N.C.A. [18F]-LABELLING OF ALIPHATIC COMPOUNDS IN HIGH YIELDS VIA AMINOPOLYETHER - SUPPORTED NUCLEOPHILIC SUBSTITUTION

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SUMMARY

The nucleophilic introduction of n.c.a. - [18F]-fluoride into alkanes and carboxylic acids using anion activation by macrocyclic polyethers was investigated.

The reactions carried out in the presence of the bicyclic aminopolyether APE 2.2.2. gave rise to high radiochemical yields (40-65 %) under mild conditions. Important for the successful 18F-labelling is a suitable cation/polyether couple, which leads to an unsolvated [18F]-fluoride in a dipolar aprotic solvent and ensures a high ion concentration, thus enhancing the reactivity. Reaction parameters, such as concentration of substrate and APE, the influence of cation, anion and water were studied and optimised.

Key words: nucleophilic radiofluorination,
            n.c.a. $^{18}F^-$, anion activation,
            aminopolyether 2.2.2.,
            [18F]-fluorofatty acid.

INTRODUCTION

[18]F-labelled compounds are gaining importance in Positron Emission Tomography (PET), particularly when the labelling is carried out under no carrier added (n.c.a.) conditions (cf.e.g.(1)).

An ideal fluorination agent under these conditions is [^{18}F]-fluoride. In principle it is possible, to label all compounds with this anion via nucleophilic substitution reactions. Carrier-free [^{18}F]-fluoride can be generated in high yields from various targets at a cyclotron (see e.g. (2,3)). But nucleophilic introduction of fluorine normally requires long reaction times and drastic reaction conditions. The yields are generally small. This is particularly true for n.c.a. reactions, which suffer from loss of activity as a result of adsorption. Dipolar aprotic solvents having good cation solvating properties thus rendering them suitable for common preparative fluorination reactions, can also be employed in ^{18}F-labelling (4-12).

[^{18}F]-tetraalkylammonium fluorides (13) and 18-crown-6 (5,14) have been employed with the aim of improving the fluoride solubility and suppressing the solvation of the fluoride ion, but 18-crown-6 has not been always successful (8,15).

Bicyclic aminopolyethers, which can be used successfully for preparative fluorination (16, 17) have not been applied to ^{18}F-labelling so far. In this paper parameters which are important for the nucleophilic introduction of [^{18}F]-fluorine under the conditions of anion activation are examined and optimised with the aim to achieve fast n.c.a. ^{18}F-labelling under mild reaction conditions. The influence of various reaction parameters on ^{18}F-labelling were studied with bromoheptane as a model system. These were used as a basis for a general method of fast nucleophilic introduction of [^{18}F]-fluoride under n.c.a. conditions.

EXPERIMENTAL

Materials and methods

All used chemicals are commercially available. The aminopolyethers (APE) (Merck, Darmstadt, FRG) and the crown ethers (Fluka, Buchs, Switzerland) were used without further purification.

The applied salts were of the highest grade of purification. The solvents were purified by ordinary methods. The substrates were distilled or recrystallized before use. Aqueous n.c.a. [^{18}F]-fluoride was produced by the $^{16}O(^{3}He, p)^{18}F$ nuclear reaction at the Philips AVF Isochron-Cyclotron of the University of Hamburg.

The ^{18}F-production was carried out by irradiation of 4 ml water, (Nanopure[R]-quality), with 40 MeV ^{3}He-ions (1 μA; 2-3h) in a target vessel (2 mm, 50 mm ϕ) made of titanium. A tantalum-foil was used as a beam window. Further details are described elsewhere (18). Small amounts of ^{7}Be were produced too. The radiochemical purity

Table 1: Separation conditions.

| Substrate | GC Carbowax 20 M; | | | HPLC Radial Pak C_{18} | | | |
	T_{column} [^{o}C]	k'_F	k'_{Br}	Eluent	k'_F	k'_{Br}	k'_{Cl}
1-bromoheptane	95	1.3	6.1	9MeOH:1H$_2$0	2.8	4.2	--
1-bromooctane	90	2.1	6.3	----	--	--	--
2-Br-CH$_2$COOCH$_3$	65	6.3	14.3	8MeOH:3H$_2$0	2.3	3.5	--
2-Br-C$_2$H$_5$COOC$_2$H$_5$	90	2.3	9.6	---	--	--	--
4-Br-(CH$_2$)$_3$COOC$_2$H$_5$	120	1.5	9.3	---	--	--	--
6-Br-(CH$_2$)$_5$COOC$_2$H$_5$	120	3.4	16.8	7MeOH:3H$_2$0	1.3	3.8	--
17-Br-(CH$_2$)$_{16}$COOC$_2$H$_5$	---			MeOH	1.4	5.2	--
6-chloronicotinic acid-N-diethylamide	---			3MeOH:7H$_2$0	1.5	--	2.5

was $^7Be/^{18}F = 2.5 \cdot 10^{-4}$. An aliquot of ^{18}F-containing water was taken for the reaction.

