Swiss Webster mice (Taconic Farms) were treated intraperitoneally with tetrabenazine methanesulfonate (50 mg/kg). The compound was solubilized in 0.9% NaCl with moderate heating. The extent of ptosis was evaluated subjectively and then scored on a rating scale of 0–4 with a score of 0 representing complete eye closure and a score of 4 representing no closure. Test compounds were dissolved in 0.9% NaCl (10 mL/kg) and administered po 30 min before tetrabenazine. Mice were evaluated for ptosis 30 min after treatment with tetrabenazine. ED_{50} values with 95% confidence limits were determined by computer analysis of quantal dose–response data as described by Tallarida and Murray.[29]

In Vivo Uptake of 5-HT in Rat Brain Using the *p*-Chloroamphetamine (PCA) Method. Modifications of published methods were used in this study to evaluate the inhibition of serotonin uptake in vivo.[32,33] Male Sprague–Dawley rats (160–200 g, Taconic Farms, Germantown, NY) were fasted overnight. They were orally medicated by intubation (10 mL/kg) with test compounds or vehicle (triple distilled water) 15 min prior to intraperitoneal injection with PCA (3 mg/kg; 1 mL/kg). This dose of PCA was chosen because it resulted in approximately 45% depletion of serotonin levels with no overt signs of toxicity. Three hours later animals were decapitated and the forebrains were quickly excised and homogenized in 10 volumes (w/v) of ice-cold acidified 1-butanol (0.85 mL of 12 N HCl/L of 1-butanol) with a 10-s burst of a Polytron homogenizer. The homogenates were centrifuged at 6000*g* for 10 min. A 4-mL aliquot of the supernatant was added to 4 mL of *n*-heptane and 1 mL of 0.1 N HCl and the mixture was transferred to glass-stoppered centrifuge tubes. Samples were shaken on a flat-bed shaker for 15 min and were then centrifuged for 5 min at 2000*g*. The organic phase (including the tissue disk at the organic/aqueous interface) was aspirated and discarded. Serotonin content in the aqueous phase was determined spectrofluorometrically after condensation with *o*-phthaladehyde (OPT). Briefly, a 0.2-mL aliquot of the aqueous phase was added with vortex mixing to 1.0 mL of a 10 mg % solution of OPT in 10 N HCl. The tubes were placed in boiling water for 10 min and were then cooled to room temperature in tap water. Fluorescence was measured on an Aminco-Bowman Spectrofluorometer (excitation, 360 nm; emission, 470 nm, uncorrected). Standard curves were generated from known amounts of 5-HT added to 4 mL of acidified 1-butanol just prior to the first shaking step. Percent recoveries were determined from 5-HT standards added to aqueous phase just prior to reaction with OPT. The percent blockade of the PCA depletion was calculated by the formula:

$$\%\ \text{block} = [(\text{5HT levels in presence of drug + PCA}) - (\text{5HT levels in presence of PCA alone})]/[(\text{control 5HT levels}) - (\text{5HT levels in presence of PCA})]$$

Data were analyzed by using a Duncan's multiple range *t* test using SAS software. A $p < 0.05$ was considered significant.

Acknowledgment. We thank the Analytical Chemistry and Chemical Development Departments of this Institute for spectral measurements and synthesis support.

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X-ray Crystal Structure of the Opioid Ligand Naltrexonazine

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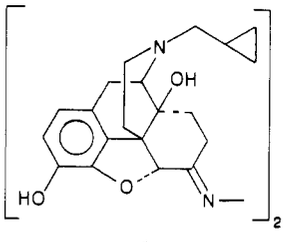
The anti–anti isomer of naltrexonazine (1) was synthesized, and its configuration was confirmed by X-ray crystallography. The syn–anti isomer is readily converted to 1 under acidic conditions. The apparent equal receptor binding of 1 and syn–anti isomer indicates that isomerization of the azine moiety may take place under the conditions of biological evaluation. Two possible explanations for wash-resistant binding of 1 to opioid receptors are presented. The first possibility involves a noncovalent interaction of the ligand with the opioid receptor, and the second considers covalent binding by a receptor-based sulfhydryl group.

Molecules that consist of two pharmacophores connected by a spacer chain have been termed bivalent ligands[3–9] and are of interest as probes for opioid receptors because of the possibility of "bridging" a subpopulation of vicinal receptors when the spacer is a specific length. The binding and biological activities of bivalent ligands derived from naloxone, naltrexone, and oxymorphone in which two alkaloid pharmacophores are connected by an azine moiety have been reported.[10–13] In this connection, the opioid receptor binding characteristics of naltrexonazine (1) and related azines to brain membranes have been investigated. On the basis of their reversible and wash-resistant binding components, Wolozin and Pasternak[14] divided μ receptors into two distinct μ subtypes. The naloxonazine-selective reversible and wash-resistant properties have been suggested as a means of distinguishing between receptors that mediate analgesia and those that produce respiratory depressant actions.[15,16]



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Two questions concerning opioid azines are addressed in this paper; the first deals with the configuration and

