arterial blood was collected in 1 mL of 3.2% sodium citrate in a polystyrene tube from Okamoto-Aoki spontaneously hypertensive rats (SHR) (Taconic Farms, Germantown, NY) between 19 and 24 weeks of age. The blood was diluted with 3 mL of cold saline and centrifuged at room temperature for 15 min at 460*g*. The platelet-rich plasma (PRP) was separated. The platelets were isolated by centrifuging the PRP at 4 °C for 10 min at 1060*g* and were washed in 4 mL of cold oxygenated Krebs phosphate buffer, pH 7.4. The chilled platelets recovered from centrifuging at 800*g* for 10 minutes were resuspended in oxygenated Krebs phosphate buffer and diluted to contain (4.5–6.0) × 10^4 platelets/μL. Platelets prepared by this procedure did not aggregate spontaneously.

The inhibition of thromboxane (TX) formation was studied by determining the concentration of thromboxane B_2 (TXB_2), the stable hydrolysis product of TXA_2 released into the incubation fluid by the platelets. Assay samples, prepared on ice, contained 200 μL of platelet suspension, 50 μL of saline, and 50 μL of vehicle or drug under study. The samples were incubated for 10 min at 37 °C in a metabolic shaker. The reaction was terminated by immersing the tubes in an ice bath and adding 50 μL of 0.5 M citric acid. The samples were centrifuged for 10 min in a refrigerated centrifuge and the supernatants thus obtained were decanted and stored at −20 °C. Controls wherein platelets, vehicle, and incubation buffer were inactivated in boiling water for 3 min prior to 37 °C incubation were run in parallel. The TXB_2 content for each sample was determined by a direct radioimmunoassay (RIA) utilizing a TXB_2 specific RIA kit purchased from New England Nuclear, Boston, MA, and instructions contained therein and expressed as picograms of TXB_2 formed per minute per sample, from which the percent inhibition of TXB_2 formation was calculated. The small amount of TXB_2 measured in the controls was considered released before incubation and was subtracted from the test samples before this calculation. The results of this test are summarized in Tables I–VI.

The inhibition of PGI_2 was similarly determined on guinea pig aortic ring preparations with $^3H_6$-keto $PGF_{1\alpha}$ (the stable hydrolysis product of PGI_2) levels as measured with a RIA method obtained from New England Nuclear. None of the test compounds altered levels of PGI_2 from control values.

Antihypertensive Activity in Spontaneously Hypertensive Rats. The test compounds were tested for antihypertensive activity by the published methods.[12] Male, 16 weeks old, spontaneously hypertensive rats of the Okamoto strain, from Taconic Farms, Germantown, NY, having an average mean arterial blood pressure of 170 ± 1.5 mmHg are used in the test. One to three rats are used per test compound. A rat is dosed by gavage with a test compound, suspended in 2% preboiled starch at a concentration of 50 mg/mL, at a dose of 100 mg/kg of body weight or less, with 0.9% sodium chloride loading at a dose of 25 mL/kg of body weight. A second identical dose of the test compound, without sodium chloride loading, is given 24 h later. At 28 h after the initial dose, the mean arterial blood pressure is measured by the method of Chan and Poorvin vide supra.[12] The procedure is repeated in a second and third rat depending on the activity found in the first rat. Compounds are considered active when blood pressure in one test SHR has been reduced to ≤116 mmHg or when the average of two test SHR has been reduced to ≤122 mmHg.

Acknowledgment. We thank L. Gehrlein and associates for elemental analyses and D. Cole, G. Morton, and co-workers for spectral studies. We also express our appreciation to Dr. Laurence Torley and Judy Lucas, Arlene Hoffman, Margaret Ronsburg, Trina Saunders, Gerald Quirk, and George Vice for determination of the biological properties of these compounds.

(12) Chan, P. S.; Poorvin, D. *Clin. Exp. Hypertension* **1979**, *1*, 817.

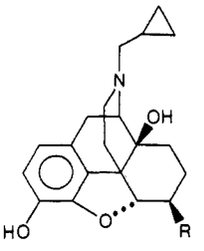
Nonequilibrium Opioid Antagonist Activity of 6,14-Dideoxynaltrexone Derivatives

J. W. Schoenecker,† A. E. Takemori,‡ and P. S. Portoghese*†

Department of Medicinal Chemistry, College of Pharmacy, and Department of Pharmacology, School of Medicine, University of Minnesota, Minneapolis, Minnesota 55455. *Received October 29, 1986*

A series of 6,14-dideoxynaltrexones that contain different electrophiles in the 6-position were synthesized and evaluated for nonequilibrium opioid antagonist activity in the guinea pig ileum and mouse vas deferens preparations. Members **3–5** of the series possessed irreversible antagonist activity profiles similar to those previously reported for the 14-hydroxy analogues. In contrast, the 14-deoxy-β-funaltrexamine (14-deoxy-β-FNA) analogue (**6**) exhibited a profile of irreversible antagonist activity that differed from that of β-FNA. It was concluded that the 14-hydroxy group is not essential for irreversible blockage when the electrophile is capable of reacting with a broad spectrum of nucleophiles. However, with a highly selective electrophile such as the fumarate group, the 14-hydroxy function appears to play a role in aligning the molecule to optimize attack by a receptor-based nucleophile.

