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Nucleophilic substitution of nitro groups by [^{18}F]fluoride in methoxy-substituted *ortho*-nitrobenzaldehydes—A systematic study

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ABSTRACT

As model reactions for the introduction of [^{18}F]fluorine into aromatic amino acids, the replacement of NO_2 by [^{18}F]fluoride ion in mono- to tetra-methoxy-substituted *ortho*-nitrobenzaldehydes was systematically investigated. Unexpectedly, the highly methoxylated precursors 2,3,4-trimethoxy-6-nitrobenzaldehyde and 2,3,4,5-tetramethoxy-6-nitrobenzaldehyde showed high maximum radiochemical yields (82% and 48% respectively). When the electrophilicity of the leaving group substituted carbon atom is expressed by its ^{13}C NMR chemical shift a good correlation with the reaction rate at the beginning of the reaction (first min) was found (R^2 = 0.89), whereas the maximum radiochemical yields correlated much poorer with this electrophilicity parameter. This may be caused by side reactions becoming influencial in the further reaction course. As possible side reactions the demethylation of methoxy groups and intramolecular redox reactions could be detected by HPLC/MS.

1. Introduction

Positron emission tomography (PET) is an important imaging technique that exhibits the unique feature of following metabolic functions in vivo. The use of radioactively labelled compounds (i.e. tracers or PET biomarkers) allows the quantitative measurement of the distribution of radioactivity in vivo according to the physiological or pathophysiological processes to be investigated.

^{18}F-labelled aromatic amino acids, like FDOPA or tyrosine derivatives, have been of interest for many years now. Commonly, they are synthesized by electrophilic substitution, using [^{18}F]F_2, [^{18}F]acetylhypofluorite etc. as reagents. Due to the type of nuclear processes that are used at the cyclotron, labelling results in products with low specific activities (ratio of ^{18}F-compound (in Bq) to ^{18}F- plus ^{19}F-compound (in mol)). At least in the case of [^{18}F]FDOPA this generates no problem for the PET investigation, but nevertheless it is a limitation. In many cases high specific activities are crucial for following up metabolic processes without altering the biochemical processes that are on-going in vivo.

In order to obtain labelled aromatic amino acids with high specific activities, the incorporation of ^{18}F into the tracer is to be carried out with no-carried-added fluorination reagents. The standard and most efficient production route for production of fluorine-18 is the ^{18}O(p,n)^{18}F nuclear reaction resulting in [^{18}F]fluoride ion with a high specific activity ready for nucleophilic substitution. Today, this reaction is available at every medical cyclotron and therefore the method of choice.

For that reason nucleophilic aromatic substitution (S_NAr) is considered to be the most desirable reaction type to introduce ^{18}F into aromatic amino acids. This approach, indeed, has become a challenging task to radiopharmaceutical groups world wide [1]. Especially the demand to make [^{18}F]FDOPA easily available became a major driving force in various attempts. Enantiomeric purity of this product often is a major problem [2], which was circumvented by e.g. the use of a chiral catalyst [3]. Still, the synthesis of [^{18}F]FDOPA via S_NAr is complex and laborious [4].

As the high electron density in the aromatic part of the amino acids is counter-productive to a nucleophilic attack, a particular interest has to be paid to the choice of leaving group in the precursors to be applied for the particular radiofluorination. Furthermore, auxiliary groups exerting an electron-withdrawing effect are required to enhance the reactivity for the nucleophilic attack of [^{18}F]fluoride ion. For the synthesis of ^{18}F-labelled arenes typical precursors contain a nitro substituent as leaving group which is activated for nucleophilic substitutions by an *ortho*- or *para*-positioned aldehydic function [5]. In addition to its role as auxiliary group in the nucleophilic introduction of ^{18}F, the formyl substituent has the advantage, that in the further course of the

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synthesis it can be used as starting point for the synthetic addition of the amino acid residue to the aromatic part of the molecule [6]. Therefore, *o*-nitrobenzaldehydes substituted by methoxy groups (as masked hydroxyl groups) have been selected as labelling precursors for the synthesis of ^{18}F-amino acids such as 2-[^{18}F]fluorotyrosine and [^{18}F]FDOPA.

In the case of 5-methoxy-2-nitrobenzaldehyde a radiochemical yield (RCY) of 5% was reported for the substitution of NO_2-group by ^{18}F [7]. The poor RCY is to be understood in terms of the strong +M-effect of the CH_3O-substitutent *p*-oriented to the leaving group. Compared to this result, the radiochemical yields obtained for 4,5-dimethoxy-2-nitrobenzaldehyde (25–55%) as labelling precursor [8,9] are considered to be unexpectedly high. That prompted us to a systematic study about the dependence of the RCY on the number and positions of methoxy substituents present in *o*-nitrobenzaldehydes.

2. Results and discussion

2.1. ^{18}F-labelling conditions

The goal of this work was to study the influence of additional methoxy groups on the reactivity of 2-nitrobenzaldehyde in the nucleophilic aromatic fluorination. In this type of reaction, the use of polar aprotic solvents is mandatory in order to take advantage of the nucleophilicity of the $^{18}F^-$anion. In literature, DMSO is commonly used as solvent in this type of labelling reactions. However, we previously observed the potential of DMSO to give rise to oxidation of the benzaldehyde precursors to benzoic acids [10]. Therefore, DMSO was replaced by DMF through this work in order to avoid oxidation as a side reaction. For ^{18}F-labelling the reaction conditions were applied as determined previously [11], i.e. the reactions were performed in 1 mL of DMF at 140 °C up to 30 min using 10 mg of precursor. RCYs were measured at different reaction times as presented in Table 1 and Fig. 1.

2.2. Effect of methoxy groups on nucleophilic aromatic substitution

The systematic study on the relationship between RCY and an increasing number of methoxy substituents in *o*-nitrobenzaldehydes was started from 2-nitrobenzaldehyde (**a_0**). In this case, a radiochemical yield of 84% was observed. The derivatives obtained by introduction of an increasing number of methoxy groups to the aromatic ring of **a_0-NO_2** can be roughly devided in three groups with respect to the incorporation yield of fluorine-18.

Remarkably high yields close to or even above the value for **a_0-NO_2** were noted for compounds **c-NO_2** (89%), **a-NO_2** (79%), **g-NO_2** (87%), **i-NO_2** (86%) and **j-NO_2** (82%) (Table 1). These compounds have a methoxy group at C-4 (*meta*-oriented with respect to NO_2) in common. Lower, however, still satisfactory radiochemical yields were found for the fluorination reaction of **f-NO_2** (69%), **d-NO_2** (70%), **b-NO_2** (57%) and **k-NO_2** (48%). Compound **h-NO_2** which is substituted by two methoxy groups in *o*- and *p*-position to the leaving group showed a significantly lower maximum yield of only 13%. Furthermore, the RCY of 2,3-dimethoxy-6-nitrobenzaldehyde (**e-NO_2**) was low (22%); the reason could be the formation of a radioactive by-product of unknown structure accounting for a labelling yield of 15%. Therefore, this compound cannot be considered to contribute to the discussion about S_NAr in multiple substituted benzaldehydes. In all other cases, no radioactive by-product was observed, neither by radio-TLC nor by radio-HPLC.

In a further set of experiments the nitro substituent was replaced by bromine as leaving group, thus resulting in identical ^{18}F-labelled products. These experiments were carried out in order to allow identification of labelled compounds (see Method 3, analytical assay, Section 4). Results similar to the nitro compounds were observed, however, due to the poorer qualities of the bromo substituent as leaving group, the radiochemical yields generally were much lower (Fig. 2).

For checking possible carrier effects (see Method 2, analytical assay, Section 4) yield curves of n.c.a. and c.a. nucleophilic radiofluorination were determined by TLC at different time points as presented in Fig. 2. For both reactions similar trends were observed indicating both the n.c.a. and c.a. substitution to proceed by principally the same substitution mechanism, but labelling yields were partly different as it is often observed in radiochemical reactions due to concentrations being different from those of typical organic reactions.

