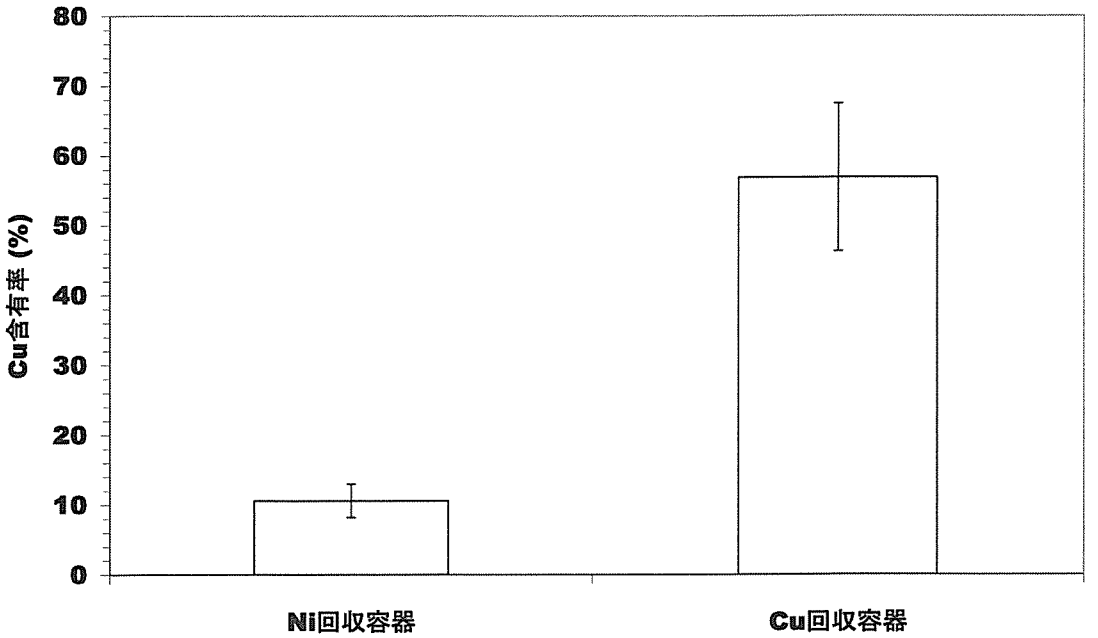

Fig.23　濃度 1 ppm における Cu 回収率（n = 5）

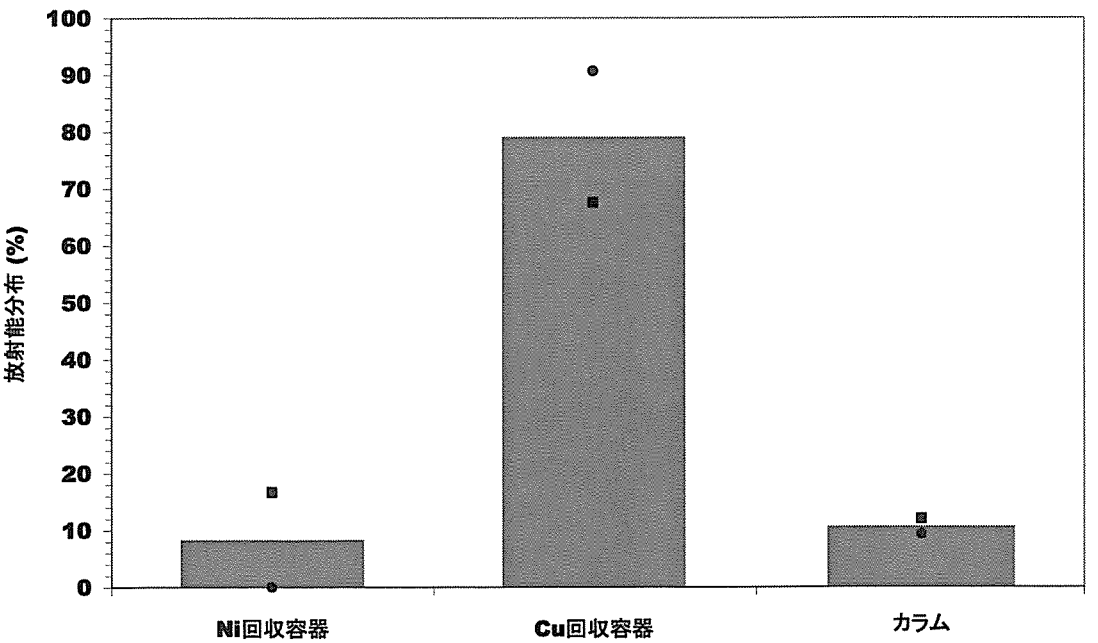

Fig.24　カラム法を用いて ^{64}Cu 精製を行ったときの放射能分布（n = 2）

4.3 考察

バンドヒータ 2 個における加熱 10 分後の水温は最高水温の 58%であり、マントルヒータの 90%に比べ大きく劣っている。これはバンドヒータ本体の温度上昇がマントルヒータ本体の温度上昇に劣るためである。バンドヒータは加熱部分が直接大気と接しており、それが温度上昇の妨げになっていると考えられた。そこで、予めバンドヒータを加熱し、バンドヒータ本体の温度を高めた後、溶液の加熱を行った。システムの精製手順として溶解のためのヒータ加熱を行う前にラインの洗浄、陰イオン交換樹脂の洗浄の工程があり、その工程に 8 分要する。この工程の 8 分間ヒータを加熱し、そこに高純度水を入れ加熱するとマントルヒータの加熱性能以上の結果が得られた。このことより、従来の精製で用いられていた溶解時間で行った場合でも十分に金ディスクからの溶解が可能であると考えられた。

バッチ法を用いた精製では 40%の Cu を安定して分離精製することができた。Cu 回収率 40%は Cu 濃度依存性が小さいため、イオン交換効率の影響だと考えられる。バッチ法は溶液と樹脂を攪拌させることで Cu を保持する。本実験では攪拌回数を 2 回とし、攪拌時間を変更しても Cu 回収率に大きな差はみられなかったが、30 分など長時間攪拌を行うことや攪拌回数を増やすことでバッチ法を用いた装置での Cu 回収率を向上させることができると考えられる。

装置の構造はバッチ法に比べ複雑となったが、カラム法を用いた精製では約 80%の Cu を精製することができ、バッチ法よりも回収率を向上させることができた。また、非放射性物質を用いた Cu 回収率よりも ^{64}Cu の精製を行ったときの Cu 回収率が上昇した。バッチ法において低濃度で Cu 回収率が上昇する傾向が見られたため、カラム法でも同様のことが考えられ、^{64}Cu 濃度は 1 ppm よりも低いため Cu 回収率が上昇したと考えられる。また、カラム法は送液が煩雑であり制御が困難であるため送液が安定していないと考えられる。

