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Asymmetric Synthesis of Diastereometric Diaminohepatanetetraols. A Proposal for the Configuration of (+)-Zwittermicin A

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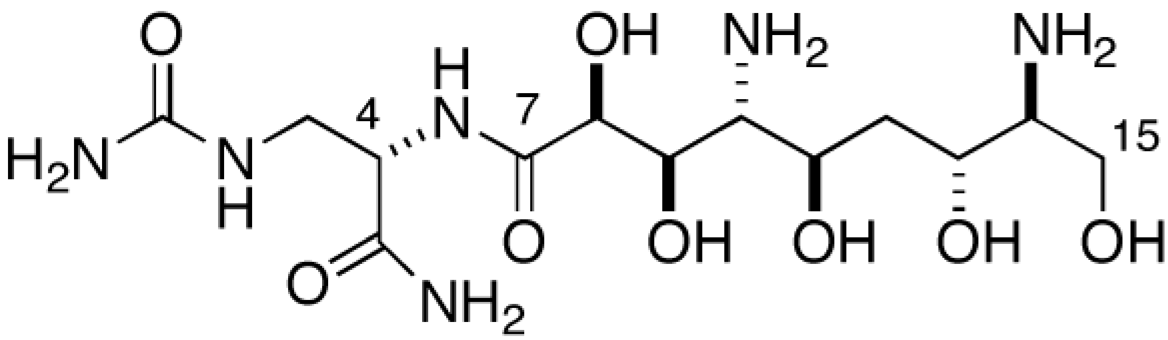
Abstract



Zwittermicin A (1)

A proposed absolute configuration for the 7 stereocenters in (+)-zwittermicin A is described based on asymmetric synthesis of six diastereomeric 2,6-diamino-1,3,5,7-heptanetetraols corresponding to the C9-C15 segment, pair-wise ^{13}C NMR chemical shift difference analysis of the models with the natural product, interpretation of enantiospecificity of serine loading domain of the zwittermicin A biosynthetic gene cluster, and degradation of the natural product.

(+)-Zwittermicin A (**1**), a water-soluble natural antibiotic isolated from fermentation of the soil-borne bacterium *Bacillus cereus*.[1] Compound **1** is of significant interest for control of crop diseases both as an antifungal agent and an adjuvant with BT toxin for biocontrol.[2]

Despite the appearance of its structure, **1** is not a sugar, but a polyketide derived from a serine starter unit followed by consecutive additions of aminomalonate, malonate and two hydroxymalonates, each with a concomitant loss of CO_2.[3] Although the original isolation and structure elucidation — with partial relative configuration at C8-C10 — were reported twelve years ago, the complete relative and absolute configuration remained unsolved. Neither the

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Supporting Information Available: Experimental procedures, X-ray data for **22** and selected ^{1}H and ^{13}C NMR. This material is available free of charge via the Internet at http://pubs.acs.org.

