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SYNTHESIS AND NMDA RECEPTOR BINDING OF 2-AMINO-7,7-DIFLUORO-7-PHOSPHONOHEPTANOIC ACID

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Abstract: In an NMDA specific receptor binding assay, **1** had lower affinity than its parent, APH. This result suggests that for competitive antagonists, diionization of the phosphonic acid moiety may be detrimental to receptor affinity.

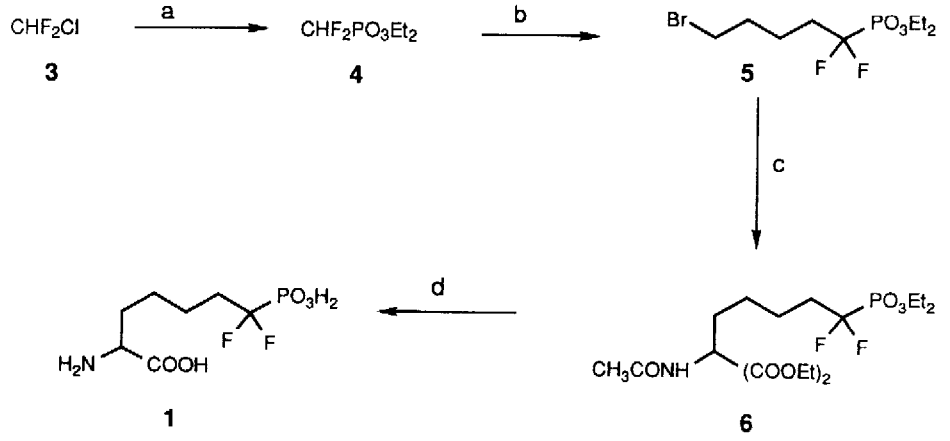
It is known that for competitive N-methyl-D-aspartic acid (NMDA) antagonists of the same overall structure a distal phosphonic acid moiety imparts a roughly ten fold increase in receptor binding affinity over that of the corresponding carboxylic acid.[1] Given the current rudimentary state of published NMDA receptor molecular modeling, the reason for this increase in affinity is unknown, but has been ascribed to the unique ability of phosphonic acid based antagonists to be diionized at physiological pH.[2] However, for a simple alkylphosphonic acid (pKa x and y) the second ionization will not be complete at physiological pH.[3] This raises the possibility that additional features of the phosphonate group, other than diionization, may be responsible for the increased NMDA receptor binding affinity of known antagonists. To explore whether complete diionization is a prerequisite for increased receptor binding, we synthesized 2-amino-7,7-difluoro-7-phosphonoheptanoic acid **1**. In this derivative, the presence of the two proximal fluorine atoms was expected to lower the second pKa close to 6, thereby ensuring complete diionization at physiological pH,[4] without significantly increasing the steric bulk α to the phosphonic acid.

Our initial attempts to prepare **1** centered on the conversion of dimethyl 1-oxo-5-chloropentylphosphonate into the corresponding α-difluoro derivative. Although precedented for α-ketoesters,[5] treatment of the corresponding α-ketophosphonates with diethylaminosulfur trifluoride (DAST) under a range of conditions did not afford identifiable fluorinated products.[6] As an alternative strategy in which both α-fluorines are already incorporated into a phosphonate synthon, we prepared diethyl (bromodifluoromethyl)phosphonate **2** via an Arbuzov reaction,[7] Eq. 1.[8,9] Although reported to undergo Reformatsky reactions with chloroacyl derivatives,[10] and more recently cadmium mediated coupling with allyl bromide,[11] in our hands, **2** proved to be a poorly reactive substrate. Indeed, we were unable to generate and couple the zinc salt even with a simple activated halide such as benzyl bromide.

Eq. 1 $Br_2F_2C + (EtO)_3P \xrightarrow[-78° \text{ to } 0° C]{} BrF_2CPO(OEt)_2$ **2**

Diethyl (difluoromethyl)phosphonate **4** provided a more versatile synthon. Treatment of chlorodifluoromethane with sodium diethylphosphonate (Scheme 1) provided **4** as a volatile oil in good yield.[12-14] Deprotonation with lithium diisopropylamide followed by addition to excess 1,4-dibromobutane at -78°C gave a moderate yield of the desired monoalkylated product **5**. Displacement of the remaining bromine with sodium acetamidomalonate proceeded smoothly to give **6**. Refluxing this product with 6 M hydrochloric acid resulted in the removal of all protecting groups and malonate decarboxylation to afford **1**.

SCHEME 1.



