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# - Supporting information -

# Click to Enter: Activation of Oligo-Arginine Cell-Penetrating Peptides by Bioorthogonal Tetrazine Ligations

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## General

Unless stated otherwise, all chemicals were purchased from commercial sources and all reactions were carried out under ambient atmosphere. Breipohl (Rink amide) resin and 2-chlorotrityl chloride resin were obtained from Bachem (Bubendorf, Switzerland). Fmoc-Arg(Pbf)-OH was purchased from Novabiochem (EMD Chemicals, Gibbstown, U.S.A.), Fmoc-6-Ahx-OH was acquired from Iris Biotech GMBH (Marktredwitz, Germany) and 4-pentynoic acid was bought from Acros Organics (Thermo Fisher Scientific, New Jersey, U.S.A.). Fluorescein isothiocyanate was obtained from Santa Cruz biotechnologies (Dallas, Texas, U.S.A.) and BCN-NHS was purchased from SynAffix (Oss, The Netherlands). Recombinant Human Serum Albumin was purchased from Sigma Aldrich. MilliQ was doubly deionized using a Labconco Water Pro PS purification system (18.1 MΩ).

## Cell culture

HeLa culture medium comprised Dulbecco's modified eagle medium (DMEM) (Gibco High Glucose) supplemented with 44 mM $NaHCO_3$, 44 mM pyruvate and 10% amino acid supplement (consisting of cysteine, alanine, asparagine, aspartic acid, proline and glutamic acid), 10% fetal bovine serum (Gibco) 100 units/mL penicillin and 100 µg/mL streptomycin (Gibco Penicillin-Streptomycin). For trypsinization of HeLa cells, a mixture of 0.05% trypsin (Gibco) and 0.68 mM ethylenediamine tetraacetic acid (EDTA, titriplex II) in PBS was used. PBS consisted of 0.14 M NaCl, 2.7 mM KCl, 1.1 mM $K_2HPO_4$ and 10 mM $Na_2HPO_4$ in MiliQ. FACS buffer consisted of 0.1% albumin from bovine serum (BSA) in PBS.

## Mass spectrometry

Low resolution mass spectra (LRMS) were recorded on a Thermo Finnigan LCQ Advantage Max electrospray (ESI) ion-trap mass spectrometer (organic synthesis). A Thermo Finnigan LCQ Fleet ESI ion-trap mass spectrometer (peptide synthesis), which is equipped with a Shimadzu HPLC (C18-column, 200 × 3 mm, particle size 3 µm, MeCN/$H_2O$ gradient 5-100%, 1-31 minutes and a flow of 0.2 mL/min) and a PDA detector, was used to record low resolution mass spectra of peptides (LCMS). In addition, a Thermo Finnigan Polaris-Q electron ionization (EI) ion-trap mass spectrometer equipped with a VF1701MS column, 30 m ×x 0.25 mm, Df 0.25 µM (GCMS) was used to measure low resolution

mass spectra of organic compounds. MALDI-TOF mass spectra were recorded on a Bruker Microflex using α-cyano-4-hydroxycinnamic acid as a matrix.

## High-performance liquid chromatography (HPLC)

Analytical HPLC spectra of peptides were recorded on a Shimadzu LC-20A Prominence system (Shimadzu,'s-Hertogenbosch, The Netherlands) equipped with a Gemini-Nx C18 column, 150 × 3 mm, particle size 3 µm, pore size 110 Å (Phenomenex, Torrance, California, U.S.A.). Peptides were eluted in an aqueous acetonitrile gradient containing 0.1% TFA (v/v) (5 - 100%, 1 - 40 min., flow 0.4 mL/min). Preparative HPLC of peptides was performed on a Shimadzu LC-20A Prominence system (Shimadzu, 's-Hertogenbosch, The Netherlands) equipped with a Gemini-Nx C18 column, 150 × 10 mm, particle size 3 µm, pore size 110 Å (Phenomenex, Torrance, California, U.S.A.), using a linear gradient of $MeCN/H_2O$ with 0.1% TFA (v/v) (5 - 100%, 1 - 50 min., unless stated otherwise) and a flow of 6.0 mL/min.

## Nuclear magnetic resonance (NMR)

For NMR analysis a Varian Inova400 (400 MHz for $^1H$), and a Bruker Avance III 500 (125 MHz for $^{13}C$) spectrometer were used. In $^1H$-NMR spectra chemical shifts ($\delta$) are listed as parts per million (ppm) relative to TMS. The following abbreviations are used to describe multiplicities: s (singlet), bs (broad singlet), d (doublet), t (triplet), dd (double doublet), and m (multiplet). Coupling constants are reported in Hertz (Hz) as a J value. Chemical shifts ($\delta$) of $^{13}C$-NMR spectrometry are listed as ppm relative to a residual solvent proton peak, $\delta$ =77.2 for $CDCl_3$ or $\delta$ = 49.0 for $CD_3OD$.

## Synthesis

### General peptide synthesis

All peptides were prepared by standard Fmoc solid phase peptide synthesis (SPPS) on Breipohl resin or 2-chlorotrityl chloride resin. Resins were swollen in DMF (Breipohl amide resin) or DCM (2-chlorotrityl chloride resin) for at least 20 minutes prior to synthesis. Fmoc deprotection was achieved by rinsing the resin once with piperidine in DMF (20%, v/v), followed by incubation with piperidine in DMF for 20 minutes. Sequence extension of peptides was performed by incubation with Fmoc-L-amino acids (3.0 eq.), DIPCDI (3.3 eq.) and HOBt (3.6 eq.) in DMF for 45 - 60 minutes. The progress of peptide couplings and Fmoc deprotections was checked with a Kaiser test.[1] When this showed that couplings were not complete, they were repeated using Fmoc- L -amino acids (2.0 eq.) preincubated with HATU (1.95 eq.) and DiPEA (4.0 eq.) for 2 minutes. Unreacted sites were capped using excess of a pyridine/$Ac_2O$ mixture (1:1, v/v) in DMF and incubating the resin for 5 minutes in this mixture. When Fmoc-deprotections were suspected to be not fully complete, resin was incubated with a mixture of 2% DBU and 2% piperidine in DMF (v/v) for 5 minutes to assure full deprotection. After the final step on the solid phase the resin was washed with DMF (3×), DCM (3×), MeOH (3×) and $Et_2O$ (2×) after which it was air-dried for at least 2 h. Cleavage of the peptide from the resin was achieved by suspending the resin in a

mixture of TFA/$H_2O$/triisopropyl-silane (95:2.5:2.5, v/v/v) for 4 to 5 h. Subsequently, the peptides were precipitated in $Et_2O$, redissolved in $H_2O$ or acetic acid and lyophilized to yield crude peptides. Purification by reversed-phase HPLC, followed by lyophilisation yielded pure peptides as powders.

*Peptides with a C-terminal carboxylic acid*

After swelling in DCM, the 2-chlorotrityl chloride resin was loaded with the first amino acid using the required amino acid (2.0 eq.) and DiPEA (3.0 eq.) in dry DCM during 1 h, after which unreacted sites on the resin were capped with DiPEA (2.0 eq.) in MeOH. After washing the resin with DCM (3×), DMF (3×), MeOH (1×) and DMF (2×), the peptide synthesis was continued as described above for "General peptide synthesis".

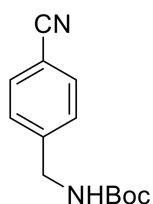
*Peptides with a C-terminal amide*

Peptides with a C-terminal amide were prepared on a Breipohl resin and loaded with the first amino acid after Fmoc deprotection following the procedure described above for "General peptide synthesis".

*Fluorescein-labeled peptides*

Fluorescein-labeled peptides were prepared by coupling FITC (1.5 eq.) after the final Fmoc deprotection in a mixture of DiPEA (2.0 eq.) DMF/DCM (7:5, v/v) o/n. Subsequently, the resin was washed with DMF (3×), *i*-PrOH (3×) and DCM (6×). Afterwards, the peptide was cleaved from the air-dried resin and handled further as described in the section "General peptide synthesis".

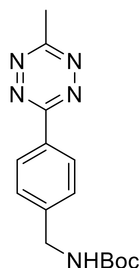
**_tert_-Butyl 4-cyanobenzylcarbamate (1)**




The synthesis was performed according to a literature procedure[2]:
4-(Aminomethyl)benzonitrile hydrochloride (1.20 g, 7.11 mmol, 1.0 eq.) in $H_2O$ (8 mL) was added dropwise to di-*tert*-butyl dicarbonate (1.8 mL, 7.82 mmol, 1.1 eq.) and sodium hydroxide (8.88 g, 22.1 mmol, 3.1 eq.) in $H_2O$ (8 mL) and stirred for 1.5 h at r.t. The white solid was filtered off and washed twice with $H_2O$ followed by lyophilisation to yield Boc-protected amine **1** (1.33 g, 5.74 mmol, 81%).

