THE LABELLING OF 2-OXOQUAZEPAM WITH ELECTROPHILIC ^{18}F.

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SUMMARY

2-Oxoquazepam, 7-chloro-1-(2,2,2-trifluoroethyl)-1,3-dihydro-5-(2-fluorophenyl)-2H-1,4-benzo-diazepine-2-one, is a benzodiazepine agonist. It has been shown to bind *in vitro* with a higher affinity to benzodiazepine type 1 receptors than to type 2 receptors. Here we report the synthesis of a trimethyltin precursor and demonstrate the feasibility of using it for radiolabelling acid- and base-sensitive benzodiazepine structures such as 2-oxoquzepam. Conversions of the electrophilic fluorine to [^{18}F]-2-oxoquazepam on the order 20-25% were obtained.

KEY WORDS: Oxoquazepam, ^{18}F, [^{18}F]-2-oxoquazepam, electrophilic radiofluorination, destannylation, benzodiazepine type 1 receptor ligand

INTRODUCTION

Positron emission tomography (PET) has become a powerful tool for non-invasive investigations of basic biological and physiological processes in man, for example: blood flow, transport and metabolism of natural substrates and localization and characterization of receptor systems. The success of receptor studies depends on such factors as site specificity of the tracer ligand's binding as well as appropriate constants for its association and dissociation. Both ^{11}C- and ^{18}F-ligands have been synthesized for a number of receptor systems such as: dopamine, benzodiazepine, opiate, histamine, nicotine, muscarine and serotonin.

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0362–4803/91/111223–17$08.50

Received 30 May, 1991
Revised 8 August, 1991

Benzodiazepines (Bzd) are widely used in the treatment of insomnia, anxiety, seizures and muscle disorders. Their pharmacological effect appears to be initiated by interaction with specific neuronal membrane receptors. Benzodiazepine receptor ligands have been labelled with positron emitting radionuclides as early as 1975 when the Orsay PET group reported the synthesis of [N-methyl-^{11}C]-diazepam (1). Since 1975 antagonists and agonists for central Bzd receptors such as [N-methyl-^{11}C]-flunitrazepam (2), [methyl-^{11}C]- (3,4) and [ethyl-^{11}C]-Ro 15-1788 (5), [^{11}C]-suriclone (6), [^{18}F]-3-fluorodiazepam (7), [^{11}C]-3-methoxybenzodiazepines (8), [^{11}C]-fludiazepam (9) and [^{11}C]-alprazolam (10) have been labelled with short-lived positron emitting radionuclides.

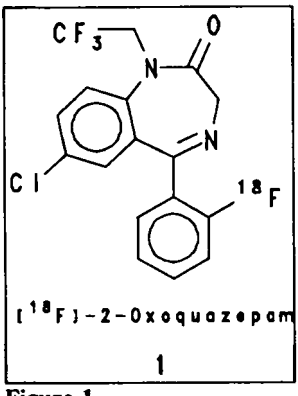


Figure 1

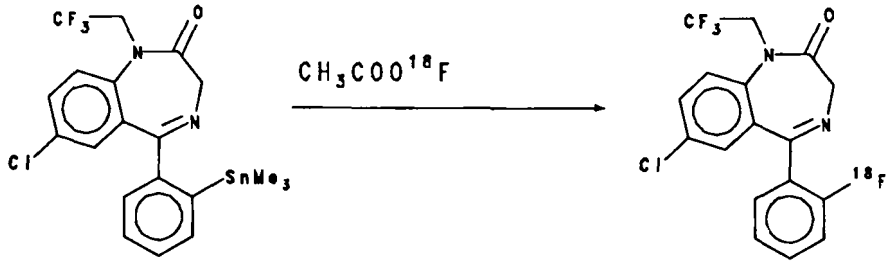
Bzd receptors in the brain have been characterized into at least two sub-types. Those found in the cerebellum are >90% Bzd-1 while those in the cortex and hippocampus are approximately 50% Bzd-1 and 50% Bzd-2 (11). The benzodiazepine receptor agonist 2-oxoquazepam, has been shown to bind *in vitro* with a higher selectivity for Bzd-1 (K_d=4.85 nM) than for Bzd-2 (K_d=198 nM) (12). 2-Oxoquazepam labelled with a short-lived positron emitting radionuclide might thus allow the *in vivo* study of the distribution of the Bzd-1 receptors in the human brain with PET.

