The Configuration of Phenoxymethyl- and 6-Epiphenoxymethylpenicillin Sulfoxides

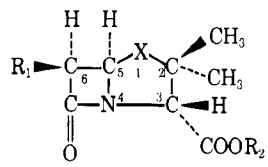
Arnold J. Vlietinck,[1a] Eugene Roets,[1a] Hubert Vanderhaeghe,*[1a] and Suzanne Toppet[1b]

Rega and Pharmaceutical Institute and the Laboratory of Macromolecular and Organic Chemistry, University of Leuven, 3000 Leuven, Belgium

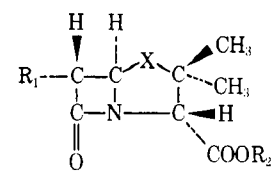
Received August 3, 1973

Phenoxymethyl- (8) and 6-epiphenoxymethylpenicillin benzyl ester (7) and their corresponding (*S*)- and (*R*)-sulfoxides were studied. The methyl signals in the nmr spectra of these compounds were assigned by nuclear Overhauser effects, and the configuration was determined by aromatic solvent induced shifts and by sulfoxide bond chemical shift perturbations. Finally, deuterium incorporation was studied in the isomerization of phenoxymethyl- (10) and 6-epiphenoxymethylpenicillin (*R*)-sulfoxide benzyl ester (3) to their corresponding (*S*)-sulfoxides 1 and 2.

In a recent publication describing the conversion of phenoxymethylpenicillin (*S*)-sulfoxide benzyl ester (1) to the corresponding 6 epimer **2**, we assumed that the latter had kept the *S* configuration of the starting product.[2] We wish now to give evidence in support of this hypothesis, by preparing the corresponding 6-epiphenoxymethylpenicillin (*R*)-sulfoxide benzyl ester (3) and using different nmr techniques for the assignment of configuration.



No.	R_1	R_2	X
1	$C_6H_5OCH_2CONH-$	$C_6H_5CH_2-$	S—O
8	$C_6H_5OCH_2CONH-$	$C_6H_5CH_2-$	S
9	$C_6H_5OCH_2CONH-$	$C_6H_5CH_2-$	S—O
10	$C_6H_5OCH_2CONH-$	H	S—O
11	$C_6H_5OCH_2CONH-$	CH_3	S—O



No.	R_1	R_2	X
2	$C_6H_5OCH_2CONH-$	$C_6H_5CH_2-$	S—O
3	$C_6H_5OCH_2CONH-$	$C_6H_5CH_2-$	S—O
4	$C_6H_4(CO)_2N-$	CH_3-	S
5	$C_6H_4(CO)_2N-$	CH_3-	S—O
6	$C_6H_4(CO)_2N-$	CH_3-	S—O
7	$C_6H_5OCH_2CONH-$	$C_6H_5CH_2-$	S

Whereas the oxidation of 6-epiphthalimidopenicillin methyl ester (4) with *m*-chloroperbenzoic acid[3] gave the (*R*)- and (*S*)-sulfoxides 5 and 6 in a ratio of 1:4, the application of the same method to 6-epiphenoxymethylpenicillin benzyl ester (7) yielded only the 6-epi (*S*)-sulfoxide[2] **2**. The 6-epiphenoxymethylpenicillin (*R*)-sulfoxide benzyl ester (3), however could be prepared by oxidation of 6-epiphenoxymethylpenicillin benzyl ester (7) with iodobenzene dichloride.[4,5] Surprisingly no corresponding 6-epi (*S*)-sulfoxide 2 was formed in this reaction, while oxidation of phenoxymethylpenicillin benzyl ester (8) with iodobenzene dichloride afforded a mixture of the (*S*)- and (*R*)-sulfoxides 1 and 9 in a ratio of approximately 1:4.

With ozone as oxidant[6,7] in acetone–water (3:2) both phenoxymethyl- (8) and 6-epiphenoxymethylpenicillin benzyl ester (7) afforded a mixture of the corresponding (*S*)- and (*R*)-sulfoxides in a ratio of about 1:1. In the former mixture the two sulfoxides 1 and 9 were isolated by column chromatography, while in the latter mixture 6-epi (*S*)-sulfoxide 2 was separated from 6-epi (*R*)-sulfoxide 3 by fractional crystallization from dry benzene, because the two isomers presented the same R_f values in our chromatographic systems.

