Organofluorine Synthesis via Photofluorination: 3-Fluoro-D-alanine and 2-Deuterio Analogue, Antibacterials Related to the Bacterial Cell Wall

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Abstract: Design and synthesis of the novel antibacterials 3-fluoro-D-alanine (FA) as well as its 2-deuterated version (DFA) are described. The design of FA exploits a fundamental divergence in biosynthesis of the peptidoglycan component of the bacterial cell wall and of the metabolic pathways in humans. This divergence suggested application of the concept of antimetabolite synthesis via the specific approach of photofluorination. Thus, photofluorination of a key component of the bacterial cell wall (D-alanine) generated FA. FA in fact displays a high degree of wide-spectrum antibacterial activity. A variant of FA with increased metabolic stability—and with unimpaired antibacterial activity—was obtained via photofluorination of 2-deuterio-D-alanine, namely 3-fluoro-D-alanine-*2-d* (DFA). (DFA is the unorthodox component of a novel, fixed-ratio antimicrobial combination, effective in vitro and in vivo against every bacterial strain tested.)

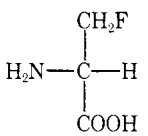
Notwithstanding the importance of organic fluorine compounds, their synthesis by direct (substitutive) fluorination is seldom practicable. To fill this gap, we have proposed an approach named *photofluorination*, which represents the first general and selective method for substitutive fluorination of organic compounds.[1a] Application of this method for C-fluorination of the amino acids D- and L-alanine as well as of 2-deuterio-D-alanine is presented now. These syntheses also illustrate the utility of photofluorination for generation of antimetabolites via fluorination of selected metabolites.[1b]

Electrophilic fluorination of olefins as well as of activated aromatics by fluoroxytrifluoromethane (CF_3OF) was first described by Porter and Cady.[2] The full potential and wide applicability of electrophilic fluorination by CF_3OF were recognized and demonstrated by D. H. R. Barton and coworkers.[3,4] Subsequently, utility of CF_3OF for fluorination of organic molecules was extended by introduction of photofluorination. This method consists of light-induced, liquid-phase interaction of CF_3OF with organic compounds; it appears to be of radical mechanism:

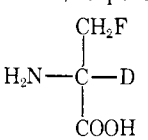
$$RH \xrightarrow{CF_3OF;\ h\nu} RF$$

Fluoro amino acids are of considerable interest in biochemistry; however, their synthesis is cumbersome, especially if optical resolution is included.[5] Photofluorination allows one-step transformation of amino acids into fluoro amino acids. There is no racemization observed, thus optically active fluoro amino acids become easily available.

The electrophilic nature of CF_3OF would not allow selective C-fluorination of amino acids without protection of the NH_2 groups.[6] However, by utilizing the earlier approach[7,8] for protection of NH_2 groups against the similarly electrophilic chlorine via protonation by strong acid solvents, this complication was avoided. Trifluoroacetic acid and especially liquid hydrogen fluoride proved to be suitable solvent for these reactions. Thus, by employing photofluorination in liquid HF solution, 3-fluoro-D-alanine (FA) [(*S*)-2-amino-3-fluoropropionic acid], 3-fluoro-L-alanine, and 3-fluoro-2-deuterio-D-alanine (DFA) were obtained in good yield from D-alanine, L-alanine, and 2-deuterio-D-alanine, respectively:



FA



DFA

The ir, ^1H and ^{19}F NMR data, as well as the mass spectra of these new compounds fully support the structures assigned. The absence of racemization during photofluorination is indicated by the optical rotation data. Rigorous proof of full retention of chirality of C(2) is provided by the ^1H and ^{19}F NMR spectra of DFA.

These results support the following mechanism for photofluorination:

$$CF_3OF \rightarrow CF_3O\cdot + F\cdot$$

$$RH + F\cdot \rightarrow R\cdot + HF$$

$$R\cdot + CF_3OF \rightarrow RF + CF_3O\cdot$$

$$CF_3O\cdot + RH \rightarrow R\cdot + COF_2 + HF$$

The first—photolytic—step has been demonstrated.[9] As F· is known to be a nonselective fluorinating species, $CF_3O\cdot$ is preferred over it as chain carrier, in light of the highly selective fluorination of alanine at C(3).

