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Reactions of the fluorinating agent CF_3OF with amino acid derivatives and peptides

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Abstract

A number of hydrophobic acyl amino acid esters were fluorinated using CF_3OF, yielding *N*-fluoroamides. The ^1H, ^{13}C and ^{19}F nuclear magnetic resonance (NMR) data for these compounds are reported. The first ^{14}N NMR spectrum of an *N*-fluoroamide is also reported, namely that of *N*-acetyl-*N*-fluoroglycine ethyl ester. The ^{14}N resonance of the fluorinated compound was shifted downfield by over 100 ppm from that of the starting material. Reaction of CF_3OF with *N*-acetylleucine ethyl ester yielded the *N*-fluoroamide, as well as the compound in which the γ-hydrogen of leucine is replaced by fluorine. Treatment of the cyclopentapeptide c-(gly-pro-gly-D-ala-pro) with CF_3OF yielded an intractable mixture of fluorinated hydrocarbons, *N*-fluoroamides and difluoroamides. Separation of the mixture resulting from treatment of *N*-acetylproline with CF_3OF led to recovery of a fraction whose ^{19}F NMR spectrum indicated fluorination at the proline nitrogen and subsequent opening of the proline ring. Treatment of the cyclopentapeptide c-(gly-leu-gly-gly-gly) with CF_3OF proceeded to a very limited extent, with the resulting mixture of *N*-fluoroamides showing no preference for fluorination at a specific site.

Keywords: Fluorinating agent; *N*-Fluoroamides; Amino acid derivatives; Peptides; NMR spectroscopy; Trifluoromethyl oxyfluoride

1. Introduction

Peptides and proteins are essential components of functioning organisms. The biological activity of proteins results from the adoption of a specific three-dimensional conformation [1]. Interest in our laboratory in the determination of the conformation of peptides led us to explore the synthesis of *N*-fluoroamides and *N*-fluoroamino acids and the use of fluorine nuclear magnetic resonance (NMR) spectroscopy as a tool to study the conformation of these amides.

Using solution-state NMR, one may obtain internuclear distances in peptides via nuclear Overhauser effects, or by use of Karplus-type equations which relate the dihedral angle and coupling constant. Unfortunately the four-bond amide-proton-α-proton interresidue coupling constant in peptides is small and of little use in structure determination. Because fluorine–proton coupling constants are generally larger than proton–proton coupling constants, we sought to introduce the fluorine atom in order to obtain information about the interresidue dihedral angle. Hammer and Chandrasegaran synthesized a number of *N*-fluoroamides of known conformation and developed Karplus equations relating the three- and four-bond proton–fluorine coupling constants to the dihedral angles between the two nuclei [2].

The equations derived are as follows:

Φ angle: $^3J_{HF} = 70.8 \cos^2\theta - 44.1 \cos\theta - 7.2$

and

Ψ angle: $^4J_{HF} = -19.5 \cos^2\theta + 8.8 \cos\theta + 4.9$

where θ is the dihedral angle between the two atoms. Typical values for the three-bond coupling were in the range of 0 to 33.8 Hz, while four-bond coupling showed a range of 0 to 10 Hz. Chemical shifts for *N*-fluoroamides ranged from 42–81 ppm downfield from external $CFCl_3$. Quadrupolar broadening from the adjacent ^{14}N concealed couplings of less than 5 Hz.

Our goal was to use this method first to ascertain the conformations of peptides of known conformation, and eventually to determine the conformation of new peptides. As an initial step in pursuit of this goal, we have synthesized a number of *N*-fluoroamino acids and studied them using NMR spectroscopy. Results of the fluorination of a number of acyl amino acids and several cyclopentapeptides are reported in this paper. We also reported the first triple-resonance NMR experiments with *N*-fluoroamides, specifically the measurement of the ^{19}F NMR spectrum while simultaneously decou-

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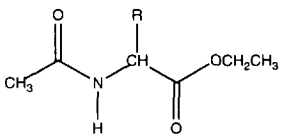
pling ^{1}H and ^{14}N. Two of the cyclopentapeptides, c-(gly-leu$_3$-gly) and c-(gly-leu-gly$_3$), are synthesized for the first time in our laboratory [3]. The low solubility of the first peptide in a variety of solvents precluded its use as a test compound, despite its rigid solution conformation. The second peptide also had a fixed conformation. We treated this peptide with CF_3OF and analyzed the product mixture using NMR.

2. Results and discussion

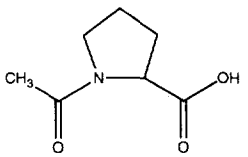
As a starting point in the investigation of peptide conformation using the fluorination method developed previously [2], a number of amino acid derivatives were fluorinated, as shown in Fig. 1, and the ^{1}H, ^{13}C, ^{14}N and ^{19}F spectra of the fluorinated derivatives measured. Generally one fluorinated product was obtained (>99%), the *N*-fluoroamide. In the case of *N*-acetylglycine-*N'*-methyl amide, several products were obtained, corresponding to the two *N*-fluoroamide isomers, the *N,N'*-difluoro compound and to the products resulting from the reaction of solvent with CF_3OF. A more detailed analysis of the results of the fluorination of these compounds follows.

