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Multisite Modification of Neomycin B: Combined Mitsunobu and Click Chemistry Approach

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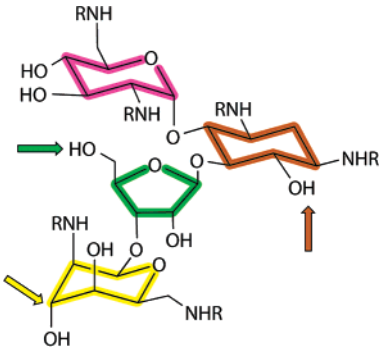

The aminoglycoside antibiotic neomycin B has been converted into several novel building blocks that can be used for the specific modification of three of the four ring systems. Under carefully controlled conditions, the Mitsunobu reaction can be used to selectively dehydrate the *ido* ring to give the *talo* epoxide. Subsequently however, under more forcing conditions, the 2-deoxy streptamine ring undergoes Mitsunobu dehydration to give an aziridine. An unusual remote neighboring group effect was observed. When the primary hydroxyl of the ribose ring was blocked, aziridine formation on the deoxystreptamine ring did not occur. Both the epoxide and epoxide-aziridine neomycin building blocks can be ring-opened with azide and subjected to "click" type chemistry with terminal alkynes to generate a series of new neomycin analogues. These reactions can all be carried out without recourse to O-protecting groups. A detailed conformational analysis by NMR revealed some unexpected conformer preferences in these systems.

Introduction

Aminoglycoside antibiotics are widely used for the treatment of a variety of infections including tuberculosis and pneumonia in particular. They exert their antibacterial activities by binding to RNA sequences in the bacterial ribosome,[1] and this binding impedes mRNA translation, results in miscoding, and leads ultimately to bacterial cell death.[2] Compounds in this class can interact with a variety of other biologically relevant RNA sequences including group I introns,[3] hammerhead ribozymes,[4] and the hepatitis delta virus (HDV) ribozyme.[5] Recently, the aminoglycoside antibiotic neomycin B, **1** (Figure 1), has also been found to be the most potent inhibitor of the proteolytic activity of anthrax lethal factor (LF).[6] Despite the broad-spectrum efficacy of these natural amino sugars, the use of these

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(1) (a) Tanaka, N. In *Handbook of Experimental Pharmacology*; Umezawa, H., Hooper, I. R., Eds.; Springer- Verlag: New York, 1982; pp 221−266. (b) Cundlife, E. In *In the Ribosome*; Hill, W. E. et al, Eds.; American Society of Microbiology: Washington, DC, 1990; pp 479−490. (c) Noller, H. F. *Annu. Rev. Biochem.* **1991**, *60*, 191−227.

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(3) Wank, H.; Rogers, J.; Devies, J.; Schroeder, R. *J. Mol. Biol.* **1994**, *236*, 1001−1010.

(4) Stage, T. K.; Hertel, K. J.; Uhlenbec, O. C. *RNA* **1995**, *1*, 95−101.

(5) Rogers, J.; Chang. A. H.; Von Ahsen, U.; Schroeder, R.; Devies, J. *J. Mol. Biol.* **1996**, *259*, 916−925.

(6) (a) Lee, L. V.; Bower, K. E.; Liang, F.-S.; Shi, J.; Wu, D.; Suchek, S. J.; Vogt, P. K.; Wong, C.-H. *J. Am. Chem. Soc.* **2004**, *126*, 4774−5. (b) Fridman, M.; Belakhov, V.; Lee, L. V.; Liang, F.-S.; Liang, F.-S.; Wong, C.-H.; Baasov, T. *Angew. Chem., Int. Ed.* **2005**, *44*, 447−452.

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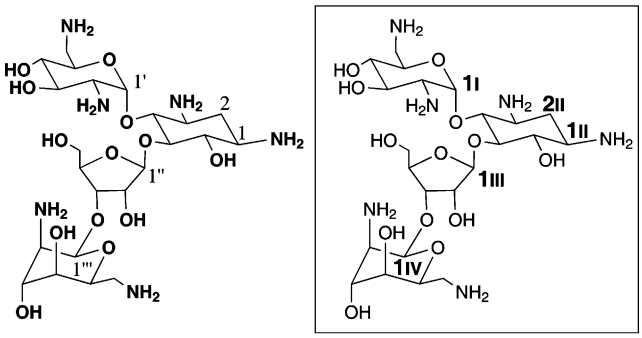


1

FIGURE 1. Conventional numbering scheme for neomycin B, **1**, and the numbering scheme adopted here for illustrative clarity (inset).

drugs is restricted due to their toxicity, poor oral bioavailability, and poor cell permeability. Consequently, semisynthetic analogues of this class of compounds are attractive targets for medicinal chemists,[7] as modified compounds of this type may demonstrate increased effectiveness in clinical applications. Although there are many examples of direct synthetic modification of aminoglycosides, these often require multiple protection/deprotection strategies using a variety of O-protecting groups, as well as N-protecting groups, to both improve the organic solubility of these hydrophilic compounds and to allow the *selective* manipulation of the numerous amino and hydroxy substituents. Furthermore, even though naturally occurring neomycin B has several potential points of attachment for the addition of substituents to attenuate the properties of the drug, little effort has been directed toward modification of all four residues of the tetrasaccharide scaffold. Indeed, although many structural analogues of this antibiotic have been synthesized over the past decade, the majority of these cases involve modification at the ribose moiety, by exploiting the differential reactivity of the single primary hydroxyl group.

"Near perfect" reactions are required to maximize the synthetic efficiency of selective modifications of complex molecules such as the amino-glycoside antibiotics. The development of the concept of "click chemistry" by Sharpless has provided the organic chemist with a first generation toolbox of such reactions.[8] Two identified by Sharpless[9] involve azides: nucleophilic opening of epoxides and coupling reactions with alkynes. Here, we describe the use of this chemistry, in conjunction with Mitsunobu dehydration reactions, for the rapid, highly selective modification of the aminoglycoside antibiotic neomycin B in three different ring systems. Having several potential points of attachment would allow addition of substituents to improve the nonspecific physicochemical properties of the drug without destroying the specific biological activity. Initially we sought a more direct and specific functionalization of the singular primary alcohol found in many members of this family through Mitsunobu esterification, previously demonstrated to be successful in this regard on disaccharides such as trehalose.[10] However, the Mitsunobu reaction has resulted in selective chemistry elsewhere in the molecule, and this has been exploited for modification of the *ido* and streptamine ring systems. We have developed highly selective chemistry on this natural product that allows for incorporation of an azide functional group in each of three rings of neomycin and further transformation via copper-catalyzed coupling reactions with hydrophobic alkynes. This chemistry precludes the need for O-protecting groups and thus allows for higher synthetic throughput.

Our initial aim was the introduction of hydrophobic esters that might serve as prodrugs to improve the parent drug's effectiveness at crossing the waxy, mycolate-rich barrier that envelops *Mycobacterium tuberculosis*.[11] Although the aminoglycoside streptomycin was the first effective drug in the treatment of tuberculosis, drug resistance to this class of compounds was observed quite early after its introduction and is a growing problem today.[12] It has recently been demonstrated that mycobacteria enter the macrophage by way of a cholesterol-dependent mechanism.[13] Membrane cholesterol depletion reduced macrophage uptake of *M. tuberculosis* by 85%. Normally, after phagocytosis mycobacteria actively recruit and retain tryptophan aspartate-containing coat protein (TACO) and this prevents delivery to lysosomes, thus protecting the pathogen from degradation. The TACO associates with the phagosome in a cholesterol-dependent manner, and it is known that mycobacteria display a high binding capacity for cholesterol itself. By modifying antitubercular drugs with mimics of this steroid,[14] it may be possible to hijack this binding mechanism as a route for targeted drug delivery.[15]

Results and Discussion

Modification of the *ido* Ring. It has been reported by Fourrey et al. in two separate publications[16,17] that under the conditions of the Mitsunobu reaction the *ido* ring of hexa-*N*-Boc-protected neomycin B forms a tricyclic aziridine-azetidine structure. We have shown[18] that this structure is incorrect, and that an epoxide is formed, as expected for the *ido* conformer with *trans*-diaxial alcohols. Thus, when **2** was treated with 2 equiv of triphenylphosphine (TPP) and diisopropyl azodicarboxylate (DIAD), the epoxide derivative of neomycin B, **3**, was formed in 40%

(7) For a review of methodologies in syntheses of aminoglycoside antiobiotics, see: Haddad, J.; Liu, M.-Z.; Mobashery, S. In *Glycochemistry: Principles, Synthesis and Applications*; Wang, P. G., Bertozzi, C. R., Eds.; Marcel Dekker: New York, 2001; pp 353−424.

(8) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004−2021.

(9) Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128−1137.

(10) (a) Bottle, S.; Jenkins, I. D. *Chem. Commun.* **1984**, 385. (b) Jenkins, I. D.; Goren, M. B. *Chem. Phys. Lipids* **1986**, *41*, 225−235.

(11) For examples of lipophilic antitubercular compounds, see: (a) Ballell, L.; Field, R. A.; Duncan, K.; Young, R. J. *Antimicrob. Agents Chemother.* **2005**, *49*, 2153−2163. (b) Jones, P. B.; Parrish, N. M.; Houston, T. A.; Stapon, A.; Bansal, N. P.; Dick, J. D.; Townsend,C. A. *J. Med. Chem.* **2000**, *43*, 3304−3314. (c) Parrish, N. M.; Houston, T.; Jones, P. B.; Townsend, C.; Dick, J. D. *Antimicrob. Agents Chemother.* **2001**, *45*, 1143−1150.

(12) Smith, C. V.; Sharma, V.; Sacchettini, J. C.; Tuberculosis **2004**, *84*, 45−55.

(13) Gatfield, J.; Pieters, J. *Science (Washington, D. C.)* **2000**, *288*, 1647−1650.

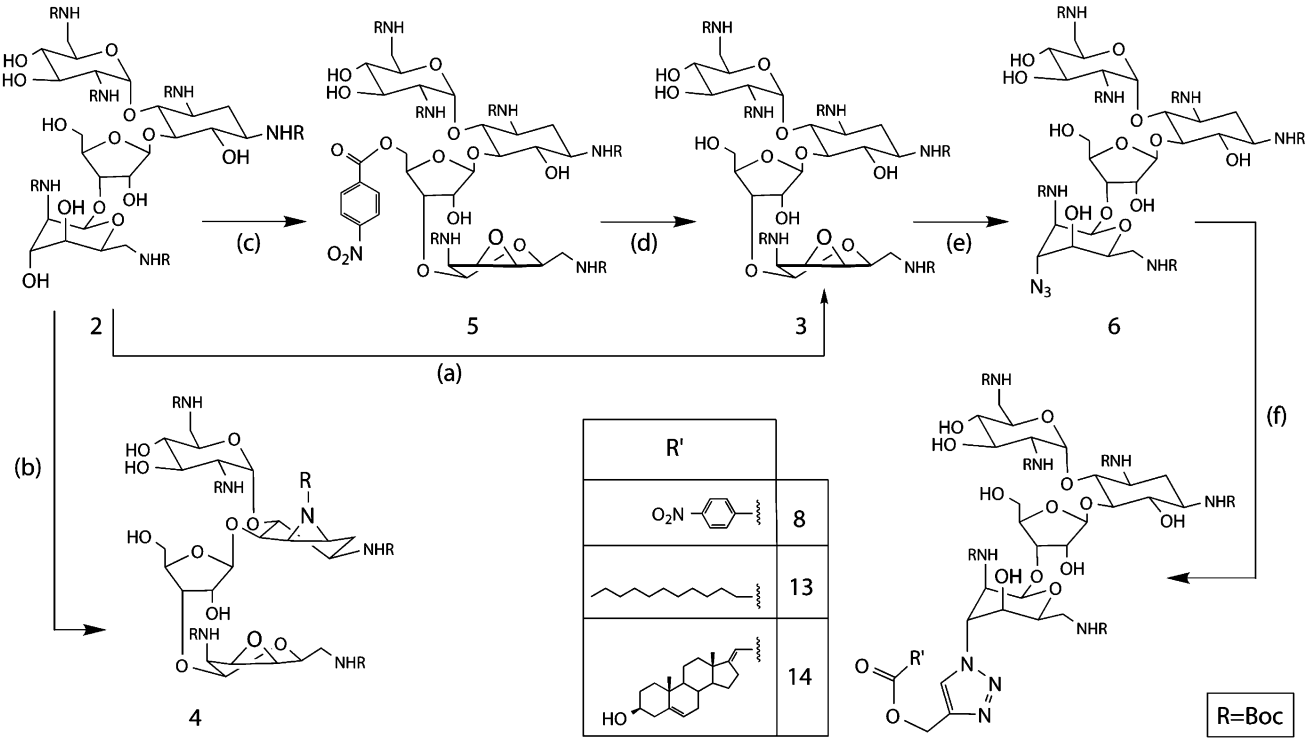
(14) For a cholesterol-derived aminoglycoside designed for gene transfection, see: Belmont, P.; Aissaoui, A.; Hauchecorne, M.; Oudrhiri, N.; Petit, L.; Vigneron, J.-P.; Lehn, J.-M.; Lehn, P. *J. Gene Med.* **2002**, *4*, 517−526.

(15) Alternatively we are interested in designing boronates to target the mannose-rich exterior of *M. tuberculosis*, see: (a) Gray, C. W., Jr.; Walker, B. T.; Foley, R.; Houston, T. A. *Tetrahedron Lett.* **2003**, *44*, 3309−3312. (b) Johnson, L. L., Jr.; Houston, T. A. *Tetrahedron Lett.* **2002**, *43*, 8905−8909.

(16) Hernandez Linares, A.; Fourmy, D.; Fourrey, J.-L.; Loukaci, A. *Org. Biomol. Chem.* **2005**, *3*, 2064−2066.

(17) Hernandez Linares, A.; Fourmy, D.; Fourrey, J.-L.; Loukaci, A. *Synth. Commun.* **2006**, *36*, 487−493.

SCHEME 1[a]



[a] Reagents and conditions: (a) TPP (2 × 2 equiv), DIAD (2 × 2 equiv), toluene, rt, N_2, 18 h; (b) TPP (4 equiv), DIAD (4 equiv), toluene, rt, N_2, 18 h; (c) 4-NO_2C_6COOH (12 equiv), TPP (10 equiv), DIAD (10 equiv), toluene, rt, N_2, 18 h; (d) MeOH/28% $NH_{3(aq)}$, 8 h, rt; (e) NaN_3, rt, DMF, 2 d; (f) R′COO-CH_2C″CH (2 equiv), *t*-BuOH/H_2O (1:1), Cu/$CuSO_4$, rt, 18 h.

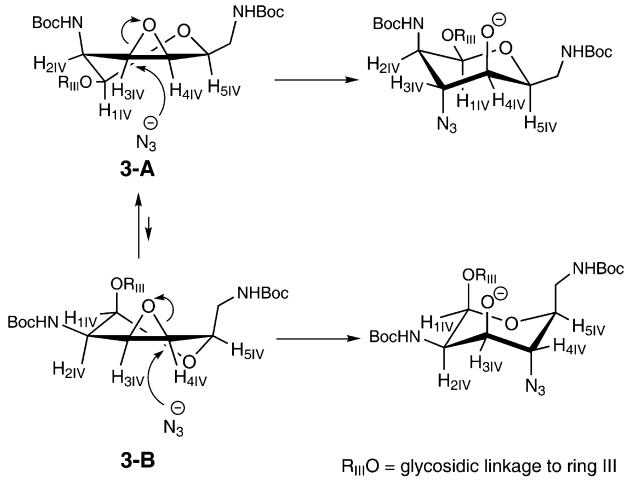
yield.[18a] Epoxide **3** represents an attractive target for subsequent selective modification of the neomycin B framework at the *ido* moiety (Scheme 1). However, under our initial synthetic conditions almost 50% of starting material, **2**, was recovered from the reaction mixture unconverted. In an effort to increase the conversion of the starting material and thus to increase the yield of the epoxide **3**, the reaction was repeated using an excess of the two reagents. Accordingly, compound **2** was treated with 4 equiv of TPP and DIAD at room temperature for 12 h in toluene. However, although the net conversion of the starting material **2** did improve, the yield of the epoxide **3** did not. After column chromatographic separation, the monoanhydro compound **3** was found to have formed in only in 33% yield. Interestingly, a bis-anhydro neomycin derivative, **4**, (HRMS $[M + H^+] = 1179.60897$) was obtained as the major product (45%). The same bis-anhydro species was found to be present as a minor product in the initial reaction. The structural characterization and chemistry of **4** is discussed further in the succeeding sections. An increased yield of the mono-anhydro species **3** (62%) was obtained when 4 equiv of DIAD and TPP was added in two portions of 2 equiv each, with a 12 h interval between additions. However, optimal yields of **3** are obtained if an alternate, two-step, procedure is employed. Treatment of **2** with 12 equiv of 4-nitrobenzoic acid, under Mitsunobu conditions (10 equiv of TPP and DIAD), affords the 5_{III}-substituted 4-nitrobenzoate ester derivative **5** in high yield (85%). Esterification of the primary hydroxyl of the ribose residue suppresses the formation of the side products (vide infra). Ester **5** was then readily converted to **3** on treatment with methanolic aqueous ammonia solution (2:1, MeOH/ 28%$NH_{3(aq)}$) by simply stirring the suspension at room temperature (8 h, quantitative). Per-acetylation of the mono-anhydro product using acetic anhydride and pyridine afforded penta-*O*-acetyl mono-anhydro neomycin B, **3**-penta-*O*-acetate.

The structure of **3**-penta-*O*-acetate was probed using high-temperature ^1H NMR spectroscopy.[18a] The formation and stereochemistry of the oxirane ring in **3** can be inferred from the structure of the product of the ring opening reaction of **3** with azide ion. Treatment of the mono-anhydro product, **3** with NaN_3 in DMF at room temperature for 2 days furnished an azido neomycin **6** (Scheme 1) as a single product (85% yield after purification by column chromatography). Alternatively, **6** could be obtained directly from the ester **5**, on treatment with NaN_3.[19] The regioselective ring opening of the *talo* epoxide in **3** and **5** at C_{3IV} by azide ion to give the trans-diaxial product (*ido* configuration) follows from the Fürst−Plattner rule and from consideration of the relative energies of the two possible epoxide

(18) (a) Quader, S.; Boyd, S. E.; Jenkins, I. D.; Houston, T. A. *Org. Biomol. Chem.* **2006**, *4*, 36−37. (b) The errors in the structures initially reported by Fourrey et al. in ref 16 are noted in footnote 11 of ref 18a (which formally corrects the aziridine/azetidine structure presented in ref 16). The subsequent errors in the similar structures reported in ref 17 are acknowledged by Fourrey et al. in the unreferenced footnote, footnote [15-(PDF version)/18(HTML version)], which renounces the aziridine/azetidine structures presented in the main body of that article. Professor Fourrey has advised us in a personal communication that after a re-examination of their NMR data, they were unable to observe the HMBC correlations between the respective Boc-carbonyl carbons and H-3‴ and H-4‴ protons, forwarded initially as diagnostic evidence for the aziridine/azetidine structure described in ref 16.

(19) Gómez-Vidal, J. A.; Forrester, M. T.; Silverman, B. *Org. Lett.* **2001**, *3*, 2477−2479.

SCHEME 2



conformations (Scheme 2).[20] The $^{O}H_1$ conformation (**3-A**) would be expected to be lower in energy than the $^{1}H_{O}$ conformation (**3-B**), as there is a severe (pseudo) 1,3-diaxial interaction between the C_6NHBoc group and the ribose moiety at C_1 in **3-B**. Conformation **3-A** may also be stabilized by a hydrogen bonding interaction between the C_2NH and the ring oxygen. Similar energetic considerations are likely to be reflected in the relative energies of the transition states leading from **3-A** and **3-B**. Additionally, attack on conformation **3-A** is likely to be further favored over **3-B** as the transition state for attack by azide ion on conformation **3-B** will be subject to an additional dipolar repulsion (field effect) between the attacking nucleophile and the ring oxygen atom.

The regiospecific opening of the epoxide **3** by azide affords a new functionality in the *ido* ring with potential for further facile modification using a click chemistry approach; in this case, the copper-catalyzed coupling of appropriately substituted acetylenes to the azide substituent. Such a utilitarian procedure offers wide scope for the attachment of lipophilic groups to the neomycin B core. In order to examine the feasibility of the click approach, a model reaction was carried out using the propargyl ester derivative of 4-nitrobenzoic acid, **7**. The Cu(I)-catalyzed coupling[21] of the peracylated azido neomycin **6** with the propargyl ester **7** (Scheme 1) was carried out in the presence of $CuSO_4$ and copper powder in 1:1 mixture of water and *t*-BuOH at room temperature. The required triazole-containing click product, **8**, was formed cleanly and isolated in good yield (79%). Expansions of the carbohydrate region of the ^{1}H NMR spectra obtained for the per-acetylated parent neomycin, **2**, the azido derivative, **6**, and the derived triazole-containing click product, **8**, are provided in Figure 2.[22] The marked upfield shift of the H_{3IV} resonance in the azido derivative, **6**, in comparison to the acetylated parent protected neomycin, **2**, is in evidence (Figure 2b). This shift to highfield is mirrored in the ^{13}C NMR spectrum of the compound as C_{3IV} resonates at 60.8 ppm in the azido derivative, **6**, some 9.2 ppm to high field of the corresponding resonance in the parent compound, **2** (70.0 ppm). These shifts are in accord with those provided in previous reports of azidosaccharide species.[23] The stereochemistry of the azide addition product, **6**, was confirmed using selective 1D NOE experiments conducted on **6**-hexa-*O*-acetate. Figure 3a shows an expansion of the ^{1}H NMR spectrum of **6**-hexa-*O*-acetate, focusing on the region containing the H_{3IV}, H_{5IV}, and H_{2IV} resonances. Selective saturation of the near co-incident H_{4IV} and H_{1IV} resonances (δ 5.13 ppm) results in NOE enhancement of the H_{3IV}, H_{5IV}, and H_{2IV} resonances, as expected.[25] Selective irradiation (δ 6.43 ppm) of the overlapping $H_{(2)NH(IV)}$ and $H_{(2)NH(I)}$ resonances results in significant enhancement of the H_{3IV} and H_{2IV} resonances, establishing the proximity of the H_{3IV} and $H_{(2)NH(IV)}$ nuclei.[26] This places the H_{3IV} proton on the top face of the *ido* ring (IV), i.e. in the equatorial position, confirming axial attachment of the azido moiety and thus, by inference, the *talo* configuration of the monoanhydro precursor, **3**.[18a] The singlet resonance expected for the aryl proton of the newly formed triazole unit is in evidence in the ^{1}H NMR spectrum (Figure 2c) of the click product, **8**-hexa-*O*-acetate, (8.40 ppm), as is the deshielded broad singlet observed for the methylene protons adjacent to the ester linkage (5.67 ppm). The resonances attributable to H_{3IV} and H_{4IV} both show distinctive downfield shifts (5.41 and 5.75 ppm) upon conversion of the azide substituent to the triazole ring (cf. Figure 2, b and c). The ^{13}C NMR chemical shifts of C_{3IV} and C_{4IV} are less sensitive to the conversion of the azide substituent to the triazole unit, however. In **8**-hexa-*O*-acetate, C_{3IV} and C_{4IV} are observed at 61.6 and 69.9 ppm, respectively, both within 2 ppm of the corresponding resonant frequencies of these carbons in the per-acetylated azido precursor, **6**-hexa-*O*-acetate.[27]

The success of the model alkyne-azide coupling reaction encouraged us to adopt this click approach as our general method of choice for lipophilic modification of the *ido* residue of the neomycin B core. The click synthons propargyl laurate,**9**, and a propargyl ester of a cholesterol derivative, **10**, illustrate the utility of the procedure for increasing the hydrophobicity and biological recognition and transport properties of the aminoglycoside. Mycobacterial interaction with membrane-bound cholesterol must involve some sort of recognition of the A-ring, as this portion of the steroid will be oriented toward

(20) Angyal, S. J. *Chem. Ind. (London, UK)* **1954**, 1230−1231. Note that a similar Fürst−Plattner analysis of the other possible diastereomer (*altro* epoxide) would result in azide attack at the 4-position.

