in vacuo. Recrystallization of the solid residue from hexane afforded **42** (4.0 g, 51%), mp 100–101 °C. Anal. ($C_{24}H_{43}N_3O$) C, H, N.

**α-[(Diisopropylamino)ethyl]-α-(2-cyclohexylidenylethyl)-o-chlorophenylacetonitrile (6).** A suspension of KH (0.45 g of a 26.3% dispersion in oil, 2.69 mmol) and compound **3b** (0.50 g, 1.79 mmol) in toluene (10 mL) was slowly heated to 90 °C and kept at that temperature for 10 min. The orange mixture was then cooled somewhat (ca. 75 °C) and a solution of 2-cyclohexylidenylethyl bromide (**5**; 0.68 g, 3.6 mmol) in toluene (10 mL) was added in dropwise portions over a 30-min period. The mixture was stirred an additional 30 min at 70–75 °C, then cooled (5 °C), and water (10 mL) was slowly added. After separating the two phases, the toluene layer was washed with dilute HCl. A dark yellow layer formed between the organic and aqueous layers, and when it was separated it solidified on standing. After washing this solid several times with $Et_2O$, a light-yellow solid was obtained (0.57 g, 75%), mp 169–173 °C. Anal. ($C_{24}H_{35}Cl$-$N_2$·HCl·0.5$H_2O$) C, H, N.

This hydrochloride salt was dissolved in water and converted to its free base in the usual manner. The free base (oil) was used in the subsequent preparation of compound **34**.

**α-[(Diisopropylamino)ethyl]-α-(2-cyclohexylethyl)-o-chlorophenylacetamide (34).** A solution of compound **6** (18.53 g, 0.048 mol) in EtOH was hydrogenated in a 500-mL Parr shaker apparatus at room temperature and 2.0 psi pressure. Platinum oxide was the catalyst used in this reduction. After 2 h, the mixture was filtered and the filtrate evaporated to dryness in vacuo, yielding a yellow oil (17.87 g, 96%). The $^1$H NMR spectrum of this oil indicated that the vinyl proton, present in **6** at δ 5.1, was no longer present. This oil was then immediately used, without further purification, as follows: 14.77 g of this oil (0.038 mol) in concentrated $H_2SO_4$ (125 mL) was heated on a steam bath for 45 min. The acidic solution was then poured onto ice, basified with 10% NaOH solution, and the alkaline mixture was extracted with $Et_2O$. After washing the $Et_2O$ extract with brine, it was dried

(MgSO₄), and the solvent was removed in vacuo. Crystallization of the residue from ether–pentane afforded **34** as an off-white solid (8.0 g, 52%), mp 125–129 °C. Anal. ($C_{24}H_{39}ClN_2O$) C, H, N, Cl.

**Biological Methods.** All test compounds (**2**) were administered by the iv route to mixed-breed or beagle dogs that had been subjected to a two-stage ligation of the left anterior descending coronary artery.[9] No dog was used if the ECG revealed more than 25% beats of sinus origin as determined by an upright QRS complex preceded by a P wave (i.e., a normally conducted ventricular complex). A minimum of two dogs were used with each test compound. Compounds were administered by the dosage regimens described previously, and the arrhythmia was then monitored at 2.5-min intervals.

For the determination of oral activity, the animals were prepared in a similar manner; however, after the oral dose (administered in a lactose-filled gelatin capsule), the ECG was taken at 15-min intervals for at least 6 h. A compound was rated active after oral administration if it produced a 25% reduction in the ectopic rhythm for at least 30 min.

For the determination of potential cardiovascular toxicity, normal dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv). The lead II ECG was monitored for heart rate and electrocardiographic intervals. A femoral artery was catheterized for the measurement of blood pressure, and compounds were continually infused at 0.5 (mg/kg)/min until the blood pressure decreased by 50% from control levels or the cumulative dose reached 50 mg/kg.

# Synthesis and Antibacterial Activity of New 1-Oxa-1-dethiacephalosporins

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A series of 3-(deacetoxymethyl)-1-oxa-1-dethiacephalosporins (**1**) bearing the 7-(α-alkoxyimino)acyl side chain were synthesized and their antibacterial properties were examined in comparison with that of FK-749 (**2**). (Z)-2-(Ethoxyimino)-2-(2-aminothiazol-4-yl)acetic acid was found to be a prefered side chain in the 1-oxa series, and the derivative **18b** with this side chain proved to be a potential broad-spectrum antibiotic not at all inferior to **2**.

Considerable efforts by a number of laboratories to prepare the 1-oxa isosteric analogues of the natural cephalosporin antibiotics have appeared in the past few years.[1] The significant antimicrobial activity of these 1-oxa analogues was first reported by Christensen et al.[1b] and, subsequently, in extensive work of Nagata et al.[1e,g] In our

continuing research for unique and potent β-lactam antibiotics, we have now synthesized a series of 3-(deacetoxymethyl)-1-oxa-1-dethiacephalosporins (**1**).[2] This work
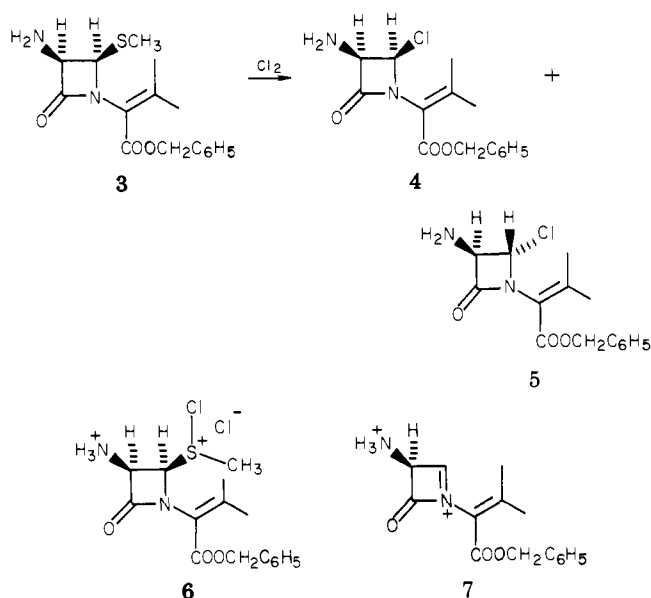