In a typical reaction 0.05 - 0.1 mmol K_2CO_3 and 0.1 - 0.2 mmol APE 2.2.2. are added to 1-4 ml irradiated target water, containing 10 - 40 mCi [(0.37 - 1.5)$\cdot 10^9$ Bq] ^{18}F. The mixture is vaporized and dried in vacuum (15 Torr) at 95 - 110°C. This procedure takes 10 - 30 minutes depending on the amount of the target water (1-4 ml).

In the vessel with the dry residue 1.5 ml acetonitrile (abs.) and 0.05 - 1 mmol substrate are given. After 10 minutes refluxing the ^{18}F-labelling is finished. The products are separated by gas chromatography or HPLC using two columns of the same type. An overview of the separation conditions is given in table 1.

The radiochemical yield was based on the activity in the reaction mixture, neglecting wall activity. Before chromatographic separation the reaction mixture (solvent CH_3CN) is filtered (0.45 μm). Filter and vessel are washed twice with 1 ml CH_3CN. The ^{18}F-activities in the CH_3CN fractions from the chromatographic separations were determined off-line after collection on charcoal using a NaJ (Tl) scintillation detector (Bicron).

RESULTS AND DISCUSSION

Effect of Polyether and Solvent

Nucleophilic substitution reactions can best be carried out in dipolar aprotic solvents, owing to their poor anion solvation properties. Macrocyclic polyethers and aminopolyethers in form of their alkali complexes enhance this effect. Therefore different dipolar aprotic solvents and commercially available mono- and bicyclic

Table 2:   Radiochemical yield of n.c.a. [^{18}F]-fluoroheptane in the presence of different polyether complexes of K_2CO_3.

Polyether	Radiochemical Yield [%]
2.2.2.	63
2.2.2.B.	52
18-crown-6	38

Reaction conditions: 1 mmol bromoheptane, 0.1 mmol polyether complex, 1.5 ml acetonitrile, 82°C, 10 min.

polyethers were investigated, especially with regard to their effectiveness in supporting n.c.a. ^{18}F-labelling. The polyethers listed in table 2 were found to be the best in combination with acetonitrile.

The bicyclic APE 2.2.2. and 2.2.2.B. gave radiochemical yields of more than 60 % within 10 minutes under relatively mild conditions (82°C). Due to the presence of a benzene ring in the aminopolyether 2.2.2.B. its reactivity is lower than that of 2.2.2. The anticipated prevention of the loss of activities due to adsorption on the walls of the vessel by use of more lipophilic APE 2.2.2.B. could not be confirmed. The activity retained by the vessel was equal to that measured for the APE 2.2.2., but the radiochemical yield was 10 % lower.

The reaction, supported by 18-crown-6, especially in acetonitrile proceeds less successfully. Compared with the bicyclic APE 2.2.2. the inclusion of the cation by the monocyclic polyether is less complete. This results in higher interactions between anion and encapsulated cation than in the system of the bicyclic APE's, and the fluoride anion appears therefore less "naked".

Effect of the added salts

For the conventional preparative nucleophilic introduction of fluoride the solubility and reactivity of the fluoride anion are highly dependent on the accompanying cation. Only large and "soft" alkali cations (K^+, Rb^+, Cs^+) are effective, since their polar interactions with the "hard" anion are weaker than those of the "hard" cations like Li^+ and Na $^+$.

This effect is more valid for polyether-assisted reaction systems, because in addition one has to look for an optimal encapsulation of the cation by the polyether. To first approximation salts of heavy alkali metals, whose cation dimensions correspond to the diameter of crown ethers or to the cavity size of bicyclic aminopolyethers are easily dissolved and more reactive. This condition is in the case of n.c.a. ^{18}F-labelling not easily fulfilled because the nature of the counter cation to ^{18}F$^-$, generated with a water target, is unknown to a great extent.

Table 3:   Influence of the anion on the n.c.a. ^{18}F-labelling of
bromoheptane.

Anion X	as [K/2.2.2.]X or [K/2.2.2.]$_2$X	Radiochemical Yield of [^{18}F]-fluoroheptane [%]
Cl^-		8-25
OH^-		34
ClO_4^-		no reaction
SO_4^{--}		5
CO_3^{--}		65

Reaction conditions: 1 mmol bromoheptane, 0.2 mmol [K/2.2.2.]X, 1.5 ml
acetonitrile, 82°C, 10 min.

