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α-Galactose Based Neoglycopeptides. Inhibition of Verotoxin Binding to Globotriosylceramide†

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Abstract—Solution and solid phase strategies for the synthesis of α-galactose based neoglycopeptide derivatives **2**–**13** were developed. Neoglycopeptides generated were tested for the inhibition of verotoxin binding to globotriosylceramide (Gb3) using ELISA. Among all of the compounds tested, only the lipid derivatives of neoglycopeptides, **11, 12** and **13** were found to be inhibitors, $IC_{50} = 2.0$ mM (**11b** and **12c**) and 0.2 mM (**11c** and **13c**). All of the inhibitors (**11b, 11c, 12c** and **13c**) have a similar branching of the two α-galactosyl units at the N-terminal glycine residue of a short peptide and a lipid moiety attached at the C-terminal site. Both of these factors seem to be crucial for the inhibition. It is interesting to note that the inhibitors have only a portion of the natural trisaccharide ligand. The secondary groups either may contribute in sub-site oriented interactions with the protein receptors or may mimic the internal sugar units of the cell-surface ligand, Gb3.

Introduction

The importance of cell-surface carbohydrates in initiating a wide variety of biological and pathological processes is now well recognized.[1–3] Since carbohydrate–protein interactions are responsible for initiating the early stages of several microbial infections, the use of a soluble form of the natural ligands as anti-adhesives may serve as useful agents in developing new therapies. For the success of this approach, there are two major challenges to consider and to overcome. First, it is well accepted that the individual interactions between carbohydrate ligands and protein receptors are weak (i.e. millimolar region), and in natural systems such interactions are applied in a cooperative manner.[4–6] Second, as we have learned from the poor in vivo stability and bioavailability of peptides and nucleotides as therapeutics, carbohydrates are also likely to suffer from the same drawbacks and are not ideal candidates.[7,8] However, unlike with carbohydrates, tremendous progress has been made in developing peptide and nucleotide mimics which can be synthesized with relative ease and possess acceptable pharmacokinetic properties. Mimics of cell-surface carbohydrates capable of competing with natural carbohydrate–protein interactions would be of interest in potentially developing anti-microbial agents.[9]

In the past several years, as an alternative to the use of antibiotics for the treatment of bacterial infections, significant efforts have been made in designing soluble multivalent carbohydrate ligands as anti-adhesive agents. Some of these approaches include carbohydrate-based polymers, dendrimers, as well as anchoring of carbohydrate ligands on various solid supports.[10–13] Due to the high molecular weight of glyco-based derivatives, it is likely that such compounds are not rapidly transported into various tissues and possess poor pharmacokinetic properties.

Results and Discussion

In recent years, there has been much emphasis on the synthesis of small molecules as analogues of cell-surface carbohydrate ligands.[14–17] With a similar goal of developing compounds that are capable of interfering with the natural carbohydrate–protein interactions, we proposed a flexible and control-oriented model for the

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synthesis of neoglycopeptides.[18,19] This model is based upon presenting terminal or exposed glycosides on a peptide/pseudopeptide template and can be achieved in a controlled manner, to allow for a high degree of flexibility in the design. For the initial studies, α-galactose derivatives were incorporated as the terminal glycoside moiety of the neoglycopeptides. α-Galactose is the terminal glycoside of the trisaccharide portion of globotriosylceramide (Gb3, Fig. 1, **1**). The glycosphingolipid ligand, Gb3, is present on cell-surfaces of human epithelial cells and is involved in the binding of Shiga toxin and Shiga-like toxin receptors produced by *Shigella dysenteriae* or by certain enterohemorrhagic *E. coli*.[20–25] Shiga and Shiga-like toxins have similar structural and functional proteins, and are commonly known as verotoxins. It is known that infection with verotoxin producing *E. coli* leads initially to hemorrhagic colitis and further to hemolytic uremic syndrome in humans.[26,27] The binding subunits (e.g. B subunits, see Fig. 1) of verotoxin are lectins (i.e. homopentameric protein) that recognize the galabiose moiety (Galα1-4Gal) of glycosphingolipid derivatives. It has been shown that cells which do not express the galabiose moiety on their surfaces are not affected by the toxins.[28] Following the carbohydrate ligand–lectin receptor recognition, in which the B subunits are involved, the subsequent step is the endocytosis of the toxic subunit, subunit A, of verotoxin.[29,30] It is, therefore, reasonable to believe that novel compounds that are capable of interfering with cell-surface glycoconjugate ligand–toxin receptor interactions may offer new therapies for the treatment of the disease initiated by ingestion of enterohemorrhagic *E. coli*. It is interesting to note that the binding constant for the soluble trisaccharide of Gb3 to the soluble form of the pentameric subunit B of a similar toxin is weak (e.g. millimolar region, Ka $0.5–1\times10^3$ M^{-1} for subunit monomer).[29] Also, derivatives of the natural ligand which contain the galabiosyl moiety attached to a short chain hydrocarbon were shown not to be inhibitors, even in the millimolar concentration range.[23]

