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Total Synthesis of Bleomycin A_2 and Related Agents. 4. Synthesis of the Disaccharide Subunit: 2-*O*-(3-*O*-Carbamoyl-α-D-mannopyranosyl)-L-gulopyranose and Completion of the Total Synthesis of Bleomycin A_2

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Abstract: The chemical synthesis of 2-*O*-(3-*O*-carbamoyl-α-D-mannopyranosyl)-L-gulopyranose and its incorporation into a total synthesis of bleomycin A_2 (**1**) readily adaptable to the preparation of structural analogs are detailed. Key strategic elements of the approach include diastereoselective α-*O*-glycosidation of *erythro*-N^{α}-CBZ-N^{π}-trityl-β-hydroxy-L-histidine methyl ester with the β-glycosyl diphenyl phosphate of hexaacetyl 2-*O*-(3-*O*-carbamoyl-α-D-mannopyranosyl)-L-gulopyranose to provide **4** (63%) with clean inversion of the glycosyl C1 stereochemistry. The subsequent convergent incorporation of **4** into bleomycin A_2 (**1**) was accomplished through sequential couplings with tetrapeptide S (94%) and N^{α}-BOC-pyrimidoblamic acid (83%), both of which proceeded in exceptionally high yield without protection of the adorning functionality. The precursor disaccharides **2** and **3** were obtained in a diastereospecific glycosidation of benzyl 3,4,6-tri-*O*-benzyl-β-L-gulopyranoside (**31**) with the α-glycosyl diphenyl phosphate of 3-*O*-carbamoyl-2,4,6-tri-*O*-acetyl-α-D-mannose (**19**), which proceeded in exceptionally good yield (93%) with net retention of the α-D-mannopyranosyl C1 stereochemistry. Both the suitably protected D-mannose and L-gulose derivatives **19** and **31** for incorporation into **1**–**4** were prepared from D-mannose with implementation of a regioselective equatorial C3 alcohol alkylation of the 2,3-*O*-dibutylstannylene derived from **13** and **23**. A tactically simple interconversion of a D-mannose to L-gulose derivative through inversion of the C5 stereochemistry by diastereoselective Rh(I)-catalyzed hydroboration of **28** provided the key transformation for the preparation of **31**.

Bleomycin A_2 (**1**)[1] is the major constituent of a family of glycopeptide antitumor antibiotics[2] that are thought to exert their biological effects through a metal-dependent oxidative cleavage of DNA[3] or RNA[4] in the presence of oxygen. In conjunction with efforts to define the structural features of **1** contributing to its biological properties and sequence-selective cleavage of DNA,[5] herein we report a concise synthesis of the protected disaccharides **2** and **3**,[6] their incorporation into **4**, and a total synthesis of bleomycin A_2[7,8] (Figure 1). Inherent in the design of the approach to **2** and **3** and its convergent incorporation into **1** was the potential extension of the technology to structurally related analogs.

Key strategic elements of the approach include a diastereoselective α-*O*-glycosidation of N^{α}-CBZ-N^{π}-trityl-*erythro*-β-hydroxy-L-histidine methyl ester (**36**) with the β-glycosyl diphenyl phosphate of hexaacetyl 2-*O*-(3-*O*-carbamoyl-α-D-mannopyranosyl)-L-gulopyranose with clean inversion of the glycosyl C1 stereochemistry (63%) and the subsequent convergent incorporation of **4** into **1** through sequential couplings with tetrapeptide S (94%) and pyrimidoblamic acid (83%) (Scheme 1). In turn, the suitably protected disaccharides **2** and **3** were obtained in a diastereospecific glycosidation coupling of benzyl 3,4,6-tri-*O*-benzyl-β-L-gulopyranoside (**31**) with the α-glycosyl diphenyl phosphate of 3-*O*-carbamoyl-2,4,6-tri-*O*-acetyl-α-D-mannose (**19**, 93%), which proceeded with retention of the α-D-mannopyranosyl C1 stereochemistry predictably directed by C2 acetoxy neighboring-group participation. The suitably protected D-mannose and

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(1) Takita, T.; Muraoka, Y.; Nakatani, T.; Fujii, A.; Umezawa, Y.; Naganawa, H.; Umezawa, H. *J. Antibiot.* **1978**, *31*, 801. Umezawa, H. *Pure Appl. Chem.* **1971**, *28*, 665.

(2) Kane, S. A.; Hecht, S. M. *Prog. Nucleic Acid Res. Mol. Biol.*, in press. Natrajan, A.; Hecht, S. M. In *Molecular Aspects of Anticancer Drug–DNA Interactions*; Neidle, S., Waring, M. J., Eds.; MacMillan Press: London, 1933; p 197. Ohno, M.; Otsuka, M. In *Recent Progress in the Chemical Synthesis of Antibiotics*; Lukas, G., Ohno, M., Eds.; Springer-Verlag: New York, 1990; p 387. Dedon, P. C.; Goldberg, I. H. *Chem. Res. Toxicol.* **1992**, *5*, 311. Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107. Hecht, S. M. *Acc. Chem. Res.* **1986**, *19*, 383.

(3) D'Andrea, A. D.; Haseltine, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 3608. Takeshita, M.; Grollman, A. P.; Ohtsubo, E.; Ohsubo, H. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5983.

(4) Magliozzo, R. S.; Peisach, J.; Ciriolo, M. R. *Mol. Pharmacol.* **1989**, *35*, 428. Holmes, C. E.; Carter, B. J.; Hecht, S. M. *Biochemistry* **1993**, *32*, 4293.

(5) (a) Boger, D. L.; Colletti, S. L.; Honda, T.; Menezes, R. F. *J. Am. Chem. Soc.*, companion paper in this issue. (b) Boger, D. L.; Menezes, R. F. *J. Org. Chem.* **1992**, *57*, 4331. (c) Boger, D. L.; Honda, T.; Dang, Q. *J. Am. Chem. Soc.*, companion paper in this issue. (d) Boger, D. L.; Honda, T.; Menezes, R. F.; Colletti, S. L. *J. Am. Chem. Soc.*, companion paper in this issue. (e) Boger, D. L.; Menezes, R. F.; Dang, Q. *J. Org. Chem.* **1992**, *57*, 4333. (f) Boger, D. L.; Menezes, R. F.; Dang, Q.; Yang, W. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 261. (g) Boger, D. L.; Honda, T.; Menezes, R. F.; Colletti, S. L.; Dang, Q.; Yang, W. *J. Am. Chem. Soc.* **1994**, *116*, 82. Boger, D. L.; Yang, W. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1649. Boger, D. L.; Dang, Q. *J. Org. Chem.* **1992**, *57*, 1631. (h) Boger, D. L.; Honda, T. *Tetrahedron Lett.* **1993**, *34*, 1567.

(6) (a) Katano, K.; Millar, A.; Pozsgay, V.; Primeau, J. L.; Hecht, S. M. *J. Org. Chem.* **1986**, *51*, 2927. (b) Minster, D. K.; Hecht, S. M. *J. Org. Chem.* **1978**, *43*, 3987. (c) Pozsgay, V.; Ohgi, T.; Hecht, S. M. *J. Org. Chem.* **1981**, *46*, 3761. (d) Katano, K.; Chang, P.-I.; Millar, A.; Pozsgay, V.; Minster, D. K.; Ohgi, T.; Hecht, S. M. *J. Org. Chem.* **1985**, *50*, 5807. (e) Millar, A.; Kim, K. H.; Minster, D. K.; Ohgi, T.; Hecht, S. M. *J. Org. Chem.* **1986**, *51*, 189. (f) Tsuchiya, T.; Miyake, T.; Kageyama, S.; Umezawa, S.; Umezawa, H.; Takita, T. *Tetrahedron Lett.* **1981**, *22*, 1413.

(7) Bleomycin A_2: (a) Aoyagi, Y.; Katano, K.; Suguna, H.; Primeau, J.; Chang, L.-H.; Hecht, S. M. *J. Am. Chem. Soc.* **1982**, *104*, 5337. (b) Takita, T.; Umezawa, Y.; Saito, S.; Morishima, H.; Naganawa, H.; Umezawa, H.; Tsuchiya, T.; Miyake, T.; Kageyama, S.; Umezawa, S.; Muraoka, Y.; Suzuki, M.; Otsuka, M.; Narita, M.; Kobayashi, S.; Ohno, M. *Tetrahedron Lett.* **1982**, *23*, 521. (c) Saito, S.; Umezawa, Y.; Yoshioka, T.; Takita, T.; Umezawa, H.; Muraoka, Y. *J. Antibiot.* **1983**, *36*, 92.

(8) Deglycobleomycin A_2: Takita, T.; Umezawa, Y.; Saito, S.; Morishima, H.; Umezawa, H.; Muraoka, Y.; Suzuki, M.; Otsuka, M.; Kobayashi, S.; Ohno, M. *Tetrahedron Lett.* **1981**, *22*, 671. Saito, S.; Umezawa, Y.; Morishima, H.; Takita, T.; Umezawa, H.; Narita, M.; Otsuka, M.; Kobayashi, S.; Ohno, M. *Tetrahedron Lett.* **1982**, *23*, 529. Aoyagi, Y.; Suguna, H.; Murugesan, N.; Ehrenfeld, G. M.; Chang, L.-H.; Ohgi, T.; Shekhani, M. S.; Kirkup, M. P.; Hecht, S. M. *J. Am. Chem. Soc.* **1982**, *104*, 5237. Boger, D. L.; Menezes, R. F.; Honda, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 273. Boger, D. L.; Honda, T.; Menezes, R. F.; Colletti, S. L. *J. Am. Chem. Soc.*, companion paper in this issue.

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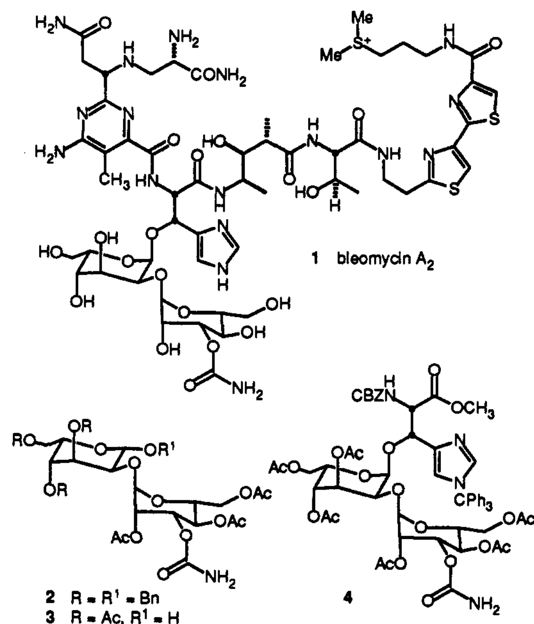
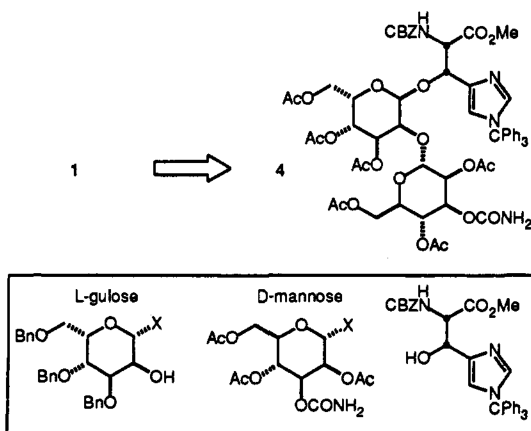


Figure 1.

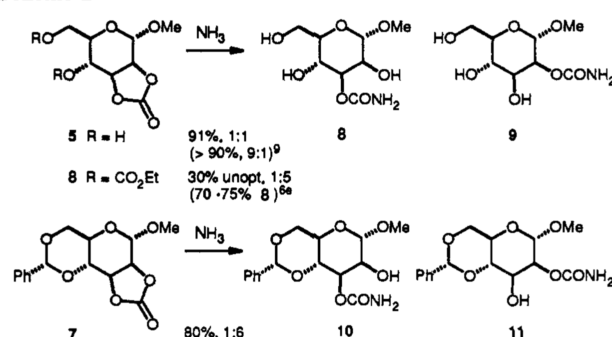
Scheme 1



L-gulose derivatives for incorporation into **2**–**4** were both prepared from D-mannose. The former was addressed through selective C3 functionalization of D-mannose employing a regioselective alkylation of the 2,3-*O*-dibutylstannylene of **13** and was strategically designed to employ the intermediate **19** as the mannose-derived glycosyl donor in which the C2 acetate serves the tactical function of controlling the stereochemistry of the glycosidation reaction. The L-gulose subunit, which functions first as a glycosyl acceptor and ultimately as a glycosyl donor, was also prepared from D-mannose. A second application of the selective alkylation of an α-D-mannose 2,3-*O*-dibutylstannylene for C3 functionalization was employed to access derivatives capable of selective C2 hydroxyl deprotection required of disaccharide coupling. A subsequent and tactically simple interconversion of a D-mannose to L-gulose derivative by inversion of the C5 stereochemistry through implementation of a diastereoselective Rh(I)-catalyzed hydroboration reaction of **28** provided the key conversion for the synthesis of **31**.

D-Mannose Subunit. Initial efforts on the preparation of a suitably protected derivative of 3-*O*-carbamoyl-α-D-mannose were based on the prior reports in which the regioselective aminolysis of a C2/C3 carbonate derivative of D-mannose was achieved selectively to provide the desired 3-*O*-carbamoyl derivatives (Scheme 2). Notably, Omoto and co-workers[9] described the aminolysis of **5** which selectively provided **8** versus **9** (9:1), and Hecht et al.[6e] have disclosed its similar preparation through aminolysis of **6** (70–75%). Our efforts to employ this regioselective generation of **8** through aminolysis of the cyclic carbonates **5**–**7**[10] were not as successful as reported although this was not extensively investigated. In contrast to the report of Omoto, the aminolysis of **5** (liquid NH_3, −78 °C, 1 h, −33 °C, 30 min, 91%) in our hands provided a 1:1 versus 9:1 (quantitative) mixture of **8** and **9**,[10] the aminolysis of **6** (10% NH_3–EtOH, 0 °C, 1 h) provided a 1:5 ratio of **8** and **9**, and the aminolysis of **7** (10% NH_3–EtOH, 0 °C, 1 h, 80%) provided a 1:6 ratio of **10** and **11**[11] (Scheme 2). The subtle experimental details responsible for the reversed regioselectivity in our hands were not investigated, but the observation of the predominant generation of the undesired 2-*O*-carbamoyl-α-D-mannose derivatives proved analogous to the results disclosed in the report of Umezawa et al.[7b] The 2- versus 3-*O*-carbamoyl derivatives (**9** and **11** versus **8** and **10**) were readily distinguishable, since the proton adjacent to the carbamoyl group in the reaction products appears at a higher chemical shift with characteristic and diagnostic coupling constants. The C3-H of the 3-*O*-carbamoyl derivative **8** was observed at δ 4.71 with coupling constants (dd, J = 2.8, 10.0 Hz) expected of an axial C3-H hydrogen. In contrast, the C2-H of the 2-*O*-carbamoyl derivatives **9** and **11** was observed at δ 4.70 and 5.08 (dd, J = 1.6, 5.2 and 1.4, 4.0 Hz, respectively) with coupling constants characteristic of an equatorial C2-H and with distinguishable and diagnostic coupling with the adjacent anomeric C1-H (d, J = 1.6 and 1.4 Hz, respectively).

