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Design and synthesis of Capecitabine-*tris*(nonofluoro *tert*-butyl) a highly symmetrical fluorinated hydrocarbons as multifunctional image-guided drug delivery vehicles using CuAAC reaction

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ABSTRACT

In the light of extensive versatility of application of controlled release and formulations, in the field of medical and pharmaceutical sciences, they are unavoidable tools for the exploitation of the modern concept of therapeutic treatment whose aim is to increase drug effectiveness and patient compliance, to reduce the administration frequency and side effects connected to dosing and toxicity of healthy cells. As a matter of fact, controlled release formulations bring engineers and pharmacists to work together with the common aim of realizing more and more effective therapeutic products. The healthy cells may also need to succeed 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. Herein we describes the novel Capecitabine-*tris*(nonofluoro *tert*-butyl) derived a highly symmetrical fluorinated hydrocarbons drug delivery system in the design of ligand-targeted drugs and ligand–drug conjugates and ligand–imaging-agent conjugates are described.

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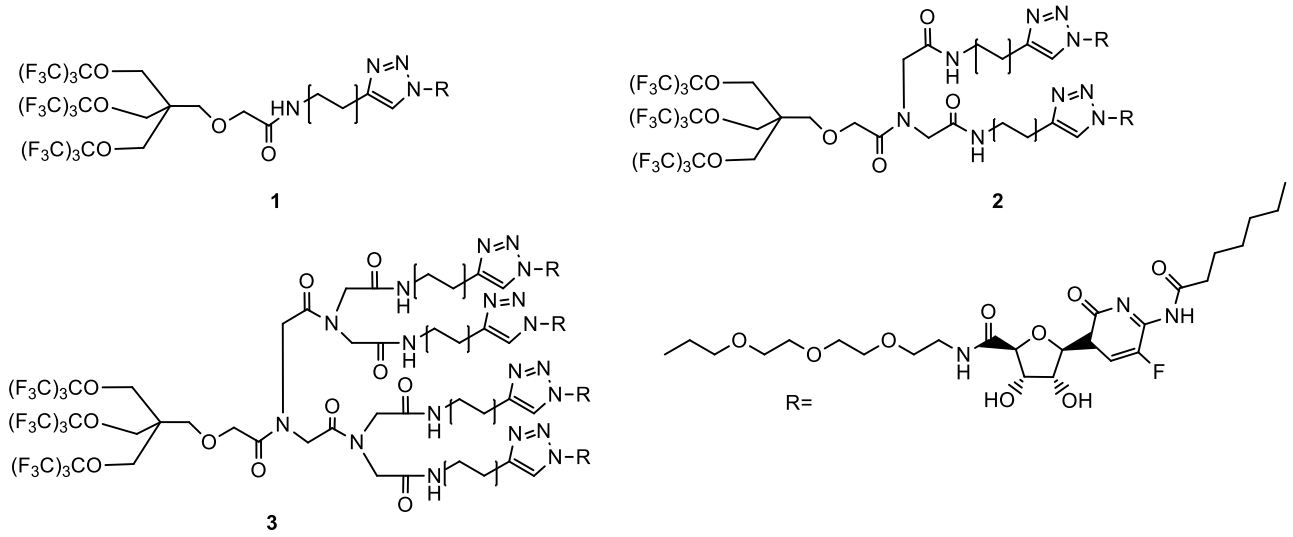
Introduction

Drug delivery systems can in principle provide enhanced efficacy and/or reduced toxicity for anticancer agents.[1] Long circulating macromolecular carriers such as liposomes can exploit the 'enhanced permeability and retention' effect for preferential extravasation from tumor vessels. Liposomal anthracyclines have achieved highly efficient drug encapsulation, resulting in significant anticancer activity with reduced cardiotoxicity, and include versions with greatly prolonged circulation such as liposomal daunorubicin and pegylated liposomal doxorubicin.[2] Pegylated liposomal doxorubicin has shown substantial efficacy in breast cancer treatment both as monotherapy and in combination with other chemotherapeutics.[3] Additional liposome constructs are being developed for the delivery of other drugs. The next generation of delivery systems will include true molecular targeting; immune-liposomes and other ligand-directed constructs represent an integration of biological components capable of tumor recognition with delivery technologies. 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] Pharmacokinetics (PK) of ^{19}F-labeled drugs can be appropriately monitored by observing ^{19}F magnetic resonance spectroscopy ^{19}F MRS even at low concentration.[6-8]

