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Research Article

^{18}F-glycosylation using Koenigs–Knorr conditions: a comparative study

Simone Maschauer, Torsten Kuwert and Olaf Prante*
Laboratory of Molecular Imaging, Clinic of Nuclear Medicine, Friedrich-Alexander University, Schwabachanlage 6, D-91054 Erlangen, Germany

Summary

We compared two ^{18}F-glycosylation methods, the BF_3-mediated ^{18}F-glycosylation versus the newly developed AgOTf-activated ^{18}F-glycosylation procedure. The AgOTf-activated ^{18}F-glycosylation makes use of 3,4,6-tri-O-acetyl-2-deoxy-2-[^{18}F]fluoro-glucopyranosyl bromide and revealed an improved radiochemical yield of $67 \pm 6\%$ in the case of a protected serinyl precursor as compared to $27 \pm 4\%$ obtained by the BF_3-method. This suggests the suitability of Koenigs–Knorr conditions for ^{18}F-glycosylation of protected bioactive peptides. Copyright © 2006 John Wiley & Sons, Ltd.

Key Words: ^{18}F-glycosylation; F-18; Koenigs–Knorr; glycosyl donor; PET

Introduction

The use of ^{18}F-labelled prosthetic groups for the radiofluorination of biomolecules has gained considerable importance for the synthesis of radiopharmaceuticals suitable for positron emission tomography (PET), since the presence of sensitive functional groups excludes a direct nucleophilic radiofluorination reaction under basic conditions. In particular, *N*-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB),[1–3] 4-nitrophenyl-2-[^{18}F]fluoropropionate,[4] 4-[^{18}F]fluorobenzaldehyde[5,6] or ^{18}F-labelled maleimide derivatives[6,7] have found widespread use for ^{18}F-labelling of peptides and proteins. As an extension of the available ^{18}F-labelling agents, we recently examined the applicability of tetra-O-acetylated 2-[^{18}F]fluoro-2-deoxy-glucopyranose (**[^{18}F]1**) as ^{18}F-glycosylation agent.[8] However, this approach implies problems

*Correspondence to: Olaf Prante, Laboratory of Molecular Imaging, Clinic of Nuclear Medicine, Friedrich-Alexander University Erlangen-Nürnberg, Schwabachanlage 6, D-91054 Erlangen, Germany.
E-mail: olaf.prante@nuklear.imed.uni-erlangen.de

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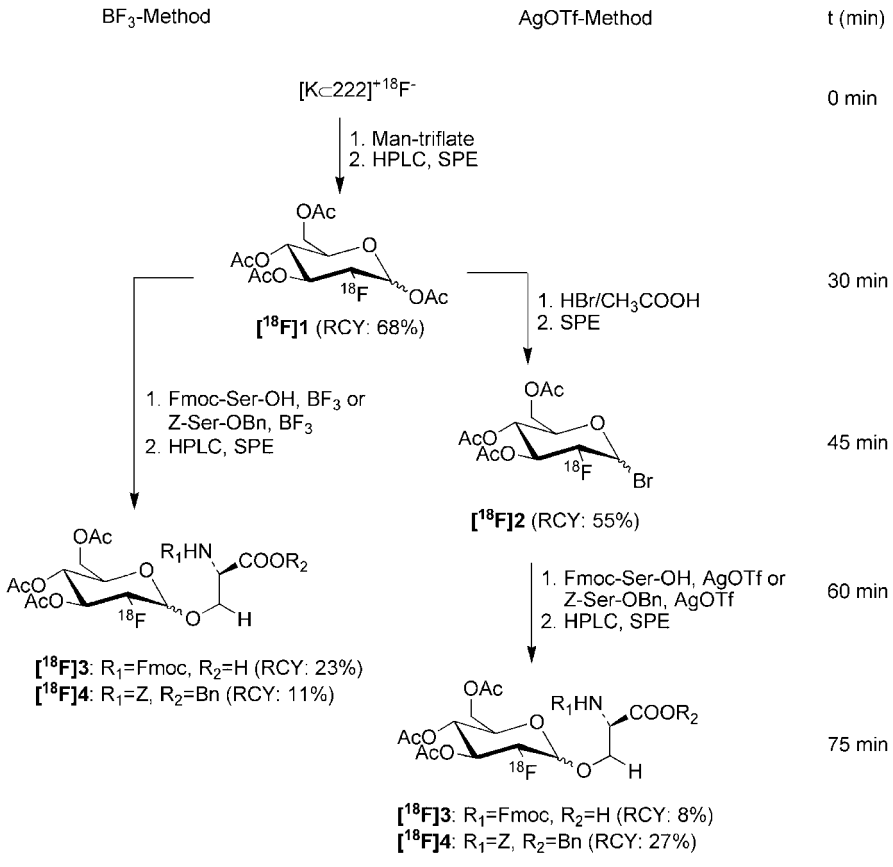
when applied to the labelling of peptides: the use of BF_3 as a Lewis acid promoter could cause side-reactions, such as cleavage of protecting groups, and limits the choice of the reaction solvent. In order to circumvent these drawbacks, we aimed at the development of an alternative ^{18}F-glycosylation procedure.

