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Conformationally restricted analogs of deoxynegamycin ☆

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Abstract—Deoxynegamycin (**1b**) is a protein synthesis inhibitor with activity against Gram-negative (GN) bacteria. A series of conformationally restricted analogs were synthesized to probe its bioactive conformation. Indeed, some of the constrained analogs were found to be equal or better than deoxynegamycin in protein synthesis assay (**1b**, $IC_{50} = 8.2\,\mu M$; **44**, $IC_{50} = 6.6\,\mu M$; **35e$_2$**, $IC_{50} = 1\,\mu M$). However, deoxynegamycin had the best in vitro whole cell antibacterial activity (*Escherichia coli,* MIC = 4–16 μg/mL; *Klebsiella pneumoniae*, MIC = 8 μg/mL) suggesting that other factors such as permeation may also be contributing to the overall whole cell activity. A new finding is that deoxynegamycin is efficacious in an *E. coli* murine septicemia model ($ED_{50} = 4.8$ mg/kg), providing further evidence of the favorable in vivo properties of this class of molecules.

1. Introduction

A surge in resistance[1] to the existing armamentarium of clinically used antibacterial agents has stimulated the search for novel antibiotics with different mechanisms of action[2] that are not subject to cross-resistance. Antibiotics, which are natural products, have played a predominant role in antibacterial therapy. Chemistry has in many cases improved on nature and semi-synthetic variants of the original antibiotic are the marketed products. Total synthesis of the typically complex antibiotic structures has been mostly an academic challenge rather than a practical alternative for their manufacture. In a limited number of cases the structure of an antibiotic has been sufficiently simple to allow reasonable synthetic access, for example, chloramphenicol is manufactured by total synthesis. Negamycin[3] is an antibiotic with activity against Gram-negative bacteria and has a mechanism involving inhibition of protein synthesis. As a starting point for making improved negamycin analogs, we chose to work with deoxynegamycin,[4] a closely related molecule that is less complex and more synthetically accessible. We report here on the synthesis and activity of several analogs that are conformationally restricted at the *N*-terminal region.

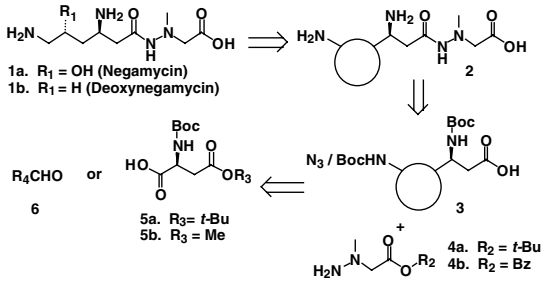

Figure 1. Design and retrosynthetic analysis.

The desired β-amino acids of type **3** can be obtained from appropriately protected aspartic acid **5** (Fig. 1) through functionalization of α-carboxyl group.[5] Alternatively, aldehyde **6** can serve as a starting material to synthesize the β-amino acids of type **3** through multicomponent condensation reaction.

2. Chemistry

Homologation of D-ornithine derivative **7** (Scheme 1) under Arndt–Eistert reaction[6] conditions followed by ester hydrolysis produced the desired β-amino acid **9**.

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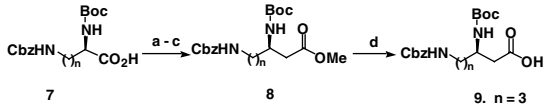
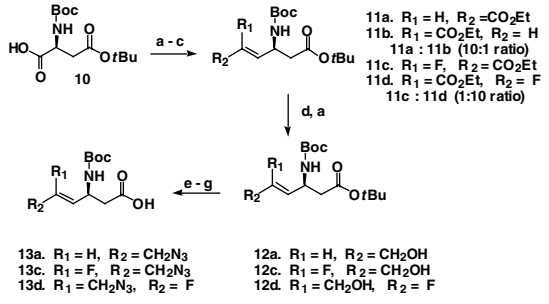


