2-Amino-2'-[^{18}F]Fluorobenzhydrols, Intermediates for the Synthesis of [2'-^{18}F]-1,4-Benzodiazepine-2-ones

Peter Johnström[a,b] and Sharon Stone-Elander*[a,c]

[a]Karolinska Pharmacy, Box 160, S-171 76 Stockholm, Sweden
[b]Dept. of Psychiatry and Psychology, Karolinska Hospital and Institute, Stockholm, Sweden
[c]Dept. of Clinical Neurophysiology, Karolinska Hospital and Institute, Stockholm, Sweden

SUMMARY

A method for synthesizing ^{18}F-labelled 2-amino-2'-fluorobenzhydrols under no-carrier-added conditions for use as radiolabelled intermediates in the synthesis of [2'-^{18}F]-1,4-benzodiazepine-2-ones is presented. Anilinodichloroborane reagents were formed by the reaction of boron trichloride with 4-chloro-*N*-methylaniline, 6a, 4-nitro-*N*-methylaniline, 6b, 4-nitro-*N*-ethylaniline, 6c, and 4-chloro-*N*-(2,2,2-trifluoroethyl)aniline, 6d. 2-[^{18}F]Fluorobenzaldehyde, 5, synthesized in 55-70% yields by the nucleophilic aromatic substitution of 2-nitrobenzaldehyde with the Kryptofix/K^+ complex of [^{18}F]F^-, was subsequently reacted with the anilinodichloroborane coupling reagents with aromatic substitution occurring ortho to the amino group. The resulting 2-amino-2'-[^{18}F]fluorobenzhydrols, 7a - 7d, were produced in conversions of 60-95% with reaction time ≤ 10 min at room temperature or 60°C, depending on the aniline used. The total synthesis time, including evaporation of the target water, was 60-65 min. The total radiochemical conversions were of the order of 50-65% for 7a - 7c and 35-45% for 7d, decay-corrected and based on [^{18}F]F^-.

Key words: fluorine-18, boron, 2-amino-2'-fluorobenzhydrol, 1,4-benzodiazepine-2-ones
*Author for correspondence

INTRODUCTION

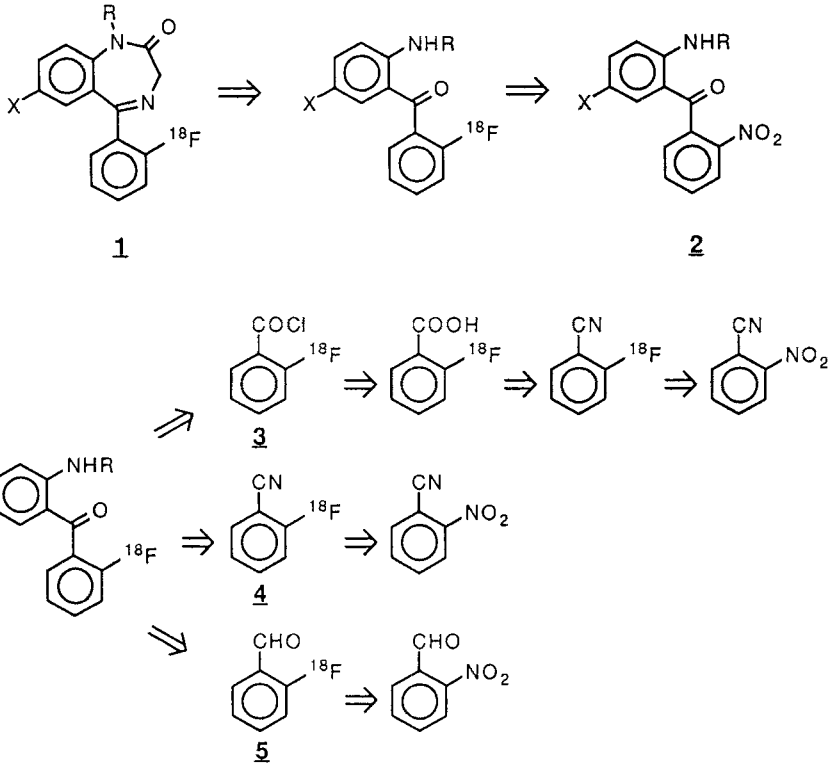
The development of methods for labelling radiopharmaceuticals in different positions in the molecule is important in the design of radiotracers for positron emission tomography (PET). Depending on the metabolic fate of the tracer *in vivo* and the position of the label, quantification of the distribution of the radiopharmaceutical can be complicated by the appearance and redistribution of labelled metabolites.

