DEVELOPMENT OF A NO-CARRIER-ADDED METHOD FOR ^{18}F-LABELLING OF AROMATIC COMPOUNDS BY FLUORODEDIAZONATION

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SUMMARY

To improve the accessibility of [^{18}F]arylfluorides a method is presented using the decomposition of aromatic diazonium salts in the presence of [^{18}F]fluoride. Several *p*-toluidyldiazonium salts have been synthesized and decomposed in the presence of fluoride. The most useful salt, *p*-toluidyldiazonium-2,4,6-tri-isopropylbenzenesulfonate, has been used to investigate several parameters influencing the fluorination. No-carrier-added labelling with [^{18}F]fluoride gave a radiochemical yield of 39% [^{18}F]p-fluorotoluene in a total synthesis time of 78 minutes.

Key words: [^{18}F]fluoride, no-carrier-added labelling, diazonium salts.

INTRODUCTION

Positron-emission-tomography (PET) is a powerful diagnostic method that is limited mainly by the availability of a useful tracer for the individual diagnostic scope. All tracers have as common features:

- A short living positron emitting radionuclide - in most cases carbon-11 ($t_{1/2}$ = 20 min), nitrogen-13 ($t_{1/2}$ = 10 min), oxygen-15 ($t_{1/2}$ = 2min) or fluorine-18 ($t_{1/2}$ = 110 min).
- A defined and in many cases complicated chemical structure and biological function.

The nuclide properties of fluorine-18 (E_{max} (β^+) = 635 keV; 97 % β^+ decay; no other gamma lines) are for many applications superior to those of the other nuclides. The low kinetic energy of

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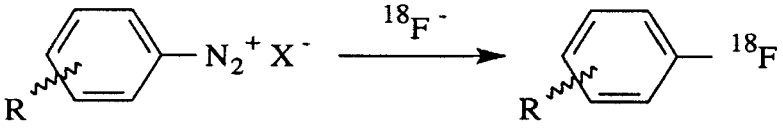
CCC 0362–4803/96/040325–12

Received 28 October 1994
Revised 17 November 1995

the positron allows high resolution scans. The longer half-life time allows either more time consuming syntheses or longer lasting diagnostics or even transport of the tracer over longer distances from the labelling site to the hospital.

Fluorine-18 labelled radiopharmaceuticals are synthesized either by electrophilic substitution with $[^{18}F]F_2$ as precursor or by nucleophilic substitution starting with $[^{18}F]F^-$ [1]. The nucleophilic pathway has two advantages compared to the eletrophilic method. Theoretically all radioactive fluorine-18 can be used for labelling the tracer, whilst 50% is lost using an eletrophilic reaction. The nucleophilic reaction can be carried out without the addition of carrier fluoridewhich is still required for an eletrophilic ^{18}F-labelling process. For some tracers for example for receptor binding studies a high specific radioactivity is required that can only be achieved with a reaction on no-carrier-added level. This reaction type is used for a large variety of aliphatic compounds (*eg.* [2]), but the direct nucleophilic aromatic ^{18}F-fluorination is restricted to electron-deficient systems, so in most cases it is necessary to synthesize the product by radiofluorination of a simple precursor followed by subsequent time consuming conversions [3].
To develop an alternative method for a no-carrier-added aromatic radiofluorination we examined the decomposition of aromatic diazonium salts (Scheme 1). This reaction allows the introduction of ^{18}F-fluoride in aromatic systems with high electronic density. The classical method for fluorodediazonations using $[^{18}F]$-BF_4 as counter ion for the diazonium salt, gives only low yields and the tetrafluoroborate anion contains large amounts of fluoride carrier [4]. The use of BCl_4^- instead of BF_4^- as counter anion allows a no-carrier-added radiofluorination but 75 % of the ^{18}F-fluoride starting material are still removed from the reaction mixture as gaseous byproducts [5].

