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Design, synthesis, and immunochemical characterization of a chimeric glycopeptide corresponding to the *Shigella flexneri* Y O-polysaccharide and its peptide mimic MDWNMHAA

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Dedicated, with respect, to Professor Dr. Hans Kamerling on the occasion of his 65th birthday

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ABSTRACT

Two glycopeptide chimeras corresponding to the *Shigella flexneri* Y O-polysaccharide and its peptide mimic were designed in an attempt to improve the binding affinity by increasing the entropy of binding relative to the original octapeptide mimic of the O-polysaccharide. The design was based on the X-ray crystal structures of a monoclonal antibody SYA/J6 in complex with its cognate ligands, a pentasaccharide corresponding to the *S. flexneri* Y O-polysaccharide and the octapeptide mimic, MDWNMHAA. Both chimeric molecules consist of a rhamnose trisaccharide linked through an α- or β-thioglycosidic linkage to a MDW moiety in which the W unit has been modified. We predicted that omission of the NMHAA moiety would obviate the bound water molecules that provided complementarity with the antibody-combining site, and the conformational restriction resulting from imposition of an α-turn at the C-terminus of the peptide. The glycopeptides were then docked into the active site of SYA/J6 using the program AUTODOCK 3.0, and the structures were optimized. The best models obtained in each case showed that the chimeric molecules, with either an α- or β-thioglycosidic linkage, might be reasonable surrogate ligands for the antibody. We report here the synthesis of the α-glycopeptide employing solution and solid-phase strategies. Immunochemical characterization indicated that the α-glycopeptide unfortunately did not inhibit binding of SYA/J6 to the *S. flexneri* Y lipopolysaccharide.

1. Introduction

Antigens that can stimulate both humoral and cellular immune responses, are crucial in the development of effective vaccines against pathogenic bacteria.[1] While carbohydrates that coat the surfaces of bacteria are capable of inducing an immune response that can recognize whole bacteria,[2–8] polysaccharide vaccines fail to provide protection in infants, the elderly, and in immunodeficient persons since the immune response induced might not involve the T-helper cells of the immune system.[2–11] Conversion of some of these cell-surface polysaccharides into T-cell-dependent antigens, by conjugation to carrier proteins, has resulted in potential vaccines that could successfully target infectious bacteria in humans.[5,8,11] However, progress in this field is challenging because the complex structures of the polysaccharide–protein conjugates make them difficult to be synthesized and characterized.[12,13] Consequently, to drive T-cell-dependent immune responses against carbohydrate antigens, other vaccination strategies need to be explored in order to overcome some of the limitations of carbohydrate vaccines.

Molecular mimics of carbohydrates that have the potential to raise antibodies that cross-react with the natural structures have been identified, and there is now a growing interest in carbohydrate–mimetic peptides as vaccines to target cell-surface polysaccharides of infectious bacteria.[14–17] In addition to their ability to elicit carbohydrate-binding antibody responses, these peptide mimics should focus the immune responses on particular epitopes since greater discrimination of the peptides for the corresponding antibody-combining sites has been observed,[18] thus minimizing autoimmune reactions with self structures where the original carbohydrate antigens would pose a threat as immunogens.[17–19]

Shigella flexneri Y is a virulent bacterium that causes bacillary dysentery by invading the colonic mucosa.[20] Screening of a phage-displayed peptide library with an anti-carbohydrate antibody SYA/J6, directed against the O-polysaccharide (Fig. 1a) of *S. flexneri* Y yielded the peptide sequence MDWNMHAA (Fig. 1b).[18] The X-ray structures and the thermodynamics of binding of the Fab complexes of SYA/J6 with a pentasaccharide portion

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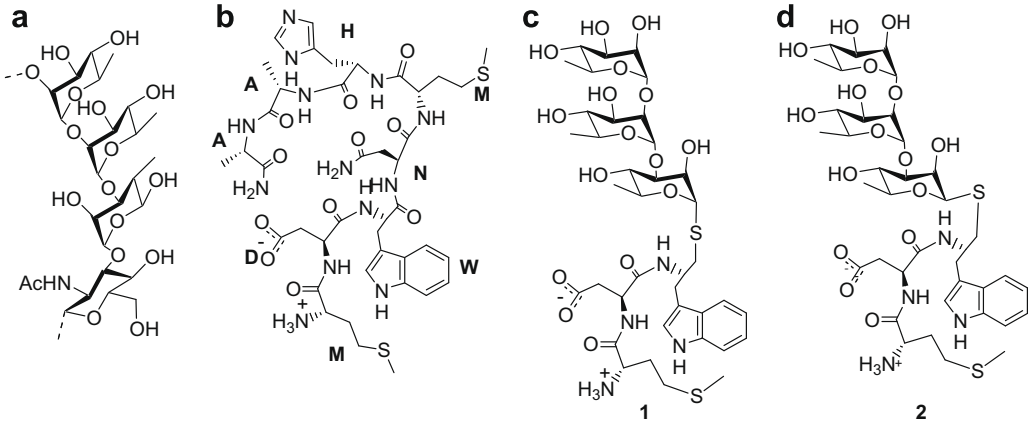


Figure 1. Structures of the (a) the O-polysaccharide of *Shigella flexneri* Y, (b) its peptide mimic (c) the chimeric α-glycopeptide **1**, and (d) the chimeric β-glycopeptide **2**.

[→2)-α-L-Rha-(1→2)-α-L-Rha-(1→3)-α-L-Rha-(1→3)-β-D-GlcNAc-(1→2)-α-L-Rha-(1→] of the O-polysaccharide (Fig. 1a) and with the octapeptide mimic, MDWNMHAA (Fig. 1b) have been studied in detail, and interesting similarities and differences between the two complexes have been revealed.[21,22]

Although the octapeptide (Fig. 1b) complements the shape of the combining site groove much better than the pentasaccharide, and makes greater contacts with the antibody-combining site, the binding affinity of the peptide is only twofold higher than that of the pentasaccharide, potentially compromising the immunogenicity of the peptide.[21,22] The affinity can be attributed to the considerable unfavorable entropy that arises from a more ordered bound peptide and the immobilization of several water molecules.[17,22] Furthermore, for a peptide to be immunogenic, it might be necessary that a sufficient population of the bound conformation be displayed in the conformational ensemble of the free peptide.[14,15,17] Since the α-helix adopted by NMHAA in the C-terminus of MDWNMHAA is only restricted to the bound conformation and not to that of the free peptide, we hypothesized that MDWNMHAA might not lead directly to a cross-reactive response against the corresponding polysaccharide.[14,15,17] Nevertheless, we believed it could still be used in prime–boost strategies to strengthen the immune responses already induced by the polysaccharide epitopes, as shown with a peptide mimic of the capsular polysaccharide of *Cryptococcus neoformans*.[23] In order to test these hypotheses, we synthesized protein conjugates of the octapeptide[24] and recently evaluated their immunogenicities.[16] Indeed, we found that, although the kinetics of the immune response in mice were slow, cross-reactivity was observed, and an effective prime–boost strategy was developed.[16]

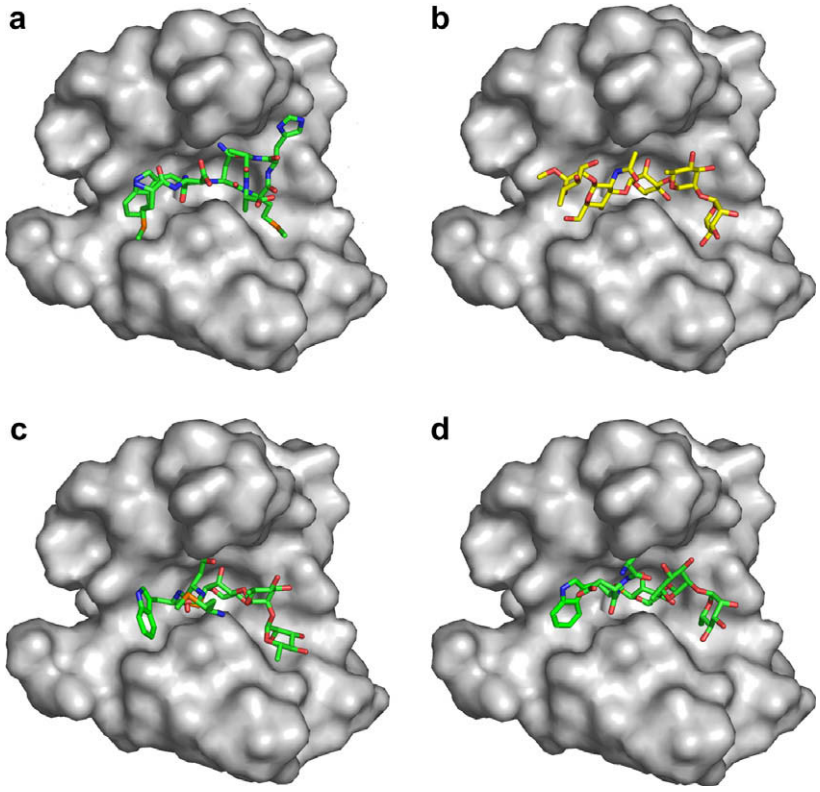


Figure 2. Structures of the Fab fragment of SYA/J6 antibody with (a) bound octapeptide MDWNMHAA, (b) bound pentasaccharide, (c) bound α-glycopeptide **1**, and (d) bound β-glycopeptide **2**.

We now report the design of two chimeric glycopeptides (Fig. 1) that might serve as surrogate haptens against *S. flexneri* Y, and the synthesis and immunochemical evaluation of the first candidate.

2. Results and discussion

Based on the information gained from the X-ray structures of the antibody–pentasaccharide and antibody–octapeptide complexes,[21,22] and by molecular modeling, we have designed two chimeric molecules (Fig. 1c and d) that contain certain elements of both the oligosaccharide and the octapeptide mimic, and this should lead to increased binding affinity by increasing the entropy of binding relative to the parent octapeptide. The glycopeptides **1** and **2** comprise the two fragments, MDW and the rhamnose trisaccharide (A–B–C), derived from the parent octapeptide and pentasaccharide, respectively, and linked as a thioglycoside; the two glycopeptides differ in the α- or β-configuration at the thioglycosidic linkage (Fig. 1). We chose to retain the MDW portion of the octapeptide to maintain the favorable hydrophobic interaction of the W moiety within the combining site. Replacement of the NMHAA unit specifically by the rhamnose trisaccharide (A–B–C) in the glycopeptides **1** and **2**, was done firstly to ensure that ring C penetrates the deep hole at the bottom of the binding site, thus obviating the engagement of water molecules that provide complementarity with the antibody-combining site in binding of the peptide MDWNMHAA, and secondly, to reduce any conformational entropy differences that result from imposition of the α-turn at the C-terminus of the parent octapeptide upon binding. The incorporation of a thioglycosidic linkage between the trisaccharide moiety and the tripeptide in the glycopeptides **1** and **2** was done to increase their stabilities compared to those of the corresponding *O*-glycosides.[25]

2.1. Molecular modeling

In order to validate the choice of the glycopeptides **1** and **2** (Fig. 1), they were each docked into the Fab fragment of SYA/J6 using AUTODOCK 3.0[26] starting from the coordinates of the two corresponding moieties in the complexes Fab-ABCDA′ and Fab-MDWNMHAA (Fig. 2a and b).[21,22] The most favored mode of binding in each case, with the lowest docked energy, showed that both **1** and **2** could fit into the binding groove without steric clashes (Fig. 2c and d). Superposition of the two binding modes of **1** and **2** on the parent octapeptide (Fig. 3a and c) and the parent pentasaccharide (Fig. 3b and d) indicated that both glycopeptides **1** and **2** superimposed well on the parent ligands; furthermore, the favorable hydrophobic interaction of the W moiety with the active site, as well as the penetration of ring C into the deep hole at the bottom of the binding site were preserved in both molecules. The interaction of the W moiety with the hydrophobic pocket within the Ab-combining site was more pronounced in the β-glycopeptide (Fig. 2c and d). Thus, the docking experiments justify the choice of **1** and **2** as reasonable surrogate ligands for the antibody SYA/J6. We report here the synthesis and immunochemical evaluation of the first candidate, namely the α-glycopeptide **1**, which lent itself to a more facile synthesis.

2.2. Synthesis of the α-glycopeptide 1

Retrosynthetic analysis indicated that the α-glycopeptide **1** could be obtained by deprotection of the glycopeptide **3**, which, in turn, could be derived from the trisaccharide **4** and the tripeptide bromide **5** (Scheme 1).

The trisaccharide **4** was prepared by methods analogous to those that we had previously developed[27,28] to prepare oligosaccharides containing α-(1→2)- and α-(1→3)-L-rhamnopyranosidic linkages. In the present case (Scheme 2), the reducing terminal rhamnose unit was introduced as a thioglycoside in order to facilitate later conversion to a 1-thiol via the corresponding glycosyl bromide. Thus, the known 2,4-dibenzoylated rhamnose thioglycoside **6**[29] was glycosylated at the 3-position with the rhamnose trichloroacetimidate donor **7**[28] to give the disaccharide derivative **8** in >90% yield. Selective methanolysis of the 2′-*O*-acetate using HCl–MeOH gave the disaccharide glycosyl acceptor **9** in only moderate yield due to an unusually low selectivity for reaction of the acetate over the benzoate esters. A second glycosylation reaction with donor **7** gave the trisaccharide **10** in 79% yield. The benzoates were replaced by acetates through base-catalyzed methanolysis, followed by acetylation under standard conditions to give the acetylated phenylthio trisaccharide **11**, in quantitative yield for the two steps. The phenylthio group was replaced by SH in a three-step procedure that was carried out without purification of intermediates. Thus, the phenylthio group was first replaced by a bromide by treatment of the thioglycoside **11** with IBr[30] to yield the glycosyl bromide **12**. Nucleophilic displacement of the bromide gave the isothiouronium salt **13** which was immediately hydrolyzed to the thiol **4**. Compound **4** was obtained as the pure α anomer by crystallization of the crude α/β product mixture from EtOAc–hexanes. Analysis by ^{1}H NMR spectroscopy indicated that the α anomer **4**, in $CDCl_3$ did not undergo significant anomerization over several days at ambient temperature.

The tripeptide **5** was prepared from the alcohol **14** by a bromination reaction using triphenylphosphine and carbon tetrabromide

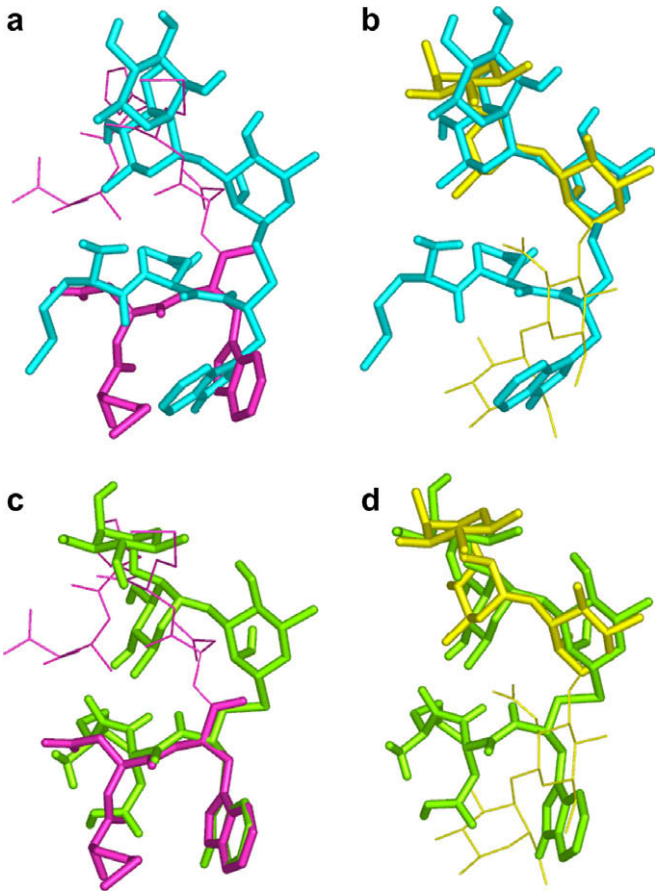
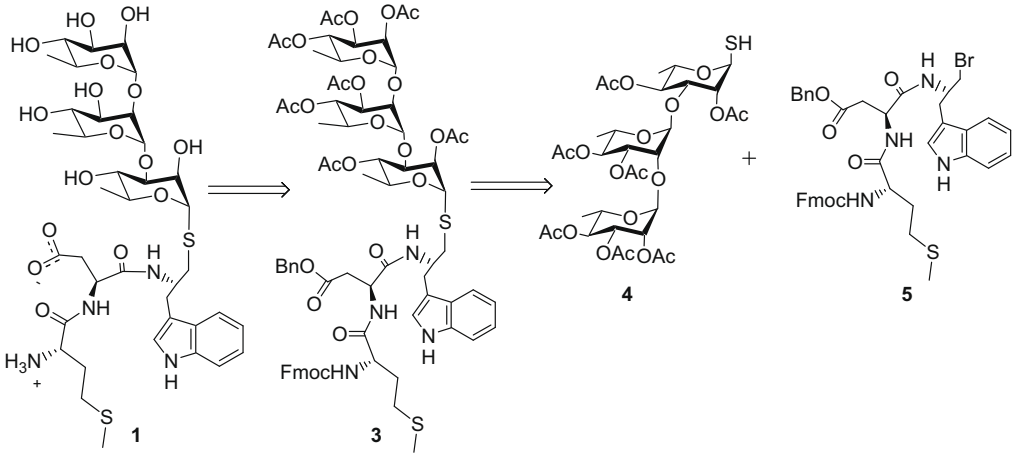
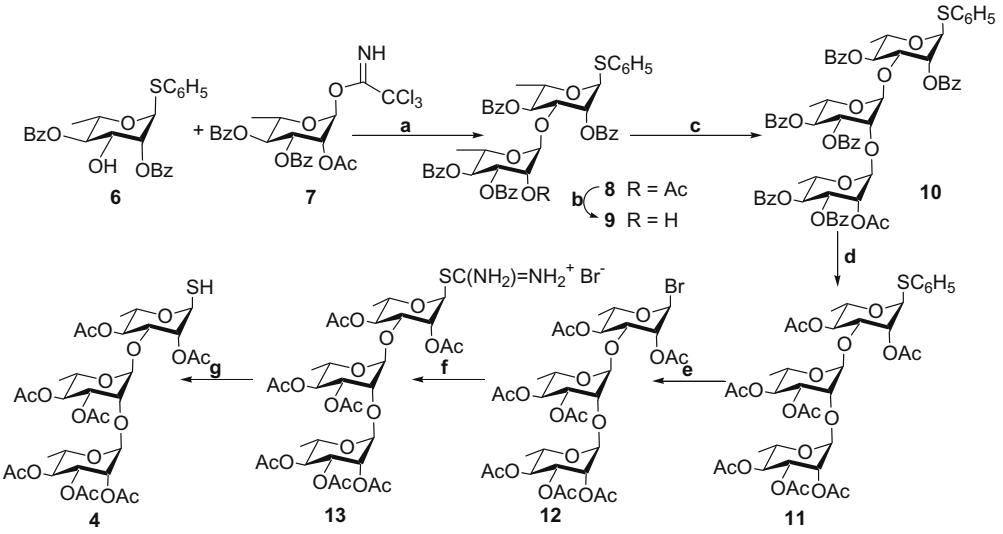