The different compounds showed the nmr signals presented in Table I.

Table I
Nmr Data[a] for Phenoxymethyl- and 6-Epiphenoxymethylpenicillin Derivatives

	H_3	H_5	H_6	2β-Me	2α-Me
8, $CDCl_3$	4.49	5.56	5.70	1.55	1.40
1, $CDCl_3$	4.70	5.00	6.08	1.67	1.07
9, $CDCl_3$	4.43	4.75	5.52	1.63	1.22
7, $CDCl_3$[b]	4.55	5.24	5.22	1.60	1.40
C_6D_6[b]	4.50	5.26	5.01	1.22	1.15
2, $CDCl_3$	4.56	5.05	5.44	1.64	1.10
C_6D_6	4.57	4.58	5.17	1.31	0.61
3, $CDCl_3$	4.52	4.79	5.54	1.46	1.34
C_6D_6	4.33	4.93	5.09	0.89	1.19

[a] In parts per million in 2.5% solution using TMS as internal reference, measured on a Varian XL-100. [b] Concentration ≪1% owing to insolubility in C_6D_6. The values of **2** in $CDCl_3$ are extrapolated to infinite solution.

Assignments for the methyl groups were made using internal nuclear Overhauser effects (NOE).[3,8,9] In 6-epiphenoxymethylpenicillin (*S*)-sulfoxide benzyl ester (2) a NOE effect of 30% (in $CDCl_3$) was observed between the 2β-methyl group (1.64 ppm) and the H_3 signal (4.56 ppm). A similar effect (19%) was also observed between the 2α-methyl group (1.10 ppm) and the H_5 (5.05 ppm) but not between H_3 and the 2α-methyl group. In 6-epiphenoxymethylpenicillin (*R*)-sulfoxide benzyl ester (3) positive effects (in $CDCl_3$ and C_6D_6) were observed between the 2β-methyl group (1.46, 0.89 ppm) and the H_3 signal (4.52, 4.33 ppm) (18 and 16%) and between the 2α-methyl group (1.34, 1.19 ppm) and the H_5 (4.79, 4.93 ppm) (10 and 8%). Finally, 6-epiphenoxymethylpenicillin benzyl ester (7) gave NOE effects (in $CDCl_3$ and C_6D_6) between H_3 (4.55, 4.50 ppm) and the 2α-methyl group (1.40, 1.15 ppm) (7 and 9%) and the 2β-methyl group (1.60, 1.22 ppm) (17 and 20%).

The high-field signal (Table I) may be assigned to the 2α-methyl protons, except for compound 3 in C_6D_6 solution. It also may be concluded that parallel conformational differences exist between both 6-epi sulfoxides 2 and 3 and the parent 6-epi sulfide 7, as has been previously re-

Table II
Solvent Shifts for 6-Epiphenloxymethylpenicillin Derivatives[a]

	H_3	H_5	H_6	2β-Me	2α-Me
7, Δ_1 [δ($CDCl_3$) − δ(C_6D_6)]	+0.05	−0.02	+0.21	+0.38	+0.25
2, Δ_2 [δ($CDCl_3$) − δ(C_6D_6)]	−0.01	+0.47	+0.27	+0.33	+0.49
$\Delta_2 - \Delta_1$	−0.06	+0.49	+0.06	−0.05	+0.24
3, Δ_3 [δ($CDCl_3$) − δ(C_6D_6)]	+0.19	−0.14	+0.45	+0.57	+0.15
$\Delta_3 - \Delta_1$	+0.14	−0.12	+0.24	+0.19	−0.10

[a] Positive values indicate upfield shifts, negative values downfield shifts.

