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The Use of γ-Turn Mimetics to Define Peptide Secondary Structure

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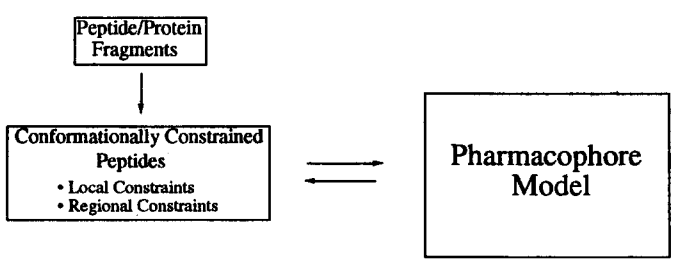
Abstract: *A novel γ-turn mimetic 2 has been prepared based on retro amide peptide design. Incorporation of this mimetic into linear peptide fibrinogen receptor antagonist 7 (GPIIb/IIIa receptor) affords the opportunity to test models of antagonist pharmacophore.*

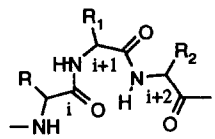
Proteins and peptides are known to interact with macromolecular receptors to initiate biological events. When it is desirable to interrupt these processes, medicinal chemists attempt to develop antagonists which block the native ligand from binding to its receptor. The rational design of such antagonists is facilitated by a knowledge of which parts of the peptide/protein interact with the receptor and in what conformation. In some instances, the first piece of information can be determined by screening regions or fragments of the peptide/protein for receptor affinity and then determining the minimum active fragment which retains affinity.[1] The second problem is somewhat more complex. By nature, linear peptide fragments are flexible and exhibit a multitude of conformations in solution and even in the solid state. Also, since many of the macromolecular receptors are found in lipid environments, it is almost impossible using current technology to determine the receptor bound conformations of these linear fragments when complexed to the receptor. However, if one can restrict the conformational freedom of these linear peptides by introducing local (e.g. N-methyl amino acids, α-methyl amino acids) and regional constraints (e.g. cyclic disulfides and amides), their solution conformations can be better determined by physical methods. This conformational knowledge about analogs which retain affinity for the receptor is then used to develop a model for the biologically active conformation of the peptide antagonist, i.e. an antagonist pharmacophore. Once a pharmacophore model is proposed, additional analogs can be designed that test the various conformational features of the model and further refinements can be made ultimately resulting in the design of novel high affinity peptide and non-peptide antagonists (Figure 1). One approach toward validation of the pharmacophore model is to replace a proposed feature of backbone secondary structure with a conformational mimetic.[2]

Figure 1

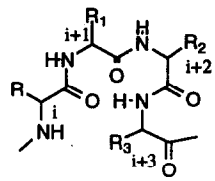


An important component of the secondary structure of small peptides is often a reverse turn (Figure 2).[3] The most common form of reverse turn found in peptides, especially small cyclic peptides, is the β-turn.

Figure 2



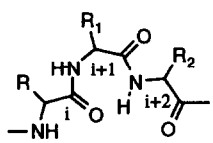
γ-Turn Conformation



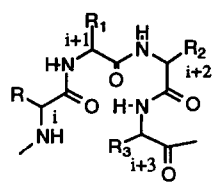
β-Turn Conformation

There has been considerable effort in recent years to either use an unusual amino acid to lock the backbone conformation into a β-turn[2,4] or to replace the residues involved in the β-turn with a conformational mimetic.[2,5] Little attention has been given however to the study of the related γ-turn. In small peptides, a γ-turn has been frequently postulated to represent an important feature of peptide secondary structure as determined by ^{1}H NMR,[6] X-ray crystallography[7] and molecular modeling.[8] In order to validate a pharmacophore model containing a γ-turn, it was necessary to develop mimetics of this secondary structural feature. The first mimetic we designed replaced the amide bond between the i and i+1 residues of the γ-turn with a *trans* olefin (Figure 3).[9] Besides locking the amide bond in a *trans* conformation, the mimetic allowed