β-Chlornaltrexamine[1] (**1**, β-CNA) and β-funaltrexamine[2] (**2**, β-FNA) are potent nonequilibrium opioid antagonists and are employed widely as tools in opioid research.[3]



1 (β-CNA), R = $N(CH_2CH_2Cl)_2$

2 (β-FNA), R = $NCOC{=}CCO_2Me$ (H, H)

† Department of Medicinal Chemistry.
‡ Department of Pharmacology.

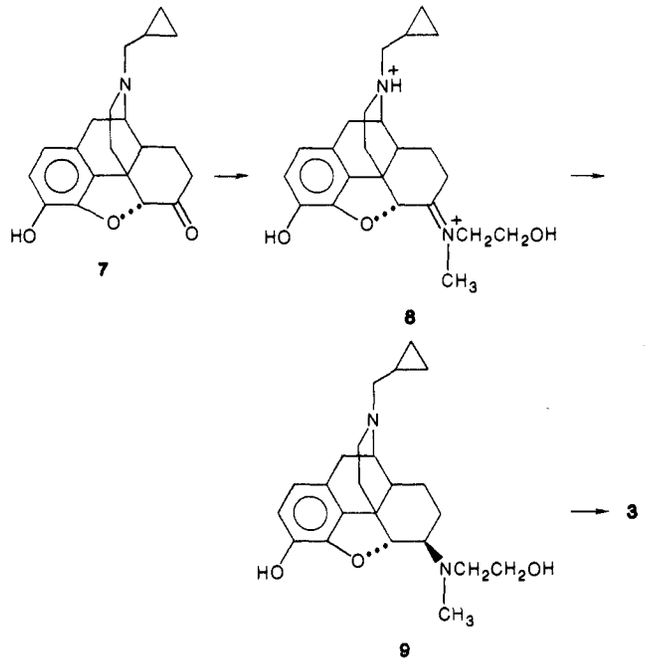
β-CNA blocks the three major opioid receptor types (μ, κ, δ), while β-FNA is highly selective for μ opioid receptors. Because these ligands become attached covalently to opioid receptors, they produce ultralong-lasting antagonism in vivo and irreversible blockage in vitro.

A variety of other electrophilic moieties have been attached to the naltrexamine pharmacophore in order to study the relationship between intrinsic reactivity and selectivity at opioid receptors.[4-6] In this paper we examine

(1) Portoghese, P. S.; Larson, D. L.; Jiang, J. B.; Takemori, A. E.; Caruso, T. P. *J. Med. Chem.* **1978**, *21*, 598.
(2) Portoghese, P. S.; Larson, D. L.; Sayre, L. M.; Fries, D. S.; Takemori, A. E. *J. Med. Chem.* **1980**, *23*, 233.
(3) Takemori, A. E.; Portoghese, P. S. *Annu. Rev. Pharmacol.* **1985**, *25*, 193.
(4) Sayre, L. M.; Larson, D. L.; Fries, D. S.; Takemori, A. E.; Portoghese, P. S. *J. Med. Chem.* **1983**, *26*, 1229.
(5) Sayre, L. M.; Larson, D. L.; Takemori, A. E.; Portoghese, P. S. *J. Med. Chem.* **1984**, *27*, 1325.

0022-2623/87/1830-1040$01.50/0

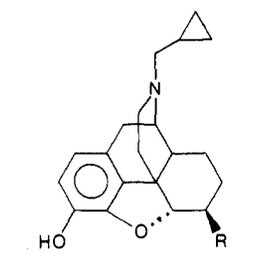
Scheme I



a closely related series that contains the 14-deoxynaltrexamine pharmacophore in order to evaluate whether or not the 14-hydroxyl group is important for nonequilibrium opioid antagonist activity.

Chemistry

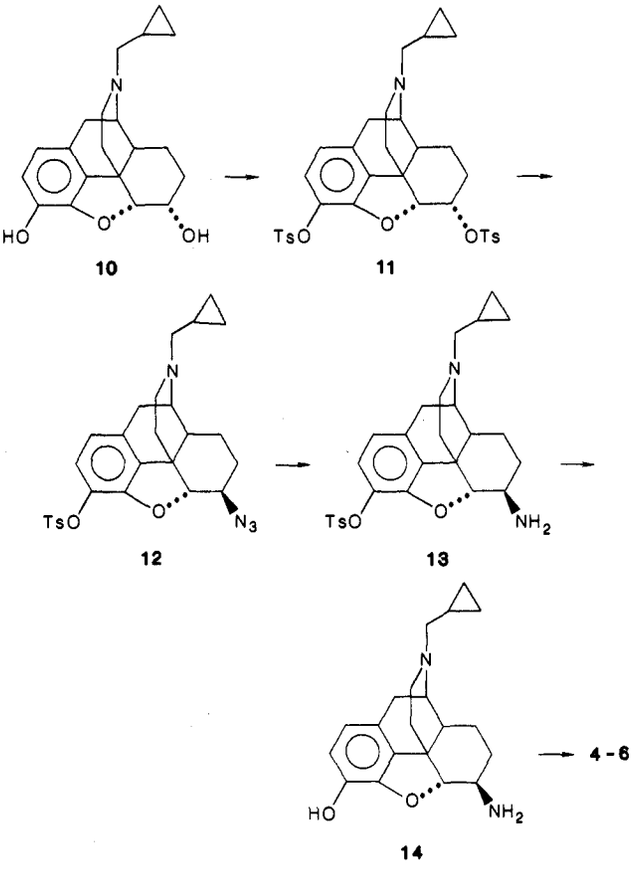
The target compounds (3–6) are related to reported[4,7] affinity labels that were found to possess irreversible opioid antagonist activity in smooth muscle preparations. The absence of the 14-OH group is the only structural difference between 3–6 and the reported series.



