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Chemo-enzymatic synthesis of tetra-*N*-acetyl-chitotetraosyl allosamizoline

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Abstract—A new compound **7**, possessing a tetra-*N*-acetyl-chitotetraosyl moiety as a constituent, was synthesized by bacterial fermentation which used allosamizoline **6** as the initial acceptor.

Chitooligosaccharides (COS) have been attracting a keen interest in their utilization because they have been reported to possess physiological activities such as antitumor activity[1] and elicitor activity for plants.[2] It is considered that the greatest physiological activities are shown by COS with a degree of polymerization (dp) greater than the chitopentaose.[1] However, chitinases and other enzymes rapidly cleave COS. Therefore, improving the stability of the active COS is the key to developing the biomedicines of COS.

Allosamidin, a *Streptomyces* metabolite, is an inhibitor of family 18 chitinases and has a pseudotrisaccharide structure consisting of two units of *N*-acetyl-D-allosamine and one unit of an aminocyclitol derivative, allosamizoline **6**.[3] And the allosamidin was synthesized mainly by chemical methods.[4]

The use of whole cells for the biotransformation of organic substrates is a useful technique which has a number of benefits over conventional, reagent-based methods. With the aim of improving the stability of active COS, herein we attempt to synthesize a new compound **7** (Scheme 1) by bacterial fermentation. It is an analogue of tetra-*N*-acetyl-chitotetraose with one unit of an allosamizoline **6** at the reducing end as the aglycone.

The cyclopentadienylthallium **1** was alkylated and then treated with thiourea and cationic methylene blue to give the *meso*-diol **2**. The optically active monoacetate **3** was derived from the *meso*-diol **2** by acetylation and the action of the acetylcholinesterase from the electric eel. The yield and the ee value of compound **3** were 89% and 95%, respectively. The carbamate **4** was formed from the alcohol **3** by the action of benzyl chloroformate followed by treatment with methanolic ammonia. The diol **5** was obtained from the intermediate **4** by O-trifluoroacetylation, cyclization, O-methylation, exposure to dimethylamine, hydroxylation with trifluoroperacetic acid, and solvolysis with aqueous trifluoroacetic acid, respectively. Catalytic hydrogenation of the diol **5** gave allosamizoline **6**.

Bacterial fermentation was carried out as previously described[5] in 10 L bioreactors containing an initial culture volume of 7 L. Compound **6** (1 g/L) was added to culture system. The *Escherichia coli* strain BL21(DE3) containing a plasmid carrying the cloned *nodC* gene from *Mesorhizobium loti* strain E1R[6] was used as the source of NodC protein. The culture time lasted 48 h.

After centrifugation of the culture broth, chitooligosaccharide was recovered exclusively in the pellet containing the bacterial cells. After disruption of the cells by boiling, cell debris was removed by centrifugation and the chitooligosaccharide was purified by activated charcoal adsorption and aq ethanol (55%, v/v) elution. The crude product was further purified by size-exclusion chromatography on Biogel P4. The ^{1}H NMR, ^{13}C NMR, and MS data confirmed the identification of the obtained sample as the new compound **7**.[7] The yield of **6** to **7** was 43%. Therefore, it indicates that **6** is clearly

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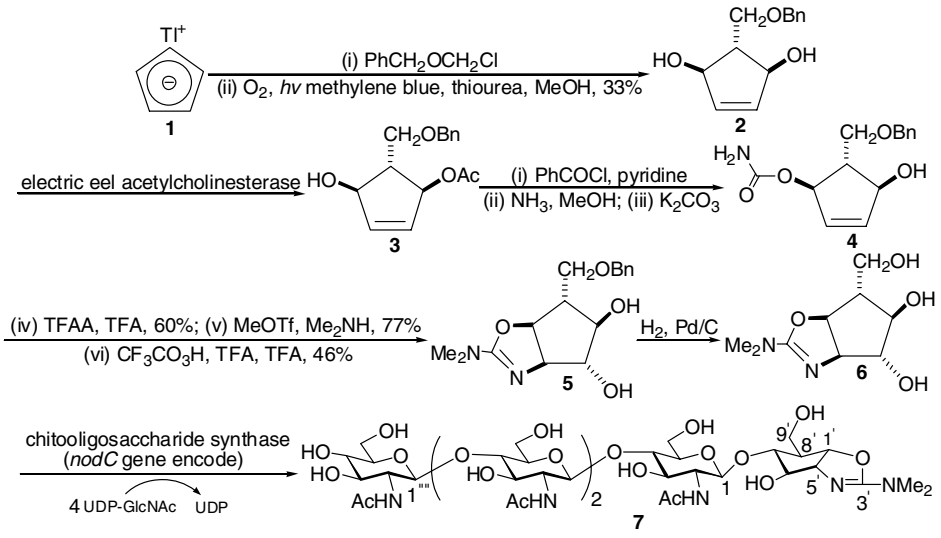

Scheme 1. Chemo-enzymatic synthesis of tetra-*N*-acetyl-chitotetraosyl allosamizoline.

used as substrate by NodC in vivo. The most obvious explanation for this phenomenon is that **6** does not influence the binding affinity of NodC for the oligosaccharide intermediate and therefore leads to an elongation with additional GlcNAc units. In our system, the exclusive formation of pentamer is probably due to the fact that the synthesis was carried out in growing *E. coli* cells in which the physiological pool of UDP-GlcNAc is maintained at a high level.

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7. Analytical data for **7**: $[\alpha]_D$ −14.2 (*c* 0.42, 0.1 M AcOH). ^{1}H NMR (300 MHz, D_2O): δ 5.30 (dd, 1H, H-1′), 4.85 (m, 4H, H-1, H-1″, H-1‴, H-1⁗), 4.53 (dd, 1H, H-5′), 4.50 (dd, 1H, H-6′), 4.45–4.14 (m, 4H, H-3, H-3″, H-3‴, H-3⁗), 4.05–3.70 (m, 23H), 3.17, 3.14 (s, 6H, NMe_2), 2.40 (m, 1H, H-8′), 2.14, 2.10 (each s, 12H, CH_3 NHAc). ^{13}C NMR (75 MHz, D_2O): δ 22.8, 23.5 (4× CH_3NHAc), 38.3 (NMe_2), 54.6 (C-8′), 55.4, 55.6 (C-2, C-2″,C-2‴, C-2⁗), 60.9 (C-1′), 62.3 (C-9′), 63.9 (C-6, C-6″, C-6‴, C-6⁗), 68.5, 69.1 (C-4″, C-4‴, C-4⁗), 71.8, 72.8 (C-3, C-3″), 75.3, 76.3 (C-5, C-5″), 79.7 (C-4), 82.3 (C-6′), 86.8 (C-7′), 92.7 (C-5′), 102.8, 103.7 (C-1, C-1″, C-1‴, C-1⁗), 162.9 (C-3′), 176.7 (C=O NHAc). ESIMS: *m*/*z* 1051.0 $[M+Na]^+$. Anal. Calcd for $C_{41}H_{68}N_6O_{24}$: C, 47.86; H, 6.66; N, 8.17. Found: C, 48.00; H, 6.39; N, 8.01.