Tetrahedron 65 (2009) 7935–7941

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Synthesis of crosslinking amino acids by click chemistry

Chun Li [a,b], Jie Tang [b], Juan Xie [a,*]

[a] PPSM, Institut d'Alembert, ENS Cachan, CNRS, UniverSud, 61 av President Wilson, F-94230 Cachan, France
[b] Institute of Medicinal Chemistry, Department of Chemistry, East China Normal University, Shanghai 200062, China

ARTICLE INFO

Article history:
Received 11 May 2009
Received in revised form 20 July 2009
Accepted 23 July 2009
Available online 26 July 2009

Keywords:
Bis-amino acids
Crosslinks
Click chemistry
Fluorescence

ABSTRACT

Crosslinking amino acids are naturally existing protein crosslinkers. Herein, we described the synthesis of several novel bis-amino acids constituted of serine, alanine, lysine, and tyrosine with click chemistry. The Huisgen 1,3-dipolar cycloadditions between azido- and alkyne-functionalized amino acids can be easily realized in the presence of catalytic amount of $CuSO_4$ and Na ascorbate. In addition, fluorescent bis-amino acid derivatives have also been prepared using anthracene, fluorescein, and benzothiadiazole as fluorophores. With symmetrical bis-alkyne benzothiadiazole, it was possible to synthesize asymmetrical derivative bearing two different amino acid moieties.

1. Introduction

Crosslinking amino acids like histidinoalanine, lysinoalanine, and pyridinoline are natural metabolism products identified from a range of sources like milk products, human tissues including connective tissue, bone, dentin, and eye cataracts, phosphoproteins of bivalve molluscs as well as bicyclic dodecapeptides theonellamides.[1] As covalent protein crosslinks, histidinoalanine and lysinoalanine have been shown to increase the stability toward proteases and modify the quaternary structure of proteins. They seem to play an important role in mineral binding in bivalve molluscs and in advanced eye cataracts. On the other hand, deoxypyridinoline and pyridinoline, two fluorescent crosslinks derived from the degradation of bone collagen, have been used as biochemical markers of collagen turnover.[2] Synthesis of these crosslinking amino acids has therefore attracted increasing attentions.[1,3] However, preparation of bis-amino acids like histidinoalanine remained difficult because of the problem of regioisomers and low yield.[4] As a continuing program on the use of click chemistry for bioorganic and analytical applications,[5] we decided to synthesize new bis-amino acid derivatives of serine, alanine, tyrosine, and lysine (compounds **1**–**10**, Fig. 1) using the Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition reaction.[6] Since the triazole ring has similar chemical properties as the imidazole ring and is resistant to hydrolysis, oxidation, reduction, or other modes of cleavage, compounds **1**–**3** can be considered as mimetics of histidinoalanine. Fluorescence methods have been widely used in the biosciences and appear to be among the most powerful analytical research tools. We have also synthesized fluorescent bis-amino acid derivatives **5**–**10** based on anthracene, fluorescein, and benzothiadiazole, which absorb at wavelengths longer than 320 nm, where interference from the tryptophan units is negligible. These molecules could then be used as multivalent organic probes for fluorescent labeling of biomolecules.[7]

2. Results and discussion

In order to obtain various bis-amino acids, we have firstly synthesized protected amino acids functionalized with azido or alkyne functions. *O*-Propargyl Boc-Ser-OMe **11**,[8] 3-azido Boc-Ala-OMe **12**,[9] 3-azido Boc-Ala-OBn **13**,[10] *O*-propargyl Boc-Tyr-OMe **14**[11] have been prepared according to the literature procedure. *O*-Propargyl Boc-Ser-OBn **15** was obtained from Boc-Ser-OH by O-alkylation and esterification reactions (Scheme 1). By a diazotransfer reaction with imidazole-1-sulfonyl azide,[12] N^{α}-Cbz-Lys-OH has been transformed into the corresponding azide **16** in 70% yield after esterification with $SOCl_2$ and MeOH.

Click reaction of azides **12** and **13** with alkynes **11**, **14**, and **15** underwent smoothly in the presence of catalytic amount of $CuSO_4$ and sodium ascorbate in a mixture of CH_2Cl_2 and H_2O (Table 1). Bis-amino esters **1**–**4** were obtained in 55–86% yield. By saponification or hydrogenolysis, these compounds can be easily transformed into the carboxylic acids **17**–**20** for further derivatization (Scheme 2).

Synthesis of anthracene and fluorescein-based bis-amino acid derivatives is depicted in the Scheme 3. Reaction of the alkyne **15** (2 equiv) with the known diazido anthracene **21**[13] (1 equiv) led to the desired compound **5** in 60% yield. However, hydrogenolysis

* Corresponding author. Tel.: +33 1 47405586; fax: +33 1 47402454.
E-mail address: joanne.xie@ens-cachan.fr (J. Xie).

0040-4020/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2009.07.054

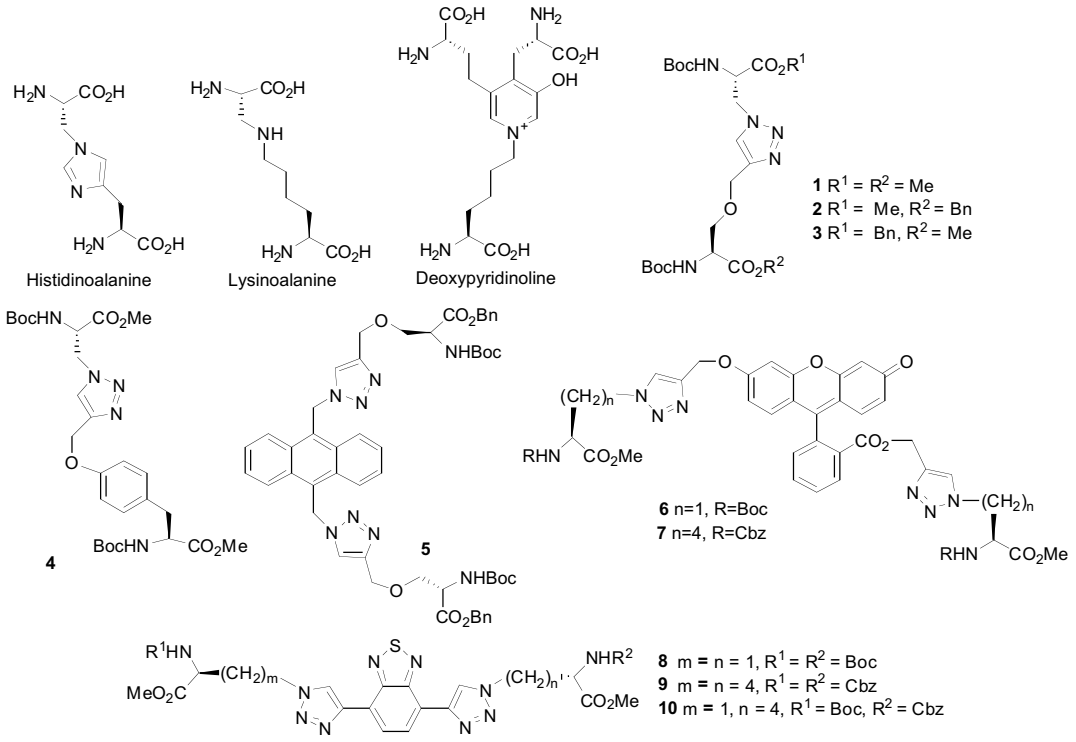
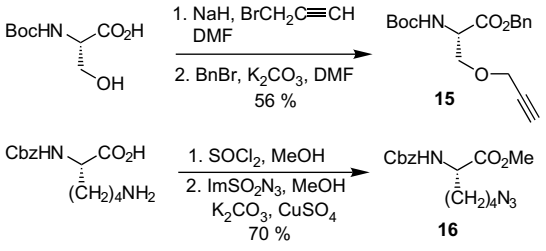


Figure 1. Structure of natural and target crosslinking amino acid derivatives.

