Synthesis, Configuration, and Evaluation of Two Conformationally Restrained Analogues of Phencyclidine

Brian R. de Costa, Clifford George,† Terrence R. Burke, Jr., Michael F. Rafferty, Patricia C. Contreras,‡ Stephen J. Mick,‡ Arthur E. Jacobson, and Kenner C. Rice*

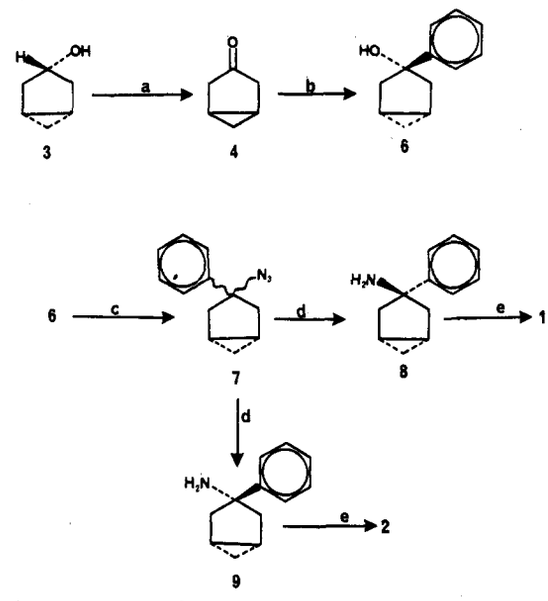
Section on Drug Design and Synthesis, Laboratory of Neuroscience, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892, Naval Research Laboratory, Code 6030, Washington, D.C. 20375, and G. D. Searle and Co., CNSDR, St. Louis, Missouri 63198. *Received November 19, 1987*

Brown oxidation of *cis*-bicyclo[3.1.0]hexan-3-ol afforded bicyclo[3.1.0]hexan-3-one in 98% yield. Treatment of this ketone with either phenyllithium or phenylmagnesium bromide in ether at room temperature followed by solvolysis of the resulting alcohol in a mixture of trifluoroacetic acid, sodium azide, and chloroform gave a mixture of *cis*- and *trans*-3-azido-3-phenylbicyclo[3.1.0]hexanes. LAH reduction of this crude mixture of azides afforded a 1:3.5 mixture of *cis*- and *trans*-3-phenyl-3-bicyclo[3.1.0]hexylamine, respectively, in 51% overall yield from the alcohol. Separation of the mixture of amines by column chromatography followed by cyclization of each by heating at 60 °C in DMF solution with 1 equiv of 1,5-dibromopentane furnished the two conformationally restrained analogues of phencyclidine (PCP), *cis*- and *trans*-3-phenyl-3-piperidinylbicyclo[3.1.0]hexane (1 and 2, respectively), in high yield. Configurations were assigned on the basis of an X-ray crystallographic analysis of the cis isomer (1). Bond lengths and angles are similar to those found in PCP and its derivatives. Binding to PCP receptors and σ sites as well as behavioral effects of 1 and 2 in rats was determined relative to PCP. In displacement of specifically bound [^{3}H]TCP (1-[1-(2-thienyl)cyclohexyl]piperidine) from PCP receptors, 1 and 2 were nearly equipotent and about one-seventh as potent as PCP. These compounds were about one-fifth as potent as PCP in displacing [^{3}H]-(+)-SKF 10,047 from its binding site. Calculation of the ED_{50} values of 1 and 2 for stereotyped behavior and ataxia indicated that they were about equipotent, and 2–3-fold less active than PCP.

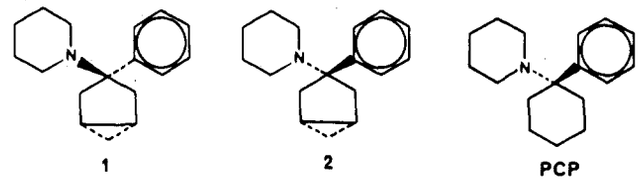
The discovery of the potentially useful pharmacological properties of phencyclidine (PCP) and PCP-like compounds, and the subsequent widespread abuse of PCP, initiated intensive research into their mode of action and into the development of potential antagonists and therapeutic agents.[1] A considerable number of analogues of PCP have now been tested for PCP activity.[1] It is evident that relatively small structural changes to either the phenyl (e.g., nitro, hydroxyl, amino substitution) or cyclohexyl portions of the PCP molecule result in significant loss or gain in its PCP-like activity. Loss of activity has been noted especially when structural changes are made to the cyclohexane ring,[2] or when electron-withdrawing moieties are added to the phenyl ring. The 2-thienyl analogue of PCP, where the phenyl ring has been replaced with a 2-thienyl ring, displays increased in vivo potency and in vitro affinity for PCP receptors.[3] We were interested in examining the effect of conformational restriction in PCP. Thus, we synthesized two "zero bridged" analogues, 1 and 2 (Scheme I). Previous work has shown that conformationally restricted analogues are valuable probes of drug–receptor steric requirements. For example, the synthesis of 9-azabicyclo[4.2.1]nona[2,3-*c*]pyridine served as the first bridged analogue of nornicotine, which combined high activity and conformational rigidity.[4] This new analogue possessed 3 times the toxicological activity and 16 times the receptor affinity of nornicotine. Both compounds 1 and 2 are semirigid and we, a priori, viewed the structural change in the cyclohexane ring as relatively minor. Because 1 and 2 are semirigid, it was hoped that they would provide information about the conformation of PCP at its receptor site. Previous work in the area of conformationally restricted PCP analogues has resulted in compounds in which the cyclohexane ring is restricted by virtue of it being part of a rigid molecule such as adamantane.[5] The adamantyl analogue of PCP did not bind to PCP receptors. However, it was found to be the most potent muscarinic antagonist yet obtained from PCP analogues.

