the above medium. The fermentation was stopped after 37 h at which time analysis by HPLC indicated 7.9 g/L of **2**; acetate, lactate, and ethanol were also produced.[18]

In practical use, the addition of resazurin and the analysis by HPLC are not necessary. To accommodate foaming, a 5-gal carboy should contain no more than 15 L of medium. The periodic addition of antifoam (FG-10, Dow-Corning, concentration ≤0.5 g/L) can control foaming.

Purification of (−)-(*R*)-1,2-Propanediol (2). Centrifugation (4000*g*, 25 min) of the cell suspension resulting from a 5-L fermentation separated the cell mass from the broth. The broth was concentrated to 880 mL by rotary evaporation at 0.1 Torr after the addition of 0.1 mL/L of silicon antifoam (FG-10, Dow-Corning). Overnight storage of the concentrate at 4 °C precipitated salts and protein that were removed by centrifugation. Buchner filtration of the concentrate and passage through a 0.45-μm filter prevented the formation of an emulsion during the subsequent continuous extraction into ether. After 120 h of continuous extraction (water:ether ≃ 1:1.4, v:v), concentration of the ethereal phase yielded 31 g of crude **2**, which was distilled at reduced pressure to yield 23 g (0.3 mol) of a clear, viscous liquid: ee >99%; $[\alpha]^{23}_{D}$ −22.0° (*c* 7.5, H_2O) [lit.[19] $[\alpha]^{20}_{D}$ −22.2° (*c* 7.5, H_2O)]; ^1H NMR spectrum agreed with that of the *S* enantiomer.[13] Further continuous extraction yielded an additional 12 g of crude **2** after nine more days.

(+)-(*R*)-Methyloxirane (4). Conversion of the diol to the epoxide proceeded via the acetoxybromopropanes according to published procedures.[13] Thus, from 25.0 g (330 mmol) of **2** was obtained 48.7 g (270 mmol, 82%) of a clear, colorless liquid whose ^1H NMR spectrum[13] identified it as mainly **3**; ^1H NMR spectroscopy indicated the presence of approximately 6% of 1-acetoxy-2-bromopropane. Conversion of the mixture (25.0 g, 138 mmol) to **4** by treatment with potassium pentanolate in pentan-1-ol followed the literature[13] to yield 6.8 g (121 mmol, 88%) of **4** as a clear, colorless liquid: bp 38–39 °C (lit.[13] bp 34–35 °C); ee >99%; $[\alpha]^{22}_{D}$ +18.0° (*c* 5.73, CCl_4) [(lit.[13] $[\alpha]^{18}_{D}$ +19.13° (*c* 5.66, CCl_4)]; ^1H NMR spectral data were in agreement with those in the literature.[13]

Registry No. **1**, 50-99-7; **2**, 4254-14-2; **3**, 99457-42-8; **4**, 15448-47-2; (*S*)-$H_3CCHBrCH_2OAc$, 109243-62-1.

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(17) Daniels, L.; Zeikus, J. G. *Appl. Microbiol.* **1975**, *29*, 710–711.

(18) The fermentation was repeated on a 15-L scale with similar results.

(19) Huff, E. *Biochim. Biophys. Acta* **1961**, *48*, 506–517.

Synthesis of Functionalized Bicyclic Dioxopiperazines via Intramolecular Epoxide Opening

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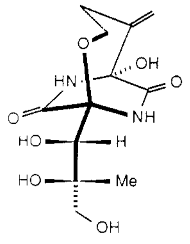
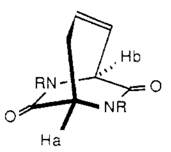
d,l-Homoserine was condensed with *N*-*p*-methoxybenzylglycine ethyl ester and alkylated to furnish (2-hydroxyethyl)piperazinedione **8**. Oxidation to the corresponding aldehyde and condensation with dimethylsulfoxonium methylide provided the epoxide **10**. Enolate formation resulted in an intramolecular epoxide opening reaction providing the bicyclo[2.2.2] (**11**) and bicyclo[3.2.2] (**12**) dioxopiperazines in a 3:1 ratio. Dehydration of **11** and **12** provided 6,8-bis(*p*-methoxybenzyl)-2-methylene-6,8-diazabicyclo[2.2.2]octane-5,7-dione (**2**) and 7,9-bis(*p*-methoxybenzyl)-7,9-diazabicyclo[3.2.2]non-2-ene-6,8-dione (**1**), respectively.

