Fluorescence Labeling of Amino Acids and Peptides with 7-Aminocoumarins

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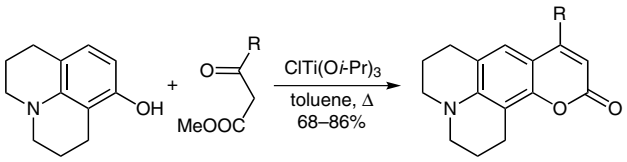
Abstract: Pechmann condensation is a straightforward protocol for the synthesis of alkynyl- and azido-substituted 7-(dialkylamino)coumarins, which can be coupled to amino acid and peptide derivatives by copper-catalyzed [3+2]-cycloaddition (click reaction) in high yield. This allows the introduction of these efficient fluorescence labels into biologically relevant molecules.

Key words: amino acids, click chemistry, cycloaddition, coumarins, fluorescence labeling, peptides

In the life sciences, fluorescence microscopy is an important and powerful tool for the three-dimensional imaging of tissues and living cells.[1] A wide range of fluorescence dyes can be used for the labeling of proteins and drugs, allowing the investigation of cellular processes and target identification.[2] Besides the BODIPYs[3] and fluoresceine derivatives,[4] coumarins also play a major role. Excellent fluorescence quantum yields are obtained with coumarin derivatives bearing electron-donating substituents at the 7-position, such as the 7-(dialkylamino)coumarins.[5] Therefore, these derivatives are appropriate candidates for the development of fluorescence labels.[6] These labels have to be coupled with biomolecules such as peptides and proteins.[7] In general, functionalized amino acid side chains, as found in cysteine,[8] lysine,[9] or tyrosine,[10] are ideal candidates for selective labeling. In addition, the Huisgen–Meldal–Sharpless reaction,[11] a copper-catalyzed [3+2]-cycloaddition between an azide and an alkyne, was developed to an attractive labeling strategy during the last years.[12] At the beginning, this technique could only be applied *in vitro*, because of the cytotoxicity of copper in the μM range. But in the meanwhile a protocol has been developed to reduce the copper levels, so that this method has become biocompatible.[13]

For an application of this click chemistry,[14] the biological target has to be modified by introduction of an alkyne group, while the fluorescence label has to contain an azide, or the other way around. Because of our previous work on natural product synthesis,[15] we became interested in the fluorescence labeling of peptides for biological studies.[16]

While a wide range of reactions can be used for the synthesis of coumarins, the Pechmann condensation is probably the most popular approach.[17] Here, a phenol reacts with a β-keto ester in the presence of a strong acid such as sulfuric acid. Although this approach is suitable for a wide range of phenols, it is critical for aminophenols, probably because of protonation and deactivation of the aromatic ring by the strong acid. Therefore, we recently developed a very mild procedure using chlorotriisopropoxytitanium(IV), which is especially suitable for the synthesis of the required 7-(dialkylamino)coumarins (Scheme 1).[18]



Scheme 1 Synthesis of 7-(dialkylamino)coumarins via modified Pechmann synthesis

Herein we report on the synthesis of coumarin-based 'clickable' fluorescence labels and their application in the modification of amino acids and peptides. The synthesis of alkyne- and azide-substituted coumarins is shown in Scheme 2. According to a procedure developed by Masamune et al.[19] pent-4-ynoic acid was first activated with 1,1′-carbonyldiimidazole (CDI) and subsequently coupled with the potassium salt of monomethyl malonate in the presence of magnesium chloride. The desired β-keto ester **1** was obtained in good yield and directly subjected to a modified Pechmann condensation using 8-hydroxyjulodin as phenol component. The Pechmann condensation delivered the alkynylated coumarin derivative **2** in high yield. For the synthesis of analogous azidocoumarin we started with the previously described aminoethyl derivative using a diazo-transfer reagent.[20] The required triflyl azide was freshly prepared before use.[21] Unfortunately, the expected azide **3** was obtained only in modest yield. Therefore, we decided to switch to a more straightforward approach using again the modified Pechmann condensation. Starting from commercially available 4-chloroacetoacetate under standard conditions, the chlorinated coumarin **4** was obtained in acceptable yield. Subsequent nucleophilic substitution with sodium azide gave rise to azidocoumarin **5**.

With these coumarin derivatives in hand we next focused on the synthesis of azide- and alkyne-substituted amino acid and peptide derivatives. Two alkynylated peptides **7** and **8** could be obtained easily starting from *N*-Boc-protected serine (Scheme 3). Deprotonation with an excess of sodium hydride and propargylation with propargyl bro-

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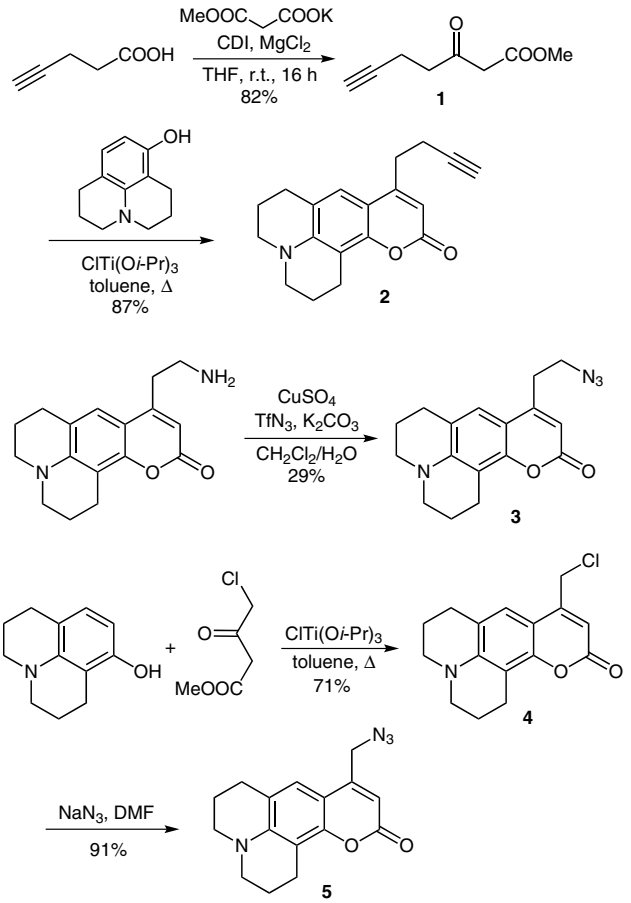
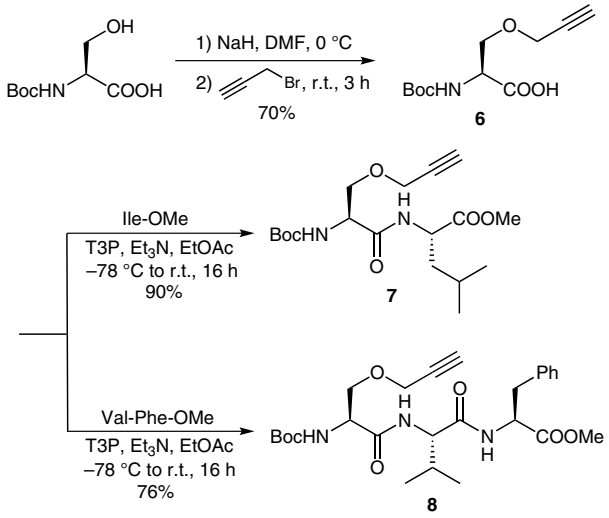


