diastereomers were separated via flash chromatography using diethyl ether. Anal. Calcd for $C_{13}H_{18}NO_5P$: C, 52.18; H, 6.06; N, 4.68. Found: C, 51.99; H, 6.29; N, 4.90. IR: 1375, 1260.

Fast band (5a, cis): R_f = 0.13 (diethyl ether), $[\alpha]^{23}_D$ -23.90° (c 2.31, $CHCl_3$). ^1H NMR: δ 1.28 (t, J = 7.1 Hz, 3 H), 3.66 (s, 3 H), 3.82 (dt, J = 8.3, 4.6 Hz, 1 H), 4.01-4.42 (m, 6 H), 7.26-7.39 (m, 5 H). ^{13}C NMR: δ 16.04, 47.56, 52.27, 56.67, 63.94, 67.27, 127.83, 128.47, 128.79, 135.63, 170.66. ^{31}P NMR: δ 20.83. HPLC: t_R = 12.4 min.

Slow band (5b, trans): R_f = 0.09 (diethyl ether), $[\alpha]^{23}_D$ -56.06° (c 1.09, $CHCl_3$). ^1H NMR: δ 1.28 (t, J = 7.0 Hz, 3 H), 3.64 (s, 3 H), 3.72 (ddd, J = 10.8, 6.8, 2.8 Hz, 1 H), 4.02-4.14 (m, 2 H), 4.06 (q, J = 7.0 Hz, 2 H), 4.21-4.36 (m, 2 H), 7.25-7.31 (m, 5 H). ^{13}C NMR: δ 16.37, 47.04, 52.55, 57.67, 64.25, 66.12, 127.89, 128.44, 128.62, 135.98, 170.78. ^{31}P NMR: δ 19.89. HPLC: t_R = 11.7 min.

Methyl (2*S*,4*S*)- and (2*R*,4*S*)-2-Phenoxy-2-oxo-3-benzyl-1,3,2-oxazaphospholidine-4-carboxylate (6). Yield: 96%. The diastereomers were separated by flash chromatography utilizing petroleum ether/ethyl acetate as the eluent. Anal. Calcd for $C_{17}H_{18}NO_4P$: C, 58.79; H, 5.22; N, 4.03. Found: C, 58.75; H, 5.29; N, 4.01. IR: 1745, 1275.

Fast band (6a, cis): R_f = 0.29 (diethyl ether). This diastereomer crystallized as white plates from ethyl acetate/petroleum ether; mp = 92-94 °C, $[\alpha]^{23}_D$ -29.27° (c 0.96, $CHCl_3$). ^1H NMR: δ 3.58 (s, 3 H), 3.80 (ddd, J = 9.3, 8.3, 4.7 Hz, 1 H), 4.21-4.40 (m, 3 H), 4.56 (dd, J = 14.5, 8.9 Hz, 1 H), 7.31-7.41 (m, 10 H). ^{13}C NMR: δ 47.83, 52.49, 55.92, 65.63, 120.92, 125.12, 128.20, 128.72, 128.91, 129.38, 135.29, 150.68 (d, J = 8.7), 170.06. ^{31}P NMR: δ 16.38. HPLC: t_R = 24.4 min.

Slow band (6b, trans): R_f = 0.22 (diethyl ether). This diastereomer recrystallized as needles from methylene chloride/ether/petroleum ether; mp = 83-85 °C, $[\alpha]^{23}_D$ -70.22° (c 1.12, $CHCl_3$). ^1H NMR: δ 3.70 (s, 3 H), 3.82 (ddd, J = 12.1, 7.4, 2.0 Hz, 1 H), 4.30 (dd, J = 15.6, 8.1 Hz, 1 H), 4.33-4.49 (m, 2 H), 4.52 (dd, J = 15.1, 7.9 Hz, 1 H), 7.14-7.33 (m, 10 H). ^{13}C NMR: δ 47.36, 52.69, 57.15, 66.66, 120.27, 125.05, 128.08, 128.58, 128.74, 129.69, 135.55, 151.06 (d, J = 8.1), 170.43. ^{31}P NMR: δ 15.01. HPLC: t_R = 23.6 min.

Methyl (2*S*,4*S*)- and (2*R*,4*S*)-2-(4-Nitrophenoxy)-2-oxo-3-benzyl-1,3,2-oxazaphospholidine-4-carboxylate (7). Yield: 78%. The diastereomers were separated via flash chromatography using a gradient from 70:30 diethyl ether–petroleum ether to 100% diethyl ether. Recrystallization was accomplished with methylene chloride/diethyl ether/petroleum ether. Anal. Calcd for $C_{17}H_{17}N_2O_7P$: C, 52.05; H, 4.37; N, 7.10. Found: C, 51.94; H, 4.34; N, 7.10. IR: 1750, 1280.

Fast band (7a, cis): R_f = 0.35 (diethyl ether), $[\alpha]^{23}_D$ -4.49° (c 1.18, $CHCl_3$), mp = 91-92 °C. ^1H NMR: δ 3.70 (s, 3 H), 3.91 (ddd, J = 11.0, 8.1, 4.6 Hz, 1 H), 4.23 (dd, J = 14.4, 9.0 Hz, 1 H), 4.30-4.49 (m, 2 H), 4.51 (dd, J = 14.5, 9.8 Hz, 1 H), 7.27-7.36 (m, 7 H), 8.20 (d, J = 7.4 Hz, 2 H). ^{13}C NMR: δ 47.99, 52.72, 56.26, 66.12, 121.41, 125.49, 128.41, 128.87, 128.93, 134.91, 155.74 (d, J = 7.3), 169.93. ^{31}P NMR: δ 15.85. HPLC: t_R = 28.9 min.