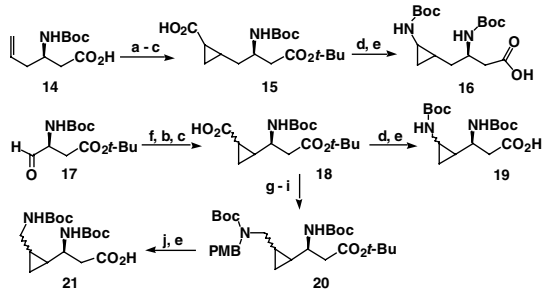
Scheme 1. Synthesis of 3,6-diaminohexanoic acid. Reagents and conditions: (a) *i*-butyl chloroformate, NMM, THF −15 °C, 45 min then CH_2N_2 in ether; (b) $C_6H_5CO_2Ag$, Et_3N, MeOH (65% for two steps); (c) LiOH, dioxane, water (85%).

The synthesis of β-amino acids **13a**, **13c**, and **13d** relied on L-aspartic acid derivative **10** as a chiral source (Scheme 2). Hence, aspartic acid **10** was reduced to corresponding alcohol and oxidized to aldehyde under Swern[7] oxidation conditions. Condensation of aldehyde with triethyl 2-fluoro-2-phosphonoacetate[8] or with triethyl 2-phosphonoacetate gave olefin derivatives **11a**–**d**, *cis*/*trans* isomers were separated by silica gel chromatography. Hydrolysis of the ethyl ester in **11a**, **11c**, and **11d** followed by reduction[9] of the acid produced alcohols **12a**, **12c**, and **12d**, respectively. These alcohols were converted to the corresponding mesylates and treated with sodium azide to furnish the azides. Removal of the *t*-butyl ester under basic conditions provided azido acids **13a**, **13c**, and **13d**.

Aspartic acid **10** and homoallylglycine **14** served as chiral source to access cyclopropane-based β-amino acids **16**, **19**, and **21** as a mixture of diastereomers (Scheme 3). Homoallylglycine **14** was converted to the corresponding *t*-butyl ester and subjected to rhodium catalyzed carbene insertion reaction.[10] The cyclopropane derivative was selectively hydrolyzed to furnish cyclopropane carboxylic acid **15**. Curtius rearrangement[11] of acyl azide derived from acid **15** in the presence of *t*-butanol as a solvent followed by removal of the *t*-butyl ester by base hydrolysis gave β-amino acid **16** as a mixture of diastereomers. Aldehyde **17**, obtained from aspartic acid as depicted in Scheme 2, was converted to an alkene derivative. Alkene was subjected to the rhodium catalyzed carbene insertion[10] reaction followed by selective removal of the ethyl ester under basic conditions to get acid **18** as a mixture of diastereomers. Acyl



Scheme 2. Synthesis of alkene-based β-amino acids. Reagents and conditions: (a) *i*-butyl chloroformate, NMM, THF −15 °C, 45 min then $NaBH_4$, H_2O, 0 °C to rt, 2 h (75%); (b) oxalyl chloride, DMSO, Et_3N, −60 to −40 °C, 1 h (95%); (c) *n*-BuLi, triethyl 2-fluoro-2-phosphonoacetate, THF, −78 °C or NaH, triethyl 2-phosphonoacetate, 0 °C to rt (65%); (d) LiOH, THF, H_2O, rt, 16 h (100%); (e) CH_3SO_2Cl, pyridine DCM, 0 °C, 2 h (100%); (f) NaN_3, DMF, 80 °C, 16 h (80%); (g) 2 M KOH in MeOH, rt, 16 h (100%).