Scheme 2 Synthesis of 'clickable' aminocoumarins

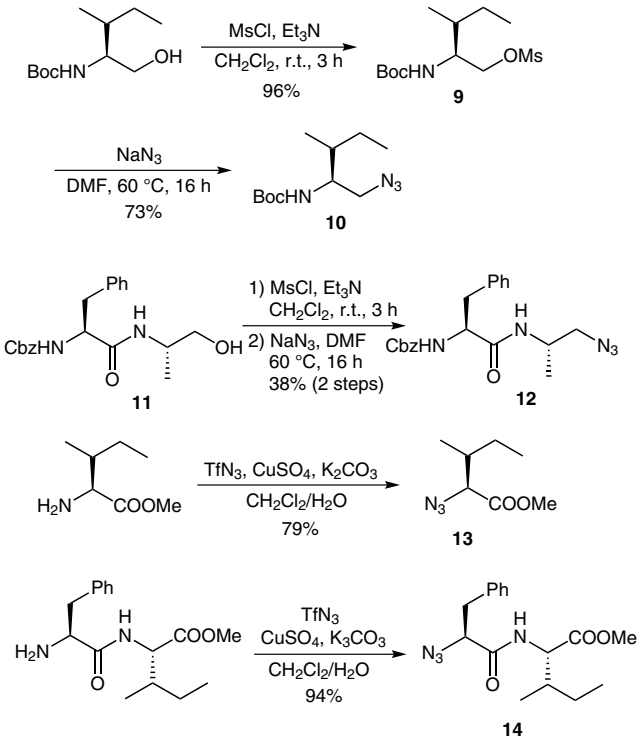


Scheme 3 Synthesis of acetylenic amino acids and peptides

mide provided ether **6** in acceptable yield, without significant amounts of propargyl ester formed.

Initial peptide couplings were performed with *N*,*N*′-dicyclohexylcarbodiimide, but here some side products were obtained, which could not be separated easily from the desired product. Therefore, we switched to propylphosphonic anhydride (T3P). In this case, possible side products can easily be removed with water, providing the required peptides in pure form.[22]

To get access to some amino acid and peptide derived azides we started on the one hand with *N*-Boc-protected leucinol and dipeptide alcohol **11** (Scheme 4). Reaction with mesyl chloride gave rise to mesylate **9** in excellent yield, which could be converted into the required azide **10**. We observed that **9** is not a very stable compound and should not be stored for long. It should be coupled directly after preparation. Even worse was the situation in the case of the dipeptide alcohol **11**. Also here, the mesylate was nicely formed, but the decomposition started immediately. Therefore, the crude product was directly reacted with sodium azide to give **12**.

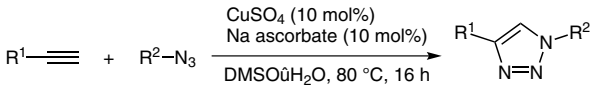


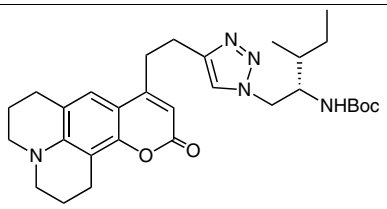
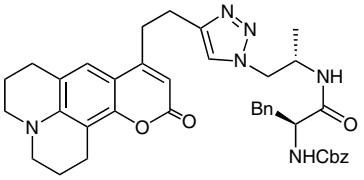
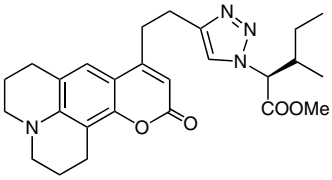
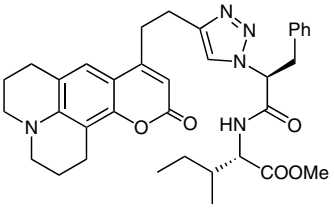
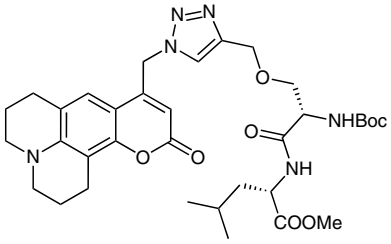
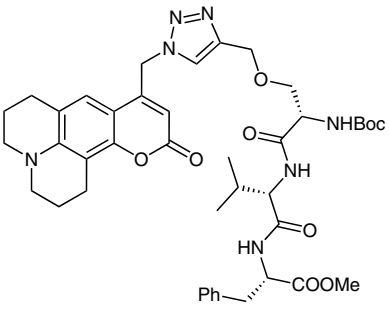
Scheme 4 Synthesis of amino acid and peptide azides

On the other hand, we introduced the azide functionality into the N-terminus of amino acids and dipeptides as illustrated for azides **13** and **14**. In contrast to the previous reaction, the diazo transfer with triflyl azide, starting from the corresponding free amines, provided the azides **13** and **14** in high yield and in pure form.

Now we had building blocks in hand with 'clickable' functionalities at the N-terminus, the previous C-terminus, as well as in the side chain. The results obtained in the subsequent click reactions are summarized in Table 1. We started our investigations with the acetylenic coumarin **2** and the leucine-derived azide **10**. Under standard coupling conditions, by using 10 mol% each of copper(II) sulfate and sodium ascorbate, a very sluggish reaction was observed at room temperature. Even after six days a yield of only 25% was obtained.

Table 1 Fluorescence Labeling of Amino Acids and Peptides via Huisgen–Meldal–Sharpless Reaction



Entry	Alkyne	Azide	Product	Yield (%)
1	**2**	**10**	**15** 	78
2	**2**	**12**	**16** 	78
3	**2**	**13**	**17** 	77
4	**2**	**14**	**18** 	75
5	**7**	**5**	**19** 	84
6	**8**	**5**	**20** 	77

The situation changed significantly when we heated the reaction mixture to 80 °C overnight. A clean conversion was observed and the triazole derivative **15** was isolated in 78% yield (entry 1). Exactly the same result was obtained with the dipeptide-derived azide **12** (entry 2). Steric hindrance does not seem to play a significant role. Even with azides **13** and **14** with the azido group at the α-position of an amino acid, comparable results were obtained (entries 3 and 4).

Last but not least, we changed the components, using azide **5** and the propargyl ethers **7** and **8**. The same picture resulted: also here the yields were in the region of 80% (entries 5 and 6). In all examples investigated so far, the yields were in the range of 75–84%, and only the 1,4-disubstituted triazoles are formed.

In conclusion, we could show that 'clickable' derivatives of coumarin fluorescence dyes are ideally suited to label amino acids and peptides in constantly high yield. Steric factors seem to play no significant role, as nearly the same results are obtained independent of the coupling position. Further applications of this protocol are currently under investigation.