(21) (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596. (b) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057. (c) For a recent review of the mechanistic and synthetic aspects of Cu(I)-catalyzed alkyne-azide couplings, including the choice of catalysts and the regiochemical outcomes of the reactions, see: Bock, V. D.; Hiemstra, H.; van Maarseveen, J. H. *Eur. J. Org. Chem.* **2006**, 51−68 and references therein.

(22) Due to the complexity observed in spectra obtained at room temperature,[18a] all 1D and 2D ^{1}H NMR studies conducted on the Boc-protected, per-acetylated neomycin B derivatives reported here have been performed in C_5D_5N solution at 363 K (or 373 K in the case of **26**). A comparison of the ^{1}H NMR spectra obtained in $CDCl_3$ and C_5D_5N solution at room and elevated temperature is provided in Supporting Information.

(23) See for example: Szilagyi, L.; Gyorgydeak, Z. *Carbohydr. Res.* **1985**, *143*, 21−41. Compare Vignon, M. R.; Vottero, P. J. A. *Tetrahedron Lett.* **1976**, *17*, 2445−8.

(24) Cyclenoe; NOE difference experiment, VNMR 6.1C, Varian Associates.

(25) Unambiguous enhancement of the H_{3III} resonance (δ 4.73 ppm) was also observed in this experiment (not shown).

(26) Unambiguous enhancement of the H_{3I} resonance (δ 5.63 ppm) was also observed in this experiment (not shown).

(27) The 1,4 regioselectivity of the Cu(I)-catalyzed alkyne-azide couplings in this work is verified by the ^{13}C and ^{1}H NMR chemical shifts of the triazole unit in the click-coupled products obtained. We find the resonance attributable to the triazole C_{ipso} consistently in the range δ 143−145 ppm in the ^{13}C NMR spectra of the compounds. Similarly, the triazole CH carbon resonates in the range δ 122−127 ppm in the ^{13}C NMR and the triazole CH proton is observed between δ 8−8.5 ppm in the ^{1}H NMR in all cases. These values are consistent with those observed previously for 1,4-subtituted triazoles (see, for example, ref 21b) and contrast with those reported previously for 1,5-subtituted triazoles (see, for example, Crandall, J. K.; Crawley, L. C.; Komin, J. B. *J. Org. Chem.* **1975**, *40*, 2045−2047).

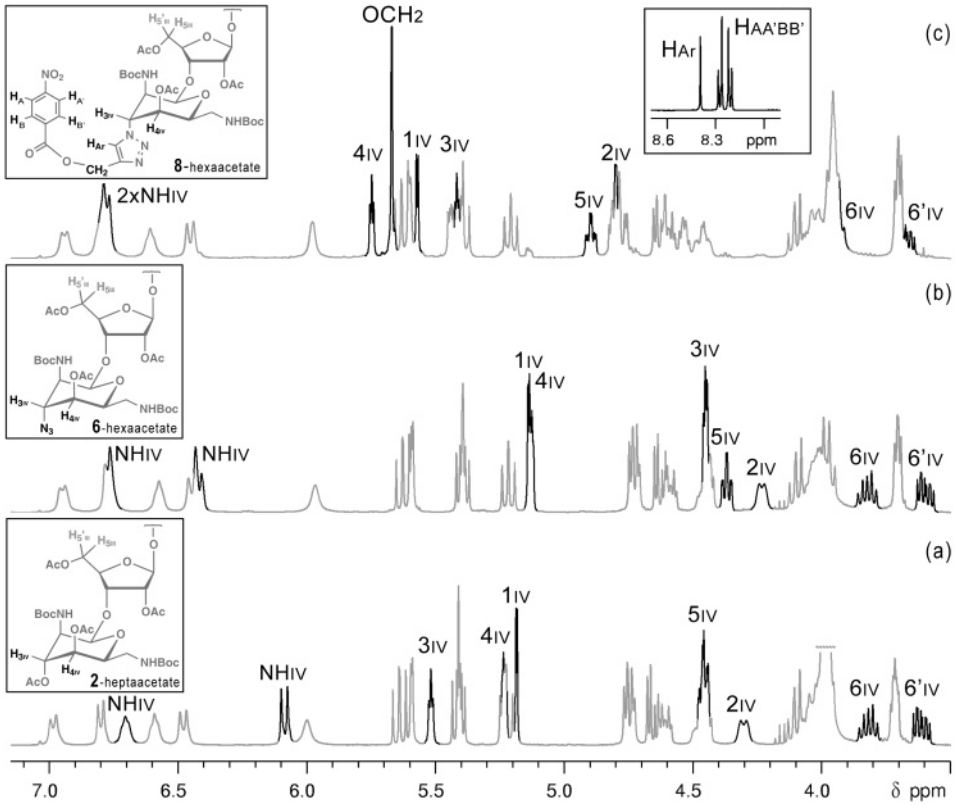
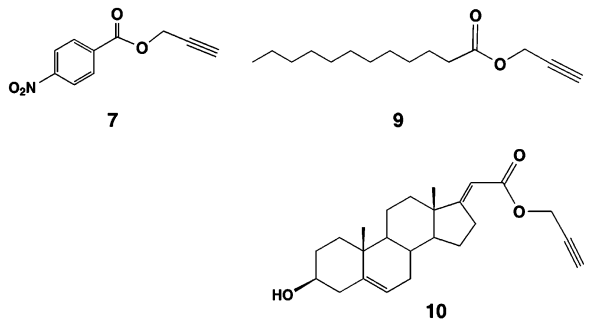


FIGURE 2. Expansions of the carbohydrate regions of the ^{1}H NMR spectra (400 MHz, 363 K, C_5D_5N) of (a) **2**-hepta-*O*-acetate, (b) **6**-hexa-*O*-acetate, and (c) **8**-hexa-*O*-acetate are shown. The changes observed in the resonances attributable to the protons of ring IV are highlighted. An expansion of the aromatic region of the spectrum of **8**-hexa-*O*-acetate is also provided as the inset in (c).

the external surface of the macrophage. Thus, we have chosen to link a steroid core structure through the D-ring of **10** to neomycin to present a mimic of the cholesterol tetracyclic system with an unmodified 3-OH group.



Propargyl laurate, **9**, was prepared using a standard DCC coupling reaction between lauric acid and propargyl alcohol. Copper(I)-catalyzed reaction of this ester with azidoneomycin **6** proceeded smoothly and afforded compound **13** (Scheme 1) in 72% yield after purification using column chromatography. Similarly, modified neomycins incorporating a steroidal lipophilic domain were achieved using a click coupling strategy employing the cholesterol derivative **10** as the terminal alkyne synthon. The propargyl ester **10** was prepared in two steps from the corresponding ethyl ester derivative, **11**, reported previously.[28] The commercially available ketone dehydroandrosterone

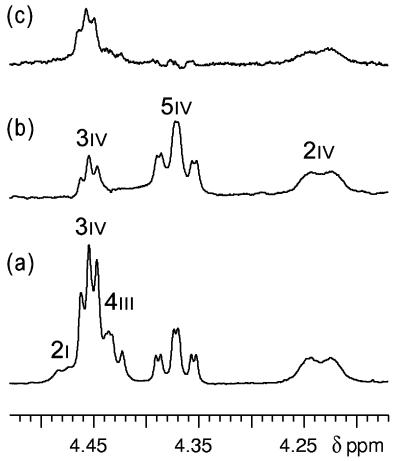


FIGURE 3. Expansions of (a) the ^{1}H NMR spectrum (400 MHz, 363 K, C_5D_5N) of **6**-hexa-*O*-acetate and the corresponding regions of the 1D NOE spectra obtained from presaturation at (b) δ 5.13 ppm and (c) δ 6.43 ppm, using the cyclenoe sequence[24] with internal subtraction (400 MHz, 363 K, C_5D_5N).

was extended using a Wittig−Horner reaction with triethyl phosphonoacetate and sodium ethoxide in ethanol. Hydrolysis of the resultant ethyl ester with KOH in isopropanol gave the acid **12** (72% yield), which was then re-esterified with propargyl alcohol, under Mitsunobu conditions, to furnish **10** in 75% yield. The Cu(I)-mediated alkyne-azide coupling reaction of **10** with the azido neomycin **6** afforded the cholesterol neomycin B hybrid **14** (Scheme 1) in 75% yield after purification.

Full assignment of the entire ^{1}H NMR spectral range of the click adducts with attached lipophilic groups is hampered by

(28) (a) Wicha, J.; Bal, K.; Piekut, S. *Synth. Commun.* **1977**, *7*, 215. (b) Quader, S.; Boyd, S. E.; Houston, T. A.; Jenkins, I. D.; Healy, P. C. *Acta Crystallogr.* **2006**, *E62*, o162−o164.

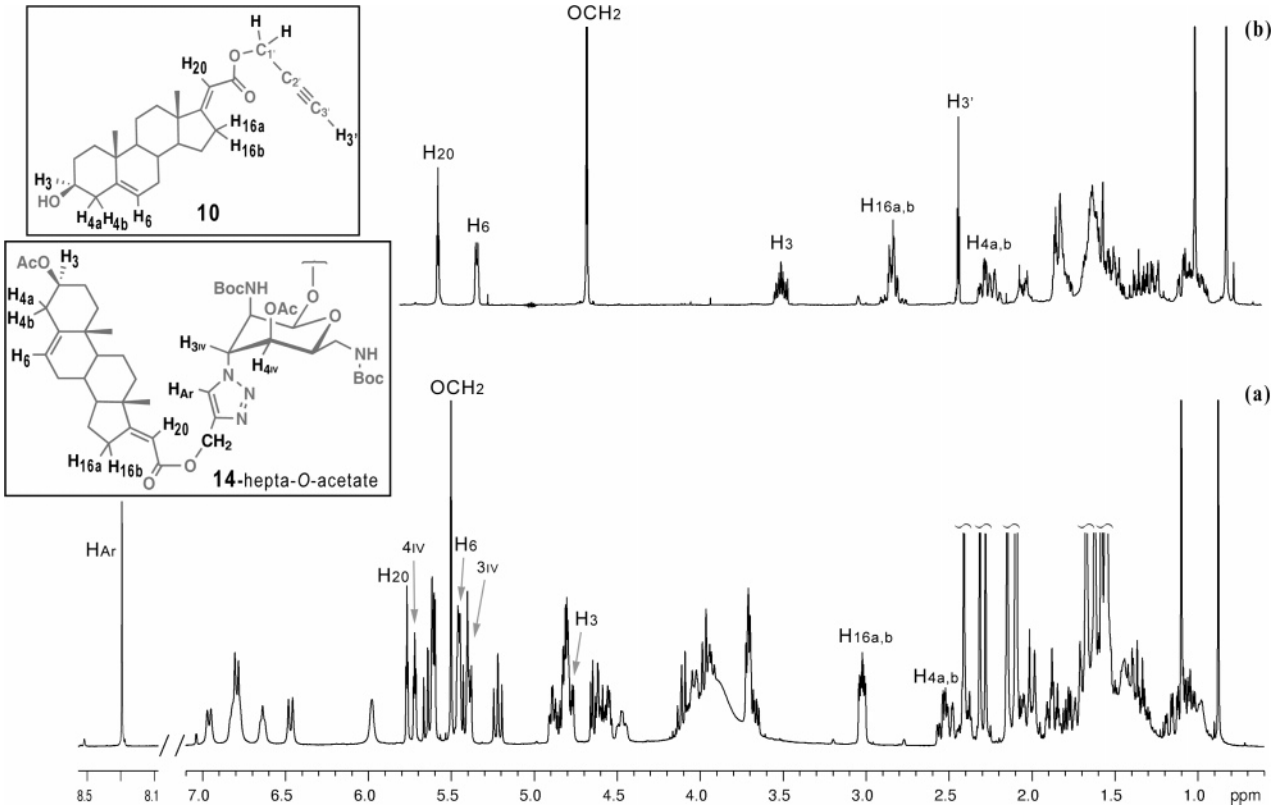


FIGURE 4. Expansions of the ^{1}H NMR spectra (400 MHz, 363 K, C_5D_5N) of (a) **14**-hepta-*O*-acetate and (b) the click synthon, **10**. Resonances attributable to the protons of the steroid, the ester linker, and the triazole are highlighted.

both the complexity of the aliphatic/alicyclic region and the relative intensity of the protecting group resonances that also occur in this region. In particular, under our experimental conditions, the elevated probe temperature dictates the use of traditional phase cycled 2D pulse sequences, instead of their cleaner gradient-filtered counterparts, and hence as would be expected, the spectral region surrounding the ^{1}H resonances of the protecting groups is adversely affected by extensive T_1 noise. This inhibits full assignment of the resonances of the hydrocarbon portions of the click adducts. However, sufficient sensitivity and dispersion is obtained in the range above 2.5 ppm (in one- and two-dimensional experiments) to facilitate explicit identification of the major diagnostic resonances expected for the compounds. As in the case of the 4-nitrobenzoic acid based click product, **8**, key resonances for the triazole aryl proton (8.25 ppm) and the methylene protons of the propargyl linker unit (5.43 ppm) are readily identified in the high-temperature ^{1}H NMR spectrum of the lauric acid adduct, **13**-hexa-*O*-acetate. Similarly, downfield shifts are observed for the resonances attributable to H_{3IV} and H_{4IV} (δ 5.38 and 5.71 ppm), and these resonances correlate with resonances at 61.3 and 69.8 ppm, respectively, in the ^{13}C NMR spectrum of the same material (HMQC). Figure 4 highlights the diagnostic resonances attributable to the cholesterol-derived portion of the per-acetylated click product, **14**-hepta-*O*-acetate. The doublet of doublets assigned to H_{20} of the steroid side-chain, the broad singlet of the OCH_2 steroid/neomycin linker group, the AB systems observed for the H_4 and H_{16} steroid methylene pairs, and the singlet resonance of the aromatic proton of the triazole unit are all clearly evidenced. The resonances attributable to H_6 and H_3 of the acetylated steroid are co-incident with aminoglycoside resonances under the experimental conditions, however. As expected, the resonances of the steroid ring protons are largely insensitive to the changes in the linker group in the click-coupled product. There is, however, a general shift to lower field in the high-temperature spectrum of the fully protected product in the C_5D_5N solvent.

Modification of the Ribose Ring. The formation of the dehydration products observed in the reaction of neomycin B with nucleophiles under Mitsunobu conditions renders this method inappropriate for the synthesis of neomycin derivatives substituted at C_{5III} unless the secondary alcohols are protected.[29] Selective lipophilic modification of the ribose residue of neomycin B, without concomitant modification of the *ido* ring, could be accomplished, however, via a procedure based in part on the previously reported selective tripsylation of the C_{5III} primary hydroxyl with 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) (Scheme 3).[30] *N*-Boc-protected neomycin B **2** (1 equiv) was treated with excess TPSCl (28 equiv) in pyridine to obtain the tripsylated derivative **15** (61%). Treatment of **15** with sodium azide in DMF at 100 °C (18 h) afforded the *ribo*-substituted azido neomycin B, **16**, in quantitative yield. Cu-catalyzed alkyne-azide coupling of the archetypal click synthons **7**, **9**, and **10** to **16** afforded the corresponding triazole derivatives **17** (75%), **18** (71%), and **19** (69%) in good yield.

The diagnostic features of the high-temperature ^{1}H NMR spectra of the *ribo*-substituted azido neomycin B, **16**-hexa-*O*-

(29) (a) Hanessian, S.; Tremblay, M.; Swayze, E. E. *Tetrahedron* **2003**, *59*, 983. (b) Liang, F.-S.; Wang, S.-K.; Nakatani, T.; Wong, C.-H. *Angew. Chem., Int. Ed.* **2004**, *43*, 6496−6500.

(30) Michael, K.; Wang, H.; Tor, Y. *Bioorg. Med. Chem.* **1999**, *7*, 1361−1371.

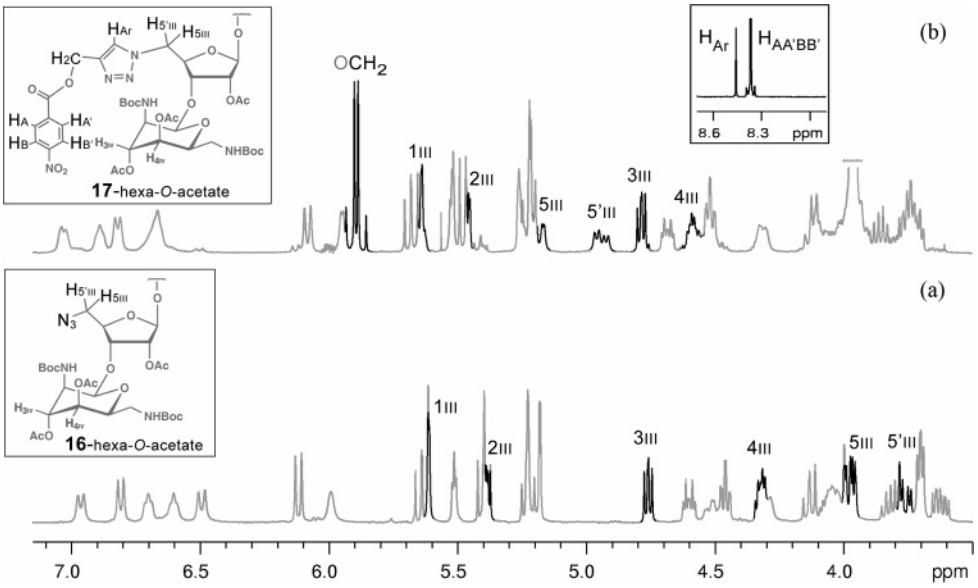
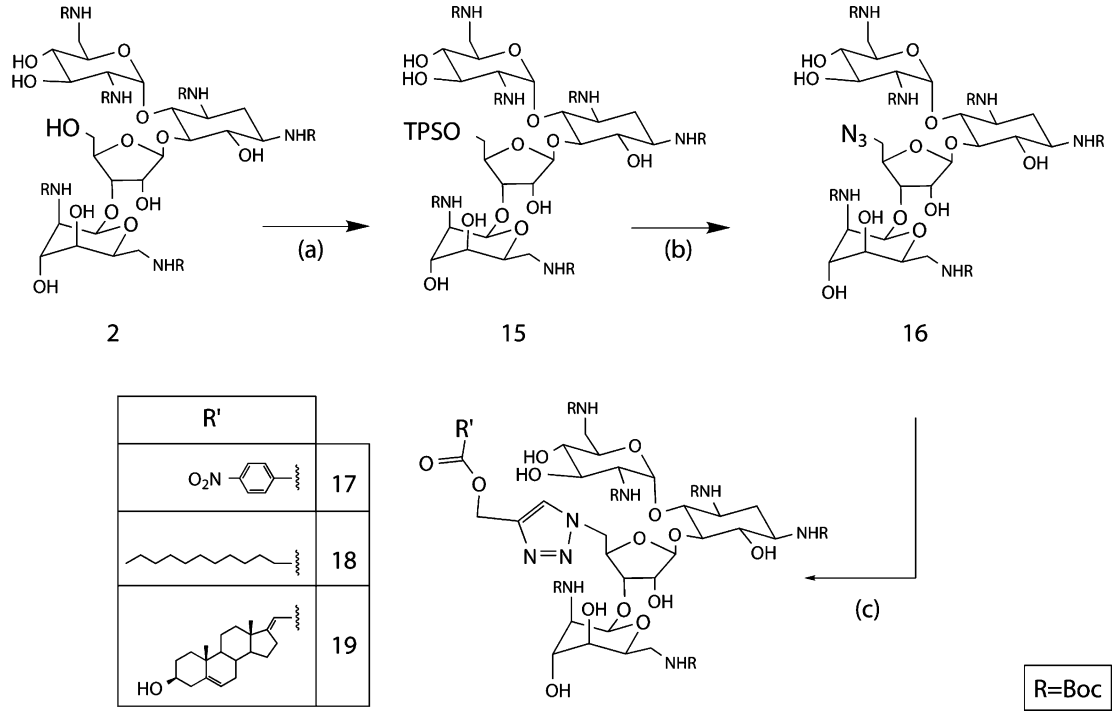


FIGURE 5. Expansions of the ^{1}H NMR spectra (400 MHz, 363 K, C_5D_5N) of (a) **16**-hexa-*O*-acetate and (b) **17**-hexa-*O*-acetate. The changes observed in the resonances attributable to the protons of ring **III** are highlighted. An expansion of the aromatic region of the spectrum of **17**-hexa-*O*-acetate is also provided as the inset in (b).

SCHEME 3[a]



[a] Reagents and conditions: (a) TPSCI (28 equiv), pyridine, rt, 18 h; (b) NaN_3, 100 °C, DMF, 8 h; (c) R′COO-CH_2C″ CH (2 equiv), *t*-BuOH/H_2O (1:1), Cu/$CuSO_4$, rt, 18 h.

acetate, and the derived click product **17**-hexa-*O*-acetate (Scheme 3) are highlighted in Figure 5. It can be seen that the resonances assignable to protons H_{5III} and $H_{5'III}$ in the azido derivative **16**-hexa-*O*-acetate (δ 3.97 and 3.76 ppm, Figure 5a) become increasingly dispersed and resonate 0.7−1 ppm upfield of the corresponding resonances in the per-acetylated parent, **2**-hepta-*O*-acetate. These shifts are consistent with the $\Delta\delta$ values observed for the H_{3IV} ring proton of azido compound **6**-hexa-*O*-acetate, when compared with the parent compound (vide supra). The ^{13}C NMR resonance attributable to C_{5III} also exhibits a dramatic upfield shift in the spectrum of the azido derivative **16**-hexa-*O*-acetate (δ 51.94 ppm, cf. C_{5III} 63.9 ppm in **2**-hepta-*O*-acetate). This shift is again consistent with the chemical shift of the azide bearing carbon in compound **6**-hexa-*O*-acetate and with literature values.

