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Synthesis and Conformational Studies of a β-Turn Mimetic Incorporated in Leu-enkephalin

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A β-turn mimetic in which the four amino acids of a β-turn have been replaced by a 10-membered ring has been designed, synthesized, and subjected to conformational studies. In the mimetic, the intramolecular $CO_i - HN_{i+3}$ hydrogen bond that is often found in β-turns has been replaced by an ethylene bridge. In addition, the amide bond between residues *i* and *i* + 1 was exchanged for a methylene ether isoster. Such a β-turn mimetic, based on the first four residues of Leu-enkephalin (*Tyr-Gly-Gly-Phe*-Leu), was prepared in 15 steps. The synthesis relied on a β-azido alcohol prepared in five steps from Cbz-Tyr(tBu)-OH as a key, *i*-position building block. *tert*-Butyl bromoacetate, glycine, and a Phe-Leu dipetide were then used as building blocks for positions *i* + 1, *i* + 2, and *i* + 3, respectively. Conformational studies based on ^{1}H NMR data showed that the β-turn mimetic was flexible, but that it resembled a type-II β-turn at low temperature. This low energy conformer closely resembled the structure determined for crystalline Leu-enkephalin.

Introduction

Turns constitute an important class of polypeptide secondary structure, defined as regions where a peptide chain reverses its overall direction.[1,2] When chain reversal occurs over four residues in such a way that the carbonyl oxygen atom of the first residue (*i*) and the amide NH proton of the fourth residue (*i* + 3) come close in space a β-turn is formed. In most cases, this involves formation of an intramolecular hydrogen bond between residues *i* and *i* + 3 to give a pseudo-ten-membered ring. Different β-turns are distinguished by the values of the Φ and Ψ torsional angles for the *i* + 1 and *i* + 2 residues[1,3] or by the topology of the side chains in the turn.[4]

β-Turns are often located at the surface of proteins where they can undergo posttranslational modification and serve as sites of recognition in interactions with receptors and antibodies.[1,2,5,6] Structural studies have also revealed that small peptides functioning as hormones, neurotransmitters, or having other regulatory roles in the organism may contain β-turn structural motifs. Examples include the hormones oxytocin,[7−10] which induces labor and milk production in mammals, vasopressin,[11,12] which regulates water readsorption in the kidneys, and LHRH,[13,14] which releases sex-specific hormones from the testes and ovaries. β-Turns have also been found for the endogenous morphine-like substance Leu-enkephalin.[15−17] In view of these, and other examples, it is not surprising that large efforts have been devoted to the design and synthesis of mimetics of β-turns.

Several requirements should be fulfilled by a successful β-turn mimetic. The conformation of the mimetic should of course resemble that of the peptide backbone in the β-turn. Because the side chains of biologically active peptides have crucial roles in receptor recognition it is

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essential that they can be introduced at desired positions in the mimetic with correct orientation. Turn mimetics, in which amide bonds have been modified, can also be expected to suffer less from the pharmacokinetic problems that affect peptide drug candidates, i.e., enzymatic degradation and low uptake.[18,19] Due to their rigid nature, mimetics are useful tools in investigating the bioactive conformation of peptides. Furthermore, since rigid analogues pay a lower entropy cost upon binding to a receptor they should be more potent and also more selective for a specific type of receptor.[20−22] It is, however, important to avoid over-rigidification resulting in compromised interactions between the ligand and the receptor.[23]

Many β-turn mimetics constitute replacements of residues $i + 1$ and $i + 2$ in β-turns by aromatic,[24,25] bicyclic,[26−32] or spirocyclic[33,34] systems (references are to selected recent publications). Such mimetics attempt to position their amino- and carboxyl-termini correctly so that a reversal of the peptide backbone is enforced when they are incorporated in a polypeptide. In general, this kind of mimetic does not allow introduction of side chains at the $i + 1$ and $i + 2$ positions. Reports of β-turn mimetics that provide close resemblance of the backbone of the turn, allow incorporation of side chains with correct topology, and also incorporation into a peptide chain, have appeared but are less frequent.[23] Herein we describe a novel type of β-turn mimetic that resembles the peptide backbone in a turn and allows ready incorporation into a peptide.[35] Introduction of different side chains is possible at the i, $i + 2$, and $i + 3$ positions, while side chains other than hydrogen and methyl at the $i + 1$ position will require larger synthetic efforts. The approach is exemplified by incorporation of a β-turn mimetic in place of the first four residues of Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu). Several mimetics of Leu-enkephalin have been synthesized earlier by others including cyclized analogues,[36] a mimetic containing a conformationally

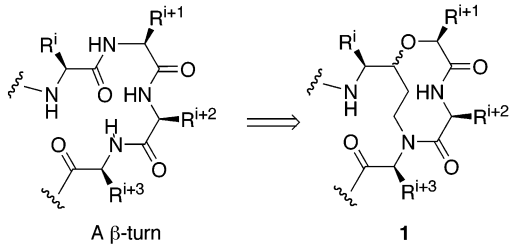
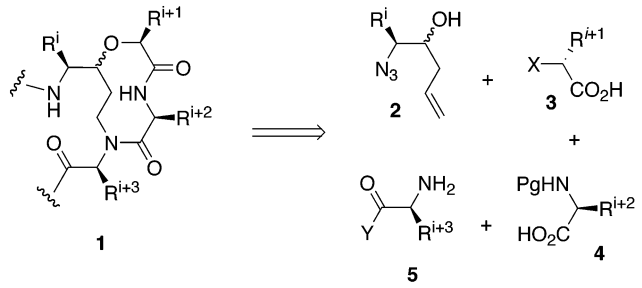


FIGURE 1. Transformation of a β-turn into conformationally restricted mimetic **1**.

SCHEME 1. Retrosynthetic Analysis Reveals That *β*-Turn Mimetic 1 Can Be Prepared from *β*-Azido Alcohols 2, α-Bromo Acids 3, Amino Acids 4, and Amino Acid Derivatives 5



restricted tyrosine analogue,[37] and those containing β-turn mimetics.[38] Conformational studies and biological evaluation of these mimetics have not produced a definite picture of the bioactive conformation of Leu-enkephalin, but the biological relevance of the β-turn observed in the crystal structure of Leu-enkephalin[15] has been questioned.[36,38]

Results and Discussion

Design of the *β*-Turn Mimetic. As discussed above, β-turns are often stabilized by an intramolecular hydrogen bond between the carbonyl group of residue i and the amino group of residue $i + 3$. The distance between the carbonyl carbon atom of residue i and the nitrogen atom of the amino group of residue $i + 3$ falls between 3.5 and 3.8 Å in type-I, -II, and -III β-turns. This indicates that the intramolecular hydrogen bond found in these turns could be replaced by an ethylene bridge. Simultaneous replacement of the amide bond between residues i and $i + 1$ with a methylene ether isostere suggested that the 10-membered ring in **1** could serve as a novel β-turn mimetic (Figure 1). Replacement of the amide bond with a methylene ether isostere generates a new stereogenic center, the configuration of which should influence the conformation of the turn mimetic.

A retrosynthetic analysis revealed that azido alcohols **2**, α-halo acids **3**, protected amino acids **4**, and peptides (or amino acids) **5** constitute suitable building blocks for synthesis of β-turn mimetic **1** (Scheme 1). The azido group in **2** serves as a precursor for the amino group of

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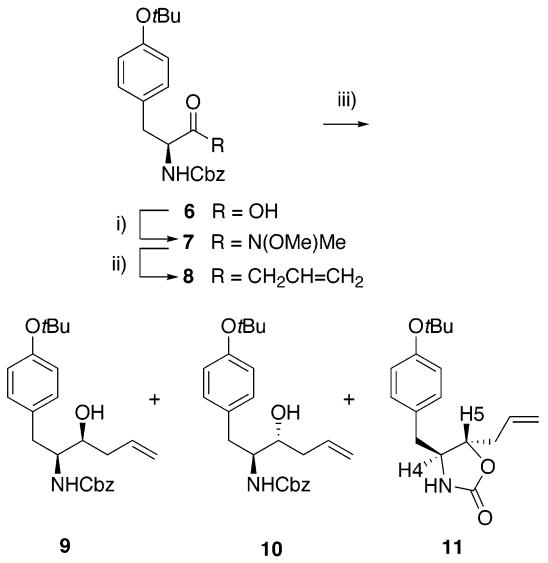
SCHEME 2[a]



