13. Synthesis and Tritium Labelling of 6β-Amino-4,5α-epoxymorphinans and Their 14-Hydroxy Derivatives as Potential Affinity Labelling Probes with μ Opioid Agonist Activity

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The synthesis of 6β-(methylfumaramido) and 6β-chloroacetamido derivatives **1b** and **1c** of 6β-amino-7,8-didehydromorphinan and the corresponding 14-hydroxy derivatives **1e** and **1f** are described. The 7,8-dihydro derivatives of these compounds were synthesized in inactive (**2b, c, e, f**) as well as in tritiated form (**3b, c, e, f**).

Introduction. – The existence of three major types of opioid receptors is now well established. For the study of these receptors, radiolabelled ligands are required with high receptor-binding affinity, high selectivity, and high specific radioactivity.

In some instances, nonequilibrium-type ligands that are capable to bind covalently to the receptor are preferred. Precise information can be obtained *in vitro* by the use of nonequilibrium ligands in the receptor-binding experiments as well as in mapping the location and distribution of type-specific sites. It is possible to label opioid receptors by electrophilic affinity radioligands [1–8], although affinity labelling of the μ opioid receptor has always been difficult due to the lack of radioligands with high selectivity and affinity necessary to obtain irreversible labelling. Usually, electrophilic groups are attached to the C(6) position of the 4,5α-epoxymorphinans, but in some cases 4,5α-epoxymorphinans contained the reactive group at position C(14) [2].

The first successful antagonist affinity label prepared was a nitrogen mustard derivative of naltrexone, β-chloronaltrexamine (β-CNA), that exhibited irreversible binding to the μ opioid receptors [3]. Its agonist analogue, β-chlorooxymorphamine (β-COA), behaved also as an irreversible opioid ligand both *in vivo* and *in vitro* [4]. In an effort to obtain affinity labels that have higher selectivity, electrophilic groups that are less reactive and more selective than the nitrogen mustard group were attached to C(6) of naltrexone. These derivatives possess fumaramate [5] [6], isothiocyanate, and iodoacetamido groups [7]. These studies resulted in the synthesis of β-funaltrexamine (β-FNA), a highly selective and irreversible μ opioid receptor antagonist. The synthesis of the ^{3}H-labelled form of β-FNA was also reported [8]. The corresponding agonist analogue (β-FOA) resulting from oxymorphone was prepared too, but this derivative proved to be a reversible μ ligand both *in vivo* and *in vitro*.

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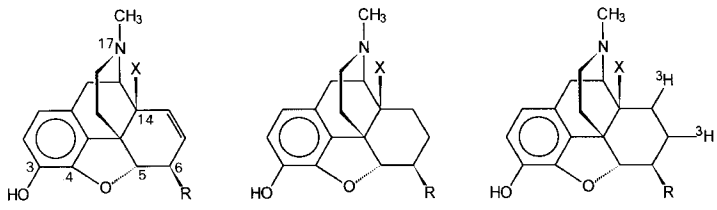

1 **2** **3**

	X	R
a	H	NH_2
b	H	(*E*)-NHC(O)CH=CHCOOMe
c	H	$NHC(O)CH_2Cl$
d	OH	NH_2
e	OH	(*E*)-NHC(O)CH=CHCOOMe
f	OH	$NHC(O)CH_2Cl$

The purpose of our work was to prepare opioid agonists containing an electrophilic group (methylfumaramido or chloroacetamido) at C(6). We also planned the radioactive labelling of these compounds, so we had to elaborate rapid and efficient synthetic methods.

Chemistry. – The 6β-amino-6-deoxymorphine (**1a**) and 6β-amino-6-deoxy-14β-hydroxymorphine (**1d**) and their dihydro derivatives **2a** and **2d** were synthesized by the method of *Simon et al.* [9] which gave configurationally pure products and better yields for 6β-aminomorphinans than described earlier [10]. Unlabelled amides **1b**, **c**, **e**, **f**, and **2b**, **c**, **e**, **f** were synthesized from the appropriate amines with chloroacetic or methyl hydrogen fumarate using *N*-hydroxysuccinimide and dicyclohexylcarbodiimide as coupling reagents.

