ORGANIC LETTERS

2002
Vol. 4, No. 20
3455−3458

Reexamination of Neomycin B Degradation: Efficient Preparation of Its CD and D Rings as Protected Glycosyl Donors

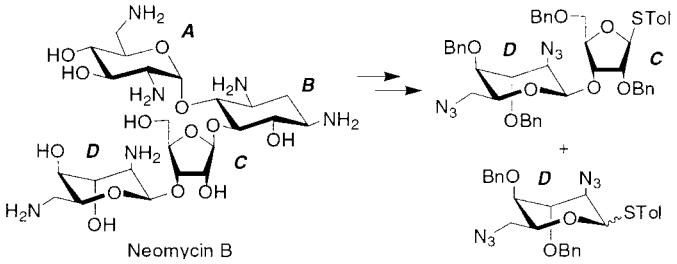
Baogen Wu,[†] Jun Yang, Yun He,*[,†] and Eric E. Swayze

Ibis Therapeutics, A Division of Isis Pharmaceuticals, Inc., 2292 Faraday Avenue, Carlsbad, California 92008

yhe@gnf.org

Received July 17, 2002

ABSTRACT



The degradation of neomycin B was reexamined, and a novel protocol was established to prepare the properly masked neomycin CD ring as a glycol donor in excellent yield. Glycosylation of the CD ring with glycol acceptors provided a facile access to versatile intermediates that could be utilized to synthesize a variety of novel neomycin B mimetics for RNA recognition.

Recently, there has been an increasing interest in the potential application of RNAs as targets for small molecular drug discovery.[1] Like proteins, RNAs offer a diversity of three-dimensional folds to allow the selective binding of effector molecules.[2] Among the drugs obtained from natural sources that have been shown to work by binding to RNA or RNA/protein complexes, special attention has been paid to the class of aminoglycoside antibiotics. These natural aminosugars have been shown to interact with a number of functional RNA molecules including group I introns,[3] hammerhead ribozymes,[4] and the human hepatitis δ virus ribozyme.[5] For example, neomycin B (Figure 1) induces translational miscoding, most likely as a result of its interaction with 16*S* rRNA in the A site of the ribosome.[6] As methods[7,8] have been developed to study small molecule−RNA interactions,

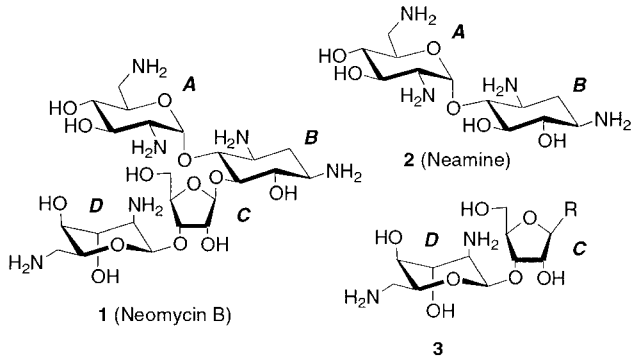


Figure 1. Structure of neomycin B.

[†] Current address: Medicinal Chemistry, GNF, 10675 Johns Hopkins Dr., San Diego, CA 92121.

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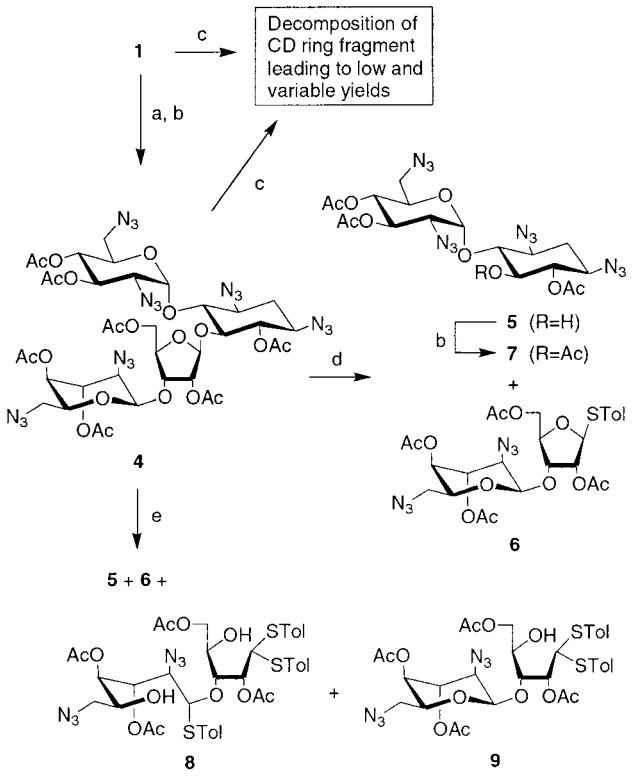
10.1021/ol026548n CCC: $22.00
Published on Web 09/07/2002