Design and synthesis of α-galactose based neoglycopeptides

With the goal of developing inhibitors of verotoxin binding to Gb3, several α-galactose based neoglycopeptides were synthesized (see Figs. 2 and 3) and tested for the inhibition of verotoxin binding to Gb3 by ELISA. Such assays have been described in detail in the literature.[29] The first strategy was developed to present bivalent carbon linked α-galactoside derivatives on different peptide/pseudopeptide based templates (Fig. 2). In the long term, this approach could be extended to obtain bivalent forms of the terminal or exposed saccharide moiety on a library of peptide/pseudopeptide templates. A combinatorial approach to optimize the presentation of bivalent α-galactoside derivatives may be used to reach two different binding domains of a multimeric protein receptor. We are working with C-linked neoglycopeptides to benefit from the advantages of structural stability in vivo, in acidic and basic media, and ease of synthesis.[31] Bivalent, α-galactose based neoglycopeptides (**2**–**3**, Fig. 2) were synthesized by solution phase chemistry, whereas a solid phase approach was developed for the synthesis of compounds **4**–**7** (Fig. 2). As a second strategy, we plan to couple one or two α-galactoside derivatives onto the N-terminal side of short peptides (see compounds **9**–**13**, Fig. 3). It is anticipated that the additional peptide/pseudopeptide/neoglycopeptide moiety may provide sub-site oriented secondary interactions with the protein receptors. Alternatively, the additional groups may mimic the internal sugars of the cell-surface ligand, Gb3. In the long term, this approach could also be developed in a combinatorial manner. Part of the second strategy consisted of coupling long-chain hydrophobic amines [i.e. $NH_2(CH_2)_{10}Me$ and $NH_2(CH_2)_{13}Me$] to the carboxyl

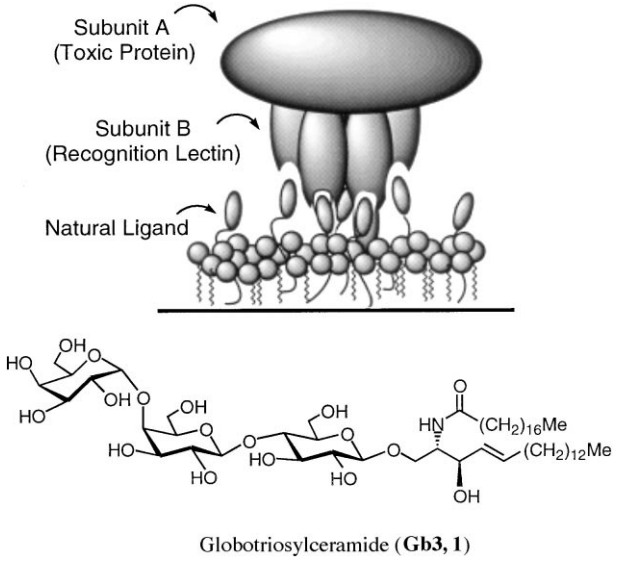


Figure 1. Model for the interaction of verotoxin to globotriosylceramide (Gb3) on cell-surfaces.

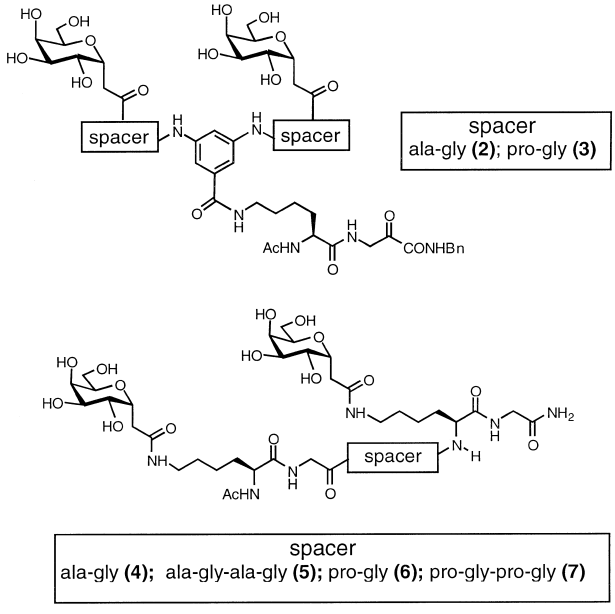


Figure 2. Bivalent species of α-galactose based neoglycopeptides.

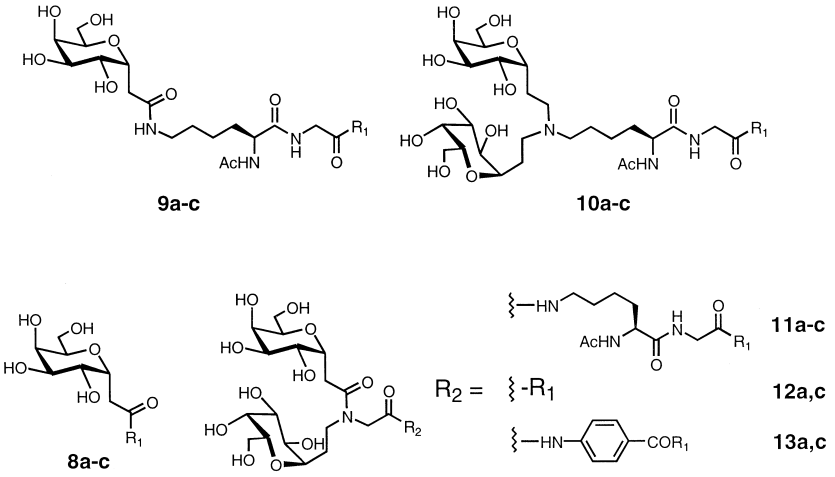


Figure 3. α-Galactose based neoglycopeptides.

terminal of the peptide moiety. The attachment of the lipid moiety was expected to provide a "cluster effect" to enhance the inhibitory effect.

