Supporting information

Polyacrylamide backbones for polyvalent bioconjugates using “post-click” chemistry

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Materials

$N,N'$-diisopropylethylamine (DIPEA, 98%), acryloyl chloride (95%), chlorotrimethylsilane (98%), and 2-bromoethanol (95.0%) were purchased from Tokyo Chemical Industry (Tokyo, Japan). 3-Butynyl amine (95%), magnesium sulfate (MgSO$_4$, 95%), 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU, 97%), acrylamide (97%), 2,2'-azobisisobutyronitrile (AIBN, 95%), and acetic acid (99.7%) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Acrylamide and AIBN were purified by recrystallization prior to being used. $N,N'$-Dimethylacetamide (DMAc, 99.0%), potassium hydroxide (86%), copper sulfate (CuSO$_4$, 97.5%) and sodium L-ascorbate (L·Asc·Na, 98%) were purchased from Kanto Chemical (Tokyo, Japan). Dimethyl sulfoxide was purchased from Kishida chemical (Osaka, Japan). Silver chloride (99.999%), tetrabutylammonium fluoride solution (1.0 M in THF), tetrabutylammonium fluoride trihydrate (97.0%), 4-Cyano-4-(phenylcarbonothioylthio)pentanoic acid (CPADB, 97%), and acetonitrile (99.9%) were purchased from Sigma Aldrich (St. Louis, USA). Triethylene glycol monoethyl ether monomethacrylate (TEG MA) was purchased from Polyscience, Inc (Warrington, USA).

Characterization

$^1$H NMR spectra were recorded on a JEOL-EC$\Theta$F400 spectrometer (JEOL, Tokyo, Japan) using CDCl$_3$, MeOD, d$_6$-DMSO or D$_2$O as a solvent. Size exclusion chromatography (SEC) with water solvent was performed on a JASCO DG-980-50 degasser equipped with a JASCO PU-980 pump (JASCO Co., Tokyo, Japan), a Shodex OH pak SB-G guard column, a Shodex OH pak SB-803 HQ column (Showa Denko, Tokyo, Japan) and a JASCO RI-2031 Plus RI detector. SEC analyses were performed by injecting 20 $\mu$L of a
polymer solution (5 g/L) in 10 mM phosphate buffer (pH 7.4). The SEC system was calibrated using a pullulan standard (Shodex). SEC with organic solvent was performed on a HLC-8320 GPC Eco-SEC equipped with a TSKgel Super AW guard column and TSKgel Super AW (4000 and 2500) columns (TOSOH, Tokyo Japan). The SEC analyses were performed by injecting 20 μL of a polymer solution (5 g/L) in DMAc buffer with 10 mM LiBr. The SEC system was calibrated using a polystyren standard (Shodex). Multiangle light scattering (MALS) was performed on a DAWN HELEOS-II spectrometer (SHOKO SCIENTIFIC, Yokohama Japan). The analysis was performed by injecting 20 μL of a polymer solution (5 g/L) in 10 mM phosphate buffer (pH 7.4). All the samples for SEC and MALS were previously been filtered through a 0.45 μm filter. The buffer solution was also used as the eluent at a flow rate of 0.5 mL/min.

Methods

Propargyl acrylamide (M1), CPBTC, 6’-SALac azide, and TBTA were synthesized according to the previous report.1 TMS PrMA was synthesized according to the procedure reported.2
Figure S1–1. $^1$H NMR spectrum of propargyl acrylamide

Figure S1–2. $^1$H NMR spectrum of trimethylsilyl propargylacrylamide (Monomer 1)
Synthesis of 3-butynyl acrylamide (M2)

3-Butynyl amine (5.5 g, 79.6 mmol) and DIPEA (13.9 mL, 79.6 mmol) were dissolved in dry dichloromethane (143 mL) and stirred in ice bath. Acryloyl chloride (7.72 mL, 95.5 mmol) was slowly dropped into the solution and the mixture was stirred for 10 h at room temperature. The progress of the reaction was confirmed by TLC (AcOEt : hexane = 2 : 1). The reactant was washed by saturated brine once. The organic phase was dried by MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by silica column chromatography (AcOEt : hexane = 2 : 1) to give butynyl acrylamide (7.94 g, 64.5 mmol, 81%).