[a] Key: (i) *N*-methylmorpholine, isobutyl chloroformate, *N*,*O*-dimethylhydroxylamine hydrochloride, CH_2Cl_2, −15 °C to rt, 81%; (ii) allylmagnesium bromide, THF −78 °C, 74%; (iii) K-Selectride, THF, −78 °C, **9** 36%, **10** 8%, **11** 14%.

the first residue of the turn, while the alkene serves as a masked aldehyde to be used in a reductive amination with **5**. Alkylation of the hydroxyl group in **2** with α-halo acids **3** links the residues found at position *i* and *i* + 1 of the turn to each other. This step should be facile for derivatives of α-halo acetic and propionic acid,[39] i.e., when glycine and alanine are found at the *i* + 1 position of the *β*-turn, but can be expected to be more complex when other amino acids are found in the *i* + 1 position. Incorporation of the *i* + 2 residue requires formation of two amide bonds. The overall success of this approach to *β*-turn mimetics relies on a route that provides ready access to building block **2** in enantiomerically pure form, as well as on procedures for linking the four building blocks to each other. The first four residues of Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu) have been shown to adopt a *β*-turn both in the crystal and in solution,[15−17] and incorporation of a *β*-turn mimetic in Leu-enkephalin was therefore chosen as our first synthetic target. This requires preparation of an azido alcohol in which R^i contains a protected phenol (cf. **13**, Scheme 3), for instance from a derivative of tyrosine. The remaining building blocks would be derivatives of α-bromo acetic acid, glycine, and the dipeptide Phe-Leu, respectively.

Synthesis of a *β*-Turn Mimetic and Its Incorporation in Leu-enkephalin. The synthesis started by conversion of protected tyrosine **6** to the corresponding Weinreb amide **7** by activation using isobutyl chloroformate and *N*-methylmorpholine followed by treatment with *N*,*O*-dimethylhydroxylamine hydrochloride (Scheme 2).[40] Treatment of **7** with allylmagnesium bromide at −78 °C afforded allyl ketone **8** in 68% yield over two steps.[41] Ketone **8** was then reduced with K-Selectride[42,43] at −78 °C, which furnished allylic syn and anti amino alcohols **9** and **10** together with oxazolidinone **11**. The latter was assumed to be formed from syn alcohol **9** by cyclization. Formation of the corresponding, diastereomeric oxazolidinone from **10** was not observed under these reaction conditions. The diastereomeric amino alcohols **9** and **10**, and oxazolidinone **11**, were separated by careful flash chromatography providing the three compounds in 36, 8, and 14% yields, respectively. Thus, the overall syn/anti ratio ((**9** + **11**)/**10**) obtained in the reduction of ketone **8** was >6:1. The stereochemistry at the newly formed stereogenic center of **9** and **10** was determined by converting them to the corresponding oxazolidinones by using aqueous potassium hydroxide in a mixture of methanol and THF (1:2:4) at room temperature. Under these conditions, syn amino alcohol **9** gave **11**, whereas anti amino alcohol **10** gave the diastereomeric oxazolidinone. Irradiation of H-4 in the oxazolidinone ring of **11** gave a 5% NOE for H-5, whereas a 14% enhancement of H-5 was obtained for the diastereomeric oxazolidinone, thereby establishing that H-4 and H-5 in **11** had an anti orientation. When reduction of **8** was performed with DIBAL or $Zn(BH_4)_2$, i.e., with reducing agents which contain chelating metals, anti amino alcohol **10** was instead obtained as the main product. Best results were obtained with $Zn(BH_4)_2$ which gave **10** in 64% yield together with 25% of **9**. No oxazolidinones were formed when using these two reducing agents. A route to **9** and **10** based on addition of allyl zinc bromide to an aldehyde corresponding to Weinreb amide **7** was investigated previously but was then found to be inferior to the above results.[35]

Removal of the carbamate protective group from the amino functionality of allylic alcohol **9** was accomplished by refluxing in a 1:1 mixture of aqueous potassium hydroxide and ethanol to give amino alcohol **12** in 80% yield (Scheme 3). Amino alcohol **12** was also prepared in 75% yield starting from oxazolidinone **11** using the same conditions. Because **11** is formed from **9** under basic conditions, oxazolidinone **11** is most likely a common reaction intermediate in the deprotection of **9**. The amino group of **12** was converted to an azido group by treatment with triflyl azide under copper sulfate catalysis in a biphasic system.[44,45] This reaction is known to proceed with retention of configuration,[44] and azide **13** was obtained in 85% yield. The hydroxyl group of **13** was alkylated under phase-transfer conditions[46] using *tert*-butyl bromoacetate to give compound **14**, which contained tyrosine and glycine equivalents at the *i* and *i* + 1 turn positions. Introduction of the azido functionality proved to be essential for a successful alkylation of syn-configurated **13**. When alkylation of Cbz-protected amino alcohol **9** was attempted under the same conditions as for **13** an intramolecular reaction involving the Cbz group occurred to give oxazolidinone **11** as the major product. Alkylated azido alcohol **14** was found to be thermally sensitive.

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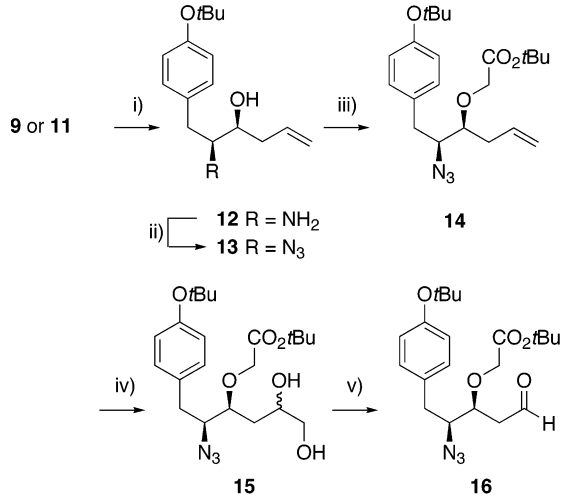
SCHEME 3[a]

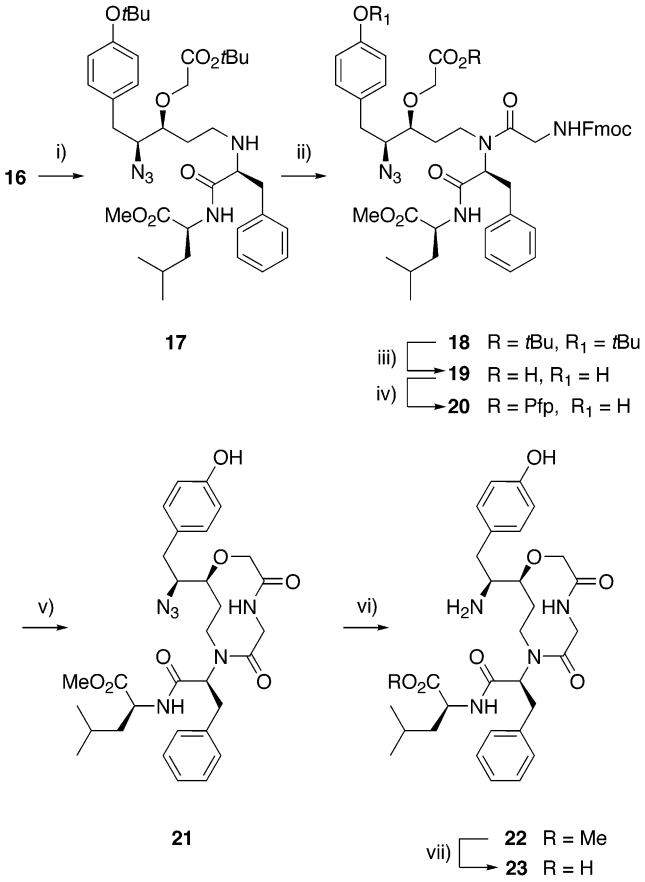


[a] Key: (i) EtOH/KOH (1 M), reflux, 81%; (ii) NaN_3, Tf_2O, H_2O, CH_2Cl_2, $CuSO_4$, DMAP, 0 °C to rt, 85%; (iii) *tert*-butyl α-bromoacetate, $QHSO_4$, 50% NaOH (aq), benzene; (iv) *N*-methylmorpholine oxide, OsO_4, H_2O, acetone, THF, 70% from **13**; (v) Na_2CO_3, $Pb(OAc)_4$, benzene, 85%.

