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Synthesis and Antinociceptive Activity of 4-(Cyclopropylmethyl)-3,4,5,6-tetrahydro-2*H*-1,5-methano-1,4-benzodiazocin-9-amine and Congeners[1]

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The synthesis of 1,4-benzodiazocines has been extended to include the 9-amines. The title compound, **6**, was prepared from the known nor-base by N-acylation with cyclopropanecarboxylic acid anhydride, followed by nitration of the aromatic ring and sequential reduction of the resulting nitroamide. Standard in vivo bioassays for antinociceptive and opiate antagonist activities indicated that **6** is an analgesic agonist mixed narcotic antagonist whose oral to parenteral activity ratio approaches unity. Resolution of **6** established that the activity of the racemate resides in the dextrorotatory antipode. Several congeners were also prepared and tested. Their synthesis and structure–activity relationships are given in this paper.

Several years ago we began a program to synthesize and investigate the pharmacological properties of two series of benzodiazocines depicted by general formulas **1** and **2**.[2]

1a, R = R' = H
b. R = H; R' = CH₂-c-C₃H₅

2a, R = R' = H
b. R = H; R' = CH₂CH=CH₂
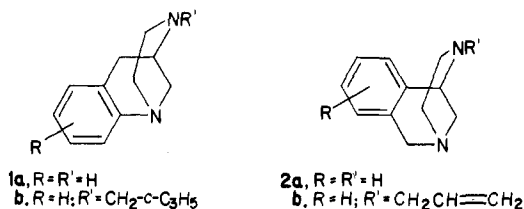
Since the benzodiazocines represented ring systems that had not been previously reported, our goal was to prepare several examples incorporating various R and R' moieties with the hope of uncovering desirable biological activity. During the course of this work, Kametani[3,4] and Mitsuhashi[5,6] independently described the synthesis of both series **1** and **2** by a progression of preparative steps very similar to the procedures we were pursuing. Later, Kametani[7] reviewed 12 types of benzodiazocine structures and stated that introduction of an additional nitrogen atom into the benzazocines, i.e., benzomorphan analgesics, appeared to have an unfavorable effect on analgesic activity. In our laboratory, preliminary test data from Harris[8] suggested that **2b** was an active opioid antagonist vs. phenazocine in the rat tail-flick test.[9] Later, we reported in a patent[2] that representatives of both series were useful as antagonists of strong antinociceptive agents such as morphine and meperidine. Consequently, we were encouraged to extend our work, particularly to undertake the preparation of phenolic benzodiazocines. Representatives of 1,5-methanobenzodiazocines (series **1**) were chosen to be studied first since this system more closely approximates the 2,6-methano-3-benzazocine ring system. Since nine steps were required to build up the desired tricyclic

structure, we decided to simplify the lengthy multistep synthesis by introducing the phenolic moiety by means of nitration/reduction to the primary aromatic amine followed by diazotization/hydrolysis. Thus, we investigated aromatic ring nitration of 4-(cyclopropylcarbonyl)-3,4,5,6-tetrahydro-2*H*-1,5-methano-1,4-benzodiazocine (**3a**). This process gave predominantly one regioisomer which upon sequential reduction provided 4-(cyclopropylmethyl)-3,4,5,6-tetrahydro-2*H*-1,5-methano-1,4-benzodiazocin-9-amine (**6**). This compound proved to have considerable activity as a mixed agonist–opioid antagonist. Finally, a total, unequivocal synthesis of the phenol 4-(cyclopropylmethyl)-3,4,5,6-tetrahydro-2*H*-1,5-methano-1,4-benzodiazocin-9-ol (**7**) was achieved,[10] providing proof that the location of the nitro group in the regioisomer obtained from direct nitration of **3a** was correctly assigned.

This synthesis also provided an abundant supply of the desired phenol **7** for biological evaluation in the various antinociceptive agonist and opioid antagonist screens. The results indicated that the phenol has opioid antagonist activity (Table I) approximately equivalent to nalorphine but with one-third the agonist activity of **6**. Our emphasis, therefore, shifted to a structure–activity study of several benzodiazocin-9-amines. Details of the synthesis and preliminary pharmacology of these compounds are presented in this paper.

Chemistry. We began by studying the effect of incorporating various substituents on the secondary nitrogen of the benzodiazocines to determine if an activity profile similar to the one observed by changing nitrogen substituents on the benzazocine analgesics would result. An abundant supply of nor-base **1a** was available to us from our earlier work.[2] The secondary nitrogen of **1a** was cyclopropanecarbonylated with either cyclopropanecarboxylic acid anhydride or chloride to give nearly quantitative yields of amide **3a** (Scheme I). Aromatic ring nitration of **3a** was performed in concentrated H_2SO_4 solution with KNO_3. Isolation of the nitro amide **4a** was accomplished in 90% yield consisting of predominantly one regioisomer. NMR protonation studies indicated that the procedure had placed the nitro group in the aromatic ring at the desired 9-position of the tricyclic benzodiazocine. Spectra were obtained for both the free base and the HCl salt of **4a**. Protonation of the basic bridgehead nitrogen causes the aromatic H's to shift downfield. The results indicated that the proton ortho to the bridgehead nitrogen (C-10) was shifted most (δ 0.57), the proton para to the bridgehead nitrogen (C-8) was shifted

