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Synthesis, NMR characterization and pharmacological evaluation of ligands derived from diprenorphine for central opioid receptors imaging

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ABSTRACT: The aim of this work was to explore the synthesis of a tosylated derivative of diprenorphine (DPN) able to be radiolabeled with either fluorine-18 or iodine-123, making it suitable for PET or SPECT imaging studies of central opioid receptors, respectively. The strategy was based on the reactivity of the C-19 alcohol tertiary function. As an unexpected deacetylation of the phenolic function of the diprenorphine occurred, the prosthetic group reacted with the deprotected C-3 phenolic function instead of the C-19 alcohol group. UV spectroscopy and ^{1}H and ^{13}C NMR studies provided good evidence for the 3-phenolic substituted diprenorphine structure. Thorough 2D NMR experiments such as ^{1}H–^{1}H COSY, ^{1}H–^{1}H TOCSY, ^{1}H–^{13}C HMQC, ^{1}H–^{13}C HMBC and ^{1}H–^{1}H NOESY allowed us to assign fully 3-*O*-[(*Z*)-4-fluorobut-2-enyl] diprenorphine (**10a**) and gave us an unambiguous proof of the C-3 prosthetic group position. *In vitro* binding studies showed low affinity for both fluoro and iodo derivatives of diprenorphine, $K_i = 0.31 \pm 0.05$ and 0.09 ± 0.03 μM, respectively, for mouse brain membranes, these inhibition constants also being in agreement with a 3-phenolic substituted structure. Copyright © 2001 John Wiley & Sons, Ltd.

KEYWORDS: radiopharmaceutical compounds; diprenorphine; opioid receptors; nucleophilic halogenation; NMR; structural determination

INTRODUCTION

In order to understand more thoroughly the role and the functions of the opioid receptors and their interactions with effectors *in vivo*, the development of metabolically stable non-peptidic ligands with high affinity and good penetration in the central nervous system is a research area in constant development. Opioid receptors are widely distributed in mammalian systems, both in the central nervous system,[1] and at the periphery.[2] It is generally thought that stimulation of μ receptors leads to analgesic effects, respiratory depression, euphoria and physical dependence,[3] whereas κ receptors, when stimulated, produce analgesia;[4] δ receptors play a role in spinal analgesia but are involved in other biological processes such as immune response.[5]

In recent years, positron emission tomography (PET) has emerged as an important technique for studying opioid receptors in animals and humans[6] to explore their clinical involvement in several diseases such as epilepsy,[7] pain,[8] drug addiction[9] and dyskinesia.[10] Diprenorphine (DPN) or 6,14-*endo*-etheno-oripavines (Fig. 1), a universal opioid antagonist displaying a high affinity for μ, δ and κ opioid receptors, has been used for imaging opioid receptors in living humans by PET using [^{11}C]diprenorphine[11] and *N*-([^{18}F]fluoropropyl) nordiprenorphine.[12] Other selective ligands, such as [^{11}C]carfentanil[13] and [^{11}C]methylnaltrindole[14] have also been used for PET imaging of μ and δ opioid receptors respectively. Studies of iodinated oripavine analogues for performing *in vivo* exploration of the opioid receptors by single photon emission computed tomography (SPECT) have been reported. Musachio and Co-workers have reported the synthesis of two 6-*O*-iodoallyl- and *N*-iodoallyldiprenorphine analogues from thebaine in eight steps for the SPECT study of opioid receptors.[15] 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.[16] On the other hand, the introduction of an iodine atom at the *ortho* position of the phenolic group in diprenorphine

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by using the classical direct electrophilic radioiodination technique resulted also in a lowered affinity and a diminished pharmacological efficiency.[17] It is well known that the free phenolic group is essential in morphine, 4,5-epoxy-6,14-ethenomorphinan and naltrindole[18] derived agents for enhancing both anti-nociceptive effects and opioid receptor binding affinity in the study of the human cerebral central nervous system with PET and SPECT imaging. Therefore, radiolabelling of sites removed from the aromatic ring seems to be the most appropriate approach for derivatization.

We report here our approach to introduce a prosthetic group at the C-19 position through the reactivity of the tertiary alcohol group. This prosthetic group functionalized by a tosyl function could be suitable for developing nucleophilic substitution for iodination (SPECT studies) and fluorination (PET studies).

