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Studies on Glycosylation of *erythro*-β-Hydroxy-L-histidine. A Key Step of Bleomycin Total Synthesis

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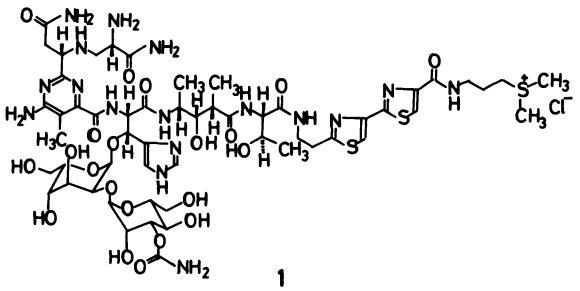
To improve the synthetic yield of bleomycin and its analogues, it is necessary to find the high yield procedure for condensation of sugar portion with the hydroxyl group of *erythro*-β-hydroxy-L-histidine moiety, preventing the *N*-glycosylation to imidazole. The present studies include the condensations of 4,6-di-*O*-acetyl-2,3-di-*O*-methyl-α-D-glucopyranosyl bromide (**10**) or 3,4,6-tri-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl bromide (**3**) and 3,4,6-tri-*O*-acetyl-2-*O*-[2,4,6-tri-*O*-acetyl-3-*O*-(*N*-acetylcarbamoyl)-α-D-mannopyranosyl]-α-L-gulopyranosyl bromide (**28**) with a variety of protected derivatives of *erythro*-β-hydroxy-L-histidine. The results revealed that a) N^{im}-tosyl group is most effective to prevent the N^{im}-glycosylation, b) use of silver carbonate and silver perchlorate at low temperature (−55 °C) gave the desired α-glycosides in satisfactory yields, and c) among the esters (methyl and *p*-nitrobenzyl) and the *N*-protecting groups (Z, Boc, and pMZ) of *erythro*-β-hydroxy-L-histidine, methyl and Z were the best groups, respectively. Using the method thus improved, the desired *O*-α-L-guloside, that is, *erythro*-β-*O*-[3,4,6-tri-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]-N^{α}-benzyloxycarbonyl-$N^{im(\tau)}$-tosyl-L-histidine *p*-nitrobenzyl ester (**31**) was successfully prepared.

Bleomycin is an antitumor antibiotic clinically used in the treatment of squamous cell carcinoma, malignant lymphomas, and testis tumors.[1] The total synthesis of bleomycin A2 (**1**) had been accomplished by our group[2,3] and Hecht et al.[4] In our first total



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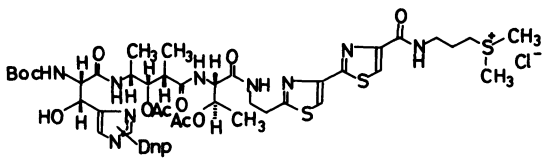

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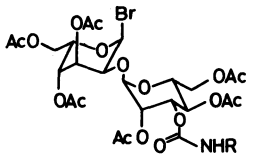
Boc: $COOC(CH_3)_3$
Dnp: $C_6H_3(NO_2)_2$(*o*, *p*)

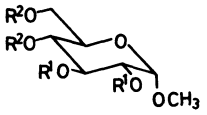
Chart 1.

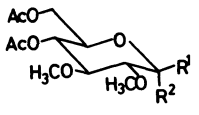
synthesis, the key step was the coupling of a protected suger, 3,4,6-tri-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl bromide (**3**), with protected pentapeptide **2**[5] at the hydroxyl group of *erythro*-β-hydroxy-L-histidine. We therefore intended to improve the yield of the desired coupling product. In this paper we describe the basic experiments for this purpose, that is, coupling reactions of protected glucosyl and 2-*O*-mannopyranosyl-α-L-gulopyranosyl bromides with several protected *erythro*-β-hydroxy-L-histidine.

Glycosylation of a Derivative (14) of β-Hydroxy-L-histidine Protected with a Dnp Group at Imidazole Moiety. As a model glycosyl bromide for bleomycin synthesis, we prepared 4,6-di-*O*-acetyl-2,3-di-*O*-methyl-α-D-glucopyranosyl bromide (**10**). Methyl 4,6-*O*-cyclohexylidene-α-D-glucopyranoside (**4**)[6] was methylated with methyl iodide in *N*,*N*-dimethylformamide (DMF) to give the 2,3-di-*O*-methyl ether **5**.



	R
3	H
28	Ac





	R^1	R^2
4	H	$R^2R^2=C(CH_2)_5$
5	CH_3	$R^2R^2=C(CH_2)_5$
6	CH_3	H
7	CH_3	Ac

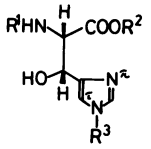
	R^1	R^2
8	H	OAc
9	OAc	H
10	H	Br

Chart 2.

Acidic hydrolysis of **5** followed by acetylation gave the 4,6-di-*O*-acetyl derivative **7**.[7] Acetolysis of **7** gave the corresponding 1-*O*-acetyl α- and β-D-glucoses **8** and **9** in 79 and 13% yield, respectively, with slightly accompanied methyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (1.4%).[8] Treatment of the mixture of **8** and **9** with hydrogen bromide in acetic

acid gave the α-1-bromide **10**.

As a protected β-hydroxy-L-histidine for the condensation with **10**, we prepared a N^{im}-(2,4-dinitrophenyl) (=Dnp)-N^{α}-benzyloxycarbonyl(=Z) derivative[9] **14** of β-hydroxy-L-histidine. Since the low yield of our first condensation[2] was considered to be correlated to the presence of the bulky N^{α}-*t*-butoxycarbonyl (Boc) group near the hydroxyl group, the Boc group was replaced by a Z group. Treatment of **11** with *O*-benzyl *S*-(4,6-dimethyl-2-pyrimidinyl) thiocarbonate (Z-S reagent)[10] gave the N^{α}-Z-derivative **12** in high yield. Treatment of **12** with hydrogen chloride in methanol (to give **13**) followed by reaction with 2,4-dinitrofluorobenzene gave **14**.



	R^1	R^2	R^3
11	H	H	H
12	Z	H	H
13	Z	CH_3	H
14	Z	CH_3	Dnp
21	Z	CH_3	Ts
22	Boc	CH_3	Ts
23	PMZ	CH_3	Ts
24	Z	H	Ts
25	Z	$CH_2C_6H_4NO_2(p)$	Ts

PMZ: $COOCH_2C_6H_4OCH_3(p)$
Z: $COOCH_2C_6H_5$

Chart 3.

The position of the Dnp group, which remained ambiguous in the previous papers,[2,5] was determined by the procedure of Bell and Jones.[11] Treatment of **14** with benzyl chloroformate and sodium hydrogen-

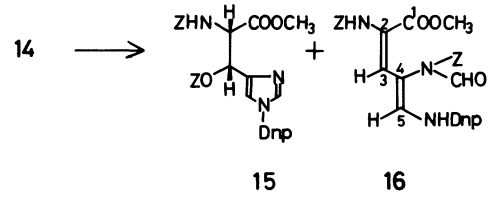


Chart 4.

carbonate in chloroform–water gave two main products. ^{1}H NMR spectroscopy provided evidences for their structural assignments: *O*-benzyloxycarbonyl derivative **15** of **14** and methyl 2,4-bis(benzyloxycarbonylamino)-4-*N*-formyl-5-(2,4-dinitroanilino)-2,4-pentadienoate (**16**). The ^{1}H NMR spectrum of **16** showed a doublet (*J*=12 Hz, NHDnp) at δ 10.64, which collapsed to a singlet on irradiation at δ 7.22 and disappeared on deuteration. The doublet at δ 7.22 (*J*=12 Hz, =C*H*-NHDnp) became a singlet on deuteration or irradiation at δ 10.64. Two singlets appeared at δ 7.49 (HC=) and 9.25 (*N*CHO), the latter being gradually disappeared on deuteration. These results indicate the hydrogen assignments to be described in the parentheses and, therefore, it was concluded that Dnp was attached to the nitrogen (*N*-5) bearing a hydrogen and a methine (C-5), not the nitrogen (*N*-4) bearing a formyl group. Thus, the structure of **16** was 2,4,5-tris(substituted amino)-2,4-diene, supporting the structure of **14**. The geometrical structure of **16** was not studied, however, from the expected reaction pathway and the NMR spectrum, a mixture of two isomers possibly composed of *E,Z* (major) and *Z,Z*-structures was presumed as follows. The compound **16** may be produced from **15** by a sequence of reactions including benzyloxycarbonylation of the $N^{im(\pi)}$ group, hydrolysis of the imidazole ring to give an *N*-formyl group,[12] and removal of a ZOH fragments (to give the 2,3-unsaturation).

