

Synthesis of Fluorine-18 Labeled GABA Uptake Inhibitors

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The first syntheses of fluorine-18 labeled inhibitors of GABA reuptake [(R,S)-1-[2-(4-[¹⁸F]fluorophenyl)phenyl]methoxyethyl]piperidine-3-carboxylic acid, (R,S)-1-[2-(4-[¹⁸F]fluorophenyl)(4-fluorophenyl)methoxyethyl]piperidine-3-carboxylic acid, and (R,S)-1-[2-[(4-[¹⁸F]trifluoromethyl)phenyl]{(4-trifluoromethyl)phenyl}-methoxyethyl]piperidine-3-carboxylic acid] are described. These *N*-substituted nipecotic acid derivatives were prepared in no-carrier-added form by the condensation of the appropriately substituted [¹⁸F]benzhydryl chlorides (prepared in three steps from [¹⁸F]fluoride ion) with *N*-(2-hydroxyethyl)nipecotic acid ethyl ester, followed by ester hydrolysis. Overall radiochemical yields were 17-28% (corrected, 150 min synthesis time). A simple new method for synthesis of a [¹⁸F]trifluoromethyl group by the nucleophilic substitution of a bromodifluoromethyl substituent has also been developed.

Introduction

Gamma aminobutyric acid (GABA) is an inhibitory amino acid neurotransmitter involved in the control of neuronal activity in the brain (Enna, 1980; Krosggaard-Larsen *et al.*, 1987). Dysfunctions of the GABA system have been implicated in a wide variety of neurological disorders, and are of particular interest in studies of epilepsy and other seizure disorders. *In vivo* approaches to the study of the GABA system in man using positron emission tomography (PET) have centered on the preparation and application of radiolabeled ligands known to bind to the GABA/benzodiazepine receptor complex (Maziere *et al.*, 1980, 1984; Frost *et al.*, 1986).

An alternative approach to the study of the role of degeneration of the GABA neurotransmitter pathway in disease would be development of *in vivo* markers of the presynaptic GABA-ergic neurons. Reasonable approaches are the development of radiolabeled synthetic precursors to GABA formation; radiolabeled inhibitors of the enzymes involved in GABA production (e.g. glutamic acid decarboxylase, GAD); or radiolabeled inhibitors of the presynaptic GABA reuptake system. This last option is particularly appealing due to the interest in developing drugs

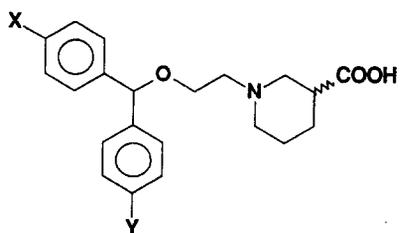
that block this process for use in treatment of epilepsy (Krosggaard-Larsen *et al.*, 1987). The mechanisms of GABA uptake are not well understood, and there is relatively little data regarding *in vivo* pharmacokinetics and specificity of GABA uptake inhibitors. We describe here the first synthesis of novel ¹⁸F labeled GABA uptake inhibitors (Fig. 1: Pavia, 1988; Taylor and Vartanian, 1989) as potential radioligands for studying the GABA system by *in vivo* methods (*ex vivo* tissue counting, *in vivo* autoradiography, and PET).

Experimental

Materials

The following were obtained from Aldrich Chemical Co. and used without further purification: terephthaldehyde, diethylaminosulfur trifluoride (DAST), 4-bromobenzotrifluoride, Kryptofix 2.2.2, lithium aluminum hydride (1 M in diethyl ether). The quaternary ammonium resin was prepared as previously described (Mulholland *et al.* 1989b). High specific activity (50,000 Ci/mmol) [¹⁸F]fluoride ion was produced by proton irradiation of [¹⁸O]water held in an all silver cyclotron target as previously described (Mulholland *et al.*, 1989a). Preparations of 4-[¹⁸F]fluorobenzhydryl chloride (**11**) (Haka *et al.*, 1989) and 4-[¹⁸F]fluoro-4'-fluorobenzhydryl chloride (**12**) (Haka and Kilbourn, 1990) were according to literature methods.

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1 X = H, Y = ^{18}F

2 X = F, Y = ^{18}F

3 X = CF_3 , Y = CF_2^{18}F

Fig. 1. Fluorine-18 labeled inhibitors of GABA reuptake.

