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Gas-phase structure and conformation of the glycosidase and ceramide glucosyltransferase inhibitor *N*-benzyl deoxynojirimycin†

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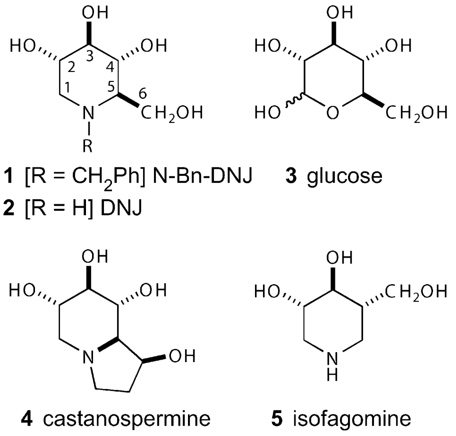
The deoxynojirimycin (DNJ) family of imino sugars are glucose analogues with an NH group replacing the oxygen atom in the pyranose ring. They are powerful inhibitors of glycosidases and ceramide glucosyltransferases. The conformation of *N*-benzyl-DNJ, isolated in the gas phase, is studied using a combination of resonant two-photon ionization (R2PI), UV/UV hole-burn, and IR ion-dip spectroscopies in conjunction with electronic structure theory calculations. Three distinct conformers, one major and two minor, are present and all three are assigned to structures in which the exocyclic hydroxymethyl group is axial to the piperidine ring (gauche- to the ring nitrogen). This contrasts with the preferred equatorial (gauche+) orientation observed for simple glucosides and may well contribute to the stronger binding of some enzymes to DNJ-based inhibitors compared to their natural glucoside substrates.

1. Introduction

N-Benzyl deoxynojirimycin (*N*-Bn-DNJ) **1**, the *N*-benzylated derivative of deoxynojirimycin (DNJ) **2**, is a mimic of glucose **3** (Scheme 1). DNJ, a member of an important family of imino sugars that function as inhibitors of glycosidases, is an inhibitor of α- and β-glucosidases.[1,2] Enzymes other than glycosidases are also inhibited by imino sugars; for example, derivatives of DNJ are powerful inhibitors of ceramide-specific glucosyltransferase[3,4] whereas DNJ itself is not an effective inhibitor of this enzyme.[5] X-ray diffraction and NMR studies of DNJ and its *N*-alkyl derivatives[6,7] have revealed differing conformational preferences. In DNJ the hydroxymethyl O(6)H group adopts an equatorial orientation with respect to the piperidine ring, possibly favoured by a weak, intramolecular NH → O6 hydrogen-bond [$r(\mathrm{N}\cdots\mathrm{O}) = 2.86$ Å, $\angle\mathrm{NH–O} = 104°$][6] but in the *N*-alkyl derivatives, where this H-bonding is no longer possible, its orientation switches to axial.[7] It has been suggested that this axial orientation is an important structural requirement for potent α-glucosidase inhibition and the consequent interference in the biosynthesis of *N*-linked glycoproteins (and anti-viral activity).[8] The enhanced potency of some α-glucosidases by castanospermine **4**, where the O(6)H is forced into an axial conformation by the fused five-membered ring, has lent support to this view.[6]

Despite the potential therapeutic uses of imino sugars, only two crystal structures of biological complexes containing DNJ as a tightly-bound inhibitor have been reported, one to an α- and one to a β-glucosidase.[9,10] In both, the O(6)H interacts with an acidic residue and is oriented axially to the piperidine ring, which is in a (distorted) $^4C_1$ chair conformation. Interestingly, both also show ordered water molecules within the active site interacting with the ring nitrogen. *N*-alkylation could serve to displace the bound water molecules, representing an entropic advantage for binding these inhibitors (an advantage also suggested for the increased potency of isofagomine[11] **5** over DNJ by Zechel and coworkers[10]); no complexes containing bound *N*-alkylated DNJ are available in the protein data bank at this time. The only available structure containing bound castanospermine shows the piperidine ring distorted into a twisted boat conformation which is suggested to be a precursor to the transition state for the cleavage of the glucoside.[12]

