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Orthogonally Protected Cyclo-β-tetrapeptides as Solid-Supported Scaffolds for the Synthesis of Glycoclusters

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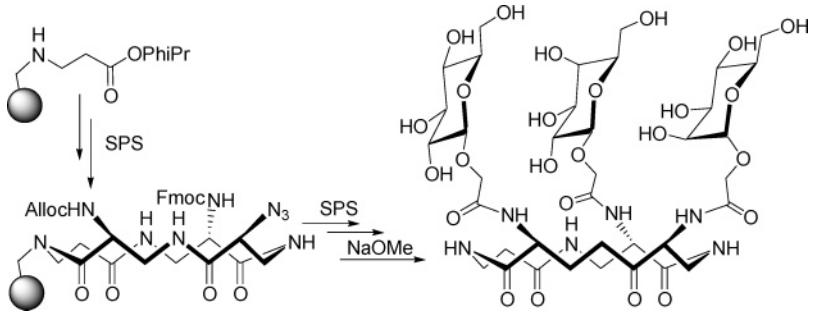

Two novel peptide scaffolds, viz. cyclo[(*N*α-Alloc)Dpr-β-Ala-(*N*α-Fmoc)Dpr-β-Ala] (**1**) and cyclo[(*N*α-Alloc)Dpr-α-azido-β-aminopropanoyl-(*N*α-Fmoc)Dpr-β-Ala] (**2**), composed of orthogonally protected 2,3-diaminopropanoyl (Dpr) and β-alanyl residues, have been described. Fmoc chemistry on a backbone amide linker derivatized resin has been used for the chain assembly. Selective removal of the 4-methyltrityl (Mtt) and 1-methyl-1-phenylethyl protections (PhiPr) exposes the β-amino and carboxyl terminus, respectively, and on-resin cyclization then gives the desired orthogonally protected cyclo-β-tetrapeptides (**1** and **2**). The α-amino groups, bearing the Fmoc and Alloc protections and the azide mask, allow stepwise orthogonal derivatization of these solid-supported cyclo-β-tetrapeptide cores (**1** and **2**). This has been demonstrated by attachments of various sugar units [viz., acetyl- or toluoyl-protected carboxymethyl α-D-glycopyranosides (**13**−**15**) and methyl 6-*O*-(4-nitrophenoxycarbonyl)-α-D-glycopyranosides (**22**−**24**)] to obtain diverse di- and trivalent glycoclusters (**33**−**42**). Acidolytic release (TFA) from the support, followed by conventional NaOMe-catalyzed transesterification (**33**−**40**) or hydrazine-induced acyl substitution in DMF (**41** and **42**), gives the fully deprotected clusters (**43**−**52**) as final products.

Introduction

Small cyclic peptides have been used as scaffolds in the construction of template assembled synthetic proteins (TASP),[1−4] where the folding of native proteins has been mimicked by appropriately aligned amphiphilic units of peptide secondary structure. The scaffold used in these studies has been a decapeptide, cyclo(KXKPGKXKPG) (X = a variable amino acid residue), incorporating two linked β-turns. Consequently, the lysine side chains used for attachment of the peptide branches are orientated to the same face of the peptide ring. The same decapeptide has also been utilized in synthesis of glycoclusters, [5−7] in which multiple carbohydrate recognition elements form a well-defined construct and offer a potential

(1) Abbreviations used: Alloc, allyloxycarbonyl; DCA, dicloroacetic acid; DCM, dichloromethane, DIEA, *N*,*N*-diisopropylethylamine; Fmoc, 9-fluorenylmethoxycarbonyl; FmocOSu, *N*-(9-fluorenylmethoxycarbonyloxy)succinimide; HATU, *N*-[(1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yloxy)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate; HFIP, 1,1,1,3,3,3-hexafluoro-isopropyl alcohol; HOBt, 1-hydroxybenzotriazole; Mtt, 4-methyltrityl; NMP, *N*-methylpyrrolidinone; Dpr, 2,3-diaminopropanoyl; PhiPr, 1-methyl-phenylethyl; Py, pyridine; PyAOP, 7-azabenzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate; PyBOP, benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate; RP, reverse phase; SPPS, solid-phase peptide synthesis; SPS, solid-phase synthesis; TASP, template assembled synthetic proteins; TBTU, 1-[bis(dimethylamino)methylene]-1*H*-benzotriazolium tetrafluoroborate 3-oxide.

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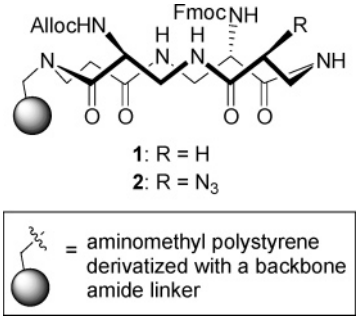


FIGURE 1. Solid-supported scaffolds **1** and **2**.

binding site for lectins. Useful tools for the cell-specific targeting or artificial receptors for soluble lectins have, hence, been obtained.

Small cyclic β-peptides (trimers and tetramers) exhibit interesting conformational properties[8−17] that make them attractive scaffolds. The additional methylene group between the amino and carboxy function in each β-amino acid residue orientates the amide moieties in a unidirectional manner, with NH and CO groups lying on opposite faces of the β-peptide ring. When the cycle is composed of homochiral residues, the structure adopts a disklike conformation with amino acid side chains orientating in an equatorial manner on the exterior of the peptide ring, the axial and interior positions remaining unobstructed.[13] For one cyclo-β-tetrapeptide, a dissenting conformation in solution has been reported by Seebach and coworkers.[15] There, a transannular H-bond bisects the macrocyclic 16-membered ring to 10- and 12-membered H-bonded rings. In addition, a homochiral cyclo-β-tetrapeptide may adopt a boat-shaped structure in solution, which fluctuates with the disklike conformation.[17] The appropriately orientated NH and CO groups in the disklike conformation is an underlying feature of cyclo-β-peptide structure that leads to vertical stacking in the solid state via backbone-backbone hydrogen bonding and, hence, to formation of nanotubular structures.[10,13] While this stacking undeniably is an interesting phenomenon, it at the same time limits the use of cyclic β-peptides as scaffolds, since problems associated with the synthesis of the so-called difficult sequences may arise. Royo et al. used solid-supported cyclo[(N^{α}-Alloc)-Dpr-β-Ala-(N^{α}-Alloc)Dpr-β-Ala] as a scaffold for a receptor recognition motif and showed it to be successfully derivatized with the RGD sequences.[18] We have utilized the same backbone in the present study by describing two novel orthogonally protected solid-supported cyclo-β-tetrapeptides, viz. cyclo[(N^{α}-Alloc)Dpr-β-Ala-(N^{α}-Fmoc)Dpr-β-Ala] (**1**) and cyclo[(N^{α}-Alloc)Dpr-α-azido-β-aminopropanoyl-(N^{α}-Fmoc)Dpr-β-Ala] (**2**) (Figure 1). The applicability of scaffolds **1** and **2** was demonstrated by the synthesis of 10 diverse di- and trivalent glyco clusters (**33**−**42**), in which the sugar units were attached to the cyclo-β-tetrapeptide cores by carbamate or amide couplings. Whereas the previously reported peptide scaffolds have either been derivatized in solution[3−7] or had only several similar units attached,[18] the present methodology allows construction of conjugates containing two or three different sugars and the synthesis starting from monomeric constituents can be carried out entirely on a single solid support.

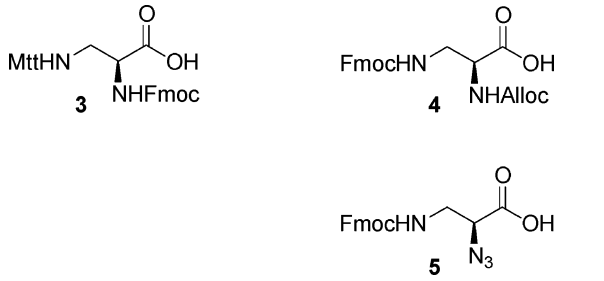
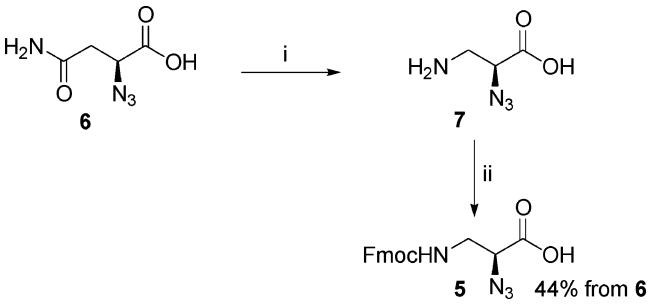


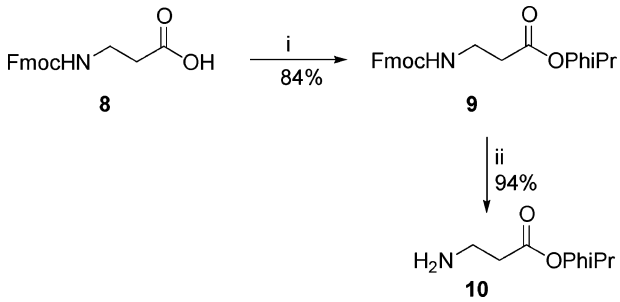
FIGURE 2. 2,3-Diaminopropionic acid derivatives (**3**−**5**) used for the syntheses of the scaffolds (**1** and **2**).

SCHEME 1. Synthesis of Branching Unit 5[a]



[a] Reagents and conditions: (i) [bis(trifluoroacetoxy)iodo]benzene, DIEA, H_2O, DMF; (ii) FmocOSu, DIEA, H_2O, acetonitrile.

