DOI 10.5012/bkcs.2010.31.04.1055

Construction of 6-Triazole-Linked Mannopyranosyl Serine and Threonine as Novel Sugar Amino Acid Mimics

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Received December 29, 2009, Accepted January 27, 2010

Key Words: Sugar amino acid, Click chemistry, Propargyl amino acid, Glycomimic

Glycopeptides, or being named more succinctly: the sugar amino acids (SAA), are a range of pivotal building blocks that could be ubiquitously found in Nature.[1] They play many central roles in living organisms governing myriad vital processes such as signal transportation, metabolic intermediation and intercellular recognition.[2] Consequently, the design and synthesis of their mimics by assembling two most fundamental natural elements, i.e., carbohydrates and amino acids *via* various chemo- or bio-methods has received explosive attention.[3]

One of the most efficiently potent tools to construct such nonnatural mimics shall be the 1,3-dipolar cycloaddition (or "Click Chemistry")[4] of organic azides with terminal alkynes. On the basis of the chemically and biologically stable property of triazole ring, countless strategies have been achieved to create solid neo-glycoconjugates.[5] In the meanwhile, expansion of this methodology toward the preparation of SAA has also been robustly progressed.[6] For example, Kuijpers and co-workers reported the achievement of 1,3-dipolar cycloaddition of azido-functionalized glycosides with acetylenic amino acids *via* click chemistry, resulting in the formation of novel stable 1-distributed SAA mimics (**A** in Figure 1 represents the general structure of this class).[6b]

Recently, mannopyranosyl azides have been independently prepared[7] given that mannopyranosyl derivatives are promising building units to further mimic certain oligosaccharides that bear biological significance.[8] In the meanwhile, serine and threonine residues are identified on an enormous amount of nuclear and cytoplasmic proteins, which could generate *in vivo* glycosylation with sugars leading to numerous essential biological processes.[9] Therefore, we decided to construct a new type of SAA mimics comprising both mannosyl scaffold and serine/threonine fragments *via* click chemistry. As shown in Figure 1, other than the previously described comparable samples where the amino acid moieties were conventionally settled on the anomeric carbon of saccharides (1-position, **A**),[6a,10] we would install in this report the serine/threonine precursors onto the 6-position of the mannosyl scaffold (**B**) for variation. Noteworthy, to the best of our knowledge, this is the first time for synthesizing such 6-distributed mannosyl serine or threonine as SAA mimics *via* click chemistry.

Experimental

Solvents were purified by standard procedures. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 spectrometer in $CDCl_3$ or DMSO-d_6 solutions. Optical rotations were measured using a SG WZZ-2A polarimeter at room temperature and a 10-cm 1-mL cell. Column chromatography was performed on E. Merck Silica Gel 60 (230 ~ 400 mesh). Analytical thin-layer chromatography was performed on E. Merck aluminum percolated plates of Silica Gel 60F-254 with detection by UV and by spraying with 6 N H_2SO_4 and heating at 300 °C. High resolution mass spectra (HRMS) were recorded on a KE465 LCT Premier/XE instrument using standard conditions (ESI, 70 eV).

Preparation of *N*-*tert*-butyloxycarbonyl-*O*-propargyl-L-threonine benzyl ester (2). To a solution of *N*-tertbutyloxycarbonyl-L-threonine (2.09 g, 9.5 mmol) in DMF (15 mL) at 0 °C, was carefully added NaH (60%, 1.1 g, 28.6 mmol), stirring for 30 min. Propargyl bromide (1.6 mL, 19.1 mmol) was then added. The mixture was stirred for 30 min at 0 °C followed by 12 h

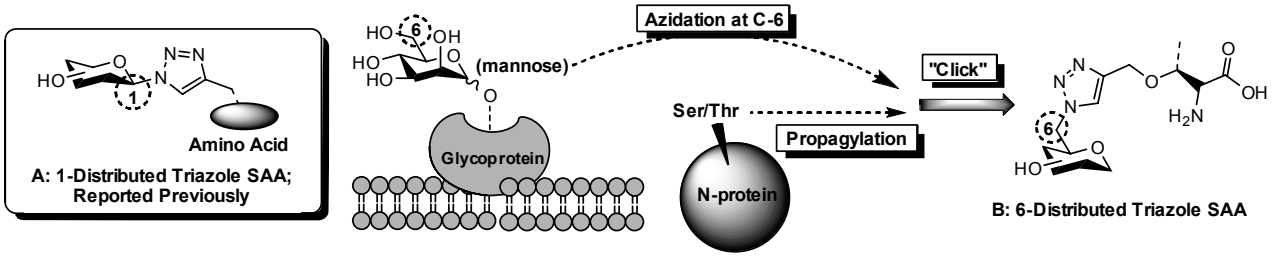


Figure 1. Previously reported SAA analogs (A) and new SAA mimics (B) presented in this paper *via* click chemistry.

