Supplementary Information

Non Catalytic Synthesis of Chromogen I and III from N-acetyl-D-glucosamine in High-temperature Water

Mitumasa OSADA a*, Kazushi KIKUTA a, Kohei YOSHIDA a, Kazuhide TOTANI a,
Makoto OGATA b, and Taichi USUI c

a Department of Chemical Engineering, Ichinoseki National College of Technology,
Ichinoseki, Iwate, 021-8511, Japan

b Department of Chemistry and Biochemistry, Fukushima National College of Technology,
Nagao 30, Kamiaraka wa, Taira, Iwaki, Fukushima, 970-8034, Japan

c Department of Bioscience, Graduate School of Science and Technology, Shizuoka
University, Ohya 836, Suruga ward, Shizuoka 422-8529, Japan

Corresponding author. Tel.: +81-191-24-4767; fax: +81-191-24-2146

E-mail osada@ichinoseki.ac.jp
List of Supplementary Information:

S1. HPLC chromatograph examples

S2. Characterization of Chromogen I, 3,6-anhydro-GNF, 1, 3,6-anhydro-MNF, 2, and Chromogen III
S1. HPLC chromatograph examples

(a) 180°C, 25 MPa, reaction time 17 sec
(b) 180°C, 25 MPa, reaction time 33 sec
When Chromogen III was high concentration as the product, the peaks of Chromogen III and 3,6-anhydro-GMF, 1 overlapped. To analyze these compounds separately, we conducted the following HPLC method too. However, GlcNAc and ManNAc were not separated and Chromogen I showed two peaks in this method.

Column: Unison US-C18 (4.6 × 250mm, Imtakt)
Mobile phase: water
Flow rate: 1.0 mL min⁻¹
Column oven temperature: 40°C

(c) 180°C, 25 MPa, reaction time 19 sec
S2. Characterization of Chromogen I, 3,6-anhydro-GNF, 1, 3,6-anhydro-MNF, 2, and Chromogen III

Chromogen I; HRESIMS: m/z 226.06893 [M+Na]$^+$ (calcd for C$_8$H$_{13}$N$_1$Na$_1$O$_5$, 226.06914).

$^1$H NMR (D$_2$O, 500 MHz) $\alpha$-anomer: $\delta$ 6.16 (1H, H-3), 6.04 (1H, H-1), 5.06 (1H, H-4), 3.83–3.57 (3H, H-5, H-6b, H-6a), 2.13 (s, 3H, CH$_3$CONH–); $\beta$-anomer: $\delta$ 6.21 (1H, H-3), 5.99 (1H, H-1), 4.83 (1H, H-4), 3.83–3.57 (3H, H-5, H-6b, H-6a), 2.13 (s, 3H, CH$_3$CONH–);

$^{13}$C NMR (D$_2$O, 125 MHz) $\alpha$-anomer: $\delta$ 176.1 (CH$_3$CONH–), 137.0 (C-2), 112.0 (C-3), 102.2 (C-1), 87.5 (C-4), 76.2 (C-5), 65.2 (C-6), 25.4 (CH$_3$CONH–); $\beta$-anomer: $\delta$ 176.1 (CH$_3$CONH–), 136.5 (C-2), 112.7 (C-3), 102.0 (C-1), 87.2 (C-4), 76.5 (C-5), 65.1 (C-6), 25.4 (CH$_3$CONH–).