Figure 3



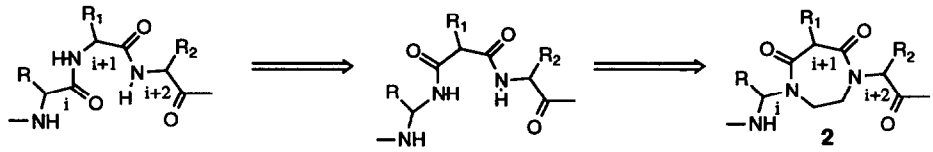
γ-Turn Conformation



β-Turn Conformation

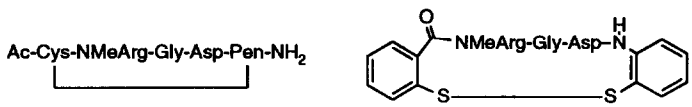
for an ethylene bridge to be substituted for the hydrogen bond between the carbonyl of the i residue to the amide hydrogen of the i+2 residue effectively locking the i+1 torsion angles into a γ-turn-like conformation. The mimetic **1** also allowed for the introduction of any side chain on the three residues of the γ-turn and for the incorporation of the mimetic into any portion of the peptide sequence. A significant limitation to this mimetic is that, even though its synthesis has been improved[10] since it was first proposed,[9] it still requires a complex multi-step asymmetric synthesis. In looking to address this limitation, we began to explore the design of more readily available γ-turn mimetics. If instead of a *trans* olefin, the amide bond between the i and i+1 residues of the γ-turn was replaced by a retro amide, an ethylene bridge could still be introduced between the carbonyl of the i residue to the amide hydrogen of the i+2 residue (Figure 4).[11] Molecular modeling studies comparing simplified versions of each of the mimetics showed that their low energy conformations mimetics effectively locked the i+1 torsion angles into a γ-turn-like conformation.[12] The major difference in the two mimetics is that the retro amide bond in **2** introduces a carbonyl oxygen and a dipole which is not present in the *trans* olefin C_7 mimetic **1**. This dipole would be the reverse of that found in the peptide that is being mimicked (Figure 4). However, the retro amide mimetic 2 allows for the introduction of the i and i+2 units as amino

Figure 4



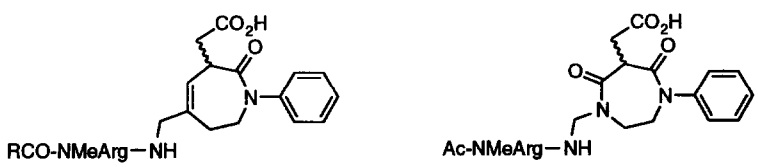
acids, thus simplifying the asymmetric synthesis of the γ-turn mimetic. In cases where the additional carbonyl does not generate a negative interaction with the receptor, this mimetic could be seen as a simplified alternative for the *trans* olefin mimetic.

In order to test the retro amide mimetic concept, we chose to incorporate mimetic **2** into a peptide antagonist whose postulated pharmacophore contained a γ-turn. The work to develop potent peptide antagonists of the binding of fibrinogen to its receptor (GPIIb/IIIa) has led, through the introduction of both local and regional constraints, to the discovery of two conformationally constrained cyclic disulfide antagonists, compounds **3**[13] and **4**.[14] Extensive study of **3** and **4** by ^{1}H NMR, X-ray crystallography and



3 **4**

molecular modeling has led to a pharmacophore model for these GPIIb/IIIa receptor antagonists.[15] This model is characterized by an extended glycine residue in the Arg-Gly-Asp region and a C_7 conformation about the aspartic acid residue. In our initial work to examine the conformation of the region about the Asp residue more closely, the *trans* olefin C_7 mimetic **1** was successfully introduced into a linear antagonist and the resulting analogs, **5** and **6**, retained high affinity for the GPIIb/IIIa receptor as well as potent inhibition of

