22a was filtered off and dried in a vacuum oven to yield 5.7 g (77%) of a colorless solid, mp 240–243 °C, which was reduced without further purification.

4,5-(Methylenedioxy)-*N*,*N*-dimethyltryptamine (1). To a stirred and cooled (ice bath) mixture of 3.8 g (0.1 mol) of $LiAlH_4$ in 100 mL of anhydrous THF was added a solution of 3.7 g (0.015 mol) of **22a** in 500 mL of anhydrous THF during 1 h. The mixture was then stirred and refluxed for 1 h, cooled in an ice bath, and hydrolyzed by the cautious sequential addition of 3.8 mL of H_2O, 3.8 mL of 5 N NaOH, and 10.4 mL of H_2O and allowed to stir for 30 min. The inorganic salts were removed by filtration, the filtrate was dried ($MgSO_4$) and filtered, and the solvent was evaporated to leave a residual yellow oil. Kugelrohr distillation (100 °C, 0.5 mm) of this oil gave, after recrystallization from benzene–petroleum ether, 250 mg (8%) of a colorless solid: mp 93–95 °C; NMR [$(CD_3)_2C{=}O$] δ 9.9 (br s, 1, NH), 7.0 (br s, 1, C-2 H), 6.7 (q, 2, C-6 H and C-7 H), 5.9 (s, 2, OCH_2O), 2.9 (br t, 2, CH_2N), 2.6 (br t, 2, CH_2CH_2), 2.2 (s, 6, NCH_3). Anal. ($C_{12}H_{16}N_2O_2$) C, H, N.

Acknowledgment. The data and analyses are taken from the Ph.D. dissertation of T.B.K. We acknowledge support provided by a UAB Faculty Research Grant and NIH Grant 1R01-DA01826. The high-affinity binding studies performed by Drs. S. Maayani and H. Weinstein were supported by NIDA Grants DA-01875 and DA-00060.

Potential Affinity Labels for the Opiate Receptor Based on Fentanyl and Related Compounds

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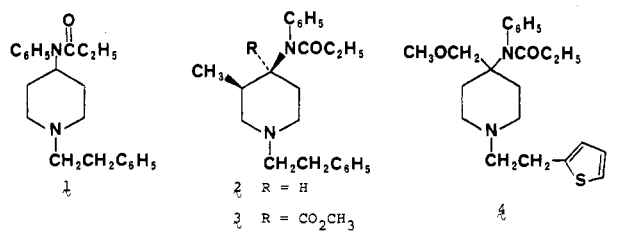
Derivatives of fentanyl, 3-methylfentanyl, sufentanil, and lofentanil, possessing chemo- or photoaffinity functionalities, were synthesized as potential affinity reagents for the opiate receptor. Opiate receptor binding constants (IC_{50}) were determined in competition experiments with [^{3}H]naloxone and [^{3}H]naltrexone. Affinity-labeling experiments were generally unsuccessful, although some irreversible attachment was achieved with α-diazoamide **17** and aryl azide **23**.

Opiate receptors, stereospecific binding sites for narcotic analgesic drugs in the central nervous system of vertebrates, have been extensively studied[1] since their initial identification[2] in 1973. Much research[1] has focused on the determination of membrane components responsible for the high-affinity binding, especially following the discovery[3] of endogenous opioid ligands, the enkephalins and endorphins. The isolation and characterization of opiate receptors would aid in understanding their function on a molecular level and their role in neuromodulation.

Attempts to isolate and purify membrane-bound opiate receptors in an active state have been plagued with problems.[1,4] Simon et al. found that preformed ligand–receptor complexes allowed receptor solubilization, but the dissociated receptor isolate was unable to bind opiate ligands.[4a] A receptor–enkephalin complex solubilized by Zukin and Kream, using the same procedure, was also inactive.[4b] More recently, active solubilized receptors have been reported by three research groups.[5] In 1977 we became interested in applying affinity-labeling methods, which had proved to be useful in the isolation of various biological macromolecules,[6] to the opiate receptor. Although several unsuccessful approaches to affinity labels for the opiate receptor had already been explored at that time,[7] we sought to synthesize a new series of compounds based on fentanyl (**1**) and its congeners [(+)-*cis*-3-methylfentanyl (**2**, R-26,800),[8] lofentanil (**3**, R-34,995),[9] and sufentanil (**4**, R-30,730)[9]], possessing either chemo- or



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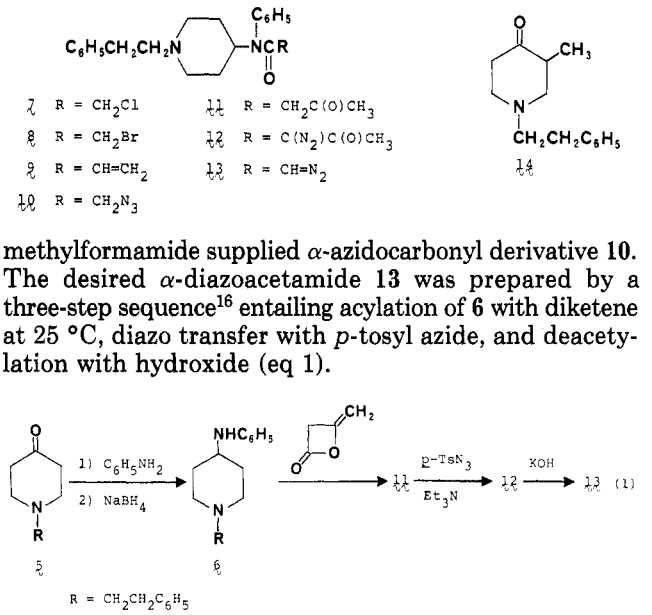
photoaffinity functional groups. Both types of labeling reagents were desired because of their complimentary applications: photoaffinity labels are reversible until photoactivation occurs, and chemoaffinity labels can be employed in vivo for pharmacological studies. We hoped that the structurally modified compounds would retain high receptor affinity, exhibit receptor specificity, and achieve irreversible attachment to the opiate–receptor macromolecular complex. During the course of our work, Portoghese and co-workers published exciting results with narcotic-agonist (chloroxymorphanamine) and narcotic-antagonist (chlornaltrexamine) chemoaffinity labels, both containing a nitrogen mustard alkylating moiety.[10] Also, Pasternak and co-workers reported on interesting long-acting opiate agonists and antagonists (morphinone hydrazone derivatives), which showed prolonged in vitro and in vivo blockade of opiate receptor binding.[11] Favorable results were also reported with photoaffinity labels based on aryl azide enkephalin analogues[12a–c] and an enkephalin chemoaffinity reagent.[12d,e] In this article we disclose the details of our research on fentanyl-related affinity reagents for the opiate receptor.