stirring at rt. After completion of the reaction monitored by TLC, the mixture was poured into brine and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, evaporated to give a liquid which was directly dissolved in DMF (10 mL). $NaHCO_3$ (1.97 g, 21.7 mmol) and BnBr (1.4 mL, 11.4 mmol) were then added, stirring for 12 h. The mixture was successively washed with brine and water, extracted with ether, dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography (petroleum ether:EtOAc; 1:1) to give **2** (1.53 g, 46.1%) as a viscous oil. TLC: R_f = 0.65 (petroleum ether:EtOAc; 5:1); $[\alpha]_D$ = +6.5 (c = 2.5, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39-7.28 (m, 5H), 5.27 (t, 1H, J = 4.8 Hz), 5.22 (d, 1H, J = 8.0 Hz), 5.17 (d, 1H, J = 8.4 Hz), 4.36 (d, 1H, J = 9.6 Hz), 4.32-4.28 (m, 1H), 4.08-3.98 (m, 2H), 2.33 (brs, 1H), 1.46 (s, 9H), 1.25 (d, 3H, J = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 156.1, 135.5, 128.5, 128.3, 79.8, 79.3, 74.6, 74.1, 67.1, 58.3, 55.9, 28.3, 15.9.

General procedure for click reaction (4 and 5). Azide **3** (1 equiv.) and the amino acid alkyne (1 equiv.) were dissolved in a mixture of CH_2Cl_2 (10 mL) and water (10 mL). With vigorous stirring, sodium ascorbate (6 equiv.) was added followed by $CuSO_4{\cdot}5H_2O$ (3 equiv.). The mixture was stirred at rt for 12 h then extracted with CH_2Cl_2 and washed with water. The combined organic layers were dried over $MgSO_4$, evaporated and purified by column chromatography.

2-(*tert*-Butoxycarbonylamino)-3-((1-(((2*R*,3*R*,4*S*,5*S*,6*S*)-3,4,5-tris(benzyloxy)-6-methoxytetrahydro-2*H*-pyran-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)propanoate benzyl ether (4): From **1** (300.0 mg, 0.89 mmol) and **3** (483.4 mg, 0.89 mmol), column chromatography (petroleumether/EtOAc, 5:1 to 1:1) afforded **4** as a colorless syrup (646.2 mg, 87.5%). $[\alpha]_D$ = −76.3 (c = 0.1, CH_2Cl_2); TLC: R_f = 0.77 (petroleum ether:EtOAc; 1:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.37-7.32 (m, 20H), 5.36 (d, 1H, J = 8.0 Hz), 5.23 (d, 1H, J = 12.4 Hz), 5.12 (d, 1H, J = 12.4 Hz), 4.95 (d, 1H, J = 11.2 Hz), 4.75 (d, 1H, J = 12.0 Hz), 4.69 (d, 1H, J = 11.6 Hz), 4.67-4.45 (m, 9H), 3.92 (dd, 2H, J = 2.8, 6.4 Hz), 3.90-3.87 (m, 1H), 3.78 (brs, 1H), 3.71-3.62 (m, 2H), 3.08 (d, 3H, J = 6.0 Hz), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 155.5, 144.0, 138.2, 138.1, 135.5, 128.6, 128.5, 128.4, 128.2, 127.8, 127.7, 124.3, 99.2, 80.2, 80.0, 75.1, 75.0, 74.9, 74.6, 73.0, 72.9, 72.1, 70.6, 70.5, 70.1, 67.1, 64.8, 64.7, 64.8, 54.1, 51.0, 29.7, 29.6, 28.3; HRMS: calcd for $C_{46}H_{54}N_4O_{10}$+H: 823.3918, found: 823.3922.