As counter cations one has to consider, for example, protons from the target water and metal ions from the target vessel and foil materials. As these cations in combination with fluoride-anions are poor fluorinating agents, a salt of the alkali cations, mentioned above, containing a more qualified anion has to be added in amounts corresponding to that of the polyether.

Now one has to consider the nature of this corresponding anion, which is introduced into the vessel together with the alkali cation, e.g. K^+. Consequences for the resulting radiochemical yield are described in table 3.

Apart from a large amount of by-products (chloropentane) the presence of Cl^--anions (as KCl) produces varying amounts of [^{18}F]-fluoroheptane. The presence of KOH in the vessel also leads to by-products, but the radiochemical yields are well over 30 % and reproducible. The yields in the presence of oxoanions like ClO_4^- and SO_4^{2-} correspond to the solubility of $KClO_4$ and K_2SO_4; these salts are not suitable for supporting the ^{18}F-labelling. Only the presence of carbonate leads to high yields of carrier-free labelled [^{18}F]-fluoroheptane.

The variation of the cation in the carbonate system shows the outstanding role of K_2CO_3 and Rb_2CO_3.

This is illustrated by the results of the experiments summarized in table 4. Li^+ is not complexed by the aminopolyether, and therefore bound in an ion-pair. Na^+ gives weak APE-complexes with great interaction to the anion. In both cases the nucleophilic activity of the ^{18}F-anion is deactivated. By the addition of K_2CO_3 or Rb_2CO_3 strong complexes of the polyether with the cation are formed. This results in a very weak interaction between the macrocation and the [^{18}F]-anion.

The amount of dissolved K_2CO_3 in the reaction medium is also important for the successful labelling reaction as shown in figure 1.

Table 4:   Influence of the cation on the n.c.a. ^{18}F-labelling of
            bromoheptane

Cation [M/2.2.2.]$_2$CO$_3$	Radiochemical Yield of [^{18}F]-fluoroheptane [%]
without any addition	2
only 2.2.2., no cation	5
Li$_2$CO$_3$	3
Na$_2$CO$_3$	7
K$_2$CO$_3$	63
Rb$_2$CO$_3$	62

Reaction conditions: 1 mmol bromoheptane, 0.2 mmol [M/2.2.2.]$_2$CO$_3$,
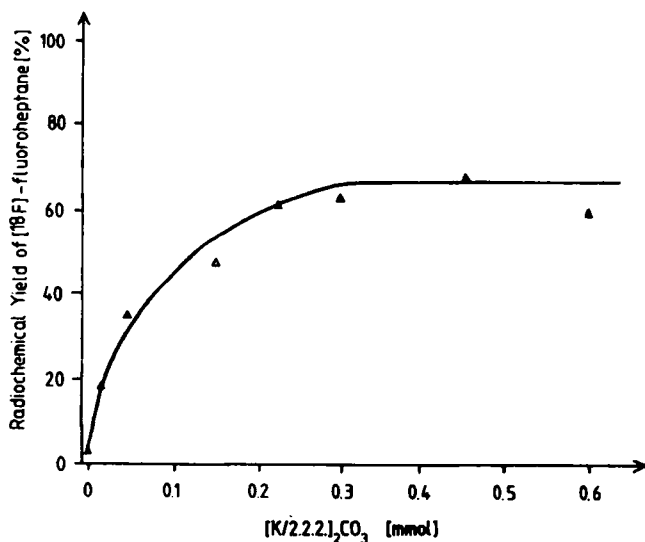   1.5 ml acetonitrile, 82 $^{\circ}$ C, 10 min.

Figure 1:   Effect of the dissolved K$_2$CO$_3$ on the yield of [^{18}F]-fluoroheptane
             (1 mmol bromoheptane, 1.5 ml acetonitrile, 82 $^{\circ}$C, 10 min).

Carbonate quantities, which greatly exceed the [^{18}F]-fluoride concentrations but are
of the same magnitude as the added polyether, lead to maximum radiochemical
yields. The positive effect of the carbonate on the reaction can be attributed not
only to the supply of a "suitable cation", but also to the basicity of the carbonate
itself. Obviously the carbonate competes successfully with the fluoride for the protic
components of the reaction system. Probably the APE complexes and solvent

molecules are forming micellar structures (19), in which protic molecules are hindered in their interaction with $^{18}F^-$. Furthermore one can assume that the micelles favour the transition states of the substitution reaction. As a result the $[^{18}F]$-anion is less solvated or protonated and remains in a "naked", reactive state.

