Supporting Information
for DOI: 10.1055/s-0035-1561970
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A facile synthesis of NODASA-Functionalized Peptide

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General information:

All the chemicals were purchased from commercial sources and used without further purification. Mono-methyl fumarate, tert-butyl bromoacetate, N,N-diisopropylethylamine (DIPEA), lithium hydroxide (LiOH), tetrahydrofuran (THF), dichloromethane (DCM), dimethylformamide (DMF) were purchased from Sigma-Aldrich. 1,4,7 triazacyclononane was purchased from Leap LabChem. Fmoc-Phe-OH, Fmoc-Gly-OH, Fmoc-Tyr (tBu)-OH, Fmoc-OSu and rinkamide MBHA were purchased from GL Biochem (Shanghai). Coupling reagents used were 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) from Luxembourg Biotech, N,N-diisopropylethylamine (DIPEA) from Sigma-Aldrich, N,N'-diisopropylcarbodiimide (DIC) (Fluka, lot number BCBK8348 V) and oxyma (Luxembourg Biotech).

All compounds were analyzed by RP-HPLC (Agilent 1100, USA). Runs were performed using a linear gradient of solvent A (0.1% TFA in H2O) and solvent B (0.1% TFA in ACN); where the gradient was 5% B to 95% B in 15 min at a flow rate of 1 ml/min. All the synthetic steps were further characterized using LCMS (Shimadzu 2020 UFLC-MS, Japan) with an YMC-Triart C18 (5 μm, 4.6 × 150 mm) column for their respective masses. NMR spectra (1H NMR, 13C NMR, HMBC and HSQC) were recorded on a Bruker AVANCE III 400 MHz.

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spectrometer using deuterated methanol as the solvent. HRMS was carried out on a Bruker microTOF-QII.

**Peptide synthesis:**

The model peptide YGGF was synthesized using Fmoc chemistry in solid phase at 0.1 mmol scale. Amino acids were dissolved in 1.0 ml of DMF along with HATU and DIPEA followed by addition to the resin. The ratio of Fmoc protected amino acid to free amine was 2:1 and each coupling was carried out for 1 hr. A ratio of 1:1:2 of amino acid: HATU: DIPEA was employed in the synthesis. Fmoc deprotection was performed in 4.0 ml of 20% piperdine in DMF for 5 minutes twice. In all the steps the resin was washed with approx. 5.0 ml of DMF (2 x), DCM (2 x) and DMF (2 x) consecutively. An aliquot of the synthesized peptide was cleaved from the resin and monitored by HPLC as well as LCMS.

**4-Methoxy-4-oxo-3-(1,4,7-triazonan-1-yl)butanoic acid (1):**

1,4,7-triazacyclononane (991.5 mg, 2.0 equiv.) and DIPEA (1.33 ml, 2.0 equiv.) were dissolved in 10 ml DCM. To this solution, 500 mg (1.0 equiv.) of Mono-methyl fumarate pre dissolved in 1.0 ml DCM was added drop-wise over a period of 10-15 min. After 2 hr of agitation, the DCM was removed in vacuo. The product was further dissolved in 4.0 ml DMF and kept overnight at -20 oC for the precipitation of the (1). The DMF was decanted and the residual DMF was co-evaporated with toluene in vacuo which gave a yield of 94%. ¹HNMR (400 MHz, MeOD): δ 3.36 (1H, t), 2.93 (2H, m), 2.74 (2H, m), 2.61-2.70 (2H, m), 2.58 (2H, m), 2.56 (3H, s), 2.36-2.51 (5H, m), 2.24 (1H, m); ¹³C NMR (100 MHz, MeOD): δ 179.4, 175.3, 64.4, 52.2 (2), 50.3, 46.8 (2), 45.8 (2), 35.1. HRMS (ESI⁺): calcd. for C₁₁H₂₂N₃O₄ [M+H] 260.1605; found 260.1612.

**3-(4,7-Bis((9H-fluoren-9-yl)methoxy)carbonyl)-1,4,7-triazonan-1-yl)-4-methoxy-4-oxobutanoic acid (2):**

Compound 1 (100 mg, 1.0 equiv.) and NaHCO₃ (162 mg, 5 equiv.) was dissolved in 60 ml of water and acetone (1:1). The mixture was cooled down to 0 oC and 286 mg (2.2 equiv.) of
Fmoc-OSu pre-dissolved in 2.0 ml acetone was added dropwise over a period of 10-15 min followed by overnight stirring. The reaction was then concentrated in vacuo and extracted with diethyl ether to remove the unreacted Fmoc-OSu. Thereafter, the resulting reaction mixture was acidified with 10 ml 1M HCl followed by extraction of the compound with 50 ml diethyl ether. The organic layer was evaporated in vacuo to give a white solid yielding 82%. LCMS (positive mode) showed desired m/z 704. The product was then used for the next step without further purification.

