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Chemical Synthesis of CD52 Glycopeptides Containing the Acid-Labile Fucosyl Linkage

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Glycopeptide **1** with the fucosylated trisaccharide, β-D-GlcNAc(1→4)[α-L-Fuc(1→6)]-β-D-GlcNAc, linked to the Asn of CD52 peptide was prepared by two methods, both of which used the free glycosyl Asn **12** and glycotripeptide **21** as key intermediates. Thus, after the trisaccharide was prepared and linked to Asn, the carbohydrate moiety was deprotected to give **12**. From **12**, **21** was constructed in homogeneous NMP solutions by elongating the peptide chain alone the *N*-terminus. Though the glycopeptides were easily soluble in NMP, they were barely soluble in diethyl ether, because of the free trisaccharide. Consequently, addition of diethyl ether to the reaction mixtures could precipitate the glycopeptides, and the products were conveniently isolated and purified in the solid form. The coupling of **21** with a free nonapeptide **24** in NMP afforded **1**. **1** was also prepared by solid-phase synthesis, using the acid-sensitive 2-chlorotrityl resin. In this case, **21** was attached to the nonapeptide on the resin, and the resulting glycopeptide was then released with dilute acetic acid. Deprotection of the peptide under moderate acidic conditions gave **1**. The acid-labile α-fucose was not affected in these syntheses.

Introduction

CD52 is a glycosylphosphatidylinositol (GPI)-anchored glycopeptide expressed by virtually all human lymphocyte and sperm cells.[1,2] Lymphocyte CD52 and sperm CD52 share an identical peptide, but they have distinct functions. The former plays an important role in the human immune system,[3] while the latter is involved in the reproduction process of human beings.[4,5] Sperm CD52-specific antibodies that did not cross-react with lymphocyte CD52 were also identified.[6,7]

The peptide of CD52 antigens is extremely short, consisting of only 12 amino acids, and it has one *N*-glycosylation site, to which complex glycans having the fucosylated core are linked.[1,2] The glycans have a decisive influence on the biological activities of CD52. For instance, it is the *N*-glycans of sperm CD52 that the sperm-specific antibodies bind to.[6,7]

Because of their short peptide, simple glycosylation pattern, and interesting bioactivities, CD52 antigens are useful models for structure−activity relationship studies of glycopeptides. To this end, pure CD52 glycopeptides are essential. The problem is that it is difficult to obtain pure CD52 glycopeptides by biological methods because of biological microheterogeneity, a common problem in glycoscience. A promising solution to the problem is chemical synthesis.

However, chemical synthesis of glycopeptides is a significant challenge.[8,9] What makes chemical synthesis of CD52 glycopeptides more complex is the presence of a fucose residue in its *N*-glycan. It is well established that the α-fucosidic linkage is particularly labile to acids. For example, the glycosidic bond of benzylated fucose is easily broken by trifluoroacetic acid (TFA) used to retrieve glycopeptides from resins in the traditional solid-phase synthesis.[10] The glycosidic bond of acylated fucose is more stable, so it is usually the acyl-protected fucose that is used to prepare fucosylated glycopeptides.[11−15] A drawback of utilizing acyl protective groups is that, to form the α-fucosidic linkage and avoid neighboring group participation in the glycosylation, the fucosyl donor must be ether-protected and later on it has to be transformed to the acylated form in several steps.[14−16] Though a one-

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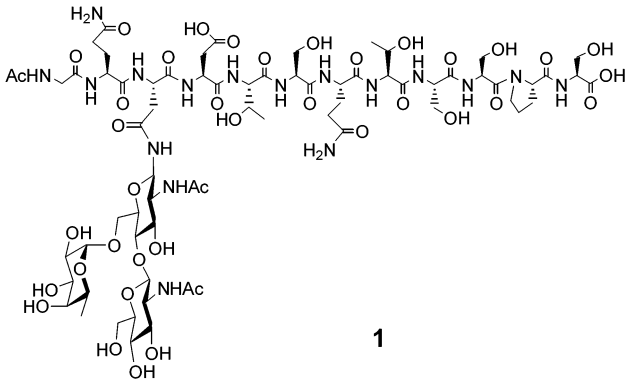

1

FIGURE 1. The synthetic target.

step procedure to prepare α-linked fucosyl amino acids from peracetylated fucose was reported,[17] to the best of our knowledge no similar method has been developed for fucosylated oligosaccharides. More importantly, the final deprotection of acyl-protected glycopeptides is tricky. While strict control of pH is critical to prevent *β*-elimination and other side reactions in the deprotection,[9] more basic conditions may be necessary for the complete removal of acyl groups in complex oligosaccharides. Another potential problem caused by the intimate core fucosyl group is the steric hindrance that may hinder the coupling reactions especially in solid-phase glycopeptide synthesis. Enzymatic and chemoenzymatic synthesis is a useful method for fucosylated glycopeptides,[18−22] while its downside is the limited availability and substrate specificity of the enzymes.

Fucosylation is very common in nature and it has a significant influence on the conformations and biological functions of natural glycoproteins. Therefore, more robust and practical synthetic methods for fucose-containing glycopeptides are highly desirable not only for the study of CD52 but also for the study of other glycoproteins.

In this paper, two related methods were exploited in the preparation of fucose-containing CD52 glycopeptides. One is solution-phase synthesis with solid-phase workup of the glycopeptide products, and the other is combined solution-phase and solid-phase synthesis with use of the acid-sensitive 2-chlorotrityl resin. Both utilized a glycosyl asparagine with its carbohydrate chain deprotected as the key building block, and both should be applicable to other glycopeptides.

Results and Discussion

One of our synthetic targets was glycopeptide **1** that has a fucosylated core trisaccharide linked to the Asn of an intact CD52 peptide (Figure 1). Though a CD52 glycopeptide was previously prepared by solid-phase synthesis,[23] its *N*-glycan did not contain the problematic fucosyl residue. Bertozzi and co-workers[24] recently achieved a more complex glycopeptide of CD52 by chemical ligation of glycans, which was not fucosylated either.

As mentioned above, the glycosidic linkage of fucose, especially if it is protected by benzyl groups, is extremely labile to acids.[10] Our studies indicated that the half-life of the core trisaccharide having a benzylated fucose was less than an hour in 10−20% TFA/dichloromethane (DCM) commonly used to deprotect peptide side chains. Therefore, conventional solid-phase synthesis with benzylated glycosyl amino acids is not applicable even if an acid-sensitive resin is utilized to facilitate the retrieval of glycopeptides. In contrast, the free trisaccharide is significantly more stable to acids. For example, we did not notice any obvious change of the free trisaccharide after it was treated with 20% TFA/DCM at room temperature for 3 h. Thus, using unprotected oligosaccharides as building blocks should be of general benefit to fucosylated glycopeptides.

To deal with the stability problem of carbohydrates, as well as the potential problems related to their final deprotection, we recently explored a new strategy for glycopeptide synthesis, namely "solution-phase synthesis with solid-phase workup", using unprotected glycosyl amino acids as the key building blocks and phase tags.[25,26] Its underlining concept is that the free oligosaccharides are used to facilitate the isolation of reaction products by the precipitation method, while neither detachment of the phase tags nor final deprotection of carbohydrates is required. This strategy should be especially suitable for synthetic targets such as **1**.

An alternative solution to the problem is solid-phase synthesis with unprotected glycosyl amino acids as building blocks,[27−29] because the free oligosaccharides are more stable to acidic conditions. Nevertheless, to avoid treating fucosylated glycopeptides with strong acids, it is beneficial to employ an acid-sensitive resin as the solid-phase support. The glycosidic linkage of free fucose can then withstand the mild acidic conditions involved in the retrieval of glycopeptides from the resin and the deprotection of peptide side chains. Moreover, the new solution-phase synthesis can be combined with solid-phase synthesis to formulate a novel and highly convergent method.