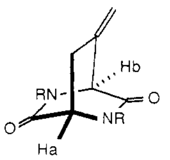
As part of a program directed at synthesizing and studying unnatural analogues of the natural antibiotic bicyclomycin,[1] the carbocyclic bicyclo[3.2.2] and bicyclo-[2.2.2] olefins **1** and **2** were chosen as potentially interesting targets. It was reasoned that omission of the bridging ether oxygen in the more highly strained bicyclo[3.2.2] and -[2.2.2] ring systems would impart both increased chemical stability[2] and increased biological reactivity to these analogues. Since neither functionalized ring system has been reported in the literature,[3,4] a new approach had to be devised that would produce useful quantities of **1** and **2**. In this paper is described a convenient and practical synthesis of these unusual bicyclic dipeptides using an intramolecular enolate epoxide cyclization reaction.



BICYCLOMYCIN



1



2

(±)-*N*-Carbobenzoxyhomoserine (**3**) is silylated (**4**) (Me_2Bu^+SiCl, DMF, Et_3N, 0 °C) and condensed with *N*-*p*-methoxybenzylglycine ethyl ester (DCC, THF, 25 °C) to afford the dipeptide **5**. Without purification, **5** was directly subjected to hydrogenolysis (10% Pd/C, H_2, EtOH, 1 atm) to afford the piperazinedione **6** in 40% overall yield from **3** (Scheme I). Alkylation of the remaining amide nitrogen with *p*-methoxybenzyl chloride in DMF in the presence of NaH furnished the fully protected substrate **7** (79%).[5] Desilylation (HF–Py complex) furnished alcohol **8** (87%) that was oxidized to the aldehyde **9** (78%). Homologation of the aldehyde with dimethylsulfoxonium methylide afforded the epoxides **10** (1:1

[†] Fellow of the Alfred P. Sloan Foundation 1986–88. NIH Research Career Development Awardee 1984–89. Eli Lilly Grantee 1986–88.

(1) (a) Williams, R. M.; Armstrong, R. W.; Dung, J.-S. *J. Am. Chem. Soc.* **1985**, *107*, 3253. (b) Williams, R. M.; Armstrong, R. W.; Dung, J.-S. *J. Med. Chem.* **1985**, *28*, 733.

(2) Bicyclomycin is known to undergo various acid catalyzed ring-opening reactions resulting from scission of bridging-ether linkage, see: Maag, H.; Blount, J. F.; Coffen, D. L.; Steppe, T. V.; Wong, F. *J. Am. Chem. Soc.* **1978**, *100*, 6786.

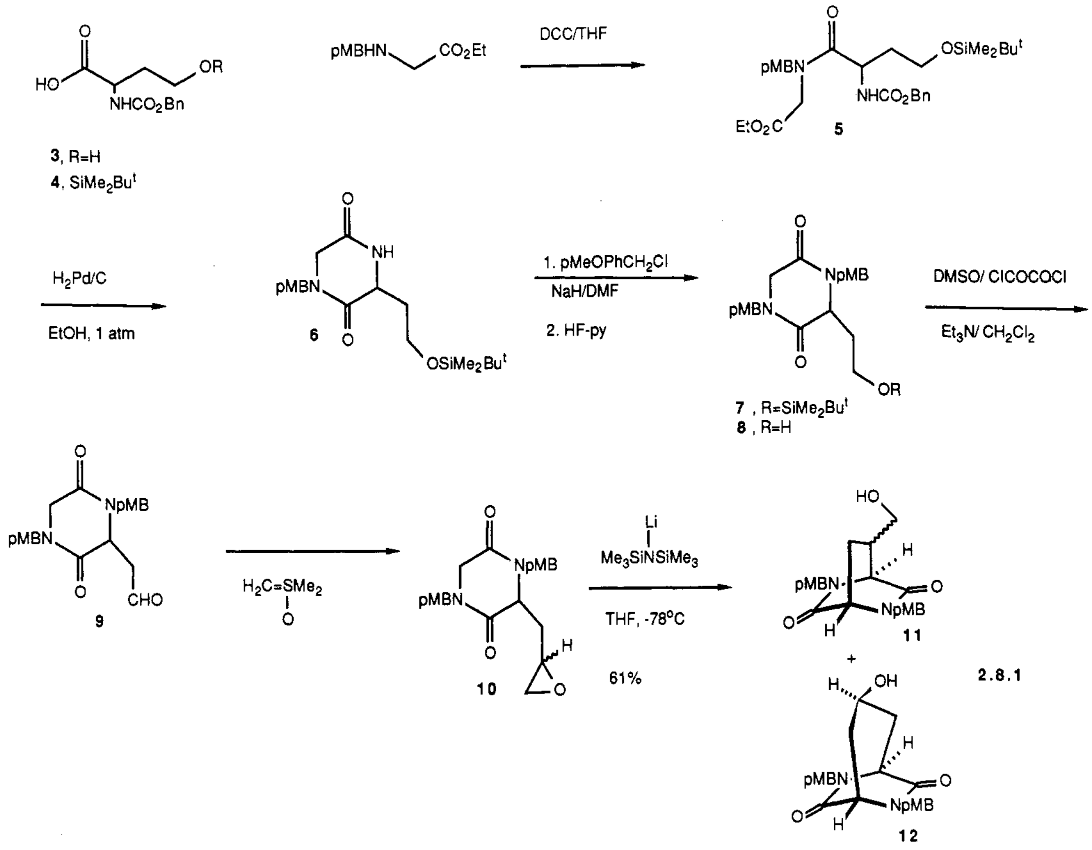
(3) For a synthesis of a simple bicyclo[2.2.2] dioxopiperazine system, see: (a) Kemp, D. S.; Sun, E. T. *Tetrahedron Lett* **1982**, *23*, 3759. (b) Eastwood, F. W.; Gunawardena, D.; Wernert, G. T. *Aust. J. Chem.* **1982**, *35*, 2289.