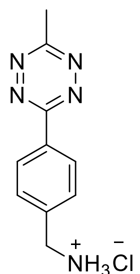
$^1$H-NMR (400 MHz, $CDCl_3$) = $\delta$: 7.61 (d, $J$ = 8.1 Hz, 2H), 7.37 (d, $J$ = 8.1 Hz, 2H), 4.98 (bs, 1H), 4.36 (d, $J$ = 5.8 Hz, 2H), 1.46 (s, 9H). $^{13}$C-NMR (125 MHz, $CDCl_3$) = $\delta$: 156.0, 144.8, 132.6, 127.9, 118.9, 111.3, 80.2, 44.3, 28.5. MS (ESI+) *m/z* calcd. for $C_{13}H_{17}N_2O_2$ [M+H]$^+$ 233.3, found 232.9. The data agrees with literature.[2]

**_tert_-Butyl 4-(6-methyl-1,2,4,5-tetrazin-3-yl)benzylcarbamate (2)**




The synthesis was performed according to a literature procedure[3] : To nitrile **1** (1.00 g, 4.31 mmol, 1.0 eq.) in dioxane (2 mL) MeCN (2.3 mL, 43.05 mmol, 10.0 eq.) and $Zn(OTf)_2$ (801 mg, 2.20 mmol, 0.51 eq.) were added. Next, hydrazine hydrate (10.4 mL, 215 mmol, 50 eq.) was added dropwise and the reaction mixture was stirred for 18 h at 60 °C. After cooling the reaction mixture to r.t., $NaNO_2$ (5.94 g, 86.1 mmol, 20 eq.) in $H_2O$ (50 mL) was added. Subsequently, aqueous HCl (1M) was added to the solution until the solution turned red, gas evolution stopped and the pH became acidic (Caution: during this reaction toxic nitrous fumes are produced). The reaction mixture was extracted six times with DCM (100 mL), followed by washing of the organic layers with brine (100 mL) and drying with $Na_2SO_4$. Volatiles were removed under reduced pressure and the crude mixture was purified with column chromatography (20% EtOAc/heptane, v/v) to yield tetrazine **2** (355 mg, 1.18 mmol, 27%).

$R_f$ = 0.25 (EtOAc/heptane, 2:8, *v/v*). $^1$H-NMR (400 MHz, $CDCl_3$) = $\delta$: 8.56 (d, $J$ = 8.6 Hz, 2H), 7.51 (d, $J$ = 8.5 Hz, 2H), 4.44 (s, 2H), 3.10 (s, 3H), 1.48 (s, 9H). $^{13}$C-NMR (125 MHz, $CDCl_3$) = $\delta$:167.4, 164.1, 156.1, 144.1, 131.08, 128.4, 128.2, 80.0, 44.6, 28.6, 21.3. MS (ESI+) *m/z* [(M-Boc)+2H]$^+$ 202.0 (calcd. 202.1). The data corresponds with literature.[3]



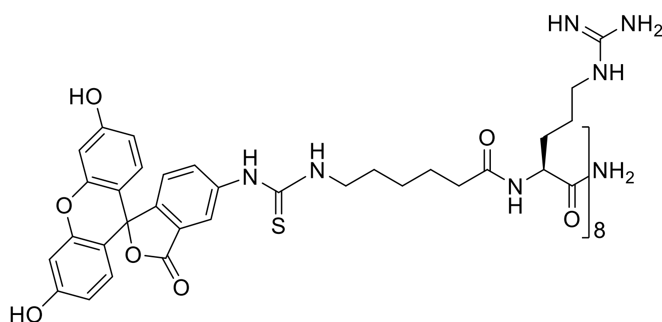

**(4-(6-Methyl-1,2,4,5-tetrazin-3-yl)phenyl)methanamine hydrochloride (3)**

To Boc-protected amine **2** (339 mg, 1.13 mmol, 1.0 eq.) in dry DCM (12 mL) 4M HCl in dioxane (8.3 mL, 33 mmol, 29 eq.) was added dropwise. The mixture was stirred for 2 h after which the volatiles were removed under reduced pressure to yield amine **3** (263 mg, 1.11 mmol, 98%).

$^1$H-NMR (400 MHz, $CD_3OD$) = $\delta$: 8.63 (d, $J$ = 8.5 Hz, 2H), 7.71 (d, $J$ = 8.6 Hz, 2H), 4.25 (s, 2H), 3.06 (s, 3H). $^{13}$C-NMR (125 MHz, $CD_3OD$) = $\delta$:169.2, 164.9, 138.7, 134.4, 130.1, 129.5, 44.0, 21.1. MS (ESI+) *m/z* [M+H]$^+$ 201.9 (calcd. 202.1). The data agrees with literature.[2]

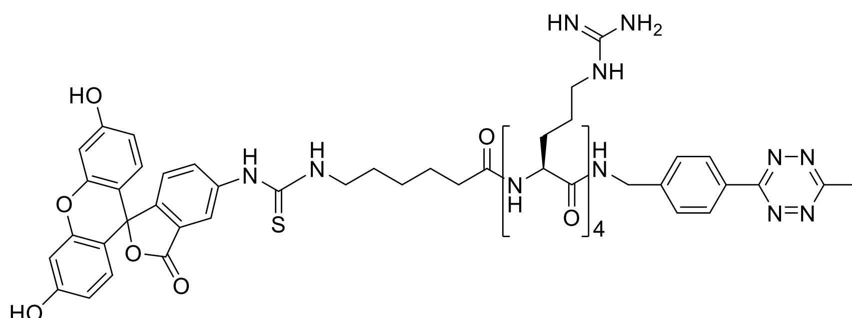
**FITC-Ahx-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-$NH_2$ (R8)**

FITC-Ahx-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-$NH_2$ was prepared following the procedures under "General peptide synthesis", "Peptides with a C-terminal amide" and "Fluorescein-labeled peptides".




HPLC: rt. 15.0 min. LCMS (ESI+) m/z [M+H]$^+$ 1769.6 (calcd.1770.9), [M+2H]$^{2+}$ 884.8 (calcd. 885.9), [M+3H]$^{3+}$590.5 (calcd. 590.9), [M+4H]$^{4+}$ 443.2 (calcd. 443.5), [M+5H]$^{5+}$ 354.8 (calcd. 354.9), [M+6H]$^{6+}$ 295.8 (calcd. 295.9). The data agrees with literature.[4]

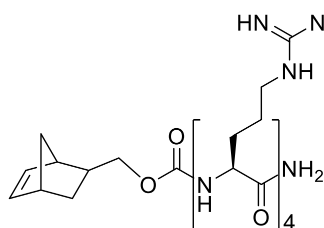
**FITC-Ahx-Arg-Arg-Arg-Arg-tetrazine (R4-Tz)**




FITC-Ahx-Arg-Arg-Arg-Arg-OH was prepared following the procedures as described in the sections "General peptide synthesis", "Peptides with a C-terminal amide" and "Fluorescein-labeled peptides". Next, the peptide was functionalized by adding tetrazine **3** (3.0 eq.), BOP (2.0 eq.) and DiPEA (4.0 eq.) in DMF. The mixture was stirred at r.t. o/n, followed by precipitation in $Et_2O$. Lyophilisation from HOAc and subsequent purification by reversed-phase HPLC (using a gradient of 10 - 70% $MeCN/H_2O$, v/v from 1 - 60 min.) yielded title compound **R4-Tz**.

HPLC: rt. 16.1 min. LCMS (ESI+) m/z $[M+H]^+$ 1328.4 (calcd. 1328.6), $[M+2H]^{2+}$ 664.8 (calcd. 664.8), $[M+3H]^{3+}$ 443.6 (calcd. 443.5), $[M+4H]^{4+}$ 333.0 (calcd. 332.9).