Synthesis of the Unprotected Trisaccharide-Asn Conjugate (Scheme 1). The monosaccharide building blocks **3**, **4**, and **8** were prepared from D-glucosamine (**2**) and L-fucose (**7**) according to the reported procedures.[30−32]

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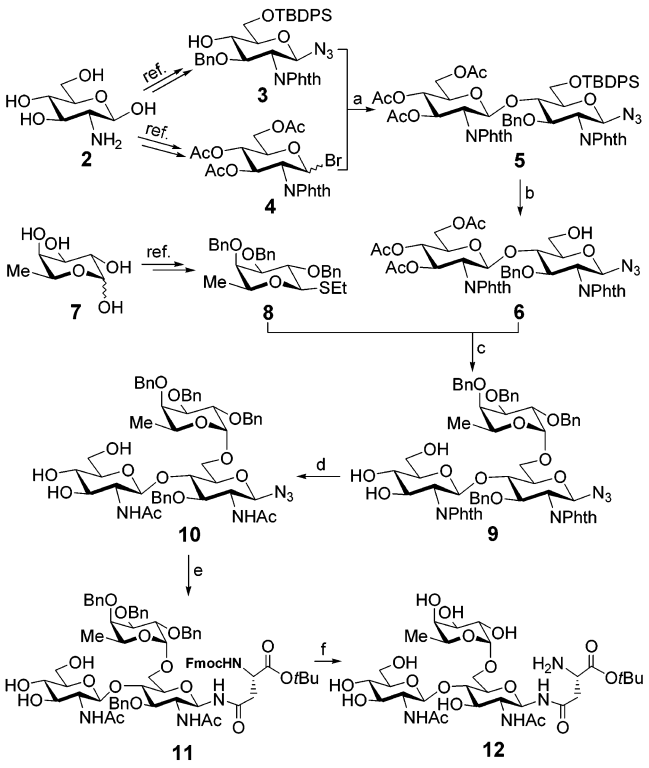
SCHEME 1[a]



[a] Reagents and conditions: (a) AgOTf, MS 4 Å, DCM, −40 °C to rt, overnight, 75%; (b) TBAF, HOAc, THF, rt, 72 h, 87%; (c) $CuBr_2$, Bu_4NBr, MS 4 Å, DCM/DMF, rt, 2 h, 85%; (d) NH_2CH_2-CH_2NH_2, BuOH, 90 °C, overnight; Ac_2O, pyr., rt, overnight; NaOMe, MeOH, rt, overnight, 86%; (e) Lindlar catalyst, H_2; Fmoc-Asp(OBt)-O*t*Bu, NMP, rt, 2 h, 87%; (f) 20% $Pd(OH)_2$/C, H_2, rt, 20 h, 96%.

Stereoselective glycosylation of **3** by **4** was achieved by using silver triflate (AgOTf) as the promoter.[33] After the removal of *tert*-butyldiphenylsilyl (TBDPS) in **5**, the α-fucose was introduced to the exposed 6-*O*-position. Several promoters were evaluated for this glycosylation, and $CuBr_2$/Bu_4NBr produced the highest stereoselectivity and yield (85%).[34,35] Then, the phthalimido groups in **9** were substituted for acetamido groups by a one-pot procedure to give **10** (86%).

The azido group at the reducing end of trisaccharide **10** was selectively reduced by hydrogenation with Lindlar catalyst to give the glycosylamine that was immediately treated with a freshly prepared active ester, Fmoc-Asp-(OBt)-O*t*Bu, to form the trisaccharide-Asn conjugate **11** (H-1: δ 4.93, J = 3.0 Hz; δ 4.76, J = 8.4 Hz; and δ 4.67, J = 8.4 Hz). No α-anomer was observed. The product was easily purified by silica gel column chromatography. The benzyl and Fmoc groups were finally removed under a H_2 atmosphere by using $Pd(OH)_2$ as the catalyst to afford **12** (96%), which had a free α-amino group and was ready for the peptide elongation.

Solution-Phase Synthesis of CD52 Glycopeptides with Solid-Phase Workups. Our synthetic plan was to construct the target glycopeptide **1** by coupling the free *N*-terminal glycopeptide segment Gly[1]-Asn[3][glycan] to the free nonapeptide segment Asp[4]-Ser[12]. This convergent design can minimize the number of *C*-terminal elongations that require acidic treatment.[25] The key elements of this synthesis are the preparation of the glycotripeptide segment **16** and the coupling between the free peptide and glycopeptide segments.

Glycotripeptide **16** was prepared from **12** by solution-phase elongation of the peptide chain alone the *N*-terminus. *N*-Methylpyrrolidinone (NMP), which could dissolve the unprotected glycopeptides easily, was used as the solvent to achieve homogeneous reactions,[25] and the commercially available pentafluorophenyl (Pfp) esters of amino acids were employed for the peptide coupling reactions.

The synthetic procedure is shown in Scheme 2. After **12** was dissolved in NMP, to the solution was added the Pfp ester of Gln at room temperature. The progress of the coupling was monitored by TLC, which indicated a very clean reaction completed in 2 h. Then, diethyl ether (10 times the volume of NMP) was added to the mixture to precipitate the expected glycopeptide **13**, while the excessive Gln-Pfp and the side products, such as pentafluorophenol, remained in solution. After the solution was removed, the precipitate was washed with diethyl ether to afford **13** (96%) that was practically pure. The less than perfect yield of this reaction might be due to the mass loss during the sample transfer. *N*-Terminal deprotection of **13** was achieved with 20% piperidine in NMP, and the product **14** was also isolated by the precipitation method and purified by washing with diethyl ether. The coupling protocol described for **13** was also applied to introduce Gly and give the glycotripeptide **15**. Finally, the *C*-terminus of **15** was deprotected with 15% TFA in DCM at room temperature to yield **16**. It was proved that this treatment did not affect the unprotected carbohydrate moiety. Glycopeptide segment **16** with a free *C*-terminus was ready to be activated and coupled to a peptide fragment.

To investigate the coupling reaction between the glycopeptide and a peptide fragment, **16** was first linked to a protected tripeptide **17**. The reaction was achieved in DMF with use of 3 equiv of **17** with DCC/HOBt as the condensation reagent. TLC indicated a clean and complete reaction. The product was again precipitated out and washed with diethyl ether, and then deprotected in two steps to give **19**, a partial structure of CD52 glycopeptide, that was finally purified by HPLC (71% yield based on **18**). DCU formed during the activation of **16** was soluble in DMF and in the mixture of DMF/ether, so it was removed from the product during the precipitation and washing process, as proved by the NMR spectra of crude **18** and **19**. The HPLC results and NMR spectra of **19** did not show any racemization of amino acids or decomposition of the glycan.

The synthesis of **1** is shown in Scheme 3. After the *N*-terminus of **15** was deprotected and capped by an acetyl group, its *tert*-butyl group, as well as the trityl group, was removed to give **21**. Meanwhile, the free nonapeptide segment **24** was prepared by the conventional solid-phase method with use of Wang resin and Fmoc chemistry. Because **24** had two free carboxyl groups that may interfere with the reaction, instead of utilizing

