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Design and synthesis of cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Xaa-] peptide analogues by click chemistry

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ABSTRACT

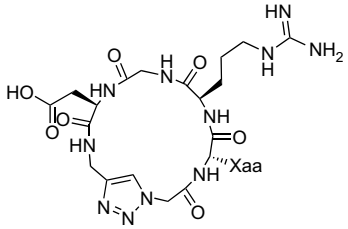
A simple and mild synthesis of a new family of cyclopeptide analogues cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Xaa-] that is obtained by cyclization with click chemistry was investigated. In addition, the method was also successfully expanded to the synthesis of their analogues of different ring sizes. The result supports the potential utility of click chemistry in the preparation of novel integrin domain-binding antagonists and other cyclopeptide analogues.

1. Introduction

Integrins are a family of membrane adhesion receptors, which are highly involved in cell–cell and cell–matrix interactions.[1] They are heterodimeric transmembrane glycolproteins composed of an α-subunit and a β-subunit. One of the most prominent members of this class of adhesion molecules is $\alpha_v\beta_3$ integrin, which plays an important role in tumor-induced angiogenesis and tumor metastasis.[2] This integrin interacts with the extracellular matrix proteins through the arginine-glycine-aspartic acid sequence (RGD).[3] During the last decade, this universal recognition site (RGD) became the basis for the development of a variety of RGD-containing peptides[4] and RGD peptidomimetics[5] with a high affinity and selectivity for $\alpha_v\beta_3$. In particular, Kessler et al. designed and synthesized a series of cyclic peptides containing the RGD to exhibit a relatively high and specific affinity toward the $\alpha_v\beta_3$ integrins.[6,4b]

In recent years, click chemistry with copper (I)-catalyzed Huisgen 1,3-dipolar azide–alkyne cycloaddition[7] producing 1,2,3-triazole[8] has exhibited increasing importance in organic chemistry especially for the synthesis of peptides. The application of 1,2,3-triazole has been reported in peptide bond isosteres, peptide nanotubes,[9] β-turn mimics,[10] protease inhibitors,[11] cyclopeptide analogues,[7b,12] and peptide chain analogues.[13]

In this study, we describe the synthesis of a new family of cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Xaa-] **7** in which an amide bond is replaced by the 1,2,3-triazole based on click chemistry (Scheme 1). It is the first application of [3+2] Huisgen cycloaddition click chemistry in solution cyclization and synthesis of integrin domain-binding peptides.



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Xaa=Gly,Ala,Leu,Ile,Cys,Met,Asp,Asn,
Glu,Lys,Arg,His,Phe,Trp,Pro,0,Gly-Gly.

Scheme 1. Structure of cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Xaa-].

2. Results and discussion

The strategy for the synthesis of cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Xaa-], where Xaa represents the residue of Gly, Ala, Leu, Ile, Cys, Met, Asp, Asn, Glu, Lys, Arg, His, Phe, Trp, Pro, and Gly-Gly,

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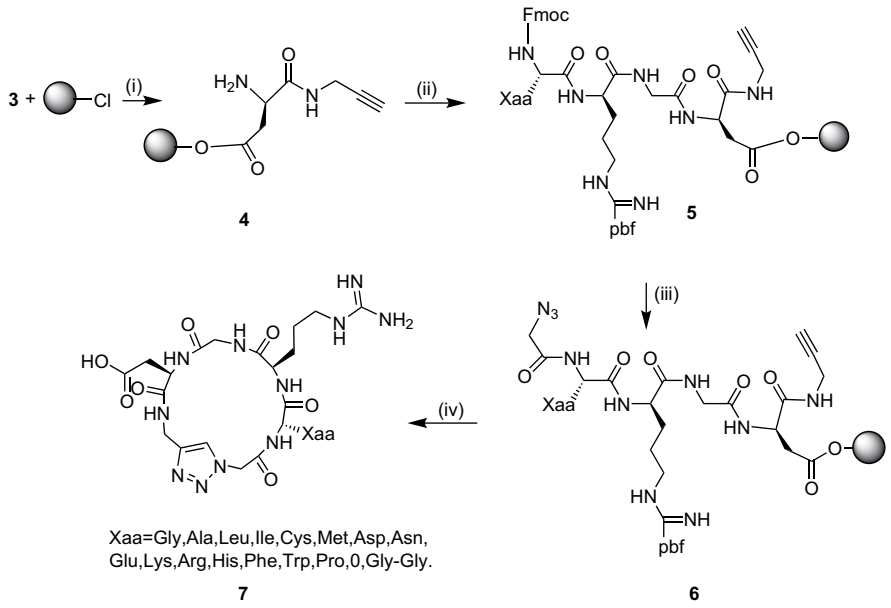


Scheme 2. Strategy for the synthesis of cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Xaa-]. Reagents and conditions: (i) (1) DCC/HOBt, THF, pH=7, 25 °C, 2 h; (2)TFA/DCM=2/3 (v/v); (ii) TBTU,NMM, 30 °C, 2–4 h; (iii) (1) 20% piperidine/DMF, 30 min; (2) EDC/HOBt, DIPEA, rt, 4–6 h; (iv) (1) CuBr/DBU=1/3, DCM, rt, 6 h; (2) TFA/H_2O/TIPS=95/2.5/2.5.

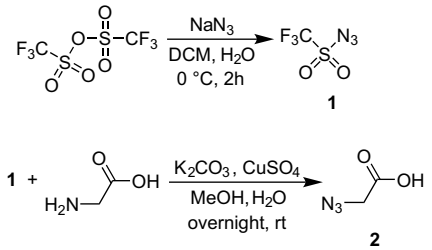
respectively, is outlined in Scheme 2. For the click chemistry 1,3-dipolar cycloaddition reaction, the first step is necessary to introduce an azide moiety on the scaffold (Scheme 3).

Preparation of azide (**2**) from glycine and sodium azide by diazo transfer using a sulfonyl azide had been reported previously in the literature.[14] The similar approach has been adopted in our study.

