Peptide Analogue Systems. 9.[1] Synthesis of 1,2,4-Triazines. 16.[2] Bridged Azapeptides, a Class of Novel 1,4,5,6-Tetrahydro-1,2,4-triazin-3(2*H*)ones

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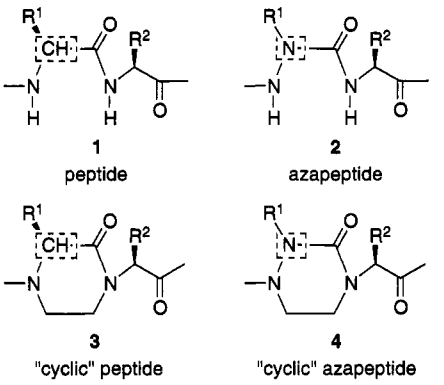
Peptide mimetics have gained considerable importance during recent years as a class of compounds with high therapeutic relevance.[3] These analogues of biologically active peptides can have several distinct advantages over the corresponding native compounds, e.g. reduced enzymatic degradability, oral bioavailability, longer duration of action, and an improved spectrum of activities. Among the peptide mimetics, azapeptides are particularly interesting.[4] This class of peptide analogues, in which an α-CH-group in a peptide chain (**1**) is isoelectronically replaced by nitrogen (**2**) (Scheme 1), display considerable biological activity in many cases. For instance, a compound of this type is marketed as a valuable drug for the treatment of prostate cancer.[5] In addition, many efforts have been made in peptide chemistry to stabilize biologically relevant conformations by incorporating cyclic building blocks into the peptide chain.[3] For instance, in the enkephalin series an ethylene bridge between the nitrogen atoms of two adjacent amino acid residues within the peptide chain leads to the corresponding piperazinones **3** (Scheme 1), which, as "bridged" peptides, represent dipeptide-mimetic units with highly interesting properties.[6]

Novel hybrids of the "aza" and "cyclic" peptide mimetics are the tetrahydro-1,2,4-triazin-3(2*H*)-one derivatives **4**. Of the basic heterocyclic system itself, only a few derivatives are known.[7]

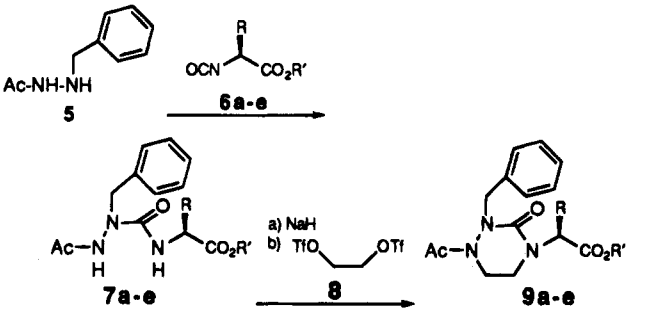
In this paper we describe the synthesis and subsequent transformations of building blocks **4** which represent the first examples of bridged azapeptides. The synthesis of bridged azapeptide mimetics **9a–e** is shown in Scheme 2 and involves a novel cyclization method for the synthesis of this heterocyclic system. Thus, reaction of the deprotonated "linear" azapeptides **7a–e** with ethylene glycol bistriflate (**8**)[8] gave the cyclized products **9a–e** directly in 52–59% yield. This "one-pot" reaction was performed by using 2 equiv of NaH in dry THF, followed by addition of the bifunctional reagent **8**. The use of ethylene dibromide in this reaction failed. Azapeptides **7a–e** were prepared by reaction of N^1-acetyl-N^2-benzylhydrazine (**5**)[9] with isocyanates **6a–e**.[10] The applicability of the new peptide mimetics for transformations is shown in Scheme 3.

