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Two Facile General Methods for the Conjugation of Three Different Molecules

Keumrok Oh,[†] Dong Seok Shin,[‡] Hyeong Baik Kim,[†] Uthaiwan Sirion,[§] and Dae Yoon Chi [†,‡,*]

[†]*Department of Chemistry, Sogang University, 35 Baekbeomro Mapogu, Seoul 04107, Korea*
[‡]*FutureChem Co., Ltd., 26 Seongsuil-ro 10-gil, Seongdong-gu, Seoul 04782, Korea.*
**E-mail: dychi@sogang.ac.kr*
[§]*Department of Chemistry, Faculty of Science, Burapha University, Chonburi 20131, Thailand*
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In the quest to synthesize compounds that are uniquely optimized for fighting each individual target disease, the importance of developing a new methodology for the bioconjugation of compounds with different functional groups or biological activities cannot be overemphasized. This study investigates two novel methods for click reaction: coupling a molecule with three functional compounds by repetitive copper(I) catalyzed azide/alkyne 1,3-dipolar cycloadditions. Method A uses three copper(I) catalyzed azide/alkyne 1,3-dipolar cycloadditions in series. Method B uses two copper(I) catalyzed azide/alkyne 1,3-dipolar cycloadditions and one conjugation reaction of amine and isothiocyanate.

Keywords: Conjugation chemistry, Bioconjugation, Tripodal conjugation, Click reaction

Introduction

As our understanding of biological functions and processes increases, so grows the need to facilitate the syntheses of big biomolecules connected with one or more different molecules. Commercial automatic synthesizers have made possible the rapid and convenient preparation of oligopeptides, oligonucleic acids, and oligosaccharides, using similar and repeated coupling methods of limited number of protected monomers.[1–4] However, the synthesis of compounds that have different functional groups remains an arduous and labor-intensive task.

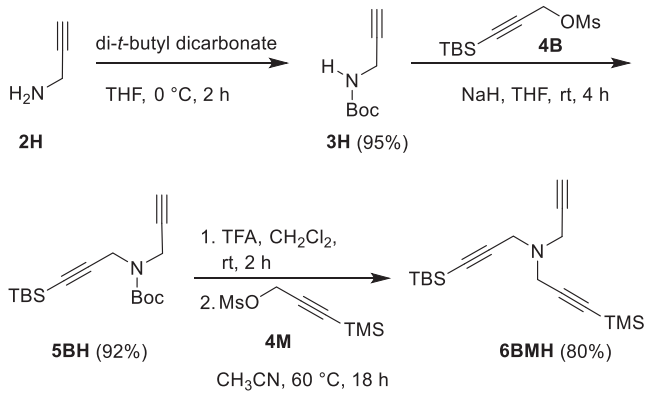
In 2001, Huisgen azide/alkyne 1,3-dipolar cycloadditions have been widely applied after these cycloaddition reactions could be proceeded regiospecifically by using Cu(I) catalyst.[5,6] The most attractive merit of these cycloaddition reactions is that the reactions go in very mild conditions, even in aqueous phase and in rt.[7] This cycloaddition reaction was also applied successfully to the synthesis of fluorine-18 labeled biomolecules in extremely low concentrations, giving various fluorine-18 labeled 1,2,3-triazoles with high radiochemical yield.[8] Conjugation of two different big molecules, one with azido group and the other with a terminal alkyne group, became feasible without complicated protective groups.[9–11]

Molecular imaging using fluorescent or radioactive labeling is a new field to understand the functions of biological systems. To incorporate label either fluorescent or radioactive group to bioconjugated molecules by Huisgen cycloaddition reactions, a new facile method that can connect three different biomolecules is needed. Arg-Gly-Asp (RGD) derivative was prepared by our group to image $\alpha_v\beta_3$ integrin protein. Introduction of carbohydrate to RGD derivative shows around 30% of cell uptake high. However, as the synthesis of F-18 labeled carbohydrate-RGD requires very complicated protection and deprotection steps, the development of this compound is not so easy.[12–15] Herein, we have described the development of a new convenient method, which can conjugate three different complicated molecules.[16,17]

Results and Discussion

Method A. Scheme 2 shows the basic concept of this method to connect three molecules X, Y, and Z having azide group. It has been found that terminal alkyne goes well while internal alkynes do not go in Huisgen cycloaddition. As the pKa of proton of terminal alkyne is around 25, terminal alkynes can be deprotonated and protected by various silyl groups. We had to find two proper silyl groups that could be discriminated in the deprotection step. In acidic media, the relative stabilities of silyl groups are: trimethylsilyl (TMS) (1) < triethylsilyl (TES) (64) < tert-butyldimethylsilyl (TBS) (20 000) < triisopropylsilyl (TIPS) (700 000) < tert-butyldiphenylsilyl (TBDPS) (5 000 000). In basic media, the relative stabilities are: TMS (1) < TES (10−100) < TBS ~ TBDPS (20 000) < triisopropylsilyl (TIPS) (100 000).[18] TMS and TBS were chosen, tested, and found to be suitable silyl groups.

Among the various methods we tried out to synthesize **6BMH**, the route shown in Scheme 1 is the most practical for mass production. Propargylamine (**2H**) was selected as the starting material of this route. Boc-protected propargylamine **3H** was successfully monoalkylated with



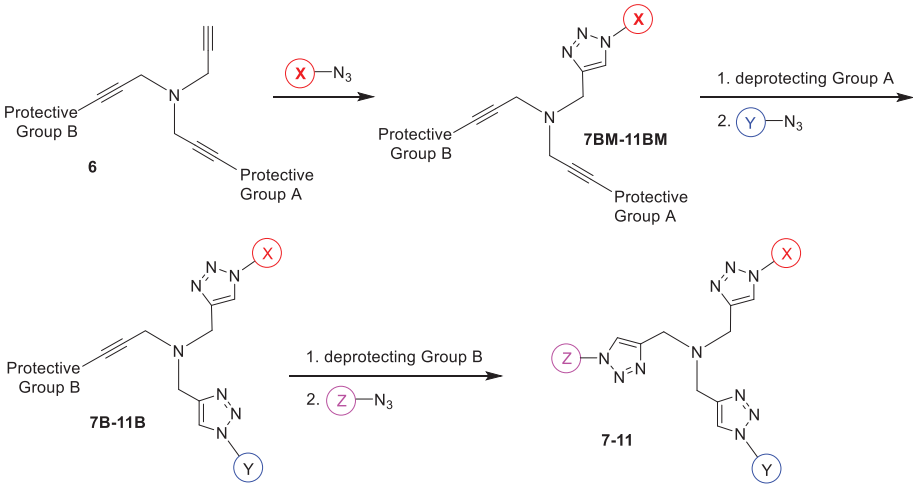
Scheme 1. Synthesis of Trialkyne **6BMH**.

TBS mesylate **4B**. Then, deprotection of boc group and alkylation with TMS mesylate **4M** provided one of the target tripodal amine **6BMH**. The overall yield to prepare **6BMH** from propargylamine (**2H**) was 70% in four steps.

