2,6-Difluorophenol as a Bioisostere of a Carboxylic Acid: Bioisosteric Analogues of γ-Aminobutyric Acid

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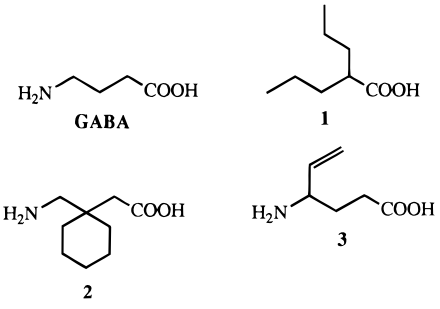
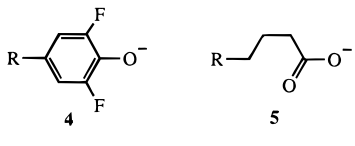
3-(Aminomethyl)-2,6-difluorophenol (**6**) and 4-(aminomethyl)-2,6-difluorophenol (**7**) were synthesized in eight and four steps, respectively, starting from 2,6-difluorophenol, to test the potential of the 2,6-difluorophenol moiety to act as a lipophilic bioisostere of a carboxylic acid. Compounds **6** and **7** are potential bioisosteric analogues of γ-aminobutyric acid (GABA). Substrate studies and inhibition studies were carried out with pig brain γ-aminobutyric acid aminotransferase; **6** and **7** are very poor substrates, but both inhibit the enzyme, indicating that the 2,6-difluorophenol moiety appears to be able to substitute for a carboxylic acid to increase the lipophilicity of drug candidates.

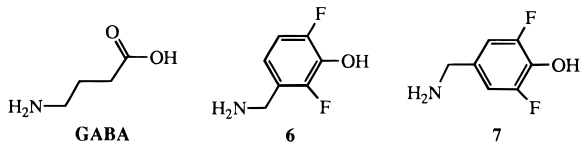
Introduction

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system (CNS).[1] The receptors to which it binds ($GABA_A$ and $GABA_B$ receptors), as well as the enzyme which degrades it (GABA aminotransferase), have been targets for drug design for decades.[2] The concentration of GABA in the brain is regulated principally by the enzyme that biosynthesizes it, L-glutamate decarboxylase, and the enzyme that degrades it, GABA aminotransferase (GABA-AT). When the GABA levels in the brain fall below a threshold level, convulsions begin.[3] These convulsions can be interrupted by injecting GABA directly into the brain,[4] but this is not a practical solution for controlling seizures. Because GABA is a small polar and hydrophilic molecule, it does not cross the blood−brain barrier; therefore, it cannot serve as an effective anticonvulsant agent. Several anticonvulsant drugs, however, that have structures similar to that of GABA, such as valproic acid (**1**), gabapentin (**2**), and vigabatrin (**3**), but which are slightly more lipophilic, do cross the blood−brain barrier (gabapentin, however, may be actively transported via an L-system amino acid transporter[5]). The mechanism of action of **1** is not clear, **2** appears to bind to the $\alpha_2\delta$ subunit of a calcium channel,[5] and **3**, after crossing the blood−brain barrier into the brain, albeit poorly, acts as a mechanism-based inactivator[6] of GABA aminotransferase,[7] thereby increasing the brain concentration of GABA, which controls the seizure. We thought that a more lipophilic bioisostere of the carboxylic acid moiety might be useful in the design of GABA mimics that could be more effective at crossing the blood−brain barrier. If this approach is successful, it could be applied as a bioisostere for any molecule containing a carboxylic acid group.



Given that the pK_a of phenol (9.8) drops to 7.1 by 2,6-difluoro substitution,[8] at pH 8.5, the pH optimum for GABA aminotransferase, 2,6-difluorophenol would be completely ionized (**4**) and may mimic a carboxylate ion (**5**). Since fluorine atoms are nearly as small as hydrogen but are capable of hydrogen bonding,[9] they may mimic the carboxylate carbonyl oxygen. However, fluorine is slightly lipophilic[10] as, of course, is benzene, so the 2,6-difluorophenol moiety should be a lipophilic isostere of a carboxylic acid. To determine the viability of **4** as a bioisostere of a carboxylic acid for drug design, 3-(aminomethyl)-2,6-difluorophenol (**6**) and 4-(aminomethyl)-2,6-difluorophenol (**7**) were synthesized as analogues of GABA and were tested as substrates and inhibitors of GABA aminotransferase.