Since 2-oxoquazepam contains a fluorine in one of the aryl groups, one possible approach for labelling the ligand with a positron emitting radionuclide could be introduction of ^{18}F in an aromatic substitution reaction to produce [^{18}F]-2-oxoquazepam (figure 1). Demetallation reactions are chemically an attractive alternative for introducing halogens via electrophilic aromatic substitutions. Use of organometallic precursors has been previously reported in the ^{18}F-labelling of model compounds (13-18). In general, radiochemical yields are good, the incorporation of ^{18}F is regioselective with only trace amounts of difluorinated products and the method is compatible with a variety of functional groups. Since carrier fluorine is added to the target gas during the radionuclide production, electrophilic fluorinating agents are presently characterized by low specific

activities and are therefore less attractive for labelling potential receptor ligands. However, efforts are being made to develop production methods for electrophilic fluorinating agents with specific activities closer to those of the ^{14}C- and ^{3}H-labelled tracers used in autoradiographic studies. Therefore, investigations into the possibility of controlling the radiolabelling selectivity of these highly reactive fluorinating agents in complicated organic molecules is of general chemical interest and of potential interest for the future production of receptor radioligands.

Here, we report the synthesis of a trialkyltin benzodiazepine and its reaction with electrophilic ^{18}F (scheme 1). 2-Oxoquazepam is sensitive to both acidic and basic conditions. Our success in making the trialkyltin analogue **2** for labelling with ^{18}F indicates the versatility of this route.

Scheme 1



2 **1**

DISCUSSION

Synthesis of trialkyltin-2-oxoquazepam.

We tried three synthetic approaches to **2** :

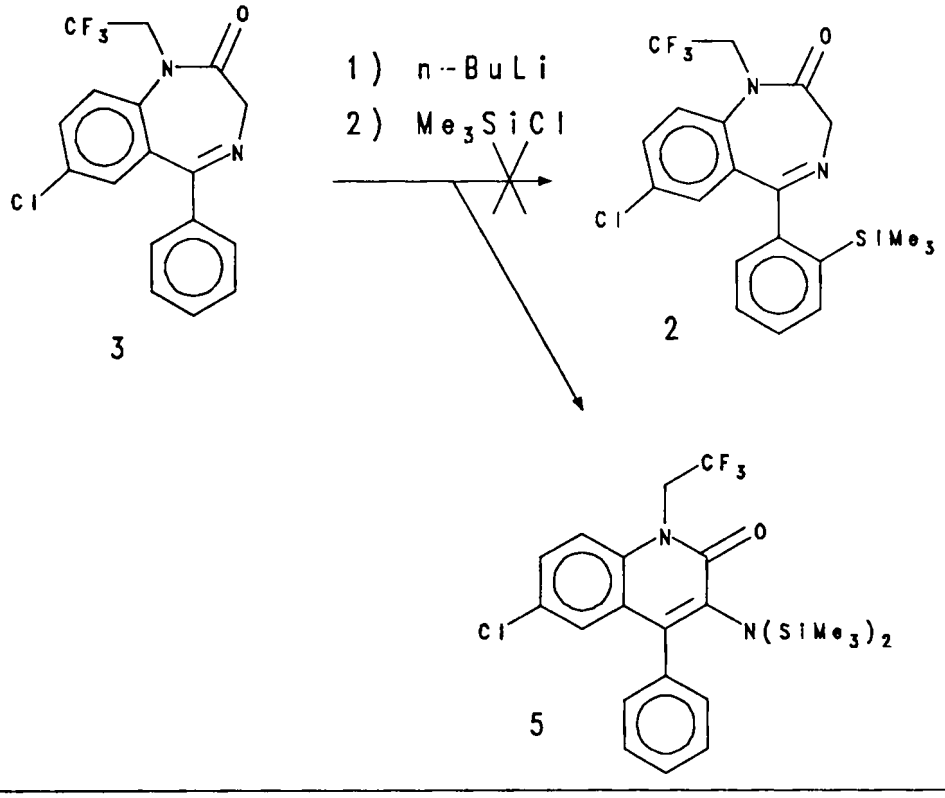
(1)-Directed lithiation of the desfluoro compound (halazepam, **3**), followed by reaction with trimethyltin chloride.

(2)-Lithiation of the bromo-desfluoro-2-oxoquazepam, **4**, followed by reaction with trimethyltin chloride.

(3)-Reaction of **4** with hexamethylditin in the presence of a palladium catalyst.

The simplest approach to **2** seemed to be treatment of **3** with butyllithium followed by trimethylstannyl chloride (scheme 2). There is considerable precedent in the literature for an imine to direct lithiation to the appropriate carbon to produce **2** (for example, 19 and 20). However, treatment of these benzodiazepines with n-butyllithium or t-butyllithium caused collapse of the benzodiazepine ring to the aminoquinolone. The reaction was first attempted with trimethylsilyl chloride, in the hope that the silane corresponding to **2** would be more stable and isolable. However, one primary product, isolated in 40% yield, was identified by electron impact and thermospray mass spectroscopy to be N-disilylaminoquinolone, **5** (scheme 2). This rearrangement has also been observed separately under acid conditions (21). It was therefore concluded that 2-oxoquazepam would not tolerate either strongly acidic or basic conditions.