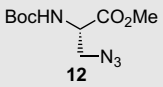
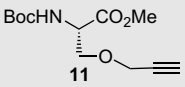
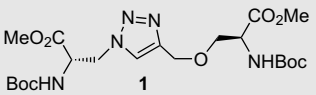
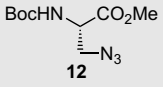
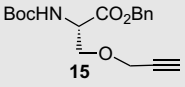
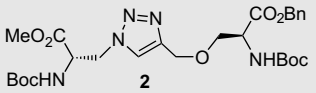
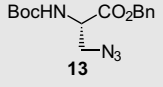
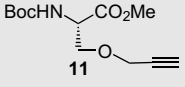
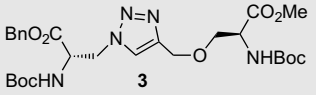
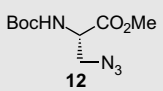
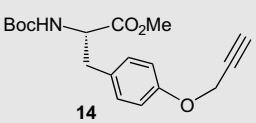
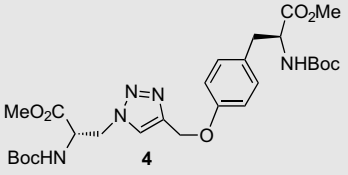


Scheme 1. Synthesis of azido or propargyl amino esters **15**, **16**.

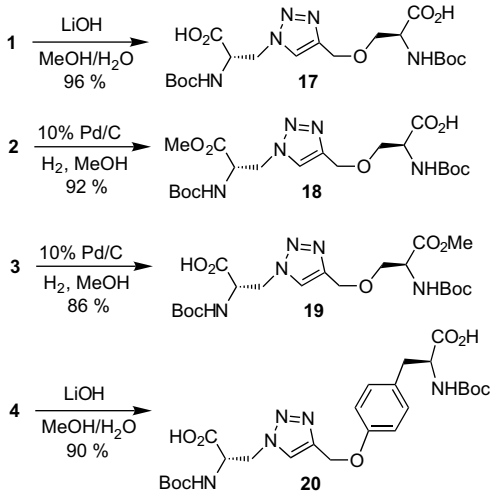
under different conditions failed to give the corresponding free acid. Treatment of the azido alanine **12** or azido lysine **16** with the bis-propargyl fluorescein **22**[14] furnished the fluorescent molecules **6** and **7** as a yellow solid in good yield (82% for **6** and 92% for **7**). Tentative saponification of **6** and **7** also hydrolyzed the ester bond of the fluorescein moiety. We then decided to deprotect the amino function of compounds **6** and **7** for further modification. Reaction of **6** with TFA led to the free amino derivative **23** in quantitative yield. Hydrogenolysis or HBr treatment of **7** failed to give the corresponding free acid.

Benzothiadiazole fluorophore was easily accessible and possessed high fluorescence efficiency.[15] Treatment of its bis-alkyne

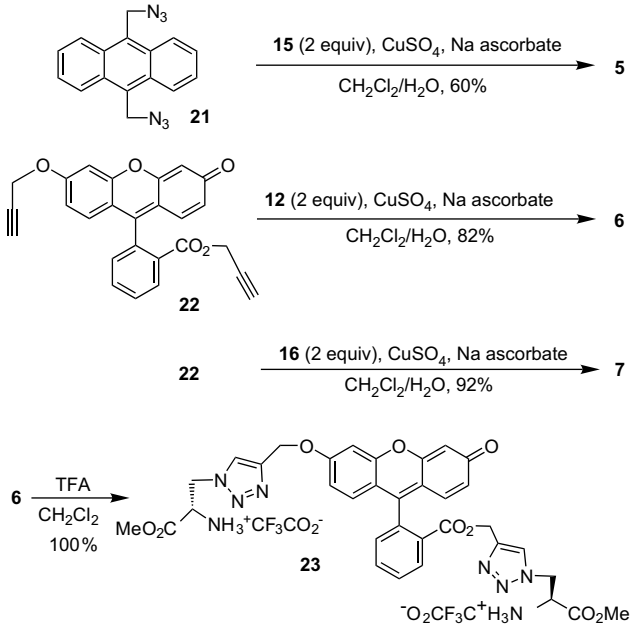
Table 1
Synthesis of bis-amino esters **1**–**4** via click chemistry[a]

Entry	Azide	Alkyne	Product	Yield[b]%
1	 **12**	 **11**	 **1**	55
2	 **12**	 **15**	 **2**	70
3	 **13**	 **11**	 **3**	86
4	 **12**	 **14**	 **4**	86

[a] The reaction was conducted in the presence of 0.01–0.1 equiv of $CuSO_4$ and 0.02–0.2 equiv of sodium ascorbate in a mixture of CH_2Cl_2/H_2O (1/1) at rt for 12–20 h.
[b] Isolated yield after column chromatography.



Scheme 2. Synthesis of bis-amino acids **17**–**20**.



Scheme 3. Synthesis of anthracene and fluorescein-based bis-amino acid derivatives.

derivative **24**[16] (1 equiv) with the azido alanine **12** or azido lysine **16** (2 equiv) in the presence of TBAF afforded, in one-pot,[5b] the bis-triazole compounds **8** and **9** in 68–73% yield (Scheme 4). Reaction of **24** with 0.44 equiv of **16** led to a mixture of mono-click compounds **25** and **26**. The TMS-protected compound **25** can be easily converted into the deprotected alkyne **26** by TBAF in 86% total yield. Further click reaction of **26** with the azide **12** led to the asymmetrical bis-amino ester **10** in 91% yield. This result is very interesting since different azido derivatives can be linked to the benzothiadiazole fluorophore through two consecutive click reactions. Saponification of compounds **8**–**10** led to the corresponding free acids **27**–**29** in good yield. The amino-protecting groups of **8** and **9** can also be easily removed with TFA (for Boc) or HBr in AcOH (for Cbz), leading to the corresponding amino esters **30** and **31**.

3. Conclusion

In summary, we have realized an efficient synthesis of new crosslinking amino esters with click chemistry. Bis-amino acids containing serine, alanine, tyrosine, and lysine can then be easily prepared. Furthermore, their fluorescent derivatives have also been prepared using azido or alkyne-functionalized anthracene, fluorescein, and benzothiadiazole as fluorophores. These bis-amino acids should be useful as protein crosslinks for various biological applications. Fluorescent bis-amino acids could also be used for biomolecules labeling or as fluorescent sensors.[17] Work is in progress in our laboratory for potential applications of these molecules.

4. Experimental

4.1. General

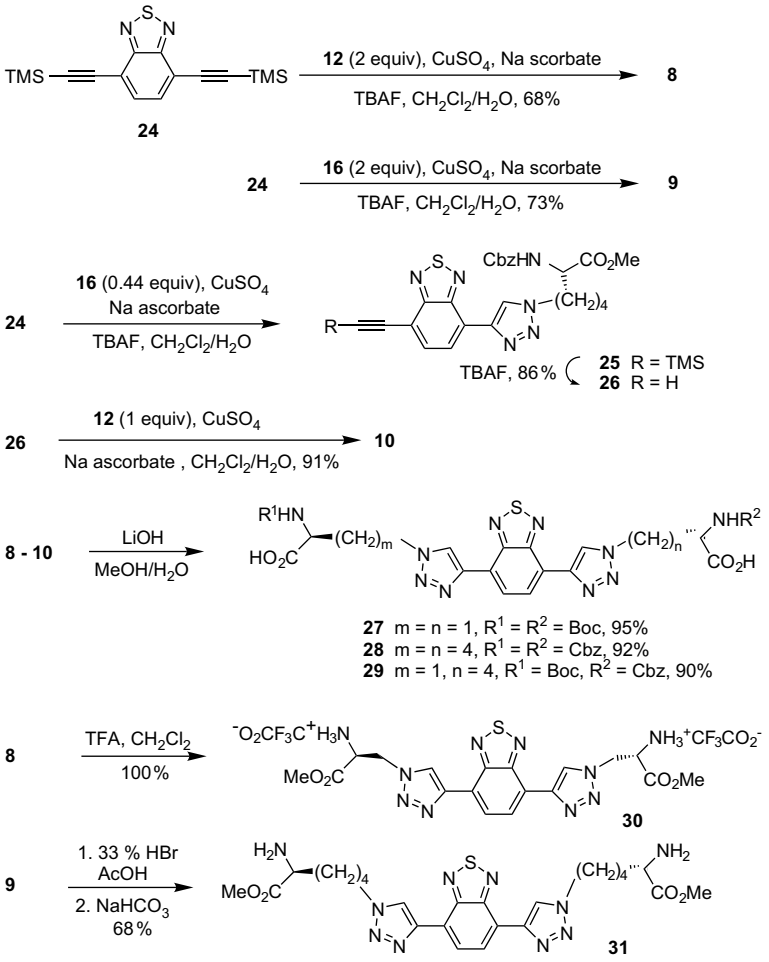
^{1}H and ^{13}C NMR spectra were recorded on Jeol 400 spectrometer in $CDCl_3$, DMSO-d_6 or CD_3OD solutions. Column chromatography was performed on E. Merck Silica Gel 60 (230–400 mesh). Analytical thin-layer chromatography was performed on E. Merck aluminum percolated plates of Silica Gel 60F-254 with detection by UV and ninhydrin. Optical rotations were measured using a Jasco P-2000 polarimeter. High-resolution mass spectra (HRMS) were recorded on a MA1212 instrument using standard conditions (ESI, 70 eV).

