Published in final edited form as:

Tetrahedron. 2016 October 6; 72(40): 6127–6135. doi:10.1016/j.tet.2016.08.015.

Synthesis and opioid receptor binding of indium (III) and [¹¹¹In]-labeled macrocyclic conjugates of diprenorphine: novel ligands designed for imaging studies of peripheral opioid receptors

Shefali Srivastava^{a,c}, Emily A. Ferguson-Cantrell^{a,c}, Roger I. Nahas^{b,c}, and John R. Lever^{a,c,*}

^a Department of Radiology, University of Missouri, Columbia, MO 65211, USA

^b Department of Chemistry, University of Missouri, Columbia, MO 65212, USA

^c Research Service, Harry S. Truman Memorial Veterans' Hospital, Columbia, MO 65201, USA

Abstract

Radiolabeled diprenorphine (DPN) and analogs are widely used ligands for non-invasive brain imaging of opioid receptors. To develop complementary radioligands optimized for studies of the peripheral opioid receptors, we prepared a pair of hydrophilic DPN derivatives, conjugated to the macrocyclic chelator DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), for complexation with trivalent metals. The non-radioactive indium (III) complexes, tethered to the C6-oxygen position of the DPN scaffold by 6- to 9-atom spacers, displayed high affinities for binding to μ , δ and κ opioid receptors in vitro. Use of the 9-atom linker conferred picomolar affinities equipotent to those of the parent ligand DPN. The [¹¹¹In]-labeled complexes were prepared in good yield (>70%), with high radiochemical purity (~99%) and high specific radioactivity (>4000 mCi/ μ mol). Their log $D_{7.4}$ values were –2.21 to –1.66. In comparison, DPN is lipophilic, with a log $D_{7.4}$ of +2.25. Further study in vivo is warranted to assess the suitability of these [¹¹¹In]-labeled DPN-DOTA conjugates for imaging trials.

Keywords

Diprenorphine; Opioid receptor; Bioconjugate; Radiometal; Imaging

1. Introduction

Non-invasive positron emission tomography (PET) imaging of the classical μ , δ and κ opioid receptors of the central nervous system (CNS) has generated a wealth of information on normal function, as well as insight into substance abuse, epilepsy, neurodegenerative disorders and the perception of pain.^{1–4} Despite thirty years of progress in brain imaging, few advances have been made in the development of radioligands optimized for imaging

* Corresponding author. leverj@health.missouri.edu (J.R. Lever).

Supplementary data

Supplementary data (¹³C NMR studies) associated with this article can be found in the online version, at <http://dx.doi.org/10.1016/j.tet.2016.08.015>.

studies of the important functional roles played by the peripheral opioid receptors. These include mediation of cardiovascular functions,⁵ visceral and inflammatory pain relief,⁶ cell death and survival,⁷ and smooth muscle contraction.⁸ For instance, there is growing interest in peripheral opioid analgesics to evade the central side effects of respiratory depression and euphoria,⁶ while peripheral antagonists are indispensable for clinical management of opioid-induced bowel dysfunction.^{8b} Moreover, opioid receptors are over-expressed by several types of cancer, and are emerging as molecular oncology targets for imaging and therapy.⁹ For illustration, PET imaging of lung cancer patients shows high levels of μ and δ opioid receptors on primary lung tumors as compared to normal lung.¹⁰

Opioid receptor radioligands designed for CNS imaging must be neutral, lipophilic and of modest molecular size in order to negotiate the tight junctions of the blood-brain barrier.¹¹ As a consequence, they typically show slow kinetics for clearance of the unbound fraction from peripheral tissues and blood, which masks the receptor-bound fraction in the periphery, reduces image quality, and leads to unfavorable internal radiation dosimetry.^{11a} Accordingly, we are working toward a new generation of hydrophilic radioligands designed for studies of the peripheral opioid receptors.^{1,9} Since opioid receptor peptides tend to show limited metabolic stability,^{12,13} our focus is on linking small molecules having a potent opioid receptor pharmacophore to a macrocyclic chelator for radiometals. In this regard, we demonstrated that the δ opioid receptor antagonist naltrindole (NTI) can be conjugated to the polyaminocarboxylate DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), such that the derived indium (III) and [¹¹¹In]-labeled complexes maintain high binding affinity.¹⁴ Indium-111 was chosen as a representative radionuclide, and is suitable for use in single photon emission computed tomography (SPECT), a nuclear imaging technique complementary to PET. DOTA is a versatile chelator that also forms stable complexes with a variety of trivalent radiometals for PET imaging (⁶⁸Ga, ⁶⁴Cu) and radionuclide therapy (⁹⁰Y, ¹⁷⁷Lu), as well as with gadolinium (III) for magnetic resonance imaging (MRI) with contrast.¹⁵

Arguably, the most widely used radioligands for opioid receptor PET imaging in the CNS are diprenorphine (DPN) isotopically labeled with carbon-11, and analogs labeled with fluorine-18 (Fig.1).¹⁻⁴ DPN congeners labeled with iodine-123 have also been developed for brain imaging by SPECT (Fig. 1).^{16,17} DPN, a well known member of the orvinol class of semi-synthetic 4,5-epoxymorphinans derived from Diels–Alder reactions with the opium alkaloid thebaine, was first prepared by Bentley and colleagues¹⁸ during seminal studies in the 1960's.¹⁹ Other prominent orvinols are buprenorphine (BPN, Suboxone®), a partial agonist used to treat opioid addiction,²⁰ and etorphine (Immobilon®), a ‘super-potent’ agonist used to immobilize large animals, such as wild elephants.²¹ In veterinary medicine, DPN (Revivon®) is employed to reverse the effects of Immobilon® when animals need to be revived.²¹ Thus, the antagonist character of DPN provides a good safety profile for clinical imaging studies. These three orvinols are universal opioid receptor ligands, in that they bind with high and nearly equal affinities to μ , δ and κ sites.²² Herein we describe the design, synthesis and opioid receptor-binding properties of hydrophilic indium (III) and [¹¹¹In]-labeled DPN-DOTA conjugates, with a view toward imaging studies of peripheral opioid receptors.

2. Results and discussion

2.1. Chemistry and radiochemistry

Preserving the opioid receptor-targeting properties of DPN, while accommodating the steric bulk imparted by a linker group attached to a large metal coordination complex, is a challenging proposition. Since radiolabeled analogs of DPN having fluoroalkyl and iodoalkyl groups at the C6-oxygen (Fig. 1) maintain high opioid receptor binding affinity,^{1–4,16} we chose to investigate conjugation of DOTA at this position. Support also comes from the high binding affinity observed for some bivalent opioid receptor ligands, developed to detect homomeric and heteromeric receptors, which are joined by long linkers through the C6-positions of oxymorphone or naltrexone.²³ The synthetic route envisioned calls for selective elaboration of a free C6-hydroxyl, by analogy to procedures first developed for efficient isotopic radiolabeling of DPN and BPN using [¹¹C]iodomethane (cf. Fig. 1).²⁴

Key features of this approach include an unusual demethylation of DPN to give triol **1**, accomplished in 95% yield by treatment of DPN with LiAlH₄ in tetrahydrofuran (THF) containing CCl₄ (Scheme 1), and recognition that alkylation can be driven selectively to the C6-hydroxyl position.²⁴ These demethylation conditions are unique to the orvinols, and offer convenient entry to the otherwise inaccessible C6-hydroxyl. They were serendipitously discovered for simpler members of the series by Kopcho and Schaeffer,²⁵ who suggested that the C6-*O*-methyl group is activated by formation of a six-membered ring complex between the C6-*O*, aluminum and an electron-rich atom (*O* or *N*) at C19, and then removed by S_N2 attack of an unknown nucleophile.

Regarding differentiation of the three hydroxyl groups of 6-*O*-desmethyl-DPN, triol **1**, previous dual labeling studies with [¹¹C/¹³C] iodomethane showed that base-promoted *O*-alkylation occurs exclusively at the tertiary C6-hydroxyl, which is less sterically hindered than the tertiary C19-hydroxyl, as long as the phenolic position is blocked.^{24a} Acid-labile *t*-butyldimethylsilyl or trityl ethers have been employed for protection of the phenol.²⁴ The more stable trityl moiety is preferred in recent fully automated radiosyntheses of DPN and other orvinols for clinical PET.²⁶ We reasoned that a tosyl protecting group would be advantageous for the present multi-step approach (vide infra) by providing stable intermediates compatible with deprotection under basic conditions. In practice, treatment of **1** with *p*-toluenesulfonyl chloride and K₂CO₃ in acetone proceeded smoothly to give novel ester **2** in 93% yield.