**1**          **2**
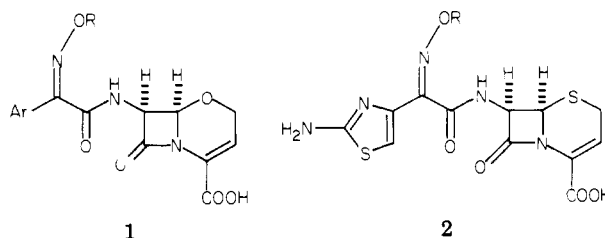
has been undertaken because of an interest in determining

(1) (a) S. Wolfe, J.-B. Ducep, K. C. Tin, and S.-L. Lee, *Can. J. Chem.*, **52**, 3996 (1974); (b) L. D. Cama and B. G. Christensen, *J. Am. Chem. Soc.*, **96**, 7582 (1974); (c) R. A. Firestone, J. L. Fahey, N. C. Maciejewicz, C. S. Patel, and B. G. Christensen, *J. Med. Chem.*, **20**, 551 (1977); (d) E. G. Brain, C. L. Branch, A. J. Eglington, J. H. C. Nayler, N. F. Osborne, M. J. Pearson, J. C. Smale, R. Southgate, and P. Tolliday, *Spec. Publ. Chem. Soc.*, no. 28, 204 (1977); (e) M. Narisada, H. Onoue, and W. Nagata, *Heterocycles*, **7**, 839 (1977); (f) C. U. Kim and D. N. McGregor, *Tetrahedron Lett.*, 409 (1978); (g) M. Narisada, T. Yoshida, H. Onoue, M. Ohtani, T. Okada, T. Tsuji, I. Kikkawa, N. Haga, H. Satoh, H. Itani, and W. Nagata, *J. Med. Chem.*, **22**, 757 (1979); (h) C. L. Branch and M. J. Pearson, *J. Chem. Soc., Perkin Trans. 1*, 2268 (1979).

(2) Two independent syntheses of this nucleus have appeared very recently: (a) ref 1h; (b) Y. Hamashima, S. Yamamoto, T. Kubota, K. Tokura, K. Ishikura, K. Minami, F. Matsubara, M. Yamaguchi, I. Kikkawa, and W. Nagata, *Tetrahedron Lett.*, 4947 (1979).

**Scheme I**



Table I. Benzyl 7-[2-(Alkoxyimino)-2-arylacetamido]-1-oxa-1-dethiaceph-3-em-4-carboxylates

| no. | yield, % | mp, °C | formula | anal. |
|---|---|---|---|---|
| 17a | 87 | amorphous | $C_{21}H_{19}N_5O_7S^b$ | C, H, N |
| 17b[a] | 99 | ~120 | $C_{22}H_{21}N_5O_7S$ | C, H, N |
| 17c | 92 | 162-166 | $C_{23}H_{23}N_5O_7S$ | C, H, S |
| 17d | 73 | ~132 | $C_{24}H_{25}N_5O_7S$ | C, H, N |
| 17e | 75 | amorphous | $C_{23}H_{21}N_5O_7S$ | |
| 17f | 84 | amorphous | $C_{23}H_{19}N_5O_7S$ | |
| 21 | 67 | 157-158.5 | $C_{23}H_{21}N_3O_6$ | C, H, N |
| 23 | 71 | amorphous | $C_{21}H_{19}N_3O_7$ | |
| 19[a] | 87 | amorphous | $C_{21}H_{19}N_5O_7S$ | C, H, N |

[a] See Experimental Section.  [b] N: calcd, 14.43; found, 13.98.

the biological properties of the 1-oxa series bearing a 7-[α-(alkoxyimino)acyl] side chain in comparison with the excellent activity of FK-749 (2), which has been recently prepared in our laboratories.[3]  The significance of the α-oxy- or α-(alkoxyimino) functionalities on the acyl side chain in the β-lactam antibiotic field has been recently disclosed by us and others, as exemplified by, in addition to 2, nocardicins,[4] cefuroxime,[5] cefotaxime (HR-756),[6] and others.[7]  In this paper, we report the synthesis of the 3-nor-1-oxacephem nucleus from penicillins and the antibacterial activity of its 7-[α-(alkoxyimino)acyl] derivatives (1).

**Chemistry.** The 3-nor-1-oxacephem nucleus (15) was synthesized by a variation of the procedures described in the literature.[1a,e]  The starting material was 1,2-seco-penicillin (3) tosylate,[8] which was chlorinated with $Cl_2$ in $CH_2Cl_2$ to give a crude mixture of two isomeric chlorides (4 and 5) in a ratio of 4:1 (Scheme I).[9]  From this mixture, we isolated in 65% yield the pure cis chloride (4) by crystallization from ether.  Interestingly, when this chlorinolysis of 3 was carried out in the presence of a small amount of MeOH (in an attempt to trap methanesulfenyl chloride and/or sulfur dichloride coproduced in the reaction), the ratio of 4 and 5 changed to 1:2.  As has been documented in analogous reactions,[10] the chlorinolysis of

3 would proceed via the resonance-stabilized carbonium ion (7) arising from the $C_4$-S bond cleavage of the initially formed sulfonium ion (6), the chloride anion attacking from either face of the β-lactam ring.  In the former reaction, the chloride anion might approach mainly from the β face by the aid of an ionic interaction with the 3β-ammonium ion, while, in the presence of an excess of chloride as in the case of the latter reaction, the competing attack from the sterically less hindered α face might occur more preferentially.[11]

Treatment of 4 with an excess of ethylene glycol in the presence of $AgBF_4$ gave a mixture of the cis and trans isomers of 4-substituted azetidinones (8a and 9a) (presumably 1:1), which was immediately acylated with phenoxyacetyl chloride to give a 1:1 mixture of the diacylated products (8b and 9b) (Scheme II).  An improved yield of the desired cis isomer (8b), on the other hand, was obtained from the 1:2 mixture of 4 and 5.  Thus, a similar treatment with ethylene glycol, followed by acylation with phenoxyacetyl chloride, produced a 2:1 ratio of the products in favor of the cis isomer (8b).  This result shows that the substitution reaction of 5 occurred to a considerable extent with inversion of configuration.  Separation of 8b and 9b was partially achieved by a careful chromatography on silica gel.  Ozonolysis of the pure cis isomer (8b) gave in 79% yield the crystalline oxalyl derivative (10).  Advantageously, this key intermediate was also isolated from the crude ozonolysis products of the mixture of 8b and 9b (2:1) in ~38% overall yield based on the starting 1,2-secopenicillin (3).

