Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 1499–1504

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS AND BIOLOGICAL EVALUATION OF α-MANNOSIDASE INHIBITORY ACTIVITY OF THREE DEOXY DERIVATIVES OF MANNOSTATIN A

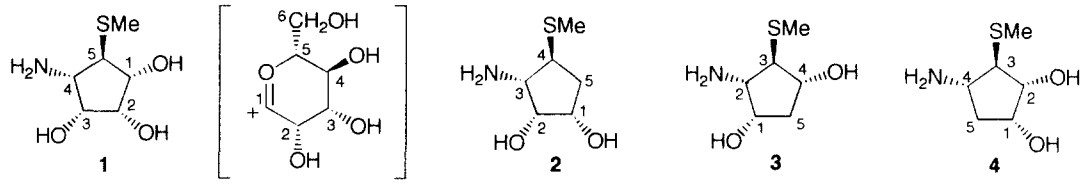
Seiichiro Ogawa* and Takayuki Morikawa

Department of Applied Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi, Kohoku-ku, Yokohama, 223-8522 Japan

Received 25 January 1999; accepted 9 April 1999

Abstract: Three deoxy derivatives of α-mannosidase inhibitor mannostatin A have been synthesized and their inhibitory activity for Jack beans α-mannosidase evaluated in order to elucidate roles of each hydroxyl groups of the inhibitor. The 1- and 2-deoxy derivatives have preserved inhibitory potentials although they lowered the activity one-hundred fold compared to the parent, but the 3-deoxy derivative lost activity.

A potent and specific α-mannosidase inhibitor mannostatin A[1,2] (**1**), 1D-(1,2,3,4/5)-4-amino-5-methylthio-1,2,3-cyclopentanetriol,[3] has prompted us to develop new glycosidase inhibitors composed of 5-amino-1,2,3,4-cyclopentanetetrols, which are thought to act as transition state mimics of glycopyranosyl cations postulated to form during hydrolysis of glycosides[4]. Concerning conformational feature of the transition state mannopyranosyl cation, it appeared rather difficult to correlate the structures of the known α-mannosidase inhibitors to that of the mannopyranosyl cation[5]. Recently, Winkler and his coworkers[6] have proposed a correlationship by comparing the structure of mannostatin A to their flap up mannopyranosyl cation model, suggesting an importance of good overlap of the 1- and 2-hydroxyl groups of 1 onto the 3- and 2-hydroxyls of the mannopyranosyl cation, respectively.