Slow band (7b, trans): R_f = 0.20 (diethyl ether), $[\alpha]^{23}_D$ -27.45° (c 0.94, $CHCl_3$), mp = 101-102 °C. ^1H NMR: δ 3.76 (s, 3 H), 3.92 (ddd, J = 12.5, 6.5, 2.9 Hz, 1 H), 4.28 (dd, J = 15.1, 9.9 Hz, 1 H), 4.43-4.53 (m, 2 H), 4.52 (dd, J = 14.6, 7.9 Hz, 1 H), 7.26-7.38 (m, 7 H), 8.19 (d, J = 9.2 Hz, 2 H). ^{13}C NMR: δ 47.46, 52.93, 57.31, 66.97, 120.76, 120.83, 125.57, 128.53, 128.90, 135.18, 155.99 (d, J = 7.4), 170.12. ^{31}P NMR: δ 14.91. HPLC: t_R = 24.6 min.

Conversion of 2-(*p*-Nitrophenoxy)- (7b) into Methyl (2*S*,4*S*)-2-Methoxy-2-oxo-3-benzyl-1,3,2-oxazaphospholidine-4-carbxoylate (4b). The starting phosphorus ester (**7b**; 0.196 g, 0.5 mmol) was dissolved in 10 mL of anhydrous methanol and chilled to 0 °C. A catalytic amount (5 mg) of sodium methoxide was added and the reaction was warmed to room temperature, stirred, and monitored by TLC and HPLC for loss of starting material and appearance of product.

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Registry No. 1, 5680-80-8; 2, 123639-56-5; **3a**, 123621-73-8; **3b**, 123672-99-1; **4a**, 123621-74-9; **4b**, 123673-00-7; **5a**, 123621-75-0; **5b**, 123673-01-8; **6a**, 123621-76-1; **6b**, 123673-02-9; **7a**, 123621-77-2; **7b**, 123673-03-0; ^{31}P, 7723-14-0.

Supplementary Material Available: Tables of bond angles and bond lengths of **4b** and details of X-ray analysis for **4b** (3 pages). Ordering information is given on any current masthead page.

Application of the Dibenzoate Chirality Method To Determine the Absolute Configuration of Glycerols and Related Acyclic Alcohols

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Circular dichroism (CD) and nuclear magnetic resonance spectroscopy (NMR) were applied to the study of various chiral 1,2- (or 2,3)-di-*O*-benzoyl-*sn*-glycerols (substituent at *sn*-C1 or -C3 = cetyloxy, OBn, OMe, hexadecanoyloxy, OAc, penta-*O*-acetyl-β-D-glucopyranosyl, H, N_3, Br, OTs, and $OSi(Me)_2{}^tBu$). All compounds gave positive or negative exciton couplet (CD) peaks, reflecting the absolute configuration at C2 and conformational preference about the vicinal di-*O*-benzoates. The results were confirmed by conformational analyses using ^1H NMR spectroscopy and led to the proposal of a new method to determine the absolute configuration of asymmetric glycerols.

Glycerol constitutes the backbone of glycerolipids. It has a symmetrical plane centered at C2 and, therefore, is achiral and optically inactive. In biological reactions, however, the prochiral positions at C1 and C3 are differentiated, giving optically active glycerolipids or other natural products.[1] Determination of their absolute con-

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No	R1	R3
1*	$OCH_2(CH_2)_{14}CH_3$	OBz
2	OBz	OCH_2Ph
3	OBz	OMe
4	OBz	$OCO(CH_2)_{14}CH_3$
5	OBz	OAc
6	OBz	Tetra-*O*-acetyl-β-D-glucopyranosyl
7*	Tetra-*O*-acetyl-β-D-glucopyranosyl	OBz
8	OBz	H
9	OBz	N_3
10	OBz	Br
11	OBz	OTs[a]
12	OBz	$OSi^tBu(CH_3)_2$
13	OBz	NHBz

a) *p*-tolunenesulfonyl

Figure 1. Structures of 1,2- or 2,3-di-O-benzoylated *sn*-glycerols 1–13.

figuration is important in studies on biosynthetic processes or the relationship between the biological activity and absolute stereochemistry.[2] Several approaches to determine the configuration of chiral glycerolipids or related glycols have been reported,[3-6] i.e., chiroptical methods[3,4] based on molecular rotations ($[M]_D$), optical rotatory dispersion (ORD) and circular dichroism (CD), chromatographic analyses using chiral columns,[5] and NMR spectral methods using chiral shift reagents.[6]

Conventional $[M]_D$ or ORD methods[3] require considerable amounts of pure materials and careful comparison with data of authentic samples with known configurations. This is mainly because the optical rotations of glycerolipids are usually small and have not yet been fully correlated with the absolute configurations in both empirical and theoretical ways. CD spectra provide a better means to determine the absolute configurations of chiral compounds having suitable UV chromophores which give Cotton effects reflecting the absolute stereochemistries. For chiral glycerols, two alternative CD methods have already been proposed;[4] one method utilizes CD[4a] induced by the complexation of a glycol with Ni(acetylacetonate)$_2$ or Pr(dipivalomethanato)$_3$, and the other[4b-d] applies a dibenzoate chirality method of Harada and Nakanishi.[7] In this paper, we apply the latter method to a series of asymmetric mono-*O*-alkyl-, -acyl-, and -glycosylglycerols as a new approach to determine the absolute configuration of glycerolipids with CD spectra.

Materials and Methods

Model Compounds 1–13. A series of chiral 1,2-di-*O*-benzoyl-*sn*-glycerols (**2–6** and **8–13**) or 2,3-di-*O*-benzoyl-*sn*-glycerols (**1** and **7**) was studied (Figure 1). They are classified into the following four types: mono-*O*-alkylglycerols (**1–3**), mono-*O*-acylglycerols (**4** and **5**), glycosylglycerols (**6** and **7**) resembling naturally occurring glycerolipids, and related acyclic 1,2-diols with various kinds of substituents at C3 (**8–13**).