SCHEME 2. Synthesis of PhiPr-Protected β-Alanine 10[a]



[a] Reagents and conditions: (i) 1-methyl-1-phenylethyl trichloroacetimidate, DCM; (ii) Et_2NH, DCM.

Results and Discussion

Amino Acid Building Blocks (3−5 and 10). The diaminopropanoic acid branching units (**3**−**5**), affording the shortest possible orthogonally protected aminoalkyl sidearms to the cyclo(tetra-β-peptide) cores, are depicted in Figure 2. N^2-(9-Fluorenylmethoxycarbonyl)-N^3-(4-methyltrityl)-2,3-diaminopropanoic acid [Fmoc-Dpr(Mtt)-OH, **3**] was commercially available

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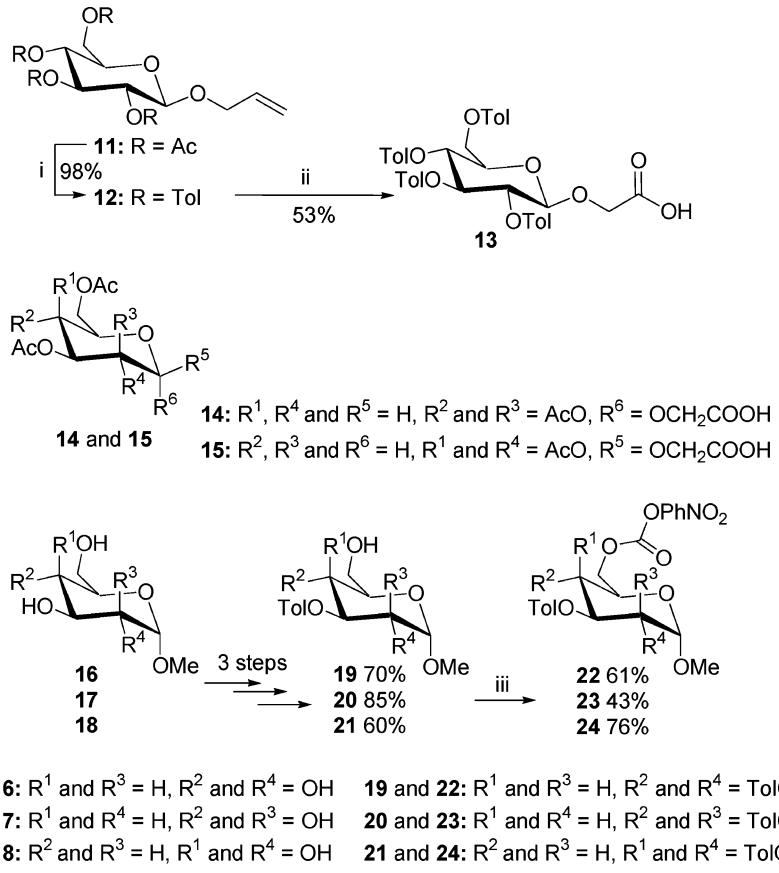
SCHEME 3. Sugar Units (13−15, 22−24) and Their Syntheses (13, 22−24)[a]



[a] Reagents and conditions: (i) (1) NaOMe, MeOH, (2) 4-toluoyl chloride, Py; (ii) $NaIO_4$, $RuCl_3$, DCM, MeCN, H_2O; (iii) 4-nitrophenylchloroformate, Py.

and N^2-(allyloxycarbonyl)-N^3-(9-fluorenylmethoxycarbonyl)-2,3-diaminopropanoic acid [Alloc-Dpr(Fmoc)-OH] was synthesized as described in the literature.[19] To provide the scaffold (**2**) with a third selectively exposable α-amino group, a novel 2,3-diaminopropanoic acid precursor (**5**) was prepared, having an azide mask at the α-position. This protection has recently been shown to be an attractive choice when a high dimensional orthogonal protection scheme is required.[20,21] The synthesis of this building block (**5**) is outlined in Scheme 1. The (2*S*)-4-amino-2-azido-4-oxobutanoic acid (**6**)[20] was first obtained in 71% yield from L-asparagine by a copper(II)-catalyzed diazo transfer method.[22] Hoffmann rearrangement of **6** using [bis-(trifluoroacetoxy)iodo]benzene as an electrophile[23,24] gave the desired β-amino acid (**7**), which was subsequently protected with *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (FmocOSu). By this simple route, the desired 2-azido-3-[(9-fluorenylmethyloxy-carbonyl)amino]propanoic acid (**5**) was obtained in 31% overall yield from L-asparagine.

Coupling of the α-azide masked amino acids has been reported to take place without racemication.[20] Care must however be exercized with this amino acid (**5**). In our preliminary attempt, a remarkable 36% racemization of **5** occurred by using HATU[25] activation in the presence of DIEA. This problem was avoided by using collidine instead of DIEA as a base.[26] The coupling procedure (5 equiv of **5**, 5 equiv of HATU, 10 equiv of collidine in DMF, at 25 °C for 1 h) reduced racemization to a negligible level and gave an acceptable 95% coupling yield. Evidently the electron-withdrawing azido group at the α-position is not the only reason for the facilitated α-proton abstraction, but the presence of the β-carbamate proton in **5** must somehow be involved in the racemization. Possibly the β-carbamate proton may stabilize the enolate oxyanion by intramolecular H-bonding.[27]

The 1-methyl-1-phenylethyl (PhiPr) protected β-alanine (**10**) was synthesized from Fmoc-β-alanine (**8**) in two steps (Scheme 2). The carboxylic acid (**8**) was treated with 1-methyl-1-phenyl-ethyl trichloroacetimidate[28−30] to obtain the acid-labile ester (**9**), and the Fmoc group was then removed by diethylamine, giving the desired building block (**10**) in 79% overall yield.

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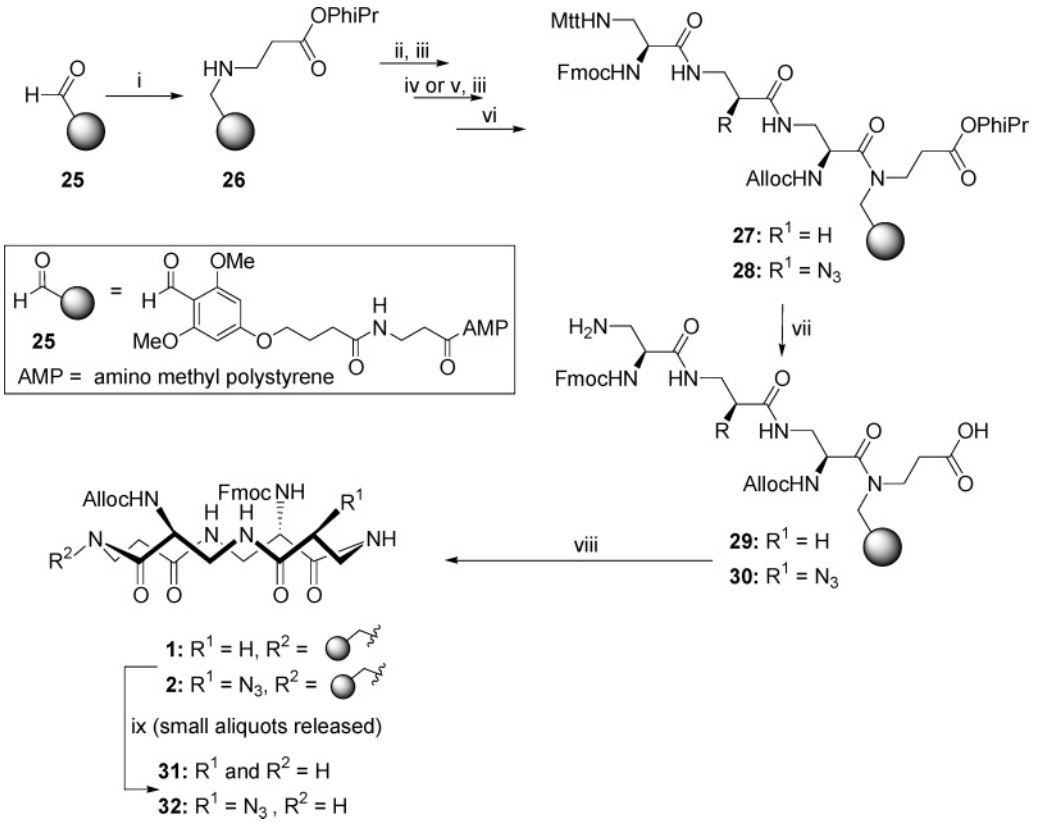
SCHEME 4. Synthesis of Solid-Supported Scaffolds 1 and 2[a]



[a] Reagents and conditions: (i) **10**, $NaCNBH_3$, AcOH, DMF; (ii) **4**, PyAOP, collidine, DMF, DCM; (iii) piperidine, DMF; (iv) Fmoc-*β*-Ala-OH, HATU, DIEA, DMF to obtain **27**; (v) **5**, HATU, collidine, DMF to obtain **28**; (vi) **3**, HATU, DIEA, DMF; (vii) (1) 1% TFA in DCM, 6 min, (2) neutralization with Py, DCM; (viii) PyAOP, DIEA, DMF, 2 h; (ix) 50% TFA in DCM.

