aminobutyl)-1-(1-naphthyl)piperazine (6) as a yellow oil, which was used in the next step without further purification.

A solution of 1-adamantanecarbonyl chloride (0.08 g, 0.4 mmol) in dry THF (5 mL) was added to a solution of 4-(4-aminobutyl)-1-(1-naphthyl)piperazine (6) (0.12 g, 0.4 mmol) and triethylamine (0.16 g, 1.6 mmol) in dry THF (5 mL) maintained at 0 °C under a nitrogen atmosphere. The reaction mixture was allowed to stir at 0 °C for 75 min and at room temperature for 2 h. The solids were removed by filtration, and the volatiles were removed under reduced pressure to yield 0.15 g of an oil, which was purified by column chromatography using a mixture of $CHCl_3$ and MeOH (9:1) as the eluent to yield 0.1 by (54%) of the desired product. The fumarate salt was prepared by the addition of an ethereal solution of the free base (0.1 g, 0.23 mmol) to an ethereal solution of fumaric acid (0.03 g, 0.23 mmol). Recrystallization from a mixture of absolute EtOH and anhydrous Et_2O afforded 0.03 g of the title compound as white crystals, mp 185-186 °C (Table I).

Forskolin-Stimulated Adenylyl Cyclase Assay. Forskolin-stimulated adenylyl cyclase assays were performed using Sprague Dawley rat hippocampi (Taconic Farms, Germantown, NY) as previously described.[7] Briefly, the assay incubation media contained 80 mM Tris-HCl (pH 7.5 at 22 °C), 100 mM NaCl, 2 mM $MgCl_2$, 5 mM creatine phosphate, 2 mM cAMP, 0.2 μg of myokinase, 10 μg of creatine phosphokinase, 3 units/mL of adenosine deaminase, 10 μM GTP, 50 μM forskolin, 60 mM sucrose, 0.2 mM EGTA, 1 mM Na_2EDTA, 0.2 mM dithiothreitol, 50-100 μg of membrane protein, and 0.1 mM ATP containing 1.5 μCi of [α-^{32}P]ATP. The conversion of [α-^{32}P]ATP to [^{32}P]cAMP was measured as described by Salomon.[22]

Radioligand Binding Assays. Brain homogenate preparation and radioligand binding assays were performed as previously described.[7] Briefly, 5-HT_{1A} receptor affinity was assayed using 0.1 nM [^{3}H]-8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin), 6 mg of rat hippocampal homogenate, and 10 μM 8-OH-DPAT to define nonspecific binding. 5-HT_{1B} receptor binding was assayed using [^{3}H]-5-HT (2.0 nM), 5 mg of rat striatal homogenate, 100 nM 8-OH-DPAT, and 100 nM mesulergine. Nonspecific binding was defined using 10 μM 5-HT. 5-HT_{1C} affinity was assayed with 1.0 nM [^{3}H]mesulergine, 10 nM spiperone, 10 mg of homogenized rat frontal cortex, and 10 μM 5-HT to define nonspecific binding. 5-HT_{1D} receptor affinity was assayed with 2 nM [^{3}H]-5-HT, 10 μM pindolol, 100 nM mesulergine, 10 mg of homogenized calf caudate, and 10 μM 5-HT to define nonspecific binding. 5-HT_2 receptor affinity was assayed using [^{3}H]DOB (0.4 nM), 15 mg of homogenized rat frontal cortex, and 1 μM cinanserin to define nonspecific binding. 5-HT_3 receptor affinity was assayed with [^{3}H]GR-65630, 5 mg of homogenized calf area postrema, and 140 mM NaCl. ICS-205-930 (1 μM) was used to define nonspecific binding. α_1-Adrenergic receptor affinity was assayed using 1.0 nM [^{3}H]WB4101, 100 nM 8-OH-DPAT, 10 mg of rat frontal cortex, and 1 μM prazosin to define nonspecific binding. β-Adrenergic affinity was assayed using 1 nM [^{3}H]DHA and 5 mg of homogenized rat frontal cortex. Nonspecific binding was defined by 1 μM propranolol. D_1-Dopamine receptor affinity was assayed using 1 nM [^{3}H]SCH-23390, 20 nM ketanserin, 2 mg of homogenized rat striatum, and 1 μM SCH-23390 to define nonspecific binding. D_2-Dopamine receptor affinity was assayed using [^{3}H]NMSP (1 nM) and 2 mg of calf caudate in the presence of 140 mM NaCl. Sulpiride (10 μM) was used to define nonspecific binding.

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Registry No. **1a**, 102392-05-2; **1b**, 134390-52-6; **1b** free base, 134390-53-7; **1c**, 134390-55-9; **1c** free base, 134390-54-8; **1d**, 134390-57-1; **1d** free base, 134390-56-0; **1e**, 115338-33-5; **1e** free base, 115338-25-5; **1f**, 134390-59-3; **1f** free base, 134390-58-2; **2a**, 134390-60-6; **2b**, 134390-61-7; **2c**, 134390-62-8; **2d**, 134390-63-9; **2e**, 134390-65-1; **2e** free base, 134390-64-0; **2f**, 134390-66-2; **2g**, 134390-67-3; **2g** free base, 134390-68-4; **2h**, 134390-70-8; **2i**, 134390-71-9; **2i** free base, 133025-01-1; **2j**, 134390-73-1; **2k**, 134390-75-3; **2k** free base, 134390-74-2; **2l**, 115338-28-8; **2m**, 134390-76-4; **3a**, 35386-24-4; **3b**, 1019-06-3; **3c**, 6269-89-2; **3d**, 59084-06-9; **3e**, 57536-86-4; **4**, 5394-18-3; **5**, 21103-33-3; **6**, 134390-77-5; 4-nitrobenzoyl chloride, 122-04-3; 4-phenylbutyric acid, 1821-12-1; 1-adamantanecarbonyl chloride, 2094-72-6; 4-(benzyloxy)benzoyl chloride, 1486-50-6.