Therefore, the solvents were removed at 0 °C after workup and the product was used without any further purification in the following oxidation step. Attempted alkylation of the anti-configurated azido alcohol generated from **10** resulted in decomposition and the diastereomer of **14** could therefore not be prepared. The alkene moiety in **14** was transformed to a diol giving **15** (70% from **13**) using catalytic amounts of osmium tetraoxide with *N*-methylmorpholine *N*-oxide as co-oxidant. Diol **15** was then cleaved by lead tetraacetate to give aldehyde **16** (85%). Somewhat surprisingly, attempts to generate aldehyde **16** directly from alkene **14** by ozonolytic cleavage failed.

Reductive alkylation[47] of the amino group of the dipeptide H-Phe-Leu-OMe[48] with aldehyde **16** using sodium triacetoxyborohydride as reducing agent gave secondary amine **17** in 82% yield (Scheme 4). In this way the C-terminal leucine, and also the phenylalanine at position $i + 3$ of the β-turn mimetic, were connected to building block **16** containing the *i* position tyrosine and the $i + 1$ glycine of the desired Leu-enkephalin mimetic. Introduction of glycine at the $i + 2$ position was achieved by amide bond formation between Fmoc-protected glycine and the secondary amine in **17**. Coupling using diisopropyl carbodiimide as activating agent gave the desired amide **18** in moderate yields. However, replacing diisopropyl carbodiimide with the more potent HATU[49,50] furnished tertiary amide **18** in 85% yield. The *tert*-butyl ester and the phenolic *tert*-butyl ether of **18** were cleaved simultaneously, without affecting the methyl ester, by treatment with formic acid to afford **19** in almost quantitative yield. Activation of the carboxylic acid in **19**, to give pentafluorophenyl ester[51] **20**, followed by treatment with DBU in refluxing dioxane under high dilution conditions gave the desired cyclized product **21** in 56% yield. Attempts to achieve this critical macrolactamisation starting with Fmoc-cleavage from **19**, followed by intramolecular formation of the amide bond promoted by coupling reagents as HATU or DIC, proved to be less successful. Finally, the N- and C-termini of **21** were deprotected in two steps. First the azide was reduced[52] using tin chloride, thiophenol and triethylamine to give amine **22**. Then the methyl ester in **22** was hydrolyzed with lithium hydroxide which gave the target β-turn mimetic **23** (57% yield from **21**). Mimetic **23** was thus prepared with an overall yield of 3.2% over the 15-step reaction sequence.

SCHEME 4[a]



[a] Key: (i) H-Phe-Leu-OMe, $NaBH(OAc)_3$, NEt_3, $ClCH_2CH_2Cl$, 82%; (ii) Fmoc-Gly-OH, HATU, DIEA, DMF, 85%; (iii) formic acid, 99%; (iv) PfpOH, DCC, EtOAc, 0 °C; (v) DBU, dioxane, reflux, 56% from **19**; (vi) $SnCl_2$, PhSH, TEA, THF; (vii) LiOH (0.1 M), THF, 57% from **21**.

Conformational Studies. A previous structure determination of a γ-turn mimetic incorporated in the peptide drug desmopressin, performed in our laboratory, was complicated by the flexibility and size of the peptidomimetic.[53] Attempted structural determination of Leu-enkephalin **23** turned out to be complex for the same

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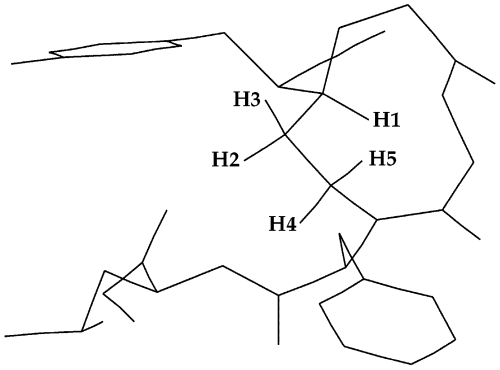
TABLE 1. ^{1}H NMR Chemical Shifts (δ, ppm) of Compound 21 in MeOH-d_3 at −70 °C



residue	NH	CαH	Cβ H	Cγ H	other
Tyr1		4.01	2.49, 2.96		7.18 (Hδ), 6.78 (Hϵ)
Gly2[a]		4.51[b]			
Gly3	8.86	3.52, 4.75			
Phe4		4.30	3.46[b]		7.28 (Hδ), 7.38 (Hϵ)
Leu5	7.32	4.69	1.65, 1.74	1.63	0.98[c] (Hδ)
bridge	H1	H2	H3	H4	H5
	3.95	2.15	1.70	2.41	3.77

[a] Refers to the methylene group corresponding to Gly2 Hα in Leu-enkephalin. [b] Degeneracy has been assumed.

reasons. Several different solvents and temperatures were explored to find suitable conditions for NMR-based structure determination of **23**, i.e., aqueous solution, mixtures of water with DMSO-d_6 (1:1) or TFE-d_3 (1:1), as well as MeOH-d_4. Although the spectrum of **23** could be assigned in aqueous solution to confirm the structure, spectral data of sufficient quality for structure determination was not obtained in any of the solvents. Broad peaks and cross-peaks with both positive and negative signs in the NOESY spectrum indicated slow to intermediate exchange rates between several conformers. This conformational mobility may be advantageous from a molecular recognition perspective since it allows some flexibility for the mimetic in receptor interactions.

Our attention then shifted to the protected mimetic **21**, since it should be able to serve as a model for the conformational preferences of the turn mimetic part which is common to **21** and **23**. We reasoned that the fact that the N- and C-termini of **21** were protected, should have no or only minor influence on the low-energy conformations of the 10-membered ring that constitutes the β-turn mimetic. Fortunately, the dynamic properties of **21** were favorable in MeOH-d_3 at −70 °C, and spectra with sufficient quality could be acquired to assign the resonances of **21** (Table 1) and extract distance restraints. By inspection of the NOE-buildup curves when mixing times ranging from 30 to 250 ms were used, it was apparent that many of the observed cross-peaks originated from spin-diffusion[54] due to the slow tumbling rate of **21**. To avoid problems with spin-diffusion, all distance restraints were extracted from the NOESY spectrum obtained with a mixing time of 30 ms. In total, 29 distance restraints for the structure calculation were derived and classified as short or long distances with 4 or 5 Å as the upper distance limit, respectively (Table 2). Simulated annealing with the X-PLOR[55] force field resulted in an ensemble of low-energy structures with a well defined backbone conformation, especially in the part of the molecule which contains the β-turn mimetic (Figure 2). The average rmsd for all heavy atoms was 2.64 Å but for the backbone of the turn mimetic it was as low as 0.23 Å. Since relatively few distance restraints were used in the structure calculation, unrestrained simulated annealing was performed. This confirmed that the extracted distance restraints provided the necessary information to find the observed low-energy conformations of **21**.

TABLE 2. Distance Restraints Used in the Structure Calculation of Compound 21

proton pair[a,b]	distance restraint[c,d] (Å)
Gly3 HN-Gly3 Hα1	2.36
Gly3 HN-Gly3 Hα2	2.98
Leu5 HN-H2	2.70
Leu5 HN-Leu5 Hβ1	2.91
Leu5 HN-Leu5 Hβ2	2.82
Phe4 Hδ-Phe4 Hβ	2.31
Tyr1 Hδ-Tyr1 Hα	3.16
Tyr1 Hδ-Tyr1 Hβ1	2.84
Tyr1 Hδ-Tyr1 Hβ2	2.58
H1-Gly2 Hα	2.19
H1-Gly3 Hα2	2.22
H5-Gly3 Hα2	2.03
Phe4 Hβ-Phe4 Hα	2.38
H4-Gly3 Hα2	2.72
H4-Phe4 Hα	2.53
H2-Phe4 Hα	2.66
Leu5 Hγ-Leu5 Hα	2.69
Leu 5 Hδ-Leu5 Hα	2.71
H2-Tyr1 Hβ1	2.81
H3-Tyr1 Hβ1	3.41
H1-Tyr1 Hβ2	2.80
H1-H2	3.19
H1-H3	2.63
Tyr1 Hα-H3	3.16
Tyr1 Hα-H2	2.93
Tyr1 Hα-Tyr1 Hβ2	2.92
H2-Tyr1 Hβ2	2.75
H2-H4	2.48
H3-H4	2.26

[a] Hydrogen atoms H1−H5 are defined in Table 1. [b] The hydrogen atoms in the ethylene bridge (H2−H5) were also treated as pseudoatoms in the structure calculations. This resulted in a similar overall conformation although a slightly larger flexibility was observed in the part of the mimetic containing the bridge. [c] Determined with the Gly3 Hα1-Hα2 cross-peak volume as reference, and a Gly3 Hα1-Hα2 reference distance of 1.772 Å. [d] In the structure calculations the upper limit was set to 4 Å for distances determined to be below 3 Å. For distances determined to be above 3 Å, the upper limit was set to 5 Å.