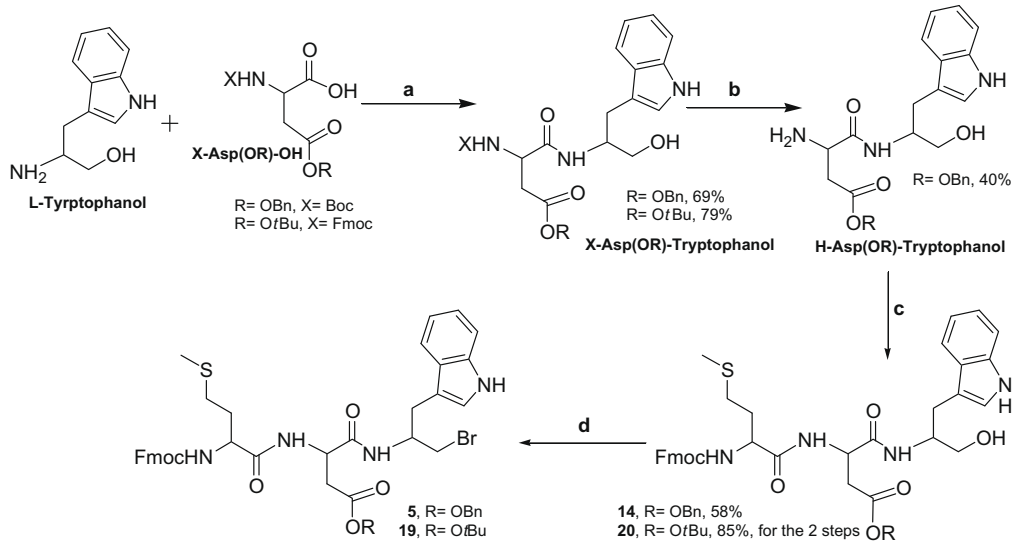
Figure 3. Superposition of the (a) α-glycopeptide **1** (cyan) on the parent octapeptide (pink), (b) α-glycopeptide **1** (cyan) on the parent pentasaccharide (yellow), (c) β-glycopeptide **2** (green) on the parent octapeptide (pink), and (d) β-glycopeptide **2** (green) on the parent pentasaccharide (yellow).



Scheme 1. Retrosynthetic analysis of the α-glycopeptide **1**.



Scheme 2. Synthesis of the precursor **4**. Reagents and conditions: TMSOTf, CH_2Cl_2, −40 °C→rt, >90%, (b) CH_3COCl, MeOH, pyridine, 45 °C→rt, 58%, (c) **7**, TMSOTf, CH_2Cl_2, −40 °C→rt, 79%, (d) NaOMe–MeOH, MeOH, Ac_2O, Pyr, quantitative, (e) IBr, CH_2Cl_2, (f) thiourea,CH_3CN, reflux, and (g) $Na_2S_2O_5$, CH_2Cl_2–H_2O, reflux, 56% over three steps.

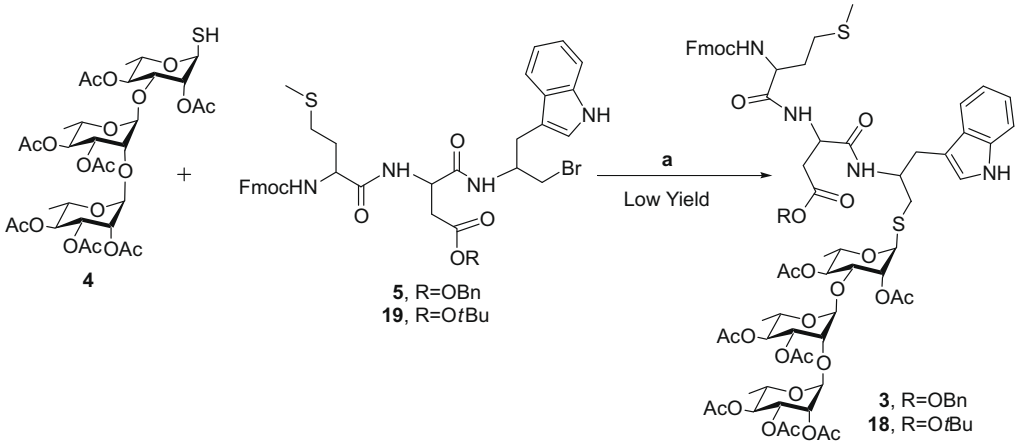


Scheme 3. Synthesis of the tripepides **5** and **19**. Reagents: (a) DCC, HOBt, (b) TFA, or piperidine, (c) Fmoc-Met-OH, DCC, HOBt, and (d) CBr_4, PPh_3, CH_2Cl_2.

(Scheme 3). The alcohol **14** was assembled using standard solution-phase peptide assembly techniques from L-tryptophanol,[31] BOC-Asp(OBn)-OH, and Fmoc-Met-OH, with dicyclohexylcarbodiimide (DCC)–1-hydroxybenzotriazole (HOBt) as the coupling agent (Scheme 3). The aspartate side-chain carboxyl group was protected as a benzyl ester, while the *N*-terminal methionine was protected as the α-*N*-Fmoc derivative since it was envisioned that a single, basic deprotection step would suffice for removal of all protecting groups from both the peptide and trisaccharide portions of the target molecule **1**.

The crude bromide **5** was then coupled with the trisaccharide thiol **4** using a two-phase reaction (Scheme 4) with the phase-transfer protocol that was recently reported to be effective for the synthesis of thio-linked glycopeptide derivatives.[32] In the present application, however, the phase-transfer reaction suffered from several competing reaction pathways and provided the glycopeptide **3** in low yield as mixtures with side products. If the processing did not involve acidic conditions, such as an aq citric acid wash, purification by flash chromatography gave the α-glycopeptide **3**, which was contaminated by sizeable portions of two impurities. One impurity was entirely made up of carbohydrate, and was tentatively identified as the disulfide **15** resulting from air-oxidation of thiol **4** (Fig. 4). The other impurity was entirely peptidic in character and was assigned to be the oxazoline derivative **16** (Fig. 4), resulting from intramolecular displacement of the primary bromide by nucleophilic attack of the oxygen atom of the neighboring amide. These impurities could be removed by HPLC, but it was eventually determined that this was not necessary. Implementation of a citric acid wash in the processing resulted in ring-cleaving hydrolysis of the oxazoline to give a product that was tentatively assigned the ester structure **17** (Fig. 4). This was much more polar than the initial peptide impurity, and was then easily separable from the glycopeptide by flash chromatography. The other acetylated carbohydrate impurity **15** was unchanged by this modified workup, and was still not removed by flash chromatography. The mixture was then used without further purification in the deprotection step.

Treatment of the protected glycopeptide **3** with NaOMe in MeOH resulted in formation of a more polar product as shown by TLC analysis of the reaction mixture. Isolation of this material by flash chromatography and analysis by HPLC, and by NMR and MALDI spectroscopy, made it apparent that the product was a mixture of several similar compounds, none of which appeared to be the desired glycopeptide **1**. The mass spectrum indicated that the major components of the mixture had masses of 18 mass units less than that of the expected glycopeptide **1**. It was concluded that the strongly basic deprotection conditions had resulted predominantly in aspartimide formation, and that partial hydrolysis must have occurred during the subsequent HPLC analysis to give mixtures comprising both α- and β-amido linkages between the aspartate and tryptophan units (Scheme 5). This is a well-known side reaction[33] in peptide synthesis and has often been observed when peptides containing aspartate side-chain esters are deprotected by treatment with base.



Scheme 4. Synthesis of the protected glycopeptide **3**. Reagents: (a) $NaHCO_3$, Bu_4NHSO_4, EtOAc–H_2O, ~16%, after HPLC.

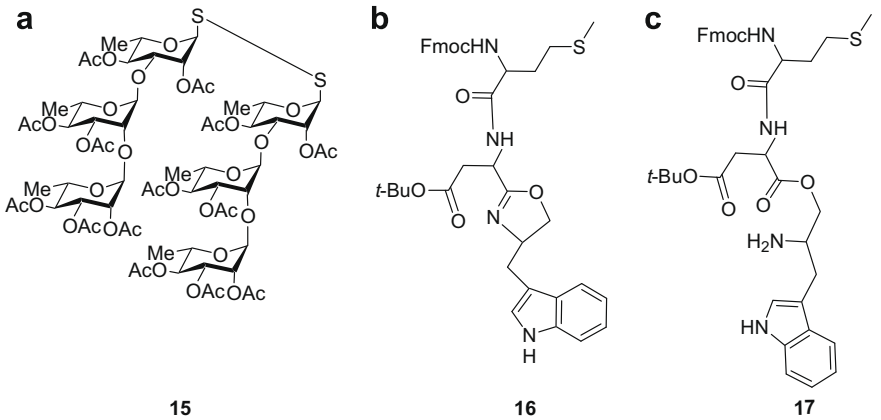
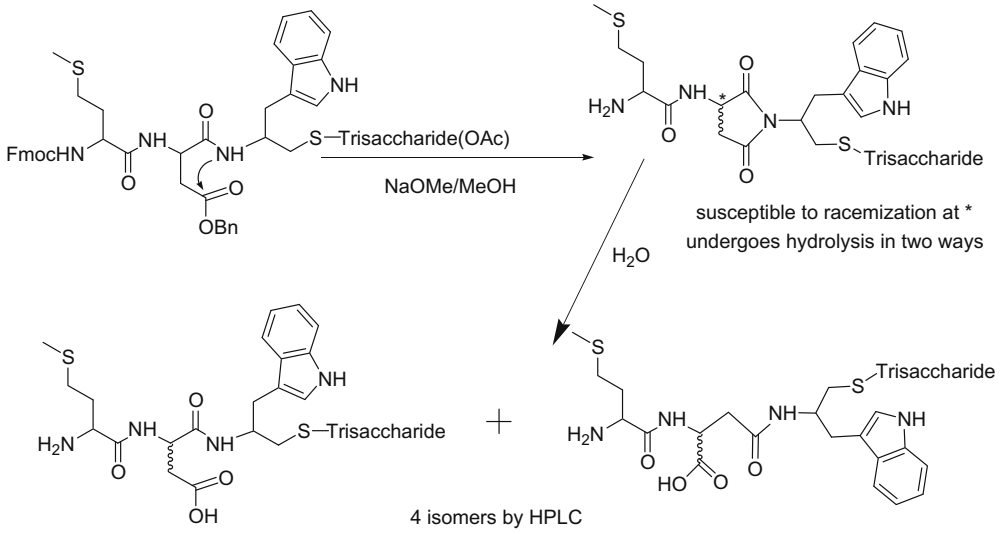


Figure 4. Structures of the (a) disulfide **15**, (b) oxazoline derivative **16**, and (c) ester **17**.

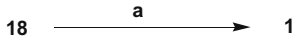


Scheme 5. Aspartimide formation in the deprotection of the protected glycopeptide **3**.

The aspartimide problem was eventually avoided by the preparation of the glycopeptide **18** in which the benzyl ester was replaced by a *tert*-butyl ester (O*t*Bu), giving a protecting group for the aspartate side chain that could be selectively removed under acidic conditions. The glycopeptide **18** was prepared by the coupling of the crude tripeptide bromide **19** with the thiol **4** (Scheme 4), using the phase-transfer protocol,[32] but the glycopeptide **18** was obtained in low yield, and the side products **15** and **16** were also detected. The bromo compound **19** was obtained by the bromination of tripeptide **20**, which in turn, was prepared from L-tryptophanol,[34] Fmoc-Met-OH, and Fmoc-Asp(O*t*Bu)-OH amino acids, following the same strategy that was used to prepare the tripeptide **14** (Scheme 3).

In the deprotection of the glycopeptide **18**, when the –O*t*Bu group was first removed using TFA–TIPSiH, the intermediate free aspartate carboxyl group was converted to a carboxylate salt by subsequent NaOMe treatment. It therefore became resistant to aspartimide formation, while the other protecting groups were smoothly removed (Scheme 6). The selective cleavage of the aspartate ester also meant that the acetylated carbohydrate disulfide impurity **15** in the glycopeptide was then considerably less polar than the free aspartate glycopeptide, and it could be readily separated in the early fractions during chromatographic purification of the partially deprotected glycopeptide. Nevertheless, after the base-catalyzed deprotection that followed, a considerable amount of sodium acetate (which was formed by neutralization of the reaction mixture with acetic acid) did co-elute with the glycopeptide **1** fraction during the final chromatographic purification.

We next examined a more efficient synthesis of the glycopeptide **18**. We reasoned that an alternative disconnection was desirable, in particular, one that avoided the presence of the aspartate and methionine moieties in the coupling reaction with **4**. Accordingly, the thiol **4** was coupled with the Fmoc-tryptophanol bromide derivative **21**; here, the two-phase reaction proceeded smoothly to give the glycopeptide **22** as the sole product in excellent yield (Scheme 7).



Scheme 6. Deprotection of **18** to the α-glycopeptide **1**. Reagents: (a) (i) TFA–TIPSiH, (ii) NaOMe–MeOH, 32%.

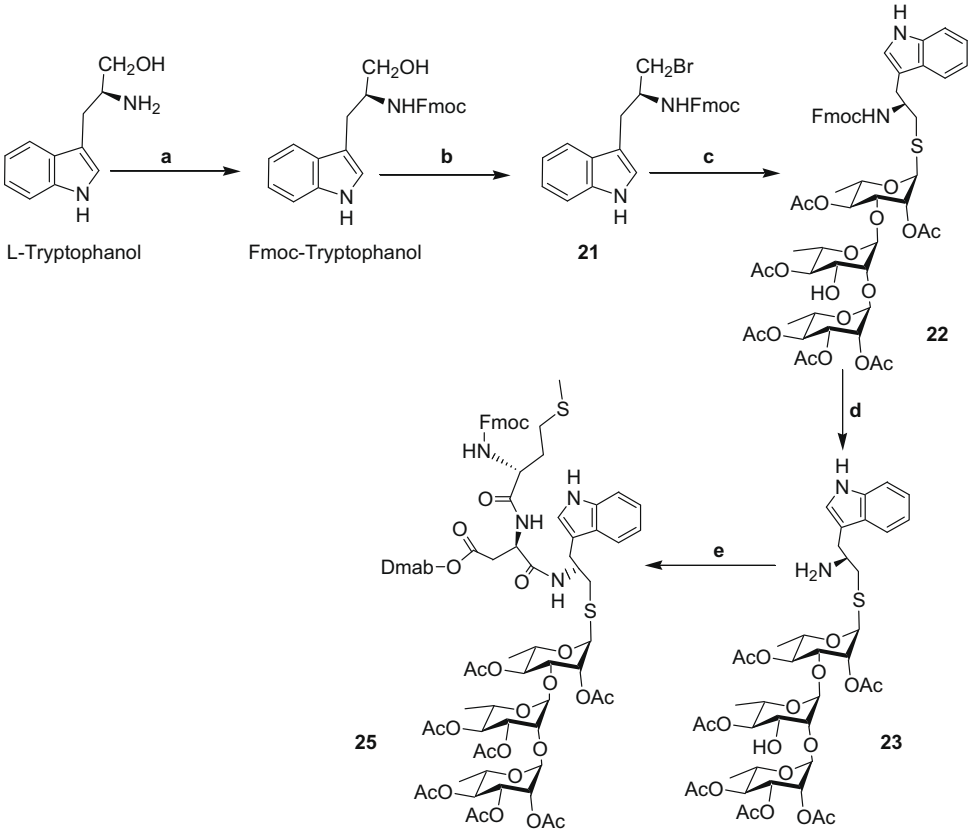
The bromide **21** was prepared from L-tryptophanol[34] in two steps (Scheme 7). L-tryptophanol was first treated with Fmoc-succinimide to yield Fmoc-tryptophanol. Bromination of the Fmoc-tryptophanol was then carried out using PPh_3–CBr_4 to afford the bromide **21** in 65% yield. Compound **21** was obtained even more efficiently (95%) when Fmoc-tryptophanol was converted into its mesylate and was subsequently treated with lithium bromide (Scheme 7).

The Fmoc protecting group in **22** was then removed using DBU–octanethiol[35] to form the amine **23**, which was coupled to the dipeptide **24** using *N*-hydroxybenzotriazole (HOBt) as coupling agent to afford the protected glycopeptide **25**, with a minor peptide impurity that was readily removed by HPLC to afford the protected glycopeptide **25** (Scheme 7).