We report here the synthesis of both *cis*- and *trans*-3-phenyl-3-(*N*-piperidinyl)bicyclo[3.1.0]hexane (1 and 2), both of which represent semirigid analogues of PCP. In

Scheme I[a]



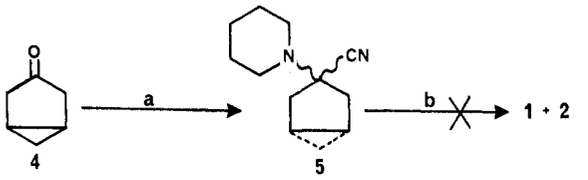
[a] (a) H_2CrO_4–Et_2O; (b) PhLi–Et_2O, −70 °C; (c) NaN_3–CF_3CO_2H–$CHCl_3$; (d) LAH–Et_2O, reflux; (e) 1,5-dibromopentane, 1.0 equiv; K_2CO_3; DMF, 60 °C.



† Naval Research Laboratory.
‡ Searle Research and Development, G. D. Searle and Co.

(1) *PCP (Phencyclidine): Historical and Current Perspectives*; Domino, E. F., Ed.; NPP Books: Ann Arbor, MI, 1981. *Phencyclidine and Related Arylcyclohexylamines*; Kamenka, J. M., Domino, E. F., Geneste, P., Domino, A. F., Eds.; NPP Books: Ann Arbor, MI, 1983.

Scheme II[a]



[a] (a) Piperidine, 1.0 equiv of aqueous HCl to pH 3; 1.05 equiv of NaCN; (b) PhLi–Et_2O or PhMgBr–Et_2O.

the naming of **1** and **2** and related compounds, "*cis*" refers to a cis configuration between the phenyl moiety and cyclopropyl methylene, and "*trans*" refers to a trans configuration between these two groups. Compounds **1** and **2** were evaluated for their affinity to PCP receptors and σ sites by displacement assays. PCP-like behavioral effects of **1** and **2** were also determined.

Chemistry

The synthetic route (Scheme I) to cis and trans **1** and **2** began with the known *cis*-bicyclo[3.1.0]hexan-3-ol (**3**), which was readily prepared from cyclopentadiene.[6] Brown oxidation[7] of **3** at room temperature over 3 h in an ether–aqueous dichromate two-phase system afforded bicyclo[3.1.0]hexan-3-one (**4**) in 98% yield. In a previously reported synthesis,[6] **4** was prepared in only 51% yield through CrO_3–pyridine oxidation of **3**.

Initially, the Bruylants reaction[8] was attempted as a potential route to **1** and **2** starting with **4** (Scheme II). Reaction between **4** and equimolar amounts each of piperidine and sodium cyanide in sufficient aqueous HCl to bring the pH to the range 3–4 furnished nitrile **5** of undetermined configuration, in 90% yield. Compound **5** gave a complex mixture on treatment with phenylmagnesium bromide. With phenyllithium, **5** reacted cleanly by addition of a phenyl moiety across the nitrile group to give, after hydrolytic workup, the corresponding ketone instead of the desired substitution; it therefore appeared that **5** was not a useful intermediate in the synthesis of **1** and **2**.

Solvolysis of alcohol **6** with hydrazoic acid offered a potential route to both cis and trans isomers **1** and **2**. Preferential attack of a bulky reagent on an intermediate with a hindered carbonyl moiety might be expected to lead to predominantly trans product. Reaction between **4** and phenylmagnesium bromide in refluxing ether, followed by NH_4Cl quench, gave *trans*-3-phenylbicyclo[3.1.0]hexan-3-ol (**6**) in 42% yield as a crystalline solid. Similarly, reaction between **4** and phenyllithium in ether at −70 °C, followed by aqueous quench, also gave **6** in 42% yield. No trace of the cis phenyl isomer could be found in either case. The configuration of **6** was tentatively assigned as trans on the basis of attack of the bulky phenylmagnesium bromide or phenyllithium on the least hindered face of the carbonyl group of **4**. Solvolysis of **6** was very slow when the conditions involved treatment of a chloroform solution with 2 molar equiv of trichloroacetic acid and 2 molar equiv of sodium azide at room temperature.[9] However, use of trifluoroacetic acid in this reaction instead of trichloroacetic acid, resulted in complete solvolysis after only 4 h at room temperature. The resulting crude mixture of azides (**7**) was not purified further but was used directly for the next reaction step.