Synthesis of the Sugar Units (13−15 and 22−24, Scheme 3). For the synthesis of glycoconjugates, six different sugar units, bearing either a glycosidic carboxymethyl moiety (**13**−**15**) or an active carbonate ester at the primary hydroxyl function (**22**−**24**), were prepared. These allow attachment to the scaffolds (**1** or **2**) via amide or carbamate bonds, respectively. In both cases a four-atomic spacer between the cyclo-*β*-tetrapeptide core and the pyranose ring is obtained. Carboxymethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (**14**)[31] and carboxymethyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranoside (**15**)[32] were synthesized according to literature[33] by the Ru(III)-catalyzed oxidation of the corresponding allyl pyranosides.[34] Carboxymethyl 2,3,4,6-tetra-*O*-toluoyl-*β*-D-glucopyranoside (**13**) was obtained similarly. The acetyl groups of the allyl glucopyranoside (**11**) were replaced by the toluoyl groups (**12**), and the *β*-glycosidic allyl group was then oxidized to a carboxymethyl group (**13**) by $NaIO_4$ in the presence of a catalytic amount of $RuCl_3$. It is worth of noting that a toluoylated sugar unit (**13**) is essential for reliable detection of the conjugates (**33**−**40**) and their released intermediates, when small aliquots are analyzed by RP HPLC during the course of the synthesis. The presence of aromatic toluoyl chromofors is not the only reason for this, but the corresponding fully acetylated conjugates tend to give broad RP HPLC signals. Methyl 6-*O*-(4-nitrophenoxycarbonyl)-2,3,4-tri-*O*-toluoyl-α-D-glycopyranosides (**22**−**24**) were synthesized from appropriate methyl α-D-glycopyranosides (**16**−**18**). A three-step procedure, including a temporary tritylation of the 6-position, pertoluoylation, and detritylation, gave first the

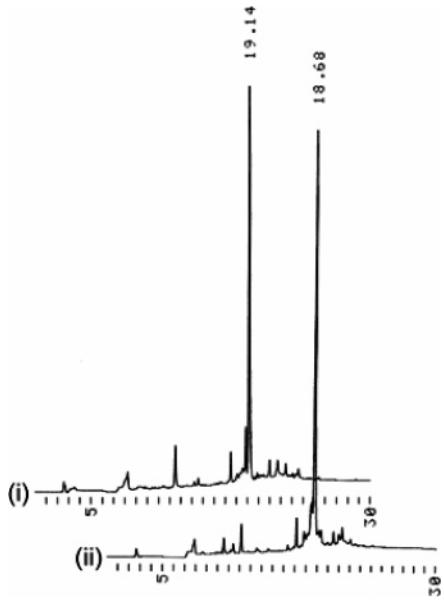


FIGURE 3. Released scaffolds **31** and **32**. Notation: (i) **31** (19.14 min), (ii) **32** (18.68 min). For the chromatographic conditions, see protocol A in Experimental Section.

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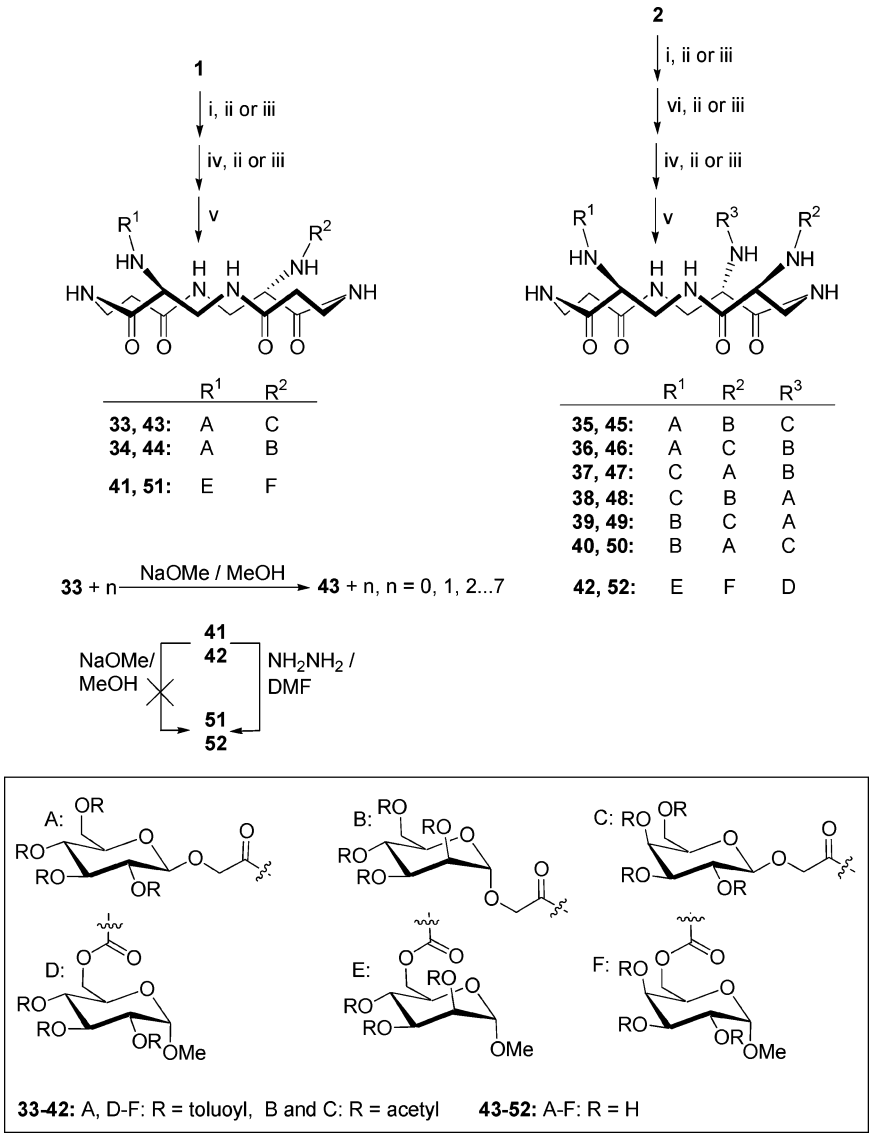
SCHEME 5. Syntheses of Glycoconjugates (33−52)[a]



[a] Reagents and conditions: (i) piperidine, DMF; (ii) **13**, **14**, or **15**, HATU, DIEA, DMF; (iii) **22**, **23**, or **24**, HOBt, DIEA, NMP; (iv) $(Ph_3P)_4Pd^0$, $PhSiH_3$, DCM; (v) TFA, DCM = release; (vi) Me_3P, toluene, H_2O, dioxane.

methyl 2,3,4-tri-*O*-toluoyl-α-D-glycopyranosides (**19**−**21**) in 60−85% overall yields, as previously reported.[35] The exposed 6-hydroxyl group of **19**−**21** was then converted to the desired active carbonate, giving the desired building blocks (**22**−**24**) in 43−76% yield.

Synthesis of the Solid-Supported Scaffolds (1 and 2, Scheme 4). Building block **10** was first attached to a resin derivatized with the 4-(4-formyl-3,5-dimethoxyphenoxy)butyrate linker[36] (**25**, L = 0.16 mmol/g). Reductive amination in the presence of $NaCNBH_3$ (4 equiv of **10**, 4 equiv of $NaCNBH_3$, in AcOH−DMF, 1:99 for 1 h at 25 °C) gave a 75% yield (L = 0.12 mmol/g). A small amount of acetic acid was observed to accelerate the reaction. Peptide chains (**27** and **28**) were then assembled using the Fmoc chemistry. HATU was used as an activator for couplings of **3**, **5** and **8** and PyAOP for **4**. Due to the potential racemization, collidine was used as a base in the coupling of **5** (cf. above). The other branching units (**3**, **4**, and **8**) were coupled in the presence of DIEA; 90% overall yields were obtained for both chain elongations (L = 0.10 mmol/g, **27** and **28**). The next step, i.e., selective removal of Mtt and PhiPr groups, was carefully optimized, since the backbone amide linker (**25**) seemed to be unexpectedly prone to premature cleavage.[37] Several acid treatments, including mixtures of TFA, DCA, and 1,1,1,3,3,3-hexafluoro-isopropyl alcohol (HFIP) with or without scavengers were tested. In addition, similar trials with the 4-(4-formyl-3-methoxyphenoxy) butyrate linker[38] were carried out. Surprisingly, the latter linker turned out to be even more labile than the former (**11**), in contrast to what is expected in the basis of Hammet σ^+ values.[39,40] The medium of choice for removal of Mtt- and PhiPr-protections was a short treatment

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with 1% TFA in DCM (at 25 °C for 6 min),[41] followed by immediate neutralization with a mixture of Py/DCM (1:5, v/v). By this procedure the premature cleavage remained at an acceptable level (20%). The loading of the deprotected linear peptides (**15** and **16**) was 0.080 mmol/g. Interestingly, a mixture of HFIP and DCM (1:3, v/v) cleaved both protections in solution, but on a solid support, only Mtt group may be removed.[42] Replacement of TFA with DCA did not result in the required selectivity.

Cyclization (**29** and **30**) under different conditions (PyAOP[43] and PyBOP[44] activation in different solvent systems) was then evaluated to minimize the expected on-resin polymerization. PyAOP as an activator in DMF (5 equiv of PyAOP, 5 equiv of DIEA in DMF, at 25 °C for 2 h) gave the best result. Small aliquots from the resins (**1** and **2**) were released and analyzed by analytical RP HPLC (Figure 3). Acceptable purities of crude mixtures and satisfactory overall isolated yields (15% for **31** and 12% for **32**) were obtained for the released scaffolds.

