Tetrahedron Letters 54 (2013) 5755–5757

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Synthesis of perfluorinated analogs of DOTA and NOTA: bifunctional chelating groups with potential applications in hybrid molecular imaging

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ARTICLE INFO

Article history:
Received 12 July 2013
Revised 7 August 2013
Accepted 10 August 2013
Available online 16 August 2013

Keywords:
PET–MRI
Multimodality imaging
Chelation
Radioactive metal ions
Fluorous chemistry

ABSTRACT

The synthesis of novel NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid) and DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) chelating groups bearing perfluorinated appendages is described. DOTA and NOTA groups are used in the production of radiopharmaceutical agents for PET and SPECT imaging (by chelation of radioactive metal ions), as well as MRI contrast agents (by chelation of lanthanide Ln^{3+} ions). The novel perfluorinated variants disclosed herein will enhance the synthesis and purification of such agents, as they are compatible with fluorous purification strategies. Moreover, the perfluorous tag is anticipated to be detectable by ^{19}F-MRI, suggesting future applications in hybrid molecular imaging such as PET–MRI.

The synthesis of novel NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid) and DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) compounds is an important area of research because of their widespread use as chelating groups in, for example, molecular imaging.[1] Such chelating groups can be attached to bioactive molecules and then used to complex lanthanide ions such as Gd^{3+} to generate contrast agents for magnetic resonance imaging (MRI).[2] Alternatively, if radioactive metal ions are complexed then radiopharmaceuticals for positron emission tomography (PET) or single photon emission computed tomography (SPECT) imaging are readily accessible.[3] In the latter case, the short-lived radionuclides typically employed in diagnostic PET and SPECT imaging studies require rapid and efficient methods for the synthesis and purification of radiopharmaceuticals. With this goal in mind, we were interested in exploiting fluorous techniques in our radiopharmaceutical manufacturing program, and the objective of this research was to synthesize chelating groups bearing perfluorinated tags. However, as there is growing interest in the use of fluorinated compounds in ^{19}F MRI and magnetic resonance spectroscopy (MRS),[4] introduction of the proposed perfluorous tags could serve a dual purpose as they should also be detectable by ^{19}F MRI. ^{19}F is the only stable isotope of fluorine and therefore 100% naturally abundant. Whilst fluorine occurs in the body, it is localized in bones and teeth and has been shown to have a very short T_2 relaxation time.[5] The combination of these factors means that exogenously administered fluorine containing compounds can be seen clearly in [^{19}F]MRI scans and there is no significant interference from background fluorine signals. Therefore we believe that bioactive molecules tagged with perfluorinated chelating groups have the potential to function as a new class of bimodal agents for PET/^{19}F-MRI or SPECT/^{19}F-MRI depending upon the choice of radioactive metal ion (Fig. 1).[6] In this Letter we report the initial chemical synthesis of NOTA and DOTA analogs functionalized with perfluorooctane side chains (C_8F_{17}– = Rf), and

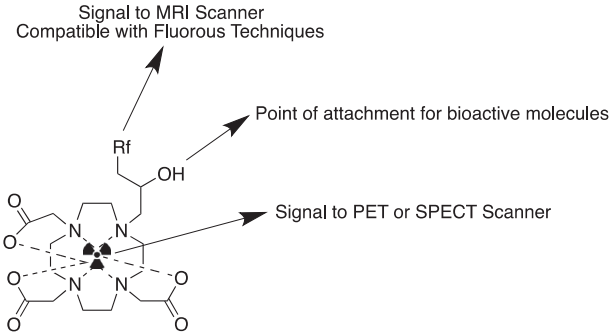

Figure 1. Concept of perfluorinated-chelating groups.

