[2'-^{18}F]-2-Oxoquazepam: Synthesis of a 5-(2-[^{18}F]Fluorophenyl)-1,4-Benzodiazepine-2-one

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SUMMARY

The use of a 2-amino-2'-[^{18}F]fluorobenzhydrol as a radiolabelling intermediate in the synthesis of a 1,4-benzodiazepine-2-one is demonstrated. 5-Chloro-2'-[^{18}F]fluoro-2-(*N*-(2,2,2-trifluoroethyl)amino)benzhydrol, 2, was synthesized by the coupling of the anilinodichloroborane reagent with 2-[^{18}F]fluorobenzaldehyde, 1 and was subsequently oxidized to the benzophenone, 3, using Jones reagent in 70-80% conversions after 10 min at 0-5°C. After solid phase extraction, 3 reacted with bromoacetyl bromide to generate the bromoamide, 4, in 90-95% conversions after 10 min at 140°C. Ring closure of 4 to the 1,4-benzodiazepine-2-one, 5, was accomplished using hexamethylenetetramine in aqueous dimethylsulfoxide. Conversions of 80-90% were obtained after 10 min at 100°C. Following preliminary cleaning by solid phase extraction, 5 was isolated by radio-HPLC. The total time of synthesis was 180-190 min and the isolated yield was on the order of 10-12% (decay-corrected) or 3-4% (not decay-corrected) and based on [^{18}F]F$^-$. The radiochemical purity of the isolated 1,4-benzodiazepine-2-one was >99% and the specific activity was ~2000 Ci/mmol at the end-of-synthesis.

Key words: fluorine-18, 2-oxoquazepam, 1,4-benzodiazepine-2-one, PET

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INTRODUCTION

1,4-Benzodiazepine-2-ones of the general structure shown in Figure 1 are widely used in the treatment of neurological disorders such as insomnia, anxiety, seizures and muscle disorders. By varying the substituents R, X and Y, a series of compounds may be synthesized with a range of binding characteristics and biological effects (1). In the 1960's Sternbach and coworkers (1)

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demonstrated that the biological activity of such compounds was increased by the presence of a halogen in the 2'-position (Y = F and Cl). A number of fluorine-substituted 1,4-benzodiazepine-2-ones have been synthesized (for example: flunitrazepam, flurazepam, elfazepam, fluotoprazepam, fludiazepam and 2-oxoquazepam). Clinically interesting drugs are usually tested by *in vitro* evaluations of their binding to the given receptor type and by *ex vivo* measurements of their biodistribution in animals. Positron emission tomography (PET) provides a powerful method for directly observing *in vivo* the distribution of the compound with time in the living animal or human being, provided there is a means for introducing the positron-emitting radionuclide into an appropriate place in the molecule. Several 5-(2-fluorophenyl)-1,4-benzodiazepine-2-ones have previously been labelled with positron-emitting radionuclides in the alkyl side chain ([*N*-methyl-^{11}C]flunitrazepam (2), [*N*-methyl-^{11}C]-fludiazepam (2), and *N*-(2-[^{18}F]fluoroethyl)-norfludiazepam (3)) and with bromine-75 in the 7-position (7-[^{75}Br]bromo-deschloro-fludiazepam (4)).

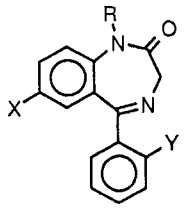


Figure 1

We have previously reported a method for labelling 1,4-benzodiazepine-2-ones with electrophilic fluorine-18 in the 2'-position *via* a destannylation reaction (5). Though attractive as a one-step radiolabelling procedure, use of this method for labelling receptor ligands with fluorine-18 will depend on access to electrophilic fluorinating agents of higher specific activity than those typically available (6,7). In an attempt to develop a route for labelling 1,4-benzodiazepine-2-ones with high specific activity fluoride ([^{18}F]F$^-$) in the 2'-position, we have adapted a method of Sugasawa and coworkers (8) in which anilinodichloroborane reagents are coupled with 2-[^{18}F]-fluorobenzaldehyde, 1, to rapidly produce 2-amino-2'-[^{18}F]fluorobenzhydrols in good yields (9). Here we demonstrate the use of one of these ^{18}F-labelled 2-aminobenzhydrols, 5-chloro-2'-[^{18}F]fluoro-2-(*N*-(2,2,2-trifluoroethyl)amino)benzhydrol, 2, in the synthesis of high specific activity [2'-^{18}F]-2-oxoquazepam, 5 (previously reported in a preliminary communication (10)). 2-Oxoquazepam binds with a higher selectivity to the benzodiazepine binding site subtype-1, BZ-1, ($\alpha1 > \alpha2 = \alpha3 = \alpha5$) (11-14). The availability of the radiolabelled ligand with appropriately high specific activity might enable studies of the distribution of these receptor subtypes *in vivo* with PET.