Synthesis of the Glycoconjugates (33−52, Scheme 5). Protected conjugates 33 and 34. To evaluate the applicability of amide coupling to construction of glycoconjugates, dipodal conjugates **33** and **34** were first synthesized on scaffold **1.** The Fmoc group was removed (20% piperidine in DMF, for 30 min at 25 °C) from the scaffold and carboxymethyl 2,3,4,6-tetra-*O*-toluoyl-*β*-D-glucopyranoside (**13**) was attached using HATU as an activator (5 equiv of **13**, 5 equiv of HATU, 10 equiv of DIEA, for 2 h at 25 °C). After the first sugar unit attachment, the Alloc group was removed by a Pd^0 treatment [0.5 equiv of $(Ph_3P)_4Pd$, 24 equiv of $PhSiH_3$ in DCM, 1 h at 25 °C under argon, the treatment was repeated], and the second carboxymethyl glycopyranoside (**14** or **15**) was attached similarly. The conjugates were released with TFA in DCM and purified by RP HPLC (Figure 4). As seen, purity of the crude products (**33** and **34**) after this multistep synthesis, including construction of the scaffold (**1**), was quite high. The yields of the desired conjugates compared were 77% (**33**) and 82% (**34**) of that of the scaffold (**31**), and the overall yields were 12% and 13%, respectively (cf. Table 1). Different orders of deprotection and coupling steps (i.e., first the Alloc- and then the Fmoc-removal and first coupling of **15**, then **13**) were additionally tested, but no remarkable differences in the RP HPLC profile were observed.

Protected Conjugates 35−40. Compared to the dipodal conjugates (**33** and **34**), construction of tripodal conjugates was more complex, and the success of the synthesis depended on the order of conjugation of sugar units. To obtain an optimal protocol, six tripodal conjugates (**35**−**40**), consisting of all possible combinations of the three different sugar units (**13**−**15**), were synthesized on scaffold **2**. The α-amino groups of **2** were exposed in the following order: Fmoc removal, demasking of the azide group, and Alloc removal. Fmoc and Alloc groups were removed as described for **33** and **34** above, and the azide group was demasked using trimethylphosphine in toluene [24 equiv of 1 mol L^{-1} Me_3P in toluene (resin suspended in H_2O−dioxane, 1:4, v/v), for 2 h at 25 °C]. Each α-amino group was acylated with an appropriate carboxymethyl glycopyranoside immediately after its exposure. A higher excess of reagents and a prolonged reaction time was used for these acylations (10 equiv of **13**, **14**, or **15**, 10 equiv of HATU, 20 equiv of DIEA in DMF, for 15 h at 25 °C), compared to preparation of the dipodal conjugates (**33** and **34**). The conjugates were released with TFA in DCM and then purified by RP HPLC (Figure 5). As seen, three of the conjugates (**35**, **39,** and **40**, iv−vi in Figure 5) were obtained in relatively high purity and good yields [**35**, **39**, and **40** vs **32** being 71−58% and the overall yields 9−7%, respectively (cf. Table 1)]. However, with conjugates **36**−**38**, the presence of one glycopyranoside markedly retarded the subsequent couplings. The low reactivity may result from increased aggregation of the scaffold (**2**). These difficult acylations could not be driven forward, even by repeated couplings. Addition of chaotropic salts (LiCl, $LiClO_4$, $NaClO_4$, KSCN, 0.4 M solutions in DMF)[45] to the coupling mixture and applicability of an elevated temperature (55 °C) were also attempted. Relative yields of the conjugates (**36**−**38** vs. **18**) remained at 33−37% and overall yields at 4%, respectively (cf. Table 1).

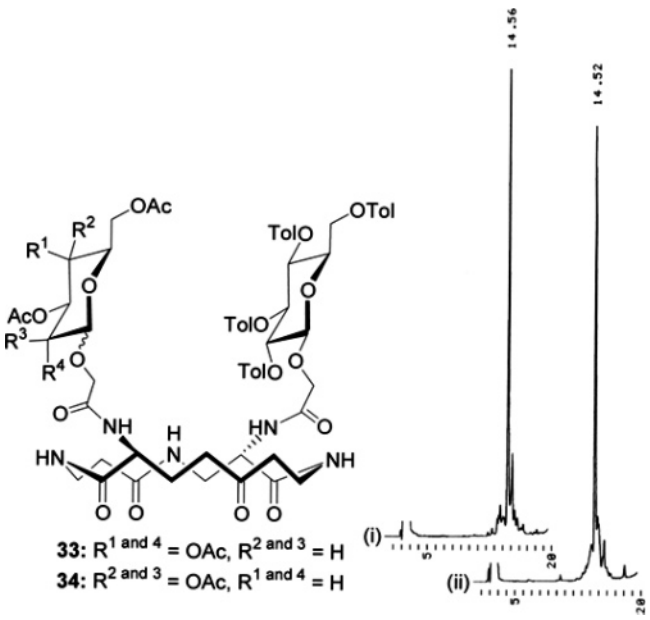

FIGURE 4. RP HPLC profiles of the crude product (**33** and **34**) mixtures. Notation: (i) **33** (14.56 min), (ii) **34** (14.52 min). For the chromatographic conditions, see protocol B in Experimental Section. [The glycosidic bond in the galactopyranosyl unit (**33**) is *β*, and in the mannopyranosyl unit (**34**) it is α.]

TABLE 1. Yields of the Clusters (33−42)

glycoconjugate	relative yield, % (**33**−**42** vs **31** or **32**)	overall yield, %
33	77	12
34	82	13
35	71	9
36	36	4
37	37	4
38	33	4
39	57	7
40	62	8
41	69	10
42	78	9

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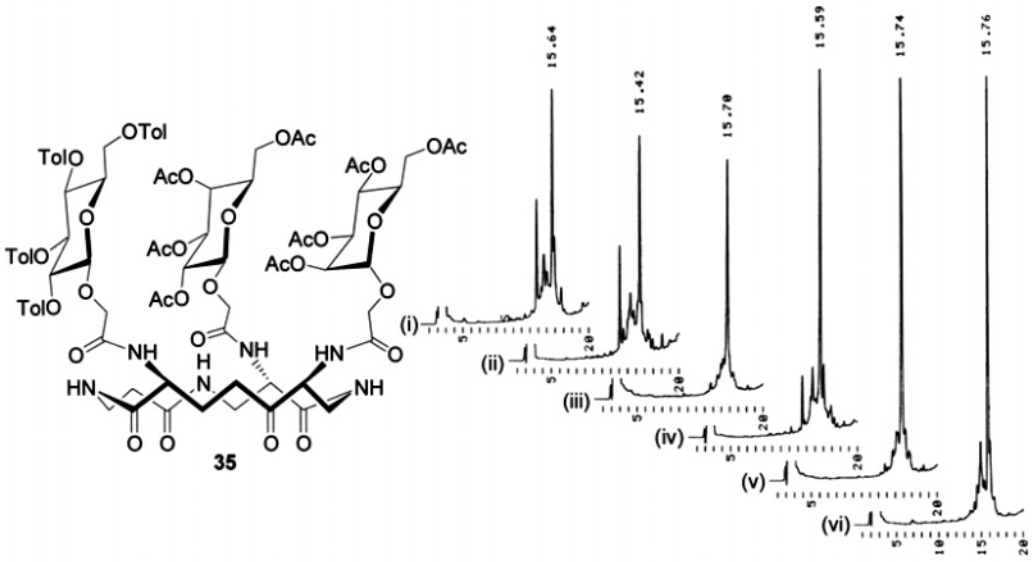

FIGURE 5. RP HPLC profiles of the crude product (**35**−**40**) mixtures. Notation (profiles in a reversed order of synthetic success): (i) **36** (15.64 min), (ii) **38** (15.42 min), (iii) **37** (15.70 min), (iv) **39** (15.59 min), (v) **40** (15.74 min), (vi) **35** (15.76 min). The main side products in the RP HPLC profiles are related to incomplete couplings. For the chromatographic conditions, see protocol B in Experimental Section. Structure of the conjugate **35** is only shown.

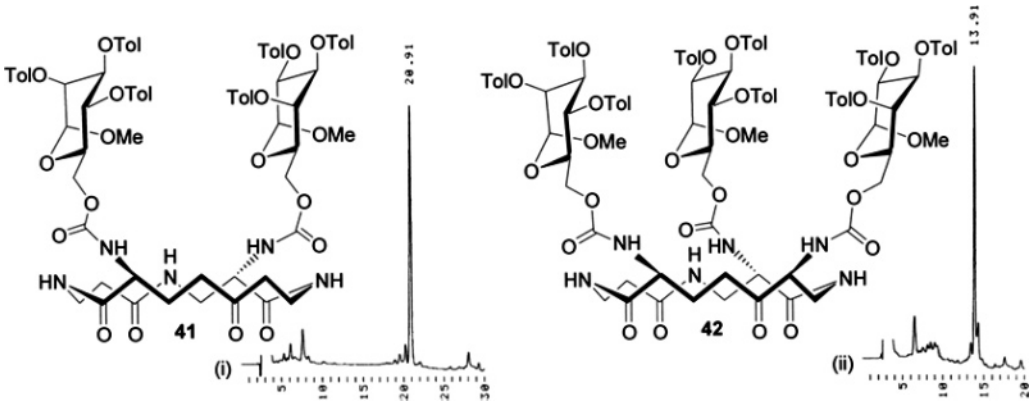

FIGURE 6. RP HPLC profiles of the crude product (**41** and **42**) mixtures. Notation: (i) **41** (20.91 min), (ii) **42** (13.91 min). For the chromatographic conditions, see protocol C and D in Experimental Section.

Deprotected Conjugates 43−50. Global deprotection (i.e., removal of acetyl and toluoyl groups) by conventional NaOMe/MeOH treatment[46] was first studied using one dipodal (**33**) and tripodal conjugate (**35**) as model compounds. By this base-catalyzed transesterification (20 mmol L^{-1} NaOMe in MeOH, 15 h at 25 °C) the desired globally deprotected conjugates were obtained in 54% (**43**) and 52% (**45**) isolated yields (RP HPLC profiles of the homogenized products, see Figure 7). Using the same treatment on conjugates **34** and **36**−**40**, which were synthesized in a small scale, deprotected conjugates **44** and **46**−**50** were obtained. No remarkable differences in product distributions, caused by degradation or incomplete deprotection, were found.

