Chemistry. Elemental analyses were performed by Preben Hansen, The H. C. Ørsted Institute, Copenhagen. All the compounds reported in Table I had analytical data of C, H, N, and Cl within ±0.4% of the calculated values, unless otherwise specified. Melting points, which are uncorrected, were obtained with a Büchi melting point apparatus. Thin-layer chromatography (TLC) was performed on premade plates (Merck) with a mixture of ethanol/acetone/triethylamine (1:1:0.05) as eluent. NMR spectra were recorded on a JEOL FX90Q spectrometer. Chemical shifts are given in δ units (parts per million) with Me_4Si as internal standard. The NMR spectra of the compounds reported in Table I were all in agreement with the assigned structures.

The absorption spectra of compounds **3a**, **3′a**, **7a**, and **7′a** in water at ambient temperature were recorded on a UNICAM SP800A spectrophotometer. Percent hypochromism (% H) was calculated from the following equation: % H = $[\epsilon_3/(0.5\epsilon_7) - 1]100$.[15] The extinction coefficients were as follows: **3a**, ϵ_{410} = 11 400; **3′a**, ϵ_{420} = 8600; **3d**, ϵ_{410} = 11 300; **7a**, ϵ_{410} = 19 100; **7′a**, ϵ_{420} = 16 200; **7d**, ϵ_{410} = 22 400.

2-Methoxy-6,9-dichloroacridine (**5′**) and all the amines, except *N*-ethylhexane-1,6-diamine (**8e**), were purchased from EGA Chemie. 9-Chloroacridine (**5**),[28] 9-phenoxyacridine (**1**),[28] 2-methoxy-6-chloro-9-phenoxyacridine (**1′**),[28] *N*-ethyl-1,6-hexanediamine (**8e**),[29] and the Boc-protected polyamines (**2a–c**), were synthesized as reported previously.[24] The diacridines **7** and **7′** and acridines **9** were prepared from the corresponding amines and 9-chloroacridine (**5**) or 2-methoxy-6,9-dichloroacridine (**5′**) in phenol, by a slightly modified procedure of that of Canellakis.[20]

For each type of compound, a representative synthetic procedure is described below.

Boc Protection. *N,N′*-Bis(3-aminopropyl)piperazine (60 g, 0.3 mol) was dissolved in Me_2SO (125 mL), and Boc-N_3 (55.5 mL of a ~3.6 M solution of Boc-N_3 in ether, 0.2 mol) was added as described previously.[24] The mixture was stirred at room temperature for 2 (**2a**·HCl; Addition of water (250 mL, pH 8–9) resulted in precipitation of crude *N,N′*-bis[3-[(*tert*-butyloxycarbonyl)amino]propyl]piperazine (17.7 g, 0.044 mol). The aqueous phase was adjusted to pH ~12–13 with NaOH and extracted continuously with ether. From the ether phase was isolated *N*-[3-[(*tert*-butyloxycarbonyl)amino]propyl]-*N′*-(3-aminopropyl)piperazine trihydrochloride (**2d**·3HCl; 44.8 g, 54.5% yield), mp 190 °C.

2-Methoxy-6-chloro-9-[(6-aminohexyl)amino]acridine Dihydrochloride (3′a·2HCl). Method A. 2-Methoxy-6,9-dichloroacridine (**5′**; 600 mg, 2.16 mmol) and 1-[(*tert*-butyloxycarbonyl)amino]-6-hexanamine hydrochloride (**2a**·HCl; 600 mg, 2.52 mmol) were stirred at room temperature for 7 days in a mixture of anhydrous potassium carbonate (1.1 g) and anhydrous magnesium sulfate (250 mg) in dimethyl sulfoxide (10 mL). The mixture was taken up in a mixture of ether (25 mL) and H_2O (50 mL). The aqueous phase was extracted with ether (3 × 50 mL). The combined ether phases were dried ($MgSO_4$) and concentrated in vacuo, and to the resulting yellow oil was added 1 M HCl in glacial acetic acid (25 mL). The mixture was stirred at room temperature for 30 min and concentrated in vacuo. Trituration with ether gave yellow crystals, which were washed with ethanol and ether to give 775 mg of **3′a**·H_2O (~80%).

Method B. 2-Methoxy-6-chloro-9-phenoxyacridine (**1′**; 600 mg, 2.16 mmol) and 1-[(*tert*-butyloxycarbonyl)amino]-6-hexanamine (**2a**·HCl; 2.16 mmol) were stirred in phenol (3 g) at 100–120 °C for 1.5 h. The mixture was cooled to room temperature, and 2-methoxy-6-chloro-9-[[6-[(*tert*-butyloxycarbonyl)amino]hexyl]-amino]acridine was precipitated by addition of ether (75 mL), giving 1.25 g (~100%).

Deprotection was achieved by treatment with 1 M HCl in AcOH, and the product was recrystallized from ethanol/ether to give **3′a**·2HCl (1.0 g, ~96% yield).