RESULTS AND DISCUSSION

The choice of a radioligand which is metabolically stable

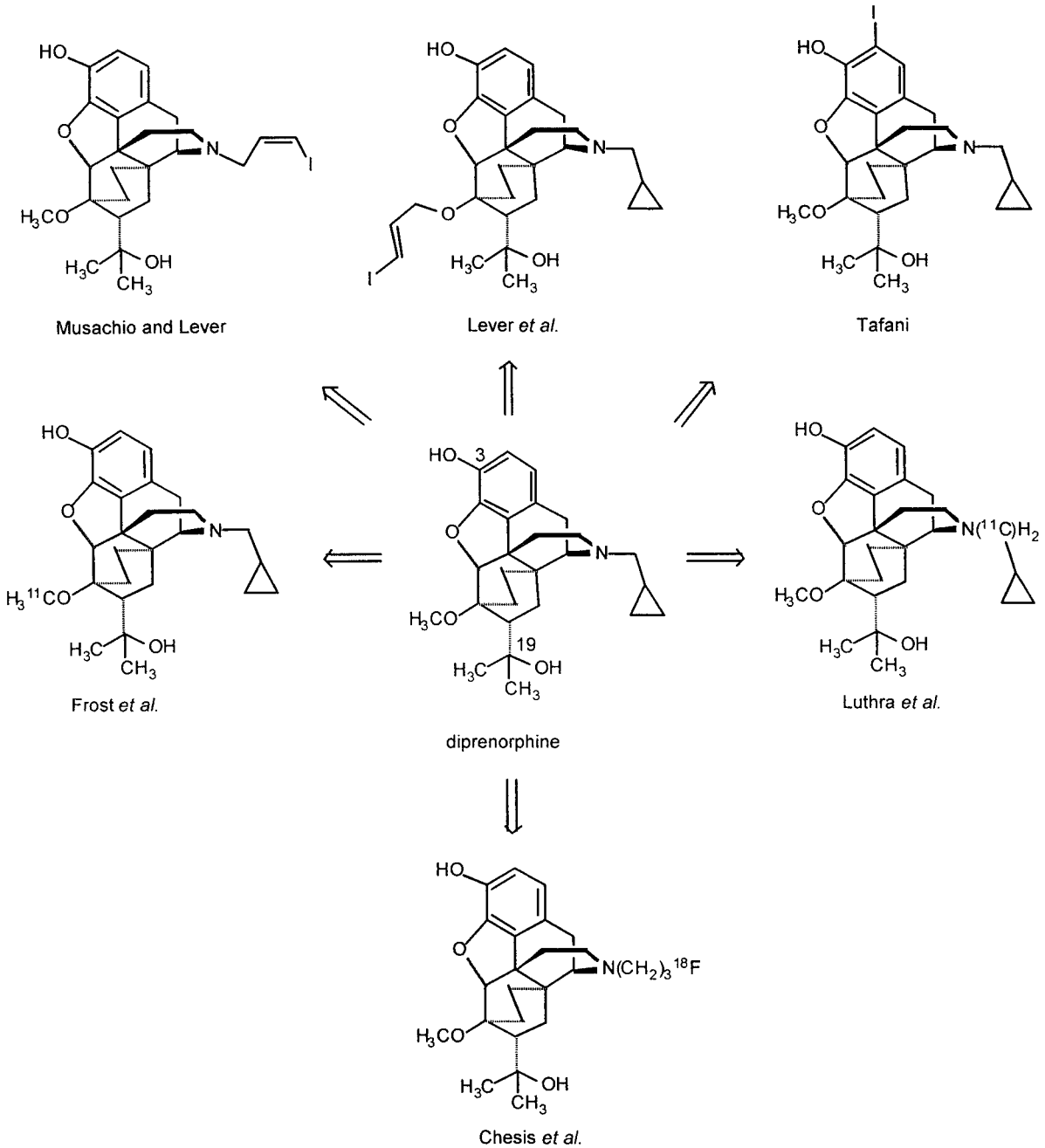


Figure 1. Previous labelled compounds derived from diprenorphine for PET and SPECT imaging

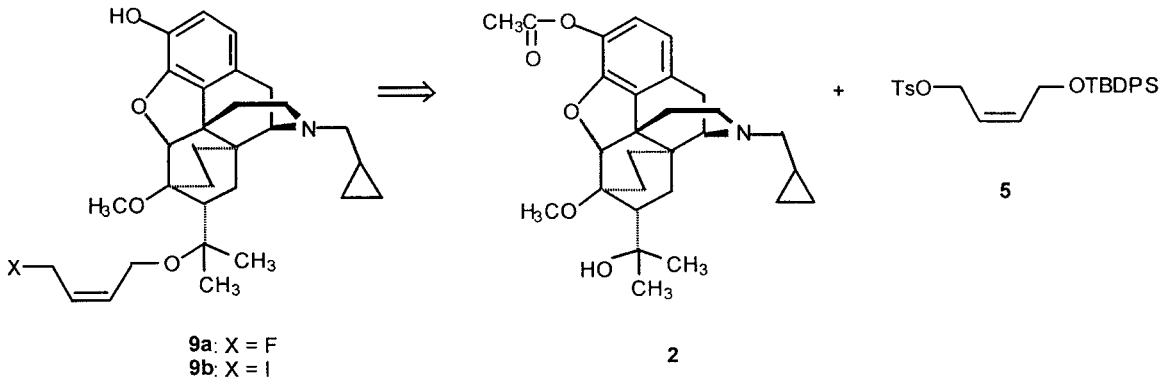


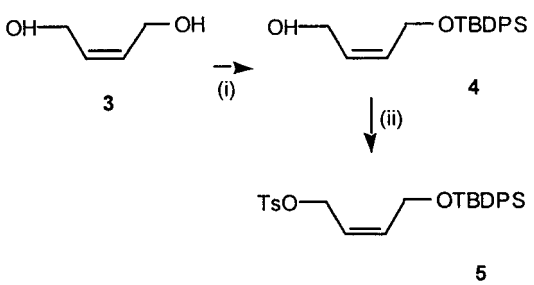
Figure 2. Retrosynthetic scheme of both 19-*O*-[(*Z*)-4-fluorobut-2-enyl]diprenorphine **9a** and 19-*O*-[(*Z*)-4-iodobut-2-enyl]diprenorphine **9b**

in neuronal tissues and exhibits a high degree of specific binding to receptors *in vivo* and low non-specific binding is an essential prerequisite for obtaining quantitative and meaningful measurements of receptor–ligand interactions from PET data.

Synthesis

The synthetic strategy is based on the functionalization of the 3-acetylated diprenorphine **2** using the (*Z*)-4-(*tert*-butyldiphenylsilyloxy)but-2-en-1-ol-*p*-toluenesulfonate **5** as prosthetic group (Fig. 2). This reactant, obtained from the commercially available (*Z*)-but-2-en-diol **3** in a two-step procedure, involved the protection of one of the two alcoholic functions to afford the (*Z*)-4-(*tert*-butyldiphenylsilyloxy)but-2-en-1-ol **4** followed by the tosylation of the second alcoholic function with *p*-toluenesulfonyl chloride (Scheme **1**). This compound **5** can be stored for up to 6 months at −20 °C without any decomposition.

Prior to carrying out the functionalization of diprenorphine, this strategy requires a regiospecific protection of the phenolic group (Scheme **2**). This was realized quantitatively by acetylation of diprenorphine **1** with a large excess of acetic anhydride in the presence of triethylamine in THF solution, leading only to the 3-acetyldiprenorphine derivative **2** as a white crystalline solid. During the key introduction step of silyloxy alkenyl moiety to the C-19 tertiary alcohol group by reaction of **2** with the tosylate **5** using NaH as a base, an unexpected deacetylation occurred, evidenced by the disappearance of the signals of the acetyl group in the ^{13}C NMR spectrum of the final product obtained after 4 h of reaction. Before performing the specific deprotection of the *tert*-butyldiphenylsilyl group with HF–pyridine mixture, a second acetylation of the phenolic group was needed to protect this phenolic function, which might react concurrently with the primary alcoholic group during the final tosylation step. The well-known acetylation procedure of the phenolic group was run unsuccessfully. The observed lack of reactivity seemed to indicate that the phenolic function was linked to the prosthetic group yielding **6**. The C-3-substituted diprenorphine will be demonstrated unequivocally further by NMR structural analysis.

