

## Synthesis and Evaluation of a Full-Agonist Orvinol for PET-Imaging of Opioid Receptors: [<sup>11</sup>C]PEO

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**Abstract:** Antagonist radiotracers have shown only a low sensitivity for detecting competition from high-efficacy agonists at opioid receptors (ORs) in vivo. We report that [<sup>11</sup>C]PEO binds with high affinity to  $\mu$  and  $\kappa$ -opioid receptors, is a full agonist, and concentrates in brain regions of rats with a high density of the  $\mu$ -OR after intravenous injection. Blocking studies with  $\mu$  and  $\kappa$ -OR selective compounds demonstrated that the binding of [<sup>11</sup>C]PEO is saturable and selective to the  $\mu$ -OR in rat brain.

The  $\mu$ -,  $\delta$ -, and  $\kappa$ -opioid receptors<sup>1</sup> are distributed in the central nervous system (CNS) of mammals. These opioid receptors (ORs) mediate the effects of the endogenous opioid peptides and are known to be involved in several physiological functions, including pain modulation<sup>2,3</sup> as well as representing molecular targets of opioid and several stimulant drugs of abuse.<sup>4,5</sup>

Noninvasive imaging of ORs by means of positron emission tomography (PET) enables investigations of the relationship between OR-mediated signaling and (patho)physiology at the molecular level.<sup>6</sup> The nonselective OR-antagonists [6-*O*-methyl-<sup>11</sup>C]diprenorphine ([<sup>11</sup>C]DPN) and [6-*O*-(2-[<sup>18</sup>F]fluoroethyl)]-diprenorphine ([<sup>18</sup>F]FDPN) are currently employed for studies in humans.<sup>7–10</sup> Despite detectable changes in OR occupancy of these orvinols in studies of chronic and experimental pain, pharmacological studies in humans<sup>11</sup> and rats<sup>12</sup> have supported minimal competition between high-efficacy agonists and [<sup>11</sup>C]DPN. This has led to the conclusion of a limited utility of DPN for monitoring OR occupancy in vivo.

Even though the  $\mu$ -OR agonist [<sup>11</sup>C]carfentanil is available for PET studies,<sup>6</sup> the employment of structurally matched agonist/antagonist pairs could simplify a test of the hypothesis whether an OR agonist PET tracer is more sensitive to competition relative to one with antagonistic properties. These investigations have so far been hampered by the lack of available tracers. Buprenorphine, a  $\mu$ -partial agonist and  $\kappa$ -antagonist, is already available as the <sup>11</sup>C-version<sup>13</sup> and could, upon availability of an agonist orvinol, complete the

spectrum of intrinsic activities at  $\mu$ -OR among these compounds (Chart 1).

(2*R*)-4,5- $\alpha$ -Epoxy-17-methyl-3-hydroxy-6-methoxy- $\alpha$ ,17-dimethyl- $\alpha$ -(2-phenylethyl)-6,14-ethenomorphinan-7-methanol (PEO<sup>a</sup>) belongs to the Bentley compounds.<sup>14</sup> In this paper, we report the synthesis of [<sup>11</sup>C]PEO and the biological evaluation of this compound as a potential PET tracer.

The TDPEO (**6**) precursor for radiolabeling was prepared from thebaine (**1**; CAS RN: [115-37-7]) in a five-step synthesis. The synthesis of [6-*O*-methyl-<sup>11</sup>C]PEO ([<sup>11</sup>C]PEO, [<sup>11</sup>C]**4**) is displayed in Scheme 1. In principle, the Diels–Alder (**DA**) reaction of morphinan-6,8-dienes with unsymmetrical dienophiles can afford eight isomers: the 7 $\alpha$ -, 7 $\beta$ -, 8 $\alpha$ -, 8 $\beta$ -substituted 6 $\alpha$ ,14 $\alpha$ -etheno in addition to the corresponding four 6 $\beta$ ,14 $\beta$ -etheno analogues.<sup>15</sup> The addition of methyl vinyl ketone to the T-shaped thebaine (**1**) molecule is regio- and stereoselective occurring on the  $\beta$ -face and gives exclusively the C-7 acetyl derivatives. The regioselectivity is induced by the 6-OCH<sub>3</sub> moiety that polarizes the diene system.<sup>16–18</sup> Thus DA addition of methyl vinyl ketone to thebaine (**1**) resulted in thevinone (**2a**; CAS RN: [15358-22-2]) as the main product (81%) and  $\beta$ -thevinone (**2b**; CAS RN: [16196-83-1]) as by-product.

