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A Facile Synthesis of NODASA-Functionalized Peptide

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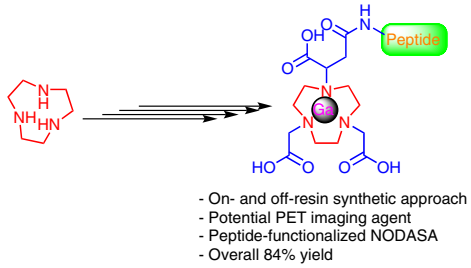

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Abstract Herein, we report a mild and efficient synthesis of a NODASA-functionalized peptide, which was initiated with a Michael addition reaction between monomethyl fumarate and 1,4,7-triazacyclononane.

Key words bifunctional chelators, NOTA, NODASA, PET, peptide

In radiopharmaceutics, chelators are small organic molecules that can form stable coordination complexes with radiometals (radioactive isotopes of various metals) and can be delivered to the target of interest in vivo.[1] Based on the type of radiometal-ion selectivity it can either be used in disease diagnosis or therapeutics.[2] In disease diagnosis; metal ions comprising of positron- or γ-emitting radionuclides and are used in positron emission tomography (PET)[3,4] and single-photon emission computed tomography (SPECT),[5] respectively. The α- and β-ion generating radioisotopes are widely employed as therapeutics in cancer treatment.[6] Due to its noninvasive, quantitative, and highly specific nature, PET imaging has become a powerful tool for diagnostic or therapeutic purposes. Some of the common radiometals which can be used in PET imaging are ^{68}Ga, ^{64}Cu, ^{86}Y, ^{89}Zr, and ^{44}Sc metal ions.[1] Among various radiometals used for PET, ^{68}Ga is of choice due to its availability from enduring ^{68}Ge/^{68}Ga generator systems which offers a cost-effective alternative to the radionuclides produced by a cyclotron.[4,7] Bifunctional chelators (BFC) are typically used for the development of radiolabeling; focusing on the in vivo target specificity and specific metal-binding capabilities. BFC contain two parts; one is a functional group which can readily react with the targeting molecule (e.g., peptides, antibodies, and nanoparticles) as well as provide a stable covalent bond to it and the other is to form a strong complex with the metal ion ensuring its undesired release within the recipient.[8]

Significant development has been achieved in the field of oncology in the context of radiolabeled peptides for tissue localization and therapy.[9] In comparison to small molecules, antibodies, and proteins as a carrier, peptides show distinctive advantages. They are reasonably lower molecular weight, easier to synthesize with the development of solid-phase synthesis, compatible to couple with various chelators, bind many significant biological targets, display excellent tumor penetration, and shows rapid clearance from the recipient. Moreover, as a therapeutic, it shows minimal side effects compared to conventional drugs and it is non immunogenic.[10]

A well-known BFC, 1,4,7-triazacyclononane-*N*′,*N*′,*N*″-triacetic acid (NOTA); is a cyclic polyamino carboxylic ligand which is one of the first and most efficient chelators of ^{64}Cu and $^{67/68}$Ga,[2,11] therefore much research and development into its derivatives has been done.[2] Contrary to its popularity

ty, a large excess of NOTA is required for the complexation with a peptide (Figure 1) as well as the high cost associated with its commercial form.[12]

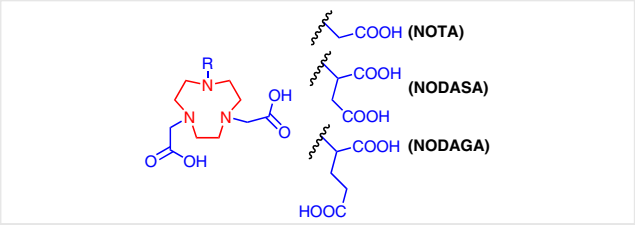
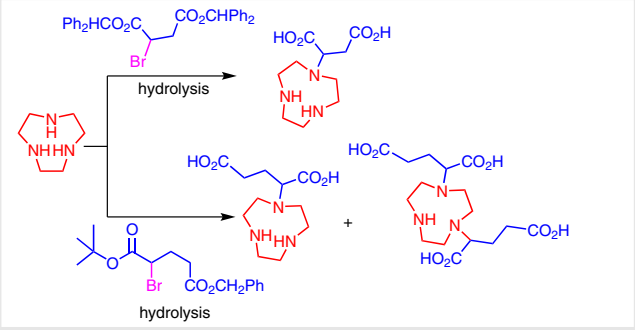


