Electronic Supporting Information

Strategy for catch and release of azide-tagged biomolecules utilizing a photolabile strained alkyne construct

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1. General methods

All chemicals were used as received unless noted otherwise. Dry solvents were prepared according to standard methods (DMF: P₂O₅; DIPEA: CaH₂; THF: sodium/benzophenone; MeOH: Mg), distilled and stored over molecular sieves 3 Å under an atmosphere of nitrogen until used.

NMR-spectra were recorded on either a Bruker Avance 400 or a Bruker ACX 250 spectrometer and calibrated for TMS (0.0 ppm) or the solvent-signal (¹H-CDCl₃: 7.26 ppm; ¹³C-CDCl₃: 77.16 ppm; ¹H-MeOH-d₄: 3.31 ppm; ¹³C-MeOH-d₄: 49.00 ppm, ¹H-DMSO-d₆: 2.50 ppm; ¹³C-DMSO-d₆: 39.52 ppm; ¹H-benzene-d₆: 7.16 ppm; ¹³C-benzene-d₆: 128.39 ppm).

FT-ICR-MS spectra were recorded on a Bruker Apex II FT-ICR-MS (FAB) spectrometer and FAB-spectra on a Finnigan model TSQ 70. Optical rotations were measured with a Perkin-Elmer Model 341 polarimeter. Melting points were determined with a Büchi Melting Point M-560 and are uncorrected. Elemental analysis was performed on a HEKAtech Euro EA Analyzer. IR-spectra were recorded on a Bruker Tensor 27. TLC-analysis was performed with either a Thermo Betasil C8 column or a ZORBAX Eclipse XDB-C8 column with a flow rate of 1.5 ml/min. of methanol/KH₂PO₃ pH 2.3 buffer. TLC-analysis was performed with Polygram SIL G/UV pre-coated polyester sheets (Macherey-Nagel). UV-spectra for photometry were recorded on a Perkin-Elmer Lambda 25 UV-Vis spectrometer using 1 cm quartz cuvettes. Absorbance was measured at 266.8 nm.

Dibenzocyclooctynol 2¹⁷ and the corresponding pNP-carbonate 8,¹¹b nitroaromatic 4,⁹ ethylene glycol linker 6²⁰ were prepared following the published procedures.

For irradiation of the photolabile samples a 150 W medium pressure mercury lamp from UV-Consulting Peschl Model TQ 150 was used. For elimination of UV light <350 nm a filter solution composed of 2 M aq. KNO₃ containing 0.02 w% 5,7-dimethyl-3,6-dihydro-2H-1,4-diazepinium perchlorate was placed between light source and sample.²¹ The filter solutions layer thickness was adjusted to ~2 cm and the samples placed perpendicular and as close as possible to the light bulb. The filter solution was replaced with a freshly prepared one every 12 h of irradiation.

Immobilization of 3 on TOYOPEARL-AF-650-amino: 1 ml of a TOYOPEARL-AF-650-amino suspension in aq. EtOH was transferred to a 5 ml PP-syringe with inserted PE-frit (35 μm pore size) and washed successively 3 times with H₂O and 5 times with dry MeCN. After re-suspending the beads in 3 ml dry MeCN, 21.4 mg of pNP-ester 3a (25 μmol) and 13.1 μl DIPEA (75 μmol) were added and the mixture shaken at 1500 rpm and rt for 72 h. Subsequently 94.5 μl Ac₂O (1 mmol, 10 eq.) and 172.3 μl DIPEA (1 mmol, 10 eq.) were added and the mixture shaken as described for additional 12 h at RT. Then the beads were washed successively 3 times each with DMSO and water. For storage at 4°C the alkyne functionalized beads were suspended in 20 % aqueous ethanol.