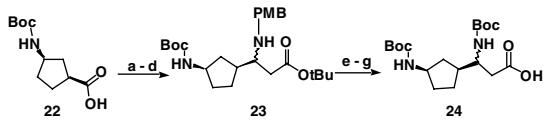


Scheme 3. Synthesis of cyclopropane-based β-amino acids. Reagents and conditions: (a) DIC, DMAP, *t*-BuOH; (b) N_2CHCO_2Et, $Rh_2(OAc)_4$, DCM, rt (27–31%); (c) LiOH, H_2O, EtOH (79–80%); (d) DPPA, Et_3N, *t*-BuOH, 110 °C (40–66%); (e) 2 M KOH in MeOH (60–97%); (f) PPh_3MeBr, KHMDS, THF, −78 °C to rt (80%); (g) *p*-methoxybenzyl amine, HATU, DIEA, DMF, 0 °C to rt (82%); (h) BH_3, THF, 0 °C to rt to 45 °C (94%); (i) $(Boc)_2O$, K_2CO_3, THF, rt (75%); (j) CAN, CH_3CN–H_2O (2:1), rt (65%).

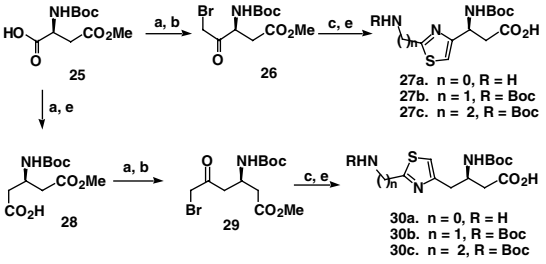
azide, obtained from acid **18**, upon Curtius rearrangement[11] using *t*-butanol as a solvent and further removal of the *t*-butyl ester group under basic conditions gave the desired β-amino acid **19** as a mixture of diastereoisomers. Acid **18** was coupled with *p*-methoxybenzyl amine and the resultant amide was reduced to yield secondary amine, which was capped with *t*-butoxycarbonyl (Boc) group. Removal of *p*-methoxybenzyl group under oxidative conditions[12] followed by base hydrolysis of the *t*-butyl ester furnished the desired β-amino acid **21** as a mixture of diastereoisomers.

The synthesis of cyclopentane-based β-amino acid is depicted in Scheme 4. γ-Amino acid **22** was reduced[9] to alcohol and then oxidized to aldehyde. Condensation of aldehyde with *t*-butyl diethylphosphonoacetate followed by 1,4-addition of *p*-methoxybenzyl amine gave β-amino ester **23** as a mixture of diastereomers. Protection of secondary amine in **23** with Boc group, removal *p*-methoxybenzyl group under oxidative conditions,[12] and hydrolysis of the *t*-butyl ester furnished the desired β-amino acid **24**.

The synthesis of thiazole-based β-amino acids was accomplished as described in Scheme 5 starting from aspartic acid **25** as a chiral synthon. Diazoketone, prepared from aspartic acid **25**, was reacted with hydrobromic acid to give the bromoketone[13] **26** (Scheme 5). On the other hand, diazoketone was subjected to Wolff



Scheme 4. Synthesis of cyclopentane-based β-amino acid. Reagents and conditions: (a) *i*-butyl chloroformate, NMM, THF −15 °C, 45 min and then $NaBH_4$, H_2O, 0 °C to rt, 2 h (75%); (b) DMSO, oxalyl chloride, TEA, −60 to −40 °C, 1 h (75%); (c) *t*-butyl diethylphosphonoacetate, NaH, THF, 0 °C to rt (63%); (d) *p*-methoxybenzyl amine, 110 °C, 60 h (42%); (e) $(Boc)_2O$, Pd–C (10%), EtOAc (65%); (f) CAN, CH_3CN–H_2O (2:1), rt, 16 h (89%); (g) 2 M NaOH in MeOH, rt, 16 h (95%).

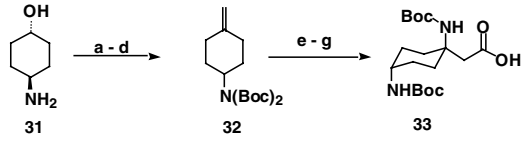


Scheme 5. Synthesis of thiazole-based β-amino acids. Reagents and conditions: (a) *i*-butyl chloroformate, NMM, THF −15 °C, 45 min then CH_2N_2 in ether; (b) HBr, AcOH, ether; (c) thiourea or Boc-$NH(CH_2)_nC(=S)NH_2$ ($n = 1$, 2), EtOH; (d) LiOH, H_2O, THF; (e) $C_6H_5CO_2Ag$, H_2O, THF.

