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ELECTROPHILIC AND NUCLEOPHILIC APPROACHES TO THE SYNTHESIS OF 3-FLUORODIAZEPAM

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SUMMARY

Electrophilic fluorination of diazepam with elemental fluorine or gas phase acetyl hypofluorite gave 3-fluorodiazepam in 20-60% yields. Nucleophilic displacement of chlorine in 3-chlorodiazepam by fluoride ion also gave 3-fluorodiazepam in modest yields (maximum: 21%). Both of these methods were used for the synthesis of ^{18}F-labelled 3-fluorodiazepam.

INTRODUCTION

1,4-Benzodiazepine derivatives [1] have enjoyed continued clinical application for their anxiolytic, anticonvulsant and hypnotic behavior in humans [2]. The presence of high affinity, stereospecific binding sites for benzodiazepines in the mammalian brain seems to be responsible for these characteristic pharmacological actions [3]. Preliminary studies in animals have shown that 3-fluorodiazepam (3) (3-fluoroderivative of Valium®) is a valuable ligand for investigating the benzodiazepine receptor system [4]. A positron emitting radiotracer such as the ^{18}F (half-life, 110 min) labelled derivative of 3-fluorodiazepam could be useful for mapping the benzodiazepine receptor sites *in vivo* by positron emission tomography (PET) [5] since (a) the potential of 3-fluorodiazepam as a benzodiazepine agonist has been confirmed by studies in rats where it has been shown to be at least 5 times more potent than the parent diazepam itself, and (b) fluorine substitution in 3 would inhibit C-3 hydroxylation, a principal pathway of benzodiazepine metabolism, thereby increasing its metabolic stability *in vivo* [4].

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3-Fluorodiazepam (3) has been previously synthesized by the reaction of diazepam or its 3-lithioenolate with perchloryl fluoride [6]. Middleton et al. [7] have recently reported a high yield (80%) synthesis of 3, by the reaction of trifluoromethyl hypofluorite with the 3-trimethylsilylenol ether of diazepam. A rearrangement of a 1-hydroxy-1-fluorohalomethylquinazoline derivative [8] and the reaction of diethylaminosulfur trifluoride (DAST) with 3-hydroxydiazepam [4] also yield 3-fluorodiazepam. A priori, these procedures can be extended to the synthesis of ^{18}F-labelled 3. However, all these methods involve either multistep syntheses, highly moisture sensitive intermediates, or require the syntheses of the starting reagents $ClO_3{}^{18}F$, $CF_3O{}^{18}F$ and ^{18}F-DAST which are rather time consuming for ^{18}F-radiolabelling. Hence we have developed two simple syntheses of 3 involving (a) an efficient, direct fluorination of diazepam and (b) nucleophilic substitution at C-3 with fluoride ion. Both reactions are also suitable for radiolabelling.

RESULTS AND DISCUSSION

The use of fluorine diluted with inert gases and a still milder fluorinating agent, acetyl hypofluorite, are rapidly gaining importance in organofluorine chemistry [9,10]. Following this trend, we have developed a novel one step synthesis for 3-fluorodiazepam (3) from the commercially available diazepam (Sigma) and fluorine. A nucleophilic approach has also been developed for the synthesis of 3.

(a) Electrophilic fluorination of diazepam (1)

Reaction of fluorine (0.5% in neon v/v) or acetyl hypofluorite generated in the gas phase [11,12] with diazepam (1) gave 3-fluorodiazepam (3). The reaction was conducted in various solvents and the results are summarized in Table I. The reaction was found to be sensitive to (a) stoichiometry of the fluorinating agent and the substrate, (b) polarity of the reaction medium, and (c) the nature of the fluorinating agent. Use of 1:1 fluorine to diazepam ratios resulted in complex mixtures which were difficult to purify. However, when a 2 to 3 molar excess of diazepam was used, formation of the by-products resulting from multisite fluorination and possible oxidation of the starting material was minimized, and 3-fluorodiazepam was isolated in good yields (see

Table I). The role of the polarity of the solvent in this reaction is not clear and is the subject of further investigation. However, solvents of low polarity ($CHCl_3$ and $CFCl_3$) gave better yields than polar solvents (HOAc, CH_3CN) in which complex product mixtures resulted.