The electronic influence of methoxy groups on aromatic rings is described by a combination of the electron-donating +M-effect and the electron-withdrawing −I-effect. With respect to the *ortho*- and *para*-positions relative to the methoxy-substituted carbon atom the mesomeric effect dominates, and, therefore, renders nucleophilic attack more difficult. Indeed, both, the 3-methoxy-(**d-NO_2**) and the 5-methoxy-substituted derivative (**b-NO_2**) gave lower yields in comparison to the standard model compound **a_0-NO_2**. The lowest yield was observed when both positions were methoxylated (**h-NO_2**). In contrast, introducing CH_3O in position 4 (**c-NO_2**) and 6 (**a-NO_2**) (both *meta* relative to NO_2) did not result in a larger change of the radiochemical yields. For *meta* positions the electron-withdrawing −I-effect becomes more important, which can result in an increasing reactivity of the nitro-substituted carbon towards nucleophilic attack. On the other hand, in this substitution pattern the methoxy group suppresses to some extend the activating effect of the *ortho*-formyl substituent by +M-donation.

Table 1
^{13}C NMR chemical shifts of the nitro-substituted carbon (ArC-NO_2) and radiochemical yields (RCY; as mean ± SD) of methoxy-substituted 2-nitrobenzaldehydes ($n \geq 3$).

Nr.	ArC-NO_2 (ppm)	k^* (min^{-1})	RCY (%)					
			1 min	3 min	7 min	10 min	20 min	30 min
c-NO_2	151.0	2.00	86.5 ± 0.6	88.3 ± 1.8	89 ± 2.4	86.3 ± 4.2	87 ± 3.7	86.6 ± 3.3
a_0-NO_2	149.1	1.62	80.3 ± 1.6	84.1 ± 0.8	84.3 ± 0.4	81.9 ± 4.4	82 ± 4.1	82.3 ± 3.6
a-NO_2	148.1	1.19	69.5 ± 0.4	72.3 ± 1.1	77.6 ± 1.4	78.5 ± 3.5	78.9 ± 4.0	78.7 ± 3.1
j-NO_2	143.8	1.09	66.3 ± 2.0	73.8 ± 6.0	80.4 ± 4.3	81.5 ± 2.4	74.0 ± 4.8	65.9 ± 4.1
g-NO_2	143.6	1.32	73.2 ± 1.7	81.5 ± 1.0	85.2 ± 2.2	86.8 ± 1.8	81.7 ± 1.0	79.7 ± 1.6
i-NO_2	142.3	1.11	67.0 ± 6.4	72.9 ± 4.7	70.7 ± 9.7	85.9 ± 4.2	84.8 ± 4.7	84.6 ± 3.6
b-NO_2	141.8	0.62	46.2 ± 1.2	53.3 ± 1.2	57.4 ± 1.0	53.1 ± 5.6	54.9 ± 4.6	53.6 ± 4
e-NO_2	139.0	0.09	8.9 ± 0.5	15.8 ± 1.8	20.6 ± 2.3	22.3 ± 2.1	21.0 ± 1.3	18.9 ± 1.0
k-NO_2	137.8	0.48	38.0 ± 2.0	43.7 ± 1.6	47.6 ± 4.5	45.3 ± 3.5	44.2 ± 5.8	41 ± 6.9
d-NO_2	137.2	0.61	45.7 ± 3.1	64.3 ± 2.1	70.1 ± 3.1	67.4 ± 5.0	67.4 ± 2.8	66.5 ± 4.7
f-NO_2	137.1	0.65	47.8 ± 2	65.2 ± 3.6	69.6 ± 1.6	67.9 ± 4.1	67.0 ± 2.5	60.8 ± 2.4
h-NO_2	131.9	0.01	1.4 ± 0.2	6.0 ± 0.9	11.2 ± 0.2	13.0 ± 1.5	11.8 ± 1.6	10.4 ± 0.7

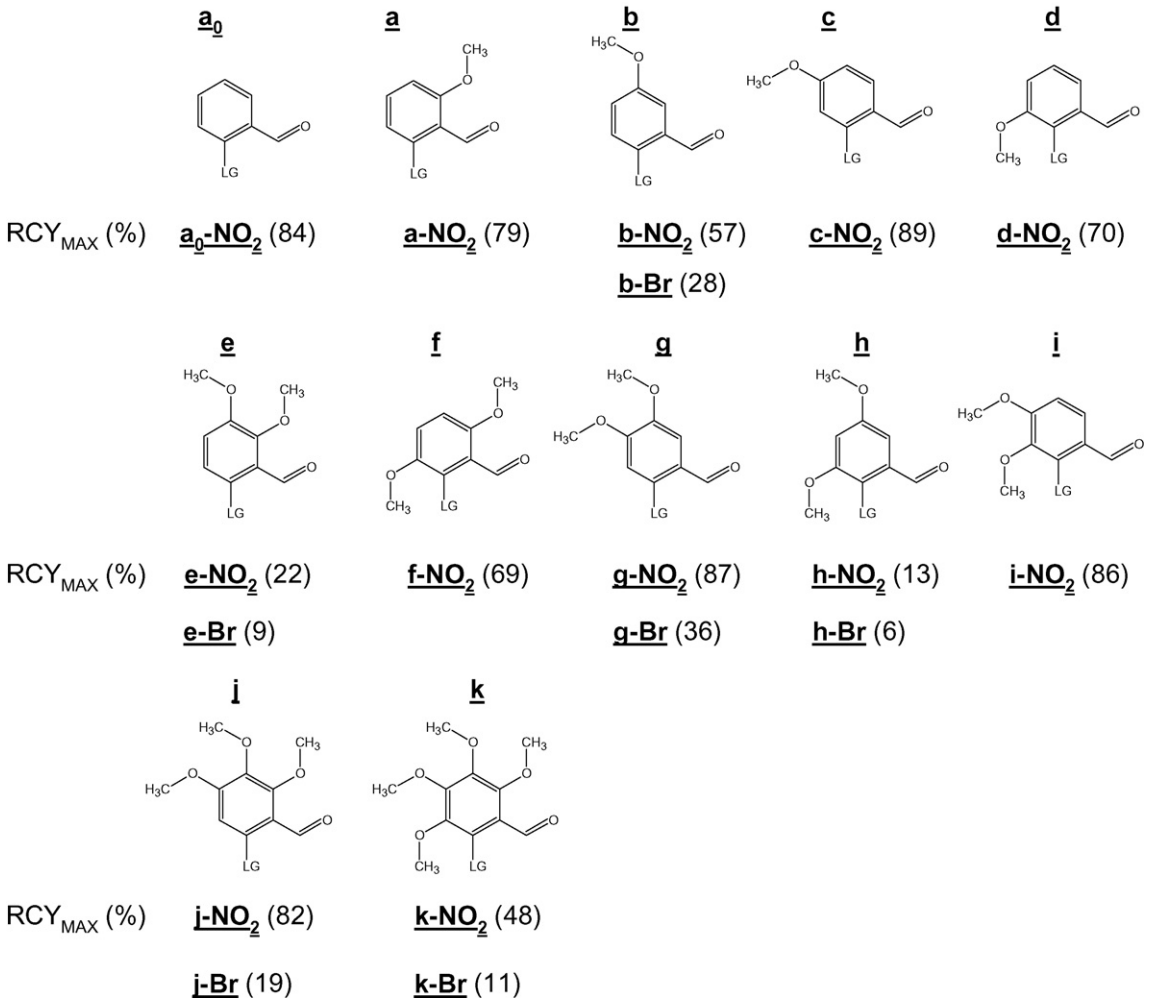

Fig. 1. Maximum radiochemical yields (RCY_{MAX}) for ^{18}F-labelling of *ortho* nitro- and bromobenzaldehyde derivatives (LG: leaving group), $n \geq 3$.

The combined action of both effects may be the reason why such derivatives give more or less the same radiochemical yield as the reference compound **a_0-NO_2**.

The overall effect of the presence of both *o*/*p*-and *m*-methoxy groups is difficult to predict, because steric effects become superimposed on the electronic effects [7]. In particular, steric hindrance works against the +M-effect as the orbital overlap between the lone pair at the oxygen atom and the aromatic π-system becomes disturbed. The total effect of all substituents and the electron density of the carbon atom bearing the leaving group may be derived from the ^{13}C-chemical shift of this carbon position.

The ^{13}C-chemical shift of the nitro-substituted C-atom of the *o*-nitrobenzaldehydes studied (Table 1) fell in the range between δ = 132 ppm (**h-NO_2**) and 151 ppm (**c-NO_2**). In similar reaction series Rengan et al. [12] and Ding et al. [7] observed an approximately linear correlation of the RCY with the deshielding of the leaving group substituted carbon atom. An analogous treatment of our results (Fig. 3) showed a trend but not a good fit (R^2 = 0.62). The deshielding of the nitro-substituted carbon atom is considered as a measure of its electrophilicity. In principle, such a reactivity parameter cannot be expected to correlate with maximum radiochemical yields obtained after different reaction times. In radiofluorination experiments the [precursor]/[^{18}F]F^- ratios applied are very high. If no product decomposition and side reactions consuming or deactivating $^{18}F^-$ intervene, this should result in a complete incorporation of $^{18}F^-$ into the reaction product. Only the time needed for the completion of the reaction should vary according to the electrophilicity of the precursor. In reality, however, side reactions and product decomposition contribute to the outcome of a labelling reaction and decrease the yield. These side effects should become more influencial the less reactive the precursor is. This may explain why the maximum radiochemical yields correlate to some extent with the ^{13}C-chemical shift values (δ) as reactivity parameter.