Methods

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded with a Varian EM-390 spectrometer using TMS as internal reference. Infrared spectra were recorded with a Nicolet XS-20 FT-IR spectrometer using KBr plates. Thin layer chromatography was done using plastic-backed silica gel plates (Merck) and the following systems: System A, silica gel, 5/2 pentane/diethyl ether; System B, silica gel, 7/3 hexane/ethylacetate; System C, 7/2.5/0.5 toluene/isopropanol/acetic acid. HPLC was done using a Phenomenex C18 10 μ 0.45 \times 10 cm column fitted with u.v. (263 nm) and radioactivity detectors, and a mobile phase of 60/40/3 acetonitrile/0.065 M aqueous ammonium acetate/tetrahydrofuran (flow rate 0.5 mL/min).

All radiochemical yields are corrected for decay of ^{18}F ($t_{1/2} = 110$ min).

4-(Difluoromethyl)benzaldehyde (5). Terephthaldehyde (**4**; 38 g, 0.28 mol) was dissolved in dichloromethane (350 mL), a nitrogen atmosphere introduced, and the mixture cooled to -10°C . A solution of DAST (38 g, 0.24 mol) in dichloromethane (50 mL) was added dropwise over 1 h. The reaction was stirred for 4 days at room temperature, quenched very carefully by slow (30 min) addition of water (25 mL), and neutralized with saturated aqueous NaHCO_3 . The aqueous layer was repeatedly extracted with CH_2Cl_2 , and the extracts dried (MgSO_4), filtered through silica and evaporated *in vacuo* at temperature $<10^\circ\text{C}$. The remaining oil (36 g) was distilled (113–115 $^\circ\text{C}$, 20 torr) to give 19.9 g (54% yield) of the desired aldehyde **5**, which was used in the next step without further purification. $^1\text{H-NMR}$ (CDCl_3) δ 9.8 (bs, 1H, CHO), 7.65–7.35 (dd, 4H, ArH), 6.68 (t, 1H, $J = 55.9$ Hz, CHF_2).

(R,S)- α -[4-(Difluoromethyl)phenyl]-4-(trifluoromethyl)benzenemethanol 6. 4-Bromobenzotrifluoride (2.25 g, 10 mmol) was added dropwise to a mixture of magnesium metal (0.24 g, 10 mmol) in diethyl ether (30 mL). The reaction was initiated (manual crushing

of Mg, addition of iodine crystal) and the 4-bromobenzotrifluoride added at a rate sufficient to maintain reflux. When the addition was complete, the reaction was refluxed for an additional 45 min and then cooled to 0°C . Approximately one-fifth of this Grignard reagent was then added to a solution of **5** (0.31 g, 2 mmol) dissolved in cold ether (2 mL, 0°C). The reaction was monitored by TLC (silica, 10% EtOAc/hexane) which indicated incomplete consumption of aldehyde **5**. A small additional amount of Grignard reagent was added and the reaction then judged complete by TLC. The mixture was diluted with ether and washed with saturated NH_4Cl solution. The organic layer was dried (MgSO_4), filtered, and evaporated *in vacuo*. Purification by flash column chromatography (silica, 15% EtOAc/hexane) and crystallization from hexane afforded 0.48 g (80% yield) of the product **6**; m.p. 81–82 $^\circ\text{C}$; $^1\text{H-NMR}$ (CDCl_3) δ 7.65–7.45 (m, 8H, ArH), 6.65 (t, 1H, $J = 56.6$ Hz, CHF_2), 5.94 (s, 1H, CHO), 2.10 (bs, 1H, OH); MS 302 (M^+), 301 (M^+-1); i.r. (cm^{-1}) 3350, 1619, 1425, 1376; Anal. Calcd for $\text{C}_{15}\text{H}_{11}\text{F}_5\text{O}$: C, 59.61; H, 3.69. Found; C, 59.82; H, 3.64.