The synthesis of α-galactose based neoglycopeptides **9a**–**13a** was developed. Compound **9a** was synthesized by attaching a C-linked α-galactose derivative having a carboxyl group, **18**, onto the side chain of the dipeptide lysine-glycine as a key step. Compounds **10a** and **11a** are branched α-galactose based neoglycopeptide derivatives. Using a bis-reductive amination approach, two units of α-*C*-galactosyl derivatives **28** were attached to the side chain of lysine in the peptide backbone, leading to the synthesis of compound **10a**. For the synthesis of compound **11a**, sequential branching of the amino group of glycine benzyl ester **35** was accomplished by reductive amination using an α-*C*-galactosyl derivative having an aldehyde group, **28**, followed by amide coupling of the monosubstituted glycine derivative to an α-*C*-galactosyl derivative with a carboxyl group, **18**. Coupling of di-substituted α-*C*-galactosyl glycine **38** to dipeptide **27** was achieved using standard peptide coupling methods, generating neoglycopeptide **11a**.

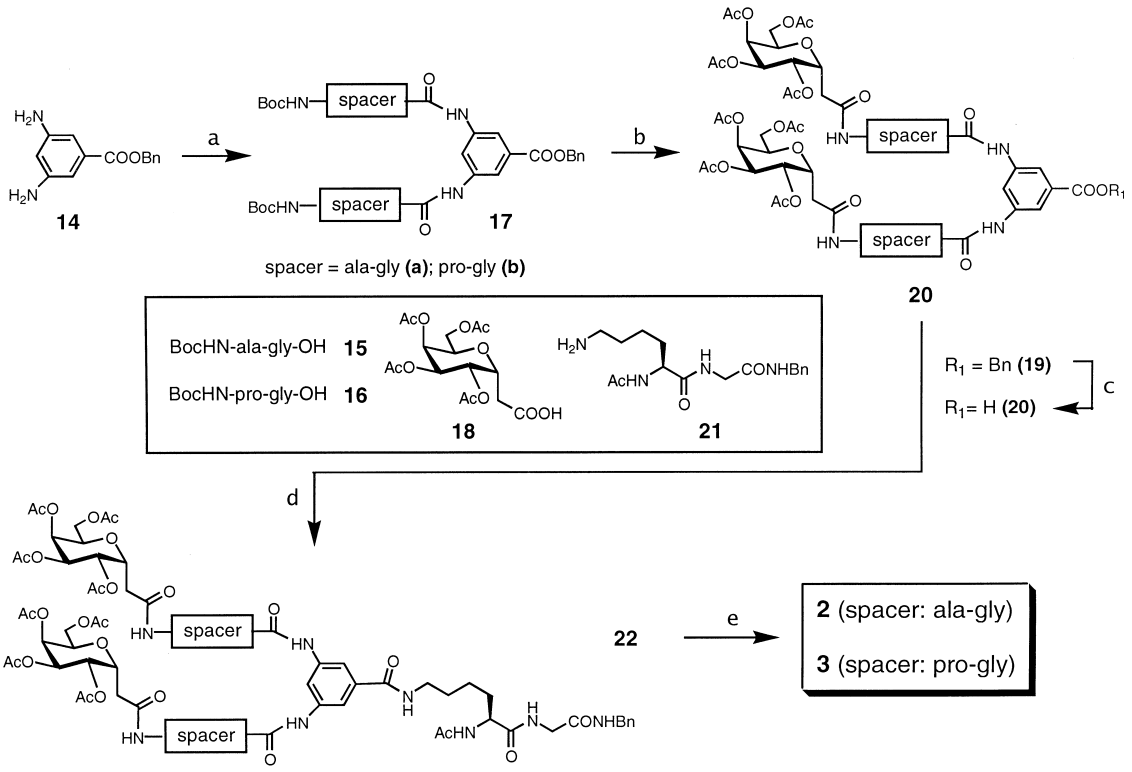
Synthesis of neoglycopeptides 2 and 3 (Scheme 1)

The free amino groups of the benzyl ester of 3,5-diaminobenzoic acid (**14**) were coupled with BocHN-ala-gly-OH (**15**) and BocHN-pro-gly-OH (**16**) respectively, using a DCC–HOBt method, to give compounds **17a** and **17b** in 90 and 93% yields after purification.

Compounds **17a** and **17b** were treated with TFA to remove the Boc-groups, and independently coupled with α-galactosyl carboxylic acid **18** to obtain compounds **19a** (70%) and **19b** (98%). Bivalent galactosyl derivative **19a** was hydrogenated, using 10% Pd/C as a catalyst, to give the free carboxylic acid **20a**. Acid **20a** was then coupled to the side-chain amino group of dipeptide **21** using a HATU coupling method to give compound **22a**. Following treatment with NaOMe/MeOH, neoglycopeptide **2** was obtained from **22a** in 60% yield, after purification. Using the same approach, compound **3** was obtained from **19b**.