Effect of water contents

An indication of the existence of micellar structures with protection properties against protic components is the very remarkable reaction behaviour in the presence of water.

Normally the presence of protic compounds like H_2O leads to a reduction of the nucleophilic reactivity as a result of the high fluoride/proton affinity. This should have a negative effect, particularly on ^{18}F-labelling under n.c.a. conditions, because, especially if $[^{18}F]$-fluoride is produced from a water target, the protic component would by far exceed the amount of the radionuclide.

Therefore the influence of water on the $^{18}F^-$-activity and the yield of $[^{18}F]$-fluoroheptane in the APE-assisted reaction system was studied, using tritium as tracer in the determination of the water content. The results are summarized in figure 2.
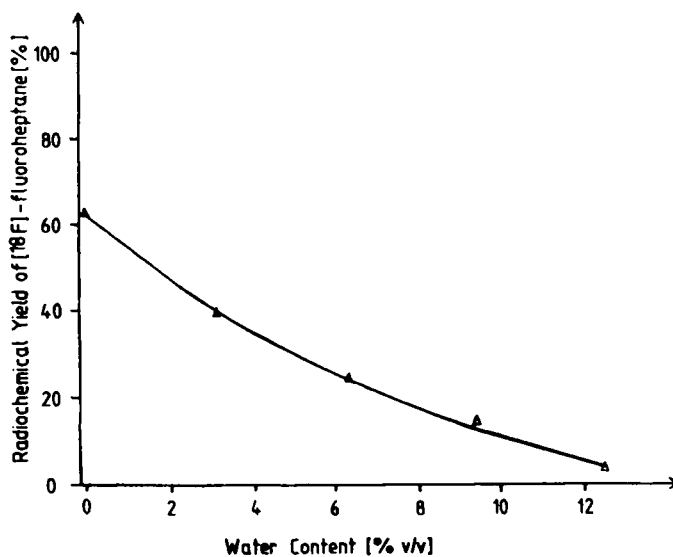

Figure 2:   Effect of water content on the course of n.c.a. ^{18}F-fluorination of bromoheptane, (1 mmol bromoheptane, 0.2 mmol $[K/2.2.2.]_2CO_3$, 1.5 ml acetonitrile, 82°C, 10 min).

A water content of 3 % leads to a decrease of the yield from 65 to 40 %. Even with a water content of 6 % in the reaction system a yield of more than 20 % is attainable. With a water content of 12 % the reaction finally comes to a standstill.

Considering the fact, that a 3 % water content corresponds to a molar excess of 10^6 relative to ^{18}F, it is remarkable that the system is very insensitive to water. It therefore takes care of the conditions, given by the water target.

Loss of $[^{18}F]$-fluoride by adsorption.

For the investigations on hand, optimizations of the vessel material concerning minimum fluoride adsorption were not carried out. But the influence of the carbonate/polyether concentration with regard to the amount of ^{18}F-activity adsorbed onto the walls of Wheaton glas vessels was examined. This relation is shown in figure 3.
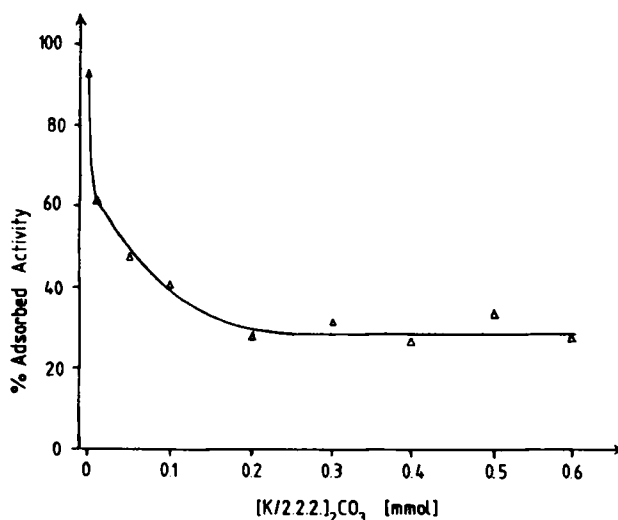

Figure 3:   Dependence of the amount of polyether complex on the activity adsorbed onto the walls of a Wheaton glas vessel (1 mmol bromoheptane, 1.5 ml acetonitrile, $82^{o}C$, 10 min).

