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Design and synthesis of dendrimeric *tris*(nonofluoro *tert*-butyl)-DOTA dual MRI agents using CuAAC reaction

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ABSTRACT

Cancer therapeutics are designed to interfere with one or more events in cell propagation or survival. However, the healthy cells may also need to thrive and avoid apoptosis, anticancer agents can be toxic to such cells. To minimize these toxicities, approaches have been developed wherein the therapeutic agent is targeted to tumor cells through conjugation to a tumor-cell-specific small-molecule ligand, thus reducing delivery to normal cells and the associated collateral toxicity. This article describes the novel tris(nonofluoro *tert*-butyl)-DOTA derived drug imaging agents in the design of ligand-targeted drugs and ligand–drug conjugates and ligand–imaging-agent conjugates.

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Introduction

Imaging biomarkers are very important and much promise for diagnosing the disease,[1] monitoring disease evolution, tracking therapeutic response, and enhancing our knowledge of physiology and pathophysiology. With the continued momentum towards specialized therapies and personalized medicine, there is an increasing need to monitor the physical state of an individual in a noninvasive manner with increased specificity.[2] The drug imaging has been put forth as one of the major players in such assessments, with an emphasis on molecular and functional imaging in addition to bodily imaging. However, Imaging technologies indeed the interaction of various forms of energy with tissues to non-invasively visualize the body. Many imaging techniques were originally developed for human use and the amount of imaging agent needed to get enough signal is crucial, but have recently been scaled-down to allow the high-resolution imaging of mice.[3]

Choice of ligand targeting allows selective delivery of therapeutic and imaging agents to cancer cells while avoiding collateral damage to healthy tissues.[4] Small-molecule–ligand conjugates more readily penetrate dense solid tumors than do high-molecular-weight conjugates. Subsequent the delivery of cytotoxic drug conjugates to their target cells, cleavage of the ligand from its therapeutic cargo and release from endosomes into the cytoplasm are necessary for optimal killing of the targeted cell. Usually, cytotoxic agents with nano-molar potencies are required in an effective ligand-targeted strategy, as receptor-mediated delivery may limit the maximum intracellular concentration of drug to 100 nM or less.[5] In current approach tris(nonofluoro tert-butyl)-DOTA as dual imaging agent is superior due to ^{19}F is the second most sensitive stable nucleus for MRI (83% of ^{1}H) with a natural abundance of 100%, and also, pharmacokinetics (PK) of ^{19}F-labeled drugs can be appropriately monitored by observing 19F magnetic resonance spectroscopy 19F MRS even at low concentration.[6-8]

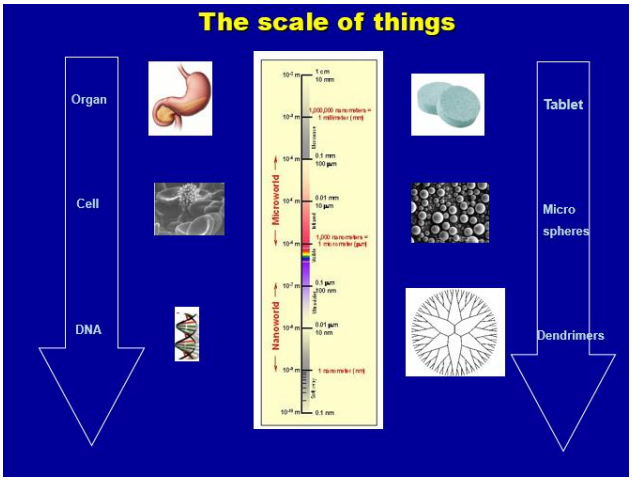
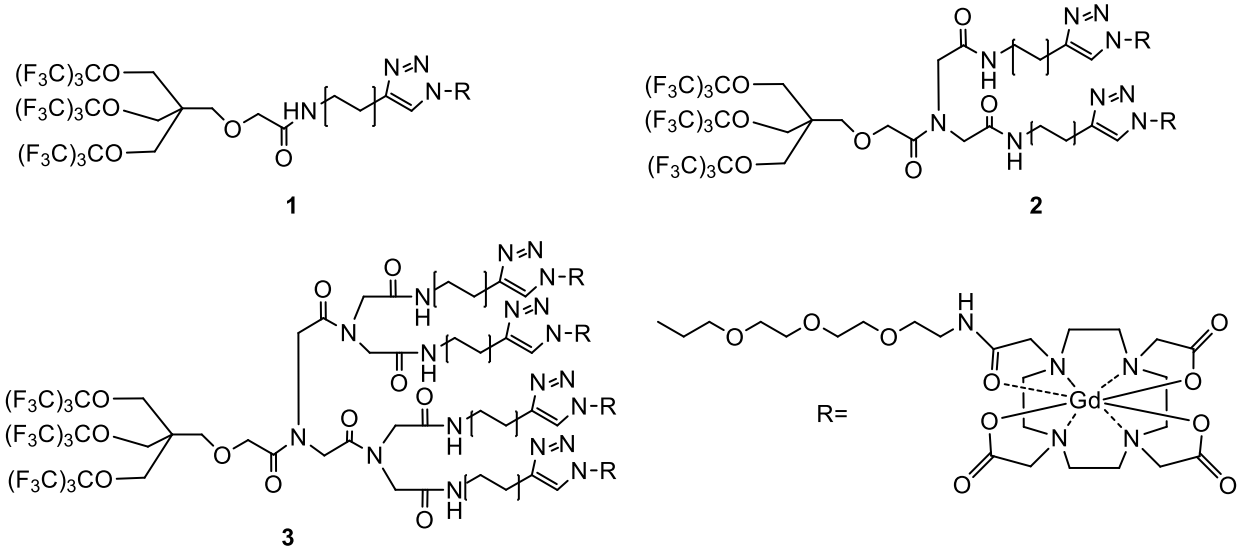


Figure 1.



Scheme 1.

