Resolved N,N-Dialkylated 2-Amino-8-hydroxytetralins: Stereoselective Interactions with 5-HT_{1A} Receptors in the Brain

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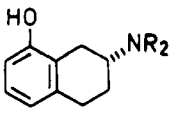
The enantiomers of the *N,N*-dimethylamino (**1**), *N,N*-diethylamino (**2**), and *N,N*-dibutylamino (**4**) derivatives of 8-hydroxy-2-(dipropylamino)tetralin (8-OH DPAT; **3**) have been synthesized. The compounds have been tested for activity at central 5-hydroxytryptamine and dopamine receptors by use of biochemical and behavioral tests in rats. In addition, the ability of the enantiomers of **1–4** to displace [^{3}H]-8-OH DPAT from 5-HT_{1A} binding sites was evaluated. Rank order of potencies in the in vivo tests corresponded to that observed in the 5-HT_{1A} binding assay. In all three tests, the enantiomeric potency ratio was about 10 for **1** and **2** and only around 2–4 for **3** and **4**. The more potent enantiomer of **1–3** had the *R* configuration. In contrast, (*S*)-**4** seemed to be slightly more potent than (*R*)-**4**.

Treatment of rats with 8-hydroxy-2-(di-*n*-propylamino)tetralin (**3**; 8-OH DPAT)[1] leads to inhibition of the biosynthesis and utilization of 5-hydroxytryptamine (5-HT) in the brain and to behavioral effects similar to those observed after administration of the 5-HT precursor 5-hydroxytryptophan (5-HTP).[1,2] In radioligand binding studies, **3** has a great affinity and selectivity for 5-HT_{1A} receptors.[3] Thus, **3** is a very interesting tool for studying serotonergic mechanisms.[4]

In biochemical and behavioral in vivo assays, (+)-(2*R*)-**3** is about twice as potent as its antipode, indicating a weak stereoselectivity.[1,2] Similarly, (*R*)-**3** binds with only slightly higher affinity to 5-HT_{1A} sites than does (*S*)-**3**.[5] In contrast, several derivatives of **3**, in which methyl substituents have been introduced in the nonaromatic ring, show a pronounced stereoselectivity in their interaction with 5-HT_{1A} receptors.[6] It is difficult to unambiguously rationalize why **3** shows such a low stereoselectivity.[7]



(*R*)-**1**: R = CH_3
(*R*)-**2**: R = C_2H_5
(*R*)-**3**: R = C_3H_7
(*R*)-**4**: R = C_4H_9

The 5-HT_{1A} receptors are stimulated also by the racemic *N,N*-dimethyl, *N,N*-diethyl, and *N,N*-dibutyl homologues of **3** (**1**, **2**, and **4**, respectively).[2] There might be differences, however, in the pharmacology of the enantiomers of these compounds, causing problems in interpreting their effects.[8] Therefore, we have synthesized and studied the pharmacology of the enantiomers of **1–4**. The compounds were investigated pharmacologically by use of biochemical and behavioral tests in rats. In addition, their affinity for [^{3}H]-8-OH DPAT labeled 5-HT_{1A} binding sites was evaluated in vitro. Results obtained demonstrate that the weak stereoselectivity of **3** is increased at least 5-fold by shortening of the *N*-alkyl substituents to methyl or ethyl.

Chemistry

Resolution of 2-(*N*-benzylamino)-8-methoxytetralin (**5**) by fractional crystallization of the diastereomeric di-*p*-toluoyltartrates followed by N-debenzylation provides facile access to the enantiomers of 2-amino-8-methoxytetralin (**6**).[9] The resolved *N,N*-diethyl, *N,N*-dipropyl, and *N,N*-dibutyl derivatives **8–10** were prepared from (*R*)- or (*S*)-**6** by N,N-dialkylation with the appropriate alkyl halide (method A; Scheme I). The *N,N*-dimethyl-substituted enantiomers were prepared from (*R*)- or (*S*)-**6** by reductive methylation (method B).