All compounds were prepared by acylation of the corresponding 1,2- or 2,3-diols with benzoyl chloride in pyridine with a catalytic amount of 4-(dimethylamino)pyridine. They were prepared almost quantitatively (90–100%) except for compound **13** (ca. 50%), which was contaminated by an di-N-benzoylated derivative. Purification was carried out for all materials with a silica gel TLC plate (Kiesel gel 60 GF_{254} (Merck), 20 × 20 cm) developed with *n*-hexane–ethyl acetate (4/1), and the purity of all compounds **1–13** was judged to be >90% by ^1H NMR spectra (e.g., Figure 6 and Table IIIb, supplementary material (see paragraph at end of paper)). UV (λ_{max} = ca. 230 nm (OOCPh)) and IR (ν = ca. 1720 cm^{-1} (OOCPh)) spectra (Tables I–III) were also used to confirm their structures.

The starting asymmetric diols were prepared according to methods in the literature.[8-10] Thus, 1,2-di-*O*-benzyl-*sn*-glycerol from D-mannitol[8] was used as a key intermediate for the preparation of **1** and **3–6**. Similarly, 3-*O*-benzyl-*sn*-glycerol derived from 1,6-anhydro-β-D-galactopyranose[9] was a key precursor of **2**, **7**, and **12**. 1,2-*O*-Isopropylidene-*sn*-glycerol[10] was used to prepare **8–11** and **13**.

Typical Procedure for the Isolation of Di-*O*-benzoylglycerols from Glycerols. A mixture of 3-*O*-benzyl-*sn*-glycerol[9] (1 mg, 5.5 μmol) and 4-(dimethylamino)pyridine (ca. 0.1 mg) in pyridine (0.2 mL, dried over KOH) was stirred for 0.5 h at room temperature. To the solution was added benzoyl chloride (3–5 times excess), and the mixture was stirred until benzoylation was completed (TLC analysis, 0.5–3 h). Excess benzoyl chloride was decomposed by adding saturated $NaHCO_3$ aqueous solution before the solution was diluted with chloroform (5 mL). The organic layer was washed successively with saturated $NaHCO_3$, water, 1% HCl aqueous solution, and then water and concentrated in vacuo to dryness. The residue was applied to a preparative TLC plate (20 × 20 cm) and developed with *n*-hexane–ethyl acetate (3/1). The fraction containing the desired material **2** (being monitored with a UV lamp at 254 nm) was extracted with a chloroform–methanol (100/1) mixture. The extract was filtered through a pad of silica gel and evaporated in vacuo to give syrupy **2** (2 mg, 95% yield).

UV, CD, $[\alpha]_D$, IR, and NMR Spectral Measurements. UV spectra were measured on Hitachi Model 200-10 spectrometer in methanol with 1-cm cell at 22 °C. CD spectra were measured on a Dichrograph Mark III-J instrument (CNSR-Roussel-Jouan) at 22 °C with concentration of ca. 0.01 mg/mL and cell path length of 1 mm. Optical rotations were measured on a JASCO J-20A spectrometer at 589-nm wavelength and temperature within 22–24

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Table I. UV and CD Data of Di-*O*-benzoyl-*sn*-glycerols 1–13 in MeOH Solution

compd	R	UV $10^{-3}\epsilon$ (λ_{max}, nm)	CD $10^{-3}[\theta]_{max}$ (λ_{max}, nm)		sign/config[a]
1*	*O*-cetyl	18 (230)	−13.6 (236)	+8.4 (220)	−/2,3(*R*)
2	*O*-Bn	23 (230)	+10.0 (234)	−4.8 (217)	+/1,2(*S*)
		15 (207)			
3	*O*-Me	25 (230)	+3.3 (234)	−1.4 (219)	+/1,2(*S*)
4	*O*-palmi	21 (230)	+1.4 (236)	−2.5 (225)	+/1,2(*S*)
5	*O*-Ac	20 (230)	+3.0 (239)	−4.9 (226)	+/1,2(*S*)
6	D-Glc (OAc)	20 (229)	+21.6 (234)	−5.6 (220)	+/1,2(*S*)
7*	D-Glc (OAc)	23 (229)	−7.5 (237)	+4.8 (222)	−/2,3(*R*)
8	H	21 (229)	+25.2 (235)	−6.9 (222)	+/1,2(*S*)
9	N_3	18 (230)	+10.2 (236)	−9.0 (222)	+/1,2(*S*)
10	Br	19 (230)	+12.2 (234)	−4.4 (220)	+/1,2(*R*)
11	OTs	29 (229)	+7.2 (235)	+2.8 (220)	+/1,2(*R*)
12	OSi ^{t}BuMe$_2$	23 (230)	+10.2 (236)		+/1,2(*R*)
13	NHBz	31 (229)	−10.5 (239)	+11.3 (218)	−/1,2(*S*)

[a] Signs of exciton couplings and the configurational assignments using the *sn* positions of di-*O*-benzoates and *R*,*S* designations in parentheses. Note that *R*,*S* assignments vary with substituents at *sn*-C3 among the compounds 8–13 with a common 1,2-di-*O*-benzoyl skeleton.

°C. The values were calibrated with a 1% sucrose aqueous solution ($[\alpha]^{22}_D$ +66.5° (c = 1)). The solvents used for the UV, CD, and $[\alpha]_D$ measurements cited in Tables I–III were purified by distillation before use. IR spectra were recorded on a JASCO A-102 spectrometer. NMR spectra were measured on a JEOL GSX-400 spectrometer in $CDCl_3$ and CD_3OD with an internal TMS standard (0.0000 ppm) and a digital resolution of 0.24 Hz.