1-(9-Acridinylamino)-6-[(2-methoxy-6-chloro-9-acridinyl)amino]hexane Dihydrochloride (4a·2HCl). Compound **3′a**·2HCl (225 mg, 0.5 mmol) and 9-phenoxyacridine (165 mg, 0.61 mmol) were stirred in phenol (1.5 g) at 80–100 °C for 2.5 h, upon which the mixture was allowed to cool to room temperature, and ether (75 mL) was added to precipitate the product. This was isolated and recrystallized from ethanol and minute amounts of ether to give **4a**·2HCl (290 mg, 95%), mp 230–237 °C dec.

Acknowledgment. This work was supported by the Danish Medical Science Research Council by Grant 12-1281 to J.B.H.

Registry No. **1′**, 7478-26-4; **2a**·HCl, 65915-94-8; **2b**·HCl, 82408-99-9; **2c**·2HCl, 82409-05-0; **2d**·3HCl, 86689-01-2; **3a**·2HCl, 86689-02-3; **3′a**·2HCl, 86689-03-4; **3′b**·2HCl, 86689-04-5; **3′c**·3HCl, 86689-05-6; **3d**·4HCl, 86689-06-7; **3′d**·4HCl, 86689-07-8; **4a**·2HCl, 86536-88-1; **4b**·2HCl, 86536-89-2; **4c**·3HCl, 86536-90-5; **4d**·4HCl, 86536-91-6; **5**, 1207-69-8; **5′**, 86-38-4; **6a**, 124-09-4; **6b**, 373-44-4; **6c**, 56-18-8; **6d**, 7209-38-3; **7a**·2HCl, 35555-85-2; **7′a**·2HCl, 75340-78-2; **7c**·3HCl, 75340-74-8; **7′c**·3HCl, 86689-08-9; **7d**·4HCl, 86689-09-0; **7′d**·4HCl, 86689-10-3; **8e**, 40043-26-3; **8f**, 140-31-8; **9e**·HCl, 86689-11-4; **9f**·2HCl, 86689-12-5; *N,N′*-bis[3-[(*tert*-butyloxycarbonyl)amino]propyl]piperazine, 86689-13-6; 2-methoxy-6-chloro-9-[[6-[(*tert*-butyloxycarbonyl)amino]hexyl]amino]-acridine, 86689-14-7; 9-phenoxyacridine, 2148-14-3.

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Optical Resolution, Absolute Configuration, and Activity of the Enantiomers of Proxyphylline

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The enantiomers of proxyphylline have been separated via their corresponding camphanates. Synthesis of (+)-proxyphylline from theophylline and (*S*)-propylene oxide derived from (*S*)-lactic acid established the absolute configuration of the (+) and (−) isomer as *S* and *R*, respectively. The activity of the enantiomers as cyclic nucleotide phosphodiesterase inhibitors was tested in human lung tissue homogenate. No differences were found either between the two enantiomers or between the enantiomers and racemic proxyphylline.

The bronchodilator proxyphylline [(±)-3,7-dihydro-7-(2-hydroxypropyl)-1,3-dimethyl-1*H*-purine-2,6-dione] was patented in 1955 as a water-soluble, stable, and neutral theophylline derivative suitable for oral and parenteral administration.[1] The potency and/or efficacy of proxyphylline, which is being used in the racemic form, is reported to vary from one-eighth to one-half of that of theophylline.[2-5] The metabolism and pharmacokinetics

† Institute of Clinical Biochemistry.
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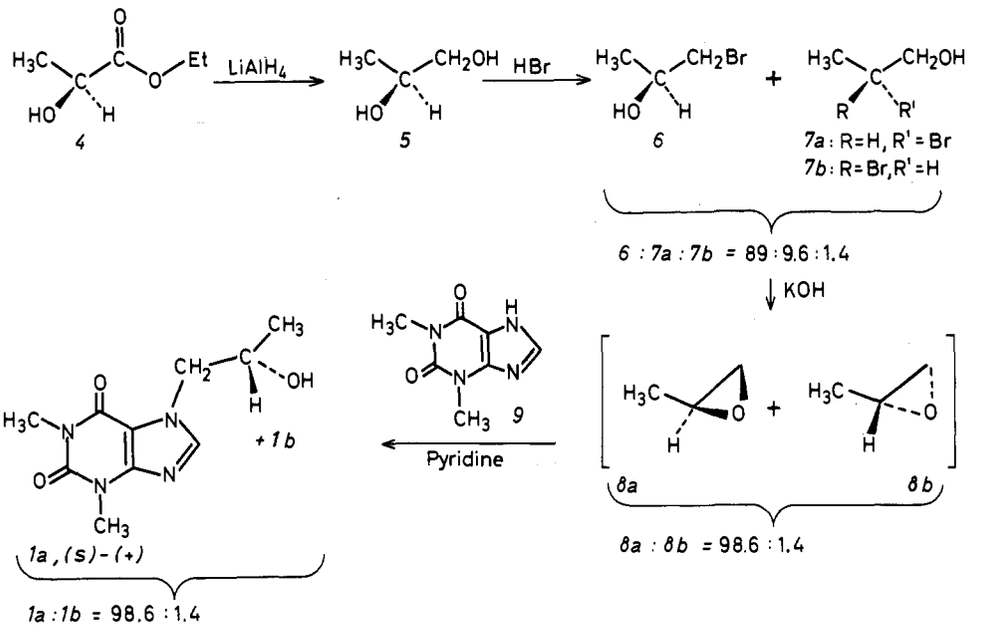
0022-2623/83/1826-1514$01.50/0