2.1. Fluorination of N-acetylamino acid esters

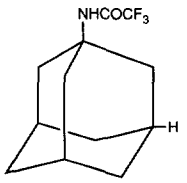
N-Acetylglycine ethyl ester (AGE) fluorinated to a single product, *N*-acetyl-*N*-fluoroglycine ethyl ester (FAGE) (Fig. 1). In contrast to the results reported by Barton et al. [4], no other products could be detected in the ^{19}F spectrum of the raw reaction mixture. However, it is possible that the side-products could be volatile (e.g. acyl fluoride which boils at 21 °C, or possibly *N,N*-difluoroglycine ethyl ester or trifluoromethyl acetate).



R = H, glycine, AGE
CH_3, alanine, AAE
$CH(CH_3)_2$, valine, AVE
$CH_2CH(CH_3)_2$, leucine, ALE



N-acetylproline, NAP



N-trifluoroacetyladamantadine

Fig. 1. Structures of amino acid derivatives which were treated with CF_3OF.

The ^{19}F spectrum of FAGE is shown in Fig. 2. Three- and four-bond coupling to ^{1}H is manifested in the triplet of a quartet structure. Three-bond coupling to the α-proton was 31.42 Hz, while four-bond coupling to the acetyl CH_3 protons was 7.28 Hz. The ^{14}N spectrum of AGE was measured (not shown). The linewidth was extremely broad, with the width at half-height equal to 2100 Hz. The ^{14}N spectrum of the fluorinated analogue (Fig. 3) had a linewidth smaller than that of the starting material, $\nu_{1/2} = 1700$ Hz. The peak is shifted strongly downfield from 102 ppm to 240 ppm by the presence of the fluorine. We believe this to be the first reported ^{14}N spectrum of an *N*-fluoroamide.

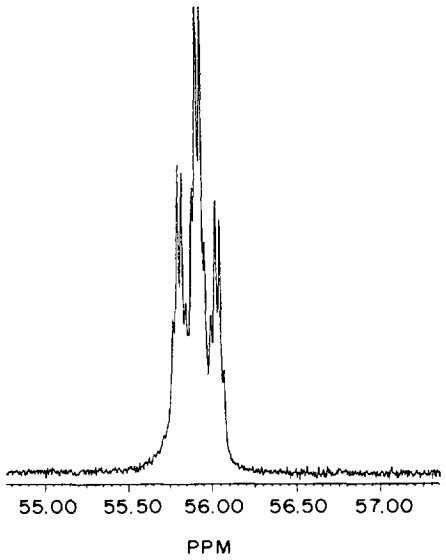


Fig. 2. The 282.407 MHz ^{19}F NMR spectrum of *N*-acetyl-*N*-fluoroglycine ethyl ester in $CDCl_3$. Three- and four-bond couplings to ^{1}H are 31.42 and 7.28 Hz, respectively.

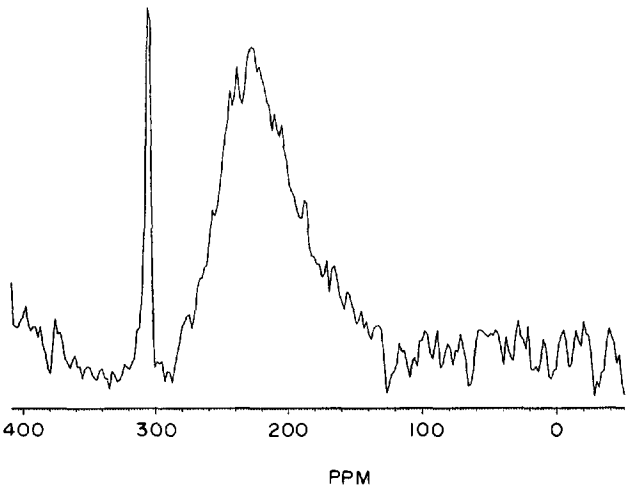


Fig. 3. The 21.683 MHz ^{14}N NMR spectrum of *N*-acetyl-*N*-fluoroglycine ethyl ester in $CDCl_3$.

A slight narrowing of the ^{1}H-decoupled line is evident in the ^{19}F spectrum of FAGE when ^{14}N decoupling is applied (not shown). The line narrows from 3.84 Hz to 2.35 Hz, a roughly 40% decrease. A similar experiment in our laboratory on the compound *N*-fluoroisoquinuclidone resulted in the observation of previously unrevealed coupling of fluorine to the H-4 proton, thereby illustrating the utility of the method [5].

Fluorination of *N*-acetylglycine-*N'*-methylamide (AGA) gave two major and several minor products. The major peaks in the ^{19}F NMR spectrum at 52.4 and 61.7 ppm are assigned to *N*-acetyl-*N*-fluoroglycine-*N'*-methylamide and *N*-acetylglycine-*N'*-fluoro-*N'*-methylamide. The ^{19}F resonance at 52.4 ppm is a triplet of quartets, the coupling constants and chemical shifts of which are nearly identical to those of FAGE ($^{3}J_{HF}=31.23$ Hz and $^{4}J_{HF}=7.12$ Hz). The other major peak at 61.7 ppm was so broad that no coupling constants could be measured. Two minor peaks are of interest. At 55.5 ppm there is a second triplet of quartets, approximately 5% of the intensity of the set at 52.4 ppm and there is a broad quartet (possibly of triplets) at 63.3 ppm of approximately the same intensity as the peak at 55.5 ppm. This pair of peaks probably corresponds to *N*-acetyl-*N*-fluoroglycine-*N'*-fluoro-*N'*-methylamide. These compounds were not isolated.