Results and Discussion

Since the usual glycerides do not have a strong UV chromophore, circular dichroism (CD) has been rarely applied to determine their absolute configuration. However, it is expected that introduction of a suitable UV chromophore selectively at *O*-1 or *O*-3 asymmetrical 1- (or 3)-mono-*O*-alkyl- or -acyl-*sn*-glycerides would cause strong Cotton effects reflecting their absolute configurations. In our preceding paper,[11] this approach was applied to determine the absolute configuration of the glycerol moiety in the glycosyl glycerols. Here, we attempt a similar approach using the dibenzoate chirality method[7] to determine the absolute configuration of asymmetric *sn*-glycerols after di-O-benzoylation.

For compounds with a rigid conformation, the dibenzoate chirality method is generally applicable. However, for flexible acyclic compounds,[4,12] its application should be approached cautiously because the helicity of the interacting chromophores might be reversed as a result of conformational changes. This might be true for *sn*-glycerols in the present study. Therefore, conformational analyses of the derived di-O-benzoylated *sn*-glycerols were undertaken together with their CD analyses. Here, we wish to report that the benzoate chirality method can be applied reliably to determine the absolute configurations of asymmetrical *sn*-glycerols, particularly with mono-1- (or 3)-*O*-alkyl- and -acyl-*sn*-glycerols.

CD Spectra of Chiral 1,2- (or 2,3)-Di-*O*-benzoyl-*sn*-glycerols. The CD spectrum of **1** with the 1-*O*-cetyl group was studied as a model for *O*-alkylglycerides, which are widely distributed in nature, e.g., PAF.[2b,c] The spectrum of **1** (Figure 2, top) gave the first band at 236 nm with a negative sign and the second band at 220 nm with a positive one, thus giving a negative exciton couplet CD. This strong and characteristic CD curve is used to determine unambiguously the absolute configurations of the related *O*-alkylglycerols. The CD peaks can be attributed to the interaction between the di-*O*-benzoyl chromophores

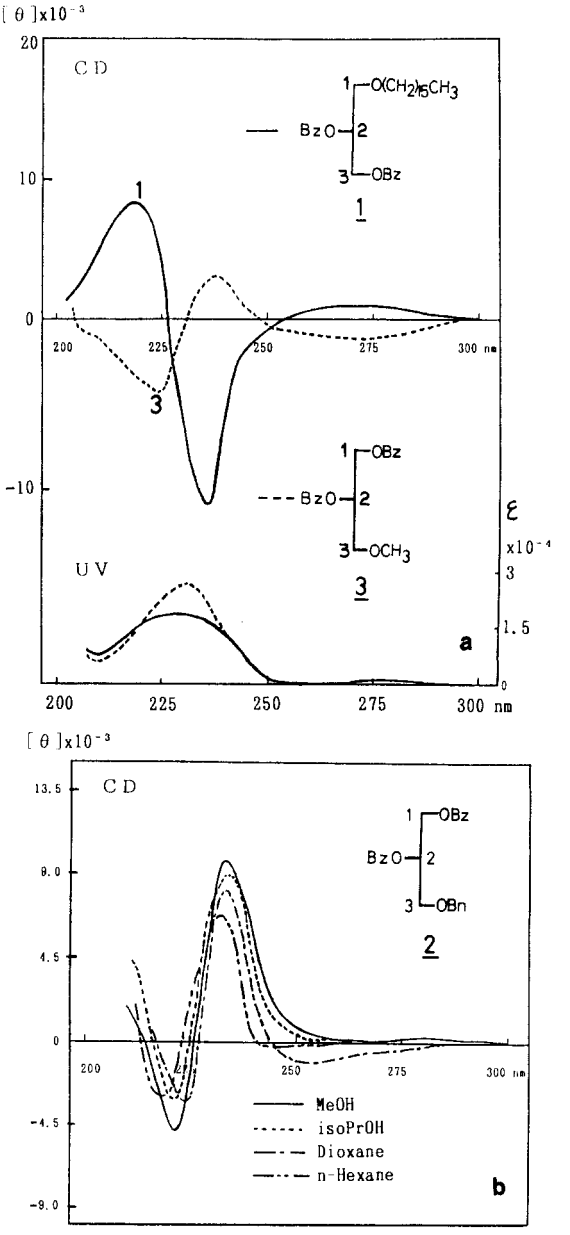

Figure 2. (a) CD and UV spectra of compounds **1** and **3** in methanol. (b) Circular dichroic (CD) spectra of a compound **2** and the solvent effect.

at *sn*-C2 and -C3 by the exciton coupling theory or the dibenzoate chirality rule.[7] The negative sign of the longer wavelength peak indicates that the two chromophores have

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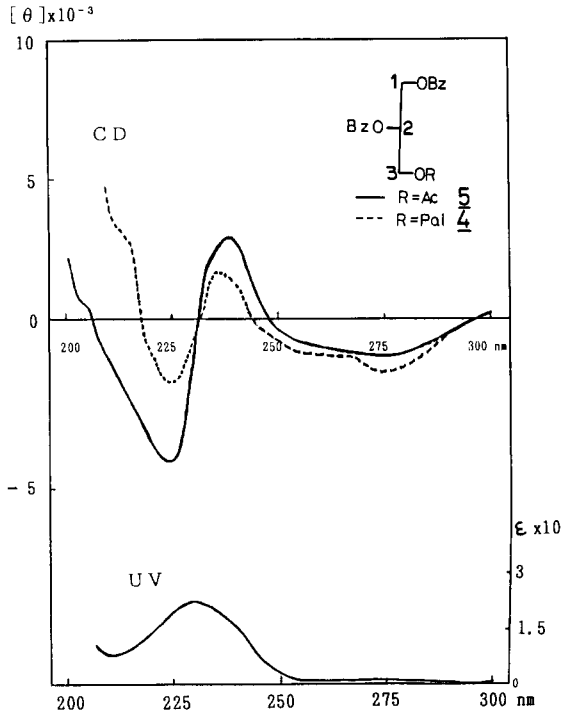


Figure 3. CD and UV spectra of compounds **4** and **5** in methanol.