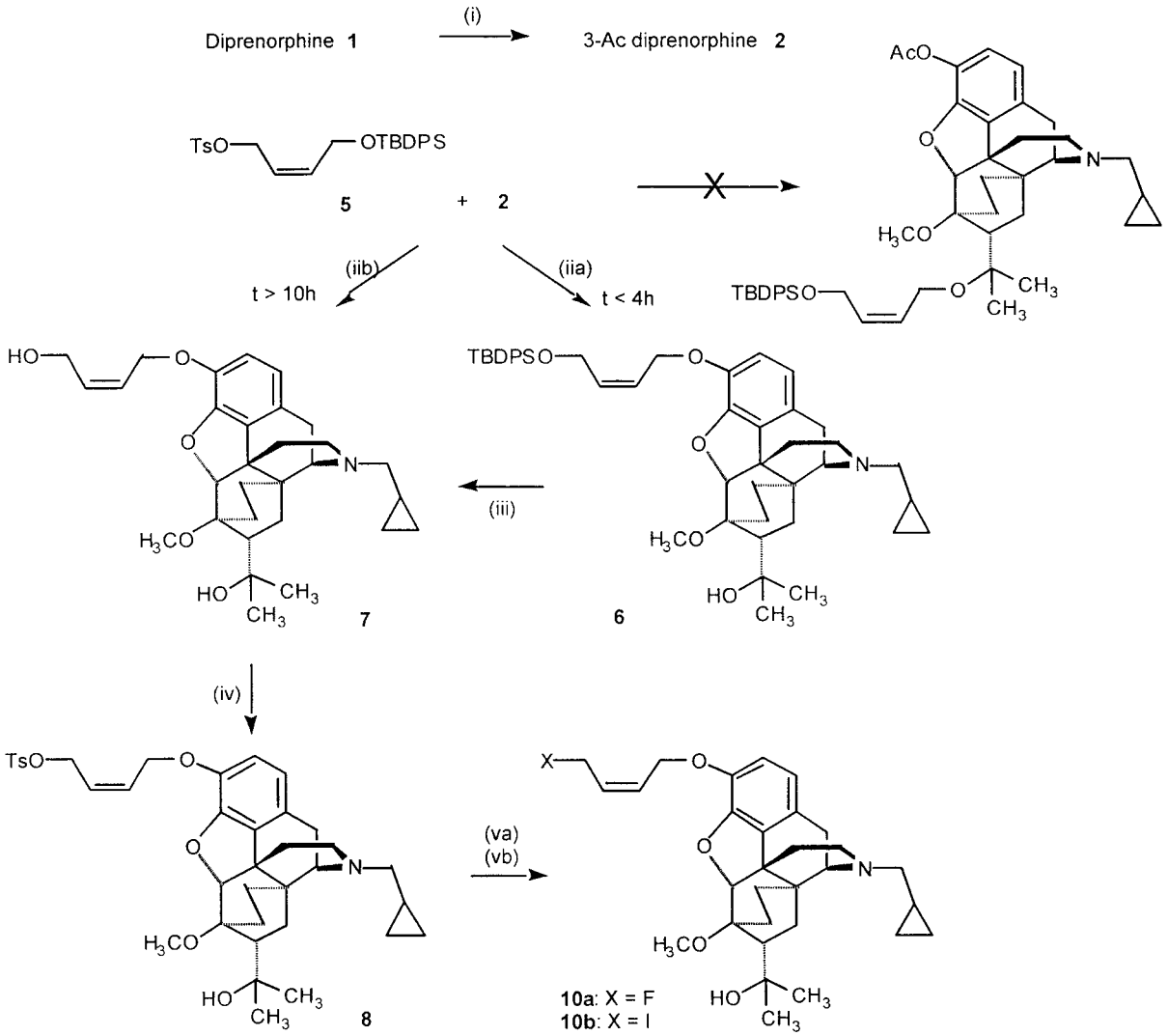


Scheme 1. Synthesis of the prosthetic group **5**. Reagents and conditions: (i) BuLi, TBDPSCl, THF, −78 °C, 86%; (ii) $(CH_3)_3SiOK$, TsCl, Et_2O, 0 °C, 76%

The 3-*O*-[(*Z*)-4-(*p*-toluenesulfonate)but-2-enyl] diprenorphine **8** was obtained from tosylation of the 3-*O*-[(*Z*)-4-hydroxy-but-2-enyl] diprenorphine **7** obtained in two different ways: either after silyl deprotection of **6** or directly using a longer reaction time (>10 h) for the coupling reaction **5** + **2** where desilylation occurred yielding compound **7** directly. The next steps were focused on the reactivity of the tosylate precursor with KF or NaI. The originality of this unique precursor permits labelling with either fluorine or iodine in good yields making it very suitable for radiolabelling with fluorine-18 or iodine-123.

UV analysis

Diprenorphine contains two ionizable sites in aqueous solution. Their pK_a values may be estimated from previous studies[18] on buprenorphine to be 8.24 and 10 for the ammonium and phenol groups, respectively. Buffer solutions were prepared to give two solutions with



Scheme 2. Experimental route leading to 3-*O*-[(*Z*)-4-fluorobut-2-enyl]diprenorphine **10a** and 3-*O*-[(*Z*)-4-iodobut-2-enyl]diprenorphine **10b** instead of the 19-*O*-substituted derivatives. Reagents and conditions: (i) $(CH_3CO)_2O$, Et_3N, THF, 30 °C, 100%; (iia) NaH, DMF, 30 °C, 99%; (iib) 91%; (iii) HF, pyridine, THF, 25 °C, 40%; (iv) TsCl, $(CH_3)_3SiOK$, CH_2Cl_2, 0 °C, 61%; (va) KF, Kryptofix, CH_3CN, 120 °C, 48%; (vb) NaI, acetone, 25 °C, 40.7%

pH 6.9 and 11.0. Diprenorphine and **10a** were diluted with ethanol to obtain 10^{-2} M solutions. Aliquots (50 µl) of the 10^{-2} M ethanolic solution of diprenorphine or **10a** were added to each buffer (3 ml). Spectra for each compound (1.7×10^{-4} M) in the two buffer solutions were recorded between 250 and 350 nm. The UV absorption spectrum of diprenorphine showed a bathochromic shift of 13.5 nm related to the two pH-dependent forms [non-ionized (λ_{max} = 286.5 nm) or ionized (λ_{max} = 300 nm)] of the phenolic group, whereas the spectrum of **10a** was independent of the pH of the buffers. These data showed that the phenolic OH function of the diprenorphine derivative **10a** is substituted, and strongly suggest that the (*Z*)-**4**-(*tert*-butyldiphenylsilyloxy)but-2-en-1-ol-*p*-toluenesulfonate prosthetic group **5** reacted with the phenolic group instead of the C-19 tertiary alcoholic function of the diprenorphine.

NMR analysis

NMR spectral assignments of fluorinated diprenorphine **10a** in deuterochloroform were mainly obtained from 2D homonuclear experiments such as ^1H–^1H correlation spectroscopy (COSY), ^1H–^1H total correlation spectroscopy (TOCSY), 2D heteronuclear ^1H–^{13}C multiple quantum correlations experiments (HMQC) ($^1J^1H$–^{13}C inverse correlations), heteronuclear multiple bond correlations (HMBC) (J^1H–^{13}C inverse correlations) and 2D ^1H–^1H nuclear Overhauser effect spectroscopy (NOESY), which allowed us to observe numerous dipolar (through-space) couplings between protons.