Chemistry

Compound **6** was synthesized by the route shown in Scheme 1, starting from 2,6-difluorophenol (**8**). Nitration of **8** at 20 °C gave 2,6-difluoro-4-nitrophenol (**9**).[11] The nitrophenol **9** was heated at reflux with CH_3I and

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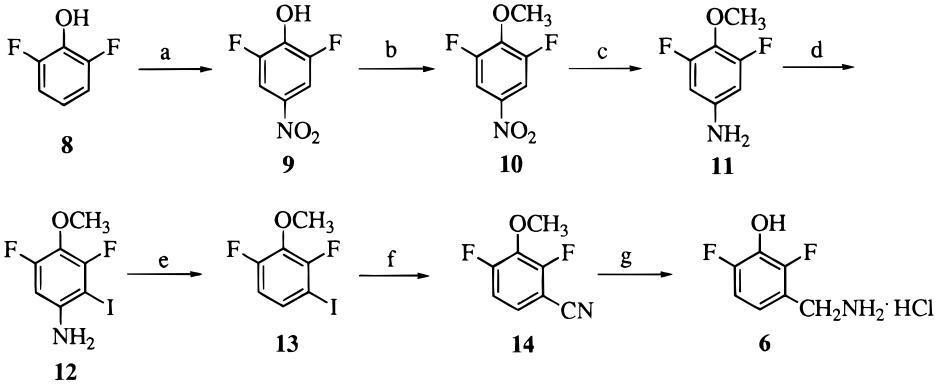
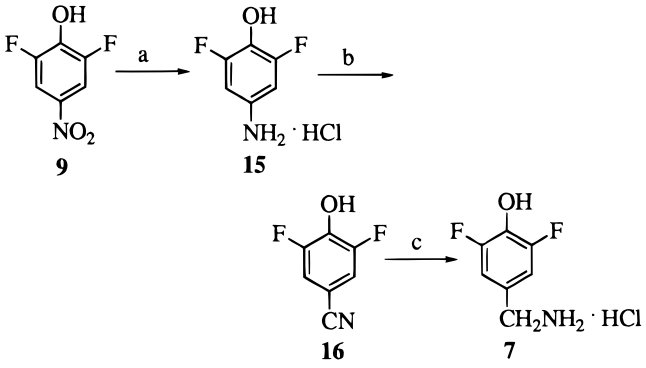
Scheme 1. Synthesis of **6**[a]



[a] (a) HNO_3, CH_3COOH, 15−20 °C, 96%; (b) CH_3I, K_2CO_3, acetone, 92%; (c) H_2, 10% Pd/C, 99%; (d) I_2, $NaHCO_3$, water, 64%; (e) 1. $NaNO_2$, H_2SO_4, 0 °C, 2. Cu powder, ethanol, 82%; (f) CuCN, DMF, 140 °C, 88%; (g) 1. BH_3, THF, reflux, 2. 6 N HCl, reflux, 3. 48% HBr, CH_3CO_2H, reflux, 4. ion-exchange chromatography, HCl, 78%.

Scheme 2. Synthesis of **7**[a]



[a] (a) 1. H_2, 10% Pd/C, 2. HCl, 99%; (b) 1. $NaNO_2$, HCl, 0 °C, 2. CuCN, KCN, 50 °C, 40%; (c) 1. BH_3, THF, reflux, 2. 6 N HCl, reflux, 71%.