This is highly relevant, because as genomics provides us with better models of disease, imaging readouts can be used to evaluate novel therapeutics. Imaging methods offer several advantages over other current practices in drug discovery, examples of which are discussed in this article. The use of imaging endpoints instead of time-consuming division and histology can significantly decrease the workload involved in tissue analysis and thereby speed up the evaluation of drug candidates. Imaging can provide biomarkers of a disease process and thus help to define stratified study groups. As imaging methods are non-invasive, they allow for longitudinal studies in a single animal. This increases the statistical relevance of a study, allows for more clinically relevant study designs and decreases the number of animals required. Imaging can also provide important information on the optimal timing and dosing of drugs. Emerging molecular-imaging tools can provide much earlier proxy markers of therapy success than is currently possible

Results and Discussion

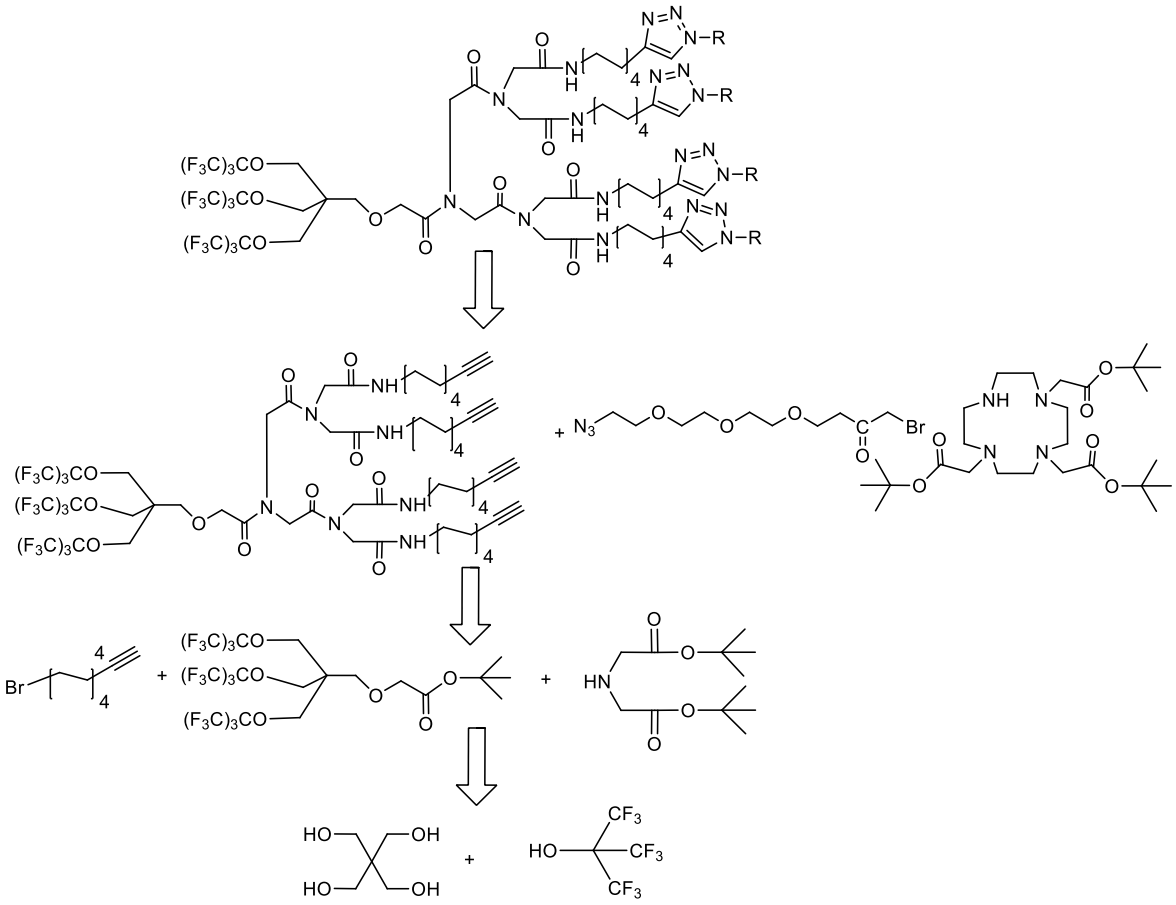
The retro synthesis analysis shows the target molecule **3** could be synthesized by conjugating with tris-(nono fluoro *tert*-butyl) and 10 bromo 1 decyne and DOTA tris-tri-*tert*-butyl acetate. The tris-(nono fluoro *tert*-butyl) compound could be achieved by coupling reaction of tris-(nono fluoro *tert*-butyl) *tert*-butyl acetate with di-*tert*-butyl iminodiacetate. The tris-(nono fluoro *tert*-butyl) which in term could be prepare by pentaerythritol and nonofluoro tert-butanol using mistunobu conditions **scheme 2**.

Our synthetic approach was started with readily available pentaerythritol **4**. The fluorinated compound **9** was prepared by selective mono benzyl protection of pentaerythritol and by the reaction of nonofluoro *tert*-butanol using mistunobu conditions followed deprotection of benzyl group. The introduction of an aliphatic chain was started with etherification of tris-(nono fluoro *tert*-butyl) ethanol with *tert*-butyl bromoacetate in dry acetone using potassium carbonate as base at 52 ^{0}C in 90% **10**. The formation of the product was confirmed by *tert* butyl signal at d 1.4 as singlet in ^{1}H NMR and molecular ion peak in spectrum. The *tert*-butyl group was deprotected in 1:1 TFA-DCM in quantitative yield **11**. In order to increase the branches to get the dendrimer structure, we chose the di-*tert*-butyl iminodiacetate as an initiator. The amide coupling reaction of **11** with di-*tert*-butyl iminodiacetate using 1,3-diisopropyliminocarbodiimide, HOBt as coupling reagent and *N*,*N*-diisopropylethyl amine as base in DMF obtained in 95% yield. Appearance of two *tert*-butyl groups at δ-1.4 and also molecular ion peak in mass spectrum was established the compound **12**. The similar steps were followed to get the compounds **13** and **15 Scheme 3**.