[4-(Difluoromethyl)phenyl]-[4-(trifluoromethyl)phenyl]methanone 7. The benzhydrol **6** (0.6 g, 2 mmol) was dissolved in acetone (30 mL) and cooled to 0°C . Jones reagent ($\text{CrO}_3/\text{H}_2\text{SO}_4/\text{H}_2\text{O}$) was added dropwise until an orange color persisted. The reaction was stirred an additional 5 min then quenched by addition of methanol. The product was poured into ether, the solid removed, and the organic layer evaporated *in vacuo*. The residue was again taken up in ether, decanted from solids, and evaporated. The white solid obtained was recrystallized from hexane to afford 0.44 g (74%) of the ketone **7**; m.p. 131–131.5 $^\circ\text{C}$; $^1\text{H-NMR}$ (CDCl_3) δ 7.92–7.65 (m, 8H, ArH), 6.74 (t, 1H, $J = 56$ Hz, CHF_2); MS 300 (M^+), 281 (M^+-19); i.r. (cm^{-1}) 1653, 1615, 1578, 1511, 1409, 1331; Anal. Calcd for $\text{C}_{15}\text{H}_9\text{F}_5\text{O}$: C, 60.01; H, 3.02. Found: C, 60.01; H, 2.98.

[4-(Bromodifluoromethyl)phenyl]-[4-(trifluoromethyl)phenyl]methanone 8. The benzophenone **7** (3.0 g, 10 mmol) was suspended in CCl_4 (40 mL) and to this added anhydrous K_2CO_3 (6.9 g, 50 mmol) and then Br_2 (1.76 g, 11 mmol). The reaction mixture was placed under an atmosphere of N_2 and illuminated with a 275W sunlamp which raised the temperature to 45°C . After 24 h additional Br_2 (1.76 g, 10 mmol) was added and the reaction continued for a total of 60 h, at which time TLC (silica, 5% EtOAc/hexane) showed complete conversion. The reaction mixture was cooled, diluted with CH_2Cl_2 , and then washed with saturated aqueous sodium sulfite and sodium chloride solutions. The organic layer was dried (MgSO_4) and evaporated to give a solid which was dissolved in 1:1 ethyl acetate/hexane and passed through a short column of silica. The solvent was evaporated and the solid remaining recrystallized from hexane to yield 3.4 g (95%) of the ketone **8**; m.p. 78–79 $^\circ\text{C}$; $^1\text{H-NMR}$ (CDCl_3) δ 7.94–7.74 (m, 8H, ArH);

MS 299 ($\text{M}^+ - 80$), 207 ($\text{M}^+ - 172$); i.r. (cm^{-1}) 1667, 1612, 1581, 1411, 1324. Anal. Calcd for $\text{C}_{15}\text{H}_8\text{BrF}_5\text{O}$: C, 47.52; H, 2.13. Found: C, 47.52; H, 2.08.

^{18}F bis(4-Trifluoromethyl)benzophenone **9**. No-carrier-added tetrabutylammonium ^{18}F fluoride was prepared by combining tetrabutylammonium hydroxide (2 μmol) and aqueous ^{18}F fluoride and evaporating the water (100°C, N_2). Traces of water were removed by azeotropic distillation with acetonitrile, and the residue dissolved in dimethylsulfoxide (200 μL). Bromobenzophenone **8** (2 mg) was added, and the solution heated at 150°C for 20 min. The mixture was cooled, diluted with water, and passed through a C-18 Sep-Pak. The solid phase was washed with water (5 mL) then the product eluted with diethylether (2 mL). Yields ranged from 20–75% with an average of 50%. The product **9** was used in the next step without further purification. TLC System A) $R_f = 0.57$.

(*R,S*)- ^{18}F bis-(4-Trifluoromethyl)benzhydrol **10**. To a solution of ketone **9** (100 μCi –5 mCi) in diethyl ether was added LAH (200 μL of 1M in THF) and the solution allowed to stand at 25°C for 5 min. Excess LAH was carefully quenched with water, 2 N H_2SO_4 added, and the mixture passed through a C-18 Sep-Pak. The product was eluted with diethyl ether (2 mL) and then ether dried (Na_2SO_4) to yield a solution of the desired alcohol **10** (yields 85–95%). This was not further purified but used in the next step in the sequence. TLC (System A) $R_f = 0.2$.

(*R,S*)- ^{18}F bis(4-Trifluoromethyl)benzhydrol chloride (**13**). Chlorination of the alcohol **10** was done in dichloromethane solution (1–1.5 mL) using 100 μL of phosphorus trichloride at 100°C (closed vessel). TLC showed complete chlorination after 35 min. The solvent and excess PCl_3 were removed by a slow stream of nitrogen, and the residue used in the next step without purification. TLC (System A) $R_f = 0.55$.