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Acyclic Analogues of 2-(4-Phenylpiperidino)cyclohexanol (Vesamicol): Conformationally Mobile Inhibitors of Vesicular Acetylcholine Transport

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Several 1,3-disubstituted propan-2-ols and one α,β-disubstituted ethanol (**11i**) were synthesized and evaluated as potential acyclic mimics of the vesicular acetylcholine transport inhibitor 2-(4-phenylpiperidinyl)cyclohexanol (**1**, vesamicol, AH5183). Analogues containing the 4-phenylpiperidyl fragment (**11a**, **11b**) were more potent than those containing the 4-phenylpiperazyl moiety (**11e**, **11f**). Substitution at the second terminal carbon of the propyl (or ethyl) fragment with simple lipophilic aryl substituents yielded potent inhibitors of vesicular acetylcholine storage, including (-)-**11a** and *d*-**11i**, which are equipotent with vesamicol. However, the activity of analogues containing bicyclic aryl groups was susceptible to aryl substitution patterns (**11g** vs **11h**), indicating a definite receptor site topography. In addition, the inhibitory activity of these acyclic analogues was enantioselective, exhibiting a preference, similar to the parent vesamicol, for the levorotatory isomer [(-)-**11a** vs (+)-**11a**]. Therefore, the simple lipophilic acyclic vicinal amino alcohols may successfully mimic the biological activity of vesamicol.

Introduction

The lipophilic amino alcohol *trans*-2-(4-phenylpiperidino)cyclohexanol (**1**, vesamicol, AH 5183) induces paralysis and death in rodents and other laboratory animals.[1,2] The biological activity of **1** is largely mediated

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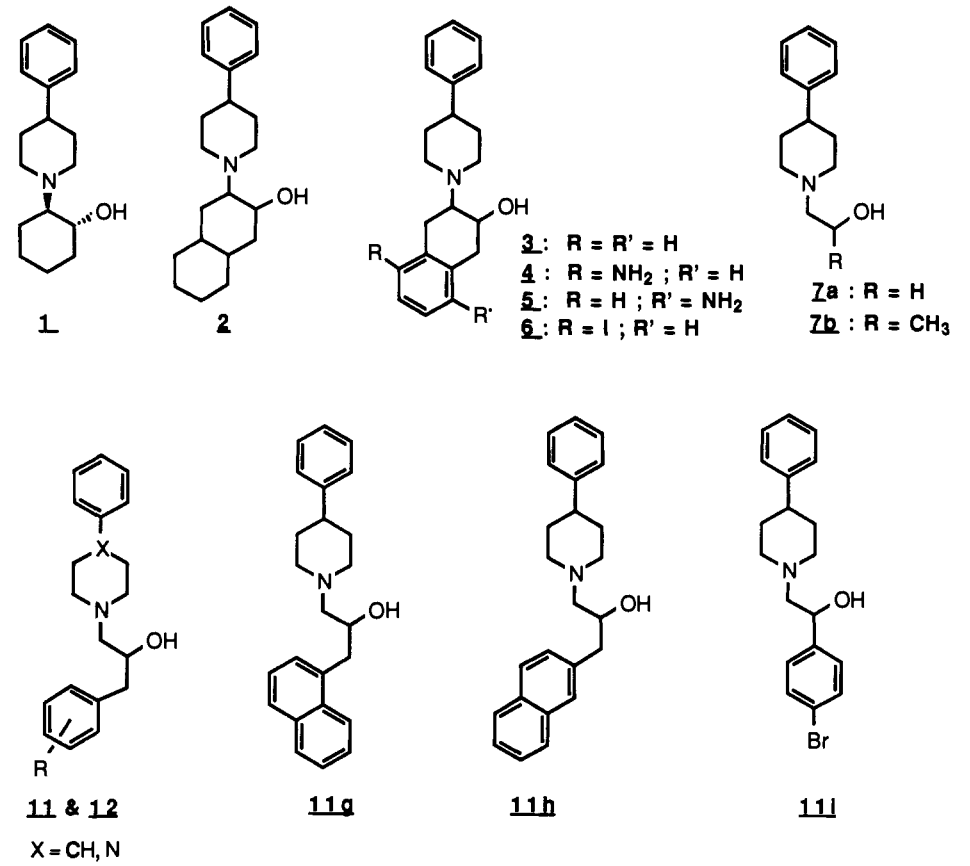

Figure 1. Vesamicol and analogues.

by its ability to inhibit both the uptake of acetylcholine (ACh) into synaptic vesicles and the release of this neurotransmitter from cholinergic terminals (for review, see ref 3). This inhibition results from the high-affinity binding of 1 to the vesamicol receptor, a cytoplasmically oriented site associated with the vesicular acetylcholine transporter.[4,5] At higher concentrations 1 also exhibits α-adrenoceptor blocking activity.[6,7] Given its presynaptic location, the vesamicol receptor may be a useful marker of cholinergic innervation. As such, this receptor may provide a suitable target for the development of novel anticholinergics and/or radiotracers for mapping central cholinergic pathways in the mammalian brain. This paper describes our attempts to develop a suitable structurally simple vesamicol analogue which may eventually be used for developing such a radiotracer.