A separate synthesis of 10 amino 1 decyne was started with 1-bromo 1-decyne 16. 10-Bromo 1-decyne on treatment with sodium azide in dimethyl formamide to yield 10-azide 1-decyne **17** in in quantitative yield **Scheme 4**. The product was confirmed by the tertiary hydrogen signal at δ-1.8 as singlet in ^{1}H NMR spectrum. The azido compound further reaction with triphenyl phosphine in THF at room temperature to yield 10 amino 1 in 95%. The product was distinct by the molecular ion in mass spectrum and also by the ^{1}H NMR.

The coupling reaction of tris(nonofluoro tert-butyl)-carboxylic acid compounds **11**, **13**, **15** with 10 amino 1 decyne using 1,3-diisopropyliminocarbodiimide, HOBt as coupling reagent and *N*,*N*-diisopropylethyl amine as base in DMF gave **18-20** in 95% yield. The compound **24** was synthesized using procedure in literature[9-10] **Scheme 5**.

After being preparation of alkyne compounds **18-20** and azide compound **24,** CuAAC reaction[11] of alkyne with corresponding 1 equiv. of azide using $CuSO_4$ and sodium ascorbate in 9/1 THF/water at rt for 24 hrs. produced, after reversed phase column chromatography, a mixture of triazole-containing compounds that differed in the number of unreacted alkynes remaining in the molecule. The principal component of this mixture tris(nonofluoro tert-butyl)-Gd-DOTA, determined by MALDI-TOF MS, was the triazole compounds **1-3 Scheme 6**.



Scheme 2.

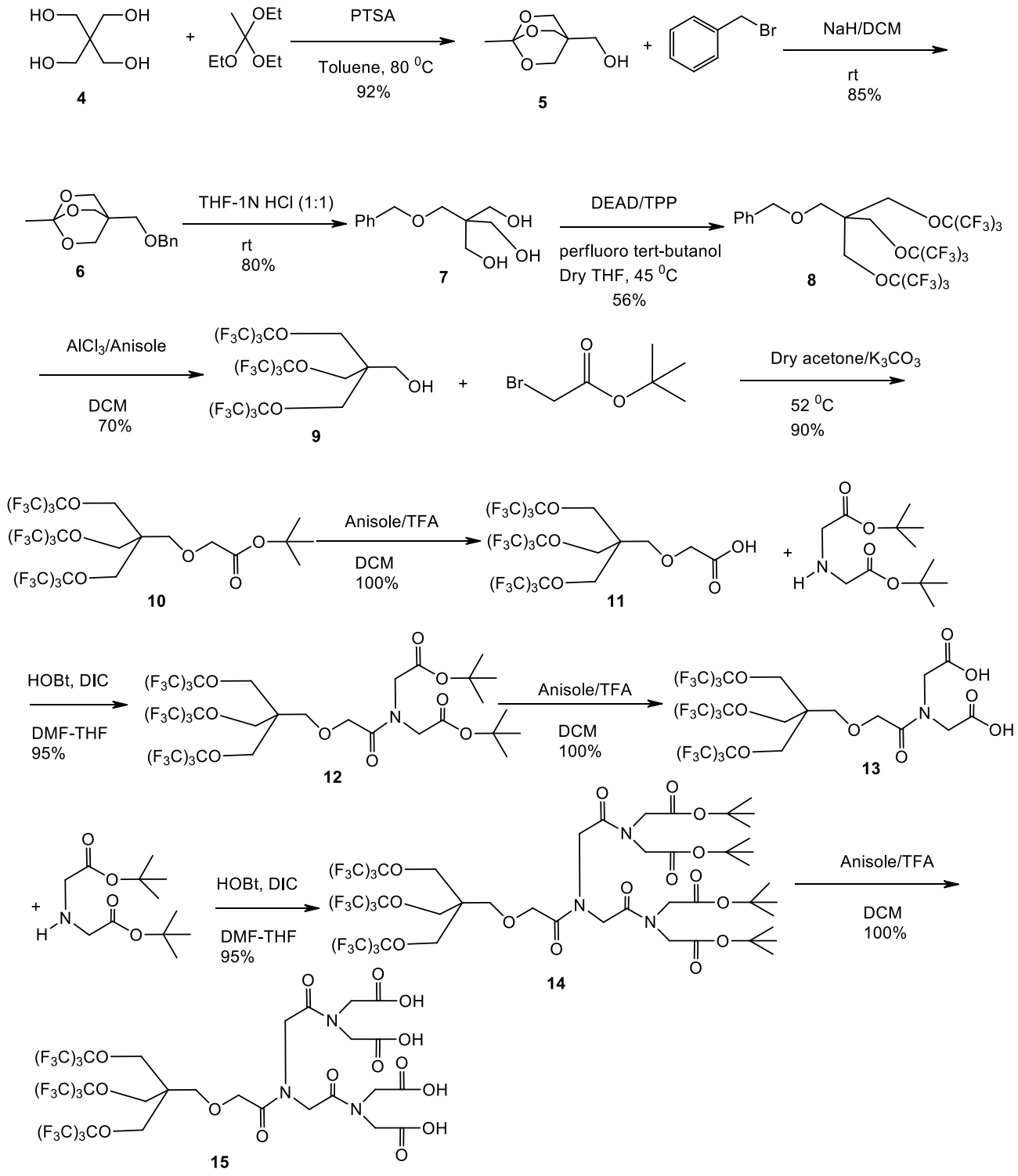
Experimental

General

Spectra were recorded with the following instruments: ^{1}H-NMR Varian INOVA 500 MHz and ^{13}C-NMR, Varian INOVA 125 MHz, ^{19}F NMR, JEOL ECX 400MHz and ESI mass spectra were recorded on TSQ LCMS and LCQ (70 eV) as Q + 1 mode. Column chromatography was performed over silica gel (Aldrich >300 mesh flash chromatography) and TLC with silica gel MERCK GF254 (pre-coated). The visualization of the spots in TLC plats was carried out either in UV light (short wave 250nm) or exposing the plates to iodine vapors or spraying with 10% sulfuric acid in methanol or developed by β-napthol or p-anisaldehyde charring solution and subsequently heating on hot plate; HPLC experiment was carried out on Agilent 1100 instrument using Zorbax XBD-C8-Reverse phase column 4.6 x 150 mm, 5um particle size and 95% methanolisocratic as eluent.