According to the cross-ring coupling constant criterion suggested by Matthews and Rapoport,[13] the coupling constant between the two CH protons of imidazole ring is 0.9—1.0 Hz for the $N^{im(\pi)}$-substituted derivatives and 1.1—1.5 Hz for the $N^{im(\tau)}$-substituted derivatives. In the ^{1}H NMR spectrum of **14**, the broad singlet at δ 7.59 (H-2 of imidazole) measured at room temperature was resolved into a sharp doublet of 1.2 Hz at 55 °C, supporting the structure of **14** deduced from the structure of **16**.

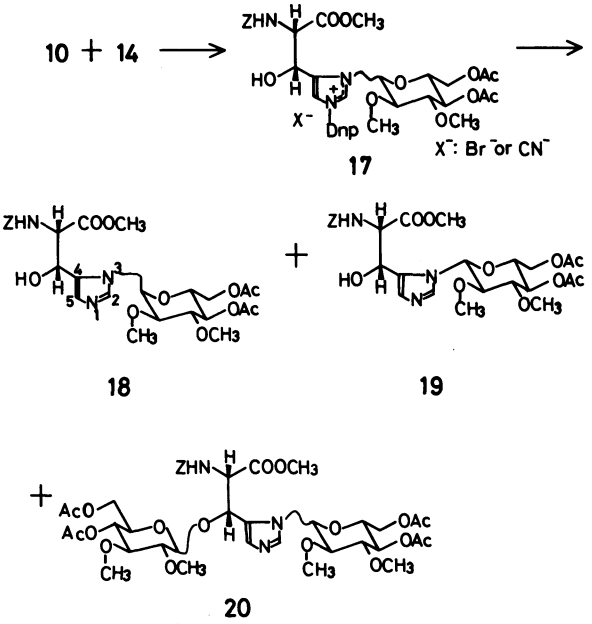


Chart 5.

Glycosylation of compound **14** with the bromide **10** according to the previous method[2] ($HgCN_2$ and molecular sieves in sulfolane, 40 °C, overnight) gave a complex mixture mainly comprised of two glucosides **17** having the Dnp groups. Since the mixture was unstable to light and cleavage of the Dnp group

occurred gradually during the purification, the Dnp group of **17** was removed beforehand by treatment with 2-mercaptoethanol to give the α- and β-$N^{im(3)}$-glucosides **18** and **19** in 34 and 26% yield (based on **14**) and a mixture of products (**20**, 6%) glucosylated both at $N^{im(3)}$ and OH-3. The positions of the glucosyl moieties in **18** and **19** were determined to be N^{im} from the low chemical shifts[14] of the anomeric protons (δ 6.30 and J=3.5 Hz, and δ 5.54 and J=9 Hz, respectively). The above results show that, glucosylation occurs at the $N^{im(3)}$ position in preference to the hydroxyl group in the presence of the $N^{im(1)}$-Dnp protecting group.

Preparation of the *O*-Glycosyl Derivatives of β-Hydroxy-L-histidine. As described previously in our communication,[3] N^{im}-tosyl group was revealed to be suitable for prevention of N^{im}-glycosylation. The *N*-tosyl compound **21** prepared from **13** showed a large

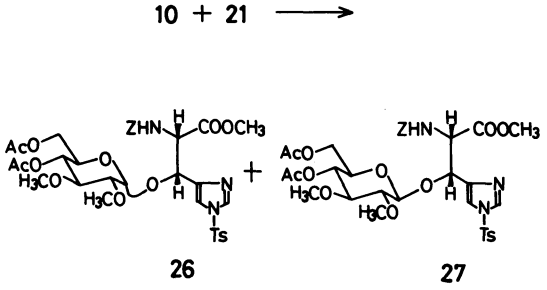
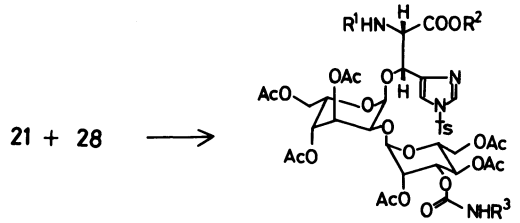


Chart 6.

cross-ring coupling constant ($J_{2,4}$=1.5 Hz) in its ^{1}H NMR spectrum, suggesting[13] the introduction of the tosyl group at $N^{im(\tau)}$. Condensation of **21** with the bromide **10** in the presence of mercury(II) cyanide and molecular sieves in dry dichloromethane at room temperature gave the desired α- and β-*O*-glucosides **26** and **27** in 21% yield in a ratio of 2:1, with the corresponding detosyl derivatives. Pure **26** and **27** were isolated by preparative high-performance liquid chromatography. The chemical shifts of the resonances of the anomeric protons of **26** and **27** in their ^{1}H NMR spectra (δ 4.89 and J=3.5 Hz for **26**, and δ 4.50 and J=7.5 Hz for **27**) supported the *O*-glucosides.

When the condensation was carried out in the presence of a mixture of silver carbonate and silver perchlorate as the catalysts at room temperature, the ratio of **26** and **27** was improved to 20:1 but without change of the yield (≈20%). Formation of the detosyl-di-N^{im},*O*-glucoside (≈25%) was also observed. However, the condensation yield was surprisingly improved to 67%, with the above ratio of **26** and **27** maintained, when the reaction was carried out at a low temperature (−55 °C). In this case, the yield of the detosyldiglucosyl derivative was lowered to 8%. It should be noted that sole lowering the reaction temperature remarkably suppressed the removal of the tosyl group and increased the yield of the desired *O*-α-glucoside. When mercury(II) cyanide was used as the catalyst, no reaction occurred at −55 °C, revealing the desirable effects of the silver catalysts.

The above reaction conditions were next applied to



	R^1	R^3	R^3
29	Z	CH_3	Ac
30	Boc	CH_3	Ac
31	Z	$CH_2C_6H_4NO_2(p)$	H

Chart 7.

the condensation of **21** and a protected 2-*O*-[3-*O*-(*N*-acetylcarbamoyl)-α-D-mannopyranosyl]-α-L-gulopyranosyl bromide (**28**) prepared from 1,3,4,6-tetra-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranosyl)-β-L-gulopyranose[2] by bromination with hydrogen bromide–acetic acid–acetic anhydride. When **28** was used in 1.4 molar equivalents for **21**, the best yield (95%) of **29** was obtained. Incidentally, when 1.1, 1.2, 1.3 molar equivalents of **28** for **21** were used, 69, 77, and 89% of **29** were obtained. The structure of **29** was confirmed by the ^{1}H NMR spectrum (Fig. 1).

N^{α}-Protecting groups were further studied. When N^{α}-Boc- (**22**) and N^{α}-(*p*-methoxybenzyloxycarbonyl)(=PMZ) derivatives (**23**) of N^{im}-tosyl-β-hydroxy-L-histidine methyl ester were condensed with **28**, the yields of the desired *O*-α-L-gulosides were lower than the case when Z-group was used. The inspection of the ^{1}H NMR spectra of the mixtures of products obtained by the both reactions revealed that considerable part of these protecting groups were removed during the coupling reaction.

Finally, in view of easy removal of protecting group, methyl ester group of **21** was replaced by a *p*-nitrobenzyl, and the *N*-acetyl of the carbamoyl group was omitted. Compound **12** was treated with tosyl chloride as described for **21** to give the N^{im}-tosyl derivative **24**, which was led to the *p*-nitrobenzyl ester **25** by treatment with *p*-nitrobenzyl bromide. Condensation of **25** and 3,4,6-tri-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl bromide (**3**) by the same procedure as above described for **29** gave the desired *O*-α-L-guloside **31** (43% from **25**) with recoverd **25** (25%). The product **31** is useful for the synthesis of bleomycin and its analogues.

