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THE SYNTHESIS OF FLUORINATED AMINOPHOSPHONIC ACID INHIBITORS OF ALANINE RACEMASE

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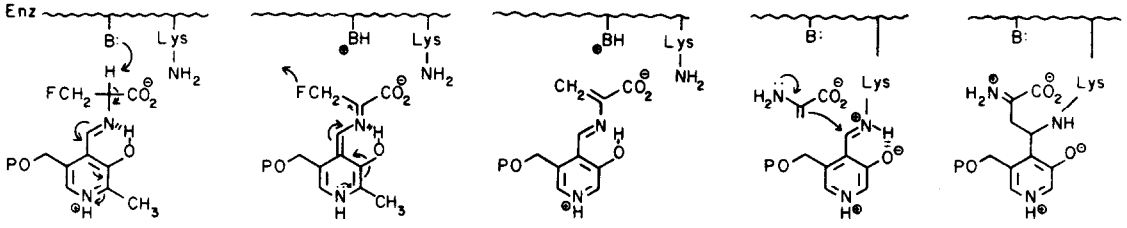
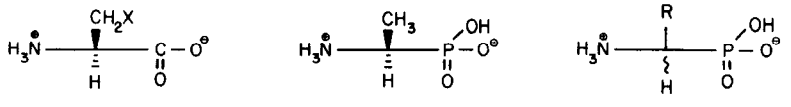
ABSTRACT: The synthesis of β-trifluoro, β-difluoro, and β-monofluoro-1-aminoethanephosphonic acids is described utilizing fluorinated acetic acids as starting materials.

The inhibition of peptidoglycan biosynthesis continues to be a primary focus of antibacterial drug design. The intrinsic property of bacteria to incorporate D-alanine into their cell wall has prompted recent efforts to inhibit the essential pyridoxal dependent racemase responsible for the production of D-alanine. Cycloserine[1,2] O-carbamoylserine[2,3] and the β-haloalanines[2,4] 1 are mechanism-based inactivators of bacterial alanine racemase whose presumed mechanism of action[5] is illustrated in Scheme I. In contrast, L-1-aminoethanephosphonic acid 2 is a potent selective competitive inhibitor of alanine racemase in vitro which when converted to the L-alanine dipeptide 5 (Alaphosphin) displays significant antibacterial properties and synergism with D-cycloserine and β-lactam antibiotics.[6,7]



SCHEME I

The fluorinated aminophosphonic acids 3a-c were considered to be attractive potential inhibitors of alanine racemase which might exhibit the enhanced binding affinity and specificity of aminophosphonic acid 2 while retaining the irreversible vector of the fluorinated alanines.[2,4]