The numbering scheme and stereochemical designations for **10a** are given in Fig. 3. Our analysis of proton–proton couplings and proton–carbon connectivities by

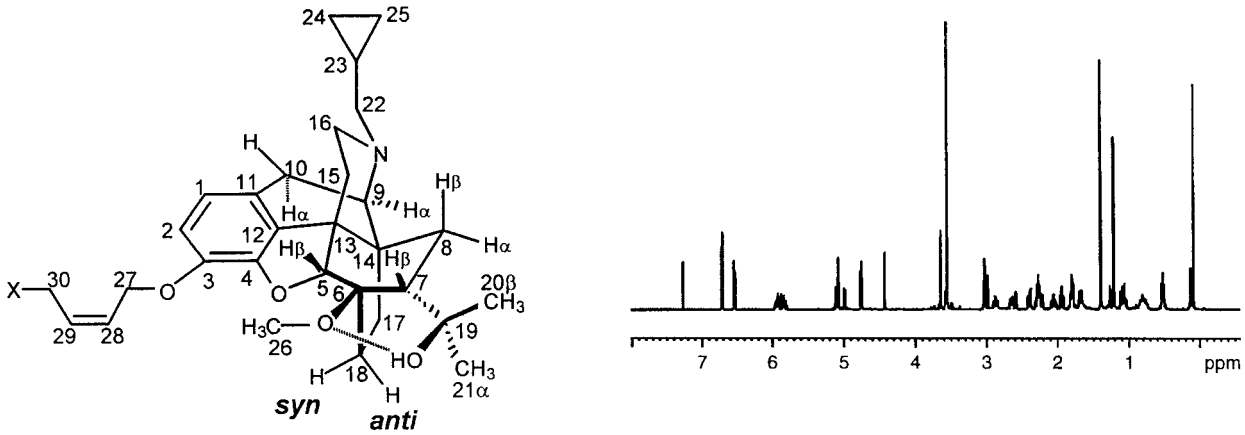


Figure 3. Atom numbering and stereochemical assignments for compounds **10a** (X = F) and **10b** (X = I)

different homo and hetero 2D scalar correlation experiments for the core of the molecule **10a** (Table 1) were in agreement and similar to those established by Mazza *et al.*[19] for the diprenorphine and are therefore not described in detail here. Stereochemical and conformational features of **10a**, in particular the piperidyl and cyclopro-

Table 1. ^{13}C and ^{1}H chemical shifts and coupling constants for selected atoms of **10a** (the positions are taken from Fig. 3)

Position	$\delta^{13}C$ (ppm)	$\delta^{1}H$ (ppm)		J(H–H), J(H–F) and J(C–F) (Hz)
1	119.3	6.71	d	J(H1–H2) = 8.1
2	116.9	6.54	d	J(H2–H1) = 8.1
3	140.0			
4	147.7			
5	97.3	4.42		
6	80.6			
7	48.0	1.93	t	J(H7–H8) = 10.8
8	32.5	1.08(α)	dd	J(H8α–H8β) = 13.4, J(H8β–H17) = 3.9
		2.87(β)	ddd	
9	58.2	3.03	d	J(H9–H10α) = 6.6
10	22.8	2.23(α)	dd	J(H10α–H10β) = 17.7
		3.00(β)	d	
11	130.0			
12	133.2			
13	47.0			
14	36.0			
15	35.7	2.04(ax)	td	J(H15eq–H15ax) = 12.8, J(H15ax–H16eq) = 5.6,
		1.67(eq)	dd	J(H15ax–H16ax) = 12.8
16	43.8	2.27(ax)	dd	J(H16eq–H16ax) = 11.9, J(H15eq–H16eq) = 1.5,
		2.64(eq)	td	J(H15eq–H16ax) = 3.1
17	30.1	1.06(*anti*)	m	
		0.76(*syn*)	m	
18	17.7	1.85-1.74	m	
19	77.6	5.08 (OH)	s	
20	30.0	1.21	s	
21	25.0	1.39	s	
22	60.0	2.39	dd	J(H22–H22′) = 12.5, J(H22–H23) = 5.9,
		2.26	dd	J(H22′–H23) = 6.9
23	9.6	0.81	m	
24	3.5	0.51(*anti*)	m	
		0.11(*syn*)	m	
		0.51(*anti*)	m	
25	4.4	0.11(*syn*)	m	
26	52.8	3.55	s	
27	66.1	4.77–4.73	m	J(H27–F) $\sim$ 3
28	130.1	5.93	m	J(C28–F) = 9.8, J(H28–F) $\sim$ 3
29	127.6	5.84	m	J(C29–F) = 19.5, J(H29–F) = 15
30	79.1	5.09–5.00	m	J(C30–F) = 160, J(H30–F) = 47

pyl rings, also confirmed the results reported by the same authors.

We then had to identify and localize the site where the prosthetic group is linked. Its identification was obtained by 2D COSY and TOCSY experiments. First, a new spin system appeared at a downfield position showing correlations from H-27 to H-30. The fluorine atom was clearly identified to be linked to the C-30 carbon which exhibit a $^{1}J_{C-F}$ coupling of 160 Hz. We also observed $^{2}J_{H30-F}$ couplings of 47 Hz. Characteristic patterns of two ethylenic protons were observed at 5.93 and 5.84 ppm, taking into account the H–F couplings. These results are summarized in Table 1. The second point was to determine unambiguously the position of this prosthetic group by using two different methods. The first was based on heteronuclear long-range correlations. The HMBC experiment exhibits a ^{3}J correlation between C-3 and H-27 (Fig. 4), justifying the incorporation of the prosthetic group at the phenolic position. We can also note a $^{2}J_{C19-OH19}$ correlation in the HMBC experiment, confirming that the C-19/OH-19 position is unmodified (Fig. 4). The second method used was based on dipolar connectivities. The NOESY experiment clearly showed H-2/H-27 dipolar correlation (Fig. 5), giving additional proof that substitution took place on the aromatic ring through the oxygen atom of the phenolic group.