As the other component for the [3+2] Huisgen 1,3-dipolar cycloaddition, the terminal alkyne group was synthesized from propargylamine. The N-terminal amino group of propargylamine condensed with the C-terminal carboxylic group of Fmoc-Asp(O^tBu)-OH via DCC/HOBt mediated the coupling reaction. The deprotection of *tert*-butyl ester group (O^tBu group) was carried out with TFA/DCM (v/v=2/3) for 1 h at room temperature to give Fmoc-Asp-propargyl (Scheme 4).

The linear peptide analogue (N_3-Gly-Gly-Arg(Pbf)-Gly-Asp-propargyl) was synthesized by standard solid-phase peptide synthesis (SPPS) strategy on resin. It should be noted that the initial experiments with Fmoc-Asp-propargyl coupled to the Wang resin were not successful. The amino acids should be easily loaded onto the 2-chlorotrityl chloride resin (2-CTC resin) and the peptides elongated more efficiently with high purity. Thus, the 2-CTC resin was chosen as a candidate supporter. For side-chain protection 2,2,4,6,7-penta methyl dihydro-benzofuran-5-sulfonyl (Pbf) was used for arginine. Coupling reactions were performed using protected amino acids, activated with HBTU or EDC/HOBt in the presence of base (NMM or DIPEA) for 4 h. Following this approach, a series of linear peptide analogues were prepared on 2-CTC resin.

Cyclization of azido-alkynl peptide analogues is considered as a most important step in the synthesis of the cyclopeptides. The



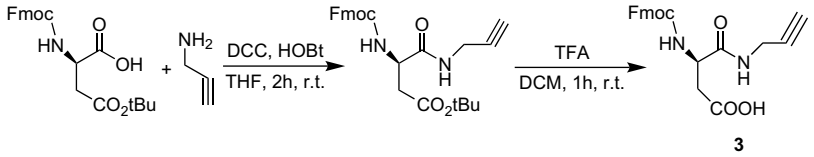
Scheme 3. Synthesis of Azido-glycine.

on-resin cyclization was performed by Vidal et al.[12c] But the completeness of solid-phase peptide synthesis may not be detected directly until after final cleavage, we employed the solution method to cyclize the linear peptides. The linear peptides in their protected forms were cleaved from the resins with mild cleavage reagent (TFE/DCM=2/8), under which the protecting groups at the side chains remained intact. Dilute conditions (about 10 mg/l) were employed to avoid intermolecular coupling of the linear peptides.

For optimization of the azide–alkyne cyclization condition by click chemistry, a variety of reaction conditions were evaluated for the best cyclization efficiency (Table 1). At first, CuBr was employed to catalyze with sodium ascorbate at room temperature for 2 h, 4 h, 8 h and 16 h. However, the desired product was not detected at all. When 1,8-diazabicyclo [5.4.0] undec-7-ene (DBU) was introduced instead of sodium ascorbate, the cyclization yield of the desired product was still poor, and only a trace amount of the desired product was detected. Thus we attempted to raise the reaction temperature, prolong the reaction time, and increase the loading of the CuBr and DBU. At last, the desired product was obtained in better yield (50%) at room temperature for 6 h (Table 1, entry 14). HPLC was used to assess the completion of the reaction. The peak of the cyclic peptide, which is much earlier than its corresponding linear one's, was monitored during RP-HPLC analysis. After 4–6 h at room temperature, the conversion of the azide and terminal alkyne in 1,2,3-triazole completed.

The peptides were deprotected and then purified by semi-preparative RP-HPLC. The results obtained by using several different amino acids are summarized in Table 2. In addition, the method was also successfully expanded to the synthesis of their analogues of different ring sizes (Table 2, entries 7p and 7q). All the cyclic peptides' structures were confirmed by ESI-MS, ^1H NMR, and ^{13}C NMR spectroscopy. The cyclization's isolated yield of the cyclopeptide was 33–62% with the solution method.

To determine the cytotoxic effect of cyclopeptide cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Xaa-], the human umbilical vein endothelial cell line (HUVEC), human lung carcinoma cell line (SPC-A-1), and hepatocellular carcinoma cell line (HepG2) were determined using MTT method and the results are summarized in Table 3. These results clearly showed that **7j**, **7q**, **7a**, **7o**, **7h**, and **7i** are the most cytotoxic compounds for all the three cell lines compared with the very well known ligand cRGDfK, and **7b**, **7p**, **7m**, **7k**, **7f**, and **7e** are



Scheme 4. Synthesis of Fmoc-Asp-propargyl.

much less cytotoxic than cRGDfK. In addition, the cytotoxicity of the same compound may be significantly different for different cell lines, such as **7g** and **7n**. At the same time, no obvious cytotoxicity was found in **7c**, **7d**, and **7l**.

3. Conclusion

In summary, we have devised a simple and mild synthesis of a new family of cyclic peptides containing the RGD tripeptide sequence cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Xaa-] that was obtained by cyclization with click chemistry. The result supports the potential utility of click chemistry in the preparation of novel cyclopeptides. Their cytotoxic activity has also been evaluated using MTT method. The further biological activity of triazole cyclopeptide analogues will be tested and reported in coming work.

4. Experimental

4.1. General

All chemicals were obtained from commercial suppliers and used without further purification. Melting points were determined using XT-4 apparatus and were not corrected. The optical rotation was measured by Atago Polax-2L polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AVANCE DMX-500 spectrometry at 500 MHz and 125 MHz in DMSO-d_6, respectively. Chemical shifts are reported in parts per million (δ), relative to the internal standard of tetramethylsilane (TMS). ^{1}H and ^{13}C NMR spectra were acquired under standard conditions (5 mm QNP probe). Mass spectra were performed on a Bruker Esquire 3000plus mass spectrometer (Bruker-Franzen Analytik GmbH Breman, Germany) equipped with ESI interface and ion trap analyzer. HRMS were obtained on a Bruker 7-T FT-ICR MS equipped with an electrospray source (Billelica, MA, USA).