Conjugates 41, 42, 51, and 52. Applicability of the scaffolds (**1** and **2**) was further evaluated by the synthesis of two glycoconjugates (**41** and **42**), in which carbamate coupling was utilized. Although coupling between 4-nitrophenoxycarbonates and amines[47] is expectedly a slower reaction than the HATU-promoted amide coupling, these two model conjugates (**41** and **42**) were obtained with rather high coupling efficiency resulting a formation of the desired conjugates in 10% (**41**) and 9% (**42**) overall yields (Figure 6). With the exception of the coupling procedure (10 equiv of **30**, **31** or **31**, 10 equiv of DIEA in 0.1 mmol L^{-1} HOBt/NMP, for 15 h at 25 °C), these conjugates were synthesized as described for **33**−**40**. However, their global deprotection by a conventional NaOMe/MeOH treatment resulted in cleavage of the carbamate bond. Sodium methoxide was found to catalyze nucleophilic attack of one of the ring amides to the carbamate carbon, which cleaves the sugar unit and results in formation of a five-membered ring (cf. the aspartimide formation in SPPS).[48,49] The use of hydrazine, being a weak Brønsted base ($pK_b = 6.05$) but a strong nucleophile, suppressed this side reaction. The conjugates (**41** and **42**) were treated with hydrazine hydrate in DMF (1:9, v/v, for 15 h at 25

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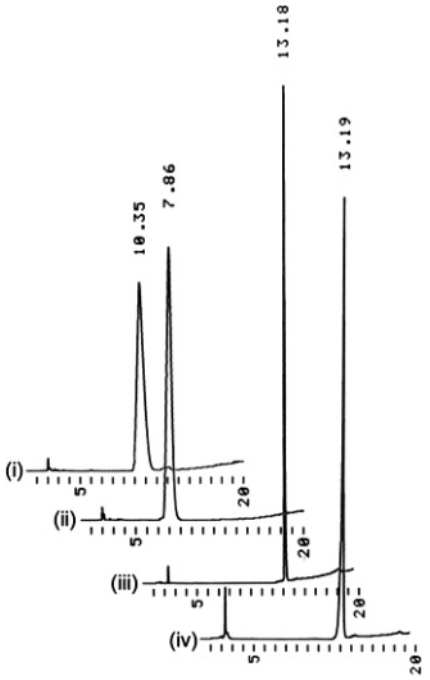

FIGURE 7. RP HPLC profiles of the homogenized conjugates **43**, **45**, **51**, and **52**. Notation: (i) **43** (10.35 min), (ii) **45** (7.86 min), (iii) **51** (13.18 min), (iv) **52** (13.19 min). For the chromatographic conditions, see protocol E in Experimental Section.

°C) and purified by RP HPLC (Figure 7) to give the globally deprotected products in 31% (**51**) and 59% (**52**) isolated yields. Shortcoming of this deprotection procedure may be the presence of toluoylhydrazide in the crude product mixture, which in contrast to methyl 4-methylbenzoate cannot be cleanly removed by simple diethyl ether extraction.

The all conjugates synthesized (**33**−**52**) were purified by RP HPLC. Authenticity of the purified products (**33**−**52**) was verified by ESI(MS). In addition, eight of the conjugates (**33**, **35**, **41**−**43**, **45**, **51**, and **52**), which were synthesized in a larger scale, were characterized by ^{1}H NMR spectroscopy (for the MS and NMR spectral data, see Supporting Information).

Conclusion

Two novel solid-supported cyclo-*β*-tetrapeptide scaffolds {viz., cyclo[(*N*^{a}-Alloc)Dpr-*β*-Ala-(*N*^{a}-Fmoc)Dpr-*β*-Ala] (**1**) and cyclo[(*N*^{a}-Alloc)Dpr-α-azido-*β*-aminopropanoyl-(*N*^{a}-Fmoc)Dpr-*β*-Ala] (**2**)} have been described. The scaffolds (**1** and **2**) are composed of *β*-alanine and orthogonally protected 2,3-diaminopropanoic acid residues. These include one novel 2,3-diaminopropanoic acid precursor (**5**), i.e., 2-azido-3-[(9-fluorenylmethyloxycarbonyl)amino]propanoic acid. Orthogonal derivatization of these cyclo-*β*-tetrapeptide cores (**1** and **2**) has been demonstrated by insertion of acetyl- or toluoyl-protected carboxymethyl α-D-glycopyranosides (**13**−**15**) and methyl 6-*O*-(4-nitrophenoxycarbonyl)-α-D-glycopyranosides (**22**−**24**) to obtain 10 diverse di- and trivalent glycoclusters (**33**−**42**). Both scaffolds (**1** and **2**) have been shown to allow efficient sugar moiety conjugation by either amide or carbamate coupling. Formation of trivalent glycoclusters (**35**−**40**) is, however, dependent on the order of the sugar unit attachment (**13**−**15**). By these approaches, diverse fully acylated conjugates (**33**−**42**) can be constructed from monomeric constituents entirely on a solid support in acceptable yield and purity. Removal of the acyl protections in solution gives the fully deprotected conjugates (**43**−**52**).

Experimental Section

2-Azido-3-[(9-fluorenylmethyloxycarbonyl)amino]-propanoic Acid (5). [Bis(trifluoroacetoxy)iodo]benzene (12 g, 27 mmol) was added to a mixture of (2*S*)-4-amino-2-azido-4-oxobutanoic acid[20] (**6**, 2.83 g, 18 mmol) in aqueous DMF (140 mL, 1:1, v/v). The mixture was agitated for 15 min, and then DIEA (6.2 mL, 36 mmol) was added. After 3 h of stirring, volatiles were removed, the residue was dissolved in water, and organic side products were removed by repeated washings with diethyl ether (4 × 50 mL). The water phase was evaporated to dryness to yield a reddish oil (crude **7**), which was then redissolved in water (15 mL). DIEA (3.1 mL, 18 mmol) and FmocOSu (5.7 g, 18 mmol) in acetonitrile (15 mL) were added, and the reaction was allowed to stir for 1.5 h. The mixture was acidified (to pH 2.0) by addition of HCl, and the product was extracted in DCM (4 × 50 mL). The organic phases were combined, dried with Na_2SO_4, and evaporated to dryness. The crude product mixture was purified by silica gel chromatography (10% MeOH in DCM). Hexane was added to the combined product fractions, and the precipitate formed was filtered, washed with hexane, and dried to yield 2.8 g (44%) of product as a white powder: $[\alpha]^{20}_D$ −42.1 (*c* 1.0, MeOH); ^{1}H NMR (DMSO-d_6, 400 MHz) *δ* 7.87 (d, 2H, *J* = 7.6 Hz), 7.68 (d, 2H, *J* = 7.6 Hz), 7.42−7.26 (m, 4H), 4.28 (m, 2H), 4.20 (t, 1H, *J* = 6.8 Hz), 3.62 (dd, 1H, *J* = 9.2 and 3.6 Hz), 3.47 (m, 1H), 3.18 (m, 1H); ^{13}C NMR (DMSO-d_6, 50 MHz) *δ* 172.2, 156.6, 144.3, 141.2, 128.1, 127.5, 125.7, 120.6, 66.0, 64.3, 47.2, 43.1; HRMS (ESI) [M + Na]$^+$ requires 375.1057, found 375.1064.

1-Methyl-1-phenylethyl 3-[(9-Fluorenylmethoxycarbonyl)-amino]propanoate (9). 1-Methyl-1-phenylethyl trichloroacetimidate was first prepared according to literature.[29] A mixture of 1-methyl-1-phenylethanol (5.1 g, 38 mmol) in diethyl ether (4.0 mL) was added dropwise to a suspension of NaH (0.10 g, 4.2 mmol) in diethyl ether (4.0 mL). The mixture was stirred at ambient temperature for 20 min and then cooled to 0 °C. Trichloroacetonitrile (3.8 mL, 38 mmol) was slowly added (upon 15 min), and the mixture was allowed to warm to ambient temperature. After 1 h of stirring, volatiles were removed, the resulted oil was dissolved in pentane (4.0 mL), and the solution was filtered. The filtrate was evaporated to dryness to yield 11 g (quant) of the crude imidate as a reddish oil, which may be used without further purification. The freshly prepared 1-methyl-1-phenylethyl trichloroacetimidate (0.9 g, 3.2 mmol) was added to a mixture of Fmoc-*β*-alanine (**8**, 0.5 g, 1.6 mmol) in DCM (4.0 mL). After overnight stirring, the precipitated trichloroacetamide was removed by filtration, and the filtrate was evaporated to dryness. The resulted crude product mixture was purified by silica gel chromatography (0−1% MeOH in DCM) to yield 0.58 g (84%) of **9** as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) *δ* 7.76 (d, 2H, *J* = 7.6 Hz), 7.57 (d, 2H, *J* = 7.5 Hz), 7.41−7.23 (m, 9H), 5.24 (b, 1H), 4.37 (d, 2H, *J* = 7.1 Hz), 4.20 (t, 1H, *J* = 7.0 Hz), 3.42 (m, 2H), 2.55 (t, 2H, *J* = 5.7 Hz), 1.78 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 171.3, 156.3, 145.5, 144.0, 141.3, 128.4, 127.7, 127.2, 127.1, 125.1, 124.2, 120.0, 82.3, 66.7, 47.3, 36.6, 35.4, 28.7; HRMS (ESI)) [M + Na]$^+$ requires 452.1832, found 452.1824.