In conclusion, the present studies revealed that (a) $N^{im(\tau)}$-tosyl group successfully prevented the glycosylation at $N^{im(\pi)}$, (b) use of a mixture of silver carbonate

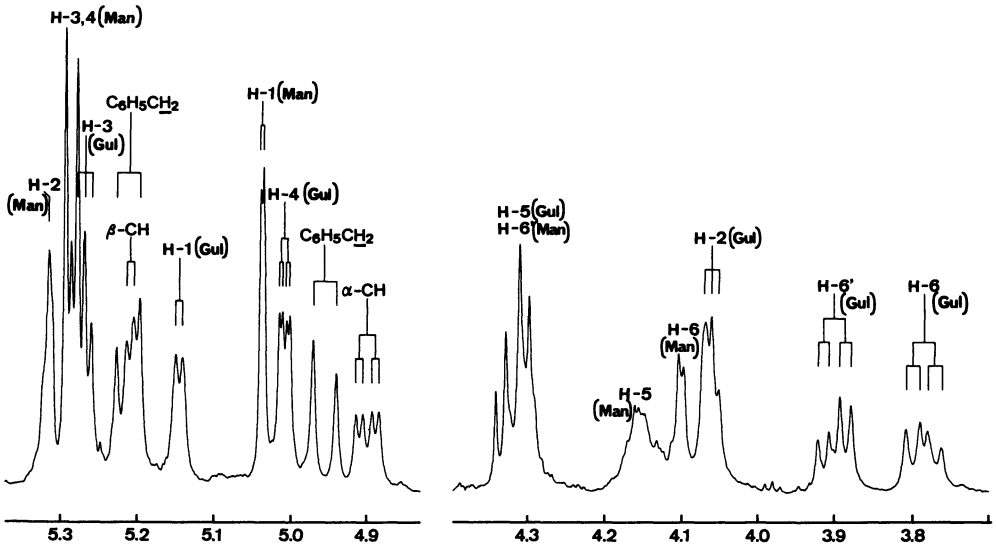


Fig. 1. Partial ^{1}H NMR spectrum of **29** (400 MHz, $CDCl_3$).

and silver perchlorate at low temperature (−55 °C) gave the desired *O*-α-glycosyl compounds almost selectively in satisfactory yields, and (c) among the protective groups of α-amino and carboxylic acid in the β-hydroxy-L-histidine portion, benzyloxycarbonyl and methyl groups, respectively, gave the best yield of the *O*-glycoside.

Experimental

IR spectra were determined in KBr disks on a Jasco A-202 infrared spectrophotometer. ^{1}H NMR spectra were recorded at 25 °C, unless otherwise stated, with Bruker WM-250 and WM-400 spectrometers (250 MHz and 400 MHz, respectively). Tetramethylsilane (δ=0.00) was used as an internal standard. Proton assignments of the products were confirmed by decoupling method if necessary. Secondary ion mass spectra (SIMS) and field desorption mass spectra (FDMS) were recorded on a Hitachi M-80 spectrometer. Optical rotations were measured in 0.1-dm cells of 0.5-ml capacity by using a Perkin-Elmer Model 241 polarimeter. Thin-layer chromatography (TLC) was carried out on E. Merck precoated TLC plates (silica gel 60 F-254). For column chromatography, silica gel (Wakogel C-200) was used, unless otherwise stated.

Methyl 4,6-*O*-Cyclohexylidene-2,3-di-*O*-methyl-α-D-glucopyranoside (5). To a solution of methyl 4,6-*O*-cyclohexylidene-α-D-glucopyranoside[6] (**4**, 505 mg) in dry *N*,*N*-dimethylformamide (DMF) (3 ml) were added silver oxide (2.15 g) and methyl iodide (0.6 ml), and the mixture was vigorously shaken at room temperature overnight. The resulting solution showed, on TLC with benzene–ethyl acetate (1:1), a single spot of **5** at R_f 0.64 (cf. **4**, R_f 0.16). The mixture was centrifuged and the residue was washed with DMF. The solutions combined were concentrated and the residue was extracted with chloroform. The organic solution was washed with water, dried ($MgSO_4$), and concentrated to give a pale yellow syrup (615 mg), that was purified by column chromatography with benzene–ethyl acetate (4:1) to give needles of **5**, 453 mg (81%), mp 82—83 °C; $[\alpha]_D^{23}$ +117° (*c* 1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ=3.23 (1H, dd, H-2), 3.41, 3.53, 3.62 (each 3H, s, OCH_3), 4.80 (1H, d, H-1); $J_{1,2}$=3.5, $J_{2,3}$=8.5 Hz.

Found: C, 59.52; H, 8.49%. Calcd for $C_{15}H_{26}O_6$: C, 59.58; H, 8.67%.

Methyl 4,6-Di-*O*-acetyl-2,3-di-*O*-methyl-α-D-glucopyranoside (7). A solution of **5** (103 mg) in 80% aqueous acetic acid (1 ml) was heated at 50 °C for 1 h. The solution showed a single spot on TLC. Concentration gave a solid of **6** (83 mg) which partly crystallized on standing. A mixture of **6** and acetic anhydride (0.16 ml) in pyridine (1 ml) was kept at room temperature overnight. Addition of water (0.05 ml) followed by concentration gave a syrup, that was dissolved in chloroform. The solution was washed successively with 5% aqueous potassium hydrogensulfate, water, 5% aqueous sodium hydrogencarbonate, and water, dried ($MgSO_4$), and concentrated to give a syrup of **7**,[7] 104 mg (quantitative), $[\alpha]_D^{23}$ +109° (*c* 1, $CHCl_3$); IR (KBr): 1740 ($\nu_{C=O}$), 1240 cm^{-1} ($\nu_{C=O}$ of OAc); ^{1}H NMR (250 MHz, $CDCl_3$): δ=2.09, 2.10 (each 3H, s, OAc), 3.32 (1H, dd, H-2), 3.45, 3.53, 3.54 (each 3H, s, OCH_3), 3.59 (1H, t, H-3), 3.86 (1H, ddd, H-5), 4.05 (1H, dd, H-6), 4.24 (1H, dd, H-6′), 4.87 (1H, d, H-1), 4.95 (1H, dd, H-4); $J_{1,2}$=3.5, $J_{2,3}$=$J_{3,4}$=9.5, $J_{4,5}$=10.5, $J_{5,6}$=2.5, $J_{5,6'}$=5.0, $J_{6,6'}$=12.5 Hz.

Found: C, 50.93; H, 7.04%. Calcd for $C_{13}H_{22}O_8$: C, 50.97; H, 7.24%.

1,4,6-Tri-*O*-acetyl-2,3-di-*O*-methyl-α- and β-D-Glucopyranose (8 and 9). Compound **7** (3.89 g) was added to a mixture of acetic acid–acetic anhydride–sulfuric acid (10:10:1) (21 ml) under stirring at 0 °C, and the mixture was stirred at the temperature for 100 min. The resulting solution showed, on TLC with benzene–ethyl acetate (4:1), two spots at R_f 0.24 (**8**) and 0.30 (**9**). After dilution with chloroform (300 ml), the solution was successively washed with water, 5% aqueous sodium hydrogencarbonate, and water, dried ($MgSO_4$), and concentrated to give a pale yellow syrup, 4.13 g. Duplicate column chromatographies with benzene–ethyl acetate (4:1) and chloroform–2-butanone (15:1) gave syrups of **8**, 3.37 g (79%), **9**, 0.57 g (13%), and methyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside,[8] 57 mg (1.4%).

8: $[\alpha]_D^{23}$ +90° (*c* 1, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): δ=2.07, 2.10, 2.18 (each 3H, s, OAc), 3.42 (1H, dd, H-2), 3.48, 3.54 (each 3H, s, OCH_3), 3,55 (1H, t, H-3), 3.96 (1H, ddd, H-5), 4.03 (1H, dd, H-6), 4.24 (1H, dd, H-6′), 5.00 (1H, dd, H-4), 6.35 (1H, d, H-1); $J_{1,2}$=3.5, $J_{2,3}$=$J_{3,4}$=9.5, $J_{4,5}$=10, $J_{5,6}$=2.5, $J_{5,6'}$=4.5, $J_{6,6'}$=10 Hz.

Found: C, 50.53; H, 6.59%. Calcd for $C_{14}H_{22}O_9$: C, 50.29; H, 6.63%.