A number of ligands for the benzodiazepine receptor have previously been labelled with carbon-11 and fluorine-18, as has been reviewed (1). The label is often introduced in a side-chain alkyl group which is a metabolically labile position (2). Depending on the time scale for this

0362–4803/94/020135–11$10.50
©1994 by John Wiley & Sons, Ltd.
Received 17 July, 1993
Revised 18 October, 1993

metabolism, such radiolabels may be lost during the PET evaluation (see review in 1). We have previously reported a method for labelling 1,4-benzodiazepine-2-ones in the 5-phenyl ring in one step using electrophilic fluorine-18 and a trialkylstannyl precursor (3). However, the specific activity of the radioligand obtained with the electrophilic fluorinating reagents available at that time was considered too low for *in vivo* studies. Progress has recently been made toward increasing the specific activity of these reagents (4), which may make this labelling approach more useful in the future for the synthesis of ^{18}F-labelled receptor ligands for PET. Here we present the design of a method for radiolabelling in the same position using nucleophilic fluoride ([^{18}F]F$^-$) (previously reported in a preliminary communication (5)).

Nucleophilic aromatic substitutions with high specific activity [^{18}F]F$^-$ have been performed on a variety of substrates (see, for example, references in 6). The substrates require good leaving groups such as, -NO_2 or -$N(CH_3)_3^+$ and activation of the aromatic ring with an appropriate electron-withdrawing group. A retrosynthetic analysis to find a method for synthesizing ^{18}F-labelled 1,4-benzodiazepine-2-ones, 1, from [^{18}F]F$^-$ gave several possible substrates for the nucleophilic procedure. The first possibility considered was a one-step labelling reaction using the corresponding 2'-nitro-1,4-benzodiazepine-2-one. This approach was, however, not considered feasible due to the tendency of 1,4-benzodiazepine-2-ones to undergo rearrangements (3,7) under basic conditions (which are typical for nucleophilic fluorinations). Rearrangement complications could be avoided if the 7-membered ring could be formed after radiolabelling. Disconnection of the 1,4-benzodiazepine-2-one thus gave the 2-amino-2'-nitrobenzophenone, 2, as a possible substrate.



Additional approaches for the introduction of [^{18}F]F$^-$ required further disconnection of the fluorobenzophenone. At least three potential fluoroaromatic precursors can be proposed that should be fairly readily labelled with [^{18}F]F$^-$ by literature methods: 2-fluorobenzoylchloride, 3, 2-fluorobenzonitrile, 4, and 2-fluorobenzaldehyde, 5.

Use of 3, 4 or 5 requires a method for synthesizing the 2-amino-2'-fluorobenzophenone in times acceptable for radiolabelling with fluorine-18. A method has been reported (8) by Sugasawa and co-workers using anilinodichloroborane reagents to specifically ortho couple with benzonitriles and/or benzaldehydes. 2-Aminobenzophenones and 2-aminobenzhydrols were obtained in good yields under mild reaction conditions. Such an approach utilizing 4 or 5 might therefore be feasible. Benzoyl halides such as 3 yield benzophenones by Friedel-Crafts acylation reactions. However, these reactions require drastic heating conditions and can potentially yield a mixture of products depending on the relative degree of activation by the aromatic ring substituents (-X and -NHR).

Based on this retrosynthetic analysis the reaction of [^{18}F]F$^-$ with a 2-amino-2'-nitrobenzophenone was first tested. Subsequently, the coupling of 2-fluorobenzonitrile, 2-fluorobenzaldehyde and 2-[^{18}F]fluorobenzaldehyde with aromatic amines by the method of Sugasawa (8) was investigated as a general means of producing ^{18}F-labelled 2-amino-2'-fluorobenzhydrols and 2-amino-2'-fluorobenzophenones.

RESULTS AND DISCUSSION

Reaction of [^{18}F]F$^-$ with a 2-amino-2'-nitrobenzophenone, 2

The first approach suggested by the retrosynthetic analysis, radiolabelling of a nitrobenzophenone with [^{18}F]F$^-$, was tested with one substrate (X=Cl, R=CH_2CF_3) using several solubilizing reagents (Kryptofix 2.2.2/K_2CO_3, Bu_4NOH, Cs_2CO_3 and Rb_2CO_3) and solvents (DMSO and sulfolane). Both the amounts of substrate and reagents as well as the reaction temperatures were varied. No 2-amino-2'-[^{18}F]fluorobenzophenone was obtained in any of the experiments. One unidentified labelled product was obtained in small yields (10-15%) but did not correspond chromatographically with the reference 2-amino-2'-fluorobenzophenone as analyzed by radio-HPLC and -TLC. This approach was therefore abandoned.

