ORGANIC LETTERS

2009
Vol. 11, No. 2
429−432

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

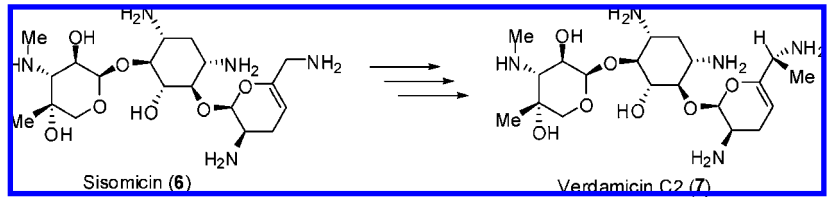
Stephen Hanessian,* Janek Szychowski, and J. Pablo Maianti

Department of Chemistry, Université de Montréal, C. P. 6128, Succ. Centre-Ville, Montréal, P.Q., Canada, H3C 3J7

stephen.hanessian@umontreal.ca

Received October 21, 2008

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[2*H*]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′-

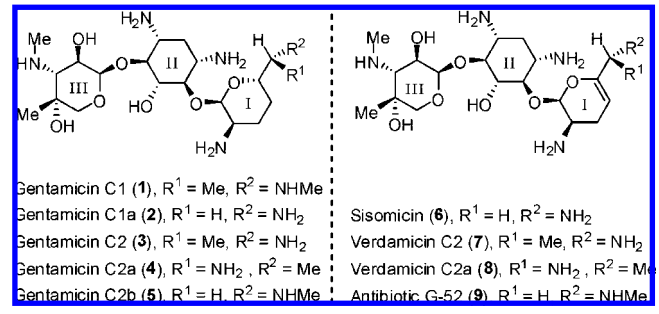


Figure 1. Structure of related aminoglycosides.

(1) (a) Jao, R. L.; Jackson, G. G. *Antimicrob. Agents Chemother.* **1963**, *161*, 148. (b) Jao, R. L.; Jackson, G. G. *J. Am. Med. Assoc.* **1964**, *189*, 817.

(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.

(3) Weinstein, M. J.; Luedemann, G. M.; Oden, E. M.; Wagman, G. H.; Rosselet, J. P.; Marquez, J. A.; Coniglio, C. T.; Charney, W.; Herzog, H. L.; Black, J. *J. Med. Chem.* **1963**, *6*, 463.

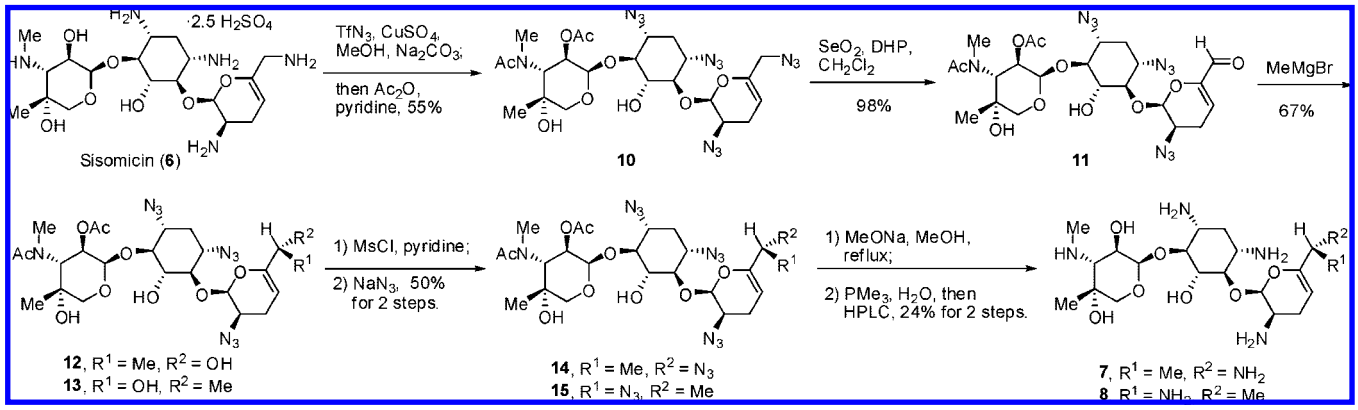
(4) Weinstein, M. J.; Marquez, J. A.; Testa, R. T.; Wagman, G. H.; Oden, E. M.; Waitz, J. A. *J. Antibiot.* **1970**, *23*, 551.