Tritiated 6β-amino-6-deoxy-7,8-dihydromorphine **3a** and its 14-hydroxy derivative **3d** were obtained by catalytic saturation of the 7,8-double bond of the unsaturated aminodeoxymorphins **1a** and **1d**, respectively. The tritiation was performed in DMF solution using PdO catalyst and $^3H_2$ gas at room temperature. Labelled amides **3b**, **c**, **e**, **f** were synthesized by the mixed anhydride method [11] using isobutyl chloroformate as coupling reagent and *N*-methylmorpholine as a base in THF solution at −5°. This method provided good yields and a negligible amount of by-products [12]. Regarding to the very small amount of the starting radioactive compounds (1 μmol or less), it was useful to apply a 5–10 fold excess of the inactive reagents. Our results showed that specific activity of the starting compounds **3a** and **3d** was not affected by the coupling reactions.

Biological studies are in progress, and results will be published elsewhere.

Experimental Part

General. $^3H_2$ Gas was purchased from *Technabexport*, Russia, and contained at least 98% of $^3H_2$. All materials were anal. grade, but DMF and Et_3N were purified by vacuum distillation and dried over molecular sieves prior to use. The amount of tritiated material was measured by UV detection on a *Shimadzu-UV-160* spectrophotometer. Radioactivity was counted in *Liquidfluor* scintillant (*BDH*, England) with a *Searle-Delta-300* liquid scintillation counter. Radiochemical purity was checked with a *Berthold Radichromatogram Tracemaster*. Anal. purity of compounds were controlled by TLC: silica gel *60* F_{254} (*Merck*, Art. No. 5554) plates; eluents (*v*/*v*):

$CHCl_3$/acetone/MeOH/NH_3 40:30:10:5 (*A*), AcOEt/MeOH/Et_3N 90:20:5 (*B*); benzene/MeOH 80:20 (*C*), $CHCl_3$/acetone/Et_2NH 50:40:10 (*D*), AcOEt/MeOH/NH_3 80:20:10 (*E*). The spots were detected by *Dragendorff* reagent (0.6% bismuth(III) nitrate basic and 1.5% KI in 15% aq. AcOH) and UV light. Column chromatography (CC): silica gel *60 M* (0.063–0.2 mm, *Reanal*); eluents *C* and *E* (see TLC). Melting points: *Electrothermal* digital instrument (type *8103*) in open capillary tubes and on a *Kofler* melting-point microscope; uncorrected. ^{1}H-NMR Spectra: *Varian-Gemini-200* instrument; δ in ppm, *J* (apparent coupling constant) in Hz. Mass spectra; *VG-Trio-2* mass spectrometer by the thermospray technique at the Analytical Laboratory of *Alkaloida Chemical Company Ltd.*, Tiszavasvári. All compounds gave satisfactory elemental analysis (C ± 0.3%, H ± 0.2%, N ± 0.3%).

Unlabelled Amides **1b, c, e, f** *and* **2b, c, e, f**: *General Procedure.* A soln. of *N*-hydroxysuccinimide (*Fluka*; 0.58 g, 5 mmol), methyl hydrogen fumarate (0.65 g, 5 mmol) or chloroacetic acid (*Fluka*; 0.48 g, 5 mmol), and dicyclohexylcarbodiimide (*Fluka*; 1.03 g, 5 mmol) in THF (25 ml) was stirred at r.t. for 0.5 h. The precipitated dicyclohexylurea was filtered off, the solvent evaporated, the residue (gum) dissolved in $CHCl_3$ (50 ml), and the primary amine **1a**, **1d**, **2a**, and **2d** (4.5 mmol) for **1b, c**, **1e, f**, **2b, c**, and **2e, f**, respectively, added. The mixture was stirred at r.t. for 3 h and then extracted with aq. Na_2CO_3 soln. and subsequently with H_2O. The org. layer was dried (Na_2SO_4) and evaporated, the gummy material dissolved in MeOH, Na_2CO_3 added, and the mixture boiled for 5 min, diluted with H_2O, and extracted with $CHCl_3$. The combined org. phase was washed first with sat. aq. NaCl soln., then with H_2O, dried (Na_2SO_4), and evaporated. Materials were purified by CC if it was needed, then crystallized.