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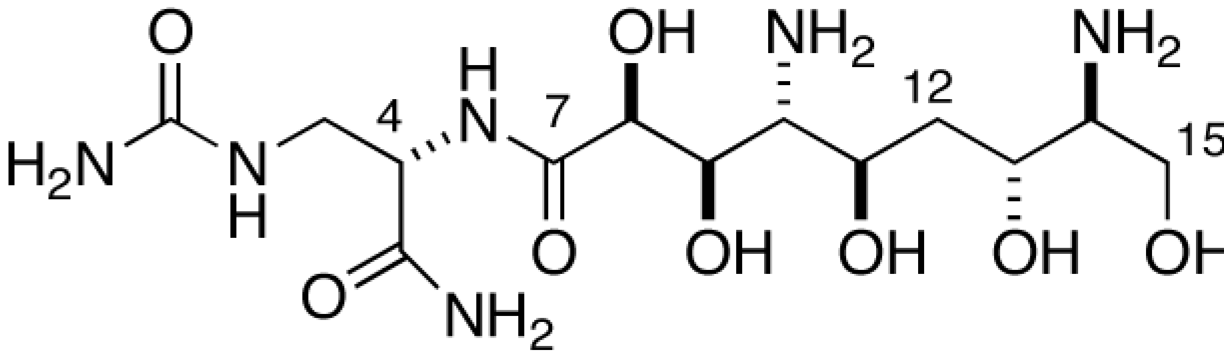
natural product or any of the derivatives prepared to date exhibit crystallinity suitable for X-ray analysis. Applications of '*J*-based' NMR methods for assignment of relative configuration in **1** failed due to lack of stereorelayed scalar couplings across the C12 methylene group; a problem related to the stereotopicity of the corresponding ^{1}H NMR signals (*vide infra*).[4] Thus, this rare *diamino*-polyol represents a significant challenge for stereochemical elucidation. Herein, we assign the configuration of **1** using a combination of model synthesis, paired ^{13}C NMR chemical shift comparisons, Marfey's analyis,[5] and a bioinformatic interpretation of the gene sequence for zwittermicin A synthase.[3] A flexible preparation of the C9-C15 core of **1** is revealed that exploits Miyashita conditions for regioselective 2,3-epoxy-1-alkanol ring opening by azide and is amenable for the total synthesis of the natural product.



Zwittermicin A (**1**)

The *pseudo*-symmetry present in the C9-C15 portion of **1** suggested a unified strategy for construction of six model compounds that embody all possible relative configurations for the pair of diads, C10,11 and C13,14.

With the exception of C11, the ^{13}C NMR chemical shifts of these remote centers are expected to be relatively independent of the remainder of the molecule. Consequently, pair-wise comparison of chemical shifts in the models with the corresponding values in **1** should converge upon a unique configurational assignment.

The six models **2-7** were synthesized starting with serine (Scheme 1). *O*-TBS-*N,N*-dibenzylserinal (**8**), prepared from L-serine, was converted to epoxide **9** using the method of Concellón.[6,7] Carbon chain extension of **9** by a BF_3•Et_2O-mediated epoxide opening with the anion derived from *O*-TBS propargyl ether afforded **10**. Protecting group adjustment followed by Red-Al reduction of the triple bond gave *E*-olefin **11**, which was treated with *m*-CPBA to give diastereomeric epoxides **12** and **13** in a ratio of 1:1.8.

Separation of the diastereomers required protection of the primary alcohol and HPLC separation followed by deprotection to give the pure epoxides. *Regioselective* elaboration of the contiguous 2-amino-1,3-diol motif was projected based on Miyashita's boron-directed azide opening of 1,2-epoxy-alkanols.[8] In the event, separate azide opening of epoxides **12** and **13** using Miyashita's method provided 1,3-diols **14** (dr 9:1) and **15** (dr 2.3:1), respectively, in good yields.[9] Acid catalyzed deprotection of **14** and **15**, with concomitant hydrogenolysis of the benzyl and azido groups afforded models **2** and **3**, respectively, as their hydrochloride salts. The configurations of the two diastereomers were readily apparent by ^{1}H and ^{13}C NMR spectroscopy which revealed C_{2v} symmetry in **2**.

The remaining four models were synthesized from the L-serine methyl ester derivative **16** (Scheme 2) using the complementary *syn*-selective epoxide formation[7] to provide **17**, the C2 epimer of **9**. Chain extension of **17** was achieved as before to give propargyl alcohol **18** which was separately converted to *E*- and *Z*-allylic alcohols **19** and **20** by Red-Al reduction or hydrogenation over Lindlar catalyst, respectively. Epoxidation of olefin **19** using *m*-CPBA was less successful due to the lability of the diastereomeric products, however, oxidation of **19** with methyltrioxorhenium gave a mixture of distereomeric epoxides which was carried forward using Miyashita's method followed by acetonide protection to give azides **21** and **22**. Since neither of the two diaminotetraols anticipated from conversion of **21** and **22** were expected to show symmetry (C_1 space group), the configurational assignments of these molecules from NMR were in doubt. Fortunately, azide **22** crystallized as colorless needles (m.p. 138 °C) and X-ray analysis (Figure 2) provided the configuration of the 4-substitued (4*R*,5*S*)-2,2-dimethyl-5-azidodioxane ring [(13*R*,14*S*), zwittermicin A numbering]. It follows that **21** is the (4*S*,5*R*)-diastereomer.