We herein report the radiosynthesis of 3,4,6-tri-O-acetyl-2-deoxy-2-[^{18}F]fluoroglucopyranosyl bromide (**[^{18}F]2**) and its use as ^{18}F-labelled glycosyl donor under Koenigs–Knorr[9] conditions in silver trifluoromethanesulphonate (AgOTf) promoted ^{18}F-glycosylation reactions on protected serinyl derivatives as model compounds. In addition, we compared this approach to the BF_3-promoted reaction sequence with regard to radiochemical yield, reaction time, mechanistic implications and dependence on the labelling precursor.

Results and discussion

Starting from the tetra-O-acetylated ^{18}F-labelled glycosyl donor **[^{18}F]1**, which was synthesized by nucleophilic ^{18}F-for-OTf substitution on the corresponding tetra-O-acetylated mannopyranoside following the method of Hamacher and others[10] and isolated by HPLC in a radiochemical yield (RCY) of 68% (decay-uncorrected) in 30 min as reported previously,[8] we developed two different routes for ^{18}F-glycosylation of amino acids: the BF_3-method and the AgOTf-method (Scheme 1). First, we reinvestigated the BF_3-method[8] using 200 mM $BF_3 \cdot Et_2O$ and 10 mM Fmoc-Ser-OH or Z-Ser-OBn as glycosyl acceptors in acetonitrile as solvent. This procedure gave a decay-uncorrected radiochemical yield (RCY) of 23% for **[^{18}F]3** after HPLC-separation in a total synthesis time of about 60 min (Scheme 1). Using Z-Ser-OBn as a more sterical demanding glycosyl acceptor, a decreased RCY of about 11% for **[^{18}F]4** was obtained. Studying the reaction of **[^{18}F]1** with Fmoc-Ser-OH at early time points (1–6 min) by analytical radio-HPLC, we observed the formation of glycoester **[^{18}F]5**, which was almost completely converted into the desired O-glycosylated product **[^{18}F]3** during the progress of the reaction (Figure 1). Thus, the use of BF_3 in high concentration (200 mM) ensured completed formation of **[^{18}F]3**, nicely confirming the observation of Salvador *et al.* for the synthesis of glycopeptide building blocks.[11] Therefore, we conclude, that glycoester **[^{18}F]5** was cleaved in the presence of BF_3 leading to an oxocarbenium intermediate as depicted in Scheme 2, which could subsequently be attacked at the electrophilic C-1 by the hydroxyl side chain of Fmoc-Ser-OH.

