(1 H, m), 1.42 (1 H, m), 1.14 ppm (10 H, m); ^{31}P NMR ($CHCl_3$) −8.94, −9.02 ppm (1:1). Anal. ($C_{19}H_{32}Cl_2N_3O_3P$) H, N; C: calcd 50.54; found: 51.04.

For kinetic studies the two major isomers could be separated by flash chromatography (CH_2Cl_2/acetone 1:1).

1-Phenyl-2-(4,4,6-trimethyltetrahydro-1,3-oxazin-2-yl)ethyl *N*,*N*-diethylphosphorodiamidate (8f) was prepared as described for **8e**. The crude oil was purified by using flash chromatography (CH_2Cl_2/acetone 1:2) to yield **8f** (353 mg, 34%) as a mixture of diastereomers: ^1H NMR ($CDCl_3$) 7.31 (5 H, m), 5.48 (1 H, m), 4.35 (0.5 H, t), 4.25 (0.5 H, t), 3.78 (1 H, m), 2.97 (4 H, m), 2.21 (1 H, m), 1.97 (1 H, m), 1.41 (1 H, m), 1.14 (10 H, m), 0.97 ppm (6 H, t); ^{31}P NMR ($CHCl_3$) −8.88, −9.02 ppm (1:1).

1-Phenyl-2-(4,4,6-trimethyltetrahydro-1,3-oxazin-2-yl)ethyl *N*,*N*-bis(2-chloroethyl)-*N'*,*N'*-morpholinophosphorodiamidate (8g) was prepared as described for **8e** with the following modifications. Morpholine (5 equiv) was added all at once to the cooled (0 °C) solution in place of ammonia, and the reaction was then stirred for 5 h at room temperature. The crude product was purified by using flash chromatography (CH_2Cl_2/acetone 2:1) to give **5g** (595 mg, 30%) as a 1:1 mixture of diastereomers: ^1H NMR ($CDCl_3$) 7.37 (5 H, m), 5.55 (1 H, m), 4.13 (0.5 H, t), 3.93 (0.5 H, dd), 3.68 (5 H, m), 3.32 (4 H, m), 3.17 (4 H, m), 3.03 (4 H, m), 2.27 (1 H, m), 2.02 (1 H, m), 1.41 (1 H, m), 1.11 ppm (10 H, m); ^{31}P NMR ($CHCl_3$) −10.10, −10.28 ppm (1:1).

1-Phenyl-2-(4,4,6-trimethyltetrahydro-1,3-oxazin-2-yl)ethyl *N*,*N*,*N'*,*N'*-tetrakis(2-chloroethyl)phosphorodiamidate (8h) was prepared as described for **8e** except that phosphoryl chloride **4b** (1.42 g, 3.9 mmol) was added to the alkoxide at 0 °C. The solution was then heated to reflux for 2 h. Ethanol (30 mL) was added to the cooled solution and the resulting mixture was reduced with sodium borohydride at −40 °C as described above. The crude product was purified by using flash chromatography (EtOAc/hexanes 1:1) to give **5h** (910 mg, 45%) as a 1:1 mixture of diastereomers: ^1H NMR ($CDCl_3$) 7.30 (5 H, m), 5.52 (1 H, m), 4.03 (0.5 H, m), 3.80 (0.5 H, m), 3.57 (4 H, m), 3.31 (8 H, m), 2.99 (4 H, m), 2.22 (1 H, m), 2.01 (1 H, m), 1.25 (1 H, m), 1.10 ppm (10 H, m); ^{31}P NMR ($CHCl_3$) −9.37, −9.45 ppm (1:1). Anal. ($C_{23}H_{38}Cl_4N_3O_3P$) H; C: calcd, 40.37; found: 40.87.

Cytotoxicity Assays. Cytotoxic activity was evaluated in vitro by using 1-h drug exposure in a clonogenic assay as previously described.[7] LC_{99} values were obtained by linear regression of log survival vs drug concentration from at least three determinations and are calculated as the drug concentration required to reduce colony formation to 1% of control values. Standard errors are within ±10%. Resistance factors were calculated from the ratio of LC_{99} values for wild-type and cyclophosphamide-resistant cell lines.

Antitumor activity in vivo was determined by using standard NCI protocols for the L1210 line. Tumors were propagated in DBA/2 mice, and drug evaluations were carried out in $B_6D_2F_1$ mice. Mice were randomly assigned to groups of 10 and were injected ip with 10^5 L1210 cells; a suspension of drug in 2% (carboxymethyl)cellulose (0.2 mL) or vehicle alone was injected ip 24 h later. Survivors were counted daily for 30 days. Response is reported as % *T*/*C*, calculated as 100 × (treatment survival time)/(control survival time). Survival times of control mice were in the range of 8–10 days for all experiments.

Acknowledgment. We thank Karen Maddison for her technical assistance and Dr. Robert F. Struck for providing the cyclophosphamide-resistant cell lines. Financial support from the National Cancer Institute, NIH (Grants CA34619 and CA11198), is gratefully acknowledged.

Registry No. 3d, 135865-43-9; **3e**, 135865-44-0; **4a**, 127-88-8; **4b**, 60106-92-5; **4c**, 1498-54-0; **5a**, 39800-29-8; **5b**, 135865-45-1; **5c**, 135887-43-3; **5d**, 135865-46-2; **6**, 49852-73-5; **7e**, 129904-24-1; **8a** (isomer 1), 135968-84-2; **8a** (isomer 2), 135968-85-3; **8b**, 135865-47-3; **8c** (isomer 1), 135968-86-4; **8c** (isomer 2), 135968-87-5; **8d**, 135865-48-4; **8e** (isomer 1), 135968-88-6; **8e** (isomer 2), 135968-89-7; **8f** (isomer 1), 135969-92-5; **8f** (isomer 2), 135865-49-5; **8g** (isomer 1), 135968-90-0; **8g** (isomer 2), 135968-91-1; **8h** (isomer 1), 135968-92-2; **8h** (isomer 2), 135968-93-3; 4-HC, 39800-16-3; 4-methyl-4-amino-2-pentanol, 4404-98-2; propionaldehyde, 123-38-6; hydrocinnamaldehyde, 104-53-0; bis(2-chloroethyl)amine hydrochloride, 821-48-7; diethylamine, 109-89-7; phosphorus oxychloride, 10025-87-3; 3-buten-1-ol, 627-27-0; benzaldehyde, 100-52-7.

Synthesis and Receptor Binding of Enantiomeric N-Substituted *cis*-*N*-[2-(3,4-Dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)cyclohexylamines as High-Affinity σ Receptor Ligands

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N-Alkyl-substituted derivatives of (+)- and (−)-*cis*-*N*-[2-(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)cyclohexylamine have been synthesized in nine steps in a stereospecific manner starting from cyclohexene oxide. The key step in the reaction sequence involved catalytic hydrogenation of oxime **8** in the presence of PtO_2 and AcOH to give the cis diamine (±)-**7**. Most of the compounds in this series exhibited very high affinity at σ receptors when tested against [^3H]-(+)-3-PPP, and in general it was observed that the 1*R*,2*S* enantiomers bound more potently to σ receptors than their corresponding 1*S*,2*R* enantiomers. The most potent σ ligand found in this class was the unsubstituted derivative (1*R*,2*S*)-(−)-**4**, which exhibited an affinity constant of 0.49 nM. This compound was also found to be very selective for σ receptors. It exhibited little or no affinity for κ opioid, PCP, and dopamine-D_2 receptors. It was also demonstrated that the cis configuration as opposed to the trans configuration of (+)- and (−)-**5** was necessary for a higher σ receptor affinity.