K_2CO_3 in dry acetone.[12] After workup and purification by flash chromatography, the anisole **10** was obtained in 92% yield. Hydrogenation of **10** afforded the methoxyaniline **11** in 99% yield. The aniline **11** was treated with Br_2 in acetic acid at room temperature and I_2 and K_2CO_3[13] in water to obtain a monohalogenated product. The iodination reaction gave better results since only one product (**12**) was obtained, whereas the bromination reaction gave both mono- and disubstituted products, which increased the complexity of the purification. The iodoaniline **12** was treated with $NaNO_2$ and diluted H_2SO_4 solution to generate the diazonium, which was immediately reduced[14] with Cu powder in ethanol to afford the iodoanisole **13**. The corresponding nitrile (**14**) was produced in 88% yield by heating **13** at 140 °C with Cu(I)CN in DMF in a Rosenmund−von Braun reaction.[15] The cyano analogue (**14**) was reduced with BH_3[16] to generate the intermediate (aminomethyl)-2,6-difluoroanisole, which was then treated with refluxing 48% HBr and acetic acid to afford the desired product **6**.

Compound **7** was synthesized from **9** by reduction with hydrogen in the presence of palladium on charcoal to give **15** quantitatively (Scheme 2). The aminophenol **15** is very sensitive to air unless it is converted to the HCl salt. 4-Cyano-2,6-difluorophenol (**16**) was obtained in a 40% yield by a Sandmeyer reaction,[17] in which the amine HCl salt (**15**) was treated with $NaNO_2$ and HCl at 0 °C to generate the diazonium salt in situ and then treated with Cu(I)CN and KCN at 50 °C. The final reduction of **16** to **7** was attempted using various reducing reagents, such as $LiAlH_4$,[18] $NaBH_4/CoCl_2$,[19] and BH_3/THF.[16] The best results were obtained with BH_3/THF. The final product **7** was purified by an AG 50W-X12 resin column eluting with HCl solution and then by recrystallization from ethanol/ethyl acetate.

Results and Discussion

Compounds **6** and **7** were tested as substrates for GABA aminotransferase from pig brain[20] by the radiochemical procedure of measuring the conversion of [^{14}C]α-ketoglutarate to [^{14}C]glutamate.[21] Both were poor substrates; only (0.07 ± 0.01)% conversion for **6** and (0.5 ± 0.04)% conversion for **7** resulted over a 22-h incubation, too slow to determine kinetic constants.

Both compounds, however, were competitive inhibitors of GABA aminotransferase, having K_i values of 6.3 ± 0.2 and 11 ± 1 mM for **6** and **7**, respectively. The K_m value for GABA under these conditions is 2.5 mM, so there is little difference in the binding of these inhibitors as compared with GABA.

These results suggest that the 2,6-difluorophenol moiety mimics the carboxylic acid functionality in the case of GABA binding to GABA aminotransferase and, therefore, acts as a lipophilic bioisostere. However, it would be very useful to determine if these analogues also mimic GABA in other biological systems, such as in their ability to bind to $GABA_A$ or $GABA_B$ receptors, to act as GABA mimetics in a functional model, for example, to induce ion current in oocytes, or to inhibit the GABA transporter. If they also show these activities, then this group should be a useful addition to the growing list of bioisosteres in the design of other enzyme inhibitor or receptor agonist/antagonists analogues.

Experimental Section

General Methods. Optical spectra and GABA-AT assays were recorded on a Perkin-Elmer Lambda 10 UV/Vis spectrophotometer. NMR spectra were recorded on a Varian Gemini 300 MHz, a Varian VXR 300 MHz, or a Varian Unity plus 400 NMR spectrometer. Chemical shifts are reported as δ values in parts per million downfield from Me_4Si as the internal standard in $CDCl_3$. IR spectra were taken with a Bio-Rad FTS60. An Orion Research model 701 pH meter with a general combination electrode was used for pH measurements. Mass spectra were obtained on a VG Instrument VG70-250SE high-resolution spectrometer with a Maspec data system. Elemental analyses were performed by the Department of Geological Sciences at Northwestern University. Melting points were

taken on a Fisher-Johns melting point apparatus and are uncorrected. Flash column chromatography was carried out with Merck silica gel 60 (230−400 mesh ASTM). TLC was run with EM Science silica gel 60 F254 precoated glass plates.

