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ω-DIFLUOROAMINO CARBOXYLIC ACIDS: READILY ACCESSIBLE FLUORINE-LABELED FATTY ACID ANALOGS

D.M. JEWETT and R.E. EHRENKAUFER

Cyclotron/PET Facility, University of Michigan Medical Center, Box 056, Ann Arbor, Michigan 48109, (U.S.A.)

SUMMARY

15-Difluoroamino-pentadecanoic acid and 12-difluoroamino-dodecanoic acid were prepared by treating the corresponding lactams with F_2 in aqueous acetonitrile. The intermediate N-fluorolactams were also isolated. Whereas isolated $-CH_2-F$ groups introduce considerable polarity into aliphatic chains, $-CH_2-NF_2$ groups did not. The $-NF_2$ group thus appears to be a suitable isostere of the $-CH_3$ group, where introduction of a ^{18}F label is required for positron-based or ^{19}F NMR imaging.

INTRODUCTION

Of the positron-emitting isotopes ^{18}F (half-life 110 min) is among the most attractive for incorporation into organic compounds for use in positron-based imaging systems. Since ^{18}F-F_2 is already available at several facilities for the production of ^{18}F-2-deoxy-2-fluoro-glucose, it is useful to find general methods for incorporating F_2 into molecules of biological interest. In particular, we required a method for introducing ^{18}F into the aliphatic chains of amino acids and long chain fatty acids that would have minimal effect on the properties of the compounds. When we substituted $-CH_2F$ groups for the terminal $-CH_3$ groups in leucine and hexadecanoic acid [1] the polarity of the compounds was markedly increased, as shown by chromatography. The low boiling points reported for alkyl difluoroamines [2] indicated that $-NF_2$

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might be more satisfactory than $-CH_2F$ as an isostere for $-CH_3$. Since radiolabeled analogs of long-chain fatty acids are becoming increasingly important as cardiac imaging agents [3], and since Grakauskas and Baum [4] synthesized 6-difluoroamino-hexanoic acid by the direct fluorination of caprolactam, we thought that the synthesis of 12-difluoroamino-dodecanoic acid and 15-difluoroamino-pentadecanoic acid as analogs of tridecanoic- and hexadecanoic acid might offer a convenient, practical test of the feasibility of substituting $-NF_2$ for $-CH_3$ in molecules of biological interest.

EXPERIMENTAL

^{19}FNMR spectra were recorded at 84.26 MHz from approx. 10% solutions in $CDCl_3$. Chemical shift values are reported in ppm downfield from an external trifluoroacetic acid standard. ^{1}HNMR were recorded at 360 MHz in $CDCl_3$, TMS internal standard. ^{13}CNMR were recorded at 90.56 MHz, TMS internal standard. IR spectra were recorded from the neat liquids in NaCl cells maintained somewhat above the melting points of the compounds.

Fluoride ion concentrations were measured with a F^- ion-specific electrode (Orion) *vs* standards prepared in the appropriate 0.2 M tris buffers.

15-Difluoroamino-pentadecanoic acid (I)

Azacyclohexadecanone [5] 1.2 g (0.005 mole) was dissolved in 90 ml MeCN and 10 ml H_2O. This solution was divided into 5 aliquots. F_2, 2% in N_2, was bubbled into each of these at room temperature, at the rate of 80 ml/min for 50 min. The aliquots were combined and evaporated to dryness. The residue was dissolved in ether, extracted with H_2O and saturated aqueous NaCl, and dried over Na_2SO_4. After evaporation of the ether the product was applied to a silica column. Elution with a gradient of hexane with increasing amounts of diethyl ether (acetic acid, 1% was added to the solvents and silica to prevent tailing of the carboxylic acid) gave a waxy solid homogenous by TLC (silica; hexane 4: ether 1; dichlorofluorescein; Rf 0.9 relative to hexadecanoic acid) and by GLC of the methyl ester (DEGS, 170°). The methyl ester was formed by coinjection of the carboxylic acid with a solution of 50% dimethyl formamide dimethyl acetal in MeCN [6]. Yield, 720 mg, 50%. See Table 1 for spectra and other analytical results.

N-Fluoro-azacyclohexadecanone (II)

The procedure was identical to that for (I) except that the F_2/N_2 was introduced at 0° for 30 min for each 20 ml aliquot. Acetic acid was omitted during column chromatography of the product. From 1.2 g azacyclohexadecanone 400 mg (31%) of a colorless oil was obtained which crystallized on standing. An analytical sample was recrystallized from MeOH at -70°. (TLC: hexane 4: ether 1; R_f 1.2 relative to hexadecanoic acid). A sample of the material was readily converted to (I) by further fluorination.