Hydrolytic removal of the *N*-acetyl protecting group from **9a** was achieved with boiling trifluoroacetic acid/water which gave **10** in 82% yield after reesterification. The common conditions for the hydrolytic cleavage of an *N*-acetyl group (e.g. with boiling hydrochloric acid/acetic acid) had failed in this case. Deprotection at the C-terminus was accomplished by alkaline saponification of **9a** and gave the carboxylic acid **11**. Interconversion of one peptide analog into another was demonstrated by catalytic hydrogenolysis of **9a** which gave cyclic azaglycine **12** in 93% yield. Finally, N-terminal chain elongation was shown to be possible by coupling **10** with *Z*-glycine (**13**) which gave cyclic azatripeptide **14** in modest yield using the mixed-anhydride method. No reaction could be observed using EDCI or BOP as coupling reagents. The relatively low yield of **14** is perhaps due to the diminished nucleophilicity of the heterocycle, which is also observed in coupling reactions of "linear" azapeptides.

Scheme 1. Different Modifications of the Peptide Backbone



Scheme 2. Synthesis of the Dipeptide Mimetics 9a–e



6, 7, 9	a	b	c	d	e
R	H	C_6H_5-CH_2	CH_3	C_2H_5-CH(CH_3)	CH_3-CH(CH_3)-CH_2
R'	C_2H_5	CH_3	CH_3	CH_3	CH_3

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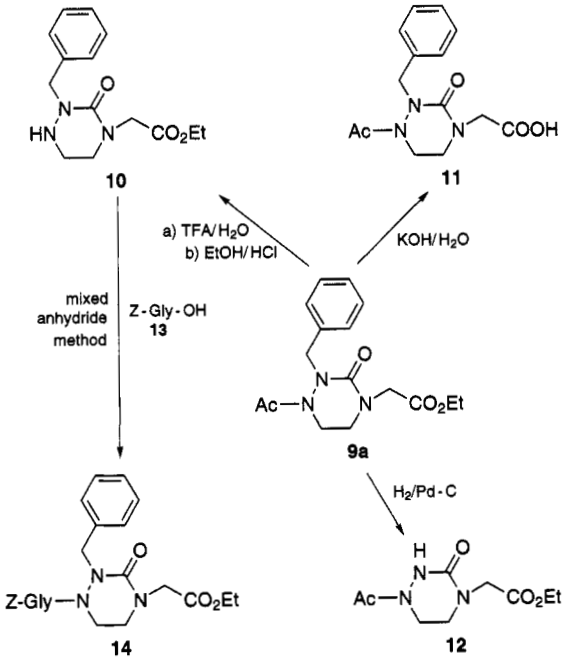
Scheme 3. Transformations in the Cyclic Azapeptide Series



In summary, the synthesis of a series of novel bridged azapeptides **9a–e** was accomplished by a direct cyclization reaction using ethylene glycol bistriflate as a two-carbon electrophile. As demonstrated, these building blocks can be incorporated into longer chains by the usual techniques of peptide synthesis. Incorporation of the new peptide-mimetizing building blocks as "surrogates" into biologically relevant peptidic structures is in progress.

Experimental Section

Melting points were determined on a Reichert melting-point microscope and are uncorrected. ^{1}H NMR spectra were recorded on Bruker AC-200 (200 MHz), Bruker AMX-300 (300 MHz), and Bruker AM-500 (500 MHz) spectrometers with TMS as internal standard. NH proton assignments were made by exchange with TFA. IR spectra were measured on a Perkin Elmer 297 spectrometer, and FAB mass spectra (MS) were recorded with a Vacuum Generator VG 70–250 SE. Optical rotations were measured on a Perkin Elmer Polarimeter 241. THF was stored over 4 Å molecular sieves prior to use. For column chromatography silica gel (0.063–0.200 mm, E. Merck) was used. Chromatographic fractions were concentrated in vacuo at 40 °C. Elemental analyses were performed in the Institut für Organische Chemie, Technische Hochschule Darmstadt (Perkin Elmer CHN 240A and 240B).