The phenols presented in Table I were prepared from the corresponding methoxy-substituted derivatives with use of 48% aqueous HBr (method C).

Pharmacological Results

Behavior. Stimulation of postsynaptic 5-HT and dopamine (DA) receptors was studied in rats in which the presynaptic monoamine stores had been depleted by re-

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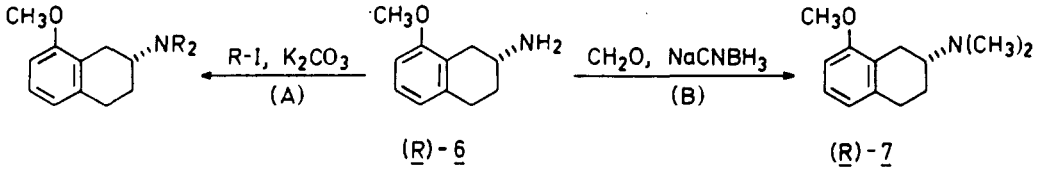
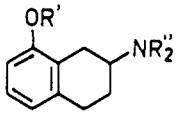
Scheme I



(R)-6 (R)-7

Table I. Physical Data of the Compounds Studied[a]



compd	R'	R''	prepn method[b]	yield, %	mp, °C	recrystn solvents[c]	$[\alpha]^{22}_D$, deg[d]	formula
(R)-1	H	Me	C	77	262–263	A	+89.7	$C_{12}H_{17}NO\cdot HCl$
(S)-1	H	Me	C	57	261–262	A	−89.7	$C_{12}H_{17}NO\cdot HCl$
(R)-2	H	Et	C	84	186–188	A	+89.6	$C_{14}H_{21}NO\cdot HCl$
(S)-2	H	Et	C	89	186–188	A	−91.0	$C_{14}H_{21}NO\cdot HCl$
(R)-3[e]	H	n-Pr	C	92	204–205	A	+77.9	$C_{16}H_{25}NO\cdot HCl$
(S)-3[e]	H	n-Pr	C	87	204–205	A	−79.2	$C_{16}H_{25}NO\cdot HCl$
(R)-4	H	n-Bu	C	80	161–163	A	+73.1	$C_{18}H_{29}NO\cdot HCl$
(S)-4	H	n-Bu	C	81	161–163	A	−74.9	$C_{18}H_{29}NO\cdot HCl$
(R)-7	Me	Me	B	62	267–268	B	+85.3	$C_{13}H_{19}NO\cdot HCl$
(S)-7	Me	Me	B	52	266–268	B	−87.7	$C_{13}H_{19}NO\cdot HCl$
(R)-8	Me	Et	A	51	171–173	A	+88.4	$C_{15}H_{23}NO\cdot HCl$
(S)-8	Me	Et	A	62	172–173	A	−88.2	$C_{15}H_{23}NO\cdot HCl$
(R)-9	Me	n-Pr	A	61	162–163[f]	A	+77.6[g]	
(S)-9	Me	n-Pr	A	67	163–164[h]	A	−75.9[i]	
(R)-10	Me	n-Bu	A	46	146–148	A	+74.2	$C_{19}H_{31}NO\cdot HCl$
(S)-10	Me	n-Bu	A	44	148–149	A	−73.5	$C_{19}H_{31}NO\cdot HCl$

[a] The elemental analyses of the compounds were within ±0.4% of the theoretical values. [b] For details, see the Experimental Section. [c] A: ethanol/ether. B: ethanol. [d] c 1.0, CH_3OH. [e] The hydrobromide salt has been reported previously (ref 1). [f] Literature[1] mp 164–165 °C. [g] Literature[1] $[\alpha]^{22}_D$ + 77.1° (c 1.0, CH_3OH). [h] Literature[1] mp 164–164.5 °C. [i] Literature[2] $[\alpha]^{22}_D$ − 76.1° (c 1.0, CH_3OH).