Molecular mechanics (MM3) and low-level *ab initio* (HF/6-31G) calculations for DNJ and castanospermine have supported the assignment of the two structures (established by diffraction and NMR measurements in the *condensed* phase) to



Scheme 1

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† Electronic supplementary information (ESI) available: Synthetic procedure for *N*-benzyl deoxynojirimycin and calculated Cartesian coordinates of its low-energy conformers. See http://www.rsc.org/suppdata/cp/b4/b413672a/

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the global minimum conformations predicted for the two molecules isolated in the gas phase.[6] To date however, there have been no experimental determinations of their conformational landscapes under isolated conditions free of environmental perturbations to compare with (higher-level) theoretical results, or of the ease of populating alternative conformers. Our recent investigations of the conformational structures of the model sugars *O*-phenyl β-D-*gluco*- or D-*galacto*-pyranoside, vaporised by heating or laser ablation and cooled in a free-jet argon expansion, probed *via* UV mass-selected resonant two-photon ionization (R2PI), resolved through 'hole-burning' techniques, and assigned through analysis of their resonant, mid-IR ion-dip spectra,[13,14] have prompted their extension to enable the first investigation of an imino sugar in the gas phase.

2. Methods

2.1. Spectroscopy

Gas-phase *N*-Bn-DNJ (synthetic details provided as electronic supplementary information (ESI)†) was produced by placing the sample in an oven attached to the expansion side of a pulsed nozzle (General Valve, 0.8 mm diameter, 4 bar argon backing pressure, 10 Hz pulse rate). The molecules, vaporized at 175 °C, were thus entrained and cooled in the pulsed expanding argon jet. The beam was skimmed and then intersected by infrared (IR) and/or ultraviolet (UV) laser beam(s) tuned to the desired wavelength(s) for excitation and ionization in the acceleration region of a time-of-flight mass spectrometer (RM Jordan) [using a resonant two-photon ionization (R2PI) scheme] enabling mass selective detection of the ions thus produced. Tuneable UV laser radiation for the R2PI experiments was generated by frequency doubling the output of a Nd:YAG-pumped pulsed dye laser [GCR-170/Laser Analytical Systems (LAS)] operated with a mixture of Coumarin 153 and Coumarin 500 dye (∼0.4–1 mJ pulse^{-1}). This laser was employed as the scanned, 'burn' laser in UV/UV hole-burn experiments: the fixed 'probe' pulse, fired ∼200 ns after the burn laser, was generated by a second, excimer-pumped pulsed dye laser (Lambda Physik FL3002), tuned to successive features of interest in the R2PI spectrum. Resonant IR ion dip scheme spectra were recorded in an analogous manner with the burn laser generating tuneable pulsed IR radiation (∼3000–4000 cm^{-1}, 0.4 cm^{-1} bandwidth, 4.5 mJ pulse^{-1}), through difference frequency mixing the output of a Nd:YAG laser (Powerlite Precision 8000) and that of a pumped dye laser (ND6000, Continuum) in a $LiNbO_3$ crystal: the UV probe pulse was provided by the LAS laser system, fired ∼150 ns after the IR pulse.

2.2. Computation

Starting structures for geometry optimization were generated using molecular mechanics (MMFF94c and MM3 forcefields) and conformation searching with the Monte Carlo multiple minimization method implemented in the MacroModel software (MacroModel v.8.5, Schrödinger, LLC);[15] 1000 structures were generated. A total of 40 conformers were selected for subsequent electronic structure theory calculations; these included all structures within 15 kJ mol^{-1} of the most stable in the conformational search, as well as other assorted structures, such as ones with the benzyl group axial to the $(^4C_1)$ piperidine ring, $^1C_4$ conformers and boat conformers. The selected conformers were further optimized using B3LYP hybrid-method density functional theory and the 6-31+G(d) basis set. Analytical frequency and thermochemistry calculations were performed on the B3LYP geometry-optimized conformations, as were single-point energy calculations, using perturbation theory (MP2) and the 6-311+G(d,p) basis set. All electronic structure theory calculations were performed using the GAUSSIAN software.[16]

