Design, Synthesis, and X-ray Crystallographic Analysis of Two Novel Spirolactam Systems as β-Turn Mimics

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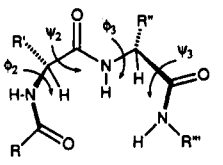
Two novel 5.4-spirolactam systems have been developed as β-turn mimics. The (*R*)-5.4-spirolactam system of **4** was designed to mimic the type-II β-turn, and the (*S*)-5.4-spirolactam system of **5** was designed to mimic the type-II′ β-turn. The 5.4-spirolactam dipeptide mimic **11** was synthesized in racemic form from pipecolic acid. This intermediate was then converted to the diastereoisomeric mixture of peptidomimetics **4** and **5**, which were separated by fractional recrystallization. X-ray crystallographic analysis of **4** and **5** indicated that both of these compounds adopted hydrogen-bonded β-turn conformations. As predicted, the (*R*)-5.4-spirolactam system of **4** induced a type-II β-turn while the (*S*)-5.4-spirolactam system of **5** induced a type-II′ β-turn. The backbone torsion angles of **4** and **5** were close to those of the classical type-II and -II′ β-turns, respectively. A computer-generated fit between nine atoms of the backbone of **4** and their counterparts in an ideal type-II β-turn yielded an RMS fit of 0.218 Å. A similar comparison between **5** and an ideal type-II′ β-turn produced an RMS fit of 0.392 Å.

The delineation of the biologically active conformation of a peptide hormone or neurotransmitter is a key step in the design of more potent and selective peptide receptor agonists and antagonists. Presently, the development of compounds designed to mimic certain secondary structural features of peptides is an important approach for elucidating the receptor bound conformation of a biologically active peptide.[1] One major secondary structural feature of many biologically active peptides is the β-turn. This type of turn is characterized by its ϕ_2, ψ_2, ϕ_3, and ψ_3 torsion angles. For example, in an ideal type-II β-turn (**1**) these angles possess values of −60°, 120°, 80°, and 0°, respectively.[2]

Several nonpeptide systems have been designed to mimic the different types of β-turns.[1b,3] Previously, the (*R*)-4.4-spirolactam system illustrated in structure **2** was shown by Hinds et al.[3f,4] to be a good mimic of the type-II β-turn. Ward et al.[5] calculated that the ϕ_2 and ψ_2 angles would be constrained to values of −75 ± 20° and +140 ± 10°, respectively, for this mimic. These workers also suggested that the (*S*)-4.4-spirolactam system illustrated in structure **3** in which the ϕ_2 and ψ_2 angles are constrained to values of +75 ± 20° and −140 ± 10°, respectively, would be a good mimic of the type-II′ β-turn conformation.



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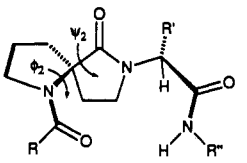

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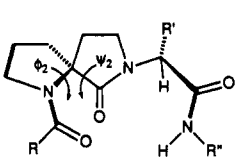

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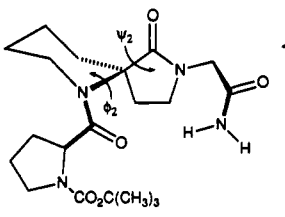

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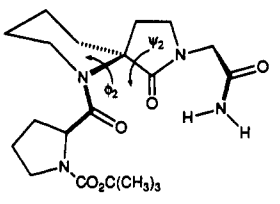

5

In light of the conformational properties of the 4.4-spirolactam systems we designed the (*R*)-5.4-spirolactam constraint illustrated in structure **4** as a mimic of the type-II β-turn and the (*S*)-5.4-spirolactam constraint illustrated in structure **5** as a mimic of the type-II′ β-turn. Like the 4.4-spirolactam constraints, the 5.4-spirolactam constraints restrict the ϕ_2 and ψ_2 torsion angles of the peptide. However, the presence of the six-membered piperidine ring system in the 5.4-spirolactam constraint allows for more conformational freedom thereby providing some interesting conformational possibilities. For example, modeling studies on the (*R*)-5.4-spirolactam system show that the ϕ_2 and ψ_2 torsion angles vary depending upon the chair form that the piperidine ring adopts. When the lactam carbonyl oxygen is axial, energy minima are

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(1) (a) Hruby, V. J. *Life Sci.* **1982**, *31*, 189–199. (b) Ball, J. B.; Alewood, P. F. *J. Mol. Recognit.* **1990**, *3*, 55–64. (c) Kemp, D. S. *Trends Biol. Sci.* **1990**, *8*, 249–255.
(2) Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1–109.
(3) (a) Freidinger, R. M.; Veber, D. F.; Hirschmann, R.; Paege, L. M. *Int. J. Peptide Protein Res.* **1980**, *16*, 464–470. (b) Krstenansky, J. L.; Baranowsky, R. L.; Currie, B. C. *Biochem. Biophys. Res. Commun.* **1982**, *109*, 1368–1374. (c) Nagai, U.; Sato, K. *Tetrahedron Lett.* **1985**, *26*, 647–650. (d) Kemp, D. S.; Stites, W. E. *Tetrahedron Lett.* **1988**, *29*, 5057–5060. (e) Kahn, M.; Wilke, S.; Chen, B.; Fujita, K.; Lee, Y.-H.; Johnson, M. *J. Mol. Recognit.* **1988**, *1*, 75–79. (f) Hinds, M. G.; Richards, N. G. J.; Robinson, J. A. *J. Chem. Soc., Chem. Commun.* **1988**, 1447–1449. (g) Olson, G. L.; Voss, M. E.; Hill, D. E.; Kahn, M.; Madison, V. S.; Cook, C. M. *J. Am. Chem. Soc.* **1990**, *112*, 323–333. (h) Genin, M. J.; Johnson, R. L. *J. Am. Chem. Soc.* **1992**, *114*, 8778–8783.
(4) Hinds, M. G.; Welsh, J. H.; Brennend, D. M.; Fisher, J.; Glennie, M. J.; Richards, N. G. J.; Turner, D. L.; Robinson, J. A. *J. Med. Chem.* **1991**, *34*, 1777–1789.
(5) Ward, P.; Ewan, G. B.; Jordan, C. C.; Ireland, S. J.; Hagan, R. M.; Brown, J. R. *J. Med. Chem.* **1990**, *33*, 1848–1851.

