Synthesis and Comparative Antibacterial Activity of Verdamicin C2 and C2a. A New Oxidation of Primary Allylic Azides in Dihydro[2H]pyrans
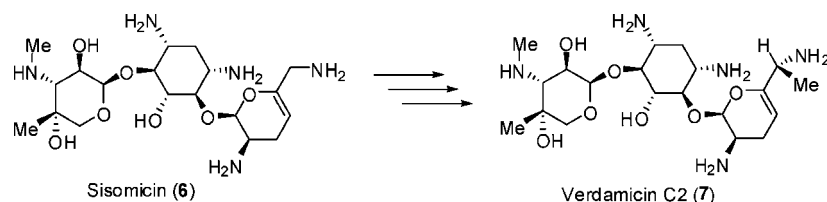
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ABSTRACT

A synthesis of verdamicin C2 and its congener C2a has been accomplished from sisomicin relying on a novel oxidative transformation of an allylic azide to the corresponding α,β-unsaturated aldehyde, and its stereocontrolled elaboration into the intended 5′ side chain of verdamicin C2 and C2a. *In vitro* antibacterial testing shows that both C6′ epimers in verdamicin C2 and C2a are equally active against a variety of bacterial strains. Oxidation of allylic primary azides, ethers, and esters of 2-substituted dihydro[2H]pyrans with SeO_2 leads directly to the corresponding aldehydes.

The Gentamicin complex of aminoglycoside antibiotics has been in clinical use for the treatment of acute Gram-negative infections since 1963.[1] The complex consists of a mixture of four major congeners (gentamicins C1, C1a, C2, and C2a) which are all equipotent as antibacterials (Figure 1).[2] In addition to the gentamicins, the genus *Micromonospora*[3] can also produce good quantities of sisomicin,[4] verdamicin C2a,[5] and lesser amounts of othercomponents like antibiotic G-52[6] (Figure 1).

These differ from gentamicin C1a, C2a, or C2b (also called sagamicin) respectively by the presence of a 4′,5′-double bond in ring I.

Although degradative work has been carried out on verdamicin, definitive stereochemical assignment of the 6′-

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(2) Sandoval, R. M.; Reilly, J. P.; Running, W.; Campos, S. B.; Santos, J. R.; Phillips, C. L.; Molitoris, B. A. *J. Am. Soc. Nephrol.* **2006**, *17*, 2697.

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(5) Weinstein, M. J.; Wagman, G. H.; Testa, R.; Marquez, J. A. German patent DE 2239964, 1973, CAN 79:51820.

(6) Weinstein, M. J.; Wagman, G. H.; Marquez, J. A. German patent DE 2334923, 1974, CAN 80:131672.

Gentamicin C1 (**1**), R^1 = Me, R^2 = NHMe
Gentamicin C1a (**2**), R^1 = H, R^2 = NH$_2$
Gentamicin C2 (**3**), R^1 = Me, R^2 = NH$_2$
Gentamicin C2a (**4**), R^1 = NH$_2$, R^2 = Me
Gentamicin C2b (**5**), R^1 = H, R^2 = NHMe