^1^H NMR (CDCl₃, δ in ppm): 6.27 (dd, J = 1.6, 16.8 Hz, trans CH₂=CH, 1H), 6.11 (dd, J = 10.4, 16.8 Hz, CH₂=CH, 1H), 5.64 (dd, J = 1.6, 10.4 Hz, cis CH₂=CH, 1H), 3.48 (ddd, J = 6.4 Hz, -HN-CH₂-CH₂-, 2H), 2.43 (ddd, J = 3.2, 6.4 Hz, -CH₂-C≡C, 2H), 2.00 (t, J = 3.2 Hz, C≡CH, 1H).

![Figure S1–3. ^1^H NMR spectrum of 3-butynyl acrylamide](image)
Synthesis of 4-trimethylsilyl-3-butynyl acrylamide (protected M2)

3-Butynyl acrylamide (2.16 g, 17.5 mmol), DBU (15 mL, 100 mmol) were dissolved in dry dichloromethane (120 mL) with silver chloride (720 mg, 5.0 mmol), and the mixture was stirred for 15 min at room temperature. The colorless solution became white. Chlorotrimethylsilane (26.4 mL, 209 mmol) was added, and the white solution became colorless. The mixture was refluxed for 22 h at 45°C. The reaction progress was determined by TLC (EtOAc : hexane = 1 : 1). Dichloromethane (120 mL) was added and the solution was washed by equal amount of a saturated NaHCO₃ (aq) twice, 1 wt% HCl aq twice, and water once. The organic phase was dried with MgSO₄, filtered and concentrated under reduced pressure. The crude product was purified by silica column chromatography (EtOAc : hexane = 1 : 1) to give 4-trimethylsilyl-3-butynyl acrylamide (2.05 g, 10.5 mmol, 60%).

¹H NMR (CDCl₃, δ in ppm): 6.28 (dd, J = 1.2, 1720 Hz, trans CH₂=CH, 1H), 6.10 (dd, J = 10.4, 17.2 Hz, CH₂=CH, 1H), 5.66 (dd, J = 1.2, 10.4 Hz, cis CH₂=CH, 1H), 3.47 (ddd, J = 6.0 Hz, -HN-CH₂-CH₂-, 2H), 2.47 (t, J = 6.0 Hz, -CH₂-C≡C, 2H), 0.142 (s, Si(CH₃)₃, 9H).

Figure S1–4. ¹H NMR spectrum of 4-trimethylsilyl-3-butynyl acrylamide
Synthesis of polyacrylamide backbones (P2 ~ P7, poly(AAm-r-TMS BtnAAm))

\[
\text{M2}, \text{ acrylamide (AAm), CPBTC, and AIBN were dissolved in 750 \, \mu L of DMSO in a glass tube. The monomer concentration was 1.0 M, and the feed ratio of monomer : RAFT agent : initiator was 100 : 1 : 0.4. The ratio of [M2] : [AAm] was summarized in Table S1. The solution was degassed by three freeze-thaw cycles, and the glass tube was sealed under vacuum and held at 70°C for 18 h. The products were purified by dialysis (Spectra/Por 7; MWCO 1000) against DMSO for 24 h. DMSO was exchanged to mixture of MeOH and acetone (MeOH : acetone = 1 : 1) subsequently and kept for 24 h and dried to give poly(AAm-r-TMS BtnAAm).}
\]

\(^1\text{H NMR ((CD}_3\text{OH, } \delta \text{ in ppm): 2.45 (brs, } -\text{CH}_2-\text{C}≡\text{C}, 2\text{H), 2.26 (brs, } -\text{CH}-), 1.67 (brs, } -\text{CH}_2-), 0.13 (s, Si(CH}_3)_3).}\\n
Table S1. RAFT copolymerization of acrylamide derivatives with acrylamide.