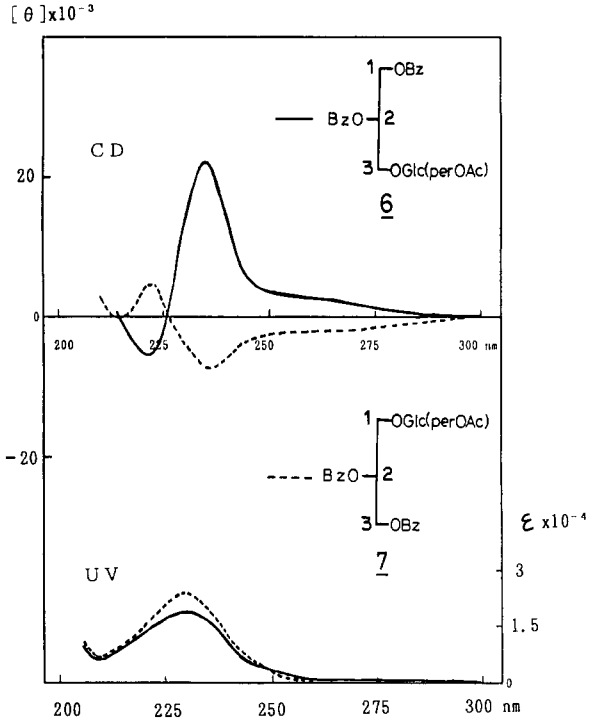


Figure 4. CD and UV spectra of compounds **6** and **7** in methanol.

a counterclockwise helicity in solution. This result is consistent with the conformational analyses in the next section.

The CD spectra of **2** with a 3-*O*-benzyl group were measured in four solvents (methanol, 2-propanol, 1,3-dioxane, and *n*-hexane). It gave positive exciton couplet CD peaks to reflect its 2*S* configuration (Figure 2, bottom) irrespective of the solvent, although the intensity increased with the solvent polarity.

Compound **3** with a 3-*O*-Me group as the smallest *O*-alkyl group also showed positive couplet CD peaks in accordance with compound **2** and the optical isomer of **1** having a 2*S* configuration. These results strongly suggest that the absolute configurations of mono-1- or -3-*O*-alkyl-*sn*-glycerides can be determined by the CD sign regardless of the length of the alkyl group after they are derivatized into chiral di-*O*-benzoates.

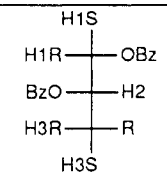
We further studied **4** and **5** with small and long *O*-acyl groups at *sn*-C3, respectively, as models of mono-*O*-acyl-*sn*-glycerides. Their CD sign is also in agreement with the CD sign of *O*-alkyl analogues **1–3** as described above. This result indicates that the absolute configurations of mono-*O*-acyl-*sn*-glycerols can be simply determined by the CD signs of their benzoates.

The CD spectra of the two diastereomeric glycosyl glycerols **6** and **7** with 2*S* and 2*R* configuration, respectively, gave positive and negative couplet CD peaks reflecting their absolute configurations at C2 of the glycerol moiety. Therefore, these results are in agreement with the rule described above for compounds **1–5**. The intensity of the CD curve of **7** was lower than that of **6**, suggesting that the conformational relationship of the two benzoyl chromophores was to some extent different in the two diastereomers. Conformational analyses of **6** and **7** by ^{1}H NMR spectroscopy confirmed this conclusion, as will be shown in Table II in the next section. Although this method is useful for determining the absolute configuration of the glycerol moiety in glycosylglycerols, conversion of glycosylglycerides into **6** or **7** seems to be difficult by the usual chemical reactions. Therefore, an alternative method described in a separate paper[11] which involves per-*O*-benzylations of a glycosyl glycerol followed by acid hydrolysis to give an optically active di-*O*-benzyl *sn*-glycerol seems to be more practical for glycosylglycerides.

The above results can be summarized by observing that 1,2-di-O-benzoylated *sn*-glycerols gave positive exciton CD spectra, while 2,3-di-O-benzoylated *sn*-glycerols gave negative exciton CD spectra reflecting the absolute configurations at C2. To ascertain the generality of this rule, we also measured the CD spectra of other types of 1,2-di-*O*-benzoyl-*sn*-glycerols (**8–13**) with various types of substituents at *sn*-C3 (H, N_3, Br, OTs, $OSi(Me)_2{}^tBu$, and NHBz). The first five compounds (**8–12**) showed positive couplet CD spectra in agreement with this rule, while compound **13** with an *N*-benzoyl chromophore gave CD peaks having the opposite sign. Here, it should be noted that *R,S* assignments of the absolute configuration are interchangable among **8–13** with a common 1,2-di-*O*-benzoyl skeleton since the substituent at *sn*-C3 has priority over that at *sn*-C1 group for **10–12**. Therefore, the above results may be better summarized by using *sn* designations rather than the *R,S* designations as follows; the exciton CD spectra of 1,2-di-O-benzoylated *sn*-glycerols are positive irrespective of the substituent at *sn*-C3 as long as the substituent is free from a strong UV chromophore like an *N*-benzoyl group. Thus, we have found that absolute configurations of chiral glycerols and related acyclic alcohols can be determined unambiguously with the dibenzoate chirality method after they are asymmetrically di-O-benzoylated.