1-Methyl-1-phenylethyl 3-Aminopropanoate (10). Diethylamine (6.0 mL) was added to a mixture of **9** (0.53 g, 1.2 mmol) in DCM (2.0 mL). After 2 h of stirring, toluene (10 mL) was added, and the mixture was evaporated to dryness. The crude product mixture was purified by silica gel chromatography (10% MeOH, 2% Et_3N in DCM) to yield 0.24 g (94%) of **10** as colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) *δ* 7.37−7.31 (m, 4H), 7.26−7.23 (m, 1H), 2.94 (b, 2H), 2.47 (t, 2H, *J* = 6.2 Hz), 1.83 (b, 2H), 1.77 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 171.4, 145.8, 128.3, 127.0, 124.2, 81.8, 38.6, 37.8, 28.7; HRMS (ESI) [M + Na]$^+$ requires 230.1152, found 230.1167.

Allyl 2,3,4,6-Tetra-*O*-toluoyl-*β*-D-glucopyranoside (12). Allyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranoside (**11**, 1.4 g, 3.6 mmol), prepared from the commercially available pentaacetyl-*β*-D-glucose,[34] was dissolved in 0.1 mol L^{-1} NaOMe/MeOH solution. The mixture was stirred at ambient temperature for 1 h, neutralized by addition of strongly acidic cation-exchange resin, and filtered. The filtrate was evaporated to dryness, the residue was dissolved in pyridine (30 mL), and then toluoyl chloride (3.8 mL, 29 mmol) was added. After overnight stirring at 50 °C, ice-water was added to quench the reaction and the product was extracted with DCM. The organic phases were combined, washed with $NaHCO_3$, dried with Na_2SO_4, and evaporated to dryness. The crude product mixture was purified by silica gel chromatography (20% EtOAc in petroleum ether) to yield 2.5 g (98%) of **12** as white foam: ^{1}H NMR ($CDCl_3$, 500 MHz) *δ* 7.93 (d, 2H, *J* = 8.2 Hz), 7.88 (d, 2H, *J* = 8.2 Hz), 7.81 (d, 2H, *J* = 8.2 Hz), 7.75 (d, 2H, *J* = 8.2 Hz), 7.22 (d, 2H, *J* = 8.1 Hz), 7.20 (d, 2H, *J* = 8.1 Hz), 7.15 (d, 2H, *J* = 8.0 Hz), 7.09 (d, 2H, *J* = 8.0 Hz), 5.90 (dd, 1H, *J* = 9.7 and 9.7 Hz), 5.82 (m, 1H), 5.65 (dd, 1H, *J* = 9.7 and 9.7 Hz), 5.56 (dd, 1H, *J* = 9.8 and 7.9 Hz), 5.24 (m, 1H), 5.14 (m, 1H), 4.90 (d, 1H, *J* = 7.9 Hz), 4.62 (dd, 1H, *J* = 12.1 and 3.2 Hz), 4.50 (dd, 2H, *J* = 12.1 and 5.6 Hz), 4.38 (ddt, 1H, *J* = 13.3, 4.9, and 1.5 Hz), 4.19 (ddt, 1H, *J* = 13.3, 6.4, and 1.3 Hz), 4.15 (m, 1H), 2.43 (s, 3H), 2.39 (s, 3H), 2.37 (s, 3H), 2.31 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 166.2, 165.8, 165.2, 165.1, 144.1, 143.9, 143.8, 133.4, 129.9, 129.9, 129.8, 129.8, 129.1, 129.0, 127.0, 126.7, 126.2, 117.9, 99.9, 72.8, 72.3, 71.7, 70.1, 69.7, 63.2, 21.7, 21.6, 21.6; HRMS (ESI) $[M + Na]^+$ requires 715.2514, found 715.2511.

Carboxymethyl 2,3,4,6-Tetra-*O*-toluoyl-*β*-D-glucopyranoside (13). $NaIO_4$ (2.0 g, 9.4 mmol) and a catalytic amount of $RuCl_3$ were added to a stirred biphasic mixture of allyl 2,3,4,6-tetra-*O*-toluoyl-*β*-D-glucopyranoside (**12**, 1.3 g, 18 mmol), DCM (6 mL), acetonitrile (6 mL), and water (9 mL). After 5 h of stirring at 40 °C, the mixture was diluted with DCM, washed with water and brine, dried with Na_2SO_4, and evaporated to dryness. The crude product mixture was purified by silica gel chromatography (2% Py, 10% MeOH in DCM). The product fractions were combined and evaporated to dryness to give an oil, which was then evaporated with toluene to yield 0.69 g (53%) of **13** as a yellowish foam: ^{1}H NMR (CD_3OD + $CDCl_3$, 500 MHz) *δ* 7.85−7.76 (m, 6H), 7.68 (d 2H, *J* = 7.9 Hz), 7.15−7.09 (m, 6H), 7.03 (d, 2H, *J* = 8.0 Hz), 5.98 (dd, 1H, *J* = 9.7 Hz and 9.7 Hz), 5.69 (dd, 1H, *J* = 9.8 and 9.8 Hz), 5.59 [dd, 1H, *J* = 8.8 Hz (average)], 5.12 (b, 1H), 4.59 (dd, 1H, *J* = 12.0 and 3.2 Hz), 4.46 (dd, 1H, *J* = 12.1 and 4.6 Hz), 4.39 (d, 1H, *J* = 15.0 Hz), 4.30 (m, 1H), 4.16 (d, 1H, *J* = 15.0 Hz), 2.33, 2.32, 2.30 and 2.22 (each s, each 3H); ^{13}C NMR (CD_3OD + $CDCl_3$, 100 MHz) *δ* 176.2, 166.7, 166.3, 165.9, 165.4, 144.4, 144.3, 144.0, 129.9, 129.6, 129.5, 129.4, 129.0, 128.9, 128.8, 125.9, 125.9, 125.8, 100.1, 72.6, 72.3, 72.1, 69.5, 67.7, 62.9, 20.8, 20.8, 20.7; HRMS (ESI) $[M + Na]^+$ requires 733.2255, found 733.2247.

Methyl 6-*O*-(4-Nitrophenoxycarbonyl)-2,3,4-tri-*O*-toluoyl-α-D-glucopyranoside (22). 4-Nitrophenylchloroformate (1.1 g, 5.1 mmol) was added to a cooled (0 °C) mixture of methyl 2,3,4-tri-*O*-toluoyl-α-D-glucopyranoside[35] (2.8 g, 5.1 mmol) in Py (30 mL). The reaction was allowed to warm to ambient temperature and then stirred overnight. The mixture was evaporated to dryness and purified by silica gel chromatography (20% EtOAc in petroleum ether) to yield 2.2 g (61%) of **22** as a white foam: ^{1}H NMR ($CDCl_3$, 500 MHz) *δ* 8.30 (d, 2H, *J* = 7.6 Hz), 7.88 (d, 2H, *J* = 6.8 Hz), 7.85 (d, 2H, *J* = 6.8 Hz), 7.77 (d, 2H, *J* = 6.8 Hz), 7.43 (d, 2H, *J* = 7.6 Hz), 7.19 (m, 4H), 7.10 (d, 2H, *J* = 6.8 Hz), 6.18 (m, 1H), 5.63 (dd, 1H, *J* = 8.2 and 8.2 Hz), 5.29−5.27 (m, 2H), 4.55 (dd, 1H, *J* = 10.0 and 3.9 Hz), 4.47 (dd, 1H, *J* = 10.0 and 1.8 Hz), 4.34 (m, 1H), 3.52 (s, 3H), 2.37 (s, 6H), 2.31 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 165.9, 165.8, 165.5, 155.5, 152.4, 145.5, 144.5, 144.3, 143.9, 130.0, 129.9, 129.7, 129.2, 129.2, 129.0, 126.4, 126.2, 125.9, 125.3, 121.9, 97.2, 71.8, 69.9, 68.8, 67.5, 66.8, 55.9, 21.7, 21.7, 21.6; HRMS (ESI) $[M + H]^+$ requires 714.2181, found 714.2202.

Methyl 6-*O*-(4-Nitrophenoxycarbonyl)-2,3,4-tri-*O*-toluoyl-α-D-mannopyranoside (23). The active carbonate (**23**) was synthesized from the corresponding methyl 2,3,4-tri-*O*-toluoyl-α-D-mannopyranoside[35] (8.3 g, 15 mmol) as described for **22**. The product (**23**) was obtained as a white foam in a 43% yield (4.7 g): ^{1}H NMR ($CDCl_3$, 500 MHz) *δ* 8.28 (d, 2H, *J* = 7.6 Hz), 8.03 (d, 2H, *J* = 7.5 Hz), 7.87 (d, 2H, *J* = 6.8 Hz), 7.73 (d, 2H, *J* = 6.8 Hz), 7.37 (d, 2H, *J* = 7.6 Hz), 7.26 (d, 2H, *J* = 6.7 Hz), 7.19 (d, 2H, *J* = 6.7 Hz), 7.07 (d, 2H, *J* = 6.8 Hz), 5.97 [dd, 1H, *J* ≈ 8.3 Hz (average)], 5.92 (dd, 1H, *J* = 8.4 and 2.6 Hz), 5.66 (m, 1H), 5.03 (d, 1H, *J* = 0.8 Hz), 4.57 (dd, 1H, *J* = 9.9 and 3.9 Hz), 4.53 (dd, 1H, *J* = 9.9 and 2.0 Hz), 4.36 (m, 1H), 3.57 (s, 3H), 2.42 (s, 3H), 2.37 (s, 3H), 2.31 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 165.7, 165.5, 165.5, 155.5, 152.5, 145.4, 144.5, 144.4, 144.0, 130.0, 129.9, 129.8, 129.3, 129.2, 129.0, 126.6, 126.3, 126.0, 125.3, 121.9, 98.7, 70.2, 69.4, 68.6, 67.3, 66.6, 55.7, 21.8, 21.7, 21.6; HRMS (ESI) $[M + H]^+$ requires 714.2181, found 714.2155.