9: $[\alpha]_D^{23}$ −16° (*c* 1, $CHCl_3$) [lit,[15] $[\alpha]_D^{22}$ −18° (*c* 1.3, $CHCl_3$)]; ^1H NMR (250 MHz, $CDCl_3$): δ=2.07, 2.10, 2.17 (each 3H, s, OAc), 3.26 (1H, d, H-2), 3.34 (1H, t, H-3), 3.54, 3.56 (each 3H, s, OCH_3), 3.67 (1H, ddd, H-5), 4.05 (1H, dd, H-6), 4.24 (1H, dd, H-6′), 4.97 (1H, dd, H-4), 5.52 (1H, d, H-1); $J_{1,2}$=7.5, $J_{2,3}$=$J_{3,4}$=9, $J_{4,5}$=10, $J_{5,6}$=2.5, $J_{5,6'}$=5, $J_{6,6'}$=12.5 Hz.

Found: C, 50.11; H, 6.39%. Calcd for $C_{14}H_{22}O_9$: C, 50.29; H, 6.63%.

4,6-Di-*O*-acetyl-2,3-di-*O*-methyl-α-D-glucopyranosyl Bromide (10). To a mixture of **8** and **9** (21.3 mg) was added a cold (0 °C) mixture of 30% hydrogen bromide in acetic acid–acetic acid-acetic anhydride (3:8:1, 0.4 ml) and the solution was kept at the temperature for 1 h, then at room temperature overnight in the dark. The resulting solution showed, on TLC with benzene–ethyl acetate (5:1), a spot at R_f 0.48. Concentration of the solution gave a syrup, that was dissolved in dichloromethane (1.5 ml) and the solution was washed successively with water, 5% aqueous sodium hydrogencarbonate, and water, dried ($MgSO_4$), and concentrated to give a syrup of **10**, 21.2 mg (94%), that was only slightly contaminated by the starting materials; ^1H NMR (250 MHz, $CDCl_3$): δ=2.08, 2.11 (each 3H, s, OAc), 3.30 (1H, dd, H-2), 3.52, 3.55 (each 3H, s, OCH_3), 3.63 (1H, dd, H-3), 4.07 (1H, dd, H-6), 4.17 (1H, ddd, H-5), 4.29 (1H, dd, H-6′), 5.03 (1H, dd, H-4), 6.55 (1H, d, H-1); $J_{1,2}$=4, $J_{2,3}$=9, $J_{3,4}$=9.5, $J_{4,5}$=10.5, $J_{5,6}$=2, $J_{5,6'}$=4.5, $J_{6,6'}$=12.5 Hz.

***erythro*-N^{α}-Benzyloxycarbonyl-β-hydroxy-L-histidine (12).** To an aqueous solution (2 ml) of *erythro*-β-hydroxy-L-histidine (**11**)[9] (305 mg, monohydrochloride) were added triethylamine (0.52 ml, 2.5 mol equiv for **11**) and a solution of *O*-benzyl *S*-(4,6-dimethyl-2-pyrimidinyl) thiocarbonate[10] (432 mg, 1.1 mol equiv for **11**) in 1,4-dioxane (2 ml) under stirring, and the solution was kept at room temperature for 2.5 h. The solution showed, on TLC with ethyl acetate–ethanol–water–formic acid (20:2:2:1), spots at R_f 0.12 (**12**) and 0 (trace, **11**). After addition of water (10 ml), the resulting solution was washed with ethyl acetate (10 ml×2) and the aqueous solution was concentrated to give a syrup, that was purified by column chromatography with the same solvent system described above to give a pale brown solid of **12**, 434 mg (97%), $[\alpha]_D^{17}$ +18° (*c* 1, CH_3OH); IR (KBr): 1705 (amide I), 1600 (aromatic), 1530 cm^{-1} (amide II); ^1H NMR (250 MHz, D_2O): δ=4.39 (1H, d, α-CH), 5.06 (2H, $C_6H_5CH_2$), 5.19 (1H, d, β-CH), 8.54 (1H, s, H-2 of Im); $J_{\alpha\text{-CH},\beta\text{-CH}}$=6.5 Hz.

Found: C, 54.11; H, 5.03; N, 13.21%. Calcd for $C_{14}H_{15}N_3O_5 \cdot 0.3\ H_2O$: C, 54.12; H, 5.06; N, 13.52%.

***erythro*-N^{α}-Benzyloxycarbonyl-β-hydroxy-L-histidine Methyl Ester (13).** Compound **12** was dissolved in 10% hydrogen chloride in methanol (6 ml) and the solution was kept at room temperature overnight, then at 40 °C for 7 h. The resulting solution showed, on TLC with chloroform–methanol (4:1), spots at R_f 0.45 (**13**) and 0.08 (trace, **12**). Concentration of the solution gave a yellow solid, that was purified by column chromatography with chloroform–methanol (5:1→5:2) to give a colorless, hygroscopic solid of **13**, 389 mg (78%, as the 0.8 hydrochloride), $[\alpha]_D^{20}$ −4° (*c* 1, CH_3OH); IR (KBr): 1720 ($\nu_{C=O}$ of ester, and amide I), 1615 (aromatic), 1525 cm^{-1} (amide II); ^1H NMR (250 MHz, CD_3OD): δ=3.74 (3H, s, $COOCH_3$), 4.60 (1H, d, α-CH), 5.05 (2H, $C_6H_5CH_2$), 5.12 (1H, d, β-CH), 7.32 (5H, $C_6H_5CH_2$), 7.35 [1H, s, H-5 of imidazole (=Im)], 8.65 (1H, s, H-2 of Im); $J_{\alpha\text{-CH},\beta\text{-CH}}$=7.5 Hz.

Found: C, 51.93; H, 5.33; N, 12.16; Cl, 8.21%. Calcd for $C_{15}H_{17}N_3O_5 \cdot 0.8$ HCl: C, 51.70; H, 5.15; N, 12.06; Cl, 8.14%.

***erythro*-N^{α}-Benzyloxycarbonyl-$N^{im(\tau)}$-(2,4-dinitrophenyl)-β-hydroxy-L-histidine Methyl Ester (14).** To a mixture of **13** (62.9 mg, 0.8 hydrochloride) and sodium hydrogencarbonate (40.0 mg, 2.6 mol equiv for **13**) in 50% aqueous 1,4-dioxane (1.2 ml) was added a solution of 2,4-dinitrofluorobenzene (49.1 mg, 1.5 mol equiv for **13**) in methanol (0.6 ml) and the solution was kept in the dark at room temperature for 3.5 h. Concentration of the solution gave a yellow solid, that was purified by column chromatography with benzene–ethyl acetate (1:2) to give a yellow solid of **14**, 74.1 mg (87%), $[\alpha]_D^{17}$ +8° (*c* 1, $CHCl_3$); IR (KBr): 1720 ($\nu_{C=O}$ of ester and amide I), 1610 (aromatic), 1540 (amide II and ν_{as} NO_2), 1500 (aromatic), 1350 cm^{-1} (ν_s NO_2); ^1H NMR (250 MHz, $CDCl_3$, at 55 °C): δ=3.75 (3H, s, $COOCH_3$), 4.86 (1H, dd, α-CH), 5.15 (2H, $C_6H_5CH_2$), 5.22 (1H, broad d, β-CH), 6.01 (1H, broad s, NH), 7.03 (1H, broad s, H-5 of Im), 7.3 (5H, $C_6H_5CH_2$), 7.56 (1H, broad d, H-2 of Im), 7.63 (1H, d, H-6 of Dnp), 8.53 (1H, dd, H-5 of Dnp), 8.81 (1H, d, H-3 of Dnp); $J_{NH,\alpha\text{-CH}}$=8.5, $J_{\alpha\text{-CH},\beta\text{-CH}}$=4, $J_{2,5(Im)}$=1.2, $J_{3,5(Dnp)}$=2.5, $J_{5,6(Dnp)}$=8.5 Hz.

Found: C, 50.18; H, 4.03; N, 13.69%. Calcd for $C_{21}H_{19}N_5O_9 \cdot H_2O$: C, 50.10; H, 4.21; N, 13.91%.

***erythro*-Bis-N^{α}:β-*O*-(benzyloxycarbonyl)-$N^{im(\tau)}$-dinitrophenyl-L-histidine Methyl Ester (15) and Methyl 2,4-Bis(benzyloxycarbonylamino)-4-*N*-formyl-5-(2,4-dinitrophenylamino)-2,4-pentadienoate (16).** A mixture of **14** (20.1 mg), sodium hydrogencarbonate (36.1 mg) and benzyl chloroformate (0.035 ml) in water–chloroform (0.6 ml each) was shaken at room temperature for 8 h. The chloroform layer showed, on TLC with benzene–ethyl acetate (3:1), two major spots at R_f 0.26 (**15**) and 0.46 (**16**). The organic layer separated was washed with water, dried ($MgSO_4$), and concentrated to give a dark-red syrup, that was column chromatographed with benzene–ethyl acetate (9:1) to give a yellow solid of **15**, 6.4 mg (26%), and a dark-red solid of **16**, 6.4 mg (26%).