Scheme 2



In the development of an alternative direct route to a 3-*O*-carbamoyl-α-D-mannose derivative requiring selective C3 functionalization, we examined potential approaches based on established protocols for the selective alkylation or acylation of the readily available diol **13**.[12] In these studies, we found that the alkylation of the 2,3-*O*-dibutylstannylene derived from reaction

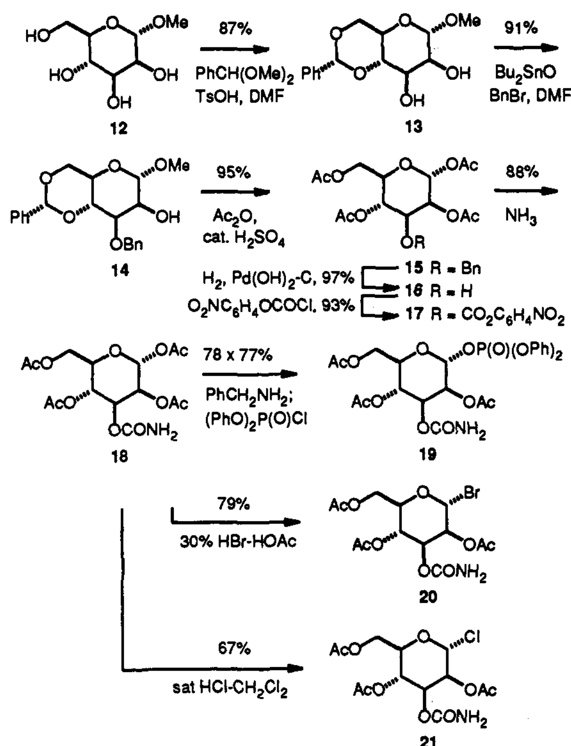
(9) Omoto, S.; Takita, T.; Maeda, K.; Umezawa, S. *Carbohydr. Res.* **1973**, *30*, 239.

(10) Cyclic carbonate **7** was prepared by treatment of **13** with phosgene (1.1 equiv of Bu_2SnO, CH_3OH, reflux, 1 h; 1.1 equiv of $COCl_2$, toluene, 0 °C, 30 min, 98%) or through acylation of the 2,3-*O*-dibutylstannylene (1.1 equiv of Bu_2SnO, CH_3OH, reflux, 1 h) with di(2-pyridyl) carbonate (1.1 equiv, toluene, 25 °C, 12 h, 69%). For **7**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30–7.50 (5H, m), 5.60 (1H, s), 5.04 (1H, s), 5.04 (1H, s), 4.86 (1H, dd, J = 7.2, 7.2 Hz), 4.71 (1H, d, J = 7.2 Hz), 3.80–4.00 (3H, m), 3.43 (3H, s). Acetal hydrolysis of **7** (1% HCl–CH_3OH, 25 °C, 30 min, 62%) provided **5**: ^{1}H NMR (CD_3OD, 400 MHz) δ 4.92 (1H, s), 4.61 (1H, dd, J = 10.0, 10.0 Hz), 4.60 (1H, d, J = 10.0 Hz), 3.78 (1H, dd, J = 2.5, 12.0 Hz), 3.60 (1H, dd, J = 7.2, 12.0 Hz), 3.54 (1H, dd, J = 6.0, 10.0 Hz), 3.47 (1H, m), 3.34 (3H, s). Cyclic carbonate **6** was prepared as described in ref 9b. For **8**: ^{1}H NMR (D_2O, 400 MHz) δ 4.71 (1H, dd, J = 2.8, 10.0 Hz, C3-H), 4.55 (1H, d, J = 1.7 Hz, C1-H), 3.87 (1H, dd, J = 1.7, 3.2 Hz), 3.40–3.80 (4H, m), 3.31 (3H, s). For **9**: ^{1}H NMR (D_2O, 400 MHz) δ 4.70 (1H, dd, J = 1.6, 5.2 Hz, C2-H), 4.61 (1H, d, J = 1.6 Hz, C1-H), 3.40–3.80 (5H, m), 3.30 (3H, s). Methyl 2-*O*-carbamoyl-6-*O*-(ethoxycarbonyl)-α-D-mannopyranoside was also isolated from studies of the aminolysis of **6**: R_f 0.09 (SiO_2 65% EtOAc–hexane); ^{1}H NMR (CD_3OD, 400 MHz) δ 4.71 (1H, dd, J = 1.7, 4.0 Hz, C2-H), 4.57 (1H, d, J = 1.7 Hz, C1-H), 4.31 (1H, dd, J = 2.0, 12.0 Hz), 4.18 (1H, dd, J = 5.6, 12.0 Hz), 4.08 (2H, q, J = 7.2 Hz), 3.72 (1H, dd, J = 4.0, 8.8 Hz), 3.50–3.65 (2H, m), 3.27 (3H, s), 1.18 (3H, J = 7.2 Hz).

(11) For **11**: R_f 0.01 (SiO_2, 65% EtOAc–hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.20–7.50 (5H, m), 5.58 (1H, s, CHPh), 5.08 (1H, dd, J = 1.4, 4.0 Hz, C2-H), 4.74 (1H, d, J = 1.4 Hz, C1-H), 4.20–4.30 (2H, m), 3.60–4.00 (3H, m), 3.38 (3H, s).

(12) Buchanan, J. G.; Schwarz, J. C. P. *J. Chem. Soc.* **1962**, 4770.

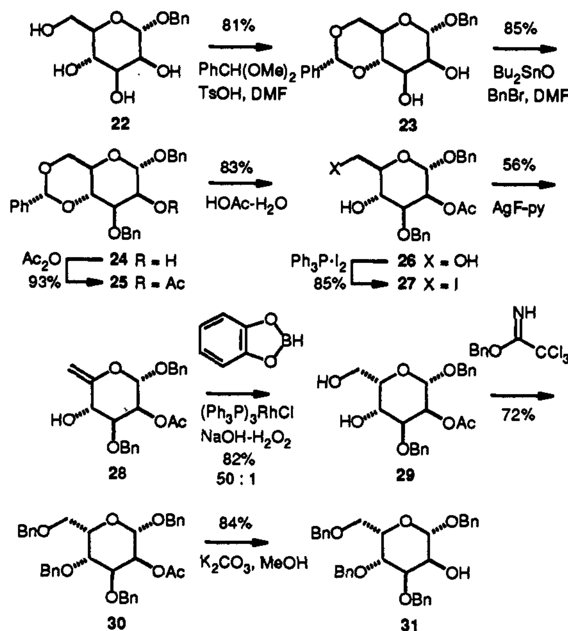
Scheme 3



of the diol **13** with Bu_2SnO (1.1 equiv, CH_3OH, reflux, 1 h)[13] with benzyl bromide (2 equiv, DMF, 100 °C, 30 min) cleanly provided **14** (91%, $[\alpha]^{25}_D$ +37.3 (*c* 0.25, EtOH) (lit[13b,14] $[\alpha]^{25}_D$ +38 (*c* 1, EtOH))) derived from exclusive alkylation of the equatorial C3 alcohol (Scheme 3). Attempts to introduce the C3 carbamoyl group directly through acylation of the diol **13** or its derived 2,3-*O*-dibutylstannylene with trimethylsilyl isocyanate, trichloroacetyl isocyanate, or chlorosulfonyl isocyanate did not prove successful. Similarly, the diol-derived dibutylstannylene cleanly provided the corresponding cyclic carbonate **7** rather than the C3 *p*-nitrophenyl carbonate upon reaction with *p*-nitrophenyl chloroformate (1.05 equiv, 1.2 equiv of Et_3N, CH_2Cl_2, 25 °C, 10 h, 72%).

Single-step peracetylation of **14** (2% H_2SO_4–Ac_2O, 25 °C, 20 min, 95%) cleanly provided the pentaacetate **15**,[15] $[\alpha]^{25}_D$ +7.2 (*c* 0.2, $CHCl_3$). Notably, **15** was secured in this approach in 75% overall yield from D-mannose, providing an improvement over its prior preparation[6e] and with an efficiency that effectively competes with the reported preparations of **18** and related agents based on the aminolysis of **5** and **6**.[6e,9] Following protocols introduced in Hecht's efforts, debenzylation of **15** was accomplished cleanly with use of Pearlman's catalyst (H_2, 0.2 wt equiv of $Pd(OH)_2$-C, CH_3OH, 25 °C, 10 h, 97%) and provided **16**, $[\alpha]^{25}_D$ +28 (*c* 0.23, $CHCl_3$). Unambiguous confirmation of the regioselectivity of the selective benzylation of **13** was obtained from the ^1H NMR COSY spectrum of **16**, which clearly indicated that the C2-H coupled to the anomeric C1-H was shifted downfield consistent with C2 versus C3 acetylation. Conversion of **16** to the *p*-nitrophenyl carbonate **17** (4.0 equiv of $ClCO_2C_6H_4NO_2$, 2 equiv of DMAP, py, 40 °C, 2 h, 93%) followed by its controlled aminolysis (10% NH_3 in THF–CH_2Cl_2 (1:2), 25 °C, 1.5 h, 88%) provided 1,2,4,6-tetra-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranose (**18**, $[\alpha]^{25}_D$ +39 (*c* 0.18, $CHCl_3$)) in 70% overall yield for the 4–5 steps from **13** and in 62% overall yield from D-mannose. Activation of **18** as a glycosyl donor was accomplished through

Scheme 4



treatment of **18** with saturated HCl–CH_2Cl_2 (25 °C, 10 h, 67%), treatment with 30% HBr–HOAc (25 °C, 2 h, 79%) or sequential treatment with benzylamine (2.5 equiv, THF, 25 °C, 20 h, 78%) and $(PhO)_2P(O)Cl$ (1.3 equiv, –78 °C, 10 min, 77%)[16] to provide the α-glycosyl chloride **21**, $[\alpha]^{25}_D$ +79 (*c* 0.3, $CHCl_3$) (lit[6e] $[\alpha]^{25}_D$ +84 (*c* 0.5, $CHCl_3$)), mp 126–128 °C (lit[6e] mp 132–134 °C), the α-glycosyl bromide **20**, or the α-glycosyl diphenyl phosphate **19**, mp 140–142 °C, $[\alpha]^{25}_D$ +25 (*c* 0.13, $CHCl_3$), respectively.

L-Gulose Subunit. Past efforts to prepare a suitably protected derivative of the L-gulose subunit of bleomycin A_2 have employed a six-step synthesis from the rare L-gulose itself (3–4% overall yield)[6c,17] and the successive head-to-tail inversion of a D-glucofuranose with interconversion of the C1/C6 oxidation states. In the initial implementation of this latter approach, the key mannose C3 carbamoylation was conducted with aminolysis of a disaccharide cyclic carbonate and provided predominantly the undesired 2-*O*-carbamoyl (28%) versus the desired 3-*O*-carbamoyl derivative (15%).[6f,7b] Subsequent improvements in both these approaches have been detailed.[6d] Complementary to the past efforts, our approach to the L-gulose subunit was based on the use of a readily available D-mannose precursor bearing the correct C1–C4 stereochemistry anticipating the development of a tactically simple inversion of the C5 stereochemistry required for interconversion of D-mannose to L-gulose.[17] We were especially attracted to this approach, since we could enlist the 2,3-*O*-dibutylstannylene-mediated selective C3 versus C2 hydroxyl alkylation for selective C2 functionalization required for eventual disaccharide coupling.

Protection of the C4 and C6 hydroxyl groups of benzyl α-D-mannopyranoside (**22**)[18] with formation of acetal **23** (1.0 equiv of $PhCH(OMe)_2$, catalytic TsOH, DMF, 50 °C, 3 h, 81%) followed by selective C3 hydroxyl benzylation through alkylation of the intermediate 2,3-*O*-dibutylstannylene[13] (1.1 equiv of Bu_2SnO, CH_3OH, reflux, 1 h; 2.0 equiv of $PhCH_2Br$, DMF, 100 °C, 30 min, 85%) provided **24** (Scheme 4). Notably, this clean alkylation of the equatorial C3 alcohol permitted selective protection of the C2 hydroxyl group in a manner which ultimately allows its selective deprotection prior to disaccharide coupling. Acetylation of the C2 hydroxyl group (3.0 equiv of Ac_2O, py, 25 °C, 3 h, 93%) followed by acetal hydrolysis of **25** (80% HOAc–

(13) (a) Auge, C.; David, S.; Veyrieres, A. *J. Chem. Soc., Chem. Commun.* **1976**, 375. (b) Nashed, M. A. *Carbohydr. Res.* **1978**, *60*, 200. (c) Nashed, M. A.; Anderson, L. *Carbohydr. Res.* **1977**, *56*, 419. (d) Srivastava, V. K.; Schuerch, C. *Tetrahedron Lett.* **1979**, 3269. (e) Review: David, S.; Hanessian, S. *Tetrahedron* **1985**, *41*, 643.

(14) Bhattacharjee, S. S.; Gorin, P. A. J. *Can. J. Chem.* **1969**, *47*, 1195.

(15) Ponpipom, M. M. *Carbohydr. Res.* **1977**, *59*, 311.

(16) Hashimoto, S.; Honda, T.; Ikegami, S. *J. Chem. Soc., Chem. Commun.* **1989**, 685.

(17) L-Gulose presently costs *ca.* \$130–150/100 mg. For an alternative synthesis of L-gulose starting from D-mannose, see: Evans, M. E.; Parrish, F. W. *Carbohydr. Res.* **1973**, *28*, 359.

(18) Gorin, P. A. J.; Perlin, A. S. *Can. J. Chem.* **1961**, *39*, 2474.