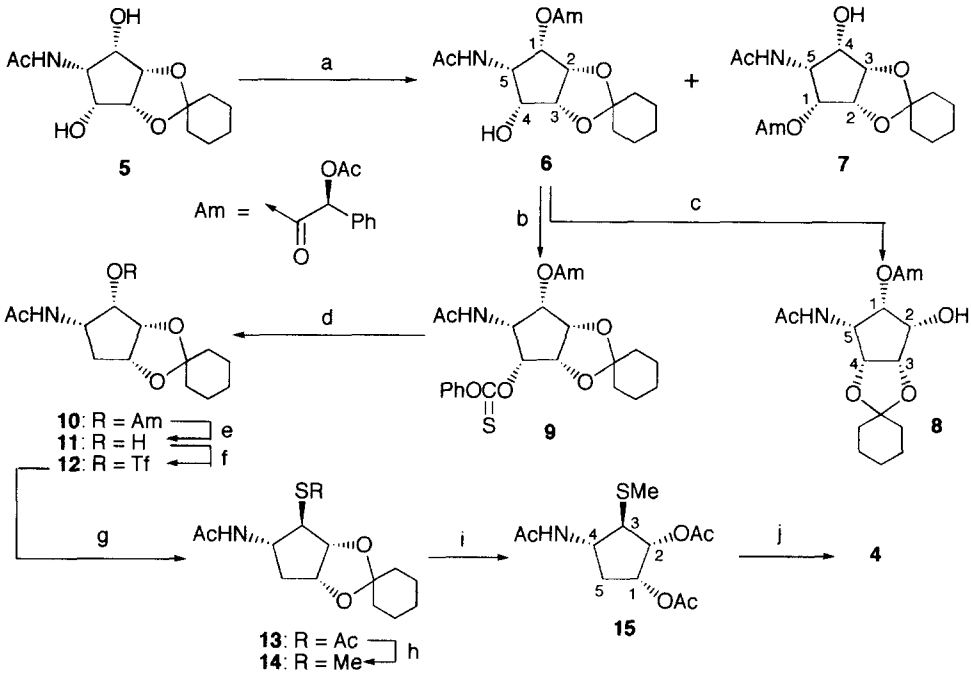


Three isomers of deoxymannostatin A

PII: S0960-894X(99)00224-3

Although **1** has a simple and unique structure, a very few chemical modification[2b] of **1** has been carried out so far. The present communication describes syntheses and evaluations of α-mannosidase inhibitory activity of the deoxy derivatives **2**, **3**, and **4** of mannostatin A, in an effort to elucidate the role of each hydroxyl group of **1** in binding to the active site of the enzyme.

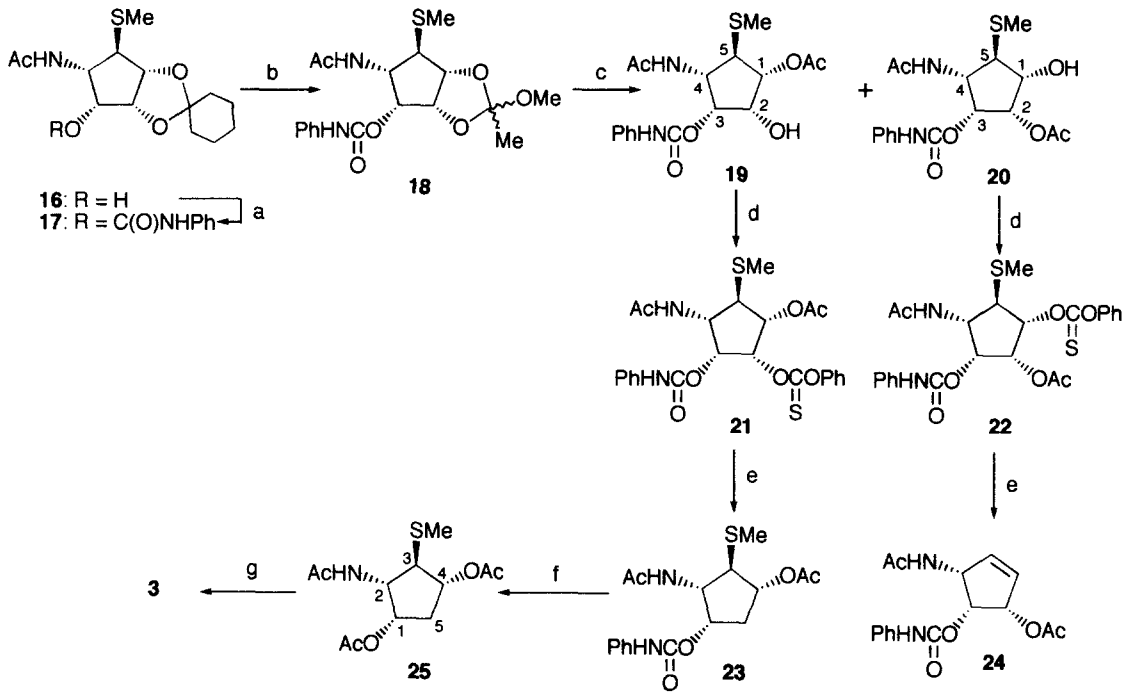
Synthesis of deoxymannostatins has been carried out by the conventional sequence of deoxygenation: phenylthiocarbonylation of unprotected hydroxyl group of intermediate protected 5-amino-1,2,3,4-cyclopentanetetrol derivatives, followed by treatment with tributyltinhydride in the presence of AIBN, de-*O*-acylation, conversion into triflates, direct nucleophilic substitution by a thioacetate anion, de-*S*-acetylation, *S*-methylation with iodomethane, and removal of protecting groups by acid hydrolysis.



