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Syntheses of All Four Stereoisomers Which Are Conformationally Constrained 1,4-Dioxanyl Analogs of the Antineoplastic Ether Lipid ET-18-OCH$_3$

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The syntheses of each of the four nearly optically pure stereoisomers of [[(5-heptadecyl-1,4-dioxan-2-yl)-methyl]oxy]phosphocholine (**2**,**3**,**2′**,**3′**) were performed by two parallel divergent sequences. Phosphocholines **2** and **3** were prepared via the corresponding 5-heptadecyl-2-(hydroxymethyl)-1,4-dioxanes **21** and **23**, respectively, from the completely regiospecific mixed-hydride reductions of (1*R*,4*S*,5*S*)-4-heptadecyl-3,6,8-trioxabicyclo[3.2.1]octane (**19**) and (1*R*,4*R*,5*S*)-4-heptadecyl-3,6,8-trioxabicyclo[3.2.1]octane (**20**), respectively. The two 4-heptadecyl-3,6,8-trioxabicyclo[3.2.1]octanes **19** and **20** were the two separable products from an intramolecular cyclization reaction. By a parallel divergent sequence from the enantiomeric starting material, 3-*O*-benzyl-*sn*-glycerol (**16′**), the other two diastereomeric [[(5-heptadecyl-1,4-dioxan-2-yl)methyl]oxy]phosphocholines **2′** and **3′** were prepared. These four monocyclic [[(5-heptadecyl-1,4-dioxan-2-yl)methyl]oxy]phosphocholines (**2**,**3**,**2′**,**3′**) are conformationally constrained analogs of the antineoplastic and immunomodulatory ether lipid *rac*-2-*O*-methyl-1-*O*-octadecylglycero-3-phosphocholine (*rac*-**1**) (*rac*-ET-18-OCH$_3$, *rac*-Edelfosine).

Ether lipids (alkyl lysophospholipids) have received considerable attention due to their antineoplastic and immunomodulatory activities, which have been the subjects of several recent reviews.[1] *rac*-2-*O*-Methyl-1-*O*-octadecylglycero-3-phosphocholine (*rac*-**1**) (*rac*-ET-18-OCH$_3$, *rac*-Edelfosine)[2,3,4] is the most studied of the ether lipids. The unique mechanism(s) of action of *rac*-ET-18-OCH$_3$ (*rac*-**1**) and related ether lipids are not completely understood. *rac*-ET-18-OCH$_3$ (*rac*-**1**) is known to accumulate selectively in neoplastic cells,[5] where it has cytostatic and cytotoxic effects which are highly selective for the neoplastic cells.[5b,6] *rac*-ET-18-OCH$_3$ (*rac*-**1**) has antimetastic[7] and antiinvasive properties[8] and also induces differentiation of mouse and human neoplastic cells to macrophages and granulocytes.[9] *rac*-ET-18-OCH$_3$ (*rac*-**1**) is known to activate tumoricidal macrophages[10] and to disrupt cellular phospholipid metabolism[6b,11] and phosphocholine synthesis.[11,12] In addition to the inhibition of lysophosphocholine-acyltransferase (LPC-acyltransferase),[11,12] a number of other membrane-linked enzymes are inhibited by *rac*-ET-18-OCH$_3$ (*rac*-**1**), including phospholipid-sensitive calcium-dependent protein kinase (PKC),[13,14] (sodium plus potassium)-activated adenosine triphosphatase (Na,K-ATPase),[14] phosphoinositol-specific phospholipase C (PI-PLC),[15] and the glucose transport protein.[16] Recently, *rac*-ET-18-OCH$_3$ (*rac*-**1**) was found to have activity against human immunodeficiency virus (HIV) replication.[17] The unique molecular mechanism(s) of action of *rac*-ET-18-OCH$_3$ (*rac*-**1**) and related ether lipids seems to originate from the plasma membrane[5c,18] of the macrophages or neoplastic cells. *rac*-ET-18-OCH$_3$ (*rac*-**1**) was first synthesized as a racemic mixture.[3,4] Recently, both the 2-*O*-methyl-1-*O*-octadecyl-*sn*-glycero-3-phosphocholine ((*R*)-ET-18-OCH$_3$, (*R*)-**1**)[5b,10b,16,19,20] and the 2-*O*-methyl-3-*O*-octadecyl-*sn*-glycero-1-phosphocholine ((*S*)-ET-18-OCH$_3$, (*S*)-**1**)[10b,16,20] enantiomers became available for biological studies. The optically active hexadecyl analogs ((*R*)-ET-16-OCH$_3$ and (*S*)-ET-16-OCH$_3$) were also recently synthesized.[21]

We have been interested in the molecular mechanism(s) of action of ether lipids within plasma membranes, such

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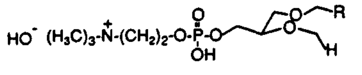
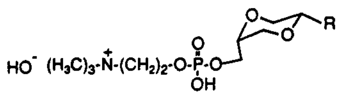
as what structural and stereochemical characteristics result in accumulation preferentially in neoplastic cells, what biophysical disturbances are caused in the cell membrane, and what is important for cell-surface recognition, metabolism, or other specific effects which result in cytotoxicity or immunomodulation. Our interest in conformationally restricted ether lipids was stimulated by previous reports of methyl analogs[22] and monocyclic analogs[23] of various ether lipids.



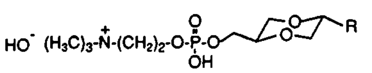
(*R*)-1 -R = -$C_{17}H_{35}$

((*R*)-ET-18-OCH$_3$)

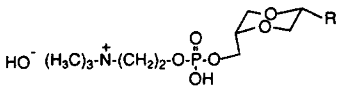


(2*R*,5*S*)-2 -R = -$C_{17}H_{35}$

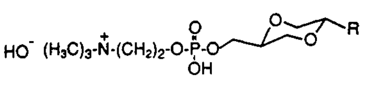
(1)



(2*R*,5*R*)-3 -R = -$C_{17}H_{35}$



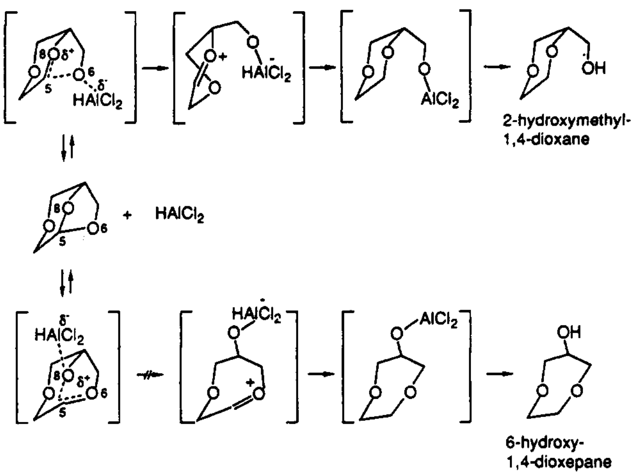
(2*S*,5*R*)-2′ -R = -$C_{17}H_{35}$



(2*S*,5*S*)-3′ -R = -$C_{17}H_{35}$

In this paper we will describe the synthesis of a series of four nearly optically pure, conformationally well-defined diastereomers which are monocyclic analogs of ET-18-OCH$_3$ (**1**). These phosphocholines **2**,**3**,**2′**,**3′** were envisioned by simply connecting the α-methylene carbon of the 1-*O*-octadecyl group to the 2-*O*-methyl carbon of ET-18-OCH$_3$ (**1**) to give cyclized, conformationally restricted 1,4-dioxanyl analogs of ET-18-OCH$_3$ (**1**). These monocyclic ether lipids **2**,**3**,**2′**,**3′** each have two chiral centers. This paper will describe the two chirospecific parallel divergent synthetic sequences which were carried out to give all four distinctly conformationally constrained monocyclic analogs of the ether lipid ET-18-OCH$_3$ (**1**). We are currently probing the molecular mechanism(s) of action of ether lipids with these four diastereomeric phosphocholines to evaluate the stereochemical and conformational requirements for selective neoplastic cytotoxicity, immunomodulation, and enzyme (PLA$_2$, PLC, LPC-acyltransferase, and PKC) inhibition.

Scheme I



Results and Discussion

Retrosynthetically, the four diastereomeric [[(5-heptadecyl-1,4-dioxan-2-yl)methyl]oxy]phosphocholines (**2**,**3**,**2′**,**3′**) were envisioned coming from the corresponding alcohols **21**, **23**, **21′**, and **23′**, respectively, via the regiospecific reductions of the C5 to O6 bonds of the 4-heptadecyl-3,6,8-trioxabicyclo[3.2.1]octane intermediates **19**, **20**, **19′**, and **20′**, respectively. While there are a variety of synthetic approaches to the required 4-heptadecyl-3,6,8-trioxabicyclo[3.2.1]octanes, we chose a straightforward intramolecular Williamson ether cyclization method in which what becomes the O3 to C4 bond was formed in the critical cyclization step for the synthesis of each of the 4-heptadecyl-3,6,8-trioxabicyclo[3.2.1]octanes. We will first discuss the regiospecific ring opening of the parent compound (±)-**7** which was used as a model. The determinations of the optical purities of the starting materials will be discussed, and then the two parallel divergent syntheses will be detailed.