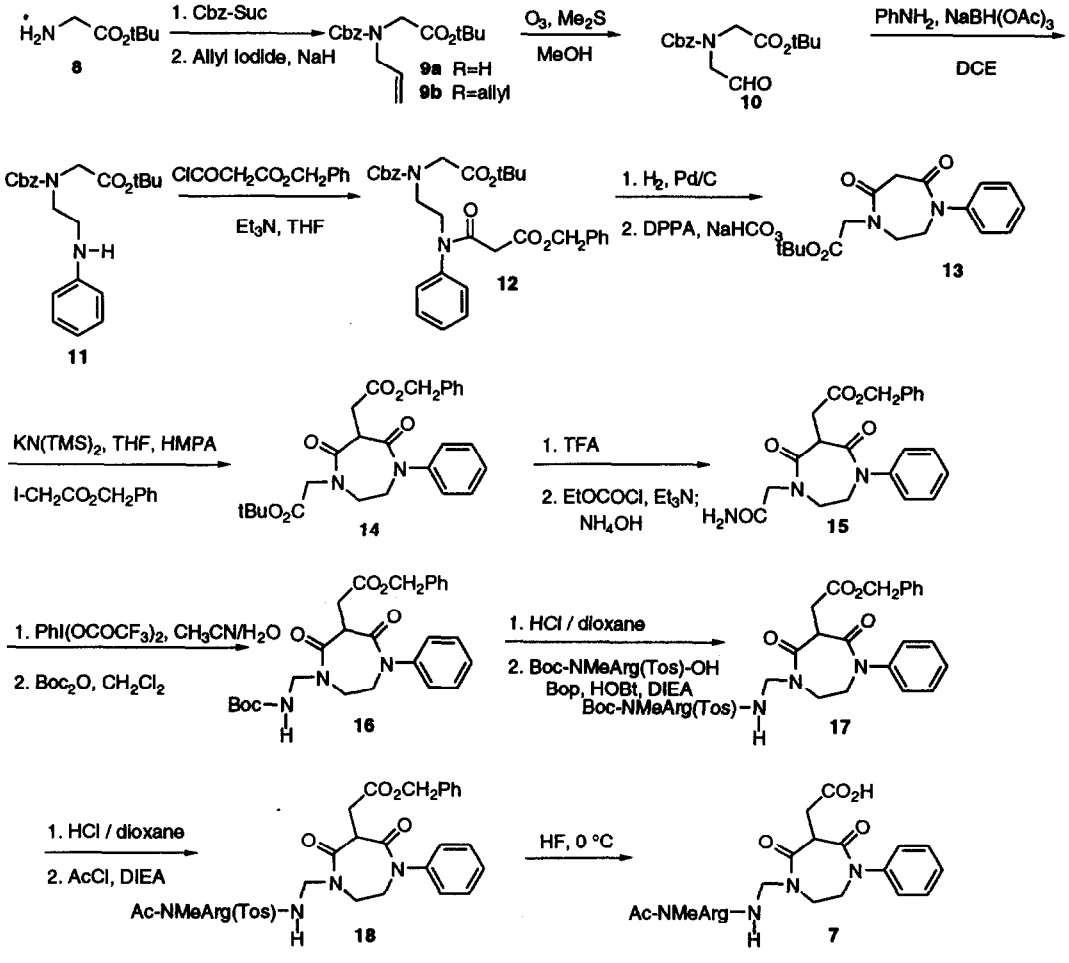


platelet aggregation *in vitro*.[16] Introduction of the new retro amide mimetic into the same linear antagonist would allow us to test the concept as well as have a comparison to the corresponding *trans* olefin mimetic.

The synthesis of the retro amide C_7 mimetic containing analog **7** is outlined in Scheme 1. The basic design of the synthesis allows for the introduction of both the i and i+2 residues as amino acids or amines and the introduction of the incipient i+1 residue as a mono-protected malonic acid derivative. Glycine t-butyl ester (**8**) was protected as its N-Cbz derivative **9a** and then N-alkylated with allyl iodide to give **9b**. Selective

ozonolysis of **9b** followed by a reductive work-up provided the aldehyde **10**. Reductive amination of **10** with aniline gave **11**, which was converted via acylation with the acid chloride of benzyl malonate to the C_7

Scheme 1



precursor **12**. Simultaneous deprotection of the amine and acid in **12** via catalytic hydrogenation followed by cyclization of the resulting amino acid with diphenylphosphoryl azide furnished **13**, which incorporated the backbone portion of the desired retro amide C_7 mimetic. The i+1 side chain was incorporated into **13** by selective deprotonation at the "malonyl" ring carbon and alkylation with benzyl iodoacetate to give **14**. Deprotection of **14** followed by aminolysis of the mixed anhydride afforded **15**, which was converted to the protected amine **16** by modified Hofmann rearrangement[17] of the primary amide followed by treatment with di-tert-butyl-dicarbonate. Treatment of **16** with 4N HCl in dioxane followed by BOP coupling[18] to Boc-NMeArg(Tos)-OH[13] gave the protected analog **17**. Selective removal of the N-terminal Boc protection

followed by treatment with acetyl chloride provided **18**. Final deprotection of **18** with anhydrous HF yielded **7** as an inseparable set of diastereoisomers.