Construction of the 1-oxacephem nucleus (15) from this intermediate (10) was carried out utilizing a route involving an intramolecular Wittig reaction.[12]  Thus, 10 was reduced with Zn-AcOH,[13] giving the epimeric alcohols (11) (1:1 ratio), which were then subjected to chlorination with $SOCl_2$ and subsequently treated with $PPh_3$ to give the phosphorane (13).  Hydrolysis of the phenoxyacetate group in 13 with dilute aqueous NaOH gave the alcohol (14), after purification by silica gel chromatography, in an overall 54% yield from 10.  The final Moffatt oxidation of 14 afforded, via a spontaneous cyclization of the resulting aldehyde, in 74% yield the 1-oxacephem (15) as an oily material, whose structure was confirmed by inspection of its physical properties.

(3) T. Kamimura, Y. Matsumoto, N. Okada, Y. Mine, T. Murakawa, M. Nishida, S. Goto, and S. Kuwahara, *Antimicrob. Agents Chemother.*, 16, 540 (1979).

(4) M. Hashimoto, T. Komori, and T. Kamiya, *J. Am. Chem. Soc.*, 98, 3023 (1976); *J. Antibiot.*, 29, 890 (1976).

(5) P. C. Cherry, M. C. Cook, M. W. Foxton, M. Gregson, G. I. Gregory, and G. B. Webb, *Spec. Publ. Chem. Soc.*, no. 28, 145 (1977).

(6) R. Bucourt, R. Heymes, A. Lutz, L. Penasse, and J. Perronet, *Tetrahedron*, 34, 2233 (1978).

(7) (a) M. Ochiai, O. Aki, A. Morimoto, J. Okada, and Y. Matsushita, *Chem. Pharm. Bull.*, 25, 3115 (1977); (b) M. Numata, I. Minamida, S. Tsushima, T. Nishimura, M. Yamaoka, and N. Matsumoto, *ibid.*, 25, 3117 (1977).

(8) E. G. Brain, I. McMillan, J. H. C. Nayler, R. Southgate, and P. Tolliday, *J. Chem. Soc., Perkin Trans. 1*, 562 (1975).

(9) This result is in good agreement with that of Nagata et al. (ref 1e).

(10) (a) S. Kukolja, *J. Am. Chem. Soc.*, 93 6267 (1971); (b) S. Wolfe, S.-L. Lee, J.-B. Ducep, G. Kannengiesser, and W. S. Lee, *Can. J. Chem.*, 53, 497 (1975).

(11) Treatment of the cis chloride (4) with HCl in the presence of MeOH resulted in the recovery of the starting material unchanged with some decomposition. This rules out a possibility that the trans chloride (5) is formed via an equilibrium with 4.

(12) R. Scartazzini, H. Peter, H. Bickel, K. Heusler, and R. B. Woodward, *Helv. Chim. Acta*, 55, 408 (1972).

(13) S. Yamamoto, N. Haga, T. Aoki, S. Hayashi, H. Tanida, and W. Nagata, *Heterocycles*, 8, 283 (1977).

**Scheme II**



8

9

a, R = H
b, R = $COCH_2OC_6H_5$



10, X = O
11, X = H, OH
12, X = H, Cl
13, X = $P(C_6H_5)_3$

14



15

16

**Chart I**



17

18

19, R = $CH_2C_6H_5$; X = CHO
20, R = H; X = H

a, R = $CH_3$      d, R = $n$-$C_4H_9$
b, R = $C_2H_5$     e, R = $CH_2CH=CH_2$
c, R = $i$-$C_3H_7$    f, R = $CH_2C\equiv CH$



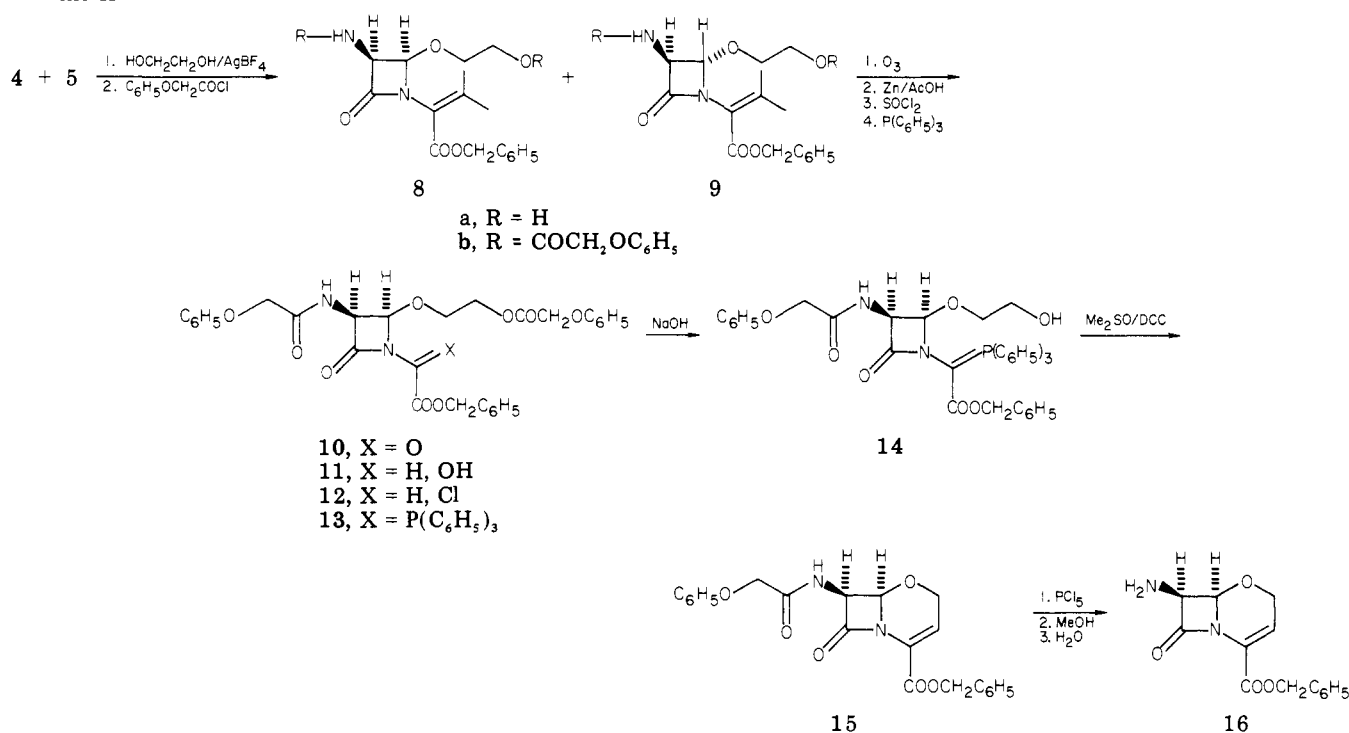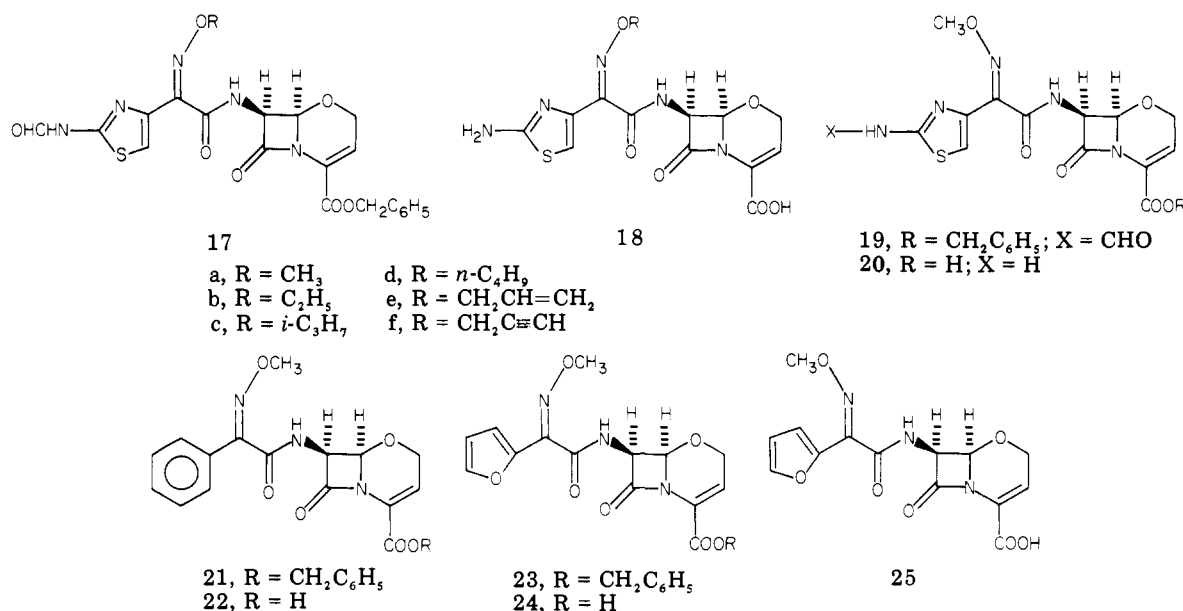21, R = $CH_2C_6H_5$
22, R = H