4.2. Preparation of *O*-propargyl Boc-Ser-OBn 15

To an ice cold solution of Boc-Serine (0.380 g, 1.86 mmol) in 10 mL DMF was added slowly NaH (60%, 107 mg, 4.45 mmol). After kept stirring for 30 min, propargyl bromide (0.23 mL, 2.61 mmol) was then added. After 12 h at rt, brine (15 mL) was added. The reaction mixture was extracted with EtOAc (2×10 mL). The water layer was acidified to pH=1–2 with 1 N $KHSO_4$, then extracted with EtOAc (3×15 mL). The combined organic layers were dried over $MgSO_4$, evaporated to give a liquid, which was dissolved in DMF (10 mL). $NaHCO_3$ (312 mg, 3.72 mmol) and BnBr (0.59 mL, 4.65 mmol) were added successively. After 12 h at rt, brine (15 mL) was added. The reaction mixture was extracted with EtOAc (3×15 mL). The combined organic layers were washed with brine and dried over anhydrous $MgSO_4$, then evaporated. Purification by column chromatography (petroleum ether/EtOAc: 10/1) afforded the title compound as a colorless liquid (0.350 g, 56%). TLC: R_f=0.6 (petroleum ether/EtOAc=4/1). $[\alpha]_D^{25}$ −2.4 (*c* 0.3, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35–7.36 (m, 5H, Ph), 5.39 (d, 1H, *J*=8.3 Hz, NH), 5.27 (d, 1H, *J*=12.4 Hz, CHPh), 5.15 (d, 1H, *J*=12.4 Hz, CHPh), 4.49 (dt, 1H, *J*=2.8, 8.7 Hz, CH), 4.10 (d, 2H, *J*=2.3 Hz, CH_2), 4.00 (dd, 1H, *J*=3.2, 9.6 Hz, CH), 3.75 (dd, 1H, *J*=3.2, 9.2 Hz, CH), 2.40 (t, 1H, *J*=2.3 Hz, C≡CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 155.6, 135.5 (Cq); 128.6, 128.4, 128.2 (Ph); 80.1 (Cq), 75.2 (CH≡); 69.8, 67.3, 58.6 (CH_2); 54.0 (CH), 28.4 (Boc). HRESIMS: calcd for $C_{18}H_{23}NNaO_5$: 356.1474; found: *m*/*z* 356.1468.

4.3. Preparation of 6-azido-2(*S*)-benzyloxycarbonylamino-hexanoic acid methyl ester 16

To precooled (ice-salt bath) absolute MeOH (5 mL) was added slowly $SOCl_2$ (0.2 mL, 2.8 mmol), then N^{α}-Cbz-Lys-OH (100 mg, 0.36 mmol) in one portion. The solution was allowed to warm to rt slowly and stirred overnight. After evaporation, N^{α}-Cbz-Lys-OMe was obtained as clear oil, which was dissolved in dry MeOH (8 mL). K_2CO_3 (248 mg, 1.8 mmol) and $CuSO_4 \cdot 5H_2O$ (23 mg, 0.09 mmol) were added to the above solution, followed by $ImSO_2N_3$ (75 mg, 0.43 mmol) 10 min later. The reaction mixture was stirred at rt for 12 h. After addition of H_2O (30 mL), the mixture was extracted with CH_2Cl_2 (3×10 mL). The organic layers were combined, washed with brine, dried over $MgSO_4$, and evaporated under vacuum. The residue was purified on silica gel with EtOAc/petroleum ether (1/4) as eluent to give 70 mg (70%) of **16** as a colorless liquid. TLC: R_f=0.5 (petroleum ether/EtOAc=4/1). $[\alpha]_D^{25}$ +109.2 (*c* 0.6, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36–7.37 (m, 5H, Ph), 5.31 (d, 1H,



Scheme 4. Synthesis of benzothiadiazole-based bis-amino acid derivatives.

J=5.1 Hz, NH), 5.10 (s, 2H, CH_2), 4.37–4.42 (m, 1H, CH), 3.72 (s, 3H, OMe), 3.26 (t, 2H, J=6.6 Hz, CH_2N_3), 1.39–1.99 (m, 6H, 3×CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.8, 155.9, 136.2 (Cq); 128.7, 128.3, 128.2 (Ph); 67.2 ($PhCH_2$), 53.7 (CH), 52.6 (OMe), 51.2 (CH_2N_3), 32.4, 28.5, 22.5 (CH_2). HRESIMS: calcd for $C_{15}H_{20}N_4NaO_4$: 343.1382; found: m/z 343.1377.

4.4. General procedure for click reaction

To a solution of alkyne (1 mmol) and azide (1 mmol) in CH_2Cl_2 (10 mL) and water (10 mL) were added $CuSO_4{\cdot}5H_2O$ (0.01–0.2 mmol) and sodium ascorbate (0.02–0.2 mmol) successively. The reaction mixture was vigorously stirred at rt for 12–20 h. CH_2Cl_2 (30 mL) was then added. After separation of the organic layer, the aqueous layer was extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were washed with brine (40 mL), dried over $MgSO_4$, evaporated and purified by column chromatography.

4.4.1. 2(S)-tert-Butoxycarbonylamino-3-[4-(2(S)-tert-butoxycarbonylamino-2-methoxycarbonyl-ethoxymethyl)-1,2,3-triazol-1-yl]-propionic acid methyl ester ***1****.* From **11** (84 mg, 0.33 mmol) and **12** (80 mg, 0.33 mmol), column chromatography (petroleum ether/EtOAc, 1/1,) afforded **1** as a colorless liquid (90 mg, 55%). TLC: R_f=0.2 (petroleum ether/EtOAc=1/1). $[\alpha]_D^{25}$ +34.6 (c 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47 (s, 1H, CH), 5.40 (d, 1H, J=6.4 Hz, NH), 5.36 (d, 1H, J=8.7 Hz, NH), 4.85 (dd, 1H, J=5.0, 14.2 Hz, CH), 4.78 (dd, 1H, J=4.1, 14.2 Hz, CH), 4.71–4.76 (m, 1H, CH), 4.65 (d, 1H, J=12.4 Hz, CH), 4.61 (d, 1H, J=12.4 Hz, CH), 4.42–4.45 (m, 1H, CH), 3.93 (dd, 1H, J=3.2, 9.6 Hz, CH), 3.79 (s, 3H, MeO), 3.75 (s, 3H, MeO), 3.73 (dd, 1H, J=3.2, 9.6 Hz, CH), 1.42 (s, 18H, 2×t-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.1, 169.5, 155.5, 155.2, 144.7 (Cq); 123.8 (CH), 80.8 (Cq), 70.4, 64.8 (CH_2), 53.9, 53.7 (MeO), 53.2, 52.5 (CH); 50.9 (CH_2), 28.3 (CH_3). HRESIMS: calcd for $C_{21}H_{35}N_5NaO_9$: 524.2333; found: m/z 524.2327.

