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Total Synthesis and Antifungal Activity of a Carbohydrate Ring-Expanded Pyranosyl Nucleoside Analogue of Nikkomycin B†

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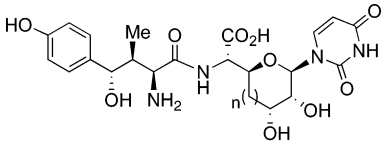
Received August 17, 2007



n = 0; **Nikkomycin B**
n = 1; **'Pyranosyl Nikkomycin B'**

In a study aimed at investigating an as yet unknown structure−activity relationship of the nikkomycin family of antifungal peptidyl nucleoside antibiotics, the present research reports the synthesis and antifungal evaluation of a carbohydrate ring-expanded pyranosyl nucleoside analogue of nikkomycin B. Employing a convergent synthetic route, independent synthesis of the *N*-terminal amino acid side chain and a stereoselective de novo construction of the desired pyranosyl nucleoside amino acid fragment was followed by peptidic coupling of the two components, leading to the first synthesis of a carbohydrate ring-enlarged pyranosyl nikkomycin B analogue. In vitro biological evaluation of the above analogue against a variety of human pathogenic fungi demonstrated significant antifungal activity against several fungal strains of clinical significance.

Introduction

The complex peptidyl nucleoside antibiotics are a unique class of secondary microbial metabolites, with potent antifungal activity against a wide spectrum of pathogenic fungi.[1] Among the various peptidyl nucleoside antibiotics, polyoxins (Figure 1) were the first family of compounds to be isolated in the 1960s from *Streptomyces cacaoi* var. *asoensis*.[2] The polyoxins were found to exhibit potent antimicrobial activity against various fungi and find use as efficient agricultural fungicides with no adverse side effects.[3] Subsequently, another class of *Streptomyces*-derived peptidyl nucleoside antibiotics, the nikkomycins (neopolyoxins) (Figure 1), displayed selective and potent inhibitory activity against fungi such as *Pyricularia oryzae* and *Rhizoctonia solani*.[4]

The original interest in the polyoxin and nikkomycin families of compounds stemmed from their highly selective activity against various fungi, while being nontoxic to bacteria, plants, and animals. Acting via a novel mode of action, these natural products were found to be strong competitive inhibitors of chitin synthases (K_i range: 0.1−1 μM),[2,4] the fungal glycosyl processing enzymes responsible for the formation of chitin, an essential fungal cell wall component. Chitin, a β-(1→4)-linked polymer of *N*-acetylglucosamine, is responsible for imparting shape, strength, and rigidity to the fungal cell wall.[5] Inhibition of chitin

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† Dedicated to the memory of Dr. C. V. Asokan, a fellow chemist, educator, and a good friend.

‡ The University of Kansas.

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10.1021/jo701814b CCC: $37.00 © 2007 American Chemical Society
Published on Web 11/15/2007

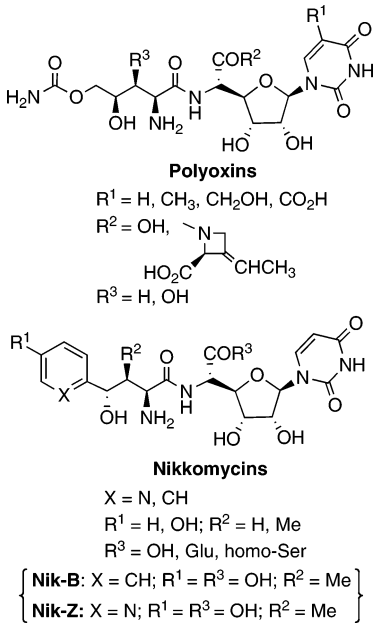

FIGURE 1. Structures of representative polyoxins and nikkomycins.

biosynthesis has been found to cause osmotic sensitivity, abnormal morphology, and fungal growth arrest, ultimately leading to cell death. As the chitin biosynthetic pathway is absent in humans and other mammals,[5c,6] chitin synthase and the cellular mechanisms that regulate the activity of this enzyme are considered to be excellent targets for pharmaceutical and agricultural pathogen management.[7]

With demonstrated in vitro and in vivo activity against various human pathogenic mycotic infections, the nikkomycin and polyoxin family of complex peptidyl nucleosides represent exciting leads in the continuing search for new, effective, and nontoxic antifungal agents.[8] However, direct clinical application of the peptidyl nucleosides has been compromised by their attenuated in vivo activity, largely due to their inefficient uptake into the fungal cell.[7] A detailed understanding of the pharmacophore of these compounds and the ability to fine-tune structural features toward optimization of antifungal potency and pharmacodynamics are therefore critical needs in accelerating the transfer of these promising antifungal peptidyl nucleoside lead compounds into clinically useful therapeutic agents.

In view of their exciting antifungal potential, nikkomycins and polyoxins continue to be the focus of total syntheses and structure−activity relationship investigations.[9,10] Most of the reported SAR studies on these compounds have, however, been directed at modification of the amino acid side chain, the nucleobase component, and replacement of the furanose ring oxygen with carbon, sulfur, or nitrogen functionalities.[9a] Surprisingly, thus far there have been no studies investigating the effect of the carbohydrate ring size of the above nucleosides on their biological activity. Interestingly, there are several examples of bioactive complex nucleoside antibiotic natural products containing a four-membered ("ring contracted") carbohydrate core (e.g., oxetanocins),[11] or a six-membered ("ring expanded") carbohydrate nucleoside core (e.g., amipurimycin, miharamycin, blasticidin, gougerotin, etc.).[12] The occurrence and impressive spectrum of biological activities of the above natural products suggest that size and conformation of the carbohydrate ring can be potentially important handles toward improving and "fine-tuning" the activity profile of the peptidyl nucleosides. Accordingly, as part of a research program directed at the synthesis, structural modification studies, and biological evaluation of the peptidyl nucleoside antibiotics,[13] we report herein the synthesis and preliminary antifungal evaluation of a hitherto unreported pyranosyl nucleoside analogue of nikkomycin B (**1** in Figure 2).

Results and Discussion

Our strategy for the synthesis of the desired pyranosyl nikkomycin B (**1**) is shown in Figure 2. The plan envisaged a convergent synthetic route based upon initial stereocontrolled synthesis of the left-hand side amino acid side chain fragment **2**, and the right-hand side α-amino acid nucleoside subunit **3**, followed by peptidic coupling of the above fragments to construct the complete structural framework of **1** (Figure 2).

