eluting with a mixture of hexane and ethyl acetate (5:1, v/v), to give an analytically pure sample of **16** (38 mg, 80%), $[\alpha]_D$ −28.8° (c 1.0, $CHCl_3$); NMR δ_H: 8.06–7.93 (6H, m, Ph), 7.59–7.35 (9H, m, Ph), 5.79 (1H, m, H-3), 4.91 (1H, m, H-4), 4.66 (1H, m, H-4′), 4.44 (1H, d, J = 12 Hz, H-1), 4.38 (1H, d, H-1′), 2.97 (1H, s, OH), 1.50 (3H, s, methyl). CD (MeOH) λ_{ext}: 240 nm (θ − 44000), 224 nm (θ − 20000). *Anal.* Found: C, 69.50; H, 5.43. Calcd. for $C_{26}H_{24}O_7$: C, 69.63; H, 5.53%.

1,2:5,6-Di-O-cyclohexylidene-α-D-xylohexofuranos-3-ulose (9). To a solution of **8** (6.8 g, 20 mmol) in DMF (150 ml) were added DMSO (7.7 ml) and P_2O_5 (6 g), and the mixture was stirred for 1 h at 50°C. After cooling, $CHCl_3$ (200 ml) was added to the mixture. The mixture was successively washed with sat. $NaHCO_3$ (50 ml × 2) and H_2O (30 ml × 3), dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to give a syrup. This syrup was purified by silica gel column chromatography, eluting with a mixture of hexane and ether (3:1, v/v), to give crystalline **9** (4.1 g, 61%), mp 125–126°C, $[\alpha]_D$ + 24.0° (c 1.4, $CHCl_3$); NMR δ_H: 6.05 (1H, d, J = 4.4 Hz, H-1), 4.45 (1H, d, H-2), 4.33 (1H, m, H-5), 4.12–4.02 (3H, m, H-4, 6, 6′), 1.79–1.27 (20H, m, cyclohexylidene). *Anal.* Found: C, 64.02; H, 7.64%. Calcd. for $C_{18}H_{26}O_6$: C, 63.89; H, 7.74%.

1,2:5,6-di-O-cyclohexylidene-3-C-methyl-α-D-gulofuranose (10). To a solution of **9** (1 g, 3 mmol) in dry THF (10 ml) under Ar was added dropwise CH_3MgCl (10 ml, 3.0 M in THF), and the mixture was stirred for 1 h at room temperature. To the ice-cooled mixture was added dropwise aq. 25% NH_4Cl. The cooled mixture was extracted with ethylacetate (10 ml × 3), and the resulting organic layer was washed with water, dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to afford crude crystals which were recrystallized from cyclohexane to give pure **10** (1 g, 97%), mp 122–123°C, $[\alpha]_D$ −32.0° (c 1.0, $CHCl_3$); NMR δ_H: 5.84 (1H, d, J = 3.9 Hz, H-1), 4.39 (1H, m, H-5), 4.18 (1H, d, H-2), 4.15 (2H, m, H-4), 3.68 (2H, m, H-6, 6′), 3.04 (1H, OH), 1.97–1.27 (23H, m, cyclohexylidene, methyl). *Anal.* Found: C, 64.17; H, 8.44%. Calcd. for $C_{19}H_{30}O_6$: C, 64.38; H, 8.53%.

3-O-Benzyl-1,2:5,6-di-O-cyclohexylidene-3-C-methyl-α-D-gulofuranose (11). A mixture of **10** (1062 mg, 3 mmol) and powered KOH (10 g) in benzyl chloride (15 ml) was refluxed while vigorously stirring for 5 h. After cooling, ether (20 ml) and H_2O (15 ml) were added to the mixture while stirring. The layers were separated, and the aqueous layer was extracted with ether (10 ml × 2). The combined organic layers were washed with water, dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to afford crude crystals which were recrystallized from cyclohexane to give **11** (1130 mg, 85%), mp 158–159°C, $[\alpha]_D$ −30.3° (c 1.5, $CHCl_3$); NMR δ_H: 7.35–7.26 (5H, m, Ph), 5.86 (1H, d, J = 3.6 Hz, H-1), 4.74 (1H, m, H-5), 4.57 (2H, Ph-CH_2-O), 4.39 (1H, d, H-2), 3.93 (2H, m, H-6), 3.75 (1H, H-4), 3.49 (2H, H-4), 3.49 (2H, m, H-6′), 1.76–1.22 (23H, m, cyclohexylidene, methyl). *Anal.* Found: C, 70.27; H, 8.12%. Calcd. for $C_{36}H_{36}H_6$: C, 70.24; H, 8.16%.