The 7 $\alpha$ -ketone (**2a**) was reacted with 2-phenylethylmagnesium bromide and gave a complex product mixture (**3a**, **3b**, **3c**, and **3d**). The reaction took place with a high degree of stereoselectivity, in accordance with Cram's rule,<sup>19</sup> and resulted in the tertiary alcohol **3a** (CAS RN: [13965-63-4]) with 2*R* absolute configuration as the main product (62%). The formation of the major byproduct (**3b**) is the result of a base-catalyzed rearrangement,<sup>20</sup> which was followed by Grignard addition. In the case of 2-phenylethylmagnesium bromide, a  $\beta$ -hydrogen transfer is also possible (Grignard reduction). A mixture of secondary alcohols **3c/3d** was isolated as minor byproducts (1.7%). We confirmed the structure of **3b** by acid catalyzed dehydration, enol-ether hydrolysis, and rearrangement<sup>20</sup> to the 5,14-bridged thebainone derivative (**7**). 3-*O*-demethylation<sup>14</sup> of (2*R*)-phenylethyl thevinol (**3a**) resulted in (2*R*)-phenylethyl orvinol (PEO, **4**; CAS RN: [14521-98-3]), and the following 6-*O*-demethylation<sup>13</sup> led to 6-*O*-desmethyl-PEO (DPEO, **5**). 3-*O*-Tritylation of **5** in dichloromethane in the presence of triethylamine provided the trityl-protected precursor (TDPEO, **6**) for radiolabeling in > 99% purity.

The radiosynthesis of [<sup>11</sup>C]**4** was accomplished by treating **6** with 8–10 equiv NaH in DMF, followed by reaction with [<sup>11</sup>C]methyl iodide (5 min, 90 °C) and removal of the trityl group by adding 1 M HCl in EtOH. The crude product was purified by HPLC, yielding [<sup>11</sup>C]**4** in a decay-corrected radiochemical yield of 57 ± 16%, based on [<sup>11</sup>C]MeI (*n* = 18), in a specific activity of 1622 ± 233 mCi/ $\mu$ mol at end-of-synthesis and in a chemical purity of > 99%.

<sup>a</sup> Abbreviations: PEO, (2*R*)phenylethyl orvinol; DPEO, (2*R*)-6-*O*-Desmethyl-phenylethyl orvinol = 6-*O*-Desmethyl-PEO; TDPEO, (2*R*)-3-*O*-Trityl-6-*O*-desmethyl-phenylethyl orvinol; CHO, Chinese hamster ovary. HEK, human embryonic kidney. GTP $\gamma$ S, guanosine-5'-*O*-(3-thio)-triphosphate. DAMGO, [D-Ala<sup>2</sup>, N-MePhe<sup>4</sup>, Gly-ol]-enkephalin; U69593, (+)-(5 $\alpha$ ,7 $\alpha$ ,8 $\beta$ )-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-benzeneacetamide.

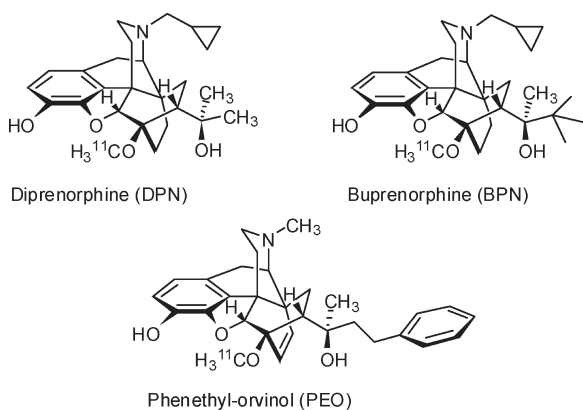
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The octanol–PBS partition coefficient ( $\log P_{\text{oct/PBS}}$ ) of [ $^{11}\text{C}$ ]4 was  $2.26 \pm 0.05$  ( $n = 4$ ).