Table 1

1.Cat. $Rh(PPh_3)_3Cl$ Reductant THF 2.NaOH-H_2O_2

L-gulose D-mannose

entry	substrate	reductant	yield (%)	ratio
1	R = Me, R^1 = TBDMS, R^2 = R^3 = CMe_2	catecholborane	nd	0:100
2	R = Me, R^1 = TBDMS, R^2 = Bn, R^3 = Ac	catecholborane	nd	50:50
3	R = Me, R^1 = R^2 = R^3 = Ac	9-BBN	85	37:63
4	R = Me, R^1 = Bn, R^2 = R^3 = Ac	catecholborane	78	95:5
5	R = Bn, R^1 = Bn, R^2 = R^3 = Ac	catecholborane	nd	95:5
6	R = Bn, R^1 = H, R^2 = Bn, R^3 = Ac	catecholborane	82	98:2

H_2O, 60 °C, 1 h, 83%) provided the diol **26**, $[\alpha]^{25}_D$ +32 (*c* 0.2, $CHCl_3$). Satisfactory but lower yields for the acetal hydrolysis were observed when the deprotection reaction was conducted with 5% HCl–CH_3OH (25 °C, 30 min, 62%). Confirmation of the selective C3 alcohol benzylation was derived from the ^1H NMR of **25**. Acetylation of the C2 alcohol provides a readily distinguishable downfield C2-H at δ 5.45, which exhibited coupling constants characteristic of an equatorial versus axial hydrogen (dd, *J* = 1.6, 2.8 Hz) and had a distinguishable and diagnostic coupling with the adjacent C1-H anomeric hydrogen (d, *J* = 1.6 Hz). This set the stage for inversion of the C5 stereochemistry with conversion from the D-mannose to L-gulose series. Conversion of the primary alcohol **26** to the corresponding primary iodide **27** (1.2 equiv of Ph_3P, 1.2 equiv of I_2, 1.5 equiv of imidazole, toluene, 100 °C, 3 h, 85%)[19] followed by elimination of HI, which was best affected by treatment with AgF–py[20] (5.0 equiv, py, 25 °C, 3 h, 56%), provided **28**.[21] Initial attempts to protect the C4 alcohol as its benzyl ether at the stage of **27** were not successful but proved to be unnecessary, and alternative approaches to conducting the elimination of HI (*e.g.*, 5.0 equiv of DBU, toluene, 90 °C, 8 h, 45%) proved less satisfactory. Rh(I)-catalyzed[22] hydroboration–oxidation of **28** cleanly provided **29**, $[\alpha]^{25}_D$ +76 (*c* 0.2, $CHCl_3$), with inversion of the C5 stereochemistry in a reaction that proceeded with a diastereoselection of ≥50:1. Optimal conversions were obtained with fresh catalyst and upon oxidative workup under neutral rather than basic conditions (pH 7 phosphate buffer–50% aqueous H_2O_2) in order to avoid inadvertent acetate hydrolysis. This hydroboration reaction was examined in some detail and the observed diastereoselection was found to be dependent on the nature of the allylic alcohol substituent (Table 1). A small allylic alcohol protecting group (CH_2Ph) or the free allylic alcohol itself was found to favor near exclusive β-face reagent delivery with clean introduction of the L-gulose C5 stereochemistry. Not surprisingly, only regeneration of the D-mannose C5 stereochemistry was observed with 2,3-*O*-acetonide derivatives and may be attributed to steric deceleration of β-face reagent delivery. Thus, appropriate selection of the C4 allylic alcohol protecting group in conjunction with appropriate C2/C3 alcohol protecting groups permits clean access to the L-gulose *or* D-mannose series. Exhaustive benzylation of **29** (10 equiv of $BnOC(=NH)CCl_3$, catalytic CF_3SO_3H, CH_2Cl_2–hexane 1:2, 25 °C, 1 h, 72%) followed by methanolysis of the C2 acetate of **30**, $[\alpha]^{25}_D$ +70 (*c* 0.13, $CHCl_3$) (lit[6c] $[\alpha]^{25}_D$ +72.7 (*c* 1.4, $CHCl_3$)), provided benzyl 3,4,5-tri-*O*-benzyl-β-L-gulopyranoside (**31**), $[\alpha]^{25}_D$ +49 (*c* 0.12, $CHCl_3$) (lit[6c] $[\alpha]^{25}_D$ +48.3 (*c* 0.7, $CHCl_3$)), and a suitable L-gulose glycosyl acceptor for disaccharide coupling.

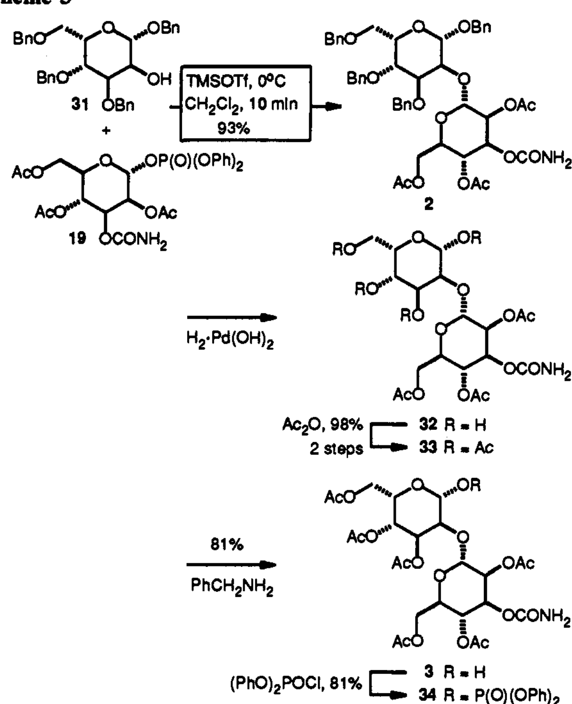
(19) Garegg, P. J.; Samuelsson, B. *J. Chem. Soc., Chem. Commun.* **1979**, 978.

(20) Cheng, T. M.; Horton, D.; Weckerl, W. *Carbohydr. Res.* **1977**, *58*, 139.

(21) The bromide corresponding to **27** was also prepared (2.0 equiv of Ph_3P and NBS, DMF, 50 °C, 45 min, 65%) but was not as effectively converted to **28** through elimination of HBr. For benzyl 2-*O*-acetyl-3-*O*-benzyl-6-bromo-6-deoxy-α-D-mannopyranoside: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.20–7.40 (10H, m), 5.41 (1H, dd, *J* = 2.0, 2.8 Hz), 4.93 (1H, d, *J* = 2.0 Hz), 4.80 (1H, d, *J* = 12.0 Hz), 4.73 (1H, d, *J* = 12.0 Hz), 4.56 (1H, d, *J* = 12.0 Hz), 4.41 (1H, d, *J* = 12.0 Hz), 3.70–3.90 (4H, m), 3.57 (1H, dd, *J* = 6.8, 10.8 Hz), 2.11 (3H, s).

(22) Evans, D. A.; Fu, G. C.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1992**, *114*, 6671.

Scheme 5



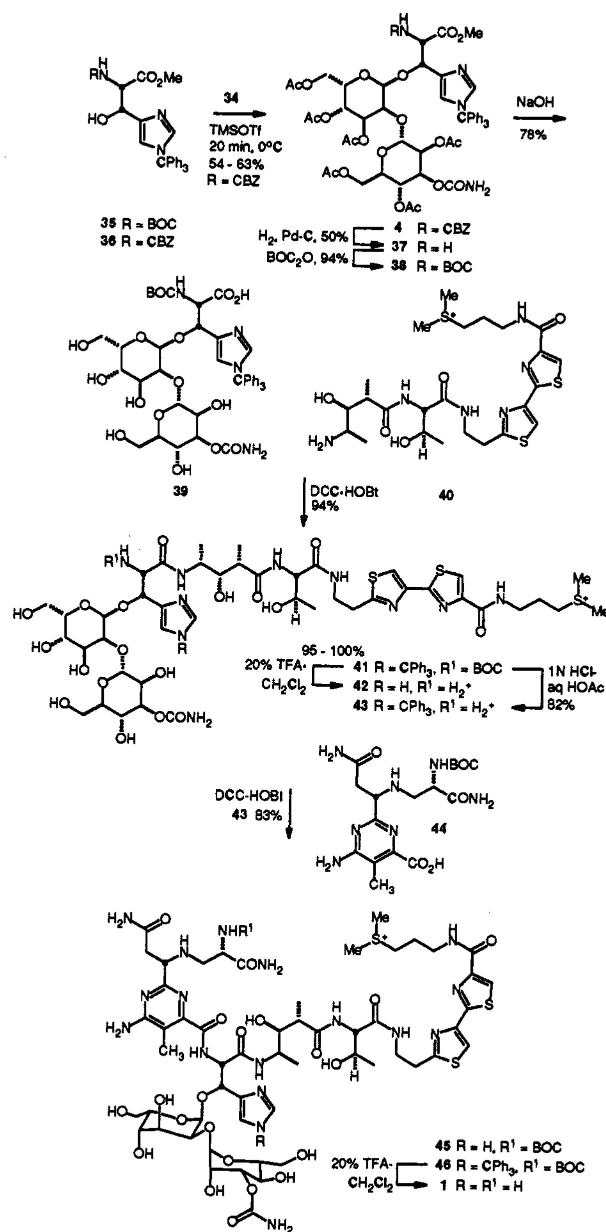
Disaccharide Formation: Preparation of 2 and 3. Disaccharide formation with **31** serving as the glycosyl acceptor and **19–21** serving as the glycosyl donors cleanly provided the key disaccharide **2**, $[\alpha]^{25}_D$ +38 (*c* 0.05, $CHCl_3$). Although the glycosidation reactions conducted with **20** (1.2 equiv of AgOTf, 3.0 equiv of TMU, CH_2Cl_2, 25 °C, 10–36 h, 74–78%) and **21** provided **2** cleanly analogous to the report of Hecht,[6c] the use of the glycosyl diphenyl phosphate **19** (1.8 equiv of TMSOTf, 0 °C, CH_2Cl_2, 10 min, 93%)[16] provided **2** under milder reaction conditions (0 versus 25 °C) in much shorter reaction times (10 min versus 10–36 h) and in higher conversions (93% versus 74–78%) (Scheme 5). Notably, the carbamoyl group could be taken through this glycosidation reaction without deliberate protection or observation of competitive reactions. The exclusive retention of the α-linkage stereochemistry may be attributed to neighboring-group participation of the mannopyranosyl C2′ acetate, providing coupling with clean retention of the mannose C1′ stereochemistry. This α-linkage was supported by ^1H NMR through observation of the C1′-H anomeric proton of **2** at δ 4.76 with a coupling constant characteristic of an equatorial proton (d, *J* = 1.6 Hz). Debenzylation of **2** (0.1 wt equiv of $Pd(OH)_2$-C, H_2, CH_3OH, 25 °C, 15 h, 98%) followed by acetylation of **32** (10 equiv of Ac_2O, py, 25 °C, 10 h, 82%) provided **33**, $[\alpha]^{25}_D$ +35.4 (*c* 0.06, $CHCl_3$), and conducting these two steps without the deliberate purification of **32** afforded **33** in much higher conversions (98% for two steps). Activation of **3** as its β-glycosyl diphenyl phosphate **34**, $[\alpha]^{25}_D$ +34 (*c* 0.17, $CHCl_3$), for coupling with a suitably protected β-hydroxy-L-histidine derivative was accomplished by sequential treatment of **33** with benzylamine (2.5 equiv, THF, 25 °C, 10–24 h, 81–84%) and $(PhO)_2P(O)Cl$ (1.5 equiv, −78 °C, 10 min, 81%)[16] with the intermediate generation of **3**. Through this sequence the β-stereochemistry of the L-gulopyranoside anomeric center was maintained and confirmed through observation of diagnostic C1-H/C2-H coupling constants (*J* = 8.0 Hz) characteristic of an axial C1-H coupled to the adjacent axial C2-H at δ 4.95 (1H, d, *J* = 8.0 Hz for **2** C1-H), 5.89 (1H, d, *J* = 8.0 Hz for **33** C1-H), 5.00 (1H, d, *J* = 8.0 Hz for **3** C1-H), and 5.71 (1H, dd, *J* = 6.8, 8.0 Hz for **34** C1-H).

Preparation of 4 and Incorporation into Bleomycin A_2. Key to the completion of the total synthesis of bleomycin A_2 was the stage and manner by which the disaccharide was incorporated and the order of the assemblage of the subunits. In initial efforts, Umezawa and co-workers found that the glycosyl bromide of disaccharide 3 failed to couple with the free or fully protected aglycone itself but could be coupled with a fully protected pentapeptide S derivative albeit with generation of the desired *O*-glycosidation product as a minor product.[7b] Competitive and preferential imidazole *N*-glycosidation was observed. Coupling of the glycosidated pentapeptide S with N^{α}-BOC-pyrimidoblamic acid and deprotection provided bleomycin A_2 in low yield. They later modified their approach to *O*-glycosidation of *erythro*-N^{α}-BOC-N^{π}-Tos-β-hydroxy-L-histidine methyl ester with the glycosyl bromide of disaccharide 3, which provided the desired α-*O*-glycosidation product in modest yield (21%).[7c] Subsequent and sequential tetrapeptide S and N^{α}-BOC-pyrimidoblamic acid couplings followed by final deprotection provided bleomycin A_2. This latter approach proved comparable to that initially described by Hecht[7a] in which the α-*O*-glycosidation of N^{α}-BOC-N^{π}-BOC-β-hydroxy-L-histidine benzyl ester with the glycosyl bromide of *N*-acetyl-3 was accomplished in modest yield (20–25%). Sequential coupling of the *O*-glycosidated histidine subunit with (2*S*,3*S*,4*R*)-4-amino-3-hydroxy-2-methylpentanoate, demethyltripeptide S, and N^{α}-BOC-pyrimidoblamic acid followed by deprotection provided demethylbleomycin A_2. Notably, the Hecht approach employed the demethyl C-terminus, requiring penultimate *S*-methylation, a key *O*-glycosidation coupling of the disaccharide with the protected β-hydroxy-L-histidine subunit which proceeded in modest conversion (20–25%), and a linear sequential introduction of the pentanoic acid, tripeptide S, and pyrimidoblamic acid subunits.

Our initial efforts at *O*-glycosidation of N^{α}-BOC-N^{π}-trityl-β-hydroxy-L-histidine methyl ester (35)[5a] with the β-glycosyl diphenyl phosphate 34 (1.5 equiv of TMSOTf, Et_2O, 0 °C, 30 min) were not especially successful and provided a mixture of at least three components. Although this was not investigated in detail, N^{α}-BOC deprotection under the reaction conditions was observed. Consequently, the *O*-glycosidation reaction was conducted with 34 and N^{α}-CBZ-N^{π}-trityl-β-hydroxy-L-histidine methyl ester (36,[5a] 4 equiv of TMSOTf, 2:1 Et_2O–CH_2Cl_2, 0 °C, 20 min, 54–63%), thereby avoiding the competitive *N*-BOC deprotection, and provided the adduct 4 in excellent conversion as a $\geq$13:1 mixture of the desired α and undesired β linked anomers (Scheme 6). In fact, the undesired β anomer was not unambiguously identified and only a trace amount (3–4%) of additional material containing the structural elements of both 34 and 36 was isolated. As such the diastereoselectivity of the glycosidation was estimated to be at least 13:1 and may in fact be higher. Confirmation of the *O*-glycosidation α-linkage arising from inversion of the disaccharide C1 stereochemistry was derived by ^1H NMR of 38 through observation of the C1-H anomeric proton at δ 5.13 (d, J = 3.6 Hz) with a distinguishable coupling constant characteristic of an equatorial proton coupled to the adjacent axial C2-H at δ 3.99 (dd, J = 3.6, 3.6 Hz), which itself exhibits a diagnostic set of coupling constants with the adjacent C1 and C3 equatorial hydrogens. The surprisingly high and welcomed diastereoselectivity of this glycosidation reaction is especially noteworthy and may be a consequence of both the low reactivity of the glycosyl acceptor, which tends to favor formation of the most stable α anomer, and mechanistic elements characteristic of a glycosyl phosphate donor which favor reaction with inversion of the stereochemistry at the reacting anomeric center.