本研究で開発したシステムにより ^{64}Cu の金ディスクからの溶解及び分離精製が自動で行うことが可能となった。本システムにより ^{64}Cu 精製時間は従来の 2 時間から 1 時間に短縮され、作業者の被曝及び負担を大いに低減した。さらに、装置の簡略化に成功し、パーツに滅菌済み使い捨てパーツを用いることで容易なメンテナンスを可能とし、精製の安定性が向上すると考えられる。また、本システムはバッチ法とカラム法の分離法 2 種類に対応することができ、高い汎用性を示すことができたと考えられる。

第5章　結語

本研究では、様々な薬剤の開発･製造を可能とする安価かつ汎用性に優れた自動合成装置の開発を目指し、ロボット制御用に市販されているマイコンボード及びサーボモータを用いて、送液及び温度コントローラ制御を可能としたシステムの応用として陽電子放出核種^{64}Cu で標識された放射性薬剤 ^{64}Cu-ATSM 製造に必要な ^{64}Cu 固体ターゲットから ^{64}Cu を分離精製する自動精製装置を開発し、性能評価を行った。

本研究により得られた結果を以下に示す。

(1) 医療用滅菌済み使い捨て製品を使用可能な送液ユニットをデザインし、ヒータ及び送液をマイコンボードにより制御するシステムの構築を行い、システムの自動化及び簡略化、作業者の被曝及び負担の低減、容易なメンテナンス、精製の安定性の向上を実現した。
(2) バッチ法を用いたシステムを構築し、非放射性物質を用いて Cu を安定して 40%回収できることを確認した。
(3) バッチ法を用いたシステムにより 40%の ^{64}Cu を安定して分離精製することができた。
(4) カラム法を用いたシステムを構築し、非放射性物質を用いて Cu を 60%回収できることを確認した。
(5) カラム法を用いたシステムにより 80%の ^{64}Cu を分離精製することができた。

以上のことから、本研究で開発した ^{64}Cu 標識放射性薬剤自動精製装置は実用可能であると考えられた。また、本システムはバッチ法とカラム法の分離法 2 種類に対応することができ、高い汎用性を示すことができたと考えられた。

本研究で開発したパーツは使用用途に合わせてレイアウトが変更でき、様々なケースの薬剤製造に対応できると考えられるため、多くの新しい薬剤の開発･製造が機械工学等の専門知識を必要とせずに可能となり、病気の早期発見や治療効果判定等に利用される PET 画像診断技術等が飛躍的に広がると予想され、医療の発展に大いに貢献できると考えられる。

参考資料

1　^{64}Cu 自動精製装置に使用したパーツ及びその仕様

1.1　ニプロエクステンションチューブ

EX2-25MH(Table.1)　×2
ET-10(Table.2)　×2

Table.1　EX2-25MH の仕様

内容量	0.5 ml
チューブ長	25 cm
チューブ内径	1.5 mm
チューブ外形	2.8 mm

Table.2　ET-10 の仕様

内容量	0.2 ml
チューブ長	10 cm

1.2　シリンジ　10 ml　B BRAUN 社製　×1

1.3　三方活栓　R1-FL-LP　トップ社製　×10

1.4　バイアル瓶　5-111-07　アズワン社製　×2

1.5　加熱用ヒータ

坂口バンドヒータ　BH4330(Table.3)　坂口電熱社製　×2

Table.3　坂口バンドヒータ BH4330 の仕様

形式	内径 (mm)	巾 (mm)	電圧 (V)	容量(W)
1 ピース	43	30	200	120

1.6　温度調節器

卓上型温度調節器　T-550-K(Table.4)　アズワン社製　×1

Table.4　卓上型温度調節器 T-550-K の仕様

項目	T-550-K
操作スイッチ	POWER スイッチ及び温度調節計のアップ・ダウンキー
電源電圧	AC100 V, 50/60 Hz 共用
許容電源電圧	上記電源電圧に対して±10%以内
出力	SSR による電圧出力、AC100 V MAX、10 A(抵抗負荷)
入出力方法	裏面端子台による接続
温度制御方式	PID オートチューニングタイプ PIDS(オーバーシュート抑制型)
センサ	Pt100　Ω
設定温度範囲	0 ~ 500℃(1℃単位で設定)

1.7　陰イオン交換樹脂カラム

Poly-Prep Column、AG 1-X8 resin(Table.5)　BIO-RED 社製　×1

Table.5　Poly-Prep Column、AG 1-X8 resin の仕様

Item	Poly-Prep Column AG 1-X8 resin
Matrix	Styrene divinylbenzene
Bed Volume	2 ml
Ion	Q (Quaternary ammonium)
pH Stability	0-14
Column Bore	8 mm
Barrel Material	polypropylene
Mesh Size	100-200
Ionic Capacity	1.2 meq / ml

2　計測に使用した装置の仕様

2.1　原子吸光光度計　Z-5300(Table.6、Table.7)　日立社製

原子吸光分析法

原子吸光分析法は水溶液中の金属元素などを定量する方法であり、原子吸光光度計が用いられる。原子吸光光度計は測定したい金属元素が陰極に付いたホローカソードランプの光をフレーム等で加熱気化させた試料に通し、吸光度を測定することで試料中の金属元素の量を測定することができる。

Table.6　原子吸光光度計 Z-5300 の仕様

項目	Z-5300
横幅寸法(タンデム)	115 cm
ホローカソードランプ	縦置き
ホローカソードランプの配置	本体右側
焦点距離	400 mm
逆線分散	1.3 nm / mm
アセチレンガス制御	マスフコーコントローラ
フレームセンサ	水素フレームチェック可
キャリアガス流量制御	0、10、30、200 ml / 分
データ通信	GP-IB

Table.7　Z-5300 のフレームの検出限界

元素	波長(nm)	検出限界(mg / l)
Cu	324.8	0.012
Ni	232.0	0.036

2.2 ラジオアイソトープキャリブレータ

CRC-712、CAPINTEC

特徴

- 最大5台までリモートチェンバーとリモートディスプレイを接続
- 測定範囲の異なる3種類にチェンバーを選択可能

  最大測定放射能　8 Ci、80 Ci、800 Ci
- スピードと使い勝手の両方を満たす、手動及び自動の測定レンジ選択
- 主ユニットでリモートチェンバーの測定値を選択表示可能
- 離れたところからも読みやすい、大きくて明るい4桁LED表示ディスプレイ