Solid phase, parallel synthesis of neoglycopeptides 4–7 (Scheme 2)

Fmoc–Rink Amide MBHA resin (Novabiochem, loading 0.56 mmol/g, **23**) was treated with 20% piperidine to effect Fmoc removal. The free amino group was coupled with neoglycopeptide building block **30** using a HATU coupling method (4.0 eq **30**, 4.0 eq HATU, 8.0 equiv DIEA). The product, **24a**, after treatment with 20% piperidine, gave compound **24b** with a free amino group on the resin. At this stage, the resin was divided into two portions. Each portion was subjected to parallel coupling either with FmocHN-ala-gly-OH (**25**) or FmocHN-pro-gly-OH (**26**) using a HATU method, followed by the removal of the Fmoc group to give resin $\mathbf{A_1}$ and $\mathbf{A_2}$. Resin $\mathbf{A_1}$ and $\mathbf{A_2}$ was divided further into two portions, and was subjected to parallel couplings. Neoglycopeptide **4** was obtained from the first half of the $\mathbf{A_1}$ batch in a sequential order: (i) coupling of the building block, **30**, using a HATU method, (ii) 20% piperidine, (iii) *N*-acetylation by Ac_2O, (iv) 95% TFA, for the resin cleavage, and (iv) NaOMe in MeOH for the deacetylation. Neoglycopeptide **4** was obtained in 42% overall yield after purification by reverse phase HPLC. From the second half of the $\mathbf{A_1}$ batch, neoglycopeptide **2** was obtained as follows: (i) coupling of Fmoc-ala-gly-OH (**25**) using a HATU method, (ii) 20% piperidine, (iii) coupling of compound **30** using a HATU method, (iv) 20% piperidine, (v) *N*-acetylation by using Ac_2O, (vi) cleavage from the resin (95% TFA), and (vii) treatment with NaOMe in MeOH for the deacetylation. Neoglycopeptide **5** was obtained in 40% overall yield after purification by reverse phase HPLC.



Scheme 1. (a) **14** (1.0 mmol), Boc-NH-ala-gly-OH (**15**, 2.0 mmol) or Boc-NH-pro-gly-OH (**16**, 2.0 mmol), DCC (2.5 mmol), HOBt (2.5 mmol), DIEA (2.5 mmol), CH_2Cl_2, room temp, 17–20 h, 90–93%. (b) (i) TFA/CH_2Cl_2, room temp, 1 h; (ii) **18** (2.0 mmol), DCC (2.5 mmol), HOBt (2.5 mmol), DIEA (2.5 mmol), CH_2Cl_2, room temp, 17–20 h, 70% (**19a**), 98% (**19b**). (c) 10% Pd/C, EtOH, room temp, 1.5 h, 97%. (d) **21** (1.0 mmol), HATU (1.4 mmol), DIEA (1.2 mmol), DMF, room temp, 18 h for **22a** (20%) and 5 h for **22b** (30%). (e) NaOMe, MeOH, room temp, 5 h, 60% (**2**) and 33% (**3**).

A similar strategy was utilized for the synthesis of neoglycopeptides **6** (51%) and **7** (46%) using resin batch $\mathbf{A}_2$.

Synthesis of neoglycopeptides 8–10 (Scheme 3)

α-Galactoside amide derivatives **8a–c** were synthesized from compound **18** as follows. α-Galactosyl carboxylic acid **18** was coupled with three different amines (e.g. $NHEt_2$, $NH(CH_2)_{10}Me$ and $NH(CH_2)_{13}Me$) using the DCC/HOBt coupling method, followed by de-*O*-acetylation (NaOMe/MeOH). Neoglycopeptides **9a–c** were obtained from dipeptide **27**. Coupling of dipeptide **27** with α-galactosyl carboxylic acid **18** using the DCC/HOBt method gave compound **29** in 87% yield, after purification. Neoglycopeptides **9a–c** (60–73%) were obtained from **29** in a number of steps: (i) hydrogenation over 10% Pd/C to give the free carboxylic acid, (ii) coupling to different amines (DCC/HOBt), (iii) 20% piperidine for Fmoc removal, (iv) Ac_2O for *N*-acetylation, and (v) deacetylation of sugar hydroxyls using NaOMe/MeOH. Dipeptide **27** was subjected to bis-reductive amination with α-galactosyl aldehyde **28** to obtain compound **32** in 65% yields. Neoglycopeptides **10a–c** were obtained from **32** by the same series of steps discussed above.