Introduction

The σ receptor was originally proposed by Martin et al.[1] based on the ability of the racemic opiate SKF10,047 to produce psychotomimetic effects in dogs. Since that time, σ receptors have undergone several revisions in definition.[2-4] This binding site has been shown to be distinct from either opiate, phencyclidine (PCP), or dopamine-D_2

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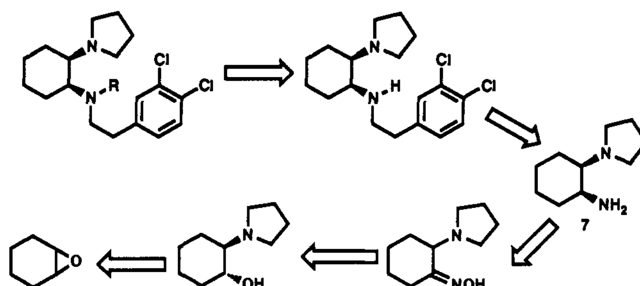
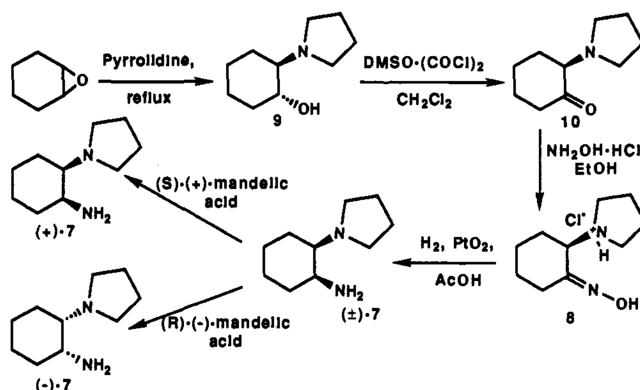
binding sites, sites which commonly cross-react with a variety of σ ligands. The clear difference between σ and these other receptors has been substantiated by differences in their autoradiographic distribution in brain,[5] separate and distinct molecular models proposed for these sites as well as differences in their binding characteristics.[4] The σ receptor has elicited considerable interest during the last few years because its functional role is not well understood. Among the numerous physiological and pharmacological systems with which the σ receptor is known to interact, the main ones are (1) negative modulation of carbachol stimulated phosphoinositide turnover,[6] (2) induction of dystonic reactions and contralateral rotation in rats,[7] (3) inhibition of smooth muscle contraction,[8] and (4) certain motor disorders.[9] The σ receptor has more recently been implicated in the neuroprotective effects of certain σ ligands, including dextromethorphan.[10]

In order to further improve our knowledge of the functional role of this receptor, we require firstly compounds exhibiting a high degree of potency and selectivity for the σ receptor and secondly a σ receptor antagonist.

Scheme I



Scheme II



The synthesis and structure–activity studies (SAR) of ligands from novel structural classes showing a high degree of selectivity and potency is the initial step in satisfying both of these requirements. Unfortunately, development of compounds with these characteristics has not been a straightforward task. For example, phenylpiperidines such as (+)-3-PPP bind to both σ and dopamine-D_2 receptors;[11] (+)-benzomorphans such as SKF10,047 cross-react with PCP as well as σ receptors.[12] However, more recently, we identified the (+)-benzomorphan [^{3}H]-(+)-pentazocine to be a highly potent and selective probe for σ receptors.[13]

In light of the different subtype selectivity and potency of various classes of σ receptor ligands, we recently explored the κ-selective agonist, U50,488 as well as related benzeneacetamides for their effect at σ receptors.[14] We found that U50,488 exhibited weak affinity for σ receptors labeled by [^{3}H]-(+)-3-PPP. To our surprise, we discovered that an alteration in the stereochemistry of U50,488 from trans to cis resulted in an almost total loss in affinity for κ receptors and a corresponding increase in affinity for σ receptors. Thus (1*S*,2*S*)-(−)-U50,488 (1) (Chart I) exhibited

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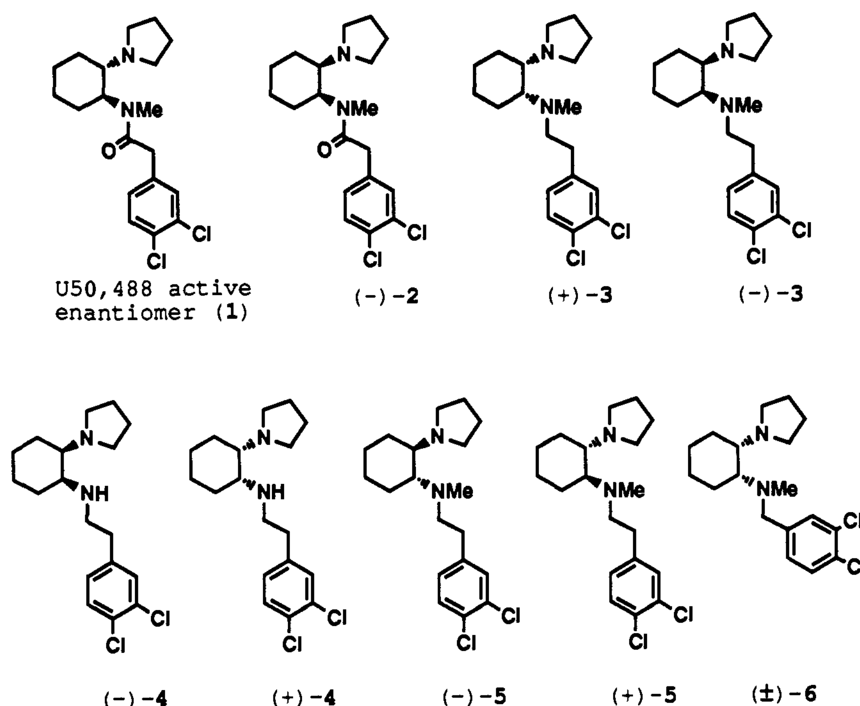
Chart I



a K_i of 594 nM for σ receptors and 44 nM for κ receptors labeled by [^{3}H]-bremazocine, while the 1*S*,2*R*-(−)-cis diastereoisomer [(−)-2] exhibited a K_i of 81 nM for σ receptors and negligible affinity for κ receptors. Similarly, the 1*R*,2*S*-(+)-cis diastereoisomer [(+)-2] showed an affinity of 221 nM for σ and negligible affinity for κ receptors. This initial finding led us to pursue a large SAR study that resulted in the discovery of (1*R*,2*S*)-(+)- and (1*S*,2*R*)-(−)-3, which proved to be extremely potent and selective ligands at σ receptors from a novel structural class.[15] Thus, (−)-3 displaced [^{3}H]-(+)-3-PPP with an affinity of 1.3 nM while (+)-3 displaced [^{3}H]-(+)-3-PPP with an affinity of 6.0 nM. (+)- and (−)-3 showed little or no affinity for either κ opiate receptor subtype (κ_1 or κ_2), PCP, or dopamine-D_2 receptors and have since proven to be valuable probes in functional studies of σ receptors. (+)- and (−)-3 were also found to exhibit σ agonist activity in their effects on motor behavior and on the cholinergic phosphoinositide response in rats.[16] With these results in hand, we wished to further investigate the SAR of (+)- and (−)-3 with the hope of increasing their efficacy and potency and perhaps discovering potential σ receptor antagonists.