<table>
<thead>
<tr>
<th>No.</th>
<th>Monomer (μmol)</th>
<th>CPBTC (μmol)</th>
<th>AIBN (μmol)</th>
<th>Conv.(^a) (%)</th>
<th>Alkyne units(^a) (%)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>750 (136 mg)</td>
<td>0 (0 mg)</td>
<td>7.5 (1.7 mg)</td>
<td>3 (0.49 mg)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No.</td>
<td>Monomer (μmol)</td>
<td>CPBTC (μmol)</td>
<td>AIBN (μmol)</td>
<td>Conv.(^a) (%)</td>
<td>Alkyne units(^a) (%)</td>
<td>Yield (%)</td>
</tr>
<tr>
<td>-----</td>
<td>----------------</td>
<td>--------------</td>
<td>-------------</td>
<td>------------------</td>
<td>-------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>P2</td>
<td>75 675</td>
<td>7.5</td>
<td>3</td>
<td>92</td>
<td>12</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>(15 mg)</td>
<td>(48 mg)</td>
<td>(1.7 mg)</td>
<td>(0.49 mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>150 525</td>
<td>7.5</td>
<td>3</td>
<td>86</td>
<td>31</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>(44 mg)</td>
<td>(37.5 mg)</td>
<td>(1.7 mg)</td>
<td>(0.49 mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P4</td>
<td>375 375</td>
<td>7.5</td>
<td>3</td>
<td>79</td>
<td>49</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>(73.5 mg)</td>
<td>(27 mg)</td>
<td>(1.7 mg)</td>
<td>(0.49 mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P5</td>
<td>525 150</td>
<td>7.5</td>
<td>3</td>
<td>75</td>
<td>68</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>(102 mg)</td>
<td>(16.5 mg)</td>
<td>(1.7 mg)</td>
<td>(0.49 mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P6</td>
<td>675 75</td>
<td>7.5</td>
<td>3</td>
<td>69</td>
<td>86</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>(132 mg)</td>
<td>(5.4 mg)</td>
<td>(1.7 mg)</td>
<td>(0.49 mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P7</td>
<td>750 0</td>
<td>7.5</td>
<td>3</td>
<td>70</td>
<td>100</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>(147 mg)</td>
<td>(0 mg)</td>
<td>(1.7 mg)</td>
<td>(0.49 mg)</td>
<td></td>
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</tr>
</tbody>
</table>

\(^a\)Monomer conversion and ratio of alkyne units were determined by \(^1\)H NMR.

Figure S1–5. \(^1\)H NMR spectrum of P2
Figure S1–6. $^1$H NMR spectrum of P3

Figure S1–7. $^1$H NMR spectrum of P4
Figure S1–8. $^1$H NMR spectrum of **P5**

Figure S1–9. $^1$H NMR spectrum of **P6**
Deprotection of polymer backbones

P2 (20 mg) and potassium hydroxide (130 mg, 2.3 mmol) was dissolved in water (3 mL), and the mixture was kept stirring for 3.5 h. The products were purified by dialysis (Spectra/Por 7; MWCO 1000) against water for 48 h and freeze-dried to get poly(AAm-r-BtnAAm).

P3 ~ P7 was dissolved in dry THF (5 mL), and 1 M TBAF in THF (1 mL) was added.
The mixture was stirred for 9 h at room temperature. The solvent was eliminated under reduced pressure. The residue was purified by dialysis against mixture of MeOH and acetone (MeOH : acetone = 1 : 1) for 36 h and dried to give poly(AAm-\text{-}r-BtnAAm).

$^1$H NMR ((CD$_3$)$_2$SO, δ in ppm): 3.16 (brs, -HN-CH$_2$-CH$_2$-), 2.81 (brs, -C≡CH, 1H), 2.31 (brs, -CH$_2$-C≡C-, 2H), 2.06 (brs, -CH-), 1.48 (brs, -CH$_2$-).

Table S2. Detailed condition of TMS deprotection.

