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Research Article

Utility of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-[^{18}F]fluoro-glucopyranoside for no-carrier-added ^{18}F-glycosylation of amino acids

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Summary

A radiochemical method for the ^{18}F-glycosylation of amino acid side chains was developed starting from peracetylated 2-deoxy-2-[^{18}F]fluoroglucopyranoside (TA-[^{18}F]FDG). *O*-(2-deoxy-2-[^{18}F]fluoro-D-glucopyranosyl)-L-serine and the corresponding threonyl compound were obtained in a radiochemical yield of 25% and 12% (related to [^{18}F]fluoride), respectively, after Zemplén deprotection within a total reaction time of 90 min. The anomeric configuration of the corresponding ^{19}F-substituted compounds revealed preferential α-stereoselectivity. The ^{18}F-glycosylation method using TA-[^{18}F]FDG is compatible with the short half-life of fluorine-18 and combines glycosylation and ^{18}F-labelling of a target compound within a single reaction step. TA-[^{18}F]FDG is a promising ^{18}F-labelled prosthetic group and could be adapted to ^{18}F-labelling of bioactive peptides to study their pharmacokinetics using positron emission tomography (PET). Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: ^{18}F-glycosylation; F-18; glycosidic linkage; positron-emission-tomography; PET

Introduction

The development of ^{18}F-labelling methods adopted to proteins and bioactive peptides for diagnostic imaging by positron emission tomography (PET) has

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gained enormous interest in the field of radiopharmaceutical sciences.[1] Particularly small radioactive labelled peptides were optimized for their pharmacological properties and show remarkable advantages over large proteins or monoclonal antibodies due to their higher uptake in target tissue and improved blood clearance. The commonly used strategy to label peptides still relies on the use of ^{18}F-labelled prosthetic groups targeting the N-terminus or lysine side chains. Up to now, *N*-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB) and 4-nitrophenyl 2-[^{18}F]fluoropropionate are amongst the most effective and suitable ^{18}F-acylation agents as indicated by their prevalent application in a large number of radiosyntheses for ^{18}F-labelled peptide-based imaging agents (i.e. [^{18}F](D-Phe1)octreotide,[2] [^{18}F]SAA-RGD,[3] [^{18}F]NT(8-13)[4] or [^{18}F](Nle^4, D-Phe^7)-α-MSH).[5] Alternative ^{18}F-labelling methods for peptides include the use of ^{18}F-labelled aldehydes,[6] photochemical conjugation[7] and ^{18}F-alkylation.[8] However, these laborious strategies require multiple-step syntheses and often suffer from low yields and the necessity of protecting groups to prevent side-reactions. Furthermore, complicated and time consuming tracer purification steps are unfavorable for routine large-scale synthesis of short-lived ^{18}F-labelled radiopharmaceuticals.

Thus, additional and improved radiofluorination techniques are urgently required in the field of molecular imaging. Such developments include the approach toward direct ^{18}F-substitution of hydroxy groups in peptides,[9] the development of a chemo-enzymatic ^{18}F-glycosylation method[10] and, more recently, the use of [^{18}F]fluorothiols for chemoselective labelling of peptides.[11] Furthermore, Poethko *et al.* developed a convenient and high yielding ^{18}F-labelling method for peptides by oxime conjugation of [^{18}F]fluorobenzaldehyde with unprotected aminooxy peptides providing an important improvement in the search for improved ^{18}F-labelling methodologies.[12]

In addition, it has been shown that glycosylation of peptides often improves their biokinetics. In the case of RGD peptides, which are antagonists of $\alpha_v\beta_3$ integrins, the methodology of glycosylation prior to ^{18}F-acylation has successfully led to the development of novel PET-radiopharmaceuticals for the imaging of tumor angiogenesis.[3] Moreover, glycosylation of peptides has been shown to enhance bioavailability and improve blood brain barrier (BBB) permeability and *in vivo* clearance properties in a large number of studies.[13–16]

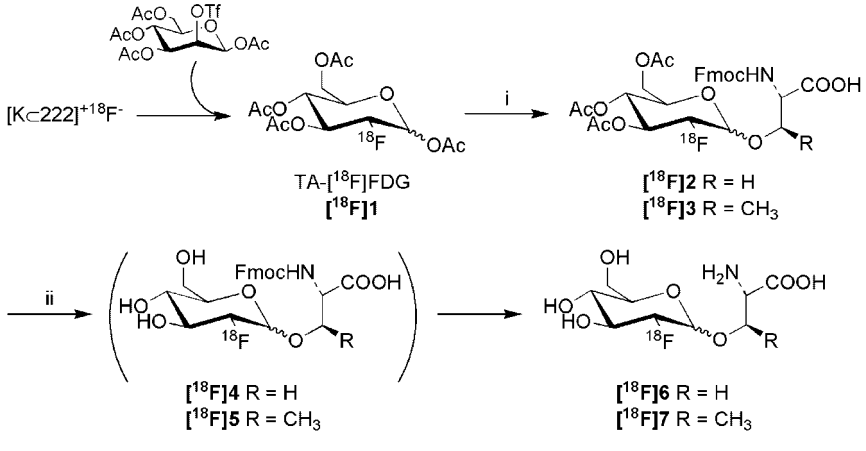
Glycosylation strategies in carbohydrate chemistry are many including syntheses of glycopeptide building blocks using peracetylated glycosides and $BF_3 \cdot Et_2O$ or glycosyl bromides and AgOTf or $HgBr_2$.[17–22] In comparison to these methods, a suitable ^{18}F-glycosylation method should apply a rapidly accessible glycosyl donor compound. Advantageously, the peracetylated intermediate of the well-known radiosynthesis of 2-deoxy-2-[^{18}F]fluoro-glucose ([^{18}F]FDG), which represents the most frequently used PET-radiopharmaceutical for tumor imaging in nuclear medicine, can be produced in

highest radiochemical yields starting from [^{18}F]fluoride and the precursor 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluormethanesulfonyl-*β*-D-mannopyranose.[23] A glycosylation reaction that made use of peracetylated [^{18}F]FDG to obtain *N*-glycosylated 2-nitroimidazole was reported by Patt *et al.*[24]

The challenge of this project was the development of a radiochemical ^{18}F-glycosylation method that combines both, *O*-glycosylation and ^{18}F-labelling of the target compound. In this study we report the optimization of a no-carrier-added ^{18}F-glycosylation method using 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-[^{18}F]fluoro-D-glucopyranose (TA-[^{18}F]FDG, **[^{18}F]1**) as ^{18}F-labelled glycosyl donor. In order to investigate the general feasibility of this ^{18}F-glycosylation strategy, we used the Fmoc-protected amino acids serine and threonine as model glycosyl acceptors. We herein also report the stereochemical outcome of glycosylation reactions of 2-deoxy-2-fluoro glycosides and the mechanistic implications of these results.