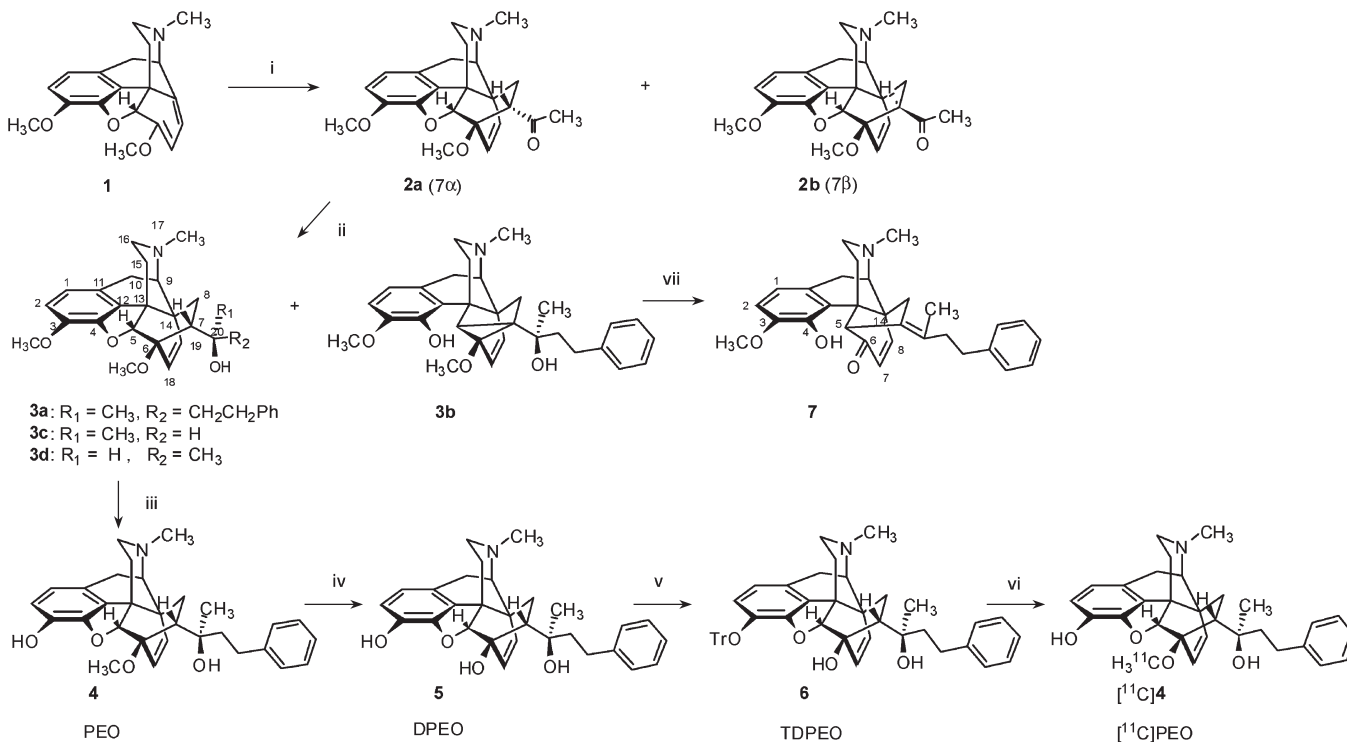
The affinities of 4 for the  $\mu$ -,  $\delta$ - and  $\kappa$ -ORs were determined in radioligand binding experiments in vitro using human cloned receptors stably expressed on CHO cells ( $\delta$  and  $\mu$ -OR) and HEK-293 ( $\kappa$ -OR) according to published procedures.<sup>21–23</sup> Compound 4 was found to possess high affinity binding to  $\kappa$ -OR ( $K_i = 0.12 \pm 0.08$  nM) and to  $\mu$ -OR ( $K_i = 0.18 \pm 0.06$  nM), and lower affinity to  $\delta$ -OR ( $K_i = 5.1 \pm 0.6$  nM).