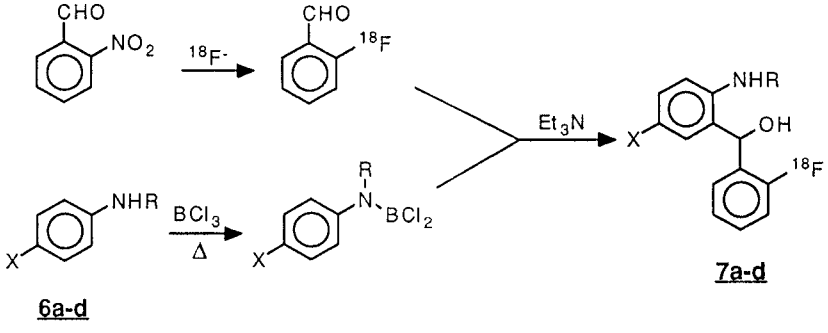
Ortho-coupling reactions

The anilinodichloroborane reagents used by Sugasawa provide a specific ortho coupling method for generating 2-aminobenzophenones in one step from benzonitriles or in two steps from benzaldehydes. According to the literature results (8), 2-aminobenzophenones were produced from benzonitriles in yields of 30-85% after heating for 3-17 h. Using benzaldehydes, 2-aminobenzhydrols were obtained in yields of 65-85% in reaction times less than one hour at room temperature. The benzhydrols were subsequently easily oxidized to the corresponding 2-aminobenzophenones (9,10).

Our initial screening with 2-fluorobenzonitrile and 2-fluorobenzaldehyde gave results that were consistent with those of Sugasawa. For example, coupling between the anilinodichloroborane reagent of 4-chloro-*N*-(2,2,2-trifluoroethyl)aniline and 2-fluorobenzonitrile produced the 2-amino-2'-fluorobenzophenone in 54% yield (185°C, 24 h) while the coupling with 2-fluorobenzaldehyde gave good conversions to the 2-amino-2'-fluorobenzhydrol (80%) at room temperature in 1 h.

These results indicated that the two-step method using benzaldehydes would probably be more adaptable to the time constraints of radiolabelling with $[^{18}F]F^-$ than the one-step procedure with benzonitriles.

To investigate the scope of the coupling reaction, anilines containing *N*-alkyl and para-substituents found in 1,4-benzodiazepine-2-ones were used to form the coupling reagents: 4-chloro-*N*-methylaniline, 6a, 4-nitro-*N*-methylaniline, 6b, 4-nitro-*N*-ethylaniline, 6c, and 4-chloro-*N*-(2,2,2-trifluoroethyl)aniline, 6d. Formation of the reagent required heating the aniline and boron trichloride (BCl_3) in dichloroethane (DCE) at reflux for at least 2.5-3 h, but the reagents could be mixed the day before and refluxed overnight prior to coupling with the ^{18}F-labelled benzaldehyde. Unreacted BCl_3 and the HCl formed during the reaction were removed during reflux by a N_2 stream at the top of the condenser. Best yields were obtained in the subsequent coupling reaction when essentially equimolar amounts of the aniline and BCl_3 had been used to form the reagent. For example, in the coupling of 6b with labelled or unlabelled 2-fluorobenzaldehyde, the presence of excess BCl_3 decreased the yields of the 2-aminobenzhydrol from 95% to 40%. Similar effects were observed for couplings with 6d. These results probably reflect the difficulty of completely removing BCl_3 under these conditions, which may complex with other functional groups in the aniline (for example, the $-NO_2$ group in 6b) or with the -CHO group in the 2-fluorobenzaldehyde, thereby reducing the yields of the aminobenzhydrol. For reproducible results, the ratio of aniline to BCl_3 used was 1:1.1.



