A SYNTHETIC MODEL APPROACH TO THE SUGAR MOIETY OF BLEOMYCIN†

Masami Otsuka,*[a] Toshiyuki Nishio,[a] Tetsuta Oshitari,[a] Takashi Owa,[a] Yukio Sugiura,[b] Kenji Maeda,[c] Masaji Ohno,[a] and Susumu Kobayashi*[a]

[a]Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan. [b]Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan. [c]Institute of Microbial Chemistry, Kamiosaki, Shinagawa-ku, Tokyo 141, Japan.

Abstract - In order to investigate the role of the sugar moiety of antitumor antibiotic bleomycin, glycosylation of *erythro*-β-hydroxy-L-histidine derivative is examined and several glycosylated model compounds are prepared by the chloroacetimidate method. These models show excellent dioxygen activating capability.

Many natural products possess sugars which often play key roles to exert the biological activities. Antitumor antibiotic bleomycin contains an unusual disaccharide, 3-*O*-carbamoyl-α-D-mannopyranosyl-α-L-gulopyranose in its *erythro*-β-hydroxy-L-histidine residue of the linear hexapeptide.[1,2] Whereas the bleomycin aglycon appears to contribute mainly to dioxygen activation (by iron complex of the pyrimidine moiety) and DNA binding (by the bithiazole moiety), the sugar moiety was hypothesized to be significant not only for the formation of a molecular cavity to accommodate dioxygen but also for the molecular recognition of bleomycin at the cell surface (Figure 1).[3-5] The disaccharide contributes significantly to the therapeutic effects of bleomycin expressed by sequence-specific degradation of cellular DNA which requires ferrous ion and molecular oxygen.

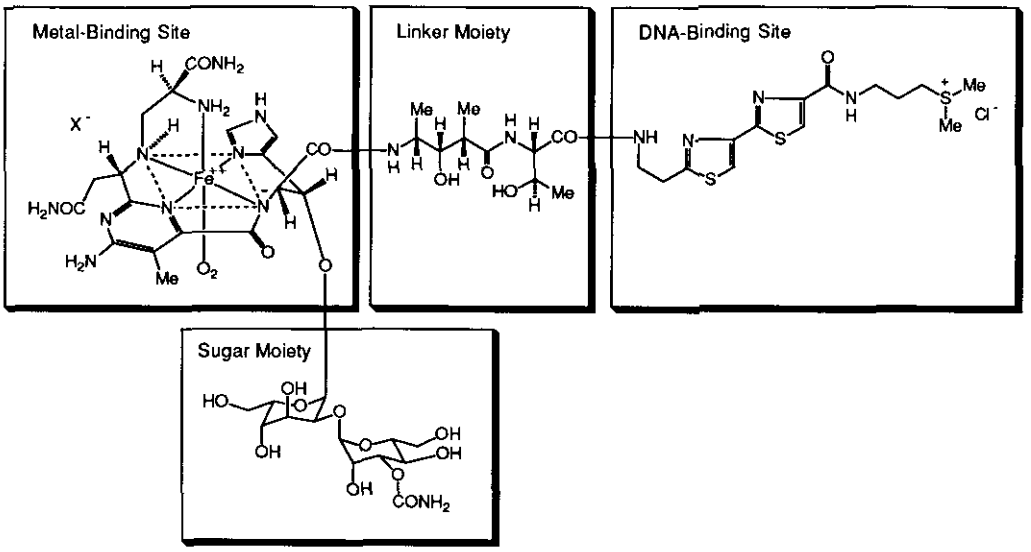
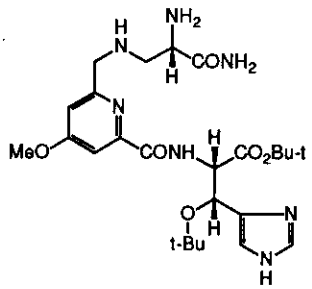


Figure 1. Proposed structure of BLM-Fe(II)-O_2 and assumed role of each functional moiety.