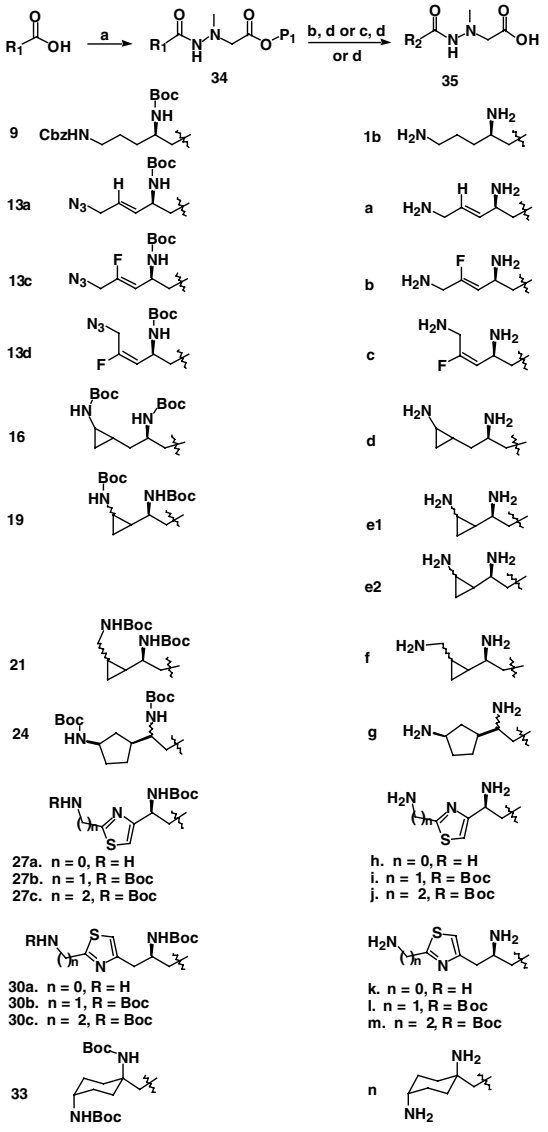
rearrangement in the presence of water to provide acid **28**. Bromoketone **29** was prepared from acid **28** as described above. Condensation of bromoketones **26** and **29** with thiourea followed by base hydrolysis of methyl ester afforded β-amino acid **27a** and **30a**, respectively. These acids were isolated as lithium salt upon lyophillization. Further, the condensation of bromoketones **26** and **29** with thioamides derived from *t*-butyloxygylcinamide and 3-(*N*-*t*-butoxycarboylamino)propionamide followed by esters hydrolysis gave acids **27b**, **27c**, **30b**, and **30c**.

Synthesis of β-amino acid **33** is outlined in Scheme 6. The protection of amine **31** with Boc group followed by oxidation of secondary alcohol gave the corresponding ketone. This ketone upon Wittig olefination followed by protection of the carbamate nitrogen with Boc group gave the cyclohexene **32**. The [2+2] cycloaddition[14] reaction between cyclohexene **32** and isocyanate gave β-lactam, which was treated with di-*tert*-butyl dicarbonate in the presence of DMAP followed by base hydrolysis to furnish β-amino acid **33**.