Our approach in the present study was to synthesize enantiomeric intermediates in optically pure form that would allow us to investigate the SAR of the *N*-alkyl substituent of (+)- and (−)-3. Compounds (+)- and (−)-4 appeared to be the best intermediates for this purpose. Additionally, we wished to investigate the effects of stereochemistry by synthesizing the 1*S*,2*S*-(−) and 1*R*,2*R*-(+) diastereoisomers of 3, compounds (−)- and (+)-5; these could be obtained directly by reduction of the enantiomers of U50,488, which we have previously reported.[14] Compounds (−)- and (+)-5 would allow us to prove whether in this class of compounds the cis configuration is more important for high σ receptor activity than the trans configuration. We also investigated the effect of reducing the distance between the *N*-methyl nitrogen atom and 3,4-dichlorophenyl ring by synthesis of the corresponding benzyl analogue (±)-6.

Chemistry

The key intermediates for the preparation of the target compounds are diamines (+)- and (−)-7 (Scheme I), which we have recently synthesized.[17] However, in order to increase the efficiency of the overall synthesis it was necessary to look for a method that would provide them in a stereospecific manner. The retrosynthetic analysis (Scheme I) shows that diamines 7 could be prepared from cyclohexene oxide if reduction of the oxime 8 would selectively give the cis stereoisomer.

Alcohol 9 (Scheme II) was obtained in quantitative yield (redistilled) by refluxing together for 4 days equimolar amounts of cyclohexene oxide and pyrrolidine. Swern oxidation[18] of 9 using activated dimethyl sulfoxide led to the corresponding ketone 10 in 72% yield, which reacted smoothly with hydroxylamine hydrochloride to give oxime 8 in 88% yield.

The key step, the selective reduction of this oxime to cis diamine 7, was accomplished by treatment with H_2 (45 psi) in glacial acetic acid in the presence of PtO_2. The solvent had a very important role in this process. Sub-

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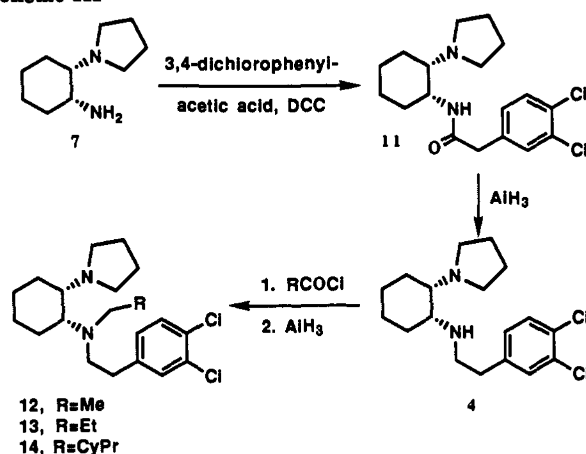
Scheme III



stitution of acetic acid by EtOH-AcOH (4:1)[19] led to no reaction and by 20% aqueous AcOH or 1 M aqueous HCl to very long reaction times. It is interesting to note that reduction with Na/EtOH gave exclusively the trans isomer, although the reaction was not very clean, and LAH gave a 1:1 mixture of the cis and trans isomers.

Diamine 7 was resolved to optical purity with (-)- and (+)-mandelic acid as we have previously described (Scheme II).[17] Coupling of each enantiomer of 7 with 3,4-dichlorophenylacetic acid was achieved in the presence of dicyclohexylcarbodiimide,[20] and the corresponding amides (1*S*,2*R*)-(+)- and (1*R*,2*S*)-(-)-11 were reduced with a 0.67 M solution of aluminum hydride[21] in THF to give intermediates (1*S*,2*R*)-(+)- and (1*R*,2*S*)-(-)-4 (Scheme III). Attempts to use lithium aluminum hydride instead of AlH_3 resulted in loss of the chlorine atoms.

Alkylation of this secondary amine was most cleanly achieved by first formation of the corresponding amide by coupling with the acid chloride or the anhydride, followed by reduction with aluminum hydride in about 60% overall yield (procedure C below). Attempts to directly alkylate (+)- and (-)-4 led to quaternization of the amino groups.

During the reduction of the acylated product with aluminum hydride a secondary product was obtained in about 10% yield, which corresponded to the secondary amine 4. This amine could not be separated from the desired compounds by simple crystallization. It was necessary to convert it to an amide by treating the entire mixture with Ac_2O (the tertiary amines would not react) and then crystallizing with, for example, fumaric acid.

Trans isomers (1*S*,2*S*)-(+)- and (1*R*,2*R*)-(-)-5 were obtained by reduction of 1*S*,2*S*-(-) and 1*R*,2*R*-(+) enantiomers of U50,488[22] respectively, following procedure B described below, and they were purified as the hydrobromide salts by recrystallization from *i*-PrOH-MeOH (4:1). The analogue (±)-6 was prepared in the same way by reduction of the corresponding benzamide[15] and purified from MeOH as its hydrobromide salt.

Table I. Binding Affinities of the Cis Isomers at σ Receptors[a]

compound	R, X	K_i (nM) [^{3}H]-(+)-3-PPP
(1*R*,2*S*)-(-)-11	H, O	11.2 ± 2.5
(1*S*,2*R*)-(+)-11	H, O	146 ± 26
(1*R*,2*S*)-(-)-4	H, H_2	0.49 ± 0.04
(1*S*,2*R*)-(+)-4	H, H_2	2.4 ± 0.8
(1*R*,2*S*)-(+)-3	Me, H_2	6.0 ± 3.0[b]
(1*S*,2*R*)-(-)-3	Me, H_2	1.3 ± 0.3[b]
(1*R*,2*S*)-(-)-12	Et, H_2	3.5 ± 1.0
(1*S*,2*R*)-(+)-12	Et, H_2	3.1 ± 0.6
(1*R*,2*S*)-(-)-13	Pr, H_2	55.7 ± 9.9
(1*S*,2*R*)-(+)-13	Pr, H_2	140 ± 17
(1*R*,2*S*)-(-)-14	CyPrMe, H_2	38 ± 13
(1*S*,2*R*)-(+)-14	CyPrMe, H_2	412 ± 69

[a] Twelve concentrations of unlabeled ligand ranging from 0.005 to 1000 nM or 0.05 to 10000 nM were incubated with 3 nM [^{3}H]-(+)-3-PPP as described in Methods. The CDATA iterative curve fitting program (EMF Software, Inc., Baltimore, MD) was used to determine IC_{50} values. Values are the result of 2-4 experiments ± SEM, each carried out in duplicate. The Cheng-Prusoff equation[25] was then used to convert IC_{50} values to apparent K_i values. The K_d for [^{3}H]-(+)-3-PPP (27.4 nM) was determined in independent experiments using guinea pig brain. [b] Previously reported data.[15]

