J Chem Crystallogr (2009) 39:494–499
DOI 10.1007/s10870-008-9506-8

ORIGINAL PAPER

Synthesis and X-ray Structure of (2*R*, 3*R*, 4*R*, 5*R*)-3,4,5-*Tris*-Benzyloxy-2-Benzyloxymethyl-Piperidin-1-ol, the *N*-Hydroxy-Analogue of 2,3,4,6-*Tetra*-*O*-Benzyl-1-Deoxymannojirimycin

Emilie Racine · Christian Philouze · Sandrine Py

Received: 7 October 2008 / Accepted: 13 November 2008 / Published online: 11 December 2008

Abstract The title compound **8** was obtained for the first time by hydride-mediated reduction of the D-fructose-derived nitrone **9**. The *N*-hydroxy piperidine **8** is a precursor of the *N*-hydroxy analogue of DMJ, a potent inhibitor of α-mannosidases. It was isolated as colorless crystals (triclinic, *P*1 space group) exhibiting the following cell parameters: $a = 9.947(2)$ Å; $b = 12.155(2)$ Å; $c = 13.864(5)$ Å; $\alpha = 100.98(3)°$; $\beta = 97.94(2)°$; $\gamma = 109.50(1)°$. The X-ray analysis of a monocrystal of **8** allowed confirmation of its relative configurations and showed the *anti* orientation of its *N*-hydroxy group. This structural feature should be useful for considering the interaction of *N*-hydroxy iminosugars with the recognition site of carbohydrate processing enzymes.

Keywords Iminosugars · *N*-Hydroxypiperidines · Deoxymannojirimycin · Glycosidase inhibitors · Crystal structure · Binding properties

Introduction

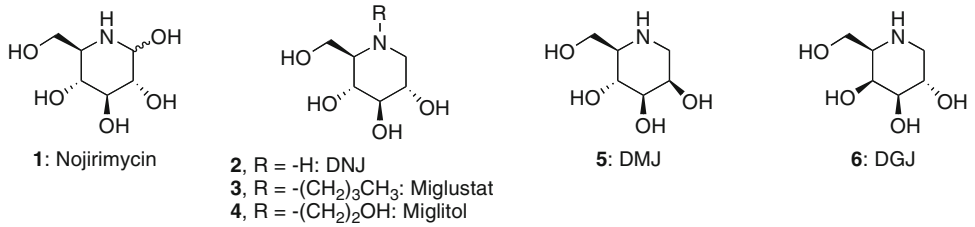
Iminosugars, also named iminocyclitols, are polyhydroxylated heterocycles containing a basic endocyclic nitrogen atom. They are one of the most important class of glycosidase inhibitors [1, 2] and, more generally, of glycoprocessing enzyme modulators [3]. Since these enzymes are involved in a number of important biological processes, iminosugars have tremendous potential as therapeutic agents against a wide range of diseases such as diabetes [4], viral infections [5], tumor metastasis [6], and lysosomal storage disorders [7, 8]. They are generally admitted to inhibit glycoprocessing enzymes by mimicking an oxonium intermediate of the catalytic process, thus being considered as transition state analogues [9].

The first iminosugar to be reported was nojirimycin (**1**), a natural product isolated from *Streptomyces roseochromogenes* [10] that demonstrated to be a powerful inhibitor of β-D-glucosidases (Fig. 1) [11]. However its short lifetime in vivo has limited its usefulness and soon, more stable analogues lacking the hemiaminal functionality such as 1-deoxynojirimycin [12] (DNJ, **2**) have been developed. Intense research in this area culminated in the approval of Zavesca® (*N*-butyl deoxynojirimycin or miglustat, **3**) for the treatment of type-1 Gaucher's disease and of Glyset® (*N*-hydroxyethyl deoxynojirimycin or miglitol, **4**) for the treatment of type-2 diabetes mellitus, both currently used as oral drugs [1–3]. Based on the hypothesis that DNJ mimics the oxonium of *gluco*- configuration and inhibits preferentially glucosidases, 1-deoxymannojirimycin (DMJ, **5**) was next considered as a mannosidase inhibitor [13–15], 1-deoxygalactonojirimycin (DGJ, **6**) as a galactosidase inhibitor [16, 17], etc. (Fig. 1).