3. Spectroscopy and structure

The lowest lying conformers of *N*-Bn-DNJ computed *ab initio* are shown in Fig. 1† The global minimum structure is calculated to lie at an energy 4.5 kJ mol^{-1} below its nearest neighbour, see Table 1. All the conformers are associated with a *gauche* (G−) [O6–C6–C5–N] conformation, creating an axially oriented O(6)H group (regardless of the orientation of the *N*-benzyl substituent): the lowest lying equatorially oriented structures, *gauche* (G+) or *trans* (T), all lie at relative energies ≥ 9 kJ mol^{-1} above the global minimum. The six most stable conformers differ primarily by the orientation of the benzyl ring (I *vs.* II *vs.* III) and the orientation of the co-operative 'daisy chain' sequence of hydrogen bonds formed by the hydroxyls on the ring carbons [(a) *vs.* (b): in conformers (a) they adopt a *gauche* orientation with respect to the C_{n+1} carbon, while conformers of type (b) present a *trans* orientation]. Conformers IIa,b and IIIa,b are stabilized by a hydrogen bond between OH6 and the ring nitrogen [r(OH6···N) ∼ 2.2–2.4 Å, ∠OH–N ∼115°], an interaction which is considerably weaker in the conformers Ia,b [r(OH6···N) ∼ 2.8–3.0 Å, ∠OH–N ∼95°] because of the position of the benzyl ring; on the other hand conformer Ia, the global minimum structure, displays the longest 'daisy chain' sequence of hydroxyl H-bonds.

Fig. 2 shows the one-colour R2PI spectrum of jet-cooled *N*-Bn-DNJ (top trace) and below it three distinct UV hole-burn spectra, labeled α, β and β′; they account for all of the intense peaks observed in the R2PI spectrum and correspond to three different conformers of the molecule. The UV contour and vibronic spacings displayed by the principal conformer, α, are quite distinct from the (nearly identical) spectra displayed by the two minor conformers, β and β′. In addition to their $S_1 \leftarrow S_0$ origin bands, centred around 37 280 cm^{-1} (α) and 37 500 cm^{-1} (β/β′), all three hole-burn spectra display a series of more intense vibronic bands located ∼530 cm^{-1} higher in energy (associated with the excitation of a benzyl ring mode, a feature commonly seen in one colour R2PI spectra of compounds containing this chromophore[17]). Each band accommodates a series of much lower frequency vibronic progressions with spacings of ∼37.5 and 41 cm^{-1} (conformer α) and ∼21 and 24 cm^{-1} (conformers β and β′). These spectra suggest that conformers β and β′ are extremely similar to each other while conformer α is somewhat different.

The mass of *N*-Bn-DNJ is 253 u but in all three conformers more than 90% of the observed ion signal is at mass 222, a loss of 31 u. Spectra measured in the fragment and parent ion mass channels show the same features, though conformers β and β′ have a greater ratio of fragment to parent ions than conformer α. The fragmentation, in all probability, corresponds to the cleavage of the C5–C6 bond and loss of the hydroxymethyl

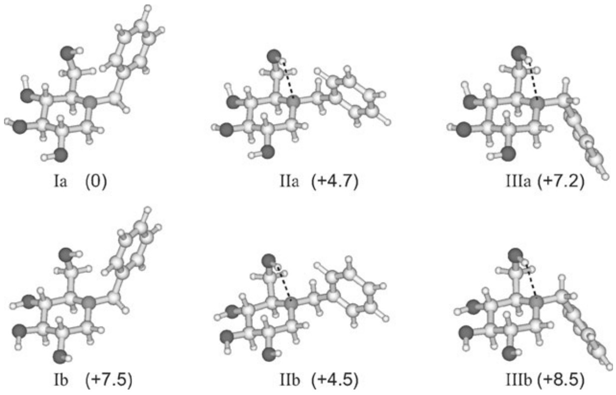

Fig. 1 Six lowest-energy conformers of *N*-benzyl deoxynojirimycin (*N*-Bn-DNJ) optimized at B3LYP/6-31+G(d). Relative energies are given in parenthesis in kJ mol^{-1} at the MP2/6-311+G(d,p)//B3LYP/6-31+G(d) level of theory and include zero-point energy corrections.