an increasing number of searches for specific RNA binders and therapeutic agents have been conducted by rational design and/or library synthesis.[9,10] Most recently, Wong and co-workers synthesized novel neamine dimers that bind to 16*S* RNA with high affinity and exhibited significantly increased antibiotic activity.[9a]

In a search for new antibacterial agents that target bacterial ribosomal RNAs, we have initiated a program to synthesize aminosugar mimetics with simpler structure and improved biological properties.[11] Although studies by Wong and co-workers have suggested that the CD ring contributes significantly to the binding affinity ($K_D = 0.019\ \mu M$ for **1** and $K_D = 7.8\ \mu M$ for **2**) and specificity (**1** is 5-fold more specific than **2**) of neomycin for the target RNA,[9e] most previous studies on neomycin B analogues have maintained the core AB ring structure and eliminated or modified the C and/or D rings.[9b,c,e,10b,e,h] To address the relative contributions to neomycin activity made by the various ring components, we became interested in the synthesis of neomycin analogues having the CD ring structure maintained and a variety of carbohydrate and non-carbohydrate moieties substituted for the A and/or B rings. Although many studies on neomycin B and related aminosugars have been reported in the literature,[12] no practical preparation of the neomycin B CD ring has been documented. We have described the preparation of the CD fragment from neomycin B with hydrochloric acid by modifying the literature procedure.[13] Unfortunately, the yield for the CD ring was variable and often accompanied by multiple byproducts when translated to non-milligram scales (Scheme 1).

Scheme 1. Degradation of **4** to CD Ring Fragment[a]



[a] Reagents and conditions: (a) TfN_3 (10 equiv), $CuSO_4$, Et_3N, MeOH, H_2O; (b) Ac_2O (50 equiv), Py, DMAP (cat.), 82% from **1**; (c) 1.0 N HCl, MeOH−dioxane, reflux; (d) TolSH (1.1 equiv), $BF_3{\cdot}OEt_2$ (3.0 equiv), 25 °C, 15 h, **6**, 82%; (e) TolSH (2.0 equiv), $BF_3{\cdot}OEt_2$ (3.0 equiv), 25 °C, 15 h, **5**, 88%; **6**, 45%; **8**, 33%; **9**, ∼5%.

We report herein a new protocol for the degradation of neomycin B that allows us to efficiently and reproducibly access the CD and D ring fragments of neomycin B in quantity. Furthermore, this protocol conveniently provides the CD and D rings as thioglycosides with proper protections for the amino and hydroxy groups in place, making them amenable to subsequent glycosylations.

Initially, perazidoperacetylneomycin (**4**) was chosen as our starting material for the study of cleavage conditions. Compound **4** can conveniently be prepared from inexpensive neomycin B by azido transfer followed by acetylation (Scheme 1).[9c] Under acidic conditions (1.0 N HCl−MeOH−dioxane, Table 1), **4** was decomposed slowly at room temperature, giving neither the desired CD ring product **5**, AB ring product **6** nor their related de-acylated products according to LC−MS analysis. Similar results were obtained by employing sulfuric acid. We then turned our attention to Lewis acid mediated degradation conditions. Treatment of **4** with 1,3-propanedithiol and $SnCl_4$ in methylene chloride resulted in slow decomposition of the starting material at −78 °C. Under similar conditions with different Lewis acids

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Table 1. Degradation of **4** under Various Conditions

conditions	5	6
1.0 N HCl−MeOH−dioxane	dec	dec
$HS(CH_2)_3SH$, $SnCl_4$	dec	dec
$HS(CH_2)_3SH$, $TiCl_4$	<10%	dec
$HS(CH_2)_3SH$, $BF_3{\cdot}OEt_2$	65%	dec
EtSH, $SnCl_4$	25%	dec
EtSH, $BF_3{\cdot}OEt_2$	30%	dec
TolSH, $SnCl_4$	70%	dec
TolSH, TMSOTf	75%	25%
TolSH, $BF_3{\cdot}OEt_2$	90%	82%