3, R = $N(CH_3)CH_2CH_2Cl$
4, R = N=C=S
5, R = $NHCOCH_2I$
6, R = $NHCOC(H)=C(H)CO_2CH_3$

Scheme I outlines the route to the β-chloroethylamine target compound **3**. 14-Deoxynaltrexone[8] (**7**) was converted to the iminium intermediate **8** by azeotropic removal of water. This was reduced in situ with sodium cyanoborohydride to afford the amino compound **9**, which was converted to **3** by treatment with thionyl chloride. The 6β stereochemistry was established from the C-5 NMR coupling constants of **9** and **3** ($J_{5,6}$ = 8.1 and 8.3 Hz), which are similar to those reported[9,10] for closely related compounds. The high 6β stereoselectivity of the reduction may be due to a boat conformation of ring C in the iminium intermediate **8**.[9,10] Such a boat conformation may be preferred due to pseudoallylic strain,[12] thereby making the α face of the iminium carbon more susceptible to attack by cyanoborohydride.

Scheme II



Target compounds **4–6** were obtained from **10** via Scheme II. Treatment of **10** with tosyl chloride in pyridine afforded the ditosyl derivative **11**, which, when heated with sodium azide in DMF, yielded the 6β-azide **12**. This nucleophilic displacement by azide proceeded with inversion of configuration at C-6, as indicated by the difference in the coupling constants in **11** ($J_{5,6}$ = 4.8 Hz) and **12** ($J_{5,6}$ = 8.35 Hz). Catalytic reduction of **12** gave the corresponding 6β-amino compound, which, after conversion by alkaline hydrolysis to **14**, was employed in the preparation of **4–6**. In this regard, the isothiocyanate **4** was obtained by reaction of **14** with thiophosgene in aqueous THF; iodoacetamide **5** was formed from reaction of the amine **14** with *N*-(iodoacetoxy)succinimide; the fumaramate methyl ester **6** was synthesized by coupling the amine with the corresponding acid chloride.

Pharmacology

Opioid agonist and antagonist activities were evaluated on the electrically stimulated guinea pig longitudinal muscle[13] (GPI) and mouse vas deferens[14] (MVD) preparations. Morphine, ethylketazocine (EK), and [D-Ala^2,D-Leu^5]enkephalin (DADLE) were employed as standard agonists because of their selectivity for μ, κ, and δ opioid

(6) Portoghese, P. S.; Rein, M. D.; Takemori, A. E. *J. Med. Chem.* **1986**, *29*, 1861.
(7) Schoenecker, J. W.; Takemori, A. E.; Portoghese, P. S. *J. Med. Chem.* **1987**, *30*, 933.
(8) Gates, M.; Montzka, T. A. *J. Med. Chem.* **1964**, *7*, 127.
(9) Sayre, L. M.; Portoghese, P. S. *J. Org. Chem.* **1980**, *45*, 3366.
(10) Jiang, J. B.; Hanson, R. N.; Portoghese, P. S.; Takemori, A. E. *J. Med. Chem.* **1977**, *20*, 1100.
(11) Mohamed, M. S.; Portoghese, P. S. *J. Org. Chem.* **1986**, *51*, 105.
(12) Johnson, F. *Chem. Rev.* **1968**, *68*, 375.
(13) Rang, H. P. *Br. J. Pharmacol.* **1964**, *22*, 356.
(14) Henderson, G.; Hughes, J.; Kosterlitz, H. N. *Br. J. Pharmacol.* **1972**, *46*, 764.

Table I. Irreversible Antagonist Activities of 6,14-Dideoxynaltrexone Derivatives on the GPI and MVD

	IC ratios[a]			
	GPI[b]		MVD[c]	
compd	morphine	EK	morphine	DADLE
2 (β-FNA)	6.0 ± 0.6[d]	1.0 ± 0.1[d]	3.9 ± 0.8[e]	1.8 ± 0.4[e]
3	125 ± 88[f]	6.27 ± 0.72	51 ± 28[g]	46.77 ± 4.82
4	4.8 ± 0.77	1.58 ± 0.73	11.50 ± 2.06	1.96 ± 0.27
	70 ± 30[g,h]	2.29 (2)[h]		
5	3.05 ± 0.79	1.03 ± 0.15	15.6 ± 2.9[g]	2.40 ± 0.20
6	1.58 (2)	1.33 (2)	4.12 ± 0.90	2.29 ± 0.52
	2.77 ± 0.83[h]	0.50 ± 0.03[h]		

[a] Agonist IC_{50} after antagonist treatment divided by the agonist control IC_{50}, unless otherwise specified. Where agonist was unable to elicit over a 65% response, an IC_{15} or IC_{20} ratio is reported. Values represent means of three separate determinations unless otherwise noted in parentheses. [b] Values from tissues treated with 20 nM of compound unless otherwise specified. [c] Values from tissues treated with 50 nM of compound unless otherwise specified. [d] Takemori, A. E.; Larson, D. L.; Portoghese, P. S. *Eur. J. Pharmacol.* 1981, *70*, 445. [e] Tissue was treated with 100 nM compound. Data is for leucine-enkephalin rather than DADLE: Ward, S. J.; Portoghese, P. S.; Takemori, A. E. *Eur. J. Pharmacol.* 1982, *80*, 377. [f] IC_{15} ratio. [g] IC_{20} ratio. [h] Tissue was treated with 200 nM compound rather than 20 nM.

