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COMPLETE STEREOSTRUCTURE OF NYSTATIN A_1: A PROTON NMR STUDY

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Summary: *Using the sugar D-mycosamine as an internal chiral probe for nmr spectroscopy, the absolute configuration at the asymmetric centers of the C10-C19 fragment of nystatin A_1, as well as the ß-configuration of the D-mycosamine unit, were assigned by a combination of 2D-proton phase-sensitive DQF-COSY, NOESY and ROESY experiments.*

In previous reports[1,2,3], the 3R, 5R and 7R absolute configurations of the C1-C10 fragment of nystatin A_1 were demonstrated on the basis of spectroscopic and chemical evidence. The structure of the sugar D-mycosamine has been known for some time[4], and the pioneering work of Borowski et al.[5] settled the absolute configuration of the C33-C37 fragment. We now report the determination of the eight remaining asymmetric centers based on recent 2D-proton nmr experiments on the natural antibiotic. The feasibility of this approach was based on the idea that the macrocyclic ring would be a rather ridig molecular assembly for at least two reasons: the C13-C17 portion is known to be cyclized in a tetrahydropyran ring in solution[6], and a restrained geometry is imposed by the C20-C33 polyene section although some considerable flexibility can be brought about by the C28-C29 saturated carbons. Once the relative configuration is set up, unambiguous proton nmr-derived distance constraints between the D-sugar and the macrocycle would then establish the absolute configuration.

Configurational and conformational features of nystatin A_1 are all derived from scalar and dipolar coupling connectivities extracted from phase-sensitive DQF-COSY[7] (15 mM DMSO-d_6 or 4 mM CD_3OD solutions), ROESY (4 mM CD_3OD solutions) and NOESY (15 mM DMSO-d_6 solution) experiments[8,10].

Information gathered from the phase-sensitive DQF-COSY is given on the Table below. The proton resonances of a CD_3OD solution of nystatin A_1 were previously assigned[11] and are confirmed here in a DMSO-d_6 solution. The phase-sensitive version allows, in this case, the assignment of the scalar coupling constants although a complete extraction of the J couplings was not possible for the methylene protons at C4, C6, C8, C9, C28 and C29 due to the complexity of the methylene region (1.4-2.4 ppm).

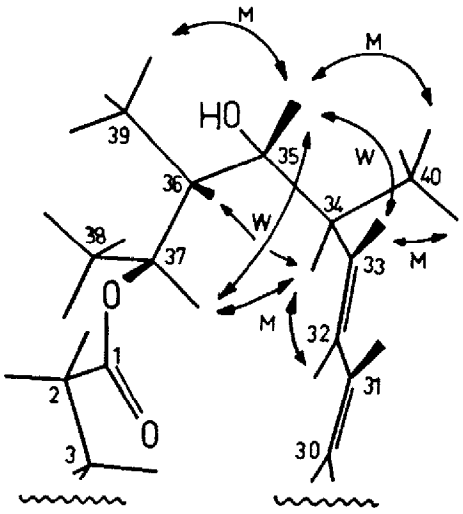
COSY (see Table) and NOESY-ROESY (Scheme 1) connectivities found for the C33-C37 segment fully confirmed the previous assignment[5] based on degradation studies.

The relative configuration of the C13-C17 region could easily be assigned from the COSY data (see Table) where substituents at C15, C16 and C17 are all equatorially disposed in a chair conformation ($J_{14e,15}$ 4.5 Hz, $J_{14a,15}$ 10.5 Hz, $J_{15,16}$ 9.5 Hz and $J_{16,17}$ 11 Hz).