However, establishing structure–activity relationship in the class of iminosugars was quickly found to not be that trivial. Crystallographic studies, combined with theoretical calculations are often needed to rationalize the inhibitory activity of iminosugars towards specific enzymes. As the available crystallographic data relating to carbohydrate-processing enzymes and their respective active sites have been recently increasing, rational inhibitor design studies can now be undertaken. For representative studies using crystallographic data of enzyme–inhibitor complexes to define and quantify the interaction of inhibitors with their active site, see [18–20]. For examples of computational

E. Racine · C. Philouze · S. Py (✉)
Département de Chimie Moléculaire (SERCO) UMR-5250, ICMG FR-2607, CNRS, Université Joseph Fourier, BP 53, 38041 Grenoble Cedex 09, France
e-mail: Sandrine.Py@ujf-grenoble.fr

Fig. 1 Piperidine iminosugars



studies by molecular dynamics of complexes of iminosugars with the active site of glycoprocessing enzymes using X-ray crystallographic data see [21–24]. For such studies, crystallographic data on iminosugar-type ligands/inhibitors are of major importance to define their favoured conformations, bond angles and coordinates of specific substituents. In this paper, we report the synthesis and crystallographical data of a novel *N*-hydroxypiperidine **8** of *manno*- configuration, which would be useful for a better understanding of the positioning of DMJ derivatives in the active site of mannosidases (Fig. 2).

The important biological activities of iminosugars have motivated great interest among synthetic chemists. However, while synthetic and biological studies on piperidines have been extensively investigated [25–27] only very few reports describe the synthesis and the properties of their *N*-hydroxy analogues. To the best of our knowledge, the only example of bioactive *N*-hydroxypiperidine reported in the literature is SB-219383 (**10**) a natural product extracted from *Micromonospora* sp. that has been developped by GlaxoSmithKline as a potent and selective inhibitor of bacterial tyrosyl *t*RNA synthetase (Fig. 3) [28–30]. In terms of crystallographic studies, the group of Jäger has published the X-ray structure of *N*-hydroxy piperidine **11** obtained by sodium borohydride reduction of a (*E,Z*) mixture of 2-*O*-*tert*-butyldimethylsilyl-3,4-*O*-isopropylidene-L-arabino-5-hexenose oxime, followed by a Cope-House cyclization [31].

In the course of an ongoing program [32–35] based on the use of carbohydrate-derived nitrones [36] as synthetic intermediates for the preparation of novel iminosugars, nitrone **9** was stereoselectively reduced to the *N*-hydroxypiperidine **8** using L-selectride as a hydride source (Scheme 1) [37]. Crystallization of **8** in a pentane/diethyl ether mixture afforded white crystals that turned out suitable for single crystal X-ray analysis, allowing not only the

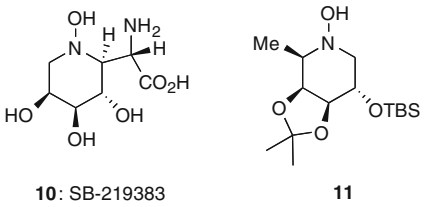
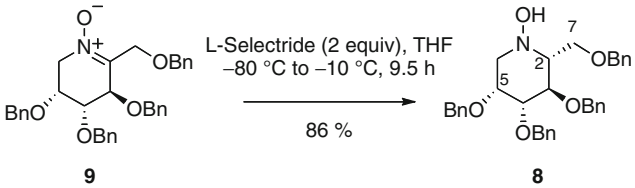


Fig. 3 Known *N*-hydroxypiperidines



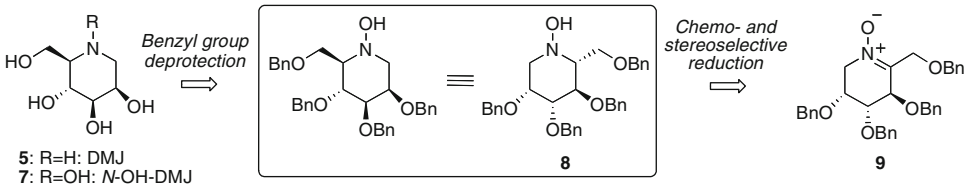
Scheme 1

confirmation of the configuration at the newly created C-2 stereogenic center, but also the spatial orientation of the N–OH bond.