The products were purified by flash chromatography on silica gel (0.063–0.2 mm) or on Flashsystem Reveleris® (UV- and ELSD-detector, Grace). Mixtures of EtOAc and hexanes were generally used as eluents. Analysis by TLC was carried out on commercially precoated silica gel 60 TLC-PET plates (Fluka). Visualization was accomplished with UV light or $KMnO_4$ solution. ^1H and ^{13}C NMR spectroscopic analysis was performed on a Bruker Avance II 400 (400 MHz) spectrometer. HRMS were measured with at Finnigan MAT 95S mass spectrometer at the Institute of Organic Chemistry at Saarland University. Elemental analyses were carried out at the Department of Chemistry at Saarland University.

3-Oxo-hept-6-ynoic Acid Methyl Ester (1)[23]

A suspension of $MgCl_2$ (938 mg, 9.86 mmol) and the potassium salt of hydrogen methyl malonate (2.22 g, 14.2 mmol) in anhyd THF (15 mL) was stirred for 4 h at 50 °C. In a second flask, CDI (1.85 g, 11.4 mmol) was added in portions to a solution of pent-4-ynoic acid (930 mg. 9.48 mmol) in anhyd THF (20 mL) at 5 °C. Then the mixture was stirred for 1 h at r.t. To the methylmagnesium malonate suspension, the aforementioned imidazolide solution was added dropwise at r.t. The reaction mixture was stirred at r.t. overnight, concentrated under reduced pressure, and dissolved in EtOAc (20 mL). The suspension thus obtained was washed with 1 M $KHSO_4$, $NaHCO_3$, and brine (15 mL each). The organic layer was dried (Na_2SO_4) and the solvent was evaporated in vacuo. The crude product was purified by flash chromatography (silica gel, hexane–EtOAc, 8:2); this gave **1** as a pale yellow oil.

Yield: 1.20 g (7.78 mmol, 82%).

^1H NMR (400 MHz, $CDCl_3$): δ = 1.96 (t, *J* = 2.7 Hz, 1 H, 1-H), 2.47 (td, *J* = 7.2, 2.7 Hz, 2 H, 3-H), 2.81 (t, *J* = 7.2 Hz, 2 H, 4-H), 3.48 (s, 2 H, 6-H), 3.74 (s, 3 H, 8-H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.8, 41.6, 48.9, 52.4, 69.0, 82.4, 167.3, 200.4.

Aminocoumarins 2 and 4; General Procedure

A 1 M solution of Ti(O*i*-Pr)$_3$Cl (2.00 equiv) was added to a suspension of 8-hydroxyjulodin (1.00 equiv) and the corresponding β-keto ester (1.00 equiv) in toluene (3 mL/mmol). The mixture was heated to reflux overnight. After the mixture had cooled to r.t., CH_2Cl_2 (10 mL/mmol) was added and the whole solution was poured into a stirred sat. potassium sodium tartrate solution (10 mL/mmol) until the phases separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were dried (Na_2SO_4), the solvent was removed in vacuo, and the crude product was purified by flash chromatography (silica gel, hexane–EtOAc).

8-But-3-ynyl-2,3,5,6-tetrahydro-1*H*,4*H*-11-oxa-3a-azabenzo[*de*]anthracen-10-one (2)

According to the general aminocoumarin procedure, 8-hydroxyjulodin (378 mg, 2.00 mmol) and β-keto ester **1** (308 mg, 2.00 mmol) were allowed to react in the presence of Ti(O*i*-Pr)$_3$Cl (4 mL, 4.00 mmol); this gave **2** after flash chromatography (silica gel, hexane–EtOAc 7:3) as a yellow solid.

Yield: 508 mg (1.73 mmol, 87%); mp 125–127 °C.

^1H NMR (400 MHz, $CDCl_3$): δ = 1.93–2.00 (m, 4 H, 3-H, 14-H), 2.04 (t, *J* = 2.6 Hz, 1 H, 19-H), 2.54 (td, *J* = 7.4, 2.6 Hz, 2 H, 17-H), 2.77 (t, *J* = 6.3 Hz, 2 H, 16-H), 2.86–2.90 (m, 4 H, 4-H, 13-H), 3.22–3.27 (m, 4 H, 2-H, 15-H), 5.94 (s, 1 H, 9-H), 6.97 (s, 1 H, 6-H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.9, 20.5, 20.6, 21.6, 27.8, 30.6, 49.5, 49.9, 69.9, 82.5, 107.0, 107.4, 107.6, 118.0, 121.0, 145.8, 151.3, 154.1, 162.4.

HRMS (CI): *m*/*z* calcd for $C_{19}H_{19}NO_2$ $[M]^+$: 293.1416; found: 293.1427.

Anal. Calcd for $C_{19}H_{19}NO_2$ (293.36): C, 77.79; H, 6.53; N, 4.77. Found: C, 77.54; H, 6.71; N, 4.69.

8-Chloromethyl-2,3,5,6-tetrahydro-1*H*,4*H*-11-oxa-3a-azabenzo[*de*]anthracen-10-one (4)

According to the general aminocoumarin procedure, 8-hydroxyjulodin (6.27 g, 33.2 mmol) and methyl 4-chloroacetoacetate (5.00 g, 33.2 mmol) were allowed to react in the presence of Ti(O*i*-Pr)$_3$Cl (66 mL, 66.0 mmol); this gave **4** after flash chromatography (silica gel, hexane–EtOAc, 7:3) as a yellow solid.

Yield: 6.83 g (23.6 mmol, 71%); mp 188–190 °C.

^1H NMR (400 MHz, $CDCl_3$): δ = 1.95–1.99 (m, 4 H, 3-H, 14-H), 2.79 (t, *J* = 6.5 Hz, 2 H, 4-H), 2.88 (t, *J* = 6.5 Hz, 2 H, 13-H), 3.24–3.29 (m, 4 H, 2-H, 15-H), 4.54 (s, 2 H, 16-H), 6.13 (s, 1 H, 9-H), 7.02 (s, 1 H, 6-H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.5, 20.6, 21.5, 27.8, 41.6, 49.5, 49.9, 106.0, 107.0, 108.3, 118.3, 121.1, 146.1, 150.0, 151.5, 162.1.

HRMS (CI): *m*/*z* calcd for $C_{16}H_{16}ClNO_2$ $[M]^+$: 289.0870; found: 289.0888.

8-Azidomethyl-2,3,5,6-tetrahydro-1*H*,4*H*-11-oxa-3a-azabenzo[*de*]anthracen-10-one (5)

NaN_3 (306 mg, 4.70 mmol) was added to a solution of the chlorinated coumarin **4** (1.14 g, 3.92 mmol) in acetone (250 mL), and the reaction mixture was stirred overnight. The reaction control showed that the reaction was incomplete. Therefore, NaN_3 (306 mg, 4.70 mmol) was added again and the mixture was stirred overnight at 35 °C. The solvent was removed under vacuum and the crude product was purified by flash chromatography (silica gel, hexane–EtOAc, 9:1, 8:2); this gave **5** as an orange solid.

Yield: 1.05 g (3.54 mmol, 91%); mp 148–152 °C.