3 **4** **5** **1** **6**

a) $NaPO_3Et_2$, THF; b) LDA, THF, Br $(CH_2)_4$Br; c) NaC(NHAc)$(COOEt)_2$, EtOH, reflux; d) 6 M HCl, reflux

The pKa values of the parent compound, 2-amino-7-phosphonoheptanoic acid (APH) and **1** were determined by potentiometric titration in water by first acidifying with HCl (aq), and then titrating with 0.2 N NaOH (aq). The observed dissociation constants for APH were pKa_1 = 2.73 and pKa_2 = 7.89, and for **1** were, as predicted, pKa_1 = 3.0 and pKa_2 = 5.7.[15] Significantly, the increased dissociation of the second phosphonate proton of **1** compared with that of APH was illustrated both by facile phosphono monoester hydrolysis and the loss of HCl upon evaporation. Further, the non-typical physical properties of **1** are noted in the use of water as a recrystallization solvent. This is unusual due to the extreme hygroscopic nature of the non-fluorinated antagonists, and high water solubility of APH itself. These observations suggest that in **1** an internal salt exists between the phosphonic acid and the α-amine. Such an association might be expected to cause the molecule to fold in a manner which presents a predominatly hydrophobic exterior to solvent, effectively screening its high intrinsic polarity.

Using [^{3}H]4-(3-phosphonopropyl)-2-piperazinecarboxylic acid (CPP) as a ligand, NMDA receptor binding was carried out as previously described.[16] In this assay, APH had an IC_{50} of 0.8 μM. Surprisingly, **1** had significantly less affinity for the NMDA receptor with an

IC_{50} of 27 μM. The weak receptor binding affinity observed for **1** suggests that diionization of the distal phosphonic acid functionality may be detrimental to NMDA receptor binding. However, other factors may be responsible for the loss of receptor affinity. Although fluorine atoms have been used widely as replacements of hydrogen to modify activity in a variety of biologically active compounds,[17,18] a change in the preferred conformation of **1** and/or adverse electronic interactions of the fluorine atoms with a receptor component may instead be responsible for this observed loss in receptor binding affinity. The existence of such an interaction cannot as yet, be determined.

Diethyl (5-bromo-1,1-difluoropentyl)phosphonic acid (5). A solution of **4** (5.6 gm, 0.03 mol) in THF (30 mL) was cooled in a dry ice-acetone bath, and treated via syringe with a solution of lithium diisopropylamide (20 mL, 1.5 M in cyclohexane). After stirring 30 min, the anion solution was cannulated into a cold solution (-78°C) of 1,4-dibromobutane (19.4 gm, 0.09 mol) in THF (70 mL). The reaction mixture was allowed to warm to room temperature, and then concentrated, quenched with 0.5 N HCl, and extracted with EtOAc (200 mL). The organic layer was washed with 1 N HCl (4 x 10 mL), saturated $NaHCO_3$ (20 mL), and saturated NaCl (20 mL). The EtOAc solution was dried over $MgSO_4$, filtered and evaporated. The oily residue was chromatographed on silica gel (50% EtOAc in heptane as eluant) to give **5** as a yellow oil (4.5 gm). MS (EI) 325 (40%), 323 (43%). ^{1}H-NMR ($CDCl_3$) 4H, m, 4.35-4.20 ppm; 2H, t, 3.42; 6H, m, 2.21-1.70; 6H, t, 1.38. Anal.($C_9H_{18}BrF_2O_3P$) C, H, N.

(Acetylamino)[5-(diethoxyphosphinyl)-5,5-difluoropentyl]propanedioic acid diethyl ester (6). Diethylacetamidomalonate (2.94 g, 13.5 mmol) was added to a solution of sodium ethoxide (13 mmol) in EtOH (10 mL). After 30 min, **5** (3.5 g, 10.8 mmol) was added neat and washed into the reaction mixture with absolute EtOH (3 mL). The reaction mixture was refluxed overnight. The solvent was removed by evaporation and the residue was dissolved in EtOAc and washed consecutively with water and saturated NaCl solution. The organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was chromatographed on silica gel (25-75% gradient of EtOAc in heptane as eluant) to give a white solid (1.8 g). The phosphonate monoethyl ester was obtained from the acidified aqueous wash as a light brown oil (1.5 g). MS (EI) 460 (82%). ^{1}H-NMR ($CDCl_3$) 1H, s, 6.78 ppm; 8H, m, 4.33-4.19; 2H, m, 1.72-1.55; 6H, t, 1.37; 6H, t, 1.25; 2H, m, 1.51-1.14. Anal. ($C_{18}H_{32}F_2NO_8P$) C, H, N.

(±)-2-Amino-7,7-difluoro-7-phosphonoheptanoic acid (1). **6** (1.5 g, 3.26 mmol) was refluxed in 6 N HCl (30 mL) overnight. The water was removed by evaporation in vacuo, and the residue was redissolved in water and lyophilyzed. The solid was washed with diethyl ether and recrystallized from water to give **1** as a white solid, (0.47 g). mp 240-241°C dec. MS (FAB) 262 (100%). ^{1}H-NMR (D_2O) 1H, t, 4.07 ppm; 4H, m, 2.18-1.92; 4H, m, 1.71-1.47. Anal. ($C_7H_{14}F_2NO_5P$) C calc'd 32.19; found 31.65, H, N.

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