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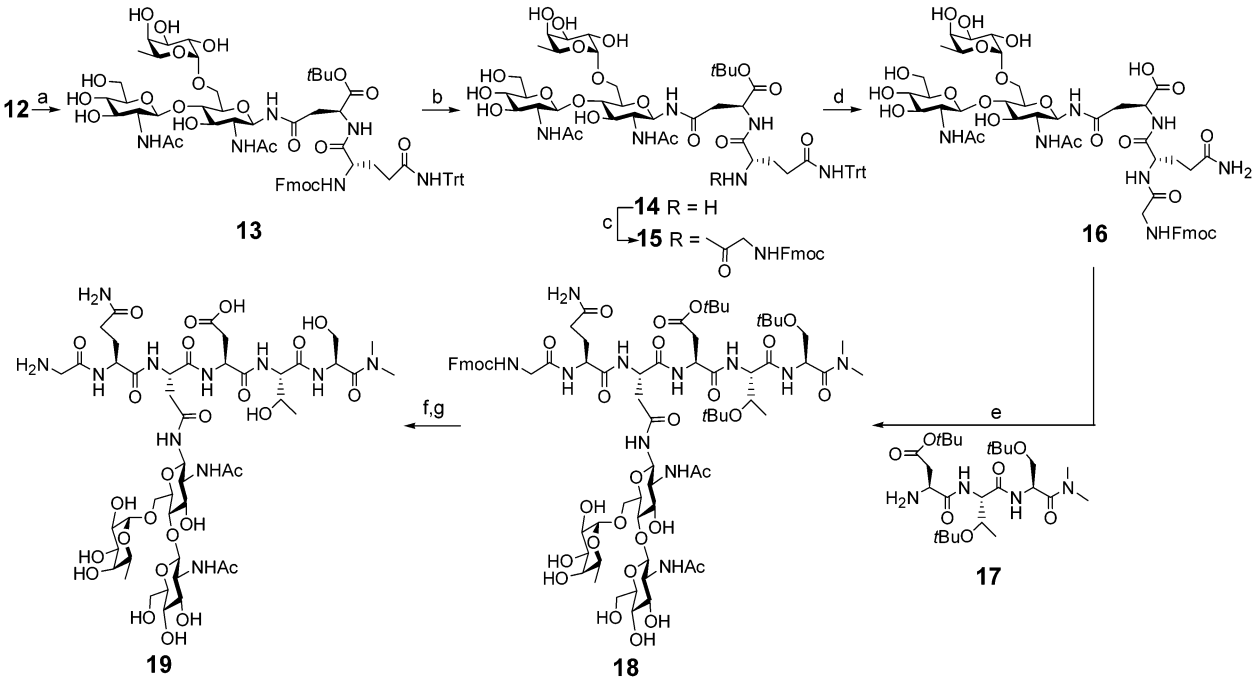
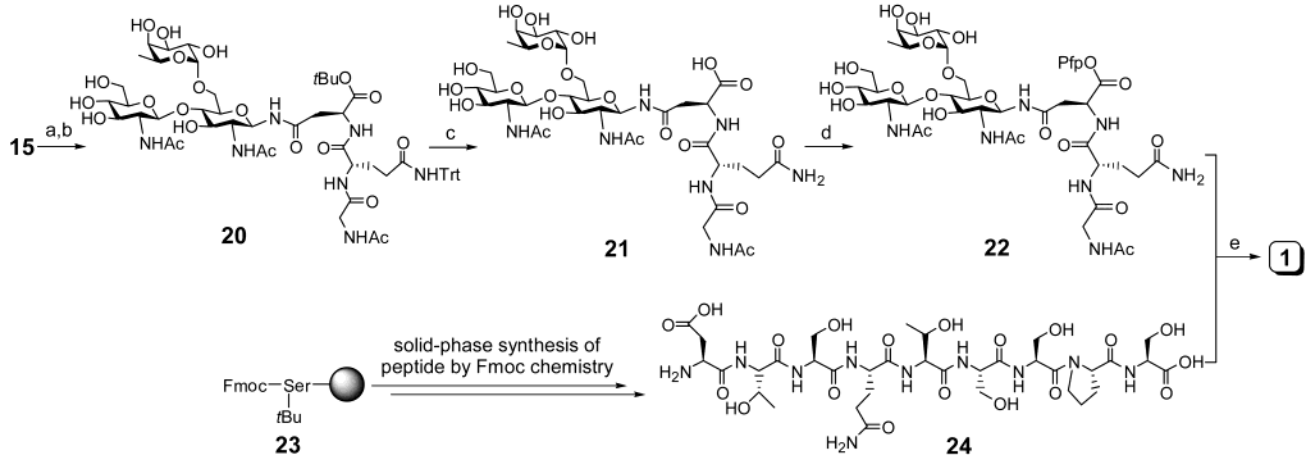
SCHEME 2[a]



[a] Reagents and conditions: (a) Fmoc-Gln(Trt)-OPfp, NMP, rt, 2 h, 96%; (b) 20% piperidine/NMP, rt, 1 h, 98%; (c) Fmoc-Gly-OPfp, NMP, rt, 2 h, 98%; (d) TFA/DCM/Et_3SiH (3/17/2), rt, 2 h, >98%; (e) DCC, HOBt, NMP, 0 °C to rt, overnight, 93%; (f) 20% TFA/DCM, rt, 2.5 h; (g) 20% piperidine/NMP, rt, 1 h, 71% (two steps overall, after HPLC).

SCHEME 3[a]



[a] Reagents and conditions: (a) 20% piperidine/NMP, rt, 1 h; (b) Ac_2O/MeOH, 91% (2 steps); (c) 20% TFA/DCM, rt, 2 h; (d) DCC, PfpOH, NMP, rt, 5 h; (e) NMP, rt, overnight, 42% (3 steps after HPLC, 30% of **21** recovered).

the one-pot coupling protocol described for **19**, we had to use the active ester of **21** free of DCC. Thus, **21** was treated with pentafluorophenol and DCC in NMP to form **22**. The reaction was monitored with TLC. After precipitation and washing with diethyl ether, the obtained product **22** was essentially DCC and DCU-free. As such, the new strategy enabled the partial purification of the active ester without passing through a column. **22** was then coupled with **24** (3 equiv) in NMP to give **1**, which was precipitated out and finally purified by HPLC. **1** was obtained in a 42% yield, and its structure was characterized by 1D, 2D NMR and MS. As shown by the HPLC diagram (Figure 2a), **1** (R_t = 25.3 min) was easily separated from the unreacted glycotripeptide **21** (30% recovered, R_t = 8.1 min), the nonapeptide **24** (R_t = 12.2 min), and a side product (R_t = 29.3 min, ca. 12%). The NMR data for **1** and **19**, including the chemical shifts and coupling constants of all reporter signals, listed in Table 1 further support the structural assignment.

On the other hand, the side product (HPLC: R_t = 29.3 min) was identified as a diastereoisomer of **1**, probably having the asparagine racemized. The racemization could happen during the activation of **21**, but it could also happen during the reaction between the active ester **22**