Results and discussion

Based on the [^{18}F]FDG-synthesis by Hamacher and others,[23] the ^{18}F-labelled glycosyl donor **[^{18}F]1** was obtained in a radiochemical yield (RCY) of 92% by aminopolyether (Kryptofix® 2.2.2) supported nucleophilic ^{18}F-for-OTf substitution on the corresponding tetra-*O*-acetylated mannopyranoside (Scheme 1). For the subsequent reaction steps the crude reaction mixture of **[^{18}F]1** was passed through a LiChrolut® Si-cartridge with CH_3CN and submitted to semipreparative reversed-phase HPLC for rapid radiochemical isolation of **[^{18}F]1**. Solid phase extraction (SPE) on a LiChrolut® RP18-cartridge and elution with CH_3CN followed by evaporation was performed in order to



Scheme 1. ^{18}F-glycosylation of serine or threonine using TA-[^{18}F]FDG ([^{18}F]1). (i) $BF_3 \cdot Et_2O$, Fmoc-Ser-OH or Fmoc-Thr-OH, CH_3CN, $T = 80°C$, 5 min; (ii) NaOMe/MeOH, $T = 60°C$, 10 min

provide **[^{18}F]1** for subsequent ^{18}F-glycosylation of the model compounds Fmoc-Ser and Fmoc-Thr (Scheme 1, step 2). The reaction parameters for the ^{18}F-glycosylation key step were investigated with respect to the choice of reaction solvent, reaction time, Lewis acid promotor (Table 1) and the concentration of Fmoc-Ser (Table 2).

As shown in Table 1, CH_3CN was convenient for *O*-glycosylation (Scheme 1, step 2), whereas CH_2Cl_2 turned out to be less suitable. However, the ^{18}F-glycosylation of Fmoc-protected lysine was hindered due to a solubility problem in CH_3CN (data not shown).

Patt *et al.* suggested $SnCl_4$ and $Hg(CN)_2$ as promoters in the radiosynthesis of an adduct of [^{18}F]FDG and 2-nitroimidazole.[24] This *N*-glycosylation used

Table 1. Radiochemical yields (RCY) for the ^{18}F-glycosylation of Fmoc-Ser/Thr using [^{18}F]1 as glycosyl donor

Product	Solvent	Lewis acid[b]	*T* (°C)	RCY (%)[a]
[^{18}F]2	CH_3CN	$BF_3 \cdot Et_2O$	rt	0
[^{18}F]2	CH_3CN	$BF_3 \cdot Et_2O$	40	0
[^{18}F]2	CH_3CN	$BF_3 \cdot Et_2O$	60	32
[^{18}F]2	CH_3CN	$BF_3 \cdot Et_2O$	70	35
[^{18}F]2	CH_3CN	$BF_3 \cdot Et_2O$	80	48
[^{18}F]2	CH_3CN	$BF_3 \cdot Et_2O$	120	0
[^{18}F]2	CH_3CN	$BF_3 \cdot Et_2O$, $Hg(CN)_2$	60	47
[^{18}F]2	CH_3CN	$Cu(OTf)_2$	60	0
[^{18}F]2	CH_3CN	$SnCl_4$	60	11
[^{18}F]2	CH_3CN	$SnBr_4$	60	0
[^{18}F]2	CH_2Cl_2	$BF_3 \cdot Et_2O$	reflux	0
[^{18}F]2	$C_2H_5Cl_2$	$BF_3 \cdot Et_2O$	60	0
[^{18}F]2	Et_2O	$BF_3 \cdot Et_2O$	reflux	0
[^{18}F]3	CH_3CN	$BF_3 \cdot Et_2O$	60	30
[^{18}F]3	CH_3CN	$SnCl_4$	60	9
[^{18}F]3	CH_3CN	$Cu(OTf)_2$	60	0

[a] $t = 10$ min, 8 mM Fmoc-Ser/Thr, $V = 400$ µl.
[b] 80 µmol BF_3; 40 µmol $Hg(CN)_2$; 20 µmol $Cu(OTf)_2$; 40 µmol $SnBr_4$; 85 µmol $SnCl_4$.

Table 2. Dependence of the radiochemical yield on the concentration of precursor for the ^{18}F-glycosylation of Fmoc-Ser using TA-[^{18}F]FDG ([^{18}F]1)

Concentration of Fmoc-Ser (mM)	RCY (%)[a]
0.5	14 ± 2
2	26 ± 3
5	31 ± 2
10	32 ± 4
25	42 ± 4
50	41 ± 4

[a] $n = 3$, $t = 10$ min, 0.2 M $BF_3 \cdot Et_2O$, $T = 60$°C, $V = 200$ µl (CH_3CN).

large amounts of $SnCl_4$ and catalytic amounts of $Hg(CN)_2$ to achieve the *N*-glycosylated product in surprisingly high radiochemical yields (80%) within 60 min. We observed only a moderate RCY of 11% for the *O*-linked tetraacetylated [^{18}F]FDG adduct **[^{18}F]2** when using $SnCl_4$ (Table 1). In contrast, our experiments revealed $BF_3 \cdot Et_2O$ as the Lewis acid promoter of choice, so that a maximum RCY of 48% (**[^{18}F]2**) and 32% (**[^{18}F]3**) was reached in a much shorter reaction time (8–10 min) at 80°C. A prolonged reaction time did not significantly lead to enhanced RCYs as determined by radio-HPLC.

As suggested by Patt *et al.*[24] we also used $Hg(CN)_2$ as a typical Helferich-promotor for glycosylation reactions. We observed a moderate increase of radiochemical yield of **[^{18}F]2** under the same reaction conditions as without $Hg(CN)_2$ (Table 1). However, we excluded $Hg(CN)_2$ from the reaction mixture due to its toxic properties which could cause problems when applying this procedure to the production of radiopharmaceuticals for human use. Yamada *et al*. used copper(II) triflate as an activator for peracetylated carbohydrates in an approach for a one-pot strategy of oligosaccharide synthesis.[25] In our attempts to utilize copper(II) triflate as a promoter we were not successful (Table 1), possibly due to the necessity to avoid moisture entirely. The copper(II) triflate used in this study was dried with P_2O_5 under reduced pressure, which may not be sufficient for subsequent reactions under no-carrier-added conditions.

When using higher reaction temperatures (> 80°C) clearly a more accelerated degradation of **[^{18}F]2** and **[^{18}F]3** was observed, accompanied by hydrolytic cleavage of the glycosidic bond, whereas at room temperature no ^{18}F-glycosylated product was detected (Table 1).

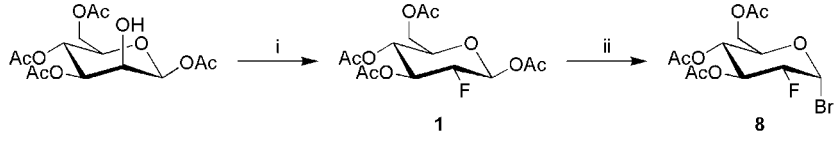
Thus, the use of BF_3 as a Lewis acid promotor and CH_3CN at 80°C turned out to be the optimum reaction. The concentration of Fmoc-protected amino acid necessary for a reliable RCY of **[^{18}F]2** was determined to be 5–10 mM (Table 2). As indicated in Table 2, a further increase in amino acid concentration did not result in distinct increased RCYs for the ^{18}F-glycosylation key step. A precursor concentration of 5–10 mM is suitable for peptide labelling, since these substrates are usually available in sufficient amounts, whereas an application of this radiosynthetic approach to the ^{18}F-glycosylation of rare (or expensive) compounds could be problematic.

