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^{1}H- AND ^{13}C-N.M.R. STUDIES ON *N*-METHYL-1-DEOXYNOJIRIMYCIN, AN α-D-GLUCOSIDASE INHIBITOR

ROBERT GLASER*
Department of Chemistry, Ben Gurion University of the Negev, Beersheva 84105 (Israel)

AND ARTHUR S. PERLIN
Department of Chemistry, McGill University, Montréal, Québec H3A 2A7 (Canada)

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ABSTRACT

The ^{13}C-{^{1}H}-n.m.r. spectra (acidic D_2O, pD ~1) of *N*-methyl-1-deoxynojirimycin (1,5-dideoxy-1,5-methyliminium-D-glucitol), an inhibitor of processing α-D-glucosidases involved in glycopeptide biosynthesis, showed two isomers (~11:1 ratio) differing in the stereochemistry of the N^+DCH_3 group. Assignments of carbon absorbances were made by ^{1}H–^{13}C heterocorrelation 2D n.m.r. spectroscopy, and DEPT techniques. For the minor (axial *N*-methyl group) species, the chemical shifts of the ring carbons in 1,4 position to the methyl group (and of the methyl carbon atom itself) were shifted characteristically upfield relative to the absorbances for the corresponding carbon atoms in the major (equatorial *N*-methyl) species. Two N^+DCH_3 singlets, in an ~11:1 ratio, were also noted in the slow-exchange limit (SEL) ^{1}H-n.m.r. spectrum recorded for a solution in acidic aqueous medium (pD ~1). Vicinal proton–proton coupling constants were consistent with a chair conformation for the SEL cationic major species, and for the free base at the fast exchange limit. The 5-hydroxymethyl *gauche–gauche* rotamer of the cationic equatorial *N*-methyl diastereomer (and of the free base) was found to be strongly preponderant [≥90%] in aqueous solution.

INTRODUCTION

N-Methyl-1-deoxynojirimycin [1,5-dideoxy-1,5-methylimino-D-glucitol; (2*R*,-3*R*,4*R*,5*S*)-3,4,5-trihydroxy-2-hydroxymethyl-1-methylpiperidine, **1**][1] inhibits the biosynthesis of glycoproteins in cells by inhibiting α-D-glucosidases involved in the *N*-linked oligosaccharide processing pathways. Also it has recently been shown to cause partial inhibition of the incorporation of labeled D-mannose into *N*-linked complex oligosaccharides in F9 teratocarcinoma cells[2]. It is the *N*-methylated derivative[1] of the catalytic or metal hydride reduction product **2** of nojirimycin (α-D-glucopiperidinose; **3**)[3], an antibiotic isolated from the fermentation broths of

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*Author for correspondence.

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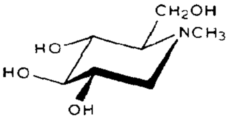
several strains of streptomyces (*Str. roseochromogenes* R-468, *Str. lavendulae* SF-425, and *Str. nojiriensis n. sp.* SF-426)[4]. This antibiotic shows activity against *Sarcina lutea, Xanthomonas oryzae,* and *Shigella flexneri*[4]. Nojirimycin (**3**) has been shown by n.m.r. spectroscopy to exist in aqueous medium as a 3:2 mixture of α and β anomers, respectively[3].



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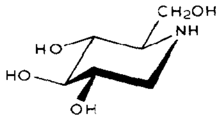

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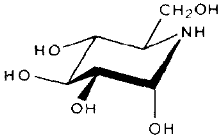

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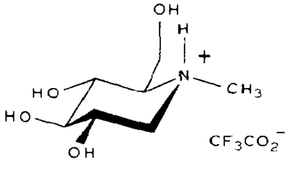
N-Methyl-1-deoxynojirimycin (**1**) can undergo a diastereomerization to yield two species (**4, 5**) in solution, differing in *N*-methyl stereochemistry, owing to the presence of the labile chirotopic stereogenic[5] nitrogen atom. Increasing the lifetimes, on the n.m.r. time scale, of these two interconverting diastereomers enables the recording of n.m.r. spectra from each isomer. Acidic media affords longer lived *N*-protonated species, and permits the attainment of slow exchange limit (SEL) ^{1}H-[6,7] and ^{13}C-n.m.r.[8] spectral parameters. We have recently reported a similar SEL n.m.r. investigation of tropane alkaloids (including cocaine[9]), and nefopam[10] (an eight-membered ring analgesic agent).