CRC-15W、CAPINTEC

特徴

- ウェルの自動キャリブレーション
- 6 ch MCA組み込みウェルカウンタ
- マイクロプロセッサコントロール
- 自動 QC 機能(ゼロ調整、バックグラウンド調整、システムテスト、正確度安定性テスト)
- 80核種以上の核種名と半減期データをメモリに内蔵

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プログラムソース

```
P
OLLLLLLLL
#LET N1 = V00
#LET N1 = V01
#LET N13 = V02
#LET N17 = V03
#LET N9 = V04
#LET N13 = V05
#LET N8 = V06
#LET N8 = V07
#LET N8 = V08
#LET N8 = V09
#LET N8 = V10
#LET N13 = V11
#LET N1 = V15
#LET N1 = V16
#LET N1 = V17
#LET N0 = V18
#LET N0 = V19
OLLLLLLLH
J MAIN

:PP
A0190
A0390
A0590
A07167
A0990
A1190
A1390
A1590
A1790
A1990
A2190
#RET

:WEND
#RET

:WC
#IF V17 V16 WEND WEND ##
#LET N3 = V15
#LET N1 = V17
#RET

:SILING_UP
#IF V15 V16 SILING_UPH
SILING_UPH SILING_UPL

:SILING_UPH
#IF V00 V01 ## ## END
#AS S00 + 5
;MAX=5
#AM 400 1 N
#LET V00 N1 + = V00
J SILING_UPH

:SILING_UPL
#IF V00 V01 ## ## END
#AS S00 + 5
;MAX=5
#AM 400 1 N
#LET V00 N1 + = V00
J SILING_UPL

:END
#LET N1 = V00
#LET V15 N1 - = V15
W100
#RET

:KEND
#LET N1 = V00
#LET V01 N1 + = V01

W100
#RET

:SILING_DOWN
#IF V15 V16 SILING_DOWNH
```

```
SILING_DOWNH SILING_DOWNL

:SILING_DOWNH
#IF V00 V01 ## ## END
#AS S00 - 5
;MAX=5
#AM 400 1 N
#LET V00 N1 + = V00
J SILING_DOWNH

:SILING_DOWNL
#IF V00 V01 ## ## END
W200
W200
W200
W200
W200
W200
W200
W200
W200
#AS S00 - 5
;MAX=5
#AM 400 1 N
#LET V00 N1 + = V00
J SILING_DOWNL

:SILING_DOWNK
#IF V00 N5 ## ## KEND
W200
#AS S00 - 1
;MAX=1
#AM 100 1 N
#LET V00 N1 + = V00
J SILING_DOWNK

:L_TO_R
#AS S07 + 156
;MAX=156
#AM 162 1 N
#JSUB SILING_UP
W200
#AS S07 - 156
;MAX=156
#AM 162 1 N
#JSUB SILING_DOWN
W200
J END

:TRANSFER_TO_R
#JSUB SILING_UP
#AS S07 - 156
;MAX=156
#AM 162 1 N
#JSUB WC
#JSUB SILING_DOWN
#RET

:TRANSFER_TO_R_C
#JSUB SILING_UP
#AS S07 - 156
;MAX=156
#AM 162 1 N
#JSUB WC
#JSUB SILING_DOWN
#RET

:STOP
#AS S11 - 77
;MAX=77
#AM 79 1 N
W200
W200
W200
#AS S11 + 77
;MAX=77
#AM 79 1 N
#RET

:COD_A
#JSUB TRANSFER_TO_R
#JSUB L_TO_R
#JSUB L_TO_R
J END

:COD_A_A
#JSUB TRANSFER_TO_R
#JSUB L_TO_R
J END
```

```
:COD_B
#AS S11 + 77
;MAX=77
#AM 161 1 N
#JSUB SILING_UP
#AS S11 - 77
;MAX=77
#AM 79 1 N
#JSUB SILING_DOWN
#RET

:COD_C
#AS S09 + 77
#AS S11 - 79
;MAX=79
#AM 81 1 N
#JSUB TRANSFER_TO_R_C
#AS S09 - 77
#AS S11 + 79
;MAX=79
#AM 81 1 N
W200
#RET

:AIR
#AS S21 - 79
;MAX=79
#AM 81 1 N
#LET V19 = V01
#JSUB SILING_UP
#AS S21 + 79
;MAX=79
#AM 81 1 N
#RET


:01N_WASH_LINE
#LET V02 = V01
#JSUB PP
#AS S03 - 79
;MAX=79
#AM 81 1 N
#JSUB COD_A
#RET

:K_WATER
#LET V03 = V01
#JSUB PP
#AS S07 - 156
;MAX=156
#AM 162 1 N
#LET N2 = V15
#JSUB COD_B
#RET

:K_6N_WASH
#LET V04 = V01
#LET N2 = V19
#JSUB PP
#AS S05 - 79
;MAX=79
#AM 81 1 N
#JSUB TRANSFER_TO_R
#AS S07 + 156
;MAX=156
#AM 162 1 N
#LET N2 = V17
#JSUB COD_C
#LET N2 = V17
#AS S07 + 156
;MAX=156
#AM 162 1 N
#JSUB COD_C
#LET N2 = V17
#AS S07 + 156
;MAX=156
#AM 162 1 N
#JSUB COD_C
#RET

:6N_WASH_LINE
#LET V05 = V01
#JSUB PP
#AS S05 - 79
;MAX=79
#AM 81 1 N
#JSUB COD_A_A
#RET
```

```
:6N_TO_TARGET
#LET V06 = V01
#JSUB PP
#AS S05 - 79
#AS S13 + 77
;MAX=79
#AM 81 1 N
#JSUB SILING_UP
#LET V11 = V01
#AS S21 - 79
;MAX=79
#AM 81 1 N
#JSUB SILING_UP
#AS S07 - 156
#AS S21 + 79
;MAX=156
#AM 161 1 N
#LET V06 V11 + = V01
#JSUB SILING_DOWN
#RET
#RET

:HETER_1
#CTCLR
#CTUP
W200
W200
:COUNT1
#LET N0 = V18

:COUNT
#LET V18 N1 + = V18
W200
W200
W200
W200
W200
W200
#IF V18 N10 COUNT ## ##
#CTUP
IA9030 COUNT1 COUNT1 ##
OLLLLLLLL
#RET

:TERGET_TO_K_TO_NI
#LET V06 V11 + = V01
#LET V01 N2 + = V01
#JSUB PP
#AS S07 - 156
#AS S13 + 77
#AS S15 + 77
;MAX=156
#AM 161 1 N
#JSUB SILING_UP
#AS S09 + 77
#AS S11 - 79
#AS S13 - 77
;MAX=79
#AM 80 1 N
#LET N2 = V15
#LET V06 = V01
#JSUB SILING_DOWN
W200
#AS S09 - 77
#AS S11 + 79
;MAX=79
#AM 81 1 N
W200
#LET V11 = V01
#LET V01 N2 + = V01
#JSUB SILING_DOWN
W200
#RET

:6N_TO_TERGET_TO_K_TO_NI
#LET V08 = V01
#JSUB 6N_TO_TARGET
W200
#JSUB TERGET_TO_K_TO_NI
#RET

:6N_TO_K_TO_NI
#LET V09 = V01
#JSUB PP
#AS S05 - 79
#AS S15 + 77
;MAX=79
#AM 81 1 N
#LET N2 = V17
#JSUB COD_C
```

```
#RET

:NI_LINE_WASH
#JSUB PP
#AS S15 + 77
#AS S21 - 79
;MAX=79
#AM 81 1 N
#LET V09 = V01
#JSUB TRANSFER_TO_R
#RET

:01N_TO_K_TO_CU
#LET V10 = V1
#LET N10 = V19
#JSUB PP
#AS S03 - 79
#AS S17 + 77
;MAX=79
#AM 81 1 N
#LET N2 = V17
#AS S09 + 77
#AS S11 - 79
;MAX=79
#AM 81 1 N
#JSUB SILING_UP
#JSUB AIR
#AS S07 - 156
;MAX=156
#AM 162 1 N
#JSUB WC
#LET V10 V19 + = V01
#JSUB SILING_DOWN
#AS S09 - 77
#AS S11 + 79
;MAX=79
#AM 81 1 N
W200
#RET

:WATER_WASH_LINE
#LET V02 = V01
#JSUB PP
#AS S01 - 79
;MAX=79
#AM 81 1 N
#JSUB COD_A
#RET

:WATER_TO_CU
#LET V10 = V01
#JSUB PP