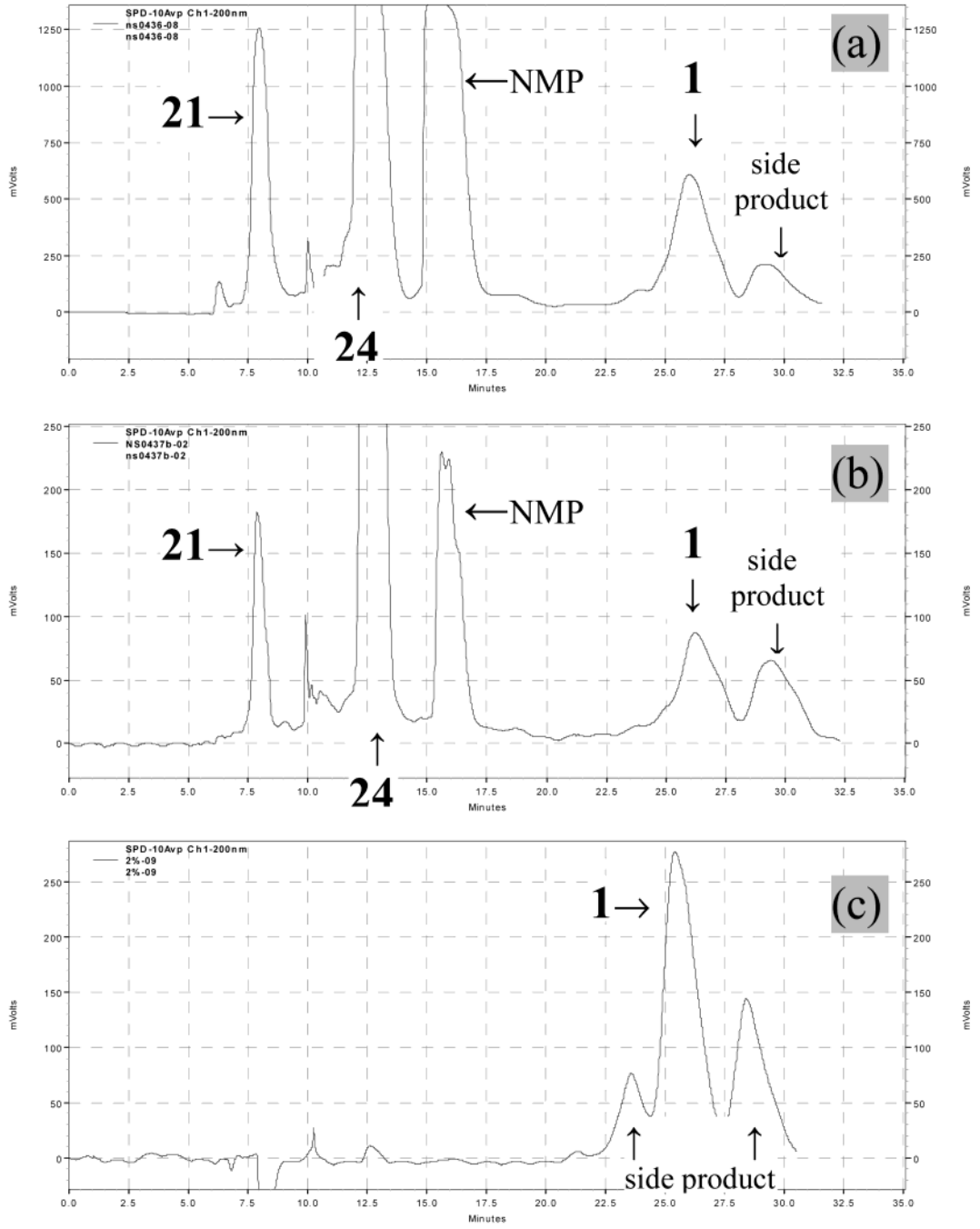


FIGURE 2. HPLC diagrams of the final product (**1**): (a) solution-phase synthesis with PfpOH/DCC as the coupling reagent; (b) solution-phase synthesis with HOBt/DCC as the coupling reagent; (c) solid-phase synthesis. HPLC conditions: semipreparative C18 column (1.5 cm × 25 cm); eluent, 0.1% TFA and 2% *i*-PrOH in H_2O; flow rate, 2 mL/min; dual wavelength detection at 200 and 220 nm.

and the free peptide **24**. Trying to answer this question, the coupling reaction between **21** and **24** with HOBt/DCC as the condensation reagent was examined.

After the reaction of **21** and HOBt/DCC was finished, the active ester was isolated by precipitation and washed with diethyl ether. It was then dissolved in NMP and reacted with **24**. HPLC analysis (Figure 2b) of the product indicated a higher conversion of **21**, while the synthetic target **1** was formed in a yield similar to that with Pfp ester. The side product was obtained in obviously higher yield (ca. 25%). The fact that the one-pot reaction between **16** and **17** with HOBt/DCC as the coupling reagent did not give the racemization product suggests that the racemization was not caused by the activation procedure. Instead, it might result from the slower reaction of the free peptide and/or the prolonged workup procedure, which can also explain why the Bt ester, which is less stable than Pfp ester, gave more of the side product.

Convergent Solid-Phase Synthesis of CD52 Glycopeptide. The target glycopeptide **1** was also prepared by coupling the unprotected glycotripeptide segment obtained via solution-phase synthesis to the nonapeptide segment on the 2-chlorotrityl resin[36] (Scheme 4). The ester bond that attaches the glycopeptide to this resin

TABLE 1. Selected NMR Data of 1, 19 and the Side Product

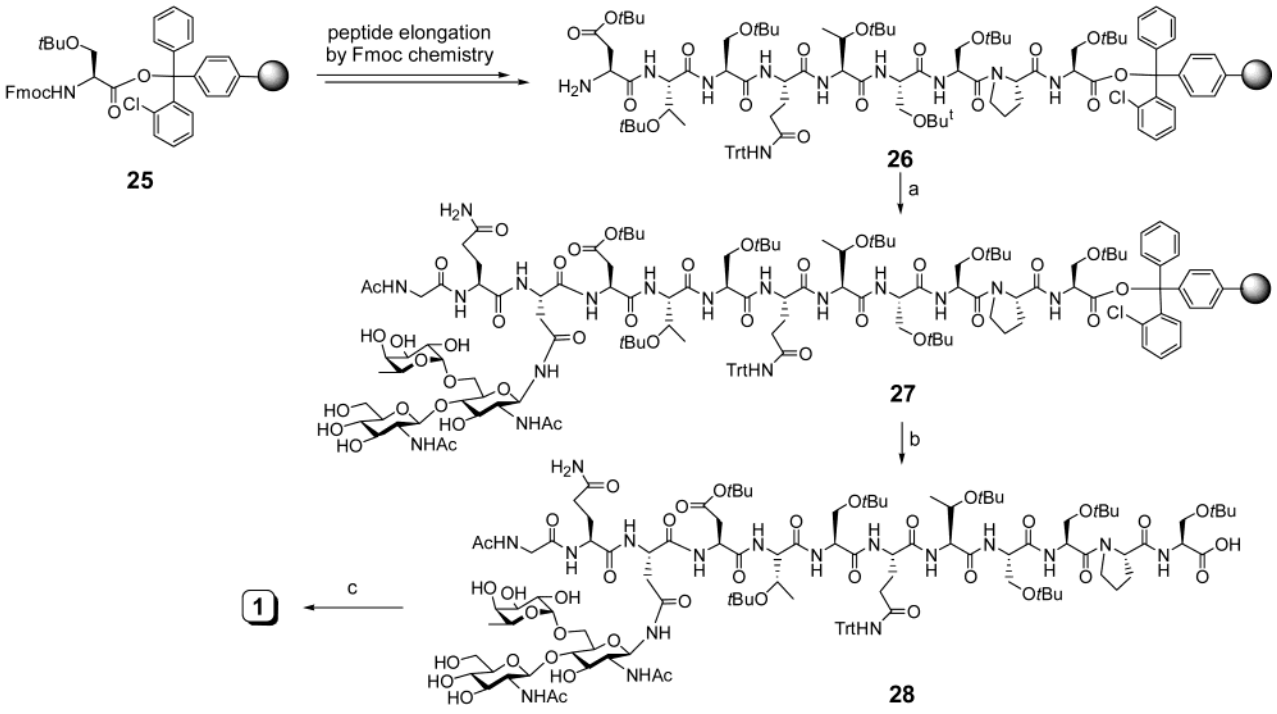
	chemical shift (*J* in Hz)		
proton signal	**1**	**19**	side product
Glc-1	5.03 (9.6)	5.04 (9.6)	5.04
Glc-1	4.66 (8.4)	4.66 (8.4)	4.66
Fuc-1	4.88 (3.6)	4.88 (4.2)	
Fuc-5	4.12 (6.0)	4.13 (6.6)	4.11
Fuc-6	1.21	1.20 (6.6)	1.21
Glu^2-α	4.31	4.33 (9.6)	
Glu^2-*β*	2.19−1.96	2.10	2.19
	2.19−1.96	2.00	2.13
Glu^2-*γ*	2.40−2.29	2.38 (7.8)	2.40
Asn^3-α	4.73 (6.0, 7.2)	4.76 (6.0, 7.2)	
Asn^3-*β*	**2.87 (6.0, 13)**	**2.85 (7.2, 17)**	**2.90**
	2.78 (7.2, 13)	**2.79 (7.2, 17)**	**2.73**
Asp^4-α	4.79 (6.0, 7.8)	4.80 (6.0, 7.2)	
Asp^4-*β*	**2.95 (6.0, 17)**	**2.93 (6.0, 16)**	**3.00**
	2.84 (7.8, 17)	**2.88 (6.0, 16)**	**2.87**
Thr^5-α	4.38 (4.2)	4.37 (4.2)	
Thr^5-*β*	4.30	4.24 (6.6, 4.2)	
Thr^5-*γ*	1.21	1.20 (6.6)	
Ser^6-α	4.82 (6.6, 6.6)	4.96 (6.0, 6.0)	
Glu^7-α	4.26	-	
Thr^8-α	4.41	-	
$Ser^{9,10,12}$-α	4.52, 4.47, 4.45	-	
Pro^{11}-α	4.50	-	
Ac	2.07, 2.07, 2.01	2.08, 2.02	

can be easily cleaved under very mild acidic conditions that would not affect oligosaccharide structures.