4.2. General procedure for the synthesis of azido-Gly (2) by diazo transfer

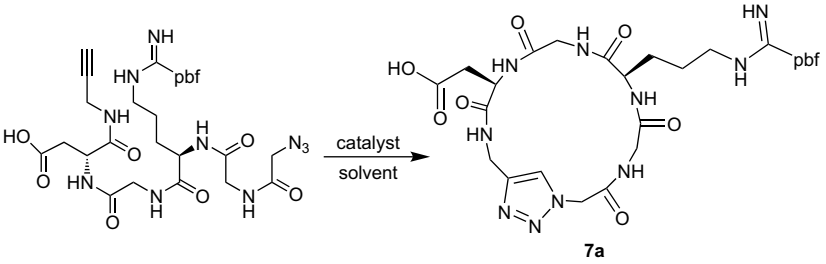
4.2.1. Preparation of triflyl azide

Sodium azide (6 g, 92.4 mmol) was dissolved in distilled water (15 mL) and then dichloromethane (DCM, 24 mL) was added. The mixture was cooled on ice bath for 20 min. Triflyl anhydride (3 mL, 18 mmol) was added slowly over 5 min and the mixture was stirred for 2 h. The mixture was extracted with DCM (2×12 mL). The organic portions, containing the triflyl azide, were pooled, washed once with saturated Na_2CO_3, and used without further purification. Cytotoxic activity assay was determined with a 96-well format using a Tecan microplate reader.

4.2.2. Preparation of azido-Gly

Glycine (0.675 g, 9 mmol) was combined with K_2CO_3 (1.783 g, 13.5 mmol) and $CuSO_4$ (0.0226 g, 90 μmol) in distilled water (30 mL) and methanol (MeOH, 60 mL). The triflyl azide in DCM (48 mL) was added and the mixture was stirred at ambient

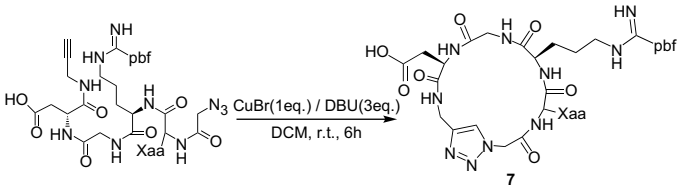
Table 1
Optimization of cyclization condition by click chemistry



Entry	Catalyst (mol %)	Solvent	Temp (°C)	Time (h)	Yield[a] (%)
1[b]	CuBr/Na ascorbate (1/3)	Toluene	rt	2	—
2[b]	CuBr/Na ascorbate (1/3)	Toluene	rt	4	—
3[b]	CuBr/Na ascorbate (1/3)	Toluene	rt	8	—
4[b]	CuBr/Na ascorbate (1/3)	Toluene	rt	16	—
5	CuBr/DBU (0.2/3)	Toluene	55	4	1
6	CuBr/DBU (0.2/3)	Toluene	55	6	1
7	CuBr/DBU (0.2/3)	Toluene	55	8	1
8	CuBr/DBU (0.2/3)	Toluene	110	4	10
9	CuBr/DBU (0.2/3)	DCM	110	6	15
10	CuBr/DBU (0.2/3)	Toluene	110	8	12
11	CuBr/DBU (1/3)	Toluene	rt	1	20
12	CuBr/DBU (1/3)	DCM	rt	2	32
13	CuBr/DBU(1/3)	DCM	rt	4	49
14	CuBr/DBU(1/3)	DCM	rt	6	50

[a] Isolated yields.
[b] No target product was observed.

Table 2
Synthesis for a series of cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Xaa-]



Entry	Xaa	Yield[a] (%)
7a	Gly	50
7b	Ala	46
7c	Leu	33
7d	Ile	53
7e	Cys	53
7f	Met	61
7g	Asp	46
7h	Asn	47
7i	Glu	52
7j	Lys	54
7k	Arg	43
7l	His	58
7m	Phe	61
7n	Trp	47
7o	Pro	55
7p[b]	0	51
7q[c]	Gly-Gly	62

[a] Isolated yields.
[b] Cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-].
[c] Cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Gly-Gly-].

temperature and pressure overnight. Subsequently, the organic solvents were removed in vacuo and the residue was diluted with water (150 mL). This was acidified to pH 6 with concd HCl, diluted with phosphate buffer (250 mM, pH 6.2, 150 mL), and extracted with ethyl acetate (EA, 4×60 mL) to remove sulfonamide by-product. The aqueous phase was then acidified to pH 2 with HCl. The product was obtained from another round of EA extractions (3×60 mL). These EA extracts were combined, dried (over $MgSO_4$), and evaporated to dryness giving pale yellow oils in 78% yield with no need for further purification.

Table 3
Cytotoxicity of various samples against human cells, HUVEC, SPC-A-1, and HepG2 in vitro (IC_{50}, μM)

Entry	The human cell lines and IC_{50} (μM)		
	HUVEC	SPC-A-1	HepG2
7a	0.28	0.35	0.32
7b	14.93	15.38	13.75
7c[a]	—	—	—
7d[a]	—	—	—
7e	32.43	33.65	33.02
7f	29.85	29.53	30.43
7g	2.58	12.65	21.61
7h	0.72	0.76	0.63
7i	0.93	0.87	0.62
7j	0.21	0.28	0.24
7k	23.70	24.56	25.70
7l[a]	—	—	—
7m	15.76	16.78	14.37
7n	18.56	37.45	24.53
7o	0.33	0.29	0.33
7p	14.63	17.57	16.34
7q	0.21	0.27	0.24
cRGDfK[b]	0.27	0.28	0.23

[a] No significant cytotoxicity and the IC_{50} value is higher than 50 μM.
[b] positive control.

4.3. General procedure for on-resin loading propargyl aspartic acid and linear sequence of amino acids

To a 100 mL round-bottomed flask equipped with a $CaCO_3$ drying tube and charged with Fmoc-Asp(O^tBu)-OH (0.93 g, 3 mmol) in freshly distilled THF (25 mL) were added *N*,*N*′-dicyclohexylcarbodimide (DCC, 2.49 g, 12 mmol) and 1-hydroxybenzotriazole (HOBt, 1.62 g, 12 mmol). Propargylamine (55 mg, 1 mmol) was then added in freshly distilled THF (5 mL). Reactions were carried out for 2 h with ninhydrin Kaiser test monitoring and recoupled as necessary. Deprotection of O^tBu group was carried out with TFA/DCM (v/v=2/3) for 1 h. This solution was extracted with EA (3×20 mL) and washed with H_2O (10 mL). The combined organics were dried, filtered, and concentrated in vacuo to yield Fmoc-Asp-propargyl (**3**) (5.1 mmol, 85%) as a white solid. The side-chain O^tBu group for propargyl-Asp (O^tBu) was removed by treatment with TFA (15 mL) for 2.5 h at room temperature. The Fmoc-Asp-propargyl was loaded on 2-CTC resin and then other amino acids were assembled on resin according to the standard SPPS procedure.