On coupling with the click synthon **7**, the initially high field resonances of H_{5III} and $H_{5'III}$ show a marked downfield shift in **17**-hexa-*O*-acetate (δ 5.15 and 4.94, Figure 5b), resonating 0.2−

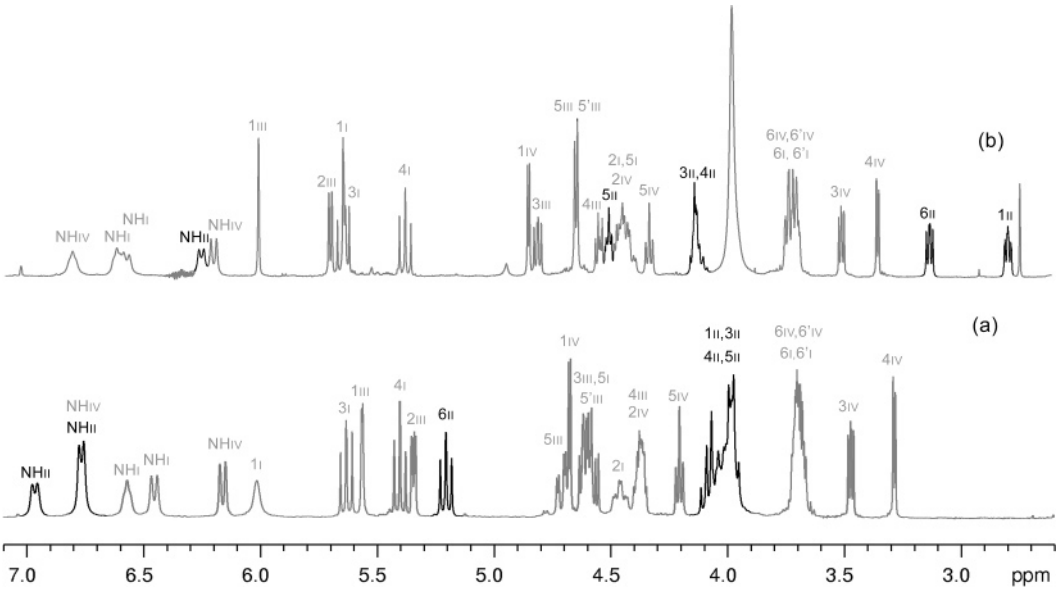


FIGURE 6. Expansions of the ^{1}H NMR spectra (400 MHz, 363 K, C_5D_5N) of (a) **3**-penta-*O*-acetate and (b) **4**-tetra-**O**-acetate. The changes observed in the resonances attributable to the protons of ring **II** are highlighted.

0.5 ppm to lower field of the antecedent resonances of the per-acetylated parent compound. The resonances in the lauric acid derived analogue, **18**-hexa-*O*-acetate (δ 5.13 and 4.93 ppm), and the cholesterol adduct, **19**-hepta-*O*-acetate (δ 5.11 and 4.98), similarly show distinctive downfield shifts, indicative of triazole formation adjacent to C_{5III}. However, as was observed in the case of the *ido* substituted analogues, the ^{13}C NMR shift of C_{5III} is less sensitive to the conversion of the azide function to a triazole derivative, C_{5III} resonating at δ51.9 and 52.4 ppm in compounds **16**-hexa-*O*-acetate and **19**-hepta-*O*-acetate, respectively.

In addition to the notable changes in the ^{1}H NMR chemical shifts of the H_{5III} and $H_{5'III}$ protons, a consistent diagnostic feature of adducts isolated from click coupling reactions conducted on the *ribo* substituted azido neomycin B, **16**, is the appearance of the resonances attributable to the OCH_2 methylene protons of the propargyl alcohol derived unit of the click synthon. In the spectra of the per-acetylated derivatives such as **17**-hexa-*O*-acetate (Figure 5b), the resonances of these diastereotopic protons diverge in chemical shift and appear as distinct AB systems (400 MHz). This contrasts with the adducts formed from *ido* substituted azido neomycin B, **6** (Figure 2c), where in all cases the methylene protons of the per-acetylated systems show co-incident resonant frequencies.

Modification of the Steptamine Ring. As discussed above, a second major dehydration product, **4**, was isolated from reaction mixtures containing the protected parent species, **2**, and the Mitsunobu reagents TPP and DIAD (Scheme 1). Mass spectral analysis of the per-acetylated derivative confirmed a tetra-*O*-acetate, supporting the formation of a stable bis-anhydro derivative of the parent aminoglycoside. Subsequent ^{1}H and ^{13}C NMR analysis of the isolated, per-acetylated aminoglycoside, **4**-tetra-*O*-acetate, revealed the formation of an aziridine ring in ring II, with a Boc-protected nitrogen bridging C_{6II} and C_{1II}, in addition to the previously observed epoxide at C_{3IV} and C_{4IV} of the *ido* ring.

Expansions of the ^{1}H NMR spectrum of **4**-tetra-*O*-acetate and its mono-anhydro precursor **3** (Scheme 1) are provided in Figure 6. It is noteworthy that an NH resonance, present in the mono-anhydro species (Figure 6a), is absent in the spectrum of the bis-anhydro derivative, **4**-tetra-*O*-acetate (Figure 6b). In this instance, the second dehydration step clearly proceeds with one of the protected amine nitrogens serving as the intramolecular nucleophile, rather than a second hydroxylic oxygen nucleophile. The marked changes observed in the ^{1}H NMR and ^{13}C NMR resonances of the streptamine residue (ring II) locates the resultant aziridine within this ring. Distinct shifts are observed for the resonances of the H_{5II} and the H_{1II} and H_{6II} protons in particular. H_{5II} resonates 0.5 ppm downfield from its antecedent, while H_{1II} and H_{6II} move 1.2 and 2.1 ppm upfield, respectively. Further evidence of aziridine ring formation at C_{6II} and C_{1II} is obtained from the ^{13}C NMR chemical shifts of the C_{6II} and C_{1II} carbons. Unambiguous cross-peaks are observed in the HMQC

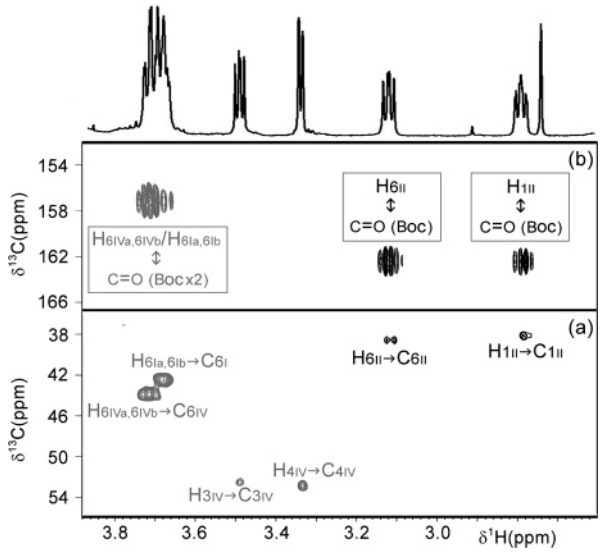
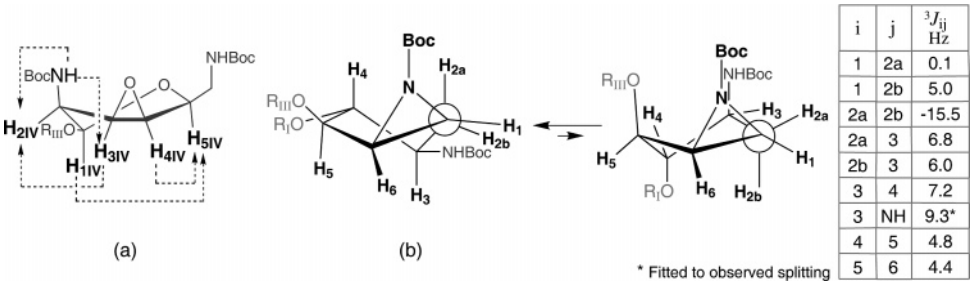


FIGURE 7. Expansions of the aziridine region of (a) the HMQC spectrum and (b) the HMBC spectrum obtained for **4**-tetra-*O*-acetate (400 MHz, C_5D_5N, 363 K). The corresponding region of ^{1}H NMR spectrum is provided as an axis reference.



i	j	$^3J_{ij}$ Hz
1	2a	0.1
1	2b	5.0
2a	2b	-15.5
2a	3	6.8
2b	3	6.0
3	4	7.2
3	NH	9.3*
4	5	4.8
5	6	4.4

FIGURE 8. (a) NOE enhancements observed in cyclenoe experiments conducted on **4**-tetra-*O*-acetate and summarizing intraresidue enhancements observed in experiments focused on ring IV. (b) Preferred conformation of ring II. $^3J_{HH}$ couplings, obtained in the simulation, are provided in the table inset. R_IO and $R_{III}O$ represent glycosidic linkages to rings I and III.

spectrum of **4**-tetra-*O*-acetate linking H_{1II} and H_{6II} with ^{13}C resonances at δ 38.1 and 38.5 ppm, respectively (Figure 7a). These shifts are in accord with those observed previously in Boc-protected aziridines.[31] Furthermore, the HMBC spectrum (Figure 7b) of **4**-tetra-*O*-acetate shows distinct cross-peaks correlating H_{1II} and H_{6II} with a single carbamate C═O function resonating at 162 ppm. This C═O resonates some 5 ppm downfield of all other carbamate (carbonyl) resonances observed both in this species and other compounds studied in this work and is consistent with literature values recorded for the carbamate functions of Boc-protected aziridines.[31] The two HMBC cross-peaks linking both H_{1II} and H_{6II} with this carbamate resonance confirms the formation of the three-membered aziridine ring at this position.

1D NOE experiments conducted on **4**-tetra-*O*-acetate (Scheme 1) support an average $^OH_1$ conformation of ring IV, as observed in the mono-anhydro precursor, **3**-penta-*O*-acetate. Enhancement of the H_{5IV} resonance results from selective saturation of either H_{4IV} or H_{1IV}. Saturation of H_{3IV} results in enhancement of H_{2IV} as expected (Figure 8a). NOE experiments conducted on ring II, however, yielded ambiguous results due to the near coincident overlap of the H_{3II} and H_{4II} resonances.[32] Simulation of the ^1H NMR resonances of the ring II spin system, for **4**-tetra-*O*-acetate and related compounds (Scheme 5), yielded $^3J_{HH}$ vicinal coupling constants similar to those observed by Crotti and co-workers in their studies[33] on aziridine-containing pyrans. In particular, we typically observe values of ~0.1 and 5.0 Hz for the $^3J_{1II,2aII}$ and $^3J_{1II,2bII}$ coupling constants of compounds synthesized that are isostructural in ring II. These distinct values suggest that ring II averages to a $^4H_3$ conformation (Figure 8b), as the alternate $^3H_4$ conformation would be expected to exhibit similar values for the $^3J_{1II,2aII}$ and $^3J_{1II,2bII}$ couplings.[33] Furthermore, the values fitted for the other ^3J couplings of ring II, for this compound and related aziridine-containing compounds synthesized in this work, are also consistent with those expected for the $^4H_3$ conformation.

An interesting observation is that the formation of the aziridine ring takes place on the deoxystreptamine ring but not on the *gluco* ring. Also, when the 5-position of the (remote) ribose is blocked (as the 4-nitrobenzoate ester), aziridine ring formation does not occur. This suggests that the 5-position of ribose takes part in the mechanism of formation of the aziridine ring. A possible explanation is that a cyclic 10-membered ring phosphorane intermediate is formed. This ring may stabilize the all-axial conformation of the deoxystreptamine required for aziridine ring formation and/or facilitate transfer of the triphenylphosphinoxy leaving group from the primary hydroxyl of ribose to the secondary hydroxyl of the deoxystreptamine (Scheme 4). From a study of molecular models, the arrangement of the two oxygen atoms about the phosphorus could be either diaxial or axial−equatorial, the latter being less crowded (these two forms can equilibrate via Berry pseudorotation[34]). Phosphoranes of this type are well-known intermediates in the Mitsunobu reaction.[35] The corresponding 14-membered ring phosphorane joining the 5-position of ribose to the 3-position of the *gluco* ring is highly strained and much less likely to form.

Attempts to improve the yield of **4**, by increasing the proportion of TPP and DIAD added to **2** from 4 to 6 equiv, resulted in essentially no change in the isolated yield of **4** (48%, 6:6:1 TPP/DIAD/**2**; cf. 45%, 4:4:1 TPP/DIAD/**2**). Mass spectral analysis of the product mixture obtained in the reaction revealed the presence of significant amounts (18%) of a compound with a molecular ion corresponding to a DIAD-derived hydrazine adduct (LRESMS $[M + Na^+] = 1387$) of the initially formed bis-anhydro derivative **4**. In order to suppress the formation of DIAD derived hydrazine adducts and thus improve the yield of **4**, the bulkier di-*tert*-butyl azodicarboxylate (DBAD) was substituted for DIAD in the synthesis of **4**. Thus, **2** was treated with 6 equiv of TPP and DBAD in toluene at room temperature (12 h). This procedure resulted in a 10% improvement in the isolated yield of **4** (55%) and only traces of putative DBAD-derived hydrazine adducts were observed in these reactions.

The bis-anhydro neomycin B derivative **4** invites further synthetic modification. The ring opening of the epoxide and/or aziridine functions permits modification of two sugar residues in the neomycin B framework. Modification of ring II, a key pathogen/target binding residue,[36] in particular offers considerable potential for significantly affecting the resultant aminoglycoside's antibiotic activity. Accordingly, we have undertaken preliminary studies exploring the ring opening of the epoxide and/or the aziridine rings of compound **4**.

HPLCMS (MeCN/H_2O 75:25) analysis of a per-acetylated product mixture obtained after treatment of the bis-anhydro

(31) See, for example: Gardiner, J. M.; Loyns, C. R. *Tetrahedron* **1995**, *51*, 11515−11530.

(32) Sample spectra obtained in the NOE experiments conducted on ring IV are provided in Supporting Information.

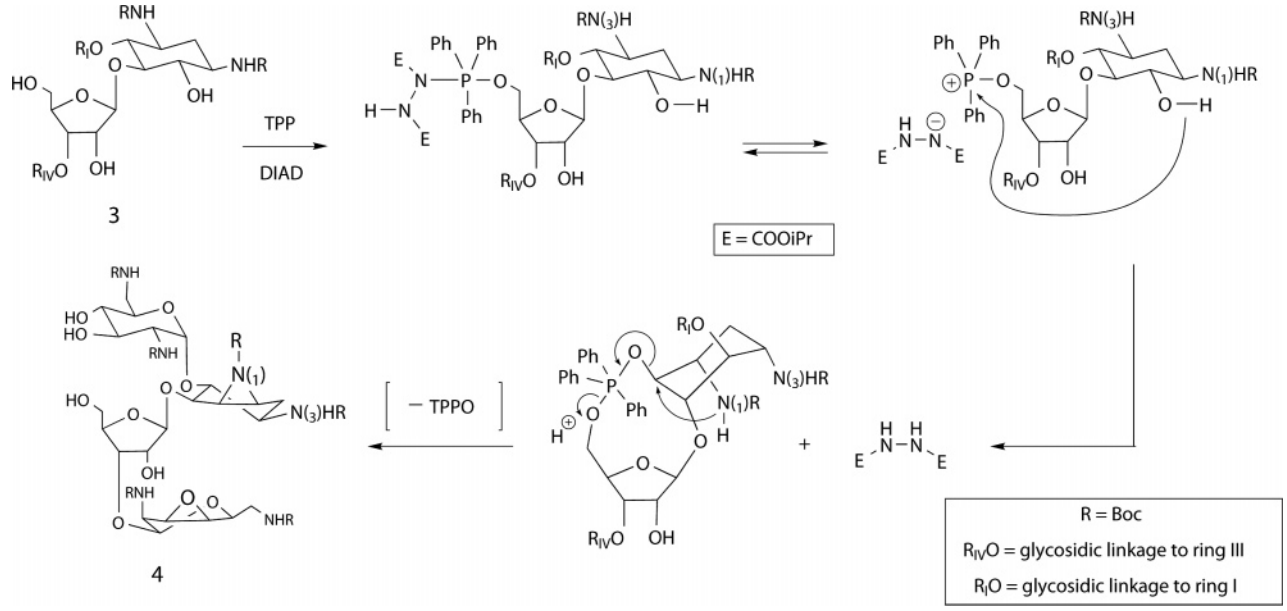
(33) Crotti, P.; Di Bussolo, V.; Favero, L.; Pineschi, M. *Tetrahedron* **1997**, *53*, 1417−1438.

(34) (a) Berry, R. S. *J. Chem. Phys.* **1960**, *32*, 933. (b) Gallagher, M. J.; Jenkins, I. D. In *Topics in Stereochemistry*; Eliel, E. L., Allinger, N. L., Eds.; New York: Wiley-Interscience, 1968; Vol. 3, p 64.

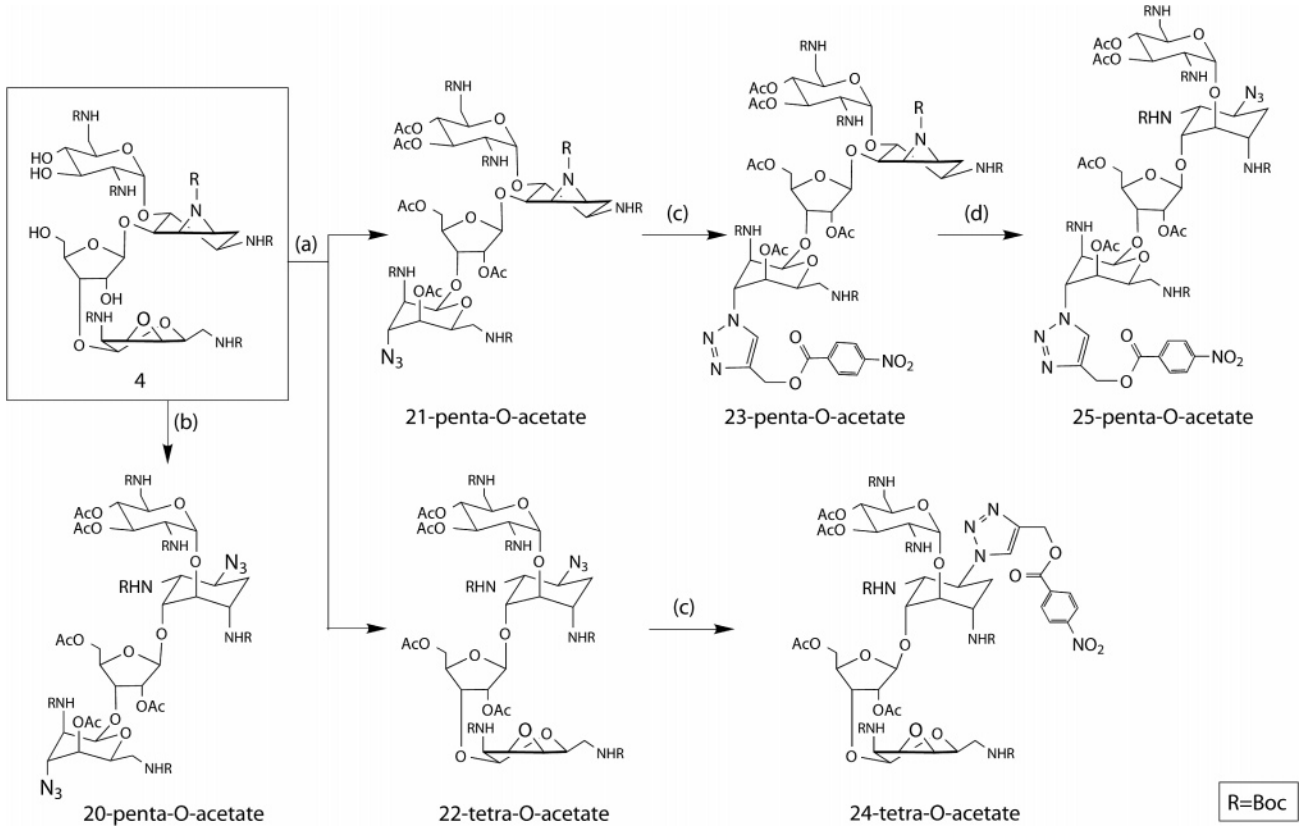
(35) (a) von Itzstein, M.; Jenkins, I. D. *Aust. J. Chem.* **1983**, *36*, 557−563. (b) von Itzstein, M.; Jenkins, I. D. *J. Chem. Soc., Perkin Trans. 1* **1986**, 437−445. (c) von Itzstein, M.; Jenkins, I. D. *J. Chem. Soc., Perkin Trans. 1* **1987**, 2057−2060. (d) Camp, D.; Jenkins, I. D. *J. Org. Chem.* **1989**, *54*, 3045−3049; 3049−3054.

(36) Magnet, S.; Blanchard, J. S. *Chem. Rev.* **2005**, *105*, 477−497.