N-Acetylalanine ethyl ester (AAE) fluorinated smoothly to a single product, *N*-acetyl-*N*-fluoroalanine ethyl ester (FAAE). The ^{19}F NMR resonance is split into a doublet by the α-proton ($^{3}J_{HF}=42.13$ Hz), and into a quartet by coupling to the acetyl CH_3 protons ($^{4}J_{HF}=7.34$ Hz). Attempts to acquire ^{14}N spectra on the fluorinated compound were unsuccessful, even with concentrations and accumulation times identical to those of the ^{14}N spectrum of FAGE. The linewidth of the ^{14}N signal of the starting material, AAE, is 2480 Hz.

Fluorination of *N*-acetylleucine ethyl ester (ALE) gave two products, one in approximately 50-fold excess over the other. The major product was the *N*-fluoroamide. The familiar doublet of quartets indicative of coupling of the fluorine to the acetyl CH_3 protons and to the α-proton is readily evaluated. Three-bond coupling was 41.80 Hz, while four-bond coupling to the acetyl CH_3 protons was 7.43 Hz. Attempts to acquire a ^{14}N spectrum of this fluorinated compound were also unsuccessful. The ^{19}F spectrum of the minor product showed a nontuplet structure, with the outermost peaks buried in noise. The only way this pattern could arise would be if the tertiary γ-proton were replaced by fluorine. The six δ-CH_3 protons and the two β-protons would split the fluorine into a nontuplet. A reaction of this sort is not unprecedented.

Barton, the first author to prepare *N*-fluoroamides, treated *N*-trifluoroacetyladamantadine with CF_3OF [6]. This species fluorinated cleanly to give 3-fluoro-*N*-trifluoroacetyladamantadine. The electron-withdrawing effect of the trifluoroacetyl group apparently deactivates the amide nitrogen. Barton initially assumed that the reaction was free radical in nature, and repeated the experiment in the presence of a variety of free-radical inhibitors, The 3-fluoro compound was produced in high yield even in the presence of these inhibitors.

Two possible mechanisms could explain the formation of the fluorinated hydrocarbon moiety. In the first case, the electron-poor hypofluorite fluorine could cause hydride-ion abstraction, leading to the formation of a carbocation, followed by immediate attack of fluoride from OCF_3^- which would lead to the formation of the C–F compound along with fluorophosgene. A second mechanism is a non-classical attack of the electrons in the C_γ–H bond on the electron poor hypofluorite fluorine, leading to the same product. In other studies of the reaction of CF_3OF with unsaturated centers [7], the cation generated by attack of the olefinic electrons on the hypofluorite fluorine is able to capture any nucleophile which may be present in solution. This leads to the formation of the trifluoromethyl ether, as well as the difluoride.

Unfortunately, no evidence of the trifluoromethyl ether was found in the ^{19}F spectrum of the raw product mixture of FALE (as it is most likely a gas). This would tend to support the hypothesis of a nucleophilic attack on CF_3OF by the electrons in the C_γ–H bond. Olah and Lin postulated this mechanism to explain the products formed in their study of the electrophilic nitration of tertiary alkanes [8]. Barton also explains the formation of 3-fluoro-*N*-trifluoroacetyladamantadine in this way [6].

N-Acetylvaline ethyl ester (AVE) fluorinated smoothly to one product. There was no evidence for replacement of the β-hydrogen with fluorine in the raw reaction mixture. Apparently, the α-carbon is not sufficiently electron-donating to render the hydrogen–carbon bond nucleophilic (at least relative to the amide nitrogen). The familiar double quartet at 75.51 ppm ($J=39.18$ and 7.32 Hz) is again evident in the ^{19}F spectrum; however, the resonance is somewhat broadened, which could be a result of long-range coupling to the ^{14}N or long-range coupling to the β-methine or γ-CH_3 groups. However, as in the case of the alanine and leucine derivatives above, we were unable to obtain the ^{14}N spectrum due to problems of low sensitivity.