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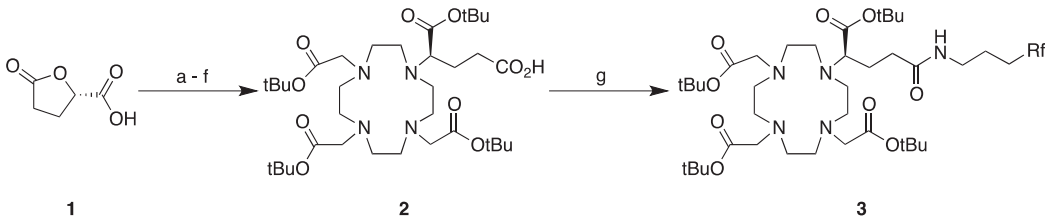
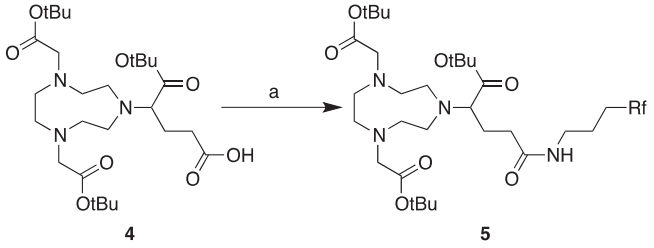
http://dx.doi.org/10.1016/j.tetlet.2013.08.035



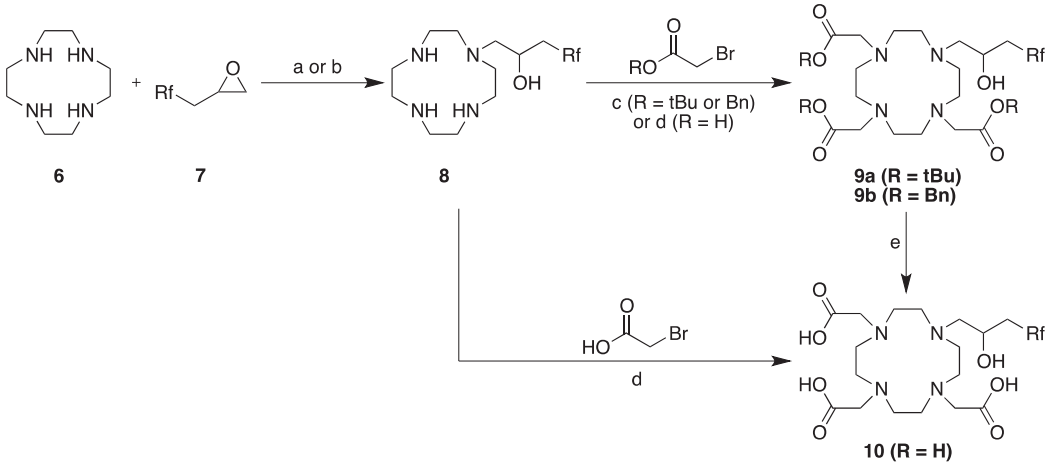
Scheme 1. Reagents and conditions: (a) $(COCl)_2$, DMF (Cat.), DCM; (b) *t*BuOH, 2,6-lutidine, DCM, −40 °C; (c) (1) KOH, THF; (2) BnBr, DMF; (d) (1). MsCl, NEt_3, DCM, 0 °C – rt, 2 h; 2. Cyclen (2 equiv), DCM, rt, −50 °C; (e) *t*Butyl 2-bromoacetate (3 equiv), K_2CO_3, MeCN, rt; (f) H_2, Pd/C, MeOH (20% from **1**); (g) (1). $C_8F_{17}(CH_2)_3NH_2$, HATU, DIPEA, DMF, rt, 24 h; (2) F-SPE (88%).