Table II. Resolved N,N-Dialkylated 2-Amino-8-hydroxytetralins: Abilities To Elicit the 5-HT Motor Syndrome in Reserpinized Rats[a,b]

	dose, μmol/kg sc						
compd	0.1	0.32	1.0	3.2	10	32	100
(R)-1			0/3	0/4	4/4*	3/3	
(S)-1					0/2	0/4	4/4*
(R)-2	0/4	4/4*	3/3				
(S)-2			0/3	0/5	4/5*		
(R)-3	0/4	4/4*					
(S)-3		0/5	4/5*				
(R)-4					0/4	4/4*	
(S)-4				0/3	0/4	4/4*	

[a] Shown are the number of rats exhibiting the 5-HT motor syndrome out of the number of rats studied. [b] The difference from the immediately lower dose was tested for statistical significance by an χ^2 test; (*) $P < 0.01$.

serpine pretreatment. Behavioral observations were made, particularly with regard to flat body posture and forepaw treading (5-HT motor syndrome).[10,11]

The R enantiomers of 2 and 3 were the most potent compounds in producing the 5-HT motor syndrome (Table II). The difference in potency between the R and S isomers was much more pronounced for 2 (N,N-diethyl) than for 3 (N,N-dipropyl). The N,N-dimethyl (1) and N,N-dibutyl (4) enantiomers were less potent. Furthermore, no stereoselectivity was observed for 4.

Rats receiving (R)-2, (S)-2, (R)-3, or (S)-3 in a dose immediately below that giving the 5-HT motor syndrome showed an increase in locomotor activity. Such doses of either of the enantiomers of 4 induced a weak increase in locomotion whereas (R)- or (S)-1 did not induce locomotion in any of the doses tested.

Biosynthesis of 5-HT and DA. It is well-known that agonists at 5-HT, dopamine (DA), and norepinephrine (NE) receptors inhibit the biosynthesis of the corresponding monoamine.[12] Thus, the monoamine synthesis can be used as an indicator of pre- and postsynaptic receptor activation. In the present study, the biosynthesis was measured indirectly by determining the accumulation of 5-hydroxytryptophan (5-HTP) in the various brain parts and of 3,4-dihydroxyphenylalanine (DOPA) in the DA-predominant (corpus striatum, limbic system) and NE-predominant (brain stem, hemispheres) rat brain regions following L-aromatic amino acid decarboxylase inhibition with 3-hydroxybenzylhydrazine (NSD 1015).[13] The rats were not pretreated with reserpine.

The R enantiomers of 2 and 3 were the most potent compounds also in inhibiting the tryptophan hydroxylase activity in the corpus striatum and the limbic system

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Table III. Resolved N,N-Dialkylated 2-Amino-8-hydroxytetralins: Effects on NSD 1015 Induced Accumulation of 5-HTP in Rats

compd	dose, μmol/kg	5-HTP,[a] ng/g striatum	5-HTP,[a] ng/g limbic
(R)-1	0.10	88 ± 0.3	172 ± 2.4
	0.32	79 ± 9.4*	130 ± 3.8**
	1.0	76 ± 4.9**	110 ± 12.5**
	3.2	67 ± 9.1**	90 ± 10.2**
	10	44 ± 1.5**	89 ± 2.5**
(S)-1	3.2	107 ± 7.5	178 ± 2.5
	10	69 ± 2.0**	162 ± 9.1
	32	66 ± 4.0**	128 ± 9.7**
	100	45 ± 6.0**	85 ± 7.6**
(R)-2	0.032	87 ± 5.7	131 ± 8.1**
	0.10	68 ± 3.8**	110 ± 4.2**
	0.32	50 ± 7.9**	70 ± 1.2**
	1.0	47 ± 2.9**	72 ± 3.8**
(S)-2	1.0	85 ± 5.1	135 ± 11**
	3.2	74 ± 7.2**	107 ± 9.5**
	10	55 ± 4.3**	86 ± 5.0**
	32	46 ± 2.7**	74 ± 14**
(R)-3	0.032	87 ± 12	163 ± 14
	0.10	78 ± 3.5*	119 ± 5.5**
	0.32	52 ± 5.4**	86 ± 14**
(S)-3	0.032	95 ± 3.5	157 ± 7.0
	0.10	94 ± 3.5	154 ± 2.2
	0.32	61 ± 4.3**	114 ± 8.4**
	1.0	53 ± 3.4**	75 ± 3.3**
(R)-4	10	80 ± 1.1*	145 ± 6.7
	32	63 ± 1.5**	106 ± 6.3**
(S)-4	3.2	90 ± 7.2	168 ± 14
	10	52 ± 4.9**	95 ± 0.6**
	32	45 ± 5.6**	112 ± 3.5**
control		107 ± 4.1	186 ± 6.7