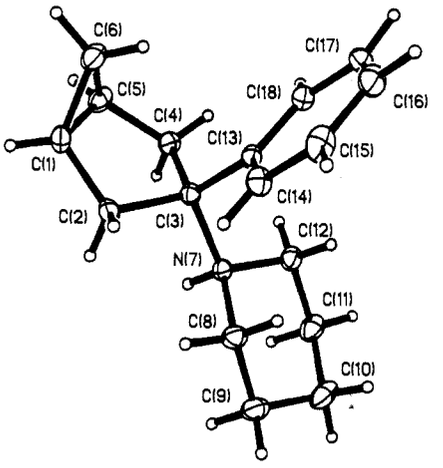


Figure 1. Thermal ellipsoid plot of the 2,4-dinitrobenzenesulfonate salt of *cis*-3-phenyl-3-(*N*-piperidinyl)bicyclo[3.1.0]hexane (**1**) with thermal ellipsoids drawn at 20% probability level. The 2,4-dinitrobenzenesulfonate anion and hydrogen bond are omitted for clarity.

Reduction of **7** by refluxing with excess LAH in ether, quenching with excess 20% aqueous HCl, and isolating the basic fraction gave a 3.5:1 ratio (as determined by capillary GC analysis at 110 °C) of *cis*-3-phenyl-3-aminobicyclo[3.1.0]hexane (**8**) to *trans*-3-phenyl-3-aminobicyclo[3.1.0]hexane (**9**). Attempted fractional crystallization of a mixture of either HCl or fumarate salts of **8** and **9** failed to afford any separation and thus chromatographic separation was used. The reaction gave major isomer **8** in 35% and minor isomer **9** in 12% overall yield from **6**, after separation by column chromatography on silica gel. The oily bases **8** and **9** both formed crystalline HCl salts. Monoalkylation of **8** in DMF solution by heating with an equimolar amount of 1,5-dibromopentane at 60 °C for 24 h, followed by dialkylation (ring closure) which occurred on addition of 1.1 molar equiv of K_2CO_3 and further heating, gave *cis*-3-phenyl-3-(*N*-piperidinyl)bicyclo[3.1.0]hexane (**1**) in 86% yield.[9] Similarly, reaction of **9** with 1,5-dibromopentane in DMF gave the trans isomer **2** in 77% yield. Use of refluxing acetone[9] in these alkylations instead of DMF resulted in substantially lower (40–60%) yields. Both compounds **1** and **2** formed crystalline, water-soluble HCl salts. From a mixture of **1** and **2**, the former could be efficiently separated through one crystallization of the 2,4-dinitrobenzenesulfonate salts to yield the corresponding salt of **1** as colorless prisms.

The configuration of the major product of the reaction sequence was initially assigned as shown (**1**) on the basis of steric considerations in the initial solvolysis reaction with hydrazoic acid.[9] The reaction is envisioned to proceed through the intermediacy of a benzylic carbonium ion[10] generated by the action of trifluoracetic acid on alcohol

(2) Kalir, A. *PCP (Phencyclidine): Historical and Current Perspectives*; Domino, E. F., Ed.; NPP books: Ann Arbor, MI 1981; pp 31–46. McQuinn, R. L.; Cone, E. J.; Shannon, H. E.; Su, T.-P. *J. Med. Chem.* **1981**, *24*, 1429.
(3) Vincent, J. P.; Kartalovsky, B.; Geneste, P.; Kamenka, J. M.; Lazdunski, M. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 4678. Geneste, P.; Kamenka, J. M.; Ung, S. N.; Herrmann, P.; Goudal, R.; Trouiller, G. *Eur. J. Med. Chem.* **1979**, *14*, 301.
(4) Gabrielevitz, A.; Kloog, Y.; Kalir, A.; Balderman, D.; Sokolovsky, M. *Life Sci.* **1980**, *26*, 89.
(5) Kanne, D. B.; Ashworth, D. J.; Cheng, M. T.; Mutter, L. C. *J. Am. Chem. Soc.* **1986**, *108*, 7864.
(6) Winstein, S.; Sonnenberg, J. *J. Am. Chem. Soc.* **1961**, *83*, 3235.
(7) Brown, H. C.; Garg, C. P.; Liu, K. T. *J. Org. Chem.* **1971**, *36*, 387.
(8) Kalir, A.; Edery, H.; Pelah, Z.; Balderman, D.; Porath, G. *J. Med. Chem.* **1969**, *12*, 473.
(9) Geneste, P.; Herrmann, P.; Kamenka, J. M.; Pons, A. *Bull. Soc. Chim. Fr.* **1975**, 7–8, 1619.
(10) Corey, E. J.; Dawson, L. *J. Am. Chem. Soc.* **1963**, *85*, 1782.

Table I. Evaluation of Binding of Compounds 1 and 2 to PCP Receptors and σ Sites By Displacement of [^{3}H]TCP and [^{3}H]-(+)-SKF 10,047

drug	K_i (apparent),[a] nM	
	[^{3}H]TCP	[^{3}H]-(+)-SKF 10,047
PCP[b]	91 ± 5	530 ± 100
1	680 ± 70	2600 ± 400
2	630 ± 50	3200 ± 570

[a] Mean ±SEM of at least three experiments. [b] Phencyclidine.