5-Fluorouracil (5FU) is an antimetabolite with activity against numerous types of neoplasms, including those of the breast, esophagus, larynx, and gastrointestinal and genitourinary tracts. However, it has a known toxicity like neutropenia, stomatitis, and diarrhea, often occur due to cytotoxic non selectivity. Capecitabine was developed as a prodrug of 5FU, with the goal of improving tolerability and intra-tumor drug concentrations through tumor-specific conversion to the active



Scheme 1.

drug. Capecitabine is an approved anti-cancer drug by the FDA for use as first-line therapy in patients with metastatic colorectal cancer when single-agent fluoropyrimidine therapy is preferred.[9-10]

Drug Discovery v/s Drug Delivery

Drug discovery is the process by which new candidate medications are discovered, in medicine, biotechnology and pharmacology. Historically, drugs were discovered by identifying the active ingredient from traditional remedies or by unexpected. There have been developed several new chemical entities for use as pharmaceutical therapies, only 8% of candidate's are eventually approved and enter the market place after surviving a process of drug development that takes an average of 13.5 years. Despite the promise of genetic technologies, proteomics, and high throughput screening, there has not been any increase in the rate of gaining marketing approval of new drugs. Therefore, there are growing concerns about the future viability of the current model of drug development. The therapeutics industry is searching for new biological targets and new approaches for generating novel pharmacotherapy. The use of imaging endpoints as an alternative 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.[11]