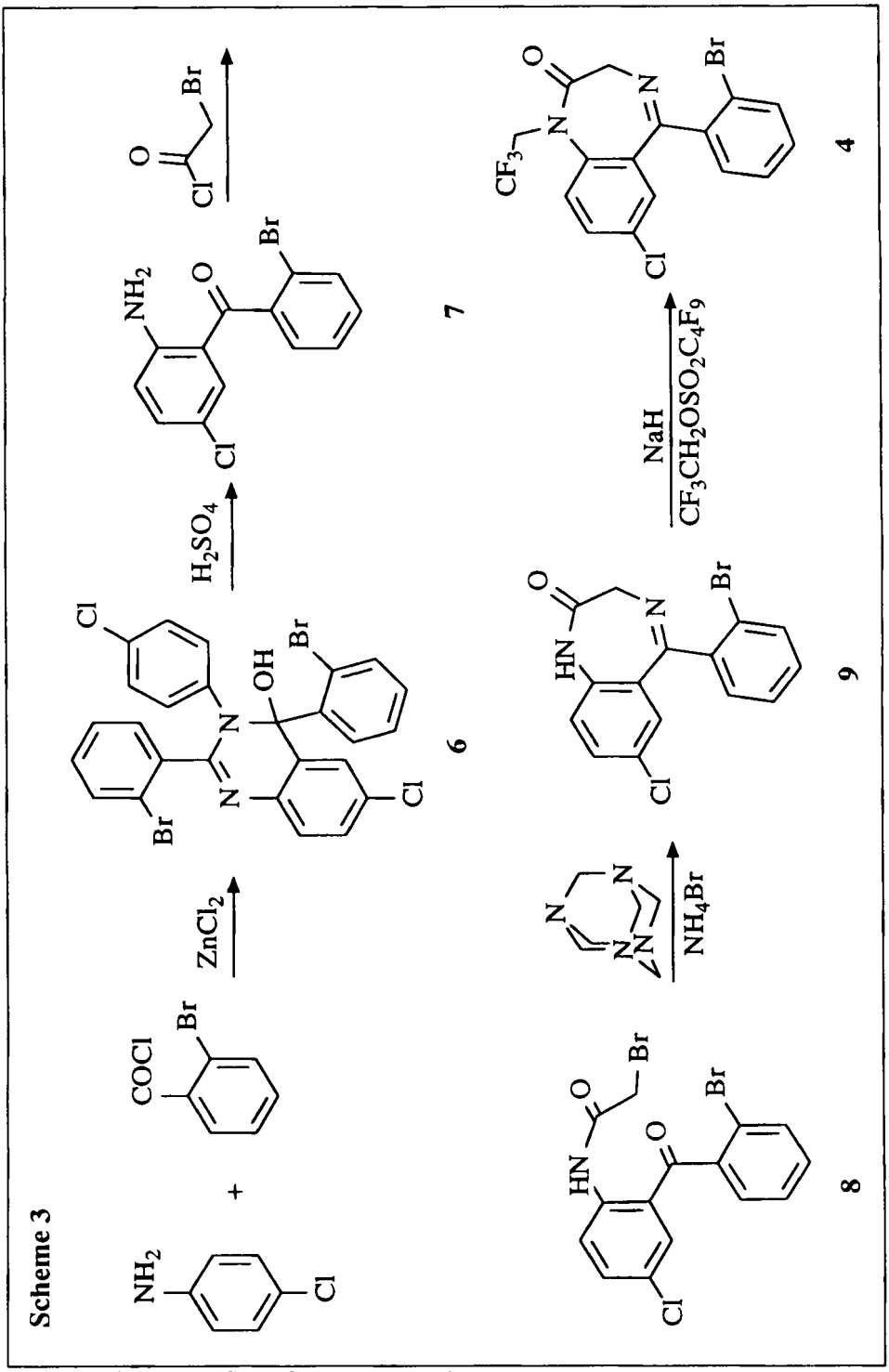
Scheme 2



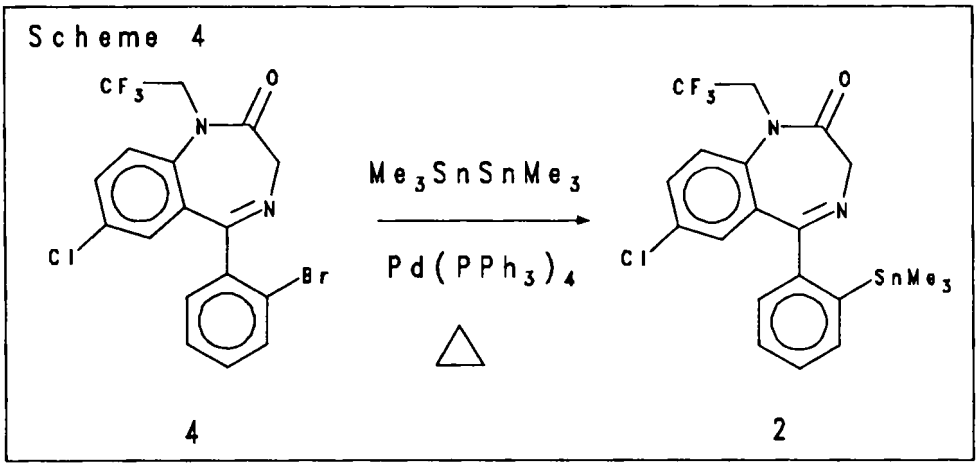
The other approaches to **2** required the synthesis of the bromo analogue, **4**. The method used is shown in scheme 3 and is similar to those used by Steinman (22) and Sugasawa (23) to make analogous compounds. 2-Bromobenzoyl chloride was reacted with 4-chloroaniline under Friedel-Crafts conditions to produce an intermediate, **6**, which was hydrolyzed to give the benzophenone **7**. The benzophenone was acylated with bromoacetyl chloride to produce **8**, which was subsequently annulated using hexamethylenetetramine and ammonium bromide. The ammonium bromide acted as a buffer to prevent rearrangement to the six membered ring, as discussed. The resulting benzodiazepine, **9** was alkylated on the amide nitrogen using the perfluorobutylsulfonate of trifluoroethanol to give bromo-desfluoro-2-oxoquazepam, **4**. Deuterated trifluoroethyl perfluorobutylsulfonate, made from commercial deuterated trifluoroethanol by a literature method (24) was used for the spectroscopic studies of these compounds. Proton NMR and mass spectra of compounds containing the trifluoroethyl moiety reflect this isotopic substitution. In ^{1}H-NMR, this methylene would appear as multiplets (overlapping doublet of quartets) at δ 4.13 and at 5.21, if these deuterons were protons.

Similar to **3**, bromo-desfluoro-2-oxoquazepam, **4**, also failed to yield the trimethylstannyl-2-oxoquazepam, **2**, on treatment with butyllithium followed by trimethyltin chloride. In light of these results and the base sensitivity of **3**, we abandoned further attempts to synthesize **2** via lithiation.

Hexaalkylditin compounds have been reported to react with aryl halides in the presence of palladium catalysts to give the corresponding aryl tin compound (25). Bromo-desfluoro-2-oxoquazepam, **4**, reacted with hexamethylditin in the presence of palladium tetrakis(triphenylphosphine) in xylene to give trimethylstannyl-2-oxoquazepam in 64% isolated yield. Thus, under these neutral conditions, the rearrangement that had thwarted the first two approaches to **2** was avoided.