To further verify stereochemical assignments of the models, azide **15** was converted to the acetonide **23** (Scheme 3). The ^{1}H NMR spectrum of **23** showed the expected large diaxial vicinal couplings (δ 4.14, ddd, *J* 10.4, 8.0, 2.4 Hz; δ 3.83, ddd, *J*= 11.6,6.4, 2.4 Hz) for a *syn*-4,6-disubstituted 1,3-dioxane and large ^{13}C chemical shift differences for the *gem* CH_3 signals of the isopropylidene group (δ 29.9, q; 19.7, q).[10]

Acid-catalyzed global deprotection and hydrogenolysis of **21** and **22** compounds provided models **4** and **5**, respectively, as their HCl salts. Models **6** and **7** were synthesized from olefin **20** using the same approach.

The diastereomeric family of model compounds comprise two *meso* compounds (**3** and **6**), two C_2 isomers (**2** and **7**) and two isomers lacking symmetry (C_1, **4** and **5**). As expected, the ^{1}H NMR signal of the C4 methylene protons in each C_2 isomer (e.g. **2**, δ 1.66 m, 2H) appeared as complex second-order multiplet owing to the fact that the H4 protons were chemical-shift equivalent but magnetically in-equivalent. Conversely, the 4-CH_2 protons in the *meso* isomer **3** are both chemical shift inequivalent and magnetically inequivalent and appear as diastereotopic protons exhibiting a first-order ABX_2 pattern (δ 1.79 dt 1H, *J*=14.4, 8.4 Hz; δ 1.84, dt, 14.4, 4.7 Hz, 1H). Analogous patterns were observed for C_2-symmetrical **7** and *meso*-**6**. Interestingly, the ^{1}H NMR signal of corresponding C12 methylene group in **1** also exhibited a complex second order pattern (400, 500 and 600 MHz) similar to those of **2** and **7**, but dissimilar to the 4-CH_2 signals of **3** and **6** of suggesting that the spin systems in **1**, **2** and **7** reflected local C_2 or *pseudo*-C_2 symmetry, largely dictated by an *anti*- relationship of the C11 and C13 OH groups.

An unequivocal assignment of relative configuration for the diaminotetraol segement in **1** was made by pairwise comparisons of the differences in the ^{13}C chemical shifts (Δδ) for C10-C15 of **1** and model compounds (Figure 1).[11] There are only six diastereomers of the symmetrically substituted diaminoheptanetetraol models but eight diastereomers of the C10-C15 segment in **1**. To complete the comparison, the ^{13}C δ assignments of C_1 isomers **4** and **5** were reversed to give the remaining two isomers — virtual compounds "**4b**" and "**5b**". The C_{2v} symmetric **2** is the only model compound with a close match to **1** for every carbon (Figure 1) except C9, which is the point of difference between **1** and the models and expected to show an 'outlying' Δδ in every case.

Importantly, the other C_{2v} isomer **7** had the largest mismatch which secures confidence for assignment of *erythro* relationships in each of the C10,11 and C13,14 diads. Elimination of the mismatched *meso* isomers **3** and **6**, as suggested by ^{1}H NMR and stereotopicity analysis of the 12-CH_2 signal (above), is now corroborated by ^{13}C NMR.

