Title: A one-pot synthesis of DOTA-hydrazide *via* HATU-mediated coupling reaction for biomacromolecule radiolabeling

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Abstract

Introduction: DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) is a common radiometal chelator used to tag biomacromolecules, such as nanoparticles, antibodies and peptides, for radiolabeling for PET and SPECT applications. DOTA forms a stable complex with the trivalent metal ions, such as ^{68}Ga (for PET), ^{111}In (for SPECT) and ^{177}Lu (theranostic). The hydrazide group has shown an advantage in bioconjugate chemistry by its ability to react with an aldehyde group without a catalyst, forming a stable hydrazone bond. The synthesis of DOTA-hydrazide starting from the cyclen has been previously reported, however, it involves multistep reactions and is not necessarily accessible for research groups without developed organic synthesis laboratories.[1] Here, we report a simple one-pot reaction of DOTA-hydrazide synthesis by the HATU (Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium) coupling reaction. Moreover, we carried out the conjugation of the DOTA-hydrazide to the aldehyde group available at the reducing end of cellulose nanocrystals (CNC) as a model compound, and subsequently labeled the DOTA-CNC with ^{111}In.

Methods: The DOTA-hydrazide synthesis scheme is shown in **Figure 1A**. First, 1,4,7,10-tetraazacyclododecane-1,4,7-tris-*tert*-butyl acetate-10-acetic acid (Boc-DOTA-COOH) and HATU (1.2 eq) were dissolved in anhydrous DMF under argon atmosphere and left stirring for 10 min at room temperature (RT). DIPEA (2 eq) was added to the reaction mixture and further stirred for 30 min. The hydrazide solution in 1.0 M THF (1.1 eq) was added and the reaction was stirred overnight at RT under argon. The Boc-DOTA-NH-NH_2 was purified by liquid-liquid extraction in ethyl acetate and dried over sodium sulfate before evaporation to dryness. The Boc-deprotection was carried out in MeOH:HCl (1:3 v/v) for 4 h, RT. The deprotected DOTA-hydrazide was washed with DCM and evaporated to dryness, yielding viscous yellow-tinted oil product. The DOTA-hydrazide was further purified on a reverse-phase Sep-Pak® Plus C18 SPE cartridge with acetonitrile:water (70:30) eluent. The DOTA-hydrazide product was characterized by ^{1}H-NMR, ^{13}C-NMR, and ESI-TOF MS. The installation of DOTA-hydrazide to the CNC was done in anhydrous DMSO at 60℃ for 4 days. The DOTA-CNC was purified with dialysis against ultrapure water and collected back by lyophilization. The DOTA-CNC were characterized by elemental analysis (EA) and ATR-FTIR. The DOTA-CNC was further radiolabeled with $^{111}InCl_3$ (30 MBq) in 0.2 M ammonium acetate buffer (pH 4.0) at 100℃ for 60 min. The ^{111}In-DOTA-CNC was purified with sequential washes in 50 mM EDTA, PBS, and ultrapure water. The radiochemical yield (RCY) and purity (RCP) were checked by dose calibrator and radio-TLC, respectively.

Results: The DOTA-hydrazide was fully characterized by ^{1}H- and ^{13}C-NMR, with the assignments show in **Figure 1B**. ^{1}H-NMR (300 MHz, *d6*-DMSO), (a) δ = 2.49 ppm (s, 16H), (b) δ = 3.01 – 3.55 ppm, br, 6H, (c) δ = 3.68 ppm, m, 2H, (d) δ = 3.83 – 4.12 ppm, t, 2H, and (e) δ = 10.24 ppm, s, 1H. ^{13}C-NMR (300 MHz, *d6*-DMSO), (a) δ = 49.58 ppm, (b) δ = 52.94 ppm, and (c) δ = 170.28 ppm. The ESI-TOF MS showed *m/z* ratio of 419.2134 (calc. 419.2210). The final yield was 72%, which was relatively high compared to the literature.[1,2] The EA revealed 0.1% increase in nitrogen content after DOTA conjugation to the CNC while undetected in unmodified CNC, also ATR-FTIR indicated the amine II (1590 cm^{-1}) and amide I (1665 cm^{-1}) bands, demonstrating successful conjugation of DOTA to CNC. For radiolabeling, the RCY was only a modest at 7% due to the limited number of the reducing-end aldehydes. The radio-TLC showed a high RCP at 99.3% after purification.

