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Boat conformations: synthesis, NMR spectroscopy, and molecular modeling of methyl 2,6-anhydro-3-deoxy-3-phthalimido-α-D-mannopyranoside and its ^{15}N-labeled analog☆

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Abstract

Methyl 2,6-anhydro-3-deoxy-3-phthalimido-α-D-mannopyranoside and its ^{15}N-labeled analog have been synthesized by reaction of methyl 2,6:3,4-dianhydro-α-D-altropyranoside with a mixture of phthalimide and potassium phthalimide or their ^{15}N-labeled analogs. The geometry of the skew conformation of the mannopyranoside derivative has been characterized by molecular dynamics with simulated annealing and correlated with vicinal and long-range ^{1}H–^{1}H and vicinal ^{1}H–^{15}N coupling constants measured by ^{1}H NMR spectroscopy. Dihedral angle comparisons have been made with derivatives of the parent 2,2,2-bicyclo-octane structure. The ^{1}H–^{15}N coupling constant data provide further values for definition of a Karplus equation for analysis of the stereochemistry of aminoglycosides. Published by Elsevier Science Ltd.

Keywords: 2,6-Anhydrides; Boat conformations; Carbon-13–proton chemical shift correlation; Carbon-13–nitrogen-15 and nitrogen-15–proton coupling constants; Gradient-enhanced 2D HSQC; Mannopyranoside; Molecular dynamics; Phthalimido derivatives; Simulated annealing

1. Introduction

Bridged pyranoid derivatives of carbohydrates are of interest as chemical intermediates in the synthesis of ^{15}N-labeled amino sugars in which the ^{15}N isotope has a defined stereochemical orientation. Such ^{15}N-labeled amino sugar derivatives are useful for basic studies of the dependence of ^{15}N–^{1}H NMR coupling constants on dihedral angle, a relationship that has not been established in the carbohydrate series, but has an application in the industrial analysis of the stereochemistry of aminoglycoside antibiotics by NMR spectroscopy. Cyclic systems having a relatively fixed geometry are also of interest for further testing of equations that relate ^{1}H–^{1}H and ^{13}C–^{1}H coupling constants to dihedral angle. In previous work [1], we studied the geometry of the skew boat conformation of 3-*O*-benzoyl-1,2,4-*O*-benzylidene-α-D-ribopyranose, and discussed the applicability of various equations [2–5] to the experimentally determined values of the $^{3}J_{H,H}$ and $^{4}J_{H,H}$ coupling constants. The present paper reports the synthesis, NMR spectroscopy, and molecular modeling of an ^{15}N-labeled, *N*-phthaloyl-

☆ Part II of a series, Boat conformations: for Part I, see Ref. [1].

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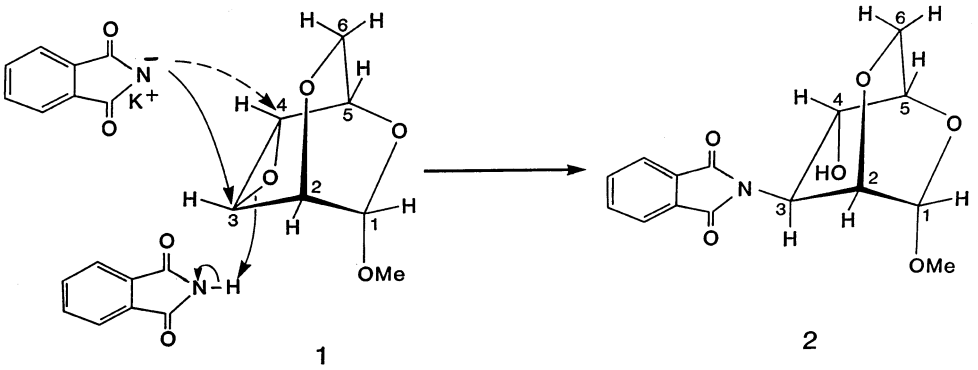

Scheme 1.

aminodeoxyglycoside derivative having a fixed, almost classical boat conformation that does not permit averaging of the observed $^{15}N–^{1}H$ coupling constants by contributions from other pyranoid conformational populations. Parameters for Karplus equations that describe the angular dependence of vicinal $^{15}N–^{1}H$ coupling constants in peptides [6] and proteins [7,8] are well established.