Sisomicin (**6**), R^1 = H, R^2 = NH$_2$
Verdamicin C2 (**7**), R^1 = Me, R^2 = NH$_2$
Verdamicin C2a (**8**), R^1 = NH$_2$, R^2 = Me
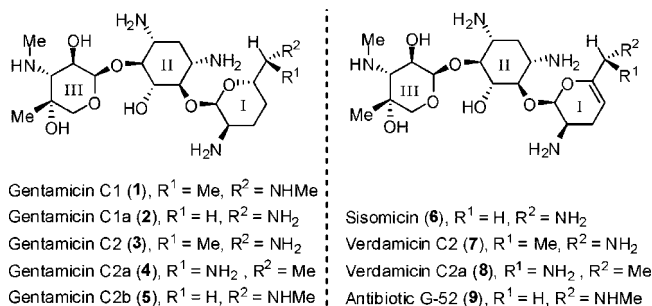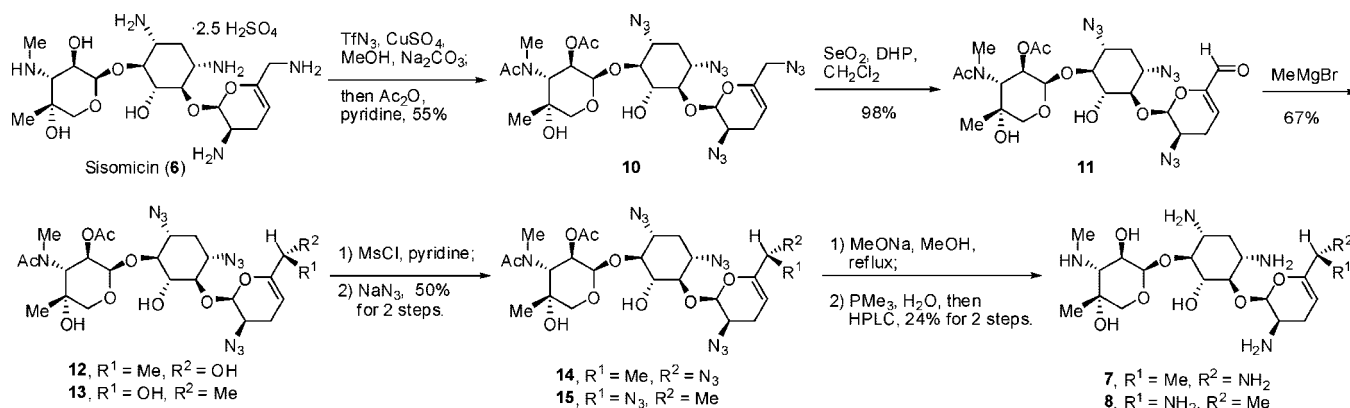Antibiotic G-52 (**9**), R^1 = H, R^2 = NHMe

Figure 1. Structure of related aminoglycosides.

Scheme 1. Preparation of Verdamicin C2 and C2a as a 1:1 Mixture

aminoethyl side chain has not been reported. In fact, both the 6′-(*R*)-aminoethyl and 6′-(*S*)-aminoethyl side chains have been cited in the literature.[7,8]

The biotransformation of the naturally occurring verdamicin to gentamicin C2a by the mutant strain KY11525 from *M. sagamiensis* suggested that verdamicin may exist as the C2a epimer.[9] However, the stereochemical identity of verdamicin has not been definitively established. Furthermore, no study has compared the in vitro antimicrobial activities of verdamicin C2a and its C6′ epimeric C2 analogue.[2]

In this letter, we describe a synthesis of verdamicin C2 and its congener C2a from sisomicin relying on a novel oxidative transformation of an allylic azide to the corresponding α,β-unsaturated aldehyde, and its stereocontrolled elaboration into the intended 5′ side chains of verdamicin C2 and C2a.
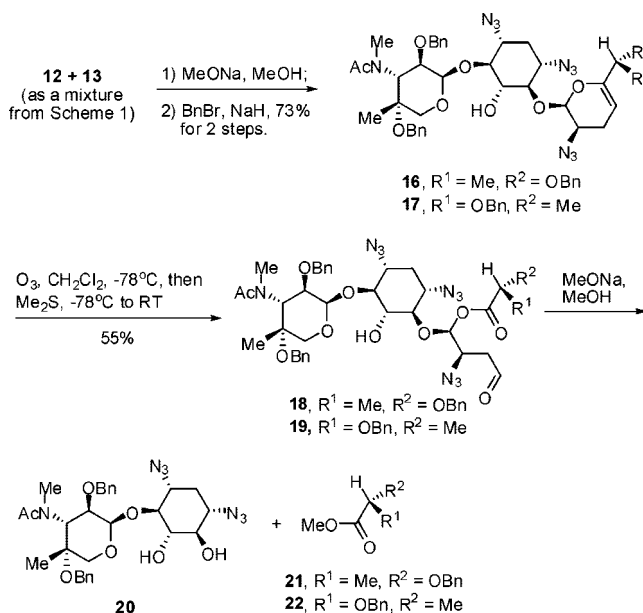
Sisomicin was converted to the tetraazido analogue **10** by a known method,[10] and the remaining secondary amino group was acetylated (Scheme 1). Treatment of **10** with stoichiometric SeO₂ in CH₂Cl₂ containing 3 equiv of dihydropyran led to the corresponding aldehyde **11** in quantitative yield.[11,12]

Addition of MeMgBr to **11** afforded a 1:1 mixture of epimers **12** and **13**. Initially, the 1:1 mixture was converted to the 6′-mesylates, then transformed to the corresponding C6′-azides **14** and **15**. This mixture was deacetylated, and reduction with PMe₃ led to verdamicin as a 1:1 mixture of