(*R,S*)-Ethyl 1-[2-(4- ^{18}F trifluoromethyl)phenyl]{(4-trifluoromethyl)phenyl}methoxyethyl]-piperidine-3-carboxylate **16**. To the residue of chloride **13** was added ethyl *N*-(2-hydroxyethyl)-piperidine-3-carboxylate (10 mg), and the mixture heated at 155°C for 25 min. The resultant brown oil was dissolved in methanol (100 μL), diluted with water (2 mL) and extracted with diethyl ether. The ether layers were combined and dried (Na_2SO_4) and the ether evaporated (N_2 flow). The residue was dissolved in dichloromethane and passed through a short column of silica gel to yield the desired crude product **16**. Yield 33%; TLC (System B) $R_f = 0.43$.

(*R,S*)-Ethyl 1-[2-(4- ^{18}F fluorophenyl)phenyl]methoxyethyl]-piperidine-3-carboxylate (**14**). This condensation was performed as per synthesis of **16**, using the chloride **11**; yield 49%; TLC (System B) $R_f = 0.375$.

(*R,S*)-Ethyl 1-[2-(4- ^{18}F fluorophenyl)(4-fluorophenyl)methoxyethyl]piperidine-3-carboxylate (**15**). This ester was prepared utilizing chloride **12** as per the synthesis of **14**; yield 31%. TLC (System B) $R_f = 0.28$; (System C) $R_f = 0.68$.

(*R,S*)-1-[2-(4- ^{18}F trifluoromethyl)phenyl]{(4-trifluoromethyl)phenyl}methoxyethyl]-piperidine-3-carboxylic acid (**3**). The crude ethyl ester **16** was dissolved in 3 drops 6 N H_2SO_4 and heated (100°C) for 30 min. The acid solution was diluted with 6 mL of 20 mM K_2HPO_3 buffer (pH 6.5) and extracted with two 2 mL portions of 4/1 chloroform/isopropanol. The organic layers were combined, dried, and evaporated *in vacuo*. Yield 83%, radiochemical purity 95%; TLC (System C) $R_f = 0.42$; HPLC $R_t = 8.88$ min.

(*R,S*)-1-[2-(4- ^{18}F fluorophenyl)phenyl]methoxyethyl]-piperidine-3-carboxylic acid (**1**). Hydrolysis of the ethyl ester **14** was done as per the synthesis of **3**. Yield 90%, radiochemical purity 97%; TLC (System B) $R_f = 0.05$, (System C) $R_f = 0.35$; HPLC $R_t = 6.83$ min.

(*R,S*)-1-[2-(4- ^{18}F fluorophenyl)(4-fluorophenyl)methoxyethyl]piperidine-3-carboxylic acid (**2**). Hydrolysis of the ester **15** was done as per synthesis of **3**. Yield 85%, radiochemical purity 96%; TLC (System B) $R_f = 0.05$; (System C) $R_f = 0.40$; HPLC $R_t = 7.12$ min.

Results and Discussion

Preparation of radiolabeled inhibitors of the high affinity GABA uptake system would form a new and potentially powerful method for examining the distribution and function of this neurotransmitter system *in vivo*. GABA uptake sites show a heterogeneous distribution in primate brain, with a more than 4-fold difference between areas of low concentration (pons, deep cerebellar nuclei) and those of high concentration (amygdala, hypothalamus, globus pallidus) (Enna, 1981). Similarly, regional variations in [^3H]nipecotic acid binding to GABA uptake sites in human brain tissue samples has been reported (Simpson *et al.*, 1988).

Guvacine (1,2,5,6-tetrahydronicotinic acid) and nipecotic acid (3-piperidine-carboxylic acid) are potent *in vitro* inhibitors of GABA uptake into both neurons and glial cells, with no affinity for GABA receptors (Krogsgaard-Larsen *et al.*, 1987). These amino acids, however, do not readily pass the blood-brain barrier (BBB) and thus are not good candidates for radiolabeling with ^{11}C or ^{18}F (^{11}C]GABA has been recently prepared (Antoni and Langstrom, 1989) but suffers the same *in vivo* limitation). Preparation of esters of such acids provides prodrugs which do pass through the BBB and are hydrolyzed in the brain to the active parent drugs (Frey *et al.*, 1979; Falch *et al.*, 1978). Application of prodrugs to PET imaging, although interesting, may be too complicated for successful analysis of *in vivo* pharmacokinetics. The placement of small alkyl groups on the nitrogen in guvacine or nipecotic acid has resulted in compounds which are less active than the parent amino acids, but surprisingly the attachment of large lipophilic groups, such as diarylbutenyl (Ali *et al.*, 1985; Braestrup *et al.*, 1990; Sonnwald,