General Procedure for the Preparation of the Azapeptides 7. To the hydrazine **5**[9] (1.00 g; 6.09 mmol) in anhydrous THF (100 mL) were added the isocyanates **6**[10] (6.20 mmol) in anhydrous THF (50 mL) dropwise with stirring at 0 °C. The solution was stirred for 1 h at rt and concentrated. The colorless oil was purified by column chromatography (CH_2Cl_2/MeOH = 95.5).

Ethyl 2-[(3-Acetyl-2-benzylcarbazoyl)amino]acetate (7a): 93%; mp 110 °C; IR 3000, 1750, 1645 cm^{-1}; ^{1}H NMR (200 MHz; DMSO-d_6) δ 1.20 (t, J = 7.0 Hz, 3H), 1.83 (s, 3H), 3.73 (d, J = 6.2 Hz, 2H), 4.08 (q, J = 7.0 Hz, 2H), 4.53 (bs, 2H), 7.07 (t, J = 6.2 Hz, 1H, exch TFA), 7.28 (m, 5H), 9.80 (s, 1H, exch TFA); MS (FAB$^+$) *m/e* 294 (M^+ + 1). Anal. Calcd for $C_{14}H_{19}N_3O_4$ (293.33): C, 57.32; H, 6.53; N, 14.33. Found: C, 57.10; H, 6.50; N, 14.20.

Methyl (S)-2-[(3-Acetyl-2-benzylcarbazoyl)amino]-3-phenylpropionate (7b): 90%, mp 139 °C; $[\alpha]^{20}_D$ −20.4° (c = 0.53, MeOH); IR 3000, 1735, 1640 cm^{-1}; ^{1}H NMR (200 MHz DMSO-d_6) δ 1.82 (s, 3H), 3.02 (d, J = 7.0 Hz, 2H), 3.60 (s, 3H), 4.40 (q, J = 7.0 Hz, 1H), 4.52 (m, 2H), 6.71 (d, J = 7.0 Hz, 1H, exch TFA), 7.24 (m, 10H), 9.77 (s, 1H, exch TFA); MS (FAB$^+$) *m/e* 370 (M^+ + 1). Anal. Calcd for $C_{20}H_{23}N_3O_4$ (369.43): C, 65.02; H, 6.45; N, 11.30. Found: C, 65.30; H, 6.45; N, 11.31.

Methyl (S)-2-[(3-Acetyl-2-benzylcarbazoyl)aminopropionate (7c): 87%; colorless oil; $[\alpha]^{20}_D$ −2.3° (c = 0.61, MeOH); IR 3010, 1740, 1650 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.28 (d, J = 7.0 Hz, 3H), 1.82 (s, 3H), 3.61 (s, 3H), 4.19 (quin, J = 7.0 Hz, 1H), 4.52 (bs, 2H) 6.88 (d, J = 7.0 Hz, 1H, exch TFA), 7.26 (m, 5H), 9.74 (s, 1H, exch TFA); MS (FAB$^+$) *m/e* 294 (M^+ + 1). Anal. Calcd for $C_{14}H_{19}N_3O_4$ (293.33); C, 57.33; H, 6.53; N, 14.33. Found: C, 56.97; H, 6.76; N, 14.08.

Methyl (2S,3R)-2-[(3-Acetyl-2-benzylcarbazoyl)amino]-3-methylpentanoate (7d): 63%; mp 108–109 °C; $[\alpha]^{20}_D$ −5.7° (c = 0.87, MeOH); IR 2965, 1730, 1640 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.82 (m, 6H), 1.26 (m, 3H), 1.84 (s, 3H), 3.65 (s, 3H), 4.12 (t, J = 7.0 Hz, 1H), 4.54 (bd, 2H), 6.40 (d, J = 7.0 Hz, 1H, exch TFA), 7.32 (m, 5H), 9.80 (s, 1H, exch TFA); MS (FAB$^+$) *m/e* 336 (M^+ + 1). Anal. Calcd for $C_{17}H_{25}N_3O_4$ (335.41): C, 60.88; H, 7.51; N, 12.53. Found: C, 60.92; H, 7.24; N, 12.51.

