Rapid Combinatorial Synthesis of Aminoglycoside Antibiotic Mimetics: Use of a Polyethylene Glycol-Linked Amine and a Neamine-Derived Aldehyde in Multiple Component Condensation as a Strategy for the Discovery of New Inhibitors of the HIV RNA Rev Responsive Element

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Abstract: A library of neomycin B mimetics has been prepared rapidly without chromatography using a neamine-derived aldehyde, *tert*-butyl isocyanide or isocyanoacetic acid methyl ester, a glycine-conjugated polyethylene glycol (PEG) methyl ether, and various Cbz-N-protected amino acids as substrates in a Ugi-type one-pot reaction. The product linked to PEG was isolated by precipitation in ether. A simultaneous base-catalyzed hydrolysis and de-O-acetylation followed by hydrogenation provided an easy access to a library of neomycin B mimetics, which were screened for binding to the Rev responsive element of HIV mRNA (RRE). Several products were found to be more active than neamine with the IC_{50} values in the micromolar range.

The replication of human immunodeficiency virus type 1 (HIV-1) is dependent on the function of the viral transactivator protein Rev.[1,2] Rev acts in the nucleus through the recognition of a highly structured target RNA sequence (Rev response element, RRE) by an arginine-rich sequence in the N-terminus of the protein ($K_d \approx 1$ nM for the protein−RNA interaction).[3] Thus blocking this highly specific interaction by a small molecule such as an aminoglycoside is an attractive strategy for the inhibition of HIV.[4] Among many aminoglycosides that have been examined, neomycin B (**1**) is by far the most effective inhibitor (IC_{50} = 0.1 to 1 μM), and this aminoglycoside antibiotic has been shown to compete with Rev for binding to RRE (Figure 1).[1]

Direct use of neomycin B as an inhibitory drug, however, has been discouraged due in large part to its toxicity.[5] In addition, neomycin B is relatively unstable (the ribosyl glycosidic bond is sensitive to acids) and, like many other aminoglycoside antibiotics, is prone to enzymatic modifications *in vivo* (e.g. phosphorylation and acetylation) that leads to the problem of drug resistance.[6a] Therefore, it is highly desirable to find compounds which are less toxic, more stable, and more active than neomycin B.[6b]

Although **1** is a fairly good inhibitor of the Rev−RRE interaction by binding to the RRE, neamine (**2**) alone interacts only weakly with the mRNA ($IC_{50} \approx 100$ μM) and neobiosamine (**3**) has no inhibitory activity (Figure 2). Furthermore, **2** contains the *trans*-1,3-hydroxy amine and 1,3-diamine components that are common structures of many aminoglycosides that exhibit inhibitory activity against other RNAs andribozymes.[6c] For these reasons we intend to develop effective and rapid synthetic methodologies for the preparation of a library of compounds containing neamine as a common core and screen this library of aminoglycoside mimetics for binding to RRE and other specific RNA sequences as an approach to the development of novel antiviral agents and ribozyme inhibitors.

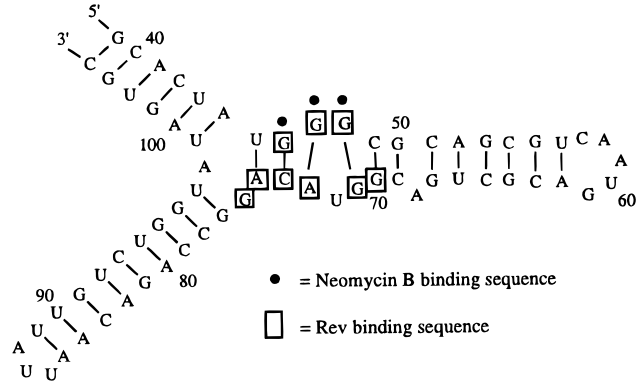

Figure 1. The RRE region of the HIV mRNA interacts competitively with Rev and neomycin B.

In a representative synthesis of a neomycin B mimetic library, refluxing neomycin B in acidic methanol (1 N HCl in methanol) gave neamine **2** (81%) and neobiosamine **3** (72%) in the

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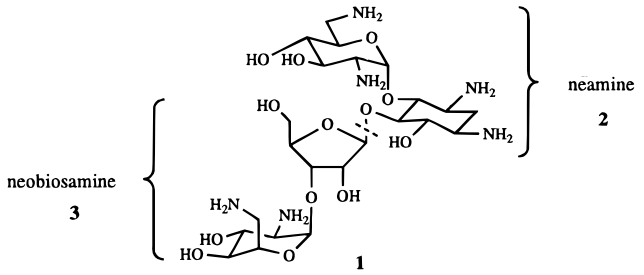


Figure 2. Structure of neomycin B (**1**) and its substructures neamine **2** and neobiosamine **3**.

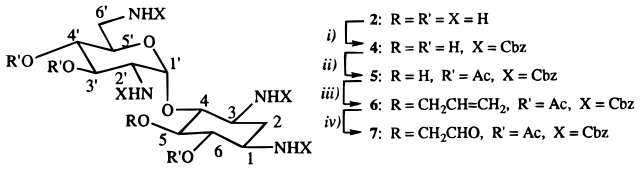


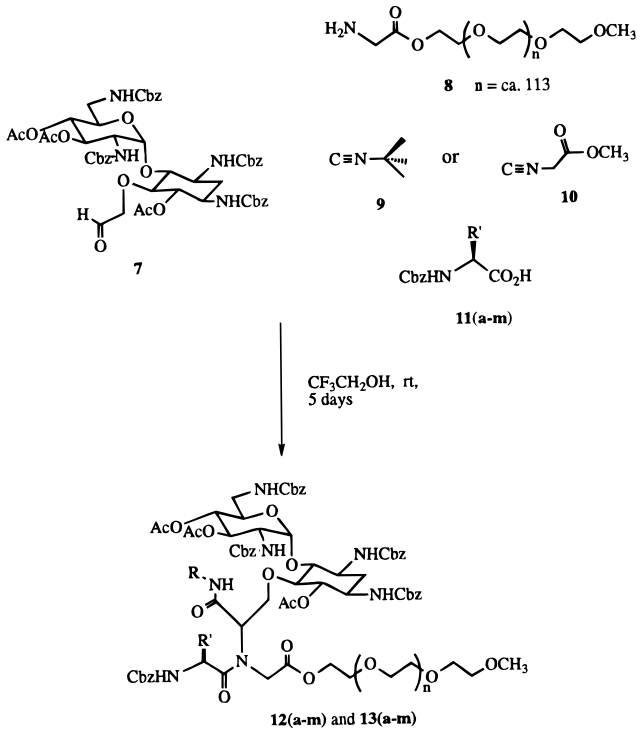
Figure 3. Conditions: (i) CbzCl, acetone, saturated Na_2CO_3(aq), toluene, 0 °C, 86%; (ii) Ac_2O, 10% (v/v) DMF/py, room temperature, overnight, 78%; (iii) allyl bromide, *n*-Bu_4NI, $(Me_3Si)_2NLi$, DMSO, room temperature, 76%; (iv) O_3, DCM, −76 °C, 95%.