Interestingly, Zemplén deacylation[26,27] of **[^{18}F]2** or **[^{18}F]3** also led to cleavage of the Fmoc-protecting group (**[^{18}F]6**, **[^{18}F]7**) under no-carrier-added reaction conditions. This observation depended largely upon reaction time. For compounds **[^{18}F]4** or **[^{18}F]5** a maximum RCY of about 40% (related to **[^{18}F]2/[^{18}F]3**) was observed after 2 min as determined by radio-HPLC. After 20 min deprotection was complete and **[^{18}F]6** or **[^{18}F]7** were obtained in 85 and 40% RCY, respectively. The major by-product was 2-deoxy-2-[^{18}F]fluoroglucose ([^{18}F]FDG), especially in the case of the threonyl compound (50% after

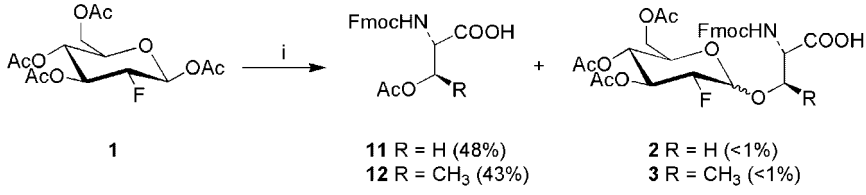
20 min, radio TLC (SiO_2, CH_3CN:H_2O, 9:1 (v/v)), that was easily separated by the subsequent cation exchange cartridge.

Optimized conditions and SPE techniques led to an experimental procedure consistent with the use of TA-[^{18}F]FDG (**[^{18}F]1**) as a ^{18}F-labelled glycosyl donor. The total synthesis time was 90 min, the overall radiochemical yield was about 25% (**[^{18}F]6**) and 12% (**[^{18}F]7**). Clearly, this two-step procedure including two HPLC separation steps could be difficult to adjust for automation in large-scale radiopharmaceutical production for human use, but for application in prior animal studies this ^{18}F-glycosylation method should be eminently practical. Therefore, we successfully utilized this laboratory ^{18}F-glycosylation procedure to obtain **[^{18}F]6** as a model compound to test the stability of the glycosidic linkage in human serum *in vitro*. Not surprisingly and as a proof of principle, **[^{18}F]6** was found to be stable in human serum at 37°C for up to 45 min (>98%, radio-TLC).

^{18}F-labelled radioactive compounds were identified by characterization of their corresponding ^{19}F-substituted analogs. Scheme 2 depicts the syntheses of the glycosyl donors 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-fluoro-*β*-D-glucopyranoside (**1**) and the corresponding α-bromide (**8**), which were obtained following literature procedures.[28–32] With these glycosyl donors in hand, we turned our attention toward the glycosylation reaction of Fmoc-Ser-OH and Fmoc-Thr-OH according to Elofsson's general procedure.[33] Activation of the anomeric *β*-acetyl of **1** (1 equiv) by $BF_3 \cdot Et_2O$ (3 equiv) to a solution of Fmoc-Ser or Fmoc-Thr (1 equiv) in dry CH_3CN provided low yields of **2** or **3**, respectively (Scheme 3). Interestingly, the main reaction was 3-*O*-acylation at the amino acid side chain (48% (**11**), 43% (**12**), Scheme 3) as similarly observed by



Scheme 2. (i) DAST, −40°C → rt; (ii) HBr/AcOH, CH_2Cl_2

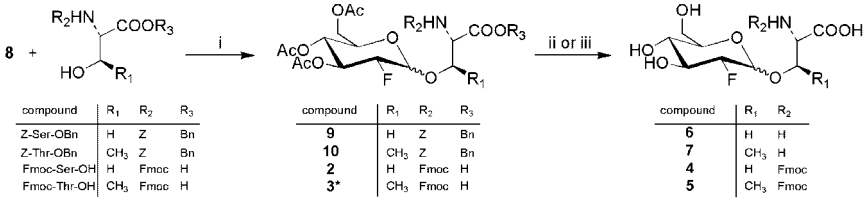


Scheme 3. Glycosylation method using boron trifluoride. (i) Fmoc-Ser-OH (for 11 and 2) or Fmoc-Thr-OH (for 12 and 3), $BF_3 \cdot Et_2O$, CH_3CN, rt, 24 h

Salvador *et al.* using pentaacetylated glycosyl donors, although to a minor extent.[18] In contrast to the reaction under no-carrier-added conditions (Scheme 1), this observation indicated preferential orthoester formation as an intermediate in the glycosylation reaction of 2-deoxy-2-fluoroglucosyl donors in the presence of BF_3, since orthoesters have been shown to rearrange with *O*-acetylation of the glycosyl acceptor under Lewis acid catalysis.[34]

In order to improve the yield of **2**, we used α-bromide **8** in a silver triflate mediated glycosylation, whereas **3** was advantageously obtained by treatment of Fmoc-Thr-OH with **1** in the presence of TMSOTf (Scheme 4). Compounds **2** and **3** were isolated by preparative reversed-phase HPLC in 7–8% yield. As the major by-products occurring during these coupling reactions, compounds **13** and **14** were isolated due to glycoester formation (Table 3, entry 7, 8). This observation was also described by Salvador *et al.*[18] and noticed by Seitz *et al.*[35] for the synthesis of *O*-glycopeptides. Subsequent Zemplén deacetylation of **2** and **3** afforded the Fmoc-protected compounds **4** and **5**, respectively, as analyzed by LC/MS. The deprotected glycosylated amino acids **6** and **7** were obtained by starting from the fully protected amino acid derivatives Z-Ser-OBn and Z-Thr-OBn using glycosyl donor **8**. AgOTf-mediated glycosylation provided **9** and **10** in improved yields, which were then deprotected by hydrogenolytic benzyl removal followed by subsequent Zemplén reaction resulting in an anomeric mixture of **6** and **7**, respectively.

The anomeric configuration of glycosylated intermediates and product compounds were analyzed by ^{1}H-NMR and ^{19}F-NMR spectroscopy, since the $^3J_{F,1}$ coupling constant is an excellent tool to distinguish between α ($^3J_{F,1}$ is negligible) and β ($^3J_{F,1} \approx 2.5$ Hz) configuration at C-1 in 2-deoxy-2-fluoro glycosides. A summary of analytical data that were used to ensure identity of the corresponding ^{18}F-labelled analogs is presented in Table 3. Although the 2-deoxy-2-fluoro glucopyranosyl donors **1** and **8** used in this study were opposite in anomeric configuration, the TMSOTf-mediated glycosylation of Fmoc-Thr



compound	R_1	R_2	R_3
Z-Ser-OBn	H	Z	Bn
Z-Thr-OBn	CH_3	Z	Bn
Fmoc-Ser-OH	H	Fmoc	H
Fmoc-Thr-OH	CH_3	Fmoc	H

compound	R_1	R_2	R_3
9	H	Z	Bn
10	CH_3	Z	Bn
2	H	Fmoc	H
3*	CH_3	Fmoc	H

compound	R_1	R_2
6	H	H
7	CH_3	H
4	H	Fmoc
5	CH_3	Fmoc

Scheme 4. Glycosylation method using silver triflate. (i) AgOTf, CH_2Cl_2, rt, 2 h; For compounds 6 or 7: (ii) 9 or 10, Pd black, $HCOONH_4$, methanol, rt, 15 min and (iii) NaOMe/MeOH, rt, 2 h. For compounds 4 or 5: (iii) 2 or 3, NaOMe/MeOH, rt, 24 h (4) or 4d (5). (* Treatment of Fmoc-Thr-OH with 1 and TMSOTf advantageously provided 3 in a α:β ratio of > 97:3; see Experimental)

