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CHEMICAL MODIFICATIONS AND STRUCTURE ACTIVITY STUDIES OF ZIRACIN AND RELATED EVERNINOMICIN ANTIBIOTICS

Ashit K. Ganguly,* Jinping L. McCormick, Anil K. Saksena, Pradip R. Das, and Tze-Ming Chan

Chemical Research, Schering-Plough Research Institute
2015 Galloping Hill Road, Kenilworth, New Jersey 07033

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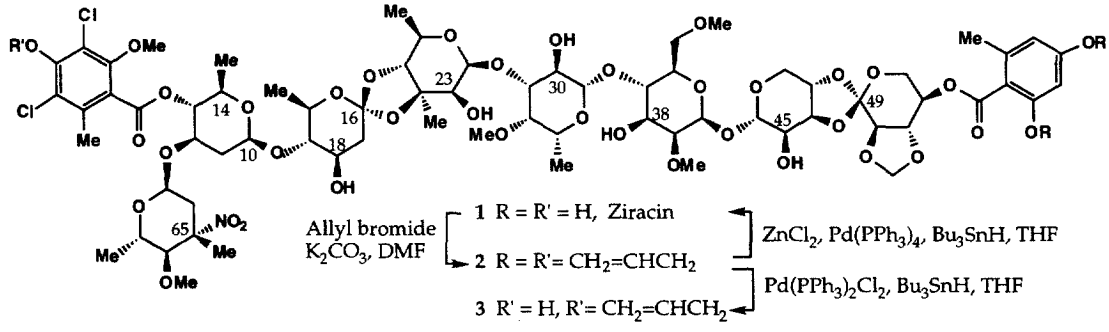
Abstract: Chemical modifications of everninomicin antibiotics, particularly ziracin (**1**), were carried out to study the SARs as well as the chemical properties of this class of compounds. Use of allyl ether group for protection and selective deprotection of phenolic groups provided access to a variety of novel analogs of the title compounds, some of which exhibited the same high *in vitro* potency as the parent compounds.

Everninomicins, a group of complex oligosaccharides produced by *Micromonospora carbonaceae*, are highly active against Gram positive bacteria including methicillin resistant *Staphylococci* and vancomycin resistant *Enterococci*. Ziracin (**1**), a member of this group of antibiotics, is undergoing extensive clinical trials to determine its activity against both sensitive and resistant strains.



We have previously reported the structures of several everninomicins,[1] including everninomicin B,[2] C,[3] D[4] and antibiotic 13-384[5] components 1 and 5 (ziracin and Sch 27900, respectively). A unique structural feature shared among these compounds is the presence of two acid sensitive orthoester linkages[4,6] at C16 and C49, the

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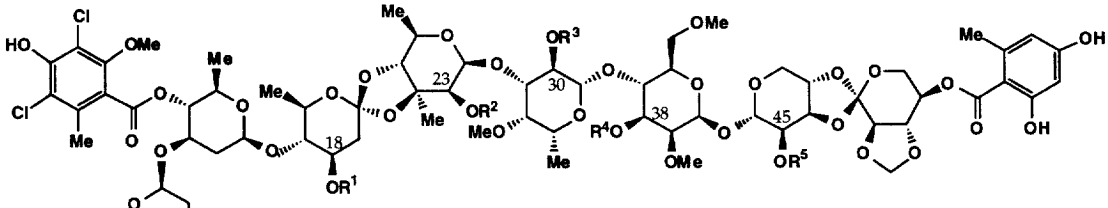
former being relatively more acid labile. The absolute configuration of C49 center was determined by X-ray crystallography;[7] and that of C16 was recently assigned by chemical and spectroscopic methods.[8]

We undertook the chemical modifications of everninomicins to study the *in vitro* SARs of this group of compounds as well as their chemical properties. The nitro group of everninomicin D has been previously reduced to nitroso, hydroxyamino, amino and alkyl amino groups, etc.[9] This paper will describe our work on (i) alkylation of the saccharide hydroxyl groups, (ii) deoxygenation of saccharide hydroxyl group, and (iii) conversion of the nitro group to the amino acid derivatives. Most of the present work was carried out on ziracin (**1**).