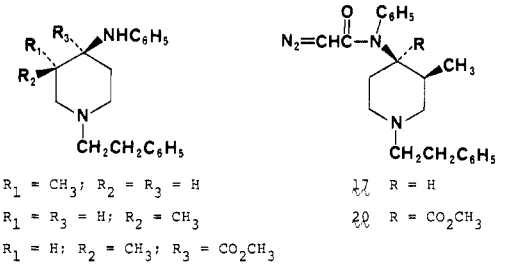
Results and Discussion

The fentanyl series of compounds was chosen for adaptation into affinity labels primarily because such compounds exhibit excellent affinities for the opiate receptor [for a range of fentanyl derivatives: IC_{50} = 0.3–25 nM (vs. [^{3}H]naltrexone);[13] IC_{50} = 0.1–2.5 nM (vs. [^{3}H]fentanyl)[14]], as well as exceedingly potent analgesic activities.[8,9] Also, synthetic routes, involving common intermediates, were envisioned for emplacement of affinity-labeling functionalities on, what we deemed, suitable loci: the amino substituent, propionyl group, aniline ring, and piperidine 4-position substituent. Our efforts to date have concentrated on the modification of two sites, the propionyl group and the amino substituent, to obtain potential chemo- and photoaffinity reagents. Thus, we have examined mainly some α-halocarbonyl, α-diazocarbonyl, β-chlorophenethyl, and *p*-azidophenethyl derivatives.

Chemistry. Commercial piperidinone **5** was converted to aniline **6**,[15] a key intermediate for the synthesis of propionyl-modified fentanyls. Acylation of **6** with chloroacetyl chloride, bromoacetyl bromide, and acryloyl chloride conveniently furnished the corresponding affinity compounds **7–9**. Reaction of **7** with sodium azide in dimethylformamide supplied α-azidocarbonyl derivative **10**. The desired α-diazoacetamide **13** was prepared by a three-step sequence[16] entailing acylation of **6** with diketene at 25 °C, diazo transfer with *p*-tosyl azide, and deacetylation with hydroxide (eq 1).



The diazoacetyl analogue of *dl-cis*-methylfentanyl (viz., **2**) was obtained in a similar manner. Thus, piperidinone **14**[17,18] was transformed into a mixture of diastereomeric anilines[8] **15** and **16** (ca. 1:1 ratio), via condensation with aniline and $NaBH_4$ reduction.[19] Cis isomer **16**, isolated



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(18) (a) An alternative synthesis of **14** was devised involving metalation of the oxime of **5** (2 *n*-BuLi, THF, −78 °C, then −10 °C), followed by methylation with CH_3I (−78 °C and then 0 °C).[18b] Deoximation to **14** was nicely effected using aqueous $NaHSO_3$.[18c] (b) Lyle, R. E.; Fribush, H. M.; Lyle, G. G.; Saavedra, J. E. *J. Org. Chem.* **1978**, *43*, 1275. (c) Pines, S. H.; Chemerda, J. M.; Kozlowski, M. A. *J. Org. Chem.* **1966**, *31*, 3446.

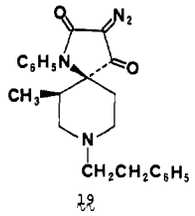
Table I. Chemical and Opiate-Receptor Binding Data

compd	mp, °C	formula	binding IC_{50}, nM [^{3}H]naloxone	[^{3}H]naltrexone
fentanyl (1)[a]			1.6	25
7	267-270 dec	$C_{21}H_{25}ClN_2O\cdot HCl$	4.4	107
8	263-267 dec	$C_{21}H_{25}BrN_2O\cdot HBr$	330	>1000
9	252-258 dec	$C_{22}H_{26}N_2O\cdot HCl$	1.4	17
10	124-129	$C_{21}H_{25}N_5O$	8.7	152
11	201-204	$C_{23}H_{28}N_2O_2\cdot HCl$	51	
12	113-114	$C_{23}H_{26}N_4O_2$	210	
13	124-130 dec	$C_{21}H_{24}N_4O$	5.5	70
(+)-2[a]			0.6	1.3
17	69-76	$C_{22}H_{26}N_4O$	0.6	2.5
i[b]	110-111 dec	$C_{24}H_{28}N_4O_2$	59	1000
(+)-3[a]				0.25[c]
(+)-19	158-160 dec	$C_{23}H_{24}N_4O$	410	>1000
4[a]				2.3
21	145	$C_{24}H_{31}ClN_2O_2\cdot HCl$		5
23	170	$C_{24}H_{31}N_5O_2\cdot HCl$		16
morphine[a]			4.2	27

[a] Reference compounds. [b] For structure of compound i, see Experimental Section. [c] This value is a correction of the reported value of 0.025 nM.[12]

from the mixture by fractional crystallization of oxalate salts, was converted to diazoacetamide **17** by the three-step process described above. In this case, since the diketene reaction was very sluggish, it had to be conducted at a higher temperature (110 °C).

The three-step process was also applied to (+)-**18**.[9a] Acylation of (+)-**18** with diketene at 100 °C and diazo transfer afforded the intermediate diazoacetoacetamide, deacetylation of which unfortunately produced spiroketodiazolactam (+)-**19** instead of desired diazoacetamide



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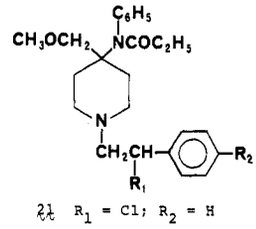

21 R_1 = Cl; R_2 = H
22 R_1 = OH; R_2 = H
23 R_1 = H; R_2 = N_3
24 R_1 = H; R_2 = NO_2
25 R_1 = H; R_2 = NH_2

20. Apparently, the carbanion entity formed in the deacetylation reaction is readily trapped by an intramolecular Claisen-type condensation with the proximate carbomethoxy group.