a: X = Cl, R = CH_3; **b**: X = NO_2, R = CH_3; **c**: X = NO_2, R = CH_2CH_3; **d**: X = Cl, R = CH_2CF_3

For the synthesis of 2-$[^{18}F]$fluorobenzaldehyde, no-carrier-added Kryptofix 2.2.2/ $K^+[^{18}F]F^-$ was prepared according to the method previously reported (11,12). The conversions obtained for the nucleophilic aromatic substitution with 2-nitrobenzaldehyde were in the same range as those previously reported for similar conditions (13-15). The yields of the coupling reaction were affected by the method used to isolate 2-$[^{18}F]$fluorobenzaldehyde. The solvent and reagents used in the nucleophilic aromatic substitution were removed by solid phase extraction on SepPak C-18. When DCE was used to elute 2-$[^{18}F]$fluorobenzaldehyde a discolored solution was obtained and the coupling yields were typically very low even when the DCE solution was dried before addition to the coupling reagent. Hexane, on the other hand, efficiently eluted the aldehyde in a colorless solution which could be adequately dried with Na_2SO_4 before addition to the coupling reagent.

Mixtures of DCE:hexane up to at least 1:1 were tolerated, although some precipitation was observed on addition of the 2-[^{18}F]fluorobenzaldehyde solution and/or during the coupling reactions.

The yields obtained for coupling reactions performed at room temperature were affected by variations in the *N*-R and 4-X aniline substituents (Table 1). The slower conversions for 6b and 6c (and, to a lesser extent, even for 6d) could be compensated by increasing the reaction temperature to 60°C. The effect of varying the 4-X substituents (6b vs 6a) is consistent with the differences in the deactivating effects of -NO_2 and -Cl for electrophilic aromatic substitution. The lower yields for 6d may be the result of an electron-withdrawing effect exerted by the -CH_2CF_3 group (see, for example, the σ* (0.87 and -0.10 for -CH_2CF_3 and -CH_2CH_3, respectively (16)) and the ^{1}H-NMR chemical shifts for the methylene groups in CH_3CH_2NHAr- vs CF_3CH_2NHAr- (δ= 3.2 and 3.7 in 7c and 7d, respectively, as given in the experimental)).

Table 1. Yields for the coupling between anilinodichloroborane reagents[a] and [^{18}F]-2-fluorobenzaldehyde

Aniline	Temperature (°C)	Time (min)	Yield (%)
6a	20	5	95
6b	20	5	55
		10	75
		20	90
	60	5	95
6c	20	5	50
		10	75
		20	85
	60	5	90
6d	20	5	10
		10	20
		20	35
		60	65
	60	10	60

[a] 0.25 mmol in DCE:hexane = 1.3:1

Consistently high yields of the 2-amino-2'-[^{18}F]fluorobenzhydrols were obtained when the ratio of 6a, 6b or 6c to the 2-nitrobenzaldehyde remaining after the SepPak extraction procedure (~0.03 mmol) was essentially one. However, for 6d, yields of the coupling reaction varied. It was found that reproducible results were achieved when a 10-fold excess of 6d (0.34-0.35 mmol) was used.

The coupling reaction was quenched and excess anilinodichloroborane reagent was hydrolyzed by either acidic (8) or basic (10) reagents. However, the organic phase was more quickly separated from the aqueous phase when aqueous K_2CO_3 instead of HCl was used. For subsequent use of the

benzhydrol as a radiolabelling intermediate, it was found that the aniline starting material could be reduced by diluting the organic phase and eluting through a silica SepPak.

The 2-amino-2'-[^{18}F]fluorobenzhydrols were not isolated here from other chemical impurities (the 2-amino-2'-nitrobenzhydrol, the aniline and 2-nitrobenzaldehyde) or radiochemical impurities (unreacted 2-[^{18}F]fluorobenzaldehyde). These components could readily be removed by preparative HPLC, if desired, since they could be separated on the reversed-phase column used here. However, for use as radiolabelling intermediates for 1,4-benzodiazepine-2-ones, it has not been found to be necessary to purify the benzhydrol obtained by other measures than those described above. The use of these benzhydrols as radiolabelling intermediates for high specific activity ligands is demonstrated in a separate communication (17) in which 7d was used to synthesize [2'-^{18}F]-2-oxoquazepam with a specific activity ~ 2000 Ci/mmol at the end-of-synthesis (190 min), indicating that the specific activity of 7d was >4000 Ci/mmol at the end-of-preparation.

