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Synthesis and Characterization of 7α-O-Iodoallyl diprenorphine : A New Ligand for Potential SPECT Imaging of Opioid Receptors

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SUMMARY : The preparation and synthesis of [^{125}I]7α-O-iodoallyl diprenorphine, a high affinity opioid receptor antagonist, is described using a versatile vinylstannane as prosthetic group for radioiodination at the tertiary alcohol group in the 7α-side chain. Radioiododestannylation with selective conditions in one step occurs under mild, no-carrier-added-conditions to give the corresponding [^{125}I]7α-O-iodoallyl diprenorphine analogue in good radiolabelled yields (70-90%) with specific radioactivity 80 TBq/mmol (2200 Ci/mmol) and radiochemical purity >95%. Iodoallyl diprenorphine exhibited *in vitro* a very high affinity (K_i=0.4 nM), so that this radioligand could be suitable for imaging opioid receptors in living humans by Single Photon Emission Computed Tomography (SPECT).

Key words : Ligand, vinylstannane, iodoallyl diprenorphine, SPECT, opioid receptor

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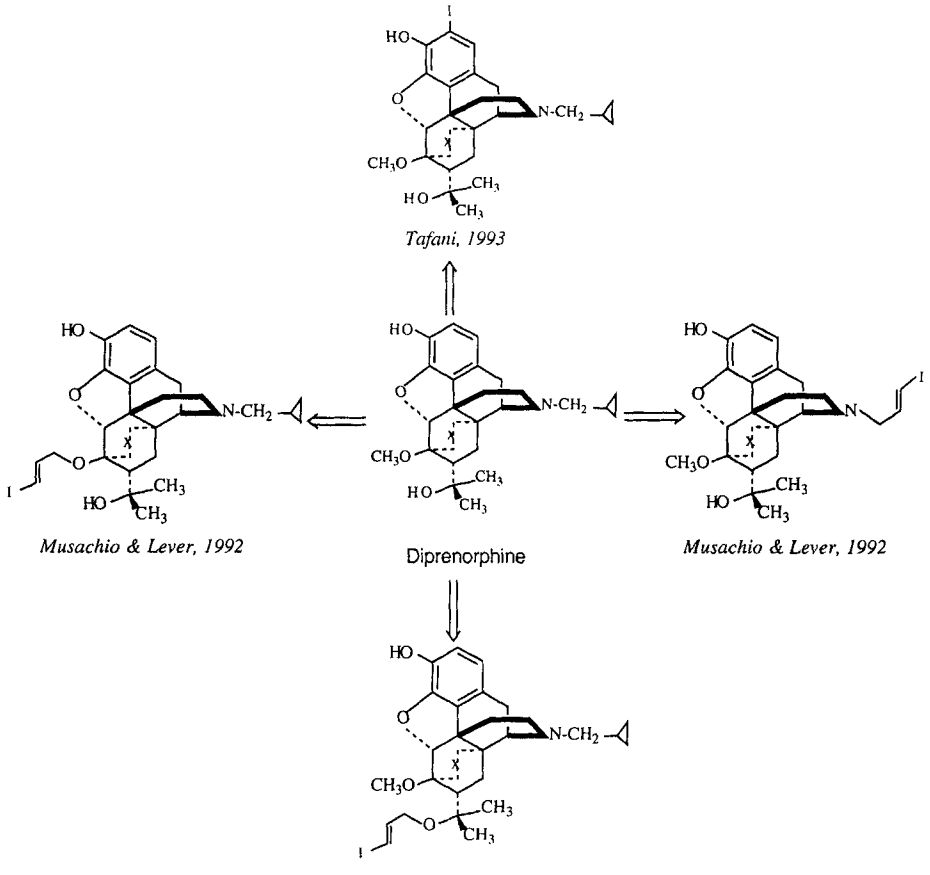
Introduction