Experimental

To a solution of nitrone **9** (50 mg, 0.09 mmol) in distilled THF (1 mL), L-selectride (1 M solution in THF, 0.186 mL, 0.18 mmol, 2 equiv) was added at −80 °C. The reaction mixture was stirred at −80 °C during 2.5 h, then at −10 °C during 7 h. Aqueous saturated solution of NH_4Cl (0.5 mL) was added. The aqueous layer was extracted three times with Et_2O. The organic phase was stirred with an aqueous saturated solution of KHF_2 (2 mL) during 1 h. The aqueous layer was extracted three times

Fig. 2 Synthesis of DMJ and *N*-OH-DMJ from nitrone **9**



with Et_2O. The organic phase was washed with brine, dried, filtered and concentrated. Purification of the residue by chromatography on silica gel (pentane/AcOEt: 3:1, 1:1, 0:1) afforded pure, colorless crystals of *N*-hydroxypiperidine **8** (43 mg, 86%).

(2*R*, 3*R*, 4*R*, 5*R*)-3,4,5-*Tris*-Benzyloxy-2-Benzyloxymethyl-Piperidin-1-ol

Colorless crystals, mp 78–80 °C; $[\alpha]_D^{20} = +1.9$ (*c* 1.00; $CHCl_3$); IR (neat) *v* (cm^{-1}) 3,393 (m), 3,026 (m), 2,857 (m), 1,494 (m), 1,449 (s), 1,351 (m), 1,095 (s); ^{1}H NMR (400 MHz, CD_3OD) δ ppm 2.43 (br d, 1 H, $J = 9.4$ Hz, ^{5}CH), 2.50 (d, 1 H, $J = 11.9$ Hz, $^7CH_2$), 3.52 (dd, 1 H, $J = 3.3$, 9.6 Hz, ^{3}CH), 3.59 (dd, 1 H, $J = 3.4$, 12.0 Hz, $^7CH_2$), 3.79 (dd, 1 H, $J = 2.6$, 10.2 Hz, $^6CH_2$), 3.89–3.98 (m, 2 H, ^{2}CH and $^6CH_2$), 4.11 (t, 1 H, $J = 9.6$ Hz, ^{4}CH), 4.44 (d, 1 H, $J = 11.8$ Hz, O$\underline{CH_2}$Ph), 4.49–4.68 (m, 5 H, O$\underline{CH_2}$Ph), 4.74 (d, 1 H, $J = 12.1$ Hz, O$\underline{CH_2}$Ph), 4.84 (d, 1 H, $J = 10.6$ Hz, O$\underline{CH_2}$Ph), 7.16–7.30 (m, 20 H, CH_{ar}); ^{13}C NMR (75 MHz, CD_3OD) δ ppm 59.5 ($^7CH_2$), 67.3 ($^6CH_2$), 72.4, 72.5, 72.8 and 72.9 (^{2}CH, ^{5}CH and 2 × O$\underline{CH_2}$Ph), 74.5 (O$\underline{CH_2}$Ph), 76.2 (O$\underline{CH_2}$Ph), 76.3 (^{4}CH), 84.3 (^{3}CH), 128.5–129.2 (CH_{ar}), 139.8, 139.9, 140.1 (Cq_{ar}); MS (ESI+) *m/z* 540 ($M + H^+$); Anal. Calcd for $C_{34}H_{37}NO_5$: C, 75.68; H, 6.92; N, 2.60; Found: C, 75.90; H, 7.03; N, 2.68.

A prismatic crystal, 0.25 × 0.12 × 0.08 mm^3 of size, was mounted on a glass fibre using epoxy resin (araldite). The crystal was then centered on a Bruker-AXS-Enraf-Nonius CAD-4 automated 4-circle diffractometer, working at room temperature and at the monochromated (graphite) CuKα radiation $\lambda = 1.54178$ Å. Data reduction, cell determination and refinement were performed using the CAD-4 software [38]. The cell parameters were determined from 24 reflections ($20.28° < \theta < 24.88°$). 6,505 Reflections were recorded for $\theta < 75°$. All the Friedel pairs were not collected since no heavy atom was suspected to be part of the structure. The two standard reflections for intensity showed a significant decay of 27.54%. The data were corrected for this decay and for the Lorenz and polarization effects. Crystal data are summarized in Table 1.