At the beginning of a labelling experiment any competing side effect lowering the yield should be less disturbing. In our experiments the course of the radiochemical yields was determined up to 30 min (Table 1 and Fig. 2). The strongest yield increase was generally observed within the first minute. Provided that the ^{13}C-chemical shifts are reliable measures of the electrophilicities, the NMR data should give a satisfactory correlation with the rate constants in this reaction interval. Because only the measurements after 1 min are available, the (pseudo)monomolecular rate constants (k^*) derived from the radiochemical yields after 1 min have to be considered with caution. Despite of the uncertainty of the k^* values a satisfactory correlation (R^2 = 0.89) resulted for the plot k^* vs. δ (Fig. 4).

As outlined before, with respect to the S_NAr reaction more reactive *o*-nitrobenzaldehydes derivatives should have better chances to escape unwanted side reactions and, therefore, attain higher yields in the radiofluorination steps. The precursor molecules 2,3,4-trimethoxy-6-nitrobenzaldehyde (**j-NO_2**) and

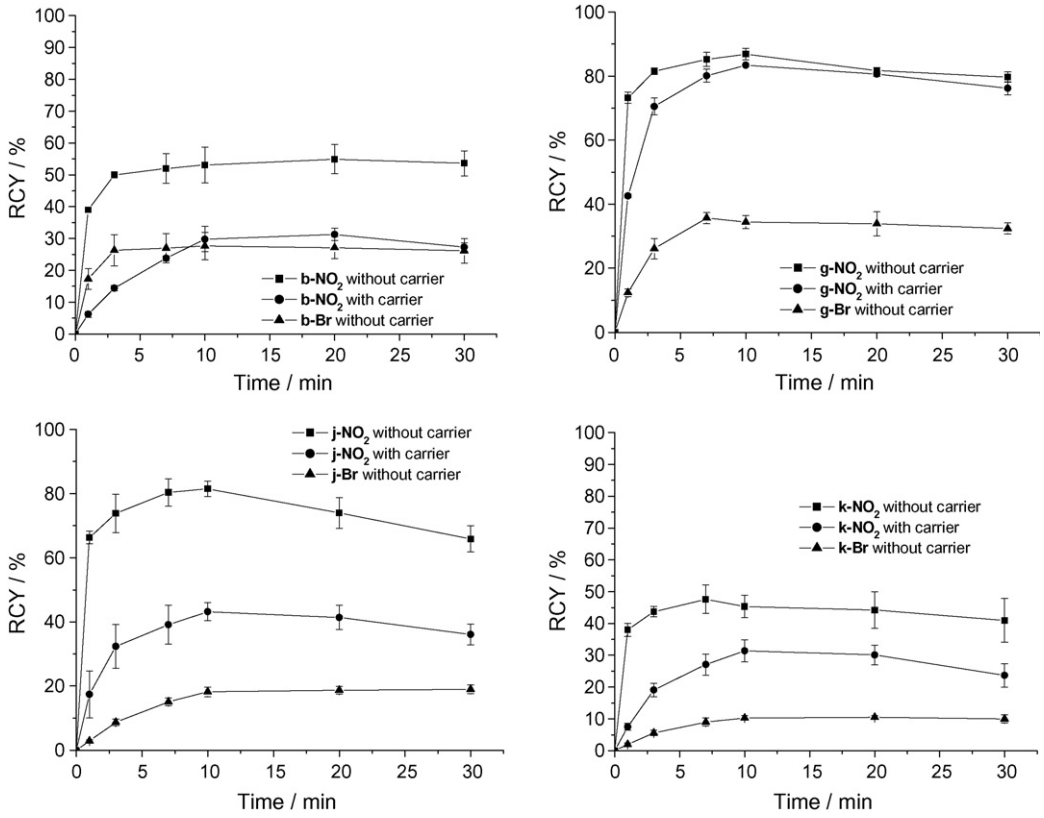

Fig. 2. Radiochemical yields (RCY) of ^{18}F-labelling of methoxy-substituted 2-nitro and 2-bromobenzaldehydes in dependence on the reaction time. Labelling conditions: precursor (10 mg), DMF (1 mL), Kryptofix®222 (15 mg), 3.5% K_2CO_3 (100 μL); 140 °C; carrier KF (50 μg).

2,3,4,5-tetramethoxy-6-nitrobenzaldehyde (**k-NO$_2$**) are a good illustration for that assumption. Compound **j-NO$_2$** (δ(ArC-NO$_2$) = 144 ppm) was about two times as reactive as compound **k-NO$_2$** (δ(ArC-NO$_2$) = 138 ppm) as the corresponding maximum radiochemical yields were 81.5% (**j-NO$_2$**) and 47.6% (**k-NO$_2$**). It should be emphasized that the yield obtained from the fully substituted derivative **k-NO$_2$** is still acceptable if discussing from a practical point of view. It is even higher than expected when compared with 3,5-dimethoxy-2-nitrobenzaldehyde (**h-NO$_2$**) (13%, δ(ArC-NO$_2$) = 132 ppm).

From the data compiled in Table 1 it can be seen that the precursors with chemical shifts of the nitro-substituted carbon atom near to the corresponding value found in 2-nitrobenzaldehyde (**a$_0$-NO$_2$**, δ(ArC-NO$_2$) = 149 ppm) gave high maximum radiochemical yields, e.g. **c-NO$_2$** (δ(ArC-NO$_2$) = 151 ppm), 89%; **j-NO$_2$** (δ(ArC-NO$_2$) = 144 ppm), 81.5%.

In these cases, because of the high reactivities of the precursors towards the S_NAr reaction, side reactions do not play an important role and high yields (≥80%) are found in the radiofluorination. However, for the less reactive precursors exhibiting δ-values in the

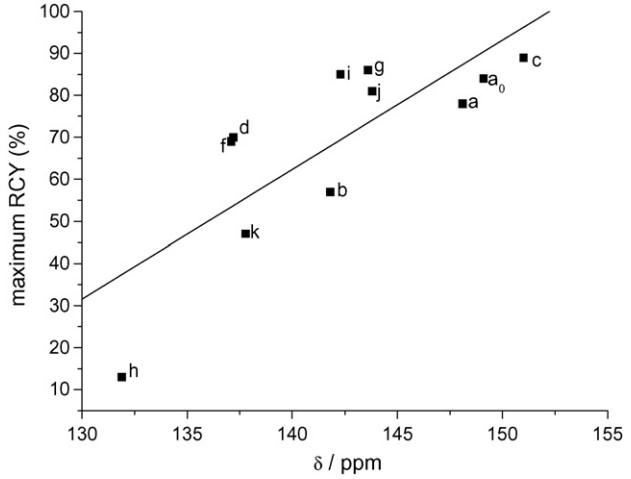

Fig. 3. Correlation of maximum radiochemical yields (RCY) with ^{13}C NMR chemical shift values (δ). Compound **e-NO$_2$** was omitted because a radioactive by-product (15%) of unknown structure was detected.

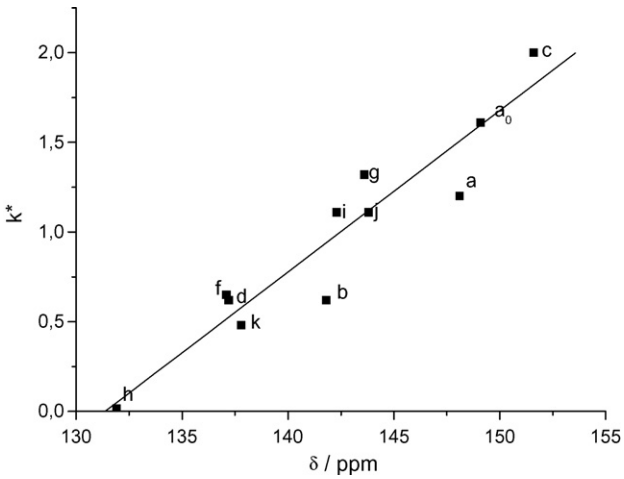

Fig. 4. Correlation between k^* and ^{13}C NMR chemical shift values (δ). Compound **e-NO$_2$** was omitted because a radioactive by-product (15%) of unknown structure was detected.