#AS S01 - 79
#AS S17 + 77
;MAX=79
#AM 81 1 N
#JSUB TRANSFER_TO_R
#RET

:MAIN
#AS S04 + 109
;MAX=109
#AM 113 1 N
#JSUB 01N_WASH_LINE
#JSUB K_6N_WASH
#JSUB 6N_WASH_LINE
#JSUB 6N_TO_TARGET
#JSUB HETER_1
#JSUB TERGET_TO_K_TO_NI
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Alkyl-fluorinated thymidine derivatives for imaging cell proliferation I. The in vitro evaluation of some alkyl-fluorinated thymidine derivatives

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Abstract

Derivatives of 2′ -deoxyuridine that contain fluoroalkyl groups at the C5 position and derivatives of thymidine that contain fluoroalkyl groups at the N3 position were synthesized and examined in three in vitro assays designed to evaluate their potential as radiopharmaceuticals for imaging cellular proliferation. Three of the former nucleosides and five of the latter were synthesized. The three assays were as follows: (a) phosphoryl transfer assay, which showed that all three of the former nucleosides and four of the latter ones were phosphorylated by recombinant human thymidine kinase 1 (TK1) and that N^3-(2-fluoroethyl)-thymidine (NFT202) was the most potent substrate of the eight nucleosides studied; (b) transport assay, which indicated that all eight nucleosides had good affinity for an 6-[(4-nitrobenzyl)thio]-9-β-D-ribofuranosylpurine-sensitive mouse erythrocyte nucleoside transporter, with inhibition constants in the range of 0.02–0.55 mM; and (c) degradation assay, which showed that all but one of the former nucleosides and none of the latter were degraded by recombinant *Escherichia coli* thymidine phosphorylase (an enzyme that catalyzes the glycosidic bond of thymidine and 2′ -deoxyuridine derivatives). From these in vitro screening assays, we selected NFT202 as a candidate for subsequent in vivo evaluation because this compound met the three minimum requirements of the in vitro screening assays and had the most potent phosphorylation activity as a substrate for recombinant human TK1.

Keywords: Nucleosides; Thymidine kinase; Thymidine phosphorylase; Nucleoside transporter

1. Introduction

The application of current nuclear imaging techniques, such as glucose metabolism, depends basically on the nonspecific phenotype of the tumor. This phenotype dependence is troublesome in several patient management situations [1–4]. Based on recent progress in tumor biology, cancer is best described as a mass of cells with highly elevated and uncontrolled proliferative potential, caused by mutations in cellular growth control genes that are partly inherited and partly generated by spontaneous as well as environmental DNA damage [5]. For that reason and because of the direct link of phenotype to genotype, we have focused on tumor-specific highly elevated cell proliferation.

DNA synthesis for cell proliferation is essential, and thymidine can be rapidly incorporated into newly synthesized DNA via a specific salvage pathway. Based on these findings, many attempts have been made to visualize tumor cell proliferation by using thymidine and 2′-deoxyuridine derivatives [6–13]. However, the rapid degradation of these tracers in vivo results in numerous labeled metabolites, which hampers the measurement of proliferation rates and compromises image quality. The recognition that an electronegative substituent at the 2′-position (α or β configuration) can stabilize thymidine analogs towards enzymatic cleavage of the nucleoside glycosidic bond led to the study of 1-(2-deoxy-2-[^{18}F]fluoro-β-D-arabinofuranosyl)-thymine ([^{18}F]FMAU) for proliferation imaging, given that [^{14}C]FMAU was known to label DNA [14–19]. The first human imaging study with [^{18}F]FMAU illustrated that it was possible to image DNA synthesis in vivo in human tumors [18]. However, an unexpectedly low uptake

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was seen for proliferative bone marrow in contrast to thymidine and 3′-[^{18}F]fluoro-3′-deoxythymidine ([^{18}F]FLT) [20]. Therefore, this unsolved mechanism of uptake and retention in tumors relative to bone marrow should be explored. From a substrate specificity point of view, these 2′-β-nucleosides, such as 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-thymine (FMAU) and 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil, are efficient substrates for thymidine kinase 2 (TK2; the mitochondrial isozyme of thymidine kinase) but show only minimal activity with thymidine kinase 1 (TK1; the key enzyme of mammalian DNA synthesis) [21,22]. In a previous report, we found no correlation between the cell proliferation (demonstrated by [^{3}H]thymidine uptake and S-phase fraction) of malignant tumor cells and the uptake of the TK2 selective substrate [^{3}H]arabinothymidine [23]. We therefore concluded that radiopharmaceuticals with a high affinity for TK2 are not suitable agents for the diagnostic imaging of proliferating tissues, despite the fact that 30% of [^{3}H]arabinothymidine was incorporated into DNA.