*Methyl 1-amino-2-benzyl-11-(4-hydroxybenzyl)-1,4,7,10,13-pentaaoxo-15-(1,4,7-triazonan-1-yl)-3,6,9,12-tetraazaheptadecan-16-oate on resin (3):*

Compound 2 was coupled to the synthesized peptide on resin at 0.0125 mmol scale. Compound (2) was dissolved in 0.5 ml of DMF along with DIC and OxymaPure followed by 2 min activation. The entire mixture was then added to the resin containing the model peptide and the reaction was carried out for 16 hr. A ratio of 1:1:1: (2):DIC:OxymaPure was used in the synthesis. Compound (2) to free amine ratio was 2:1. In all the steps resin was washed with about 5.0 ml each of DMF (2 x), DCM (2 x) and DMF (2 x). The Fmoc group was removed using 4.0 ml of 20% piperdine in DMF along with 0.5M HOBt to produce compound 3. The resin was washed using approx. 5.0 ml of DMF (2 x) and DCM (2 x) consecutively. An aliquot of the synthesized peptide was cleaved from the resin and monitored by analytical HPLC as well as LCMS. LCMS (positive mode) showed desired m/z 683.

*Tert-butyl 2,2’-(7-(18-amino-17-benzyl-8-(4-hydroxybenzyl)-3,6,9,12,15,18-hexaaoxo-2-oxa-7,10,13,16-tetraazaoctadecan-4-yl)-1,4,7-triazonane-1,4-diyl)diacetate on resin (4):*

Compound (3); 0.0125 mmol was swelled with 1 ml DCM for 5 min followed by filtration, to which tert-butyl bromoacetate (5.5 µl, 3.0 equiv.) and DIPEA (6.5 µl, 3.0 equiv.) in 0.4 ml of NMP was added and the reaction mixture left to stir for 2.5 hr. The resin was washed with approx. 5.0 ml of DMF (2x) and DCM (2x) consecutively. An aliquot of the synthesized
compound was cleaved from the resin and monitored by analytical HPLC. LCMS (positive mode) showed its desired m/z 799.

**1-amino-2-benzyl-15-(4,7-bis(2-tert-butoxy-2-oxoethyl)-1,4,7-triazonan-1-yl)-11-(4-hydroxybenzyl)-1,4,7,10,13-pentaaxo-3,6,9,12-tetraazahexadecan-16-oic acid (5):**

The functionalized peptide on resin (4); 0.0125 mmol was swelled in 1.0 ml DCM for 5 min followed by filtration, and 1.0 ml of 1M LiOH (dissolved in methanol and THF in 1:1 ratio) was added. The reaction was carried out for a period of 30 min at room temperature. The completion of the reaction was monitored by cleaving an aliquot of the compound from the resin and checked by LCMS as well as analytical HPLC. The resin was washed with about 5.0 ml of THF (2x), DMF (2x), and DCM (2x) consecutively. Compound 5 (0.0125 mmol) was deprotected and cleaved from the resin using a cocktail of 1.0 ml TFA:H₂O:thioanisole (95:2.5:2.5) over 2 hr. The resin was removed by filtration and washed with 1 ml TFA. Further, TFA was evaporated with the aid of N₂ gas bubbling through the mixture. The peptide was then precipitated in 5.0 ml of ice-cold diethyl ether. The precipitated peptide was centrifuged and the diethyl ether solution was decanted. It was then dissolved in 1.0 ml of water and freeze dried without further purification which gave a yield of 84%. The purity of the synthesis was checked by analytical RP-HPLC which showed 100% purity and characterized by LCMS. HRMS (ESI⁺): calcd. for C₃₆H₄₉N₈O₁₂ [M+H] 785.3464; found 785.3434.

**Conjugation of cold Ga to NODASA-Peptide:**

Cold Ga was labelled to NODASA peptide as described before by Peter A. Knetsch et. al ². 1.0 mg of NODASA-peptide (6) was dissolved in 828 µl of water; 167 µl 1.9M sodium acetate and 5 µl of 0.7M ⁶⁹GaCl₃. The solution was allowed to react for 15 min at room temperature. Excess ⁶⁹GaCl₃ was filtered through a SPE C18 cartridge and conjugated NODASA-Peptide was eluted in 50% methanol. The ⁶⁹Ga conjugation was further confirmed by ESI-LCMS. HRMS (ESI⁺): calcd. for C₃₆H₄₆GaN₈O₁₂ [M+H] 851.2486; found 851.2534.
Transchelation assay:

The stability of $^{69}$Ga NODAGA complexation was carried out by the EDTA challenge as described by previous researchers $^3$. $^{69}$Ga [NODASA]-peptide was incubated with 500 fold excess of EDTA at room temperature. Samples were tested for transchelation at six different time points i.e. 0 min, 30 min, 60 min, 120 min, 180 min and 240 min respectively and monitored using Q-TOF LCMS.

Reference:

Spectral Data