1987) or diarylmethoxyethyl groups, (Pavia, 1988; Falch and Krogsgaard-Larsen, 1989), provides compounds which are actually more active as GABA inhibitors (IC_{50} values of 67–30 nM) than the parent amino acids, and do show potent *in vivo* activity after systemic administration. One such compound, NO 328 ((R)-N-[4,4-bis(3-methyl-2-thienyl)but-3-en-1-yl]nipecotic acid), has been recently prepared in tritiated form and its *in vitro* and *in vivo* behavior examined in some detail (Braestrup *et al.*, 1990). Their results ($K_D = 18$ nM, and regional variation in binding to brain synaptosomes) are encouraging for further development of radioligands for GABA uptake sites. As we have recently developed general routes to ^{18}F labeled diarylmethanols (4- ^{18}F fluorobenzhydryl, Haka *et al.*, 1989; 4- ^{18}F fluorophenyl) (2-thienyl)methanol, Kilbourn, 1989; 4- ^{18}F fluoro-4'-fluorobenzhydryl, Haka and Kilbourn, 1990), the application of such precursors to synthesis of ^{18}F labeled derivatives of these cyclic amino acids appeared promising. A large number of substituted diphenylmethoxyethyl derivatives of nipecotic acid

and guvacine have been recently reported (Pavia, 1988) and we chose to prepare fluoroaryl (1,2) and trifluoromethyl (3) substituted diarylmethoxyethyl derivatives to test the feasibility of our synthetic approach. The reported IC_{50} for GABA uptake for compound 3 (as the R(-) stereoisomer) is 200 nM (Taylor and Vartanian, 1989); data for the other two compounds have not been reported. The K_D values, which for NO 328 (18 nM) is significantly lower than the IC_{50} for GABA uptake (67 nM), have also not yet been reported.

Synthesis of the target molecules was broken down into two parts, the preparation of the appropriately labeled benzhydryl chlorides (11,12,13), followed by condensation with the ethyl ester of N-(2-hydroxyethyl)nipecotic acid. High specific activity 4- ^{18}F fluorobenzhydryl chloride (11) and 4- ^{18}F fluoro-4'-fluorobenzhydryl chloride (12) were prepared by minor modifications of previously described methods. The ^{18}F labeled bis(trifluoromethyl)benzhydryl chloride (13) was prepared by the sequence shown in Fig. 2. Terephthalaldehyde was