Table III
Proton Resonance Shifts Induced by the Sulfoxide Bond (Δ_{SO})[a] in Penicillins and 6-Epipenicillins

	H_3	H_5	H_6	2β-Me	2α-Me
Sulfide **8** → sulfoxide **1**, $CDCl_3$	−0.21	+0.56	−0.38	−0.12	+0.33
Sulfide **8** → sulfoxide **9**, $CDCl_3$	+0.06	+0.81	+0.18	−0.08	+0.18
Sulfide **7** → sulfoxide **2**, $CDCl_3$	−0.01	+0.19	−0.22	−0.04	+0.30
C_6D_6	−0.07	+0.68	−0.16	−0.09	+0.54
Sulfide **7** → sulfoxide **3**, $CDCl_3$	+0.03	+0.45	−0.32	+0.14	+0.06
C_6D_6	+0.17	+0.33	−0.08	+0.33	−0.04

[a] $\Delta_{SO} = \delta_{sulfide} - \delta_{sulfoxide}$. Positive and negative signs indicate upfield and downfield shifts, respectively, resulting from the process sulfide → sulfoxide.

ported[8-10] for penicillins with a normal configuration and their sulfoxides.

From the chemical shifts of Table I, the aromatic solvent induced shift (ASIS) values were calculated (Table II). These data indicate that H_5 and the 2α-methyl group are shielded (0.49 and 0.24 ppm, respectively) in **2**, and that H_3, H_6, and the 2β-methyl group are shielded (0.14, 0.24, and 0.19 ppm, respectively) in **3**. Therefore **2** is assigned the *S* configuration and **3** the *R* configuration. Our figures are in good agreement with the ASIS values obtained for the epiphthalimidopenicillin sulfoxides **5** and **6**.[3]

Sulfoxide bond chemical shift perturbations have also been useful in assigning configuration of sulfoxides.[3,8,11,12] Table III summarizes the chemical shift changes in 6-epiphenoxymethyl- (**7**) and phenoxymethylpenicillin benzyl ester (**8**) upon oxidation to their corresponding sulfoxides. The data show that the sulfoxide bond in sulfoxides **1** and **2** exerts shielding effects on H_5 and the 2α methyl group and exerts deshielding effects on H_3, H_6, and the 2β-methyl group. Consequently, in the light of the presently accepted model for the screening environment around the sulfoxide bond,[8] these sulfoxides should have the *S* configuration. The deshielding effects on H_3, H_6, and the 2β-methy group in (*S*)-sulfoxides have been explained by the occurrence of a syn-axial effect.[11] The shielding of H_5 and also of the 2α-methyl group in (*R*)-sulfoxides has been explained[9,11] by the influence of the lone-pair electrons of the (*R*)-sulfoxide function on any α-anti-axial group. However, the upfield shift of H_6 in the *R* sulfoxide **9** and the 2β-methyl group in the 6-epi (*R*)-sulfoxide **2** are in disagreement with calculated shifts[8] and suggest that the sulfoxide-bond anisotropy might be more complicated than previously assumed.

It should be noted that the difference in chemical shifts between the two methyl groups is more pronounced in the (*S*)-sulfoxide than in the corresponding (*R*)-sulfoxide (Table I). Similar differences are observed for other penicillin esters where the epimeric sulfoxides are available.[3,8,9]

The determination of the configuration of penicillin (*S*)-sulfoxides proves that base-catalyzed epimerization occurs without change of the configuration of the sulfoxide function. This was further confirmed by the epimerization of phenoxymethylpenicillin (*R*)-sulfoxide benzyl ester (**9**) in methylene chloride with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) for 10 min at 0° to the corresponding 6-epiphenoxymethylpenicillin (*R*)-sulfoxide benzyl ester (**3**). It was estimated from tlc that about 50% of the starting product was epimerized and that several less polar products were formed.

The transformation of the (*R*)-sulfoxides of different penicillin esters into their *S* isomers in refluxing solvent has been described. We examined the same isomerization reaction for the (*R*)-sulfoxides prepared in this study.