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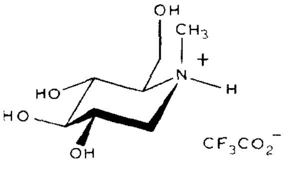

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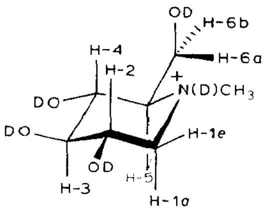
RESULTS AND DISCUSSION

The SEL ^{1}H- and ^{13}C-n.m.r. (D_2O, pD 1) spectral parameters of (*NR,S*) *N*-methyl-1-deoxynojirimycin hydrotrifluoroacetate equatorial, axial *N*-methyl diasteoreomers (**4–5**) are listed in Table I and II (proton and carbon labels are shown in structures **6** and **7**). The fast-exchange-limit (FEL), averaged ^{1}H-n.m.r. spectral parameters of *N*-methyl-1-deoxynojirimycin free base (**1**) are compared in Table I to those of the parent desmethyl (**2**) free base.



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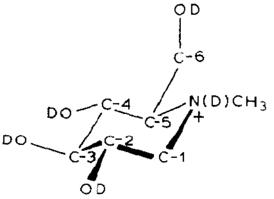

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TABLE I

^{1}H-N.M.R. SLOW-EXCHANGE LIMIT SPECTRAL PARAMETERS FOR (*NR*)-*N*-METHYL-1-DEOXYNOJIRIMYCIN HYDROTRIFLUOROACETATE (**4**); AND FAST-EXCHANGE LIMIT SPECTRAL PARAMETERS FOR *N*-METHYL-1-DEOXYNOJIRIMYCIN FREE BASE (**1**) AND 1-DEOXYNOJIRIMYCIN FREE BASE (**2**)[a]

δ_H	**4**[b]	**1**	**2**[c]
H-1*a*	3.14	2.68	2.46
H-1*e*	3.57	3.26	3.13
H-2	3.79	3.58	3.51
H-3	3.53	3.41	3.33[d]
H-4	3.66	3.53	3.24[d]
H-5	3.10	2.56	2.55
H-6a	4.10	3.95	3.84
H-6b	4.01	3.95	3.64
CH_3	3.00 (2.95)[e]	2.67	
$J_{H,H'}$[f]			
1*a*,1*e*	−12.1(1)	−11.7(3)	−11.9(4)
1*a*,2	11.3(1)	11.2(3)	10.5(5)
1b,2	5.0(1)	5.0(1)	5.3(4)
2,3	9.4(1)	9.3(2)	9.1(4)
3,4	9.3(1)	9.5(2)	8.0(9)
4,5	10.4(1)	9.9(3)	8.5(9)
5,6a	0.9(2)	2.2(2)	3.1(3)
5,6b	3.0(1)	2.2(2)	6.1(2)
6a,6b	−13.0(1)	[g]	−11.6(2)

[a] δ Values downfield from the signal of sodium 4,4-dimethyl-4-silapentane-1-sulfonate at 300 MHz, for a solution in D_2O. [b] D_2O contains trifluoroacetic acid, pD ~1. [c] Spectral parameters measured from the 220 MHz spectrum in ref. 4. [d] H-3,4 values in spectrum of **2** were assigned as in ref. 4 (criteria for assignment were not provided). [e] δ Value for axial *N*-methyl protons in diastereomer **5** given in parentheses, **4**-to-**5** ratio ~11:1. [f] In Hz, standard deviation given in parentheses. [g] Not determined.

TABLE II

$^{13}C\{^1H\}$-N.M.R. SLOW-EXCHANGE LIMIT SPECTRAL PARAMETERS FOR EQUATORIAL (**4**) AND AXIAL (**5**) *N*-METHYL DIASTEREOMERIC MIXTURES OF *N*-METHYL-1-DEOXYNOJIRIMYCIN HYDROTRIFLUOROACETATE[a]

δ_C	**4**	**5**
C-1	59.76	59.97
C-2	69.05	67.46
C-3	78.90	79.49
C-4	70.01	67.46
C-5	70.39	69.44
C-6	56.77	57.85
CH_3	43.70	38.37

[a] δ Values downfield from the signal of sodium 4,4-dimethyl-4-silapentane-1-sulfonate at 75 MHz, for a solution in D_2O with trifluoroacetic acid, pD ~1, **4**-to-**5** ratio ~11:1.