Therefore, compounds **1** and **2** share the same relative configuration at the stereogenic centers corresponding to C10, C11, C13 and C14 of **1**. The data in Figure 1, in conjunction with the relative configurations at C8-C10[1] now allow us to extend the assignment of relative configuration of **1** to C8-C15.

Although no direct evidence for the absolute configuration of C8-C15 is yet available, analysis of the published sequence of the gene cluster for biosynthesis of zwittermicin A highly suggests that the C14 shares the same configuration as L-serine.[3] Zwittermicin A is synthesized by a hybrid polyketide synthase-nonribosomal peptide synthase (PKS-NRPS) that comprises nine open reading frames including a loading domain for the starter unit that is homologous with serine adenylation domains found in gene clusters for biosynthesis of iturin A and mycosubtilin. Since the proposed gene sequence for production of **1** shows C13-C15 originating from L-serine and epimerase domains are absent, it is highly likely that C14 is L- and the absolute stereochemistry for C8-C14 in **1** is as depicted.

The absolute configuration at the remaining C4 stereocenter in **1** was determined as 4*S* by Marfey's analysis.[5] Acid hydrolysis of authentic **1** (6 N HCl, 24 h, 110 °C) and derivatization of the products with 2,4-dinitrophenyl-5-fluoro-L-alaninamide (Marfey's reagent) under standard conditions, followed by analysis (C_{18} HPLC-MS) gave one peak that matched the peak (coinjection, MS spectrum) obtained by similar treatment of commercially available (-)-(*S*)-N^3-ureido-2,3-diaminopropionic acid (*S*-albizziin).

In conclusion we have assigned the configuration of **1** as (4*S*,8*S*,9*R*,10*R*,11*R*,13*R*,14*S*). using an integrated approach based on synthesis and pairwise comparisons with model compounds, Marfey's analysis, and published data. This sets the stage for completion of **1** by chain extension of a suitably protected derivative of **2** and attachment of the N^3-ureido-2,3-diaminopropionamide side chain, which is the subject of current research in our laboratories.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

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C12-C13 sequence, while ^1H-^1H couplings to the C12 methylene group showed second-order effects as discusssed later in the text.

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(9). Under standard conditions, in the absence of $B(OMe)_3$ yields and regioselectivity were poor (e.g. **12**-> **14**, (NH_4Cl, NaN_3, DMF, 44%, dr 1:1.4)