4 $R^1=R^2=R^3=R^4=R^5=Me$

5 $R^2=R^4=R^5=CH_2COOMe$, $R^1=R^3=H$

6 $R^5=CH_2CH_2OMe$, $R^1=R^2=R^3=R^4=H$

7 $R^5=CH_2CH_2OH$, $R^1=R^2=R^3=R^4=H$

8 $R^5=CH_2CO_2Bn$, $R^1=R^2=R^3=R^4=H$

9 $R^5=CH_2COOH$, $R^1=R^2=R^3=R^4=H$

Alkylation of the saccharide hydroxyl groups. Ziracin (**1**) consists of eight saccharides and two highly substituted aromatic ester moieties. The saccharide hydroxyl groups were one area for modification that would alter the hydrogen bonding properties of the molecules and in some cases, water solubility, which consequently may effect their pharmacokinetic properties.

First, the three phenolic hydroxyl groups needed to be selectively protected. Several protecting groups, including allyloxycarbonyl, SEM and allyl were used and evaluated. Allyl group was selected because of its stability, ease and selectivity in removal.[10] Therefore, 1, 57, 59-triallylether **2** was prepared and used as a common substrate for further reactions. Allyl group at O-1 was selectively removed to give **3** using Bu_3SnH, $Pd(PPh_3)_2Cl_2$ in THF in good yield (70-90 %), and a combination of anhydrous $ZnCl_2$, $Pd(PPh_3)_4$ and Bu_3SnH cleaved all three allyl groups of **2** in quantitative yield.

Alkylation of triallyl ziracin **2** was typically accomplished by controlled deprotonation using appropriate amount of NaH or $NaN(TMS)_2$ followed by addition of excess amount of appropriate alkylating agents. Employing alkylating reagents of varying reactivity, we were able to probe the relative reactivity of the five hydroxyl groups.

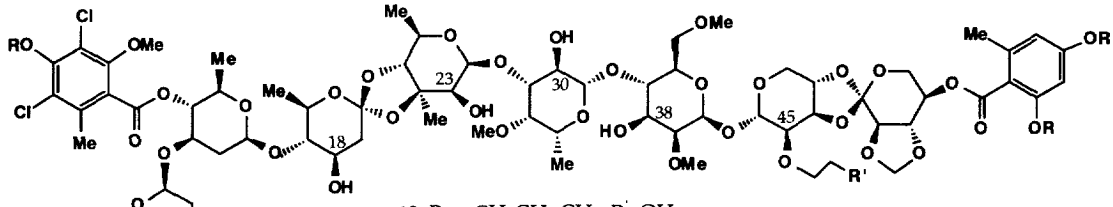
A variety of modifications of hydroxyl groups, from nonpolar pentamethylether **4** to hydroxylethyl ether **7** to polar carboxylic acid **9** have been carried out. When **2** was treated with NaH and excess methyl

