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

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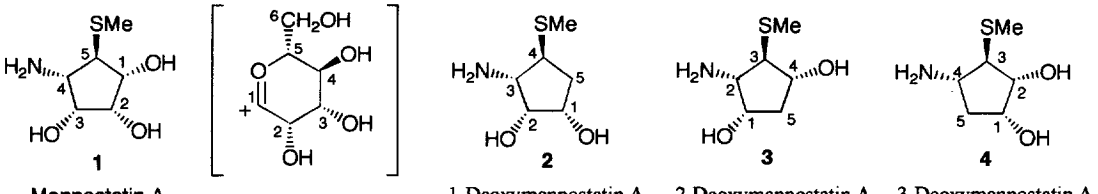
Abstract: Three deoxy derivatives of α-mannosidase inhibitor mannostatin A have been synthesized and their inhibitory activity for Jack beans α-mannosidase evaluated in order to elucidate roles of each hydroxyl groups of the inhibitor. The 1- and 2-deoxy derivatives have preserved inhibitory potentials 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

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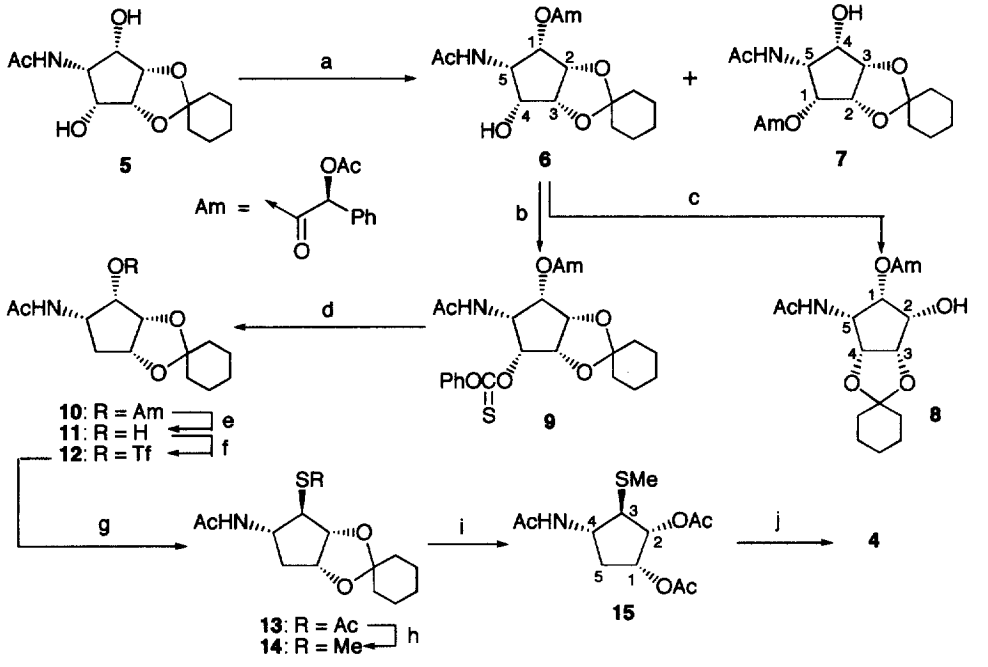
Although **1** has a simple and unique structure, a very few chemical modification[2b] of **1** has been carried out so far. The present communication describes syntheses and evaluations of α-mannosidase inhibitory activity of the deoxy derivatives **2**, **3**, and **4** of mannostatin A, in an effort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