^1H NMR (400 MHz, $CDCl_3$): δ = 1.94–2.00 (m, 4 H, 3-H, 14-H), 2.77 (t, *J* = 6.3 Hz, 2 H, 4-H), 2.89 (t, *J* = 6.3 Hz, 2 H, 13-H), 3.24–3.29 (m, 4 H, 2-H, 15-H), 4.40 (s, 2 H, 16-H), 6.09 (s, 1 H, 9-H), 6.92 (s, 1 H, 6-H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.4, 20.6, 21.5, 27.8, 49.5, 49.9, 51.0, 106.1, 107.0, 107.8, 118.3, 120.9, 146.1, 148.7, 151.5, 162.0.

HRMS (CI): *m*/*z* calcd for $C_{16}H_{16}N_4O_2$ $[M]^+$: 296.1273; found: 296.1294.

(*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-(prop-2-ynyloxy)propanoic Acid (6)

N-Boc-L-serine (5.00 g, 24.4 mmol) was dissolved in DMF (37 mL) and the solution was cooled to 0 °C. NaH (60% w/w) dispersion in mineral oil (2.16 g, 53.7 mmol) was added over 15 min and the reaction mixture was stirred for 1 h at 0 °C. 3-Bromopropyne (3.00 mL, 3.99 g, 26.8 mmol) was added dropwise over 15 min and the reaction mixture was stirred for 1 h at 0 °C. Afterwards the ice bath was removed and stirring continued for 3 h at r.t. The solvent was evaporated and the residue was dissolved in H_2O (50 mL). The solution was washed with Et_2O (3 × 20 mL) and acidified to pH 2 by addition of 3 M aq HCl. The resulting mixture was extracted with EtOAc (5 × 20 mL). The combined organic extracts were dried (Na_2SO_4) and the solvent was evaporated. The crude product was purified by flash chromatography (silica gel, hexane–EtOAc–AcOH, 1:1:0.01); this afforded **6** as a pale yellow, viscous oil.

Yield: 4.15 g (17.1 mmol, 70%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.44 (s, 9 H, 1-H), 2.46 (t, *J* = 2.3 Hz, 1 H, 8-H), 3.80 (dd, *J* = 9.3, 3.4 Hz, 1 H, 5-H), 3.98 (dd, *J* = 9.3, 2.4 Hz, 1 H, 5-H), 4.17 (d, *J* = 2.3 Hz, 2 H, 6-H), 4.48 (m, 1 H, 4-H), 5.41 (d, *J* = 8.4 Hz, 1 H, NH), 10.03 (br s, 1 H, COOH).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.3, 53.7, 58.7, 69.5, 75.3, 78.7, 80.4, 155.7, 175.1.

(*S*)-2-{(*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-(prop-2-ynyloxy)propanoylamino}-4-methylpentanoic acid Methyl Ester (7)

A 50% solution of T3P in EtOAc (9.2 mL, 4.93 g, 15.5 mmol) was added to a solution of **6** (2.50 g, 10.3 mmol), leucine methyl ester (1.50 g, 10.3 mmol), and DIPEA (5.4 mL, 3.99 g, 30.9 mmol) in EtOAc (150 mL) at 0 °C. The reaction mixture was stirred overnight at r.t. For workup, the mixture was quenched with brine and the aqueous layer was extracted with CH_2Cl_2 (2 × 25 mL). The combined organic extracts were washed with 1 M HCl and brine (25 mL each) and dried (Na_2SO_4). The solvent was removed under vacuum and the crude product was purified by flash chromatography (silica gel, hexane–EtOAc, 7:3); this gave **7** as a colorless, viscous oil.

Yield: 3.42 g (9.23 mmol, 90%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.91 (d, *J* = 6.5 Hz, 6 H, 13-H), 1.43 (s, 9 H, 1-H), 1.52–1.64 (m, 3 H, 11-H, 12-H), 2.44 (t, *J* = 1.4 Hz, 1 H, 8-H), 3.63 (dd, *J* = 9.3, 6.4 Hz, 1 H, 5-H), 3.70 (s, 3 H, 15-H), 3.90 (dd, *J* = 9.3, 4.0 Hz, 1 H, 5-H), 4.16 (d, 2 H, 6-H), 4.28 (br s, 1 H, 10-H), 4.60 (m, 1 H, 4-H), 5.39 (d, *J* = 8.7 Hz, 1 H, NH_{Ser}), 6.85 (br s, 1 H, NH_{Ile}).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.9, 22.8, 24.7, 28.2, 41.5, 50.6, 52.2, 53.6, 58.6, 69.3, 75.1, 78.8, 80.3, 155.4, 169.9, 172.9.

HRMS (CI): *m*/*z* calcd for $C_{18}H_{30}N_2O_6$ $[M + H]^+$: 371.2182; found: 371.2215.

Anal. Calcd for $C_{18}H_{30}N_2O_6$ (370.44): C, 58.36; H, 8.16; N, 7.56. Found: C, 57.86; H, 7.68; N, 7.29.

(*S*)-2-{(*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-(prop-2-ynyloxy)propanoylamino}-3-methylbutanoylamino]-3-phenylpropanoic Acid Methyl Ester (8)

A 50% solution of T3P in EtOAc (4.6 mL, 2.43 g, 7.65 mmol) was added to a solution of **6** (1.24 g, 5.10 mmol), valyl-phenylalanine methyl ester (1.42 g, 5.10 mmol), and DIPEA (2.7 mL, 1.98 g, 15.3 mmol) in EtOAc (45 mL) at 0 °C. The reaction mixture was stirred overnight at r.t. For workup, the mixture was quenched with brine (25 mL) and the aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic extracts were washed with 1 M HCl and brine (25 mL each) and dried (Na_2SO_4). The solvent was removed under vacuum and the crude product was purified by flash chromatography (silica gel, hexane–EtOAc, 7:3, 6:4); this gave **8** as a colorless solid.

Yield: 1.95 g (3.87 mmol, 76%); mp 87–90 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.84 und 0.90 (2d, *J* = 6.8 Hz, 6 H, 12-H, 13-H), 1.44 (s, 9 H, 1-H), 2.15 (m, 1 H, 11-H), 2.46 (t, *J* = 2.3 Hz, 1 H, 8-H), 3.06 (dd, *J* = 13.9, 6.4 Hz, 1 H, 16-H), 3.06 (dd, *J* = 13.9, 6.0 Hz, 1 H, 16-H), 3.63 (dd, *J* = 9.3, 6.4 Hz, 1 H, 5-H), 3.69 (s, 3 H, 22-H), 3.89 (dd, *J* = 9.3, 4.3 Hz, 1 H, 5-H), 4.10 (d, *J* = 2.3 Hz, 2 H, 6-H), 4.25–4.30 (m, 2 H, 4-H, 10-H), 4.84 (br s, 1 H, 15-H), 5.40 (br s, 1 H, NH_{Ser}), 6.55 (br s, 1 H, NH_{Phe}), 6.83 (d, *J* = 8.6 Hz, 1 H, NH_{Val}), 7.08–7.29 (m, 5 H, 18-H, 19-H, 20-H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.6, 19.1, 28.2, 30.5, 37.8, 52.2, 53.2, 54.0, 58.5, 58.6, 69.4, 75.3, 78.9, 80.4, 127.1, 128.6, 129.2, 135.7, 155.5, 170.0, 170.3, 171.