(1) Presented at the 182nd National Meeting of the American Chemical Society, New York, Aug 1981, by Michael A. Eissenstat.
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(10) Details of the synthesis of phenol **7** will be submitted for publication in *J. Heterocycl. Chem.*
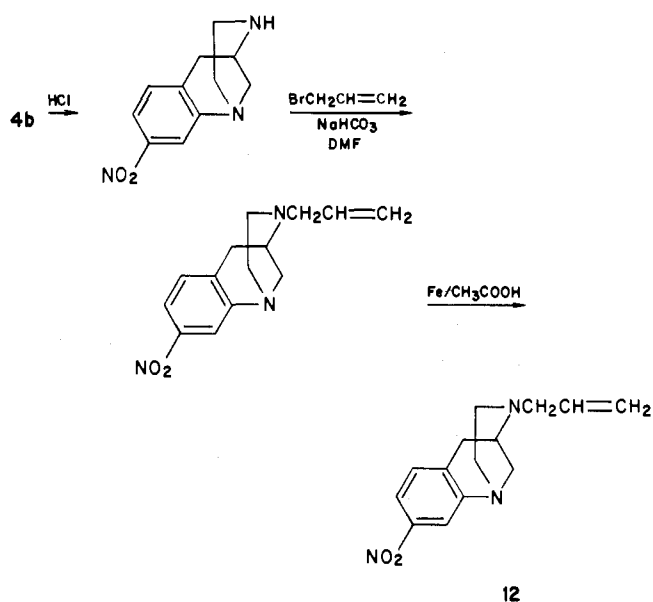
Scheme I
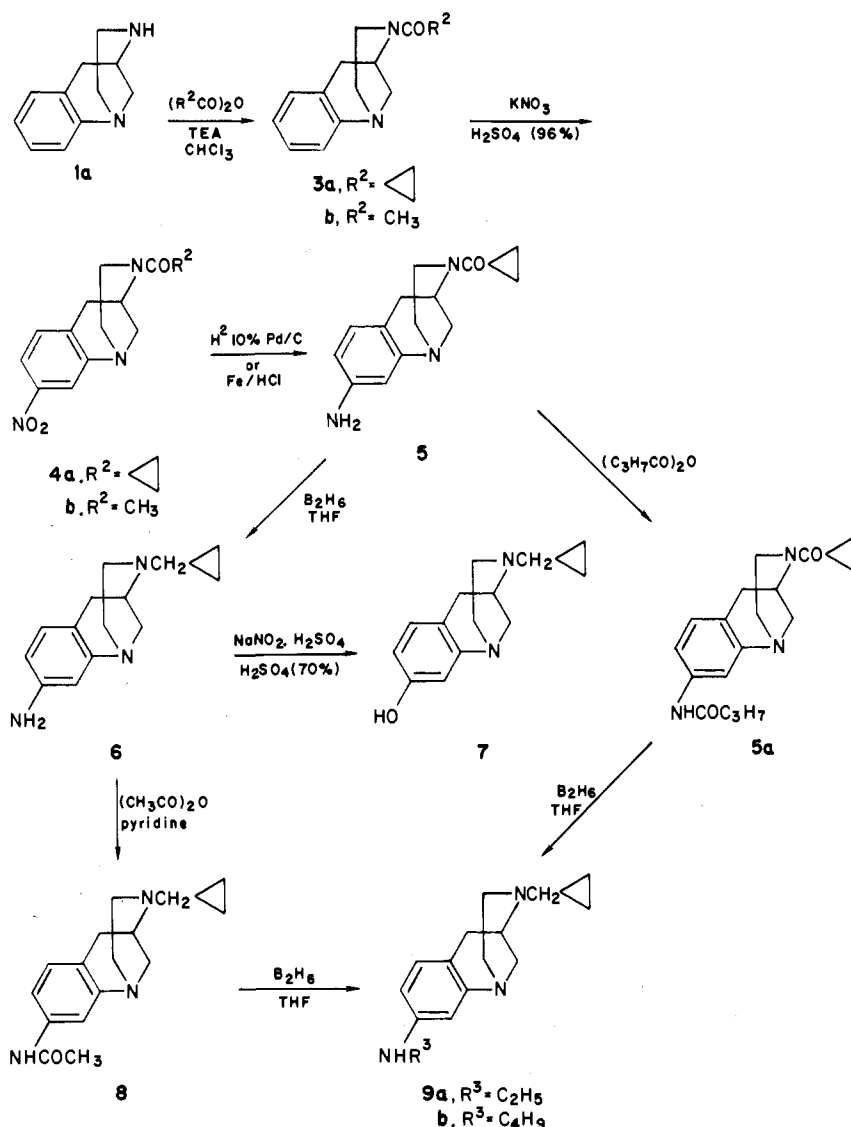
less (δ 0.45), and the proton meta to the bridgehead nitrogen (C-7) was shifted least (δ 0.32). Therefore, the proton (C-10) that showed the largest shift downfield has meta coupling only, indicating nitration had occurred in the position meta (C-9) to the bridgehead nitrogen, i.e., in the 9-position of 4-(cyclopropylcarbonyl)-3,4,5,6-tetrahydro-2H-1,5-methano-1,4-benzodiazocine (3a). Subsequent reduction of the nitro group either by catalytic hydrogenation (10% Pd/C) or by reduction with Fe/HCl gave 60–70% yields of amino amide 5. Diborane or LiAlH₄ reduction of the amide carbonyl provided 6 in greater than 90% yield. Phenol 7 obtained by sulfuric acid hydrolysis of the diazonium sulfate derivative of 6 furnished material identical in all respects (mmp, NMR, IR, MS, and TLC) to phenol provided by an alternative total synthesis.[10]
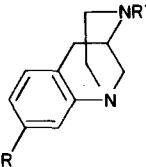
In Scheme I, compounds 5a and 8 were made by straightforward N-acylation of the primary amino group of 5 or 6 with acid anhydrides, followed by reduction of the amides (5a and 8) with diborane to 9a and 9b. Finally, in Scheme II, 4b was deacetylated by hydrolysis in HCl to nor-base 10, which provided an alternative intermediate for direct alkylation of the secondary nitrogen with allyl bromide to give 11. Hydrogen from iron and acetic acid was used to reduce the nitro group of 11 to the 9-amine 12.