In 1998, a biologically stable radiofluorine-labeled thymidine analog, [^{18}F]FLT, was developed as a candidate for cell proliferation imaging [20,24,25]. FLT is phosphorylated by TK1, a cell-cycle-regulated isozyme, and it is metabolically retained as 5′-phosphate. This is because FLT lacks a 3′-hydroxyl structure necessary for the polymerase reactions of oligonucleotide synthesis and because FLT 5′-triphosphate can only terminate newly synthesized DNA strands. These characteristics of FLT trapping will induce a discrepancy between FLT uptake and the DNA synthesis phenomenon [26].

Our group has reported on another strategy. Noting the report of Rahim et al. [27] on 5-iodo-4′-thio-2′-deoxyuridine (ITdU), we conducted an experimental study on ^{125}I-labeled ITdU [28,29]. It was preclinically confirmed that ITdU, in which the 4′-oxo of 5-iodo-2′-deoxyuridine had been replaced by 4′-sulfur, is resistant to metabolic decomposition by thymidine phosphorylase (TP) and is an agent that directly reflects DNA synthesis. The results of that study suggested that this tactic could be used to produce a new 4′-thio derivative to supplement the research on the 2′-arabino-F and 3′-F derivative previously developed. As mentioned, in spite of these studies on nucleoside-based imaging agents, there is still a need for a thymidine analog that might prove simpler to use for imaging DNA synthesis and stimulates a more widespread use of such agents. That is why we further tested several thymidine derivatives and introduced a new approach to drug design.

In this paper, we describe studies with thymidine derivatives that contain fluoroalkyl groups at the N3 position and 2′ -deoxyuridine derivatives that contain fluoroalkyl groups at the C5 position. The structures of these derivatives and R designations are shown in Fig. 1 and in the tables, respectively. The chemical structures of 5-(2-fluoroethyl)-2′-deoxyuridine (**1b**; FT202), 5-(fluoromethyl)-4′-thio-2′-deoxyuridine (**1c**; FTS101), 5-(2-fluoroethyl)-4′-thio-2′-deoxyuridine (**1d**; FTS202), N^3-(fluoromethyl)-thymidine (**1e**; NFT201) and N^3-(2-fluoroethyl)-thymidine (**1f**; NFT202) are already known and have been used as antiviral agents, but the corresponding ^{18}F-labeled compounds are not known and have not been used as imaging agents [27,30–33]. We have compared the in vitro phosphorylation rates of these nucleosides with those of recombinant human TK1 by phosphoryl transfer assay. Nucleoside transport inhibition constants (K_i) were measured to evaluate their interaction with the nucleoside transporter. Their stability toward the phosphorolytic enzyme, TP, has been used as an indicator of their metabolic stability. From these in vitro screening assays, we selected NFT202 (**1f**) as a candidate for subsequent in vivo evaluation because this compound surmounted the three minimum requirements of in vitro screening assays and also had the most potent phosphorylation activity as a substrate for recombinant human TK1. In addition, this compound might be amenable to labeling with ^{18}F by the use of a known method.

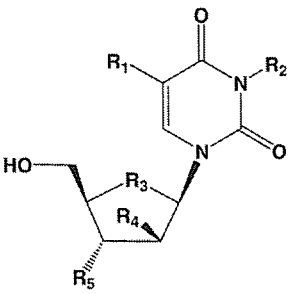

Fig. 1. Structures of evaluated nucleosides. R designations are described in the tables.

However, our companion work describes an in vitro and an in vivo evaluation of NFT202 (**1f**) and reveals that NFT202 was less effective than 3′-deoxy-3′-fluorothymidine (**1j**; FLT). We discuss the pitfalls of our limited selection criteria in our companion paper.

2. Materials and methods

2.1. Chemicals

Thymidine (**1a**), FLT (**1j**) and 5-fluoro-2′ -deoxyuridine (**1k**; FdUrd) were purchased from Sigma-Aldrich Japan KK (Tokyo, Japan). Other reagents for synthesis were purchased from Sigma-Aldrich Japan KK, Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and Tokyo Kasei Kogyo, Co., Ltd. (Tokyo, Japan).

2.2. Synthesis

A summary of the various syntheses is given below. Experimental details and the characterization of compounds are described in succeeding sections.