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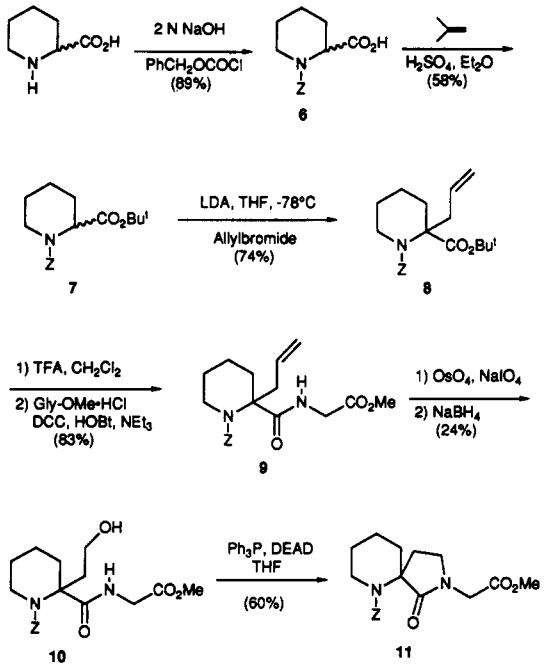
Scheme I



observed in which the ϕ_2 and ψ_2 torsion angles are about −68° and +133°, respectively, while when the carbonyl group is in the equatorial position the corresponding torsion angles are around −37° and +109°. In the present study, we describe the synthesis and X-ray crystallographic analysis of the novel 5.4-spirolactam peptidomimetics **4** and **5**.

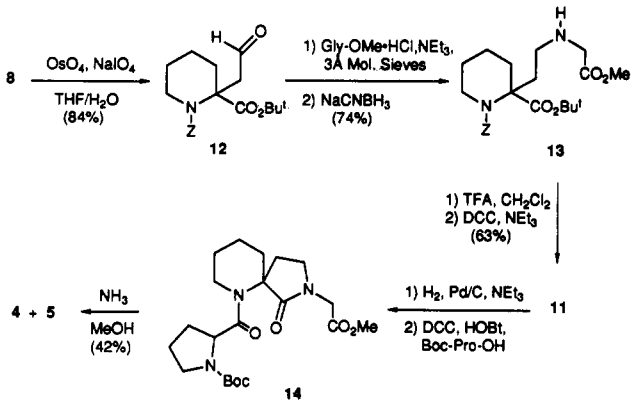
Results and Discussion

Syntheses. Our first synthesis of the 5.4-spirolactam system is outlined in Scheme I. Fully protected DL-pipecolic acid **7** was alkylated with allyl bromide to give the α-allyl derivative **8**. Removal of the *tert*-butyl ester of **8** followed by coupling of the resulting acid to Gly-OMe·HCl afforded dipeptide ester **9**. Oxidative cleavage of the double bond of **9** (OsO_4 and $NaIO_4$) followed by immediate reduction of the resulting aldehyde gave alcohol **10**. This alcohol was cyclized to the desired 5.4-spirolactam system **11** under Mitsunobu conditions (Ph_3P, DEAD).

A drawback to the above synthetic approach into the 5.4-spirolactam system was that the overall yield for the sequence of reactions was low and the oxidation and reduction steps led to very complex mixtures. Thus, an alternate route (Scheme II) was tried in order to obtain spirolactam **11** in higher yields. In this approach **8** was oxidized to aldehyde **12**, which was then converted to secondary amine **13** via a reductive amination reaction with Gly-OMe·HCl. Deprotection of the *tert*-butyl ester of **13** followed by activation of the carboxyl group with dicyclohexylcarbodiimide (DCC) to initiate cyclization gave spirolactam **11**. This approach gave much cleaner reactions and a higher yield of **11** than the method depicted in Scheme I.

Spirolactam **11** was deprotected via catalytic hydrogenation and the resulting product coupled to Boc-L-Pro-OH using DCC and HOBt to give a diastereoisomeric mixture of peptidomimetic esters (**14**). This mixture was treated with methanolic ammonia to give the corresponding mixture of peptidomimetic amides **4** and **5**. Diastereoisomer **5**, which had the lower R_f value on TLC,

Scheme II



was separated from **4** by fractional recrystallization from EtOAc. The filtrate from this crystallization, now enriched with the diastereoisomer **4**, was subjected to silica gel column chromatography. After this chromatographic procedure, **4** also readily crystallized from EtOAc. The crystals obtained for **4** and **5** were each found to be suitable for X-ray crystallography. This allowed for the determination of the absolute stereochemistry of the spiro linkage in **4** and **5**. Diastereoisomer **4** was found to possess the 5.4-spirolactam with the (*R*)-configuration, while **5** was determined to contain the 5.4-spirolactam system with the (*S*)-configuration. The crystal structure analysis of both of these compounds is discussed below in detail.

X-ray Analysis. The molecular structures of **4** and **5** with their atomic numbering schemes are shown in Figures 1 and 2, respectively.[6] Data collection information is shown in Table I. In the molecular structure of compound **5** the C20 atom was found to be disordered with 18.3% occupancy for the minor component. The major component was refined with anisotropic thermal parameters, while the minor component was refined with an isotropic thermal parameter. Hydrogen atoms were included only for the major component and were included at idealized positions except for H2, which was refined.

In the crystal state, both compounds were observed to adopt hydrogen-bonded β-turn conformations. The (*S*,*R*)-spirolactam peptidomimetic **4** forms a classical type-II β-turn in the solid state, as illustrated in the stereoview of this compound in Figure 3. This turn is stabilized by an intramolecular hydrogen bond between the trans carboxamide hydrogen and the prolyl carbonyl oxygen. The distance between the hetero atoms involved in the hydrogen bond, O17···N15, is 3.01 Å. This distance is in the range observed for similar hydrogen-bonded β-turns in peptides.[7] The ϕ_2, ψ_2, ϕ_3, and ψ_3 torsion angles for **4** are −47.8°, +132.8°, +81.3°, and +3.3°, respectively. As shown in Table II, these angles are in very good agreement with those of the classical type-II β-turn. A fit of the backbone atoms of **4** with the corresponding backbone atoms of a classical type-II β-turn gave an RMS fit of 0.218 Å (Figure 4).[8] These results support our proposal that the (*R*)-5.4-spirolactam constraint is a very good mimic of the type-II β-turn.

(6) The authors have deposited atomic coordinates for these structures 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, UK.

(7) (a) Taylor, R.; Kennard, O. *Acc. Chem. Res.* **1984**, *17*, 320–326. (b) Gorbitz, C. H. *Acta Cryst.* **1989**, *B45*, 390–395.

(8) RMS fits were performed using the Alchemy III software, TRIPOS Associates, Inc., 1992, on a Macintosh Quadra 700 computer.