In order to provide an alternative ^{18}F-glycosylation method, we developed and optimized a procedure based on the classical Koenigs–Knorr conditions using **[^{18}F]2** as glycosyl donor, AgOTf as activator and Fmoc-Ser-OH or Z-Ser-OBn as glycosyl acceptor. Peracetylated 2-deoxy-2-[^{18}F]fluoroglucopyranose (**[^{18}F]1**) was completely converted into its anomeric bromide **[^{18}F]2** with HBr/CH_3COOH in a reaction time of 5 min followed by solid phase extraction



Scheme 1. Time course of the BF_3-mediated ^{18}F-glycosylation method starting from [^{18}F]1 compared to the AgOTf-activated ^{18}F-glycosylation (Koenigs–Knorr method) using glycosyl donor [^{18}F]2. RCY are expressed as decay-uncorrected yields of the HPLC-isolated ^{18}F-labelled products and referred to [^{18}F]fluoride

on a RP-18 cartridge (Scheme 1). The AgOTf-method was optimized in regard to solvent, temperature, reaction time, activator and the ratio of glycosyl acceptor (Z-Ser-OBn) to activator (AgOTf) (Table 1). The most favourable reaction parameters turned out to be the ratio of labelling precursor Z-Ser-OBn to AgOTf of 0.5–6 and dichloromethane as solvent affording **[^{18}F]4** in a RCY of 67% after a reaction time of 1–2 min at room temperature (Table 1). Applying these Koenigs–Knorr reaction conditions to the ^{18}F-glycosylation of Fmoc-Ser-OH to give **[^{18}F]3**, the RCY decreased to about 21% (Table 2), due to the formation of glycoester **[^{18}F]5** as major by-product in equivalent RCY compared to the desired product **[^{18}F]3**. HPLC analysis showed the formation of a radiolabeled hydrophilic compound, possibly indicating the presence of an oxocarbenium trifluoromethanesulphonate intermediate, which equably

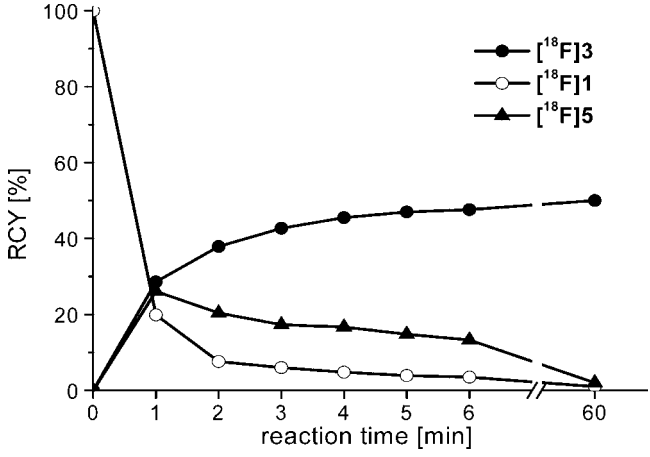
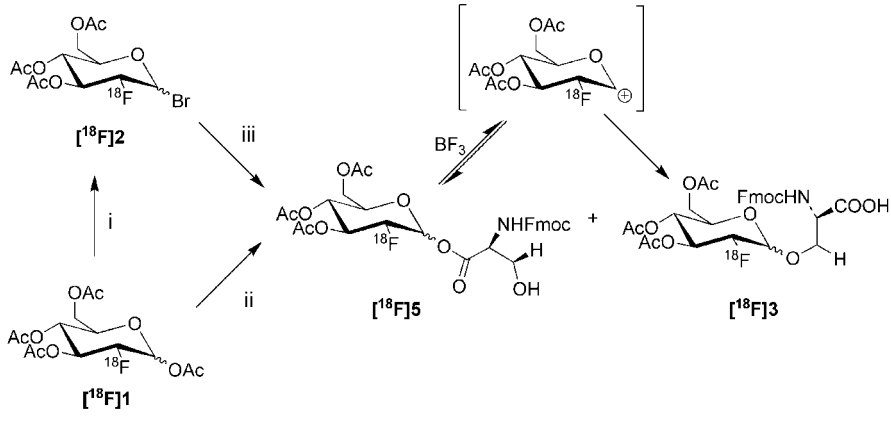