3-O-Benzyl-1,2-O-cyclohexylidene-3-C-methyl-α-D-lyxofuranose (12). A solution of **11** (1.33 g, 3 mmol) in 70% acetic acid (30 ml) was kept for 1 h at 70°C and then evaporated under reduced pressure to give a syrup. This syrup was dissolved in $CHCl_3$ (30 ml). The $CHCl_3$ solution was successively washed with 1 N NaOH (10 ml) and water (10 ml), dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to afford a syrup. This syrup was purified by silica gel column chromatography, eluting with a mixture of hexane and ethyl acetate (1:1, v/v), to give crystalline 3-*O*-benzyl-1,2-*O*-cyclohexylidene-3-*C*-methyl-α-D-gulofuranose (890 mg, 82 %), mp 128–129°C, $[\alpha]_D$ − 11.3° (c 1.0, $CHCl_3$). To a solution of this gulofuranose (1.1 g, 3 mmol) in EtOH (30 ml) were added dropwise H_2O (10 ml) and $NaIO_4$ (1.26 g in 50 ml of H_2O) at room temperature. After the mixture had been stirred for 2 h at the temperature, $NaBH_4$ (220 mg) was added, and the mixture was stirred for a further 2 h. The mixture was concentrated undrer reduced pressure, and the resulting residue was dissolved in ethyl acetate (30 ml). The ethyl acetate solution was washed with water (10 ml),

dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to give crude crysatals which were recrystallized from cyclohexane to give analytically pure **12** (862 mg, 86%), mp 84–85°C, $[\alpha]_D - 10.6°$ (c 1.0, $CHCl_3$); NMR δ_H: 7.41–7.28 (5H, m, Ph), 5.83 (1H, d, J = 3.9 Hz, H-1), 4.64 (2H, Ph-CH_2-O), 4.40 (1H, d, H-2), 4.12 (1H, m, H-5), 3.94 (1H, m, H-4), 3.80 (1H, m, H-5′), 2.40 (1H, OH), 1.93–1.36 (13H, m, cyclohexylidene, methyl). *Anal.* Found: C, 68.41; H, 7.77%. Calcd. for $C_{19}H_{26}O_5$: C, 68.24; H, 7.84%.

*3,5-Di-O-benzyl-1,2-O-cyclohexylidene-3-C-methyl-β-*L-lyxofuranose (*13*). A mixture of **12** (1.0 g, 3 mmol) and powdered KOH (10 g) in benzyl chloride (20 ml) was refluxed for 5 h. After being cooled, to the mixture were added ether (20 ml) and H_2O (20 ml). The organic layer and aqueous layer were separated, and the aqueous layer was extracted with ether (10 ml × 2). The combined organic layers were washed with water (10 ml), dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to afford a syrup. This syrup was purified by silica gel column chromatography, eluting with a mixture of hexane and ethyl acetate (10:1, v/v), to give analytically pure **13** (114 mg, 90%) as a syrup, $[\alpha]_D - 82.8°$ (c 1.0, $CHCl_3$); NMR δ_H: 7.37–7.24 (10H, m, Ph), 5.82 (1H, d, J = 3.5 Hz, H-1), 4.60 (4H, Ph-CH_2-O), 4.36 (1H, d, H-2), 4.10 (1H, m, H-4), 3.94–3.87 (2H, m, H-5, 5′), 1.70–1.25 (13H, m, cycliohexylidene, methyl). *Anal.* Found: C, 73.43; H, 7.77%. Calcd. for $C_{26}H_{32}O_5$: C, 73.56; N, 7.60%.