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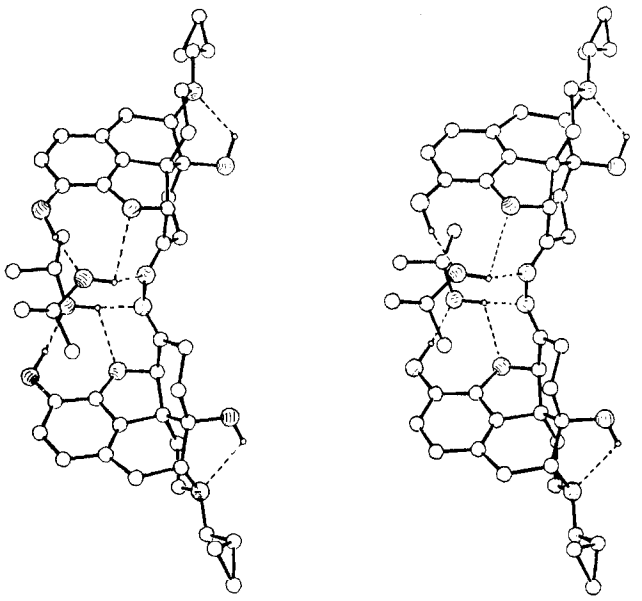

Figure 1. Stereo ORTEP view of naltrexonazine showing the molecule–isopropyl alcohol interactions.

conformation of one of these azines, naltrexonazine, and the second is concerned with the type of binding that makes one of the azine-receptor binding components so stable. Previous studies suggested the possibility that such ligands can exist as configurational isomers involving the azine linkage.[17] In this paper we present the results of chemical, X-ray, and receptor binding studies of the predominant isomer of naltrexonazine. We also propose a new interpretation of the wash-resistant phenomenon of opioid azines and related analogues.

Results

Chemistry. Naltrexonazine (1) prepared from hydrazine hydrochloride in refluxing methanol afforded the anti–anti isomer in 93% yield. The hydrochloride salt was converted to the free base and crystallized from isopropyl alcohol solution. The elemental analysis suggested that the crystals were solvated with isopropyl alcohol, and this was verified by X-ray crystallography. When the crystalline base was examined in chloroform solution, its ^{13}C NMR spectrum exhibited resonances corresponding to nonequivalent isopropyl methyl groups ($\Delta\delta$ = 0.23 ppm) of equal intensity. Since addition of isopropyl alcohol to the solution reduced the magnitude of δ while retaining the equality of the intensities, this suggests that the added isopropyl alcohol exchanges rapidly on the NMR time scale with the solvent associated with the ligand–isopropyl alcohol complex.

X-ray Crystal Structure Determination. Crystals of naltrexonazine base were investigated by X-ray diffraction methods. Figure 1 shows a stereoscopic view of the molecule, which was found to be a symmetrical dimer of two naltrexone units connected by an anti–anti azine

Table I. Bond Lengths (Å) for the First and Second Halves of the Molecule

	bond length			bond length	
atom	first half	second half	atoms	first half	second half
C1–C2	1.393 (6)	1.398 (7)	C9–N17	1.483 (5)	1.482 (5)
C1–C11	1.389 (5)	1.413 (5)	C10–C11	1.504 (6)	1.507 (6)
C2–C3	1.383 (6)	1.372 (7)	C11–C12	1.388 (5)	1.370 (6)
C3–C4	1.375 (5)	1.377 (5)	C12–C13	1.499 (5)	1.507 (5)
C3–O1	1.379 (5)	1.374 (5)	C13–C14	1.524 (5)	1.521 (5)
C4–C12	1.368 (5)	1.378 (6)	C13–C15	1.549 (5)	1.540 (5)
C4–O2	1.393 (5)	1.402 (5)	C14–O3	1.442 (4)	1.429 (4)
C5–C6	1.524 (5)	1.519 (5)	C15–C16	1.521 (5)	1.529 (5)
C5–C13	1.547 (5)	1.547 (5)	C16–N17	1.467 (5)	1.471 (5)
C5–O2	1.477 (4)	1.464 (4)	N17–C18	1.474 (5)	1.468 (5)
C6–C7	1.509 (5)	1.503 (5)	C18–C19	1.501 (6)	1.512 (6)
C6–N1	1.269 (5)	1.273 (5)	C19–C20	1.513 (6)	1.517 (6)
C7–C8	1.541 (6)	1.523 (6)	C19–C21	1.506 (6)	1.498 (6)
C8–C14	1.505 (5)	1.520 (5)	C20–C21	1.497 (6)	1.497 (6)
C9–C10	1.558 (5)	1.561 (5)	C9–C14	1.546 (5)	1.550 (5)
		N1–N1A	1.418 (4)		

Table II. Selected Bond Angles and Torsion Angles for the C Ring

atoms	bond angle,[a] deg	
C5–C6–C7	117.4 (4)	119.1 (4)
C5–C6–N1	116.7 (4)	115.5 (4)
C7–C6–N1	125.9 (4)	125.3 (4)
C6–N1–N1A	116.0 (3)	115.4 (3)
atoms	**torsion angle,[a] deg**	
C13–C5–C6–C7	31.3	26.6
C5–C6–C7–C8	−45.9	−42.9
C6–C7–C8–C14	59.0	57.8
C7–C8–C14–C13	−57.3	−57.8
C8–C14–C13–C5	43.2	42.9
C14–C13–C5–C6	−29.7	−26.6
asymmetry parameter $C_s(5–8)$[b]	2.1	0.0

[a] Second column shows values for the second half of the molecule. [b] Duax, W. L.; Weeks, C. M.; Rohrer, D. C. *Top. Stereochem.* **1976**, *9*, 1354.