As shown in Schemes 2 1) click reaction, 2) deprotection of protection group A, 3) click reaction, 4) deprotection of protection group B, and 5) click reaction through the reaction until a total of five steps was synthesized trifunctionalized molecule. To selectively deprotect the silyl group, **6BMH** (A = TMS, B = TBS) was selected in accordance with the relative stability of the silyl group in basic conditions.

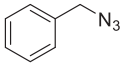
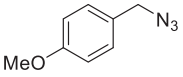
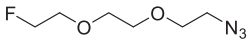
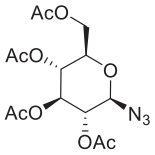
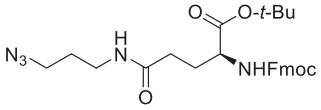
Azido compounds to the Cu(I)-catalyzed alkyne/azide 1,3-dipolar cycloadditions were used to **12a**-**12f**, as shown in Table 1. Using compound **6BMH** and the three selected azides in Table 1, compounds **7**, **8**, **9**, **10**, and **11** were synthesized in the order as shown in Scheme 1. As an example, compound **7** was prepared in five steps via **7BM** (79%), **7H** (98%), **7B** (87%), **7H** (95%), and **7** (95%) according to the series of sequence as shown in Scheme 1 using **12a**, **12b**, and **12c**. Compound **8** was also used in the same manner from **6BMH** using **12a**, **12b**, and **12d** via **7BM** (79%), **7H** (98%), **7B** (87%), **7H** (95%), and **8** (87%). Table 1 shows the yields and the azides used for the synthesis of the compounds **9**, **10**, and **11**.

Method B. In Table 1, the yield of synthesizing **11** from **11H** and **12c** is as low as 23%. Except for this reaction, the rest reaction proceeded generally in high yield. The last reaction to prepare **11** is depending on the size of azide. Between center of tripodal amine and triazoles, the number of carbons will be important. In this case, as the spacer carbon from the center of tripodal amine to the triazoles is only one, the length of the space may be short, resulting in arising steric hinder after three sequential click reactions. In the case of a large molecule such as RGDfK, we found that the yield decreased. The stepwise click reaction of (tripropargyl)amines such as **6BMH** tends to decrease the yield due to steric hindrance as the click reaction proceeds. In the case of introducing a large molecule, we designed **13** as shown in Scheme 3. Compound **13** has only two propargylic groups that are used, and the other one is connected to amine using the sulfonamide group. Sulfonamide group has three methylene as spacer and protected amine by boc group in ω-position. By using these three methylene groups, steric hindrance could be reduced. In the case of a compound with a larger steric hindrance, a longer aliphatic chain could be used instead of the three methylene spaces. If boc is used as a protecting agent in amine, it can be removed relatively easily. When this unprotected amine in ω-position reacts with a molecule having an isothiocyanate group, it will be able to conjugate easily by the formation of thiourea. Scheme 3 shows an example of Method B that efficiently attaches three very polar compounds, such as glucose, RGDfK, and DOTA. To obtain the structure–activity relationships (SAR) of these synthesized tripod

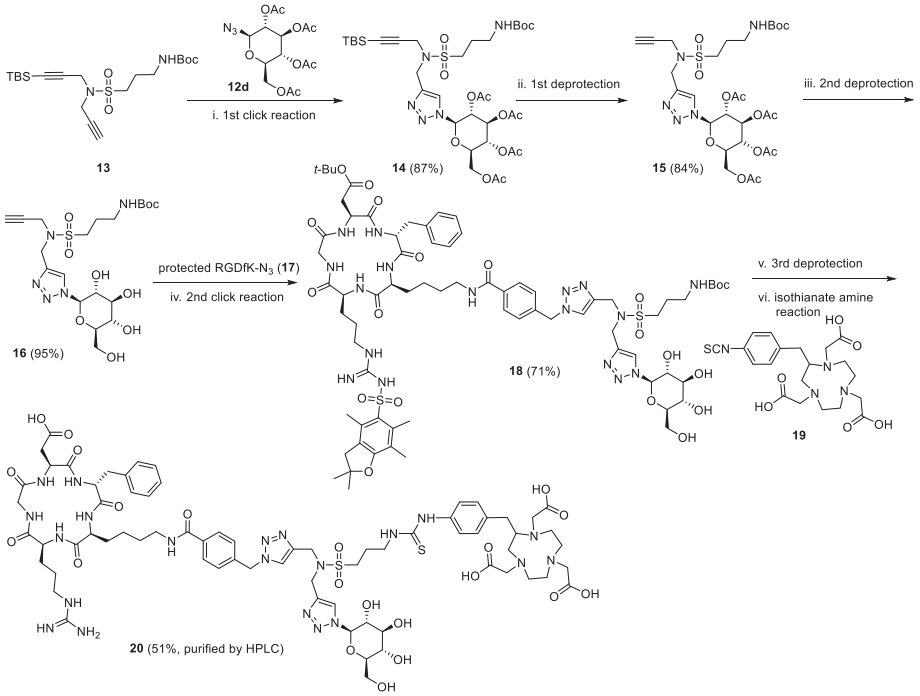


Scheme 2. Schematic of tripod compound (Method A). Compound number notation in all schemes: **7BM-11BM**, group A = TMS, B = TBS; **7BH-11BH**, Group A = H, B = TBS; **7B—11B**, X,Y-clicked, Group B = TBS; **7H—11H**, X,Y-clicked, Group B = H; **7–11**, X,Y,Z-clicked. Silyl group notation: B = TBS (t-butyldimethylsilyl); M = TMS (trimethylsilyl); E = TES (triethylsilyl); I = TIPS (triisopropylsilyl); D = TBDPS (t-butyldiphenylsilyl); P = TPS (triphenylsilyl).

Table 1. Various azido compounds and reaction yields of each Product[a].

Compd	X—N_3	Y—N_3	Z—N_3
7			 **12c** (**7**, 95%)
8	**12a** (**7BM**, 79%; **7BH**, 98%)	**12ba** (**7B**, 87%; **7H**, 95%)	 **12d** (**8**, 87%)
9	**12a** (**7BM**, 79%; **7BH**, 98%)	**12b** (**7B**, 87%, **7H**, 95%)	Rhodamine-N_3 **12e** (**9, 33%**)
10	**12a** (**7BM**, 79%; **7BH**, 98%)	**12d** (**10B**, 68%; **10H**, 71%)	**12c** (**10,** 90%)
11	**12a** (**7BM**, 79%; **7BH**, 98%)		**12c** (**11**, 23%)

[a] Product numbers and yields in parenthesis.