SCHEME 4



SCHEME 5[a]



[a] Reagents and conditions: (a) (i) 1 equiv NaN_3, 50 °C, DMF, 2 d, (ii) Ac_2O, C_5H_5N, DMAP; (b) (i) excess NaN_3, 50 °C, DMF, 1 d, (ii) Ac_2O, C_5H_5N, DMAP; (c) $O_2NC_6H_4COO\text{-}CH_2C''CH$ (2 equiv), *t*-BuOH/H_2O (1:1), Cu/$CuSO_4$, rt, 18 h; (d) excess NaN_3, 50 °C, DMF, 1 d.

neomycin derivative **4** with 1 equiv of sodium azide in DMF (Scheme 5) revealed a product mixture comprising **4**-tetra-*O*-acetate (32%), a diazido adduct, **20**-penta-*O*-acetate (11%), and two possible mono-azido adducts, **21**-penta-*O*-acetate and **22**-tetra-*O*-acetate (40% and 15%, respectively). Subsequent attempts at product purification showed that although the diazido adduct **20** and the mono-azido adduct **21** could be isolated, the second mono-azido adduct **22** coeluted with the residual starting material in all solvent systems examined. Attempts to separate the species after per-acetylation of the crude reaction mixture met with only limited success. The diazido adduct **20**-penta-*O*-acetate was again successfully isolated; however, analytical quantities of the mono-azido adducts were obtained only after preparative HPLC. The mono-azido species resulting from ring

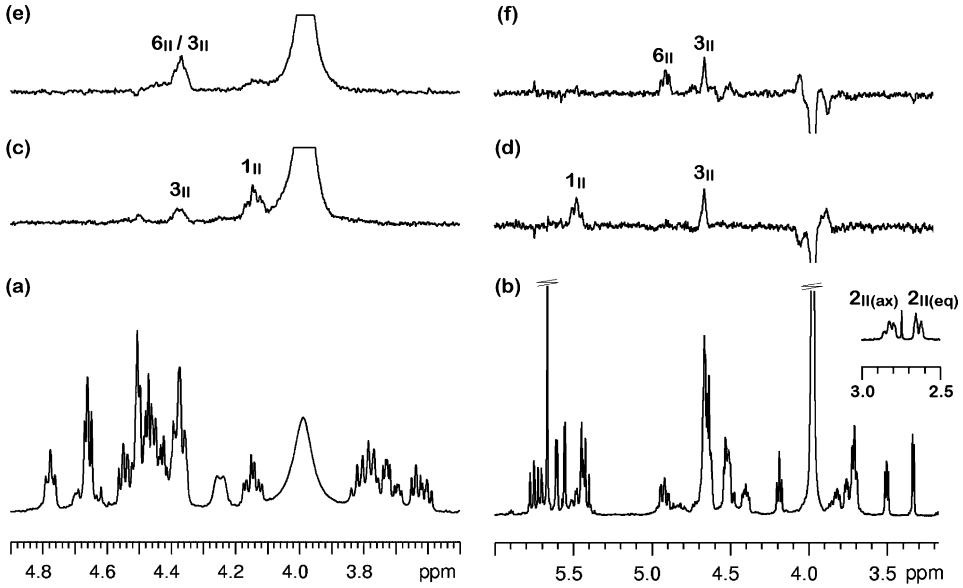


FIGURE 9. Expansions of the carbohydrate regions of the ^{1}H NMR spectrum (400 MHz, C_5D_5N, 363 K) of (a) **20**-penta-*O*-acetate and (b) **24**-tetra-*O*-acetate. NOE enhancements resulting from selective saturation of H_{2IIeq} are provided for each compound in (c) and (d). NOE enhancements resulting from selective saturation of H_{2IIax} are provided for each compound in (e) and (f).

opening of the ring II aziridine, **22**-tetra-*O*-acetate, has not been obtained in sufficient quantity for full characterization (^{1}H NMR only). However, a separable mixture of the bis-anhydro derivate, **4**-tetra-*O*-acetate, and the click adducts **23**-penta-*O*-acetate and **24**-tetra-*O*-acetate was obtained when a partially purified mixture of **4**-tetra-*O*-acetate, **21**-penta-*O*-acetate, and the unisolated **22**-tetra-*O*-acetate was treated with the click synthon **7** under our standard conditions. The click coupling proceeds quantitatively with respect to the initial concentration of azido species present in the reaction mixture in both cases. No degradation of the aziridine-containing ring II was observed in the formation of **23**-penta-*O*-acetate, and no degradation of the epoxide-containing ring IV was observed in the formation of **24**-tetra-*O*-acetate under the conditions of the click coupling reaction.

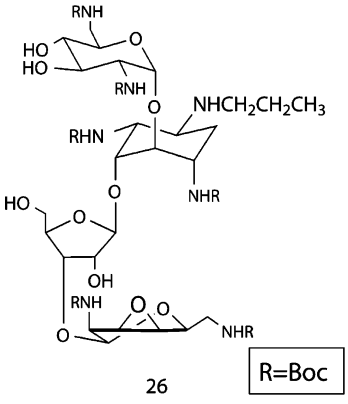
The ring opening of the epoxide function of ring IV by azide ion in both the mono-azido adduct **21** and the diazido adduct **20** (Scheme 5) proceeded in a manner directly analogous to that observed in the reaction of the mono-anhydro neomycin derivative **4**, as expected. The *talo* epoxide of ring IV of **4** undergoes regioselective ring opening by azide ion at C_{3IV} in both cases. The ^{1}H and ^{13}C NMR resonances of the ring IV fragments of the per-acetylated derivatives of **20** and **22** are in close agreement with those observed for **6**-hexa-*O*-acetate. Similarly, chemical shift, coupling constant, and NOE experiments focused on the aziridine containing ring II of the **21**- and **23**-penta-*O*-acetates showed results in accord with those observed for the bis-anhydro parent species **4**-tetra-*O*-acetate. The $^4H_3$ conformations are observed for ring II in both cases.

Analysis of the ^{1}H and ^{13}C NMR spectra obtained for diazido adduct **20**-penta-*O*-acetate[37] revealed an interesting outcome in the ring opening of the aziridine function of ring II by azide ion. Azide substitution occurs at C_{1II}, resulting in a net migration of the amine function from C_{1II} in the parent neomycin to C_{6II} in these derivatives. Azide substitution at C_{1II} is supported by the shift of the H_{1II} resonance 1.35 ppm downfield with respect to the aziridine precursor. Furthermore, C_{1II} resonates at δ 57.3 ppm in the ^{13}C NMR (cf. 50.5 ppm in **2**-hepta-*O*-acetate). The migration of the Boc protected amine to C_{6II} is confirmed by the cross-peak correlating H_{6II} and the NH proton in the COSY spectrum.[38] The attack by azide at C_{1II}, rather than C_{6II}, is not surprising. C_{1II} is the least hindered site for attack, and moreover, being adjacent to the methylene group, this site would be favored on electronic grounds as the transition state for attack by azide would result in the development of a small positive charge at the aziridine carbon. Attack would therefore be favored by an adjacent CH_2 rather than a CHOR group. It was surprising, however, that the product of azide attack did not retain the $^4C_1$ conformation expected from the opening of the $^4H_3$ conformer. The initial trans-diaxial arrangement of the C_{1II} azido and C_{6II} NHBoc groups is unobserved in the product. Instead, ring II of **20**-penta-*O*-acetate averages to a $^1C_4$ conformation, in which the C_{1II} azido and C_{6II} NHBoc groups are diequatorial, while the substituents on C_{3II}, C_{4II} and C_{5II} are all axial. The $^1C_4$ conformation of ring II of **20**-penta-*O*-acetate is evidenced in the ^{1}H NMR spectrum. The H_{1II} resonance of **20**-penta-*O*-acetate is well-separated from surrounding resonances in the C_5D_5N solvent at 363 K and shows couplings of 4, 10, and 10 Hz to neighboring protons, suggesting an axial disposition of the proton. Furthermore, selective irradiation of the H_{2IIax} (δ 2.12 ppm) and H_{2IIeq} (δ 2.36 ppm) resonances of **20**-penta-*O*-acetate engenders NOE at H_{3II} in both cases (Figure 9). Significant enhancement of the H_{1II} and $NH_{(3II)}$ resonances is only observed in the case of selective irradiation of the H_{2IIeq} resonance; however, further supporting the mutually trans-diaxial dispositions of H_{1II}, H_{2IIax}, and the $NH_{(3II)}$Boc group. It is also noteworthy that the resonances of H_{1III} and H_{2III} show marked shifts in **20**-penta-*O*-acetate relative to their antecedents in the

(37) The diazido adduct **20**, obtained in low yield in the reaction of **4** with equimolar sodium azide, was obtained quantitatively on treatment of **4** with excess sodium azide.

(38) (a) Separate crosspeaks for the $H_{6II}-NH_{(6II)}$ and $H_{3II}-NH_{(3II)}$ correlations are clearly evident in the 2D COSY experiment (400 MHz, 363 K, C_5D_5N); see Supporting Information. (b) Migratory, aziridine formation and ring opening has been observed in streptamine systems before: Ikeda, D.; Horiuchi, Y.; Kondo, S.; Umezawa, H. *J. Antibiot.* **1980**, *33*, 1281−1288.

parent neomycin, although no direct chemical modification has taken place in this ring. A similar picture emerges from NMR spectroscopic studies conducted on derivatives that are *iso*-structural at ring II, i.e., **22**-tetra-*O*-acetate and **25**-penta-*O*-acetate, as well as the triazole derivative **24**-tetra-*O*-acetate (Figure 9). The body of data suggests a significant conformational change accompanies the migration of the amine function in ring II, with this ring averaging to a $^1C_4$ conformation, in contrast to the $^4C_1$ conformation of the neomycin parent, even though this conformation places three bulky substituents in axial positions. We suggest that the $^1C_4$ conformation is stabilized by a H-bonding interaction between the axial NHBoc group on C_{3II} and the axial oxygen on C_{5II}. Recent theoretical calculations[39] and a literature example[40] suggest that H-bonding interactions can affect conformational preferences in these systems.



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Attempts were made to improve the regioselectivity of the initial azide addition to **4**. However, essentially no improvement in yield or selectivity was observed when either catalytic quantities of cerium chloride were added to the reaction mixture or when the reactions were carried out using trimethylsilylazide (TMSA)[41] in place of NaN_3.[42] Treatment of **4** with neat propylamine at room temperature for 16 h did, however, afford a 47% yield of the product obtained from selective ring opening of the aziridine function of ring II, **26**. The starting material, **4**, was recovered in 50% yield after purification. Only traces of the product resulting from ring opening of both the aziridine and epoxide functions were detected in the product mix. The alternate epoxide opened species was undetected in the mixture. Investigations into alternate methods for improving the yield and regioselectivity of the ring opening reactions are ongoing in our laboratory.

Conclusion

We have demonstrated the selective modification of the *ido*- (IV), *ribo*- (III), and streptamine (II) rings of the neomycin B framework utilizing two different yet complementary synthetic approaches. Utilizing these strategies we have prepared a number of derivatives of neomycin B. Indeed, by employing a strategy based on the bis-anhydro derivative **4**, highly modified derivatives of the neomycin B parent framework can be obtained. For example, treatment of the isolated click product **23**-penta-*O*-acetate (Scheme 5) with excess sodium azide under the standard conditions yields an aminoglycoside derivative, **25**-penta-*O*-acetate, bearing a hydrolyzable lipophilic function at C_{3IV} and a transformable azido functionality located in ring II. Moreover, ring II has an orientation of functional groups (three axial and two equatorial) different from that of the parent deoxystreptamine ring (all groups equatorial) of neomycin. Furthermore, the anhydro neomycin **3** can now be produced efficiently and in high yield and provides an excellent building block for the preparation of a library of neomycin analogues bearing a range of functionality at the 3-position of the *ido* moiety. The biological activity of the modified neomycins reported here is currently being investigated.

Experimental Section

1,3,2′,6′,2‴,6‴-Hexa-*N*-(*tert*-butoxycarbonyl)-neomycin B (2). Triethylamine (TEA) (7 mL) and methanol (10 mL) were added to a stirred solution of neomycin B sulfate **1** (2 g, 2.2 mmol) in water (10 mL). Di-*tert*-butyl dicarbonate (5 g, 22 mmol) was then added, and the resultant reaction mixture was stirred at elevated temperature (55 °C, 16 h). Methanol was removed by evaporation and the residue was partitioned between ethyl acetate (200 mL) and water (100 mL). The aqueous layer was extracted with fresh ethyl acetate (2 × 50 mL) and the combined organic layers were dried (Na_2SO_4) and concentrated in vacuo. Purification by flash chromatography on silica (DCM/acetone 3:2) afforded **2** as an amorphous white solid (2.42 g, 90%). LRMS (ESI): calculated for $(M + Na^+)$ $C_{53}H_{94}N_6O_{25}$ 1237.61, found 1237.6.

General Procedure for the Per-acetylation. 2-Hepta-*O*-acetate.[18a] Compound **2** (25 mg, 0.02 mmol) was treated with acetic anhydride and pyridine (1:1, v/v, 2 mL) in the presence of a catalytic amount of *N*,*N*-dimethylaminopyridine (DMAP) at room temperature (4 h). The solvent was removed in vacuo and the residue was purified by flash chromatography (DCM/MeOH 98:2). This afforded **2**-hepta-*O*-acetate as a white amorphous solid (30 mg, 98%). ^{1}H NMR (400 MHz, pyridine-d_5, 363 K): δ 6.97 (1H, d, J = 8 Hz, NH_{II}), 6.79 (1H, d, J = 8 Hz, NH_{II}), 6.70 (1H, br dd, NH_{IV}), 6.57 (1H, br dd, NH_I), 6.48 (1H, d, J = 10 Hz, NH_I), 6.09 (1H, d, J = 9.5 Hz, NH_{IV}), 6.00 (1H, br s, H_{1I}), 5.64 (1H, dd, J = 9.5, 10.8 Hz, H_{3I}), 5.60 (1H, d, J = 2.5 Hz, $H_{1\ III}$), 5.52 (1H, dd, J = 3, 3 Hz, H_{3IV}), 5.41 (1H, dd, J = 9.5, 10 Hz, H_{4I}), 5.41 (1H, dd, J = 2.5, 5 Hz, $H_{2\ III}$), 5.24 (1H, ddd, J = 3, 2, 1 Hz, H_{4IV}), 5.23 (1H, dd, J = 10.3, 9.3 Hz, H_{6II}), 5.19 (1H, d, J = 2 Hz, H_{1IV}), 4.75 (2H, m, $H_{3\ III}$, $H_{5\ III}$), 4.66 (1H, dd, J = 11.9, 5.3 Hz, $H_{5III'}$), 4.61 (1H, ddd, J = 4, 4, 10 Hz, H_{5I}), 4.51−4.43 (3H, br m, H_{2I}, H_{5IV}, H_{4III}), 4.30 (1H, br m, H_{2IV}), 4.14−3.9 (4H, br m, H_{1II}, H_{3II}, H_{4II}, H_{5II}), 3.82 (1H, ddd, J = 7, 7, 14 Hz, H_{6IV}), 3.72 (2H, m, H_{6I}, $H_{6I'}$), 3.61 (1H, ddd, J = 5, 7, 14 Hz, $H_{6IV'}$), 2.41 (3H, s, -$OCOCH_3$), 2.40 (1H, m, H_{2II} eq), 2.35 (3H, s, -$OCOCH_3$), 2.29 (3H, s, -$OCOCH_3$), 2.15 (3H, s, -$OCOCH_3$), 2.14 (3H, s, -$OCOCH_3$), 2.10 (3H, s, -$OCOCH_3$), 2.04 (3H, s, -$OCOCH_3$), 2.0 (1H, m, H_{2II} ax), 1.68 (9H, s, -$COC(CH_3)_3$), 1.63 (9H, s, -$COC(CH_3)_3$), 1.58 (9H, s, -$COC(CH_3)_3$), 1.57 (18H, s, 2 × -$COC(CH_3)_3$), 1.56 (9H, s, -$COC(CH_3)_3$). ^{13}C {^{1}H} NMR (100 MHz, pyridine-d_5, 363 K): δ 171.5−169.3[43] (7 × OAc C═O), 157.5−156.7[43] (6 × Boc C═O), 108.0 (C_{1III}), 99.2 (C_{1IV}), 98.3 (C_{1I}), 83.5 (C_{5II}/C_{4II}), 79.9 (C_{4II}/C_{5II}), 79.9 (C_{4III}), 79.6−78.8[43] (6 × Boc C_q), 76.9 (C_{6II}), 76.5 (C_{3III}), 75.2 (C_{2III}), 73.7 (C_{5IV}), 73.0 (C_{3I}), 70.9 (C_{4I}), 70.0 (C_{3IV}), 69.8 (C_{5I}), 67.5 (C_{4IV}), 63.9 (C_{5III}), 54.3 (C_{2I}), 50.5 (C_{2IV}, C_{1II}, C_{3II}), 41.9 (C_{6I}), 41.4 (C_{6IV}), 35.4 (C_{2II}), 28.9−27.6[43] (6 × Boc $(CH_3)_3$), 21.8−20.7[43]

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(43) Recorded at room temperature.

(7 × OAc CH_3). LRMS (ESI): calculated for (M + Na^+) $C_{67}H_{108}N_6O_{32}$ 1531.6, found 1531.6.

3‴,4‴-Anhydro-1,3,2′,6′,2‴,6‴-hexa-*N*-(*tert*-butoxycarbonyl)-neomycin B (3)[18a] and 3‴,4‴-Anhydro-3,2′,6′,2‴,6‴-penta-*N*-(*tert*-butoxycarbonyl)-1,6-[*N*-(*tert*-butoxycarbonyl)epimino]-1-deamino-6-deoxyneomycin B (4). DIAD (330 μL, 1.6 mmol) was added slowly to an ice-cold solution of **2** (500 mg, 0.41 mmol) and TPP (430 mg, 1.6 mmol) in dry toluene (5 mL) under a dry N_2 atmosphere. The mixture was stirred at room temperature (18 h) and then evaporated to dryness. Column chromatography on silica (DCM/MeOH 97:3 → 95:5) afforded **4** (220 mg, 45%) [HRMS (ESI) calculated for (M + H^+) $C_{53}H_{90}N_6O_{23}$ 1179.61356, found 1179.60897] and **3** (160 mg, 33%) [HRMS (ESI) calculated for (M + Na^+) $C_{53}H_{92}N_6O_{24}$ 1219.60607, found 1219.60032] as a white amorphous solid.

4-Tetra-*O*-acetate. Per-acetylation of compound **4** was carried out exactly as described above for **2**-hepta-*O*-acetate, affording **4**-tetra-*O*-acetate as an amorphous white solid in quantitative yield. ^{1}H NMR (400 MHz, pyridine-d_5, 363 K): δ 6.79 (1H, br dd, NH_{IV}), 6.60 (1H, br dd, NH_I), 6.55 (1H, d, J = 9 Hz, NH_I), 6.26 (1H, d, J = 8.5 Hz, NH_{II}), 6.18 (1H, d, J = 9.5 Hz, NH_{IV}), 6.01 (1H, br s, H_{1III}), 5.69 (1H, d, J = 5.2 Hz, H_{2III}), 5.64 (1H, d, J = 3.8 Hz, H_{1I}), 5.54 (1H, dd, J = 9.4, 10.8 Hz, H_{3I}), 5.37 (1H, dd, J = 10, 9.5 Hz, H_{4I}), 4.84 (1H, d, J = 3.3 Hz, H_{1IV}), 4.80 (1H, dd, J = 5.3, 7.1 Hz, H_{3III}), 4.64 (2H, m, H_{5III}, $H_{5'III}$), 4.54 (1H, ddd, J = 4.6, 4.6, 7 Hz, H_{4III}), 4.50 (1H, m, H_{5II}), 4.48−4.37 (3H, m, H_{2IV} H_{2I},H_{5I}), 4.33 (1H, dd, J = 6.3,6.3 Hz, H_{5IV}), 4.12 (2H, m, H $_{3II,}$ H_{4II}), 3.76−3.67 (4H, m, H_{6IV}, $H_{6IV'}$, H_{6I}, $H_{6I'}$), 3.50 (1H, dd, J = 5.3, 3.8 Hz, H_{3IV}), 3.34 (1H, d, J = 3.8 Hz, H_{4IV}), 3.12 (1H, dd, J = 6.4, 4.7 H_{6II}), 2.80 (1H, dd, J = 5.1, 6.4 Hz, H_{1II}), 2.42 (3H, s, -$OCOCH_3$), 2.30 (1H, m, H_{2IIb}), 2.28 (3H, s, -$OCOCH_3$), 2.15 (3H, s, -$OCOCH_3$), 2.06 (3H, s, -$OCOCH_3$), 2.05 (1H, m, H_{2IIa}), 1.65 (9H, s, -$COC(CH_3)_3$), 1.61 (9H, s, -$COC(CH_3)_3$), 1.60 (9H, s, -$COC(CH_3)_3$), 1.58 (18H, s, 2 × -$COC(CH_3)_3$), 1.53 (9H, s, -$COC(CH_3)_3$). ^{13}C {^{1}H} NMR (100 MHz, pyridine-d_5, 363 K): δ 171.4−170.0[43] (4 × OAc C═O), 162.4 (Boc C═O), 159.5−154.4 (5 × Boc C═O), 105.0 (C_{1III}), 99.7 (C_{1IV}), 99.0 (C_{1I}), 81.6 (C_{4III}), 80.6−78.0[43] (6 × Boc C_q), 77.0 (C_{3III}), 76.6 (C_{4II}), 76.4 (C_{2III}), 74.1 (C_{5II}), 73.0 (C_{3I} ,C_{5IV}), 71.4 (C_{4I}), 70.8 (C_{5I}), 65.0 (C_{5III}), 54.9 (C_{2I}), 52.8 (C_{4IV}), 52.5 (C_{3IV}), 49.4 (C_{3II}), 46.6 (C_{2IV}), 43.7 (C_{6IV}), 42.6 (C_{6I}), 38.5 (C_{6II}), 38.1 (C_{1II}), 28.4 (C_{2II}), 29.3−28.0 (6 × Boc $(CH_3)_3$), 20.8−20.6 (4 × OAc CH_3). HRMS (ESI): calculated for (M + H^+) $C_{61}H_{98}N_6O_{27}$ 1347.65581, found 1347.65496.

3-Penta-*O*-acetate. Per-acetylation of compound **3** according to the general procedure afforded **3**-penta-*O*-acetate. ^{1}H NMR (400 MHz, pyridine-d_5, 363 K): δ 6.96 (1H, d, J = 9 Hz, NH_{II}), 6.77 (2H, br m, NH_{II}, NH_{IV}), 6.57 (1H, br dd, NH_I), 6.46 (1H, d, J = 10 Hz, NH_I), 6.16 (1H, d, J = 9.3 Hz, NH_{IV}), 6.02 (1H, br s, H_{1I}), 5.63 (1H, dd, J = 9.5, 10.6 Hz, H_{3I}), 5.57 (1H, d, J = 2.0 Hz, H_{1III}), 5.40 (1H, dd, J = 9.5, 10 Hz, H_{4I}), 5.35 (1H, dd, J = 2.4, 5.3 Hz, H_{2III}), 5.21 (1H, dd, J = 10.2, 9.4 Hz, H_{6II}), 4.71 (1H, m, $H_{5\ III}$), 4.68 (1H, d, J = 3.4 Hz, H_{1IV}), 4.66−4.55 (3H, br m, H_{3III}, H_{5I}, H_{5III}), 4.46 (1H, ddd, J = 10.4,10.4, 4.3 Hz, H_{2I}), 4.38 (2H, m, H_{2IV}, H_{4III}), 4.22 (1H, dd, J = 6, 6 Hz, H_{5IV}) 4.14−3.9 (4H, br m, H_{1II}, H_{3II}, H_{4II}, H_{5II}), 3.70 (4H, H_{6IV}, $H_{6IV'}$, H_{6I}, $H_{6I'}$) 3.48 (1H, dd, J = 5.4, 3.8 Hz, H_{3IV}), 3.30 (1H, d, J = 3.8 Hz, H_{4IV}), 2.45 (3H, s, -$OCOCH_3$), 2.40 (3H, s, -$OCOCH_3$), 2.40 (1H, m, H_{2II} eq), 2.28 (3H, s, -$OCOCH_3$), 2.15 (3H, s, -$OCOCH_3$), 2.09 (3H, s, -$OCOCH_3$), 2.0 (1H, m, H_{2II} ax), 1.68 (9H, s, -$COC(CH_3)_3$), 1.64 (9H, s, -$COC(CH_3)_3$), 1.60 (9H, s, -$COC(CH_3)_3$), 1.58 (18H, s, 2 × -$COC(CH_3)_3$), 1.56 (9H, s, -$COC(CH_3)_3$). ^{13}C {^{1}H} NMR (100 MHz, pyridine-d_5, 363 K): δ 171.3−170.0[43] (5 × OAc C═O), 157.2−156.3[43] (6 × Boc C═O), 108.8 (C_{1III}), 99.8 (C_{1IV}), 98.6 (C_{1I}), 83.3 (C_{5II}/C_{4II}), 80.2 (C_{5II}/C_{4II}), 80.4 (C_{4III}), 79.3−79.0[43] (6 × Boc C_q), 77.5 (C_{6II}), 75.8 (C_{3III}, C_{2III}), 73.4 (C_{3I}), 73.0 (C_{5IV}), 71.4 (C_{4I}), 70.2 (C_{5I}), 64.1 (C_{5III}),), 54.8 (C_{2I}), 52.7 (C_{4IV}), 52.5 (C_{3IV}), 51.1 (C_{1II}, C_{3II}), 46.4 (C_{2IV}), 43.7 (C_{6IV}), 42.3 (C_{6I}), 35.8 (C_{2II}), 29.2−29.0[43] (6 × Boc $(CH_3)_3$), 22.0−21.3[43] (5 × OAc CH_3). HRMS (ESI): calculated for (M + H^+) $C_{63}H_{102}N_6O_{29}$ 1407.67695, found 1407.67615.