Table III. Important Contacts within the Asymmetric Unit

atoms		distance, Å	atoms		distance, Å
(a) Nonbonding Intramolecular Distances in the Naltrexonazine Molecule					
CEN1[a]	N17	4.515	CEN2[a]	N17A	4.521
CEN1	N17A	11.721	CEN2	N17	11.838
CEN1	O1A	7.732	CEN2	O1	7.710
CEN1	N1	4.362	CEN2	N1A	4.558
CEN1	N1A	5.355	CEN2	N1	5.567
CEN1	CEN2	8.903			
O1	N17	7.037	O1A	N17A	7.067
O1	N17A	10.837	O1A	N17	11.081
O1	N1	4.446	O1A	N1A	4.641
O1	N1A	5.394	O1A	N1A	5.601
(b) Distances Showing Position of Isopropyl Alcohols Relative to Naltrexonazine Molecule					
CEN1	C22A	4.177	CEN2	C22	5.423
CEN1	C23A	4.626	CEN2	C23	3.635
CEN1	C24A	3.931	CEN2	C24	5.832

[a] CEN1 and CEN2 are geometric centers of corresponding phenol rings.

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Table IV. Hydrogen Bonding between Naltrexonazine and the Two Isopropyl Alcohol Molecules

donor–acceptor	distance, Å	D–H, Å	H···A, Å	D–H···A, deg	type
O3–N17	2.726 (4)	1.04	2.05	120.5	intra
O3A–N17A	2.642 (4)	0.93	2.18	109.9	intra
O1–O4	2.674 (4)	1.09	1.61	162.4	inter
O1A–O4A	2.730 (5)	1.06	1.72	158.1	inter
O4–N1	3.109 (5)	1.08	2.21	139.6	inter
O4A–N1A	3.033 (4)	1.12	1.98	156.2	inter
O4–O2	2.864 (4)	1.09	2.49	98.7	inter
O4A–O2A	2.850 (4)	1.06	2.26	110.3	inter

moiety. In all essential details the bond lengths (Table I) of the naltrexone unit are similar to those of naloxone,[18] α-oxymorphamine,[19] and both epimers of funaltrexamine.[20]

The N1–N1A bond length of 1.418 (4) Å compares well with 1.417 Å for the acyclic 4,4′-dihydroxy-α,α′-dimethylbenzalazine,[21] which indicates pure N(sp^2)–N(sp^2) bond character. The dihedral angle C6=N1—N1A=C6A is 118.6 (9)° compared to 148° found in the α,α′-dimethyl-α,α′-azinobis(p-cresol) monohydrate.[22] The conformation of the corresponding C rings is a chair (C) flattened at one end with carbon C5 located closer to the four-atom plane C6,C7,C13,C14 (for torsional angles see Table II). The conformation found for the C ring is identical with that found in naloxone. The nonbonding distances listed in Table III illustrate that the distribution of corresponding atoms on both sides of the azine moiety is nearly symmetrical and that mutual stereochemical relations exist between the two pharmacophores and isopropyl alcohol solvent molecules. Distance between geometric centers of phenyl rings (8.903 Å) and dihedral angle (5 (1)°) between their least-squares planes indicate that the central part of the molecule forms a cavity that is accessible to interactions with hydrogen-bonding acceptor or donor molecules. Indeed, strong intermolecular hydrogen bonding (see H···acceptor distances in Table IV) between the phenolic hydroxyl protons and the isopropyl alcohol hydroxyl oxygen is seen for each half of the molecule. The isopropyl alcohol hydroxyls are proton donors as well as acceptors, forming a second pair of hydrogen bonds with electron lone pairs from azine nitrogens. Two more short intermolecular distances between each of the isopropyl alcohols and either phenolic oxygen also are seen, considering their rather long H···acceptor distances and O–H···O angles (98.9° and 110.3°), are probably the best geometry to fulfill the bifurcated hydrogen bond conditions. Each of the hydroxyl groups at C14 is involved in an intramolecular hydrogen bond with a lone pair of the piperidine nitrogen. The H-bond geometries presented in Table IV show that there are some differences between distances in corresponding pairs of bonds in either half of the dimer. Another difference was found for nonbonding interactions between isopropyl methyl groups and phenol rings. Each phenol ring is close to one of the isopropyl alcohol molecules. The distances between each of the phenol rings and the methyl groups of each of the neighboring isopropyl alcohol molecules are different (Table III).