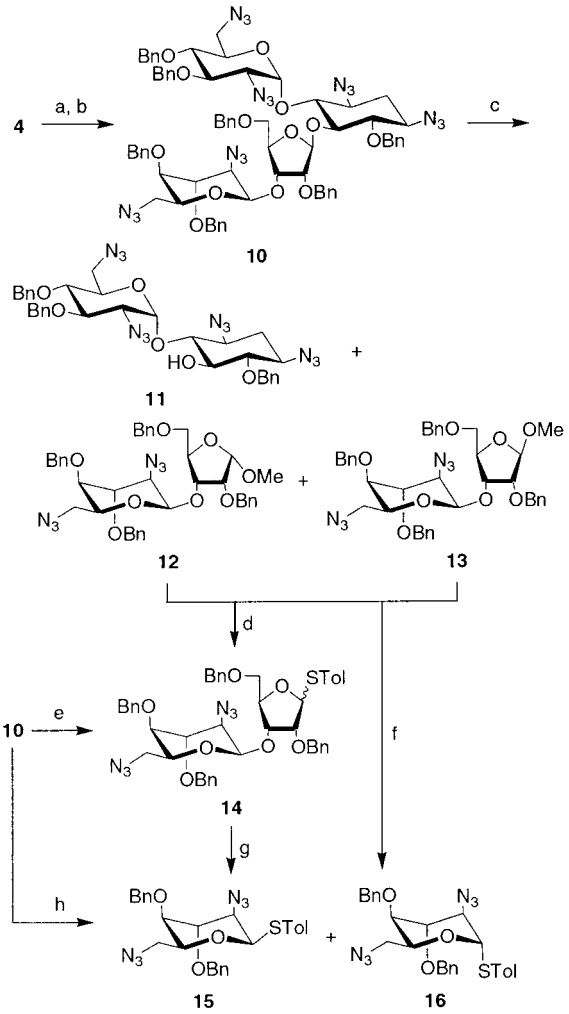
such as $TiCl_4$, $SnCl_4$, and $BF_3{\cdot}OEt_2$ using either dithiol, ethylthiol, or TolSH gave only AB ring product at −78 °C with CD ring fragment decomposed. When TMSOTf was employed, the reaction provided the desired CD ring product in 25% yield at −30 °C. The best result was obtained by treating **4** with 3.0 equiv of $BF_3{\cdot}OEt_2$ and 1.1 equiv of TolSH in methylene chloride (see the Supporting Information). Under these conditions, the reaction proceeded smoothly to give both the AB (**5**) and CD (**6**) ring fragments in excellent yields.

Since the products have very similar R_f values on TLC, the crude reaction mixture was acetylated to convert the AB ring fragment **5** to **7**. Compounds **6** and **7** could then be readily separated by silica column chromatography. The chemical structure of **6** was established by various spectroscopic analyses, and the chemical shifts of protons in **6** were assigned according to its 2D NMR spectrum (see the Supporting Information). Following this procedure, glycosyl donor **6** could be prepared in gram quantities. Interestingly, if 2.0 equiv of TolSH was used, the CD ring fragment **6** began to degrade, and a mixture of **5** (88%), **6** (45%), **8** (33%), and **9** (∼5%) was obtained.

Wong et al.[9c] mentioned the formation of the CD ring as a minor byproduct when degrading perazidoperbenzylneomycin **10** under acidic conditions. In our hands, when **10** was refluxed in a HCl solution of MeOH and dioxane, it gave the AB ring fragment **11** as well as the CD ring fragment as a mixture of two anomers, which could be separated via careful silica gel chromatography (**12** and **13**; Scheme 2). This is in contrast to the methanolysis of **1** or **4**, which resulted in decomposition of the CD ring fragment. As expected, prolonged heating of the reaction mixture resulted in decomposition of CD ring products and gave lower yields.