The biological results for the retro amide C_7 mimetic containing analog **7** are summarized in Table 1. The data indicate that while the olefin C_7 mimetic containing analogs **5** and **6** are potent inhibitors of *in vitro* platelet aggregation and retain high affinity for the GPIIb/IIIa receptor,[16] the retro amide C_7 mimetic

Table 1

Compound	Antiaggregatory Activity Canine PRP/ADP[13] IC_{50} (μM)[a]	Binding Inhibition Human GPIIb/IIIa[13] K_i (μM)[b]
3	0.36 ± 0.04	0.175 ± 0.025
4	0.09 ± 0.02	0.004
5	1.11 ± 0.16	0.106 ±0.007
6	0.72 ± 0.28	0.271 ± 0.003
7	~200	>100

[a] Inhibition of platelet aggregation in canine platelet-rich plasma induced by ADP.

[b] Inhibition of [^{3}H]-107260 binding to purified GPIIb/IIIa isolated from human platelets that were reconstituted in liposomes.

containing analog **7** exhibits a significant reduction of receptor affinity and *in vitro* platelet anti-aggregatory activity. Due to the lower level of affinity exhibited by **7**, no attempt was made to separate the diastereoisomers generated from the i+1 side chain.[19] In the *trans* olefin C_7 mimetic containing analog **6**, each diastereomer generated from the i+1 side chain was shown to exhibit similar affinity. Two possible reasons for the loss of affinity of the retro amide C_7 mimetic containing analog could be that: (1) the retro amide C_7 mimetic does not effectively mimic the γ-turn conformation found in the biologically active conformation of the peptide antagonists **3** and **4** or (2) the polar carbonyl group, which is not present in **5** and **6**, somehow interferes with the ligand/receptor interaction. X-ray crystallographic analyses of similar *trans* olefin and retro amide C_7 compounds show that each mimetic successfully constrains the torsion of the i+1 residue to a γ-turn conformation.[20] Taking this into consideration, the data implies that the carbonyl of the retro amide C_7 mimetic interferes with the binding of **7** to the GPIIb/IIIa receptor. Other work to examine this region of the receptor is in progress.

In conclusion, the work on the retro amide C_7 mimetic has provided a novel γ-turn mimetic that complements the previously disclosed *trans* olefin C_7 mimetic. As in all cases of peptidomimetic design, variation of functional groups inherent in the design of the mimetic may either eliminate an important binding element of the peptide that is being mimicked or introduce a new, unfavorable interaction with the receptor. In this case, the introduction of the carbonyl group may interfere with the binding of **7** to the GPIIb/IIIa receptor. Future applications of this mimetic, especially in conjunction with the *trans* olefin C_7 mimetic, will better enable one to elaborate a peptide antagonist pharmacophore and, from that, design non-peptide drugs.

Acknowledgments: The authors which to thank Mary Mentzer, Walt Johnson, Gerald Roberts, Mark Huddleston and Mark Bean of the Department of Physical and Structural Chemistry for mass spectral analyses.

Experimental Section

^{1}H NMR spectra were recorded at 90 MHz on a Varian EM 390 spectrometer. Chemical shifts are reported in δ units from the internal standard tetramethylsilane. Mass spectra were taken on either VG 70 FE, PE Syx API III or VG ZAB HF instruments. TLC were taken on Analtech Silica Gel GF plates or E. Merck Silica Gel 60-F-254 plates. Flash chromatography was carried out on E. Merck 60 (230-400 mesh) silica gel. Tetrahydrofuran was distilled from sodium ketyl immediately before use. Analytical hplc was carried out on a Beckman Chromatograph using a 5μ (4.6x250 mm) Apex-ODS column manufactured by Jones Chromatography. Semi-preparative hplc was carried out on a Beckman Chromatograph using a 5μ (10x250 mm) IBM-ODS column distributed by IBM.