Methyl (S)-2-[(3-Acetyl-2-benzylcarbazoyl)amino]4-methylpentanoate (7e): 88%; mp 121 °C; $[\alpha]^{20}_D$ −17.6° (c = 0.97; MeOH); IR 2960, 1740, 1640 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.88 (t, J = 6.0 Hz, 6H), 1.55 (m, 3H), 1.82 (s, 3H), 3.62 (s, 3H), 4.23 (m, 1H), 4.53 (bd, 2H), 6.80 (d, J = 7.0 Hz, 1H, exch TFA), 7.28 (m, 5H), 9.73 (s, 1H, exch TFA); MS (FAB$^+$) *m/e* 336 (M^+ + 1). Anal. Calcd for $C_{17}H_{25}N_3O_4$ (335.41): C, 60.88; H, 7.51; N, 12.53. Found: C, 61.00; H, 7.39; N, 12.60.

General Procedure for the Preparation of 1,4,5,6-Tetrahydro-1,2,4-triazin-3(2*H*)-ones (9). To a solution of the azapeptides **7** (3.41 mmol) in anhydrous THF (200 mL) was added NaH (180 mg, 7.49 mmol). The suspension was stirred at rt for 45 min. Bis-triflate **8**[8] (866.5 mg, 3.41 mmol) in anhydrous THF (100 mL) was added dropwise at 0 °C. It was stirred for 5 h at 0 °C and 12 h at rt. The solution was evaporated, and the residue was dissolved in water (20 mL) and extracted with ethyl acetate (4 × 40 mL). The extracts were dried over anhydrous sodium sulfate and evaporated, and the residue was purified by column chromatography (CH_2Cl_2/MeOH = 96/4).

Ethyl 2-(1-Acetyl-2-benzyl-1,2,3,4,5,6-hexahydro-3-oxo-1,2,4-triazin-4-yl)acetate (9a): 57%; colorless oil; IR 2980, 1740, 1450 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) 1.20 (t, J = 7.0 Hz, 3H), 1.97 (s, 3H), 3.32 (bt, 2H), 4.12 (m, 6H), 5.03 (bs, 2H) 7.34 (bs, 5H); MS (FAB$^+$) *m/e* 320 (M^+ + 1). Anal. Calcd for $C_{16}H_{21}N_3O_4$ (319.37): C, 60.17; H, 6.63; N, 13.16. Found: C, 60.10; H, 6.83; N, 13.20.

Methyl (S)-2-(1-Acetyl-2-benzyl-1,2,3,4,5,6-hexahydro-3-oxo-1,2,4-triazin-4-yl)-3-phenylpropionate (9b): 52%; mp 92 °C; $[\alpha]^{20}_D$ −100.8° (c = 0.91, MeOH); IR 2950, 1730, 1440 cm^{-1}; ^{1}H NMR (200 MHz; DMSO-d_6) δ 2.35 (s, 3H), 3.49 (m, 4H), 3.82 (s, 3H), 4.33 (bs, 2H), 5.35 (s, 2H), 5.73 (m, 1H), 7.29 (m, 10H); MS (FAB$^+$) *m/e* 396 (M^+ + 1). Anal. Calcd for $C_{22}H_{25}N_3O_4$ (395.47): C, 66.82; H, 6.37; N, 10.63. Found: C, 66.80; H, 6.54; N, 10.50.

Methyl (S)-2-(1-Acetyl-2-benzyl-1,2,3,4,5,6-hexahydro-3-oxo-1,2,4-triazin-4-yl)propionate (9c): 58%; mp 100–101 °C; $[\alpha]^{20}_D$ −23.6° (c = 0.68, MeOH); IR 2960, 1740; 1440 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-$_{d6}$) δ 1.37 (d, J = 7.0 Hz, 3H), 1.97 (s, 3H), 3.15 (bt, 2H), 3.61 (s, 3H), 4.23 (bt, 2H), 4.61 (q, J = 7.0 Hz, 1H), 5.02 (m, 2H), 7.33 (bs, 5H); MS (FAB$^+$) *m/e* 320 (M^+ + 1). Anal. Calcd for $C_{16}H_{21}N_3O_4$ (319.37): C, 60.18; H, 6.63; N, 13.16. Found: C, 60.40; H, 6.77; N, 12.90.