In earlier studies, a large number of vesamicol analogues were examined, and several (e.g., **2**, **3**, **4**) were identified as potent inhibitors of vesicular acetylcholine storage.[8,9] All of the potent analogues that were identified contain more than one stereogenic center. In addition, the synthesis of substituted benzovesamicol analogues such as **4** yields a mixture of the positional isomers **4** and **5**, which differ in potency.[9] These must be separated to provide the active isomer **4**, which in turn must be resolved to yield the active enantiomer. A structurally simple but potent vesamicol analogue would eliminate the need for multiple separation steps. To develop such an analogue, a reexamination of existing structure–activity data was undertaken. In an earlier study, the authors noted that the vicinal amino alcohol functionality was important for potent vesamicol-like inhibitory activity.[9] In addition, replacement of the cyclohexyl moiety with fused bicyclic fragments yielded potent analogues such as **2**, **3**, and **4** (Figure 1), suggesting that the "benzo" fragment fulfilled some steric requirement at the receptor. In contrast, substitution of the cyclohexyl moiety with two- or three-carbon fragments yielded the weak inhibitors **7a** and **7b**. These observations point to a "minimum steric bulk" requirement for this region of the vesamicol receptor. Such a requirement may be attributed to essential nonbonded hydrophobic interactions within this region of the receptor. Based on the foregoing observations, we postulated (a) that the minimum bioactive fragment at the vesamicol receptor

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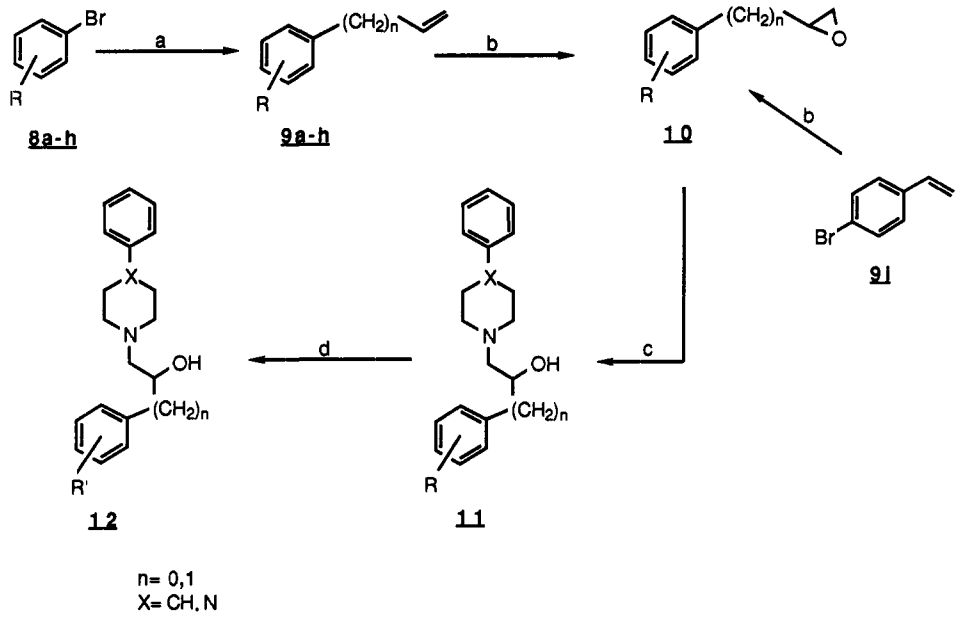
Scheme I.[a] Synthesis of Vesamicol Analogues



[a] (a) Mg, THF, reflux; allyl bromide; (b) *m*-CPBA, CH_2Cl_2; (c) 4-phenylpiperidine or 1-phenylpiperazine, EtOH, reflux; (d) BBr_3, CH_2Cl_2, −60 °C.

is represented by **7a**, and (b) that simple analogues of **4** which satisfy the steric requirement at the vesamicol receptor would be potent ligands for this receptor site.

In this paper, we describe the synthesis and the vesamicol receptor binding properties of these structurally simple analogues.

Chemistry

The target compounds **11a–j** were obtained from the corresponding alkenes via epoxidation and subsequent reaction with 4-phenylpiperidine (Scheme I). The yields for this two-step sequence ranged between 50 and 80%. The alkene precursors were obtained by reaction of allyl bromide with the corresponding Grignard reagent prepared in situ. The substituted ethanol, **11i**, was obtained from 4-bromostyrene, following the sequence outlined in Scheme I. Finally, the phenols **12a** and **12b** were obtained by dealkylation of the corresponding anisoles. All target compounds were found to be homogeneous on HPLC, although some of these samples were not analytically pure. The assignment of structure for the target amino alcohols is based on the preferential attack of the secondary amine at the less hindered carbon of the epoxide. In the NMR spectrum of **11b**, the methine proton at the C2 position of the propyl fragment appears as a multiplet at δ 3.91. On acetylation (Ac_2O, Et_3N) this signal shifts to δ 5.23, confirming the assignment of structure. The racemate **11a** was successfully resolved by fractional crystallization of the diastereomeric tartrates to yield (+)-**11a** and (−)-**11a** in 97% and 88% ee, respectively. The enantiomeric purity was determined by HPLC on a Chiralcel OD column which provides facile separation of (+)-**11a** (12.3 min) and (−)-**11a** (7.2 min).

Results and Discussion

The tertiary amino alcohol **1** (vesamicol) represents an interesting class of neutral lipophilic anticholinergics which has not been fully explored. Recent attempts to identify more potent and selective analogues of vesamicol have yielded a number of potent compounds.[8,9] In these studies, it was observed that all potent vesamicol analogues contain the cyclohexyl ring. Replacement of this ring with either an acyclic or cyclopentyl fragment resulted in significant reductions in potency. However, the addition of a fused bicyclic moiety yielded some of the most potent analogues known. Based on these observations, the authors concluded that the cyclohexyl ring was necessary for potent inhibition of vesicular acetylcholine storage. In our search for a potent structurally simple vesamicol analogue, a reexamination of the data indicated that (a) the ethanol analogue **7a** was a very weak inhibitor of vesicular acetylcholine storage and (b) the addition of an α-methyl group, to yield **7b**, resulted in a 30-fold increase in activity. Based on the totality of available evidence, we suggested (1) that the minimum bioactive fragment at the vesamicol receptor was represented by the substituted ethanol **7a**, (2) that region of the binding site which accommodates the cyclohexyl (or fused bicyclic) moiety is characterized by a minimum steric bulk requirement, and (3) that this requirement may be satisfied by bulky groups linked to the hydroxyl-containing acyclic fragment (as in **11a** and its analogues). The biological data obtained in the present study supports these propositions.