Compound **21** was obtained by reaction of parent alcohol **22**[9a] with thionyl chloride. Azide **23** was prepared from nitro compound **24** by a series of steps involving reduction of **24** to aniline **25** (H_2/Raney nickel), diazotization of **25**, and treatment of the diazonium salt with sodium azide.

Opiate-Receptor Binding. Each fentanyl analogue was tested in the opiate-receptor binding assay[20] (Table I) to establish the receptor affinity and to extend the in vitro structure-activity relationship (SAR) for fentanyl-like analgesics.[13,14] The affinities, determined by competition with radioligands [^{3}H]naloxone and/or [^{3}H]naltrexone in rat-brain homogenates, are presented as concentrations (nanomolar) required for displacement of 50% of radioligand (Table I).

It is evident that photoaffinity analogue **17** has a very high affinity for the receptor [cf. (+)-**2**] and so should be especially suitable for affinity-labeling studies. The other diazo compound (**13**), the α-chlorocarbonyl analogue (**7**), and the α,β-unsaturated analogue (**9**) had high affinities [cf. fentanyl (**1**)], making them suitable for affinity-labeling work. From a SAR point of view, replacement of the methyl group of fentanyl (**1**) with isosteric Cl (**7**) resulted in a small (3-fold) loss in affinity, which may be connected with the electron-withdrawing property of the Cl substituent, and replacement with Br (**8**) resulted in a large (200-fold) loss in affinity, attributable to both electronic and steric effects. Replacement of the methyl group with N_3 (**10**) or acetyl (**11**) also diminished affinity (5- and 32-fold, respectively). Replacement of the ethyl group of fentanyl with vinyl (**9**) retained the same affinity for the opiate receptor, whereas substitution of CH=N_2 (**13**) caused about a 3-fold loss in affinity. In the *cis*-methylfentanyl series, the diazo derivative had nearly the same excellent affinity as its prototype [cf. (+)-**2** and **17**].

Opiate-receptor binding for spiro compound (+)-**19** was substantially less (>1000 times lower) than that for lofentanil [(+)-**3**]. Sufentanil analogues **21** and **23** showed good receptor affinities, albeit 2 and 7 times less, respectively, than sufentanil (**4**).[21]

Affinity-Labeling Experiments. The functionalized fentanyl derivatives were studied as potential agents for irreversible inactivation and modification of brain opiate receptor. Affinity-labeling experiments with most of the compounds described herein have met with limited success.

As predictable from the high IC_{50}, α-bromocarbonyl derivative **8**, even at high concentrations (40 μM), was completely washed out without any detectable effect. Binding of ^{3}H-labeled ligand to membranes treated with high concentrations of the α-chlorocarbonyl compound (**7**)

(19) In an attempt to find a cis-stereoselective reduction of the intermediate phenylimine, lithium tri-*sec*-butylborohydride (L-Selectride) was employed as a reducing agent in tetrahydrofuran. However, no aniline products were formed even after prolonged reflux (2 days).

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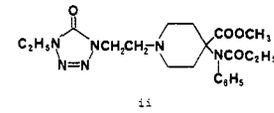
(21) We also had the opportunity to determine IC_{50} values ([^{3}H]-naltrexone) for two other fentanyl analogues of therapeutic interest. Carfentanil, which is 3-demethyllofentanil, had an IC_{50} of 0.2 nM, and alfentanil (ii), a relative of sufentanil, had an IC_{50} of 440 nM.



ii

reached a plateau below that of controls (50–60% of control), and no further increase in binding was seen even after prolonged incubation (37 °C) to dissociate and wash out any reversibly bound drug. The positive results with **7**, however, were produced in the presence of excessively large amounts of drug (>70 μM) and may be associated with a high degree of nonspecific effects. Vinyl derivative **9** (a potential Michael-acceptor alkylating agent) and nitrogen mustard alkylating agent **21** did not exhibit any irreversible binding, nor did photoreactive agents **10** and **13**.

Two photoreactive compounds, **17** and **23**, looked promising in preliminary experiments. The extent of covalent attachment to membranes incubated with photoreactive drug, upon photolysis, was evaluated by comparing the degree of binding of radioligand in the experimental sample to the amount of binding in (1) membranes photolyzed in the absence of affinity reagent and (2) membrane samples bound with label, but not photolyzed, and then washed free of drug. Photolysis reduced the binding activity by 26% in the presence of α-diazocarbonyl compound **17** and 16% in the presence of aryl azido compound **23** (Gioannini et al., unpublished results). This loss cannot be recovered even after extensive washing and may be the result of occupation of the binding site by the now covalently bound label. Our study of **17** is still in an early stage; because of the very high affinity of **17**, additional experiments will be pursued.

What could account for the limited success with the photogenerated carbene (from **13**, **17**) or nitrene (from **10**, **23**) species in forming a covalent bond between the fentanyl derivative and the receptor? The efficiency and overall success of the photoaffinity labeling technique depends on the concentration and photolability of receptor protein, the strength of ligand–receptor interaction, and the nature of the photogenerated species. The most successful labeling events have been achieved on relatively pure proteins in moderately high concentrations. In the brain membranes, the opiate receptor represents a small percentage of protein (femtomoles per milligram of protein) of a very crude mixture. For a chemically reactive species, this greatly escalates the probability of nonspecific labeling. Success depends on the ability of the compound to (1) bind with the chemically reactive species intact, (2) be in the proximity of a reactive nucleophile on the receptor, and (3) be bound long enough and be reactive enough for covalent bond formation to occur. With a photoreactive analogue of very high affinity, washing of the membranes after reversible binding can reduce nonspecific labeling. The extent of labeling then depends on the efficiency of the reactive intermediate produced. The photoactivated carbene or nitrene, generated from the α-diazocarbonyl or azido compounds, can undergo undesirable side reactions, such as rearrangement to a less reactive species or reaction with solvent or a group outside of the actual receptor binding site.[6c,22]

Another problem is photodecomposition of opiate receptor during photoaffinity experiments. Since irradiated receptor preparations were found to lose 50% of binding activity in about 5 min, a large percentage of the photodecomposition of affinity label must be accomplished within this time frame to afford an opportunity for reasonable levels of irreversible binding. Compounds **13**, **17**, and **23** met this requirement, but compound **10** did not (see Experimental Section).[23]

The low concentration of opiate binding sites (receptors) in the crude brain membrane preparations may pose a serious obstruction to successful affinity labeling with certain types of ligands. Thus, one might consider avoiding membrane-bound receptor systems altogether. Solubilized active opiate receptor is available for study,[5] and it may provide a favorable system with which to pursue chemical and photochemical receptor-labeling experiments.