CONCLUSIONS

A series of 2-amino-2'-[^{18}F]fluorobenzhydrols 7a - 7d have been prepared in a two-step radiolabelling procedure from [^{18}F]F$^-$. The method demonstrates the use of a [^{18}F]fluorobenzaldehyde, one of the most commonly synthesized types of ^{18}F-labelled aromatic substrates, in a regioselective electrophilic coupling with activated aromatic amines. Reaction conditions were found for good conversions in short reaction times for anilines 6a - 6d, indicating that certain variations in the amino alkyl group as well as in the aromatic substituents are tolerated. The full extent of the substituent variation in the anilines and benzaldehydes which can be used in this reaction has yet to be determined. The use of one of these labelled aminobenzhydrols to synthesize a 1,4-benzodiazepine-2-one, 2-oxoquazepam, labelled in the 2-position in the 5-phenyl ring with no-carrier-added [^{18}F]F$^-$ has now been demonstrated (17).

EXPERIMENTAL

General

All solvents used were commercially available and were of analytical grade. Tetrahydrofuran (THF) was distilled from sodium/benzophenone before use. Dimethylsulfoxide (DMSO) was distilled from BaO under reduced pressure and stored over molecular sieves (4Å) in the refrigerator. Dichloroethane (DCE) was stirred with H_2SO_4 (18 M), dried with $CaCl_2$, distilled and stored over molecular sieves (4Å). Acetonitrile (CH_3CN) was stored over molecular sieves (4Å). Potassium carbonate (K_2CO_3), Kryptofix 2.2.2 and lithium aluminum hydride (LAH) were obtained from Merck. Triethylamine, obtained from Merck, was stirred with KOH, distilled and stored over molecular sieves (4Å). Boron trichloride (BCl_3) was obtained from Alphagaz/Alfax AB, Sweden. For use in the synthesis of the anilinodichloroborane reagents, BCl_3 was trapped in DCE and the content determined gravimetrically. Trifluoroacetic anhydride, 4-chloroaniline, 4-chloro-*N*-methylaniline, 4-nitro-*N*-methylaniline, 4-nitro-*N*-ethylaniline, 2-fluorobenzonitrile, 2-fluorobenzaldehyde and 2-nitrobenzaldehyde were obtained from Aldrich. 5-Chloro-2-(*N*-(2,2,2-trifluoroethyl)amino)-2'-nitrobenzophenone and 5-chloro-2-(*N*-(2,2,2-trifluoroethyl)amino)-2'-fluorobenzophenone were kindly supplied by Schering Plough Corp.

IR spectra were run on a Perkin-Elmer 377 instrument and ^{1}H-NMR on a Varian XL-300 NMR at 300 MHz. GC-MS analyses were performed using an LKB 2091 at 70 eV using a CP-Sil 5 column (5 m, i.d. = 0.53 mm, injector temperature 250°C, ionization source 270°C, temperature program 120-250°C, 10°C/min.). HRMS analyses were performed on a VG Analytical 70-250 HF in the EI mode.

Analytical radio-HPLC was performed using a μ-Bondapak C-18 column (Waters 300 x 7.8 mm, 10 μm) and an LDC Constametric III pump (flow 4 ml/min). An Erma ERC 7210 UV-spectrophotometer and a Beckman model 170 β-flow radiodetector were used to monitor the UV-absorption (λ=254 nm) and radioactivity, respectively. A Shimadzu C-R4A integrator was used for peak processing. The mobile phase was CH_3CN:H_3PO_4 (0.01 M) 50:50.

TLC and radio-TLC were performed using Merck 60 F_{254} silica plates. A Bioscan imaging scanner, system 200, was used when scanning the TLC plates for radioactivity. The eluents were:

TLC system 1: CH_2Cl_2:petroleum ether (bp 60-80°C) 2:1

TLC system 2: toluene:ethyl acetate 9:1

TLC system 3: toluene:ethyl acetate 3:1

TLC system 4: toluene

Synthesis of precursor and reference compounds

4-Chlorotrifluoroacetanilide

Trifluoroacetic anhydride (10 ml, 71.4 mmol) in diethyl ether (25 ml) was slowly added under N_2 atmosphere to 4-chloroaniline (18.1 g, 142.5 mmol) dissolved in diethyl ether (200 ml) while stirring at 0°C. A precipitate formed during the addition and the reaction mixture was stirred vigorously for 1 h at room temperature. The reaction was quenched by washing with HCl (2 M) and water and the ether phase was dried with Na_2SO_4. After filtration the ether solution was decolorized with charcoal. Subsequent evaporation of the ether gave white crystals (13.5 g, yield 84%). One spot on TLC system 3, R_f=0.7. IR: 1700 cm^{-1}, C=O. MS: *m/z* (relative intensity, %) 223 (100) and 225 (43). This compound was used without further purification.

