Synthesis and Crystal Structure of a Peptidomimetic Containing the (*R*)-4.4-Spiro Lactam Type-II β-Turn Mimic

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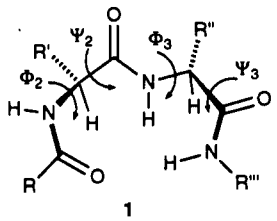
The secondary structure of peptides is known to play a major role in their receptor recognition and biological activity. In an effort to delineate the biologically active conformation of a given bioactive peptide, a considerable amount of effort has gone into the design and synthesis of peptidomimetic constraints which when incorporated into a peptide will induce it to adopt a particular secondary structure.[1] One of the most common types of secondary structures found in peptides is the β-turn.[2] Several conformational constraints have been designed to mimic the different kinds of β-turns, including the type-II β-turn illustrated in structure **1**.[1b,3] One example of a type-II β-turn mimic is the (*R*)-4.4-spiro lactam constraint developed by Hinds et al.[3f] that is illustrated in structure **2**. In this paper we report, what is to our knowledge, the first crystal structure of a peptidomimetic, compound **3**, that contains the (*R*)-4.4-spiro lactam constraint.



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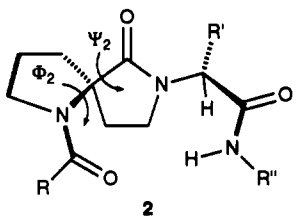

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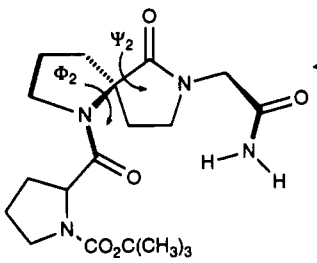

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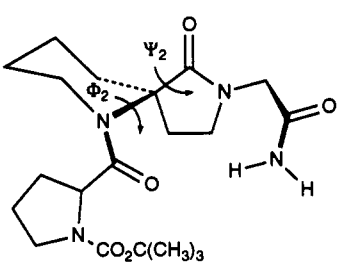

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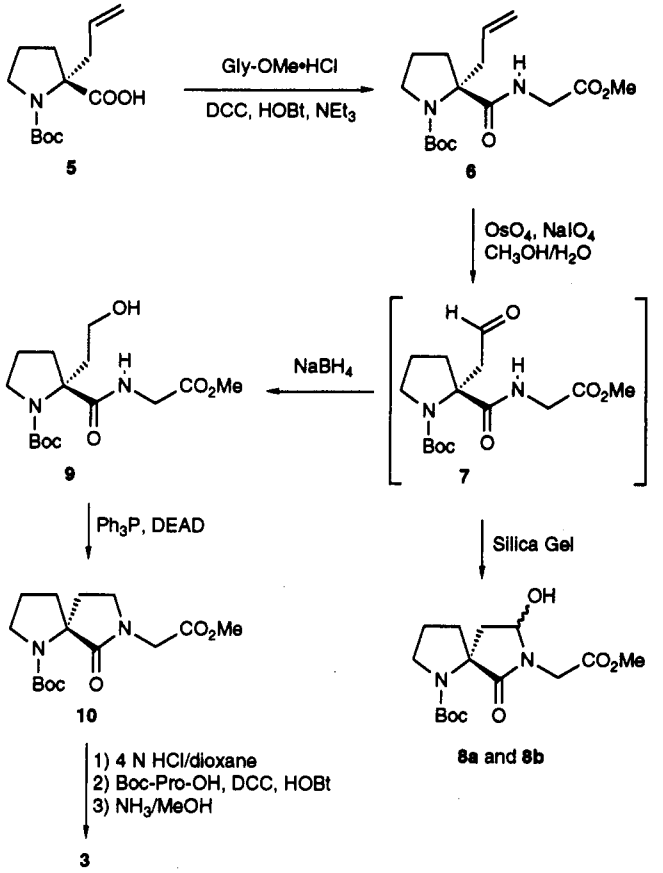
Results