Methyl 6-*O*-(4-Nitrophenoxycarbonyl)-2,3,4-tri-*O*-toluoyl-α-D-galactopyranoside (24). The active carbonate (**24**) was synthesized from the corresponding methyl 2,3,4-tri-*O*-toluoyl-α-D-galactopyranoside[35] (4.2 g, 7.6 mmol) as described for **22**. The product (**24**) was obtained as a white foam in a 76% yield (4.2 g): ^{1}H NMR ($CDCl_3$, 500 MHz) *δ* 8.26 (d, 2H, *J* = 7.7 Hz), 7.98 (d, 2H, *J* = 6.8 Hz), 7.87 (d, 2H, *J* = 6.8 Hz), 7.67 (d, 2H, *J* = 6.8 Hz), 7.35 (d, 2H, *J* = 7.7 Hz), 7.28 (d, 2H, *J* = 6.7 Hz), 7.17 (d, 2H, *J* = 6.8 Hz), 7.04 (d, 2H, *J* = 6.8 Hz), 5.95−5.93 (m, 2H), 5.65 (dd, 1H, *J* = 8.6 and 3.0 Hz), 5.33 (d, 1H, *J* = 2.9 Hz), 4.55 [dd, 1H, *J* ≈ 5.2 Hz (average)], 4.45 (d, 2H, *J* = 4.9 Hz), 3.50 (s, 3H), 2.44 (s, 3H), 2.35 (s, 3H), 2.29 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 166.2, 165.7, 165.6, 155.4, 152.2, 145.5, 144.5, 144.2, 144.0, 130.0, 129.9, 129.7, 129.4, 129.2, 129.0, 126.4, 126.4, 126.3, 125.3, 121.8, 97.8, 69.1, 68.9, 68.0, 66.8, 66.6, 55.9, 21.8, 21.7, 21.6; HRMS (ESI) $[M + H]^+$ requires 714.2181, found 714.2205.

Synthesis of the Solid-Supported Scaffolds (1 and 2). The aldehyde derivatized resin (**25**) was prepared by the following procedure. A mixture of Boc- and Fmoc-*β*-alanine (2.0 equiv of both, 4.0 equiv of TBTU, 8 equiv of DIEA in DMF, 1 h at 25 °C) was first coupled to an aminomethyl polystyrene resin (1.0 g, a loading of 0.5 mmol/g). The Boc groups were removed with 50% TFA in DCM, and the exposed amino groups were then capped with an acetanhydride treatment. In this manner loading was reduced to 0.16 mmol/g (determined by the release of benzofulvene). The Fmoc groups were then removed with 20% piperidine in DMF and 4-(4-formyl-3,5-dimethoxyphenoxy)butyric acid was attached by HATU-promoted coupling to obtain resin **25**. Resin **25** (1.0 g, a loading of 0.16 mmol/g) was treated for 1 h at room temperature with a mixture of 1-methyl-1-phenylethyl 3-aminopropanoate (**10**, 160 mg, 4 equiv) and $NaCNBH_3$ (48 mg, 4 equiv) in DMF, containing 1% (v/v) AcOH (16 mL). The resin was washed with DMF, DCM, and MeOH and dried on a filter. A small aliquot of **26** was acylated with excess of $(Fmoc\text{-}\beta\text{-}Ala)_2O$ in DCM, to determine the loading of the acylated secondary amine by the release of benzofulvene. A loading of 0.12 mmol/g (a 75% yield) was obtained. The following protocols for the peptide chain elongations (**27** and **28**) were then applied. The secondary amine of **26** was acylated with Alloc-Dpr(Fmoc)-OH (5.0 equiv of **4**, 5 equiv of PyAOP, 10 equiv of DIEA in DMF−DCM, 1:9, v/v, 2 h at 25 °C), the Fmoc group was removed (piperidine−DMF, 1:4, v/v, for 20 min at 25 °C), and then the resin was divided into two batches (2 × 0.5 g). To obtain resin **27**, chain elongation was continued by couplings of Fmoc-*β*-Ala-OH and Fmoc-Dpr(Mtt)-OH [5 equiv of amino acid (**8** or **3**), 5 equiv of HATU, and 10 equiv of DIEA in DMF, 1 h at 25 °C]. With the other batch (to obtain resin **28**), chain elongation was continued by couplings of 2-azido-3-[(9-fluorenylmethyloxycarbonyl)amino]propanoic acid and Fmoc-Dpr-(Mtt)-OH (5 equiv of **5**, 5 equiv of HATU, 10 equiv of collidine

in DMF, 1 h at 25 °C, **3** was coupled as above); 90% yields (loadings of 0.10 mmol/g, **27** and **28**) for both chain elongations were obtained. To remove Mtt and PhiPr protections, the resins **27** and **28** were treated with a solution of TFA in DCM (1:99, v/v, for 6 min at 25 °C), followed by immediate neutralization by washings with a mixture of Py in DCM (1:5, v/v). After the Mtt/PhiPr deprotection, loadings of 0.080 mmol/g were obtained for the linear peptides (**29** and **30**). Cyclizations were then performed using PyAOP as an activator (5 equiv of PyAOP, 5 equiv of DIEA in DMF for 2 h at 25 °C). After each coupling (including the cyclization step), potentially remaining free amino groups were capped by an acetic anhydride treatment. Since cyclizations of the solid-supported scaffolds (**1** and **2**) could not be monitored by loading, small aliquots of the resins (10.0 mg samples of **1** and **2**) were treated with TFA in DCM (1:1, v/v, 30 min at 25 °C), and the released product (**31** and **32**) mixtures were analyzed by analytical RP HPLC (Figure 3). Acceptable purities for the crude released scaffolds (**31** and **32**) were obtained. Isolated yields of the released scaffolds (**31** and **32**) were determined as follows. Product (**31** and **32**) fractions were collected, evaporated to dryness, and then treated with a known (1.0 mL) volume of piperidine in DMF (1:4, v/v); 22% (**31**) and 17% (**32**) yields were obtained, according to the extent of benzofulvene released (calculated from **29** and **30**). The overall yields were 15% (**31**) and 12% (**32**), respectively. Authenticity of the released scaffolds was verified by HRMS (ESI). **31**: $[M + H]^+$ requires 621.2667, found 621.2659. **32**: $[M + H]^+$ requires 662.2681, found 662.2644.

Protected Conjugate 33. The Fmoc group of the supported scaffold (**1**) (200 mg batch, a loading of 0.080 mmol/g) was removed with piperidine in DMF (1:4, v/v, 30 min), and the first sugar unit was coupled to the exposed amino group (5 equiv of **13**, 10 equiv of DIEA in DMF, 2 h at 25 °C). The potentially remaining free amino groups were capped with an acetic anhydride treatment, and then the Alloc group was removed. The resin was suspended in DCM, 0.5 equiv of $(Ph_3P)_4Pd$ and 24 equiv of $PhSiH_3$ were added, and the suspension was stirred for 1 h at 25 °C under argon. The treatment was repeated, and then the second sugar unit (**15**) was coupled in a similar manner. After each step above, the resin was washed with DMF, DCM, and MeOH and dried on filter. The protected dipodal glycogluster **33** was released with TFA in DCM (1:1, v/v, for 30 min at 25 °C), and the crude product mixture was evaporated to dryness, dissolved in aqueous EtOH (1:1, v/v), and subjected to RP HPLC [(i) in Figure 4]. The combined product fractions were lyophilized to give the desired product **33** in a 12% (3.8 mg) overall isolated yield. The relative yield (**33** vs **31**) was 77%: ^{1}H NMR (CD_3CN, 500 MHz) δ 7.92 (m, 3H), 7.85 (d, 2H, J = 8.2 Hz), 7.76 (m, 3H), 7.66 (d, 2H, J = 8.2 Hz), 7.30 (d, 2H, J = 8.0 Hz), 7.26 (d, 2H, J = 8.1 Hz), 7.24 (d, 2H, J = 8.1 Hz), 7.19 (d, 2H, J = 8.1 Hz), 7.14 (b, 1H), 7.08 (b, 1H), 6.94 (b, 1H), 6.89 (b, 1H), 5.90 (dd, 1H, J = 9.6 and 9.6 Hz), 5.74 (dd, 1H, J = 9.5 and 7.9 Hz), 5.72 (dd, 1H, J = 9.7 and 9.7 Hz), 5.33 (d, 1H, J = 3.5 Hz), 5.26 (dd, 1H, J = 10.5 and 8.0 Hz), 5.12 (d, 1H, J = 7.9 Hz), 5.06 (dd, 1H, J = 10.5 and 3.5 Hz), 4.64 (d, 1H, J = 8.0 Hz), 4.53 (b, 2H), 4.39 (m, 1H), 4.35−4.29 (m, 2H), 4.27 (d, 1H, J = 15.6 Hz), 4.20 (d, 1H, J = 15.6 Hz), 4.19 (dd, 1H, J = 9.7 and 5.5 Hz), 4.12 (d, 1H, J = 15.1 Hz), 4.04−3.99 (m, 3H), 3.60−3.31 (m, 8H), 2.43 (s, 3H), 2.38 (s, 3H), 2.37 (s, 3H), 2.39−2.30 (m, 4H), 2.33 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.94 (s, 3H); MS (ESI) $[M + H]^+$ requires 1395.5, found 1395.5.