Experimental Section

General Procedures. Melting points are corrected. UV data were collected on a Cary 14 spectrophotometer. IR spectra were obtained on a Perkin-Elmer 521 or 283 spectrophotometer. ^{1}H NMR spectra were measured on a Perkin-Elmer R-32 (90 MHz) instrument with $(CH_3)_4Si$ as an internal reference. TLC separations were performed on silica gel plates (250 μm) and visualized with UV and iodine vapor. Chemical microanalyses were determined by Atlantic Microlab, Inc., Atlanta, GA.

2-Chloro-*N*-phenyl-*N*-[1-(2-phenylethyl)-4-piperidinyl]-acetamide (7) Hydrochloride. Aniline **6**[15] (1.40 g, 5.0 mmol) in 8 mL of dry CH_2Cl_2 was treated slowly with a solution of chloroacetyl chloride (565 mg, 5.5 mmol) in 5 mL of CH_2Cl_2, while cooling the reaction in a water bath at room temperature. After 6 h, 75 mL of dry Et_2O was added, and the mixture was filtered. The tan solid was rinsed with dry Et_2O and dried in air (1.90 g, 100%). The material was recrystallized from methanol/ethyl acetate (1:1) to give 1.16 g of cream prisms: mp 267–270 °C (dec w/intumescence); IR (KBr) ν_{max} (C=O) 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$/Me_2SO-d_6, 2:1) δ 1.7–2.2 (m, 4, CCH_2CCH_2C), 2.9–3.9 (m, 10, s at δ 3.75 for CH_2Cl), 4.5–4.9 (m, 1, CH), 7.1–7.6 (m, 10). Anal. ($C_{21}H_{25}ClN_2O$·HCl) C, H, Cl.

2-Bromo-*N*-phenyl-*N*-[1-(2-phenylethyl)-4-piperidinyl]-acetamide (8) Hydrobromide. Aniline **6**[15] (1.05 g, 4.0 mmol) in 6 mL of dry CH_2Cl_2 was treated slowly at 0 °C with a solution of bromoacetyl bromide (835 mg, 4.12 mmol) in 4 mL of CH_2Cl_2. The reaction was allowed to warm to room temperature. It was diluted with 15 mL of dry Et_2O and filtered. The dry solid (1.60 g) was recrystallized from 30 mL of methanol/ethyl acetate (2:1) to give 0.99 g of cream crystals: mp 263–267 °C dec; IR (KBr) ν_{max} (C=O) 1656 cm^{-1}; ^{1}H NMR ($CDCl_3$/Me_2SO-d_6, 2:1) δ 1.6–2.2 (m, 4), 2.8–3.8 (m, 10, s at δ 3.57 for CH_2Br), 4.5–5.0 (m, 1), 7.1–7.7 (m, 10) 9.4–9.9 (br s, 1, NH^+). Anal. ($C_{21}H_{25}BrN_2O$·HBr) C, H, Br.

***N*-Phenyl-*N*-[1-(2-phenylethyl)-4-piperidinyl]-2-propenamide (9) Hydrochloride.** Aniline **6**[15] (1.00 g, 3.57 mmol) in 4 mL of dry CH_2Cl_2 was cooled to 0 °C and treated slowly with a solution of acryloyl chloride (325 mg, 3.6 mmol) in 1 mL of CH_2Cl_2. The reaction was allowed to warm to room temperature and stirred for 2 h. It was diluted with 10 mL of CH_2Cl_2 and basified with 10% NaOH. The CH_2Cl_2 solution was separated, rinsed with water, dried (Na_2SO_4), and concentrated to a dark tan solid. The material was mixed with 25 mL of dry Et_2O and 1 mL of dry methanol. The solution was decanted from some brown gum and treated with ethereal HCl. Methanol (5 mL) was added, and the mixture was filtered. The solid (1.2 g) was recrystallized twice from 2-propanol to give 0.34 g of ivory crystals: mp 252–258 °C (turned orange); IR (KBr) ν_{max} (C=O) 1649 cm^{-1}; ^{1}H NMR ($CDCl_3$/Me_2SO-d_6, 2:1) δ 1.8–2.2 (m, 4), 2.9–3.8 (m, 8), 4.85 (m, 1, $w_{1/2}$ = 32 Hz), 5.35–5.95 (m, 2, vinyl), 6.1–6.4 (d of d, 1, J = 3 and 16 Hz, vinyl), 7.05–7.6 (m, 10), 10.2 (br s, 1, NH^+). Anal. ($C_{22}H_{26}N_2O$·HCl) C, H, Cl.

2-Diazo-*N*-phenyl-*N*-[1-(2-phenylethyl)-4-piperidinyl]-acetamide (13). Aniline **6**[15] (2.10 g, 7.5 mmol) in 7.5 mL of dry CH_2Cl_2 was treated with diketene (750 mg, 8.9 mmol, distilled) and heated at gentle reflux for 4 h. The mixture was concentrated on a steam bath and then dried under high vacuum for 30 min. The syrup (**11**), one spot by TLC ($CHCl_3$ saturated with NH_3), was dissolved in 10 mL of dry acetonitrile and treated with 0.75 g of triethylamine and then a solution of *p*-tosyl azide[24] (1.5 g, 7.6 mmol) in 2 mL of acetonitrile. After 10 h at 23 °C, the acetonitrile was evaporated at 35 °C (15 torr). The residue was treated with 15 mL of extraction ether and 5 mL of ethyl acetate.

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The solution was rinsed with a solution of 0.45 g of KOH (85% assay) in 10 mL of water, a solution of 0.10 g of KOH in 10 mL of water, and deionized water (2 mL). The organic solution was dried (Na_2SO_4) and concentrated to dryness [35 °C (15 torr)]. A sample (0.40 g) was dissolved in dry ether, decanted from some residue, diluted with petroleum ether, and let stand at 0 °C. A pale yellow solid (0.23 g, **12**) was obtained: mp 111 °C (softened), 113–114 °C (gas evolution); IR (KBr) ν_{max} 2105 (C=N_2), 1637 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.9–3.3 (m, 15), 4.4–4.8 (m, 1), 7.0–7.5 (m, 10); UV (CH_3OH) λ_{max} 267 mm (ϵ 5710, sh), 232 (16175). Anal. ($C_{21}H_{24}N_4O$) C, H, N.