Figure 1. Dependence of the radiochemical yield of the ^{18}F-labelled products on reaction time for the BF_3-mediated ^{18}F-glycosylation of Fmoc-Ser-OH using [^{18}F]1 as glycosyl donor (200 mM $BF_3 \cdot Et_2O$ in CH_3CN, $V = 500\,\mu l$, 8 mM Fmoc-Ser-OH, $T = 80^\circ C$)



Scheme 2. (i) HBr/CH_3COOH, CH_2Cl_2; (ii) $BF_3 \cdot Et_2O$, CH_3CN, Fmoc-Ser-OH; (iii) AgOTf, CH_2Cl_2, Fmoc-Ser-OH

decreased during the progress of the reaction in support of both products, the ^{18}F-glycosylated product (**[^{18}F]3** or **[^{18}F]4**) and the by-product **[^{18}F]5**. In contrast to the BF_3-mediated glycosylation method, no consequential conversion of **[^{18}F]5** into product **[^{18}F]3** occurred under Koenigs–Knorr conditions (Scheme 2).

In summary, the two investigated ^{18}F-glycosylation methods differed in maximum RCY for the ^{18}F-glycosylation key step depending on the labelling precursor (Table 2). The Koenigs–Knorr ^{18}F-glycosylation method revealed an improved radiochemical yield of $67 \pm 6\%$ in the case of the N^{α}- and

Table 1. Optimization of the Koenigs–Knorr ^{18}F-glycosylation of Z-Ser-OBn using [^{18}F]2

Activator	Ratio/(Z-Ser-OBn activator)[a]	Solvent	Temperature	Reaction time (min)	RCY[b] (%)
$Hg(CN)_2$	5	CH_2Cl_2	rt	10	7
$HgBr_2$	1	CH_2Cl_2	rt	2	0
AgOTf	0.1–0.25	CH_2Cl_2	rt	1–2	22 ± 10[c]
AgOTf	0.5–6	CH_2Cl_2	rt	1–2	67 ± 6[c]
AgOTf	10	CH_2Cl_2	rt	1–2	55 ± 13[c]
AgOTf	24–120	CH_2Cl_2	rt	1–2	0
AgOTf	3	Et_2O	rt	2	30
AgOTf	3	dichloroethane	80°C	2	38
AgOTf	3	CH_3NO_2	90°C	2	7
AgOTf	1	CH_3CN	rt	5	7
AgOTf	1	DMF	130°C	5	0
AgOTf	3	CH_2Cl_2	0°C	5	46
AgOTf	1	CH_2Cl_2	−20°C	1	22
AgOTf	1	CH_2Cl_2	−20°C	10	53

[a] n (Z-Ser-OBn) = 1.5–50 μmol, V = 500 μl.
[b] RCY are expressed as mean of two independent experiments ($n = 2$) determined by analytical radio-HPLC from a sample withdrawn from the reaction mixture and referred to the ^{18}F-labelled glycosyl donor **[^{18}F]2**.
[c] Mean ± standard error of the mean (SEM, $n = 4$).

Table 2. BF_3-method vs AgOTf-method: comparison of maximum radiochemical yields (RCY) of the ^{18}F-glycosylation key step

Precursor	Product	AgOTf-method using glycosyl donor **[^{18}F]2**		BF_3-method using glycosyl donor **[^{18}F]1**	
		RCY[a] (%)	t (min)	RCY[a] (%)	t (min)
Fmoc-Ser-OH	**[^{18}F]3**	21 ± 3	2	47 ± 8	5
Z-Ser-OBn	**[^{18}F]4**	67 ± 6	1–2	27 ± 4	10

[a] RCY are expressed as mean ± standard error (SEM, $n = 4$) determined by analytical radio-HPLC from a sample withdrawn from the reaction mixture and referred to the ^{18}F-labelled glycosyl donor.