Scheme 3. Typical example of Method B: Synthesis of RGDfK-NOTA-Glucose **20**. Reaction conditions: i. $CuSO_4$, NaASC, *t*-BuOH, water, rt, 18 h, 87%; ii. TBAF, THF, 0 °C, 1 h, 84%; iii. 7 N NH_3 in MeOH, rt., 18 h, 95%; iv. $CuSO_4$, NaASC, MeOH, water, rt, 2 h, 71%; v. 50% TFA in CH_2Cl_2, rt, 2 h; vi. 0.1 M Na_2CO_3 (aq):DMF = 1:4, rt, 20 h, 51%.

compounds, the number of compounds should be prepared. For example, after metal chelated compound **20** with diagnostic and/or therapeutic radioisotope could be an excellent candidate of cancer treatment based on protein receptor radionuclide therapy (PRRT).[19] The moiety of carbohydrate will increase pharmacokinetic by interaction with glycoproteins in endothelial cell, and RGD will be binding to integrin expressed from cancer.[12–14]

To reduce the steric effects, the tripod synthesis for the bioconjugation started with the sulfonamide **13**, having one TBS protected propargylic group and one propargyl group. Click reaction of the sulfonamide **13** and protected azidoglucose **12d** followed by deprotection of the TBS ether produced compound **14** in 87% yields. After deprotection of TBS group and tetraacetate groups, a second click reaction of **16** with protected RGDfK **17** produced **18** in 71% yield. Then, deprotection of the boc, pbf and *t*-butoxy groups with 50% trifluoroacetic acid in dichloromethane provided a deprotected amine compound. The conjugation reaction of primary amine to 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) isothiocyanate **19** produced thiourea **20** under basic condition, purified in 51% yield after isolation by HPLC.

Conclusion

Until now, linking compounds with three different functional groups such as **20** has been a notoriously long and laborious process. We sought to develop an efficient and elegant method of synthesis. We first designed Method A and it proceeds well as we desired. However, as the three different functional groups become larger, there may be limitations due to steric hindrance of this method, so we went back to the drawing table and designed Method B. The synthesis of the very complicated compound **20** containing carbohydrate, RGD derivative and NOTA demonstrated high yield. Both of these methods can be applied to synthesize compounds having multimodality in the field of molecular imaging.

Experimental

General Information. All commercial reagent and solvents were used without further purification unless otherwise specified. Reagents and solvent were commercially purchased form Sigma-Aldrich (MO, USA), Merck (Darmstadt, Germany), TCI (Tokyo, Japan), and FutureChem. Reaction progress was monitored by analytical thin layer chromatography (TLC) with Merck 60 F-254 silica plates and visualized by UV light (254 nm) or PMA indicator. Flash column chromatography was performed on silica gel (Kieselgel 60; 230–400 mesh). ^{1}H and ^{13}C NMR spectra were recorded on 400-MR (400 MHz-^{1}H, 100 MHz-^{13}C) or Varian Inova-500 (500 MHz-^{1}H, 125 MHz-^{13}C) and chemical shift (δ, ppm) are reported in parts per million downfield from tetramethylsilane. The hard copies of NMR spectra of all compounds is available in Supplementary Information. The melting point was determined by fisher US/9300 and high-resolution mass obtained on a 4.7 Tesla IonSpec Electrospray Ionization-Fourier transform mass spectrometry (ESI-FTMS) or Micromass LCT ESI-Time of Fright (ESI-TOF) mass spectrometer.

***tert*-Butyl prop-2-yn-1-ylcarbamate** (3H)**.** To a solution of di-t-butyl dicarbonate (37.7 g, 173 mmol) in THF (30 mL) at 0 °C was added solution of propargylamine (**2H**, 10.0 mg, 181 mmol) dissolved in THF (10 mL), dropwisely. The reaction mixture was stirred for 2 h at rt and concentrated to give yellow oil. Purification with silica column chromatography (EtOAc:hexane = 1:9) gave a pale white solid (25.6 g, 95%): mp. 37–38 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.46 (s, 9H), 2.24 (t, J = 3.0 Hz, 1H), 3.92 (s, 2H), 4.98 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.3, 30.3, 71.2, 79.9, 80.2, 155.4. HRMS (ESI$^+$): *m/z* calcd. for $C_8H_{13}NNaO_2^+$ $[M + Na]^+$: 178.0838, found 178.0839.

***tert*-Butyl(3-methoxyprop-1-yn-1-yl)dimethylsilane** (4B)**.** To a solution of *t*-butyldimethylsilylated propargyl alcohol (1.7 g, 9.98 mmol) in anhydrous dichloromethane at 0 °C was added triethylamine (1.81 mL, 13.0 mmol). After being stirred for 15 min at the same temperature, methanesulfonyl chloride (0.86 mL, 11.0 mmol) was added dropwisely and stirred for 1 h. The reaction mixture was quenched by addition of water. The aqueous layer was extracted three times with dichloromethane, the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford the mesylate **4B** as a colorless oil (2.38 g, 88%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.13 (s, 6H), 0.93 (s, 9H), 3.12 (s, 3H), 4.86 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.0, 16.4, 25.9, 39.1, 58.3, 94.2, 97.3. HRMS (ESI$^+$): *m/z* calcd. for $C_{10}H_{20}NaO_3SSi^+$ $[M + Na]^+$: 271.0795, found 271.0797.

(3-Methoxyprop-1-yn-1-yl)trimethylsilane (4M). To a solution of trimethylsilylated propargyl alcohol (3.57 g, 27.8 mmol) in anhydrous dichloromethane at 0 °C was added triethylamine (5.04 mL, 36.2 mmol). After being stirred for 15 min at the same temperature, methanesulfonyl chloride (2.38 mL, 30.6 mmol) was added dropwisely and stirred for 1 h. The reaction mixture was quenched by addition of water. The aqueous layer was extracted three times with dichloromethane, the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford the mesylate **4M** as a colorless oil (5.66 g, 89%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.18 (s, 9H), 3.11 (s, 3H), 4.82 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −0.6, 39.0, 58.2, 95.7, 96.7. HRMS (ESI$^+$): *m/z* calcd. for $C_7H_{14}NaO_3SSi^+$ $[M + Na]^+$: 229.0325, found 229.0327.

***tert*-Butyl (3-(*tert*-butyldimethylsilyl)prop-2-yn-1-yl) (prop-2-yn-1-yl)carbamate** (5BH)**.** To the solution of **3H** (155 mg, 1.0 mmol) in anhydrous THF (3 mL) was added 60% NaH (48 mg, 1.2 mmol) at 0 °C and stirred for 15 min. **4B** (238 mg, 0.96 mmol) in anhydrous THF (4 mL) was dropwise at 0 °C and stirred for 4 h at rt. Reaction mixture was washed with saturated aqueous $NaHCO_3$ and then extracted with EtOAc, dried, filtered, and concentrated. Silica column chromatography provided **5BH** as colorless oil (304 mg, 92%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.10 (t, J = 0.5 Hz, 6H), 0.93 (s, 9H), 1.48 (d, J = 0.5 Hz, 9H), 2.20 (s, 1H), 4.17 (br, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −4.8, 16.4, 26.0, 28.2, 35.0, 36.3, 71.8, 78.9, 80.8, 86.7, 101.2, 154.2. HRMS (ESI$^+$): *m/z*

calcd. for $C_{17}H_{29}NNaO_2Si^+$ $[M + Na]^+$: 330.1860, found 330.1860.