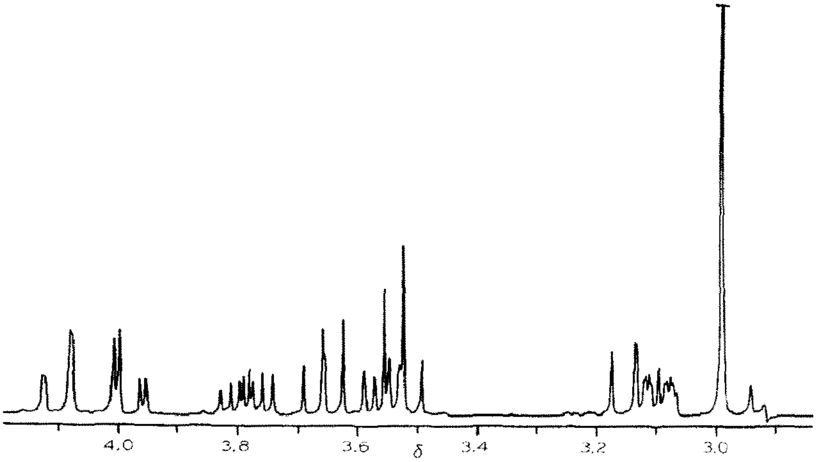

Fig. 1. ^{1}H-N.m.r. spectrum (300 MHz, acidic D_2O, pD ~1) for a diastereomeric mixture (~11:1, respectively) of equatorial and axial *N*-methyl-1-deoxynojirimycin hydrotrifluoroacetate (**4** and **5**).

Two species (~11:1) were observed in both the ^{1}H- and ^{13}C-n.m.r. spectrum of the hydrofluoroacetate of **1** recorded for solutions in acidic deuterium oxide (pD ~1). Stereochemical descriptors for the major and minor species were assigned on the basis of the ^{13}C-n.m.r. spectrum. For the (*NS*) axial *N*-methyl minor diastereomer **5**, the absorbances of the two ring carbon atoms (C-2,4) located at a 1,4-position relative to the methyl group (and the methyl carbon atom itself) were shifted characteristically to higher field, as compared to values for corresponding carbon atoms in the (*NR*) equatorial *N*-methyl epimer[11] **4**.

SEL and FEL ^{1}H-n.m.r. spectra. — Two $N^{+}DCH_3$ singlets at δ 3.00 (equatorial *N*-methyl group) and δ 2.95 (axial *N*-methyl group) were found for the hydrofluoroacetate of **1** in the ratio of ~11:1, respectively (see Fig. 1). Stereochemical descriptors for these two absorbances were assigned *via* a ^{1}H–^{13}C 2D n.m.r. heterocorrelation spectroscopy experiment in which the methyl carbon absorbances were identified in a prior experiment by use of the DEPT[12] pulse sequence. The axial *N*-methyl singlet was the only absorbance of the minor species readily identified in the ^{1}H-n.m.r. spectrum.

Two protons (H-2 and -5) are expected to exhibit an eight-line pattern of eight unit-intensity transitions both for the hydrotrifluoroacetate salt and for the free base. They are readily differentiated because the former is geminal to the oxygen atom (and hence expected to be relatively downfield, *e.g.*, δ 3.79 in **4**), whereas the latter is geminal to the nitrogen atom (and hence relatively upfield, *e.g.*, δ 3.10 in **4**)*.

Although the H-5 absorbance of the salt **4** is an eight-line pattern of eight unit-intensity transitions, the corresponding signal (δ 2.56) for the free base **1** is a six-line pattern of eight unit-intensity transitions in an arrangement of 1:2:1:1:2:1 relative intensities, *vide infra***.