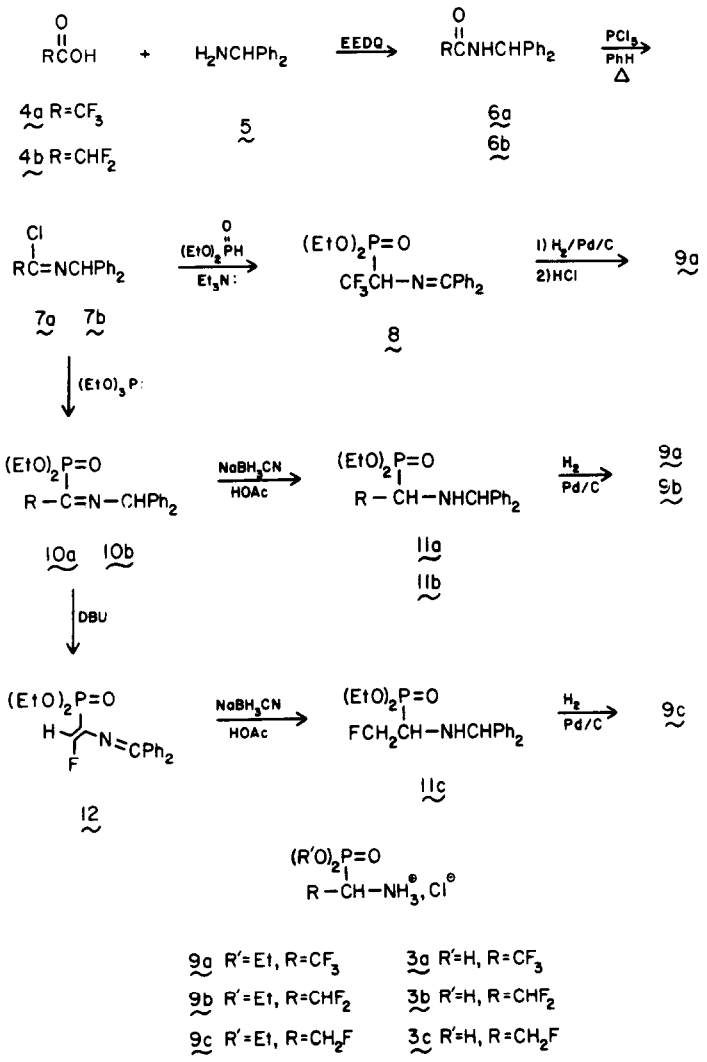


1 2

3a R=CF_3
3b R=CHF_2
3c R=CH_2F

Our general synthetic approach to all three fluorinated aminophosphonic acids 3a-c (Scheme II) would, in principle, utilize the corresponding trifluoro, difluoro, and monofluoroacetic acids 4a-c as starting materials. Due to the extreme toxicity of fluoroacetic acid 4c (LD_{50}=7mg/kg), our exploratory efforts began with the condensation of trifluoroacetic anhydride with 1-aminodiphenylmethane in pyridine (90% yield). The resulting amide 6a was converted to imidoyl chloride 7a (1 eq. PCl_5, PhH, reflux, 18 h) in 62% yield[8] after Kugelrohr distillation (125°C at 2 mm Hg). Heating of equivalent amounts of imidoyl chloride, diethyl phosphite, and triethylamine in the absence of solvent at 60°C for 20 hours gave a 30-40% yield of isomerized imine 8 after chromatography (Rf=0.20, 20% EtOAc/hexane).[9] Assignment of the isomerized structure 8 to this imine was supported by downfield aromatic protons in the NMR.

SCHEME II



Hydrogenation of 8 followed by acidification with HCl gave the analytically pure hydrochloride salt 9a as a sublimable white solid, mp. 117-118°C in 30% yield. The poor yield reported for this last step reflects partial phosphonic ester hydrolysis. Direct hydrolysis of the crude hydrogenation product without intermediate isolation (6N HCl, reflux, 6 h) provided the targeted aminophosphonic acid hydrochloride salt 3a in 84% yield.

Application of this initial route to the synthesis of the difluoro analog 3b proceeded smoothly to the imidoyl chloride stage. Difluoroacetic acid 4b was coupled to amine 5 (EEDQ, CH_2Cl_2, 95%) to give amide 6b which was quantitatively converted to the thermally unstable imidoyl chloride 7b. Treatment of 7b with diethyl phosphite under a variety of basic conditions failed to yield any tractable product. We surmised that products from this reaction might be unstable to the basic conditions employed and that loss of HF and subsequent polymerization had occurred. Imidoyl chloride 7b was successfully condensed with triethyl phosphite under the more neutral Arbuzov conditions [1 eq. $(EtO)_3P$, neat, 80°C, 18 h] to give the nonisomerized iminophosphonate 10b in 45-50% yield after chromatography (Rf=0.35, 20% EtOAc/hexane).

In contrast to isomerized imine 8, the relatively hindered imine 10b was resistant to catalytic hydrogenation and was converted to complex mixtures with severe material loss under forcing conditions. Enamino imine 12 was an interesting by-product isolated in small amounts from several of these attempts. Elimination of HF in this system is apparently a major pathway to decomposition.

The analogous Arbuzov reaction of trifluoroimidoyl chloride 7a with triethyl phosphite afforded iminophosphonate 10a (56% yield, Rf=0.35, 20% EtOAc/hexane) which was clearly different from imine 8 previously obtained.

Trifluoroimine 10a and difluoroimine 10b underwent smooth reduction with sodium cyanoborohydride in acetic acid to give benzhydrylamines 11a and 11b in 95% and 100% yields, respectively. These benzhydrylaminophosphonic esters were hydrogenated (H_2, 10% Pd/C, EtOH, HCl) and hydrolyzed (6N HCl, reflux, 6 h) to give the corresponding aminophosphonic acid hydrochloride salts 3a and 3b in excellent overall yield. Compound 3a obtained from the Arbuzov sequence was identical to that obtained from our initial approach.

Our efforts to prepare the monofluoro analog 3c by simple modification of either of the methods described thus far were unsuccessful presumably due to the reactivity of the fluoroacetate moiety. These results and the toxicity of fluoroacetic acid and its derivatives led us to look for alternate methods of generating monofluorinated molecules.

The key observation that enamino imine 12 was formed as a by-product during the attempted catalytic hydrogenation of iminophosphonate 10b prompted us to examine the effects of various dehydrohalogenation conditions on 10b in hopes of developing by-product 12 into an intermediate to the monofluoro analog 3c. We generally observed that conditions basic enough to promote elimination also led to unacceptable levels of further decomposition. Even lithium diisopropyl amide at -78°C gave complex mixtures in low yields. Clean conversion of 10b to 12 was ultimately achieved with DBU[10] (THF, 25°C, 5H). The sensitive enamino imine 12 could be isolated in 55-65% yield after chromatography (Rf=0.40, 1:1 EtOAc/hexane) provided that the

temperatures during workup did not exceed 35°C. Treatment of 12 with $NaBH_3CN$ in acetic acid affected quantitative reduction of both the enamine and imine moieties to give 11c. Hydrogenolysis of 11c (H_2, Pd/C) followed by ester hydrolysis (6N HCl, reflux, 6 h) provided β-fluoro-1-aminoethanephosphonic acid 3c in 90% overall yield.

All three fluorinated aminoethanephosphonic acids have been prepared in good yield using related synthetic techniques. The unusually complex ^{1}H, ^{19}F, and ^{31}P spectra of these compounds are consistent with their structures. The preparation of the monofluoro analog avoids the use of potentially toxic fluoroacetate derivatives. All three compounds are time-dependent inactivators of alanine racemase[11] with the monofluoro analog being the most potent. A detailed account will be discussed elsewhere.

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