Table II. Opioid Agonist Activities of 6,14-Dideoxynaltrexone Derivatives in the GPI and MVD

	potency ratio[a] ± SE	
compd	GPI[b]	MVD[c]
2 (β-FNA)[d]	5.0 (5)	4.4 ± 1.8
3	154 ± 68	380 ± 150
4	3.1 ± 0.9[e]	4.5 ± 1.1[f]
5	6.27 ± 0.93	25.3 (2)
6	17.62 ± 6.02	18.0 ± 3.3

[a] Mean values from three determinations except where noted in parentheses. [b] Compared to morphine = 1.0. Calculated from IC_{50} values of morphine and respective agents unless otherwise specified (morphine $IC_{50} = (2.92 \pm 0.71) \times 10^{-7}$ M, $n = 17$). [c] Compared to DADLE = 1000. Calculated from IC_{50} values of DADLE and respective agents unless otherwise specified (DADLE $IC_{50} = (3.57 \pm 0.47) \times 10^{-10}$ M, $n = 30$). [d] See footnote *d*, Table I. [e] Since agent was a partial agonist, potency ratio is based on IC_{10} values for agent and morphine. [f] Since agent was a partial agonist, potency ratio based on IC_{10} values for agent and DADLE.

receptors, respectively. The agonist response to these standard ligands is expressed as the concentration required to reduce the muscle twitch to half of the maximal response (IC_{50}).

All target compounds (**3–6**) were tested on the GPI for irreversible antagonism of the agonist effect of morphine or EK and on the MVD for irreversible blockage of morphine or DADLE (Table I). This testing involved the determination of the IC_{50} value of the standard agonist, washing (3 × 10 mL), and then redetermining the IC_{50} value in the same tissue after incubation (30 min) with the target compound followed by thorough washing (20 × 10 mL). The degree of irreversible antagonism is expressed as an IC_{50} ratio when the concentration–response curve is shifted in a parallel fashion. The IC_{50} ratio is defined as the IC_{50} of the agonist after the preparation had been incubated with target compound divided by the IC_{50} of this agonist in the untreated preparation. Antagonism is expressed as an IC_{20} ratio when the dose–response curve in the treated preparation was shifted to higher concentration with a decline in its maximum.

The agonist potencies for the target compounds (**3–6**) are listed in Table II. On the GPI, all compounds except **4** were substantially more potent than morphine. A similar agonist profile was observed on the MVD. Compound **4** behaved as a partial agonist. In order to determine whether or not the agonist effect of **6** was mediated through κ opioid receptors, it was tested on a β-FNA-treated GPI preparation.[15] The agonism of **6** was unchanged by such treatment. Since such a treated preparation is devoid of functional μ receptors, the lack of significant change implicates κ receptors in the agonist response.

The target compounds appear to possess varying degrees of irreversible antagonism toward the standard agonists in both smooth muscle preparations (Table I). The nitrogen mustard **3** blocked the effects of all three standard agonists, with the most potent antagonism toward morphine. The isothiocyanate **4** was morphine-selective, with little, if any, antagonism toward EK or DADLE. The iodoacetamide **5** blocked morphine more strongly in the MVD than in the GPI, and weak antagonism was observed toward DADLE. The β-FNA analogue **6** showed little antagonism toward morphine or EK in the GPI, although some weak antagonism toward morphine and DADLE was noted in the MVD.

Discussion

It appears that the more chemically reactive members of the 14-deoxy series were capable of irreversibly blocking opioid receptors in the GPI and MVD preparations. Thus, compounds **3–5** have antagonist potencies and selectivities that are similar to those of the corresponding 14-hydroxy analogues.[4,7] In this connection, the β-chloroethylamine **3** blocked μ, κ, and δ receptors, while the isothiocyanate **4** and iodacetamide **5** are selective for μ opioid receptors in the lower concentration range.

On the other hand, comparison of 14-deoxy-β-FNA analogue (**6**) with β-FNA reveals a substantial difference in irreversible blockage at μ receptors. In fact, there appears to be no relationship between concentration and antagonism in the GPI preparation. We are uncertain if the κ agonism of **6**, which is not easily removed on washing, has contributed to the low antagonist potency.

The results of these studies suggest that the 14-hydroxy group is not essential for the irreversible antagonism or selectivity in the irreversible blockage of opioid receptors when the electrophilic moiety is capable of reacting with a broad spectrum of nucleophiles. However, when the electrophilic moiety is highly selective, such as a fumarate group, the absence of a 14-hydroxy group compromises irreversible antagonist potency. It is possible that this reflects the critical nature of the alignment between a receptor-based sulfhydryl group and the fumaramate moiety.[16] Thus, one possible explanation for the apparent absence of irreversible antagonism by **6** in the GPI could be that the presence of a 14-hydroxyl group provides an

(15) Ward, S. J.; Portoghese, P. S.; Takemori, A. E. *Eur. J. Pharmacol.* 1982, *85*, 163.