2.2. *Fluorination of proline-containing species*

The cyclopentapeptide, c-(gly-pro-gly-D-ala-pro) (CGPGAP) was previously synthesized by Pease and Watson [9]. This compound was chosen as a model for the study of conformation using fluorine NMR spectroscopy because of its rigid solution conformation. CGPGAP was fluorinated as described under experimental details and a ^{19}F spectrum run on the raw product mixture. The ^{19}F spectrum was extremely complex, showing peaks due not only to *N*-fluoroamides, but to difluoroamines and to fluorinated hydrocarbon moieties as well. The experimental was repeated with a 100-fold decrease in the amount of CF_3OF. Despite the use of a much smaller amount of reagent, the ^{19}F spectrum of the raw product mixture remained largely unchanged. Attempted separation of the fluorinated peptide mixture on silica using hexane, ethyl

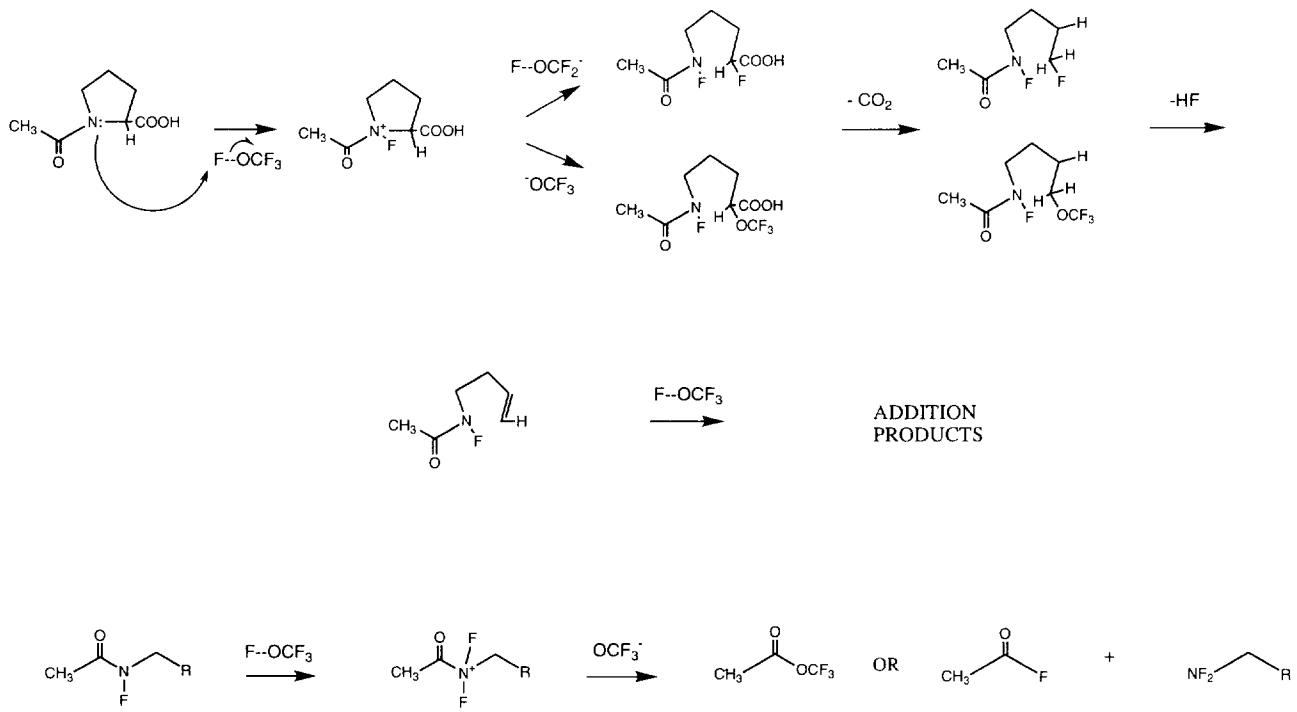


Fig. 4. Proposed mechanistic description of the fate of fluorinated *N*-acetylproline species.

acetate and acetone as eluents was unsuccessful. Reverse-phase chromatography was also unsuccessful.

Further experiments showed that the rate at which the CF_3OF was added is crucial to the amounts of side-product formed. Rapid addition of the gas increased the amounts of difluoroamines relative to *N*-fluoroamide. The amount of reagent added seemed to have little effect on the relative amounts of side-products formed. Use of a variety of drying agents and solvents had little effect on the product distribution of the peptide reaction mixture. Because both AGE and AAE fluorinated smoothly to a single product, the probable culprit for the proliferation of products when CGPGAP was fluorinated was proline. *N*-Acetylproline (NAP) was synthesized without incident [10] and subjected to a variety of experiments to ascertain the exact nature of the reaction of proline with CF_3OF.

Barton suggests [6] that nitrobenzene is an ideal inhibitor of free-radical reactions. NAP was fluorinated in the presence and absence of nitrobenzene, and the ^{19}F spectrum of the residues from the two experiments measured. When NAP was fluorinated in the absence of nitrobenzene, a wide variety of products resulted. Unfortunately, the addition of nitrobenzene had no effect on the product distribution. We conclude, therefore, that the mechanism responsible for the complex mix of products was not free radical in nature.

HPLC of the raw product residue on silica gel using 100% $CHCl_3$ as eluent showed a multitude of products. The ^{19}F spectrum of two of the fractions collected (retention times 15.0 and 19.0 min, respectively) showed very interesting peaks. A set of peaks split into triplets and further into quartets occurred in each of these. One possible way this splitting pattern could occur is if the proline nitrogen is fluorinated. The δ-protons of proline split the fluorine into a triplet, which is further split by the acetyl protons into quartets. The splittings, 32.99 and 7.16 Hz, are comparable to those found in the other fluorinated amino acid derivatives.