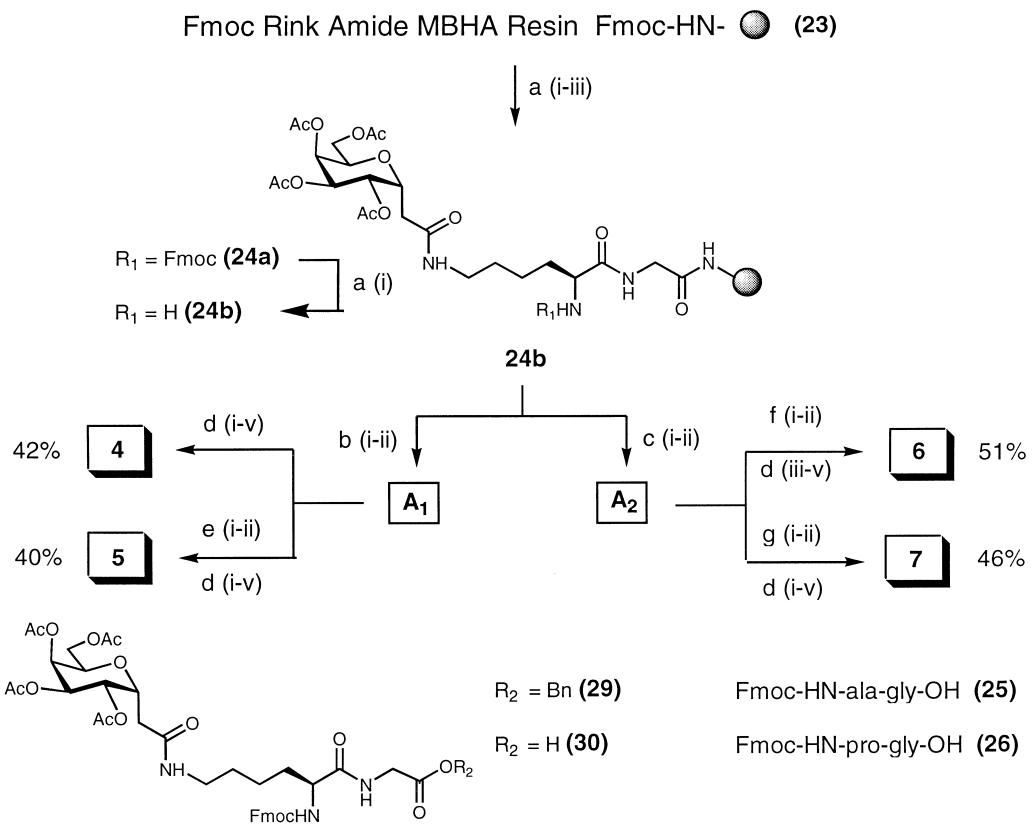
Synthesis of neoglycopeptides 11–13 (Scheme 4)

Neoglycopeptides **11–13** were synthesized as follows. Monoreductive amination of glycine benzyl ester **35** with aldehyde **28** gave mono-glycosylated compound **36** in 85% yields after purification. Compound **36** was then coupled with α-galactosyl carboxylic acid **18** using the DCC/HOBt method. The coupled product, **37**, was obtained in 80% yield after purification.

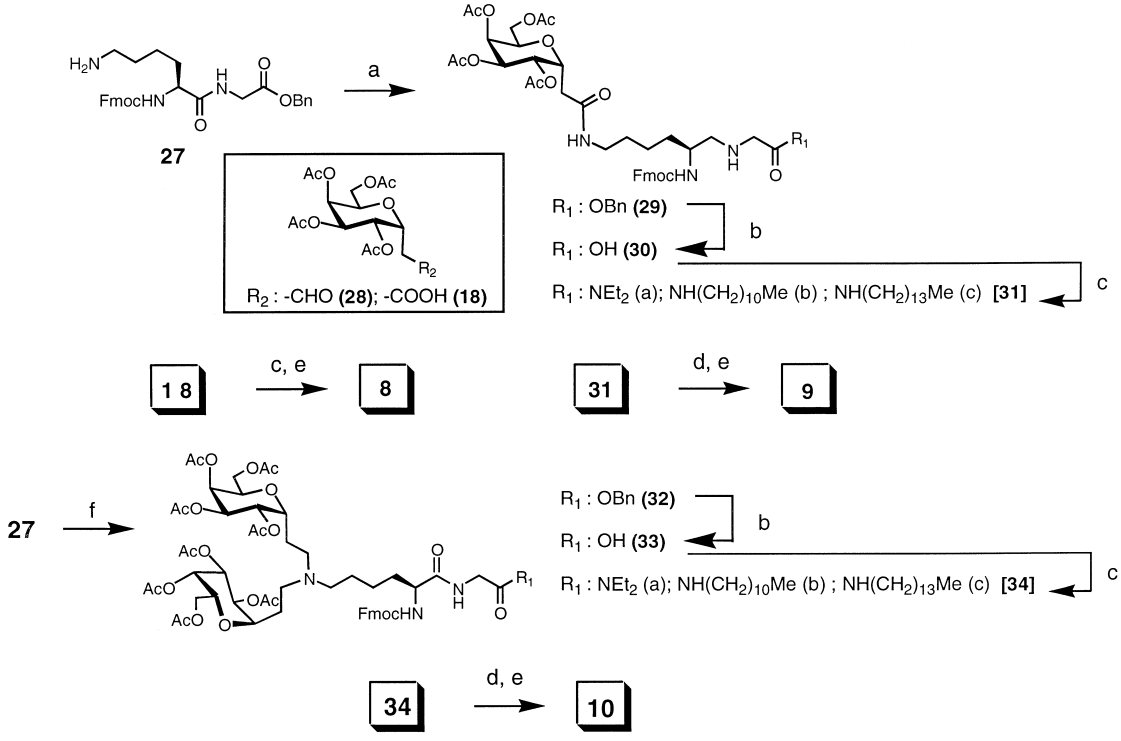
Hydrogenation of **37** gave the carboxylic acid **38**. Coupling of acid **38** with either dipeptide **27** or the benzyl ester of *p*-aminobenzoic acid using DCC/HOBt gave compounds **40** and **43** respectively. Neoglycopeptides **12a,c** were obtained from compound **38** as follows. Compound **38** was coupled with two different amines using DCC/HOBt to give **39a,c** and then subjected to de-*O*-acetylation conditions. To generate neoglycopeptides **11a–c** and **13a,c**, compounds **41** and **44** underwent lipid coupling and de-*O*-acetylation in a manner analogous to that described for compounds **12a,c**.