Table II. Behavioral Assays of Compounds 1 and 2 after Intracerebroventricular Introduction in the Rat

drug	ED_{50},[a] nmol/rat	
	stereotyped behavior	ataxia
PCP[b]	150 (120–180)	210 (170–180)
1	310 (254–370)	300 (260–360)
2	400 (350–460)	450 (380–520)

[a] Parenthesized values represent 95% confidence intervals. [b] Phencyclidine.

(6). Attack of the azide anion on the less hindered face of this carbocation would be expected to lead to 1 as the major product, regardless of the initial configuration of alcohol (6). The initially assigned cis configuration of 1 was confirmed by single-crystal X-ray analysis (Figure 1) of its 2,4-dinitrobenzenesulfonate salt.

Results and Discussion

The configuration of 1 is shown in Figure 1. The cyclopropane ring is fused in an orientation cis to the phenyl substituent. The cyclopentane ring is in an envelope conformation in the crystal, with C(3) bent out of the plane of the other four atoms. The phenyl ring is axially orientated with respect to this plane, and the chair-shaped piperidinium ring is in an equatorial position. Bond distances and angles for these two rings are normal and similar to those found in PCP[11] and PCP derivatives.[12,13] In the "zero-bridged" cyclohexane ring, the dihedral angle between the plane through the cyclopropane ring and that formed by the planar four atom group composed of the two bridgehead carbons and adjacent carbons is 114°. This is comparable to the value of 110° found for the equivalent angle in 2-methoxy-6-methyl(trichloromethyl)bicyclo-[3.1.0]hexan-3-one[14] (the maximum deviation from the least-squares plane for the four atoms is 0.02 Å). The cyclopropane ring has a near 3-fold symmetry with respect to the average C–C bond distances and C–C–C angles, 1.500 (7) Å and 60.0 (4)°, respectively. Hydrogen bonding occurs between the quadrivalent nitrogen and one of the sulfonate oxygens with bond parameters of N···O = 2.84 Å, O···H = 1.97 Å, and angle N–H···O = 167.5°.

Both isomers were about equipotent, but less potent than PCP in both the [^{3}H]TCP[15] and [^{3}H]-(+)-SKF 10,047 displacement assays (Table I). In the behavioral assays (Table II),[16,17] the peak effect of all three drugs occurred 5 min after intracerebroventricular administration. Like PCP, 1 and 2 produced exploratory behavior at low doses. At moderate doses, circling, head weaving, and back peddling behavior was evident and at very large doses, continuous circling, head weaving, or dyskinetic extension of head and limbs was evident. Both drugs also produced dose-dependent ataxia. Compounds 1 and 2 were both less potent than PCP in receptor binding, stereotyped behavior, and ataxia.

Similarity between bond lengths and angles in 1 and 2 and those found in PCP are good indications that they are accurate representations of two possible conformations of PCP (pseudoboat with the phenyl ring axial as in 1 and equatorial as in 2). These data, coupled with the results from the binding experiments and the behavioral effects of 1 and 2 in the rat, suggest that the cyclopropyl moiety in the cyclohexane ring, which projects above or below the plane of the cyclohexane ring, may sterically interfere with receptor interaction. Apparently, even minor changes in the cyclohexane ring of PCP are sufficient to lessen PCP-like activity. The results are consistent with former work.[2]

Experimental Section

Chemistry. General Comments. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were obtained in $CDCl_3$ with a Varian XL-300 MHz spectrometer and chemical shift values (δ) are given in ppm. Electron-ionization mass spectra (EIMS) were obtained with a Hitachi Perkin-Elmer RMU-6E spectrometer (70 eV). IR spectra were obtained on a Beckmann 4230 instrument. Chemical-ionization mass spectra (CIMS) were obtained with a Finnigan 1015D spectrometer with a Model 6000 data collection system. GC was performed on a Hewlett-Packard 5880A instrument having a 12-m methyl silicone fused silica capillary column (Hewlett-Packard SP-2100), with a flame-ionization detector. Column chromatography was performed with Merck EM reagent Kieselgel 60 (0.040–0.063 mm, 230–400 mesh). Elemental analyses were performed by Atlanta Microlabs, Atlanta, GA, and results were within ±0.4% of the calculated values for all new compounds.

Bicyclo[3.1.0]hexan-3-one (4). Ether (10 mL) and *cis*-bicyclo[3.1.0]hexan-3-ol[6] (3) (2.00 g, 20.4 mmol) were placed in a three-necked flask fitted with a stirrer, addition funnel, condenser, and thermometer. Chromic acid solution (prepared according to procedure A in ref 7) (10.2 mL, 6.8 mmol) was added to the stirred solution over a period of 15 min while the temperature was maintained between 25 and 30 °C. After the mixture was stirred for 2.5 h at room temperature, capillary GC (oven temperature 40 °C) of the reaction mixture indicated that less than 3% of the starting material remained. The reaction was found to be complete after 3 h. The upper ethereal layer was separated and the aqueous phase extracted with ether (2 × 10 mL). The combined ether extracts were washed once with saturated $NaHCO_3$ (30 mL) and water (30 mL) and then dried (Na_2SO_4). Ether was distilled off through a short Vigreux column and the residue was distilled (50 °C/17 mm; lit.[6] bp 51–52 °C/18 mm) to yield 1.92 g (98%) of 4 as a pleasant-smelling liquid, 97% pure by GC (40 °C): NMR δ 0.9–0.93 (m, 1 H) 1.53–1.56 (m, 1 H), 2.13–2.2 (d, 2 H), 2.62–2.64 (sym dm, 2 H).