C-protected serinyl precursor (Z-Ser-OBn) as compared to 27 ± 4% obtained by the BF_3-method (Table 2). In contrast to this result, a serinyl labelling precursor containing a free carboxyl group (Fmoc-Ser) was advantageously labelled by ^{18}F-glycosylation applying the reaction conditions of the BF_3-method. Thus, the use of **[^{18}F]2** for ^{18}F-glycosylation under Koenigs–Knorr conditions should particularly be of interest for labelling precursors without interfering free C-terminus, such as bioactive cyclic peptides.

As shown in Scheme 1, we also performed a comparison of both ^{18}F-glycosylation methods with regard to the reaction time and overall decay-uncorrected radiochemical yields. Starting from [^{18}F]fluoride, the BF_3-method

gave **[^{18}F]3** or **[^{18}F]4** in an overall decay-uncorrected RCY of 23 and 11%, respectively, within a total reaction time of 60 min including 2 HPLC purification steps (Scheme 1). The AgOTf-method permitted the use of dichloromethane as solvent, lasted 15 min longer affording **[^{18}F]3** in an overall RCY of 8%, but was more advantageous for the radiosynthesis of **[^{18}F]4** revealing an overall decay-uncorrected RCY of 27% (Scheme 1). Experiments concerning the deacetylation of the ^{18}F-glycosylated compounds (**[^{18}F]3**, **[^{18}F]4**) were beyond the scope of the present study, since this issue was successfully addressed elsewhere.[8]

Experimental

General

N^{α}-(9-Fluorenylmethoxycarbonyl)-L-serine (Fmoc-Ser-OH) and N^{α}-(phenylmethoxycarbonyl)-L-serine phenylmethyl ester (Z-Ser-OBn) were purchased from Bachem (Germany). [^{18}F]Fluoride was obtained from PET Net GmbH (Erlangen, Germany). Radio-thin layer chromatography (radio-TLC) was carried out on plastic sheets (Polygram®, Sil G/UV$_{254}$, Macherey Nagel) using ethyl actetate/*n*-hexane (1:1, v/v) as eluent. Analytical HPLC was performed on the following system: HPLC Agilent 1100 with a quarternary pump and variable wavelength detector and radio-HPLC-detector D505TR (Canberra Packard). Computer analysis of the HPLC data was performed using FLO-One software (Canberra Packard). Electronic autoradiography was used to analyse radio-TLC data (Instant Imager™, Canberra Packard). The syntheses of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-fluoro-D-glucopyranose (**[^{18}F]1**, **[^{19}F]1**), 1,3,4,6-tetra-O-acetyl-2-O-trifluoromethanesulfonyl-β-D-mannopyranose and all other reference compounds (**[^{19}F]2**, **[^{19}F]3**, **[^{19}F]4**, **[^{19}F]5**) were described elsewhere.[8] Each ^{18}F-labelled compound was identified by k' values on the radio-HPLC system and co-injection of the corresponding reference compound.

3,4,6-Tri-O-acetyl-2-deoxy-2-[^{18}F] fluoroglucopyranosyl bromide (**[^{18}F]2**)

Three hundred microlitre 33% HBr/CH_3COOH were added to a reaction vessel containing **[^{18}F]1** in 50 µl dichloromethane at room temperature. The reaction was monitored by radio-TLC. After 5 min conversion was complete and the mixture was diluted with 10 ml H_2O and passed through a C18-cartridge (Merck, 100 mg). The cartridge was washed with H_2O, dried and eluted with 1 ml CH_3CN. Starting from 250 MBq **[^{18}F]1** this procedure yielded 205 MBq **[^{18}F]2** within 15 min. Radio-TLC: $R_f = 0.77$. HPLC (Kromasil C8, 250×4.6, 1.5 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min): $k' = 6.2$.

