Electronic Supplementary Material

Analytical and Bioanalytical Chemistry

Identification Of New O-GlcNAc Modified Proteins Using A Click Chemistry Based Tagging

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Synthesis

All chemical reagents were purchased from Sigma and used as received.

\textit{a) GlcNAz 1 and peracetylated GlcNAz 2}


\textit{b) GlcNAk 3 and peracetylated GlcNAk 4}

\begin{itemize}
  \item[i)] p-anisaldehyde; NaOH; 0-10°C; 3h; 60% - Anhydride acetic; pyridine; r.t., 1 night; 90%
  \item[ii)] NaHCO₃; H₂O; r.t.; 20 min; 94%
  \item[iii)] 4-pentynoic acid; DCC; DMAP; anh. CH₂Cl₂; r.t.; 1 day, 72%
  \item[iv)] Aq. NH₃; MeOH; -15°C 24h; 58%
\end{itemize}


Coupling between peracetylated GlcNH₂ and pentynoic acid (4): the acid compound (98 mg, 1 mmol) and peracetylated GlcNH₂ (350 mg, 1 mmol) were dissolved in anhydrous dichloromethane (CH₂Cl₂) (10 mL) under inert atmosphere; the solution was cooled in an ice-water bath. Dicyclohexyl carbodiimide (DCC) (249 mg, 1.2 mmol) and 4-dimethylaminopyridine (DMAP) (12 mg, 0.1 mmol) were dissolved in a minimum of anhydrous CH₂Cl₂ and added slowly to the acid-amine mixture at 0°C. The solution was allowed to warm to r.t. and stirred 20h. The suspension was filtered twice and evaporated. The crude product was purified by flash chromatography (silica gel column, CH₂Cl₂/MeOH 95:5) to obtain peracetylated GlcNac Alkyne (4) as a white powder (310 mg, 73% yield). ¹H NMR (300 MHz, DMSO d-6, 25°C, TMS): δ=8.05 ppm (d, J(H,H)=9.2 Hz, 1H; NH), 5.7 ppm (d,
\(J(H,H)=8.8\) Hz, \(1H;\) Glc), \(5.17\) ppm (t, \(J(H,H)=10.3\) Hz, \(1H;\) Glc), \(4.88\) ppm (t, \(J(H,H)=9.8\) Hz, \(1H;\) Glc), \(4.18\) ppm (dd, \(J(H,H)=8.1\) Hz, \(J(H,H)=15\) Hz, \(1H;\) Glc), \(3.98\) ppm (m, \(3H;\) Glc), \(2.74\) ppm (s, \(1H;\) Alkyne), \(2.32\) ppm (t, \(J(H,H)=6.4\) Hz, \(2H;\) CH2), \(2.20\) ppm (t, \(J(H,H)=6.4\) Hz, \(2H;\) CH2), \(2.04\) ppm (s, \(3H;\) Ac), \(2.01\) ppm (s, \(3H;\) Ac), \(1.97\) ppm (s, \(3H;\) Ac), \(1.92\) ppm (s, \(3H;\) Ac).

Desacetylation (3): peracetylated GlcNAc Alkyne (4) (150 mg, 0.35 mmol) was dissolved in methanol (MeOH) (2mL) at r.t. and cooled at -15°C. Aqueous ammonia (13.5 M, 450 µL, 5.26 mmol) was added drop wise under stirring. The solution was kept at low temperature during 24h. The solvent was then evaporated and the crude material was chromatographed (silica gel column, CH2Cl2/MeOH 95:5) to give the modified sugar (3) as a white powder (53 mg, 58% yield). \(^1\)H NMR (300 MHz, DMSO d-6, 25°C, TMS): \(\delta=7.73\) ppm (d, \(J(H,H)=7.8\) Hz, \(1H;\) NH), \(6.46\) ppm (d, \(J(H,H)=3.9\) Hz, \(1H;\) Glc), \(4.96\) ppm (q, \(J(H,H)=5.1\) Hz, \(2H;\) Glc), \(4.64\) ppm (d, \(J(H,H)=5.4\) Hz, \(1H;\) Glc), \(4.46\) ppm (m, \(1H;\) Glc), \(3.65\) ppm (m, \(2H;\) OH), \(3.52\) ppm (m, \(2H;\) OH), \(3.17\) ppm (m, \(2H;\) Glc), \(2.79\) ppm (s, \(1H;\) Alkyne), \(2.43\) ppm (m, \(4H;\) CH2); MS (Electrospray positive): \(m/z: 260\ [M+H]^+, 282\ [M+Na]^+, 541\ [2M+Na]^+.

