Biomimetic synthesis and structural refinement of the macrocyclic dimer aminoglycoside 66-40C—the remarkably selective self-condensation of a putative aldehyde intermediate in the submerged culture medium producing sisomicin†

Stephen Hanessian* and Juan Pablo Maianti

Received (in Cambridge, UK) 8th December 2009, Accepted 2nd February 2010
First published as an Advance Article on the web 15th February 2010
DOI: 10.1039/b925668g

Aminoglycoside 66-40C, an unprecedented 16-membered *bis*-azadiene macrocyclic natural product isolated from the *Micromonospora* producer of the antibiotic sisomicin, was synthesized following a biomimetic strategy which definitively established its origin as arising from a remarkably selective non-enzymatic macro-dimerization.

The submerged fermentation of *Micromonospora inyoensis* gives the broad-spectrum aminoglycoside antibiotic sisomicin (**1**) as the principal product (Fig. 1).[1,2] Among the minor components produced by the same medium is a 4′,5′-unsaturated pseudotrisaccharide which was designated as aminoglycoside 66-40C.[3] Degradative studies by Mallams and co-workers, then at the Schering-Plough Corporation, established the structure and stereochemistry of aminoglycoside 66-40C as the dimer shown as expression **2** (Fig. 1).[3] Critical experiments included careful osmometric determination of the molecular weight of aminoglycoside 66-40C compared to the parent sisomicin and its degradation to the monomer aldehyde **3** under strongly acidic conditions.[3] However, the authors made no allusion to the possibility of self-condensation of this monomeric aldehyde to regenerate aminoglycoside 66-40C. The dimer exhibited no antibacterial activity, instead it was used to access several 6′*N*-substituted sisomicins by reductive amination from the 6′-aldehyde (**3**).[4]

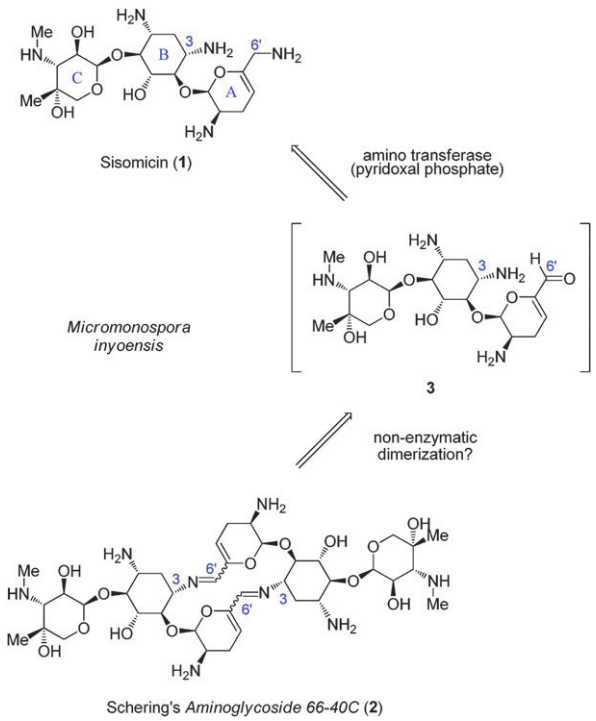


Fig. 1 Sisomicin (**1**), aminoglycoside 66-40C (**2**) and their hypothetical biosynthetic relationship through a putative 6′-aldehyde monomer (**3**).