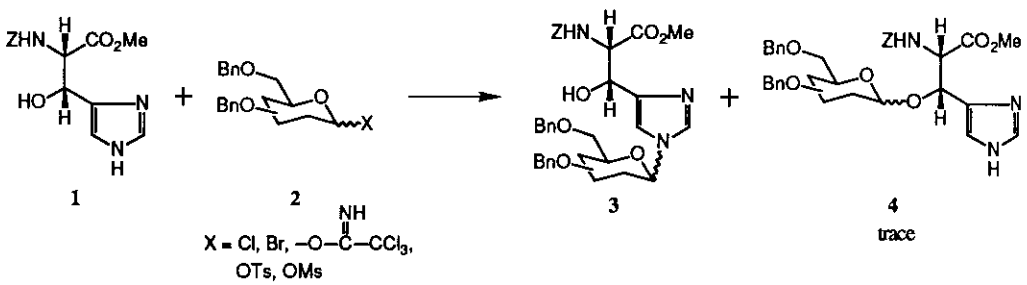
Previously we have reported several synthetic models for the metal binding site of bleomycin in which the pyrimidine ring and the disaccharide moiety were replaced by a 4-substituted pyridine and a *tert*-butyl group, respectively.[6] In particular, a model ligand PYML-6 possessing a 4-methoxypyridine showed dioxygen-activating capability virtually equivalent to that of bleomycin.[7,8] The efficient oxygen-activating power of PYML-6 is presumably due to the electron-donating nature of the 4-methoxyl group and the bulkiness of the *tert*-butyl group. All of the bleomycin models we prepared so far have a *tert*-butyl group instead of the disaccharide, and by these models we showed the role of the sugar moiety as a bulky steric factor to accommodate oxygen.[6] Now we intended to prepare advanced models having a real sugar in the place of the *tert*-butyl group in order to examine another role of the sugar moiety in relation to the membrane permeability of the drug. However, introduction of a sugar into the β-hydroxyhistidine moiety was not an easy task due to the problematic arrangement of the functional groups, i. e., amino, carboxyl, hydroxyl, and imidazolyl, in the small molecule. Previously Miyake *et al.* intensively studied the glycosylation of *erythro*-β-hydroxy-L-histidine and successfully found out a specific condition to introduce 3-*O*-carbamoyl-D-mannopyranosyl-L-gulopyranose which greatly promoted the total synthesis of bleomycin.[9] Herein we examined introduction of various sugars into *erythro*-β-

hydroxy-L-histidine, aiming at a structure-function study of the sugar moiety of bleomycin models and prepared several glycosylated derivatives of PYML-6.