Figure 1 NOTA, NODASA, and NODAGA

NOTA derivatives namely NODASA and NODAGA (Figure 1) contain an additional carboxylic acid moiety within the macrocycle allowing the core to better saturate the hexadentate coordination of the metal ion as well as provide a site for attachment of a peptide.[9]

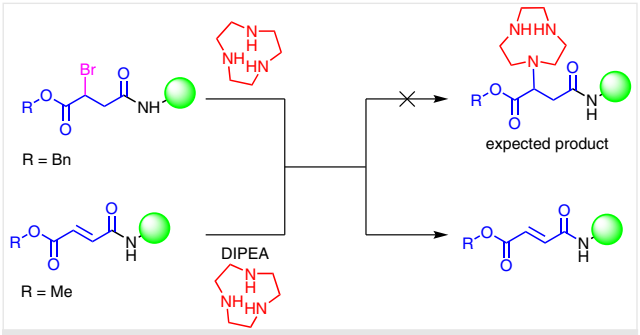


Scheme 1 NODASA[13] and NODAGA[14] synthesis

In literature noncommercially available bis(diphenyl methyl) *d,l*-bromosuccinate[13] and α-bromoglutaric acid 1-*tert*-butyl ester 5-benzyl[14] are typically described for the synthesis of NODASA and NODAGA, respectively (Scheme 1). Hence, there is a need to provide a more convenient synthetic approach for these functionalized chelator derivatives and their coupling to peptides. Herein, we report a new and facile synthetic route for the preparation of peptide-functionalized NODASA on solid phase from readily available and cheaper starting material.

The model peptide, YGGF from the parent peptide YGGFL, is a part of the enkephalin sequence. Coupling of each amino acid was carried out with HBTU/DIPEA in DMF and each step was monitored by the ninhydrin test. Single coupling for one hour with excess of Fmoc-protected amino acids were sufficient for completion of the reaction. The pure peptide product was observed in analytical HPLC when a small portion of the peptide was cleaved from the resin.

Initially, the NODASA synthesis was carried out with the coupling of 4-(benzyloxy)-3-bromo-4-oxobutanoic acid to a peptide on resin followed by addition of 1,4,7-triazacyclononane; this was unsuccessful due to the HBr elimination reaction (Scheme 2). Subsequently, monomethyl fumarate was attached to the peptide on resin, and the addition with 1,4,7-triazacyclononane was attempted, however, this reaction did not yield the product.