<table>
<thead>
<tr>
<th>No.</th>
<th>Alkyne groups in the backbones (%)</th>
<th>Polymer (mg)</th>
<th>TMS moieties (μ)</th>
<th>KOH (mmol)</th>
<th>H$_2$O (mL)</th>
<th>Time (h)</th>
<th>Yield (mg)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P2</td>
<td>12</td>
<td>20</td>
<td>24</td>
<td>2.3</td>
<td>3</td>
<td>3.5</td>
<td>17</td>
<td>93</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No.</th>
<th>Alkyne groups in the backbones (%)</th>
<th>Polymer (mg)</th>
<th>TMS (μmol)</th>
<th>TBAF in THF 1M</th>
<th>dry THF (mL)</th>
<th>Time (h)</th>
<th>Yield (mg)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P3</td>
<td>32</td>
<td>60</td>
<td>166</td>
<td>1</td>
<td>5</td>
<td>9</td>
<td>30</td>
<td>63</td>
</tr>
<tr>
<td>P4</td>
<td>49</td>
<td>70</td>
<td>263</td>
<td>1</td>
<td>5</td>
<td>9</td>
<td>51</td>
<td>73</td>
</tr>
<tr>
<td>P5</td>
<td>68</td>
<td>50</td>
<td>250</td>
<td>1</td>
<td>5</td>
<td>9</td>
<td>31</td>
<td>91</td>
</tr>
<tr>
<td>P6</td>
<td>86</td>
<td>50</td>
<td>246</td>
<td>1</td>
<td>5</td>
<td>9</td>
<td>32</td>
<td>100</td>
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<tr>
<td>P7</td>
<td>100</td>
<td>70</td>
<td>358</td>
<td>1</td>
<td>5</td>
<td>9</td>
<td>42</td>
<td>95</td>
</tr>
</tbody>
</table>
Figure S1–11. $^1$H NMR spectrum of deprotected $P_2$

Figure S1–12. $^1$H NMR spectrum of deprotected $P_3$
Figure S1–13. $^1$H NMR spectrum of deprotected \textbf{P4}

Figure S1–14. $^1$H NMR spectrum of deprotected \textbf{P5}
Figure S1–15. $^1$H NMR spectrum of deprotected P6

Figure S1–16. $^1$H NMR spectrum of deprotected P7
Synthesis of 2-azido ethanol

2-Bromo ethanol (22 mL, 308 mmol) and sodium azide (32.5 g, 500 mmol) were dissolved in water (100 mL). The mixture was refluxed for 20 h at 80°C with reflux. The product was extracted by diethyl ether (100 mL, three times). The organic phase was dried with NaSO₄, filtered, and concentrated under reduced pressure to get 2-azidoethanol (18.3 g, 68%).

Precaution: This compound is potentially explosive.

¹H NMR (CDCl₃, δ in ppm): 3.77 (t, J = 5.2 Hz, N₃-CH₂-, 2H), 3.44 (t, J = 5.2 Hz, -CH₂-OH, 2H).

Figure S1-17. ¹H NMR spectrum of 2-azide ethanol
Synthesis of methacrylate backbones (P8, poly(TMS PrMA-\(r\)-TEG MA))

\[
\text{CH}_2=\text{CHCO}_2\text{Si}- + \text{CH}_2\text{CHCO}_2\text{Si}- \xrightarrow{\text{CPADB, AIBN, Toluene, 60°C, 15 h}} \text{CH}_2=\text{CHCO}_2\text{Si}-\text{CH}_2\text{CHCO}_2\text{Si}-\text{TEG MA}
\]

TMS PrMA (196 mg, 1 mmol), TEG MA (246 mg, 1 mmol), CPADB (5.6 mg, 20 μmol), and AIBN (0.66 mg, 4 μmol) were dissolved in toluene, and the mixture was held at 60°C for 15 h. The conversion percentage was determined by \(^1\)H NMR (79%). The product was purified by reprecipitation with heptane twice to get P8 (248 mg, 56%).

Figure S1–18. \(^1\)H NMR spectrum of poly(TMS PrMA-\(r\)-TEG MA)
The TMS protected backbones were deprotected by TBAF. \textbf{P8} (240 mg) was dissolved in dry THF (10 mL) with TBAF trihydrate (257 mg) and acetic acid (49 \( \mu \)L). The mixture was kept stirring at room temperature for 2 h, and passed through packed silica. The eluent was concentrated under reduced pressure to get deprotected \textbf{P8} (155 mg, 77\%).