A key reaction in the formation of the side chain amino acid **2** envisaged a stereoselective crotylboration reaction between 4-pivaloyloxybenzaldehyde (**4**) and an appropriate chiral borane reagent. On the other hand, following our earlier reported protocol,[13b] the pivotal pyranosyl nucleoside amino acid **3** could be synthesized from the D-serine-derived chiral pyranone intermediate **5**.

Accordingly, following Barrett's procedure,[14] a highly enantio- and diastereoselective addition of (−)-*E*-crotyldiisopi-

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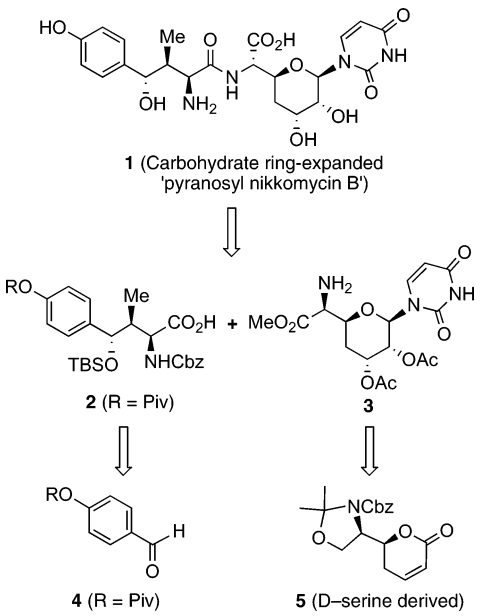
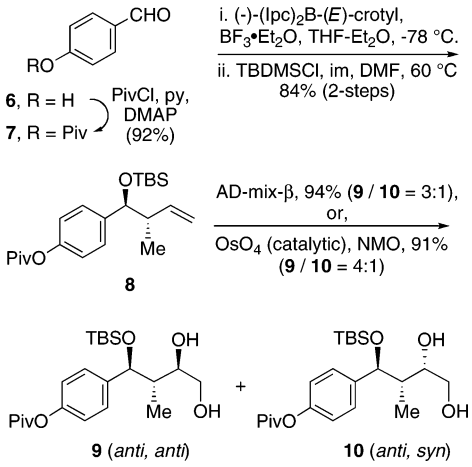


FIGURE 2. Pyranosyl nikkomycin B (**1**): retrosynthetic strategy and approach.

nocampheylborane to 4-pivaloyloxybenzaldehyde **7** (Scheme 1) and subsequent protection of the resulting secondary hydroxy group as its TBS-ether resulted in the expected homoallylic alcohol derivative **8** (Scheme 1) as the only product. NMR (^{1}H and ^{13}C) spectral studies and HPLC analysis of **8** confirmed its structural and stereochemical integrity.

SCHEME 1



Toward introduction of the required α-amino acid functionality as present in the nikkomycin B side chain, stereoselective dihydroxylation of the terminal olefinic moiety of **8** was next investigated. Disappointingly, attempted Sharpless asymmetric dihydroxylation of **8** with AD-mix-β (a matched pair)[15] resulted in a mixture of the expected 2,4-*syn*-diol **9** along with the unwanted 2,4-*anti*-diol **10** with only a moderate selectivity (3:1, by ^{1}H NMR) in favor of **9**.

In retrospect, the unsatisfactory selectivity in the above reaction can probably be attributed to possible unfavorable steric interactions between the bulky dihydroxylating reagent and the β-configured substituent at the benzylic stereocenter, situated two carbons away from the olefinic reactive site. Interestingly, dihydroxylation of **8** with osmium tetroxide (OsO_4) under standard conditions afforded the required 2,4-*syn*-diol **9** with somewhat improved selectivity (4:1, by ^{1}H NMR). It is assumed that, compared to AD-mix-β, the relatively smaller size of OsO_4 probably reduces the steric interaction with the distal benzylic stereocenter, resulting in the observed improvement in selectivity.

Employing Rychnovsky's ^{13}C NMR method for stereochemical determination of 1,3-diol systems,[16] the assigned stereochemistry of the 1,3-*syn*- and 1,3-*anti*-diols **9** and **10** was confirmed via analysis of the ^{13}C NMR spectra of the corresponding acetonide derivatives **9A** and **10A**, respectively (Figure 3).

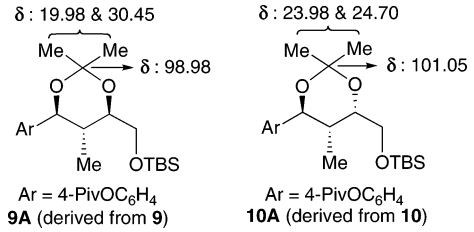
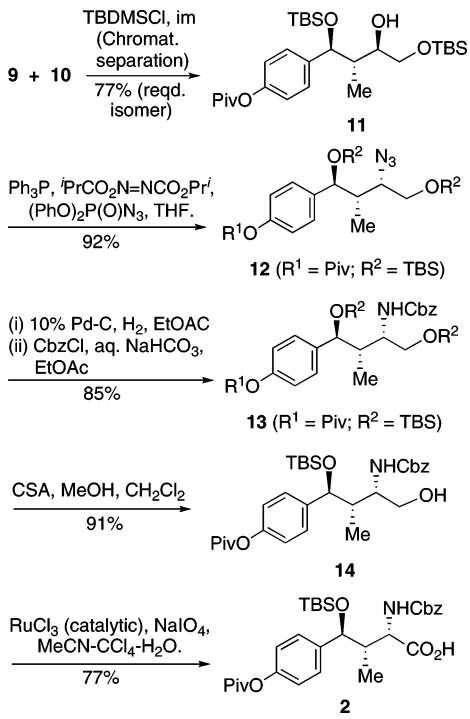


FIGURE 3. Diagnostic ^{13}C NMR shifts of the acetonides derived from the diols **9** and **10**.