4-Chloro-N-(2,2,2-trifluoroethyl)aniline, 6d

4-Chlorotrifluoroacetanilide (13.5 g, 60.5 mmol) was dissolved in THF (50 ml) and added dropwise to LAH (5 g, 131.9 mmol) in THF (100 ml) under N_2 at room temperature. The mixture was heated at reflux overnight. After cooling to room temperature, the mixture was carefully hydrolysed with HCl (2 M) until dissolution. The phases were separated and the aqueous phase was extracted with diethyl ether (3x20 ml). The combined organic phases were dried with Na_2SO_4 and the solvent evaporated. The residual oil was distilled *in vacuo* giving a colorless oil (9.9 g, yield 78%), bp: 87-88°C at 3 mm Hg (116-119°C at 16 mm Hg (18)). One spot on TLC system 4, R_f=0.8. ^{1}H-NMR ($CDCl_3$): δ 7.2 (m, 2H, ArH ortho to Cl); 6.6 (m, 2H, ArH ortho to NH); 3.9 (broad s, 1H, NH); 3.7 (m, 2H, CH_2). MS: *m/z* (%) 209 (53), 211 (22) and 140 (100).

Synthesis of reference 2-amino-2'-fluorobenzhydrols (general procedure)

The *N*-alkylaniline, 7a-d, (1 equiv) dissolved in DCE was added dropwise at 0°C to a solution of BCl_3 (1.1 equiv) in DCE with stirring and under N_2 atmosphere. The solution was refluxed for 2.5-3 h under a stream of N_2 (at the top of the cooler) to remove unreacted BCl_3 and the HCl

formed. The solution of the anilinodichloroborane reagent was cooled to 0°C prior to the addition of a mixture of 2-fluorobenzaldehyde (1 equiv) and triethylamine (1.2 equiv) in DCE and then reacted at room temperature for 1.5 h. K_2CO_3 (2 M) was added. The aqueous and organic phases were separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were dried with Na_2SO_4 and then evaporated. The residual oil was purified on a silica gel column.

5-Chloro-2'-fluoro-2-(N-methylamino)benzhydrol, 7a

Silica gel chromatography was performed with CH_2Cl_2:petroleum ether 2:1 as the eluent. The collected fractions were evaporated giving an oil (85%). The product was one spot on TLC system 2, R_f=0.4. ^{1}H-NMR ($CDCl_3$): δ 7.4-7.0 (m, 5H, ArH); 6.9 (d, 1H, ArH); 6.6 (d, 1H, ArH ortho to NH); 6.1 (s, 1H, benzyl-H); 2.8 (s, 3H, CH_3); 4.7 (broad s, 1H) and 2.5 (broad s,1H) (OH and NH). MS: *m/z* (%) 265 (30), 267 (9) and 246 (100). HRMS: calcd for ^{35}Cl: 265.0670, found: 265.0616; calcd for ^{37}Cl: 267.0640, found: 267.0612.

2'-Fluoro-5-nitro-2-(N-methylamino)benzhydrol, 7b

Silica gel chromatography was performed with toluene:CH_2Cl_2 3:2 as eluent. The collected fractions were evaporated yielding yellow crystals (75%). The product was one spot on TLC system 3, R_f=0.4. Mp: 155-157°C. ^{1}H-NMR ($CDCl_3$): δ 8.1 (m, 1H, ArH ortho to NO_2); 7.8 (d, 1H, ArH ortho to NO_2); 7.4-7.1 (m, 4H, ArH); 6.6 (d, 1H, ArH ortho to NH); 6.1 (d, 1H, benzyl-H); 2.9 (d, 3H, CH_3); 5.9 (broad s, 1H) and 2.6 (broad s, 1H) (OH and NH). MS: *m/z* (%) 276 (15) and 257 (100). HRMS: calcd: 276.0910, found: 276.0912.

2'-Fluoro-5-nitro-2-(N-ethylamino)benzhydrol, 7c

Silica gel chromatography was performed with toluene:CH_2Cl_2 3:2 as eluent. The collected fractions were evaporated giving yellow crystals (yield 87%). The product was one spot on TLC system 3, R_f=0.6. Mp: 129-130°C. ^{1}H-NMR ($CDCl_3$): δ 8.1 (m, 1H, ArH ortho to NO_2); 7.9 (d, 1H, ArH ortho to NO_2); 7.4-7.1 (m, 4H, ArH); 6.6 (d, 1H, ArH ortho to NH); 6.1 (d, 1H, benzyl-H); 3.2 (m, 2H, CH_2); 1.3 (t, 3H, CH_3); 5.7 (broad s, 1H) and 2.6 (broad s, 1H) (OH and NH). MS: *m/z* (%) 290 (20) and 227 (100). HRMS: calcd: 290.1067, found: 290.1057.