hydrochloride forms.[7] The acid lability of the α-glycosidic linkage between **2** and **3** comes from the lack of the stabilization effect assisted by hydrogen bonding from the neighboring amino group as seen in the other α-glycosidic linkages of neomycin B.[5] Upon evaporation, neamine **2** crystallized out while neobiosamine **3** remained in solution. The amino groups of neamine were then protected with the benzyloxycarbonyl (Cbz) group to give **4**. Acetylation of **4** was performed in a 1:9 mixture of DMF and pyridine to give a single product with only the 5-OH group free (**5**) in 78% yield. This regioselective acetylation is believed to originate from both steric hindrance of the OH at C-5 and its hydrogen bonding with the NH at C-2′ (Figure 3). Allylation of **5** in the presence of lithium bis-(trimethylsilyl)amide as base in DMSO gave the desired allylated product **6**.[8] This alkylation step facilitates not only further functionalization of the allyl group of the neamine derivative but also provides a new opportunity for the synthesis of neamine derivatives which may be screened for stable neomycin B mimetics.

Further transformation of **6** to aldehyde **7** was achieved by ozonolysis in 95% yield. An Ugi-type of multiple component condensation (MCC)[9,10] was then used to construct the library. Boc-N-protected glycine was coupled to the PEG methyl ether (MW *ca.* 5000) with DCC in the presence of DMAP to give the product in 88% yield. Treatment of this product with TFA followed by a brief exposure to the basic resin Amberlite 400 (OH^- form) in methanol gave the free amine-containing compound **8**.

Two isocyanides were used in two sets of the MCC: *tert*-butyl isocyanide **9** and isocyanoacetic acid methyl ester **10**. The latter was prepared by N-formylation of glycine followed by dehydration.[11] In the first MCC set, the neamine derivative **7**,

Scheme 1. Synthesis of Neomycin B Mimetics Using Four-Component Condensation[a]



[a] **11a** Gly, **11b** Ala, **11c** Val, **11d** Phe, **11e** Trp, **11f** His, **11g** Tyr, **11h** Thr, **11i** Ser, **11j** Asp, **11k** Gln, **11l** Lys, and **11m** Arg (side chains of some amino acids were also protected with Cbz, e.g. Lys or Arg). **12**: R = *tert*-butyl. **13**: R = $CH_2C(O)OCH_3$ and R′ = side chain of the corresponding amino acid (**a**−**m**).

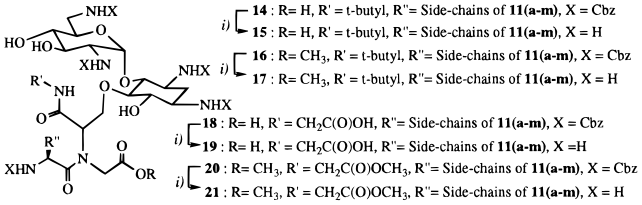


Figure 4. Structures of the peptido aminoglycosides **15a**−**m**, **17a**−**m**, **19a**−**m**, and **21a**−**m** as neomycin B mimetics. The removal of Cbz (i) group was carried out by hydrogenation (10% Pd−C in AcOH) as the final step.

tert-butyl isocyanide **9**, and the glycine-PEG derivative **8** were reacted with various N-protected amino acids **11a**−**m** (Scheme 1). Similarly, a second library was prepared using isocyanoacetic acid methyl ester **10** instead of **9**.

The individual condensation products **12a**−**m** and **13a**−**m** in the reaction mixture were easily isolated by precipitation with ether and then treated with either LiOH in 10% (v/v) H_2O/MeOH or NaOMe solution in methanol (1 M) to give **14a**−**m** and **18a**−**m** or **16a**−**m** and **20a**−**m**, respectively. This treatment conveniently released the peptido aminoglycosides from the PEG as well as the acetyl groups. The PEG units were removed from the desired products by precipitation, and the filtrates were then concentrated by evaporation. These products were hydrogenated with 10% Pd−C in AcOH to give the corresponding peptido aminoglycosides **15a**−**m**, **17a**−**m**, **19a**−**m**, and **21a**−**m**, respectively in the AcOH salt forms as shown in Figure 4.

Chromatography was not used in the isolation of products from these reactions. Each of the final products **15a**−**m**, **17a**−**m**, **19a**−**m**, and **21a**−**m** was relatively pure (>90% purity) based on the mass analysis. Without further purification, each

(7) Rinehart, K. L., Jr. *The Neomycins and Related Antibiotics*; John Wiley and Sons, Inc.: New York, 1964; p 93.

(8) Allylation of **5** with $H_2C{=}CHCH_2Br$ in the presence of NaOH and DMF at refluxing temperature for 4 h or in the presence of NaH and DMF for 1 h failed. Allylation of **5** with $H_2C{=}CHCH_2Br$ in the presence of Bu_4NI, $LiN(SiMe_3)_2$, and DMF at rt for 1 h gave predominantly the de-O-acetylated products.

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Table 1. Percent Inhibition of the Rev−RRE Interaction with 200 μM Concentration of Peptido Aminoglycosides **15a**−**m**, **17a**−**m**, **19a**−**m**, and **21a**−**m**,[13] 70.5% inhibition was observed with 200 μM **1**

	a	b	c	d	e	f	g	h	i	j	k	l	m
15	<5	14.5	12	55	8	37	32	39	58	91	41	52	47.5
17	<5	17.5	<5	36	<5	<5	66.5	39	48.5	<5	<5	<5	<5
19	74.5	24.5	72	36.5	78	71	54	87	60.5	81.5	81	24	19
21	70.5	<5	53	<5	<5	41.5	55	65	41.5	51	56	16.5	<5

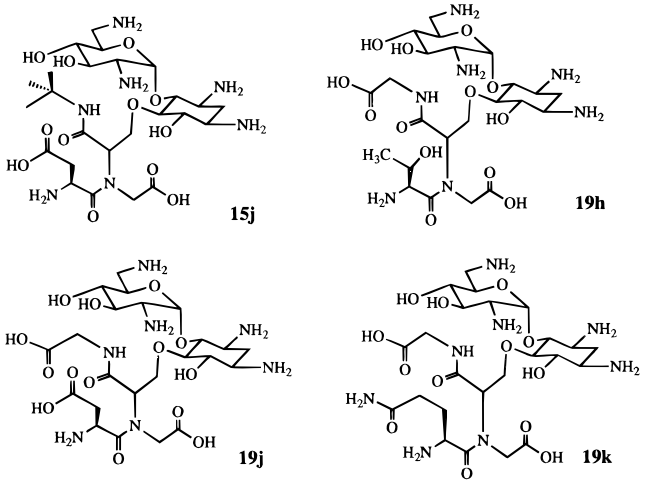

Figure 5. Structures of the peptido aminoglycosides **15j** and **19h**,**j**,**k** which are active to inhibit the interaction between Rev and mRNA of HIV.