Optimal Conditions for 3‴,4‴-Anhydro-1,3,2′,6′,2‴,6‴-hexa-*N*-(*tert*-butoxycarbonyl)-neomycin B (3). DIAD (170 μL, 0.82 mmol) was added slowly to a stirred ice-cold solution of **2** (500 mg, 0.41 mmol) and TPP (215 mg, 0.82 mmol) in dry toluene (3 mL) and the resultant reaction mixture was stirred at room temperature under a dry N_2 atmosphere (12 h). A second aliquot of a toluene solution of TPP (215 mg, 0.82 mmol, 2 mL) was then added, followed by the addition of a second portion of DIAD (170 μL, 0.82 mmol) and the reaction was stirred for a further 12 h prior to removal of the solvent in vacuo. Column chromatography of the residue on silica (DCM/MeOH 97:3 → 95:5) afforded **3** as an amorphous white solid (305 mg, 62%).

Optimal Condition for 3‴,4‴-Anhydro-3,2′,6′,2‴,6‴-penta-*N*-(*tert*-butoxycarbonyl)-1,6-[*N*-(*tert*-butoxycarbonyl)epimino]-1-deamino-6-deoxyneomycin B (4). DBAD (227 mg, 0.99 mmol) was added slowly, under a dry N_2 atmosphere, to a stirred ice-cold solution of **2** (200 mg, 0.16 mmol) and TPP (258 mg, 0.99 mmol) in dry toluene (3 mL). The reaction mixture was stirred overnight at room temperature and then concentrated in vacuo. Column chromatography of the resultant residue on silica (DCM/MeOH 97:3 → 95:5) afforded compound **4** as an amorphous white solid (107 mg, 55%).

3‴,4‴-Anhydro-1,3,2′,6′,2‴,6‴-hexa-*N*-(*tert*-butoxycarbonyl)-5″-deoxy-5″-(4-nitrobenzoyl)-neomycin B (5). DIAD (330 μL, 1.65 mmol) was added slowly to a cold solution of **2** (200 mg, 0.165 mmol), TPP (430 mg, 1.65 mmol), and 4-nitrobenzoic acid (330 mg, 2.0 mmol) in dry solvent (2 mL toluene + 1 mL THF) under a dry N_2 atmosphere. The mixture was stirred at room temperature (18 h) and then evaporated to dryness. Column chromatography on silica (DCM/MeOH 97:3 → 95:5) afforded **5** (187 mg, 85%) as a white amorphous solids. HRMS (ESI): calculated for (M + Na^+) $C_{60}H_{95}N_7O_{27}$ 1368.61736, found 1368.61214.

5-Tetra-*O*-acetate. Per-acetylation of compound **5,** according to the general procedure, provided **5**-tetra-*O*-acetate. ^{1}H NMR (400 MHz, pyridine-d_5, 363 K): δ 8.57 (2H, m, ArH $_{AA'}$), 8.49 (2H, m, ArH $_{XX'}$), 7.03 (1H, d, J = 8.4 Hz, NH_{II}), 6.84 (2H, br dd, NH_{IV}), 6.72 (1H, d, J = 7.9 Hz, NH_{II}), 6.52 (1H, d, J = 9.8 Hz, NH_I), 6.36 (2H, br dd, NH_I), 6.16 (1H, d, J = 9.6 Hz, NH_{IV}), 6.04 (1H, br s, H_{1I}), 5.61 (1H, d, J = 1.9 Hz, H_{1III}), 5.59 (1H, dd, J = 9.5, 10.6 Hz, H_{3I}), 5.47 (1H, dd, J = 1.6, 5.1 Hz, H_{2III}), 5.20 (2H, m, $H_{6II,}$ H_{4I}), 4.71 (1H, dd, J = 3.0, 12.0 Hz,H_{5III}), 4.80−4.78(2H, m, $H_{5III'}$, H_{3III}), 4.74 (1H, d, J = 3.3 Hz, H_{1IV}), 4.62−4.48 (2H, m, H_{5I}, H_{4III}), 4.46−4.35 (2H, m, H_{2I} ,H_{2IV}), 4.25 (1H, dd, J = 6, 6 Hz, H_{5IV}), 4.12−3.91 (4H, br m, H_{1II}, H_{3II}, H_{4II}, H_{5II}), 3.70−3.55 (4H, H_{6IV}, $H_{6IV'}$, H_{6I}, $H_{6I'}$), 3.48 (1H, dd, J = 5.2, 4 Hz, H_{3IV}), 3.32 (1H, d, J = 4 Hz, H_{4IV}), 2.48 (3H, s, -$OCOCH_3$), 2.42 (3H, s, -$OCOCH_3$), 2.30 (1H, m, H_{2II} eq), 2.09 (3H, s, -$OCOCH_3$), 2.05 (3H, s, -$OCOCH_3$), 1.96 (1H, m, H_{2II} ax), 1.67 (9H, s, -$COC(CH_3)_3$), 1.65 (9H, s, -$COC(CH_3)_3$), 1.58 (27H, m, 3 × -$COC(CH_3)_3$), 1.56 (9H, s, -$COC(CH_3)_3$). ^{13}C {^{1}H} NMR (100 MHz, pyridine-d_5, 363 K): δ 131.9 ($ArC_{AA'}$), 124.3 ($ArC_{XX'}$), 109.3 (C_{1III}), 99.6 (C_{1V}), 98.2 (C_{1I}), 84.3 (C_{5II}/C_{4II}), 80.0 (C_{4III}), 79.9 (C_{5II}/C_{4II}), 77.0 (C_{6II}), 75.8 (C_{3III}), 75.3 (C_{2III}), 73.2 (C_{3I}), 72.7 (C_{5IV}), 71.1 (C_{4I}), 69.9 (C_{5I}), 65.6 (C_{5III}), 54.5 (C_{2I}), 52.5 (C_{4IV}), 52.3 (C_{3IV}), 50.8 (C_{1II}, C_{6II}), 46.1 (C_{2IV}), 43.0−42.0 (C_{6I}, C_{6IV}), 35.4 (C_{2II}), 30−27 (6 × Boc $(CH_3)_3$), 22.0−19.0 (4 × OAc CH_3). LCMS (ESI): calculated for (M + Na^+) $C_{68}H_{103}N_7O_{31}$ 1536.66, found 1536.7.

3‴-Azido-1,3,2′,6′,2‴,6‴-hexa-*N*-(*tert*-butoxycarbonyl)-3‴-deoxy-neomycin B (6).[18a] NaN_3 (130 mg, 2 mmol) was added to a solution of **3** (400 mg, 0.33 mmol) in DMF (10 mL) and the mixture was stirred at room temperature (2 d). The reaction mixture was then diluted with ethyl acetate (200 mL) and washed with water (2 × 100 mL). The ethyl acetate layer was dried (Na_2SO_4), concentrated in vacuo, and purified by flash chromatography (DCM/MeOH 97:3) yielding **6** as a white amorphous solid (352 mg, 85%).

IR (KBr disk) 2109.9 cm^{-1} (N_3). HRMS (ESI): calculated for (M + Na^+) $C_{53}H_{93}N_9O_{24}$ 1262.62311, found 1262.62303.

6-Hexa-*O*-acetate. Per-acetylation of **6**, using the general procedure above, afforded **6**-hexa-*O*-acetate as a white amorphous solid in quantitative yield. ^{1}H NMR (400 MHz, pyridine-d_5, 363 K): δ 6.95 (1H, d, J = 8 Hz, NH_{II}), 6.78 (2H, br dd, NH_{II}, NH_{IV}), 6.58 (1H, br dd, NH_I), 6.43 (2H, m, NH_I, NH_{IV}), 5.97 (1H, br s, H_{1I}), 5.63 (1H, dd, J = 9.5, 10.7 Hz, H_{3I}), 5.59 (1H, d, J = 2.8 Hz, H_{1III}), 5.40 (2H, m, H_{4I}, H_{2III}), 5.22 (1H, dd, J = 10, 9 Hz, H_{6II}), 5.14 (1H, d, J = 2.3 Hz, H_{1IV}), 5.13 (1H, ddd, J = 3, 2, 1 Hz, H_{4IV}), 4.73 (2H, m, H_{5III}, H_{3III}), 4.63 (1H, dd, J = 11.8, 5.2 Hz, $H_{5III'}$), 4.59 (1H, ddd, J = 4, 4, 10 Hz, H_{5I}), 4.49−4.42 (3H, br m, H_{2I}, H_{3IV}, H_{4III}), 4.37 (1H, ddd, J = 2.6, 5, 7 Hz, H_{5IV}), 4.24 (1H, br m, H_{2IV}), 4.10 (1H, dd, J = 8.5,10 Hz, H_{4II}), 4.07−3.97 (2H, br m, $H_{1II,}$ H_{3II}), 3.97 (1H, dd, J = 9, 9 Hz, H_{5II}), 3.83 (1H, ddd, J = 7, 7, 14 Hz, H_{6IV}), 3.71 (2H, m, H_{6I}, $H_{6I'}$), 3.60 (1H, ddd, J = 5, 6.5, 14 Hz, $H_{6IV'}$), 2.39 (3H, s, -$OCOCH_3$), 2.39 (1H, m, H_{2II} eq), 2.34 (3H, s, -$OCOCH_3$), 2.28 (3H, s, -$OCOCH_3$), 2.14 (3H, s, -$OCOCH_3$), 2.13 (3H, s, -$OCOCH_3$), 2.09 (3H, s, -$OCOCH_3$), 2.01 (1H, m, H_{2II} ax), 1.67 (9H, s, -$COC(CH_3)_3$), 1.61 (9H, s, -$COC(CH_3)_3$), 1.58 (18H, s, 2 × -$COC(CH_3)_3$), 1.57 (9H, s, -$COC(CH_3)_3$), 1.56 (9H, s, -$COC(CH_3)_3$). ^{13}C {^{1}H} NMR (100 MHz, pyridine-d_5, 363 K): δ 171.3−170.2[43] (6 × OAc C═O), 157.4−156.4[43] (6 × Boc C═O), 107.9 (C_{1III}), 98.9 (C_{1IV}), 98.7 (C_{1I}), 83.6 (C_{5II}), 80.4 (C_{4II}), 80.3 (C_{4III}), 79.8−78.8[43] (6 × Boc C_q), 77.2 (C_{6II}), 76.9 (C_{3III}), 75.5 (C_{2III}), 73.3 (C_{5IV}, C_{3I}), 71.3 (C_{4I}), 70.0 (C_{5I}), 68.0 (C_{4IV}), 64.1 (C_{5III}), 60.8 (C_{3IV}), 54.7 (C_{2I}), 51.3 (C_{2IV}), 50.9 (C_{1II}, C_{3II}), 42.2 (C_{6I}), 41.6 (C_{6IV}), 35.4 (C_{2II}), 29.1−28.9[43] (6 × Boc $(CH_3)_3$), 21.9−21.1[43] (6 × OAc CH_3). HRMS (ESI): calculated for (M + Na^+) $C_{65}H_{105}N_9O_{30}$ 1514.68650, found 1514.68447.

Prop-2-ynyl 4-Nitrobenzoate (7). Propargyl alcohol (50 μL, 0.86 mmol) was added to a stirred solution of 4-nitrobenzoyl chloride (100 mg, 0.5 mmol) in pyridine (1 mL) and the solution was stirred at room temperature (4 h). Aqueous HCl acid solution (1 N, 12 mL) was added to the reaction mixture and the resultant mixture was extracted with ethyl acetate (50 mL). The organic layer was washed with $Na_2CO_{3(sat)}$ solution (15 mL) and water (3 × 15 mL) and then dried (Na_2SO_4). Concentration in vacuo afforded prop-2-ynyl 4-nitrobenzoate **7** (105 mg, 94%) as a brown solid which, in general, was used in succeeding steps without further purification. Recrystallization of the crude product from hexane afforded **7** as fine white needles, mp 90−92 °C {lit.[44] mp 89−90 °C}. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 8.36 (4H, m), 4.95 (2H, d, J = 2.5 Hz) 2.49 (1H, t, J = 2.5 Hz). ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$, 298 K): δ 164.5 (C═O), 150.6 (ipso), 135.0 (ipso), 131.2 (Ar), 123.8 (Ar), 77.2(Cq), 75.9 (CH), 53.5 (-OCH_2).

6,3′,4′,2″,5″,4‴-Hexa-*O*-acetyl-1,3,2′,6′,2‴,6‴-hexa-*N*-(*tert*-butoxycarbonyl)-3‴-deoxy-3‴-[4-(4-nitrobenzoyloxymethyl)-[1,2,3]-triazol-1-yl)-neomycin B (8-hexa-*O*-acetate). A mixture of *tert*-butanol and water (1:1, 2 mL) was added to the mixture of **6**-hexa-*O*-acetate (50 mg 0.04 mmol), prop-2-ynyl 4-nitrobenzoate, **7** (10 mg, 0.05 mmol), $CuSO_4$·$5H_2O$ (1 mg), and copper powder (30 mg). The resultant reaction mixture was stirred vigorously at room temperature (18 h) and then diluted with ethyl acetate (20 mL). Residual copper powder was removed by filtration and the filtrate was dried (Na_2SO_4) and concentrated in vacuo. Purification by flash chromatography on silica (DCM/MeOH 98:2) afforded **8**-hexa-*O*-acetate (46 mg, 79%) as an amorphous white solid. ^{1}H NMR (400 MHz, pyridine-d_5, 363 K): δ 8.40 (1H, s, ArH, triazole), 8.28 (2H, m, $ArH_{XX'}$), 8.21 (2H, m, $ArH_{AA'}$), 6.94 (1H, d, J = 8.5 Hz, NH_{IV}), 6.79 (3H, m, 2 × NH_{II}, NH_{IV}), 6.61 (1H, br dd, NH_I), 6.40 (1H, d, J = 10 Hz, NH_I), 5.98 (1H, br s, H_{1I}), 5.75 (1H, dd, J = 3, 3 Hz, H_{4IV}), 5.67 (2H, s,OCH_2), 5.63 (1H, dd, J = 9.5, 10.5 Hz, H_{3I}), 5.60 (1H, d, J = 2.5 Hz, H_{1III}), 5.57 (1H, d, J = 3 Hz, H_{1IV}), 5.44 (1H, dd, J = 2.5, 5 Hz, H_{2III}), 5.41 (1H, dd, J = 3.5, 6 Hz, H_{3IV}), 5.40 (1H, dd, J = 9.5, 9.5 Hz, H_{4I}), 5.21 (1H, dd, J = 10, 10 Hz, H_{6II}), 4.90 (1H, ddd, J = 2.5, 6.7, 6.7 Hz, H_{5IV}), 4.83−4.75 (3H, m, H_{2IV}, H_{5III}, H_{3III}), 4.63 (1H, dd, J = 11.8, 5 Hz, $H_{5III'}$), 4.60 (1H, ddd, J = 4, 4, 10 Hz, H_{5I}), 4.54 (1H, ddd, J = 3.8, 5.6, 5.6 Hz, H_{4III}), 4.46 (1H, ddd, J = 3.5, 10, 10 Hz, H_{2I}), 4.13−3.91 (5H, m, $H_{1II,}$ H_{3II} $H_{4II,}$ H_{5II} ,H_{6IV}), 3.71 (2H, m, $H_{6I,}$ $H_{6I'}$), 3.67 (1H, m, $H_{6IV'}$), 2.39 (3H, s, -$OCOCH_3$), 2.37 (1H, m, H_{2II} eq), 2.28 (3H, s, -$OCOCH_3$), 2.27 (3H, s, -$OCOCH_3$), 2.14 (6H, m, 2 × -$OCOCH_3$), 2.10 (3H, s, -$OCOCH_3$), 2.0 (1H, m, H_{2II} ax), 1.67 (9H, s, -$COC(CH_3)_3$), 1.62 (9H, s, -$COC(CH_3)_3$), 1.58 (9H, s, -$COC(CH_3)_3$), 1.55 (18H, s, 2 × -$COC(CH_3)_3$), 1.51 (9H, s, -$COC(CH_3)_3$). ^{13}C {^{1}H} NMR (100 MHz, pyridine-d_5, 363 K): δ 171.4−170.0[43] (6 × OAc C═O), 165.0[43] (Ar_{ipso}), 157.4−156.4[43] (6 × Boc C═O), 151.34[43] (Ar_{ipso}), 143.15[43] ($triazole_{ipso}$), 131.3 ($ArC_{AA'}$), 126.27 (CH, triazole), 124.0 ($ArC_{XX'}$), 108.1 (C_{1III}), 99.0 (C_{1IV}), 98.7 (C_{1I}), 83.5 (C_{5II}), 80.4 ($C_{4II,}$ C_{4III}), 79.9−78.8[43] (6 × Boc C_q), 77.0 (C_{6II}), 76.5 (C_{3III}), 75.4 (C_{2III}), 73.8 (C_{5IV}), 73.3 (C_{3I}), 71.5 (C_{4I}), 70.2 (C_{5I}), 69.9 (C_{4IV}), 64.4 (C_{5III}), 61.7 (C_{3IV}), 59.6 (OCH_2, linker), 54.7 (C_{2I}), 52.5 (C_{2IV}), 50.9 (C_{1II}, C_{3II}), 42.3 (C_{6I}), 41.9 (C_{6IV}), 35.5 (C_{2II}), 29.4−28.4[43] (6 × Boc $(CH_3)_3$), 22.0−21.0[43] (6 × OAc CH_3). HRMS (ESI): calculated for (M + Na^+) $C_{75}H_{112}N_{10}O_{34}$ 1719.72401, found 1719.72346.

Prop-2-ynyl Dodecanate (9).[45] Propargyl alcohol (60 μL, 1 mmol) was added to a stirred solution of lauric acid (100 mg, 0.5 mmol) and DCC (120 mg, 0.58 mmol) in dry DCM (3 mL), and the resultant mixture was stirred at room temperature (4 h). The reaction mixture was then diluted with acetone and filtered. The filtrate was concentrated, diluted with ethyl acetate (50 mL), and washed with saturated aqueous Na_2CO_3 solution followed by water (3 × 15 mL).The organic phase was dried (Na_2SO_4) and evaporated, leaving prop-2-ynyl dodecanate **9** as a light yellow oil (110 mg, 92%). The crude product was found to be of sufficient purity for use in the next step without need for further purification. ^{1}H NMR (400 MHz, $CDCl_{3,}$ 298 K): δ 4.65 (2H, d, J = 2.5 Hz) 2.44 (1H, t, J = 2.5 Hz), 2.36 (2H, t, J = 7.5 Hz), 1.62 (2H, m), 1.34−1.18 (16H, m), 0.86 (3H, m). ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$, 298 K): δ 173.2 (C═O), 78.0 (q C, -C≡CH), 74.9(CH), 52.0 (-OCH_2), 34.2, 32.1, 29.8−29.3, 25.0, 22.9, 14.3.

1,3,2′,6′,2‴,6‴-Hexa-*N*-(*tert*-butoxycarbonyl)-3‴-deoxy-3‴-(4-dodecanoyloxymethyl-[1,2,3]-triazol-1-yl)-neomycin B (13). A mixture of *tert*-butanol and water (1:1, 2 mL) was added to a mixture of the 3‴-azidoneomycin **6** (50 mg 0.04 mmol), prop-2-ynyl dodecanate **9** (12 mg, 0.05 mmol), $CuSO_4$·$5H_2O$ (1 mg), and copper powder (30 mg). The reaction mixture was stirred vigorously at room temperature (18 h) and then diluted with ethyl acetate (20 mL). Copper powder was removed by filtration and the filtrate was dried (Na_2SO_4) and concentrated in vacuo. Purification by flash chromatography on silica (DCM/MeOH 95:5) afforded **13** (43 mg, 72%) as an amorphous white solid. HRMS (ESI): calculated for (M + Na^+) $C_{68}H_{119}N_9O_{26}$ 1500.81639, found 1500.81614.