To find out how well the 10-membered ring of **21** mimics a β-turn, we superimposed the β-turn mimetic part in the low energy conformers with different idealized β-turns. This revealed that the backbone of the β-turn in **21**, and the position of the $i + 2$ side chain, superimposes well with an idealized type-II β-turn (Figure 3). The backbone rmsd from C_α of residue i to C_α of residue $i + 3$ is only 0.43 Å for the ensemble of low energy conformers. The only larger discrepancy is the orientation of the $i + 1$ side chain that differs approximately 60°

(54) Spin-diffusion refers to transfer of magnetization through alternative pathways in a network of nuclei in close proximity to each other and reveals itself as delayed NOEs.

(55) Brünger, A. T. *X-PLOR, Version 3.1. A system for X-ray crystallography and NMR*; Yale Universtity Press: New Haven, CT, 1992.

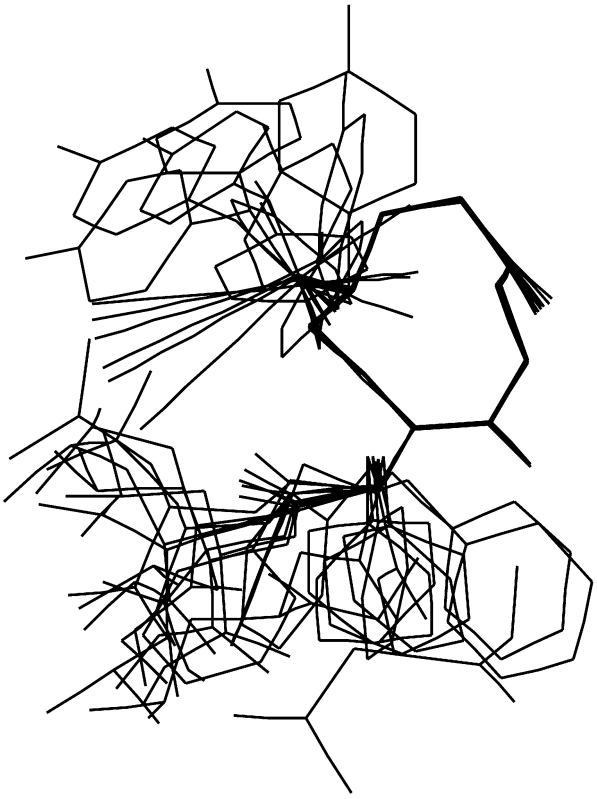


FIGURE 2. Superimposition of the 10 lowest energy conformers of Leu-enkephalin mimetic **21**. The backbone atoms for residues $i \rightarrow i + 2$ were used for the superimposition.

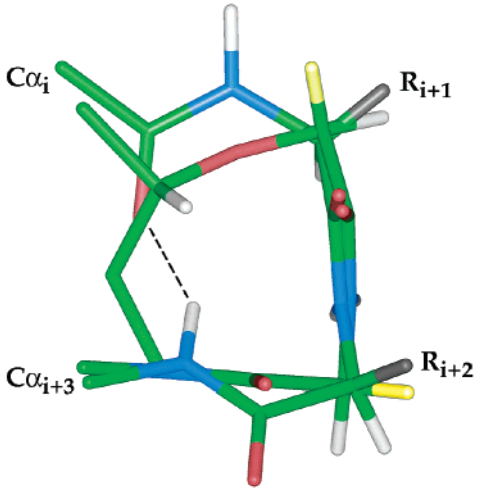


FIGURE 3. Superimposition of the β-turn mimetic part from the lowest energy conformer of **21** with a tetrapeptide oriented as an idealized type-II β-turn. Backbone atoms from Tyr1 Cα to Phe4 Cα in **2** were used for the superimposition. The dashed line indicates the hydrogen-bond found in the ideal β-turn. The location of the side-chains of residues $i + 1$ and $i + 2$ in the tetrapeptide are marked in gray. Positions for side chains at these positions in mimetic **21** are marked in light yellow.

between **21** and a type-II β-turn. Since **21** has a glycine in this position, this deviation should not be critical for the receptor interactions of the present Leu-enkephalin mimetic. If a properly oriented side-chain is required in the $i + 1$ position, incorporation of a D-amino acid equivalent should provide a better agreement with the i

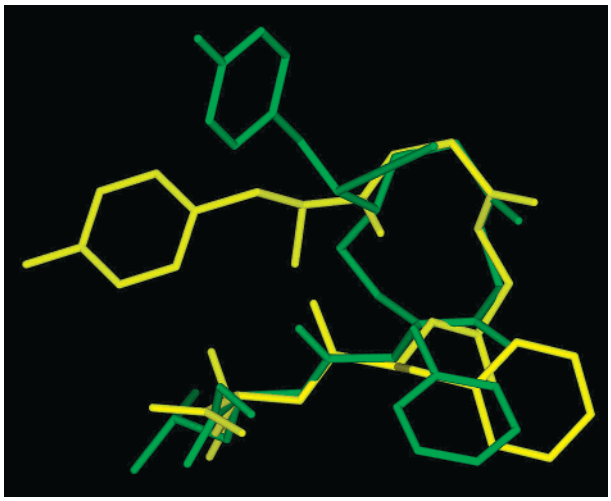


FIGURE 4. Superimposition of the structure of crystalline Leu-enkephalin (yellow) and one of the 10 lowest energy conformers of **21** (green). The conformer of **21** having the lowest rmsd value (0.37 Å) when compared to crystalline Leu-enkephalin was chosen for the superimposition.

$+ 1$ side chain of a type-II β-turn. The conformation of the β-turn mimetic in **21** also coincides well with the β-turn found in the crystal structure of Leu-enkephalin (Figure 4).[15] The average rmsd for the heavy atoms in the turn is 0.55 Å for the ensemble of conformers for **21** compared to the structure of crystalline Leu-enkephalin. The rmsd for the whole backbone when comparing **21** and Leu-enkephalin ranges from 0.61 to 1.68 Å, a value that is strongly correlated to the orientation about the flexible Phe φ-angle.

Conclusions

A novel mimetic of a β-turn has been designed. In the mimetic, the intramolecular hydrogen bond that is often found between residues i and $i + 3$ of a β-turn has been replaced by an ethylene bridge to give a 10-membered ring. In addition, the amide bond between residues i and $i + 1$ was exchanged for a methylene ether isoster. A synthetic route to such a β-turn mimetic corresponding to the first four residues of Leu-enkephalin was developed based on assembly of building blocks. The key, i-position building block was prepared from Cbz-Tyr(tBu)-OH in five steps and contained a β-azido alcohol moiety as well as an ethylene bridge equivalent. Assembly of the β-turn mimetic then begun by alkylation of the hydroxyl group of the i position tyrosine building block with *tert*-butyl bromoacetate thus incorporating a glycine equivalent at the $i + 1$ position. The $i + 3$ phenylalanine in the turn, together with the C-terminal leucine of Leu-enkephalin, were incorporated by reductive amination. Finally, glycine at position $i + 2$ of the β-turn mimetic was introduced by formation of two amide bonds, one of which closed the 10-membered ring. It is envisioned that this approach to β-turn mimetics should allow facile incorporation of different side chains at positions i, $i + 2$, and $i + 3$ of a β-turn. However, it is likely that, in its present form, the approach will be restricted to incorporation of glycine and alanine equivalents at the $i + 1$ position.[39] Conformational studies showed that the β-turn mimetic

was flexible when incorporated in Leu-enkaphlin but that a type-II β-turn was adopted in methanol-d_3 at low temperature. This low energy conformer closely resembled the structure found for crystalline Leu-enkephalin. It is suggested that the inherent flexibilty of this type of β-turn mimetics could be an advantage in ligand−receptor interactions.