Recently, diprenorphine (DPN) of 6,14-endo-etheno-oripavines, a universal opioid antagonist showing a high affinity for μ, δ and κ opioid receptors, has been used for imaging opioid receptors in living humans by Positron Emission Tomography (PET) studies using [^{11}C]-diprenorphine (^{11}C-DPN) [1, 2, 3, 4, 5], and [^{18}F]-Fluoropropyl-N-nordiprenorphine (^{18}F-FDPN) [6]. On the other hand, studies of iodinated oripavine analogues for performing *in vivo* exploration of the opioid receptor system by Single Photon Emission Computed Tomography (SPECT) have not been reported so widely. There is therefore considerable interest in the development of an efficient synthesis of diprenorphine radio-labelled with ^{123}I, a short-lived radionuclide, as a potential SPECT imaging agent for non-invasive visualisation and quantification of human opioid receptors because this imaging technique would be more suitable than PET for widespread clinical application.

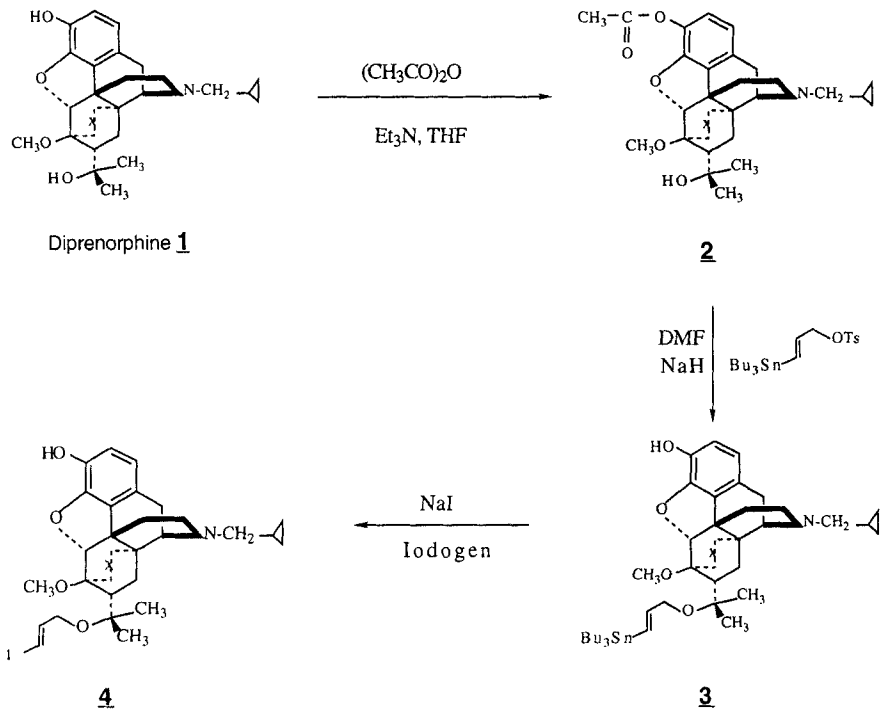
Musachio and Lever [7] have reported the synthesis of two 6-O-iodoallyl and N-iodoallyl diprenorphine analogues from thebaine in eight steps for SPECT study of opioid receptors [Scheme I]. Nevertheless, this approach implies modification of the environment of the 6-methoxy and 17-cyclopropylmethyl groups which play a very important role in pharmacological effects of 6,14-endo-ethenomorphinan derivatives or oripavines [8, 9]. In addition, the classically direct electrophilic radioiodination technique [Scheme I] resulted in a lowered affinity (20-fold less for diprenorphine) and a diminished pharmacological efficiency [11]. In order to avoid significant modifications in receptor binding capacity or biological activity as well as the use of tedious synthetic procedures, we selected diprenorphine (DPN) as a template for the development of a new O-iodoallyl analogue at the less crucial tertiary alcohol group position in the 7α-side chain.



Scheme I. Iodinated analogues of diprenorphine as radioligands for potential SPECT study of opioid receptors

Our strategy was based on the derivatization of the alcohol group by a vinylstannane moiety which permits an easy iodination outside the aromatic ring in 6,14-endo-ethenomorphinan derivatives or oripavines, leading to the radiolabelled target in good radiochemical yields with high specific radioactivities [10]. In this paper, we report the design, synthesis, radiolabelling and *in vitro* opioid receptor (μ, δ and κ) binding characteristics of this new original agent (7α -O-iodoallyl diprenorphine).

