Glycoconjugates

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# RDC-Enhanced NMR Spectroscopy in Structure Elucidation of Sucro-Neolambertellin**

*Anne Schuetz, Takanori Murakami, Noboru Takada, Jochen Junker, Masaru Hashimoto,* and Christian Griesinger**

In the course of mechanistic investigations of mycoparasitism by *Lambertella* species the novel glycoside sucro-neolambertellin (**1**) was found. In this work, the configuration of **1** (Figure 1) was determined using NMR spectroscopy. Whereas

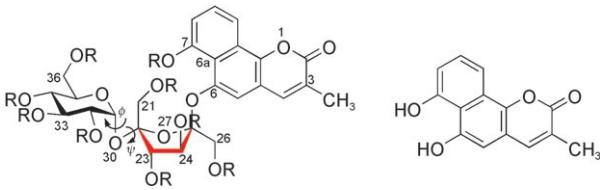


***Figure 1.*** Sucro-neolambertellin (**1**) with correct configuration of the central five-membered ring (C22 to C25 as indicated in red: *RSSR*). The dihedral angles around the glycosidic linkage are defined as $\phi$: H31-C31-O30-C22 and $\psi$: C31-O30-C22-O27. The existence of the chromophore neolambertellin (**3**) in the molecule was deduced from the UV spectrum.

the conventional NMR spectroscopic parameters, namely *J* couplings and NOEs, did not suffice to determine the configuration, we report herein the additional use of residual dipolar couplings (RDCs) together with a structure calculation protocol that uses floating chirality to unequivocally establish the configuration of **1**. RDCs are inherently different restraints from *J* couplings and NOEs, and therefore restrict conformation and configuration more than NOEs and *J* couplings alone. While this difference could be shown recently for examples on cyclic systems with naturally restricted conformations, the expansion to open-chain systems underpins the general applicability of the approach.[1] With the established configuration in the central furanose ring, sucro-neolambertellin is the first natural product identified to have a sucrose moiety that is oxidized at the 5′-carbon atom of the fructose group.

Sucro-neolambertellin was discovered within culture broth of *Lambertella* sp. 1346[2] during studies of the relationship between the production of lambertellols,[3] candidate substances of mycoparasitism on apple fruit, and the culture conditions.[4]

Negative fast atom bombardment mass spectroscopy (negative-FABMS) of **1** provided a pseudomolecular ion signal $[M-H]^-$ at $m/z$ 581.1525, thus implying that the molecular formula is $C_{26}H_{29}O_{16}$. The photo diode array (PDA) spectrum of **1** as well as $^1$H and $^{13}$C NMR spectra (Supporting Information) identify neolambertellin (**3**[5], Figure 1) as the aglycon.

The $^1$H NMR spectra reveal a unique hexofuranose moiety carrying an α-D-glucopyranoside unit at C22 (the numbering is shown in Figure 1). The α-D-glucopyranoside unit was identified from the characteristic *J* coupling pattern of the protons from C31 to C36. The absolute configuration was established by acidic hydrolysis, perbenzoylation, and circular dichroism (CD) spectroscopy. The constitution of the linkage between the aglycon and the hexofuranose moiety was established in the following way: The C7−OH group was assigned by observing HMBC correlations between this exchangeable proton in [D$_6$]DMSO at $\delta = 10.28$ ppm and $^{13}$C resonances for C6a and C7. As there is no C6−OH resonance in the $^1$H NMR spectra, the hexofuranose moiety must be connected through the C6 oxygen atom to the neolambertellin aglycon.

More information concerning the configuration of the five-membered ring could not be derived owing to signal overlap. Therefore, **1** was converted into its nona-acetate **2**, which considerably reduced the signal overlap in the proton dimension. All NMR spectroscopic analyses and structure calculations were then performed on the peracetate **2** (Figure 1). At this stage the absolute configuration of the glucose unit and the connectivity of the three moieties neolambertellin, hexofuranose, and α-D-glucose are clear,

[*] T. Murakami, N. Takada, M. Hashimoto
Faculty of Agriculture and Life Sciences
Hirosaki University
3-Bunkyo-Cho, Hirosaki, 036-8561 (Japan)
Fax: (+81) 172-39-3782
E-mail: hmasaru@cc.hirosaki-u.ac.jp
A. Schuetz, J. Junker,[+] Prof. C. Griesinger
Abteilung für NMR-basierte Strukturbiologie
Max-Planck-Institut für Biophysikalische Chemie
Am Fassberg 11, 37077 Göttingen (Germany)
Fax: (+49) 551-201-2202
E-mail: cigr@nmr.mpibpc.mpg.de
[+] Present address: Fundção Oswaldo Cruz—CDTS
Rua Sizenando Nabuco 100, CEP 21040-250 Rio de Janeiro, RJ (Brazil)

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whereas the configuration of the stereocenters of the hexofuranose unit need to be established.