4.4. General procedure for cyclization of peptides in solution by click chemistry

The linear, side-chain-protected peptide was cleaved from the resin using mild cleavage reagent TFE/DCM (2/8, 15 mL) with three drops of AcOH for 2 h and evaporated. The products were purified by preparative RP-HPLC on an Agilent C_{18} column (5 μm, 4.6×250 mm) with a gradient program (solvent A is water with 0.1% trifluoroacetic acid and solvent B is acetonitrile with 0.1% trifluoroacetic acid) at a flow rate of 1 mL/min with UV detection at 210 nm and obtained as white solid. The peptide (0.4 mmol) was located in distilled DCM (25 mL) on a flame-dried round-bottomed flask, and CuBr (0.286 g, 2 μmol) and DBU (0.912 g, 6 μmol) were added. The suspension was stirred in DCM at room temperature for 4–6 h, depending on peptide sequence, until the completeness of the cyclization reaction was monitored by HPLC.

The solvent was evaporated in vacuo and side chains of cyclopeptide were deprotected using cleavage reagent for 2 h at room temperature, and then continued to evaporate. The crude cyclopeptide was isolated by precipitation with dry-ice cold Et_2O and centrifugation. The products were purified by preparative RP-HPLC on an Agilent C_{18} column (5 μm, 4.6×250 mm) with a gradient program (solvent A is water with 0.1% trifluoroacetic acid and solvent B is acetonitrile with 0.1% trifluoroacetic acid) at a flow rate of 1 mL/min with UV detection at 210 nm and obtained as white solid.

4.5. Cytotoxic activity assay

The three cell lines used for the tests were human umbilical vein endothelial cell line (HUVEC), human lung carcinoma cell line (SPC-A-1), and hepatocellular carcinoma cell line (HepG2), and the MTT method was carried out. Briefly, the cells in exponential phase were placed in a 96-well plate, containing 5000 cells/well. After incubating overnight to allow the cells to attach, the serial concentrations of various test samples were added to each well. The cells were continually incubated for 72 h. The groups treated by the corresponding culture media were used as control groups. To each well was added 20 μL of thiazolyl blue 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) solution and the incubation was continued for 4 h. The formazane crystals thus formed were dissolved by 150 μL DMSO. After 15 min, the absorbance was detected in the microplate reader (TECAN, Austria) at wavelengths of 570 nm. The data reported represent the means of triplicate measurement; the standard errors of the mean were less than 15%.

*4.5.1. Cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Gly-] (**7a**)*

White solids, mp: 162–164 °C; $[\alpha]_D^{25}$ +23.2 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, DMSO-d_6): δ 1.40 (2H, d), 1.50–1.56 (1H, m), 1.71–1.79 (1H, m), 2.71–2.77 (2H, m), 3.07–3.11 (2H, m), 3.48 (1H, *J*=5.0 Hz, d), 3.69 (1H, *J*=5.8 Hz, d), 3.79–3.89 (2H, m), 4.26 (2H, *J*=14.2, 6.9 Hz, dd), 4.45 (2H, s), 4.54 (2H, *J*=6.3 Hz, d), 5.05 (1H, s), 5.29 (1H, s), 7.50 (1H, s), 7.68 (2H, s), 7.83 (1H, *J*=5.9 Hz, d), 8.03 (1H, *J*=8.7 Hz, t), 8.28 (1H, *J*=5.9 Hz, d), 8.42 (1H, *J*=7.1 Hz, t), 8.84 (1H, *J*=5.8 Hz, t); ^{13}C NMR (125 MHz, DMSO-d_6): δ 26.2, 26.6, 29.6, 35.3, 35.9, 36.5, 42.9, 43.3, 44.3, 51.5, 52.8, 124.4, 157.8, 167.8, 169.7, 170.0, 171.7, 172.5, 172.8; IR (KBr, cm^{-1}): 3393, 3073, 2932, 1667, 1538, 1417, 1331, 1201, 1136, 1058, 1024; HRMS calcd for $C_{19}H_{30}N_{11}O_7$ $(M+H)^+$ 524.2341, found 524.2369.

*4.5.2. Cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Ala-] (**7b**)*

White solids, mp: 164–165 °C; $[\alpha]_D^{25}$ +56.3 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, DMSO-d_6): δ 1.23 (1H, *J*=6.3 Hz, d), 1.28 (2H, *J*=7.0 Hz, d), 1.38–1.45 (2H, m), 1.87–1.93 (2H, m), 2.67–2.75 (2H, m), 2.89 (2H, s), 4.12–4.19 (3H, m), 4.21 (1H, *J*=5.0 Hz, d), 4.28 (1H, *J*=6.3 Hz, d), 4.39 (1H, *J*=14.3, 7.2 Hz, dd), 4.40 (1H, *J*=7.1 Hz, d), 5.07 (1H, *J*=6.3 Hz, d), 5.27 (1H, *J*=16.6 Hz, d), 6.92 (2H, s), 7.36 (2H, s), 7.63 (1H, s), 7.88 (1H, *J*=7.8 Hz, d), 8.26 (1H, *J*=8.5 Hz, d), 8.35 (1H, *J*=6.9 Hz, t), 8.48 (1H, *J*=7.5 Hz, d), 8.78 (1H, *J*=5.8 Hz, d), 12.38 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 18.6, 24.5, 26.2, 27.1, 29.4, 30.0, 32.9, 36.1, 43.0, 49.1, 51.2, 54.6, 124.5, 157.8, 169.9, 170.2, 171.7, 172.8, 173.0, 173.6; IR (KBr, cm^{-1}): 3361, 3063, 2980, 2936, 1673, 1538, 1426, 1384, 1326, 1202, 1133, 1058; HRMS calcd for $C_{20}H_{32}N_{11}O_7$ $(M+H)^+$ 538.2481, found 538.2468.