Selective CBZ deprotection (0.35 wt equiv, 10% Pd-C, H_2, EtOAc–CH_3OH 2:1, 25 °C, 5 h, 50%; 69% based on consumed 4) without competitive hydrogenolysis of the trityl protecting group followed by reprotection of the free amine of 37 (3.0 equiv of BOC_2O, 6.0 equiv of $NaHCO_3$, THF–H_2O 3:1, 25 °C, 10 h, 94%) provided 38 suitably protected for sequential couplings with tetrapeptide S and N^{α}-BOC-pyrimidoblamic acid. Exhaustive

Scheme 6



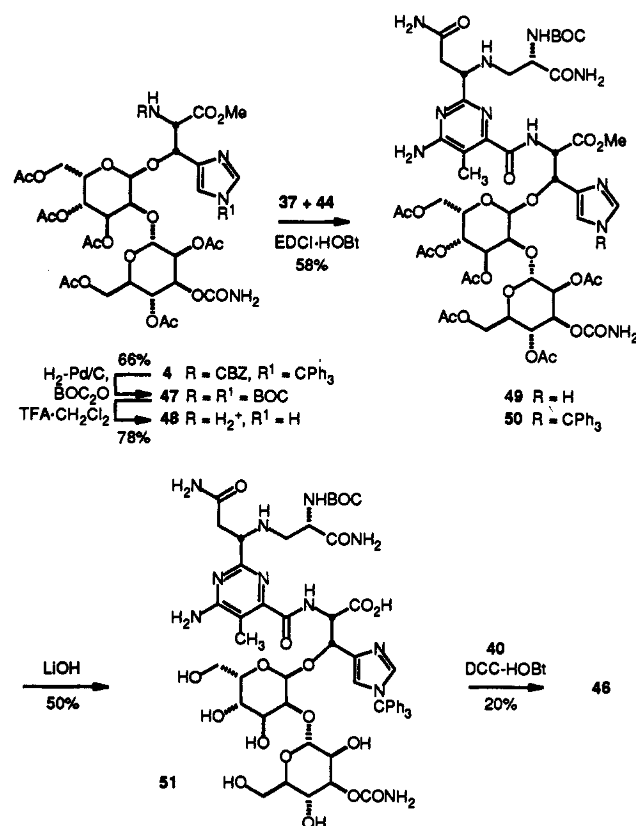
ester hydrolysis of 38 (0.1 N aqueous NaOH–CH_3OH 1:1, 0 °C, 12 h, 78%) provided 39. Coupling of 39 with the free amine of tetrapeptide S (40,[5a] 1.5 equiv, 2.0 equiv of DCC, 1.0 equiv of HOBt, 1.5 equiv of $NaHCO_3$, DMF, 25 °C, 48 h, 94%) provided 41 in superb yield. Notably, this exceptionally effective coupling was conducted without deliberate protection of the disaccharide or tetrapeptide S hydroxyl groups and was conducted with the tetrapeptide S sulfonium salt installed in the substrate. Initial attempts to follow this same approach utilizing 47, in which the imidazole was protected as its N^{π}-BOC derivative, were found to suffer deprotection under the ester hydrolysis conditions. Acid-catalyzed deprotection of 41 (20% TFA–CH_2Cl_2, 0 °C, 3 h, 95–100%) served to remove both the BOC and trityl protecting groups cleanly without deglycosidation and provided 42. However, attempts to couple 42 with N^{α}-BOC-pyrimidoblamic acid (1.0 equiv of 44,[5c] 2.0 equiv of DCC, 1.0 equiv of HOBt, DMF, 25 °C, 36 h) in close albeit not identical analogy to the efforts disclosed by Hecht et al.[7a] provided only small amounts of N^{α}-BOC-bleomycin A_2 (45) and provided principally the product derived from acylation of the free imidazole.[23] Although it is possible that use of the free base of 42 or the deliberate addition of base to the coupling reaction mixture might improve this coupling of 42 with 44 and that longer reaction times might result in imidazole to N^{α}-amine acyl transfer, the solution to this final

coupling reaction was more effectively addressed through the surprisingly selective deprotection of **41**. Mild acid treatment of **41** (1 N HCl in 90% aqueous HOAc, 25 °C, 15 min) under conditions defined by Sieber and Riniker[24] cleanly provided **43** (82%) derived from removal of the BOC protecting group without deglycosidation or removal of the trityl protecting group. Subsequent coupling of **43** with N^{α}-BOC-pyrimidoblamic acid (1.0 equiv of **44**,[5c] 3 equiv of DCC, 1 equiv of HOBt, DMF, 25 °C, 48 h) cleanly provided N^{α}-BOC-N^{π}-tritylbleomycin A_2 (**46**, 83%). Similar to the reaction of **39** with **40**, this final coupling was conducted without protection of the disaccharide or tetrapeptide S hydroxyl groups with the terminal sulfonium salt intact in exceptionally good conversion. Final acid-catalyzed deprotection of **46** (20% TFA–CH_2Cl_2, 0 °C, 2.5 h) provided bleomycin A_2 (**1**) identical in all compared respects with natural material (^1H NMR, IR, MS, $[\alpha]_D$, TLC, HPLC).[25]

Concurrent with the successful incorporation of **4** into bleomycin A_2 detailed in Scheme 6, we also examined the alternative order of couplings employing first N^{α}-BOC-pyrimidoblamic acid and finally tetrapeptide S. This latter approach, which proved less successful, was viewed initially as more attractive since the incorporation of the tetrapeptide S sulfonium salt would occur at the penultimate step potentially simplifying the purification of the preceding intermediates. Removal of the CBZ and trityl protecting groups of **4** (H_2, 0.8 wt equiv, 10% Pd-C, CH_3OH, 25 °C, 8 h) in the presence of BOC_2O (10 equiv) provided **47** (60–66%) cleanly (Scheme 7). *N*-BOC deprotection of **47** (20% TFA–CH_2Cl_2, 0 °C, 2 h, 75% or TFA, 25 °C, 30 min, 78%) provided **48**, and in contrast to the use of TFA, the attempted deprotection of **47** with HCl (4 M HCl–EtOAc) led to competitive deglycosidation. Like the attempted coupling of **42** with **44**, coupling of **48** with N^{α}-BOC-pyrimidoblamic acid (**44**,[5c] 1.1 equiv of EDCI, 1.0–1.2 equiv of HOBt, DMF, 25 °C, 2–3 days, 64–73%) did not provide **49**, but rather preferential and clean imidazole *N*-acylation was observed.[26]

However, coupling of **37** with N^{α}-BOC-pyrimidoblamic acid (1.0 of equiv **44**,[5c] 1.1 equiv of EDCI, 1.0 equiv of HOBt, DMF, 25 °C, 48 h, 58% unoptimized) cleanly provided **50**. Exhaustive ester hydrolysis of **50** (15 equiv of LiOH, H_2O–CH_3OH 1:1, 0 °C, 12 h, 50% unoptimized) followed by coupling of **51** with the free amine of tetrapeptide S **40**[5a] (1.5 equiv, 3.0 equiv of DCC, 1.0 equiv of HOBt, DMF, 25 °C, 72 h) provided **46** (20%) identical to the material previously prepared albeit in modest conversions. The major product observed in several attempts proved to be dehydro-N^{α}-BOC-N^{π}-trityldeglycobleomycin A_2 (40%)[27] derived from deglycosidation of **51** via elimination of the disaccharide followed by coupling with **40**. While the origin of this facile deglycosidation was not established, it is tempting to suggest that the increased acidity of the α-proton in the N^{α}-acyl (*i.e.* **51**) versus N^{α}-BOC/CBZ derivatives of the β-hydroxy-L-histidine subunit including **39** upon carboxylate activation is sufficient to promote β-elimination of the disaccharide. More importantly and more assuredly, these latter studies clearly illustrate that the order of assemblage of the bleomycin A_2 subunits is critical and that the approach detailed in Scheme 6 is optimal.

Scheme 7



The extension of the work detailed herein to the preparation and evaluation of key structural analogs of bleomycin A_2 is underway and will be reported in due course.

Experimental Section

Methyl 4,6-*O*-Benzylidene-3-*O*-benzyl-α-D-mannopyranoside (14). A suspension of methyl 4,6-*O*-benzylidene-α-D-mannopyranoside[12] (**13**, 0.35 mmol, 100 mg) and dibutyltin oxide (0.38 mmol, 96 mg, 1.1 equiv) in CH_3OH (3 mL) was warmed at reflux for 1.5 h to provide a clear solution, cooled to 25 °C, and the solvent was removed under reduced pressure. The resulting methyl 4,6-*O*-benzylidene-2,3-*O*-(dibutylstannylene)-α-D-mannopyranoside was dried under vacuum for 6 h and taken up in DMF (3 mL). The solution was treated with $PhCH_2Br$ (0.71 mmol, 120 mg, 2.0 equiv) and warmed at 100 °C with stirring for 30 min. The cooled reaction mixture was poured into a two phase solution of EtOAc (5 mL) and saturated aqueous $NaHCO_3$ (3 mL) with vigorous stirring. The organic layer was washed with saturated aqueous NaCl (3 mL) and dried (Na_2SO_4). Chromatography (SiO_2, 1 × 6 cm, 20% EtOAc–hexane) afforded **14** (120 mg, 131 mg theoretical, 91%; typically 80–85%, 12.0 mmol scale) as a colorless syrup: R_f 0.30 (SiO_2, 30% EtOAc–hexane); $[\alpha]^{25}_D$ +37.3 (*c* 0.25, EtOH) (lit[14] $[\alpha]^{25}_D$ +38 (*c* 1, EtOH)); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.20–7.70 (10H, m), 5.61 (1H, s), 4.86 (1H, d, *J* = 12.0 Hz), 4.76 (1H, d, *J* = 1.2 Hz), 4.73 (1H, d, *J* = 12.0 Hz), 4.28 (1H, dd, *J* = 3.6, 9.2 Hz), 4.09 (1H, dd, *J* = 9.2, 9.2 Hz), 4.05 (1H, dd, *J* = 1.2, 3.6 Hz), 3.80–3.95 (3H, m), 3.38 (3H, s).

1,2,4,6-Tetra-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranose (15). A solution of 2% H_2SO_4–Ac_2O (6 mL) was added to **14** (1.02 mmol, 380 mg), and the reaction mixture was stirred for 20 min at 25 °C. The reaction mixture was poured into a two-layer solution of EtOAc (20 mL) and saturated aqueous $NaHCO_3$ (10 mL). The organic layer was washed with saturated aqueous NaCl (10 mL), dried (Na_2SO_4), and concentrated

(23) The imidazole *N*-acylation product was isolated as a thin film (reverse-phase C-18, 10–20% CH_3OH–H_2O gradient elution 88%): R_f 0.33 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.21 (1H, s), 8.10 (1H, s), 7.76 (1H, br s), 7.22 (1H, br s), 5.30 (1H, m), 5.15 (1H, m), 4.30 (1H, d, *J* = 4.5 Hz), 3.30–4.20 (22H, m), 2.93 (6H, s), 2.10 (5H, m), 1.43 (9H, s), 1.05–1.15 (9H, m); IR (neat) ν_{max} 3361, 1700, 1650, 1391, 1157, 903 cm^{-1}; FABMS (NBA) *m/e* 1514 (M^+, $C_{60}H_{92}N_{17}O_{23}S_3$). Acid-catalyzed deprotection of the crude reaction product (20% CF_3CO_2H–CH_2Cl_2, 0 °C, 1.5 h) provided only small quantities of bleomycin A_2 and provided principally the deprotection product derived from the presumed imidazole *N*-acylation with pyrimidoblamic acid.

(24) Sieber, P.; Riniker, B. *Tetrahedron Lett.* **1987**, *28*, 6031.

(25) Boger, D. L.; Menezes, R. F.; Yang, W. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 959. Freyder, C. P.; Zhou, W.; Doetsch, P. W.; Marzilli, L. G. *Prep. Biochem.* **1991**, *21*, 257.

(26) For the imidazole *N*-acylation product: a colorless foam; R_f 0.42 (SiO_2, 20% CH_3OH–$CHCl_3$); $[\alpha]^{25}_D$ –6.7 (*c* 0.06, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 7.71 (1H, s), 7.23 (1H, s), 5.15–5.30 (5H, m), 5.00 (1H, d, *J* = 1.6 Hz), 4.10–4.30 (5H, m), 4.03 (1H, dd, *J* = 3.6, 3.6 Hz), 3.95 (1H, m), 3.89 (1H, dd, *J* = 7.0, 12.8 Hz), 3.79 (3H, s), 3.59 (1H, m), 2.13 (3H, s), 2.15 (3H, s), 2.09 (3H, s), 2.06 (3H, s), 2.05 (3H, s), 1.97 (3H, s), 1.43 (9H, s); IR (neat) ν_{max} 3400, 1735, 1705, 1650, 1440, 1235, 1018 cm^{-1}; FABHRMS (NBA) *m/e* 1212.4462 (M^+ + H, $C_{49}H_{69}N_{11}O_{25}$ requires 1212.4465).

(27) For dehydro-N^{α}-BOC-N^{π}-trityldeglycobleomycin A_2: a colorless foam; R_f 0.44 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.25 (1H, s), 8.14 (1H, s), 7.63 (1H, s), 7.28 (1H, s), 7.12–7.37 (15H, s), 6.94 (1H, s), 4.33 (1H, d, *J* = 4.0 Hz), 4.14 (1H, m), 4.08 (2H, m), 3.70 (2H, m), 3.60 (3H, m), 3.30–3.55 (5H, m), 2.97 (6H, s), 2.80 (2H, m), 2.65 (3H, m), 2.33 (3H, s), 2.18 (2H, m), 1.45 (9H, s), 1.10–1.30 (9H, m); FABMS (NBA) *m/e* 1372 (M^+, $C_{66}H_{83}N_{16}O_{11}S_3$).

in vacuo. Chromatography (SiO_2, 3 × 6 cm, 20% EtOAc–hexane) gave **15**[6e] (405 mg, 446 mg theoretical, 91%; typically 80–95%, 1–1.5 mmol scale) as a syrup: R_f 0.31 (SiO_2, 30% EtOAc–hexane); $[\alpha]^{25}_D$ +7.2 (*c* 0.21, $CHCl_3$), +4.8 (*c* 1.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.10–7.40 (5H, m), 6.10 (1H, d, *J* = 2.4 Hz), 5.35 (1H, dd, *J* = 2.4, 3.6 Hz), 5.28 (1H, dd, *J* = 10.0, 10.0 Hz), 4.69 (1H, d, *J* = 12.4 Hz), 4.44 (1H, d, *J* = 12.4 Hz), 4.24 (1H, dd, *J* = 5.2, 12.4 Hz), 4.09 (1H, dd, *J* = 2.4, 12.4 Hz), 3.95 (1H, ddd, *J* = 2.4, 5.2, 10.0 Hz), 3.87 (1H, dd, *J* = 3.6, 10.0 Hz), 2.17 (3H, s), 2.12 (3H, s), 2.09 (3H, s), 2.03 (3H, s); IR ($CHCl_3$) ν_{max} 1750, 1733, 1519, 1419, 1217, 1043, 924 cm⁻¹; FABHRMS (NBA–CsI) *m/e* 571.0565 (M^+ + Cs, $C_{21}H_{26}O_{10}$ requires 571.0580).