Scheme 1: Introduction of ^{18}F-Fluoride into aromatic compounds via dediazonation



This paper presents the results of our experiments dealing with different counter anions that should not give major side reactions with $[^{18}F]$fluoride (an abstract of this work has been presented at the 9th International Symposium On Radiopharmaceutical Chemistry [6]). To enhance the yield further parameters that might influence the radiofluorination were checked using the p-toluidyldiazonium ion as model compound.

EXPERIMENTAL

Materials and Methods: All reagents used were of analytical grade or purified by the usual methods [7]. Diazotisations were carried out under nitrogen employing the usual techniques [8]. It is necessary to use reagents of the highest purity for the diazotisation, for it is extremely difficult to remove impurities from the diazonium salts. These impurities have an extreme negative influence on the yield of products from radiofluorination. Labelling experiments were carried out in boronsilicate vials from Wheaton. The vials had been boiled 30 minutes in nanopure water after the usual cleaning procedure.

General procedure for the preparation of *p*-toluidyldiazonium salts in glacial acetic acid: 20 Mmol of the anion acid, whose diazonium salt is to be synthesized, is dissolved in 5-10 ml glacial acetic acid. To this solution a solution of 1 g (9 mmol) *p*-toluidine in 5 ml glacial acetic acid is added while stirring. After dropwise addition of 2 ml (15 mmol) isoamyl-nitrite in 5 ml glacial acetic acid at room temperature the mixture is quenched with 15 ml ether and cooled to –20°C. Further ether is added slowly until the product precipitates. The product is washed with ether and petrol-ether and dried in vacuo.

This method has been used for the preparation of diazonium salts (2), (3), (5) - (8). The results are given in Table 1.

Preparation of *p*-toluidyldiazonium tetraphenylborate (1): 0.3 g (2.7 mmol) *p*-toluidine are dissolved in 10 ml water containing 12 mmol hydrochloric acid and cooled 0-5 °C. 1 g (15 mmol sodium nitrite) in 10 ml water and 1 g (3 mmol) sodium tetraphenylborate in 10 ml water are added simultaneously under stirring. After 10 min stirring the precipitate is sucked off, washed three times with water and dried in vacuo.

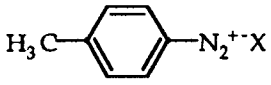
The results are given in Table 1.

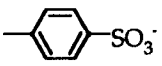
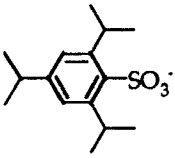
Preparation of *p*-toluidyldiazonium tetrachlorozincate (3): 1g (2.7 mmol) *p*-toluidine are dissolved in 6.6 ml water containing 12 mmol hydrochloric acid and cooled 0 °C. 1.1 g (16 mmol sodium nitrite) in 5 ml water are added slowly under stirring. After 10 min stirring 2 g (15 mmol) zinc chloride in 4 ml water are added. The precipitate is sucked off, and dried in vacuo.

The results are given in Table 1.

Table 1

Analytical data of some p-toluidyldiazonium salts for the fluorodediazonation



Anion X		Yield [%]	mp. [° C]	Elemental Analysis C calc. (found)	H (found)	N (found)	Remark
BPh_4^-	(1)	42	61-63				a,b
HSO_4^{2-}	(2)	75	102-103	38,89 (38,89)	3,73 (3,83)	12,96 (13,54)	
$H_2PO_4^-$	(3)	94	92-93	26,76 (26,83)	3,85 (3,90)	8,92 (9,43)	c
$ZnCl_4^{2-}$	(4)	34	98-99	37,75 (36,91)	3,17 (3,80)	12,58 (12,55)	b,d
CF_3COO^-	(5)	28	65-66				a
$CF_3SO_3^-$	(6)	56	82-83	35,83 (35,56)	2,63 (2,58)	10,44 (10,45)	
	(7)	41	88	57,92 (57,52)	4,86 (5,25)	9,64 (10,07)	
	(8)	47	112 (64,55)	64,69 (7,89)	7,92 (4,05)	4,07 c	

remarks:
a) not stable enough for elemental analysis
b) diazotisation carried out in water
c) 1 M free acid in the diazonium salt
d) small amounts of $ZnCl_2$ as impurity