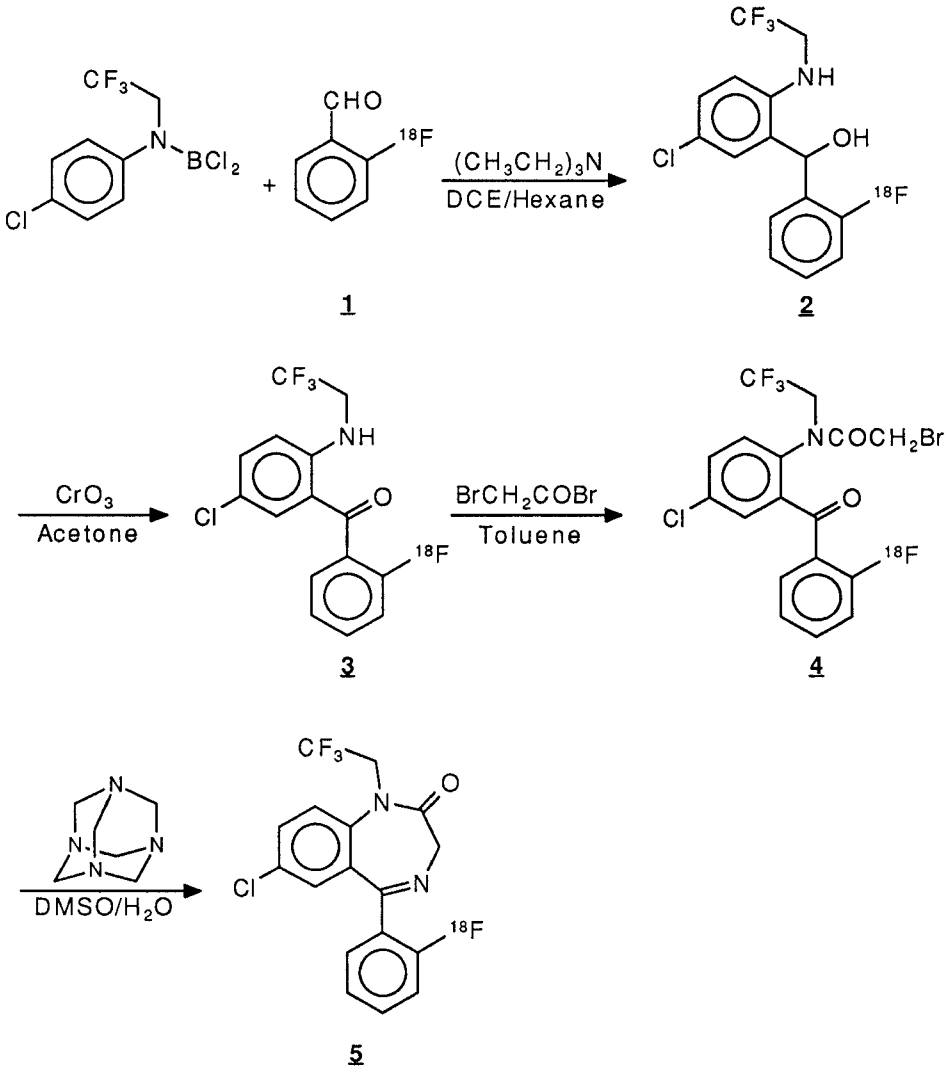
The method used is shown in Figure 2. The 3-step procedure from 2 consists of (**A**) oxidation of 2 to the corresponding benzophenone, 5-chloro-2'-[^{18}F]fluoro-2-(*N*-(2,2,2-trifluoroethyl)amino)-benzophenone, 3, (**B**) reaction with an α-haloacetyl halide to form the α-haloamide, 5-chloro-2'-[^{18}F]fluoro-2-(*N*-(2,2,2-trifluoroethyl)bromoacetamido)benzophenone, 4, and, finally, (**C**) ring closure to the 1,4-benzodiazepine-2-one, 7-chloro-1,3-dihydro-5-(2-[^{18}F]fluorophenyl)-1-(2,2,2-trifluoroethyl)-2H-1,4-benzodiazepine-2-one, 5. Despite the number of radiolabelling steps involved, the ability to optimize to short times and good conversions demonstrates that the use of 2-amino-2'-[^{18}F]fluorobenzhydrols as radiolabelling intermediates is a feasible approach for synthesizing [2'-^{18}F]-1,4-benzodiazepine-2-ones of high specific activity.