Scheme 1. *Reagents and conditions*: (a) (*S*)-*O*-Acetylmandelic acid, DMAP, CH_2Cl_2, 0 °C; (b) DMAP (6 molar equiv), PhOC(S)Cl (5 molar equiv), CH_3CN, 3 h, rt; (c) PhOC(S)Cl (7 molar equiv), DMAP (6 molar equiv), CH_3CN, 11 d, rt; (d) *n*-Bu_3SnH, AIBN, toluene, reflux, 1 h; (e) 1 M NaOMe/MeOH, rt, 3 h; (f) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, 10 min, –15 °C; (g) AcSK, 18-crown-6 ether, benzene, rt, overnight; (h) 1 M NaOMe/MeOH, rt; CH_3I (I) 2 M HCl, reflux, 3 h; Ac_2O, pyridine, rt; (j) 2 M HCl, 80 °C, 12 h.

Reaction of the 2,3-*O*-cyclohexylidene derivative[7] **5** of (1,2,3,4,5/0)-5-acetamido-1,2,3,4-cyclopentanetetrol with (*S*)-*O*-acetylmandelic acid in the presence of DCC and DMAP in CH_2Cl_2 gave diastereoselectively the 1*S*-ester[8] **6** (56%), together with the 1*R*-ester **7** (5%). Compound **6** was converted

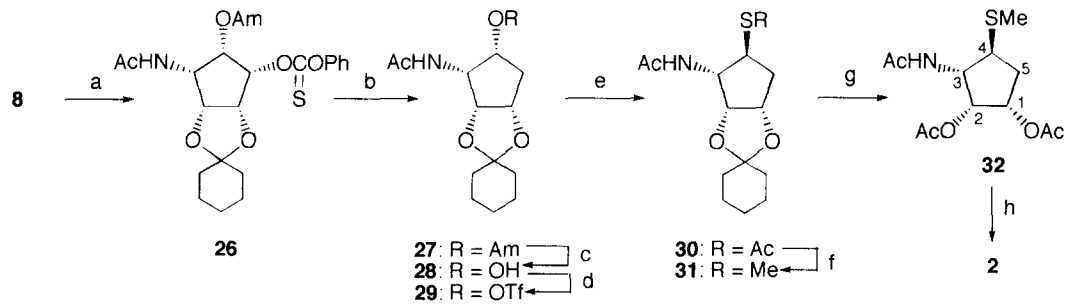
into the phenylthiocarbonyl ester **9** (70%) by treatment in turn with DMAP (6 molar equiv) and phenyl chlorothionocarbonate (5 molar equiv) in CH_3CN at room temperature for 3 h. When phenyl chlorothionocarbonate (7 molar equiv) and DMAP (6 molar equiv) were added in turn reversely, a migration of the cyclohexylidene group occurred slowly to give mainly **8** (56%), together with **9** (9%). Treatment of **9** with tributyltinhydride in the presence of AIBN gave **10** (71%). The Zemplén de-*O*-acylation of **10** gave **11** (82%). Compound **11** was converted into **12** , which was treated with potassium thioacetate in benzene in the presence of 18-crown-6 ether to give **13** (71% over-all yield). De-*S*-acylation of **12** with methanolic sodium methoxide and the subsequent treatment with iodomethane, afforded **14** (83%). The structure was established on the basis of the ^1H NMR spectrum. Hydrolysis of **14** with 2 M HCl at reflux, followed by treatment with acetic anhydride in pyridine, gave **15**[9] (70%). Similar hydrolysis of **15** and purification over a column of Dowex 50W ×2 (H^+) resin with 1% aq ammonia gave **4** (~100%) ($[\alpha]_D$ +7°, MeOH) (Scheme 1).



Scheme 2. *Reagents and conditions:* (a) PhNCO, pyridine, 6 h, rt; (b) 60% aq AcOH, 9 h, 80 °C; $(MeO)_3CMe$, TsOH, C_6H_6, rt; (c) 80% aq AcOH, 0.5 h, rt; (d) PhOC(S)Cl, DMAP, CH_3CN, 0.5 h, rt; (e) *n*-Bu_3SnH, AIBN, toluene, 2 h, reflux; (f) 1 M NaOMe/ MeOH, 2 h, reflux; Ac_2O, pyridine; (g) 2 M HCl, 1.5 h, 80 °C.