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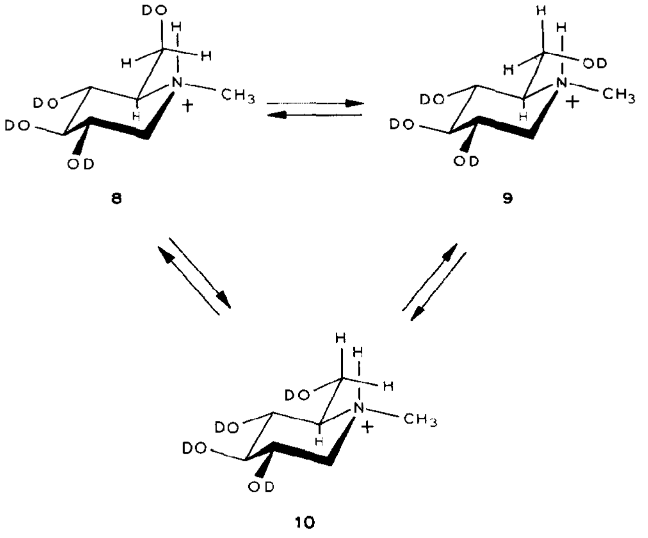
*There is an inversion in the order of transitions 4,5 for the H-2 absorbances of the salt **4**, the free base **1**, and the desmethyl free base **7** owing to $^{3}J_{1a,2} < (^{3}J_{1e,2} + {}^{3}J_{2,3})$.

The two overlaps (transitions 2 and 3, and 6 with 7) arose owing to $^{3}J_{5,6a} \sim {}^{3}J_{5,6b}$ in **1.

In all three cases (the salt **4**, the free base **1**, and the desmethyl free base **2**), the axial H-1*a* proton absorbed at a field higher than that of its internally diastereotopic[13] counterpart H-1*e* (*e.g.*, δ 3.15 *vs.* δ 3.57, respectively for **4**). At 300 MHz (as well as at 200 MHz), the H-1*a*, -1*e*,-2 protons afforded an AMX subsystem. Also in all three compounds, the diastereotopic H-6a, -6b pair absorbed at the lowest field (*e.g.*, δ 4.01 and δ 4.10, respectively for **4**), and formed the AB part of an ABX subsystem. The corresponding AB absorbance for **1** appeared as a deceptively simple two-line pattern ($\Delta\nu$ 2.5 Hz).

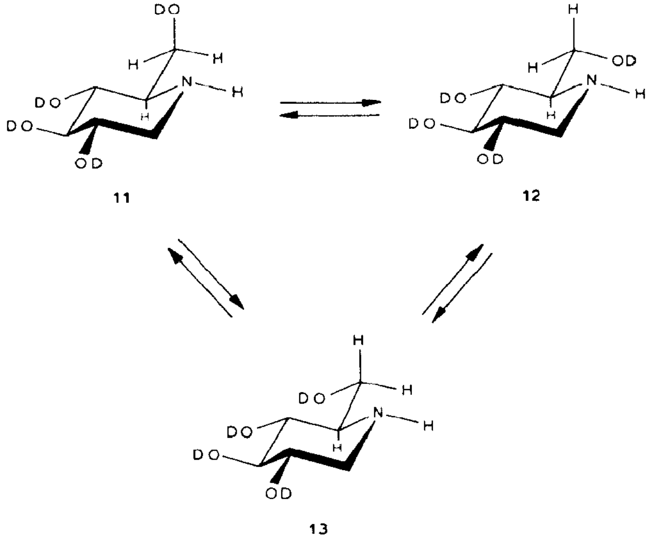
For the major diastereomer **4**, the axial H-3 and -4 absorbances were three- (δ 3.53, ~1:2:1 relative intensity) and four-line (δ 3.66) AMX-type patterns, respectively. Selective homonuclear-decoupling experiments were utilized to assign these two absorbances, and to confirm all the proton assignments. The differences in magnitude between vicinal coupling constants (${}^3J_{4,5} > {}^3J_{3,4}$ *vs.* ${}^3J_{3,4} \sim {}^3J_{2,3}$) that were observed in the ^{1}H-n.m.r. spectrum of salt **4** were also noted in the spectrum for the corresponding free base **1**. In the spectrum of **1**, the relative intensities of the H-4 three-line patterns at δ 3.53 were distorted owing to differences in the ${}^3J_{4,5}$, ${}^3J_{3,4}$ coupling constants [relative broader middle line ($w_{1/2}$ ~1.7 Hz, *vis-a-vis* ~1.2 Hz for outer lines)], whereas the H-3 absorbance at δ 3.41 showed ~1:2:1 relative intensities similar to its counterpart in the spectrum of **4**.