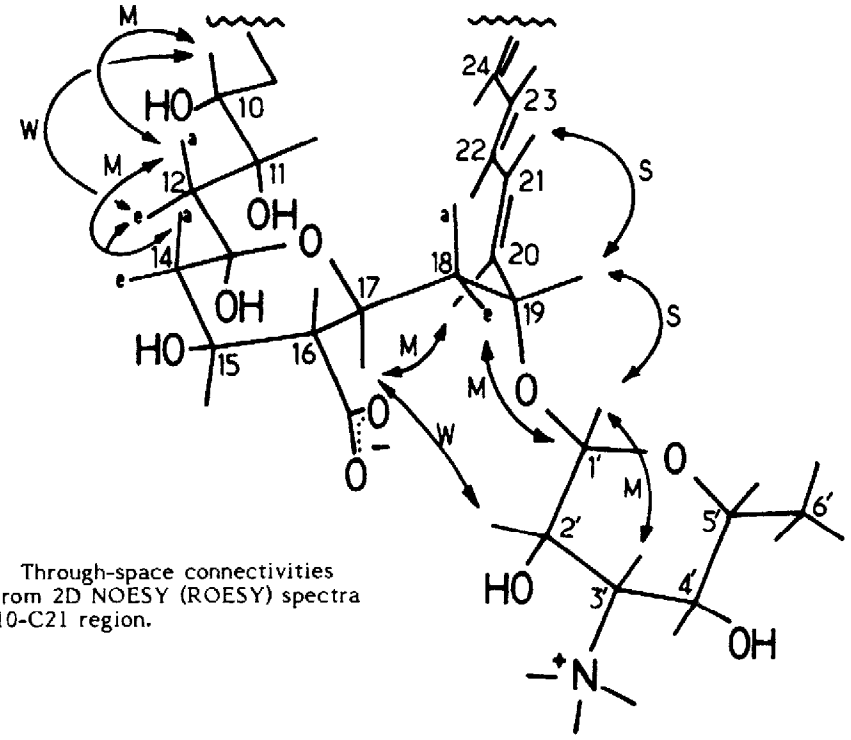
Definition of the local conformation of the C17-C21 portion, crucial for the determination of the absolute configurations because of the proximity of this structural segment to the D-sugar,

Proton	δ, ppm	Coupling partner (J, Hz)
8-9	1.45-1.55	
10	3.23	H9 (3)', H9" (8.5), H11 (4)
11	3.91	H10 (4)', H12a (1), H12e (10.5)
12a	1.55	
12e	1.69	
14a	1.17	H14e (12.5), H15 (10.5)
14e	1.89	H14a (12.5), H15 (4.5)
15	3.96	H14a (10.5), H14e (4.5), H16 (9.5)
16	1.89	H15 (9.5), H17 (11)
17	3.98 (4.26)*	H16 (11), H18a (8), H18e (1)
18a	1.70 (1.68)*	H17 (8), H18e (13), H19 (1.5)
18e	1.86 (2.11)*	H17 (1), H18a (13), H19 (6)
19	4.36	H18a (1.5), H18e (6), H20 (7.5)
20	5.77	H19 (7.5), H21 (15)
21	6.23 (6.17)*	H20 (15), H22 (10.5*)
22	6.25 (6.31)*	
33	5.52	H32 (15), H34 (8.5)
34	2.255	H33 (8.5), H35 (8.5) 40CH_3 (6.5)
35	3.14	H34 (8.5), H36 (4)
36	1.81	H35 (4), H37 (4.5), 39CH_3 (6.5)
37	5.08	H36 (4.5), 38CH_3 (6.5)
1'	4.51	H2' (0-1)
2'	3.73	H1' (0-1), H3' (3.5)
3'	2.77	H2' (3.5), H4' (9.5)
4'	3.12	H3' (9.5), H5' (9.5)
5'	3.15	H4' (9.5), 6'CH_3 (6.5)
6'CH_3	1.16	H5' (6.5)

Table: Selected proton assignments from phase-sensitive DQF-COSY of nystatin A_1 (DMSO-d_6, 298°K; solvent as internal reference = 2.49 ppm); *in CD_3OD (CD_3OH as internal reference = 4.82 ppm).