HRMS (CI): *m*/*z* calcd for $C_{26}H_{38}N_3O_7$ $[M + H]^+$: 504.2710; found: 504.2713.

Anal. Calcd for $C_{26}H_{37}N_3O_7$ (503.59): C, 62.01; H, 7.41; N, 8.34. Found: C, 62.08; H, 7.28; N, 8.21.

(*S*)-2-[(tert-Butoxycarbonyl)amino]-3-methylpentyl Methanesulfonate (9)

A solution of *N*-Boc-protected leucinol (1.00 g, 4.60 mmol) and Et_3N (1.45 g, 14.3 mmol) in absolute CH_2Cl_2 (23 mL) was cooled to 0 °C and MsCl (790 mg, 6.90 mmol) was added dropwise. The mixture was stirred 3 h at 0 °C. After dilution with CH_2Cl_2 (25 mL), the mixture was washed with 1 M $KHSO_4$ (20 mL), sat. aq $NaHCO_3$ (2 × 20 mL), H_2O, and brine (25 mL each). The organic layer was dried (Na_2SO_4) and the solvent was removed in vacuo. The crude product was purified by flash chromatography (silica gel, hexane–EtOAc, 7:3); this gave **9** as a colorless oil (unstable compound).

Yield: 1.30 g (4.42 mmol, 96%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.90–0.96 (m, 6 H, 6-H, 8-H), 1.16 (m, 1 H, 5-H), 1.45 (s, 9 H, 1-H), 1.51–1.66 (m, 2 H, 7-H), 3.02 (s, 3 H, 10-H), 3.70 (br s, 1 H, 4-H), 4.24–4.31 (m, 2 H, 9-H), 4.63 (d, *J* = 7.7 Hz, 1 H, NH).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.1, 15.4, 25.2, 28.3, 35.6, 37.4, 53.8, 69.7, 79.8; Boc carbonyl not observed.

HRMS (CI): *m*/*z* calcd for $C_{12}H_{24}NO_5S$ $[M]^+$: 295.1453; found: 293.1474.

***tert*-Butyl (*S*)-1-Azido-3-methylpentan-2-ylcarbamate (10)**

NaN_3 (2.22 g, 34.2 mmol) was added to a solution of **9** (1.30 g, 4.40 mmol) in absolute DMF (44 mL). After the reaction mixture had been stirred for 15 h at 60 °C, the cooled mixture was diluted with H_2O (100 mL) and EtOAc (100 mL). The aqueous layer was extracted with EtOAc (3 × 20 mL). Afterwards the combined organic layers were washed with H_2O (3 × 20 mL) and brine (20 mL). The organic layer was dried (Na_2SO_4) and the solvent was removed under vacuum. The crude product was purified by flash chromatography (silica gel, hexane–EtOAc, 8:2); this gave **10** as a colorless solid.

Yield: 782 mg (3.23 mmol, 73%); mp 48–50 °C. The azide is stable and can be stored at r.t.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.89–0.93 (m, 6 H, 6-H, 8-H), 1.12 (m, 1 H, 5-H), 1.45–1.58 (m, 11 H, 1-H, 7-H), 3.42 (m, 2 H, 9-H), 3.58 (br s, 1 H, 4-H), 4.55 (d, *J* = 7.3 Hz, 1 H, NH).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.3, 15.5, 25.2, 28.4, 36.3, 52.9, 54.4, 79.6, 155.5.

HRMS (CI): *m*/*z* calcd for $C_{11}H_{22}N_4O_4$ $[M]^+$: 243.1821; found: 243.1793.

Anal. Calcd for $C_{11}H_{22}N_4O_4$ (242.32): C, 54.52; H, 9.15; N, 23.12. Found: C, 54.52; H, 8.77; N, 22.94.

Benzyl (*S*,*S*)-1-(1-Hydroxypropan-2-ylamino)-1-oxo-3-phenylpropan-2-ylcarbamate (11)

$NaBH_4$ (446 mg, 11.8 mmol) and LiCl (500 mg, 11.8 mmol) in EtOH (14 mL) were added to a solution of *N*-Cbz-phenylalanylalanine methyl ester (2.27 g, 5.90 mmol) in THF (7 mL). The reac-

tion mixture was stirred overnight at r.t. For workup, the mixture was hydrolyzed with 10% aq citric acid (40 mL) and the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (50 mL) and washed with H_2O and brine (20 mL each). The organic layer was dried (Na_2SO_4) and the solvent was removed in vacuo. The crude product was purified by flash chromatography (silica gel, hexane–EtOAc, 1:1, EtOAc); this gave **11** as a colorless solid.

Yield: 1.68 g (4.71 mmol, 80%); mp 110–114 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.04 (d, *J* = 6.8 Hz, 3 H, 15-H), 2.15 (br s, 1 H, OH), 2.99 (dd, *J* = 13.5, 8.1 Hz, 1 H, 8-H), 3.11 (dd, *J* = 13.5, 6.3 Hz, 1 H, 8-H), 3.22 (dd, *J* = 11.2, 5.2 Hz, 1 H, 16-H), 3.41 (dd, *J* = 11.2, 3.6 Hz, 1 H, 16-H), 3.94 (m, 1 H, 14-H), 4.36 (m, 1 H, 7-H), 5.08 (s, 2 H, 5-H), 5.51 (d, *J* = 5.2 Hz, 1 H, NH_{Phe}), 5.86 (d, *J* = 5.9 Hz, 1 H, NH_{Ala}), 7.07–7.37 (m, 10 H, 1-H, 2-H, 3-H, 10-H, 11-H, 12-H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.7, 39.0, 47.6, 56.6, 66.1, 67.1, 127.1, 128.1, 128.2, 128.5, 128.8, 128.8, 136.1, 136.5, 156.0, 170.8.

HRMS (CI): *m*/*z* calcd for $C_{20}H_{24}N_2O_4$ $[M]^+$: 356.1735; found: 356.1707.

Anal. Calcd for $C_{20}H_{24}N_2O_4$ (356.42): C, 67.40; H, 6.79; N, 7.86. Found: C, 67.17; H, 6.94; N, 7.71.

Benzyl (*S*,*S*)-1-(1-Azidopropan-2-ylamino)-1-oxo-3-phenylpropan-2-ylcarbamate (12)

A solution of **11** (500 mg, 1.40 mmol) and Et_3N (440 mL, 4.34 mmol) in absolute CH_2Cl_2 (27 mL) was cooled to 0 °C before MsCl (241 mg, 2.10 mmol) was added dropwise. The mixture was stirred for 3 h at 0 °C. After dilution with CH_2Cl_2, the mixture was washed with 1 M $KHSO_4$ (29 mL), sat. $NaHCO_3$ (2 × 29 mL), H_2O (29 mL), and brine (29 mL). The organic layer was dried (Na_2SO_4) and the solvent was removed under vacuum. The methanesulfonate was instable. Therefore, the crude product (200 mg, 0.46 mmol) was subsequently dissolved in absolute DMF (5 mL), and NaN_3 (232 mg, 3.58 mmol) was added. After the reaction mixture had been stirred for 15 h at 60 °C, the cooled mixture was diluted with H_2O and EtOAc (10 mL each). The aqueous layer was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with H_2O (3 × 20 mL) and brine (25 mL). The organic layer was dried (Na_2SO_4) and the solvent was removed under vacuum. The crude product was purified by flash chromatography (silica gel, hexane–EtOAc, 7:3, 6:4); this gave **12** as a colorless, viscous oil.