*4.5.3. Cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Leu-] (**7c**)*

White solids, mp: 167–169 °C; $[\alpha]_D^{25}$ −61.2 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, DMSO-d_6): δ 0.86 (3H, *J*=6.5 Hz, d), 0.91 (3H, *J*=6.5 Hz, d), 1.47–1.55 (5H, m), 1.69–1.76 (2H, m), 2.74–2.82 (2H, m), 3.09 (2H, *J*=6.8 Hz, t), 3.94 (1H, *J*=6.8 Hz, d), 4.05 (2H, s), 4.21 (1H, s), 4.44 (1H, *J*=14.1, 7.1 Hz, dd), 4.65 (1H, *J*=16.3 Hz, d), 5.03 (1H, *J*=6.3 Hz, d), 5.28 (1H, *J*=6.3 Hz, d), 6.93 (2H, s), 7.37 (2H, s), 7.61 (1H, s), 7.86 (1H, *J*=7.5 Hz, d), 8.09 (1H, *J*=6.0 Hz, t), 8.35 (1H, *J*=6.6 Hz, d), 8.47 (1H, *J*=7.7 Hz, d), 8.63 (1H, *J*=6.6 Hz, d), 12.22 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 22.1, 23.7, 24.9, 25.7, 30.1, 35.7, 36.3, 42.6, 50.7, 52.6, 52.7, 53.8, 123.7, 147.0, 157.4, 167.0, 169.4, 171.2, 172.3, 172.5, 172 .8; IR (KBr, cm^{-1}): 3404, 3084, 2959, 2871, 1667, 1538, 1470, 1417, 1384, 1202, 1138, 1057, 1019; HRMS calcd for $C_{23}H_{38}N_{11}O_7$ $(M+H)^+$ 580.2939, found 580.2975.

*4.5.4. Cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Ile-] (**7d**)*

Yellow solids, mp: 167–168 °C; $[\alpha]_D^{25}$ +25.3 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, DMSO-d_6): δ 0.82–0.91 (6H, m), 1.40–1.47 (4H, m), 1.62–1.74 (2H, m), 1.80–1.87 (1H, m), 2.74–2.81 (2H, m), 3.09 (2H, *J*=6.3 Hz, d), 3.95 (1H, *J*=6.4 Hz, d), 4.02 (2H, *J*=7.0 Hz, d), 4.10 (1H, *J*=5.3 Hz, d), 4.44 (1H, *J*=14.2, 7.5 Hz, dd), 4.88 (1H, s), 5.11 (1H, s), 5.28 (1H, *J*=16.1 Hz, d), 6.95 (2H, s), 7.37 (2H, s), 7.63 (1H, s), 8.11 (1H, *J*=6.3 Hz, d), 8.27 (1H, *J*=8.4 Hz, t), 8.36 (1H, *J*=6.9 Hz, d), 8.44 (1H, *J*=7.9 Hz, d), 8.52 (1H, *J*=6.6 Hz, d), 12.35 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 12.4, 12.5, 25.9, 29.7, 30.3, 36.4, 42.9, 50.8, 51.0, 53.1, 53.4, 53.7, 59.3, 60.2, 124.2, 147.4, 157.8, 167.1, 169.8, 171.6, 172.3, 172.9, 173.0; IR (KBr, cm^{-1}): 3404, 3084, 2959, 2871, 1667, 1538, 1470, 1417, 1384, 1202, 1138, 1057, 1019; HRMS calcd for $C_{23}H_{38}N_{11}O_7$ $(M+H)^+$ 580.2950, found 580.2946.

*4.5.5. Cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Cys-] (**7e**)*

White solids, mp: 189–190 °C; $[\alpha]_D^{25}$ −47.5 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, DMSO-d_6): δ 1.36 (2H, *J*=6.5 Hz, d), 1.44–1.52 (1H, m), 1.62–1.71 (1H, m), 2.53–2.64 (2H, m), 2.89 (2H, *J*=6.2 Hz, d), 3.69 (1H, *J*=6.1 Hz, d), 3.72 (1H, *J*=6.4 Hz, d), 4.31 (H, *J*=6.6 Hz, d), 4.39 (2H, *J*=14.3, 7.1 Hz, dd), 4.56 (2H, *J*=6.3 Hz, d), 4.61 (2H, *J*=6.3 Hz, d), 6.16 (2H, *J*=7.5 Hz, d), 6.79 (2H, s), 7.23 (2H, s), 7.80 (1H, s), 8.12 (1H, *J*=6.7 Hz, d), 8.29 (1H, *J*=7.1 Hz, t), 8.47 (1H, *J*=6.6 Hz, d), 8.54 (1H, *J*=6.7 Hz, t), 8.75 (1H, *J*=6.8 Hz, t), 12.36 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 24.2, 26.3, 27.4, 27.7, 29.2, 30.5, 32.3, 36.1, 43.3, 49.3, 51.7, 56.3, 124.8, 156.8, 169.3, 170.5, 171.7, 172.3, 173.2, 173.9; IR (KBr, cm^{-1}): 3373, 3079, 2926, 2849, 1667, 1538, 1417, 1384, 1331, 1201, 1136, 1057, 1019; HRMS calcd for $C_{20}H_{32}N_{11}O_7S$ $(M+H)^+$ 570.2183, found 570.2142.

*4.5.6. Cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Met-] (**7f**)*

Yellow solids, mp: 193–195 °C; $[\alpha]_D^{25}$ −85.6 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, DMSO-d_6): δ 1.42–1.49 (2H, m), 1.60–1.67 (1H, m), 1.76–1.84 (1H, m), 1.97 (1H, s), 2.02 (1H, s), 1.92–2.05 (3H, m), 2.34–2.42 (2H, m), 2.67 (1H, s), 3.09 (3H, *J*=6.0 Hz, d), 3.72–3.83 (2H, m), 4.28 (2H, *J*=15.3, 6.7 Hz, dd), 4.41 (1H, s), 4.57 (1H, *J*=5.4 Hz, d), 5.17 (2H, *J*=14.8 Hz, d), 7.49 (1H, s), 7.79 (1H, *J*=8.9 Hz, d), 8.21–8.27 (1H, m), 8.33 (1H, s), 8.42 (1H, s), 8.60 (1H, s), 12.38 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 15.8, 26.2 29.4, 30.6, 33.5, 35.7, 37.5, 41.2, 41.6, 43.1, 50.7, 53.3, 53.5, 125.5, 129.6, 145.7, 157.9, 166.6, 169.8, 171.4, 171.9, 172.8, 172.9; IR (KBr, cm^{-1}): 3383, 3073, 2932, 2849, 1661, 1538, 1434, 1384, 1201, 1137, 1047; HRMS calcd for $C_{22}H_{36}N_{11}O_7S$ $(M+H)^+$ 598.2435, found 598.2471.