Synthesis. The synthetic route to peptidomimetic **3** (Scheme I) was analogous to that previously reported by Hinds et al.[3f] (*R*)-*N*-(*tert*-Butoxycarbonyl)-2-allylproline (**5**), which served as the starting material for our synthesis, was made as described previously.[3h] This material was coupled to Gly-OMe·HCl to give the protected dipeptide ester **6**. Oxidative cleavage of the double bond of **6** provided aldehyde **7**. Initially, we attempted to purify this intermediate by silica gel chromatography. This procedure, however, yielded the diastereoisomeric spiro carbinolamides **8a** and **8b** as the major products.[4] The structures of **8a** and **8b** were delineated through their ^{13}C and COSY spectra. In the case of **8b**, the ^{13}C NMR spectrum showed the presence of a new ketal-like carbon at 81.5 ppm, while in the COSY spectrum, the resonance at 5.59 ppm, which was exchangeable with D_2O, was coupled only to the signal at 5.10 ppm. The fact that this exchangeable hydrogen was not coupled to the glycine α-CH_2 was clear evidence that the amide hydrogen was no longer present. It was thus assigned as the hydroxyl hydrogen. In addition to being coupled to the hydroxyl group, the spiro carbinolamide methine hydrogen (NC-*H*OH) at 5.10 ppm also showed cross peaks with the resonance at 2.5 ppm that corresponded to the 4-CH_2 of the γ-lactam ring. Similar correlations were seen for spiro carbinolamide **8a**.

The formation of **8a** and **8b** could be avoided by immediately reducing, after workup, aldehyde **7** to alcohol **9**. Cyclization of **9** to (*R*)-4.4-spiro lactam **10** was accomplished under Mitsunobu reaction conditions. The deprotected product obtained after the deprotection of **10** was

Scheme I



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‡ Biomedical Engineering Center and Department of Laboratory Medicine and Pathology.

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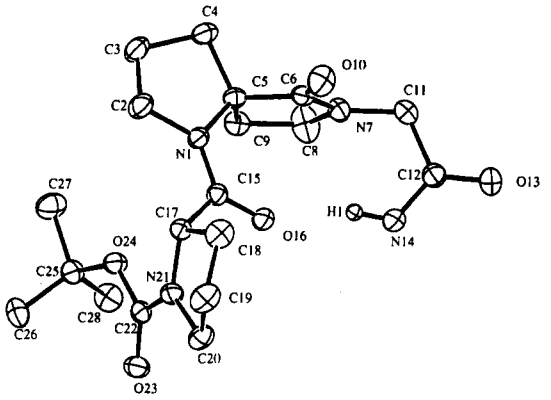

Figure 1. ORTEP representation of **3** with crystallographic numbering system.

Table I. Crystal Data for Peptidomimetic 3

molecular formula	$C_{19}H_{30}N_4O_5$
molecular wt (amu)	394.47
d_{calcd}, g/cm^3	1.281
crystal system	orthorhombic
space group	$P2_12_12_1$
Z	4
a, Å	9.170(2)
b, Å	10.632(2)
c, Å	20.971(2)
V, Å^3	2044
crystal size (mm)	0.4 × 0.2 × 0.1
total reflections	3248
unique reflections	2622
observed ($I > 3.00\sigma(I)$)	2329
no. of variables	343
final R	0.036
final R_w	0.042
goodness of fit	1.48

coupled to Boc-Pro-OH to give an ester, which when treated with methanolic ammonia gave peptidomimetic **3**.

X-ray Analysis. The X-ray crystal structure of the (*R*)-4.4-spiro lactam peptidomimetic **3** with its atomic numbering scheme is shown in Figure 1, while the data collection information is listed in Table I.[5] In the crystal state, **3** is observed to adopt a classical type-II β-turn which is stabilized by an intramolecular hydrogen bond between the trans carboxamide hydrogen and the prolyl carbonyl oxygen. This type-II β-turn is clearly illustrated in the stereoview of the crystal structure of **3** in Figure 2.