Table 3. Analytical data of the various glycosylated compounds including α/β anomeric ratios determined by ^{1}H- and ^{19}F-NMR spectroscopy

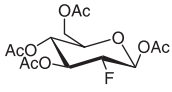
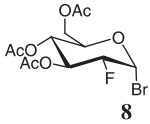
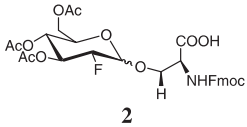
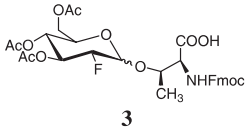
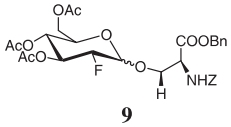
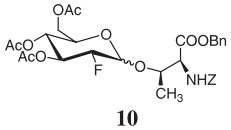
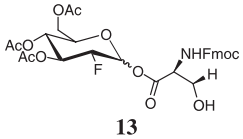
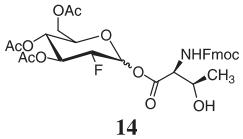
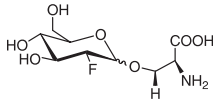
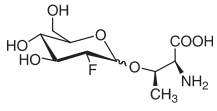
Entry	compound	yield (%)	R_f	k'[a]	^{1}H-NMR chemical shift of H-1 (ppm)	α/β[‡]
1	 **1**	33	0.70[b]	4.5	5.75 (β)[f]	pure β
2	 **8**	88	0.77[b]	6.2	n.d.	pure α
3	 **2**	8	0.20[d]	10.3	5.17 (α), 4.94 (β)[g]	66/34
4	 **3**	7	0.18[d]	11.2	5.24 (α)[g]	>97/3
5	 **9**	35	0.71[b]	13.8	4.91 (α), 4.75 (β)[f]	97/3
6	 **10**	43	0.86[c]	14.7	4.95 (α), 4.70 (β)[f]	97/3
7	 **13**	5	n.d.	10.8	6.30 (α), 6.05 (β)[g]	70/30
8	 **14**	40	n.d.	12.0	6.31 (α), 6.08 (β)[g]	80/20

Table 3. continued

Entry	compound	yield (%)	R_f	k'[a]	^{1}H-NMR chemical shift of H-1 (ppm)	α/β ‡
9	 **6**	11	0.14[e]	0.2	5.06 (α), 4.68 (β)[h]	66/34
10	 **7**	7	0.20[e]	0.2	5.13 (α)[h]	67/33

[a] Kromasil C8, 250 × 4.6, 1.5 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min.
[b] Ethyl acetate/*n*-hexane 1:1.
[c] CH_2Cl_2/MeOH 95:5.
[d] Ethyl acetate/*n*-hexane 7:3.
[e] CH_3CN/9.5mM tetrabutylammonium hydroxide 8:2.
[f] In $CDCl_3$.
[g] In DMSO-d_6.
[h] In D_2O.
[i] determined by ^{19}F-NMR.

using **1** (Table 3, entry 4) and also the silver-promoted glycosylation reactions (Scheme 4, Table 3, entry 3, 5, 6) proceeded with preferential α-stereoselectivity. Thus, the product distribution obtained was consistent with an S_N1-type reaction mechanism due to the thermodynamic effect (anomeric effect) in tetrahydropyrans which generally favors axial linkage of electronegative functional groups to C-1.[36] In contrast to 2-deoxy-2-iodo glycosyl donors used for selective β-glycosylation,[37] we conclude that glycosylation reactions employing 2-deoxy-2-fluoro glucopyranosyl donors **1** or **8** are stereoelectronically controlled and proceed via an fluoroglycosyl oxocarbonium intermediate with excellent α-stereoselectivities. However, reaction conditions for the syntheses of standard compounds were not in accordance with those of the ^{18}F-labelled analogs **[^{18}F]2** and **[^{18}F]3**, so that further studies are required to gain precise information about the α/β ratio under no-carrier-added reaction conditions. Noteworthy, the decrease of α/β ratio for compounds **6** and **7** after Zemplén deprotection (Table 3, entry 9, 10) could not be attributed to epimerization of amino acid stereocenters or β-elimination, since these side-reactions were excluded by a systematic study of Sjölin *et al.* on a model compound.[38] It is tempting to speculate that anomerization occurred during the work-up procedure for deprotected anomeric mixtures of **6** and **7**, respectively.

Experimental

General

All chemicals and reagents were of analytical grade and obtained from commercial sources. [^{18}F]Fluoride was obtained from PET Net GmbH (Erlangen, Germany). Solid phase cartridges (Merck LiChrolut® Si 200 mg, LiChrolut® RP-18 100 mg and LiChrolut® SCX 100 mg) were purchased from VWR International. Thin layer chromatography (TLC) was carried out on silica gel-coated aluminum plates (Alugram®, Sil G/UV$_{254}$, Macherey Nagel); for radio-TLC plastic sheets (Polygram®, Sil G/UV$_{254}$, Macherey Nagel) were used. Compounds were visualized by UV light (254 nm), charring with anisaldehyde solution (anisaldehyde/sulfuric acid/acetic acid, 1:2:97 (v/v/v)) or charring with ninhydrin reagent (1% ninhydrin in ethanol). Analytical HPLC was performed on the following system: HPLC Hewlett Packard (HP 1100) with a quarternary pump and variable wavelength detector (HP 1100) and radio-HPLC-detector D505TR (Canberra Packard). Computer analysis of the HPLC data was performed using FLO-One software (Canberra Packard). Preparative HPLC was performed on a Knauer system (HPLC pump 64, LC photometer at 254 nm) using columns and conditions as specified in the text. Electronic autoradiography (Instant Imager™, Canberra Packard) was used to analyze radio-TLC data. NMR spectra were recorded on a Varian Gemini-300 spectrometer, operating at 300.18 MHz for ^{1}H and 282.41 MHz for ^{19}F by Deutero GmbH (Kastellaun, Germany). ESI-MS and LC-MS analysis were performed on an Agilent 1100 Series analytic HPLC system with a VWL detector, coupled to a Bruker esquire 2000 mass spectrometer with electron spray ionization. 1,3,4,6-tetra-*O*-acetyl-β-D-mannopyranose,[28,29] 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluormethanesulfonyl-β-D-mannopyranose[30] and 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl bromide[31] (**8**) were synthesized following literature procedures. ^{19}F-NMR of **8** confirmed the pure α-anomer (^{19}F-NMR ($CDCl_3$): δ −189.27 (dd, $J_{F,2} = 49.4$ Hz, $J_{F,3} = 11.0$ Hz)).

