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A PROSTHETIC GROUP FOR THE RAPID INTRODUCTION OF FLUORINE INTO PEPTIDES AND FUNCTIONALIZED DRUGS

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SUMMARY

Fluoride ion as the tetrabutylammonium salt displaces bromide in para-substituted benzyl bromides in acetonitrile or dimethylformamide. The p-bromomethyl benzoyl (BMB) group has been coupled to amino groups, including peptide amino groups, via its N-hydroxysuccinimide ester. In a subsequent step, the facile displacement of bromide by fluoride occurred under conditions compatible for use with ^{18}F radiotracers.

INTRODUCTION

Prosthetic groups have been described [1] which may be attached readily to biologically active molecules, such as drugs and proteins, and which are designed for the facile introduction of radioisotopes. ^{18}Fluorine is a positron-emitting isotope having a half-life of 110 minutes which has been used extensively as a non-invasive *in vivo* tracer [2-5]. ^{18}F-Positron emission tomography has been paricularly effective in the study of brain

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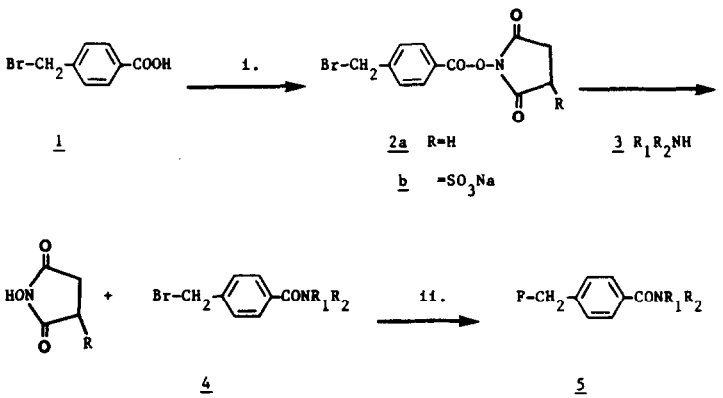
metabolism and in scanning receptors and sites of drug uptake. Reactions through which ^{18}F is introduced include nucleophilic displacement of alkyl triflates [2], aromatic nucleophilic attack [3], and halogenation of phenols [4], olefins [5] and organometallics [6].

Benzylic electrophilic centers are subject to attack by active nucleophiles, including 'naked' fluoride ion in non-aqueous medium [7,8]. We have found that benzyl bromide substituted in the para position by an electron-withdrawing substituent is subject to rapid displacement by fluoride, and that the resulting benzyl fluoride (Scheme 1) is relatively stable in aqueous medium. Moreover, benzyl halides are not subject to elimination, which has been noted [2] as a side reaction with other methods of introducing ^{18}F. We have used these observations as the basis for the development of a prosthetic group for use in a general approach to fluorine labelling of peptide derivatives and 'functionalized congeners' of drugs [9].



Scheme 1. reagents: i. N-hydroxysuccinimide or N-hydroxysulfosuccinimide dicyclohexylcarbodiimide, DMF; ii. $F^- N^+(Bu)_4$ / CH_3CN.

RESULTS AND DISCUSSION

p-Bromomethylbenzoic acid (1) was preactivated as an active ester [11] prior to coupling to amines. In stoichiometrically equivalent amounts, this active ester efficiently and selectively acylated aliphatic and aromatic amino groups, with little competing N-alkylation at room temperature. The more water-soluble sulfosuccinimidyl ester (2b), was also prepared,[11] and found effective for reaction with amines in aqueous medium. Following coupling to amines as above, the bromide of the resulting amides (4) was displaced in dilute solution (less than 1mM) in refluxing acetonitrile in the presence of excess tetraalkylammonium fluoride giving the corresponding fluoromethylbenzoyl (FMB) derivative (5).

Studies at 50° using the N-methylamide (4a) at 0.2 mg/ml and a five-fold excess of fluoride showed the $t_{\frac{1}{2}}$ of conversion to the benzyl fluoride to be less than 1 minute. The reaction rate at 23° was studied under pseudo first order conditions, using an excess of fluoride (Fig. 1.) A rate constant (k_2) of 0.554 M/min was obtained. The $t_{\frac{1}{2}}$ with a concentration of tetrabutylammonium fluoride of 0.5 M was 2.5 min.