Structure Determination and Refinement

The structure was solved using SIR-92, [39] and refined using TeXsan. [40] C, N and O atoms were refined anisotropically by the full matrix least-squares method. H atoms were generally set geometrically and recalculated before the last refinement cycle, however the hydrogen atoms from the hydroxyl groups were located from the Fourier Map. Figures were created using ORTEP [41] and Insight II [42].

Table 1 Crystal data and structure refinement

Compound **8**	(2*R*, 3*R*, 4*R*, 5*R*)-3,4,5-*tris*-benzyloxy-2-benzyloxymethyl-piperidin-1-ol
CCDC deposit no.	683365
Color/shape	Colorless/prism
Chemical formula	$C_{34}H_{37}NO_5$
Formula weight	539.67
Temperature, K	293
Crystal system	Triclinic
Space group	*P*1
Unit-cell dimensions (24 reflections)	$a = 9.947(2)$ Å $b = 12.155(2)$ Å $c = 13.864(5)$ Å $\alpha = 100.98(3)°$ $\beta = 97.94(2)°$ $\gamma = 109.50(1)°$
Unit-cell volume [$Å^3$]	1514.0(8)
Z	2
Density (calculated) (g/cm^3)	1.184
Absorption coefficient (mm^{-1})	0.632
Diffractometer/scan	Bruker–Enraf-Nonius CAD4/omega
θ range for data collection (°)	3.33–74.90
Reflections measured	6,505
Independent/observed reflections	6,237 ($R_{int} = 0.013$)/4,620 [$I > 2\sigma(I)$]
Data/restraints/parameters	4,620/0/722
Goodness of fit on *F*	1.945
Final *R* indices [$I > 2\sigma(I)$]	$R_1 = 0.0541$, $wR_2 = 0.0735$
R indices (all data)	$R_1 = 0.0643$, $wR_2 = 0.0765$

Results and Discussion

Since compound **8** crystallized in the *P*1 space group, the cell and the asymmetric unit are identical; the cell displays two molecules **8**-I and **8**-II which are chemically identical even though there is no symmetry link between them. The secondary structure results from two intermolecular hydrogen bonds based on the *N*-hydroxyl groups (Table 2) and from phenyl group π-stackings. Whilst N(36) of **8**-II is the acceptor of H–bond from H(1) of **8**-I, the acceptor of H-bond from H(38) of **8**-II is O(2) of the molecule **8**-I.

Table 2 Hydrogen bonds between molecules **8**-I and **8**-II

A	H	B	B-adc	A···B	A–H	H···B	A–H···B
O(1)	H(1)	N(36)	55401	2.845(4)	1.02	1.85	161.8
O(6)	H(38)	O(2)	55601	2.917(3)	1.13	1.96	139.3