Although none of these new compounds contain the cyclohexyl ring, some are nevertheless fairly potent ligands for the vesamicol receptor (Table I). The levorotary isomer (−)-**11a**, a structural mimic of benzovesamicol, **3**, is essentially equipotent with vesamicol. In spite of the inherent flexibility of **11a**, receptor binding is stereoselective, the levorotatory isomer being more potent than its corresponding antipode. Since the cyclohexyl-containing analogues such as **1**, **4**, and **6** also exhibit a similar levo/dextro enantioselectivity,[8-10] **11a** and its analogues would appear to be suitable mimics of benzovesamicol. The observed enantioselectivity also suggests similarities in ligand stereochemistry. Since the absolute configuration of the hydroxyl-substituted carbon of (−)-vesamicol is *R*, we suggest that the levorotatory enantiomer (−)-**11a** may be designated (*R*)-**11a**.

The binding data also reveals a number of other trends. Replacement of the piperidyl fragment with a piperazyl moiety is accompanied by significant reductions in binding

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Table I. Binding Affinities of Amino Alcohols at the Vesamicol Receptor[a]

compound	R	R'	X	IC_{50}[c] (nM)
(*dl*)-vesamicol[b]	-	-	-	34 ± 6
(+)-**11a**	*p*-Br	-	CH	328 ± 108
(-)-**11a**	*p*-Br	-	CH	36 ± 5
(*dl*)-**11a**	*p*-Br	-	CH	170 ± 20
11b	*m*-Br	-	CH	73 ± 17
11c	*p*-OMe	-	CH	ND
11d	*m*-OMe	-	CH	115 ± 14
11e	*p*-Br	-	N	1540 ± 260
11f	*m*-Br	-	N	990 ± 102
11g	2,3-benzo	-	CH	1400 ± 300
11b	3,4-benzo	-	CH	145 ± 15
11i	*p*-Br	-	CH	30 ± 5
11j	*m*-I	-	CH	73 ± 11
12a	-	*p*-OH	CH	220 ± 54
12b	-	*m*-OH	CH	520 ± 30

[a] The compounds were tested as the corresponding hydrochlorides, using highly purified synaptic vesicles obtained from the electric organ of *Torpedo californica*, following a procedure described earlier.[8] [b] Data obtained from ref 4. [c] Data provided is for the corresponding hydrochlorides. IC_{50} values are quoted ±1 standard deviation.

affinity (**11a** vs **11e** and **11b** vs **11f**). This observation is consistent with an earlier report on vesamicol analogues.[9] On the whole, most single-point substitutions on the pendant phenyl ring appear to have only minimal effects on binding affinity. Halogen substitution at the meta position is favored over the para position (**11b** and **11j** vs **11a**). In contrast, hydrophilic substituents are better tolerated at the para position (**12a** vs **12b**). The presence of a second fused ring results in variable effects on binding affinity. Although the β-naphthyl analogue **11h** is essentially equipotent with **11a**, the corresponding α-naphthyl isomer **11g** is substantially less potent. The differential affinity of these isomeric naphthalenes, which may be partly attributed to the rigidity of the fused bicyclic system, suggests a preferred orientation for bulky substituents in this region of the receptor site. Interestingly, racemic **11i** is equipotent with *dl*-vesamicol and thus provides a new lead for subsequent investigation. While it may be argued that the activity of **11a** and the other propyl analogues could be anticipated on the basis of the close structural analogy with benzovesamicol, the potent activity of **11i** strongly supports the view that neither a cyclohexyl group nor an obvious mimic thereof is necessary for high-affinity binding to this receptor. We have therefore demonstrated that potent but structurally simple vesamicol analogs may be developed to probe this area of cholinergic pharmacology. Finally, some of these potent analogues (e.g., **11a**, **11i**, and **11j**) will be subsequently utilized to develop new radiotracers for evaluating cholinergic function.

Experimental Section

General. Synthetic intermediates were purchased from Aldrich, Inc. (Milwaukee, WI) and were used as received. Solvents were distilled immediately prior to use. Commercially available reagents were used without subsequent purification.

All air-sensitive reactions were carried out under nitrogen. Standard handling techniques for air-sensitive materials were employed throughout this study. Melting points were determined on a Mel-Temp melting point apparatus and are uncorrected. The specific rotation was determined on an automatic polarimeter (Autopol III, Rudolph Research, Flanders, NJ). ^1H NMR spectra were recorded on an IBM-Bruker spectrometer at 200 MHz. NMR spectra are referenced to the deuterium lock frequency of the spectrometer. Under these conditions, the chemical shifts (in ppm) of residual solvent in the ^1H NMR spectra were found to be, respectively, $CHCl_3$, 7.26; DMSO, 2.56; HOD, 4.81. The following abbreviations are used to describe the peak patterns when appropriate: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Both low- and high-resolution MS were performed on an AEI MS-30 instrument. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Unless otherwise indicated, these values are within ±0.4% of the theoretical.