***3-(tert*-Butyldimethylsilyl)-*N*-(prop-2-yn-1-yl)-*N*-(3-(trimethylsilyl)prop-2-yn-1-yl)prop-2-yn-1-amine (6BMH).** To the solution of **5BH** (5.7 g, 18.5 mmol) in dichloromethane (30 mL) was added TFA (10 mL) and stirred for 2 h at rt. Reaction mixture was washed with saturated aqueous $NaHCO_3$ and then extracted with dichloromethane, dried, filtered, and concentrated to boc-deprotected compound. Deprotected compound was dissolved in acetonitrile (30 mL), to which was added **4M** (4.58 g, 22.2 mmol) in acetonitrile (10 mL), then stirred for 18 h at 60 °C. The reaction mixture was washed with saturated aqueous $NaHCO_3$ and then extracted with ethyl acetate, dried, filtered, and concentrated. Silica column chromatography provided **6BMH** as an oil (4.71 g, 80%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.11 (s, 6H), 0.17 (s, 9H), 0.94 (s, 9H), 2.25 (t, J = 2.5 Hz, 1H), 3.46 (s, 2H), 3.47 (d, J = 2.5 Hz, 2H), 3.50 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −4.6, −0.1, 16.5, 26.0, 41.7, 42.9, 42.9, 73.2, 78.6, 88.4, 90.0, 100.6, 100.9. HRMS (ESI^+): *m/z* calcd. for $C_{18}H_{32}NOSi_2^+$ $[M + H]^+$: 318.2068, found 318.2068.

***N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-3-(*tert*-butyldimethylsilyl)-*N*-(3-(trimethylsilyl)prop-2-yn-1-yl) prop-2-yn-1-amine (7BM).** To a solution of **6BM** (73 mg, 0.23 mmol) in acetonitrile (0.5 mL) were added benzyl azide (32 μL, 0.25 mmol), solution of CuI (460 μL, 0.1 M in acetonitrile) and diisopropylethyl amine (460 μL, 0.1 M in acetonitrile). The reaction mixture was stirred for 3 h and concentrated under reduced pressure to afford a crude oil. The organic layer was extracted three times with ethyl acetate, dried over Na_2SO_4, and absorbed on silica for column chromatography (EtOAc:hexane = 1:5). After purification, **7BM** was obtained as a white solid (82 mg, 79%): mp. 72–74 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.09 (s, 6H), 0.15 (s, 9H), 0.93 (s, 9H), 3.43 (s, 2H), 3.46 (s, 2H), 3.82 (s, 2H), 5.51 (s, 2H), 7.25–7.29 (m, 2H), 7.33–7.40 (m, 3H), 7.42 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −4.6, −0.1, 16.4, 26.0, 43.0, 43.1, 48.1, 54.1, 88.3, 89.9, 100.8, 101.2, 122.6, 128.1, 128.7, 129.1, 134.5, 145.2. HRMS (ESI^+): *m/z* calcd. for $C_{25}H_{38}N_4NaSi_2^+$ $[M + Na]^+$: 473.2527, found 473.2525.

***N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-3-(*tert*-butyldimethylsilyl)-*N*-(prop-2-yn-1-yl)prop-2-yn-1-amine (7BH).** To a solution of **7BM** (596 mg, 1.32 mmol) dissolved in methanol (8 mL) was added NaOMe (2.64 mL, 1.32 mmol, 0.5 M solution in methanol) and stirred for 1 h at rt. Then the mixture was concentrated under reduced pressure and dissolved again in EtOAc for filtration over pad of silica. Resulting solution was evaporated to afford a white solid **7BH** (490 mg, 98%): mp. 52–53 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.07 (s, 6H), 0.90 (s, 9H), 2.20 (t, J = 2.5 Hz, 1H), 3.40 (d, J = 2.5 Hz, 2H), 3.44 (s, 2H), 3.81 (s, 2H), 5.48 (s, 2H), 7.20–7.25 (m, 2H), 7.29–7.36 (m, 3H), 7.41 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −4.7, 16.3, 26.0, 41.8, 43.0, 47.9, 54.0, 73.1, 78.6, 88.4, 101.0, 122.6, 128.0, 128.6, 129.0, 134.5, 144.9. HRMS (ESI^+): *m/z* calcd. for $C_{22}H_{30}N_4NaSi^+$ $[M + Na]^+$: 401.2132, found 401.2135.

***N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-3-(*tert*-butyldimethylsilyl)-*N*-((1-(4-methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)prop-2-yn-1-amine (7B).** To a solution of **7BH** (450 mg, 1.19 mmol) in acetonitrile (16 mL) were added *p*-methoxybenzyl azide (233 mg, 1.43 mmol), diisopropylethylamine (0.02 mL, 0.12 mol) and CuI (22 mg, 0.115 mmol). The reaction mixture was stirred 2 h and concentrated under reduce pressure to afford crude oil. Silica column chromatography (EtOAc:hexane =1:1) provided **7B** as a yellow oil (561 mg, 87%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.08 (s, 6H), 0.91 (s, 9H), 3.34 (br, 2H), 3.78 (s, 3H), 3.64–3.98 (m, 4H), 5.40 (s, 2H), 5.48 (s, 2H), 6.87 (d, J = 8.5 Hz, 2H), 7.20 (d, J = 8.5 Hz, 2H), 7.24 (d, J = 7.5 Hz, 2H), 7.31–7.37 (m, 3H), 7.46 (s, 1H), 7.47 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −4.5, −0.1, 16.4, 26.1, 29.6, 43.2, 47.9, 54.6, 54.1, 55.3, 88.8, 114.4, 122.6, 122.9, 126.5, 128.0, 128.7, 128.9, 129.0, 129.6, 134.6, 145.0, 159.8. HRMS (ESI^+): *m/z* calcd for $C_{30}H_{39}N_7NaOSi^+$ $[M + Na]^+$: 564.2878, found 564.2876.

***N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-*N*-((1-(4-methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl) prop-2-yn-1-amine (7H).** To the **7B** (470 mg, 0.87 mmol) in THF (25 mL) was added 1 M TBAF (0.9 mL, 9 mmol) at 0 °C. The reaction was stirred for 30 min and then washed with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layers were dried, filtered and concentrated. Silica column chromatography (EtOAc:hexane = 1:2) provided the **7H** as a yellow oil (343 mg, 95%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.21 (s, 1H), 3.29 (s, 2H), 3.76 (s, 3H), 3.79 (br, 4H), 5.39 (s, 2H), 5.46 (s, 2H), 6.85 (d, J = 8.5 Hz, 2H), 7.18 (d, J = 8.5 Hz, 2H), 7.21–7.34 (m, 5H), 7.45 (s, 1H), 7.49 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 41.9, 47.6, 53.5, 53.9, 55.2, 73.7, 78.2, 114.3, 122.7, 123.0, 126.5, 127.9, 128.5, 128.9, 129.5, 134.6, 144.4, 144.5, 159.7. HRMS (ESI^+): *m/z* calcd. for $C_{24}H_{25}N_7NaO^+$ $[M + Na]^+$: 450.2013, found 450.2016.