$\kappa$ -OR agonist stimulation of [ $^{35}\text{S}$ ]GTP $\gamma$ S binding was measured for 4 using cloned receptors stably expressed on CHO cells for  $\mu$ -OR and HEK-293 cells for  $\kappa$ -OR according to published procedures.<sup>24,25</sup> The agonist efficacy of 4 was 105% relative to the maximal effect of the full  $\mu$ -agonist DAMGO<sup>25</sup> and 113% relative to that of the full  $\kappa$ -agonist U69593<sup>22</sup> at

### Chart 1. Selected $^{11}\text{C}$ -Labeled Orvinols



### Scheme 1. Synthesis of [6-*O*-Methyl- $^{11}\text{C}$ ]PEO ([ $^{11}\text{C}$ ]PEO, [ $^{11}\text{C}$ ]4<sup>a</sup>)



<sup>a</sup> Reagents and conditions: (i) methyl vinyl ketone, reflux, 2 h; (ii) 2-phenylethylmagnesium bromide, toluene-THF, 2 h; (iii) KOH, diethyleneglycol, 201 °C; (iv) LiAlH<sub>4</sub>, THF, chlorinated methane; (v) triphenylmethyl chloride, CH<sub>2</sub>Cl<sub>2</sub>, Et<sub>3</sub>N; (vi) [ $^{11}\text{C}$ ]methyl iodide, NaH, DMF, 2:1 M HCl, EtOH; (vii) 1 M HCl, EtOH, reflux, 4 h.

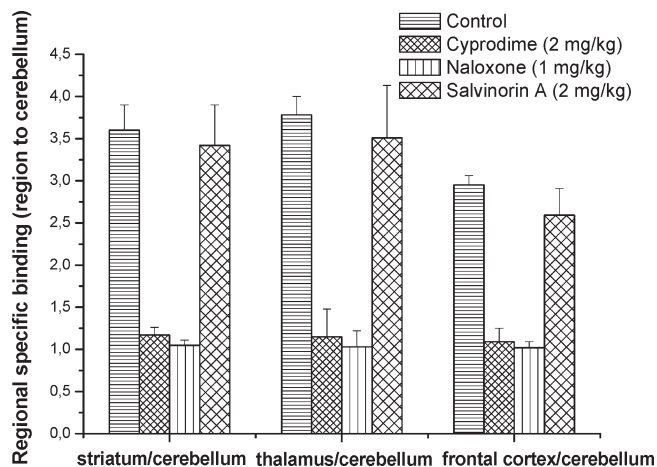
1 nM concentration of the ligand in question. The high potency of 4 found in the present work is consistent with the original report on the high in vivo potency of this compound.<sup>14</sup> To determine the regional brain uptake kinetics of [ $^{11}\text{C}$ ]4, male Wistar rats were injected intravenously (iv) with the radiotracer and sacrificed at different time points after injection. Dissected brain regions were measured for their content of radioactivity. The results from this ex vivo biodistribution study are listed in Table 1.

Initial uptake of [ $^{11}\text{C}$ ]4 in the brain was high, with the percent injected dose per gram of brain tissue (%ID/g) in various brain regions ranging from  $0.82 \pm 0.14\%$  in the frontal cortex to  $0.67 \pm 0.08\%$  in the cerebellum at 5 min postinjection (pi). Over time, radioactivity was highly concentrated in the frontal cortex, thalamus, and striatum, where the  $\mu$ -OR expression is highest. To obtain a measure of the specific binding to  $\mu$ -OR, the radioactivity in these regions relative to that in cerebellum was determined. The ratios (region/cerebellum) were in the range  $0.94 \pm 0.14$ – $1.22 \pm 0.16$  at 5 min pi,  $2.95 \pm 0.18$ – $3.78 \pm 0.22$  at 20 min pi, and  $4.47 \pm 0.23$ – $5.23 \pm 0.27$  at 60 min pi.