2-(*tert*-Butoxycarbonylamino)-3-((1-(((2*R*,3*R*,4*S*,5*S*,6*S*)-3,4,5-tris(benzyloxy)-6-methoxytetrahydro-2*H*-pyran-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)butanoate benzyl ether (5): From **2** (500.0 mg, 1.43 mmol) and **3** (602.7 mg, 1.43 mmol), column chromatography (petroleumether/EtOAc, 5:1 to 1:1) afforded **5** as a colorless syrup (868.4 mg, 86.8%). $[\alpha]_D$ = +5.7 (c = 0.8, CH_2Cl_2); TLC: R_f = 0.61 (petroleum ether/EtOAc; 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (s, 1H), 7.36-7.26 (m, 20H), 5.24 (d, 1H, J = 9.6 Hz), 5.16 (d, 1H, J = 11.6 Hz), 5.10 (d, 1H, J = 12.4 Hz), 4.96 (d, 1H, J = 11.2 Hz), 4.74 (d, 1H, J = 12.0 Hz), 4.68 (d, 1H, J = 12.4 Hz), 4.67-4.56 (m, 6H), 4.40-4.31 (m, 3H), 4.32 (dd, 1H, J = 6.0, 9.2 Hz), 4.18-4.10 (m, 1H), 3.92-3.85 (m, 2H), 3.78 (t, 1H, J = 2.0 Hz), 3.66 (t, 1H, J = 9.6 Hz), 3.07 (s, 3H), 1.45 (s, 9H), 1.22 (d, 3H, J = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 156.1, 144.5, 138.2, 138.1, 135.6, 128.6, 128.5, 128.3, 128.2, 127.8, 127.7, 126.1, 125.8, 124.2, 99.2, 80.2, 79.8, 75.1, 75.0, 74.7, 74.6, 73.0, 72.1, 70.7, 67.0, 62.5, 60.3, 58.5, 54.9, 51.0, 28.3, 21.0, 16.3, 14.2; HRMS: calcd for $C_{47}H_{56}N_4O_{10}$+Na: 859.3894, found: 859.3901.

General procedure for the deprotection (6 and 7). To a solution of benzyl ester in MeOH (15 mL), was added 10% Pd/C (5% wt.), stirring for 15 min. This resulting mixture was filtered and concentrated. The obtained residue was directly dissolved in MeOH (15 mL). $PdCl_2$ (0.5 equiv.) and TFA (0.8 mL) were then added under hydrogen, stirring at rt for another 12 h. Following filtration and concentration afforded the unique product without further purification.

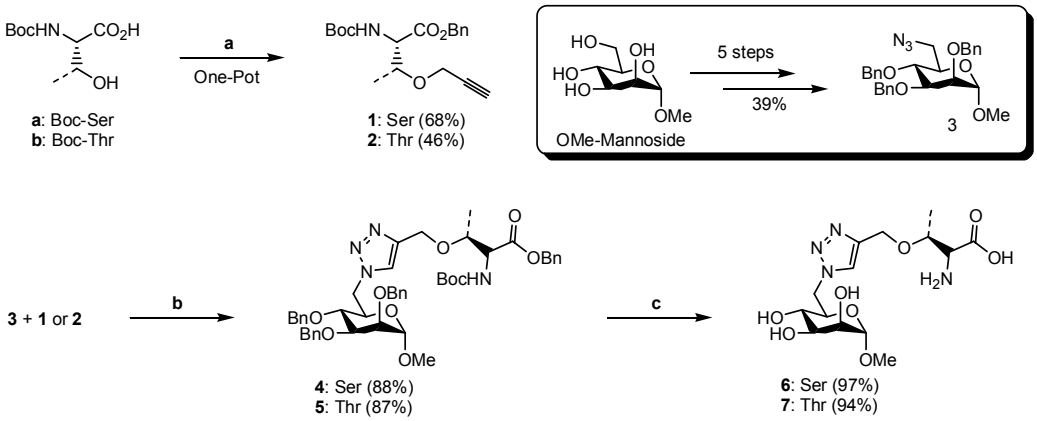
(*R*)-2-Amino-3-((1-(((2*R*,3*S*,4*S*,5*S*,6*S*)-3,4,5,6-tetrahydroxytetrahydro-2*H*-pyran-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)propanoic acid (6): From **4** (190.0 mg, 0.23 mmol) afforded **6** as a yellow power (81.4 mg, 97.3%). $[\alpha]_D$ = +52.8 (c = 0.6, MeOH); TLC: R_f = 0.11 (Water/AcOH; 1:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 (brs, 3H), 8.12 (s, 1H), 4.75 (dd, 1H, J = 2.0, 14.0 Hz), 4.63 (d, 1H, J = 12.4 Hz), 4.58 (d, 1H, J = 12.4 Hz), 4.47 (brs, 1H), 4.41 (dd, 1H, J = 8.8, 14.0 Hz), 4.17 (brs, 1H), 3.84 (dd, 1H, J = 4.4, 10.4 Hz), 3.81 (dd, 1H, J = 3.2, 10.4 Hz), 3.64 (dd, 1H, J = 2.0, 9.2 Hz), 3.61-3.60 (m, 1H), 3.47 (dd, 1H, J = 3.2, 9.2 Hz), 3.39 (t, 1H, J = 9.2 Hz), 2.94 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.9, 142.8, 125.2, 101.0, 71.6, 70.7, 70.0, 68.0, 67.1, 63.8, 53.8, 52.3, 51.0; HRMS: calcd for $C_{13}H_{22}N_4O_8$+Na: 385.1335, found: 385.1334.