*Radiosynthesis of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-fluoro-*D*-glucopyranose (*[^{18}F]**1**)

For the preparation of **[^{18}F]1** a QMA-cartridge with [^{18}F]fluoride was eluted with a solution of 15 mg Kryptofix® 2.2.2/15 µl 1 M K_2CO_3 in 1 ml acetonitrile/water (8:2). The solution was evaporated using a stream of nitrogen at 85°C and co-evaporated to dryness with CH_3CN (2 × 200 µl). According to the FDG-synthesis by Hamacher *et al.*,[23] 9 mg precursor (1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluormethanesulfonyl-β-D-mannopyranose) in 450 µl anhydrous acetonitrile was added. This mixture was stirred for 5 min at 85°C and passed through a SiO_2-cartridge (Merck, 200 mg). The radiochemical yield of TA-[^{18}F]FDG was 93% as determined by analytical HPLC from a sample withdrawn from the reaction mixture. After elution with 1.0 ml acetonitrile and evaporation of

the solvent the residue was taken up in 500 µl acetonitrile/water (30:70) and submitted to semipreparative HPLC (Kromasil C8, 125 × 8, 3.5 ml/min, acetonitrile/ water 30:70). The product fraction was diluted 1:10 with water and fixed on a C18-cartridge (Merck, 100 mg), dried in a nitrogen stream and eluted with 1 ml acetonitrile in a reaction vessel. Starting from 370 MBq [^{18}F]F^- this procedure yielded 250 MBq **[^{18}F]1** in a reaction time of 30 min.

^{18}F-glycosylation procedure

Radiosyntheses of N^{α}-(9-fluorenylmethoxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-deoxy-2-[^{18}F]fluoro-D-glucopyranosyl)-L-serine ([^{18}F]2) and N^{α}-(9-fluorenylmethoxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-deoxy-2-[^{18}F]fluoro-D-glucopyranosyl)-L-threonine ([^{18}F]3)

Fmoc-protected serine or threonine (10 mM) in 200 µl anhydrous CH_3CN and 5 µl BF_3 etherate were added to a reaction vessel containing dry **[^{18}F]1** at 80°C. After the reaction time of 5 min the mixture was diluted with H_2O 1:10 and passed through a C18-cartridge (Merck, 100 mg). The cartridge was washed with CH_3CN/H_2O (30:70) to remove hydrolytic by-products, dried and eluted with 1.0 ml CH_3CN. The solvent was evaporated and the residue (diluted in 500 µl acetonitrile/water (40:60)) was transferred to the semipreparative HPLC-system. The radioactive products (**[^{18}F]2** or **[^{18}F]3**) were isolated by gradient reversed-phase HPLC (Kromasil C8, 250 × 8 mm, 4 ml/min, 40–100% CH_3CN in H_2O (0.1% TFA) within 50 min) in a radiochemical yield of 32% for the threonine derivative or 48% for the serine derivative. The product fraction was diluted with water (1:10), fixed on a C18-cartridge (Merck, 100 mg), dried in a stream of nitrogen and eluted with 1 ml acetonitrile in a reaction vessel. Starting from 250 MBq **[^{18}F]1** this procedure yielded 85 MBq **[^{18}F]2** or 55 MBq **[^{18}F]3** within 30 min.

Optimization of the ^{18}F-glycosylation procedure

The ^{18}F-glycosylation procedure was optimized by repeating the reaction with varying parameters as indicated in Table 1.

Radiosynthesis of O-(2-deoxy-2-[^{18}F]fluoro-D-glucopyranosyl)-L-serine ([^{18}F]6)

The solution of **[^{18}F]2** in CH_3CN was evaporated to dryness as described before. Subsequently, 50 mM NaOMe in dry methanol was added and the mixture was stirred 20 min at 60°C leading to deprotection of the glucose unit and of the Fmoc-protecting group to afford **[^{18}F]6**. The radiochemical yield of this cleavage was 85%. **[^{18}F]6** was fixed on a SCX-cartridge (100 mg) and eluted with 600 µl PBS. Starting from 85 MBq **[^{18}F]2** this procedure yielded 55 MBq **[^{18}F]6** in 27 min. The total synthesis time (starting from [^{18}F]F^-) was about 90 min, the overall radiochemical yield was about 25% for **[^{18}F]6**.

*Radiosynthesis of O-(2-deoxy-2-[^{18}F]fluoro-D-glucopyranosyl)-L-threonine ([^{18}F]**7**)*

The solution of **[^{18}F]2** in CH_3CN was evaporated. Subsequently, 20 mM NaOMe in dry methanol was added and the mixture was stirred 20 min at 60°C leading to deprotection of the glucose unit and of the Fmoc-protecting group to afford **[^{18}F]7**. The radiochemical yield of this cleavage was 40%.

*1,3,4,6-Tetra-O-acetyl-2-deoxy-2-fluoro-β-D-glucopyranose (**1**)*

1 was synthesized by fluorination of 1,3,4,6-tetra-*O*-acetyl-*β*-D-mannopyranose (3.45 g, 10.35 mmol) using diethylamino sulfur trifluoride (DAST) according to the method described by Card.[32] The crude product was purified by column chromatography (silica gel, ethyl acetate/*n*-hexane 7:3) to give 33% (1.2 g, 3.4 mmol) after crystallization in diethylether. ^{1}H- and ^{19}F-NMR were consistent with the literature data.[39,40]

*N^{α}-(Phenylmethoxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-D-glucopyranosyl)-L-serine phenylmethyl ester (**9**)*

138 mg (539 μmol) silver triflate were added to a cooled (0°C) solution of 200 mg (539 μmol) **8** and 177 mg (539 μmol) N^{α}-(phenylmethoxycarbonyl)-L-serine phenylmethyl ester (Bachem, Germany) in 2 ml CH_2Cl_2. The cooling bath was removed and the suspension was stirred at room temperature. The reaction was monitored by TLC. After 2 h the suspension was filtered through Celite®, washed with saturated $NaHCO_3$ and water, dried with Na_2SO_4 and concentrated. The residue was purified by column chromatography (silica gel, ethyl acetate/*n*-hexane 1:1) to give 35% (117 mg , 189 μmol) of **9**. TLC (ethyl acetate/*n*-hexane 1:1): R_f = 0.71. HPLC (Kromasil C8, 250 × 4.6, 1.5 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min): k' = 13.8. ^{1}H-NMR ($CDCl_3$): δ 7.40-7.23 (m, 10H, CH-benzyl), 5.84 (d, 1H, NH, J = 8.1 Hz), 5.39 (dt, 1H, H-3, $J_{3,F}$ = 11.6 Hz, $J_{3,4}$ = 9.5 Hz), 5.21-5.08 (m, 4H, 2 × CH_2-benzyl), 4.92 (t(dd), 1H, H-4, $J_{4,5}$ = 9.5 Hz), 4.91 (d, 1H (α), H-1, $J_{1,2}$ = 3.6 Hz), 4.75 (dd, 1H, H-1 (*β*), $J_{1,2}$ = 9.6 Hz, $J_{1,F}$ = 4.0 Hz), 4.59 (dt, α-CH (Ser)), 4.40 (ddd, 1H, H-2, $J_{2,F}$ = 48.6 Hz, $J_{2,3}$ = 9.5 Hz), 4.20-4.09 (m, 2H, H-6a, H-6b), 4.02-3.97 (m, 2H, *β*-CH_2(Ser)), 3.95-3.87 (ddd, 1H, H-5), 2.06 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.01 (s, 3H, OAc). ^{19}F-NMR ($CDCl_3$): δ −201.93 (dd, $J_{F,2}$ = 48.6 Hz, $J_{F,3}$ = 11.6 Hz, α-anomer), −200.16 (ddd, $J_{F,2}$ = 50.1 Hz, $J_{F,3}$ = 14.6 Hz, $J_{F,1}$ = 2.6 Hz, *β*-anomer); α/*β* = 97/3.