Conformational Analyses by NMR Spectroscopy. The preceding CD study showed that 1,2-di-*O*-benzoyl-*sn*-glycerols consistently gave positive exciton CD spectra. This indicates that the C1–O1 and C2–O2 bonds adopt a positive helicity, namely, *gt* conformation (Figure 5) in solution; there exist three kinds of staggered conformers, namely, *gg*, *gt*, and *tg* about the C1–C2 or C2–C3 axis. Conformer *gt* of the C1–C2 bond should have a positive contribution, while conformer *gg* should have a negative contribution to the exciting coupling. (Here, the contribution of conformer *tg* with the anti-periplanar disposition of the di-*O*-benzoates will be negligible.)

Table II. ^{1}H NMR Spectral Data of 1,2-Di-*O*-benzoyl-*sn*-glycerols 1–12 (400 MHz)



	solv	chem shifts, ppm	assgn	^2J,[a] Hz	^3J,[a] Hz	population,[b] % gg	gt	tg
1*, R = *O*-cetyl[c]	CD_3OD	4.697	H1-*S*	12.1	3.7	37	54	9
		4.588	H1-*R*		6.6			
	$CDCl_3$	4.680	H1-*S*	12.1	3.9	39	49	12
		4.605	H1-*R*		6.2			
2, R = *O*-Bn	CD_3OD	4.735	H1-*S*	12.7	3.9	34	53	13
		4.616	H1-*R*		6.6			
	$CDCl_3$	4.695	H1-*S*	12.1	4.0	38	49	13
		4.633	H1-*R*		6.2			
3, R = *O*-Me	CD_3OD	4.681	H1-*S*	12.1	3.7	35	56	9
		4.562	H1-*R*		6.8			
4, R = *O*-Palmi	CD_3OD	4.700	H1-*S*	11.8	4.2	41	44	15
		4.602	H1-*R*		5.8			
5, R = *O*-Ac	CD_3OD	4.702	H1-*S*	11.9	4.2	38	47	15
		4.602	H1-*R*		6.1			
6, R = D-Glc (OAc)	CD_3OD	4.696	H1-*S*	12.0	3.6	37	55	8
		4.600	H1-*R*		6.6			
	$CDCl_3$	4.646	H1-*S*	12.0	3.9	42	46	12
		4.501	H1-*R*		5.9			
7*, R = L-Glc (OAc)[c]	CD_3OD	4.669	H1-*S*	12.1	3.9	39	50	11
		4.533	H1-*R*		6.2			
	$CDCl_3$	4.642	H1-*S*	12.1	3.9	39	50	11
		4.535	H1-*R*		6.2			
8, R = H	CD_3OD	4.603	H1-*S*	11.8	3.3	35	60	5
		4.493	H1-*R*		7.0			
	$CDCl_3$	4.525	H1-*S*	11.7	4.2	38	47	15
		4.488	H1-*R*		6.1			
9, R = N_3	CD_3OD	4.714	H1-*S*	12.1	4.2	34	51	15
		4.573	H1-*R*		6.5			
	$CDCl_3$	4.654	H1-*S*	12.0	4.6	39	39	22
		4.595	H1-*R*		5.6			
10, R = Br	CD_3OD	4.784	H1-*S*	12.0	3.9	39	48	13
		4.663	H1-*R*		6.1			
	$CDCl_3$	4.720	H1-*S*	11.8	4.6	42	37	21
		4.676	H1-*R*		5.4			
11, R = OTs	CD_3OD	4.629	H1-*S*	11.9	4.5	36	44	20
		4.541	H1-*R*		6.0			
	$CDCl_3$	4.596	H1-*S*	11.9	5.0	37	37	26
		4.437	H1-*R*		5.6			
12, R = OSi^tBuMe_2	CD_3OD	4.705	H1-*S*	11.7	3.7	37	54	9
		4.607	H1-*R*		6.6			

[a] Observed first-order couplings ($J \pm 0.2$ Hz). [b] Calculated from J(H1*S*,H2) and J(H1*R*,H2) values according to our preceding ways.[9b,11] [c] Data of the enantiomer were given to circumvent complexity.

In our preceding papers,[9] we have prepared stereospecifically deuteriated *sn*-glycerols ((1*R*)- and (1*S*)-[1-^2H]-*sn*-glycerols) and used them to determine the conformation of glycerol in aqueous solution and triacylglycerides (acetyl, palmitoyl, and benzoyl) in chloroform solution by high-resolution NMR spectroscopy (400 MHz). These selectively deuteriated *sn*-glycerols enabled us to differentiate the two prochiral protons at C1 and C3 (H1-*pro*-*R* and H1-*pro*-*S* or H3-*pro*-*R* and H3-*pro*-*S*), vicinal couplings of which provide information about the conformation about the C1–C2 or C2–C3 bonds of *sn*-glycerols.[13] Our data[9] have shown that H1-*pro*-*R* and H1-*pro*-*S* signals could be differentiated with the rules of δ(H1-*pro*-*R*) < δ(H1-*pro*-*S*) (ppm) and J(H1-*pro*-*R*,H2) > J(H1-*pro*-*S*,H2) (hertz).

A similar NMR study with selectively deuteriated analogues was carried out with compound **2** to ascertain the applicability of the NMR rule for di-O-benzoylated glycerols. The ^{1}H NMR spectra shown in Figure 6 indicate that the proton at higher field and with larger vicinal coupling constant is assignable to the H1-*pro*-*R* since the missing signal in the 1*S* deuteriated analogue of a compound **2** is assignable to H1-*pro*-*S*. This result completely agrees with the NMR rule described above.