Table 2. Percent Inhibition of the Resynthesized and Purified Active Peptido Aminoglycosides **15j**, **19h**, **19j**, and **19k** at 200 μM

compd	% inhibn	compd	% inhibn
15j	91	**19j**	85
19h	87	**19k**	81

product was initially tested for its potential to inhibit the interaction of the full-length RRE (252 nucleotides) with HIV-1 Rev in a standard "filterbinding assay", in which recombinant Rev protein (13 kDa) was bound to the radio labeled RRE RNA transcripts.[12] The results are shown in Table 1.

These results suggest that the most potent compounds of the series require a free carboxylic acid moiety instead of a methyl ester at position R. A smaller functionality at R′ is preferred to the *tert*-butyl group. This result also indicates that the side chain R″ may interact more efficiently with the RRE if R′ is less hindered: Thr, Asp, and Gln in series **19** (Figure 5) were found to be active while only Asp in series **15** showed more than 80% inhibition. In comparison, of the 52 compounds tested, nine of them showed activity as good as or better than neomycin B (70.5%) and were much better than neamine and 4′-deoxyneamine (10.5% and <5%, respectively, at 200 μM in the filterbinding assay).

In order to confirm these initial screening results compounds **15j**, **19h**, **19j**, and **19k** were resynthesized, purified, and retested for their binding affinities. The results are tabulated in Table 2.

The percent inhibitions of the resynthesized compounds are consistent with those of the earlier results as shown in Table 1. All of theses compounds showed slightly better activity than that of neomycin B.

In summary we have presented a new method for the rapid synthesis of aminoglycoside mimetics using a neamine-derived aldehyde and a PEG-linked amine as key substances in the multiple component Ugi condensation. No chromatography is necessary, and each final product is pure enough for characterization and evaluation of biological activity. The new stereogenic center formed in the reaction is greater than 90% diasteromerically pure. The procedure reported here is superior to solution- or solid-phase methods.[13] The use of polyethylene glycol holds the advantages of both solution- and solid-phase chemistry, and the desired product can be easily isolated by precipitation with ether.[14] Monitoring the progress of the reaction is also easy because the NMR spectral data can be obtained without cleaving the product from the polymer.[15] In addition several different solvents such as trimethyl orthoformate,[16] 2,2,2-trifluoroethanol, or methanol[10] can be used as reaction media. This is particularly beneficial for the Ugi-type reactions since the solvents are known to promote the Schiff base formation.

Work is in progress to screen other members of the library and study the detailed course of actions of the active peptido aminoglycosides.

General Methods

A Bruker AMX-400 spectrometer was used for 400 MHz ^{1}H NMR and 100 MHz ^{13}C NMR analyses. High-resolution mass spectra (HRMS) were obtained on a VG ZAB-ZSE mass spectrometer. For the MS of the compounds that are obtained from the MCC, normal molecular ion peaks ($M + H^+$, $M - H^+$, $M + Na^+$, or $M + Cs^+$) were recorded.

A representative procedure of the filterbinding assay used in the study is described as follows: Rev−RRE complexes were detected on nitrocellulose filters and were subsequently quatitated by measuring the retained radioactivity on the filter. The peptido aminoglycosides were dissolved in a 1:1 mixture of DMSO and H_2O at a concentration of 10 mM. The inhibitory potency of the neomycin B mimetic on the Rev−RRE interaction was analyzed by preincubation of the RRE (8 pM) with 200 μM testing compounds. After 10 min Rev protein was added to a final concentration of 3.6 nM in a total volume of 250 μL (2% DMSO). The incubation proceeded for additional 15 min. Subsequently an aliquot of 200 μL was filtered through a prewetted nitrocellulose filter using a S&S MINIFOLD filtration unit without

(12) (a) Daly, T. J.; Cook, K. S.; Gray, G. S.; Maione, T. E.; Rusche, J. R. *Nature* **1989**, *342*, 816. (b) Daly, T. J.; Doten, R. C.; Rennert, P.; Auer, M.; Jaksche, H.; Donner, A.; Fisk, G.; Rusche, J. R. *Biochemistry* **1993**, *32*, 10479.

(13) (a) For comparison, the four-component condensation reaction was carried out using a β-alanine-linked Wang resin as the amine component, **7** as an aldehyde, and *tert*-butyl isocyanide and Cbz-N-protected lysine as an acid in MeOH, trimethyl orthoformate, or 2,2,2-trifluoroethanol. None of the solvents provided an adequate solvation for the reaction. The same reaction was then carried out in 30% (v/v) of each of the above solvents in DMF and a significantly lowered reactivity was observed. Because DMF is not a good solvent for the reaction as a Schiff base formation is required. After 7 days ca. 30% of the unreacted β-alanine was recovered from the cleaved products. In addition, it is difficult to monitor the reaction progress using the solid-phase method unless the intermediate is cleaved from the support. The solution-phase MCC requires chromatography to isolate the desired product. (b) For recent developments on solid-phase combinatorial chemistry, see: Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555. Armstrong, R. W.; Combs, A. P.; Tempest, P. A.; Brown, S. D.; Keating, T. A. *Acc. Chem. Res.* **1996**, *29*, 123. Keating, T. A.; Armstrong, R. W. *J. Am. Chem. Soc.* **1996**, *118*, 2574.

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(15) Douglas, S. P.; Whitfield, D. M.; Krepinsky, J. J. *J. Am. Chem. Soc.* **1991**, *113*, 5095.

(16) Look, G. C.; Murphy, M. M.; Campbell, D. A.; Gallop, M. A. *Tetrahedron Lett.* **1995**, *36*, 2937.

washing. After filtration the membrane was removed, dried at 45 °C for 20 min, and counted using a Packard Matrix 96 direct beta counter. All measurements were performed in 96-well microtiter plates and duplicated minimum values.