5-Chloro-2'-fluoro-2-(N-(2,2,2-trifluoroethyl)amino)benzhydrol, 7d

Silica gel chromatography was performed with CH_2Cl_2:petroleum ether 1:2 as eluent. The collected fractions were evaporated to an oil (yield 82%). The product was one spot on TLC system 1, R_f=0.4. ^{1}H-NMR ($CDCl_3$): δ 7.4-7.05 (m, 5H, ArH); 6.9 (d, 1H, ArH); 6.7 (d, 1H, ArH ortho to NH); 6.1 (s, 1H, benzyl-H); 3.7 (m, 2H, CH_2); 5.3 (broad s, 1H) and 2.6 (broad s, 1H) (OH and NH). MS: *m/z* (%) 333 (15), 335 (6) and 181 (100). HRMS: calcd for ^{35}Cl: 333.0544, found: 333.0547; calcd for ^{37}Cl: 335.0514, found: 335.0528.

Radiolabelling

Radionuclide production

No-carrier-added [^{18}F]F$^-$ was produced via the ^{18}O(p,n)^{18}F reaction using the Scanditronix MC 16 cyclotron at the Karolinska Hospital. [^{18}O]Water (95-98% enrichment, Isotec, obtained

from Campro Scientific, Veenendaal, Netherlands) was diluted to 10-50% enrichment using 18 MΩ water produced from a Milli-Q water system. A total volume of 1.8-1.9 ml was irradiated in a high-purity silver target (19) with 17 MeV protons (15 μA) for 30-45 min. After waiting for the decay of ^{13}N also produced during the irradiation, the contents of the target were emptied through silicon tubing into a sterile injection vial for transportation to the radiochemistry laboratory.

2-[^{18}F]Fluorobenzaldehyde, 5

The aqueous solution of [^{18}F]F$^-$ was added to Kryptofix 2.2.2 (20 mg, 0.053 mmol) and K_2CO_3 (3.7 mg, 0.027 mmol) and the water was azeotropically evaporated with CH_3CN at 120°C under a stream of N_2. 2-Nitrobenzaldehyde (16 mg, 0.106 mmol) dissolved in DMSO (0.5 ml) was added and the solution was heated at 120°C for 10 min. The reaction mixture was cooled, water (5 ml) was added and the solution was passed through a SepPak C-18. The SepPak was rinsed with additional water (5 ml) prior to elution of 2-[^{18}F]fluorobenzaldehyde with hexane (1.5 ml). The hexane was dried through a Na_2SO_4 column (0.6 g) before use in the next step. This procedure removed ~70% of the 2-nitrobenzaldehyde starting material, as determined gravimetrically. Analysis of the hexane solution showed one radioactive product (conversion 55-70%) which coeluted with the 2-fluorobenzaldehyde reference on analytical HPLC (5 min) and TLC system 1 (R_f= 0.6).

2-amino-2'-[^{18}F]fluorobenzhydrol, 7a-d

Triethylamine (1.2 equiv, based on the aniline) was added to the hexane mixture prior to dropwise addition at 0°C to the anilinodichloroborane reagent prepared as above (using 0.03 mmol for anilines 6a-6c and 0.35 mmol for aniline 6d in 2 ml DCE). The reaction mixture was stirred at room temperature or 60°C for 5-10 min (see Table 1). After completed reaction, the solution was cooled and aqueous K_2CO_3 (2M, 2 ml) was added. The organic and aqueous phases were separated and the radioactivity in the phases was counted in a Capintec radioisotope calibrator. Essentially all radioactivity was found in the organic phase. The product distribution in the organic phase was analyzed by radio-HPLC and -TLC. One major new radioactive product was obtained in the four coupling reactions and was in all cases well-separated from the 2-[^{18}F]fluorobenzaldehyde (>3 min). The product solution was analyzed without and with references 7a-7d. The new radioactive products coeluted with the corresponding reference samples on the HPLC and TLC systems used. One other major non-radioactive product was formed during each of the coupling reactions, which though unidentified, was assumed to be the corresponding 2-amino-2'-nitrobenzhydrol, based on the amounts formed, their shorter retention times on the HPLC column and their smaller R_fs on the TLC analyses. With the chromatographic conditions used here, the ^{18}F-labelled products 7a-7d eluted last of all the other detectable components in the product mixture. Adjustments of the mobile phase would be expected to allow the use of the same separation system for a preparative isolation of the products, if desired. The yields of 7a-7d and their elution properties on the HPLC and TLC systems used were as follows:

7a: 95% after 5 min at room temperature; HPLC (10 min); TLC system 2 (R_f= 0.4).