Protection of pentaerythritol with triethyl orthoacetate, preparation of 5: To a suspension of pentaerythritol (68 g, 0.5 mol) in toluene (50 mL) was added triethyl orthoacetate (81 g, 0.5 mol) and *p*-toluenesulphonic acid monohydrate (300 mg). The ethanol was distilled at 80 ^{0}C overnight. After all ethanol had been distilled away, the toluene was evaporated under reduced pressure. The crude product was purified by neutral alumina column chromatography using 1:1 hexane and ethyl acetate to give mono hydroxyl compound 5 as white solid (72.8 g, 91%).

Protection of mono hydroxyl compound as benzyl derivative; preparation of 6: To a solution of mono hydroxy compound **5** (73.6 g, 460 mmol) in dichloromethane (200 mL) was added sodium Hydride (60% in mineral oil (19.1 g, 0.5 mol) portion wise at 0 ^{0}C, and benzyl bromide (94.7 g 554. mmol) was added drop wise, the reaction was stirred at RT overnight. The reaction was monitored by TLC, quenched with ice cold water (20 mL). The organic layer was separated, washed with brine and dried over anhydrous sodium sulphate. The solvent was evaporated under reduced presser to give crude product, which was purified by neutral alumina column chromatography using 1:1 hexane and ethyl acetate to give mono hydroxyl compound 5 as white solid (72.0 g, 91%).


PTSA
Toluene, 80 °C
92%
NaH/DCM
rt
85%
THF-1N HCl (1:1)
rt
80%
DEAD/TPP
perfluoro tert-butanol
Dry THF, 45 °C
56%
AlCl3/Anisole
DCM
70%
Dry acetone/K2CO3
52 °C
90%
Anisole/TFA
DCM
100%
HOBt, DIC
DMF-THF
95%
Anisole/TFA
DCM
100%
HOBt, DIC
DMF-THF
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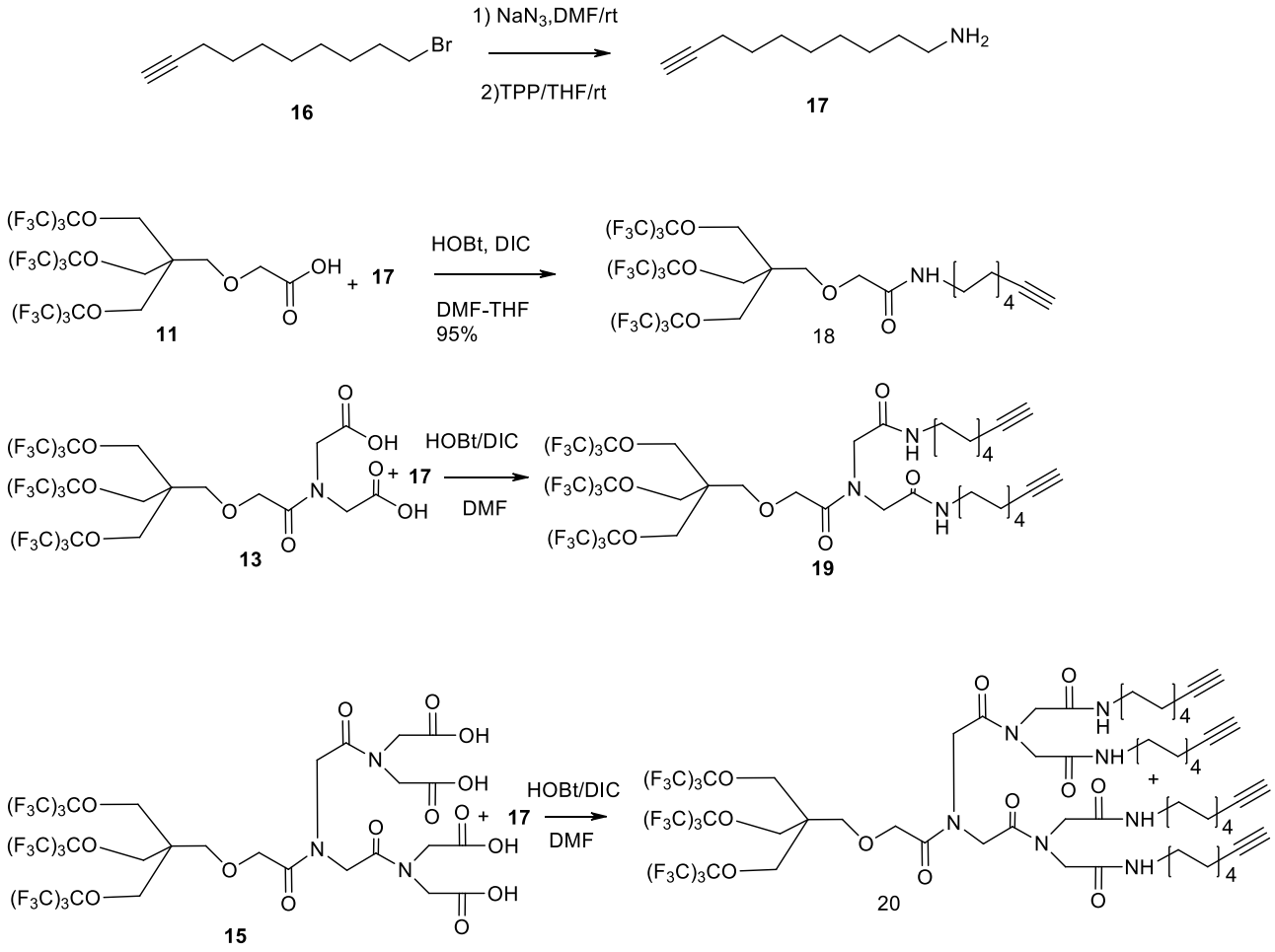
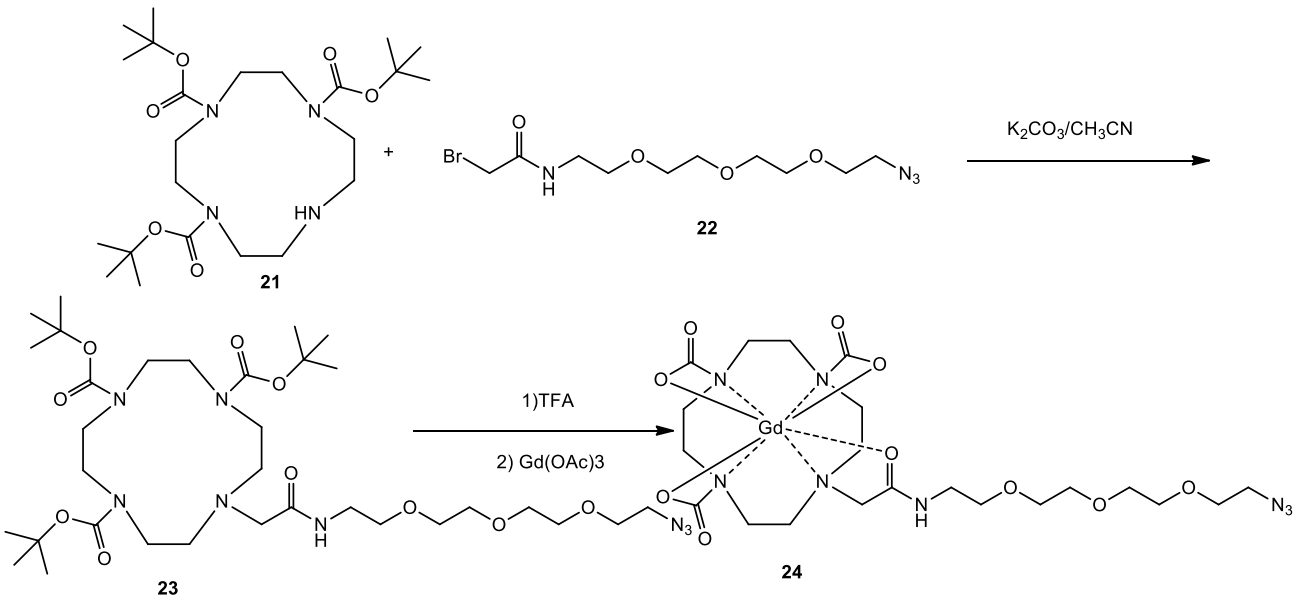
Scheme 3.