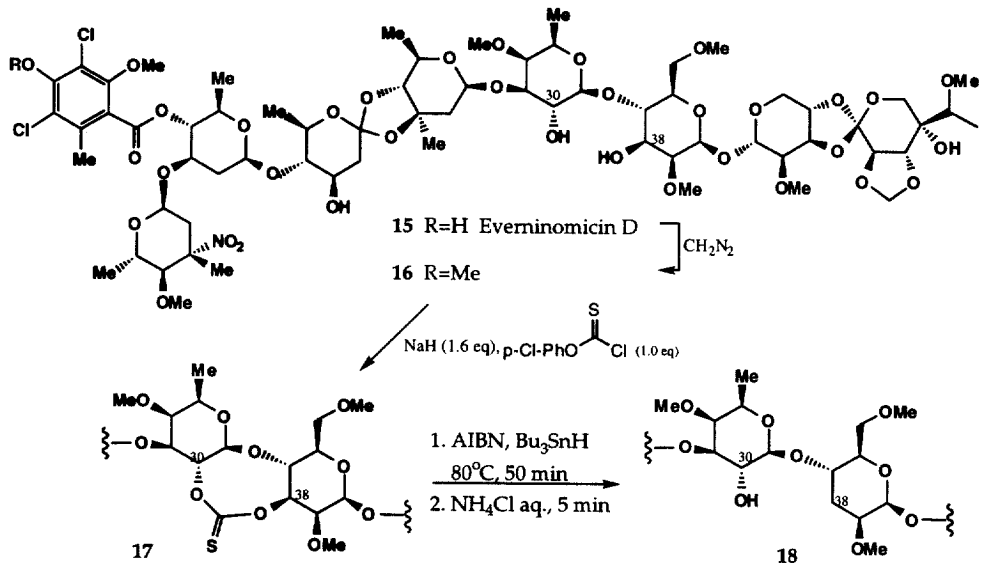
bromoacetate, 23, 38, 45-trialkylated product **5** was isolated as the major product along with minor amount of both di- and tetra-substituted products. Limiting the amount of benzyl bromoacetate to 1.5 eq resulted in 45-O- monoalkylated ziracin **8** after removal of allyl groups. Hydrogenolysis of **8** over 10% Pd on carbon in pyridine gave the desired acid **9**. The use of neat pyridine as solvent suppressed one unidentified byproduct which occurred when several other solvents (ethanol, ethanol/pyridine, etc) were used. Alkyaltion of **2** with excess 2-bromo (or iodo)-TBDMS ethanol gave a single product (43% yield) which by deprotection of the allyl groups yielded 45-monosubstituted derivative **7**.

The variations in selectivity of alkylation suggest differring reactivities of the five hydroxyl groups, of which 45-OH is the most accessible, followed by 23-OH (observed in acylation) and 38-OH, whereas 18-OH is the least reactive. This order of reactivity was also observed in other acylation reactions such as those with thionochloroformate reported later in this paper. Examination of a three dimensional molecular model of **1** reveals that the axial hydroxyl groups at 45-OH and 23-OH are oriented out of the rigid planes defined by H-I rings and D-E rings respectively. On the other hand, 18-OH is in perfect position to hydrogen bond with the ring oxygen atom between C-10 and C-14, making it less accessible.

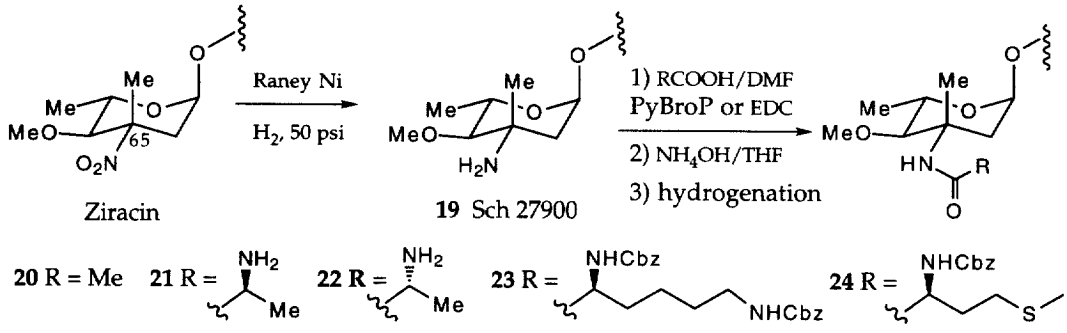
Compound **10**, obtained from alkylation of **2** with 2-bromo-TBDMS ethanol and treatment with TBAF, was further modified to obtain azido and amino derivatives. Thus, the primary hydroxyl group was carefully converted to tosylate **11**, which was displaced with NaN_3 to give **12**. Hydrogenation of **12** in the presence of acetic anhydride over 10% Pd on carbon followed by ammonia hydrolysis (to remove any esters) afforded the acetamide **13**.