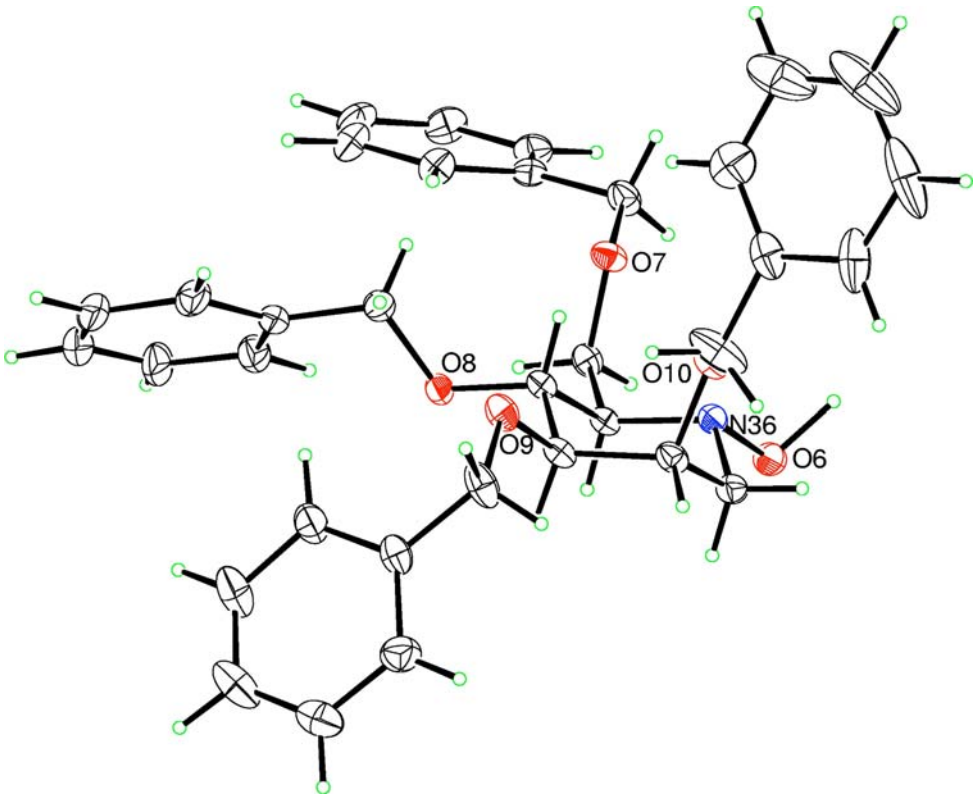


Fig. 4 Ortep drawing for conformer II of compound **8** (10% probability for thermal ellipsoids)

Each of the two molecules from the cell are composed of saturated six-membered rings containing a nitrogen atom (piperidinic rings) on which positions 3, 4 and 5 are functionalized by benzyloxy groups, position 2 by a benzyloxymethyl (Fig. 4). The nitrogen atoms are functionalized with a hydroxy group, with N–O bond lengths of 1.444 Å in both molecules. The nitrogen atoms are pyramidal as demonstrated by the bond angles around the nitrogen atom (for example, in molecule **8**-II: O(6)–N(36)–C(37) = 106.7(2); O(6)–N(36)–C(41) = 105.3(2)°; C(37)–N(36)–C(41) = 111.2(2)°). The observed bond lengths and bond angles are within the regular range observed for organic compounds [43].

The conformations of the piperidinic rings in **8**-I and **8**-II display very slight differences which can be analyzed with the Cremer and Pople parameters [44] which are $Q = 0.590(4)$ Å, $\Phi 2 = 201{,}4(3)°$, $\Theta = 175.7(3)°$ and $Q = 0.576(4)$ Å, $\Phi 2 = 135.3(3)°$, $\Theta = 177.0(3)°$, respectively. These indicate that the piperidinic rings are very close to a perfect chair conformation. Direct superposition of the six-membered rings of the two molecules using Insight II evidences that the main differences between the conformations consist in the folding of the benzyloxy groups (Fig. 5). This superposition of the two conformers **8**-I and **8**-II shows also two main orientations of the phenyl groups for π-stacking.

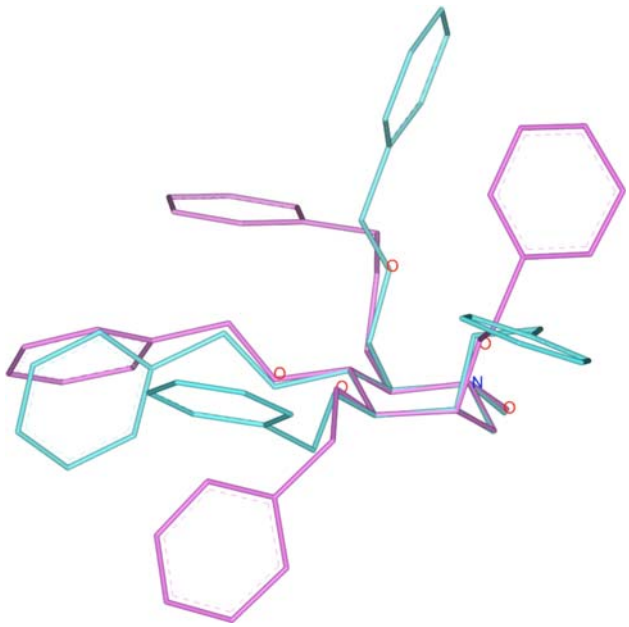


Fig. 5 Insight II drawing of the superposition of conformers **8**-I (blue) and **8**-II (pink) [Refer online version for color figure]