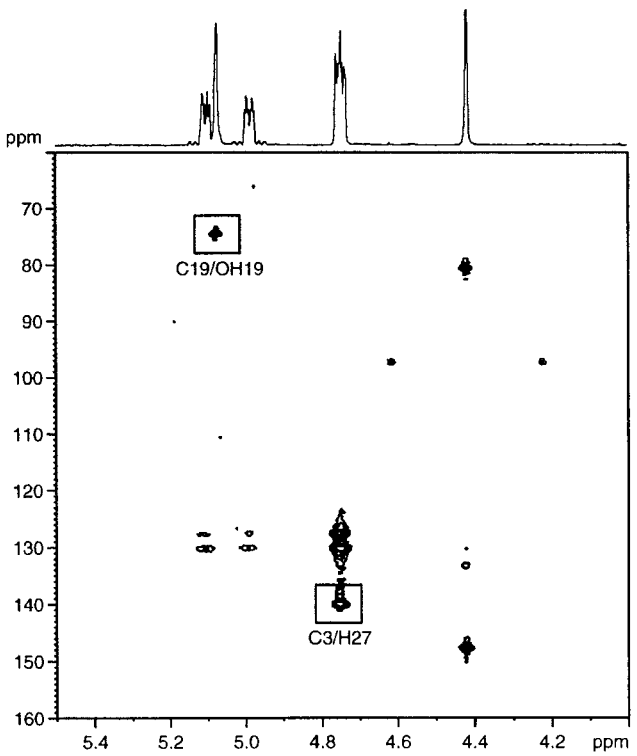


Figure 4. Part of the HMBC spectrum of 3-*O*-[(*Z*)-4-fluoro-but-2-enyl]diprenorphine **10a** ($CDCl_3$, 400.13 MHz, 298 K) showing pertinent correlations to determine unambiguously the position of the prosthetic group

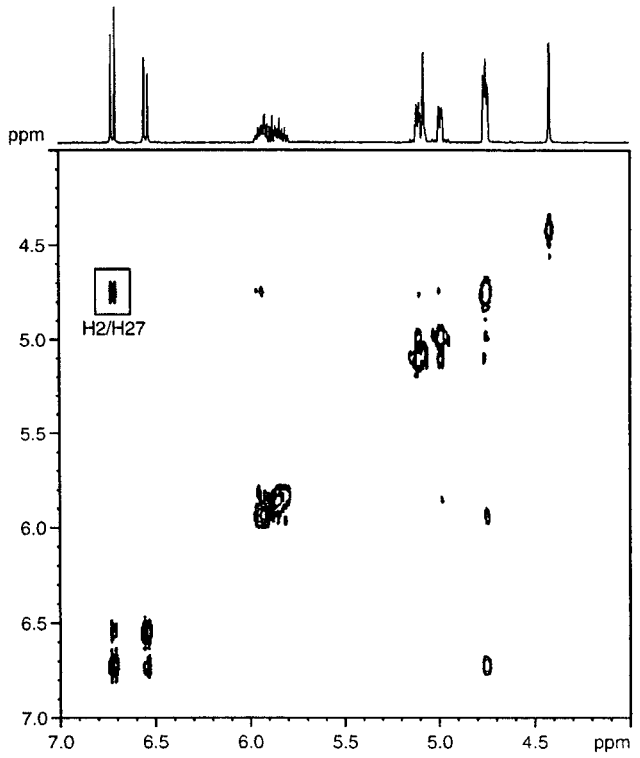


Figure 5. Part of the NOESY spectrum of 3-*O*-[(*Z*)-4-fluoro-but-2-enyl]diprenorphine **10a** ($CDCl_3$, 400.13 MHz, 298 K) showing the correlation between H-2 and H-27

Receptor binding assays

The inhibition constant for the binding affinity of diprenorphine to opioid receptors in mouse brain membrane preparations have been reported to be 0.2 ± 0.16 nM[20] (in this report it is claimed that successful attachment at C-19 of an iodo-containing prosthetic group leads to a derivative with a high affinity to opioid receptors; thorough rexamination of these results led to conclusions in perfect agreement with those reported in this paper both on the regioselectivity of the attachment and on the affinity), while the derivatives **10a** and **10b** displayed a very reduced affinity $K_i = 0.31 \pm 0.05$ and 0.09 ± 0.03 μM, respectively. These results are in agreement with the studies on naltrindole reported by Rice and coworkers,[21,22] where the masking of the 3-phenol caused a decrease (1/1000) in the binding affinity of opioid receptors.

CONCLUSION

The choice of a tosylated derivative of DPN seems to offer a very good opportunity to develop radioligands able to be labelled with either iodine-123 or fluorine-18 for SPECT or PET imaging.

Unfortunately, the attachment of the prosthetic group at the 3-position of diprenorphine led to dramatic

reduction in the binding ability of the opioid receptors, indicating the essential role of the phenolic group for the ligand binding to the opioid receptors.[19] The phenolic group seems to be a message component that is responsible for signal transduction in the message-address concept reported by Portoghese.[23] The very low specific binding demonstrated by this compound indicates that it may be not useful for *in vivo* imaging of opioid receptors with PET.

New synthetic strategies are currently under way in order to establish the synthesis of compounds functionalized at the C-19 carbon atom of the diprenorphine.

EXPERIMENTAL

General. IR spectra were recorded on a Perkin-Elmer 1725X apparatus as KBr pellets or in $CHCl_3$ solution. ^1H NMR spectra were recorded at 200.13 MHz, ^{13}C spectra at 50.32 MHz and ^{19}F spectra at 372.42 MHz using Bruker spectrometers. Chemicals shifts are given in parts per million (δ) downfield from tetramethylsilane as internal standard and coupling constants are given in hertz. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad. Mass spectra were recorded using a VG 2AB-SE double-focusing mass spectrometer and chemical ionization mass spectra (CIMS) were obtained using a Nermag R-10 mass spectrometer. Microanalyses were obtained using a Perkin-Elmer 2400 CHN elemental analyser. Analytical TLC was carried out on Merck Kieselgel 60_{254} plates and the spots visualized using a Vilbert–Lourmat UV lamp. Flash chromatography was effected using Merck Kielselgel 60 (15–40 μm).

NMR. A 4.7 mg amount of **10a** was dissolved in 0.5 ml of 100% deuterated chloroform (Eurisotop, CEA, Saclay, France). Spectra were recorded on a Bruker ARX-400 NMR spectrometer operating at 400.13 MHz for ^1H and 100.61 MHz for ^{13}C, equipped with a Bruker Aspect Station 1 computer and an inverse ^1H/broad band *z*-gradient probe. Data were processed on an SGT 02 workstation using Bruker WINNMR 2.1 software. All NMR experiments were performed at 298 K using standard pulse sequences (using *z*-gradient when possible) of the Bruker library. ^1H spectra were referenced to the $CHCl_3$ residual solvent resonance taken at 7.27 ppm, downfield from $SiMe_4$ (TMS). TOCSY experiments using the MLEV17 sequence for spin locking and mixing times of 50 and 100 ms (9 kHz) were performed to identify short- and long-range coupling. NOESY experiments was carried out with a mixing time of 1 s. 2D spectra (except COSY) were obtained with quadrature phase detection in both dimensions using the TPPI method in the indirect dimension. 4096 points were acquired in the F_2 dimension for each 256 (512 for COSY experiments) complex points in the F_1 dimension, and 16 free induction decays (four for COSY) were accumulated with a relaxation delay of 2 s. Spectra were apodized with differents functions (COSY with a non-shifted sine-bell function in both dimensions, TOCSY and the NOESY with a $\pi/2$-shifted sine squared function in both dimensions). ^{13}C spectra were referenced to the central $CDCl_3$ resonance taken at 77.2 ppm, downfield from TMS. For the HMQC experiment the following parameters were used: 128 experiments with 4096 data points and 16 scans each were recorded. The linear prediction method was used to increase the F_1 dimension resolution giving rise after zero filling to a 4096×1024 point matrix. $\pi/4$-shifted sine squared functions were applied to both dimensions before Fourier transformation.