Protected Conjugate 35. The Fmoc group of the scaffold (**2**) (200 mg batch, a loading of 0.080 mmol/g) was removed (as described for **33**), and the first carboxymethyl glycopyranoside (10 equiv of **13**, 10 equiv of HATU, 20 equiv of DIEA in DMF, 15 h at 25 °C) was coupled. The potentially remaining free amino groups were capped, and the resin was suspended in a mixture of H_2O in dioxane (1:4, v/v). A solution of 1mol L^{-1} Me_3P (24 equiv) was added, and the suspension was allowed to stir for 2 h at 25 °C under nitrogen. After filtration and washings, the second sugar unit (**14**) was attached as described for the first one (**13**). The unreacted free amino groups were capped, the Alloc group was removed (as described for **33**), and the last sugar unit (**15**) was attached as described above (**13**). After each step, the resin was washed with DMF, DCM, and MeOH and dried on a filter. The protected tripodal glycocluster **35** was released with TFA in DCM (1:1, v/v, for 30 min at 25 °C), purified by RP HPLC [(vi) in Figure 5], and lyophilized to give the desired product (**35**) in a 9% (3.7 mg) overall isolated yield. Relative yield (**35** vs **32**) was 71%, respectively: ^{1}H NMR (CD_3CN, 500 MHz) δ 7.92 (d, 2H, J = 8.2 Hz), 7.83 (d, 2H, J = 8.2 Hz), 7.77 (d, 2H, J = 8.2 Hz), 7.72 (b, 1H), 7.67 (d, 2H, J = 8.2 Hz), 7.61 (b, 1H), 7.31−7.19 (m, 12H), 7.11 (b, 1H), 5.90 (dd, 1H, J = 9.6 and 9.6 Hz), 5.72 (dd, 1H, J = 9.7 and 9.7 Hz), 5.61 (dd, 1H, J = 9.6 and 8.0 Hz), 5.42 (m, 1H), 5.35 (b, 1H), 5.30 (dd, 1H, J = 10.0 and 3.4 Hz), 5.26 (dd, 1H, J = 10.0 and 10.0 Hz), 5.14 (dd, 1H, J = 10.5 and 7.8 Hz), 5.14 (d, 1H, J = 8.0 Hz), 5.08 (dd, 1H, J = 10.5 and 3.5 Hz), 4.97 (d, 1H, J = 1.2 Hz), 4.67 (d, 1H, J = 7.8 Hz), 4.54 (b, 2H), 4.49−4.41 (m, 2H), 4.35−4.04 (m, 14H), 3.70−3.28 (m, 8H), 2.43 (s, 3H), 2.38 (s, 3H), 2.37 (s, 3H), 2.32 (s, 3H), 2.40−2.32 (m, 2H), 2.14 (s, 3H), 2.11 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.98−1.96 (s, 3H), 1.95 (s, 3H); MS (ESI) $[M + H]^+$ requires 1798.6, found 1799.7.

Protected Conjugates 34 and 36−40. The other conjugates (**34**, **36**−**40**), constructed by amide linkages, were synthesized as described for **33** and **35** using a smaller scale (10 mg resin batches). Yields of these compounds (listed in Table 1) were determined by RP HPLC (Figures 4 and 5) comparing the product peak (**34**, **36**−**40**) areas to the isolated standards of **33** and **35**. Authenticity of the purified products was verified by MS (ESI). **34**: $[M + H]^+$ requires 1395.50, found 1395.49. **36**, **38**−**40**: $[M + H]^+$ requires 1798.62, found 1799.70 (**36**), 1799.70 (**38**), 1799.69 (**39**) and 1799.69 (**40**). **37**: $[(M + K + H)/2]^+$ requires 918.79, found 918.80.

Protected Conjugate 41. With the exception of coupling (10 equiv of **23** or **24**, 10 equiv of DIEA in 0.1 mol L^{-1} HOBt/NMP, 15 h at 25 °C), the synthetic details to obtain conjugate **41** were the same as described for **33**. The purified [(i) in Figure 6] conjugate **41** was obtained in a 10% yield. Relative yield (**41** vs **31**) was a 69%, respectively. For ^{1}H NMR (CD_3CN, 500 MHz) spectrum, see Supporting Information; HRMS (ESI) $[M + H]^+$ requires 1463.5, found 1463.5.

Protected Conjugate 42. Except for coupling (10 equiv of **22**, **23**, or **24**, 10 equiv of DIEA in 0.1 mol L^{-1} HOBt/NMP, 15 h at 25 °C), the synthetic details to obtain conjugate **42** were the same as described for **35**. The purified [(ii) in Figure 6) conjugate **42** was obtained in a 9% yield. Relative yield (**42** vs **32**) was 78%, respectively. For ^{1}H NMR ($CD_3CN + CDCl_3$, 500 MHz) spectrum, see Supporting Information; MS(ESI) $[M + H]^+$ requires 2051.7, found 2053.7.

Deprotected Conjugates 43 and 45. A 3.0 mg portion of the conjugate (**33** or **35**) was treated with a solution of 20 mmol L^{-1} NaOMe in MeOH (0.5 mL) for 15 h at 25 °C, and then strong cation-exchange resin was added to neutralize the mixture. The mixture was filtered, the filtrate was evaporated to dryness, and the resulted residue was dissolved in a biphasic mixture of Et_2O and water (1:1, v/v, 1.0 mL). The organic layer was removed, the water phase was lyophilized, and the crude mixture was purified/desalted by RP HPLC to give the homogenized product (**43** or **45**) (Figure 7). The product fractions were combined and lyophilized to give the desired globally deprotected conjugate in 54% (0.86 mg **43**) and 52% (0.86 mg **45**) isolated yields. **43**: ^{1}H NMR (D_2O, 500 MHz) δ 4.46 (d, 1H, J = 7.5 Hz), 4.43−4.39 (m, 3H), 4.37 (d, 1H, J = 16.0 Hz), 4.36 (d, 1H, J = 15.5 Hz), 4.25 (2 × d, b, 2H), 3.86−3.82 (m, 2H), 3.74−3.33 (m, 18H), 2.44−2.34 (m, 4H); HRMS (ESI) $[M + H]^+$ requires 755.2941, found 755.2927. **45**: ^{1}H NMR (D_2O, 500 MHz) δ 4.85 (s, 1H), 4.45 (d, 1H, J = 7.9 Hz), 4.46−4.40 (m, 3H), 4.38 (d, 1H, J = 7.9 Hz), 4.37 (d, 1H, J = 16.1 Hz), 4.36 (d, 1H, J = 16.3 Hz), 4.27 (d, b, 1H), 4.23 (2 × d, b, 2H), 4.10 (d, 1H, J = 15.5 Hz), 4.02 (m, 1H), 3.88−3.80 (m,

4H), 3.74−3.49 (m, 13H), 3.45−3.26 (m, 7H), 2.40 (m, 2H); HRMS (ESI) $[(M + 2Na)/2]^+$ requires 517.6673, found 517.6697.

Deprotected Conjugates 44, 46−50. Small samples of the conjugates (**34**, **36**−**40**) were treated similarly as described for **43** and **45**. According to RP HPLC profiles (comparing to profiles of crude **43** and **45** mixtures), no remarkable differences in product distributions were found. Authenticity of the purified products was verified by MS (ESI). **44**: HRMS (ESI) $[M + Na]^+$ requires 777.2761, found 777.2790. **46**−**50**: MS (ESI) $[M + Na]^+$ requires 1012.35, found 1012.32 (**46**), 1012.33 (**47**), 1012.33 (**48**), 1012.32 (**49**), and 1012.32 (**50**).

Deprotected Conjugates 51 and 52. A 3.0 mg portion of the conjugates (**41** or **42**) was treated with a solution of hydrazine hydrate in DMF (1:9, v/v, 0.5 mL) for 15 h at at 25 °C. The mixture was evaporated to dryness, and the residue was subjected to RP HPLC to give the homogenized product (**51** or **52**) (Figure 7). The product fractions were combined and lyophilized yielding the desired globally deprotected product **51** (0.47 mg) and **52** (0.85 mg) in 31% (**51**) and 59% (**52**) yields. **51**: ^{1}H NMR (D_2O, at 45 °C, 500 MHz) δ 5.00 (d, 1H, $J = 3.1$ Hz), 4.91 (s, 1H), 4.52−4.41 (m, 6H), 4.24 (m, 1H), 4.16 (b, 1H), 4.11 (m, 1H), 4.01−3.84 (m, 5H), 3.78−3.54 (m, 8H), 3.55 (s, 3H), 3.55 (s, 3H), 2.61 [2 × (t, 4H, $J = 5.3$ Hz, average)]; HRMS (ESI) $[M + H]^+$ requires 755.2941, found 755.2950. **52**: ^{1}H NMR (D_2O, at 45 °C, 500 MHz) δ 5.00 (d, 1H, $J = 2.8$ Hz), 4.96 (d, 1H, $J = 3.7$ Hz), 4.90 (s, 1H), 4.58−4.36 (m, 9H), 4.24 (m, 1H), 4.16 (b, 1H), 4.10 (m, 1H), 4.01−3.55 (m, 17H), 3.57 (s, 3H), 3.56 (s, 3H), 3.55 (s, 3H), 2.76−2.54 (m, 2H); HRMS (ESI) $[(M + 2Na)/2]^+$ requires 517.6673, found 755.6704.

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Supporting Information Available: General procedure and spectral data for compounds **5**, **9**, **10**, **12**, **13 22**−**24**, and **33**−**52**. This material is available free of charge via the Internet at http://pubs.acs.org.

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