Figure S1–19. \textsuperscript{1}H NMR spectrum of poly(PrMA-\textit{r}-TEG MA)
Synthesis of glycoconjugates using CuAAC reaction (acrylamide polymer backbones)

Poly(AAm-r- BtnAam) (P2 ~ P7) or poly(PrMA-r-TEG MA) were dissolved with 6’-SAlac azide, CuSO₄, TBTA, and sodium-L-ascorbate into mixture of water and CH₃CN, and N₂ bubbled for 6 h at 60°C (Table S3). The mixture was freeze-dried, and 2-azido ethanol (500 μL), CuSO₄ (2.6 mg), TBTA (8.7 mg), and sodium-L-ascorbate (16 mg) were added with water (1.9 mL) and CH₃CN (600 μL). The mixture was kept stirring for 9 h at room temperature. The products were purified by dialysis (Spectra/Por 7; MWCO 3,500) against water with hydrochloric acid (pH = 4) for 24 h. Water was changed to pure water (pH = 7) subsequently and kept for 12 h, and dried to give poly(AAm-r-6’-SALac).

Poly(AAm-r-6’-SALac)

¹H NMR (D₂O, δ in ppm): 8.1 (triazole), 5.7-5.6 (H-1 of 6’-sialyllac), 4.5-4.3 (N-CH₂, H-1’ of 6’-sialyllac), 4.2-3.4 (sugar-H), 2.7-2.6 (H-3”eq of 6’-sialyllac), 2.3-1.9 (-CH of backbone), 1.9 (Me of NHAc), 1.8-1.3 (-CH₂ of backbone).
Figure S1–20. $^1$H NMR spectrum of GP2

Figure S1–21. $^1$H NMR spectrum of GP3
Figure S1–22. $^1$H NMR spectrum of GP4

Figure S1–23. $^1$H NMR spectrum of GP5
Figure S1–24. $^1$H NMR spectrum of GP6

Figure S1–25. $^1$H NMR spectrum of GP7
PolyEtOH was synthesized by the same procedure without 6'-SAlac aizde. P7 (4 mg), 2-azido ethanol (500 μL), CuSO₄ (2.6 mg), TBTA (8.7 mg), and sodium-L-ascorbate (16 mg) were added with water (1.5 mL) and CH₃CN (1.0 mL). The mixture was kept stirring for 144 h at room temperature. The products were purified by dialysis (Spectra/Por 7; MWCO 3,500) against water with hydrochloric acid (pH = 4) for 24 h. Water was changed to pure water (pH = 7) subsequently and kept for 12 h, and dried to give polyEtOH.

PolyEtOH

¹H NMR (D₂O, δ in ppm): 7.8 (triazole), 4.4 (-CH₂-CH₂-OH), 3.9 (-CH₂-CH₂-OH), 3.4-3.2 (HN-CH₂-), 2.8 (-CH₂-CH₂-triazole), 2.0 (-CH- of backbone), 1.7-1.3 (-CH₂- of backbone).

Figure S1–26. ¹H NMR spectrum of polyEtOH
Figure S1–27. \(^1\)H NMR spectrum of glycoconjugates introduced both of 6'-SALac and 2-azidoethanol units (GP3 whose alkyne conversion was not 100%).

Figure S2. SEC chromatograph. P2 was calibrated by pullulan standard after deprotection (solvent: 10 mM PBS(-)). P3–P7 were calibrated by polystyrene standard before deprotection (solvent: DMAc with 10 mM LiBr).
Figure S3. SEC chromatograph of glycoconjugates.
(calibrated by pullulan standard, solvent: 10 mM PBS(-)).