The distance between the hetero atoms involved in the hydrogen bond, O16···N14 is 2.88 Å, a value well within the range observed for hydrogen-bonded β-turns in peptides.[6] In addition, the backbone Φ_2, Ψ_2, Φ_3, and Ψ_3 torsion angles of −50.9°, 128.7°, 91.1°, and −5.4°, respectively, agree closely with the corresponding torsion angles of −60°, 120°, 80°, and 0° that characterize a classical type-II β-turn.[2]

Discussion

In their studies, Ward et al.[7] calculated that the (*R*)-4.4-spiro lactam constraint would restrict the Φ_2 and Ψ_2 angles to values of −75 ± 20° and +140 ± 10°, respectively. These values are slightly higher than those found in the crystal structure of peptidomimetic **3**. Although the (*R*)-4.4-spiro lactam constraint restricts only two of the four torsion angles that describe a type-II β-turn, the crystallographic results obtained in this study provide direct evidence that a peptidomimetic containing the (*R*)-4.4-spiro lactam constraint can adopt a type-II β-turn. Recently, we synthesized and obtained the crystal structure of the corresponding (*R*)-5.4-spiro lactam analogue of **3**, compound **4**.[8] This peptidomimetic also adopts a type-II β-turn and its Φ_2, Ψ_2, Φ_3, and Ψ_3 torsion angles of −47.8°, 132.8°, 81.3°, and 3.3°, respectively, are very close to those found for **3** in this study.

Previously, Hinds et al.[9] showed through modeling studies that when the (*R*)-4.4-spiro lactam system is incorporated into a peptide, energy minima are obtained wherein the distance and torsion angles are in good agreement with those predicted for the classical type-II β-turn. In contrast, Ward et al.[7] found that when this constraint was incorporated into a neurokinin analogue it did not induce a β-turn. Instead, modeling studies suggested that an extended conformation was preferred. This conclusion was supported by the fact that no stable secondary structure could be detected by NMR. Although our crystallographic study supports the proposition originally set forth by Hinds et al.[3f] that the (*R*)-4.4-spiro lactam constraint can serve as an effective type-II β-turn mimic, the different results obtained by Hinds et al.[9] and Ward et al.[7] with this constraint indicate that further studies are needed with other model peptides to determine how various conformational properties intrinsic to a particular peptide will affect the ability of the (*R*)-4.4-spiro lactam constraint to induce a type-II β-turn.

Experimental Section

General Aspects. Unless otherwise noted, organic extracts were washed with H_2O and saturated NaCl solution, dried over $MgSO_4$, and concentrated under reduced pressure with the aid of a rotary evaporator. Flash column chromatography was carried out on silica gel, Merck, grade 60 (240–400 mesh, 60 Å) from Aldrich Chemical Co., Inc. ^1H and ^{13}C NMR spectra were measured in $CDCl_3$ at 300 MHz and 75.5 MHz, respectively, with the following internal references: tetramethylsilane (δ 0.00) for ^1H and $CHCl_3$ (δ 77.06) for ^{13}C. J values are in hertz. For those compounds where rotamers about the carbamate bond were observed, both ^1H resonances are listed in the ^1H spectra, while in the ^{13}C spectra the rotameric resonance is placed in parentheses after the first resonance.

Methyl *N*-(*tert*-Butoxycarbonyl)-2(*R*)-allylprolylglycinate (6). (*R*)-*N*-Boc-2-allylproline[3h] (0.90 g, 3.53 mmol), Gly-OMe·HCl (0.44 g, 3.53 mmol), HOBt (0.48 g, 3.53 mmol), and NEt_3 (0.49 mL, 3.53 mmol) were dissolved in dry $CHCl_3$ (10 mL). A solution of DCC (0.73 g, 3.53 mmol) in $CHCl_3$ (10 mL) was added and the reaction was stirred at room temperature under N_2 overnight. The precipitate of dicyclohexylurea was removed by filtration and the filtrate was diluted with $CHCl_3$ (20 mL) and washed with 1 M $NaHCO_3$, 10% citric acid, and saturated NaCl solution. The organic layer was dried ($MgSO_4$) and then stripped of solvent in vacuo. The residue was chromatographed on a 3- × 40-cm column with EtOAc/hexane (1:3) as the eluting solvent. The product was isolated as a colorless oil in a yield of 1.1 g (95%): $[\alpha]_D$ +16.3° (*c* 0.70, MeOH); ^1H NMR δ 1.33 and 1.36 (s, 9), 1.54–1.81 (m, 3), 2.00–2.15 and 2.41–2.44 (m, 1), 2.57–2.64 (m, 1), 2.85–2.98 (m, 1), 3.15–3.25 (m, 1), 3.40–3.45 (m, 1), 3.62 (s, 3),

(5) The authors have deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, U.K.