Regiospecific Ring Opening of (±)-3,6,8-Trioxabicyclo[3.2.1]octane ((±)-7). We first prepared (±)-3,6,8-trioxabicyclo[3.2.1]octane ((±)-**7**), the parent bicyclic compound for our key synthetic intermediates, under conditions similar to the previously reported methods for the synthesis of this bicyclic acetal (±)-**7**.[24] The lithium aluminum hydride–aluminum chloride reduction of (±)-3,6,8-trioxabicyclo[3.2.1]octane ((±)-**7** had previously been

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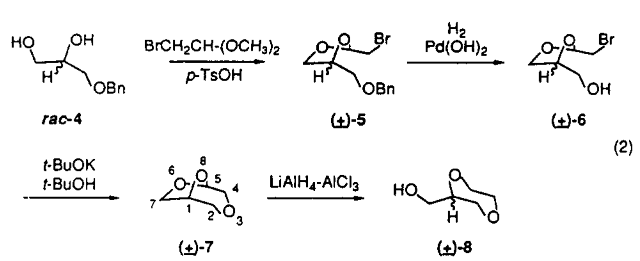
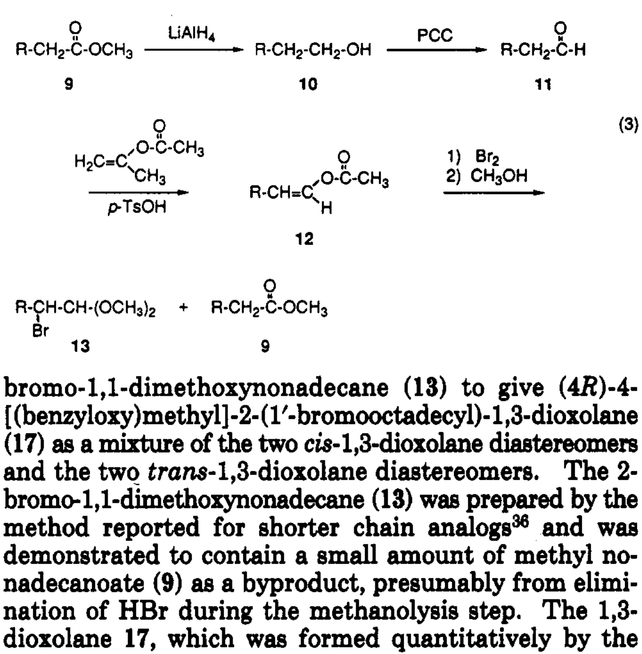


reported to give only (±)-2-(hydroxymethyl)-1,4-dioxane ((±)-8) resulting from reductive cleavage of the C5 to O6 bond of the bicyclic acetal and none of the 1,4-dioxepane which would have resulted from reduction of the C5 to O8 bond (see Scheme I).[25] The mixed anhydride reductions of (±)-3,6,8-trioxabicyclo[3.2.1]octane ((±)-7) and its derivatives[25] and the related bicyclic acetal (±)-6,8-dioxabicyclo[3.2.1]octane[26] and its derivatives[26b,27] in which the 1,3-dioxolane ring has been tied back into a bicyclic structure always gave the corresponding (±)-2-(hydroxymethyl)-1,4-dioxanes and (±)-2-(hydroxymethyl)tetrahydropyrans, respectively, as the exclusive products. The explanation for this absolute regiospecificity, which is not determined by steric or electronic effects of substituents, must involve the clear preference for the lower energy oxonium intermediate leading to the cleavage of the 5-membered ring of the bicyclic structure[25] and from the relief of ring strain.[28] We confirmed that the mixed hydride reduction of (±)-3,6,8-trioxabicyclo[3.2.1]octane ((±)-7) proceeded regiospecifically to give (±)-2-(hydroxymethyl)-1,4-dioxane ((±)-8) in 66% yield. The complete regiochemical control of the reduction of 3,6,8-trioxabicyclo[3.2.1]octanes was then applied to the mixed hydride reductions of 4-heptadecyl analogs and gave us the desired 5-heptadecyl-2-(hydroxymethyl)-1,4-dioxane derivatives as the exclusive reaction products.

Determinations of Optical Purities. The phosphocholines 2 and 3 were synthesized by a divergent synthetic sequence starting from 2,3-*O*-isopropylidene-*sn*-glycerol (14), and the phosphocholines 2′ and 3′ were synthesized by the parallel divergent synthetic sequence starting from 1,2-*O*-isopropylidene-*sn*-glycerol (14′). The 2,3-*O*-isopropylidene-*sn*-glycerol (14) and the 1,2-*O*-isopropylidene-*sn*-glycerol (14′) were each demonstrated to be nearly enantiomerically pure by the quantitative NMR method that we had previously reported.[29] Briefly, the 2,3-*O*-isopropylidene-*sn*-glycerol (14) was converted to 1-*O*-octadecyl-2,3-*O*-isopropylidene-*sn*-glycerol, transacetalated to 2,3-*O*-benzylidene-1-*O*-octadecyl-*sn*-glycerol, and then reduced with lithium aluminum hydride–aluminum chloride. The 3-*O*-benzyl-1-*O*-octadecyl-*sn*-glycerol, the major product of the regioselective reduction, was converted to its Mosher's ester derivative.[30] We unambiguously determined the optical purity of this Mosher's ester to be 99.0 ± 0.5% ee from the integrations of the two AB quartets for the benzyl protons in the ^{1}H NMR spectra. The corresponding Mosher's ester of 1-*O*-benzyl-3-*O*-octadecyl-*sn*-glycerol, which was prepared from 1,2-*O*-isopropylidene-*sn*-glycerol (14′), was determined to be 99.5 ± 0.5% ee. The appropriate doping experiments permitted us to quantitatively determine optical purities as percent enantiomeric excesses. The optical purities of 2,3-*O*-isopropylidene-*sn*-glycerol (14) and 1,2-*O*-isopropylidene-*sn*-glycerol (14′) can unambiguously be determined by a capillary GC method for the separation of their diastereomeric Mosher's ester derivatives[31] or by the HPLC separation of their benzoate ester derivatives on a chiral stationary phase.[32] We felt that it was better to determine the optical purities of 3-*O*-benzyl-*sn*-glycerol derivatives or 1-*O*-benzyl-*sn*-glycerol derivatives which must be completely stable to racemization. Alternatively, the 1-*O*-benzyl-*sn*-glycerol (16)[33] and 3-*O*-benzyl-*sn*-glycerol (16′)[34] could be converted to their corresponding stearylaldehyde acetals and reduced[35] to give 1-*O*-benzyl-3-*O*-octadecyl-*sn*-glycerol and 3-*O*-benzyl-1-*O*-octadecyl-*sn*-glycerol, respectively, the precursors to the Mosher's ester derivatives which we used to determine optical purities. The determinations of the optical purities of 1-*O*-benzyl-*sn*-glycerol (16) and 3-*O*-benzyl-*sn*-glycerol (16′) allowed the assignments of optical purities for all subsequent intermediates, and ultimately phosphocholines 2, 3, 2′, and 3′.

The Divergent Synthesis of Phosphocholines (2*R*,5*S*)-2 and (2*R*,5*R*)-3. The synthesis of phosphocholines 2 and 3 began with the conversion of 2,3-*O*-isopropylidene-*sn*-glycerol (14) via 1-*O*-benzyl-2,3-*O*-isopropylidene-*sn*-glycerol (15) to 1-*O*-benzyl-*sn*-glycerol (16).[33a] The diol 16 was then transacetalated with 2-bromo-1,1-dimethoxynonadecane (13) to give (4*R*)-4-[(benzyloxy)methyl]-2-(1′-bromooctadecyl)-1,3-dioxolane (17) as a mixture of the two *cis*-1,3-dioxolane diastereomers and the two *trans*-1,3-dioxolane diastereomers. The 2-bromo-1,1-dimethoxynonadecane (13) was prepared by the method reported for shorter chain analogs[36] and was demonstrated to contain a small amount of methyl nonadecanoate (9) as a byproduct, presumably from elimination of HBr during the methanolysis step. The 1,3-dioxolane 17, which was formed quantitatively by the transacetalation reaction under thermodynamic control, was determined to be 3:1 ratio of the cis to trans isomers by ^{1}H NMR. A 1.4:1 cis to trans ratio was reported for 4-[(benzyloxy)methyl]-2-(bromomethyl)-1,3-dioxolanes ((±)-5),[24b] the analogous synthetic intermediate in the synthesis of the parent 3,6,8-trioxabicyclo[3.2.1]octane



