Design and Synthesis of a Potential Endoglycosidase Inhibitor: Chemical Conversion of *N,N'*-Diacetylchitobiose into Novel Pseudodisaccharide Containing a Fivemembered Cyclic *N,N*-Dimethylguanidine

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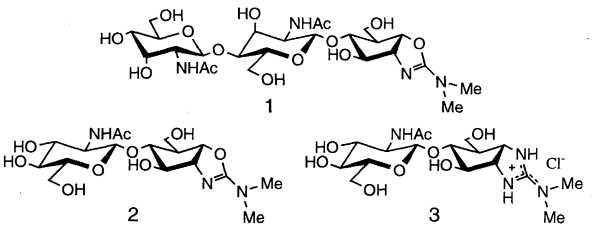
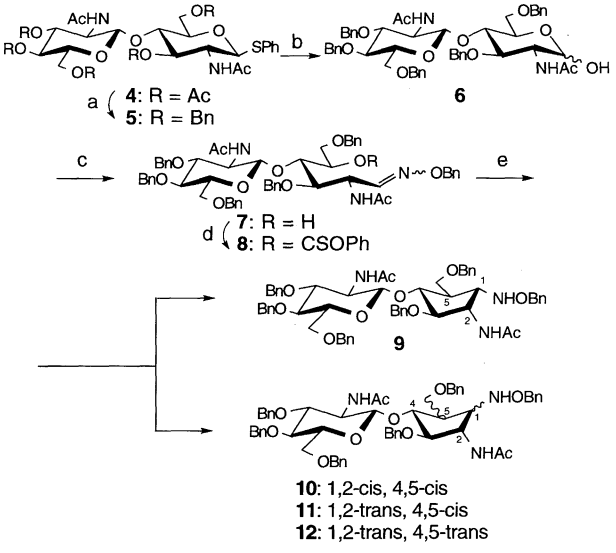
(3aS,4R,5R,6R,6aS)-2-Dimethylamino-3a,5,6,6a-tetrahydro-4-hydroxy-6-hydroxymethyl-4*H*-cyclopentimidazole-5-yl 2-Acetamido-2-deoxy-β-D-glucopyranoside hydrochloride was designed as a potential inhibitor against endoglycosidases like lysozyme and chitinase, and was synthesized from *N,N'*-diacetylchitobiose by a series of reactions including radical cyclization of oxime ethers and cyclic guanidine-formation.

In the preceding paper,[1] we reported total synthesis of a natural chitinase inhibitor, allosamidin **1**,[2] and have continued to develop new chitinase inhibitors through a study of the relationship between structure and inhibitory activity thereof.[3] Nishimoto et al.[4] found that a pseudodisaccharide **2** obtained by acidic hydrolysis of a congener of **1** was a potent inhibitor against the chitinase from a pathogenic yeast, *Candida albicans*. This finding prompted us to design a novel pseudodisaccharide **3** containing *N,N*-dimethylguanidine as an inhibitor against an endoglycosidase like the chitinase. The guanidine moiety[5] was expected to show a stronger affinity for a carboxyl group in an active site in the enzyme than the core structure of **2**. We have already disclosed a novel methodology employing oligosaccharides as a key starting material to construct a pseudooligosaccharide framework.[6] Described herein is the synthesis of **3** by this method including a first radical cyclization reaction of disaccharide-derived oxime ethers **8**.