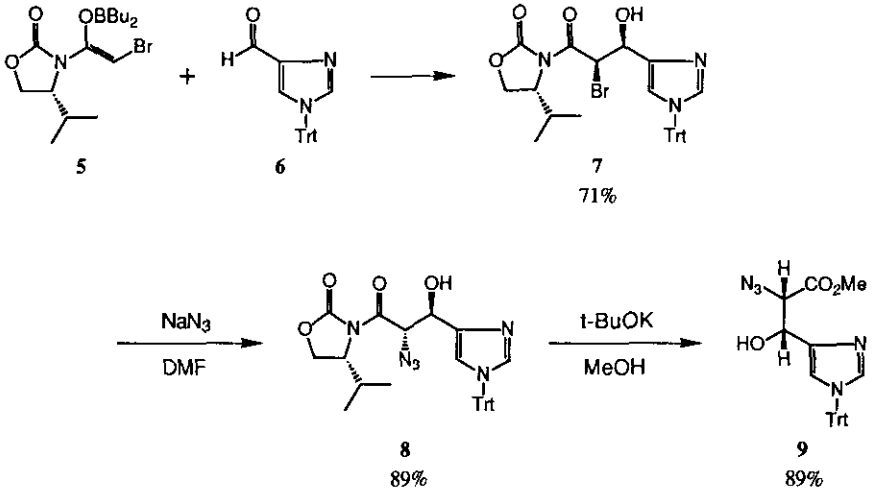
PYML-6

In a preliminary experiment, we attempted reaction of *N*-benzyloxycarbonyl-*erythro*-β-hydroxy-L-histidine methyl ester (**1**)[9] with halogeno sugars (**2**); X = Cl, Br (the Koenigs-Knorr method, Ag_2CO_3, $AgClO_4/CH_2Cl_2$), trichloroacetimidate (**2**); X = $OC(=NH)CCl_3$ (Schmidt method, $BF_3{\cdot}OEt_2/CH_2Cl_2$ or toluene), or sulfonates (**2**); X = OTs, OMe. We found that the reaction did not proceed well only to give a material which seems to be an imidazolyl glycoside (**3**). *N*-Glycoside structure of **3** was supported by the nmr chemical shifts of the imidazole protons and also by the Pauly test. The desired *O*-glycosyl product (**4**) could hardly be obtained. This indicated that an appropriate protection, in particular, of the imidazole ring is essential for successful glycosylation.



In our previous synthesis of *erythro*-β-hydroxy-L-histidine based on the Evans' chiral oxazolidinone procedure,[10] we employed a trityl group for the protection of the imidazole nitrogen owing to the convenience of the removal and achievement of stereoselection of the reaction by its bulkiness.[11] The Evans' chiral boron enolate (**5**) and tritylated formylimidazole (**6**) were reacted to give bromohydrin (**7**) in 71% yield as a virtually enantiomerically

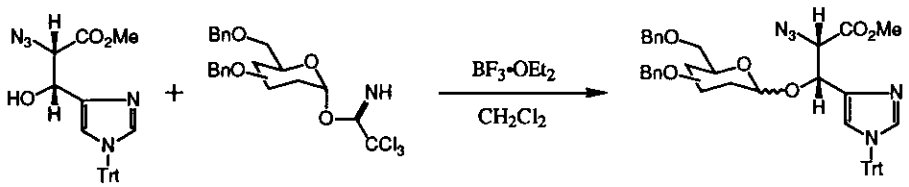
pure material. Introduction of a nitrogen substiuent was accomplished by the S_N2 reaction of **7** with sodium azide to afford azido derivative (**8**) in 89% yield. The stereochemical integrity of **8** has been established by converting **8** into *erythro*-β-hydroxy-L-histidine.[11] Taking advantage of this synthesis, we intended to make use of a synthetic intermediate with a trityl group for the purpose of glycosylation. Thus, the oxazolidinone derivative (**8**) was treated with potassium *tert*-butoxide in MeOH to give trityl-azido-ester (**9**) in 89% yield, $[\alpha]_D^{24}$ +9.31° (c = 1.60, $CHCl_3$), FABms m/z 454 (MH^+).



5 **6** **7** 71%

8 89% **9** 89%

We found that the Schmidt's procedure[12] was successfully applied to *erythro*-β-hydroxy-L-histidine derivative (**9**) after extensive investigation. Thus, 2,3,4,6-tetra-*O*-benzyl-α-D-mannnopyranosyl trichloroacetimidate (**10a**), 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl trichloroacetimidate (**10b**), and 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl trichloroacetimidate (**10c**), prepared according to the procedure of Schmidt,[13] were allowed to react with alcohol (**9**) in the presence of $BF_3{\cdot}OEt_2$ in CH_2Cl_2 at 0 °C for 30 minutes. Extractive work up of the reaction mixture followed by silica gel column chromatography afforded anomeric mixture of *O*-glycosides (**11a**), (**11b**), and (**11c**). These anomers were perfectly separated each other by a Lobar Column™ (LiChroprep Si60, Merck, 40-63 μm, 25×310 mm, eluted with hexane:AcOEt = 2:1→3:2). The results of the glycosylation are shown in Table 1.[14] *O*-Glycosides were obtained in 60 to 70% yields in contrast to the case of the imidazole-free derivative (**1**). Whereas α-anomer was exclusively formed in the case of D-mannose, D-galactose and D-gulose furnished α- and β-anomers in the ratio of 4:1 and 1:1, respectively.



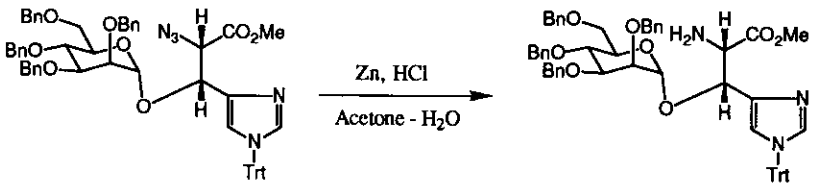
9 **10a, 10b, 10c** **11a, 11b, 11c**

Table 1. Glycosylation of *erythro*-β-hydroxy-L-histidine derivative.

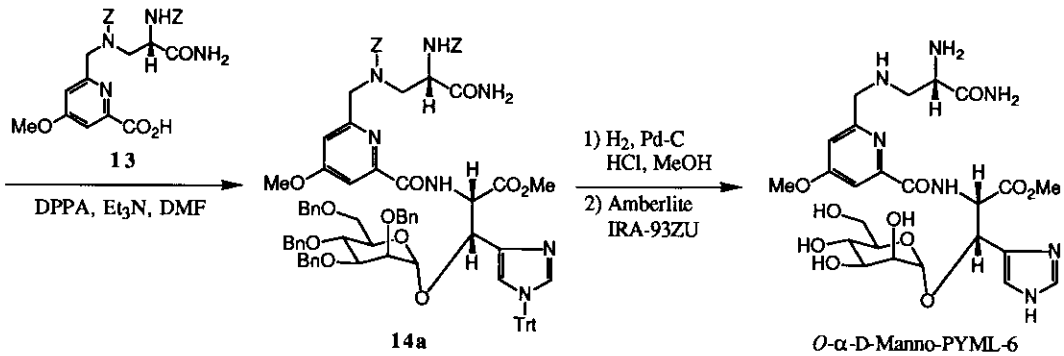
Monosaccharide	Yield (%)	α : β	
Mannose (**10a**)	68	>99	<1
Glucose (**10b**)	65	48	52
Galactose (**10c**)	56	79	21

O-Glyco-hydroxyhistidines thus obtained were converted into *O*-D-glyco-PYML-6. Synthesis of *O*-α-D-Manno-PYML-6 is described in detail. The azido group of the *O*-α-D-manno-histidine derivative (**11a**) was reduced (zinc powder, HCl, aqueous acetone, 0 °C, 2 hours) to give amine (**12a**) quantitatively. Coupling of amine (**12a**) and pyridinecarboxylic acid (**13**) was facilitated by the DPPA method[15] (DPPA; 1.1 eq., Et_3N; 1.3 eq., DMF, 0 °C, 8 hours) to give peptide (**14**) in 76% yield. The Z, benzyl, and trityl groups were simultaneously removed by catalytic hydrogenation (H_2; 50 atm, 10% Pd-C, HCl, MeOH, room temperature, 2 days) and the material obtained was purified by Amberlite IRA-93ZU chromatography (eluted with MeOH) to give *O*-α-D-Manno-PYML-6 in 93% yield.[16] *O*-α-D-Gluco-PYML-6 and *O*-β-D-Gluco-PYML-6 were prepared by the same procedure.[16]

Esr spin trapping experiments revealed that oxygen-activating capability of *O*-α-D-Manno-PYML-6-Fe(II), *O*-α-D-Gluco-PYML-6-Fe(II), and *O*-β-D-Gluco-PYML-6-Fe(II) are 91%, 88%, and 83% of that of bleomycin, respectively. These model ligands produced low spin ferric complex. This demonstrated the fairly good steric effect of the sugar moiety to facilitate the dioxygen activation. The present study provided a basis for the introduction of various sugar to PYML-6 and related model ligands. Synthesis of model ligands with natural type saccharide for the investigation of the interaction with the components of tumor cell surface is currently actively under way.

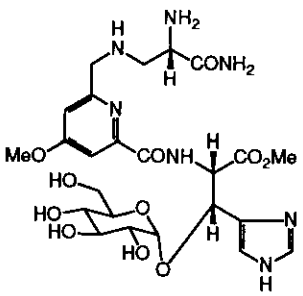


11a **12a**
quant.

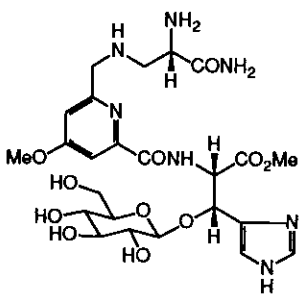


14a
76%

O-α-D-Manno-PYML-6
93%



O-α-D-Gluco-PYML-6



O-β-D-Gluco-PYML-6

ACKNOWLEDGMENT

This study was financially supported in part by Grant-in-Aid for Scientific Research on Priority Areas (Dynamic Aspects of Natural Product Chemistry) from Ministry of Education, Science, and Culture, Japan, and the Sapporo Bioscience Foundation.

REFERENCES AND NOTES

† Dedicated to Professor Masatomo Hamana on the occasion of his 75th birthday.

1. S. Omoto, T. Takita, K. Maeda, H. Umezawa, and S. Umezawa, *J. Antibiot.*, **1972**, *25*, 752.
2. T. Takita, Y. Muraoka, T. Nakatani, A. Fujii, Y. Umezawa, H. Naganawa, and H. Umezawa, *J. Antibiot.*, **1978**, *31*, 801.
3. Y. Sugiura, T. Takita, and H. Umezawa, 'Metal Ions in Biological Systems,' ed. by H. Sigel, Marcel Dekker, New York, 1985, pp. 81 - 108.
4. S. M. Hecht, *Acc. Chem. Res.*, **1986**, *19*, 383.
5. J. Stubbe and J. W. Kozarich, *Chem. Rev.*, **1987**, *87*, 1107.
6. M. Ohno and M. Otsuka, 'Recent Progress in the Chemical Synthesis of Antibiotics,' ed. by G. Lukacs and M. Ohno, Springer-Verlag, Berlin Heidelberg, 1990, pp. 387 - 414 and references cited therein.
7. Y. Sugano, A. Kittaka, M. Otsuka, M. Ohno, Y. Sugiura, and H. Umezawa, *Tetrahedron Lett.*, **1986**, *27*, 3635.
8. A. Kittaka, Y. Sugano, M. Otsuka, and M. Ohno, *Tetrahedron*, **1988**, *44*, 2821.
9. T. Miyake, T. Tsuchiya, S. Umezawa, S. Saito, and H. Umezawa, *Bull. Chem. Soc. Japan*, **1986**, *59*, 1387.
10. D. A. Evans, E. B. Sjogren, A. E. Weber, and R. E. Conn, *Tetrahedron Lett.*, **1987**, *28*, 39.
11. T. Owa, M. Otsuka, and M. Ohno, *Chemistry Lett.*, **1988**, 1873.
12. R. R. Schmidt and J. Michel, *Angew. Chem., Int. Ed. Engl.*, **1980**, *19*, 731.
13. R. R. Schmidt, J. Michel, and M. Ross, *Liebigs Ann. Chem.*, **1984**, 1343.
14. Physicochemical data for *O*-glycosides are as follows. **11a** (α-anomer): $[\alpha]_D^{24}$ +25.3° (c = 2.25, $CHCl_3$); ^{1}H-nmr ($CDCl_3$) δ 4.50 (1H, d, J = 8.0, N_3CHCO_2), 4.91 (1H, d, J = 1.5, H-1), 4.98 (1H, d, J = 8.0, C*H*Im). **11b** (α-anomer): $[\alpha]_D^{23}$ +35.4° (c = 3.65, $CHCl_3$); ^{1}H-nmr ($CDCl_3$) δ 4.60 (1H, d, J = 8.0, N_3CHCO_2), 4.94 (1H, d, J = 3.5, H-1), 5.02 (1H, d, J = 8.0, C*H*Im). **11b** (β-anomer): $[\alpha]_D^{23}$ +9.81° (c = 3.65, $CHCl_3$); ^{1}H-nmr ($CDCl_3$) δ 4.55 (1H, d, J = 6.0, N_3CHCO_2), 4.57 (1H, d, J = 7.5, H-1), 5.27 (1H, d, J = 6.0, C*H*Im). **11c** (α-anomer): $[\alpha]_D^{23}$ +28.7° (c = 2.80, $CHCl_3$); ^{1}H-nmr ($CDCl_3$) δ 4.59 (1H, d, J = 7.3, N_3CHCO_2), 4.91 (1H, d, J = 4.0, H-1), 5.03 (1H, d, J = 7.3, C*H*Im). **11c** (β-anomer):

$[\alpha]_D^{23}$ +6.16° (c = 1.44, $CHCl_3$); ^{1}H-nmr ($CDCl_3$) δ 4.52 (1H, d, J = 6.0, N_3CHCO_2), 4.53 (1H, d, J = 7.5, H-1), 5.21 (1H, d, J = 6.0, CHIm).