An equilibrium mixture of the (*S*)-sulfoxide **1** and the (*R*)-sulfoxide **9** in a ratio of about 4:1 and of the 6-epi (*S*)-sulfoxide **2** and the 6-epi (*R*)-sulfoxide **3** in a ratio of about 1:1 was obtained by refluxing a solution of phenoxymethyl- (**9**) and 6-epiphenoxymethylpenicillin (*R*)-sulfoxide benzyl ester (**3**) for 3 hr in benzene containing *tert*-butyl[^{2}H] alcohol. In both the (*S*)-sulfoxide **1** and the 6-epi (*S*)-sulfoxide **2**, the 2β-methyl group contained an average of one deuterium atom with a deuterium incorporation of about 47%. The recovered 6-epi (*R*)-sulfoxide **3** showed no deuterium incorporation, while in the (*R*)-sulfoxide **9** the 2α-methyl group contained an average of one deuterium atom with an incorporation of about 19%. These results clearly indicate a greater thermodynamic stability not only of the (*S*)-sulfoxide **1**, which possesses a strong hydrogen bond[8] to the side-chain amide proton, but also of the 6-epi (*S*)-sulfoxide **2**, which lacks this intramolecular hydrogen bond. However, the equilibrium ratio in the latter is less favored for the (*S*)-sulfoxide than in the former epimerization mixture.

Similar treatment of phenoxymethyl- (**1**) and of 6-epiphenoxymethylpenicillin (*S*)-sulfoxide benzyl ester (**2**) gave only starting products, which nevertheless contain an average of one deuterium located in the 2β-methyl group. The deuterium incorporation was established from nmr and mass spectra, around 10%. Prolonged heating (24 hr) in benzene containing *tert*-butyl[^{2}H] alcohol increases the deuterium incorporation in the 2β-methyl group to about 47% in **1** and 70% in **2**.

It has been reported that prolonged heating of **11** in benzene containing deuterium oxide is required before deuterium incorporation into the 2β-methyl group is observed.[13]

These experiments confirm the involvement of a sulfenic acid[5,7,13] in the thermal epimerization of (*R*)-sulfoxides to (*S*)-sulfoxides.

Experimental Section

Melting points were determined on a Büchi-Tottoli apparatus and are corrected. The ir spectra were recorded on a Perkin-Elmer 257 spectrometer. The nmr spectra were run on a Varian XL-100 spectrometer with tetramethylsilane (TMS) as internal

standard. Mass spectra were recorded on a single focusing AEI-MS 12 apparatus, operating at 8-kV accelerating voltage, 100-μA trap current, and 70 eV ionization energy. Deuterium incorporation percentages are calculated on the $M^+ - H_2O$ peak by a temperature of 170–180° and are minimum values, because some HOD may be split off. The ozone oxidations were run using a J. W. Towers (Gallenkamp) ozonizer with an output of 0.6 g of O_3/hr.

Phenoxymethylpenicillin (*R*)-Sulfoxide Benzyl Ester (9). A. Oxidation of Phenoxymethylpenicillin Benzyl Ester (8) with Ozone. Into a cooled (0°) solution of phenoxymethylpenicillin benzyl ester (8, 3.0 g, 6.82 mmol) in 50 ml of 3:2 acetone–water mixture was bubbled ozone for 3.0 hr. Evaporation of acetone from the reaction mixture gave an emulsion which was extracted three times with 100 ml of EtOAc. Extracts were washed with water, dried (Na_2SO_4), and evaporated to an oil, which was a mixture of the two sulfoxides 1 and 9 in a ratio of ±1:1 (nmr). As crystallization in methanol failed, the oil was chromatographed over silica gel (40.0 g) using a gradient of benzene changing to benzene–acetone (80:20) as eluent. Fractions (10 ml) 30–47 were evaporated by dryness, and the product was crystallized from anhydrous methanol (5 ml), yielding 1.21 g (39%) of the (*S*)-sulfoxide 1. Fractions 52–74 were evaporated to dryness, dissolved in 10 ml of anhydrous benzene, and lyophilized, yielding 0.87 g (28%) of the amorphous (*R*)-sulfoxide 9: mp about 80° dec; $[\alpha]^{20}D$ +118° (*c* 0.5, acetone); *m/e* 456; ir (KBr) 3460–3120, 1675, 1510 (amide), 1790 (β-lactam C=O), 1750, 1210 (ester), 1055 cm^{-1} (S=O); nmr ($CDCl_3$) δ 1.22 (s, CH_3), 1.63 (s, CH_3), 4.43 (s, 3-H), 4.56 (s, OCH_2CO), 4.75 (d, J = 4.5 Hz, 5-H), 5.23 (AB, CH_2), 5.52 (dd, J = 4.5, 10.5 Hz, 6-H), 6.92–7.42 (m, phenyl and amide).