***1-(1-Benzyl-1H*-1,2,3-triazol-4-yl)-*N*-((1-(2-(2-(2-fluoroethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-*N*-((1-(4-methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl) methanamine (7).** To the **7H** (34 mg, 0.08 mmol) in acetonitrile (2 mL) was added **12c** (17 mg, 0.096 mmol), 0.1 M diisopropylethylamine in acetonitrile (0.16 mL, 0.016 mmol) and 0.1 M CuI in acetonitrile (0.16 mL, 0.016 mmol). After 2 h, reaction mixture was concentrated. Silica column chromatography (2–8% methanol in dichloromethane) provide **7** as a sticky green oil (46 mg, 95%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.61–3.67 (m, 6H), 3.71–3.73 (m, 7H), 3.80 (s, 3H), 3.87 (t, J = 8.5 Hz, 2H), 4.46 (t, J = 4.0 Hz, 1H), 4.53 (t, J = 4.5–5.5 Hz, 2H), 4.56 (t, J = 4.0 Hz, 1H), 5.30 (s, 1H), 5.44 (s, 2H), 5.51 (s, 2H), 6.88 (d, J = 8.5 Hz, 2H), 7.22 (d, J = 8.5 Hz, 2H), 7.26 (d, J = 6.5 Hz, 2H), 7.32–7.38 (m, 3H), 7.64 (s, 1H), 7.69 (s, 1H), 7.86 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 50.5,

55.1, 69.1, 69.9, 70.1, 70.2, 70.2, 70.3, 70.4, 70.5, 70.5, 70.6, 70.6, 71.2, 82.2, 82.3, 83.6, 83.6, 114.2, 126.4, 127.9, 128.5, 128.8, 129.6, 134.4, 159.6. HRMS (ESI^+): *m/z* calcd. for $C_{30}H_{37}FN_{10}NaO_3^+$ $[M + Na]^+$: 627.2926, found 627.2929.

***(2R,3R,4S,5R,6R)*-2-(Acetoxymethyl)-6-(4-((((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)((1-(4-methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (8).** To the **7H** (122 mg, 0.29 mmol) in acetonitrile (2 mL) was added protected azidoglucose **12d** (118 mg, 0.32 mmol), 0.1 M diisopropylethylamine in acetonitrile (0.31 mL, 0.031 mmol) and 0.1 M CuI in acetonitrile (0.31 mL, 0.031 mmol). After 3 h, reaction mixture was concentrated. Silica column chromatography (5% methanol in dichloromethane) provide **8** as an oil (200 mg, 87%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.80 (s, 3H), 2.03 (s, 3H), 2.07 (s, 3H), 2.09 (s, 3H), 3.64–3.77 (m, 6H), 3.80 (s, 3H), 3.99–4.01 (m, 1H), 4.16 (d, *J* = 12.5 Hz, 1H), 4.30 (dd, *J* = 12.5, 5.0 Hz, 1H), 5.26 (t, *J* = 9.0 Hz, 1H), 5.40–5.47 (m, 4H), 5.52 (s, 2H), 5.83 (d, *J* = 8.5 Hz, 1H), 7.23 (d, *J* = 9.0 Hz, 2H), 7.26–7.38 (m, 5H), 7.64 (s, 1H), 7.68 (s, 1H), 8.00 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.0, 20.4, 20.5, 20.6, 46.9, 47.0, 53.6, 54.0, 55.2, 61.4, 67.6, 70.4, 72.5, 75.0, 85.7, 114.3, 122.4, 123.4, 123.7, 126.7, 127.9, 128.6, 129.0, 129.5, 134.7, 144.0, 144.1, 144.6, 159.7, 168.7, 169.3, 169.9, 170.5. HRMS (ESI^+): *m/z* calcd. for $C_{38}H_{44}N_{10}NaO_{10}^+$ $[M + Na]^+$: 823.3134, found 823.3136.

***N*-(9-(3-((2-(2-(2-(2-(4-((((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)((1-(4-methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)ethyl)carbamoyl)phenyl)-6-(diethylamino)-3*H*-xanthen-3-ylidene)-*N*-ethylethanaminium chloride (9).** To azidorhodamine **12e** (100 mg, 0.157 mmol) in acetonitrile (2 mL) was added **7H** (74 mg, 0.173 mmol), diisopropylethylamine (5 μL, 0.052 mmol) and CuI (3 mg, 0.016 mmol). After 12 h, the reaction mixture was concentrated. Silica column chromatography (5% methanol in dichloromethane) provide **9** as a white solid (60 mg, 33%): mp. 69–71 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.12 (t, *J* = 7.0 Hz, 12H), 3.12 (t, *J* = 7.0 Hz, 2H), 3.27–3.32 (m, 13H), 3.41–3.42 (m, 2H), 3.50 (s, 4H), 3.58–3.86 (m, 11H), 4.46 (br, 2H), 6.23 (dd, *J* = 9.0, 2.0 Hz, 2H), 6.34 (d, *J* = 2.0 Hz, 2H), 6.38 (s, 1H), 6.40 (s, 1H), 6.84 (d, *J* = 7.0 Hz, 2H), 6.88 (d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 9.0 Hz, 2H), 7.20 (d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 7.0 Hz, 2H), 7.28–7.85 (m, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.4, 39.1, 44.2, 55.2, 64.7, 67.6, 69.2, 69.8, 70.2, 70.3, 97.6, 105.3, 107.9, 114.3, 122.5, 123.6, 126.6, 127.8, 127.9, 128.5, 128.6, 128.9, 129.5, 130.7, 132.3, 134.6, 148.6, 153.1, 153.6, 159.7, 168.1. HRMS (ESI^+): *m/z* calcd. for $C_{60}H_{72}N_{13}NaO_6^+$ $[M + Na]^+$: 1093.5615, found 1093.5617.

***N*-(1-Benzyl-1,2,3-triazol-4-yl)methyl-*N*-[1-(*O*-tetraacetyl-1-deoxyglucose-1-yl)-1,2,3-triazol-4-yl] methyl-*N*-[3-(*t*-butyldimethylsilyl)propargyl]-*N*-propargylamine (10B).** To a solution of **7BH** (100 mg, 0.26 mmol) in acetonitrile (2 mL) were added protected azidoglucose **12d** (118 mg, 0.32 mmol), 0.1 M diisopropylethylamine in acetonitrile (0.26 mL, 0.026 mmol) and 0.1 M CuI in acetonitrile (0.26 mL, 0.026 mmol). The reaction mixture was stirred 3 h and concentrated under reduce pressure to afford a crude oil. Silica column chromatography (EtOAc:hexane = 1:1) provided **10B** as a yellow oil (136 mg, 68%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.05 (s, 6H), 0.88 (s, 9H), 1.95 (s, 3H), 1.99 (s, 3H), 2.00 (s, 3H), 3.30 (br, 2H), 3.76 (br, 2H), 3.78 (br, 2H), 3.97–3.99 (m, 1H), 4.08 (d, *J* = 12.5 Hz, 1H), 4.24 (dd, *J* = 12.5, 5.0 Hz, 1H), 5.17 (t, *J* = 9.5 Hz, 1H), 5.31–5.39 (m, 2H), 5.45 (s, 2H), 5.82 (d, *J* = 8.0 Hz, 1H), 7.19 (d, *J* = 6.5 Hz, 2H), 7.25–7.30 (m, 3H), 7.45 (s, 1H), 7.74 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −4.7, 13.9, 16.2, 19.9, 20.3, 20.4, 25.9, 43.0, 47.6, 53.8, 61.3, 67.4, 70.2, 72.3, 74.8, 85.4, 88.7, 100.8, 121.3, 122.8, 127.8, 128.4, 128.8, 134.5, 144.8, 145.2, 168.5, 169.1, 169.6, 170.2. HRMS (ESI) *m/z* calcd. for $C_{36}H_{49}N_7NaO_9Si$ $[M + Na]^+$ 774.3259, found 774.3260.