Selective TBS-protection of the primary hydroxy group allowed easy chromatographic separation of the product mixture, affording the required triol derivative **11** in good overall yield (Scheme 2). Following a standard Mitsunobu procedure,[17] the free hydroxy group of **11** was converted to the corresponding

SCHEME 2



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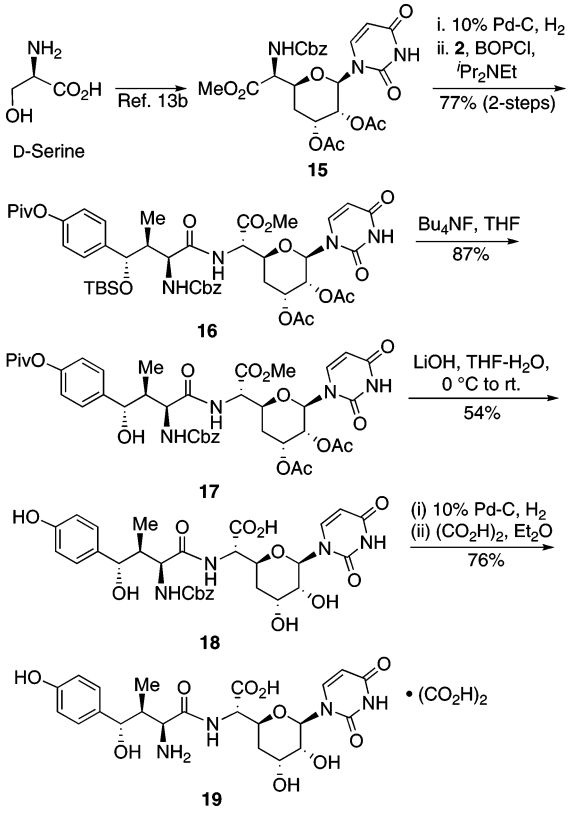
azide **12**, with concomitant inversion of the stereocenter involved. Reduction of the azide to amine and protection of the resulting amine uneventfully afforded the corresponding carbamate derivative **13** in high overall yields. Camphorsulfonic acid assisted selective deprotection at the primary silylether linkage[18] to generate **14**, followed by oxidation of the resulting free hydroxy group to carboxylic acid culminated in an efficient route to the orthogonally protected nikkomycin B side chain *N*-terminal amino acid derivative **2**. It is worth mentioning that, in terms of brevity and efficiency, the above synthesis of the strategically protected nikkomycin B side chain (**2**) compares well with the other literature reported methods,[9a] and is expected to be an attractive alternative for the synthesis of this unusual amino acid and related analogues.

Following our earlier reported method, a de novo synthesis of the fully protected right-hand side pyranosyl nucleoside amino acid fragment **15** (Scheme 3) was achieved utilizing D-serine as a chiral starting material.[13b] Subsequent hydrogenolytic removal of the Cbz-functionality generated the free amine **3**. A BOP-Cl mediated peptidic coupling between **3** and the side chain amino acid **2** provided the fully protected pyranosyl nikkomycin B analogue **16**.

SCHEME 3



Standard removal of the silyl protecting group to form **18**, followed by hydrolytic cleavage of the four ester functionalities yielded the polyhydroxylated carboxylic acid derivative **18**. Finally, hydrogenolysis of the amine protecting group followed by crystallization induced purification of the resulting product via oxalate salt formation culminated in the desired carbohydrate ring expanded nikkomycin B pyranosyl analogue **19**.

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Biological Evaluation

To evaluate the effect of the above carbohydrate ring enlargement on the antifungal activity of nikkomycins, in vitro susceptibility testing of the pyranosyl analogue **19** against clinical isolates of five different fungi, *Candida albicans*, *Candida glabrata*, *Aspergillus fumigatus*, *Cryptococcus neoformans*, *Coccidioides immitis*, and *Blastomyces dermatitidis*, were performed. The antifungal drug amphotericin B (Ampho-B) and commercially available nikkomycin Z (Nik-Z) (Sigma) were used as reference standards in these antifungal assays. The results (MIC) of the biological evaluation are given in Table 1. The MIC was designated as the lowest concentration where visible growth was absent.

TABLE 1. Antifungal Activity (MIC) of Pyranosyl Nikkomycin B (19)

fungal species (no. of isolates)	pyranosyl Nik-B (**19**) [MIC = μg/mL]	Nik-Z [MIC = μg/mL]	Ampho-B [MIC = μg/mL]
C. albicans (3)	>16	4, 8, and >16	0.125
C. glabrata (2)	>16	>16	0.125 and 0.25
A. fumigatus (3)	>16	2−4	0.25−0.5
C. neoformans (1)	≤0.03	≤0.03	**0.25**
C. immitis (1)	≤0.03	**0.25**	**0.25**
B. dermatitidis (1)	**0.5**	**0.03**	**0.25**

As evident, the pyranosyl nikkomycin B analogue **19** was found to be inactive against *Candida albicans*, *Candida glabrata*, and *Aspergillus fumigatus.* This is not entirely unexpected, as nikkomycin Z (the most potent among the natural nikkomycins) itself is not a very efficient antifungal agent against most strains of *Candida* and *Aspergillus*. Gratifyingly, against human pathogenic fungal strains of *Cryptococcus neoformans* and *Coccidioides immitis*, the analogue **19** exhibited strong inhibitory activity. Thus, while the antifungal activity of **19** against *Cryptococcus neoformans* was equipotent to that of nikkomycin Z (and significantly better than that of amphotericin B), the MIC of **19** against *Coccidioides immitis* was much lower than both the references amphotericin B and nikkomycin Z. Although not as active as amphotericin B or nikkomycin Z, the pyranosyl analogue **19** also displayed some inhibitory activity against *Blastomyces dermatitidis*.

It is worth mentioning that, although relatively less common, fungal infections caused by *C. neoformans* (cryptococcosis), *C. immitis* (coccidiodomycosis), and *B. dermatitidis* (blastomycosis) are nonetheless pathogenic systemic (lungs, brain, bone, GI tract, etc) fungal infections of increasingly serious concern. Interestingly, while Amphotericin B is a commonly used agent for the treatment of these infections, in our antifungal assay, involving clinical isolates of the above human pathogenic strains, the newly synthesized pyranosyl nik-B analogue **19** was found to be about 10 times more active than the Amphotericin B standard. We believe that this is a significant observation and can provide important design parameters toward future optimization of the nikkomycin structural lead.

In conclusion, the present total synthesis and structure−activity relationship study aimed at a previously unexplored structural component of the natural nikkomycins indicate that, carbohydrate core ring-enlargement of the nikkomycin family of compounds could be a potentially beneficial strategy toward optimizing the antifungal potency of the above peptidyl nucleosides. Studies are currently underway toward the synthesis of further modified analogues based on the above pyranosyl nucleoside scaffold and eventual antifungal evaluation of these

analogues against various pathogenic fungi. Results from the above studies are expected to provide a better understanding of the role of the carbohydrate core ring size in the biological activity of the nikkomycin family of antifungal antibiotics. We hope that continued investigation of the pyranosyl nikkomycin analogues will help us fully explore and optimize the structural features of the nikkomycins family of novel lead compounds toward the potential development of a new class of therapeutically useful antifungal agents.

Experimental Section

4-Pivaloyloxybenzaldehyde (7). 4-Hydroxybenzaldehyde **6** (12 g, 98.8 mmol) was dissolved in anhydrous CH_2Cl_2 (120 mL), followed by addition of anhydrous pyridine (13.5 mL, 167 mmol) and a catalytic amount of DMAP. The reaction mixture was cooled to 0 °C, and freshly distilled pivaloyl chloride (18.5 mL, 150 mmol) was added dropwise with stirring. After completion of the addition, the reaction mixture was brought to room temperature and stirred for another hour. The reaction was quenched by the addition of ice-cold water (100 mL) and stirred for 5 min. The two layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and the solvent was removed in vacuo. The residual oil was purified by flash chromatography (hexane/EtOAc = 19:1) to yield 4-pivaloyloxybenzaldehyde (**7**) as a low melting solid (21.0 g, 92%): IR (neat) 1752, 1680 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.38 (s, 9H), 7.27 (d, J = 6.0 Hz, 2H), 7.92 (d, J = 6.0 Hz, 2H), 10.0 (s, 1H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 26.9, 39.1, 122.2, 131.0, 133.7, 155.8, 176.2, 190.8. HRMS calcd for $C_{12}H_{15}O_3$ m/z $(M + H)^+$ 207.1021, found 207.1012.

(3*S*,4*S*)-4-[(*tert*-Butyldimethylsilyl)oxy]-3-methyl-4-[4-(pivaloyloxy) phenyl]butene (8). Step 1: To a well-stirred solution of *t*-BuOK (1 M solution in THF, 38 mL, 38 mmol) at − 78 °C was added *trans*-2-butene (7 mL, 74.7 mmol), followed by dropwise addition of *n*-BuLi (1.6 M in hexane, 24 mL, 38 mmol). After completion of addition, the reaction mixture was warmed to −40 °C, stirred for 30 min, then recooled to −78 °C, then (−)-*B*-methoxydiisopinocampheyl-borane (1 M in ether, 44.5 mL, 44.5 mmol) was added to it dropwise. After stirring at −78 °C for 30 min, $BF_3{\cdot}OEt_2$ (6.4 mL, 51 mmol) was added to the reaction mixture, followed by the slow addition of a solution of the aldehyde **7** (5 g, 21.2 mmol) in anhydrous ether (30 mL). After stirring at the same temperature for another 4.5 h, the reaction was quenched by careful addition of MeOH (3 mL) and the mixture was allowed to attain room temperature. After removal of the volatile components in vacuo, the residue was dissolved in MeOH (50 mL), and 8-hydroxyquinoline (7 g) was added to the solution. After stirring at room temperature for 12 h, the precipitated 8-HQ-B(Ipc)$_2$ crystals were filtered off and the filtrate was concentrated to afford the crude adduct as a thick syrup along with some pinene-derived impurities.

Step 2: The crude product obtained from step 1 above was dissolved in anhydrous DMF (20 mL), followed by the addition of imidazole (3.5 g, 51.4 mmol), TBDMSCl (5 g, 31.1 mmol), and a catalytic amount of DMAP. The mixture was stirred at 60 °C for 12 h, cooled to room temperature, and diluted by the addition of ice-cold water (30 mL) and ether (100 mL). The two layers were separated and the aqueous layer was extracted with ether (3 × 25 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, the solvent was removed under vacuum, and the residue was purified by flash column chromatography (hexanes/EtOAc = 97:3) to provide the TBS-protected olefin **8** as a viscous oil (6.8 g, 84% over two steps): $[\alpha]_D$ −36.7 (*c* 0.96, $CHCl_3$); IR (neat) 1752 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ −0.18 (s, 3H), 0.03 (s, 3H), 0.90 (m, 12H), 1.33 (s, 9H), 2.42 (m, 1H), 4.48 (d, J = 5.8 Hz, 1H), 4.97 (m, 2H), 5.82 (m, 1H), 7.01 (d, J = 8.5 Hz, 2H), 7.27 (d, J = 8.5 Hz, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ −4.6, −4.2, 16.4, 18.6, 26.2, 27.6, 39.5, 46.8, 78.9, 115.1, 121.0, 128.0, 141.1, 141.4, 150.3, 177.5; HRMS calcd for $C_{22}H_{40}NO_3Si$ m/z $(M + NH_4)^+$ 394.2777, found 394.2797. HPLC: CHIRALPAK AD-RH, 95% MeOH/H_2O, 0.8 mL/min, 254 nm, retention time = 4.55 min (>95%).

(1*S*,2*S*,3*R*)-1-[(*tert*-Butyldimethylsilyl)oxy]-2-methyl-1-[4-(pivaloyloxy)phenyl]butane-3,4-diol (9) and (1*S*,2*S*,3*S*)-1-[(*tert*-Butyldimethylsilyl)oxy]-2-methyl-1-[4-(pivaloyloxy)phenyl]butane-3,4-diol (10). To an ice-cooled, well-stirred solution of the olefin **8** (4 g, 10.6 mmol) and 4-methylmorpholine *N*-oxide (3.7 g, 31.6 mmol) in acetone (80 mL) and H_2O (20 mL) was added a solution of OsO_4 (4 mL, 5% solution in toluene) dropwise. The resulting solution was stirred at 0 °C for 12 h. The reaction was quenched by the addition of saturated aqueous Na_2SO_3 solution (15 mL), diluted by EtOAc (100 mL), and stirred at room temperature for 30 min. The two layers were separated and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and the solvent removed in vacuo. The residue was purified by flash column chromatography (hexanes/EtOAc = 4:1) to yield a mixture of the diols **9** and **10** (4:1 by ^1H NMR) as viscous oil (4 g, 91%).