The synthesis started from the commercially available Ser-loaded resin **25**. Conventional Fmoc chemistry was utilized to construct the nonapeptide on an automatic peptide synthesizer with 2-(1*H*-9-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) as the condensation reagent. Thereafter, the glycotripeptide **21** was coupled to **26** under the influence of DCC/HOBt in NMP by the one-pot protocol to afford the resin-bound glycopeptide **27**. The coupling reaction yield was no less than 76%, based on comparing the weight gain of the resin, which reflected the quantity of **21** attached to the resin, to the actual peptide loading on the resin. **27** was then treated with HOAc/TFE/DCM (1/1/8) to release the glycopeptide **28** (92%). This condition was mild enough to leave the *t*Bu and Trt groups intact, so the product could be purified by silica gel column chromatography. Finally, treatment of **28** with TFA/DCM/Et_3SiH afforded **1** in a good yield (70% after HPLC). Its spectroscopic and HPLC properties were identical with those of the product prepared by solution-phase synthesis. HPLC (Figure 2c) showed the same racemization product obtained above with solution-phase synthesis. Its proportion was between that shown in panels a and b of Figure 2, which may suggest that the slower reaction between the active ester and resin-bound peptide is the main cause of active ester reacemization.

In summary, CD52 glycopeptides with a fucosylated trisaccharide were prepared by convergent solution-phase and solid-phase syntheses. Homogeneous CD52 glycopeptide samples were obtained in sufficient amounts for their structural and structure−activity relationship studies. The acid-labile fucose linkage proved to be stable to all reactions involved. The results of coupling between the glycopeptide and peptide segments varied. The reaction of the glycotripeptide and a protected peptide **17** in solution with HOBt/DCC as the coupling reagent gave excellent yield of the expected product, while a similar reaction in solid-phase synthesis resulted in some racemization products. Glycopeptide activation with PfpOH/DCC seemed to cause fewer problems than that with DCC/HOBt. Nonetheless, the final products were easily purified. The advantage of the solution-phase synthetic

SCHEME 4[a]



[a] Reagents and conditions: (a) **21**, DCC, HOBt, NMP, rt, overnight, 76%; (b) HOAc/TFE/DCM (1/1/8), rt, 2 h, 92%; (c) TFA/DCM/Et_3SiH (4/16/2), rt, 3 h, 70% (after HPLC).

method is that it can avoid operations such as the retrieval of glycopeptides and the deprotection of peptide side chains, and it is applicable to the preparation of large glycoproteins. The advantage of solid-phase glycopeptide−peptide coupling is that the unreacted glycotripeptide can be readily recovered and reused. The best result was obtained with a one-pot protocol of solution-phase coupling between the glycopeptide and a protected peptide. Nevertheless, the optimization of the coupling protocols and the application of solution-phase glycopeptide−peptide coupling to the preparation of complex glycoproteins is under further study.

Experimental Section

***O*-(2,3,4-Tri-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl)-(1→4)-6-*O*-(*tert*-butyldiphenylsilyl)-3-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl Azide (5).** To a solution of 2,3,4-tri-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl acetate (3.74 g, 7.8 mmol) in DCM (20 mL) was added 33% HBr in HOAc (3.0 mL) at 0 °C. After being stirred at room temperature for 4 h, the reaction mixture was washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated in a vacuum to give the glycosyl bromide **4** as a syrup that was directly used without further purification. After a mixture of **4**, **3** (2.5 g, 3.8 mmol), and MS 4 Å (4 g) in dry DCM (20 mL) was stirred at room temperature for 2 h, it was cooled to −40 °C. To this mixture was added AgOTf (2.33 g, 9.0 mmol). The mixture was then warmed to room temperature and stirred overnight. The solution was filtered off, washed with brine, dried over Na_2SO_4, and then concentrated in a vacuum. Column chromatography of the residue afforded **5** as a white foam (3.0 g, 75%). $[\alpha]_D$ +13.6 (*c* 1.0, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.88−7.65 (m, 13 H), 7.60−7.40 (m, 5 H), 7.07−7.02 (m, 2 H), 6.88−6.82 (m, 3 H), 5.90 (dd, J = 9.0, 10.8 Hz, 1 H), 5.72 (d, J = 8.4 Hz, 1 H), 5.24−5.14 (m, 2 H), 4.88 (d, J = 12.4 Hz, 1 H), 4.57−4.34 (m, 4 H), 4.20 (dd, J = 8.4, 10.6 Hz, 1 H), 4.07 (dd, J = 2.0, 12.2 Hz, 1 H), 4.01 (dd, J = 9.4, 10.5 Hz, 1 H), 3.90−3.70 (m, 3 H), 3.28 (d, J = 9.5 Hz, 1 H), 2.02 (s, 3 H), 1.87 (s, 3 H), 1.15 (s, 9 H).

***O*-(2,3,4-Tri-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl)-(1→4)-3-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl Azide (6).** After **5** (3.0 g, 2.8 mmol) was dissolved in THF (15 mL), to the solution were added TBAF (1 M solution in THF, 5.0 mL, 5.0 mmol) and acetic acid (300 μL, 5.0 mmol) at 0 °C. The solution was then warmed to room temperature and stirred for 72 h. The reaction mixture was diluted with EtOAc (200 mL), and the organic layer was washed with saturated $NaHCO_3$ (150 mL) and water (150 mL), dried over Na_2SO_4, and finally concentrated under vacuum to dryness. The residue was purified on a silica gel column to afford **6** as white foam (2.05 g, 87%). $[\alpha]_D$ +10.8 (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94−7.65 (m, 8 H), 7.14−6.98 (m, 2 H), 6.91−6.82 (m, 3 H), 5.81 (dd, J = 9.1, 10.6 Hz, 1 H), 5.67 (d, J = 8.4 Hz, 1 H), 5.17 (dd, J = 9.2, 9.2 Hz, 1 H), 4.87 (d, J = 12.6 Hz, 1 H), 4.44 (d, J = 12.7 Hz, 1 H), 4.39−4.26 (m, 3 H), 4.14−3.96 (m, 3 H), 3.87−3.81 (m, 1 H), 3.74−3.69 (m, 1 H), 3.50−3.40 (m, 2 H), 2.01 (s, 3 H), 1.99 (s, 3 H), 1.85 (s, 3 H). HRFAB-MS calcd for $C_{41}H_{39}N_5O_{15}Na$ (M + Na^+) 864.2340, found 864.2332.

***O*-(2,3,4-Tri-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl)-(1→4)-[*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→6)]-3-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl Azide (9).** After the mixture of **8** (957 mg, 2.0 mmol), **6** (670 mg, 0.8 mmol), Bu_4NBr (650 mg, 2.0 mmol), and MS 4 Å (4 g) in dry CH_2Cl_2/DMF (18 mL, 5/1) was stirred at room temperature for 2 h, $CuBr_2$ (450 mg, 2.0 mmol) was added and the reaction mixture was stirred at room temperature overnight. The solution was filtered off, washed with brine, and dried and then concentrated in a vacuum. Column chromatography of the residue gave **9** as a white solid (1.06 g, 85%). $[\alpha]_D$ −58.1 (*c* 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.91−7.90 (m, 2 H), 7.78−7.76 (m, 3 H), 7.68−7.64 (m, 2 H), 7.56−7.54 (m, 3 H), 7.48−7.40 (m, 4 H), 7.36−7.23 (m, 9 H), 6.98−6.96 (m, 2 H), 6.79−6.78 (m, 3 H), 5.69 (dd, J = 9.6, 10.8 Hz, 1 H), 5.60 (d, J = 8.4 Hz, 1 H), 5.15−5.12 (m, 3 H), 5.10 (d, J = 3.6 Hz, 1 H), 5.05 (dd, J = 9.6, 10.2 Hz, 1 H), 4.98 (d, J = 11.4 Hz, 1 H), 4.95 (d, J = 11.4 Hz, 1 H), 4.87 (d, J = 11.4 Hz, 1 H), 4.82 (dd, J = 11.4 Hz, 12.0, 2 H), 4.63 (d, J = 11.4 Hz, 1 H), 4.44 (d, J = 13.2 Hz, 1 H), 4.30−4.26 (m, 2 H), 4.16−4.08 (m, 3 H), 4.02−3.95 (m, 3 H), 3.90 (dd, J = 2.4, 12.0 Hz, 1 H), 3.78 (d, J = 10.2 Hz, 1 H), 3.70 (d, J = 2.4 Hz, 1 H), 3.41−3.37 (m, 2 H), 3.30 (dd, J = 2.4, 10.8 Hz, 1 H), 1.90 (s, 3 H), 1.85 (s, 3 H), 1.82 (s, 3 H), 1.07 (d, J 6.6 Hz, 3 H). FAB-MS calcd for $C_{68}H_{67}N_5O_{19}$ 1257.4, found 1256.7 (M + H^+).