(4) Kemp et al. have used a bicyclo[2.2.2] dioxopiperazine as an intermediate in the preparation of β-turn-inducing dipeptide analogues, see ref 3a and Kemp, D. S.; McNamara, P. E. *J. Org. Chem.* **1985**, *50*, 5834. Syntheses of the bicyclic materials reported herein have potential application in this area.

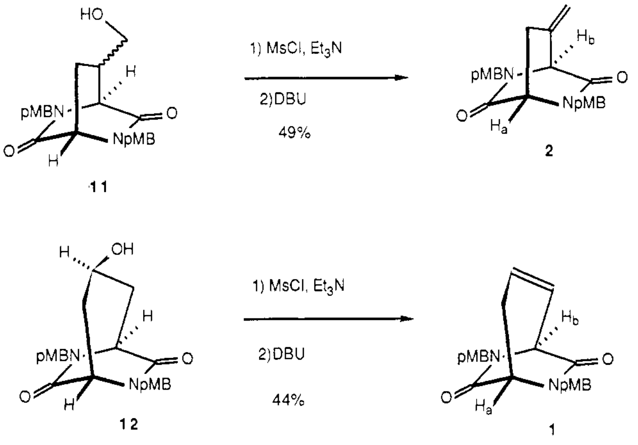
(5) Williams, R. M.; Glinka, T. *Tetrahedron Lett.* **1986**, *27*, 3581; complete experimental details for the preparation of **3**–**7** will appear elsewhere (Williams, R. M.; Glinka, T.; Kwast, E., manuscript in preparation).

0022-3263/87/1952-4044$01.50/0

Scheme I

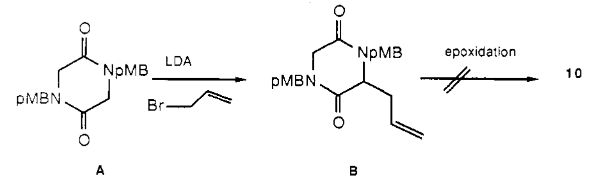


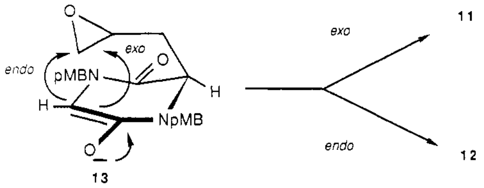
Scheme II



mixture of diastereomers 91%).