RESULTS AND DISCUSSION

(A) Oxidation

Conversion of 2 to 3 was readily achieved when performed as in the literature method (15) with Jones reagent (aqueous chromic acid, CrO_3, in sulfuric acid and acetone). Conversions were followed by radio-HPLC and -TLC for reaction times up to 15 min. All benzhydrol had reacted after 10 min at 0°C with conversions to the benzophenone on the order of 70-80%. To remove the oxidizing agent quickly and efficiently, solid phase extraction procedures with SepPak columns (normal phase Al and Si) were investigated. It was found that the alumina cartridge adequately retained the red chromium reagent while 3 was readily eluted with acetone. The acetone (4 - 5 ml) was quickly evaporated by heating in an oil bath (100°C) under N_2 gas flow.

Figure 2



(B) Condensation with the haloacetyl halide

To form the α-haloamide 4, 3 was reacted with bromoacetyl chloride ($BrCH_2COCl$) or with bromoacetyl bromide ($BrCH_2COBr$). The results (as evaluated by radio-HPLC and -TLC) for one comparative study are given in Table 1. Lower conversions were obtained with $BrCH_2COCl$ at longer reaction times, although these results were improved when the reagent was freshly distilled before use. With $BrCH_2COBr$ good conversions were obtained after 10 min at both temperatures investigated. Repeated experiments indicated that the yields with $BrCH_2COBr$ were not as sensitive to its storage time and this reagent was therefore chosen for its reliability.

Table 1. Evaluation of reaction conditions for the conversion of 3 to 4

Haloacetyl halide	Temp (°C)	Conversions (%)		
		10 min	20 min	30 min
$BrCH_2COCl$	100	15%	33%	42%
	140	18%	46%	67%
$BrCH_2COCl$[a]	140	69%	95%	-
$BrCH_2COBr$	100	90%	-	-
	140	95%	-	-

[a]Reagent was freshly distilled prior to use

(C) Ring closure

Before performing the cyclization to the 1,4-benzodiazepine-2-one, unreacted $BrCH_2COBr$ from step (B) was removed as completely as possible. Variations in yields in step (C) caused by residual acetyl halide were confirmed by the observation that when additional $BrCH_2COBr$ was intentionally added to the reaction mixture, no 5 was obtained at all. $BrCH_2COBr$ was removed by evaporating the toluene solution from step (B) to dryness (140-150°C and N_2) and then adding toluene twice to the reaction vessel with evaporation after each addition.

The ring closure of 4 to 5 was accomplished using hexamethylenetetramine (16). The results obtained with several solvents under otherwise identical conditions are given in Table 2. The

Table 2. Effect of solvent on the conversion of 4 to 5 at 100°C

Solvent	Time (min)	Conversions (%)
Ethanol (99%)	20	36
	60	73
Isopropanol:H_2O = 85:15	10	46
	20	77
DMSO	10	33
	20	78
DMSO:H_2O = 88:12	10	85
DMSO:H_2O = 70:30	10	60

alcoholic solvents previously used in this type of reaction, ethanol (16) and aqueous isopropanol (5), were initially tested. Good conversions could be obtained with both solvents though the reaction times required with ethanol were longer than for aqueous isopropanol. This reaction is proposed to proceed *via* an S_N2 type mechanism (17), a type of reaction which often may be accelerated by the use of dipolar aprotic solvents. When DMSO was used as the solvent, similar reaction times to those observed with aqueous isopropanol were required for comparable yields. Including small amounts of water in the DMSO increased the yields at a given reaction time, with the best results being obtained for DMSO:H_2O = 88:12.