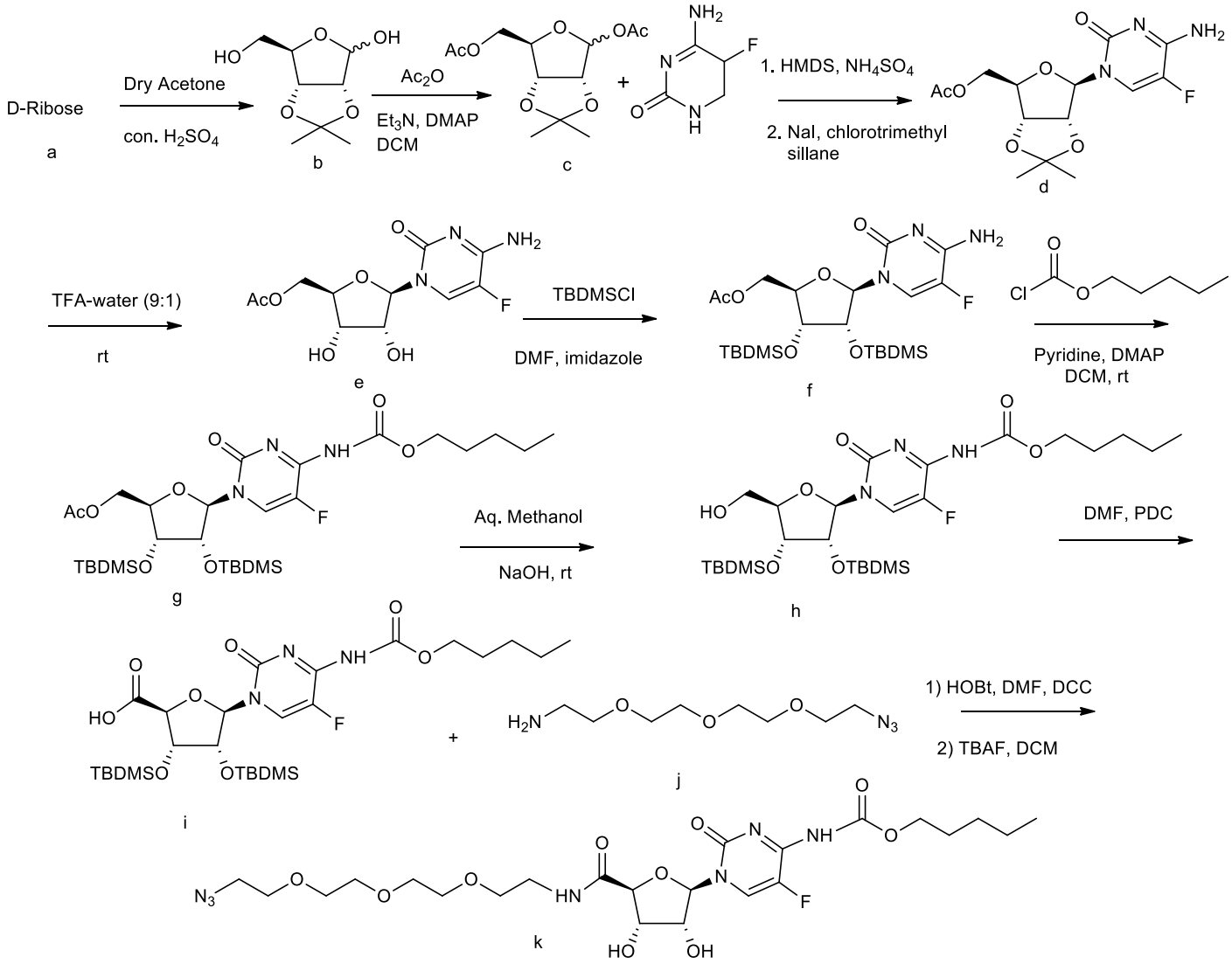
Drug delivery is the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals. Drug delivery is becoming a whole interdisciplinary and independent field of research and is gaining the much attention of pharmaceutical makers, medical doctors and pharmaceutical industry. A targeted and safe drug delivery could improve the performance of some classical medicines already on the market, furthermore, will have implications for the development and success of new therapeutic strategies, such as peptide and protein delivery, gene therapy and RNA interference. Recently many innovative technologies for effective drug delivery have been developed, including implants, nanotechnology, cell and peptide encapsulation, microfabrication, chemical modification and others. On the long way from the clinic to market, however, several issues will have to be addressed, including suitable scientific development, specific financial support as a result of altered scientific policy, government regulations and markets.[1,2] In current approach tris(nonofluoro *tert*-butyl)-capecitabine highly fluorinated hydrocarbon compound and conjugated to capecitabine anticancer drug.

Results and Discussion

Selection of tris(nonafluoro *tert*-butyl) group: We chose the tris(perfluoro *tert*-butyl) moiety as fluorocarbon tag, which is highly spherically symmetric and give the all 27 fluorine atoms as sharp singlet. To the best our knowledge tris(nonafluoro *tert*-butyl) fluorocarbon tag is the only gives the all 27 fluorine atoms as high instance sharp singlet, in which hydroxyl group as a functionality and could be identified in small quantity of the compound.[6-8]

Selection of tetraethylene glycol: The drug delivery systems conjugating with inactive polymers, such as polyethylene glycol (PEG), drastically increase their ability to avoid recognition by the immune system. However, tetraethylene glycol found to be smallest molecule which having the sufficient hydrophilicity and biodegradability. And also one of the major idea of keeping the tetraethylene glycol, it releases the drug easily in the animal body.[12]

The active ingredient in capecitabine is 5-fluorouracil, hence we decided to conjugate the drug delivery system to lactone part of the drug, hoping the activity of the capecitabine intact. Our synthetic approach was started with readily available D-ribose **a**,



Scheme 2.

reaction of D-ribose in dry acetone, catalytic amount of concentrated sulfuric acid was added slowly at 0 ^{0}C. The reaction was stirred at room temperature for 2.5 h. Solid sodium bicarbonate was added slowly at 0 ^{o}C to neutralize the sulfuric acid and filtered through sintered funnel and evaporated. The residue was extracted into dichloromethane and dried over anhydrous sodium sulfate and evaporated under reduced pressure to give the crude product and purified by silica gel flash chromatography using 20% ethyl acetate in hexane obtained the acetonide product in 90% **b**. The formation of the product was confirmed by di methyl signal at d 1.4 as singlet in ^{1}H NMR and molecular ion peak in spectrum.