Synthesis of bromo-desfluoro-2-oxoquazepam, **4**



^{18}F-Labelling of 2-oxoquazepam.

Electrophilic aromatic substitution on **2** was performed with both [^{18}F]CH_3COOF and [^{18}F]F_2. [^{18}F]-2-Oxoquazepam was obtained in both reactions. According to radio-TLC and -HPLC (figures 2 and 3) the reaction with [^{18}F]CH_3COOF gave essentially one major radioactive product which corresponded to 2-oxoquazepam. The conversion of electrophilic fluorine to product was 20-25% (radio-TLC), out of a maximum of 50% possible.

After waiting until the sample was no longer radioactive, the isolated product from the [^{18}F]CH_3COOF reaction was analyzed by ^{1}H- and ^{19}F-NMR and by C.I. mass spectroscopy. The chemical composition was determined from these data. Considerable destannylation had occurred producing **3**, as indicated by the proton NMR. The ratio of **1**:**3** was 3:2 based on integration of the ring methylene resonances (for **1**: δ 3.82, d, and δ 4.87, d, J= -9 Hz; for **3**: δ 3.87, d, and δ 4.92, d, J = -9 Hz). No doublet representing the difluorinated compound was observed, indicating that difluorination was less than 5% of the reaction product. The ^{19}F-NMR showed resonances of δ -7.5285 and -7.5463 (vs. trifluoromethylbenzene) for the CF_3 moieties of **1** and **3**, respectively. No resonance attributible to the CF_3 of the ring difluorinated species was found. This result is consistent with less than 5% electrophilic difluorination in the labelling reaction. The CF_3 moiety was examined because it is common to all three compounds and the carbon adjacent to it bears deuterium which does not split the signal. The aryl fluorines of the products were not chosen

because those of the difluorinated compound would be coupled making it difficult to detect them as a few percent of the reaction mixture.