Diols 9 and 10: IR (neat) 3430, 1752, cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, diastereomeric mixture) δ −0.26 and −0.19 (2s, 3H), 0.06 and 0.09 (2s, 3H), 0.65 and 0.99 (2d, J = 8, 3H), 0.89 and 0.93 (2s, 9H), 1.37 (s, 9H), 1.82 and 2.0 (2 m, 1H), 2.2 (br t, 1H), 3.56−3.93 (m, 3H), 4.31 and 4.42 (2s, 1H), 4.60 and 4.78 (2d, J = 8 Hz, 1H), 7.03 (d, J = 6.7 Hz, 2H), 7.33 (d, J = 6.6 Hz, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$, diastereoisomeric mixture) δ −4.8, −4.7, −4.1, −3.9, 12.3, 12.4, 18.4, 26.1, 26.2, 27.5, 39.5, 42.7, 44.0, 64.9, 65.6, 71.6, 75.4, 80.1, 80.6, 121.3, 121.5, 121.6, 127.6, 128.6, 140.6, 140.9, 150.9, 177.5; HRMS calcd for $C_{22}H_{42}NO_5Si$ m/z $(M + NH_4)^+$ 428.2832, found 428.2859.

(1*S*,2*S*,3*R*)-1,4-Bis[(*tert*-butyldimethylsilyl)oxy]-2-methyl-1-[4-(pivaloyloxy)phenyl]butane-3-ol (11) and (1*S*,2*S*,3*S*)-1,4-Bis[(*tert*-butyldimethylsilyl)oxy]-2-methyl-1-[4-(pivaloyloxy)phenyl]butane-3-ol (minor isomer). A solution of the mixture of the diols **9** and **10** (4.0 g, 9.8 mmol), imidazole (1.0 g, 14.7 mmol), a catalytic amount of DMAP, and TBDMSCl (1.8 g, 11.9 mmol) in anhydrous CH_2Cl_2 (40 mL) was stirred at room temperature for 12 h. Upon completion of the reaction (tlc monitoring), ice-cold water (100 mL) was added to the reaction mixture. The two layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated. Purification of the residue by flash column chromatography (hexanes/EtOAc = 97:3) yielded the required alcohol isomer **11** as a colorless oil (3.94 g, 77%): $[\alpha]_D$ −12.6 (*c* 1.02, $CHCl_3$); IR (neat) 3462, 1745 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ −0.15 (s, 3H), 0.05, 0.08 and 0.11 (3s, 9H), 0.70 (d, J = 10.4 Hz, 3H), 0.91 (s, 18 H), 1.37 (s, 9H), 2.02 (m, 1H), 2.97 (d, J = 3.0 Hz, 1H), 3.33 (m, 1H), 3.50 (dd, J = 7, 10 Hz, 1H), 3.69 (dd, J = 3.2, 10 Hz, 1H), 5.01 (d, J = 5.4 Hz, 1H), 7.02 (d, J = 9.0 Hz, 2H), 7.35 (d, J = 8.5 Hz, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ −5.0, −4.9, −4.7, −4.2, 10.4, 18.5, 18.7, 26.2, 26.3, 27.5, 39.5, 44.2, 65.5, 73.1, 75.5, 121.0, 128.4, 140.0, 150.4, 177.6; HRMS calcd for $C_{28}H_{52}O_5Si_2Li$ m/z $(M + Li)^+$ 531.3513, found 531.3521.

Minor alcohol isomer. Colorless oil (0.99 g, 19%): $[\alpha]_D$ −30.6 (*c* 1.00, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ −0.19 (s, 3H), 0.02 (s, 3H), 0.04 (s, 3H), 0.08 (s, 3H), 0.86 and 0.91 (2 br s, 21H), 1.38 (s, 9H), 1.59−1.83 (m, 1H), 3.00 (d, J = 2.0 Hz, 1H), 3.51−3.57 (m, 2H), 4.01 (t, J = 6.5 Hz, 1H), 4.74 (d, J = 6.0 Hz, 1H), 7.03 (d, J = 8.5 Hz, 2H), 7.31 (d, J = 8.5 Hz, 2H); ^{13}C NMR (100.6 MHz, $CHCl_3$) δ −5.0, −4.9, −4.8, −4.2, 11.0, 18.5, 18.6, 26.2, 26.3, 27.5, 39.5, 42.4, 65.6, 70.5, 79.0, 121.4, 127.8, 141.5, 150.5, 177.4.

(1*S*,2*S*,3*S*)-3-Azido-1,4-bis[(*tert*-butyldimethylsilyl)oxy]-2-methyl-1-[4-(pivaloyloxy)phenyl]butane (12). To a well-stirred solution of alcohol **11** (0.10 g, 0.2 mmol) in dry THF (1 mL) at 0 °C was added diisopropyl azodicarboxylate (0.05 mL, 0.25 mmol) and triphenylphosphine (0.063 g, 0.24 mmol), followed by dropwise addition of diphenylphosphoryl azide (0.05 mL, 0.23 mmol). After

completion of addition, the reaction mixture was brought to room temperature and stirred for 1 h. Upon completion of the reaction (monitored by tlc), excess solvent was removed under vacuum and the residue was purified by flash chromatography (hexanes/EtOAc = 98:2) to obtain the azide **12** as an oil (0.096 g, 92%): $[\alpha]_D$ −22.5 (*c* 1.04, $CHCl_3$); IR (neat) 2094, 1752 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ −0.29 (s, 3H), 0.10, 0.11, and 0.12 (3s, 9H), 0.49 (d, *J* = 6.8 Hz, 3H), 0.89 (s, 9H), 0.94 (s, 9H), 1.37 (s, 9H), 1.78 (m, 1H), 3.71 (dd, *J* = 4.4, 10.4 Hz, 1H), 3.82 (dd, *J* = 8.9, 10.2 Hz, 1H), 4.21 (m, 1H), 4.41 (d, *J* = 9.2 Hz, 1H), 7.05 (d, *J* = 8.3 Hz, 2H), 7.29 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ −5.1, −5.0, −4.9, −4.0, 10.6, 18.5, 18.6, 26.2, 26.3, 27.5, 39.5, 42.7, 64.0, 66.5, 76.8, 121.6, 128.5, 141.5, 150.8, 177.5; HRMS calcd for $C_{28}H_{55}N_4O_4Si_2$ *m*/*z* $(M + NH_4)^+$ 567.3762, found 567.3780. HPLC: IProSIL 120-5 Si 5.0 μm, 98% hexanes/2% EtOAc, 1.0 mL/min, 254 nm, retention time = 6.23 min.