C2 and C2a congeners (**7** and **8**), which was found to be equally active as sisomicin against *E. coli* and *S. aureus*. Likewise, addition of EtMgBr and *i*PrMgBr, followed by mesylation and azide displacement afforded the ethyl and isopropyl analogues (**S31−32** and **S37−38**) which were found to be less active (see the Supporting Information).[12]

Since the C6′ alcohols **12** and **13** were inseparable, we deemed it necessary to first confirm our configurational assignments which were based on a Mosher ester analysis.[13] Thus, the mixture containing a 1:1 ratio of (*R*)- and (*S*)-isomers **12** and **13** was deacetylated and *O*-benzylated to **16** and **17**, then this mixture was subjected to ozonolysis to give the dicarbonyl products **18** and **19** (Scheme 2). Cleavage

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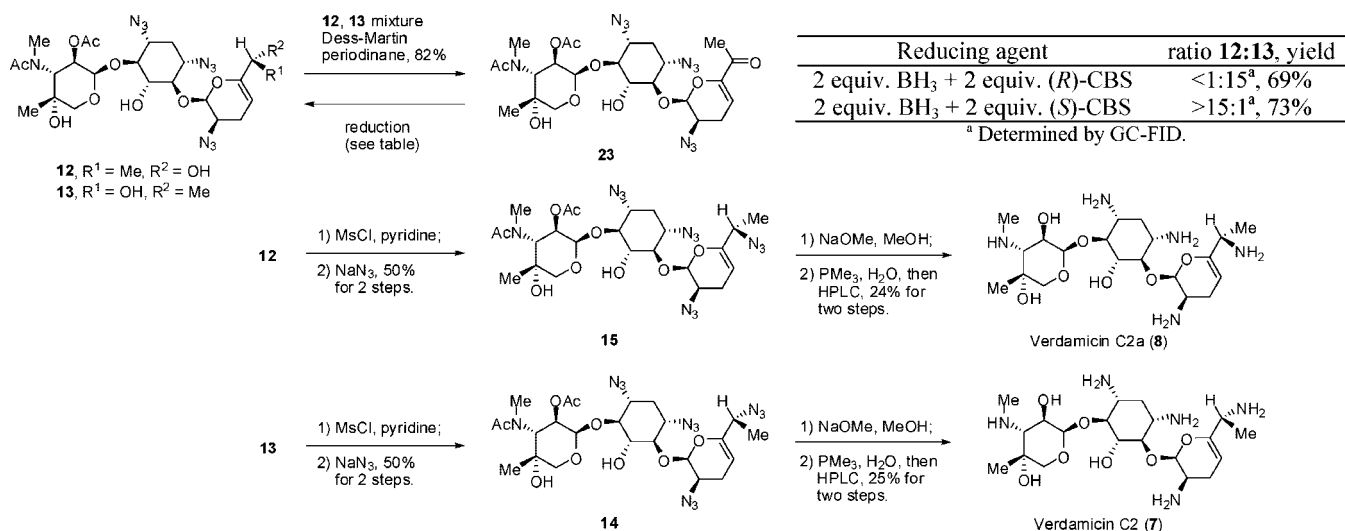(9) Kase, H.; Shimura, G.; Iida, T.; Nakayama, K. *Agric. Biol. Chem.* **1982**, *46*, 515.

(10) (a) Nyffeler, P. T.; Liang, C.-H.; Koeller, K. M.; Wong, C.-H. *J. Am. Chem. Soc.* **2002**, *124*, 10773. (b) Alper, P. B.; Hung, S.-C.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 6029. (c) Vasella, A.; Witzig, C.; Chiara, J.-L.; Martin-Lomas, M. *Helv. Chim. Acta* **1991**, *74*, 2073. (d) Zaloom, J.; Roberts, D. C. *J. Org. Chem.* **1981**, *46*, 5173. (e) Cavender, C. J.; Shiner, V. J. *J. Org. Chem.* **1972**, *37*, 3567. (f) Fischer, W.; Anselme, J.-P. *J. Am. Chem. Soc.* **1967**, *89*, 5284.

(11) For an example of SeO₂ oxidation of 6′-*N*-(2,4-dinitrophenyl)sisomicin derivatives into the corresponding aldehydes, see: Nagabhushan, T. L. U.S. patent 3997524, 1976, CAN 88:51133.

(12) See the Supporting Information for details.