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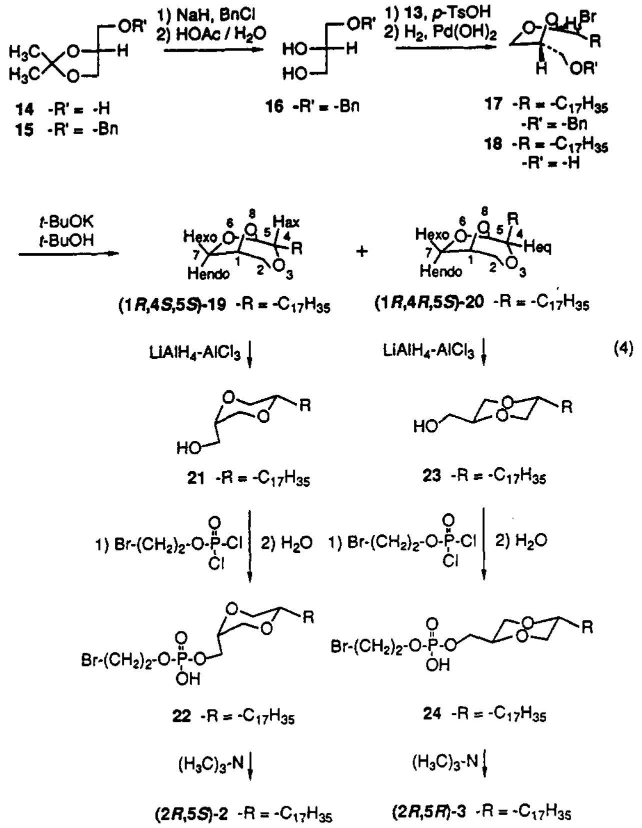

(±)-7), and we had observed a 1.1:1 cis to trans ratio for 1,3-dioxolane (±)-5 under our reaction conditions. Our ^{1}H NMR assignments were made by analogy with earlier ^{1}H NMR studies of some related *rac*-1,2-*O*-alkylideneglycerols.[24b,37] The two acetal proton doublets of the trans-diastereomers of 1,3-dioxolane **17** were downfield from the two acetal proton doublets of the cis-diastereomers of 1,3-dioxolane **17**. A high cis to trans ratio was advantageous, since only the cis isomer of **17** would be able to undergo the desired intramolecular cyclization reaction.

The precursor for the intramolecular cyclization was prepared by hydrogenolysis of **17** to give a mixture of the four diastereomeric (4*R*)-1,3-dioxolanes **18**. The cyclization reaction was performed with potassium *tert*-butoxide under conditions which have been discussed earlier for the synthesis of the racemic parent compound (±)-**7** and gave a 1:4 mixture of the high-R_f 4-heptadecyl-3,6,8-trioxabicyclo[3.2.1]octane (**19**) to the low-R_f 4-heptadecyl-3,6,8-trioxabicyclo[3.2.1]octane (**20**), based on the integration of the C5 acetal protons of the two diastereomers at δ 5.11 and δ 5.18, respectively. The two diasteriomeric bicyclic acetals **19** and **20** were isolated chromatographically in 5.7% yield and 25% yield, respectively.

The trans-diastereomers of **18**, as well as the cis-diastereomers of **18**, were actually each 1:1 mixtures of the (1'*R*)-1'-bromooctadecyl diastereomer and the (1'*S*)-1'-bromooctadecyl diastereomer since the C1' centers were racemic. The two *trans*-**18** diastereomers were unable to undergo intramolecular cyclization and almost exclusively underwent HBr elimination. The two *cis*-**18** diastereomers underwent cyclizations to different extents. While (2*S*,4*R*,1'*R*)-2-(1'-bromooctadecyl)-4-(hydroxymethyl)-1,3-dioxolane ((2*S*,4*R*,1'*R*)-**18**) was inefficiently cyclized to give the (1*R*,4*S*,5*S*)-4-heptadecyl-3,6,8-trioxabicyclo[3.2.1]octane (**19**) with the endo or equatorial 4-heptadecyl group due to the competing mechanism of HBr elimination, (2*S*,4*R*,1'*S*)-2-(1'-bromooctadecyl)-4-(hydroxymethyl)-1,3-dioxolane ((2*S*,4*R*,1'*S*)-**18**) underwent the intramolecular nucleophilic substitution reaction to the extent of about 50% to give the cyclization product (1*R*,4*R*,5*S*)-4-heptadecyl-3,6,8-trioxabicyclo[3.2.1]octane (**20**) with the exo or axial 4-heptadecyl group. Clearly the required intramolecular S_N2 transition state conformation of (2*S*,4*R*,1'*S*)-**18**, with the bromide in position for leaving during backside attack of the alkoxide oxygen, has less steric interaction between the heptadecyl group and the rest of the molecule relative to the corresponding conformation of (2*S*,4*R*,1'*R*)-**18** with its crowded transition state during the course of this kinetically controlled reaction mechanism. Epimerization at C4 of the 4-heptadecyl-3,6,8-trioxabicyclo[3.2.1]octane cyclization products was not possible under the basic reaction conditions nor during the isolations.

The structures of (1*R*,4*S*,5*S*)-4-heptadecyl-3,6,8-trioxabicyclo[3.2.1]octane (**19**), which has the equatorial 4-heptadecyl group, and (1*R*,4*R*,5*S*)-4-heptadecyl-3,6,8-trioxabicyclo[3.2.1]octane (**20**), which has the axial 4-heptadecyl group, were suggested by ^{1}H NMR spectroscopy. The assignments of the proton resonances for both compounds were first made based on the assignments of related 3,6,8-trioxabicyclo[3.2.1]octanes[38] and were confirmed with magnitude-mode 2D ^{1}H COSY spectroscopy. We then ran phase-sensitive 2D ^{1}H NOESY spectra of the high R_f **19** and the low R_f **20**. We were particularly interested in the NOE crosspeaks between H4 (H4ax for **19** and H4eq for **20**) and both H2ax and H2eq. However, for the low R_f diastereomer **20**, the H4eq and the H2eq resonances overlapped completely and the NOE crosspeak with H2ax appeared to be only from NOE contact of the two geminal H2 protons, H2ax and H2eq. For the high R_f diastereomer **19**, the resonances of H2ax, H2eq, and H4ax were clearly resolved, and a strong NOE contact between H4ax and H2ax was observed relative to little or no NOE contact between H4ax and H2eq, suggesting that H4 was axial and that C4 had the *S* absolute configuration with the 4-heptadecyl group equatorial. An X-ray crystal structure of at least one of the diastereomeric 4-heptadecyl derivatives of 3,6,8-trioxabicyclo[3.2.1]octane was necessary to assign the structures definitively. The single-crystal X-ray structures of both **19** and **20** were performed and are being published separately.[39]

Having both diastereomeric bicyclic acetals represented a useful divergence in this synthesis since **19** was then converted to phosphocholine **2**, while **20** was converted to phosphocholine **3**. The regiospecific reduction of **19** with lithium aluminum hydride–aluminum chloride under the conditions used for the parent compound (±)-**7** gave the 2-(hydroxymethyl)-1,4-dioxane derivative **21**. Conversion of **21** via the corresponding β-bromoethyl phosphodiester **22** to phosphocholine **2** was performed under conditions reported for other phosphocholines.[40] Similarly, phosphocholine **3** was prepared from **20**. Phosphocholines **2** and **3** were prepared in overall yields of 2.4% and 9.8%, respectively, by this divergent synthesis starting from 1-*O*-benzyl-*sn*-glycerol (**16**). The low intramolecular cyclization yields might be improved by chromatographic separation of the *cis*-1,3-dioxolanes *cis*-**17** from the

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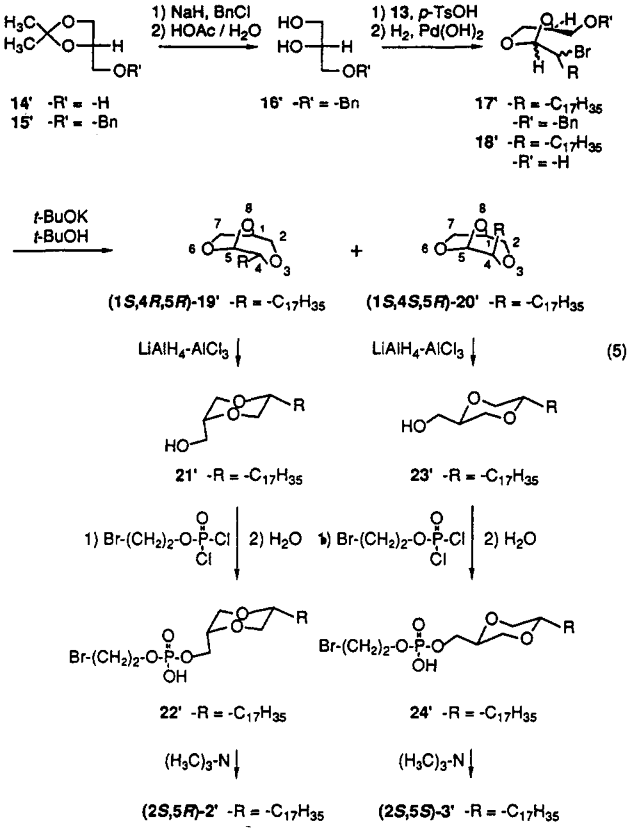
trans-1,3-dioxolanes *trans*-**17**, which could be reequilibrated and recycled. Both phosphocholines **2** and **3** were 99.0 ± 0.5% ee. The diastereomeric phosphocholines **2** and **3** have conformationally restricted structures due to their cyclic structures and due to the heptadecyl and the (methyloxy)phosphocholine groups. The monocyclic phosphocholines **2** and **3** are relatively constrained analogs of two possible conformations of 2-*O*-methyl-1-*O*-octadecyl-*sn*-glycero-3-phosphocholine ((*R*)-**1**). For example, ether lipid **3** is locked into a chair conformation with both the 2-(methyloxy)phosphocholine and the 5-heptadecyl groups equatorial.