***N*-(1-Benzyl-1,2,3-triazol-4-yl)methyl-*N*-[1-(*O*-tetraacetyl-1-deoxyglucose-1-yl)-1,2,3-triazol-4-yl] methyl-*N*-propargylamine (10H).** To **10B** (136 mg, 0.18 mmol) in THF (9 mL) was added 1 M TBAF (0.02 mL, 0.2 mmol) at 0 °C. The reaction was stirred for 2 h and then concentrated. Silica column chromatography (2% methanol in dichloromethane) provided **10H** as a yellow oil (82 mg, 71%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.76 (s, 3H), 1.97 (s, 3H), 2.01 (s, 3H), 2.02 (s, 3H), 2.24 (s, 1H), 3.29 (br, 2H), 3.78 (br, 4H), 3.97–4.00 (m, 1H), 4.10 (d, *J* = 12.5 Hz, 1H), 4.25 (dd, *J* = 12.5, 5.0 Hz, 1H), 5.19 (t, *J* = 9.5 Hz, 1H), 5.33–5.40 (m, 2H), 5.47 (s, 2H), 5.83 (d, *J* = 8.0 Hz, 1H), 7.22 (d, *J* = 7.5 Hz, 2H), 7.27–7.33 (m, 3H), 7.50 (s, 1H), 7.79 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.9, 20.3, 20.4, 20.5, 41.8, 47.4, 47.6, 53.9, 60.2, 61.3, 67.5, 70.3, 72.3, 73.7, 74.9, 85.5, 121.5, 122.9, 127.9, 128.5, 128.9, 134.6, 144.7, 145.0, 168.7, 169.2, 169.7, 170.3. HRMS (ESI) *m/z* calcd. for $C_{30}H_{35}N_7NaO_9$ $[M + Na]^+$ 660.2394, found 660.2394.

***(2S,3S,4R,5S,6R)*-2-(Acetoxymethyl)-6-(4-((((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)((1-(2-(2-(2-fluoroethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (10).** To **10H** (65 mg, 0.10 mmol) in acetonitrile (2 mL) was added **12c** (22 mg, 0.12 mmol), 0.1 M diisopropylethylamine in acetonitrile (0.1 mL, 0.01 mmol) and 0.1 M CuI in acetonitrile (0.1 mL, 0.01 mmol). After 1.5 h, reaction mixture was concentrated. Silica column chromatography (2–5% methanol in dichloromethane) provide **10** as a yellow solid (75 mg, 90%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.79 (s, 3H), 1.99 (s, 3H), 2.03 (s, 3H), 2.05 (s, 3H), 3.60 (br, 6H), 3.69 (br, 2H), 4.01 (br, 3H), 4.14 (d, *J* = 11.5 Hz, 1H), 4.28 (d, *J* = 9.5 Hz, 1H), 4.44 (br, 1H), 4.53 (br, 3H), 5.23 (br, 1H),

5.40 (br, 1H), 5.49 (br, 3H), 5.87 (br, 1H), 7.23–7.32 (m, 5H), 8.04 (br, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.1, 20.4, 20.5, 20.6, 29.6, 50.6, 61.4, 67.6, 69.1, 70.0, 70.2, 70.3, 70.4, 70.4, 70.5, 70.6, 70.7, 72.6, 75.1, 82.4, 83.7, 128.1, 128.6, 128.9, 134.5, 168.7, 169.2, 169.8, 170.5. HRMS (ESI^+) *m/z* calcd. for $C_{36}H_{47}FN_{10}NaO_{11}^+$ [M + Na]$^+$: 837.3302, found 837.3301.

***N*-(1-Benzyl-1,2,3-triazol-4-yl)methyl-*N*-[(3-(t-butyldimethylsilyl)propargyl)-1,2,3-triazol-4-yl]methyl-*N*-{1-[3-(*N*-Fmoc-*O*-*t*-butylglutamin-*N*'-yl)propyl]-1,2,3-triazol-4-yl}methylamine** (11B)**.** To a solution of **7BH** (71 mg, 0.20 mmol) in acetonitrile (2 mL) were added **12f** (148 mg, 0.29 mmol), 0.1 M diisopropylethylamine in acetonitrile (0.01 mL, 0.01 mmol) and 0.1 M CuI in acetonitrile (0.2 mL, 0.02 mmol). The reaction mixture was stirred 0.5 h and concentrated under reduce pressure to afford a crude oil. Silica column chromatography (2% methanol in dichloromethane) provided **11B** as oil (110 mg, 64%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.09 (s, 6H), 0.92 (s, 9H), 1.44 (s, 9H), 1.88–1.94 (m, 1H), 2.05–2.31 (m, 5H), 3.12–3.27 (m, 2H), 3.36 (s, 2H), 3.79 (s, 2H), 3.83 (s, 2H), 4.19–4.24 (m, 2H), 4.34–4.42 (m, 4H), 5.48 (s, 2H), 5.75 (d, J = 8.0 Hz, 1H), 6.63 (m, 1H), 7.23–7.41 (m, 9H), 7.47 (s, 1H), 7.58–7.59 (m, 2H), 7.65 (s, 1H), 7.75 (d, J = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.5, 16.5 26.1, 28.0, 28.9, 30.0, 32.5, 36.3, 43.4, 47.1, 47.5 (2C), 48.2, 54.0, 67.0, 82.4, 88.9, 101.0, 120.0, 123.0, 123.6, 125.1, 125.2, 127.1, 127.7, 128.1, 128.7, 129.1, 134.6, 141.3, 143.7, 143.9, 144.8, 145.0, 156.5, 171.2, 172.6. HRMS (ESI) *m/z* calcd. for $C_{49}H_{63}N_9NaO_5Si$ [M + Na]$^+$ 908.4614, found 908.4616.

***N*-(1-Benzyl-1,2,3-triazol-4-yl)methyl-*N*-{1-[3-(*N*-*O*-*t*-butylglutamin-*N*'-yl)propyl]-1,2,3-triazol-4-yl}methyl-*N*-propargylamine** (11H)**.** To **11B** (100 mg, 0.11 mmol) in THF (2 mL) was added 1 M TBAF (0.033 mL, 0.33 mmol) at rt. The reaction was stirred for 2 h and then concentrated. Silica column chromatography (5–10% methanol in dichloromethane) provided the **11H** as a yellow oil (50 mg, 81%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 9H), 1.76–1.84 (m, 1H), 1.92 (br, 2H), 2.07–2.14 (m, 3H), 2.27 (t, J = 2.4 Hz, 1H), 2.36 (t, J = 8.0 Hz, 2H), 3.18–3.24 (m, 2H), 3.35–3.36 (m, 3H), 3.82 (s, 2H), 3.85 (s, 2H), 4.40 (t, J = 6.8 Hz, 2H), 5.52 (s, 2H), 6.71 (t, J = 5.6 Hz, 1H), 7.26–7.28 (m, 2H), 7.35–7.40 (m, 3H), 7.53 (s, 1H), 7.68 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.0, 30.1, 30.2, 32.9, 36.2, 42.1, 47.6, 47.7, 47.9, 54.1, 54.4, 73.8, 78.3, 81.4, 123.1, 123.6, 128.0, 128.7, 129.1, 134.7, 144.5, 144.9, 173.1, 174.7. HRMS (ESI) *m/z* calcd. for $C_{28}H_{39}N_9NaO_3$ [M + Na]$^+$ 572.3068, found 572.3069.

