Evaluation of an Integrin $\alpha_v\beta_3$ and Aminopeptidase N Dual-receptor Targeting Tracer for Breast Cancer Imaging

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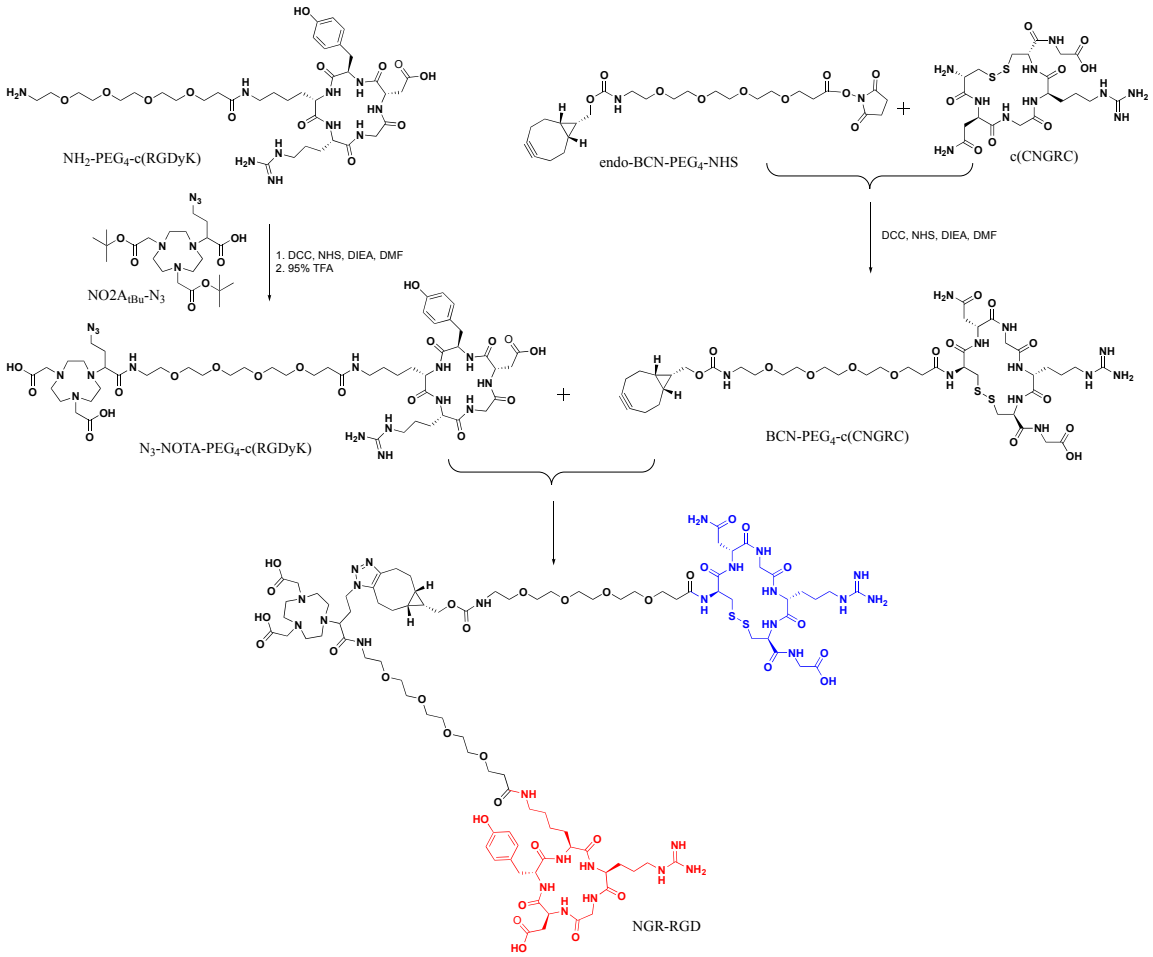

Scheme S1: synthetic route for NGR-RGD

Synthesis of N_3-NOTA-PEG_4-c(RGDyK)

N,N'-dicyclohexylcarbodiimide (DCC, 2.2 mg) and N-Hydroxy succinimide (NHS, 1.2 mg) was added to a solution of $NO2A_{tBu}$-N_3 in DMF (200 μL, 25 mg/mL). After reaction at room temperature for 1 h, the insoluble solid was removed by centrifugation. Peptide NH_2-PEG_4-c(RGDyK) (5 mg) and DIEA (10 μL) were added to the supernatant and further reacted for 4 h. After HPLC purification and lyophilization, N_3-NOTA-PEG_4-c(RGDyK) was obtained as a white solid (3.7 mg, 52%).

Synthesis of BCN-PEG_4-c(CNGRC)

Endo-BCN-PEG_4-NHS (5 mg) and DIEA (10 μL) was added to a solution of Cyclic peptide c(CNGRC) (5 mg) in DMF (200 μL). The mixture was reacted for 4 h at room temperature.

Pure BCN-PEG_4-c(CNGRC) was obtained after HPLC purification and lyophilization (4.2 mg, 50%).

Synthesis of c(RGDyK)-PEG_4- NOTA-click-PEG_4-c(CNGRC) (NGR-RGD)

100 μL N_3-NOTA-PEG_4-c(RGDyK) in water (10 mg/mL) and 120 μL N_3-NOTA-PEG_4-c(RGDyK) in water (10 mg/mL) were mixed and reacted for 2 h in room temperature. The reaction was monitored by HPLC. NGR-RGD was obtained after HPLC purification and lyophilization (1.8 mg, 82%). High resolution mass spectrometry (HRMS) (Bruker SolariX 7.0T, Bruker Daltonik, Bremen, Germany): *m/z* calcd for $C_{94}H_{151}N_{27}O_{33}S_2$ $[M+2H]^{2+}$ 1126.0283; found 1126.0427.