The Divergent Synthesis of Phosphocholines (2*S*,5*R*)-2′ and (2*S*,5*S*)-3′. The synthesis of phosphocholines **2′** and **3′** began with transacetalation of 3-*O*-benzyl-*sn*-glycerol (**16′**)[34] with 2-bromo-1,1-dimethoxynonadecane (**13**) to give (4*S*)-4-[(benzyloxy)methyl]-2-(1′-bromooctadecyl)-1,3-dioxolane (**17′**) as a 2.4:1 mixture of the two *cis*-1,3-dioxolane diastereomers to the two *trans*-1,3-dioxolane diastereomers by ^{1}H NMR. Hydrogenolyis of **17′** gave the corresponding mixture of the four diastereomeric (4*S*)-1,3-dioxolanes **18′**, having both the bromo and alcohol functional groups. The intramolecular cyclization was performed under conditions which have been discussed earlier for the synthesis of the racemic parent compound (±)-**7** as well as for the synthesis of **19** and **20**. The cyclization gave a 1:5 mixture of the high-R_f 4-heptadecyl-3,6,8-trioxabicyclo[3.2.1]octane (**19′**) to the low-R_f 4-heptadecyl-3,6,8-trioxabicyclo[3.2.1]octane (**20′**) based on the integration of the C5 acetal protons, and the two diastereomers were isolated in 5.5% yield and 28% yield, respectively. Again, the two separable diastereomeric cyclization products **19′** and **20′** represented a useful divergence in this parallel synthesis since **19′** was then converted to phosphocholine **2′** while **20′** was converted to phosphocholine **3′** exactly as described for the synthesis of their enantiomers **2** and **3**, respectively. Phosphocholines **2′** and **3′** were prepared in overall yields of 1.7% and 11%, respectively, by this divergent synthesis starting from 3-*O*-benzyl-*sn*-glycerol (**16′**). Again, the low intramolecular cyclization yields might be improved by chromatographic separation of the *cis*-1,3-dioxolanes *cis*-**17′** from the *trans*-1,3-dioxolanes *trans*-**17′**, which could be reequilibrated and recycled. Both phosphocholines **2′** and **3′** were 99.5 ± 0.5% ee. The diastereomeric phosphocholines **2′** and **3′** have conformationally restricted structures and are relatively constrained analogs of two possible conformations of the ether lipid 2-*O*-methyl-3-*O*-octadecyl-*sn*-glycero-1-phosphocholine ((*S*)-**1**).



(5)

Experimental Section

General Methods. Petroleum hydrocarbons (pet) used were bp 40–60 °C range. The *p*-TsOH was crystallized from PhH prior to use. The CCl_4 (from CaH_2), *t*-BuOH (from 5 mol % Na), and Et_2O (from benzophenone ketyl) were distilled prior to use. Reactions were carried out under a N_2 atmosphere with magnetic stirring at ambient temperature. Reaction temperatures were reported as bath temperatures (bT). Organic phases were dried over Na_2SO_4 and evaporated under reduced pressure. Silica gel (grade 60, 230–400 mesh, E. Merck) was used for column chromatography. All compounds were demonstrated to be homogeneous by analytical TLC on precoated silica gel TLC plates (grade 60, F254, E. Merck), and chromatograms were visualized by iodine staining. Phosphorus-containing products were also checked by staining duplicate chromatograms with molybdic acid reagent.[41] A μ-Porasil column (10 μm, 3.9-mm i.d. × 30 cm), a flow rate of 1.0 mL min^{-1}, and a refractive index detector were used for HPLC (t_R was typically 3 min 20 s). Melting points of all solids were determined in open Pyrex capillaries. All ^{1}H NMR spectra of all compounds were recorded in $CDCl_3$ at 200 MHz with TMS as an internal reference. Exchangeable resonances, unless noted, are not reported. In addition, the spectra of the phosphocholines were run in $CDCl_3/CD_3OD$ 2:1 (or 1:1) v/v, and the $CHCl_3$ resonance was then observed at δ 7.51. Magnitude-mode COSY and phase-sensitive NOESY were performed with Bruker software, and NOESY had time-proportional phase increments (TPPI) modified to include a variable delay (e.g., 800, 804, 817, and 811 ms for an 800-ms mixing time) to minimize correlation cross peaks. All reported solvent ratios are by volume. Specific rotations were determined using a 1.00-dm cell.

(±)-4-[(Benzyloxy)methyl]-2-(bromomethyl)-1,3-dioxolane ((±)-5). A stirred mixture of 19.0 g (104 mmol) of *rac*-1-*O*-benzylglycerol (*rac*-**4**), 17.6 g (104 mmol) of bromoacetaldehyde dimethyl acetal, and 0.17 g (1.0 mmol) of *p*-TsOH was gradually heated to 100 °C (bT) over 30 min during which time the MeOH byproduct distilled. The reaction mixture was maintained at 100 °C (bT) for an additional 20 h and then cooled, and 200 mL of Et_2O was added. The organic phase was washed with 0.05 M NaOH (200 mL) and H_2O (200 mL) and then dried and evaporated, and the residue was distilled [bp 160–170 °C (0.6 mm) (lit.[24b] bp 190–198 °C (12 mm))] to give 26.2 g (91.2 mmol, 88%) of (±)-**5** as a clear colorless liquid: TLC ($MeOH/CHCl_3$ (5:95)) R_f 0.72; ^{1}H NMR (cis/trans 1.1:1) δ (cis-diastereomers) 7.34 (s, 10 H), 5.16 (t, 2 H, J = 4.0 Hz), 4.58 (s, 4 H), 4.25–4.50 (m, 2 H), 4.03 (dd, 2 H, J = 8.1, 6.6 Hz), 3.89 (dd, 2 H, J = 8.1, 5.9 Hz), 3.45–3.65 (m, 4 H), 3.35–3.45 (m, 4 H), (trans-diastereomers) 7.34 (s, 10 H), 5.27 (t, 2 H, J = 4.0 Hz), 4.58 (s, 4 H), 4.25–4.50 (m, 2 H), 4.19 (dd, 2 H, J = 8.1, 6.4 Hz), 3.81 (dd, 2 H, J = 8.1, 6.4 Hz), 3.45–3.65 (m, 4 H), 3.35–3.45 (m, 4 H).

(±)-2-(Bromomethyl)-4-(hydroxymethyl)-1,3-dioxolane ((±)-6. To a solution of 26.0 g (90.5 mmol) of (±)-**5** in 150 mL of MeOH was added 1.3 g (1.8 mmol $Pd(OH)_2$) of palladium hydroxide (20% on carbon), and the mixture was mechanically shaken under 50 psi of H_2 on a Parr apparatus for 3 h. The catalyst was removed by millipore filtration, and the solvent was evaporated to give 17.7 g (89.8 mmol, 99%) of (±)-**6**[24a,b] as a slightly yellow liquid: TLC ($MeOH/CHCl_3$ (5:95)) R_f 0.43; ^{1}H NMR (cis/trans 1.1:1) δ (cis-diastereomers) 5.13 (t, 2 H, J = 3.4 Hz), 4.25–4.45 (m, 2 H), 4.01 (dd, 2 H, J = 14.9, 8.1 Hz), 3.99 (dd, 2 H, J = 14.2, 8.1 Hz), 3.84 (dd, 4 H, J = 11.7, 4.5 Hz), 3.50 (d, 4 H, J = 3.4 Hz), (trans-diastereomers) 5.30 (t, 2 H, J = 4.1 Hz), 4.25–4.45 (m, 2 H), 4.19 (dd, 2 H, J = 8.2, 6.4 Hz), 3.78 (dd, 2 H,

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[41] Dittmer, J. C.; Lester, R. L. *J. Lipid Res.* **1964**, *5*, 126–127.

J = 8.2, 3.7 Hz), 3.65 (dd, 4 H, *J* = 11.6, 4.8 Hz), 3.39 (d, 4 H, *J* = 4.1 Hz).