The above described examples of photofluorination illustrate the feasibility of the original concept: synthesis of antimetabolites (drugs) by fluorination of selected key metabolites.[1] Design of new types of drugs, in contrast to the so-called "molecular manipulation" of existing drugs,[10] is a rarely successful venture. In fact, FA and DFA represent the first instance of conception of a novel *type* of an effective antibacterial. This design rests on considerations of "molecular physiology" of bacteria.[11,12]

D-Alanine was selected as substrate for photofluorination, because it is the one and only constituent of the bacterial cell wall for which each one of the following statements is applicable:

1. It is an amino acid constituent which occurs in the peptidoglycan of every and any of the *bacterial* species: In other words, it is a *ubiquitous* component.[13]

2. It does not play a role, as far as it is known, in *human* metabolism.[14]

3. It is endogenously synthesized by the bacteria, preponderantly by racemization of L-alanine.[15]

4. Interference with various biosynthetic steps, all involving—directly or indirectly—D-alanine, has been invoked to explain antibacterial effects of some of the so-called "cell-wall active" antibiotics.[16]

On the basis of the premises listed above, it was projected that 3-fluoro-D-alanine could represent an antimetabolite directed exclusively against bacteria.

FA in fact displayed wide-spectrum antibacterial activity.

In vivo activity in infected mice was comparable with tetracycline and chloramphenicol, respectively; toxicity (mice) was remarkably low[17,18] (LD_{50} 3.4 g/kg, per oral). Its L-antimer [3-fluoro-L-alanine, (*R*)-2-amino-3-fluoropropionic acid] also displayed a high degree of antibacterial activity; however, it was toxic to mice (lethal at 250 mg/kg). DFA was designed to enhance the in vivo (metabolic) stability of FA, by exploitation of a $^2H/^1H$ deuterium isotope effect.[19] As expected, DFA was found to have substantially higher in vivo metabolic stability in animals than FA; however, its antibacterial activity was identical with the protio prototype.[20] DFA is the unorthodox component of a "fixed-ratio" antimicrobial combination,[20] the other component of which is the hemihydrate of sodium D-4-[(2-oxo-3-penten-4-yl)amino]-3-isoxazolidinone (PCS). PCS is a "pro-drug" of cycloserine (CS).[21] The DFA/PCS combination is an improved version—in respect to pharmacokinetics—of the prototype FA/CS combination. The FA/CS combination[22,23] was shown to display in vivo and in vitro antibacterial activity against every bacterial species tested, thus representing a "first" in antibacterial chemotherapy. The mechanism of antibacterial action of FA involves irreversible inhibition of alanine racemase.[22]

Our studies on photofluorination of other components of peptidoglycan will be the subject of a forthcoming publication.

Experimental Section

^1H NMR spectra were determined with Varian T-60 and HA-100 instruments; apparent splittings are given in all cases. Chemical shifts are reported in parts per million (δ) downfield from internal $(CH_3)_4Si$ standard. Mass spectra were measured with an LKB-9000 GC-MS spectrometer at 70 eV. The ^{19}F NMR spectra were determined with a Varian Model T-60 operating at 56.4 MHz. Fluorine chemical shifts (ϕ^*) are reported in ppm, external standard $FCCl_3$ = 0.0 ppm. Infrared spectra were recorded on a Perkin-Elmer Model 137 spectrometer. Melting points (hot stage) are uncorrected. Optical rotations were determined on a Zeiss polarimeter. ORD–CD spectra were determined with a Cary-60 spectropolarimeter. Amino acid analyses were carried out on a Spinco-Beckman automatic amino acid analyzer by Mr. Carl F. Homnick. Elemental analyses were performed by Mr. R. N. Boos and associates.