Scheme 2. Chemical Degradation for Diastereoselectivity Determination of the Grignard Addition Products

with NaOMe in MeOH afforded the pseudodisaccharide **20** and a mixture of (*R*)- and (*S*)-*O*-benzyl methyl lactates in a

Scheme 3. Diastereoselective Reduction of Ketone **23** and Preparation of the Individual Verdamicins C2 or C2a

Reducing agent	ratio 12:13, yield
2 equiv. BH$_3$ + 2 equiv. (R)-CBS	<1:15[a], 69%
2 equiv. BH$_3$ + 2 equiv. (S)-CBS	>15:1[a], 73%
	[a] Determined by GC-FID.

1:1 ratio as determined by gas chromatography by using enantiopure reference compounds **21** and **22** which were independently prepared.[14] Enhancement of individual peak areas by doping with the (R)- and (S)-lactates respectively confirmed the configurational identities of the 6′-(R)- and 6′-(S)-alcohols in the mixture.

Our next goal was to prepare the individual verdamicin C2 and C2a congeners. Oxidation of the mixture containing **12** and **13** using Dess−Martin periodinane reagent[15] gave the ketone **23** (Scheme 3). Reduction with borane under the Corey−Bakshi−Shibata conditions[16] gave a >15:1 ratio of the 6′-(R)-alcohol **12** over the epimeric **13** in the presence of a stoichiometric amount of (S)-CBS. The 6′-(S)-alcohol **13** was obtained in a >15:1 ratio by the use of the (R)-CBS reagent. These ratios were determined by GC analysis of the corresponding alcohols **21** and **22** obtained by the above-described chemical degradation of the CBS reduction products.

Transformation of **12** to the mesylate and displacement with sodium azide gave the (S)-azide **15** (Scheme 3). Deacetylation and reduction of the azide under Staudinger conditions led to verdamicin C2a (**8**) having a 6′-(S)-aminoethyl configuration. Verdamicin C2 (**7**), having the 6′-(R)-aminoethyl configuration, was obtained by using the same approach starting with the epimeric 6′-(S)-alcohol **13** via the azide **14**.

We also prepared the so-called antibiotic G-52[6] (**9**, 6′-N-methyl sisomicin) which is the sisomicin congener related to gentamicin C2b (**5**). Treatment of the aldehyde **11** with methylamine under conditions of reductive amination gave

24, which was then deacetylated and reduced to afford **9** (Scheme 4). Treatment of the aldehyde **11** with dimethylamine followed by deacetylation and reduction led, likewise, to a new congener (**26**, 6′-N,N-dimethyl sisomicin). Antibacterial activities against a selected panel of strains were maintained in **9**, but significantly reduced in **26**.[12]

Scheme 4. Preparation of Antibiotic G-52 (**9**) and Compound **26**
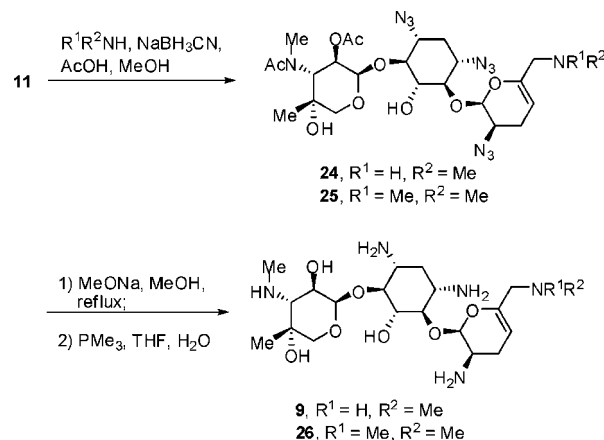
Verdamicin C2 (**7**) and C2a (**8**) exhibited essentially similar in vitro inhibitory activity compared to gentamicin complex.[12]

The venerable SeO$_2$ oxidation of allylic methyl or methylene groups to the corresponding alcohol or carbonyl derivative is a time honored and tested reaction.[17] The ready availability of the 6′-azido derivative **10** of sisomicin as described above gave us the incentive to test its direct oxidation with SeO$_2$. The successful conversion to aldehyde

(13) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512.

(14) Dubost, C.; Leroy, B.; Marko, I. E.; Tinant, B.; Declercq, J.; Bryans, J. *Tetrahedron* **2004**, *60*, 7693.

(15) (a) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155. (b) Meyer, S. D.; Schreiber, S. L. *J. Org. Chem.* **1994**, *59*, 7549.