All isolated materials were shown to be pure by nuclear magnetic resonance (NMR; free of obvious impurities) and by thin-layer chromatography (TLC; homogeneous material).

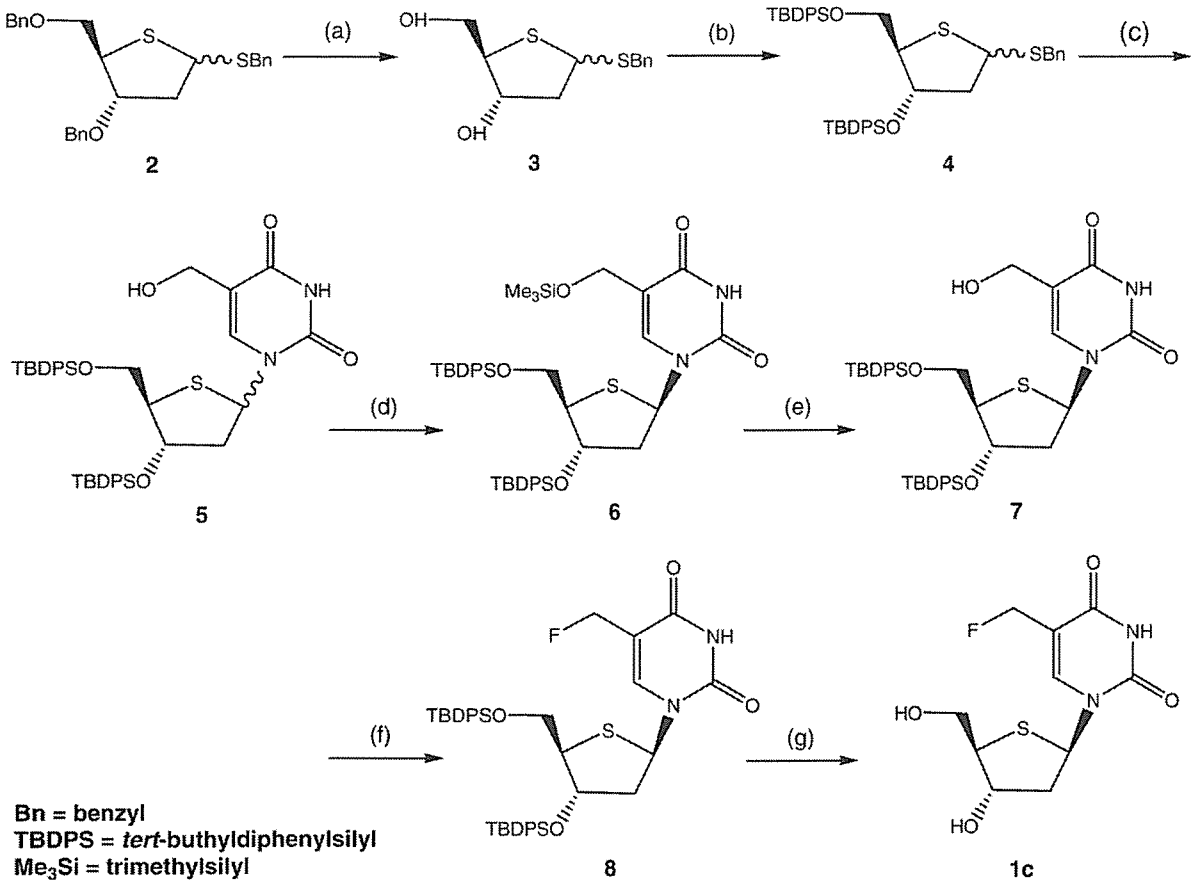

Fig. 2. Synthesis of FTS101. Conditions: (a) BCl_3, CH_2Cl_2, −78°C. (b) TBDPSCl, imidazole, DMF, 0°C. (c) (i) 5-Hydroxymethyluracil, BTMSA, MS4Å, DMF, 0°C; (ii) NIS, RT. (d) TMSCl, TEA, CH_3CN, 0°C. (e) 6 N HCl, $CHCl_3/CH_3CN$, 0°C. (f) DAST, CH_2Cl_2, −20°C. (g) TBAF, THF, RT.

Compound **1b** was synthesized by a previously described method [30]. The synthetic procedures of Compounds **1c** and **1d** differed from that of 5-fluoro-4′-thio-2′-deoxyuridine (**1l**). The coupling reaction of benzyl 3,5-di-*O*-benzyl-2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside (**2**) with a silylated base in the presence of *N*-iodosuccinimide (NIS) and molecular sieves was processed successfully [34]. However, subsequent debenzylation by Lewis acid hardly proceeded, and the desired compounds (**1c** and **1d**) were not obtained. As shown in Figs. 2 and 3, Compounds **1c** and **1d** were obtained by replacing the protecting groups of Compound **2** with di-*O*-*tert*-butyldiphenylsilyl. Moreover, separation of the α/β mixture of Compound **1c** was conducted by trimethylsilylation of the hydroxylmethyl residue of 3′,5′-di-*O*-*tert*-butyldiphenylsilyl-5-hydroxymethyl-4′-thio-2′-deoxy-β-uridine (**7**). Compound **1e** was synthesized by a previously described method [32]. The N^3 alkyl-fluorination of thymidine derivatives [**1f**, N^3-(3-fluoropropyl)-thymidine (**1g**), N^3-(2-fluoroethyl)-4′-thio-2′-deoxyuridine (**1h**) and 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-N^3-(2-fluoroethyl)-thymine (**1i**)] was conducted in a one-step reaction from the corresponding nucleoside (β anomer) by adapting

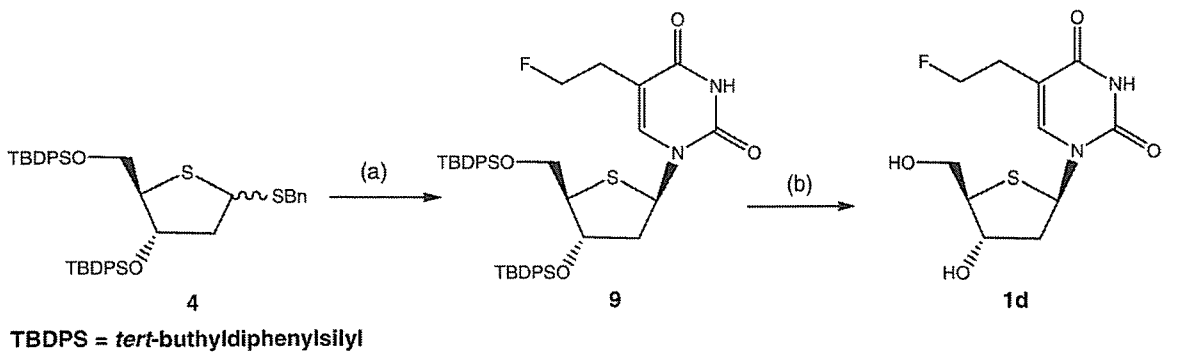

Fig. 3. Synthesis of Compound **1d** (FTS202). Conditions: (a) (i) 5-(2-Fluoroethyl)uracil, BTMSA, CH_3CN, RT; (ii) MS4Å, NIS, DMF, RT. (b) TBAF, THF, RT.

previously described procedures [35,36]. Fluoroalkyl-tosylate or fluoroalkyl-bromide was added to a solution containing a weak base and a nucleoside in either a *N,N*-dimethylformamide (DMF)/acetone mixture, DMF, or tetrahydrofuran (THF) at a mild reaction temperature [from room temperature (RT) to 80°C], yielding the target compounds at high yields (72–100%). While alkylations at other positions on the nucleoside are possible, selective alkylation on the N3 position of pyrimidine was reported [35,37,38].

2.3. 5-(2-Fluoroethyl)-2′-deoxyuridine (**1b**; FT202)

5-(2-Acetylhydroxyethyl)uracil was synthesized using γ-butyrolactone as a starting material, according to the method of Fissekis et al. [39]. Furthermore, FT202 was prepared from 5-(2-acetylhydroxyethyl)uracil according to the method of Griengl et al. [30]. The pure β anomer of 3′,5′-di-*O*-*p*-toluoyl-5-(2-fluoroethyl)-2′-deoxyuridine was crystallized in ethanol at 4°C, as reported in the literature [30]. After deprotection, the resultant compound (**1b**) was obtained. The melting point of Compound **1b** was in agreement with the previously reported temperature [30]. Its purity was assessed by a conspicuous absence of impurities in the ^{1}H NMR spectrum and in high-performance liquid chromatography (HPLC; purity, 96.9%):

^{1}H NMR (CD_3OD, 500 MHz) δ 7.92 (s, 1H), 6.26 (t, J=6.5 Hz, 1H), 4.53 (dtd, J=47.0, 5.5 and 3.0 Hz, 2H), 4.37 (quint, J=3.0 Hz, 1H), 3.92 (q, J=3.0 Hz, 1H), 3.90 (dd, J=12.0 and 3.0 Hz, 1H), 3.73 (dd, J=12.0 and 3.5 Hz, 1H), 2.71 (td, J=5.5 and 2.0 Hz, 1H), 2.68 (td, J=5.5 and 1.5 Hz, 1H), 2.26 (m, 1H), 2.21 (m, 1H)
UV λ_{max} (CH_3OH)=266 nm
m_p=153°C
fast atom bombardment mass spectroscopy (FABMS), m/z=297 [M+Na].

The conditions of HPLC analysis are described in Section 2.15.

2.4. 5-(Fluoromethyl)-4′-thio-2′-deoxyuridine (**1c**; FTS101)

The schematic diagram for the synthesis of FTS101 is depicted in Fig. 2. Compound **2** was prepared with a seven-step synthesis starting from 2-deoxy-D-*erythro*-pentose, following the procedure of Dyson et al. [40]. To a dichloromethane solution (25 ml) of Compound **2** (3.85 g, 8.8 mmol) was added borontrichloride (1.0 M in dichloromethane, 40 ml, 40 mmol) at −78°C. The resultant mixture was stirred at −78°C for 4 h. The mixture was added to a methanol–aqueous solution of ammonia (1:1 vol/vol, 40 ml), then the organic layer was separated. The aqueous layer was rinsed with chloroform. The combined organic layer was dried with sodium sulfate, filtered and then concentrated in vacuo. The crude material was purified by silica gel column chromatography (eluent, acetone:hexane=1:1) to give benzyl-2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside (**3**) (1.5 g, 66%).

