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N.C.A. ^{18}F-FLUOROACYLATION VIA FLUOROCARBOXYLIC ACID ESTERS

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SUMMARY

The preparation and introduction of fluoroacyl moieties as prosthetic groups is described for n.c.a. labelling with fluorine-18. Activation by the aminopolyether 2.2.2./K_2CO_3 complex was used for the nucleophilic ^{18}F-exchange in α-substituted acid esters. Increasing yields were found in the sequence: iodo << chloro < tosyloxy < bromo. The methylester of α-bromopropionic acid proved to be the best precursor for acylation. The [2-^{18}F]fluoropropionic acid methylester was prepared with radiochemical yields of > 90% within 10 minutes. It reacted effectively with primary alcohols in the presence of 3% methane sulfonic acid. Using n-butylamine as model compound and 0.1% NH_4Cl as acid in aqueous solution, n.c.a. N-fluoroacylation was also performed with > 80% radiochemical yield. This reaction is relevant to ^{18}F-labelling of biomolecules such as peptides.

Key words: aminopolyether 2.2.2.,
nucleophilic n.c.a. ^{18}F-fluorination,
^{18}F-fluoroacylation,
[2-^{18}F]fluoroacetic acid esters,
[2-^{18}F]fluoropropionic acid esters

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INTRODUCTION

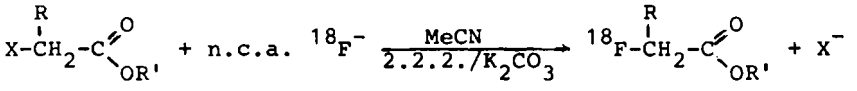
^{18}F-Fluoroalkylation has recently been suggested (1) and described by several groups (2-5) as a longer-lived alternative to ^{11}C-alkylation. This approach provides a new possibility for n.c.a. labelling with fluorine-18 in cases where direct nucleophilic fluorination is not possible. The method has greatly increased the potential variety of fluorine-18 radiopharmaceuticals for positron emission tomography (PET). Unfortunately, fluoroalkylation is generally not applicable to molecules which are only soluble in water. It was therefore highly desirable to develop a similar method of introducing fluorine-18 into such compounds via small prosthetic groups. This would open the route to ^{18}F-labelling of peptides. A first approach along this line was carried out by our laboratory (6) by using [2-^{18}F]fluoroacetate which was prepared from the ethyl ester as an intermediate which was activated with the water-soluble (N-ethyl-N'-(dimethylamino)propyl)carbodiimide and then coupled covalently to a free amino group of a protein. Another approach is the introduction of larger aromatic prosthetic groups such as 3-fluoro-5-nitrobenzimidate and 4-fluorophenacylbromide (7).

A simpler way of introducing n.c.a. fluorine-18 is fluoroacylation using esters of α-substituted carboxylic acids directly. Our recently described method of n.c.a. nucleophilic fluorination in high yields using aminopolyether (2.2.2.) supported preparation techniques (1,4,8) lends itself to an efficient preparation of fluoroacylating agents. We describe here the preparation of n.c.a. [2-^{18}F]fluorocarboxylic acid esters and their use for fluoroacylating simple model compounds such as alcohols and amines.

RESULTS AND DISCUSSION

N.c.a. ^{18}F-fluorination of the acylation reagent. α-Fluoro substituted acetic and propionic acid esters were obtained by

aminopolyether (APE 2.2.2.) supported nucleophilic exchange according to the general reaction:



X = Cl, Br, I, OTos; R = H, CH_3;

R' = methyl, ethyl, phenyl, p-anisyl, p-toluyl, p-chlorophenyl, p-nitrophenyl

The influence of the leaving group is shown in Table 1 for the case of the acetic acid methylesters.