(1*S*,2*S*,3*S*)-3-[(Benzyloxycarbonyl)amino]-1,4-bis[(*tert*-butyldimethylsilyl)oxy]-2-methyl-1-[4-(pivaloyloxy)phenyl]butane (13). Step 1: To a well-stirred room temperature solution of the azide **12** (5.3 g, 9.65 mmol) in anhydrous EtOAc (50 mL) was added 10% palladium on activated carbon (1 g) and the mixture was stirred under H_2 atmosphere for 4 h. After completion of the reaction (tlc monitoring), the catalyst was filtered off and washed thoroughly with EtOAc. The combined filtrate was concentrated under vacuum and then dried under high vacuum for 1 h to yield the crude amine as a light yellow oil.

Step 2: The crude amine (5.04 g, 9.63 mmol) obtained from step 1 above was dissolved in a 4:1 mixture of EtOAc (40 mL) and saturated aqueous $NaHCO_3$ solution (10 mL), and cooled in an ice-bath, followed by dropwise addition of benzylchloroformate (1.64 mL, 11.55 mmol) with vigorous stirring. After completion of addition, the reaction mixture was allowed to come to room temperature and stirred for 3 h. The aqueous and organic layers were separated and the aqueous layer was extracted with EtOAc (2 × 10 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (hexanes/EtOAc = 97:3) to yield the desired carbamate **13** as a clear viscous oil (5.38 g, 85% over two steps): $[\alpha]_D$ −34.3 (*c* 1.00, $CHCl_3$); IR (neat) 3416, 1757, 1721, 1705 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) −0.32 (s, 3H), 0.27 (s, 9H), 0.72 (d, *J* = 6.6 Hz, 3H), 0.73 and 0.88 (2s, 18H), 1.38 (s, 9H), 2.17 (m, 1H), 3.59 (t, *J* = 8.4 Hz, 1H), 3.76 (br s, 1H), 4.01 (br s, 1H), 4.53 (d, *J* = 7.4 Hz, 1H), 5.07−5.17 (m, 3H), 7.0 (d, *J* = 8.1 Hz, 2H), 7.24 (m, 2H), 7.33−7.40 (m, 5H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ −5.1, −4.9, −4.8, −4.1, 12.2, 18.4, 18.5, 26.2, 27.5, 39.5, 41.8, 52.7, 64.1, 66.9, 77.9, 121.5, 128.1, 128.4, 128.6, 128.9, 137.1, 141.4, 150.7, 156.5, 177.4; HRMS calcd for $C_{36}H_{59}NO_6Si_2Na$ *m*/*z* $(M + Na)^+$ 680.3779, found 680.3787.

(1*S*,2*S*,3*S*)-3-[(Benzyloxycarbonyl)amino]-1-[(*tert*-butyldimethylsilyl)oxy]-2-methyl-1-[4-(pivaloyloxy)phenyl]butan-4-ol (14). To an ice-cooled solution of the carbamate **13** (2.46 g, 3.7 mmol) in MeOH/CH_2Cl_2 (1:1, 30 mL) was added with stirring small portions of camphorsulfonic acid (0.007 g, 0.032 mmol) until pH 3. Stirring was continued at 0 °C for 1 h. Upon completion of the reaction (tlc monitoring), saturated $NaHCO_3$ solution (10 mL) was added to the mixture, the two layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined extracts were dried over anhydrous Na_2SO_4 and the solvent was removed in vacuo. The residue was purified by flash column chromatography (hexanes/EtOAc = 7:3) to yield the free primary alcohol derivative **14** as a viscous low melting solid (1.75 g, 91%): $[\alpha]_D$ −52.0 (*c* 1.00, $CHCl_3$); IR (neat) 3421, 3385, 1746, 1705 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, rotameric mixture) δ −0.26 and 0.30 (2s, 3H), 0.02 and 0.06 (2s, 3H), 0.90 (br s, 12H), 1.37 (s, 9H), 2.07 (m, 1H), 3.45 (br s, exchangeable with D_2O, 1H), 3.61 (t, *J* = 7.0 Hz, 1H), 3.77 (br s, 2H), 4.60−4.62 (2d, *J* = 6.0 Hz, 1H), 5.11 (m, 2H), 6.02 (br s, 1H), 7.01 (d, *J* = 8.5 Hz, 2H), 7.24 (d, *J* = 8.5, 2H), 7.35 (m, 5H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ −4.9, −4.1, −3.9, 13.6, 18.4, 26.2, 27.5, 39.5, 42.6, 55.6, 66.0, 67.3, 78.6, 121.4, 121.7 127.6, 127.9, 128.5, 128.6, 128.7, 128.8, 128.9, 136.8, 140.5, 150.8, 177.4; HRMS calcd for $C_{30}H_{45}NO_6SiNa$ *m*/*z* $(M + Na)^+$ 566.2914, found 566.2772.

(2*S*,3*S*,4*S*)-2-[(Benzyloxycarbonyl)amino]-4-[(*tert*-butyldimethylsilyl)oxy]-3-methyl-4-[4-(pivaloyloxy)phenyl]butyric Acid (2). To a stirring, room temperature solution of $CH_3CN/CCl_4/H_2O$ (1.5:0.5:1, 3 mL) was added $NaIO_4$ (0.08 g, 0.36 mmol) and $RuCl_3 \cdot 3H_2O$ (0.005 g, 0.018 mmol) sequentially. The resulting mixture was stirred for 30 min, after which it was added into a solution of the alcohol **14** (0.1 g, 0.18 mmol) dissolved in CH_3CN (3 mL). To the resulting dark solution an additional lot of $NaIO_4$ (0.04 g, 0.18 mmol) was added and the mixture was stirred at room temperature for 30 min. The inorganic solids were removed by filtration through a celite pad, washing thoroughly with EtOAc. The combined filtrate was concentrated and the residue purified by flash chromatography (hexanes/EtOAc = 6:4) to obtain the carboxylic acid **2** as a foamy white solid (0.079 g, 77%): mp 71−74 °C; $[\alpha]_D$ −20.0 (*c* 0.50, $CHCl_3$); IR (neat) 3308, 1746, 1726 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ −0.33 (s, 3H), 0.03 (s, 3H), 0.75 (d, *J* = 6.8 Hz, 3H), 0.88 (s, 9H), 1.38 (s, 9H), 2.41 (m, 1H), 4.5 (d, *J* = 8.2 Hz, 1H), 4.71 (br s, 1H), 5.12 (d, *J* = 12.2 Hz, 1H), 5.23 (d, *J* = 12.2 Hz, 1H), 5.67 (br d, *J* = 8.1 Hz, 1H), 7.03 (d, *J* = 8.2 Hz, 2H), 7.28 (d, *J* = 7.8 Hz, 2H), 7.49 (m, 5H), 9.27 (br s, 1H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ −5.5, −4.6, 12.3, 17.9, 25.7, 27.0, 38.9, 42.9, 55.0, 67.0, 76.7, 121.3, 127.6, 128.0, 128.1, 128.4, 136.2, 140.0, 150.5, 156.5, 176.9, 177.2; HRMS calcd for $C_{30}H_{47}N_2O_7Si$ *m*/*z* $(M + NH_4)^+$ 575.3153, found 575.3127.