The acetylation of ribose acetonide **b,** on reaction with acetic anhydride and triethyl amine in dichloromethane using DMAP as catalyst gave the diacetyl ribose acetonide in 95% yield. The formation of product was acetyl protons at δ-2.2 and also molecular ion peak in mass spectrum was established the compound **c**, it was further reacted with 5-fluoro uracil in presence of HMDS and sodium sulphate and subsequently sodium iodide and chloromethylsillane yielded in 80% yield **d**.

Removal of acetonide protection group by the reaction of **d** with TFA-water (9:1) gave the **e** then re-protected hydroxyl groups with TBDMSCl **f** followed by reaction with pentyle chloroformate in dichloromethane using catalytic amount of DMAP gave the **g** in 85% yield. The formation of product was confirmed, presence of molecular ion peak in mass spectrum and ^{1}H NMR spectroscopy. De-protection of acetyl group using methanol and NaOH at room temperature **h** and followed by oxidation of primary alcohol by pyridinium dichromate and in DMF gave the compound **i** in 80% yield, which was coupled with **j**[14] using HOBt as coupling reagent and *N,N*-diisopropylethyl amine as base in DMF gave **k** in 90% yield **Scheme 2**. A separate synthesis of tris(nonofluoro *tert*-butyl) alkyne compound **l, m, n** were synthesized using procedure in literature[13]

After being preparation of alkyne compounds and azide compound**,** CuAAC reaction[16] of alkyne **l** with 1 equiv. of azide **k** using $CuSO_4$ and sodium ascorbate in 9/1 THF/water at rt for