***O*-(2-Deoxy-2-acetamido-*β*-D-glucopyranosyl)-(1→4)-[*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→6)]-3-*O*-benzyl-2-deoxy-2-acetamido-*β*-D-glucopyranosyl Azide (10).** After the mixture of **9** (1.06 g, 0.78 mmol), ethylenediamine (2 mL, 29 mmol), and *n*-butanol (10 mL) was stirred at 90 °C overnight, it was concentrated to dryness under vacuum. The residue was dissolved in Ac_2O/pyridine (16 mL, 1/2) and stirred overnight at room temperature. After the reaction mixture was diluted with EtOAc (150 mL), the solution was washed with saturated $NaHCO_3$ (80 mL) and water (50 mL), dried over Na_2SO_4, and concentrated in a vacuum to dryness. The residue was purified on a silica gel column. After the product was treated with MeOH (10 mL) containing NaOMe (0.05 M) at room temperature for 3 h, it was neutralized to pH 6−7 with Amberlyst 15 resin. The resin was filtered off and washed with MeOH, and the washings were combined and concentrated. The crude product was purified on a silica gel column to give **10** as a white foam (640 mg, 86%). $[\alpha]_D$ −64.5 (*c* 1.0, CH_3OH). ^{1}H NMR (600 MHz, CD_3OD) δ 7.40−7.21 (m, 20 H), 5.04 (d, J = 10.8 Hz, 1 H), 4.89 (d, J = 3.6 Hz, 1 H), 4.86−4.76 (m, 6 H), 4.62 (d, J = 11.4 Hz, 1 H), 4.54 (d, J = 10.8 Hz, 1 H), 4.48 (d, J = 9.0 Hz, 1 H), 4.10 (q, J = 6.6 Hz, 1 H), 4.04 (dd, J = 9.6, 9.6 Hz, 1 H), 4.01 (dd, J = 3.0, 10.8 Hz, 1 H), 3.96 (dd, J = 3.0, 10.2 Hz, 1 H), 3.88−3.72 (m, 6 H), 3.60−3.40 (m, 5 H), 3.25 (dd, J = 9.6, 9.6 Hz, 1 H), 1.97 (s, 3 H), 1.91 (s, 3 H), 1.10 (d, J = 6.6 Hz, 3 H). HRFAB-MS calcd for $C_{50}H_{62}O_5N_{14}$ (M + H^+) 956.4293, found 956.4307.

***N*$^{\alpha}$-(9-Fluroenylmethoxycarbonyl)-*N*$^{\gamma}$-[*O*-(2-deoxy-2-acetamido-*β*-D-glucopyranosyl)-(1→4)-[*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→6)]-3-*O*-benzyl-2-deoxy-2-acetamido-*β*-D-glucopyranosyl]-L-asparagine *tert*-Butyl Ester (11).** To a solution of Fmoc-Asp-O*t*Bu in CH_2Cl_2 (10 mL) and NMP (1 mL) was subsequently added HOBt and DCC at room temperature. The mixture was stirred at room temperature for 1 h to give the active ester solution after filtration to remove the solid DCU. A mixture of **10** (110 mg, 0.115 mmol), Lindlar catalyst (110 mg), and MeOH (5 mL) was stirred at room temperature for 2.5 h under a H_2 atmosphere. After filtration through Celite, the solution was concentrated in a vacuum, and to the residue were added CH_2Cl_2 (2 mL) and the freshly prepared active ester. The mixture was stirred at room temperature overnight, concentrated, and purified on a silica gel column to give **11** as a white foam (132 mg, 87%). $[\alpha]_D$ −26.2 (*c* 1.0, CH_2Cl_2/MeOH 1/1). ^{1}H NMR (600 MHz, CD_3OD) δ 7.77 (d, J = 7.2 Hz, 2 H), 7.61 (d, J = 7.8 Hz, 2 H), 7.38−7.22 (m, 24 H), 5.04 (d, J = 10.8 Hz, 1 H), 4.97 (d, J = 10.2 Hz, 1 H), 4.93 (d, J = 3.0 Hz, 1 H), 4.88−4.74 (m, 4 H), 4.76 (d, J = 8.4 Hz, 1 H), 4.67 (d, J = 8.4 Hz, 1 H), 4.62 (d, J = 11.4 Hz, 1 H), 4.54 (d, J = 11.4 Hz, 1 H), 4.42 (dd, J = 5.4, 6.0 Hz, 1 H), 4.33 (dd, J = 7.2, 10.8 Hz, 1 H), 4.24 (dd, J = 7.2, 10.8 Hz, 1 H), 4.16 (dd, J = 7.2, 6.6 Hz, 1 H), 4.10 (q, J = 6.6 Hz, 1 H), 3.98−3.90 (m, 4 H), 3.86−3.74 (m, 5H), 3.58 (dd, J = 9.6, 9.6 Hz, 1 H), 3.53−3.17 (m, 5 H), 2.63 (d, J = 6.6 Hz, 2

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H), 1.93 (s, 3 H), 1.85 (s, 3 H), 1.41 (s, 9 H), 1.12 (d, J = 6.6 Hz, 3 H). FAB-MS calcd for $C_{73}H_{86}O_4N_{19}$ 1322.6, found 1323.3 ($M + H^+$).

***N*$^{\gamma}$-[*O*-(2-Deoxy-2-acetamido-*β*-D-glucopyranosyl)-(1→4)-[*O*-(α-L-fucopyranosyl)-(1→6)]-2-deoxy-2-acetamido-*β*-D-glucopyranosyl]-L-asparagine *tert*-Butyl Ester (12).** After **11** (132 mg, 0.1 mmol) in MeOH (6 mL) and H_2O (2 mL) was stirred with $Pd(OH)_2$ (50 mg) under a H_2 atmosphere at room temperature for 20 h, the mixture was filtered off through a Celite pad, and the catalyst was washed with water. The filtrates were combined and concentrated in a vacuum to remove MeOH. The resulting water solution (5 mL) was washed with Et_2O (10 mL) twice and lyophilized to afford **12** (71 mg, 96%) as a white solid. ^{1}H NMR (300 MHz, D_2O) δ 5.04 (d, J = 9.6 Hz, 1 H), 4.89 (d, J = 3.9 Hz, 1 H), 4.67 (d, J = 8.5 Hz, 1 H), 4.29 (dd, J = 4.4, 4.7 Hz, 1 H), 4.12 (q, J = 6.4 Hz, 1 H), 4.95−3.46 (m, 15 H), 3.10 (dd, J = 4.9, 17.5 Hz, 1 H), 2.92 (dd, J = 4.5, 17.5 Hz, 1 H), 2.08 (s, 3 H), 2.03 (s, 3 H), 1.48 (s, 9 H), 1.21 (d, J = 6.6 Hz, 3 H). HRFAB-MS calcd for $C_{30}H_{53}O_4N_{17}$ ($M + H^+$) 741.3406, found 741.3411.

Glycopeptide 16. To the solution of **12** (92 mg, 0.125 mmol) in NMP (2 mL) was added Fmoc-Gln(Trt)-OPfp (284 mg, 0.375 mmol), and the solution was stirred at room temperature for 2 h. Et_2O (20 mL) was added to the solution with gentle stirring to get a white precipitate. The mixture was then centrifuged and the solvent removed by pipet. The precipitate was washed with Et_2O (5 mL) twice. After being dried under a flash of nitrogen, the crude product **13** (160 mg, 96%) was dissolved in NMP (1.6 mL) and piperidine (0.4 mL). The reaction mixture was stirred at room temperature for 1 h. The deprotection product was again precipitated and washed following the same procedure described above to give the crude product as a white solid (130 mg, 97.5%). This product was again dissolved in NMP (2 mL) and Fmoc-Gly-OPfp (216 mg, 0.466 mmol) was added. Two hours later precipitation of the product gave glycotripeptide **15** as a white solid (160 mg, 98%). After the crude **15** (10 mg) was dissolved in TFA/CH_2Cl_2/Et_3-SiH (0.3/1.7/0.2 mL) and stirred at room temperature for 2 h, toluene (4 mL) was added. The mixture was concentrated under vacuum, and the residue was dissolved in H_2O (5 mL) and washed with Et_2O (5 mL) twice. The water solution was finally lyophilized to give **16** as a white solid, which was used in the next step of reaction without further purification.