Treatment of either **12** or **13** with $BF_3{\cdot}OEt_2$ and 1.1 equiv of TolSH at 0 °C for 15 min led to the isolation of **14** (α/β = 1:2) in over 88% yield. We subsequently discovered that donor **14** could be obtained directly from **10** by using the same conditions as for the degradation of **4**. Thus, treatment of **10** with $BF_3{\cdot}OEt_2$ and 1.2 equiv of TolSH led to the formation of **14** (α/β = 1:2) in 66% yield in one simple operation. Use of excess $BF_3{\cdot}OEt_2$ (5 equiv) reduced the yield of **14** due to the opening of D ring. When **14** was allowed to react with $BF_3{\cdot}OEt_2$ and TolSH at 25 °C, the thioglycosides **15** and **16** derived from D ring were then obtained. Similarly, **15** and **16** were obtained in nearly identical yield from a mixture of **12** and **13**. Furthermore, **15** and **16** could be obtained directly from **10**, albeit in slightly reduced yields.

Scheme 2. Degradation of **10** to CD and D Ring Fragments[a]



[a] Reagents and conditions: (a) NaOMe, MeOH; (b) BnBr, NaH, DMF, 76% from **4**; (c) 1.0 M HCl, MeOH, dioxane, reflux, 14 h, **11**, 82%; **12**, 20%; **13** (α/β = 1:2), 56%; (d) TolSH (1.2 equiv), $BF_3{\cdot}OEt_2$ (3.0 equiv), 0 °C, 15 min, 88%; (e) TolSH (1.5 equiv), $BF_3{\cdot}OEt_2$ (3.0 equiv), 25 °C, 10 h, **14** (α/β = 1:2), 66%; (f) TolSH (2.0 equiv), $BF_3{\cdot}OEt_2$ (3.0 equiv), 25 °C, 2 h, **15**, 37%; **16**, 25%; (g) TolSH (3.0 equiv), $BF_3{\cdot}OEt_2$ (5.0 equiv), 25 °C, 4 h, **15**, 34%; **16**, 23%; (h) TolSH (3.0 equiv), $BF_3{\cdot}OEt_2$ (3.0 equiv), 25 °C, 4 h, **15**, 23%; **16**, 18%.

We then tested the reactivity of donor **6** toward a simple glycosyl acceptors (Scheme 3). The glycosylation reaction of **6** with methyl α-hydroxyacetate in the presence of NIS and a catalytic amount of AgOTf proceeded smoothly to give product **17** as the only anomer in 76% yield.[14] Derivatives such as **17** may serve as appropriate key intermediates in the future library synthesis of neomycin mimetics with modified AB rings.

In summary, we have established a simple and efficient method for the preparation of the CD and D ring derivatives

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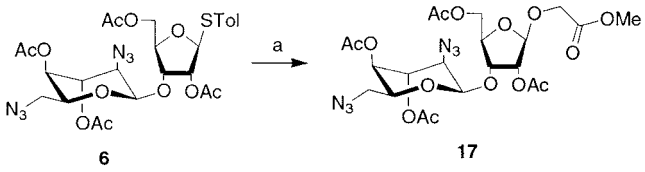
Scheme 3. Glycosylation of **6**[a]



[a] Reagents and conditions: (a) NIS (1.3 equiv), AgOTf (cat.), $HOCH_2CO_2Me$ (10 equiv) methylene chloride, 4 Å MS, 0 °C, 2 h, 76%.

of neomycin B. Protected derivatives of neomycin such as **4** and **10** have been prepared on a multigram scale and extensively utilized as a source of the AB ring fragments by the Wong group.[9c] The degradation conditions described herein now allow access to the CD ring derivative **6** and **14** in gram quantity. These synthons react readily with glycosyl acceptors and could serve as key intermediates for the synthesis of neomycin mimetics with modifications on the A and AB ring moieties. Additionally, the protected neomycin B D ring deriviatives **15** and **16** were obtained in a single step from the readily available **10**. Compounds of this type are relatively difficult to access otherwise. The use of Lewis acids in concert with thiol scavengers may also be applicable to the preparation of other aminosugar derivatives via the degradation of aminoglycosides. The use of these intermediates for the synthesis of libraries of neomycin mimetics will be reported in due course.

Supporting Information Available: Experimental procedures, spectral data for representative compounds (**6**, **8**, and **12**−**17**). This material is available free of charge via the Internet at http://pubs.acs.org.

OL026548N