Preparations of the 1- and 2-deoxymannostatins were started from the protected derivative[8] **16** of mannostatin A derived from **8**. Thus, the hydroxyl group of **16** was first protected to generate **17** (90%) in a

usual manner. Treatment of **17** with 80% aq acetic acid at 80 °C gave the diol, which was treated with trimethyl orthoacetate in the presence of TsOH in benzene to afford a mixture of the epimeric orthoacetates **18**. The mixture was treated with aq 80% acetic acid at room temperature to give a mixture (~60%) of **19** and **20**, which were similarly converted into the respective phenylthiocarbonates **21** (58%) and **22** (15%). Compound **21** was easily converted into the 2-deoxymannostatin A (**3**) through treatment with *n*-Bu_3SnH-AIBN [→**23** (70%)], and conventional deprotection and acetylation [→**25** (~35%)]. The tri-*N,O*-acetyl derivative[10] **25** gave the free base **3** (79%), $[\alpha]_D$ +22° (MeOH). On the other hand, under the influence of *n*-Bu_3SnH, **22** was found to give rise to the elimination product **24** instead of the desired deoxy derivative.



Scheme 3. *Reagents and conditions:* (a) PhOC(S)Cl, DMAP, CH_3CN, 1.5 h, rt; (b) *n*-Bu_3SnH, AIBN, toluene, 0.5 h, reflux; (c) 1 M NaOMe/MeOH, 0.5 h, rt; (d) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, 20 min, -15 °C; (e) AcSK, 18-crown-6 ether, benzene, rt, 2 days; (f) 1 M NaOMe/MeOH, 10 min, rt; MeI, 2 h, rt; (g) 2 M HCl, 2 h, reflux; Ac_2O, pyridine; (h) 2 M HCl, 2 h, 80 °C.

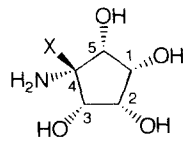
For the synthesis of **2**, **26** that was derived from **8** in 91% yield was treated with *n*-Bu_3SnH to afford the deoxy derivative **27** (77% overall yield), to which a methylthio function was incorporated similarly as in the preparation of **14**, giving **31** (68% overall yield) via the alcohol **28**, the triflate **29**, and the acetylthiolate **30**. De-*O*-cyclohexylidenation of **31** followed by acetylation gave **32**[11] (80%), the structure of which was estasblished by the NMR spectrum. The free base **2** (56%), $[\alpha]_D$ +29° (MeOH), was obtained by the treatment of **32** with 2 M HCl.

The inhibitory activities of **2**, **3**, and **4** are listed in Table 1. The 1-deoxy **2** and 2-deoxy derivatives **3** preserved the inhibitory activity although lowered by one-hundred fold compared to the parent compound **1**. The 3-deoxy derivative **4** lost activity.

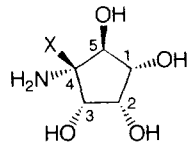
Table 1. Inhibitory activity[a] [IC_{50} (M)] of three deoxy derivatives **2**–**4** of mannostatin A (**1**) against α-mannosidase[b] (Jack bean)

Compound	**1**	**2**	**3**	**4**	Nojirimycin B bisulfite[c]
Inhibitory activity	2.4×10^{-7}	2.8×10^{-5}	3.1×10^{-5}	$>10^{-4}$	4.2×10^{-5}

[a] 2.0 mM *p*-nitrophenyl α-D-mannopyranoside, 0.1 M acetate buffer, pH 4.5.[12]; [b] α-Mannosidase (Jack bean) and nitrophenyl mannopyranoside were purchased from SIGMA; [c] ref. [13].