Inhibition of verotoxin binding to Gb3 by ELISA

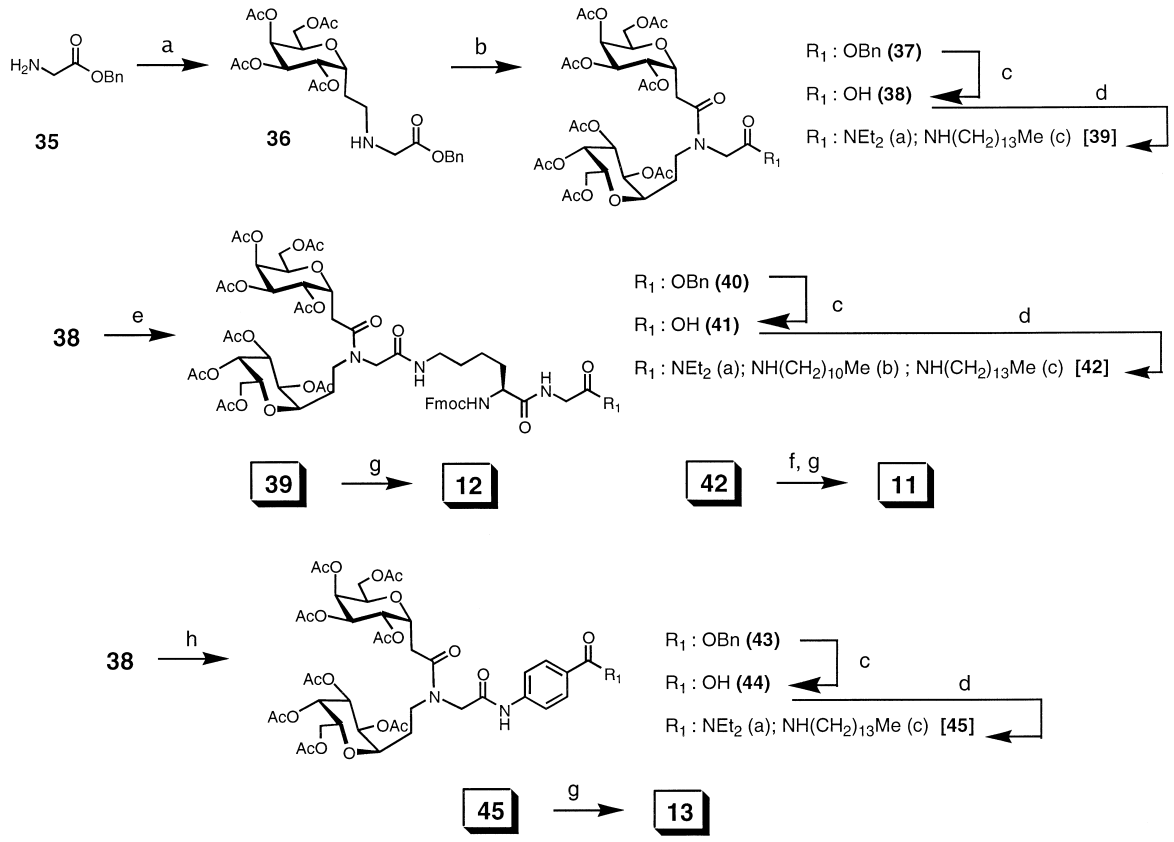
To screen for the inhibition of verotoxin binding to Gb3 using α-galactose based neoglycopeptides, a microtiter plate assay was established. Gb3 isolated from human kidney was immobilized onto 96-well plates and the amount of verotoxin bound was measured against increasing concentrations of neoglycopeptide derivatives using a receptor–ELISA based system. None of the α-galactose based bivalent neoglycopeptides (**2–7**, for inhibition results see Table 1) showed any inhibition. From all of the derivatives (**8–11**) tested, only the lipid derivatives of neoglycopeptide **11** exhibited inhibitory