The dimeric 16-membered C_2-symmetrical *bis*-Schiff's base structure of aminoglycoside 66-40C is unprecedented among members of the aminoglycoside antibiotic family. Moreover, it is the only minor fermentation product which appears to be biosynthetically derived through additional reactions on the parent antibiotic (Fig. 1).[2,3,5]

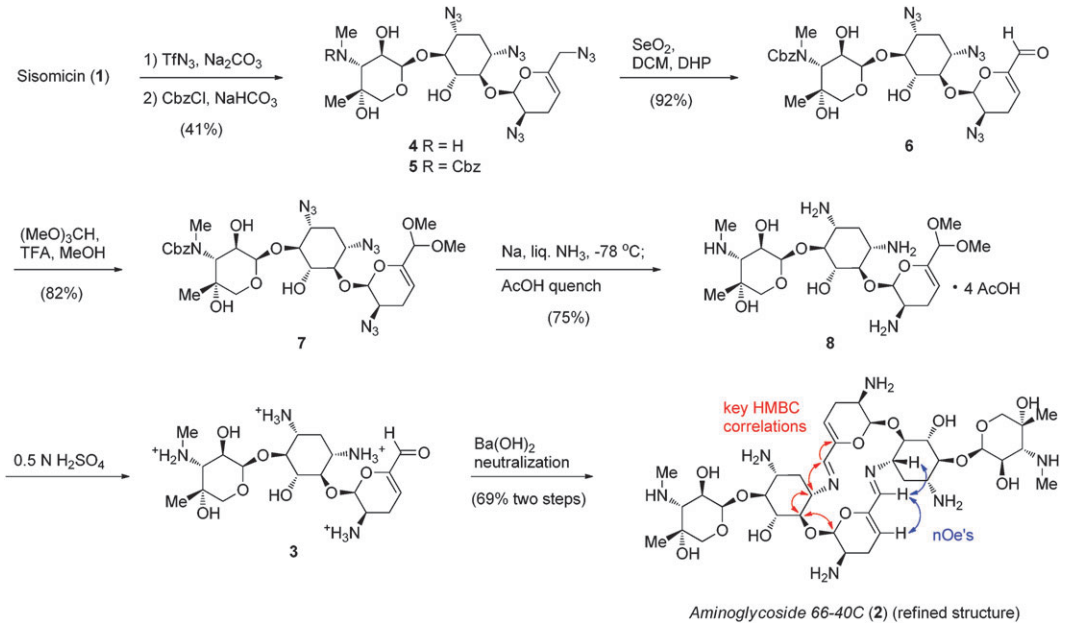
The unique features of aminoglycoside 66-40C make it an intriguing synthetic target. Detailed structural consideration suggests its formation from a non-enzymatic intermolecular double condensation of a putative 6′-aldehyde intermediate **3** onto the 3-amino group of a congener.

We report herein the validation of this hypothesis through the first concise biomimetic synthesis, and structural refinements of aminoglycoside 66-40C, starting with the readily available sisomicin sulfate (Scheme 1).[1] Conversion of the 3″′*N*-Cbz tetra-azido derivative **5** to the 6′-aldehyde (**6**) was achieved in excellent yield, following our recently reported oxidation of dihydropyran allylic azides with SeO_2.[6]

Treatment with trimethylorthoformate and TFA led to the dimethyl acetal intermediate **7**, as a key latent form of the 6′-aldehyde. Cleavage of the *N*-Cbz group with concomitant reduction of the azide groups under Birch conditions afforded masked sisomicin 6′-acetal **8**. Treatment with dilute sulfuric acid immediately liberated the monomer aldehyde **3**, which upon neutralization with barium hydroxide underwent smooth self-condensation. The dimerization event could be followed by sequential H-NMR owing to the individual signals of the functionalities involved (Fig. 2). Filtration of the barium sulfate precipitate followed by lyophilization gave essentially pure aminoglycoside 66-40C, which was purified from residual salts by bench-top ammoniacal silica-gel column

Department of Chemistry, Université de Montréal, C. P. 6128, Succ. Centre-Ville, Montréal, QC, Canada H3C 3J7. E-mail: stephen.hanessian@umontreal.ca; Fax: +1-514-343-5728; Tel: +1-514-343-6738

† Electronic supplementary information (ESI) available: Experimental details, spectroscopic data and a model of aminoglycoside 66-40C in MOL format. See DOI: 10.1039/b925668g