Table 1: Influence of the leaving group on n.c.a. ^{18}F-fluorination of α-substituted acetic acid methylester

Leaving group	Radiochemical yield (%)
chloride	12 ± 3
bromide	45 ± 4
iodide	< 0.1
tosylate	22 ± 2

Exp. conditions: 1 ml MeCN, 82 °C, 10 min,
0.25 mmol educt
0.02 mmol [2.2.2./K]$_2CO_3$

The iodo compound does not have sufficient thermal stability and the yields are correspondingly small. It should be noted that the α-substituted acetic acid esters in general exhibit little thermal stability. Competing reactions of the decomposition products are therefore taking place. The optimal educt concentration is around 0.2-0.3 M. At higher concentra-

tions the radiochemical yields start to decrease. The highest radiochemical yields are obtained with Br as leaving group. The tosylate exhibits lower yields despite its higher nucleofugicity, probably due to an unfavourable sterical hindrance. It must be noted that α-substituted carboxylic acid esters exhibit two nucleophilic centers at the α- and the carbonyl-carbon atoms. Thus, nucleophilic attack is determined by the nucleofugicities of the substituent and the ester component as well as by different steric hindrance, a fact which favours substitution at the carbonyl group.

The influence of the ester component has also been studied using α-bromoacetic acid esters and the results are shown in Table 2. It can be seen that the radiochemical yields decrease with increasing activating influence of the alcohol component

Table 2: Influence of ester components on the radiochemical yield of n.c.a. ^{18}F-fluorination of α-bromoacetic acid esters and comparison with relative hydrolysis constants.

Ester component	Radiochemical yield (%)	Rel. hydrolysis constant (from ref. 9)
ethyl	81 ± 7	1
methyl	45 ± 4	2
p-anisyl	9 ± 3	-
p-toluyl	7 ± 1	17
phenyl	6 ± 3	34
p-chlorophenyl	≤ 0.5	140
p-nitrophenyl	< 0.1	4200

Exp. conditions: 1 ml MeCN, 82 °C, 10 min
0.20-0.25 mmol educt
0.02 mmol $[2.2.2./K]_2CO_3$

of the ester. This is probably due to substitution on the carbonyl carbon atom. A comparison with the relative hydrolysis constants taken from the literature (9) clearly shows that the radiochemical yield decreases with increasing hydrolysis constants. The most unreactive ester with respect to its acyl reactivity is the α-bromoacetic acid ethylester, the only one that can be fluorinated in high yields. As expected, the more reactive esters have a stronger tendency for substitution at the carbonyl group. The yields cannot be increased by longer reaction times since the α-fluoroacetic acid esters formed are rather unstable and decompose. The optimal reacion time is observed to be about 10 min in refluxing acetonitril. Also, an increase of the reaction temperature does not change the ratio of carbonyl-to-α-substitution which should lead to the thermodynamically more stable 2-fluoroacetic ester.

The relatively low stability, together with the high toxicity of the halogen acetates, prompted us to use the corresponding propyl derivatives as ^{18}F-fluoroacetylation reagent even though they would represent a somewhat larger prosthetic group to be introduced. We have therefore studied the fluorination of 2- and 3-bromopropionic acid esters. The results are shown in Fig. 1. As expected, the 3-bromopropionic acid ester does not give rise to the [3-^{18}F]fluoropropionic acid ester. This is due to the instability of the precursor with respect to elimination reactions to form acrylic acid within 5 minutes under the basic conditions used. N.c.a. ^{18}F-fluorination of the 2-bromopropionic acid ester, on the other hand, almost proceeds quantitatively. Optimal substrate concentrations, however, have to be 6 times higher than for bromoacetic acid esters, indicating the lower reactivity of the secondary carbon in S_N2 reactions. We have therefore used the [2-^{18}F]fluoropropionic acid methylester as the fluoroacylation agent of choice.