4.4.2. 2(S)-tert-Butoxycarbonylamino-3-[4-(2(S)-tert-butoxycarbonylamino-2-methoxycarbonyl-ethoxymethyl)-1,2,3-triazol-1-yl]-propionic acid benzyl ester ***2****.* From **12** (80 mg, 0.33 mmol) and **15** (110 mg, 0.33 mmol), column chromatography (petroleum ether/EtOAc, 1/1) afforded **2** as a colorless liquid (134 mg, 70%). TLC: R_f=0.2 (petroleum ether/EtOAc=1/1). $[\alpha]_D^{25}$ +16.6 (c 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34 (s, 1H, CH), 7.33–7.34 (m, 5H, Ph), 5.38 (m, 2H, 2×NH), 5.25 (d, 1H, J=12.4 Hz, CHPh), 5.11 (d, 1H, J=12.4 Hz, CHPh), 4.78–4.81 (m, 1H, CH), 4.69–4.73 (m, 2H), 4.63 (d, 1H, J=12.4 Hz, CH), 4.55 (d, 1H, J=12.4 Hz, CH), 4.48 (td, 1H, J=3.7, 9.2 Hz, CH), 3.92 (dd, 1H, J=3.2, 9.2 Hz, CH), 3.76 (s, 3H, MeO), 3.73 (dd, 1H, J=3.2, 9.6 Hz, CH), 1.44 (s, 18H, 2×t-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 169.5, 155.5, 155.2, 144.5, 135.5 (Cq); 128.6, 128.3, 128.1 (Ph), 123.8 (CH), 80.8 (Cq), 70.5, 67.2, 64.8 (CH_2), 54.1 (MeO), 53.7, 53.1 (CH); 50.9 (CH_2), 28.3 (CH_3). HRESIMS: calcd for $C_{27}H_{39}N_5NaO_9$: 600.2646; found: m/z 600.2640.

4.4.3. 3-[4-(2(S)-Benzyloxycarbonyl-2-tert-butoxycarbonylamino-ethoxymethyl)-1,2,3-triazol-1-yl]-2(S)-tert-butoxycarbonylamino-propionic acid methyl ester ***3****.* From **11** (187 mg, 0.583 mmol) and **13** (150 mg, 0.583 mmol), column chromatography (petroleum ether/

EtOAc, 1/1) afforded **3** as a colorless liquid (280 mg, 86%). TLC: R_f=0.3 (petroleum ether/EtOAc=1/1). $[\alpha]_D^{25}$ +10.8 (*c* 0.3, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ 7.33–7.35 (m, 5H, Ph), 7.25 (s, 1H, CH), 5.42 (d, 1H, *J*=7.3 Hz, NH), 5.35 (d, 1H, *J*=8.7 Hz, NH), 5.20 (d, 1H, *J*=12.4 Hz, CHPh), 5.15 (d, 1H, *J*=12.4 Hz, CHPh), 4.82 (dd, 1H, *J*=4.6, 14.2 Hz, CH), 4.76 (dd, 1H, *J*=4.1, 14.2 Hz, CH), 4.66–4.71 (m, 1H), 4.58 (d, 1H, *J*=12.4 Hz, CH), 4.52 (d, 1H, *J*=12.4 Hz, CH), 4.41 (td, 1H, *J*=3.7, 8.7 Hz, CH), 3.88 (dd, 1H, *J*=3.2, 9.6 Hz, CH), 3.71 (s, 3H, OMe), 3.70 (dd, 1H, *J*=3.2, 9.2 Hz, CH), 1.41 (s, 18H, 2×*t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.1, 168.9, 155.5, 155.2, 144.5, 134.8 (Cq); 128.9, 128.8 (Ph), 123.8 (CH), 80.8 (Cq), 70.4, 68.2, 64.7 (CH_2), 54.0, 53.9 (CH); 52.6 (OMe), 50.9 (CH_2), 28.3 (CH_3). HRESIMS: calcd for $C_{27}H_{39}N_5NaO_9$: 600.2645; found: *m*/*z* 600.2640.

4.4.4. 2(S)-tert-Butoxycarbonylamino-3-{4-[4-(2(S)-tert-butoxycarbonylamino-2-methoxycarbonyl-ethyl)-phenoxymethyl]-1,2,3-triazol-1-yl}-propionic acid methyl ester ***4***. From **12** (22 mg, 0.09 mmol) and **14** (30 mg, 0.09 mmol), column chromatography (petroleum ether/EtOAc, 1/1) afforded **4** as a white crystal (45 mg, 86%). TLC: R_f=0.4 (petroleum ether/EtOAc=1/1). $[\alpha]_D^{25}$ +54.6 (*c* 0.5, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ 7.55 (s, 1H, CH), 7.01 (d, 2H, *J*=8.7 Hz, Tyr), 6.88 (d, 2H, *J*=8.7 Hz, Tyr), 5.36 (d, 1H, *J*=7.3 Hz, NH), 5.16 (s, 2H, CH_2), 4.94 (d, 1H, *J*=7.8 Hz, NH), 4.80 (dd, 1H, *J*=4.6, 14.2 Hz, CH), 4.78 (dd, 1H, *J*=4.6, 13.8 Hz, CH), 4.68–4.71 (m, 1H, CH), 4.50–4.55 (m, 1H, CH), 3.75 (s, 3H, OMe), 3.70 (s, 3H, OMe), 3.02 (dd, 1H, *J*=5.5, 14.2 Hz, CH), 2.99 (dd, 1H, *J*=6.0, 13.8 Hz, CH), 1.42 (s, 9H, *t*-Bu), 1.40 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.4, 169.5, 157.3, 155.1 (Cq); 130.4 (CH), 128.7 (Cq), 129.3 (CH), 114.9 (CH), 80.8 (Cq), 62.1 (CH_2), 54.6, 53.8 (CH); 53.2, 52.3 (OMe), 51.0, 37.5 (CH_2), 28.4 (CH_3). HRESIMS: calcd for $C_{27}H_{39}N_5NaO_9$: 600.2646; found: *m*/*z* 600.2647.

4.4.5. 3-(1-{10-[4-(2(S)-Benzyloxycarbonyl-2-tert-butoxycarbonylamino-ethoxymethyl)-1,2,3-triazol-1-ylmethyl]-anthracen-9-ylmethyl}-1H-1,2,3-triazol-4-ylmethoxy)-2(S)-tert-butoxycarbonylamino-propionic acid benzyl ester ***5***. From diazido anthracene **21** (20 mg, 0.07 mmol) and **15** (47 mg, 0.14 mmol), column chromatography (petroleum ether/EtOAc, 1/1) afforded **5** as a colorless liquid (40 mg, 60%). TLC: R_f=0.1 (petroleum ether/EtOAc=1/2). $[\alpha]_D^{25}$ −4.2 (*c* 0.5, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ 8.41 (dd, 4H, *J*=3.2, 6.9 Hz, H-Ar), 7.66 (dd, 4H, *J*=3.2, 6.9 Hz, H-Ar), 7.20–7.25 (m, 10H, Ph), 7.09 (s, 2H, 2×CH), 6.52 (s, 4H, 2×CH_2), 5.28 (d, 2H, *J*=8.7 Hz, 2×NH), 5.02 (d, 2H, *J*=12.4 Hz, 2×CHPh), 4.95 (d, 2H, *J*=12.4 Hz, 2×CHPh), 4.36–4.45 (m, 6H, 2×CH, 2×CH_2), 3.84 (dd, 2H, *J*=3.2, 9.2 Hz, 2×CH), 3.65 (dd, 2H, *J*=3.2, 9.2 Hz, 2×CH), 1.39 (s, 18H, 2×*t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 155.4, 144.7, 135.4, 130.7 (Cq); 128.5, 128.3, 127.7, 127.1, 124.2 (CH), 122.2 (CH), 80.1 (Cq), 70.5, 67.0, 64.7 (CH_2); 54.1 (CH), 46.6 (CH_2), 28.4 (CH_3). HRESIMS: calcd for $C_{52}H_{58}N_8NaO_{10}$: 977.4174; found: *m*/*z* 977.4168.