The light source[1] and the special Kel-F reactor[24] have been described. Hydrogen fluoride was from Matheson Gas Products; CF_3OF was from PCR Inc., Gainesville, Fla. D-Alanine and L-alanine were from Cyclo (NRC Grade). D-Alanine-*2-d* was from Merck Sharp & Dohme of Canada, Ltd., Pointe Clare-Dorval 700, Quebec, Canada. Early samples of the latter compound were prepared by H. Kropp and F. M. Kahan, by resolution of D,L-alanine-*2-d* via N-acetylation followed by enzymatic deacetylation with hog renal acylase I (as it is described for the protio D-alanine[25]). D,L-Alanine-*2-d* was prepared by treatment of L-alanine in D_2O with a crude alanine racemase preparation made from *Staphylococcus aureus*[26] (see also ref 19 and 27).

3-Fluoro-D-alanine (FA). D-Alanine (17.9 g, 0.2 mol) was placed into a Kel-F reactor[28] under slight positive pressure of dry N_2 and cooled to −78 °C (dry ice/acetone bath). Hydrogen fluoride gas was passed in until ~200 ml condensed. The crystal-clear, colorless solution was vigorously stirred and irradiated with light, while fluoroxytrifluoromethane (CF_3OF) gas (28.5 g) was passed in at −78 °C, in the course of about 19 h. To measure it, CF_3OF gas, taken from the cylinder, was liquified into a graduated glass trap, cooled in liquid N_2 (bp of CF_3OF −95 °C; mp −215 °C; thus it does not freeze in liquid N_2; 15.3 ml of $CF_3OF \simeq 28.5$ g $\simeq 0.264$ mol). The rate of evaporation of CF_3OF was controlled by careful regulation of the depth of immersion of the CF_3OF trap in the Dewar holding the liquid N_2. (The trap was not *immersed* into liquid N_2.) The reaction was interrupted overnight and the system was kept at −78 °C while slight pressure of dry N_2 gas was maintained. [In other experiments, CF_3OF was taken directly from the cylinder. The rate of addition was followed then by observing the pressure decrease on a gauge (Matheson B15F-679) attached to the CF_3OF cylinder.[29]] After two more hours of irradiation, the HF solvent was blown off with N_2 and the pale yellow residue dissolved in water. After evaporation in vacuo to dryness, it was redissolved in water and chromatographed on Dowex 50-X-8 analytical grade cation exchange resin column (200–400 mesh; 1.4 l. resin); elution with water (1.4 l.), 0.5 N HCl (~2.5 l.) and 1 N HCl (~5 l.). Fractions (25 ml) were collected. FA was found (by ninhydrin spray of small samples on silica-coated TLC plates) in tubes no. 247–293. These were combined and evaporated in vacuo to give FA hydrochloride. This was dissolved in water, the pH was adjusted with pyridine to 4, then cold 2-propanol (100 ml) was added. After aging in an ice bath, the FA was filtered, washed with H_2O–2-propanol and diethyl ether, and dried in vacuo: 12.21 g (57% of theory) of snow-white crystals of FA. It can be recrystallized from four volumes of H_2O, recovery 80%.[30]

The compound gives a single symmetrical peak in the amino acid analyzer: mp 168 °C dec; NMR (D_2O) 4.08 (apparent d of t, $J_{H\text{-}F}$ = 29.5 Hz, $J_{1,2}$ = 3.5 or 4.5 Hz, $J_{2,3} \simeq$ 4.5 or 3.5 Hz, 1 H), 4.83 and 4.85 (2 m, $J_{2,3} \simeq 0$ Hz, $J_{1,2} \simeq$ 3.5 or 4.5 Hz, $J_{1,3} \simeq$ 4.5 or 3.5 Hz, $J_{2,F} \simeq$ 46 or 47 Hz, $J_{3,F} \simeq$ 47 or 46 Hz, 2 H); ^{19}F NMR (H_2O/DCl) 2 triplets at +233.2 and +233.5 ($J_{H\text{-}Fvic}$ = 29.5 Hz and $J_{H\text{-}Fgem}$ = 46 Hz); $[\alpha]^{25}D$ −10.0 ± 0.1° (c, 6 in 1 N HCl); ORD (in 1 N HCl) $[\Phi]_{224}$ −1410° (trough); $[\Phi]_{210}$ 0 (cross-over) [for D-alanine $[\Phi]_{224}$ −1830 (trough)]; CD, − maximum at 206 nm. The mass spectrum of trimethylsilylated FA showed peaks at *m/e* 134 [100%; M − 117 = M − $COOSi(CH_3)_3$], 208 (2.85%; M − 43 = M − CH_3 + CO), 218 (1%; M − 33 = M − CH_2F), and 236 (2.13%; M − 15 = M − CH_3); ir spectrum (KBr pellet) NH_3^+ nitrogen–hydrogen stretch at 3.45 and 4.8 μ (med. asym deformation), C=O stretch of carboxylate anion 6.3 μ, sym CO_2 stretch 7.1 μ, CH deformation 7.46 μ s, CF stretch 9.20 μ m.