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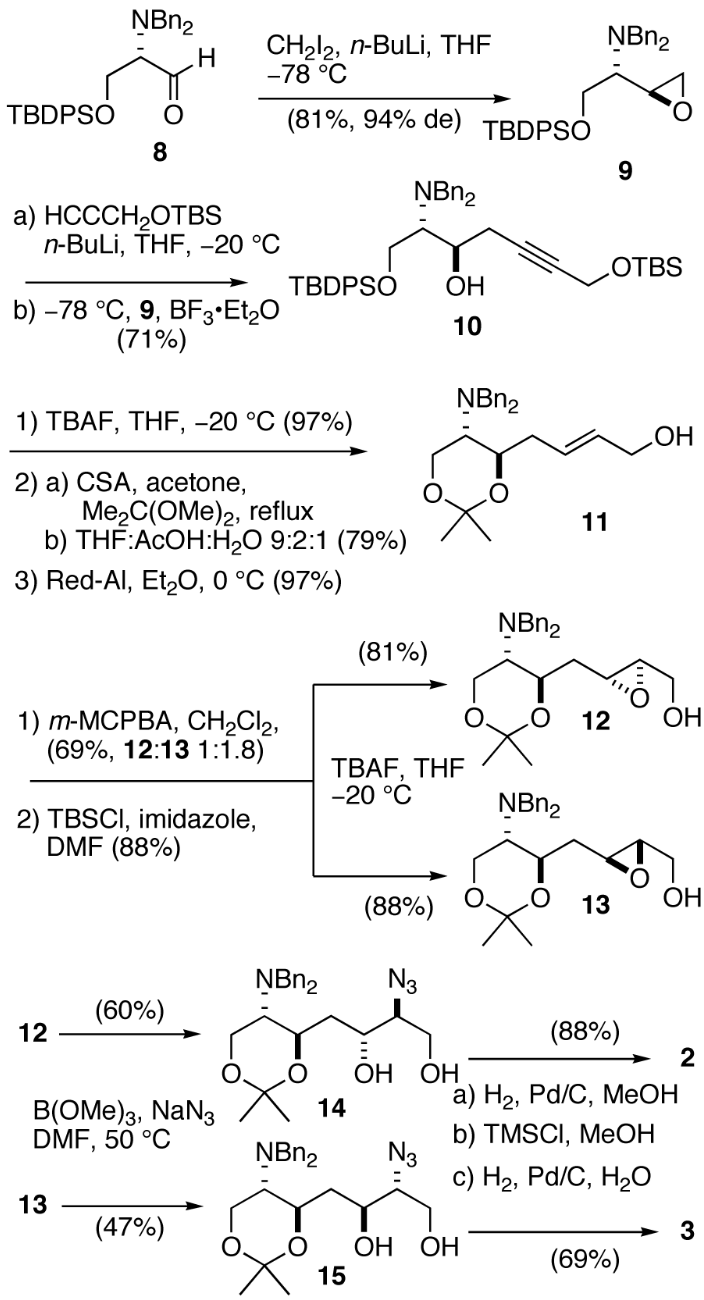
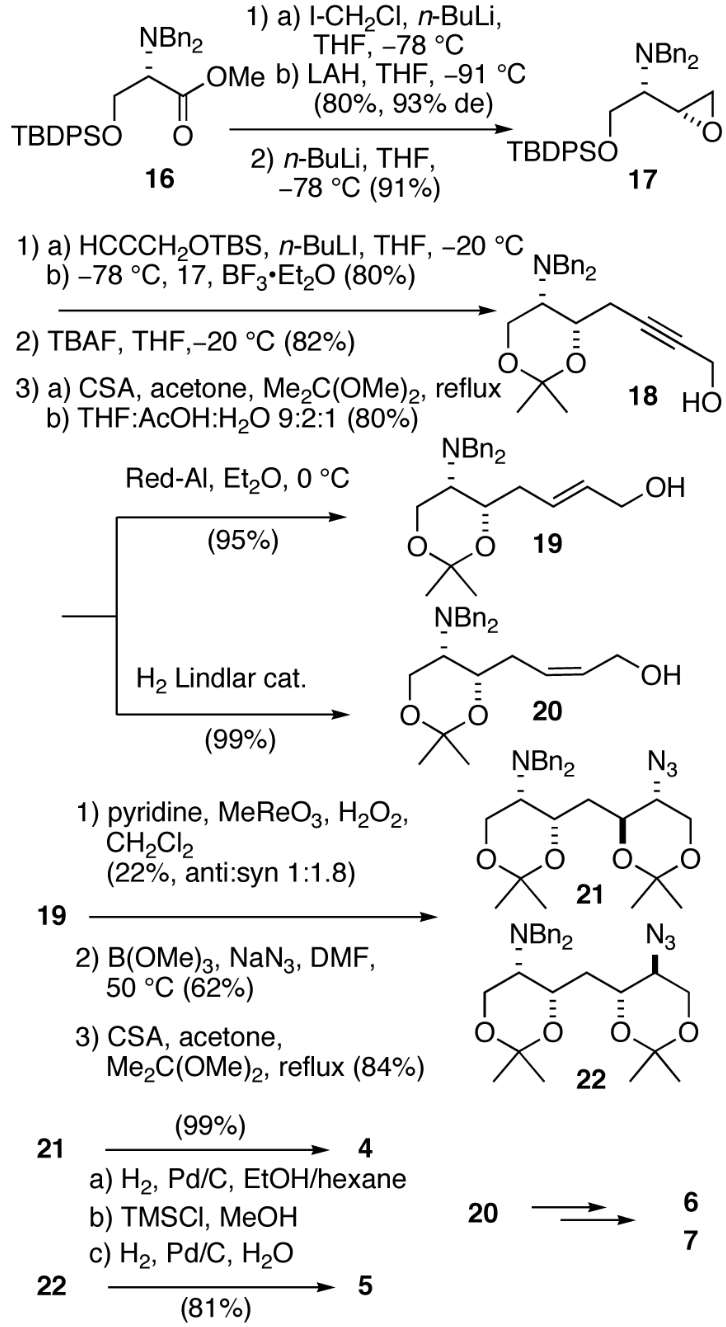
(11). ^{13}C NMR measurements of the of the hydrochloride salts of **1** and models **2**-**7** were carried out under essentially identical conditions (D_2O, 25 °C) with internal CH_3CN as reference.