Production of [^{18}F]fluoride: [^{18}F]fluoride was generated in an $H_2{}^{18}O$ target by an $^{18}O(p,n)^{18}F$ nuclear reaction with the baby cyclotron at the KFA-Forschungszentrum Jülich. 10 - 300 µl of target solution containing 40 to 400 MBq [^{18}F]fluoride were adjusted to 300 µl. Samples of 20 - 50 µl were added to a solution of the phase transfer catalyst used in the reaction.

Drying of the fluoride source: The fluoride or the solution containing [^{18}F]F$^-$fluoride is dried in the reaction vessel under a nitrogen stream (300 - 600 ml/min) at 110° C. To remove water traces,

300 µl acetonitrile are added twice and re-evaporated. After removing the solvent for the last time the residue is dried for a further minute under a nitrogen stream.

Thermal decomposition: After drying the fluoride source, 300 µl solvent, a stirring bar and 70 - 130 µmol diazonium salt are added. The reaction vessel is closed (dust, but not gas sealed) with a reflux condenser and heated for 30 minutes at 110° C.

Gas-Chromatographic Analysis: After cooling the reaction mixture to room temperature 300 µl pentane are added. The mixture is washed once with 1.0 M sodium hydroxide, three times with water and adjusted to 1 ml with pentane. A sample of 0.5 µl is analyzed by GC using a 2 m x 4 mm Chromosorb W column coated with 5 % diisodecylphthalate and 5 % Bentone 34. The retention times are 4.7 minutes for p-fluorotoluene and 4.2 minutes for toluene respectively, at a flow rate of 65 ml/min and a temperature of 75° C. Gas-Chromatographic analysis was not carried out with samples containing ^{18}F.

HPLC-Analysis: After cooling the reaction mixture to room temperature 300 µl methanol are added. The reaction apparatus is rinsed three times and a sample of 100 µl is adjusted to 10 ml with methanol. 50 µl of this solution are analyzed by HPLC. Usually a Nucleosil ODS-5 25 cm x 4 mm column was used. The retention times are 6.7 minutes for p-fluorotoluene and 7.0 minutes for toluene with a methanol/water mixture (80:20) at a flow rate of 0.8 ml/min. A Nucleosil 5 NO_2 column (20 cm x 4 mm) acted as reference system with a methanol/tetrahydrofuran/water mixture (10.5:18.5:71) as eluent. Using this system the retention time for toluene is 10.6 minutes and for p-fluorotoluene 12.9 minutes at a flow rate of 0.8 ml/min. Compounds were detected by their absorbance at 265 nm and by γ - measurement at 511 keV.

RESULTS AND DISCUSSION

Investigations with fluoride: The experiments concerning the influence of various reagents were carried out with inactive fluoride.

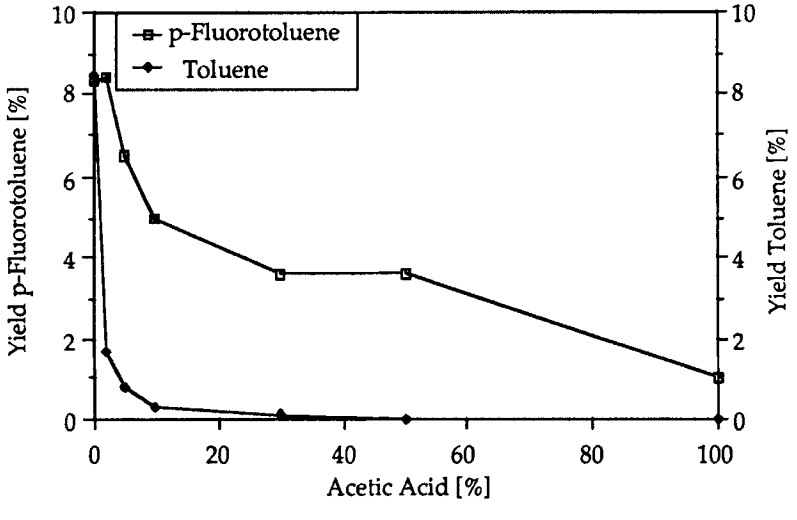
Diazonium cations can decompose either by a heterolytic pathway with a cationic intermediate or by a homolytic pathway with a radical intermediate. Which reaction actually occurs depends on a number of influences such as the other substitutents of the diazonium ion, the solvent, the acidity/basicity of the reaction medium and the presence of radical sources or redox systems.