33: X = H
34: X = CH_3



35: X = H
36: X = CH_3

We have demonstrated that, among twenty four stereoisomers[14] of 5-amino-1,2,3,4-cyclopentanetetrols, only 1L-(1,2,3,5/4)- **33** and (1,2,3,4,5/0)-isomers **35**, and the corresponding 5-C-methyl derivatives[15] **34** and **36**, bear weak inhibitory activity for Jack bean α-mannosidase (IC_{50} = 1–3 × 10^{-5} M). Their structures resemble that of mannostatin A, which contains four contiguous 1-, 2-, and 3-hydroxyl, and 4-amino groups in all-cis relationships, suggesting that these core structures are essential for inhibitory activity against α-mannosidase.

The fact that the 3-deoxy derivative **4** lost the inhibitory activity demonstrated that the 3-hydroxyl function of **1** is the most essential group for binding to the enzyme, which indicated that, when binding to the enzyme, it should conceivably be correlated to the 2-hydroxyl group of the mannopyranosyl cation and the amino group be located around the carbocation atom. Accordingly, in addition to the Winkler's model,[6] the other candidate where the 1- and 2-hydroxyl groups of **1** are roughly corresponding to the hydroxymethyl and the 3-hydroxyl groups of mannopyranosyl cation, respectively, may be proposed for molecular model study.

It is reasonable to consider that compound **4** can bind to the enzyme through hydrogen bonding of the 1- and 2-hydroxyl groups, but it loses the activity due to lack of the 3-hydroxyl group. On the other hand, it may be possible to correlate the two hydroxyl groups of **2** for overlapping onto the 3- and 2-hydroxyls of the mannopyranosyl cation. Interestingly, compound **3** has shown to preserve the moderate inhibitory activity, showing that the presence of a pair of cis hydroxyl groups considered to correspond to the 2- and 3-hydroxyls of mannopyranosyl cation is not always indispensable for mannosidase inhibitors.

Acknowledgments

The authors thank Dr. Eijiro Umemura (Meiji Seika Kaisha Ltd., Yokohama) for providing us with an authentic sample of nojirimycin B (mannonojirimycin) bisulfite.