[a] Mean ± SEM (n = 15 and 3–5 in the control and experimental groups, respectively). Statistics: one-way analysis of variance followed by Dunnett's t test; (**) $P < 0.01$, (*) $P < 0.05$ vs controls.

(Table III). In agreement with the results from the behavioral studies, the difference in potency between the R and S enantiomers was much more pronounced for the N,N-diethyl derivative **2** than for the N,N-dipropyl derivative **3**. However, whereas the enantiomers of **4** appeared to be of similar potency in the behavioral studies, (S)-**4** was more potent than its antipode in reducing the biosynthesis of 5-HT. In addition, all rats exhibited seizures and died following 100 μmol/kg of (R)-**4**.

The following approximate dose levels for a half-maximal reduction of the 5-HTP levels (ED_{50} values) were obtained graphically from the data presented in Table III: (R)-**1**, 1 μmol/kg; (S)-**1**, 20 μmol/kg; (R)-**2**, 0.05 μmol/kg; (S)-**2**, 3 μmol/kg; (R)-**3**, 0.1 μmol/kg; (S)-**3**, 0.2 μmol/kg; (R)-**4**, 20 μmol/kg; (S)-**4**, 5 μmol/kg. It should, however, be noted that the spread of the data does not allow a precise estimation of the ED_{50} values.

The effects of the compounds on the biosynthesis of 5-HT in the hemispheres and in the brain stem were not significantly different from those in the corpus striatum and in the limbic system (data are presented in the supplementary material).

The accumulation of DOPA in the dopamine-rich areas (corpus striatum and limbic system) was enhanced by (S)-**1**. The increase was more than 50% following 32 and 100 μmol/kg. No clearcut changes in the DOPA accumulation were observed following the other compounds (data are presented in the supplementary material).

Affinity for 5-HT_{1A} Binding Sites in Vitro. The enantiomers of **1**–**4** were also evaluated for their direct effects on cortical 5-HT_{1A} binding sites in the rat brain. (R)-**2** and (S)- and (R)-**3** had high affinities for the 5-HT_{1A} sites (Table IV). In agreement with the in vivo studies, **2** was considerably more stereoselective than **3**. The relative difference in affinity for the 5-HT_{1A} sites between the enantiomers of **1** and **2** was 9–10-fold. In contrast, the enantiomeric affinity ratio of **3** and **4** was 1.4 and 2.4, respectively. In agreement with the biochemical in vivo assay, (S)-**4** was more potent than (R)-**4**. Thus, the stereoselectivity is reversed when the N,N-dipropyl groups of **3** are extended to N,N-dibutyl groups. As observed in a previous investigation,[6c] the rank order of potencies for 5-HT_{1A} binding correlate well with that for decreasing the accumulation of 5-HTP and for eliciting the 5-HT syndrome.