Scheme 1: Through-space connectivities obtained from 2D NOESY (ROESY) spectra for the C32-C37 region. For S, M, W see Reference 12.



Scheme 2: Through-space connectivities obtained from 2D NOESY (ROESY) spectra for the C10-C21 region.

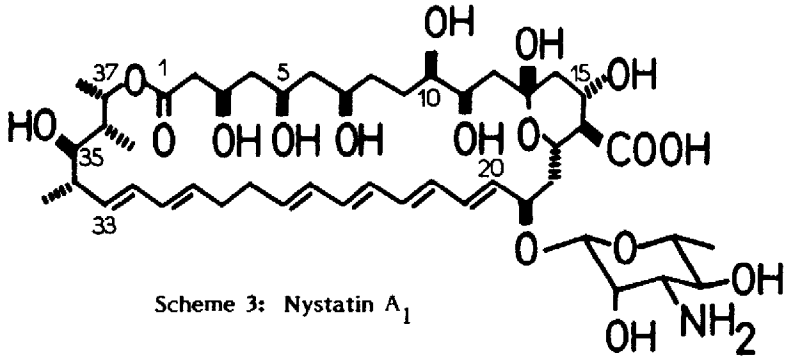
was derived from COSY and NOESY(ROESY) data. Analysis of a COSY experiment in CD_3OD gave the complete ^1H-^1H coupling pattern of the C17-C21 fragment ($J_{17,18a}$ 8 Hz, $J_{17,18e}$ 1 Hz, $J_{18a,19}$ 1.5 Hz, $J_{18e,19}$ 6 Hz, $J_{19,20}$ 7.5 Hz and $J_{20,21}$ 15 Hz) which shows the topicity of the protons at C18 (H18a,18e are not resolved in a DMSO-d_6 solution), hence the orientation of the OH at C19 (Scheme 2). This configurational assignment was validated by the analysis of the NOE(ROE) connectivities depicted in Scheme 2, especially for the proton pairs H17-H20 (M)[12] and H19-H21 (S). This led us to the relative configurations R/S (S/R) for C17-C19 and at this stage, the conclusion was reinforced by COSY experiments conducted under similar conditions (DMSO-d_6, 313°K) on amphotericin B (17S, 19R configurations) which furnished very similar coupling patterns in the C17-C21 portion.

Taking into account the overall geometry imposed by the macrocycle for the C1-C13 polyol chain, the relative configurations at C10 and C11 could be given as syn based on the COSY data (Table, $J_{9,10}$ 3 Hz, $J_{9',10}$ 8.5 Hz, $J_{10,11}$ 4 Hz, $J_{11,12e}$ ~1 Hz and $J_{11,12a}$ 10.5 Hz). At this point these two centers had to be connected to the C13-C17 cyclic portion of the molecule and the inter-residue contacts were derived from the NOESY spectrum (DMSO at 298°K). Starting from H14a, four cross-peaks can be labeled with confidence: H14a-H12a (M), H12a-H10 (M), H14a-H12e (W), and H12e-H10 (W). The intensity of the cross-peaks defines the diastereotopicity of the protons at C12 and the results imply that H10 is on the same face of the molecule as H14a allowing the configuration given in Scheme 2. The H12a, H12e and H14e protons are unfortunately too close together (see Table) so that the H12e-H14e NOE connection is lost in the diagonal peaks.

After the C10 to C19 relative stereostructure was set up, we turned our attention to the through-space contacts between the D-mycosamine residue and the macrocycle. The NOE map first established the unknown β-configuration of the anomeric linkage (H1'-H3', H3'-H5' connectivities) of the D-mycosamine in a $^4C_1$ conformation. Dipolar coupling connectivities between H1'-H19 (S) and H1'-H18e (M) define well the space location of the anomeric proton H1' relative to nystatin A_1 aglycone. *A third NOE — H2'-H17 (W) — observable in ROESY (CD_3OD solution) and NOESY (DMSO-d_6 solution) at various mixing times, then requires that the configuration at C19 be R.* Further, the interactions found satisfy the exo-anomeric effect and make possible the electrostatic interaction of the NH_3^+ at C3' and the COO^- at C16.