N-Allyl-N-benzyloxycarbonyl-glycine t-butyl ester (9b):

A solution of glycine t-butyl ester (**8**) (15 g, 114 mmol) in CH_2Cl_2 (250 mL) was cooled to 0 °C and treated with N-(benzyloxycarbonyloxy)succinimide (35 g, 137 mmol) at room temperature for 5 h. The reaction mixture was washed with 1 N HCl (aqueous), 5% $NaHCO_3$ (aqueous) dried over $MgSO_4$ and evaporated at reduced pressure. The residue was purified by flash chromatography (silica gel, 6x20 cm column, elution with 30 % ethyl acetate in hexane) to give 27.27 g (90%) of **9a**. ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 3.70-3.95 (m, 2H), 5.12 (s, 2H), 5.43 (br s, 1H), 7.33 (s, 5H).

A solution of **9a** (27.27 g, 103 mmol) in anhydrous tetrahydrofuran (200 mL) was treated with allyl iodide (37.6 mL, 411 mmol) and cooled to 0 °C. Sodium hydride (60% in oil, 6.15 g, 154 mmol) was added slowly to the reaction mixture and the resulting suspension was stirred at room temperature for 24 h. The reaction mixture was evaporated at reduced pressure and ethyl acetate carefully added to the residue. This was then washed with saturated $Na_2S_2O_3$ (aqueous) and water, dried over $MgSO_4$, filtered and evaporated at reduced pressure. The residue was purified by flash chromatography (silica gel, 6x20 cm column, elution with 5 to 10 % ethyl acetate in hexane) to give 18.51 g (60%) of **9b**. ^{1}H NMR spectroscopy indicates an interconverting mixture of carbamates (cis/trans). ^{1}H NMR ($CDCl_3$) δ.1.40/1.43 (2s, 9H), 3.70-4.10 (m, 4H), 4.90-5.37 (m, 4H), 5.53-6.17 (m, 1H), 7.32 (s, 5H).

N-[1-(2-Oxoethyl)]-N-benzyloxycarbonyl-glycine t-butyl ester (10):

A solution of **9b** (18.51 g, 60.8 mmol) in methanol (100 mL) was treated with O_3 at -70 °C for approximately 1.25 h (until the solution was blue indicating excess O_3). The excess O_3 was purge with argon, methyl sulfide was added and the resulting solution was slowly warmed to room temperature and stirred for 24 h. The reaction mixture was evaporated at reduced pressure and the residue was purified by flash chromatography (silica gel, 6x20 cm column, elution with 35 % ethyl acetate in hexane) to give 15.1 g (81%) of **10**. ^{1}H NMR spectroscopy indicates an interconverting mixture of carbamates (cis/trans). ^{1}H NMR ($CDCl_3$) δ.1.40/1.45 (2s, 9H), 3.87-4.20 (m, 4H), 5.15 (br s, 2H), 7.33 (s, 5H), 9.65 br s, 1H).

N-[1-(2-Phenylaminoethyl)]-N-benzyloxycarbonyl-glycine t-butyl ester (11):

A solution of **10** (13.2 g, 42.9 mmol) and aniline (4.31 mL, 47.3 mmol) in dichloroethane (200 mL) was cooled to 0 °C and treated sequentially with sodium triacetoxyborohydride (13.6 g, 64.5 mmol) and acetic acid (2.45 mL) and the resulting mixture was stirred at room temperature for 24 h. The reaction was diluted with chloroform, washed 2 times with 5% $NaHCO_3$ (aqueous), dried over anhydrous $MgSO_4$, filtered and evaporated at reduced pressure. The residue was purified by flash chromatography (silica gel, 6x20 cm column, elution with 15 % ethyl acetate in hexane) to give 12.47 g (76%) of **11**. ^{1}H NMR spectroscopy indicates an interconverting mixture of carbamates (cis/trans). ^{1}H NMR ($CDCl_3$) δ.1.37/1.45 (2s, 9H), 3.13-3.67 (m, 4H), 3.77-3.95 (m, 2H), 4.25 (br s, 1H), 5.12 (s, 2H), 6.33-6.83 (m, 3H), 6.97-7.27 (m, 2H), 7.30 (s, 5H); MS (FAB) m/e 385 $(M+H)^+$.