The X-ray structure displays unambiguously the relative configuration of the molecule, compound **8** exhibiting a trans, trans relationship of the substituents on N-1 and C-3 compared to the benzyloxymethyl group at C-2. Since there

is no heavy atom and since all the Friedel pairs have not been measured, it was not possible to deduce the absolute configuration from the data: the calculated Flack parameters [45] are meaningless and there is no significative change in R values for both possible structures. However the correct configuration (2*R*, 3*R*, 4*R*, 5*R*)-3,4,5-*tris*-benzyloxy-2-benzyloxymethyl-piperidin-1-ol **8** was assigned from the starting material with the chiral centers at C-3, C-4 and C-5 in D-fructose left unchanged by the chemical reactions used to prepare nitrone **9** and thus, *N*-hydroxypiperidine **8**. Interestingly, the equatorial orientation of the *N*-hydroxy group is also noticeable in the solid state preferred conformations of **8** ($^4C_1$ chair conformations by analogy with carbohydrates).

Conclusion

The 2,3,4,6-*tetra*-*O*-benzyl protected derivative **8** of *N*-hydroxy-1-deoxymannojirimycin (*N*-OH-DMJ, **7**) was prepared for the first time from a D-fructose-derived nitrone. Using L-selectride as the reductant, the *N*-hydroxy piperidine **8** was isolated in a good yield (86%) and as a single diastereomer. Its crystallographic data, and in particular the evidence for the equatorial orientation of its *N*-hydroxy substituent, should be useful for future molecular modeling studies and for designing novel inhibitors of specific glycoprocessing enzymes.

Supplementary Material

Crystallographic data (CIF) for (2*R*, 3*R*, 4*R*, 5*R*)-3,4,5-*tris*-benzyloxy-2-benzyloxymethyl-piperidin-1-ol have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC-683365. This material is available free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK. Fax: +44-1223-336033. E-mail: deposit@ccdc.cam.ac.uk).

Acknowledgments We thank Mrs Marie-Louise Dheu-Andries for her helpful support. E. R. is grateful to the French Ministry of Education, Research and Technology (MENRT) for a doctoral fellowship. This work was supported by the CNRS, the Université Joseph Fourier and the Agence Nationale pour la Recherche (Grant No. ANR-05-JCJC-0130-01).