(±)-3,6,8-Trioxabicyclo[3.2.1]octane ((±)-7). To a stirred solution of 7.80 g (69.5 mmol) of *t*-BuOK in 30 mL of *t*-BuOH was added a solution of 11.4 g (57.9 mmol) of (±)-**6** in *t*-BuOH (30 mL) over 10 min. The reaction mixture was then warmed to 65 °C (bT) for 1 h. The reaction mixture was cooled and then partitioned between 100 mL of Et_2O and 40 mL of H_2O. The aqueous phase was washed with additional Et_2O (3 × 100 mL), and then the organic extracts were combined, dried, and evaporated. Two distillations [bp 80–85 °C (25 mm) (lit.[24a] bp 68–70 °C (12 mm), lit.[24b] bp 67–70 °C (12 mm), lit.[24c,d] bp 65–67 °C (11 mm))] gave 1.72 g (14.8 mmol, 26%) of (±)-**7** as a white crystalline solid: mp 56 °C (lit.[24a] mp 56–57 °C, lit.[24c,d] mp 55–56 °C); TLC ($MeOH/CHCl_3$ (5:95)) R_f 0.60; ^1H NMR δ 5.35 (s, 1 H), 4.43 (d, 1 H, *J* = 5.3 Hz), 4.29 (d, 1 H, *J* = 6.7 Hz), 3.97 (d, 1 H, *J* = 11.5 Hz), 3.86 (dd, 1 H, *J* = 6.7, 5.3 Hz), 3.67 (d, 1 H, *J* = 11.3 Hz), 3.64 (d, 1 H, *J* = 11.5 Hz), 3.56 (d, 1 H, *J* = 11.3 Hz); mass spectrum *m/z* 116 (M^+), 87, 70, 57; HRMS calcd for $C_5H_8O_3$ (M^+) *m/z* 116.0473, found *m/z* 116.0469.

(±)-2-(Hydroxymethyl)-1,4-dioxane ((±)-8). To a stirred, gently refluxing solution of 0.52 g (4.5 mmol) of (±)-**7** and 0.24 g (6.3 mmol) of $LiAlH_4$ in 5 mL of Et_2O was added a solution of 0.78 g (5.8 mmol) of $AlCl_3$ in 5 mL of Et_2O dropwise (exothermic) over 5 min. The reaction mixture was refluxed vigorously overnight. The reaction mixture was cooled to 0 °C (bT) and quenched cautiously by the dropwise addition of EtOAc, and then 10 mL of H_2O was added. The organic phase was separated, and the aqueous phase was extracted exhaustively with Et_2O. The organic extracts were all combined, dried, and evaporated. Chromatography ($CHCl_3$) gave 0.35 g (3.0 mmol, 67%) of (±)-**8**[25,42] as a clear colorless liquid: ^1H NMR δ 3.40–3.90 (m, 9 H); mass spectrum *m/z* 118 (M^+), 87, 74, 57; HRMS calcd for $C_5H_{10}O_3$ (M^+) *m/z* 118.0630, found *m/z* 118.0625.

Nonadecanol (10). The $LiAlH_4$ reduction of methyl nonadecanoate (**9**) gave **10** after chromatography (EtOAc) as a white solid: mp 62–63 °C; TLC (EtOAc) R_f 0.43; ^1H NMR δ 3.46 (t, 2 H, *J* = 6.6 Hz), 1.50–1.70 (m, 2 H), 1.26 (br s, 32 H), 0.88 (t, 3 H, *J* = 6.6 Hz).

Nonadecanal (11). The PCC[43] oxidation of **10** gave **11** after chromatography on Florisil (100–200 mesh, Aldrich, EtOAc) as a white solid which was used immediately: TLC (EtOAc) R_f 0.71.

Nonadec-1-en-1-yl Acetate (12). To a stirred gently refluxing mixture of 1.85 g (10.7 mmol) of *p*-TsOH in 50 mL of isopropenyl acetate at 110 °C (bT) was added a solution of 30.6 g (108 mmol) of **11** in 100 mL of isopropenyl acetate dropwise over 10 min. The reaction mixture was maintained at 110 °C (bT) for 19 h and then cooled, washed with 0.15 M $NaHCO_3$ (1 × 150 mL) and H_2O (2 × 150 mL), and evaporated. Chromatography (Et_2O/pet (2:98)) gave 27.0 g (83.2 mmol, 77%) of **12** as a white solid: mp 41 °C (soften) 44–46 °C (melt); TLC (Et_2O/pet 5:95) R_f 0.36; ^1H NMR (trans/cis 9:1) δ (trans) 6.99 (dt, 1 H, *J* = 6.5, 1.5 Hz), 4.86 (dt, 1 H, *J* = 6.5, 7.5 Hz), 2.15 (s, 3 H), 1.50–1.70 (m, 2 H), 1.26 (br s, 30 H), 0.88 (t, 3 H, *J* = 6.6 Hz), (cis) 7.06 (dt, 1 H, *J* = 12.4, 1.5 Hz), 5.41 (dt, 1 H, *J* = 12.4, 7.5 Hz), 2.11 (s, 3 H), 1.50–1.70 (m, 2 H), 1.26 (br s, 30 H), 0.88 (t, 3 H, *J* = 6.6 Hz); mass spectrum *m/z* 324 (M^+), 282, 264, 236; HRMS calcd for $C_{21}H_{40}O_2$ (M^+), *m/z* 324.3028, found *m/z* 324.3035. Anal. Calcd for $C_{21}H_{40}O_2$: C, 77.72; H, 12.42. Found: C, 78.00; H, 12.70.

2-Bromo-1,1-dimethoxynonadecane (13). To a stirred solution of 10.1 g (31.1 mmol) of **12** in 350 mL of CCl_4 at 0 °C (bT) was added a solution of 5.23 g (32.7 mmol) of Br_2 in 50 mL of CCl_4 dropwise over 30 min. The reaction mixture was refluxed for 1 h and then cooled, and 200 mL of anhydrous MeOH and 0.43 g (2.5 mmol) of *p*-TsOH were added. The reaction mixture was refluxed for 18 h, and then the solvent was evaporated. Chromatography (Et_2O/pet (2:98)) gave 9.75 g of a white solid which contained 8.65 g (21.2 mmol, 86%) of product acetal **13** and 1.10 g (3.52 mmol, 14%) of ester **9** and was used without further purification: TLC (Et_2O/pet (5:95)) R_f 0.40; ^1H NMR δ (acetal **13**) 4.38 (d, 1 H, *J* = 5.6 Hz), 3.97 (ddd, 1 H, *J* = 9.9, 5.6, 3.2 Hz), 3.44 (s, 6 H), 1.30–2.00 (m, 2 H), 1.26 (br s, 30 H), 0.88 (t, 3 H, *J* = 6.6 Hz), (ester **9**) 3.66 (s, 3 H), 2.30 (t, 2 H, *J* = 7.5 Hz), 1.45–1.80 (m, 2 H), 1.26 (br s, 30 H), 0.88 (t, 3 H, *J* = 6.6 Hz); mass spectrum *m/z* 405 and 407 [acetal **13**, $(M - H)^+$], 375 and 377, 326, 312, (ester **9**, M^+), 298, 281; HRMS calcd for $C_{21}H_{42}O_2Br$ [$(M - H)^+$] *m/z* 405.2368, found *m/z* 405.2384.

1-*O*-Benzyl-*sn*-glycerol (16). The conversion of 8.22 g (62.2 mmol) of 2,3-*O*-isopropylidene-*sn*-glycerol (**14**) (α^{25}_D −14.0° (neat), d^{25}_4 1.05, $[\alpha]^{25}_D$ −13.3° (neat)) to 9.45 g (51.9 mmol, 93%) of **16** was performed according to the reported method:[33a] ^1H NMR δ 7.34 (br s, 5 H), 4.55 (s, 2 H), 3.85–3.95 (m, 1 H), 3.50–3.75 (m, 4 H); $[\alpha]^{25}_D$ −5.5° (neat), d^{25}_4 1.1, $[\alpha]^{25}_D$ −5.0° (neat), $[\alpha]^{25}_D$ −1.7° (*c* 7.5, $CHCl_3$) (lit.[33a,b] $[\alpha]^?_D$ −5.85° (neat); lit.[33c] $\alpha^?_D$ −6.5° (neat), $[\alpha]^?_D$ −5.7° (neat); lit.[33d] $\alpha^?_D$ −5.73° (neat), $[\alpha]^?_D$ −5.01° (neat)).