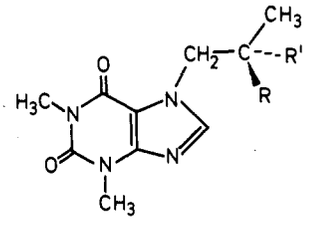
Scheme I



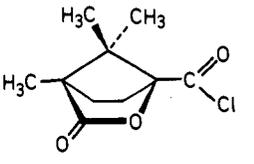
of racemic proxyphylline have recently been published.[6-8]

Theoretically, both the pharmacodynamics and the pharmacokinetics of the proxyphylline enantiomers may differ from the racemic form. In the present paper, the optical resolution and absolute configuration of the proxyphylline enantiomers are described, together with a comparison of their inhibition of human lung cyclic nucleotide phosphodiesterases.

Chemistry. Racemic proxyphylline (1) was reacted with



1a; R = H, R' = OH
1b; R = OH, R' = H
2a; R = H, R' = OCfn
2b; R = OCfn, R' = H



3 (Cfn Cl)

(−)-camphanoyl chloride (**3**)[9] to the diastereoisomeric esters **2**, which could be separated by preparative thin-layer chromatography. The pure esters revealed solubility differences in methanol, permitting an alternative mode of isolation of the less soluble isomer, (−)-proxyphylline camphanate (**2b**), which could be obtained in the pure state after four recrystallizations. Careful hydrolysis of the esters furnished the proxyphylline enantiomers exhibiting opposite rotations and being identical in all respects with racemic proxyphylline except for melting points, optical activities, and IR spectra measured for crystalline samples in KBr. The significant dissimilarities between the identical IR spectra of the enantiomers **1a** and **1b** and that of (±)-proxyphylline (**1**) established the nature of the latter as a true racemate and not a conglomerate.[10]

Since the absolute configuration of (−)-camphanoyl chloride (**3**) is known,[9] it was envisaged that X-ray diffraction analysis of one of the diastereoisomeric esters would allow configurational assignments of (+)- and (−)-proxyphylline. However, this approach was precluded because both esters crystallized as extremely thin needles. Consequently, a more indirect stereochemical proof was realized by preparing (*S*)-proxyphylline (**1a**) from theophylline (**9**) and (*S*)-propylene oxide (**8a**) derived from ethyl (*S*)-lactate[11] (**4**).

The ester **4** was reduced with $LiAlH_4$ to the corresponding diol **5**, which was subsequently treated with hydrogen bromide to yield an optically active mixture of primary and secondary bromides (**6** and **7**) (Scheme I). Based on the integral in the 200-MHz ^1H NMR spectrum of the mixture, the ratio of **6** to **7** was estimated to be 89:11, which is somewhat different from that reported by Franzus and Surridge,[12] who found 66:34 by NMR and 70:30 by GLC for a mixture similarly prepared from (*S*)-1,2-propanediol (**5**) and hydrogen bromide. Treatment of the bromohydrins **6** and **7** with strong base afforded the propylene oxides **8**, which were allowed to react[13] with theophylline (**9**) without prior isolation to give (*S*)- and (*R*)-proxyphylline (**1a, b**) in a 98.6:1.4 ratio as judged from the 400-MHz ^1H NMR spectrum of the mixture of the corresponding camphanates **2a,b**. Assuming the conversion of the secondary bromides **7** to the propylene oxides **8** involved *only* inversion of configuration, the ratio between (2*R*)- and (2*S*)-2-bromo-1-propanol (**7a,b**) was 87:13. On the basis of optical activities, Franzus and Surridge[12] estimated the corresponding ratio to be 59:41. This divergency is probably due to the fact that product composition,

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Table I. Inhibition Constants of Proxyphylline on Human Lung High- and Low-Affinity cAMP and cGMP Phosphodiesterases[a]

inhibitor	K_I, mmol/L			
	cAMP concn		cGMP concn	
	0.5–2 μmol/L	10–50 μmol/L	0.5–2 μmol/L	10–50 μmol/L
(*S*)-proxyphylline (1a)	0.4, 0.5	1.2, 1.3	0.6, 0.7	0.7, 0.7
(*R*)-proxyphylline (1b)	0.5, 0.6	1.1, 1.3	0.6, 0.7	0.6, 0.7
(±)-proxyphylline (1)	0.5, 0.5	1.0, 1.2	0.6, 0.6	0.6, 0.6

[a] The assays were performed in triplicate, and the results of two separate experiments are given.