Table IV. Resolved N,N-Dialkylated 2-Amino-8-hydroxytetralins: Potencies at 5-HT_{1A} Binding Sites[a]

compd	IC_{50},[a] nM	slope	N
(R)-1	75.0 ± 10.0	0.97 ± 0.16	3
(S)-1	675 ± 64.2	0.92 ± 0.17	3
(R)-2	9.65 ± 2.98	1.00 ± 0.04	3
(S)-2	114.5 ± 32.1	0.87 ± 0.11	3
(R)-3	4.84 ± 0.42	0.96 ± 0.06	3
(S)-3	6.54 ± 0.15	1.01 ± 0.07	3
(R)-4	108.9 ± 9.61	1.42 ± 0.35	3
(S)-4	46.2 ± 7.90	0.97 ± 0.09	3

[a] By simple t test (2-tailed) the IC_{50} values for each enantiomeric pair were significantly different except for **3**: (R)-**1** vs (S)-**1**, $p < 0.01$; (R)-**2** vs (S)-**2**, $p < 0.05$; (R)-**4** vs (S)-**4**, $p < 0.01$.

Discussion

The R enantiomer of **3** was only about 3 times as potent as its antipode in producing the 5-HT motor syndrome and in inhibiting the biosynthesis of 5-HT (Tables II and III). This difference between the stereoisomers is in fair agreement with previous findings.[1] The enantiomers of **3** behaved similarly in vivo and in vitro; (R)-**3** was somewhat more potent than (S)-**3** in the radioligand assay.

The R enantiomer of **2** was approximately as potent as (R)-**3** in the behavioral, the 5-HT synthesis, and the [^{3}H]-8-OH DPAT binding experiments. The S enantiomer of **2** was much less potent. The difference in potency between the enantiomers was greater for **2** than for **3**. In fact, the enantiomeric affinity ratios for **2** and **3** differ almost 10-fold (Table IV).

The enantiomers of the N,N-dimethyl (**1**) and the N,N-dibutyl (**4**) derivatives were much less potent than those of **3**. These findings agree with previous reports on the racemates.[1b] The R enantiomer of **1** was more potent than its antipode in the three tests performed (Tables II–IV). In contrast, (R)-**4** was not more potent than the (S)-**4**. Actually, (R)-**4** was somewhat less potent in two of the models.

The potencies of the compounds in the three different tests correlate fairly well. Thus, they seem to have similar pharmacological profiles. However, the results from the present investigation clearly demonstrate that the N-alkyl substituents affect the potencies of the enantiomers differently; the R and the S enantiomers show parabolic but different relationships between lipophilicity and potency/affinity. The fact that the 5-HT_{1A} receptor affinity of (R)-**1** is about 10-fold greater than that of (S)-**1** whereas the enantiomeric affinity ratio (R)-**4**/(S)-**4** is 0.39 might indicate that the affinity of the S enantiomers is increased by favorable interactions between large N-alkyl groups and lipophilic structures at the receptor which are not equally accessable to the R enantiomers. In the absence of large N-alkyl groups, however, the (R)-2-aminotetralin moiety appears to interact more favorably with the 5-HT_{1A} receptors.

The observation that large doses of (S)-**1** considerably increased the rat brain DOPA levels and decreased the 5-HTP levels may indicate a more complex pharmaco-

logical profile; (S)-1 might, for example, be an agonist at 5-HT_{1A} receptors and an antagonist at DA D_2 receptors. In fact, several 2-aminotetralin derivatives were recently reported to be DA D_2 receptor antagonists with preferential actions at presynaptic receptors.[15]

On the basis of structural comparisons of (R)- and (S)-3, we have previously discussed several tentative rationalizations of the small stereoselectivity of 3.[7] The present data seem to support models in which the enantiomers of 3 interact differently with 5-HT_{1A} receptors. In addition, the results from this study emphasize the importance of studying the enantiomers when evaluating the pharmacology of analogues to 3. It is apparent that erroneous conclusions may be drawn from results obtained with the racemates.