N-[1-(2-Phenyl-(N-benzylmalonyl)-aminoethyl)]-N-benzyloxycarbonyl-glycine t-butyl ester (12):

A solution of 2,2-dimethyl-1,3-dioxane-4,6-dione (25 g, 174 mmol) and benzyl alcohol (36 mL, 347 mmol) in toluene (100 mL) was heated at 106 °C for 24 h. The solution was poured into 5% Na_2CO_3 (aqueous), the organic layer separated and the aqueous layer was washed with ether (3 times). The aqueous layer was then acidified with 1 N HCl (aqueous) and extracted with ethyl acetate (2 times). The combined ethyl acetate extracts were dried over anhydrous MgSO4, filtered and evaporated at reduced pressure to give 11.42 g (34%) of malonic acid monobenzyl ester. The malonic acid monobenzyl ester (11.42 g, 60 mmol) was dissolved in toluene and treated with excess oxalyl chloride (15 mL) at 35 °C for 24 h. Evaporation of the solvent, along with the excess oxalyl chloride, gave 11.4 g of the acid chloride of malonic acid monobenzyl ester.

A solution of **11** (1.9 g, 4.97 mmol) in chloroform was treated with pyridine (1 mL) and the acid chloride of malonic acid monobenzyl ester (2.13 g, 10 mmol) and stirred at room temperature for 24 h. The reaction mixture was evaporated at reduced pressure and the residue was dissolved in ethyl acetate; washed with 5% $NaHCO_3$ (aqueous) (2 times), 1N HCl (aqueous) (2 times) and saturated NaCl (aqueous); dried over anhydrous $MgSO_4$, filtered and evaporated at reduced pressure. The residue was purified by flash chromatography (silica gel, 2x20 cm column, elution with 20 to 25 % ethyl acetate in hexane) to give 1.86 g (67%) of **12**. ^1H NMR spectroscopy indicates an interconverting mixture of carbamates (cis/trans). ^1H NMR ($CDCl_3$) δ.1.38/1.43 (2s, 9H), 3.18 (s, 2H), 3.33-4.00 (m, 6H), 5.00-5.13 (m, 4H), 6.90-7.43 (m, 15H); MS (DCI/NH_3) m/e 561 $(M+H)^+$.

1-Phenyl-2,4-dioxo-5-[carbo(t-butyloxy)]methyl-hexahydro-1,5-diazepine (13):

A solution of **12** and 5% Pd/C (220 mg) in methanol was treated with H_2 at 50 psi (Parr apparatus) at room temperature for 4 h. The reaction mixture was filtered through Celite® to remove the catalyst and then evaporated at reduced pressure. The residue was dissolved in N,N-dimethylformamide (300 mL) and the resulting solution was treated with $NaHCO_3$ (1.60 g, 19 mmol) and diphenylphosphoryl azide (1.73 mL, 7.98 mmol) at 0 °C and stirred with slow warming to room temperature over 72 h. The reaction mixture was evaporated under vacuum and the residue was purified by flash chromatography (silica gel, 6x20 cm column, elution with 75 % ethyl acetate in hexane) to give 540 mg (44%) of **13**. ^1H NMR ($CDCl_3$) δ1.47 (s, 9H), 3.50-4.23 (m, 8H), 7.03-7.57 (m, 5H); MS (DCI/NH_3) m/e 319 $(M+H)^+$.