Receptor binding assays. Mouse brains minus the cerebellum obtained by decapitation were dissected and homogenized (Potter) in 12 ml of ice-cold Tris–HCl buffer (50 nM, pH 7.4). The cerebral homogenates were centrifuged at 100 000 *g* at 4 °C for 35 min and the resultant pellets were rehomogenized in the same buffer and volume and recentrifuged. The final pellets were resuspended in Tris–HCl buffer and kept at −80 °C. The binding assay was performed by incubating 100 μl of the crude mouse brain membrane preparation containing a fixed amount of protein (0.5 mg) with different concentrations of the labelled 3-*O*-[(*Z*)-4-fluorobut-2-enyl] diprenorphine **10a** and 3-*O*-[(*Z*)-4-iodobut-2-enyl] diprenorphine **10b** by competition–inhibition with 1.17 nM [^3H]diprenorphine (specific activity 66 Ci mmol^{-1}) in a total volume of 1 ml of Tris–HCl buffer. The assay mixture was incubated for 60 min at 25 °C, and the samples were rapidly filtered through Whatman GF/B glass-fibre filters prewetted with assay buffer and washed twice with 5 ml of ice-cold assay buffer. The filters were then added to 5 ml of scintillation cocktail and the samples were allowed to equilibrate for 6 h before counting. The non-specific binding was defined in the presence of 10 μM of levorphanol. The amount of bound radioactivity was determined by liquid scintillation spectrometry using a TR1-CARB 1600 TR liquid scintillation counter (Packard) with an efficiency for tritium of approximately 80%. Saturation bindings, Scatchard and competition experiments were analysed with the integrative non-linear least-squares curve-fitting program Prism. All binding assay values were determined by using the same mouse brain preparation performed with each tube in triplicate. Scatchard's transformation of the binding data was treated to obtain values for the inhibition constant (K_i). 3-*O*-[(*Z*)-4-Fluorobut-2-enyl] diprenorphine **10a** and 3-*O*-[(*Z*)-4-iodobut-2-enyl] diprenorphine **10b** at different concentrations, ranging from 10^{-6} to 10^{-12} M, were studied to assess their suitability for binding opioid receptors by competition–inhibition with 1.17 nM [^3H]DPN in mouse brain membrane homogenates.

*(Z)-4-(tert-Butyldiphenylsilyloxy)but-2-en-1-ol (**4**).* Under a nitrogen atmosphere, *n*-butyllithium 1.6 M in hexane (23 ml, 36.8 mmol) was added with stirring to a cooled (−78 °C) solution of (*Z*)-butene-1,4-diol (3.2 g, 36.3 mmol) in THF (56 ml). When the initial vigourous reaction had subsided, *tert*-butyldiphenylsilyl chloride (10 g, 36.4 mmol) was added dropwise over 5 min. The resulting mixture was then allowed to warm to room temperature and stirred for 30 min. The white mixture was boiled under reflux for 4 h. The THF was removed under reduced pressure and the product was extracted with diethyl ether (2 × 20 ml), dried over magnesium sulfate and concentrated under reduced pressure. Purification by flash chromatography gave (*Z*)-4-(*tert*-butyldiphenylsilyloxy)but-2-en-1-ol (10.2 g, 86%) as a thick, colourless oil, $R_f = 0.25$ (70% light petroleum–30% diethyl ether). $C_{20}H_{26}O_2Si$ requires C, 73.6; H, 7.97%. Found C, 72.57; H, 7.64%. ^{1}H NMR (200.13 MHz, $CDCl_3$): δ_H 1.16 [9H, s, $(CH_3)_3C$], 2.36 (1H, s, CH_2OH), 4.04 (2H, d, $J = 5.6$, $=CHCH_2OH$), 4.32 (2H, $J = 5.4$, $CH_2OTBDPS$), 5.66–5.79 (2H, m, $CH_2CH{=}CHCH_2$), 7.41–7.49 (6H, m, 2 × Ar*H*), 7.74–7.79 (4H, m, 2 × Ar*H*). ^{13}C NMR (50.32 MHz, $CDCl_3$): δ_C 19.27, 26.97, 58.61, 60.40, 127.91, 129.94, 130.18, 130.81, 133.57, 135.73. MS (DCI/NH_3) *m/z* (%): $C_{20}H_{26}O_2Si$ requires 326, 344 (MNH_4^+, 37.7), 327 (MH^+, 100), 309 (6.04), 249 (5.43), 216 (19.29).

*(Z)-4-(tert-Butyldiphenylsilyloxy)-1-p-toluenesulfonyloxy-but-2-ene (**5**).* Tosyl chloride (2.10 g, 11 mmol) was added under a nitrogen atmosphere to a solution of (*Z*)-4-(*tert*-butyldiphenylsilyloxy)but-2-en-1-ol (3.26 g, 10 mmol) in diethyl ether (20 ml) at 0 °C. Potassium trimethylsilanoate (6.55 g, 51.3 mmol) was added dropwise over 30 min. The resulting mixture was mechanically stirred at 0 °C for a further 30 min. The reaction mixture was then allowed to warm to room temperature. The reaction was quenched with cold water (20 ml) and extracted with diethyl ether (2 × 20 ml). The combined organic layers were dried over magnesium sulfate and concentrated under reduced pressure. Purification by flash chromatography (70% light petroleum–30% dichloromethane) gave (*Z*)-4-(*tert*-butyldiphenylsilyloxy)-1-*p*-toluenesulfonyloxybut-2-ene (3.8 g, 79%) as a lightly yellow oil, $R_f = 0.35$. $C_{27}H_{32}O_4SSi$ requires C, 67.48; H, 6.66%. Found C, 67.18; H, 6.09%. ^{1}H NMR (200.13 MHz, $CDCl_3$) δ_H: 1.08 [9H, s, $(CH_3)_3C$], 2.41 (3H, s, CH_3-Ar), 4.21 (2H, d, $J = 5.6$, $=CHCH_2OTs$), 4.57 (2H, d, $J = 6.6$, $TBDPSOCH_2CH{=}$), 5.54 (1H, m, $TBDPSO–CH_2–CH{=}CH$), 5.84 (1H, m, $CH{=}CH–OTs$), 7.27 (2H, d, $J = 8.6$, $ArCH_3$), 7.39–7.49 (6H, m, *Ph*), 7.67–7.77 (6H, m, *Ph* + $ArCH_3$). ^{13}C NMR (50.32 MHz, $CDCl_3$) δ_C: 19.18, 21.72, 26.86, 60.49, 66.34, 122.69, 127.95, 129.96, 129.98, 133.22, 134.82, 135.59, 144.84. MS (DCI/NH_3) *m/z* (%): $C_{27}H_{32}O_4Si$ requires 480.18, 498 (MNH_4^+, 100), 481 (MH^+, 4.18), 344 (8.02), 327 (8.37), 309 (16.08), 274 (5.42), 174 (7.59).