Column chromatography was performed using "Baker Analyzed" silica gel (60–200 mesh). Preparative chromatography was performed on either a Harrison Research Chromatotron using Merck 60 PF_{254} silica gel or a preparative HPLC (Rainin Instrument Co.) using a 41.1-mm i.d. Dynamax silica gel column (at a solvent delivery rate of 80 mL/min). Enantiomeric purity was determined by HPLC with a Chiralcel OD column (isopropyl alcohol–hexane–Et_3N, 10:89:1; flow rate 1 mL/min). Analytical TLC was performed on Analtech glass TLC plates coated with silica gel GHLF and were visualized with UV light and/or methanolic iodine. All target compounds were checked for purity by HPLC (silica gel, 10–20% isopropyl alcohol–hexanes, trace Et_3N). Representative procedures for the steps shown on Scheme I are provided below as procedures A–D.

Procedure A. 2-[3-(1-Propenyl)]naphthalene (9h). Allyl bromide (4.8 mL, 55 mmol) in THF (25 mL) was added to a solution of the Grignard reagent prepared by adding 2-bromonaphthalene (7.0 mL, 50 mmol) in THF (25 mL) to a suspension of magnesium (1.2 g, 50 mmol) in THF (50 mL). The reaction mixture was refluxed for 5 h, allowed to cool, and quenched with a saturated solution of NH_4Cl (50 mL). After dilution with H_2O (100 mL), the mixture was extracted with CH_2Cl_2 (3 × 100 mL). The combined extracts were dried over Na_2SO_4 and concentrated to a residue. The latter was subsequently distilled (86° C (1.4 torr)) to give 6.1 g (75.0%) of a colorless oil: ^1H NMR ($CDCl_3$) δ 3.68 (d, 2 H, J = 6.6 Hz), 5.24 (m, 1 H), 5.32 (m, 1 H), 6.20 (m, 1 H), 7.53 (m, 3 H), 7.76 (s, 1 H), 7.93 (m, 3 H); CIMS (NH_3) m/e (intensity) 170.2 ($(M + 2H)^+$, 100.0).

Procedure B. 2-[3-(1,2-Epoxypropyl)]naphthalene (10h). A solution of 2-[3-(1-propenyl)]naphthalene (5.9 g, 35 mmol) in CH_2Cl_2 (200 mL) was cooled in an ice bath. *m*-CPBA (50%, 13.3 g, 38.5 mmol) was added in small portions over 5 min. The solution was allowed to come to room temperature, stirred for 16 h, and subsequently concentrated under reduced pressure. The residue was triturated with CCl_4 and filtered to remove *m*-chlorobenzoic acid. The filtrate was washed with 5% $NaHSO_3$–5% $NaHCO_3$ (100 mL, 1:1). The aqueous layer was subsequently washed with CCl_4 (2 × 100 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give 6.7 g (quantitative) of a yellow oil, which was carried on without further purification: ^1H NMR ($CDCl_3$) δ 2.62 (dd, 1 H, J = 2.7 Hz, J = 5.0 Hz), 2.85 (m, 1 H), 3.06 (m, 2 H), 3.27 (m, 1 H), 7.48 (m, 3 H), 7.74 (s, 1 H), 7.85 (m, 2 H); CIMS (NH_3) m/e (intensity) 185.9 ($(M + 2H)^+$, 100.0).

Procedure C. (±)-2-Hydroxy-3-(2-naphthyl)-1-[1-(4-phenylpiperidinyl)]propane (11h). 2-[3-(1,2-Epoxypropyl)]-naphthalene (2.8 g, 15 mmol), **10h**, and 4-phenylpiperidine (2.4 g, 15 mmol) were dissolved in EtOH (30 mL) and refluxed for 3 h. The solution was concentrated under reduced pressure, and the residue was purified by HPLC on SiO_2 (hexane–i-PrOH–Et_3N, 90:10:1). The desired fractions were collected and concentrated under reduced pressure to give 10.4 g (80%) of a dark yellow oil. The hydrochloride was prepared by treating an ethanolic solution of the amine with HCl gas and precipitating out the salt with Et_2O to give off-white cubic crystals (mp 213–15 °C): ^1H NMR (DMSO-d_6) δ 1.89 (m, 2 H), 2.16 (m, 2 H), 2.78 (m, 1 H), 2.95 (d, 2 H, J = 6.0 Hz), 3.14 (m, 2 H), 3.47 (br s, 2 H), 3.63 (m, 2 H), 4.47 (m, 1 H), 5.81 (br s, 1 H), 7.29 (m, 5 H), 7.49 (m, 3 H), 7.86 (m, 4 H), 10.33 (br s, 1 H). Anal. ($C_{24}H_{27}NO$·HCl) C, H, N.

3-(4-Bromophenyl)-2-hydroxy-1-[1-(4-phenylpiperidinyl)]propane (11a). Compound **9a** was prepared in 80% yield as previously described.[12] Starting with 4.0 g (20 mmol) of **9a**, the crude epoxide **10a** was obtained, according to procedure A, in 83% yield. The latter was reacted with 2.42 g (15 mmol) of 4-phenylpiperidine (procedure B) to yield, after chromatography, 2.7 g (48%) of crystalline **11a**: ^1H NMR ($CDCl_3$) δ 1.65 (m, 4 H), 2.01 (t, 1 H), 2.32–2.93 (m, 7 H), 3.08 (d, 1 H), 3.78 (br s, 1 H), 3.92 (m, 1 H), 7.13–7.36 (m, 7 H), 7.46 (d, 2 H). The hydrochloride was obtained, as described in procedure C, as a white solid: mp 194–198 °C. Anal. ($C_{20}H_{24}BrNO$·HCl) C, H, N.