Deoxygenation study of the saccharide hydroxyl groups. To further study the SAR around the saccharide hydroxyl groups, we set out to selectively remove the hydroxyl groups of everninomicin antibiotics including ziracin. When the methyl ether of everninomicin D **16** was treated with NaH (1.6eq) and aryl thionochloroformate (1.0eq), an eight-membered cyclic thiocarbonate **17**, to our surprise, was isolated as the





major product. The structure of **17** was confirmed by extensive 2D-NMR experiments including HMQC and HMBC. Treatment of **17** with Bu_3SnH and AIBN at 80°C followed by mild hydrolysis in aqueous NH_4Cl gave 38-deoxy everninomicin D methyl ether **18** as the only isolable compound in modest yield (15%). So far the above deoxygenation experiments did not succeed when applied to ziracin. Further work is in progress.



Preparation of amino acid derivatives at C65. It has been reported from our laboratory that the nitro group in everninomicins could be converted to the amino, hydroxylamino and nitroso derivatives without complete loss of microbiological activity. Indeed all of these compounds are also found in the fermentation broth in varying amounts. In the present studies, ziracin was reduced to its amino derivative **19** using Raney Ni in nearly quantitative yield. Reaction of **19** with acetic acid catalyzed by EDC/pyridine went smoothly to give the acetamide **20**. However, coupling with Cbz-protected amino acids did not proceed under similar conditions presumably due to steric hindrance around the amino group. Instead, we found PyBroP a very efficient

coupling reagent to prepare compounds **21** to **24**. Mild alkaline hydrolysis selectively cleaved all esters formed during the coupling steps. The sites of amino acid attachment were vigorously proven by MS and NMR experiments.[11]

Table 1. MIC (μg/mL) ranges of selected everninomicin derivatives

Compd#	*Staphylococci*	*Enterococci*	Compd#	*Staphylococci*	*Enterococci*
1 (ziracin)	2-4 0.25-0.5[a]	0.25-4 0.25-0.5[a]	**3**	4-16	4-8
4	16-64	4-128	**5**	32-64	4-64
7	1-2	0.5-2	**10**	16-64	8-64
12[a]	2-8	2	**13**[a]	2-4	4-8
19	2-16	4-16	**20**	1-4	2-16
21	4-8	4-32	**23**	32-64	8-32
24	2-16	2-8			

(1)[a] All samples were tested in the same batch against 4 species (6 strains each) except for those labelled with[a] which were tested in second batch (3 strains each).
(2) All samples were tested as N-methyl glucamine salts.

***In vitro* antibacterial assay results.** The ziracin derivatives reported in the present paper showed (Table 1) similar activities against methicillin-resistant and methicillin-susceptible *Staphylococci,* and vancomycin-resistant and vancomycin-susceptible *Enterococci.* From the microbiological results it can be concluded that

- monosubstituted hydroxyethyl compound **7** was as potent as ziracin, but azido and acetamido analogs (**12, 13**) were an order of magnitude less active, while an acid (**10**) lost most of the activity.
- capping multiple hydroxyl groups with hydrophobic groups (**4, 5**) resulted in loss of activity.
- capping of 57,59-phenolic hydroxyl groups reduced activity by 2-10 fold. (**3** and corresponding diallyl compounds of **4, 5, 7** not shown here).
- among the amino acids derivatives, acetamide **20** exhibited the best *in vitro* activity, about 2-4 fold less active than **1**. However, structurally quite diverse (in terms of size, polarity and charge) amino acid modifications yielded similar activities suggesting that the nitro group in ziracin could be modified without loss of biological activity.