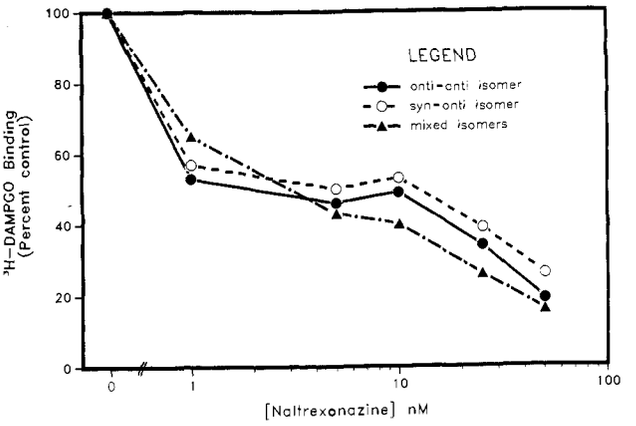

Figure 2. Wash-resistant inhibition of [^{3}H]DAMPGO binding by naltrexonazine isomers. Tissue was incubated with the stated isomer for 30 min at 25 °C, after which it was centrifuged, washed twice, and assayed with [^{3}H]DAMPGO (1 nM) at 25 °C. For each wash, the tissue was centrifuged (49000*g* for 20 min), resuspended, incubated at 25 °C for 15 min, and resuspended in fresh buffer. Results are the means of triplicate samples from a single experiment. Similar results were observed in two separate experiments.

Receptor Binding Assays. The affinities of the pure anti–anti and syn–anti isomers were compared to that of a 2:1 mixture of the anti–anti/syn–anti isomers[17] in binding studies. First, we compared the ability of the compounds to inhibit the binding of [^{3}H][D-Ala^2,MePhe^4,Gly(ol)^5]-enkephalin ([^{3}H]DAMPGO) (Table VI). Since the compounds remained during the assay, this approach assessed both reversible and wash-resistant inhibition of binding. All compounds potently lowered binding, with IC_{50} values under 0.5 nM; an analysis of variance revealed no statistical difference among them. Wash-resistant binding was determined by incubating tissue with the isomers for 30 min and then washing the membranes (Figure 2). Both isomers and the mixture clearly lowered binding with similar potencies. The biphasic nature of the curve implied that [^{3}H]DAMPGO binds at least two sites with differing sensitivities toward naloxonazine-induced wash-resistant inhibition of binding, similarly to previous studies with naloxonazine.[12,23]

Discussion

The X-ray analysis has unequivocally assigned the anti–anti geometry to the free base of naltrexonazine obtained from its hydrochloride salt. The fact that under acidic conditions an isomeric mixture was equilibrated to the anti–anti isomer with no detectable amount of other configurational isomers suggests that it is the most stable form of the azine in acidic medium.

We observed that under the conditions reported[12] for introducing the ^{3}H-labeled pharmacophore (through exchange under acidic conditions) the anti–anti azine isomer also was formed. Previously, synthesis of the azine under basic conditions afforded a mixture containing approximately 30% of an isomer, which was assigned the syn–anti configuration.[17] It therefore appears likely that under the acidic conditions necessary for solubilizing the azine as well as for synthesis of [^{3}H]azines[12] other geometric isomers would be converted to the anti–anti form.

Generally, the binding results with naltrexonazine closely parallel those previously seen with naloxonazine.[12] Under

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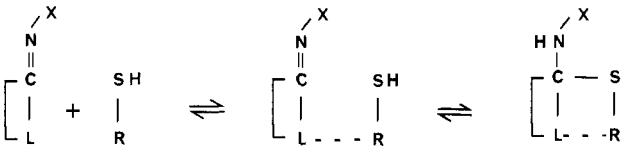
Scheme I. Interaction of the Imino-Containing Ligand L with a SH at a Receptor R[a]



[a] The reaction is presumed to take place via a noncovalent complex.

conditions measuring both reversible and wash-resistant binding, the compounds potently inhibited the binding of [^{3}H]DAMPGO. When wash-resistant binding was examined separately, naltrexonazine lowered [^{3}H]DAMPGO binding in a manner suggesting at least two binding sites. Binding, corresponding to μ_1 sites, was quite sensitive while the remainder required far greater concentrations of drug. These findings illustrate several important issues. First, the reversibly bound form of naltrexonazine will not discriminate between μ_1 and non-μ_1 sites. Second, the selectivity of wash-resistant inhibition for μ_1 sites is concentration dependent. Non-μ_1 sites will be inhibited in a wash-resistant manner if the concentration of naltrexonazine is sufficiently high. Also the fact that both the anti–anti and the syn–anti isomers have similar potencies in both reversible and wash-resistant inhibition of binding suggests that the anti–anti isomer might arise from conversion of the syn–anti isomer to the anti–anti isomer.

In the conformation adopted by the anti–anti naltrexonazine, the two pharmacophores are related by pseudo twofold symmetry located at the center of the azine bond. The geometry and ring conformation of the naltrexone pharmacophore in each half are identical within statistical error. Both halves also show the same intra- and almost the same intermolecular-hydrogen-bond pattern, but some minor differences in the geometry of bonding of isopropyl alcohol solvent were observed. The central part of the molecule contains a cavity with hydrogen-bonding donor and acceptor groups. The fact that the cavity contains heteroatoms with formal charges (ether oxygen −0.784, phenol oxygen −0.392, and azine nitrogens −0.116 as calculated by the program ChemGraf[24]) implicates these atoms in H-bonding type interactions with isopropyl alcohol.