Results and Discussion

In our earlier study,[14] the corresponding acetamide precursors to (-)- and (+)-3 exhibited affinities of 81 and 221 nM while (-)- and (+)-3 exhibited affinities of 1.3 and 6.0 nM, respectively, at the σ receptor.[15] These results indicated marginal enantioselectivity with the 1*S*,2*R* absolute configuration favoring higher σ receptor affinity. Examination of the results in Table I indicates that the 1*R*,2*S* configuration generally yields enantiomers with the highest potency at the σ receptor. The results of this study varying the *N*-alkyl substituents of (-)- and (+)-3 revealed that the methyl group is not optimal for highest affinity and enantioselectivity of this class of compounds at sigma receptors. Thus, further reduction in the size of the *N*-alkyl substituents of (-)- and (+)-3 gives rise to (+)- and (-)-4 where R = H; (-)-4 [K_i = 0.49 nM (Table I)] proved to be 2.7-fold more potent than our best previously reported σ ligand (-)-3. In this assay (+)-pentazocine exhibited K_i = 1.2 ± 0.2 nM, haloperidol 3.8 ± 2.9 nM, DTG 20.5 ± 3.6 nM, (+)-SKF10,047 62.5 ± 11.6 nM, dextrallorphan 16.1 ± 1.8 nM, and (+)-3-PPP 27.4 ± 2.0 nM.[23] The 1*S*,2*R* enantiomer (+)-4 exhibited an affinity of 2.4 nM (5-fold enantioselectivity) and with 2-fold decreased affinity compared with its *N*-methyl homologue. Interestingly, the corresponding amide precursors of (-)- and (+)-4, compounds (-)- and (+)-11, also favored the 1*R*,2*S* enantiomer in terms of σ receptor affinity [(-)-11, K_i = 11.2 nM and (+)-11, K_i = 146 nM]. As we have observed previously[15] for related σ receptor ligands, these amides showed a better enantioselectivity ratio (13-fold) than their corresponding amines (-)- and (+)-4 (5-fold), presumably due to increased rigidity of the amide side chain. Further

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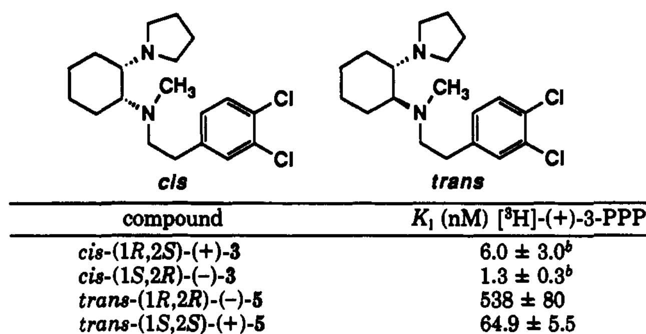
Table II. Binding Affinities for Cis and Trans Isomers at σ Receptors[a]



compound	K_i (nM) [^{3}H]-(+)-3-PPP
cis-(1*R*,2*S*)-(+)-**3**	6.0 ± 3.0[b]
cis-(1*S*,2*R*)-(−)-**3**	1.3 ± 0.3[b]
trans-(1*R*,2*R*)-(−)-**5**	538 ± 80
trans-(1*S*,2*S*)-(+)-**5**	64.9 ± 5.5

[a] Same as Table I. [b] Previously reported data.[15]

increase in size of the *N*-alkyl group to ethyl [compounds (−)- and (+)-**12**] resulted in a further reduction in affinity (3.5 and 3.1 nM, respectively). Unlike the other compounds in this series, the ethyl homologues showed no significant enantioselectivity. Further increase to propyl [(−)- and (+)-**13**] revealed a boundary condition for the *N*-alkyl group in this class of compounds. In the 1*R*,2*S* series, going from ethyl [(−)-**12**] to propyl [(−)-**13**] produces a 16-fold loss in affinity, compared to a more gradual loss in going from H to ethyl. This effect is even more dramatic with the 1*S*,2*R* isomers. Going from ethyl [(+)-**12**] to propyl [(+)-**13**] produces a 45-fold loss in affinity, while there is only a minor affinity change in going from H to ethyl. Further increase to cyclopropylmethyl [(−)-**14** and (+)-**14**] indicated no further improvement in affinity over the *N*-propyl compounds but revealed an improvement in enantioselectivity (11-fold) compared with the other compounds in the series.

Therefore, from this study, it is clear that optimal affinity in this class of compounds is obtained by substitution of the *N*-alkyl group with NH. A surprising feature in the SAR of these compounds is that whereas the (+)-benzomorphans (e.g. (+)-pentazocine),[24] (+)-morphinans (e.g. dextromethorphan),[24] 3-phenyl piperidines (e.g. (+)-3-PPP),[11] and octahydrobenzo[*f*]quinolines (OHBQ's)[11] exhibit substantial increases in their affinity at σ receptors with increasing size of their *N*-alkyl substituents, the *cis*-*N*-[2-(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)cyclohexylamines show decreases in affinity. It is possible that increase in size of the *N*-alkyl group of (−)- and (+)-**3** actually sterically impedes access to the receptor site, or the effect could be more subtle involving changes in the electron density on the second nitrogen atom.

In order to definitively prove that the cis configuration of these ligands is better than the trans configuration for increased σ receptor affinity, we examined enantiomeric *trans*-*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)cyclohexylamines, (+)- and (−)-**5** for affinity at the sigma receptor (Table II). The 1*R*,2*R* diastereoisomer (−)-**5** displaced [^{3}H]-(+)-3-PPP with an affinity of 538 nM; although the only difference between (−)-**5** and (−)-**3** is a change in one stereocenter, the affinity dropped by 414-fold. The corresponding 1*S*,2*S* diastereoisomer, (+)-**5** exhibited an affinity of 64.9 nM corresponding to a

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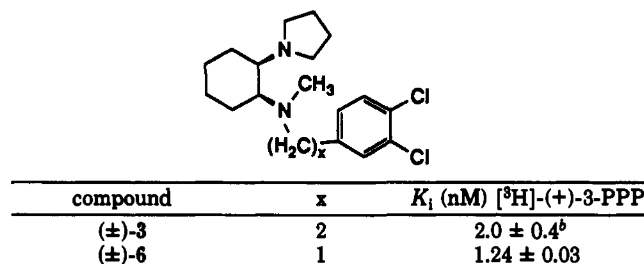
Table III. Binding Affinities for Homologous Compounds at σ Receptors[a]



compound	x	K_i (nM) [^{3}H]-(+)-3-PPP
(±)-**3**	2	2.0 ± 0.4[b]
(±)-**6**	1	1.24 ± 0.03

[a] Same as Table I. [b] Previously reported data.[15]

Table IV. Affinity of (1*R*,2*S*)-(−)-**4** across Receptors[c]

radioligand/receptor	K_i (nM)
[^{3}H]-(+)-3-PPP/σ	0.49 ± 0.04
[^{3}H]-bremazocine/κ opiate	no inhib[d]
[^{3}H]-TCP/phencyclidine	6880 ± 1095
[^{3}H]-(−)-sulpiride/dopamine-D_2	8514 ± 66

[c] Twelve concentrations of unlabeled ligand ranging from 0.5 to 100 000 nM were incubated with the indicated radioligand for dopamine-D_2, κ opiate, or phencyclidine receptors. Assay conditions were as described in Methods. Data was analyzed as described in the legend of Tables I–III. Values are the result of 2–3 experiments ± SEM, each carried out in duplicate. The following K_d values (as determined in independent experiments) were employed to calculate K_i: [^{3}H]-(−)-sulpiride (rat brain), K_d = 10.3 nM; [^{3}H]-bremazocine (guinea pig brain), K_d = 0.64 nM; [^{3}H]-TCP (guinea pig brain), K_d = 25 nM. [d] No IC_{50} or K_i value was determined since the compound produced less than 30% inhibition of control binding at a concentration of 10 000 nM. σ data is taken from Table I.

50-fold drop in affinity compared with (−)-**3** and 11-fold lowered compared with (+)-**3**. These results clearly show that in this class of σ ligands those with the cis configuration are more potent at the σ receptor than those with the trans configuration. This observation is in concordance with the related but much less potent benzeneacetamide[14] class of σ ligands where the cis isomers exhibit greater affinity and selectivity for sigma receptors than those with the trans configuration.