A comment regarding the choice of this particular route is in order. Initially, the conceptually much shorter route to **10** via the allyl derivative B was investigated. Allyl bromide alkylation of dioxopiperazine A could be accomplished in modest yield, but all attempts to epoxidize B → **10** failed; this route was unhappily abandoned. Fortunately, the sequence from homoserine could be conveniently carried out on a large scale and has furnished multigram quantities of **10**. In the key step, treatment of **10** with lithium hexamethyldisilazane furnished a 2.8:1 mixture of **11** and **12** in 61% combined yield. The cyclization reaction is noteworthy for several reasons. It seems that the entropically favored reaction at the more substituted position of the epoxide moiety is governed primarily by nucleophilic approach vector constraints.[6] Although six- and seven-membered rings are respectively formed for **11** and **12**, the conformational constraints imposed by the dioxopiperazine ring changes the analysis to better approximate four- and five-membered ring closures, respectively. On the basis of this assumption, the closure of enolate **13** to form **11** can be geometrically analyzed as approximating a *4-exo-tet* ring closure[6] and the closure of **13** to form **12** can be similarly analyzed as approximating a *5-endo-tet* ring closure.[6] An analogy between these results and the observations of Stork[7] on the epoxy nitrile cyclizations **14** → **15** can be made. Baldwin[6] has also pointed to the general preference for three-membered rings to undergo intramolecular ring-opening via the exo mode. Our results which exhibit a nearly 3:1 preference for exo attack corroborate these geometrical[6,7] considerations.





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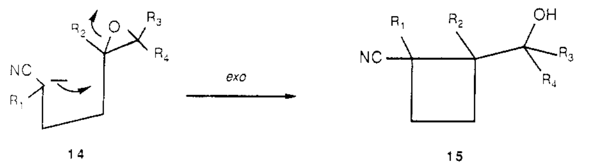
[6] Baldwin, J. E. *J. Chem. Soc., Chem. Commun.* **1976**, 734 and references cited therein.

[7] Stork, G.; Cohen, J. F. *J. Am. Chem. Soc.* **1974**, *96*, 5270.



14 15

Separation of **11** and **12** by silica gel chromatography and treatment of each isomer with methanesulfonyl chloride followed by elimination mediated by DBU furnished the olefins **1** and **2** (R = *p*-methoxybenzyl) in 49% and 44% yields, respectively (Scheme II). The spectroscopic properties of these materials are fully consistent with the assigned structures. In particular, the infrared absorptions of the amide carbonyls for **1** were at 1695 and 1675 cm^{-1} and for **2** were at 1690 cm^{-1}, indicative of considerable ring strain imposed by the bicyclic geometries.

Studies are in progress to elaborate these unusual bicyclic dipeptides and to further elucidate their fundamental chemical, structural, and possibly biological properties. It is also anticipated that the enolate epoxide-opening strategy employed herein will lead to more efficient syntheses of other bicyclo[*n*.2.2] dioxopiperazines related to and including the bicyclomycin ring system.

Experimental Section

1,4-Bis(*p*-methoxybenzyl)-3-(2-hydroxyethyl)-2,5-piperazinedione (8). To the silyl ether **7** (4.0 g, 7.8 mmol) in THF (45 mL) was added excess HF pyridine complex. After being stirred for 1 h at 25 °C, the reaction mixture was poured into 0.1 N NaOH and extracted 3 times with CH_2Cl_2. The organic extracts were combined, dried over anhydrous Na_2SO_4, filtered, concentrated, and separated by flash chromatography (eluted with EtOAc) to afford 2.7 g (87%) of the alcohol, mp 131–132 °C (recrystallized from EtOAc/hexanes).

^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 1.78–1.90 (1 H, m), 2.03–2.15 (1 H, m), 2.74 (1 H, s), 3.64 (2 H, m), 3.76 (6 H, s), 3.80–3.95 (3 H, m), 4.00–4.09 (1 H, m), 4.24 (1 H, $^1/_2$ AB q, J = 14.32 Hz), 4.67 (1 H, $^1/_2$ AB q, J = 14.32 Hz), 5.14 (1 H, $^1/_2$ AB q, J = 14.68 Hz), 6.80–7.16 (8 H, m). IR (NaCl, neat): 3420, 3025, 2940, 1660, 1615, 1465, 1170 cm^{-1}. Anal. ($C_{22}H_{26}N_2O_5$) Calcd: C, 66.31; H, 6.58; N, 7.08. Found: C, 66.03; H, 6.44; N, 6.88.