TABLE 1
Electrophilic fluorinations of diazepam

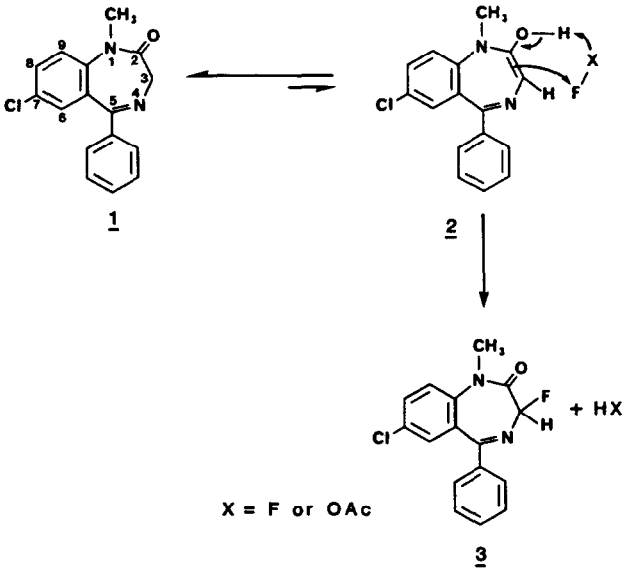
Fluorinating agent	Solvent	Yield, %
F_2	Glacial HOAc	24
F_2	CH_3CN	21
F_2	$CHCl_3$	63
F_2	$CFCl_3$	57
F_2 (in the presence of oxygen)	$CFCl_3$	45
AcOF[a]	Glacial HOAc	15
AcOF[a]	$CHCl_3$	21
AcOF[a]	$CFCl_3$	18
$[^{18}F]F_2$	$CHCl_3$	25[b]

[a] Generated in gas phase from $NaOAc.3H_2O$[12].
[b] Radiochemical yield relative to the initial amount of $[^{18}F]F_2$ (Maximum possible: 50%).

Site specific fluorinations with fluorine or acetyl hypofluorite are rare [13]. The use of elemental fluorine diluted with inert gases in organic synthesis is a relatively recent concept, and the literature on site-selective fluorinations with elemental fluorine, the most reactive element known, is not very extensive [9,10]. The more recently prepared reagent acetyl hypofluorite [14] has been characterized only recently [15], and its use is now gaining popularity.

To the best of our knowledge, the present work represents the first direct and controlled fluorination with fluorine of one of the most widely used pharmaceutical systems —— 1,4-benzodiazepines. Since the presently reported fluorination of diazepam (1) to produce 3-fluorodiazepam (3) involves electrophilic attack at a carbon alpha to a carbonyl group, it is worth noting that only recently the first examples of direct α-fluorination of carbonyl derivatives, such as pyruvic acid derivatives, using fluorine have been published [16]. Based on these studies [16] and the direct fluorination of phenols with elemental fluorine [17], a possible concerted cyclic mechanism for the reaction of fluorine or acetylhypofluorite with the enol form [18] of

diazepam (1) is given in Scheme 1. A probable radical pathway for the reaction could be reasonably excluded based on the success of the reaction in the presence of a radical inhibitor such as oxygen [19] (See Experimental Section).



Scheme 1

It has been observed that the milder fluorinating agent, acetyl hypofluorite, generally gave better yields than fluorine in several reactions [9]. However, it is rather surprising that a reverse in that trend was observed in the reaction of diazepam with fluorine and acetyl hypofluorite. In both $CHCl_3$ as well as $CFCl_3$, better yields of 3 were obtained using fluorine rather than acetyl hypofluorite as the fluorinating agent. In this particular case, this can be rationalized by comparing the hardness of F^- and OAc^- (the conjugate bases in the acid HX, Scheme I). With fluoride ion being the harder base, the abstraction of the enol proton (a hard acid) by fluorine should be more efficient than by the acetate group [20] and, hence, the higher yields of 3 with fluorine.