(D) Isolation

Solid phase extraction procedures were used to reduce the mass to be injected on the HPLC column. Dilution of the reaction mixture and elution through a SepPak C-18 removed the hexamethylenetetramine and DMSO from reaction (C). Lipophilic reagents and byproducts from the previous steps were subsequently reduced by elution through a SepPak Si cartridge.

Isolation of 5 was first attempted using a reversed-phase column (μ-Bondapak C-18, Waters). However, with all solvent systems tested, the ^{18}F-labelled product obtained was contaminated to varying degrees by an unidentified chemical impurity and large amounts of radioactive product were lost (> 75%) when the mobile phase was adjusted to minimize this chemical impurity. A straight-phase system suggested for the separation of benzodiazepines (18) was found to work well (μ-Bondapak-NH_2 column, Waters, mobile phase = ethyl acetate:hexane). [2'-^{18}F]-2-oxoquazepam, 5, was thus isolated in high radiochemical purity (> 99%) with much lower losses of radioactivity (< 30%).

Analysis on the reversed-phase system following isolation by the straight-phase system revealed no detectable amounts of chemical impurities (the UV-trace at λ = 222 nm for the isolated product solution was identical to that of a standard solution of reference 2-oxoquazepam). A major chemical component which might be expected to cause separation problems would be any amounts of the 2'-nitro-1,4-benzodiazepin-2-one co-synthesized, by the same reactions as for 5, from the 2-nitrobenzaldehyde used in the synthesis of 2. We were, however, unsuccessful in synthesizing reference 2'-nitro-1,4-benzodiazepin-2-one by this method nor has it, to our knowledge, been reported in the literature. 1,4-Benzodiazepine-2-ones with nitro groups in the 5-aryl ring have been prepared by direct nitration, with substitution occurring primarily in the meta position (19). On the straight-phase system used for the preparative separation (18) it was found that a relationship between the capacity factor, k', and the σ_m values could be used to predict the relative retention times for the benzodiazepines. A base-line separation was obtained for 5 (2'=F) and halazepam, the corresponding defluorinated compound (2'=H), with these chromatographic conditions. The difference between the σ_m values for the -F and -NO_2 groups is of the same magnitude as between -F and -H. We therefore predict that 5 would also have separated from any 2'-nitro-1,4-benzodiazepin-2-one formed in the cyclization reaction since the relative order of elution on this column should have been aryl-H < aryl-F < aryl-NO_2.

After removal of the mobile phase by evaporation, a lipophilic media was required for dissolution of the residue prior to sterile filtration. Use of saline alone resulted in large losses of radioactivity by absorption on the Millipore filter. The product was radiochemically stable in the

mobile phase as well as in the formulation media (no radiochemical impurities observed after 4 h, according to radio-TLC). The specific activity obtainable for this no-carrier-added synthesis depends to a large extent on that of the [^{18}F]F$^-$ used, which with radionuclide production systems similar to the one used here is usually very high (6, 20). A typical value obtained for [2'-^{18}F]-2-oxoquazepam was ~ 2000 Ci/mmol at end-of-synthesis (180-190 min after start-of-synthesis with 150-170 mCi [^{18}F]F$^-$).

CONCLUSIONS

The labelling of a 1,4-benzodiazepine-2-one, 2-oxoquazepam, using no-carrier-added [^{18}F]F$^-$ has been accomplished *via* the corresponding 2-amino-2'-[^{18}F]fluorobenzhydrol, 2. The entire sequence consists of the evaporation of the target water, five chemical transformations, four solid phase extraction procedures, two solvent evaporations and a final isolation by radio-HPLC. The conditions used as well as the individual and cumulative results obtained are summarized in Table 3. The conversions given indicate the range observed in repeated experiments (n>10).

Table 3. Procedure for the production of 5

	Reaction Conditions	Conversions[a] %	Total Time[b] (min)	Radioactivity[c] (% of Total)
Evaporation of target water (1.2 ml)	120°, 25 min	-	25	85
[^{18}F]F$^-$ to 1	120°, 10 min	55-70	50	40-50
1 to 2	60°, 10 min	50-70	80	17-22
2 to 3	0°, 10 min	70-80	100	13-15
3 to 4	140°, 10 min	90-95	120	10-12
4 to 5	100°, 10 min	80-90	135	8-10
Isolation	50 min	-	185	3-4

[a]Based on analyses by radio-HPLC and -TLC

[b]From start of synthesis, including times for individual isolations and/or clean-up procedures