*4.5.7. Cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Asp-] (**7g**)*

White solids, mp: 193–195 °C; $[\alpha]_D^{25}$ +23.4 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, DMSO-d_6): δ 1.41–1.44 (2H, m), 1.56 (2H, *J*=9.4 Hz, d), 2.57–2.63 (1H, m), 2.76–2.83 (1H, m), 3.07–3.11 (2H, m), 3.90 (2H, *J*=6.3 Hz, d), 4.13 (1H, s), 4.25 (1H, *J*=4.7 Hz, d), 4.40 (1H, *J*=14.7, 7.7 Hz, dd), 4.56–4.51 (1H, m), 5.11 (1H, *J*=16.7 Hz, d), 5.26 (1H, *J*=16.6 Hz, d), 7.01 (2H, s), 7.26 (1H, s), 7.51 (1H, s), 7.65 (1H, s), 7.89 (1H, *J*=8.0 Hz, d), 7.99 (1H, *J*=5.6 Hz, t), 8.35 (1H, *J*=6.7 Hz, d), 8.47 (1H, *J*=7.4 Hz, d), 8.86 (1H, *J*=6.6 Hz, d), 12.38 (2H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 26.0, 30.3, 36.1, 36.6, 36.9, 40.2, 43.0, 51.3, 52.4, 53.0, 53.1, 124.4, 147.3, 157.8, 167.6, 169.7, 171.6,172.5, 172.6, 172.9; IR (KBr, cm^{-1}): 3375, 3216, 3073, 2936, 1667, 1538, 1416, 1384, 1331, 1200, 1137, 1058, 1024; HRMS calcd for $C_{21}H_{32}N_{11}O_9$ $(M+H)^+$ 582.5467, found 582.5423.

*4.5.8. Cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Asn-] (**7h**)*

White solids, mp: 187–190 °C; $[\alpha]_D^{25}$ +42.8 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, DMSO-d_6): δ 1.43 (2H, *J*=6.8 Hz, d), 1.52–1.59 (1H, m), 1.78–1.84 (1H, m), 2.76–2.87 (2H, m), 3.01–3.09 (4H, m), 3.53–3.58 (2H, m), 3.89 (1H, s), 4.13 (1H, s), 4.38 (1H, *J*=14..4, 7.1 Hz, dd), 4.56 (1H, s), 5.13 (1H, *J*=16.6 Hz, d), 5.25 (1H, *J*=16.7 Hz, d), 7.01 (2H, s), 7.45 (2H, s), 7.64 (1H, s), 7.82 (1H, s), 7.97 (1H, s), 8.33 (1H, *J*=5.6 Hz, t), 8.46 (1H, *J*=7.4 Hz, d), 8.71 (1H, *J*=6.8 Hz, d), 12.31 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 25.9, 30.2, 36.0, 36.5, 37.4, 41.5, 42.9, 51.3, 52.4, 52.9, 53.1, 124.4, 147.2, 157.8, 167.5, 169.7, 171.6, 172.0, 172.2, 172.6, 172.9; IR (KBr, cm^{-1}): 3350, 3210, 3073, 2931, 1669, 1538, 1417, 1386, 1336, 1201, 1136, 1059, 1030; HRMS calcd for $C_{21}H_{33}N_{12}O_8$ $(M+H)^+$ 581.2568, found 581.2562.

*4.5.9. Cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Glu-] (**7i**)*

Yellow solids, mp: 197–199 °C; $[\alpha]_D^{25}$ −108.4 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, DMSO-d_6): δ 1.42 (1H, s), 1.54 (1H, s), 1.80 (1H, s), 2.01 (1H, s), 2.34–2.39 (1H, m), 2.79 (1H, *J*=6.4 Hz, t), 3.07–3.13 (2H, m), 3.45–3.53 (1H, m), 3.95 (1H, *J*=6.7 Hz, d), 4.02–4.09 (1H, m), 4.13 (1H, *J*=14.3, 6.6 Hz, dd), 4.24 (1H, *J*=5.0 Hz, d), 4.41 (1H, *J*=7.1 Hz, d), 4.58 (1H, *J*=6.3 Hz, d), 5.07 (1H, s), 5.33 (1H, *J*=16.5 Hz, d), 7.57 (2H, s), 7.87 (1H, s), 8.01 (1H, *J*=5.5 Hz, d), 8.34 (1H, *J*=5.7 Hz, t), 8.46 (1H, *J*=7.4 Hz, d), 8.71 (1H, *J*=6.2 Hz, d), 12.36 (2H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 26.1, 27.6, 30.5, 31.4, 36.1, 36.6, 42.9, 51.2, 52.9, 53.1, 55.3, 124.3, 147.3, 157.9, 159.3, 159.5, 167.7, 169.8, 171.6, 172.7, 172.9, 174.8; IR (KBr, cm^{-1}): 3419, 3073, 2948, 2558, 1668, 1652, 1557, 1538, 1413, 1380, 1178, 1052, 1024; HRMS calcd for $C_{22}H_{34}N_{11}O_9$ $(M+H)^+$ 596.2546, found 596.2544.