Recently it has been reported that two groups of related azines, diacylhydrazides[25] and phenylhydrazones,[26] show wash-resistant antagonist binding similar to that of the azines. Features common to these analogues having this wash-resistant property are a 6-amino function connected to a lipophilic moiety. One possibility that might explain this avidity is a combination of a hydrogen bonding and hydrophobic bonding involving the constellation of the electronegative substituents and lipophilic moieties in the molecule. The finding that hydrogen bonding of isopropyl alcohol to naltrexonazine appears to occur both in chloroform solution and in the crystal could mean that hydrogen bonding in conjunction with hydrophobic bonding might afford a tight ligand receptor complex. Another possibility is the formation of an additional product with the receptor-based sulfhydryl group (Scheme I). This reaction would be analogous to that reported[27] for the reaction of mercaptans with imines and could conceivably be facilitated by hydrophobic bonding of the lipophilic moiety to a sulfhydryl-containing cleft of the receptor.

Experimental Section

Elemental analyses were performed by M-H-W, Phoenix, AZ, and are within 0.4% of the theoretical values. TLC data were obtained by using Analtech silica gel plates. NMR spectra were recorded at ambient temperature on a Nicolet 300 (300 MHz) spectrometer. All reagents and solvents were reagent grade and were used without purification. Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected.

***anti,anti*-Naltrexonazine (1).** Naltrexone hydrochloride (377 mg, 1 mmol) and hydrazine hydrochloride (105 mg, 1 mmol) were dissolved in methanol and stirred under reflux for 35 min. The solid that precipitated during the reaction was filtered and washed with cold methanol to yield 342 mg (93%) of the azine hydrochloride, which was homogeneous by TLC (R_f(1) 0.22, 1-butanol/acetic acid/water, 3:1:1; R_f(2) 0.62, ethyl acetate/methanol/NH_4OH, 45:5:1) and HPLC (t_R = 6.42 min, 0.1% F_3COOH/CH_3OH, 4:6, v/v; flow rate 1 mL/min); mp > 280 °C. The azine hydrochloride was dissolved in water (2 mL), 10% $NaHCO_3$ was added (25 mL), and the solution was extracted with ethyl acetate (3 × 25 mL). The pooled organic extracts were dried ($MgSO_4$), and the solvent was removed by evaporation in vacuo. The oily residue was crystallized from boiling isopropyl alcohol: yield 257 mg (87%); mp 182–186 °C (121–122 °C desolv); TLC R_f(1) 0.22, R_f(2) 0.61; HPLC t_R = 6.42 min.; ^{1}H NMR ($CDCl_3$) δ 4.87 (s, C_5); ^{13}C NMR ($CDCl_3$) δ 119.62 (C1), 118.46 (C2), 139.47 (C3), 143.58 (C4), 87.55 (C5), 161.94 (C6), 21.85 (C7), 29.83 (C8), 61.93 (C9), 22.57 (C10), 123.84 (C11), 129.51 (C12), 49.64 (C13), 70.05 (C14), 30.43 (C15), 43.95 (C16), 59.17 (C18), 9.41 (C19), 4.06 (C20), 3.74 (C21), 24.80, 25.03 (*i*-PrOH, CH_2), 64.19 (CH_3). Anal. ($C_{40}H_{46}N_4O_4 \cdot 2C_3H_8O$) C, H, N.

X-ray Crystal Structure Determination. Single crystals of the azine base were obtained as needles from isopropyl alcohol solution. The unit cell parameters were obtained by least-squares analysis of 25 reflections in the 2θ range 8–16°. The crystals are orthorhombic, with a = 10.282 (3) Å, b = 17.228 (4) Å, c = 23.963 (4) Å, space group $P2_12_12_1$, V = 4244 (3) Å^3, Z = 4; $C_{46}H_{62}N_4O_8$, M_r 799. The density measured by flotation method in a benzene/bromoform system (d_o = 1.27 g cm^{-3}) suggested the presence of the two isopropyl alcohol molecules per asymmetric unit. X-ray data were collected at −86 °C, on an Enraf-Nonius CAD4 diffractometer with graphite monochromatized Mo Kα radiation (λ = 0.7108 Å); $\theta/2\theta$ scan technique was used. Three standard reflections showed less than 1% change in the scattering power during data collection. The 3768 independent reflections were measured up to $2\theta_{max}$ = 24°; 2600 of these were corrected for Lorentz and polarization factors. The structure was solved by direct methods (MULTAN 80) and subsequent Fourier syntheses. Atomic coordinates were refined by full-matrix least-squares procedures with anisotropic thermal parameters for all non-hydrogen atoms. The function minimized was $\sum w_i(F_o - F_c)^2$, $w = 4F^2/\sigma(F^2)$ (σ from counting statistics). The H atoms for the solvent molecules and all hydroxyls were found from difference syntheses and were included in final refinement with the isotropic temperature factors. The refinement converged at R = 0.043 and R_w = 0.043. Maximum shift/error 0.08, esd's of an observation of the unit weight 1.509. Structure factors were not corrected for absorption (μ(Mo Kα) = 0.798 cm^{-1}) or extinction. The residual electron density fluctuation on final maps was less than 0.22 e/Å^3. All crystallographic calculations were carried out on a PDP11/34 computer. Atomic scattering factors were used as implemented in ref 28. Atomic coordinates and B_{eq} for naltrexonazine are listed

(24) ChemGraf, created by E. K. Davies, Chemical Crystallography Laboratory, Oxford University, developed and distributed by Chemical Design Ltd. Oxford, U.K.