\[\text{c) Biotin azido probe 5}\]

\[
\begin{align*}
\text{H} & \quad \text{O} \quad \text{O} \quad \text{N} \quad \text{H} \\
\text{H} & \quad \text{N} \quad \text{O} \quad \text{N} \quad \text{N} \\
\text{H} & \quad \text{N} \quad \text{O} \quad \text{N} \\
\end{align*}
\]

i) \(\text{Boc}_2\text{O}; \text{CHCl}_3; \text{r.t.}; 24\text{h}; 16\%\)
ii) \(\text{TFN}_3; \text{CuSO}_4; \text{TEA}; \text{H}_2\text{O}/\text{MeOH} 1:5; \text{r.t.}; 1\text{ day}; \text{quantitative}\)
iii) \(\text{TFA}; \text{anh. CH}_2\text{Cl}_2; \text{r.t.}; 2\text{h}; \text{quantitative}\)
iv) \(\text{d-biotin}; \text{DCC}; \text{DMAP}; \text{anh. DMF}; \text{r.t.}; 48\text{h}; 70\%\)

Protected diamine synthesis (11): Diamine (4.08 g, 20 mmoles) was dissolved in chloroform (CHCl3) (100 mL). At r.t. under inert atmosphere, triethylamine (TEA) (2.8 ml, 20 mmol) was added and the solution was cooled to 0°C using an ice-water bath. \(\text{t-Butyl dicarbonate} (\text{Boc}_2\text{O}) (4.36g, 20\text{mmoles})\) was dissolved in CHCl3 (50 ml) and then added drop wise to the diamine. The suspension was allowed to warm to r.t., stirred 48h, filtered twice, and concentrated. The residue was dissolved in ethyl acetate (EtOAc) and washed three times
with water. The aqueous layer was extracted with EtOAc. The combined organic layers were dried, filtered, and evaporated to give a colourless oil. The crude product was purified by flash chromatography (silica gel column, CH₂Cl₂/MeOH 9:1 then 8:2) to give a viscous oil (1.29 g, 21% yield). ¹H NMR (300 MHz, CDCl₃, 25°C, TMS): δ=4.95 ppm (s, 1H; NH), 3.49-3.37 ppm (m, 8H; CH₂-O), 3.21 ppm (t, J(H,H)=6.2 Hz, 2H; CH₂-N), 2.78 ppm (t, J(H,H)=6.8 Hz, 2H; CH₂-N), 1.77-1.56 ppm (m, 8H; CH₂), 1.43 ppm (s, 9H; Boc).

Amine to azide (12): see the general procedure for the diazotransfer reaction: Nyffeler PT, Liang CH, Koeller KM and Wong CH (2002) J Am Chem Soc, 124:10773-10778. ¹H NMR (300 MHz, DMSO d-6, 25°C, TMS): δ=8.90 ppm (s, 1H; NH), 3.49-3.36 ppm (m, 8H; CH₂-O), 3.00 ppm (q, J(H,H)=6.2 Hz, 2H; CH₂-N), 1.80 ppm (t, J(H,H)=6.8 Hz, 2H; CH₂-N), 1.66-1.55 ppm (m, 8H; CH₂), 1.43 ppm (s, 9H; Boc). MS (Electrospray positive): m/z: 353 [M+Na]⁺, 369 [M+K]⁺