13-Hexa-*O*-acetate. Per-acetylation of compound **13** using the general method above afforded **13**-hexa-*O*-acetate as an amorphous white solid. ^{1}H NMR (400 MHz, pyridine-d_5, 363 K): δ 8.25 (1H, s, ArH, triazole), 6.94 (1H, d, J = 8.5 Hz, NH_{IV}), 6.78 (3H, m, 2 × NH_{II}, NH_{IV}), 6.62 (1H, br dd, NH_I), 6.46 (1H, d, J = 9.6 Hz, NH_I), 5.98 (1H, br s, H_{1I}), 5.71 (1H, dd, J = 3, 3 Hz, H_{4IV}), 5.64 (1H, dd, J = 9.3, 10.5 Hz, H_{3I}), 5.61 (1H, d, J = 2.4 Hz, H_{1III}), 5.58 (1H, d, J = 2.7 Hz, H_{1IV}), 5.45 (1H, dd, J = 2.6, 5 Hz, H_{2III}), 5.43 (2H, s, OCH_2), 5.40 (1H, dd, J = 9.6, 9.6 Hz, H_{4I}), 5.38 (1H, dd, J = 3.5, 5.7 Hz, H_{3IV}), 5.21 (1H, dd, J = 9.5, 10.4 Hz, H_{6II}), 4.88 (1H, ddd, J = 2.5, 6.5, 6.5 Hz, H_{5IV}), 4.82−4.77 (3H, m, $H_{2IV,}$ $H_{5III,}$ H_{3III}), 4.64 (1H, dd, J = 11.8, 5.3 Hz, $H_{5III'}$), 4.59 (1H, ddd, J = 4, 4, 10 Hz, H_{5I}), 4.54 (1H, ddd, J = 3.8, 5.6, 5.6 Hz, H_{4III}), 4.46 (1H, ddd, J = 3.7, 10.3, 10.3 Hz, H_{2I}), 4.12−3.88 (5H, m, H_{1II}, H_{3II}, H_{4II}, H_{5II}, H_{6IV}), 3.71 (2H, m, H_{6I}, $H_{6I'}$), 3.67 (1H, m, $H_{6IV'}$), 2.41 (2H, t, J = 7.4 Hz, -$COCH_2$, H_D*), 2.40 (3H, s, -$OCOCH_3$), 2.38 (1H, m, H_{2II} eq), 2.32 (3H, s, -$OCOCH_3$), 2.28 (3H, s, -$OCOCH_3$), 2.15 (6H, m, 2 × -$OCOCH_3$), 2.10 (3H, s, -$OCOCH_3$), 2.0 (1H, m, H_{2II} ax), 1.74 (2H, m, H_D*), 1.67 (9H, s,

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-COC(CH_3)$_3$), 1.62 (9H, s, -COC(CH_3)$_3$), 1.58 (9H, s, -COC(CH_3)$_3$), 1.56 (9H, s, -COC(CH_3)$_3$), 1.55 (9H, s, -COC(CH_3)$_3$), 1.51 (9H, s, -COC(CH_3)$_3$), 1.47−1.32 (16H, m, H_D*), 0.97 (3H, distorted t, H_D*). ^{13}C {^{1}H} NMR (100 MHz, pyridine-d_5, 363 K): δ 125.25 (CH, triazole), 107.8 (C_{1III}), 98.8 (C_{1IV}), 98.4 (C_{1I}), 83.3 (C_{5II}), 80.3 (C_{4III}) ,80.0 (C_{4II}), 76.7 (C_{6II}), 76.4 (C_{3III}), 75.22 (C_{2III}), 75.5 (C_{5IV}), 73.1 (C_{3I}), 71.1 (C_{4I}), 69.9 (C_{5I}), 69.8 (C_{4IV}), 64.1 (C_{5III}), 61.3 (C_{3IV}), 58.0 (OCH_2, linker), 54.5 (C_{2I}), 52.2 (C_{2IV}), 50.8 (C_{1II}, C_{3II}), 42.0 (C_{6I}), 41.6 (C_{6IV}), 35.7 (C_{2II}), 34.4 (C_D*, -CH_2-CO-), 25.5 (C_D*), 30.0−27.0 (6 × Boc (CH_3)$_3$), 22.7−20.0 (6 × OAc CH_3), 14.0 (C_D*, -CH_3). HRMS (ESI): calculated for (M + Na^+) $C_{80}H_{131}N_9O_{32}$ 1752.87978, found 1752.87986. $_D$* = dodecanoyl

3β-Hydroxypregna-5,17(20)-diene-21-oic Acid (12).[46] Powdered KOH (70 mg) was added to a stirred solution of ethyl 3β-hydroxypregna-5,17(20)-diene-21-oate **11** (200 mg, 0.55 mmol) in isopropanol (3 mL) and the reaction mixture was refluxed for 2 h. The mixture was then cooled to room temperature and evaporated to dryness. The residue was partitioned between ethyl acetate and 1 M aqueous HCl acid solution. The ethyl acetate layer was washed with water, dried (Na_2SO_4), and concentrated in vacuo. Flash column chromatography (DCM/MeOH 98:2) afforded product 3β-hydroxypregna-5,17(20)-diene-21-oic acid **12** (130 mg, 72%) as a white crystalline solid, mp 253−255 °C {lit.[46] mp 252−254 °C}. ^{1}H NMR (400 MHz, pyridine-d_5, 298 K): δ 6.00 (1H, dd, J = 2.5, 2.5 Hz, H20), 5.43 (1H, m, H6), 3.86 (1H, dddd, J = 4.1, 6.1, 10.5, 10.5 Hz, H3), 3.18−3.12 (2H, m, H16a,b), 2.64−2.58 (2H, m, H4a,b), 2.11 (1H, m, H2a), 2.01 (1H, m, H7a), 1.88−1.78 (3H, br m, H12a, H1a, H2b), 1.73 (1H, m, H15a), 1.65−1.54 (2H, br m, H11a, H7b), 1.54−1.40 (2H, br m, H8, H11b), 1.35−1.23 (2H, br m, H15b, H12b), 1.12 (1H, ,H1b), 1.06−0.95 (2H, m, H14, H9), 1.06 (3H, s, CH_3, H19), 0.81 (3H, s, CH_3, H18). ^{13}C{^{1}H} NMR (100 MHz, pyridine-d_5, 298 K): δ 175.5 (C17), 170.3 (C═O, C21), 142.5 (C5), 121.5 (C6), 111.0 (C20), 71.7 (C3), 54.6 (C14), 51.1 (C9), 46.6 (C13), 44.0 (C4), 38.3 (C1), 37.5 (C10), 36.1 (C12), 33.1 (C2), 32.5 (C7), 32.4 (C8), 31.3 (C16), 25.2 (C15), 21.8(C11), 20.1 (C19), 18.9 (C18).

Prop-2-ynyl 3β-Hydroxypregna-5,17 (20)-diene-21-oate (10). DIAD (65 μL, 0.34 mmol) was added slowly to a stirred solution of 3β-hydroxypregna-5,17(20)-diene-21-oic acid **12** (75 mg, 0.23 mmol), TPP (90 mg, 0.34 mmol), and anhydrous propargyl alcohol (30 μL, 0.44 mmol) in dry THF (1 mL) under N_2 atmosphere and the mixture was stirred at room temperature (12 h). The solvent was removed in vacuo and the residue was purified by column chromatography (DCM/MeCN 95:5) affording ester **10** (65 mg, 75%) as white solid, mp 158−159 °C . ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 5.61 (1H, dd, J = 2.5, 2.5 Hz, H20), 5.37 (1H, m, H6), 4.71 (2H, d, J = 2.5, OCH_2, H1′), 3.54 (1H, dddd, J = 4, 5, 11, 11 Hz, H3), 2.90−2.83 (2H, m, H16a,b), 2.47 (1H, t, J = 2.5 Hz, H3′) 2.36−2.20 (2H, m, H4a,b), 2.03 (1H, m, H7a), 1.92−1.77 (4H, br m, H12a, H15a, H1a, H2a), 1.74−1.46 (5H, br m, H11a,b, H8, H2b, H7b), 1.44−1.22 (2H, br m, H15b, H12b), 1.16−0.96 (3H, m, H1b, H14, H9), 1.0 (3H, s, CH_3, H19), 0.85 (3H, s, CH_3, H18); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$, 298 K): δ 178.5 (C17), 166.5 (C═O, C21), 141.0 (C5), 121.5 (C6), 107.8 (C20), 78.5 (C 2′), 74.6 (C3′), 71.9 (C3), 54.0 (C14), 51.4 (C1′), 50.4 (C9), 46.5 (C13), 42.4 (C4), 37.4 (C1), 36.8 (C10), 35.3 (C12), 31.9−31.8 (C7, C2, C8), 30.8 (C16), 24.7 (C15), 21.2 (C11), 19.6 (C19), 18.4 (C18). HRMS (EI): calculated for (M^+) $C_{24}H_{32}O_3$ 368.23514, found 368.23541.

1,3,2′,6′,2‴,6‴-Hexa-*N*-(*tert*-butoxycarbonyl)-3‴-deoxy-3‴-[4-(3β-hydroxypregna-5,17(20)-diene-21-oyloxymethyl)- [1,2,3]-triazol-1-yl]-neomycin B (14). The 3‴-azidoneomycin **6** (50 mg 0.04 mmol) was treated with ester **10** (18 mg, 0.05 mmol), according to the procedure described for the preparation of compound **13**. Purification by flash chromatography on silica (DCM/MeOH 97:3) afforded the product **14** as an amorphous white solid (48 mg, 75%). HRMS (ESI): calculated for (M + Na^+) $C_{77}H_{125}N_9O_{27}$ 1630.85826, found 1630.86152.

14-Hepta-*O*-acetate. Per-acetylation of compound **14** according to the general procedure above afforded **14**-hexa-*O*-acetate as an amorphous white solid in quantitative yield. ^{1}H NMR (400 MHz, pyridine-d_5, 363 K): δ 8.29 (1H, s, ArH, triazole), 6.95 (1H, d, J = 8.4 Hz, NH_{IV}), 6.84−6.74 (3H, m, 2 × NH_{II}, NH_{IV}), 6.63 (1H, br dd, NH_I), 6.46 (1H, d, J = 10.5 Hz, NH_I), 5.98 (1H, br s, H_{1I}), 5.77(1H, t, J = 2.5 Hz ,H_{21S}*), 5.72 (1H, dd, J = 3, 3 Hz, H_{4IV}), 5.64 (1H, dd, J = 9.4, 10.7 Hz, H_{3I}), 5.62 (1H, d, J = 2.4 Hz, H_{1III}), 5.60 (1H, d, J = 2.7 Hz, H_{1IV}), 5.50 (2H, s, OCH_2), 5.45-(1H, m, H_{6S}*), 5.43 (1H, m, H_{2III}), 5.41 (1H, dd, J = 9.7, 9.7 Hz, H_{4I}), 5.39 (1H, dd, J = 3.5, 5.5 Hz, H_{3IV}), 5.22 (1H, dd, J = 9.4, 10.2 Hz, H_{6II}), 4.89 (1H, ddd, J = 2.6, 6.7, 6.7 Hz, H_{5IV}), 4.85−4.76 (4H, m, H_{2IV}, $H_{5III,}$ $H_{3III,}$ H_{3S}*), 4.65 (1H, dd, J = 11.7, 5.0 Hz, $H_{5III'}$), 4.62 (1H, ddd, J = 3.9, 3.9, 8.0 Hz, H_{5I}), 4.55 (1H, ddd, J = 3.8, 5.3, 5.3 Hz, H_{4III}), 4.47 (1H, ddd, J = 4.0, 10.5, 10.5 Hz, H_{2I}), 4.14−3.88 (5H, m, $H_{1II,}$ H_{3II} $H_{4II,}$ H_{5II} ,H_{6IV}), 3.71 (2H, m, $H_{6I,}$ $H_{6I'}$), 3.68 (1H, m, $H_{6IV'}$),3.03 (2H, m, $H_{16a,bS}$*), 2.53 (2H, m, $H_{4a,bS}$), 2.40 (3H, s, -OCOCH_3), 2.38 (1H, m, H_{2II} eq), 2.31 (3H, s, -OCOCH_3), 2.28 (3H, s, -OCOCH_3), 2.14 (6H, m, 2 × -OCOCH_3), 2.10 (6H, s, 2 × -OCOCH_3), 2.0 (1H, m, H_{2II} ax), 1.67 (9H, s, -COC(CH_3)$_3$), 1.62 (9H, s, -COC(CH_3)$_3$), 1.58 (9H, s, -COC-(CH_3)$_3$), 1.57−1.53 (27H, m, 3 × -COC(CH_3)$_3$), 1.10 (3H, s, H_{19S}*, CH_3), 0.88 (3H, s,H_{18S}*, CH_3). ^{13}C {^{1}H} NMR (100 MHz, pyridine-d_5, 363 K): δ 177.6[43] (C_{17S}*), 171.4−170.1[43] (7 × OAc C═O), 167.2 (C═O, C_{21S}*) ,157.5−156.3[43] (6 × Boc C═O), 144.1[43] (ipso, triazole), 140.5[43] (C_{5S}*), 125.3 (CH, triazole), 122.7 (C_{6S}*) 109.3 (C_{20S}*) 108.0 (C_{1III}), 99.0 (C_{1IV}), 98.6 (C_{1I}), 83.4 (C_{5II}), 80.5 (C_{4III}) ,80.3 (C_{4II}), 80−78.7[43] (6 × Boc C_q), 77.0 (C_{6II}), 76.6 (C_{3III}), 75.5 (C_{2III}), 74.4 (C_{3S}*), 73.3 (C_{3I}), 72.6 (C_{5IV}), 71.2 (C_{4I}), 71.1 (C_{5I}), 70.0 (C_{4IV}), 64.4 (C_{5III}), 61.5 (C_{3IV}), 57.7 (OCH_2, linker), 54.8 (C_{2I}), 52.6 (C_{2IV}) 52.0−48.0 (C_{1II}, C_{3II}), 41.9 (C_{6I}), 41.73 (C_{6IV}), 38.7 (C_{4S}*), 35.67 (C_{2II}), 31.2 (C_{16S}*), 29.3−28.7[43] (6 × Boc (CH_3)$_3$), 21.8−21.0[43] (7 × OAc CH_3), 19.77(C_{19S}*), 18.58(C_{18S}*). HRMS (ESI): calculated for (M + Na^+) $C_{91}H_{139}N_9O_{34}$ 1924.93221, found 1924.93161. $_S$* = steroid

1,3,2′,6′,2‴,6‴-Hexa-*N*-(*tert*-butoxycarbonyl)-5‴-*O*-(2,4,6-triisopropylbenzenesulfonyl)-neomycin B (15).[30] A solution of **2** (1 g, 0.82 mmol) and excess 2,4,6-triisopropylbenzenesulfonyl chloride (7 g, 23 mmol) in dry pyridine (20 mL) was stirred at room temperature (18 h). Pyridine was removed in vacuo by coevaporation with toluene. The crude residue was then dissolved in ethyl acetate (200 mL) and washed with water (2 × 100 mL). The aqueous layers were combined and extracted with ethyl acetate (2 × 100 mL). The combined organic layers were dried (Na_2SO_4), concentrated in vacuo, and subjected to flash chromatography. Unreacted 2,4,6-triisopropylbenzenesulfonyl chloride (6 g, mp identical with that of authentic sample) was recovered using DCM as eluent. The residue (1.2 g) was eluted from the silica column (DCM/MeOH 5:1) and then rechromatographed (DCM/MeOH 98:2 → 95:5) affording **15** as an amorphous white solid (0.75 g, 61%). ^{1}H NMR (400 MHz, methanol-d_4, 298 K): δ 7.33 (s, 2H), 5.52 (br s, 1H), 5.22 (br s, 1H), 4.99 (br s, 1H), 4.39 (m, 1H), 4.30 (m, 2H), 4.19 (m, 4H), 3.91 (m, 1H), 3.82 (m, 1H), 3.77 (m, 2H), 3.62 (m, 1H), 3.54 (m, 4H), 3.47−3.36 (m, 4H), 3.24 (m, 2H), 2.96 (m, 2H), 1.96 (m, 1H), 1.51−1.40 (m, 54H), 1.31 (m, 18H). LRMS (ESI): calculated for (M + Na^+) $C_{68}H_{116}N_6O_{27}S$ 1504, found 1504.

5‴-Azido-1,3,2′,6′,2‴,6‴-hexa-*N*-(*tert*-butoxycarbonyl)-5‴-deoxy-neomycin B (16). A solution of **15** (500 mg, 0.3 mmol) and NaN_3 (150 mg, 2.3 mmol) in DMF (5 mL) was stirred at elevated temperature (100 °C, 8 h). After cooling to room temperature the reaction mixture was diluted with ethyl acetate (100 mL) and washed with water (2 × 20 mL). The ethyl acetate layer was dried (Na_2SO_4) and evaporated, yielding **16** (365 mg, 98%) as a white amorphous solid. The crude product was of sufficient purity to use in the next step without need for further purification. IR (KBr disk) 2106.3 cm^{-1} (N_3). HRMS (ESI): calculated for (M + Na^+) $C_{53}H_{93}N_9O_{24}$ 1262.62311, found 1262.62835.

(46) Sondheimer, F.; Mancera, O.; Urquiza, M.; Rosenkranz, G. *J. Am. Chem. Soc.* **1955**, *77*, 4145−4149.

16-Hexa-*O*-acetate. Per-acetylation of the 5″-azido neomycin B **16** according to the general method afforded **16**-hexa-*O*-acetate as an amorphous white solid, in quantitative yield. ^{1}H NMR (400 MHz, pyridine-d_5, 363 K): δ 6.96 (1H, d, J = 8.7 Hz, NH_{II}), 6.80 (1H, d, J = 8.7 Hz, NH_{II}), 6.70 (1H, br dd, NH_{IV}), 6.60 (1H, br dd, NH_I), 6.49 (1H, d, J = 10 Hz, NH_I), 6.12 (1H, d, J = 10 Hz, NH_{IV}), 5.99 (1H, br s, H_{1I}), 5.64 (1H, dd, J = 9.4, 10.7 Hz, H_{3I}), 5.61 (1H, d, J = 2 Hz, H_{1III}), 5.51 (1H, dd, J = 3, 3 Hz, H_{3IV}), 5.40 (1H, dd, J = 9.5, 10.1 Hz, H_{4I}), 5.39 (1H, dd, J = 2.5, 5 Hz, H_{2III}), 5.25 (1H, m, H_{6II}), 5.23 (1H, m, H_{4IV}), 5.18 (1H, d, J = 2.12 Hz, H_{1IV}), 4.76 (1H, dd, J = 6.6, 5.3 Hz, H_{3III}), 4.60 (1H, ddd, J = 4, 4,10 Hz, H_{5I}), 4.51 (1H, ddd, J = 3.8, 10.5, 10.5 Hz, H_{2I}), 4.46 (1H, ddd, J = 1.8, 6.7, 6.7 Hz, H_{5IV}), 4.32 (1H, ddd, J = 3.8, 4.6, 6.6 Hz, H_{4III}), 4.30 (1H, br m, H_{2IV}), 4.13 (1H, dd, J = 8.6, 10 Hz, H_{4II}), 4.10−4.01 (2H, m, H_{3II}, H_{1II}), 3.98 (1H, m, H_{5II}), 3.97 (1H, dd, J = 8.6, 8.6 Hz, H_{5III}), 3.82 (1H, ddd, J = 6.6, 6.6, 13.7 Hz, H_{6IV}), 3.76 (1H, dd, J = 8.6, 8.6 Hz, $H_{5'III}$), 3.70 (2H, m, $H_{6I,}$ H_{6I}), 3.62 (1H, ddd, J = 4.8, 6.8, 13.7 Hz, $H_{6'IV}$), 2.40 (3H, s, -$OCOCH_3$), 2.40 (1H, m, H_{2II}eq), 2.33 (3H, s, -$OCOCH_3$), 2.14 (3H, s, -$OCOCH_3$), 2.13 (3H, s, -$OCOCH_3$), 2.08 (3H, s, -$OCOCH_3$), 2.04 (3H, s, -$OCOCH_3$), 2.0 (1H, m, H_{2II}ax), 1.66 (9H, s, -$COC(CH_3)_3$), 1.62 (9H, s, -$COC(CH_3)_3$), 1.57 (18H, s, 2 × -$COC(CH_3)_3$), 1.57 (9H, s, -$COC(CH_3)_3$), 1.55 (9H, s, -$COC(CH_3)_3$). ^{13}C {^{1}H} NMR (100 MHz, pyridine-d_5, 298 K): δ 171.5−169.2 (6 × OAc C═O), 157.6−156.6 (6 × Boc C═O), 108.4 (C_{1III}), 99.0 (C_{1IV}), 98.4 (C_{1I}), 83.5 (C_{5II}/C_{4II}), 80.2 (C_{4II}/C_{5II}), 79.5 (C_{4III}), 79.0−78.7 (6 × Boc C_q), 77.3 (C_{6II}), 76.1 (C_{3III}), 74.9 (C_{2III}), 73.8 (C_{5IV}), 73.3 (C_{3I}), 70.5 (C_{4I}), 69.9 (C_{3IV}), 69.8 (C_{5I}), 66.8 (C_{4IV}), 54.1 (C_{2I}), 51.9 (C_{5III}), 50.2 (C_{2IV}, C_{1II}, C_{3II}), 41.3 (C_{6I}), 40.8 (C_{6IV}), 35.9 (C_{2II}), 29.5−28.5 (6 × Boc $(CH_3)_3$), 22.2−21.0[43] (6 × OAc CH_3). LRMS (ESI): calculated for (M + Na^+) $C_{65}H_{105}N_9O_{30}$ 1514.7, found 1514.7.

1,3,2′,6′,2‴,6‴-Hexa-*N*-(*tert*-butoxycarbonyl)-5″-deoxy-5″-[4-(4-nitrobenzoyloxymethyl)-[1,2,3]triazol-1-yl]-neomycin B (17). The synthetic procedure for compound **13** was repeated using the 5″-azido neomycin **16** (50 mg 0.04 mmol) and ester **7** (10 mg, 0.05 mmol). Purification of the crude product by flash chromatography on silica (DCM/MeOH 97:3) afforded **17** (45 mg, 75%) as an amorphous white solid. HRMS (ESI): calculated for (M + Na^+) $C_{63}H_{100}N_{10}O_{28}$ 1467.66062, found 1467.65659.