Reagents. All reagents were purchased from Aldrich Chemical Co. and used without further purification except anhydrous ether or tetrahydrofuran, which was distilled over sodium metal under nitrogen, and anhydrous dichloromethane, which was distilled over calcium hydride.

2,6-Difluoro-4-nitrophenol (9). Following a published procedure,[11] 2,6-difluorophenol (**8**) (5.0 g, 3.8 mmol) was treated with fuming nitric acid (2 mL). After removal of solvents under reduced pressure, the residue was purified by recrystallization from hexane to give the nitrophenol **9** (6.40 g, 96%) as pale-yellow needles: mp 103−105 °C (lit.[11] mp 105−105.5 °C); TLC (hexanes−ethyl acetate, 4:1) R_f 0.15; IR ($CHCl_3$) 3200b, 3002, 3104, 1612, 1516, 1347 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.92 (m, 2H), 6.0 (s, 1H, −OH); ^{19}F NMR ($CDCl_3$) −131.5; ^{13}C NMR ($CDCl_3$) 151 (dd, J = 246.5, 6.1 Hz), 140.4 (m), 109.8 (dd, J = 17.5, 9.1 Hz), 102.5 (C_1, m); EI-MS m/z 175, 159, 145, 129, 101, 81; HRMS calcd for $C_6H_3F_2NO_3$ M 175.0081, found M 175.0081.

2,6-Difluoro-4-nitroanisole (10). K_2CO_3 (3.5 g, 22.8 mmol) was added to **9** (2.0 g, 11.4 mmol) and CH_3I (4.6 g, 32.4 mmol) in dry acetone (15 mL). The mixture was stirred and heated at reflux for 6 h. After removal of acetone under reduced pressure, the resultant residue was washed with ether (50 mL). The organic solution was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel eluting with hexane/ether (4:1) to give the desired anisole **10** as a colorless crystalline solid (2.04 g, 94%): mp 33−35 °C (lit.[11] mp 33−35.5 °C); TLC (hexanes−ethyl acetate, 4:1) R_f 0.78; IR ($CHCl_3$) 3095, 3014, 2964, 1614, 1534, 1506 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.85 (m, 2H), 4.17 (s, 3H); ^1F NMR ($CDCl_3$) −124.9 (m); ^{13}C NMR ($CDCl_3$) 154 (dd, J = 246.5, 6.1 Hz), 143 (t, J = 12.9 Hz), 142.0 (m), 110 (dd, J = 18.9, 9.8 Hz), 62.8; EI-MS m/z 189, 159, 143, 128, 113, 100, 81; HRMS calcd for $C_7H_5F_2NO_3$ M 189.0237, found M 189.0238.

3,5-Difluoro-4-methoxyaniline (11). Compound **10** (2.96 g, 15.7 mmol) in ethyl acetate (15 mL) was hydrogenated over a catalytic amount of 10% Pd/charcoal until no more hydrogen was consumed. After removal of the catalyst by filtration, the solvent was evaporated under reduced pressure to give the pure aniline **11** (2.42 g, 99%) as a colorless solid: mp 72−74 °C; TLC (hexanes−ethyl acetate, 4:1) R_f 0.18; IR ($CHCl_3$) 3095, 3014, 2964, 1614, 1534, 1506 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.20 (m, 2H), 3.75 (s, 3H); ^{19}F NMR ($CDCl_3$) −129.2 (d, J = 9.3 Hz); ^{13}C NMR ($CDCl_3$) 154 (dd, J = 244, 7.5 Hz), 142 (m), 99 (dd, J = 17, 9.0 Hz), 62.4 (t, J = 2.47 Hz); EI-MS m/z 159, 144, 116; HRMS calcd for $C_7H_7F_2NO$ M 159.0496, found M 159.0496.