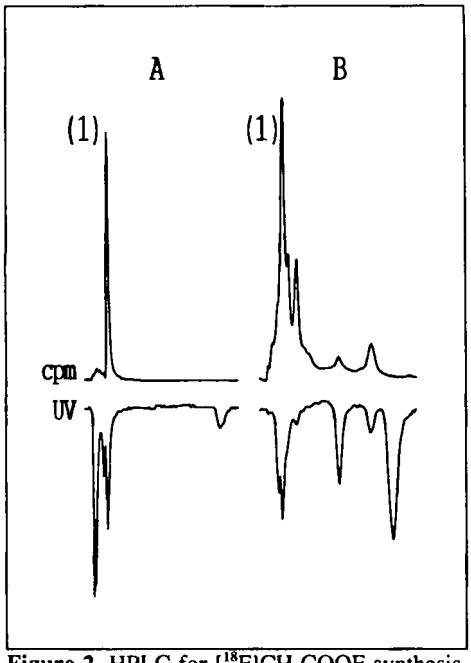


Figure 2. HPLC for $[^{18}F]CH_3COOF$ synthesis (A) and $[^{18}F]F_2$ synthesis (B). 1: $[^{18}F]$-2-oxoquazepam.

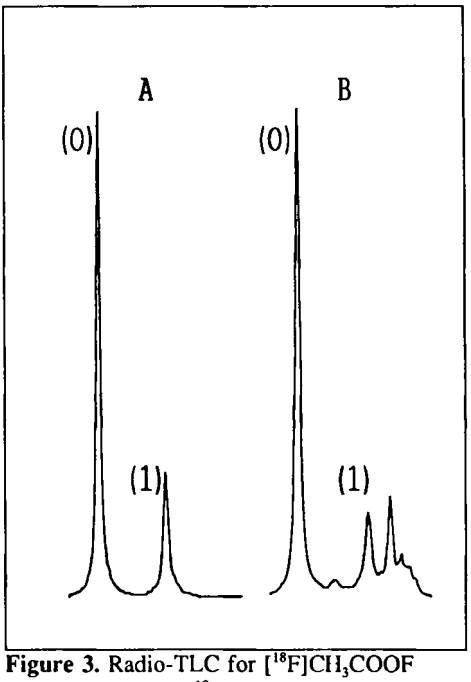


Figure 3. Radio-TLC for $[^{18}F]CH_3COOF$ synthesis (A) and $[^{18}F]F_2$ synthesis (B). O: origin, 1: $[^{18}F]$-2-oxoquazepam.

In the mass spectrum a peak at M+19 with an intensity of 3% of the 2-oxoquazepam peak was observed. Since the C.I. mass spectrum peak intensities for the mono- and difluorination products assume similar ionization efficiencies, the M.S. data are approximate.

Therefore it was concluded that the main side-reaction in radiolabelling with $[^{18}F]CH_3COOF$ yielded the chemical contaminant **3** via destannylation reaction and that any radiochemical contaminant via difluorination was less than 5%. These results are in agreement with observations by Adam et al. (15) and Coenen et al. (17). The defluorinated compound, **3**, binds to the Bzd-1 receptor although its affinity is one tenth that of **1**. In this investigative study, the chemical contamination could be reduced to 33% by manipulating the acetonitrile content of the mobile phase in the semi-preparative run. For biological studies when fluorine of higher specific activity is available, a larger column or alternative separation system should be used.

In the reaction of **2** with $[^{18}F]F_2$ the total incorporation of ^{18}F was essentially the same as for

the [^{18}F]CH_3COOF reaction. Though the main product was [^{18}F]-2-oxoquazepam, a number of other ^{18}F-compounds were also obtained as shown in figures 2 and 3. In retrospect, acetonitrile was probably not the optimal reaction solvent for this reaction (17). However, the high reactivity of fluorine gas may be expected to result in labelling in a number of positions when using complex starting materials (14). For example, Luxen et al. (7) successfully incorporated ^{18}F in the 3-position in the benzodiazepine ring of diazepam by using [^{18}F]F_2, which, in our case, could be a possible side reaction.

In the preparative run, the total synthesis time of [^{18}F]-2-oxoquazepam was 75 minutes from the end of [^{18}F]CH_3COOF production, including HPLC isolation. The incorporation of ^{18}F was 20% (radio-TLC, maximum 50% possible). Analysis by radio-HPLC indicated that the radiochemical purity was better than 95% and that the specific activity was 0.5 Ci/mmol (18.5 MBq/mmol).

Labelling 2-oxoquazepam by the reaction in scheme 1 has several advantages over other approaches. It requires only one labelling step from the appropriate starting material, trimethyltin-2-oxoquazepam, **2**. Since the ^{18}F replaces the trimethyltin moiety, it is directed to the correct position in the molecule, with good radiolabelling yields. This demonstrates that trialkylmetal leaving groups can successfully be introduced into molecules that are both sensitive to acidic and basic conditions, thereby generating precursors for subsequent use for regioselective electrophilic aromatic radiofluorinations. Investigation of a method for labelling 2-oxoquazepam by nucleophilic aromatic substitution with high specific activity [^{18}F]fluoride is currently being pursued.