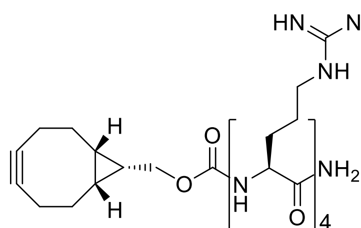
**Norbornene-Arg-Arg-Arg-Arg-$NH_2$ (norb-R4)**




H-Arg-Arg-Arg-Arg-Arg-$NH_2$ was prepared following the procedures in the sections "General peptide synthesis" and "Peptides with a C-terminal amide". Next, the peptide (25 mg, 39 µmol, 1.0 eq.) was dissolved in phosphate buffer of pH 7.5 (5 mL) and bicyclo[2.2.1]hept-5-en-2-ylmethyl (2,5-dioxopyrrolidin-1-yl) carbonate (53 mg, 201 µmol, 5.2 eq.) was added. The mixture was stirred at r.t. o/n., followed by purification of the norbornene-functionalized peptide by preparative reversed-phase HPLC. The peptide was lyophilised to yield title compound **norb-R4** (5.5 mg, 6.94 µmol, 18%).

HPLC: rt. 13.0 min. LCMS (ESI+) m/z [M+H]+ 792.7 (calcd. 792.5), $[M+2H]^{2+}$ 396.9 (calcd. 396.8), $[M+3H]^{3+}$ 264.9 (calcd. 264.8).

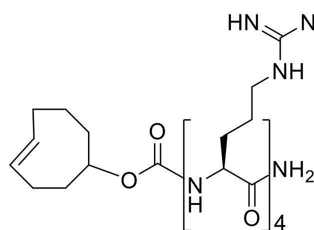
**BCN-Arg-Arg-Arg-Arg-$NH_2$ (BCN-R4)**




H-Arg-Arg-Arg-Arg-Arg-$NH_2$ was prepared following the procedures in the sections "General peptide synthesis" and "Peptides with a C-terminal amide". Next, the peptide (24 mg, 37 µmol, 1.0 eq.) was dissolved in phosphate buffer of pH 7.5 (5 mL) and BCN-NHS (58 mg, 198 µmol, 5.3 eq.) was added. The mixture was stirred at r.t. o/n, followed by purification of the BCN-functionalized peptide by preparative reversed-phase HPLC. The peptide was lyophilised to yield title compound **BCN-R4** (6.87 mg, 8.40 µmol, 22%).

HPLC: rt. 13.4 min. LCMS (ESI+) m/z $[M+H]^+$ 818.7 (calcd. 818.5), $[M+2H]^{2+}$ 409.8 (calcd. 409.8), $[M+3H]^{3+}$ 273.5 (calcd. 273.5).

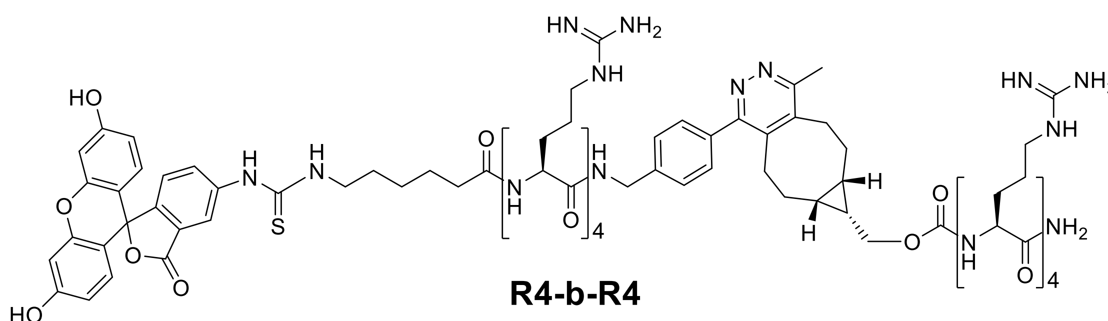
**TCO-Arg-Arg-Arg-Arg-$NH_2$ (TCO-R4)**



H-Arg-Arg-Arg-Arg-Arg-$NH_2$ was prepared following the procedures in the sections "General peptide synthesis" and "Peptides with a C-terminal amide". Next, the peptide (29 mg, 45 μmol, 1.2 eq.) was dissolved in a 50 mM phosphate buffer of pH 8 (5 mL) and TCO-NHS (10 mg, 37 μmol, 1.0 eq.) was added. The mixture was stirred at r.t. o/n, followed by purification of the TCO-functionalized peptide by preparative reversed-phase HPLC (5%-40% 25 mM $(NH_4)HCO_3$ in $H_2O$/MeCN in 50 minutes). The peptide was lyophilised to yield title compound **TCO-R4**.

HPLC: rt. 13.4 min. MS (ESI+) m/z $[M+H]^+$ 794.2 (calcd. 794.5), $[M+2H]^{2+}$ 397.6 (calcd. 397.6).

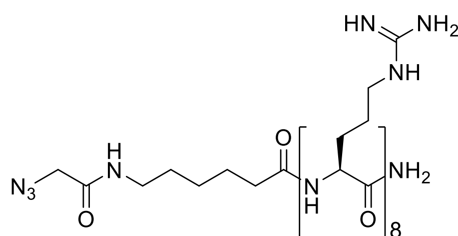
**FITC-Ahx-Arg-Arg-Arg-Arg-tetrazine-BCN-Arg-Arg-Arg-Arg-$NH_2$ (R4-b-R4)**




**BCN-R4** (1.2 mg, 1.44 μmol, 1.0 eq.) and **R4-Tz** (1.9 mg, 1.41 μmol, 1.0 eq.) were mixed in MilliQ (1.0 mL). This mixture was stirred at r.t. o/n, followed by purification of the ligated peptide by reversed-phase HPLC and subsequent lyophilisation yielded title compound **R4-b-R4** (1.8 mg, 0.83 μmol, 59%).

HPLC: rt. 14.4 min. LCMS (ESI+) m/z $[M+2H]^{2+}$ 1060.1 (calcd. 1059.6), $[M+3H]^{3+}$ 707.0 (calcd. 706.7), $[M+4H]^{4+}$ 530.7 (calcd. 530.3), $[M+5H]^{5+}$ 424.8 (calcd. 424.4), $[M+6H]^{6+}$ 354.2 (calcd. 353.9), $[M+7H]^{7+}$ 303.6 (calcd. 303.4).

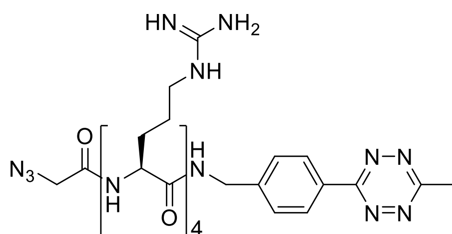
**Azidoacetyl-Ahx-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-$NH_2$ ($N_3$-R8)**



Azidoacetyl-Ahx-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-$NH_2$ was made following the procedures under "General peptide synthesis", "Peptides with a C-terminal amide" and in the final coupling 2-azidoacetic acid was employed with HATU and DIPEA as coupling reagents.

HPLC: rt. 11.6 min. MS m/z $[M+H]^+$ 1463.6 (calcd.1462.9)

**Azidoacetyl-Arg-Arg-Arg-Arg-tetrazine ($N_3$-R4-Tz)**




Azidoacetyl-Ahx-Arg-Arg-Arg-Arg-OH was prepared following the procedures as described in the sections "General peptide synthesis", "Peptides with a C-terminal carboxylic acid" after which 2-azidoacetic acid was coupled using HATU and DIPEA. Next, the purified peptide was functionalized by adding tetrazine **3** (3.0 eq.), BOP (2.0 eq.) and DiPEA (4.0 eq.) in DMF. The mixture was stirred at r.t. o/n, followed by precipitation in $Et_2O$. After redissolving it in acetic acid, lyophilisation and subsequent purification by reversed-phase HPLC (using a gradient of 5 - 15% MeCN/$H_2O$, v/v in 40 min.) yielded title compound **$N_3$-R4-Tz**.
HPLC: rt. 11.3 min. LCMS (ESI+) m/z $[M+2H]^{2+}$ 456.1 (calcd. 455.3), $[M+3H]^{3+}$ 304.4 (calcd. 303.8).

**HSA-DBCO**

To a solution of 1.0 mL of 0.225 mM HSA (0.225 µmol) in PBS was added 2.0 eq. maleimide-DBCO (0.45 mmol, 45 µL of 10 mM in 10% DMSO/PBS) and the solution was agitated for 2h at rt. Subsequently, 5.7 eq. FITC (1.28 mmol, 5 µL of 257 mM in DMSO) was added and the solution was stirred for 1h at rt. Using a spin filter (MWCO = 10 kDa) the solution was dialyzed 3× with PBS and concentrated to 500 µL (final concentration: 0.45 mM) of HSA-DBCO solution. Next, 4.4 eq. **$N_3$-R4-Tz** or **$N_3$-R8** (0.40 mmol, 40 µL of 10 mM in DMSO) was added to 200 µL of 0.45 mM HSA-DBCO (0.09 mmol) in PBS and the solution was agitated overnight at rt. The **HSA-R4-Tz** and **HSA-R8** samples were dialyzed 3× with PBS using a spin filter (MWCO = 10 kDa) and concentrated to 100 µL in PBS to a final concentration of 0.18 mM.