Capture of azide 10 onto the alkyne functionalized solid support: The alkyne functionalized beads from above were washed 3 times with dioxane/PBS 2:3 v/v and then re-suspended in 3 ml of the named solvent mixture containing 31.3 mg of azide 10 (50 μmol, 2 eq.). The resulting suspension was shaken at RT or 0°C and 1500 rpm for 3 h and the reaction progress tracked photometrically. After 30 min. no more progression of the reaction was observed.
Afterwards the beads were washed 5 times with dioxane/PBS 2:3 v/v, 3-times with water, re-suspended in 20% aqueous ethanol and stored at 4°C until used further. Quantitative analysis of the UV-spectra and subtraction of a relative amount of 10 non-specifically bound to the beads (see below) indicated an effective loading of the beads with 12.5 µmol of the corresponding triazole (uncorrected 14.7 µmol).

Non-specific binding of azide 10 on 2-azidoethanol-blocked alkyne functionalized solid support: The alkyne functionalized beads from above were washed 3 times with dioxane/PBS 2:3 v/v and then re-suspended in 3 ml of the named solvent mixture containing 100 mg of 2-azidoethanol (1.15 mmol, 46 eq. based on 3a). The resulting suspension was shaken at rt and 1500 rpm for 12 h. Afterwards the beads were washed 5 times with dioxane/PBS 2:3 v/v, 3-times with water. Then the beads were re-suspended in 3 ml dioxane/PBS 2:3 v/v containing 31.3 mg of azide 10 (50 µmol, 2 eq.). The resulting suspension was shaken at RT and 1500 rpm for 90 min. and the reaction progress tracked photometrically. Quantitative analysis revealed approx. 2.2 µmol non-specific binding to the blocked beads. The assumption was confirmed by subjecting the beads to the cleavage conditions described below. No 11 could be detected in the supernatant as indicated by HPLC-analysis, precluding that 10 was immobilized due to reaction with residual non-blocked 3b.

UV light induced liberation of the immobilized model compound 11: The triazole-loaded beads from above were washed 5-times either with dioxane/PBS 2:3 v/v or dioxane/Na-phosphate pH 6.3 buffer 3:2 v/v containing 10 mM DTT. Then the beads were re-suspended in 10 ml of one of the named solvent mixtures, transferred to a transparent 20 ml PP-tube and subjected to irradiation >350 nm for 4 h at RT while being shaken at 1500 rpm. The reaction was monitored photometrically and after 120 min. and 40 min. no more progression of the cleavage reaction could be observed, respectively. Quantitative analysis of the supernatant indicated 85% recovery of triazole 11 in an HPLC-purity of 98.2%.

2. Synthesis and characterization of the compounds 5, 7, 9, 3 and 10, 11

![Scheme 5: Synthesis of model compound 10.](image)

**General Procedure for HBTU-mediated amide coupling A:** In a round bottom flask equipped with a gas inlet and a stirring bar the corresponding free base or ammonium derivative was dissolved in dry DMF at a concentration of 0.18 M under an atmosphere of nitrogen. After cooling the solution to 0°C HOBt (1.5 eq.), DIPEA (1.5 eq. for free bases; 3 eq. for ammonium derivatives), the free carboxylic acid (1 eq.) and subsequently HBTU (1.5 eq.) were added. Stirring was continued for 2 h at 0°C and at rt for 14 h. Then, the solution was diluted with ethyl acetate, transferred to a separatory funnel, the organic layer separated and washed twice each with 1 M NaHSO₃-soln. and sat. NaHCO₃-soln. and once with brine. After drying the organic layer with Na₂SO₄ the solvent was removed under reduced pressure and
the residue subjected to column chromatography, eluting with the solvent mixtures indicated below.

\[
\text{C}_4\text{H}_8\text{O}_2\text{N}_3\text{Cl}^+ \quad \text{m/z} \quad 313 \quad \text{[M-Cl]}^+ 
\]