3,5-Difluoro-2-iodo-4-methoxyaniline (12). Compound **11** (0.8 g, 5.0 mmol) was stirred with iodine (1.6 g, 6.4 mmol) and $NaHCO_3$ (9.0 g, 9.0 mmol) in water (15 mL) at room temperature overnight. The resultant mixture was extracted with ethyl acetate (4 × 25 mL). The combined organic extracts were washed with 20% $Na_2S_2O_3$ (30 mL) and brine (25 mL) and were dried over anhydrous $NaSO_4$. After removal of solvents, the residue was purified by flash chromatography on a silica gel column eluting with hexanes−ethyl acetate (6:1) to afford the iodoaniline **12** (0.90 g, 64%) as a colorless solid: mp 50−51 °C; TLC (hexanes−ethyl acetate, 4:1) R_f 0.31; IR ($CHCl_3$) 3440, 3379, 3296, 2947, 1630, 1570 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.38 (dd, J = 10, 1.8 Hz, 1H), 3.86 (s, 3H); ^{19}F NMR ($CDCl_3$) −105.7 (d, J = 7.8 Hz), −129.2 (t, J = 10 Hz); ^{13}C NMR ($CDCl_3$) 158 (dd, J = 253, 6.8 Hz), 157 (dd, J = 233, 8.3 Hz), 144.2 (dd, J = 6.1, 12.9 Hz), 129.2 (m), 98.2 (dt, J = 20.5, 2.3 Hz), 63.6 (d, J = 19.7 Hz, OCH_3); EI-MS m/z 285, 270, 143, 115, 88; HRMS calcd for $C_7H_6F_2INO$ M 284.9464, found M 284.9463.

2,6-Difluoro-3-iodoanisole (13). $NaNO_2$ (0.3 g, 4.3 mmol) in water (5 mL) was added to a solution of **12** (0.82 g, 2.9 mmol) and concentrated H_2SO_4 (1 mL) in ice-cold water (15 mL). After the mixture was stirred for 30 min, Cu powder (0.2 g, prewashed with ether) and ethanol (15 mL) were added to the above reaction mixture. The resultant mixture was stirred and heated at 75 °C for 1 h and then at 60 °C for 2 h. After being cooled, the resultant aqueous solution was extracted with ether (3 × 20 mL). The combined organic extracts were washed with brine (25 mL) and dried over $MgSO_4$. After removal of the solvent, the residue was purified by flash chromatography on silica gel eluting with hexane to afford the iodoanisole **13** (0.64 g, 82%) as colorless needles: mp 28−29 °C; TLC (hexanes−ethyl acetate, 4:1) R_f 0.63; IR ($CHCl_3$) 3010, 2946, 1652, 1575, 1487, 1453 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.37 (m, 2H), 6.74 (dt, J = 6.0, 1.6 Hz, 1H), 4.0 (s, 3H); ^{19}F NMR ($CDCl_3$) −106.9 (m), −128.3 (m); ^{13}C NMR ($CDCl_3$) 158 (m) 157 (m), 138 (m), 132 (m), 115 (m), 111, 63 (m); EI-MS m/z 270, 229, 144, 131, 103, 77; HRMS calcd for $C_7H_5F_2IO$ M 269.9355, found M 269.9349.

3-Cyano-2,6-difluoroanisole (14). Compound **13** (0.37 g, 1.42 mmol) was stirred with CuCN (0.18 g, 2.03 mmol) in DMF (15 mL) and heated to 120 °C for 18 h. Then the mixture was heated at 140 °C for an additional 4 h. The resultant mixture was diluted with water (40 mL) and extracted with ether (4 × 25 mL). The combined organic phases were washed with brine and dried over $MgSO_4$. After removal of the solvent under reduced pressure, the residue was purified by flash chromatography on silica gel eluting with hexanes−ethyl acetate (4:1) to afford the cyanoanisole **14** (0.2 g, 83%) as a colorless solid: mp 41−42 °C; TLC (hexanes−ethyl acetate, 4:1) R_f 0.36; IR (KBr disk) 3073, 2996, 2960, 2241(sharp), 1618, 1580, 1476 cm^{-1}; ^1H NMR (CD_3OD) δ 7.29 (ddd, J = 9.1, 6.6, 5.4 Hz, 1H), 7.10 (ddd, J = 9.9, 9.0, 1.6 Hz, 1H), 4.0 (s, 3H); ^{19}F NMR (CD_3OD) −121.0 (m), −117.4 (m); ^{13}C NMR ($CDCl_3$) 159.8 (d, J = 256 Hz), 158.1 (d, J = 258 Hz), 138.6 (m), 127.6 (dd, J = 17.5, 9.1 Hz), 114.6 (m), 99.8 (d, J = 13.7 Hz), 63.1 (m); EI-MS m/z 169, 154, 126, 100, 88, 75; HRMS calcd for $C_7H_7F_2NO$ M 169.0339, found 169.0332.