Neamine 2. A solution of **1** (20.0 g, 20.8 mmol) in methanol (500 mL) was brought to boil, to which a concentrated HCl (18 g, 12.1 N) was added dropwise. The solution was refluxed for 6 h before all reactants were completely consumed. The reaction solution was cooled to room temperature to give a light-yellow solution. This solution was then evaporated to approximately half of its volume and cooled to 0 °C. The solid precipitate was filtered to give the desired product **2**: yield 7.8 g, 17.1 mmol, 82%; ^{1}H NMR (D_2O) δ 5.94 (d, $J_{1',2'} = 3.8$ Hz, 1H, H-1′), 4.01−4.06 (m, 3H, H-5′,5,3′), 3.47−3.53 (m, 1H, H-4), 3.36 (m, 1H, H-1), 3.28 (ddd, $J_{3,4} = 4.5$ Hz, 1H, H-3), 2.52 (ddd, $J_{2eq,1} = J_{2eq,3} = 2.6$ Hz, 1H, H-2$_{eq}$), 1.94 (ddd, $J_{2ax,1} = J_{2ax,3} = 7.9$, $J_{2ax,2eq} = 15.7$ Hz, 1H, H-2$_{ax}$); ^{13}C NMR (D_2O) δ 102.0, 83.53, 81.36, 78.65, 76.98, 75.38, 59.74, 55.94, 54.66, 46.45, 34.43; ESI for $C_{12}H_{26}N_4O_6$ calcd 323 (M + H$^+$), found 323.

Compound 4. To an aqueous saturated Na_2CO_3 solution (20 mL) of **2** (3.9 g, 8.36 mmole) was dropwise added at 0 °C over a half hour period a solution of CbzCl (6.40 g, 37.6 mmol) in acetone (15 mL) and toluene (2 mL). This reaction mixture was vigorously stirred overnight in a cold room (4 °C) to give a white precipitate. The precipitate was then filtered and pulverized with an HCl solution (1 N) until the filtrate became neutral. The white solid was dried for 2 days *in vacuo*: yield 6.40 g, 7.44 mmol, 86%; ^{1}H NMR (DMSO-d_6) δ 7.33−7.39 (broad m, 20 H, aromatic), 6.86−7.19 (broad s, 4H, NH), 4.89−5.20 (broad m, 9H, benzyl 4 × CH_2, H-1′), 3.11−3.73 (broad m, 16H), 1.76 (broad m, 1H, H-2eq), 1.34 (broad dd, $J_{2ax,1} = J_{2ax,3} = 8.25$, $J_{2ax,2eq} = 15.9$ Hz, 1H, H-2ax); ^{13}C NMR (DMSO-d_6) δ 156.6, 156.3, 155.8, 142.6, 137.4, 137.3, 137.1, 128.7, 128.6, 128.4, 128.1, 127.8, 127.5, 126.7, 126.5, 98.7, 76.6, 74.2, 71.5, 70.7, 65.4, 65.3, 65.2, 62.9, 56.2, 51.3, 50.4, 42.1, 36.1; HRMS for $C_{44}H_{50}N_4O_{14}$ (M + Cs) calcd 991.2378, found 991.2348.

Compound 5. A solution of acetic anhydride (0.808 mL, 8.57 mmol, 3.6 equiv) in DMF (3 mL) was added through a pressure-equalized dropping funnel to a solution of **4** (2.04 g, 2.38 mmol) in pyridine (4 mL, 20 equiv) at ambient temperature. The solution was stirred overnight to result in a light-yellow solution. This solution mixture was acidified with an aqueous HCl solution (1 N), diluted with EtOAc (30 mL), neutralized with a saturated Na_2CO_3 solution (2 × 10 mL), and washed with water (2 × 10 mL) and a saturated NaCl solution (10 mL). The organic layer was then dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure to give a white precipitate before all the sovent was removed. This white solid was isolated by filtration: yield 1.82 g, 1.85 mmol, 78%; ^{1}H NMR ($CDCl_3$) δ 7.20−7.31 (m, 20 H, aromatic), 5.48, 5.65, 5.83 (broad s, each 1H, NH), 4.94−5.34 (m, 10 H), 4.84 (m, 3H), 4.91 (m, 1H), 4.70 (m, 1H), 3.65 (m, 3H), 3.94 (m, 2H), 3.48 (m, 1H), 3.34 (m, 1H), 3.24 (m, 1H), 2.96 (m, 1H), 2.12 (broad d, 1H, H-2$_{eq}$), 1.74, 1.82, 1.93 (s, each 3H, CH_3), 1.48 (broad m, 1H, H-2$_{ax}$); ^{13}C NMR ($CDCl_3$) δ 169.9, 169.8, 169.2, 156.4, 156.0, 155.7, 137.4, 137.3, 137.2, 137.0, 128.4, 127.8, 127.7, 127.5, 127.4, 98.2, 81.4, 76.4, 73.6, 71.7, 68.7, 68.0, 65.4, 65.3, 65.1, 53.7, 50.0, 48.8, 34.1, 20.9, 20.5, 20.4; HRMS for $C_{50}H_{56}N_4O_{17}$ (M + Cs) calcd 1117.2695, found 1117.2664.

Compound 6. To a solution of **5** (1.0 g, 1.02 mmol) and tetrabutyl ammonium iodide (420 mg, 1.13 mmol) in DMSO (3 mL) was added allyl bromide (112 μL, 1.20 equiv). The reaction mixture was stirred for 5 min at room temperature. While the solution was vigorously stirred, lithium bis(trimethylsilyl)amide (1.3 mL, an 1 M solution in THF) was added at once. The reaction was complete within half an hour. The reaction mixture was then diluted with EtOAc (30 mL) and washed with HCl (1 N, 2 × 20 mL), saturated $NaHCO_3$ (2 × 20 mL), water (2 × 20 mL), and saturated NaCl (20 mL). The organic phase was then dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure to give a crude product. The desired product was isolated by column chromatography using a 1:9 mixture of actone and toluene: yield 0.790 g, 0.771 mmol, 76%; ^{1}H NMR ($CDCl_3$) δ 7.26−7.33 (m, 20H, aromatic), 5.48, 5.73 (s, each 1H, NH), 4.70−5.15 (m, 16 H), 3.85−4.10 (m, 7H), 2.90−3.48 (m, 4H), 2.06 (m, 1H, H-2$_{eq}$), 1.81, 1.87, 1.93 (s, each 3H, CH_3), 1.28 (m, 1H, H-2$_{ax}$); ^{13}C NMR ($CDCl_3$) δ 171.1, 170.6, 169.8, 156.6, 155.8, 155.6, 136.3, 136.2, 133.4, 128.6, 128.5, 128.4, 128.1, 128.0, 117.7, 98.3, 81.6, 79.4, 77.6, 75.7, 74.1, 70.9, 69.8, 68.8, 66.8, 66.7, 54.0, 50.2, 50.0, 41.6, 34.1, 20.8, 20.5, 20.4; HRMS for $C_{53}H_{60}N_4O_{17}$ (M + Cs) calcd 1157.3008, found 1157.3050.