Therefore, it is important to investigate the conditions needed for the fluorination. The occurence of toluene is used as an indicator for the radical reaction.

We started to look for a diazonium salt containing an anion that would not interfere with the fluorination. To check this, diazonium salts listed in table 1 were decomposed in various solvents in the presence of equimolar amounts of Tetraethylammonium fluoride (TEAF) and Tetra-n-butylammonium fluoride (TBAF), respectively. The best results were obtained with p-toluidyldiazonium-2,4,6-triisobutylbenzenesulfonate (8) and TBAF in p-chlorotoluene giving 6 % p-fluorotoluene. The use of polar aprotic solvents like 1,4-dioxane, ethylacetate and tetramethylurea respectively, enhances the toluene yield and lowers the fluorination yield, in most cases below the detection limit. Hashida et al. recommend the use of 2,2,2-trifluoroethanol or 1,1,1,3,3,3-hexafluoroisopropanol for the heterolytic decomposition [9]. Indeed, we received up to 33 % p-fluorotoluene, but this was primarily formed from the solvent by abstraction of fluorine. Therefore, these solvents are not suitable for [^{18}F]labelling runs.

Since the mechanism of the dediazonation can be influenced by the pH of the solution, we investigated the fluorination and the reduction behavior after adding acetic acid or triethylamine to the reaction mixture using p-chlorotoluene as cosolvent (fig. 1 and 2). Small amounts of acetic acid

Figure 1: Decomposition products under acidic conditions

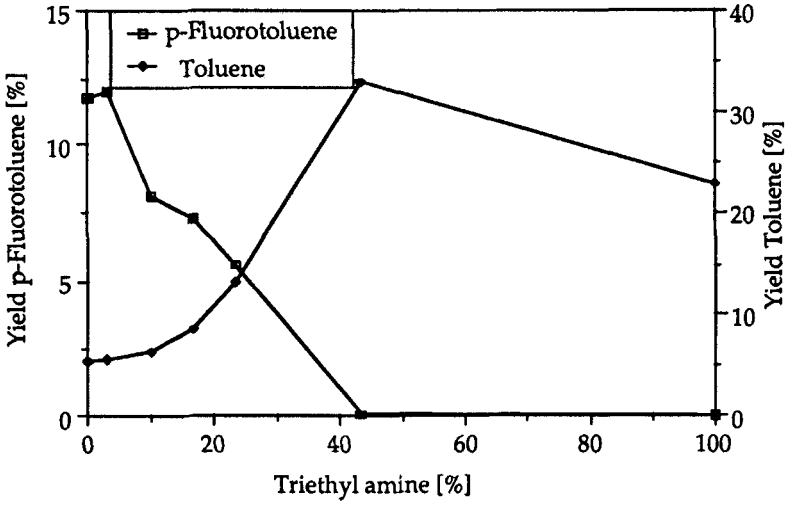


Reaction conditions: 70 µmol diazonium salt (8), 35 µmol TBAF, 300 µl p-chlorotoluene containing various amounts of acetic acid, 30 min stirring at 110 °C.

lower the reduction yield, while the fluorination yield is not affected. Large amounts of acetic acid reduce the yield of both products and the decomposition remains uncomplete. Addition of an excess of triethylamine strongly favours the reduction product, while no p-fluorotoluene at all can be found.