Yield: 204 mg (0.53 mmol, 38%). The azide is stable and can be stored at r.t.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.06 (d, *J* = 6.8 Hz, 3 H, 15-H), 2.96–3.32 (m, 3 H, 8-H, 16-H), 3.80 (m, 1 H, 16-H), 4.07 (m, 1 H, 14-H), 4.41 (m, 1 H, 7-H), 5.08 (s, 2 H, 5-H), 5.44 (d, *J* = 6.6 Hz, 1 H, NH_{Phe}), 5.79 (br s, 1 H, NH_{Ala}), 7.11–7.35 (m, 10 H, 1-H, 2-H, 3-H, 10-H, 11-H, 12-H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 17.2, 38.5, 47.4, 56.8, 65.1, 66.1, 126.7, 128.0, 128.2, 128.5, 128.9, 129.6, 137.5, 138.2, 156.0, 171.1.

HRMS (CI): *m*/*z* calcd for $C_{20}H_{23}N_5O_3$ $[M]^+$: 381.1801; found: 381.1819.

Wong Method for the Preparation of Azides 3, 13, and 14; General Procedure

TfN_3 was freshly prepared prior to each reaction. NaN_3 (9.80 equiv) was dissolved in H_2O (0.16 mL/mmol). At 0 °C an equal volume of CH_2Cl_2 was added. Afterwards Tf_2O (1.99 equiv) was added dropwise to the vigorously stirred solution. After stirring of the mixture for 2 h at 0 °C, the aqueous phase was extracted with CH_2Cl_2 (2 × 1 mL/mmol Tf_2O). The combined organic phases were washed with sat. aq $NaHCO_3$ and used without further purification. The amine (1.00 equiv), 1 M $CuSO_4$ in H_2O (1 mol%), and K_2CO_3 (1.50 equiv) were dissolved/suspended in H_2O (3 mL/mmol amino acid) and MeOH (6 mL/mmol amino acid). After addition of the TfN_3 solution, the reaction was stirred overnight at r.t. The organic solvents were removed under reduced pressure. The residue was extracted with EtOAc (3 × 20 mL), the combined organic layers were dried (Na_2SO_4), and the solvent was evaporated under vacuum.

8-(2-Azidoethyl)-2,3,5,6-tetrahydro-1*H*,4*H*-11-oxa-3a-azabenzo[*de*]anthracen-10-one (3)

According to the Wong procedure, 8-(2-aminoethyl)-2,3,4,5-tetrahydro-1*H*,4*H*-11-oxa-3a-azabenzo[*de*]anthracen-10-one (533 mg, 1.87 mmol) and TfN_3 were allowed to react in the presence of 1 M $CuSO_4$ solution (20 μL, 1 mol%) and K_2CO_3 (388 mg, 2.81 mmol). After workup, **3** was obtained without further purification as a yellow oil.

Yield: 169 mg (0.94 mmol, 29%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.93–2.00 (m, 4 H, 3-H, 14-H), 2.77 (t, *J* = 6.3 Hz, 2 H, 4-H), 2.85–2.93 (m, 4 H, 13-H, 16-H), 3.23–3.28 (m, 4 H, 2-H, 15-H), 3.58 (t, *J* = 7.3 Hz, 2 H, 17-H), 5.92 (s, 1 H, 9-H), 6.95 (s, 1 H, 6-H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.4, 20.5, 21.5, 27.7, 31.0, 49.4, 49.9, 49.9, 107.0, 107.5, 107.8, 118.2, 121.0, 146.0, 151.3, 152.4, 162.3.

HRMS (CI): *m*/*z* calcd for $C_{17}H_{18}N_4O_2$ $[M]^+$: 310.1430; found: 310.1437.

(*S*)-2-Azido-3-methylpentanoic Acid Methyl Ester (13)

According to the Wong procedure, isoleucine methyl ester (145 mg, 1.00 mmol) and TfN_3 were allowed to react in the presence of 1 M $CuSO_4$ solution (10 μL, 1 mol%) and K_2CO_3 (207 mg, 1.50 mmol). After workup, **13** was obtained without further purification as a colorless oil.

Yield: 133 mg (0.79 mmol, 79%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.88–0.96 (m, 6 H, 3-H, 5-H), 1.23 (m, 1 H, 4-H), 1.51 (m, 1 H, 4-H) 1.95 (m, 1 H, 2-H), 3.73 (s, 3 H, 7-H), 3.78 (br s, 1 H, 1-H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.5, 15.8, 25.0, 37.1, 51.8, 67.2, 170.7.

(*S*,*S*)-2-(2-Azido-3-phenylpropanoylamino)-3-methylpentanoic Acid Methyl Ester (14)

According to the Wong procedure, phenylalanyl isoleucine methyl ester (292 mg, 1.00 mmol) and TfN_3 were allowed to react in the presence of 1 M $CuSO_4$ solution (10 μL, 1 mol%) and K_2CO_3 (207 mg, 1.50 mmol). After workup, **14** was obtained without further purification as a colorless oil.

Yield: 299 mg (0.94 mmol, 94%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.69 (d, *J* = 6.9 Hz, 3 H, 10-H), 0.81 (t, *J* = 7.4 Hz, 3 H, 12-H), 0.98 (m, 1 H, 11-H), 1.25 (m, 1 H, 11-H,) 1.71 (m, 1 H, 9-H), 2.99 (dd, *J* = 14.1, 7.8 Hz, 1 H, 2-H), 2.26 (dd, *J* = 14.1, 4.1 Hz, 1 H, 2-H), 3.66 (s, 3 H, 14-H), 4.18 (dd, *J* = 7.8, 4.1 Hz, 1 H, 1-H), 4.44 (dd, *J* = 8.7, 5.0 Hz, 1 H, 8-H), 6.66 (d, *J* = 8.4 Hz, 1 H, NH), 7.18–7.24 (m, 5 H, 4-H, 5-H, 6-H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.5, 15.2, 25.0, 37.7, 38.4, 52.2, 56.4, 65.4, 127.2, 128.6, 129.6, 135.9, 168.1, 171.8.

HRMS (CI): *m*/*z* calcd for $C_{16}H_{23}N_4O_3$ $[M]^+$: 319.1770; found: 319.1784.