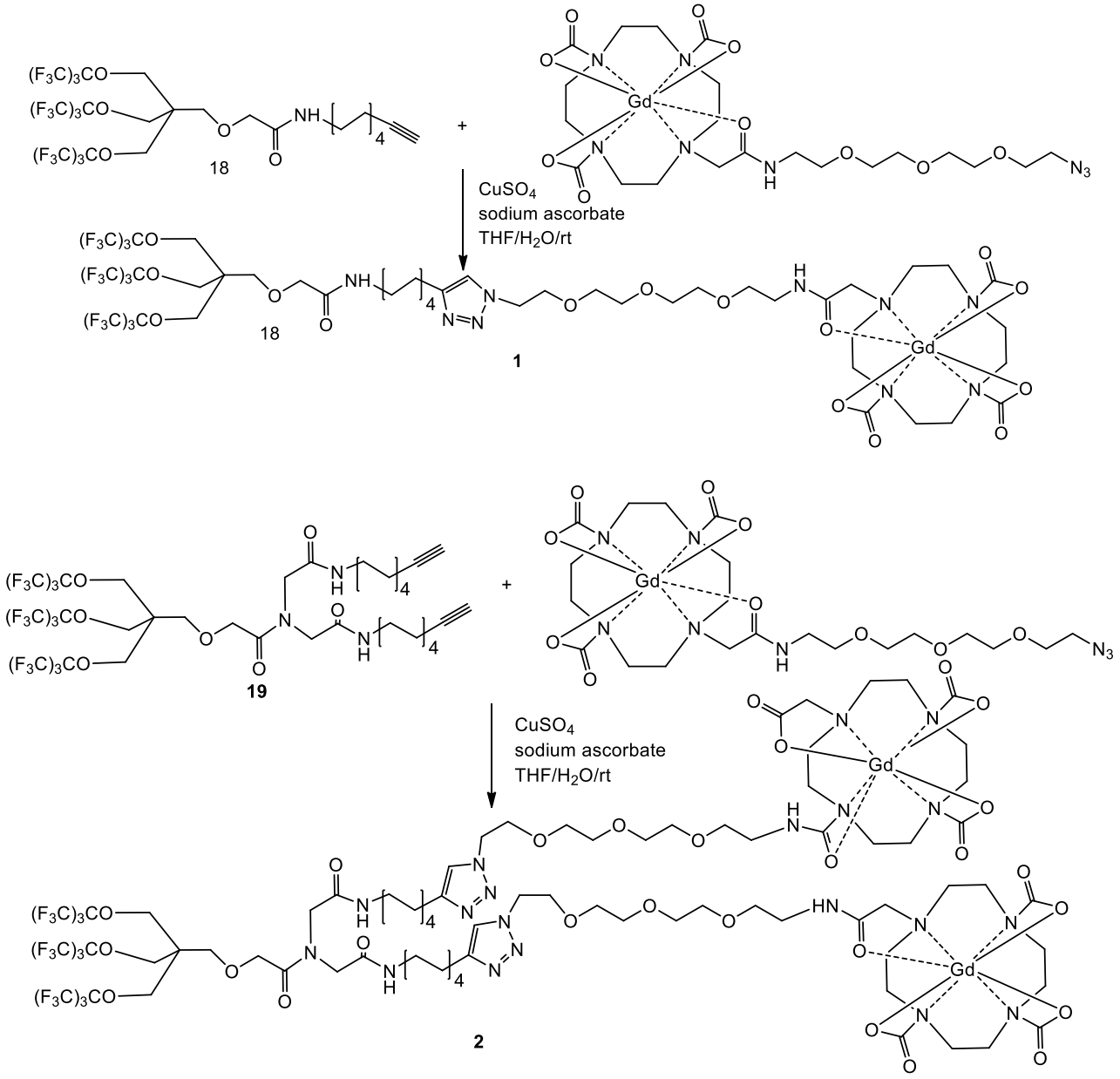
Scheme 4.