Table II. Optical Activities

compound	$[\alpha]^{20}_{589}$, deg	$[\alpha]^{20}_{578}$, deg	$[\alpha]^{20}_{564}$, deg	$[\alpha]^{20}_{436}$, deg	$[\alpha]^{20}_{365}$, deg	solvent	*c*
(*S*)-proxyphylline	+64.8	+67.7	+77.8	+143	+252	$CHCl_3$	4.5
(*R*)-proxyphylline	−63.8	−65.7	−76.2	−139	−244	$CHCl_3$	0.42
(*S*)-proxyphylline ester[a]	+85.6	+89.6	+103	+184	+314	$CHCl_3$	3.3
(*R*)-proxyphylline ester[a]	−86.2	−90.3	−104	−189	−321	$CHCl_3$	2.5
(*S*)-1,2-propanediol	+26.2[b]	+27.3	+30.8	+50.8	+86.2	$CHCl_3$	2.2
propylene bromohydrins[c]	+9.7[d]	+10.3	+12.3	+24.1	+45.0	CH_2Cl_2	3.6

[a] Camphanate. [b] Literature[23] $[\alpha]^{24.2}_D$ +30.0° ($CHCl_3$), the authors[23] erroneously refer to this diol as "D-propylene glycol"; $[\alpha]^{24.4}_D$ −28.6° ($CHCl_3$) for (*R*)-1,2-propanediol. [c] Mixture of (2*S*)-1-bromo-2-propanol (6), (2*R*)-2-bromo-1-propanol (7a), and (2*S*)-2-bromo-1-propanol (7b) in the ratio 89:9.6:1.4. [d] Literature[23] $[\alpha]^{24}_D$ +9.90° ($CHCl_3$) for a mixture prepared similarly.

and presumably degree of racemization, is sensitive to the reaction temperature and time,[12] which in our case was considerably shorter (4 vs. 43 h at ca. 60 °C).

Thus, the absolute configuration of (+)-proxyphylline has been established as *S*.

Biological Results

The inhibitory effect of the two proxyphylline enantiomers on human lung cyclic nucleotide hydrolysis was compared with racemic proxyphylline. Human lung tissue was obtained from a patient undergoing total pulmectomy due to a pulmonary carcinoma. Unaffected tissue was excised and homogenized.[5] The phosphodiesterase activity in the lung cytosol was measured essentially according to Thompson and Appleman.[5,14] The concentration range of cAMP and cGMP was 0.5–2 and 10–50 μmol/L when examining the inhibition constants for the high- and low-affinity phosphodiesterase, respectively. Proxyphylline was added in concentrations of 0.25–1.0 mmol/L. K_I values were calculated from Dixon plots by using initial substrate concentrations and initial reaction rate velocities.

The apparent inhibition constants of the compounds are compared in Table I. No statistical differences were found between the two proxyphylline enantiomers and the racemic mixture. The calculated constants were in the same range as has been reported previously.[5]

The mechanism of action of the methylxanthines in the treatment of obstructive lung disease has so far not been resolved. The degree of cyclic nucleotide phosphodiesterase inhibition may, however, be a useful parameter to evaluate the clinical potency of the methylxanthines, since the potency of the drug as a phosphodiesterase inhibitor has been observed to correlate to its relaxing effect on tracheal smooth muscle.[15–17] The methylxanthines are competitive inhibitors of the cyclic nucleotide phosphodiesterases. The stereochemistry of the side chain in proxyphylline is obviously of less importance for this effect, since no differences were found in the inhibition constants between the enantiomers and racemic proxyphylline. Further studies, including direct measurement of the bronchodilating effect of the compounds on bronchial smooth muscle, have to be performed in order to evaluate any pharmacologically important differences between the two proxyphylline enantiomers.

Experimental Section

Materials and Techniques. Melting points were determined on a Reichert melting point apparatus and are uncorrected. Analyses were performed by Ilse Beetz Mikroanalytisches Laboratorium, Kronach, West Germany. Optical rotations, infrared (IR) spectra, and mass spectra were recorded on Perkin-Elmer 141, Beckman Acculab 2, and Micromass 7070F instruments, respectively. Gas chromatography in combination with mass spectrometry (GC–MS) was carried out with a packed 3% SP 2100 column (2 mm × 1 m). Chemical-ionization (CI) mass spectra were obtained by the direct-inlet method employing isobutane as ionizing gas. ^1H NMR spectra were measured on Bruker CXP-200 (200 MHz) and WM-400 (400 MHz) spectrometers, respectively, and ^{13}C NMR spectra were measured on a Bruker CXP-200 (50 MHz) instrument using $CDCl_3$ as solvent and Me_4Si as internal reference. Spin–spin coupling constants (*J*) given in the descriptions of ^1H NMR spectra are observed splittings of signals and should not be regarded as the exact coupling constants. Analytical thin-layer chromatography (TLC) was performed on Merck's HPTLC Kieselgel 60 F_{254} with CH_3OH–$CHCl_3$ (4:96) as mobil phase. Preparative TLC was accomplished on Merck's Kieselgel 60 F_{254}, 20 × 20 × 0.025 cm, with CH_3OH–$CHCl_3$ (2:98) as the mobil phase and employing CH_3OH–$CHCl_3$ (1:1) to elute the esters from the silica gel.

(±)-Proxyphylline (1) was purchased through Norsk Medisinaldepot, Oslo, Norway: mp 136 °C [lit.[1] 135-136 °C]; R_f 0.27; EIMS, *m/z* 238 (M^+, 41), 194 (100), 180 (85), 109 (69), 193 (47), 95 (24), 137 (23), 81 (21), 181 (18); the spectrum was in good agreement with that previously reported by Kamei et al.;[18] IR (KBr) 3505 (s), 1699 (s), 1660 (s), 1553 (s), 1472 (m), 1465 (m), 1459 (m), 1406 (m), 1292 (m), 1255 (m), 752 (s).

Optical Resolution of (±)-Proxyphylline. (*S*)- and (*R*)-Proxyphylline Camphanates (2a,b). A mixture of (±)-

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proxyphylline (1; 229 mg, 0.96 mmol) and (−)-camphanoyl chloride (3; 272 mg, 1.26 mmol) in dry pyridine (3.5 mL) was kept at room temperature for 24 h. $CHCl_3$ (100 mL) was added, and the organic phase was successively washed with aqueous 10% $NaHCO_3$ (20 mL), H_2O (20 mL), 0.5 M HCl (25 mL), and twice with H_2O (20 mL). The $CHCl_3$–extract was dried over anhydrous Na_2SO_4 and evaporated in vacuo, affording the two diastereoisomeric esters as a solid residue (376 mg, 94%). Each ester was obtained in the pure state by preparative TLC (ca. 20 mg per plate), followed by crystallization from MeOH.

Pure (*R*)-proxyphylline camphanate (**2b**) could also be obtained by subjecting the mixture of diastereoisomeric esters to fractional crystallization from MeOH (535 mg of esters afforded 71 mg pure **2b** following four recrystallizations from 30, 45, 50, and 10 mL, respectively).

(*S*)-Proxyphylline camphanate (2a): mp 204–204.5 °C; R_f 0.58; for $[\alpha]^{20}$, see Table II; EIMS, *m/z* 418 (M^+, 60), 180 (100), 220 (68), 237 (57), 83 (49), 41 (42), 221 (31), 55 (30), 136 (23); IR (KBr) 1788 (s), 1740 (s), 1710 (s), 1671 (s), 1270 (m), 1173 (m), 1108 (m), 1061 (m), 749 (m); ^{1}H NMR (400 MHz) δ 0.92 (s, 3 H), 0.98 (s, 3 H), 1.11 (s, 3 H), 1.40 (d, 3 H, *J* = 6.4 Hz), 1.63–1.70 (m, 1 H), 1.85–1.94 (m, 2 H), 2.25–2.33 (m, 1 H), 3.41 (s, 3 H), 3.58 (s, 3 H), 4.25–4.30 (dd, 1 H, *J* = 8.7 and 14.3 Hz), 4.69–4.73 (dd, 1 H, *J* = 2.6 and 14.3 Hz), 5.40–5.47 (m, 1 H), 7.65 (s, 1 H). For analysis, see synthesis of (*S*)-proxyphylline (**1a**) below.

(*R*)-Proxyphylline camphanate (2b): mp 228–230 °C; R_f 0.52; for $[\alpha]^{20}$, see Table II; EIMS, *m/z* 418 (M^+, 66), 180 (100), 220 (66), 237 (57), 83 (45), 41 (37), 221 (29), 55 (28), 136 (25); IR (KBr) 1787 (s), 1766 (s), 1720 (s), 1675 (s), 1561 (m), 1480 (m), 1464 (m), 1280 (m), 1271 (m), 1241 (m), 1125 (m), 1081 (m), 754 (m); ^{1}H NMR (400 MHz) δ 0.78 (s, 3 H), 1.00 (s, 3 H), 1.10 (s, 3 H), 1.41 (d, 3 H, *J* = 6.4 Hz), 1.65–1.72 (m, 1 H), 1.87–2.02 (m, 2 H), 2.29–2.36 (m, 1 H), 3.42 (s, 3 H), 3.57 (s, 3 H), 4.32–4.38 (dd, 1 H, *J* = 8.6 and 14.4 Hz), 4.60–4.64 (dd, 1 H, *J* = 2.7 and 14.3 Hz), 5.39–5.46 (m, 1 H), 7.59 (s, 1 H). Anal. ($C_{20}H_{26}N_4O_6$) C, H, N.

(*S*)- and (*R*)-Proxyphylline (1a,b). The camphanates **2a,b** (70 mg) were hydrolyzed with KOH (10% excess) in a MeOH–H_2O mixture (6:1; 7 mL) during 22 h at 5 °C. $NaHCO_3$ (3%, 5.5 mL) was then added, and the aqueous phase was extracted three times with $CHCl_3$ (40 mL). The combined extracts were dried over anhydrous Na_2SO_4 and evaporated, and the oily residue was recrystallized from ethyl acetate, yielding 38 mg (95%; the yield varied between 64 and 95%).

(*S*)-Proxyphylline (1a): mp 150.5–151.5 °C; cochromatography with (±)-proxyphylline (1), no separation; for $[\alpha]^{20}$, see Table II; EIMS, *m/z* 238 (44), 194 (100), 180 (87), 109 (66), 193 (45), 137 (24), 95 (22), 81 (19), 181 (18); IR (KBr) 3440 (m), 1712 (s), 1660 (s), 1646 (sh), 1552 (s), 1480 (m), 1415 (m), 1408 (m), 1379 (m), 1332 (m), 1119 (m), 768 (m), 756 (m); ^{1}H NMR (200 MHz) δ 1.27 (d, 3 H, *J* = 6.1 Hz), 2.75 (d, 1 H (OH), *J* = 4.4 Hz), 3.41 (s, 3 H), 3.60 (s, 3 H), 4.07–4.3 (m, 2 H), 4.47–4.54 (dd, 1 H, *J* = 1.8 and 12.8 Hz), 7.62 (s, 1 H).

(*R*)-Proxyphylline (1b): mp 151–151.5 °C; cochromatography with (±)-proxyphylline (1), no separation; for $[\alpha]^{20}$, see Table II; EIMS, *m/z* 238 (M^+, 42), 194 (100), 180 (82), 109 (61), 193 (45), 137 (21), 95 (20), 81 (18), 181 (17); IR spectrum (KBr) indistinguishable from that of (*S*)-proxyphylline (**1a**; vide supra); ^{1}H NMR spectrum (200 MHz) superimposable on that of (*S*)-proxyphylline (**1a**) and (±)-proxyphylline (1).

Synthesis of (*S*)-Proxyphylline (1a). (*S*)-1,2-Propanediol (5). A solution of ethyl (*S*)-lactate (4; 20.15 g, 171 mmol; Fluka AG) in Et_2O (25 mL) was added dropwise during 1.5 h to a stirred suspension of $LiAlH_4$ (5.4 g, 142 mmol) in Et_2O (150 mL), and the mixture was stored at ambient temperature overnight. Et_2O (125 mL) and H_2O (15 mL) were added, and the resulting mixture was filtered. The solid material was washed with Et_2O (100 mL), the combined solutions were dried over anhydrous Na_2SO_4 and filtered, and the filtrate was evaporated in vacuo to yield an oil (4.6 g), which was distilled under reduced pressure. The diol **5** was obtained as a colorless oil (3.07 g, 24%): bp 94–96 °C (12 mm) [lit.[19] bp 95–96 °C (15 mm)]; for $[\alpha]^{20}$, see Table II; EIMS, *m/z* 76 (M^+, 0.5), 45 (100), 61 (4.3); CIMS, *m/z* 77 ($M^+ + 1$, 100); ^{1}H NMR (200 MHz) δ 1.14 (d, 3 H, CH_3, *J* = 6.4 Hz), 3.3–3.6 (m, 4 H, CH_2OH and CHO*H*), 3.8–4.0 (m, 1 H, C*H*OH), the spectrum agreed with that of (±)-1,2-propanediol except for a downfield shift of the two O*H* protons to δ 3.9; ^{13}C NMR (50 MHz) δ 18.65 (q), 67.86 (t), 68.18 (d).