Tripeptide 17. Tripeptide **17** was prepared by conventional solution-phase synthesis using the DCC/HOBt protocol and Fmoc chemistry. $[\alpha]_D$ +38.5 (*c* 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C) δ 7.75 (d, J = 7.2 Hz, 2 H), 7.68 (d, J = 7.2 Hz, 1 H), 7.59 (dd, J = 6.6, 6.6 Hz, 2 H), 7.48 (d, J = 5.4 Hz, 1 H), 7.39 (dd, J = 7.2, 7.8 Hz, 2 H), 7.32−7.30 (m, 2 H), 5.98 (d, J = 8.4 Hz, 1 H), 5.01 (dd, J = 7.8, 13.2 Hz, 1 H), 4.58 (br s, 1 H), 4.39 (br s, 2 H), 4.31 (dd, J = 3.6, 6.0 Hz, 1 H), 4.24 (dd, J = 6.6, 7.2 Hz, 1 H), 4.16 (br s, 1 H), 3.54 (dd, J = 5.4, 8.4 Hz, 1 H), 3.39 (dd, J = 7.8, 8.4 Hz, 1 H), 3.09 (s, 3 H), 2.95 (s, 3 H), 2.95−2.92 (m, 2 H), 2.64 (dd, J = 5.4, 16.8 Hz, 1 H), 1.45 (s, 9 H), 1.27 (s, 9H), 1.11 (s, 9 H), 1.03 (d, J = 6.0 Hz, 3 H). HRFAB-MS calcd for $C_{40}H_{59}O_9N_4$ ($M + H^+$) 739.4282, found 739.4286.

Glycopeptide 19. To a mixture of glycopeptide **16** (5 mg, 0.0046 mmol), tripeptide **17** (15 mg, 0.029 mmol), and HOBt (7 mg, 0.052 mmol) in DMF (1 mL) was added DCC (10 mg, 0.048 mmol) at 0 °C under N_2 atmosphere. After the solution was stirred at room temperature overnight, the product was precipitated out by Et_2O to give **18** as a white solid (6.77 mg, 93%). It was then treated with TFA/CH_2Cl_2 (0.4/1.6 mL) at room temperature for 2.5 h, whereupon toluene (4 mL) was added and the solution was concentrated in a vacuum. The residue was treated with piperidine/DMF (0.2/0.8 mL) at room temperature for 1 h, and addition of Et_2O (10 mL) to the reaction mixture afforded the crude product that was further purified by HPLC with use of a C18 column (1.5 cm × 25 cm) with 1.5% *i*-PrOH in water containing 0.1% TFA as the eluent (2 mL/min) to give **19** as a white solid (3.6 mg, 71%) after freeze-drying. HPLC: R_t = 16.5 min. ^{1}H NMR (600 MHz, D_2O, DHO at δ 4.79 as reference, 25 °C) δ 5.04 (d, J = 9.6 Hz, 1 H, GlcNAc-1), 4.96 (dd, J = 6.0, 6.0 Hz, 1 H, Ser-α), 4.88 (d, J = 4.2 Hz, 1 H, Fuc-1), 4.80 (dd, J = 6.0, 7.2 Hz, 1 H, Asp-α), 4.76 (dd, J = 6.0, 7.2 Hz, 1 H, Asn-α), 4.66 (d, J = 8.4 Hz, 1 H, GlcNAc-1), 4.37 (d, J = 4.2 Hz, 1 H, Thr-α), 4.33 (dd, J = 6.0, 8.4 Hz, 1 H, Glu-α), 4.24 (dt, J = 6.0, 11.4 Hz, 1 H, Thr-*β*), 4.13 (t, J = 6.6 Hz, 1 H, Fuc-5), 3.94−3.73 (m, 13 H), 3.70−3.65 (m, 2 H), 3.56 (dd, J = 4.8, 6.6 Hz, 1 H), 3.51−3.45 (m, 2 H), 3.16 (s, 3 H, NMe), 2.96 (s, 3 H, NMe), 2.93 (dd, J = 6.0, 16.2 Hz, 1 H, Asp-*β*), 2.85 (dd, J = 7.2, 16.8 Hz, 1 H, Asn-*β*), 2.88 (dd, J = 6.0, 16.2 Hz, 1 H, Asp-*β*), 2.79 (dd, J = 7.2, 16.8 Hz, 1 H, Asn-*β*), 2.38 (t, J = 7.8 Hz, 2 H, Glu-*γ*), 2.10 (m, 1 H, Glu-*β*), 2.08 (s, 3H, Ac), 2.02 (s, 3 H, Ac), 2.00 (m, 1 H, Glu-*β*), 1.20 (2 d, J = 6.6 Hz, 6 H, Thr-*γ* and Fuc-6). MALDI-TOF-MS calcd for $C_{46}H_{77}O_{11}N_{26}$ 1200.5, found 1223.8 ($M + Na^+$), 1239.6 ($M + K^+$).

Glycopeptide 20. Glycopeptide **15** (16 mg, 0.0115 mmol) was treated with piperidine/DMF (1 mL) at room temperature for 1 h, and the deprotected product was precipitated out by Et_2O (10 mL). To the crude product were added MeOH (2 mL) and Ac_2O (0.1 mL), and the reaction mixture was stirred at room temperature for 2 h. Addition of Et_2O (20 mL) to the reaction mixture gave **20** as a white precipitate (12.6 mg, 91%).

Nonapeptide 24. An Fmoc-protected nonapeptide linked to resin (648 mg) was synthesized after eight cycles of the standard synthesizer program of condensation with HATU-DIEA activated Fmoc amino acid (1 mmol each) starting from Fmoc-Ser-loaded HMP resin (325 mg, 0.25 mmol). A part of the resin (34 mg) was treated with 20% piperidine in NMP at room temperature for 2 h, and then the resin was washed with NMP, DCM, and Et_2O. It was treated with a mixture of TFA (1.9 mL), $HSCH_2CH_2SH$ (0.05 mL), and water (0.05 mL) at room temperature for 2 h. The mixture was filtered off and the filtrate was concentrated under vacuum. The residue was suspended in water (5 mL) and extracted with Et_2O (3 × 5 mL). The water solution was concentrated and dissolved in methanol/AcOH (0.5 mL/0.1 mL). On addition of Et_2O (15 mL), **24** was obtained as a white solid (14 mg) after washing with Et_2O and drying. ^{1}H NMR (600 MHz, D_2O, DHO at δ 4.79 as reference, 25 °C) δ 4.83 (m, 2 H), 4.54−4.40 (m, 9 H), 4.29−4.25 (m, 2 H), 3.96−3.73 (m, 10 H), 3.01 (dd, J = 5.4, 18.0 Hz, 1 H), 2.93 (dd, J = 7.2, 18.0 Hz, 1 H), 2.39 (dd, J = 7.2, 7.8 Hz, 2 H), 2.32 (m, 1 H), 2.17 (m, 1 H), 2.07−1.99 (m, 4 H), 1.25 (d, J = 6.0 Hz, 3 H), 1.21 (d, J = 6.0 Hz, 3 H). MALDI-TOF-MS calcd for $C_{34}H_{56}O_{19}N_{10}$ 908.9, found 932.3 ($M + Na^+$).