Deprotection (13): the previous product (12) (430mg, 1.3mmoles) was dissolved in anhydrous CH₂Cl₂. The solution was cooled to 0°C and placed under inert atmosphere. Trifluoroacetic acid (TFA) (6ml) was added and the solution was stirred 45 min. The reaction was quenched with ice-water. Sodium bicarbonate was added slowly to pH 7. The aqueous layer was extracted by EtOAc (3*100ml). The combined organic layers were dried, filtered and evaporated to give a colourless oil (430 mg, quantitative yield). ¹H NMR (300 MHz, CD₃OD, 25°C, TMS): δ=3.60-3.47 ppm (m, 8H; CH₂-O), 3.40 ppm (m, 2H; CH₂-N), 3.07 ppm (t, J(H,H)=7.0 Hz, 2H; CH₂-N), 1.98-1.80 ppm (m, 4H; CH₂), 1.67 ppm (m, 4H; CH₂). MS (Electrospray positive): m/z: 231 [M+H]⁺, 261 [2M+H]⁺

Coupling between d-biotin and azido linker (5): under inert atmosphere d-biotin (106 mg, 0.43 mmol) and the linker (13) (100 mg, 0.43 mmol) were dissolved in anhydrous dimethylformamide (DMF) (3 mL). The solution was cooled to 0°C. DCC (97.5 mg, 0.47 mmol) and DMAP (5 mg, 0.04 mmol) were added. The suspension was allowed to warm to r.t., stirred 48h, filtered and concentrated. The residue was triturated with diethyl ether (Et₂O), filtered and dried to obtain a white powder (5) (130 mg, 69%). ¹H NMR (300 MHz, CD₃OD, 25°C, TMS): δ=4.52 ppm (dd, J₁(H,H)=7.8 Hz, J₂(H,H)=5.0 Hz, 1H; CH Biotin), 4.33 ppm (dd, J₁(H,H)=7.8 Hz, J₂(H,H)=4.7 Hz, 1H; CH Biotin), 3.48 ppm (m, 3H; CH₂), 3.41 ppm (t, J(H,H)=6.6 Hz, 1H; CH Biotin), 3.33 ppm (m, 6H; CH₂), 3.28 ppm (m, 3H; CH₂), 2.95 ppm (dd, J₁(H,H)=12.8 Hz, J₂(H,H)=4.9 Hz, 1H; CH₂ Biotin), 2.73 ppm (d, J(H,H)=12.8 Hz, 1H;
CH$_2$ Biotin), 1.85-1.25 ppm (m, 14H; CH$_2$). $^{13}$C NMR (200 MHz, CD$_3$OD, 25°C, TMS): δ=176.3 ppm (1C; C=O), 166.4 ppm (1C; C=O Biotin), 72.1 ppm (2C; O-CH$_2$), 69.8 ppm (1C; O-CH$_2$), 68.8 ppm (1C; O-CH$_2$), 63.7 ppm (1C, CH Biotin), 61.9 ppm (1C, CH Biotin), 57.3 ppm (1C, CH Biotin), 41.3 ppm (1C, CH$_2$ Biotin), 38.1 (1C, N-CH$_2$), 37.1 (1C, N-CH$_2$), 35.0 (1C, CH$_2$), 30.8 (1C, CH$_2$), 30.5 (1C, CH$_2$), 30.0 (1C, CH$_2$), 29.8 (1C, CH$_2$), 27.8 (1C, CH$_2$), 27.2 (1C, CH$_2$), 26.3 (1C, CH$_2$).

d) Biotin alkyne probe 6

Coupling between pentynioc acid and the protected diamine (14): pentynoic acid (257 mg, 2.62 mmol) and protected diamine (11) (0.8 mg, 2.62 mmol) was dissolved in anhydrous CH$_2$Cl$_2$ under inert atmosphere; the solution was cooled at 0°C using a ice-water bath. DCC (648 mg, 3.14 mmol) and DMAP (32 mg, 0.262 mmol) was added and the mixture was allowed to warm to r.t. and stirred 24h. The suspension was filtered twice and then evaporated. The crude product was purified by flash chromatography (silica gel column, Toluene/AcOEt 3:7) to give a white viscous oil (852 mg, 85% yield). $^1$H NMR (300 MHz, DMSO d-6, 25°C, TMS): δ=7.87 ppm (d, J(H,H)=5.1 Hz, 1H; NH), 6.77 ppm (d, J(H,H)=5.5 Hz, 1H; NH), 5.35 (m, 8H; CH$_2$-O), 3.08 ppm (q, J(H,H)=6.6 Hz, 2H; CH$_2$-N), 2.95 ppm (q, J(H,H)=6.4 Hz, 2H; CH$_2$-N), 2.75 ppm (t, J(H,H)=2.5 Hz, 1H; Alkyne), 2.37-2.22 ppm (m, 4H; CH$_2$), 1.59 ppm (q, J(H,H)=6.8 Hz, 4H; CH$_2$), 1.51 ppm (m, 4H; CH$_2$), 1.38 ppm (s, 9H; Boc). MS (Electrospray positive): m/z: 385[M+H]$^+$, 407 [M+Na]$^+$, 423 [M+K]$^+$