(4*R*)-4-[(Benzyloxy)methyl]-2-(1′-bromooctadecyl)-1,3-dioxolane (17). Transacetalation of 7.00 g (15.2 mmol **13** with 2.5 mmol **9**) of **13** with 2.92 g (16.0 mmol) of **16** and 0.040 g (0.23 mmol) of *p*-TsOH was performed as described for (±)-**5**, and chromatography (Et_2O/pet (2:98) gave 8.01 g (15.2 mmol, 100%) of **17** as a clear faint yellow liquid: TLC (Et_2O/pet (20:80)) R_f 0.38 and 0.33; ^1H NMR (cis/trans 3:1) δ (cis-diastereomers) 7.33 (s, 10 H), 5.02 and 4.99 (d, 1 H, *J* = 4.1 Hz, and d, 1 H, *J* = 4.6 Hz), 4.58 (s, 4 H), 4.25–4.45 (m, 2 H), 4.02 (dd, 1 H, *J* = 9.0, 7.0 Hz, and dd, 1 H, *J* = 7.0, 7.0 Hz), 3.70–3.95 (m, 2 H), 3.40–3.70 (m, 6 H), 1.60–2.05 (m, 4 H), 1.26 (br s, 60 H), 0.88 (t, 6 H, *J* = 6.6 Hz), (trans-diastereomers) 7.33 (s, 10 H), 5.12 and 5.09 (d, 1 H, *J* = 3.8 Hz, and d, 1 H, *J* = 4.4 Hz), 4.58 (s, 4 H), 4.25–4.45 (m, 2 H), 4.19 (dd, 1 H, *J* = 9.8, 6.4 Hz, and dd, 1 H, *J* = 6.4, 6.4 Hz), 3.70–4.05 (m, 4 H), 3.40–3.70 (m, 4 H), 1.60–2.05 (m, 4 H), 1.26 (br s, 60 H), 0.88 (t, 6 H, *J* = 6.6 Hz); mass spectrum *m/z* 523 and 525 [$(M - H)^+$], 444; HRMS calcd for $C_{29}H_{48}O_3Br$ [$(M - H)^+$] *m/z* 523.2786, found *m/z* 523.2801. Anal. Calcd for $C_{29}H_{49}O_3Br$: C, 66.27; H, 9.40. Found: C, 66.28; H, 9.56.

(4*R*)-2-(1′-Bromooctadecyl)-4-(hydroxymethyl)-1,3-dioxolane (18). The hydrogenolysis of 8.00 g (15.2 mmol) of **17** was performed as described for (±)-**6** and gave 6.50 g (14.9 mmol, 98%) of **18** as a light yellow solid: mp 43–44 °C; TLC (Et_2O/pet (20:80)) R_f 0.05; ^1H NMR (cis/trans 3:1) δ (cis-diastereomers) 4.92 and 4.93 (d, 1 H, *J* = 3.6 Hz, and d, 1 H, *J* = 3.3 Hz), 4.20–4.40 (m, 2 H), 4.00 (d, 4 H, *J* = 6.5 Hz), 3.75–3.95 (m, 2 H), 3.55–3.75 (m, 4 H), 1.60–2.15 (m, 4 H), 1.26 (br s, 60 H), 0.88 (t, 6 H, *J* = 6.6 Hz), (trans-diastereomers) 5.13 and 5.12 (d, 1 H, *J* = 4.1 Hz, and d, 1 H, *J* = 4.2 Hz), 4.20–4.40 (m, 2 H), 4.20 (dd, 2 H, *J* = 6.6, 6.3 Hz), 3.75–4.10 (m, 4 H), 3.55–3.75 (m, 4 H), 1.60–2.15 (m, 4 H), 1.26 (br s, 60 H), 0.88 (t, 6 H, *J* = 6.6 Hz); mass spectrum *m/z* 433 and 435 [$(M - H)^+$], 403 and 405, 385 and 387, 354, 311, 298, 281; HRMS calcd for $C_{22}H_{42}O_3Br$ [$(M - H)^+$] *m/z* 433.2317, found *m/z* 433.2320. Anal. Calcd for $C_{22}H_{43}O_3Br$: C, 60.68; H, 9.95. Found: C, 60.81; H, 10.16.

(1*R*,4*S*,5*S*)- and (1*R*,4*R*,5*S*)-4-Heptadecyl-3,6,8-trioxabicyclo[3.2.1]octanes (19) and (20). The cyclization of 6.45 g (14.8 mmol) of **18** was performed as described for (±)-**7**, and chromatography ($CHCl_3$) first eluted the minor product which was demonstrated to be homogeneous by TLC and analytical HPLC. Crystallization (pet) gave 0.30 g (0.85 mmol, 5.7%) of **19** as colorless plates: mp 65–66 °C; TLC ($CHCl_3$) R_f 0.35; HPLC ($CHCl_3$) t_R 3 min 45 s; ^1H NMR δ 5.11 (s, 1 H, H5), 4.40 (d, 1 H, $J_{1,7exo}$ = 5.2 Hz, H1), 4.17 (d, 1 H, $J_{7endo,7exo}$ = 6.6 Hz, $H7_{endo}$), 3.94 (d, 1 H, $J_{2ax,2eq}$ = 11.5 Hz, $H2_{ax}$), 3.81 (dd, 1 H, $J_{7exo,7endo}$ = 6.6 Hz, $J_{7exo,1}$ = 5.2 Hz, $H7_{exo}$), 3.63 (d, 1 H, $J_{2eq,2ax}$ = 11.5 Hz, $H2_{eq}$), 3.45–3.55 (m, 1 H, $H4_{ax}$), 1.35–1.60 (m, 2 H, C4-CH_2), 1.26 (br s, 30 H, $(CH_2)_{15}$), 0.88 (t, 3 H, *J* = 6.6 Hz, CH_3); $[\alpha]^{25}_D$ +34° (*c* 5.5, $CHCl_3$); mass spectrum *m/e* 354 (M^+), 308, 267, 250; HRMS calcd for $C_{22}H_{42}O_3$ (M^+) *m/z* 354.3136, found *m/z* 354.3139. Anal. Calcd for $C_{22}H_{42}O_3$: C, 74.52; H, 11.94. Found: C, 74.80; H, 11.93. The major product then eluted from the column and was demonstrated to be homogeneous by TLC and analytical HPLC. Crystallization (EtOAc/pet (25:75)) gave 1.29 g (3.64 mmol, 25%) of **20** as colorless plates: mp 74–75 °C; TLC ($CHCl_3$) R_f 0.20; HPLC ($CHCl_3$) t_R 4 min 30 s; ^1H NMR δ 5.18 (s, 1 H, H5), 4.39 (d, 1 H, $J_{1,7exo}$ = 5.2 Hz, H1), 4.23 (d, 1 H, $J_{7endo,7exo}$ = 6.6 Hz, $H7_{endo}$), 4.05 (d, 1 H, $J_{2ax,2eq}$ = 11.6 Hz, $H2_{ax}$), 3.81 (dd, 1 H, $J_{7exo,7endo}$ = 6.6 Hz, $J_{7exo,1}$ = 5.2 Hz, $H7_{exo}$), 3.41 (d, 1 H, $J_{2eq,2ax}$ = 11.6 Hz, $H2_{eq}$), 3.35–3.50 (m, 1 H, $H4_{eq}$), 1.60–2.00 (m, 2 H, C4-CH_2), 1.26 (br s, 30 H, $(CH_2)_{15}$), 0.88 (t, 3 H, *J* = 6.6 Hz, CH_3); $[\alpha]^{25}_D$ +44° (*c* 5.5, $CHCl_3$); mass spectrum *m/z* 354 (M^+) 308, 267, 250; HRMS calcd for $C_{22}H_{42}O_3$ (M^+) *m/z* 354.3136, found *m/z* 354.3143. Anal. Calcd

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for $C_{22}H_{42}O_3$: C, 74.52; H, 11.94. Found: C, 74.79; H, 11.87.

(2*S*,5*S*)-5-Heptadecyl-2-(hydroxymethyl)-1,4-dioxane (21). The reduction of 0.26 g (0.73 mmol) of **19** was performed as described for (±)-**8**, and chromatography (EtOAc/CH_2Cl_2 (20:80)) gave 0.19 g (0.53 mmol, 73%) of **21** as a white solid: mp 66–67 °C; TLC (EtOAC/CH_2Cl_2 (20:80)) R_f 0.15; ^{1}H NMR δ 3.80–4.05 (m, 1 H), 3.45–3.80 (m, 7 H), 1.86 (br t, 1 H, exchangeable), 1.35–1.60 (m, 2 H), 1.26 (br s, 30 H), 0.88 (t, 3 H, J = 6.6 Hz); $[\alpha]^{25}{}_D$ −9.1° (c 3.5, $CHCl_3$); mass spectrum *m/z* 356 (M^+), 352, 325, 307, 265; HRMS calcd for $C_{22}H_{44}O_3$ (M^+) *m/z* 356.3292, found *m/z* 356.3289. Anal. Calcd for $C_{22}H_{44}O_3$: C, 74.10; H, 12.44. Found: C, 74.24; H, 12.48.