Scheme 2. (a) (i) 20% piperidine in DMF; (ii) 4.0 equiv **30**, 4.0 equiv HATU, 8.0 equiv DIEA in DMF, 16 h; (iii) same as (a) (i). (b) (i) 4.0 equiv **25**, 4.0 equiv HATU, 8.0 equiv DIEA in DMF, 6 h; (ii) same as (a) (i). (c) (i) 4.0 equiv **26**, 4.0 equiv HATU, 8.0 equiv DIEA in DMF, 6 h; (ii) same as (a) (i). (d) (i) 4.0 equiv **30**, 4.0 equiv HATU, 8.0 equiv DIEA in DMF, 16 h; (ii) same as (a) (i); (iii) Ac_2O, DMF, 3 h; (iv) 95% TFA; (v) NaOMe, MeOH. (e) (i) 4.0 equiv **25**, 4.0 equiv HATU, 8.0 equiv DIEA in DMF, 6 h; (ii) same as (a) (i). (f) (i) 4.0 equiv **30**, 4.0 equiv HATU, 8.0 equiv DIEA in DMF, 16 h; (ii) same as (a) (i); (d) iii–v. (g) (i) 4.0 equiv **26**, 4.0 equiv HATU, 8.0 equiv DIEA in DMF, 16 h; (ii) same as (a) (i).



Scheme 3. (a) **18** (1.0 equiv), DCC (1.0 equiv), HOBt (1.0 equiv), DIEA (1.0 equiv), 87%. (b) H_2, 10% Pd/C, 95% EtOH. (c) $NHEt_2$ or $NH_2(CH_2)_{10}Me$ or $NH_2(CH_2)_{13}Me$ (1.5 equiv), DCC (1.0 equiv), HOBt (1.0 equiv), DIEA (1.0 equiv), 60–73% for **9a–c** from **29**. (d) (i) 20% piperidine in DMF; (ii) Ac_2O, DMAP. (e) NaOMe, MeOH. (f) **28** (2.2 equiv), THF, $NaB(OAc)_3H$, AcOH, pH 5.0, 65%.



Scheme 4. (a) **28** (0.9 equiv), THF, $NaB(OAc)_3H$, AcOH, pH 5.0, 0°C, 85%. (b) **18** (1.0 equiv), DCC (1.0 equiv), HOBt (1.0 equiv), DIEA (1.0 equiv), 80%. (c) H_2 10% Pd/C, 95% EtOH. (d) $NHEt_2$ or $NH_2(CH_2)_{10}Me$ or $NH_2(CH_2)_{13}Me$ (1.5 equiv), DCC (1.0 equiv), HOBt (1.0 equiv), DIEA (1.0 equiv). (e) **27** (1.0 equiv), DCC (1.0 equiv), HOBt (1.0 equiv), DIEA (2.0 equiv), 83–85%. (f) (i) 20% piperidine in CH_2Cl_2; (ii) Ac_2O, DMAP. (g) NaOMe, MeOH. (h) Benzyl ester of *p*-aminobenzoic acid (1.0 equiv), DCC (1.0 equiv), HOBt (1.0 equiv), DIEA (1.0 equiv).

Table 1. ELISA-inhibition of verotoxin binding to immobilized Gb3

Compound	IC_{50}
2–7	No inhibition
8a–c to **10a–c**	No inhibition
11a–c	No inhibition (**11a**), 2.0 mM (**11b**), 0.2 mM (**11c**)
12a,c	No inhibition (**12a**), 2.0 mM (**12c**)
13a,c	No inhibition (**13a**), 0.2 mM (**13c**)

effects ($IC_{50} = 2$ mM for **11b** and 0.2 mM for **11c**) on verotoxin binding to Gb3. It is interesting to note that a subtle change in the lipid moiety can influence the inhibitory action. Lipid derivatives **8a–c**, **9a–c** and **10a–c** were not found to be inhibitors. It seems likely that the appropriate branching of the two α-galactosyl units and the attachment of the lipid moiety in compounds **11b** and **11c** are critical for the inhibition. Alternatively, the secondary groups may mimic the internal sugars of the natural ligand, Gb3.

Lead compound derivatives and inhibition studies

To determine the elements of compound **11c** that are necessary for the inhibitory effect, neoglycopeptides **12a,c** and **13a,c** were synthesized. Compound **12c** is the lipid derivative of the branched α-galactosyl based glycine. Analogue **13c** has the same branched α-galactosyl glycine structure, but to which an unnatural amino acid residue, *p*-NH-Ph-CO-, has been added between glycine and the lipid moiety. These two analogues were designed to explore the importance of the tripeptide moiety of neoglycopeptide **11c** for the inhibition of verotoxin/Gb3 bindings. As with compound **11a**, compound **12a** did not show any inhibition. The lipid derivative of the branched α-galactosyl glycine amino acid, **12c**, inhibited the Gb3–verotoxin interaction ($IC_{50} = 2$ mM). Interestingly, removal of the lys-gly dipeptide moiety from neoglycopeptide **11c** showed a weak inhibition. The addition of an unnatural amino acid at the C-terminal of compound **12** enhanced the inhibition ($IC_{50} = 0.2$ mM for compound **13c**). Compound **13c** has a similar inhibitory effect to that shown by neoglycopeptide **11c** but it is a simpler compound, with a more facile synthesis.