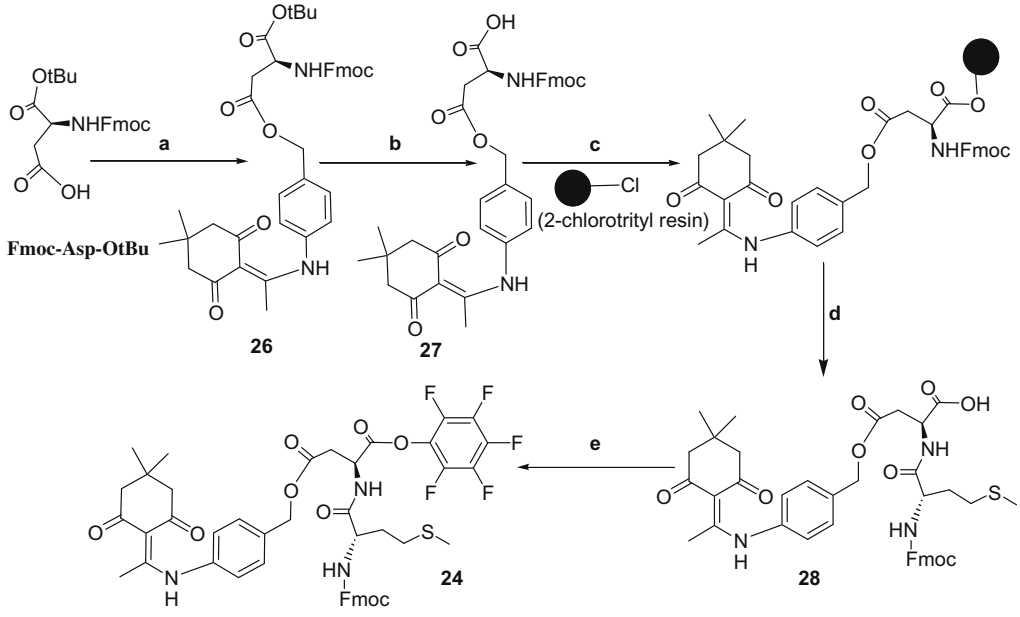
The dipeptide **24**, in turn, was constructed on solid support employing Fmoc chemistry[36] and an HBTU–HOBt–DIPEA coupling strategy[37–39] as shown in Scheme 8. In this method, we chose the protecting group *N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]aminobenzyl ester (Dmab)[40] on the side chain of Fmoc-aspartic acid because it is stable to piperidine, one of the requirements for the solid-phase synthesis of dipeptide **24**. Fmoc-Asp(ODmab)-OH (**27**) and the commercially available Fmoc-Met-OH were used in the solid-phase synthesis, and since a free carboxylic acid group was required at the C-terminus in the synthesis of the dipeptide **24**, a 2-chlorotrityl resin[41] was chosen as the solid support. The Fmoc-Asp(ODmab)-OH (**27**) was successfully prepared in two steps starting from the commercially available Fmoc-Asp-O*t*Bu (Scheme 8). The Dmab protecting group was first introduced on the side chain of Fmoc-Asp-O*t*Bu by reacting the amino acid with Dmab-OH in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP), HOBt, and DIC, to afford the product **26** in 71% yield. Treatment of **26** with 50% TFA in CH_2Cl_2 gave the desired product **27** in nearly quantitative yield (Scheme 8).

After cleavage from the resin with dilute acid, and purification by column chromatography, the dipeptide **28** was obtained in 80% yield (Scheme 8). The conversion of the carboxylic acid of **28** to the more active pentafluorophenol ester **24** was done to increase the coupling efficiency between the precursors **23** and **24**.

The Fmoc protecting group on the fully protected glycopeptide **25** was then removed selectively using DBU–octanethiol[35] so that the side product dibenzofulvene that formed could be scavenged by the octanethiol, and the complex that formed could be easily



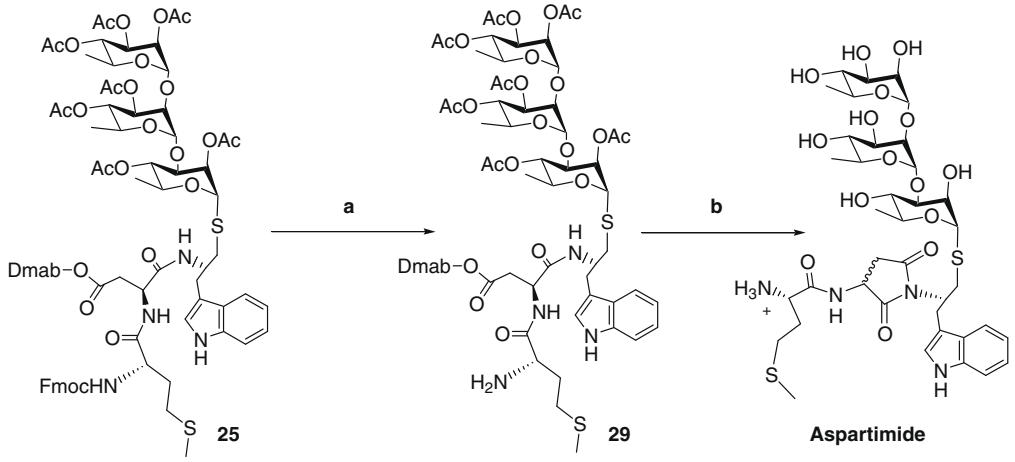
Scheme 7. Synthesis of the protected glycopeptide **25**. Reagents: (a) Fmoc-OSu, $NaHCO_3$, acetone–H_2O, 95%, (b) (i) MsCl–py, (ii) LiBr, refluxed, 95% for the two steps, (c) **4**, $NaHCO_3$, Bu_4NHSO_4, EtOAc–H_2O, 92%, (d) DBU, octanethiol, THF, quantitative, and (e) **24**, HOBt, THF, 72%.



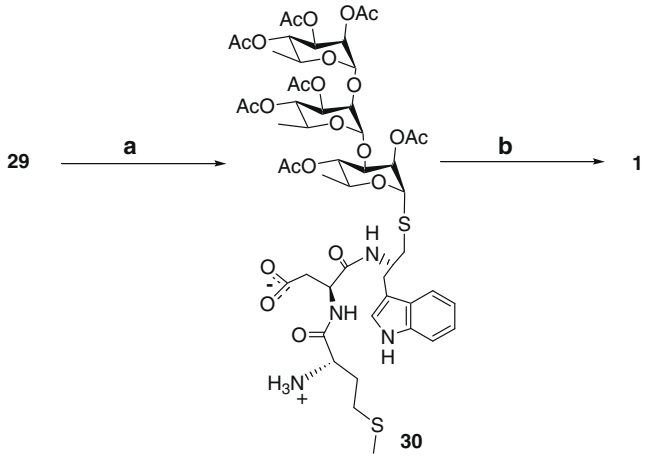
Scheme 8. Synthesis of the dipeptide **24**. Reagents: (a) Dmab-OH, DTBMP, HOBt, DIC, 71%, (b) 50% TFA in CH_2Cl_2, 97%, (c) DIPEA, DMF, (d) (i) 25% piperidine–DMF, (ii) Fmoc-Met-OH, HBTU–HOBt–DIPEA, DMF, (iii) 5% TFA in CH_2Cl_2, 80%, and (e) pentafluorophenol, DIC, 73%.

removed in ether to give **29** (Scheme 9). Treatment of the amine **29** with NaOMe–MeOH, however, did not afford the desired glycopeptide **1** (Scheme 9), but resulted in the formation of the same aspartimide side product that was formed in the deprotection of the glycopeptide **3** (Scheme 5).

However, when the Dmab and the acetate protecting groups in **29** were sequentially removed, using 2% hydrazine–THF to first afford **30**, followed by NaOMe–MeOH, the desired glycopeptide **1** was obtained in 68% yield, after treating the mixture first with Rexyn 101 H^+ to make the mixture slightly basic, followed by



Scheme 9. Aspartimide formation in the deprotection of the glycopeptide **25**. Reagents: (a) DBU–octanethiol, THF, quantitative and (b) NaOMe, MeOH, 88%.



Scheme 10. Deprotection of **29** to the α-glycopeptide **1**. Reagents: (a) 2% hydrazine, THF, quantitative and (b) NaOMe–MeOH, 68%.

further neutralization with acetic acid, and purification by HPLC (Scheme 10).

2.3. Immunochemistry

The binding of the glycopeptide to the monoclonal antibody SYA/J6 was investigated by competitive inhibition ELISA studies, using *S. flexneri* Y LPS as coating antigen. The purified glycopeptide was analyzed as an inhibitor of the binding of monoclonal antibody SYA/J6, while the LPS, the purified O-polysaccharide, and the mimetic-octapeptide, were used as control inhibitors. No inhibitory activity was observed with the glycopeptide even at the highest concentration tested (200 μg/mL). Both the LPS and O-polysaccharide showed IC_{50} values <0.7 μg/mL and the mimetic-octapeptide showed an IC_{50} value of 6.25 μg/mL (data not shown). The relative inhibitory activities of multivalent carbohydrate ligand and monovalent peptide ligand differ from those obtained by microcalorimetry for monovalent pentasaccharide and octapeptide ligands (twofold difference);[21,22] we attribute these differences to the effect of multivalent presentation of carbohydrate ligands. The reason for the disappointing lack of inhibition by the glycopeptide is not at all obvious and will require further investigation through synthesis and immunochemical evaluation of its stereoisomer, the β-linked glycopeptide.

3. Experimental

3.1. Synthesis

3.1.1. General methods

The Fmoc amino acids that were used were purchased from Novabiochem, and the other reagents were purchased from Aldrich Chemical Co. DMF was freed of amines by concentrating it under high vacuum, and it was then distilled and stored over 4 Å molecular sieves, whereas the other solvents were distilled according to standard procedures.[42] The dipeptide **3** was synthesized on 2-chlorotrityl resin[41] (Novabiochem) (1.64 mmol/g substitution level), using standard 9-fluorenylmethoxycarbonyl (Fmoc) chemistry[36] employing the 2-[1*H*-Benzotriazole-1-yl]-1.1.13.3-tetramethyluronium hexafluorophosphate–1-hydroxybenzotriazole (HBTU–HOBt) coupling strategy.[37–39] The Kaiser ninhydrin (5% ninhydrin in EtOH, 80% phenol in EtOH, and 2% 0.001 M aq KCN in pyridine) assay for the amino group[43] was used to monitor both the Fmoc-amino acid coupling and the Fmoc deprotection reactions. 1D- and 2D-NMR spectra were recorded on 400-, 500 and 600-MHz spectrometers. Chemical shifts were referred to internal $CHCl_3$ or external DSS [3-(trimethylsilyl)-1-propanesulfonic acid] and were reported in δ units relative to tetramethylsilane. Coupling constants were obtained from a first-order analysis of one-dimensional spectra, and spectral assignments were based on COSY, HMQC, and TOCSY experiments. MALDI-TOF mass spectra were obtained for samples dispersed in a 2,5-dihydroxybenzoic acid matrix on a Perspective Biosystems Voyager-DE instrument. High resolution mass spectra were obtained using LSIMS (FAB), run on a Kratos Concept H double focusing mass spectrometer at 10,000 RP and by the electrospray ionization method, using an Agilent 6210 TOF LC/MS high resolution magnetic sector mass spectrometer.

3.1.2. Phenyl 2-*O*-acetyl-3,4-di-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzoyl-1-thio-α-L-rhamnopyranoside (8)

A mixture of the thioglycoside **6**[29] (1.33 g, 2.86 mmol) and the trichloroacetimidate **7**[28] (1.52 g, 2.72 mmol) in anhyd CH_2Cl_2 (30 mL) was stirred with freshly activated, crushed 4 Å molecular sieves (1.3 g) under N_2 for 10 min at room temperature. The mixture was cooled in a −40 °C bath, and trimethylsilyl triflate (40 μL, 0.22 mmol) was added. After 15 min, the cooling bath was removed, and the mixture was allowed to warm to room temperature over a period of 40 min. Et_3N (0.2 mL) was added, and the molecular sieves were removed by filtration through Celite, with the aid of additional CH_2Cl_2 (100 mL). The filtrate was washed with

satd $NaHCO_3$ (30 mL), dried over $MgSO_4$, and concentrated to a syrup. Flash chromatography (3:1 to 2:1 hexanes–EtOAc) gave the disaccharide **8** (2.25 g, >90%) as a colorless, hard foam containing approximately 10 mol % of the byproduct trichloroacetamide (^{1}H NMR, broad singlet at δ 6.6). The mixture was used directly in the next reaction. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25–7.25 (25 H, m, Ar), 5.74 (1H, dd, $J_{1,2}$ = 1.9, $J_{2,3}$ = 3.3 Hz, H-2), 5.71 (1H, d, H-1), 5.63 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4), 5.47 (1H, dd, $J_{2',3'}$ = 3.4, $J_{3',4'}$ = 9.9 Hz, H-3′), 5.36 (1H, t, $J_{4',5'}$ = 9.8 Hz, H-4′), 5.11 (1H, dd, $J_{1',2'}$ = 2.0 Hz, H-2′), 5.08 (1H, d, H-1′), 4.54 (1H, dq, H-5), 4.42 (1H, dd, H-3), 4.06 (1H, dq, H-5), 1.95 (3H, s, OAc), 1.37 (3H, d, $J_{5,6}$ = 6.3 Hz, H-6), 1.33 (3H, d, $J_{5',6'}$ = 6.3 Hz, H-6′). ^{13}C NMR (150 MHz, $CDCl_3$): δ 169.4 (1C, OAc), 166.0–164.7 (4C, 4 × OBz), 133.6–127.9 (30C, Ar), 99.4 (1C, C-1′), 85.6 (1 C, C-1), 76.6 (1C, C-3), 73.8 (1C, C-2), 73.2 (1C, C-4), 71.5 (1C, C-4′), 69.9 (1C, C-2′), 69.3 (1C, C-3′), 68.3 (1C, C-5), 67.6 (1C, C-5′), 20.6 (1C, OAc), 17.6 (1C, C-6), 17.3 (1C, C-6′). MALDI-TOFMS: *m/z* 883.3 (M+Na).

3.1.3. Phenyl 3,4-di-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzoyl-1-thio-α-L-rhamnopyranoside (9)

The disaccharide **8** (8.15 g, 9.95 mmoL) was dissolved in MeOH (250 mL), and the solution was stirred rapidly. AcCl (4 mL) was added dropwise, and the mixture was stirred for 4 h at rt and then at 45 °C for 3.5 h. The solution was cooled to rt and neutralized by the dropwise addition of pyridine. The solvents were evaporated, and the residue was partitioned between dichloromethane (200 mL) and satd $NaHCO_3$ solution (100 mL). The organic phase was dried over $MgSO_4$ and was concentrated to give a syrup. Purification by column chromatography (hexanes:EtOAc; 2:1) gave the disaccharide hemiacetal **9** (4.54 g, 58%) as a colorless foam containing a trace of the starting 2′-*O*-acetate, as indicated by TLC. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.25–7.25 (25H, m, Ar), 5.75 (1H, dd, $J_{1,2}$ = 1.5, $J_{2,3}$ = 2.4 Hz, H-2), 5.71 (1H, d, H-1), 5.62 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4), 5.42 (1H, t, $J_{3',4'}$ = $J_{4',5'}$ = 9.5 Hz, H-4′), 5.37 (1H, dd, $J_{2',3'}$ = 2.9 Hz, H-3′), 5.09 (1H, d, $J_{1',2'}$ = 1.5 Hz, H-1′), 4.56 (1H, dq, H-5), 4.45 (1H, dd, H-3), 4.08 (1H, dq, H-5′), 3.98 (1H, br s, H-2′), 2.05 (1H, br s, 2′-OH) 1.35 (3H, d, $J_{5,6}$ = 6.4 Hz, H-6), 1.17 (3H, d, $J_{5',6'}$ = 6.4 Hz, H-6′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.0, 165.5 (2C) and 164.9 (4C, 4 × OBz), 133.5–127.9 (30C, Ar), 101.5 (1C, C-1′), 85.6 (1C, C-1), 76.1 (1C, C-3), 73.9 (1C, C-2), 73.4 (1C, C-4), 72.1 (1C, C-4′), 71.2 (1C, C-2′), 69.4 (1C, C-3′), 68.1 (1C, C-5), 67.5 (1C, C-5′), 17.6 (1C, C-6), 17.3 (1C, C-6′). MALDI-TOFMS: *m/z* 841.3 (M+Na). Anal. Calcd for $C_{46}H_{42}O_{12}S$: C, 67.46; H, 5.17. Found: C, 67.67; H, 5.11.

3.1.4. Phenyl 2-*O*-acetyl-3,4-di-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzoyl-1-thio-α-L-rhamnopyranoside (10)

A mixture of the disaccharide **9** (4.54 g, 5.54 mmol) and the trichloroacetimidate **7** (3.34 g, 5.98 mmol) in anhyd CH_2Cl_2 (90 mL) was stirred with freshly activated, crushed 4 Å molecular sieves (4 g) under N_2 for 10 min at room temperature. The mixture was cooled in a −40 °C bath, and Et_3N (80 μL, 0.45 mmol) was added. After 30 min, the cooling bath was removed and the mixture was allowed to warm to room temperature over a period of 0.5 h. Et_3N (0.3 mL) was added and the sieves were removed by filtration through Celite, with the aid of additional CH_2Cl_2 (100 mL). The filtrate was washed with satd $NaHCO_3$ (50 mL), dried over $MgSO_4$, and concentrated to a syrup. Purification by column chromatography (20:1 toluene–EtOAc;) gave the trisaccharide **10** (5.36 g, 79%) as a colorless, hard foam. $[\alpha]_D^{25}$ + 51 (*c* 1.1, $CHCl_3$). ^{1}H NMR (600 MHz, CD_2Cl_2): δ 8.25–7.29 (40H, m, Ar), 5.79 (1H, dd, $J_{1,2}$ = 1.8, $J_{2,3}$ = 3.4 Hz, H-2), 5.73 (1H, d, H-1), 5.67 (1H, dd, $J_{2'',3''}$ = 3.5, $J_{3'',4''}$ = 10.0 Hz, H-3″), 5.59 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 5.56 (1H, dd, $J_{2',3'}$ = 3.3, $J_{3',4'}$ = 10.1 Hz, H-3′), 5.46 (1H, t, $J_{4',5'}$ = 9.8 Hz, H-4′), 5.45 (1H, dd, $J_{1'',2''}$ = 1.7, Hz, H-2″), 5.37 (1H, t, $J_{4'',5''}$ = 9.9 Hz, H-4″), 5.23 (1H, d, $J_{1',2'}$ = 1.8 Hz, H-1′), 4.60 (1H, d, H-1″), 4.58 (1H, dq, H-5), 4.50 (1H, dd, H-3), 4.11 (1H, dq, H-5′), 4.04 (1H, dq, H-5″), 3.98 (1H, dd, H-2′), 2.00 (3H, s, OAc), 1.34 (3H, d, $J_{5,6}$ = 6.3 Hz, H-6), 1.23 (3H, d, $J_{5',6'}$ = 6.3 Hz, H-6′), 1.11, (3H, d, $J_{5'',6''}$ = 6.3 Hz, H-6″). ^{13}C NMR (100 MHz, CD_2Cl_2): δ 169.6 (1C, OAc), 166.3–165.3 (6C, 6 OBz), 133.9–128.4 (42C, Ar), 100.9 (1C, C-1′), 99.5 (1C, C-1″), 86.2 (1C, C-1), 76.8 (1C, C-2′), 76.3 (1C, C-3), 74.1 (1C, C-2), 73.8 (1C, C-4), 71.7 (1C, C-4′), 71.6 (1C, C-4″), 70.9 (1C, C-3′), 69.9 (1C, C-3″), 69.8 (1C, C-2″), 68.5 (1C, C-5), 68.1 (1C, C-5′), 67.8 (1C, C-5″), 20.8 (1C, *C*H$_3$, OAc), 17.8, 17.6 and 17.5 (3C, 3 × *C*H$_3$, C-6, C-6′, C-6″). MALDI-TOFMS: *m/z* 1237.4 (M+Na). Anal. Calcd for $C_{68}H_{62}O_{19}S$: C, 67.20; H, 5.15. Found: C, 67.11; H, 5.13.