3-(Aminomethyl)-2,6-difluorophenol Hydrochloride (6). BH_3·THF (1 M, 12 mL, 12 mmol) was added carefully to a solution of **14** (0.18 g, 1.06 mmol) in anhydrous THF (10 mL). The resultant solution was stirred and heated at reflux for 10 h. After the mixture had cooled, 6 N HCl was carefully added to the solution and heating at relux was continued for 30 min. The resultant solution was evaporated in vacuo to afford 3-(aminomethyl)-2,6-difluoroanisole hydrochloride: ^1H NMR (D_2O) δ 7.18 (m, 1H), 7.10 (m, 1H), 4.2 (s, 2H), 3.9 (s, 3H).

Without purification, the 3-(aminomethyl)-2,6-difluoroanisole hydrochloride was added to a solution of 48% HBr (4 mL) and HOAc (4 mL) and heated at 120 °C for 12 h. After removal of all solvents in vacuo, the resultant residue was purified by ion-exchange chromatography (AG 50W-X8) eluting with a gradient between 0.1 and 3 N HCl to give the desired HCl salt **6** as a colorless solid (0.18 g, 84%): mp >173 °C dec; IR (KBr disk) 3320b, 3075, 3019, 2927, 2811, 1627, 1612, 1548, 1494 cm^{-1}; ^1H NMR (CD_3OD) δ 6.95 (m, 2H), 4.13 (s, 2H); ^{19}F NMR ($CDCl_3$) −134.9, −129.5; ^{13}C NMR (D_2O) 153.5 (d, J = 206 Hz), 151.8 (d, J = 236 Hz), 133.8 (t, J = 16.6 Hz) 121.6 (dd, J = 9.1, 3.8 Hz), 117.4 (d, J = 9.9 Hz), 112.9 (dd, J = 18.9, 3.7 Hz), 37.8 (d, J = 3.8 Hz). Anal. ($C_7H_8ClF_2NO$) C, H, N.

4-Amino-2,6-difluorophenol (15). 2,6-Difluoro-4-nitrophenol (**9**) (0.52 g, 0.30 mmol) was dissolved in ethanol and hydrogenated over 10% Pd/charcoal catalyst (20 mg) for 2 h. After addition of concentrated HCl (0.5 mL) and removal of the catalyst by filtration, the residue was purified by recrystallization from ethanol/ether to give the aminophenol·HCl salt **15** as a gray solid (0.54 g, 99%): mp >230 °C dec; IR ($CHCl_3$) 3200b, 3180, 3045, 2887, 1565, 1537 cm^{-1}; ^1H NMR (DMSO) δ 7.55 (m, 2H); ^{19}F NMR ($CDCl_3$) −131.5; ^{13}C NMR (D_2O) 153.5 (dd, J = 244, 7.6 Hz), 134.5 (t, J = 100 Hz), 121.8 (t, J = 122 Hz), 108.7 (dd, J = 17.4, 9.1 Hz); EI-MS m/z 145, 124, 116, 97, 70, 36; HRMS calcd for $C_6H_5F_2NO$ M 145.0339, found M 145.0339.