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(10) See Kocienski, P.J. in "Protecting Groups" Georg Thieme Verlag, New York, 1994, pp 61-68.
(11) a) All new compounds were fully characterized using FAB-MS, 2D-NMR and ^{13}CNMR.
b) Chan, T.-M.; Osterman, R. M.; Morton, J. B.; Ganguly, A. K. *Magn. Res. Chem.* **1997**, *35*(8), 529.
(12) Representative experimental procedures:

Allylation of ziracin. To a dry DMF solution of ziracin (4.9667 g, 3.049 mmol) was added solid anhydrous K_2CO_3 (2.53 g, 18.294 mmol). In 5 minutes, allyl bromide (1.58 mL, 18.294 mmol) was added dropwise to the stirring suspension. The mixture was allowed to stir for 23 hours, then diluted with water (200 mL) and extracted with EtOAc (3x200 mL). The combined organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo to afford a white solid **2** (5.33 g, 100% yield).

Mono-deallylation. A solution of **2** (80.0 mg, 0.044 mmol) in THF at room temperature was treated with $(PPh_3)_2PdCl_2$ (3 mg, 0.004 mmol), followed by dropwise addition of Bu_3SnH (35.5 μL, 0.113 mmol). In 15 min, TLC indicated completion of reaction. The mixture was concentrated to dryness. After being triturated with hexane, the crude material was purified on SiO_2 column using 0 - 5 % MeOH-CH_2Cl_2 to afford a white solid **3** (52.7 mg, 67 %).

Complete deallylation. A solution of triallyl ether of **4** (101.7 mg, 0.055 mmol) in THF was treated with $(PPh_3)_4Pd$ (11 mg, 0.009 mmol), followed by dropwise addition of 0.5 M $ZnCl_2$ in THF (335 μL, 0.167 mmol) at room temperature. In 5 min, Bu_3SnH (45 μL, 0.167 mmol) was added to the above solution and stirred for additional 25 min. The reaction was quenched by addition of aqueous NH_4Cl solution and extracted with EtOAc. The organic layer was concentrated to dryness. After being triturated with hexane, the crude material was purified on SiO_2 column using 0 - 5 % MeOH-CH_2Cl_2 to afford a white solid **4** (93.7 mg, 98 %).

Alkylation of 2. Preparation of 45-O-(2-hydroxyethyl)-triallyl ziracin (10). To an ice-chilled suspension of NaH (310 mg, 7.750 mmol, 60% dispersion in mineral oil) in dry DMF (10 mL) was added **2** (2.02 g, 1.155 mmol) in DMF (14 mL). The mixture was stirred at room temperature for 1 hour and treated with 2-bromo-O-*tert*-butyldimethylsilyl ethanol (2.0 g, 8.368 mmol) over night. The reaction was quenched with aqueous NH_4Cl and partitioned between EtOAc and water. Purification of the crude product on SiO_2 using 0 - 5% MeOH-CH_2Cl_2 gave a white solid (943 mg, 43% yield) which was deprotected with TBAF/THF to afford **10**.

Preparation of amino acid derivative of ziracin (21). A solution of **19** (101 mg, 0.0632 mmol) and N-Cbz-L-Ala (15.9 mg, 0.0713 mmol) in DMF (2 mL) was treated with PyBroP (32 mg, 0.0686 mmol) and (*i*-Pr)$_2$NEt (22 μL, 0.126 mmol) at room temperature for 24 hours. The reaction mixture was diluted with THF (10 mL) and treated with concentrated aqueous NH_4OH (0.8 mL). In 75 min, the reaction was quenched with citric acid. The mixture was concentrated in vacuo and partitioned between EtOAc and aqueous NH_4Cl. The crude material was purified on SiO_2 to afford N-Cbz protected **21** (62.2 mg, 55 %) as a white solid which was hydrogenated over 10%Pd/C (15 mg) in EtOH to give **21** (32 mg, 58%).