3.1.5. Phenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-1-thio-α-L-rhamnopyranoside (11)

A solution of NaOMe–MeOH (1 M, 10 mL) was added to a suspension of trisaccharide **10** (3.71 g, 3.05 mmol) in anhyd MeOH (100 mL), and the mixture was stirred at room temperature until the starting material had dissolved (∼1 h). The solution was kept in a closed flask for 20 h and was then neutralized by stirring with Rexyn 101 H^+ ion-exchange resin. The resin was removed by filtration, and the filtrate was concentrated by rotary evaporation to give a syrupy residue. The residue was extracted by triturating with hexanes (3 × 50 mL) to remove MeOBz. The insoluble trisaccharide was acetylated with Ac_2O (20 mL) and pyridine (40 mL) for 3 h at rt. The excess reagents were removed by rotary evaporation under high vacuum. The gummy residue was dissolved in Et_2O (150 mL) and was washed with satd $NaHCO_3$ (50 mL), dried over $MgSO_4$, and concentrated to give the trisaccharide **11** as a hard foam (2.72 g, quantitative). $[\alpha]_D^{25}$ − 71 (*c* 1.2, $CHCl_3$). ^{1}H NMR (600 MHz, CD_2Cl_2): δ 7.51–7.29 (5H, m, Ar), 5.44–5.39 (2H, m, H-1,H-2), 5.28 (1H, dd, $J_{2'',3''}$ = 3.5, $J_{3'',4''}$ = 10.0 Hz, H-3″), 5.25 (1H, dd, H-2″), 5.10 (1H, t, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4), 5.06–4.99 (3H, m, H-3′, H-4′, H-4″), 4.96 (1H, d, $J_{1',2'}$ = 1.8 Hz, H-1′), 4.79 (1H, d, H-1″), 4.27 (1H, dq, H-5), 4.10 (1H, dd, $J_{2,3}$ = 3.1 Hz, H-3), 3.97 (1H, dq, $J_{4'',5''}$ = 9.9 Hz,H-5″), 3.92 (1H, dd, $J_{2'3'}$ = 1.9 Hz, H-2′), 3.86 (1H, dq, $J_{4',5'}$ = 9.9 Hz, H-5′), 2.16, 2.13, 2.11, 2,05, 2.04, 2.03, 1.97 (each 3H, 7 s, 7 × OAc), 1.22 (3H, d, $J_{5'',6''}$ = 6.3 Hz, H-6″), 1.20 (3H, d, $J_{5,6}$ = 6.2 Hz, H-6), 1.19 (3H, d, $J_{5',6'}$ = 6.3 Hz, H-6′); ^{13}C NMR (100 MHz, CD_2Cl_2): δ 170.5–170.1 (7C, 7 × OAc), 133.7–128.3 (6C, Ar), 100.5 (1C, C-1′), 99.9 (1C, C-1″), 86.2 (1C, C-1), 77.7 (1C, C-2′), 75.4 (1C, C-3), 73.1 (2C, C-2 and C-4), 71.1, 71.0 and 70.5 (3C, C-3′, C-4′ and C-4″), 70.1 (1C, C-2′), 69.0 (1C, C-3″), 68.3 (1C, C-5), 67.8 (1C, C-5′), 67.6 (1C, C-5″), 21.1–20.9 (7C, 7 × OAc), 17.66, 17.59 and 17.48 (3C, 3 × *C*H$_3$, C-6, C-6′, C-6″). MALDI-TOFMS: *m/z* 865.2 (M+Na). Anal. Calcd for $C_{38}H_{50}O_{19}S$: C, 54.14; H, 5.98. Found: C, 54.02; H, 6.24.

3.1.6. 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-1-thio-α-L-rhamnopyranose (4)

A solution of the trisaccharide phenyl thioglycoside **11** (2.99 g, 3.17 mmol) in CH_2Cl_2 (60 mL) was cooled in an ice-bath while a 1 M solution of IBr in CH_2Cl_2 (40 mL) was added dropwise. The mixture was stirred for 20 min, then diluted with additional cold CH_2Cl_2 (60 mL), and washed with cold 5% aq $Na_2S_2O_3$ solution (60 mL) and cold satd $NaHCO_3$ solution (50 mL). The organic phase was dried over $MgSO_4$, and was then filtered and evaporated to give the trisaccharide glycosyl bromide **12** as a colorless foam. The bromide **12** was immediately dissolved in anhyd CH_3CN (40 mL) and was slowly warmed to reflux along with thiourea (0.41 g, 5.4 mmol). After 1.5 h at reflux temperature under a N_2 atmosphere, the mixture was cooled and concentrated by rotary evaporation. This yielded the crude isothiouronium salt **13** as a mixture, together with the byproduct (diphenyl disulfide)

and excess thiourea. Without further purification, the crude product was dissolved in a two-phase mixture of CH_2Cl_2 (40 mL) and water (10 mL) containing $Na_2S_2O_5$ (1.5 g). Hydrolysis of the isothiouronium salt **13** over 0.5 h was brought about by rapid stirring of the heterogeneous reaction mixture while refluxing under N_2. The mixture was cooled to rt and was diluted with additional CH_2Cl_2 (100 mL). The organic phase was washed with water (50 mL) and satd NaCl solution (50 mL), dried over $MgSO_4$, and concentrated to give a syrupy residue. Purification by chromatography on silica gel (1:1 hexanes–EtOAc) gave an α/β mixture of the trisaccharide glycosyl thiol **4** as a colorless syrup (1.52 g, 56% for three steps). Selective crystallization of the α anomer from EtOAc–hexanes gave pure **4** (857 mg) as colorless fine needles. mp 202–204° C; $[\alpha]_D^{25} - 63$ (*c* 1,$CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.50 (1H, dd, $J_{1,2}$ = 1.6, $J_{1,SH}$ = 7.0 Hz, H-1), 5.32 (1H, dd, $J_{2'',3''}$ = 3.5, $J_{3'',4'}$ 10.1 Hz, H-3″), 5.27–5.25 (2H, m, H-2, H-2″), 5.09 (1H, t, $J_{3,4} = J_{4,5}$ = 9.8 Hz, H-4), 5.06 (1H, t, $J_{3'',4''} = J_{4'',5''}$ = 9.8 Hz, H-4″), 5.06–5.01 (2H, m, H-3, H-4), 4.91 (1H, d, $J_{1',2'}$ = 1.8 Hz, H-1′), 4.76 (1H, d, $J_{1'',2''}$ = 1.8 Hz, H-1″), 4.25–4.08 (2H, m, H-3, H-5), 3.96 (1H, dq, H-5″), 3.92 (1H, dd, $J_{2'3'}$ = 2.1 Hz, H-2′), 3.83 (1H, dq, $J_{4',5'}$ = 9.9 Hz, H-5′), 2.21 (1H, d, SH), 2.20, 2.13, 2.12, 2.07, 2.06, 2.04 and 2.00 (each 3H, 7 s, 7 × OAc), 1.22 (3H, d, $J_{5'',6''}$ = 6.3 Hz, H-6″), 1.20 (3H, d, $J_{5,6}$ = 6.3 Hz, H-6), 1.19 (3H, d, $J_{5',6'}$ = 6.3 Hz, H-6′). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.3, 170.3, 170.1, 169.8, 169.8, 169.6, 169.6 (7C, 7 × OAc), 99.9 (1C, C-1′), 99.6 (1C, C-1″), 77.5 (1C, C-2′), 76.6 (1C, C-1), 73.8 (1C, C-2″), 73.7 (1C, C3), 72.8 (1C, C-4), 70.9 (1C, C-4″), 70.8 (1C, C-4′) 70.02 (1C, C-3′), 70.0 (1C, C-3′), 68.8 (1C, C-2′), 68.5 (1C, C-3″), 67.9 (1C, C-5), 67.4 (1C, C-5′), 67.2 (1C, C-5″), 20.9, 20.8 (2C), 20.8 (3C), 20.7 (7C, 7 × OAc), 17.4, 17.4 and 17.3 (3C, 3 × CH_3, C-6, C-6′, C-6″). MALDI-TOFMS: *m/z* 789.2 (M+Na). Anal. Calcd for $C_{32}H_{46}O_{19}S$: C, 50.12; H, 6.05. Found: C, 50.24; H, 6.17.

3.1.7. Fmoc-Met-Asp(OBn)-tryptophanol (14)

L-Tryptophanol[34] (1.97 g, 10.4 mmol), BOC-Asp(OBn)-OH (3.23 g, 10.0 mmol), and HOBt (1.35 g, 10.0 mmol) were combined in dry THF (60 mL). DCC (2.06 g, 10.0 mmol) was added, and the mixture was stirred at rt for 1 h. After filtration to remove dicyclohexylurea (DCU), the solvent was removed by rotary evaporation, and the residue was partitioned between EtOAc (150 mL) and satd aq $NaHCO_3$ (50 mL). The organic phase was washed with 10% citric acid (50 mL), and then with satd aq $NaHCO_3$ (2 × 50 mL) and satd aq NaCl (30 mL). The solution was dried over $MgSO_4$ and was evaporated to an amorphous solid. The crude product was purified by flash chromatography on silica gel (1:2 hexanes–EtOAc) to give compound BOC-Asp(OBn)-tryptophanol as a colorless, hard foam (3.42 g, 69%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07 (1H, br s, NH-Trp ring), 7.65 (1H, br d, $J_{4,5}$ = 7.9 Hz, H4-Trp), 7.40–7.28 (6H, m, H7-Trp and Bn Ar), 7.20 (1H, ddd, $J_{6,7}$ = 8.0, $J_{5,6}$ = 7. 1, $J_{4,6}$ = 1.2 Hz, H6-Trp), 7.13 (1H, ddd, $J_{4,5}$ = 8.0, $J_{5,7}$ = 1.1 Hz, H5-Trp), 7.06 (1H, d, $J_{1,2}$ = 2.3 Hz, H2-Trp), 6.65 (1H, br d, $J_{NH,\alpha}$ = 7.7 Hz, NH-Trp), 5.54 (1H, br d, $J_{NH,\alpha}$ = 7.8 Hz, NH-Asp), 5.12 and 5.07 (1H each, 2d, $J_{A,B}$ = 12.3 Hz, -OCH_2- Bn), 4.47 (1H, br m, α-H-Asp), 4.23 (1H, m, α-H-Trp), 3.66 (1H, dd, $J_{A,B}$ = 11.2, $J_{A,\alpha}$ = 3.8 Hz, β-CH_2- Trp), 3.58 (1H, dd, $J_{B,\alpha}$ = 5.3 Hz, β-CH_2- Trp), 3.06–2.94 (3H, m, β-CH_2- Asp, and -CH_2OH- Trp), 2.73 (1H, dd, $J_{A,B}$ = 17.0, $J_{B,\alpha}$ = 6.5 Hz, β-CH_2- Asp), 2.45 (1H, br s, OH), 1.40 (9H, s, 3 × BOC). ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.6 (2C, 2 × *C*=O), 155.5 (1C, *C*=O-BOC), 136.4–111.3 (14C, Ar), 78.7 (1C, -O*C*(CH_3)$_3$- BOC), 65.9 (1C, -OCH_2- Bn), 62.1 (1C, -OCH_2- Trp), 52.0 (1C, Cα- Trp), 51.4 (1C, Cα- Asp), 36.7 (1C, β-CH_2- Asp), 28.4 (1C, -OC(CH_3)$_3$- BOC), 26.6 (1C, β-CH_2- Trp). HRMS: Calcd for $C_{27}H_{33}N_3O_6$: *m/z* 496.2447. Found: *m/z* 496.2458.

Dipeptide BOC-Asp(OBn)-Tryptophanol (2.30 g, 4.64 mmol) was dissolved in TFA (20 mL) and was left at rt for 10 min. The TFA was removed by rotary evaporation, and the residue was partitioned between EtOAc (100 mL) and satd aq $NaHCO_3$ solution. The EtOAc phase was again washed with aq $NaHCO_3$ solution (30 mL) and then with satd aq NaCl solution (20 mL). The solution was dried over $MgSO_4$ and was evaporated to give a colorless syrup.

The crude product H-Asp(OBn)-tryptophanol (0.730 g) was dissolved in dry THF (20 mL). Fmoc-Met (0.693 g, 1.87 mmol) was added, followed by HOBt (0.253 g, 1.87 mmol) and DCC (0.386 g, 1.87 mmol). The mixture was stirred at rt for 1 h and was then filtered to remove DCU. The THF was removed to leave a residue, which was only sparingly soluble in EtOAc. Most of the material was brought into solution by rapid stirring with a mixture of EtOAc (150 mL) and satd aq $NaHCO_3$ solution for 0.5 h. The EtOAc layer was then separated and washed with 10% citric acid (30 mL), satd $NaHCO_3$ solution (30 mL), and satd NaCl solution (20 mL). The solution was dried ($MgSO_4$) and concentrated to give the crude tripeptide as a pale-yellow solid. Attempted purification by flash chromatography (1:2 hexanes:EtOAc to EtOAc) resulted in only a marginal improvement in purity with the major contaminant (DCU) co-eluting with the product. A suitable solvent for recrystallization could not be found, but eventually a jelly-like solid, with acceptable purity, was obtained by slow cooling of a hot EtOH solution. Pumping of the gel on high vacuum gave the tripeptide **14** as a white solid (0.810 g, 58%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.8 (1H, br s, NH-Trp ring), 8.35 (1H, d, $J_{\alpha,NH}$ = 8.0 Hz, NH- Asp), 7.90–7.24 (17H, m, 13 Ar-H [Fmoc and Bn], H4-, H7-Trp, NH- Met, NH- Trp), 7.10 (1H, d, $J_{1,2}$ = 1.9 Hz, H2-Trp), 7.04 (1H, br t, $J_{5,6} \approx J_{6,7} \approx$ 7.5 Hz, H6-Trp), 6.95 (1H, br t, $J_{5,6} \approx J_{4,5} \approx$ 7.5 Hz, H5-Trp), 5.06 (2H, br s, -OCH_2- Bn), 4.68 (1H, t, *J* = 5.5 Hz, -O*H*- Trp), 4.64 (1H, m, α-H- Asp), 4.34–4.16 (3H, m, CH_2-Fmoc and C-9H-Fmoc), 4.08 (1H, m, α-H- Met), 3.92 (1H, m, α-H- Trp), 3.32 (2H, m, β-CH_2- Trp), 2.86 (2H, br dd, β-CH_2- Trp, and β-CH_2- Asp), 2.74 (1H, dd, $J_{A,B}$ = 14.4, $J_{\alpha,B}$ = 5.9 Hz, β-CH_2- Trp), 2.66 (1H, dd, $J_{A,B}$ = 11.1, $J_{\alpha,B}$ = 8.3 Hz, β-CH_2- Asp), 2.49 (2H, br t, *J* = 7.4 Hz, -SCH_2- Met), 2.01 (3H, s, -SCH_3- Met), 1.84 (2H, m, β–CH_2- Met). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.5, 170.1 169.6 (3C, 3 × C=O), 156.1 (1C, C=O- Fmoc), 143.9–111.0 (26C, Ar), 65.7 (2C, -OCH_2- Fmoc and -OCH_2- Bn), 61.8 (1C, -OCH_2- Trp), 54.0 (1C, Cα-Met), 51.9 (1C, Cα-Trp), 49.6 (1C, Cα-Asp), 46.6 (1C, C9-Fmoc), 36.1 (1C, Cβ-Asp), 31.4 (1C, t, Cβ-Met), 29.5 (1C, SCH_2-Met), 26.3 (1C, Cβ-Trp), 14.6 (1C, SCH_3-Met). HRMS Calcd for $C_{39}H_{46}N_4O_7S$: *m/z* 749.3008. Found: *m/z* 749.3014.

3.1.8. Fmoc-Met-Asp(OBn)-tryptophanyl bromide (5)

The tripeptide alcohol **14** (0.157 g, 0.210 mmol) was dispersed with stirring in CH_2Cl_2 (5 mL). Triphenylphosphine (0.121 g, 0.461 mmol) and CBr_4 (0.163 g, 0.491 mmol) were added, which resulted in the peptide dissolving within 10 min to give a homogeneous yellow solution. After a further 10 min, the solvent was removed to yield the crude product as a sticky yellow gum. This was purified by flash chromatography on silica gel (1:1 to 1:2 hexanes–EtOAc) to provide the bromide **5** as a pale-yellow solid. This material was only of limited stability at rt and was generally used immediately in the next step. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (1H, br s, NH-Trp ring), 7.78–7.22 (17H, m, Ar-[Fmoc and Bn], H4-, H7-Trp, NH-Met, NH-Asp) 7.19 (1H, ddd, $J_{6,7}$ = 8.0, $J_{5,6}$ = 7.1, $J_{4,6}$ = 1.2 Hz, H6-Trp), 7.13 (1H, ddd, $J_{4,5}$ = 8.0, $J_{4,6}$ = 1.0 Hz, H5-Trp), 7.05 (1H, d, $J_{1,2}$ = 2.3 Hz, H2-Trp), 6.81 (1H, br d, $J_{NH,\alpha}$ = 7.7 Hz, NH- Trp), 5.34 (1H, br d, $J_{NH,\alpha}$ = 7.7 Hz, NH Met), 5.10 (2H, br s, -OCH_2- Bn), 4.79 (1H, m, α-H- Asp), 4.48–4.37 (3H, m, CH_2-Fmoc and α-H-Trp), 4.27–4.17 (2H, m, H9-Fmoc and α-H-Met), 3.51 (1H, dd, $J_{A,B}$ = 10.3, $J_{\alpha,A}$ = 4.8 Hz, -CH_2Br- Trp), 3.39 (1H, dd, $J_{\alpha,B}$ = 4.1 Hz, -CH_2Br- Trp), 3.11–2.99 (3H, m, β-CH_2- Trp and β-CH_2- Asp), 2.71 (1H, dd, $J_{A,B}$ = 17.2, $J_{\alpha,B}$ = 6.4 Hz, Asp β-CH_2- Asp), 2.45 (2H, br m, -SCH_2- Met), 2.07 (4H, br s, β-CH_2- Met, and

-SCH_3- Met), 1.84 (1H, m, β-CH_2- Met). MALDI-TOFMS: *m/z* 833.4 (M+Na).

3.1.9. Protected glycopeptide 3

The trisaccharide thiol **4** (0.098 g, 0.13 mmol) and the tripeptide bromide **5** (0.103 g, 0.127 g) were combined in EtOAc (15 mL) and were stirred with satd aq $NaHCO_3$ (2 mL). Bu_4NHSO_4 (0.032 g) was added, and the mixture was stirred rapidly at rt for 5 h. The reaction mixture was diluted with EtOAc (50 mL) and water (20 mL), and the separated aq phase was further extracted with EtOAc (30 mL). The combined extracts were dried over $MgSO_4$ and were concentrated to dryness. The residue was purified by flash chromatography on silica gel (1:2 hexanes–EtOAc) to give the product as a colorless solid (43 mg). Analysis by ^{1}H NMR spectroscopy indicated a purity of approximately 80%, along with one major, unidentified contaminant that exhibited only peptide resonances. Further purification by preparative HPLC (C-18 reversed phase, 80:20–5:95 H_2O–CH_3CN over 30 min) gave the pure glycopeptide **3** as a colorless solid (30 mg, 16%). ^{1}H NMR (400 MHz, CD_2Cl_2): δ 8.31 (1H, br s, NH- Trp ring), 7.81–7.28 (15H, m, 13 Ar-[Fmoc and Bn], H4-, H7-Trp), 7.20 (1H, br d, $J_{\alpha,NH}$ = 8.5 Hz, NH -Asp), 7.15 (1H, ddd, $J_{6,7}$ = 8.1, $J_{5,6}$ = 7.3, $J_{4,6}$ = 1.0 Hz, H6-Trp), 7.08 (1H, ddd, $J_{4,5}$ = 7.9, $J_{4,6}$ = 0.9 Hz, H5-Trp), 7.01 (1H, d, $J_{1,2}$ = 2.2 Hz, H2-Trp), 6.72 (1H, br d, $J_{\alpha,NH}$ = 8.0 Hz, NH-Trp), 5.38 (1H, br d, $J_{\alpha,NH}$ = 7.1 Hz, NH-Met), 5.29–5.20 (4H, m, H2-, H2′′-, H3′-, H3′′-rha), 5.11–4.99 (6H, m, -OCH_2- Bn, H1-, H4-, H4′-, H4′′-rha), 4.92 (1H, d, $J_{1,2}$ = 1.6 Hz, H1′-rha), 4.77(1H, d, $J_{1,2}$ = 1.5 Hz, H1′′-rha), 4.70 (1H, m, α-H-Asp), 4.47 (1H, dd, $J_{A,B}$ = 10.5, $J_{A,9}$ = 6.7 Hz, CH_2-Fmoc), 4.41–4.29 (2H, m, CH_2-Fmoc, α-H- Trp), 4.22 (1H, t, J = 6.7 Hz, H9-Fmoc), 4.17 (1H, m, α-H- Met), 4.10 (1H, m, H5-rha), 4.02 (1H, dd, $J_{2,3}$ = 3.4, $J_{3,4}$ = 9.8 Hz, H3-rha), 3.95 (1H, m, H5-rha), 3.89 (1H, dd, $J_{1',2'}$ = 1.6, $J_{2'3'}$ = 3.4 Hz, H2′-rha), 3.83 (1H, m, H5-rha), 3.04–2.92 (3H, m, β-CH_2- Trp, and β-CH_2- Asp), 2.80–2.69 (3H, m, -SCH_2- Trp, and β-CH_2- Asp), 2.44 (2H, br t, J = 6.8 Hz, -SCH_2- Met), 2.16 (3H, s, OAc), 2.12 (3H, s, CH_3–OAc), 2.08 (3H, s, CH_3–OAc), 2.06 (3H, s, -SCH_3- Met), 2.07 (9H, s, 3 × OAc), 2.01 (1H, m, β-CH_2- Met), 1.98 (3H, s, OAc), 1.77 (1H, m, β-CH_2- Met), 1.19 (3H, d, J = 6.3 Hz, CH_3-rha) 1.16 (3H, d, J = 6.3 Hz, CH_3-rha) 1.13 (3H, d, J = 6.2 Hz, CH_3-rha). ^{13}C NMR (100 MHz, CD_2Cl_2): δ 171.9–169.8 (11C, 11 × C═O), 156.8 (1C, C═O-Fmoc), 144.2–111.2, (26C, Ar), 100.4 (1C, C1′′-rha), 99.9 (1C, C1′-rha), 83.5 (1C, C1-rha), 77.8 (1C, C2′-rha), 75.4 (1C, C3′-rha), 73.3 (1C, C2-rha), 73.2 (1C, C3′-rha), 71.1 (2C, 2 × C4-rha), 70.5 (1C, C4-rha), 70.1 (1C, C2′′-rha), 69.1(1C, C3′′-rha), 67.8 (2C, 2 × C5-rha), 67.6 (1C, C5-rha), 67.5 (1C, CH_2-Fmoc), 67.1 (1C, OCH_2-Bn), 55.0 (1C, Cα-Met), 50.6 (1C, Cα-Trp), 50.0 (1C, Cα-Asp), 47.6 (1C, C9-Fmoc), 36.0 (1C, SCH_2-Trp), 35.7 (1C, Cβ-Asp), 31.1 (1C, Cβ-Met), 30.5 (1C, SCH_2-Met), 29.4 (1C, Cβ-Trp), 21.1–20.9 (7C, 7 × OAc) 17.7, 17.6, 17.4, (3C, 3 × C6-rha), 15.5 (1C, SCH_3-Met). MALDI-TOFMS: *m/z* 1519.9 (M+Na).

3.1.10. Attempted deprotection of glycopeptide 3

The protected glycopeptide **3** (30 mg) was treated with 0.1 M NaOMe in MeOH (4 mL) for 5 h at rt. The reaction mixture was neutralized by addition of HOAc, filtered, and concentrated to a solid residue. Flash chromatography on silica gel (4:2:1 EtOAc–MeOH–H_2O) and combining of those fractions that appeared as a single spot on TLC gave a product free from protecting-group remnants. However, analysis by HPLC showed this to be a mixture of four closely eluting compounds with a ratio of 2:2:1:1. The MALDI mass spectrum of the mixture showed a single, major mass corresponding to loss of 18 mass units from the expected glycopeptide **1** (calcd for $C_{38}H_{58}N_4O_{16}S_2Na$ (M+Na) 913.31, found 895.15). The ^{1}H NMR spectrum in CD_3OD also indicated a mixture of compounds, as judged by the presence of several SCH_3 singlets near δ 2.0 as well as of a complicated pattern of overlapping rhamnose CH_3 resonances near δ 1.3.

3.1.11. Fmoc-Met-Asp(O*t*Bu)-tryptophanol 20

L-Tryptophanol[34] (3.77 g, 19.8 mmol), Fmoc-Asp(O*t*-Bu)-OH (8.09 g, 19.7 mmol), and HOBt (2.67 g, 19.7 mmol) were combined in dry THF (70 mL). DCC (4.07 g, 19.7 mmol) was added, and the mixture was stirred at rt for 1 h. After filtration to remove DCU, the solvent was removed by rotary evaporation, and the residue was partitioned between EtOAc (250 mL) and satd aq $NaHCO_3$ (50 mL). The organic phase was washed with 10% citric acid (50 mL), and then with satd aq $NaHCO_3$ (2 × 50 mL) and satd aq NaCl (50 mL). The solution was dried over $MgSO_4$ and was evaporated to an amorphous solid. The crude product was purified by flash chromatography on silica gel (1:2 hexanes–EtOAc) to give Fmoc-Asp(O*t*Bu)-Tryptophanol as a colorless hard foam (9.11 g, 79%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07 (1H, br s, NH-Trp ring), 7.82–7.22 (10H, m, H4-, H7-Trp, Ar-Fmoc), 7.16 (1H, br t, $J_{5,6} \approx J_{6,7} \approx$ 7.4 Hz, H6-Trp), 7.13 (1H, ddd, $J_{4,5}$ = 7.9, $J_{5,7}$ = 0.8 Hz, H5-Trp), 7.06 (1H, d, $J_{1,2}$ = 1.5 Hz, H2-Trp), 6.61 (1H, br d, $J_{NH,\alpha}$ = 7.0 Hz, NH–Trp), 5.76 (1H, br d, $J_{NH,\alpha}$ = 8.4 Hz, NH–Asp), 4.47 (1H, br m, α-H- Asp), 4.40 (1H, dd, $J_{A,B}$ = 10.5, $J_{A,9}$ = 7.2 Hz, CH_2-Fmoc), 4.33 (1H, dd, $J_{B,9}$ = 7.0 Hz, CH_2-Fmoc), 4.23 (1H, m, αH-Trp), 4.19 (1H, br t, $J \approx$ 7.0 Hz, H9-Fmoc), 3.70 (1H, dd, $J_{A,B}$ = 11.2, $J_{A,\alpha}$ = 3.4 Hz, -CH_2OH- Trp), 3.61 (1H, dd, $J_{B,\alpha}$ = 5.6 Hz, -CH_2OH- Trp), 3.03 (1H, dd, $J_{A,B}$ = 17.4, $J_{A,\alpha}$ = 7.0 Hz, β-CH_2- Trp), 2.98 (1H, dd, $J_{B,\alpha}$ = 7.0 Hz, β-CH_2- Trp), 2.84 (1H, dd, $J_{A,B}$ = 17.1, $J_{A,\alpha}$ = 7.1 Hz, β-CH_2- Asp), 2.61 (1H, dd, $J_{B,\alpha}$ = 6.8 Hz, β-CH_2- Asp), 2.02 (1H, br s, OH), 1.41 (9H, s, *t*Bu). ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.5, 169.7 (2C, 2 × C═O), 156.1 (1C, C═O-Fmoc), 144.1–111.4 (20C, Ar), 80.4 (1C, -OC$(CH_3)_3$–*t*-Bu), 66.1 (1C, -OCH_2- Fmoc), 62.3 (1C, -OCH_2- Trp), 52.1 (1C, Cα- Trp), 51.9 (1C, Cα- Asp), 46.9 (1C, C9-Fmoc), 38.2 (1C, β-CH_2- Asp), 28.0 (1C, -OC$(CH_3)_3$- *t*-Bu), 26.6 (1C, β-CH_2 -Trp). HRMS: Calcd for $C_{34}H_{37}N_3O_6$: *m/z* 584.2760. Found: *m/z* 584.2758.

The Fmoc-protected dipeptide Fmoc-Asp(O*t*Bu)-Tryptophanol (8.31 g, 14.2 mmol) was dissolved in dry DMF containing 20% v/v piperidine (100 mL). After 10 min at rt, the solvents were removed by rotary evaporation under high vacuum to give the free amino compound H-Met-Asp(O*t*Bu)-Tryptophanol as a crystalline solid residue. This was dissolved in dry THF (100 mL) and Fmoc-Met-OH (5.34 g, 14.3 mmol) was added, followed by HOBt (1.90 g, 14.1 mmol) and DCC (2.97 g, 14.3 mmol). The mixture was stirred at rt for 15 h and was then filtered to remove DCU. The THF was removed to leave a residue that was partitioned between EtOAc (250 mL) and satd aq $NaHCO_3$ (50 mL). The EtOAc layer was separated and was washed with 10% citric acid (50 mL), satd aq $NaHCO_3$ (2 × 50 mL), and satd aq NaCl (50 mL). The solution was dried ($MgSO_4$) and concentrated to give the crude tripeptide as a pale-yellow solid. Purification by flash chromatography (EtOAc) gave the protected tripeptide **20** as a white solid (8.65 g, 85%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.12 (1H, br s, NH- Trp ring), 7.80–7.28 (11H, m, 8 × Ar Fmoc, H4-, H7-Trp, NH- Asp), 7.17 (1H, br t, $J_{5,6} \approx J_{6,7} \approx$ 7.5 Hz, H6-Trp), 7.10 (1H, br t, $J_{5,6} \approx J_{4,5} \approx$ 7.5 Hz, H5-Trp), 7.04 (1H, br s, H2-Trp), 6.84 (1H, d, $J_{\alpha,NH}$ = 8.0 Hz, NH-Trp), 5.57 (1H, d, $J_{\alpha,NH}$ = 6.8 Hz, NH- Met), 4.70 (1H, m, α-H- Asp), 4.46 (1H, dd, $J_{A,B}$ = 10.7, $J_{A,9}$ = 6.9 Hz, CH_2-Fmoc), 4.42 (1H, dd, $J_{B,9}$ = 6.9 Hz, CH_2-Fmoc), 4.28–4.18 (3H, m, α-H- Met, α-H- Trp, H9-Fmoc), 3.70 (1H, dd, $J_{A,B}$ = 11.4, $J_{A,\alpha}$ = 3.4 Hz, -CH_2OH- Trp), 3.60 (1H, dd, $J_{B,\alpha}$ = 5.3 Hz, -CH_2OH-Trp), 3.02 (1H, dd, $J_{A,B}$ = 14.7, $J_{\alpha,A}$ = 7.5 Hz, β-CH_2-Trp), 2.97 (1H, dd, $J_{\alpha,B}$ = 6.4 Hz, β-CH_2- Trp), 2.90 (1H, dd, $J_{A,B}$ = 16.8, $J_{\alpha,A}$ = 4.3 Hz, β-CH_2-Asp), 2.59 (1H, dd, $J_{\alpha,B}$ = 6.4 Hz, β-CH_2-Asp), 2.48 (2H, m, SCH_2- Met), 2.30 (1H, br s, OH), 2.08 (3H, s, SCH_3- Met), 2.05 (1H, m, β-CH_2- Met), 1.86 (1H, m, β-CH_2–Met), 1.37 (9H, s, O*t*Bu). ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.4, 169.7 169.4 (3C, 3 × C═O), 156.1 (1C, C═O-Fmoc),

143.9–111.1 (20C, Ar), 80.5 (1C, O*t*Bu), 65.7 (1C, *C*H_2-Fmoc), 61.9 (1C, O*C*H_2-Trp), 54.0 (1C, Cα-Met), 51.9 (1C, Cα-Trp), 49.7 (1C, Cα-Asp), 46.6 (1C, C9-Fmoc), 37.3 (1C, β-*C*H_2–Asp), 31.5 (1C, β-*C*H_2–Met), 29.5 (1C, S*C*H_2–Met), 27.6 (1C, O*t*Bu), 26.3 (1C, β-*C*H_2–Trp), 14.6 (1C, S*C*H_3–Met). HRMS: Calcd for $C_{39}H_{46}N_4O_7S$: 715.3165. Found: 715.3159.

3.1.12. Glycopeptide 18

The protected tripeptide **20** (1.08 g, 1.51 mmol) was dissolved in CH_2Cl_2 (30 mL) and was stirred at rt while triphenylphosphine (0.550 g, 1.10 mmol) and CBr_4 (0.709 g, 2.14 mmol) were added. After 1 h, additional CH_2Cl_2 (50 mL) was added and the solution was washed with satd aq $NaHCO_3$ (20 mL), dried over $MgSO_4$, and concentrated. The residue was purified by flash chromatography (1:2 hexanes–EtOAc) to give the tripeptide bromide **19** as a pale-yellow foam (1.24 g). This material was only of limited stability at rt and was used immediately in the next step.