*3,5-Di-O-benzyl-3-C-methyl-*L*-lyxitol (14).* A solution of **13** (828 mg, 2 mmol) in 30% CF_3COOH (50 ml) was stirred overnight at room temperature and then neutralized with 4 N NaOH. The solution was extracted with $CHCl_3$ (20 ml 3), the resulting extract being washed with water (10 ml), dried over $MgSO_4$ and filtered. The filtrate was concentrated to give a mixture of 3,5-di-*O*-benzyl-3-*C*-methyl-L-lyxose and cyclohexanone. The residue was dissolved in EtOH (50 ml), and $NaBH_4$ was then added. The mixture was stirred overnight at room temperature. After acetone (5 ml) had been added, the mixture was evaporated under reduced pressure to give a syrup. This syrup was purified by silica gel column chromatography, eluting with a mixture of hexane and ethyl acetate (1:1, v/v), to give **14** (595 mg, 86%) as a syrup, $[\alpha]_D - 20.8°$ (c 0.5, $CHCl_3$); NMR δ_H: 7.33–7.21 (10H, m, Ph), 4.57–4.40 (4H, m, Ph-CH_2-O), 4.13 (1H, CH), 3.99–3.95 (1H, H-2), 3.90–3.87 (1H, H-2′), 3.80–3.60 (4H, m, OH × 2, CH × 2), 2.79 (1H, s, OH), 1.29 (3H, s, methyl). *Anal.* Found: C, 69.47; H, 7.78%. Calcd. for $C_{20}H_{26}O_5$: C, 69.34; H, 7.56%.

*2,4-Di-O-benzyl-2-C-methyl-*L*-threitol (15).* To a solution of **14** (692 mg, 2 mmol) in MeOH (30 ml) were added dropwise H_2O (30 ml) and $NaIO_4$ (675 mg in 70 ml of H_2O). After the mixture had been stirred for 2 h at room temperature, $NaBH_4$ (165 mg) was added and the mixture was stirred for 2 h more at room temperature. The mixture was evaporated under reduced pressure, and the resuting residue was extracted with ethyl acetate (50 ml). The extract was washed with water, dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure to give a syrup. This syrup was purified by silica gel column chromatography, eluting with a mixture of hexane and ethyl acetate (1:1, v/v), to give **15** (493 mg, 78%) as a syrup, $[\alpha]_D - 10.7°$ (c 0.5, $CHCl_3$); NMR δ_H: 7.36–7.23 (10H, m, Ph), 4.56–4.49 (4H, Ph-CH_2-O), 3.97 (1H, H-3), 3.73–3.65 (4H, m, H-1, H-4, $CH_2 \times 2$), 2.93 (2H, s, OH × 2), 1.24 (3H, s, methyl). *Anal.* Found: C, 72.33; H, 7.78%. Calcd. for $C_{19}H_{24}O_4$: C, 72.13; H, 7.65%.

*2-C-Methyl-*L*-threitol (B).* A mixture of **7** (670 mg, 2 mmol) and 10% Pd-C (15 mg) in MeOH (15 ml) was stirred undera a H_2 atmosphere for 3 h at room temperature. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure to give **B** (234 mg, 86%) as a syrup, $[\alpha]_D - 16.1°$ (c 0.5, H_2O), $-11.7°$ (c 0.5, MeOH); NMR δ_H (DMSO-d_6): 3.75 (1H, m, H-4), 3.62 (1H, m, H-3), 3.52–3.45 (3H, m, H-1, H-1′, H-4′), 1.08 (3H, s, methyl). *Anal.* Found: C, 44.08; H, 8.76%. Calcd. for $C_5H_{12}O_4$: C, 44.11; H, 8.88%.

*1,3,4-Tri-O-benzoyl-2-C-methyl-*L*-threitol (17).* To a solution of **B** (35 mg) in dry pyridine (5 ml) was added benzoyl chloride (500 μl), and the mixture was stirred overnight at room temperature. After MeOH (15 ml) had been added, the mixture was evaporated under reduced pressure. The residue was dissolved in a mixture of ethyl acetate (30 ml) and water (10 ml), and the aqueous layer was extracted with ethyl acetate (10 ml). The combined ethyl acetate extracts were washed with water, dried over $MgSO_4$ and filtered. The filtrate was concentrated to give a syrup. This syrup was purified by silica gel column chromatography, eluting with amixture of hexane and ethyl acetate (5:1, v/v), to give **17** (80 mg, 70%) as a syrup, $[\alpha]_D - 6.27°$ (c 1.5, $CHCl_3$); NMR δ_H: 8.06–7.34 (15H, m, Bz), 5.75 (1H, m, H-3), 4.86 (1H, m, H-4), 4.69 (1H, m, H-4′),4.50 (1H, J = 12 Hz, H-1), 4.40 (1H, J = 12 Hz, H-1′), 2.77 (1H, s, OH), 1.50 (3H, s, methyl); CD (MeOH) λ_{ext}: 238 nm ($\theta + 32000$), 224 nm ($\theta - 50000$). *Anal.* Found: C, 69.55; H, 5.47%. Calcd. for $C_{26}H_{24}O_7$: C, 69.63; H, 5.39%.