(16) Griffin, J. F.; Larson, D. L.; Portoghese, P. S. *J. Med. Chem.* 1986, *29*, 778.

additional point of attachment that orients the molecule more favorably for nucleophilic attack of the receptor-bound ligand.

Experimental Section

General Procedures. Melting points were determined in open capillary tubes with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, and were within ±0.4% of the theoretical values. IR spectra were obtained on a Perkin-Elmer 281 instrument. NMR spectra were recorded at ambient temperature on Varian A-60, T-60, and FT-80 or JEOL FX90Q instruments using tetramethylsilane as an internal standard. Optical rotations were determined with a 1-dm cell on a Perkin-Elmer 141 polarimeter. Mass spectra were determined on AE 1 MS-30 or Finnegan 4000 instruments. Electron-ionization mass spectra were conducted at 20 or 70 eV, and chemical-ionization mass spectra were conducted with either ammonia or methane as reagent gases. All TLC data were determined with Eastman "Chromagram" 13181 plastic-backed sheets (silica gel) with fluorescent indicator. HPLC separation was performed on a Beckman Model 110-A system using a 10-μm (Rsil, Alltech) semipreparative (10 mm × 250 mm) silica gel column. HPLC grade solvents were obtained from MCB, Cincinnati, OH. Unless otherwise stated, all reagents and solvents were reagent grade and were used without prior purification. All chemicals were obtained from Aldrich Chemical Co., Milwaukee, WI. Gifts of drugs that were gratefully received were naltrexone hydrochloride from the National Institute on Drug Abuse, Rockville, MD. Hydrochloride salts were precipitated from solutions of free bases in nonpolar solvents by the addition of ethanolic HCl, followed by filtering and drying.

17-(Cyclopropylmethyl)-4,5α-epoxy-6β-[*N*-(2-hydroxyethyl)-*N*-methylamino]morphinan-3-ol (9). The ketone 7[8] (0.922 g, 2.83 mmol), 2-(methylamino)ethanol (0.425 g, 5.66 mmol), and benzoic acid (1.036 g, 8.49 mmol) were refluxed in benzene (50 mL) for 18 h with a Dean–Stark trap for azeotropic removal of water to yield the iminium benzoate intermediate 8. This solution was added to a stirred mixture of $NaCNBH_3$ (0.214 g, 2.83 mmol) in dry methanol (80 mL) over molecular sieves (3 Å). The reaction mixture was stirred for 15 min and allowed to stand for 48 h under dry nitrogen at 23 °C. The mixture was filtered and evaporated to dryness in vacuo, and the residue was dissolved in dilute aqueous HCl. The acidic aqueous solution was washed with CH_2Cl_2 (2 × 25 mL), basified with NH_4OH, and extracted with CH_2Cl_2 (3 × 75 mL). The basic CH_2Cl_2 layers were pooled, washed with brine (30 mL), dried (Na_2SO_4), filtered, and evaporated in vacuo to afford **9** (0.852 g, 78% yield) as an amorphous solid: mp 70–73 °C; $[\alpha]^{25}_D$ −176.8° (*c* 1.0, MeOH); TLC, R_f 0.25 (EtOAc–MeOH–NH_4OH, 90:10:3); IR (KBr) 1615 (m), 1450 (s) cm^{-1}; NMR ($CDCl_3$, δ 6.74–6.47 (2 d, 1 H each, Ar *H*), 4.39 (d, *J* = 8.13, C-5H), 2.33 (s, 4 H, NCH_2CH_2O); EIMS, *m/e* 384 (M^+). Anal. ($C_{23}H_{32}N_2O_3$) C, H, N.

6β-[*N*-(2-Chloroethyl)-*N*-methylamino]-17-(cyclopropylmethyl)-4,5α-epoxymorphinan-3-ol (3). To the primary alcohol **9**·HCl (90 mg, 0.196 mmol) in acetonitrile (5 mL) at 25 °C was added $SOCl_2$ (0.23 g, 0.14 mL, 1.96 mmol) dropwise with stirring. After 3 h the solvent and remaining $SOCl_2$ were evaporated in vacuo. The residue was dissolved in ethanol. Toluene was added, and the solvents were evaporated in vacuo to leave chromatographically pure **3**·2HCl (100%) as a white amorphous solid: mp 195 °C dec; $[\alpha]^{25}_D$ −100.7° (*c* 1.0, MeOH); TLC, R_f (EtOAc–MeOH–NH_4OH, 90:10:2); IR (KBr) 2800–2200 (s, broad, $^+$N–H stretching) cm^{-1}; NMR (D_2O) δ 6.89 (s, 2 H, Ar *H*), 5.12 (d, *J* = 8.35, C-5*H*); EIMS, *m/e* 402 (M^+). Anal. ($C_{23}H_{31}N_2O_2Cl$·2HCl·1.5H_2O) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,6α-bis(tosyloxy)morphinan (11). The diol[17] **10** (0.75 g, 2.29 mmol) in pyridine (5 mL) at 0 °C was added to a solution of *p*-toluenesulfonyl chloride (1.75 g, 9.16 mmol) in dry pyridine (5 mL). After 15 min the ice bath was removed, stirring was discontinued, and the mixture was allowed to stand at 25 °C for 48 h. The mixture was diluted with water (50 mL) and extracted with ether (5 × 30 mL). The ether extracts were pooled, washed with brine (20 mL), dried (Na_2SO_4), filtered, and evaporated in vacuo. Addition of toluene and evaporation of the toluene–pyridine azeotrope in vacuo followed by removal of residual solvent under high vacuum for 12 h left 1.37 g (94% yield) of chromatographically pure **11** as an amorphous solid: mp 95–97 °C; $[\alpha]^{25}_D$ −98.7° (*c* 1.0, MeOH); TLC, R_f 0.6 (EtOAc–MeOH–NH_4OH, 90:10:2); IR (CCl_4) 1180 (s, S=O stretch), 1190 (s, S=O stretch) cm^{-1}; NMR ($CDCl_3$) δ 7.82–7.19 (4 d, 2 H each, tosyl Ar *H*), 6.87–6.49 (2 d, 1 H each, Ar *H*), 4.36 (d, *J* = 4.84 Hz, C-5*H*); CIMS (CH_4), *m/e* 635 (M^+). Anal. ($C_{34}H_{37}NO_7S_2$·H_2O) C, H, N, S.