B. Oxidation of Phenoxymethylpenicillin Benzyl Ester (8) with Iodobenzene Dichloride. A solution of iodobenzene dichloride (1.65 g, 6 mmol) in 4 ml of anhydrous pyridine was added at once to a stirred solution of phenoxymethylpenicillin benzyl ester (8, 1.76 g, 4 mmol) in 4 ml of 20% aqueous pyridine. The mixture was diluted with 40 ml of methylene chloride, washed once with 40 ml of 1 *M* sulfuric acid and three times with 40 ml of ice–water, dried (Na_2SO_4), and evaporated to an oil. The residue was dissolved in 10 ml of dry benzene and chromatographed as described under A, yielding 1.08 g (59%) of starting product, 75 mg (4%) of crystalline (*S*)-sulfoxide 1, and 302 mg (16%) of amorphous (*R*)-sulfoxide 9.

C. Esterification of Phenoxymethylpenicillin (*R*)-Sulfoxide Free Acid (10). To a cooled (0°) solution of phenoxymethylpenicillin (*R*)-sulfoxide (10, 1.0 g, 2.73 mmol) in 10 ml of anhydrous acetone, a solution of phenyldiazomethane[14] in n-pentane was added dropwise until an orange color persisted. The mixture was kept for a further 30 min at 0°, and the excess of phenyldiazomethane was destroyed by addition of 0.5 ml of acetic acid. The solution was diluted with 50 ml of methylene chloride, washed twice with 20 ml of 1 *M* $NaHCO_3$ and twice with 20 ml of H_2O, dried (Na_2SO_4), and evaporated to an oil. The residue was chromatographed as described under A, yielding 631.0 mg (51%) of amorphous (*R*)-sulfoxide.

6-Epiphenoxymethylpenicillin (*R*)-Sulfoxide Benzyl Ester (3). A. Oxidation of 6-Epiphenoxymethylpenicillin Benzyl Ester (7) with Ozone. The 6-epiphenoxymethylpenicillin benzyl ester (7, 660.0 mg, 1.5 mmol) was oxidized with ozone as described for 8 under A, yielding 386.0 mg (56%) of amorphous solid, which contained the two sulfoxides 2 and 3 in a ratio of about 1:1 (nmr). The mixture was crystallized from 3 ml of anhydrous benzene, yielding 177.0 mg (26%) of 6-epi (*S*)-sulfoxide 2. Lyophilization of the filtrate gave 194.0 mg (28%) of noncrystalline 6-epi (*R*)-sulfoxide 3: mp about 70° dec; $[\alpha]^{20}D$ +116° (*c* 0.5, acetone); *m/e* 456; ir (KBr) 3360, 1680, 1515 (amide), 1782 (β-lactam C=O), 1747, 1212 (ester), 1050 cm^{-1} (S=O); nmr ($CDCl_3$) δ 1.34 (s, CH_3), 1.46 (s, CH_3), 4.52 (s, 3-H), 4.56 (s, OCH_2), 4.79 (d, J = 1.5 Hz, 5-H), 5.22 (AB, CH_2), 5.54 (dd, J = 1.5, 9 Hz, 6-H), 6.84–7.48 (m, phenyl and amide).

B. Oxidation of 6-Epiphenoxymethylpenicillin Benzyl Ester (7) with Iodobenzene Dichloride. 6-Epiphenoxymethylpenicillin benzyl ester (880.0 mg, 2 mmol) was oxidized with iodobenzene dichloride as described for 8 under B, yielding an oil which contained only the 6-epi (*R*)-sulfoxide 3. Lyophilization of the residue from anhydrous benzene gave 524.0 mg (57%) of amorphous 6-epi (*R*)-sulfoxide 3.