1-Phenyl-2,4-dioxo-3-(carbobenzyloxy)methyl-5-[carbo(t-butyloxy)]methyl-hexahydro-1,5-diazepine (14):

A solution of **13** (1.98 g, 6.22 mmol) in a mixture of tetrahydrofuran (30 mL) and hexamethylphosphoramide (10 mL) was cooled to -78 °C and treated with 13.5 mL of 0.5M potassium bis (trimethylsilyl)amide for 20 min. Then benzyl iodoacetate (3.46 g, 12.4 mmol) was added and the reaction brought to room temperature over 2 h. The reaction mixture was quenched with saturated NH_4Cl (aqueous) followed by 1N HCl (aqueous) and then extracted with ethyl acetate. The combined organic extracts were washed with saturated NaCl (aqueous), dried over anhydrous $MgSO_4$, filtered and evaporated at reduced pressure. The residue was purified by flash chromatography (silica gel, 4x20 cm column, elution with 50 % ethyl acetate in hexane) to give 1.33 g (46%) of **14**. ^1H NMR ($CDCl_3$) δ.1.43 (s, 9H), 3.13 (d, 2H, J=7.5 Hz), 3.33-4.83 (m, 7H), 5.13 (s, 2H), 7.10-7.47 (m, 10H); MS (DCI/NH_3) m/e 467 $(M+H)^+$.

1-Phenyl-2,4-dioxo-3-(carbobenzyloxy)methyl-5-(carboxyamido)methyl-hexahydro-1,5-diazepine (15):

A solution of **14** in methylene chloride was treated with trifluoroacetic acid (60 mL) at room temperature for 3 h. The reaction mixture was evaporated at reduced pressure and the residue was evaporated from a mixture of toluene and chloroform to remove any trace of water. The resulting acid was dissolved in tetrahydrofuran (60 mL), cooled to -20 °C and treated with N-methylmorpholine (747 μL, 6.8 mmol) and ethyl chloroformate (650 μL, 6.8 mmol) for 30 min. The reaction mixture was then treated with a mixture of

saturated NH_4OH (aqueous) (4.5 mL) and tetrahydrofuran (45 mL) and warmed over 1 h to room temperature.The reaction mixture was then poured into cold 3N HCl (aqueous) and extracted with ethyl acetate. The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and evaporated at reduced pressure. The residue was purified by flash chromatography (silica gel, 2x20 cm column, elution with 5 % methanol in chloroform) to give 850 mg (73%) of **15**. ^1H NMR ($CDCl_3$) δ3.10 (d, 2H, J=7.5 Hz), 3.50-4.00 (m, 3H), 4.03 (s, 2H), 4.20-4.83 (m, 2H), 5.10 (s, 2H), 7.07-7.50 (m, 12H); MS (DCI/NH_3) m/e 410 $(M+H)^+$.

1-Phenyl-2,4-dioxo-3-(carbobenzyloxy)methyl-5-[t-butyloxycarbonyl-aminomethyl]-hexahydro-1,5-diazepine (16):

A solution of **15** in a mixture of acetonitrile and water (4:1, 80 mL) was treated with [bis(trifluoroacetoxy)iodo]benzene (1.8 g, 4.04 mmol) at room temperature for 5 h. The reaction mixture was evaporated at reduced pressure and the residue was evaporated from toluene (2 times) at reduced pressure. The resulting material was dissolved in methylene chloride and was treated with di-t-butyl dicarbonate (913 mg, 4.14 mmol) and triethylamine (585 µL, 4.14 mmol) at room temperature for 18 h. The reaction mixture was evaporated at reduced pressure and the residue was purified by flash chromatography (silica gel, 2x20 cm column, elution with 30 to 50 % ethyl acetate in hexane) to give 347 mg (35%) of **16**. ^1H NMR ($CDCl_3$) δ 1.40 (s, 9H), 3.10 (d, 2H, J=7.5 Hz), 3.60-4.83 (m, 7H), 5.15 (s, 2H), 5.70 (br t, 1H, J=7.5 Hz), 7.10-7.53 (m, 10H); MS (FAB) m/e 482 $(M+H)^+$.