4-Cyano-2,6-difluorophenol (16). Sodium nitrate (0.51 g, 7.4 mmol) in water (10 mL) was added dropwise to an ice-cold solution of **15** (1.2 g, 6.7 mmol) and concentrated HCl (3 mL) in water (8 mL) until the resultant solution was tested for excess nitrous acid with KI-starch paper. Then the diazotized

solution was carefully neutralized with sodium carbonate and was added to a suspended solution of KCN (2.1 g, 33.5 mmol) and CuCN (2.4 g, 26.8 mmol) in water (15 mL) at room temperature. The resultant solution was gradually heated to 50 °C. After being stirred for 2 h, the resultant solution was extracted with $CHCl_3$ (4 × 25 mL). The combined organic extracts were washed with brine, dried over sodium sulfate, and evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel eluting with hexane/ethyl acetate (4:1) to give the cyano product **16** (0.51 g, 40%) as a colorless solid: mp 89−92 °C; TLC (hexane−ethyl acetate, 4:1) R_f 0.10; IR (KBr disk) 3200b, 2245, 1624, 1603, 1527, 1448 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.28 (m, 2H); ^{19}F NMR ($CDCl_3$) −132; ^{13}C NMR (D_2O) 157 (d, J = 244 Hz), 140, 118 (m), 116 (m); EI-MS *m*/*z* 155, 154, 126, 100, 88, 75; HRMS calcd for $C_7H_3F_2NO$ M 155.0183, found M 155.0195.

4-(Aminomethyl)-2,6-difluorophenol Hydrochloride (7). BH_3·THF (1 M, 6.4 mL, 6.4 mmol) was added carefully to a solution of **16** (0.25 g, 1.61 mmol) in anhydrous THF (15 mL). The resultant solution was stirred and heated to reflux for 10 h. After the mixture had cooled, 6 N HCl was carefully added to the solution, and heating was continued at reflux for 30 min. The resultant solution was evaporated in vacuo. The residue was purified by ion-excahnge chromatography (AG 50W-X8), eluting with a gradient between 0.1 and 3 N HCl, to give the desired (aminomethyl)phenol **7** (0.22 g, 71%) as a colorless solid: mp >176 °C dec; IR (KBr disk) 3300b, 3100, 2733, 1610, 1574, 1534 cm^{-1}; ^1H NMR (CD_3OD) δ 7.07 (m, 2H) 4.02 (s, 2H); ^{19}F NMR ($CDCl_3$) −132 (m); ^{13}C NMR (D_2O) 153 (d, J = 243 Hz), 134.2 (m), 124.7 (m), 113.7 (m), 43.2. Anal. ($C_7H_8ClF_2NO$) C, H, N.

Enzymes and Assays. Pig brain GABA aminotransferase (specific activity 3.9 units/mg), GABAse, and succinic semialdehyde dehydrogenase were obtained and assayed as previously described.[21]

Determination of Substrate Activity for 6 and 7. Substrate activity was determined in duplicate by a radiochemical assay, measuring the conversion of [U-^{14}C]α-ketoglutarate to [U-^{14}C]L-glutamate, as previously described.[21] Compound **6** (7.3 mM) or **7** (6.9 mM) was incubated in potassium phosphate buffer (100 mM, pH 7.4) at 25 °C for 22 h with GABA aminotransferase (0.02 unit) in the presence of [U-^{14}C]α-ketoglutarate (4.7 mM, 120 μCi/mmol) and β-mercaptoethanol (5 mM). A control experiment also was carried out with the entire incubation mixture except substrate.

Inhibition of GABA Aminotransferase by 6 and 7. The competitive inhibition of GABA aminotransferase by **6** and **7** was carried out and analyzed as previously described.[22] Pig brain GABA aminotransferase (0.004 unit) was incubated at 25 °C with α-ketoglutarate (5 mM), $NADP^+$ (1 mM), β-mercaptoethanol (5.0 mM), excess succinic semialdehyde dehydrogenase, and varying amounts of **6**, **7**, and GABA in potassium phosphate buffer (100 mM) at pH 7.4. Initial rates were measured spectrophotometrically, and kinetic parameters were derived from Dixon[23] and Cornish−Bowden[24] plots with four different concentrations of **6** and **7** (0.0, 3.5, 5.8, and 8.2 mM) for every concentration of GABA (0.68, 1.7, and 3.5 mM). Both analogues are competitive inhibitors of GABA aminotransferase.

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