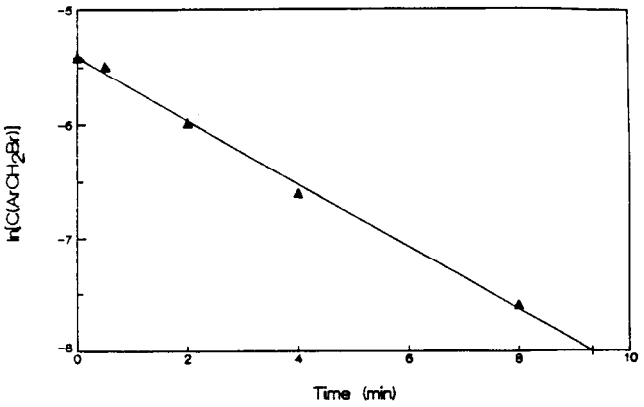


Fig. 1. Reaction rate plot.

Kinetics of displacement by fluoride ($[Bu_4N^+F^-]$ = 0.5 M) of p-bromomethylbenzoyl methylamide (4a, initial conc. = 4.4 mM) in acetonitrile at 23°.

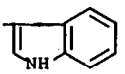
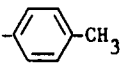
A variety of p-bromobenzoyl amides derived from alkyl and aryl amines were prepared as model compounds. Fluorine substitution was carried out efficiently (Table) at 50°C, as determined by gas or high pressure liquid chromatography. The resulting p-fluoromethylbenzoylamino derivatives were not active alkylating agents, as evidenced by non-reactivity towards aryl thiolates [12]. The rapid rate of the fluorination reaction combined with the stability of the product suggest that this fluorination scheme is suitable for radiotracer studies.

Several FMB-amino acid and peptide derivatives (4k-m) have been prepared in this study. An alternative route to the precursor BMB derivatives of peptides involves initial coupling of 4-hydroxymethylbenzoic acid (Chemical Dynamics Corp., South Plainfield, NJ) to the peptide followed by conversion to the p-bromomethylbenzoyl derivative by short exposure to 30% HBr in acetic acid at room temperature.

Certain peptide functional groups present complications when exposed to a bromomethylbenzoyl group or to nucleophilic fluoride. For example, the presence of a cysteinyl residue would not allow isolation of a reactive bromomethylbenzoyl derivative. In such cases an alternative procedure involves initial fluorination of a BMB derivative to be coupled subsequently to a functionalized drug or peptide. For example (Scheme 2), α-bromo-p-toluic acid t-butyl ester (6) reacts with tetrabutylammonium fluoride in acetonitrile giving α-fluoro-p-toluic acid (7) in 90% yield. Quantitative cleavage of the t-butyl ester by brief (10 min) exposure to trifluoroacetic acid gives α-fluoro-p-toluic acid (8). This acid, already containing a fluorine atom, is then coupled to an amine.

Table

Yields for acylation of amines by 2 and for fluoride displacement.

	3 =	4: % yield	mp °C	5: % yield	mol. ion[A]
a	H_2NCH_3	81	136-140	95[C]	168
b	$H_2N(CH_2)_3CH_3$	89	110	79[C]	210
c	$H_2N(CH_2)_2$- 	91	125-129	>80	258
d	$H_2N(CH_2)_2$- 	66	144-147	65	274
e	$H_2N(CH_2)_2$- 	45[B]	oil	75	297
f	H_2N- 	94	184-186	63	232
g	$H_2NCH_2CHOHCH_3$ (R)	69[B]	88-90	34	212
h	$H_2NCHCH_3CH_2OH$ (S)	76[B]	130-1	89	212
i	$H_2NCH(C_6H_5)CH_2OH$ (R)	84[B]	171-3	51	274
j	$H_2NCH(C_6H_5)CH_3$ (R)	63[B]	137-8	>90	258
k	H-Gly-NH_2	60[B]	oil	>90	211
l	H-Leu-NH_2	50[B]	oil	54	267
m	H-Ala-Gly-OH	63[B]	290d	50	343

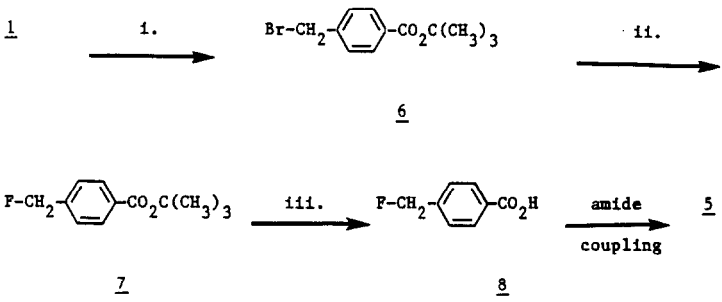
A By CI mass spectrometry (NH_3), value equals m + 1 unless noted.

B From (2b), aqueous reaction medium.

C Yield determined by GC.