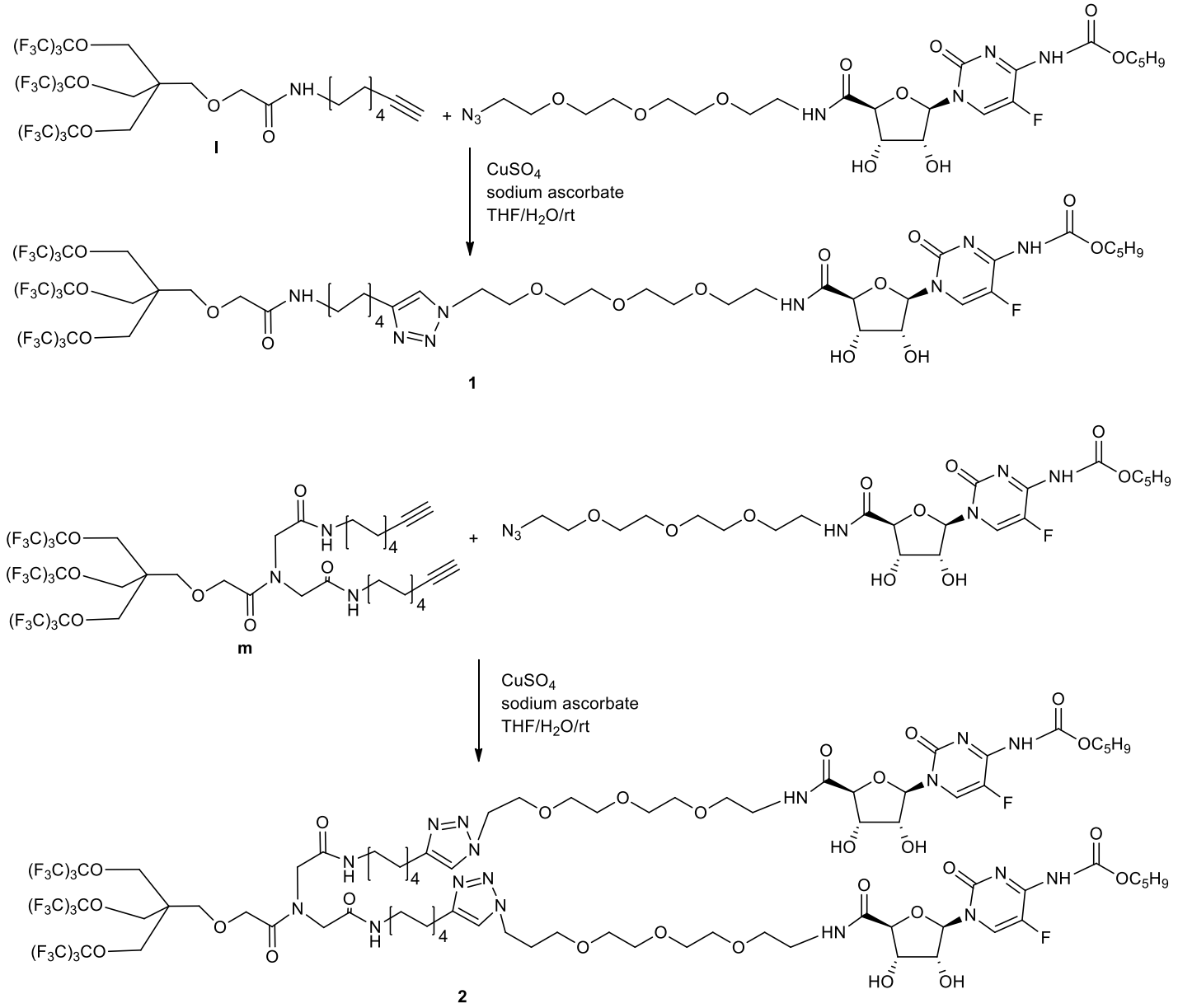
24 hrs produced, after reversed phase column chromatography, triazole-containing compound. The target component of this tris(nonofluoro *tert*-butyl)-capecitabine, determined by ^{1}H NMR and molecular ion peak in spectrum, was the triazole compounds **1.** Similarly compounds **2**, **3** were synthesized using corresponding stoichiometric equivalent of azide compound **Scheme 3**.