23, R = $CH_2C_6H_5$
24, R = H

25

Cleavage of the phenoxyacetyl side chain by the traditional imino chloride procedure gave a 54% yield of the crystalline 7-amino-1-oxacephem (16) (tosylate). From this intermediate, several new 1-oxacephem derivatives with the 7-[α-(alkoxyimino)]acyl side chain were prepared by a conventional acylating method (DMF–POCl₃).[14] This acyl side chain includes 2-(alkoxyimino)-2-(2-amino-thiazol-4-yl)acetic acids which have been recently shown to confer high antimicrobial activity on cephalosporins (as described above). The amino group of these acids was protected with the formyl group, which was then removed at the final stage. When acylation was carried out using α-(alkoxyimino)acyl chloride prepared from the corresponding acid by treatment with SOCl₂, the syn (Z) acid

was isomerized to the anti (E) acid chloride[15] and the acylation product obtained was exclusively the anti (E) isomer (19; Chart I), as exemplified by the case of 2-(methoxyimino)-2-(2-aminothiazol-4-yl)acetic acid (see Experimental Section).

The configuration of the alkoxyimino group (syn or anti) of the condensation products was assigned by means of ¹H NMR spectroscopy, which we have previously shown to be useful for the determination of the oxyimino configuration in the nocardicin case.[3] Namely, in comparing the spectra (in Me₂SO-d₆) of the isomeric oxacephem oximes, the amide proton of the syn (Z) isomer always occurs at a lower field than that for the anti (E) isomer (as summarized in Table II). In addition, in the aminothiazolyl series the proton on the thiazole ring (C-5) for the syn

(14) See, e.g., T. Takaya, T. Masugi, H. Takasugi, and H. Kochi, U.S. Patent 4 166 115.

(15) T. Takaya et al., an unpublished result.

Table II.  7-[2-(Alkoxyimino)-2-arylacetamido]-1-oxa-1-dethiaceph-3-em-4-carboxylic Acids

| no. | mp, °C | formula | anal. | NMR (Me₂SO-d₆) | |
| --- | --- | --- | --- | --- | --- |
| | | | | amide NH | 5-H (thiazole) |
| 18a | amorphous | $C_{13}H_{13}N_5O_6S$ | C, H, N | 9.34 | 6.78 |
| 18b | 150-158 (dec) | $C_{14}H_{15}N_5O_6S \cdot HCl \cdot H_2O$ | C, H, N [b] | 9.79 | 6.96 |
| 18c | ~140 (dec) | $C_{15}H_{17}N_5O_6S \cdot HCl$ | C, H, S | 9.41 | 6.86 |
| 18d | >200 | $C_{16}H_{19}N_5O_6S$ | C, H, N | 9.28 | 6.75 |
| 18e | amorphous | $C_{15}H_{15}N_5O_6S$ | C, H, N | 9.35 | 6.80 |
| 18f | ~160 (dec) | $C_{15}H_{13}N_5O_6S \cdot HCl$ | C, H, N [c] | 9.61 | 7.00 |
| 22 | 80-95 (dec) | $C_{16}H_{15}N_3O_6$ | C, H, N | 9.50 | |
| 24 [a] | 197 (dec) | $C_{14}H_{13}N_3O_7$ | C, H, N | 9.50 | |
| 20 | amorphous | $C_{13}H_{13}N_5O_6S$ | C, H, N | 8.99 | 7.37 |

[a] Deprotection of the benzyl ester group in 23 gave a mixture of 24 and its *E* isomer (25) in a ratio of 2:1. Recrystallization from AcOEt gave pure 24.  [b] C: calcd, 38.58; found, 38.17.  [c] N: calcd, 16.37; found, 15.86.