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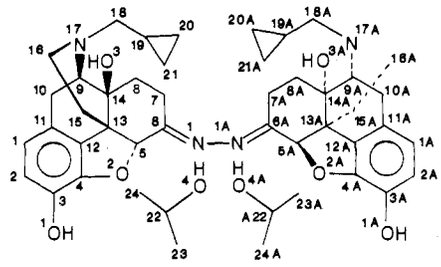
Table V. Final Fractional Atomic Coordinates and B_{eq} with ESD's in Parentheses



atom	x	y	z	B, Å^2	atom	x	y	z	B, Å^2
C1	0.8024 (5)	0.1447 (2)	0.8599 (2)	3.1 (1)	C12A	0.1499 (5)	0.0214 (2)	0.5742 (2)	2.20 (9)
C2	0.6852 (5)	0.1851 (2)	0.8590 (2)	2.9 (1)	C13A	0.1836 (4)	−0.0635 (2)	0.5787 (2)	1.85 (9)
C3	0.5664 (5)	0.1476 (2)	0.8565 (2)	2.34 (9)	C14A	0.0788 (4)	−0.1035 (2)	0.6131 (2)	1.97 (8)
C4	0.5694 (4)	0.0678 (2)	0.8581 (2)	2.11 (9)	C15A	0.1887 (4)	−0.1002 (2)	0.5202 (2)	2.12 (9)
C5	0.5203 (4)	−0.0556 (2)	0.8355 (2)	2.03 (9)	C16A	0.0542 (4)	−0.1001 (3)	0.4929 (2)	2.35 (9)
C6	0.5173 (4)	−0.0555 (2)	0.7720 (2)	1.90 (9)	N17A	−0.0443 (3)	−0.1323 (2)	0.5309 (1)	2.15 (8)
C7	0.5256 (5)	−0.0974 (3)	0.7420 (2)	2.5 (1)	C18A	−0.1695 (5)	−0.1431 (3)	0.5025 (2)	2.9 (1)
C8	0.7591 (5)	−0.0769 (3)	0.7672 (2)	2.4 (1)	C19A	−0.2705 (4)	−0.1831 (3)	0.5382 (2)	2.6 (1)
C9	0.8951 (4)	−0.0756 (2)	0.8567 (2)	2.5 (1)	C20A	−0.4113 (5)	−0.1775 (3)	0.5196 (2)	3.2 (1)
C10	0.9237 (5)	0.0132 (3)	0.8563 (2)	3.1 (1)	C21A	−0.3670 (5)	−0.1378 (3)	0.5719 (2)	3.4 (1)
C11	0.8052 (4)	0.0641 (2)	0.8603 (2)	2.27 (9)	N1	0.4227 (4)	−0.0205 (2)	0.7490 (1)	2.09 (7)
C12	0.6844 (4)	0.0281 (2)	0.8608 (2)	1.90 (9)	N1A	0.4210 (4)	−0.0212 (2)	0.6898 (1)	2.31 (7)
C13	0.6584 (4)	−0.0575 (2)	0.8612 (2)	1.79 (8)	O1	0.4531 (3)	0.1903 (2)	0.8552 (1)	3.22 (7)
C14	0.7656 (4)	−0.0978 (2)	0.8281 (2)	1.95 (9)	O2	0.4629 (3)	0.0180 (2)	0.8553 (1)	2.11 (6)
C15	0.6565 (4)	−0.0896 (2)	0.9215 (2)	2.19 (9)	O3	0.7508 (3)	−0.1810 (2)	0.8295 (1)	2.42 (6)
C16	0.7897 (4)	−0.0806 (2)	0.9483 (2)	2.4 (1)	O1A	0.3648 (4)	0.1919 (2)	0.5738 (1)	4.28 (8)
N17	0.8919 (3)	−0.1129 (2)	0.9124 (1)	2.15 (8)	O2A	0.3719 (3)	0.0184 (2)	0.5836 (1)	2.58 (6)
C18	1.0187 (4)	−0.1132 (3)	0.9411 (2)	3.0 (1)	O3A	0.0982 (3)	−0.1856 (2)	0.6143 (1)	2.36 (6)
C19	1.1228 (5)	−0.1540 (3)	0.9084 (2)	3.0 (1)	O4	0.2622 (3)	0.1105 (2)	0.8058 (1)	3.37 (7)
C20	1.2634 (5)	−0.1405 (3)	0.9243 (2)	4.2 (1)	O4A	0.5478 (4)	0.1208 (2)	0.6386 (1)	5.5 (1)
C21	1.2115 (5)	−0.1082 (3)	0.8707 (2)	3.8 (1)	C22	0.2161 (6)	0.1642 (3)	0.7647 (2)	4.0 (1)
C1A	0.0207 (5)	0.1339 (3)	0.5635 (2)	3.4 (1)	C23	0.1399 (7)	0.1269 (3)	0.7202 (2)	5.5 (2)
C2A	0.1361 (6)	0.1768 (2)	0.5636 (2)	4.0 (1)	C24	0.1446 (6)	0.2263 (3)	0.7954 (2)	4.4 (1)
C3A	0.2570 (5)	0.1449 (2)	0.5717 (2)	3.0 (1)	C22A	0.5942 (6)	0.1486 (3)	0.6920 (2)	4.4 (1)
C4A	0.2619 (5)	0.0652 (2)	0.5758 (2)	2.4 (1)	C23A	0.5687 (6)	0.2321 (3)	0.6961 (3)	5.2 (1)
C5A	0.3209 (4)	−0.0555 (2)	0.6044 (2)	2.23 (9)	C24A	0.7362 (7)	0.1302 (3)	0.6974 (2)	6.2 (2)
C6A	0.3226 (4)	−0.0541 (2)	0.6678 (2)	1.88 (9)	H(O1)	0.3632	0.1640	0.8398	4*[a]
C7A	0.2150 (4)	−0.0936 (3)	0.6993 (2)	2.6 (1)	H(O3)	0.7949	−0.1953	0.8671	4*
C8A	0.0821 (5)	−0.0775 (2)	0.6737 (2)	2.6 (1)	H(O1A)	0.4472	0.1621	0.5898	4*
C9A	−0.0531 (4)	−0.0881 (2)	0.5839 (2)	2.29 (9)	H(O3A)	0.0507	−0.2128	0.5878	4*
C10A	−0.0879 (5)	−0.0004 (2)	0.5775 (2)	2.8 (1)	H(O4)	0.2773	0.0507	0.7929	4*
C11A	0.0276 (5)	0.0525 (2)	0.5703 (2)	2.6 (1)	H(O4A)	0.5156	0.0605	0.6484	4*