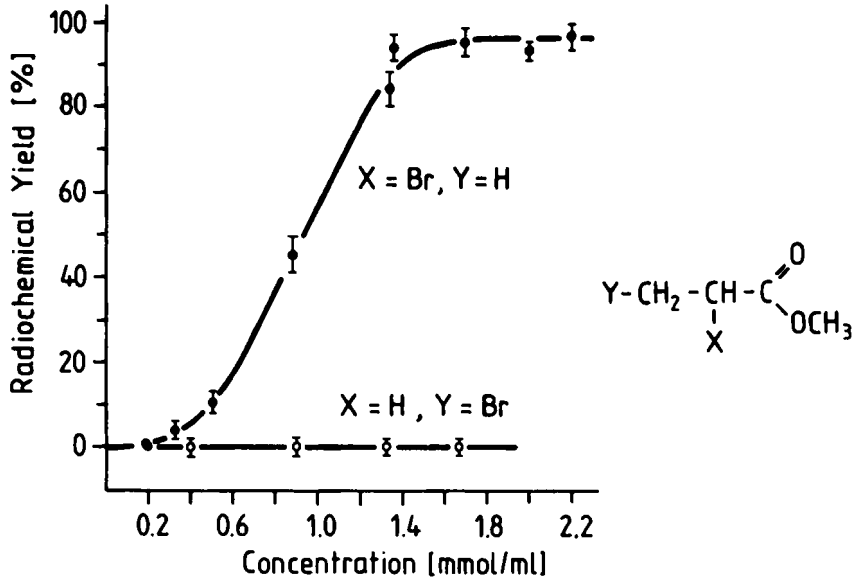


Fig. 1 Radiochemical yields of n.c.a. ^{18}F-fluorination of 2- and 3-bromopropionic acid methylester as depending on the educt concentration.
Reaction conditions: 1 ml MeCN, 82 °C, 10 min
0.02 mmol [2.2.2./K]$_2$CO$_3$

N.c.a. ^{18}F-fluoroacylation of ethanol. Simple aliphatic alcohols are ill suited for ^{18}F-fluoroalkylation under basic conditions. We have therefore chosen ethanol as the first model system to study the introduction of fluorine-18 by fluoroacylation with [2-^{18}F]fluoropropionic acid methylester according to the transesterification equation:

$$H_3C\text{-}\underset{\underset{^{18}F}{|}}{\overset{\overset{H}{|}}{C}}\text{-}COOCH_3 + C_2H_5OH \xrightarrow{H^+} H_3C\text{-}\underset{\underset{^{18}F}{|}}{\overset{\overset{H}{|}}{C}}\text{-}COOC_2H_5 + CH_3OH$$

The reaction can in principle be carried out as a quasi one-pot-process starting with the 2-bromopropionic ester and the reactive [^{18}F]fluoride system. However, it was found in optimization reactions that concentrations higher than 2 mM of APE 2.2.2./K$_2$CO$_3$

had a negative effect on the acylation reaction. In the acylation experiments only 50 µl of the acetonitrile solution at the first fluorination step was added to the substrate to be acylated. For labelling purposes the separation of the intermediate [2-^{18}F]fluoropropionic acid ester from the APE 2.2.2./K_2CO_3 is recommended. The radiochemical yields of the [2-^{18}F]fluoropropionic acid ethylester exhibit a maximum at a molar ethanol-to-ester ratio of about 200. The transesterification reaction only proceeds in the absence of water due to competing hydrolysis reactions.

The fluoroacylation reaction is catalysed by protons which increase the nucleophilicity of the carbonyl carbon. Methanesulfonic acid was used, and the dependence of the radiochemical yield on its concentration is shown in Fig. 2.