*3-Acetyldiprenorphine (**2**).* A mixture of diprenorphine **1** (85.1 mg, 0.2 mmol) and triethylamine (24.3 mg, 0.24 mmol) in THF (3 ml) was heated on an oil-bath and stirred for 5 min at 30 °C under a nitrogen atmosphere. Then freshly distilled 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 (96:3.7:0.3, v/v/v) as eluent. The reaction was run until TLC indicated complete reaction (single uniform spot, $R_f = 0.51$). The solvent was evaporated under reduced pressure to obtain 3-acetyldiprenorphine **2** as a white crystalline solid (93.7 mg, 100%). ^{1}H NMR (200.13 MHz, $CDCl_3$) δ_H: 2.25 (3H, s, CH_3CO_2). The other proton assignments are in agreement with a previous report by Mazza *et al.*[19] ^{13}C NMR (50.32 MHz, $CDCl_3$) δ_C: 3.39, 4.26, 9.35, 17.07, 20.56, 23.12, 24.86, 29.76, 30.01, 32.3, 35.25, 35.96, 44.93, 46.99, 47.1, 52.70, 58.12, 59.91, 74.50, 80.10, 98.27, 119.23, 122.10, 131.45, 133.52, 134.41, 149.68, 166.71. IR (KBr) ν_{max} (cm^{-1}): 3467 (aliphatic OH), 1768 (C=O). MS (DCI/NH_3) *m/z* (%): $C_{28}H_{37}NO_5$ requires 467.56, 468 (MH^+, 100), 450 (46).

*3-O-[(Z)-4-(tert-Butyldiphenylsilyloxy)-but-2-enyl]diprenorphine (**6**).* To a 50 ml biconical vial containing **2** (147.2 mg, 0.314 mmol) in DMF (6 ml) was added, under a nitrogen atmosphere, NaH (75.3 mg, 3.14 mmol) prewashed with light petroleum. The mixture was stirred for 30 min prior to dropwise addition of (*Z*)-4-(*tert*-butyldiphenylsilyloxy)-1-*p*-toluenesulfonyloxybut-2-ene (448 mg, 0.92 mmol) in DMF (2 ml). After stirring for 4 h at 30 °C, the reaction was quenched by addition of NH_4Cl (5 ml). The mixture was extracted with diethyl ether (3 × 5 ml), the extracts were combined and dried over magnesium sulfate and the solvent was removed under vacuum. Purification by flash chromatography (95% light petroleum–4% ethyl acetate–0.9% methanol–0.1% NH_3) gave 3-*O*-[(*Z*)-4-(*tert*-butyldiphenylsilyloxy)but-2-enyl]-diprenorphine (230 mg, 99%) as a yellow oil, $R_f = 0.50$. ^{1}H NMR (200.13 MHz, $CDCl_3$) [only reported are the proton assignments of the (*Z*)-4-(*tert*-butyldiphenylsilyloxy)but-2-en-1-oxy moiety], δ_H: 1.04 [9H, s, $(CH_3)_3C$], 4.28 (2H, d, $J = 5.4$, $=CHCH_2ODPN$), 4.56 (2H, d, $J = 4$, $=CHCH_2OTBDPS$), 5.62–5.87 (2H, m, $CH{=}CH$), 7.33–7.41 (6H, m, *Ph*), 7.65–7.69 (4H, m, *Ph*); the other proton assignments of DPN are in agreement with previous studies.[19] ^{13}C NMR (50.32 MHz, $CDCl_3$) δ_C: 3.38, 4.29, 9.47, 17.53, 19.17, 22.65, 24.94, 26.80, 29.72, 29.95, 32.36, 35.65, 35.89, 43.76, 46.88, 47.89, 52.66, 58.14, 59.92, 60.53, 66.14, 74.42, 80.44, 97.00, 116.74, 119.10, 126.49, 127.79, 129.47, 129.77, 132.38, 132.87, 133.50, 135.58, 140.15, 147.59. MS (DCI/NH_3) *m/z* (%); $C_{46}H_{59}NO_5Si$ requires 733.42, 751 (MNH_4^+, 98.05), 734 (MH^+, 61.53), 716 ($MH^+ - H_2O$, 30.19).

*3-O-[(Z)-4-hydroxy-but-2-enyl]diprenorphine (**7**).* Under

a nitrogen atmosphere, HF–pyridine complex 65% (667 mg, 8 equiv.) was added dropwise to 3-*O*-[(*Z*)-4-(*tert*-butyldiphenylsilyloxy)but-2-enyl]diprenorphine (307 mg, 0.42 mmol) in THF–pyridine (3.7:2). After stirring at room temperature for 1.5 h, the reaction mixture was diluted with diethyl ether (2 ml). Hydrolysis was performed by adding carefully a saturated aqueous solution of $NaHCO_3$. The mixture was extracted with diethyl ether (3×10 ml), dried with $MgSO_4$ and evaporated. Purification of the crude reaction mixture by flash chromatography (50% light petroleum–49% ethyl acetate–1% NH_3) gave 3-*O*-[(*Z*)-[4-hydroxy-but-2-enyl]diprenorphine (79 mg, 40%) as a lightly yellow oil, $R_f = 0.15$. ^{1}H NMR (200.13 MHz, $CDCl_3$) [only reported are the proton assignments of the (*Z*)-4-hydroxy-but-2-en-1-oxy moiety], δ_H: 4.21 (2H, d, $J = 4.1$, =CHCH_2OH), 4.31 (1H, s, =CHCH$_2$O*H*), 4.70 (2H, d, $J = 4.8$, =CHCH_2ODPN), 5.71–5.86 (2H, m, C*H*=C*H*); the other proton assignments of DPN are in agreement with previous studies.[19] ^{13}C NMR (50.32 MHz, $CDCl_3$) δ_C: 3.39, 4.23, 9.43, 17.67, 22.73, 24.94, 29.82, 29.97, 32.33, 35.64, 35.92, 43.72, 46.89, 47.80, 52.69, 58.17, 58.71, 59.91, 66.00, 74.49, 80.50, 97.01, 116.98, 119.22, 127.59, 129.87, 132.74, 133.03, 139.98, 147.73. MS (DCI/NH_3) *m/z* (%); $C_{30}H_{41}NO_5$ requires 495.30, 513 (MNH_4^+, 26.19), 496 (MH^+, 69.37), 478 ($MH^+ - H_2O$, 100).