(2*R*,5*S*)-*O*-[[[(5-Heptadecyl-1,4-dioxan-2-yl)methyl]oxy]phospho]-β-bromoethanol (22). To a stirred solution of 0.39 g (1.6 mmol) of β-bromoethyl dichlorophosphate[40] in 5 mL of Et_2O was added 0.6 mL of pyridine dropwise over 2 min. After 15 min, a solution of 0.16 g (0.45 mmol) of **21** in 5 mL of Et_2O was added to the slightly heterogeneous reaction mixture over 2 min. The reaction mixture was warmed to 50 °C (bT) for 4 h. The reaction mixture was then cooled to 0 °C (bT), 2 mL of H_2O was added, and the reaction mixture was stirred overnight to give a clear colorless homogeneous solution which was partitioned between 4 mL of 3 M HCl and 25 mL of MeOH/$CHCl_3$ (5:95). The aqueous phase was extracted with additional MeOH/$CHCl_3$ (5:95) (3 × 25 mL), and the organic extracts were all combined, dried, and evaporated. Chromatography (MeOH/$CHCl_3$ (5:95) gradually increased to MeOH/$CHCl_3$ (15:85)) gave 0.17 g (0.31 mmol, 69%) of **22** as a white amorphous solid: mp 190–195 °C; TLC ($CHCl_3$/MeOH/H_2O (60:30:4)) R_f 0.41; ^{1}H NMR δ 3.00–4.50 (br m, 12 H), 1.35–1.60 (m, 2 H), 1.26 (br s, 30 H), 0.88 (t, 3 H, J = 6.6 Hz); FAB mass spectrum *m/z* 543 and 545 (MH^+).

(2*R*,5*S*)-[[(5-Heptadecyl-1,4-dioxan-2-yl)methyl]oxy]phosphocholine (2). To a stirred solution of 0.16 g (0.29 mmol) of **22** in 20 mL of $CHCl_3$/*i*-PrOH/DMF (2:2:1) in a glass pressure bottle was added excess trimethylamine and the reaction mixture heated at 50 °C (bT) for 48 h. The excess trimethylamine was evaporated with a stream of N_2, and the reaction mixture was then concentrated. Chromatography ($CHCl_3$/MeOH/H_2O (60:30:0) increased stepwise to $CHCl_3$/MeOH/H_2O (60:30:4)) and evaporation followed by millipore filtration of a $CHCl_3$ solution of the product then gave 0.13 g (0.25 mmol, 86%) of **2** as a white solid. An analytical sample was prepared by precipitation from $CHCl_3$ solution with acetone: mp 230 °C; TLC ($CHCl_3$/MeOH/H_2O (60:30:4)) R_f 0.06; ^{1}H NMR ($CDCl_3$/CD_3OD (50:50)) δ 4.20–4.30 (m, 2 H), 3.90–4.20 (m, 2 H), 3.65–3.85 (m, 2 H), 3.45–3.65 (m, 6 H), 3.23 (s, 9 H), 1.40–1.65 (m, 2 H), 1.26 (br s, 30 H), 0.88 (t, 3 H, J = 6.6 Hz); $[\alpha]^{25}{}_D$ −6.2° (c 0.18 $CHCl_3$/MeOH (50:50)); FAB mass spectrum *m/z* 522 (MH^+), 224, 184, 166; FAB HRMS calcd for $C_{27}H_{57}NO_6P$ (MH^+) *m/z* 522.3924, found *m/z* 522.3920. Anal. Calcd for $C_{27}H_{56}NO_6P{\cdot}H_2O$: C, 60.08; H, 10.83; N, 2.60. Found: C, 59.91; H, 10.99; N, 2.42.

(2*S*,5*R*)-5-Heptadecyl-2-(hydroxymethyl)-1,4-dioxane (23). The reduction of 1.25 g (3.52 mmol) of **20** was performed as described for **21** and gave 0.960 g (2.69 mmol, 76%) of **23** as a white solid: mp 83–84 °C; TLC (EtOAc/CH_2Cl_2 (20:80)) R_f 0.16; ^{1}H NMR δ 3.70–3.85 (m, 2 H), 3.55–3.70 (m, 2 H), 3.29–3.55 (m, 4 H), 1.83 (br t, 1 H, exchangeable), 1.30–1.50 (m, 2 H), 1.26 (br s, 30 H), 0.88 (t, 3 H, J = 6.6 Hz); $[\alpha]^{25}{}_D$ +0.86° (c 3.5, $CHCl_3$); mass spectrum *m/z* 356 (M^+), 352, 325, 307, 265; HRMS calcd for $C_{22}H_{44}O_3$ (M^+) *m/z* 356.3292, found *m/z* 356.3306. Anal. Calcd for $C_{22}H_{44}O_3$: C, 74.10; H, 12.44. Found: C, 74.25; H, 12.33.

(2*R*,5*R*)-*O*-[[[(5-Heptadecyl-1,4-dioxan-2-yl)methyl]oxy]phospho]-β-bromoethanol (24). The β-bromoethyl phosphorylation of 0.20 g (0.56 mmol) of **23** was performed as described for **22** and gave 0.18 g (0.33 mmol, 59%) of **24** as a white amorphous solid: mp 190–195 °C; TLC ($CHCl_3$/MeOH/H_2O (60:30:4)) R_f 0.42; ^{1}H NMR δ 3.00–4.50 (br m, 12 H), 1.35–1.60 (m, 2 H), 1.26 (br s, 30 H), 0.88 (t, 3 H, J = 6.6 Hz); FAB mass spectrum *m/z* 543 and 545 (MH^+).

(2*R*,5*R*)-[[(5-Heptadecyl-1,4-dioxan-2-yl)methyl]oxy]phosphocholine (3). The quaternization of trimethylamine with 0.15 g (0.28 mol) of **24** was performed as described for **2** and gave 0.13 g (0.25 mmol, 89%) of **3** as a white solid: mp 231 °C; TLC ($CHCl_3$/MeOH/H_2O (60:30:4)) R_f 0.06; ^{1}H NMR ($CDCl_3$/CD_3OD (50:50)) δ 4.20–4.30 (m, 2 H), 3.40–3.95 (m, 10 H), 3.22 (s, 9 H), 1.40–1.65 (m, 2 H), 1.26 (br s, 30 H), 0.88 (t, 3 H, J = 6.6 Hz); $[\alpha]^{25}{}_D$ +3° (c 0.18, $CHCl_3$/MeOH (50:50)); FAB mass spectrum *m/z* 522 (MH^+), 224, 184, 166; FAB HRMS calcd for $C_{27}H_{57}NO_6P$ (MH^+) *m/z* 522.3924, found *m/z* 522.3925. Anal. Calcd for $C_{27}H_{56}NO_6P{\cdot}H_2O$: C, 60.08; H, 10.83; N, 2.60. Found: C, 59.79; H, 10.73; N, 2.31.

3-*O*-Benzyl-*sn*-glycerol (16′). 3-*O*-Benzyl-*sn*-glycerol (**16′**) was purchased commercially or prepared[33a,34] from 1,2-*O*-isopropylidene-*sn*-glycerol (**14′**): $\alpha^{25}{}_D$ +6.2° (neat), $d^{25}{}_4$ 1.1, $[\alpha]^{25}{}_D$ +5.6° (neat) (lit.[33a] $[\alpha]^{?}{}_D$ +5.85° (neat); lit.[34] $[\alpha]^{20}{}_D$ +6.5° (neat)).

(4*S*)-4-[(Benzyloxy)methyl]-2-(1′-bromooctadecyl)-1,3-dioxolane (17′). The transacetalation of 9.75 g (21.2 mmol **13** with 3.5 mmol **9**) of **13** with 4.25 g (23.3 mmol) of **16′** was performed as described for **17** and gave 10.87 g (20.7 mmol, 98%) of a light yellow liquid which was a 2.4:1 ratio of the two cis-diastereomers to the two trans-diastereomers: HRMS calcd for $C_{29}H_{48}O_3Br$ [$(M - H)^+$] *m/z* 523.2786, found *m/z* 523.2794. Anal. Calcd for $C_{29}H_{49}O_3Br$: C, 66.27; H, 9.40. Found: C, 65.89; H, 9.12.

(4*S*)-2-(1′-Bromooctadecyl)-4-(hydroxymethyl)-1,3-dioxolane (18′). The hydrogenolysis of 8.38 g (15.9 mmol) of **17′** was performed as described for **18** and gave 6.94 g (15.9 mmol, 100%) of a light yellow solid which was a 2.4:1 ratio of the two cis-diastereomers to the two trans-diastereomers: HRMS calcd for $C_{22}H_{42}O_3Br$ [$(M - H)^+$] *m/z* 433.2317, found *m/z* 433.2320. Anal. Calcd for $C_{22}H_{43}O_3Br$: C, 60.68; H, 9.95. Found: C, 60.52; H, 10.07.

(1*S*,4*R*,5*R*)- and (1*S*,4*S*,5*R*)-4-Heptadecyl-3,6,8-trioxabicyclo[3.2.1]octanes (19′) and (20′). The cyclization of 6.91 g (15.9 mmol) of **18′** was performed as described for **19** and **20**. The 0.31 g (0.87 mmol, 5.5%) of **19′** was otherwise identical to the previously described enantiomer **19**: $[\alpha]^{25}{}_D$ −34° (c 5.5, $CHCl_3$); HRMS calcd for $C_{22}H_{42}O_3$ (M^+) *m/z* 354.3136, found *m/z* 354.3139. Anal. Calcd for $C_{22}H_{42}O_3$: C, 74.52; H, 11.94. Found: C, 74.40; H, 12.05. The 1.55 g (4.37 mmol, 28%) of **20′** was otherwise identical to the previously described enantiomer **20**: $[\alpha]^{25}{}_D$ −44° (c 5.5, $CHCl_3$); HRMS calcd for $C_{22}H_{42}O_3$ (M^+) *m/z* 354.3136, found *m/z* 354.3135. Anal. Calcd for $C_{22}H_{42}O_3$: C, 74.52; H, 11.94. Found: C, 74.65; H, 12.13.