By imposing the proximity of H1' to both H19 and H18e as found in NOESY and ROESY, no reasonable conformer would place H2' close enough to H17 in modelling the interactions of the D-sugar with an enantiomeric aglycone. In this situation, cross-peaks should be detected rather between H5' (and/or 6'CH_3) and H17 (and/or H18e).

Based on this study, the 10R, 11R, 13S, 15S, 16R, 17S, 19R, 1'R configurations are assigned for nystatin A_1 (Scheme 3). It is interesting to note that with the experimental protocols used, the structural assignment only required an intermediate field spectrometer (300 MHz ^1H). The asymmetric centers found for the C13-C19 structural segment of nystatin A_1 are the same as those found in amphotericin B and, in closely related polyenes (pimaricin, tetrins, partricins...), a similar chiral pattern may be anticipated as well. A detailed comparison of the spectral data of amphotericin B and nystatin A_1 revealed conformational differences with a possible biological significance. We plan to evaluate these differences by using mechanical and/or restrained dynamic calculations.



Scheme 3: Nystatin A_1

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References and notes

1. J.-M. Lancelin, F. Paquet and J.-M. Beau, Tetrahedron Lett., 29 (1988) 2827-2830.
2. J. Prandi and J.-M. Beau, preceding paper in this issue.
3. K.C. Nicolaou and K.H. Ahn, Tetrahedron Lett., 30 (1989) 1217-1220.
4. J.D. Dutcher, D.R. Walters and D. Wintersteiner, J. Org. Chem., 28 (1963) 995-999; M. Von Saltza, J.D. Dutcher, J. Reid and O. Wintersteiner, J. Org. Chem., 28 (1963) 999-1004.
5. E. Jereczek, P. Sowinski, J. Zielinski and E. Borowski, Inst. Nucl. Phys. Cracow Rep., (1973) 232-240.
6. R.C. Pandey and K.L. Rinehart, Jr., J. Antibiotics, 24 (1976) 1035-1042.
7. U. Piantini, O.W. Sørensen and R.R. Ernst, J. Am. Chem. Soc., 104 (1982) 6800-6801; D. Marion and K. Wüthrich, Biochem. Biophys. Res. Commun., 113 (1983) 967-974.
8. ^{1}H-N.m.r. spectroscopy was performed at 300 MHz on a Bruker AM series spectrometer equipped with an Aspect 3000 computer. Resonance and coupling assignments were achieved with the aid of the double-quantum filtered (DQF) COSY, NOESY and/or ROESY spectra, run in the phase-sensitive mode with time-porportional phase incrementation (TPPI)[9].
9. R.R. Ernst, G. Bodenhausen and A. Wokaun, Principles of Nuclear Magnetic Resonance in One and Two Dimensions, Oxford: Claredon, 1987; H. Kessler, M. Gehrke and C. Griesinger, Angew. Chem. Int. Ed. Engl., 27 (1988) 490-536.
10. Nystatin in saturation at room temperature in CD_3OD was found to be about 4 mM. No longitudinal NOEs were found under these conditions by NOESY experiment ($\tau_c \simeq 1.12\ \omega_o^{-1} \simeq 0.5$ ns), while negative longitudinal NOEs could be obtained in DMSO-d_6 ($\tau_c >> 0.5$ ns), characteristic of a decrease of the molecular motion.
11. J.M. Brown, A.E. Derome and S.J. Kimber, Tetrahedron Lett., 26 (1985) 253-256.
12. Classification of the inter-residue NOE(ROE) cross-peak intensities are as follows: S (strong), M (medium) and W (weak); the proton pair H1'-H3' in the D-sugar is taken as a reference for medium intensity (M, 2.7 Å). Distance constraints imposed by nmr observations need not be precise in order to define a reasonable conformational domain.

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