In separate experiments, the binding specificity and selectivity of the radioligand were evaluated by pretreating the

**Table 1.** Regional Brain Uptake Kinetics (% Dose/g) of [ $^{11}\text{C}$ ]4 in Rats (Mean  $\pm$  SD,  $N = 4$ –5)

region	time after injection		
	5 min	20 min	60 min
striatum	$0.63 \pm 0.11$	$0.83 \pm 0.27$	$0.89 \pm 0.25$
thalamus	$0.73 \pm 0.12$	$0.87 \pm 0.18$	$0.78 \pm 0.16$
frontal cortex	$0.82 \pm 0.14$	$0.68 \pm 0.12$	$0.76 \pm 0.21$
cerebellum	$0.67 \pm 0.08$	$0.23 \pm 0.14$	$0.17 \pm 0.11$



**Figure 1.** Regional specific binding of [ $^{11}\text{C}$ ]4 in rat brain (radioactivity in region/radioactivity in cerebellum).

animals with different pharmacological agents. When the rats were treated with  $\mu$ -antagonist cyprodime<sup>26</sup> (2 mg/kg, iv) 10 min before the injection of radioligand [ $^{11}\text{C}$ ]4 and sacrificed 20 min after, specific binding of the radioligand in the striatum, thalamus, and frontal cortex was reduced by 86, 84, and 82% when compared with the control group (Figure 1).

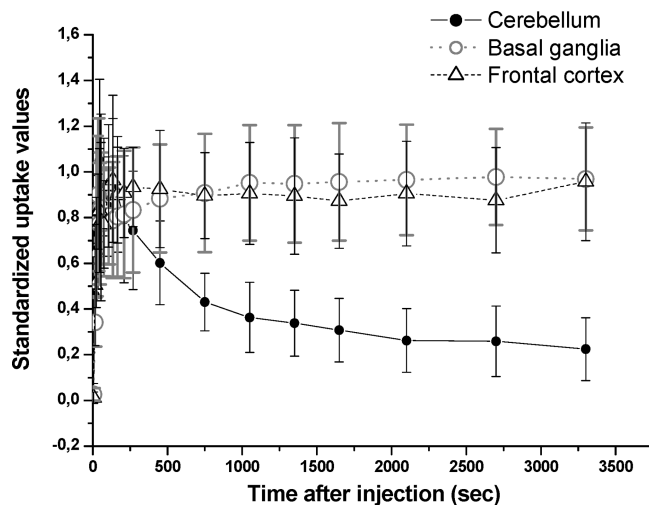
On the other hand, when the rats were treated with salvinorine A, a  $\kappa$ -OR selective agonist<sup>27,28</sup> at a dose of 2 mg/kg, binding of [ $^{11}\text{C}$ ]4 in the striatum, thalamus, and frontal cortex was reduced by only 5, 7, and 11%, respectively (Figure 1).

Blocking with naloxone (1 mg/kg body weight) reduced the binding of [ $^{11}\text{C}$ ]4 in striatum, thalamus, and frontal cortex by > 95% (Figure 1). Taken together, the results from these *ex vivo* studies in rats indicate that [ $^{11}\text{C}$ ]4 has an excellent brain uptake and a saturable and selective binding to the  $\mu$ -OR in rat brain. The reason for the selective binding of [ $^{11}\text{C}$ ]4 *in vivo* in rats may be related to the higher concentration of  $\mu$ -OR (41%) relative to that of  $\kappa$ -OR (9%) in rat brain.<sup>29</sup>

Experiments were conducted to investigate whether or not radioactive metabolites were contributing to the activity in brain of rats receiving [ $^{11}\text{C}$ ]4. To characterize and quantify radioactive species, rats ( $n = 4$ ) were injected with the tracer and their brains were removed 40 min after injection and homogenized. Radiolabeled species were extracted from the homogenate with  $88 \pm 3\%$  efficiency and analyzed using reverse-phase HPLC. Radioactivity in the extracted supernatants was determined to be  $\geq 92\%$  intact tracer at 40 min pi, based on the limits of detection, thus representing a conservative estimate of potential metabolization. These experiments indicate high metabolic stability of [ $^{11}\text{C}$ ]4 in brain and negligible blood–brain barrier permeability and binding in the brain of peripherally generated metabolites.