The structurally diverse set of β-amino acids synthesized (Schemes 1–6) in the present study were incorporated in deoxynegamycin template as summarized in Scheme 7. β-Amino acid **9** was coupled with hydrazine **4a** and the resultant product was subjected to catalytic hydrogenation followed by acidic treatment to afford **1b**. Azido β-amino acids **13a**, **13c**, and **13d** were coupled with hydrazine **4a**, the azide was reduced to amine and the resultant products were treated with hydrochloric acid in dioxane to furnish **35a**–**c**, respectively. β-Amino acid **19** was coupled with hydrazine **4a** and the diastereomers were separated by silica gel chromatography.[15] Global



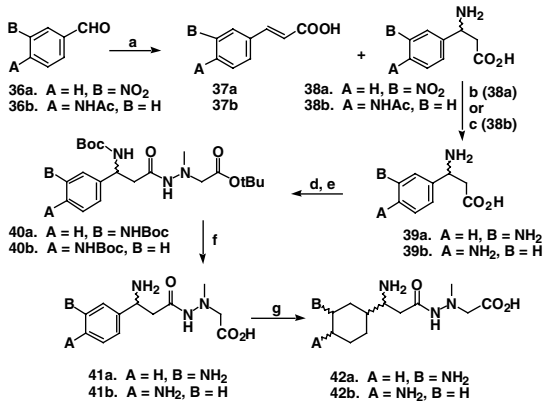
Scheme 6. Synthesis of cyclohexane-based β-amino acid. Reagents and conditions: (a) Boc_2O, Et_3N, THF (95%); (b) $(ClCO)_2$, DMSO, CH_2Cl_2, Et_3N (94%); (c) H_3C–PPh_3·Br, KHMDS, THF (91%); (d) LiHMDS, Boc_2O, THF (99%); (e) $ClSO_2NCO$, Et_2O (5%); (f) Boc_2O, DMAP, MeCN (93%); (g) LiOH, THF/H_2O (65%).



Scheme 7. Conformationally restricted deoxynegamycin analogs. Reagents and conditions: (a) HATU, DIEA, DMF, **4a**, 3 h, 0 °C to rt (70–90%); (b) PPh_3, THF, H_2O (65–80%); (c) H_2, Pd–C, EtOAc; (d) 4 M HCl in dioxane (quantitative).

removal of protecting groups under acid conditions furnished the desired analogs **35e$_1$** and **35e$_2$**. β-Amino acids **16**, **21**, **24**, **27a**–**c**, **30a**–**c**, and **33** were coupled with hydrazine **4a** and the resultant products were treated with hydrochloric acid in dioxane to give **35d** and **35f**–**n**, respectively.

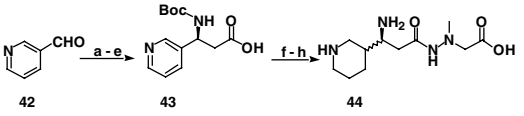
In the synthesis of cyclohexyl-based deoxynegamycin analog, reduction of aryl ring in the penultimate step is the key reaction (Scheme 8). Thus, a three component condensation of aldehyde (**36a** or **36b**), malonic acid, and ammonium acetate gave β-amino acids **38a** and **38b** along with cinnamic acids **37a** and **37b**, respectively. The reduction of nitro group in **38a** under catalytic hydrogenation conditions furnished diamine **39a**. Acetyl group in **38b** was removed under acidic conditions to get diamine **39b**. Diamines **39a** and **39b** were protected with Boc group then the resultant diBoc derivatives were



Scheme 8. Synthesis of cyclohexane-based β-amino acids. Reagents and conditions: (a) malonic acid, NH_4OAc, EtOH, reflux, 24 h (70%); (b) H_2, Pd–C, MeOH, rt 50 psi, 16 h (100%); (c) 6 M HCl reflux, 20 h (99%); (d) $(Boc)_2O$, Na_2CO_3, H_2O, rt (5%); (e) hydrazine, HATU, DIEA, DMF, 0 °C to rt, 12 h (60%); (f) 4 M HCl in dioxane, H_2O, rt 4 h (100%); (g) H_2, PtO_2, AcOH, H_2O, rt, 50 psi (50%).

coupled with hydrazine **4a** using HATU as coupling reagent to give **40a** and **40b**, respectively. Removal of the protecting groups from **40a** and **40b** under acidic conditions gave **41a** and **41b**, respectively. Reduction of the aromatic ring of **41a** and **41b** gave the corresponding cyclohexane derivatives **42a** and **42b**, respectively.