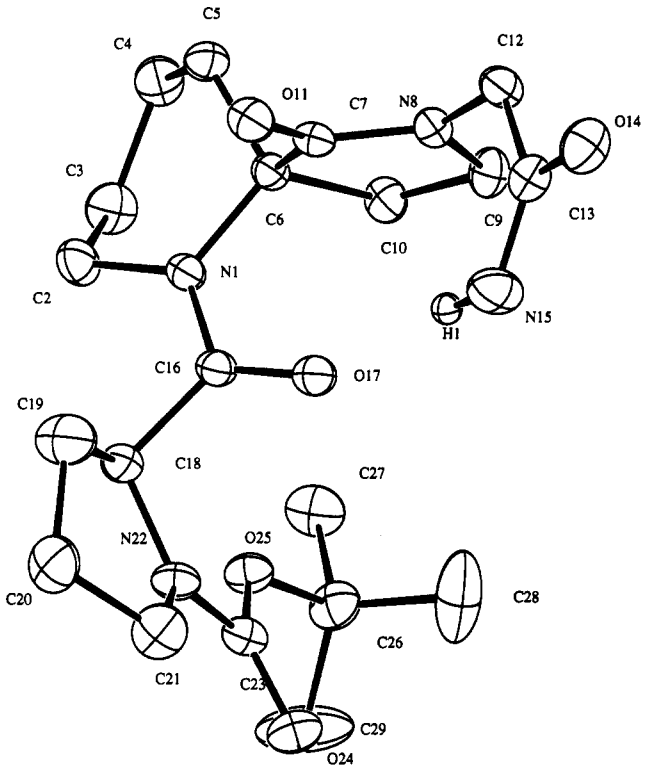


Figure 1. ORTEP representation of 4 with crystallographic numbering system.

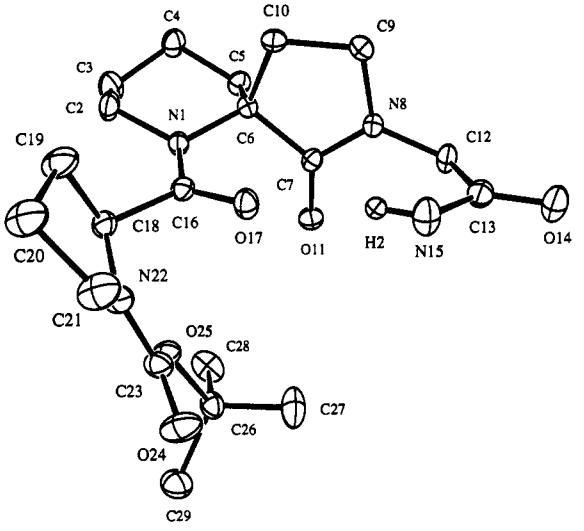


Figure 2. ORTEP representation of 5 with crystallographic numbering system.

Hinds et al.[3f,4] and Ward et al.[5] have previously employed the (*R*)-4.4-spirolactam constraint (**2**) as a mimic of the type-II β-turn. The ϕ_2 and ψ_2 torsional angles for this constraint have been calculated to be −75 ± 20° and +140 ± 10°, respectively. When comparing these values to the corresponding torsion angle values for the (*R*)-5.4-spirolactam system of **4**, it is clear that both of these spirolactam systems are capable of restricting the ϕ_2 and ψ_2 torsion angles to values in the vicinity of those found in a type-II β-turn.

The (*S*,*S*)-spirolactam peptidomimetic **5**, like its diastereoisomer **4**, adopts a β-turn conformation in the crystal state. However, because of the (*S*)-stereochemistry at the spiro carbon atom, the β-turn conformation that is adopted is of the type-II′ type. A stereoview of this type-II′ β-turn

Table I. Crystal Data for Peptidomimetics 4 and 5

parameter	4	5
molecular formula	$C_{20}H_{32}N_4O_5$	$C_{20}H_{32}N_4O_5$
MW (amu)	408.50	408.50
d(cald), g/cm^3	1.264	1.241
crystal system	orthorhombic	monoclinic
space group	$P2_12_12_1$	$P2_1$
Z	4	2
a, Å	9.661 (4)	8.786 (5)
b, Å	10.042 (3)	10.127 (2)
c, Å	22.115 (4)	12.548 (6)
α, deg	90.00	90.00
β, deg	90.00	101.69 (4)
γ, deg	90.00	90.00
V, Å^3	2146	1093
crystal size (mm)	0.3 × 0.5 × 0.6	0.4 × 0.4 × 0.3
total reflections	6742	5529
unique reflections	3548	3355
obsd ($I > 2.00\sigma(I)$)	2841	2861
no. of variables	276	274
final R	0.040	0.042
final R_w	0.047	0.051
GOF	1.10	1.28

conformation of **5** is illustrated in Figure 5. This structure is also stabilized by an intramolecular hydrogen bond between the trans carboxamide hydrogen and the prolyl carbonyl oxygen. The distance between the hetero atoms involved in the hydrogen bond, O17···N15, is 3.00 Å. This distance is essentially the same as that observed in **4**. The ϕ_2, ψ_2, ϕ_3, and ψ_3 torsion angles of the (*S*)-spirolactam constraint of **5** are +45.7°, −135.1°, −101.4°, and +23.8°, respectively. These values are, in general, close to those observed in an ideal type-II′ β-turn as shown in Table II and illustrated in Figure 6 where a fit of the backbone atoms of **5** with the corresponding atoms in a classical type-II′ β-turn gave an RMS fit of 0.392 Å.[8] These results demonstrate that like the previously described (*S*)-4.4-spirolactam constraint (**3**)[5] the (*S*)-5.4-spirolactam constraint of **5** is a good mimic of the type-II′ β-turn.

An interesting aspect of the crystal structures of **4** and **5** is the conformation of the six-membered piperidine ring in these two 5.4-spirolactam molecules. It is known that peptides in which the pipecolyl moiety is incorporated as an internal residue have properties different from their prolyl counterparts, since it is observed that the C-2 pipecolyl–CONH– must lie in an axial orientation[9] because of pseudoallylic strain.[10,11] Although pseudoallylic strain should be in effect in the 5.4-spirolactam derivatives **4** and **5**, the situation with these two molecules is more complicated than in the pipecolyl case because of the disubstituted nature of the C-2 carbon of the piperidine ring. The question becomes one of whether the pseudoallylic strain is greater when the lactam ring carbonyl group, C7=O11, is equatorial or when the methylene at position 4 of the lactam ring, C10, is equatorial. When the carbonyl group of the lactam ring, C7=O11, is equatorial, steric interference and π cloud crowding can occur with the carbonyl oxygen, O17, of the prolyl residue. The distance between the two carbonyl oxygens, O17···O11, is about 2.2 Å. When the methylene on the 4-position of the lactam ring, C10, is equatorial the hydrogens point directly into the area of the prolyl carbonyl oxygen, O17. Models show an oxygen–hydrogen, O17···HC10, distance of about 1.2 Å.

(9) Hruby, V. J.; Kazmierski, W.; Kawasaki, A. M.; Matsunaga, T. O. In *Peptide Pharmaceuticals*; Ward, D., Ed.; Elsevier Science: New York, 1991; pp 135–177.
(10) Johnson, F. *Chem. Rev.* **1968**, *68*, 375–411.
(11) Sugg, E. E.; Griffin, J. F.; Portoghese, P. S. *J. Org. Chem.* **1985**, *50*, 5032–5037.