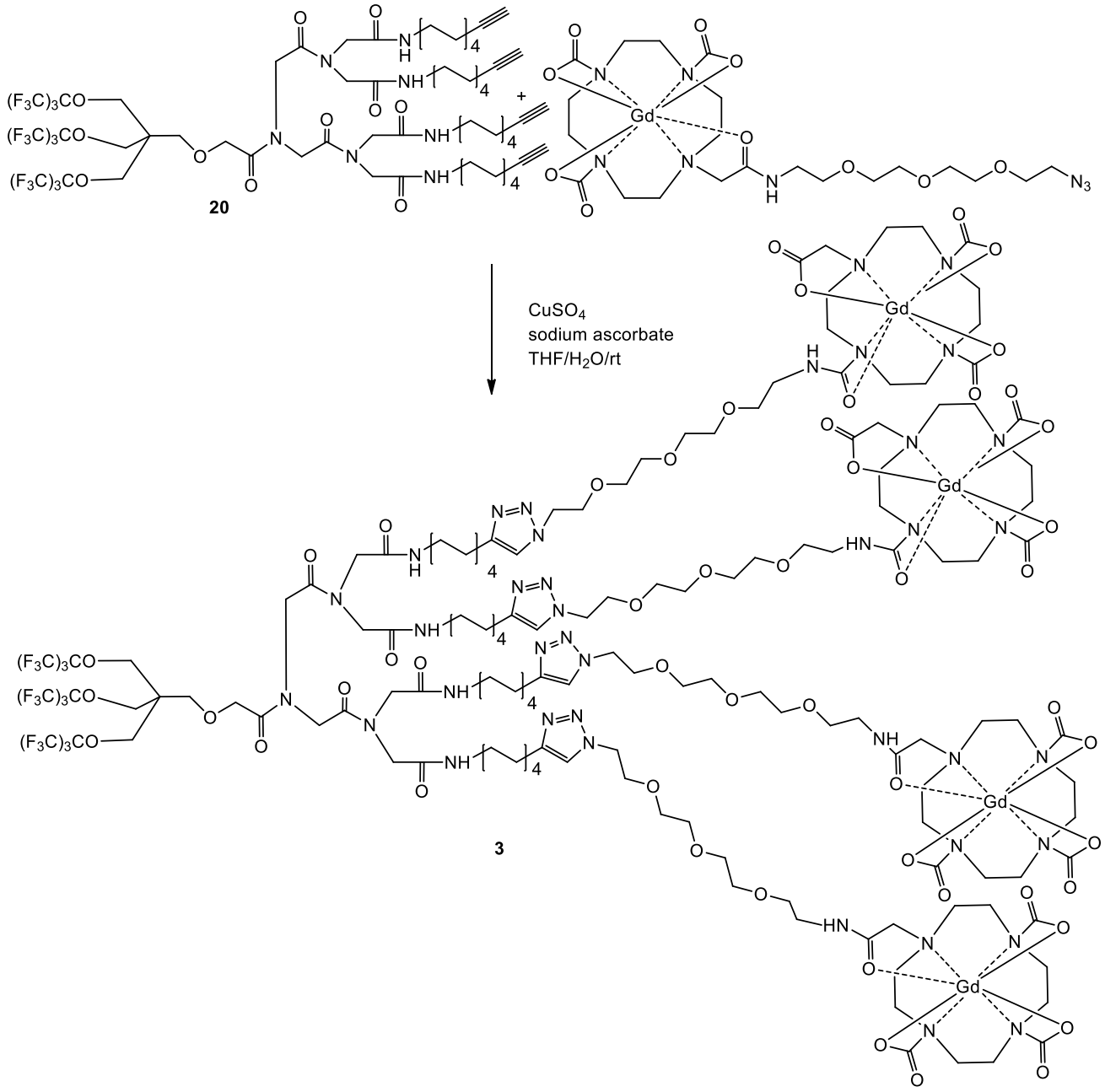


Scheme 5.

Deprotection 7: To the above compound was added 1:1 THF and 1N HCl, and stirred at rt overnight, the reaction was monitored on TLC. After completion of the reaction initially the organic lawyer (THF) was separated with separating funnel and aqueous lawyer was again extracted with dichloromethane. The THF was evaporated and mixed to the dichloromethane extract. The combined extract was washed with brine and dried over anhydrous sodium sulphate to give the crude product **7** as heavy liquid in 80% yield.

Preparation of tris(tri fluoro *tert*-butyl) compound 8 using mistunobu conditions: To a stirred suspension of **7** (9.04 g, 40 mmol), triphenyl phosphine (41.92 g, 160 mmol) and 4 A^0 molecular sieves (10 g) in dry THF (100 mL) at 0 ^{0}C was added dropwise diethylazodicarboxylate (72.8 mL, 40% w/w in toluene solution, 160 mmol). Afterward, the reaction mixture was allowed to warm to room temperature and was stirred for an additional 20 min. Then perfluoro-*tert*-butanol (33.14 mL, 240 mmol) was added in one portion and the resulting mixture was stirred for 48 h at 45 ^{0}C in a sealed vessel. Water (20 mL) was added to the reaction mixture and stirred for an additional 10 min. Then the mixture was transferred to a separating funnel and it was extracted with perfluorohexanes (FC72). The lower fluorous phase was collected. Removal of the solvent and excess





Scheme 6.

amount of perfluoro-*t*-buty1 alcohol under vacuum gave the product 7 as clear oil (9.84 g, 56%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35-7.25 (m, 5H), 4.47 (s, 2H), 4.08 (s, 6 H), 3.41 (s, 2H). The perfluorohexanes (FC72) was recovered below -30 ^{0}C and reused for the same.