17-Hexa-*O*-acetate. Per-acetylation of **17**, according to the general procedure, afforded **17**-hexa-*O*-acetate, as an amorphous white solid in quantitative yield. ^{1}H NMR (400 MHz, pyridine-d_5, 363 K): δ 8.46 (1H, s, ArH, triazole), 8.37 (2H, m, $ArH_{XX'}$), 8.36 (2H, m, $ArH_{AA'}$), 7.04 (1H, d, NH_{II}), 6.9 (1H, br dd, NH_{IV}), 6.83 (1H, d, J = 8 Hz, NH_{II}), 6.69 (2H, m, 2 × NH_I), 6.08 (1H, d, J = 9.8 Hz, NH_{IV}), 5.95 (1H, br s, H_{1I}), 5.90 (2H, s, OCH_2), 5.68 (1H, dd, J = 9.5, 10.5 Hz, H_{3I}), 5.64 (1H, d, J = 2.0 Hz, H_{1III}), 5.52 (1H, dd, J = 3, 3 Hz, H_{3IV}), 5.49 (1H, dd, J = 9.5, 9.5 Hz, H_{4I}), 5.46 (1H, dd, J = 2.0, 5.5 Hz, H_{2III}), 5.27−5.15 (4H, m, $H_{4IV'}$, H_{1IV}, H_{6II}, H_{5III}), 4.94 (1H, dd, J = 6.5, 14.5 Hz, $H_{5III'}$), 4.79 (1H, dd, J = 6.6, 5.5 Hz, H_{3III}), 4.68 (1H, ddd, J = 3.8, 3.8, 10 Hz, H_{5I}), 4.63−4.53 (2H, m, $H_{4III,}$ H_{2I}), 4.51 (1H, ddd, J = 2.0, 6.5, 6.5 Hz, H_{5IV}), 4.31 (1H, m, H_{2IV}), 4.09−4.0 (4H, m H_{1II}, H_{3II}, H_{4II}, H_{5II}), 3.86 (1H, m, H_{6IV}), 3.75 (2H, m, H_{6I}, $H_{6I'}$), 3.73 (1H, m, $H_{6IV'}$), 2.45 (3H, s, -$OCOCH_3$), 2.4 (1H, m, H_{2II} eq), 2.35 (3H, s, -$OCOCH_3$), 2.17 (3H, s, -$OCOCH_3$), 2.15 (3H, s, -$OCOCH_3$), 2.07 (3H, s, -$OCOCH_3$), 2.05 (3H, s, -$OCOCH_3$), 2.0 (1H, m, H_{2II} ax), 1.69 (9H, s, -$COC(CH_3)_3$), 1.57 (45H, m, 5 × -$COC(CH_3)_3$). ^{13}C {^{1}H} NMR (100 MHz, pyridine-d_5, 363 K): δ 171.4−169.0[43] (6 × OAc C═O), 165.4[43](ipso, Ar) 157.5−156.4[43] (6 × Boc C═O), 151.4[43](ipso, Ar) 143.3[43](ipso, triazole) 131.5 ($ArC_{AA'}$), 126.3 (CH, triazole), 124.5 ($ArC_{XX'}$), 108.1 (C_{1III}), 99.6 (C_{1IV}), 99.0 (C_{1I}), 82.9 (C_{5II}), 80.6 (C_{4III}), 80.3 (C_{4II}), 79.9−78.8[43] (6 × Boc C_q), 77.8 (C_{3III}), 76.9 (C_{6II}), 74.9 (C_{2III}), 74.3 (C_{5IV}), 73.1 (C_{3I}), 71.2 (C_{4I}), 70.5 (C_{3IV}), 70.4 (C_{5I}), 67.8 (C_{4IV}), 59.9 (OCH_2, linker), 54.7 (C_{2I}), 52.7 (C_{5III}), 50.7 (C_{2IV}, C_{1II}, C_{3II}), 42.2 (C_{6I}), 41.5 (C_{6IV}), 35.8 (C_{2II}), 29.2−28.7[43] (6 × Boc $(CH_3)_3$), 22.2−21.8[43] (6 × OAc CH_3). HRMS (ESI): calculated for (M + H^+) $C_{75}H_{112}N_{10}O_{34}$ 1697.74207, found 1697.74399.

1,3,2′,6′,2‴,6‴-Hexa-*N*-(*tert*-butoxycarbonyl)-5″-deoxy-5″(4-dodecanoyloxymethyl-[1,2,3]triazol-1-yl)-neomycin B (18). 5″-Azido neomycin **16** (50 mg 0.04 mmol) was treated with ester **9** as per the method described for the synthesis of compound **13**. Chromatographic purification of the residue on silica (DCM/MeOH 96:4) afforded **18** (42 mg, 71%) as an amorphous white solid. HRMS (ESI): calculated for (M + Na^+) $C_{68}H_{119}N_9O_{26}$ 1500.81639, found 1500.81215.

18-Hexa-*O*-acetate. Per-acetylation of **18**, according to the general procedure, afforded **18**-hexa-*O*-acetate as an amorphous white solid in quantitative yield. ^{1}H NMR (400 MHz, pyridine-d_5, 363 K): δ 8.34 (1H, s, ArH, triazole), 7.01 (1H, d, J = 8.5 Hz NH_{II}), 6.89 (1H, br dd, NH_{IV}), 6.80 (1H, d, J = 7.7 Hz, NH_{II}), 6.67 (2H, m, 2 × NH_I), 6.06 (1H, d, J = 9.8 Hz, NH_{IV}), 5.92 (1H, d, J = 3.5 Hz H_{1I}), 5.67 (1H, dd, J = 9.3, 10.6 Hz, H_{3I}), 5.65 (2H, s, OCH_2), 5.63 (1H, d, J = 2.0 Hz, H_{1III}), 5.52 (1H, dd, J = 3, 3 Hz, H_{3IV}), 5.49 (1H, dd, J = 9.8, 9.8 Hz, H_{4I}), 5.45 (1H, dd, J = 2.2, 5.3 Hz, H_{2III}), 5.25 (1H, br dd, H_{4IV}), 5.20 (1H, dd, J = 10, 10 Hz, H_{6II}), 5.20 (1H, d, J = 2 Hz, H_{1IV}), 5.13 (1H, dd, J = 3.8, 14.5 Hz, H_{5III}), 4.93 (1H, dd, J = 6.5, 14.5 Hz, $H_{5III'}$), 4.79 (1H, dd, J = 6.8, 5.0 Hz, H_{3III}), 4.68 (1H, ddd, J = 3.6, 3.6, 9.6 Hz, H_{5I}), 4.58 (2H, m, $H_{4III,}$ H_{2I}), 4.51 (1H, ddd, J = 1.5, 6.5, 6.5 Hz, H_{5IV}), 4.30 (1H, m, H_{2IV}), 4.14−3.9 (4H, m $H_{1II,}$ H_{3II} $H_{4II,}$ H_{5II}), 3.86 (1H, m, H_{6IV}), 3.75 (3H, m, H_{6I}, $H_{6I'}$, $H_{6IV'}$), 2.57 (2H, t, J = 7.5 Hz, -$COCH_2$, H_D),2.46 (3H, s, -$OCOCH_3$), 2.4 (1H, m, H_{2II} eq), 2.35 (3H, s, -$OCOCH_3$), 2.19 (3H, s, -$OCOCH_3$), 2.17 (3H, s, -$OCOCH_3$), 2.10 (3H, s, -$OCOCH_3$), 2.06 (3H, s, -$OCOCH_3$), 2.0 (1H, m, H_{2II} ax), 1.83 (2H, m, H_D), 1.70 (9H, s, -$COC(CH_3)_3$), 1.61 (18H, s, 2 × -$COC(CH_3)_3$), 1.58 (27H, s, 3 × -$COC(CH_3)_3$), 1.46−1.36 (16H, m, H_D), 0.98 (3H, distorted t, H_D). ^{13}C {^{1}H} NMR (100 MHz, pyridine-d_5, 363 K): δ 125.8 (CH, triazole), 108.0 (C_{1III}), 99.5 (C_{1IV}), 98.6 (C_{1I}), 82.9 (C_{5II}), 80.6 (C_{4III}), 80.3 (C_{4II}), 77.5 (C_{3III}), 76.8 (C_{6II}), 74.6 (C_{2III}), 74.2 (C_{5IV}), 73.1 (C_{3I}), 70.8 (C_{4I}), 70.3 (C_{3IV}), 70.3 (C_{5I}), 67.2 (C_{4IV}), 58.4 (OCH_2, linker), 54.5 (C_{2I}), 52.3 (C_{5III}), 50.9 (C_{1II}, C_{3II}), 50.6 (C_{2IV}), 42.1 (C_{6I}), 41.7 (C_{6IV}), 35.7 (C_{2II}), 34.8 ($C_{D,}$ -CH_2-CO-), 25.5 (C_D) 31.0−28.0 (6 × Boc $(CH_3)_3$), 23.0−19.0 (6 × OAc CH_3) ,14.0 (C_D, -CH_3). HRMS (ESI): calculated for (M + Na^+) $C_{80}H_{131}N_9O_{32}$ 1752.87978, found 1752.88177.

1,3,2′,6′,2‴,6‴-Hexa-*N*-(*tert*-butoxycarbonyl)-5″-deoxy-5″-[4-(3β-hydroxypregna-5,17(20)-diene-21-oyl-oxymethyl)-[1,2,3]triazol-1-yl]-neomycin B (19). The azido neomycin **16** (50 mg 0.04 mmol) was treated with ester **10** (18 mg, 0.05 mmol) in accordance with the procedure described for the preparation of compound 13. Purification by flash chromatography on silica (DCM/MeOH, 97:3) afforded **19** (45 mg, 69%) as an amorphous white solid. HRMS (ESI): calculated for (M + Na^+) $C_{77}H_{125}N_9O_{27}$ 1630.85826, found 1630.86482.

19-Hepta-*O*-acetate. Per-acetylation of **19**, according to the general procedure, provided **19**-hepta-*O*-acetate. ^{1}H NMR (400 MHz, pyridine-d_5, 363 K): δ 8.38 (1H, s, ArH, triazole), 7.01 (1H, d, J = 9.5 Hz NH_{II}), 6.88 (1H, br dd, NH_{IV}), 6.78 (1H, d, J = 8.5 Hz, NH_{II}), 6.71 (1H, br dd, NH_I), 6.63 (1H, d, J = 9 Hz, NH_I), 6.05 (1H, d, J = 9.4 Hz, NH_{IV}), 5.92 (1H, d, J = 3.5 Hz H_{1I}), 5.86 (1H, t, J = 2.4, H_{21S}), 5.73 (2H, s, OCH_2), 5.68 (1H, dd, J = 9.4, 10.4 Hz, H_{3I}), 5.64 (1H, d, J = 2.0 Hz, H_{1III}), 5.52 (1H, dd, J = 3, 3 Hz, H_{3IV}), 5.49 (1H, dd, J = 10, 10 Hz, H_{4I}), 5.86 (1H, m, H_{6S}), 5.45 (1H, m, H_{2III}), 5.25 (1H, br dd, H_{4IV}), 5.20 (1H, dd, J = 9.5, 9.5 Hz, H_{6II}), 5.20 (1H, d, J = 2.3 Hz, H_{1IV}), 5.11 (1H, dd, J = 4, 14.2 Hz, H_{5III}), 4.98 (1H, dd, J = 5.8, 14.2 Hz, $H_{5III'}$), 4.83 (1H, m, H_{3S}), 4.79 (1H, dd, J = 6.5, 5.3 Hz, H_{3III}), 4.67 (1H, ddd, J = 4, 4, 10 Hz, H_{5I}), 4.61−4.52 (2H, m, $H_{4III,}$ H_{2I}), 4.49 (1H, ddd, J = 1.6, 6.5, 6.5 Hz, H_{5IV}), 4.30 (1H, m, H_{2IV}), 4.17−3.97 (4H, m H_{1II}, H_{3II}, $H_{4II,}$ H_{5II}), 3.87 (1H, m, H_{6IV}), 3.77 (1H, m, H_{6I}), 3.72 (2H, m, $H_{6I'}$ $H_{6IV'}$), 3.10 (2H, m, H_{16abS}), 2.53 (2H, m, H_{4abS}), 2.44 (3H, s, -$OCOCH_3$), 2.38 (1H, m, H_{2II} eq), 2.35 (3H, s, -$OCOCH_3$), 2.19 (3H, s, -$OCOCH_3$), 2.16 (3H, s, -$OCOCH_3$), 2.09 (6H, s, 2 × -$OCOCH_3$), 2.06 (3H, s, -$OCOCH_3$), 2.0 (1H, m, H_{2II} ax), 1.69

(9H, s, -COC(CH$_3$)$_3$), 1.61 (9H, s, -COC(CH$_3$)$_3$), 1.60 (9H, s, -COC(CH$_3$)$_3$), 1.58 (9H, s, -COC(CH$_3$)$_3$), 1.57 (9H, s, -COC(CH$_3$)$_3$), 1.56 (9H, s, -COC(CH$_3$)$_3$), 1.10 (3H, s, -CH$_3$ H$_{19S}$), 0.91 (3H, s, -CH$_3$ H$_{18S}$). ^{13}C {^{1}H} NMR (100 MHz, pyridine-d_5, 363 K): δ 177.7[43] (C$_{17S}$), 171.4−169.0[43] (7 × OAc C═O), 167.2[43] (C═O, C$_{21S}$), 157.5−156.4[43] (6 × Boc C═O), 144.0[43](ipso, triazole) 140.5[43] (C$_{5S}$), 122.7 (CH, triazole), 109.4 (C$_{20S}$) 108.2 (C$_{1III}$), 99.8 (C$_{1IV}$), 98.7 (C$_{1I}$), 82.8 (C$_{5II}$), 80.8 (C$_{4III}$) ,80.6 (C$_{4II}$), 79.9−78.8[43] (6 × Boc C$_q$), 77.8 (C$_{3III}$), 77.0 (C$_{6II}$), 74.9 (C$_{2III}$), 74.4 (C$_{5IV}$, C$_{3S}$), 73.3 (C$_{3I}$), 70.8 (C$_{4I}$), 70.4 (C$_{3IV}$, C$_{5I}$), 67.9 (C$_{4IV}$) ,57.9 (OCH$_2$, linker), 54.8 (C$_{2I}$), 52.4 (C$_{5III}$), 50.7 (C$_{2IV}$), 50.0 (C$_{1II}$, C$_{3II}$), 42.4 (C$_{6I}$), 41.9 (C$_{6IV}$), 38.9 (C$_{4S}$), 35.9 (C$_{2II}$), 31.3 (C$_{16S}$), 29.2−28.7[43] (6 × Boc (CH$_3$)$_3$), 22.0−20.8[43] (7 × OAc CH$_3$) 19.7 (C$_{19S}$), 18.8 (C$_{18S}$). HRMS (ESI): calculated for (M + Na$^+$) $C_{91}H_{139}N_9O_{34}$ 1924.93221, found 1924.92960.

1,3‴-Diazido-6-*tert*-butoxycarbonylamino-3,2′,6′,2‴,6‴-penta-*N*-(*tert*-butoxycarbonyl)-1-deamino-6,3‴-di-deoxyneomycin B (20), 3‴-Azido-3,2′,6′,2‴,6‴-penta-*N*-(*tert*-butoxy-carbonyl)-1,6-[*N*-(*tert*-butoxycarbonyl)epimino]-1-deamino-6,3‴-di-deoxyneomycin B (21), and 3′,4′,2″,5″-Tetra-*O*-acetyl-1-azido-6-*tert*-butoxycarbonylamino-3‴,4‴-anhydro-3,6,2′,6′,2‴,6‴-penta-*N*-(*tert*-butoxycarbonyl)-1-deamino-6-deoxyneomycin B (22). NaN_3 (11 mg, 0.17 mmol) was added to a solution of **4** (200 mg, 0.17 mmol) in DMF (5 mL) and the resultant mixture was stirred at 50 °C (24 h). The mixture was then diluted with ethyl acetate (50 mL) and washed with water (2 × 25 mL). The ethyl acetate layer was dried (Na_2SO_4) and concentrated in vacuo. An aliquot of the crude reaction (40 mg) was per-acetylated via the general procedure outlined above and subjected to semipreparative HPLC (MeCN/H_2O 75:25) to isolate **4**-tetra-*O*-acetate (12 mg), **20**-penta-*O*-acetate (5 mg) [LRMS (ESI) calculated for (M + Na$^+$) $C_{63}H_{102}N_{12}O_{28}$ 1497.68, found 1497.94], **21**-penta-*O*-acetate (15 mg) [LRMS (ESI) calculated for (M + Na$^+$) $C_{63}H_{101}N_9O_{28}$ 1454.67, found 1454.92], and **22**-tetra-*O*-acetate (3 mg) [LRMS (ESI) calculated for (M + Na$^+$) $C_{61}H_{99}N_9O_{27}$ 1412.65, found 1412.7]. Careful column chromatography of remaining crude reaction mixture (≈160 mg) on silica column (DCM/MeOH 98:2 → 95:5) afforded **20** (16 mg, ∼10%) [HRMS (ESI) calculated for (M + Na$^+$) $C_{53}H_{92}N_{12}O_{23}$ 1287.62960, found 1287.62327] and **21** (20 mg) [HRMS (ESI) calculated for (M + Na$^+$) $C_{53}H_{91}N_9O_{23}$ 1244.61255, found 1244.615243] as white amorphous solids along with some mixed fraction containing compounds **4**, **21**, and **22**. These fractions were combined and concentrated and the resulting mixture (120 mg) was used for the subsequent reactions.

NMR Spectroscopic Data for 20-Penta-*O*-acetate. ^{1}H NMR (400 MHz, pyridine-d_5, 363 K): δ 6.89 (1H, d, J = 7.8 Hz, NH$_{II}$), 6.83 (1H, br dd, NH$_{IV}$), 6.77 (1H, d, J = 8 Hz, NH$_I$), 6.51 (1H, br dd, NH$_I$), 6.44 (2H, m, NH$_{II}$, NH$_{IV}$), 5.70 (1H, dd, J = 9.4, 10.7 Hz, H$_{3I}$), 5.55 (1H, d, J = 4 Hz, H$_{1I}$), 5.51 (2H, m, H$_{1III}$,H$_{2III}$), 5.42 (1H, dd, J = 9.4, 10 Hz, H$_{4I}$), 5.15 (2H, m, H$_{1IV,}$ H$_{4IV}$), 4.78 (1H, dd, J = 6, 5 Hz, H$_{3III}$), 4.68 (2H, m, H$_{5III}$, H$_{5III'}$), 4.56 (1H, m, H$_{4III}$), 4.52 (2H, m, H$_{4II,}$ H$_{5II}$), 4.48 (1H, m, H$_{3IV}$), 4.46 (1H, m, H$_{2I}$), 4.44 (1H, m, H$_{5I}$), 4.38 (3H, m, H$_{3II}$, H$_{6II}$, H$_{5IV}$), 4.25 (1H, m, H$_{2IV}$), 4.15 (1H, ddd, J = 3.9, 9.4,9.4 Hz, H$_{1II}$), 3.78 (1H, m, H$_{6IV}$), 3.73 (2H, m, H$_{6I}$, H$_{6I'}$), 3.62 (1H, m, H$_{6IV'}$), 2.36 (1H, ddd, dd, J = 4.3,4.3,14 Hz, H$_{2II}$), 2.24 (3H, s, -OCOCH$_3$), 2.23 (3H, s, -OCOCH$_3$), 2.14 (3H, s, -OCOCH$_3$), 2.13 (3H, s, -OCOCH$_3$), 2.05 (3H, s, -OCOCH$_3$), 2.12 (1H, m, H$_{2II'}$), 1.63 (9H, s, -COC(CH$_3$)$_3$), 1.61 (9H, s, -COC(CH$_3$)$_3$), 1.59 (18H, s, 2 × -COC(CH$_3$)$_3$), 1.56 (18H, s, 2 × -COC(CH$_3$)$_3$). ^{13}C {^{1}H} NMR (100 MHz, pyridine-d_5, 363 K): δ 171.0−169.5[43] (5 × OAc C═O), 157.0−155.5[43] (6 × Boc C═O), 108.0 (C$_{1III}$), 99.1 (C$_{1IV}$, C$_{1I}$),81.2 (C$_{4III}$), 79.4−78.4[43] (6 × Boc C$_q$), 78.8 (C$_{4II}$), 77.2 (C$_{3III}$), 76.5 (C$_{5II}$), 75.8 (C$_{2III}$), 73.2 (C$_{5IV}$), 72.5 (C$_{3I}$), 70.9 (C$_{4I}$), 70.5 (C$_{5I}$), 68.2 (C$_{4IV}$), 64.6 (C$_{5III}$), 60.6 (C$_{3IV}$), 57.3 (C$_{1II}$) 54.6 (C$_{2I}$), 53.6 (C$_{6II}$), 51.3 (C$_{2IV}$), 50.0 (C$_{3II}$), 42.0 (C$_{6I}$), 41.3 (C$_{6IV}$), 32.0 (C$_{2II}$), 28.7−28.3[43] (6 × Boc (CH$_3$)$_3$), 21.0−20.3[43] (5 × OAc CH$_3$).

NMR Spectroscopic Data for 21-Penta-*O*-acetate. ^{1}H NMR (400 MHz, pyridine-d_5, 363 K): δ 6.79 (1H,br dd, NH$_{IV}$), 6.60 (1H, br dd, NH$_I$), 6.54 (1H, d, J = 8.5 Hz, NH$_I$), 6.44 (1H, d, J = 9 Hz, NH$_{IV}$), 6.18 (1H, m, NH$_{II}$), 6.05 (1H, s, H$_{1III}$), 5.76 (1H, d, J = 5.2 Hz, H$_{2II}$), 5.66 (1H, dd, J = 9.4, 10.8 Hz, H$_{3I}$), 5.64 (1H, d, J = 4 Hz ,H$_{1I}$), 5.39 (1H, dd, J = 9.4, 10 Hz, H$_{4I}$), 5.34 (1H, d, J = 2.4 Hz, H$_{1IV}$), 5.17 (1H, m, H$_{4IV}$) 4.95 (1H, dd, J = 7, 5.3 Hz, H$_{3III}$), 4.71 (2H, m, H$_{5III}$, H$_{5III'}$), 4.0 (1H,, ddd, J = 4.4, 4.4, 6.7 Hz, H$_{4III}$), 4.55−4.38 (5H, m, H$_{5II}$, H$_{5IV}$, H$_{3IV}$, H$_{2I}$, H$_{5I}$), 4.30 (1H, m, H$_{2IV}$), 4.14 (2H, m, H$_{3II}$, H$_{4II}$), 3.84 (1H, m, H$_{6IV}$), 3.76−3.60 (3H, m, H$_{6I}$, H$_{6I'}$, H$_{6IV'}$), 3.17 (1H, dd, J = 6, 4.6 Hz, H$_{6II}$), 2.80 (1H, dd, J = 6, 6 Hz, H$_{1II}$), 2.4 (1H, m, H$_{2IIb}$), 2.32 (3H, s, -OCOCH$_3$), 2.31 (3H, s, -OCOCH$_3$), 2.16 (3H, s, -OCOCH$_3$), 2.15 (3H, s, -OCOCH$_3$), 2.09 (3H, s, -OCOCH$_3$), 2.12 (1H, m, H$_{2IIa}$), 1.66 (9H, s, -COC(CH$_3$)$_3$), 1.61 (9H, s, -COC(CH$_3$)$_3$), 1.60 (9H, s, -COC(CH$_3$)$_3$), 1.59 (9H, s, -COC(CH$_3$)$_3$), 1.58 (9H, s, -COC(CH$_3$)$_3$), 1.53 (9H, s, -COC(CH$_3$)$_3$). ^{13}C {^{1}H} NMR (100 MHz, pyridine-d_5, 363 K): δ 171.5−170.0[43] (5 × OAc C═O),162.4[43] (Boc C═O), 157.4−156.4[43] (5 × Boc C═O), 104.6 (C$_{1III}$), 99.0 (C$_{1IV}$,C$_{1I}$), 81.34 (C$_{4III}$), 79.7−79.0[43] (6 × Boc C$_q$), 77.9 (C$_{3III}$), 76.9 (C$_{4II}$), 76.5 (C$_{2III}$), 74.0 (C$_{5II}$), 73.2 (C$_{5IV}$), 72.9 (C$_{3I}$), 71.1 (C$_{4I}$), 70.7 (C$_{5I}$), 70.2 (C$_{4IV}$), 64.9 (C$_{5III}$), 60.8 (C$_{3IV}$), 54.9 (C$_{2I}$), 51.4 (C$_{2IV}$), 49.4 (C$_{3II}$), 42.2 (C$_{6I}$), 41.8 (C$_{6IV}$), 38.5 (C$_{6II}$), 37.8 (C$_{1II}$), 28.6 (C$_{2II}$), 29.2−28.6[43] (6 × Boc (CH$_3$)$_3$), 21.7−21.0[43] (5 × OAc CH$_3$).