The equatorial *N*-methyl diastereomer of (*NR*)-*N*-methyl-1-deoxynojirimycin hydrotrifluoroacetate (**4**) exists in aqueous medium in the chair conformation, as shown by the magnitudes of the four antiperiplanar vicinal-coupling constants, ${}^3J_{1a,2}$ 11.3(1), ${}^3J_{2,3}$ 9.4(1), ${}^3J_{3,4}$ 9.3(1), and ${}^3J_{4,5}$ 10.4(1) Hz. Similar FEL averaged magnitudes of the four abovementioned coupling constants were also noted for the free base **1**, and for the desmethyl free base **2**.



Scheme 1. Rotamer conformations for the 5-hydroxymethyl group in the equatorial (*NR*)-*N*-methyl-1-deoxynojirimycin hydrotrifluoroacetate diastereomer (**4**).

The major differences in the three sets of ^{1}H-n.m.r. spectral parameters listed in Table I center on the averaged $^3J_{5,6a}$, $^3J_{5,6b}$ coupling constants, which relate to various rotamer populations about the C-5–C-6 bond. Examination of the structures **8**–**10** (Scheme 1) showed that the *gauche–gauche* rotamer **8** should be the most stable species for **4**, because the other two rotamers suffer nonbonding *cis*-1,3-diaxial interactions (O-6 · · · Me in *gauche–trans* **9**, and O-4 · · · O-6 in *trans–gauche* **10**). In the desmethyl free base **2**, the major destabilizing interaction is the O-4 · · · O-6 *cis*-1,3-diaxial interaction in *trans–gauche* **13** (see Scheme 2). Thus,

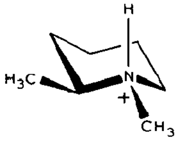


Scheme 2. Rotamer conformations for the 5-hydroxymethyl group in 1-deoxynojirimycin free base (**2**).

based on this conformational analysis, one should expect a larger amount of the *gauche–trans* rotamer for the desmethyl free base **2** than would be found for either the salt **4** or for the free base **1**. The rotamer population of the 5-hydroxymethyl group may be estimated by use of the two averaged $^3J_{5,6a}$, $^3J_{5,6b}$ coupling constants. This estimation was made on the following basis[14,15]: (a) Only fully staggered rotamers (*gauche–gauche, gauche–trans*, and *trans–gauche*) were considered; (b) an observed coupling constant was taken to be the sum of *gauche* (synclinal) and *trans* (antiperiplanar) couplings, weighted by the relative contributions of the three staggered rotamers; and (c) $^3J_{trans}$ 10 Hz and $^3J_{gauche}$ 2 Hz. The *gauche–gauche* rotamer of the equatorial *N*-methyl diastereomer **4** (and of the free base **1**) was found to strongly preponderate ($\geq$90%) in aqueous solution. By contrast, there is a much larger contribution from the *gauche–trans* rotamer in the case of the desmethyl free base **2** [estimated to be ~0.35:0.50:0.15 for *gauche–gauche* (**11**):*gauche–trans* (**12**):*trans–gauche* (**13**), if H-6a,6b are assigned as pro-*S* and pro-*R*, respectively], which is consistent with the observation that rotamer **12** does not incorporate the destabilizing O-6 · · · Me interaction present in the *gauche–trans* rotamer **9**. MMX87 molecular mechanics calculations[16] are in qualitative accord with the ^{1}H-n.m.r. estimated population rankings for rotamer models depicted in Schemes 1 and 2 (*i.e.*, minimized total energy for model **8** < **9** < **10**; and model **12** < **11** < **13**).

^{13}C-N.m.r. spectral parameters. — As noted above, the DEPT pulse sequence was utilized to obtain methyl-, methylene-, and methine-carbon subspectra. Assignments were based upon a ^{1}H–^{13}C 2D n.m.r. heterocorrelation spectroscopy experiment. Integration of externally diastereotopic[13] pairs of carbon atoms showed the major and minor species to be present in amounts similar to the ~11:1 ratio noted in the ^{1}H-n.m.r. spectrum.