References

1. Asano N (2003) Glycobiology 13:93. doi:10.1093/glycob/cwg090
2. Stütz AE (1999) Iminosugars as glycosidase inhibitors: nojirimycin and beyond. Wiley-VCH, Weinheim
3. Compain P, Martin OR (2007) iminosugars: from synthesis to therapeutic applications. Wiley, Chichester
4. Somsak L, Nagy V, Hadady Z, Docsa T, Gergely P (2003) Curr Pharm Des 9:1177. doi:10.2174/1381612033454919
5. Durantel D, Branza-Nichita N, Carrrouee-Durantel S, Butters TD, Dwek RA, Zitzmann N (2001) J Virol 75:8987. doi:10.1128/JVI.75.19.8987-8998.2001
6. Goss PE, Baker MA, Carver JP, Dennis JW (1995) Clin Cancer Res 1:935
7. Butter TD, Dwek RA, Platt FM (2000) Chem Rev 100:4683
8. Fan JQ (2003) Trends Pharmacol Sci 24:355
9. Heightman TD, Vasella A (1999) Angew Chem Int Ed 38:750, see also refs. 1–3
10. Inouye S, Tsuruoka T, Niida T (1966) J Antibiot Ser A 19:288
11. Niwa TS, Miyata S (1970) Agric Biol Chem 34:966
12. Schmidt DD, Frommer W, Müller L, Truscheit E (1979) Naturwiss 66:584
13. Bischoff J, Jornfeld R (1984) Biochem Biophys Res Commun 125:324. doi:10.1016/S0006-291X(84)80371-X
14. Winkler DA, Holan G (1989) J Med Chem 32:2084. doi:10.1021/jm00129a011
15. van den Elsen JMH, Kuntz DA, Rose DR (2001) EMBO J 20:3008. doi:10.1093/emboj/20.12.3008
16. Asano N, Oseki K, Kisu H, Matsui K (1994) J Med Chem 37:3701. doi:10.1021/jm00048a006
17. Legler G, Pohl S (1986) Carbohydr Res 155:119. doi:10.1016/S0008-6215(00)90138-1
18. Williams SJ, Notenboom V, Wicki J, Rose DR, Withers SG (2000) J Am Chem Soc 122:4229
19. Zechel DL, Boraston AB, Gloster T, Boraston CM, Macdonald JM, Tilbrook DMG, Stick RV, Davies GJ (2003) J Am Chem Soc 125:14313. doi:10.1021/ja036833h
20. Yu Z, Sawkar AR, Whalen LJ, Wong C-H, Kelly JW (2007) J Med Chem 50:94, see also ref. 15
21. Cardona F, Goti A, Brandi A, Scarselli M, Niccolai N, Mangani S (1997) J Mol Model 3:249
22. Ruiz FM, Grigera JR (2005) Med Chem 1:455
23. Zhou J-M, Zhou J-H, Meng Y, Chen M-B (2006) J Chem Theory Comput 2:157
24. Oikonomakos NG, Tiraidis C, Leonids DD, Zographos SE, Kristiansen M, Jessen CU, Norskov-Lauritsen L, Agius L (2006) J Med Chem 49:5687
25. Afarinkia K, Bahar A (2005) Tetrahedron Asymmetry 16:1239. doi:10.1016/j.tetasy.2005.02.020
26. Pearson MSM, Mathé-Allainmat M, Fargeas V, Lebreton J (2005) Eur J Org Chem 2159. doi:10.1002/ejoc.200400823
27. Ayad T, Genisson Y, Baltas M (2004) Curr Org Chem 8:1211. doi:10.2174/1385272043370005
28. Stefanska AL, Coates NJ, Mensah LM, Pope AJ, Ready SJ, Warr SR (2000) J Antibiotics 53:345
29. Berge JM, Copley CB, Eggleston DS, Hamprecht DW, Jarvest RL, Mensah LM, O'Hanlon PJ, Pope AJ (2000) Bioorg Med Chem Lett 10:1811. doi:10.1016/S0960-894X(00)00348-6
30. Berge JM, Catherine SV, Houge-Frydrych CSV, Jarvest RL (2001) J Chem Soc, Perkin Trans 1 20:2521. doi:10.1039/b105965n
31. Pothier J, Frey W, Jager V, Kristallogr Z (2002) New Cryst Struct 21:401
32. Desvergnes S, Py S, Vallée Y (2005) J Org Chem 70:1459. doi:10.1021/jo048237r
33. Desvergnes S, Desvergnes V, Martin OR, Itoh K, Liu H-W, Py S (2007) Bioorg Med Chem 15:6443. doi:10.1016/j.bmc.2007.06.059
34. Pillard C, Desvergnes V, Py S (2007) Tetrahedron Lett 48:6209. doi:10.1016/j.tetlet.2007.06.083

35. Desvergnes S, Py S, Vallée Y (2008) Org Lett 10:2967. doi: 10.1021/ol8007759
36. Revuelta J, Cicchi S, Goti A, Brandi A (2007) Synthesis 485
37. Racine E, Py S Manuscript in preparation
38. Enraf-Nonius C AD-4 Software (1988) Bruker-AXS-Enraf-Nonius. Delft, The Netherlands
39. Altomare A, Cascarano G, Giacovazzo C, Guagliardi A (1993) J Appl Cryst 26:343. doi:10.1107/S0021889892010331
40. Molecular Structure Corporation TeXsan. Single Crystal Structure Analysis Software. Version 1.7. MSC, 3200 Research Forest Drive, The Woodlands, TX 77381, USA. 1992–1997
41. Johnson CK (1976) ORTEPII. Report ORNL-5138. Oak Ridge National Laboratotry, Oak Ridge
42. Insight II Accelerys Inc 10188 Telesis Court, San Diego, CA 92121, USA, 2005
43. Allen FH, Kennard O, Watson DG, Brammer L, Orpen AG, Taylor R (1987) J Chem Soc Perkin Trans II:S1
44. Cremer D, Pople JA (1975) J Am Chem Soc 97:1354. doi: 10.1021/ja00839a011
45. Flack HD (1983) Acta Crystallogr A 39:876. doi:10.1107/S0108767383001762