Synthesis of the Fully Protected Pyranosyl Nikkomycin B (16). Step 1: To a solution of the nucleoside amino acid derivative **15** (0.62 g, 1.16 mmol)[13b] in anhydrous EtOAc (10 mL) was added 10% palladium on activated carbon (0.7 g), and the mixture was stirred under a H_2 atmosphere for 3 h. The reaction mixture was filtered through celite, and the residue was washed with EtOAc and MeOH. The filtrate was concentrated to yield the amine as a foamy solid (0.45 g, 97% crude), which was taken on to the next step without further purification.

Step 2: The carboxylic acid **2** (0.75 g, 1.35 mmol) was dissolved in anhydrous CH_2Cl_2 (8 mL) and cooled to 0 °C, followed by the addition of *N*,*N*-diisopropylethylamine (0.23 mL, 1.35 mmol) and BOP-Cl (0.34 g, 1.35 mmol). The reaction was stirred at 0 °C for 45 min. The crude amine (0.45 g, 1.12 mmol) as obtained from step 1 above was dissolved in anhydrous CH_2Cl_2 (7 mL) and was added to the activated acid, followed by *N*,*N*-diisopropylethylamine (0.23 mL, 1.35 mmol). The reaction was allowed to come to rt and stirred for 12 h. After the reaction was quenched with H_2O (10 mL) the two layers were separated, and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (1 × 15 mL), dried (Na_2SO_4), and concentrated under vacuum, and the residue was purified by flash chromatography (EtOAc/hexanes = 6:4 to 8:2) to yield the fully protected pyranosyl nikkomycin analogue **16** as a white solid (0.83 g, 77%): mp 134−136 °C; $[\alpha]_D$ −18.8 (*c* 1.05, $CHCl_3$); IR (neat) 3293, 1752, 1696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ −0.28 (s, 3H), 0.03 (s, 3H), 0.80 (d, *J* = 7.1 Hz, 3H), 0.85 (s, 9H), 1.36 (s, 9H), 1.97−2.07 (m, 4H), 2.18−2.25 (m, 4H), 2.33 (br s, 1H), 3.78 (s, 3H), 4.28−4.35 (m, 2H), 4.66−4.73 (m, 2H), 4.90 (d, *J* = 7.2 Hz, 1H), 4.90−5.20 (m, 2H), 5.57 (s, 1H), 5.73 (d, *J* = 6.5 Hz, 1H), 6.03 (d, *J* = 9.6 Hz, 1H), 6.10 (br s, 1H), 6.96 (d, *J* = 8.5 Hz, 2H), 7.18−7.37 (m, 9H), 9.10 (br s, 1H); ^{13}C NMR (100.6 MHz, $CHCl_3$) δ −4.8, −4.2, 12.8, 18.4, 20.9, 21.5, 26.2, 27.5, 32.0, 39.5, 43.2, 53.2, 55.2, 56.4, 67.3, 67.6, 68.9, 74.9, 78.9, 103.9, 121.8, 127.7, 128.7, 128.9, 136.5, 139.5, 140.2, 150.5, 150.9, 157.1, 163.0, 169.1, 170.1, 170.3, 172.1, 177.5; HRMS calcd for $C_{46}H_{66}N_5O_{15}Si$ *m*/*z* $(M + NH_4)^+$ 956.4325, found, 956.4293. HPLC: Agilent ZORBAX SB-C18, 3.0 × 150 mm, 3.5 μm, 2% acetonitrile/water containing 0.1% TFA and TEA, 0.4 mL/min, 225 nm, retention time = 7.57 min.

Silyl Deprotection of the Coupled Product 16 to the Alcohol 17. To a stirring solution of the pyranosyl analogue **16** (0.745 g,

0.79 mmol) in anhydrous THF (12 mL) at 0 °C was added TBAF (1 M in THF, 1.6 mL, 1.6 mmol). The reaction was stirred at 0 °C for 2 h, and then quenched with saturated aqueous ammonium chloride (5 mL), followed by addition of EtOAc (20 mL). The two layers were separated, and the aqueous layer was extracted with EtOAc (3 × 5 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under vacuum, and the residue was purified by flash chromatography (EtOAc/hexanes = 7:3 to 8:2) to afford the deprotected alcohol **17** as a white solid (0.535 g, 87%): mp 154−156 °C; $[\alpha]_D$ 22.7 (*c* 1.00, $CHCl_3$); IR (neat) 3298, 1747, 1699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.53 (d, *J* = 6.7 Hz, 3H), 1.35 (s, 9H), 1.87 (s, 3H), 2.04−2.10 (m, 4H), 2.17−2.28 (m, 3H, 1H exchangeable with D_2O), 3.84 (s, 3H), 4.21 (dd, *J* = 3.8 and 9.7 Hz, 1H), 4.40 (d, *J* = 12.1 Hz, 1H), 4.71 (d, *J* = 3.8 Hz, 1H), 4.89 (d, *J* = 9.0 Hz, 1H), 4.98 (br s, 1H), 5.11 and 5.20 (2d, *J* = 12.3 Hz, 2H), 5.58 (d, *J* = 2.8 Hz, 1H), 5.71 (d, *J* = 6.5 Hz, 1H), 6.14 (d, *J* = 7.9 Hz, 1H), 6.20 (d, *J* = 9.8 Hz, 1H), 6.98 (d, *J* = 8.4 Hz, 2H), 7.17 (d, *J* = 8.2 Hz, 1H), 7.23 (d, *J* = 8.4 Hz, 2H), 7.35−7.39 (m, 5H), 8.20 (d, *J* = 8.7 Hz, 1H), 9.52 (s, exchangeable with D_2O, 1H); ^{13}C NMR (125 MHz, $CHCl_3$) δ 11.0, 20.3, 20.7, 27.0, 31.8, 39.0, 45.0, 53.1, 54.4, 54.8, 66.7, 67.4, 67.6, 74.8, 75.3, 78.6, 103.8, 121.3, 127.9, 128.0, 128.2, 128.5, 136.0, 138.7, 139.7, 150.4, 150.7, 157.3, 162.2, 169.5, 169.9, 170.1, 170.9, 176.9; HRMS calcd for $C_{40}H_{49}N_4O_{15}$ *m*/*z* $(M + H)^+$ 825.3194, found 825.3195.