*4.5.10. Cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Lys-] (**7j**)*

White solids, mp: 178–180 °C; $[\alpha]_D^{25}$ +78.5 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, DMSO-d_6): δ 1.42–1.49 (4H, m), 1.59 (4H, *J*=4.7 Hz, d), 2.50–2.53 (2H, m), 2.78–2.85 (4H, m), 3.13 (2H, *J*=6.2 Hz, d), 4.08 (1H, *J*=6.2 Hz, d), 4.12 (1H, s), 4.26 (1H, s), 4.47 (2H, *J*=14.2, 7.0 Hz, dd), 5.12 (2H, *J*=16.3 Hz, d), 5.21 (2H, s), 6.96 (2H, s), 7.35 (2H, s), 7.79 (1H, s), 8.08 (1H, *J*=5.4 Hz, t), 8.22 (1H, *J*=8.4 Hz, d), 8.32 (1H, s), 8.44 (1H, *J*=7.6 Hz, t), 8.78 (1H, *J*=6.7 Hz, t), 12.37 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 22.3, 24.6, 26.4, 27.3, 28.9, 29.4, 30.6, 32.5, 32.9, 36.4, 43.0, 43.8, 49.4, 51.3, 54.5, 124.3, 157.3, 169.8, 170.4, 171.5, 172.3, 173.2, 173.5; IR (KBr, cm^{-1}): 3393, 3073, 2932, 1667, 1538, 1417, 1338, 1201, 1136, 1058, 1024; HRMS calcd for $C_{23}H_{39}N_{12}O_7$ $(M+H)^+$ 595.3056, found 595.3085.

*4.5.11. Cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Arg-] (**7k**)*

White solids, mp: 162–164 °C; $[\alpha]_D^{25}$ +58.9 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, DMSO-d_6): δ 1.41–1.50 (2H, m), 1.55–1.63 (4H, m), 1.71–1.80 (2H, m), 2.74–2.83 (1H, m), 3.12 (4H, *J*=5.8 Hz, d), 3.45–3.52 (1H, m), 3.94–4.00 (2H, m), 4.14 (1H, *J*=4.7 Hz, d), 4.23 (1H, *J*=5.5 Hz, d), 4.43 (1H, *J*=14.4, 7.0 Hz, dd), 5.06 (1H, *J*=16.5 Hz, d), 5.31 (1H, s), 7.63 (1H, s), 7.70 (2H, s), 7.91 (1H, *J*=7.5 Hz, d), 8.05 (1H, *J*=5.6 Hz, t), 8.36 (1H, *J*=6.0 Hz, t), 8.49 (1H, *J*=7.5 Hz, d), 8.76 (1H, *J*=6.4 Hz, d), 12.36 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 24.6, 27.7, 27.9, 29.5, 31.0, 32.3, 37.8, 43.8, 52.7, 52.3, 54.8, 124.8, 157.9, 169.9, 170.2, 171.2, 172.5, 173.3, 173.9; IR (KBr, cm^{-1}): 3393, 3212, 3073, 2932, 1667, 1538, 1417, 1331, 1201, 1136, 1058, 1024; HRMS calcd for $C_{23}H_{39}N_{14}O_7$ $(M+H)^+$ 623.3121, found 623.3120.

*4.5.12. Cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-His-] (**7l**)*

White solids, mp: 176–178 °C; $[\alpha]_D^{25}$ −104.6 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, DMSO-d_6): δ 1.41 (2H, *J*=5.9 Hz, d), 1.51–1.58 (1H, m), 1.79 (1H, s), 2.76 (1H, *J*=5.8 Hz, d), 3.09–3.16 (2H, m), 3.56 (1H, *J*=4.9 Hz, d), 3.90–3.96 (1H, m), 4.16 (1H, *J*=5.3 Hz, d), 4.25 (1H, *J*=5.3 Hz, d), 4.41 (2H, *J*=7.4 Hz, d), 4.53 (1H, *J*=14.2, 7.0 Hz, dd), 5.06 (1H, *J*=16.6 Hz, d), 5.29 (2H, *J*=16.4 Hz, d), 7.45 (1H, s), 7.61 (2H, *J*=6.9 Hz, t), 7.94 (1H, *J*=7.5 Hz, d), 8.08 (1H, *J*=5.8 Hz, t), 8.36 (1H, *J*=6.0 Hz, d), 8.47 (1H, *J*=7.2 Hz, d), 8.85 (1H, *J*=7.2 Hz, d), 8.99 (1H, *J*=6.3 Hz, d), 12.40 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 26.0, 27.4, 30.4, 36.1, 36.7, 41.4, 43.0, 51.3, 53.0, 53.2, 54.8, 118.3, 124.3, 135.2, 147.3, 157.8, 167.6, 169.7, 171.0, 171.7, 172.5, 172.8; IR (KBr, cm^{-1}): 3393, 3073, 2932, 1667, 1538, 1417, 1331, 1201, 1136, 1058, 1024; HRMS calcd for $C_{23}H_{34}N_{13}O_7$ $(M+H)^+$ 604.2653, found 604.2672.

*4.5.13. Cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Phe-] (**7m**)*

Yellow solids, mp: 187–189 °C; $[\alpha]_D^{25}$ +54.8 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, DMSO-d_6): δ 1.52–1.61 (2H, m), 1.88–1.97 (2H, m), 2.63–2.75 (2H, m), 2.89 (1H, s), 3.11 (3H, *J*=5.9 Hz, d), 3.82 (1H, *J*=14.3, 6.3 Hz, dd), 4.19 (1H, *J*=14.2, 6.5 Hz, dd), 4.23 (1H, *J*=5.8 Hz, d), 4.30 (1H, *J*=7.1 Hz, d), 4.58 (1H, s), 5.01 (1H, s), 5.04 (1H, s), 5.16 (1H, s), 7.18–7.27 (5H, m), 7.61 (1H, *J*=7.0 Hz, d), 8.15 (1H, s), 8.34 (1H, *J*=6.0 Hz, t), 8.46 (1H, *J*=7.6 Hz, d), 8.96 (1H, s), 12.35 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 26.1, 28.5, 29.4, 30.4, 36.1, 36.7, 37.8, 50.5, 50.6, 51.1, 52.6, 53.0, 53.2, 55.2, 124.2, 127.6, 129.4, 129.7, 130.3, 130.4, 138.7, 167.4, 169.8, 171.6, 172.7, 173.5; IR (KBr, cm^{-1}): 3352, 3068, 2931, 2849, 1667, 1538, 1457, 1434, 1384, 1331, 1201, 1136, 1084, 1033; HRMS calcd for $C_{26}H_{36}N_{11}O_7$ $(M+H)^+$ 614.2794, found 614.2758.