Experimental Section

[(*S*)-2-(4-*tert*-Butoxyphenyl)-1-(methoxymethylcarbamoyl)ethyl]carbamic Acid Benzyl Ester (7). Cbz-Tyr(tBu)-OH*DCHA (**6**, 5.00 g, 9.05 mmol) was dissolved in EtOAc (250 mL), and 10% aqueous citric acid (150 mL) was added. The mixture was stirred for 30 min at room temperature. The two phases were separated, and the aqueous phase was extracted with EtOAc. The combined organic phases were washed with 10% aqueous citric acid and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2 (72 mL) and cooled to −20 °C. *N*-Methylmorpholine (2.50 mL, 22.6 mmol) was added followed by slow addition of isobutyl chloroformate (1.67 mL, 11.3 mmol), and the reaction was stirred for 15 min. *N*,*O*-Dimethylhydroxylamine HCl (1.10 g, 11.3 mmol) was added in one portion,[40] and after 15 min the cooling bath was removed and the reaction was stirred for another 3 h at room temperature. The reaction was quenched with water and the phases were separated. The aqueous phase was extracted with CH_2Cl_2 and the combined organic layers were washed with satd. aqueous $NaHCO_3$, brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (heptane/ethyl acetate 3:1) to give **7** (3.18 g, 85%) as a colorless oil: $[\alpha]^{20}_D$ = +2.4 (c = 0.25 in $CHCl_3$); IR (neat) 1716, 1658, 1506 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.34−7.31 (m, 5H), 7.04 (d, J = 8 Hz, 2H), 6.89 (d, J = 8 Hz, 2H), 5.39 (d, J = 9 Hz, 1H), 5.08 (d, J = 12 Hz, 1H), 5.02 (d, J = 12 Hz, 1H), 5.02−4.93 (m, 1H), 3.62 (s, 3H), 3.13 (s, 3H), 3.01 (dd, J = 6.4 and 6.5 Hz, 1H), 2.87 (dd, J = 6.4 and 6.5 Hz, 1H), 1.31 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 171.9, 155.6, 154.1, 136.3, 131.1, 129.7, 128.3, 127.9, 127.8, 124.0, 78.1, 66.6, 61.3, 52.0, 38.0, 31.8, 28.7; HRMS (FAB) calcd for $C_{23}H_{30}N_2O$ (M + H) 415.2233, found 415.2244.

[(*S*)-1-(4-*tert*-Butoxybenzyl)-2-oxopent-4-enyl]carbamic Acid Benzyl Ester (8). Weinreb amide **7** (5.69 g, 13.7 mmol) was dissolved in THF (100 mL) and cooled to −78 °C. Allylmagnesium bromide (41 mL, 41.0 mmol, 1 M solution in THF) was added slowly,[41] and after complete addition stirring was continued for 4 h at −78 °C. The reaction was quenched with saturated aqueous NH_4Cl followed by extraction with CH_2Cl_2. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography (heptane/ethyl acetate 15:1 → 6:1) to give **8** (4.34 g, 80%) as a white solid: $[\alpha]^{20}_D$ = +64.7 (c = 0.6 in $CHCl_3$); IR (neat) 1707, 1504 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.38−7.27 (m, 5H), 7.02 (d, J = 8 Hz, 2H), 6.80 (d, J = 8 Hz, 2H), 5.82 (m, 1H), 5.38 (d, J = 7.5 Hz), 5.16 (d, J = 10.2 Hz, 1H), 5.08 (s, 2H), 5.04 (d, J = 16.2 Hz, 1H), 4.62 (dd, J = 7.3 and 6.8 Hz, 1H), 3.15 (dd, J = 6.8 and 17.5 Hz, 1H), 3.05 (dd, J = 6.8 and 17.5 Hz, 1H), 3.00 (d, J = 6.6 Hz, 2H), 1.31 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 206.7, 155.6, 154.3, 136.1, 130.5, 129.6, 129.4, 128.4, 128.0, 127.9, 124.2, 119.2, 78.3, 66.8, 60.0, 45.5, 37.0, 28.7; HRMS (FAB) calcd for $C_{24}H_{29}NO_4$ (M + H) 396.2175, found 396.2188.

[(1*S*,2*S*)-1-(4-*tert*-Butoxybenzyl)-2-hydroxypent-4-enyl]carbamic Acid Benzyl Ester (9), [(1*S*,2*R*)-1-(4-*tert*-Butoxybenzyl)-2-hydroxypent-4-enyl]carbamic Acid Benzyl Ester (10), and (4*S*,5*S*)-5-Allyl-4-(4-*tert*-butoxybenzyl)-oxazolidin-2-one (11). Allyl ketone **8** (4.34 g, 11 mmol) was dissolved in THF (80 mL) and cooled to −78 °C. K-Selectride[42,43] (20 mL, 20 mmol, 1 M solution in THF) was added slowly, and the reaction was stirred for 4 h. Water and 10% aqueous citric acid were added to quench the reaction and adjust pH > 4. The aqueous phase was extracted with CH_2Cl_2. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography (heptane/ethyl acetate 10:1 → 2:1) to yield **9** (1.55 g, 36%) as a colorless oil, **10** (0.37 g, 8.5%) as a white solid, and **11** (0.45 g, 14%) as a colorless oil.

Compound **9**: $[\alpha]^{20}_D$ = −17.6 (c = 0.42 in $CHCl_3$); IR (neat) 3419, 3343, 1691 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.38−7.28 (m, 5H), 7.11 (d, J = 8.3 Hz, 2H), 6.90 (d, J = 8.3 Hz, 2H), 5.79−5.64 (m, 1H), 5.25 (d, J = 7.5 Hz 1H), 5.10−5.00 (m, 4H), 3.87−3.78 (m, 1H), 3.63−3.56 (m, 1H), 2.89 (dd, J = 13.7 and 7.4 Hz, 1H), 2.84 (dd, J = 13.7 and 8.1 Hz, 1H), 2.52 (sb, 1H), 2.24 (dd, J = 4.9 and 13.6 Hz, 1H), 2.19 (dd, J = 8.1 and 13.6 Hz, 1H), 1.32 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 156.4, 153.6, 136.4, 134.2, 133.0, 129.6, 128.4, 127.9, 127.8, 124.1, 118.2, 78.2, 69.6, 66.6, 55.6, 39.1, 38.0, 28.7; HRMS (FAB) calcd for $C_{24}H_{30}NO_4$ (M+H) 398.2331, found 398.2336. Anal. Calcd for $C_{24}H_{29}NO_4$: C, 72.52; H, 7.86; N, 3.52. Found: C, 72.83; H, 7.99; N, 3.46.

Compound **10**: $[\alpha]^{20}_D$ = −14.7 (c = 0.075 in $CHCl_3$); IR (neat) 3318, 1687, 1535, 1504 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.35−7.20 (m, 5H), 7.07 (d, J = 8.3 Hz, 2H), 6.90 (d, J = 8.3 Hz, 2H), 5.88−5.77 (m, 1H;), 5.24 (d, J = 7.5 Hz, 1H), 5.20−4.96 (m, 4H), 3.93−3.83 (m, 1H), 3.76−3.67 (m, 1H), 3.05 (sb, 1H), 2.92 (dd, J = 14.2 and 4.6 Hz, 1H), 2.77−2.66 (m, 1H) 2.36−2.18 (m, 2H), 1.32 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 156.2, 153.5, 136.3, 134.4, 132.8, 129.5, 128.3, 127.8, 127.7, 124.0, 117.9, 78.1, 72.4, 66.4, 56.1, 38.1, 34.5, 28.6; HRMS (FAB) calcd for $C_{24}H_{30}NO_4$ (M + H) 398.2331, found 398.2331. Anal. Calcd for $C_{24}H_{29}NO_4$: C, 72.52; H, 7.86; N, 3.52. Found: C, 72.38; H, 8.07; N, 3.60.