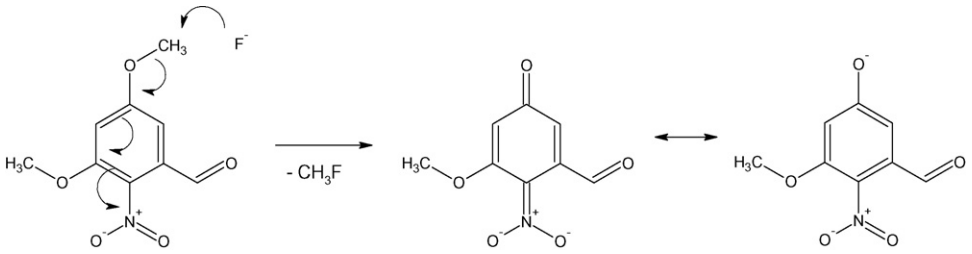

Fig. 5. Proposed mechanism for the demethylation of the methoxy group as a possible side reaction of methoxylated *o*-nitrobenzaldehydes.

range of 132–137 ppm a decrease in the radiofluorination yields (13–70%) is observed. Because the yields have a maximum within the reaction interval of 30 min, the reduced yields cannot be explained by insufficiently long reaction times. Obviously, with compounds less prone to the S_NAr reaction other processes consuming $^{18}F^-$ become important.

In the course of our experiments with methoxylated *o*-nitrobenzaldehydes we indeed obtained indication of possible side reactions. HPLC/APCI-MS measurements revealed the formation of phenolates which could be formed in an S_N2-like demethylation by attack of F^- at the methoxy group (Fig. 5).

In all cases a HPLC-peak eluting faster than the starting compound with a mass corresponding to the $[M-H]^-$ ion of the phenolic demethylation product could be detected. For 2,3,4-trimethoxy-6-nitrobenzaldehyde (**j-NO$_2$**) the phenolic product was isolated and characterised.

The proximity of a nitro and an aldehyde group in the precursor molecules could give rise to an intramolecular redox process resulting in *o*-nitrosobenzoic acids. If *o*-nitrosobenzoic acids are formed as side products, they deactivate the nucleophilic power of [^{18}F]fluoride ion by interacting with the acidic hydrogens potentially ending up in strong hydrogen bond formations. Such intramolecular redox-processes are well known in photochemistry [13] and electron-impact induced fragmentation (mass spectrometric *ortho*-effect) [14]. Under these reaction conditions the intramolecular process is initiated by abstraction of the aldehydic hydrogen by an unpaired electron at a nitro oxygen formed by $n\pi^*$ excitation and ionisation, respectively. An analogous thermal process could be started by intramolecular nucleophilic addition of a nitro oxygen atom to the neighbouring carbonyl group (Fig. 6).

To test a thermal intramolecular redox reaction as a possible side reaction intervening in our labelling experiments, *o*-nitrobenzaldehyde was heated under conditions identical with the labelling experiments except that no fluoride ion was present. On heating in DMF in the presence of the K_2CO_3–crown ether complex a bluish colour was built up. Subsequent HPLC-APCI-MS analysis indicated the formation of an isomeric compound eluting faster than the starting material. The mass spectrum (negative mode) exhibits strong $[M-H]^-$(*m*/*z* 150) and $[M-H-CO_2]^-$(*m*/*z* 106) ions. In contrast, the negative ion mass spectrum of the starting compound is dominated by the radical anion $M^{\bullet-}$ at *m*/*z* 151. The blue colour (typical for C-nitroso compounds) and the mass spectral data are in accordance with the formation of *o*-nitrosobenzoic acid.

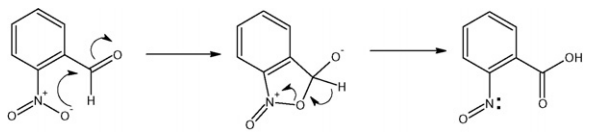

Fig. 6. Intramolecular redox-process in *ortho*-nitrobenzaldehyde by intramolecular nucleophilic addition of a nitro oxygen atom to the neighbouring carbonyl group.

3. Conclusions

For the nucleophilic aromatic fluorination of methoxylated *o*-nitrobenzaldehydes by [^{18}F]fluoride ion a correlation was found between the downfield shift of the nitro-substituted aromatic carbon atom in the ^{13}C NMR spectrum and the reaction rate at the beginning of the reaction. Side reactions and product decomposition decreased the radiochemical yields to some extent the longer the reaction proceeded and became relevant in the case of less reactive precursor molecules.

4-Methoxy-2-nitrobenzaldehyde (**c-NO$_2$**), 4,5-dimethoxy-2-nitrobenzaldehyde (**g-NO$_2$**) and 3,4-dimethoxy-2-nitrobenzaldehyde (**i-NO$_2$**) are potential candidates for the synthesis of ^{18}F-labelled tyrosine or DOPA, respectively, when the aldehydic function is utilised for connecting the aromatic substructure with the amino acid residue. All the three *o*-nitrobenzaldehyde precursors gave high maximum radiochemical yields (>85%). Even the threefold and fourfold methoxylated precursors **j-NO$_2$** and **k-NO$_2$** reacted in remarkably good yields to the corresponding ^{18}F-labelled products (82% and 48%, respectively).

4. Experimental

4.1. General

For performing the labelling reactions, as solvent DMF (stored over molecular sieve) was purchased from Fluka (Germany). Acetonitrile (for DNA synthesis) and Kryptofix 222 were obtained from Merck (Darmstadt, Germany). For the middle pressure liquid chromatography system (MPLC, Büchi, Switzerland) silica gel 60 (0.040–0.063 mm, Merck) or reverse phase material (POPLYGO-PREP 60-50, C18, MN, Germany) was used, eluents were mixtures of petroleum ether (60/90 °C) and ethyl acetate or water and acetonitrile. Precursors and reference standards were characterised by their melting point (Gallenkamp MPG 350, Germany, values uncorrected), IR (Spectrum One FT-ATR-IR, PerkinElmer, Boston, USA), MS (Finnigan-MAT TSQ 70, Bremen, Germany) and NMR (Bruker Avance 400, Rheinstetten). For NMR measurements, as internal standards the deuterated solvents were used (DMSO-d6: δ = 2.49 in ^{1}H and δ = 39.5 in ^{13}C; $CDCl_3$: δ = 7.25 in ^{1}H and δ = 77.0 in ^{13}C). The chemical shift δ in ppm was referred to the internal standard. All radiochemical yields given in this work represent an average of 3–5 experiments.

4.2. Precursors and reference standards

As precursors and reference standards, the following compounds were of the highest purity available from either Sigma-Aldrich, Fluka, ABCR, Alfa Aesar, AVOCADO or Fluorochem and were used as received (authenticity of the fluoro compounds used as standards was confirmed by NMR and MS): 3-methoxy-2-nitrobenzaldehyde (**d-NO$_2$**, >97%), 2-fluoro-3-methoxybenzaldehyde (**d-F**, 97%), 2-fluoro-4-methoxybenzaldehyde (**c-F**, 98%),

2-fluoro-5-methoxybenzaldehyde (**b-F**, 98%), 2-fluoro-6-methoxybenzaldehyde (**a-F**, 98%), 2-nitrobenzaldehyde (**a_0-NO_2**, 98%), 2-fluorobenzaldehyde (**a_0-F**, 97%), 2-nitro-4,5-dimethoxybenzaldehyde (**g-NO_2**, 96%), 2-fluoro-4,5-dimethoxybenzaldehyde (**g-F**, 98%).

a-NO_2, **b-NO_2**, **c-NO_2**, **e-NO_2**, **e-Br** and **e-F** were obtained according to literature [10,11,15]. All other precursors in Fig. 1 were synthesized as described below.