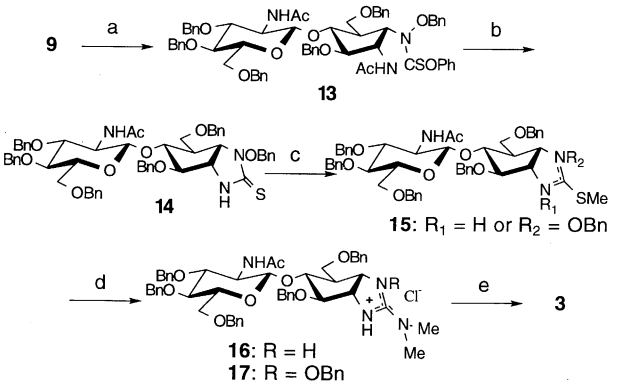
Chitobiose heptaacetate **4**[1] was hydrolyzed, and then benzylated with benzyl bromide, barium oxide and barium hydroxide in *N,N*-dimethylformamide (DMF) to provide benzyl ether **5**, mp 217 °C (dec.), $[\alpha]_D^{25}$ -32° (*c* 0.92),[7] in 69% yield (Scheme 1). Hydrolysis of **5** with *N*-bromosuccinimide in aqueous tetrahydrofuran (THF) gave hemiacetal **6** in 70% yield. This was condensed with *O*-benzyl hydroxylamine hydrochloride in pyridine-CH_2Cl_2 at room temperature (r.t.) to afford oxime ethers **7**, as an unseparable mixture of stereoisomers (85% yield; anti/syn = 5/1 by NMR analyses). Upon treatment with chloro phenylthionoformate in pyridine-CH_2Cl_2, **7** provided thiocarbonates **8** in 71% yield. The radical cyclization[8] of **8** was carried out with 4.0 equiv of tributyltin hydride (Bu_3SnH) and a catalytic amount of azobis(isobutyronitrile) (AIBN) as an initiator in toluene at 100~110 °C, giving desired hydroxylamine **9**, $[\alpha]_D^{23}$ - 6.2° (*c* 0.41), as a major product (31%), along with other three isomers {**10**, $[\alpha]_D^{24}$ - 1.0° (*c* 0.75), (~2%), **11**, $[\alpha]_D^{24}$ - 2.9° (*c* 0.42), (23%) and **12**, $[\alpha]_D^{24}$ - 9.6° (*c* 0.53), (15%) }. The stereochemistry of each isomer, separated by silica gel chromatography, was established by the NMR analyses together with the difference NOE experiments and by chemical derivations such as an acetonide formation. For example, in **9**, a strong NOE was observed for the signals of N*H*Ac, N*H*OBn, and H_6 upon irradiation of H_5.[9] Likewise, irradiation of H_1 caused enhancement of signal due to H_2. These data are consistent with the assigned 1,2-*cis*:4,5-*trans* structure for **9**. The isomer ratio of the products derived from **8** was compared with the result obtained with a monosaccharide.[8b] There was no significant difference in the stereoselectivity at C-1 (1,2-*cis* vs. 1,2-*trans*). In the case of 1,2-*trans* isomers, however, an increase of 4,5-*cis* selectivity on the cyclization of **8** was observed; 4,5-*cis* / 4,5-*trans* = 61/39 (lit.[8b] 31/69). Simple procedure and readily access from **4** made a moderate yield[10] of **9** no problem for further transformation.



Reagents and conditions: a) NaOMe, MeOH, rt, quant., then BnBr, $Ba(OH)_2 \cdot 7H_2O$, BaO, DMF, rt, 69%; b) NBS, aq. THF, rt, 70%; c) $BnONH_2 \cdot HCl$, pyridine-CH_2Cl_2, rt, 81%; d) PhOCSCl, pyridine-CH_2Cl_2, rt, 71%; e) Bu_3SnH, AIBN, toluene, 100~110 °C, 31% for **9**, ~2% for **10**, 23% for **11**, and 15% for **12**.

Scheme 1.

Next phase in this synthesis was to construct a cyclic guanidine moiety on the cyclopentane ring of **9**. Attempts to remove selectively acetyl and benzyloxy groups in the nitrogen functions of this ring were unsuccessful. Therefore, we adopted a stepwise procedure as follows (Scheme 2). Thus, **9** reacted



Reagents and conditions: a) PhOCSCl, pyridine-CH_2Cl_2, rt, 74%; b) NaH, DMF, 0 °C, 81%; c) CH_3I, reflux, quant; d) $Me_2NH_2^+OAc^-$, 120 °C, then aq. HCl, 69% for **16** and 12% for **17**; e) H_2, 10% Pd/C, EtOH-AcOH-H_2O (1:1:1), 81% from **16**, 75% from **17**.