In the minor (*NS*) axial *N*-methyl diasteeomer **5**, the methyl carbon atom and C-2, -4 in 1,4-position to the methyl group were more strongly shielded ($\Delta\delta$ 5.38, 1.59, and 2.55, respectively) *vis-a-vis* the corresponding carbon atoms in the major (*NR*) equatorial *N*-methyl diastereomer **4**. Comparison of the chemical shifts for C-1, -5 in 1,3-position to the methyl group showed little, if any, upfield shift upon placement of the methyl group in an axial orientation relative to the values for the methyl group in equatorial orientation. However, as the rotamer population about the C-5–C-6 bond is likely to differ considerably in the two *N*-methyl epimers **4** and **5**, different effects upon the chemical shifts of externally diastereotopic carbons may arise. By contrast, in the *cis*- and *trans*-1,2-dimethylpiperidinium chloride epimers **14** and **15**, the carbon atoms, both in 1,3- and 1,4-position to the methyl group, showed larger upfield shifts for the axial than for the equatorial epimers*.



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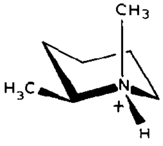

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For tropane alkaloids[9], a prototropic shift together with nitrogen inversion in the free base provides the diastereomerization process for the interconversion of the major equatorial *N*-methyl and minor axial *N*-methyl diastereomers in acidic D_2O. The presence of the ethano bridge in these bicyclo[3.2.1]octane systems precludes interconversion of ring substituents from equatorial to axial orientation (and *vice-versa*) *via* a ring inversion mechanism[9]. In *N*-methyl-1-deoxynojirimycin hydrotrifluoroacetate (**4**, **5**), the presence of equatorial hydroxyl groups, and the equatorial hydroxymethyl substituent are expected, similarly, to make inversion of the piperidine ring a relatively high energy process as compared to the prototropic shift–nitrogen inversion.

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*In the comparison[17] of diastereomers **15** *vs.* **14**, the signals for the carbon atoms in 1,3-position, C-2, -6, were shifted upfield by 4.38 and 5.13 p.p.m., respectively; the signals for the carbon atoms in 1,4-positions C-3, -5, and C–CH_3 were shifted upfield by 4.46, 3.05, and 4.39 p.p.m., respectively; the signal for the carbon atom in 1,5-position, C-4 was shifted upfield by 3.05 p.p.m.; and the signal for N-CH_3 was shifted upfield by 4.41 p.p.m.

The n.m.r. investigations described herein have shown that the two *N*-methyl epimers **4** and **5** are present in unequal amounts. However, the Curtin–Hammett principle[18] warns us that the more stable diastereomer need not be the more biologically active species.

EXPERIMENTAL

N-Methyl-1-deoxynojirimycin was obtained as a gift from Dr. Annette Herscovics, McGill University Cancer Centre. Acidic solutions of the hydrotrifluoroacetate salt were prepared in D_2O pretreated with trifluoroacetic anhydride (pD ~1). Aqueous solutions of the free base were prepared in deuterium oxide treated with excess K_2CO_3. ^{1}H- and ^{13}C-n.m.r. spectra (7.0 tesla) were recorded at 299.9 MHz and 75.4 MHz, respectively, with a Varian XL-300 F.t. n.m.r. spectrometer. The broad-band proton-decoupling technique was used for the ^{13}C-n.m.r. spectra, the D_2O solvent as an internal lock, and sodium 4,4-dimethyl-4-silapentane-1-sulfonate as an internal reference. MMX87 program[16] is an enhanced version of the MM2 program[19] with MMP1 π-subroutines[20] incorporated for localized π-electron systems. MMX87 molecular mechanics energy minimizations[16] include an N-H, O-H to lone pair $1/R^2$ attraction term, and were performed with a VAXstation-II computer. The following minimized total energies were calculated: for **8** 2.49, **9** 2.53, **10** 2.70, **11** 1.89, **12** 1.46, and **13** 2.06 kJ.

ACKNOWLEDGMENTS

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