Table III.  Antibacterial Activities of 1-Oxa-1-dethiacephalosporin Derivatives[a]

| no. | S. aureus 209P JC-1 | B. subtilis ATCC 6633 | E. coli NIHJ JC-2 | K. pneumoniae 12 | P. mirabilis 1 | Ps. aeruginosa NCTC 10490 | S. marcescens 35 |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 2 | 6.25 | 25 | 0.05 | 0.05 | 0.025 | 25 | 50 |
| 18a | 25 | 100 | 0.2 | 0.78 | 0.2 | 800 | 100 |
| 20 | 12.5 | 100 | 6.25 | 1.56 | 3.13 | 800 | 100 |
| 18b | 6.25 | 50 | 0.1 | 0.1 | 0.05 | 3.13 | 12.5 |
| 18c | 12.5 | 50 | 0.78 | 0.78 | 0.2 | 12.5 | 25 |
| 18d | 12.5 | 50 | 0.78 | 3.13 | 0.78 | 12.5 | 100 |
| 18e | 6.25 | 100 | 3.13 | 1.56 | 0.1 | 50 | 100 |
| 18f | 12.5 | 25 | 1.56 | 0.2 | 0.1 | 400 | 100 |
| 22 | 1.56 | 25 | 25 | 12.5 | 6.25 | 800 | 100 |
| 24 | 12.5 | 50 | 6.25 | 1.56 | 0.78 | 800 | 100 |

[a] The in vitro antibacterial activities are given as minimum inhibitory concentrations (MIC) in μg/mL.

isomer resonates contrariwise at a higher field than that of the anti isomer. This latter observation is in good agreement with the previously reported results.[6,7]

The carboxylic acid protective group was removed by treatment with AlCl₃[16] to give the corresponding 1-oxa-1-dethiacephalosporanic acids. An exceptional and undesired result encountered on this treatment was in the case of the furyl derivative (23), in which a partial isomerization of the oxime group occurred during deprotection of the ester group to form a mixture of the syn and anti acids (24 and 25) in a ratio of 2:1. This isomerization is probably facilitated by AlCl₃ via its coordination between the furyl oxygen and the oxime nitrogen or oxygen. The final deprotection of the formyl group of the aminothiazolyl derivatives was achieved by treatment with methanolic HCl.

**Biological Results.**[17]  The new 1-oxacephalosporin derivatives were tested in vitro against several strains of Gram-positive and Gram-negative bacteria, and the MIC values are shown in comparison with that of 2 in Table III. In the aminothiazolyl series, the *syn*-methoxyimino derivative (18a), which is the isosteric counterpart of 2, was fairly less active than 2, and the anti isomer (20) showed considerably less activity even compared to 18a. The ethoxyimino (syn) derivative (18b), on the other hand, displayed activity of the same order as 2 and, in fact, was rather superior to 2, especially against *Pseudomonas aeruginosa* and *Serratia marcescens*. The isopropoxyimino (syn) derivative (18c) was moderately active but slightly less than 2 or 18b against Gram-positive bacteria and Gram-negative organisms of *Escherichia coli, Klebsiella pnemoniae*, and *Proteus mirabilis*. Against *Ps. aeruginosa* and *S. marcescens*, however, 18c was again more potent

than 2, though less than 18b. The other three aminothiazolyl derivatives (18d–f) were substantially less active than 2 and 18b. The phenyl and furyl derivatives (22 and 24) also showed no significant activity.

In conclusion, the (*Z*)-2-(ethoxyimino)-2-(2-aminothiazol-4-yl)acetyl side chain was found to be the most preferable in the 1-oxa series, and compound 18b with this side chain proved to be a potential broad-spectrum antibiotic not at all inferior to 2.

**Experimental Section**

Melting points were determined on a Thomas-Hoover capillary melting point apparatus. IR spectra were taken on a Hitachi 215 spectrometer. ¹H NMR spectra were recorded at 100 MHz on a JEOL PS-100 spectrometer and at 60 MHz on a JNM-PMX 60 NMR spectrometer. High-pressure LC separations were performed on a Waters Associates chromatograph instrument equipped with a Model 6000 A pump, a Series 440 refractometer detector, and a μ-Porasil (30 cm × 4 mm) column. Mass spectra were measured with a Hitachi RMU-6M mass spectrometer. The results of the elemental analyses were within ±0.4% of the theoretcial values, except where noted.

**Chlorinolysis of 3. Method 1.** A solution of 3 (tosylate; 24.60 g, 49.94 mmol) in $CH_2Cl_2$ (290 mL) was cooled to –65 °C and a solution of $Cl_2$ (4.20 g, 59 mmol) in $CCl_4$ (30 mL) was added. After stirring for 1 h at the same temperature, the mixture was warmed slowly to 0 °C and evaporated to give an oil (ratio of 4 and 5, 4:1): NMR ($CDCl_3$) δ 6.29 (d, *J* = 5 Hz, 3-H of 4), 6.17 (d, *J* = 1.5 Hz, 3-H of 5). Crystallization from $Et_2O$ and filtration gave 15.65 g (65% yield) of 4 (TsOH salt): mp 125–129 °C (dec); IR (Nujol) 1778, 1720 cm⁻¹; NMR ($CDCl_3$) δ 2.00 (s, 3 H), 2.20 (s, 3 H), 2.28 (s, 3 H), 5.11 (d, 1 H, *J* = 5 Hz), 5.20 (s, 2 H), 6.29 (d, 1 H, *J* = 5 Hz), 7.13 (d, 2 H, *J* = 8 Hz), 7.36 (s, 5 H), 7.52 (d, 2 H, *J* = 8 Hz). Anal. ($C_{15}H_{17}N_2O_3Cl \cdot C_7H_8O_3S$) C, H, N, S, Cl.

**Method 2.** A solution of 3 (tosylate; 11.50 g, 23.35 mmol) in $CH_2Cl_2$ (150 mL) containing MeOH (1.5 mL) was treated with a solution of $Cl_2$ (3.32 g, 46.8 mmol) in $CCl_4$ (20 mL) in a similar way to that described in method 1 to give an oil (13.81 g, 1:2 ratio of 4 and 5 tosylates).