Conducting the reaction under identical conditions but for shorter reaction periods provided **15** in lower conversions (71–81%).

1,2,4,6-Tetra-*O*-acetyl-α-D-mannopyranose (16). A solution of **15** (2.9 mmol, 1.27 g) in CH_3OH (30 mL) was hydrogenated over 10% Pd-$(OH)_2$-C (240 mg) under H_2 (1 atm) at 25 °C for 10 h. The reaction mixture was filtered through a Celite pad and concentrated in vacuo. Chromatography (SiO_2, 3 × 10 cm, 40% EtOAc–hexane) provided **16**[6e] (980 mg, 1.01 g theoretical, 97%; typically 97–99%) as a colorless syrup: R_f 0.10 (SiO_2, 50% EtOAc–hexane); $[\alpha]^{25}_D$ +28 (*c* 0.23, $CHCl_3$); ^1H NMR (CD_3OD, 400 MHz) δ 5.93 (1H, d, *J* = 1.6 Hz), 5.10 (1H, dd, *J* = 10.0, 10.0 Hz), 5.01 (1H, dd, *J* = 1.6, 3.6 Hz), 4.17 (1H, dd, *J* = 4.8, 12.4 Hz), 4.04 (1H, dd, *J* = 3.6, 10.0 Hz), 3.98 (1H, dd, *J* = 2.4, 12.4 Hz), 3.91 (1H, m), 2.09 (3H, s), 2.07 (3H, s), 2.03 (3H, s), 1.97 (3H, s); IR ($CHCl_3$) ν_{max} 3498, 1742, 1519, 1419, 1217, 1151, 1047, 968, 924 cm⁻¹; FABHRMS (NBA–CsI) *m/e* 481.0125 (M^+ + Cs, $C_{21}H_{20}O_{10}$ requires 481.0111).

1,2,4,6-Tetra-*O*-acetyl-3-*O*-((*p*-nitrophenyl)carbamoyl)-α-D-mannopyranose (17). A solution of **16** (8.64 mmol, 3.0 g) in pyridine (30 mL) in the presence of (*N*,*N*-dimethylamino)pyridine (DMAP, 34.6 mmol, 4.3 g) was treated with *p*-nitrophenyl chloroformate (34.6 mmol, 7.0 g). The reaction mixture was stirred at 40 °C for 2 h and poured into a two-phase solution of EtOAc (30 mL) and H_2O (10 mL) with vigorous stirring. The organic layer was washed with 10% aqueous HCl (3 × 10 mL), saturated aqueous $NaHCO_3$ (10 mL), and saturated aqueous NaCl (10 mL), dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 3 × 10 cm, 25% EtOAc–hexane) gave **18**[6e] (3.8 g, 4.4 g theoretical, 86%; typically 75–93%, 10–0.3 mmol scale) as a syrup: R_f 0.58 (SiO_2, 50% EtOAc–hexane); $[\alpha]^{25}_D$ +1.4 (*c* 0.21, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.26 (2H, m), 7.41 (2H, m), 6.16 (1H, d, *J* = 2.0 Hz), 5.48 (1H, dd, *J* = 2.0, 3.2 Hz), 5.45 (1H, dd, *J* = 9.2, 9.2 Hz), 5.22 (1H, dd, *J* = 3.2, 9.2 Hz), 4.33 (1H, dd, *J* = 4.8, 12.4 Hz), 4.16 (1H, dd, *J* = 2.4, 12.4 Hz), 4.09 (1H, m), 2.24 (3H, s), 2.18 (3H, s), 2.13 (3H, s), 2.11 (3H, s); IR ($CHCl_3$) ν_{max} 1751, 1738, 1522, 1423, 1259, 1210, 1046, 1030, 926 cm⁻¹; FABHRMS (NBA–CsI) *m/e* 646.0160 (M^+ + Cs, $C_{21}H_{23}NO_{14}$ requires 646.0173).

1,2,4,6-Tetra-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranose (18). A solution of **17** (0.71 mmol, 360 mg) dissolved in CH_2Cl_2 (20 mL) was treated with a solution of 10% NH_3–THF (10 mL). The combined solution was stirred for 1.5 h at 25 °C and concentrated in vacuo. Flash chromatography (SiO_2, 1 × 8 cm, 50% EtOAc–hexane) afforded **18**[6e] (240 mg, 276 mg theoretical, 88%) as a colorless syrup: R_f 0.10 (SiO_2, 50% EtOAc–hexane); $[\alpha]^{25}_D$ +39 (*c* 0.18, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 6.10 (1H, d, *J* = 1.6 Hz), 5.34 (1H, dd, *J* = 9.6, 10.0 Hz), 5.25–5.31 (2H, m), 4.71 (2H, br s), 4.31 (1H, dd, *J* = 4.8, 12.4 Hz), 4.04–4.15 (2H, m), 2.18 (3H, s), 2.16 (3H, s), 2.10 (3H, s), 2.06 (3H, s); IR ($CHCl_3$) ν_{max} 3488, 1749, 1738, 1579, 1519, 1421, 1366, 1213, 1050, 973 cm⁻¹; FABHRMS (NBA–CsI) *m/e* 524.0150 (M^+ + Cs, $C_{15}H_{21}NO_{11}$ requires 524.0169).

2,4,6-Tri-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranosyl Diphenyl Phosphate (19). A solution of **18** (1.49 mmol, 550 mg) and benzylamine (3.72 mmol, 380 mg, 2.5 equiv) in THF (3 mL) was stirred for 24 h at 25 °C and concentrated in vacuo. Chromatography (SiO_2, 1 × 12 cm, 50% EtOAc–hexane) gave 2,4,6-tri-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranose (405 mg, 520 mg theoretical, 78%) as a colorless syrup: R_f 0.25 (SiO_2, 75% EtOAc–hexane): ^1H NMR ($CDCl_3$, 400 MHz) δ 5.20–5.40 (4H, m), 4.85 (2H, br s), 4.20–4.30 (2H, m), 4.15–4.20 (1H, m), 2.15 (3H, s), 2.10 (3H, s), 2.07 (3H, s).

A solution of *n*-BuLi (1.8 mmol, 0.8 mL, 2.3 M in hexane) was added to a stirred solution of 2,4,6-tri-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranose (1.49 mmol, 520 mg) in anhydrous THF (2.5 mL) at −78 °C. The resulting solution was stirred for 10 min at −78 °C before the addition of diphenyl chlorophosphate (1.8 mmol, 0.37 mL, 483 mg). After stirring for 10 min at −78 °C, the reaction mixture was poured into a two-phase solution of EtOAc (8 mL) and saturated aqueous $NaHCO_3$ (5 mL) with vigorous stirring. The organic layer was washed with saturated aqueous NaCl (2 × 3 mL) and dried (Na_2SO_4). Chromatography (SiO_2, 1 × 15 cm, 30% EtOAc–hexane in the presence of 5% Et_3N) afforded **19** (656 mg, 865 mg theoretical, 77%) as a white solid: mp 140–142 °C dec; R_f 0.42 (SiO_2, 60% EtOAc–hexane); $[\alpha]^{25}_D$ +25 (*c* 0.13, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.10–7.40 (10H, m), 5.89 (1H, dd, *J* = 1.8, 7.2 Hz), 5.25–5.40 (3H, m), 4.75 (2H, br s), 4.20 (1H, dd, *J* = 4.8, 12.4 Hz), 4.10 (1H, m), 3.93 (1H, dd, *J* = 2.0, 12.4 Hz), 2.16 (3H, s), 2.05 (3H, s), 1.98 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.5, 169.8, 169.5, 154.9, 130.0, 129.9, 125.8, 125.7, 120.2, 120.1, 120.0, 96.2, 70.7, 69.1, 69.0, 65.2, 61.6, 20.7, 20.6, 20.5; IR ($CHCl_3$) ν_{max} 3364, 1718, 1598, 1462, 1220, 958 cm⁻¹; FABHRMS (NBA–CsI) *m/e* 714.0366 (M^+ + Cs, $C_{25}H_{28}NO_{13}P$ requires 714.0353).

2,4,6-Tri-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranosyl Chloride (21). A solution of **18** (0.032 mmol, 12.4 mg) in CH_2Cl_2 (3 mL) was treated with HCl(g) at −10 °C for 10 min. The reaction flask was stoppered tightly, and the reaction mixture was maintained at 25 °C for 10 h. The solution was concentrated in vacuo, and the residue was purified by flash chromatography (SiO_2, 1 × 2 cm, 25% EtOAc–hexane) to provide **21** (5.5 mg, 11 mg theoretical, 48%; typically 45–67%) as a colorless syrup, which was determined not to be completely stable to the chromatographic purification: R_f 0.42 (SiO_2, 50% EtOAc–hexane); mp 126–128 °C (lit[6e] mp 132–134 °C); $[\alpha]^{25}_D$ +79 (*c* 0.3, $CHCl_3$) (lit[6e] $[\alpha]^{25}_D$ +83.6 (*c* 0.5, $CHCl_3$)); ^1H NMR ($CDCl_3$, 400 MHz) δ 5.93 (1H, d, *J* = 1.2 Hz), 5.46 (1H, dd, *J* = 3.2, 10.0 Hz), 5.34 (1H, dd, *J* = 1.2, 3.2 Hz), 5.27 (1H, dd, *J* = 10.0, 10.0 Hz), 4.60 (2H, br s), 4.25 (1H, dd, *J* = 12.0, 5.0 Hz), 4.21 (1H, ddd, *J* = 10.0, 5.0, 2.0 Hz), 4.08 (1H, dd, *J* = 2.0, 12.0 Hz), 2.11 (3H, s), 2.04 (3H, s), 2.02 (3H, s).

Benzyl 4,6-*O*-Benzylidene-α-D-mannopyranoside (23). A solution of **22**[18] (3.63 mmol, 972 mg) in DMF (8 mL) was treated with benzaldehyde dimethylacetal (3.63 mmol, 552 mg) and *p*TsOH (0.26 mmol, 50 mg) and warmed to 60 °C for 1 h under reduced pressure (20 mm Hg) to remove the liberated CH_3OH. The reaction mixture was poured into a two-phase solution of EtOAc (10 mL) and saturated aqueous $NaHCO_3$ (8 mL) with vigorous stirring. The organic layer was washed with saturated aqueous NaCl (2 × 5 mL) and dried (Na_2SO_4). Chromatography (SiO_2, 3 × 8 cm, 30% EtOAc–hexane) afforded **23** (990 mg, 1.30 g theoretical, 76%; typically 76–81%, 40 mmol scale): R_f 0.28 (SiO_2, 50% EtOAc–hexane); $[\alpha]^{25}_D$ +31.7 (*c* 0.23, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.20–7.50 (10H, m), 5.55 (1H, s), 4.92 (1H, s), 4.73 (1H, d, *J* = 12.0 Hz), 4.52 (1H, d, *J* = 12.0 Hz), 4.25 (1H, dd, *J* = 3.4, 9.5 Hz), 4.10 (1H, m), 4.05 (1H, m), 3.80–3.95 (3H, m); IR ($CHCl_3$) ν_{max} 3430, 1519, 1418, 1217, 1028, 927 cm⁻¹; FABHRMS (NBA–CsI) *m/e* 491.0470 (M^+ + Cs, $C_{20}H_{22}O_6$ requires 491.0471).

Benzyl 4,6-*O*-Benzylidene-3-*O*-benzyl-α-D-mannopyranoside (24). A suspension of **23** (1.56 mmol, 560 mg) and Bu_2SnO (1.71 mmol, 426 mg, 1.1 equiv) in CH_3OH (10 mL) was warmed at reflux for 1.5 h to provide a clear solution, cooled to 25 °C, and the solvent was removed under reduced pressure. The resulting benzyl 4,6-*O*-benzylidene-2,3-*O*-(dibutylstannylene)-α-D-mannopyranoside was dried under vacuum for 6 h and taken up in DMF (8 mL). The solution was treated with $PhCH_2Br$ (3.12 mmol, 533 mg, 2.0 equiv) and warmed at 100 °C with stirring for 30 min. The cooled reaction mixture was poured into a two-phase solution of EtOAc (15 mL) and saturated aqueous $NaHCO_3$ (10 mL) with vigorous stirring. The organic layer was washed with saturated aqueous NaCl (10 mL) and dried (Na_2SO_4). Chromatography (SiO_2, 3 × 6 cm, 25% EtOAc–hexane) afforded **24** (597 mg, 700 mg theoretical, 85%) as a colorless syrup: R_f 0.25 (SiO_2, 30% EtOAc–hexane); $[\alpha]^{25}_D$ +53 (*c* 0.47, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.20–7.60 (15H, m), 5.62 (1H, s), 4.97 (1H, s), 4.87 (1H, d, *J* = 12.0 Hz), 4.72 (1H, d, *J* = 12.0 Hz), 4.71 (1H, d, *J* = 12.0 Hz), 4.52 (1H, d, *J* = 12.0 Hz), 4.27 (1H, m), 4.12 (2H, m), 3.80–4.05 (3H, m); IR ($CHCl_3$) ν_{max} 3462, 1518, 1371, 1211, 1041 cm⁻¹; FABHRMS (NBA–CsI) *m/e* 581.0940 (M^+ + Cs, $C_{27}H_{28}O_6$ requires 581.0940).

Benzyl 4,6-*O*-Benzylidene-2-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranoside (25). A solution of **24** (1.13 mmol, 510 mg) in pyridine (5 mL) was treated with Ac_2O (3.4 mmol, 346 mg, 3.0 equiv). The reaction mixture was stirred at 25 °C for 4 h and poured into a two-phase solution of EtOAc (15 mL) and H_2O (8 mL) with vigorous stirring. The organic layer was washed with 10% aqueous HCl (2 × 10 mL), saturated aqueous $NaHCO_3$ (8 mL), and saturated aqueous NaCl (2 × 6 mL), dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 3 × 5 cm, 15% EtOAc–hexane) gave **25** (516 mg, 556 theoretical, 93%) as a syrup: R_f 0.76 (SiO_2, 60% EtOAc–hexane); $[\alpha]^{25}_D$ +29 (*c* 0.55, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.20–7.60 (15H, m), 5.64 (1H, s), 5.45 (1H, dd, *J* = 1.6, 2.8 Hz), 4.87 (1H, d, *J* = 1.6 Hz), 4.71 (2H, d, *J* = 12.0 Hz), 4.66 (1H, d, *J* = 12.0 Hz), 4.53 (1H, d, *J* = 12.0 Hz), 4.24 (1H,

dd, J = 2.8, 9.6 Hz), 4.05–4.10 (2H, m), 3.90 (1H, m), 3.85 (1H, dd, J = 9.6, 10.0 Hz), 2.15 (3H, s); IR ($CHCl_3$) ν_{max} 3014, 1728, 1518, 1422, 1371, 1211, 1041, 922 cm^{-1}; FABHRMS (NBA–CsI) m/e 623.1050 (M^+ + Cs, $C_{29}H_{30}O_7$ requires 623.1046).