4.4.6. 2-{6-[1-(2(S)-tert-Butoxycarbonylamino-2-methoxycarbonyl-ethyl)-1H-1,2,3-triazol-4-ylmethoxy]-3-oxo-3H-xanthen-9-yl}-benzoic acid 1-(2(S)-tert-butoxycarbonylamino-2-methoxycarbonyl-ethyl)-1H-1,2,3-triazol-4-ylmethyl ester ***6***. From **22** (41 mg, 0.1 mmol) and **12** (49 mg, 0.2 mmol), column chromatography (petroleum ether/EtOAc, 1/3) afforded **6** as a brown solid (74 mg, 82%). TLC: R_f=0.3 (petroleum ether/EtOAc=1/2). $[\alpha]_D^{25}$ +2.8 (*c* 0.5, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ 8.21 (d, 1H, *J*=7.3 Hz, H-Ar), 7.62–7.71 (m, 3H, H-Ar), 7.27 (d, 1H, *J*=6.8 Hz, H-Ar), 7.18 (s, 1H, CH), 7.04 (d, 1H, *J*=1.8 Hz, H-Ar), 6.73–6.83 (m, 3H, H-Ar), 6.47 (d, 1H, *J*=9.6 Hz, H-Ar), 6.38 (s, 1H, CH), 5.47–5.56 (m, 2H, 2×NH), 5.29 (m, 2H, CH_2), 5.03–5.12 (m, 2H, CH_2), 4.84 (m, 2H, CH_2), 4.67–4.76 (m, 6H, 2×CH, 2×CH_2), 3.76 (s, 6H, 2×OMe), 1.40 (s, 9H, *t*-Bu), 1.38 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 185.6, 169.6, 169.5, 165.2, 162.6, 162.5, 158.8, 155.2, 154.1, 149.9, 142.8, 141.5, 141.4, 134.2 (Cq); 132.9, 131.4, 130.5, 130.3, 129.8, 129.0, 124.8, 124.7 (CH); 117.8, 115.2 (Cq); 113.8, 105.7, 101.5 (CH), 80.7 (Cq), 62.4, 58.2 (CH_2), 53.8, 53.5 (CH); 53.2 (OMe), 51.1, 51.0 (CH_2), 28.3 (CH_3). HRESIMS: calcd for $C_{44}H_{49}N_8O_{13}$: 897.3419; found: *m*/*z* 897.3414. Calcd for $C_{44}H_{48}N_8NaO_{13}$: 919.3239; found: *m*/*z* 919.3233.

4.4.7. 2-{6-[1-(5(S)-Benzyloxycarbonylamino-5-methoxycarbonyl-pentyl)-1H-1,2,3-triazol-4-ylmethoxy]-3-oxo-3H-xanthen-9-yl}-benzoic acid 1-(5(S)-benzyloxycarbonylamino-5-methoxycarbonyl-pentyl)-1H-1,2,3-triazol-4-ylmethyl ester ***7***. From **22** (30 mg, 0.073 mmol) and **16** (45 mg, 0.145 mmol), compound **7** was obtained as a brown solid (69 mg, 92%). TLC: R_f=0.2 (petroleum ether/EtOAc=1/2). $[\alpha]_D^{25}$ −2.4 (*c* 0.5, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ 8.22–8.25 (m, 1H, H-Ar), 7.62–7.71 (m, 3H, H-Ar), 7.28–7.35 (m, 11H, H-Ar), 7.11 (s, 1H, CH), 7.03 (s, 1H, CH), 6.85 (dd, 1H, *J*=2.8, 9.2 Hz, H-Ar), 6.76–6.80 (m, 2H, H-Ar), 6.49 (d, 1H, *J*=9.6 Hz, H-Ar), 6.39 (s, 1H, CH), 5.76 (d, 0.5H, *J*=8.2 Hz, NH), 5.73 (d, 0.5H, *J*=8.2 Hz, NH), 5.41 and 5.43 (2d, 1H, *J*=7.8 Hz, NH), 5.27 (s, 2H, CH_2), 5.01–5.12 (m, 6H, 3×CH_2), 4.18–4.33 (m, 6H, 2×CH, 2×CH_2), 3.76 (s, 6H, 2×OMe), 1.66–1.99 (m, 12H, 6×CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.8, 172.6, 165.3, 162.7, 156.1, 156.0, 154.1, 150.1, 136.3, 136.2, 134.2 (Cq); 132.9, 131.5, 130.4 (CH); 130.2 (Cq), 129.9, 129.1, 128.6, 128.5, 128.3, 128.1, 123.3 (CH); 117.6, 115.1 (Cq); 113.9, 101.5 (CH); 67.1, 62.5, 58.5 (CH_2); 53.6, 53.5 (CH); 52.6 (OMe), 50.2, 50.0 (CH_2); 32.1, 31.8, 29.7, 22.5, 22.4, 22.2 (CH_2). HRESIMS: calcd for $C_{56}H_{57}N_8O_{13}$: 1049.4045; found: *m*/*z* 1049.4046.

4.4.8. 2(S)-tert-Butoxycarbonylamino-3-(4-{7-[1-(2(S)-tert-butoxycarbonylamino-2-methoxycarbonyl-ethyl)-1H-1,2,3-triazol-4-yl]-2,1,3-benzothiadiazol-4-yl}-1,2,3-triazol-1-yl)-propionic acid methyl ester ***8***. Compound **8** was prepared from **24** (11 mg, 0.032 mmol) and **12** (17 mg, 0.064 mmol) according to the general procedure, in the presence of nBu$_4$NF·3H$_2$O (40 mg, 0.128 mmol). After the completion of the reaction, the crude mixture was diluted in a mixture of 10 mL CH_2Cl_2/0.1 N EDTA solution (1/1) to remove the copper complexes. Column chromatography (petroleum ether/EtOAc, 1/1) afforded **8** as a yellow solid (15 mg, 68%). TLC: R_f=0.2 (petroleum ether/EtOAc=1/2). $[\alpha]_D^{25}$ +45.6 (*c* 0.5, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ 8.70 (s, 2H, 2×CH), 8.58 (s, 2H, 2×CH), 5.51 (d, 2H, *J*=7.3 Hz, 2×NH), 4.98 (d, 4H, *J*=3.6 Hz, 2×CH_2), 4.79 (m, 2H, 2×CH), 3.83 (s, 6H, 2×OMe), 1.46 (s, 18H, 2×*t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.1, 155.0, 142.9 (Cq); 125.1, 124.6 (CH); 122.4, 80.9 (Cq); 53.8 (CH), 53.2 (OMe), 51.1 (CH_2), 28.3 (CH_3). HRESIMS: calcd for $C_{28}H_{36}N_{10}NaO_8S$: 695.2336; found: *m*/*z* 695.2331.

4.4.9. 2(S)-Benzyloxycarbonylamino-6-(4-{7-[1-(5(S)-benzyloxycarbonylamino-5-methoxycarbonyl-pentyl)-1H-1,2,3-triazol-4-yl]-2,1,3-benzothiadiazol-4-yl}-1,2,3-triazol-1-yl)-hexanoic acid methyl ester ***9***. Compound **9** was prepared from **24** (33 mg, 0.1 mmol) and **16** (60 mg, 0.2 mmol) in the presence of nBu$_4$NF·3H$_2$O (567 mg, 1.8 mmol), followed by treatment as for compound **8**. Column chromatography (petroleum ether/EtOAc, 1/3) afforded **9** as a yellow solid (60 mg, 73%). TLC: R_f=0.2 (petroleum ether/EtOAc=1/2). $[\alpha]_D^{25}$ +4.0 (*c* 0.3, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ 8.68 (s, 2H, 2×CH), 8.60 (s, 2H, 2×CH), 7.26–7.32 (m, 10H, Ph), 5.37 (d, 2H, *J*=8.2 Hz, 2×NH), 5.04 (s, 4H, 2×CH_2), 4.43 (t, 4H, *J*=6.9 Hz, 2×N–CH_2), 4.35 (m, 2H, 2×CH), 3.70 (s, 6H, 2×OMe), 1.38–2.08 (m, 12H, 6×CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.7, 155.9, 152.3, 143.2, 136.2 (Cq); 128.6, 128.3, 128.1, 126.1, 123.8 (CH); 122.7 (Cq), 67.1 (CH_2), 53.6 (CH), 52.6 (OMe), 50.1, 32.1, 29.8, 22.3 (CH_2). HRESIMS: calcd for $C_{40}H_{44}N_{10}NaO_8S$: 847.2962; found: *m*/*z* 847.2957.