Experimental Section

Chemistry. General Comments. Melting points (uncorrected) were determined in open glass capillaries on a Thomas-Hoover apparatus. ^1H and ^{13}C NMR spectra were recorded at 90 MHz and 22.5 MHz, respectively, on a JEOL FX 90Q spectrometer and were referenced to internal tetramethylsilane. IR spectra (recorded on a Perkin-Elmer 157 G spectrometer) and mass spectra[14] (recorded at 70 eV on a 9000 LKB spectrometer using a direct insertion probe) were all in accordance with the assigned structures. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter. The elemental analyses (C, H, and N), which were performed by Micro Kemi AB, Uppsala, Sweden, were within ±0.4% of the theoretical values. For purity tests, TLC was performed on fluorescent silica gel or alumina plates. For all the compounds, only one spot (visualized by UV light and I_2 vapor) was obtained.

Synthesis. Below are given representative examples of the reactions presented in Table I.

(+)-(R)-2-(Diethylamino)-8-methoxytetralin [(+)-(R)-8]. Method A. A mixture of (+)-(R)-2-amino-8-methoxytetralin hydrochloride[9] [(+)-(R)-6·HCl; 0.8 g, 3.7 mmol], iodoethane (0.7 mL, 8 mmol), and potassium carbonate (1.3 g, 9 mmol) in acetonitrile (15 mL) under nitrogen was stirred at room temperature for 2 days. Ether was added and the reaction mixture was filtered and concentrated. The residue was passed through an alumina column with ether as eluant. The crude amine was converted into the hydrochloride and recrystallized from ethanol/ether to afford 0.5 g (51%) of (+)-(R)-8·HCl: ^1H NMR (methanol-d_4) δ 1.32–1.48 (t, 6 H), 1.73–3.20 (m, 6 H), 3.27–3.48 (q, 4 H), 3.55–3.90 (m, 1 H), 3.83 (s, 3 H), 6.67–6.81 (m, 2 H), 7.05–7.23 (m, 1 H); MS m/z 233 (93), 218 (80), 161 (100).

(+)-(R)-8-Methoxy-2-(dimethylamino)tetralin [(+)-(R)-7]. Method B. Aqueous formaldehyde (37%; 2.2 mL, 30 mmol) and 90% sodium cyanoborohydride (1.9 g, 20 mmol) were added to a solution of (+)-(R)-6·HCl (0.8 g 3.7 mmol) in methanol (10 mL). The pH was adjusted to 6 by addition of acetic acid. The mixture was stirred at room temperature for 2 days. The methanol was evaporated and the residue was treated with 2 M aqueous sodium hydroxide. The aqueous solution was extracted with ether (3 × 25 mL) and the organic layer was dried (potassium carbonate), filtered, and concentrated. The crude product was purified on an alumina column eluted with ether. The resulting amine was treated with ethereal hydrochloric acid and then recrystallized from ethanol, yielding 0.6 g (62%) of pure (+)-(R)-7·HCl: ^1H NMR (methanol-d_4) δ 1.67–3.20 (m, 7 H), 2.95 (s, 6 H), 3.36–3.75 (m, 1 H), 3.83 (s, 3 H), 6.67–6.81 (m, 2 H), 7.06–7.22 (m, 1 H); MS m/z 205 (85), 190 (23), 160 (19).

(+)-(R)-2-(Diethylamino)-8-hydroxytetralin [(+)-(R)-2]. Method C. A solution of (+)-(R)-8·HCl (0.40 g 1.4 mmol) in freshly distilled 48% aqueous hydrogen bromide (15 mL) was stirred for 2 h at 120 °C under nitrogen. The mixture was concentrated and the residue was partitioned between ether and saturated aqueous sodium bicarbonate. The ether layer was dried (sodium sulfate), filtered, and concentrated. Ethereal hydrogen chloride was added to a solution of the aminophenol in ether and the precipitate was recrystallized from ethanol/ether to give 0.3 g (84%) of pure (+)-(R)-2·HCl: ^1H NMR (methanol-d_4) δ 1.32–1.48 (t, 6 H), 1.72–3.17 (m, 6 H), 3.23–3.48 (q, 4 H), 2.55–3.91 (m, 1 H), 6.56–6.65 (d, 2 H), 6.86–7.06 (m, 1 H); MS m/z 219 (100), 204 (87), 147 (97).