1-Phenyl-2,4-dioxo-3-(carbobenzyloxy)methyl-5-[t-butyloxycarbonyl-(N-methyl-tosyl-arginyl)-aminomethyl]-hexahydro-1,5-diazepine (17):

Compound **16** was treated with 4N HCl in dioxane at room temperature for 1.5 h. The reaction mixture was then evaporated at reduced pressure and the residue was evaporated from toluene to remove traces of water and HCl. The resulting material was dissolved in N,N-dimethylformamide and the pH was adjusted to 7 (moist pH paper) with triethyl amine. This solution was then treated with Boc-N^{α}-Me-Arg(Tos)-OH (Bachem, 143 mg, 0.327 mmol), 1-hydroxybenzotriazole (44.2 mg, 0.327 mmol) and N,N-dicyclohexylcarbodiimide (67.4 mg, 0.327 mmol) and the resulting mixture was stirred at room temperature for 16 h. The reaction mixture was evaporated under vacuum and the residue was purified twice by flash chromatography (silica gel, 2x20 cm column, elution with 3 % methanol in chloroform; silica gel, 2x20 cm column, elution with 75 % ethyl acetate in hexane) to give 123 mg (49%) of **17** as an inseparable mixture of two diastereomers. ^1H NMR ($CDCl_3$) δ.1.03-1.99 (m, 14H), 2.37 (s, 3H), 2.58-3.32 (m, 8H), 3.55-4.87 (m, 7H), 5.03-5.16 (m, 2H), 6.16-6.49 (m, 1H), 6.91-7.38 (m, 14H), 7.61-7.78 (m, 2H); MS (ES) m/e 806 $(M+H)^+$.

1-Phenyl-2,4-dioxo-3-(carbobenzyloxy)methyl-5-[(N-acetyl-N-methyl-tosyl-arginyl)aminomethyl]-hexahydro-1,5-diazepine (18):

Compound **17** was treated with 4N HCl in dioxane at room temperature for 3 h. The reaction mixture was evaporated at reduced pressure and the residue was evaporated from toluene to remove any trace of water. The material from above was dissolved in N,N-dimethylformamide (5 mL) and treated with triethylamine (55.6 µL, 0.399 mmol) and acetyl chloride (29 µL, 0.399 mmol) at room temperature for 24 h. The reaction mixture was evaporated under vacuum and the residue was purified by flash chromatography (silica gel, 2x20 cm column, elution with 3 % methanol in chloroform; silica gel, 2x20 cm column, elution with 2-10 % methanol in chloroform) to give 102 mg (89%) of **18** as an inseparable mixture of two diastereomers. MS (ES) m/e 748 $(M+H)^+$.

1-Phenyl-2,4-dioxo-3-carboxymethyl-5-[(N-acetyl-N-methyl-arginyl)-aminomethyl]-hexahydro-1,5-diazepine (7):

A solution of **18** (140 mg, 0.192 mmol) from above in dichloromethane was transferred to a Kel-F® HF vessel and the solvent evaporated under a stream of argon. Anhydrous HF (10 mL) was condensed into the vessel at -78 °C and the reaction mixture stirred for 1 h at 0 °C. The HF was evaporated at reduced pressure and the residue taken into 10% acetic acid (aqueous) and lyophilized to give 78 mg of crude product. A portion of the crude product (32 mg) was dissolved in aqueous acetonitrile and purified by repeated runs on a semi-preparative reverse phase hplc column [5μ ODS: IBM, 10x250 mm, flow = 4.0 mL/min, 85:15 (0.1% trifluoroacetic acid (aqueous) : 0.1% trifluoroacetic acid in acetonitrile)] to give, after evaporation and lyophilization from 1% acetic acid (aqueous), 9 mg of **7** as an inseparable mixture of two diastereomers. HRMS(FAB) calcd for $C_{23}H_{33}N_7O_6$ 504.2571, found 504.2569; HPLC k' 1.54 [5μ Apex-ODS: Jones Chromatography, 4.6x250 mm, flow = 1.5 mL/min, UV detection at 220 nm, 80:20 (0.1% trifluoroacetic acid (aqueous) : 0.1% trifluoroacetic acid in acetonitrile)]; HPLC k' 3.43 [5μ Apex-ODS: Jones Chromatography, 4.6x250 mm, flow = 1.5 mL/min, UV detection at 220 nm, gradient elution (0.1% trifluoroacetic acid (aqueous) : 0.1% trifluoroacetic acid in acetonitrile) start at 90:10, to 20 min. 50:50, hold for 5 min, return in 5 min. to 90:10]; TLC R_f 0.17 (silica gel, 4:1:1 butanol : acetic acid : water); TLC R_f 0.37 (silica gel, 1:1:1:1 butanol : acetic acid : water : ethyl acetate).

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