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(17) Bulman, P. C.; McCarthy, T. J. *Comp. Org. Synth.* **1991**, *7*, 83.

11 led us to study the analogous reaction in a series of simple dihydropyrans. Surprisingly, no examples of related SeO_2 oxidations of allylic azides have been recorded in the literature. In fact, the only example of an allylic oxidation of a carbon—nitrogen bond to the carbonyl derivative appears to be the above-mentioned example by the Schering group.[11] Therefore, we undertook a preliminary comparative study of the oxidation of a series of 6-methyl-substituted dihydro[2*H*]pyrans and 1-methyl-substituted cyclohexenes. As representative model substrates we chose 6-methyl-dihydro[2*H*]pyrans containing N_3, OMe, and OAc functional groups on the primary carbon. On the basis of preliminary observations, we ran the oxidations with and without additives in the presence of 5 equiv of SeO_2.[12]

The methyl ether was oxidized much faster to the aldehyde, although the yield was modest compared to the azide (Table 1, entries 1—3). Addition of Bu_4NN_3 (TBAA)

Table 1. Allylic Oxidation of 6-Methyl-Substituted Dihydro[2*H*]pyrans

entry	R	additive (equiv)	time	isolated yield (%)
1	N_3		3 h	70
2	OMe		45 min	47
3	OAc		6 h	41
4	N_3	TBAA (1)	1 h	70
5	OMe	TBAA (1)	<30 min	65
6	OAc	TBAA (1)	4 h	60
7	N_3	TBACN (1)	1.5 h	71
8	OMe	TBACN (1)	45 min	65
9	OAc	TBACN (1)	5 h	69
10	N_3	DMAP (1)	4 h	81
11	OMe	DMAP (1)	1.5 h	80
12	OAc	DMAP (1)	16 h	77
13	N_3	DHP (3)	2.5 h	98
14	OMe	DHP (3)	30 min	82
15	OAc	DHP (3)	17 h	86

rendered the reaction mixture homogeneous with improved yields (Table 1, entries 4—9). On a preparative scale, addition of 1 equiv of DMAP was beneficial, although reaction times were somewhat longer.[12,18]

A study of the nature of the base on the time and efficiency of oxidation to the aldehyde showed DMAP to be the most effective (Table 1, entries 10—12).[12] However, addition of 3 equiv of dihydropyran as a scavenger proved to be most beneficial with regard to yield and reaction time (Table 1, entries 13—15).[12] Preliminary experiments with 1-azidom-ethyl, 1-methoxymethyl, and 1-acetoxymethyl 1-cyclo-hexenes under the same oxidation conditions, with or without additives, produced mixtures of the expected aldehydes and respective allylic endocyclic alcohols.[12]

Clearly, the oxidation of 6-methyl-substituted dihydro-[2*H*]pyrans is highly regioselective and influenced by the nature of the substituent on the primary carbon atom with respect to reaction rate. We defer from speculative mecha-nistic discussions favoring a concerted or stepwise pro-cess,[19,20] although the involvement of oxocarbenium ion intermediates may be highly favored.

In conclusion, we have devised a practical synthesis of verdamicin as a mixture of 5′-aminoethyl substituents and as the individual 6′-(*R*)- and 6′-(*S*)-isomers which correspond to the analogous components in the gentamicin complex. Although there is biogenetic evidence that verdamicin is related to gentamicin C2a,[2] the results of the present work demonstrate that the stereochemistry at C6′ is not critical for antibacterial activity in this series.
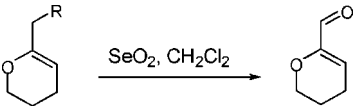
With the exception of gentamicin/tobramycin resistant *P. aeruginosa*, and the gentamicin acetylating strains, verdami-cin has been shown to possess potent *in vitro* and *in vivo* (mice) activity.[7] Comparative acute toxicity administered via parenteral (intravenous or subcutaneous) routes showed an improved performance for verdamicin compared to gentami-cin or tobramycin.[7] With the facile access to the natural verdamicin C2 and its congener C2a by semisynthesis from the readily available sisomicin, more detailed studies can be carried out for broader antibacterial testing, and to evaluate levels of toxicity of individual epimers.

Supporting Information Available: Experimental pro-cedures, full spectroscopic data for all new compounds, and antimicrobial activities. This material is available free of charge via the Internet at http://pubs.acs.org.

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