To a DMF (30 ml) solution of Compound **3** (1.5 g, 5.85 mmol) was added *tert*-butyldiphenylsilylchloride (TBDPSCl; 3.8 ml, 11.7 mmol) and imidazole (1.79 g, 23.4 mmol) at 0°C. The resultant mixture was stirred overnight at RT. The mixture was concentrated in vacuo to remove DMF. The residue was extracted with ethyl acetate. The organic layer was washed with water, dried with sodium sulfate, filtered and then concentrated in vacuo. The crude material was purified with silica gel column chromatography (eluent, hexane:ethyl acetate=10:1) to give benzyl-3,5-di-*O*-*tert*-butyldiphenylsilyl-2-deoxy-1,4-dithio-D-*erythro*-pentofuranoside (**4**) (3.88 g, 90%).

To a DMF (28 ml) suspension of 5-hydroxymethyluracil (737 mg, 5.18 mmol) were added bis(trimethylsilyl)acetylene (BTMSA; 2.56 ml, 11.3 mmol), Compound **4** (3.8 g, 5.18 mmol) and molecular sieves 4 Å (MS4Å; ca. 460 mg) at 0°C, and the mixture was stirred for 30 min. The mixture was then added to a DMF (10 ml) solution of NIS (1.23 g, 5.18 mmol) and stirred at RT overnight. The mixture was concentrated in vacuo to remove DMF. The residue was added to ice water, and an aqueous solution of 5% sodium thiosulfate then was extracted with ethyl acetate. The organic layer was washed with 5% aqueous solution of sodium thiosulfate, 5% aqueous solution of sodium hydrogen carbonate and a saturated aqueous solution of sodium chloride. It was then dried with sodium sulfate, filtered and concentrated in vacuo. The crude material was purified by silica gel column chromatography (eluent, hexane:ethyl acetate=1:1) to give a mixture of Compound **5** (1.2 g, 31%).

To an acetonitrile (40 ml) solution of Compound **5** (2.69 g, 3.58 mmol) were added triethylamine (TEA; 3 ml, 21.5 mmol) and trimethylsilylchloride (TMSCl; 1.36 ml, 10.7 mmol) at 0°C. After stirring for 5 min, the mixture was concentrated in vacuo. The residue was extracted with ethyl acetate. The organic layer was washed with a diluted aqueous solution of sodium hydrogen carbonate and a saturated aqueous solution of sodium chloride, dried with sodium sulfate, filtered and concentrated in vacuo. The crude material was purified by silica gel column chromatography (eluent, hexane:ethyl acetate=2:1) to give 3′,5′-di-*O*-*tert*-butyldiphenylsilyl-5-trimethylsilyloxymethyl-4′-thio-2′-deoxyuridine (**6**) (1.15 g, 39%) and its anomer (1.4 g, 47%):

Compound **6**
^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.52 (br.s, 1H), 7.63–7.27 (m, 21H), 6.56 (dd, J=8.8 and 6.2 Hz, 1H), 4.48 (dd, J=3.0 and 2.0 Hz, 1H), 4.30 (s, 2H), 3.68–3.63 (m, 2H), 3.49 (dd , J=12.4 and 10.4 Hz, 1H), 2.14–2.07 (m, 1H), 1.44–1.38 (m, 1H), 1.07 (s, 9H), 0.94 (s, 9H), 0.01 (s, 9H).

To a chloroform (10 ml)–acetonitrile (20 ml) solution of Compound **6** (1.15 g, 1.39 mmol) was added 6 N hydrochloric acid (0.6 ml) at 0°C. The mixture was stirred for 10 min, neutralized with 5% aqueous solution of sodium hydrogen carbonate and concentrated in vacuo to remove the organic solvent. The residue was extracted with ethyl

acetate. The organic layer was washed with water, dried with sodium sulfate, filtered and concentrated in vacuo. The crude material was purified by silica gel column chromatography (eluent, hexane:ethyl acetate=1:1) to give 3′,5′-di-*O*-*tert*-butyldiphenylsilyl-5-hydroxymethyl-4′-thio-2′-deoxy-β-uridine (**7**; 1.01 g, 97%):

^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.54 (br.s, 1H), 7.64–7.26 (m, 21H), 6.53 (dd, J=8.1 and 6.3 Hz, 1H), 4.43 (br.s, 1H), 4.11 (d, J=2.4 Hz, 1H), 2.28–2.21 (m, 1H), 1.75–1.60 (m, 1H), 1.07 (s, 9H), 0.96 (s, 9H).

To a dichloromethane (12 ml) solution of Compound **7** (1.0 g, 1.33 mmol) was added a dichloromethane (12 ml) solution of (dimethylamino)sulfur trifluoride (DAST; 264 μl, 2.0 mmol) at −20°C, followed by stirring for 20 min. The mixture was poured onto crushed ice then extracted with chloroform. The organic layer was dried with sodium sulfate, filtered and concentrated in vacuo. The crude material was purified by silica gel column chromatography (eluent, hexane:ethyl acetate=2:1) to give 3′,5′-di-*O*-*tert*-butyldiphenylsilyl-5-fluoromethyl-4′-thio-2′-deoxyuridine (**8**) (740 mg, 74%).