3-(3-Bromophenyl)-2-hydroxy-1-[1-(4-phenylpiperidinyl)]propane (11b). Following Kugelrohr distillation (50–80 °C (0.3 mmHg)), compound **9b** was obtained, as a colorless liquid, in 87% yield from 1,3-dibromobenzene (procedure A): ^{1}H NMR ($CDCl_3$) δ 3.42 (m, 2 H), 5.15 (m, 2 H), 6.00 (m, 1 H), 7.28 (m, 4 H). Compound **11b** was obtained as a white crystalline hydrochloride in 35% yield after two steps (procedures B and C): mp 161–163 °C; ^{1}H NMR (DMSO-d_6) δ 1.90 (m, 2 H), 2.16 (m, 2 H), 2.78 (m, 2 H), 3.10 (m, 4 H), 3.38 (br s, 1 H), 3.63 (m, 2 H), 4.35 (br s, 1 H), 5.72 (m, 1 H), 7.37 (m, 9 H), 10.30 (br s, 1 H). Anal. ($C_{20}H_{24}BrNO$·HCl) N, H, C: calcd 58.48, found 60.27.

^{1}H NMR ($CDCl_3$) for the free base, **11b**, δ 1.81 (m, 4 H), 2.02 (dt, 1 H, J = 2.7 Hz, J' = 11.3 Hz), 2.42 (m, 4 H), 2.66 (dd, 1 H, J = 5.2 Hz, J' = 13.7 Hz), 2.79 (dd, 1 H, J = 7.1 Hz, J' = 13.9 Hz), 2.92 (br d, 1 H, J = 10.9 Hz), 3.08 (d, 1 H, J = 11.0 Hz), 3.91 (m, 2 H), 7.28 (m, 9 H). A small quantity of the free base was treated with Ac_2O/Et_3N to yield to acetate of **11b**: ^{1}H NMR ($CDCl_3$) δ 1.78 (m, 4 H), 2.05 (s, 3 H, acetyl), 2.18 (m, 2 H), 2.37–2.59 (m, 3 H), 2.80–3.03 (m, 4 H), 5.23 (m, 1 H), 7.15–7.43 (m, 9 H).

2-Hydroxy-3-(4-methoxyphenyl)-1-[1-(4-phenylpiperidinyl)]propane (11c). Starting with 4-bromoanisole (15.0 g, 80 mmol), procedure A yielded 9.0 g (76%) of **9c** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 3.41 (d, 2 H), 3.82 (s, 3 H), 5.06–5.16 (m, 2 H), 5.90–6.10 (m, 1 H), 6.88 (d, 2 H, J = 8.6 Hz), 7.15 (d, 2 H, J = 8.6 Hz).

The product **9c** was subjected to procedure B to yield 6.5 g (79%) of the epoxide **10c** as a colorless liquid: ^{1}H NMR ($CDCl_3$) δ 2.50–3.16 (m, 5 H), 3.78 (s, 3 H), 6.85 (d, 2 H, J = 8.6 Hz), 7.17 (d, 2 H, J = 8.6 Hz).

Compound **10c** was reacted with 4-phenylpiperidine (procedure C) to yield 3.0 g (42%) of the hydrochloride **11c**: mp 207.1 °C; ^{1}H NMR (DMSO-d_6) δ 1.93–3.04 (m, 9 H), 3.58 (d, 4 H, J = 11.0 Hz), 3.73 (s, 3 H), 4.29 (br s, 1 H), 5.66 (d, 1 H, J = 5.1 Hz), 6.85–7.37 (m, 9 H). Anal. ($C_{21}H_{27}NO_2$·HCl) C, H, N.

2-Hydroxy-3-(3-methoxyphenyl)-1-[1-(4-phenylpiperidinyl)]propane (11d). 3-(3-Methoxyphenyl)propene, **9d**, was obtained from 3-bromoanisole, according to procedure A, in 85% yield: ^{1}H NMR ($CDCl_3$) δ 3.44 (d, 2 H, J = 6.7 Hz), 3.85 (s, 3 H), 5.13–5.29 (m, 2 H), 5.94–6.15 (m, 1 H), 6.81–7.33 (m, 4 H).

The epoxide **10d** was subsequently obtained in 80% yield from **9d** (procedure B): ^{1}H NMR ($CDCl_3$) δ 2.54–3.27 (m, 5 H), 3.80 (s, 3 H), 6.76–7.27 (m, 4 H).

The target hydrochloride **11d** was finally obtained as a white solid, following procedure C, in 33% yield: mp 180 °C (isopropyl alcohol); ^{1}H NMR (DMSO-d_6) δ 1.93–3.07 (m, 9 H), 3.60 (d, 2 H, J = 13.0 Hz), 3.75 (s, 3 H), 4.32 (br s, 1 H), 5.87 (br s, 1 H), 6.74–7.27 (m, 9 H), 10.06 (br s, 1 H). Anal. ($C_{21}H_{27}NO_2$·HCl) C, H, N.

3-(4-Bromophenyl)-2-hydroxy-1-[1-(4-phenylpiperazinyl)]propane (11e). The reaction of **10a** with 1-phenylpiperazine, as described in procedure C, provided the white crystalline hydrochloride **11e** in 13% yield: mp 235.5 °C; ^{1}H NMR (DMSO-d_6) δ 2.73 (t, 2 H, J = 5.7 Hz), 3.16–3.25 (m, 6 H), 3.62–3.76 (m, 4 H), 4.31 (br s, 1 H), 6.87 (t, 1 H, J = 7.2 Hz), 7.01 (d, 2 H, J = 8.0 Hz), 7.26 (m, 4 H), 7.58 (d, 2 H), 7.94 (br s, 2 H). Anal. ($C_{19}H_{23}BrN_2O$·2HCl) H, N, C: calcd 50.91, found 51.48.