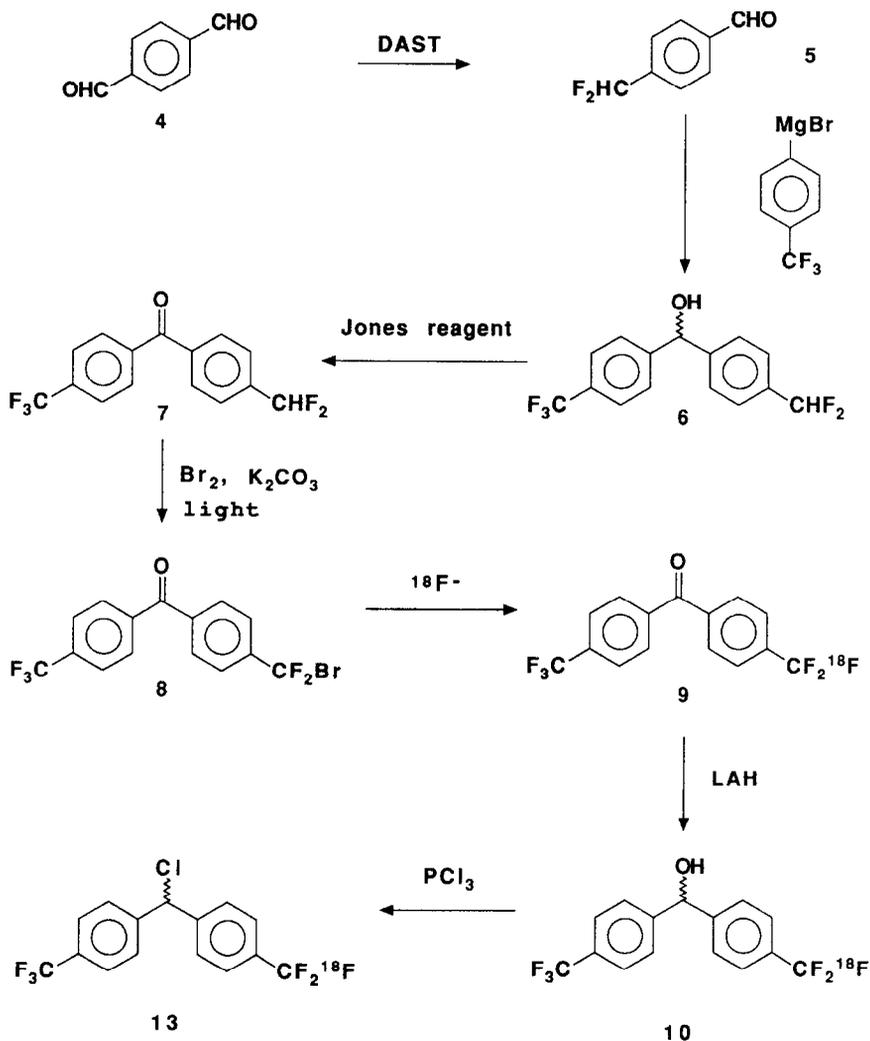


Fig. 2. Synthesis of ^{18}F bis(4-trifluoromethyl)benzhydryl chloride.

fluorinated (DAST; 54% yield) and the intermediate 4-difluoromethylbenzaldehyde (**5**) reacted with 4-trifluoromethylphenylmagnesium bromide to give the benzhydrol **6** in 80% yield. The alcohol was oxidized to the ketone (**7**, 74% yield) using Jones reagent under standard conditions. Bromination of the difluoromethyl group was performed using bromine in CCl_4 under photolytic conditions (90% yield), with solid potassium carbonate added to consume HBr formed in the reaction. This bromodifluoromethyl group was then converted to a ^{18}F labeled trifluoromethyl group by a simple reaction with no-carrier-added [^{18}F]fluoride ion (as salt with tetrabutylammonium, K^+ /Kryptofix, or quaternary ammonium resin (Mulholland *et al.*, 1989b) counterion) in 50% yield (average; 25 min reaction time). The ease of this synthesis of a [^{18}F]trifluoromethyl group was surprising since previous attempts at ^{18}F -for- ^{19}F (Ido *et al.*, 1987) or ^{18}F -for-Cl (Angelini *et al.*, 1986) substitutions required high temperature and/or Lewis acid catalysts, and products were obtained in only moderate yields. The ketone **9** was then reduced (**10**: LAH; 90% yield) and chlorinated (PCl_3 in CH_2Cl_2 , or neat SOCl_2 ; 95–100% yield) to give the required benzylic chloride **13**.

The benzylic chlorides **11,12,13** were reacted with (*N*-2-hydroxyethyl)nipecotic acid ethyl ester (Fig. 3) to yield the intermediate ethers **14,15,16** in 33–49% yields. The products were separated from unreacted benzylic chlorides by extraction of the piperidine derivatives into dilute aqueous acid, and from excess *N*-hydroxynipecotic acid ester by a short column of silica gel. Finally, the ester groups were hydrolyzed under acidic conditions to yield the free amino acids **1,2,3**. Products were obtained in high radiochemical purities (>95% by TLC and/or HPLC) but all contained trace amounts of chemical impurities. Small amounts of chemically pure products were obtained after injection of portions of the crude products on the analytical HPLC column. All of the final product acids **1,2** and **3** have been obtained as racemic mixtures (mixtures of isomers at the carboxylic acid position) and compound **1** as also a mixture of isomers at the benzylic position.