In a limited test of the importance of the (3,4-dichlorophenyl)ethyl side chain on σ receptor affinity, we examined the 3,4-dichlorobenzyl analogue, (±)-**6**. This compound showed a comparable potency (K_i = 1.24 nM) with (±)-**3** (K_i = 2.0 nM) (Table III), which indicates that the ethylene spacing between the NMe and 3,4-dichlorophenyl is not important for high potency at this receptor. This is in contrast to results with the corresponding benzeneacetamides, where shortening the side chain by one carbon atom caused a 40-fold loss in σ binding affinity.[15] Perhaps the rigidity of the amide linkage plays some role in the chain length requirement. Further testing of propylene, butylene, and perhaps longer spacer groups will be necessary before an exact answer to this question is known.

Because of the very high σ affinity of (−)-**4**, this compound was investigated for selectivity at σ receptors. The ability of this compound to displace [^{3}H]-(−)-sulpiride, [^{3}H]-TCP, and [^{3}H]-bremazocine from dopamine-D_2, phencyclidine and κ opiate receptors, respectively, was evaluated. The results are shown in Table IV. This compound lacks affinity for kappa opiate receptors labeled by [^{3}H]-bremazocine and exhibits only very weak affinity for dopamine and PCP receptors. σ selectivity ratios of 14 000- and 17 000-fold are obtained for PCP and dopamine-D_2 receptors, respectively. This is comparable to the σ selectivity exhibited by (−)- and (+)-**3** in our previous study.[15]

Conclusion

We have successfully developed a synthetic strategy leading to optically pure enantiomeric *N*-alkyl-substituted homologues of (−)- and (+)-**3**, a novel class of highly potent and specific σ receptor ligands. Because these compounds are of known absolute configuration, they should prove useful in molecular modeling studies of this pharmacophore. We have shown that in general, compounds with the 1*R*,2*S* configuration are better ligands at the sigma receptor than those with the 1*S*,2*R* absolute configuration within this series. By manipulation of the *N*-alkyl group, we have discovered (1*R*,2*S*)-(+)-*cis*-*N*-[2-(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)cyclohexylamine [(−)-**4**] (K_i = 0.49 nM). Since this compound lacks high affinity for PCP, κ opiate and dopamine-D_2, it is to our knowledge, the most potent and selective known σ receptor ligand. We have proved that the cis configuration is more efficacious than the trans configuration for highest affinity at this receptor. This class of compounds will provide new tools with which to more effectively study the function of σ receptors and their possible involvement in dystonia and other motor disorders. Selected compounds from this class are presently being evaluated as agonists or antagonists at the σ receptor.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Specific rotation determinations at the sodium-D line were obtained in a 1-dm cell using a Perkin-Elmer 241-MC polarimeter. Elemental analyses were performed at Atlantic Microlabs, Atlanta, GA. Chemical ionization mass spectra (CIMS) were obtained using a Finnigan 1015 mass spectrometer. Electron ionization mass spectra (EIMS) and high-resolution mass measurements (HRMS) were obtained using a VG-Micro Mass 7070F mass spectrometer. ^{1}H NMR spectra were obtained from $CDCl_3$ solutions using a Varian XL-300 spectrometer. Infrared (IR) spectra were obtained using a Beckman 4230 IR spectrometer. Gas chromatography was performed on a Hewlett-Packard 5890 gas chromatograph. Thin-layer chromatography (TLC) was performed on 250 μm Analtech GHLF silica gel plates. No attempt was made to optimize the yields reported.

2-(1-Pyrrolidinyl)cyclohexanol [(±)-9]. A stirred mixture of cyclohexene oxide (196 g, 2.0 mol) and pyrrolidine (142 g, 2.0 mol) was boiled under reflux under a nitrogen atmosphere. Examination of the reaction temperature indicated a gradual elevation of the boiling point as the reaction proceeded. After 18 h, the reaction mixture stopped boiling and reached equilibrium at 150 °C. The reaction was kept at this temperature for 4 days or until TLC ($CHCl_3$–MeOH–NH_4OH, 80:18:2) indicated the reaction to be complete. The oily reaction mixture was distilled in vacuo to give (±)-**9** in quantitative yield as a colorless oil: bp 76 °C (0.25 mmHg); IR (neat) 3450, 2920, 2860, 2800, 1445 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.32 (m, 4 H), 1.82 (m, 7 H), 2.19 (m, 1 H), 2.54 (m, 1 H), 2.65 (m, 2 H), 2.75 (m, 2 H), 3.42 (m, 1 H), 4.12 (s, 1 H).

2-(1-Pyrrolidinyl)cyclohexanone (10). A solution of oxalyl chloride (50 mL, 0.57 mol) in 200 mL of freshly distilled CH_2Cl_2 was cooled down to −70 °C, and DMSO (80 mL, 1.13 mol) was carefully added dropwise. After 15 min 2-(1-pyrrolidinyl)cyclohexanol (**9**) (70 g, 0.41 mol) was added to the reaction mixture, followed by Et_3N (350 mL). The cold bath was then removed, and the mixture was stirred at room temperature for 2.5 h. Progress of the reaction was followed by gas chromatography. The solvent was partially removed in vacuo, and ethyl ether (200 mL) was added. This organic layer was washed with brine (3 × 100 mL) and dried (Na_2SO_4), and the solvent was removed in vacuo. The crude product was distilled under high vacuum (bp 84 °C (0.5 mmHg)) to yield 49.5 g of the ketone **10** as a colorless oil (72%): bp 84 °C (0.5 mmHg); IR (neat) 2940, 2860, 2800, 1715, 1440 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.53–1.63 (m, 1 H), 1.72 (m, 4 H), 1.76–1.93 (m, 4 H), 1.94–2.04 (m, 1 H), 2.21 (m, 1 H), 2.52 (m, 5 H), 2.83 (dd, 1 H, J_1 = 8.6, J_2 = 4.5 Hz); HRMS, M$^+$ calcd for $C_{10}H_{17}NO$ 167.1310, found 167.1310.

2-(1-Pyrrolidinyl)cyclohexanone Oxime (8). 2-(1-Pyrrolidinyl)cyclohexanone (**10**) (46 g, 0.28 mol) was dissolved in EtOH (175 mL), and $NH_2OH·HCl$ (23.4 g, 0.34 mol) was added. Reaction progress was followed by gas chromatography. The solvent was removed in vacuo, the residue was dissolved in MeOH, and the solution was poured into an Erlenmeyer flask. EtOAc was added to replace the MeOH while the mixture was boiling. In this way, crystallization of the hydrochloride of the oxime occurred, and 53 g of salt was obtained (88%): mp (HCl salt) 170–171 °C; IR (neat) 3400, 2960, 1640, 1450 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.36–1.58 (m, 3 H), 1.73 (m, 4 H), 1.79–1.88 (m, 2 H), 2.02–2.15 (m, 2 H), 2.46–2.54 (m, 2 H), 2.69 (m, 1 H), 3.02 (bd, 1 H, J = 13.4 Hz), 8.35 (bs, 1 H); EIMS (70 eV, *m/e* (rel int)) 182 (15, M$^+$), 166 (100), 148 (29), 136 (27), 124 (29), 110 (100), 97 (100), 70 (60). Anal. ($C_{10}H_{19}ClN_2O$) C, H, N, Cl.