Preparation of tris(tri fluoro *tert*-butyl) compound 9: To a stirred solution of 8 (9.84 g, 11.2 mmol) and anisole (4.9 mL, 44.8 mmol) in CH_2Cl_2 (100 mL) at 0 ^{0}C was added aluminum chloride (6.75 g, 50.04 mmol) slowly. The resulting mixture was stirred at 0 ^{0}C for 1 h and then water (100 mL) was added slowly. The mixture was extracted with FC72. The lower layer was collected and it was concentrated to give a clear oil of **9** (25.9 g, 99%). ^{1}H NMR (500 MHz, acetone-d_6) δ 4.27 (s, 6H), 3.74 (s, 2H).

Preparation of tris(tri fluoro *tert*-butyl) compound 10: To a solution of compound 9 (7.05 g, 11.18 mmol) in dry acetone was added potassium carbonate (2.42 g, 18 mmol) and to this *t*-butyl bromoacetate (1.30, 11.8 mmol) was added. The resulting mixture was stirred for overnight at 52 ^{0}C. The reaction was monitored on TLC, after completion of the reaction the solvent

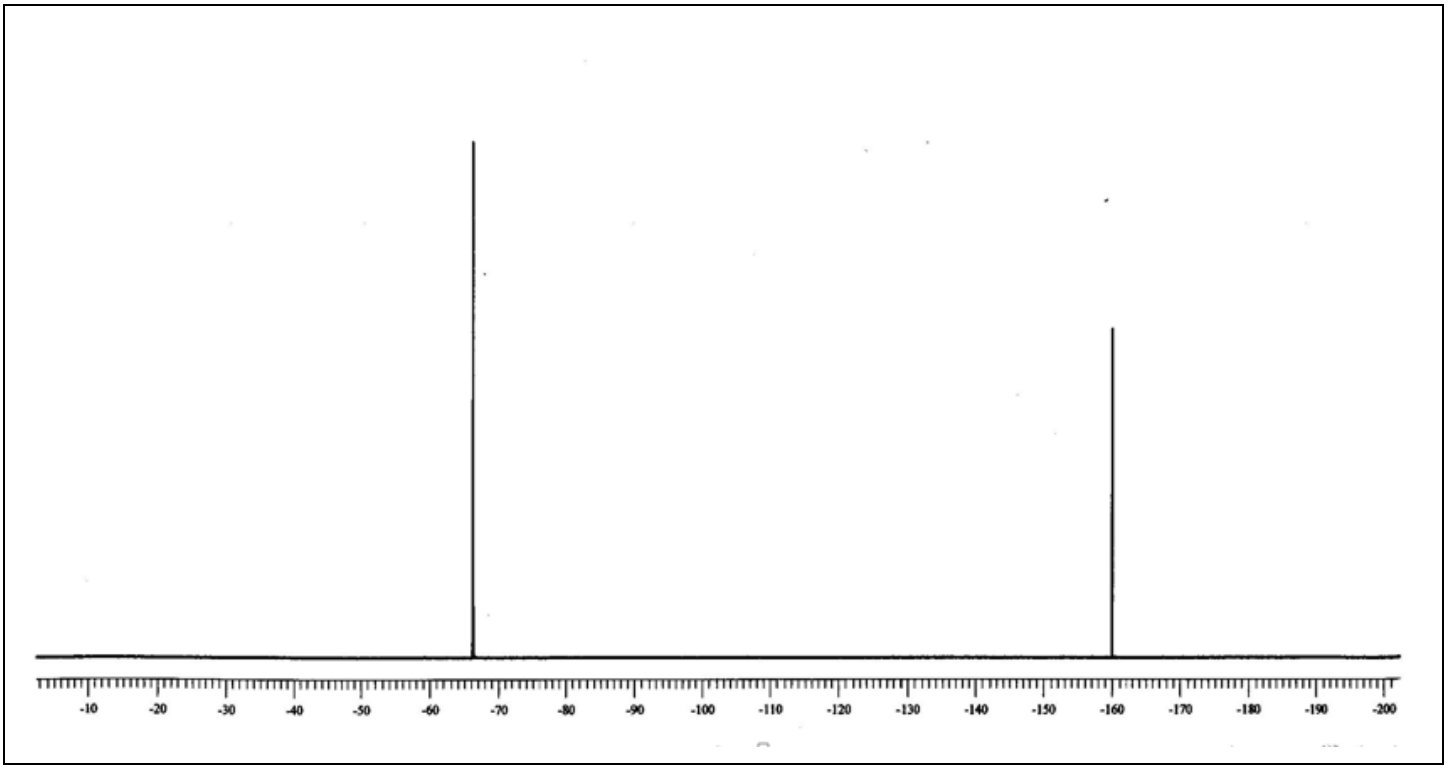


Figure 2. ^{19}F NMR of tris(nonofluoro *tert*-butyl) fluorinated hydrocarbon compound **2**

was removed under reduced pressure. After being quenched with 2 mL of water, the mixture was extracted with FC72. Removed of the solvent afforded the ester **10** (3.88 g, 72% yield) as a clear oil. ^1H NMR (500 MHz, $CDCl_3$) δ 4.14 (s, 2H), 3.91 (s, 2 H), 3.57 (s, 2H), 1.46 (s, 9H).

Preparation of tris(nonofluoro *tert*-butyl)-carboxylic acid compound 11: To a stirred solution of F–ester 10 (5.82 g, 6.45 mmol) and anisole (0.56 mL, 5.2 mmol) in methylene chloride (10 mL) was added trifluoroacetic acid (10 mL) at rt. After being stirred for 1 h, the mixture was evaporated to dryness under vacuum to give 5.50 g, as oil, Yield 100%. ^1H NMR (500 MHz, Acetone-d_6) δ 4.29 (s, 6H), 4.14 (s, 2H), 3.73 (s, 2H).