*N^{α}-(Phenylmethoxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-D-glucopyranosyl)-L-threonine phenylmethyl ester (**10**)*

A mixture of 200 mg (539 μmol) **8** and 185 mg (539 μmol) N^{α}-(phenylmethoxycarbonyl)-L-threonine phenylmethyl ester in 2 ml CH_2Cl_2 was treated as described for the preparation of glycoside **9**. The residue was eluted from a

column of silica gel with CH_2Cl_2/methanol (95:5) to give 43% (147 mg, 108 μmol) of **10**. TLC (CH_2Cl_2/methanol 95:5): R_f = 0.86. HPLC (Kromasil C8, 250 × 4.6, 1.5 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min): $k' = 14.7$. ^{1}H-NMR ($CDCl_3$): δ 7.36-7.25 (m, 10H, CH-benzyl), 5.51 (d, 1H, NH, $J = 9.6$ Hz), 5.37 (dt, 1H, H-3, $J_{3,F} = 12.9$ Hz, $J_{3,4} = 9.6$ Hz), 5.17-5.05 (m, 4H, 2 × CH_2-benzyl), 4.95 (d, 1H, H-1 (α), $J_{1,2} = 3.6$ Hz), 4.91 (t(dd), 1H, H-4, $J_{4,5} = 9.6$ Hz), 4.70 (dd, 1H, H-1 (β), $J_{1,2} = 9.5$ Hz, $J_{1,F} = 3.9$ Hz), 4.47 (dd, α-CH (Thr), $J_{H,NH} = 9.6$ Hz, $J_{H,CH} = 2.2$ Hz), 4.38 (m, 1H, β-CH (Thr)), 4.32 (ddd, 1H, H-2, $J_{2,F} = 49.3$ Hz, $J_{2,3} = 9.6$ Hz), 4.19 (dd, 1H, H-6a, $J_{6a,6b} = 12.4$ Hz, $J_{6a,5} = 5.4$ Hz), 4.02 (dd, 1H, H-6b, $J_{6b,5} = 2.2$ Hz), 4.01 (ddd, 1H, H-5), 2.05 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.34 (d, 3H, CH_3 (Thr), $J = 6.7$ Hz). ^{19}F-NMR ($CDCl_3$): δ −200.68 (dd, $J_{F,2} = 49.3$ Hz, $J_{F,3} = 12.9$ Hz, α-anomer), −200.78 (ddd, $J_{F,2} = 52.2$ Hz, $J_{F,3} = 14.6$ Hz, $J_{F,1} = 3.3$ Hz, β-anomer); α/β 97/3. ESI-MS: 633.9 $[M+H]^+$.

*N^{α}-(9-Fluorenylmethoxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-*D*-glucopyranosyl)-*L*-serine (**2**)*

Method A: Glycosylation method using AgOTf. A mixture of 100 mg (270 μmol) **8** and 88 mg (270 μmol) N^{α}-(9-fluorenylmethoxycarbonyl)-L-serine in 1 ml CH_2Cl_2 was treated as described for the preparation of glycoside **9**. The residue was isolated by preparative HPLC (Kromasil C8, 250 × 20, 15 ml/min, CH_3CN/H_2O 1:1 (0.1% TFA)) to give 8% (13 mg, 21 μmol) of **2**. TLC (ethyl acetate/*n*-hexane 7:3): R_f = 0.20. HPLC (Kromasil C8, 250 × 4.6, 1.5 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min): $k' = 10.3$. ^{1}H-NMR (DMSO-d_6): δ 7.88-7.24 (m, 8H, CH-Fmoc), 5.38 (dt, 1H, H-3, $J_{3,F} = 12.5$ Hz, $J_{3,4} = 9.6$ Hz), 5.17 (d, 1H, H-1 (α), $J_{1,2} = 3.6$), 4.94 (dd, 1H, H-1 (β), $J_{1,2} = 7.4$ Hz, $J_{1,F} = 2.1$ Hz), 4.87 (t(dd), 1H, H-4, $J_{4,5} = 9.6$ Hz), 4.67 (ddd, 1H, H-2, $J_{2,F} = 48.5$ Hz, $J_{2,3} = 9.6$ Hz), 4.34-4.19 (m, 4H, α-CH (Ser), CH_2-Fmoc, CH-Fmoc), 4.15-4.05 (m, 2H, H-6a, H-6b), 4.04-3.92 (m, 3H, H-5, β-CH_2 (Ser)), 2.00 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.95 (s, 3H, OAc). ^{19}F-NMR (DMSO-d_6): δ −201.1 (dd, $J_{F,2} = 48.5$ Hz, $J_{F,3} = 12.5$ Hz, α-anomer), −199.6 (ddd, $J_{F,2} = 51.2$ Hz, $J_{F,3} = 14.3$ Hz, $J_{F,1} < 2.5$ Hz, β-anomer); α/β = 66/34. MS (APCI): $m/z = 617.9$ $[M+H]^+$, ESI-MS: 639.9 [M + Na], 635.0 $[M+H_2O]^+$, 615.9 $[M-H]^-$.

As a major by-product 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-glucopyranosyl N^{α}-(9-fluorenylmethoxycarbonyl)-L-serinoate (**13**) was characterized as follows: HPLC (Kromasil C8, 250 × 4.6, 1.5 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min): $k' = 10.8$. ^{1}H-NMR (DMSO-d_6): δ 7.88-7.24 (m, 8H, CH-Fmoc), 6.30 (d, 1H, H-1 (α), $J_{1,2} = 3.8$ Hz), 6.05 (dd, 1H, H-1 (β), $J_{1,2} = 7.9$ Hz, $J_{1,F} = 3.3$ Hz), 5.40 (dt, 1H, H-3, $J_{3,F} = 12.2$ Hz, $J_{3,4} = 10.1$ Hz), 4.96 (t(dd), 1 H, H-4, $J_{4,5} = 10.1$ Hz), 4.93 (ddd, 1 H, H-2, $J_{2,F} = 48.6$ Hz, $J_{2,3} = 10.1$ Hz), 4.34-4.19 (m, 4H, α-CH (Ser), CH_2-Fmoc, CH-Fmoc),

4.15-4.05 (m, 2H, H-6a, H-6b), 4.04-3.92 (m, 3H, H-5, β-CH_2 (Ser)), 2.00 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.95 (s, 3H, OAc). ^{19}F-NMR (DMSO-d_6): δ −202.3 (dd, $J_{F,2} = 48.6$ Hz, $J_{F,3} = 12.2$ Hz, α-anomer), −201.2 (ddd, $J_{F,2} = 51.9$ Hz, $J_{F,3} = 12.0$ Hz, $J_{F,1} < 3.5$ Hz, β-anomer); $\alpha/\beta = 70/30$. MS (APCI): m/z = 617.9 $[M+H]^+$, ESI-MS: 635.0 $[M+H_2O]^+$, 615.9 $[M-H]^-$.