*Methyl (*E*)-4-[(7,8-Didehydro-4,5α-epoxy-3-hydroxy-17-methylmorphinan-6β-yl)amino]-4-oxobut-2-enoate* (**1b**): Yield 37% after CC (*E*). M.p. 124–127° (Et_2O). R_f 0.21 (*C*), 0.31 (*D*), 0.77 (*E*). ^{1}H-NMR ($CDCl_3$): 2.5 (*s*, Me–N(17)); 3.8 (*s*, COOMe); 4.1 (br., OH–C(3)); 4.5 (*m*, H_α–C(6)); 4.8 (*s*, H_β–C(5)); 5.7 (*d*, H–C(8)); 5.8 (*m*, H–C(7)); 6.4 (*d*, NH–C(6)); 6.6 ('*q*', *AB*, 2 arom. H); 6.8, 7.0 ((*E*)-CH=CH). MS: 397 (45, $[M + 1]^+$).

*2-Chloro-*N*-(7,8-didehydro-4,5α-epoxy-3-hydroxy-17-methylmorphinan-6β-yl)acetamide* (**1c**): Yield 55% after CC (*E*). M.p. > 250° (dec.; EtOH). R_f 0.15 (*C*), 0.27 (*D*), 0.68 (*E*). ^{1}H-NMR ((D_6)DMSO): 2.3 (*s*, Me–N(17)); 4.1 (*s*, CH_2Cl); 4.2 (*m*, H_α–C(6)); 4.5 (*s*, H_β–C(5)); 5.7 (*m*, H–C(7), H–C(8)); 6.5 ('*q*', *AB*, 2 arom. H); 8.3 (*d*, NH–C(6)); 8.9 (*s*, OH–C(3)). MS: 361 (100, $[M + 1]^+$).

*Methyl (*E*)-4-[(7,8-Didehydro-4,5α-epoxy-3,14β-dihydroxy-17-methylmorphinan-6β-yl)amino]-4-oxobut-2-enoate* (**1e**): Yield 40%. M.p. 216–218° (AcOEt). R_f 0.33 (*C*), 0.54 (*D*), 0.97 (*E*). ^{1}H-NMR ((D_6)DMSO): 2.3 (*s*, Me–N(17)); 3.8 (*s*, COOMe); 4.3 (*m*, H_α–C(6)); 4.5 (*s*, H_β–C(5)); 5.8 (*m*, H–C(7), H–C(8)); 6.5 ('*q*', *AB*, 2 arom. H); 6.6, 7.2 ((*E*)-CH=CH); 8.4 (*d*, NH–C(6)); 9.0 (br., OH–C(3)). MS: 413 (100, $[M + 1]^+$).

*2-Chloro-*N*-(7,8-didehydro-4,5α-epoxy-3,14β-dihydroxy-17-methylmorphinan-6β-yl)acetamide* (**1f**): Yield 30%. M.p. > 250° (dec.; AcOEt). R_f 0.39 (*C*), 0.55 (*D*), 0.97 (*E*). ^{1}H-NMR ((D_6)DMSO): 2.3 (*s*, Me–N(17)); 4.2 (*s*, CH_2Cl); 4.3 (*m*, H_α–C(6)); 4.5 (*s*, Hβ–C(5)); 5.2 (br. OH–C(14)); 5.8 (*m*, H–C(7), H–C(8)); 6.5 ('*q*', *AB*, 2 arom. H); 7.9 (*d*, NH–C(6)); 9.1 (*s*, OH–C(3)). MS: 377 (100, $[M + 1]^+$).