6β-Azido-17-(cyclopropylmethyl)-4,5α-epoxy-3-(tosyloxy)morphinan (12). To a solution of **11** (0.99 g, 1.57 mmol) in DMF (20 mL) was added a solution of NaN_3 (2.04 g, 31.4 mmol) in water (10 mL). The mixture was heated to 100 °C for 12 h with stirring. Water (50 mL) was added, and the mixture was basified with NH_4OH and extracted with ether (3 × 50 mL). The ether extracts were pooled, washed with brine (10 mL), dried (Na_2SO_4), filtered, and evaporated in vacuo. The residue was dissolved in CH_2Cl_2 (20 mL) and ether (200) mL). The solution was concentrated to 30 mL by removal of an ether–CH_2Cl_2 azeotrope in vacuo, and crystallization ensued (69% yield). Recrystallization from ether gave analytically pure **12**: mp 182–183 °C. The product was converted to its HCl salt to increase methanol solubility: $[\alpha]^{25}_D$ −139° (HCl salt, *c* 1.11, MeOH); TLC, R_f 0.69 (EtOAc–MeOH–NH_4OH, 90:10:2); IR (KBr, free base) 2090 (s, N_3 asymmetric stretching), 1180 (s, S=O stretch) cm^{-1}; NMR ($CDCl_3$) δ 7.86–7.77 (d, *J* = 8.35, 2 H, tosyl Ar *H*), 7.34–7.24 (d, *J* = 8.35, 2 H, tosyl Ar *H*), 6.99–6.57 (2 d, 1 H each, Ar *H*), 4.31 (d, *J* = 7.47, C-5*H*); CIMS (CH_4), *m/e* 506 (M^+). Anal. ($C_{27}H_{30}N_4O_4S$·0.5H_2O) C, H, N, S.

6β-Amino-17-(cyclopropylmethyl)-4,5α-epoxy-3-(tosyloxy)morphinan (13). A solution of **12** (1.86 g, 3.67 mmol) in methanol (20 mL) was acidified by the addition of concentrated HCl (2 mL). Palladium-on-carbon (10%, 0.19 g) was added as a slurry in water (1 mL). The mixture was hydrogenated at 50 psi for 48 h at 25 °C. Filtration and removal of solvent in vacuo afforded 2.1 g (99.6%) of **13**·2HCl. Conversion to the free base left chromatographically pure **13** (35%) as an amorphous solid: mp 58–60 °C; $[\alpha]^{25}_D$ −144° (*c* 1.0, MeOH); TLC, R_f 0.54 (EtOAc–MeOH–NH_4OH, 90:10:2); IR (KBr) 3370 (w, N—H symmetric stretch), 1598 (m, N—H in-plane bending), 1178 (s, S=O stretch) cm^{-1}; NMR ($CDCl_3$) δ 7.81–7.24 (2 d, 2 H each, tosyl Ar *H*), 6.83–6.49 (2 d, 1 H each, Ar *H*), 4.07–4.16 (d, *J* = 7.47 Hz, C-5*H*), 2.43 (s, 3 H, tosyl CH_3); EIMS, *m/e* 480 (M^+). Anal. ($C_{27}H_{32}N_2O_4S$) C, H, N, S.