[c]Actual yields, not decay-corrected, including physical losses for the separate handling procedures

In spite of the rather large number of steps involved, this entire procedure was accomplished in ~1.5 half-lives of the radionuclide. The total yields obtained were most affected by variations in the yields of the nucleophilic aromatic substitution with [^{18}F]F$^-$ and in the coupling of 2-[^{18}F]fluorobenzaldehyde to generate the ^{18}F-labelled benzhydrol, 2. The anilinodichloroborane reagent used here with *N*-alkyl = -CH_2CF_3 was previously observed to be the least reactive and most sensitive to the coupling conditions of all the anilines tested (9). The successful use of this reagent to generate the 2-amino-2'-[^{18}F]fluorobenzhydrol in sufficient quantities for the next three reactions demonstrates the feasibility of this approach for synthesizing 5-(2-[^{18}F]fluorophenyl)-1,4-benzodiazepine-2-ones of high specific activity. The conversions of the five-step method ranged

from 15-25%, based on [^{18}F]F$^-$ and according to analyses by radio-HPLC. However, the physical losses associated with the individual isolation procedures decreased this value to the actual isolated yields of 10-12% (decay-corrected) or 3-4% (uncorrected for decay).

EXPERIMENTAL

General methods

All solvents used were commercially available and were of analytical grade. Dimethylsulfoxide (DMSO) was distilled from BaO under reduced pressure and stored over molecular sieves (4 Å) in the refrigerator. Toluene was stirred with H_2SO_4 (18 M), dried with $CaCl_2$, distilled and stored over molecular sieves (4 Å). CrO_3 was obtained from Merck, $BrCH_2COCl$ from Aldrich and $BrCH_2COBr$ and hexamethylenetetramine from Janssen Chimica. Reference 5-chloro-2'-fluoro-2-(*N*-(2,2,2-trifluoroethyl)amino)benzophenone and 2-oxoquazepam were supplied by Schering Plough Corp. SepPak (C-18, Al (N) and Si) cartridges were obtained from Waters.

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 and 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 or 222 nm) and radioactivity, respectively. A Shimadzu C-R4A integrator was used for peak processing.

Isolation by HPLC was performed using a μ-Bondapak-NH_2 column (Waters 300 x 3.9 mm, 10 μm) and a Shimadzu LC-6A pump (flow 1.0 ml/min). An LDC Spectromonitor II coupled in series with a GM tube were used to monitor the UV-absorption (λ=254 nm) and radioactivity, respectively. The mobile phases used were:

Analytical HPLC: CH_3CN:H_3PO_4 (0.01 M) 50:50

Semi-preparative HPLC: Ethyl acetate:hexane 23:77

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: CH_2Cl_2

TLC system 3: toluene:ethyl acetate 2:1

Synthesis of reference compound for 4

5-Chloro-2'-fluoro-2-(*N*-(2,2,2-trifluoroethyl)amino)benzophenone (103.3 mg, 0.31 mmol) was dissolved in toluene (10 ml). $BrCH_2COBr$ (136 μl, 1.56 mmol) was added dropwise under N_2 atmosphere at room temperature. The resulting solution was heated at reflux with stirring. The progress of the reaction was followed by TLC (system 1) to completion at 2 hr. The solution was cooled to room temperature and then washed with saturated $NaHCO_3$ (3x2 ml) and H_2O (2x2 ml).

The organic phase was dried over Na_2SO_4. After removal of the drying agent by filtration, the solvent was evaporated. Purification of the residue on silica gel using CH_2Cl_2:petroleum ether 3:1 as eluent yielded a slightly yellow oil (114.5 mg, yield 82%). The product was one spot on TLC system 2 R_f=0.45 and TLC system 3 (R_f= 0.78). IR: 1680 cm^{-1}, N-C=O; 1665 cm^{-1}, $(Ar)_2C$=O. ^{1}H-NMR ($CDCl_3$): δ 7.78-7.1 (m, 7H, ArH); 4.77 (m, 1H) and 3.73 (m, 1H) (-CH_2CF_3); 3.81 (d, 1H) and 3.64 (d, 1H) (-CH_2Br). MS: *m/z* (relative intensity,%) 452 (32), 454 (41), 456 (13) and 330 (100). HRMS: calcd for ^{35}Cl, ^{79}Br: 450.9598, found: 450.9624; calcd for ^{35}Cl, ^{81}Br: 452.9578, found: 452.9608