## Cell experiments

*Cell culture*

HeLa cells were grown in a culture flask and incubated in a humidified incubator with 7.5% $CO_2$ at 37 $^{o}$C in the presence of cell culture medium. The cells were passaged every two to three days at 80% confluency, maintaining HeLa cells until their growth reduced. For passaging, the culture medium was removed and the cells were rinsed twice with 5 mL PBS, followed by incubation with 0.05% trypsin in EDTA during 5 minutes. Fresh culture medium was added to inactivate the trypsin and cells were suspended by trituration with a pipette. An appropriate number of HeLa cells was then added to a new culture flask.

*Confocal laser scanning microscopy (CLSM)*

HeLa cells were seeded in 8-well chambered coverslips (Nunc, Wiesbaden, Germany) at a density of 40,000 cells per well one day prior to the experiment. Cells were then incubated with 200 µL peptide solution in HeLa culture medium for 30 minutes (pre-ligated peptides) or 30, 60 or 90 minutes (*in situ* activation) in a humidified atmosphere with 7.5% $CO_2$ at 37 $^{o}$C. After incubation, peptide solutions were removed, cell culture medium was added and cells were visualized immediately with confocal microscopy using a Leica TCS SP2 AOBS Confocal Laser Scanning Microscope (Leica Microsystems,

Mannheim, Germany) in combination with Leica Confocal Software. A HCX apo Long working distance Ultraviolet (VI) 40x water immersion lens was used and fluorescein was excited by an argon laser at 488 nm. The emission was collected between 500 and 550 nm.

*Flow cytometry*

HeLa cells were seeded in 24-well plates (Sarstedt, Numbrecht, Germany) at a density of 60,000 cells per well one day prior to the experiment. Cells were then washed with HeLa culture medium, followed by 30 minutes (pre-ligated peptides) or 30, 60 or 90 minutes (*in situ* activation) of incubation with 400 µL of peptide solutions in a humidified atmosphere with 7.5% $CO_2$ at 37 ºC. Subsequently, the cells were washed twice with PBS, after which they were detached from the wells by treatment with trypsin/EDTA for 5 minutes. Cells were removed from the wells after trituration in the trypsin/EDTA solution and rinsing with culture medium, after which they were spun down at 1000 rpm for 5 minutes. After resuspension of the cells in 200 µL of FACS buffer, the fluorescence of fluorescein was measured using a Beckman Coulter FC500 flow cytometer (Beckman Coulter, Brea, California, U.S.A.) after excitation at 488 nm. Results were based on gating for 1 minute.

## Supporting figures:

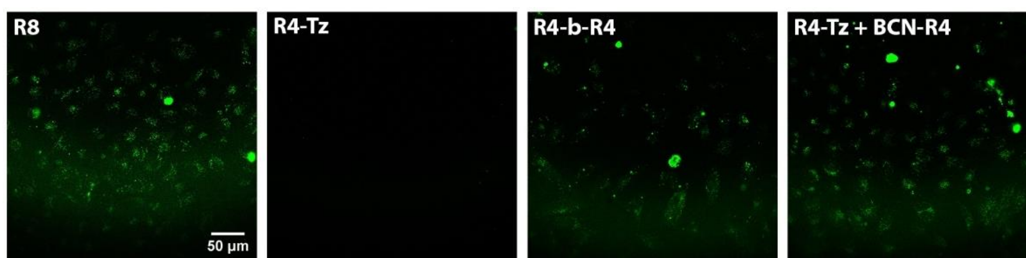



**Figure S1** – CLSM micrographs for HeLa cell uptake studies to test *in situ* activation. Confocal FITC fluorescence images are depicted for positive control R8, negative control **R4-Tz**, pre-ligated **R4-b-R4** and *in situ* activated **R4-b-R4**. For the *in situ* activation HeLa cells were incubated with 5 µM **R4-Tz** and 5 eq. of **BCN-R4** for 90 minutes.

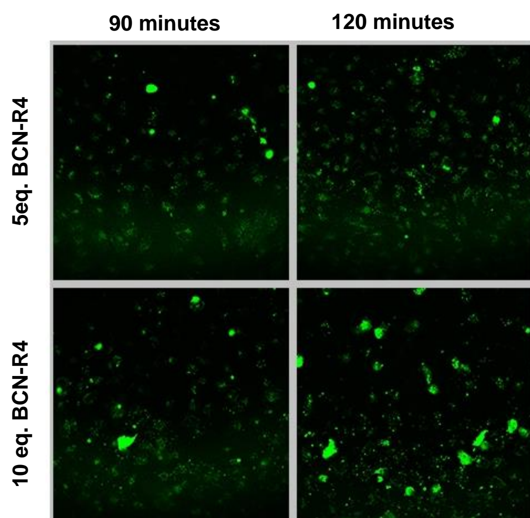



**Figure S2** - CLSM micrographs for HeLa cell uptake studies to test *in situ* activation at 5 µM **R4-Tz** with 5 or 10 eq. **BCN-R4** in serum-containing medium, incubation time of 90 or 120 minutes.

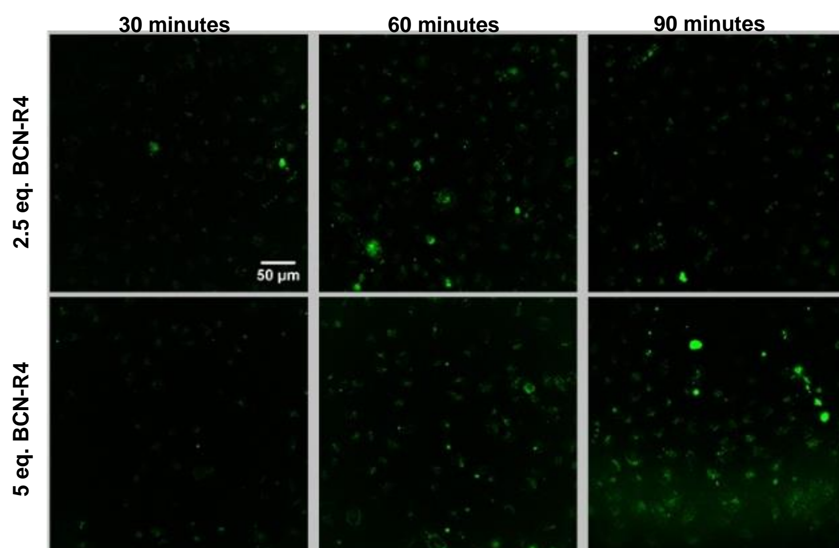



**Figure S3** - CLSM micrographs for HeLa cell uptake studies to test *in situ* activation at 5 µM **R4-Tz** with 2.5 or 5 eq. **BCN-R4** in serum-containing medium, incubation time of 30, 60 or 90 minutes.

## References

[1] E. Kaiser, R. L. Colescott, C. D. Bossinger, P. I. Cook, *Anal. Biochem.* **1970**, *34*, 595-598.