15. T. Shioiri, K. Ninomiya, and S. Yamada, *J. Am. Chem. Soc.*, **1972**, *94*, 6203.

16. Physicochemical data for *O*-D-glyco-PYML-6 are as follows. *O*-α-D-Manno-PYML-6: $[\alpha]_D^{24}$ +63.3° (c = 0.655, MeOH); ir (KBr) 3411, 2930, 1669, 1602, 1522, 1436 cm^{-1}; ^{1}H-nmr (D_2O+CD_3OD) δ 2.97 (1H, dd, J = 6.0, 13.0, $CH_2CHCONH_2$), 3.12 (1H, dd, J = 6.0, 13.0, $CH_2CHCONH_2$), 3.60-3.79 (5H, m), 3.81 (3H, s, $PyOCH_3$), 3.83-3.89 (2H, m), 3.93 (3H, s, CO_2CH_3), 4.04-4.15 (2H, d×2, J = 12.0, 16.0, $PyCH_2$), 4.68 (1H, s, H-1), 5.20 (1H, d, J = 6.8, $CONHCHCO_2$), 5.32 (1H, d, J = 6.8, CHIm), 7.15 (1H, s, Py), 7.33 (1H, s, Im), 7.48 (1H, s, Py), 7.78 (1H, s, Im); FABms m/z 598 (MH^+). *O*-α-D-Gluco-PYML-6: $[\alpha]_D^{24}$ +86.8° (c = 0.600, MeOH); ir (KBr) 3382, 2925, 1669, 1602, 1521, 1437 cm^{-1}; ^{1}H-nmr (D_2O+CD_3OD) δ 3.03 (1H, dd, J = 6.0, 13.0, $CH_2CHCONH_2$), 3.17 (1H, dd, J = 6.0, 13.0, $CH_2CHCONH_2$), 3.41 (1H, dd, J = 9.2, 9.6, H-4), 3.46 (1H, dd, J = 9.8, 4.4, H-2), 3.63-3.85 (4H, m), 3.75-3.85 (4H, broad, $PyOCH_3$ and $CH_2CHCONH_2$), 3.93 (3H, s, CO_2CH_3), 4.13 (2H, s, $PyCH_2$), 4.70-4.90 (H-1), 5.24 (1H, d, J = 7.2, $CONHCHCO_2$), 5.33 (1H, d, J = 7.2, CHIm), 7.14 (1H, s, Py), 7.33 (1H, s, Im), 7.49 (1H, s, Py), 7.78 (1H, s, Im); FABms m/z 598 (MH^+). *O*-β-D-Gluco-PYML-6: $[\alpha]_D^{24}$ +25.6° (c = 0.570, MeOH); ir (KBr) 3367, 2920, 1669, 1602, 1521, 1437 cm^{-1}; ^{1}H-nmr (D_2O+CD_3OD) δ 3.07 (1H, dd, J = 6.0, 13.0, $CH_2CHCONH_2$), 3.16 (1H, dd, J = 6.0, 13.0, $CH_2CHCONH_2$), 3.27 (1H, dd, J = 8.8, 8.8, H-4), 3.29-3.41 (3H, m), 3.48 (1H, dd, J = 8.8, 8.8), 3.60-3.92 (2H, m), 3.75 (3H, s, $PyOCH_3$), 3.95 (3H, s, CO_2CH_3), 4.12 (2H, s, $PyCH_2$), 4.68 (1H, d, J = 8.0, H-1), 5.35 (1H, d, J = 5.6, $CONHCHCO_2$), 5.53 (1H, d, J = 5.6, CHIm), 7.17 (1H, d, J = 2.0, Py), 7.27 (1H, s, , Im), 7.55 (1H, d, J = 2.0, Py), 7.79 (1H, s, Im); FABms m/z 598 (MH^+).

Received, 2nd September, 1991