Compound 7. A solution of **6** (1.00 g, 0.977 mmol) in dichloromethane (20 mL) was cooled to −76 °C. Ozone gas was passed through the solution at that temperature until it became a light-blue color. The color was diminished as oxygen gas was bubbled through the solution. The reaction was quenched with triphenyl phosphine (0.384 g, 1.47 mmol, 1.5 equiv), and the resutant reaction solution was evaporated under reduced pressure to give a white solid. The desired product was obtained by column chromatography using a 7:3 mixture of toluene and acetone as eluent: yield 0.952 g, 0.928 mmol, 95%; ^{1}H NMR ($CDCl_3$) δ 9.24 (s, 1H, HC(O)), 7.24−7.30 (m, 20 H, aromatic), 5.42 (m, 1H, NH), 4.70−5.15 (m, 11H), 2.98−4.40 (m, 11H), 2.10 (m, 1H, H-2$_{eq}$), 1.74, 1.81, 1.84 (s, each 3H, CH_3), 1.28 (m, 1H, H-2$_{ax}$); HRMS for $C_{52}H_{58}N_4O_{18}$ (M + Cs) calcd 1159.2800, found 1159.2844.

Compound 8. To a solution of polyethylene glycol methyl ether (MW *ca.* 5000, 1.12 g) in DCM was added *t*-BocGlyOH (38.5 mg, 0.22 mmol), DCC (45.4 mg, 0.22 mmol), and DMAP (26.9 mg, 0.22 mmol). The reaction mixture was stirred at room temperature overnight. A white solid that formed during the reaction was filtered, and the filtrate was evaporated under reduced pressure to give a fluffy white solid. This solid (1.15 g) was then redissolved in DCM and treated with 95% TFA (2 mL) at ambient temperature for 1 h. The reaction mixture was evaporated and further dried *in vacuo* overnight to give **8** as a white solid in a TFA salt form:[17] yield 1.15 g; ^{1}H NMR ($CDCl_3$) δ 3.68 (2H, glycyl CH_2), 3.66 (broad s, *ca*. 450H, PEG-CH_2), 3.33 (s, 3H, OCH_3).

Compound 10. To an ethyl formate solution (120 mL) containing glycine methyl ester monohydrochloride (20.0 g, 0.159 mol) was added *p*-TsOH (20 mg), and the solution was brought to boil. Boiling triethylamine (1.1 equiv) was added dropwise, and the reaction mixture was refluxed overnight. The reaction mixture was then cooled to room temperature. A white triethylamine hydrochloride salt was filtered, and the filtrate was concentrated to ca. 30 mL. The resultant solution was further cooled to −5 °C and filtered. The filtrate was concentrated to afford a light brown liquid. Vacuum distillation of this liquid gave formylacetic acid methyl ester as a colorless oil at 105 °C, 0.15 mmHg: yield 17.7 g, 0.151 mol, 95%. To the solution of formyl acetic acid methyl ester (10.0 g, 85.4 mmol) and triethylamine (2.5 equiv) in DCM (200 mL) was added $POCl_3$ (1.0 equiv) dropwise at 0 °C. The solution turned red immediately. After all of the $POCl_3$ was added, the reaction mixture was stirred for an additional 1 h at room temperature. To the reaction mixture was slowly added a Na_2CO_3 solution (10 g, 200 mL of H_2O). The reaction mixture was stirred for $^1/_2$ h. The organic phase was separated from aqueous phase, washed with a saturated NaCl solution, and dried over K_2CO_3. The resultant solution was then filtered, and the filtrate was evaporated under reduced pressure to give a dark brown oil. The final product (liquid) was obtained from a fractional distillation (54 °C, 0.15 mmHg): yield 6.22 g, 61.5 mmol, 72%; ^{1}H NMR ($CDCl_3$) δ 4.21 (s, 3H, CH_3), 3.77 (d, 2H, glycyl CH_2); ^{13}C NMR ($CDCl_3$) δ 161.1, 164.4, 53.3, 43.3; FABMS for $C_4H_5NO_2$ (M + Cs$^+$) calcd 231.95, found 231.

A representative MCC: **7** (70 mg, 0.0682 mmol) and **8** (300 mg, ∼0.060 mmol) were dissolved in degassed 2,2,2-trifluoroethanol (500 mL), and the mixture was stirred at room temperature for 2 h. To this solution were added **9** (5.7 mg, 0.0682 mmol) and **11a** (14.3 mg, 0.0682 mmol). The reaction mixture was stirred at ambient temperature for 5 days. The isolation of the desired product was accomplished by precipitation and filtration with cold ether. The precipitated product was dried *in vacuo* to give **12a** as a white solid (290 mg). At this stage the product was divided into two portions to carry out further modifications.

(i) **12a** (140 mg) was dissolved in THF (1 mL) at 0 °C, and LiOH solution (0.2 mL, 1 N, 10% (v/v) H_2O/MeOH) was added to the solution. The mixture was then stirred for 4 h while the temperature was slowly elevated to room temperature. After the solution was neutralized with acidic resin (Amberlite IR 120) and filtered, the filtrate

(17) Trifluoroacetic acid as a salt was removed by treating the PEG derivative with a basic ion-exchange resin (Amberlite IRA-400(OH)) in methanol for less than 5 min.

was evaporated under reduced pressure to afford a white solid. The solid was then washed with cold ether (2 × 10 mL), and insoluble PEG material was removed by filtration. The filtrate was then concentrated and further dried *in vacuo* to give **14a** as a white solid (17 mg). This product was then directly used for the next step without further purification. ESI for $C_{64}H_{77}N_7O_{20}$ ($M - H^+$), calcd 1234, found 1234.

To a solution of **14a** (15 mg, 0.0259 mmol) in acetic acid (glacial, 2 mL) was added 10% Pd/C (10 mg). The solution was stirred under hydrogen at ambient temperature for 3 h. The reaction mixture was then filtered through a Celite bed, and the filtrate was concentrated under reduced pressure to give **15a** as light-yellow syrup: yield 20 mg, 0.0228 mmol, 88% (based on the assumtion that five acetic acid molecules are complexed with a molecule of **15a**); ESI for $C_{23}H_{45}N7O_{10}$ ($M - H^+$) calcd 578.3, found 578.

(ii) To the PEG-linked compound **12a** (140 mg) in MeOH (1 mL) was added NaOMe (100 mL, 1 M, MeOH) at 0 °C for 3 h. After the solution was neutralized with an acidic resin (Amberlite IR 120) and filtered, the filtrate was evaporated under reduced pressure to afford a white solid. The solid was then washed with cold ether (2 × 10 mL), and insoluble PEG material was removed by filtration. The filtrate was then concentrated and further dried *in vacuo* overnight to give **16a** as a white solid (13 mg, 0.0103 mmol). FABMS for $C_{64}H_{78}N_7O_{20}$ ($M + Cs^+$) calcd 1396.9, found 1396. The solution of **16a** was stirred under hydrogen at ambient temperature for 3 h. The reaction mixture was filtered through a Celite bed, and the filtrate was concentrated under reduced pressure to give **17a** as light-yellow syrup: yield 9 mg, 0.0101 mmol, 98% (based on the assumption that five acetic acid molecules are complexed with a molecule of **17a**); FABMS for $C_{24}H_{47}N_7O_{10}$ ($M + Cs^+$) calcd 725, found 725.