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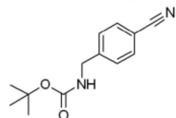
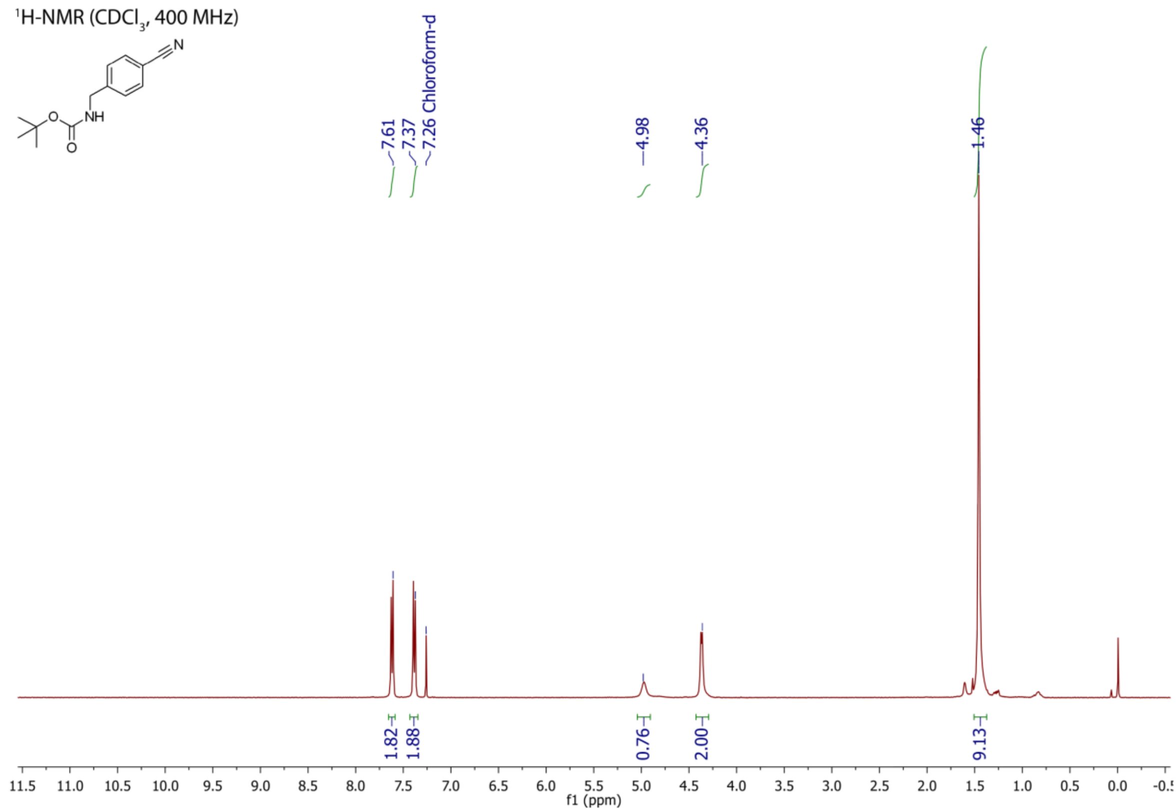
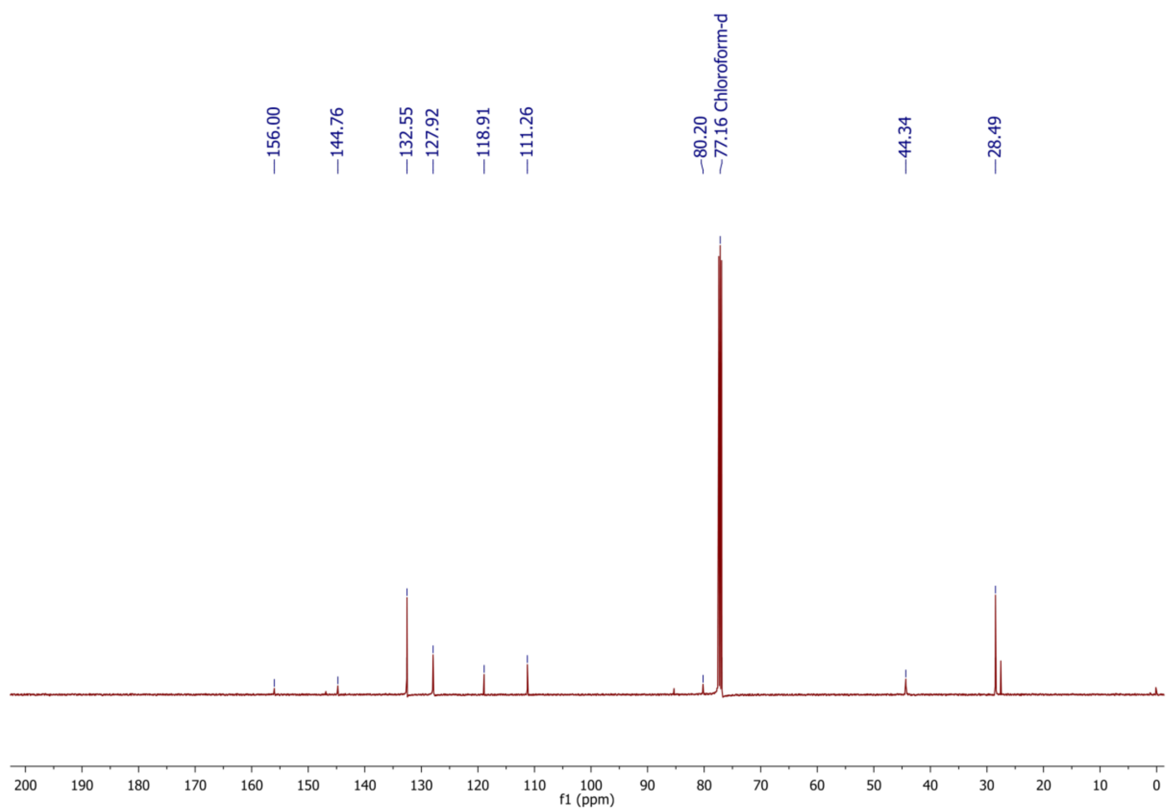
## NMR spectra

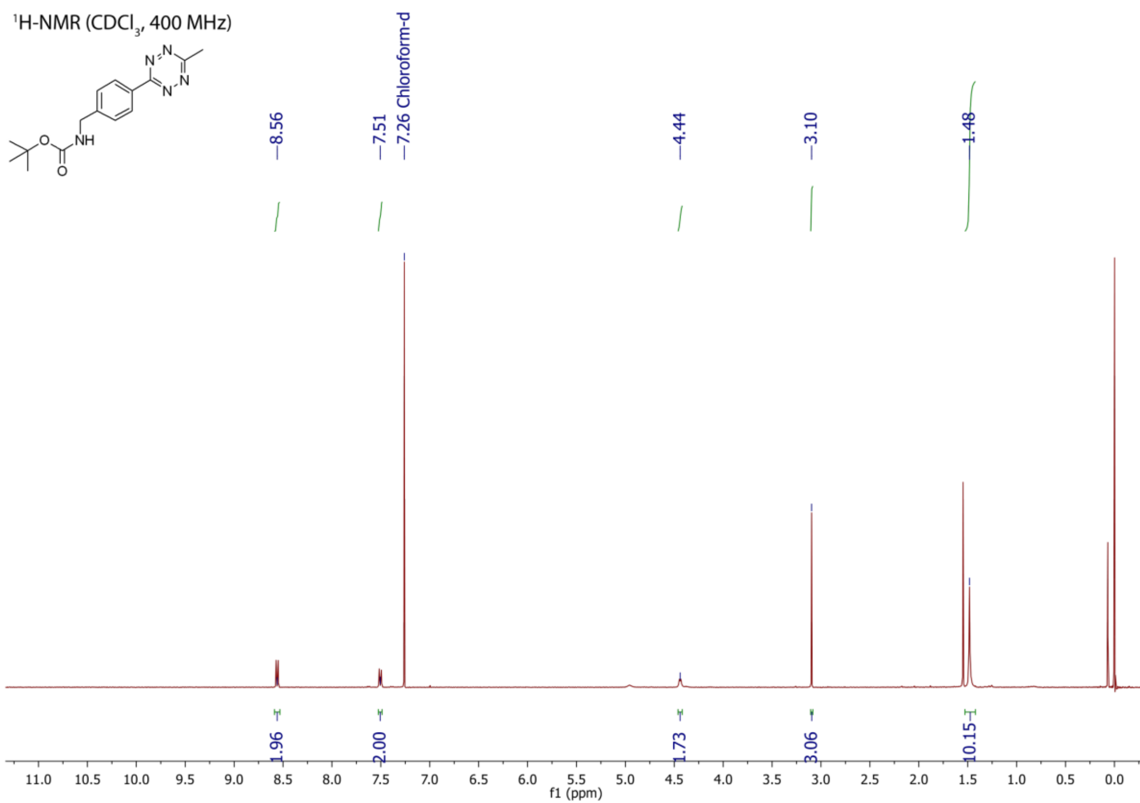
### *tert*-Butyl 4-cyanobenzylcarbamate (1)