C. Epimerization of Phenoxymethylpenicillin (*R*)-Sulfoxide Benzyl Ester (9). A solution of DBN (80.7 mg, 0.65 mmol) in 0.5 ml of methylene chloride was added to a cooled (0°) solution of phenoxymethylpenicillin (*R*)-sulfoxide benzyl ester (9, 296.0 mg, 0.65 mmol) in 6 ml of anhydrous methylene chloride. The reaction was allowed to proceed at 0° for 20 min. The mixture was evaporated to near dryness, and preparative tlc of the residue (benzene–EtOAc, 50:50) gave, after elution with acetone, evaporation to dryness, and lyophilization from dry benzene, 86.0 mg (29%) of starting material 9 and 115.0 mg (39%) of 6-epiphenoxymethylpenicillin (*R*)-sulfoxide benzyl ester (3). The product was identical in all respects with the compound described under A.

Incorporation of Deuterium. A. Phenoxymethylpenicillin (*R*)-sulfoxide benzyl ester (9, 300.0 mg, 0.66 mmol) was heated in refluxing benzene (12 ml) containing *tert*-butyl[^{2}H] alcohol (6 ml) for 3 hr. The residue obtained after removal of solvents was dissolved in methanol (2 ml), and the solution was set aside for 16 hr at room temperature. The crystals of the corresponding (*S*)-sulfoxide 1 thus obtained were collected and washed with methanol to yield 188 mg (63%). Preparative tlc of the filtrate gave 48.0 mg (16%) of unchanged (*R*)-sulfoxide 9.

The (*S*)-sulfoxide 1 showed in its integrated nmr spectrum a ratio of the two methyl signals (2β:2α) of 2.52:3.00, indicating an incorporation of 48% deuterium; the amide proton integrated as 1.0 H. In its mass spectrum this material showed a mixture of 53.7% d_0, 39.1% d_1, 6.8% d_2, and 0.3% d_3 products. The recovered (*R*)-sulfoxide 9 showed in its integrated nmr spectrum a ratio of the two methyl signals (2β:2α) of 2.81:3.00, indicating an incorporation of 19% deuterium. Its mass spectrum showed a mixture of 82.0% d_0, 16.9% d_1, and 1.1% d_2 products.

B. Phenoxymethylpenicillin (*S*)-sulfoxide benzyl ester (1, 100.0 mg, 0.22 mmol) was heated in refluxing benzene (4 ml) containing *tert*-butyl[^{2}H] alcohol (2 ml) for 3 hr. Treatment of the product as in A gave 89.0 mg (89%) recovery of starting material. The methyl ratio in its integrated nmr spectrum was for 2β:2α 2.89:3.00, indicating a deuterium incorporation of 11%. The mass spectrum showed a mixture of 91.5% d_0, 6.6% d_1, and 1.9% d_2 products.

When heating was continued for 24 hr and the product was treated as in A, 56 mg (56%) of starting material 1 was recovered, showing in its nmr spectrum a methyl ratio (2β:2α) of 2.53:3.00. This corresponds with a deuterium incorporation of 47%. The mass spectrum showed a mixture of 60.4% d_0, 32.0% d_1, and 7.5% d_2 products.

C. 6-Epiphenoxymethylpenicillin (*R*)-sulfoxide benzyl ester (3, 55.0 mg, 0.12 mmol) was heated in refluxing benzene (2 ml) containing *tert*-butyl[^{2}H] alcohol (1 ml) for 3 hr. After the ^{2}H of the amide group was reexchanged with methanol, the solvent was removed and the residue was lyophilized from dry benzene, yielding 40.0 mg (73%). Preparative tlc of the residue (20:80 acetone–benzene) gave 15.0 mg (25%) of a mixture of the (*R*)- and (*S*)-sulfoxide in a ratio of 46:54. The integrated nmr spectrum of the mixture showed a ratio of the two methyl groups (2β:2α) of 2.54:3.00 in the 6-epi (*S*)-sulfoxide 2, indicating an incorporation of 46% deuterium. The 6-epi (*R*)-sulfoxide showed no deuterium incorporation. The two products could not be separated. The mass spectrum of the mixture showed 82.4% d_0, 14.2% d_1, and 3.4% d_2 products.