To further evaluate the brain uptake kinetics of [ $^{11}\text{C}$ ]4, we conducted small-animal PET imaging experiments in rats. These experiments confirmed the rapid entry of radioactivity into the brain and subsequent concentration in brain regions containing a high concentration  $\mu$ -OR, such as the basal ganglia and frontal cortex (Figure 2).

In summary, an orvinol  $\mu$ - and  $\kappa$ -agonist radioligand, [ $^{11}\text{C}$ ]PEO, has been successfully prepared. Evaluation of this radioligand for its potential to label  $\mu$ -OR in rats was carried out in *ex vivo* biodistribution studies and *in vivo* micro-PET imaging experiments in rats. [ $^{11}\text{C}$ ]PEO displays a highly



**Figure 2.** Regional time–activity curves of [ $^{11}\text{C}$ ]4 in rat brain (mean  $\pm$  SD,  $N = 8$ ). Points are activities measured in the cerebellum, basal ganglia, and frontal cortex. Activity data were normalized for injected dose and body weight.

selective and specific binding to the  $\mu$ -OR in striatum, thalamus, and frontal cortex. Micro-PET imaging experiments confirm the specific binding of [ $^{11}\text{C}$ ]4 *in vivo* in rats. Blocking of  $\mu$ -OR prior to injection of the tracer reduced the specific uptake effectively while blocking of  $\kappa$ -OR reduced the uptake only slightly. A study on the use of [ $^{11}\text{C}$ ]4 for detecting changes to the availability of opioid receptors will be published separately.<sup>30</sup>

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**Supporting Information Available:** General methods, experimental procedures,  $^1\text{H}$ - and  $^{13}\text{C}$  NMR shift values of the prepared compounds, radiolabeling, analytical HPLC, log  $P$  measurement, radioligand binding assays, animal experiments (PET/CT, determination of regional brain distribution, speciation of radioactivity in brain tissue). This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References

- (1) Dhawan, B. N.; Cesselin, F.; Raghurir, R.; Reisine, T.; Bradley, P. B.; Portoghese, P. S.; Hamon, M. International Union of Pharmacology. XII. Classification of opioid receptors. *Pharmacol. Rev.* **1996**, *48*, 567–592.
- (2) Willoch, F.; Schindler, F.; Wester, H. J.; Empl, M.; Straube, A.; Schwaiger, M.; Conrad, B.; Tolle, T. R. Central poststroke pain and reduced opioid receptor binding within pain processing circuitries: a [ $^{11}\text{C}$ ]diprenorphine PET study. *Pain* **2004**, *108*, 213–220.
- (3) Sprenger, T.; Valet, M.; Boecker, H.; Henriksen, G.; Spilker, M. E.; Willoch, F.; Wagner, K. J.; Wester, H. J.; Tolle, T. R. Opioidergic activation in the medial pain system after heat pain. *Pain* **2006**, *122*, 63–67.
- (4) Prisinzano, T. E. Natural products as tools for neuroscience: discovery and development of novel agents to treat drug abuse. *J. Nat. Prod.* **2009**, *72*, 581–587.
- (5) Henriksen, G.; Willoch, F.; Talbot, P. S.; Wester, H. J. Recent development and potential use of  $\mu$ - and  $\kappa$ -opioid receptor ligands in positron emission tomography. *Drug Dev. Rev.* **2006**, *67*, 890–904.
- (6) Henriksen, G.; Willoch, F. Imaging of opioid receptors in the central nervous system. *Brain* **2008**, *131*, 1171–1196.