N-Fluoro-azacyclotridecane (III)

The procedure was the same as for (II), except that F_2/N_2 was introduced for 25 min for each 20 ml aliquot at 0°, and 5 ml hexane was maintained in each aliquot during the addition to trap the N-fluorolactam and prevent further fluorination. Column chromatography yielded an oil which solidified on standing. From 1.0 g (0.005 mole) azacyclotridecanone was obtained 570 mg (53%) product.

12-Difluoroamino-dodecanoic acid (IV)

A sample of the fluorolactam (III) was treated with F_2/N_2 as described above until TLC indicated complete conversion to a single product (Hexane 4: ether 1; R_f 0.9 relative to dodecanoic acid).

RESULTS AND DISCUSSION

Conditions for synthesis

The direct fluorination of readily available lactams was found to give the desired $-NF_2$ fatty acids in satisfactory yields. The introduction of the ^{18}F label as the last step in a sequence of reactions is consistent with the limitations imposed by the 110 min half-life of the isotope. The use of aqueous acetonitrile as a solvent allowed for the hydrophobic lactams to dissolve readily, while avoiding the formation of acyl fluorides which would be expected to occur in anhydrous systems [4]. The formation of the products could be readily followed by TLC on silica or by GLC after converting the free acids to their methyl esters. In the case of the 12-carbon analog,

formation and isolation of the intermediate N-fluorolactam was favored by the addition of hexane as a second phase during the fluorination to act as a trap. This approach did not work for the 15-carbon analog, since the lactam itself was quite soluble in hexane.

The direct fluorination of a lactam with 2 equivalents of F_2, while satisfactory for preparative synthesis, is not suitable for labeling at high specific activity with ^{18}F. The requirement for 2 moles of F_2 per mole of lactam is inconsistent with the complete incorporation of label, which is, rather, favored by an excess of substrate relative to F_2. This difficulty can be avoided by radiofluorination of the unlabeled intermediate N-fluoro lactam, the preparation, isolation and properties of which we have described above. The ease with which the fluorolactam and difluoroamino carboxylic acid can be separated chromatographically permit the use of the desired excess of fluoro lactam relative to F_2.

Physical and chromatographic properties of the difluoroamino carboxylic acids

Table 1 summarizes the analytical results and spectra for the difluoroamino carboxylic acids and intermediate fluorolactams. NMR chemical shifts and coupling constants for nuclei at or adjacent to the NF_2 groups are consistent with earlier work [4].

Of particular interest is the chromatographic behavior as an indication of the polarity and partitioning behavior of the $-CH_2-NF_2$ group. Table 2 gives R_f values (silica gel) for analogs of hexadecanoic acid. 15-Difluoroamino-pentadecanoic acid is, for the purpose of this discussion, an analog of hexadecanoic acid, while the other compounds are analogs of heptadecanoic acid. Hence the effects of the $-NF_2$ group *vs* the iodo and bromo groups on polarity are somewhat obscured. Nevertheless, the advantage of the $-NF_2$ group with respect to its effect on the polarity of the aliphatic chain in this respect is clear. 15-Difluoroamino-pentadecanoic acid was also compared to hexadecanoic and 16-bromohexadecanoic acids by HPLC in a reverse phase system (gradient on ODS, A=.01% $HClO_3$, B=90% aqueous MeCN). All three compounds had identical retention times indicating similar lipid-water partition coefficients.

TABLE I

Analytical and spectral results for the difluoroamino carboxylic acids and intermediate fluorolactams