Small amounts of $[K/2.2.2.]_2CO_3$ lead to a drastic decrease of the $[^{18}F]$-fluoride adsorption.
However the extent of adsorption remained constant after the addition of more than 0.2 mmol additives, giving the described radiochemical yield of 65 % $[^{18}F]$-fluoroheptane.

Effect of leaving groups and the amount of substrates.

Investigations on the reactivity of the leaving group under the described conditions of anion activation were carried out with chloro-, bromo-, iodo- and tosylheptane as standard substrates.
In contrast to the classical leaving group reactivity no statistically significative differences could be found in the polyether-supported synthesis between bromo-, iodo-

and tosylheptane. The influence of the amount of substrate in the radiochemical yield in the case of bromoheptane is shown in figure 4.

The amount of bromoheptane was varied from 1:1 stoichiometry to a 1:12 stoichiometry, relative to $[K/2.2.2.]_2CO_3$.

Substrate concentrations under 0.2 mmol gave a yield of 50 %. Higher concentrations ($\sim$ 0.8 mmol) improved the yield to 65 %. A further increase of the substrate concentration caused a decrease of the yields. No product was registrated when the reaction was carried out without a solvent in pure bromoheptane. This can be traced back to the solvent properties of bromoheptane, which is less polar than acetonitrile.
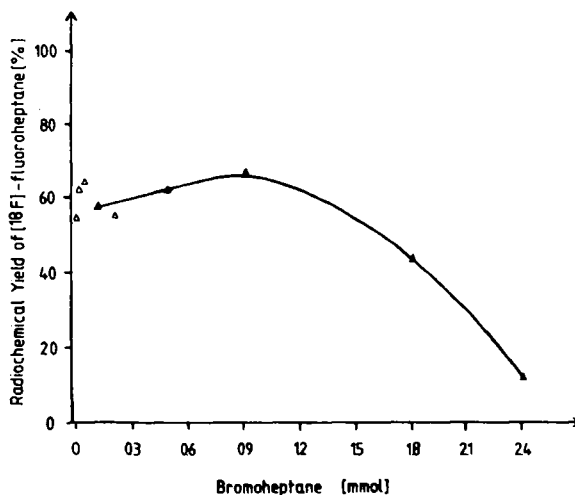

Figure 4: Influence of the amount of substrate on the radiochemical yields (0.2 mmol $[K/2.2.2.]_2CO_3$, 1.5 ml acetonitrile, 82°C, 10 min).

Extension of the method.

The polyether assisted ^{18}F-labelling is in principle applicable to all substrates, which undergo nucleophilic substitution reactions. The results of labelling experiments with substrates, which differ both structurally and in reactivity from bromoheptane, are listed in table 5.

As expected, the reactions of 1-bromoheptane and 1-bromooctane lead to results which are indentical with that of the standard substrate within the experimental error. Free carboxylic acids such as bromoacetic and bromohexanoic acid do not react under these conditions. But the conversion of reactive esters with the leaving group in the α-position, like α-bromoacetic acid methylester and 2-bromopropionic acid ethylester was achieved with the same good results as the labelling of 6-bromohex-anoic acid ethylester and 17-bromoheptadecanoic acid ethylester, which can easily be converted by saponification to the $[^{18}F]$-fluorofatty acid. This compound is of high relevance to PET-studies of heart metabolism. The n.c.a. preparation of this compound is published elsewhere (20).

The procedure is also suitable for the introduction of $^{18}F^-$ in aromatic compounds,

Table 5:   Total radiochemical yield of the ^{18}F-labelling of different substrates.

Substrate	Total yield [%]
1-bromohexane	57 ± 9
1-bromoheptane	62 ± 11
1-bromooctane	66 ± 5
2-Br-CH_2COOCH_3	44 ± 7
2-Br-$C_2H_5COOC_2H_5$	56 ± 8
4-Br-$(CH_2)_3COOC_2H_5$	47 ± 8
6-Br-$(CH_2)_5COOC_2H_5$	51 ± 10
17-Br-$(CH_2)_{16}COOC_2H_5$	45 ± 8
6-chloronicotinicacid-N-diethylamide	19 ± 3

Reaction conditions: 1 mmol substrate, 0.2 mmol [K/2.2.2.]$_2$CO$_3$, 1.5 ml
acetonitrile, 82°C, 10 min.

which undergo nucleophilic substitution. An example is the labelling of 6-chloronico-
tinic acid N-diethylamide, a potential pharmaceutical for the studies of brain
functions (21).
The conversion of this nicotinic acid derivative according to our general labelling
method leads to yields of nearly 20% of the n.c.a. product.
Analogously ^{18}F-labelled steroids and carbohydrates can be synthesized.

ACKNOWLEDGEMENTS

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