Benzyl 2-O-Acetyl-3-O-benzyl-α-D-mannopyranoside (26). A solution of **25** (0.81 mmol, 400 mg) in 80% HOAc–H_2O (8 mL) was stirred for 1 h at 60 °C. The reaction mixture was poured into a two-phase solution of EtOAc (15 mL) and H_2O (10 mL) with vigorous stirring. The organic layer was washed with saturated aqueous $NaHCO_3$ (3 × 8 mL) and saturated aqueous NaCl (2 × 5 mL) and dried (Na_2SO_4). Chromatography (SiO_2, 3 × 5 cm, 50% EtOAc–hexane) afforded **26** (270 mg, 325 mg theoretical, 83%) as a syrup: R_f 0.10 (SiO_2, 50% EtOAc–hexane); $[\alpha]^{25}_D$ +31.9 (c 0.19, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.10–7.50 (10H, m), 5.41 (1H, dd, J = 2.0, 3.0 Hz), 4.91 (1H, d, J = 2.0 Hz), 4.74 (1H, d, J = 12.0 Hz), 4.76 (1H, d, J = 12.0 Hz), 4.51 (1H, d, J = 12.0 Hz), 4.43 (1H, d, J = 12.0 Hz), 3.60–4.00 (5H, m), 2.11 (3H, m); IR ($CHCl_3$) ν_{max} 3450, 1728, 1518, 1472, 1421, 1376, 1211, 1041, 928 cm^{-1}; FABHRMS (NBA–CsI), m/e 535.0730 (M^+ + Cs, $C_{22}H_{26}O_7$ requies 535.0733).

Benzyl 2-O-Acetyl-3-O-benzyl-6-iodo-6-deoxy-α-D-mannopyranoside (27). A solution of **26** (4.9 mmol, 1.97 g), Ph_3P (5.85 mmol, 1.53 g, 1.2 equiv), and imidazole (7.4 mmol, 500 mg, 1.5 equiv) in toluene (30 mL) was treated with I_2 (5.85 mmol, 1.48 g, 1.2 equiv) at 25 °C. The reaction mixture was stirred for 3 h at 100 °C and poured into a two-phase solution of EtOAc (30 mL) and saturated aqueous $NaHCO_3$ (20 mL) with vigorous stirring. The organic layer was washed with saturated aqueous NaCl (2 × 10 mL) and dried (Na_2SO_4). Chromatography (SiO_2, 3 × 8 cm, 17% EtOAc–hexane) gave **27** (2.14 g, 2.52 g theoretical, 85%) as a syrup: R_f 0.45 (SiO_2, 30% EtOAc–hexane); $[\alpha]^{25}_D$ +21 (c 1.07, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.20–7.50 (10H, m), 5.40 (1H, dd, J = 1.8, 3.2 Hz), 4.91 (1H, d, J = 1.8 Hz), 4.84 (1H, d, J = 12.0 Hz), 4.72 (1H, d, J = 12.0 Hz), 4.57 (1H, d, J = 12.0 Hz), 4.40 (1H, d, J = 12.0 Hz), 3.82 (1H, dd, J = 3.2, 8.8 Hz), 3.70 (1H, ddd, J = 2.0, 8.8, 9.0 Hz), 3.60 (2H, m), 3.32 (1H, dd, J = 8.0, 9.0 Hz), 2.11 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.6, 137.8, 136.2, 128.6, 128.5, 128.4, 128.2, 128.17, 128.13, 96.7, 77.3, 71.7, 71.6, 70.4, 69.2, 67.8, 21.3, 6.6; IR ($CHCl_3$) ν_{max} 3410, 1729, 1518, 1371, 1248, 1211, 1042, 928 cm^{-1}; FABHRMS (NBA–CsI) m/e 644.9750 (M^+ + Cs, $C_{22}H_{25}O_6I$ requires 644.9750).

Benzyl 2-O-Acetyl-3-O-benzyl-6-deoxy-α-D-*erythro*-hex-5-enopyranoside (28). A solution of **27** (13.6 mmol, 7.0 g) in pyridine (50 mL) was treated with AgF (68 mmol, 8.6 g, 5 equiv). The reaction mixture was stirred at 25 °C for 3 h and poured into a two-phase solution of EtOAc (100 mL) and H_2O (50 mL) with vigorous stirring. The organic layer was washed with 10% aqueous HCl (3 × 40 mL), saturated aqueous $NaHCO_3$ (30 mL), and saturated aqueous NaCl (2 × 30 mL), dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 3 × 30 cm, 10% EtOAc–hexane) gave **28** (2.9 g, 5.2 g theoretical, 56%) as a syrup: R_f 0.37 (SiO_2, 20% EtOAc–hexane); $[\alpha]^{25}_D$ –4.2 (c 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.20–7.40 (10H, m), 5.45 (1H, dd, J = 1.8, 3.2 Hz), 4.95 (1H, d, J = 1.8 Hz), 4.91 (1H, dd, J = 1.0, 1.0 Hz), 4.84 (1H, d, J = 12.0 Hz), 4.77 (1H, dd, J = 1.0, 2.0 Hz), 4.71 (1H, d, J = 12.0 Hz), 4.53 (1H, d, J = 12.0 Hz), 4.44 (1H, d, J = 12.0 Hz), 4.42 (1H, m), 3.83 (1H, dd, J = 3.2, 10.0 Hz), 2.11 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.6, 155.1, 137.3, 136.7, 128.6, 128.5, 128.2, 128.15, 128.12, 128.07, 97.6, 95.8, 77.6, 71.8, 69.2, 68.1, 67.7, 20.9; IR ($CHCl_3$) ν_{max} 3430, 3018, 1724, 1519, 1422, 1211, 1045, 907 cm^{-1}; FABHRMS (NBA–CsI) m/e 517.0630 (M^+ + Cs, $C_{22}H_{24}O_6$ requires 517.0627).

Benzyl 2-O-Acetyl-3-O-benzyl-β-L-gulopyranoside (29). Catecholborane (8.72 mmol, 1.0 mL, 1.05 g, 4 equiv) was added to a mixture of **28** (2.18 mmol, 840 mg) and $Rh(PPh_3)_3Cl$ (0.11 mmol, 106 mg, 0.05 equiv) in a solution of dry THF (18 mL) at –78 °C. The resulting solution was warmed to 25 °C and stirred for 16 h. The reaction mixture was treated with 1:1 EtOH–THF (4 mL), pH 7.0 buffer (4 mL, 0.05 M potassium phosphate monobasic–sodium hydroxide), and then 50% aqueous H_2O_2 (4 mL) at 0 °C. The reaction mixture was stirred at 25 °C for 3 h and poured into a two-phase solution of EtOAc (30 mL) and 30% aqueous $NaHSO_3$ (20 mL) with vigorous stirring. The organic layer was washed with saturated aqueous $NaHCO_3$ (20 mL) and saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 3 × 15 cm, 25–60% EtOAc–hexane gradient elution) gave **29** (720 mg, 878 mg theoretical, 82%) as a syrup: R_f 0.21 (SiO_2, 60% EtOAc–hexane); $[\alpha]^{25}_D$ +75.9 (c 0.20, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.10–7.40 (10H, m), 5.18 (1H, dd, J = 2.8, 8.0 Hz), 5.00 (1H, d, J = 8.0 Hz), 4.91 (1H, d, J = 12.0 Hz), 4.66 (1H, d, J = 12.0 Hz), 4.63 (1H, d, J = 12.0 Hz), 4.60 (1H, d, J = 12.0 Hz), 3.80–4.00 (5H, m), 2.03 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.9, 137.8, 137.5, 128.4, 128.3, 127.9, 127.7 (2C), 127.5, 98.6, 76.7, 73.3, 72.7, 70.9, 70.1, 69.7, 63.3, 20.9; IR ($CHCl_3$) ν_{max} 3463, 1734, 1521, 1420, 1046, 928, 763 cm^{-1}; FABHRMS (NBA–CsI) m/e 535.0730 (M^+ + Cs, $C_{22}H_{26}O_7$ requires 535.0733).

Benzyl 2-O-Acetyl-3,4,6-tri-O-benzyl-β-L-gulopyranoside (30). A solution of **29** (1.78 mmol, 720 mg) in 30% CH_2Cl_2–hexane (15 mL) was treated with benzyl trichloroacetimidate (17.8 mmol, 4.5 g, 10 equiv) and CF_3SO_3H (0.6 mmol, 53 μL, 90 mg, 0.3 equiv) at 25 °C. After 1 h at 25 °C, the reaction mixture was poured into a two-layer solution of EtOAc (15 mL) and saturated aqueous $NaHCO_3$ (10 mL) with vigorous stirring. The organic layer was washed with saturated aqueous NaCl, dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 3 × 10 cm, 5–10% EtOAc–hexane gradient elution) afforded **30** (745 mg, 1.04 g theoretical, 72%) as a syrup: R_f 0.48 (SiO_2, 20% EtOAc–hexane); $[\alpha]^{25}_D$ +70 (c 0.13, $CHCl_3$) (lit[6c] $[\alpha]^{25}_D$ +72.7 (c 1.36, $CHCl_3$)); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.10–7.40 (20H, m), 5.09 (1H, d, J = 3.2, 8.4 Hz), 4.95 (1H, d, J = 8.4 Hz), 4.92 (1H, d, J = 12.0 Hz), 4.40–4.65 (7H, m), 4.16 (1H, ddd, J = 12, 6.4, 6.4 Hz), 4.02 (1H, dd, J = 3.2, 3.6 Hz), 3.69 (1H, dd, J = 6.5, 9.6 Hz), 3.64 (1H, dd, J = 6.4, 9.6 Hz), 3.53 (1H, dd, J = 1.2, 3.6 Hz), 2.01 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.9, 138.1, 137.8 (2 C), 137.6, 128.8, 128.4, 128.3, 128.2 (2 C), 127.9 (2 C), 127.8, 127.7, 127.6, 127.5, 127.4, 97.7, 73.84, 73.75, 73.5, 73.3, 72.5, 72.4, 71.1, 70.3, 68.8, 21.0; IR ($CHCl_3$) ν_{max} 1737, 1452, 1365, 1230, 1077, 910 cm^{-1}; FABHRMS (NBA–CsI), m/e 715.1665 (M^+ + Cs, $C_{36}H_{38}O_7$ requires 715.1672).

Benzyl 3,4,6-Tri-O-benzyl-β-L-gulopyranoside (31). A solution of **30** (1.28 mmol, 745 mg) in CH_3OH (30 mL) was treated with K_2CO_3 (0.86 mmol, 120 mg). The reaction mixture was stirred at 25 °C for 2 h and poured into a two-phase solution of EtOAc (40 mL) and saturated aqueous NaCl (20 mL) with vigorous stirring. The organic layer was washed with saturated aqueous NaCl (2 × 20 mL), dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 3 × 7 cm, 10–15% EtOAc–hexane gradient elution) gave **31** (580 mg, 691 mg theoretical, 84%) as a syrup, which could not be induced to crystallize: R_f 0.37 (SiO_2, 20% EtOAc–hexane); $[\alpha]^{25}_D$ +49 (c 0.12, $CHCl_3$) (lit[6c] $[\alpha]^{25}_D$ +48.3 (c 0.7, $CHCl_3$)); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.10–7.40 (20H, m), 4.95 (1H, d, J = 12.0 Hz), 4.72 (1H, d, J = 8.0 Hz), 4.40–4.60 (7H, m), 4.08 (1H, ddd, J = 1.2, 6.4, 6.4 Hz), 3.88 (1H, dd, J = 3.2, 4.6 Hz), 3.85 (1H, m), 3.69 (1H, dd, J = 6.4, 9.6 Hz), 3.64 (1H, dd, J = 6.4, 9.6 Hz), 3.56 (1H, dd, J = 1.2, 3.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 137.9, 137.6, 137.2, 136.8, 128.5, 128.4 (4 C), 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 100.0, 75.9, 73.6, 73.44, 73.36, 72.8, 72.3, 70.6, 69.5, 68.8; IR ($CHCl_3$) ν_{max} 3405, 1521, 1441, 1215, 1092 cm^{-1}; FABHRMS (NBA–CsI) m/e 673.1556 (M^+ + Cs, $C_{34}H_{36}O_6$ requires 673.1566).

1,3,4,5-Tetra-O-benzyl-2-O-(2,4,6-tri-O-acetyl-3-O-carbamoyl-α-D-mannopyranosyl)-β-L-gulopyranoside (2). A solution of **31** (0.07 mmol, 38 mg) and **19** (0.084 mmol, 49 mg, 1.2 equiv) in dry CH_2Cl_2 (1 mL) was treated with TMSOTf (0.126 mmol, 25 μL, 30 mg, 1.8 equiv) at 0 °C. The reaction mixture was stirred at 0 °C for 10 min and poured into a two-phase solution of EtOAc (5 mL) and saturated aqueous $NaHCO_3$ (3 mL) with vigorous stirring. The organic layer was washed with saturated aqueous NaCl (2 × 2 mL), dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 1 × 10 cm, 25% EtOAc–hexane) gave **2** (56 mg, 60 mg theoretical, 93%) as a syrup: R_f 0.38 (SiO_2, 50% EtOAc–hexane); $[\alpha]^{25}_D$ +37.8 (c 0.12, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.10–7.50 (20H, m), 5.29 (1H, dd, J = 3.6, 10.0 Hz), 5.23 (1H, dd, J = 10.0, 10.0 Hz), 5.19 (1H, dd, J = 1.6, 3.6 Hz), 4.95 (1H, d, J = 8.0 Hz), 4.93 (1H, d, J = 12.0 Hz), 4.76 (1H, d, J = 1.6 Hz), 4.30–4.70 (7H, m), 4.30 (1H, m), 4.15 (1H, m), 3.94 (1H, dd, J = 3.2, 12.8 Hz), 3.90 (1H, dd, J = 3.2, 8.0 Hz), 3.80 (1H, dd, J = 3.2, 3.2 Hz), 3.73 (1H, dd, J = 2.0, 14.8 Hz), 3.66 (1H, dd, J = 6.4, 12.8 Hz), 3.59 (1H, dd, J = 8.0, 12.8 Hz), 3.52 (1H, dd, J = 1.2, 3.2 Hz), 2.17 (3H, s), 2.02 (3H, s), 1.98 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.6, 170.1, 169.9, 155.0, 138.1, 137.7, 137.6, 137.4, 128.9 (2 C), 128.5, 128.44, 128.40, 128.37, 127.28, 128.1, 128.0, 127.9, 127.7, 98.4, 94.3, 77.2, 74.2, 73.6, 73.4, 73.1, 72.8, 72.1, 71.6, 71.2, 70.3, 70.1, 68.7, 68.2, 65.5, 61.4, 21.0, 20.8, 20.7; IR ($CHCl_3$) ν_{max} 3501, 2957, 1743, 1602, 1363, 1263, 1230, 1091, 1047 cm^{-1}; FABHRMS (NBA–CsI) m/e 1004.2433 (M^+ + Cs, $C_{47}H_{53}NO_{15}$ requires 1004.2470).

1,3,4,6-Tetra-O-acetyl-2-O-(2,4,6-tri-O-acetyl-3-O-carbamoyl-α-D-mannopyranosyl)-β-L-gulopyranoside (33). A solution of **2** (0.61 mmol, 530 mg) in CH_3OH (40 mL) was hydrogenated over 10% $Pd(OH)_2$-C (100 mg) under an atmosphere of H_2 (1 atm) at 25 °C for 15 h. The reaction mixture was filtered through a Celite pad and concentrated in vacuo.