3-Phenyl-3-cyanobicyclo[3.1.0]hexane (5). A mixture of piperidine (0.88 g, 10.42 mmol), ice water (2 mL), and 37% HCl (0.87 mL) was adjusted to pH 3 by dropwise addition of either a 10% HCl or a 10% NaOH solution. To this mixture was added 4 (1 g, 10.42 mmol), followed by sodium cyanide (0.524 g, 10.90 mmol), and the reaction mixture was stirred at room temperature for 48 h. Two phases were present after this time. Capillary GC (100 °C) of the upper organic phase indicated complete consumption of 4 and formation of a single product (ca. 99% pure). The reaction was diluted with water (50 mL) and extracted with

(11) Geneste, P.; Kamenka, J. M.; Roques, R.; Declercq, J. P.; Germain, G. *Tetrahedron Lett.* **1981**, *22*, 949.
(12) Briard, P.; Roques, R.; Kamenka, J. M.; Geneste, P.; Declercq, J. P.; Germain, G. *Cryst. Struct. Commun.* **1982**, *11*, 231.
(13) Argos, P.; Barr, R. E.; Weber, A. H. *Acta Cryst., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1970**, *B26*, 53.
(14) Brisimitzakis, A. C.; Schuster, J. M.; van der Veen, J. M. *Can. J. Chem.* **1985**, *63*, 685.
(15) Contreras, P. C.; Rice, K. C.; Jacobson, A. E.; O'Donohue, T. L. *Eur. J. Pharmacol.* **1986**, *121*, 9.
(16) Sturgeon, R. D.; Fessler, R. G.; Meltzer, H. Y. *Eur. J. Pharmacol.* **1979**, *59*, 169.
(17) Finney, D. J. *Statistical Methods in Biological Assays*, 2nd ed.; Hafner Publishing Co., New York, 1964.

CH_2Cl_2 (4 × 50 mL). The organic extract was washed once with water (50 mL) and dried (Na_2SO_4), and the solvent was evaporated to give a quantitative yield of crude **5** as a colorless oil. Crystallization from aqueous methanol afforded colorless plates: mp 56 °C; NMR δ 0.50–0.53 (m, 1 H), 0.76–0.78 (m, 1 H), 1.41–1.49 (m, 4 H), 1.49–1.62 (m, 4 H), 2.07–2.13 (dd, 2 H, J = 13, 3.5 Hz), 2.35–2.39 (d, 2 H, J = 13 Hz), 2.45 (m, 4 H); IR (KBr) 2210 cm^{-1} (very weak CN str). Anal. ($C_{12}H_{18}N_2$) C, H, N.

***trans*-3-Phenylbicyclo[3.1.0]hexan-3-ol (6).** A 2 M solution of phenyllithium in ether–cyclohexane (3:7) (7.2 mL, 1.5 equiv) was added over 5 min to a stirred solution of **4** (0.92 g, 9.58 mmol) in dry ether (10 mL) at −70 °C, under nitrogen. The solution was stirred for 10 min at −70 °C and then 20 min at 20 °C and poured into 100 mL of ice water. The ether layer was diluted to 100 mL and separated, and the aqueous layer was extracted with ether (2 × 100 mL). The combined ethereal solution was dried over Na_2SO_4 and solvent was evaporated. Column chromatography of the residue (ethyl acetate–hexane, 1:9) afforded 0.71 g (42%) of crystalline **6**: mp 68 °C; NMR δ 0.53–0.55 (m, 1 H), 1.03–1.04 (m, 1 H), 1.43–1.52 (m, 2 H), 2.06–2.11 (d, 2 H, J = 14 Hz), 2.41–2.47 (dd, 2 H, J = 14 Hz, 4 Hz), 7.23–7.47 (m, 5 H, ArH). Anal. ($C_{12}H_{14}O$) C, H.

***cis*- and *trans*-3-Phenyl-3-azidobicyclo[3.1.0]hexane (7).** A solution of **6** (0.9 g, 5.17 mmol) in pentene-stabilized $CHCl_3$ (6 mL) was added slowly to a stirred suspension (at 0 °C) of sodium azide (0.65 g, 10 mmol) and trifluoroacetic acid (1.15 mL, 14.9 mmol) in pentene-stabilized $CHCl_3$ (4 mL). Reaction was complete after stirring at room temperature for 4 h. After dilution to 100 mL with $CHCl_3$, the reaction mixture was washed with saturated $NaHCO_3$ (3 × 100 mL) and dried over Na_2SO_4. Evaporation of the solvent yielded 1.0 g of an oily mixture consisting of azides (**7**) together with olefinic byproducts: IR (film) 2100 cm^{-1} (very strong N_3 stretch). No attempt was made to further purify this mixture of azides.