D. 6-Epiphenoxymethylpenicillin (*S*)-sulfoxide benzyl ester (2, 100.0 mg, 0.22 mmol) was heated in refluxing benzene (4 ml) containing *tert*-butyl[^{2}H] alcohol (2 ml) for 3 hr. Work-up as in A gave 60.0 mg (60%) of starting material. The integrated nmr showed a ratio of the two methyl groups (2β:2α) of 2.90:3.00, indicating an incorporation of deuterium of 10%. Its mass spectrum showed a mixture of 89.0% d_0 and 11.0% d_1 products.

When heating was continued for 24 hr and the product was treated as in A, 20 mg (20%) of starting material 2 was recovered, showing in its nmr spectrum a methyl ratio (2β:2α) of 2.30:3.00. This corresponds with a deuterium incorporation of 70%. The mass spectrum showed a mixture of 34.0% d_0, 48.0% d_1, 17.5% d_2, and 0.5% d_3 products.

Acknowledgments. The authors are grateful to the Belgian Fonds voor het Wetenschappelijk Geneeskundig Onderzoek for financial support. We are indebted to Dr. G. Janssen for the mass spectral determinations and the calculations of the deuterium incorporations.

Registry No. 1, 42879-04-9; 2, 42879-05-0; 3, 42879-06-1; 7, 36416-52-1; 8, 1256-06-0; 9, 42879-09-4.

References and Notes

(1) (a) Rega and Pharmaceutical Institute; (b) Laboratory of Macromolecular and Organic Chemistry.
(2) P. Claes, A. Vlietinck, E. Roets, H. Vanderhaeghe, and S. Toppet, *J. Chem. Soc., Perkin Trans. 1*, 932 (1973).

(3) R. D. G. Cooper, P. V. DeMarco, and D. O. Spry, J. Amer. Chem. Soc., 91, 1528 (1969). According to a personal communication of R. D. G. Cooper the chemical shifts of the methyl groups of compound 4 in $CDCl_3$ and of compound 6 in C_6D_6 should be inverted in Table II of this publication. After correction of Δ_4 and Δ_6 for these groups, one obtains $\Delta_5 - \Delta_4$ for 2β-Me −0.07 and for 2α-Me +0.21 and $\Delta_6 - \Delta_4$ for 2β-Me +0.29 and for 2α-Me −0.04. The values for the methyl groups in Table III also should be corrected.
(4) G. Barbieri, M. Cinquini, S. Colonna, and F. Montanari, J. Chem. Soc. C, 659 (1968).
(5) D. H. R. Barton, D. G. T. Greig, G. Lucente, S. G. Sammes, M. V. Taylor, C. M. Cooper, C. Hewitt, and W. G. E. Underwood, J. Chem. Soc. C, 3540 (1971).
(6) D. O. Spry, J. Org. Chem., 37, 793 (1972).
(7) D. O. Spry, J. Amer. Chem. Soc., 92, 5006 (1970).
(8) R. D. G. Cooper, P. V. DeMarco, J. C. Cheng, and N. D. Jones, J. Amer. Chem. Soc., 91, 1408 (1969).
(9) R. A. Archer and P. V. DeMarco, J. Amer. Chem. Soc., 91, 1530 (1969).
(10) E. H. Flynn, Ed., "Cephalosporins and Penicillins: Chemistry and Biology," Academic Press, New York, N. Y., 1972, p 342.
(11) D. H. R. Barton, F. Comer, and P. G. Sammes, J. Amer. Chem. Soc., 91, 1529 (1969), and references cited therein.
(12) R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanlon, and S. L. Andrews, J. Amer. Chem. Soc., 91, 1401 (1969).
(13) R. D. G. Cooper, J. Amer. Chem. Soc., 92, 5010 (1970).
(14) P. Yates and B. L. Shapiro, J. Org. Chem., 23, 759 (1958).

o-Nitrophenyl Esters of Benzyloxycarbonylamino Acids and Their Application in the Synthesis of Peptide Chains by the *in Situ* Technique[1]