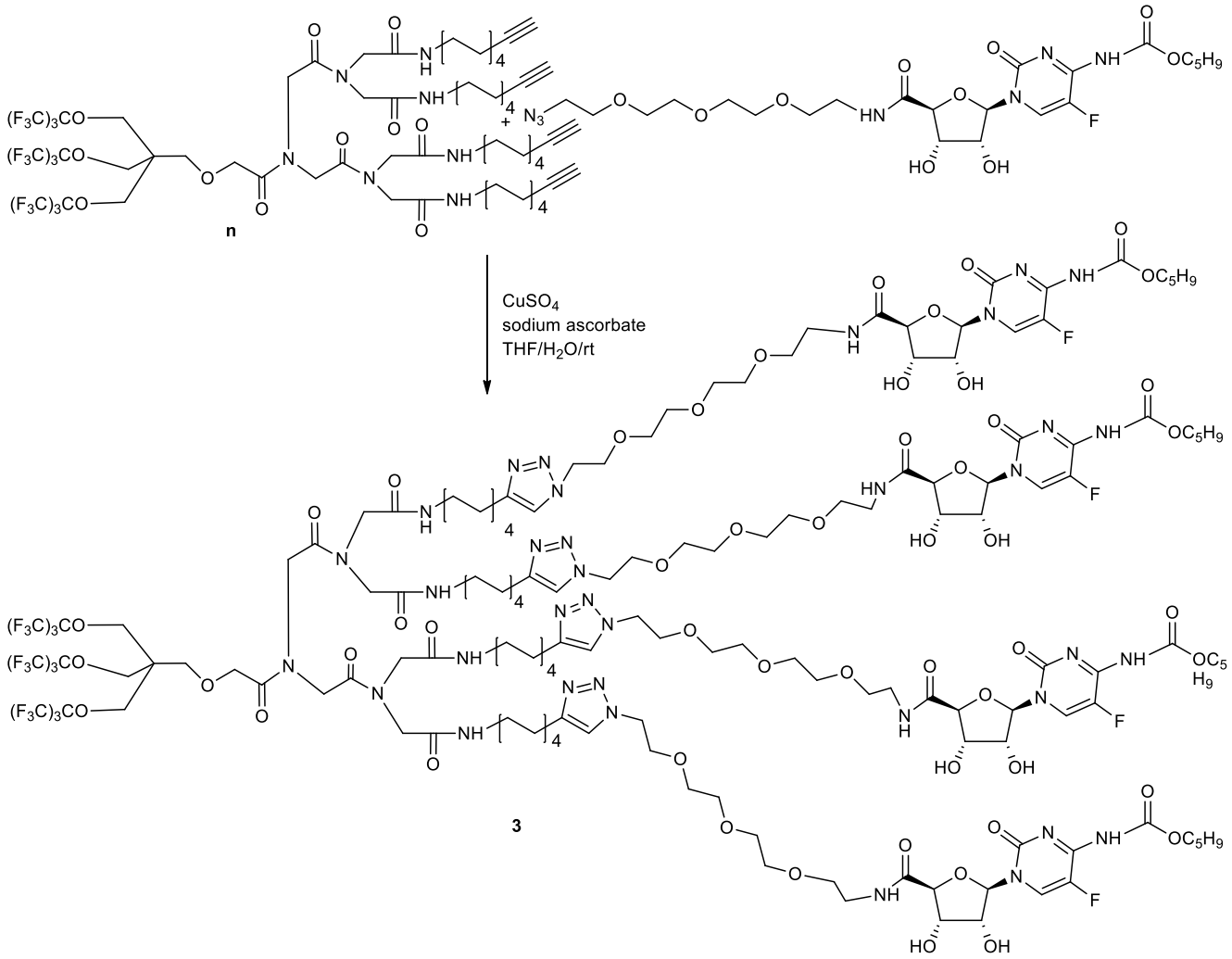
Experimental

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 colum 4.6 x 150 mm, 5um particle size and 95% methanolisocratic as eluent.

Acetonide Protection of Ribose **b:** To a stirred solution of D-ribose **a** (1.5 g, 10 mmol) in dry acetone (50 mL), catalytic amount of concentrated sulfuric acid was added slowly at 0 ^{0}C. The reaction was stirred at room temperature for 2.5 h. Solid sodium





Scheme 3.