EXPERIMENTAL

General methods.

All chemicals were purchased from Aldrich Chemical Co. except for the D_2-trifluoroethanol which was supplied from Cambridge Isotopes. Electron impact mass spectra were run on an Finigan MAT-CH5. Chemical ionization mass spectra were run on an Extrel model 401. Fast atom bombardment mass spectra were run on a Finigan MAT 312. Thermospray mass spectra were run

on an Extrel model ELQ 400-1 with a Vestec thermospray interface. Tables of isotopic abundances used in interpreting mass spectra are found in McLafferty's text (26). IR spectra were run on a Nicolet 10-MX FT-IR. Liquids were run as neat films and solids as nujol mulls on KBr plates. ^{1}H-NMR were run on a Varian 200 MHz NMR, model XL-200 or on a Varian 400 MHz model XL-400. ^{19}F-NMR were run on an XL-400.

The TLC systems used are as follows:

System 1: CH_2Cl_2 on Whatman LK6DF silica plates

System 2: Toluene:Hexane:EtOAc (50:50:10) on Whatman LK6DF silica plates

System 3: MeCN:0.2 M NH_4OAc (50:50) on Whatman KC18 silica plates

System 4: Hexane:EtOAc (50:50) on Whatman LK6DF silica plates

System 5: CH_3Cl:Acetone (90:10) on Merck 60 F_{254} silica plates

Liquid chromatography (HPLC) was performed using an Altex 110 A pump with a Knauer UV-spectrophotometer (λ =222 nm) and a sodium iodide crystal to monitor the UV-absorption and radioactivity of the eluent, respectively.

Analytical HPLC was performed on a µ-Bondapak C-18 column (Waters 300 x 7.8 mm, 10 µm) with a 50:50 mixture of acetonitrile:phosphoric acid (0.01 M) as eluent. The flow was 3.0 ml/min. Semi-preparative HPLC was performed on a Radialpak C-18 (100 x 8 mm, 10 µm) with acetonitrile:phosphoric acid (0.01 M) 40:60 as eluent. The flow was 4.0 ml/min.

Radio-TLC was performed on TLC system 5 and the plates were scanned for radioactivity with a plastic scintillator device (27).

Precursor synthesis.

2-Amino-5-chloro-2'-bromobenzophenone, **7**.

2-Bromobenzoyl chloride (22.4 g, 0.102 mol) was heated to 120°C and 4-chloroaniline (5.78 g, 0.045 mol) was added in portions while stirring. The mixture was heated to 180°C and zinc chloride (8.722 g, 0.064 mol) added in one portion. The temperature was gradually increased to 200°C -205°C and the mixture heated for 2 h. The mixture was cooled to 120°C and 3N HCl (50ml)

was cautiously added. The mixture was heated to reflux. The hot acid layer was decanted. This process was repeated three times. To the remaining residue 75% (v/v) H_2SO_4 (30 ml) was added. The solution was heated to reflux for 40 min, poured over ice (100 g) and diluted with H_2O (50 ml). The solution was extracted with CH_2Cl_2 (70 ml then 3 x 40 ml). The combined CH_2Cl_2 fractions were concentrated, then purified on a silica gel column eluted with CH_2Cl_2. A total of 3.451 g of yellow needles was obtained (yield 24.7%). TLC system 1, Rf = 0.51. IR: 3400 and 3350 cm^{-1}, NH; 1640 cm^{-1}, C=O. ^{1}H-NMR: (D_6-DMSO) δ 10.6 (s, 2H, NH_2); 8.2 (m, 1H, ArH); 7.8 (m, 2H, ArH ortho to Cl); remaining ArH appear as a complex multiplet at 7.5. This compound was used in the next step without further purification.

2-Bromoacetamido-5-chloro-2'-bromobenzophenone, **8**.

The benzophenone, **7**, (3.451 g, 11.12 mmol) was dissolved in toluene (86 ml) under N_2 and bromoacetyl chloride (968 µl, 11.12 mmol) was added. The solution was refluxed for 1 h. The reaction mixture was cooled to room temperature, then washed with saturated $NaHCO_3$ (3 x 25 ml) and dried over Na_2SO_4. The organic layer was evaporated to dryness. A total of 4.204 g of a cream colored powder was obtained for a yield of 87.7%. The compound was one spot in TLC system 1, Rf = 0.41 and TLC system 2, Rf= 0.50. IR: 3200 cm^{-1}, N-H; 1684 cm^{-1}, -NHC=O; 1652 cm^{-1}, $(Ar)_2$-C=O. ^{1}H-NMR: ($CDCl_3$) δ 3.32 (s, 2H, $-CH_2Br$); 8.21 (m, 2H ArH ortho to Cl,); remaining ArH were found in two complex multiplets at 7.77 and 7.51; 11.25 (s, 1H, -NHC=O). CI Mass Spec: The ratio of molecular ion peaks was consistent with two bromines and one chlorine:

M/e :	429	431	433	435
Rel. intensity:	0.43	1.00	0.71	0.131
Theory:	0.43	1.00	0.69	0.137

7-Chloro-1,3-dihydro-5-(2-bromophenyl)-2H-1,4-benzodiazepin-2-one, **9**.