Methyl (2S,3R)-2-(1-Acetyl-2-benzyl-1,2,3,4,5,6-hexahydro-3-oxo-1,2,4-triazin-4-yl)-3-methylpentanoate ((9d): 59%; mp 117–118 °C; $[\alpha]^{20}_D$ −49.3° (c = 0.56, MeOH); IR: 2960, 1730, 1450 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.86 (t, J = 7.0 Hz, 3H), 0.94 (d, J = 7.0 Hz, 3H), 1.07 (m, 1H), 1.34 (m, 2H), 1.93 (s, 3H), 3.17 (bs, 2H), 3.29 (bs, 2H), 3.64 (s, 3H), 4.49 (d, J = 7.0 Hz, 1H), 4.73 (bs, 2H), 7.32 (m, 5H), MS (FAB$^+$) *m/e* 362 (M^+ + 1). Anal. Calcd for $C_{19}H_{27}N_3O_4$ (361.45): C, 63.14; H, 7.53; N, 11.63. Found: C, 62.93; H, 7.66; N, 11.24.

Methyl (S)-2-(1-Acetyl-2-benzyl-1,2,3,4,5,6-hexahydro-3-oxo-1,2,4-triazin-4-yl)-4-methylpentanoate (9e): 55%; mp 64 °C; $[\alpha]^{20}_D$ −45.1° (c = 0.48, MeOH); IR 2950, 1720, 1430 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85 (d, J = 3.0 Hz, 3H), 0.88 (d, J = 3.0 Hz, 3H), 1.29 (s, 3H), 1.62 (m, 2H), 2.13 (m, 1H), 2.94 (m, 1H), 3.14 (m, 1H), 3.65 (s, 3H), 3.69 (m, 1H), 3.83 (t, J = 7.0 Hz, 1H), 4.12 (m, 1H), 4.38 (m, 2H), 7.29 (m, 5H); MS

(FAB$^+$) *m/e* 362 ($M^+ + 1$). Anal. Calcd for $C_{19}H_{27}N_3O_4$ (361.45): C, 63.14; H, 7.53; N, 11.63. Found: C, 62.90; H, 7.42; N, 11.50.

Ethyl 2-(2-Benzyl-1,2,3,4,5,6-hexahydro-3-oxo-1,2,4-triazin-4-yl)acetate (10). A solution of **9a** (1.20 g, 3.76 mmol) in a mixture of TFA (40 mL) and H_2O (7 mL) was refluxed for 0.5 h. The solution was evaporated to dryness, and 6 N HCl in EtOH (50 mL) was added. After 48 h at rt and evaporation to dryness, H_2O (30 mL) was added and the mixture extracted with ethyl acetate (5 × 40 mL). After drying (Na_2SO_4) and evaporation the residue was purified by column chromatography (CH_2Cl_2/MeOH = 97.5/2.5) to give **10** (844 mg, 82%) as a colorless oil: IR 3240, 1745, 1640 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 1.22 (t, J = 7.0 Hz, 3H), 2.92 (q, J = 7.0 Hz, 2H), 3.35 (t, J = 7.0 Hz, 2H), 4.03 (s, 2H), 4.13 (q, J = 7.0 Hz, 2H), 4.48 (s, 2H), 5.34 (t, J = 7.0 Hz, 1H, exch TFA) 7.25 (m, 5H); MS (FAB$^+$) *m/e* 278 ($M^+ + 1$). Anal. Calcd for $C_{14}H_{19}N_3O_3$ (277.32): C, 60.63; H, 6.91; N, 15.15. Found: C, 60.51; H, 6.99; N, 14.94.