Reduction of pyridine ring in the penultimate step is the key step in the synthesis of piperidine based deoxynegamycin analog.[16] Hence, pyridine-3-carboxaldehyde (Scheme 9) was condensed with the *t*-butyl diethylphosphonoacetate under Wittig–Horner conditions to give α,β-unsaturated ester. 1,4-Addition of α,β-unsaturated ester with chiral lithium amide, derived from (*R*)-(+)-*N*-benzyl-α-methylbenzylamine and *n*-butyllithium, gave the enatiomerically pure β-amino ester. Benzyl protecting groups from β-amino ester were removed under catalytic transfer hydrogenation conditions. The resultant primary amine was protected with Boc group and base hydrolysis of *t*-butyl ester gave β-amino acid **43**. β-Amino acid **43** was coupled with hydrazine **4a**, the protecting groups removed under acidic conditions, and the aromatic ring reduced under catalytic hydrogenation conditions to produce the desired compound **44**.



Scheme 9. Synthesis of piperidine-based β-amino acid. Reagents and conditions: (a) $(EtO)_2P(O)CH_2CO_2tBu$, NaH, DMF (99%); (b) (*R*)-(+)-*N*-benzyl-α-methylbenzylamine *n*-BuLi, THF (88%); (c) 1,4-cyclohexadiene, Pd–C (10%), AcOH (93%); (d) $(Boc)_2O$, aq Na_2CO_3, THF (84%); (e) 2 M NaOH in MeOH, rt (86%); (f) hydrazine, HATU, DIEA, DMF, 0 °C to rt (54%); (g) 4 M HCl in dioxane, rt (96%); (h) $H_2{\cdot}PtO_2$, AcOH, 55 psi (71%).

3. Results and discussion

Methods were developed to synthesize a number of conformationally restricted β-amino acids. Incorporation of these β-amino acids into deoxynegamycin template has resulted in structurally novel and conformationally restricted analogs. These analogs were evaluated in whole cell assays[17] and as inhibitors of cell-free protein synthesis.[18] Deoxynegamycin showed good antibacterial activity (*E. coli*, MIC = 4–16 μg/mL; *K. pneumoniae*, MIC = 8 μg/mL) in the whole cell assay and also good protein synthesis inhibition in cell-free protein synthesis assay ($IC_{50} = 8.2\ \mu M$). However, to the best of our knowledge there is no in vivo data reported for deoxynegamycin in the literature. Therefore, we evaluated deoxynegamycin in an *E. coli* murine septicemia model[19] and the efficacy was comparable to that of negamycin (Deoxynegamycin: $ED_{50} = 4.8$ mg/kg; Negamycin $ED_{50} = 5.1$ mg/kg). Thus, the combined in vitro and in vivo antibacterial profile of deoxynegamycin validates it as a good starting point for the synthesis of a structurally novel analogs with improved properties.

The conformational constraint introduced through a double bond, as in analogs **35a**, **35b**, and **35c**, in the *N*-terminal region of deoxynegamycin has resulted in either complete loss or significant decrease in antibacterial activity (Table 1). Although the activity of the alkene analog **35c** in cell-free protein synthesis is very similar to that of deoxynegamycin, it has no antibacterial activity

Table 1. MICs[17] and protein synthesis inhibition data (TC/TL)[18]