1,4-Bis(*p*-methoxybenzyl)-3-(formylmethyl)-2,5-piperazinedione (9). To a stirred solution of oxalyl chloride (0.89 mL, 10.2 mmol, 1.5 equiv) in CH_2Cl_2 (50 mL) at −78 °C was added Me_2SO (1.45 mL, 20.4 mmol, 3.0 equiv). After 30 min, the alcohol **8** (2.70 g, 6.8 mmol, 1.0 equiv) in CH_2Cl_2 (50 mL) was added at −78 °C. After 1 h Et_3N (4.76 ml, 34.0 mmol, 5.0 equiv) was added and the cooling bath was removed. The reaction mixture was stirred for 15 min, concentrated, and redissolved in THF. The insoluble $Et_3N{\cdot}HCl$ salt was filtered off and the crude residue purified by flash chromatography (silica gel) (eluted 100% EtOAc) to yield 2.1 g (78%) of aldehyde **9**, mp 100–104 °C (recrystallized from EtOAc/hexanes).

^1H NMR (270 MHz) ($CDCl_3$) δ $CHCl_3$: 2.88–3.12 (3 H, m), 3.75 (3 H, s), 3.76 (3 H, s), 3.82 (1 H, $^1/_2$ AB q, J = 17.15 Hz), 4.14 (1 H, s), 4.23 (1 H, $^1/_2$ AB q, J = 14.94 Hz), 4.38 (1 H, $^1/_2$ AB q, J = 14.38 Hz), 4.62 (1 H, $^1/_2$ AB q, J = 14.38 Hz), 4.79 (1 H, $^1/_2$ AB q, J = 14.94 Hz), 6.79–6.86 (4 H, m), 7.08–7.16 (4 H, m), 9.49 (1 H, s). IR (NaCl, neat): 1665, 1615, 1515, 1215, 750 cm^{-1}. Anal. ($C_{22}H_{24}N_2O_5$) Calcd: C, 66.65; H, 6.10; N, 7.07. Found: C, 66.82; H, 6.20; N, 7.17.

1,4-Bis(*p*-methoxybenzyl)-3-(2,3-epoxypropyl)-2,5-piperazinedione (10). NaH (15.4 mg 0.320 mmol; 1.2 equiv) was washed several times with hexane. To this was added trimethylsulfoxonium iodide (70.5 mg, 0.320 mmol, 1.2 equiv) and then Me_2SO (2 mL). After 20 min, the aldehyde **9** (106 mg, 0.267 mmol, 1.0 equiv) in Me_2SO (2.6 mL) was added to the ylide. The reaction mixture was stirred for 1 h and was then diluted with EtOAc and poured into water. After extracting several times with EtOAc, the combined organic extracts were washed with water two times and dried over $MgSO_4$. Filtration and concentration of the material yielded 100 mg (91%) of oil, which by ^1H NMR proved to be a clean, 1:1 mixture of diastereomeric epoxides (**10**).

^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 1.80–1.89 (2 H, m), 2.03–2.12 (2 H, m), 2.33–2.36 (1 H, m), 2.39–2.42 (1 H, m), 2.59–2.62 (1 H, m), 2.69–2.73 (1 H, m), 2.78–2.86 (2 H, m), 3.76 (12 H, s), 3.79–4.09 (8 H, m), 4.19 (1 H, $^1/_2$ AB q, J = 14.23 Hz), 4.36 (1 H, $^1/_2$ AB q, J = 14.34 Hz), 4.60 (1 H, $^1/_2$ AB q, J = 14.34 Hz), 4.78 (1 H, $^1/_2$ AB q, J = 14.23 Hz), 5.10 (1 H, $^1/_2$ AB q, J = 14.74 Hz), 5.18 (1 H, $^1/_2$ AB q, J = 14.74 Hz), 6.80–7.18 (16 H, m). IR: 2930, 2835, 1665, 1610, 1583, 1510, 1460, 1240, 1170, 1025 cm^{-1}. Mass spectrum, *m/e* (relative intensity): 410 (M^+, 3.7), 354 (2.3), 289 (2.1), 121 (100).