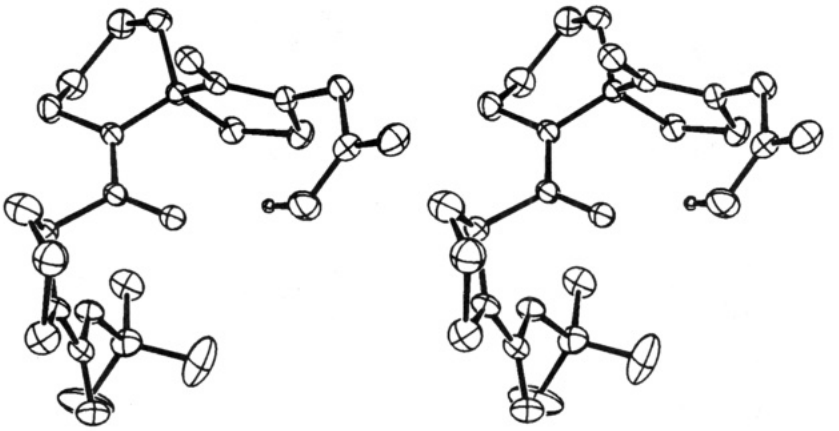


Figure 3. ORTEP stereoview of the type-II β-turn of **4**.

Table II. Comparison of the Backbone Torsion Angles (deg) and Intramolecular Hydrogen Bond Distances (Å) for the Type-II and -II′ β-Turns and the 5.4-Spirolactam Peptidomimetics 4 and 5

system	ϕ_2	ψ_2	ϕ_3	ψ_3	N15···O17 distance
(*R*)-5.4-spirolactam **4**	−47.8	132.8	81.3	3.3	3.01
ideal type-II β-turn[a]	−60	120	80	0	
(*S*)-5.4-spirolactam **5**	45.7	−135.1	−101.4	3.8	3.00
ideal type-II′ β-turn[a]	60	−120	−80	0	

[a] Data from ref 2.

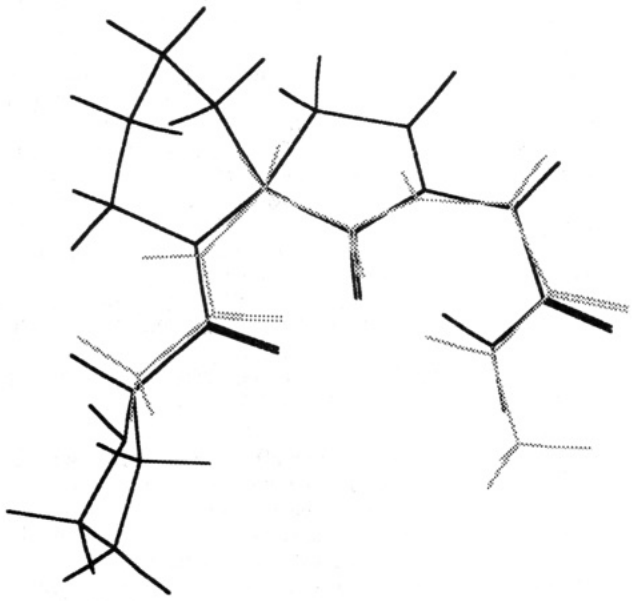


Figure 4. Fit of the molecular structure of **4** (black) with a model of a classical type-II β-turn (gray). The *tert*-butoxycarbonyl group of **4** has been removed for visual clarity. The comparison was made by fitting nine atoms of the backbone of **4** with the corresponding amide backbone atoms of an ideal type-II β-turn. RMS fit = 0.218 Å.[8]

A comparison of these two possible conformations of the 5.4-spirolactam systems suggests that the steric interference between the lactam methylene group, C10, and the prolyl carbonyl oxygen, O17, is greater than that between the lactam carbonyl oxygen, O11, and the prolyl carbonyl oxygen, O17. This supposition is supported by the fact that in the crystal structures of **4** and **5** the lactam carbonyl group, C7=O11, is pseudoequatorial/equatorial, while the C10 methylene resides in an pseudoaxial/axial orientation.

Another point worth noting about the conformations of the piperidine rings of the 5.4-spirolactam systems is that in the (*S*)-5.4-spirolactam system of **5** the piperidine ring adopts a chair conformation as predicted. In contrast, the piperidine ring in the (*R*)-5.4-spirolactam system of **4** adopts a twist-boat conformation. Also, the exocyclic amide bond, O17–C16–N1–C2, of **4** is trans (ω = 171.4°) as expected, since resonance stabilization in the amide system requires planarity. The exocyclic amide bond, O17–C16–N1–C2, of **5**, on the other hand, is in a somewhat twisted conformation (ω = −156.2°). As a result, the degree of resonance stabilization in this system will be less due to the loss of planarity. It is unclear why these differences in piperidine conformation occur in **4** and **5**. There is, however, precedence for pipecolyl derivatives to exist in either chair or twist boat conformations with planar or twisted amide bonds occurring in either.[12]

Conclusion

The present study has shown the rational design and synthesis of two novel 5.4-spirolactam systems as β-turn mimics. X-ray crystallography revealed that the (*R*)-5.4-spirolactam system of **4** and the (*S*)-5.4-spirolactam system of **5** are able to constrain peptides into type-II and type-II′ β-turns, respectively. These systems restrict the ϕ_2 and ψ_2 torsion angles of the peptide backbone. The (*R*)-5.4-spirolactam holds these angles to −47.8° and 132.8° while the (*S*)-5.4-spirolactam restricts them to 45.7° and −135.1°. These numbers agree closely with those of the classical type-II (−60° and 120°) and type-II′ (60° and −120°) β-turns. These systems should, thus, prove to be valuable tools for the design and synthesis of peptidomimetics of biologically active peptides for investigating structure–activity relationships and bioactive conformations.

Experimental Section

General Aspects. Melting points are uncorrected. 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 and gravity column chromatography were 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. Chemical shifts are expressed in ppm downfield from internal tetramethylsilane or relative to internal $CHCl_3$. *J* values are in hertz. For those

(12) (a) Holmes, A. B.; Raithby, P. R.; Russell, K.; Stern, E. S.; Stubbs, M. E.; Wellard, N. K. *J. Chem. Soc., Chem. Commun.* **1984**, 1191–1192. (b) Muhlebach, A.; Lorenzi, G. P.; Gramlich, V. *Helv. Chim. Acta* **1986**, *69*, 389.