Table 1 Relative energies (in kJ mol^{-1}) of low-energy conformers of *N*-Bn-DNJ calculated at the MP2/6-311+G(d,p)//B3LYP/6-31+G(d) level of theory

Name	Conformation[a]	U	E_0	$T\Delta S$(175 °C)	ΔG(175 °C)
Ia	*ggg*G-g+_I	**0.0**	**0.0**	0.0	1.3
IIa	*ggg*G-g+_II	5.3	4.7	6.7	**0.0**
IIIa	*ggg*G-g+_III	6.1	7.2	0.7	7.8
Ib	*ttt*G-g+_I	8.7	7.5	1.9	7.7
IIb	*ttt*G-g+_II	5.1	4.5	6.2	0.3
IIIb	*ttt*G-g+_III	7.9	8.5	0.8	9.2

[a] The naming system used follows that used in other reports on sugar molecule conformation (see, for example, refs. 13 and 14). The letters refer to the position of the rotatable dihedral angles. The first three letters (all *ttt* or *ggg* here) indicate the *trans* or *gauche* orientations of the H_n–O_n–C_n–C_{n+1}, $n = 2$–4 dihedral angles. The next (capital) letter indicates value of the O_6–C_6–C_5–O_5 dihedral which defines the position of the hydroxy methyl group (all conformers here are *Gauche−*: angles between 0° and 120°). The following letter indicates the C_5–C_6–O_6–H_6 dihedral (all here are *gauche+*: angles between 240° and 360°). The final roman numerals refer to the orientation of the benzyl ring.

radical. Although the benzyl ring is ionized in the R2PI scheme used, the ionization energy of tertiary amines is ~1 eV lower than benzyl compounds[18] and migration of the charge from the benzyl ring chromophore to the amine will endow the molecular ion with significant internal energy and promote its fragmentation, further promoted by excitation of the ring deformation mode at ~530 cm^{-1}. The difference in fragmentation ratios among the conformers may simply be due to the larger excess energy in the vibrationally excited ions, but it may also be due to differences in Franck–Condon overlap between the excited and ionized states in the different conformers α and β/β′.

Ion-dip spectra of the three conformers, measured in the O–H stretching region of the infrared are shown in Fig. 3. In each case all four O–H modes are visible although the central pair lie very close in energy and therefore appear as a partially resolved doublet. The close similarity between the minor conformers, β and β′, is again apparent; each one presents a very similar vibrational spectrum with the O–H band at lowest wavenumber more strongly shifted than for conformer α.

The O–H stretching modes are very sensitive to the local environment and provide good indicators of conformation. In the monosaccharides[13,14] *O*-phenyl β-D-*gluco*- and D-*galacto*-pyranoside, vibrational bands appearing in the region 3630–3650 cm^{-1} are associated with a sequence of weakly bonded hydroxyl groups arranged in an equatorial fashion around the pyranoside sugar ring. Stronger H-bonded interactions shift the O–H bands to lower wavenumber and broaden them; those associated with relatively 'free' (not H-bond donating) OH groups appear at slightly higher wave number. All three conformers of *N*-Bn-DNJ exhibit one relatively free O–H mode, two bands consistent with a weakly hydrogen bonded and one band associated with a more strongly bonded OH group. In conformers β and β′, particularly, this latter band is considerably shifted (~80 cm^{-1} below the central doublet) indicating a strong perturbation of one of the OH stretches in both these conformers.

As the hydroxyl groups attached directly to the ring (in its $^4C_1$ chair conformation) are geometrically constrained and