4.4.10. 6-[4-(7-Ethynyl-2,1,3-benzothiadiazol-4-yl)-1,2,3-triazol-1-yl]-2(S)-benzyloxycarbonylamino-hexanoic acid benzyl ester

26. Click reaction of **24** (53 mg, 0.16 mmol) with **16** (22 mg, 0.07 mmol) in the presence of nBu$_4$NF·3H$_2$O (202 mg, 0.64 mmol), followed by treatment as for compound **8** afforded a mixture of **25** and **26**, which was further treated with t-Bu$_4$NF·3H$_2$O (200 mg in CH_2Cl_2) to give **26** (30 mg, 86%) as a red oil. TLC: R_f=0.3 (petroleum ether/EtOAc=1/2). $[\alpha]_D^{25}$ +2.4 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.73 (s, 1H, CH), 8.51 (d, 1H, *J*=7.4 Hz, CH), 7.90 (d, 1H, *J*=7.4 Hz, CH), 7.30–7.34 (m, 5H, Ph), 5.30 (d, 1H, *J*=8.5 Hz, NH), 5.06 (s, 2H, CH_2), 4.47 (t, 2H, *J*=6.8 Hz, N–CH_2), 4.30–4.38 (m, 1H, CH), 3.71 (s, 3H, OMe), 3.62 (s, 1H, CH≡), 1.42–2.05 (m, 6H, 3×CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.6, 155.9, 155.3, 151.4, 142.8, 136.2 (Cq); 134.5, 128.6, 128.3, 128.1 (CH); 125.1 (CH), 124.5 (Cq), 124.3 (CH), 114.7 (Cq); 84.0 (CH≡), 67.2 (CH_2), 53.5, 52.6 (CH); 50.2 (CH_2), 32.2, 29.8, 22.2 (CH_2). HRESIMS: calcd for $C_{25}H_{24}N_6NaO_4S$: 527.1477; found: *m*/*z* 527.1472.

*4.4.11. 6-(4-{7-[1-(2(S)-tert-Butoxycarbonylamino-2-methoxycarbonyl-ethyl)-1H-1,2,3-triazol-4-yl]-2,1,3-benzothiadiazol-4-yl}-1,2,3-triazol-1-yl)-2(S)-benzyloxycarbonylamino-hexanoic acid benzyl ester **10**.* From **26** (15 mg, 0.03 mmol) and **12** (8 mg, 0.03 mmol), column chromatography (petroleum ether/EtOAc, 1/3) afforded compound **10** as a yellow solid (20 mg, 91%). TLC: R_f=0.2 (petroleum ether/EtOAc=1/2). $[\alpha]_D^{25}$ +22.4 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.69 (s, 2H, 2×CH), 8.59 (s, 2H, 2×CH), 7.26–7.32 (m, 5H, Ph), 5.50 (d, 1H, *J*=6.9 Hz, NH), 5.35 (d, 1H, *J*=8.2 Hz, NH), 5.05 (s, 2H, CH_2), 4.95 (m, 2H, CH_2), 4.77 (m, 1H, CH), 4.44 (t, 2H, *J*=6.9 Hz, CH_2), 4.36 (m, 1H, CH), 3.82 (s, 3H, OMe), 3.70 (s, 3H, OMe), 1.38–2.06 (m, 6H, 3×CH_2), 1.43 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.7, 169.6, 155.9, 155.2, 152.2, 143.2, 136.2 (Cq); 128.6, 128.3, 128.1, 126.2, 126.0, 125.2, 123.9 (CH); 122.8, 122.3, 80.8 (Cq); 67.1 (CH_2), 53.9, 53.5 (CH); 53.2, 52.6 (OMe); 50.9, 50.1, 32.1, 29.8 (CH_2), 28.3 (CH_3), 22.3 (CH_2). HRESIMS: calcd for $C_{34}H_{40}N_{10}NaO_8S$: 771.2649; found: *m*/*z* 771.2644.

4.5. General procedure for the saponification

To a solution of methyl ester in MeOH (1 mL) and water (0.3 mL) were added LiOH (1.2 equiv/ester). The reaction mixture was stirred at rt for 12–24 h, then acidified with resine H^+, filtered, evaporated directly to get the free acid without purification.

*4.5.1. 2(S)-tert-Butoxycarbonylamino-3-[4-(2(S)-tert-butoxycarbonylamino-2-carboxy-ethoxymethyl)-1,2,3-triazol-1-yl]-propionic acid **17**.* Saponification of **1** (28 mg, 0.06 mmol) afforded **17** as a white solid (26 mg, 96%). TLC: R_f=0.1 (CH_2Cl_2/MeOH=3/1). $[\alpha]_D^{25}$ +8.3 (*c* 1.4, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 7.85 (s, 1H, CH), 4.84 (dd, 1H, *J*=4.2, 14.2 Hz, CH), 4.60 (dd, 1H, *J*=7.4, 12.8 Hz, CH), 4.57 (s, 2H, CH_2), 4.40 (s, 1H, CH), 4.38–4.42 (m, 1H, CH), 3.70 (m, 2H, CH_2), 1.41 (s, 9H, *t*-Bu), 1.37 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, CD_3OD): δ 173.5, 172.9, 156.5, 156.0, 144.2 (Cq); 124.4 (CH), 79.2, 79.2 (Cq), 70.3, 63.8 (CH_2), 55.6, 54.6 (CH); 51.6 (CH_2), 27.4 (CH_3).

*4.5.2. 2(S)-tert-Butoxycarbonylamino-3-{4-[4-(2(S)-tert-butoxycarbonylamino-2-carboxy-ethyl)-phenoxymethyl]-1,2,3-triazol-1-yl}-propionic acid **20**.* Saponification of **4** (21 mg, 0.04 mmol) afforded **20** as a white solid (18 mg, 90%). TLC: R_f=0.1 (CH_2Cl_2/MeOH=3/1). $[\alpha]_D^{25}$ +25.3 (*c* 1.2, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 7.92 (s, 1H, CH), 7.12 (d, 2H, *J*=8.2 Hz, Tyr), 6.86 (d, 2H, *J*=8.2 Hz, Tyr), 5.07 (s, 2H, CH_2), 4.87 (dd, 1H, *J*=6.4, 13.7 Hz, CH), 4.58 (dd, 1H, *J*=6.4, 13.7 Hz, CH), 4.33 (m, 1H, CH), 4.13 (m, 1H, CH), 3.05 (dd, 1H, *J*=4.6, 13.8 Hz, CH), 2.80 (dd, 1H, *J*=7.3, 13.3 Hz, CH), 1.37 (s, 9H, *t*-Bu), 1.36 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, CD_3OD): δ 157.1, 156.1, 143.6, 130.8 (Cq); 130.3 (CH), 124.5 (CH), 114.1 (CH), 79.2, 78.7 (Cq), 61.1 (CH_2), 57.1, 56.0 (CH), 52.1, 37.5 (CH_2), 27.4 (CH_3).