**Preparation of 8b and 9b from 4.** A mixture of 4 (tosylate; 14.79 g, 30.75 mmol) and ethylene glycol (19.10 g, 308 mmol) in $CH_2Cl_2$ (80 mL) was cooled to –45 °C and AgBF₄ (12.12 g, 88

(16)  T. Tsuji, T. Kataoka, M. Yoshioka, Y. Sendo, Y. N. Nishitani, S. Hirai, T. Maeda, and W. Nagata, *Tetrahedron Lett.*, 2793 (1979).

(17)  Minimum inhibitory concentration (MIC) was determined by the agar dilution method using heart infusion agar (HI agar, Difco, Detroit).

mmol) was added. The mixture was stirred at -45 to -20 °C for 45 min, at -20 to 0 °C for 45 min, and at 0 °C for 80 min, and 15% aqueous NaHCO$_3$ (100 mL) and saturated aqueous NaCl (60 mL) were added. After stirring for 1 h at 0 °C, the mixture was filtered by the aid of Celite and the filtrate was separated. The organic layer was washed with H$_2$O, dried over MgSO$_4$, and filtered.

The filtrate was cooled to -45 °C and pyridine (7.50 g, 95 mmol) was added. The mixture was stirred and phenoxyacetyl chloride (12.80 g, 75 mmol) was added dropwise. After stirring for 80 min at 0 °C, the mixture was washed successively with 0.5 N HCl, brine, saturated aqueous NaHCO$_3$, and H$_2$O, dried over MgSO$_4$, and evaporated to give an oil. Chromatography on a short silica gel column eluting with AcOEt gave 14.54 g of a mixture of **8b** and **9b** (1:1 ratio on high-pressure LC). This mixture was again chromatographed on a silica gel column, eluting with benzene–AcOEt (gradient elution), and gave 1.14 g of **9b**, 6.57 g of the mixture, and 1.75 g of **8b**. For **8b**: NMR (CDCl$_3$) δ 2.00 (s, 3 H), 2.25 (s, 3 H), 3.5–3.7 (m, 2 H), 4.0–4.3 (m, 2 H), 4.55 (s, 4 H), 5.1–5.5 (m, 4 H), 6.7–7.5 (m, 15–16 H). For **9b**: NMR (CDCl$_3$) δ 2.02 (s, 3 H), 2.25 (s, 3 H), 3.6–3.9 (m, 2 H), 4.1–4.4 (m, 2 H), 4.50 (s, 2 H), 4.63 (s, 2 H), 5.0–5.4 (m, 4 H), 6.8–7.5 (m, 15–16 H).

**Ozonolysis of 8b.** A solution o    (1.63 g) in AcOEt (20 mL) was cooled to -65 °C and O$_3$ w    ubbled until the starting material disappeared on TLC. The reaction mixture was purged with N$_2$, allowed to warm to 0 °C, and treated with dilute aqueous NaHSO$_3$. The organic layer was washed with brine, dried over MgSO$_4$, and evaporated to give a crystalline solid, which was washed with Et$_2$O to give 1.23 g (79%) of **10**: mp 102–103 °C (dec); IR (Nujol) 1810, 1750, 1735, 1710, 1670 cm$^{-1}$; NMR (CDCl$_3$) δ 3.9–4.4 (m, 4 H), 4.56 (s, 2 H), 4.59 (s, 2 H), 5.35 (s, 2 H), 5.5–5.8 (m, 2 H), 7.8–8.5 (m, 15–16 H). Anal. (C$_{30}$H$_{28}$N$_2$O$_{10}$) C, H, N.

**Preparation of 10 from the 1:2 Mixture of 4 and 5.** A mixture of **4** and **5** (tosylates; 13.81 g, 1:2 ratio) was subjected to a similar treatment to that described above (for the preparation of **8b** and **9b** from **4**) to give a crude mixture of **8b** and **9b** (2:1 ratio on high-pressure LC). This mixture was then treated with O$_3$ in a similar way to that described above (for the preparation of **10** from **8b**) to give 5.10 g of **10** (38% based on **3**).

**Preparation of 14.** A solution of **10** (8.97 g, 15.56 mmol) in a mixture of CH$_2$Cl$_2$ (45 mL) and AcOH (18 mL) was cooled to 5 °C and Zn dust (9.0 g, 138 mmol) was added; then the mixture was stirred for 2 h at the same temperature. After filtration of the reaction mixture, the filtrate was washed successively with dilute aqueous HCl, brine, saturated aqueous NaHCO$_3$, and brine, dried over MgSO$_4$, and evaporated to give an oil (9.46 g) of **11** (ca. 1:1 ratio on TLC).

This oil was dissolved in CH$_2$Cl$_2$ (75 mL) and 2,6-lutidine (2.50 g, 23.33 mmol) was added. This was cooled to 0 °C and SOCl$_2$ (2.78 g, 23.33 mmol) was added. After stirring for 1 h at the same temperature, the mixture was washed with brine, dried over MgSO$_4$, and filtered.

To the filtrate was added PPh$_3$ (6.14 g, 23.4 mmol) and the mixture was refluxed for 4 h under an atmospheric pressure of N$_2$. The reaction mixture was washed with saturated aqueous NaHCO$_3$ and brine, dried over MgSO$_4$, and evaporated to give **13** as an oil.

This oil was dissolved in MeOH (120 mL) and cooled to 0 °C. With stirring, 1 N NaOH (8.7 mL) was added dropwise during a 15-min period and the mixture was stirred for a further 15 min. The reaction mixture was concentrated to about 20 mL and AcOEt (100 mL) was added. The mixture was washed with brine, dried over MgSO$_4$, and evaporated to give an oil, which was purified by chromatography on a silica gel column eluting with benzene–AcOEt (gradient elution) to give 5.76 g of **14** (54% yield from **10**): IR (CH$_2$Cl$_2$) 1760, 1730, 1690, 1630 cm$^{-1}$; NMR (CDCl$_3$) δ 3.2–3.7 (m, 4 H), 4.24 (s, 2 H), 4.76 (s, 2 H), 4.9–5.2 (m, 2 H).