NMR Spectroscopic Data for 22-Tetra-*O*-acetate: ^{1}H NMR (400 MHz, pyridine-d_5, 363 K): δ 6.87 (2H, br m, NH$_{II,}$ NH$_{IV}$), 6.79 (1H, b, J = 7.8 Hz NH$_{II}$), 6.53 (1H,br dd, NH$_I$), 6.40 (1H, m, NH$_I$), 6.21 (1H, d, J = 9.0 Hz, NH$_{IV}$), 5.70 (1H, dd, J = 9.4, 10.8 Hz, H$_{3I}$), 5.57 (1H, d, J = 3.4 Hz, H$_{1I}$), 5.53 (1H, d,, J = 3.4 Hz, H$_{1III}$), 5.45 (1H, dd, J = 2.2, 5.5 Hz, H$_{2III}$), 5.35 (1H, dd, J = 9.6, 9.6 Hz, H$_{4I}$), 4.71 (1H, d, J = 3.2 Hz, H$_{1IV}$), 4.69−4.57 (3H,m, H$_{3III}$, H$_{5III}$, H$_{5'III}$), 4.53−4.35 (8H, m, H$_{3II}$, H$_{4II}$, H$_{5II}$, H$_{6II}$, H$_{2I}$, H$_{5I}$, H$_{4III}$, H$_{2IV}$), 4.23 (1H, dd, J = 6, 6 Hz, H$_{5IV}$), 4.14 (1H, ddd, J = 4.3, 9.3, 9.4 Hz, H$_{1II}$), 3.78 (1H, m, H$_{6I}$), 3.76−3.67 (3H, m, H$_{6IV}$, H$_{6I'}$,H$_{6IV'}$), 3.50 (1H, dd, J = 5, 4 Hz, H$_{3IV}$), 3.33 (1H, d, J = 4 Hz, H$_{4IV}$), 2.4 (1H, m, H$_{2II}$ eq), 2.24 (3H, s, -OCOCH$_3$), 2.14 (6H, s, 2 × -OCOCH$_3$), 2.05 (3H, s, -OCOCH$_3$), 2.0 (1H, m, H$_{2II}$ ax), 1.65 (9H, s, -COC(CH$_3$)$_3$), 1.64 (9H, s, -COC(CH$_3$)$_3$), 1.63 (9H, s, -COC(CH$_3$)$_3$), 1.62 (18H, s, 2 × -COC(CH$_3$)$_3$), 1.60 (9H, s, -COC(CH$_3$)$_3$).

3′,4′,2″,5″,4‴-Penta-*O*-acetyl-3,2′,6′,2‴,6‴-penta-*N*-(*tert*-butoxycarbonyl)-1,6-[*N*-(*tert*-butoxycarbonyl)epimino]-1-deamino-6,3‴-dideoxy3‴-[4-(4-nitrobenzoyloxymethyl)-[1,2,3]triazol-1-yl]-neomycin B (23) and 3′,4′,2″,5″-Tetra-*O*-acetyl-3‴,4‴-anhydro-6-*tert*-butoxycarbonylamino-3,6,2′,6′,2‴,6‴-penta-*N*-(*tert*-butoxycarbonyl)-1-deamino-6-deoxy-1-[4-(4-nitrobenzoyloxymethyl)-[1,2,3]triazol-1-yl]-neomycin B (24). A partially purified mixture of **4**, **21**, and **22** was subjected to per-acetylation according to the general procedure. The mixture was then treated with prop-2-ynyl 4-nitrobenzoate **7** according to the procedure outlined in the preparation of compound **8**. Column chromatographic separation afforded **23**-penta-*O*-acetate (44 mg) [HRMS (ESI) calculated for (M + H$^+$) $C_{73}H_{108}N_{10}O_{32}$ 1637.72094, found 1637.72108] and **24**-tetra-*O*-acetate (20 mg) [HRMS (ESI) calculated for (M + Na$^+$) $C_{71}H_{106}N_{10}O_{31}$ 1617.69232, found 1617.69440] as amorphous white solids.

NMR Spectroscopic Data for 23-Penta-*O*-acetate. ^{1}H NMR (400 MHz, pyridine-d_5, 363 K): δ 8.41 (1H, s, ArH, triazole), 8.28 (2H, m, ArH$_{XX'}$), 8.22 (2H, m, ArH$_{AA'}$), 6.81 (1H, br dd, NH$_{IV}$), 6.74 (1H, d, J = 7 Hz, NH$_{IV}$), 6.60 (1H, br dd, NH$_I$), 6.55 (1H, d, J = 8.6 Hz, NH$_I$), 6.61 (1H, d, J = 9 Hz, NH$_{II}$), 6.02 (1H, br s, H$_{1III}$), 5.81 (1H, dd, J = 3, 3 Hz, H$_{4IV}$), 5.75 (1H d, J = 6.2 Hz, H$_{2III}$), 5.69 (2H, s, -OCH$_2$), 5.65 (1H, dd, J = 9.5, 10.6 Hz, H$_{3I}$), 5.65 (2H, m, H$_{1IV}$, H$_{1I}$), 5.42 (1H, m, H$_{3IV}$), 5.37 (1H, dd, J = 9.5, 10 Hz, H$_{4I}$), 4.98 (1H, dd, J = 3.5, 6.2 Hz, H$_{3III}$), 4.96 (1H, m, H$_{5IV}$), 4.86 (1H, ddd, J = 2.8, 6.5, 9 Hz, H$_{2IV}$), 4.80−4.66 (3H, m, H$_{5III}$, H$_{5'III}$, H$_{4III}$), 4.52 (1H, dd, J = 4.4, 4.4 Hz, H$_{5II}$), 4.48−4.38 (2H, m, H$_{2I}$, H$_{5I}$), 4.19−4.07 (2H, m, H$_{3II}$, H$_{4II}$), 3.97 (2H, m, H$_{6I,}$ H$_{6IV}$), 3.77−3.63 (2H, m, H$_{6'I}$, H$_{6'IV}$), 3.14 (1H, dd, J = 6.3, 5 Hz, H$_{6II}$), 2.78 (1H, dd, J = 5, 5 Hz, H$_{1II}$), 2.39 (1H, m, H$_{2IIb}$), 2.31 (3H, s, -OCOCH$_3$), 2.27 (3H, s, -OCOCH$_3$), 2.15 (6H, m, 2 ×

-OCOCH$_3$), 2.08 (3H, s, -OCOCH$_3$), 2.11 (1H, m, H$_{2IIa}$), 1.67 (9H, s, -COC(CH$_3$)$_3$), 1.62 (9H, s, -COC(CH$_3$)$_3$), 1.58 (9H, s, -COC-(CH$_3$)$_3$), 1.55 (18H, s, 2 × -COC(CH$_3$)$_3$), 1.51 (9H, s, -COC(CH$_3$)$_3$). ^{13}C {^{1}H} NMR (100 MHz, pyridine-d_5, 363 K): δ 171.0−169.0[43] (5 × OAc C═O), 165.0[43](Ar$_{ipso}$) 162.4[43] (Boc C═O), 157.5−156.2[43] (5 × Boc C═O), 151.35[43] (Ar$_{ipso}$) 143.13[43] (triazole$_{ipso}$) 131.3 (ArC$_{AA'}$), 125.58 (CH, triazole), 124.0 (ArC$_{XX'}$), 104.7 (C$_{1III}$), 99.1 (C$_{1IV}$, C$_{1I}$), 81.9 (C$_{4III}$), 79.9−78.9[43] (6 × Boc C$_q$), 77.5 (C$_{3III}$),-76.6 (C$_{4II}$), 76.34 (C$_{2III}$), 73.57 (C$_{5II}$), 73.56 (C$_{5IV}$), 72.7 (C$_{3I}$), 71.2 (C$_{4I}$), 70.8 (C$_{5I}$), 70.3 (C$_{4IV}$), 65.0 (C$_{5III}$), 61.5 (C$_{3IV}$), 59.57 (OCH$_2$, linker), 54.6 (C$_{2I}$), 52.7 (C$_{2IV}$) 49.2 (C$_{3II}$), 42.1 (C$_{6I}$), 41.9 (C$_{6IV}$), 38.1 (C$_{6II}$), 37.88 (C$_{1II}$), 28.0 (C$_{2II}$), 29.3−28.7[43] (6 × Boc (CH$_3$)$_3$), 22.0−21.0[43] (5 × OAc CH$_3$).

NMR Spectroscopic Data for 24-Tetra-*O*-acetate. ^{1}H NMR (400 MHz, pyridine-d_5, 363 K): δ 8.31 (2H, m, ArH$_{XX'}$), 8.26 (2H, m, ArH$_{AA'}$), 8.06 (1H, s, ArH, triazole), 6.95 (1H, m, NH$_{II}$), 6.87 (3H, br m, NH$_I$), 6.80 (1H, m, NH$_I$), 6.63 (2H, m, NH$_{II,}$ NH$_{IV}$), 6.21 (1H, d, J = 9.4 Hz, NH$_{IV}$), 5.63 (1H dd, J = 9.5, 10.8 Hz, H$_{3I}$), 5.66 (2H, s,OCH$_2$), 5.60 (1H, d, J = 3.8, Hz, H$_{1I}$), 5.55 (1H, d, J = 2.3 Hz, H$_{1III}$), 5.47 (1H, ddd, J = 3.9, 10.4, 10.4 Hz, H$_{1II}$), 5.44 (1H, dd,, J = 2.4, 5.3 Hz, H$_{2III}$), 5.42 (1H, dd, J = 9.5, 9.5 Hz, H$_{4I}$), 4.91 (1H, ddd, J = 2.9, 9.2, 11.6 Hz, H$_{6II}$), 4.68−4.60 (7H, m, H$_{1IV,}$ H$_{3III}$ H$_{5III}$ H$_{5'III}$ H$_{3II}$ H$_{4II}$ H$_{5II}$), 4.54−4.46 (3H, m, H$_{4III}$, H$_{2I}$, H$_{5I}$), 4.39 (1H, ddd,, J = 9, 4.9, 3.6, Hz, H$_{2IV}$), 4.22 (1H, dd,, J = 6, 6 Hz, H$_{5IV}$), 3.81 (1H, m, H $_{6I}$), 3.75−3.71 (3H, m, H$_{6IV,}$ H$_{6I'}$ H$_{6IV'}$), 3.49 (1H, dd, J = 5.2, 3.9 Hz, H$_{3IV}$), 3.33 (1H, d, J = 3.8 Hz, H$_{4IV}$), 2.82 (1H, ddd, J = 13.7, 10.4, 4.3 Hz, H$_{2IIax}$), 2.65 (1H, ddd, J = 3.8, 3.8, 13.7 Hz, H$_{2IIeq}$), 2.32 (3H, s, -OCOCH$_3$), 2.29 (3H, s, -OCOCH$_3$), 2.12 (3H, m, -OCOCH$_3$), 2.03 (3H, s, -OCOCH$_3$), 1.64 (9H, s, -COC(CH$_3$)$_3$), 1.62 (18H, s, 2 × -COC(CH$_3$)$_3$), 1.69 (27H, m, 3 × -COC(CH$_3$)$_3$). ^{13}C {^{1}H} NMR (100 MHz, pyridine-d_5, 363 K): δ 171.0−170.0[43] (4 × OAc C═O), 165.0[43] (Ar$_{ipso}$) 157.4−156.4[43] (6 × Boc C═O), 151.4[43] (Ar$_{ipso}$) 143.1[43] (triazole$_{ipso}$) 131.6 (ArC$_{AA'}$), 123.9 (ArC$_{XX'}$), 123.3 (CH, triazole), 108.7 (C$_{1III}$), 99.7 (C$_{1IV}$) 99.0 (C$_{1I}$), 81.1 (C$_{4III}$), 80.1 (C$_{5II}$), 79.9−78.9[43] (6 × Boc C$_q$), 76.5 (C$_{4II}$,C$_{3III}$), 75.8 (C$_{2III}$), 72.5 (C$_{5IV}$), 72.2 (C$_{3I}$), 70.8 (C$_{4I}$, C$_{5I}$), 64.2 (C$_{5III}$), 59.9 (OCH$_2$, linker), 56.9 (C$_{1II}$), 54.9 (C$_{2I}$), 53.2 (C$_{6II}$), 52.6 (C$_{4IV}$), 51.9 (C$_{3IV}$), 50.6 (C$_{3II}$), 46.3 (C$_{2IV}$), 42.9 (C$_{6IV}$), 41.9 (C$_{6I}$), 34.3 (C$_{2II}$), 29.14−28.6[43] (6 × Boc (CH$_3$)$_3$), 21.5−21.1[43] (4 × OAc CH$_3$).

3′,4′,2″,5″,4‴-Penta-*O*-acetyl-1-azido-6-*tert*-butoxycarbonyl-amino-3,2′,6′,2‴,6‴-penta-*N*-(*tert*-butoxycarbonyl)-1-deamino-6,3‴-dideoxy-3‴-[4-(4-nitrobenzoyloxymethyl)-[1,2,3]-triazol-1-yl]-neomycin B (25). NaN$_3$ (3 mg) was added to a solution of 23-penta-*O*-acetate (20 mg, 0.17 mmol) in DMF (1 mL) and the mixture was stirred at 50 °C (12 h). The reaction mixture was diluted with ethyl acetate (10 mL) and washed with water (2 × 10 mL). The ethyl acetate layer was dried (Na$_2$SO$_4$) and concentrated in vacuo. Flash column chromatography afforded **25**-penta-*O*-acetate (20 mg, 98%) as an amorphous white solid. ^{1}H NMR (400 MHz, pyridine-d_5, 363 K): δ 8.40 (1H, s, ArH, triazole), 8.28 (2H, m, ArH$_{XX'}$), 8.22 (2H, m, ArH$_{AA'}$), 6.84 (1H, d, J = 7 Hz, NH$_{IV}$), 6.74 (3H, m, NH$_{IV,}$ 2XNH$_{II}$), 6.61 (1H, br dd, NH$_I$), 6.46 (1H, d, J = 8.0 Hz, NH$_I$), 5.76 (1H, dd, J = 3, 3 Hz, H$_{4IV}$), 5.71 (1H dd, J = 9.2, 10.8 Hz, H$_{3I}$), 5.69 (2H, s,OCH$_2$), 5.58 (1H, d, J = 2.8, Hz, H$_{1I}$), 5.54 (1H, d, J = 3.7 Hz, H$_{1IV}$), 5.51 (2H, m, H$_{1III}$, H$_{2III}$), 5.44 (1H, m, H$_{3IV}$), 5.41 (1H, dd, J = 9.4, 10 Hz, H$_{4I}$), 4.92 (1H, ddd, J = 2.6, 6.6, 6.6 Hz, H$_{5IV}$), 4.86 (1H, m, H$_{2IV}$), 4.84 (1H, m, H$_{3III}$), 4.73 (1H, m, H$_{5III}$), 4.63 (2H, m, H$_{5'III}$, H$_{4III}$), 4.52−4.34 (6H, m, H$_{2I}$, H$_{3II}$, H$_{4II}$, H$_{5II}$, H$_{6II}$, H$_{5I}$), 4.46 (1H, ddd, J = 4, 9.6, 9.6 Hz, H$_{1II}$), 3.93 (1H, m, H$_{6IV}$), 3.71 (3H, m, H$_{6I}$, H$_{6I'}$, H$_{6IV'}$), 2.35 (1H, m, H$_{2II}$ eq), 2.26 (3H, s, -OCOCH$_3$), 2.21 (3H, s, -OCOCH$_3$), 2.16 (3H, m, -OCOCH$_3$), 2.14 (3H, s, -OCOCH$_3$),), 2.06 (3H, s, -OCOCH$_3$), 2.0 (1H, m, H$_{2II}$ ax), 1.66 (9H, s, -COC(CH$_3$)$_3$), 1.63 (9H, s, -COC(CH$_3$)$_3$), 1.62 (9H, s, -COC(CH$_3$)$_3$),1.61 (9H, s, -COC-(CH$_3$)$_3$), 1.55 (9H, s, -COC(CH$_3$)$_3$), 1.50 (9H, s, -COC(CH$_3$)$_3$); ^{13}C {^{1}H} NMR (100 MHz, pyridine-d_5, 363 K): δ 129.6 (ArC$_{AA'}$), 123.8 (CH, triazole), 122.2 (ArC$_{XX'}$), 108.2 (C$_{1III}$), 99.2 (C$_{1I}$), 98.9 (C$_{1IV}$), 81.3 (C$_{4III}$), 79.2 (C$_{4II}$), 76.8 (C$_{3III}$), 76.5 (C$_{5II}$), 76.0 (C$_{2III}$), 73.3 (C$_{5IV}$), 72.5 (C$_{3I}$), 70.8 (C$_{4I}$), 70.8 (C$_{5I}$), 70.2 (C$_{4IV}$), 63.1 (C$_{5III}$), 61.4 (C$_{3IV}$), 59.5 (OCH$_2$, linker), 57.2 (C$_{1II}$), 54.5 (C$_{2I}$), 53.6 (C$_{6II}$), 52.5 (C$_{2IV}$), 50.2 (C$_{3II}$), 42.0 (C$_{6I}$, C$_{6IV}$), 32.4 (C$_{2II}$), 30−27 (6 × Boc (CH$_3$)$_3$), 21.0−19.0 (5 × OAc CH$_3$). HRMS (ESI): calculated for (M + H$^+$) C$_{73}$H$_{109}$N$_{13}$O$_{32}$ 1680.73798, found 1680.74466.

3‴,4‴-Anhydro-6-*tert*-butoxycarbonylamino-3,2′,6′,2‴,6‴-penta-*N*-(*tert*-butoxycarbonyl)-6-deoxy-1-*N*-(propyl)-neomycin B (26). The dianhydro neomycin **4** (50 mg 0.04 mmol) was stirred at room temperature in neat propylamine (1 mL) for 16 h. The mixture was then evaporated to dryness and column chromatographic separation (DCM/MeOH 97:5 → 95:8) afforded **26** (25 mg, 47%) as a white amorphous solid. LRMS (ESI): calculated for (M + H$^+$) C$_{56}$H$_{99}$N$_7$O$_{23}$ 1238.69, found 1239.0.

***N*-Acetyl-26-tetra-*O*-acetate.** Per-acetylation of compound **26** according to the general method afforded *N*-acetyl-**26**-tetra-*O*-acetate. ^{1}H NMR (400 MHz, pyridine-d_5, 373 K): δ 6.77 (1H, br dd, NH$_{IV}$), 6.53 (1H, br dd, NH$_I$), 6.44 (2H,m, NH$_I$, NH$_{II}$), 6.25 (1H, m, NH$_{II}$), 6.10 (1H, J = 9.5 Hz, NH$_{IV}$), 5.72 (1H, dd, J = 9.4, 10.9 Hz, H$_{3I}$), 5.58 (1H, dd, J = 2, 5 Hz, H$_{2III}$), 5.53 (1H, d, J = 4 Hz, H$_{1I}$), 5.48 (1H, br s, H$_{1III}$), 5.38 (1H, dd, J = 9.5, 910 Hz, H$_{4I}$), 4.81 (1H, m, H$_{3III}$), 4.79 (1H, d, J = 3.3 Hz, H$_{1IV}$), 4.71−4.68 (4H,br m, H$_{1II}$, H$_{3II}$, H$_{5III}$, H$_{5'III}$), 4.61−4.49 (3H, br m, H$_{4III}$, H$_{6II}$, H$_{2IV}$), 4.49−4.39 (4H, br m, H$_{5II}$, H$_{4II}$, H$_{2I}$, H$_{5I}$), 4.32 (1H, dd,, J = 6, 6 Hz, H$_{5IV}$), 3.77 (4H, m, H$_{6I}$, H$_{6IV}$, H$_{6I'}$, H$_{6IV'}$), 3.55 (1H, dd, J = 5, 4 Hz, H$_{3IV}$), 3.37 (1H, d, J = 4 Hz, H$_{4IV}$), 3.34 (2H, m, -*N*Ac-CH$_2$-), 2.57 (1H, m, H$_{2II}$ eq), 2.37 (3H, s, -OCOCH$_3$), 2.36 (1H, m, H$_{2II}$ ax), 2.33 (2H,br s, -CH$_2$-), 2.18 (6H, s,2 × -OCOCH$_3$), 2.15 (3H, s, -OCOCH$_3$), 2.07 (3H, s, -OCOCH$_3$), 1.67 (9H, s, -COC(CH$_3$)$_3$), 1.64 (9H, s, -COC(CH$_3$)$_3$), 1.64 (2H, s, (-*N*Ac-CH$_2$-CH$_2$-),1.63 (27H, s, 3 × -COC(CH$_3$)$_3$), 1.60 (9H, s, -COC-(CH$_3$)$_3$), 1.0 (-CH$_3$); ^{13}C {^{1}H} NMR (100 MHz, pyridine-d_5, 363 K): δ 173.66[43] (*N*Ac, C═O),171.21−170.0[43] (4 × OAc C═O), 157.2−155.3[43] (6 × Boc C═O), 108.4 (C$_{1III}$), 99.9 (C$_{1IV}$), 98.9 (C$_{1I}$), 81.0 (C$_{4III}$), 79.6 (C$_{4II}$), 76.1 (C$_{5II}$), 79.7−78.0[43] (6 × Boc C$_q$), 76.0 (C$_{3III}$), 75.4 (C$_{2III}$), 72.9 (C$_{5IV}$), 72.3 (C$_{3I}$), 71.1 (C$_{5I}$), 70.9 (C$_{4I}$), 70.2 (C$_{5I}$), 64.2 (C$_{5III}$), 54.7 (C$_{2I}$), 52.7 (C$_{4IV}$), 52.2 (C$_{3IV}$), 51.8 (C$_{6I!}$), 50.8 (C$_{1II}$, C$_{3II}$), 47.5 (-*N*Ac-CH$_2$-), 46.3 (C$_{2IV}$), 43.7 (C$_{6IV}$), 42.1 (C$_{6I}$), 31.5 (C$_{2II}$), 29.2−27.2[43] (6 × Boc (CH$_3$)$_3$), 22.0−20.5[43] (5 × Ac, CH$_3$), 24.2 (-*N*Ac-CH$_2$-CH$_2$-), 11.4 (-CH$_3$). HRMS (ESI): calculated for (M + Na$^+$) C$_{66}$H$_{109}$N$_7$O$_{28}$ 1470.72183, found 1470.72365.

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Supporting Information Available: General experimental procedures, 1D and 2D NMR spectra of the per-acetylated aminoglycosides **2**, **4**, **5**, **8**, **13**, **14**, and **16**−**26** and the starting synthons **9** and **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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