- (7) Hammers, A.; Asselin, M. C.; Turkheimer, F. E.; Hinz, R.; Osman, S.; Hotton, G.; Brooks, D. J.; Duncan, J. S.; Koeppe, M. J. Balancing bias, reliability, noise properties and the need for parametric maps in quantitative ligand PET: [<sup>11</sup>C]diprenorphine test–retest data. *Neuroimage* **2007**, *38*, 82–94.
- (8) Schreckenberger, M.; Klega, A.; Grunder, G.; Buchholz, H. G.; Scheurich, A.; Schirmacher, R.; Schirmacher, E.; Muller, C.; Henriksen, G.; Bartenstein, P. Opioid receptor PET reveals the psychobiologic correlates of reward processing. *J. Nucl. Med.* **2008**, *49*, 1257–1261.
- (9) Hammers, A.; Asselin, M. C.; Hinz, R.; Kitchen, I.; Brooks, D. J.; Duncan, J. S.; Koeppe, M. J. Upregulation of opioid receptor binding following spontaneous epileptic seizures. *Brain* **2007**, *130*, 1009–1016.
- (10) Boecker, H.; Sprenger, T.; Spilker, M. E.; Henriksen, G.; Koppenhoefer, M.; Wagner, K. J.; Valet, M.; Berthele, A.; Tolle, T. R. The runner's high: opioidergic mechanisms in the human brain. *Cereb. Cortex* **2008**, *18*, 2523–2531.
- (11) Melichar, J. K.; Hume, S. P.; Williams, T. M.; Daghli, M. R.; Taylor, L. G.; Ahmad, R.; Malizia, A. L.; Brooks, D. J.; Myles, J. S.; Lingford-Hughes, A.; Nutt, D. Using [<sup>11</sup>C]diprenorphine to image opioid receptor occupancy by methadone in opioid addiction: clinical and preclinical studies. *J. Pharm. Exp. Ther.* **2005**, *312*, 309–315.
- (12) Hume, S. P.; Lingford-Hughes, A. R.; Nataf, V.; Hirani, E.; Ahmad, R.; Davies, A. N.; Nutt, D. J. Low sensitivity of the positron emission tomography ligand [<sup>11</sup>C]diprenorphine to agonist opiates. *J. Pharmacol. Exp. Ther.* **2007**, *322*, 661–667.
- (13) Luthra, S. K.; Brady, F.; Turton, D. R.; Brown, D. J.; Dowsett, K.; Waters, S. L.; Jones, A. K. P.; Matthews, R. W.; Crowder, J. C. Automated radiosyntheses of 6-*O*-methyl-<sup>11</sup>C]diprenorphine and 6-*O*-methyl-<sup>11</sup>C]buprenorphine from 3-*O*-tritryl protected precursors. *Appl. Radiat. Isot.* **1994**, *45*, 857–873.
- (14) Bentley, K. W.; Hardy, D. G. Novel Analgesics and Molecular Rearrangements in the Morphine-Thebaine Group. III. Alcohols of the 6,14-*endo*-Ethenotetrahydrooripavine Series and Derived Analogs of *N*-Allylnormorphine and -norcodeine. *J. Am. Chem. Soc.* **1967**, *89*, 3281–3292.
- (15) Linders, J. T. M.; Maat, L. Face selectivity of the Diels–Alder reaction of thebaine-like morphinandienes, a computational approach. *Bull. Soc. Chim. Belg.* **1989**, *98*, 265–276.
- (16) Bentley, K. W.; Hardy, D. G. Novel Analgesics and Molecular Rearrangements in the Morphine-Thebaine Group. I. Ketones Derived from 6,14-*endo*-Ethenotetrahydrothebaine. *J. Am. Chem. Soc.* **1967**, *89*, 3267–3273.
- (17) Ghosh, A. C.; Portlock, D. E.; Dalzell, H. C.; Malmberg, C.; Herlihy, P.; Razdan, R. K.; Duax, W. L.; Smith, D. Diels–Alder Reaction of  $\beta$ -Dihydrothebaine and Its 4-Phenyl Ether with Methyl Vinyl Ketone: Synthesis of 6,14-*exo*-Ethenomorphinans. *J. Org. Chem.* **1983**, *48*, 4137–4139.
- (18) Linders, J. T. M.; Kokje, J. P.; Overhand, M.; Lie, T. S.; Maat, L. Diels–Alder reaction of 6-demethoxy- $\beta$ -dihydrothebaine with methyl vinyl ketone using microwave heating; preparation and pharmacology of 3-hydroxy- $\alpha$ , $\alpha$ -17-trimethyl-6 $\beta$ ,14 $\beta$ -ethenomorphinan-7 $\beta$ -methanol, a novel deoxygenated diprenorphine analog. *Recl. Trav. Chim. Pays-Bas.* **1988**, *107*, 449–454.