(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.

10.1021/ol802421d CCC: $40.75
Published on Web 12/10/2008

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]

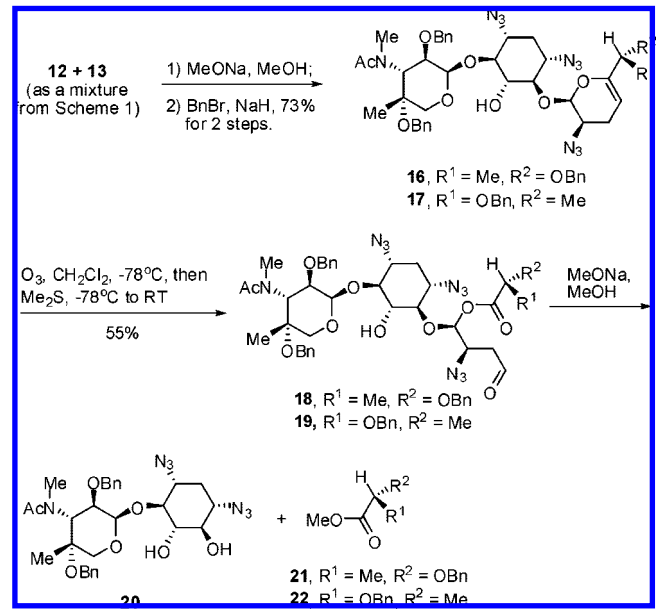
In this letter, we describe a synthesis of verdamicin C2 and its congener C2a from sisomicin relying on a novel oxidative transformation of an allylic azide to the corresponding α,*β*-unsaturated aldehyde, and its stereocontrolled elaboration into the intended 5′ side chains of verdamicin C2 and C2a.

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 SeO_2 in CH_2Cl_2 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 PMe_3 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

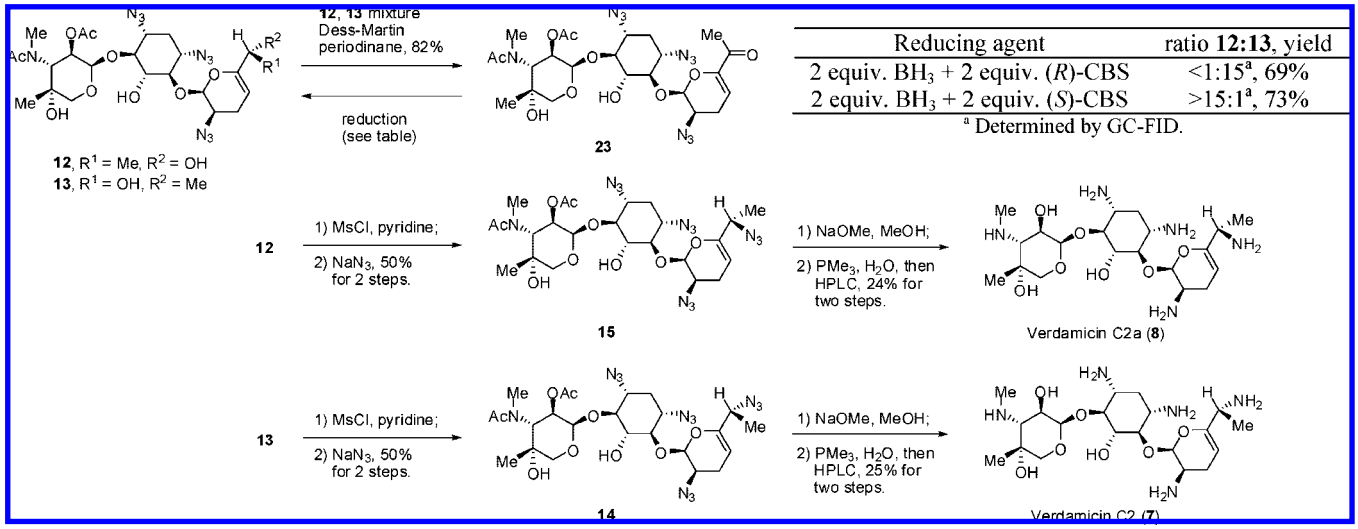
Scheme 2. Chemical Degradation for Diastereoselectivity Determination of the Grignard Addition Products