*4.2.1. 2-Bromo-5-methoxybenzaldehyde (**b-Br**)*

To a stirred solution of 3-methoxybenzaldehyde (3.35 g, 24.6 mmol) in dry $CHCl_3$ (200 mL) bromine (1.89 mL, 3.69 mmol) was introduced slowly during 20 min. The reaction solution was stirred at room temperature for 0.5 h. Then the resulting solution was poured into diethyl ether (100 mL) and the mixture was washed with satd. $Na_2S_2O_3$ (2 × 50 mL) and water (50 mL). The organic layer was separated and dried over Na_2SO_4. After evaporation of the solvent, the crude product was purified on MPLC (silica gel, petroleum ether:ethyl acetate, 1:1, v:v). 4.37 g **b-Br** were obtained (82%). mp 77 °C (reported [16] 78–80 °C). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 10.30 (*s*, 1H, –C*H*O), 7.50 (*d*, ^3J = 8.6 Hz, 1H, H_{arom}), 7.39 (*d*, $^4J_{H,H}$ = 3.3 Hz, 1H, H_{arom}), 7.02 (*dd*, $^3J_{H,H}$ = 8.7 Hz, $^4J_{H,H}$ = 3.1 Hz, 1H, H_{arom}), 3.83 (*s*, 3H, –OCH_3) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 55.7, 112.6, 117.9, 123.1, 133.9, 134.5, 159.2, 191.7 ppm. MS-EI (70 eV): *m*/*z* (%) = 216 (95) $[M]^{\bullet+}$, $C_8H_7{}^{81}BrO_2$; 214 (100) $[M]^{\bullet+}$, $C_8H_7{}^{79}BrO_2$.

*4.2.2. 3,5-Dimethoxy-2-nitrobenzaldehyde (**h-NO_2**)*

3,5-Dimethoxybenzaldehyde (2.0 g, 12 mmol) was portion-wise added to 90% HNO_3 (10 M eq. based on 3,5-dimethoxybenzaldehyde) while stirring at 0 °C. After this addition, the reaction solution was stirred at 0 °C and monitored by TLC. 1 h later the starting compound was completely consumed. The resulting mixture was poured into water (100 mL) and extracted with diethyl ether (100 mL). The organic layer was washed with satd. $NaHCO_3$ (2 × 50 mL) and brine (50 mL). Then the organic layer was separated, dried over Na_2SO_4 and evaporated. 0.41 g **h-NO_2** (17%) was obtained as yellow crystals after separation on the MPLC (silica gel, petroleum ether:ethyl acetate, 1:1, v:v). mp 104–106 °C (reported [17] 104–106 °C). ^{1}H NMR (DMSO-d6, 400 MHz): δ = 9.88 (*s*, 1H, –C*H*O), 7.18 (*d*, $^4J_{H,H}$ = 2.5 Hz, 1H, H_{arom}), 7.14 (*d*, $^4J_{H,H}$ = 2.3 Hz, 1H, H_{arom}), 3.92 (*s*, 3H, –OCH_3), 3.92 (*s*, 3H, –OCH_3) ppm. ^{13}C NMR (DMSO-d6, 100 MHz): δ = 56.4, 57.2, 104.9, 109.1, 129.2, 131.9, 152.3, 161.6, 189.4 ppm. MS-EI (70 eV): *m*/*z* (%) = 211 (48) $[M]^{\bullet+}$, $C_9H_9NO_5$.

*4.2.3. 2-Bromo-3,5-dimethoxybenzaldehyde (**h-Br**)*

To a stirred solution of 3,5-dimethoxybenzaldehyde (1.5 g, 9 mmol) in dry $CHCl_3$ (90 mL) the bromine (0.6 mL, 11.7 mmol) was introduced slowly over 20 min. This reaction solution was stirred at room temperature for 0.5 h. Then the resulting solution was poured into diethyl ether (100 mL) and the mixture was washed with satd. $Na_2S_2O_3$ (2 × 50 mL) and water (50 mL). The organic layer was separated and dried over Na_2SO_4. After evaporation of the solvent, the crude product was purified on MPLC (silica gel, petroleum ether:ethyl acetate, 5:1, v:v). 1.86 g **h-Br** was obtained (84%). mp 114–115 °C (reported [18] 115–116 °C). ^{1}H NMR ($CDCl_3$, 250 MHz): δ = 10.24 (*s*, 1H, –C*H*O), 6.97 (*d*, $^4J_{H,H}$ = 2.8 Hz, 1H, H_{arom}), 6.93 (*d*, $^4J_{H,H}$ = 2.7 Hz, 1H, H_{arom}), 3.89 (*s*, 3H, –OCH_3), 3.82 (*s*, 3H, –OCH_3) ppm. ^{13}C NMR ($CDCl_3$, 60 MHz): δ = 55.8, 56.8, 104.4, 105.7, 107.0, 134.3, 156.9, 159.7, 191.7 ppm. MS-EI (70 eV): *m*/*z* (%) = 246 (97) $[M]^{\bullet+}$, $C_9H_9{}^{81}BrO_3$; 244 (100) $[M]^{\bullet+}$, $C_9H_9{}^{79}BrO_3$.

*4.2.4. 3,6-Dimethoxy-2-nitrobenzaldehyde (**f-NO_2**)*

To a solution of 2,5-dimethoxybenzaldehyde (1.0 g, 6 mmol) in AcOH (5 mL) was added 70% HNO_3 (1–1.5 M eq. based on 2,5-dimethoxy-benzaldehyde) at 0 °C within 5 min and then this solution was stirred at room temperature for 1 h. The resulting mixture was poured into water (50 mL) and was extracted with diethyl ether (2 × 50 mL). The combined organic layers were washed with satd. $NaHCO_3$ (50 mL) and brine (50 mL). Then the organic layer was separated, dried over Na_2SO_4 and the solvent was evaporated. 0.53 g **f-NO_2** (42%) was obtained as yellow crystals after separation on the MPLC (silica gel, petroleum ether:ethyl acetate, 2:1, v:v). mp 172 °C (reported [19] 171–172 °C). ^{1}H NMR (DMSO-d6, 400 MHz): δ = 10.24 (*s*, 1H, –C*H*O), 7.67 (*d*, $^3J_{H,H}$ = 9.3 Hz, 1H, H_{arom}), 7.45 (*d*, $^3J_{H,H}$ = 9.3 Hz, 1H, H_{arom}), 3.94 (*s*, 3H, –OCH_3), 3.85 (*s*, 3H, –OCH_3) ppm. ^{13}C NMR (DMSO-d6, 100 MHz): δ = 57.2, 57.3, 114.9, 116.0, 121.7, 137.0, 143.7, 155.1, 186.7 ppm. MS-EI (70 eV): *m*/*z* (%) = 211 (18) $[M]^{\bullet+}$, $C_9H_9NO_5$.

*4.2.5. 3,4-Dimethoxy-2-nitrobenzaldehyde (**i-NO_2**)*

Into the stirred solution of 4-formyl-2-methoxy-3-nitrophenyl benzenesulfonate [20] (2.80 g, 8.3 mmol) in ethanol (70 mL), aqueous NaOH solution (2 mL, 1 g/mL) was added. Then this solution was refluxed for 0.5 h and ethanol was evaporated. To the residue water (100 mL) was added and pH was adjusted to 6 by 2N HCl. The resulting mixture was extracted with diethyl ether (200 mL) and the organic layer was washed with satd. $NaHCO_3$ (50 mL), water (50 mL) and brine (50 mL) respectively, then dried over Na_2SO_4 and concentrated to afford 0.86 g 4-hydroxy-3-methoxy-2-nitrobenzaldehyde crude product, which was used directly in further synthetic procedure.

4-Hydroxy-3-methoxy-2-nitrobenzaldehyde (0.86 g, 4.4 mmol), K_2CO_3 (0.72 g, 5.2 mmol) and MeI (0.32 mL, 5.2 mmol) were stirred in DMF (15 mL) at room temperature for 2 h. The reaction mixture was poured into water and extracted with diethyl ether (3 × 50 mL). The combined organic layers were washed with water (100 mL) and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by MPLC (silica gel, petroleum ether:ethyl acetate, 1:1, v:v) to yield 0.81 g **i-NO_2** was obtained (46%, based on 4-formyl-2-methoxy-3-nitrophenyl benzenesulfonate). mp 60–62 °C (reported [21] 58–61 °C). ^{1}H NMR (DMSO-d6, 400 MHz): δ = 9.79 (*s*, 1H, –C*H*O), 7.90 (*d*, $^3J_{H,H}$ = 8.6 Hz, 1H, H_{arom}), 7.51 (*d*, $^3J_{H,H}$ = 8.6 Hz, 1H, H_{arom}), 4.01 (*s*, 3H, –OCH_3), 3.83 (*s*, 3H, –OCH_3) ppm. ^{13}C NMR (DMSO-d6, 100 MHz): δ = 57.2, 62.0, 114.2, 119.5, 131.3, 140.2, 142.3, 158.1, 188.3 ppm. MS-EI (70 eV): *m*/*z* (%) = 211 (18) $[M]^{\bullet+}$, $C_9H_9NO_5$.

*4.2.6. 2,3,4-Trimethoxy-6-nitrobenzaldehyde (**j-NO_2**)*

The nitration of 2,3,4,-trimethoxybenzaldehyde is similar to the nitration of 2,5 dimethoxybenzaldehyde.

2,3,4-trimethoxybenzaldehyde (2 g, 10.2 mmol) and a mixture of 100% HNO_3 and 100% AcOH (10 mL, 1:1, v:v) were stirred at 0 °C for 10 min. After extraction with diethyl ether and purification on the MPLC (silica gel, petroleum ether:ethyl acetate, 3:1, v:v), 1.62 g **j-NO_2** was obtained as yellow crystals (66%) mp 83–84 °C (reported [22] 80–82 °C). ^{1}H NMR (DMSO-d6, 400 MHz): δ = 10.12 (*s*, 1H, –C*H*O), 7.51 (*s*, 1H, H_{arom}), 3.94 (*s*, 3H, –OCH_3), 3.93 (*s*, 3H, –OCH_3), 3.86 (*s*, 3H, –OCH_3) ppm. ^{13}C NMR (DMSO-d6, 100 MHz): δ = 57.4, 60.9, 62.7, 104.2, 117.3, 143.7, 144.7, 153.8, 156.7, 187.4 ppm. MS-EI (70 eV): *m*/*z* (%) = 241 (34) $[M]^{\bullet+}$, $C_{10}H_{11}NO_6$.

*4.2.7. 6-Bromo-2,3,4-trimethoxybenzaldehyde (**j-Br**)*

j-Br was synthesized from 5-bromo-2,3-dimethoxybenzaldehyde [20] by the following steps. Baeyer Villiger reaction: 30% H_2O_2 (4 mL) and formic acid (11 mL) with catalytic amount of concentrated H_2SO_4 were stirred at room temperature for 1 h. The solution of 5-bromo-2,3-dimethoxybenzaldehyde (4.05 g, 16.5 mmol) in formic acid (25 mL) was added dropwise into the solution at 0 °C, then the mixture was kept stirring at 0 °C

overnight. TLC analysis indicated complete conversion of the starting material. The reaction was quenched with water (50 mL) and extracted with diethyl ether (200 mL). The organic layer was washed with satd. $NaHCO_3$ (100 mL) and brine (50 mL). Then the organic layer was separated, dried over Na_2SO_4 and evaporated to afford 5.5 g 5-bromo-2,3-dimethoxyphenol crude product as red oil which was used directly in the next synthesis step.

5-Bromo-2,3-dimethoxyphenol was formylated by Duff reaction. 5-Bromo-2,3-dimethoxyphenol (4.0 g, 17.1 mmol) and hexamethylenetetramine (2.3 g, 17.2 mmol) were refluxed at 130 °C in TFA (40 mL) for 4 h. The reaction solution was cooled down and quenched by adding 6N HCl (120 mL). After 20 min stirring, the resulting solution was extracted with DCM (2 × 100 mL). The combined organic layers were washed with satd. $NaHCO_3$ solution (2 × 50 mL), water (50 mL) and brine (50 mL). After evaporation of the solvent, 1.55 g crude product 6-bromo-2-hydroxy-3,4-dimethoxybenzaldehyde was afforded and used directly in the next synthesis step.

6-Bromo-2-hydroxy-3,4-dimethoxybenzaldehyde (1.55 g, 6 mmol), K_2CO_3 (0.8 g, 6 mmol) and MeI (0.36 mL, 6 mmol) were stirred in DMF (20 mL) at room temperature for 3 h. After extraction with diethyl ether and purification on the MPLC (silica gel, petroleum ether:ethyl acetate, 2:1, v:v), 0.41 g **j-Br** as yellow oil was obtained (13%, based on 5-bromo-2,3-dimethoxybenzaldehyde). ^{1}H NMR (DMSO-d6, 400 MHz): δ = 10.09 (*s*, 1H, –C*H*O), 7.19 (*s*, 1H, H_{arom}), 3.90 (*s*, 3H, –OCH_3), 3.86 (*s*, 3H, –OCH_3), 3.76 (*s*, 3H, –OCH_3) ppm. ^{13}C NMR (DMSO-d6, 100 MHz): δ = 56.7, 60.6, 62.4, 113.9, 117.6, 120.9, 141.4, 156.5, 157.8, 188.8 ppm. MS-EI (70 eV): *m*/*z* (%) = 276 (93) $[M]^{\bullet+}$, $C_{10}H_{11}{}^{81}BrO_4$; 274 (100) $[M]^{\bullet+}$, $C_{10}H_{11}{}^{79}BrO_4$.

4.2.8. 2-Hydroxy-3,4,5-trimethoxybenzaldehyde

30% H_2O_2 (15 mL) was added to a stirred solution of 2,3,4-trimethoxybenzaldehyde (7.50 g, 38.4 mmol) in methanol (75 mL) with a catalytic amount of concentrated H_2SO_4 (0.2 mL). After 2 h TLC analysis indicated complete conversion of the starting material. The reaction was quenched with water (50 mL) and extracted with diethyl ether (200 mL). The organic layer was washed with satd. $NaHCO_3$ (50 mL) and brine (50 mL). The organic layer was separated, dried over Na_2SO_4, evaporated and purified on MPLC (silica gel, petroleum ether:ethyl acetate, 2:1, v:v) to afford 4.44 g 2,3,4-trimethoxyphenol and used directly in the next synthesis step.

2,3,4-Trimethoxyphenol (3.40 g, 18.5 mmol) and hexamethylentetramine (3.15 g, 22.5 mmol) were refluxed at 130 °C in TFA (20 mL) for 24 h. After extraction with DCM, the crude product was chromatographied on the MPLC (silica gel, petroleum ether:ethyl acetate, 2:1, v:v) to yield 2.0 g 2-hydroxy-3,4,5-trimethoxybenzaldehyde as colorless oil (51%, based on 2,3,4-trimethoxyphenol). ^{1}H NMR (DMSO-d6, 400 MHz): δ = 10.12 (*s*, 1H, –C*H*O), 7.01 (*s*, 1H, H_{arom}), 3.87 (*s*, 3H, –OCH_3), 3.77 (*s*, 3H, –OCH_3), 3.76 (*s*, 3H, –OCH_3) ppm. ^{13}C NMR (DMSO-d6, 100 MHz): δ = 55.9, 60.7, 60.9, 105.6, 117.1, 141.4, 146.0, 149.0, 150.0, 190.6 ppm. MS-EI (70 eV): *m*/*z* (%) = 212 (100) $[M]^{\bullet+}$, $C_{10}H_{12}O_5$.

4.2.9. 2,3,4,5-Tetramethoxybenzaldehyde

2-Hydroxy-3,4,5-trimethoxybenzaldehyde (1.70 g, 8 mmol), K_2CO_3 (1.24 g, 9 mmol) and MeI (0.56 mL, 9 mmol) were stirred in DMF (30 mL) at room temperature overnight. After extraction with diethyl ether and purification on MPLC (silica gel, petroleum ether:ethyl acetate, 2:1, v:v), 1.45 g 2,3,4,5-tetramethoxybenzaldehyde was obtained (80%, based on 2-hydroxy-3,4,5-trimethoxybenzaldehyde). ^{1}H NMR (DMSO-d6, 400 MHz): δ = 10.17 (*s*, 1H, –C*H*O), 7.02 (*s*, 1H, H_{arom}), 3.88 (*s*, 3H, –CH_3), 3.86 (*s*, 3H, –OCH_3), 3.85 (*s*, 3H, –OCH_3), 3.80 (*s*, 3H, –OCH_3) ppm. ^{13}C NMR (DMSO-d6, 100 MHz): δ = 55.9, 60.8, 61.1, 62.8, 103.7, 123.7, 146.5, 148.8, 149.6, 151.5, 188.4 ppm. MS-EI (70 eV): *m*/*z* (%) = 226 (100) $[M]^{\bullet+}$, $C_{11}H_{14}O_5$.