Scheme 1 Synthesis and biomimetic dimerization of aminoglycoside 66-40C (**2**), refined structure and key nOe (blue) and HMBC correlations (red).

chromatography. The physical, spectroscopic and chemical characteristics of the product were identical to those reported.[3]

A quasi first order proton spectrum of aminoglycoside 66-40C in D_2O is indicative of the prevalence of a preferred rigid macrocycle conformer. As originally presumed, homo- and heteronuclear correlation experiments attest to the imine location on N-3 of the deoxystreptamine ring B.[3] In addition, nuclear Overhauser effect (nOe) spectroscopy revealed spatial proximity between the 6′-imine proton and both the vinylic 4′-H and the axial proton 3-H of ring B refining the originally proposed structure with an in-line arrangement for the *trans*, *trans-bis*-azadiene linkers bridging rings A and B (Scheme 1 and Fig. 3).

Intrigued by this unique macrocyclic dimerization event, we envisioned the analogous process for a nominal motif, namely the 6′-aldehyde of sisamine, a pseudodisaccharide which could be derived by periodate degradation of the vicinal amino alcohol in sisomicin derivative **4** (Scheme 2).[7]

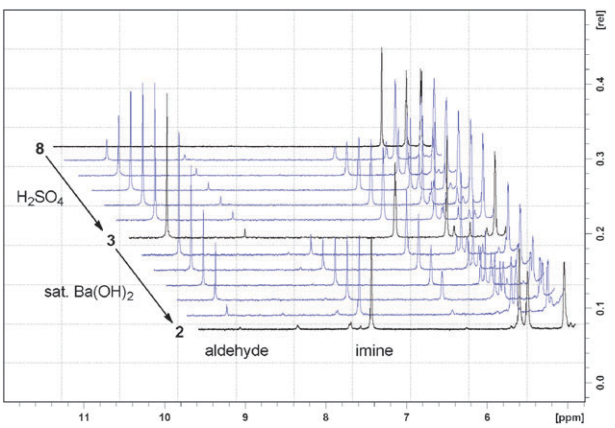


Fig. 2 Sequential H-NMR spectra in D_2O revealing the conversions from acetal **8** to aldehyde **3**, and finally self-condensation to dimer **2**.

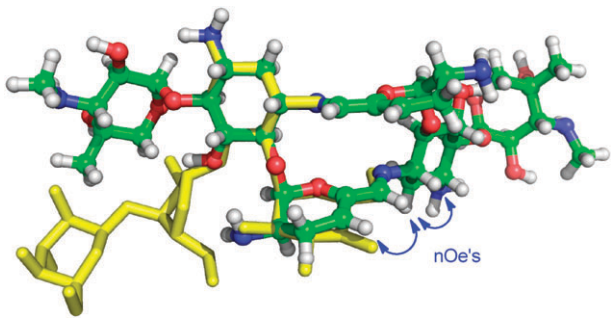
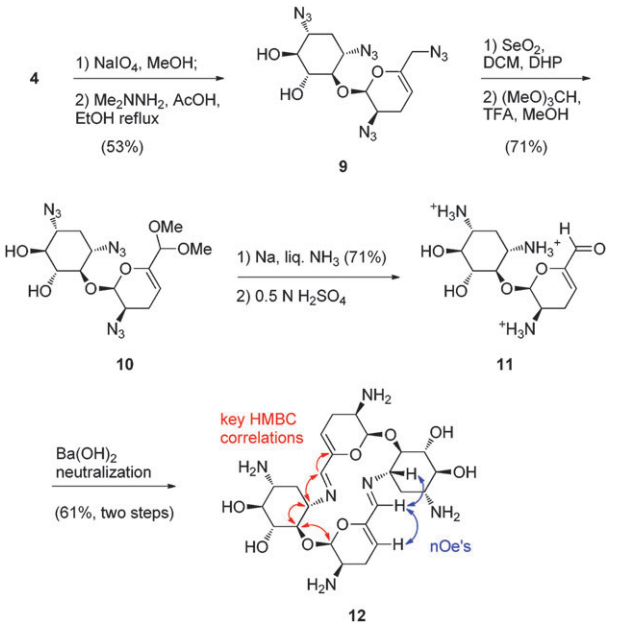