The residue containing **32** was dissolved in pyridine (20 mL) and treated with Ac_2O (80 mmol, 810 mg, 10 equiv). The reaction mixture

was stirred at 25 °C for 10 h and poured into a two-phase solution of EtOAc (40 mL) and H_2O (20 mL) with vigorous stirring. The organic layer was washed with 10% HCl (2 × 20 mL), saturated aqueous $NaHCO_3$ (15 mL), and saturated aqueous NaCl (2 × 15 mL), dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 3 × 10 cm, 50% EtOAc–hexane) gave **33** (405 mg, 414 mg theoretical, 98%) as a colorless foam: R_f 0.28 (SiO_2, 60% EtOAc–hexane); $[\alpha]^{25}_D$ +35.4 (c 0.07, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 5.89 (1H, d, J = 8.0 Hz), 5.44 (1H, dd, J = 3.6, 3.6 Hz), 5.27 (1H, dd, J = 10.0, 10.0 Hz), 5.12 (1H, dd, J = 1.6, 3.6 Hz), 5.08 (1H, dd, J = 3.6, 10.0 Hz), 5.02 (1H, dd, J = 1.2, 3.6 Hz), 4.99 (1H, d, J = 1.6 Hz), 4.62 (2H, br s), 4.36 (1H, m), 4.05–4.30 (5H, m), 4.00 (1H, dd, J = 3.6, 8.0 Hz), 2.20 (3H, s), 2.16 (3H, s), 2.14 (3H, s), 2.13 (3H, s), 2.12 (3H, s), 2.06 (3H, 2), 2.05 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.5, 170.4, 169.8, 169.4, 169.3 (2 C), 168.7, 155.0, 95.0, 90.6, 71.3, 69.8, 69.5, 69.2, 69.0, 67.7, 65.9, 65.5, 62.0, 61.3, 20.8 (3 C), 20.7 (4 C); IR ($CHCl_3$) ν_{max} 3425, 1744, 1371, 1209, 1077, 1053, 758 cm^{-1}; FABHRMS (NBA–CsI) m/e 812.1014 (M^+ + Cs, $C_{27}H_{37}NO_{19}$ requires 812.1014).

3,4,6-Tri-O-acetyl-2-O-(2,4,6-tri-O-acetyl-3-O-carbamoyl-α-D-mannopyranosyl)-β-L-gulopyranosyl Diphenyl Phosphate (34). A solution of **33** (0.10 mol, 70 mg) and benzylamine (0.26 mmol, 28 mg, 2.5 equiv) in THF (1 mL) was stirred for 17 h at 25 °C. The reaction mixture was directly evaporated in vacuo. Chromatography (SiO_2, 1 × 8 cm, 1.5–2.5% CH_3OH–CH_2Cl_2 gradient elution) gave 3,4,6-tri-O-acetyl-2-O-(2,4,6-tri-O-acetyl-3-O-carbamoyl-α-D-mannopyranosyl)-β-L-gulopyranose **3** (52 mg, 64 mg theoretical, 81%) as a colorless foam: R_f 0.11 (SiO_2, 60% EtOAc–hexane); ^1H NMR ($CDCl_3$, 400 MHz) δ 5.39 (1H, dd, J = 3.6, 3.6 Hz), 5.27 (1H, dd, J = 10.0, 10.0 Hz), 5.19 (1H, dd, J = 3.2, 10.0 Hz), 5.13 (1H, dd, J = 1.6, 3.2 Hz), 5.00 (1H, d, J = 8.0 Hz), 4.98 (1H, dd, J = 1.2, 3.6 Hz), 4.96 (1H, d, J = 1.6 Hz), 4.76 (2H, br s), 4.40 (1H, m), 4.10–4.30 (5H, m), 3.77 (1H, dd, J = 3.6, 8.0 Hz), 2.17 (3H, s), 2.14 (3H, s), 2.12 (3H, s), 2.11 (3H, s), 2.07 (3H, s), 2.06 (3H, s).

A solution of n-BuLi (0.176 mmol, 76 μL of 2.3 M in hexane) was added to a stirred solution of **3** (0.082 mmol, 52 mg) in anhydrous THF (1.2 mL) at –78 °C. The resulting solution was stirred for 10 min at –78 °C before the addition of diphenyl chlorophosphate (0.132 mmol, 27 μL, 35 mg). After being stirred for 10 min at –78 °C, the reaction mixture was poured into a two-phase solution of EtOAc (4 mL) and saturated aqueous $NaHCO_3$ (2 mL) with vigorous stirring. The organic layer was washed with saturated aqueous NaCl (2 × 2 mL) and dried (Na_2SO_4). Chromatography (SiO_2, 1 × 8 cm, 1–2% CH_3OH–CH_2Cl_2 gradient elution) afforded **34** (50 mg, 70 mg theoretical, 71%) as a colorless foam: R_f 0.21 (SiO_2, 10% CH_3OH–$CHCl_3$); $[\alpha]^{25}_D$ +33.7 (c 0.17, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.10–7.40 (10H, m), 5.71 (1H, dd, J = 6.8, 8.0 Hz), 5.46 (1H, m), 5.26 (1H, dd, J = 10.0, 10.0 Hz), 5.18 (1H, dd, J = 1.6, 3.6 Hz), 5.15 (1H, dd, J = 3.6, 10.0 Hz), 5.02 (1H, dd, J = 1.2, 3.6 Hz), 4.99 (1H, d, J = 1.6 Hz), 4.00–4.40 (7H, m), 2.22 (3H, s), 2.16 (3H, s), 2.14 (3H, s), 2.08 (3H, s), 2.00 (3H, s), 1.72 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.6, 170.4, 169.8, 169.4, 169.3, 169.2, 154.9, 129.9, 129.7, 128.7, 128.3, 127.9, 127.6, 125.7, 125.6, 96.2, 95.4, 77.2, 71.6, 70.0, 69.12, 69.07, 67.4, 65.5, 65.4, 61.8, 61.2, 20.8, 20.7 (2 C), 20.64, 20.59, 20.3; IR ($CHCl_3$) ν_{max} 3018, 1751, 1521, 1424, 1213, 1046, 928, 777 cm^{-1}; FABHRMS (NBA–CsI) m/e 1002.1191 (M^+ + Cs, $C_{37}H_{44}NO_{21}P$ requires 1002.1198).

erythro-N^α-((Benzyloxy)carbonyl)-N^{im}-(triphenylmethyl)-β-[3,4,6-tri-O-acetyl-2-O-(2,4,6-tri-O-acetyl-3-O-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]-L-histidine Methyl Ester (4). A solution of **36** (0.15 mmol, 83 mg) and **34** (0.17 mmol, 153 mg, 1.1 equiv) in dry CH_2Cl_2–Et_2O (1:2, 3 mL) was treated with TMSOTf (0.70 mmol, 140 μL, 166 mg, 4.2 equiv) at 0 °C. The reaction mixture was stirred at 0 °C for 20 min and poured into a two phase solution of EtOAc (10 mL) and saturated aqueous $NaHCO_3$ (5 mL) with vigorous stirring. The organic layer was washed with saturated aqueous NaCl (2 × 5 mL), dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 1 × 14 cm, 1.4–3.2% CH_3OH–CH_2Cl_2 gradient elution) afforded **4** (104 mg, 173 mg theoretical, 60%; typically 54–63%, 0.03–0.05 mmol scale) as a colorless foam: R_f 0.35 (SiO_2, 10% CH_3OH–$CHCl_3$); $[\alpha]^{25}_D$ –4.7 (c 0.43, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.43 (1H, br s), 7.05–7.40 (20H, m), 6.82 (1H, br s), 6.56 (1H, br d, J = 8.4 Hz), 5.00–5.30 (9H, m), 4.99 (1H, d, J = 1.6 Hz), 4.90 (1H, m), 4.67 (2H, br s), 4.10–4.40 (4H, m), 3.99 (1H, dd, J = 3.6, 3.6 Hz), 3.89 (1H, dd, J = 6.2, 12.8 Hz), 3.67 (1H, dd, J = 7.5, 12.8 Hz), 3.63 (3H, s), 2.12 (3H, s), 2.09 (3H, s), 2.05 (3H, s), 1.99 (3H, s), 1.88 (3H, s), 1.80 (3H, s); IR ($CHCl_3$) ν_{max} 3005, 1744, 1523, 1420, 1214, 1047, 929 cm^{-1}; FABHRMS (NBA–CsI) m/e 1313.3050 (M^+ + Cs, $C_{59}H_{64}N_4O_{22}$ requires 1313.3067).

erythro-N^α-((tert-Butyloxy)carbonyl)-N^{im}-(triphenylmethyl)-β-[3,4,6-tri-O-acetyl-2-O-(2,4,6-tri-O-acetyl-3-O-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]-L-histidine Methyl Ester (38). A solution of **4** (0.016 mmol, 19 mg) in CH_3OH–EtOAc (1:2, 3 mL) was hydrogenated over 10% Pd-C (7 mg) under H_2 (1 atm) at 25 °C for 5 h. The reaction mixture was filtered through a Celite pad and concentrated in vacuo. Chromatography (SiO_2, 0.5 × 5 cm, 5–10% CH_3OH–CH_2Cl_2 gradient elution) gave **37** (8.0 mg, 16 mg theoretical, 50%) as a colorless foam and 5.0 mg (26%) of recovered **4**. For **38**: R_f 0.27 (SiO_2, 10% CH_3OH–$CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.42 (1H, br s), 7.10–7.40 (15H, m), 6.87 (1H, br s), 5.10–5.30 (6H, m), 4.99 (1H, d, J = 4.0 Hz), 4.98 (1H, d, J = 1.6 Hz), 4.90 (1H, dd, J = 1.2, 3.6 Hz), 4.62 (2H, br s), 4.30 (2H, m), 4.10 (2H, m), 3.99 (1H, dd, J = 3.6, 3.6 Hz), 3.95 (1H, dd, J = 6.2, 12.8 Hz), 3.72 (1H, dd, J = 7.5, 12.8 Hz), 3.69 (3H, s), 2.13 (3H, s), 2.10 (3H, s), 2.08 (3H, s), 2.07 (3H, s), 1.89 (3H, s), 1.80 (3H, s).

A solution of **37** (0.006 mmol, 6.0 mg) in THF–H_2O (3:1, 0.4 mL) was treated with di-tert-butyl dicarbonate (0.017 mmol, 3.7 mg, 3 equiv) at 25 °C, and the mixture was allowed to stir for 10 h. The reaction mixture was poured into a two-phase solution of saturated aqueous NaCl (0.5 mL) and EtOAc (1 mL). The organic phase was dried (Na_2SO_4) and concentrated in vacuo. Chromatography (SiO_2, 0.5 × 5 cm, 1.5–3.0% CH_3OH–CH_2Cl_2 gradient elution) afforded **38** (5.5 mg, 6.5 mg theoretical, 85%; typically 85–94%) as a colorless foam: R_f 0.35 (SiO_2, 10% CH_3OH–$CHCl_3$); $[\alpha]^{25}_D$ –20 (c 0.06, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.44 (1H, d, J = 1.2 Hz), 7.10–7.40 (15H, m), 6.81 (1H, d, J = 1.2 Hz), 6.19 (1H, br d, J = 7.0 Hz), 5.15–5.30 (4H, m), 5.13 (1H, d, J = 3.6 Hz), 5.10 (1H, m), 4.98 (1H, d, J = 1.6 Hz), 4.96 (1H, m), 4.84 (1H, br s), 4.60 (2H, br s), 4.40 (2H, m), 4.11 (1H, dd, J = 2.4, 12.8 Hz), 3.99 (1H, dd, J = 3.6, 3.6 Hz), 3.90 (1H, dd, J = 6.2, 12.8 Hz), 3.70 (1H, dd, J = 7.5, 12.8 Hz), 3.63 (3H, s), 2.13 (3H, s), 2.09 (3H, s), 2.08 (3H, s), 1.91 (3H, s), 1.89 (3H, s), 1.72 (3H, s), 1.43 (9H, s); IR ($CHCl_3$) ν_{max} 3005, 1745, 1521, 1418, 1215 cm^{-1}; FABHRMS (NBA–CsI) m/e 1147.4275 (M^+ + H, $C_{56}H_{66}N_4O_{22}$ requires 1147.4247).

erythro-N^α-((tert-Butyloxy)carbonyl)-N^{im}-(triphenylmethyl)-β-[2-O-(3-O-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]-L-histidine (39). A solution of **38** (0.004 mmol, 4.0 mg) in CH_3OH (0.7 mL) was cooled at 0 °C and treated with aqueous 0.1 N NaOH (0.7 mL, 0.07 mmol, 20 equiv), and the reaction mixture was stirred at 0 °C for 12 h. After evaporation of the CH_3OH under a N_2 stream, the aqueous phase was neutralized to pH 7 with the addition of HOAc, and the mixture was concentrated under a N_2 stream. Chromatography (reverse-phase C-18, 15–25% CH_3OH–H_2O gradient elution) afforded **39** (2.4 mg, 3.1 mg theoretical, 78%) as a colorless film: R_f 0.31 (SiO_2, 8:2:1 t-BuOH–HOAc-H_2O); $[\alpha]^{25}_D$ +20 (c 0.03, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.39 (1H, s), 7.15–7.38 (15H, m), 7.00 (1H, s), 5.13 (2H, m), 4.91 (1H, m), 4.60–4.50 (2H, m), 3.40–4.10 (11H, m), 1.37 (9H, s); IR (neat) ν_{max} 3400, 2938, 1700, 1611, 1491, 1156, 1057 cm^{-1}; FABHRMS (NBA–CsI) m/e 1013.2460 (M^+ + Cs, $C_{43}H_{52}N_4O_{16}$ requires 1013.2433).

N^α-((tert-Butyloxy)carbonyl)-1-[amido-[3-[2'-(2'''''-((N-(4''(R)-amino-3''(S)-hydroxy-2''(S)-methylpentanoyl)-L-threonyl)amino)ethyl)-2,4'-bithiazole-4-carboxamido]propyl]dimethylsulfonium iodide]-N^{im}-(triphenylmethyl)-erythro-β-[2'''-O-(3''''-O-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]-L-histidine (41). A solution of **39** (0.0033 mmol, 2.9 mg) in DMF (20 mL) was treated sequentially with DCC (0.0066 mmol, 1.4 mg, 2.0 equiv), HOBt (0.0036 mmol, 0.50 mg, 1.1 equiv), $NaHCO_3$ (0.005 mmol, 0.4 mg, 1.5 equiv), and **40** (0.005 mmol, 3.1 mg, 1.5 equiv) dissolved in DMF (70 μL), and the mixture was stirred under Ar at 25 °C for 48 h. After removal of the solvent in vacuo, the crude residue was dissolved in CH_3OH (0.3 mL), and the insoluble salts were removed by centrifugation. The CH_3OH solution was evaporated, and the sample was triturated with $CHCl_3$ (3 × 0.2 mL). Chromatography (reverse-phase C-18, 10–90% CH_3OH–H_2O gradient elution) afforded **41** (4.4 mg, 4.7 mg theoretical, 94%) as a thin film: R_f 0.45 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); $[\alpha]^{25}_D$ +26.6 (c 0.03, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.20 (1H, s), 8.10 (1H, s), 7.43 (1H, br s), 7.10–7.40 (15H, m), 7.02 (1H, br s), 5.08 (1H, br s), 4.97 (1H, br s), 4.80–4.90 (3H, m), 3.40–4.30 (23H, m), 2.93 (6H, s), 2.59 (1H, dq, J = 7.0, 7.0 Hz), 2.15 (2H, tt, J = 7.0, 7.0 Hz), 1.41 (9H, s), 1.00–1.20 (9H, m); IR (neat) ν_{max} 3330, 1708, 1643, 1545, 1441, 1249, 1056 cm^{-1}; FABHRMS (NBA) m/e 1449.5499 (M^+, $C_{67}H_{89}N_{10}O_{20}S_3$ requires 1449.5417).

