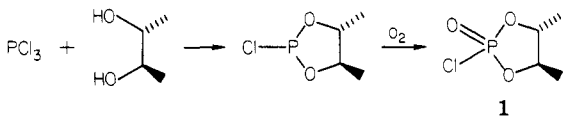
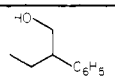
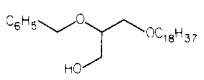
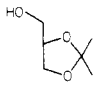
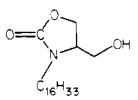
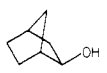
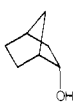
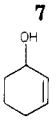
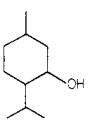
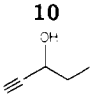
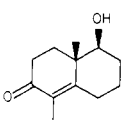
Scheme I



1

Table I. ^{31}P NMR Nonequivalences and Enantiomeric Ratios

structure	$\Delta\delta$,[a] Hz	ratio of enantiomers[b]
 2	5.5	51:49
 3	2.40	50:50
 4	0	
 5	5.61	48:52
 6	6.11	50:50
 7	0	
 8	3.7	48:52
 9	0.5	*c*
 10	12.2	68.43:31.57[d] (68.38:31.62)[e]
 11	10.13	51:49
 12	8.3	48:52

[a] 88.9 mHz. [b] Determined from ratio of peak intensities or by integration of peak area and are believed to be accurate to ±1%. [c] Base-line resolution not obtained. [d] Preweighed from pure (+) and (−) enantiomers. [e] Calculated value.

NMR and Mosher's reagent. Furthermore, the potential for asymmetric induction is in principle diminished due to decreased interactions between asymmetric centers during derivatization using CDA 1. In this regard, racemic **12** is reported in the literature[3] as yielding a 63:37 ratio upon use of Mosher's reagent, whereas the results obtained by using CDA 1 are considerably better. The utility of CDA 1 for the direct analysis of primary alcohols such as **2**, **3**, and **5** is also demonstrated. In the past, enantiomeric purity of primary alcohols *has required the use of lanthanide shift reagents in conjunction with the CDA.*[10]

In conclusion, it has been shown that **1** is capable of the direct in situ determination of enantiomeric purity by ^{31}P NMR spectroscopy without the use of auxillary agents for both primary and secondary alcohols.

Experimental Section

2-Chloro-4(*R*),5(*R*)-dimethyl-2-oxo-1,3,2-dioxaphospholane (1). Literature synthesis for the racemic dioxaphospholane[6] was followed by using (*R*,*R*)-(−)-2,3-butandiol[7] to produce enantiomerically pure **1**. This material was stored at 5 °C in a tightly sealed flask within a jar containing a dessicant and showed no signs of decomposition over an 8-month period: bp 105–110 °C at 500 mT; ^{31}P NMR δ 17.4, d = 1.5 g/mL.

^{31}P NMR spectra were obtained on a JEOL FX-200 spectrometer system with the following spectral conditions. A 5-mm NMR tube in a 15-mm broad-band probe tuned to ^{31}P and internal C_6D_6 lock, sweep width of 1 kHz, pulse width of 45°, 16K real data points with no exponential window processing. All shifts obtained are reported by using external H_3PO_4 as reference standard, δ = 0.0.

A typical NMR experiment is as follow: the substrate to be derivatized (1.0 equiv) was dissolved in dry benzene[8] (1.0 mmol/mL) in a vial, and then dry triethylamine (1.5 equiv) and 4-(dimethylamino)pyridine (DMAP) (~0.1 equiv) were added. Then the CDA **1** (1.05 equiv) was added and the vial was shaken for 30 s. After standing for about 15 min, a small amount of C_6D_6, for NMR locking purposes, was added, and the mixture was filtered through cotton into a NMR tube and spectra were taken.

Acknowledgment. We thank Mr. M. X. Kolpak for obtaining the ^{31}P NMR spectra and Professor R. Boeckman for helpful discussions and for compound **12**.

Registry No. **1**, 89104-48-3; (±)-**2**, 89104-46-1; (*R*)-**2** (1 deriv), 89065-35-0; (*S*)-**2** (1 deriv), 89104-36-9; (±)-**3**, 89104-47-2; (*R*)-**3** (1 deriv), 89065-36-1; (*S*)-**3** (1 deriv), 89104-37-0; (±)-**4**, 22323-83-7; (±)-**5**, 89065-43-0; (*R*)-**5** (1 deriv), 89065-37-2; (*S*)-**5** (1 deriv), 89104-38-1; (±)-**6**, 60133-16-6; (*R*)-**6** (1 deriv), 89065-38-3; (*S*)-**6** (1 deriv), 89104-39-2; (±)-**7**, 24325-44-8; (±)-**8**, 62860-38-2; (*R*)-**8** (1 deriv), 89065-39-4; (*S*)-**8** (1 deriv), 89104-40-5; (±)-**9**, 15892-23-6; (*R*)-**9** (1 deriv), 89104-41-6; (*S*)-**9** (1 deriv), 89104-42-7; (1*R*)-**10**, 2216-51-5; (1*S*)-**10**, 15356-60-2; (1*R*)-**10** (1 deriv), 89065-40-7; (1*S*)-**10** (1 deriv), 89104-43-8; (±)-**11**, 65253-21-6; (*R*)-**11** (1 deriv), 89065-41-8; (*S*)-**11** (1 deriv), 89104-44-9; (±)-**12**, 24138-10-1; (*R*)-**12** (1 deriv), 89065-42-9; (*S*)-**12** (1 deriv), 89104-45-0; PCl_3, 7719-12-2; (*R*,*R*)-2,3-butanediol, 24347-58-8; (4*R*,5*R*)-2-chloro-4,5-dimethyl-1,3,2-dioxaphospholane, 89104-49-4.