Fig. 3 Model of aminoglycoside 66-40C (**2**, green), overlaid on the skeleton of paromomycin (yellow),[10] RMS error 0.23 Å.[9]

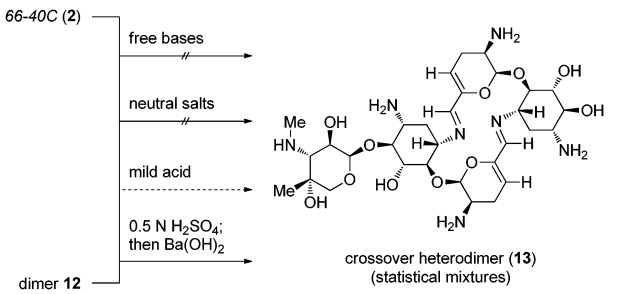
The reliable transformations we developed were applied unexceptionally to tetra-azido sisamine **9**, to access a sisamine core aldehyde **11** which behaved essentially like **2**, self-condensing to yield sisamine dimer **12**.

Likewise, we observed that the self-condensation is slow for acidic to neutral salt solutions of the monomeric aldehydes (**3** and **11**), but occurs readily in mild alkaline solutions which warrant the presence of the 3-amino group in its nucleophilic form (approx. $pK_a \approx 7$ to 8).[2,8] These results, the similarity of nOe, and all other spectroscopic characteristics of dimers **2** and **12**, suggest that the sisamine core imparts the remarkable selectivity over the vast space of mono-, di- and oligomeric imine variations available.

In order to further explore the solution properties of aminoglycoside 66-40C, we studied various pH conditions to determine if it exists in dynamic equilibria with partially open or monomeric species. We performed crossover experiments using sisamine dimer **12**, as a labeling entity which permitted monitoring the behavior of the mixtures by LCMS (Scheme 3). Testament to the stability of aminoglycoside 66-40C, negligible amounts of crossover heterodimer product (**13**) were detected when dimers were incubated as their free-base or neutral



Scheme 2 Degradation of tetra-azido sisomicin ring C and synthesis of the minimal dimer motif (**12**). Key nOe and HMBC correlations.



Scheme 3 Crossover experiments between aminoglycoside 66-40C (**2**) and its truncated sisamine analog **12**, monitored by LCMS.

acetate salt forms, in aqueous solutions over periods of > 72 h. Under slight excess of acetic acid we observed slow hydrolysis to aldehydes **3** and **11** (approx. 25% over 72 h, H-NMR), nonetheless, scrambling between dimers was incomplete. These results indicate that aminoglycoside 66-40C is a resilient dimer, and does not readily undergo dynamic equilibration even under acid catalysis. On the other hand, the crossover heterodimer (**13**) could be readily generated by neutralizing a mixture of aldehydes **3** and **11** obtained by sulfuric acid treatment of the corresponding homodimers (Scheme 3).

Models of the structure of dimers **2** and **12**,[9] respecting the azadiene conformation indicated from nOe's, suggest that the conformations of rings A and B are unperturbed compared to aminoglycosides co-crystallized within A-site RNA oligonucleotide models,[10,11] also believed to be the lowest energy conformation in solution (Fig. 3).[12] In fact, the innate conformation of aminoglycosides might be the determining factor for the spontaneous self-selection of the putative 6′-aldehyde intermediate (**3**), responsible for the prevalence of this unusual macrocyclic motif in a complex biological medium, where a plethora of other potential nucleophiles are also present.