Glycopeptide 1. The *tert*-butyl and trityl groups of **20** (5 mg, 0.005 mmol) were removed with 20% TFA/DCM as described for **16** to afford **21**, which was directly used in the next step of reaction without further purification. To the mixture of **21** obtained above and pentafluorophenol (9 mg, 0.05 mmol) in NMP (1 mL) was added DCC (21 mg, 0.1 mmol), and the solution was stirred at room temperature for 5 h. The reaction product was precipitated out by Et_2O followed by washing. After the crude product was dissolved in NMP, the free nonapeptide **24** (10 mg, 0.011 mmol) was added, and the reaction mixture was stirred at room temperature for 12 h. Et_2O was added to the reaction mixture to give the solid product, which was finally purified by HPLC with use of a C18 column (1.5 cm × 25 cm) with 2% *i*-PrOH in water containing 0.1% TFA as the eluent (2 mL/min). A part of the nonapeptide **24** (5 mg, 0.005 mmol, R_t = 12.2 min) and the glycotripeptide **21** (1.9 mg, 0.0015 mmol, R_t = 8.1 min) were recovered. In addition to the synthetic target **1** (R_t = 25.3 min) obtained as a white solid after freeze-drying (3.8 mg, 0.0021 mmol, 42%), HPLC also gave another product (R_t = 29.3 min, 1.0 mg, 12%) identified as a stereoisomer of **1**. **1**: ^{1}H NMR (600 MHz, D_2O, DHO at δ 4.79 as reference, 25 °C): δ 5.03 (d, J = 9.6 Hz, 1 H, GlcNAc-1), 4.88 (d, J = 3.6 Hz, 1 H, Fuc-1), δ 4.82 (dd, J = 6.6, 6.6 Hz, 1 H, Ser-α), 4.79 (dd, J = 7.8, 7.8 Hz, 1 H, Asp-α), 4.73 (dd, J = 6.0, 7.2 Hz, 1 H, Asn-α), 4.66 (d, J = 8.4 Hz, 1 H, GlcNAc-1), 4.52 (dd, J = 5.4, 10.8 Hz, 1 H, Ser-α), 4.50 (dd, J

= 4.2, 3.6 Hz, 1 H, Pro-α), 4.47 (dd, *J* = 5.4, 5.4 Hz, 1 H, Ser-α), 4.45−4.43 (m, 2 H, Thr-*β* and Ser-α), 4.41 (d, *J* = 4.2 Hz, 1 H, Thr-α), 4.38 (d, *J* = 4.2 Hz, 1 H, Thr-α), 4.31−4.29 (m, 2 H, Thr-*β* and Glu-α), 4.26 (dd, *J* = 4.8, 6.0 Hz, 1 H, Glu-α), 4.12 (q, *J* = 6.0 Hz, 1 H, Fuc-5), 3.97−3.45 (m, 29H), 2.95 (dd, *J* = 6.0, 16.8 Hz, 1 H, Asp-*β*), 2.89−2.82 (m, 2 H, Asn-*β* and Asp-*β*), 2.78 (dd, *J* = 7.2, 12.8 Hz, 1 H, Asn-*β*), 2.40−2.29 (m, 5 H), 2.19−1.96 (m, 7 H), 2.07 (s, 3 H, Ac), 2.07 (s, 3H, Ac), 2.01 (s, 3 H, Ac), 1.22−1.19 (m, 9 H, 2 Thr-*γ* and Fuc-6). HMQC (^{13}C 150 MHz, ^{1}H 600 MHz, D_2O, 25 °C) *δ* 80.6/5.03 (GlcNAc-1), 101.5/4.88 (Fuc-1), 55.8/4.82 (Ser-α), 53.0/4.79 (Asp-α), 52.2/4.73 (Asn-α), 103.1/4.66 (GlcNAc-1), 57.6/4.52 (Ser-α), 62.9/4.50 (Pro-α), 58.2/4.47 (Ser-α), 55.2/4.45 (Thr-*β*), 61.3/4.43 (Ser-α), 61.5/4.41 (Thr-α), 58.7/4.38 (Thr-α), 69.1/4.32 (Gln-α), 55.5/4.31 (Thr-*β*), 69.2/4.26 (Gln-α), 69.0/4.12 (Fuc-5), 38.6/2.87 (Asp-*β*), 38.5/2.79 (Asn-*β*), 33.2/2.37 (Gln-*γ*), 31.4/2.31 (Pro), 28.9/2.18 (Gln-*γ*), 28.8/2.09 (Gln-*β*), 24.3/2.07 (Ac), 23.8/2.07 (Ac), 26.6/2.05 (Pro), 31.4/2.03 (Pro), 24.2/2.01 (Ac), 28.8/1.99 (Gln-*β*), 20.8/1.23 (Thr-*γ*), 17.5/1.22 (Thr-*γ*), 28.4/1.19 (Fuc-6). MALDI-TOF-MS calcd for $C_{69}H_{111}O_{39}N_{17}$ 1825.6, found 1825.4 ($M + Na^+$). The side product: ^{1}H NMR (600 MHz, D_2O, DHO at *δ* 4.79 as reference, 25 °C) *δ* 5.04 (d, *J* = 9.0 Hz, 1 H), 4.88 (br, 1 H), 4.70 (m, 1 H), 4.66 (d, *J* = 8.4 Hz, 1 H), 4.54−4.51 (m, 2 H), 4.48 (t, *J* = 5.4 Hz, 1 H), 4.45 (dd, *J* = 5.4, 7.2 Hz, 1 H), 4.41 (br d, *J* = 4.2 Hz, 1 H), 4.38 (br d, *J* = 4.2 Hz, 1 H), 4.31−4.25 (m, 3 H), 4.12 (q, *J* = 6.0 Hz, 1 H), 3.98 (dd, *J* = 4.8, 3.0 Hz, 1 H), 3.92 (br d, *J* = 3.6 Hz, 2 H), 3.91−3.68 (m, 28 H), 3.66 (t, *J* = 9.0 Hz, 1 H), 3.49 (m, 1 H), 3.00 (dd, *J* = 6.0, 17.9 Hz, 1 H), 2.90−2.86 (m, 2 H), 2.73 (dd, *J* = 8.4, 16.2 Hz, 1 H), 2.41−2.31 (m, 6 H), 2.18 (m, 1 H), 2.11−1.96 (m, 6 H), 2.08 (s, 3 H), 2.07 (s, 3H), 2.02 (s, 3 H), 1.22−1.19 (m, 9 H).

Resin-Bonded Glycopeptide (27). Starting from Fmoc-Ser-loaded 2-chlorotrityl resin (417 mg, 0.25 mmol), a non-apeptide linked to the resin (661 mg) was prepared on an automatic peptide synthesizer after eight cycles of peptide elongation following the standard Fmoc chemistry with HATU-DIEA as the condensation regent. A part of the resin (54.3 mg) was treated with 20% piperidine/NMP at room temperature for 2 h. After the mixture was washed and dried, the resin was mixed with **21** (15 mg, 0.016 mmol) and HOBt (15 mg, 0.11 mmol) in NMP (1 mL). The mixture was cooled to 0 °C under N_2, and then DCC (30 mg, 0.14 mmol) was added. The reaction mixture was allowed to warm to room temperature and stirred overnight. The resin was washed with NMP and Et_2O thoroughly and dried under vacuum to give a yellowish resin **27** (58 mg, 76%).

Glycopeptide 28. After the resin-bonded glycopeptide **27** was treated with HOAc/TFE/DCM (0.1 mL/0.1 mL/0.8 mL) at room temperature for 2 h, it was filtered off and the filtrate was concentrated. The residue was purified on a silica gel column (eluent MeOH/DCM/EtOAc 1/1/1) to give glycopeptide **28** (28 mg, 92%). MALDI-TOF-MS calcd for $C_{116}H_{181}O_{39}N_{17}$ 2436.3, found 2459.4 ($M + Na^+$).

Glycopeptide 1. After glycopeptide **28** (4 mg, 1.64 *μ*mol) was treated with TFA/DCM/Et_3SiH (0.4/1.6/0.2 mL) at room temperature for 3 h, to the mixture was added toluene (5 mL). The mixture was concentrated in a vacuum, and the residue was dissolved in water (5 mL) and extracted with Et_2O (3 × 3 mL). After the water solution was lyophilized, the product was purified by HPLC as described above to give glycopeptide **1** (2 mg, 70%) as a white solid.

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Supporting Information Available: The general methods of the experiments and selected ^{1}H, COSY and HMQC NMR and MS spectra of the intermediates and the final product. This material is available free of charge via the Internet at http://pubs.acs.org.

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