Scheme II. Synthetic route to 7α -O-iodoallyl diprenorphine



Results and discussion

The general synthetic outlines for this work are shown in scheme II. The synthetic strategy is based on the functionalization of the tertiary alcohol group of the diprenorphine using the [E]-3-(tri-n-butylstannyl)prop-2-en-1-ol-p-toluenesulfonate as prosthetic group. This reactant obtained from propargyl alcohol in a two step procedure involved an addition of tributyltin hydride [12] followed by the tosylation of the alcoholic function with p-toluenesulfonyl chloride according to the approach of Musachio and Lever [7]. This compound can be stored for at least six months at -20° without any decomposition.

Before carrying out this functionalization, this strategy requires a regiospecific protection of the phenolic group. It was realized quantitatively by acetylation with acetic anhydride in the presence of

triethylamine in THF solution, leading to a white crystalline solid. Under these conditions, no detectable reaction was observed on the tertiary alcohol group in the 7α-side chain.

During the key step of introducing of the vinylstannane moiety to the tertiary alcohol group in the 7α-side chain by reaction of 3-acetyldiprenorphine 2 with the tosylate using NaH as a base, a concomitant deacetylation led to a 90 % yield of the desired precursor 3. The deprotection of the phenolic group was an essential prerequisite to the use of such compounds for imaging. Indeed, it is well known that the free phenolic group is essential in morphine and 4,5-epoxy-6,14-ethenomorphinan derived agents for enhancing both antinociceptive potency as well as opioid receptor binding affinity in the study of human cerebral central nervous system with PET and SPECT imaging [13]. Thus this protection of the phenolic group as an acetate in our synthetic pattern proved to be superior to other alternatives used in morphinan chemistry [13, 14] by virtue of the high yields obtained and by avoiding a specific deprotection step.

Radiosynthesis. Even in the presence of the highly activated aromatic ring, treatment of compound 3 with NaI and iodogen in CH_3CN/10% H_2SO_4 (65/35, v/v) gave exclusively by ipso-substitution the iodoallyl diprenorphine 4 in 70-90% yield. Mass spectroscopy (CIMS) gave m/z calcd, 591.52; found, 592.52 (MH^+).

Radioiododestannylation of compound 3 with [^{125}I]NaI and iodogen at room temperature in CH_3CN/10% H_2SO_4 for 2 min provided [^{125}I]7α-O-iodoallyl diprenorphine 4 of 80 TBq/mmol (2200 Ci/mmol) in 70-90% radiochemical yield with more than 95% radiochemical purity after HPLC purification.

The most attractive feature of this synthesis of universal opioid ligand [^{125}I]7α-O-iodoallyl diprenorphine is its rapidity and regioselectivity. The tributylstannyl group allows one to iodinate selectively the vinyl substituent in the presence of an unprotected

phenolic group in contrast to other electrophilic radiohalogenations in the 6,14-endo-ethenomorphinan derivatives or oripavines which require the protection of the phenolic group either as the acetate [15, 16, 17] or the methyl ether [7]. Since no protecting groups are used, the radiosynthesis is reduced to a simple step. The physico-chemical properties of the starting material and the final product differing substantially, the purification of the final product can be easily performed by reverse-phase chromatography. Electrophilic destannylation provides an easy and selective method for radiolabelling organic compounds and is well suited for selective labelling of small molecules with radioisotopes without modifying the molecular biochemical activity in the study of neurotransmission by SPECT [18, 19, 20].

Receptor binding assays. To determine the opioid receptor binding affinity of the prepared compounds, their K_i values were measured by competition with [^{3}H]diprenorphine (0.2 nM) in mice brain membrane preparation (7 mg/mL). Hill's transformation of the binding data was treated to obtain values for the inhibition constant (K_i). The results obtained for these competition binding experiments are given in Table I.