6β-Amino-17-(cyclopropylmethyl)-4,5α-epoxymorphinan-3-ol (14). To 1 N methanolic KOH (50 mL) was added **13** (2.1 g, 3.79 mmol) and the resulting solution was allowed to stand at 25 °C. After 2 h, the solution was acidified with aqueous HCl, and the solvents were evaporated in vacuo. The residue was dissolved in water (75 mL), and the aqueous solution was washed with CH_2Cl_2 (2 × 10 mL). After basification with NH_4OH, the product was extracted with CH_2Cl_2 (4 × 50 mL). The pooled extract was dried (Na_2SO_4), filtered, and evaporated to leave 0.93 g of crude **14**. The residue was dissolved in absolute ethanol and concentrated to induce crystallization. A total of 0.75 g (61% yield) of crystalline **14** was collected in three crops of EtOH-solvated product: mp 115 °C. Slow heating at 110–120 °C afforded the unsolvated compound: mp 216–217 °C; $[\alpha]^{25}_D$ −138.4° (*c* 1.0, MeOH); TLC, R_f 0.10 (EtOAc–MeOH–NH_4OH, 90:10:3); IR (KBr) 1608 (s, NH_2, in-plane bending), 1330 (m, phenolic O–H bending), 1250 (s, phenolic C–O stretching) cm^{-1}; NMR ($CDCl_3$, solvated) δ 6.69–6.43 (2 d, 1 H each, Ar *H*), 4.21 (d, *J* = 6.81 Hz, C-5*H*); EIMS, *m/e* 327 (M^+). Anal. ($C_{20}H_{26}N_2O_2$·C_2H_5OH) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-6β-isothiocyanomorphinan-3-ol (4). To a stirred solution of **14** (60.4 mg, 0.185 mmol) and $NaHCO_3$ (31 mg, 0.37 mmol) in water (0.5 mL) and THF (1 mL) was added a solution of thiophosgene (26.7 mg, 0.318 mmol) in THF (0.2 mL) dropwise at 25 °C. After 30 min the reaction mixture was extracted with toluene (3 × 2 mL). The toluene extracts were pooled, dried (Na_2SO_4), filtered, and acidified with ethanolic HCl. Removal of solvent in vacuo left an oily residue that was digested in ether (2 mL) for 12 h to give a white solid. The solid was collected by filtration and washed with ether

(17) Meltzer, R. I. U.S. Patent 3 880 862, April 29, 1975.

(3 mL) to yield 68 mg of crude 4·HCl. The HCl salt was dissolved in water. The aqueous solution was basified with $NaHCO_3$ and extracted with $CHCl_3$. The $CHCl_3$ extracts were pooled and concentrated to a small volume, with care being taken not to bring the solution to dryness. The solution was loaded to a silica gel column, which was eluted at 10 psi ($CHCl_3$–acetone–Et_3N, 90:10:3). The eluate fractions containing pure product were pooled. An equal volume of toluene was added, and the solvents were partially evaporated, with care being taken not to bring the solution to dryness. The addition and evaporation of toluene was repeated several times to insure that all the triethylamine had been removed. The solution was acidified with ethereal HCl and evaporated to dryness in vacuo to leave 38 mg (51% yield) of 4·HCl: mp 93 °C dec; $[\alpha]^{25}_D$ −157.2° (c 0.5, MeOH); TLC, R_f 0.69 (ether–acetone–Et_3N, 90:10:3); IR (KBr) 2090 (s, N=C=S stretching) cm^{-1}; NMR ($CDCl_3$, free base) δ 6.77–6.52 (2 d, 1 H each, Ar *H*), 4.49 (d, *J* = 7.47 Hz, C-5*H*); EIMS, *m/e* 368 (M^+). Anal. ($C_{21}H_{24}N_2O_2S$·HCl) C, H, N.

***N*-[17-(Cyclopropylmethyl)-4,5α-epoxy-3-hydroxymorphinan-6β-yl]iodoacetamide (5).** To the primary amine 14 (50 mg, 0.153 mmol) in 2-propanol (2.5 mL) and THF (5 mL) at −70 °C was added a solution of *N*-(iodoacetoxy)succinimide[18] (48 mg, 0.17 mmol) in THF (2.5 mL). The mixture was stored at −8 °C for 10 h and at 25 °C for an additional 8 h. A solution of methanesulfonic acid in methanol then was added to pH 1.5. Toluene (10 mL) was added, and all of the solvents were evaporated in vacuo to leave an oily residue, which was crystallized from acetone (5 mL) to afford 49 mg (54%) of 5·CH_3SO_3H. The product was recrystallized from toluene and ethanol: mp 272–273 °C dec; $[\alpha]^{25}_D$ −115.8 °C (c 0.5, MeOH); TLC, R_f 0.58 (EtOAc–MeOH–NH_4OH, 90:10:3); IR (KBr) 1640 (s, amide C=O) cm^{-1}; NMR ($CDCl_3$, free base) δ 6.82 (d, *J* = 8.24 Hz, amide N*H*), 6.74–6.49 (2 d, 1 H each, Ar *H*), 4.38 (d, *J* = 7.69 Hz, C-5*H*), 3.70 (s, 2 H, CH_2I); EIMS, *m/e* 494 (M^+). Anal. ($C_{22}H_{27}N_2O_3I$·CH_3SO_3H) C, H, N.

(18) *Methods Enzymol.* **1977**, *46*, 157.

***(E)*-4-[[17-(Cyclopropylmethyl)-4,5α-epoxy-3-hydroxymorphinan-6β-yl]amino]-4-oxo-2-butenoic Acid Methyl Ester (6).** To the primary amine 14 (54 mg, 0.165 mmol) and K_2CO_3 (0.11 g, 0.825 mmol) in water (0.5 mL) and THF (1.5 mL) at 25 °C was added dropwise a solution of (*E*)-4-chloro-4-oxo-2-butenoic acid methyl ester (24.5 mg, 0.165 mmol) in THF (3 mL) over a 5-min period. After 48 h the solvent was removed in vacuo, and the residue was dissolved in water (25 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The CH_2Cl_2 layers were pooled, dried (Na_2SO_4), filtered, and evaporated to leave 50 mg of crude **6**. The residue was dissolved in methanol (1 mL) and triethylamine (0.2 mL) and stirred for 48 h. Removal of solvents followed by silica gel column chromatography (ether–MeOH–NH_4OH, 95:5:2) left 18 mg (25%) of pure **6**: mp 159–161 °C; $[\alpha]^{25}_D$ −149° (c 0.5, MeOH); TLC, R_f 0.49 (EtOAc–MeOH–NH_4OH, 90:10:3); IR (KBr, HCl salt) 1744 (s, ester C=O) 1679 (s, amide C=O) cm^{-1}; NMR ($CDCl_3$) δ 7.25–6.64 (2 d, 1 H each, olefinic *H*), 6.96–6.45 (2 d, 1 H each, Ar *H*), 4.48 (d, *J* = 7.69 Hz, C-5*H*); EIMS, *m/e* 438 (M^+). Anal. ($C_{25}H_{30}N_2O_5$·$0.5H_2O$) C, H, N.