Scheme 2.

with chloro phenylthionocarbonate in pyridine-CH_2Cl_2, to provide thiocarbonate **13**, $[\alpha]_D^{23}$ +15° (*c* 0.87), in 74% yield. When **13** was treated with sodium hydride in DMF, a cyclization concomitant with de-*N*-acetylation took place to give thiourea **14**, $[\alpha]_D^{23}$ -10° (*c* 0.62), in 81% yield. A solution of **14** in methyl iodide was heated under reflux to afford a mixture of iminothioethers, **15**, in high yield. This reacted smoothly with dimethylammonium acetate at 120 °C under argon atmosphere,[11] followed by treatment with hydrochloric acid, giving guanidine hydrochloride **16**, $[\alpha]_D^{24}$ -15° (*c* 0.53, CH_2Cl_2), in 69 % yield along with its *N*-benzyloxy derivative **17** (12%). Finally, all benzyl groups in **16** were removed by hydrogenation in the presence of 10% palladium on carbon under hydrogen atmosphere in acetic acid-ethanol-water to give **3**, $[\alpha]_D^{24}$ +13° (*c* 0.20, H_2O), in high yield. Similarly, **17** was also converted into **3**. The bioassay of **3** for lysozyme and chitinase is under investigation.

In conclusion, the facile synthesis of a novel pseudodisaccharide **3** without glycosidation reaction was achieved employing chitobiose as a key starting material.