The active carbon center generated by the ring-opening would explain the proliferation of products. The α-carbon cation, with the electron-withdrawing carboxy group as a neighbor, would react with the nearest nucleophile, F^- or CF_3O^-, and possibly decarboxylate (Fig. 4). This could be followed by elimination, yielding a double bond which would be more reactive than the original amide function. Additional CF_3OF would react with this center yielding further elimination/addition products. The presence of fluorine on the proline nitrogen activates that nitrogen toward further substitution. All the fractions examined had peaks in the ^{19}F spectrum characteristic of difluoroamines. The combination of reactions between the two sites explains the multitude of peaks observed. Thus, the reactivity of proline precludes use of CF_3OF to prepare *N*-fluoroamides in peptides containing this residue. This reactivity stems apparently from the presence of a second carbon atom bonded to the nitrogen. The additional electron density afforded increases the reactivity of the nitrogen toward CF_3OF.

2.3. Fluorination of peptides which do not contain proline

Subsequently, two new cyclopentapeptides were synthesized which did not contain proline. The two new peptides, c-(gly-leu$_3$-gly) (CG2L3) and c-(gly-leu-gly$_3$) (CG4L), were synthesized in a straightforward manner [3], and their

suitability for conformation studies determined using NMR spectroscopy. Both compounds were rigid with fixed conformations on the NMR timescale, but the low solubility of CG2L3 precluded further study.

CG4L was treated with CF_3OF, the residue dissolved in CD_3OD and the ^{19}F NMR spectrum measured. Five singlets were apparent, three clustered at 154 ppm while the other two resonated at 73 and 149 ppm, with no evidence of the formation of *N*-fluoroamides. Examination of the ^{19}F spectrum of the raw reaction mixture resulting from treatment of CH_3OH alone with CF_3OF showed that the CH_3OH had reacted. Because the solubility of CG4L in CH_2Cl_2 and $CHCl_3$ was low, other solvents were explored for their suitability for fluorination reactions.

Tetrahydrofuran and dioxan reacted extensively with CF_3OF, yielding mainly fluorinated hydrocarbons. Dimethylformamide reacted to yield a product whose ^{19}F spectrum showed a broad singlet at 180 ppm, while the spectrum of dimethyl sulfoxide treated with CF_3OF showed a broad singlet at 173 ppm. All of these solvents reacted preferentially with CF_3OF over the peptide. When CG4L was dissolved at high dilution in CH_2Cl_2 and treated with CF_3OF, a small amount of fluorinated material resulted which apparently contained *N*-fluoroamides.

Unfortunately, all the amide nitrogens had a similar reactivity toward CF_3OF. No single set of peaks resulted which could be used for conformational studies. The overlap of these peaks in the 60 ppm range precluded analysis without separation of the various isomers. The low solubility of the starting material, combined with the inefficiency of the reaction made this an impossible task.

3. Experimental details

Melting points were measured on a Gallenkamp melting point apparatus and are corrected. Thin layer chromatography (TLC) was performed using BakerFlex plates coated with Aluminum Oxide 1B-F (200 μm coating with fluorescent indicator) or Silica Gel 1B-F (200 μm coating with fluorescent indicator). High performance liquid chromatography (HPLC) was performed on a Waters system. Columns used were a Waters uBondapack C_{18} reverse-phase semipreparative column (25 cm × 1.0 cm) and a Dupont Zorbax Sil column (25 cm × 6.2 mm). Solvents were Fischer HPLC grade.

Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker AM-300WB NMR spectrometer equipped with an Aspect 3000 computer. Quadrature detection with Bruker Cyclops phase cycling was used in all experiments. Additional data manipulation was accomplished on a Silicon Graphics 4D25 workstation using the FELIX program (Biosym/MSI, Inc.). Internal lock was provided by use of deuterated solvents. Tetramethylsilane was used as an internal reference for ^1H spectra and for some carbon spectra. $CDCl_3$ at 77.096 ppm was also used as a reference for carbon. $CFCl_3$ was used as internal reference for the ^{19}F spectra, while external formamide was used as a reference for ^{14}N. The chemical shift of formamide was then related to neat liquid ammonia at 0 ppm. ^{14}N was decoupled from ^1H and ^{19}F using the NWALTZ sequence, written by Mike Geckel of Bruker Instruments, Inc.