***cis*- and *trans*-3-Phenyl-3-aminobicyclo[3.1.0]hexane (8 and 9).** The solution of azides **7** (3.6 g) in dry ether (10 mL) was added dropwise to a stirred a solution of LAH (1.8 g) in ether (25 mL) at 0 °C. After refluxing for 3 h under a nitrogen atmosphere, the solution was carefully poured into 100 mL of an ice-cold solution of 20% HCl. The mixture was washed with ether (2 × 200 mL) and the ether extract was discarded. The aqueous layer was made alkaline with concentrated aqueous ammonia and extracted with ether (4 × 100 mL). Drying (Na_2SO_4) and evaporation of solvent yielded 1.76 g (51% from **6**) of a 3.5:1 mixture (capillary GC at 110 °C) of isomeric amines **8** and **9**. Column chromatography of this mixture (concentrated aqueous NH_3–MeOH–$CHCl_3$, 0.2:1.8:98) yielded in the earlier fractions the minor isomer **9** (0.43 g, 12% overall from **6**): NMR (base) δ 0.53–0.60 (m, 1 H), 1.13–1.17 (m, 1 H), 1.41–1.46 (m, 4 H), 1.90–1.94 (d, 2 H, J = 14 Hz), 2.43–2.49 (dd, 2 H, J = 14 Hz, 3.8 Hz), 7.15–7.40 (m, 5 H, ArH); mp **9**·HCl 214–215 °C. Anal. ($C_{12}H_{16}ClN$) C, H, N. Later column fractions yielded the major isomer **8** (1.22 g, 35% overall from **6**): NMR (base) δ 0.25–0.29 (m, 1 H), 0.96–1.02 (m, 1 H, 1.55–1.60 (m, 2 H), 1.60–1.67 (br s, 2 H, NH_2), 1.82–1.87 (d, 2 H, J = 14 Hz), 2.16–2.23 (dd, 2 H, J = 14 Hz, 4 Hz), 7.17–7.36 (m, 5 H, ArH); mp **8**·HCl 205–207 °C. Anal. ($C_{12}H_{16}ClN$) C, H, N.

***cis*-3-Phenyl-3-(*N*-piperidinyl)bicyclo[3.1.0]hexane (1).** A solution of **8** (0.85 g, 4.91 mmol), and 1,5-dibromopentane (1.13 g, 1 equiv) in dry DMF (21 mL) as heated under a nitrogen atmosphere at 60 °C for 24 h. Anhydrous K_2CO_3 (748 mg, 1.1 equiv) was added, and the reaction mixture heated at 60 °C for a further 48 h. The reaction mixture was poured into 50 mL of 10% aqueous HCl and washed with ether (4 × 30 mL). After the ether phase was discarded the aqueous phase was made alkaline with excess concentrated aqueous NH_3, and reextracted with ether (4 × 100 mL). Drying (Na_2SO_4) and evaporation of the solvent afforded 1.01 g (86%) of **1** as an oil: NMR (base) δ −0.25 to −0.23 (m, 1 H), 0.10–0.15 (m, 1 H), 1.24–1.33 (m, 4 H), 1.43–1.53 (m, 4 H), 2.16–2.29 (m, 4 H), 2.62–2.66 (dd, 2 H, J = 14 Hz, 3 Hz), 2.88–2.96 (d, 2 H, J = 14 Hz), 7.25–7.34 (m, 5 H, ArH). Treatment of an ethyl acetate solution of **1** with a 2-propanol solution of HCl afforded **1**·HCl: mp 204–205 °C. Anal. ($C_{17}H_{24}ClN$) C, H, N. Addition of an equimolar amount of 2,4-dinitrobenzenesulfonic acid dihydrate to 0.18 g of a 3.7:1 mixture of **1** and **2** (obtained by execution of the reaction sequence without prior separation of intermediates **8** and **9**), followed by one recrystallization of the resultant salt from 2-propanol, yielded the pure 2,4-dinitrobenzenesulfonate salt of **1** (0.18 g, 64%) (as seen by GC, 150 °C): mp 172–173 °C. Anal. ($C_{23}H_{27}N_3O_7S$) C, H, N.

***trans*-3-Phenyl-3-(*N*-piperidinyl)bicyclo[3.1.0]hexane (2).** The procedure used for **1** was repeated, starting with a mixture of **9** (0.34 g, 1.96 mmol) and 1,5-dibromopentane (452 mg, 1.0 equiv) in dry DMF (9 mL). After 24 h at 60 °C, anhydrous K_2CO_3 (300 mg, 1.1 equiv) was added, and heating at 60 °C was continued for a further 48 h. Workup as described for **1** afforded 0.36 g (77%) of **2** as an oil: NMR (base) δ 0.64–0.65 (m, 1 H), 0.82–0.84 (m, 1 H), 1.90–1.96 (dd, 2 H, J = 14 Hz, 6 Hz), 2.17–2.33 (m, 4 H), 7.18–7.37 (m, 5 H, ArH). Treatment of an ethyl acetate solution of **2** with HCl in 2-propanol afforded **2**·HCl: mp 215–215.5 °C. Anal. ($C_{17}H_{24}ClN$) C, H, N.