**Preparation of 15.** A solution of **14** (14.10 g, 20.5 mmol) in benzene (23 mL) was added to a mixture of pyridine (1.62 g, 20.5 mmol) and dicyclohexylcarbodiimide (12.70 g, 61.5 mmol) in Me$_2$SO (45 mL)–benzene (25 mL), and a solution of trifluoroacetic acid (1.17 g, 10.25 mmol) in benzene (5 mL) was added dropwise at room temperature. After stirring overnight, the mixture was cooled and 1 N HCl (50 mL) was added. The precipitate was removed by filtration and the filtrate was diluted with AcOEt, washed successively with 1 N HCl and brine, dried over MgSO$_4$,

and evaporated to give an oil. Chromatography on silica gel, eluting with benzene–AcOEt (10:1), gave 6.20 g (74%) of **15** as an oil: IR (CHCl$_3$) 1795, 1727, 1690 cm$^{-1}$; NMR (CDCl$_3$) δ 4.52 (d, 2 H, $J$ = 2 Hz), 4.57 (s, 2 H), 5.06 (d, 1 H, $J$ = 4 Hz), 5.28 (s, 2 H), 5.76 (dd, 1 H, $J$ = 4 and 10 Hz), 6.50 (t, 1 H, $J$ = 2 Hz), 6.9–7.5 (m, 11 H); MS *m/e* 408 (M$^+$).

**Deacylation of 15.** A mixture of **15** (10.40 g, 25.46 mmol) and *N*,*N*-dimethylaniline (6.18 g, 51.0 mmol) in CH$_2$Cl$_2$ (250 mL) was cooled to -35 °C and PCl$_5$ (10.60 g, 51.0 mmol) was added. After stirring for 2 h at the same temperature, the mixture was cooled to -50 °C and MeOH (23 mL) was added dropwise. The temperature was gradually raised to 0 °C during a period of 80 min and H$_2$O (23 mL) was added. After stirring for 80 min at the same temperature, the mixture was extracted with cold H$_2$O, and the aqueous layer was adjusted to pH 7, saturated with NaCl, and extracted with AcOEt. The extract was concentrated to about 40 mL and a solution of TsOH·H$_2$O (4.84 g, 25.5 mmol) in AcOEt (60 mL) was added under ice-bath cooling. After standing for 1 h, the resulting crystalline precipitate was filtered and washed successively with AcOEt and Et$_2$O to give 6.15 g (54%) of **16** (TsOH salt): mp 152–158 °C (dec); IR (Nujol) 1800, 1730 cm$^{-1}$; NMR (Me$_2$SO-$d_6$) δ 2.30 (s, 3 H), 4.67 (d, 2 H, $J$ = 3 Hz), 5.03 (d, 1 H, $J$ = 4 Hz), 5.30 (d, 1 H, $J$ = 4 Hz), 5.32 (s, 2 H), 6.70 (t, 1 H, $J$ = 3 Hz), 7.10 (d, 2 H, $J$ = 8 Hz), 7.38 (s, 5 H), 7.52 (d, 2 H, $J$ = 8 Hz). Anal. (C$_{14}$H$_{14}$N$_2$O$_3$·C$_7$H$_8$O$_3$S) C, H, N, S.

**General Procedure of the Preparation of 17a–f, 21, and 23.** A typical procedure is described for the preparation of **benzyl 7-[(*Z*)-2-(ethoxyimino)-2-[2-(formylamino)thiazol-4-yl]-acetamido]-1-oxa-1-dethiaceph-3-em-4-carboxylate (17b)**. Phosphoryl chloride (368 mg, 2.40 mmol) was added to a mixture of DMF (280 mg, 3.83 mmol) and AcOEt (4 mL) at 0 °C, and the mixture was stirred for 85 min at the same temperature. To this mixture was added (*Z*)-2-(ethoxyimino)-2-[2-formylamino)thiazol-4-yl]acetic acid (432 mg, 2.41 mmol) and the mixture was stirred for an additional 70 min at the same temperature. This mixture was added to a cooled (-50 °C) mixture of **16** (free amine), prepared from **16** (TsOH salt; 725 mg, 1.62 mmol) and pyridine (0.3 mL) in AcOEt (10 mL), and the mixture was stirred for 70 min, during which time the temperature was allowed to warm to 0 °C. The reaction mixture was washed successively with 1 N HCl, dilute aqueous NaHCO$_3$, and brine, dried over MgSO$_4$, and evaporated to give 800 mg (99%) of **17b** as a crystalline solid (see Table I).

**General Procedure for the Removal of the Carboxylic Acid and/or Amine Protective Groups.** A typical procedure is described for the preparation of 7-[(*Z*)-2-(ethoxyimino)-2-(2-aminothiazol-4-yl)acetamido]-1-oxa-1-dethiaceph-3-em-4-carboxylic acid (**18b**). To a mixture of **17b** (440 mg, 0.88 mmol) and anisole (1.43 g, 13.2 mmol) in CH$_2$Cl$_2$ (10 mL) was added a solution of AlCl$_3$ (587 mg, 4.4 mmol) in nitromethane (4 mL) under ice-bath cooling, and the mixture was stirred for 1.5 h at room temperature. The reaction mixture was poured into a mixture of AcOEt (40 mL) and acetate buffer (pH 4; 20 mL) and the organic layer was separated. The aqueous layer was acidified to pH 3 with dilute aqueous HCl and extracted with AcOEt. The combined organic layer was extracted with dilute aqueous NaHCO$_3$, and the aqueous layer was acidified to pH 3 with dilute aqueous HCl and extracted with AcOEt. The organic layer was washed with brine, dried over MgSO$_4$, and evaporated to give 232 mg of a crystalline solid.

To a solution of this solid in a mixture of MeOH (5 mL) and THF (6 mL) was added concentrated HCl (0.06 mL) under ice-bath cooling and the mixture was stirred for 4 h at room temperature. The reaction mixture was concentrated to leave a crystalline solid, which was washed successively with cold MeOH and Et$_2$O to give 147 mg of **18b** (HCl salt) (see Table II).

**Preparation of Benzyl 7-[(*E*)-2-(Methoxyimino)-2-[2-(formylamino)thiazol-4-yl]acetamido]-1-oxa-1-dethiaceph-3-em-4-carboxylate (19).** (*Z*)-2-(Methoxyimino)-2-[2-(formylamino)thiazol-4-yl]acetic acid (2.0 g) was suspended in SOCl$_2$ (30 mL), 2 drops of DMF was added, and the mixture was stirred at 60 °C for 15 min. The excess SOCl$_2$ was removed by evaporation, and the residue was washed with benzene to give (*E*)-2-(methoxyimino)-2-[2-(formylamino)thiazol-4-yl]acetyl chloride (1.38 g), mp 225 °C (dec). A 1.12-g (4.5 mmol) portion of this acid chloride was suspended in CH$_2$Cl$_2$ (50 mL) and **16** (TsOH salt; 1.78 g, 3.99

mmol) was added. This mixture was cooled to 0 °C and pyridine (0.81 mL, 10 mmol) was added dropwise. After stirring for 10 min at the same temperature, the mixture was concentrated and diluted with AcOEt. The solution was washed successively with dilute aqueous $NaHCO_3$, 1 N HCl, and brine, dried over $MgSO_4$, and evaporated to give an oil, which was chromatographed on a silica gel column, eluting with $CH_2Cl_2$–AcOEt (gradient elution),

to give 1.69 g (87%) of **19** as an amorphous solid (see Table I).