[a] Starred atoms were refined isotropically. Anisotropically refined atoms are given in the form of the isotropic equivalent thermal parameter defined as $^4/_3[a^2B_{11} + b^2B_{22} + c^2B_{33} + ab(\cos\gamma)B_{12} + ac(\cos\beta)B_{13} + bc(\cos\alpha)B_{23}]$.

Table VI. Inhibition of [^{3}H][D-Ala^2,MePhe^4,Gly(ol)^5]enkephalin Binding by Naltrexonazine Isomers in Standard Binding Assays[a]

isomer	IC_{50}, nM
anti-anti isomer	0.45 ± 0.05 (4)
syn-anti isomer	0.47 ± 0.05 (3)
mixture of isomers	0.35 ± 0.06 (4)

[a] Tissue was prepared and incubated with nothing or the stated isomers at concentrations ranging from 0.5 through 50 nM and the radioligand (1 nM) for 60 min at 25 °C in triplicate, after which the tubes were filtered and counted. the results are the means of the indicated number of separate determinations. The mixture of isomers was approximately 2:1 anti-anti/syn-anti. No syn-syn has been identified.

in Table V (for H-atomic positions, full geometry tables, etc., see paragraph at the end of this paper concerning supplementary material).

***syn,anti*-Naltrexonazine.** Naltrexone base (750 mg, 2.2 mmol) was dissolved in methanol (25 mL) and stirred at room temperature while anhydrous hydrazine (76 mg, 2.4 mmol) was added in portions over 24 h. Prior to each subsequent addition of hydrazine, the reaction was filtered to remove *anti,anti*-naltrexonazine that had precipitated. After the final addition, the reaction mixture was stirred for another 6 h, filtered, and evaporated in vacuo. The residue was a mixture of naltrexonazine isomers that was composed of *syn,anti*-naltrexonazine.[14] Pure syn-anti isomer was isolated by preparative TLC on silica gel plates (500 μm) using $CHCl_3$/MeOH/NH_4OH (95:5:1) as the solvent system. The R_f values of *syn,anti*- and *anti,anti*-naltrexonazine isomers were 0.55 and 0.42, respectively. The eluted product was obtained as an oil and was shown to convert with time, especially on heating, to the anti-anti isomer. Therefore, minimum heat was employed during handling of the compound, which precipitated from chloroform using petroleum ether (60–70 °C). The total yield of azine product ranged from 82% to 90%, with the approximate ratio of anti-anti to syn-anti isomer being 4:1. A mass spectrum (CI) gave a M + 1 ion at 679. The IR spectrum showed the absence of carbonyl, while the NMR spectrum was similar to that recorded for the anti-anti isomer.

Receptor Binding Assays. Receptor binding assays were performed by methods similar to those described previously.[23] For characterization of total specific binding to opioid receptors, rat brain homogenates were incubated with nothing or the stated azine at concentrations ranging from 0.5 to 50 nM and [^{3}H][D-Ala^2,MePhe^4,Gly(ol)^5]enkephalin ([^{3}H]DAMPGO) (1 nM) for 60 min at 25 °C in triplicate, after which the contents of the tubes were filtered and the residue was counted. The mean IC_{50} values from three or four separate determinations are shown in Table VI.