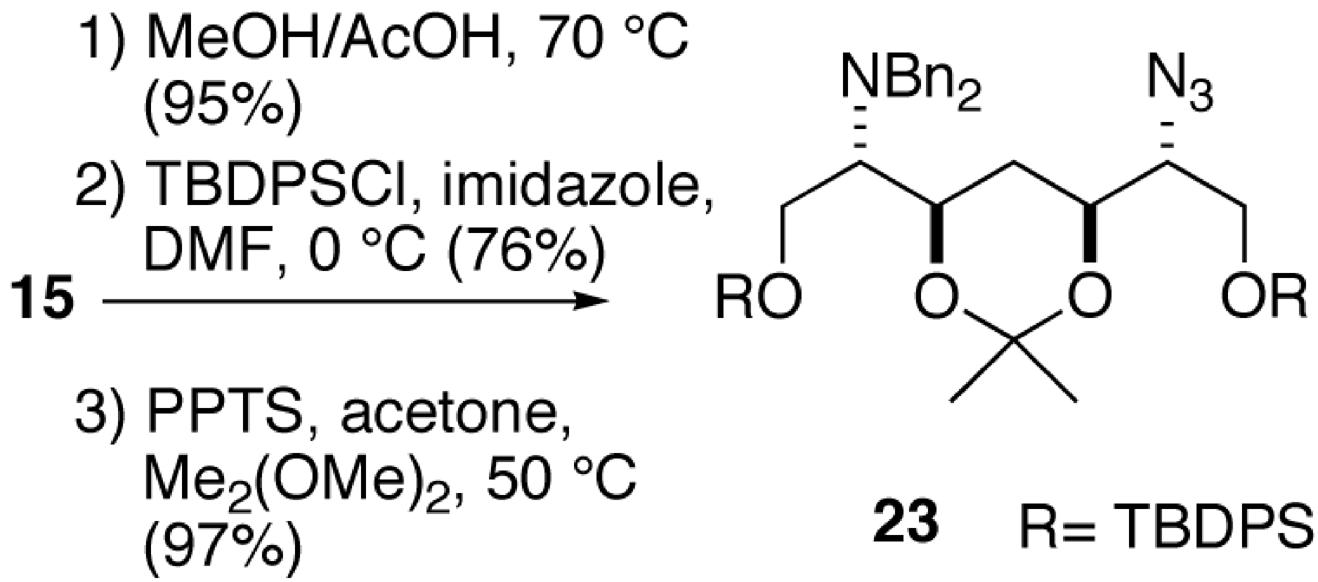


NBn2
H
TBDPSO
O
8
CH2I2, n-BuLi, THF
−78 °C
(81%, 94% de)
NBn2
TBDPSO
O
9
a) HCCCH2OTBS
n-BuLi, THF, −20 °C
b) −78 °C, 9, BF3•Et2O
(71%)
NBn2
TBDPSO
OH
OTBS
10
1) TBAF, THF, −20 °C (97%)
2) a) CSA, acetone,
Me2C(OMe)2, reflux
b) THF:AcOH:H2O 9:2:1 (79%)
3) Red-Al, Et2O, 0 °C (97%)
NBn2
OH
O
O
11
1) m-MCPBA, CH2Cl2,
(69%, 12:13 1:1.8)
2) TBSCl, imidazole,
DMF (88%)
(81%)
TBAF, THF
−20 °C
(88%)
NBn2
O
O
O
12
OH
NBn2
O
O
O
13
OH
12
(60%)
B(OMe)3, NaN3
DMF, 50 °C
13
(47%)
NBn2
N3
O
O
OH
OH
14
NBn2
N3
O
O
OH
OH
15
(88%)
2
a) H2, Pd/C, MeOH
b) TMSCl, MeOH
c) H2, Pd/C, H2O
3
(69%)

Scheme 1.


NBn2
OMe
TBDPSO
O
16
1) a) I-CH2Cl, n-BuLi, THF, −78 °C
b) LAH, THF, −91 °C
(80%, 93% de)
2) n-BuLi, THF, −78 °C (91%)
NBn2
TBDPSO
O
17
1) a) HCCCH2OTBS, n-BuLI, THF, −20 °C
b) −78 °C, 17, BF3•Et2O (80%)
2) TBAF, THF,−20 °C (82%)
3) a) CSA, acetone, Me2C(OMe)2, reflux
b) THF:AcOH:H2O 9:2:1 (80%)
18
HO
Red-Al, Et2O, 0 °C
(95%)
OH
19
H2 Lindlar cat.
(99%)
20
N3
21
19
1) pyridine, MeReO3, H2O2, CH2Cl2
(22%, anti:syn 1:1.8)
2) B(OMe)3, NaN3, DMF, 50 °C (62%)
3) CSA, acetone, Me2C(OMe)2, reflux (84%)
22
21
(99%)
4
a) H2, Pd/C, EtOH/hexane
b) TMSCl, MeOH
c) H2, Pd/C, H2O
22
5
(81%)
20
6
7

Scheme 2.


1) MeOH/AcOH, 70 °C (95%)
2) TBDPSCl, imidazole, DMF, 0 °C (76%)
15
3) PPTS, acetone, Me2(OMe)2, 50 °C (97%)
NBn2
N3
RO
O
O
OR
23
R= TBDPS

Scheme 3.