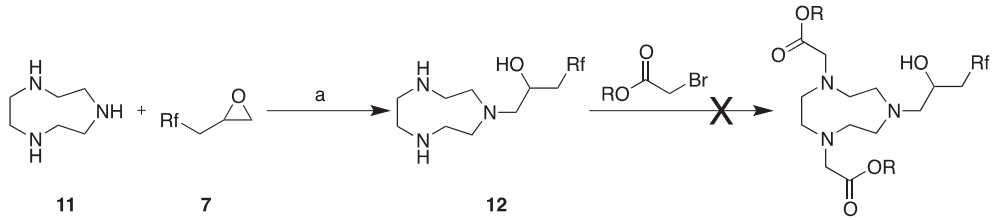
Scheme 2. Reagents and conditions: (a) (1). $C_8F_{17}(CH_2)_3NH_2$, HATU, DIPEA, DMF, rt, 24 h; (2). F-SPE (92%).

demonstrate their compatibility with fluorous chemistry. Evaluation of bioactive molecules tagged with the new chelating groups as bimodal agents for hybrid molecular imaging is ongoing, and will be reported in due course.

Potential chelating groups must have a site for attaching a bioactive molecule as well as a site for attachment of the perfluorous tag (Fig. 1). Initial investigations focused upon two strategies for the introduction of a perfluorous group along with a point of attachment for a bioactive molecule. In the first approach, chelating groups were prepared initially and the perfluorous functionality was introduced as the last step using commercially available 3-(perfluorooctyl)propylamine (4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoroundecylamine, Sigma–Aldrich) (Scheme 1). Thus, *R*-DOTA-AMP (**2**) was prepared in 6-steps and 20% overall yield from (*S*)-5-oxotetrahydrofuran-2-carboxylic acid (**1**) as previously described by Levy et al.[7] With gram quantities of *R*-DOTA-AMP (**2**) in hand, we initially attempted to couple it with 3-(perfluorooctyl)propylamine under standard peptide coupling conditions (*N*,*N*′-dicyclohexylcarbodiimide or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide), but the reaction proved sluggish and difficult to purify. A much faster and cleaner coupling reaction proceeded when *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) was used as the activating agent. Purification of the crude reaction mixture by fluorous solid-phase extraction (F-SPE) provided the perfluorinated analog of DOTA (**3**) in 88% yield.[8] In the case of the corresponding NOTA-analog, commercially available starting material **4** (ChemTek) was treated



Scheme 3. Reagents and conditions: (a) K_2CO_3, MeCN, 50 °C, overnight (48%); (b) $BF_3{\cdot}Et_2O$, MeCN, 50 °C, 22 h (65%); (c) α-bromo ester, K_2CO_3, MeCN, rt, overnight (67%, R = *t*Bu; 51%, R = Bn); (d) bromo acetic acid, NaOH, THF/H_2O, rt, overnight (43%); (e) 1M NaOH (aq), THF, 55 °C, 6 h (100%).



Scheme 4. Reagents and conditions: (a) K_2CO_3, MeCN, 50 °C, overnight (60%).

in an analogous fashion to yield a perfluorinated analog of NOTA (**5**) in 92% yield (Scheme 2).

The second approach utilized perfluorous epoxide **7** to introduce the perfluorous tag at the start of the synthesis. Thus, treatment of commercially available cyclen (**6**) with perfluorinated epoxide **7** in the presence of potassium carbonate or boron trifluoride etherate yielded DOTA intermediate **8** in 48% or 65% yield, respectively, (Scheme 3).[9] DOTA derivative **8** was allowed to react with α-bromo esters to generate the *tert*-butyl- and benzyl-protected perfluorinated analogs of DOTA **9a** and **9b** in 67% and 51% yield, respectively. Alternatively, **8** could be coupled directly with bromo acetic acid to provide the fully deprotected chelating group **10** in 43% yield.[10] These compounds were all fragile white powders most readily purified by fluorous solid-phase extraction (F-SPE) and isolated by lyophilization. Treating NOTA **11** with epoxide **7** also yielded the expected perfluorinated-NOTA intermediate **12** in 60% yield. Surprisingly however, analogous coupling reactions between NOTA-intermediate **12** and α-bromo esters did not yield the desired products, although there is no obvious explanation for the decomposition products formed instead (Scheme 4).