*4.5.3. 2(S)-tert-Butoxycarbonylamino-3-(4-{7-[1-(2(S)-tert-butoxycarbonylamino-2-carboxy-ethyl)-1H-1,2,3-triazol-4-yl]-2,1,3-benzothiadiazol-4-yl}-1,2,3-triazol-1-yl)-propionic acid **27**.* Saponification of **8** (44 mg, mmol) afforded **27** as a yellow solid (39 mg, 95%). TLC: R_f=0.3 (CH_2Cl_2/MeOH=3/1). $[\alpha]_D^{25}$ +29.4 (*c* 0.5, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 8.99 (s, 2H, 2×CH), 8.50 (s, 2H, 2×CH), 5.06 (m, 2H, 2×CH), 4.75 (m, 2H, 2×CH), 4.52 (m, 2H, 2×CH), 1.32 (s, 18H, 2×*t*-Bu). ^{13}C NMR (100 MHz, CD_3OD): δ 172.6, 156.1, 151.9, 142.4 (Cq); 125.6, 125.0 (CH); 122.5, 79.3 (Cq); 55.3 (CH), 51.7 (CH_2), 27.3 (CH_3).

*4.5.4. 2(S)-Benzyloxycarbonylamino-6-(4-{7-[1-(5(S)-benzyloxycarbonylamino-5-carboxy-pentyl)-1H-1,2,3-triazol-4-yl]-2,1,3-benzothiadiazol-4-yl}-1,2,3-triazol-1-yl)-hexanoic acid **28**.* Saponification of **9** (50 mg, 0.06 mmol) afforded **28** as a yellow solid (44 mg, 92%). TLC: R_f=0.3 (CH_2Cl_2/MeOH=3/1). $[\alpha]_D^{25}$ +12.0 (*c* 0.3, DMSO); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.93 (s, 2H, 2×CH), 8.48 (s, 2H, 2×CH), 7.18–7.28 (m, 10H, Ph), 6.74 (d, 2H, *J*=6.4 Hz, 2×NH), 4.86 (m, 4H, 2×CH_2), 4.43 (t, 4H, *J*=6.7 Hz, 2×N–CH_2), 3.67 (m, 2H, 2×CH), 1.25–1.85 (m, 12H, 6×CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.0, 156.0, 152.0, 142.2, 137.7 (Cq); 128.8, 128.2, 128.0, 125.8, 125.1 (CH); 122.8 (Cq), 65.6 (CH_2), 55.4 (CH), 50.2, 32.0, 30.3, 22.8 (CH_2).

*4.5.5. 6-(4-{7-[1-(2(S)-tert-Butoxycarbonylamino-2-carboxy-ethyl)-1H-1,2,3-triazol-4-yl]-2,1,3-benzothiadiazol-4-yl}-1,2,3-triazol-1-yl)-2(S)-benzyloxycarbonylamino-hexanoic acid **29**.* Saponification of **10** (12 mg, 0.02 mmol) afforded **29** as a yellow solid (10 mg, 90%). TLC: R_f=0.3 (CH_2Cl_2/MeOH=3/1). $[\alpha]_D^{25}$ +10.1 (*c* 0.7, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 8.94 (s, H, CH), 8.91 (s, H, CH), 8.41 (s, 2H, 2×CH), 7.15–7.27 (m, 5H, Ph), 5.03 (dd, 1H, *J*=3.6, 13.7 Hz, CH), 4.94 (s, 2H, CH_2), 4.72 (dd, 1H, *J*=6.9, 13.8 Hz, CH), 4.50 (t, 2H, *J*=6.9 Hz, CH_2), 4.41 (m, 1H, CH), 4.01 (m, 1H, CH), 1.41–2.06 (m, 6H, 3×CH_2), 1.30 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, CD_3OD): δ 178.0, 173.5, 156.8, 156.6, 152.0, 142.6, 136.9 (Cq); 128.0, 127.5, 127.2, 125.5, 125.2, 125.1, 125.0, 124.8 (CH); 122.6, 122.3, 79.1 (Cq); 66.0 (CH_2), 56.1, 56.0 (CH); 52.3, 50.1, 32.2, 29.7 (CH_2), 27.4 (CH_3), 22.3 (CH_2).

4.6. General procedure for the hydrogenolysis of benzyl ester

Benzyl ester (0.55 mmol) in MeOH (15 mL) was hydrogenated over 10% Pd–C (5 wt %) at rt for 12 h. After filtration, the filtrate was evaporated to get the pure product.

*4.6.1. 2(S)-tert-Butoxycarbonylamino-3-[4-(2(S)-tert-butoxycarbonylamino-2-methoxycarbonyl-ethoxymethyl)-1,2,3-triazol-1-yl]-propionic acid **18**.* Hydrogenolysis of **2** (320 mg, 0.55 mmol) afforded **18** as a colorless oil (266 mg, 92%). TLC: R_f=0.1 (CH_2Cl_2/MeOH=3/1). $[\alpha]_D^{25}$ −9.2 (*c* 0.5, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 7.89 (s, 1H, CH), 4.80 (dd, 1H, *J*=4.2, 13.8 Hz, CH), 4.66 (m, 1H, CH), 4.62 (m, 1H, CH), 4.58 (s, 2H, CH_2), 4.16 (s, 1H, CH), 3.71 (s, 3H, MeO), 3.70 (m, 2H, CH_2), 1.40 (s, 9H, *t*-Bu), 1.37 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, CD_3OD): δ 170.0, 156.4, 156.1, 144.5 (Cq); 124.6 (CH), 79.8, 79.0 (Cq), 70.9, 63.8 (CH_2), 53.8 (CH); 51.9 (MeO), 50.1 (CH_2), 27.4, 27.3 (CH_3).

*4.6.2. 3-[4-(2(S)-tert-Butoxycarbonylamino-ethoxymethyl)-2-carboxy-1,2,3-triazol-1-yl]-2(S)-tert-butoxycarbonylamino-propionic acid methyl ester **19**.* Hydrogenolysis of **3** (180 mg, 0.31 mmol) afforded **19** as a colorless oil (130 mg, 86%). TLC: R_f=0.1 (CH_2Cl_2/MeOH=3/1). $[\alpha]_D^{25}$ −6.9 (*c* 0.9, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 7.88 (s, 1H, CH), 4.84 (d, 1H, *J*=4.1 Hz, CH), 4.65 (dd, 1H, *J*=7.8, 13.8 Hz, CH), 4.50

(s, 3H, CH and CH_2), 4.29 (t, 1H, J=3.1 Hz, CH), 3.80 (dd, 1H, J=4.6, 9.6 Hz, CH), 3.68 (s, 4H, CH and OMe), 1.42 (s, 9H, *t*-Bu), 1.31 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, CD_3OD): δ 171.2, 156.6, 156.5, 144.0 (Cq); 124.5 (CH), 79.6, 79.5 (Cq); 69.5, 63.8 (CH_2), 54.0 (CH); 51.6 (OMe), 50.8 (CH_2), 27.3 (CH_3).

4.7. 2-{6-[1-(2(*S*)-Amino-2-methoxycarbonyl-ethyl)-1*H*-1,2,3-triazol-4-ylmethoxy]-3-oxo-3*H*-xanthen-9-yl}-benzoic acid 1-(2(*S*)-amino-2-methoxycarbonyl-ethyl)-1*H*-1,2,3-triazol-4-ylmethyl ester, compound with trifluoroacetic acid 23

To a solution of **6** (12 mg, 0.013 mmol) in CH_2Cl_2 (10 mL) was added CF_3CO_2H (0.1 mL, 1.4 mmol). The reaction mixture was stirred at rt for 12 h, then evaporated to get red oil (12 mg, 100%). TLC: R_f=0.2 (CH_2Cl_2/MeOH=3/1). $[\alpha]_D^{25}$ +5.0 (*c* 0.8, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 8.32 (dd, 1H, J=1.4, 9.2 Hz, H-Ar), 8.29 (s, 1H, H-Ar), 7.80–7.91 (m, 3H, H-Ar), 7.68 (s, 1H, CH), 7.45 (dd, 1H, J=1.4, 7.8 Hz, H-Ar), 7.30–7.34 (m, 2H, H-Ar), 7.16–7.19 (m, 1H, H-Ar), 7.07 (s, 1H, CH), 6.96–6.99 (m, 1H, H-Ar), 5.53 (s, 2H, CH_2), 5.00–5.08 (m, 4H, 2×CH_2), 4.91–4.97 (m, 3H, CH and CH_2), 4.68–4.77 (m, 1H, CH), 3.80 (s, 6H, 2×OMe). ^{13}C NMR (100 MHz, CD_3OD): δ 180.0, 168.1, 161.4, 158.5, 143.8, 143.3, 134.5, 134.4 (Cq); 133.1, 132.4, 131.1, 130.9, 130.8, 130.2, 126.3, 125.9 (CH); 118.7, 118.6 (Cq); 117.7, 104.3, 102.5 (CH), 63.7, 58.8 (CH_2), 54.3 (OMe), 53.6, 53.5 (CH); 48.6, 48.5 (CH_2).