Preparation of compound 12: To a stirred solution of tris(nonofluoro tert-butyl)-carboxylic acid compound **11** (1 equiv.) in DMF-THF (1:0.1) was added DIPEA (1.5 equiv.), HOBt (1.5 equiv.) and di-*tert*-butyl iminodiacetate (1.5 equiv.). To this reaction mixture was added 1, 3-diisopropylcarbodiimide (1.5 equiv.). The reaction was stirred at room temperature overnight. The THF was removed under reduced pressure. The product was extracted with perfluorohexanes. The fluorous layer was evaporated under reduced pressure at below -30 ^0C to give the product in 90%. ^1H NMR (500 MHz, $CDCl_3$) δ 3.82 (s, 6H), 3.58 (s, 2H), 3.42 (s, 2H), 3.30 (s, 2H), 1.12 (s, 6H), 1.13 (s, 6H). The perfluorohexanes was recovered and reused.

Deprotection of tert-butyl ester; Preparation of compound tris(nonofluoro tert-butyl)-carboxylic acid compound 13: To a stirred solution of ester **12** (3.88 g, 4.3 mmol) and anisole (0.56 mL, 5.2 mmol) in methylene chloride (10 mL) was added trifluoroacetic acid (10 mL) at rt. After being stirred for 1 h, the mixture was evaporated to dryness under vacuum to give 3.67 g as oil, Yield 100%. ^1H NMR (500 MHz, $CDCl_3$) δ 4.29 (s, 6H), 4.14 (s, 2H), 3.73 (s, 2H).

Preparation of compound 14: To a stirred solution of tris(nonofluoro *tert*-butyl) -carboxylic acid compound 13 (1 equiv.) in DMF-THF (1:0.1) was added DIPEA (1.5 equiv.), HOBt (1.5 equiv.) and di-*tert*-butyl iminodiacetate (1.5 equiv.). To this reaction mixture was added 1,3-diisopropylcarbodiimide (1.5 equiv.). The reaction was stirred at room temperature overnight. The THF was removed under reduced pressure. The product was extracted with perfluorohexanes. The fluorous layer was evaporated under reduced pressure at below -30 ^0C to give the product in 90%. The perfluorohexanes was recovered and reused.

Preparation of compound tris(nonofluoro tert-butyl)-carboxylic acid compound 15: To a stirred solution of ester compound **14** (3.88 g, 4.3 mmol) and anisole (0.56 mL, 5.2 mmol) in methylene chloride (10 mL) was added trifluoroacetic acid (10 mL) at rt. After being stirred for 1 h, the mixture was evaporated to dryness under vacuum to give 3.67 g, as oil, yield 100%.

10 Azido 1 decyne 16: To a stirred solution of 10-bromo 1-decyne in DMF (21.6 g 10 mmol) added slowly NaN_3 (9.75 g 15 mmol). The reaction was stirred at room temperature for 3 hrs. The reaction was monitored on TLC, after completion of the reaction. To the reaction mixture was quenched with ice cold water (50 mL) and the organic lawyer was separated. The aqueous lawyer was extracted with dichloro methane (2 x 50 mL), the combined organic lawyer was washed with brine and dried on anhydrous sodium sulphate and concentrated under reduced pressure to give crude product and purified on silica gel column chromatography using 1:1 hexane and ethyl acetate to give title compound 16 as a clear oil (16.9 g, 90%).

10 Amino 1 decyne 17: To a stirred solution of 10 azido 1 decyne in THF (17.9 g 10 mmol) added slowly triphenyl phosphine (26.2 g 10 mmol) at 0 ºC. The reaction was stirred at room temperature for 3 hrs. The reaction was monitored on TLC, after completion of the reaction. To the reaction mixture was quenched with ice cold water (50 mL) and the organic lawyer was separated. The aqueous lawyer was extracted with dichloro methane (2 x 50 mL), the combined organic lawyer was washed with 2N HCl (10 mL) brine and dried on anhydrous sodium sulphate and concentrated under reduced pressure to give crude product and purified on silica gel column chromatography using 1:1 hexane and ethyl acetate to give title compound **17** as a clear oil (13.7 g, 90%).

Preparation of compound 18: To a stirred solution of **11** (1 equiv.) in DMF-THF (1:0.1) was added DIPEA (1.5 equiv.), HOBt (1.5 equiv.) and 10 Amino 1 Decyne 17 (1.5 equiv.). To this reaction mixture was added 1, 3-diisopropylcarbodiimide (1.5 equiv.). The reaction was stirred at room temperature overnight. The THF was removed under reduced pressure. The reaction mixture was quenched with ice cold water (50 mL) and the organic lawyer was separated. The aqueous lawyer was extracted with dichloro methane (2 x 50 mL), the combined organic lawyer was washed with 1N HCl (10 mL) brine and dried on anhydrous sodium sulphate and concentrated under reduced pressure to give crude product and purified on silica gel column chromatography using 1:1 hexane and ethyl acetate to give title compound 18 as a clear oil. In similar procedure the reaction of **13** and **15** with 10 amino 1 decyne **17**, products **19**, and **20** were prepared.

CuAAC reaction of alkyne **18** with 1 equiv. of azide **24** using $CuSO_4$ and sodium ascorbate in 9/1 THF/water at rt for 24 hrs produced, after reversed phase column chromatography, a mixture of triazole-containing compounds that differed in the number of unreacted alkynes remaining in the molecule. The principal component of this mixture, determined by MALDI-TOF MS, was the triazole compound **1**. Similar procedure the reaction of alkyne compounds **19** and **20** with azide compound 24, products **2**, and **3** were prepared respectively.

Conclusion

Highly symmetrical fluorinated hydrocarbons, conjugated with DOTA, a synthesis of dendrimeric tris(nonofluoro tert-butyl)-DOTA dual MRI agents using CuAAC reaction is described.

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