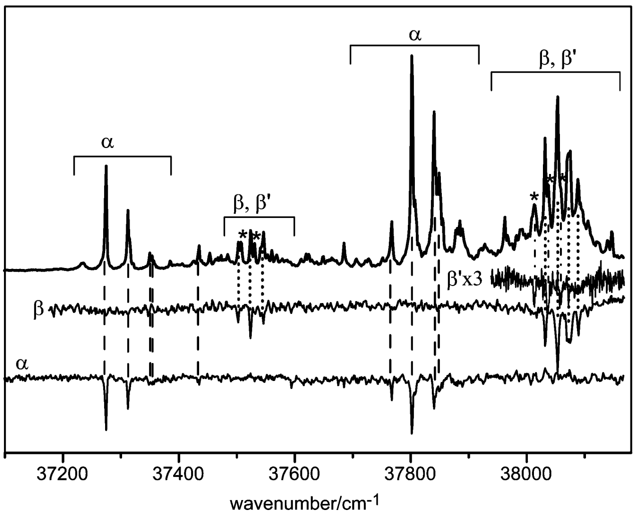

Fig. 2 Resonant two-photon ionization (R2PI) and UV/UV hole-burn spectra of *N*-Bn-DNJ. Peaks marked with * burned with β′.

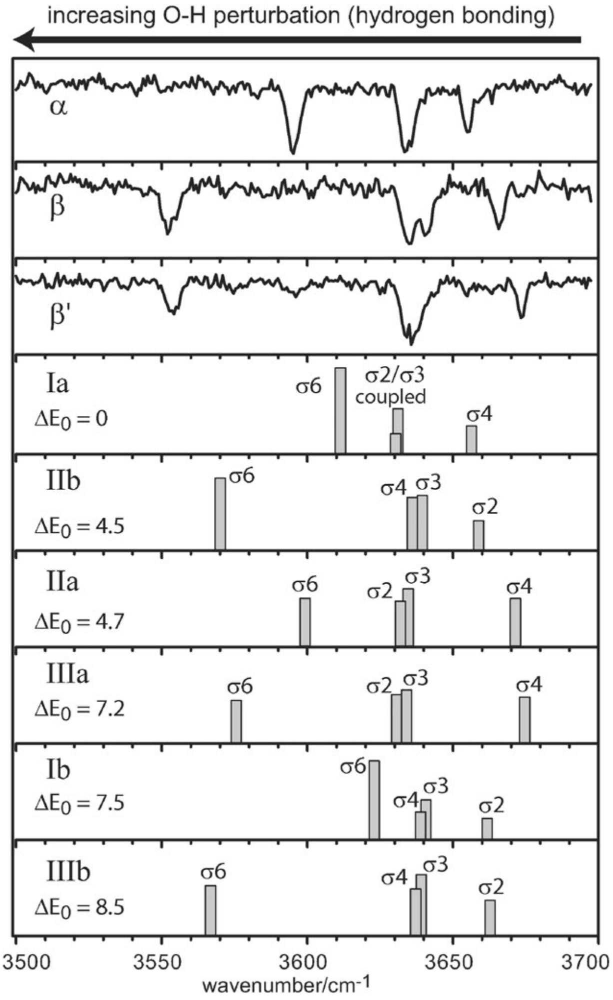

Fig. 3 Experimental and computed infrared ion-dip spectra of *N*-Bn-DNJ in the O–H stretching region. Computed bands are labeled with the (C_n-) O–H stretching motion to which they correspond according to the normal coordinate analysis.

cannot form strong interactions with each other, this implies a strong interaction between the hydroxymethyl group and most likely, the ring nitrogen in the two conformers, β and β′.§

Infrared spectra calculated for each of the six lowest-lying conformers (see Fig. 1) are also shown in Fig. 3. Conformers Ia and Ib, differing in the orientation of the ring hydroxyl groups (note the effect on the relative positions of the alternately 'free' or hydrogen-bonded O–H stretch modes, σ4 and σ2, in each one of the three 'a,b' conformer pairs) and conformer IIa all present very similar IR spectra and each could provide a reasonable match for the recorded spectrum of the principal conformer α. On balance however, its assignment to the structure Ia is strongly favoured for the following reasons:

(i) Calculations at 0 K place conformer Ia at the global minimum,¶ lying 4.5 kJ mol^{-1} below the energy of conformer IIa and 7.5 kJ mol^{-1} below Ib.

(ii) There is a very close correspondence between the spacings in the vibronic progressions in its R2PI spectrum, ~37.5 and 41.0 cm^{-1} (in the S_1 state) and the in-plane and out-of-plane benzyl ring wag modes of conformer Ia calculated (in the S_0 state) at 37.6 and 42.3 cm^{-1}. The equivalent lowest wavenumber modes in conformer IIa are calculated at 20.6 and 24.1 cm^{-1}.