The physical data of some other products obtained from the multiple-component condensation are as follows.

15k: FABMS for $C_{26}H_{50}N_8O_{11}$ ($M + Cs$) calcd 782, found 782; ($M + H^+$) 650.

16a: FABMS for $C_{64}H_{78}N_7O_{20}$ ($M + Cs$) calcd 1396.9, found 1396.

16b: FABMS for $C_{65}H_{79}N_7O_{20}$ ($M + Cs$) calcd 1410.4, found 1410.

16c: FABMS for $C_{67}H_{83}N_7O_{20}$ ($M + Cs$) calcd 1438.5, found 1438.

16d: FABMS for $C_{71}H_{83}N_7O_{20}$ ($M + Cs$) calcd 1486.5, found 1486.

16e: FABMS for $C_{73}H_{83}N_8O_{20}$ ($M + Cs$) calcd 1524.5, found 1525.

16f: FABMS for $C_{68}H_{81}N_9O_{20}$ ($M + Cs$) calcd 1476.5, found 1476.

16j: FABMS for $C_{67}H_{81}N_7O_{22}$ ($M + Cs$) calcd 1468.0, found 1468.

16k: FABMS for $C_{67}H_{82}N_8O_{21}$ ($M + Cs$) calcd 1467.5, found 1467.

16l: FABMS for $C_{76}H_{92}N_8O_{22}$ ($M + Cs$) calcd 1601.5, found 1601.

16m: FABMS for $C_{84}H_{98}N_{10}O_{24}$ ($M + Cs$) calcd 1630.6, found 1630.

17a: FABMS for $C_{24}H_{47}N_7O_{10}$ ($M + Cs$) calcd 725, found 725.

17c: FABMS for $C_{26}H_{50}N_8O_{11}$ ($M + Cs$) calcd 767, found 736 ($M + Cs^+ - OCH_3$).

17g: FABMS for $C_{31}H_{52}N_7O_{11}$ ($M + Cs$) calcd 831, found 831.

21b: FABMS for $C_{64}H_{75}N_7O_{22}$ ($M + Cs$) calcd 1426, found 1426.

21c: FABMS for $C_{66}H_{79}N_7O_{22}$ ($M + Cs$) calcd 1454, found 1454.

21d: FABMS for $C_{70}H_{79}N_7O_{22}$ ($M + Cs$) calcd 1502, found 1502.

21e: FABMS for $C_{72}H_{79}N_8O_{22}$ ($M + Cs$) calcd 1541, found 1409 ($M + H^+$).

21f: FABMS for $C_{66}H_{75}N_9O_{22}$ ($M + Cs$) calcd 1478, found 1478.

21i: FABMS for $C_{64}H_{75}N_7O_{23}$ ($M + Cs$) calcd 1310.5, found 1310.

21k: ESI for $C_{66}H_{78}N_8O_{23}$ ($M + H^+$) calcd 1352, found 1353 ($M + H^+$); 1375 ($M + Na^+$).

21l: FABMS for $C_{74}H_{85}N_8O_{24}$ ($M + Cs$) calcd 1603.5, found 1603.

15e: ^{1}H NMR (D_2O) δ 7.12−7.51 (m, 4H, aromatic), 5.61 (d, $J_{1',2'}$ = 2.5 Hz, 1H, H-1′), 2.36−2.40 (m, 1H, H-2_{eq}), 1.73 (m, 1H, H-2_{ax}), 1.28−1.32 (m, 2H, indolyl CH_2), 1.21−1.27 (m, 9H, *t*-Bu).

15l: ^{1}H NMR (D_2O) δ 5.75 (d, $J_{1',2'}$ = 2.5 Hz, 1H, H-1′), 2.95−3.00 (m, 2H, lysyl CH_2), 2.40−2.43 (m, 1H, H-2_{eq}), 1.79−1.86 (m, 2H, lysyl CH_2), 1.66−1.72 (m, 1H, H-2_{ax}), 1.45 (m, 2H, lysyl CH_2), 1.27−1.31 (m, 11H, *t*-Bu, lysyl CH_2).

17b: ^{1}H NMR (D_2O) δ 5.77 (d, $J_{1',2'}$ = 2.5 Hz, 1H, H-1′), 3.28 (s, 3H, OCH_3), 2.36−2.40 (ddd, $J_{2eq,2ax}$ = 14.4 Hz, 1H, H-2_{eq}), 1.71−1.78 (m, 1H, H-2_{ax}), 1.42−1.46 (m, 3H, alanyl CH_3), 1.29−1.31 (m, 9H, *t*-Bu).

17m: ^{1}H NMR (D_2O) δ 5.74 (d, $J_{1',2'}$ = 2.5 Hz, 1H, H-1′), 3.28 (s, 3H, OCH_3), 2.36−2.40 (m, 1H, H-2_{eq}), 2.03 (m, 2H, guanidyl CH_2), 1.73 (m, 1H, H-2_{ax}), 1.67−1.70 (m, 2H, guanidyl CH_2), 1.25−1.34 (m, 11H, *t*-Bu, guanidyl CH_2).

Preparation of Active Compounds 15j, 19h, 19j, and 19k.[18] **Compound 16j.** To a solution of **7** (200 mg, 0.195 mmol) in 2,2,2-trifluoroethanol (1.0 mL) was added glycine methyl ester (14 mg, 0.156 mmol) at room temperature. The solution was stirred for 2 h before *tert*-butyl isocyanide (13 mg, 18 μL, 0.156 mmol) and CbzAsp(OBn)-OH (**11j**, 55.7 mg, 0.156 mmol) were added. The reaction mixture was stirred for additional 5 days at room temperature to complete. The mixture was then poured into a 1 N HCl and extracted from ethyl acetate. The organic layer was washed with 1 N $NaHCO_3$ solution and water followed by saturated NaCl. The organic layer was dried over $MgSO_4$ and filtered. The filtrate was then concentrated under reduced pressure to give a light-yellow crude product. The desired product **16j** was isolated by silica gel column chromatography using 9:1 and 8:2 gradient solvent mixtures of toluene and acetone: yield 170.6 mg, 0.114 mmol, 73%; ^{1}H NMR (CD_3OD) δ 7.20−7.64 (m, 30 H, Ph), 4.69−5.42 (m, 15H), 3.55−4.26 (m, 8 H), 3.77 (broad s, 3H, OCH_3), 3.29−3.30 (broad s, 3H, OCH_3), 1.88 (s, 3H, C(O)CH_3), 1.80 (s, 3H, C(O)CH_3), 1.28−1.33 (s, 9H, *t*-Bu); ^{13}C NMR (CD_3OD) δ 173.1, 172.8, 171.9, 171.7, 171.5, 168.2, 163.1, 159.2, 158.9, 158.4, 158.0, 157.8 (C═O), 138.4, 138.1, 138.0, 137.2, 133.8, 133.1, 133.0, 132.3, 130.1, 130.0, 129.9, 129.8, 129.6, 129.5, 129.3, 129.2, 129.1, 128.9, 128.7, 98.92, 84.9, 78.4, 78.2, 73.1, 72.9, 72.0, 71.1, 69.7, 68.7, 38.5, 68.1, 67.9, 67.8, 67.7, 67.3, 66.7, 62.7, 54.7, 53.6, 52.0, 50.9, 50.8, 49.6, 49.4, 49.2, 41.9, 37.7, 34.5, 30.8, 29.9, 29.0, 28.91, 28.9, 28.8, 28.7, 28.7, 28.6, 21.0, 20.8, 20.7; HRMS for $C_{79}H_{91}N_7O_{25}$ ($M + Cs$) calcd 1670.5119, found 1670.5139.