Compound **11**: $[\alpha]^{20}_D$ = −64.1 (c = 0.105 in $CHCl_3$); IR (neat) 1753, 1508 cm^{-1}, ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 6.99 (d, J = 8.5 Hz, 2H), 6.90 (d, J = 8.5 Hz, 2H), 6.62 (s, 1H), 5.57−5.44 (m, 1H), 5.00−4.91 (m, 2H), 4.25−4.19 (m, 1H), 3.64−3.58 (m, 1H; C*H*NH), 2.80 (dd, J = 13.5 and 6.1 Hz, 1H), 2.63 (dd, J = 13.5 and 7.5 Hz, 1H), 2.23−2.07 (m, 2H), 1.25 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 158.8, 154.0, 131.1, 130.4, 129.4, 124.2, 118.7, 80.1, 78.1, 57.7, 40.3, 38.2, 28.4; HRMS (FAB) calcd for $C_{17}H_{24}O_3N$ (M + H) 290.1756, found 290.1727.

(2*S*,3*S*)-2-Amino-1-(4-*tert*-butoxyphenyl)hex-5-en-3-ol (12). Amino alcohol **9** (1.47 g, 3.70 mmol) was dissolved in a mixture of 1 M aqueous KOH (30 mL) and ethanol (30 mL). The reaction was heated to reflux for 5 h. The solvent volume was reduced to ∼35 mL under reduced pressure. Water and CH_2Cl_2 were added, and the two phases were separated. The aqueous layer was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, concentrated under reduced pressure, and purified by flash chromatography (toluene/ethanol 4:1 → 2:1) to give **12** (0.78 g, 80%) as colorless oil. Compound **12** (0.26 g, 75%) was also prepared starting from oxazolidinone **11** (0.35 g, 1.2 mmol) following the procedure above: $[\alpha]^{20}_D$ = −29.9 (c = 0.107 in $CHCl_3$); IR (neat) 3357, 1504 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 6.96 (d, J = 8.4 Hz, 2H), 6.80 (d, J = 8.4 Hz, 2H), 5.82−5.71 (m, 1H), 5.03−4.91 (m, 2H), 3.39−3.32 (m, 1H), 2.81−2.68 (m, 2H), 2.41−2.11 (m, 6H), 1.21 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 153.3, 134.8, 133.5, 129.2, 123.8, 116.6, 77.7, 72.0, 55.7, 39.6, 38.6, 28.4; HRMS (FAB) calcd for $C_{16}H_{26}NO_2$ (M+H) 264.1964, found 264.1978. Anal. Calcd for $C_{16}H_{25}NO_2$: C, 72.97; H, 9.57; N, 5.32. Found: C, 73.17; H, 9.38; N, 5.28.

(2*S*,3*S*)-2-Azido-1-(4-*tert*-Butoxyphenyl)hex-5-en-3-ol (13). A solution of NaN_3 (5.00 g, 76.9 mmol) in H_2O (11.5 mL) was treated with CH_2Cl_2 (19 mL) at 0 °C. To this vigorously stirred solution was added triflic anhydride (2.60 mL, 15.4 mmol, freshly distilled from P_2O_5), and stirring was continued

for 2 h. The two phases were separated, and the aqueous layer was extracted with CH_2Cl_2 to give a triflyl azide solution of a total volume of ~30 mL (do not evaporate to dryness; explosions have been reported). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and dried over Na_2SO_4 to give a triflyl azide solution. The triflyl azide solution was carefully added to amino alcohol **12** (0.91 g, 3.46 mmol), DMAP (0.24 g, 1.96 mmol), and $CuSO_4$ (0.03 g, 0.19 mmol) in CH_2Cl_2 (10 mL).[44,45] The reaction was stirred for 2 h and quenched with 10% aqueous citric acid. The aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash chromatography (heptane/ethyl acetate 9:1 → 4:1) to furnish **13** (0.85 g, 85%) as a colorless oil: $[\alpha]^{20}_D$ = +18.8 (*c* = 0.18 in $CHCl_3$); IR (neat) 3425, 2102, 1504 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.11 (d, *J* = 8.4 Hz, 2H), 6.92 (d, *J* = 8.4 Hz, 2H), 5.80−5.68 (m, 1H), 5.15−5.05 (m, 2H), 3.59−3.53 (m, 1H), 3.43−3.48 (m, 1H), 2.94 (dd, *J* = 13.8 and 6.0 Hz, 1H), 2.85 (dd, *J* = 13.8 and 8.7 Hz, 1H), 2.34−2.28 (m, 2H), 1.32 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 153.74, 133.7, 132.2, 129.5, 124.2, 118.0, 78.2, 71.4, 66.9, 38.7, 36.3, 28.5; HRMS (FAB) calcd for $C_{16}H_{24}N_3O_2$ (M + H) 290.1869, found 290.1889.

(4*S*,5*S*)-5-Azido-6-(4-*tert*-butoxyphenyl)-4-(oxyacetic acid *tert*-butyl ester)hexane-1,2-diol (15). Azido alcohol **13** (0.800 g, 2.76 mmol) was dissolved in benzene (30 mL) and added to 50% aqueous NaOH (30 mL). NBu_4HSO_4 (165 mg, 0.49 mmol) was added followed by addition of *tert*-butyl bromoacetate (0.73 mL, 1.67 mmol), and the reaction was vigorously stirred.[46] After 1 h, H_2O (30 mL) and CH_2Cl_2 (30 mL) were added, and the two phases were separated. The aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure at 0 °C to give the sensitive alkylated azide **14.** The freshly generated **14** (0.85 g, 1.98 mmol), OsO_4 (cat. amount), and *N*-methylmorpholine *N*-oxide (0.50 g, 4.27 mmol) were immediately dissolved in a mixture of acetone (16 mL), THF (16 mL), and H_2O (16 mL). After the mixture was stirred for 10 h, 0.2 M aqueous HCl was added to adjust the pH to ~2. The aqueous layer was extracted with EtOAc. The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash chromatography (heptane/ethyl acetate 1:1 → 1:2) to give **15** (0.88 g, 73%, from **13**) as a 1:1 diastereomeric mixture: IR (neat) 3428, 2110, 1730 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.14−7.09 (m, 2H), 6.94 (d, *J* = 8.6 Hz, 2H), 4.40 (s, 0.5H), 4.24 (s, 0.5H), 4.24 (d, *J* = 16.5 Hz, 0.5H), 4.22 (d, *J* = 16.9 Hz, 0.5H), 4.17 (d, *J* = 16.9 Hz, 0.5H), 4.17−4.09 (m, 0.5H), 4.04 (d, *J* = 16.5 Hz, 0.5H), 3.98−3.90 (m, 0.5H), 3.78−3.72 (m, 0.5H), 3.69−3.59 (m, 2.5H), 3.56−3.47 (m, 1H), 3.00 (dd, *J* = 2.6 and 14.2 Hz, 0.5H), 2.96 (dd, *J* = 3.2 and 14.2 Hz, 0.5H), 2.67−2.56 (m, 1H), 2.44 (s, 0.5H), 2.39 (s, 0.5H), 1.82−1.56 (m, 2H), 1.49 (s, 4.5H), 1.48 (s, 4.5H), 1.32 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 170.6, 170.0, 153.9, 132.6, 132.5, 129.5, 124.3, 82.4, 82.3, 82.0, 78.5, 78.2, 70.5, 67.9, 67.8, 66.5, 66.5, 65.7, 60.2, 35.6, 35.3, 34.1, 33.5, 28.6, 27.9; HRMS (FAB) calcd for $C_{22}H_{35}N_3O_6$ (M + H) 438.2604, found 438.2598.