Among the compounds evaluated, the 7α-O-iodoallyl diprenorphine has a high receptor binding affinity, similar to that of the initial compound 1. The affinity of 4 was 21.5-fold higher than that obtained by direct electrophilic iodination in the aromatic ring (K_i=8.6 nM) [11]. The 6-fold lower K_i values of stannylDPN 3 as compared to 7α-O-iodoallylDPN 4, could be explained by the presence of a bulky tri-n-butylstannyl group in the C-7α-side chain [9]. More interestingly, this new ligand is 3.4 and 58.5-fold more potent respectively than 6-O-iodoallylDPN (K_i=1.35 nM) and N-iodoallylDPN (K_i=23.4 nM) as recently reported by Lever et al. [21].

Table I. Physicochemical properties (formula, CIMS) and comparative affinity (K_i, nM) of DPN 1, stannylDPN 3 and 7α-O-iodoallylDPN 4 for opioid receptor binding in mice brain membranes.

Compound	Formula (formula wt)	CIMS m/z, (MH^+)	K_i, nM (mean±SD, n=3)
DPN (1)	$C_{26}H_{35}NO_4$ (425.56)	426.56 (100%)	0.21±0.16
StannylDPN (3)	$C_{41}H_{65}NSnO_4$ (754.15)	755.15 (100%)	2.33±0.62
7α-O-iodoallyl DPN (4)	$C_{29}H_{38}NIO_4$ (591.52)	592.52 (100%)	0.40±0.03

CONCLUSION

We prepared [E]-17-(cyclopropylmethyl)-4,5α-epoxy-18,19-dihydro-3-hydroxy-6-methoxy-7α-[1-(3-iodoallyl)oxy-1-methylethyl]-6,14-endo-ethenomorphinan (7α-O-iodoallyl diprenorphine) through a iododestannylation approach using a vinylstannanyl precursor as prosthetic group. The radiolabelling of the stannylDPN 3 was carried out in one step using iodogen as the oxidizing agent to afford compound 4 ([^{125}I]7α-O-iodoallylDPN) with high specific activity and high radiochemical yield. The high affinity of 7α-O-iodoallyl diprenorphine (K_i=0.4 nM) strongly suggests that this new radioprobe may be a useful agent for future SPECT studies of opioid receptors.

Experimental Section

DPN was purchased from Reckitt and Colman Ltd., Kingston-upon-Hill, England, [^{3}H]diprenorphine from Amersham Buchler, Braunschweig,

Germany and [^{125}I]NaI from Cis Biointernational, France. Tetrahydrofuran (THF) was freshly distilled before use. Dimethylformamide (DMF), acetonitrile (anhydrous, gold standard), propargyl alcohol, tri-n-butyltin hydride, azobis-isobutyronitrile (AIBN), tosyl chloride, potassium trimethylsilanolate and sodium hydride were purchased from the Aldrich Chemical Co. All other chemicals were of reagent grade and used without further purification, unless otherwise specified. Infrared spectra were recorded on a Perkin-Elmer 883 apparatus as KBr pellets or in $CHCl_3$ solutions and NMR spectra were carried out on a Bruker-200 spectrometer using $CDCl_3$ solutions. Fast-atom bombardment mass spectra (FABMS) were recorded with a VG 2AB-SE double focusing mass spectrometer and chemical-ionization mass spectra (CIMS) were obtained using a Nermag R-10 mass spectrometer both by direct insertion probe. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel plates. Flash chromatography was performed using Merck silica gel 60 (230-400 mesh). The HPLC system used included a Waters 600E pump, a linear UV-450 spectrometer (285 nm), a NaI (Tl) radioactivity detector ST7, S. A. I. P. with associated electronics, and a Macherey Nagel nucleosil column C18 (20 mmx30 cm). The column was eluted with CH_3OH/H_2O/AcOH (65/34/1, v/v/v) at a flow rate of 4.0 mL/min. All reactions were run under an argon atmosphere.

3-Acetyldiprenorphine (2) : A mixture of diprenorphine 1 (base) (85.1 mg, 0.2 mmol) and Et_3N (24.3 mg, 0.24 mmol) in THF (3 mL) was heated with an oil bath and stirred for 5 min at 30 °C under argon atmosphere. Then acetic anhydride (194 mg, 1.9 mmol) was added dropwise over a 10-min period and the mixture was stirred for 90 min. The reaction was monitored by TLC on silica gel using $CHCl_3$/MeOH/NH_3 (99/1/0.3, v/v/v) as eluent. The reaction was not stopped until TLC indicated complete reaction (single uniform spot, R_f=0.51). The solvent was evaporated *in vacuo* to furnish quantitatively compound 2

as a white crystalline solid. **^{1}H NMR (80 MHz, $CDCl_3$)** : δ 2.24 (s, 3H, C*H*$_3$CO). The other proton assignments were made by analogy with a previous report by Mazza et al. [22] : 0.08-0.85 (m, 6H, cyclopropyl H and H18*syn*), 1.02-1.35 (m, 8H, H18*anti*, H8α, CH_3 α and β), 1.72-2.35 (m, 9H, H15*eq*, H19, H7β, H15*ax*, CH_2N, H10α, H16*ax*), 2.26-3.03 (m, 4H, H16*eq*, H8β, H10β and H9), 3.44 (s, 3H, OCH_3), 4.37 (s, 1H, H5), 5.06 (broad s, 1H, OH), 6.57 (d, 1H, J=8 Hz, H1), 6.75 (d, 1H, J=8 Hz, H2). **^{13}C NMR (62.90 MHz, $CDCl_3$)** : δ 166.71 (C=O), 20.56 (*C*H$_3$C=O). The other carbon signals were identified by analogy to previous studies [22] : 149.68 (C), 134.41 (C), 133.52 (C), 131.45 (C), 122.10 (CH), 119.23 (CH), 98.27 (*C*HO), 80.40 (C), 74.50 (C), 59.91 (CH_2), 58.12 (CH), 52.70 (*C*H$_3$O), 47.91 (CH), 47.00 (C), 43.62 (CH_2), 35.95 (C), 35.25 (CH_2), 32.30 (CH_2), 30.01 (CH_2), 29.79 (CH_3), 24.60 (CH_3), 23.10 (CH_2), 17.06 (CH_2), 9.35 (CH cyclopropyl), 4.25 (CH_2 cyclopropyl), 3.40 (CH_2 cyclopropyl). **IR (KBr, cm^{-1})** : 3413 (aliphatic OH), 1766 (C=O). **CIMS ($C_{28}H_{37}NO_5$)** : m/z calcd, 467.56; found, 468.56 (MH^+).

Synthesis of [E] 7α-O-[[(3-tri-butylstannyl)prop-2-enyl]oxy]-diprenorphine (3) : To a 25 mL biconical vial containing 35 mg (0.07 mmol) of 3-acetyldiprenorphine 2 in DMF (3 mL) was added NaH (17 mg, 0.71 mmol) prewashed with petroleum ether. The mixture was stirred for 2 minutes prior to addition of [E]-3-(tri-butylstannyl)prop-2-en-1-ol-p-toluenesulfonate (180 mg, 0.35 mmol) in DMF (1 mL). After stirring for 4 h at 30 °C, saturated NH_4Cl (1 mL) was added when TLC on silica gel using $CHCl_3$/MeOH/NH_3 (99/1/0.3, v/v/v) as eluent indicated complete reaction. The mixture was extracted with ether (3x3 mL), the extracts were combined, and the solvent was removed under vacuum. The residue chromatographed on silica gel (eluting with petroleum ether/ethyl acetate; 95/5, v/v) afforded compound 3 (56 mg, 0.105 mmol) in 90% yield as a thick and brown color oil. For the ^{1}H NMR spectral data only the signals assigned to the vinylstannane moiety linked to diprenorphine are listed. **^{1}H NMR (80**

MHz, $CDCl_3$) : 6.22 (overlapping multiplet, 2H, vinyl protons), 4.63 (d, 2H, J=3.6 Hz, $OCH_2CHCHSn$), 0.95, 0.94, 0.91 and 0.86 (CH_2 and CH_3 groups of the butyl chain). **^{13}C NMR (62.90 MHz, $CDCl_3$)** : δ 143.49 (*C*H allyl), 131.67 (*C*H allyl), 73.40 (CH_2O), 29.73 and 26.92 (CH_2 butyl), 13.73 (CH_3 butyl), 9.47 (CH_2 butyl). The other carbon signals were attributed from a 2D NMR study of diprenorphine [22] : 147.56 (C), 140.47 (C), 132.63 (C), 127.23 (C), 119.00 (CH), 116.74 (CH), 97.01 (CHO), 80.51 (C), 74.43 (C), 59.93 (CH_2), 58.22 (CH), 52.75 (CH_3O), 47.93 (CH), 46.94 (C), 43.77 (CH_2), 35.95 (C), 35.66 (CH_2), 32.39 (CH_2), 30.00 (CH_2), 29.78 (CH_3), 24.93 (CH_3), 22.69 (CH_2), 17.62 (CH_2), 12.90 (CH cyclopropyl), 4.24 (CH_2 cyclopropyl), 3.37 (CH_2 cyclopropyl). **IR ($CHCl_3$, cm^{-1})** : 3447 (phenolic OH), 1671 (C=C allyl). **CIMS ($C_{41}H_{65}NO_4Sn$)** : m/z calcd, 754.15; found, 755.15 (MH^+).

[E]-17-(cyclopropylmethyl)-4,5α-epoxy-18,19-dihydro-3-hydroxy-6-methoxy-7α-[1-(3-iodoallyl)oxy-1-methylethyl]-6,14-endo-ethenomorphinan (4) : To a solution of **3** (1 mg, 1.33 μmol) in MeOH (100 μL) in a glass tube was added NaI (1 mg in solution of 100 μL H_2O, 6.6 nM) followed by 70μL of CH_3CN containing 10% aqueous H_2SO_4 (65/35, v/v). A bead of solid Iodogen (Simiod®, Cerebio) was added, and the contents gently stirred at ambient temperature for 5 min. HPLC purification (Waters, 600A, C-18) using MeOH/H_2O/AcOH (60/39/1, v/v/v; 4 mL/min) as eluent provided compound **4** (t_R=8 min). Concentration under N_2 and then lyophilisation gave 300 μg as a white powder in 70% yield. CIMS ($C_{29}H_{38}INO_4$) : m/z calcd, 591.52; found, 592.52(MH^+).

[^{125}I]7α-O-iodoallyl diprenorphine (4') : To a solution of **3** (100 μg, 0.133 μmol) in MeOH (10μL) in a glass tube was added [^{125}I]NaI (10 μL, Ca. 0.5 nmol; 1 mCi) followed by 120 μL of CH_3CN containing 10% aqueous H_2SO_4 (65/35, v/v). A bead of solid Iodogen was added, and then the contents gently stirred at ambient temperature

for 2 minutes. HPLC purification (Waters, 600A, C-18) using MeOH/H_2O/AcOH (65/34/1, v/v/v; 4 mL/min) as eluent provided [^{125}I]7α-O-iodoallyl diprenorphine (t_R=8 min). Concentration under N_2 gave compound 4' in 90% labelled yield. An aliquot was reconstituted in mobile phase (MeOH/H_2O/AcOH; 65/34/1, v/v/v) for examination by analytical reverse-phase HPLC at 4 mL/min. The labelled product (t_R=8 min) was greater than 95% radiochemically pure with a specific activity of 80 TBq/mmol (2200 Ci/mmol).

Receptor Binding Assays : Binding assays were performed in homogenates of mice (CDF1 male, 20-25 g) brain preparation. Protein concentrations of 7 mg/mL were determined by the method of Lowry using BSA as standard [23]. Receptor preparations were routinely used at a final protein concentration of 0.20 mg/mL. [^{3}H]diprenorphine (31.04 Ci/mmol) binding assays were performed in a final volume of 0.5 mL 50 mM Tris-HCl (pH=7.4 at 25 °C) with different concentrations of the unlabelled DPN, stannylDPN and labelled [^{127}I]7α-O-iodoallylDPN. K_i values were calculated from IC_{50} values according to the Cheng-Prusoff equation [24]. All binding assays were conducted in three separate determinations using a single mice brain membrane preparation performed with each tube in triplicate.

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