Anal. Calcd for $C_3H_6NO_2F$: C, 33.65; H, 5.65; N, 13.08; F, 17.74. Found: C, 33.61; H, 5.79; N, 12.98; F, 17.45.

Photofluorination in Trifluoroacetic Acid. D-Alanine (1.0 g, 0.011 mol) was dissolved in 50 ml of trifluoroacetic acid. Under cooling in a −2 °C bath and with vigorous stirring, CF_3OF gas (4.2 g measured as 2.2 ml of liquid in liquid N_2 bath, 0.041 mol) was passed in 4 h while irradiating it with the 1000-W light source. The solvent was removed in vacuo, water was added and distilled off in vacuo. The residue was chromatographed on resin column (150 ml of Dowex 50-X-8), as it was described above, to give 0.25 g of FA (yield, 20% of theory).

3-Fluoro-L-alanine. L-Alanine (9.1 g, 0.102 mol) was photofluorinated, in similar manner as it has been described for FA. HF solvent employed was 100 ml. CF_3OF (19.5 g = 10.5 ml, 0.191 mol) was passed in the course of 15 h, while under concurrent irradiation. Isolation (via chromatography) gave 5.91 g (54% of theory) of 3-fluoro-L-alanine. Single symmetrical peak in the amino acid analyzer; mp 167–168 °C dec, $[\alpha]^{20}D$ + 10.0 ± 0.1° (c, 6 in 1 N HCl); ORD (in 1 N HCl) $[\Phi]_{225}$ +1440° (+ peak), $[\Phi]_{210}$ 0 (cross-over); CD + maximum at 206 nm.

Anal. Calcd for $C_3H_6NO_2F$: C, 33.65; H, 5.65; N, 13.08; F, 17.74. Found: C, 33.59; H, 5.53; N, 12.99; F, 17.56.

3-Fluoro-D-alanine-*2-d* (DFA). D-Alanine-*2-d* (4 g, 0.0445 mol) was photofluorinated in 50 ml of liquid HF, employing 4.45 g ($\simeq$2.3 ml, 0.043 mol) of CF_3OF, added in 2 h, followed by 1 h more irradiation. The usual workup gave 2.84 g (yield: 59%) of colorless crystals.

$[\alpha]^{20}D$ −10.0 ± 0.1° (c, 6 in 1 N HCl); ^1H NMR exhibits an ABX pattern with an apparent singlet at 5.40 ppm and an apparent AB pattern at 4.63 ppm ($J_{H,F}$ = 47 or 46 Hz, J_{H_3F} = 46 or 47 Hz); ^{19}F NMR (in Me_2SO/DCl/D_2O) triplet at 227.3 ± 0.9 ppm.

Anal. Calcd for $C_3H_5DFNO_2$: C, 33.33; N, 12.96; F, 17.58. Found: C, 33.28; N, 12.96; F, 17.46.

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(29) This and other improvements in scaling up the method up to 1 g mol of D-alanine were contributed by Dr. S. Marburg and Mr. L. M. Perkins of these laboratories.
(30) Note: About other products of photofluorination of alanine, see our forthcoming paper (manuscript under preparation).