(±)-*cis*-2-(1-Pyrrolidinyl)cyclohexylamine ((±)-7). 2-(1-Pyrrolidinyl)cyclohexanone oxime (**8**) (22.9 g, 0.11 mol) was dissolved in glacial acetic acid (120 mL), PtO_2 (1g) was added, and the mixture was hydrogenated at 45 psi for 96 h. During this time, the catalyst was filtered and renewed every 24 h. The reaction was followed by gas chromatography. The PtO_2 was removed by filtration of the reaction mixture through Celite, and excess concentrated HCl was added. The solvent was removed in vacuo at 40–50 °C, and the resulting hydrochloride salt was dissolved in 100 mL of water. This aqueous solution was carefully basified to 30% w/v with pellets of NaOH and extracted with $CHCl_3$ (5 × 50 mL). The organic layer was dried (Na_2SO_4), and the solvent was removed in vacuo. The crude amine was distilled under high vacuum to yield 13.7 g of (±)-**7** (78%) as a clear liquid. This compound proved to have identical properties to the one obtained previously by us,[17] and it was resolved to optical purity following the procedure therein described.

(1*R*,2*S*)-(−)-*cis*-(3,4-Dichlorophenyl)-*N*-[2-(1-pyrrolidinyl)cyclohexyl]acetamide [(−)-11]. Procedure A. 3,4-Dichlorophenylacetic acid (7.3 g, 0.36 mol) was dissolved in CH_2Cl_2 (50 mL), and dicyclohexylcarbodiimide (7.4 g, 0.036 mol) was added. The mixture was stirred at room temperature for half an hour. (−)-*cis*-2-(1-Pyrrolidinyl)cyclohexylamine ((−)-**7**) (3.0 g, 0.018 mol) and pyridine (0.7 mL) were then added. After 5 min, the dicyclohexylurea was filtered off, and the solvent was concentrated in vacuo. The residue was diluted with Et_2O (200 mL), and the resulting organic layer extracted with 5% citric acid (4 × 50 mL). The aqueous layer was washed with Et_2O (4 × 50 mL), basified with concentrated NH_4OH, and extracted with CH_2Cl_2 (3 × 80 mL). The organic layer was washed with brine (50 mL) and dried (Na_2SO_4), and the solvent was removed in vacuo. A total of 6.3 g (99%) of crude crystalline product was obtained. The amide was recrystallized as its fumarate salt from EtOAc to yield 7.7 g (92%) of pure (−)-**11**·fumarate·hemihydrate: mp (free base) 110–111 °C; mp (fumarate salt) 159–160 °C; $[\alpha]^{20}_D$ (fumarate salt) −12.7° (0.11, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.16–1.35 (m, 4 H), 1.43 (m, 1 H), 1.70 (m, 5 H), 1.85 (m, 1 H), 2.11 (m, 1 H), 2.29 (bd, 1 H, J = 13.4 Hz), 2.41–2.49 (m, 4 H), 3.51 (s, 2 H), 4.01 (s, 1 H), 6.28 (bs, 1 H), 7.15 (dd, 1 H, J_1 = 8.3, J_2 = 2.0 Hz), 7.39 (d, 1 H, J = 8.3 Hz), 7.41 (d, 1 H, J = 2.0 Hz); CIMS (NH_3) 355 (MH$^+$). Anal. ($C_{18}H_{24}Cl_2N_2O$) (free base) C, H, N, Cl. Anal. ($C_{22}H_{28}Cl_2N_2O_5·{}^1/_2H_2O$) (fumarate salt) C, H, N, Cl.

(1*S*,2*R*)-(+)-*cis*-(3,4-Dichlorophenyl)-*N*-[2-(1-pyrrolidinyl)cyclohexyl]acetamide [(+)-11]: mp 159–160 °C; $[\alpha]^{20}_D$ (fumarate salt) +9.2° (0.22, MeOH). Anal. ($C_{22}H_{28}Cl_2N_2O_5·{}^1/_2H_2O$) (fumarate salt) C, H, N, Cl.

(1*R*,2*S*)-(−)-*cis*-*N*-[2-(3,4-Dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)cyclohexylamine [(−)-4]. Procedure B. Aluminum hydride solution[21] in THF (0.67 mmol/mL) (80 mL, 0.053 mol) was stirred at room temperature under argon, and (1*R*,2*S*)-(−)-*cis*-(3,4-dichlorophenyl)-*N*-[2-(1-pyrrolidinyl)cyclohexyl]acetamide [(−)-**11**] (3.8 g, 0.011 mol) in THF (30 mL) was added slowly. After 5 min the mixture was poured into ice-cooled 15% NaOH solution (100 mL). An oily compound separated from this aqueous layer, which was extracted with Et_2O (3 × 100 mL). The organic layers were combined, washed with brine (2 × 50 mL), and dried (Na_2SO_4), and the solvent was removed in vacuo. A yellowish oil was obtained, which was purified by crystallization with HBr in EtOH to yield 3.2 g (61%) of (−)-**4**·HBr: mp (HBr salt) 274–275 °C; $[\alpha]^{20}_D$ −11.1° (0.32, MeOH); ^{1}H NMR ($CDCl_3$)

δ 1.11–1.28 (m, 4 H), 1.45–1.77 (m, 8 H), 1.81–1.95 (m, 2 H), 2.28–2.44 (m, 4 H), 2.58–2.69 (m, 1 H), 2.71–2.80 (m, 3 H), 2.89–2.97 (m, 1 H), 7.07 (dd, 1 H, J_1 = 8.2, J_2 = 2.0 Hz), 7.32 (d, 1 H, J = 8.2 Hz), 7.33 (d, 1 H, J = 2 Hz); CIMS (NH_3) 341 (MH^+). Anal. ($C_{18}H_{28}Br_2Cl_2N_2$) (HBr salt) C, H, N, Cl. Anal. ($C_{18}H_{26}Cl_2N_2$) (free base) C, H, N, Cl.

(1*S*,2*R*)-(+)-*cis*-*N*-[2-(3,4-Dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)cyclohexylamine [(+)-4]: mp (HBr salt) 274–275 °C; $[\alpha]^{20}_D$ +11.5° (0.57, MeOH). Anal. ($C_{18}H_{28}Br_2Cl_2N_2$) (HBr salt) C, H, N.

(1*R*,2*S*)-(−)-*cis*-*N*-[2-(3,4-Dichlorophenyl)ethyl]-*N*-(*n*-propyl)-2-(1-pyrrolidinyl)cyclohexylamine [(−)-13]. Procedure C. (1*R*,2*S*)-(−)-*cis*-*N*-[2-(3,4-Dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)cyclohexylamine [(−)-4] (1.0 g, 0.0029 mol) was dissolved in $CHCl_3$ (10 mL) and stirred in the presence of anhydrous K_2CO_3 (2.0 g, 0.015 mol). Propionyl chloride (0.54 g, 0.0059 mol) was then added, and the reaction was followed by TLC. After completion, the mixture was diluted with $CHCl_3$ (10 mL) and extracted with 10% NaOH (10 × 10 mL). The organic layer was dried, and the solvent was removed in vacuo to give 1.1 g of crude product, which was used in the next step without further purification. Crude amide (0.2 g) was reduced following procedure B to give 0.18 g of product, which contained 10% of diamine 4 as an impurity. The entire mixture was acylated with Ac_2O, and then the desired diamine 13 was purified by crystallization with fumaric acid in EtOAc: mp 168–169 °C; $[\alpha]^{20}_D$ −11.1° (0.38, MeOH). Anal. ($C_{25}H_{36}Cl_2N_2O_4 \cdot H_2O$) (fumarate salt) C, H, N.