NWALTZ microprogram: for accumulation of any nucleus with heteronuclear decoupling

```
1 ZE
2 D1
  P5 PH1
  D5
  D5 PHO
  D6 ADC
3 P3:C10:C11 P4:C10 P2:C10:C11 P3:C10
  P1:C10:C11 P2:C10 P4:C10:C11 P2:C10: P3:C10:C11
4 P3:C10 P4:C10:C11 P2:C10 P3:C10:C11
  P1:C10 P2:C10:C11 P4:C10 P2:C10:C11 P3:C10
  LO TO 4 TIMES 2
  P3:C10:C11 P4:C10 P2:C10:C11 P3:C10
  P1:C10:C11 P2:C10 P4:C10:C11 P2:C10 P3:C10:C11
  L1 TO 3 TIMES UPR
5 RCYC=2 PH5 6 EXIT

PH0=0
PH1=0 0 2 2 1 1 3 3
PH5=R0 R0 R2 R2 R1 R1 R3 R3 D1=OBSERVE 5*T1
P5=90 DEG. PULSE FOR OBSERVE NUC.
D5=DE/2 D6=2USEC
P1, P2, P3 AND P4 ARE 90, 180, 270, AND 360 DEG.
PULSES FOR HETERONUCLEUS
UPR=AQ/96*P1
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3.1. Synthetic methods

3.1.1. General fluorinating procedure

Warning: hypofluorites, including CF_3OF, constitute an extremely hazardous class of compounds. Use utmost caution when performing experiments involving these compounds.

CF_3OF was purchased from PCR, Inc., and also kindly prepared for us by Prof. Darryl DesMarteaux of Clemson University. Following the procedure of Barton et al. [7], the compound to be fluorinated was dissolved in $CHCl_3$, cooled to −20 °C and the desired amount of CF_3OF, diluted several-fold with N_2, bubbled through the solution via Teflon tubing transfer lines. Any unreacted gas was destroyed with an aqueous KI trap.

3.1.2. N-Fluoroamino acid derivatives

N-Acetyl-N-fluoroglycine ethyl ester

AGE (0.500 g, 3.44 mmol) was dissolved in 100 ml of $CHCl_3$ and the solution cooled to −20 °C using a CCl_4/Dry Ice bath. A small amount of anhydrous Na_2CO_3 was added. Over a period of several hours, 1.1 mmol CF_3OF was bubbled through the solution. During the addition of the gas, the solu-

tion was kept at −20 °C. The solution was allowed to return to room temperature and stirred overnight. The solution was then washed with 2×100 ml of dilute $NaHCO_3$ solution, followed by 2×100 ml of H_2O and then dried over $MgSO_4$. The solvent was then removed by use of a rotary evaporator. The resulting pale yellow oil was chromatographed on a 1 g silica (60–200 mesh) column using 5 ml of 4:1 hexane/ethyl acetate to elute the product. The starting material was eluted with 5 ml of ethyl acetate. Yield, 0.032 g (5.7%). The product was an oil at room temperature.

^{1}H NMR in $CDCl_3$ (δ 7.33 ppm) δ: 1.20 (CH_3CH_2, 3H, t, J=6.95 Hz); 2.21 (CH_3C=O, 3H, d, $^4J_{HF}$=7.31 Hz); 4.14 (CH_3CH_2, 2H, q, J=7.07 Hz); 4.47 (CH_2N, 2H, d, $^3J_{HF}$=31.43 Hz) ppm. ^{13}C NMR in $CDCl_3$ (δ 77.00 ppm) δ: 13.40 (1.41, CH_3CH_2); 19.78 (0.63, CH_3C=O); 51.81 (0.74, 0.081, CH_2N, d, $^2J_{CF}$=11.10 Hz); 61.30 (1.14, CH_3CH_2); 165.59 (0.08, 0.06, C=O, amide, d $^2J_{CF}$=2.78 Hz); 170.33 (0.09, C=O, ester) ppm. ^{19}F NMR in $CDCl_3$ δ: 56.02 (NF, tq, $^3J_{HF}$=31.42 Hz, $^4J_{HF}$=7.28 Hz) ppm. ^{14}N NMR in $CDCl_3$ δ: 240 (NF, $\nu_{1/2}$=1700 Hz) ppm.

N-Acetylglycine-N-methylamide

N-Acetylglycine-*N*-methylamide [11] (0.500 g, 3.84 mmol) was dissolved in 20 ml of CH_3OH and to this was added 120 ml of $CHCl_3$. The solution was cooled to −20 °C and treated with 50 psi (1.25 mmol) of CF_3OF. The solution was stirred overnight at room temperature. The solvent was removed by rotary evaporation. The fluorinated products were not isolated.

N-Acetyl-N-fluoroalanine ethyl ester

N-Acetylalanine ethyl ester (0.500 g, 3.14 mmol) was dissolved in 125 ml of $CHCl_3$ and the solution cooled to −20 °C. A small amount of anhydrous Na_2CO_3 was added. Dry N_2 gas was bubbled through the solution during the addition of 2.5 mmol of CF_3OF. The temperature was maintained at −20 °C during the addition of the gas. The solution was stirred overnight. Work-up as above yielded a pale yellow oil. Four additional batches were combined and the residue applied to a 5 g silica column. The product was eluted with 10 ml of a 4:1 mixture of hexane/ethyl acetate. Starting material began eluting after an additional 5 ml of the solvent mixture had passed through the column. Yield for the combined five runs, 0.128 g (4.6% per run).