The acylated bromobenzophenone, **8**, (2.000 g, 4.63 mmol), hexamethylenetetramine (2.600 g, 18.54 mmol) and ammonium bromide (1.816 g, 18.54 mmol) were dissolved in a mixture of

isopropanol (10.4 ml) and H_2O (2.1 ml). The solution was refluxed for 2 h. The hot mixture was poured over ice (18.6 g) and stirred vigorously. Toluene (14 ml) was added and the mixture stirred until the ice melted. The organic layer was removed, washed with H_2O (3 x 20 ml), stirred with Na_2SO_4 and decolorizing carbon for 30 min, then filtered through celite. The organic layer was concentrated to give 1.572 g of crude product. It was purified on a chromatotron (Harrison Research, model 7924T) using a 4000 μ silica rotor which was eluted with a gradient of hexane, hexane:EtOAc (75:25), hexane:EtOAc (50:50), hexane:EtOAc (25:75) and EtOAc. The product fractions were pooled and evaporated to dryness. A total of 1.163 g of a light yellow oil was obtained for a yield of 71.8%. The compound was one spot in TLC systems 2, Rf= 0.57 and system 3, Rf= 0.56. IR: 3250 cm^{-1}, N-H; 1690 cm^{-1}, C=O. ^{1}H-NMR:(D_6-DMSO) δ 4.20 (s, 2H, -CH_2-); 6.85-7.55 (m, 7H, Ar-H); 10.0 (br s, 1H, -NHC=O). EI Mass spectrum is consistent with one chlorine and one bromine:

M/e :	348	350	352
Rel. intensity:	0.777	1.00	0.244
Theory:	0.766	1.00	0.244

7-Chloro-1-(2,2,2-trifluorethyl)-1,3-dihydro-5-(2-bromophenyl)-2H-1,4-benzodiazepin-2-one, **4.**

The benzodiazepin-2-one, **9**, (1.138g, 3.255 mmol) and NaH (179 mg, 3.730 mmol) were dissolved in DMF (1.6 ml). The mixture was cooled in an ice bath and toluene (11.4 ml) added slowly. The mixture was stirred for 2 h at 0°C, 2 h at room temperature and 10 min at 45°C. A solution of $CF_3CD_2OSO_2C_4F_9$ (1.628 g, 3.776 mmol) in 1 ml toluene was added dropwise over a period of 2-3 min. The mixture was stirred at 40-45°C for 4 h and subsequently cooled to room temperature and poured into ice water (25 ml). After drying over Na_2SO_4, the organic layer was evaporated to dryness. A total of 1.173 g of crude product was obtained. The crude product was purified in a silica column eluted with CH_2Cl_2:EtOAc (85:15). The product fractions were pooled and evaporated to dryness in vacuo. A total of 848 mg of a light yellow crystalline product was obtained for a yield of 60.4%. The compound was one spot in TLC system 4, Rf= 0.65 and was

used in the next step without further purification. IR: 1690 cm^{-1}, C=O. ^{1}H-NMR (D_6-DMSO): δ 3.78 (d, 1H, J= -9 Hz, $COCH_2$); 4.75 (d, 1H, J= -9 Hz, $COCH_2$); 7.18- 7.76 (m, 7H, ArH). EI Mass spectrum is consistent with one chlorine and one bromine:

M/e :	432	434	436
Rel. intensity:	0.830	1.00	0.251
Theory:	0.766	1.00	0.244

7-Chloro-1-(2,2,2-trifluorethyl)-1,3-dihydro-5-(2-trimethylstannylphenyl)-2H-1,4-benzodiazepin-2-one, **2.**

Bromo-desfluoro-2-oxoquazepam, **4**, (100 mg, 0.232 mmol) was added to xylene (3 ml) and palladium tetrakis(triphenylphosphine) (35 mg, 0.03 mmol). Hexamethylditin (375 mg, 1.14 mmol) was introduced. The reaction was protected from light, then heated under N_2 to 115°C for 15 hr. TLC showed that the reaction was complete. The reaction mixture was cooled to room temperature and filtered. The filter cake was washed with xylene (9 ml in three portions) and benzene (2 ml). The combined filtrate was evaporated in vacuo and purified by chromatography on silica gel using EtOAc:hexane (1:1). Fractions containing the product were pooled and evaporated in vacuo to yield 74.97 mg (0.150 mmol) of a viscous yellow oil (64%). The product was one spot by TLC system 3, Rf= 0.77 and system 4, Rf= 0.10. IR: 1700 cm^{-1}, C=O; 1330 cm^{-1}, CF_3. ^{1}H-NMR: δ 0.18 (s, 9H, $Sn(CH_3)_3$); 3.74 (d, 1H, J= -9 Hz, $COCH_2$); 4.71 (d, 1H, J= -9 Hz, $COCH_2$); 7.18-7.76 (m, 7H, ArH).