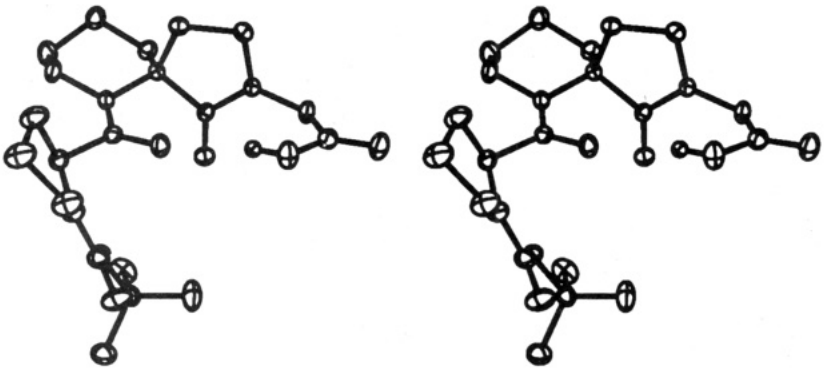


Figure 5. ORTEP stereoview of the type-II′ β-turn of **5**.

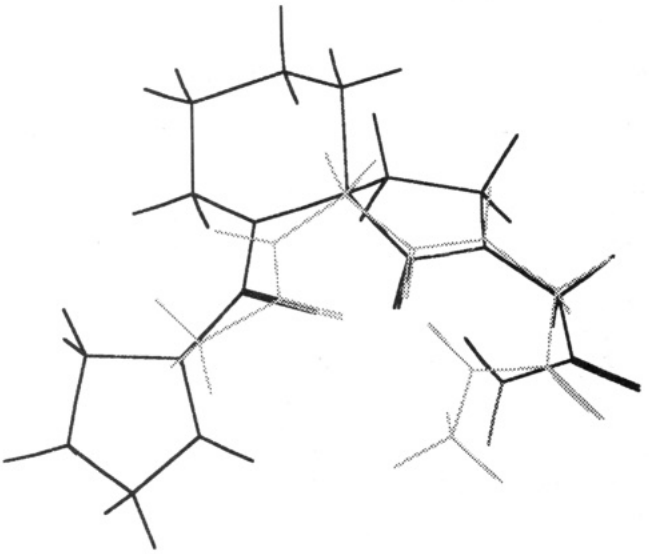


Figure 6. Fit of the molecular structure of **5** (black) with a model of a classical type-II′ β-turn (gray). The *tert*-butoxycarbonyl group of **5** has been removed for visual clarity. The comparison was made by fitting nine atoms of the backbone of **5** with the corresponding amide backbone atoms of an ideal type-II′ β-turn. RMS fit = 0.392 Å.[8]

compounds where rotamers about the carbamate bond were observed both ^{1}H resonances are listed, while in the ^{13}C spectra the rotameric resonance is placed in parentheses after the first resonance.

***N*-(Benzyloxycarbonyl)-DL-pipecolic Acid (6).** A solution of DL-Pip-OH (5.0 g, 38.7 mmol) in 2 N NaOH (19 mL) was cooled to 0 °C. Benzyl chloroformate (7.0 mL, 40.0 mmol) and 2 N NaOH (19 mL) in separate addition funnels were added at a rate that kept the reaction temperature below 5 °C. After the addition of the reagents was complete, the mixture was allowed to warm to room temperature over a 1-h period. The reaction mixture was washed with Et_2O (20 mL), and the aqueous layer was acidified with 10% citric acid and then extracted with EtOAc (3 × 50 mL). The combined extracts were washed, dried, and concentrated to give 9.1 g (89%) of product as a colorless oil which crystallized on standing: mp = 83–84 °C; ^{1}H NMR δ 1.24–1.47 (m, 2), 1.64–1.73 (m, 3 H), 2.26 (br t, J = 14.1, 1), 2.96–3.12 (m, 1), 4.04–4.17 (m, 1), 4.91 and 5.02 (br d, J = 3.6, 1), 5.17 (m, 2), 7.33–7.37 (m, 5), 9.73 (br s, 1); ^{13}C NMR δ 21.29 (21.34), 25.14 (25.24), 27.22 (27.34), 42.44 (42.57), 54.85 (55.06), 68.13 (68.22), 128.45, 128.71, 129.16, 137.12, 157.41, 178.08; FAB MS m/z 264 $[MH]^+$. Anal. Calcd for $C_{14}H_{17}NO_4$: C, 63.86; H, 6.51; N, 5.32. Found: C, 63.80; H, 6.66; N, 5.23.

***tert*-Butyl *N*-(Benzyloxycarbonyl)-DL-pipecolate (7).** A mixture of Z-DL-Pip-OH (**6**, 5.0 g, 19.0 mmol), Et_2O (15 mL), and H_2SO_4 (1 mL) in a pressure bottle was cooled in a dry ice/*i*-PrOH bath. Isobutylene (25 mL) was added via a cannula. The bottle was capped and then shaken at room temperature for 24 h. The solution was poured into saturated $NaHCO_3$ (100 mL). The Et_2O layer was saved, and the aqueous phase was extracted with Et_2O (2 × 75 mL). The combined Et_2O extracts were washed, dried, and concentrated to yield 3.5 g (58%) of **7** as a colorless oil: ^{1}H NMR δ 1.09–1.20 (m, 2), 1.28 and 1.32 (s, 9), 1.40–1.60 (m, 3), 2.06 (br t, J = 12.3, 1), 2.86 and 2.98 (br t, J = 12.2, 1), 3.87–3.99 (m, 1), 4.56 and 4.71 (d, J = 5.1, 1), 4.90 and 5.11 (d, J = 12.3, 1), 5.02 (s, 1), 7.11–7.21 (m, 5); ^{13}C NMR δ 21.10 (21.21), 25.17 (25.37), 27.32 (27.40), 28.45 (28.52), 42.31, 55.40 (55.66), 67.45 (67.63), 81.73 (81.83), 128.32, 128.42, 128.93, 137.38, 156.55 (156.99), 171.08 (171.13); FAB MS m/z 320 $[MH]^+$. Anal. Calcd for $C_{18}H_{25}NO_4$: C, 67.69; H, 7.89; N, 4.39. Found: C, 67.49; H, 7.98; N, 4.35.

***tert*-Butyl *N*-(Benzyloxycarbonyl)-2(*RS*)-allylpipecolate (8).** A solution of Z-DL-Pip-OBu^t (**7**, 3.0 g, 9.39 mmol) in dry THF (75 mL) under N_2 was cooled to −78 °C. A 2 M LDA solution in THF/hexane/EtPh (5.6 mL, 11.3 mmol) was added dropwise. After 20 min, allyl bromide (1.0 mL, 11.3 mmol) was added slowly. The solution was stirred at −78 °C for 20 min, and then it was allowed to warm to room temperature. The reaction was partitioned between CH_2Cl_2 and H_2O. The organic layer was washed, dried, and concentrated to give a yellow oil which was chromatographed on a 5- × 40-cm flash column using hexane as the eluting solvent. The product was isolated in a yield of 2.5 g (74%) as a colorless oil which crystallized on standing: mp 64–65 °C; ^{1}H NMR δ 1.34 (s, 9), 1.42–1.57 (m, 4), 1.60–1.75 (m, 1), 1.79–1.90 (m, 1), 2.47 (dd, J = 14.0 and 8.0, 1), 2.98 (dd, J = 14.1 and 6.9, 1), 3.13–3.20 (m, 1), 3.70–3.80 (m, 1), 4.99 (br d, J = 12.3, 3), 5.12 (d, J = 12.0, 1), 5.70–5.85 (m, 1), 7.18–7.28 (m, 5); ^{13}C NMR δ 17.20, 22.22, 24.22 (24.42), 27.42, 31.15, 41.27 (41.38), 54.45 (54.67), 66.52, 80.33, 117.81, 127.48, 127.53, 127.99, 133.82, 136.39, 155.64, 172.39; FAB MS m/z 360 $[MH]^+$. Anal. Calcd for $C_{21}H_{29}NO_4$: C, 70.17; H, 8.13; N, 3.90. Found: C, 70.19; H, 8.17; N, 3.84.