^{1}H NMR in $CDCl_3$ (δ 7.31 ppm) δ: 1.20 (CH_3CH_2, 3H, t, J=7.18 Hz); 1.51 (CH_3CH, 3H, d, J=7.35 Hz); 2.20 (CH_3C=O, 3H, d, $^4J_{HF}$=7.35 Hz); 4.13 (CH_3CH_2, 2H, dq, J=7.35, 1.05 Hz); 5.01 (CHN, 1H, dq, $^2J_{HF}$=41.24 Hz, J=7.41 Hz) ppm. ^{13}C NMR in $CDCl_3$ (δ 76.98 ppm) δ: 13.46 (8.89, CH_3CH_2); 13.95 (10.00, CH_3CH); 20.67 (4.53, CH_3=O); 58.57 (4.63, 4.36, CHN, d, $^2J_{CF}$=11.10 Hz); 61.54 (7.30, CH_3CH_2); 168.64 (0.84, C=O, amide); 177.26 (0.43, C=O, ester) ppm. ^{19}F NMR in $CDCl_3$ δ: 74.95 (NF, dq, $^3J_{HF}$=42.13 Hz, $^4J_{HF}$=7.34 Hz) ppm.

N-Acetyl-N-fluoroleucine ethyl ester

N-Acetylleucine ethyl ester (0.500 g, 2.48 mmol) was dissolved in 150 ml of $CHCl_3$. A small amount of anhydrous Na_2CO_3 was added and the solution cooled to −20 °C. CF_3OF (3.75 mol) diluted about 10-fold with dry N_2 was added over a period of 6 h with stirring. Work-up as usual gave a colorless oil. This procedure was repeated four times and the combined mixture applied to a 5 g silica gel chromatography column. The elution solvent was a 5% solution of ethyl acetate in hexane. The fluorinated product was eluted with 35 ml of solvent. The starting material was recovered with 10 ml of ethyl acetate. Additional purification was afforded by use of a 1 g silica gel column in a disposable pipette using 2.5% ethyl acetate in hexane as eluent. The pure *N*-fluorinated product eluted with 15 ml of solvent. Yield for combined runs, 0.189 g (6.9% per run). An additional product, in which the leucine γ-proton was replaced with fluorine, was detected by ^{19}F NMR spectroscopy. This product was not isolated.

^{1}H NMR in $CDCl_3$ (δ 7.29 ppm) δ: 0.95 (CH_3CH, 3H, d, J=6.30 Hz); 0.99 (CH_3CH, 3H, d, J=6.30 Hz); 1.28 (CH_3CH_2, 3H, t, J=7.16 Hz); 1.71–2.00 ($CH_2CH+CH(CH_3)_2$, 3H, m); 2.18 (acetone impurity); 2.30 (CH_3C=O, 3H, d, $^4J_{HF}$=7.45 Hz); 4.21 (CH_3CH_2, 2H, q, J=7.07 Hz); 5.05 (CHN, 1H, ddd, $^3J_{HF}$=41.84 Hz, J=10.89, 4.01 Hz) ppm. ^{13}C NMR in $CDCl_3$ (δ 76.98 ppm) δ: 13.96 (8.87, CH_3CH_2); 20.66 (4.24, CH_3C=O, br); 20.87, 22.94 (10.00, 9.31, CH_3CH); 24.68 [8.38, $CH(CH_3)_2$]; 36.00 (8.05, CH_2CH); 61.20 (4.26, 4.59, CHN, d, $^2J_{CF}$=11.10 Hz); 61.81 (7.75, CH_3CH_2); 168.86 (1.01, C=O, amide); 177.46 (0.65, C=O, ester) ppm. ^{19}F NMR in $CDCl_3$ δ: 75.44 (NF, dq, $^3J_{HF}$=41.80 Hz, $^4J_{HF}$=7.43 Hz) ppm and for the compound not isolated, 136.76 [$CH_2CF(CH_3)_2$, nontuplet, $^3J_{HF}$=21.17 Hz] ppm.

N-Acetyl-N-fluorovaline ethyl ester

N-Acetylvaline ethyl ester (0.500 g, 2.67 mmol) was dissolved in 125 ml of $CHCl_3$ and a small amount of anhydrous Na_2CO_3 added. The solution was cooled to −20 °C and treated with 3.75 mmol of CF_3OF over a period of 2 h. After working-up as usual, the residual oil was combined with that from four other runs and the mixture applied to a 5 g column of activated neutral alumina with 100% hexane used as eluent; 10 ml was sufficient to elute the product while another 20 ml eluted the starting material. Yield for the combined runs, 0.157 g (5.8% for each run).

^{1}H NMR in $CDCl_3$ (δ 7.27 ppm) δ: 0.95 (CH_3CH, 3H, d, J=6.43 Hz); 0.99 (CH_3CH, 3H, d, J=6.44 Hz); 1.18 (CH_3CH_2, 3H, t, J=7.32 Hz); 2.12 [$CH(CH_3)_2$, 1H, m]; 2.25 (CH_3C=O, 3H, d, $^4J_{HF}$=7.35 Hz); 4.13 (CH_3CH_2, 2H, q, J=7.32 Hz); 5.01 (CHN, 1H, dd, $^3J_{HF}$=39.22 Hz, J=6.92 Hz) ppm. ^{13}C NMR in $CDCl_3$ (δ 76.99 ppm) δ: 13.83 (CH_3CH_2); 17.65, 18.51 [$(CH_3)_2CH$]; 20.45 (CH_3C=O); 26.93 [$CH(CH_3)_2$]; 61.11 (CH_3CH_2); 64.25 (CHN, $^2J_{CF}$=10.97 Hz); 168.43 (C=O, amide); 177.77 (C=O, ester) ppm. ^{19}F NMR in $CDCl_3$ δ: 75.51 (NF, tq, $^3J_{HF}$=39.18 Hz, $^4J_{HF}$=7.32 Hz) ppm.

3.1.3. Proline-containing peptides and c-(gly-leu-gly$_3$)

c-(Gly-pro-gly-D-ala-pro)

c-(Gly-pro-gly-D-ala-pro) (CGPGAP) [9] (100 mg, 0.26 mmol) was dissolved in 125 ml of $CHCl_3$ which had