Organisms/Compounds	MIC (μg/mL)											
	1b	**35a**	**35b**	**35c**	**35d**	**35e$_1$**	**35e$_2$**	**35f**	**35g**	**42a**	**42b**	**44**
E. coli ATCC 25922	16	>256	>256	>256	64	>128	>128	>128	128	256	128	>128
E. coli MG1655	8	>256	>256	>256	64	>128	>128	>128	128	128	64	>128
E. coli MG1655 *tolC*	4	>256	>256	>256	32	>128	>128	>128	64	64	32	>128
K. pneumoniae ATCC13882	8	>256	>256	>256	32	>128	>128	>128	64	64	32	>128
Enterobacter cloacae ATCC35030	4	>256	>256	>256	128	>128	>128	>128	128	256	128	>128
Pseudomonas aeruginosa PA01	64	>256	>256	>256	256	>128	>128	>128	256	>256	256	>128
Staphylococcus aureus ATCC25293	64	>256	>256	>256	256	>128	>128	>128	128	256	128	>128
Streptococcus pneumoniae 1005	64	256	256	256	128	ND	ND	ND	128	128	128	>128
TC/TL IC_{50} (μM)	8.2	>80	>80	11	11	>82	1	>82	>82	10.7	13.2	6.6

Analogs **35h**–**n** are inactive (MIC>256 μg/mL and IC_{50}>80 μM).

in the whole cell assay. Introduction of a cyclopropyl ring adjacent to the *N*-terminal amine, as in analog **35d**, resulted in significant loss in whole cell antibacterial activity although the activity of this analog in cell-free protein assay is very similar to that of deoxynegamycin (**1b**). On the other hand, introduction of cyclopropyl ring adjacent to the internal amine (**35e1**, **35e2**, and **35f**) has resulted in complete loss of antibacterial activity. However, the cyclopropyl analog **33e2** is 8-fold more active than deoxynegamycin in the cell-free protein synthesis assay. The cyclohexyl analog **35n** was inactive. Insertion of five and six member ring in the N-terminal region of deoxynegamycin resulted in analogs (**35g**, **42a**, and **42b**), which are less active than deoxynegamycin (**1b**). Piperidine-based analog **44** has no appreciable antibacterial activity in the whole cell assay but its activity in cell-free protein synthesis assay is comparable to that of deoxynegamycin. The conformational constraint introduced by insertion of aryl or heteroaryl rings (analogs **27a**–**c**, **30a**–**c**, **41a**, and **41b**) has resulted in complete loss of antibacterial activity.

Our earlier studies[20] were indicative of an active-transport mediated event for permeability of highly charged molecules such as negamycin and related analogs across the outer membrane of Gram-negative bacteria. In the present study we were able to walk through the conformational space by the insertion of a set of conformationally restricted β-amino acids in the N-terminal region of deoxynegamycin. Among these analogs, **35e2** is 8-fold more active than deoxynegamycin (**1b**) in the cell-free protein synthesis assay (Table 1). However, this analog has no antibacterial activity in the whole cell assay, suggesting that it may not be getting transported efficiently into the bacterial cell. In conclusion, the transport structure–activity relationship (SAR) may not be in parallel with the target SAR for this class of molecules. These observations may play a critical role in the future discovery of new deoxynegamycin based antibacterial agents.

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15. Acid **19** is an inseparable mixture of diastereomers. Upon coupling with the hydrazine derivative, the diastereomers were separated by silica gel column chromatography. Removal of protecting groups from higher R_f diastereomer under acidic conditions afforded **35e$_1$**. Similar transformation on lower R_f diastereomer resulted in **35e$_2$**. The stereochemistry of these analogs at cyclopropane juncture cannot be assigned by NMR due to the presence of rotomers.
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17. Microdilution MICs were determined in Mueller–Hinton broth supplemented with 50% human serum and an inoculum size of 5×10^5 cfu/mL.
18. Inhibition of protein synthesis was measured with a cell-free transcription/translation coupled assay (*E. coli* S30 Extract System, Promega, Madison, WI) using pGEMbgal as a DNA template. Compounds were pre-incubated with the reaction mixture for 10 min prior to the addition of template. After 1 h incubation, ONPG was added and β-galactosidase activity was monitored at 420 nm.
19. In vivo efficacy: Mice were infected ip with approximately 1×10^5 cfu of *E. coli* ATCC25922. The compound was administered iv at 1 and 5 h after infection. Survival was monitored for 7 days and the ED_{50} calculated by nonlinear regression. Ampicillin was used as a standard in this study ($ED_{50} = 2.3$ mg/kg).
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