3-(3-Bromophenyl)-2-hydroxy-1-[1-(4-phenylpiperazinyl)]propane (11f). 1-Phenylpiperazine and the epoxide **10b** were reacted (procedure C) to yield the white crystalline hydrochloride **11f** in 13% yield: mp 216.9 °C; ^{1}H NMR (DMSO-d_6) δ 2.73 (t, 2 H, J = 5.7 Hz), 3.16–3.25 (m, 6 H), 3.62–3.76 (m, 4 H), 4.35 (br s, 1 H), 6.89 (t, 1 H, J = 7.2 Hz), 7.04 (d, 2 H, J = 8.1 Hz), 7.28 (m, 4 H), 7.40–7.51 (m, 2 H), 8.73 (br s, 2 H). Anal. ($C_{19}H_{23}BrN_2O$·2HCl) H, N, C: calcd 50.91, found 51.95.

2-Hydroxy-3-(1-naphthyl)-1-(4-phenylpiperidinyl)propane (11g). The reaction of 2-bromonaphthalene, **8g**, and allyl bromide (procedure A) yielded **9g**, as a colorless liquid, in 75% yield: bp 86 °C (1.5 mmHg); ^{1}H NMR ($CDCl_3$) δ 3.68 (d, 2 H, J = 6.6 Hz), 5.24 (m, 1 H), 5.32 (m, 1 H), 6.20 (m, 1 H), 7.53 (m, 3 H), 7.76 (s, 1 H), 7.93 (m, 3 H); CIMS (NH_3) m/e (intensity) 170.2 (M + $2H^+$, 100.0).

The arylalkene **9g** was subjected to procedure B to provide a quantitative yield of the yellow liquid epoxide **10g**: ^{1}H NMR ($CDCl_3$) δ 2.62 (dd, 1 H, J = 2.7 Hz, J' = 5.0 Hz), 2.85 (m, 1 H), 3.06 (m, 2 H), 3.27 (m, 1 H), 7.48 (m, 3 H), 7.74 (s, 1 H), 7.85 (m, 2 H); CIMS (NH_3) m/e (intensity) 185.9 (M + $2H^+$, 100).

The hydrochloride **11g** was obtained, as a white crystalline solid, in 80% yield (procedure C): mp 213–215 °C; ^{1}H NMR (DMSO-d_6) δ 1.89 (m, 2 H), 2.16 (m, 2 H), 2.78 (m, 1 H), 2.95 (d, 2 H, J = 6.0 Hz), 3.14 (m, 2 H), 3.47 (br s, 2 H), 3.63 (m, 2 H), 4.47 (m, 1 H), 5.81 (br s, 1 H), 7.29 (m, 5 H), 7.49 (m, 3 H), 7.86 (m, 4 H), 10.33 (br s, 1 H). Anal. ($C_{24}H_{27}NO$·HCl) C, H, N.

***dl*-1-(4-Bromophenyl)-2-(4-phenylpiperidinyl)ethanol (11i).** Compound **11i** was obtained from the epoxidation of 4-bromostyrene and subsequent reaction of the epoxide with 4-phenylpiperidine in ethanol (procedures B and C). The product was purified by HPLC (20% isopropyl alcohol–hexanes, trace Et_3N; silica gel) to yield a white solid (48%): ^{1}H NMR ($CDCl_3$) δ 1.86 (m, 4 H), 2.17 (t, 1 H), 2.37–2.55 (m, 4 H), 2.93 (d, 1 H), 3.26 (d, 1 H), 4.26 (br s, 1 H), 4.72 (dd, 1 H), 7.22–7.36 (m, 7 H), 7.48 (d, 2 H). The hydrochloride was recrystallized from isopropyl alcohol, mp 233–236 °C. Anal. ($C_{19}H_{22}BrNO$·HCl·$^1/_2H_2O$) C, H, N.

Procedure D. 2-Hydroxy-3-(4-hydroxyphenyl)-1-[1-(4-phenylpiperidinyl)]propane (12a). Compound **11c** (1.0 g, 3.1 mmol) was dissolved in 60 mL of dry CH_2Cl_2, and the solution was cooled to −60 °C (dry ice–acetone–CCl_4). A 1 M solution of BBr_3 (3.82 mL) was then added dropwise, under nitrogen, while the temperature was maintained at −60 °C. After 1 h, the reaction mixture was allowed to warm up to room temperature and stirred for an additional 12 h. The reaction mixture was subsequently treated with 10 mL of MeOH and concentrated under reduced pressure. The residue was diluted with 10% aqueous NaOH (100 mL) and extracted with EtOAc (150 mL). The product was converted into the corresponding hydrochloride by bubbling HCl(g) into this solution. The precipitated salt was finally recrystallized from isopropyl alcohol to yield 0.8 g (75%) of **12a**: mp 190 °C; ^{1}H NMR (DMSO-d_6) δ 1.85–3.63 (m, 14 H), 4.24 (br s, 1 H), 5.65 (br s, 1 H), 6.73 (d, 2 H, J = 8.3 Hz), 7.05 (d, 2 H, J = 8.3 Hz), 7.21–7.36 (m, 5 H), 9.40 (s, 1 H), 10.18 (br s, 1 H). Anal. ($C_{20}H_{25}NO_2$·HCl·$^1/_{10}H_2O$) C, H, N.

2-Hydroxy-3-(3-hydroxyphenyl)-1-[1-(4-phenylpiperidinyl)]propane (12b). Starting with 0.85 g (2.2 mmol) of **11d**, the demethylation was carried out, according to procedure D, to yield 0.35 g (46%) of the hydrochloride **12b**: mp 216.5 °C; ^{1}H NMR (DMSO-d_6) δ 1.94–3.61 (m, 14 H), 4.25 (br s, 1 H), 5.68 (d, 1 H, J = 5.2 Hz), 6.63–7.33 (m, 9 H), 9.41 (s, 1 H), 9.98 (br s, 1 H). Anal. ($C_{20}H_{25}NO_2$·HCl·$^1/_5H_2O$) C, H, N.