References and Notes

1. *Isolation:* Aoyagi, T.; Yamamoto, T.; Kojiri, K.; Morishima, H.; Nagai, M.; Hamada, M.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1989**, *42*, 883. *Structure:* Morishima, H.; Kojiri, K.; Yamamoto, T.; Aoyagi, T.; Nakamura, H.; Iitaka, Y. *J. Antibiot.* **1989**, *42*, 1008.
2. *Total synthesis:* a) Ogawa, S.; Yuming, Y. *J. Chem. Soc., Chem. Commun.* **1991**, 890; *Biorg. Med. Chem.* **1995**, *3*, 939. b) King, S. B.; Ganem, B. *J. Am. Chem. Soc.* **1991**, *113*, 5089; *J. Am. Chem. Soc.* **1994**, *116*, 562. c) Knapp, S.; Murali Dhar, T. G. *J. Org. Chem.* **1991**, *56*, 4096. d) Trost, B. M.; Van Vranken, D. L., *J. Am. Chem. Soc.***1991**, *113*, 5089. For a review article, see Ganem, B. *Carbohydrate Mimics*; Chapleur, Y. Ed.; Wiley-VCH: Weinheim, 1998; pp. 239–258.
3. In this paper, nomenclature of cyclitols follows IUPAC-IUB 1973 Recommendations for Cyclitols [*Pure Appl. Chem.* **1974**, *37*, 285].
4. Legler, G. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 319. Legler, G. *Carbohydrate Mimics*; Chapleur, Y. Ed.; Wiley-VCH: Weinheim, 1998; pp. 463–490.
5. Look, G. C.; Fotsch, C. H.; Wong, C. H. *Acc. Chem. Res.* **1993**, *26*, 182.
6. Winkler, D. A. *J. Med. Chem.* **1996**, *39*, 4332.
7. Suami, T.; Tadano, K.; Nishiyama, S.; Lichtenthaler, F. W. *J. Org. Chem.* **1973**, *38*, 3691. Uchida, C.; Yamagishi, T.; Ogawa, S. *J. Chem. Soc., Perkin Trans 1* **1994**, 589.
8. Ogawa, S.; Kimura, H.; Uchida, C.; Ohashi, T. *J. Chem. Soc. Perkin Trans 1*, **1995**, 1695.
9. **15**: $[\alpha]_D^{22}$ +4° ($CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ = 5.69 (1 H, d, $J_{4,MH}$ 8.6, NH), 5.34 (1 H, ddd, $J_{1,2}$ 4.6, $J_{1,5a}$ 6.4, $J_{1,5b}$ 3.9 Hz, 1-H), 5.05 (1 H, dd, $J_{1,2}$ 4.6, $J_{2,3}$ 8.1 Hz, 2-H), 4.27 (1 H, dddd, $J_{3,4}$ 8.1, $J_{4,5a}$ 14.9, $J_{4,5b}$ 5.6, $J_{4,NH}$ 8.6 Hz, 4-H), 2.97 (1 H, dd, $J_{2,3} = J_{3,4}$ = 8.1 Hz, 5-H), 2.62 (1 H, ddd, $J_{1,5a}$ 6.4, $J_{4,5a}$ 14.9, J_{5gem} 9.0 Hz, 5a-H), 2.15, 2.09, 2.08, and 2.02 (each 3 H, 4 s, 3 Ac and SMe), 1.71 (1 H, ddd, $J_{1,5b}$ 5.4, $J_{4,5b}$ 5.4, J_{5gem} 9.0 Hz, 5b-H).
10. **25**: $[\alpha]_D^{27}$ +13.5° ($CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ = 5.63 (1 H, d, $J_{2,NH}$ 11.1 Hz, NH), 5.11 (1 H, ddd, $J_{1,2} = J_{1,5b}$ = 5.9, $J_{1,5a}$ 1.5 Hz, 1-H), 5.08 (1 H, ddd, $J_{3,4}$ 7.1, $J_{4,5a}$ 4.9, $J_{4,5b}$ 8.8 Hz, 4-H), 4.35 (1 H, ddd, $J_{1,2}$ 5.9, $J_{2,3}$ 11.5, $J_{2,NH}$ 11.3 Hz, 2-H), 3.09 (1 H, dd, $J_{2,3}$ 11.5, $J_{3,4}$ 7.1 Hz, 3-H), 2.61 (1 H, ddd, $J_{1,5a}$ 5.9, $J_{4,5a}$ 8.8, J_{5gem} 6.4 Hz, 5a-H), 2.12, 2.11, 2.08, and 2.05 (each 3 H, 4 s, 3 Ac and SMe), 1.78 (1 H, ddd, $J_{1,5b}$ 1.5, $J_{4,5b}$ 4.9, J_{5gem} 6.4 Hz, 5b-H).
11. **32**: $[\alpha]_D^{22}$ +15° ($CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ = 5.74 (1 H, d, $J_{3,NH}$ 8.8 Hz, NH), 5.37 (1 H, m, 2-H), 5.35 (1 H, m, 1-H), 4.52 (1 H, m, 3-H), 3.15 (1 H, ddd, $J_{3,4}$ 6.8, $J_{4,5a}$ 10.0, $J_{4,5b}$ 6.8 Hz, 4-H), 2.28 (1 H, m, 5a-H), 2.07 (1 H, m, 5b-H), 2.18, 2.11, 2.05, and 2.02 (each 3 H, 4 s, 3 Ac and SMe).
12. Suzuki, H.; Li, S.-C.; Li, Y.-T, *Biol. Chem.* **1970**, *245*, 781.
13. Niwa, T.; Tsuruoka, T.; Goi, H.; Kodama, Y.; Itoh, T.; Inouye, S.; Yamada, Y.; Niida, T.; Nobe, M.; Ogawa, Y. *J. Antibiot.* **1984**, *37*, 1579.
14. Uchida, C.; Kimura, H.; Ogawa, S. *Bioorg. Med. Chem.* **1997**, *5*, 921.
15. Ogawa, S.; Washida, K. *Eur. J. Org. Chem.*, **1998**, 1929.