(10) (a) Yasuhara, F.; Yamaguchi, S. *Tetrahedron Lett.* **1977**, 4085–4088. (b) Merck, E. M.; Lepoiuse, J. A.; Lemiere, G. L.; Alderweireldt, F. C. *Org. Magn. Reson.* **1983**, *21*, 380–387.

Methotrexate Analogues. 22. Selective Cleavage of 2-(Trimethylsilyl)ethyl Esters in the Presence of *N*-*tert*-Butyloxycarbonyl Groups during the Synthesis of Protected Dilysine and Trilysine Derivatives of Methotrexate

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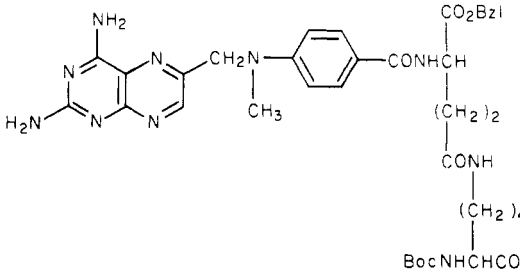
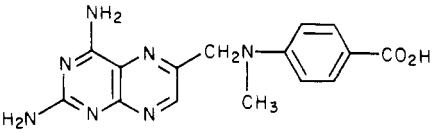
In connection with a larger project involving γ-substituted derivatives of the antitumor agent methotrexate



1, Bzl = benzyl; Boc = *tert*-butyloxycarbonyl; Cbz = benzyloxycarbonyl; Tmse = 2-(trimethylsilyl)ethyl



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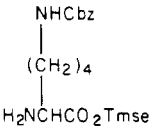

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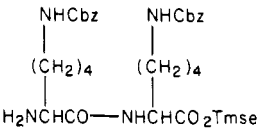
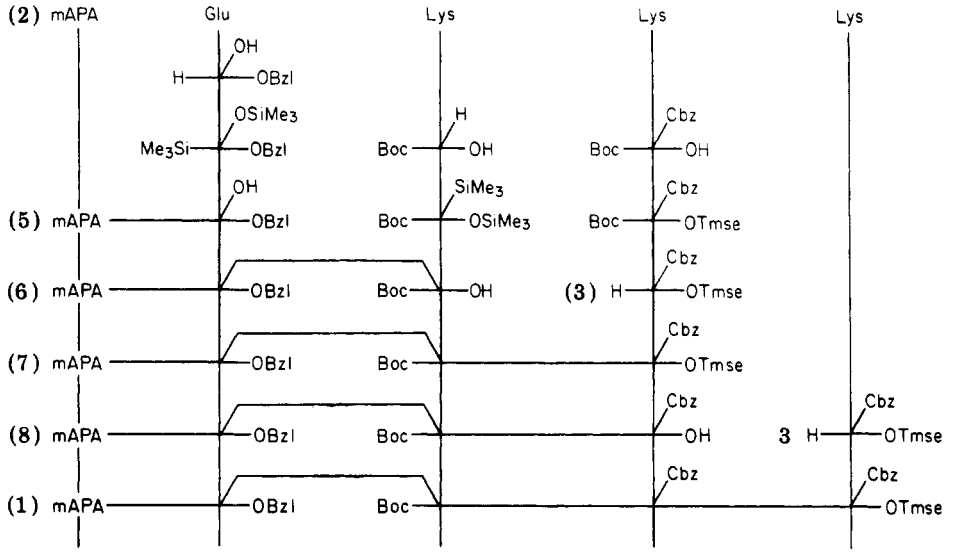
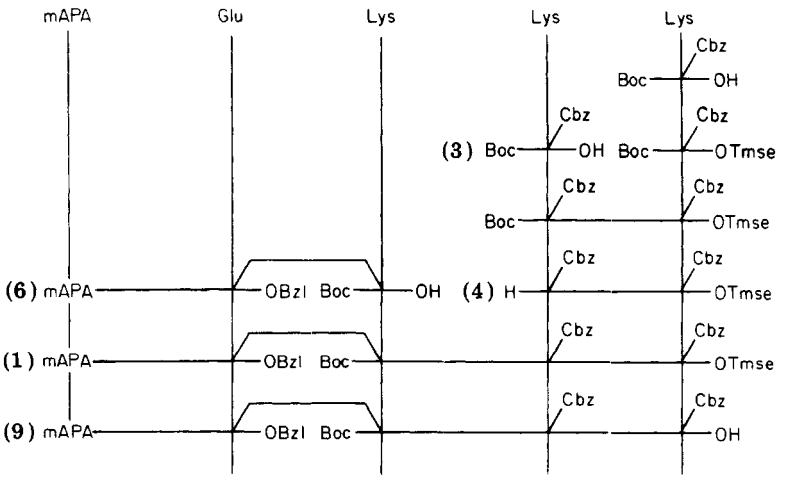