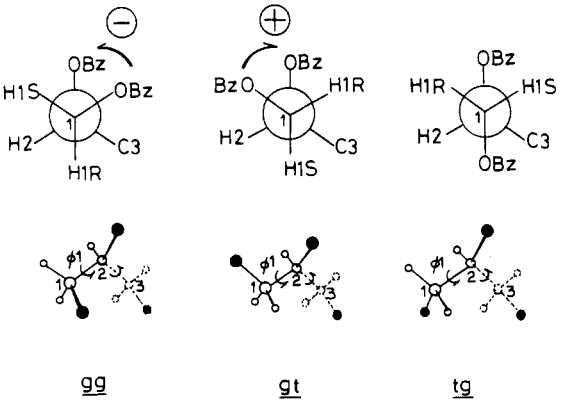


Figure 5. Three possible conformers, *gg*, *gt* and *tg*, about the C1–C2 bond of a 1,2-di-*O*-benzoyl-*sn*-glycerol.

Calculations of the time-averaged populations of the three rotamers for 1–12 were carried out on the basis of

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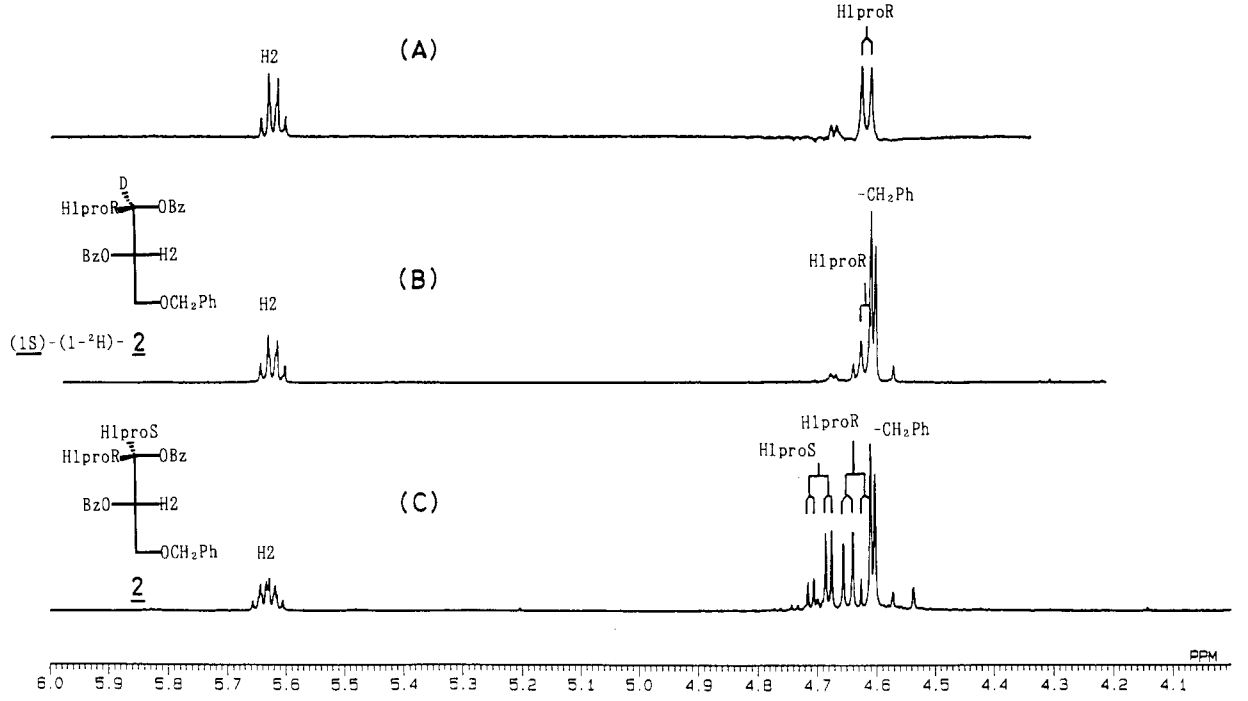

Figure 6. The 400-MHz ^1H NMR spectra of a compound **2** (spectrum C) and its 1*S* deuterated analogue (spectra A and B) in $CDCl_3$ solution. Inversion recovery method with a pulse sequence of [180°–*t*(=900 ms)–90°] was used for the partially relaxed spectrum (A) to separate the methine proton signals from the methylene proton signals in the spectrum B.

these assignments of the two prochiral protons at C1 or C3 and also on the Karplus type equation proposed by Haasnoot et al.,[14] which is widely used to determine the conformations of carbohydrate molecules[15] (Table II). The results show that all compounds studied here exist predominantly in a *gt* conformer, although its proportion varies somewhat. The preference for the *gt* conformer was stronger in the polar solvent methanol than in the less polar medium chloroform. This trend corresponds well with the solvent effects on the CD spectra of the compound **2** (Figure 2b) as described above. Moreover, the compounds with larger CD peaks in methanol tended to show higher *gt* population and/or lower *gg* population in the NMR analyses in the same solvent (compared between **1** and **3**, **4** and **5**, and **6** and **7** or among **8**–**12**). Thus, these NMR analyses and the previous CD spectra show good agreement. The conformational preference of *gt* > *gg* ≫ *tg* among **1**–**12** also agrees with the conformations of glycerol in water and tri-*O*-acylglycerides in chloroform solution in our previous paper[9] and seems to be a common feature of glycerolipids. The *gg* and *gt* predominance over *tg* could be partly rationalized by the "gauche effect", which stabilizes gauche conformers of vicinal diols or halogens particularly in polar solvents.[16] The weak but consistent preference for the *gt* conformer over the *gg* alternative in polar solvents may be ascribed to the higher steric hindrance in the latter.