7b: 95% after 5 min at 60°C; HPLC (8 min); TLC system 3 (R_f= 0.4).

7c: 90% after 5 min at 60°C; HPLC (10 min); TLC system 3 (R_f= 0.6).

7d: 60% after 10 min at 60°C; HPLC (12 min); TLC system 1 (R_f= 0.4).

ACKNOWLEDGEMENTS

The authors would like to thank Dr. T. Sugasawa for valuable discussions concerning the coupling reaction, Schering Plough Corp. for supplying reference substances, Mr. W. Pulka for technical assistance with the synthesis of reference substances, Dr. Hugh Webb and Dr. Gary Rogers, Department of Chemistry, U.C.S.B. and Dr. M. Hassan for the mass spectra, Dr. K-O. Schoeps for NMR spectra, Mr. G. Printz and Mr. P. Söderholm for technical assistance with the radionuclide production. This project has been partially supported by research grants from the Karolinska Institute, which is gratefully acknowledged.

REFERENCES

1. Pike V.W., Halldin C., Crouzel C., Barré L., Nutt D.J., Osman S., Shah F., Turton D.R. and Waters S.L. - Nucl. Med. Biol. 20: 503 (1993)

2. Kaplan S.A. and Jack M.L. - In: *The Benzodiazepines: From Molecular Biology to Clinical Practice* (edited by E. Costa), pp. 173-199, Raven Press, New York (1983)

3. Duelfer T., Johnström P., Stone-Elander S., Holland A., Halldin C., Haaparanta M., Solin O., Bergman J., Steinman M. and Sedvall G. - J. Label. Compounds Radiopharm. 29: 1223 (1991)

4. Bergman J., Källman K-M., Solin O., DeJesus O.T., Oakes T.R. and Nickels R.J. - J. Nucl. Med. 34: 69P (1993)

5. Johnström P. and Stone-Elander S. - J. Label. Compounds Radiopharm. 32: 134 (1993)

6. Kilbourn M.R. - *Fluorine-18 Labeling of Radiopharmaceuticals*, Nuclear Science Series NAS-NS-3203, National Academy Press, Washington, D.C. (1990) and references therein

7. Fryer R.I. - J. Heterocycl. Chem. 9: 747 (1972)

8. Sugasawa T., Toyoda T., Adachi M. and Sasakura K. - J. Am. Chem. Soc. 100: 4842 (1978)

9. Sugasawa T., Adachi M., Toyoda T. and Sasakura K. - J. Heterocycl. Chem. 16: 445 (1979)

10. Sugasawa T., personal communication

11. Hamacher K., Coenen H.H. and Stöcklin G. - J. Nucl. Med. 27: 235 (1986)

12. Coenen H.H., Klatte B., Knöchel A., Schüller M. and Stöcklin G. - J. Label. Compounds Radiopharm. 23: 455 (1986)

13. Ding Y-S., Shiue C-Y., Fowler J.S., Wolf A.P. and Plenevaux A. - J. Fluor. Chem. 48: 189 (1990)

14. Hwang D-R., Dence C.S., McKinnon Z.A., Mathias C.J. and Welch M.J. - Nucl. Med. Biol. 18: 247 (1991)

15. Lemaire C., Damhaut P., Plenevaux A., Cantineau R., Christiaens L. and Guillaume M. - Appl. Radiat. Isot. 43: 485 (1992)

16. Perrin D.D., Dempsey B. and Serjeant I.P. - *pK_a Predictions for Organic Acids and Bases*, Chapman and Hall, London (1981)

17. (a) Johnström P., Stone-Elander S., Halldin C. and Duelfer T. - *Synthesis and Applications of Isotopically Labelled Compounds,1991*, (edited by E. Buncel and G.W. Kabalka) pp 644-647 Elsevier Science Publishers B.V., Amsterdam (1992).
(b) Johnström P., Stone-Elander S. and Duelfer T. - J. Label. Compounds Radiopharm., in press

18. Topliss J.G. - U.S. Patent No. 3,641,147 (1972)

19. Printz G. and Solin O. - Acta Radiol., Suppl. 376: 59 (1991)