Amine deprotection (15): the previous product (14) (420 mg, 1.09 mmoles) was dissolved in anhydrous CH$_2$Cl$_2$ (15 ml). The solution was cooled to 0°C and placed under inert atmosphere. TFA (1.3 ml) was added. The solution was allowed to warm to r.t. and
stirred 2h30. The solvent was evaporated to give a light-browned oil. The product was solubilized once again in CH₂Cl₂ and washed by saturated NaCl. The organic layer was dried, filtered and evaporated to give the attending product as a viscous oil (240 mg, 78% yield). ¹H NMR (300 MHz, DMSO d-6, 25°C, TMS): δ=7.95 ppm (s, 1H; NH), 6.80 ppm (s, 1H; NH), 3.49-3.38 (m, 8H; CH₂-O), 3.14 ppm (q, J(H,H)=6.8 Hz, 2H; CH₂-N), 2.77 ppm (q, J(H,H)=7.0, Hz, 2H; CH₂-N), 2.80 ppm (t, J(H,H)=2.4 Hz, 1H; Alkyne), 2.40 ppm (m, 2H; CH₂), 2.30 ppm (m, 2H; CH₂), 1.82 ppm (qt, J(H,H)=6.8 Hz, 2H; CH₂), 1.66 ppm (qt, J(H,H)=6.6 Hz, 2H; CH₂), 1.57 ppm (t, J(H,H)=2.5 Hz, 4H; CH₂).

Coupling between d-biotin and alkyne linker (6): d-biotin (292 mg, 1.19 mmol) and the previous product (15) (340 mg, 1.19 mmol) were dissolved together in anhydrous DMF (10 mL); the solution was cooled to 0°C. Under inert atmosphere DCC (294 mg, 1.42 mmol) and DMAP (15 mg, 0.12 mmol) were added. The suspension was allowed to warm to r.t., stirred 5 days, filtered twice and concentrated. The crude mixture was purified on column (silica gel column, CH₂Cl₂/MeOH 8:2) to give a white powder (250 mg, 41% yield). ¹H NMR (300 MHz, DMSO d-6, 25°C, TMS): δ=7.86 ppm (t, J(H,H)=5.3 Hz, 1H; NH), 7.76 ppm (t, J(H,H)=5.5 Hz, 1H; NH), 6.44 ppm (s, 1H; NH Biotin), 6.37 ppm (s, 1H; NH Biotin), 4.30 ppm (t, J(H,H)=4.9 Hz, 1H; CH Biotin), 4.13 ppm (t, J(H,H)=7.5 Hz, 1H; CH Biotin), 3.07 ppm (qt, J(H,H)=10.6 Hz, 5H; CH Biotin + CH₂-N), 3.33 (m, 8H; O-CH₂), 2.81 ppm (dd, J₁(H,H)=12.4 Hz, J₂(H,H)=5.1 Hz, 1H; CH₂ Biotin), 2.75 ppm (t, J(H,H)=2.6 Hz, 1H; Alkyne), 2.57 (d, J(H,H)=12.4 Hz, 1H, CH₂ Biotin), 2.33 (m, 2H; CO-CH₂), 2.22 (m, 2H; CO-CH₂), 2.04 ppm (t, J(H,H)=7.3 Hz, 2H; CH₂-Alkyne), 1.73-1.24 ppm (m, 14H; CH₂). MS (Electrospray positive): m/z: 511 [M+H]⁺, 533 [M+Na]⁺.

e) Tris(triazolyl)amine (ligand)