Attempts to fluorinate α-bromo-p-toluic acid under basic conditions using tetrabutylammonium fluoride or using KF/18-crown-6 resulted in the formation of insoluble poly(oxycarbonyl-1,4-phenylenemethylene) [13]. Attempted fluorination of the active ester (2a) resulted in decomposition.

For peptides and other substrates of limited solubility, the fluorination reaction may be carried out in other polar aprotic solvents such as THF and DMF. An apparent reaction of DMSO with the benzylic bromide [14] precluded the use of this solvent.



Scheme 2. reagents: i. isobutylene; ii. $F^- N^+(Bu)_4$ / CH_3CN; iii. CF_3COOH.

We have synthesized high affinity ligands for adenosine receptors incorporating the bromomethylbenzoyl group [15] and are studying displacement on these substrates using radioactive fluoride.

EXPERIMENTAL

Preparation of active esters of p-bromomethylbenzoic acid

N-Succinimidyl p-(bromomethyl)benzoate, BMB-OSu (2a) was prepared by condensing N-hydroxysuccinimide and α-bromo-p-toluic acid (1, Aldrich

Chemical Co., St. Louis, MO) in DMF/ethyl acetate (1:1) using one equivalent of dicyclohexylcarbodiimide. After filtration of an insoluble urea, addition of petroleum ether caused precipitation of the product [10] (2a), mp. 150-153°, in 70% yield. Analysis ($C_{12}H_{10}NO_4Br$): calc. 46.18%C, 3.23%H, 4.49%N; found 46.24%C, 3.25%H, 4.49%N.

N-Sulfosuccinimidyl p-(bromomethyl)benzoate (2b), mp $>$ 300°, was prepared in 89% yield by condensing N-hydroxysulfosuccinimide and α-bromo-p-toluic acid (1, Aldrich Chemical Co., St. Louis, MO) in DMF by the method of Staros [11].

Coupling of active ester to amine

In a typical coupling experiment, BMB-OSu, 2a (162 mg, 0.52 mmol) and 2-phenylethylamine (65 μL, 0.52 mmol) were combined in 3 ml DMF. After one hour the product (4c) was extracted into ethyl acetate, washed with acid/base, and recrystallized. A typical NMR spectrum in CD_3CN showed resonances at δ 7.74 and 7.49 (each d, 2H, J=8Hz, aryl) and 4.61 (s, 2H, benzylic) ppm.

Fluoride substitution reaction

A BMB-amide (4) was dissolved in anhydrous acetonitrile (1 mg/ml) and treated with two equivalents of tetrabutylammonium fluoride (THF solution, Aldrich Chemical Co.). This acetonitrile solution was then dried by evaporation of the azeotrope. The anhydrous solution was heated for three minutes at 50° under a nitrogen atmosphere. The reaction could be followed by thin layer chromatography (silica, ethyl acetate:petroleum ether, 1:1, R_f values for BMB-methylamide, 4a, and FMB-methylamide, 5a, = 0.48 and 0.43, respectively). Yields of FMB-amides (5) for the Table were determined by

gas chromatography (OV-1 capillary column, J&W Scientific, Folson, CA) or by HPLC (Waters μPorasil, 4.6X25 mm, using 20% EtOAc/hexane or Beckman Ultrasphere ODS, medium, for 5k to 5m). Typical NMR spectrum (for 5a, CD_3CN) δ 7.78 and 7.43 (each d, 2H, J=8Hz), 5.40 (d, 2H, J=47Hz), 2.83 (d, 3H, J=4.5 Hz). Mp of 5a 113-115°.

Preparation of α-fluorotoluic acid

4-Bromomethylbenzoic acid was converted to t-butyl 4-bromomethylbenzoate using isobutylene in sulfuric acid [16], in 50% yield. The product, an oil, had the following NMR spectrum in $CDCl_3$: δ 7.96 and 7.43 (each d, 2H, J=8Hz), 4.50 (s, 2H), 1.59 (s, 9H, t-Bu). The chemical ionization mass spectrum (NH_3) showed peaks at 211 (m + 1), 228. The bromomethyl compound was fluorinated as above using tetrabutylammonium fluoride in acetonitrile, in 90% yield. NMR spectrum in $CDCl_3$: δ 8.01 and 7.41 (each d, 2H, J=8Hz), 5.44 (d, 2H, J=47Hz), 1.60 (s, 9H, t-Bu). The t-butyl ester was removed quantitatively upon 10 min exposure to neat trifluoroacetic acid. The product, α-fluorotoluic acid, was obtained as a white solid, melting at 178-181°. The chemical ionization mass spectrum (NH_3) showed a peak at 172 = (m + 1 + 17). NMR spectrum in $CDCl_3$: δ 8.12 and 7.47 (each d, 2H, J=8Hz), 5.47 (d, 2H, J=47Hz).

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