***N*-(1-Benzyl-1,2,3-triazol-4-yl)methyl-*N*-[1-(8-fluoro-3,6-dioxaoctyl)-1,2,3-triazol-4-yl]methyl-*N*-{1-[3-(*N*-*O*-*t*-butylglutamin-*N*'-yl)propyl]-1,2,3-triazol-4-yl}methylamine** (11)**.** To the **11H** (100 mg, 0.18 mmol) in acetonitrile (2 mL) was added **12f** (64 mg, 0.36 mmol), diisopropylethylamine (0.01 mL, 0.05 mmol) and CuI (2 mg, 0.009 mmol). After 3 h, reaction mixture was concentrated. Silica column chromatography (2–5% methanol in dichloromethane) provide **11** as an oil (30 mg, 23%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 9H), 1.82–1.84 (m, 1H), 1.99–2.14 (m, 4H), 2.38–2.40 (m, 2H), 3.18 (q, J = 6.0 Hz, 2H), 3.45 (m, 1H), 3.60–3.64 (m, 6H), 3.70–3.76 (m, 7H), 3.85 (t, J = 5.0 Hz, 2H), 4.39–4.44 (m, 2H), 4.51–4.56 (m, 2H), 5.51 (s, 2H), 7.17 (t, J = 5.6 Hz, 1H), 7.16–7.34 (m, 5H), 7.12 (s, 1H), 7.89 (s, 1H), 7.96 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.0, 10.9, 14.1, 22.7, 23.0, 23.7, 28.0, 28.9, 29.3, 29.7, 30.0, 32.8, 36.2, 38.7, 47.4, 47.6, 47.7, 50.2, 53.4, 54.2, 61.0, 69.4, 70.2, 70.4, 70.6, 71.8, 77.2, 81.7, 82.3, 83.9, 123.7, 124.5, 124.8, 128.1, 128.7, 129.1, 134.6, 143.7, 143.9, 144.4, 173.3, 181.0. HRMS (ESI^+) *m/z* calcd. for $C_{34}H_{51}FN_{12}NaO_5^+$ [M + Na]$^+$: 749.3982, found 749.3986.

***β*-1-*N*-Boc-TBS-tripod-2,3,4,6-tetra-*O*-acetyl-D-glucose** (14)**.** A solution of protected azidoglucose **12d** (550 mg, 1.47 mmol) and **13** (632 mg, 1.47 mmol) in a 4:1 mixture of t-BuOH–water (30 mL) was treated with $CuSO_4{\cdot}5H_2O$ (184 mg, 0.74 mmol) and sodium ascorbate (NaASC, 174 mg, 0.88 mmol). The reaction mixture was stirred at 23 °C for 18 h. The reaction mixture was diluted with EtOAc (300 mL), washed with water (3 × 30 mL) and saturated aqueous NaCl (30 mL), dried (Na_2SO_4) and concentrated under reduced pressure to provide the crude product. Flash column chromatography (EtOAc:hexane = 1:1) afforded bis-protected tripod-glucose **14** as a white solid (1.02 g, 87%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H), 5.79 (d, J = 9.2 Hz, 1H), 5.54 (d, J = 3.1 Hz, 1H), 5.44 (t, J = 9.7 Hz, 1H), 5.24 (dd, J = 10.3 Hz, 2.9 Hz, 1H), 4.74 (m, 1H), 4.62 (s, 2H), 4.23–4.11 (m, 3H), 4.06 (d, J = 5.2 Hz, 2H), 3.32–3.24 (m, 2H), 3.21–3.14 (m, 2H), 2.24 (s, 3H), 2.09–2.04 (m, 5H), 2.00 (s, 3H), 1.88 (s, 3H), 1.43 (s, 9H), 0.94 (s, 9H), 0.13 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 170.0, 169.7, 168,9, 155.8, 143.8, 121.1, 99.6, 89.6, 86.2, 79.2, 73.9, 70.4, 68.2, 66.8, 61.2, 49.5, 41.5, 38.8, 37.0, 28.3, 26.0, 23.8, 20.61, 20.56, 20.4, 20.1, 16.4, −4.8. HRMS (ESI^+): *m/z* calcd. for $C_{34}H_{55}N_5NaO_{13}SSi^+$ [M + Na]$^+$: 824.3179, found 824.3179.

***β*-1-*N*-Boc-tripode-2,3,4,6-tetra-*O*-acetyl-D-glucose** (15)**.** A solution of **14** (900 mg, 1.12 mmol) in THF (20 mL) at 0 °C under Ar was treated with a solution of TBAF in THF (1.4 mL, 1.0 M). The reaction mixture was stirred at 0 °C for 1 h and quenched with saturated aqueous NH_4Cl (10 mL). Resulting mixture was treated with EtOAc (100 mL) and stirred for 1 h. Phases were separated, and the organic phase was washed with water (3 × 20 mL) and saturated aqueous NaCl (30 mL), dried (Na_2SO_4) and concentrated under reduced pressure to provide the crude product. Flash column chromatography (EtOAc:hexane = 4:1) afforded **15** as a white solid (647 mg, 84%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (s, 1H), 5.80 (d, J = 9.1 Hz, 1H), 5.51 (s, 1H), 5.41 (t, J = 9.7 Hz, 1H), 5.24 (dd, J = 10.3 Hz, 1.8 Hz, 1H), 4.87 (m, 1H), 4.61 (s, 2H), 4.24–4.10 (m, 3H), 4.01 (s, 2H), 3.26–3.22 (m, 2H),

3.21–3.13 (m, 2H), 2.40 (s, 1H), 2.21 (s, 3H), 2.05–2.01 (m, 5H), 1.97 (s, 3H), 1.84 (s, 3H), 1.40 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 170.0, 169.8, 169.0, 155.9, 143.8, 121.9, 86.3, 79.4, 77.4, 74.4, 74.1, 70.5, 68.2, 66.8, 61.2, 49.9, 41.7, 38.8, 36.0, 28.3, 24.1, 20.7, 20.6, 20.5, 20.2. HRMS (ESI^+): *m/z* calcd. for $C_{28}H_{41}N_5NaO_{13}S^+$ $[M + Na]^+$: 710.2314, found 710.2314.