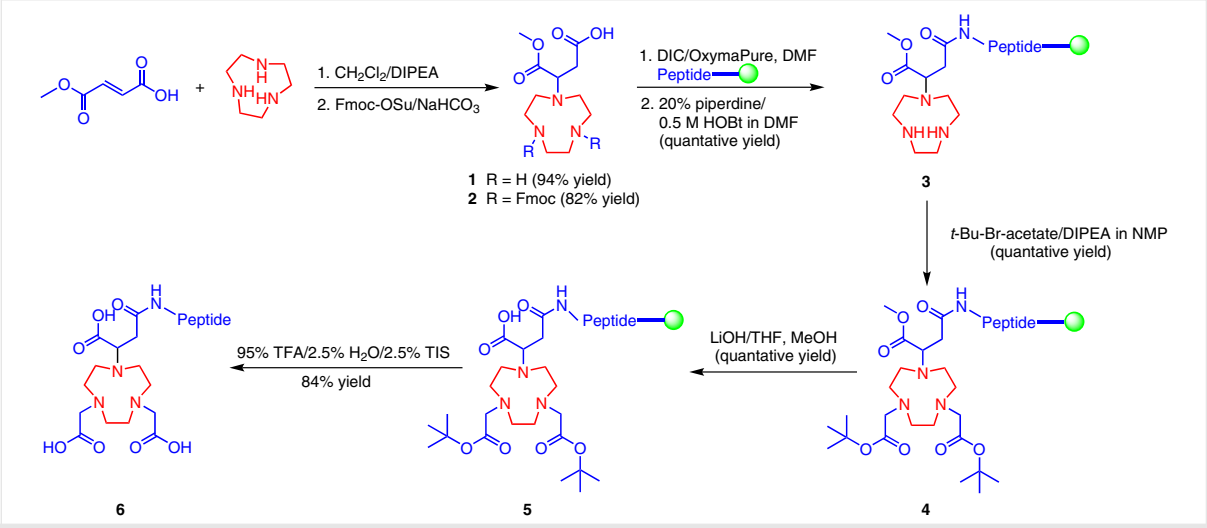
Scheme 2

Next, a Michael addition reaction between monomethyl fumarate and 1,4,7-triazacyclononane (Scheme 3) was carried out in solution. To our delight, the reaction proceeded to the desired product and proved to be regioselective for the C3 position of the alkane and was confirmed using 2D NMR spectroscopy. It was recrystallized in DMF with a yield of 94%. This product (**1**) was also confirmed by LC–MS (positive mode, at *m/z* = 260). In order to prevent self-polymerization of **1** during the amide coupling reaction, protection of the free secondary amines with Fmoc-OSu was carried out.

Compound **2** was then easily coupled to the model peptide YGGF with standard coupling reagents, DIC/OxymaPure. The reaction was monitored by cleaving a small portion of the resin and further analysis via HPLC. Complete conversion into the desired diastereomeric product was observed at *m/z* = 1127. It was then subject to a standard Fmoc deprotection to yield compound **3** which was monitored by HPLC and confirmed by LC–MS.

The resulting free secondary amines were then successfully alkylated on resin with *tert*-butyl bromoacetate in the presence of DIPEA. Complete conversion into the product was observed using analytical HPLC and LC–MS (positive mode) and showed its corresponding *m/z* = 799 for compound **4**. Thereafter, base hydrolysis of the ester was achieved on resin with LiOH in THF–MeOH to furnish derivative **5** which was confirmed from analytical HPLC with 100% conversion of the ester. The *tert*-butyl groups were removed simultaneously with cleavage of the peptide from the resin using TFA/H_2O/TIS. The final product **6** was analyzed by analytical HPLC followed by LC–MS characterization. Analytical HPLC showed 100% conversion into the product with its corresponding *m/z* = 785 and an isolated yield of 84%.[15]

An application of the conjugated functionalized peptide product was metal chelation with cold gallium. A period of 30 minutes at room temperature was adequate for $GaCl_3$ in



Scheme 3

the presence of NaOAc for the complete complexation of NODASA–YGGF (**6**). The stability of gallium complexation was further analyzed by 500-fold excess of EDTA. However, EDTA was challenged for 0, 30, 60, 120, 180, and 240 min showing no significant release of gallium from the complex confirming the resistance towards *trans* chelation.

In this study we described a facile seven-step synthesis and purification of NODASA with a model peptide. The combination of on- and off-resin synthetic approach was employed. We also successfully demonstrated the efficient conjugation of cold gallium to this NODASA–YGGF chelator as a potential PET imaging agent. The synthetic route provides a cheap and simple alternative to commercially available functionalized NODASA in the current market and could be applied to various peptides of choice.

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Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0035-1561970.

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(15) **General Procedure for the Synthesis of 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-pentaoxo-3,6,9,12-tetraazahexadecan-16-oic Acid (5)**
The functionalized peptide on resin **4**; 0.0125 mmol was swelled in 1.0 ml CH_2Cl_2 for 5 min followed by filtration, and 1.0

mL of 1 M LiOH (dissolved in MeOH 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 LC–MS as well as analytical HPLC. The resin was washed with about 5.0 mL of THF (2×), DMF (2×), and CH_2Cl_2 (2×) consecutively. Compound **5** (0.0125 mmol) was deprotected and cleaved from the resin using a cocktail of 1.0 mL TFA/H_2O/thioanisole (95:2.5:2.5) over 2 h. The resin was removed by filtration and washed with 1 mL TFA. Further, TFA was evaporated with the aid of N_2 gas bubbling through the mixture. The peptide was then precipitated in 5.0 mL of ice-cold Et_2O. The precipitated peptide was centrifuged and the Et_2O 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 LC–MS. HRMS (ESI^+): *m/z* calcd. for $C_{36}H_{49}N_8O_{12}$ [M + H]: 785.3464; found: 785.3434.