Method B: Glycosylation method using BF_3. 108 µl (0.86 mmol) $BF_3 \cdot Et_2O$ were added to a solution of 100 mg (285 µmol) **1** and 93 mg (285 µmol) N^{α}-(9-fluorenylmethoxycarbonyl)-L-serine in 2 ml dry acetonitrile. The mixture was stirred at room temperature and the reaction was monitored by gradient HPLC (Kromasil C8, 250 × 4.6, 1.5 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min). After 24 h the solution was washed with saturated $NaHCO_3$ and water, dried with Na_2SO_4 and concentrated. The residue was purified by preparative HPLC (Kromasil C8, 250 × 20, 15 ml/min, CH_3CN/H_2O 1:1 (0.1% TFA)) to give only <1% (<2 mg, <3 µmol) of **2**. HPLC (Kromasil C8, 250 × 4.6, 1.5 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min): $k' = 10.3$. The main reaction was *O*-acylation at the amino acid side chain yielding 48% of N^{α}-(9-fluorenylmethoxycarbonyl)-*O*-acetyl-L-serine (**11**). This product was confirmed by ^{1}H-NMR and HPLC (Kromasil C8, 250 × 4.6, 1.5 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min): $k' = 6.6$) as compared to the commercially available reference (Bachem No.: B-1010). ^{1}H-NMR (DMSO-d_6): δ 13.0 (1H, COOH), 7.9-7.3 (m, 9H, NH, CH-Fmoc), 4.4-4.2 (m, 6H, α-CH, CH_2-Fmoc, CH-Fmoc, β-CH_2), 2.0 (s, 3H, OAc).

*N^{α}-(9-Fluorenylmethoxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-deoxy-2-fluoro-*D*-glucopyranosyl)-*L*-threonine (**3**)*

Method A: Glycosylation method using AgOTf. A mixture of 100 mg (270 µmol) **8** and 92 mg (270 µmol) N^{α}-(9-fluorenylmethoxycarbonyl)-L-threonine in 2 ml CH_2Cl_2 was treated as described for the preparation of glycoside **9**. The residue was isolated by preparative HPLC (Kromasil C8, 250 × 20, 15 ml/min, CH_3CN/H_2O 60:40 (0.1% TFA)) to give only <1% (<2 mg, <3 µmol) of **3**.

As a major by-product (25%) 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-glucopyranosyl N^{α}-(9-fluorenylmethoxycarbonyl)-L-threoninoate (**14**) was characterized as follows: HPLC (Kromasil C8, 250 × 4.6, 1.5 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min): $k' = 12.0$. ^{1}H-NMR (DMSO-d_6): δ 7.88-7.25 (m, 8H, CH-Fmoc), 6.31 (d, 1H, H-1 (α), $J_{1,2} = 3.9$ Hz), 6.08 (dd, 1H, H-1 (β), $J_{1,2} = 8.1$ Hz, $J_{1,F} = 3.9$ Hz), 5.47 (dt, 1H, H-3, $J_{3,F} = 12.4$ Hz, $J_{3,4} = 9.5$ Hz), 4.96 (t(dd), 1H, H-4, $J_{4,5} = 9.9$ Hz), 4.93 (ddd, 1H, H-2 (α), $J_{2,F} = 47.6$ Hz, $J_{2,3} = 9.7$ Hz), 4.55 (ddd, 1H, H-2 (β), $J_{2,F} = 50.8$ Hz, $J_{2,3} = 9.0$ Hz, $J_{2,1} = 8.0$ Hz), 4.32-3.95 (m, 8H, α-CH (Thr), CH_2-Fmoc,

CH-Fmoc, β-CH (Thr), H-6a, H-6b, H-5), 2.02 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.93 (s, 3H, OAc), 1.14 (d, 3H, CH_3 (Thr), $J = 6.3$ Hz). ^{19}F-NMR (DMSO-d_6): δ −202.4 (dd, $J_{F,2} = 48.2$ Hz, $J_{F,3} = 12.6$ Hz, α-anomer), −201.3 (ddd, $J_{F,2} = 51.4$ Hz, $J_{F,3} = 14.6$ Hz, $J_{F,1} = 3.5$ Hz β-anomer); $\alpha/\beta =$ 80/20. ESI-MS: 649.0 $[M + H_2O]^+$.

Method B: Glycosylation method using BF_3. 108 µl (855 µmol) $BF_3 \cdot Et_2O$ were added to a solution of 100 mg (285 µmol) **1** and 97 mg (285 µmol) N^{α}-(9-fluorenyl-methoxycarbonyl)-L-threonine in 2 ml dry acetonitrile. The mixture was stirred at room temperature and the reaction was monitored by gradient HPLC (Kromasil C8, 250 × 4.6, 1.5 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min). After 24 h the solution was washed with saturated $NaHCO_3$ and water, dried with Na_2SO_4 and concentrated. The residue was purified by preparative HPLC (Kromasil C8, 250 × 20, 15 ml/min, CH_3CN/H_2O 1:1 (0.1% TFA)) to give only <1% (<2 mg, <3 µmol) of **3**.

The main reaction was *O*-acylation at the amino acid side chain yielding 43% of N^{α}-(9-fluorenylmethoxycarbonyl)-*O*-acetyl-L-threonine (**12**). HPLC: (Kromasil C8, 250 × 4.6, 1.5 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min): $k' = 6.6$). ^{1}H-NMR (DMSO-d_6): δ 12.8 (1H, COOH), 7.9-7.3 (m, 9 H, NH, CH-Fmoc), 5.2 (m, 1H, β-CH_2), 4.4-4.2 (m, 4H, α-CH, CH_2-Fmoc, CH-Fmoc), 2.0 (s, 3H, OAc), 1.2 (d, 3H, CH_3). LC-MS (APCI): 406.0 $[M + Na]^+$.

Method C: Glycosylation method using TMSOTf. 250 µl (2.2 mmol) TMSOTf were added to a solution of 33 mg (94 µmol) **1** and 36 mg (105 µmol) N^{α}-(9-fluorenylmethoxycarbonyl)-L-threonine in 3 ml dry CH_2Cl_2. The mixture was stirred at room temperature and the reaction was monitored by gradient HPLC (Kromasil C8, 250 × 4.6, 1.5 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min). After 2 days the solution was washed with saturated $NaHCO_3$ and water, dried with Na_2SO_4 and concentrated. The residue was purified by semipreparative HPLC (Kromasil C8, 125 × 8, 4 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min) to give 7% (4,5 mg, 7 µmol) of **3**. TLC (ethyl acetate/*n*-hexane 7:3): $R_f = 0.18$. HPLC (Kromasil C8, 250 × 4.6, 1.5 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min): $k' = 11.2$. ^{1}H-NMR (DMSO-d_6): δ 7.92-7.26 (m, 8H, CH-Fmoc), 5.46 (dt, 1H, H-3, $J_{3,F} = 11.8$ Hz, $J_{3,4} = 9.6$ Hz), 5.24 (d, 1H, H-1(α), $J_{1,2} = 3.8$ Hz), 4.84 (t(dd), 1H, H-4, $J_{4,5} = 9.8$ Hz), 4.61 (ddd, 1H, H-2, $J_{2,F} = 49.0$, $J_{2,3} = 9{,}7$ Hz), 4.31-3.97 (m, 8H, α-CH (Thr), CH_2-Fmoc, CH-Fmoc, β-CH (Thr), H-6a, H-6b, H-5), 2.00 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.26 (d, 3H, CH_3 (Thr), $J = 6.3$ Hz). ^{19}F-NMR (DMSO-d_6): δ −200.07 (dd, $J_{F,2} = 48.8$ Hz, $J_{F,3} = 12.3$ Hz, α-anomer); $\alpha/\beta > 97/3$. ESI-MS: $m/z = 631.9$ $[M + H]^+$, 649.0 $[M + H_2O]^+$.