Compound 15j. After the above methyl ester **16j** (100 mg, 0.0650 mmol) was dissolved in THF (1 mL), a LiOH solution (0.2 mL, 1 N, 10% (v/v) H_2O/MeOH) was added to the solution at 0 °C. The mixture was then stirred for 12 h while the temperature was slowly elevated to room temperature. After the solution was neutralized with acidic resin (H^+, Amberlite IR 120) and filtered, the filtrate was evaporated under reduced pressure to afford **14j** as a white solid. The product was then isolated by a column chromatography using a 9:1 mixture of $CHCl_3$ and CH_3OH as eluent: yield 70 mg, 0.053 mmol, 82%; ESI for $C_{65}H_{77}N_7O_{22}$ ($M - H^+$) calcd 1306, found 1306. To a solution of **14j** (45 mg, 0.0344 mmol) in acetic acid (glacial, 2 mL) was added 10% Pd/C (10 mg). The solution was stirred under hydrogen at ambient temperature for 3 h. The reaction mixture was then filtered through a Celite bed, and the filtrate was concentrated under reduced pressure to give **15j** as a white solid: yield 31 mg, 0.0331 mmol, 96%; ^{1}H NMR (D_2O) δ 5.84 (d, $J_{1',2'}$ = 4 Hz, 1H, H-1′), 3.64−4.36 (m, 10 H), 2.83 (m, 1H), 2.61, (m, 1H), 2.42 (m, 1H, H-2_{eq}), 1.76 (ddd, $J_{2ax,3} = J_{2ax,3}$ = 7.9 Hz, $J_{2ax,2eq}$ = 15.6 Hz, 1H, H-2_{ax}), 1.27 (s, 9H, *t*-Bu); ^{13}C NMR (D_2O) δ 171.6, 170.9, 170.4, 170.3 (C═O), 135.6, 134.4, 131.4, 98.5, 97.2, 94.9, 80.5, 77.6, 76.6, 74.9, 74.8, 73.1, 71.9, 71.6, 70.7, 61.7, 55.9, 54.9, 52.1, 50.7, 24.8; ESI for $C_{25}H_{47}N_7O_{12}$ calcd 638 ($M + H^+$), found 638.

Compound 20h. To a solution of **7** (250 mg, 0.244 mmol) in 2,2,2-trifluoroethanol (1.0 mL) was added glycine methyl ester (22 mg, 0.244 mmol) at room temperature. The solution was stirred for 2 h before **10** (50 mg, 0.448 mmol) and **11h** CbzThr(OBn)OH (123.6 mg, 0.488 mmol) were added. After the reaction, the desired product **20h** was isolated similarly by silica gel column chromatography using 9:1 and 8:2 gradient solvent mixtures of toluene and acetone: yield 210 mg, 0.136 mmol, 56%; ^{1}H NMR (CD_3OD) δ 7.67−7.64 (m, 30H, Ph), 4.69−5.42 (m, 15H), 3.55−4.26 (m, 8 H), 3.77 (broad s, 3H, OCH_3), 1.89 (s, 3H, C(O)CH_3), 1.77 (s, 3H, C(O)CH_3), 1.59−1.61 (m, 1H), 1.27 (1H, H-2a); ^{13}C NMR (CD_3OD) δ 174.2, 172.6, 172.0, 171.5, 171.3, 171.1, 164.2, 159.1, 158.9, 158.5, 158.1, 157.8 (C═O), 139.6, 138.4, 138.3, 138.1, 133.8, 133.1, 132.2, 130.0, 129.9, 129.8, 129.5, 129.4, 129.3, 129.1, 128.9, 128.7, 99.4, 98.3, 84.7, 84.3, 83.8, 81.4, 78.6, 78.4, 78.0, 77.2, 76.5, 76.0, 75.7, 73.0, 72.9, 72.8, 72.3, 72.3, 72.2, 70.9, 70.7, 70.4, 69.9, 69.7, 69.3, 68.4, 68.1, 68.0, 67.9, 67.8, 67.7, 67.6, 67.4, 62.7, 60.7, 52.6, 50.9, 50.8, 41.9, 34.9, 34.5, 33.1, 30.4, 21.4, 21.3, 21.0, 20.8, 20.7, 17.2, 16.9, 16.7; HRMS for $C_{78}H_{89}N_7O_{26}$ ($M + Cs$) calcd 1672.4912, found 1672.4840.

(18) For the purpose of characterizations of the MCC products before deprotection, glycine methyl ester was used as the amine component instead of the PEG attached glycine.

Compound 19h. The methyl ester of **20h** was deprotected as usual to give **18h** as a white solid. The product was then isolated by a column chromatography using a 9:1 mixture of $CHCl_3$ and CH_3OH as eluent. Yield: 82 mg, 0.0592 mmol, 91%; ESI for $C_{70}H_{80}N_7O_{23}$ ($M - H^+$), calcd 1385, found 1385. To a solution of **18h** (50 mg, 0.0361 mmol) in acetic acid (glacial, 2 mL) was added 10% Pd/C (10 mg). The solution was stirred under hydrogen at ambient temperature overnight. The reaction mixture was then filtered through a Celite bed, and the filtrate was concentrated under reduced pressure to give **19h** as a white solid: yield 28 mg, 0.0303 mmol, 84%; ^{1}H NMR (D_2O) δ 5.80 (d, $J_{1',2'} = 4$ Hz, 1H, H-1′), 3.27−4.59 (m, 24 H), 2.64−2.72 (m, 3H), 2.35 (m, 1H, H-2_{eq}), 1.76 (m, 1H, H-2_{ax}); ^{13}C NMR (D_2O) δ 179.1, 178.4, 172.9, 172.0 (C═O), 98.5, 96.5, 85.2, 84.9, 80.8, 77.6, 75.9, 75.5, 74.9, 73.2, 71.7, 70.9, 70.8, 55.9, 52.4, 51.1, 50.9, 45.9, 42.5, 25.4; ESI for $C_{23}H_{49}N_7O_{13}$ calcd 626 ($M + H^+$), found 626.