Elemental Anal:

Calc'd: C: 46.59 H: 3.91 N: 5.41

Found: C: 46.99 H: 3.96 N: 4.99

FAB Mass spectrum indicates one chlorine and one tin (with the loss of CH_3)

M/e :	499	500	501	502	503	504	505	506	507
Rel. intensity:	0.283	0.267	0.742	0.448	1.00	0.263	0.338	0.080	0.164
Theory:	0.256	0.256	0.713	0.408	1.00	0.275	0.383	0.079	0.172

Radiolabelling.

Radionuclide production.

$[^{18}F]F_2$ was produced by irradiating a Ne + 0.5% F_2 gas mixture in a nickel target with deuterons (10 μA, 10 MeV, 1 h) using the Åbo Akademi 103 cm AVF isochronous cyclotron. The $[^{18}F]F_2$ gas was either used directly or converted to $[^{18}F]CH_3COOF$ by reaction with ammonium acetate in glacial acetic acid.

Labelling with $[^{18}F]F_2$.

The $[^{18}F]F_2$ (7.0 μmol) gas was trapped at 0°C in acetonitrile (10 ml) containing the trimethylstannyl precursor **2** (4.0 mg, 7.8 μmol). The reaction was immediate and the solution was subsequently evaporated to dryness. The residue obtained was redissolved in acetonitrile (1 ml) prior to radio-TLC and -HPLC analysis. The elution time (8-10 min) and Rf= 0.56 (system 5) of the major product were consistent with those of a reference sample of 2-oxoquazepam.

Labelling with $[^{18}F]CH_3COOF$.

The $[^{18}F]F_2$ was trapped in a solution of ammonium acetate (15 mg, 0.19 mmol) in glacial acetic acid (10 ml). A portion (0.9 ml) of the CH_3COOF formed corresponding to 6.3 μmol was added to the trimethylstannyl precursor **2** (4.0 mg, 7.8 μmol). The reaction mixture was ultrasonicated for a few seconds, water was added and the solution extracted with CH_2Cl_2. The organic layer was evaporated to dryness and the product was dissolved in acetonitrile (1 ml) prior to analysis by HPLC and radio-TLC. Essentially one labelled product was observed which eluted with the same retention time on HPLC and with the same Rf-value as 2-oxoquazepam.

For preparation of the sample for spectroscopic analysis of the reaction product, the trimethylstannyl precursor **2** (15.5 mg, 30.1 μmol) was reacted with 3.7 ml of the $[^{18}F]CH_3COOF$ (26.3 μmol). The product was purified by injecting aliquots of the reaction mixture onto the HPLC column (to prevent over-loading). The radioactive fractions corresponding to $[^{18}F]$-2-oxoquazepam were collected, combined and evaporated to dryness.

In a preparative run, the trimethylstannyl precursor **2** (36.9 mg, 71.7 μmol) was placed in a Lidex Mixxor, liquid-liquid extraction system, equipped with tubing for the addition and removal of reagents. [^{18}F]CH_3COOF (61 μmol) in acetic acid (7.5 ml) was added and mixture was agitated until the precursor dissolved by pneumatically forcing the plunger up and down. Water (10 ml) and CH_2Cl_2 (10 ml) were added and the product was extracted into the organic phase. The aqueous phase was removed and CH_2Cl_2 was washed twice with H_2O (5 ml). The remaining organic phase was concentrated by flushing with N_2 and heating.

The residue obtained was injected onto the semi-preparative HPLC. To minimize chemical contamination by the defluorinated product **3**, only the major part of the fraction corresponding to 2-oxoquazepam was collected. The mobile phase was removed by evaporation with a rotary evaporator. Dissolution of [^{18}F]-2-oxoquazepam required a lipophilic medium consisting of propylene glycol:ethanol:physiological saline.

ACKNOWLEDGEMENTS

This work has been supported by grants from the Bank of Sweden's Tercentenary Foundation (project nr 427) and the Swedish Medical Research Council (project nr 3560) which are gratefully acknowledged.

The authors also wish to thank Mr. K. Aho for assistance with the radionuclide production; Dr. T. M. Chan and Dr. M. Puar for NMR spectra; Dr. B. Paraminik, Mr. R. Malchow, Mr. R. Parks, Ms. S. Hendershot, Mr. P. Bartner and Ms. R. Osterman for mass spectra; and Mrs. B. Evans for elemental analysis.

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