*N^{α}-(9-Fluorenylmethoxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-deoxy-2-[^{18}F]fluoro-*D*-glucopyranosyl)-*L*-serine (**[^{18}F]3**) and N^{α}-(phenylmethoxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-deoxy-2-[^{18}F]fluoro-*D*-glucopyranosyl)-*L*-serine phenylmethyl ester (**[^{18}F]4**)*

^{18}F-glycosylation using BF_3. A solution of the labelling precursor (10 mM Fmoc-Ser-OH or Z-Ser-OBn) 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. The radioactive products (**[^{18}F]3** or **[^{18}F]4**) were obtained in a radiochemical yield of 47% (**[^{18}F]3**) or 27% (**[^{18}F]4**) after a reaction time of 5–10 min. The reaction 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 separated by gradient reversed-phase HPLC (Kromasil C8, 125 × 8 mm, 4 ml/min, 40–100% CH_3CN in H_2O (0.1% TFA) within 50 min). The product fraction was diluted with water (1:10), fixed on a C18-cartridge (Merck, 100 mg) and eluted with 1 ml acetonitrile in a reaction vessel. Starting from 250 MBq **[^{18}F]1** this procedure yielded 85 MBq of **[^{18}F]3** or 40 MBq of **[^{18}F]4** within 30 min.

^{18}F-glycosylation using AgOTf. A solution of the labelling precursor (10 mM Fmoc-Ser-OH or Z-Ser-OBn) and 10 mM AgOTf in 500 µl anhydrous CH_3CN were added to a reaction vessel containing dry **[^{18}F]2** at room temperature. The radioactive products (**[^{18}F]3** or **[^{18}F]4**) were obtained in a radiochemical yield of 21% (**[^{18}F]3**) or 67% (**[^{18}F]4**) after a reaction time of 1–2 min. The reaction mixture was passed through a Si-cartridge (Merck, 200 mg) and seperated by gradient reversed-phase HPLC (Kromasil C8, 125 × 8 mm, 4 ml/min, 40–100% CH_3CN in H_2O (0.1% TFA) within 50 min). The product fraction was diluted with water (1:10), fixed on a C18-cartridge (Merck, 100 mg) and eluted with 1 ml acetonitrile in a reaction vessel. Starting from 205 MBq **[^{18}F]2** this procedure yielded 30 MBq **[^{18}F]3** or 100 MBq **[^{18}F]4** within 30 min.

[^{18}F]3 and **[^{18}F]4** were characterized by radio-HPLC and radio-TLC for both methods (**[^{18}F]3**: Radio-TLC: $R_f = 0.10$. HPLC (Kromasil C8, 250 × 4.6, 1.5 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min): $k' = 10.3$. **[^{18}F]4**: Radio-TLC: $R_f = 0.71$. HPLC (Kromasil C8, 250 × 4.6, 1.5 ml/min, 40–100% CH_3CN in water (0.1% TFA) in 50 min): $k' = 13.8$).

Optimization of the ^{18}F-glycosylation procedure using the AgOTf-method

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

Conclusion

The ^{18}F-labelled glycosyl bromide **[^{18}F]2** was easily available without the necessity of an additional HPLC purification step as compared to the radiosynthesis of the corresponding tetra-O-acetylated glycosyl donor **[^{18}F]1**. Applying Koenigs–Knorr reaction conditions, **[^{18}F]2** proved its suitability for the AgOTf-activated ^{18}F-glycosylation of Z-Ser-OBn with improved radiochemical yield. Therefore, the ^{18}F-glycosylation agent **[^{18}F]2** should be preferentially applied to O-glycosylation reactions on suitably protected bioactive peptides in further studies, in order to develop ^{18}F-glycopeptides as potential PET radiopharmaceuticals.

Acknowledgements

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