[(1*S*,2*S*)-2-Azido-3-(4-*tert*-butoxyphenyl)-1-(2-oxoethyl)propoxy]acetic Acid *tert*-Butyl Ester (16). The diastereomeric mixture of diols **15** (0.87 g, 2.0 mmol) was dissolved in benzene (60 mL) followed by addition of Na_2CO_3 (0.53 g, 5.0 mmol). The reaction was cooled to 0 °C, and $Pb(OAc)_4$ (1.4 g, 3.16 mmol) was added. The reaction was kept at 0 °C for 10 min and then allowed to reach room temperature and stirred for another 3 h. The reaction was quenched with ethylene glycole (20−25 drops), and after 5 min Et_2O and saturated aqueous $NaHCO_3$ were added. The aqueous phase was extracted with Et_2O. The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash chromatography (heptane/ethyl acetate 3:1) to give **16** (0.69 g, 85%) as a colorless oil: $[\alpha]^{20}_D$ = −17.5 (*c* 0.21, $CHCl_3$); IR (neat) 2728, 2112, 1747, 1726 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 25 °C) δ 9.75 (s, 1H), 7.09 (d, *J* = 8.4 Hz, 2H), 6.88 (d, *J* = 8.4 Hz, 2H), 4.03 (s, 2H), 3.98−3.91 (m, 1H), 3.61−3.54 (m, 1H), 3.01 (dd, *J* = 4.8 and 14.3 Hz, 1H), 2.84−2.68 (m, 3H), 1.42 (s, 9H), 1.27 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 199.7, 168.9, 154.0, 132.1, 129.4, 124.1, 81.5, 78.0, 76.5, 68.6, 65.6, 45.3, 35.4, 28.6, 27.8. Anal. Calcd for $C_{37}H_{56}O_7N_5$: C, 62.20; H, 7.71; N, 10.36. Found: C, 62.47; H, 7.88; N, 10.17.

(*S*)-2-[(*S*)-2-[(3*S*,4*S*)-4-Azido-3-*tert*-butoxycarbonylmethoxy-5-(4-*tert*-butoxyphenyl)pentylamino]-3-phenylpropionylamino]-4-methylpentanoic Acid Methyl Ester (17). Triethylamine (0.32 mL, 2.22 mmol) and H-Phe-Leu-OMe[48] (0.72 g, 2.16 mmol) were added to a solution of aldehyde **16** (0.67 g, 1.66 mmol) in 1,2-dichloroethane (25 mL). The solution was stirred for 5 min, after which time $NaBH(OAc)_3$ (0.57 g, 2.67 mmol) was added in one portion.[56] Stirring was continued for 45 min followed by addition of saturated aqueous $NaHCO_3$ and separation of the two phases. The aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash chromatography (heptane/ethyl acetate 3:1 → 1:1) to give **17** (0.93 g, 82%) as a slightly yellow oil: $[\alpha]^{20}_D$ = −47.6 (*c* = 0.17 in $CHCl_3$); IR (neat) 3329, 2107, 1743, 1673 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.60 (d, *J* = 9.1 Hz, 1H), 7.29−7.15 (m, 5H), 7.08 (d, *J* = 8.2 Hz, 2H), 6.88 (d, *J* = 8.2 Hz, 2H), 4.64−4.56 (m, 1H), 3.90 (d, *J* = 16.3 Hz, 1H), 3.84 (d, *J* = 16.3 Hz, 1H), 3.64 (s, 3H), 3.49 (dt, *J* = 10.2 and 4.1 Hz, 1H), 3.30 (dd, *J* = 9.3 and 3.8 Hz, 1H), 3.23 (dt, *J* = 8.0 and 4.3 Hz, 1H), 3.14 (dd, *J* = 13.6 and 3.8 Hz, 1H), 2.92 (dd, *J* = 14.0 and 3.6 Hz, 1H), 2.78−2.55 (m, 4H), 1.76−1.46 (m, 6H), 1.43 (s, 9H), 1.28 (s, 9H), 0.90 (d, *J* = 5.8 Hz, 3H), 0.88 (d, *J* = 5.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 173.4, 173.1, 169.1, 153.9, 137.4, 132.6, 129.4, 129.0, 128.5, 126.7, 124.1, 81.4, 80.5, 78.0, 68.0, 65.7, 63.7, 51.9, 49.8, 44.7, 41.2, 39.2, 35.3, 30.2, 28.6, 27.9, 24.6, 22.7, 21.5; HRMS (FAB) calcd for $C_{37}H_{56}N_5O_7$ (M + H) 682.4180, found 682.4175. Anal. Calcd for $C_{37}H_{55}N_5O_7$: C, 65.18; H, 8.13; N, 10.27. Found: C, 65.42; H, 8.35; N, 10.44.

(*S*)-2-((*S*)-2-[[(3*S*,4*S*)-4-Azido-3-*tert*-butoxycarbonylmethoxy-5-(4-*tert*-butoxyphenyl)pentyl]-[2-(9*H*-fluoren-9-ylmethoxycarbonylamino)acetyl]amino]-3-phenylpropionylamino)-4-methylpentanoic Acid Methyl Ester (18). Amine **17** (0.85 g, 1.25 mmol) was dissolved in DMF (5 mL) and added to a mixture of diisopropylethylamine (0.56 mL, 3.23 mmol), HATU[49,50] (*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, 0.62 g, 1.64 mmol), and Fmoc-Gly-OH (0.53 g, 1.78 mmol) in DMF (3 mL) at 0 °C. The reaction was allowed to reach room temperature, and stirring was continued for another 15 h. To the reaction were added saturated aqueous $NaHCO_3$ and EtOAc. The two phases were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash chromatography (heptane/ethyl acetate 2:1 → 1:1) to give **18** (1.06 g, 85%) as white solid of a ~4:1 mixture of rotamers: $[\alpha]^{20}_D$ = −57.0 (*c* = 0.32 in $CHCl_3$); IR (neat) 2108, 1724, 1678, 1651 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.79−7.73 (m, 2H), 7.65−7.54 (m, 2H), 7.43−7.36 (m, 2H), 7.35−7.18 (m, 7H), 7.18−7.09 (m, 2H), 6.99 (d, *J* = 8.4 Hz, 1H), 6.93 (d, *J* = 7.0 Hz, 2H), 5.74 (t, *J* = 4.3 Hz, 0.8H), 5.62 (t, *J* = 4.3 Hz, 0.2H), 4.76−4.62 (m, 0.8H), 4.60−4.47 (m, 1.2H), 4.42−4.30 (m, 2H), 4.28−4.17 (m, 1H), 4.15−3.94 (m, 4H), 3.71 (s, 0.5H), 3.67 (s, 2.5H), 3.64−3.45 (m, 2H), 3.43−3.30 (m, 3H), 3.21−3.08 (m, 1H), 3.05 (dd, *J* = 3.3 and 14.0 Hz, 0.2H), 2.96 (dd, *J* = 3.3 and 14.0 Hz, 0.8H), 2.70 (dd, *J* = 10.4 and 13.9 Hz, 0.2H), 2.55 (dd, *J* = 10.4 and 13.9 Hz, 0.8H), 1.80−1.52 (m, 5H), 1.49

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(s, 1.5H), 1.46 (s, 7.5H), 1.33 (s, 9H), 0.93 (d, $J = 2.5$ Hz, 2.5H), 0.92 (d, $J = 2.5$ Hz, 2.5H), 0.89−0.85 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C, $CHCl_3$) δ 173.1, 170.3, 170.1, 169.3, 156.3, 154.3, 144.0, 141.4, 137.3, 132.6, 129.8, 129.1, 128.8, 127.8, 127.2, 126.9, 125.2, 124.5, 124.4, 120.1, 82.0, 79.8, 78.5, 68.0, 67.3, 65.9, 52.3, 51.0, 47.2, 43.3, 41.1, 35.4, 34.2, 30.3, 28.9, 25.0, 22.9, 21.9; HRMS (FAB) calcd for $C_{54}H_{69}N_6O_{10}$ (M + H) 961.5075, found 961.5048. Anal. Calcd for $C_{54}H_{68}N_6O_{10}$: C, 67.48; H, 7.13; N, 8.74. Found: C, 67.81; H, 7.28; N, 8.55.