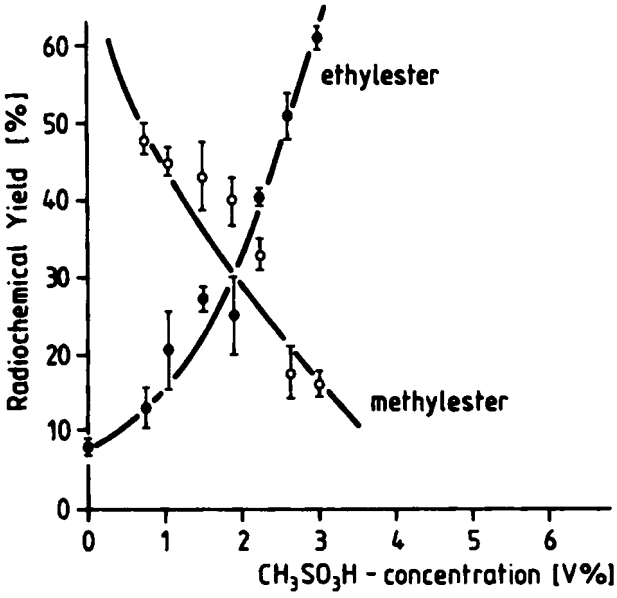
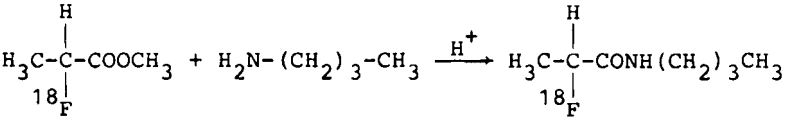


Fig. 2 Dependence of the radiochemical yield of [2-^{18}F]fluoropropionic acid ethylester from transesterification on the concentration of methane sulfonic acid. Reaction conditions: 500 µl EtOH, 80 °C, 10 min; for addition of ester see experimental.

It can be seen that the product [2-^{18}F]fluoropropionic acid ethylester increases rapidly with increasing acid concentration while a concomitant decrease of the methylester is observed. The high acid concentration necessary for obtaining high acylation yields, indicate that the acid does not act as a catalyst but rather it converts a high fraction of the acylation reagent into its protonated form which seems to be required for a fast reaction. On statistical grounds the back-reaction can be neglected for the n.c.a. product obtained.

Fluoroacylation of other primary alcohols such as n-propanol and n-butanol in the presence of H_3CSO_3H show similar radiochemical yields with 32 and 22%, respectively. As expected, secondary alcohols (2-propanol, 2-butanol) exhibit only low yields (< 10%) and tertiary alcohols can hardly be acylated via the above route. The acylation therefore is mainly restricted to primary aliphatic alcohols. Secondary alcohols can undergo elimination under acidic conditions and aromatic alcohols form instable arylesters.

N.c.a. fluoroacylation of n-butylamine. n-Butylamine was used as a model compound to study fluoroacylation of the amine functionality according to the equation



Ammonium chloride was used as the acid. Fig. 3 shows the influence of the concentration of added NH_4Cl on the ^{18}F-fluoroacylation yield in water.

Different from the acylation of the alcohol (Fig. 2), Fig. 3 shows that the highest radiochemical yields are obtained at a small NH_4Cl concentration of less than 1%. Obviously, an increase