previously been distilled from P_2O_5. A small amount of anhydrous Na_2CO_3 was added and the solution cooled to −20 °C using a Dry Ice/CCl_4 bath. CF_3OF (0.75 mmol) diluted approximately 10-fold with dry N_2 was bubbled slowly into the solution over a period of 3 h. The solution was allowed to stir overnight, during which time its temperature rose to 25 °C. The residue was subjected to ^1H and ^{19}F NMR spectroscopy. A large number of fluorinated products resulted. The amount of CF_3OF was reduced 10-fold and 100-fold, and the experiment repeated. Again, a large number of fluorinated products resulted despite the decrease amounts of CF_3OF. The residue of the reaction of CF_3OF and a 100-fold excess of peptide was dissolved in $CHCl_3$ and applied to a 5 g silica column. Fractions were eluted with various ethyl acetate/hexane mixtures. Both difluoroamines and fluorinated hydrocarbons, in addition to *N*-fluoroamides, were present in all fractions containing fluorine.

N-Acetylproline

N-Acetylproline (NAP) [10] (300 mg, 2.00 mmol) was dissolved in 125 ml of $CHCl_3$ and a small amount of anhydrous Na_2CO_3 added. The solution was cooled to −78 °C and 100 psi (2.5 mmol) of CF_3OF, diluted with dry N_2, added in the dark. The reaction was allowed to proceed for 1 h. Solvent was removed at room temperature in the dark using a rotary evaporator. The residue was subjected to HPLC on a silica column using $CHCl_3$ as eluent at a flow rate of 1 ml min^{-1}. A similar experiment was performed in the presence of 0.1 equiv. of nitrobenzene.

c-(Gly-leu-gly$_3$)

CG4L (5 mg, 0.025 mmol) was dissolved in 800 ml of CH_2Cl_2 which had previously been distilled from P_2O_5. A small amount of anhydrous Na_2CO_3 was added and the solution cooled to −20 °C. CF_3OF (0.25 mmol) was diluted with dry N_2 and slowly bubbled into the solution. The reaction was allowed to proceed overnight, during which time the temperature of the mixture gradually rose to 25 °C. The mixture was filtered and the solvent removed by use of a rotary evaporator at room temperature. The ^{19}F NMR spectrum of the residue was then measured.

4. Conclusions

In summary, *N*-fluoroamides were easily prepared from *N*-acetylamino acid ester derivatives. Two derivatives yielded minor products. AGA fluorinated to two major components which correspond to *N*-acetyl-*N*-fluoroglycine-*N'*-methylamide and *N*-acetylglycine-*N'*-fluoro-*N'*-methylamide, and a minor component which corresponds to the *N,N'*-difluoroamide derivative. Other products present in the reaction mixture result from reaction of CF_3OF with solvent. ALE fluorinated to the *N*-fluoroamide and to a minor (<2%) component in which the γ-proton was replaced by fluorine. This reaction did not occur in the case of valine.

The ^{14}N NMR spectrum of *N*-acetyl-*N*-fluoroglycine ethyl ester was measured. To our knowledge, this represents the first ^{14}N spectrum reported for an *N*-fluoroamide. In addition, ^{14}N-decoupled ^{19}F spectra and ^1H-/^{14}N-decoupled ^{19}F spectra are also a first.

The multiplicity of products resulting from treatment of peptides containing proline with CF_3OF precluded separation. Evidently, the lone pair of the amide nitrogen in proline attacks the electrophilic center in CF_3OF, causing a subsequent ring-opening of proline. Evidence for this process was obtained by analysis of the ^{19}F spectrum of fractions obtained in a preliminary separation of the product mixture. The *N*-fluoroamide formed undergoes further addition/substitution reactions to fluorinate the aliphatic side-chain and/or form difluoroamines. The multitude of products, each present in small amounts, made any separation of the products impractical.

The reactivity of CGPGAP as a result of the proline residues was in direct contrast to the nonreactivity of CG4L toward CF_3OF. The low solubility of the latter peptide also presented some difficulty. There was also no selectivity in the reaction of the amide protons in the peptide with CF_3OF, in contrast to the differences in reactivity of the amide nitrogens in AGA. Attempts to enhance the reactivity of CG4L using metal hydrides were fruitless. Additionally, several solvents, tetrahydrofuran, dioxan, dimethylformamide, dimethyl sulfoxide, and to a lesser extent, methanol, were shown to undergo reactions with CF_3OF.

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