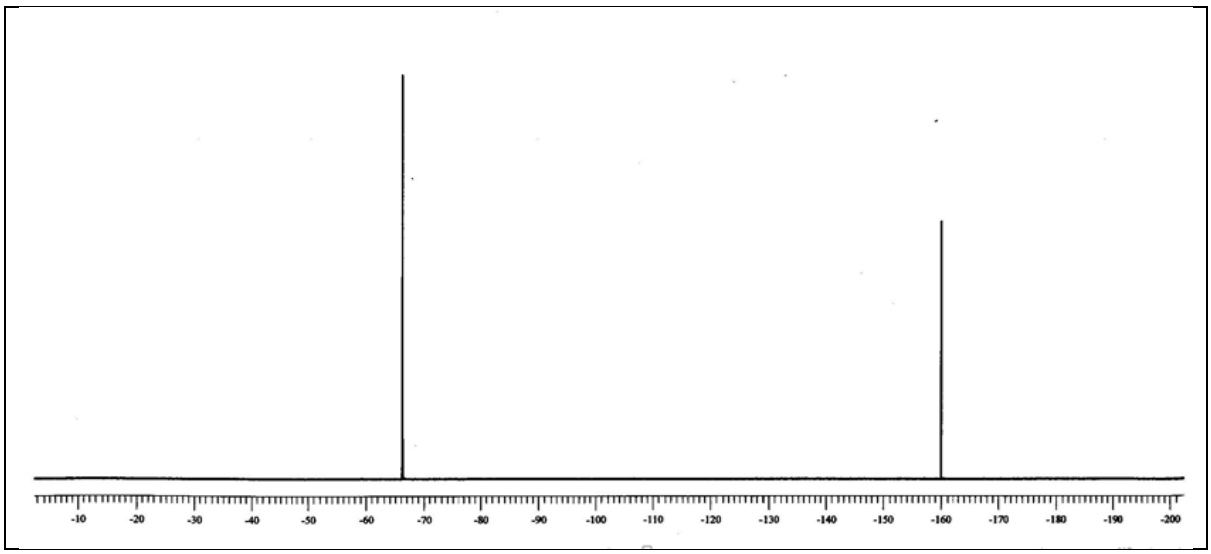


Figure 1. ^{19}F NMR of *tris*(nonofluoro *tert*-butyl) fluorinated probe compound **2**

bicarbonate was added to neutralize the sulfuric acid and filtered through sintered funnel and evaporated. The residue was extracted into dichloromethane and dried over anhydrous sodium sulfate and evaporated under reduced pressure to give the crude product and purified by silica gel flash chromatography using 20% ethyl acetate in hexane obtained title compound yield 17.1 g, 90%. EIMS (m/z): 191 (M^+ H).

Acetylation of acetonide ribose **c:** To a solution of Ribose acetonide **b** (1.7 g, 9 mmol) in dry dichloromethane (50 mL) was added triethyl amine (1.0 g, 10 mmol) and catalytic amount of dimethyl amino **pyridine**, then acetic anhydride (2.0 g, 20 mmol) was added at 0 ^{0}C. The reaction was stirred with a magnetic stir bar at room temperature. The reaction was monitored by TLC, after completion of the reaction, the 1N HCl was added at 0 ^{0}C, the organic lawyer was separated and washed with brine. The organic layer was dried over anhydrous sodium sulfate and evaporated the organic solvent under reduced pressure to give crude product which was purified by silica gel flash chromatography obtained title compound yield 2.16 g, 90%. EIMS (m/z): 275 (M^+H)

Sillylated 5- fluorouracil: A mixture of 5-fluorocytosine (1.2 g, 10 mmol), HMDS (hexamethyldisillylazine) (2.4 g, 15 mmol) and added catalytic amount of ammonium sulfate heated at 100 ^{0}C for 3-5h, and then the excess HMDS was evaporated under reduced pressure to give sillylated 5-fluorocytosine.

Reaction of diacetylation of acetonide Ribose with 5-fluorocytosine **d:** NaI (1.4 g, 10 mmol) was taken in round bottomed flask along with magnetic stir bar and closed with rubber septa. 50 ML of dry acetonitrile was injected and stirred for 5 min to dissolve NaI completely, then the chlorotrimethylsilane (1.08 g, 10 mmol) was injected at 0 ^{0}C, then after 5 min we can find the NaCl deposition. A solution of ribose di acetyl acetonide **c** (2.16 g, 8 mmol) in acetonitrile (10 mL) was injected and stirred for 30 min. Then a solution of freshly prepared sillylated 5-fluorocytosine in acetonitryl (10 mL) was injected and the reaction was stirred with a magnetic stir bar at room temperature overnight. The organic solvent was evaporated and the residue was dissolved in minimum quantity of methanol and an aqueous solution of hypo was added and the content was extracted thrice with 10% methanol in dichloromethane. The organic layer was washed with brine and dried over anhydrous sodium sulphate and concentrated to give the crude product and purified by silica gel flash chromatography using 5% methanol in dichloromethane, obtained title compound yield 2.1 g, 80%. EIMS (m/z): 345 (M^+H).