The bromide (1.24 g, 1.59 mmol) was combined with the trisaccharide thiol **4** (0.771 g, 1.01 mmol) in EtOAc (50 mL) and was stirred with satd aq $NaHCO_3$ (20 mL). Bu_4NHSO_4 (0.223 g) was added, and the mixture was stirred rapidly at rt for 6 h. The reaction mixture was diluted with EtOAc (50 mL) and water (20 mL), and the separated aq phase was further extracted with EtOAc (30 mL). The combined extracts were washed with 10% citric acid (30 mL) and satd aq $NaHCO_3$ (2 × 30 mL), and were then dried over $MgSO_4$ and concentrated to dryness. The residue was purified by flash chromatography on silica gel (1:2–100% hexanes–EtOAc) to give the product as a colorless, hard foam (1.01 g). Analysis by ^{1}H NMR spectroscopy indicated that this material was a mixture of the expected glycopeptide **18** (>80%) along with a single impurity that exhibited only typical rhamnose resonances. An analytical sample was further purified by preparative HPLC (C-18 reversed phase, 95:5–5:95 H_2O–CH_3CN over 40 min) to give first, an impurity, a compound that was tentatively identified as the disulfide **15** resulting from oxidation of the trisaccharide thiol **4**, and then, the pure glycopeptide **18** as a pure colorless solid (16%). ^{1}H NMR (500 MHz, CD_2Cl_2): δ 8.34 (1H, br s, NH-Trp ring), 7.82–7.30 (10H, m, 8× Ar-Fmoc, H4-, H7-Trp), 7.26 (1H, br d, $J_{\alpha,NH}$ = 8.3 Hz, NH-Asp), 7.16 (1H, br t, $J_{5,6} \approx J_{6,7} \approx$ 7.5 Hz, H6-Trp), 7.09 (1H, br t, $J_{5,6} \approx J_{4,5} \approx$ 7.5 Hz, H5-Trp), 7.05 (1H, d, $J_{1,2}$ = 2.1 Hz, H2-Trp), 6.77 (1H, br d, $J_{\alpha,NH}$ = 8.2 Hz, NH- Trp), 5.50 (1H, br d, $J_{\alpha,NH}$ = 7.2 Hz, NH- Met), 5.30–5.23 (3H, m, H2-, H2″-, H3″-rha), 5.22 (1H, br s, H1-rha), 5.11–4.99 (4H, m, H-3′, H4-, H4′-, H4″-rha), 4.93 (1H, d, $J_{1,2}$ = 1.5 Hz, H1′-rha), 4.78 (1H, d, $J_{1,2}$ = 1.4 Hz, H1″-rha), 4.65 (1H, m, α-H-Asp), 4.47 (1H, dd, $J_{A,B}$ = 10.5, $J_{A,9}$ = 7.1 Hz, C*H*$_2$-Fmoc), 4.41 - 4.33 (2H, m, C*H*$_2$-Fmoc, α-H-Trp), 4.26–4.19 (2H, m, H9-Fmoc, α-H-Met), 4.11 (1H, m, H5-rha), 4.03 (1H, dd, $J_{2,3}$ = 3.4, $J_{3,4}$ = 9.8 Hz, H3-rha), 3.96 (1H, m, H5-rha), 3.90 (1H, br m, H2′-rha), 3.84 (1H, m, H5-rha), 3.02 (2H, d, J = 6.3 Hz, β-C*H*$_2$- Trp), 2.83 (1H, dd, $J_{A,B}$ = 16.8, $J_{\alpha,A}$ = 4.8 Hz, β-C*H*$_2$- Asp), 2.78 (2H, d, J = 6.4 Hz, -SC*H*$_2$- Trp), 2.60 (1H, dd, $J_{\alpha,B}$ = 6.1 Hz, β-C*H*$_2$- Asp), 2.49 (2H, br t, J = 6.8 Hz, -SC*H*$_2$- Met), 2.17 (3H, s, OAc), 2.13 (3H, s, C*H*$_3$-OAc), 2.10 (3H, s, C*H*$_3$-OAc), 2.08 (3H, s, -SC*H*$_3$- Met), 2.05 (1H, m, β-C*H*$_2$- Met), 2.04 (9H, s, 3 × OAc), 1.99 (3H, s, OAc), 1.85 (1H, m, β-C*H*$_2$- Met), 1.41 (9H, s, O*t*-Bu), 1.21 (3H, d, J = 6.3 Hz, C*H*$_3$-rha) 1.18 (3H, d, J = 6.3 Hz, C*H*$_3$-rha) 1.14 (3H, d, J = 6.2 Hz, C*H*$_3$-rha). ^{13}C NMR (100 MHz, CD_2Cl_2): δ 171.3–169.7 (11C, 11 × C═O), 156.5 (1C, C═O-Fmoc), 144.0–111.2, (20C, Ar), 100.2 (1C, C1″-rha), 99.8 (1C, C1′-rha), 83.4 (1C, C1-rha), 77.7 (1C, C2′-rha), 75.3 (1C, C3′-rha), 73.2 (1C, C2-rha), 73.1 (1C, C3′-rha), 71.0 (2C, 2 × C4-rha), 70.5 (1C, C4-rha), 70.1 (1C, C2″-rha), 69.0(1C, C3″-rha), 67.8 (1C, C5-rha), 67.7 (2C, 2 × C5-rha), 67.6 (1C, *C*H_2-Fmoc), 54.9 (1C, Cα- Met), 50.5 (1C, Cα- Trp), 49.9 (1C, Cα- Asp), 47.5 (1C, C9-Fmoc), 36.7 (1C, β-*C*H_2- Asp), 35.9 (1C, -S*C*H_2- Trp), 31.3 (1C, β-*C*H_2- Met), 30.5 (1C, -S*C*H_2- Met), 29.4 (1C, β-*C*H_2-Trp), 28.1 (1C, O*t*Bu), 21.1–20.8 (7C, 7 × OAc) 17.7, 17.6, 17.4, (3C, 3 × C6-rha), 15.5 (1C, -SCH_3-Met). HRMS: Calcd for $C_{71}H_{90}N_4O_{25}S_2$: *m/z* 1463.5413. Found: *m/z* 1463.5393.

3.1.13. Deprotection of glycopeptide 18

The partially purified glycopeptide **18** (>80% pure, 0.510 g, ~0.279 mmol) was dissolved in CH_2Cl_2 (20 mL). Tri-isopropylsilane (0.3 mL) and TFA (6.0 mL) were added, and the mixture was kept at rt for 2.5 h. The solvents were evaporated, and the residue was partitioned between CH_2Cl_2 (80 mL) and satd aq $NaHCO_3$ (30 mL). The emulsified organic phase was separated and was washed with 2 M HCl (30 mL) and satd aq NaCl (2 × 30 mL). The solvents were removed, and the residue was purified by flash chromatography (10:1 to 4:1 EtOAc–MeOH) to give the purified, free β-carboxylic acid-aspartate, glycopeptide. At this stage the impurity that was present in the protected glycopeptide was easily separated as a faster running component and was characterized as the acetylated trisaccharide 1,1′-disulfide **15** by NMR and MALDI spectra (data not shown).

The glycopeptide fraction was dissolved in 0.2 M NaOMe in MeOH (12 mL) and was kept at rt for 6 h. The base was neutralized by addition of HOAc and the volatile material was removed under reduced pressure. Purification by flash chromatography (4:2:1 EtOAc–MeOH–H_2O) gave the glycopeptide **1** as a colorless solid. Analysis of the product by ^{1}H NMR spectroscopy indicated the presence of NaOAc (19 wt %, singlet at δ 1.78). This led, after allowing for this impurity, to an estimate of 32% for the yield of the deprotection sequence. ^{1}H NMR (500 MHz, D_2O): δ 7.69 (1H, d, $J_{4,5}$ = 7.5 Hz, H4-Trp), 7.47 (1H, d, $J_{6,7}$ = 8.0 Hz, H7-Trp), 7.24 (1H, br s, H2-Trp), 7.21 (1H, br t, $J_{5,6} \approx J_{6,7} \approx$ 8.0 Hz, H6-Trp), 7.14 (1H, br t, H5-Trp), 5.13 (2H, br s, 2 × H1-rha), 4.93 (1H, d, $J_{1,2}$ = 1.5 Hz, H1-rha), 4.63 (1H, dd, α-H Asp), 4.26 (1H, m, α-H Trp), 4.08–4.01 (4H, m, 3 × H2-rha, α-H Met), 3.96 (1H, m, H5-rha), 3.89 (1H, dd, $J_{2,3}$ = 3.4, $J_{3,4}$ = 9.9 Hz, H3-rha), 3.76 (1H, dd, $J_{2,3}$ = 3.4, $J_{3,4}$ = 9.8 Hz, H3-rha), 3.73 (1H, m, H5-rha), 3.69 (1H, dd, $J_{2,3}$ = 3.3, $J_{3,4}$ = 9.7 Hz, H3-rha), 3.67 (1H, m, H5-rha), 3.51(t, 1H, J = 9.6, H4-rha), 3.45(1H, t, J = 9.7 Hz, H4-rha), 3.41(1H, t, J = 9.7 Hz, H4-rha), 3.09 (1H, dd, $J_{A,B}$ = 14.7 Hz, $J_{A,\alpha}$ = 5.8 Hz, β-C*H*$_2$ Trp), 2.96 (1H, dd, $J_{B,\alpha}$ = 7.3 Hz, β-C*H*$_2$ Trp), 2.92 (1H, dd, $J_{A,B}$ = 13.7, $J_{A,\alpha}$ = 4.3 Hz, -SC*H*$_2$ Trp), 2.79 (1H, dd, $J_{B,\alpha}$ = 9.2 Hz, -SC*H*$_2$ Trp), 2.63 (1H, dd, $J_{A,B}$ = 16.0, $J_{\alpha,A}$ = 4.6 Hz, β-C*H*$_2$ Asp), 2.49 (1H, dd, $J_{\alpha,B}$ = 9.9 Hz, β-C*H*$_2$ Asp), 2.44 (1H, dt, $J_{A,B}$ = 13.5, $J_{\beta,A}$ = 7.4 Hz, -SC*H*$_2$ Met), 2.33 (1H, dt, $J_{\beta,B}$ = 7.2 Hz, -SC*H*$_2$ Met), 2.02 (2H, m, β-C*H*$_2$ Met), 1.96 (3H, s, -SC*H*$_3$ Met), 1.25 (3H, d, J = 6.3 Hz, C*H*$_3$- rha) 1.23 (3H, d, J = 6.4 Hz, C*H*$_3$-rha) 1.22 (3H, d, J = 6.3 Hz, C*H*$_3$-rha). ^{13}C NMR (125 MHz, D_2O): δ 177.3, 172.2, 168.7 (each 1C, 3 × C═O), 136.2, 127.3, 124.4, 121.9, 119.4, 118.7, 112.0, 110.3 (each, 1C, 8 × Ar Trp), 102.4 (1C, C1″-rha), 100.9 (1C, C1′-rha), 86.6 (1C, C1-rha), 78.2 (1C, C2′-rha), 77.8 (1C, C3-rha), 72.2, 72.1, 71.9 (each 1C, 3 × C4-rha), 70.2, 70.1, 70.0 (each 1C, 2 × C3-, C2-rha), 69.5, 69.4, 69.3 (each 1C, 3 × C5-rha), 52.3 (1C, Cα Met), 52.0 (1C, Cα Asp), 51.3 (1 C, Cα Trp), 39.4 (1C, β-*C*H_2 Asp), 36.1 (1C, -S*C*H_2Trp), 30.0 (1C, β-*C*H_2 Met), 29.4 (1C, β-*C*H_2 Trp), 28.2 (1C, -SCH_2 Met), 16.8, (2C, 2 × C6-rha), 16.7 (1C, C6-rha), 14.2 (1C, -SCH_3 Met). MALDI-TOFMS: Calcd for $C_{38}H_{58}N_4O_{16}S_2Na$ (M+Na) *m/z* 913.31. Found: *m/z* 913.11.

3.1.14. Bromo compound 21

To a stirred mixture of L-tryptophanol[34] (3.04 g, 16 mmol) and $NaHCO_3$ (1.34 g) in water and acetone (50 mL, 1:1) was added Fmoc-OSu (5.3 g, 16 mmol). After 5 min, a thick white precipitate formed, and the reaction mixture was stirred at rt for 1 h. The solid was then collected by filtration, and was washed with hot H_2O (100 mL) and hot Et_2O (100 mL) to afford Fmoc-tryptophanol (6.3 g, 95%) as a white solid. ^{1}H NMR (500 MHz, acetone-d_6): δ 10.02 (1H, br s, NH-Trp ring), 7.85 (2H, d, J = 7.6 Hz, H4-, H5-Fmoc), 7.72 (1H, d, J = 7.7 Hz, H4-Trp), 7.68 (2H, d, J = 6.9 Hz, H1-,

H8-Fmoc), 7.41 (2H, d, H3-, H6-Fmoc), 7.36 (1H, d, J = 7.8 Hz, H7-Trp), 7.30 (2H, dd, H2-, H7-Fmoc), 7.20 (1H, br s, H2-Trp), 7.09 (1H, br t, J = 7.3 Hz, H6-Trp), 7.02 (1H, br t, J = 7.3 Hz, H5-Trp), 6.31 (1H, d, J = 8.4 Hz, NH-Trp), 4.34 (1H, dd, J = 11.0, 7.3 Hz, CH_2-Fmoc), 4.27 (1H, dd, CH_2-Fmoc), 4.20 (1H, dd, H9-Fmoc), 4.08–3.96 (1H, m, α-H-Trp), 3.68–3.58 (2H, m, CH_2OH-Trp), 3.09 (1H, dd, J = 13.9, 6.9 Hz, β-CH_2-Trp), 3.00 (1H, dd, J = 7.3 Hz, β-CH_2-Trp), 2.89 (1H, br s, CH$_2$OH-Trp). ^{13}C NMR (150 MHz, acetone-d_6): δ 156.3 (1C, C=O-urethane), 144.5 (2C, C8a-, C9a-Fmoc), 141.4 (2C, C4a-, C4b-Fmoc), 137.0 (1C, C3a-Trp), 128.3 (1C, C7a-Trp), 127.8 (2C, C3-, C6-Fmoc), 127.2 (2C, C2-, C7-Fmoc), 125.5 (2C, C1-, C8-Fmoc), 123.4 (1C, C2-Trp), 121.4 (1C, C5-Trp), 120.1 (2C, C4-, C5-Fmoc), 118.9 (1C, C4-Trp), 118.8 (1C, C6-Trp), 112.1 (1C, C3-Trp), 111.4 (1C, C7-Trp), 66.1 (1C, CH$_2$-Fmoc), 63.5 (1C, CH$_2$OH), 54.2 (1C, Cα-Trp), 47.6 (1C, C9-Fmoc), 27.1 (1C, βCH$_2$-Trp). MALDI-TOFMS: m/z 435.7 (M+Na), 413.4 (M+H). Anal. Calcd for $C_{26}H_{24}N_2O_3$: C, 75.71; H, 5.86; N 6.79. Found: C, 76.02; H, 5.93; N 6.54.

Method 1: To a stirred mixture of Fmoc-tryptophanol (0.98 g, 2.4 mmol) and CBr_4 (1.34 g, 4 mmol) in dry CH_2Cl_2 at 0 °C was added PPh_3 (1.25 g, 4.8 mmol) in portions. The reaction mixture was stirred at 0 °C for 2 h and was then concentrated under reduced pressure at rt. The residue was then purified by flash chromatography (3:1 hexanes–EtOAc) to afford the bromide **21** as a pale-yellow powder (0.79 g, 65%). The spectral data were the same as those for the compound obtained with method 2 (see below).

Method 2: To a stirred solution of Fmoc-tryptophanol (3 g, 7.3 mmol) in pyridine (150 mL) at 0 °C under a N_2 atmosphere was added MsCl (0.7 mL, 11 mmol). The reaction mixture was stirred at 0 °C for 1 h and at rt for 4 h. The reaction mixture was then quenched with ice, and the solvent was evaporated. The residue was then dissolved in EtOAc (500 mL), washed with water (300 mL) and satd aq $NaHCO_3$ (300 mL), and dried over Na_2SO_4, and the solvent was evaporated. The crude material containing LiBr (5.1 g, 58.4 mmol) in THF (75 mL) was refluxed under a N_2 atmosphere for 4 h. The solvent was then evaporated, and the mixture was diluted with EtOAc (500 mL), washed with water (5 × 300 mL) and brine (200 mL), dried over Na_2SO_4, and the solvent was evaporated. The residue was then purified by column chromatography (3:1 hexanes–EtOAc) to give the bromide **21** (3.3 g, 95% over the two steps), as a pale-yellow solid. ^{1}H NMR (500 MHz, acetone-d_6): δ 10.1 (1H, br s, NH-Trp ring), 7.87 (2H, d, J = 7.8 Hz, H4-, H5-Fmoc), 7.68 (1H, d, J = 6.3 Hz, H4-Trp), 7.67 (2H, d, J = 7.3 Hz, H1-, H8-Fmoc), 7.43–7.38 (2H, m, H3-, H6-Fmoc, H7-Trp), 7.31 (2H, dd, H2-, H7-Fmoc), 7.26 (1H, br s, H2-Trp), 7.12–7.07 (1H, br t, J = 7.3 Hz, H6-Trp), 7.04 (1H, br t, J = 7.3 Hz, H5-Trp), 6.65 (1H, d, J = 7.8 Hz, NH-Trp), 4.34 (2H, d, J = 7.3 Hz, CH_2-Fmoc), 4.24–4.15 (2H, m, α-H-Trp, H9-Fmoc), 3.69 (1H, dd, J = 10.3, J = 4.88 Hz, CH_2Br-Trp), 3.69 (1H, dd, J = 10.3 and 4.88 Hz, CH_2Br-Trp), 3.60 (1H, dd, J = 5.86 Hz, β-CH_2-Trp), 3.19–3.08 (1H, m, β-CH_2-Trp). ^{13}C NMR (150 MHz, acetone-d_6): δ 156.0 (1C, C=O-urethane), 144.4 (2C, C8a-, C9a-Fmoc), 141.4 (2C, C4a-, C4b-Fmoc), 137.0 (1C, C3a-Trp), 127.9 (1C, C7a-Trp), 127.8 (2C, C3-, C6-Fmoc), 127.2 (2C, C2-, C7-Fmoc), 125.5 (2C, C1-, C8-Fmoc), 123.7 (1C, C2-Trp), 121.6 (1C, C6-Trp), 120.1 (2C, C4-, C5-Fmoc), 119.0 (1C, C5-Trp), 118.6 (1C, C4-Trp), 111.6 (1C, C7-Trp), 110.9 (1C, C3-Trp), 66.2 (1C, CH$_2$-Fmoc), 53.1 (1C, Cα-Trp), 47.4 (1C, C9-Fmoc), 36.9 (1C, CH$_2$Br), 28.7 (1C, βCH$_2$-Trp). MALDI-TOFMS: m/z 498.23, 497.3 (M+Na), 476.8, 475.3 (M+H). Anal. Calcd for $C_{26}H_{23}BrN_2O_2$: C, 65.69; H, 4.88; N, 5.89. Found: C, 66.02; H, 5.04; N 5.59.