4

Scheme I



Scheme II



(4-amino-4-deoxy-N^{10}-methylpteroyl-L-glutamic acid, MTX),[1] we had a need to prepare the fully protected L-γ-glutamyl-L-lysyl-L-lysyl-L-lysyl derivative 1. The choice of blocking groups in 1 was dictated by a requirement for subsequent synthetic flexibility, as it was our intent to ultimately be able to deprotect either the carboxyl group in the C-terminal lysine or the α-amino group in the *N*-terminal lysine while retaining the benzyl ester and *N*-benzyloxycarbonyl (Cbz) groups intact. This note describes the synthesis of 1 from 4-amino-4-deoxy-N^{10}-

(1) For other recent papers in this series on methotrexate analogues, see: Rosowsky, A.; Moran, R. G.; Forsch, R.; Colman, P.; Wick, M. *Biochem. Pharmacol.* **1984**, *33*, 155. Rosowsky, A.; Forsch, R.; Yu, C.-S.; Lazarus, H.; Beardlsey, G. P. *J. Med. Chem.*, in press. Rosowsky, A.; Forsch, R.; Freisheim, J. H.; Moran, R. G.; Wick, M. *Ibid.*, in press.

methylpteroic acid (2).[2-4] Two key intermediates were the heretofore unreported salts 3·TsOH and 4·TsOH, which were obtained from the corresponding *N*-*tert*-butyloxycarbonyl (Boc) derivatives in excellent yield by reaction with *p*-toluenesulfonic acid.[5] Selective removal of the 2-(trimethylsilyl)ethyl ester (Tmse) ester group in 1 was achieved with fluoride ion.[6] The ability to perform these deprotection steps with complete selectivity illustrates the utility of the Tmse ester blocking group in peptide chemistry.[7]

Two approaches were used to prepare the tetrapeptide 1. In one of them (Scheme I) the substituted L-glutamyl-L-lysine intermediate 6 was elaborated stepwise via the dilysine 7, whereas in the other (Scheme II) a convergent synthesis was carried out, with the two N-terminal lysines being joined to 6 in one step. In principle the latter approach, which embodies the "backing off" concept of peptide synthesis,[8] should be the one less likely to involve partial racemization during peptide bond formation via an activated ester.

The Tmse ester 3 was prepared by (a) carbodiimide-mediated esterification of N^{α}-(*tert*-butyloxycarbonyl)-N^{ϵ}-(benzyloxycarbonyl)-L-lysine with 2-(trimethylsilyl)-ethanol in the presence of 4-(dimethylamino)pyridine,[9] followed by (b) selective N^{α}-deprotection with 1.1 mol equiv of *p*-toluenesulfonic acid in boiling benzene.[5] The overall yield was quantitative. The product was isolated and characterized as a crystalline tosylate salt (3·TsOH). Similarly, the crystalline Tmse ester salt 4·TsOH was prepared from 3 and a second molecule of N^{α}-(*tert*-butyloxycarbonyl)-N^{ϵ}-(benzyloxycarbonyl)-L-lysine by diphenylphosphoryl azide (DPPA) coupling (86% yield) followed by deblocking with *p*-toluenesulfonic acid (81% yield). Both 3·TsOH and 4·TsOH showed the expected NMR signals for the aliphatic protons of the Tmse group and the aromatic protons of the Cbz and tosylate groups, and showed disappearance of the Boc group on acidolysis. These heretofore unknown tosylate salts appear to be stable derivatives that are readily dissolved in organic solvents and are well suited for use in peptide synthesis.

Methotrexate α-benzyl ester (5), another key intermediate in the synthesis, was prepared in 94% yield by condensation of 2 with the N,O^{γ}-bis(trimethylsilyl) derivative of α-benzyl L-glutamate in the presence of diethyl phosphorocyanidate (DEPC).[10] We have previously found DEPC to be highly effective in activating the otherwise rather inert carboxyl group in 2.[11-13] The product of reaction of α-benzyl L-glutamate with 2.25 mol equiv each of Me_3SiCl and Et_3N was determined to be the N,O^{γ}-bis(silylated) derivative from its NMR spectrum, which revealed two closely spaced singlets for the *N*- and O^{γ}-trimethylsilyl groups respectively. The silylated compound was a moisture-sensitive oil and was used immediately for coupling. Desilylation of the coupling product occurred during aqueous workup. The α-benzyl ester 5 was also used promptly in order to preclude isomerization to the γ-isomer or cleavage to free MTX, a problem we have encountered with other MTX α-esters.[14]

The γ-L-glutamyl-L-lysine intermediate 6 was obtained from 5 and the N^{ϵ},O-bis(trimethylsilyl) derivative of N^{α}-(*tert*-butyloxycarbonyl)-L-lysine by mixed anhydride coupling (93% yield). The silylated lysine derivative, which was prepared by reaction of the N^{α}-blocked amino acid with 2.2 mol equiv each of Me_3SiCl and Et_3N, was a moisture-sensitive oil but its NMR spectrum showed the expected pair of N^{ϵ}- and *O*-trimethylsilyl singlets. Further condensation of 6 with 3·TsOH in the presence of DPPA and Et_3N in DMF afforded the tripeptide 7 (75% yield). The same reaction with 4·TsOH led to 1 (83% yield).

Selective cleavage of the Tmse ester in 7 was achieved with tetrabutylammonium fluoride generated in situ in DMF from tetrabutylammonium chloride and KF·$2H_2O$.[15] The product (8, 42% yield) was condensed with another molecule of 3·TsOH via the DPPA method to give 1 (56% yield). The product was indistinguishable from the material prepared from 6 and 4·TsOH.

When 1 was treated with fluoride ion in the same manner as 7, selective ester cleavage again occurred. As we had hoped, the product (9, 62% yield) retained not only the Boc and Cbz groups, but also the α-benzyl ester on the MTX moiety. These findings suggest that further exploration of the use of the Tmse group in peptide syntheses where more than one carboxyl group has to be protected and selectively deprotected would be of interest.

Experimental Section

Infrared spectra were obtained on a Perkin-Elmer Model 137B double-beam recording spectrophotometer. NMR spectra were recorded on a Varian T60A instrument with Me_4Si as the internal reference except in the case of compounds already containing one or more Me_3Si groups. In those instances, one of the Me_3Si groups in the compound was used as the internal reference, and was set at δ 0.00. TLC was carried out on Eastman 13181 silica gel or Eastman 13254 cellulose sheets, with a fluorescent indicator. Spots were visualized under 254 nm illumination in a viewing chamber. Column chromatography was performed on Baker 3405 silica gel (60–200 mesh). Melting points were measured in Pyrex capillary tubes in a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, MA) and are not corrected. Elemental microanalyses were performed by Galbraith Laboratories, Knoxville, TN, and were within ±0.4% of theoretical values unless otherwise indicated.

α-Benzyl L-glutamate, N^{α}-(*tert*-butyloxycarbonyl)-L-lysine, and N^{α}-(*tert*-butyloxycarbonyl)-N^{ϵ}-(benzyloxycarbonyl)-L-lysine were purchased from Chemical Dynamics Corporation, South Plainfield, NJ. 2-(Trimethylsilyl)ethanol, 4-(dimethylamino)pyridine, and diphenylphosphoryl azide were from Aldrich, Milwaukee, WI. Cesium carbonate was from Alfa, Beverly, MA. 4-Amino-4-deoxy-N^{10}-methylpteroic acid and diethyl phosphorocyanidate were prepared as previously described.[11-13] Unless otherwise specified, solutions in organic solvents were dried over anhydrous Na_2SO_4 before being evaporated.

N^{ϵ}-(Benzyloxycarbonyl)-L-lysine 2-(Trimethylsilyl)ethyl Ester *p*-Toluenesulfonate (3·TsOH). A solution of N^{α}-(*tert*-butyloxycarbonyl)-N^{ϵ}-(benzyloxycarbonyl)-L-lysine (4.58 g, 0.012 mol), 2-(trimethylsilyl)ethanol (1.42 g, 0.012 mol), and 4-(dimethylamino)pyridine (0.74 g, 0.006 mol) in CH_2Cl_2 (60 mL) was cooled to 0 °C and treated with *N*,*N*′-dicyclohexylcarbodiimide (2.48 g, 0.012 mol). After being stirred in ice for 1 h and then left in the refrigerator for 3 days, the mixture was filtered, and

(2) Elliott, R. D.; Temple, C.; Jr.; Montgomery, J. A. *J. Org. Chem.* **1970**, *35*, 1676.
(3) Chaykovsky, M.; Rosowsky, A.; Papathanasopoulos, N.; Chen, K. K. N.; Modest, E. J.; Kisliuk, R. L.; Gaumont, Y. *J. Med. Chem.* **1974**, *17*, 1212.
(4) Piper, J.; Montgomery, J. A. *J. Org. Chem.* **1977**, *42*, 208.
(5) Rosowsky, A.; Wright, J. E. *J. Org. Chem.* **1983**, *48*, 1539.
(6) Sieber, P. *Helv. Chim. Acta* **1977**, *60*, 2711.
(7) For a recent review on carboxyl group protection, see: Haslam, E. *Tetrahedron* **1980**, *36*, 2409.
(8) Goodman, M.; Stueben, K. C. *J. Am. Chem. Soc.* **1959**, *81*, 3980.
(9) Dhaon, M. K.; Olsen, R. K.; Ramasamy, K. *J. Org. Chem.* **1982**, *47*, 1962.
(10) Shioiri, T.; Hamada, Y. *J. Org. Chem.* **1978**, *43*, 3631.
(11) Rosowsky, A.; Forsch, R.; Uren, J.; Wick, M. *J. Med. Chem.* **1981**, *24*, 1450.
(12) Rosowsky, A.; Wright, J. E.; Ginty, C.; Uren, J. *J. Med. Chem.* **1982**, *25*, 960.
(13) Rosowsky, A.; Forsch, R.; Uren, J.; Wick, M.; Kumar, A. A.; Freisheim, J. H. *J. Med. Chem.* **1983**, *26*, 1719.
(14) Rosowsky, A.; Beardsley, G. P.; Ensminger, W. D.; Lazarus, H.; Yu, C.-S. *J. Med. Chem.* **1978**, *21*, 380.
(15) Carpino, L. A.; Sau, A. C. *J. Chem. Soc., Chem. Commun.* **1979**, 514.