Finally, with a range of protected perfluorinated-chelating groups in hand, conditions were explored for removal of the ester protecting groups as the final step. Initial efforts focused upon acid-mediated hydrolysis but this was quickly found to be ineffective and resulted in elimination of the perfluorous tag (and other decomposition products) in the case of trifluoroacetic acid (TFA), or recovery of starting material in the case of HCl. Therefore attention was turned to ester saponification using sodium hydroxide which, in the case of protected-intermediates **9a** and **9b**, provided perfluorinated-DOTA analog **10** in 67–100% yield (Scheme 3).

In conclusion, syntheses of novel NOTA and DOTA chelating groups bearing perfluorinated appendages have been developed. All compounds were characterized by NMR spectroscopy, elemental analysis, and/or high-resolution mass spectrometry, and gram quantities are available for evaluation in hybrid molecular imaging applications.

Acknowledgments

Financial support of this research from the Office of Biological and Environmental Research (BER) of the Office of Science (SC), at the US Department of Energy (DE-FG02-08ER64645), is gratefully acknowledged.

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8. Carboxylic acid **2** (98 mg, 0.14 mmol, 1.2 equiv) and HATU (55 mg, 0.14 mmol, 1.2 equiv) were dissolved in DMF (1.5 mL). To the homogenous yellow solution was added DIPEA (37 μL, 0.21 mmol, 1.8 equiv) and 3-(perfluorooctyl)propylamine (57 mg, 0.12 mmol). The reaction was stirred under nitrogen at rt for 24 h. After this time the reaction mixture was poured into a mixture of CH_2Cl_2/H_2O (50/50 mL). The organic layer was washed with brine, dried ($MgSO_4$) and concentrated. Perfluorous SPE (2 g-cartridge) gave compound **3** as a light yellow amorphous solid (143 mg, 88%). ^{1}H NMR ($CDCl_3$, 400 MHz) ∂ in ppm: 6.73 (1H, t, J = 8.0 Hz), 3.43–3.26 (7H, m), 2.80–1.81 (26H, m), 1.45 (36H, s); HRMS: M+Na$^+$ measured 1182.4810; calculated 1182.4794.
9. A mixture of cyclen **6** (364 mg, 2.11 mmol, 2.1 equiv), K_2CO_3 (708 mg, 5.12 mmol, 5.0 equiv) and CH_3CN (2 mL) was heated to 50 °C. To this was added a solution of perfluorous epoxide **7** (488 mg, 1.02 mmol) in CH_3CN, and the reaction was stirred overnight at 50 °C. After this time the solvent was evaporated under vacuum, and the resulting yellow residue was dissolved in DMF/H_2O (9/1 v/v, 2 mL) and purified by Fluorous-SPE (2 g, elution with acetone 50 mL) to give **8** as a white powder (318 mg, 48%). ^{1}H NMR ($CDCl_3$, 400 MHz) ∂ in ppm: 4.21–4.14 (m, 1H), 3.56–2.50 (m, overlay, 20H), 2.38–2.07 (m, 4H); ^{1}HRMS: M+H$^+$ measured 649.1816; calculated 649.1835.
10. Perfluorinated cyclen **8** (100 mg, 0.15 mmol), 2-bromoacetic acid (85 mg, 0.61 mmol, 4.1 equiv) and sodium hydroxide (57 mg, 1.42 mmol, 9.2 equiv) were dissolved in H_2O/THF (1/3 v/v, 8 mL) and stirred for 20 h at rt. The solution was diluted with aq. ammonium formate (20 mM, 50 mL) and passed through a fluorous SPE cartridge (2 g). The cartridge was washed twice with aq ammonium formate (20 mM, 12 mL). Compound **10** was then eluted off with acetone (40 mL) and methanol (40 mL). After concentration under vacuum, the residue was dissolved with water (70 mL), frozen and lyophilized to give **10** as a white fluffy powder (61 mg, 43%). ^{1}H NMR ($CDCl_3$, 400 MHz) ∂ in ppm: 4.60 (s, 8H), 3.80–3.30 (m, 1H), 3.06–2.60 (m, 10H), 2.65–1.90 (m, 12 H); HRMS: M+H$^+$ measured 823.1984; calculated 823.1994.