3,6-anhydro-GNF, 1; HRESIMS: m/z 226.07013 [M+Na]$^+$ (calcd for C$_8$H$_{13}$N$_1$Na$_1$O$_5$, 226.06914). $^1$H NMR (D$_2$O, 500 MHz) $\alpha$-anomer: $\delta$ 5.62 (d, 1H, J$_{1,2}$ = 5.0 Hz, H-1), 4.76 (t, 1H, J$_{3,4}$ = 5.0 Hz, H-5), 4.66 (t, 1H, J$_{2,3}$ = 5.0 Hz, J$_{3,4}$ = 5.0 Hz, H-3), 4.33–4.29 (1H, H-4), 4.27 (t, 1H, J$_{1,2}$ = 5.0 Hz, J$_{2,3}$ = 5.0 Hz, H-2), 4.00 (dd, 1H, J$_{5,6b}$ = 6.5, J$_{6a,6b}$ = 8.5 Hz, H-6b), 3.66 (t, 1H, J$_{5,6a}$ = 8.5, J$_{6a,6b}$ = 8.5 Hz, H-6a), 2.06 (s, 3H, CH$_3$CONH–); $\beta$-anomer: $\delta$ 5.44 (1H, H-1), 4.79 (t, 1H, J$_{3,4}$ = 5.0 Hz, J$_{4,5}$ = 5.0 Hz, H-4), 4.52 (d, 1H, J$_{3,4}$ = 5.0 Hz, H-3), 4.33–4.29 (1H, H-5), 4.18 (1H, H-2), 3.96 (t, 1H, J$_{5,6b}$ = 8.0, J$_{6a,6b}$ = 8.0 Hz, H-6b), 3.88 (t, 1H, J$_{5,6a}$ = 8.0, J$_{6a,6b}$ = 8.0 Hz, H-6a), 2.02 (s, 3H, CH$_3$CONH–); $^{13}$C NMR (D$_2$O, 125 MHz) $\alpha$-anomer: $\delta$ 177.1 (CH$_3$CONH–), 100.4 (C-1), 88.5 (C-3), 81.7 (C-4), 73.0 (C-5, C-6), 61.5
(C-2), 24.56 (CH$_3$CONH–); β-anomer: δ 176.8 (CH$_3$CONH–), 105.2 (C-1), 88.6 (C-3), 85.6 (C-4), 73.8 (C-6), 73.5 (C-5), 64.7 (C-2), 24.60 (CH$_3$CONH–).

3,6-anhydro-MNF, 2; HRESIMS: m/z 226.06938 [M+Na]$^+$ (calcd for C$_8$H$_{13}$N$_1$Na$_1$O$_5$, 226.06914). $^1$H NMR (D$_2$O, 500 MHz) α-anomer: δ 5.53 (d, 1H, J$_{1,2}$ = 5.5 Hz, H-1), 4.70 (t, 1H, J$_{3,4}$ = 5.5, J$_{4,5}$ = 5.5 Hz, H-4), 4.65–4.62 (1H, H-3), 4.42–4.38 (1H, H-5), 4.35 (t, 1H, J$_{1,2}$ = 5.5, J$_{2,3}$ = 5.5 Hz, H-2), 3.96–3.89 (2H, H-6b, H-6a), 2.07 (s, 3H, CH$_3$CONH–); β-anomer: δ 5.31 (d, 1H, J$_{1,2}$ = 6.0 Hz, H-1), 4.81 (t, 1H, J$_{3,4}$ = 4.6, J$_{4,5}$ = 4.6 Hz, H-4), 4.65–4.62 (1H, H-3), 4.42–4.38 (1H, H-5), 4.25 (t, 1H, J$_{1,2}$ = 6.0, J$_{2,3}$ = 6.0 Hz, H-2), 4.02 (dd, 1H, J$_{5,6b}$ = 6.7, J$_{6a,6b}$ = 8.4 Hz, H-6b), 3.55 (t, 1H, J$_{5,6a}$ = 8.4, J$_{6a,6b}$ = 8.4 Hz, H-6a), 2.05 (s, 3H, CH$_3$CONH–); $^{13}$C NMR (D$_2$O, 125 MHz) α-anomer: δ 177.0 (CH$_3$CONH–), 98.3 (C-1), 84.7 (C-4), 83.0 (C-3), 73.95 (C-5), 73.5 (C-6), 57.5 (C-2), 24.4 (CH$_3$CONH–); β-anomer: δ 177.2 (CH$_3$CONH–), 103.7 (C-1), 83.5 (C-4), 82.7 (C-3), 74.3 (C-5), 73.90 (C-6), 61.8 (C-2), 24.5 (CH$_3$CONH–).

Chromogen III; HRESIMS: m/z 393.12810 [2M+Na]$^+$ (calcd for C$_{16}$H$_{22}$N$_2$Na$_1$O$_8$, 393.12739). $^1$H NMR (D$_2$O, 270 MHz) δ 7.68 (1H, H-1), 6.25 (1H, H-3), 4.59 (1H, H-5), 3.69–3.67 (2H, H-6b, H-6a), 2.00 (s, 3H, CH$_3$CONH–).