The investigation of some radical initiators or traps like iodine or 2,2´bipyridine (example fig. 3) produce no significant effects, however, after addition of traces of copper to 2,2´-bipyridyl we observed large amounts of toluene (fig. 3B), but no fluorination product.

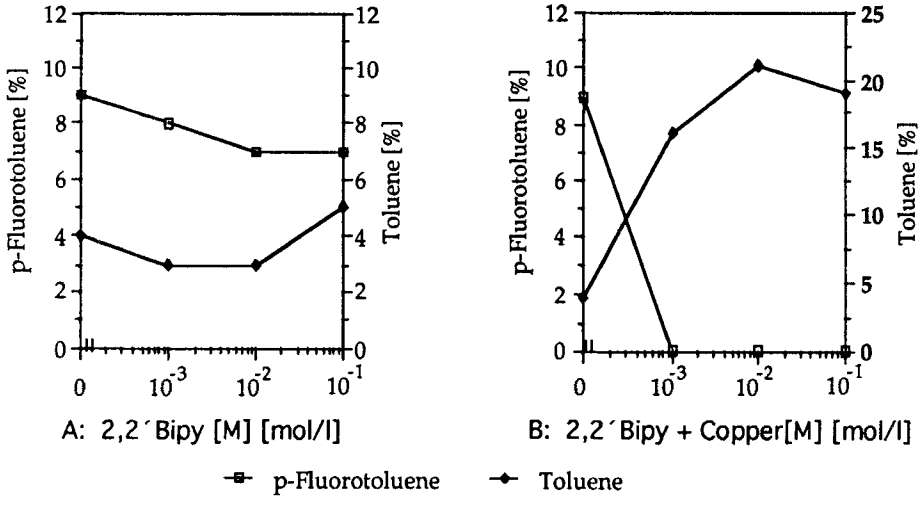
Figure 2: Decomposition products under basic conditions



Reaction conditions: 70 µmol diazonium salt (8), 35 µmol TBAF, 300 µl p-chlorotoluene containing various amounts of triethylamine, 30 min stirring at 110 °C.

To make the fluoride soluble in a reactive form an anion activating agent is needed. Therefore, we examined the effect of the fluoride source in the presence of different macrocyclic polyethers, tetraalkylammonium salts and caesium fluoride (table 2). While the best results are obtained with 15-crown-5 or benzo-15-crown-5 in the presence of sodium fluoride, the well known aminopolyether 2.2.2./ potassium fluoride system produces no fluorination product. The poor fluorination rate of 18-crown-6 and APE 2.2.2. with potassium fluoride is related to their ability to complex the diazonium ion [10]. The complexation has two negative effects. First, the solubilisation of the fluoride is reduced since parts of the crown ether react with the diazonium ion instead of mobilizing the potassium fluoride. Secondly, the complexed diazonium ion is stabilized [10, 11] and may react differently, but not decompose.

Figure 3: Influence of trace amounts of copper on the fluoro-dediazonation



Reaction conditions:

A: 70 µmol diazonium salt (8), 40 µmol TBAF, 300 µl p-chlorotoluene containing various amounts of 2,2´-bipyridyl, 30 min stirring at 110 °C.

B: 70 µmol diazonium salt (8), 40 µmol TBAF, 300 µl p-chlorotoluene containing various amounts of 2,2´-bipyridyl, 30 min stirring at 110 °C in presence of trace amounts of copper.

The ratio between the 15-crown-5/sodium fluoride complex and the diazonium salt was investigated to obtain information about the behaviour of a large excess of the diazonium ion. The yield of p-fluorotoluene increases from 9 % at equimolar amounts to 23 % using a tenfold excess of the diazonium ion.

Table 2.