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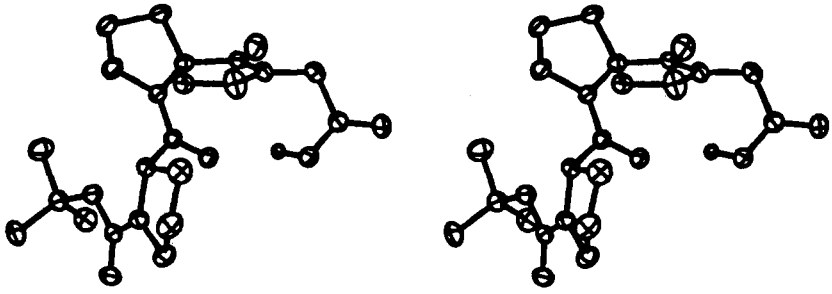

Figure 2. ORTEP stereoview of the type-II β-turn of **3**.

3.81–3.97 (m, 2), 5.01–5.06 (m, 2), 5.49–5.58 (m, 1), 6.55 and 7.89 (br s, 1); ^{13}C NMR δ 21.62 (21.86), 27.79 (27.86), 34.14 (37.07), 37.78 (38.23), 40.72 (40.94), 48.41 (49.05), 51.61, 67.58 (69.25), 79.68 (80.02), 119.00, 132.22 (132.54), 153.11 (154.59), 169.77, 174.12 (174.69); FAB-MS *m/z* 327 [MH]$^+$. Anal. Calcd for $C_{16}H_{26}N_2O_5$: C, 58.87; H, 8.03; N, 8.58. Found: C, 58.83; H, 8.14; N, 8.56.

Methyl (*R*)-1-(*tert*-Butoxycarbonyl)-1,7-diaza-6-oxo-8-(*RS*)-hydroxyspiro[4.4]nonane-7-acetate (8a and 8b). To a solution of **6** (0.52 g, 1.6 mmol) in THF/H_2O (3:1, 15 mL) was added OsO_4 (10 mg). The reaction was stirred at room temperature under N_2 for 10 min, and then finely powdered $NaIO_4$ (1.0 g, 4.68 mmol) was added. After 2 h, the light yellow mixture was poured into H_2O and extracted with EtOAc (3 × 100 mL). The combined EtOAc extracts were washed, dried, and concentrated to give a brown oil. Diastereoisomers **8a** and **8b** were separated on a 1.5- × 45-cm column with EtOAc/hexane (2:1) as the eluting solvent and were isolated as white solids in a total yield of 0.21 g (41%). Each diastereoisomer was crystallized from $CHCl_3$.

8a: mp = 150–151 °C; $[\alpha]_D$ −15.1° (*c* 0.65, MeOH); TLC R_f (EtOAc/hexane (1:1)) = 0.18; ^{1}H NMR δ 1.35 and 1.41 (s, 9), 1.80–2.03 (m, 3), 2.17–2.28 (m, 2), 2.63 and 2.82 (dd, *J* = 13.5 and 6.0, 1), 3.44–3.54 (m, 2), 3.77 (s, 3), 3.78 and 3.84 (d, *J* = 6.8, 1), 4.24 and 4.27 (d, *J* = 4.2, 1, exchanged with D_2O), 4.60 and 4.74 (d, *J* = 17.6, 1), 5.20–5.27 (m, 1); ^{13}C NMR δ 23.62 (24.30), 28.93 (29.11), 39.71 (40.66), 40.84 (41.84), 44.67 (45.32), 48.21 (48.39), 53.51, 66.01 (66.16), 80.52 (80.89), 81.86 (82.55), 154.00 (154.22), 172.07 (172.81), 176.35 (176.51); FAB MS *m/z* 329 [MH]$^+$. Anal. Calcd for $C_{15}H_{24}N_2O_6$: C, 54.86; H, 7.37; N, 8.53. Found: C, 55.09; H, 7.38; N, 8.45.