4-[2-Methoxy-5-nitro-4-(1-aminoethyl)phenoxy]butanoic acid methyl ester hydrochloride 5: In a 250 ml round bottom flask equipped with a gas inlet and a stirring bar 8.2 g of trifluoroacetamide 4 (20.1 mmol) were dissolved in 100 ml of a 0.36 M soln. of HCl in dry MeOH under an atmosphere of nitrogen. The resulting mixture was refluxed for 36 h, cooled to rt and the volatiles removed in a stream of air. The residual white solid was triturated with diethyl ether, filtered and crystallized from ethanol to yield 6.77 g of pure title compound 5 (19.4 mmol, 97%) as woolly colorless crystals. mp: 191.5°C (EtOH). Anal. calcd for C_{14}H_{21}ClN_{2}O_{5}: N, 8.03; C, 48.21; H, 6.07; found: N, 7.87; C, 48.25; H, 6.21. IR (NaBr): 175.3 (COOMe), 155.6, 149.8, 142.6, 128.2, 110.8, 110.6 (aryl), 69.6 (aryl-O), 57.3 (aryl-OCH), 52.2 (COOCH), 47.6 (CHN), 31.2 (Bu-CH), 25.5 (Bu-CH), 20.0 (NCHCH).
containing 1 v% MeOH yielding 1.52 g of pure title compound 7 (2.60 mmol, 91 %) as pale yellow viscous oil. Rf: 0.32 (CHCl₃+v1% MeOH). IR (neat): 3357, 2974, 2935, 2875, 1712, 1518, 1456, 1366, 1274, 1176, 870, 758 cm⁻¹. FT-ICR-MS: m/z [M+Na]+ cale for C₂₇H₄₃N₂O₁₂Na: 624.2739 found: 624.2743. ¹H-NMR (400.1 MHz, CDCl₃): δ 7.63-7.57 (m, 1H, CONH), 7.52 (s, 1H, aryl), 6.96 (s, 1H, aryl), 5.65-5.56 (m, 1H, ary-CNOH), 4.96 (s, broad, 1H, OCONH), 4.06 (t, 2H, J = 6.2 Hz, aryl-OCH₂), 4.01 (d, 1H, J = -15.8 Hz, OCH₂CO), 3.89 (s, 3H, CH₃), 3.89 (d, 1H, J = -15.8 Hz, OCH₂CON), 3.74-3.58 (m, 8 H, EG-chain-CH₂), 3.66 (s, 3H, CH₃), 3.48 (t, 2H, J = 5.2 Hz, EG-chain-CH₂), 3.27-3.21 (m, 2H, CH₂NBoc), 2.51 (t, 2H, J = 7.2 Hz, CH₂COOMe), 2.18-2.10 (m, 2H, CH₂), 1.53 (d, 3H, J = 6.9 Hz, CH₃CHN), 1.40 (s, 9H, t-Bu). ¹³C-NMR (100.6 MHz, CDCl₃): δ 173.4 (COOMe), 169.3 (CON), 156.0 (OCONH), 153.9, 147.0, 140.6, 134.3, 110.4, 109.9 (aryl), 79.3 (t-Bu), 71.1, 70.6, 70.4, 70.4, 70.3, 70.2 (EG-CH₂), 68.3 (aryl-OCH₂), 56.4 (aryl-OCH₃), 51.7 (COOCH₃), 46.1 (CHN), 40.4 (CH₂NBoc), 30.4 (Bu-CH₂), 28.5 (t-Bu), 24.3 (Bu-CH₂), 21.4 (aryl-NCHCH₃).