To a THF (6.5 ml) solution of Compound **8** (1.03 g, 1.36 mmol) was added tetrabutylammonium fluoride (TBAF; 1.0 M in THF, 4.1 ml, 4.1 mmol), followed by stirring at RT for 35 min. The mixture was purified by silica gel column chromatography (prepacked by dichloromethane eluent, THF:dichloromethane=2:1) to give Compound **1c** (FTS101, 300 mg, 80%). The purity of Compound **1c** was assessed by the conspicuous absence of impurities in the ^{1}H NMR spectrum and in HPLC (retention time, 13.6 min; purity, 98.7%). HPLC was performed with a C18 (5-μm) analytical column [150×4.6 (i.d.) mm, Mightysil RP-18 GP Aqua; Kanto Chemical, Tokyo, Japan]. Elution was conducted by CH_3OH:H_2O:trifluoroacetic acid (TFA)=10:90:0.1 at a flow rate of 0.8 ml/min and was monitored at 254 nm:

^{1}H NMR [dimethyl sulfoxide (DMSO)-d_6, 400 MHz] δ 11.58 (s, 1H), 8.33 (d, J=4.0 Hz, 1H), 6.25 (t, J=7.2 Hz, 1H), 5.27 (d, J=4.0 Hz, 1H), 5.19 (t, J=5.6 Hz, 1H), 5.08 (d, J=88 Hz, 2H), 4.37 (m, 1H), 3.59 (m, 1H), 3.58 (m, 1H), 3.29 (m, 1H), 2.20 (dd, J=7.2 and 4.0 Hz, 2H)
UV λ_{max} (CH_3OH)=267 nm
FABMS, m/z=299 [M+Na].

*2.5. 5-(2-Fluoroethyl)-4′-thio-2′-deoxyuridine (**1d**; FTS202)*

The schematic diagram for the synthesis of FTS202 is depicted in Fig. 3. 5-(2-Acetylhydroxyethyl)uracil was synthesized, using γ-butyrolactone as a starting material, according to the method of Fissekis et al. [39]. Furthermore, 5-(2-fluoroethyl)uracil was prepared from 5-(2-acetylhydroxyethyl)uracil, according to the method of Griengl et al. [30]. To an acetonitrile (2.5 ml) suspension of 5-(2-fluoroethyl)uracil (237 mg, 1.50 mmol) was added BTMSA (630 mg, 3.10 mmol), and the resultant mixture was stirred at RT for 1 h. The reaction mixture was added to MS4Å (ca. 1.0 g), a DMF (2.5 ml) solution of Compound **4** (733 mg, 1.0 mmol) and NIS (270 mg, 1.20 mmol). The resultant mixture was stirred at RT for 24 h and filtered, and the filtrate was extracted with diethyl ether. The combined organic layer was washed with a 5% aqueous solution of sodium thiosulfate and a saturated aqueous solution of sodium hydrogen carbonate and brine. It was then dried with sodium sulfate, filtered and concentrated in vacuo. The crude material was purified by silica gel column chromatography (eluent, hexane:ethyl acetate=3:7→2:3→1:1) and silica gel recycle HPLC to give 3′,5′-di-*O*-*tert*-butyldiphenylsilyl-5-(2-fluoroethyl)-4′-thio-2′-deoxyuridine (**9**) (180 mg, 24%) and its anomer (227 mg, 30%):

Compound **9**
^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.67 (s, 1H), 7.67–7.29 (m, 21H), 6.54 (dd, J=8.6 and 6.2 Hz, 1H), 4.49–4.42 (m, 2H), 4.38–4.31 (m, 1H), 3.62–3.03 (m, 3H), 2.49 (t, J=5.9 Hz, 1H), 2.42 (t, J=5.5 Hz, 1H), 2.22 (ddd, J=13.2, 6.2 and 3.5 Hz, 1H), 1.68–1.61 (m, 1H), 1.04 (s, 9H), 0.96 (s, 9H)
^{13}C NMR (100 MHz, $CDCl_3$) δ 162.7, 150.2, 138.2, 135.7, 135.7, 135.6, 135.4, 133.1, 133.1, 133.0, 132.6, 130.0, 130.0, 129.9, 127.9, 127.8, 127.8, 110.4, 82.1, 80.4, 77.2, 76.0, 65.9, 61.0, 58.8, 42.5, 28.3, 28.1, 26.9, 26.8, 19.2, 19.1
FABMS, m/z=789 [M+Na]
High-resolution mass spectroscopy (HRMS) for $C_{43}H_{51}FN_2O_4SSi_2Na$: calculated=789.2220, found=789.2994.

Anomer
^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.01 (s, 1H), 8.14 (s, 1H), 7.60–7.28 (m, 20H), 6.22 (dd, J=8.1 and 3.1 Hz, 1H), 4.56 (t, J=6.1 Hz, 1H), 4.46–4.42 (m, 2H), 3.76 (td, J=6.0 and 2.4 Hz, 1H), 3.38 (dd, J=10.6 and 6.5 Hz, 1H), 3.26 (dd, J=10.6 and 6.5 Hz, 1H), 2.72–2.38 (m, 3H), 2.19 (dt, J=14.3 and 2.8 Hz, 1H), 1.07 (s, 9H), 0.96 (s, 3H)
^{13}C NMR (100 MHz, $CDCl_3$) δ 163.1, 150.7, 140.5, 135.7, 135.7, 135.6, 135.5, 132.8, 132.8, 132.7, 130.2, 130.1, 129.8, 127.9, 127.9, 127.7, 109.7, 109.6, 82.3, 80.7, 77.6, 77.2, 65.7, 62.5, 60.5, 44.6, 28.6, 28.4, 27.0, 26.7, 19.1.

To a THF (17.5 ml) solution of Compound **9** (1.34 g, 1.75 mmol) was added TBAF (1.0 M in THF, 6.99 ml, 6.99 mmol) at 0°C, and the resultant mixture was stirred at RT. After 2 h, the reaction mixture was concentrated in vacuo. The crude material was purified by silica gel column chromatography (eluent, chloroform:methanol=9:1) and was recrystallized from methanol to give FTS202 (403 mg, 79%). The purity of Compound **1d** was assessed by the conspicuous absence of impurities in the ^{1}H NMR spectrum and in HPLC (purity, 99.7%):

^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.4 (s, 1H), 7.91 (s, 1H), 6.27 (t, J=7.4 Hz, 1H), 5.26 (d, J=3.8 Hz, 1H),