*4.2.10. 2,3,4,5-Tetramethoxy-6-nitrobenzaldehyde (**k-NO$_2$**)*

2,3,4,5-Tetramethoxybenzaldehyde (0.5 g, 2.2 mmol) and 70% HNO_3 (1.25 M eq. based on 2,3,4,5-tetramethoxybenzaldehyde) were stirred in acetic acid (5 mL) at 0 °C for 10 min, then the ice bath was removed and the reaction solution was stirred for 1 h at room temperature. After extraction with diethyl ether and purification on MPLC (silica gel, petroleum ether:ethyl acetate, 3:1, v:v), 0.23 g **k-NO$_2$** was obtained as yellow crystals (39%, based on 2,3,4,5-tetramethoxybenzaldehyde) mp 67–70 °C. ^{1}H NMR (DMSO-d6, 400 MHz): δ = 10.09 (*s*, 1H, –C*H*O), 4.03 (*s*, 3H, –OCH_3), 3.98 (*s*, 3H, –OCH_3), 3.92 (*s*, 3H, –OCH_3), 3.81 (*s*, 3H, –OCH_3) ppm. ^{13}C NMR (DMSO-d6, 100 MHz): δ = 61.4, 61.5, 62.6, 63.0, 115.0, 137.7, 140.9, 148.0, 152.7, 153.1, 186.2 ppm. HRMS (EI): *m*/*z* $[M]^{\bullet+}$ calculated for $C_{11}H_{13}NO_7$: 271.069167; found 271.07104 MS-EI (70 eV): *m*/*z* (%) = 271 (31) $[M]^+$, $C_{11}H_{13}NO_7$; 254 (100) $[M-OH]^+$, $C_{11}H_{12}NO_6$; 224 (30) $[M-OH-NO]^+$, $C_{11}H_{12}O_5$; 211 (82), 195 (42), 181 (46), 167 (27), 152 (33), 137 (35), 127 (19), 125 (16), 95 (10), 93 (8), 65 (5) $[C_5H_5]^+$, 53 (10). IR: ν, cm^{-1} (*T*%) = 2998 (87%), 2952 (77%), 2876 (80%), 1692 (26%), 1591 (60%), 1537 (39%), 1465 (28%), 1444 (48%), 1404 (32%), 1383 (29%), 1364 (29%), 1336 (17%), 1267 (44%), 1194 (44%), 1125 (34%), 1082 (22%), 1054 (21%), 1011 (17%), 963 (25%), 943 (30%), 901 (47%), 878 (44%), 823 (50%), 787 (54%), 745 (35%), 684 (59%).

*4.2.11. 2-Bromo-3,4,5,6-tetramethoxybenzaldehyde (**k-Br**)*

Into a solution of 2,3,4,5-tetramethoxybenzaldehyde (0.89 g, 4 mmol) in trifluoroacetic acid (5 mL) *N*-bromosuccinimide (NBS, 0.84 g, 4.8 mmol) was added over 15 min at 0 °C. The reaction was monitored by TLC. After 0.5 h the starting compound was completely reacted. The resulting mixture was poured into water and extracted with diethyl ether (100 mL). The combined organic layer was washed with water (100 mL) and dried over Na_2SO_4. After evaporation of solvent, the residue was purified on MPLC (silica gel, petroleum ether:ethyl acetate, 5:1, v:v) to yield 0.91 g **k-Br** as yellow oil (75%). ^{1}H NMR (DMSO-d6, 400 MHz): δ = 10.13 (*s*, 1H, –C*H*O), 3.96 (*s*, 3H, –OCH_3), 3.85 (*s*, 3H, –OCH_3), 3.84 (*s*, 3H, –OCH_3), 3.75 (*s*, 3H, –OCH_3) ppm. ^{13}C NMR (DMSO-d6, 100 MHz): δ = 60.8, 61.1, 61.3, 62.5, 111.7, 123.6, 146.2, 147.1, 151.8, 152.8, 189.5 ppm. MS-EI (70 eV): *m*/*z* (%) = 306 (95) $[M]^{\bullet+}$, $C_{11}H_{13}{}^{81}BrO_5$; 304 (100) $[M]^{\bullet+}$, $C_{11}H_{13}{}^{79}BrO_5$.

4.2.12. 6-Fluoro-2,3,4-trimethoxybenzaldehyde (j-F)

The mixture of KF (0.24 g, 8.2 mmol, spray dried) and Kryptofix 222 (1.16 g, 6.3 mmol) was heated at 140 °C under Argon for 20 min, then **j-NO$_2$** 0.50 g (4.2 mmol) in DMF (20 mL) was added and the reaction solution was heated for 15 min. The reaction solution was poured into water (25 mL) and this mixture was extracted with diethyl ether (2 × 25 mL). The combined organic layer was washed with 1N NaOH (25 mL) (this basic washing solution was kept for isolation of phenolic by-product), water (25 mL) and brine (25 mL), then was dried over Na_2SO_4, filtered and evaporated to afford 0.22 g crude product. After purification on MPLC (reverse phase material, CH_3CN:water, 30:70, v:v) 25 mg product **j-F** was obtained. This 25 mg product was purified again on preparative HPLC (Luna, phenyl-hexyl 5 μm, 150 mm × 10 mm, Phenomenex, USA; CH_3CN:water, 3:1, v:v; 5 mL/min) to yield 12 mg pure **j-F** (1.3%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 10.21 (*s*, 1H, –C*H*O), 6.45 (*d*, $^3J_{H,F}$ = 8.4 Hz, 1H, H_{arom}), 3.99 (*s*, 3H, –OCH_3), 3.90 (*s*, 3H, –OCH_3), 3.82 (*s*, 3H, –OCH_3) ppm. ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 56.3 (–OCH_3), 61.1 (–OCH_3), 62.2 (–OCH_3), 96.1 (*d*, *J* = 26.3 Hz, C_{arom}-H), 112.0 (*d*, ^2J = 8.8 Hz, C_{arom}-CHO), 138.4 (*d*, *J* = 3.7 Hz, C_{arom}-OCH$_3$), 156.0 (*d*, *J* = 7.3 Hz, C_{arom}-OCH$_3$), 159.3 (*d*, *J* = 13.9 Hz, C_{arom}-OCH$_3$), 159.9 (*d*, $^1J_{C,F}$ = 259.8 Hz, C_{arom}-F), 186.3 (*d*,

^3J = 2.2 Hz, –CHO) ppm. ^{19}F NMR ($CDCl_3$, 400 MHz): 117.1 (C_{arom}-*F*) ppm. HRMS (EI): *m*/*z* $[M]^{\bullet+}$ calculated for $C_{10}H_{11}FO_4$: 214.064116; found 214.06256 MS-EI (70 eV): *m*/*z* (%) = 214 (100) $[M]^{\bullet+}$, $C_{10}H_{11}FO_4$; 199 (79) $[M-CH_3]^+$, $C_9H_8FO_4$; 181 (66) $[M-CH_3-H_2O]^+$, $C_9H_6FO_3$; 171 (16) $[M-CH_3-CO]^+$, $C_8H_8FO_3$; 153 (30) $[M-H_2O-CO]^+$, $C_9H_9FO_2$; 138 (18), 113 (8), 97 (8), 83 (9), 57 (10). IR: ν, cm^{-1} (*T*%) = 2942 (95%), 1691 (82%), 1604 (76%), 1574 (91%), 1492 (88%), 1457 (88%), 1404 (92%), 1386 (87%), 1342 (83%), 1251 (84%), 1198 (87%), 1134 (80%), 1096 (85%), 1028 (91%), 989 (88%), 925 (95%), 820 (94%), 800 (92%).

4.2.13. Phenolic by-product

The basic washing solution was acidified by 2N HCl (50 mL) and extracted with diethyl ether (2 × 50 mL). The ether layer was washed with water (50 mL) and brine (50 mL). The organic layer was separated, dried over Na_2SO_4, and evaporated to afford 0.08 g phenol by product. The position of the hydroxyl group was not determined. ^{1}H NMR (DMSO-d6, 400 MHz): δ = 10.13 (*s*, 1H, –C*H*O), 7.23 (*d*, *J* = 8.2 Hz, 1H, H_{arom}), 3.92 (*s*, 3H, –OCH_3), 3.77 (*s*, 3H, –OCH_3) ppm. ^{13}C NMR (DMSO-d6, 100 MHz): δ = 56.9 (–OCH_3), 60.6 (–OCH_3), 100.9 (C_{arom}-H), 109.9 (C_{arom}-CHO), 138.9 (C_{arom}-NO_2), 145.5 (C_{arom}-OCH_3), 154.1 (C_{arom}-OH), 156.6 (C_{arom}-OCH_3), 188.2 (–CHO) ppm. IR: ν, cm^{-1} (*T*%) = 3095 (84%), 2948 (75%), 2850 (81%), 2324 (91%), 1632 (45%), 1508 (35%), 1460 (54%), 1445 (49%), 1416 (56%), 1389 (35%), 1317 (38%), 1283 (33%), 1250 (21%), 1203 (29%), 1139 (18%), 1035 (42%), 982 (42%), 961 (29%), 896 (32%), 856 (41%), 775 (23%), 728 (32%), 686 (40%).