8b: mp = 136–137 °C; $[\alpha]_D$ −54.4° (*c* 0.45, MeOH); TLC R_f (EtOAc/hexane (1:1)) = 0.39; ^{1}H NMR δ 1.39 (s, 9), 1.79–1.88 (m, 2), 2.05–2.15 (m, 1), 2.23 (d, *J* = 14.7, 1), 2.29–2.40 (m, 1), 2.53 (dd, *J* = 14.7 and 7.2, 1), 3.40–3.55 (m, 2), 3.67 (s, 3), 3.92 (d, *J* = 17.1, 1), 4.43 (d, *J* = 18.3, 1), 5.10 (dd, *J* = 12.3 and 7.2, 1), 5.59 (d, *J* = 12.0, 1, exchanged with D_2O); ^{13}C NMR δ 24.20, 28.87 (28.97), 40.74, 41.73, 43.35, 48.85, 52.75, 65.01, 80.79, 81.52, 154.98, 170.12, 174.85; FAB MS *m/z* 329 [MH]$^+$. Anal. Calcd for $C_{15}H_{24}N_2O_6$: C, 54.86; H, 7.37; N, 8.53. Found: C, 55.38; H, 7.61; N, 8.62.

Methyl *N*-(*tert*-Butoxycarbonyl)-2(*R*)-(2-hydroxyethyl)-prolylglycine (9). To a solution of **6** (1.4 g, 4.3 mmol) in MeOH/H_2O (2:1, 50 mL) was added OsO_4 (50 mg). The reaction was stirred at room temperature under N_2 for 10 min, and then finely powdered $NaIO_4$ (2.7 g) was added in batches over a 20-min period. After 2 h, the light yellow mixture was poured into H_2O and extracted with EtOAc (3 × 100 mL). The combined EtOAc extracts were washed, dried, and concentrated to give a brown oil. A solution of this material in dry EtOAc (50 mL) was cooled to −78 °C, after which time a solution of $NaBH_4$ (160 mg, 4.3 mmol) in *i*-PrOH (10 mL) was added. The reaction was stirred at −78 °C for 1 h after which time it was allowed to warm to room temperature. The reaction was poured into H_2O and extracted with EtOAc (3 × 100 mL). The combined organic extracts were washed, dried, and concentrated to give a yellow oil which was chromatographed on a 2.5- × 45-cm column with CH_2Cl_2/MeOH (20:1) as the eluting solvent. The product was isolated as a colorless oil in a yield of 0.61 g (43%): $[\alpha]_D$ −46.5° (*c* 2.25, $CHCl_3$); ^{1}H NMR δ 1.38 (s, 9), 1.60–2.22 (m, 4), 2.40–2.60 (m, 2), 3.20–3.60 (m, 2), 3.62 (br s, 2), 3.67 (s, 3), 3.80–4.10 (m, 2), 6.87 and 7.87 (br s, 1); ^{13}C NMR δ 23.14 (br), 28.95, 36.98 (38.26), 38.64, 42.05 (br), 48.68 (49.60), 52.88, 59.32 (br), 69.08 (69.77), 81.18 (br), 154.34 (155.93), 170.94 (170.98), 175.65 (175.70); FAB-MS *m/z* 331.1 [MH]$^+$. Anal. Calcd for $C_{15}H_{26}N_2O_6$: C, 54.53; H, 7.93; N, 8.48. Found: C, 54.77; H, 7.88; N, 8.56.

Methyl (*R*)-1-(*tert*-Butoxycarbonyl)-1,7-diaza-6-oxospiro-[4.4]nonane-7-acetate (10). Alcohol **9** (300 mg, 0.91 mmol) was reacted with PPh_3 (0.49 g, 1.88 mmol) and diethyl azodicarboxylate (0.19 mL, 1.10 mmol) in dry THF (5 mL). The solution was stirred at room temperature for 2 h. The solvent was removed in vacuo to give a yellow oil which was chromatographed on a 2.5- × 45-cm silica gel flash column with EtOAc/hexane (3:1) as the eluting solvent. Ph_3PO, which was isolated along with **10**, was separated from the product by crystallization from Et_2O. The product was subsequently isolated as a colorless oil in a yield of 240 mg (84%): $[\alpha]_D$ −47.1° (*c* 1.40, MeOH); ^{1}H NMR δ 1.37 and 1.42 (s, 9), 1.74–2.04 (m, 4), 2.07–2.17 (m, 1), 2.43–2.53 and 2.61–2.71 (m, 1), 3.28–3.56 (m, 4), 3.56 and 3.69 (d, *J* = 18.3, 1), 3.70 and 3.71 (s 3), 4.42 and 4.64 (d, *J* = 18.3, 1); ^{13}C NMR δ 23.28 (23.91), 28.84 (29.05), 30.58 (31.62), 37.12 (37.74), 44.27 (44.52), 45.04 (45.30), 48.21 (48.56), 52.62 (52.67), 67.06 (67.17), 80.08 (80.47), 153.81 (153.94), 169.42 (169.68), 175.83 (175.86); FAB MS *m/z* 313 [MH]$^+$. Anal. Calcd for $C_{15}H_{24}N_2O_5$: C, 57.67; H, 7.74; N, 8.97. Found: C, 57.42; H, 7.65; N, 8.80.