(2*R*,5*R*)-5-Heptadecyl-2-(hydroxymethyl)-1,4-dioxane (21′). The reduction of 0.30 g (0.85 mmol) of **19′** gave 0.23 g (0.64 mmol, 75%) of a white solid: $[\alpha]^{25}{}_D$ +9.1° (c 3.5, $CHCl_3$); HRMS calcd for $C_{22}H_{44}O_3$ (M^+) *m/z* 356.3292, found *m/z* 356.3306. Anal. Calcd for $C_{22}H_{44}O_3$: C, 74.10; H, 12.44. Found: C, 74.13; H, 12.67.

(2*S*,5*R*)-*O*-[[[(5-Heptadecyl-1,4-dioxan-2-yl)methyl]oxy]phospho]-β-bromoethanol (22′). The β-bromoethyl phosphorylation of 0.19 g (0.53 mmol) of **21′** gave 0.17 g (0.31 mmol, 58%) of a white amorphous solid.

(2*S*,5*R*)-[[(5-Heptadecyl-1,4-dioxan-2-yl)methyl]oxy]phosphocholine (2′). The quaternization of trimethylamine with 0.17 g (0.31 mmol) of **22′** gave 0.12 g (0.23 mmol, 74%) of a white solid: $[\alpha]^{25}{}_D$ +6.2° (c 0.18, $CHCl_3$/MeOH (50:50)), $[\alpha]^{25}{}_D$ +3° (c 0.20, $CHCl_3$); FAB HRMS calcd for $C_{27}H_{57}NO_6P$ (MH^+) *m/z* 522.3924, found *m/z* 522.3928. Anal. Calcd for $C_{27}H_{56}NO_6P{\cdot}H_2O$: C, 60.08; H, 10.83; N, 2.60. Found: C, 59.76; H, 10.67; N, 2.34.

(2*R*,5*S*)-5-Heptadecyl-2-(hydroxymethyl)-1,4-dioxane (23′). The reduction of 1.51 g (4.26 mmol) of **20′** gave 1.28 g (3.59 mmol, 84%) of a white solid: $[\alpha]^{25}{}_D$ −0.86° (c 3.5, $CHCl_3$); HRMS calcd for $C_{22}H_{44}O_3$ (M^+) *m/z* 356.3292, found *m/z* 356.3308. Anal. Calcd for $C_{22}H_{44}O_3$: C, 74.10; H, 12.44. Found: C, 73.93; H, 12.72.

(2*S*,5*S*)-*O*-[[[(5-Heptadecyl-1,4-dioxan-2-yl)methyl]oxy]phospho]-β-bromoethanol (24′). The β-bromoethyl phosphorylation of 0.22 g (0.62 mmol) of **23′** gave 0.21 g (0.39 mmol, 63%) of a white amorphous solid.

(2*S*,5*S*)-[[(5-Heptadecyl-1,4-dioxan-2-yl)methyl]oxy]phosphocholine (3′). The quaternization of trimethylamine with 0.17 g (0.31 mmol) of **24′** gave 0.12 g (0.23 mmol, 74%) of a white solid: $[\alpha]^{25}{}_D$ −3° (c 0.18, $CHCl_3$/MeOH (50:50)), $[\alpha]^{25}{}_D$ −3° (c 0.20, $CHCl_3$); FAB HRMS calcd for $C_{27}H_{57}NO_6P$ (MH^+) *m/z* 522.3924, found *m/z* 522.3926. Anal. Calcd for $C_{27}H_{56}NO_6P{\cdot}H_2O$: C, 60.08; H, 10.83; N, 2.60. Found: C, 59.89; H, 10.86; N, 2.30.

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Registry No. (*R*)-**1**, 143680-83-5; **2**, 143680-84-6; **2′**, 143730-66-9; **3**, 143730-65-8; **3′**, 143730-67-0; (±)-**4**, 13071-59-5; *cis*-(±)-**5**, 143669-37-8; *trans*-(+)-**5**, 143669-36-7; *cis*-(±)-**6**, 143669-39-0; *trans*-(±)-**6**, 143669-38-9; (±)-**7**, 143669-40-3; (±)-**8**, 143669-41-4; **9**, 1731-94-8; **10**, 1454-84-8; **11**, 17352-32-8; (*E*)-**12**, 143669-34-5; (*Z*)-**12**, 143669-33-4; **13**, 143669-35-6; **14**, 14347-78-5; **14′**, 22323-82-6; **16**, 17325-85-8; **16′**, 56552-80-8; (2*R*,4*R*,1′*R*)-**17**, 143669-19-6; (2*R*,4*R*,1′*S*)-**17**, 143669-20-9; (2*S*,4*R*,1′*R*)-**17**, 143669-17-4; (2*S*,4*R*,1′*S*)-**17**, 143669-18-5; (2*R*,4*S*,1′*R*)-**17′**, 143669-28-7; (2*R*,4*S*,1′*S*)-**17′**, 143669-29-8; (2*S*,4*S*,1′*R*)-**17′**, 143680-76-6; (2*S*,4*S*,1′*S*)-**17′**, 143669-30-1; (2*R*,4*R*,1′*R*)-**18**, 143669-23-2; (2*R*,4*R*,1′*S*)-**18**, 143669-24-3; (2*S*,4*R*,1′*R*)-**18**, 143669-21-0; (2*S*,4*R*,1′*S*)-**18**, 143669-22-1; (2*R*,4*S*,1′*R*)-**18′**, 143669-31-2; (2*R*,4*S*,1′*S*)-**18′**, 143705-56-0; (2*S*,4*S*,1′*R*)-**18′**, 143680-75-5; (2*S*,4*S*,1′*S*)-**18′**, 143669-32-3; **19**, 143669-25-4; **19′**, 143730-09-0; **20**, 143730-06-7; **20′**, 143730-10-3; **21**, 143669-26-5; **21′**, 143730-11-4; **22**, 143669-27-6; **22′**, 143730-13-6; **23**, 143730-07-8; **23′**, 143730-12-5; **24**, 143730-08-9; **24′**, 143730-14-7; isopropenyl acetate, 108-22-5; β-bromoethyl dichlorophosphate, 4167-02-6; bromoacetaldehyde dimethyl acetal, 7252-83-7.

Synthesis of Diribonucleoside Phosphorothioates via Stereospecific Sulfurization of H-Phosphonate Diesters

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Sulfurization of diribonucleoside H-phosphonates with elemental sulfur was found to be a stereospecific reaction. With this finding as the basis, an efficient method for the preparation of stereochemically homogeneous diribonucleoside phosphorothioates has been developed. The procedure consists of the synthesis and separation of the diastereomeric (R_p and S_p) pairs of the corresponding H-phosphonate diesters, followed by their stereospecific sulfurization and a single deprotection step using fluoride ion. The methodology has been used in synthesis of eight diribonucleoside phosphorothioates (four pairs of R_p and S_p diastereomers).

Introduction

Dinucleoside phosphorothioates are chiral analogues of phosphodiesters in which one of the nonbridging oxygens has been replaced by sulfur. These compounds are important research tools in stereochemical investigations, in mechanistic studies of various enzymatic reactions, and in other biochemical studies.[1-3] Different affinities of enzymes for compounds with opposite stereochemistry at the phosphorus center usually necessitate the use of optically pure phosphorothioates in biological experiments. Several synthetic methods, based on the phosphodiester,[4] phosphotriester,[5] and phosphite[6] approaches have been designed for the preparation of deoxyribonucleoside phosphorothioates. They involve either separation of phosphorothioate diesters after a final deprotection step or separation of chiral precursors followed by their stereospecific conversion into phosphorothioate diesters. A new promising approach to the chemical synthesis of phosphorothioate oligodeoxyribonucleotides has been reported by Stec et al.[7] It involves separation of suitably protected 2-(deoxyribonucleosid-3′-yloxy)-2-thio-1,3,2-oxathiaphospholanes into diastereomers, followed by their stereospecific reactions with another nucleoside.

There have, however, only been a few methods reported for the preparation of ribonucleoside phosphorothioates,[8-11] and these are quite laborious and inefficient by comparison to methods available for deoxyribonucleoside phosphorothioate synthesis. Only lately have oligoribonucleotides containing stereochemically defined phosphorothioate functions been produced by chemical means.[12,13]

Recent studies[14-16] have shown that the H-phosphonate approach can be a method of choice for the preparation of phosphorothioate diesters. In this paper we describe an efficient procedure for the preparation of stereochem-

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