Triazoles 15–20 by Copper-Catalyzed Click Chemistry; General Procedure

A freshly prepared 1 M aq solution of sodium ascorbate (10 mol%) and 1 M $CuSO_4$ solution (10 mol%) were added to a suspension of the appropriate azide (1.00 equiv) and alkyne (1.50 equiv) in DMSO–H_2O (4:1; 4 mL). The heterogeneous mixture was stirred vigorously overnight at 80 °C. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and afterwards washed with H_2O and brine (20 mL each). The organic layer was dried (Na_2SO_4), the solvent

was evaporated under vacuum, and the crude product was purified by flash chromatography (silica gel or flash system, hexane–EtOAc).

tert-Butyl (*S*)-2-Methyl-1-{4-[2-(10-oxo-2,3,5,6-tetrahydro-1*H*,4*H*-11-oxa-3a-azabenzo[*de*]anthracen-8-yl)ethyl]-1,2,3-triazol-1-ylmethyl}butylcarbamate (15)

According to the general click-chemistry procedure, azide **10** (97.0 mg, 0.40 mmol) and fluorescent alkyne **2** (176 mg, 0.60 mmol) were allowed to react. After workup, the crude product was purified by flash chromatography (silica gel, hexane–EtOAc, 9:1, 7:3, 1:1); this gave **15** as a pale brown solid.

Yield: 168 mg (0.31 mmol, 78%); mp 205–208 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.92 (t, *J* = 7.3 Hz, 3 H, 25-H), 0.99 (d, *J* = 6.7 Hz, 3 H, 23-H), 1.18 (m, 1 H, 22-H), 1.38 (s, 9 H, 28-H), 1.51 (m, 2 H, 24-H), 1.94–2.00 (m, 4 H, 3-H, 14-H), 2.77 (t, *J* = 6.3 Hz, 2 H, 4-H), 2.88 (t, *J* = 6.5 Hz, 2 H, 13-H), 2.98–3.07 (m, 4 H, 16-H, 17-H), 3.22–3.27 (m, 4 H, 2-H, 15-H), 3.83 (m, 1 H, 21-H), 4.39–4.49 (m, 2 H, 20-H), 4.67 (d, *J* = 8.7 Hz, 1 H, NH), 5.86 (s, 1 H, 9-H), 7.06 (s, 1 H, 6-H), 7.35 (s, 1 H, 19-H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.2, 15.6, 20.5, 20.7, 21.6, 24.7, 25.0, 27.7, 28.3, 31.3, 36.3, 49.4, 49.9, 51.3, 55.0, 79.7, 107.0, 107.1, 107.8, 118.1, 121.4, 121.8, 145.8, 146.1, 151.3, 155.5, 155.5, 162.5; Boc carbonyl not observed.

HRMS (CI): *m*/*z* calcd for $C_{30}H_{41}N_5O_4$ $[M]^+$: 535.3159; found: 535.3129.

Benzyl (*S*,*S*)-1-(1-Methyl-2-{4-[2-(10-oxo-2,3,5,6-tetrahydro-1*H*,4*H*-11-oxa-3a-azabenzo[*de*]anthracen-8-yl)ethyl]-1,2,3-triazol-1-yl}ethylcarbamoyl)-2-phenylethylcarbamate (16)

According to the general click-chemistry procedure, azide **12** (114.0 mg, 0.30 mmol) and fluorescent alkyne **2** (132 mg, 0.45 mmol) were allowed to react. After workup, the crude product was purified by flash chromatography (silica gel, hexane–EtOAc, 1:1, EtOAc); this gave **16** as a yellow-brown, viscous oil.

Yield: 158 mg (0.23 mmol, 78%)

^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.97 (m, 3 H, 22-H), 1.87–1.96 (m, 4 H, 3-H, 14-H), 2.73–2.86 (m, 4 H, 4-H, 13-H), 2.93–3.10 (m, 6 H, 16-H, 17-H, 25-H), 3.18–3.25 (m, 4 H, 2-H, 15-H), 4.15–4.39 (m, 4 H, 20-H, 21-H, 24-H), 4.97 (s, 2 H, 31-H), 5.52 (d, *J* = 7.3 Hz, 1 H, NH_{Phe}), 5.66 (s, 1 H, 9-H), 6.55 (d, *J* = 6.9 Hz, 1 H, NH_{Ala}), 7.04 (s, 1 H, 6-H), 7.18–7.29 (m, 11 H, 19-H, 27-H, 28-H, 29-H, 33-H, 34-H, 35-H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 17.2, 19.9, 20.1, 20.9, 24.1, 26.9, 30.5, 37.4, 44.9, 48.6, 49.1, 53.1, 56.1, 65.1, 105.6, 106.1, 107.0, 117.7, 121.5, 122.8, 126.1, 126.9, 127.8, 129.1, 137.0, 138.1, 145.2, 145.4, 150.6, 155.6, 155.8, 160.8, 171.0.

HRMS (CI): *m*/*z* calcd for $C_{39}H_{42}N_6O_5$ $[M]^+$: 674.3217; found: 674.3176.

Methyl (*S*)-3-Methyl-2-{4-[2-(10-oxo-2,3,5,6-tetrahydro-1*H*,4*H*-11-oxa-3a-azabenzo[*de*]anthracen-8-yl)ethyl]-1,2,3-triazol-1-yl}pentanoate (17)

According to the general click-chemistry procedure, azide **13** (51 mg, 0.30 mmol) and fluorescent alkyne **2** (132 mg, 0.45 mmol) were allowed to react. After workup, the crude product was purified by flash chromatography (silica gel, hexane–EtOAc, 7:3, 1:1); this gave **17** as a yellow-brown, viscous oil.

Yield: 108 mg (0.23 mmol, 77%).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.77 (d, *J* = 7.4 Hz, 3 H, 24-H), 0.88 (t, *J* = 6.8 Hz, 3 H, 22-H), 0.95 (m, 1 H, 23-H), 1.08 (m, 1 H, 23-H), 1.86–1.89 (m, 4 H, 3-H, 14-H), 2.27 (m, 1 H, 21-H), 2.68–2.74 (m, 4 H, 4-H, 13-H), 2.97–3.03 (m, 4 H, 16-H, 17-H), 3.20–3.24 (m, 4 H, 2-H, 15-H), 3.69 (s, 3 H, 26-H), 5.23 (d, *J* = 8.7 Hz, 1 H, 20-H), 5.75 (s, 1 H, 9-H), 7.15 (s, 1 H, 6-H), 8.01 (s, 1 H, 19-H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 10.5, 15.1, 20.0, 20.1, 20.9, 24.2, 24.4, 27.0, 30.3, 36.6, 42.0, 48.6, 49.1, 52.5, 66.5, 105.6, 106.4, 107.0, 117.7, 121.6, 122.2, 145.4, 145.5, 150.6, 155.7, 160.7, 168.8.

HRMS (CI): *m*/*z* calcd for $C_{26}H_{32}N_4O_4$ $[M]^+$: 464.2424; found: 464.2430.

Methyl (*S*,*S*)-3-Methyl-2-({4-[2-(10-oxo-2,3,5,6-tetrahydro-1*H*,4*H*-11-oxa-3a-azabenzo[*de*]anthracen-8-yl)ethyl]-1,2,3-triazol-1-yl}-3-phenylpropanoylamino)pentanoate (18)

According to the general click-chemistry procedure, azide **14** (96.0 mg, 0.30 mmol) and fluorescent alkyne **2** (132 mg, 0.45 mmol) were allowed to react. After workup, the crude product was purified by flash chromatography (silica gel, hexane–EtOAc, 7:3, CH_2Cl_2–EtOAc, 98:2); this gave **18** as a yellow-brown, viscous oil.