The *trans* configuration of the ring protons H23 and H24 ($^3J_{H,H} = 9.7$ Hz) in combination with the similar chemical shifts of the carbon atoms and protons, which are related by a pseudo $C_2$-symmetry axis through the middle of the C23–C24 bond and O27, is compatible only with four configurations *RRRR, RSSR*, *SRRS*, and *SSSS* (where for example, *RRRR* means C22/*R*,C23/*R*,C24/*R*,C25/*R*).[6] Thus, the challenge was to find the correct configuration for these stereocenters that are remote from the known stereocenters of the α-D-glucose unit. Therefore, long-range structural restraints relating conformation and configuration of the hexofuranose moiety to the known α-D-glucopyranoside unit were required. Such restraints are provided by quantitative NOEs as well as by RDCs. The latter reflect orientations of bond vectors in molecules relative to a global-alignment tensor.[7] They have only recently become accessible for natural compounds through the availability of alignment media that are compatible with organic solvents such as DMSO.[8]

Quantitative NOE build-up curves for eight different mixing times varying from 55 to 1000 ms were derived from a NOESY experiment that suppresses zero-quantum artifacts almost completely.[9] The build-up curves for protons with fixed distances were compatible with correlation times that varied by less than 20 %. This would be either compatible with more or less free motion of all three moieties with respect to each other or a rather rigid molecule. Since there are 18 specific NOEs between the 3 moieties, it was concluded that the molecule shows little internal dynamics. A total of 49 NOEs were integrated. Owing to signal overlap and strong coupling, 22 were treated as unambiguous and the rest as ambiguous restraints.

Although only the four configurations for the hexofuranose ring mentioned above need to be distinguished, structure calculations with floating chirality[10–13] for all centers in the furanose ring were performed by using X-PLOR NIH[14] with the above-mentioned ambiguous and unambiguous NOEs (see also the Supporting Information). Although floating chirality protocols are frequently used for proteins, so far they have rarely been applied to small molecules. Whereas the stereocenters in the hexofuranose ring were left floating, those of the α-D-glucopyranoside moiety were fixed. For each of the 16 possible configurations the energy penalty associated with NOE violations (averaged over the 10 lowest-energy structures) is shown as filled bars in Figure 2. The NOE violations are not significant enough to single out one configuration. Therefore, the structures for all 16 configurations were subjected to cross-validation filtering by RDCs.

RDCs $D(C_iH_i)^{exp}$, which are the dipolar couplings between carbon and proton *i*, were obtained by aligning **2** in three different polymer-based media (listed in the Supporting Information). RDCs were extracted from $t_2$-coupled $^1$H and $^{13}$C HSQC spectra by superimposing and fitting $\omega_2$ traces from isotropic and anisotropic spectra. The RDCs were used to cross validate the above-mentioned 10 structures for the 16 different configurations using the SANI module[15,16] from X-PLOR with the standard force constant of 50 kcal mol$^{-1}$ Hz$^{-2}$ that is also used for protein structure calculations. The derived RDC energy penalty for each configuration is given as open bars in Figure 2.

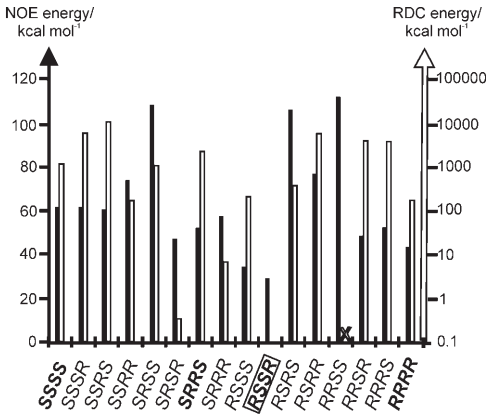


***Figure 2.*** Energy penalties associated with NOE and RDC violations. The configurations that are in agreement with the H23 and H24 $^3J_{H,H}$ coupling and the symmetry of chemical shifts are given in bold letters. The **x** denotes that for this configuration, the RDC energy minimization failed. The optimal configuration is highlighted within a box.