Both optical antipodes of racemic nor-base 1a were provided by crystallization of the mandelic and tartaric

Scheme II

acid salts followed by liberation of the optically active free bases. Identical stepwise procedures were then utilized (1a–6, Scheme I) to give the enantiomorphs (–)-6 and

Table I. Physical Properties and Biological Activities of 4-Substituted-3,4,5,6-tetrahydro-2H-1,5-methano-1,4-benzodiazocin-9-amines

no.[a]	R	R[1]	formula[b]	route	ED$_{50}$[c]	AD$_{50}$[d]	ED$_{50}$[e]	ED$_{50}$[f]
1b	H	CH$_2$◁	C$_{15}$H$_{20}$N$_2$·2C$_2$H$_5$SO$_3$H	sc	12 (7.4–20)	34 (20–58)	I[g] at 120	21 (8.8–46)
				po	32 (21–47)			
6	NH$_2$	CH$_2$◁	C$_{15}$H$_{21}$N$_3$	sc	3.2 (2.0–4.8)	1.6 (1.0–2.6)	1 at 120	2.6 (1.5–4.1)
				po	5.9 (3.9–8.7)	3.5 (2.3–5.2)	1 at 200	4.2 (2.1–6.2)
(–)-6	NH$_2$	CH$_2$◁	C$_{15}$H$_{21}$N$_3$	sc	40[h]	25% at 80		
(+)-6	NH$_2$	CH$_2$◁	C$_{15}$H$_{21}$N$_3$	sc	1.00 (0.62–2.0)	0.31 (0.15–0.60)		
7	OH	CH$_2$◁	C$_{15}$H$_{20}$N$_2$O	sc	10 (4.9–19)	0.02 (0.01–0.038)	1 at 120	37 (6.4–9.6)
				po		3.2 (2.1–4.8)		
9a	NHC$_2$H$_5$	CH$_2$◁	C$_{17}$H$_{25}$N$_3$·2HCl	sc	3.8 (2.8–5.0)	4.4 (2.9–6.6)	17% at 60, lethal at 120	3.4 (1.6–6.1)
9b	NH(CH$_2$)$_3$CH$_3$	CH$_2$◁	C$_{19}$H$_{29}$N$_3$	sc	1.6 (1.0–2.8)	1 at 10	1 at 10, convulsions at 20	2.9 (1.0–6.0)
12	NH$_2$	CH$_2$CH=CH$_2$	C$_{14}$H$_{19}$N$_3$·2C$_2$H$_5$SO$_3$H·$^1/_2$H$_2$O	sc	30[i]	1.6 (1.0–2.6)		
morphine sulfate				sc	0.21 (0.12–0.35)			
nalorphine hydrochloride				sc	2.8 (1.5–7.7)	0.032 (0.018–0.057)		0.24 (0.039–0.51)

[a] Compounds are racemic except (+)- and (–)-6. [b] All compounds were analyzed for C, H, and N. Elemental analyses were within ±4% of the theoretical values. [c] Acetylcholine writhing test (mouse), milligram of base/kilogram sc (95% confidence limits). [d] Phenazocine antagonism (rat) milligram of base/kilogram sc (95% confidence limits). [e] Tail-flick test (rat), milligram of base/kilogram sc ± SE. [f] Intracarotid bradykinin test (rat), milligrams/kilogram sc (95% confidence limits). [g] Inactive. [h] Maximum effect obtained = 67% at 75 mg/kg. [i] Estimated from only three dosage levels.