Miklos Bodanszky,* Michio Kondo,[2] Cynthia Yang Lin, and Gerald F. Sigler

Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106

Received September 4, 1973

Synthesis of *S*-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (*S,S'*-dibenzyloxytoceine) is described. The partially protected tetrapeptide derivative, *S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide, was acylated with the *o*-nitrophenyl ester of benzyloxycarbonyl-L-asparagine and the chain was lengthened in the same manner, with *o*-nitrophenyl esters of benzyloxycarbonylamino acids, until the fully protected nonapeptide was secured. The latter was partially deprotected with HBr in acetic acid and *S,S'*-dibenzyloxytoceine was obtained as the hydrobromide. All of the operations were carried out in the same vessel from which the intermediates were not removed throughout the synthesis. The preparation and properties of *o*-nitrophenyl esters of benzyloxycarbonylamino acids are also reported.

In a recent publication[3] from this laboratory, the preparation of *o*-nitrophenyl esters of *tert*-butyloxycarbonylamino acids and their application in the synthesis of a protected nonapeptide, corresponding to the C-terminal sequence of a secretin analog, was described. An additional characteristic feature of the synthesis was that the intermediates remained in the same vessel throughout the chain lengthening steps. The expression "*in situ* peptide synthesis" was proposed for the new technique. This report deals with an extension of the new approach to benzyloxycarbonylamino acid *o*-nitrophenyl esters and includes also the preparation and properties (Table I) of a number of such active esters. The intriguing questions, why *o*-nitrophenyl esters are more reactive[4] than their para isomers, why their reaction rates in aminolysis are less solvent dependent,[5] and why these rates remain less effected by steric hindrance, such as encountered in solid phase peptide synthesis,[6] are the subject of a separate study.[7]

The preparation of *o*-nitrophenyl esters of benzyloxycarbonylamino acids followed the procedure used for the para isomers.[8,9] However, in the case of hindered amino acids such as isoleucine or valine, pyridine rather than ethyl acetate had to be applied as solvent. Otherwise, the relatively poor reactivity of *o*-nitrophenol resulted in the formation of appreciable amounts of *N*-acyldicyclohexylureas and it was necessary to purify the products by chromatography. In pyridine, the nucleophilic character of *o*-nitrophenol is more pronounced; the esterification proceeds at a faster rate as shown by the disappearance of the 4.8-μ band of DCC in the ir spectra. Details of the preparation of the active esters are described in the Experimental Section, their properties in Table I.

For the examination of the usefulness of the new group of active esters for chain lengthening, especially by the *in situ* technique, the partially protected nonapeptide *S*-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (*S,S'*-dibenzyloxytoceine)[10] was selected. This choice was dictated in part by the familiarity of one of the authors (M. B.) with the synthesis of this peptide, including the properties of the intermediates. The *in situ* approach[3] requires a suitable solvent for the acylation reaction and a precipitant for the selective separation of the intermediate acyl peptides from the by-products, such as salts of triethylamine or diisopropylamine,[11] nitrophenol, and also from the excess[12] of the acylating reagent, the active ester. For some peptide derivatives it may be difficult to find a proper solvent–precipitant combination, and information on the solubility properties of the expected products is indeed desirable. In the present synthesis of *S,S'*-dibenzyloxytoceine, the protected tetrapeptide derivative *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide[13] was used as starting material, because the shorter chain intermediates are too soluble in common organic solvents that can keep the by-product, etc., in solution. The benzyloxycarbonyl group was removed with HBr in acetic acid, the hydrobromide precipitated and washed with ether, and the resulting amine allowed to react with the *o*-nitrophenyl ester of the next residue, benzyloxycarbonyl-L-asparagine, in dimethylformamide in the presence of tertiary base. The protected pentapeptide was isolated by dilution of the reaction mixture with 95% ethanol. The same combination, dimethylformamide as solvent and 95% ethanol as precipitant, was used through the rest of the synthetic procedure. The reactions were carried out in a centrifuge tube, *from which the intermediates were not removed.* The yields in this facile technique were found to be comparable with those of the conventional approach in which the intermediates were transferred from the reaction vessel to a filter, etc. These intermediates (Table II) were obtained mostly in satisfactory purity; yet, when necessary, they could be purified by recrystallization, still *in situ*, in the same centrifuge tube.

Because of some obvious limitations, such as the availability of a suitable solvent–precipitant combination or