(2*R*)-2-Amino-3-((1-(((2*R*,3*S*,4*R*,5*S*,6*S*)-3-(benzyloxy)-4,5-dihydroxy-6-methoxytetrahydro-2*H*-pyran-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methoxy)butanoic acid (7): From **5** (250.0 mg, 0.30 mmol), afforded **7** as a yellow power (106.0 mg, 94.3%). $[\alpha]_D$ = +33.1 (c = 0.8, CH_2Cl_2); TLC: R_f = 0.17 (Water/AcOH; 1:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.06 (s, 1H), 4.74 (d, 1H, J = 14.0 Hz), 4.64 (d, 1H, J = 12.4 Hz), 4.53 (d, 1H, J = 12.4 Hz), 4.47 (brs, 1H), 4.38 (dd, 1H, J = 8.8, 13.6 Hz), 4.08 (brs, 1H), 3.88 (brs, 1H), 3.67-3.60 (m, 2H), 3.46 (dd, 1H, J = 2.4, 9.2 Hz), 3.39 (t, 1H, J = 9.2 Hz), 2.93 (s, 3H), 1.23 (d, 3H, J = 6.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.2, 143.4, 125.0, 101.0, 72.1, 71.7, 71.6, 70.7, 69.9, 68.0, 61.8, 56.8, 53.9, 51.0, 16.3; HRMS: calcd for $C_{14}H_{24}N_4O_8$+H: 377.1672, found: 377.1667.

Result and Discussion

In order to obtain the desired amino acid alkynes (Scheme 1), commercially available Boc-Ser (**a**) and Boc-Thr (**b**) were employed as starting materials. According to the known literature procedure,[11] they were facilely converted to the corresponding *O*-Propargyl Boc-Ser-OBn (**1**)[11] and *O*-Propargyl Boc-Thr-OBn (**2**) in one pot with 68.4% and 46.1% yields, respectively. The azido-mannoside could be readily prepared from α-D-methyl mannopyranoside *via* multiple synthetic steps. TBDMSCl has been utilized to regioselectively protect 6-OH of α-D-methyl mannopyranoside. The following benzylation with NaH and benzyl bromide gave the fully protected sugar which was directly desilylated under acidic condition (AcCl in MeOH). After mesylation followed by azide substitution, the known sugar azide **3**[12] was furnished in a 5-step yield of 39.2%.

With terminal alkynes (**1** and **2**) and azide (**3**) in hand, we



Scheme 1. Synthetic route of SAA: a) NaH, propargyl bromide, then BnBr, K_2CO_3, DMF; b) $CuSO_4 \cdot 5H_2O$, VcNa, CH_2Cl_2/water; c) Pd/C, H_2 then $PdCl_2$/TFA, H_2, MeOH.

subsequently studied the 1,3-dipolar cycloaddition. The click reaction was proceeded in the presence of $CuSO_4 \cdot 5H_2O$ (3 equiv.) and sodium ascorbate (6 equiv.) in a mixture of dichloromethane and water. At room temperature, both reactions were completed in 10 h with considerable yields (87.5% for **4**, 86.8% for **5**). Deprotection of the click products was then easily realized. Hydrogenolysis step was conducted separately to uniquely obtain the desired products. The SAA benzyl esters were first converted to the corresponding free acids *via* H_2 over Pd/C in methanol within 15 min. The crude products were then directly transferred to a $PdCl_2/H_2$ promoted system in order to debenzylate the highly hindered protecting groups on sugar moiety. Notably, TFA was added to the latter process as the co-catalyst for simultaneously removing the Boc group. Eventually, the desired sugar amino acids **6** and **7** were successfully obtained with excellent yields of 97.3% and 94.3%, respectively.

In summary, we have effectively synthesized two novel sugar amino acid mimics bearing either a serine or a threonine precursor at C-6 position of the mannopyranosyl scaffold *via* click chemistry. It is believed that with the assist of such simply accessible method, various other SAA mimics of this category could be abundantly disclosed in the near future. Indeed, such work together with the evaluation of biological applications[13] toward these nonnatural but nature-like structures is currently underway by us and our co-workers.

Acknowledgments. This work was supported by National Natural Science Foundation of China (Grant No.20876045).

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