*Methyl (*E*)-4-[(4,5α-Epoxy-3-hydroxy-17-methylmorphinan-6β-yl)amino]-4-oxobut-2-enoate* (**2b**): Yield 23%. M.p. 172–175° (AcOEt). R_f 0.07 (*C*), 0.34 (*D*), 0.85 (*E*). ^{1}H-NMR ((D_6)DMSO): 2.3 (*s*, COOMe); 3.7 (*s*, Me–N(17)); 4.4 (*d*, H_β–C(5)); 6.5–6.7 (*m*, 2 arom. H, 1 H of (*E*)-CH=CH); 7.0 (*d*, 1 H of (*E*)-CH=CH); 8.8 (*d*, NH–C(6)); 9.0 (br. OH–C(3)). MS: 399 (85, $[M + 1]^+$).

*2-Chloro-*N*-(4,5α-epoxy-3-hydroxy-17-methylmorphinan-6β-yl)acetamide* (**2c**): Yield 37%. M.p. > 250° (dec., EtOH). R_f 0.07 (*C*), 0.34 (*D*), 0.55 (*E*). ^{1}H-NMR ($CDCl_3$): 2.4 (*s*, Me–N(17)); 3.7 (*m*, H_α–C(6)); 4.0 (*q*, CH_2Cl); 4.4 (*d*, H_β–C(5)); 6.7 ('*q*', *AB*, 2 arom. H); 6.8 (*d*, NH–C(6)). MS: 363 (100, $[M + 1]^+$).

*Methyl (*E*)-4-[(4,5α-Epoxy-3,14β-dihydroxy-17-methylmorphinan-6β-yl)amino]-4-oxobut-2-enoate* (**2e**): Yield 26%. M.p. 137–141° ([5]: 149–153°). R_f 0.22 (*C*), 0.48 (*D*), 0.90 (*E*). ^{1}H-NMR ($CDCl_3$): 2.4 (*s*, Me–N(17)); 3.8 (*s*, COOMe); 4.5 (*d*, H_β–C(5)); 4.6 (br. OH–C(14)); 6.7 ('*q*', *AB*, 2 arom. H); 6.8–7.0 (*m*, (*E*)-CH=CH); 7.3 (*d*, NH–C(6)). MS: 415 (100, $[M + 1]^+$).

*2-Chloro-*N*-(4,5α-epoxy-3,14β-dihydroxy-17-methylmorphinan-6β-yl)acetamide* (**2f**): Yield 20% after CC (*C*). M.p. > 250° (dec.; AcOEt). R_f 0.18 (*C*), 0.54 (*D*), 0.97 (*E*). ^{1}H-NMR ((D_6)DMSO): 2.3 (*s*, Me–N(17)); 4.0 (*s*, CH_2Cl); 4.5 (*d*, H_β–C(5)); 6.6 ('*q*', *AB*, 2 arom. H); 8.5 (*d*, NH–C(6)); 9.1 (br. OH–C(3)). MS: 379 (100, $[M + 1]^+$).

6β-Amino-4,5α-epoxy-17-methyl[7,8-$^3H_2$]morphinan-3-ol (**3a**). A mixture of **1a** (2.0 mg, 7 μmol), DMF (0.9 ml), and PdO catalyst (5.3 mg; *Merck*) was treated with 15 Ci (555 GBq) of $^3H_2$ gas at r.t. in vacuum manifold [13]. After 2.5 h of tritiation, the excess of $^3H_2$ was removed by absorption on pyrophoric uranium, the catalyst filtered off using *Whatman-GF/C* glass-fiber filter, and the filtrate evaporated. Labile ^3H was removed by repeated evaporation with EtOH/H_2O 1:1. The radioactivity of the crude material was 134 mCi (4.96 GBq). Radiochemical purity of **3a** was > 95% by TLC (R_f 0.18 (*A*), 0.10 (*B*)), so **3a** was used for coupling reactions without purification. Specific activity: 24.8 Ci/mmol (0.918 TBq/mmol).