Alkaline Hydrolysis of the Ester Linkages of 17 to the Hydroxy Acid 18. The pyranosyl nucleoside derivative **17** (0.214 g, 0.25 mmol) was dissolved in THF (12 mL) and cooled to 0 °C, followed by addition of a solution of $LiOH·H_2O$ (0.1 g, 2.6 mmol) in H_2O (3 mL). The reaction was stirred at 0 °C for 2.5 h, and then diluted with H_2O (2 mL) and EtOAc (15 mL). The aqueous layer was acidified to pH 2−3 with 10% $KHSO_4$. The two layers were separated, and the aqueous layer was extracted with EtOAc (5 × 10 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography ($CHCl_3$/MeOH/H_2O = 7:2.8:0.2 to 5:4.5:0.5) to yield the polyhydroxy acid **18** as a white solid (0.088 g, 54%): mp >185 °C dec; $[\alpha]_D$ 13.8 (*c* 0.55, MeOH); IR (Teflon film) 3348, 1693, 1614 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 0.58 (d, *J* = 6.8 Hz, 3H), 1.94 (d, *J* = 12.5 Hz, 1H), 2.18 (t, *J* = 12.2 Hz, 1H), 2.40 (br s, 1H), 3.61 (d, *J* = 8.7 Hz, 1H), 4.23 (d, *J* = 9.2 Hz, 2H), 4.44 (br d, *J* = 10.6 Hz, 1H), 4.51 (s, 1H), 4.82 (s, 1H), 5.09 and 5.19 (2d, *J* = 12.4 Hz, 2H), 5.68 (d, *J* = 7.9 Hz, 1H), 5.92 (d, *J* = 7.3 Hz, 1H), 6.74 (d, *J* = 8.3 Hz, 2H), 7.12 (d, *J* = 8.2 Hz, 2H), 7.31−7.43 (m, 5H), 7.61 (d, *J* = 7.9 Hz, 1H); ^{13}C NMR (100.6 MHz, CD_3OD) δ 11.0, 33.9, 43.0, 48.9, 56.2, 57.6, 67.1, 68.0, 69.9, 74.2, 75.4, 81.0, 102.4, 115.1, 128.0, 128.1, 128.3, 128.6, 134.2, 137.0, 142.0, 151.9, 157.0, 158.1, 165.1, 172.5, 174.2; HRMS calcd for $C_{30}H_{38}N_5O_{12}$ *m*/*z* $(M + NH_4)^+$ 660.2517, found 660.2523.

Pyranosyl Nikkomycin B Oxalate Salt (19). To a room temperature solution of the hydroxy acid **18** (0.052 g, 0.08 mmol) in anhydrous MeOH (3 mL) was added 10% palladium on activated carbon (0.06 g), and the mixture was stirred under a H_2 atmosphere for 2 h. The reaction mixture was filtered through celite, and the residue was washed with MeOH. The filtrate was concentrated to yield the free amine as a white solid (0.04 g, 95%, crude). Toward further purification via crystallization, a saturated solution of oxalic acid (0.1 g) in ether (5 mL) was added to the above amine dissolved in a minimum amount of MeOH. The white precipitate was collected by filtration to yield the fully deprotected pyranosyl nikkomycin B (**19**) as its oxalic acid salt (0.036 g, 76%): mp >200 °C dec; $[\alpha]_D$ −13.8 (*c* 0.13, H_2O); IR (KBr) 3447, 1670 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 0.79 (d, *J* = 7.1 Hz, 3H), 1.93 (d, *J* = 15 Hz, 1H), 2.10 (t, *J* = 12.9 Hz, 1H), 2.48 (t, *J* = 5.2 Hz, 1H), 3.81 (dd, *J* = 2.3 and 9.6 Hz, 1H), 4.31−4.42 (m, 4H), 4.55 (d, *J* = 8.1 Hz, 1H), 5.81−5.86 (m, 2H), 6.86 (d, *J* = 8.3 Hz, 2H), 7.23 (d, *J* = 8.3 Hz, 2H), 7.68 (d, *J* = 8.1 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 11.2, 33.0, 40.7, 54.3, 57.3, 67.3, 68.6, 72.4, 75.1, 80.8, 102.9, 115.5, 128.1, 133.7, 142.2, 152.1, 155.4, 165.9, 168.7, 172.9; HRMS calcd for $C_{22}H_{29}N_4O_{10}$ (free amine) *m*/*z* $(M + H)^+$ 509.1884, found 509.1880. HPLC: ZORBAX SB-C18 column (3.5 μm; 3.0 × 150 mm), 2% MeCN/H_2O (water contained 0.1% TFA and 0.1% Et_3N), 0.4 mL/min, 225 nm, retention time = 7.57 min (100%).

Acknowledgment. We thank the Herman Frasch Foundation (American Chemical Society) for financial support. Pre-doctoral research fellowships to C.S.S, from the American Chemical Society Medicinal Chemistry Division (2004−2005) (sponsored by Aventis), and the American Foundation for Pharmaceutical Education (2005−2006) are also gratefully acknowledged.

Supporting Information Available: General experimental details, copies of NMR spectra (^1H and ^{13}C) of all the new compounds, and a copy of the HPLC chromatogram of compound **19**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO701814B