With **2** in hand as a common intermediate, we advanced toward the C6-*O*-alkylamino derivatives of DPN, **5** and **8** (Scheme 2), needed for building the DPN-DOTA conjugates. Alkylation of **2** using *N*-(3-bromopropyl)phthalimide and powdered NaH in *N,N*-dimethylformamide (DMF) at room temperature for 2 h furnished **3** (53%). Hydrazine hydrate at 70 °C for 2 h removed the amine protecting group to produce **4** (42%), with the phenolic protecting group intact. Hydrolysis of the tosyl moiety, using aqueous NaOH in isopropanol at reflux overnight, afforded a 73% yield of C6-*O*-propylamino **5**. In the second case, ester **2** was treated with 6-bromohexanenitrile and NaH in DMF for 2.5 h to give hexanenitrile **6** (57%). Hydrolysis of the tosyl moiety then gave phenolic nitrile **7** (50%).

Complete nitrile reduction, using LiAlH_4 in THF at 40 °C for 1 h, delivered C6-*O*-hexylamino **8** (70%).

The synthesis of our target pair of DPN-DOTA conjugates having either 6- or 9-atom linkers is shown in Scheme 3. Primary amines **5** and **8** were coupled with the activated *N*-hydroxysuccinimidyl ester of DOTA (DOTA-NHS) using Et_3N and catalytic 4-*N,N'*-dimethylaminopyridine (DMAP) in CH_3CN to give **9** (82%) and **10** (57%), respectively. These materials were secured as the trifluoroacetic acid (TFA) salts after purification by column chromatography over C_{18} reversed-phase silica gel. Treatment of DPN-DOTA conjugates **9** and **10** with InCl_3 in water for 45 min at reflux, followed by reversed-phase column chromatography and lyophilization, provided good isolated yields of indium (III) complexes **11** (83%) and **12** (87%), respectively, as TFA salts. Structures portrayed (Scheme 3) are for seven-coordinate indium (III)-DOTA complexes. For simple indium (III)-DOTA complexes, the seven-coordinate geometry is known to be favored in solution over the eight-coordinate geometry, involving the carboxamide oxygen, which is observed in the solid state.²⁷

DPN-DOTA conjugates **9** and **10** were readily radiolabeled under standard conditions using no-carrier-added $[^{111}\text{In}]\text{Cl}_3$ in NH_4OAc buffer at 100 °C for 30 min (Scheme 3). Purification was achieved by reversed-phase high-performance liquid chromatography (HPLC) under conditions that fully resolved the precursors from the radiometal complexes. Good isolated yields (64–81%) of $[^{111}\text{In}]\textbf{11}$ and $[^{111}\text{In}]\textbf{12}$, as concentrated solutions in ethanol, were obtained after solid-phase extraction. Both radioligands were of high purity (~99%), and proved to be stable during overnight storage. $[^{111}\text{In}]\textbf{11}$ and $[^{111}\text{In}]\textbf{12}$ coeluted on reversed-phase HPLC with the fully characterized samples of **11** and **12**, respectively. Specific radioactivities were over 4000 mCi/ μmol , based upon UV (254 nm) absorption detection limits. Representative analytical HPLC data for $[^{111}\text{In}]\textbf{11}$ are shown in Fig. 2.

2.2. Lipophilicity measurements

Distribution coefficients were determined for $[^{111}\text{In}]\textbf{11}$, $[^{111}\text{In}]\textbf{12}$ and $[^3\text{H}]\text{DPN}$ by the shake-flask technique using *n*-octanol and pH 7.4 phosphate buffer. The results are recorded in Table 1, along with calculated ClogP values for non-complexed conjugates **9** and **10**. Measured log $D_{7.4}$ values for the $[^{111}\text{In}]$ -labeled complexes were negative, ranging from –2.21 to –1.66. As expected based upon substituent constant additivity,²⁸ $[^{111}\text{In}]\textbf{11}$ having an acetamidopropyl linker proved more hydrophilic than $[^{111}\text{In}]\textbf{12}$ having an acetamidohexyl linker. ClogP values for **9** and **10** showed a similar relative differentiation. As intended, the $[^{111}\text{In}]$ -labeled DPN-DOTA conjugates are much less lipophilic than DPN (log $D_{7.4}$ +2.25). Lipophilicity influences drug absorption, distribution, metabolism and excretion, and the value we find for DPN is optimal for CNS penetration.¹¹ The low lipophilicity of $[^{111}\text{In}]\textbf{11}$ and $[^{111}\text{In}]\textbf{12}$, together with molecular weights near 1000 g/mol, should preclude brain access. Further, we would expect their hydrophilicity to promote fast kidney clearance, mitigate binding to plasma proteins, and reduce non-specific uptake in peripheral organs such as lung and liver.^{11a,14}

2.3. Opioid receptor binding

Opioid receptor binding data for DPN, as well as the DPN-DOTA conjugates and their indium (III) complexes, are summarized in Table 1. Although our focus is on peripherally restricted opioid receptor ligands, assays were performed using membranes from rodent brain tissues where the binding parameters are well established. DPN bound with picomolar apparent affinity (K_i) to μ , δ and κ opioid receptors. These values are in good agreement with previous determinations for DPN with rodent brain membranes,^{22a} and with individually cloned receptors.^{22c} All of the novel conjugates and complexes showed high affinity for μ , δ and κ sites, with K_i values <10 nM (Table 1). A key finding is the picomolar affinity of indium (III) complex **12** for binding to μ and δ opioid receptors, which is equipotent to that of DPN. Complex **12** displayed slightly less affinity for κ sites, providing a modest degree of selectivity for binding to the μ and δ sites.

Across the three main types of opioid receptor, complex **12** proved 2- to 8-fold more potent than complex **11**. The shorter linker of **11** may place the bulky metal coordination sphere too close to a critical part of the opioid receptor-binding pharmacophore of DPN, or may elicit a clash with critical amino acid residues within the receptor binding pockets. For both linkers (Table 1), higher binding affinity for a given opioid receptor type was typical for the metal complex with respect to non-complexed conjugate (e.g., **12** vs **10**). Similar findings were noted for the opioid receptor binding of complexed compared to non-complexed NTI-DOTA conjugates.¹⁴ In addition to their differing electronics, the metal complexes are more compact since free rotations of acetate groups, as well as macrocyclic ring inversions, are not as likely.²⁷

Crystal structures for the μ , δ and κ opioid receptors, each bound to a representative antagonist, are available,^{29–31} and the similarities and differences between the three ligand binding pockets have been reviewed.³² As pointed out by Manglik and colleagues,²⁹ nine amino acid residues of the μ opioid receptor pocket interact directly with morphinans, and are conserved in the δ and κ binding pockets. These include an acidic Asp residue that forms a salt-bridge with the morphinan nitrogen, a His residue that participates in hydrogen-bonding, mediated by two water molecules, to the phenolic hydroxyl, and several more subtle interactions that help to confer high binding affinity. While the discrete features of the pendant groups that modulate affinity are not known, the strong opioid binding message of the DPN scaffold remains intact for DPN-DOTA metal complex **12** having an acetamidoethyl linker.

3. Conclusions

Indium (III)–DOTA complexes can be conjugated to the C6-oxygen position of the universal opioid receptor antagonist DPN with preservation of high binding affinity for μ , δ and κ sites in vitro. Use of a 9-atom, acetamidoethyl linker is preferred over a 6-atom, acetamidopropyl linker, and leads to an indium (III) complexed DPN-DOTA conjugate (**12**) having picomolar affinity for μ and δ opioid receptors, accompanied by a low nanomolar affinity for the κ type. The DPN-DOTA conjugates are readily radiolabeled with indium-111 under no-carrier-added conditions, and can be isolated in good yield and with high purity. These [¹¹¹In]-labeled DPN-DOTA conjugates are hydrophilic by design, and warrant investigation in vivo.

The linkers should serve as pharmacokinetic modifiers, and could be varied to adjust bioavailability. Within the constraints imposed by simultaneous visualization of multiple opioid receptors, [^{111}In]-labeled DPN-DOTA conjugates may prove useful for functional imaging studies of the peripheral opioid receptors expressed by normal gut and heart, and over-expressed by certain cancers.