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7 Values of $[\alpha]_D$ and δ_H (400MHz) or δ_C (100MHz) were measured for a solution in $CHCl_3$ and $CDCl_3$, respectively, at 23±2 °C, unless otherwise noted. The carbon-numbering system is conveniently according to one shown in Scheme 1. **9**: δ_H: 2.04 (1H, m, H-5), 3.35 (1H, dd, J = 7.6 and 7.0 Hz, H-1), 3.95 (2H, brs, H-3, 4); δ_C: 48.0 (C-5), 53.9 (C-2), 62.7 (C-1), 71.9 (C-6), 85.2 (C-4), 86.3 (C-3); Anal. Found: C, 71.44; H, 6.74; N, 4.30%. Calcd for $C_{58}H_{65}O_{10}N_3 \cdot 0.5H_2O$: C, 71.58; H, 6.84; N, 4.32%. **10**: δ_H: 2.52 (1H, m, H-5), 3.63 (1H, q, $J_{1,NH}$ = 10 and $J_{1,5}$ = $J_{1,2}$ = 7.0 Hz, H-1), 3.76 (1H, dd, $J_{2,3}$ = 3.6 and $J_{3,4}$ = 1.3 Hz, H-3), 4.10 (1H, brd, $J_{4,5}$ = 4.9 Hz, H-4), 4.45 (1H, m, $J_{2,NH}$ = 9.2 Hz, H-2); δ_C: 44.7 (C-5), 56.1 (C-2), 62.0 (C-1), 65.3 (C-6), 82.1 (C-4), 88.9 (C-3). **11**: δ_H: 2.54 (1H, m, H-5), 2.82 (1H, dd, $J_{1,5}$ = 9.2 and $J_{1,2}$ = 3.1 Hz, H-1), 3.73 (1H, brs, H-3), 4.20 (1H, brs, H-4); δ_C: 45.9 (C-5), 56.3 (C-2), 67.5 (C-6), 70.0 (C-1), 81.0 (C-4). 86.3 (C-3). **12**: δ_H: 2.38 (1H, m, H-5), 3.42 (1H, dd, $J_{1,2}$ = 8.4 and $J_{1,5}$ = 6.3 Hz, H-1), 3.78 (1H, dd, $J_{2,3}$ = 4.3 and $J_{3,4}$ = 4.0 Hz, H-3), 4.18 (1H, dd, $J_{4,5}$ = 3.7 Hz, H-4), 4.28 (1H, brq, H-2); δ_C: 46.3 (C-5), 55.9 (C-2), 66.3 (C-1), 68.9 (C-6), 85.7 (C-4), 86.5 (C-3). **13**: Anal. Found: C, 70.95; H, 6.55; N, 3.77; S, 2.72%. Calcd for $C_{65}H_{69}O_{11}N_3S$: C, 70.95; H, 6.32; N, 3.82; S, 2.91%. **14**: Anal. Found: C, 71.23; H, 6.46; N, 4.26; S, 3.06%. Calcd for $C_{57}H_{61}O_9N_3S$: C, 71.01; H, 6.38; N, 4.36; S, 3.33%. **16**: δ_H: 2.80 (1H, m, H-5), 2.97 (6H, brs, NMe_2), 4.11 (1H, brs, H-3), 4.23 (2H, brs, H-1, 2), 4.28 (1H, brt, H-4), 4.65 (1H, d, $J_{1',2'}$ = 7.8 Hz, H-1'); δ_C: 39.2 (NMe), 158.6 (C=N). **17**: δ_H: 2.98 (6H, brs, NMe_2), 4.32 (1H, dd, $J_{1,2}$ = 7.9 and $J_{2,3}$ = 3.1 Hz, H-2). **3**: δ_H (D_2O, DHO=4.80): 2.07 (3H, s, NAc), 2.24 (1H, m, H-5), 3.02 (6H, brs, NMe_2), 3.44 (1H, dd, $J_{4',5'}$ = 9.8 and $J_{3',4'}$ = 8.8 Hz, H-4'), 3.52 (1H, ddd, $J_{6'a,5'}$ = 6.6 and $J_{6'b,5'}$ = 2.2 Hz, H-5'), 3.58 (1H, dd, $J_{2',3'}$ = 10 Hz, H-3'), 3.64 (1H, dd, $J_{6a,6b}$ = 11 and $J_{6a,5}$ = 6.3 Hz, H-6a), 3.73 (1H, dd, $J_{1',2'}$ = 8.5 Hz, H-2'), 3.74 (1H, dd, $J_{6a',6'b}$ = 12 Hz, H-6'a), 3.79 (1H, dd, $J_{6b,5}$ = 4.4 Hz, H-6b), 3.82 (1H, dd, $J_{4,5}$ = 8.7 and $J_{3,4}$ = 4.8 Hz, H-4), 3.98 (1H, dd, H-6'b), 4.18 (1H, dd, $J_{1,2}$ = 9.6 and $J_{2,3}$ = 4.8 Hz, H-2), 4.20 (1H, t, H-3), 4.31 (1H, dd, $J_{1,5}$ = 6.0 Hz, H-1), 4.55 (1H, d, H-1'); δ_C(D_2O, dioxane=67.4): 22.0 (AcN), 37.8 (NMe), 52.1 (C-5), 55.5 (C-2'), 57.5 (C-1), 59.5 (C-6), 60.7 (C-6'), 63.2 (C-2), 70.0 (C-4'), 73.4 (C-3'), 75.7 (C-5'), 81.5 (C-3), 84.8 (C-4), 101.7 (C-1'), 158.2 (C=N), 174.4 (C=O); HR-FABMS m/z: 419.2138, Calcd for $C_{17}H_{31}O_8N_4$: 419.2142 (M+H).

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9 The data of NOE in other compounds are as follows; **10**: $H_1 \rightarrow H_2$, H_5; $H_5 \rightarrow H_1$, H_4; **11**: $H_1 \rightarrow NHAc$, H_6, $H_{6'}$; $H_5 \rightarrow H_4$; **12**: $H_1 \rightarrow NHAc$, H_5; $H_6 \rightarrow H_2$, H_4, H_5 (Irradiation of the proton on the left of the allow caused NOE of the one on the right.).

10 The use of a combination of Bu_3SnH and triethyl borane[12] at r.t. resulted in a low stereoselectivity as follows; THF, **9/10/11/12** = 38/8/24/30 (total 66% yield), toluene-THF (6:1), **9/10/11/12** = 35/21/23/21 (total 69% yield).

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