*4.5.14. Cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Trp-] (**7n**)*

Yellow solids, mp: 173–175 °C; $[\alpha]_D^{25}$ +69.7 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, DMSO-d_6): δ 1.38 (1H, s), 1.51–1.58 (2H, m), 1.77 (1H, s), 3.04–3.10 (2H, m), 3.16 (1H, s), 3.44 (1H, s), 3.50–3.57 (1H, m), 3.65 (1H, *J*=5.2 Hz, d), 4.23 (1H, *J*=5.7 Hz, d), 4.35 (1H, s), 4.43 (2H, *J*=14.2, 6.6 Hz, dd), 4.72 (1H, s), 5.05 (1H, s), 5.17 (1H, s), 6.99–7.10 (2H, m), 7.33–7.37 (2H, m), 7.50–7.56 (2H, m), 7.60 (1H, s), 7.93 (1H, s), 8.10 (1H, s), 8.33 (1H, *J*=9.4 Hz, d), 8.48 (1H, *J*=7.5 Hz, t), 8.67 (1H, s), 10.91 (1H, *J*=12.6 Hz, d), 12.37 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 26.0, 28.2, 30.0, 36.7, 42.9, 51.1, 53.0, 53.2, 56.7, 110.8, 112.6, 119.2, 119.6, 122.2, 124.2, 124.8, 28.2, 137.3, 147.3, 157.8, 167.5, 169.8, 171.7, 172.1, 172.6, 172.7; IR (KBr, cm^{-1}): 3393, 3073, 2932, 1667, 1538, 1417, 1331, 1201, 1136, 1058, 1024; HRMS calcd for $C_{28}H_{37}N_{12}O_7$ $(M+H)^+$ 653.2846, found 653.2812.

*4.5.15. Cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Pro-] (**7o**)*

White solids, mp: 162–164 °C; $[\alpha]_D^{25}$ +86.7 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, DMSO-d_6): δ 1.34–1.41 (4H, m), 1.65–1.74 (6H, m), 2.53–2.64 (4H, m), 2.89 (2H, s), 3.68 (1H, *J*=7.2 Hz, d), 4.10 (1H, *J*=6.6 Hz, d), 4.27 (1H, s), 4.77 (2H, *J*=14.2, 6.7 Hz, dd), 5.31 (2H, *J*=17.3 Hz, d), 5.58 (2H, *J*=17.3 Hz, d), 7.30 (1H, s), 7.35 (1H, *J*=7.7 Hz, t), 7.74 (1H, s), 8.15 (1H, *J*=8.7 Hz, d), 8.38 (1H, *J*=4.7 Hz, t), 8.69 (1H, *J*=7.2 Hz, d), 12.39 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 24.2, 25.7, 27.3, 27.9, 29.4, 30.6, 31.4, 32.7, 37.9, 43.9, 47.4, 49.3, 52.6, 64.8, 124.4, 157.3, 169.7, 170.5, 171.8, 172.9, 173.5, 173.8; IR (KBr, cm^{-1}): 3385, 2946, 2746, 1629, 1585, 1451, 1376, 1317, 1267, 1170, 1037; HRMS calcd for $C_{22}H_{34}N_{11}O_7$ $(M+H)^+$ 564.2637, found 564.2622.

*4.5.16. Cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-] (**7p**)*

White solids, mp: 153–155 °C; $[\alpha]_D^{25}$ +35.8 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, DMSO-d_6): δ 1.51–1.63 (2H, m), 1.73–1.85 (2H, m), 2.69–2.79 (2H, m), 3.11 (2H, *J*=4.5 Hz, d), 3.72 (2H, s), 3.87 (1H, s), 4.35 (2H, *J*=14.2, 6.9 Hz, dd), 4.50 (1H, s), 4.60 (1H, s), 5.15 (1H, s), 7.21 (1H, s), 7.54 (2H, *J*=5.1 Hz, s), 7.81 (1H, s), 8.24 (1H, *J*=7.8 Hz, d), 8.37 (1H, s), 8.65 (1H, *J*=4.9 Hz, d), 12.39 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 24.8, 27.6, 28.6, 29.6, 31.1, 32.4, 37.9, 49.8, 52.4, 54.7, 124.2, 157.6, 169.1, 171.3, 172.4, 173.5, 173.8; IR (KBr, cm^{-1}): 3393, 3073, 2932, 1667, 1538, 1417, 1331, 1201, 1136, 1058, 1024; HRMS calcd for $C_{17}H_{27}N_{10}O_6$ $(M+H)^+$ 467.2039, found 467.2056.

*4.5.17. Cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Gly-Gly-] (**7q**)*

White solids, mp: 201–203 °C; $[\alpha]_D^{25}$ +35.3 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, DMSO-d_6): δ 1.10 (2H, *J*=9.5 Hz, d), 1.31–1.42 (1H, m), 1.60–1.71 (1H, m), 2.56–2.62 (2H, m), 2.76 (2H, *J*=4.8 Hz, d), 3.69 (1H, *J*=5.1 Hz, d), 3.75 (1H, s), 3.86 (2H, *J*=6.8 Hz, d), 4.35 (3H, *J*=14.2, 6.7 Hz, dd), 4.46 (2H, *J*=4.9 Hz, d), 5.22 (3H, *J*=10.9 Hz, d), 6.72 (2H, s), 7.34 (2H, *J*=8.4 Hz, d), 7.84 (1H, s), 8.07 (1H, s), 8.35 (1H, *J*=7.6 Hz, t), 8.41 (1H, *J*=5.7 Hz, t), 8.55 (1H, *J*=6.0 Hz, t), 9.09 (1H, *J*=5.3 Hz, t), 12.41 (1H, s); ^{13}C NMR (125 MHz, DMSO-d_6): δ 25.9, 29.1, 36.1, 36.7, 41.5, 43.6, 44.2, 44.9, 51.3, 52.4, 52.7, 125.1, 146.3, 157.7, 168.7, 170.2, 170.5, 171.6, 173.2; IR (KBr, cm^{-1}): 3393, 3073, 2932, 1667, 1538, 1417, 1331, 1201, 1136, 1058, 1024; HRMS calcd for $C_{21}H_{33}N_{12}O_8$ $(M+H)^+$ 581.2597, found 581.2569.

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Supplementary data

Chemical structures for products associated with this article can be found in the online version. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.08.090.

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