In previous studies, we found that potassium phthalimide-^{15}N, or mixtures of potassium phthalimide–^{15}N and phthalimide–^{15}N are convenient sources of the ^{15}N isotope for the synthesis of ^{15}N-labeled amino sugars via deoxyphthalimido derivatives. A number of these derivatives have been synthesized by reaction of labeled phthalimide with sulfonyl ester [9–13], deoxyiodo [9–13], or epoxide [14] derivatives of carbohydrates. The free aminodeoxy sugar derivatives may be prepared in quantitative yield from the deoxyphthalimido derivatives by treatment of the latter with boiling, ethanolic hydrazine hydrate [10,13,14]. In the present study, we were interested in the construction of ^{15}N-labeled aminodeoxyglycoside derivatives in the geometrically less mobile, 2,6-anhydrohexopyranoside series.

2. Results and discussion

In previous work [14], we found that addition of the elements of phthalimide to methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside catalyzed by potassium phthalimide in hexamethylphosphoric triamide at 152 °C yielded a mixture of methyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-α-D-altropyranoside and methyl 4,6-*O*-benzylidene-3-deoxy-3-phthalimido-α-D-glucopyranoside, in a ratio of ~3:1. In accordance with the Fürst–Plattner rule [15], the preponderant product was the 2,3-diaxial addition product having the α-D-altro configuration. Therefore, a suitable starting point for synthesis of aminoglycosides in the 2,6-anhydrohexopyranoside series appeared to be methyl 2,6:3,4-dianhydro-α-D-altropyranoside (**1**), which had been synthesized by Sinclair [16]. However, we found that **1** did not react with a mixture of phthalimide (1.25 mol equiv) and potassium phthalimide (0.25 mol equiv) in hexamethylphosphoric triamide, even when heated for 28 h at 180 °C. The unreactivity of **1** under these conditions was overcome by heating a solid mixture of **1**, phthalimide, and potassium phthalimide in an evacuated, sealed tube at 240 °C (i.e., above the melting point of phthalimide) in the absence of hexamethylphosphoric triamide. A single phthalimido-substituted product (**2**) was obtained, whose structure has been determined by NMR spectroscopy.

According to the Fürst–Plattner rule [15], the epoxide ring of **1** would be expected to undergo rearside attack by nucleophiles (see Scheme 1) to give one or more trans products, namely, either methyl 2,6-anhydro-3-deoxy-3-phthalimido-α-D-mannopyranoside (**2**) or methyl 2,6-anhydro-4-deoxy-4-phthalimido-α-D-idopyranoside. When labeled with ^{15}N, either of these products would be useful for the purpose of helping to define the angular dependence of $^{3}J_{N\text{-}15,H}$ in a fixed conformation, with uncommon dihedral angles for some of

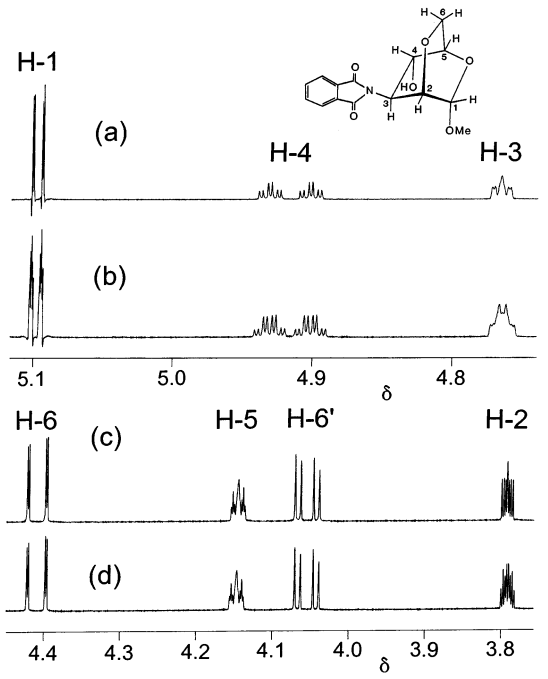

Fig. 1. ^{1}H NMR spectra at 400 MHz: (a) and (c), partial spectra of methyl 2,6-anhydro-3-deoxy-3-phthalimido-α-D-mannopyranoside (**2**); (b) and (d), partial spectra of methyl 2,6-anhydro-3-deoxy-3-phthalimido-α-D-mannopyranoside-3-^{15}*N* (**2-^{15}*N***), showing the additional splittings due to the presence of the ^{15}N isotope.

the vicinal, exocyclic atoms. In $CDCl_3$ solution, the ^{1}H NMR spectrum of the phthalimido product showed a wide doublet (J 11.6 Hz) at 2.9 ppm, which disappeared on equilibration of the solution with deuterium oxide, and which, therefore, was assigned as a hydroxyl proton signal. The H-6 and H-6′ signals were readily identified as wide quartets at 4.4 and 4.05 ppm, and sequential spin decoupling experiments starting at H-1 verified the assignments of H-2, H-3, H-4, H-5, H-6, and H-6′ (see Fig. 1(a) and (c)). The dodecet at 4.9 ppm (Fig. 1(a)) is the only multiplet that contains the large 11.6 Hz coupling constant, and is therefore assigned as a proton attached to the carbon atom that bears the hydroxyl group. The 2,6-anhydro ring of the phthalimido product clearly remained intact during the drastic conditions of reaction, because the spectrum of **2** resembles the spectra of a range of methyl 2,6-anhydro-α-D-altropyranoside derivatives [17]. If the 2,6-anhydro ring had opened, then large changes in the ring proton coupling constants would have been expected, and this was not observed. Therefore, the hydroxyl group is located at C-4, and the phthalimido group at C-3.

Repetition of the synthesis with ^{15}N-labeled phthalimides yielded **2-^{15}*N***, whose ^{1}H NMR spectrum (see Fig. 1(b) and (d)) shows a number of additional coupling constants due to the presence of the spin 1/2 ^{15}N isotope. Complete analysis of the ^{1}H NMR spectra of **2** and **2-^{15}*N*** yielded the ^{1}H chemical shifts reported in Table 1, and the ^{1}H coupling constants in Tables 2 and 3. Three long-range ^{1}H–^{1}H couplings ($^{4}J_{1,3}$, $^{4}J_{2,4}$, and $^{4}J_{3,5}$, see Table 2) were assigned by spin decoupling and matching of spectral splittings. From the small value $^{4}J_{1,3}$ 0.3 Hz, it may be inferred that H-3 is not in a planar 'W' arrangement with H-1, and is therefore axial at C-3. On the other hand, the magnitude of $^{4}J_{2,4}$ (1.0–1.1 Hz) is consistent with a planar 'W' arrangement of H-2 and H-4, which means that H-4 is equatorial. In a range of methyl 2,6-anhydro-α-D-altropyranoside derivatives, $^{4}J_{1,3}$ is 1.3–1.6 Hz when H-3 is equatorial [17].

Analysis of the ^{1}H and ^{13}C NMR spectra of **2-^{15}*N*** yielded the heteronuclear coupling constants shown in Table 3, and the ^{13}C chemical shifts for **2** and **2-^{15}*N*** are reported in Table 1. The observation of the long-range coupling $^{4}J_{1,N\text{-}15}$ 0.6 Hz as an extra splitting in the H-1 signal of **2-^{15}*N*** (see Fig. 1(b)) supports the conclusion that the ^{15}N atom of **2-^{15}*N*** is equatorial. This unusual 'W' type coupling involving a ^{15}N nucleus is surprisingly large in view of the fact that the magnetogyric ratio of the ^{15}N nucleus is a factor of 10 smaller than that of the proton, which normally attenuates the magnitudes of ^{15}N coupling constants substantially. The ^{1}H NMR spectrum of **2-^{15}*N*** (Fig. 1(b) and (d)) also displays couplings of the ^{15}N nucleus with H-2, H-3, and H-4 (see Table 3).

The ^{13}C NMR spectrum of **2** displayed a C=O resonance and three aromatic carbon resonances that are characteristic of the phthalimido group [13,14,18] and also the seven resonances required for a methyl glycoside moiety. One of the latter resonances was found just upfield from the methoxyl carbon signal and was assigned as C-3 on the basis of the ^{13}C NMR spectrum of **2-^{15}*N***, which showed the C-3 resonance as a doublet with

Table 1
^{1}H and ^{13}C chemical shifts [a] (ppm) of methyl 2,6-anhydro-3-deoxy-3-phthalimido-α-D-mannopyranosides

Compound	Larmor frequency (MHz)	Temperature (K)	Position											
			1	2	3	4	5	6	6′	OMe	OH	Ar-1,6	Ar-2,5	Ar-3,4	C=O
			^{1}H chemical shifts [b]												
2	400	297	5.098	3.791	4.767	4.917	4.146	4.408	4.054	3.577	2.899		7.846	7.731	
2-^{15}*N*	400	297	5.098	3.790	4.765	4.917	4.146	4.408	4.054	3.577	2.901		7.846	7.731	
2	600	300	5.092	3.783	4.762	4.912	4.142	4.398	4.048	3.574	2.946		7.836	7.725	
2-^{15}*N*	600	300	5.093	3.783	4.762	4.912	4.142	4.399	4.049	3.575	2.936		7.837	7.725	
			^{13}C chemical shifts [b]												
2	151	300	100.42	70.00	56.14	66.85	72.02	65.81		56.21		131.89	123.50	134.33	168.65
2-^{15}*N*	151	300	100.42	70.00	56.12 [c]	66.85	72.02	65.81		56.22		131.89 [c]	123.50	134.33	168.65 [c]

[a] In ppm from internal tetramethylsilane.
[b] For measurements of the chemical shifts, the digital resolution was 0.000277 ppm/point for ^{1}H at 400 MHz, 0.000132 ppm/point for ^{1}H at 600 MHz, and 0.00548 ppm/point for ^{13}C.
[c] Doublet.

$^1J_{\text{C-3,N-15}}$ 9.3 Hz. This doublet was completely dispersed from the methoxyl carbon signal at 151/600 MHz, but not at 101/400 MHz. The presence of the ^{15}N isotope at C-3 was also confirmed by observation of the $^2J_{\text{3,N-15}} = 1.4$ Hz coupling constant in the ^{1}H NMR spectrum of **2-^{15}*N***. In the ^{13}C NMR spectrum of **2-^{15}*N***, additional ^{15}N couplings appeared in the C=O and aromatic C-1,6 resonances, with values (see Table 3) that agree with those reported earlier for other phthalimido sugar derivatives [13,14,18]. The ^{13}C NMR assignments for **2** and **2-^{15}*N*** (see Table 1) were determined by heteronuclear chemical shift correlation with the ^{1}H assignments, using the sensitivity-improved, *z*-gradient-enhanced 2D HSQC method [19,20]. Based on the foregoing evidence, the structure of **2** is assigned as methyl 2,6-anhydro-3-deoxy-3-phthalimido-α-D-mannopyranoside. The fact that this was the only product isolated may be attributed to steric hindrance of rearside attack by phthalimide ion at C-4 of **1** by the buttressing effect of the adjacent axial methylene group at C-5 (see Scheme 1). Because the possible repulsion of the approaching negatively charged phthalimide ion by the lone pairs of electrons on O-6(2) does not appear to influence the preference for nucleophilic attack at C-3 of the epoxide **1**, it may be surmised that the steric effect of the methylene group is more important than the electronic effect of the bridging oxygen atom.

Since the ^{1}H chemical shifts of the H-3 protons of **2** and **2-^{15}*N*** measured at 400 MHz appeared to be slightly different (Table 1), the spectra of these compounds were re-measured at 600 MHz. At this frequency, any isotope effects on chemical shifts (when expressed in Hz) would be magnified by a factor of 1.5 over those measured at 400 MHz. The difference in H-3 chemical shifts was not reproduced in the 600 MHz data (Table 1), and we conclude that there is no evidence for any significant ^{15}N isotope effect on the ^{1}H chemical shifts of **2**. The occasional slight differences (0.001–0.002 ppm) observed for non-exchangeable protons cannot be considered significant. Although the ^{13}C-3 chemical shift of **2** differs slightly from that of **2-^{15}*N*** when measured at 151/600 MHz (see Table 1), these data could not readily be compared with

Table 2
Homonuclear coupling constants (Hz) [a] of methyl 2,6-anhydro-3-deoxy-3-phthalimido-α-D-mannopyranosides

Compound	Vicinal and geminal								Long-range (> 3 bonds)		
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$	$J_{4,OH}$	$J_{1,3}$	$J_{2,4}$	$J_{3,5}$
2	2.8	1.9	2.6	2.6	0.8	3.0	9.6	11.6	0.3	1.0	0.8
2-^{15}N	2.8	1.9	2.5	2.5	0.8	2.9	9.7	11.6	0.3	1.1	0.8

[a] Measured at 400 MHz with a digital resolution of 0.1 Hz.

Table 3
Heteronuclear coupling constants (Hz) [a] of methyl 2,6-anhydro-3-deoxy-3-phthalimido-α-D-mannopyranoside-*3*-^{15}N (**2-^{15}N**)

$^4J_{1,N\text{-}15}$	$^3J_{2,N\text{-}15}$	$^2J_{3,N\text{-}15}$	$^3J_{4,N\text{-}15}$	$^1J_{C\text{-}3,N\text{-}15}$	$^1J_{C=O,N\text{-}15}$	$^2J_{C\text{-}1(6),N\text{-}15}$
0.6	1.1	1.4	2.3	9.3	12.4	7.8

[a] $^1H,^{15}N$ coupling constants were measured at 400 MHz with a digital resolution of 0.1 Hz, and $^{13}C,^{15}N$ couplings at 151 MHz with a digital resolution of 0.8 Hz.

data at 101/400 MHz, because of overlap of the C-3 doublet of **2-^{15}N** with the methoxyl carbon signal at the latter frequency.

The significance of the vicinal, heteronuclear coupling constants $^3J_{2,N\text{-}15}$ 1.1 Hz and $^3J_{4,N\text{-}15}$ 2.3 Hz was assessed by molecular modeling of the structure of **2**. During molecular dynamics/simulated annealing of **2,** several phthalimido/hydroxyl/methoxyl group rotamers were encountered (for example, see Fig. 2) that differed in energy by < 7.9 kJ/mol (1.9 kcal/mol). The more stable rotamers contained the methoxyl group in an exo orientation, in which the methyl group was disposed in an almost trans arrangement with C-2. The phthalimido group rotamer of lowest energy (see Fig. 2(a)) had one carbonyl oxygen atom of the phthalimido group located near to an H-6 proton, and showed dihedral angles $\phi_{C=O,N\text{-}3,C\text{-}3,C\text{-}2}$ 76.3°, $\phi_{2,N\text{-}3}$ 51.6°, $\phi_{4,N\text{-}3}$ 10.2°, and $\phi_{4,OH}$ 172.1°. The latter value reflects an almost true trans orientation of H-4 and HO-4, and is consistent with the experimentally observed large value $^3J_{4,OH}$ 11.6 Hz. Other rotamers of the phthalimido group (for example, see Fig. 2(b) and (c)) had one of their carbonyl oxygen atoms in the vicinity of the hydroxyl group. The molecular model of one of these rotamers (see Fig. 2(b)) displayed a slight overlap of the carbonyl oxygen atom and hydroxyl hydrogen atom envelopes, suggesting hydrogen bonding of these atoms. Surprisingly, however, this rotamer was 7.5 kJ/mol (1.8 kcal/mol) less stable than that (Fig. 2(a)) having the carbonyl oxygen atom close to an H-6, and the less stable rotamers of the type shown in Fig. 2(b) also possessed dihedral angles $\phi_{4,OH}$ -59.9 or 61.5° that were inconsistent with the large value of $^3J_{4,OH}$ observed.

Similar dihedral angles were obtained by molecular dynamics/simulated annealing of 2,2,2-bicyclo-octane analogs of **2**. Under these conditions, the parent 2,2,2-bicyclo-octane showed dihedral angles $\phi_{1,2e}$ 53.8° and $\phi_{2e,3e}$ 10.3°, or $\phi_{1,2e}$ 65.3° and $\phi_{2e,3e}$ -10.3°, apparently indicating alternative boat conformations that are slightly twisted in opposite senses. For (*S*)-2-phthalimido-2,2,2-bicyclo-octane, the most stable phthalimido rotamer computed was that in which a phthalimido carbonyl oxygen atom lies between an H-7 and an H-8 atom, i.e., in an equivalent orientation to the phthalimido group of **2** (the numbering of the atoms is different in the 2,2,2-bicyclo-octane and carbohydrate systems). This rotamer showed dihedral angles $\phi_{C=O,N\text{-}2,C\text{-}2,C\text{-}1}$ 83.6°, $\phi_{1,N\text{-}2}$ 49.7°, and $\phi_{3,N\text{-}2}$ 9.5°, in rough agreement with the corresponding angles of the most stable rotamer of **2**, and with 2,2,2-bicyclo-octane. For (*S*)-2-phthalimido-2,2,2-bicyclo-octane, the rotamer with a carbonyl oxygen atom located between H-2a and H-3a was 2.5 kJ/mol (0.6 kcal/mol) less stable than the aforementioned rotamer.

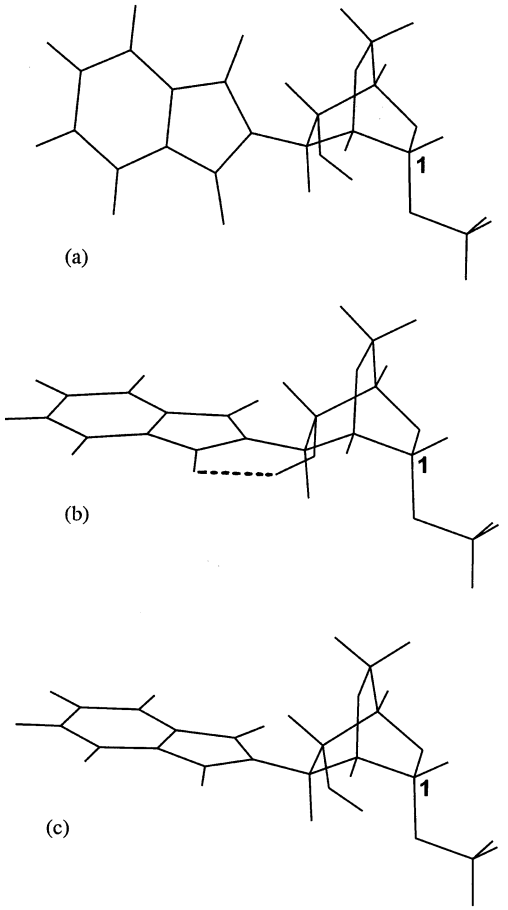

Fig. 2. Geometries of phthalimido/hydroxyl/methoxyl group rotamers computed for methyl 2,6-anhydro-3-deoxy-3-phthalimido-α-D-mannopyranoside (**2**) by molecular dynamics with simulated annealing: (a) the most stable rotamer having a phthalimido carbonyl oxygen atom close to an H-6; (b) a less stable rotamer showing hydrogen bonding (dashed line) between a phthalimido carbonyl oxygen atom and the hydroxyl hydrogen atom; and (c) a phthalimido rotamer similar to (b), but with a hydroxyl rotamer in a non-hydrogen bonding orientation.

By contrast, the most stable rotamer computed for (*R*)-2-phthalimido-(*R*)-3-hydroxy-2,2,2-bicyclo-octane was that in which the hydroxyl hydrogen atom was hydrogen bonded to a carbonyl oxygen atom. This rotamer showed dihedral angles $\phi_{C=O,N\text{-}2,C\text{-}2,C\text{-}1}$ −179.6°, $\phi_{1,N\text{-}2}$ 47.8°, $\phi_{3,N\text{-}2}$ 24.1°, and $\phi_{3,OH}$ −60.9°, i.e., with substantial distortion of the six-membered rings. In this case, a non-hydrogen-bonded hydroxyl rotamer with $\phi_{3,OH}$ 179.7° was computed to be 4.6 kJ/mol (1.1 kcal/mol) less stable than its hydrogen-bonded rotamer. Also, the phthalimido rotamer having a carbonyl oxygen atom located between an H-7 atom and an H-8 atom was found to be just 1.7 kJ/mol (0.4 kcal/mol) less stable than its hydrogen-bonded rotamer. The molecular modeling results for **2** and its 2,2,2-bicyclo-octane analogs suggest that the classical boat conformations of these compounds undergo slight twisting, presumably to relieve eclipsed, non-bonded interactions.

In summary, we believe that the values of the heteronuclear coupling constants $^3J_{2,N\text{-}15}$ 1.1 Hz and $^3J_{4,N\text{-}15}$ 2.3 Hz observed for **2-^{15}*N*** may be associated with ^{1}H–^{15}N dihedral angles of 52 and 10°, respectively, thus providing additional data for definition of a Karplus equation for aminoglycosides.

3. Experimental

General methods.—Concentrations were conducted under reduced pressure. Melting points are uncorrected.

NMR spectroscopy.—NMR spectroscopy was conducted by using solutions in chloroform-*d* contained in 5-mm sample tubes, with tetramethylsilane as an internal reference. 1D ^{1}H NMR spectra were recorded at 400 MHz and 297 K, and at 600 MHz and 300 K by use of Bruker Instruments[1] WM-400 and DRX-600 NMR spectrometers, respectively. At 400 MHz, a spectral width of 3.62 kHz was used with a data size of 65,536 points, a 90° pulse width of 13 μs, and a data acquisition time of 9.04 s. Spectra at 600 MHz were measured with a spectral width of 5.21 kHz, a data size of 65,536 points, a 90° pulse width of 8.0 μs, and a data acquisition time of 6.29 s. The spectra were resolution enhanced by Gaussian filtering of the free induction decay signal, using a Gaussian truncation fraction of 0.5 and a line broadening of −0.3 Hz at 400 MHz, and a truncation fraction of 0.3 and a line broadening of −2.15 Hz at 600 MHz. ^{1}H chemical shift assignments and short- and long-range ^{1}H–^{1}H spin coupling constant assignments were confirmed by homonuclear

[1] Certain commercial equipment, instruments, or material are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation by the National Institute of Standards and Technology, nor does it imply that the materials are necessarily the best available for the purpose.

spin decoupling at 400 MHz. The hydroxyl proton assignment was also confirmed by exchange with deuterium oxide.

1D ^{13}C NMR spectra of solutions of 25 mg of compound in 0.4 mL of solvent were measured at 151 MHz and 300 K, by use of a Bruker DRX-600 spectrometer, equipped with a triple resonance, triple gradient inverse probe. A spectral width of 27.1 kHz was used, together with a 16,384 point data set zero-filled to 32,768 points, a 45° pulse width of 8.35 μs, and WALTZ-16 ^{1}H decoupling. $^{13}C/^{1}H$ chemical shift correlation was performed by sensitivity-enhanced, *z*-gradient 2D HSQC [19,20] at 150.9/600.13 MHz and 300 K. Spectral widths of 22.6 and 5.21 kHz, and data sizes of 1,024 and 2,048 points were used in the F_1 and F_2 dimensions, respectively, together with 90° pulse widths of 17 and 8 μs for the ^{13}C and ^{1}H frequencies, respectively. Each free induction decay was acquired with either 4 or 24 scans, with either 16 or 48 dummy scans, respectively. An echo–anti-echo data processing protocol [20] was used with a sine-bell window in the F_1 dimension, shifted by 90°, and Gaussian multiplication in the F_2 dimension, using a line broadening of -1.45 Hz and a Gaussian truncation fraction of 0.3.

Methyl 2,6-anhydro-3-deoxy-3-phthalimido-α-D-mannopyranoside (**2**).—A mixture of methyl 2,6:3,4-dianhydro-α-D-altropyranoside (**1**) [16] (1.58 g, 10 mmol), phthalimide (1.83 g, 12.4 mmol), and potassium phthalimide (0.464 g, 2.50 mmol) was intimately ground in a mortar, dried under vacuum at 60 °C for 45 min, and then heated in an evacuated, sealed tube at 240 °C for 2 h. The resulting black residue was then extracted with CH_2Cl_2 (250 mL), the extract decolorized by addition of activated charcoal, followed by filtration through Celite, and the light amber solution washed with aliquots of water (3×175 mL). After drying (Na_2SO_4), the solution was concentrated, unchanged phthalimide was filtered off, and the solution was evaporated to dryness. The light brown solid was dissolved in hot MeOH, and the solution was decolorized again. Crystallization from MeOH, followed by washing of the crystals with cold EtOH, yielded crude **2** (1.65 g, 54%) that was homogeneous (R_f 0.6) according to TLC on silica gel G eluted with 1:2 v/v toluene–EtOAc. A further decolorization and recrystallization from EtOH gave **2** as colorless rods, mp 196–197 °C. Anal. Calcd for $C_{15}H_{15}NO_6$: C, 59.01; H, 4.95, N, 4.59. Found: C, 59.24; H, 5.17; N, 4.57.

Methyl 2,6-anhydro-3-deoxy-3-phthalimido-α-D-mannopyranoside-3-^{15}N (**2-^{15}N**).—The reaction of **1** with phthalimide-^{15}N (99 atom% ^{15}N) and potassium phthalimide-^{15}N (99 atom% ^{15}N) and isolation of the product in the same way yielded **2-^{15}N** as colorless rods, mp 195–197 °C. Anal. Calcd for $C_{15}H_{15}^{15}NO_6$: C, 58.82; H, 4.94; N, 4.90. Found: C, 58.95; H, 5.12; N, 4.57.

Molecular modeling.—Molecular modeling computations were performed with a Silicon Graphics Inc. Indigo 2 R-4400 workstation equipped with a 200 MHz CPU and MSI/Biosym Insight II/Discover software, versions 950 and 970. Three-dimensional structures were built in the MSI/Biosym Sketcher program, version 950 running on a Silicon Graphics workstation. Absolute configurations of bicyclo-octane derivatives were defined by the Insight II program. Energy computations were performed by using the AMBER forcefield with Homans' modifications [21] of the parameters for the anomeric atoms, together with a distance-dependent, effective dielectric constant of 4.0. Initially, a steepest-descent energy minimization was conducted for 1000 steps, followed by a VA09A minimization, until an rms derivative of < 0.001 was reached. Molecular dynamics with simulated annealing was then performed over the temperature range 1000–300 K, with 50 K decrements. At each temperature, 1000 equilibration steps of 1 fs each were run, followed by 5000 dynamics steps of 1 fs each, and then a VA09A energy minimization, typically with < 200 iterations. Dihedral angles and the energy value were inspected after each minimization.

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