4-[4-[[14-(11,12-didehydro-5,6-dihydrodibenzo[a,e]cycloocten-5-oxycarbonylamino)-4-oxo-6,9,12-trioxa-3-azatetradec-2-yl]-2-methoxy-5-nitrophenoxy]butanoic acid methyl ester 9: In a 50 ml round bottom flask equipped with a stirring bar 1.45 g of nitroaromatic 7 (2.41 mmol) were N-Boc-deprotected using 20 v% TFA in dry DCM for 1 h and the volatiles subsequently removed in a stream of air. After drying in vacuum over night the corresponding ammonium derivative was dissolved in 10 ml dry DMF in a round bottom flask equipped with a gas inlet and a stirring bar under an atmosphere of nitrogen. To the solution were added 1.26 ml DIPEA (7.23 mmol, 3 eq.) and 929 mg of activated carbonate 8¹b (2.41 mmol, 1 eq.) and the mixture stirred at rt for 24 h. Thereafter the shiny yellow solution was diluted with ethyl acetate, transferred to a separatory funnel, washed twice with 1 M NaHSO₃-soln., three times with 5% Na₂CO₃-soln. and once with brine, dried over Na₂SO₄ and the solvent removed under reduced pressure. The residue was subjected to column chromatography eluting with CHCl₃ containing 1.5 v% MeOH yielding 1.62 g of pure title compound 9 (2.17 mmol, 90%) as pale yellow foam. Rf: 0.43 (CHCl₃+v2% MeOH). IR (NaBr): 3365, 2934, 1729, 1672, 1520, 1336, 1273, 1216, 1104, 1029, 760 cm⁻¹. FT-ICR-MS: m/z [M+Na]+ cale for C₃₀H₄₅N₂O₁₂Na: 770.2896 found: 770.2894. ¹H-NMR from the mixture of diastereomers (400.1 MHz, Benzol-d₈): δ 7.77 (t, 1H, J = 8.2 Hz, CONH), 7.71-7.62 (m, 1H, aryl), 7.34 (s, 1H, aryl), 7.30-7.11 (m, 4H, aryl), 7.08-6.91 (m, 4H, aryl), 6.41-6.34/5.83-5.74 (m/m, 1H, BN-CHO), 6.35/5.91 (s/s, broad, 1H, OCONH), 6.05-5.94 (m, 1H, BN-CHN), 4.10-2.71 (m, 22H), 3.90 (dd, 1H, J = -15.7 Hz/7.3 Hz, BN-CH₂), 2.95 (dd, 1H, J = -15.7 Hz/3.8 Hz, BN-CH₂), 2.29-2.22 (m, 2H, CH₂COOMe), 1.88-1.79 (m, 2H, CH₂), 1.51/1.56-1.37 (d/m, 3H, J = 6.6 Hz, CH₃CHN). ¹³C-
NMR from the mixture of diastereomers (100.6 MHz, Benzol-d$_6$): $\delta$ 172.8 (COOMe), 169.3 (CON), 155.7 (OCONH), 154.1, 153.0, 151.6, 147.5, 141.4, 134.6, 130.4, 128.1, 127.9, 127.3, 127.2, 127.0, 126.5, 126.2, 124.3, 124.2, 121.9, 113.6, 110.8, 110.6, 109.6 (aryl), 77.8, 77.1 (benzyl-CHO), 70.9, 70.5, 70.1, 70.0 (EG-CH$_2$), 67.8 (aryl-OCH$_2$), 55.8/55.8 (aryl-OCH$_3$), 51.1 (COOCH$_3$), 46.8, 46.2 (CH$_3$/benzyl-CH$_2$), 41.1 (CH$_2$NHBOc), 30.4 (Bu-CH$_2$), 24.6 (Bu-CH$_2$), 21.4 (CH$_3$).