4. Experimental section

4.1. General methods

Solvents and reagents were the best commercial grades available, and were used as received. DPN was supplied, as the free base, by Macfarlan Smith Ltd. (Edinburgh, United Kingdom) under a Schedule II controlled substance import approval from the United States Drug Enforcement Administration. Triol **1**, 6-*O*-desmethyl-diprenorphine, was prepared from DPN as previously reported and displayed appropriate ^1H NMR spectral data.^{24a,c} DOTA-NHS was purchased from Macrocyclics, Inc. (Plano, TX). DAMGO, NTI and U69,593 were from Sigma-RBI (St. Louis, MO). [^3H]DAMGO, [^3H]NTI and [^3H]U69,593, as well as [^3H]DPN, were from Perkin Elmer, Inc. (Waltham, MA). [^{111}In] Cl_3 was from Mallinckrodt, Inc. (St. Louis, MO). High-resolution mass spectroscopy (HRMS), using electro-spray ionization mode, was performed at the University of Minnesota Mass Spectrometry Laboratory on a Bruker BioTOF II. NMR spectra were acquired at the University of Missouri, Department of Chemistry. ^1H NMR spectra were recorded on a Bruker DRX 300 MHz instrument. Chemical shifts are parts per million (δ) relative to residual solvent (CHCl_3 , 7.24 ppm; HOD, 4.80 ppm; CD_2HCN , 1.94 ppm), with coupling constants (J) in hertz (Hz). ^{13}C NMR spectra were recorded for **9**, **10**, **11** and **12** in D_2O (99.96 atom% D) at either 298 K or 343 K on Bruker Avance III spectrometers at 150.8 MHz or 201.2 MHz. Chemical shifts are reported relative to an external standard of 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS, $\delta=0$ ppm). Structural assignments and the atom-numbering scheme were based upon previous homonuclear and heteronuclear correlation NMR studies of DPN,³³ with prime numbers employed for the C6-*O*-alkyl side chains. DOTA resonances are not numbered in the line notation, and were assigned, where possible, by reference to previous ^1H and ^{13}C NMR studies.²⁷ Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA). Normal-phase silica gel (<230 mesh; Merck 7729) and reversed-phase C_{18} (35–75 μm ; Analtech) column chromatography were done under nitrogen pressure. TLC was performed using Macherey–Nagel silica gel 60 UV254 (250 μm) or Whatman reversed-phase MKC $_{18}\text{F}$ 60 Å plates. Melting points (mp) are uncorrected.

4.2. Synthesis

4.2.1. 3-O-Tosyl-6-O-desmethyl-diprenorphine (2)—*p*-Toluenesulfonyl chloride (200 mg, 1.04 mmol) was added to a solution of triol **1** (360 mg, 0.87 mmol) in dry acetone (30 mL) containing anhydrous K_2CO_3 (603 mg, 1.04 mmol) and stirred overnight at room temperature. The mixture was then filtered through Celite, eluting with acetone, and solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using cyclohexane:ethyl acetate (1:1) containing 1% Et_3N to give 460 mg (93%) of **2** as white solid: mp 108–110 $^\circ\text{C}$; TLC R_f 0.3 (cyclohexane:ethyl acetate, 40:60; 1% Et_3N); ^1H NMR (CDCl_3) δ 7.83 (d, $J=8.1$ Hz, 2 H, H-2', H-6'), 7.32 (d, $J=8.1$ Hz, 2 H,

H-3', H-5'), 6.87 (d, $J=8.1$ Hz, 1 H, H-1), 6.55 (d, $J=8.1$ Hz, 1 H, H-2), 4.07 (s, 1 H, H-5), 3.06 (d, $J=5.4$ Hz, 1 H, H-9), 2.99 (d, $J=14.1$ Hz, 1 H, H-10), 2.89–2.74 (m, 1 H, H-8), 2.66 (dd, $J=12$ Hz, $J=6.0$ Hz, 1 H, H-16), 2.46 (s, 3 H, H-7'), 2.23 (dd, $J=12.9$ Hz, $J=5.4$ Hz, 1 H, H-16), 2.35–2.15 (m, 3 H, H-10, H-22), 2.10–1.95 (m, 1 H, H-15), 1.87 (t, $J=10.2$ Hz, 1 H, H-7), 1.80–1.70 (m, 1 H, H-18), 1.70–1.50 (m, 2 H, H-15, H-18), 1.42 (s, 3 H, H-21), 1.22 (s, 3 H, H-20), 1.15–0.90 (m, 2 H, H-8, H-17), 0.90–0.70 (m, 1 H, H-23), 0.65–0.40 (m, 3 H, H-17, H-24, H-25), 0.15–0.11 (m, 2 H, H-24, H-25); HRMS m/z calcd for $[M+Na]^+$ 588.2395, found 588.2390; Anal. Calcd for $C_{32}H_{39}NO_6S \cdot 0.25H_2O$: C, 67.40; H, 6.98; N, 2.46; found: C, 67.38; H, 7.28; N, 2.28.

4.2.2. 3-O-Tosyl-6-O-propylphthalimido-6-O-desmethyl-diprenorphine (3)—A mixture of **2** (100 mg, 0.176 mmol), *N*-(3-bromopropyl) phthalimide (363 mg, 1.32 mmol) and powdered NaH (95%; 33 mg, 1.31 mmol) in dry DMF (1.0 mL) was stirred at room temperature under argon for 2 h. Saturated Na_2CO_3 buffer (5 mL) was added, and the mixture extracted with ethyl acetate (15 mL). The organic layer was washed with buffer (3×5 mL), dried over anhydrous Na_2SO_4 , filtered and concentrated under reduced pressure. The yellow syrup was purified by column chromatography on silica gel using cyclohexane:ethyl acetate (1:1) containing 1% Et_3N to give 70 mg (53%) of **3** as white solid: mp 58–60 °C; TLC R_f 0.3 (cyclohexane:ethyl acetate, 40:60; 1% Et_3N); 1H NMR ($CDCl_3$): δ 7.80 (m, 2 H, H-7', H-8'), 7.70 (d, $J=8.1$ Hz, 2 H, H-2', H-6'), 7.64 (m, 2 H, H-6', H-9'), 7.28 (d, $J=9.0$ Hz, 2 H, H-3', H-5'), 6.74 (d, $J=8.1$ Hz, 1 H, H-1), 6.49 (d, $J=8.1$ Hz, 1 H, H-2), 4.24 (s, 1 H, H-5), 3.84–3.57 (m, 4 H, H-1', H-3'), 2.99 (d, $J=4.5$ Hz, 1 H, H-9), 2.96 (d, $J=14.1$ Hz, 1 H, H-10), 2.90–2.75 (m, 1 H, H-8), 2.70–2.55 (m, 1 H, H-16), 2.43 (s, 3 H, H-7'), 2.35 (dd, $J=13.2$ Hz, $J=6.6$ Hz, 2 H, H-16, H-22), 2.28–2.12 (m, 2 H, H-10, H-22), 2.09–1.84 (m, 2 H, H-7, H-15), 1.83–1.48 (m, 5 H, H-15, H-18, H-2'), 1.43 (s, 3 H, H-21), 1.18 (s, 3 H, H-20), 1.10–1.00 (m, 2 H, H-8, H-17), 0.93–0.72 (m, 1 H, H-23), 0.70–0.56 (m, 1 H, H-17), 0.56–0.41 (m, 2 H, H-24, H-25), 0.019–0.01 (m, 2 H, H-24, H-25); HRMS m/z calcd for $[M+Na]^+$ 775.3024, found 775.3048; Anal. Calcd for $C_{43}H_{48}N_2O_8S \cdot 0.25H_2O$: C, 68.19; H, 6.45; N, 3.70; S, 4.23; found: C, 68.00; H, 6.69; N, 3.59; S, 3.93.