Conclusion: The synthesis of DOTA-hydrazide presented in this work is a simple workup with facile purification and good yield prompting its use for radiolabeling of biomacromolecules through the hydrazone linkage.

References:

(1) Fuge, F.; et al., *Tetrahedron Lett.* **2013**, 54, 918-920.

(2) Li, C.; et al., *Tetrahedron Lett.* **2009**, 50, 2929-2931.

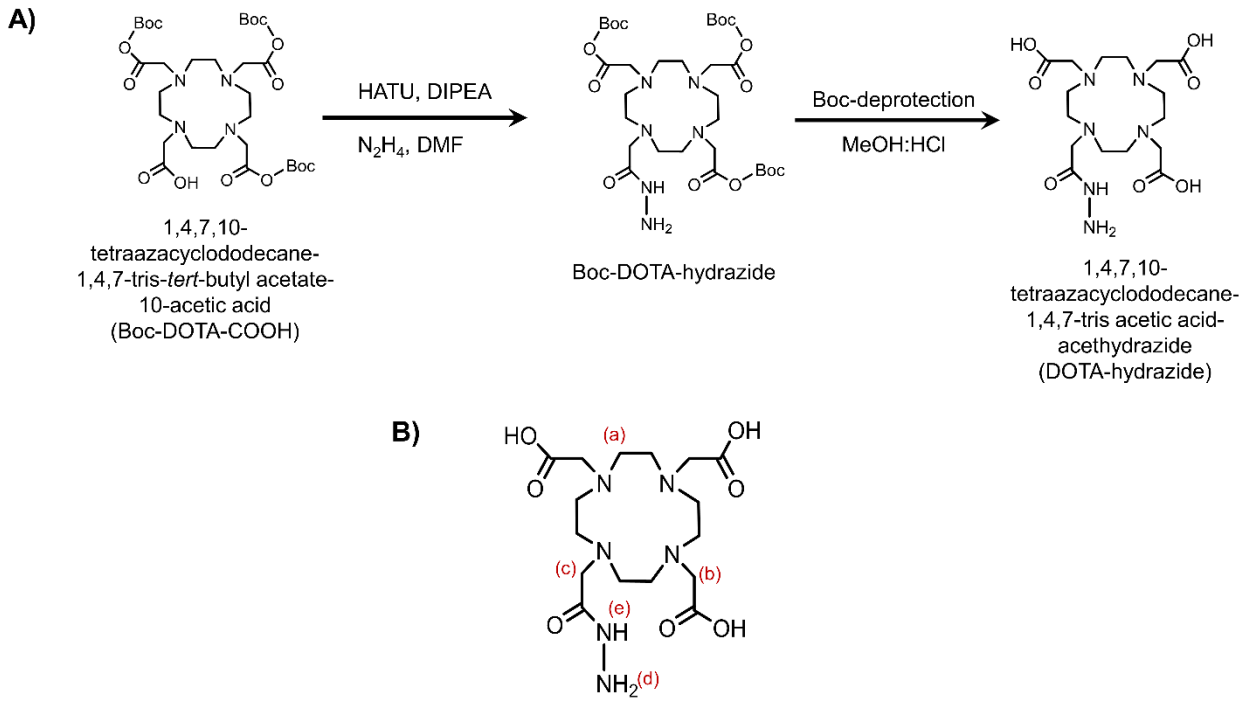

Figure 1: (A) synthesis scheme of DOTA-hydrazide, and (B) NMR assignations of DOTA-hydrazide