(*S*)-2-((*S*)-2-[[(3*S*,4*S*)-4-Azido-3-carboxymethoxy-5-(4-hydroxyphenyl)pentyl][2-(9*H*-fluoren-9-ylmethoxycarbonylamino)acetyl]amino]-3-phenylpropionylamino)-4-methylpentanoic Acid Methyl Ester (19). Compound **18** (1.10 g, 1.14 mmol) was dissolved in formic acid (19 mL) and stirred at room temperature for 20 h. Toluene (3 × 5 mL) was added, and the solvents were removed under reduced pressure. The residue was purified by flash chromatography (EtOAc/heptane/formic acid 2:1:0 → 2:1:0.05) to give **19** (0.97 g, 99%) as a white solid:. $[\alpha]^{20}_D = -50.4$ ($c = 0.14$ in $CHCl_3$); IR (neat) 3322, 2106, 1723, 1649 cm^{-1}; ^{1}H NMR (400 MHz, $(CD_3)_2S{=}O$, 70 °C) δ 7.85 (d, $J = 7.3$ Hz, 2H), 7.68 (d, $J = 7.1$ Hz, 2H), 7.40 (t, $J = 7.4$ Hz, 2H), 7.34−7.28 (m, 2H), 7.27−7.10 (m, 5H), 7.06 (d, $J = 8.4$ Hz, 2H), 6.72−6.67 (m, 2H), 4.33−4.17 (m, 4H), 4.15−4.10 (m, 2H), 3.63−3.56 (m, 1H), 3.58 (s, 3H), 3.52−3.46 (m, 2H), 3.31−3.24 (m, 2H), 3.03−2.86 (m, 2H), 2.64−2.54 (m, 1H), 1.81−1.64 (m, 2H), 1.62−1.48 (m, 3H), 0.84 (d, $J = 6.2$ Hz, 3H), 0.81 (d, $J = 6.2$ Hz, 3H); HRMS (FAB) calcd for $C_{46}H_{53}N_6O_{10}$ (M + H) 849.3823, found 849.3835.

(*S*)-2-((*S*)-2-[(*S*)-10-[(*S*)-1-Azido-2-(4-hydroxyphenyl)-ethyl]-3,6-dioxo[1,4,7]oxadiazecan-7-yl]-3-phenylpropionylamino)-4-methylpentanoic Acid Methyl Ester (21). Acid **19** (0.3 g, 0.35 mmol) was dissolved in EtOAc (3 mL). Dicyclohexylcarbodiimide (0.11 g, 0.52 mmol) and pentafluorophenol (0.1 g, 0.52 mmol) were then added at 0 °C.[51] The reaction was stirred for 2 h and then filtered through Celite. The solvent was then removed under reduced pressure, and the residue was filtered through silica (heptane/ethyl acetate 2:1 → 1:1) to yield activated ester **20** (0.35 g, ∼0.35 mmol) still containing a small amount of dicyclohexyl urea. A solution of **20** (0.35 g, ∼0.35 mmol) in dioxane (10 mL) was added, via a syringe pump, to a refluxing solution of 1,8-Diazabicyclo[5.4.0]-undec-7-ene (0.15 mL, 1.0 mmol) in dioxane (52 mL) over a period of 12 h. After complete addition, the reaction was allowed to reflux for another 15 min before the solvent was removed under reduced pressure and the residue was purified by flash chromatography (toluene/ethanol 20:1 → 10:1) to give **21** (0.12 g, 56%, from **19**) as a white solid: $[\alpha]^{20}_D = -76.9$ ($c = 0.1$ in $CHCl_3$); IR (neat) 3509−3087, 2106, 1738, 1649 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OH, −70 °C) δ 7.37−7.10 (m, 8H), 6.79−6.70 (m, 2H), 4.73−4.60 (m, 2H), 4.54−4.43 (m, 2H), 4.35−4.22 (m, 1H), 4.01−3.95 (m, 1H), 3.93−3.87 (m, 1H), 3.77−3.66 (m, 1H), 3.68 (s, 3H), 3.55−3.40 (m, 3H), 2.95−2.86 (m, 1H), 2.49−2.31 (m, 2H), 2.15−2.04 (m, 1H), 1.81−1.45 (m, 4H), 1.07−0.88 6H); HRMS (FAB) calcd for $C_{31}H_{41}N_6O_7$ (M + H) 609.3037, found 609.3026.

(*S*)-2-((*S*)-2-[(*S*)-10-[(*S*)-1-Amino-2-(4-hydroxyphenyl)-ethyl]-3,6-dioxo[1,4,7]oxadiazecan-7-yl]-3-phenylpropionylamino)-4-methylpentanoic Acid (23). Compound **21** (0.06 g, 0.1 mmol) was treated with a mixture of $SnCl_2$ (0.07 g, 0.37 mmol), triethylamine (0.15 mL, 1.1 mmol), and thiophenol (0.15 mL, 1.5 mmol) in THF (6 mL) at room temperature.[52] After 2 h, the solvent was removed under reduced pressure, and the residue was purified by HPLC and lyophilized to give amine **22** (0.04 g, 0.07 mmol) which was directly dissolved in THF (10 mL) and treated with 0.1 M aqueous LiOH (1.9 mL, 0.19 mmol). After 3 h the reaction was quenched with an excess of acetic acid (∼ 0.1 mL). The solvent was removed under reduced pressure, and the residue was purified by HPLC and lyophilized to give **23** (0.031 g, 57%, from **21**) as a white solid: $[\alpha]^{20}_D = -47.6$ ($c = 0.59$ in MeOH); IR (neat) 3261, 2958, 1658, 1616 cm^{-1}; ^{1}H NMR (600 MHz, H_2O, 33 °C, H_2O) δ 8.60−8.49 (m, 0.6H), 8.12−8.05 (m, 0.2H), 7.43−7.08 (m, 8H), 6.93−6.84 (m, 2H), 4.27−4.17 (m, 3H), 4.00−3.91 (m, 1H), 3.89−3.81 (m, 1H), 3.52−3.45 (m, 1H), 3.42−3.30 (m, 3H), 3.26−3.17 (m, 1H), 2.90−2.81 (m, 1H), 2.79−2.71 (m, 1H), 2.47−2.36 (m, 1H), 1.59−1.43 (m, 2H), 1.41−1.20 (m, 2H), 0.90−0.79 (m, 6H), 0.79−0.69 (m, 1H); HRMS (FAB) calcd for $C_{30}H_{41}N_4O_7$ (M + H) 569.2975, found 569.2964.

NMR Experiments. All spectra of **23** were recorded on a Bruker DRX 600 MHz spectrometer equipped with a TXI cryoprobe. The spectra obtained at −70 °C for **21** were recorded on a Bruker AMX 500 MHz spectrometer equipped with a TXI probe. The NMR sample of **23** was prepared by dissolving 2.3 mg in phosphate-buffered aqueous solution at pH 5.5 to give 9 mM final concentration. The NMR sample of **21** was prepared by dissolving 2.8 mg of **21** in MeOH-d_3 giving a 10 mM solution. TOCSY,[57] NOESY,[58] and DQF−COSY[59] 2D experiments were all recorded with 2048 complex data points in t_2 and 512 increments in t_1. Zero-filling resulted in datasets with 2048 × 1024 points. A DIPSI spin-lock was used in the TOCSY spectra with a mixing time of 85 ms. Apodization with a shifted squared sine-bell function was applied in both dimensions prior to Fourier transformation except in the DQF−COSY experiment where an unshifted sine-bell function was used. A 250 ms mixing time was used in the NOESY spectrum for **23** and 30 ms for **21**.

Structure Calculations. Assignment and peak picking were performed in Felix (Accelrys Inc.). Distance restraints were extracted from the NOESY spectrum of **21** obtained at −70 °C. NOESY cross-peak volumes were converted to inter-proton distance restraints, d_{ij}, by using the isolated spin-pair approximation

$$d_{ij} = d_{\mathrm{ref}}(V_{\mathrm{ref}}/V_{ij})^{1/6}$$

where d_{ref} corresponds to a reference distance in the molecule with the known cross-peak volume V_{ref}. The cross-peak between the α-protons of Gly2 was used as the reference volume with a reference distance of 1.772 Å. The upper limit for the distance restraints were set to 4 Å for all d_{ij} shorter than 3 and 5 Å for all d_{ij} exceeding 3 Å.

Starting structures for **21** were prepared with an unrestrained MD simulation. The two structures with the largest conformational difference were subjected to restrained simulated annealing with the 29 experimentally derived distance restraints. The initial temperature in the simulated annealing protocol was set to 1000 K and the final temperature was 100 K. A soft square-welled potential with a force constant of 50 kcal mol^{-1} Å^{-2} was used for the distance restraints. All structures were minimized with a 200 step Powell minimization after the refinement. Structures were accepted if they did not contain any distance restraint violations larger than 0.3 Å and no dihedral angle violations larger than 5°. X-PLOR[55] was used for all structure calculations.

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Supporting Information Available: Copies of ^{1}H NMR and ^{13}C NMR spectra for compounds **7**−**13**, **15**−**19**, **21** and **23**. This material is available free of charge via the Internet at http://pubs.acs.org.

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