4.3. Production of [^{18}F]fluoride ion

No-carrier-added (n.c.a.) [^{18}F]fluoride ion was produced at the PETtrace cyclotron (General Electric Healthcare, Uppsala, Sweden) via the ^{18}O(p,n)^{18}F nuclear reaction by irradiating 1.5 mL of >95% enriched [^{18}O]water (Rotem, Israel) with 16.5 MeV protons.

4.4. Labelling

4.4.1. n.c.a. (no-carrier-added) ^{18}F-fluorination

The activity (50–100 MBq) was introduced into a 5.0 mL sealed vial (Supelco, graduated screw top V-Vials®, autoclavable borosilicate USP Type 1 glass) containing 100 μL of 3.5% aqueous K_2CO_3 and 15.0 mg Kryptofix 222. The [^{18}F]fluoride ion solution was dried for 20 min under a mild stream of argon (ca. 2 mL/min) at 140 °C by azeotropic distillation with acetonitrile (2 × 1 mL). Then, precursor (10 mg) dissolved in DMF (1.0 mL) was added into the vial containing the [Kryptofix 222] $K^+{}^{18}F^-$ complex. The sealed vial was kept heating at 140 °C. A sample (1–5 μL) was withdrawn for determination of the radiochemical yield at 1, 3, 7, 10, 20 and 30 min. For a number of experiments (n = 20), the amount of radioactivity that remained adsorbed in the reaction vial after removing the reaction solution and washing with water (3 × 3 mL) was determined as 12 ± 8% (based on the total activity in the vial after labelling).

4.4.2. c.a. (carrier-added) ^{18}F-fluorination

The activity was introduced into a 5.0 mL sealed vial containing KF (50 μg), 3.5% aqueous K_2CO_3 (100 μL) and Kryptofix 222 (15.0 mg), the following step was the same as for n.c.a. ^{18}F-fluorination.

4.5. Analytical assay

The radiochemical standard protocol for checking identity and purity of ^{18}F-labelled products in analytical radiochemistry is done by radio-TLC and radio-HPLC (Method 1). In this work, for compounds **f**, **h**, **i** and **k** no reference standards were synthesized, therefore only purity could be checked by Method 1, identity was assured by Method 2 or Method 3 (see Table 2).

Method 1: ^{18}F-labelled products were identified and checked for purity by both radio-TLC and radio-HPLC using the non-radioactive reference compounds for detection of the UV signals in comparison to the corresponding radioactive signals. For product purity control, an aliquot of the reaction mixture was taken at time of maximum RCY (see Table 1) and injected onto HPLC. After separation, the product fraction was collected and measured by means of a gamma-counter (1480 Wallac WIZARD 3″, PerkinElmer, USA). The radiochemical product yield was calculated by relating the radioactivity of the product peak with the amount of radioactivity injected onto HPLC.

Table 2
R_f values and retention times (R_t) of the ^{18}F-labelled products as analysed by TLC and HPLC.

Precursor	R_f (TLC) of ^{18}F-labelled product	R_t on HPLC			LC/MS mass peak
		[^{19}F]fluoro standard (min)	^{18}F-labelled product (min)	k'	
a_0-NO_2	0.69	7.49	8.02[a]	3.22	
a-NO_2	0.44	6.44	7.00[a]	2.68	
b-$NO_2^{*,+}$	0.68	8.82	9.36[a]	3.93	M + 1, M + 15
b-Br^+	0.68	8.82	9.37[a]		
c-NO_2	0.61	7.94	8.47[a]	3.46	
d-NO_2	0.59	7.75	8.28[a]	3.36	
j-$NO_2^{*,+}$	0.52	7.49	8.05[a]	3.24	M + 1
j-$Br^{*,+}$	0.52	7.49	8.05[a]		
k-$NO_2^{*,+}$	0.54		10.15[a]		M + 1
k-$Br^{*,+}$	0.54		10.16[a]		
f-NO_2^*	0.26		9.71[b]		M + 1, M + 15
g-$NO_2^{*,+}$	0.45	8.59	8.88[b]	4.92	M + 1, M + 15
g-$Br^{*,+}$	0.45	8.59	8.89[b]		
i-NO_2^*	0.30		9.48[b]		M + 1, M + 15
h-NO_2^+	0.61		17.86[b]		
h-Br^+	0.61		17.87[b]		
e-NO_2^+	0.40	10.45	10.74[b]	6.16	
e-Br^+	0.40	10.45	10.78[b]		

[a] HPLC conditions A.
[b] HPLC conditions B (see Section 4.5.2).
* Method 2.
+ Method 3 (see analytical assay; Method 1 was used for all compounds in Table 2).

Method 2: Labelling reactions were performed as described above but in the presence of 8.6 μmol KF (spray-dried) carrier (c.a. reaction). In this case, the carrier added nucleophilic fluorination allowed analyses by LC/MS in addition to the detection by Radio-HPLC. Thus, by finding the mass of the ^{19}F-product within the fraction of the eluted ^{18}F-product characterised by the retention time, the identification was independently assured.

Method 3: Precursors were chosen having the same chemical structure but a different leaving group, so that they resulted in the same ^{18}F-labelled product, i.e. the substitution of the Br- or NO_2-group ended in the same ^{18}F-product, which was identified by the corresponding retention time on HPLC. In the labelling reactions the nitro compounds (**b-NO$_2$**, **e-NO$_2$**, **g-NO$_2$**, **h-NO$_2$**, **j-NO$_2$** and **k-NO$_2$**) and the corresponding brominated compounds (**b-Br**, **e-Br**, **g-Br**, **h-Br**, **j-Br** and **k-Br**) were studied and the analytical data of ^{18}F-products are shown in Table 2. The retention times of radioactive product peaks were found to match exactly with each other (error < 0.05 min).

4.5.1. TLC analysis

An aliquot of the reaction solution on a silica gel plate (Polygram® Silica G/UV$_{254}$, 8 cm × 4 cm, Macherey&Nagel, Germany) was developed with petroleum ether/ethyl acetate (3/1, v/v). The radioactive spots were quantitatively assessed by means of electronic autoradiography (Instant Imager, Canberra Packard, USA). The R_f values are presented in Table 2. The size of the TLC plate and the location of the reference standard were marked by radioactive spots on the plate, thus a correlation between radioactive labelled product and non-radioactive standards was assured.

4.5.2. HPLC analysis

HPLC was applied for identification of radiolabelled product. HPLC was carried out by means of a Hewlett-Packard Model 1050 equipped with an UV detector and a NaI(Tl)-scintillation detector in series. Retention times of products were in agreement with the UV peaks of the reference compounds, R_t and k' values are presented in Table 2. KF was added to the eluent as carrier in order to assure radioactive [^{18}F]fluoride ions to be eluted as a sharp peak (k' = 1.0).

Conditions A: A C18 column (Luna, C18 5 μm, 250 mm × 4.6 mm, Phenomenex, USA) was used with a flow rate of 1 mL/min. Eluent was acetonitrile/water (50/50, v/v) containing 0.1% KF (UV detection at 254 nm).

Conditions B: A C18 column (Luna, C18 5 μm, 250 mm × 4.6 mm, Phenomenex, USA) was used with a flow rate of 2 mL/min. Eluent was acetonitrile/water (30/70, v/v) containing 0.1% KF (UV detection at 254 nm).

4.5.3. LC/MS analysis

LC/MS measurements were performed on a quadrupole LC/MS (Agilent Technologies, Model 6120) with an HPLC (Agilent Technologies, Model 1200). All solvents (methanol and water) were of HPLC-grade purity.

For identification of the product in the c.a. ^{18}F-labelling reaction (Method 2): A HP ODS (100 mm × 2.1 mm, 5 μm) column was used. Eluent was methanol/water (40/60, v/v) buffered with ammonium acetate (144 mg/L).

For the detection of by-product in the labelling reaction: A Phenomenex Luna (100 mm × 2.0 mm, 5 μm) C18 (2) column was used. Eluent was a gradient from 100% water to 100% methanol (within 30 min) buffered with ammonium acetate (144 mg/L).

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