Effect of different fluorination sources

Fluorination source	Yield p-fluorotoluene [%]
Caesium fluoride	4
Benzo-15-crown-5 / sodium fluoride	15
15-crown-5 / sodium fluoride	14
15-crown-5 / potassium fluoride 2:1	5
18-crown-6 / potassium fluoride	1
Kryptofix 2.2.2. / potassium fluoride	-
Tetraethylammonium fluoride	9
Tetrabutylammonium fluoride	12

Reaction conditions: 70 µmol diazonium salt (8), different fluorides each containing 35 µmol F^-, 300 µl p-chlorotoluene, 30 min stirring at 110 °C.

Labelling experiments: The usefulness of the phase transfer catalysts, described above, is given on the no-carrier-added level too, as the results obtained in inactive and active runs show (see table 3):

- Tetra-n-butyl ammoniumhydroxide gives 15 % [^{18}F]p-fluorotoluene.
- Complexes of crown ethers and cryptands with potassium carbonate, where the ligands are able to complex the diazonium cation produce only small amounts of labelling product (3-5 % [^{18}F]p-fluortoluene).
- 15-crown-5 in the presence of sodium carbonate was found to be the best [^{18}F]F$^-$ activating agent for the fluoro-dediazonation, giving 32 % p-fluorotoluene.

Table 3.
Labelling experiments with different phase transfer catalysts

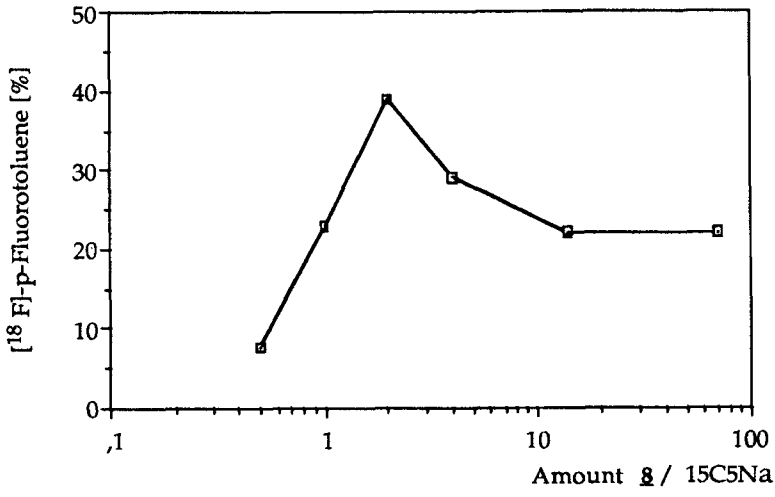
Phase Transfer Catalyst	Decay corrected Yield of [^{18}F]p-Fluorotoluene [%]
Caesium carbonate	2
Benzo-15-crown-5 / sodium carbonate	11
15-crown-5 / sodium carbonate	32
Poly-dibenzo-18-crown-6 / potassium carbonate	1
Kryptofix 2.1.1. / sodium carbonate	5
Kryptofix 2.1.1.D. / sodium carbonate	3
Kryptofix 2.2.2. / potassium carbonate	4
Tetraethylammonium hydroxide	8
Tribenzylmethylammonium hydroxide	3
Tetrabutylammonium hydroxide	15

Reaction conditions: 10 MBq [^{18}F]fluoride, 70 µmol diazonium salt (8), 35 µeq phase transfer catalyst, 300 µl p-chlorotoluene, 30 min stirring at 110 °C.

With the 15-crown-5/sodium carbonate system we examined the influence of temperature, reaction time and the amount of phase transfer catalyst on the yield of [^{18}F]p-fluorotoluene at the no-carrier-added level. An excess of carbonate carrier lowers the labelling yield to 8 % (fig. 4). This is caused by the excess of base in the reaction system. Too small amounts of 15-crown-5/sodium carbonate also reduce the yield. The best results are obtained by a twofold excess of diazonium salt.

Temperatures below 100° C reduce the rate of decomposition in such a way that complete reaction does not occur within 30 minutes. Decomposition at 115° C gives the best yield of labelled product (39 % [^{18}F]p-fluorotoluene), while higher temperatures again reduce the yield.