3.1.15. Glycopeptide 22

To a stirred mixture of the bromide **21** (46.4 mg, 98 μmol) and trisaccharide glycosyl thiol **4** (75 mg, 98 μmol) in EtOAc (3 mL), satd aq $NaHCO_3$ (3 mL), followed by tetrabutylammonium hydrogen sulfate (TBAHS) (133 mg, 39.2 μmol) was added. The reaction mixture was stirred vigorously at rt for 12 h. It was then diluted with EtOAc and was washed successively with satd aq $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, concentrated, dissolved in CH_2Cl_2, and purified by flash chromatography (1:1 hexanes–EtOAc) to afford the glycopeptide **22** (102 mg, 92%) as a white crystalline solid. ^{1}H NMR (500 MHz, CD_2Cl_2): δ 8.35 (1H, br s, NH- Trp ring), 7.80 (2H, d, J = 7.55 Hz, H4-, H5-Fmoc), 7.68 (1H, d, J = 7.78 Hz, H4-Trp), 7.64–7.57 (2H, m, H1-, H8-Fmoc), 7.47–7.38 (3H, m, H3-, H6-Fmoc, H4-Trp), 7.36–7.30 (2H, m, H2-, H7-Fmoc), 7. 23–7.18 (1H, m, H6-Trp), 7.17–7.10 ((1H, m, H5-Trp), 7.07 (1H, br s, H2-Trp), 5.37–5.33 (2H, m, H2-rha, NH-Dmab), 5.32–5.28 (2H, m, NH-Trp, H3′′-rha), 5.27 (1H, dd, $J_{2",3"}$ = 3.52, $J_{2",1"}$ = 1.82 Hz, H2"-rha), 5.22 (1H, s, H1-rha), 5.12–5.06 (2H, m, H4-, H4"-rha), 5.05–5.03 (2H, m, H3′-, H4′-rha), 4.95 (1H, d, $J_{1',2'}$ = 1.68 Hz, H1′-rha), 4.81 (1H, d, $J_{1",2"}$ = 1.53 Hz, H1"-rha), 4.45–4.36 (2H, m, CH_2-Fmoc), 4.28–4.14 (3H, m, α-H-Trp, H9-Fmoc, H5-rha), 4.07 (1H, dd, $J_{3,4}$ = 9.78 Hz, $J_{3,2}$ = 3.48 Hz, H3-rha), 4.02–3.95 (1H, m, H5"-rha), 3.94–3.91 (1H, m, H2′-rha), 3. 90–3.83 (1H, m, H5′-rha), 3.07 (2H, br t, β -CH_2-Trp) 2.85 (2H, br t, CH_2S), 2.22–1.98 (21H, 7 × OAc), 1.34–1.15 (9H, m, 3 × CH_3-rha). ^{13}C NMR (150 MHz, CD_2Cl_2): δ 170.5–169.9 (7C, 7 × OAc), 155.9 (1C, C=O-urethane), 144.3 (2C, C8a-, C9a-Fmoc), 141.5 (2C, C4a-, C4b-Fmoc), 136.5 (1C, C3a-Trp), 127.8 (1C, C7a-Trp), 127.8 (3C, C3-, C6-Fmoc), 127.2 (2C, C2-, C7-Fmoc), 125.3 (2C, C1-, C8-Fmoc), 123.2 (1 C, C2-Trp), 122.3 (1 C, C5-Trp), 120.1 (2 C, C4-, C5-Fmoc), 119.7 (1 C, C6-Trp), 118.9 (1C, C7-Trp), 111.4 (1C, C4-Trp), 111.4 (1C, C3-Trp), 100.3 (1C, C1"-rha), 99.8 (1C, C1′-rha), 83.9 (1C, C1-rha), 77.5 (1C, C2′-rha), 75.1 (1C, C3-rha), 73.1 (1C, C2-rha), 72.9 (1C, C4"-rha), 70.9 (1C, C3′-rha), 70.8 (1C, C4-rha), 70.3 (1C, C4′-rha), 69.9 (1C, C2"-rha), 68.9 (1C, C3"-rha), 67.8 (1C, C5′-rha), 67.6 (1C, C5-rha), 67.4 (1C, C5"-rha), 66.6 (1C, CH$_2$-Fmoc), 51.5 (1C, Cα-Trp), 47.5 (1C, C9-Fmoc), 36.9 (1C, CH$_2$S), 29.6(1C, β-CH$_2$-Trp), 21.1–20.6 (7C, 7 × OAc), 17.6–17.2 (3C, 3 × CH$_3$-rha). MALDI-TOFMS: m/z 1183.9 (M+Na), 1199.9 (M+K), 1160.1 (M^+). Anal. Calcd for $C_{58}H_{68}N_2O_{21}S$: C, 59.99; H, 5.90; N, 2.41. Found: C, 59.70; H, 6.06; N, 2.37.

3.1.16. Fmoc-Asp(ODmab)-O*t*Bu (26)

To a mixture of Fmoc-Asp-O*t*Bu (1.0 g, 2.4 mmol), Dmab-OH (1.6 g, 4.9 mmol), DTBMP (0.5 g, 2.4 mmol), and HOBt (0.6 g, 3.6 mmol) in dry THF (20 ml), DIC (0.37 mL, 2.4 mmol) was added dropwise. The reaction mixture was stirred for 12 h at rt, concentrated to dryness under vacuum, dissolved in CH_2Cl_2, and purified by flash chromatography (2:1 hexanes–EtOAc) to afford the product **26** as a white solid (1.25 g, 71%) after lyophilization in dioxane. ^{1}H NMR (500 MHz, CD_3OD): δ 7.75 (2H, d, J = 7.5 Hz, H4-, H5-Fmoc), 7.66–7.59 (2H, m, H1-,H8-Fmoc), 7.41 (2H, d, J = 8.0 Hz, H-Dmab), 7.35 (3H, t, J = 7.5 Hz, H3-, H6-Fmoc), 7.26 (2H, t, J = 7.4 Hz, H2-, H7-Fmoc), 7.09 (2H, d, J = 8.0 Hz, Ar-Dmab), 4.52 (1H, t, J = 6.4 Hz, αH-Asp), 4.33 (1H, dd, J = 11.2 and 7.0 Hz, CH_2-Fmoc), 4.25 (1H, dd, J = 11.2, 7.3 Hz, CH_2-Fmoc), 4.16 (1H, t, J = 6.9 Hz, H9-Fmoc), 3.04–2.89 (3H, m, CH_2CH-Dmab, β−CH2-Asp), 2.83 (1H, dd, J = 16.1 and 7.3 Hz, β−CH_2-Asp), 2.45–2.39 (4H, m, 2 × CH_2 ring-Dmab), 2.01–1.99 (4H, m, 2 × CH_2 ring-Dmab), 1.82–1.69 (1H, m, CH$_2$CH-Dmab), 1.41 (9H, s, O*t*Bu), 1.04 (6H, s, 2 × CH_3 ring-Dmab), 0.74–0.67 (6H, m, CH$_2$CH(CH_3)$_2$-Dmab). ^{13}C NMR (150 MHz, CD_3OD): δ 176.7 (2C, C=O ring-Dmab), 171.7 (1C, C=CN Dmab), 170.5 (1C, COODmab), 170.2 (1C, COO*t*Bu), 157. 0 (1C, C=O-urethane), 144.0 (2C, C8a-, C9a-Fmoc), 143.9 (1C, C_{Ar}-N Dmab), 141.4 (2C, C4a-, C4b-Fmoc), 136.3 (1C, C_{Ar}-CH$_2$O Dmab), 129.0 (2C, C_{Ar}-Dmab), 127.7 (2C, C3-, C6-Fmoc), 127.0 (2C, C2-, C7-Fmoc), 126.5 (2C, C_{Ar}-Dmab), 125.1 (2C, C1-, C8-Fmoc), 119.9 (2C, C4-, C5-Fmoc), 107.4 (1C, C=CN), 82.1 (1C, COOC(CH$_3$)$_3$-O*t*Bu), 66.9 (1C, CH$_2$-Fmoc), 65.5 (1C, C_{Ar}-CH$_2$O-Dmab), 52.4 (2C, 2 × CH$_2$ ring-Dmab), 51.5 (1C, Cα-Asp), 47.1 (1C, C9-Fmoc), 38.2 (1C, CH$_2$CH-Dmab), 36.4 (1C, βCH$_2$-Asp), 29.8 (1C, CH$_2$$C$H-Dmab),

27.1 (2C, 2 × CH_3 ring-Dmab), 27.3 (3C, O*t*Bu), 21.8 (2C, $CH_2CH(CH_3)_2$-Dmab). MALDI-TOFMS: *m/z* 745.2 (M+Na), 722.7 (M+H). Anal. Calcd for $C_{43}H_{50}N_2O_8$: C, 71.45; H, 6.97; N, 3.88. Found: C, 71.14; H, 7.24; N, 3.62.

3.1.17. Fmoc-Asp(ODmab)-OH (27)

Fmoc-Asp(ODmab)-O*t*Bu (**26**) (1.0 g, 1.4 mmol) was dissolved in a mixture of 1:1 CH_2Cl_2–TFA (10 mL), and the reaction mixture was stirred for 1 h. The volatiles were then removed under vacuum, and the residue was co-evaporated five times with toluene, dissolved in CH_2Cl_2, and purified by flash chromatography (97:3 CH_2Cl_2–MeOH) to give the product **27** as a pale-yellow solid (0.9 g, 97%). ^{1}H NMR (500 MHz, CD_3OD): δ 7.77 (2H, d, *J* = 7.5 Hz, H4-, H5-Fmoc), 7.69–7.61 (2H, m, H1-, H8-Fmoc), 7.44 (2H, d, *J* = 8.1 Hz, ArH-Dmab), 7.37 (3H, t, *J* = 7.4 Hz, H3-, H6-Fmoc), 7.28 (2H, t, *J* = 7.4 Hz, H2-, H7-Fmoc), 7.08 (2H, d, *J* = 8.1 Hz, ArH-Dmab), 5.17 (2H, d, *J* = 3.5 Hz, Ar-C*H*$_2$O-Dmab), 4.52 (1H, t, *J* = 6.4 Hz, α−H-Asp), 4.33 (1H, dd, *J* = 11.2, 7.0 Hz, C*H*$_2$-Fmoc), 4.25 (1H, dd, *J* = 11.2, 7.3 Hz, C*H*$_2$-Fmoc), 4.16 (1H, t, *J* = 6.9 Hz, H9-Fmoc), 3.04–2.89 (3H, m, C*H*$_2$CH-Dmab, β−C*H*$_2$-Asp), 2.83 (1H, dd, *J* = 16.1, 7.3 Hz, β−C*H*$_2$-Asp), 2.45–2.39 (4H, m, 2 × C*H*$_2$ ring-Dmab), 2.01–1.99 (4H, m, 2 × CH_2 ring-Dmab), 1.82–1.69 (1H, m, CH_2C*H*-Dmab), 1.41 (9H, s, O*t*Bu), 1.04 (6H, s, 2 × C*H*$_3$ ring-Dmab), 0.74–0.67 (6H, m, $CH_2CH(CH_3)_2$-Dmab). ^{13}C NMR (150 MHz, CD_3OD): δ 176.7 (2C, C=O ring-Dmab), 171.7 (1C, *C*OODmab), 170.5 (1C, C=*C*N-Dmab), 170.2 (1C, *C*OO*t*Bu), 157. 0 (1C, C=O-urethane), 144.0 (2C, C8a-, C9a-Fmoc), 143.9 (1C, C_{Ar}-N Dmab), 141.4 (2C, C4a-, C4b-Fmoc), 136.3 (1C, C_{Ar}-CH_2ODmab), 129.0 (2C, C_{Ar}-Dmab), 127.7 (2C, C3-, C6-Fmoc), 127.0 (2C, C2-, C7-Fmoc), 126.5 (2C, C_{Ar}-Dmab), 125.1 (2C, C1-, C8-Fmoc), 119.9 (2C, C4-, C5-Fmoc), 107.4 (1C, *C*=CN), 82.1 (1C, COO*C*$(CH_3)_3$-O*t*Bu), 66.9 (1C, *C*H_2-Fmoc), 65.5 (1C, C_{Ar}-*C*H_2O-Dmab), 52.4 (2C, 2 × *C*H_2 ring-Dmab), 51.5 (1C, Cα-Asp), 47.1 (1C, C9-Fmoc), 38.2 (1C, *C*H_2C*H*-Dmab), 36.4 (1C, β−*C*H_2-Asp), 29.8 (1C, CH_2*C*H-Dmab), 27.1 (2C, 2 × *C*H_3 ring-Dmab), 27.3 (3C, O*t*Bu), 21.8 (2C, $CH_2CH(CH_3)_2$-Dmab). MALDI-TOFMS: *m/z* 945.2 (M+Na), 722.7 (M+H). Anal. Calcd for $C_{43}H_{50}N_2O_8$: C, 71.45; H, 6.97; N, 3.88. Found: C, 71.14; H, 7.24; N, 3.62.

3.1.18. Fmoc-Met-Asp(ODmab)-OH (28)

2-Chlorotrityl resin (0.3 g, loading: 1.69 mmol) was swelled in CH_2Cl_2 (10 mL) for 5 min. The resin was then washed with DMF (10 mL). DIPEA (3 mL) was added, followed by the addition of Fmoc-Asp(ODmab)-OH **27** (0.5 g, 0.75 mmol) dissolved in DMF (4 mL). The flask was shaken for 1 h and the resin was washed with DMF (3 × 10 mL) and CH_2Cl_2 (10 mL). A mixture of 80:16:4 CH_2Cl_2–MeOH–DIPEA (10 mL) was then introduced, and the flask was shaken for 15 min. The operation was repeated once. The resin was then washed with DMF (3 × 10 mL), and 25% piperidine in DMF (10 mL) was added, and the flask was shaken for 10 min. The operation was repeated for 30 min. The resin was washed with DMF (3 × 10 mL), and Fmoc-Met-OH (0.57 g, 1.5 mmol), HBTU (0.54 g, 1.4 mmol), HOBt (0.22 g, 1.4 mmol), and DIPEA (3 mL) dissolved in DMF (7 mL) were added. The flask was then shaken for 1 h. The resin was washed with DMF (3 × 10 mL) and CH_2Cl_2 (3 × 10 mL). TFA (5% in CH_2Cl_2, 10 mL) was added to the resin and the flask was shaken for 1 h. The solution was collected in a round-bottomed flask and the resin was washed with CH_2Cl_2 (3 × 5 mL) and MeOH (3 × 5 mL). The solvent was then removed under vacuum, and the residue was azeotroped three times with toluene, dissolved in CH_2Cl_2, and purified by flash chromatography (1:1 hexanes–EtOAc) to afford the protected dipeptide **28** as a yellow powder (0.4 g, 80%) after lyophilization in dioxane. ^{1}H NMR (500 MHz, CD_3OD): δ 7.76 (2H, d, *J* = 7.5 Hz, H4-, H5-Fmoc), 7.67–7.59 (2H, m, H1-, H8-Fmoc), 7.42–7.32 (4H, m, Dmab-ArH, H3-, H6-Fmoc), 7.28 (2H, t, *J* = 7.4 Hz, H2-, H7-Fmoc), 7.11 (2H, d, *J* = 7.9 Hz, Ar-Dmab), 5.09 (2H, s, Ar-C*H*$_2$O-Dmab), 4.40–4.14 (5H, m, C*H*$_2$-Fmoc, α-H-Asp, α-H-Met, H9-Fmoc), 3.06–2.84 (4H, m, C*H*$_2$CH-Dmab, β-C*H*$_2$-Asp), 2.60–2.46 (2H, m, δ-C*H*$_2$-Met), 2.42 (2H, s, C*H*$_2$ ring-Dmab), 2.06 (2H, s, C*H*$_2$ ring-Dmab), 1.99 (3H, s, SC*H*$_3$-Met), 1.95–1.83 (2H, m, βC*H*$_2$-Met), 1.81–1.71 (1H, m, CH_2C*H*-Dmab), 1.05 (6H, s, 2 × C*H*$_3$ ring-Dmab), 0.71 (6H, d, *J* = 6.6 Hz, $CH_2CH(CH_3)_2$-Dmab). ^{13}C NMR (150 MHz, CD_3OD): δ 176.7 (2C, C=O ring-Dmab), 172.95 (1C, CO-amide), 171.7 (1C, *C*OODmab), 170.5 (1C, C=*C*N Dmab), 157.2 (1C, C=O-urethane), 144.1 (2C, C8a-, C9a-Fmoc), 143.9 (1C, C_{Ar}-N-Dmab), 141.4 (2C, C4a-, C4b-Fmoc), 136.3 (1C, C_{Ar}-CH_2O-Dmab), 128.9 (2C, C_{Ar}-Dmab), 127.6 (2C, C3-, C6-Fmoc), 127.0 (2C, C2-, C7-Fmoc), 126.5 (2C, C_{Ar}-Dmab), 125.1 (2C, C1-, C8-Fmoc), 119.8 (2C, C4-, C5-Fmoc), 107.4 (1C, *C*=CN), 66.9 (1C, *C*H_2-Fmoc), 65.5 (1C, C_{Ar}-*C*H_2O-Dmab), 54.8 (1C, Cα-Met), 54.3 (1C, Cα-Asp), 52.4 (2C, 2 × *C*H_2 ring-Dmab), 47.2 (1C, C9-Fmoc), 39.0 (1C, *C*H_2CH-Dmab), 36.2 (1C, *C*β-Asp), 31.3 (1C, β-C*H*$_2$-Met), 29.9 (1C, Cδ-Met), 29.4 (1C, CH_2*C*H-Dmab), 27.2 (2C, 2 × *C*H_3 ring-Dmab), 21.7 (2C, $CH_2CH(CH_3)_2$-Dmab), 19.8 (1C, S*C*H_3-Met. MALDI-TOFMS: *m/z* 706.4 (M+K), 690.1 (M+Na), 667.9 (M+H). Anal. Calcd for $C_{44}H_{51}N_3O_9S$: C, 66.23; H, 6.44; N, 5.27. Found: C, 66.07; H, 6.31; N, 5.36.