Methyl *N*-(Benzyloxycarbonyl)-2(*RS*)-allylpipecolylglycinate (9). To a solution of **8** (0.95 g, 2.64 mmol) in CH_2Cl_2 was added CF_3CO_2H (5 mL). The solution was stirred for 3 h at room temperature. Removal of solvent in vacuo gave the acid as a light brown oil which was dried under vacuum overnight. To a solution of this material along with Gly-OCH_3·HCl (0.33 g, 2.64 mmol), HOBt (0.36 g, 2.64 mmol), and NEt_3 (0.37 mL, 2.64 mmol) in dry $CHCl_3$ (10 mL) was added a solution of DCC (0.54 g, 2.64 mmol) in $CHCl_3$ (10 mL). The reaction was stirred at room temperature under N_2 overnight. The DCU was removed by filtration, and the filtrate was diluted with $CHCl_3$ (20 mL). This solution was washed with 1 M $NaHCO_3$, 10% citric acid, and saturated NaCl solution. The organic layer was dried and concentrated to give a residue which was chromatographed on a 3- × 40-cm flash column using EtOAc/hexane (1:3) as the eluting solvent. The product was isolated as a colorless oil in a yield of 0.82 g (83%): ^{1}H NMR δ 1.43–1.71 (m, 4), 1.73–1.82 (m, 1), 1.87–1.96 (m, 1), 2.61 (dd, J = 14.1 and 7.8, 1), 2.94 (dd, J = 13.5 and 7.2, 1), 3.13 (dt, J = 13.5 and 6.9, 1), 3.65 (s, 3), 3.67 (dd, J = 18.3 and 4.8, 1), 3.85 (dt, J = 13.5 and 4.1, 1), 4.01 (dd, J = 18.2 and 6.2, 1), 4.97 (d, J = 12, 1), 4.99 (br s, 1), 5.03 (br s, 1), 5.14 (d, J = 12, 1), 5.73–5.87 (m, 1), 6.55 (br t, J = 4.8, 1), 7.25–7.28 (m, 5); ^{13}C NMR δ 16.90, 22.30, 31.99, 38.58, 40.89, 41.67, 51.88, 63.85, 67.03, 118.50, 127.81, 127.84, 128.18, 133.46, 135.93, 156.16, 170.21, 174.33; FAB MS m/z 375 $[MH]^+$. Anal. Calcd for $C_{20}H_{26}N_2O_5$: C, 64.15; H, 7.00; N, 7.48. Found: C, 64.13; H, 6.89; N, 7.62.

Methyl *N*-(Benzyloxycarbonyl)-2(*RS*)-(hydroxyethyl)-pipecolylglycinate (10). To a solution of **9** (300 mg, 0.80 mmol) in MeOH/H_2O (2:1, 20 mL) was added OsO_4 (20 mg). The reaction was stirred at room temperature under N_2 for 10 min, and then finely powdered $NaIO_4$ (0.50 g) was added in batches over a 10-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 a brown oil. A solution of this material in dry EtOAc (30 mL) was cooled to −78 °C. A solution of $NaBH_4$ (30 mg, 0.8 mmol) in *i*-PrOH (10 mL) was added, and 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 × 50 mL). The combined organic extracts were washed, dried, and concentrated to give a yellow oil which was chromatographed on a 1.5- × 45-cm flash column with EtOAc as the eluting solvent. Compound **10** was isolated as a colorless oil in a yield of 70 mg (24%): ^{1}H NMR δ 1.40–1.85 (m, 6), 1.90–2.05 and 2.10–2.30 (m, 2), 3.00–3.10 (m, 1), 3.35 (s, 3), 3.40–3.80 (m, 4), 3.90–4.00 (m, 1), 4.95 (br s, 1), 5.05 (s, 2), 7.28–7.40 (m, 5); FAB MS *m/z* 379 [MH]$^+$. Anal. Calcd for $C_{19}H_{26}N_2O_6$: C, 60.30; H, 6.93; N, 7.40. Found: C, 60.26; H, 6.72; N, 7.40.

***tert*-Butyl *N*-(Benzyloxycarbonyl)-2(*RS*)-(formylmethyl)pipecolate (12).** To a solution of **8** (0.50 g, 1.39 mmol) in THF/H_2O (4:1, 20 mL) under N_2 was added OsO_4 (20 mg). After 5 min, $NaIO_4$ (750 mg) was added in three batches over a 10-min period. The reaction was stirred for 2.5 h and then diluted with Et_2O (20 mL) and H_2O (30 mL). The aqueous layer was extracted with Et_2O (3 × 50 mL), and the combined Et_2O layers were washed, dried, and concentrated to give a tan oil which was chromatographed on a 2.5- × 45-cm flash column using EtOAc/hexane (1:2) as the eluting solvent. The product was isolated as a colorless oil in a yield of 420 mg (84%): ^{1}H NMR δ 1.40 (s, 9), 1.56–1.70 (m, 4), 1.89–2.00 (m, 2), 2.91 (d, *J* = 2.4, 2), 3.28–3.33 (m, 1), 3.77 (dt, *J* = 13.2 and 5.2, 1), 5.03 (d, *J* = 12.3, 1), 5.16 (d, *J* = 12.3, 1), 7.31–7.33 (m, 5), 9.84 (s, 1); ^{13}C NMR δ 17.94, 22.98, 27.70, 33.29, 41.54, 47.16, 62.96, 67.23, 81.83, 127.92, 128.00, 128.39, 137.43, 156.21, 171.67, 200.34; FAB MS *m/z* 362 [MH]$^+$. Anal. Calcd for $C_{20}H_{27}NO_5$: C, 66.46; H, 7.53; N, 3.88. Found: C, 66.59; H, 7.42; N, 3.80.