For characterization of wash-resistant inhibition of binding, rat brain homogenates were incubated with the stated azine (from 1 to 50 nM) for 30 min at 25 °C, then centrifuged, washed twice, and assayed against [^{3}H]DAMPGO (1 nM) at 25 °C. For each wash, the tissue was centrifuged (49000*g* for 20 min), resuspended, incubated at 25 °C for 15 min, and resuspended in fresh buffer. The results shown in Figure 2 are the means of triplicate samples

of one experiment, which has been duplicated.

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Supplementary Material Available: Tables of calculated H-atom coordinates, anisotropic temperature factors U_{ij}, full list of bond lengths, bond angles, and torsion angles (14 pages); observed and calculated structure factors (13 pages). Ordering information is given on any current masthead page.

Notes

Synthesis and Biological Activity of 5-(2,2-Difluorovinyl)-2′-deoxyuridine†

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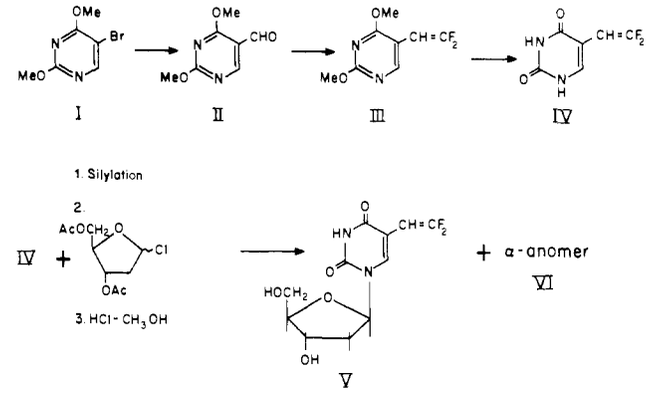
5-(2,2-Difluorovinyl)uracil (IV) was synthesized from 2,4-dimethoxy-5-bromopyrimidine by sequential formylation, difluoromethylenation, and removal of the 2- and 4-methyl groups. Condensation of the trimethylsilyl derivative of IV with protected D-*erythro*-pentofuranosyl chloride followed by separation of anomers and deblocking gave 5-(2,2-difluorovinyl)-2′-deoxyuridine (V). Compound V was active against herpes simplex virus type 1 (HSV-1) infection as well as tumor cells transformed by the HSV-1 thymidine kinase gene.

2′-Deoxy-5-vinyluridine and some related 5-halovinyl analogues exhibit potent activity against herpes simplex virus type I and type II.[1a] Among more than 100 5-vinylpyrimidine nucleosides reported so far in the literature,[1b] (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) has emerged as the most potent anti-HSV-1 agent. A fluoro analogue of BVDU, (*E*)-5-(2-fluorovinyl)-2′-deoxyuridine showed much reduced anti-HSV-1 activity[1c] and a dibromo analogue, 5-(2,2-dibromovinyl)-2′-deoxyuridine (DBVDU), was essentially inactive.[1b] A pronounced reduction in the antiviral activity[1b] also resulted from changing the *E* geometry of the halovinyl group to *Z*. While these results suggested that 5-(2,2-difluorovinyl)-2′-deoxyuridine (DFVDU) may lack strong anti-HSV-1 activity, other considerations, such as the strong electronegative nature and the chemical reactivity of the 2,2-difluoro group, raised the possibility that DFVDU may be capable of acting as an irreversible inhibitor of thymidylate synthase and/or of other enzymes in the DNA path. This paper reports the synthesis and antiherpes activity of 5-(2,2-difluorovinyl)-2′-deoxyuridine (V, Scheme I) and some reactions of the 5-(2,2-difluorovinyl) group in 2,4-dimethoxy-5-(2,2-difluorovinyl)pyrimidine.

Our initial attempts to prepare 5-(2,2-difluorovinyl)uracil (IV, Scheme I) by condensation of in situ generated (difluoromethylene)triphenylphosphorane with readily available 5-formyluracil failed to produce significant amounts of IV. We, therefore, used a protected form of 5-formyluracil, 2,4-dimethoxy-5-formylpyrimidine (II),[2] which was prepared from the corresponding 5-bromo derivative I by a modified procedure, employing *N*-formylpyperidine[3] in toluene in place of dimethylformamide in THF.[2] Reaction of II with the (difluoromethylene)-phosphorane generated from lithium chlorodifluoroacetate[4]

Scheme I



in the presence of triphenylphosphine gave 2,4-dimethoxy-5-(2,2-difluorovinyl)pyrimidine (III). Treatment of III with hexamethyldisilane in the presence of iodine[5] removed the protective methyl groups to give IV. For the preparation of the 5-(2,2-difluorovinyl)uracil nucleoside V (Scheme I), IV was sequentially silylated with a 1:1 mixture of chlorotrimethylsilane and hexamethyldisilazane in re-

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