(*R*)-1-[[1-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]-1,7-diaza-6-oxospiro[4.4]nonane-7-acetamide (3). Ester **10** (250 mg, 0.80 mmol) was deprotected in 4 N HCl/dioxane (5 mL) for 30 min after which time the dioxane and excess HCl were removed in vacuo. The residue obtained was dissolved in CH_2Cl_2 and this solution was stripped of solvent in vacuo. The residue was dried in vacuo overnight and then was dissolved in dry DMF (10 mL) along with Boc-Pro-OH (340 mg, 1.60 mmol), HOBt (430 mg, 3.2 mmol), and NEt_3 (0.11 mL, 0.80 mmol). While the solution was stirred at room temperature under N_2 a solution of DCC (250 mg, 1.2 mmol) in dry DMF (7 mL) was added. The reaction mixture was stirred for 3 days under N_2. The mixture was poured into H_2O and extracted with EtOAc (3 × 50 mL). The combined EtOAc extracts were washed, dried and concentrated. The residue was chromatographed on a 2.5- × 40-cm column using CH_2Cl_2/MeOH (20:1) as the eluting solvent. The ester which was obtained as a colorless oil (170 mg) was directly treated with a saturated methanolic ammonia solution (10 mL) and the solution then stirred overnight. The excess NH_3 and methanol were removed in vacuo and the residue was chromatographed on a 1.5- × 40-cm column with CH_2Cl_2/MeOH (20:1) as the eluting solvent. The product was initially isolated as a colorless oil which subsequently crystallized from EtOAc to give 150 mg (48%) of pure **3**: mp 235–237 °C; $[\alpha]_D$ −55.3° (*c* 1.50, MeOH); ^{1}H NMR δ 1.42 and 1.44 (s, 9), 1.80–2.27 (m, 9), 2.50–2.62 (m, 1), 3.37 (d, *J* = 16.8, 1), 3.31–3.68 (m, 5), 3.78–3.82 and 3.90–4.20 (m, 1), 4.38 and 4.45 (dd, *J* = 8.7 and 3.6, 1), 4.57 and 4.58 (d, *J* = 17.1, 1), 5.46 and 5.49 (br s, 1), 7.80 and 7.88 (br s, 1); ^{13}C NMR δ 23.38 (24.14), 24.21 (24.25), 28.18 (28.21), 29.00 (29.88), 30.43 (30.46), 36.65 (36.82), 44.16 (44.37), 46.52 (46.60), 46.62 (46.69), 47.63, 57.47, 67.40 (67.45), 79.29 (79.34), 153.41 (154.37), 170.31 (170.54), 171.51 (171.75), 173.17 (173.51); FAB MS *m/z* 395 [MH]$^+$. Anal. Calcd for $C_{19}H_{30}N_4O_5$: C, 57.85; H, 7.67; N, 14.20. Found: C, 58.10; H, 7.61; N, 14.21.

X-ray Crystallography. Diffraction data were collected using an Enraf-Nonius CAD-4 diffractometer operating in the ω–2θ mode. Crystal temperature was maintained at 173(1) K with a

Molecular Structure Corporation low temperature device. The structure was solved by the direct methods program SHELXS.[10] TEXSAN[11] was used for structure refinement and absorption corrections were made using DIFABS.[12] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were located in difference maps and refined with fixed, isotropic, temperature factors. Crystal data are given in Table I.

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