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

(7) Weinstein, M. J.; Wagman, G. H.; Marquez, J. A.; Testa, R. T.; Waitz, J. A. *Antimicrob. Agents Chemother.* **1975**, *7*, 246.
(8) Davies, D. H.; Mallams, A. K. *J. Med. Chem.* **1978**, *21*, 189.
(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 SeO_2 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 3. Diastereoselective Reduction of Ketone **23** and Preparation of the Individual Verdamicins C2 or C2a



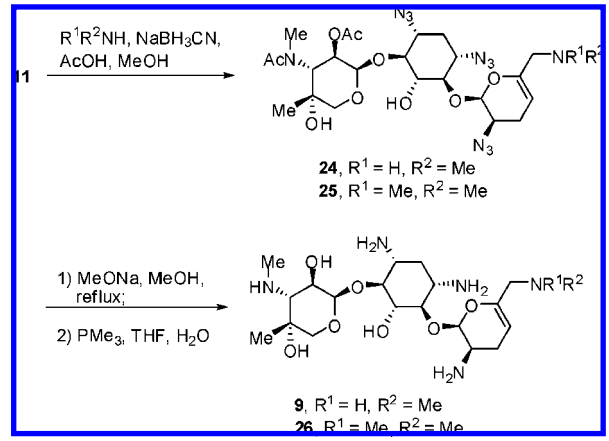
1:1 ratio as determined by gas chromatography by using enantiopure reference compounds **21** and **22** which were independently prepared.[14] Enhancement of individual peak areas by doping with the (*R*)- and (*S*)-lactates respectively confirmed the configurational identities of the 6′-(*R*)- and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

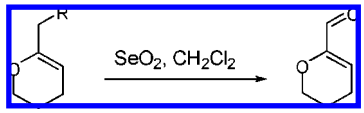
The venerable SeO_2 oxidation of allylic methyl or methylene groups to the corresponding alcohol or carbonyl derivative is a time honored and tested reaction.[17] The ready availability of the 6′-azido derivative **10** of sisomicin as described above gave us the incentive to test its direct oxidation 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.
(16) (a) Corey, E. J.; Helal, C. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 1986. (b) Corey, E. J.; Bakshi, R. K.; Shibata, S.; Chen, C. P.; Singh, V. K. *J. Am. Chem. Soc.* **1987**, *109*, 7925.
(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) 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]

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

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-azidomethyl, 1-methoxymethyl, and 1-acetoxymethyl 1-cyclohexenes 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 mechanistic discussions favoring a concerted or stepwise process,[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, verdamicin 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 gentamicin 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.

Acknowledgment. We thank NSERC, FQRNT, FRSQ fellowships, and Achaogen Inc. for financial assistance and antibacterial testing.

Supporting Information Available: Experimental procedures, 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.

OL802421D

(18) Internal salts between SeO_2 and amines have been reported: Touzin, J.; Neplechova, K.; Zak, Z.; Cernik, M. *Collect. Czech. Chem. Commun.* **2002**, *67*, 577.
(19) Stephenson, L. M.; Speth, D. R. *J. Org. Chem.* **1979**, *44*, 4683.
(20) Singleton, D. A.; Hang, C. *J. Org. Chem.* **2000**, *65*, 7554.