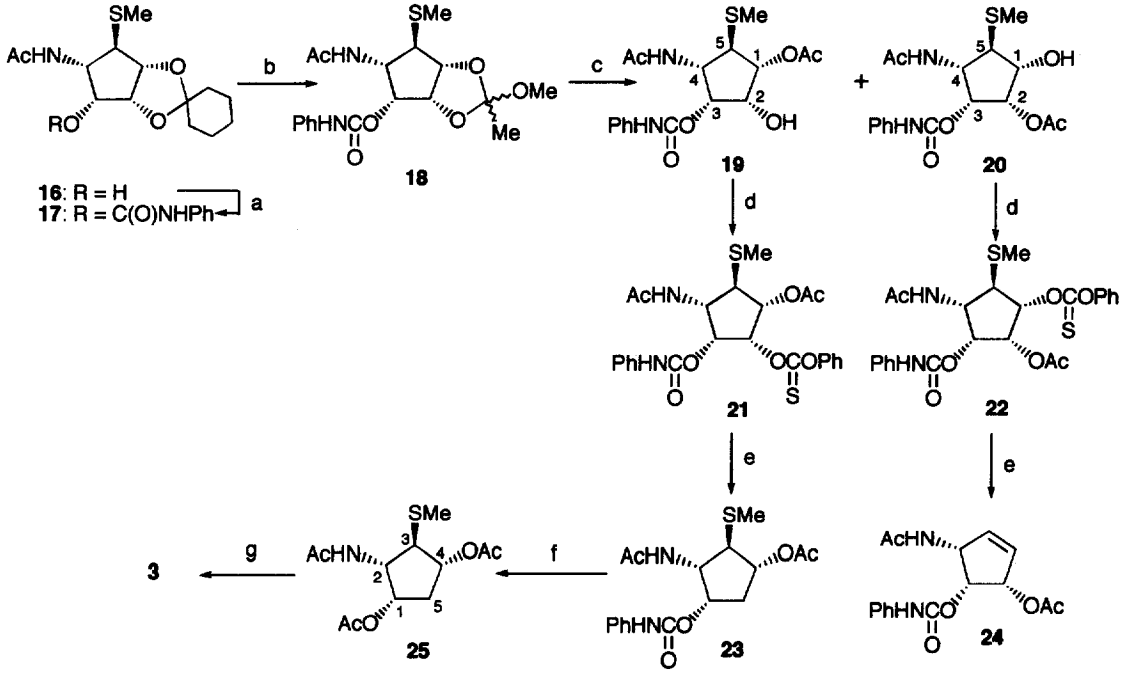
Synthesis of deoxymannostatins has been carried out by the conventional sequence of deoxygenation: phenylthiocarbonylation of unprotected hydroxyl group of intermediate protected 5-amino-1,2,3,4-cyclopentanetetrol derivatives, followed by treatment with tributyltinhydride in the presence of AIBN, de-*O*-acylation, 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

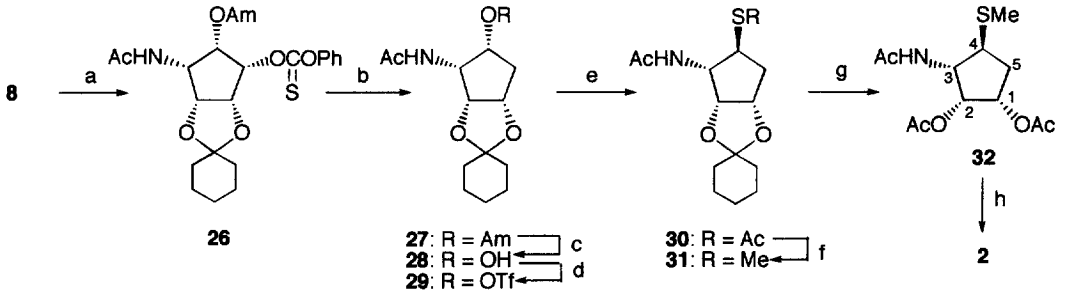
into the phenylthiocarbonyl ester **9** (70%) by treatment in turn with DMAP (6 molar equiv) and phenyl chlorothionocarbonate (5 molar equiv) in CH_3CN at room temperature for 3 h. When phenyl chlorothionocarbonate (7 molar equiv) and DMAP (6 molar equiv) were added in turn reversely, a migration of 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.

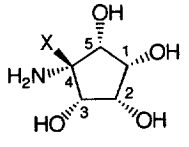
For the synthesis of **2**, **26** that was derived from **8** in 91% yield was treated with *n*-Bu_3SnH to afford the deoxy derivative **27** (77% overall yield), to which a methylthio function was incorporated similarly as in the preparation of **14**, giving **31** (68% overall yield) via the alcohol **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.

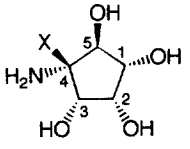
Table 1. Inhibitory activity[a] [IC_{50} (M)] of three deoxy derivatives **2**–**4** of mannostatin A (**1**) against α-mannosidase[b] (Jack bean)

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

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9. **15**: $[\alpha]_D^{22}$ +4° ($CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ = 5.69 (1 H, d, $J_{4,NH}$ 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).
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