4.2.3. 3-O-Tosyl-6-O-propylamino-6-O-desmethyl-diprenorphine (4)—A mixture of **3** (20 mg, 0.026 mmol) and hydrazine hydrate (2.7 μ L, 0.053 mmol) in ethanol (0.1 mL) was heated at 70 °C under nitrogen for 2 h, and then concentrated under reduced pressure. The residue was taken up in chilled CH_2Cl_2 and filtered through a bed of Celite, eluting with additional chilled CH_2Cl_2 (3×5 mL). Solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using methanol:ethyl acetate (15:85) containing 1% Et_3N to give 7.0 mg (42%) of **4** as a white foam: 1H NMR ($CDCl_3$): δ 7.80 (d, $J=8.1$ Hz, 2 H, H-2', H-6'), 7.34 (d, $J=8.1$ Hz, 2 H, H-3', H-5'), 6.72 (d, $J=8.1$ Hz, 1 H, H-1), 6.52 (d, $J=8.1$ Hz, 1 H, H-2), 4.30 (s, 1 H, H-5), 3.98–3.93 (m, 1 H, H-1'), 3.74–3.70 (m, 1 H, H-1'), 3.02 (d, $J=6.6$ Hz, 1 H, H-9), 2.99 (d, $J=10.8$ Hz, 1 H, H-10), 2.93–2.74 (m, 3 H, H-8, H-2'), 2.65 (dd, $J=12$ Hz, $J=4.8$ Hz, 1 H, H-16), 2.48 (s, 3 H, H-7'), 2.36 (dd, $J=12.6$ Hz, $J=6.0$ Hz, 1 H, H-16), 2.32–2.09 (m, 3 H, H-10, H-22), 2.08–1.95 (m, 1 H, H-15), 1.90 (t, $J=9.9$ Hz, 1 H, H-7), 1.86–1.66 (m, 4 H, H-18, H-3'), 1.57 (dd, $J=12.9$ Hz, $J=2.1$ Hz, 1 H, H-15), 1.39 (s, 3 H, H-21), 1.20 (s, 3 H, H-20), 1.14–0.98 (m, 2 H, H-8, H-17), 0.89–0.74 (m, 1 H, H-23), 0.73–0.58 (m, 1 H, H-17), 0.57–0.44 (m, 2 H, H-24,

H-25), 0.16–0.05 (m, 2 H, H-24, H-25); HRMS m/z calcd for $[M+Na]^+$ 623.3149, found 623.3129; Anal. Calcd for $C_{35}H_{46}N_2O_6S \cdot H_2O$: C, 65.60; H, 7.55; N, 4.37; S, 5.00; found: C, 65.71; H, 7.21; N, 4.15; S, 4.63.

4.2.4. 6-O-Propylamino-6-O-desmethyl-diprenorphine (5)—

A stirred solution of **4** (33 mg, 0.052 mmol) in isopropanol (1.2 mL) containing NaOH (1.0 N, 1.0 mL) was refluxed overnight under argon, and allowed to cool to ambient temperature. $NaHCO_3$ (8.4%, 3.0 mL) was added, and the mixture was partitioned with isopropanol (2×10 mL) followed by ethyl acetate (2×10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 , filtered and concentrated under reduced pressure. The residue was dissolved in water (5 mL), and applied to an activated Sep-Pak[®] Plus $t-C_{18}$ cartridge. The cartridge was eluted with a gradient of acetonitrile and water (10:90 to 50:50), and finally with methanol (30 mL). Appropriate methanol fractions were combined, and concentrated under reduced pressure to give 18.0 mg (73%) of **5** as a white solid: mp 114–117 °C (dec); 1H NMR ($CDCl_3$): δ 6.69 (d, $J=7.8$ Hz, 1 H, H-1), 6.51 (d, $J=8.1$ Hz, 1 H, H-2), 4.37 (s, 1 H, H-5), 4.25–3.54 (m, 1 H, H-1'), 3.85–3.55 (m, 1 H, H-1'), 3.03 (d, $J=5.2$ Hz, 1 H, H-9), 2.98 (d, $J=18.9$ Hz, 1 H, H-10), 2.93–2.69 (m, 3 H, H-8, H-3'), 2.64 (dd, $J=10.8$ Hz, $J=3.9$ Hz, 1 H, H-16), 2.47–2.14 (m, 4 H, H-10, H-16, H-22), 2.12–1.87 (m, 2 H, H-7, H-15), 1.85–1.50 (m, 5 H, H-5, H-18, H-2'), 1.40 (s, 3 H, H-21), 1.26 (s, 3 H, H-20), 1.15–0.95 (m, 2 H, H-8, H-17), 0.94–0.62 (m, 2 H, H-17, H-23), 0.60–0.35 (m, 2 H, H-24, H-25), 0.21–0.05 (m, 2 H, H-24, H-25); HRMS m/z calcd for $[M+Na]^+$ 469.3061, found 469.3085; Anal. Calcd for $C_{28}H_{40}N_2O_4 \cdot 1.25CH_3OH$: C, 69.06; H, 8.92; N, 5.51; found: C, 69.03; H, 8.42; N, 5.07.

4.2.5. 6-O-PropylamidoDOTA-6-O-desmethyl-diprenorphine (9)—

A mixture of **5** (50 mg, 0.11 mmol), DOTA-NHS (133 mg, 0.160 mmol), DMAP (2.5 mg, 0.02 mmol) and Et_3N (0.15 μ L, 1.0 mmol) in CH_3CN (0.8 mL) was stirred 3 h at room temperature under nitrogen, and then concentrated under reduced pressure. The residue was diluted with water (5 mL), brought to pH 4 by dropwise addition of 30% TFA, and loaded onto a reversed-phase C_{18} column (1×10 cm). Elution with $CH_3CN:H_2O$ (20:80) containing 0.1% TFA, followed by lyophilization, gave 70 mg (82%) of **9** as a white powder: mp 159–162 °C (dec); 1H NMR (CD_3CN+D_2O): δ 6.74 (d, $J=8.1$ Hz, 1 H, H-1), 6.58 (d, $J=8.1$ Hz, 1 H, H-2), 4.49 (s, 1 H, H-5), 4.10–3.50 (m, 4 H, H-1', CH_2CON), 3.40–3.00 (m, 10 H, H-9, H-10, H-3', NCH_2CO), 2.82 (m, 18 H, H-8, H-10, $N-CH_2$), 2.65 (m, 2 H, H-22), 2.40–2.24 (m, 1 H, H-16), 1.90 (m, 2 H, H-18), 1.85 (m, 1 H, H-7), 1.75–1.50 (m, 4 H, H-2', H-15), 1.34 (s, 3 H, H-21), 1.40–1.30 (m, 2 H, H-17), 1.29 (m, 1 H, H-8), 1.28–1.20 (m, 1 H, H-16), 1.12 (s, 3 H, H-20), 0.55–0.45 (m, 3 H, H-23, H-24, H-25), 0.18 (m, 2 H, H-24, H-25); ^{13}C NMR (150.8 MHz, D_2O): δ (298 K) 148.36 (C-4), 140.34 (C-3), 133.11 (C-12), 127.47 (C-11), 123.27 (C-1), 120.68 (C-2), 97.41 (C-5), 82.17 (C-6), 78.25 (C-19), 61.63, 61.51 (2C, 2s: C-22; C-1', C-O), 57.89 (C-9), 48.52 (C-7), 47.98 (C-13), 47.74 (C-16), 38.86 (C-14), 38.32 (C-15), 34.19 (C-8), 32.46 (C-3', C-N), 31.80 (C-2'), 31.49 (C-17), 30.73 (C-20), 27.09 (C-21), 26.43 (C-10), 20.18 (C-18), 8.00 (C-23), 7.34 (C-25) 5.18 (C-24); δ (343 K) new DOTA resonances at 173.28, 173.26, 173.23, 173.15 (C=O), 64.66 ($N-CH_2-C=O$), 57.74, 56.95, 52.89, 52.65, 52.11, 51.84 (6 br s, cyclen C); HRMS m/z calcd for $[M$

+Na]⁺ 877.4682, found 877.4693; Anal. Calcd for C₄₄H₆₆N₆O₁₁·4.5H₂O·3.5TFA: C, 45.88; H, 5.93; N, 6.29; found: C, 45.56; H, 5.60; N, 6.35.

4.2.6. 6-O-Propylamido(In(III)DOTA)-6-O-desmethyl-diprenorphine (11)—A

solution of **9** (40 mg, 33 μmol) and InCl₃ (18.2 mg, 82 μmol) in water (7.0 mL) was refluxed for 45 min, cooled to ambient temperature, and quenched with disodium ethylenediaminetetraacetic acid (EDTA; 0.1 M, 1.0 mL). The reaction mixture was loaded onto a reversed-phase C₁₈ column (1×10 cm). Elution with CH₃CN:H₂O (30:70) containing 0.1% TFA, followed by combination of similar fractions and lyophilization, gave 38 mg (83%) of **11** as a white powder: mp>230 °C; ¹H NMR (CD₃CN+D₂O): δ 6.74 (d, *J*=8.1 Hz, 1 H, H-1), 6.56 (d, *J*=8.1 Hz, 1 H, H-2), 4.51 (s, 1 H, H-5), 3.93–3.88 (m, 2 H, H-1'), 3.67–3.50 (m, 2 H, NCH₂CON), 3.45–2.60 (m, 29 H, H-8, H-9, H-10, NCH₂CO, N-CH₂, H-22, H-3'), 2.50–2.20 (m, 1 H, H-16), 2.10–1.50 (m, 9 H, H-7, H-10, H-15, H-16, H-18, H-2'), 1.50–1.20 (m, 5 H, H-8, H-17, H-21), 1.20–1.00 (m, 4 H, H-17, H-20), 0.80–0.50 (m, 3 H, H-23, H-24, H-25), 0.50–0.20 (m, 2 H, H-24, H-25); ¹³C NMR (201.2 MHz, D₂O): δ (298 K) 179.79, 179.70, 179.68 (O-C=O), 174.63 (N-C=O), 148.48 (C-4), 140.49 (C-3), 133.22 (C-12), 127.55 (C-11), 123.36 (C-1), 120.80 (C-2), 97.07 (C-5), 82.20 (C-6), 78.15 (C-19), 64.80 (N-CH₂-C=O), 61.72, 61.62 (2C, 2s: C-22; C-1', C-O), 59.49 (C-9, visible upon 20 Hz line broadening), 48.33 (C-7), 48.07 (C-13), 47.75 (C-16), 40.27 (C-14), 38.38 (C-15), 34.30 (C-8), 32.58 (C-3', C-N), 31.77 (C-2'), 31.58 (C-17), 30.74 (C-20), 27.56 (C-21), 26.54 (C-10), 20.56 (C-18), 8.09 (C-23), 7.43 (C-25) 5.27 (C-24); δ (343 K) new DOTA resonances at 62.09, 62.06, 61.92 (3 br s, N-CH₂-C=O), 59.57 (C-9), 53.78, 53.51, 53.16 (3 br s, cyclen C); HRMS *m/z* calcd for [M+Na]⁺ 989.3486, found 989.3523; Anal. Calcd for C₄₄H₆₃ InN₆O₁₁·3.5H₂O·3.0TFA: C, 43.77; H, 5.36; N, 6.13; found: C, 43.97; H, 5.41; N, 6.15.

4.2.7. 3-O-Tosyl-6-O-hexanenitrile-6-O-desmethyl-diprenorphine (6)—A mixture

of **2** (200 mg, 0.34 mmol), 6-bromohexanenitrile (180 mg, 1.0 mmol) and powdered NaH (95%; 17 mg, 0.67 mmol) in dry DMF (2.0 mL) was stirred at room temperature under nitrogen for 2.5 h. Saturated Na₂CO₃ buffer (6 mL) was added, and the mixture extracted with ethyl acetate (25 mL). The organic layer was washed with buffer (4×5 mL), dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using cyclohexane: ethyl acetate (1:1) containing 1% Et₃N to give 133 mg (57%) of **6** as a white foam: ¹H NMR (CDCl₃): δ 7.83 (d, *J*=7.2 Hz, 2 H, H-2', H-6'), 7.38 (d, *J*=7.8 Hz, 2 H, H-3', H-5'), 6.69 (d, *J*=8.1 Hz, 1 H, H-1), 6.54 (d, *J*=8.1 Hz, 1 H, H-2), 4.35 (s, 1 H, H-5), 4.05–3.85 (m, 1 H, H-1'), 3.80–3.57 (m, 1 H, H-1'), 3.05 (d, *J*=6.3 Hz, 1 H, H-9), 3.03 (d, *J*=12 Hz, 1 H, H-10), 2.98–2.79 (m, 1 H, H-8), 2.69 (dd, *J*=11.7 Hz, *J*=4.8 Hz, 1 H, H-16), 2.52 (s, 3 H, H-7'), 2.40 (t, *J*=7.2 Hz, 2 H, H-5'), 2.32–2.17 (m, 4 H, H-10, H-16, H-22), 2.15–2.00 (m, 1 H, H-15), 1.98–1.50 (m, 9 H, H-7, H-15, H-18, H-2', H-4'), 1.43 (s, 3 H, H-21), 1.33–1.28 (m, 2 H, H-3'), 1.23 (s, 3 H, H-20), 1.18–1.00 (m, 2 H, H-8, H-17), 0.93–0.79 (m, 1 H, H-23), 0.78–0.62 (m, 1 H, H-17), 0.61–0.46 (m, 2 H, H-24, H-25), 0.2–0.07 (m, 2 H, H-24, H-25); HRMS *m/z* calcd for [M+H]⁺ 661.3306, found 661.3328; Anal. Calcd for C₃₈H₄₈N₂O₆S: C, 69.06; H, 7.32; N, 4.24; found: C, 69.32; H, 7.20; N, 4.34.

4.2.8. 6-O-Hexanenitrile-6-O-desmethyl-diprenorphine (7)—A stirred solution of **6** (40 mg, 0.06 mmol) in isopropanol (1.6 mL) containing NaOH (1.0 N, 1.4 mL) was refluxed 7 h under nitrogen, and allowed to cool to ambient temperature. NaHCO₃ (8.4%, 4.0 mL) was added, and the mixture was partitioned with isopropanol (3×10 mL) followed by ethyl acetate (3×10 mL). The combined organic layers were dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using cyclohexane:ethyl acetate (1:1) containing 1% Et₃N to give 15 mg (50%) of **7** as white solid: mp 80–83 °C; ¹H NMR (CDCl₃): δ 6.70 (d, *J*=8.1 Hz, 1 H, H-1), 6.51 (d, *J*=8.1 Hz, 1 H, H-2), 4.42 (s, 1 H, H-5), 4.05–3.86 (m, 1 H, H-1'), 3.73–3.57 (m, 1 H, H-1'), 3.02 (d, *J*=6.0 Hz, 1 H, H-9), 2.99 (d, *J*=18.6 Hz, 1 H, H-10), 2.94–2.80 (m, 1 H, H-8), 2.64 (dd, *J*=12 Hz, *J*=6.9 Hz, 1 H, H-16), 2.46–2.15 (m, 6 H, H-10, H-16, H-22, H-5'), 2.30–2.00 (ddd, 1 H, H-15), 1.95 (t, *J*=10.2 Hz, 1 H, H-7), 1.86–1.50 (m, 9 H, H-15, H-18, H-2', H-3', H-4'), 1.40 (s, 3 H, H-21), 1.21 (s, 3 H, H-20), 1.15–0.96 (m, 2 H, H-8, H-17), 0.94–0.65 (m, 2 H, H-17, H-23), 0.60–0.40 (m, 2 H, H-24, H-25), 0.21–0.03 (m, 2 H, H-24, H-25); HRMS *m/z* calcd for [M+H]⁺ 507.3217, found 507.3215; Anal. Calcd for C₃₁H₄₂N₂O₄: C, 73.49; H, 8.36; N, 5.53; found: C, 73.38; H, 8.54; N, 5.45.

4.2.9. 6-O-Hexylamino-6-O-desmethyl-diprenorphine (8)—An oven-dried flask, chilled in an ice bath and under nitrogen, was charged with THF (2.0 mL) and 1.0 M LiAlH₄ in THF (1.97 mL, 1.97 mmol). A solution of **7** (100 mg, 0.197 mmol) in THF (0.6 mL) was then added dropwise. The cooling bath was removed, and the mixture heated at 40 °C for 1 h. The mixture was allowed to cool to room temperature, quenched with half-saturated NaCl (2.0 mL) and adjusted to pH 9 using NaOH (1.0 M). Extraction with ethyl acetate (3×10 mL), followed by drying over Na₂SO₄, filtration and concentration under reduced pressure, provided a residue that was purified by column chromatography on silica gel using ethyl acetate: methanol: (3:1) containing 1% Et₃N to give 70 mg (70%) of **8** as a viscous oil: ¹H NMR (CDCl₃): δ 6.68 (d, *J*=8.1 Hz, 1 H, H-1), 6.50 (d, *J*=8.1 Hz, 1 H, H-2), 4.37 (s, 1 H, H-5), 4.11–3.97 (m, 1 H, H-1'), 3.90–3.34 (m, 1 H, H-1'), 3.02 (d, *J*=6.6 Hz, 1 H, H-9), 2.98 (d, *J*=18.9 Hz, 1 H, H-10), 2.93–2.69 (m, 3 H, H-8, H-6'), 2.63 (dd, *J*=12 Hz, *J*=5.1 Hz, 1 H, H-16), 2.47–2.15 (m, 4 H, H-10, H-16, H-22), 2.13–2.09 (m, 1 H, H-15), 1.90–1.74 (m, 1 H, H-7), 1.73–1.45 (m, 7 H, H-15, H-18, H-2', H-5'), 1.40 (s, 3 H, H-21), 1.33–1.16 (m, 6 H, H-20, H-3', H-4'), 1.14–0.95 (m, 2 H, H-8, H-17), 0.89–0.68 (m, 2 H, H-17, H-23), 0.60–0.40 (m, 2 H, H-24, H-25), 0.19–0.04 (m, 2 H, H-24, H-25); HRMS *m/z* calcd for [M+H]⁺ 511.3530, found 511.3526; Anal. Calcd for C₃₁H₄₆N₂O₄·0.25H₂O: C, 72.27; H, 9.10; N, 5.44; found: C, 72.11; H, 9.09; N, 5.26.

4.2.10. 6-O-(HexylamidoDOTA)-6-O-desmethyl-diprenorphine (10)—A mixture of **8** (50 mg, 0.10 mmol), DOTA-NHS (122 mg, 0.15 mmol), DMAP (2.4 mg, 0.02 μmol) and Et₃N (0.15 μL, 1.0 mmol) in CH₃CN (0.7 mL) was stirred 3 h at room temperature under nitrogen, and then concentrated under reduced pressure. The residue was diluted with water (5 mL), brought to pH 4 by dropwise addition of 30% TFA, loaded onto a reversed-phase C₁₈ column (1×10 cm), and eluted with a gradient of CH₃CN:H₂O (10:90 to 40:60) containing 0.1% TFA. This was followed by combination of similar fractions, and lyophilization. This material was purified again, in the same fashion, to give 76 mg (57%) of **10** as a white powder: mp 140–142 °C; ¹H NMR (CD₃CN+D₂O): δ 6.73 (d, *J*=8.1 Hz, 1 H,

H-1), 6.57 (d, $J=8.1$ Hz, 1 H, H-2), 4.46 (s, 1 H, H-5), 4.00–3.30 (m, 6 H, H-9, H-10, H-1', NCH₂CON), 3.20–2.20 (m, 28 H, H-8, H-6', H-16, H-22, CH₂COOH, NCH₂), 2.10–1.60 (m, 6 H, H-7, H-10, H-15, H-16, H-18), 1.50–1.00 (m, 18 H, H-8, H-15, H-17, H-20, H-21, H-2', H-3', H-4'), 0.80–0.60 (m, 3 H, H-23, H-24, H-25), 0.50–0.30 (m, 2 H, H-24, H-25); ¹³C NMR (201.2 MHz, D₂O): δ (298 K) 148.50 (C-4), 140.43 (C-3), 133.15 (C-12), 127.56 (C-11), 123.31 (C-1), 120.77 (C-2), 97.49 (C-5), 82.16 (C-6), 78.40 (C-19), 67.44 (*N*-CH₂-C=O), 61.72, 61.63 (2C, 2s: C-22; C-1', C-O), 57.97 (C-9), 48.58 (C-7), 48.07 (C-13), 47.83 (C-16), 42.26 (C-14), 38.39 (C-15), 34.29 (C-8), 32.52 (C-6', C-N), 32.18, 30.82 (2C, 2s: C-5', C-2'), 31.60 (C-17), 30.98 (C-20), 28.54, 27.87 (2C, 2s: C-4', C-3'), 27.09 (C-21), 26.52 (C-10), 20.27 (C-18), 8.09 (C-23), 7.43 (C-25), 5.27 (C-24); δ (343 K) new DOTA resonances at 57.29, 52.56, 52.39, 52.26 (4 br s, cyclen C); HRMS m/z calcd for [M+H]⁺ 897.5332, found 897.5338; Anal. Calcd for C₄₇H₇₂N₆O₁₁·4.0TFA: C, 48.82; H, 5.66; N, 6.21; found: C, 48.95; H, 5.86; N, 6.35. *Note*: Additional material was gained by performing semi-preparative reversed-phase HPLC on aliquots (3–4 mg) of the impure fractions remaining from column chromatography. A Waters Nova-Pak[®] C₁₈ column (7.8×300 mm, 6 μ m) was used with a ternary mobile phase, consisting of CH₃OH (12.5%), CH₃CN (12.5%) and H₂O (75%, containing 2.1% Et₃N and 2.8% acetic acid), at a flow rate of 8.0 mL/min with UV (254 nm) detection to give pure **10** having a retention time (t_R) of 13.4 min.

4.2.11. 6-O-Hexylamido(In(III)DOTA)-6-O-desmethyl-diprenorphine (**12**)—A

solution of **10** (34 mg, 25 μ mol) and InCl₃ (13.8 mg, 62 mmol) in water (7.0 mL) was refluxed for 45 min, cooled to ambient temperature, and quenched with disodium EDTA (0.1 M, 0.8 mL). The reaction mixture was loaded onto a reversed-phase C₁₈ column (1×10 cm). Elution with CH₃CN:H₂O (25:75) containing 0.1% TFA, followed by combination of similar fractions and lyophilization, gave 30 mg (87%) of **12** as a white powder: mp>210 °C; ¹H NMR (CD₃CN+D₂O): δ 6.74 (d, $J=8.1$ Hz, 1 H, H-1), 6.56 (d, $J=8.1$ Hz, 1 H, H-2), 4.51 (s, 1 H, H-5), 4.00–3.70 (m, 2 H, H-1'), 3.65–2.90 (m, 13 H, H-8, H-9, H-10, NCH₂CON, NCH₂CO, H-6'), 2.85–2.30 (m, 23 H, H-10, H-15, H-16, H-18, H-22, NCH₂), 2.10–1.60 (m, 4 H, H-7, H-15, H-5'), 1.50–0.90 (m, 3 H, H-23, H-24, H-25), 0.50–0.20 (m, 2 H, H-24, H-25); ¹³C NMR (150.8 MHz, D₂O): δ (298 K) 179.75, 179.64 (O-C=O), 174.11 (N-C=O), 148.42 (C-4), 140.33 (C-3), 133.06 (C-12), 127.45 (C-11), 123.21 (C-1), 120.70 (C-2), 97.29 (C-5), 82.04 (C-6), 78.26 (C-19), 67.27 (*N*-CH₂-C=O), 61.62, 61.52 (2C, 2s: C-22; C-1', C-O), 48.43 (C-7), 47.98 (C-13), 47.72 (C-16), 43.01 (C-14), 38.29 (C-15), 34.20 (C-8), 32.43 (C-6', C-N), 32.03, 31.50 (2C, 2s: C-5', C-2'), 30.70 (2C: C-17, C-20), 28.42, 27.69 (2C, 2s: C-4', C-3'), 27.08 (C-21), 26.43 (C-10), 20.25 (C-18), 7.99 (C-23), 7.34 (C-25), 5.17 (C-24); new DOTA resonances visible upon 20 Hz line broadening: 59.41 (C-9), 51–57 (broad cyclen C); HRMS m/z calcd for [M+H]⁺ 1009.4136, found 1009.4126; Anal. Calcd for C₃₁H₄₆N₂O₄·H₂O·3.0TFA: C, 46.50; H, 5.45; N, 6.14; found: C, 46.73; H, 5.80; N, 6.30.

4.3. Radiosynthesis

4.3.1. General radiolabeling procedure—[¹¹¹In]**11**/[¹¹¹In]**12** were prepared in septum-sealed glass vials by treating a water solution (50 μ L) containing the respective DPN-DOTA conjugate precursor **9/10** (50 μ g) with 0.4 M NH₄OAc (30 μ L), followed by

addition of [^{111}In]InCl₃ (100–200 μL , 0.05 N HCl; 0.5–2.0 mCi). The mixtures were then heated at 100 °C for 30 min, allowed to cool to room temperature, and quenched by addition of disodium EDTA (20 μL , 1.0 mM). Vessel contents were taken up in a syringe, along with rinses (3 $\times$ 50 μL) of the reaction vial with the ternary mobile phase to be used for reversed-phase HPLC purification. HPLC was performed on a system equipped with UV (254 nm) and radioactivity (NaI/Tl crystal) detectors at a flow rate of 4.0 mL/min using a radial compression module (Nova-Pak[®] C₁₈ cartridge; 8 $\times$ 100 mm, 6 μm). Conditions were used where the [^{111}In]-labeled complexes were resolved from the non-radioactive precursors. Incorporations of indium-111 were nearly complete. Eluent containing radioligand was collected, and diluted with an equal volume of water. These mixtures were loaded on an activated solid-phase extraction cartridge (Sep-Pak[®] Light, *t*-C₁₈), which was flushed with water (2.0 mL) to remove residual salts and solvents, and then flushed with air to eliminate the void volume of water. Elution with 70% ethanol (0.6–1.5 mL) gave the [^{111}In]-labeled complexes in 64–81% isolated radiochemical yields. The average for [^{111}In]**11** was 79% ($n=3$), while the average for [^{111}In]**12** was 74% ($n=3$). Both radioligands were pure (~99%) by HPLC, and coeluted with the respective non-radioactive complex **11/12**. Specific radioactivities were >4000 mCi/ μmol based upon the minimum detectable mass (HPLC, 254 nm) in samples of known radioactivity (Capintec, Inc. CRC-15W; Ramsey, NJ). Both radioligands were stable for at least 15 h when stored in 70% ethanol at –20 °C in the dark, and for at least 12 h when stored at room temperature in saline containing 5% ethanol. The HPLC mobile phase compositions used, along with the retention times (t_R) and capacity factors (k') observed, follow for each radiometal complex and its non-complexed precursor.

[^{111}In]**11**: $t_R=12.9$ min, $k'=21$; **9**: $t_R=18.2$ min, $k'=30$; ternary mobile phase: CH₃OH (6.5%), CH₃CN (6.5%); H₂O (87%, containing 2.1% Et₃N and 2.8% acetic acid).

[^{111}In]**12**: $t_R=15.5$ min, $k'=26$; **10**: $t_R=21.6$ min, $k'=36$; ternary mobile phase: CH₃OH (10%), CH₃CN (10%); H₂O (80%, containing 2.1% Et₃N and 2.8% acetic acid).

4.4. Lipophilicity measurements

Log D_{7.4} values were determined using methods previously reported.^{14,34} In brief, [^{111}In]**11** and [^{111}In]**12** (6 μCi) were each partitioned between equal volumes (3.5 mL) of *n*-octanol and phosphate buffered saline (0.1 M, pH 7.4; PBS). Each phase had been presaturated with the other. Mixtures were vortexed, and then centrifuged (1500 rpm, 5 min). Radioactivity from triplicate samples of *n*-octanol (0.5 mL) and PBS (0.1 mL), determined using an automated gamma counter, provided a distribution coefficient (*D*) as the ratio of mean counts per min/mL in *n*-octanol compared to PBS. Log D_{7.4} for [^3H]DPN was determined similarly, except the initial *n*-octanol phase was washed three times with PBS to remove potential hydrophilic contaminants, and radioactivity was measured by liquid scintillation counting. The process was repeated 4 to 5 times for each radiolabeled compound. For non-radioactive compounds, estimates of lipophilicity were calculated using ClogP software (ChemDraw[®], Perkin Elmer, Inc.), which treats the materials as being neutral.

4.5. Opioid receptor binding

Assays were conducted in membranes prepared from fresh mouse (δ) or frozen Hartley guinea pig (μ , κ) brains using published procedures.^{14,35,36} Brains from adult male CD-1[®] mice (Charles River Laboratories International, Inc.; Wilmington, MA) were collected humanely in compliance with National Institutes of Health guidelines, and with Institutional Animal Care and Use Committee approvals. Guinea pig brains were obtained from Rockland Immunochemicals Inc. (Limerick, PA). In brief, conditions were [³H] DAMGO (0.6 nM, 75 min, 25 °C; μ), [³H]U69,593 (0.6 nM, 90 min, 25 °C; κ) and [³H]NTI (0.1 nM, 90 min, 37 °C; δ). Non-specific binding was defined by DAMGO (5 μ M), U69, 593 (10 μ M) and NTI (1.0 μ M), respectively. Assays used ten inhibitor concentrations equally spaced on log scale. Brain protein concentrations were 0.3–0.5 mg/test tube. Tris–HCl buffer (50 mM; pH 7.4, 25 °C) was used for μ and κ assays, and was supplemented with protease inhibitors and bovine serum albumin (0.1%) for δ assays. Assays were terminated by addition of ice-cold buffer, and vacuum filtered through glass fiber papers (GF/B) pretreated with polyethyleneimine (0.5%). Tubes and filter papers were washed with cold buffer (3×5 mL), and the papers dried under reduced pressure. Papers were then incubated with cocktail, and liquid scintillation counting used to detect bound radioactivity (47% efficiency). Data were analyzed using Prism 4.0 (GraphPad Software, Inc.; La Jolla, CA). K_i values were calculated from IC₅₀ values by the Cheng-Prusoff equation,³⁷ using K_d values for [³H]DAMGO (0.60 nM), [³H]U69,593 (0.60 nM) and [³H]NTI (0.061 nM) previously measured under these conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Drs. Wei Wycoff, Patrick Cavins and Susan Lever for assistance with acquisition and interpretation of ¹³C NMR data. We thank the National Institutes of Health, National Cancer Institute (P50CA103130: Center for Single Photon-Emitting Cancer Imaging Agents) for support of this research. We also acknowledge resources and facilities provided by the Harry S. Truman Memorial Veterans' Hospital, the University of Missouri Life Sciences Mission Enhancement Program, and NSF (CHE-95-31247; DBI-0070359) and NIH (S10RR11962; S10RR022341) awards for NMR instrumentation. This work does not represent the views of the United States Department of Veterans Affairs.

References and notes

1. Lever JR. Curr. Pharm. Des. 2007; 13:33–49. [PubMed: 17266587]
2. Henriksen G, Willoch F. Brain. 2008; 131:1171–1196. [PubMed: 18048446]
3. Dannals RF. J. Label. Comp. Radiopharm. 2013; 56:187–195.
4. van Waarde, A., Absalom, AR., Visser, AK., Dierckx, RA. PET and SPECT of Neurobiological Systems. DierckxRudi, AJO.Otte, A.de Vries, EFJ.van Waarde, A., Luiten, PGM., editors. Springer; Berlin, Heidelberg: 2014. p. 585-623.
5. Headrick JP, See Hoe LE, Du Toit EF, Peart JN. Br. J. Pharmacol. 2015; 172:2026–2050. [PubMed: 25521834]
6. a Sehgal N, Smith HS, Manchikanti L. Pain Physician. 2011; 14:249–258. [PubMed: 21587328] b Albert-Vartanian, A., Boyd, MR., Hall, AL., Morgado, SJ., Nguyen, E., Nguyen, VP., Patel, SP., Russo, LJ., Shao, AJ., Raffa, RB. J. Clin. Pharm. Ther. 2016. <http://dx.doi.org/10.1111/jcpt.12404>
7. Tegeder I, Geisslinger G. Pharmacol. Rev. 2004; 56:351–369. [PubMed: 15317908]

8. a Sobczak M, Salaga M, Storr MA, Fichna J. J. Gastroenterol. 2014; 49:24–45. [PubMed: 23397116] b Nelson AD, Camilleri M. Ther. Adv. Chronic Dis. 2016; 7:121–134. [PubMed: 26977281]
9. Lever JR. Medchem. 2012; 2:142–147.
10. Madar I, Bencherif B, Lever J, Heitmiller RF, Yang SC, Brock M, Brahmer J, Ravert H, Dannals R, Frost JJ. J. Nucl. Med. 2007; 48:207–213. [PubMed: 17268016]
11. a Waterhouse RN. Mol. Imaging Biol. 2003; 5:376–389. [PubMed: 14667492] b Rankovic Z. J. Med. Chem. 2015; 58:2584–2608. [PubMed: 25494650]
12. Aldrich JV, McLaughlin JP. Drug Discov. Today Technol. 2012; 9:e23ee31. [PubMed: 23316256]
13. Remesic M, Lee YS, Hruby VJ. Curr. Med. Chem. 2016; 23:1288–1303. [PubMed: 27117332]
14. Duval RA, Allmon RL, Lever JR. J. Med. Chem. 2007; 50:2144–2156. [PubMed: 17402725]
15. a Lever, SZ., Lydon, JD., Cutler, CS., Jurisson, SS. Comprehensive Coordination Chemistry II: From Biology to Nanotechnology. Ward, MD., editor. Elsevier, Ltd.; Amsterdam: 2003. p. 883-911. b Liu S. Chem. Soc. Rev. 2004; 33:445–461. [PubMed: 15354226]
16. a Musachio JL, Lever JR. Bioconjug. Chem. 1992; 3:167–175. [PubMed: 1515469] b Lever JR, Ilgin N, Musachio JL, Scheffel U, Finley PA, Flesher JE, Natarajan TK, Wagner HN Jr. Frost JJ. Synapse. 1998; 29:172–182. [PubMed: 9593107]
17. a Wang RF, Tafani JA, Frances B, Bergon M, Coulais Y, Zajac JM, Guiraud R. Nucl. Med. Biol. 1997; 24:553–558. [PubMed: 9316084] b Wang RF, Tafani JA, Zajac JM, Guiraud R. Neuropeptides. 1999; 33:498–502. [PubMed: 10657531]
18. Bentley KW, Hardy DG. J. Am. Chem. Soc. 1967; 89:3281–3292. [PubMed: 6042764]
19. a Casy, AF., Parfitt, RT. Opioid Analgesics: Chemistry and Receptors. Plenum; New York: 1986. b Lewis JW, Husbands SM. Curr. Pharm. Des. 2004; 10:717–732. [PubMed: 15032698]
20. Soyka M. Subst. Abuse Rehabil. 2015; 6:1–14. [PubMed: 25610012]
21. Dangolla A, Silva I, Kuruwita VY. Vet. Anaesth. Analg. 2004; 31:276–279. [PubMed: 15509293]
22. a Chang KJ, Hazum E, Cuatrecasas P. Proc. Natl. Acad. Sci. U.S.A. 1981; 78:4141–4145. [PubMed: 6270660] b Sadee W, Rosenbaum JS, Herz A. J. Pharmacol. Exp. Ther. 1982; 223:157–162. [PubMed: 6288917] c Lee KO, Akil H, Woods JH, Traynor JR. Eur. J. Pharmacol. 1999; 378:323–330. [PubMed: 10493109] d Pasternak GW, Pan YX. Pharmacol. Rev. 2013; 65:1257–1317. [PubMed: 24076545]
23. a Portoghesi PS. J. Med. Chem. 2001; 44:2259–2269. [PubMed: 11428919] b Harvey JH, Long DH, England PM, Whistler JL. ACS Med. Chem. Lett. 2012; 3:640–644. [PubMed: 23585918]
24. a Lever JR, Dannals RF, Wilson AA, Ravert HT, Wagner HN Jr. Tetrahedron Lett. 1987; 28:4015–4018. b Lever JR, Mazza SM, Dannals RF, Ravert HT, Wilson AA, Wagner HN Jr. Appl. Radiat. Isot. 1990; 41:745–752. c Luthra SK, Brady F, Turton DR, Brown DJ, Dowsett K, Waters SL, Jones AK, Mathews RW, Crowder JC. Appl. Radiat. Isot. 1994; 45:857–873.
25. Kopcho JJ, Schaeffer JC. J. Org. Chem. 1986; 51:1620–1622.
26. For recent examples, see. a Schoultz BW, Reed BJ, Marton J, Willoch F, Henriksen GA. Molecules. 2013; 18:7271–7278. [PubMed: 23787515] b Fairclough M, Prenant C, Brown G, McMahon A, Lowe J, Jones A. J. Label. Comp. Radiopharm. 2014; 57:388–396. c Schoultz BW, Hjørnevik T, Reed BJ, Marton J, Coello CS, Willoch F, Henriksen G. J. Med. Chem. 2014; 57:5464–5469. [PubMed: 24933507]
27. a Liu S, Pietryka J, Ellars CE, Edwards DS. Bioconjug Chem. 2002; 13:902–913. [PubMed: 12121149] b Liu S, He Z, Hsieh W-Y, Fanwick PE. Inorg. Chem. 2003; 42:8831–8837. [PubMed: 14686864] c Hsieh W-Y, Liu S. Inorg. Chem. 2004; 43:6006–6014. [PubMed: 15360250]
28. Hansch C. Acc. Chem. Res. 1969; 2:232–239.
29. Manglik A, Kruse AC, Kobilka TS, Thian FS, Mathiesen JM, Sunahara RK, Pardo L, Weis WI, Kobilka BK. Granier S. Nat. 2012; 485:321–326.
30. Granier S, Manglik A, Kruse AC, Kobilka TS, Thian FS, Weis WI, Kobilka BK. Nature. 2012; 485:400–404. [PubMed: 22596164]
31. Wu H, Wacker D, Mileni M, Katritch V, Han GW, Vardy E, Liu W, Thompson AA, Huang XP, Carroll FI, Mascarella SW, Westkaemper RB, Mosier PD, Roth BL, Cherezov V, Stevens RC. Nature. 2012; 485:327–332. [PubMed: 22437504]

32. a Cox BM. Mol. Pharmacol. 2013; 83:723–728. [PubMed: 23249538] b Filizola M, Devi LA. Trends Pharmacol. Sci. 2013; 34:6–12. [PubMed: 23127545]
33. Mazza SM, Erickson RH, Blake PR, Lever JR. Magn. Reson. Chem. 1990; 28:675–681.
34. Lever SZ, Xu R, Fan K-H, Ferguson-Cantrell EA, Carmack TL, Watkinson LD, Lever JR. Nucl. Med. Biol. 2012; 39:401–414. [PubMed: 22172395]
35. a Fang L, Knapp RJ, Horvath R, Matsunaga TO, Haaseth RC, Hruby VJ, Porreca F, Yamamura HI. J. Pharmacol. Exp. Ther. 1994; 268:836–846. [PubMed: 8113996] b Contreras PC, Tam L, Drower E, Rafferty MF. Brain Res. 1993; 604:160–164. [PubMed: 8384509]
36. Heyl DL, Mosberg HI. J. Med. Chem. 1992; 35:1535–1541. [PubMed: 1315870]
37. Cheng Y, Prusoff WH. Biochem. Pharmacol. 1973; 22:3099–3108. [PubMed: 4202581]

Fig. 1. Structures of diprenorphine (DPN), representative radiolabeled analogs, and a cartoon of a hypothetical radiometal-labeled DPN conjugate binding to μ , δ and κ opioid receptors.

Scheme 1.

Synthesis of triol **1** from DPN, and conversion to tosyl-protected **2**.

Scheme 2. Synthesis of C6-*O*-alkylamino-DPN derivatives **5** and **8**.

Scheme 3.

Synthesis of DPN-DOTA conjugates **9** and **10**, and their conversion to indium (III) and [¹¹¹In]-labeled complexes **11** and **12**.

Fig. 2. Top panel: Reversed-phase HPLC chromatograms of purified $[^{111}\text{In}]\mathbf{11}$. Bottom panel: Purified $[^{111}\text{In}]\mathbf{11}$ coinjected with a standard mixture prepared from $\mathbf{11}$ and precursor $\mathbf{9}$ to demonstrate coelution, as well as resolution of metal complex from precursor (see Experimental 4.3 for conditions).

Table 1

Opioid receptor binding affinity, type selectivity and lipophilicity of DPN, DPN-DOTA conjugates (**9**, **10**) and their indium (III) complexes (**11**, **12**)

Compound	K_i (nM) ^a			Selectivity ^b		Lipophilicity
	μ	δ	κ	δ/μ	κ/μ	LogD _{7.4} ^c or CLogP ^d
DPN	0.30±0.06	0.54±0.04	0.28±0.07	1.8	0.9	+2.25±0.02
9	3.4±0.3	3.0±0.3	8.2±1.2	0.9	2.4	-2.42
11	1.0±0.1	2.4±0.3	4.2±0.7	2.3	4.0	-2.21±0.07
10	1.3±0.1	0.28±0.04	3.2±0.4	0.2	2.5	-1.74
12	0.32±0.02	0.33±0.04	1.7±0.3	1.0	5.2	-1.66±0.05

^a Means±SEM, *n*=3–5. μ : [³H]DAMGO, guinea pig brain; δ : [³H]NTI, mouse brain; κ : [³H]U69,593, guinea pig brain.

^b K_i ratios.

^c Measured values±SEM (*n*=4–5) for [³H]DPN, [¹¹¹In]**11** and [¹¹¹In]**12**.

^d Calculated values using ClogP software for **9** and **10**.