3.1.19. Fmoc-Met-Asp(ODmab)-Opfp (24)

Fmoc-Met-Asp(ODmab)-OH (**28**) (0.3 g, 0.4 mmol) and pentafluorophenol (0.1 g, 0.56 mmol) were dissolved in dry CH_2Cl_2 (7 mL), DIC (58 μL, 0.4 mmol) was added dropwise, and the reaction mixture was stirred for 3 h at rt. The mixture was then cooled on ice for 1 h and the diisopropylurea was filtered. The filtrate was concentrated, and the crude product was purified by flash chromatography (3:1→2:1 hexanes–EtOAc) to afford the activated dipeptide **24** as a white, crystalline solid (0.26 g, 73%). This material was only of limited stability at rt and was generally used immediately in the next step. ^{1}H NMR (500 MHz, CD_3OD): δ 7.78 (2H, d, *J* = 7.5 Hz, H4-, H5-Fmoc), 7.68–7.61 (2H, m, H1-, H8-Fmoc), 7.42–7.32 (4H, m, ArH-Dmab, H3-, H6-Fmoc), 7.28 (2H, t, *J* = 7.4 Hz, H2-, H7-Fmoc), 7.11 (2H, d, *J* = 7.8 Hz, Ar-Dmab), 5.09 (2H, s, Ar-C*H*$_2$O-Dmab), 4.40–4.14 (5H, m, C*H*$_2$-Fmoc, α-H-Asp, α-H-Met, H9-Fmoc), 3.06–2.84 (4H, m, C*H*$_2$CH-Dmab, β-C*H*$_2$-Asp), 2.60–2.46 (2H, m, δ-C*H*$_2$-Met), 2.42 (2H, s, C*H*$_2$ ring-Dmab), 2.06 (2H, s, C*H*$_2$ ring-Dmab), 1.99 (3H, s, SC*H*$_3$-Met), 1.95–1.83 (2H, m, β-C*H*$_2$-Met), 1.81–1.71 (1H, m, CH_2C*H*-Dmab), 1.05 (6H, s, 2 × C*H*$_3$ ring-Dmab), 0.71 (6H, d, *J* = 6.6 Hz, $CH_2CH(CH_3)_2$-Dmab). ^{13}C NMR (150 MHz, CD_3OD): δ 176.7 (2C, C=O ring-Dmab), 173.3 (1C, *C*OOPfp), 170.1 (1C, CO-amide), 169.9 (1C, *C*OODmab), 167.1 (1C, C=*C*N-Dmab), 157.2 (1C, C=O-urethane), 144.2 (2C, C8a-, C9a-Fmoc), 143.9 (1C, C_{Ar}-N-Dmab), 142.1 (1C, C_{Ar}-OPfp), 141.4–141.3 (3C, C_{Ar}-OPfp, C4a-, C4b-Fmoc), 140.2, 138.9 (1C each, C_{Ar}-OPfp), 136.4 (2C, C_{Ar}-OPfp), 136.1 (1C, C_{Ar}-CH_2O-Dmab), 129.2 (2C, C_{Ar}-Dmab) 127.6 (2C, C3-, C6-Fmoc), 127.0 (2C, C2-, C7-Fmoc), 126.6 (2C, C_{Ar}-Dmab), 125.1 (2C, C1-, C8-Fmoc), 119.8 (2C, C4-, C5-Fmoc), 107.4 (1C, *C*=CN), 66.8 (1C, *C*H_2-Fmoc), 65.9 (1C, C_{Ar}-*C*H_2O-Dmab), 54.1 (1C, Cα-Met), 52.4 (2C, 2 × *C*H_2 ring-Dmab), 48.8 (1C, Cα-Asp), 47.2 (1C, C9-Fmoc), 39.2 (1C, *C*H_2CH-Dmab), 35.4 (1C, *C*β-Asp), 31.4 (1C, β-C*H*$_2$-Met), 29.8 (1C, Cδ-Met), 29.5 (1C, CH_2*C*H-Dmab), 27.2 (2C, 2 × *C*H_3 ring-Dmab), 21.7 (2C, $CH_2CH(CH_3)_2$-Dmab), 14.1 (1C, S*C*H_3-Met). MALDI-TOFMS: *m/z* 986.1 (M+Na), 963.6 (M+H).

3.1.20. Glycopeptide 25

To a stirred mixture of the glycopeptide **22** (75 mg, 65 μmol) and octanethiol (0.11 mL, 0.65 mmol), DBU (5 μL, 33 μmol) was added, and the reaction mixture was stirred for 1 h. The solvent was evaporated to dryness, and the residue was washed several times with warm Et_2O. The amine product **23** was not purified further but was used directly in the coupling reaction with the protected dipeptide **24**.

To a mixture of the amine **23** (20.6 mg, 22 μmol) and Fmoc-Met-Asp(ODmab)-Opfp (**24**) (21 mg, 22 μmol) in dry THF, HOBt (5 mg, 33 μmol) was added, and the reaction mixture was stirred at rt for 3 h. The solvent was then removed under vacuum, and the residue was purified by flash chromatography (2:1 hexanes–EtOAc) to afford the protected glycopeptide **25** as a white, crystalline solid containing a minor peptide impurity. Further purification by preparative HPLC (C-18 reversed phase, 80:20 to 5:95 H_2O–CH_3CN over 30 min) gave the pure glycopeptide **25** as a colorless solid (28 mg, 72%). ^{1}H NMR (500 MHz, CD_2Cl_2): δ 8.45 (1H, br s, NH-Trp ring), 7.82–7.76 (2H, m, H4-, H5-Fmoc), 7.65–7.55 (3H, m, H1-, H8-Fmoc, H7-Trp), 7.45–7.38 (3H, m, ArH-Dmab, H3-, H6-Fmoc), 7.38–7.30 (4H, m, H2-, H7-Fmoc, H4-Trp), 7.29–7.26 (1H, br d, J = 8.7 Hz, NH-Asp), 7.19–7.12 (2H, m, H5-Trp, ArH-Dmab), 7.12–7.07 (2H, m, H6-Trp, ArH-Dmab) 7.04 (1H, br s, H2-Trp), 6.77 (1H, br d, J = 7.8 Hz, NH-Trp), 5.34 (1H, br s, NH-Dmab), 5.32–5.24 (3H, m, H2-, H2", H3′′-rha), 5.23 (1H, s, H1-rha), 5.17–4.97 (6H, m, H3′-, H4-, H4′-, H4"-rha, Ar-CH_2O-Dmab), 4.94 (1H, d, J = 1.50 Hz, H1′-rha), 4.79 (1H, br s, H1"-rha), 4.77–4.72 (1H, m, αH-Asp), 4.47 (1H, dd, J = 10.7 and 6.4 Hz, CH_2-Fmoc), 4.43–4.32 (2H, m, CH_2-Fmoc, α-H-Trp), 4.24 (1H, dd, J = 7.32 and 6.4 Hz, H9-Fmoc), 4.18–4.07 (2H, m, H5-rha, α-H-Met), 4.04 (1H, dd, J = 9.28 and 3.42 Hz, H3-rha), 3.97 (1H, m, H5"-rha), 3.91 (1H, br s, H2′-rha), 3.84 (1H, m, H5′-rha), 3.07–2.89 (4H, m, β-CH_2-Trp, CH_2CH-Dmab, β-CH_2-Asp), 2.88–2.72 (3H, m, β-CH_2-Asp, CH_2S), 2.51–2.49 (2H, m, δ-CH_2-Met), 2.48 (2H, s, CH_2 ring-Dmab), 2.36 (2H, s, CH_2 ring-Dmab), 2.19–1.98 (26H, m, SCH_3-Met, β-CH_2-Met, 7 × OAc), 1.87–1.77 (1H, m, CH_2CH-Dmab), 1.21–1.13 (9H, m, 3 × CH_3-rha), 1.07 (6H, s, 2 × CH_3 ring-Dmab), 0.71 (6H, d, J = 6.8 Hz, $CH_2CH(CH_3)_2$-Dmab). ^{13}C NMR (150 MHz, CD_2Cl_2): δ 176.3 (2C, C=O ring-Dmab), 171.8–169.5 (10C, 11 × C=O), 156.7 (1C, C=O-urethane), 144.0 (2C, C8a-, C9a-Fmoc), 143.9 (1C, C_{Ar}-N-Dmab), 141.5 (2C, C4a-, C4b-Fmoc), 137.3 (1C, C3a-Trp), 136.4 (1C, C_{Ar}-CH_2O- Dmab), 129.1 (2C, C_{Ar}-Dmab), 128.0 (1C, C7a-Trp), 127.9 (3C, C3-, C6-Fmoc), 127.3 (2C, C2-, C7-Fmoc), 126.9 (2C, C_{Ar}-Dmab), 125.2 (2C, C1-, C8-Fmoc), 123.3 (1 C, C2-Trp), 122.2 (1 C, C6-Trp), 120.2 (2 C, C4-, C5-Fmoc), 119.6 (1 C, C5-Trp), 118.9 (1C, C7-Trp), 111.4 (1C, C4-Trp), 111.4 (1C, C3-Trp), 100.2 (1C, C1"-rha), 99.8 (1C, C1′-rha), 83.2 (1C, C1-rha), 77.6 (1C, C2′-rha), 75.2 (1C, C3-rha), 73.1 (1C, C2-rha), 72.9 (1C, C4"-rha), 70.9 (1C, C3′-rha), 70.3 (1C, C4-rha), 70.3 (1C, C4′-rha), 69.9 (1C, C2"-rha), 68.9 (1C, C3"-rha), 67.6 (1C, C5′-rha), 67.4 (1C, C5-rha), 67.3 (1C, C5"-rha), 67.2 (1C, CH_2-Fmoc), 66.0 (1C, C_{Ar}-CH_2O-Dmab), 54.9 (1C, Cα-Met), 53.9 (1C, CH_2 ring-Dmab), 52.4 (1C, CH_2 ring-Dmab), 50.4 (1C, Cα-Trp), 49.8 (1C, Cα-Asp), 47.4 (1C, C9-Fmoc), 38.2 (1C, CH_2CH-Dmab), 35.7 (1C, Cβ-Asp), 31.7 (1C, β-CH_2-Met), 29.9 (1C, Cδ-Met), 29.6 (1C, -CH_2CH-Dmab), 29.3 (1C, β-CH_2-Trp), 28.1 (2C, 2 × CH_3 ring-Dmab), 22.5 (2C, $CH_2CH(CH_3)_2$-Dmab), 21.1–20.7 (7C, 7 × CH_3-OAc), 17.5–17.1 (3C, 3 × CH_3-rha), 15.3 (1C, SCH_3-Met), MALDI-TOFMS: m/z 1741.7 (M+Na), 1757.1 (M+K), 1718.6 (M+H). Anal. Calcd for $C_{87}H_{107}N_5O_{27}S_2$: C, 60.79; H, 6.27; N, 4.07. Found: C, 60.66; H, 6.45; N, 3.71.

3.1.21. Aspartimide 36

To a stirred mixture of **25** (13 mg, 7.6 μmol) and octanethiol (13 μL, 76 μmol), DBU (0.6 μL, 33 μmol) was added, and the reaction mixture was stirred for 1 h. The solvent was evaporated to dryness, and the residue was washed several times with warm Et_2O. The amine product **29** was then dissolved in MeOH (3 mL) and MeONa–MeOH (five drops) was added. The reaction mixture was stirred overnight at rt. Rexin H^+ 101 was then added until the pH was neutral. The resin was removed by filtration, the solvent was concentrated, and the crude product was purified by flash chromatography (10:3:1 EtOAc–MeOH–H_2O) to afford a 2:1 α/β mixture of the aspartimide side product (6.4 mg, 88%) as a white solid, after lyophilization in dioxane. ^{1}H NMR (500 MHz, MeOD) of the major isomer: δ 7.60 (1H, d, J = 7.8 Hz, H4-Trp), 7.32 (1H, d, J = 8.8 Hz, H7-Trp), 7.11–7.05 (2H, m, H2-, H6-Trp), 7.01 (1H, br t, J = 7.1 Hz, H5-Trp), 5.17 (2H, br s, H1′-rha), 5.11 (1H, br s, H1-rha), 4.91 (1H, br s, H1"-rha), 4.40–4.31 (1H, m, α-H-Trp), 4.26 (1H, dd, J = 4.9 Hz, α-H-Asp), 4.14 (1H, dd, J = 6.4 Hz, α-H-Met), 4.02 (1H, br s, H2-rha), 3.97 (2H, br s, H2′-, H2"-rha), 3.93 (1H, m, H5-rha), 3.86 (1H, dd, J = 9.5 and 3.2 Hz, H3′-rha), 3.75–3.63 (4H, m, H5′-, H5", H3, H3"-rha), 3.52 (1H, dd, J = 9.7, H4-rha), 3.40–3.31 (2H, m, H4′-, H4"-rha), 3.09–2.67 (2H, m, β-CH_2-Trp), 2.86–2.72 (3H, m, β-CH_2-Asp, CH_2S-Trp), 2.68–2.52 (3H, m, β-CH_2-Asp, δCH_2-Met), 2.19–2.10 (2H, m, βCH_2-Met), 2.09 (3H, s, SCH_3-Met), 1.32–1.20 (9H, m, 3 × CH_3-rha). ^{13}C NMR (125 MHz, MeOD) of the major isomer: δ 177.4, 174.2, (3C, 3 × C=O), 136.5, 127.8, 124.7, 123.1, 121.0, 118.6, 118.2, 111.1 (each, 1C, 8 × C_{Ar}-Trp), 102.7 (1C, C1′′-rha), 101.3 (1C, C1′-rha), 86.6 (1C C1-rha,), 78.2 (1C, C2′-rha), 73.2, 72.9, 72.5, 72.3 (each 1C, 3 × C4-, C2-rha), 71.1, 70.9, 70.7, 70.6, 70.1, 69.1, 68.9, (10C, C2, 3 × C3-, 3 × C4-rha, 3 × C5-rha), 53.4, 52.3 51.7 (each 1C, Cα-Met, Cα-Asp, Cα-Trp), 50.1 (1C, β-CH_2-Asp), 35.5 (1C, SCH_2-Trp), 30.9 (1C, β-CH_2-Met), 29.6 (1C, β-CH_2-Trp), 28.3 (1C, SCH_2-Met), 16.9, ((1C, C6-rha), 16.7 (2C, 2 × C6-rha), 13.9 (1C, SCH_3-Met). HRMS: Calcd for [$C_{38}H_{56}N_4O_{15}S_2$ + Na]: m/z 895.3076. Found: m/z 895.3075.

3.1.22. Glycopeptide 1

The amine product **29** (11 mg, 7.3 μmol) was dissolved in 2% NH_2NH_2 in THF (5 mL), and the mixture was stirred for 2 h at rt. The solvent was evaporated, and the residue **30** was dissolved in MeOH (5 mL) and MeONa–MeOH (five drops) was added. The reaction mixture was stirred overnight at rt. Rexin H^+ 101 was then added until the pH was slightly basic, the resin was filtered, and the filtrate was further neutralized with HOAc. The solvent was evaporated, and the crude product was washed successively with hexanes (10 mL), CH_2Cl_2 (10 mL), and EtOAc (10 mL). The crude material was further purified by HPLC (C-18 reversed phase, 95:5–5:95 H_2O–CH_3CN over 15 min) to afford the desired glycopeptide **1** (6.5 mg, 68%) as a white solid. ^{1}H NMR (see above): HRMS: Calcd for $C_{38}H_{58}N_4O_{16}S_2$: m/z 891.3367. Found: m/z 891.3356.

3.2. Molecular modeling

The molecular structures of the glycopeptides **1** and **2** were constructed using SYBYL 6.6 (Tripos, Inc.) with the coordinates of the two 1.8 Å resolution crystal structures of the Fab fragment of SYA/J6 complexed with the octapeptide and the pentasaccharide (PDB entry 1PZ5).[22] The structures of **1** and **2** were then optimized by energy minimization within the binding site to achieve a reasonable structure in each case, employing the standard Tripos molecular mechanics force field[49] and Gasteiger–Marsili charges,[50,51] with a 0.001 kcal/mol energy gradient convergence criterion. The atom charges were retained on both **1** and **2** for the docking calculations. The water was removed. Only polar hydrogens were added to the protein, and Kollman united-atom partial charges[52] were assigned. Compounds **1** and **2** were then docked into the antibody-combining site of the Fab fragment of SYA/J6 using AUTODOCK 3.0.[26] All active torsions of **1** and **2** were selected to be fully flexible during the docking experiments. The grid maps were constructed using 70 × 70 × 70 points, with grid point spacing of 0.375 Å. A Lamarckian Genetic Algorithm (LGA) was used to search each conformation space for low-energy binding orientations. The default setting was adopted, and 500 LGA docking runs were performed.

3.3. Immunochemical analysis

3.3.1. Lipopolysaccharide, and O-polysaccharide from *S. flexneri* Y, peptide MDWNMHAA, and monoclonal antibody SYA/J6, used in ELISA

The lipopolysaccharide (LPS) and O-polysaccharide from *S. flexneri* Y and monoclonal antibody SYA/J6 (IgG3) were provided by Dr. D. R. Bundle[21,22,31,44–48] and were used as antigens/inhibitors. The mimetic-peptide MDWNMHAA,[24] was used as an inhibitor in ELISA.

3.3.2. Competitive-inhibition ELISA studies with lipopolysaccharide and O-polysaccharide of *S. flexneri* Y, peptide MDWNMHAA, and glycopeptide as inhibitors

Competitive–inhibition ELISA was performed in 96-well plates (NUNC-MaxiSorp, Rochester, NY), coated overnight with 100 μL/well of LPS from *S. flexneri* Y (10 μg/mL in carbonate buffer pH 9.6). Wells were blocked by the addition of 1% BSA for 2 h at room temperature. The plates were washed three times with a washing solution of 0.05% Tween 20 and 0.9% NaCl. Monoclonal antibody SYA/J6 (~1 μg/mL) was pre-incubated in duplicate with twofold serial dilutions of inhibitors in PBS-T, starting at an initial concentration of 400 μg/mL, for 1 h at room temperature. Then, 100 μL of the mixtures was transferred to plates, and the plates were incubated for 3 h at room temperature. After washing four times as before, 100 μL per well of alkaline phosphatase-labeled goat anti-mouse IgG (Caltag Laboratories, San Francisco, CA) diluted 1:3000 in PBS-T was added. The plates were incubated overnight at room temperature, and were again washed four times. Substrate solution (100 μL) containing *p*-nitrophenyl phosphate (1 mg/mL, Kirkegaard & Perry lab, Gaithersburg, MD) was added to the wells. After 25–30 min at room temperature, the plates were scanned at 405 nm in a SpectraMax 340 microplate reader. The percentage of inhibition was determined by the decrease in A_{405} value in wells with inhibitors as compared with those without inhibitors.

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