***tert*-Butyl *N*-(Benzyloxycarbonyl)-2(*RS*)-[1-[*N*-[(methoxycarbonyl)methyl]amino]ethyl]pipecolate (13).** Aldehyde **12** (630 mg, 1.74 mmol) in MeOH (20 mL) was added to a solution of Gly-OMe·HCl (870 mg, 7.0 mmol) in anhyd MeOH (20 mL) which was under N_2 and contained 3-Å molecular sieves. This was followed by the addition of $NaCNBH_3$ (112 mg, 1.74 mmol) in one portion. The mixture was stirred under N_2 for 24 h after which time the sieves were filtered off and the solvent was removed in vacuo. The residue was chromatographed on a 1.5- × 45-cm flash column using Et_2O as the eluting solvent to give **13** as a colorless oil in a yield of 560 mg (74%): ^{1}H NMR δ 1.34 (s, 9), 1.40–1.70 (m, 4), 1.80–1.90 (m, 2), 1.90–2.05 (m, 1), 2.32–2.40 (m, 1), 2.55–2.68 (m, 2), 2.89 (br s, 1), 3.20–3.28 (m, 1), 3.34 (br s, 2), 3.69 (s, 3), 3.71–3.78 (m, 1), 5.02 (d, *J* = 13.5, 1), 5.15 (d, *J* = 12.3, 1), 7.28–7.36 (m, 5); ^{13}C NMR δ 18.12, 22.97, 27.73, 32.36, 32.46, 41.81, 44.87, 50.51, 51.67, 62.98, 66.95, 80.86, 127.86, 127.94, 128.32, 136.56, 156.24, 172.56, 172.60; FAB MS *m/z* 435 [MH]$^+$.

(*RS*)-1-(Benzyloxycarbonyl)-1,8-diaza-7-oxospiro[5.4]-decane-8-acetic Acid Methyl Ester (11). Method 1. Alcohol **10** (30 mg, 0.08 mmol) was reacted with Ph_3P (42 mg, 0.16 mmol) and diethyl azodicarboxylate (0.02 mL, 0.09 mmol) in dry THF (1 mL). The solution was stirred under Ar at room temperature for 2 h. The solvent was removed in vacuo to give a residue which was chromatographed on a 1.5- × 45-cm flash column with EtOAc as the eluting solvent to give **11** as a colorless oil in a yield of 15 mg (52%).

Method 2. Amine **13** (460 mg, 1.06 mmol) was dissolved in CH_2Cl_2 (10 mL), and to this solution was added CF_3CO_2H (10 mL). The reaction was stirred under N_2 for 3 h after which time the solvent was removed in vacuo. The residue was dissolved in CH_2Cl_2. This solution was stripped of solvent to give a tan oil which was dried under vacuum. To a solution of this material in $CHCl_3$ (10 mL) was added a solution of DCC (230 mg, 1.12 mmol) in $CHCl_3$ (10 mL) followed by NEt_3 (0.16 mL, 1.12 mmol). The reaction was stirred under N_2 for 24 h. The solvent was removed in vacuo, and the residue was chromatographed on a 1.5- × 45-cm flash column using EtOAc/hexane (3:1) as the eluting solvent. The product was isolated as a colorless oil in a yield of 240 mg (63%): ^{1}H NMR δ 1.40–1.53 (m, 1), 1.64–1.90 (m, 5), 2.11–2.26 (m, 2), 2.94–3.03 (m, 1), 3.30–3.47 (m, 2), 3.70 (s, 3), 3.98 and 4.02 (dt, *J* = 12.0 and 4.3, 1), 5.07 (s, 2), 7.28–7.37 (m, 5); ^{13}C NMR δ 18.58, 23.53, 27.79, 31.15, 42.61, 43.46, 44.11, 52.03, 62.02, 67.40, 128.00, 128.08, 128.37, 136.12, 155.95, 169.20, 175.40; FAB MS *m/z* 361 [MH]$^+$. Anal. Calcd for $C_{19}H_{24}N_2O_5$: C, 63.32; H, 6.71; N, 7.77. Found: C, 63.49; H, 6.85; N, 7.66.

(*R*)- and (*S*)-1-[[1-(*tert*-Butoxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]-1,8-diaza-7-oxospiro[5.4]decane-8-acetamide (4 and 5). Spirolactam **11** (380 mg, 1.05 mmol) and NEt_3 (0.14 mL, 1.05 mmol) were dissolved in MeOH (10 mL) and deprotected via catalytic hydrogenation with 10% Pd/C at room temperature for 4 h. The catalyst was filtered off, and the solvent was removed in vacuo to give a tan oil. This material was dried in vacuo overnight and then dissolved in dry DMF (10 mL) along with Boc-Pro-OH (450 mg, 1.05 mmol), HOBt (540 mg, 4.0 mmol), and NEt_3 (0.14 mL, 1.05 mmol). The solution was stirred at room temperature under N_2, and to it was added a solution of DCC (210 mg, 1.0 mmol) in dry DMF (5 mL). This reaction 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, and the residue obtained was chromatographed on a 1.5- × 45-cm flash column using $CHCl_3$/MeOH (20:1) as the eluting solvent. The mixture of peptidomimetic esters (**14**) was treated with a saturated methanolic ammonia solution (10 mL). The reaction was stirred overnight, and then the excess NH_3 and MeOH were removed in vacuo. The residue that remained was chromatographed on a 1.5- × 45-cm flash column with EtOAc/MeOH (20:1) as the eluting solvent. The mixture of **4** and **5** was isolated as a colorless oil in a yield of 180 mg (42%). Fractional crystallization of this mixture from EtOAc yielded the (*S,S*)-diastereoisomer **5**. The filtrate, now enriched in **4**, was chromatographed on a 1.5- × 45-cm gravity column with EtOAc/MeOH (20:1) as the eluting solvent. Slow elution allowed the separation of the (*S,R*)-diastereoisomer **4** from the small amount of **5** left in the filtrate after the initial crystallization. The (*S,R*)-diastereoisomer **4** isolated from this chromatographic procedure was crystallized from EtOAc.

(*S,R*)-Diastereoisomer 4: mp 179–181 °C; $[\alpha]_D$ −11.7° (*c* 0.95, MeOH); TLC R_f ($CHCl_3$/MeOH (20:1)) = 0.22; ^{1}H NMR δ 1.39 and 1.42 (s, 9), 1.50–1.70 (m, 2), 1.70–2.00 (m, 7), 2.00–2.38 (m, 3), 3.21–3.46 (m, 4), 3.31 (d, *J* = 16.8, 1), 3.51–3.61 (m, 1), 3.70–3.89 (m, 1), 4.58 (d, *J* = 17.1, 1), 4.54–4.63 (m, 1), 5.34 (br s, 1), 7.91 and 8.02 (br s, 1); ^{13}C NMR δ 17.15 (17.78), 23.12 (23.25), 23.41 (24.30), 28.39 (28.42), 28.58 (28.83), 29.73 (30.49), 30.93 (31.61), 42.59 (43.43), 43.82 (44.01), 46.50 (46.60), 46.81, 56.92 (57.31), 62.06 (62.23), 79.31 (79.42), 154.69, 170.92 (171.33), 173.80, 174.30; FAB MS *m/z* 409 [MH]$^+$. Anal. Calcd for $C_{20}H_{32}N_4O_5$: C, 58.80; H, 7.90; N, 13.72. Found: C, 58.79; H, 7.74; N, 13.61.