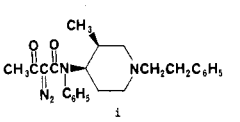
Diazoacetoacetamide **12** (975 mg, crude) in 3.5 mL of acetonitrile was added to a solution of 0.154 g of KOH in 2.5 mL of water. The heterogeneous mixture, stirring at 23 °C, became homogeneous slowly. After 5 h, TLC (ethyl acetate/95% ethanol, 5:1) indicated that the reaction was completed. The mixture was extracted with ether; the ethereal extract was rinsed with water and dried (Na_2SO_4). Removal of solvent [35 °C (15 torr)] gave a residue, which was recrystallized from ether/petroleum ether. After cooling to −78 °C, diazoacetamide **13** was collected (0.37 g) as a tannish yellow solid. This material, combined with a 0.10-g second crop, was dissolved in 15 mL of warm dry ether and filtered. The filtrate was concentrated to half volume, diluted with 1 mL of petroleum ether, and let stand at 5 °C. TLC-homogeneous yellow prisms were collected (0.28 g): mp 124–130 °C (gas evolution); IR (KBr) ν_{max} 2110 (C=N_2), 1610 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.2–3.2 (m, 12), 4.14 (s, 1, CH=N_2), 4.5–4.9 (m, 1), 7.0–7.5 (m, 10); UV (CH_3OH) λ_{max} 260 nm (ϵ 20470). Anal. ($C_{21}H_{24}N_4O$) C, H, N.

In a separate experiment, a sample of acetoacetamide **11** was purified. Thus, a sample of crude **11** was dissolved in dry ether and treated with ethereal HCl. The light tan solid (0.34 g) was recrystallized from ethyl acetate/methanol (10:1) to afford 0.22 g of colorless crystals: mp 201–204 °C (dec, turned ocher with intumescence); IR (KBr) ν_{max} 2280 (NH^+), 1708 (C=O), 1633 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.7–2.5 (s at δ 1.73 and 1.95 for CH_3, 7, CH_3 + CCH_2 + CCH_2C), 2.6–3.7 (m, 10), 4.5–5.0 (m, 1), 6.9–7.6 (m, 10). Anal. ($C_{23}H_{28}N_2O_2$·HCl) C, H, Cl.

2-Azido-*N*-phenyl-*N*-[1-(2-phenylethyl)-4-piperidinyl]-acetamide (10). Crude chloroacetamide (**7**) hydrochloride (ca. 12 mmol) was partitioned between CH_2Cl_2 and 5% Na_2CO_3. The CH_2Cl_2 solution (40 mL) was rinsed with water, dried (Na_2SO_4), and combined with 15 mL of dry dimethylformamide. Sodium azide (2.0 g, 3 mmol) was added, and the CH_2Cl_2 was evaporated to get a concentrated DMF solution, which was heated at 70 °C for 15 h. The cooled reaction mixture was treated with an equal volume of water and extracted with CH_2Cl_2. The extract was rinsed with water, dried (Na_2SO_4), and concentrated to dryness. The residue, freed of DMF under high vacuum, was dissolved in methanol. Dilution with water caused crystallization of a solid. The cooled solution was filtered to give 2.15 g of tan solid, which was extracted with 25 mL of dry ether/methanol (3:1) to give a dark brown residue and a light red solution. The solution was decanted and concentrated on a steam bath to 10 mL. Methanol (5 mL) was added, and the solution was filtered through Celite, diluted with 5 mL of water, and let stand at 5 °C. The tan prismatic needles (0.83 g) were collected and chromatographed on a dry column of silica gel. The desired band was extracted with CH_2Cl_2, and evaporation of the column extract gave 0.46 g of material, which was recrystallized from ethyl acetate/hexane to get tan crystals (0.27 g): mp 124–129 °C; IR (KBr) ν_{max} 2100 (N_3), 1657 (C=O) cm^{-1}; ^1H NMR δ 1.2–3.2 (m, 12), 3.43 (s, 2, N_3CH_2), 4.64 (m, 1), 7.0–7.5 (m, 10). Anal. ($C_{21}H_{25}N_5O$) C, H, N.

***cis*-2-Diazo-*N*-phenyl-*N*-[1-(2-phenylethyl)-3-methyl-4-piperidinyl]acetamide (17).** A mixture of amino ketone **14**[17,18] (8.2 g, 38 mmol), 4.0 g of aniline, and 0.01 g of tosic acid monohydrate in 50 mL of toluene was heated at reflux under nitrogen with azeotropic removal of water. After 50 h, the reaction was cooled, concentrated to near dryness, and diluted with 50 mL of dry methanol. To the stirred solution at 0 °C was added 0.9 g of $NaBH_4$ powder, portionwise. The reaction was allowed to warm to room temperature and was stirred for 2 h. Water and CH_2Cl_2 were added. The organic layer was separated, and the aqueous phase was extracted with CH_2Cl_2. The combined CH_2Cl_2 solution was rinsed with dilute aqueous NaCl, dried (Na_2SO_4), and concentrated to an amber oil, a mixture of cis and trans isomers in nearly 1:1 ratio (TLC; slightly more cis isomer). The oil, which weighed 10.0 g after being kept under high vacuum for 18 h, was dissolved in 100 mL of methanol/dry ether (2:1) and treated with a solution of 2.8 g of oxalic acid dihydrate in a minimum amount of methanol. A solid started to separate at 23 °C, and the mixture was cooled at 5 °C. The material, largely (ca. 95%) cis isomer, was collected (2.8 g). A 1.9-g second crop containing about 75% cis isomer was obtained on further cooling at −20 °C. TLC of a cis/trans mixture, using $CHCl_3$ saturated with NH_3, gave a good separation (ΔR_f 0.2) but variable location on the silica gel plate (cis isomer was front-running). A small sample of *cis*-oxalate was recrystallized from 2-propanol/acetone to give isomerically pure (TLC) material: mp 153–154 °C (lit.[8] mp for *trans*-oxalate 168–169 °C); ^1H NMR (**16**, free base, $CDCl_3$) δ 0.98 (d, 3, J = 7 Hz), 1.6–1.9 (m, 2), 2.0–3.0 (m, 9), 3.4–3.6 (m, 1, H_4, d of d pattern centered at δ 3.50, J = 4, 4, and 5 Hz, $w_{1/2} \simeq$ 16 Hz), 4.62 (s, 1, NH), 6.5–6.75 (m, 3), 7.0–7.4 (m, 7).