Pharmacology. Materials and Methods. Male Sprague–Dawley rats (Alab, Stockholm) weighing 180–230 g were used. Reserpine was dissolved in a minimal quantity of glacial acetic acid and made up to volume with 5.5% glucose solution. The other substances were dissolved in 0.9% NaCl. Throughout, injection volumes were 5 mL/kg. Substances to be tested were given to rats as the hydrochlorides, subcutaneously in the neck region.

Behavior. The rats were pretreated with reserpine (5 mg/kg, i.e. 8.2 μmol/kg sc). The experimental drugs were given 6 h later. The rats were placed in circular plastic cages and observed for behavioral changes. Proper dose levels were identified in preliminary experiments in which the observer did not know which dose or compound that had been administered. Throughout, attention was particularly paid at flat body posture and forepaw treading since these signs are considered as typical elements for the so-called 5-HT motor syndrome.[10,11]

Biosynthesis of 5-HT and DA. The tryptophan and tyrosine hydroxylase activities were determined from the accumulation of 5-HTP and DOPA following inhibition of the L-aromatic amino acid decarboxylase by 3-hydroxybenzylhydrazine (NSD 1015; 60 mg/kg, i.e. 287 μmol/kg sc, 30 min before killing).[13] The experimental drugs were given sc 30 min prior to NSD 1015.

The rats were killed by decapitation. The brain was taken out quickly and placed on an ice-cooled petri dish. The corpus striatum, the limbic system, the brain stem (thalamus, hypothalamus, mesencephalon, pons, medulla oblongata), and the hemispheres (cerebral neocortex and cerebellum) were separated. The rhinal fissures were used as a landmark in the dissection of the limbic system. The concentrations of 5-HTP and DOPA were determined electrochemically following ion-pair, reversed-phase HPLC (for details, see ref 6c).

5-HT_{1A} Binding Assay. The measurement of 5-HT_{1A} binding sites, with use of [^3H]-8-hydroxy-2-(di-n-propylamino)tetralin ([^3H]-8-OH-DPAT; New England Nuclear Corp., Boston, MA), was carried out essentially as described previously.[16] Male Sprague–Dawley rats (150–225 g; Harlan Sprague–Dawley, Indianapolis, IN) were decapitated, and the brains were rapidly chilled and dissected to obtain the cerebral cortex dorsal to the rhinal sulcus. The tissue was homogenized in 40 volumes of ice-cold Tris·HCl buffer (50 mM, pH 7.4 at 22 °C) with a Brinkmann Polytron (setting 5 for 15 s), and the homogenate was centrifuged at 48000g for 10 min. The resulting pellet was then resuspended in the same buffer, and the centrifugation and resuspension process was repeated three additional times to wash the membranes. Between the second and third washes the resuspended membranes were incubated for 10 min at 37 °C to facilitate the removal of endogenous 5-HT.[17] The final pellet was resuspended in the buffer to a final concentration of 10 mg of tissue (original wet weight)/mL for use in the binding assay. To each assay tube the following were added: 0.1 mL of drug dilution (or water if no competing drug was added), 0.9 mL of [^3H]-8-OH DPAT in buffer (containing Tris, $CaCl_2$, and pargyline to achieve final assay concentrations of 50 mM, 3 mM, and 100 μM, respectively, pH 7.4), and 1 mL of resuspended membranes. The final concentration of [^3H]-8-OH DPAT in the assays was 1 nM. The tubes were incubated for 15 min at 37 °C, and the incubations were terminated by vacuum filtration through Whatman GF/B filters (pretreated by soaking for 2 h in a 0.1% v/v solution of polyethylenimine and then dried), followed by two 4-mL rinses with ice-cold 50 mM phosphate buffer. The filters

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were dried, and the radioactivity bound to the filters was measured by liquid scintillation spectrometry. Specific [^{3}H]-8-OH-DPAT binding was defined as the difference between binding in the absence and presence of 10 μM 5-HT. IC_{50} and slope values from the competition assays were determined by nonlinear regression analysis by using the program PCNONLIN and the four-parameter logistic function described by De Lean et al.[18]

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Supplementary Material Available: Effects of the enantiomers of 1–4 on the NSD 1015 induced accumulation of 5-HTP (brain stem, hemispheres) and DOPA (striatum, limbic system, brain stem, hemispheres) in rats (2 pages). Ordering information is given on any current masthead page.