$\delta$ 4.08-2.70 (m, 19H), 3.88 (dd, 1H, $J = 15.8$ Hz/5.1 Hz, Bn-CO), 7.28-7.09 (m, 4H, aryl), 7.06-6.89 (m, 4H, aryl), 6.79-6.73 (m, 2H, pNP) 6.38-6.33/5.75-5.66 (m/m, 1H, Bn-CHO), 6.30/5.85 (s/s, broad, 1H, OCONH), 6.03-5.93 (m, 1H, Bn-CNH), 4.08-2.70 (m, 19H), 3.88 (dd, 1H, $J = 15.8$ Hz/5.1 Hz, Bn-CH$_2$), 2.92 (dd, 1H, $J = 15.8$ Hz/3.8 Hz, Bn-CH$_2$), 2.41 (t, 2H, $J = 7.1$ Hz, CH$_2$COOMe), 1.94-1.80 (m, 2H, CH$_2$), 1.50/1.56-1.34 (d/m, 3H, $J = 6.6$ Hz, CH$_3$CH$_2$).

$\delta$ 170.2 (COOMe), 169.4 (CON), 155.8, 155.5, 154.1 (OCONH/aryl), 153.0, 151.6, 147.4, 145.5, 141.4, 135.1, 130.4, 128.1, 127.9, 127.4, 127.3, 126.6, 126.3, 125.1, 124.3, 124.2, 122.4, 121.9, 113.6, 110.7, 110.6, 109.7 (aryl), 77.8, 77.2 (benzyl-CHO), 70.9, 70.8, 70.6, 70.5, 70.1, 70.1 (EG-CH$_2$), 67.7 (aryl-OCH$_2$), 55.8 (aryl-
OCH₃), 46.7, 46.1 (CHN/benzyl-CH₂), 41.2 (CH₂NHBoc), 31.0 (Bu-CH₂), 24.5 (Bu-CH₂), 21.5 (CH₃).

(S)-N-α-(9-Fluorenyl)methoxycarbonyl-N'-β-tert-butoxycarbonyl-β-aminoalanyl-11-azido-3,6,9-trioxaundecyl amide 10: In a 100 ml round bottom flask equipped with a gas inlet and a stirring bar 5 g of α-Fmoc-β-Boc-β-β-aminoalanine (7.98 mmol) were dissolved in 50 ml dry THF under an atmosphere of nitrogen. Then the solution was cooled to 0°C and 1.62 g PFP (8.78 mmol, 1.1 eq.) were added followed by 2.15 g DCC (10.4 mmol, 1.3 eq.) and stirring continued for 6 h at 0°C. Thereafter 1.92 g of 2-{2-[2-(Azidoethoxy)ethoxy]ethoxy}amine (8.78 mmol, 1.1 eq.) and 2.08 ml DIPEA (12.0 mmol, 1.5 eq.) were added and the slurry stirred for additional 14 h at rt. After filtration from the DC-urea precipitate the solution was diluted with ethyl acetate and transferred to a separatory funnel. The organic layer was washed twice each with 1 M NaHSO₃-soln. and sat. NaHCO₃-soln., once with brine, dried with Na₂SO₄ and the solvent removed under reduced pressure. The residue was purified by column chromatography eluting with toluene/acetone 3:1-7:3 v/v yielding 3.2 g of pure title compound 10 (5.11 mmol, 64%) as colorless resin. R₁: 0.29 (toluene/acetone 3:1 v/v). [α]D²₀ = -12.5 (c=1.0, CHCl₃). IR (NaBr): 3319, 2871, 2108, 1689, 1661, 1536, 1450, 1307, 1163, 739 cm⁻¹. FT-ICR-MS: m/z [M+Na]+ calcd for C₃₁H₄₂N₆O₈Na: 649.2956 found: 649.2950.¹H-NMR (400.1 MHz, CDCl₃): δ 7.75 (d, 2H, J = 7.5 Hz, Fmoc), 7.62-7.57 (m, 2H, Fmoc), 7.39 (t, 2H, J = 7.5 Hz, Fmoc), 7.30 (dt, 2H, J = 7.5 Hz/0.9 Hz, Fmoc), 6.96 (s, broad, 1H, CONH), 6.32 (s, broad, 1H, CONH), 5.29 (s, broad, 1H, CONH), 4.44-4.24 (m, 2H, Fmoc-CH₂O/α-CH₂), 4.20 (t, 1H, J = 7.2 Hz, Fmoc-CH), 3.65-3.40 (m, 16H, EG-chain-CH₂/β-CH), 3.34 (t, 2H, J = 5.2 Hz, CH₂N₃), 1.44 (s, 9H, tBu). ¹³C-NMR (100.6 MHz, CDCl₃): δ 170.2 (CONH), 157.2, 156.6 (OCONH), 143.9, 143.8, 141.4, 127.8, 127.2, 125.2, 120.1 (aryl), 80.1 (tBu), 70.7, 70.7, 70.6, 70.4, 70.0, 69.6 (EG-CH₂), 67.3 (Fmoc-CH₂O), 56.4 (α-CH), 50.7 (CH₂N₃), 47.2 (Fmoc-CH), 42.9, 39.5 (CH₂N), 28.4 (tBu).