(2*S*)-1-Bromo-2-propanol (6) and 2-Bromo-1-propanol (7). The reaction of HBr with 1,2-propanediol has previously been described by several authors using varying reaction times and temperatures.[12,19,20] In the present study, anhydrous HBr (J. T. Baker) was bubbled into stirred (*S*)-1,2-propanediol (**5**; 2.73 g, 36 mmol) for 4 h and 45 min at 0 °C. The reaction flask was stoppered and left at room temperature overnight. The viscous, yellow oil was then heated at 57–60 °C for 4 h. The resulting dark brown liquid (5.46 g) was transferred to a separatory funnel with $CHCl_3$ (50 mL), and 25% NaCl (5 mL) was added. Aqueous NaOH (25%) was added until pH 4. After separation of the organic layer, the aqueous phase was extracted twice with $CHCl_3$ (2 × 30 mL). The combined extracts were dried over anhydrous Na_2SO_4 and evaporated at atmospheric pressure to an oil, which was distilled in vacuo. The bromides **6** and **7** (2.06 g, 41%) were collected between 65 and 84 °C at 40 mm [lit.[19] bp 59–60 °C (25 mm)]; for $[\alpha]^{20}$, see Table II. GC–MS of the mixture of propylene bromohydrins was as follows: for (2*S*)-1-bromo-2-propanol (**6**), EIMS, *m/z* 125 (4) and 123 (4) ($M^+ - 15$), 95 (1) and 93 (1) ($M^+ - 45$), 45 (100); CIMS, *m/z* 141 (3.3) and 139 (4.0) ($M^+ + 1$), 123 (41) and 121 (43) ($M^+ - 18$), 55 (51), 44 (100); for 2-bromo-1-propanol (**7**), EIMS, *m/z* 109 (5) and 107 (4) ($M^+ - 31$), 59 (100) ($M^+ - Br$); CIMS, *m/z* 123 (23) and 121 (23) [($M^+ + 1$) − 18], 69 (28), 55 (84), 44 (100). ^{1}H NMR (200 MHz) of the mixture of propylene bromohydrins: assignments of the signals of (2*S*)-1-bromo-2-propanol (**6**) and 2-bromo-1-propanol (**7**) are based on integrals (**6**/**7** = 89:11) and spin-decoupling experiments.[21] (2*S*)-1-Bromo-2-propanol (**6**): δ 1.31 (d, 3 H, CH_3, *J* = 6.3 Hz), 2.26 (d, 1 H, CHO*H*, *J* = 3.8 Hz), 3.37 (dd, 1 H, CH_AH_BBr, *J* = 7.1 and 10.2 Hz), 3.51 (dd, 1 H, CH_AH_BBr, *J* = 3.6 and 10.2 Hz), 3.9–4.1 (m, 1 H, C*H*OH); on irradiation at δ 4.0, the doublet at δ 1.31 collapsed to a broad singlet; on addition of D_2O, the signal at δ 2.26 disappeared, and the multiplet at δ 3.9–4.1 was simplified to a doublet of quintets with *J* = 3.5 and 6.4 Hz. 2-Bromo-1-propanol (**7**): δ 1.70 (d, 3 H, CH_3, *J* = 6.8 Hz), 2.16 (br t, 1 H, CH_2OH), 3.66–3.79 (m, 2 H, CH_2OH), 4.17–4.33 (doublet of quintets, 1 H, C*H*Br, *J* = 4.4 and 6.8 Hz); irradiation at δ 1.72 effected some changes in the multiplet at δ 4.25; irradiation at δ 3.73 simplified the triplet at δ 2.16 and the doublet of quintets at δ 4.17–4.33 to a broad singlet and quartet with *J* = 6.6 Hz, respectively; irradiation at δ 4.25 furnished a singlet at δ 1.70 and effected some changes in the multiplet at δ 3.66–3.79; on addition of D_2O, the signal at δ 2.16 disappeared and effected some changes in the multiplet at δ 3.66–3.79; on irradiation of this sample at δ 4.25, the latter multiplet was simplified to an AB system with broad lines, and the doublet at δ 1.70 collapsed to a broad singlet.

(*S*)-Proxyphylline (1a). Chilled (−10 °C) 50% aqueous KOH (2.2 mL) was added to the bromides **6** and **7** (1.48 g, 19.5 mmol), which had been cooled to −10 °C in a test tube (20 mL). The test tube was then connected via a glass tube to a two-necked flask (100 mL) containing a suspension of theophylline (**9**; 633 mg, 3.5 mmol) in 2-propanol (6.5 mL) and catalytic[13] amounts of pyridine (0.07 mL). This flask was fitted with a magnetic stirrer and a dry ice/acetone condenser. On gentle heating of the test tube and the connecting glass tube with a hair-dryer, propylene oxide distilled into the main reaction vessel, whose contents were refluxed on a water bath for 3 h and 40 min. The flask containing a dark brown solution was stoppered and kept at room temperature for 2 h and then at 5 °C overnight. TLC [C_6H_6–Me_2CO–concentrated NH_3 (30:70:2)][22] revealed traces only of theophylline

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(21) Franzus and Surridge[12] appear to have exchanged and displaced the chemical shifts (ca. 0.4-ppm downfield relative to ours) of the primary and secondary bromides **6** and **7** (information about solvent and reference is lacking). Nevertheless, the product distribution given by the authors is presumably correct, since identification of the bromides **6** (which is expected to predominate) and **7** was also based on other data.

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[9: R_f 0.07; (S)-proxyphylline (1a): R_f 0.55]. The solvent was removed in vacuo, and the residue was transferred to a separatory funnel with CH_2Cl_2 (50 mL), which was washed with 0.5 M HCl (4 mL) to remove pyridine and colored, water-soluble material. The aqueous phase was extracted twice with CH_2Cl_2 (75 mL), and the combined extracts were washed with 1 M KOH (3 mL) to remove remaining theophylline (9). The extract was dried over anhydrous Na_2SO_4 and evaporated in vacuo to afford a solid residue (774 mg), which was recrystallized from absolute ethanol (2 mL): yield 588 mg (70%); mp 139–149 °C. A second recrystallization from absolute ethanol (3 mL) yielded 162 mg: $[\alpha]^{20}_{589}$ +67.3°, $[\alpha]^{20}_{578}$ +70.3°, $[\alpha]^{20}_{546}$ +80.0°, $[\alpha]^{20}_{436}$ +137°, $[\alpha]^{20}_{365}$ +250° (c 3.0, $CHCl_3$). The crystalline material and the combined, concentrated, mother liquors (576 mg) were separately esterified with (−)-camphanoyl chloride (3; 195 and 737 mg, respectively) in pyridine (2.4 and 8.8 mL, respectively) as previously described, affording 265 (93) and 972 mg (96%) crude product, respectively.

A portion (20.8 mg) of the camphanates present in the former lot was purified by preparative TLC, and the esters were eluted together (18.9 mg). Only traces of the more polar ester corresponding to that from (R)-proxyphylline (1b) could be detected on the TLC plate. The ^1H NMR spectrum (400 MHz) of the purified diastereoisomeric esters allowed an assessment of the ratio of the two esters. A comparison of the integral of the CH_3 singlet at δ 0.92 with that of a small CH_3 singlet at δ 0.78 indicated a ratio 2a to 2b of 98.6:1.4. The remainder of the former lot of camphanates and the latter (972 mg) were recrystallized separately from MeOH (15 and 60 mL, respectively), furnishing chromatographically pure (S)-proxyphylline camphanate (2a): yield 906 mg; mp 203–204.5 °C; for $[\alpha]^{20}$, see Table II; for MS, ^1H NMR, and R_f, see above under (S)-proxyphylline camphanate (2a). Anal. ($C_{20}H_{26}N_4O_6$) C, H, N. The camphanate 2a (873 mg, 2.09 mmol) was hydrolyzed [129.3 mg of KOH (2.31 mmol), 67 mL of MeOH; 12 mL of H_2O] and worked up as previously described, except for one additional washing of the $CHCl_3$–extract (270 mL) with 3% $NaHCO_3$ (10 mL). Crystallization from ethyl acetate furnished (S)-proxyphylline (1a) (416 mg, 84%): mp 150–151.5 °C; for $[\alpha]^{20}$, see Table II; for MS, ^1H NMR, and R_f, see above under (S)-proxyphylline.

Acknowledgment. The authors are indebted to the Norwegian Research Council for Science and the Humanities for providing mass spectroscopy and NMR facilities. The technical assistance of Kari Frogner is gratefully acknowledged.

Registry No. (±)-1, 86480-51-5; 1a, 86540-95-6; 1b, 86540-96-7; 2a, 86480-52-6; 2b, 86540-97-8; 4, 687-47-8; 5, 4254-15-3; 6, 16088-60-1; 7a, 16088-61-2; 7b, 60434-72-2; 8a, 16088-62-3; 8b, 15448-47-2; 9, 58-55-9; cyclic 3′,5′-nucleotide phosphodiesterase, 9040-59-9.

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Design of Anticandidal Agents: Synthesis and Biological Properties of Analogues of Polyoxin L†

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Six analogues of polyoxin L were synthesized from uridine. All of these analogues inhibited chitin synthetase from *Candida albicans*. Derivatization of the amine terminus of the polyoxin analogues resulted in loss of activity, and analogues containing aromatic amino acid residues were the most efficient inhibitors of chitin synthetase. The concentration of tryptophanyl uracil polyoxin C, 8, which caused 50% inhibition of chitin synthetase activity, was 1.6×10^{-6} M. This was virtually identical with the activity found for polyoxin D. None of the inhibitors effectively competed with the entry of $(Met)_3$ into *C. albicans*. All of the analogues caused severe morphological distortions of the yeast in culture, and a number of analogues killed *C. albicans* at millimolar concentrations. The results suggest that chitin synthetase inhibitors may have potential as anticandidal drugs.

The polyoxins are a series of peptidyl nucleoside antibiotics that strongly inhibit chitin synthetase from a spectrum of fungi[1,2] and arthropods.[3,4] They are known to be highly toxic against both phytopathogenic fungi[5] and insects[4] but are not toxic to vertebrates.[6] The absence of detectable toxicity in mammalian systems makes the polyoxins attractive as potential agents against systemic fungal infections. However, previous workers concluded that polyoxins are not active against medically important fungi, such as *Candida albicans*.[6,7] Two reports[8,9] suggested that this lack of activity might result from the inability of the antibiotic to penetrate the cell to the site of chitin synthetase. Support for this suggestion is found in the observations that the polyoxins exhibit similar activities against chitin synthetase preparations from a variety of yeasts, including *Saccharomyces cerevisiae*, but only affect *S. cerevisiae* in culture when present at high concentration.[10] Furthermore, when used in combination

† Abbreviations used in this paper are as follows: Z, benzyloxycarbonyl; DMF, *N,N*-dimethylformamide; ONp, *p*-nitrophenyl ester; NMM, *N*-methylmorpholine; TLC, thin-layer chromatography; *n*-BuOH, *n*-butyl alcohol; HOAc, acetic acid; TFA, trifluoroacetic acid.

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0022-2623/83/1826-1518$01.50/0