	$F_2N-(CH_2)_{14}-COOH$ (I)		$F_2N-(CH_2)_{11}-COOH$ (IV)		$-(CH_2)_{14}-C(=O)-N(F)-$ (II)		$-(CH_2)_{11}-C(=O)-N(F)-$ (III)	
	$C_{15}H_{29}F_2NO_2$		$C_{12}H_{23}F_2NO_2$		$C_{15}H_{28}FNO$		$C_{12}H_{22}FNO$	
	293.41		251.32		257.40		215.32	
Molecular Wt. Microanalysis	Calc	Found	Calc	Found	Calc	Found	Calc	Found
% C	61.4	61.5	57.4	57.6	70.0	70.0	66.9	67.1
% H	10.0	10.1	9.2	9.3	11.0	11.0	10.3	10.4
% N	4.8	4.9	5.6	5.7	5.4	5.4	6.5	6.6
% F	13.0	12.9	15.1	15.1	7.4	7.3	8.8	8.9
Melting point	49-50°		31-34°		approx 24°		< 20°	
Infra red spectrum	—		—		carbonyl, 1692 cm^{-1}		carbonyl, 1685 cm^{-1}	
^{19}FNMR								
Chemical shift downfield from TFA ext. std.	$-CH_2-NF_2$, triplet 132.9 ppm		$-CH_2-NF_2$, triplet 128.1 ppm		$-CH_2-NF-C(=O)-$, triplet 7.21 ppm		$-CH_2-NF-C(=O)-$ riplet 6.8[illegible] ppm	
J_{HF}	30.0 Hz		30.0 Hz		30.0 Hz		30.0 HZ	
^{1}HNMR								
Chemical shift vs TMS int. std.	$-CH_2-NF_2$ triplet of triplets 3.46 ppm		$-CH_2-NF_2$ triplet of triplets 3.46 ppm		$-CH_2-NF-$ doublet of triplets 3.89 ppm		$-CH_2-NF-$ multiplet 3.9 ppm	
J_{HH}	7.4 Hz		7.4 Hz		approx. 7 Hz		-	
J_{HF}	29.8 Hz		29.8 Hz		31.6 Hz		-	
^{13}CNMR								
Chemical shift vs TMS int. std.	$-CH_2-NF_2$ 66.0 ppm		$-CH_2-NF_2$ 66.0 ppm		$-CH_2-NF-$ 49.6 ppm		$-CH_2-NF-$ 49.1 ppm	

TABLE 2

R_f values for fatty acid analogs (TLC on silica; hexane 2: ether 1, HOAc 1%)

Compound	R_f relative to hexadecanoic acid
Hexadecanoic acid	1.00
15-Difluoroamino hexadecanoic acid	0.93
16-Bromo hexadecanoic acid	0.88
16-Iodo hexadecanoic acid	0.88
16-Fluoro hexadecanoic acid	0.70
16-Hydroxy hexadecanoic acid	0.24

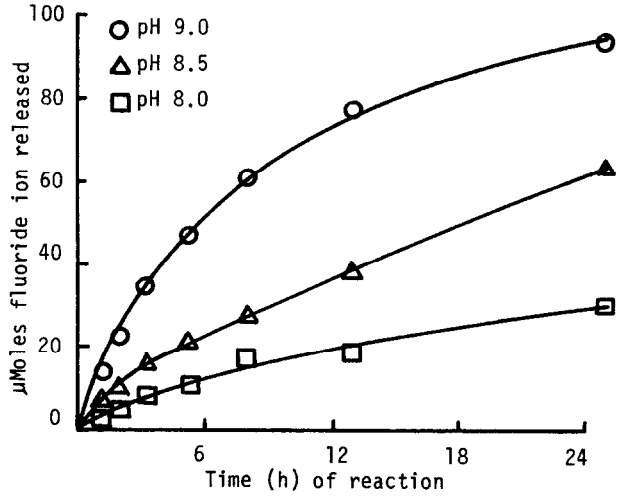

Fig. 1. Release of fluoride ion from 50 µmoles 15-difluoroaminopentadecanoic acid in 50 ml tris buffer as a function of time and pH.

Stability in aqueous media

The difluoroamino group is relatively stable to aqueous acid (viz the conditions for synthesis). However, in aqueous base decomposition is quite rapid, involving two successive dehydrofluorinations, with R-CN as final product [7]. It was thus necessary to obtain an indication of the stability of the difluoroamino fatty acids in aqueous buffers in the pH range encountered in in vivo experiments. Figure 1 shows the release of fluoride ion from 15-difluoroamino-pentadecanoic acid as a function of time and pH in 0.2 M tris buffers. The behavior in bicarbonate buffer at pH 8.0 was identical to that for the tris buffer at pH 8.0. Since this method does not take into account possibly different rates for the loss of two different F^- ions from the same molecule, it cannot give a precise measurement of the amount of the original compound remaining. Nevertheless, even for the worst case, $t_{1/2}$ for decomposition at pH 8.0 is very long (approximately 56 h by extrapolation) relative to the half-life of the isotope itself (110 min). Hence, while it will be desirable to avoid alkaline conditions in the formulation of these materials, base catalyzed decomposition should not be the limiting factor in their use as imaging agents.

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