2-(1-Acetyl-2-benzyl-1,2,3,4,5,6-hexahydro-3-oxo-1,2,4-triazin-4-yl)acetic Acid (11). **9a** (159 mg, 0.50 mmol) and KOH (56 mg, 1.0 mmol) in ethanol (20 mL) and water (10 mL) were refluxed for 1 h. The reaction mixture was evaporated and the residue dissolved in water (10 mL), acidified with 1 N HCl (0.5 mL, 0.5 mmol), and extracted with ether (5 × 20 mL). The extracts were dried over anhydrous sodium sulfate and evaporated. Recrystallization from diisopropyl ether gave **11** (123 mg, 85 %) as colorless crystals: mp 116–117 °C; IR 2940, 1720, 1620 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 1.99 (s, 3H), 3.34 (bt, 2H), 3.93 (s, 2H), 4.23 (bt, 2H), 5.01 (bs, 2H), 7.33 (bs, 5H), 12.69 (bs, 1H exch TFA); MS (FAB$^+$) *m/e* 292 ($M^+ + 1$). Anal. Calcd for $C^{14}H^{17}N_3O_4$ (291.30): C, 57.72; H, 5.88; N, 14.43. Found: C, 57.50; H, 6.14; N, 14.40.

Ethyl 2-(1-Acetyl-1,2,3,4,5,6-hexahydro-3-oxo-1,2,4-triazin-4-yl)acetate (12). **9a** (245 mg, 0.77 mmol) in methanol (30 mL) was hydrogenated in the presence of 5% Pd/C (300 mg) at rt for 19.5 h. The solution was filtered and evaporated affording **12** (164 mg, 93%) as colorless crystals: mp 76 °C; IR 3200, 1750, 1670 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 1.20 (t, J = 7.0 Hz, 3H), 2.03 (s, 3H), 3.27 (t, J = 5.5 Hz, 2H), 3.70 (t, J = 5.5 Hz, 2H), 4.01 (s, 2H), 4.12 (q, J = 7.0 Hz, 2H), 9.12 (s, 1H, exch TFA); MS (FAB$^+$) *m/e* 230 ($M^+ + 1$). Anal. Calcd for $C_9H_{15}N_3O_4$ (229.24): C, 47.16; H, 6.59; N, 18.33. Found: C, 47.30; H, 6.85; N, 18.10.

Ethyl 2-(2-Benzyl-1-(Z-Gly)-1,2,3,4,5,6-hexahydro-3-oxo-1,2,4-triazin-4-yl)acetate (14). To a cooled (−15 °C) solution of the amino acid derivative **13** (286 mg, 1.36 mmol) and triethylamine (0.19 mL, 1.36 mmol) in dry THF (10 mL) was added ethyl chloroformate (0.13 mL, 1.36 mmol), and the mixture was stirred at −10 °C for 45 min. The mixture was cooled to −24 °C and a solution of the ester **10** (375 mg, 1.35 mmol) in dry THF (8 mL) was slowly added. After slow warm-up to rt (90 min), stirring for 20 h, and filtration, the solution was evaporated to dryness. The residue was dissolved in CH_2Cl_2 (10 mL) and the organic phase was washed with water (3 × 5 mL) and dried over Na_2SO_4. After evaporation of the solvent the residue was purified by column chromatography (CH_2Cl_2/MeOH = 99/1) to give the tripeptide analogue **14** (178 mg, 28%) as a viscous oil (145 mg of unreacted starting material **10** was also isolated): IR 3420, 1730, 1660 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 1.22 (t, J = 7.0 Hz, 3H), 3.27 (m, 4H), 3.83 (s, 2H), 4.01 (m, 2H), 4.15 (q, J = 7.0 Hz, 2H), 4.66 (bs, 2H), 5.07 (s, 2H), 7.03 (bs, 1H, exch TFA), 7.33 (m, 10H); MS (FAB$^+$) *m/e* 469 ($M^+ + 1$). Anal. Calcd for $C_{24}H_{28}N_4O_6$ (468.51): C, 61.53; H, 6.02; N, 11.96. Found: C, 61.40; H, 6.12; N, 11.95.