X-ray Crystallography. X-ray crystallographic data were obtained on the 2,4-dinitrobenzenesulfonate salt of *cis*-3-phenyl-3-(*N*-piperidinyl)bicyclo[3.1.0]hexane, $C_{17}H_{24}N^+ \cdot C_6H_3N_2O_7S^-$, FW = 489.55. Crystals of the 2,4-dinitrobenzenesulfonate salt of **1** were grown in 2-propanol by allowing the solvent to evaporate slowly at room temperature. A clear, colorless crystal, 0.12 mm × 0.22 × 0.34 mm, was used for the structural determination. A least-squares refinement using 25 centered reflections within $50 < 2\theta < 65°$ gave the triclinic $P\bar{1}$ cell a = 8.443 (1) Å, b = 10.646 (1) Å, c = 13.195 (2) Å, α = 82.01 (1)°, β = 75.87 (1)°, γ = 89.13 (1)°, V = 1138.7 (3) Å^3, Z = 2, and D_{calcd} = 1.428 gm/cm^3. A computer-controlled diffractometer (Nicolet R3mμ, with Cu Kα radiation, wavelength = 1.541 78 Å), with an incident beam graphite monochromator was used for data collection. A total of 3413 reflections were measured in the $\theta/2\theta$ mode to $2\theta_{max}$ = 112°. Corrections were applied for Lorentz and polarization effects, but not for absorption. The structure was solved by direct methods with the aid of the program SHELXS[18] and refined by using full-matrix least-squares program SHELXLS.[18] The 415 parameters refined include the coordinates and anisotropic thermal parameters for all nonhydrogen atoms. Hydrogen atoms were refined isotropically. The final R factors for the 3039 observed reflections ($|F_o| > 3\sigma |F_o|$) were R = 0.047 and R_w = 0.053. Tables of coordinates and bond distances and angles have been deposited with the Crystallographic Data Center, Cambridge CB2 1EW, England.

Inhibition Assays. Membrane preparations[15] were used to examine the interaction of **1** and **2** with PCP receptors and σ binding sites. Test tubes prepared in triplicate contained 0.1 mL of tissue suspension, 2 nM of [^{3}H]TCP (New England Nuclear) or 6 nM of [^{3}H]-(+)-SKF 10,047, and varying amounts of displacing ligand and sufficient buffer (5 mM Tris·HCl, pH 7.4) for a final volume of 0.5 mL. The test tubes were incubated for 1 h at room temperature and then the contents of the test tubes were filtered through Schleicher Schuell, No. 32 filters, which were presoaked in 0.5% polyethylenimine for at least 1 h. The test tubes were rinsed twice and the filters once with 4 mL of Tris buffer. The radioactivity on the filters was counted by liquid scintillation counting. The total amount bound minus the amount bound in the presence of 10 μM of TCP or 30 μM of (+)-SKF 10,047 was used to define specific binding to PCP receptors or σ binding sites, respectively.

Behavioral Assays. For the behavioral experiments, Sprague–Dawley rats weighing approximately 200 g were placed individually into rat cages and allowed at least 1 h to adapt to their surroundings. Each animal was used only once. Drugs were administered intracerebroventricularly and the behaviors were rated by use of the PCP rating scale of Sturgeon et al.[16] Ratings were taken immediately after the injection, 2.5, 5, and every 5 min thereafter, until behaviors returned to control values. With use of the ratings determined at the time of peak effect, dose–response curves were determined. At least 18 rats were used to determine each dose–response curve and ED_{50} value (Table II). The ED_{50} values and confidence limits were calculated by using a computerized Finney assay.[17]

Acknowledgment. The financial support of the Fo-

(18) Sheldrick, G. M., SHELXTL80. Minicomputer Programs for Structure Determination, University of Gottingen, Sweden, 1980.

garty Foundation (B.D.) is gratefully acknowledged. We thank Noel Whittaker and Wesley White of the Laboratory of Analytical Chemistry (LAC), NIDDK, for mass spectral analysis.

Registry No. 1, 114200-20-3; 1·HCl, 114200-30-5; 1 (2,4-dinitrobenzenesulfonate salt), 114200-29-2; 2, 114200-21-4; 3, 694-43-9; 4, 1755-04-0; 5, 114200-22-5; 6, 71194-15-5; 7 (isomer 1), 114200-23-6; 7 (isomer 2), 114200-26-9; 8, 114200-24-7; 8·HCl, 114200-28-1; 9, 114200-25-8; 9·HCl, 114200-27-0; PCP, 77-10-1; piperidine, 110-89-4; phenyllithium, 591-51-5; 1,5-dibromopentane, 111-24-0.