References

1) Duvold, T., Bravo, J-MC., Grosdemange, P., and Rohmer, M., Biosynthesis of 2-*C*-methyl-D-erythritol, a putative C_5 intermediate in the mevalonate independent pathway for isoprenoid biosynthesis. *Tetrahedron Lett.*, **38**, 4769–4772 (1997).
2) Anthonsen, T., Hagen, S., Kazi, S. M. A., Shah, S. W., and Tagar, S., 2-*C*-methyl-erythritol, a new branched alditol from convolvulus glomerratus. *Acta Chem. Scand. B*, **30**, 91–93 (1976).
3) Dittrich, D. and Angyal, S. J., 2-*C*-methyl-D-erythritol in leaves of liriodendron tulipifera. *Phytochemistry*, **27**, 935–936 (1988).
4) Ahmed, A. A., Mohamed, H., Effat, A., Mostafa, A., William, H. J., Scott, A. I., Reibenspies, J. H., and Mabry, T. M., A new derivative of glucose and 2-*C*-methyl-D-erythritol from ferulasinaica. *J. Nat. Prod.*, **59**, 1171–1173 (1996).
5) Shah, S. W., Brandange, S., Behr, D., Dahmen, D., Hagen, S., and Anthonsen, T., Absolute configuration of 2-*C*-methylerythritol from convolvulus glomeratus. *Acta Chem. Scand. B*, **30**, 903 (1976).
6) a) Anthonsen, T., Hagen, S., and Sallam, M. A. E., Synthesis and spectroscopic studies of 2-*C*-methylerythritol and 2-*C*-methylthreitol. *Phytochemistry*, **19**, 2375–2377 (1980); b) Ford, C. W., A new lactone from water-stressed Chickpea. *Phytochemistry*, **20**, 2019–2020 (1981); c) Yoshimura, J., Hara, K., Yamamura, M., Mikamine, K., and Hashimoto, K., Branched-chain sugars. XXVIII. Synthesis of 2-*C*-methyl-L-glyceraldehyde, 2-*C*-methyl-D-erythrose, and 3-*C*-methyl-L-erythrose derivatives. *Bull. Chem. Soc. Jpn.*, **55**, 933–937 (1982); d) Teresa, J. D. P., Hernandes, J. C., Feliciano, A. S., and Migel del Corral, J. M., Saccharinic acid lactone from *Astragallus Lusitanicus Lam. Tetrahedron Lett.*, **21**, 1359–1360 (1980); e) Kobayashi, S., Horibe, H., and Saito, Y., Enantioselective synthesis of both diastereomers, including the α-alkoxy-β-hydroxy--methyl(phenyl) units, by chiral tin(II) lewis acid-mediated asymmetric aldol reactions. *Tetrahedron*, **50**, 9629–9642 (1994).
7) Kis, K., Wungsintaweekul, J., Eisenreich, W., Zenk, M. H., and Bacher, A., An efficient preparation of 2-*C*-methyl-D-erythritol 4-phosphoric acid and uts derivatives. *J. Org. Chem.*, **65**, 587–592 (2000).
8) Onodera, K., Kashimura, N., and Miyazaki, N., Synthesis of 3-amino-3-deoxy-D-ribose and D-ribose derivatives by means of methylsulfoxide-phosphorus pentaoxide and periodate oxidation. *Carbohydr. Res.*, **21**, 159–165(1972).
9) Sakamoto, I. and Ohrui, H., *Biosci. Biotechnol. Biochem.*, in press.
10) a) Uzawa, H., Nishida, Y., Ohrui, H., and Meguro, H., Application of the diibenzoate chirality method to determine the absolute configuration of glycerols and related acyclic alcohols. *J. Org. Chem.*, **55**, 116–122 (1990); b) Harada, N., Saito, A., Ono, A., Gawronski, J., and Gawronski, K., A CD method for determination of the absolute stereochemistry of acyclic glycols. 1. Application of the CD exciton chirality method to acyclic 1,3-dibenzoate sysytem. *J. Am. Chem. Soc.*, **113**, 3842–3850 (1991).