Acknowledgment. This work was supported by the National Institute of Drug Abuse. We thank Victoria Darrow Elliott and Mary Schwartz for capable technical assistance.

Registry No. **3**, 107819-63-6; **3**·2HCl, 107819-74-9; **4**, 107819-64-7; **4**·HCl, 107819-77-2; **5**, 107819-65-8; **5**·CH_3SO_3H, 107819-78-3; **6**, 107819-66-9; **7**, 16590-46-8; **8**, 107819-67-0; **9**, 107819-68-1; **9**·HCl, 107846-39-9; **10**, 107819-69-2; **11**, 107819-70-5; **12**, 107819-71-6; **12**·HCl, 107819-75-0; **13**, 107819-72-7; **13**·2HCl, 107819-76-1; **14**, 107819-73-8; $MeNH(CH_2)_2OH$, 109-83-1; (*E*)-ClC(O)CH=CHC(O)OMe, 17081-97-9; *N*-(iodoacetoxy)succinimide, 39028-27-8.

Synthesis, Structure, and Biological Activity of Certain 2-Deoxy-β-D-*ribo*-hexopyranosyl Nucleosides and Nucleotides[1]

L. Dee Nord,*[2] N. Kent Dalley, Patricia A. McKernan,[2] and Roland K. Robins[2]

Department of Chemistry, Brigham Young University, Provo, Utah 84602, and Nucleic Acid Research Institute, Costa Mesa, California 92626. *Received August 13, 1986*

2-Deoxy-β-D-*ribo*-hexopyranosyl nucleosides with adenine (**2**), hypoxanthine (**17**), guanine (**23**), cytosine (**13**), and uracil (**7**) as the aglycon were synthesized by the Lewis-acid-catalyzed condensation of an appropriate trimethylsilylated heterocyclic base and 2-deoxy-1,3,4,6-tetrakis-*O*-(4-nitrobenzoyl)-β-D-*ribo*-hexopyranose (**5**) to provide the desired β anomers in good yield. When the synthesis of **7** via an S_N2 displacement was attempted by reaction between silylated uracil and 2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-α-D-*ribo*-hexopyranosyl bromide (**8**), the major product, 1-(2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-α-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (**9**), had retained the α configuration at the anomeric carbon. The structures of both anomers of 1-(2-deoxy-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione were assigned by single-crystal X-ray methods. The anomeric configuration and conformation of other nucleosides were determined by proton magnetic resonance analysis of the 4-nitrobenzoylated nucleosides. Nucleoside 6′-monophosphates of **7**, **13**, and **2** and the 4′,6′-cyclic monophosphate of **2** were also prepared. All 2′-deoxy-D-*ribo*-hexopyranosyl nucleosides and 6′-monophosphate derivatives were tested in vitro for antiviral and antitumor activity. The guanosine analogue **23** was moderately active against HSV-2 virus. The UMP analogue, 1-(2-deoxy-6-*O*-phosphono-β-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (**28**), demonstrated moderate activity against HSV-2 and parainfluenza 3 virus and was also active against L1210 (ID_{50} = 39 μM) and P388 (ID_{50} = 33 μM) leukemic cell lines. Two compounds, 6-amino-9-(2-deoxy-β-D-*ribo*-hexopyranosyl)purine (**2**) and 9-(2-deoxy-β-D-*ribo*-hexopyranosyl)-2,6-diaminopurine (**24**), were substrates for adenosine deaminase (EC 3.5.4.4) with K_m values of 57 and 90 μM, respectively. 6-Amino-7-(2-deoxy-β-D-*ribo*-hexopyranosyl)purine, **18**, was a competitive inhibitor of ADase (K_i = 0.1 mM).

Certain hexopyranosyl nucleoside analogues have been useful as probes of enzymes involved in nucleic acid metabolism. The nucleoside 1-(2-deoxy-β-D-*erythro*-hexopyranosyl)thymine[3] (**1**) is a potent and specific inhibitor of mammalian uridine phosphorylase (K_i = 12 μM)[4] and has recently been utilized to characterize various mammalian pyrimidine phosphorylase activities.[5,6]

(1) This report is taken, in part, from the Ph.D. Dissertation of L.D.N., Brigham Young University, 1985.
(2) Present address: Nucleic Acid Research Institute, Costa Mesa, CA 92626.
(3) Zorbach, W. W.; Durr, G. J. *J. Org. Chem.* **1962**, *27*, 1474.
(4) Langen, P.; Etzold, G. *Biochem. Z.* **1963**, *339*, 190.

0022-2623/87/1830-1044$01.50/0