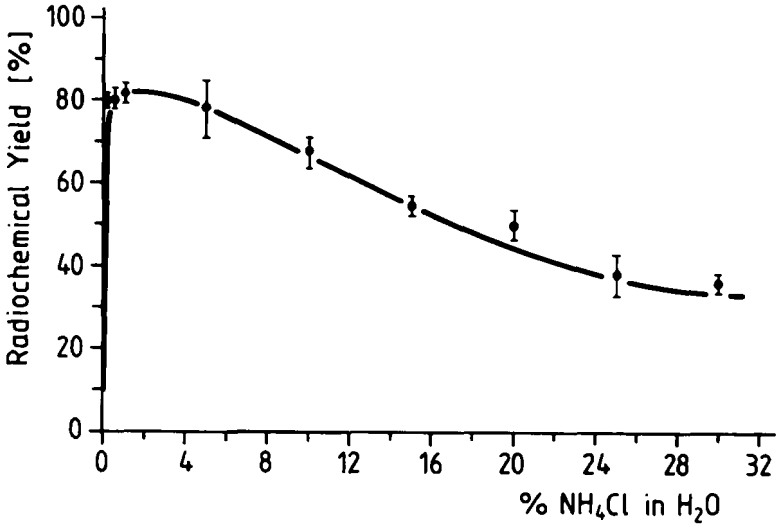


Fig. 3 Dependence of the radiochemical yield of [2-^{18}F]fluoropropionic acid n-butylamide on NH_4Cl concentration. Reaction conditions: 4 mmol amine/ml, 110 °C, 10 min; for addition of ester see experimental.

of the NH_4Cl-concentration leads to a masking of the nucleophilicity of the amine by formation of an alkylammonium salt and hence to a decrease in yield. This effect will strongly depend on the nucleophilicity of the substrate to be acylated.

Also of importance is the nucleophilicity of the solvent which should not exceed that of the substrate. This is indicated in Fig. 4 which shows that the differences for the polar protic solvents ethanol and water using 0.1% NH_4Cl-solutions are negligible within the experimental error, while considerably smaller yields are obtained in the dipolar aprotic solvent acetonitril. For peptides or other biomolecules acetonitril would be a poor solvent. For biomolecules such as antibodies or neuropeptides it is important that the acylation can be carried out in aqueous solution.

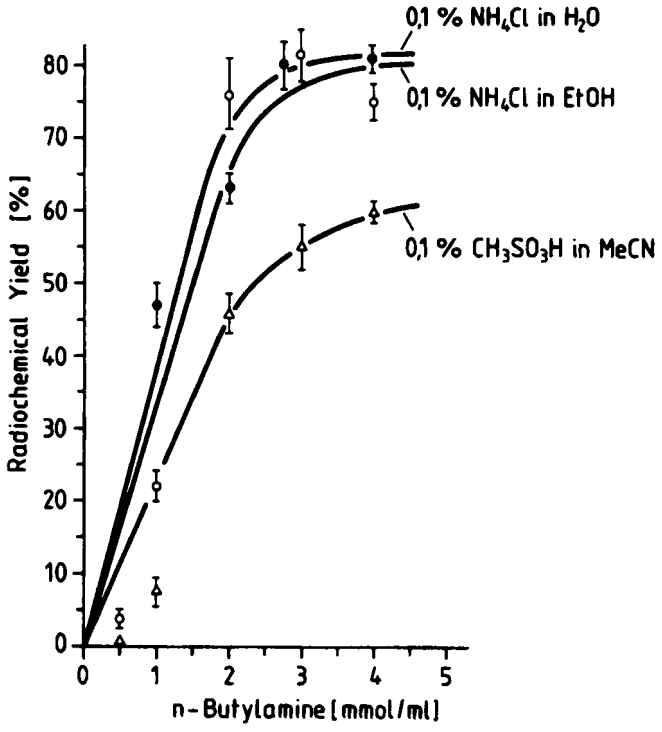


Fig. 4 The influence of different solvents on the radiochemical yield of ^{18}F-fluoroacylation of n-butylamine with [2-^{18}F]fluoropropionic acid methylester. Reaction conditions: Reflux, 10 min, 0.1% NH_4Cl, (0.1% CH_3SO_3H for MeCN); addition of ester see experimental.

CONCLUSION

[2-^{18}F]Fluoropropionic acid methylester is well suited as fluoroacylation agent for primary alcohols and amines. It can be prepared in high yields by the APE 2.2.2. supported nucleophilic ^{18}F-for-Br exchange on the corresponding 2-bromo compound and is the smallest useful fluoroacylating agent with respect to stability and yield.