# Novel Analogues of Enkephalin: Identification of Functional Groups Required for Biological Activity

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Novel tri- and tetrapeptide analogues of enkephalin, in conjunction with earlier structure–activity data, confirm that chemical substituents present in the first and fourth residues of enkephalin are required for in vitro biological activity. A class of arylamino and alkylamino derivatized tripeptides also were found to have signficiant in vitro opioid-like activity indistinguishable from [D-Ala$^2$,D-Leu$^5$]enkephalin and morphine.

The enkephalins are the smallest members of a family of opioid-like peptides endogenous to the mammalian central nervous system. The in vivo pharmacology of the enkephalins and opiates is complex, and there is growing evidence that these compounds act in vivo at several types of receptors.[1-3] In in vitro systems such as the guinea pig ileum,[4] mouse vas deferens,[5] neuroblastoma glioma NG108-15 cell line,[6] and rat brain binding assays,[7] opiates and the enkephalins produce analogous pharmacologic effects which are reversibly blocked by the opiate antagonist naloxone. These in vitro observations have motivated investigators to compare chemical and conformational similarities between the enkephalins and opiate compounds. Numerous conformational studies have proposed receptor-bound conformations for enkephalins whereby these mammalian pentapeptides and plant opiate alkaloids pharmacologically compete for the same in vitro receptors.[8]

This article describes novel, conformationally constrained analogues of enkephalin which are evaluated pharmacologically in the guinea pig ileum and which displace [$^3$H]naloxone in the rat brain binding assay. There is no assurance that the pharmacologic receptors in these two in vitro assays are equivalent, and recent investigation has suggested the existence of separate high affinity "enkephalin receptors" and "morphine ($\mu$) receptors" present in the rat brain binding assay.[2,3,9] It

appears that naloxone binds to the morphine ($\mu$) binding site with 30-fold greater affinity than the enkephalin binding site.[3] The analogues of enkephalins cited in this article have been evaluated in both bioassay systems, and excellent correlation has been found between the ranked potencies of compounds relative to [D-Ala$^2$,D-Leu$^5$]enkephalin tested in the two in vitro systems. This article uses the information derived from the structure–activity data of these analogues of enkephalin to deduce requirments about the morphine ($\mu$) receptor.

## Experimental Section

**Materials.** All Boc[10] amino acids, except Boc-Tyr-OH, Boc-Tyr($\alpha$Me)-OH, and Boc-Phe($\alpha$Me)-OH, were purchased from Bachem. L-Tyr was obtained from Fluka. The preparation of Boc-Phe($\alpha$Me)-OH is described elsewhere.[11a] The Boc-Leu-resin was prepared by the method of Gisin[11b] using Lab Systems 1% cross-linked polymer (0.90 mequiv/g). Di-*tert*-butyl dicarbonate was obtained from Fluka. 1-Aminoindan, 2-aminoindan, benzylamine, 1-(aminomethyl)cyclopropane, and 1-(aminomethyl)cyclobutane were purchased from Aldrich. $\beta$-Phenylethylamine was purchased from Sigma. [$^3$H]Naloxone (26 Ci/mmol) was obtained from New England Nuclear, and bacitracin was purchased from P. L. Biochemicals. [Aib$^2$,Met-NH$_2$$^5$]Enkephalin was obtained from Peninsula Laboratories.

**Analytical Methods.** Melting points are uncorrected. DMF was purified according to the procedure described by Stewart and Young.[12] Ascending TLC was performed on 0.25-mm silica gel G plates (Analtech) using the following solvent systems: (I) chloroform–acetone, 15:1; (II) chloroform–acetone, 7:1; (III) chloroform–acetone, 7:2; (IV) chloroform–acetone, 1:1; (V) chlo-

(1) J. N. Jaffe and W. R. Martin, in "The Pharmacological Basis of Therapeutics", L. S. Goodman and A. Gilman, Eds., MacMillan, New York, 1975, p 245.

(2) J. A. Lord, A. A. Waterfield, J. Hughes, and H. W. Kosterlitz, *Nature (London)*, **267**, 495 (1977), and references cited within.

(3) K.-J. Chang and P. Cuatrecasas, *J. Biol. Chem.*, **254**, 2610 (1979).

(4) H. W. Kosterlitz and A. J. Watt, *Br. J. Pharmacol.*, **33**, 266 (1968).

(5) G. Henderson, J. Hughes, and H. W. Kosterlitz, *Br. J. Pharmacol.*, **46**, 764 (1972).

(6) A. L. Lampert, M. Nirenberg, and W. A. Klee, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 3165 (1976).

(7) C. B. Pert and S. H. Snyder, *Mol. Pharmacol.*, **10**, 868 (1974).

(8) F. A. Gorin, T. M. Balasubramanian, C. D. Barry, and G. R. Marshall, *J. Supramol. Struct.*, **9**, 27 (1978), and references cited within.

(9) P. E. Gilbert and W. R. Martin, *J. Pharmacol. Exp. Ther.*, **198**, 66 (1976).

(10) Abbreviations used are: Boc, *tert*-butyloxycarbonyl; TLC, thin-layer chromatography; LC, liquid chromatography; DCC, dicyclohexylcarbodiimide; Phe($\alpha$Me), $\alpha$-methylphenylalanine (yl-); DMF, dimethylformamide; TFA, trifluoroacetic acid; Bzl, benzyl; TEA, triethylamine; DPPA, diphenylphosphoryl azide; HOBT, 1-hydroxybenzotriazole; DEPC, diethylphosphoryl cyanidate; Cha, L-cyclohexylalanine; Aib, aminoisobutyric acid.

(11) (a) J. Turk, P. Needleman, and G. R. Marshall, *Mol. Pharmacol.*, **12**, 217 (1976); (b) B. F. Gisin, *Helv. Chim. Acta*, **56**, 1476 (1973).

(12) J. M. Stewart and J. D. Young, "Solid Phase Peptide Syntheses", W. H. Freeman, San Francisco, 1969.