*3-O-[(Z)-4-(p-Toluenesulfonyl)-but-2-enyl]diprenorphine (**8**).* To a 25 ml biconical vial containing 3-*O*-[(*Z*)-4-hydroxy-but-2-enyl]diprenorphine (37.5 mg, 0.075 mmol) in anhydrous diethyl ether (2 ml) which was maintained at 0 °C, *p*-toluenesulfonyl chloride (15.8 mg, 0.083 mmol) was added. Potassium trimethylsilanoate (56.2 mg, 0.513 mmol) was slowly added to the mixture over 15 min. After the addition was complete, the mixture was rigorously stirred for exactly 15 min at 0 °C. The solution was allowed to warm to room temperature and the reaction was quenched by the addition of cooled NH_4Cl (2 ml). The mixture was extracted with diethyl ether (3×2 ml), the ether layers were combined, dried over $MgSO_4$, concentrated under vacuum and purified by flash chromatography (40% light petroleum–59% ethyl acetate–1% NH_3) to afford 3-*O*-[(*Z*)-4-(*p*-toluenesulfonyl)but-2-enyl] diprenorphine (23.6 mg, 61%), $R_f = 0.51$. ^{1}H NMR (200.13 MHz, $CDCl_3$) [only reported are the proton assignments of the (*Z*)-4-(*p*-toluenesulfonyl)but-2-en-1-oxy moiety], δ_H 2.42 (3H, s, CH_3Ar), 4.61 (2H, d, $J = 6$, =CHCH_2OTs), 4.66 (2H, d, $J = 6.6$, =CHCH_2ODPN), 5.60–5.68 (1H, m, CH=C*H*), 5.82–5.91 (1H, m, C*H*=CH), 7.33 (2H, d, $J = 8$, CH_3–Ar*H*), 7.75 (2H, d, $J = 8$, CH_3–Ar*H*); the other proton assignments of DPN are in agreement with previous studies.[19] ^{13}C NMR (50.32 MHz, $CDCl_3$) δ_C: 3.38, 4.30, 9.46, 17.56, 21.75, 22.67, 24.94, 29.87, 29.97, 32.34, 35.63, 35.90, 43.74, 46.91, 47.88, 52.74, 58.06, 59.91, 65.74, 65.83, 74.43, 80.46, 97.20, 116.72, 119.27, 125.11, 127.97, 129.95, 130.04, 131.58, 133.11, 133.20, 139.73, 144.97, 147.55. MS (DCI/NH_3) *m/z* (%): $C_{37}H_{47}NO_7S$ requires 649.06, 667 (MNH_4^+, 35.25), 650 (MH^+, 100).

*3-O-[(Z)-4-Fluorobut-2-enyl]diprenorphine (**10a**).* Potassium fluoride (6.2 mg, 0.108 mmol, PM = 59) and K222 Kryptofix (33.5 mg, 0.108 mmol) were added with stirring to distilled water (1 ml). The mixture was heated on an oil-bath at 120 °C for 10 min under a nitrogen flow. The white residue was dissolved with 2 ml of anhydrous acetonitrile and 3-*O*-[(*Z*)-4-(*p*-toluenesulfonyl)but-2-enyl]diprenorphine (35 mg, 0.054 mmol) in acetonitrile (2 ml) was added to the previous mixture under a nitrogen atmosphere. The resulting mixture was heated at reflux temperature for 30 min. After evaporation of the acetonitrile under reduced pressure, the residue was dissolved with chloroform and washed with water, purified by reversed-phase HPLC with Hypersil in a Hyperprep HS C18 column (3 cm × 1.2 mm i.d., 8 film thickness) eluted with 20% water–80% acetonitrile, and monitored by UV detection at 215 nm. With a flow-rate of 0.6 ml min^{-1}, 3-(*Z*)-(4-fluorobut-2-enyl)oxy]diprenorphine (13.1 mg, 48%) was collected after 3.7 min. ^{1}H and ^{13}C NMR assigments are given in Table 1. ^{19}F NMR (376.42 MHz, $CDCl_3$) δ_F: −134.61 (dt, $^2J_{F-CH_2} = 45.18$, $^3J_{F-CH_2-CH=} = 15.06$). MS (DCI/NH_3) *m/z* (%): $C_{30}FH_{40}NO_4$ requires 497.29, 515 (MNH_4^+, 20.82), 498 (MH^+, 76.83), 480 ($MH^+ - H_2O$, 100).

*3-O-[(Z)-4-Iodobut-2-enyl]diprenorphine (**10b**).* To 3-*O*-[(*Z*)-4-(*p*-toluenesulfonyl)but-2-enyl] diprenorphine (42 mg, 0.0647 mmol) dissolved in 2 ml of anhydrous acetone, sodium iodide (16 mg, 1.66 equiv.) was added under a nitrogen atmosphere. The mixture was maintained under stirring for 1 h and the reaction was followed with TLC with light petroleum–ethyl acetate (60:40) as mobile phase. The acetone was evaporated under reduced pressure and the yellow mixture was dissolved with diethyl ether and washed with distilled water. The organic phase was dried with $MgSO_4$ and purified by flash chromatography (60% light petroleum–40% ethyl acetate) to afford 3-*O*-[(*Z*)-4-iodobut-2-enyl]diprenorphine (16 mg, 40%), $R_f = 0.53$. ^{1}H NMR (200.13 MHz, $CDCl_3$) [only reported are the proton assignments of the (*Z*)-4-iodobut-2-en-1-oxy moiety], δ_H: 3.93 (2H, d, $J = 8.8$, =CHCH_2I), 4.73 (2H, d, $J = 6$, =CHCH_2*O*DPN), 5.63–5.75 (1H, m, CH=C*H*), 5.82–6.1 (1H, m, C*H*=CH); the other proton assignments of DPN are in agreement with those of **10a**. ^{13}C NMR (50.32 MHz, $CDCl_3$) δ_C: (−1.11), 3.38, 4.30, 9.46, 17.60, 22.68, 24.59, 29.88, 29.99, 32.37, 35.65, 35.92, 43.75, 46.94, 47.91, 52.88, 57.41, 58.10, 65.10, 74.42, 80.49, 97.17, 116.89, 119.26, 129.16, 129.35, 130.00, 133.11, 139.99, 147.67. MS (DCI/NH_3) *m/z* (%): $C_{30}H_{40}INO_4$ requires 605.20, 623 (MNH_4^+, 95.98), 606 (MH^+, 62.70), 588 ($MH^+ - H_2O$, 19.53).

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