N^{α}-(9-Fluorenylmethoxycarbonyl)-O-(2-deoxy-2-fluoro-D-*glucopyranosyl)*-L-*serine* (**4**)

Deacetylation of **2** was performed following a procedure firstly described by Zemplén.[26] Briefly, 13 mg (21 μmol) of **2** were stirred at room temperature in a solution of 25 mM NaOMe in anhydrous methanol (1 ml) for 24 h. The mixture was neutralized with Amberlite® IR-120 (H^+) and separated by semipreparative HPLC (Kromasil C8, 125 × 8, 4 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min) to yield 40% (3.9 mg, 8.4 μmol) of **4**. HPLC (Kromasil C8, 250 × 4.6, 1.5 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min): $k' = 2.6$. ESI-MS: $m/z = 514.0$ $[M + Na]^+$.

N^{α}-(9-Fluorenylmethoxycarbonyl)-O-(2-deoxy-2-fluoro-D-*glucopyranosyl)*-L-*threonine* (**5**)

34 mg (54 μmol) **3** were stirred at room temperature in a solution of 6 mM NaOMe in anhydrous methanol (3 ml) for 4 days. The mixture was neutralized with Amberlite® IR-120 (H^+) and separated by semipreparative HPLC (Kromasil C8, 125 × 8, 4 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min) to yield 10% (2.7 mg, 5.4 μmol) of **5**. HPLC (Kromasil C8, 250 × 4.6, 1.5 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min): $k' = 4.2$. ESI-MS: $m/z = 505.9$ $[M + H]^+$.

O-(2-Deoxy-2-fluoro-D-*glucopyranosyl)*-L-*serine* (**6**)

124 mg (3.8 mmol) ammonium formate and 78 mg (0.73 mmol) palladium black were added to a solution of 117 mg (189 μmol) N^{α}-(phenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-glucopyranosyl)-L-serine phenylmethyl ester (**9**) in 8 ml methanol under a nitrogen atmosphere. The suspension was stirred at room temperature for 15 min, filtered through Celite® and concentrated. The residue was dissolved in 15 ml 50 mM NaOMe in methanol for 2 h. The mixture was neutralized with Amberlite® IR-120 (H^+) and concentrated. Demineralization and isolation was realized by passing the reaction through a BioGel® P2 column (Biorad) and afterwards a PD-10 Sephadex® G-25 M column (Pharmacia). The aqueous solution was lyophilized giving 11% (5.6 mg, 21 μmol) of **6**. TLC (CH_3CN/9.5 mM tetrabutylammonium hydroxide 8:2): R_f = 0.14. HPLC (Kromasil C8, 250 × 4.6, 1.5 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min): $k' = 0.2$. ^{1}H-NMR (D_2O): δ 8.35 (s, 2H, NH_2), 5.06 (d, 1H, H-1 (α), $J_{1,2} = 3.8$ Hz), 4.68 (dd, 1H, H-1 (*β*), $J_{1,2} = 7.4$ Hz, $J_{1,F} = 3.0$ Hz), 4.33(ddd, 1H, H-2, $J_{2,F} = 48.0$ Hz, $J_{2,3} = 9.6$ Hz), 4.10 (dd, 1H, H-6a, $J_{6a,6b} = 10.5$ Hz, $J_{6a,5} = 3.1$ Hz), 3.94-3.35 (m, 6H, H-3, H-6b, H-5, H-4, CH_2 (Ser)). ^{19}F-NMR (D_2O): δ −196.13 (ddd, $J_{F,2} = 50.8$ Hz, $J_{F,3} = 15.9$ Hz, $J_{F,1} = 1.7$ Hz, *β*-anomer), −197.5 (dd, $J_{F,2} = 48.0$ Hz, $J_{F,3} = 13.6$ Hz, α-anomer); $\alpha/\beta = 66/34$.

*O-(2-Deoxy-2-fluoro-*D*-glucopyranosyl)-*L*-threonine (**7**)*

147 mg (108 μmol) N^{α}-(phenylmethoxycarbonyl)-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-glucopyranosyl)-L-threonine phenylmethyl ester (**10**) were treated as described for the preparation of **6** yielding 7% (2 mg, 7.5 μmol) of **7**. TLC (CH_3CN/9.5 mM tetrabutyl-ammonium hydroxide 8:2): R_f = 0.20. HPLC (Kromasil C8, 250 × 4.6, 1.5 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min): $k' = 0.2$. ^{1}H-NMR (D_2O): δ 8.35 (s, 2H, NH_2), 5.13 (d, 1H, H-1 (α), $J_{1,2} = 3.9$ Hz), 4.28 (ddd, 1H, H-2, $J_{2,F} = 48.5$ Hz, $J_{2,3} = 9.3$ Hz), 3.94-3.30 (m, 6H, H-3, H-6a, H-6b, H-5, H-4, CH (Thr)), 1.30 (d, 3H, CH_3 (Thr), $J = 6.3$ Hz). ^{19}F-NMR (D_2O): δ −195.76 (ddd, $J_{F,2} = 49.1$ Hz, $J_{F,3} = 14.3$ Hz, $J_{F,1} = 2.6$ Hz, β-anomer), −196.00 (dd, $J_{F,2} = 47.4$ Hz, $J_{F,3} = 12.8$ Hz, α-anomer); α/β = 67/33.

Metabolic stability of [^{18}F]**6** *in human serum*

200 μl of **[^{18}F]6** were dissolved in 2 ml of human serum and incubated at 37°C. Aliquots (100 μl) were taken at various time intervals (5–45 min) and quenched in 500 μl methanol/CH_2Cl_2 (1:1). The samples were centrifuged and the supernatants analyzed by radio-TLC using CH_3CN/9.5 mM tetrabutylammonium hydroxide (8:2) as eluant.

Conclusion

In summary, we developed a two-step ^{18}F-glycosylation method based on the commonly available intermediate 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-[^{18}F]fluoro-glucopyranose (**[^{18}F]1**), which occurs as an intermediate in the [^{18}F]FDG synthesis. This prosthetic group was successfully coupled to the side chains of Fmoc-protected serine and threonine by the use of boron trifluoride as promotor. The ^{18}F-glycosylation method afforded a radiochemical yield of about 25% for the serinyl compound within a total synthesis time of 90 min. Thus, **[^{18}F]1** is a promising ^{18}F-labelled prosthetic group suitable for ^{18}F-glycosylation of amino acid side chains and could be adapted for ^{18}F-labelling of bioactive peptides to study their pharmacokinetics using positron emission tomography (PET). Further studies have to investigate the scope of this methodology particularly with regard to its potential to provide not only a method for ^{18}F-glycosylation but also the opportunity to improve the biokinetics of PET-radiophamaceuticals.

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