Compound 20j. To a solution of **7** (165 mg, 0.161 mmol) in 2,2,2-trifluoroethanol (1.0 mL) was added glycine methyl ester (22 mg, 0.244 mmol) at room temperature. The solution was stirred for 2 h before **10** (50 mg, 0.448 mmol) and **11j** CbzAsp(OBn)OH (174.4 mg, 0.488 mmol) were added. The reaction mixture was stirred for 7 days at room temperature to complete. Following the same workup, **20j** was isolated by using 9:1 and 8:2 gradient solvent mixtures of toluene and acetone: yield 185 mg, 0.119 mmol, 71%; ^{1}H NMR (CD_3OD) δ 7.24−7.64 (m, 30H, Ph), 4.92−5.10 (m, 15H), 3.55−4.26 (m, 8H), 3.64 (broad s, 3H, OCH_3), 3.59 (broad s, 3H, OCH_3), 2.92−3.02 (m, 2H), 2.78−2.81 (m, 1H), 1.97 (s, 3H, C(O)CH_3), 1.89 (s, 3H, C(O)CH_3), 1.77 (s, 3H, C(O)CH_3), 1.21−1.26 (m, 3H), 0.78−0.88 (m, 4H); ^{13}C NMR (CD_3OD) 173.2, 172.4, 172.3, 172.2, 171.9, 171.6, 171.5, 171.0, 169.2, 164.1, 158.9, 158.6, 158.5, 158.4, 158.1, 138.6, 138.4, 138.1, 138.0, 137.9, 137.3, 137.2, 133.8, 133.2, 133.1, 130.2, 130.1, 130.0, 129.9, 129.7, 129.6, 129.3, 129.1, 129.0, 126.4, 97.74, 84.2, 77.7, 77.5, 77.0, 76.1, 75.3, 72.7, 72.4, 71.9, 70.8, 70.6, 69.8, 68.2, 68.1, 68.0, 68.0, 65.3, 62.4, 53.2, 52.8, 51.1, 51.0, 43.1, 42.5, 40.7, 37.5, 37.1, 36.8, 34.7, 33.1, 30.8, 30.5, 30.4, 28.2, 27.9, 24.7, 21.6, 21.5, 20.9, 20.8, 20.4; HRMS for $C_{78}H_{87}N_7O_{27}$ ($M + Cs$) calcd 1686.4704, found 1686.4755.

Compound 19j. After **20j** (60 mg, 0.0386 mmol) was treated with base as usual, compound **18j** was obtained (50 mg, 0.0337 mmol) and hydrogenated for 3 h. The reaction mixture was then filtered through a Celite bed, and the filtrate was concentrated under reduced pressure to give **19j** as a white solid: yield 22 mg, 0.0234 mmol, 70%; ^{1}H NMR (D_2O) δ 5.79 (d, $J_{1',2'} = 4$ Hz, 1H, H-1′), 3.99−4.59 (m, 2H), 3.00−4.20 (m, 21H), 2.64−2.72 (m, 3H), 2.35 (m, 1H, H-2_{eq}), 1.70 (m, 1H, H-2_{ax}); ^{13}C NMR (D_2O) δ 179.1, 178.4, 176.9 (C═O), 159.9, 97.6, 85.6, 79.7, 78.8, 75.7, 75.1, 73.2, 73.0, 71.6, 70.7, 56.0, 54.6, 45.4, 42.4, 38.9, 31.8; ESI for $C_{23}H_{41}N_7O_{14}$ calcd 640 ($M + H^+$), found 640.

Compound 20k. To a solution of **7** (150 mg, 0.146 mmol) in 2,2,2-trifluoroethanol (1.0 mL) was added glycine methyl ester (22 mg, 0.244 mmol) at room temperature. The solution was stirred for 2 h before **10** (50 mg, 0.448 mmol) and **11k** CbzGlnOH (137 mg, 0.488 mmol) were added. The reaction mixture was stirred for 7 days at room temperature to complete. After workup and isolation, the desired product **20k** was obtained: yield 123 mg, 0.0833 mmol, 57%; ^{1}H NMR (CD_3OD) δ 6.93−7.48 (m, 30H, Ph), 4.81−5.23 (m, 16H), 3.29−4.06 (m, 28H), 3.68, 3.66 (s, 3H ea, C(O)OCH_3), 3.14−3.18 (m, 1H), 1.54−1.95 (m, 6H), 1.96 (s, 3H, C(O)CH_3), 1.77 (s, 3H, C(O)CH_3), 0.78−1.30 (m, 9H); ^{13}C NMR (CD_3OD) δ 174.8, 172.6, 172.1, 171.5, 171.3, 171.1, 164.2, 159.1, 158.9, 158.5, 158.1, 157.8 (C═O), 139.6, 138.4, 138.3, 138.1, 133.8, 133.1, 132.2, 130.0, 129.9, 129.8, 129.5, 129.4, 129.3, 129.1, 128.9, 128.7, 99.4, 98.3, 84.7, 84.3, 83.8, 81.4, 78.6, 78.4, 78.0, 77.2, 76.5, 76.0, 75.7, 73.0, 72.9, 72.8, 72.3, 72.3, 72.2, 70.9, 70.7, 70.4, 69.9, 69.7, 69.3, 68.4, 68.1, 68.0, 67.9, 67.8, 67.7, 67.6, 67.4, 62.7, 60.7, 52.6, 50.9, 50.8, 41.9, 34.9, 34.5, 33.1, 30.4, 21.4, 21.3, 21.0, 20.8, 20.7, 17.2, 16.9, 16.7; ESI for $C_{72}H_{84}N_8O_{26}$ ($M + Cl^-$), calcd 1514, found 1514, 1327, 1249.

Compound 19k. After **20k** (65 mg, 0.0462 mmol) was hydrolyzed and neutralized as usual, **18k** was obtained as a white solid, which (50 mg, 0.0336 mmol) was hydrogenated for 3h to give **19k** as a white solid: yield 30 mg, 0.0336 mmol, quantitative; ^{1}H NMR (D_2O) δ 5.67 (d, $J_{1',2'} = 4$ Hz, 1H, H-1′), 4.30−4.65 (m, 1H), 3.14−4.22 (m, 18H), 2.38 (m, 1H, H-2_{eq}), 1.70−1.76 (m, 1H, H-2_{ax}); ^{13}C NMR (D_2O) δ 179.1, 178.5, 172.9, 172.1 (C═O), 98.5, 84.8, 80.8, 77.6, 75.9, 75.5, 74.9, 73.2, 72.9, 71.7, 71.5, 70.9, 70.8, 55.9, 52.4, 51.1, 50.9, 50.8, 45.9, 42.4, 20.2; ESI for $C_{24}H_{44}N_8O_{13}$ calcd 653 ($M + H^+$), found 651.

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