6β-Amino-4,5α-epoxy-17-methyl[7,8-$^3H_2$]morphinan-3,14β-diol (**3d**). A mixture of **1d** (4.0 mg, 13.2 μmol) DMF (0.8 ml), and PdO catalyst (11 mg; *Merck*) was treated with 15 Ci (555 GBq) of $^3H_2$ gas at r.t. for 2.5 h. Workup of the mixture was the same as described for **3a**. The radioactivity of the crude material was 473 mCi (17.5 GBq). Purification was not needed as shown by TLC (R_f 0.35 (*A*), 0.12 (*B*); purity > 95%). Specific activity: 37.9 Ci/mmol (14.7 TBq/mmol).

Labelled Amides: General Procedure. To a soln. of 5 μmol of acid (methyl hydrogen fumarate or chloroacetic acid), isobutyl chloroformate (5 μmol), and 4-methylmorpholine (5 μmol) in purified and dried (Na wire) THF (0.3 ml), 25 mCi (925 MBq) of labelled amine **3a** or **3d** in THF (0.2 ml) was added at −15°. The temp. was allowed to reach −5° and maintained for 40 min, then MeOH (0.5 ml) was added and the solvent evaporated. The crude product was purified by TLC (*A*). After developing the chromatogram, components were localized by autoradiography and the spots scraped and extracted by EtOH (spectrosc. grade).

Methyl (E)-4-[(4,5α-Epoxy-3-hydroxy-17-methyl[7,8-$^3H_2$]morphinan-6β-yl)amino]-4-oxobut-2-enoate (**3b**). According to the *General Procedure* with methyl hydrogen fumarate (0.65 mg, 5 μmol), isobutyl chloroformate, 4-methylmorpholine, and 25 mCi (925 MBq, 1 μmol) of **3a**. After purification, 9.94 mCi (368 GBq) of **3b** were recovered. Radiochemical purity > 95% by TLC (R_f 0.32 (*A*); 0.18 (*B*)), yield 40%. Specific activity: 24.6 Ci/mmol (0.910 TBq/mmol).

*2-Chloro-*N*-(4,5α-epoxy-3-hydroxy-17-methyl[7,8-$^3H_2$]morphinan-6β-yl)acetamide* (**3c**). According to the *General Procedure*, with chloroacetic acid (0.47 mg, 5 μmol) isobutyl chloroformate, 4-methylmorpholine, and 25 mCi (925 MBq, 1 μmol) of **3a**. After purification, 10.8 mCi (400 GBq) of **3c** were recovered. Radiochemical purity > 95% by TLC (R_f 0.37 (*A*); 0.11 (*B*)), yield 43%. Specific activity: 24.8 Ci/mmol (0.918 TBq/mmol).

Methyl (E)-4-[(4,5α-Epoxy-3,14β-dihydroxy-17-methyl[7,8-$^3H_2$]morphinan-6β-yl)amino]-4-oxobut-2-enoate (**3e**). According to the *General Procure*, with methyl hydrogen fumarate (0.65 mg, 5 μmol), isobutyl chloroformate, 4-methylmorpholine, and 25 mCi (925 MBq, 0.63 μmol) of **3d**. After purification, 19.0 mCi (703 GBq) of **3e** were recovered. Radiochemical purity > 95% by TLC (R_f 0.63 (*A*), 0.38 (*B*)), yield 76%. Specific activity: 39.7 Ci/mmol (1.47 TBq/mmol).

*2-Chloro-*N*-(4,5α-epoxy-3,14β-dihydroxy-17-methyl[7,8-$^3H_2$]morphinan-6β-yl)acetamide* (**3f**). According to the *General Procedure*, with chloroacetic acid (0.94 mg, 10 μmol) isobutyl chloroformate, 4-methylmorpholine and 50 mCi (1850 MBq, 1.26 μmol) of **3d**. After purification, 27.6 mCi (1020 GBq) of **3f** were recovered. Radiochemical purity > 95% by TLC (R_f 0.59 (*A*), 0.38 (*B*), yield 55%. Specific activity: 24.6 Ci/mmol (0.910 TBq/mmol).

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