Aniline salt (2.0 g, ca. 95% cis isomer **16**) was partitioned between CH_2Cl_2 and 10% NaOH. The organic solution was dried (Na_2SO_4), and evaporated to 1.4 g of yellow oil. The oil was dissolved in 5 mL of dry toluene and treated with 480 mg of diketene. The solution was heated at 100 °C for 16 h, whereupon TLC showed about 50% starting material and 50% product. Additional (200 mg) diketene was added, and the solution was heated for 20 h, whereupon it was 75% complete. Removal of solvent gave a brown resin (2.5 g), which was dissolved in 6 mL of acetonitrile and treated with 0.5 mL of triethylamine and a solution of 1.0 g of *p*-tosyl azide[24] in 1.5 mL of acetonitrile. After 20 h at room temperature, the acetonitrile was evaporated in vacuo. Ethyl acetate (10 mL) was added, and the solution was washed with a solution of 0.3 g of KOH in 6 mL of water, 0.05 g of KOH in 5 mL of water, and deionized water (2 mL). The organic solution was dried (Na_2SO_4) and concentrated to a brown oil (2.5 g). The material was chromatographed on a dry column of silica gel (125 g) using ethyl acetate/hexane (2:1). The yellow, front-running band was extracted (CH_2Cl_2) to furnish 0.64 g of pale yellow solid. A sample (180 mg) of diazoacetoacetamide was recrystallized from CH_2Cl_2/hexane to give a 60-mg first crop of pale yellow crystals (compound **i**): mp 80–110 °C (softened), 110–111 °C dec. Anal. ($C_{24}H_{28}N_4O_2$) C, H, N.



i

Diazoacetoacetamide (0.363 g, 0.90 mmol) was dissolved in 1.0 mL of dry methanol and 0.4 mL of dry CH_2Cl_2. A solution of sodium hexamethyldisilazide (202 mg, 1.10 mmol) in 2.0 mL of methanol was added, and the reaction was stirred under nitrogen overnight. TLC (ethyl acetate, R_f 0.24) showed complete reaction. Dilute NaCl solution was added, and the mixture was extracted with CH_2Cl_2. The CH_2Cl_2 solution was rinsed with deionized water, dried (Na_2SO_4), and concentrated. The yellow-brown oil (0.31 g) was chromatographed on a dry column of silica gel (40 g) using petroleum ether/ethyl acetate (1:1). The yellow band was excised and extracted with CH_2Cl_2. Evaporation of the extract gave 110 mg of yellow syrup, which was dissolved in 10 mL of dry ether, filtered, and concentrated with a stream of nitrogen. The yellow syrup was dried under high vacuum for 18 h to give 120 mg of soft yellow crystalline solid (**17**): mp 62–69 °C (softened), 69–76 °C; IR (KBr) ν_{max} 2090 (C=N_2), 1610 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.0–2.9 (m, 14, d for CH_3 at δ 1.12), 4.13 (s, 1, CH=N_2), 4.46 (d of d of d, 1, J = 5, 5, and 12 Hz),[25] 6.8–7.6 (m, 10); UV (CH_3OH) λ_{max} 254.5 nm (ϵ 19130). Anal. ($C_{22}H_{26}N_4O$) C, H.

(+)-*cis*-3-Diazo-6-methyl-1-phenyl-8-(2-phenylethyl)-1,8-diazaspiro[4.5]decane-2,4-dione [(+)-19]. Aniline (+)-18[9a] (1.06

(25) For the trans isomer the expected coupling constants would be J = 5, 12, and 12 Hz. It should be noted that the propionanilido substituent has a large A value in the range of 3.5–4.0 kcal/mol.[26] This is sufficient to displace the conformational equilibrium for cis isomer **17** strongly toward the axial methyl/equatorial amido conformer (ca. 95%).

g, 3.0 mmol) and diketene (270 mg, 3.2 mmol) in 7.5 mL of dry toluene were heated at 100 °C for 10 h. The solvent was evaporated to give a brown syrup. Crude acetoacetamide in 4 mL of acetonitrile was treated with 0.3 g of triethylamine and then a solution of *p*-tosyl azide[24] (0.6 g) in 1 mL of acetonitrile. After 48 h at room temperature, most of the solvent was evaporated. The residue was dissolved in 10 mL of ethyl acetate and rinsed as before. The organic solution was dried (Na_2SO_4) and concentrated to an orange-brown oil (1.5 g). The oil was chromatographed on a dry column of silica gel (150 g) using ethyl acetate/petroleum ether (2:1). The yellow band was extracted (CH_2Cl_2) to give 0.99 g of TLC-homogeneous yellow syrup. The material was dissolved in dry ether, filtered, concentrated, and dried under high vacuum to give 0.87 g of light amber syrup. The diazo compound (0.69 g, 1.5 mmol) in dry methanol (3 mL) was added to a solution of sodium hexamethyldisilazide (0.36 g, 1.6 mmol) in dry methanol (2 mL) at 0 °C, under nitrogen. After 1 h at 5 °C, the orange mixture was treated with water (10 mL) and extracted with ether (50 mL). The ethereal solution was rinsed with dilute brine and then water, dried (Na_2SO_4), stirred with 1 g of silica gel, and filtered. The silica was rinsed with a little CH_2Cl_2. The combined organic solution was dried (Na_2SO_4), filtered, and concentrated to give 0.41 g of TLC-homogeneous, tan crystalline solid. The compound was dissolved in 40 mL of boiling dry ether, filtered, concentrated to 20 mL, and cooled stepwise to 23, 5, and −20 °C. The first crop of tan solid weighed 210 mg: mp 136–138 °C (partially fused and resolidified), 158–160 °C (turned red and evolved gas); IR (KBr) ν_{max} 2115 (C≡N_2), 1683 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.12 (d, 3, J = 7 Hz), 1.4–2.8 (m, 11), 6.9–7.6 (m, 10), no OCH_3 or CH=N_2; UV (CH_3OH) λ_{max} 290 nm (br sh, ϵ 1970), 221 (17 630); $[\alpha]^{26}_D$ +6.3° (±1.5°), $[\alpha]^{26}_{436}$ −19.0° (±1.5°), $[\alpha]^{26}_{365}$ +231.7° (±3.2°) (*c* 0.063, methanol). Anal. ($C_{23}H_{24}N_4O$) C, H, N.

***N*-[1-(2-Chloro-2-phenylethyl)-4-(methoxymethyl)-4-piperidinyl]-*N*-phenylpropanamide (21) Hydrochloride.** A mixture of $SOCl_2$ (4.5 g, 0.036 mol) and **22**[9a] (13 g, 0.033 mol) in 200 mL of dichloromethane was refluxed for 2 h. The solvent was evaporated in vacuo, and the residue was dissolved in acetone, treated with charcoal and filtered. The salt crystallized on addition of diisopropyl ether, yielding **21**·HCl (9.2 g, 61.7%): mp 145.3 °C. Anal. ($C_{24}H_{31}ClN_2O_2$·HCl) C, H, Cl, N.

***N*-[4-(Methoxymethyl)-1-[2-(4-nitrophenyl)ethyl]-4-piperidinyl]-*N*-phenylpropanamide (24).** A mixture of *p*-nitrophenylethyl bromide (20 g, 0.087 mol), *N*-[4-(methoxymethyl)-4-piperidinyl]-*N*-phenylpropanamide[9a] (21.8 g, 0.07 mol), triethylamine (74.4 g, 0.747 mol), and dimethylacetamide (300 mL) was stirred at 70 °C for 5 h. After cooling, the mixture was poured into water and extracted with toluene. The organic layer was washed with water, dried ($MgSO_4$), and filtered. The solvent was evaporated in vacuo, and the residue was triturated in hexane and crystallized from diisopropyl ether to yield **24** (16.8 g, 50%), mp 98.7 °C. Anal. ($C_{24}H_{31}N_3O_4$) C, H, N.

***N*-[1-[2-(4-Aminophenyl)ethyl]-4-(methoxymethyl)-4-piperidinyl]-*N*-phenylpropanamide (25).** A solution of **24** (14.8 g, 0.035 mol) in 250 mL of methanol was hydrogenated over Raney nickel (3 g). After uptake of 3 equiv of hydrogen, the catalyst was filtered off and the solvent was evaporated in vacuo. The residual oil was purified on silica gel (eluent $CHCl_3$/MeOH, 95:5) and crystallized from diisopropyl ether to yield **25** (9.5 g, 69%), mp 106.2 °C. Anal. ($C_{24}H_{33}N_3O_2$) C, H, N.

***N*-[1-[2-(4-Azidophenyl)ethyl]-4-(methoxymethyl)-4-piperidinyl]-*N*-phenylpropanamide (23) Hydrochloride.** A solution of **25** (1 g, 0.025 mol) in 2 N HCl (7.5 mL) was cooled to 0 °C. A solution of $NaNO_2$ (0.31 g, 0.0045 mol) in 5 mL of water was added dropwise while keeping the temperature below 3 °C. The mixture was stirred at this temperature for 20 min and vacuum was applied for 10 min. Then a solution of NaN_3 (0.34 g, 0.0052 mol) in 10 mL of water was added. A precipitate formed, which was filtered off after stirring for an additional 20 min at 0 °C. The solid was dried in vacuo and then dissolved in acetone and filtered, and the filtrate was concentrated again. The residual oil was recrystallized twice from an acetone/hexane mixture and dried in the dark at room temperature to yield **23**·HCl (1.1 g, 94.7%), mp 169.6 °C. Anal. ($C_{24}H_{31}N_5O_2$·HCl) C, H, Cl, N.

[^{3}H]Naloxone Binding Assay. Brains, minus cerebellum, were removed from Sprague–Dawley rats (after decapitation) and homogenized in 20 volumes ice-cold 0.05 M Tris buffer, pH 7.4, with a motor-driven Potter Elvehjem tissue homogenizer. The homogenate was then centrifuged for 10 min at 30000*g*, and the pellet resuspended in 100 volumes of Tris buffer using a Tissuemizer tissue homogenizer. Binding assay samples contained 1.6 mL (~1.0 mg of protein) of crude tissue preparation and 1.0 nM [^{3}H]naloxone (K_D = 2.3 nM) in a total volume of 2.0 mL. Nonspecific binding was determined as the binding in the presence of 200 nM levorphanol. Specific binding was determined as the total binding minus the nonspecific binding. Samples containing test compound or levorphanol were preincubated for 10 min at 35 °C prior to the addition of [^{3}H]naloxone, and after an additional 15-min incubation period, binding was halted by filtration through Whatman GF/B glass micro fiber filters. Binding was measured by liquid scintillation spectrometry using a Tracor Mark III scintillation spectrometer. IC_{50} values were determined by linear least-square analysis of at least 20 data points from at least three independent experiments.

[^{3}H]Naltrexone Binding Assay. As previously described,[20b] the brains, minus the cerebellum, were removed from Sprague–Dawley rats (after decapitation) and were homogenized in 10 volumes of 50 mM Tris, pH 7.4, 1 mM EDTA with a Brinkmann polytron set at 6 for 20–30 s. The homogenate was centrifuged for 20 min at 20000*g*, and the pellets were resuspended in 6 volumes of 0.32 M sucrose.[26] The homogenate was stored at −70 °C until needed. For the [^{3}H]naltrexone (K_D = 1.1 nM) binding assay and labeling experiments, the homogenate was thawed and diluted with 10 volumes of 50 mM Tris, pH 7.4, 1 mM K_2EDTA (which gives a 1:60 dilution, with a protein content of about 1 mg/mL[27]).