Radiolabelling

Synthesis of 2

Optimization of the production of 2-amino-2'-[^{18}F]fluorobenzhydrols from [^{18}F]F^- has been described in detail in a separate communication (9). Briefly, the Kryptofix 2.2.2 / K_2CO_3 complex of [^{18}F]F^- was reacted with 2-nitrobenzaldehyde in DMSO for 10 min at 120°C. After isolation by SepPak C-18 and drying through a Na_2SO_4 column, the hexane solution of 1 was added to the dichloroethane solution (1.5 ml) of the anilinodichloroborane reagent (0.35 mmol) formed from 4-chloro-*N*-(2,2,2-trifluoroethyl)aniline. After reaction at 60°C for 10 min, the reaction was quenched by basic hydrolysis and the organic phase was separated. Analysis of the product mixture indicated one major radioactive product (conversions = 50-70%) which coeluted with the reference compound on analytical HPLC (ret. time: 12 min) and TLC system 1 (R_f= 0.4). The labelled benzhydrol 2 was subsequently separated from the reagents and unreacted 1 using a solid phase extraction procedure as follows: the organic phase was diluted in hexane (18 ml) and passed through a SepPak Si. The cartridge was rinsed with additional hexane (20 ml) and 2 was subsequently eluted with acetone (3 ml).

Oxidation of 2 to 3

The acetone solution of 2 was cooled to 0°C. Jones reagent (CrO_3 32.2 mg, (0.32 mmol); 27.5 µl H_2SO_4 (18 M); 93 µl H_2O) in acetone (0.5 ml) was added dropwise while stirring. After completion of the addition the solution was allowed to stand for 10 min and then eluted through an alumina SepPak to remove CrO_3. The SepPak was rinsed with acetone (2 ml) and the combined fractions were evaporated (100°C, N_2). The residue was used in next step without further purification. Analysis of the product mixture indicated one major radioactive product (conversions = 70-80%) which coeluted with the reference compound on analytical HPLC (ret. time: 20 min) and TLC system 1 (R_f= 0.8).

Condensation of 3 with $BrCH_2COBr$ to form 4

The residue containing 3 was dissolved in toluene (1 ml) and $BrCH_2COBr$ (30 µl, 0.34 mmol) was added. The reaction mixture was stirred at 140°C for 10 min and subsequently evaporated to dryness (140-150°C, N_2). To ensure that all $BrCH_2COBr$ was removed, toluene (1 ml) was added twice followed by evaporation to dryness after each addition. The time required for the three evaporations was on the order of 5 min. The residue obtained was used in the next step without

further purification. Analysis of the product mixture indicated one major radioactive product (conversions = 90-95%) which coeluted with the reference compound on analytical HPLC (ret. time: 15 min) and TLC system 1 (R_f= 0.15) and TLC system 3 (R_f= 0.78).

Ring closure of 4 to 5

The residue containing 4 was dissolved in DMSO (1.05 ml) and hexamethylenetetramine (48 mg, 0.34 mmol) dissolved in H_2O (0.15 ml) was added. The reaction mixture was stirred for 10 min at 100°C. Analysis of the product mixture gave one major new radioactive product (conversions = 80-90%) which coeluted with the reference compound on analytical HPLC (ret. time: 9 min) and TLC system 3 (R_f= 0.48).

Isolation

The DMSO:H_2O solution was diluted with aqueous CH_3CN (20%, 10 ml) and eluted through a SepPak C-18. After rinsing with additional aqueous CH_3CN (20%, 10 ml), the product was eluted with CH_2Cl_2 (4 ml). Hexane (4 ml) was added to the eluent and the solution was passed through a SepPak Si, followed by CH_2Cl_2:hexane (1:1, 10 ml) before eluting the radioactive product with CH_3CN (3 ml). The CH_3CN was evaporated to dryness (100°C, N_2) and the residue dissolved in mobile phase (0.2 ml) prior to isolation by HPLC. The fraction corresponding to [2'-^{18}F]-2-oxoquazepam eluted after 19.5-21 min. The organic solvents were rapidly removed by evaporation on a rotary evaporator. The residue was dissolved in propylene glycol: ethanol: physiologically buffered saline (2.1: 0.9: 5) and filtered through a Millex-GV filter (Millipore).

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