In conclusion, our synthetic efforts have uncovered the remarkable tendency of the putative sisomicin 6′-aldehyde **3**, and related congeners such as **11** to undergo non-enzymatic self- and/or cross-dimerization leading to exquisitely C_2-symmetrical macrocyclic Schiff's bases such as aminoglycoside 66-40C. As a result, the structure and solution conformation of the latter were refined over the original proposal.[3] Although the biological function of aminoglycoside 66-40C remains speculative, such a metabolite is conceivable as a pathway feedback regulator or an extracellular signal in *M. inyoensis*.

We thank Fonds de Recherche en Santé du Québec (FRSQ) for a fellowship, and continued support from the Natural Sciences and Engineering Research Council of Canada (NSERC).

Notes and references

1 M. J. Weinstein, J. A. Marquez, R. T. Testa, G. H. Wagman, E. M. Oden and J. A. Waitz, *J. Antibiot.*, 1970, **23**, 551; G. H. Wagman and M. J. Weinstein, *Annu. Rev. Microbiol.*, 1976, **9**, 537.
2 D. P. Arya, *Aminoglycoside Antibiotics: From Chemical Biology to Drug Discovery*, Wiley, Hoboken, New Jersey, 2007; C. Walsh, *Antibiotics: Actions, Origins, Resistance*, ASM Press, Washington, DC, 2003.
3 D. H. Davies, A. K. Mallams, J. McGlotten, J. B. Morton and R. W. Tkach, *J. Chem. Soc., Perkin Trans. 1*, 1977, 1407; D. H. Davies, D. Greeves, A. K. Mallams, J. B. Morton and R. W. Tkach, *J. Chem. Soc., Perkin Trans. 1*, 1975, 814.
4 D. H. Davies, A. K. Mallams, M. Counelis, D. Loebenberg, E. L. Moss Jr. and J. A. Waitz, *J. Med. Chem.*, 1978, **21**, 189.
5 R. T. Testa and B. C. Tilley, *J. Antibiot.*, 1976, **29**, 140; E. J. Hessler, H. K. Jahnke, J. H. Robertson, K. Tsuji, K. J. Rinehart Jr. and W. T. Shier, *J. Antibiot.*, 1970, **23**, 464; G. H. Wagman and J. Weinstein, *Journal of Chromatography Library, Antibiotics-Isolation, Separation, Purification*, Elsevier, Amsterdam, 1978, vol. 15.
6 S. Hanessian, J. Szychowski and J. P. Maianti, *Org. Lett.*, 2009, **11**, 429. DHP = 3,4-dihydro-[2*H*]-pyran.
7 H. Paulsen, R. Jansen and P. Stadler, *US pat.*, 4 195 173, 1980; H. Paulsen and R. Jansen, *Carbohydr. Res.*, 1981, **92**, 305.
8 J. R. Cox and E. H. Serpersu, *Biochemistry*, 1997, **36**, 2353; R. S. Kane, P. T. Glink, R. G. Chapman, J. C. McDonald, P. K. Jensen, H. Gao, L. Pasa-Tolić, R. D. Smith and G. M. Whitesides, *Anal. Chem.*, 2001, **73**, 4028.
9 Hyperchem 8 suite, geometry optimizations and molecular dynamics calculations run using amber99 parameter set. Pair fitting RMS error for all 16 sp^3 matching atoms of rings A and B of paromomycin. Fig. 3 image produced in PyMol.
10 Q. Vicens and E. Westhof, *Structure (London)*, 2001, **9**, 647.
11 For review see: B. François, R. J. Russell, J. B. Murray, F. Aboul-ela, B. Masquida, Q. Vicens and E. Westhof, *Nucleic Acids Res.*, 2005, **33**, 5677.
12 D. H. Fong and A. M. Berghuis, *EMBO J.*, 2002, **21**, 2323.