1-[Amido-[3-[2'-(2'''''-((N-(4''(R)-amino-3''(S)-hydroxy-2''(S)-methylpentanoyl)-L-threonyl)amino)ethyl)-2,4'-bithiazole-4-carboxamido]propyl]dimethylsulfonium iodide]-erythro-β-[2'''-O-(3''''-O-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]-L-histidine (42). Compound **41** (0.0013 mmol, 1.8 mg) was treated with 20% TFA–CH_2Cl_2 (1 mL), and the mixture was stirred at 0 °C for 3 h under Ar. The solvent was

evaporated in vacuo to give an oily solid. The sample was triturated with $CHCl_3$ (2 × 0.5 mL). Chromatography (reverse-phase C-18, 0–10% CH_3OH–H_2O gradient elution) afforded **42** (1.4 mg, 1.47 mg theoretical, 95%) as a thin film: R_f 0.15 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); $[\alpha]^{25}_D$ +10 (*c* 0.03, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.22 (1H, s), 8.12 (1H, s), 7.67 (1H, br s), 7.13 (1H, br s), 4.60–5.00 (5H, m), 4.29 (1H, d, *J* = 4.5 Hz), 3.30–4.20 (22H, m), 2.93 (6H, s), 2.40 (1H, m), 2.16 (2H, tt, *J* = 7.0, 7.0 Hz), 1.15 (9H, m); IR (neat) ν_{max} 3333, 1738, 1707, 1671, 1548, 1384, 1133, 1061 cm^{-1}; FABHRMS (NBA) *m/e* 1107.3877 (M^+, $C_{43}H_{67}N_{10}O_{18}S_3$ requires 1107.3797).

1-[Amido-[3-[2′-(2″‴-((*N*-(4″(*R*)-amino-3″(*S*)-hydroxy-2″(*S*)-methylpentanoyl)-L-threonyl)amino)ethyl)-2,4′-bithiazole-4-carboxamido]propyl]dimethylsulfonium Iodide]-N^{im}-(triphenylmethyl)-*erythro*-β-[2‴-*O*-(3⁗-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]-L-histidine (43). Compound **41** (0.003 mmol, 4.4 mg) was treated with 1 N HCl in 90% aqueous HOAc (1.0 mL), and the mixture was stirred at 25 °C for 15 min under Ar. The solvent was evaporated in vacuo to give a solid. Chromatography (reverse-phase C-18, 0–40% CH_3OH–H_2O gradient elution) afforded **43** (3.3 mg, 4.0 mg theoretical 83%) as a thin film: R_f 0.20 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); $[\alpha]^{25}_D$ + 14.2 (*c* 0.02, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.20 (1H, s), 8.11 (1H, s), 7.46 (1H, br s), 7.10–7.40 (15H, m), 7.05 (1H, br s), 4.80–5.10 (5H, m), 4.29 (1H, d, *J* = 4.3 Hz), 3.00–4.20 (22H, m), 2.93 (6H, s), 2.59 (1H, m), 2.15 (2H, m), 1.15–1.30 (9H, m); IR (neat) ν_{max} 3410, 1700, 1652, 1480, 1210 cm^{-1}; FABHRMS (NBA) *m/e* 1350.4920 (M^+ + H, $C_{62}H_{81}N_{10}O_{18}S_3$ requires 1350.4971).

N^{α}-((*tert*-Butyloxy)carbonyl)-N^{im}-(triphenylmethyl)bleomycin A_2 (46). A solution of **44** (0.002 mmol, 0.9 mg) in DMF (20 μL) was treated sequentially with DCC (0.006 mmol, 1.6 mg, 3.0 equiv), HOBt (0.002 mmol, 0.33 mg, 1.0 equiv), and **43** (0.002 mmol, 2.8 mg, 1.0 equiv) dissolved in DMF (20 μL), and the mixture was stirred at 25 °C for 48 h under Ar. The crude mixture was dissolved in CH_3OH (1 mL), and the insoluble inorganic salts were removed by centrifugation. The CH_3OH solution was evaporated and the sample was triturated with $CHCl_3$ (2 × 0.3 mL). The solid was purified by chromatography (reverse-phase C-18, 10–20% CH_3OH–H_2O gradient elution) to afford **46** (2.9 mg, 3.5 mg theoretical, 83%) as a thin film: R_f 0.46 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); $[\alpha]^{25}_D$ –16.7 (*c* 0.01, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.20 (1H, s), 8.08 (1H, s), 7.00–7.40 (17H, m), 4.80–5.10 (5H, m), 4.20 (1H, m), 3.00–4.10 (26H, m), 2.93 (6H, s), 2.50–2.80 (5H, m), 2.28 (3H, s), 2.15 (2H, m), 1.42 (9H, s), 1.02–1.14 (9H, m); IR (neat) ν_{max} 3430, 1705, 1640, 1480, 1210 cm^{-1}; FABMS (NBA) *m/e* 1757 (M^+, $C_{79}H_{106}N_{17}O_{23}S_3$).

Bleomycin A_2 (1). The compound **46** (0.0016 mmol, 2.8 mg) was treated with 20% TFA–CH_2Cl_2 (0.5 mL), and the mixture was stirred at 0 °C for 2.5 h under Ar before the solvent was evaporated in vacuo to give an oily solid. The sample was triturated with $CHCl_3$ (0.2 mL), and the remaining solid was purified by preparative TLC (SiO_2, CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH, 10:9:1) to afford **1** (1.1 mg, 2.2 mg theoretical, 50%) as a thin film: R_f 0.43 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); $[\alpha]^{25}_D$ –16 (*c* 0.03, 0.1 N HCl); ^1H NMR (D_2O, 400 MHz) δ 8.07 (1H, s), 7.87 (1H, s), 7.68 (1H, s), 7.10 (1H, s), 5.08 (2H, m), 4.87 (1H, d, *J* = 3.6 Hz), 4.78 (1H, d, *J* = 1.8 Hz), 4.63 (1H, m), 3.98 (1H, d, *J* = 4.5 Hz), 3.20–3.90 (22H, m), 3.10 (1H, m), 2.80 (1H, m), 2.75 (6H, s), 2.50–2.70 (4H, m), 2.40 (1H, m), 2.10 (2H, m), 1.90 (3H, s), 0.97 (3H, d, *J* = 6.0 Hz), 0.95 (3H, d, *J* = 6.0 Hz), 0.93 (3H, d, *J* = 6.0 Hz); IR (neat) ν_{max} 3341, 1723, 1658, 1406, 1082, 777 cm^{-1}; FABMS (NBA) *m/e* 1415 (M^+, $C_{55}H_{84}N_{17}O_4S_3$).

Alternatively, the crude bleomycin A_2 could be purified by reverse phase chromatography (C-18, 0–20% CH_3OH–H_2O gradient elution) with greater recovery (95%) but of a slightly lower purity.

***erythro*-N^{α}-((*tert*-Butyloxy)carbonyl)-N^{im}-((*tert*-butyloxy)carbonyl)-β-[3,4,6-tri-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]-L-histidine Methyl Ester (47).** A solution of **4** (0.007 mmol, 8.2 mg) in CH_3OH (2 mL) was hydrogenated over 10% Pd-C (6 mg) under H_2 (1 atm) in the presence of di-*tert*-butyl dicarbonate (0.07 mmol, 15 mg, 10 equiv) at 25 °C for 17 h. The reaction mixture was filtered through a Celite pad and concentrated in vacuo. Chromatography (SiO_2, 1 × 10 cm, 1.5–2% CH_3OH–CH_2Cl_2 gradient elution) afforded **47** (4.2 mg, 7.0 mg theoretical, 60%) as a thin film: R_f 0.33 (SiO_2, 10% CH_3OH–$CHCl_3$); $[\alpha]^{25}_D$ –1.7 (*c* 0.18, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.02 (1H, s), 7.43 (1H, s), 6.00 (1H, d, *J* = 8.0 Hz), 5.05–5.30 (5H, m), 5.02 (1H, d, *J* = 1.6 Hz), 4.88 (1H, m), 4.43 (1H, ddd, *J* = 1.2, 5.2, 5.2 Hz), 4.38 (1H, dd, *J* = 5.2, 12.8 Hz), 4.16 (1H, m), 4.10 (1H, dd, *J* = 1.2, 12.8 Hz), 4.05 (1H, dd, *J* = 3.6, 3.6 Hz), 4.01 (1H, m), 3.90 (1H, m), 3.70 (3H, s), 2.23 (3H, s), 2.15 (3, s), 2.12 (3H, s), 2.09 (3H, s), 2.08 (3H, s), 2.03 (3H, s), 1.62 (9H, s), 1.45 (9H, s); IR ($CHCl_3$) ν_{max} 3455, 3025, 1743, 1712, 1517, 1425, 1220, 1046, 928 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 1137.2670 (M^+ + Cs, $C_{42}H_{60}N_4O_{24}$ requires 1137.2652).

***erythro*-β-[3,4,6-tri-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]-L-histidine Methyl Ester (48).** The compound **47** (0.004 mmol, 4.2 mg) was treated with 20% TFA–CH_2Cl_2 (0.5 mL), and the mixture was stirred at 0 °C for 2 h under Ar. The solvent was evaporated in vacuo to give an oily solid. Chromatography (SiO_2, 10–20% CH_3OH–CH_2Cl_2 gradient elution) afforded **48** (2.6 mg, 3.3 mg theoretical, 78%) as a thin film: R_f 0.35 (SiO_2, 20% CH_3OH–$CHCl_3$); ^1H NMR (CD_3OD, 400 MHz) δ 7.69 (1H, d, *J* = 1.0 Hz), 7.22 (1H, d, *J* = 1.0 Hz), 5.24 (1H, dd, *J* = 3.6, 3.6 Hz), 5.21 (1H, d, *J* = 8.0 Hz), 5.10 (2H, m), 5.07 (1H, dd, *J* = 3.2, 10.0 Hz), 4.98 (1H, d, *J* = 1.6 Hz), 4.90 (1H, dd, *J* = 1.2, 3.6 Hz), 4.47 (1H, d, *J* = 8.0 Hz), 4.31 (1H, ddd, *J* = 1.2, 6.4, 6.4 Hz), 4.15 (1H, dd, *J* = 6.4, 12.8 Hz), 4.07 (1H, dd, *J* = 1.2, 12.8 Hz), 4.05 (1H, dd, *J* = 3.6, 3.6 Hz), 4.05 (1H, m), 3.90 (1H, dd, *J* = 6.0, 12.8 Hz), 3.72 (1H, dd, *J* = 7.0, 12.8 Hz), 3.71 (3H, s), 2.07 (3H, s), 2.03 (3H, s), 1.99 (3H, s), 1.98 (3H, s), 1.97 (3H, s), 1.88 (3H, s).

N^{α}-((*tert*-Butyloxy)carbonyl)-N^{β}-[1-amino-3(*S*)-[4-amino-6-[amido-N^{im}-(triphenylmethyl)-*erythro*-β-[3,4,6-tri-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]-L-hystidyl methyl ester]-5-methylpyrimidin-2-yl]propion-3-yl]-(*S*)-β-aminoalanine Amide (50). A solution of **44** (0.0074 mmol, 3.2 mg) in DMF (100 μL) was treated with **37** (0.0074 mmol, 7.7 mg), HOBt (0.0090 mmol, 1.2 mg, 1.1 equiv), and EDCI (0.0090, 1.75 mg, 1.1 equiv), and the mixture was stirred under Ar at 25 °C for 48 h. The reaction mixture was concentrated in vacuo to give an oily solid. Chromatography (SiO_2, 0.5 × 5 cm, 10–20% CH_3OH–$CHCl_3$ gradient elution) afforded **50** (6.2 mg, 10.7 mg theoretical, 58%) as a foam: R_f 0.18 (SiO_2, 20% CH_3OH–$CHCl_3$); $[\alpha]^{25}_D$ –13.8 (*c* 0.13, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 7.47 (s, 1H), 7.00–7.50 (m, 16H), 5.10-5.40 (m, 7H), 4.80 (m, 2H), 3.80–4.30 (m, 7H), 3.80 (s, 3H), 3.62 (m, 2H), 2.80 (m, 2H), 2.60 (m, 1H), 2.45 (m, 1H), 2.26 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 1.99 (s, 3H), 1.89 (s, 3H), 1.85 (s, 3H), 1.42 (s, 9H); IR (neat) ν_{max} 3453, 1762, 1492, 1418, 1210 cm^{-1}; FABHRMS (NBA) *m/e* 1454.5620 (M^+ + H, $C_{68}H_{83}N_{11}O_{25}$ requires 1454.5640).

N^{α}-((*tert*-Butyloxy)carbonyl)-N^{β}-[1-amino-3(*S*)-[4-amino-6-[amido-N^{im}-(triphenylmethyl)-*erythro*-β-[2-*O*-(3-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]-L-histidyl Methyl Ester]pyrimidin-2-yl]propion-3-yl]-(*S*)-β-aminoalanine Amide (51). A solution of **50** (0.0026 mmol, 3.7 mg) in CH_3OH (0.5 mL) was cooled at 0 °C and treated with aqueous 0.1 N NaOH (0.5 mL, 0.005 mmol, 20 equiv), and the reaction mixture was stirred at 0 °C for 12 h. After evaporation of the CH_3OH under a N_2 stream, the aqueous phase was neutralized to pH 7 with the addition of HOAc, and the mixture was concentrated under a N_2 stream. Chromatography (reverse-phase C-18, 0–50% CH_3OH–H_2O gradient elution) afforded **51** (1.5 mg, 3.0 mg theoretical, 50%) as a foam: R_f 0.21 (SiO_2, 4:1:1 *t*-BuOH–H_2O–HOAc); $[\alpha]^{25}_D$ +33.3 (*c* 0.16, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 6.90–7.40 (m, 17H), 4.80–5.30 (m, 5H), 3.30–4.30 (m, 13H), 2.80 (m, 2H), 2.60 (m, 1H), 2.35 (m, 1H), 2.31 (s, 3H), 1.43 (s, 9H); IR (neat) ν_{max} 3367, 1730, 1685, 1631, 1509, 1395, 1200 cm^{-1}; FABHRMS (NBA) *m/e* 1188.4815 (M^+ + H, $C_{55}H_{69}N_{11}O_{19}$ requires 1188.4849).

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