15: $[\alpha]_D^{20}$ 0° (*c* 0.5, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): δ=3.73 (3H, s, $COOCH_3$), ≈5.15 (4H, $2C_6H_5CH_2$), 5.22 (1H, dd, α-CH), 6.19 (1H, d, β-CH), 6.47 (1H, d, NH), 7.13 [1H, broad s (d on irradiation at δ 6.19), H-5 of Im], ≈7.35 (10 H, $2C_6H_5CH_2$), 7.58 (1H, d, H-2 of Im), 7.66 (1H, d, H-6 of Dnp), 8.57 (1H, dd, H-5 of Dnp), 8.86 (1H, d, H-3 of Dnp); $J_{NH,\alpha\text{-CH}}$=9.5, $J_{\alpha\text{-CH},\beta\text{-CH}}$=4, $J_{2,5(Im)}$=1.3, $J_{3,5(Dnp)}$=2.5, $J_{5,6(Dnp)}$=8.5 Hz.

16: ^1H NMR (250 MHz, $CDCl_3$): δ=3.66 (1H, s, $COOCH_3$), ≈5.2 (4H, $2C_6H_5CH_2$), 5.71 [1H, broad s (disappeared on deuteration), NHZ], 7.22 [1H, d, *J*=12 Hz (s on irradiation at δ=10.64 or on deuteration), H-5], 7.29 (1H, d, H-6 of Dnp), ≈7.35 (10 H, $2C_6H_5CH_2$), 7.49 (1H, s, H-3) 8.40 (1H, dd, H-5 of Dnp), 9.16 (1H, d, H-3 of Dnp), 9.25 (1H, s, CHO), 10.64 [1H, d, *J*=12 Hz (s on irradiation

at δ=7.22, or on deuteration), NHDnp]; $J_{3,5(Dnp)}$=2.5, $J_{5,6(Dnp)}$=9.5 Hz; FDMS: *m/z* 620 (MH^+), 619 (M^+), 591 ([M−CHO+H]$^+$).

***erythro*-$N^{im(3)}$-(4,6-Di-*O*-acetyl-2,3-di-*O*-methyl-α- and β-D-Glucopyranosyl)-N^{α}-benzyloxycarbonyl-β-hydroxyl-L-histidine Methyl Esters (18 and 19).** A mixture of **14** (41.0 mg), the bromide **10** (99 mg, ≈2.5 mol equiv for **14**) and powdered molecular sieves 4A (270 mg) in dry sulfolane (1 ml) was stirred in the dark at 40 °C for 3 h. Mercury(II) cyanide (170 mg) was added and stirring continued overnight. The mixture was charged on the top of a column with aid of benzene and chromatographed with benzene (120 ml)→benzene–ethyl acetate (1:2, 100 ml)→ethyl acetate–ethanol–water–formic acid (20:2:2:1, 120 ml) to give a mixture of products **17**, 56.4 mg, ^{1}H NMR (250 MHz, CD_3OD with slight CD_3COOD): δ=7.38 (H-5 of Im), 8.06 (H-6 of Dnp), 8.18 (H-2 of Im), 8.82 (H-5 of Dnp), 9.20 (H-3 of Dnp). To a solution of the solid in methanol (1 ml) was added 2-mercaptoethanol (10 mg) and the solution was kept at room temperature for 30 min. The resulting solution showed, on TLC with ethyl acetate–ethanol–water–formic acid (20:1:1:0.2), major three spots at R_f 0.20 (**18**), 0.30 (**19**), and 0.40 (**20**) (cf. **17**: R_f 0.05). The solution was concentrated and the syrup was chromatographed on a Sephadex LH-20 column with methanol and the products further purified by two silica-gel columns with chloroform–acetone (1:1) and ethyl acetate–ethanol–water–formic acid (20:1:1:0.2) to give solids of **18**, 16.8 mg (34% based on **14**), **19**, 12.8 mg (26% based on **14**), and **20**, 4.2 mg (6%).

18: Mp 69—71 °C, $[\alpha]_D^{21}$ +42° (*c* 0.5, $CHCl_3$); IR (KBr): 1740, 1530, 1235 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$): δ=2.00, 2.09 (each 3H, s, OAc), 3.40 (3H, s, OCH_3), 3.5 [1H, H-5 of glucoside portion (=G)], 3.59 (3H, s, OCH_3), 3.72 (3H, s, $COOCH_3$), 3.8 (2H, H-2,3 of G), 4.1 (2H, H-6,6′ of G), 4.71 (1H, dd, α-CH), 4.94 (1H, dd, H-4 of G), 5.1 (2H, $C_6H_5CH_2$), 5.34 (1H, d, β-CH), 5.79 (1H, d, NH), 6.30 (1H, d, H-1 of G), 7.08 (1H, s, H-5 of Im), 7.35 (5H, $C_6H_5CH_2$), 7.97 (1H, s, H-2 of Im); $J_{NH,\alpha\text{-}CH}$=8.5, $J_{\alpha\text{-}CH,\beta\text{-}CH}$=6, $J_{1,2(G)}$=3.5 Hz; SIMS: *m/z* 594 (MH^+), 320 ([**13**+H]$^+$), 302 ([**13**−OH]$^+$), 275 (G^+).

Found: C, 52.97; H, 5.73; N, 7.03%. Calcd for $C_{27}H_{35}N_3O_{12}\cdot H_2O$: C, 53.02; H, 6.10; N, 6.87%.

19: Mp 77—79 °C, $[\alpha]_D^{21}$ +20° (*c* 0.5, $CHCl_3$); IR (KBr): 1740, 1535, 1240 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$): δ=2.02, 2.12 (each 3H, s, OAc), 3.24 (3H, s, OCH_3), 3.34 (1H, t, H-3 of G), 3.39 (3H, s, OCH_3), 3.47 (1H, t, H-2 of G), 3.66 (1H, ddd, H-5 of G), 3.70 (1H, s, $COOCH_3$), 4.05 (1H, dd, H-6 of G), 4.16 (1H, dd, H-6′ of G), 4.95 (1H, dd, α-CH), 5.01 (1H, dd, H-4 of G), 5.07, 5.23 (each 1H, d, $C_6H_5CH_2$), 5.33 (1H, d, β-CH), 5.54 (1H, d, H-1 of G), 6.43 (1H, d, NH), 6.88 (1H, s, H-5 of Im), 7.38 (5H, $C_6H_5CH_2$), 7.70 (1H, s, H-2 of Im); $J_{NH,\alpha\text{-}CH}$=9, $J_{\alpha\text{-}CH,\beta\text{-}CH}$=5, $J_{1,2(G)}$=$J_{2,3(G)}$=$J_{3,4(G)}$=9, $J_{4,5(G)}$=10, $J_{5,6(G)}$=2, $J_{5,6'(G)}$=5, $J_{6,6'(G)}$=12.5, $J_{AB\ of\ C_6H_5CH_2}$=12 Hz; SIMS: *m/z* 594 (MH^+), 320 ([**13**+H]$^+$), 302 ([**13**−OH]$^+$), 275 (G^+).

Found: C, 52.73; H, 5.70; N, 6.83%. Calcd for $C_{27}H_{35}N_3O_{12}\cdot H_2O$: C, 53.02; H, 6.10; N, 6.87%.

20: ^{1}H NMR (250 MMz, $CDCl_3$): δ=4.52 and 4.58 [≈1H in total, each d, *J*=8 Hz, H-1 of *O*-G(β-anomer)], 4.82 and 4.92 [each d, *J*=4 Hz, H-1 of *O*-G (α-anomer)?], 5.50 and 5.59 [≈1H in total, each d, *J*=9 Hz, H-1 of *N*-G(β-anomer)], 6.31 and 6.41 [≈1H in total, each d, *J*=3.5 Hz, H-1 of *N*-G(β-anomer)].