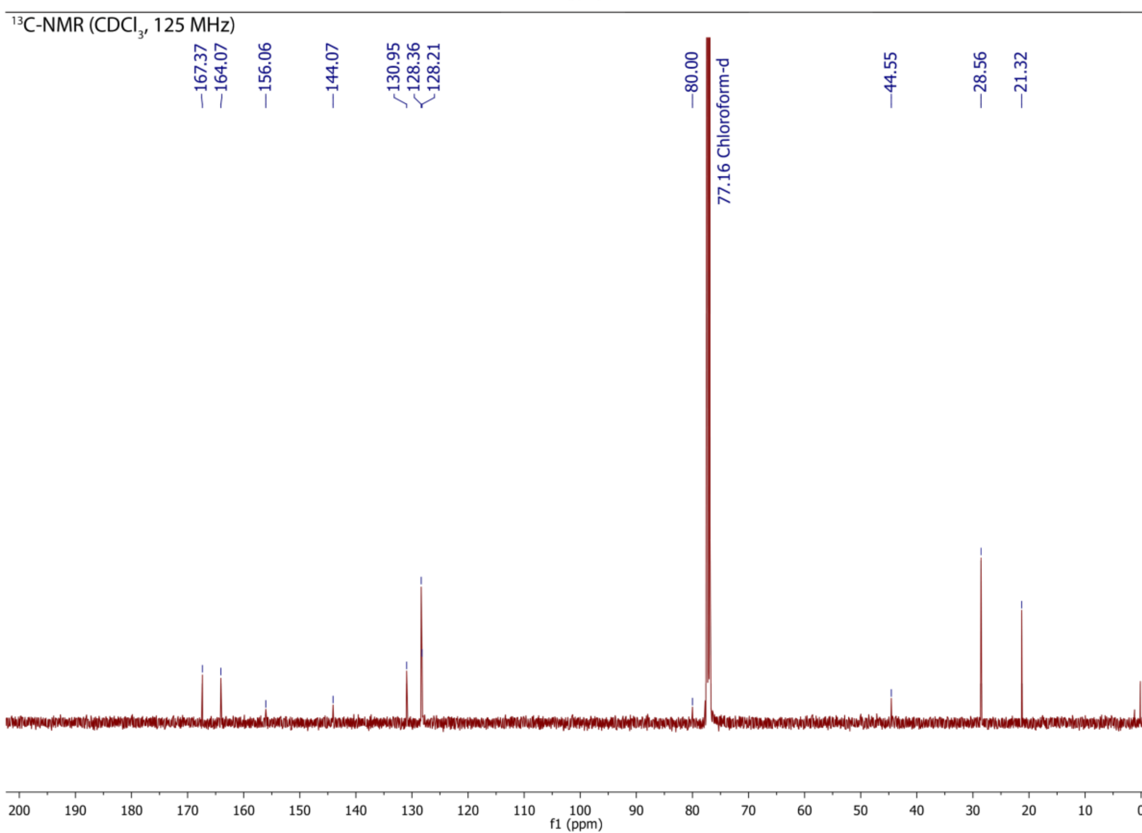


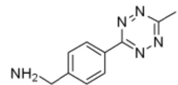



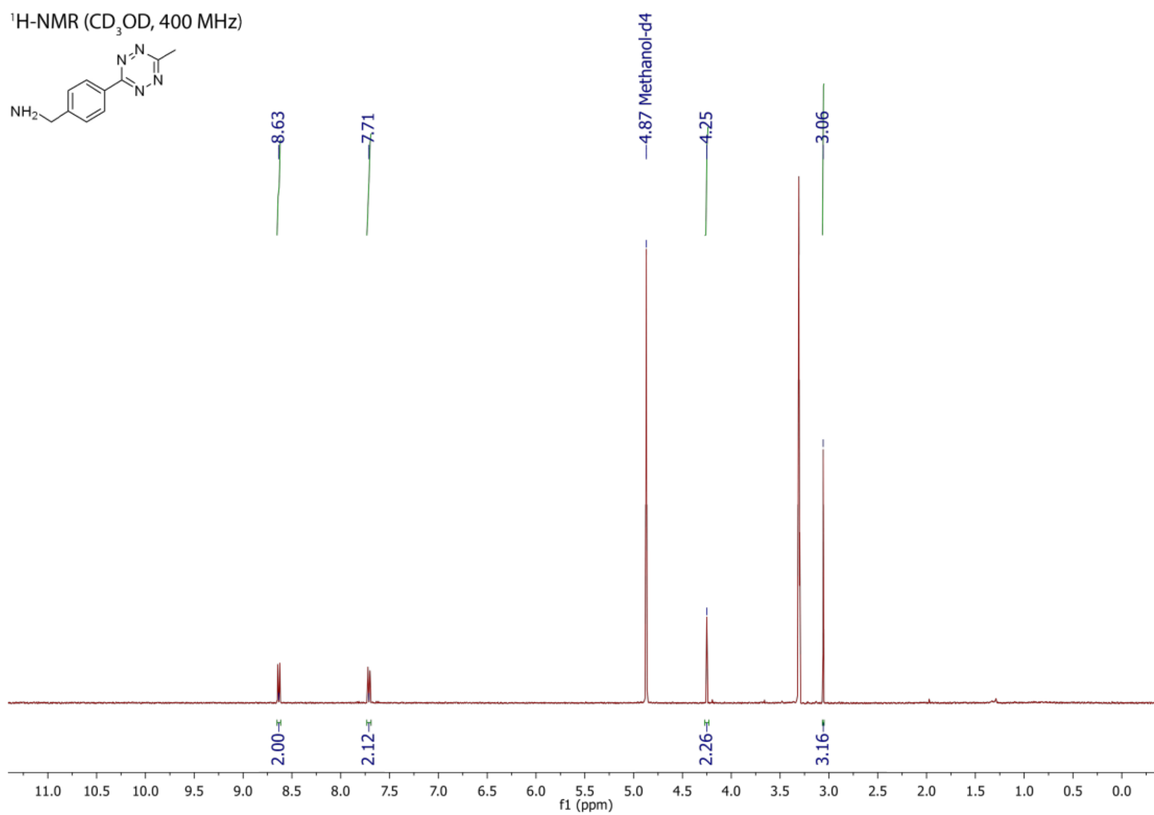
***tert*-Butyl 4-(6-methyl-1,2,4,5-tetrazin-3-yl)benzylcarbamate (2)**