Table II. Agonist and Narcotic Antagonist Activities of 6, (–)-6, and (+)-6

test[a]	route	6	(–)-6	(+)-6
acetylcholine,	sc	3.2 (2.0–4.8)	40[b]	1.0 (0.62–
ED$_{50}$	po	5.9 (3.9–8.7)		2.0)
rat bradykinin,	sc	2.6 (1.5–4.1)		
ED$_{50}$	po	4.2 (2.1–6.2)		
rat tail-flick,	sc	1[c] at 120		
ED$_{50}$	po	1 at 200		
phenylquinone,	sc	1.8 (1.1–2.9)		
ED$_{50}$	po	5.7 (4.5–7.1)		
AD$_{50}$ vs.	sc	1.6 (1.0–2.6)	25%[d]	0.31 (0.15–
phenazocine	po	3.5 (2.3–5.2)	at 80	0.60)
AD$_{50}$ vs.	sc	8.6 (5.7–13)		
morphine	po	13 (8.6–19)		
AD$_{50}$ vs.	sc	3.3 (2.1–5.3)		
meperidine	po	4.8 (3.5–6.5)		

[a] Test results reported in milligrams/kilogram, calculated as the free base (95% confidence limits). [b] Maximum effect obtained = 67% at 75 mg/kg. [c] Inactive. [d] 25% antagonism.

(+)-6. Excellent agreement was provided by the data obtained from ^{13}C NMR spectra of racemic **1a** and the isomers generated with europium(III) shift reagent combined with the two resolved norbases (−)-**1a** and (+)-**1a** and data obtained from GC analysis of the same isomers, (−)-**1a** and (+)-**1a**, combined with Mosher's reagent,[11] i.e., 97–98% optical purity or 96% enantiomer excess.

Pharmacology. The compounds of Table I were assayed for analgesic agonist activity by three in vivo antinociceptive bioassay procedures, i.e., (a) the modified D'Amour–Smith rat tail-flick test,[9] (b) the mouse acetylcholine writhing test,[12] and (c) the rat intracarotid bradykinin writhing test.[13,14] Narcotic antagonist activity was determined in vivo by the rat tail-flick vs. phenazocine test.[9] All compounds were administered subcutaneously or orally either in an aqueous solution of their acid addition salt or a solution of the free base in lactic acid. Compound 6 (Win 42,610) was established to be approximately equipotent to pentazocine[15] subcutaneously and about 10 times as potent as pentazocine orally in test animals. Structure–activity data for 6 and its congeners **9a**, **9b**, **12**, and the phenol 7 are listed vs. morphine and nalorphine in Table I. The compound had an agonist/antagonist potency ratio of approximately 1. It was nearly one-half as potent orally as it was subcutaneously in tests for analgesic and narcotic antagonist properties. Compound 6 protected guinea pigs in the ethylenediaminetetraacetic acid (EDTA) antinociceptive tests.[16] Its physical-dependence profile is similar to that of cyclazocine, i.e., a low physical dependence potency, therefore, a low, predicted abuse liability. The compound exacerbated abstinence in both weakly and strongly morphine dependent rats. Values derived for comparison from the phenylquinone writhing test[17] and the rat tail-flick antagonist test[9] vs. phenazocine, meperidine, and morphine are summarized in Table II. Data for racemic 6 and the optical isomers (−)-6 and (+)-6 derived from these tests are also summarized in Table II. The dextrorotatory enantiomer (+)-6 was the source of agonist/narcotic antagonist activity in racemic 6. Compound 6 does not have the marked depressant characteristics of aminocyclazocine.[18]

Conclusions

No interesting antinociceptive activity was reported by either Kametani[3,4,7] or Mitsuhashi[5,6] for any of the benzodiazocines they had synthesized. Preclinical animal studies have discovered, however, antinociceptive and narcotic antagonist activity in a series of 1,4-benzodiazocines in which nitrogen has been substituted for oxygen in the aromatic ring and for the quaternary carbon atom common to so many natural morphine alkaloids, semisynthetic morphine surrogates, and synthetic meperidine congeners. Introduction of a primary amine into the 9-position of the 1,4-benzodiazocine series enhances antinociceptive and narcotic antagonist potency compa-

rable to the good mixed agonist–opioid antagonist activity and favorable oral/parenteral ratio reported by Albertson[18] for 8-amino-3-(cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-cis-6,11-dimethyl-2,6-methano-3-benzazocine (aminocyclazocine). Alkylation of the primary amine increases toxicity. These primary amino derivatives exhibit parenteral/oral potency ratios of approximately 2. The 4-allyl congener is a less potent agonist than the cyclopropylmethyl compound. The biological activity is associated largely with the (+) isomer. Absolute stereochemistry of these compounds is unknown.