***erythro*-N^{α}-Benzyloxycarbonyl-β-hydroxy-$N^{im(\tau)}$-tosyl-L-histidine Methyl Ester (21).** To a solution of **13** (317 mg, 0.8 hydrochloride) in 50% aqueous 1,4-dioxane (9 ml) were added sodium carbonate (116 mg) and then tosyl chloride (190 mg) in 1,4-dioxane (2 ml) and the mixture was stirred at 0 °C for 30 min, then at room temperature for 2 h. The solution showed, on TLC with chloroform–methanol (20:1), a major spot at R_f 0.44 (**21**). The solution was poured into a mixture of ether (5 ml) and water (3 ml) with stirring. The organic layer separated and the ether washings of the residual aqueous layer combined were dried ($MgSO_4$), and concentrated to give a solid of **21**, 415 mg (98%). Recrystallization from dichloromethane–ether gave needles, mp 112—114 °C, $[\alpha]_D^{24}$ +15° (*c* 1, $CHCl_3$); IR (KBr): 1755, 1720 ($\nu_{C=O}$ of ester, and amide I), 1600 (aromatic), 1530 (amide II), 1380 (ν_{as} SO_2), 1175 cm^{-1} (ν_s SO_2); ^{1}H NMR (250 MHz, $CDCl_3$): δ=2.42 (3H, s, CH_3 of Ts), 3.66 (3H, s, $COOCH_3$), 4.79 (1H, dd, α-CH), 5.10 (2H, s, $C_6H_5CH_2$), 5.15 [1H, broad s (d, after deuteration), β-CH], 5.85 (1H, d, NH), 7.24 (1H, broad s, H-5 of Im), 7.35 (7H, $C_6H_5CH_2$ and *m*-H of Ts), 7.79 (2H, *o*-H of Ts), 7.90 (1H, d, H-2 of Im); $J_{NH,\alpha\text{-}CH}$=8, $J_{\alpha\text{-}CH,\beta\text{-}CH}$=4, $J_{2,5(Im)}$=1.5 Hz; SIMS: *m/z* 474 (MH^+), 456 ([**21**−OH]$^+$), 320 ([**13**+H]$^+$), 302 ([**13**−OH]$^+$).

Found: C, 55.07; H, 4.96; N, 9.02; S, 6.58%. Calcd for $C_{22}H_{23}N_3O_7S\cdot 0.5\ H_2O$: C, 54.76; H, 5.01; N, 8.71; S, 6.64%.

***erythro*-β-Hydroxy-N^{α}-(*p*-methoxybenzyloxycarbonyl)-$N^{im(\tau)}$-tosyl-L-histidine Methyl Ester (23).** To an aqueous solution (0.5 ml) of **11**[9] (102 mg, monohydrochloride) were added triethylamine (0.2 ml) and a solution of *O*-(*p*-methoxybenzyl) *S*-(4,6-dimethyl-2-pyrimidinyl) thiocarbonate (PMZ-S reagent)[10] (184 mg) in 1,4-dioxane (0.5 ml), and the mixture was stirred for 3 h. Work-up as described for **12** gave a syrup, that was column-chromatographed with ethyl acetate–ethanol–water–formic acid (20:2:2:1) to give a solid of the corresponding N^{α}-PMZ derivative (185 mg). A mixture of the solid, sodium carbonate (72 mg), and tosyl chloride (116 mg) in H_2O–1,4-dioxane (3:4, 7 ml) was stirred at room temperature overnight. Concentration of the solution gave a residue, an aqueous solution of which was washed with ether and then ethyl acetate. The aqueous solution was concentrated to give a solid (263 mg), that was purified by column chromatography with chloroform–methanol (5:1) to give a solid of $N^{im(\tau)}$-tosyl derivative (163 mg). To a methanol solution (1.2 ml) of the solid was added a solution of ≈0.3 M† diazomethane in ether until the reaction mixture became yellow (≈0.5 ml). After concentration, the residue was column-chromatographed with chloroform–methanol (30:1) to give a solid of **23**, 35.9 mg (15% from **11**), ^{1}H NMR (250 MHz, $CDCl_3$): δ=2.45 (3H, s, CH_3 of Ts), 3.67 (3H, s, $COOCH_3$), 3.73 [1H, d (s on irradiation at δ 5.15; disappeared on deuteration), OH], 3.82 (3H, s, CH_3O of PMZ), 4.80 (1H, dd, α-CH), 5.04 (2H, s, CH_2 of PMZ), 5.15 (1H, dd, β-CH), 5.80 (1H, d, NH, disappeared on deuteration), 6.90 (2H, *m*-H of PMZ), 7.24 (1H, broad s, H-5 of Im), 7.30 (2H, *o*-H of PMZ), 7.35 (2H, *m*-H of Ts), 7.81 (2H, *o*-H of Ts), 7.90 (1H, d, H-2 of Im); $J_{NH,\alpha\text{-}CH}$=8, $J_{\alpha\text{-}CH,\beta\text{-}CH}$=3.5, $J_{\beta\text{-}CH,OH}$=7.5, $J_{2,5(Im)}$=1.5 Hz.

† 1 M=1 mol dm^{-3}.

***erythro*-N^{α}-Benzyloxycarbonyl-β-hydroxy-$N^{im(\tau)}$-tosyl-L-histidine *p*-Nitrobenzyl Ester (25).** To an aqueous solution (1 ml) of **12** (107 mg) and sodium carbonate (45 mg) was added tosyl chloride (80.6 mg) in 1,4-dioxane (1 ml) and the resulting clear solution was kept at room temperature for 5 h. The solution showed, on TLC with ethyl acetate–ethanol–water–formic acid (20:2:2:1), a spot at R_f 0.78 (**24**). Concentration gave a solid, that was column-chromatographed with chloroform–methanol (5:1) to give a solid of **24**, 164 mg, ^{1}H NMR (250 MHz, CD_3OD): δ=2.40 (3H, s, CH_3 of Ts), 4.53 (1H, d, J=5.5 Hz, α-CH), 7.30 (5H, $C_6H_5CH_2$), 7.42 (2H, *m*-H of Ts), 7.46 (1H, broad s, H-5 of Im), 7.90 (2H, *o*-H of Ts), 8.15 (1H, d, J=1.2 Hz, H-2 of Im). To a solution of **24** (88.6 mg) in dry DMF (1.7 ml) were added triethylamine (0.028 ml, dried over CaH_2, 1.1 mol equiv for **24**) and *p*-nitrobenzyl bromide (44.2 mg, 1.1 mol equiv for **24**) and the solution was kept at room temperature overnight. Concentration gave a residue, the solution of which was washed with water, dried ($MgSO_4$), and concentrated. The residue was column-chromatographed with chlorofom–methanol (30:1) to give needles of **25**, 53.6 mg (46% from **12**), mp 159—160 °C, $[\alpha]_D^{20}$ −6° (*c* 0.5, $CHCl_3$); IR (KBr): 1725, 1710, 1520 (amide II and $\nu_{as}NO_2$), 1380 ($\nu_{as}SO_2$), 1350 (ν_sNO_2), 1175 cm^{-1} (ν_sSO_2); ^{1}H NMR (250 MHz, $CDCl_3$): δ=2.42 (3H, s, CH_3 of Ts), 3.53 (1H, d, OH), 4.88 (1H, dd, α-CH), 5.89 (1H, d, NH), 7.18 (1H, broad s, H-5 of Im), 7.33 (2H, *m*-H of Ts), 7.34 (5H, s, $C_6H_5CH_2$), 7.41 (2H, broad d, *o*-H of $NO_2C_6H_4CH_2$), 7.78 (2H, dt, *o*-H of Ts), 7.84 (1H, d, H-2 of Im), 8.18 (2H, dt, *m*-H of $NO_2C_6H_4CH_2$); $J_{NH,\alpha\text{-}CH}$=8.5, $J_{\alpha\text{-}CH,\beta\text{-}CH}$=4, $J_{OH,\beta\text{-}CH}$=8, $J_{2,5(Im)}$=1.4 Hz.

Found: C, 56.31; H, 4.46; N, 9.22; S, 5.52%. Calcd for $C_{28}H_{26}N_4O_9S$: C, 56.56; H, 4.41; N, 9.42; S, 5.39%.

***erythro*-β-*O*-(4,6-Di-*O*-acetyl-2,3-di-*O*-methyl-α- and β-D-glucopyranosyl)-N^{α}-benzyloxycarbonyl-$N^{im(\tau)}$-tosyl-L-histidine Methyl Esters (26 and 27). A) Reaction Using Mercury(II) Cyanide as the Catalyst.** To a solution of **21** (20.8 mg) and the bromide **10** (26.0 mg, ≈1.5 mol equiv for **21**) in dry dichloromethane (0.5 ml) was added powdered molecular sieves 4A (50 mg) and the mixture was stirred in the dark at room temperature for 2 h. Mercury(II) cyanide (51 mg, 3 mol equiv for **10**) was added and the mixture was stirred at room temperature for 48 h (even after 24 h, **10** and **21** still remained). The resulting solution showed, on TLC with chloroform–methanol (20:1), several spots at R_f 0.48 (**26** and **27**), 0.30 (**21**), 0.22 (detosyldi-N^{im},*O*-glucosyl derivatives) and 0.12 (N^{im}-glucosyl derivatives) (cf. **10**: R_f 0.68). The mixture was centrifuged, and the supernatant solution and the dichloromethane washings of the residue combined were washed with water, dried ($MgSO_4$), and concentrated to give a pale yellow solid (42.6 mg), that was twice column-chromatographed with chloroform–methanol (50:1) to give a mixture of **26** and **27** (2:1, determined by the ^{1}H NMR spectrum), 5.8 mg (21% from **21**), a mixture of detosylglucosyl derivatives, 14.9 mg, and **21**, 3.2 mg (15%). Compounds **26** and **27** showed the same R_f value with several kinds of solvent systems tested but was separated by means of analytical high-pressure liquid chromatography (Waters Model 6000A, μ-porasil silica column) using chloroform as the developer. The physicochemical data of **26** and **27** described next were taken by the isolated products.

B) Reaction Using Silver Carbonate-Silver Perchlorate as the Catalyst. A mixture of **21** (43.6 mg), the bromide **10** (51 mg, ≈1.4 mol equiv for **21**) and powdered molecular sieves 4A (90 mg) in dry dichloromethane (1.8 ml) was stirred in the dark at room temperature for 2 h, then cooled to −55 °C. Silver carbonate (48.3 mg, 1.3 mol equiv for **10**) and silver perchlorate (43.1 mg, 1.4 mol equiv for **10**) were added and the mixture was stirred at −55 °C for 5.5 h. Work-up as described for A) gave a pale brown syrup (82 mg), that was thrice column-chromatographed with benzene–ethyl acetate (2:1), chloroform–methanol (50:1), and chloroform–acetone (10:1) to give a mixture of **26** and **27** (≈20:1, determined by the ^{1}H NMR spectrum), 45.9 mg (67% based on **21**), a mixture of detosyldiglucosyl derivatives, 6.0 mg (8%), and **21**, 3.0 mg (7%).

26: $[\alpha]_D^{19}$ +71° (*c* 0.5, $CHCl_3$), IR (KBr): 1745, 1600, 1520, 1380, 1240, 1175 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$): δ=2.07, 2.09 (each 3H, s, OAc), 2.43 (3H, s, CH_3 of Ts), 3.24 (1H, dd, H-2 of G), 3.31 and 3.50 (each 3H, s, OCH_3), 3.54 (1H, t, H-3 of G), 3.63 (3H, s, $COOCH_3$), ≈4.0 (2H, H-5,6 of G), 4.23 (1H, dd, H-6′ of G), 4.89 (1H, d, H-1 of G), 4.90 (1H, t, H-4 of G), ≈4.9 (1H, α-CH), ≈5.1 (3H, $C_6H_5CH_2$ and β-CH), 5.79 (1H, d, NH), 7.35 (7H, $C_6H_5CH_2$ and *m*-H of Ts), 7.45 (1H, apparent t, H-5 of Im), 7.80 (2H, *o*-H of Ts), 7.93 (1H, d, H-2 of Im); $J_{NH,\alpha\text{-}CH}$=9, $J_{1,2(G)}$=3.5, $J_{2,3(G)}$=$J_{3,4(G)}$=$J_{4,5(G)}$=9.5, $J_{5,6'(G)}$=5, $J_{6,6'(G)}$=13, $J_{2,5(Im)}$=1.5, $J_{\beta\text{-}CH,5(Im)}$=0.5 Hz; SIMS: *m/z* 748 (MH^+), 594 ([M−Ts+2H]$^+$), 4.74 ([**21**+H]$^+$), 456 ([**21**−OH]$^+$), 320 ([**13**+H]$^+$), 302 ([**13**−OH]$^+$), 275 (G^+).

Found: C, 53.32; H, 5.37; N, 5.14; S, 4.16%. Calcd for $C_{34}H_{41}N_3O_{14}S\cdot H_2O$: C, 53.33; H, 5.66; N, 5.49; S, 4.19%.

27: $[\alpha]_D^{22}$ +6° (*c* 0.2, $CHCl_3$); IR (KBr): 1740, 1510, 1375, 1230, 1175 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$): δ=2.05, 2.09 (each 3H, s, OAc), 2.43 (3H, s, CH_3 of Ts), 3.09 (1H, dd, H-2 of G), 3.26 (1H, t, H-3 of G), 3.48 and 3.51 (each 3H, s, OCH_3), 3.56 (3H, s, $COOCH_3$), 3.98 (1H, dd, H-6 of G), 4.07 (1H, dd, H-6′ of G), 4.50 (1H, d, H-1 of G), 4.87 (1H, apparent t, H-4 of G), 5.00 (1H, dd, α-CH), 5.11 (2H, $C_6H_5CH_2$), 5.17 (1H, broad d, β-CH), 6.18 (1H, d, NH), 7.33 (7H, $C_6H_5CH_2$ and *m*-H of Ts), 7.36 (1H, broad s, H-5 of Im), 7.81 (2H, *o*-H of Ts), 7.88 (1H, d, H-2 of Im); $J_{NH,\alpha\text{-}CH}$=9, $J_{\alpha\text{-}CH,\beta\text{-}CH}$=4, $J_{1,2(G)}$=7.5, $J_{2,3(G)}$=$J_{3,4(G)}$=9, $J_{4,5(G)}$=10, $J_{5,6(G)}$=2.5, $J_{5,6'(G)}$=5, $J_{6,6'(G)}$=12, $J_{2,5(Im)}$=1.5 Hz; SIMS: *m/z* 748 (MH^+), 594 ([M−Ts+2H]$^+$), 474 ([**21**+H]$^+$), 456 ([**21**−OH]$^+$), 320 ([**13**+H]$^+$), 302 ([**13**−OH]$^+$), 275 (G^+).

3,4,6-Tri-*O*-acetyl-2-*O*-[2,4,6-tri-*O*-acetyl-3-*O*-(*N*-acetylcarbamoyl)-α-D-mannopyranosyl]-α-L-gulopyranosyl Bromide (28). 1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranosyl)-β-L-gulopyranose[2] (31.3 mg) was dissolved in a cold (0 °C) mixture of 30% hydrogen bromide in acetic acid–acetic acid–acetic anhydride (3:8:1) (0.4 ml) and the solution was kept at the temperature for 1 h, then at room temperature in the dark overnight. The solution showed, on TLC with benzene–ethyl acetate (1:2), a single spot at R_f 0.44 (**28**; cf. the starting sugar: R_f 0.28). Work-up as described for **10** gave a pale brown solid of **28**, 32.3 mg (94%), ^{1}H NMR (250 MHz, $CDCl_3$): δ=2.09 (6H, s, 2OAc), 2.12, 2.14, 2.15, 2.23 (each 3H, s, OAc), 2.41 (3H, s, NAc), 4.05 [1H, dd, H-2 of gulose(=Gul)], 4.63 (1H, ddd, H-5 of Gul), 5.06 [1H, d, H-1 of mannose(=Man)], 5.11 (1H, dd, H-4 of Gul), 5.18 (1H, dd, H-3 of Man), 5.24 (1H, dd, H-2 of Man), 5.28 (1H, t, H-4 of Man), 5.36 (1H, dt, H-3 of Gul), 6.42 (1H, broad d, H-1 of Gul), 7.47 (1H, s, NH);

$J_{1,2(Gul)}$=4.5, $J_{1,3(Gul)}$=1, $J_{2,3(Gul)}$=$J_{3,4(Gul)}$=3.5, $J_{4,5(Gul)}$=1, $J_{5,6(Gul)}$≈5.5, $J_{5,6'(Gul)}$≈7, $J_{1,2(Man)}$=2, $J_{2,3(Man)}$=3, $J_{3,4(Man)}$=$J_{4,5(Man)}$=9.5 Hz.