Stable Fluorinated Cyclobutenyl Cations

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Contribution No. 2357 from the Central Research and Development Department, E. I. du Pont de Nemours and Company, Experimental Station, Wilmington, Delaware 19898. *Received February 19, 1976*

Abstract: A series of remarkably stable 1-methoxy-2-R-3,4,4-trifluorocyclobutenyl cations (**2**, R = F, Cl, OCH_3) have been prepared and studied by ^1H and ^{19}F NMR. The hexafluoroantimonate salts of **2** are isolable solids which are stable at room temperature. In solution, each cation exists as an equilibrating pair of isomers which differ only by the 1-methoxy group conformation. From a complete line-shape analysis of the temperature-dependent ^1H NMR, $\Delta G^{\ddagger} = 16.4$ kcal/mol for the barrier to rotation of the methoxy group in **2** (R = F); similarly, $\Delta G^{\ddagger} = 15.9$ and 15.7 kcal/mol for **2** (R = Cl) and **2** (R = OCH_3), respectively. The NMR data also establish the importance of 1,3-π overlap in these cations. The cyclobutenyl cations are excellent alkylating agents which can be employed in the synthesis of novel fluorinated cyclobutenones.

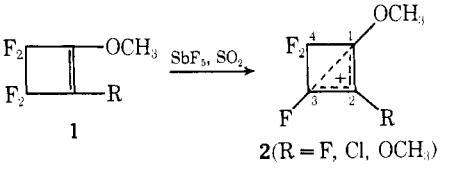
Both acyclic and cyclic allyl carbocations are now a class of well-characterized stable ions.[1] Although stable polyhalogenated allyl cations, e.g., the perchloroallyl ion,[2] have been studied, most attempts to generate stable polyfluoroallyl cations have been unsuccessful. Hexafluorocyclobutene is inert to SbF_5–SO_2ClF at −10 °C[3] and hexafluoropropene reacts with SbF_5 to give a product proposed to arise by an electrophilic dimerization, but no long-lived cations are observed.[4] To our knowledge, the 1-anisyl tetrafluoroallyl cation studied by Chambers and co-workers[4] is the only reported long-lived polyfluoroallyl cation.

In carbocations it is now well established that fluorine is conjugatively stabilizing when substituted at the cation center, but is destabilizing when substituted at adjacent or further removed positions. Apparently the fluorine inductive effect dominates conjugative stabilization and results in net destabilization of perfluoroallyl cations. A substituent which can further conjugatively stabilize positive charge, e.g., the anisyl group, is required for polyfluoroallyl cation stability.

We report here our results concerning the reaction of SbF_5 with 1-methoxy polyfluorinated cyclobutenes. The methoxy substituent is anticipated to provide the necessary stabilization required for reactivity and carbocation stability. Furthermore, the cyclobutenyl cations produced may further benefit from 1,3-π participation not available to their acyclic counterparts. The importance of 1,3-π overlap in hydrocarbon cyclobutenyl cations has been thoroughly examined by Olah and co-workers.[1b]

Results

1. Ion Production and NMR Spectra. The cyclobutenes **1** readily react with SbF_5 in SO_2 at −78 to −10 °C to produce the cyclobutenyl cations **2.**



The ^{19}F and ^1H NMR spectra of these cations at low temperature (−35 °C) indicate the presence of two isomeric species in each case (Table I). For example, at −35 °C the 1-methoxy-2-chlorotrifluorocyclobutenyl cation (**2**, R = Cl) shows a broad singlet at δ 5.22 and poorly resolved doublet ($J \simeq 0.5$ Hz) at δ 5.07. The ^{19}F NMR spectrum consists of two sets of multiplets; a 8.8-Hz triplet (1 F) at ϕ −24.8 and a 8.8-Hz doublet (2 F) at ϕ −104.6, and a 8.4-Hz triplet (1 F) at ϕ −26.4 and a 8.4-Hz doublet (2 F) at ϕ −98.1. The isomers are present in approximately equal amounts (determined by integration of the ^1H and ^{19}F NMR signals). Both the ^1H and ^{19}F NMR spectra are temperature dependent; upon warming