4.8. Preparation of 2(*S*)-amino-3-(4-{7-[1-(2(*S*)-amino-2-methoxycarbonyl-ethyl)-1*H*-1,2,3-triazol-4-yl]-2,1,3-benzothiadiazol-4-yl}-1,2,3-triazol-1-yl)-propionic acid methyl ester, compound with trifluoroacetic acid 30

To a solution of **8** (26 mg, 0.04 mmol) in CH_2Cl_2 (10 mL) was added CF_3CO_2H (0.1 mL, 1.4 mmol). The reaction mixture was stirred at rt for 12 h, then evaporated to get red oil (26 mg, 100%). TLC: R_f=0.4 (CH_2Cl_2/MeOH=3/1). $[\alpha]_D^{25}$ +25.8 (*c* 0.5, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 9.05 (s, 2H, 2×CH), 8.48 (s, 2H, 2×CH), 5.10–5.22 (m, 4H, 2×CH_2), 4.83 (m, 2H, 2×CH), 3.91 (s, 6H, 2×OMe). ^{13}C NMR (100 MHz, CD_3OD): δ 168.4, 153.3, 144.4 (Cq); 127.3, 126.8 (CH); 123.7 (Cq); 54.3 (OMe), 53.9 (CH_2), 50.2 (CH). HRESIMS: calcd for $C_{24}H_{33}N_{10}O_4S$: 557.2407; found: *m*/*z* 557.2402.

4.9. Preparation of 2(*S*)-amino-6-(4-{7-[1-(5(*S*)-amino-5-methoxycarbonyl-pentyl)-1*H*-1,2,3-triazol-4-yl]-2,1,3-benzothiadiazol-4-yl}-1,2,3-triazol-1-yl)-hexanoic acid methyl ester 31

To precooled (ice bath) AcOH (0.5 mL) was added **9** (22 mg, 0.03 mmol) and 33% HBr/AcOH (0.2 mL) successively, the solution was stirred for 12 h, then poured into 10 mL water, neutralized with solid $NaHCO_3$, extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were washed with brine and dried over anhydrous $MgSO_4$, then evaporated. Purification by column chromatography (CH_2Cl_2/MeOH=15/1) afforded yellow oil (10 mg, 68%). TLC: R_f=0.1 (CH_2Cl_2/MeOH=15/1). $[\alpha]_D^{25}$ −8.0 (*c* 0.1, DMSO); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.73 (s, 2H, 2×CH), 8.66 (s, 2H, 2×CH), 4.48 (t, 4H, J=7.1 Hz, 2×N–CH_2), 3.70 (s, 6H, 2×OMe), 3.43 (m, 2H, 2×CH), 1.46–2.08 (m, 12H, 6×CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.4, 152.4, 143.2 (Cq); 126.2, 123.9 (CH); 122.7 (Cq), 54.2 (CH), 52.2 (OMe), 50.3, 34.2, 30.2, 22.8 (CH_2). HRESIMS: calcd for $C_{18}H_{21}N_{10}O_4S$: 473.1468; found: *m*/*z* 473.1463.

Acknowledgements

C.L. thanks the China Scholarship Council for a Doctorate scholarship.

References and notes

1. Taylor, C. M.; Wang, W. *Tetrahedron* **2007**, *63*, 9033–9047.
2. (a) James, I. T.; Walne, A. J.; Perrett, D. *Ann. Clin. Biochem.* **1996**, *33*, 397–400; (b) Christenson, R. H. *Clin. Biochem.* **1997**, *30*, 573–593.
3. (a) Allevi, P.; Longo, A.; Anastasia, M. *Chem. Commun.* **1999**, 559–560; (b) Adamczyk, M.; Johnson, D. D.; Reddy, R. E. *Tetrahedron: Asymmetry* **2000**, *11*, 2289–2298; (c) Allevi, P.; Cribiù, R.; Giannini, E.; Anastasia, M. *Bioconjugate Chem.* **2005**, *16*, 1045–1048; (d) Allevi, P.; Femia, E. A.; Rota, P.; Costa, M. L.; Anastasia, M. *Tetrahedron: Asymmetry* **2008**, *19*, 2470–2478.
4. (a) Patchornik, A.; Sokolovsky, M. J. *J. Am. Chem. Soc.* **1964**, *86*, 1860–1861; (b) Tohdo, K.; Hamada, Y.; Shiori, T. *Pept. Chem.* **1992**, 7–12; (c) Tohdo, K.; Hamada, Y.; Shioiri, T. *Synlett* **1994**, 247–249.
5. (a) David, O.; Maisonneuve, S.; Xie, J. *Tetrahedron Lett.* **2007**, *48*, 6527–6530; (b) Maisonneuve, S.; Fang, J.; Xie, J. *Tetrahedron* **2007**, *64*, 8716–8720; (c) Lin, L.; Shen, Q.; Chen, Q.-R.; Xie, J. *Bioorg. Med. Chem.* **2008**, *16*, 9757–9763; (d) Cheng, K. G.; Liu, J.; Liu, X. F.; Li, H. L.; Sun, H. B.; Xie, J. *Carbohydr. Res.* **2009**, *344*, 841–850.
6. (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004–2021; (b) Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057–3069.
7. Gonçalves, M. S. T. *Chem. Rev.* **2009**, *109*, 190–212.
8. Wei, Z.-L.; Petukhov, P. A.; Bizik, F.; Teixeira, J. C.; Mercola, M.; Volpe, E. A.; Glazer, R. I.; Willson, T. M.; Kozikowski, A. P. *J. Am. Chem. Soc.* **2004**, *126*, 16714–16715.
9. Otsuka, M.; Kittaka, A.; Iimori, T.; Yamashita, H.; Kobayashi, S.; Ohno, M. *Chem. Pharm. Bull.* **1985**, *33*, 509–514.
10. Gajewski, M.; Seaver, B.; Esslinger, C. S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4163–4166.
11. Bew, S. P.; Brimage, R. A.; L'Hermite, N.; Sharma, S. V. *Org. Lett.* **2007**, *9*, 3713–3716.
12. Goddard-Borger, E. D.; Stick, R. V. *Org. Lett.* **2007**, *9*, 3797–3800.
13. Varazo, K.; Xie, F.; Gulledge, D.; Wang, Q. *Tetrahedron Lett.* **2008**, *49*, 5293–5296.
14. DaSilveira Neto, B. A.; Sant'Ana Lopes, A.; Ebeling, G.; Gonçalves, R. S.; Costa, V. E. U.; Ouina, F. H.; Dupont, J. *Tetrahedron* **2005**, *61*, 10975–10982.
15. (a) Raimundo, J.-M.; Blanchard, P.; Brisset, H.; Akoudad, S.; Roncali, J. *Chem. Commun.* **2000**, 939–940; (b) Huang, J.; Li, C.; Xia, Y.-J.; Zhu, X.-H.; Peng, J.; Cao, Y. *J. Org. Chem.* **2007**, *72*, 8580–8583.
16. Chen, L.; Hu, T.-S.; Zhu, J.; Wu, H.; Yao, Z.-J. *Synlett* **2006**, 1225–1229.
17. Callan, J. F.; de Silva, A. P.; Magri, D. C. *Tetrahedron* **2005**, *61*, 8551–8588.