Experimental Section

Elemental analyses for C, H, and N were obtained for all new compounds reported and are within ±0.4% of the theoretical calculated values. Analyses were obtained from Instranal Laboratories of Rensselaer, NY. New compound structures were consistent with NMR (Varian HA-100), IR (Perkin-Elmer 21), and MS (JOELCO JMS-1-OCS) data. GC (Varian 3700) substantiation of optical isomer composition derived from Mosher's reagent[11] was obtained isothermally at 190 °C on an OV-101, 10-m capillary column. Preparative HPLC on prepacked silica gel columns was carried out on a Waters LC500 instrument. Preparative plate chromatography was done with Brinkmann PF_{254} silica gel in 1–1.5-mm thickness on 20 × 40 cm glass plates. A Rudolph polarimeter was used to measure optical rotations on solutions containing 2% of the free base in $CHCl_3$. All melting points were obtained uncorrected by the capillary-immersion technique in a magnetically stirred melting point apparatus of our own design.[19]

Resolution of (±)-3,4,5,6-Tetrahydro-2H-1,5-methano-1,4-benzodiazocine ((−)-1a). A warm H_2O (70 °C, 400 mL) solution of 105 g (0.6 mol) of (±)-**1a** and 45.7 g (0.3 mol) of *l*-mandelic acid was left to cool at room temperature. During a period of several days, two crops of *l*-mandelate salt were collected with mp 195–198 °C and 194–197 °C. Extraction of the free base with $CHCl_3$ was accomplished from saturated K_2CO_3 solution, affording $[\alpha]^{25}_D$ −199.5° and −188.3°, respectively (2% $CHCl_3$ solution of free base), Crop I material (mp 195–198 °C, $[\alpha]^{25}_D$ −199.5°) was 96% optically pure as determined by quantitation of the two GC peaks obtained from separation of the isomers prepared by treating the nor-base with Mosher's reagent.[11] ^{13}C NMR analysis of racemic **1a** and crop I combined with tris[3-(trifluromethyl)hydroxymethylene-*d*-camphorato]europium(III) derivative (chiral shift reagent) supported the GC results obtained with use of Mosher's reagent;[11] i.e., the quaternary asymmetric C-5 carbon atom was clearly delineated by two peaks for the optical enantiomers in the racemic material, and ^{13}C analysis of the free base from crop I indicated clearly 97–98% purity or 96% enantiomer excess.

(+)-3,4,5,6-Tetrahydro-2H-1,5-methano-1,4-benzodiazocine ((+)-1a). This compound was prepared from the optically enriched free base left after removal of the *l*-mandelate salt.

Free base with $[\alpha]^{25}_D$ +105.7° (59 g, 0.34 mol) in 200 mL of EtOH was treated with *d*-tartaric acid (50.8 g, 0.34 mol). Three crops were collected and combined (71.1 g total), which were recrystallized from aqueous alcohol and assayed by preparing isomers with Mosher's reagent[11] (99% optically pure). Calculation of the GC peaks indicated the racemate to be 75% (+) and 25% (−). Thus, 26.3 g (0.17 mol) of *d*-mandelic acid was reacted with 37.6 g (0.22 mol) of the 75:25 racemate to give after purification by recrystallization from EtOH–H_2O the *d*-mandelate salt of (+)-**1a** with mp 185–187 °C (with intumescense). Liberation with K_2CO_3 solution to the free base afforded 13 g of material, $[\alpha]^{25}_D$ +218°, which was 97% optically pure by GC separation of the Mosher's reagent[11] derived isomers.

4-(Cyclopropylcarbonyl)-3,4,5,6-tetrahydro-2H-1,5-methano-1,4-benzodiazocine (3a). A mixture of racemic 3,4,5,6-tetrahydro-2H-1,5-methano-1,4-benzodiazocine[2] (**1a**; 5.63 g 0.03 mol) and cyclopropanecarboxylic acid anhydride in excess was heated on a steam bath for 2 h. After evaporation of excess

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anhydride, a CHCl₃ solution of the residue was dried over K₂CO₃, charcoaled, filtered, and stripped of solvent in vacuo. Distillation of the residual, viscous oil under vacuum afforded **3a** (6.6 g, 85%), bp 160–165 °C (0.03 mm), of yellow amorphous glass. Anal. (C₁₅H₁₈N₂O) C, H, N.

4-Acetyl-3,4,5,6-tetrahydro-2*H*-1,5-methano-1,4-benzodiazocine (3b) was prepared similarly to **3a** from 10.5 g (0.06 mol) of **1a** and 25 mL of Ac₂O, affording 12.25 g (94% yield, recrystallized from acetone–ether) of product, mp 105–106 °C. Anal. (C₁₃H₁₆N₂O) C, H, N.