6,8-Bis(*p*-methoxybenzyl)-2-(hydroxymethyl)-6,8-diazabicyclo[2.2.2]octane-5,7-dione (11) and 7,9-Bis(*p*-methoxybenzyl)-3-hydroxy-7,9-diazabicyclo[3.2.2]nonane-6,8-dione (12). To a stirred solution of 1,1,1,3,3,3-hexamethyldisilazane (0.155 mL, 0.735 mmol, 5.5 equiv) at 0 °C in THF (2.5 mL) was added *n*-BuLi (0.345 mL, 0.805 mmol, 6.0 equiv). After 20 min an aliquot of the lithium base (0.6 mL, 0.147 mmol, 1.1 equiv) was added to a solution of epoxides **10** (55 mg, 0.134 mmol, 1.0 equiv) in THF (1.0 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 20 min and then at 25 °C for an additional 2 h. The reaction mixture was then diluted with CH_2Cl_2, poured into water, and extracted with CH_2Cl_2 three times. The combined extracts were dried over anhydrous Na_2SO_4, filtered, concentrated, and separated by PTLC (silica gel) (eluted 2:1 EtOAc/hexanes) to afford **11** (24.6 mg of diastereomeric mixture) and **12** (8.9 mg), bicyclic alcohols, in a 2.8:1 ratio (combined yield 61%).

11 (single isomer of unassigned relative stereochemistry). ^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 1.82–1.91 (1 H, m), 2.13–2.18 (1 H, m), 3.15–3.21 (2 H, m), 3.35–3.39 (1 H, m), 3.72 (3 H, s), 3.74 (3 H, s), 3.92 (1 H, s), 4.09 (1 H, m), 4.24 (1 H, $^1/_2$ AB q, J = 14.59 Hz), 4.42 (2 H, s), 4.48 (1 H, $^1/_2$ AB q, J = 14.59 Hz), 6.76–7.16 (8 H, m). IR (NaCl, neat): 3410, 2925, 2830, 1655, 1580, 1480, 1210, 993 cm^{-1}. mp: 98–100 °C (diastereomeric mixture) (recrystallized from EtOAc/hexanes). Anal. ($C_{23}H_{26}N_2O_5$) Calcd: C, 67.30; H, 6.39; N, 6.83. Found: C, 67.39; H, 6.20; N, 6.75.

12. ^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 1.32–1.36 (1 H, m), 1.48–1.56 (1 H, m), 1.78–1.89 (1 H, m), 2.14–2.19 (1 H, m), 3.63–3.71 (1 H, m), 3.75 (6 H, s), 3.81–3.89 (1 H, m), 3.92–3.95 (1 H, m), 4.22 (1 H, $^1/_2$ AB q, J = 14.66 Hz), 4.30 (1 H, $^1/_2$ AB q, J = 14.38 Hz), 4.58 (1 H, $^1/_2$ AB q, J = 14.66 Hz), 4.62 (1 H, $^1/_2$ AB q, J = 14.66 Hz), 6.78–7.17 (8 H, m). IR (NaCl, neat): 3410, 2923, 1665, 1245 cm^{-1}. mp: 145–147 °C (recrystallized from EtOAc/hexanes). Anal. ($C_{23}H_{26}N_2O_5$) Calcd: C, 67.30; H, 6.39; N, 6.83. Found: C, 67.05; H, 6.37; N, 6.67.

7,9-Bis(*p*-methoxybenzyl)-7,9-diazabicyclo[3.2.2]non-2-ene-6,8-dione (1). To a stirred solution of alcohol **12** (0.064 g, 0.157 mmol, 1.0 equiv) in THF (2.0 mL) at 0 °C was added Et_3N (0.110 mL, 0.785 mmol, 5.0 equiv) and MsCl (0.024 mL, 0.314 mmol, 2.0 equiv). After 14 h the reaction mixture was filtered and washed with cold THF. After concentration and redissolution in toluene (5.0 mL), DBU (0.143 mL, 0.955 mmol, 5.0 equiv) was added and the reaction mixture refluxed for 5 h. The mixture was then diluted with CH_2Cl_2, poured into 0.1 N HCl, and exhaustively extracted with CH_2Cl_2. The combined organic extracts were washed with $NaHCO_3$, dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by PTLC silica gel (eluted EtOAc) yielded 30 mg (48.7% two-step yield) of olefin: mp 147–148 °C (recrystallized from EtOAc/hexanes).