***erythro*-β-*O*-[3,4,6-Tri-*O*-acetyl-2-*O*-[2,4,6-tri-*O*-acetyl-3-*O*-(*N*-acetylcarbamoyl)-α-D-mannopyranosyl]-α-L-gulopyranosyl]-N^{α}-benzyloxycarbonyl-$N^{im(\tau)}$-tosyl-L-histidine Methyl Ester (29).** A mixture of **21** (12.8 mg), the bromide **28**, (32.3 mg, ≈1.4 mol equiv for **21**) and powdered molecular sieves 4A (40 mg) in dry dichloromethane (0.6 ml) was stirred in the dark at room temperature for 2 h, then cooled to −55 °C. Silver carbonate (12.5 mg, 1.1 mol equiv for **21**) and silver perchlorate (10.5 mg, 1.1 mol equiv for **21**) were added and the mixture was stirred at −55 °C overnight. The solution showed, on TLC with benzene–ethyl acetate (1:2), a spot at R_f 0.30 (**29**; cf. **21**: R_f 0.55; **28**: R_f 0.42). Work-up as described for **26** gave a pale brown solid (40.4 mg), that was twice column-chromatographed with chloroform–methanol (30:1) and chloroform–acetone (3:1) to give a solid of **29**, 29.0 mg (95% based on **21**), mp 89—91 °C $[\alpha]_D^{27}$ −12° (*c* 0.5, $CHCl_3$); IR (KBr): 1740, 1600, 1510, 1375, 1220, 1175 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ=1.97, 2.00, 2.08, 2.10, 2.11, 2.14 (each 3H, s, OAc), 2.35 (3H, s, NAc), 2.43 (3H, s, CH_3 of Ts), 3.55 (3H, s, $COOCH_3$), 3.79 (1H, dd, H-6 of Gul), 3.90 (1H, dd, H-6′ of Gul), 4.06 (1H, t, H-2 of Gul), 4.1 (1H, H-6 of Man), 4.15 (1H, H-5 of Man), 4.3 [2H, H-5(Gul) and H-6′(Man)], 4.90 (1H, dd, α-CH), 4.96 (1H, d, one of $C_6H_5CH_2$), 5.01 (1H, dd, H-4 of Gul), 5.01 (1H, d, H-1 of Man), 5.15 (1H, d, H-1 of Gul), 5.210 (1H, d, β-CH), 5.213 (1H, d, one of $C_6H_5CH_2$), 5.27 (1H, t, H-3 of Gul), 5.29 (2H, H-3,4 of Man), 5.31 (1H, H-2 of Man), 6.08 (1H, d, NH), 7.33 (5H, s, $C_6H_5CH_2$), 7.37 (2H, *m*-H of Ts), 7.47 (1H, broad s, H-5 of Im), 7.83 (2H, *o*-H of Ts), 7.97 (1H, d, H-2 of Im); $J_{NH,\alpha\text{-}CH}$=8.5, $J_{\alpha\text{-}CH,\beta\text{-}CH}$=3.5, $J_{1,2(Gul)}$=$J_{2,3(Gul)}$=$J_{3,4(Gul)}$=3.5, $J_{4,5(Gul)}$=1.5, $J_{5,6(Gul)}$=7, $J_{5,6'(Gul)}$=6, $J_{6,6'(Gul)}$=11.5, $J_{1,2(Man)}$=1, $J_{AB\ C_6H_5CH_2}$=12, $J_{2,5(Im)}$=1.5 Hz; SIMS: *m/z* 1135 (MH^+), 980 ([M−Ts+H]$^+$), 662 ([**28**−Br]$^+$), 474 ([**21**+H]$^+$), 456 ([**21**−OH]$^+$), 374 (mannose moiety$^+$), 320 ([**13**+H]$^+$), 302 ([**13**−OH]$^+$), 289 (gulose moiety$^+$).

Found: C, 51.73; H, 5.34; N, 4.69; S, 2.78%. Calcd for $C_{49}H_{58}N_4O_{25}S$: C, 51.85; H, 5.15; N, 4.94; S, 2.82%

***erythro*-β-*O*-[3,4,6-Tri-*O*-acetyl-2-*O*-[2,4,6-tri-*O*-acetyl-3-*O*-(*N*-acetylcarbamoyl)-α-D-mannopyranosyl]-α-L-gulopyranosyl]-N^{α}-*t*-butoxycarbonyl-$N^{im(\tau)}$-tosyl-L-histidine Methyl Ester (30).** Condensation of **22**[3] (6.8 mg) with **28** (15.7 mg) was carried out in the presence of powdered molecular sieves 4A (30 mg), silver carbonate (7.1 mg), and silver perchlorate (6.2 mg) in dry dichloromethane (0.5 ml) in a manner described for **29**. The solution showed, on TLC with benzene–ethyl acetate (1:2), a spot at R_f 0.20 (**30**). Usual work-up including column chromatography as described for **29** gave a solid of **30**, 3.2 mg (19% based on **22**), ^1H NMR (250 MHz, $CDCl_3$): δ=1.41 (9H, s, *t*-Butyl), 5.03 (1H, d, *J*=1 Hz, H-1 of Man), 5.13 (1H, d, *J*=3.5 Hz, H-1 of Gul).

***erythro*-β-*O*-[3,4,6-Tri-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]-N^{α}-benzyloxycarbonyl-$N^{im(\tau)}$-tosyl-L-histidine *p*-Nitrobenzyl Ester (31).** A mixture of **25** (27.2 mg), the bromide **3** (52 mg, ≈1.3 mol equiv for **25**), and powdered molecular sieves 4A (115 mg) in dry dichloromethane (1.6 ml) was stirred in the dark at room temperature for 2 h, then, after cooling to −55 °C, silver carbonate (18.6 mg) and silver perchlorate (14.8 mg) were added and the mixture was stirred at −55 °C overnight. The solution showed, on TLC with benzene–ethyl acetate (1:2), two spots at R_f 0.30 (**31**) and R_f 0.65 (**25**). Usual work-up as described for **26** gave a solid (66 mg), that was column-chromatographed with benzene–ethyl acetate (1:2) to give a solid of **31**, 24.4 mg (43% based on **25**) and **25**, 6.7 mg (25% recovered).

31: Mp 94—98 °C, $[\alpha]_D^{20}$ −12° (*c* 0.5, $CHCl_3$); IR (KBr): 1740, 1600, 1520, 1365, 1345, 1220, 1175 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ=1.93, 1.99, 2.02, 2.06, 2.10, 2.14 (each 3H, s, OAc), 2.41 (3H, s, CH_3 of Ts), 3.75 (1H, dd, H-6 of Gul), 3.91 (1H, dd, H-6′ of Gul), 4.06 (1H, t, H-2 of Gul), ≈4.1 (2H, H-5, 6 of Man), ≈4.3 (2H, H-5 of Gul and H-6′ of Man), 4.61 (2H, broad s, $CONH_2$), 5.01 (1H, dd, H-4 of Gul), 5.03 (1H, d, H-1 of Man), 5.11 (1H, d, H-1 of Gul), ≈5.25 (H-3,4 of Man), 5.27 (1H, t, H-3 of Gul), 5.31 (1H, H-2 of Man), 6.10 (1H, d, NH), ≈7.3 (10H, $C_6H_5CH_2$, *o*-H of $NO_2C_6H_4CH_2$, H-5 of Im, and *m*-H of Ts), 7.79 (2H, dt, *o*-H of Ts), 7.90 (1H, d, H-2 of Im), 8.15 (2H, d, *m*-H of $NO_2C_6H_4CH_2$); $J_{NH,\alpha\text{-}CH}$=8, $J_{1,2(Gul)}$=$J_{2,3(Gul)}$=$J_{3,4(Gul)}$=3.5, $J_{4,5(Gul)}$=2, $J_{5,6(Gul)}$=7.5, $J_{5,6'(Gul)}$=6, $J_{6,6'(Gul)}$=11.5, $J_{1,2(Man)}$=1.5, $J_{2,5(Im)}$=1.3 Hz.

Found: C, 51.33; H, 4.94; N, 5.10; S, 2.27%. Calcd for $C_{55}H_{61}N_5O_{27}S\cdot 2H_2O$: C, 51.12; H, 5.07; N, 5.42; S, 2.48%.

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