(1*S*,2*R*)-(+)-*cis*-*N*-[2-(3,4-Dichlorophenyl)ethyl]-*N*-(*n*-propyl)-2-(1-pyrrolidinyl)cyclohexylamine [(+)-13]: mp 168–169 °C; $[\alpha]^{20}_D$ +15.0° (0.31 MeOH). Anal. ($C_{25}H_{36}Cl_2N_2O_4$) (fumarate salt) C, H, N, Cl.

(1*R*,2*S*)-(−)-*cis*-*N*-Ethyl-*N*-[2-(3,4-Dichlorophenyl)-ethyl]-2-(1-pyrrolidinyl)cyclohexylamine [(−)-12]. Procedure C was followed, except that Ac_2O was used instead of acetyl chloride to obtain the amide. Diamine 12 was purified as the HI salt and recrystallized from MeOH: mp 215.5–216.5 °C; $[\alpha]^{20}_D$ −8.2° (0.09, MeOH). Anal. ($C_{20}H_{32}Cl_2I_2N_2$) (HI salt) C, H, N, Cl.

(1*S*,2*R*)-(+)-*cis*-*N*-Ethyl-*N*-[2-(3,4-Dichlorophenyl)-ethyl]-2-(1-pyrrolidinyl)cyclohexylamine [(+)-12]: mp 215–215.5 °C; $[\alpha]^{20}_D$ +7.8° (0.32, MeOH). Anal. ($C_{20}H_{32}Cl_2I_2N_2$) (HI salt) C, H, N, Cl.

(1*R*,2*S*)-(−)-*cis*-*N*-[2-(3,4-Dichlorophenyl)ethyl]-*N*-(cyclopropylmethyl)-2-(1-pyrrolidinyl)cyclohexylamine [(−)-14]. Compound (−)-14 was prepared via procedure C, purified as the fumarate salt, and recrystallized from EtOAc: mp 183–184 °C; $[\alpha]^{20}_D$ −28.3° (0.30, MeOH). Anal. ($C_{26}H_{36}Cl_2N_2O_4$) (fumarate salt) C, H, N.

(1*S*,2*R*)-(+)-*cis*-*N*-[2-(3,4-Dichlorophenyl)ethyl]-*N*-(cyclopropylmethyl)-2-(1-pyrrolidinyl)cyclohexylamine [(+)-14]: mp 183–184 °C; $[\alpha]^{20}_D$ +29.9° (0.35, MeOH). Anal. ($C_{26}H_{36}Cl_2N_2O_4$) (fumarate salt) C, H, N, Cl.

(1*S*,2*S*)-(+)-*trans*-*N*-[2-(3,4-Dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)cyclohexylamine [(+)-5]. Compound (+)-5 was obtained by reduction of (1*S*,2*S*)-(−)-U50,488[22] via procedure B: mp 212–213 °C; $[\alpha]^{20}_D$ +12.8° (0.57, MeOH). Anal. ($C_{19}H_{30}Br_2Cl_2N_2 \cdot {}^1/_2H_2O$) (HBr salt) C, H, N, Cl.

(1*R*,2*R*)-(−)-*trans*-*N*-[2-(3,4-Dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)cyclohexylamine [(−)-5]: mp 212–213 °C; $[\alpha]^{20}_D$ −13.9° (0.28, MeOH). Anal. ($C_{19}H_{30}Br_2Cl_2N_2 \cdot {}^1/_2H_2O$) (HBr salt) C, H, N, Cl.

(±)-*cis*-*N*-[(3,4-Dichlorophenyl)methyl]-*N*-methyl-2-(1-pyrrolidinyl)cyclohexylamine [(±)-6]. Compound (±)-6 was prepared by reduction of the corresponding benzamide[15] via procedure B: mp 186–187 °C. Anal. ($C_{18}H_{28}Br_2Cl_2N_2$) (HBr salt) C, H, N.

Biological Materials and Methods. Membrane Preparation. Receptor binding assays were performed using the crude synaptosomal (P_2) membrane fraction of guinea pig brain (σ, κ, and PCP receptors) or rat brain (dopamine-D_2 receptors).

Crude P_2 membrane fractions were prepared from frozen (−80 °C) guinea pig brains (Pel-Freeze, Rogers, AK), minus cerebella. After removal of cerebella, brains were allowed to thaw slowly on ice and placed in ice-cold 10 mM Tris-HCl, pH 7.4, containing 320 mM sucrose (Tris-sucrose buffer). Brains were then homogenized in a Potter-Elvehjem homogenizer by 10 strokes of a motor-driven Teflon pestle in a volume of 10 mL/g tissue net weight. The homogenate was centrifuged at 1000*g* for 10 min at 4 °C, and the supernatants were saved. The pellets were resuspended by vortexing in 2 mL/gm ice-cold Tris-sucrose and centrifuged again at 1000*g* for 10 min. The combined 1000*g* supernatants were centrifuged at 31000*g* for 15 min at 4 °C. The pellets were resuspended by vortexing in 3 mL/g 10 mM Tris-HCl, pH 7.4, and the suspension was allowed to incubate at 25 °C for 15 min. Following centrifugation at 31000*g* for 15 min, the pellets were resuspended by gentle Potter-Elvehjem homogenization to a final volume of 1.53 mL/g in 10 mM Tris-HCl pH 7.4. Aliquots were stored at −80 °C until use. Protein concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin (BSA) as standard.

To prepare rat brain crude P_2 membranes, male Sprague-Dawley rats (150–200 g, Charles River, Boston, MA) were killed by decapitation. Brains (minus cerebella) were then treated as described above.

Receptor Binding. σ Receptors. σ receptors were labeled with [^{3}H]-(+)-3-PPP (98.9 Ci/mmol). Incubations were carried out in 50 mM Tris-HCl, pH 8.0, for 120 min at 25 °C in a volume of 0.5 mL with 500 μg of membrane protein and 3 nM [^{3}H]-(+)-3-PPP. Nonspecific binding was determined in the presence of 1 μM haloperidol. Assays were terminated by the addition of 5 mL of ice-cold 10 mM Tris-HCl, pH 8.0, and filtration through glass fiber filters (Schleicher and Schuell) using a Brandel cell-harvester (Gaithersburg, MD). Filters were then washed twice with 5 mL of ice-cold Tris-HCl. Filters were soaked in 0.5% polyethyleneimine for at least 30 min at 25 °C prior to use.

κ Opiate Receptors. κ receptors were labeled with [^{3}H]bremazocine (17.3 Ci/mmol) in the presence of [D-Ala^2,*N*-methyl-Phe^4,Gly-ol^5]enkephalin (DAGO) and [D-Ser^2,Leu^5,Thr^6]enkephalin (DSTLE) as μ and δ opiate receptor blockers, respectively. Incubations were carried out in 0.5 mL of 10 mM Tris-HCl, pH 7.4, for 90 min at 25 °C with 500 μg of membrane protein, 100 nM DAGO, 100 nM DSTLE, and 2 nM [^{3}H]bremazocine. Assays were terminated by the addition of 5 mL of ice-cold buffer and filtration through glass fiber filters (Schleicher and Schuell) under reduced pressure. Filters were then washed twice with 5 mL of ice-cold buffer. Nonspecific binding was determined in the presence of 10 μM levallorphan.