Opiate Receptor Affinity Labeling Experiments. In photoaffinity labeling experiments, various concentrations of photoreactive drug were added to the [^{3}H]naltrexone-assay homogenate and it was allowed to bind either during incubation at 25 °C for 30–45 min or 37 °C for 15 min in the dark. Samples were cooled for 10 min in an ice bath and then either photolyzed directly or diluted 1:1 (to provide a more transparent solution). For photolysis, samples were placed in open petri dishes in an ice bath equipped with a magnetic stirrer and photolyzed with a UVSL-25 hand-held mineral lamp (254 nm) at a distance of 2.0 cm for various time periods. The duration of the photolyses was kept under 180 s. After photolysis, samples were further diluted twofold with buffer and centrifuged for 15 min at 20000*g*. The supernatant was removed, and the pellet was resuspended in 3–4 times the original volume, incubated at 37 °C for 10 min, and spun again. The washing process was repeated two more times. Pellets were then resuspended in the original volume and bound as indicated below. Control samples containing no drug and samples bound with naloxone were treated in the same manner. Photolysis of membranes alone showed the loss of binding capacity due to photodestruction of receptor protein.[28] For example, one control run showed the following loss of binding vs. time: loss = 1, 16, 27, 40, and 46% at time = 30, 60, 120, 180, and 300 s, which was, more or less, the norm.[28] Thus, the photoaffinity experiments were conducted with less than 180 s of total exposure to UV light. Samples photolyzed in the presence of drug usually showed less of a loss in binding capacity, compared to no-drug controls, suggesting some protection by drug against photodecomposition. Photoaffinity reagents were photolyzed under standard conditions in phosphate buffer (pH 7.4) with 1 mM K_2EDTA to check that photodecomposition was taking place at a suitable rate relative to the time scale of the photoaffinity experiments (usually 120–150 s). The rate of disappearance of substrate was monitored by UV spectra on samples removed at 30-s time intervals until photodestruction was nearly complete. Approximate times for 50%

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(28) The sensitivity of the opiate receptor to short-wavelength (254 nm) UV light was recently studied (Glasel, J. A.; Venn, R. F. *Life Sci.* **1981**, *29*, 221); a $t_{1/2}$ of about 7 min (420 s) was observed. Glasel and Venn pointed out that the photolability of the opiate receptor must be taken into account in photoaffinity experiments to establish their meaningfulness.

photodestruction of photoaffinity reagents were (compound/time): 23/45 s, 10/>120 s (inefficient photolysis), 13/40 s, 17/45 s.

Experiments with chemically reactive affinity labels followed a similar pattern. Homogenate was bound with affinity label, spun at 20000g for 15 min, and then resuspended in the original volume. The bound membranes were incubated for various periods of time (up to 60 min) at 37 °C. Samples were then diluted and washed in the same manner as indicated for photolyzed samples. Control samples, which were processed in the same manner, consisted of membranes treated with no drug and samples bound with naloxone or fentanyl citrate. After resuspension in the original volume, samples were bound with tritiated drug.

Stereospecific binding of [^{3}H]naltrexone or [^{3}H]etorphine on samples treated and resuspended in the original volume was assayed on duplicate 2-mL samples as previously described.[20] Binding was assayed in the presence or absence of 1 μM naltrexone to obtain specific binding. Samples were incubated for 15 min at 37 °C, cooled for 10 min in an ice bath, and then filtered through Whatman GF/B filters. Filters were rinsed twice with 4 mL of buffer, dried, and counted in a toluene-based scintillation cocktail. Protein content was determined by the Lowry method.[27]

Acknowledgment. We thank William Baldy, Jr. (McNeil), and Lloyd Angel (NYU) for superb technical assistance. Dr. Simon's research was supported by Grant DA-00017 from the National Institute on Drug Abuse (NIDA). Dr. Gioannini is a recipient of a NIDA postdoctoral traineeship.

(±)-2-Depentylperhydrohistrionicotoxin: A New Probe for a Regulatory Site on the Nicotinic Acetylcholine Receptor–Channel

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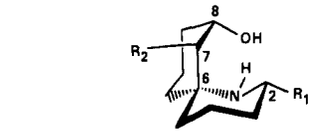
Received November 30, 1981

(±)-2-Depentylperhydrohistrionicotoxin (4), several of its analogues, and N- and O-substituted derivatives were prepared and tested for their effects on the neuromuscular transmission of the frog sartorius muscle. Compound 4, its *N*-methyl derivative 5, the *O*-acetyl derivative 9, and the quaternary methiodides 19 and 20 blocked the indirectly elicited twitch. The oxidation of 4 and 5 to ketones 12 and 14 and their reduction to the epimeric alcohols 17 and 18 afforded materials with substantially reduced activity. N-Acetylation of 4 to 11 changed the course of the activity to a transient potentiation of muscle twitch. Both 4 and 5 were not very toxic to mice after subcutaneous administration. (±)-7-*n*-Butyl-1-azaspiro[5.5]undecan-8-one (12) epimerized readily at room temperature to afford the epimer 13, and preparation of the hydrochloride of its N-methylated derivative 14 was accompanied by a retro-Michael reaction, affording the 2-*n*-butyl-3-[4-(methylamino)butyl]cyclohexene-2-one (22). The strongly hydrogen-bonded alcohol 4 was analyzed as the hydrobromide by a single-crystal X-ray analysis, confirming its structure.

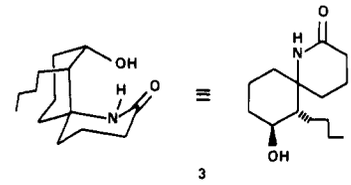
Natural histrionicotoxin (–)-1[1] and its fully hydrogenated, naturally derived congener perhydrohistrionicotoxin [(–)-H_{12}-HTX, (–)-2] are important biochemical tools for studying the mechanism of action of cholinergic agonists in the neuromuscular system (Chart I).[2a] Histrionicotoxin does not block interaction of acetylcholine with the nicotinic receptor, but instead interacts with a site on the associated ion channel. The interaction with histrionicotoxin appears to cause the channel to assume an inactive and nonconducting state. Binding of radioactive perhydrohistrionicotoxin is very slow unless a nicotinic agonist is present, indicating that binding of histrionicotoxin occurs to the channel primarily in the open configuration.[2b] Affinity of nicotinic agonists for its receptor and the extent of desensitization of the receptor are increased in the presence of isodihydrohistrionicotoxin.[2c] Histrionicotoxin enhances the conversion of acetylcholine receptor–channel complex to an inactive (desensitized) state. The receptor–channel is blocked in adrenal medulla cells where it serves to control catecholamine secretion.[2d] The unavailability of the natural toxin and congeners derived from it has been a serious handicap in exploring the biological potential of these spiroamines and performing a structure–activity relationship study. This problem can now be considered to have been solved with the several syntheses of (±)-2[3] and particularly with the recently

Chart I



1 $R_1 = CH_2CH=CH-C\equiv CH$, $R_2 = CH=CH-C\equiv CH$

2 $R_1 = n\text{-}C_5H_{11}$, $R_2 = n\text{-}C_4H_9$



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