(b) Nucleophilic approach to the synthesis of 3-fluorodiazepam (3)

The marked reactivity of C-3 in 3-chlorodiazepam (4) has been earlier recognized [21]. Thus, we attempted the nucleophilic displacement of chlorine

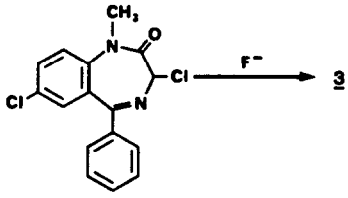
in 4 by fluoride ion to gain access to 3 (Scheme 2). Results are summarized in Table 2. From the data, it can be seen that the solvent plays a major role in this reaction. Very low yields of 3 and significant formation of by-products were observed with DMF and DMSO as the reaction medium. In the case of DMSO, this may be primarily attributed to thermal oxidative dechlorination [22] of the starting chlorodiazepam, since 3-hydroxydiazepam was the main product of the reaction. Thus, improved yields of 3 were realized in acetonitrile using tetra-n-butylammonium fluoride as a source of fluoride ion to increase its solubility.

TABLE 2

Nucleophilic substitution of 3-chlorodiazepam with fluoride ion

Source of fluoride ion	Solvent	Temp. °C/time	Yield, %
CsF	DMSO	70°/4h	7
CsF	DMSO	130°/1h	0
CsF	DMF	120°C/2h	0
$n\text{-}Bu_4NF$	CH_3CN	80°/4h	21
$K^{18}F$	CH_3CN/Kryptofix 222	80°/0.5h	15[a]

[a] Radiochemical yield based on $K^{18}F$.



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Scheme 2

(c) Syntheses of 3-^{18}F-fluorodiazepam

The electrophilic as well as the nucleophilic methods described above were extended to the synthesis of ^{18}F-labelled 3 with equal ease. For example, the nucleophilic substitution of chlorine in 4 was advantageously carried out with

no-carrier-added (nca) $K^{18}F$ complexed with the cryptand Kryptofix 222 in acetonitrile [23]. Since no-carrier-added $K^{18}F$ can be prepared with very high specific activities (10^4 Ci/mmol) [24], the nucleophilic method gave 3-^{18}F-fluorodiazepam with a specific activity of 2-10 Ci/μmol. On the other hand electrophilic fluorination of diazepam with ^{18}F-labelled fluorine gave the radiolabelled 3 with a specific activity of ~5 Ci/mmol since $[^{18}F]F_2$ can be prepared only in low specific activities (5-10 Ci/mmol) [24]. Preliminary studies of the kinetic behavior of 3-^{18}F-fluorodiazepam (both low and high specific activity) in the brain of nemistrina monkeys evaluated with PET [5] are indicative of the potential of the compound for human application [25]. Complete details of these studies will be reported elsewhere.

CONCLUSION

A novel and simple one step electrophilic method for the synthesis of 3-fluorodiazepam using fluorine or acetyl hypofluorite was developed. A nucleophilic route involving the reaction of 3-chlorodiazepam with fluoride ion also gave the required fluoro derivative. Both of these methodologies were easily extended to the synthesis of ^{18}F-labeled 3-fluorodiazepam.

EXPERIMENTAL

Melting points reported are uncorrected. The IR spectra were recorded on a Perkin-Elmer Model 710B infrared spectrometer. Proton NMR spectra were recorded on a Bruker WP-200 (200 MHz) spectrometer and the chemical shifts are expressed in ppm (δ) from TMS as internal standard. High-pressure liquid chromatography (HPLC) was carried out on a Waters M-45 instrument equipped with an Altex Model 153 UV detector and a Beckman model 170 Radioisotope detector. Fluorine (0.5% in neon) was purchased from Matheson. Diazepam (1) was purchased (Sigma) and 3-chlorodiazepam (4) was synthesized as reported in the literature [26,27]. Kryptofix 222 (4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]hexacosan) was obtained from Fluka.

Caution: Fluorine and acetyl hypofluorite are highly reactive and toxic. Thus proper care should be exercised while handling them [28]. All processes with fluorine-18 should be carried out using remote procedures [29].

General procedure for the electrophilic fluorination of diazepam (1)

Fluorine (100 μmol, 0.5% in neon) or gas phase acetyl hypofluorite (prepared from 150 μmol of fluorine in neon) [11,12] was bubbled over a period of 20 min into a solution of diazepam (1) (0.085g, 300 μmol) in 15 mL of appropriate solvent taken in a glass reaction vessel at room temperature. After evaporation of the solvent under vacuum, the residue was subjected to silica gel column chromatography. Elutions with dichloromethane: ethylacetate (5:1) gave 3-fluorodiazepam (3) in the initial fractions; mp 135-137°C (lit. [4] mp 138-140°C); IR ($CHCl_3$) 1705 cm^{-1}(C=O); ^1H NMR ($DCCl_3$): δ3.39(s,3H,CH_3), 5.51 [d, 1H,H(3),J(^1H-^{19}F)=57.0Hz], 7.20-7.60(m,8H,ArH). Unreacted diazepam (1) was generally recovered quantitatively in the latter chromatographic fractions. The isolated yields of 3 are reported in Table I.

Fluorine-18 labelled 3-fluorodiazepam (specific activity ~5 Ci/mmol) was synthesized from $[^{18}F]F_2$ as follows: Fluorine-18 labelled F_2 was produced by the $^{20}Ne(d,\alpha)^{18}F$ nuclear reaction by deuteron irradiation (9.4 MeV on target at 30 μA for 1 hr) of 0.2% fluorine (100-200 μmol) in neon [30]. At the end of bombardment, the $[^{18}F]F_2$ contained in the target chamber was purged into the reaction vessel containing diazepam in the appropriate solvents (Table I). Processing and work-up were similar to that described above for the unlabelled 3. Thus starting from 100 mCi of ^{18}F-fluorine, 25 mCi of ^{18}F-fluorodiazepam were isolated in a synthesis time of 40 min. The chemical and radiochemical purities of the final product were checked by HPLC (Beckman Ultrasil octyl column, 10 μm, 4.6 X 250 mm, 60% methanol and 40% water, flow rate 1.0 mL/min) monitoring the UV absorption of the effluent at 254 nm and the 511 keV radioactivity. Both the chemical and radiochemical purities of the final product were >99%. Knowing the mass associated with ^{18}F-fluorodiazepam from the UV absorption, the specific activity of the final product was calculated [24] to be ~5 Ci/mmol.

Reaction of fluorine with diazepam in the presence of oxygen

Into a solution of diazepam (0.057g, 200 μmol) in freon (15 mL), dry oxygen gas was bubbled for about 15 min at room temperature to remove any dissolved gases. This was followed by simultaneous bubbling of fluorine (100 μmol, 0.5% in neon) and dry oxygen. Workup of the reaction mixture was performed as described above.

Nucleophilic substitution of 3-chlorodiazepam (4) with fluoride ion

3-Chlorodiazepam (0.064g, 200 μmol) was reacted with 200 μmol of the appropriate fluoride salt in a dry solvent (5 mL) under argon. The product was isolated after evaporation of the solvent under vacuum and purified by column chromatography as described above. The final product melted at 135-137°C (lit. [4] mp 138-140°C) and was characterized by ^{1}H NMR as given above. Relevant details are provided in Table 2.

Preparation of no carrier added 3-^{18}F-fluorodiazepam

No-carrier-added (nca) potassium [^{18}F]fluoride was prepared by the addition of potassium acetate (1.0 mg, 10 μmol) and Kryptofix 222 (4.0 mg, 10.6 μmol) to proton irradiated O-18 enriched water [31]. The water was evaporated at 105°C with a stream of dry nitrogen and the last traces of moisture were removed by azeotropic distillation with acetonitrile (2 X 1 mL). A solution of 3-chlorodiazepam (0.010g, 31 μmol) in acetonitrile (0.8 mL) was added to the dry nca K^{18}F and the reaction mixture was heated for 30 min at 75°C under nitrogen. The solvent was evaporated using a stream of nitrogen at 75°C and the residue dissolved in dichloromethane (0.5 mL) and purified by HPLC (Alltech Silica Column, 10 μm, 10 X 250 mm, 90% dichloromethane and 10% ethyl acetate, flow rate 5.0 mL/min). Retention time for the starting material was 5.1 min and the 3-^{18}F-fluorodiazepam fraction that eluted at 6.7 min was collected and further checked for the chemical and radiochemical purities by analytical HPLC as described above and found to be >99%. The specific activity of the final product was determined as described above and found to be 2-10 Ci/μmol.

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