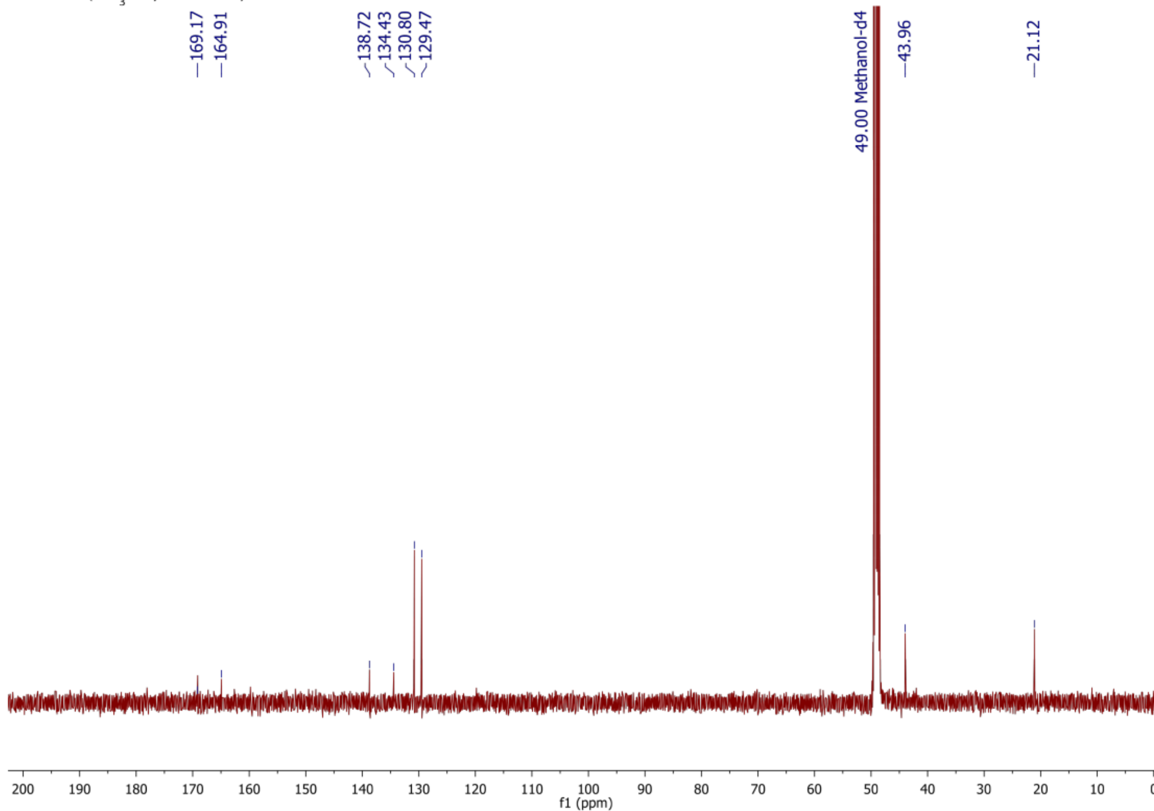


**(4-(6-Methyl-1,2,4,5-tetrazin-3-yl)phenyl)methanamine hydrochloride (3)**

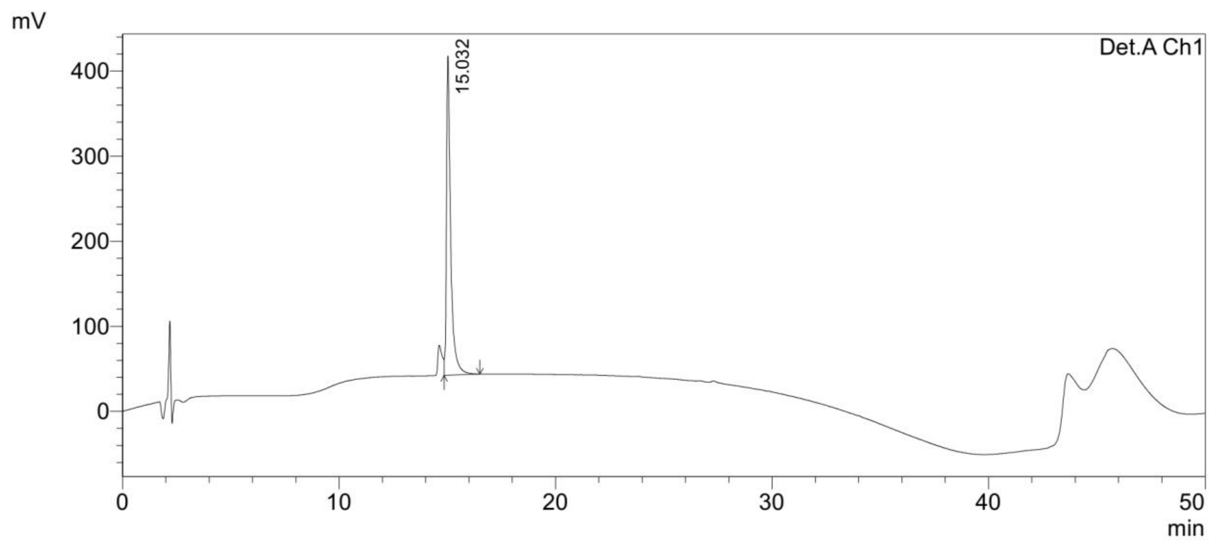


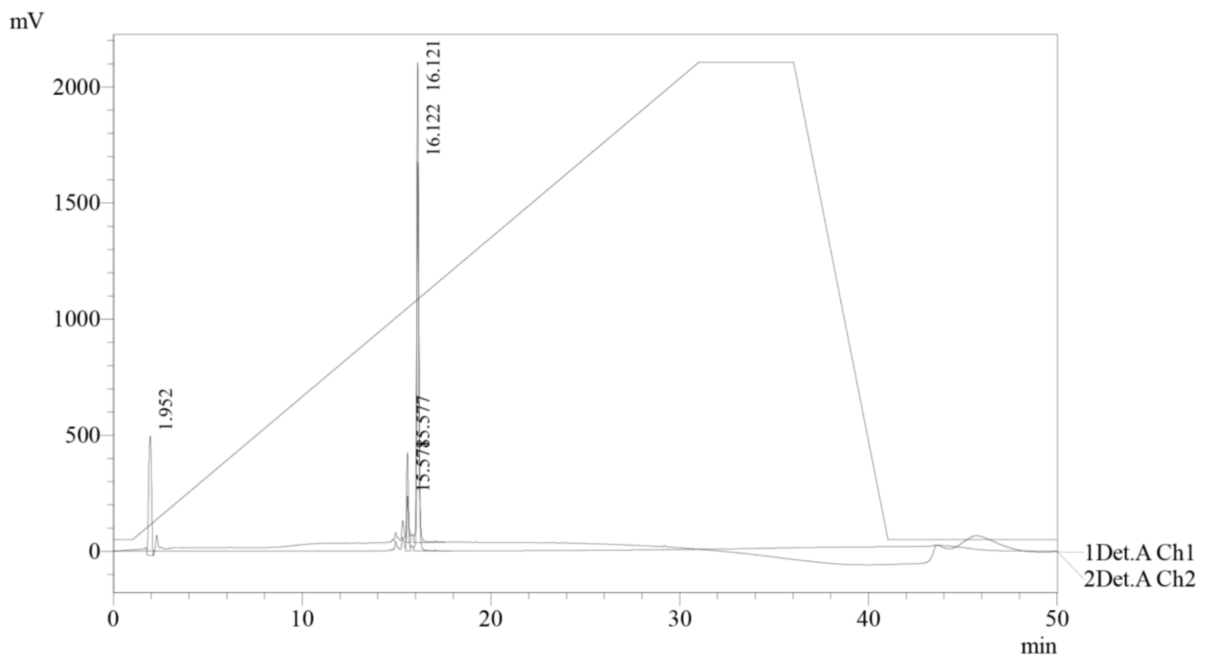



## HPLC data

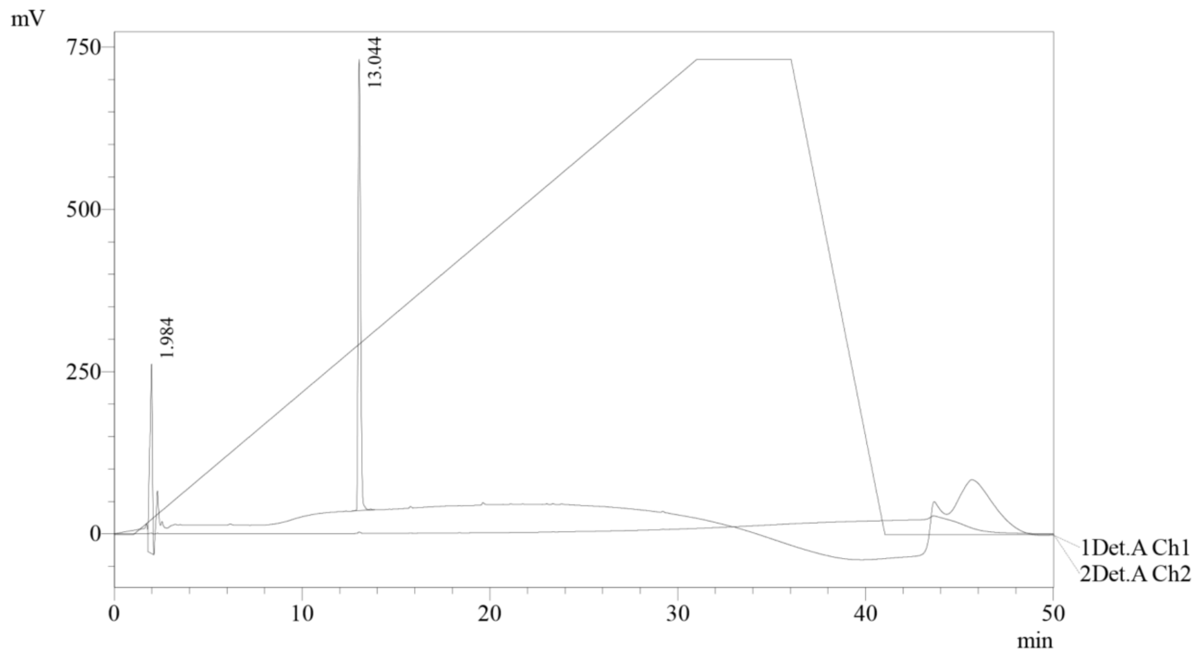