^1H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 1.98 (1 H, d, J = 19.34 Hz), 2.41 (1 H, d, J = 19.34 Hz), 3.76 (6 H, s), 3.96 (1 H, s), 3.98 (1 H, s), 4.38 (1 H, $^1/_2$ AB q, J = 14.67 Hz), 4.40 (1 H, $^1/_2$ AB q, J = 14.61 Hz), 4.55 (1 H, $^1/_2$ AB q, J = 14.61 Hz), 4.65 (1 H, $^1/_2$ AB q, J = 14.67 Hz), 5.51–5.55 (1 H, m), 5.86–5.94 (1 H, m), 6.80–7.15 (8 H, m). IR (NaCl, neat): 3048, 2935, 1695, 1675, 1615, 1510, 1452 cm^{-1}. Anal. ($C_{23}H_{24}N_2O_4$) Calcd: C, 70.39; H, 6.16; N, 7.14. Found: C, 70.09; H, 6.28; N, 7.02.

6,8-Bis(*p*-methoxybenzyl)-2-methylene-6,8-diazabicyclo[2.2.2]octane-5,7-dione (2). To a stirred solution of alcohols **11** (0.260 g, 0.633 mmol, 1.0 equiv) in THF (10 mL) at 0 °C was added Et_3N (0.354 mL, 2.53 mmol, 4.0 equiv) and then MsCl (0.098 mL, 1.27 mmol, 2.0 equiv). After 2 h the reaction was filtered and washed with cold THF. After concentration and redissolution in toluene (6.0 mL), DBU (0.283 mL, 1.90 mmol, 5.0 equiv) was added and the reaction mixture refluxed for 17 h. The mixture was then diluted with CH_2Cl_2, poured into 0.1 N HCl, and exhaustively extracted with CH_2Cl_2. The combined organic extracts were washed with $NaHCO_3$, dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by flash chromatography (silica gel) (eluted with 2:1 EtOAc/hexanes) yielded 110 mg of olefin **2** (44% two-step yield), mp 117–118 °C (recrystallized from EtOAc/hexanes).

^{1}H NMR (270 MHz, $CDCl_3$) δ $CHCl_3$: 2.22–2.47 (2 H, m), 3.73 (3 H, s), 3.74 (3 H, s), 3.96 (1 H, s), 4.18 (1 H, s), 4.23 (1 H, $^1/_2$ AB q, J = 14.62 Hz), 4.44 (2 H, s), 14.60 (1 H, $^1/_2$ AB q, J = 14.62 Hz), 4.87 (1 H, s), 4.99 (1 H, s), 6.77–7.13 (8 H, m). IR (NaCl, neat): 2923, 2825, 1690, 1605, 1503, 1437, 1240, 1021 cm^{-1}. Anal. ($C_{23}H_{24}N_2O_4$) Calcd: C, 70.39; H, 6.16; N, 7.14. Found: C, 70.21; 5.87; 7.09.

Acknowledgment. This work was supported by the National Institutes of Health PHS Grant #AI-18957.

Registry No. 1, 109392-75-8; 2, 109392-76-9; 3, 79074-03-6; 4, 109392-77-0; 5, 109164-76-3; 6, 109164-77-4; 7, 109392-78-1; 8, 109392-79-2; 9, 109392-80-5; 10 (isomer 1), 109392-81-6; 10 (isomer 2), 109392-84-9; 11 (isomer 1), 109392-82-7; 11 (isomer 2), 109525-98-6; 12, 109392-83-8; *N*-(*p*-methoxybenzyl)glycine ethyl ester, 60857-16-1; *p*-methoxybenzyl chloride, 824-94-2; trimethylsulfoxonium iodide, 1774-47-6.

Synthesis from Pregnenolone of Fluorescent Cholesterol Analogue Probes with Conjugated Unsaturation in the Side Chain

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Fluorescent sterol probes which resemble cholesterol closely in both molecular geometry and amphipathic nature have been synthesized by the introduction of hydrophobic side chains embodying aryl diene or triene units. A direct synthesis of one of these fluorescent cholesterol analogues **6a** was possible by a phosphonate Wittig reaction on pregnenolone protected at C-3. However, the other cholesterol analogues **7a** and **8a** were prepared by phosphorane and phosphonate Wittig reactions on the more reactive and less sterically hindered 20(22)*E*-α,β-unsaturated aldehyde **5a**, which could be obtained by two different routes. The first route involved a Grignard reaction with vinylmagnesium bromide followed by oxidative rearrangement with pyridinium chlorochromate, which resulted in an 80:20 ratio of the diastereomeric aldehydes **5a**/**5b**. A more stereoselective synthesis of **5a** over **5b** (96:4) was achieved by a phosphonate Wittig reaction of pregnenolone protected at C-3 with the carbanion generated from diethyl [2-(cyclohexylimino)vinyl]phosphonate. Yields and ratios of all Wittig reactions performed are presented as are preliminary absorption and fluorescence data for the cholesterol analogue probes.