4-(Cyclopropylcarbonyl)-3,4,5,6-tetrahydro-9-nitro-2*H*-1,5-methano-1,4-benzodiazocine (4a). A mixture of **3a** (7.07 g, 0.03 mol) and 25 mL of 96% H₂SO₄ was stirred at ambient temperature until all the **3a** had dissolved. KNO₃ (3.53 g, 0.35 mol) was added in portions to the H₂SO₄ solution at room temperature. The solution was stirred overnight and then quenched in ice (about 250 mL). The cold mixture was basified with aqueous 35% NaOH. Solid K₂CO₃ was added until crystalline Na₂SO₄ and K₂SO₄ began to separate, and then the mixture was extracted twice with CHCl₃. The combined CHCl₃ extracts were dried over K₂CO₃ and filtered, and the CHCl₃ was stripped in vacuo. The residual free base crystallized and was recrystallized from a mixture of 2-PrOH and isopropyl acetate to give 7.9 g (91% yield) of shiny, yellow platelets: mp 97–99 °C; NMR [(CF₃COOD), (CH₃)₄Si] δ 11.3 (s, 1 H), 8.94 (d, 1 H, *J* = 2 Hz), 8.28 (dd, 1 H, *J* = 9, 2 Hz), 7.65 (d, 1 H, *J* = 9 Hz), 5.9–1.6 (m, 10 H), 1.4–0.9 (m, 4 H). Anal. C₁₅H₁₇N₃O₃) C, H, N.

4-Acetyl-3,4,5,6-tetrahydro-9-nitro-2*H*-1,5-methano-1,4-benzodiazocine (4b). KNO₃ (13.14 g, 0.13 mol) was added in portions to a solution of **3b** (26.43 g, 0.12 M) in 100 mL of 96% H₂SO₄. A procedure identical with the one described above (for **4a**) was used to obtained 22 g (70%) of **4b**. Recrystallization from 2-PrOH gave bright, yellow prisms with double melting; mp 142–144 °C with resolidification and remelting, mp 162–164 °C. Anal. (C₁₃H₁₅N₃O₃) C, H, N.

4-(Cyclopropylcarbonyl)-3,4,5,6-tetrahydro-2*H*-1,5-methano-1,4-benzodiazocin-9-amine (5). Iron powder (104 g, 1.9 mol) was added to a warm (50 °C) solution of **4a** (75 g, 0.26 mol) in ethanol (600 mL) and water (300 mL). The mixture was heated to reflux, and then a solution of concentrated HCl (45 mL), EtOH (90 mL), and H₂O (90 mL) was added dropwise. Stirring was continued for 4 h after the addition was complete. The mixture was allowed to cool, and then NaHCO₃ (75 g) was cautiously added, and the brown mixture was filtered. The filtrate was concentrated nearly to dryness, and the residue was partitioned between H₂O and CHCl₃. The CHCl₃ layer was washed with brine, dried over K₂CO₃, charcoaled, filtered, and evaporated in vacuo. Two recrystallizations of the crude tan product from AcOEt gave **5** as a light off-white crystalline powder, mp 142–144 °C. The yield was 32.2 g (57%). Anal. (C₁₅H₁₉N₃O) C, H, N.

Alternatively, 28.73 g (0.1 mol) of **4a** was reduced in MeOH with H₂ at 50–60 psi in a Parr shaker with 1 g of 10% Pd/C. Recrystallization from MeOH gave solvated product, mp 103–105 °C, 21.74 g (84%). Crystallization of this material from AcOEt afforded product **5** free from MeOH, mp 142–144 °C.

***N*-[4-(Cyclopropylcarbonyl)-3,4,5,6-tetrahydro-2*H*-1,5-methano-1,4-benzodiazocin-9-yl]butanamide (5a).** Amino amide **5** (8 g, 0.03 mol) in 70 mL of butyric anhydride was heated on a steam bath for 3 h, and the resulting light-amber solution was poured into a mixture of 50 mL of pyridine in ice–water. The crude, oily product was extracted into CHCl₃. The CHCl₃ was dried (K₂CO₃), charcoaled, filtered, and evaporated in vacuo to a yellow, amorphous gum. The gummy diamide **5a** was then dissolved in THF and subsequently reduced with diborane to the diamine **9b** without further purification.

4-(Cyclopropylmethyl)-3,4,5,6-tetrahydro-2*H*-1,5-methano-1,4-benzodiazocin-9-amine (6). To a suspension of 322 g (1.11 mol) of amino amide **5** and 190 g (5 mol) of NaBH₄ in 8 L of THF was added with stirring 990 g (7 mol) of BF₃·(EtO)₂ in 1.5 L of THF over a period of 5 h. The reaction was then stirred and refluxed overnight. Upon cooling, the suspension was quenched by the dropwise addition of 4 L of H₂O. The THF was removed by distillation at atmospheric pressure, leaving a pale yellow, oily residue, which was acidified with 2 N HCl solution and then heated at 90–95 °C for 3 h to decompose the boramine complex. The cooled reaction mixture was rendered strongly basic

with 35% NaOH solution and filtered to remove borate salts, and the filtrate was extracted three times with CHCl₃. The combined extracts were washed with H₂O, brine, dried over K₂CO₃, charcoaled, filtered, and concentrated in vacuo to dryness. The solid crude product was recrystallized from CH₃CN to give 232 g (86% yield) of light tan material with mp 143–145 °C. Anal. (C₁₅H₂₁N₃) C, H, N.