Resolution of (*dl*)-11a. Compound **11a** was resolved according to a procedure described earlier for vesamicol.[9] Di-*p*-toluoyl-L-tartaric acid monohydrate (4.72 g, 11.67 mmol) was added portionwise, at room temperature, to a stirring solution of *dl*-**11a** (4.0 g, 10.7 mmol) in 50 mL of acetone. Following the addition, stirring was continued at room temperature for 12 h. At this time the resulting crystals were collected by filtration, dried, and weighed to yield 4.8 g of the crude product. The latter was dissolved in 220 mL of boiling acetonitrile and cooled to 4 °C. After 12 h, the product was collected by filtration and dried to yield 3.3 g of tartrate; $[\alpha]_D$ = −48.68° (c = 0.025, MeOH). Subsequent recrystallization of this product from 190 mL of boiling acetonitrile provided 2.6 g (63%) of the (−)-tartrate of **11a**; mp 184.9 °C. The free base (−)-**11a** was obtained by treatment of the tartrate with saturated $NaHCO_3$ and subsequent extraction into CH_2Cl_2 (3 × 30 mL). The enantiomeric purity of (−)-**11a** estimated by HPLC using a Chiralcel OD column (10% isopropyl alcohol–hexanes; flow rate 1 mL/min; retention time 7.2 min) was 94%. All the mother liquors from above were combined and concentrated in vacuo to a residue. The latter was treated with 1 M NaOH (70 mL), and the resulting mixture was extracted with EtOAc (250 mL). The organic extract was dried over Na_2SO_4 and subsequently concentrated to provide 2.34 g of free base enriched in (+)-**11a**. The latter sample was dissolved in acetone (25 mL) and treated dropwise with a solution of 2.76 g (7.14 mmol) of (+)-di-*p*-toluolyltartaric acid monohydrate in an equal volume of acetone. After stirring for 16 h, the crystals were collected, dried, and recrystallized from boiling acetonitrile (2 × 200 mL) to yield 1.85 g (40%) of (+)-**11a** tartrate: mp 186 °C; $[\alpha]_D$ = +49.5° (c = 0.023, MeOH). The enantiomeric purity of the free base (+)-**11a** was estimated, as outlined above, to be 98.6% (retention time 12.3 min).

Biological. The compounds were evaluated in a purified preparation of cholinergic synaptic vesicles isolated from the electric organ of *Torpedo californica*. A full description of this assay was described earlier.[8] However, the present study was carried out in the absence of ATP and acetylcholine, with a trace amount of [^{3}H]vesamicol. The data reported are averages of duplicates, which exhibit a relative range of less than 5%. Nonlinear regression analysis was carried out with MINSQ (MicroMath Scientific Software, Salt Lake City, UT). Protein content was determined by the method of Bradford,[11] using a bovine serum albumin standard.

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Synthesis of 2-Deoxy-2-[(2,2-difluoro-3-hydroxytetradecanoyl)amino]-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-glucopyranose 4-Phosphate

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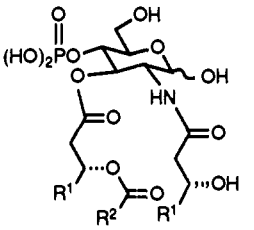
2-Deoxy-2-[(2,2-difluoro-3-hydroxytetradecanoyl)amino]-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-glucopyranose 4-phosphates (**9H,L**) were synthesized from allyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (1), (±)-3-[(benzyloxycarbonyl)oxy]-2,2-difluorotetradecanoic acid, and (*R*)-3-(tetradecanoyloxy)tetradecanoic acid. Both compounds **9H** and **9L** were more active than GLA-60 for the prostaglandin D_2 releasing test on macrophages.

Lipopolysaccharide (LPS), an outer-membrane component of Gram-negative bacterial cells, causes fever and lethal shock in higher animals. This toxic principle is called "endotoxin". Westphal et al.[1] isolated lipid A, which is the lipophilic part of LPS. Lipid A shows most of the endotoxic activities of LPS, and it was first chemically synthesized by Shiba et al.[2] Also, Raetz et al.[3] isolated lipid X from a mutant of *Escherichia coli*. Lipid X, which is the reducing sugar part of lipid A, is also one biosynthetic precursor of lipid A. In a series of investigations on the active center of the biological activities of LPS, Hasegawa and Kiso have demonstrated that the nonreducing-sugar subunit analogues of lipid A (namely, some 4-*O*-phosphonoglucosamine derivatives)[4] expressed several kinds of biological activities of endotoxin and was the least active center of LPS. In particular, 2-deoxy-2-[(*R*)-3-hydroxytetradecanoyl)amino]-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-glucopyranose 4-phosphate (GLA-60) (Chart I), one of the 4-phosphonoglucosamine analogues, possesses potent features as a therapeutic agent. In view of this result, we synthesized some compounds related to GLA-60. In this paper, we would like to describe the syntheses of 2-deoxy-2-[((3*R* and 3*S*)-2,2-difluoro-3-hydroxytetradecanoyl)amino]-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-glucopyranose 4-phosphates **9**.

Chart I



$R^1 = n\text{-}C_{11}H_{23}$
$R^2 = n\text{-}C_{13}H_{27}$
GLA-60

Chemistry

The starting allyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (1), which was obtained from allyl 2-deoxy-4,6-*O*-isopropylidene-2-trifluoroacetamido-β-D-glucopyranoside,[5] was treated with (±)-3-[(benzyloxycarbonyl)oxy]-2,2-difluorotetradecanoyl chloride, which was obtained from the corresponding carboxylic

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