In this work we have demonstrated the feasibility of preparing 0.1–1.0 mCi amounts of ^{18}F labeled derivatives of compounds known to be systemically active as GABA uptake inhibitors. Overall radiochemical yields (5 steps from resolubilized [^{18}F]fluoride ion) ranged from 17–28% (corrected, 150 min synthesis

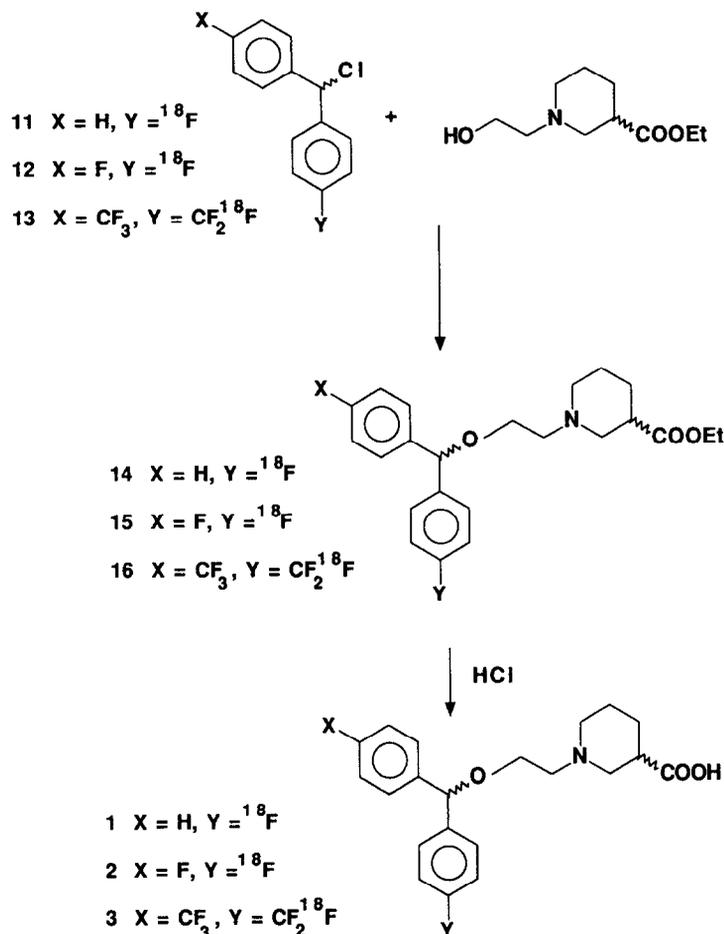


Fig. 3. Synthesis of ^{18}F labeled GABA uptake inhibitors.

time). The mono- and bisfluoro derivatives **1** and **2** have been obtained in high specific activity (> 2000 Ci/mmol). The bis(trifluoromethyl) substituted derivative, **3**, has also been obtained in no-carrier-added form, but it requires a separation from small amounts of the compound bearing the original bromodifluoromethyl group; without this separation, the effective specific activity should be more properly described as lower (about 1 Ci/mmol) as the chemical contaminant is likely to be biologically active. These specific activities have been sufficient to begin *in vivo* testing of the compounds in small animals. The effect of stereochemistry at the benzylic position in **1** is unknown, but the R(−) isomer of nipecotic acid and its derivatives are more potent inhibitors of GABA uptake (Pavia, 1988; Taylor and Vartanian, 1989). Syntheses of the R(−) isomers (carboxylic acid position) should provide better radiotracers, and are currently underway in our laboratories, as is the preparation of the corresponding guvacine derivatives (e.g. 1-{2-[bis(trifluoromethyl)phenyl]methoxy}ethyl)-1,2,5,6-tetrahydro-3-pyridine carboxylic acid, CI-966 (Pavia, 1988; Brahce *et al.*, 1989) and thienyl-substituted derivatives (Kilbourn, 1989). Preliminary *in vivo* studies of regional mouse brain distribution of **1** have been encouraging, with a cortex/striatum ratio (% injected dose per gram) of 1.44 + 0.36 at 1 h after injection; this compares favorably with the ratio of 1.53 for specific binding of [³H]NO 328 to rat brain synaptosomes determined *in vitro* (Braestrup *et al.*, 1990). The availability of radiolabeled ligands will allow examination of *in vivo* characteristics of this class of compounds (brain extraction; regional distribution and specificity of radiotracer binding in brain; kinetics of radiotracer accumulation and washout from brain; pharmacological specificity of binding; neuronal vs glial specificity).

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