^{18}F-Fluoroacylation is of interest for introducing fluorine-18 into alcohols which are often ill suited for both direct fluorination and fluoroalkylation. The method, however, is limited to

OH-group containing molecules which are soluble in non-aqueous solvents which have a significantly smaller nucleophilicity than the alcohol itself or which are sterically hindered towards the reaction with the acylating agent. Steric hindrance and elimination occuring in secondary alcohols also confines the method to primary OH group containing molecules.

Primary amines can also be conveniently ^{18}F-fluoroacylated using small amounts of NH_4Cl as acid. The reaction can be carried out in aqueous solution with high yields and should therefore be applicable to peptides. Different from fluoroalkylation, fluoroacylation lends itself to fluorination of a different group of substrates and therefore is a complementary method.

EXPERIMENTAL

Materials. The aminopolyether (Kryptofix® 2.2.2.), the solvents and other reagents, if not specified below, were obtained from Merck (Darmstadt, FRG). The organic solvents used for n.c.a. labelling procedures were appropriately dried. The aliphatic 2-fluoro-, chloro-, bromo- and iodoacetic acid esters and the 2- and 3-bromopropionic acid methylesters were obtained commercially (Merck, Fluka) and used without further purification.

The 2-tosyloxyacetic acid methylester was prepared from the 2-bromoacetic acid ester by refluxing a solution of 0.025 mmol of the bromoester with 0.03 mmol of the silver salt of p-toluenesulfonic acid in dry acetonitril for 20 hours (10). The solution was concentrated under reduced pressure and the products separated by column chromatography on silica gel Si60 and ether-hexane as eluant. The yield was 45% of the theory.

The bromoacetic acid arylesters were prepared by refluxing 0.01 mol of the corresponding phenol and 0.015 mmol of the 2-bromo-

acetic acid bromide in 10 ml CCl_4 for 10 hours. The cold reaction mixture was washed twice with K_2CO_3-solution and with water and the CCl_4 was removed at a rotation evaporator. The ester was purified before ^{18}F-fluorination using a silicagel Si60 column and hexane-chloroform mixtures as eluant. The yields were 60-80% of the theory.

The 2-fluoroacetic acid arylesters used as chromatographic standards were prepared in the same manner as the bromoacetic arylesters starting from fluoroacetic acid chloride and the corresponding phenols. The yields were 50-75% of the theory.

The n-butylamide and the aliphatic esters of 2-bromopropionic acid were synthesized by careful addition of 0.11 mmol of 2-bromopropionic acid bromide to 0.1 mmol of the alcohol or amine in 10 ml CCl_4 at 0 °C. Stirring was continued for 30 min at 0 °C and then the solution warmed to room temperature. After 2 hours the reaction mixture was separated as described for acetic acid aryl esters. The yields were 40-55% with secondary alcohols and butylamine and 70-85% with primary alcohols of the theory.

2-Fluoropropionic acid methylester and other 2-fluoropropionic acid derivatives were prepared by nucleophilic exchange, refluxing 3 mmol of the corresponding 2-bromopropionic acid compound with 3.5 mmol KF in presence of 3 mmol APE 2.2.2. in 5 ml of absolute acetonitrile for 5 hours. The 2-fluoro derivatives were also purified by column chromatography on Si60. The yields were 20-35% of the theory.

The identity of the prepared compounds was checked by comparison with ^1H-NMR literature data. The ^1H-NMR data for the new compounds in $CDCl_3$ are: fluoroacetic acid p-anisyl-ester: δ(ppm) = 7.20-6.80 4H; 5.08 2H (d. 46.6 Hz); 3.8 3H (s), fluoroacetic acid p-nitrophenylester: δ(ppm) = 8.43-7.25 4H; 5.02 2H (d. 47.8 Hz), and 2-fluoropropionic acid N-butylamide:

δ(ppm) = 8.3-7.8 1H (s); 5.81 1H (d. of q. 48 Hz); 3.4-3.05 2H (m); 1.75-1.15 7H (m); 1.0-0.74 3H (m).