Introduction

Cholesterol is an important lipid component of many membranes, but its role in influencing the structure and function of membranes is not fully understood.[1] This is in part due to the very few methods that can be used to follow the properties of cholesterol when it is in such an inhomogeneous environment as a membrane. Hence, an external material with an easily measurable property is usually added to the system under question in order to probe the effect of cholesterol on the system.

Fluorescence is one of the most sensitive probe techniques available. Very little external probe material needs to be added, thereby minimizing any possible perturbations of the system by the probe. Many fluorescent probes bearing no resemblance to natural membrane constituents have been utilized in cholesterol/membrane studies, particularly the olefin diphenylhexatriene (DPH).[2] The latter has been found to partition into the lipid acyl chain region of a membrane. For our projected cholesterol/membrane studies, we wished to have a probe molecule which would partition into the cholesterol-rich domains of a membrane. To achieve this, it was decided to synthesize a fluorescent probe molecule with a structure as close as possible to that of cholesterol and retaining cholesterol's amphipathic nature.

Three main classes of fluorescent cholesterol-like probes have previously been synthesized: those derivatized[3] in the 3β-OH position (e.g., **1a**), those containing extra unsaturation in the ring system[4] (e.g., **2a** and **2b**), and those with a modified C-17 side chain[5] (e.g., **1b**). In some of the 3β-substituted cholesterol derivatives the amphipathic property[3c] is lost, while in all cases the interaction of the 3β-substituent with the head group of the phospholipids in membranes is significantly different from that of a 3β-hydroxy group. The ring unsaturated cholesterol-type molecules suffer from having a different geometry than

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(1) Schroeder, F. *Prog. Lipid Res.* **1984**, *23*, 97.

(2) (a) Veatch, W. R.; Stryer, L. *J. Mol. Biol.* **1977**, *117*, 1109. (b) Kawato, S.; Kinoshita, K.; Ikegami, A. *Biochemistry* **1978**, *17*, 5026. (c) Lentz, B. R.; Barrow, D. A.; Hoechli, M. *Ibid.* **1980**, *19*, 1943. (d) Vincent, M.; Gallay, J. *Biochem. Biophys. Res. Commun.* **1983**, *13*, 799. (e) Straume, *M.*; Litman, B. J. *Biophys. J.* **1986**, *49*, 310a.

(3) (a) Waggoner, A. S.; Stryer, L. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *67*, 579. (b) Rando, R. R.; Bangerter, F. W.; Alecio M. R. *Biochim. Biophys. Acta* **1982**, *684*, 12. (c) Cranney, M.; Cundall, R. B.; Jones, G. R.; Richards, J. T.; Thomas, E. W. *Ibid.* **1983**, *735*, 418.

(4) (a) Smith, R. J. M.; Green, C. *FEBS Lett.* **1974**, *42*, 108. (b) Rogers, J.; Lee, A. G.; Wilton, D. S. *Biochim. Biophys. Acta* **1979**, *552*, 23. (c) Bergeron, R.; Scott, J. *Anal. Biochem.* **1982**, *119*, 128. (d) Muczynski, K. A.; Stahl, W. L. *Biochemistry* **1983**, *22*, 6037. (e) Fischer, R. T.; Stephenson, F. A.; Shafiee, A.; Schroeder, F. *Chem. Phys. Lipids* **1984**, *36*, 1. (f) Fischer, R. T.; Stephenson, F. A.; Shafiee, A.; Schroeder, F. *J. Biol. Phys.* **1985**, *13*, 13. (g) Smutzer, G.; Yeagle, P. L. *Biochim. Biophys. Acta* **1985**, *814*, 274. (h) Chong, P. L. G.; Thompson, T. E. *Biophys. J.* **1986**, *49*, 306a.

(5) Kao, Y. J.; Soutar, A. K.; Hong, K.-Y.; Pownall, H. J.; Smith, L. C. *Biochemistry* **1978**, *17*, 2689. (b) Craig, I. F.; Via, D. P.; Mantulin, W. W.; Pownall, H. J.; Gotto, A. M.; Smith, L. C. *J. Lipid Res.* **1981**, *22*, 687. (c) Kao, Y. J.; Doody, M. C.; Smith, L. C. *J. Lipid Res.* **1986**, *27*, 781.

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