Figure 4: [^{18}F]-Labelling yield with different amounts of diazonium salt and phase transfer catalyst



Reaction conditions: 10 MBq [^{18}F]fluoride, 70 µmol diazonium salt (8), various amounts of 15-crown-5 / sodium carbonate, 300 µl p-chlorotoluene, 30 min stirring at 115 °C.

To evaluate the optimal reaction time it is necessary to distinguish between the decay corrected (EOB) yield and the decay non-corrected (EOS) yield. While for the EOB-yield the best result is received after 60 minutes (39 % [^{18}F]p-fluorotoluene), the EOS based yield achieved maximum results after 30 minutes (28 % [^{18}F]p-fluorotoluene). A further 8 minutes are required for drying the complex and 10 minutes are needed for the HPLC separation, giving a total synthesis time of 48 minutes. The specific radioactivity is calculated to be 1 GBq/µmol using about 10 MBq per run.

The major sources for losses of ^{18}F radioactivity are:

- about 20 % is adsorbed on the reaction vessel
- about 30 % decays during synthesis

Labelled byproducts were observed only in a few cases and in amounts smaller than 5 %. The remaining ^{18}F radioactivity consists of unreacted [^{18}F]F$^-$-fluoride.

CONCLUSION

The preparation of [^{18}F]p-fluorotoluene has been tested as model for the no-carrier-added radiofluorination of aromatic compounds using the thermal decomposition of an aromatic diazonium salt. To avoid the limitations of the "classical" Balz-Schiemann-Decomposition a diazonium 2,4,6-tri-isopropylbenzenesulfonate has been used instead of a diazonium tetrafluoroborate salt. The decay corrected radiochemical yield of 39 % [^{18}F]p-fluorotoluene is lower than yields achieved by aliphatic nucleophilic subtitutions but much higher than yields possible with the "classical" Balz-Schiemann-Decomposition.

While direct nucleophilic aromatic [^{18}F]F$^-$fluorinations are restricted to activated, electron deficient substrates, the fluorodediazonation is not limited in this manner and therefore may be an interesting new route for [^{18}F]labelling of aromatic compounds.

ACKNOWLEDGEMENT

We greatfully acknowledge the financial support of the Bundesminister für Forschung and Technologie as well as the Verband der Chemischen Industrie - Fond der Chemischen Industrie. Especially we want to thank Prof. Dr. G. Stöcklin and his team, who enabled us to do the radioactive research work in his laboratories at the KFA-Forschungszentrum Jülich.

REFERENCES

1) M. S. Berridge, C. Crouzel, D. Comar, Journ. Lab. Comp. Radiopharm., 22, 687 (1985).

2) D. Block, B. Klatte, A. Knöchel, R. Beckmann, U. Holm, Journ. Lab. Comp. Radiopharm., 23, 467 (1986).

3) C. Lemaire, M. Guillaume, R. Cantineau, L. Christiaens, Journ. Nucl. Med., 31, 1247 (1990).

4) T. Nozaki, Y. Tanaka, Int. Journ. Appl. Radiat. Isot., 18, 111, (1967).

5) A. Knöchel, O. Zwerneman, Appl. Radiat. Isot., 42, 1077, (1991).

6) D. D. Perrin, W. L. F. Amarego, D. R. Perrin, Purification of Laboratory Chemicals, 2nd ed., Pergamon Press, Oxford (1980).

7) R. Pütter, in Houben Weil, Methoden der Organischen Chemie, Band X/3, 4. Aufl., Georg Thieme Verlag, Stuttgart 1965.

8) Y. Hashida, et al., Journ. Am. Chem. Soc., 100, 2816, (1978).

9) R. A. Bartsch, Complexation of Aryldiazonium Ions by Polyethers, in Crown Ethers and Analogs (Ed. S. Patai, Z. Rappoport), John Wiley & Sons, Chichester, 1989, 491.

10) R. A. Bartsch, H. Chien, N. F. Haddock, P. N. Juri, Journ. Am. Chem. Soc., 98, 6753 (1976).