Production of reactive n.c.a. [^{18}F]fluoride. The radionuclide was produced by bombardment of a Ne/H_2 gas target with 14 MeV deuterons via the ^{20}N(d,α)^{18}F nuclear reaction at the Jülich CV 28 compact cyclotron (11). To the aqueous solution of n.c.a. $^{18}F^-$ in the reaction vessel 0.02 mmol APE 2.2.2./K_2CO_3 in 0.1 ml H_2O was added and dried for 10 minutes at 120 °C in a stream of dry helium gas as described previously (1,8,12).

N.c.a. radiofluorination of α-substituted acid ester. Typically 0.05 to 2.5 mmol of the α-substituted acid ester in 1 ml of dry acetonitrile was added to about 10 MBq of n.c.a. APE 2.2.2./K^{18}F in the dry reaction vessel. The reaction vessels were closed with a septum which allowed sample taking with exclusion of moisture. The solution was vigorously stirred for typically 10 min at reflux temperature (82 °C). 50 µl aliquots of this mixture were analyzed for radioactivity and ^{18}F-products formed and/or used for the subsequent acylation reaction.

Fluoroacylation of alcohols and n-butylamine with [2-^{18}F]-fluoropropionic methylester. The desired amount of substrate to be acylated was dissolved in water or acetonitrile containing a given amount of acid (NH_4Cl or CH_3SO_3H). In the case of alcohols 1 ml of the substrate was used as solvent. 50 µl of the fluorination solution containing 0.05 mmol of the propionic ester and 0.001 mmol APE 2.2.2./K_2CO_3 was added to the substrate solution. The mixture again was refluxed under vigorous stirring for 10 min.

Analysis of products. All product mixtures were analysed by separation of aliquots via high pressure liquid chromatography (HPLC). The n.c.a. [^{18}F]fluorinated products were

Table 3: HPLC-conditions and k'-values of α-fluoroacetic- and propionic acid esters and amides

Compound	Eluant	Column Material	k'-Value
fluoroacetic acid methylester	H_2O 0.1% HAc	RP-18	2.2
fluoroacetic acid ethylester	MeOH:H_2O 1:9 0.1% HAc	RP-18	1.8
fluoroacetic acid phenylester	ether:hexane 1:5	Si60	1.7
fluoroacetic acid p-nitrophenylester	ether:hexane 1:5	Si60	1.5
fluoroacetic acid p-chlorophenylester	ether:hexane 1:5	Si60	1.4
fluoroacetic acid p-anisylester	ether:hexane 1:5	Si60	0.9
fluoroacetic acid p-toluylester	ether:hexane 1:5	Si60	0.9
2-fluoropropionic acid methylester	MeOH:H_2O 1:9 0.1% HAc	RP-18	3.0
2-fluoropropionic acid ethylester	MeOH:H_2O 1:9 0.1% HAc	RP-18	10.0
2-fluoropropionic acid propylester	MeOH:H_2O 1.5:8.5 0.1% HAc	RP-18	15.0
2-fluoropropionic acid isopropylester	MeOH:H_2O 1.5:8.5 0.1% HAc	RP-18	12.0
2-fluoropropionic acid butylester	MeOH:H_2O 1.5:8.5 0.1% HAc	RP-18	16.3
2-fluoropropionic acid isobutylester	MeOH:H_2O 1.5:8.5 0.1% HAc	RP-18	13.6
2-fluoropropionic acid N-butylamide	MeOH:H_2O 4.5:5.5 0.1% HAc	RP-18	2.0

identified by comparison of k'-values of radioactive and cold standard compounds. The radiochromatography was performed on 250 x 4 mm LiChrosorb Si60 or LiChrosorb RP-18 columns from Merck. The corresponding eluants and k'-values of the reference compounds are listed in Table 3. The radiochemical yields were determined by comparison of aliquots measured directly in a Packard Auto-Gamma scintillation counter with discontinuously taken eluant fractions from the HPLC separation of a second sample of the reaction solution.

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