***β*-1-*N*-Boc-tripode-D-glucose (16).** Glucose derivative **15** (600 mg, 0.87 mmol) in a vial was treated with a solution of NH_3 in methanol (23 mL, 7 N). The reaction mixture was stirred at 23 °C for 18 h in a sealed condition. Volatiles were removed under a stream of N_2, and the residue was subject to flash column chromatography (2–10% methanol in dichloromethane) to provide **16** as a white film (430 mg, 95%): ^{1}H NMR (400 MHz, CD_3OD) δ 8.25 (s, 1H), 5.55 (d, J = 9.2 Hz, 1H), 4.63 (s, 2H), 4.11 (t, J = 9.4 Hz, 1H), 4.05 (s, 2H), 3.95 (s,1H), 3.84–3.64 (m, 5H), 3.21–3.13 (m, 4H), 3.21–3.13 (m, 2H), 2.87 (s, 1H), 1.95–1.91 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD) δ 158.3, 144.4, 124.2, 90.0, 80.1, 79.8, 78.4, 76.0, 75.0, 71.4, 70.2, 62.3, 50.7, 42.5, 39.7, 37.0, 28.8, 25.0. HRMS (ESI^+): *m/z* calcd. for $C_{20}H_{33}N_5NaO_9S^+$ $[M + Na]^+$: 542.1891, found 542.1893.

***β*-1-*N*-Boc-tripod-D-glucose-protected RGDfK (18).** A solution of **16** (119 mg, 0.23 mmol) and commercially available **17** (200 mg, 0.22 mmol) in a 3:1 mixture of MeOH-water (30 mL) was treated with $CuSO_4{\cdot}5H_2O$ (28 mg, 0.11 mmol) and sodium ascorbate (27 mg, 0.13 mmol). The reaction mixture was stirred at 23 °C for 2 h and concentrated under reduced pressure to provide crude product. The crude product was purified with HPLC (Alltima 5 μm C18, 250 × 10 mm, gradient, 25–100% MeCN-0.1% aqueous TFA buffer, 30 min, 4 mL/min, t_R 16–19 min, broad peak) to afford **18** as a white solid (205 mg, 71%): ^{1}H NMR (400 MHz, CD_3OD) δ 8.22 (s, 1H), 7.95 (s, 1H), 7.83 (d, J = 8.3 Hz, 2H), 7.36 (d, J = 8.3 Hz, 2H), 7.21–7.14 (m, 5H), 5.63–5.58 (m, 3H), 4.76–4.70 (m, 1H), 4.62–4.56 (m, 1H), 4.49 (br, 4H), 4.21–4.16 (m, 3H), 4.01–3.97 (m, 2H), 3.88–3.85 (m, 1H), 3.80–3.72 (m, 3H), 3.37–3.28 (m, 2H), 3.17–3.08 (m, 7H), 2.98–2.91 (m, 5H), 2.77–2.72 (m, 1H), 2.54–2.47 (m, 8H), 2.04 (s, 3H), 1.87–1.81 (m, 3H), 1.72–1.68 (m, 1H), 1.61–1.49 (m, 4H), 1.45–1.39 (m, 24H), 1.12–1.09 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ174.8, 173.8, 173.5, 172.4, 172.1, 171.1, 169.4, 161.2, 160.9, 158.4, 144.9, 144.6, 140.2, 137.8, 135.7, 130.3, 129.6, 129.2, 129.0, 127.9, 125.8, 124.4, 118.6, 90.1, 87.8, 82.3, 80.1, 79.9, 75.2, 71.5, 70.4, 62.5, 56.5, 56.3, 54.4, 54.0, 51.4, 51.0, 44.8, 43.9, 42.7, 40.5, 39.8, 38.2, 37.5, 31.9, 31.1, 29.8, 29.2, 28.8, 28.7, 28.3, 25.1, 24.3, 19.7, 18.4, 12.6. HRMS (ESI^+): *m/z* calcd. for $C_{72}H_{104}N_{17}O_{20}S_2^+$ $[M + H]^+$: 1590.7079, found 1590.7079.

NOTA-RGDfK-D-glucose Tripod (20). RGD derivative **18** (43 mg, 0.033 mmol) in a vial was treated with a solution of 95:2.5:2.5 = TFA:TIS:dichloromethane (2.0 mL). The reaction mixture was stirred at 23 °C for 2 h in a sealed condition. Volatiles were removed under a stream of N_2, and the residue was dried under reduced pressure to provide deprotected compound, which used in the next step without purification. Crude deprotected compound from the previous step was dissolved in 4:1 = 0.1 M aqueous Na_2CO_3-DMF (4 mL) and treated with commercially available NOTA-isothiocyanate **19** (20 mg, 0.036 mmol). The reaction mixture was stirred at 23 °C for 40 h under Ar and then concentrated under reduced pressure. The residue was purified with HPLC (Alltima 5 μm C18, 250 × 10 mm, gradient, 5–50% MeCN-0.1% aqueous TFA buffer, 20 min, 4 mL/min, t_R 23 min). The collected fraction was lyophilized to afford the TFA salt of **20** as a white solid (28 mg, 51%): ^{1}H NMR (500 MHz, DMSO) δ 9.63 (br, 1H), 847–8.44 (m, 1H), 8.37 (s, 1H), 8.24 (s, 1H), 8.18 (s, 1H), 8.17–8.11 (m, 2H), 8.00 (d, J = 7.1 Hz, 1H), 7.94 (s, 1H), 7.83 (d, J = 8.1 Hz, 2H), 7.65 (br, 2H), 7.41–7.36 (m, 4H), 7.24–7.18 (m, 4H), 7.16–7.11 (m, 4H) 5.66 (s, 2H), 5.50 (d, J = 9.1 Hz, 1H), 4.66–4.61 (m, 2H), 4.48–4.37 (m, 6H), 4.19–4.14 (m, 2H), 4.06–4.00 (m, 3H), 3.97–3.91 (m, 2H), 3.82–3.68 (m, 5H), 3.57–3.47 (m, 5H), 3.28–3.15 (m, 10H), 2.92–2.88 (m, 3H), 2.83–2.68 (m, 6H), 2.63–2.60 (m, 1H), 2.38–2.34 (m, 1H), 1.93–1.87 (m, 2H), 1.75–1.69 (m, 1H), 1.60–1.56 (m, 1H), 1.47–1.34 (m, 6H), 1.11–1.02 (m, 2H); ^{13}C NMR (125 MHz, DMSO) δ 180.5, 173.4, 172.2, 171.7, 171.5, 171.2, 170.6, 170.0, 169.6, 169.3, 165.8, 162.3, 158.6, 158.4, 156.7, 142.7, 142.6, 138.9, 137.7, 137.3, 134.4, 129.2, 129.1, 128.1, 127.7, 127.6, 126.3, 124.5, 122.9, 117.8, 115.5, 88.2, 78.5, 73.6, 69.5, 68.5, 60.5, 58.7, 54.6, 54.4, 54.3, 53.2, 52.5, 51.8, 51.5, 50.9, 49.8, 48.9, 44.6, 43.3, 42.9, 42.2, 41.0, 40.3, 37.5, 35.8, 35.1, 33.5, 31.0, 30.8, 28.5, 25.2, 22.9, 22.8. HRMS (ESI^+): *m/z* calcd. for $C_{70}H_{99}N_{21}O_{21}S_2^{2+}$ $[M + 2H]^{2+}$: 816.8377, found 816.8375.

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Supporting Information. Additional supporting information is available in the online version of this article.

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