The three configurations *SRRR*, *SRSR*, and *RSSR* have the lowest RDC energies of less than 10 kcal mol$^{-1}$. Only the *RSSR* configuration is among those possible as determined from the chemical-shift symmetry and the *trans* configuration of H23 and H24. It should be mentioned that the *RRRR* and *SRRS* configurations are incompatible with the RDCs, whereas they could not be excluded by the NOE analysis. The *RSSR* configuration suggests that the hexofuranose ring is derived from D-fructose.

The 10 lowest-energy structures found for the *RSSR* configuration have an average pairwise root mean square deviation (rmsd)[17] of only 0.106 Å (Figure 3), which is not surprising because of the similar correlation times and the abundant NOEs between the three moieties. For the conformation of the C22–C31 glycosidic linkage we find $\phi = -70.1°$ and $\psi = -63.7°$, which matches extremely well with the values $\phi = -55°$and $\psi = -58°$ obtained for sucrose in DMSO.[18] The H31 and C22 $^3J_{C,H}$ coupling constant measured by HMBC[19] suggests $\phi = \pm 55°$ or $\pm 117°$ using a Karplus calibration.[20]

In conclusion, the conformation and the absolute configuration of sucro-neolambertellin could be determined from NMR spectroscopy, chemical degradation, and CD spectroscopy. From the chemical shifts and the *trans* configuration of the H23 and H24 protons, only four configurations are feasible: *RRRR*, *RSSR*, *SRRS*, and *SSSS*. The quantitative NOE analysis does not distinguish between these. The cross-validation filtering against RDCs (Figure 4) clearly, and with a large margin, isolates *RSSR* among the other possibilities. This result is also biosynthetically sensible because this moiety could then be derived from the natural D-fructose. We believe that with the described methodology, the configuration of a wide range of natural compounds can be determined and the requirement for stereoselective synthesis to prove configuration can be reduced. In addition, the cross-validation filtering of conformations and configurations by

  

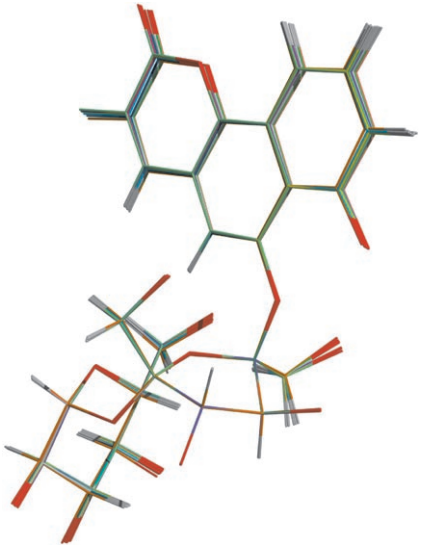

***Figure 3.*** The 10 conformers of **2** (*RSSR* configuration) with lowest energies found in floating chirality calculations (acetate moieties are not shown). The structures have been superimposed on the carbon atoms of the furanose ring. The average pairwise rmsd[17] is 0.106 Å.

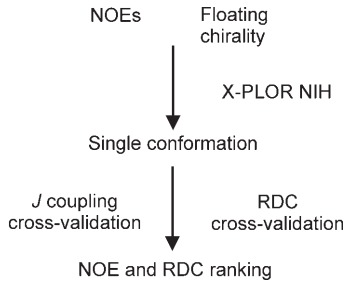


***Figure 4.*** Schematic representation of the procedure used for RDC-enhanced structure elucidation of sucro-neolambertellin (**1**).

RDCs is not limited to rather rigid molecules but can also be applied to more flexible molecules.[21] Also, in light of reference [22], RDCs may also prove to be a sensitive NMR spectroscopic parameter to detect differences in configurations of synthetic and natural products with very similar chemical shifts.

## *Experimental Section*

Experimental procedures regarding the isolation of **1**, its derivatization, CD and NMR spectroscopic measurements, preparation of alignment media, and details of NOE analysis as well as structure calculation are given in the Supporting Information.

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