### R8



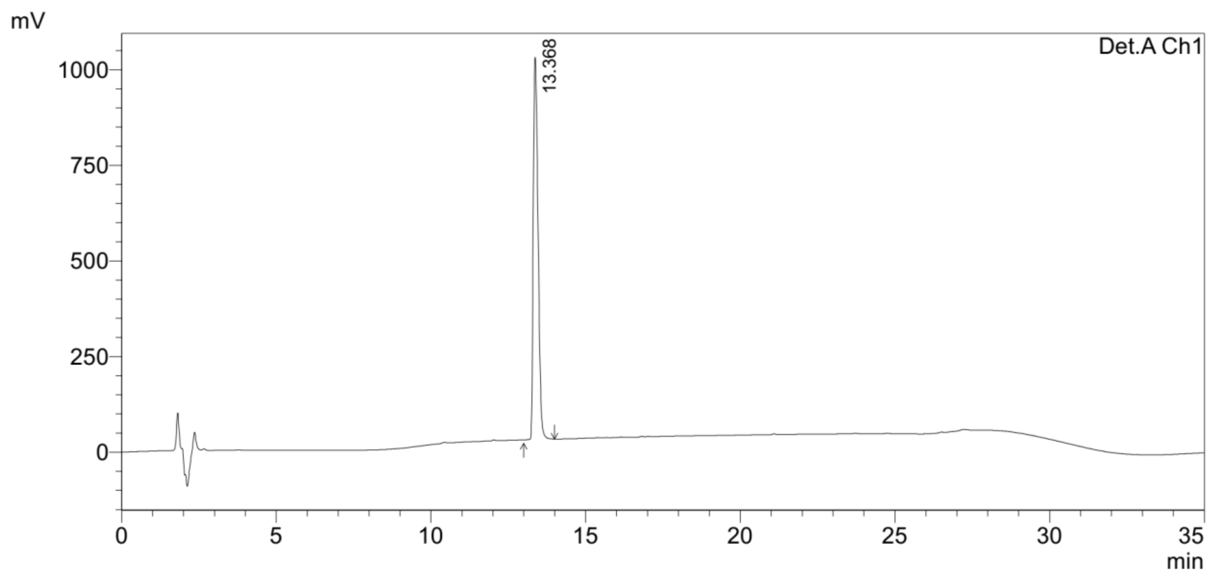

### R4-Tz



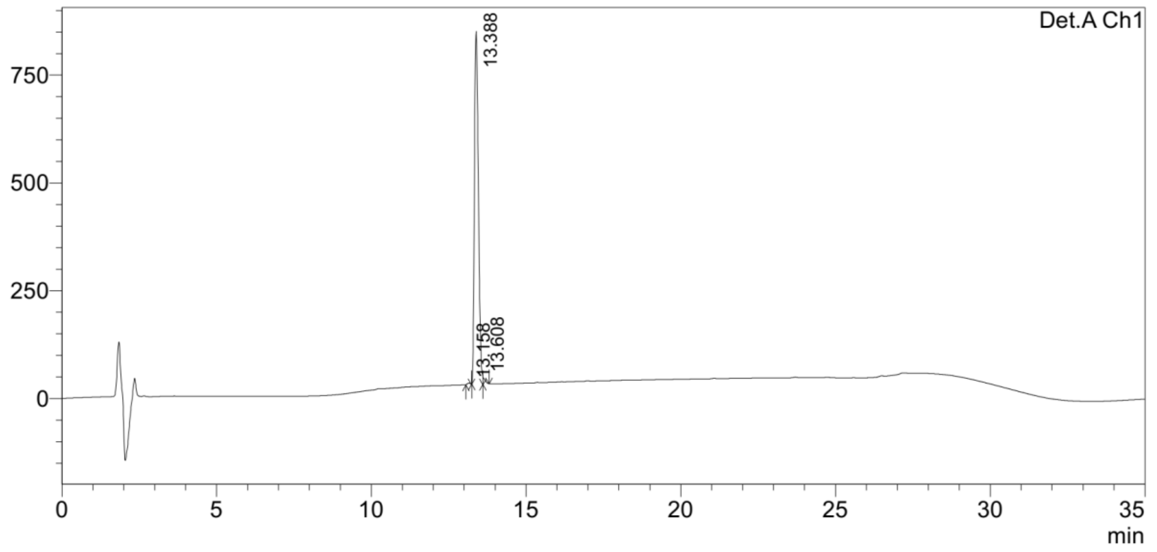
**norb-R4**



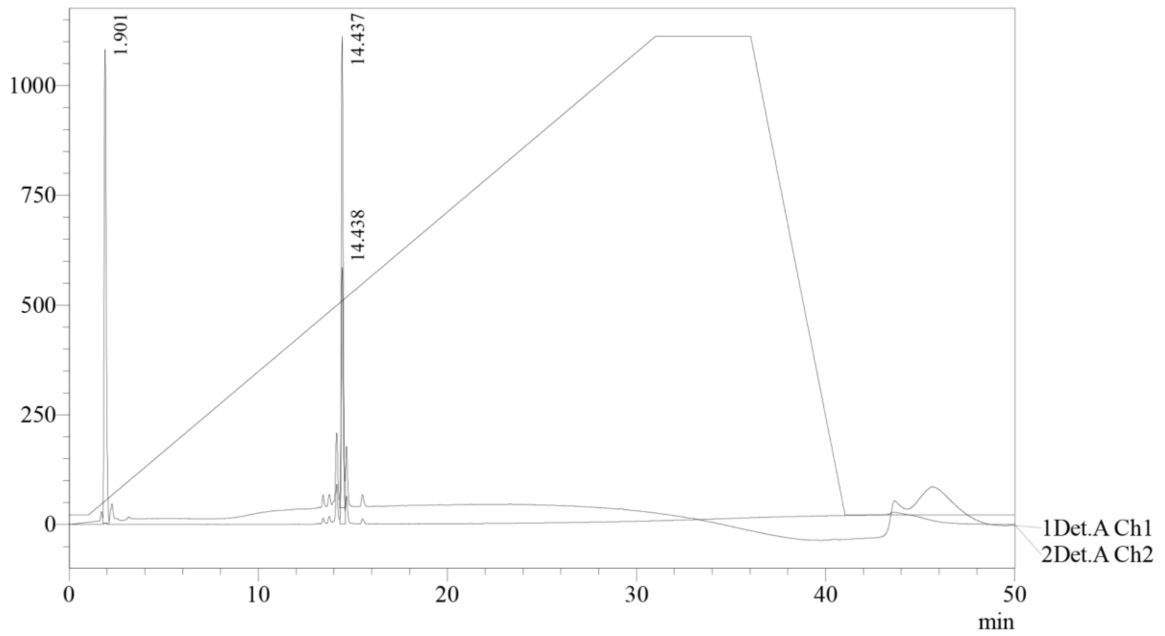

**BCN-R4**



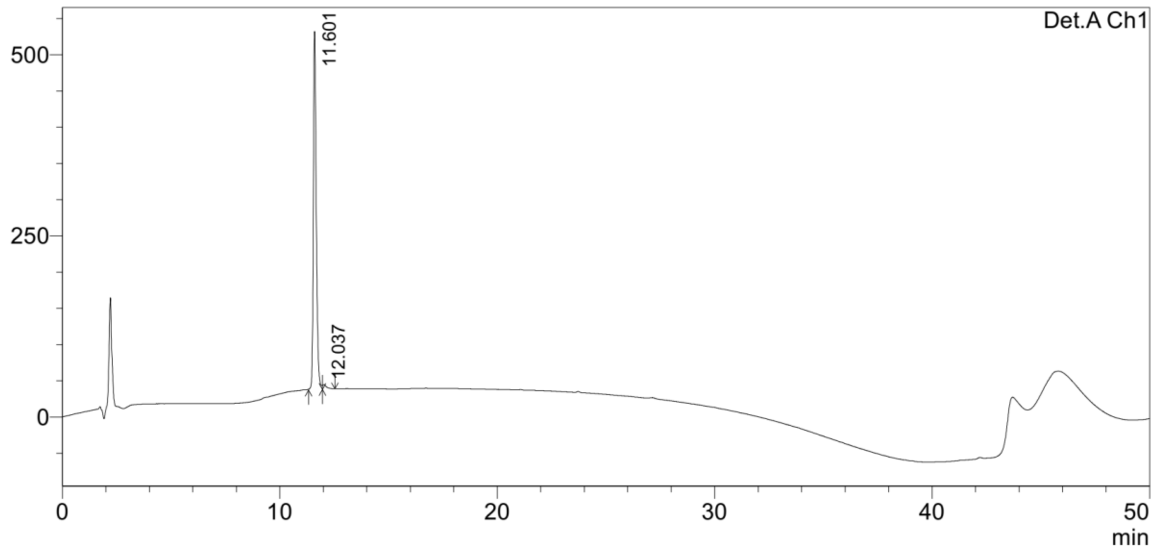
**TCO-R4**




**R4-b-R4**



**$N_3$-R8**




**$N_3$-R4-Tz**