Deprotection of secondary acetonide **e:** To a solution of acetonide protected acetyl 5-fluorocytodine **d** (2.0 g, 6 mmol) in TFA-water (10 mL) (9:1) stirred for 1h, the TFA was evaporated under reduced pressure. The crude product was extracted with dichloromethane, dried over anhydrous sodium sulfate evaporated under reduced pressure to give crude product and purified by column chromatography using 5% methanol in DCM to give pure diol compound, yield 1.4 g, 80%. EIMS (m/z): 304 (M^+H).

TBDMS protection **f:** To a solution of diol compound **e** (1.2 g, 4 mmol) in dry dimethyformamide (10 mL) was added imidazole (1.0 g, 16 mmol) 4 equiv., and stirred for 15 min then TBDMS chloride (1.5 g, 10 mmol) was added and stirring was continued for overnight. The DMF was removed under reduced pressure and the product was extracted into ethyl acetate to get crude product and purified by column chromatography using 5% methanol in DCM, to give pure compound, yield 1.9 g, 90%. EIMS (m/z): 532 (M^+H).

Reaction of acetyl diTBDMS cytodine with n-pentanoyl chloroformate **g:** To a solution of mono acetyl **di-TBDMS** cytodine **f** (1.50 g, 3 mmol) in dry dichloromethane (15 mL) was added pyridine at -20 ^{0}C, then the n-pentanoyl chloroforamte (0.75 g, 5 mmol) was slowly added. The reaction was stirred with a magnetic stir bar for one h. The completion of reaction was checked by the TLC, the water was added at 0 ^{0}C and the organic layer was separated. The organic layer was washed with brine and dried over anhydrous sodium sulfate and evaporated under reduced pressure to give crude product and purified with silica gel flash to obtain title compound yield 1.74 g, 90%. EIMS (m/z): 646 (M^+H)

Acetyl group Deprotection **h:** To a cooled solution of above compound **g** (1.20 g, 2 mmol) in methanol (10 mL), 1.5 equiv. of NaOH (0.12 g, 3 mmol) in equal volume of water was added slowly and stirred at -20 ^{0}C, the reaction was monitored by TLC. Then the contents were extracted with dichloromethane and washed with brine and evaporated under reduced pressure to get crude product and purified by silica gel column chromatography using 5% methanol in DCM, to obtain title compound yield 1.08 g, 90%. EIMS (m/z): 604 (M^+H).

PDC oxidation of primary hydroxyl group in above compound **i:** To a solution of above compound **h** (0.90 g, 1.5 mmol) in dry DMF (minimum quantity that is 2 mL per one gram of PDC) and powdered MS 4 ^{0}A, and PDC (2.2 g, 9 mmol) (6 equiv.) was added. The reaction was stirred with a magnetic stir bar for overnight. Then water (6 times volume to the DMF) was added and extracted into diethyl ether 3-4 times and washed with brine evaporated to give crude product and purified with column chromatography using 15% methanol in DCM to obtain title compound yield 0. 60 g, 66%. EIMS (m/z): 618 (M^+H).

Preparation of compound **1**: CuAAC reaction of alkyne **l**[14] (0.89 g, 1 mmol) (1 equiv.) with 1 equiv. of azide **k**[14-16] (0.58 g, 1mmol) using $CuSO_4$ (0. 15 g, 1 mmol) and sodium ascorbate (0.19 g, 1mmol) in 9/1 THF/water at room temperature for 24 hrs produced, after reversed phase column chromatography, a mixture of triazole-containing compound. The principal component of this mixture, determined by MALDI-TOF MS, was the triazole compound **1**. Similar procedure the reaction of alkyne compounds **m** and **n** with azide compound **k**, products **2**, and **3** were prepared respectively.

Conclusion

A novel class of highly symmetrical fluorinated hydrocarbons, conjugated with Capecitabine pro-drug as challenging multifunctional drug image-guided vehicles has been synthesized efficiently targeted therapy. The conventional phase separation of the fluorinated products were obtained in pure form in maximum yields and in vivo evaluation to be performed

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