The optical antipodes of **6** were prepared from the resolved optical isomers of **1a** by the same procedures used for the preparation of racemic **6**. Crystallization of the free base from isopropyl acetate gave (−)-**6**: 1.5 g, mp 94–95 °C; [α]²⁵_D −80.5°. Column chromatography on neutral alumina (Brockman Scale III) eluted with ether–2-propylamine (98:2) afforded (+)-**6**: mp 93–94 °C; [α]²⁵_D +80.7°.

4-(Cyclopropylmethyl)-3,4,5,6-tetrahydro-2*H*-1,5-methano-1,4-benzodiazocin-9-ol (7). Diazotization of the primary amino group of 14.05 g (0.06 mol) of **6** was carried out with 4.5 g (0.065 mol) of NaNO₂ in 55 mL of 20% H₂SO₄ solution at −15 °C. The dark brown crystals of diazonium sulfate were collected and added in portions to a boiling solution (70 mL) of 70% H₂SO₄. Nitrogen gas was evolved, and after ¹/₂ h the reaction solution was cooled and poured into a solution of 293.2 g (1.2 mol) of BaCl₂·2H₂O in 1 L of H₂O. The BaSO₄ precipitate was digested for 5 h on the steam bath and then filtered hot. The filtrate was evaporated in vacuo and made basic with 2 N K₂CO₃ solution, and the product **7** was extracted into CHCl₃ solution. After drying (K₂CO₃) and removal of CHCl₃, crude **7** was recrystallized from 250 mL of CH₃CN to give 9.5 g (65%) of colorless microprisms having mp 170–172 °C. Anal. (C₁₅H₂₀N₂O) C, H, N.

***N*-[4-(Cyclopropylmethyl)-3,4,5,6-tetrahydro-2*H*-1,5-methano-1,4-benzodiazocin-9-yl]acetamide (8).** A mixture of **6** (3 g, 0.01 mol), Ac₂O (10 mL), and pyridine (25 mL) was let stand for 2 h at room temperature and then concentrated to dryness. The residue was partitioned between aqueous NH₄OH and CHCl₃ and the CHCl₃ layer dried (K₂CO₃), filtered, and concentrated to dryness. Recrystallization of the residue from Et₂O afforded **8**: 2.5 g (88%); mp 131–132 °C. Anal. (C₁₇H₂₃N₃O) C, H, N.

4-(Cyclopropylmethyl)-*N*-ethyl-3,4,5,6-tetrahydro-2*H*-1,5-methano-1,4-benzodiazocin-9-amine Dihydrochloride (9a). Borane in THF (150 mL, 0.15 mol) was added slowly to a solution of **8**, 8 g (0.03 mol) in 150 mL of THF, and the resulting mixture was heated to reflux overnight. Upon cooling, H₂O was added dropwise to decompose the excess diborane and then the THF was boiled off. The mixture was acidified with HCl, heated on a steam bath, made basic with 35% NaOH solution, and extracted with CHCl₃. After drying, filtration, and evaporation of the CHCl₃, the base was taken up in Et₂O and filtered and the product precipitated as the dihydrochloride. Recrystallization from 2-PrOH gave 6.2 g (60%), mp 252–254 °C. Anal. (C₁₇H₂₅N₃·2HCl) C, H, N.

***N*-Butyl-4-(cyclopropylmethyl)-3,4,5,6-tetrahydro-2*H*-1,5-methano-1,4-benzodiazocin-9-amine (9b).** Borane in THF (1 mol/L, 250 mL) was added slowly to a mixture of **5a** in 200 mL of THF and the resulting mixture heated to reflux for 2 h. After cooling, H₂O was added dropwise and the THF evaporated. Residual free base was dissolved in 2 N HCl, heated on the steam bath to decompose boramine complex, basified with NaOH solution, and extracted into CHCl₃. After drying (K₂CO₃), crystallization of the orange oil left from evaporation of the CHCl₃ in vacuo was achieved from n-hexane solution. The product (light tan crystals, 4.8 g (53% from amino amide **5**), gave mp 84–85 °C. Anal. (C₁₉H₂₉N₃) C, H, N.

3,4,5,6-Tetrahydro-9-nitro-2*H*-1,5-methano-1,4-benzodiazocine Dihydrochloride (10). A solution of 37.5 g (0.14 mol) of **4b** and 200 mL of concentrated HCl was heated on a steam bath for 4 h and then concentrated. The solid residue obtained was slurried in 100 mL of EtOH, and the solid was collected, affording 30 g (71%) of the dihydrochloride salt of **10**, mp 255–260 °C. Anal. (C₁₁H₁₃N₃O₂·2HCl) C, H, N.