Figure S4. UV-vis spectra of (A) P2 after deprotection and (B) GP2.
Figure S5. Picture of a gel (after CuAAC reaction with polymethacrylate backbones in 60°C).
Table S3. Detailed condition of CuAAC with polyacrylamide backbones

<table>
<thead>
<tr>
<th>No.</th>
<th>Alkyne units (%)</th>
<th>Backbones (Alkyne)</th>
<th>6'-SALac azide</th>
<th>CuSO₄</th>
<th>TBTA</th>
<th>L-Asc-Na</th>
<th>Solvents (mL)</th>
<th>Yield (%)</th>
<th>Alkyne conv. (%)</th>
<th>Sugar units (%)</th>
<th>Mₜ, SEC (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP2</td>
<td>12%</td>
<td>10 mg (13 μmol)</td>
<td>26 mg (39 μmol)</td>
<td>0.83 mg (5.2 μmol)</td>
<td>2.8 mg (5.2 μmol)</td>
<td>5.2 mg (26 μmol)</td>
<td>H₂O 0.2</td>
<td>44</td>
<td>10</td>
<td>12</td>
<td>18 000</td>
</tr>
<tr>
<td>GP3</td>
<td>31%</td>
<td>20 mg (69 μmol)</td>
<td>68 mg (104 μmol)</td>
<td>4.4 mg (28 μmol)</td>
<td>15 mg (28 μmol)</td>
<td>28 mg (140 μmol)</td>
<td>H₂O 2.5</td>
<td>0.5</td>
<td>88</td>
<td>100</td>
<td>31 000</td>
</tr>
<tr>
<td>GP4</td>
<td>49%</td>
<td>10 mg (51.5 μmol)</td>
<td>51 mg (77 μmol)</td>
<td>3.2 mg (18 μmol)</td>
<td>11 mg (20 μmol)</td>
<td>20 mg (103 μmol)</td>
<td>H₂O 2.2</td>
<td>0.8</td>
<td>57</td>
<td>100</td>
<td>49 000</td>
</tr>
<tr>
<td>GP5</td>
<td>68%</td>
<td>10 mg (67 μmol)</td>
<td>66 mg (100 μmol)</td>
<td>4.3 mg (27 μmol)</td>
<td>14 mg (27 μmol)</td>
<td>26 mg (134 μmol)</td>
<td>H₂O 2.2</td>
<td>0.8</td>
<td>57</td>
<td>100</td>
<td>68 000</td>
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<tr>
<td>GP6</td>
<td>86%</td>
<td>10 mg (76.3 μmol)</td>
<td>150 mg (228 μmol)</td>
<td>4.9 mg (31 μmol)</td>
<td>16 mg (31 μmol)</td>
<td>30 mg (152 μmol)</td>
<td>H₂O 1.5</td>
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<td>52</td>
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<td>86 000</td>
</tr>
<tr>
<td>GP7</td>
<td>100%</td>
<td>10 mg (81.2 μmol)</td>
<td>80 mg (122 μmol)</td>
<td>5.2 mg (33 μmol)</td>
<td>17 mg (33 μmol)</td>
<td>32 mg (162 μmol)</td>
<td>H₂O 1.5</td>
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<td>57</td>
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<tr>
<td>GP8</td>
<td>50%</td>
<td>5 mg (13.5 μmol)</td>
<td>27 mg (41 μmol)</td>
<td>0.86 mg (5.4 μmol)</td>
<td>2.9 mg (5.4 μmol)</td>
<td>5.3 mg (27 μmol)</td>
<td>H₂O 2.2</td>
<td>0.8</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
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</table>

*Conversion was determined by ¹H NMR. The value was calculated using the following equation: Alkyne conv. = [ʃ]anomer / [ʃ]triazole × 100, where [ʃ]anomer and [ʃ]triazole correspond to integral value of anomer proton and that of triazole proton, respectively. "n.d." means "not determined".
Hemagglutinin inhibition (HI) assay

The PBS(-) buffer was added into a 96 plate (25 μL/well). Sample solution (25 μL) was injected in the first lane. The solution in the first lane were diluted by two steps. Influenza virus solution (4 HAU) was injected in each well (25 μL/well). The 96 plate was incubated for 1 h at 4°C. Blood cell suspension (from a guinea pig, 0.5% in PBS(-), 50 μL) was injected in each well. The 96 plate was incubated for 2 h at 4°C. Hemagglutinin inhibition was observed.

Reference: