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A new oligosaccharide synthesis using special hydroxy protecting group

Shiro Komba,* Motomitsu Kitaoka and Takafumi Kasumi*

Enzyme Laboratory, National Food Research Institute, 2-1-12 Kannondai, Tsukuba 305-8642, Japan

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Abstract—A new hydroxy protecting group for convenient preparation of oligosaccharide was developed using uni-chemo protection (UCP) concept. The UCP group was comprised of polymerized amino acid derivatives and protecting each hydroxyl groups by ester linkage. Depending on the polymerization degree, the hydroxyl groups were characterized and controlled. Using this protecting group, two kinds of sialyl-T analogues were successfully synthesized from same sugar parts merely by repeating Edman degradation and coupling.

Various protecting groups for the synthesis of oligosaccharides have been developed and selectively used for desired synthesis.[1–4] The selection of protecting groups, however, is quite laborious. If the oligosaccharide library is tried to synthesize, each hydroxyl groups on mono-saccharide have to be protected orthogonally. The number of these orthogonal protecting groups increase with each additional glycosidic linkage. It is not so easy to apply these methods to the complicated oligosaccharide synthesis, since there are only limited number of such orthogonal protecting groups. On resolving this problem, hydroxyl protecting groups for the saccharide should be one dimensional for the syntheses of oligosaccharide library and complicated branched oligosaccharide. If it is possible to apply only one kind of protecting group and one de-protecting method, the syntheses of oligosaccharide library and complicated branched oligosaccharide become much more easy. Furthermore, the headache problem for the selection of the orthogonal protecting group can be reduced. Recently, Les P. Miranda and Morten Meldal reported a uni-chemo protection concept.[5] Uni-chemo protection (UCP) comprises of an amino acid polymer that can attach and protect amino groups. Each amino groups were protected individually different degrees of polymerized amino acid. De-protection of this protecting group is performed simply using the Edman degradation method[6] that removes only N-terminal mono-amino acid from every UCP groups. Depending on the polymerization degree of UCP groups, each amino groups could be characterized and controlled. We applied this idea to the hydroxyl protection for oligosaccharide synthesis. To protect individual hydroxyl groups on carbohydrates, individually different degree of polymerized amino acid derivatives were introduced by ester linkage. Subsequently, the polymerized amino acid protecting groups were removed one by one from N-terminal using Edman degradation. Each hydroxyl groups were characterized and controlled using only one kind of protecting group and only one de-protecting method for applied oligosaccharide synthesis.

We attempted to synthesize one of the important cell-surface tumor antigens, sialyl-T antigen, to demonstrate the validity of these idea. It is a common marker of epithelial tumors (e.g., prostate and breast carcinomas) and some blood cell tumors.[7] Two kinds of sialyl-T antigens are α (2–3) and α (2–6) sialyl-T antigen. Using this method, both analogues were satisfactory synthesized from the same sugar parts merely by repeating Edman degradation and coupling.

N-1-Ethyl propyl glycine was chosen as a UCP group to avoid the existing chiral center of carbon compared with *N*-1-methyl propyl glycine, which was used by Miranda and Meldal[5] (Scheme 1). The NMR peaks were very complicated despite use of the *N*-1-ethyl propyl group

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* Corresponding authors. Tel.: +81-29-838-8071; fax: +81-29-838-7321; e-mail addresses: skomba@nfri.affrc.go.jp; kasumi@nfri.affrc.go.jp

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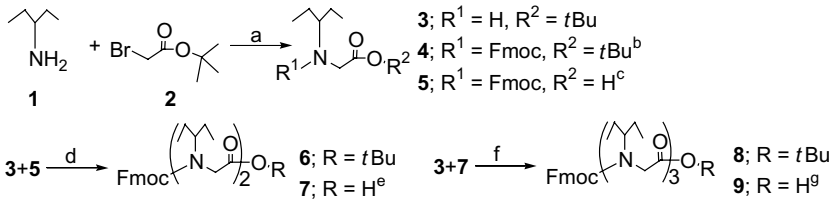
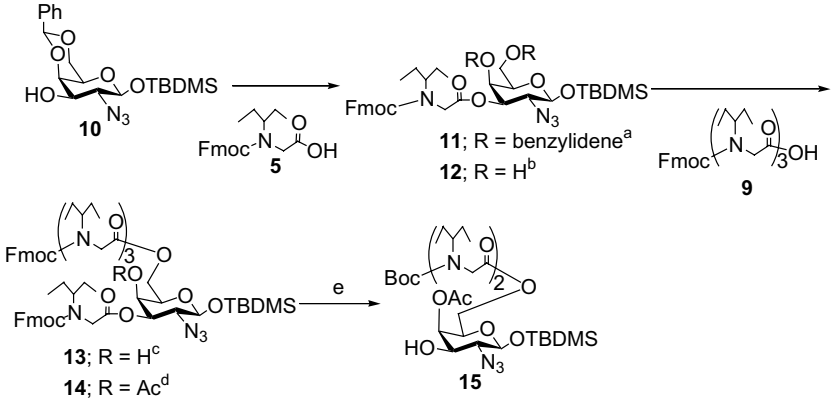


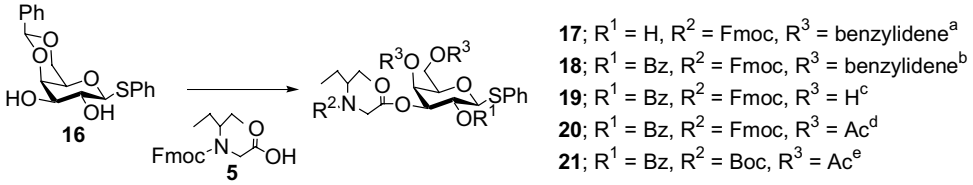
Scheme 1. Preparation of UCP group. Reagents and conditions: (a) THF, rt, 4 h, 98%; (b) 1.0 equiv Fmoc–Cl, 1,4-dioxane/satd aq $NaHCO_3$, rt, 2 h, 86%; (c) 90% formic acid, 40 °C, 7 h, 90%; (d) 2 equiv DIPEA, 1.5 equiv PyBroP, DMF, rt, 30 min, 91%; (e) 90% formic acid, 50 °C, 3 h, 93%; (f) 2 equiv DIPEA, 1.5 equiv PyBroP, DMF, rt, 30 min, 85%; (g) 90% formic acid, 50 °C, 2 h, 98%.



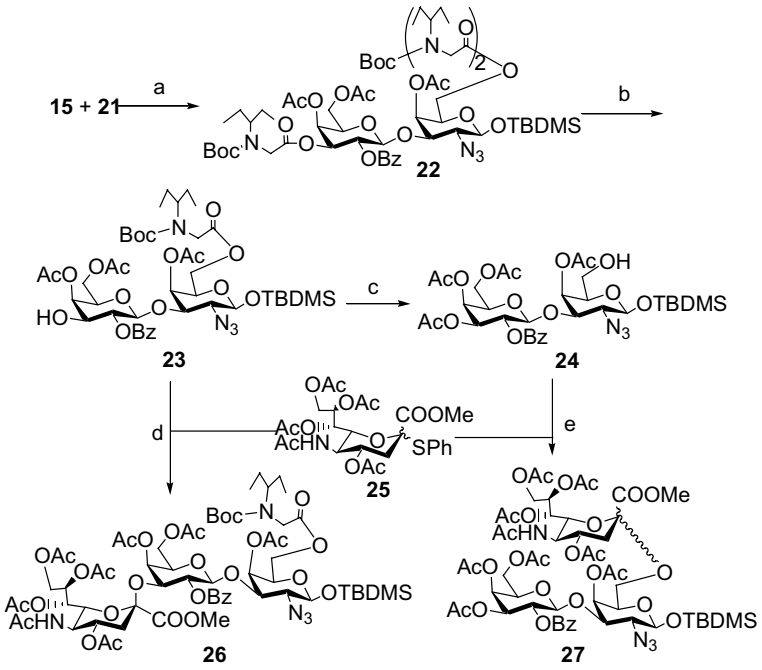
Scheme 2. Preparation of GalNAc acceptor. Reagents and conditions: (a) 1.5 equiv **5**, 5 equiv cyanuric chloride, py, 40 °C, 2 h, 90%; (b) 80% aq AcOH, 60 °C, 2 h, 79%; (c) 1.1 equiv **9**, 5 equiv cyanuric chloride, py, 40 °C, 2 h, 65%; (d) Ac_2O, py, 40 °C, 2 h, 91%; (e) (i) 20% piperidine in DMF, rt, 20 min. (ii) PITC, *N*-methyl morpholine (pH 8–9), rt, 30 min. (iii) TFA, CH_2Cl_2, rt, 30 min. (iv) Boc_2O, satd aq $NaHCO_3$, DMF, rt, 30 min, 69% (four steps).

because of the rotamers of carbamate. GalNAc residue was designed as Scheme 2.[8] The 2-position was used with N_3 group for coupling Ser or Thr linked with α configuration for future steps. The 3-position was protected by the mono-UCP group where the Gal residue was introduced in the next step. The 4-position was protected by the acetyl group as permanent protection. The 6-position was protected by the tri-UCP group where the sialic acid residue was introduced at final step. The Gal residue was designed as Scheme 3.[9] The 2-position was protected by the benzoyl group, which could make the glycoside linkage to a β configuration through neighboring effect. The 3-position was protected by the mono-UCP group where sialic acid residue was introduced at final step. The 4 and 6 positions were protected by the acetyl group as permanent protection. In addition, the sialic acid residue was used as the most common thiophenyl donor (**25**).[10]

For synthesis of selectively 3-position free GalNAc residue (**15**), compound **14** was treated by one cycle of Edman degradation (Scheme 2). The one cycle of Edman degradation was comprised of total four steps, first step: de-protection of Fmoc or Boc group by using 20% piperidine in DMF or TFA in CH_2Cl_2, respectively, second step: coupling PITC (phenyl isothiocyanate; Edman reagent) with basic condition to the all amino terminal, third step: cleaving of the N-terminal phenyl thiocarbamoyl mono-amino acid derivative from all UCP group by using TFA in CH_2Cl_2, fourth step: re-protection by Boc group to the newly appeared all N-terminal amino group. As a footnote, the mono-UCP group was immediately removed during PITC coupling step. The phenyl thiocarbamoyl mono-amino acid was not stable under the such basic condition, because of electron withdrawing effect in phenyl thiocarbamoyl moiety. Also, the Boc re-protecting step is important



Scheme 3. Preparation of Gal donor. Reagents and conditions: (a) 1.1 equiv **5**, 5 equiv cyanuric chloride, py, 0 °C, 1 h, 73%; (b) 1.5 equiv BzCl, py, rt, 2 h, 97%; (c) 80% aq AcOH, 60 °C, 2 h, 65%; (d) Ac_2O, py, rt, 2 h, 76%; (e) 20% piperidine in DMF, rt, 20 min then Boc_2O, satd aq $NaHCO_3$, rt, 1 h, 83% (two steps).



Scheme 4. Preparation of two kinds of sialyl-T antigen analogues. Reagents and conditions: (a) 2 equiv NIS, 0.3 equiv TfOH, MS 4 Å, CH_2Cl_2, −40 °C, 2 h, 89%; (b) (i) TFA, CH_2Cl_2, rt, 30 min. (ii) PITC, *N*-methyl morpholine (pH 8–9), rt, 30 min. (iii) TFA, CH_2Cl_2, rt, 30 min. (iv) Boc_2O, satd aq $NaHCO_3$, DMF, rt, 30 min, 70% (four steps); (c) (i) Ac_2O, py, rt, 2 h, 99%. (ii) TFA, CH_2Cl_2, rt, 30 min. (iii) PITC, *N*-methyl morpholine (pH 8–9), rt, 30 min, 98% (two steps); (d) 2 equiv NIS, 0.2 equiv TfOH, MS 3 Å, CH_3CN, −30 °C, 48 h, 13%; (e) 2 equiv NIS, 0.3 equiv TfOH, MS 3 Å, CH_3CN, −30 °C, 10 h, 67% (α:β = 71:29).

step. If amino free UCP protecting group was used for next coupling, the target di-saccharide was not obtained (unpublished data). Furthermore, if Fmoc group was used for amino protection for UCP group instead of Boc group, the di-UCP group was immediately cyclized and then, removed from the hydroxyl group during Fmoc cleaving condition at the next step. After treating one cycle of Edman degradation, the polymerizing degree of all UCP group equally degraded one degree, tri-UCP on 6-position was degraded to di-UCP, mono-UCP on 3-position was degraded to free hydroxyl group. The yield of this one cycle of Edman degradation was totally 69%, which was acceptable for applying oligosaccharide synthesis. This selectively 3-position free GalNAc acceptor (**15**) was coupled with Gal donor (**21**) by using NIS and TfOH activating method, to obtain selectively β and 3-position glycosidic di-saccharide (**22**; T-antigen) as 89% yield (Scheme 4). This di-saccharide (**22**) was merely treated one cycle of Edman degradation, successfully converted to 3′-position free di-saccharide acceptor (**23**) as 70% yield. This acceptor (**23**) was for synthesis of sialyl (2–3) T antigen. To convert the compound **23** into selectively 6-position free di-saccharide (**24**) that was the acceptor for the synthesis of sialyl (2–6) T antigen, successive 3′-position acetylation and one cycle of Edman degradation (which exclude third step and fourth step) were carried out. These two kinds of di-saccharide acceptor (**23** and **24**) were coupled with sialic acid donor (**25**) by using NIS and TfOH activating method in acetonitrile,[11] to obtain sialyl (2–3) T antigen (**26**) and sialyl (2–6) T antigen (**27**) derivatives, respectively. Unfortunately, only β-glycosidic sialyl (2–3) T antigen analogue was obtained instead of α-glycosidic one that was essential configuration for the natural sialyl (2–3) T antigen. Also, for the synthesis of sialyl (2–6) T antigen, the anomeric nature was mixed as α:β = 71:29. In spite of using acetonitrile as the solvent at both coupling, α selectivity was lost, due to sterical problem and high reactivity of the primary hydroxyl group, respectively.[12,13]

Nevertheless, this new method for oligosaccharide synthesis using UCP group is quite promising because of selectively cleavage of desired hydroxyl group and stepwise de-protection using only one de-protecting method. This may be the proof, the UCP method here is the basic technique to the oligosaccharide library synthesis.

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13. Compound **26**, ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm); 1.39, 1.45 (2s, 9H, Boc), 1.72 (br t, 1H, H-3ax. SA), 2.43 (dd, 1H, $J_{3eq.,4}$ 4.6 Hz, J_{gem} 13.3 Hz, H-3eq. SA), 3.11, 3.12 (2s, 3H, COO*Me*), 3.83 (m, 2H, H-9 SA), 3.97 (m, 1H, H-5 SA), 4.47, 4.47 (2d, 1H, $J_{1,2}$ 7.7, 7.8 Hz, H-1 $GalN_3$), 4.65 (dd, 1H, $J_{5,6}$ 10.5 Hz, $J_{6,7}$ 1.5 Hz, H-6 SA), 4.89, 4.89 (2d, 1H, $J_{1,2}$ 7.9, 8.0 Hz, H-1 Gal), 4.94, 4.95 (2dd, 1H, $J_{2,3}$ 10.1, 9.7 Hz, $J_{3,4}$ 3.5, 3.1 Hz, H-3 Gal), 5.04 (ddd, 1H, $J_{3ax.,4} = J_{4,5}$ 11.0 Hz, $J_{3eq.,4}$ 4.5 Hz, H-4 SA), 5.27 (m, 1H, H-8 SA), 5.37 (m, 1H, H-7 SA). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm); −5.18, −4.35 (Me_2Si), 10.99, 11.01, 11.19 (2*C*H_3CH$_2$), 17.97 (Me$_3$*C*Si), 20.52, 20.53, 20.66, 20.69, 20.83, 20.96, 20.99, 21.04, 21.06 (7*C*H_3COO), 23.26 (*C*H_3CON), 25.58, 25.59 (*Me*$_3$CSi), 25.91, 25.96, 26.39 (2CH$_3$*C*H_2), 28.25, 28.40 (*Me*$_3$CO), 37.26, 37.34 (C-3 SA), 43.54 (N*C*H_2CO), 48.57 (C-5 SA), 52.40 (COO*Me*), 58.31, 59.99 ((CH$_3$CH$_2$)$_2$*C*H), 60.95, 61.10 (C-6 Gal), 62.76 (C-6 $GalN_3$), 62.96, 63.01 (C-7 SA and C-9 SA), 64.95, 65.00 (C-2 $GalN_3$), 67.70 (C-4 SA), 68.41, 68.47 (C-4 $GalN_3$), 69.03, 69.06, 69.51, 70.01, 70.07, 70.12, 70.19, 70.34, 70.38 (C-4 Gal, C-5 Gal, and C-3 Gal), 71.60, 71.62 (C-5 $GalN_3$), 71.71 (C-2 Gal), 72.54 (C-8 SA), 73.16 (C-6 SA), 75.08, 75.24 (C-3 $GalN_3$), 79.95, 80.04 (Me$_3$*C*O), 97.55, 97.61 (C-1 $GalN_3$), 98.93 (C-2 SA), 100.90, 100.93 (C-1 Gal), 128.61, 129.81, 130.14, 130.17, 133.28, 133.31 (*Ph*COO), 155.66, 156.30 (C═O Boc), 165.71 (C═O Bz), 166.51 (C-1 SA), 169.77, 169.84, 169.86, 169.93, 170.08, 170.27, 170.67, 170.71, 171.17, 171.25, 171.27, 172.35, 172.38 (NCH$_2$*C*O and C═O 8Ac). ESI-FT-MS, calcd for $C_{63}H_{93}N_5O_{29}SiNa^+$ (M+Na$^+$): 1434.56177, found: 1434.56135. $[\alpha]_D$ 17.6 (*c* 0.5, $CHCl_3$).
Compound **27** α configuration, ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm); 1.88 (t, 1H, $J_{3ax.,4} = J_{gem}$ 12.7 Hz, H-3ax. SA), 2.52 (dd, 1H, $J_{3eq.,4}$ 4.6 Hz, J_{gem} 12.7 Hz, H-3eq. SA), 3.25 (dd, 1H, $J_{5,6}$ 3.7 Hz, J_{gem} 10.1 Hz, H-6 $GalN_3$), 3.76 (dd, 1H, $J_{5,6'}$ 2.4 Hz, J_{gem} 10.1 Hz, H-6′ $GalN_3$), 4.00 (m, 1H, H-9 SA), 4.04 (m, 1H, H-9′ SA), 4.04 (m, 1H, H-5 SA), 4.45 (d, 1H, $J_{1,2}$ 7.7 Hz, H-1 $GalN_3$), 4.25 (dd, 1H, $J_{5,6}$ 12.3 Hz, $J_{6,7}$ 2.3 Hz, H-6 SA), 4.93 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1 Gal), 4.82 (m, 1H, H-4 SA), 5.32 (m, 1H, H-8 SA), 5.32 (dd, 1H, $J_{6,7}$ 2.3 Hz, $J_{7,8}$ 4.9 Hz, H-7 SA). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm); 37.63 (C-3 SA), 49.33 (C-5 SA), 52.85 (COO*Me*), 60.92 (C-6 Gal), 62.28 (C-6 SA and C-9 SA), 63.57 (C-6 $GalN_3$), 65.29 (C-2 $GalN_3$), 66.92 (C-4 Gal), 67.11 (C-7 SA), 67.63 (C-8 SA), 68.42 (C-4 $GalN_3$), 69.04 (C-4 SA), 69.40 (C-2 Gal), 70.58 (C-3 Gal), 70.74 (C-5 Gal), 72.86 (C-5 $GalN_3$), 75.90 (C-3 $GalN_3$), 97.36 (C-1 $GalN_3$), 98.45 (C-2 SA), 101.32 (C-1 Gal), 165.11 (C═O Bz), 167.75 (C-1 SA). ESI-FT-MS, calcd for $C_{53}H_{75}N_4O_{27}Si^+$ (M+H$^+$): 1227.43825, found: 1227.43891. The α to β ratio was calculated from H-3 equatorial proton of NMR.