- (19) Bentley, K. W.; Hardy, D. G.; Meek, B. Novel Analgesics and Molecular Rearrangements in the Morphine-Thebaine Group. II. Alcohols Derived from 6,14-*endo*-Etheno- and 6,14-*endo*-Ethanotetrahydrothebaine. *J. Am. Chem. Soc.* **1967**, *89*, 3273–3280.
- (20) Bentley, K. W.; Hardy, D. G.; Crocker, H. P.; Haddlesey, D. I.; Mayor, P. A. Novel Analgesics and Molecular Rearrangements in the Morphine-Thebaine Group. VI. Base-Catalyzed Rearrangements in the 6,14-*endo*-Ethenotetrahydrothebaine Series. *J. Am. Chem. Soc.* **1967**, *89*, 3312–3321.
- (21) Simonin, F.; Befort, K.; Gaveriaux-Ruff, C.; Matthes, H.; Nappey, V.; Lannes, B.; Micheletti, G.; Kieffer, B. The human  $\delta$ -opioid receptor: genomic organization, cDNA cloning, functional expression, and distribution in human brain. *Mol. Pharmacol.* **1994**, *46*, 1015–1021.
- (22) Simonin, F.; Gaveriaux-Ruff, C.; Befort, K.; Matthes, H.; Lannes, B.; Micheletti, G.; Mattei, M.-G.; Charron, G.; Bloch, B.; Kieffer, B. Kappa-opioid receptor in humans: cDNA and genomic cloning, chromosomal assignment, functional expression, pharmacology and expression pattern in the central nervous system. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7006–7010.
- (23) Wang, J. B.; Johnson, P. S.; Persico, A. M.; Hawkins, A. L.; Griffin, C. A.; Uhl, G. R. Human mu opiate receptor: cDNA and genomic clones, pharmacologic characterization and chromosomal assignment. *FEBS Lett.* **1994**, *338*, 217–222.
- (24) Zhu, J.; Luo, L. Y.; Li, J. G.; Chen, C.; Liu-Chen, L. Y. Activation of the cloned human kappa opioid receptor by agonists enhances [<sup>35</sup>S]GTP $\gamma$ S binding to membranes: determination of potencies and efficacies of ligands. *J. Pharmacol. Exp. Ther.* **1997**, *282*, 676–684.
- (25) Alt, A.; Mansour, A.; Akil, H.; Medzihradsky, F.; Traynor, J. R.; Woods, J. H. Stimulation of guanosine-5'-*O*-(3-[<sup>35</sup>S]thio)triphosphate binding by endogenous opioids acting at a cloned mu receptor. *J. Pharmacol. Exp. Ther.* **1998**, *286*, 282–288.
- (26) Schmidhammer, H.; Burkard, W. P.; Eggstein-Aeppli, L.; Smith, C. F. Synthesis and biological evaluation of 14-alkoxymorphinans. 2. (–)-*N*-(Cyclopropylmethyl)-4,14-dimethoxymorphinan-6-one, a selective mu opioid receptor antagonist. *J. Med. Chem.* **1989**, *32*, 418–421.
- (27) Hooker, J. M.; Patel, V.; Kothari, S.; Schiffer, W. K. Metabolic Changes in the Rodent Brain after Acute Administration of Salvinorin A. *Mol. Imaging Biol.* **2009**, *11*, 137–143.
- (28) Munro, T. A.; Rizzacasa, M. A.; Roth, B. L.; Toth, B. A.; Yan, F. Studies toward the pharmacophore of salvinorin A, a potent kappa opioid receptor agonist. *J. Med. Chem.* **2005**, *48*, 345–348.
- (29) Mansour, A.; Khachaturian, H.; Lewis, M. E.; Akil, H.; Watson, S. J. Anatomy of CNS opioid receptors. *Trends Neurosci.* **1988**, *11*, 308–314.
- (30) Hjernevik, T.; Schoultz, B. W.; Marton, J. Gjerstad, J.; Drzezga, A.; Henriksen, G.; Willoch F. C-fibre LTP is associated with reduced opioid neurotransmission in rat brain. Submitted for publication.