Conclusion

The consistent CD and NMR spectral data in the present study provide the basis for a new method to determine the absolute configurations of chiral glycerols by the sign of their CD spectra: 1,2- or 2,3-di-O-benzoylated *sn*-glycerols existing predominantly in the *gt* conformation give positive and negative exciton coupling CD peaks, respectively. These results are in agreement with the results of acyclic di-*N*-benzoylamino acids reported by Kawai et al.[17] and chiral glycols reported by Yamamoto et al.[4b] and Harada et al.[4c] and confirm that the dibenzoate chirality method is applicable to asymmetric diols with a glycerol skeleton.

Absolute configurations of mono-*O*-alkylglycerides like PAF and mono-*O*-acylglycerides may be simply and sensitively determined by this method after they are converted into the corresponding di-O-benzoylated derivatives (entries **1** and **4**, respectively). For 1,2- or 2,3-di-*O*-acyl-*sn*-glycerides, the CD spectrum of a monosilyl compound (entry **12**) will be used since they may be converted into **12** by the following reactions: selective 1-O- or 3-O-silylation[9b] with *tert*-butyldimethylsilyl chloride and triethylamine in dimethylformamide, deacylations with sodium methoxide in methanol, and benzoylation in the previously described manner. This method enables us to determine the absolute configurations of chiral natural glycerolipids without comparison to compounds of known absolute stereochemistry since the sign of the observed exciton depends only on the relative disposition of the two benzoyl groups.

About 0.01 μmol of dibenzoates is required, at least for the CD measurements, but this method is more than 100 times as sensitive as the $[M]_D$ measurements. Applications of this method to the study of the absolute stereochemistry of the products from lipase action under various conditions are in progress, and the results will be reported elsewhere.

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Supplementary Material Available: Optical rotations, physical states, melting points, and IR and ^{1}H NMR spectral data for compounds 1-13 (4 pages). Ordering information is given on any current masthead page.

Conformation of the Phosphate-Methylated DNA Dinucleotides d(CpC) and d(TpC). Formation of a Parallel Miniduplex Exclusively for the *S* Configuration at Phosphorus

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It has been shown that the phosphate-methylated DNA dinucleotides d(CpC) (1) and d(TpC) (2) form a parallel miniduplex exclusively for the *S* configuration on phosphorus, which corresponds with outward orientation of the methyl group from the helix into the solvent. The melting temperatures (T_m values) of the parallel duplexes of (S_P)-1 and (S_P)-2 are 33 °C and 26 °C, respectively. The phosphate-sugar backbone strands adopt a standard right-handed geometry. The imino resonances in the 600-MHz ^{1}H NMR spectra strongly point at C-C base pairing via two symmetry-related NH_2···N hydrogen bonds, as was also observed in the X-ray crystal structure of 2'-deoxycytidine. From our previous work, it is known that phosphate-methylated d(TpT) forms parallel miniduplexes with an equal stability for the S_P and R_P diastereoisomers. Therefore, it is concluded that the R_P configuration (which is associated with location of the methyl group inside the helix groove) is unfavorable in the case of C-C base pairing. This conclusion was corroborated by AMBER molecular mechanics calculations, which showed that the larger propeller twist angle for C-C base pairing (≈41°), in comparison with T-T base pairing (≈25°), results in narrowing of the helix groove.

Introduction

Methylation of the phosphate groups in d(T_n) DNA oligomers (n = 2-8) results in the formation of a double helix with T-T base pairs and *parallel* orientation of the backbone strands.[1] In our previous work, we have focussed on the stability and molecular structure of these parallel duplexes.[1,2] In studying the stability, it was found that the T_m values rise linearly with increasing duplex length. This is explained by the lack of electrostatic phosphate-phosphate repulsion, i.e., elongation of the backbone strands leads to an increased stability since hydrogen bonding and base stacking are increased without the introduction of repulsive forces.[3] Our structural model was based on experimental data (i.e., X-ray crystal structure of 3',5'-di-*O*-acetylthymidine,[1,4] detailed ^{1}H NMR investigations of phosphate-methylated d(TpT)$_2$ in stereochemically pure form, imino proton chemical shifts for longer systems), as well as on AMBER molecular mechanics calculations.[2] This led to a symmetrical parallel duplex structure with a relatively small helix diameter (16 Å vs 21 Å for Watson-Crick B DNA), right-handed backbone strands, and 8 base pairs per full turn. Molecular models showed that the parallel duplex can easily accommodate the methyl groups, both for the S_P configuration (methyl pointing away from the duplex) and the R_P configuration (methyl located inside the helix groove).[2] Thus, the different configurations of the methylated phosphate groups in the backbone have no impact on the stability of the duplex. This readily explains why well-defined sharp melting transitions were observed for all parallel T-T duplexes.[3]

We have subsequently addressed the question of whether parallel duplex formation is restricted to T-T base pairing. The X-ray crystal structures of 2'-deoxy-3',5'-di-*O*-acetyladenosine[5a] and 2'-deoxy-3',5'-di-*O*-acetylguanosine[5b] showed highly complex hydrogen-bonded networks, from which we concluded that parallel A-A or G-G base pairing is rather unlikely. Thus, we focussed on dC structures, for which it was known from literature that parallel C-C base pairing can occur. For example, the X-ray crystal structure of 2'-deoxycytosine shows parallel C-C base pairing in which the two C bases are linked via two identical NH_2···N hydrogen bonds.[6] These data prompted us to synthesize the phosphate-methylated dinucleotides d(CpC) (1) and d(TpC) (2) in stereochemically pure form and to investigate possible duplex formation in these structures.[7] In this paper we describe a conformational study of the R_P and S_P diastereoisomers of 1 and 2, on the basis of variable-temperature high-field NMR and

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