the filtrate was washed (0.1 N HCl followed by H_2O), dried (K_2CO_3), and evaporated to give the Tmse ester as an oil (5.77 g, 100%): NMR (CCl_4) δ 0.00 (s, 9 H, Me_3Si), 0.60–1.02 (m, 2 H, CH_2Si), 1.12–1.78 (m with a singlet protruding at 1.33, 15 H, $CH_2CH_2CH_2$ and Me_3CO), 3.16 (m, 2 H, CH_2N), 4.16 (m, 3 H, CH_2O and α-CH), 4.90 (m overlapping a singlet, 3 H, NH and benzylic CH_2), 7.20 (s, 5 H, aryl). A solution of the freshly prepared ester (480 mg, 1 mmol) in dry benzene (10 mL) containing *p*-toluenesulfonic acid monohydrate (210 mg, 1.1 mmol) was refluxed for 1 h. After evaporation to dryness under reduced pressure, the residue was triturated for 15–30 min with hexane, during which solidification occurred: yield 553 mg (100%); mp 85–87 °C; NMR (CCl_4) δ 0.00 (s, 9 H, Me_3Si), 0.72–2.00 (m, 8 H, $CH_2CH_2CH_2$ and CH_2Si), 2.34 (s, 3 H, aromatic Me), 2.95 (m, 2 H, CH_2N), 4.05 (m, 3 H, CH_2O and α-CH), 4.99 (s, 2 H, benzylic CH_2), 7.22 (br s, 5 H, aryl), 7.01 (poorly resolved d, 2 H, aryl protons ortho to Me), 7.70 (poorly resolved d, 2 H, aryl protons ortho to SO_3^-). Anal. Calcd for $C_{19}H_{31}N_2O_4Si{\cdot}CH_3C_6H_4SO_3H$: C, 56.60; H, 7.12; N, 5.08. Found: C, 56.67; H, 7.26; N, 5.30.

N^{ϵ}-(Benzyloxycarbonyl)-L-lysyl-N^{ϵ}-(benzyloxycarbonyl)-L-lysine 2-(Trimethylsilyl)ethyl Ester *p*-Toluenesulfonate (4·TsOH). A solution of N^{α}-(*tert*-butyloxycarbonyl)-N^{ϵ}-(benzyloxycarbonyl)-L-lysine (551 mg, 1 mmol) and 3·TsOH (380 mg, 1 mmol) in dry DMF (10 mL) at 0 °C was treated with DPPA (275 mg, 1 mmol) and Et_3N (303 mg, 3 mmol). After being left at 0 °C for 3 h and at room temperature overnight, the mixture was evaporated. The residue was taken up in CH_2Cl_2, and the solution was successively washed (dilute ammonia, H_2O, 0.1 N HCl and H_2O), dried (K_2CO_3), and evaporated to an oil, which was chromatographed on silica gel (95:5 $CHCl_3$–MeOH): yield 635 mg (86%); NMR (CCl_4) δ 0.00 (s, 9 H, Me_3Si of Tmse), 0.73–1.83 (m with a singlet protruding at 1.36, 23 H, CH_2Si, Me_3CO, and two $CH_2CH_2CH_2$), 3.04 (m, 4 H, two CH_2N), 4.06 (m, 4 H, ester CH_2O and two α-CH), 4.95 (s, 4 H, two benzylic CH_2), 7.20 (s, 10 H, aryl).

A solution of the preceding compound (605 mg, 0.8 mmol) and *p*-toluenesulfonic acid monohydrate (168 mg, 0.88 mmol) in dry benzene (10 mL) was refluxed for 1 h and worked up as in the preparation of 3·TsOH: yield 532 mg (81%); mp 92–94 °C; NMR (CCl_4) δ 0.00 (s, 9 H, Me_3Si), 0.73–1.90 (m, 14 H, CH_2Si and two $CH_2CH_2CH_2$), 2.22 (s, 3 H, aromatic Me), 2.93 (m, 4 H, two CH_2N), 4.20 (m, 2 H, ester CH_2O), 4.99 (s, 4 H, two benzylic CH_2), 6.89 (d, J = 8 Hz, 2 H, two aryl protons ortho to Me), 7.20 (s, 10 H, aryl), 7.69 (d, J = 8 Hz, 2 H, aryl protons ortho to SO_3^-). Anal. Calcd for $C_{33}H_{50}N_4O_7Si{\cdot}CH_3C_6H_4SO_3H$: C, 58.94; H, 7.17; N, 6.87. Found: C, 58.91; H, 7.24; N, 7.15.

N-(4-Amino-4-deoxy-N^{10}-methylpteroyl)-L-glutamic Acid α-Benzyl Ester (5). A suspension of α-benzyl L-glutamate (1.42 g, 6 mmol) in dry benzene (10 mL) was treated with Et_3N (1.33 g, 13.2 mmol) and Me_3SiCl (1.66 mL, 1.42 g, 13.2 mmol), stirred at room temperature overnight, diluted with hexane (20–30 mL), and filtered quickly under suction. The filter cake was washed with hexane and the combined filtrates were evaporated to obtain α-benzyl N,O-bis(trimethylsilyl)-L-glutamate as a moisture-sensitive oil (2.25 g, 99%): NMR (CCl_4) δ −0.03 (s, 9 H, Me_3SiN), 0.20 (s, 9 H, Me_3SiO), 1.4–2.4 (m, 4 H, CH_2CH_2), 3.35 (m, 1 H, α-CH), 5.04 (s, 2 H, benzylic CH_2), 7.26 (s, 5 H, aryl).

4-Amino-4-deoxy-N^{10}-methylpteroic acid (2, 1.44 g, 4 mmol) was added in small portions to a stirred solution of DEPC (1.75 g, 12 mmol) and Et_3N (1.20 g, 12 mmol) in dry DMF (140 mL) at room temperature, and the mixture was left to stand overnight. The freshly made silylated amino ester (2.25 g, 6 mmol) was added in a little DMF and the solution was left at room temperature for 2–3 days. A small volume of H_2O was then added, and the mixture was evaporated to dryness. The residue was resuspended in H_2O, and the pH was adjusted to 9. A small amount of insoluble material consisting mainly of 2 (TLC) was quickly filtered off. The filtrate was acidified with 10% AcOH, and the precipitate was filtered, washed with cold H_2O, and dried to obtain a yellow solid (2.15 g, 94%): R_f 0.43 (cellulose, pH 7.4 phosphate buffer); R_f 0.62 (silica gel, 15:5:1 $CHCl_3$–MeOH–AcOH). Anal. Calcd for $C_{27}H_{28}N_8O_5{\cdot}1.5H_2O$: C, 56.74; H, 5.47; N, 19.60. Found: C, 56.55; H, 5.52; N, 19.98.

N^{α}-(*tert*-Butyloxycarbonyl)-N^{ϵ}-[N-(4-amino-4-deoxy-N^{10}-methylpteroyl)-γ-L-glutamyl]-L-lysine Benzyl ($O^{\alpha\text{-glu}}$) Ester (6). A suspension of N^{α}-(*tert*-butyloxycarbonyl)-L-lysine (738 mg, 3 mmol) in dry benzene (6 mL) was treated with Et_3N (666 mg, 6.6 mmol) and Me_3SiCl (840 μL, 714 mg, 6.6 mmol), stirred at room temperature overnight, and worked up as in the silylation of α-benzyl L-glutamate to obtain the N^{ϵ},O-bis(trimethylsilyl) derivative as a moisture-sensitive oil (1.16 g, 99%): NMR (CCl_4) δ 0.01 (s, 9 H, Me_3SiN), 0.25 (s, 9 H, Me_3SiO), 1.05–1.93 (m, 6 H, $CH_2CH_2CH_2$), 1.38 (s, 9 H, Me_3CO), 2.80 (m, 2 H, CH_2N), 4.10 (m, 1 H, α-CH), 6.20 (m, 2 H, NH).

A solution of 5 (1.42 g, 2.5 mmol) in dry DMF (50 mL) was treated with Et_3N (303 mg, 3 mmol) and *i*-BuOCOCl (390 μL, 408 mg, 3 mmol), and the mixture was stirred at room temperature for 20 min. The freshly made silylated lysine derivative (1.16 g, 3 mmol) was added in a small volume of DMF, and the solution was left at room temperature overnight. A small amount of H_2O was added, and the mixture was evaporated to dryness. The residue was dissolved in dilute ammonia, the solution was acidified with 10% AcOH, and the precipitate was collected, dried, and chromatographed (silica gel, 85:15 $CHCl_3$–MeOH) to obtain a yellow solid (1.06 g, 52%). Additional material of slightly lower purity (0.84 g) was recovered from the column by subsequent elution with 85:15 $CHCl_3$–MeOH containing 5% Et_3N. Anal. Calcd for $C_{38}H_{48}N_{10}O_8{\cdot}2.5H_2O$: C, 55.80; H, 6.53; N, 17.13. Found: C, 55.49; H, 6.34; N, 17.22.

N^{α}-(*tert*-Butyloxycarbonyl)-N^{ϵ}-[N-(4-amino-4-deoxy-N^{10}-methylpteroyl)-L-γ-glutamyl]-L-lysyl-N^{ϵ}-(benzyloxycarbonyl)-L-lysine Benzyl ($O^{\alpha\text{-glu}}$) 2-(Trimethylsilyl)ethyl (O^{lys}) Ester (7). To a solution of 6 (1.23 g, 1.5 mmol) and 3·TsOH (1.0 g, 1.8 mmol) in dry DMF (30 mL) at 0 °C was added DPPA (495 mg, 1.8 mmol) and Et_3N (485 mg, 4.8 mmol). After being left to stand 3 h at 0 °C and overnight at room temperature, the reaction was worked up as in the coupling of 3·TsOH and N^{α}-(*tert*-butyloxycarbonyl)-N^{ϵ}-(benzyloxycarbonyl)-L-lysine. Appropriate fractions from the column were pooled and evaporated, and the residue was triturated with ether to obtain a yellow solid (1.28 g, 75%): mp 95–104 °C after drying in vacuo at 60 °C (P_2O_5). Anal. Calcd for $C_{57}H_{78}N_{12}O_{11}Si{\cdot}0.5H_2O$: C, 59.82; H, 6.96; N, 14.69; Si, 2.45. Found: C, 59.77; H, 7.02; N, 14.76; Si, 2.35.

N^{α}-(*tert*-Butyloxycarbonyl)-N^{ϵ}-[N-(4-amino-4-deoxy-N^{10}-methylpteroyl)-L-γ-glutamyl]-L-lysyl-N-(benzyloxycarbonyl)-L-lysine Benzyl ($O^{\alpha\text{-glu}}$) Ester (8). A solution of 7 (227 mg, 0.2 mmol), tetrabutylammonium chloride (167 mg, 0.6 mmol), and KF·$2H_2O$ (79 mg, 0.84 mmol) in dry DMF (5 mL) was stirred at 50–55 °C for 3 days. After rotary evaporation, the residue was triturated with 10% AcOH until solidification occurred. The product was filtered, dried, and chromatographed (silica gel, 9:1 followed by 85:15 $CHCl_3$–MeOH): yield 86 mg (42%). Anal. Calcd for $C_{52}H_{66}N_{12}O_{11}{\cdot}3H_2O$: C, 57.35; H, 6.66; N, 15.43. Found: C, 57.40; H, 6.48; N, 15.21.

N^{α}-(*tert*-Butyloxycarbonyl)-N^{ϵ}-[N-(4-amino-4-deoxy-N^{10}-methylpteroyl)-L-γ-glutamyl]-L-lysyl-N^{ϵ}-(benzyloxycarbonyl)-L-lysyl-N^{ϵ}-(benzyloxycarbonyl)-L-lysine Benzyl ($O^{\alpha\text{-glu}}$) 2-(Trimethylsilyl)ethyl (O^{lys}) Ester (1). A. A solution of 8 (702 mg, 0.65 mmol) and 3·TsOH (397 mg, 0.72 mmol) in dry DMF (15 mL) at 0 °C was treated with DPPA (198 mg, 0.72 mmol) and Et_3N (212 mg, 2.1 mmol), and the reaction was worked up as in the preparation of the dilysyl derivative 7 to obtain a yellow solid (501 mg, 56%): mp 103–112 °C. Anal. Calcd for $C_{71}H_{96}N_{14}O_{14}Si{\cdot}0.5H_2O$: C, 60.62; H, 6.95; N, 13.94. Found: C, 60.66; H, 6.94; N, 13.96.

B. To a solution of 6 (408 mg, 0.5 mmol) and 4·TsOH (443 mg, 0.55 mmol) in dry DMF (10 mL) at 0 °C were added DPPA (152 mg, 0.55 mmol) and Et_3N (161 mg, 1.6 mmol). After being left at 0 °C for 3 h and room temperature overnight, the reaction was worked up as in the synthesis of 7. The product was eluted from the silica gel column with 95:5 $CHCl_3$–MeOH followed by the same solvent combination with 5% Et_3N added: total yield 579 mg (83%); mp 99–107 °C. Material obtained by this method was spectroscopically identical with that prepared from 8 and 3·TsOH.

N^{α}-(*tert*-Butyloxycarbonyl)-N^{ϵ}-[N-(4-amino-4-deoxy-N^{10}-methylpteroyl)-L-γ-glutamyl]-L-lysyl-N^{ϵ}-(benzyloxycarbonyl)-L-lysyl-N^{ϵ}-(benzyloxycarbonyl)-L-lysine Benzyl ($O^{\alpha\text{-glu}}$) Ester (9). A solution of the diester 1 (350 mg, 0.25 mmol), tetrabutylammonium chloride (208 mg, 0.75 mmol), and KF·$2H_2O$ (99 mg, 1.05 mmol) in dry DMF was stirred at 55 °C for 36 h, and the product was isolated as in the preparation of 8. The silica

gel column was eluted successively with 9:1 and 85:15 $CHCl_3$–MeOH, and then with 85:15 $CHCl_3$–MeOH containing 5% Et_3N: total yield 208 mg (62%). Anal. Calcd for $C_{66}H_{84}N_{14}O_{14}\cdot 3H_2O$: C, 58.65; H, 6.71; N, 14.51. Found: C, 58.45; H, 6.63; N, 14.12.

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Registry No. 1, 89106-02-5; 2, 19741-14-1; 3·TsOH, 89106-04-7; 4·TsOH, 89121-06-2; 5, 89106-05-8; 6, 89106-06-9; 7, 89106-07-0; 8, 89106-08-1; 9, 89106-09-2; Boc-Lys(Cbz)-OH, 2389-45-9; Boc-Lys(Cbz)-OTmse, 89121-14-2; Boc-Lys(Cbz)-Lys(Cbz)-OTmse, 89121-15-3; H-Glu-OBzl, 13030-09-6; Me_3Si-Glu($OSiMe_3$)-OBzl, 89106-10-5; Boc-Lys-OH, 13734-28-6; Boc-Lys($SiMe_3$)-$OSiMe_3$, 89106-11-6; 2-(trimethylsilyl)ethanol, 2916-68-9.

Preparation of Alkyl Aryl Sulfides via Phase-Transfer Catalyzed Displacement of Aromatic Chloride by Alkyl Thiolates

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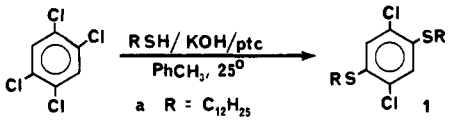
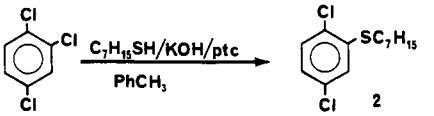
Although a number of procedures have been developed for the preparation of alkyl aryl sulfides, displacement of unactivated aryl chlorides with alkyl mercaptans was not reported until recently.[1-4] Tiecco et al. have utilized nucleophilic displacement of aromatic chlorides with alkyl sodium thiolates in hexamethylphosphoric triamide (HMPA) solution.[5] A recent report by Rolla et al.[6] on the reaction of alkyl thiolates with dichlorobenzenes in water, catalyzed by dicyclohexano-18-crown-6 prompts us to disclose results in a similar area. We have found that reaction occurs between alkyl thiolates and aromatic di-, tri-, and tetrachlorides with surprisingly high regioselectivity under phase transfer catalysis (PTC) conditions in toluene solution, thus allowing high yield preparation of alkyl aryl sulfides from a variety of chloro aromatics and alkyl thiols.



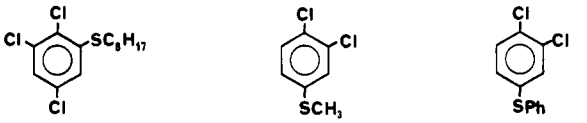
We became interested in PTC reactions of aryl chlorides because of the difficulties associated with the reaction: unactivated aryl halides normally require reaction at high temperatures in polar solvents (e.g., 200 °C in dimethylformamide[7]) or special solvents such as HMPA. Our results with a variety of phase-transfer catalysts are summarized in Table I. Phosphonium salts, crown ethers, and polyethylene glycols were effective catalysts for the conversion. Furthermore, we found it was not necessary to use strong bases, such as KH or *t*-BuOK, to form the thiolate; ground 85% KOH pellets were equally effective.

Reaction of 1,2,4,5-tetrachlorobenzene provides the para disulfide 1 in 89% yield; 1–4% of the 2,4- and 4,5-disulfides were also detected by VPC. Preparation of the monosulfide is possible by use of 1 equiv of thiol. Reaction of 1,2,4-trichlorobenzene provides almost exclusive substitution at the 2-position, providing sulfide 2 in 92% yield.



Reaction of the trichlorobenzene is substantially slower than that of the tetrachlorobenzene and requires reaction at ambient temperature for 24 h or warming to 50 °C for 3 h to effect complete reaction.

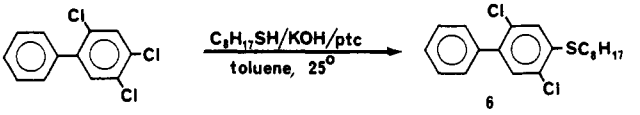
We have found, contrary to literature predictions,[8] that chlorine acts primarily as a meta and ortho activator. Thus, a competition experiment using limited thiolate in reaction with the three isomeric trichlorides showed 73% reaction of the 1,3,5-isomer, 50% reaction of the 1,2,3-isomer, and only 37% reaction of the 1,2,4-isomer. Additionally, we have found that reaction occurs predominantly at positions that have no para chloride. Thus, 1,2,3,5-tetrachlorobenzene provides sulfide 3 as the major



product; displacement at the 1-position is activated by two meta chlorides and has no deactivating para chlorides. Chloride is a more efficient activator than a sulfide or a phenyl group. Thus, sulfides 4 and 5 react to only about 5% completion under conditions that consume 1,2,4-trichlorobenzene.

All the dichlorobenzenes react significantly more slowly than the more highly chlorinated compounds, and reflux in toluene is necessary. These conditions roughly parallel those used by Rolla et al.[6] for the reaction of dichlorobenzenes in water.

We have found that polychlorinated biphenyls (PCB's) are quite reactive under our conditions. For example, 2,4,5-trichlorobiphenyl is converted to sulfide 6 in 90%



yield at ambient temperature in 5 h. The unsymmetrical PCB 2,3,4,5,6-pentachlorobiphenyl reacts to form a 40:60 mixture of di- and trisulfides.[9]

The most efficient PTC was a hindered phosphonium salt, (tricyclohexyl-*n*-dodecyl)phosphonium bromide.[10] Use of 0.1 equiv of this catalyst at 25 °C afforded complete reaction of tetrachlorobenzene in 2 h. A hindered phosphonium salt is preferred, to prevent destruction of the salt via S_N2 reaction by thiolate (butyl alkyl sulfides have

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(10) This salt has been used for the alkylation of ketones: Tamai, Y.; Nishida, Y.; Fujita, Y.; Ohmura, Y.; Hosogai, T.; Ninagawa, Y.; Itoi, K. Japan Kokai 75 96 514, 1976; *Chem. Abstr.* **1976**, *84*, 4483s.

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