described above and the remaining solid support filtered off. Then dioxane was removed under reduced pressure and the aqueous residue transferred to a separatory funnel. The aq. layer was extracted five times with DCM, the combined organic layers dried over Na₂SO₄ and the solvent removed under reduced pressure leaving 11 as colorless foam in analytical purity. Rf: 0.34 (CHCl₃+6v%MeOH). [α]D²⁰= -5.3 (c=1.0, CHCl₃). FT-ICR-MS: m/z [M+Na]⁺ calcd for C₅₆H₇₀N₈O₁₄Na: 1101.4904 found: 1101.4900. HPLC: 8.65 min., 98.2 %. 

\[^{1}H\text{-NMR}\] from the mixture of regioisomers/diastereomers (400.1 MHz, CDCl₃): δ 7.74 (d, 2H, J = 7.6 Hz, Fmoc), 7.62-7.46 (m, 4H, Fmoc/aryl), 7.45-7.34 (m, 2H, aryl), 7.38 (t, 2H, J = 7.4 Hz, Fmoc), 7.33-6.99 (m, 7H, Fmoc/aryl/CONH), 6.37 (s, broad, 1H, CONH), 5.71-5.62/5.60-5.49 (m, 1H, OCONH), 4.76-4.67/4.63-4.41 (m, 2H, Bn-CHO/α-CH), 4.41-4.24 (m, 3H, Fmoc-OCH₂CH₂), 4.19 (t, 1H, J = 7.2 Hz, Fmoc-CH), 4.05-3.84 (m, 4H, OCH₂CONH₂/Bn-CH₂), 3.74-3.17/3.01 (m/t, 28H, J = 12.1 Hz, EG-C₂H₂/β-CH₂), 1.42 (s, 9H, tBu).

\[^{13}C\text{-NMR}\] from the mixture of regioisomers/diastereomers (100.6 MHz, CDCl₃): δ 173.4, 170.3 (CONH), 157.2, 156.7, 156.0, 155.4 (OCONH), 147.9, 146.3 (aryl), 143.9/143.9, 141.4 (Fmoc), 137.3, 135.8, 135.6, 134.6, 134.4, 133.6, 132.8, 132.2, 131.5, 130.3, 129.9, 129.6, 129.4, 129.1, 128.9, 128.7, 128.6 (aryl), 127.8 (Fmoc), 127.7, 127.3 (aryl), 127.2 (Fmoc), 126.8, 126.5, 125.3 (Fmoc), 124.9, 124.5 (aryl), 120.1 (Fmoc), 79.9 (tBu), 71.7, 71.1, 71.0, 70.7, 70.6, 70.4, 70.3, 70.2, 70.0, 69.7, 69.2 (EG-CH₂), 67.3 (Fmoc-OCH₂), 56.1 (α-CH), 48.6, 48.3, 48.2 (CH₂N), 47.2 (Fmoc-CH), 43.3, 42.9, 40.9, 39.4, 37.9, 37.3 (CH₂N), 28.4 (tBu).

4. References


5. NMR-Spectra of compounds prepared