Phencyclidine (PCP) Receptors. PCP receptors were labeled using [^{3}H]-1-[1-(2-thienyl)cyclohexyl]piperidine ([^{3}H]TCP) (48.9 Ci/mmol). Incubations were carried out in 5 mM Tris-HCl, pH 7.4, for 60 min at 4 °C in a volume of 0.5 mL with 500 μg of membrane protein and 5 nM [^{3}H]TCP. Assays were terminated by addition of 5 mL of ice-cold buffer and filtration through glass fiber filters under reduced pressure. Filters were then washed twice with 5 mL of ice-cold buffer. Filters were soaked in 0.3% polyethyleneimine for at least 30 min at 25 °C prior to use. Nonspecific binding was determined in the presence of 10 μM cyclazocine.

Dopamine-D_2 Receptors. Dopamine-D_2 receptors were labelled with 5 nM (−)-[^{3}H]sulpiride (78.6 Ci/mmol) using rat brain membranes. Incubations were carried out for 60 min at 25 °C in 0.5 mL of 50 mM Tris-HCl, pH 7.7, containing 120 mM NaCl and 500 μg of membrane protein. Nonspecific binding was determined in the presence of 1 μM haloperidol. Assays were terminated by the addition of ice-cold incubation buffer and vacuum filtration through glass fiber filters (Schleicher and Schuell). Filters were then washed twice with ice-cold incubation buffer.

Chemicals. All scintillation counting was performed with a Packard Model 4450 scintillation spectrometer using Ecoscint cocktail (National Diagnostics, Manville, NJ) after an overnight extraction of the counts from the filters. Radioligands were purchased from DuPont/New England Nuclear (Boston, MA). Haloperidol, polyethyleneimine and Tris were purchased from Sigma Chemicals (St. Louis, MO). Cyclazocine and levallorphan were obtained from the National Institute on Drug Abuse (Rockville, MD).

Acknowledgment. B.d.C. acknowledges financial support from the Fogarty Foundation. L.R. was supported as an IRTA Fellow. Portions of this work were supported by PHS grant no. NS26746 from the National Institute

of Neurological Disorders and Stroke (W.D.B.) and no. DA04988 from the National Institute on Drug Abuse (W.D.B.). We wish to express our sincere appreciation to Noel Whittaker and Wesley White for performing mass and ^{1}H NMR spectral analyses of all compounds and intermediates synthesized in this study.

Registry No. (−)-1, 67198-19-0; (+)-4, 135211-15-3; (−)-4, 135093-19-5; (+)-4·HBr, 135093-12-8; (−)-4·HBr, 134970-03-9; (+)-5, 135211-17-5; (−)-5, 135211-16-4; (+)-5·HBr, 135093-13-9; (−)-5·HBr, 135093-14-0; (±)-6, 134970-10-8; (±)-6·HBr, 134970-09-5; (±)-6 benzamide, 128387-88-2; (±)-7, 133488-96-7; 8·HCl, 135093-09-3; (±)-9, 69420-67-3; 10, 118207-34-4; (−)-11, 135093-10-6; (+)-11, 135093-11-7; (−)-11 fumarate, 135211-10-8; (+)-11 fumarate, 135211-11-9; (−)-12, 135093-15-1; (+)-12, 135093-16-2; (−)-12·HI, 134970-06-2; (+)-12·HI, 135211-13-1; (−)-13, 134970-04-0; (+)-13, 135093-17-3; (−)-13 fumarate, 134970-05-1; (+)-13 fumarate, 135211-12-0; (−)-14, 134970-07-3; (+)-14, 135093-18-4; (−)-14 fumarate, 134970-08-4; (+)-14 fumarate, 135211-14-2; cyclohexene oxide, 286-20-4.

Analogues of Oxybutynin. Synthesis and Antimuscarinic and Bladder Activity of Some Substituted 7-Amino-1-hydroxy-5-heptyn-2-ones and Related Compounds

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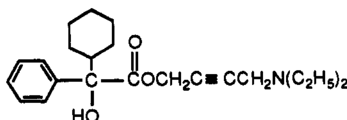
Oxybutynin chloride [4-(diethylamino)-2-butynyl α-cyclohexyl-α-hydroxybenzeneacetate hydrochloride, Ditropan] is widely used for the relief of symptoms in neurogenic bladder. This is a result of its combined anticholinergic, antispasmodic, and local anesthetic activities. In a study directed toward development of agents possessing the beneficial properties of oxybutynin, but having a longer duration of action, a series of metabolically more stable keto analogues of the parent ester, i.e. substituted 7-amino-1-hydroxy-5-heptyn-2-ones along with some analogues and derivatives, was prepared and evaluated for in vitro and in vivo antimuscarinic action in guinea pig preparations. Several members of the series were potent antimuscarinics having a longer duration of activity than that of oxybutynin in a guinea pig cystometrogram model. On the basis of its in vitro and in vivo antimuscarinic activity, coupled with a 5-fold greater duration of action than that of oxybutynin, 1-cyclobutyl-7-(dimethylamino)-1-hydroxy-1-phenyl-5-heptyn-2-one (**14b**) was selected for clinical evaluation.

Oxybutynin (1)[1,2] is extensively utilized for relief of urinary urgency, frequency, and urge incontinence in patients with uninhibited and reflex neurogenic bladder.[3–5] The effectiveness of this agent is attributed to a combination of M_3[6–8] selective muscarinic receptor subtype antagonism and antispasmodic,[9,10] local anesthetic, and calcium channel blocking actions.[11,12] The duration of action of 1 in man is about 6 h. In rats, it reaches a peak blood level about 2 h after dosing and a minimally detectable amount is present for 72 h.[4] The recommended clinical dosing regimen is 5 mg two to four times a day.[13] As this product is generally administered chronically, synthesis and identification of a similar substance having an increased duration of action was initiated. An understanding of the metabolism of oxybutynin is important for the design of such compounds. Unfortunately, the metabolic fate of 1 in humans has not been described; however, studies in rats indicate comparatively rapid metabolism that involves ester hydrolysis, N-deethylation, 4-hydroxylation of the cyclohexane ring, and conjugation.[14] Although a different metabolic route is followed in rat microsomes, where N-deethylation and *N*-oxide formation predominate,[15a] and in rabbits, where the hydrolysis product is not the major metabolite,[4] structural modification of 1 to give hydrolysis-resistant analogues was undertaken.



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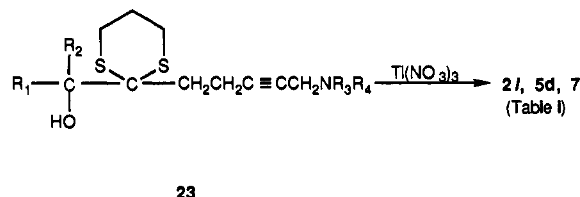
Scheme I. Method A



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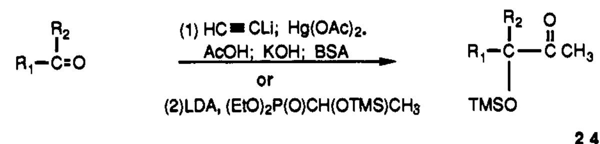
Scheme II. Method B



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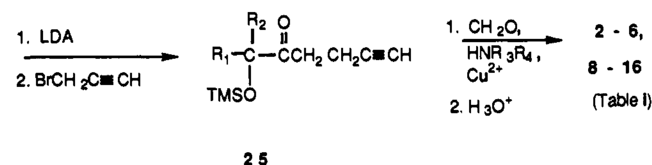

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A series of substituted 7-amino-1-hydroxy-5-heptyn-2-ones (**2–16**, Table I), as well as some analogues and de-

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0022-2623/91/1834-3065$02.50/0 © 1991 American Chemical Society