(*S,S*)-Diastereoisomer 5: mp 238–239 °C; $[\alpha]_D$ −129.6° (*c* 0.50, MeOH); TLC R_f ($CHCl_3$/MeOH (20:1)) = 0.15; ^{1}H NMR δ 1.43 and 1.45 (s, 9), 1.50–2.00 (m, 9), 2.00–2.23 (m, 3), 3.19–3.31 (m, 2), 3.30 (d, *J* = 16.8, 1), 3.49 (t, *J* = 6.8, 2), 3.59 (dt, *J* = 9.6 and 3.2, 1), 3.70–3.80 (m, 1), 4.56 (d, *J* = 17.1, 1), 4.54–4.57 (m, 1), 5.32 (br s, 1), 7.91 (br s, 1); ^{13}C NMR δ 16.88, 23.03 (23.32), 24.90 (25.56), 27.86, 29.30 (29.76), 31.54, 33.92, 42.15, 44.01, 46.34, 46.69, 57.65, 61.73, 80.34, 153.99, 171.04, 172.94, 173.38; FAB MS *m/z* 409 [MH]$^+$. Anal. Calcd for $C_{20}H_{32}N_4O_5$: C, 58.80; H, 7.90; N, 13.72. Found: C, 58.98; H, 7.90; N, 13.52.

X-ray Analysis. All measurements were made at −101 °C on an Enraf-Nonius CAD-4 diffractometer with graphite-monochromated Mo K_α radiation. All calculations were performed using the TEXSAN[13] crystallographic software package of Molecular Structure Corporation.

Molecular Structure of 4. Data Collection. A colorless prism of **4** ($C_{20}H_{32}N_4O_5$) was obtained from EtOAc at room temperature by slow evaporation. The crystal (0.3 × 0.5 × 0.6 mm) was mounted on a glass fiber.

Cell constants and an orientation matrix for data collection, obtained from a least-squares refinement using the setting angles

(13) TEXAN-TEXRAY Structure Analysis Package, Molecular Structure Corporation, 1985.

of 22 reflections in the range $18.00 < 2\theta < 42.00°$ correspond to an orthorhombic cell with the following dimensions: $a = 9.661$ (4) Å; $b = 10.042$ (3) Å; $c = 22.115$ (4) Å; $V = 2146$ (2) Å^3. For $Z = 4$ and FW = 408.50, the calculated density is 1.264 g/cm^3. On the basis of the systematic absences of $h00$: $h \neq 2n$, $0k0$: $k \neq 2n$, 001: $l \neq 2n$, the space group was determined to be $P2_12_12_1$ (#19).

The data were collected at −101 ± 1 °C using the ω–2θ scan technique to a maximum 2θ value of 59.9°. Scans of (0.80 + 0.35 tan θ)° were made at speeds ranging from 16.5 to 1.1°/min (in ω).

Data Reduction. Of the 6742 reflections which were collected, 3548 were unique (R_{int} = 0.042); equivalent reflections were merged. The intensities of three representative reflections, measured every 60 min, remained constant throughout data collection and no decay correction was necessary.

The linear absorption coefficient for Mo K_α is 0.9 cm^{-1}. Azithumal scans of several reflections indicated no need for an absorption correction. The data were corrected for Lorentz and polarization effects. A correction for secondary excitation was applied (coefficient = 0.841 22 × 10^{-6}).

Structure Solution and Refinement. The structure was solved by direct methods. The non-hydrogen atoms were refined anisotropically. The final cycle of full-matrix least-squares refinement was based on 2841 observed reflections ($I > 2.00\sigma(I)$) and 276 variable parameters and converged (largest parameter shift was 0.01 times its esd) with unweighted and weighted agreement factors of: $R = \sum||F_o|-|F_c||/\sum|F_o| = 0.040$; $R_w = [(\sum w(|F_o| - |F_c|)^2/\sum wF_o^2)]^{1/2} = 0.047$.

The standard deviation of an observation of unit weight was 1.10. The weighting scheme was based on counting statistics and included a factor (p = 0.05) to downweight the intense reflections. The maximum and minimum peaks on the final difference Fourier map correspond to 0.25 and −0.18 e$^-$/Å^3, respectively.

Molecular Structure of 5. Data Collection. A colorless prism of **5** ($C_{20}H_{32}N_4O_5$) was obtained from EtOAc at room temperature by slow evaporation. The crystal (0.4 × 0.4 × 0.3 mm) was mounted on a glass fiber.

Cell constants and an orientation matrix for data collection, obtained from a least-squares refinement using the setting angles of 22 reflections in the range $28.00 < 2\theta < 50.00°$, correspond to a monoclinic cell with the following dimensions: $a = 8.786$ (5) Å; $b = 10.127$ (2) Å; $c = 12.548$ (6) Å; $V = 1093$ (2) Å^3; $\beta = 101.69°$. For $Z = 2$ and FW = 408.50, the calculated density is 1.241 g/cm^3. On the basis of the systematic absences of $0k0$: $k \neq 2n$, the space group was determined to be $P2_1$ (#4).

The data were collected at −101 ± 1 °C using the ω–2θ scan technique to a maximum 2θ value of 59.9°. Scans of (0.80 + 0.35 tan θ)° were made at speeds ranging from 1.5 to 16.5°/min (in ω).

Data Reduction. Of the 5529 reflections which were collected, 3355 were unique (R_{int} = 0.041); equivalent reflections were averaged. The intensities of three representative reflections, measured every 50 min, remained constant throughout data collection and no decay correction was necessary.

The linear absorption coefficient for Mo K_α is 0.8 cm^{-1}. Azithumal scans of several reflections indicated no need for an absorption correction. The data were corrected for Lorentz and polarization effects. A correction for secondary excitation was applied (coefficient = 0.222 90 × 10^{-6}).

Structure Solution and Refinement. The structure was solved by direct methods. The non-hydrogen atoms were refined anisotropically. The final cycle of full-matrix least-squares refinement was based on 2861 observed reflections ($I > 2.00\sigma(I)$) and 274 variable parameters and converged (largest parameter shift was 0.01 times its esd) with unweighted and weighted agreement factors of: $R = \sum||F_o|-|F_c||/\sum|F_o| = 0.042$; $R_w = [(\sum w(|F_o| - |F_c|)^2/\sum wF_o^2)]^{1/2} = 0.051$.

The standard deviation of an observation of unit weight was 1.28. The weighting scheme was based on counting statistics and included a factor (p = 0.05) to downweight the intense reflections. The maximum and minimum peaks on the final difference Fourier map correspond to 0.046 and −0.20 e$^-$/Å^3, respectively.

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