Nucleosides of 1,4-Thiazin-3-one and Derivatives as Tetrahedral Intermediate Analogues of Enzymes in Pyrimidine Nucleoside Metabolism

Ted E. Marcus,[†,‡] Afaf Gundy,[‡] Corey H. Levenson,[†,§] and Rich B. Meyer, Jr.*[,†,‡,⊥]

College of Pharmacy, Washington State University, Pullman, Washington 99164, Nucleic Acid Research Institute, 3300 Hyland Ave., Costa Mesa, California 92626, and MicroProbe Corporation, 1725 220th Street SE, Bothell, Washington 98021.
Received July 6, 1987

Reaction of the trimethylsilylated derivative of 1,4-thiazin-3-one with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose in the presence of $SnCl_4$ gave, after deblocking, 4-β-D-ribofuranosyl-1,4-thiazin-3-one (**8**). Treatment of 1,4-thiazin-3-one with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose in the presence of sodium hydride provided, after deblocking, the corresponding 2-deoxy-β-D-ribofuranosyl derivatives (**19**). Oxidation of 4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,4-thiazin-3-one (**7**) with 1 equiv of *m*-chloroperbenzoic acid resulted in 4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,4-thiazine-2,3-dione (**9**) and 4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,4-thiazin-3-one 1-oxide (**10**). Evidence is presented that indicates that the oxidation of the thiazine at the 2-position is due to a Pummerer rearrangement. The new compounds failed to show significant activity against tumor cell lines in culture, L1210 cells in vivo, virus cytotoxicity in cell culture, or cytidine deaminase.

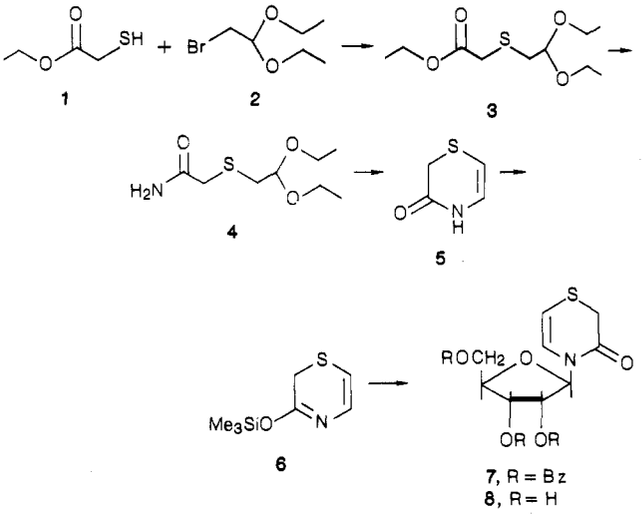
Stable analogues of tetrahedral intermediates formed in the course of enzymic reactions can be potent inhibitors of those enzymes. Classes of enzymes susceptible to inhibition by these analogues are those hydrolases and ligases that catalyze an $sp^2 \rightarrow sp^3 \rightarrow sp^2$ conversion. Among those enzymes in these classes that catalyze this type of transformation at the 4-position of the pyrimidine nucleosides and nucleotides are cytidine deaminase and CTP synthetase. Bartlett and co-workers[1] have shown that a tetrahedral intermediate analogue based on a 4-phosphapyrimidine nucleoside is a potent inhibitor of the former enzyme. The latter enzyme, thought to involve a phosphorylated tetrahedral intermediate in the transition state,[2] should be susceptible to inhibition by a suitable tetrahedral intermediate analogue, in analogy with the inhibition of the ligase glutamine synthetase by the tetrahedral sulfur compound methionine sulfoximine.[3]

As part of a program to prepare pyrimidine nucleoside derivatives with tetrahedral atoms in the 4-position, we have prepared several nucleosides of 1,4-thiazin-3-one, its oxides, and its 5-methyl and 5-carboxy derivatives. Such analogues may inhibit nucleoside-utilizing enzymes (such as cytidine deaminase) directly, or, after in vivo conversion to their triphosphates, inhibit CTP synthetase. Inhibition of this enzyme, known to occur in highly elevated levels in cancer cells,[4] could provide agents of interest in cancer chemotherapy. Additionally, the 5-carboxy congeners are analogues of orotidine.

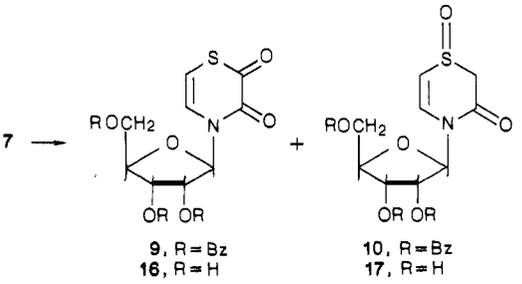
Chemistry

The nucleoside 4-β-D-ribofuranosyl-1,4-thiazin-3-one (**8**) was prepared in good yield in six steps as shown in Scheme I. Treatment of ethyl thioglycolate (**1**) with bromoacetaldehyde diethyl acetal (**2**) in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) gave ethyl 2-[(2,2-diethoxyethyl)thio]acetate (**3**). Ammonolysis of the ester **3** gave

Scheme I



Scheme II



* Address correspondence to this author at MicroProbe Corp.
† Washington State University.
‡ Nucleic Acid Research Institute.
§ Present address: Cetus Corp., Emeryville, CA.
⊥ MicroProbe Corporation.

(1) Ashley, G. W.; Bartlett, P. A. *Biochem. Biophys. Res. Commun.* **1982**, *108*, 1467.
(2) Levitzki, A.; Koshland, D. E., Jr. *Biochemistry* **1972**, *11*, 241.
(3) Ronzio, R. A.; Rowe, W. B.; Meister, A. *Biochemistry* **1969**, *8*, 1066.

0022-2623/88/1831-1575$01.50/0