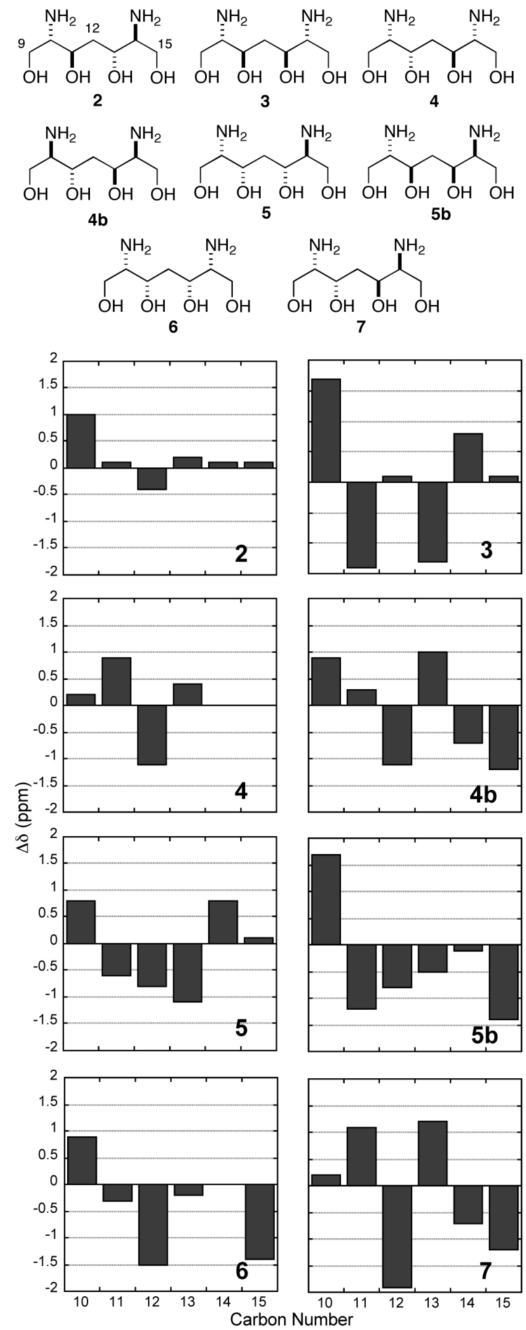


Figure 1.
^{13}C NMR (100 MHz, D_2O, ref. internal CH_3CN, δ1.47 ppm) Δδ values (δ_C model — δ_C **1**) of model compounds **2**-**7**. "**4b**" and "**5b**" are 'virtual isomers' of **4** and **5**, respectively, by reversing the order of ^{13}C δ assignments for the purpose of comparison with **1**.

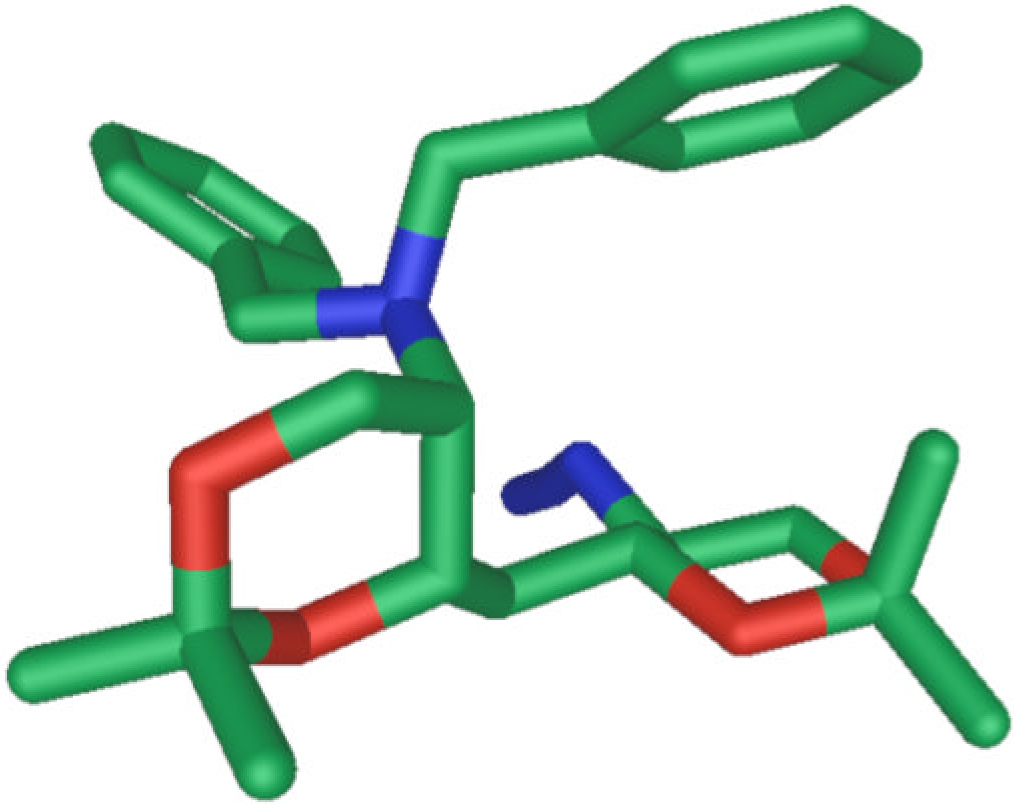


Figure 2.
X-ray structure of **22**.