Yield: 138 mg (0.23 mmol, 75%)

^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.75–0.80 (m, 6 H, 29-H, 31-H), 1.06 (m, 1 H, 30-H), 1.29 (m, 1 H, 30-H), 1.77 (m, 1 H, 28-H), 1.86–1.91 (m, 4 H, 3-H, 14-H), 2.69 (t, *J* = 6.4 Hz, 2 H, 4-H), 2.77 (t, *J* = 6.4 Hz, 2 H, 13-H), 2.91–2.98 (m, 4 H, 16-H, 17-H), 3.14–3.19 (m, 4 H, 2-H, 15-H), 3.26 (dd, *J* = 14.0, 9.2 Hz, 1 H, 21-H), 3.44 (dd, *J* = 14.0, 6.1 Hz, 1 H, 21-H), 3.60 (s, 3 H, 33-H), 4.39 (dd, *J* = 8.2, 5.4 Hz, 1 H, 27-H), 5.57 (dd, *J* = 9.0, 6.3 Hz, 1 H, 20-H), 5.79 (s, 1 H, 9-H), 6.97–7.14 (m, 6 H, 6-H, 23-H, 24-H, 25-H), 7.56 (s, 1 H, 19-H), 7.70 (d, *J* = 8.2 Hz, 1 H, NH).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 11.1, 15.3, 20.0, 20.1, 21.0, 24.0, 24.7, 27.0, 30.3, 36.3, 37.5, 48.6, 49.2, 51.8, 56.6, 63.2, 105.7, 106.2, 107.1, 117.8, 121.6, 121.7, 126.7, 128.2, 128.9, 136.0, 145.3, 145.5, 150.7, 155.7, 160.9, 167.9, 171.4.

HRMS (CI): *m*/*z* calcd for $C_{35}H_{41}N_5O_5$ $[M]^+$: 611.3108; found: 611.3091.

Methyl (*S*)-2-((*S*)-2-*tert*-Butoxycarbonylamino-3-{1-[(10-oxo-2,3,5,6-tetrahydro-1*H*,4*H*-11-oxa-3a-azabenzo[*de*]anthracen-8-yl)methyl]-1*H*-1,2,3-triazol-4-ylmethoxy}propanoylamino)-4-methylpentanoate (19)

According to the general click-chemistry procedure, azide **5** (116 mg, 0.39 mmol) and fluorescent alkyne **7** (111 mg, 0.30 mmol) were allowed to react. After workup, the crude product was purified by flash chromatography (flash system, hexane–EtOAc, gradient 7:3 to 0:1); this gave **19** as a yellow-brown, viscous oil.

Yield: 168 mg (0.25 mmol, 84%)

^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.85 (d, *J* = 6.5 Hz, 6 H, 29-H), 1.36 (s, 9 H, 24-H), 1.46–1.62 (m, 3 H, 27-H, 28-H), 1.87–1.91 (m, 4 H, 3-H, 14-H), 2.70–2.73 (m, 4 H, 4-H, 13-H), 3.23–3.28 (m, 4 H, 2-H, 15-H), 3.51–3.64 (m, 5 H, 16-H, 31-H), 4.21 (m, 1 H, 21-H), 4.29 (m, 1 H, 26-H), 4.56 (br s, 2 H, 20-H), 5.33 (s, 1 H, 9-H), 5.79 (s, 2 H, 19-H), 6.67 (d, *J* = 8.2 Hz, 1 H, NH_{Ser}), 7.02 (s, 1 H, 6-H), 7.94 (s, 1 H, 17-H), 8.20 (d, *J* = 7.7 Hz, 1 H, NH_{Ile}).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 19.8, 19.9, 20.8, 21.1, 22.7, 23.9, 27.0, 28.0, 48.6, 49.0, 49.1, 50.1, 51.7, 54.1, 63.5, 69.7, 78.1, 105.2, 105.3, 105.6, 117.9, 121.3, 124.7, 144.2, 145.9, 150.3, 150.6, 155.0, 160.4, 169.9, 172.6.

HRMS (CI): *m*/*z* calcd for $C_{34}H_{46}N_6O_8$ $[M]^+$: 666.3377; found: 666.3375.

Methyl (*S*)-2-[(*S*)-2-((*S*)-2-*tert*-Butoxycarbonylamino-3-{1-[(10-oxo-2,3,5,6-tetrahydro-1*H*,4*H*-11-oxa-3a-azabenzo[*de*]anthracen-8-yl)methyl]-1*H*-1,2,3-triazol-4-ylmethoxy}propanoylamino)-3-methylbutanoylamino]-3-phenylpropanoate (20)

According to the general click-chemistry procedure, azide **5** (116 mg, 0.39 mmol) and fluorescent alkyne **8** (151 mg, 0.30 mmol) were allowed to react. After workup, the crude product was purified by flash chromatography (flash system, hexane–EtOAc, gradient 7:3 to 0:1); this gave **20** as a yellow-brown, viscous oil.

Yield: 185 mg (0.23 mmol, 77%)

^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.74 und 0.79 (2d, *J* = 6.6 Hz, 6 H, 28-H, 29-H), 1.36 (s, 9 H, 24-H), 1.88–1.94 (m, 5 H, 3-H, 14-H, 27-H), 2.69–2.73 (m, 4 H, 4-H, 13-H), 2.91 (dd, *J* = 13.8, 8.7 Hz, 1 H, 32-H), 2.99 (dd, *J* = 13.8, 6.1 Hz, 1 H, 32′-H), 3.23–3.27 (m, 4 H, 2-H, 15-H), 3.54–3.58 (m, 5 H, 16-H, 38-H), 4.17–4.23 (m, 2 H, 21-H, 26-H), 4.45 (m, 1 H, 31-H), 4.53 (br s, 2 H, 20-H), 5.33 (s, 1 H, 9-H), 5.78 (s, 2 H, 19-H), 7.09 (d, *J* = 8.2 Hz, 1 H, NH_{Ser}), 7.16–7.26 (m, 6 H, 6-H, 34-H, 35-H, 36-H), 7.57 (d, *J* = 8.9 Hz, 1 H, NH_{Val}), 8.15 (s, 1 H, 17-H), 8.45 (d, *J* = 7.1 Hz, 1 H, NH_{Phe}).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 17.5, 18.8, 19.8, 19.9, 20.8, 27.0, 28.0, 48.6, 49.0, 49.1, 51.6, 53.4, 54.5, 56.8, 63.5, 78.3, 105.2, 105.3, 105.6, 117.9, 121.3, 124.8, 126.4, 128.1, 128.8, 136.9, 144.2, 145.8, 150.3, 150.6, 155.2, 160.4, 169.4, 170.7, 171.6.

HRMS (CI): *m*/*z* calcd for $C_{42}H_{54}N_7O_9$ $[M + H]^+$: 800.3978; found: 800.3980.

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Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synthesis.

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