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Synthesis and Biological Evaluation of De(acetylglucosaminyl)didehydrodeoxy Derivatives of Teicoplanin Antibiotics

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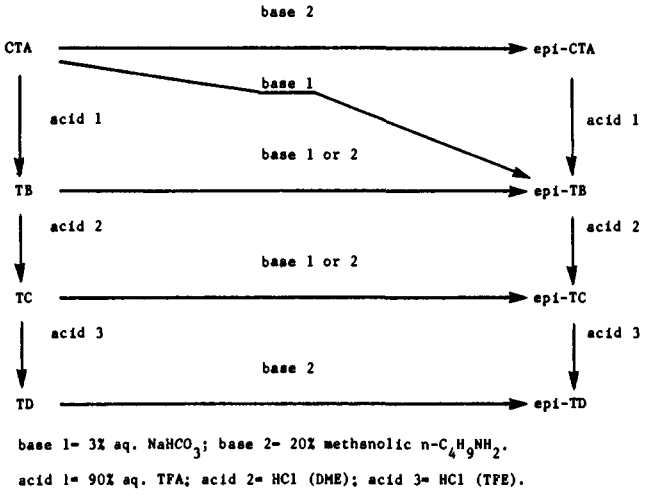
A series of 34-de(acetylglucosaminyl)-34-deoxy derivatives of 34,35- and 35,52-didehydro teicoplanin antibiotics have been synthesized from teicoplanin and its *N*-acetylglucosamine containing pseudoaglycons under basic conditions. The structures of these compounds have been determined by ^{1}H NMR, UV, and FAB-MS. 35,52-Unsaturated derivatives maintained in vitro and in vivo antimicrobial activity to a different extent as well as the ability for binding to Ac_2-L-Lys-D-Ala-D-Ala, a bacterial cell-wall model for the site of action of glycopeptide antibiotics. In contrast, 34,35-unsaturated compounds were markedly less active and possessed a negligible affinity for the synthetic tripeptide.

Teicoplanin[1] is a glycopeptide antibiotic produced by *Actinoplanes teichomyceticus* ATCC 31121.[2] It belongs to the vancomycin–ristocetin family and it is active against Gram-positive bacteria.[3] Teicoplanin is a complex consisting (Figure 1)[4] of five major closely related factors (CTA) differing in the *N*-acyl aliphatic chain linked with β-D-glucosamine at position 56 and of one pseudoaglycon (TB) deriving from CTA by loss of the above N-acylated amino sugar. Both CTA and TB contain one α-D-mannose and one *N*-acetyl-β-D-glucosamine at the 42- and 34-positions,[5] respectively, which are removed in that order by selective acidic hydrolysis, thus obtaining a second pseudoaglycon (TC) and the aglycon (TD).[6,7]

In the course of structural studies of teicoplanin, basic transformation products were also investigated. In particular, reaction with aqueous bicarbonate or methanolic amines resulted in the epimerization at C-3 (epiteicoplanins).[8] The relationship between the species involved in the acidic and basic treatments is outlined in Scheme I.

Scheme I



The glycosidic linkages of carbohydrates are generally hydrolyzed under acidic conditions but are relatively stable in diluted alkali,[9] with the exception of α-D-mannosyl phenyl glycosides.[10] Sometimes, sugars attached to the hydroxyl group of serine can be removed by alkaline treatment through a β-elimination mechanism.[11] The 34-(*N*-acetyl-β-D-glucosamine) of teicoplanin is, in principle, amenable to undergo β-elimination on treatment with alkalies to produce new pseudoaglycons or aglycon.

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