The assignment of the two minor conformers, β and β′, is less straightforward; the strong shift of the lowest wavenumber OH band provides an important clue but *each* one of the conformer pairs IIa/b and IIIa/b exhibits a hydrogen bond between the hydroxymethyl group and the ring nitrogen which significantly lowers the calculated wave number of the O–H6 band, σ6. Nonetheless, one of the remaining conformers, β or β′, is most likely to be associated with the conformation IIb, since this is the second most stable conformer at 0 K and its calculated IR spectrum provides an excellent match to the measured IR spectra of both β and β′. Assignment of the partner conformer to the structure IIIb (which differs from IIb only in the orientation of the benzyl group) is tempting but at 0 K it is calculated to lie ~4 kJ mol^{-1} higher in energy than conformer IIa [and at the temperature of the oven IIa becomes the *lowest* energy conformer while IIIb is the highest of the six (Table 1)]. Assignment of conformers β/β′ to the IIa/IIb pair is favoured by their similar low frequency vibration/internal rotation modes (calculated to be a twist of the benzyl group) lying at 24.1 and 20.6 cm^{-1}, respectively (in the ground state, S_0); these match very well the measured (S_1) vibronic progressions of 24 and 21 cm^{-1}. In contrast, normal mode analysis of the conformers IIIa/b show the lowest energy mode (also a benzyl twist though with some benzyl wag coupled in) at 34.5 and 35.3 cm^{-1}. Additional evidence favouring assignment to the IIa/IIb pair is provided by the extreme similarity of their R2PI spectra. The environment of the benzyl ring chromophore of conformer IIb is closer to that of conformer IIa (which differs in the orientation of the chain of hydroxyl groups) than conformer IIIb (which differs in the orientation of the benzyl ring). On balance, therefore the assignment of β and β′ to conformers IIa/IIb is the most favoured. Most importantly, both the major (α) and the two minor (β, β′) conformers are associated with structures in which the OH6 group is *axial* (G−) to the ring nitrogen.

The conserved G− orientation of the hydroxymethyl group of *N*-Bn-DNJ differs from the preferred orientation in simple glucosides. In 1-*O*-phenyl β-D-*gluco*-pyranoside, only ~25% of the observed population adopts a G− orientation; the most populated conformation (~70%) is G+.[13] In the corresponding *galacto*-pyranoside, the preference for G+ is even stronger (~90%).[14] This innate preference for the G− orientation in the imino sugars may well contribute to their strong binding compared to the natural glucoside substrate. Although the present investigation has focused on a neutral *N*-alkylated imino sugar, which is not thought to be protonated in its anti-ceramide glucosyltransferase activity, the ring nitrogen atom of DNJ-based inhibitors is probably protonated in the active sites of glycosidases.[5] A study of the conformation of protonated DNJ in the gas phase may provide further insight into the mechanism of such inhibition.

Acknowledgements

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§ The hydroxymethyl group could also form a strong hydrogen bond with O4, forming a *ggg*Tt structure. This would also result in a single O–H mode which is shifted to lower wavenumbers, but IR ion-dip spectra of the monosaccharide mannose (manuscript in preparation) in which this conformer is seen, show this mode to be ~35 cm^{-1} higher in energy than that measured for *N*-Bn-DNJ. Also, the most stable *ggg*Tt conformer is calculated to be 4.5 kJ mol^{-1} (about 390 cm^{-1}) higher in energy than conformers IIa and b.

¶ Conformer Ia benefits from an extended chain of hydrogen bonds encircling the piperidine ring; the stability conferred by this arrangement has become a recurring theme in gas phase studies of analogous sugar molecules [see refs. 13, 14]. The entropic constraint provided by this arrangement disfavours conformation Ia at higher temperatures; free energy calculations (harmonic approximation, treating internal rotations as vibrations) place conformer Ia slightly higher in energy than conformer IIa (by 1.5 kJ mol^{-1} at 448 K, the temperature of the oven used to vaporize the sample).

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