3,4,5,6-Tetrahydro-9-nitro-4-(2-propenyl)-2*H*-1,5-methano-1,4-benzodiazocine Dihydrochloride (11). A mixture of the free base of **10** (17.6 g, 0.08 mol), allyl bromide (7.6 mL), NaHCO₃ (1 teaspoon), and DMF (200 mL) was stirred at room temperature overnight. After evaporation in vacuo, the residue was partitioned into 0.1 N NaOH solution and i-PrAc. The organic phase was washed with NaOH and brine solutions before drying

(K_2CO_3) and evaporating in vacuo. Crystallization of the orange free base was accomplished from 2-PrOH. The dihydrochloride salt was prepared from the base in EtOAc solution by addition of anhydrous HCl in Et_2O. Ten grams (38%) of pale yellow needles gave mp 231–233 °C (with intumescense). Anal. ($C_{14}H_{17}N_3O_2\cdot2HCl$) C, H, N.

3,4,5,6-Tetrahydro-4-(2-propenyl)-2*H*-1,5-methano-1,4-benzodiazocin-9-amine Diethanesulfonate Hemihydrate (12). Reduction of the nitro group of 11 was accomplished by the addition (without the application of external heat) of four 2-g portions of Fe powder to a solution of 10 g (0.03 mol) of the HCl salt of 11 in 100 mL of HOAc and 10 mL of H_2O. After overnight stirring and then evaporation to dryness, the residue was partitioned between dilute aqueous NaOH solution and $CHCl_3$. Evaporation of the dried (K_2CO_3) $CHCl_3$ left a tan powder, which was converted into the diethanesulfonate in 2-PrOH. Recrystallization from 2-PrOH gave 1.0 g (7%), mp 218–220 °C, of product 12. Anal. ($C_{14}H_{19}N_3\cdot2C_2H_5SO_3H\cdot^1/_2H_2O$) C, H, N.

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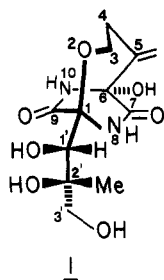
Synthesis and Antimicrobial Evaluation of Bicyclomycin Analogues

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The synthesis and antimicrobial evaluation of novel bicyclomycin analogues are described. The series of analogues were prepared from the basic 8,10-diaza-2-oxabicyclo[4.2.2]decane-7,9-dione (8), 7,9-diaza-2-oxabicyclo[3.2.2]nonane-6,8-dione (9), 8,10-diaza-5-methylene-2-oxabicyclo[4.2.2]decane-7,9-dione (10), and 7,9-diaza-4-methylene-2-oxabicyclo[3.2.2]nonane-6,8-dione (11) nuclei. For compounds where R_1 = *p*-methoxybenzyl, deprotection of the lipophilic amides with ceric ammonium nitrate affords the corresponding lipophobic free amides. The basic bicyclic nucleus of bicyclomycin (**8h**, $R_1 = R_2 = R_3 = R_4 = H$) has been synthesized for the first time as well as increasingly more complex congeners bearing the C-6 OH, 5-methylene; C-1′–C-3′ trihydroxyisobutyl group. In general, it has been found that the bicyclic nucleus of bicyclomycin is devoid of antimicrobial activity, the entire structure of bicyclomycin being generally obligate for activity. In one instance, the racemic analogue **10c** ($R_1 = CH_2Ph$, $R_2 = OH$, $R_3 = H$) showed interesting antimicrobial activity against several Gram-positive organisms; the minimum inhibitory concentrations were of the same order of magnitude as bicyclomycin displays toward Gram-negative organisms. Totally synthetic (±)-bicyclomycin was half as active as the natural antibiotic. The design, synthesis, and antimicrobial activity (and/or lack thereof) of bicyclomycin and the analogues are discussed in the context of a proposed chemical mechanism of action.

Bicyclomycin[1] (bicozamycin, **1**) is an antibiotic obtained from cultures of *Streptomyces sapporonensis*[2] and *Streptomyces aizunensis*.[3] Bicyclomycin is biosynthesized[4] from leucine and isoleucine and possesses a unique chemical structure amongst the known classes of antibiotics. The low toxicity and ready availability of bicyclomycin from fermentation have resulted in the recent commercial introduction of bicozamycin[5] as an effective agent against nonspecific diarrhea for humans and bacterial diarrhea of calves and pigs.[6] The mechanism of action of bicyclomycin is also thought to be unique and has been the subject of several accounts.[7]

1

Scheme I

1 ⇌ **2** → **3**
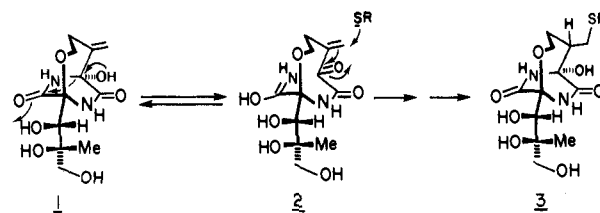
Bicyclomycin is active against Gram-negative bacteria such as *Escherichia coli*, *Klebsiella*, *Shigella*, *Salmonella*,

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(2) References 1a–d.

(3) References 1e and 1f.

(4) (a) Miyoshi, T.; Iseki, M.; Konomi, T.; Imanaka, H. *J. Antibiot.* 1980, *33*, 480; 1980, *33*, 488.