Conclusion

Several solution and solid phase approaches for the synthesis of α-galactose based neoglycopeptides were developed and neoglycopeptides generated were tested for the inhibition of verotoxin binding to Gb3. Some of these approaches can be modified for the assembly of combinatorial libraries of neoglycopeptides. Of all the compounds tested, **11c** and **13c** were found to be the

best inhibitors ($IC_{50} = 0.2$ mM). To our knowledge, this is the first example of small-molecule inhibitors of verotoxin binding to Gb3 that have only a portion of the recognition element of the trisaccharide ligand, and are capable of exhibiting inhibitory effects at a sub-millimolar concentration. Although it is too early to provide any explanation for this behavior, it is reasonable to believe that the appropriate positioning of the two α-*C*-galactose derivatives may play a crucial role in generating this effect. The secondary groups may contribute to the overall binding by participating in sub-site oriented interactions with the protein receptors or may mimic the internal sugars of the cell-surface ligand, Gb3. Further work is in progress to develop a better understanding of the importance of the peptide backbone and of the lipid moiety in their assistance in amplifying inhibitory effects.

Experimental

Synthesis of 2 and 3 (Scheme 1). Compounds 17a and 17b. To a solution of Boc-NH-ala-gly-OH (**15**, 2.0 mmol) in CH_2Cl_2 (50 mL) was added DCC (2.5 mmol) and HOBt (2.5 mmol). The reaction mixture was stirred for 15 min, followed by the addition of the benzyl ester of 3,5-diaminobenzoic acid **14** (1.0 mmol) and DIEA (2.5 mmol). It was further stirred at room temperature for 17 h, solvent evaporated in vacuo and the product purified by flash chromatography over silica gel. Compound **17a** was obtained in 90% yield after the removal of the Boc group. Similar reaction conditions were utilized to obtain compound **17b** from Boc-NH-pro-gly-OH (**16**)

Compounds 20a and 20b. DCC (2.5 mmol) and HOBt (2.5 mmol) were added to a solution of α-galactosyl carboxylic acid, **18** (2.0 mmol) in CH_2Cl_2 (50 mL) and stirred for 15 min. To this mixture, compound **17a** (1.0 mmol) and DIEA (2.5 mmol) was added and then stirred for 17 h. After the removal of the solvent in vacuo, the product was purified (**19a**, 70% yield), and subjected to hydrogenation to remove the benzyl group leading to compound **20a** in 97% yield. Synthesis of compound **20b** was achieved in a similar manner. **19a**: ^{1}H NMR (400 MHz, MeOH-d_4): δ 1.35–1.43 (d, $J = 7.0$ Hz, 6H), 1.91, 1.98, 2.05, 2.07 (4s, 24H), 2.45–2.90 (m, 4H), 3.85–4.38 (m, 12H), 4.63–4.77 (m, 2H), 5.25–5.50 (m, 8H), 7.30–7.50 (m, 5H), 8.18–8.65 (m, 3H). ^{13}C NMR (100 MHz, MeOH-d_4): δ 14.9, 18.3, 18.4, 32.4, 42.0, 49.3, 60.1, 65.7, 66.8, 67.8, 69.1, 71.1, 72.8, 73.4, 114.9, 115.8, 126.8, 127.0, 127.4, 130.0, 135.5, 138.0, 165.1, 167.7, 169.1, 169.4, 170.6, 173.6. LRMS (Electrospray, positive ion mode, *m*/*z*) for $C_{56}H_{70}N_6O_{26}$: 1243.4 (MH^+). **20a**: ^{1}H NMR (400 MHz, MeOH-d_4): δ 1.25–1.40 (m, 8H), 1.85–2.25 (m, 24H), 2.60–2.90 (m, 4H), 3.15–4.35 (m, 18H), 5.20–5.52 (m, 8H), 7.30–7.60 (m, 5H), 7.90–8.60 (m, 3H). ^{13}C NMR (100 MHz, MeOH-d_4): δ 12.0, 17.2, 17.5, 19.8, 20.5, 32.5, 43.5, 48.5, 49.2, 55.2, 61.3, 62.1, 68.2, 70.8, 114.5, 115.6, 127.0, 138.2, 165.3, 173.5. LRMS (Electrospray, positive ion mode, *m*/*z*) for $C_{60}H_{74}N_6O_{26}$: 1295.4 (MH^+).

Compounds 22a and 22b. To a solution of the carboxylic acid derivative **20a** (1.0 mmol) in DMF (20 mL) was added HATU (1.2 mmol), DIEA (1.2 mmol) and dipeptide **21** (1.0 mmol). The mixture was stirred at room temperature for 18 h. Following the work-up, purified product **22a** was obtained in 20% yield after flash chromatography. Compound **22b** was synthesized using similar reaction conditions. **22a**: ^{1}H NMR (400 MHz, MeOH-d_4): δ 1.30–2.20 (m, 39H), 2.45–3.10 (m, 4H), 3.30–3.45 (m, 2H), 3.80–4.45 (m, 15H), 4.65–4.75 (m, 2H), 5.00–5.50 (m, 8H), 7.15–7.35 (m, 5H), 7.80–8.32 (m, 3H). ^{13}C NMR (100 MHz, MeOH-d_4): δ 16.2, 16.4, 19.6, 19.7, 21.4, 23.2, 29.1, 31.0, 33.6, 42.7, 42.9, 43.3, 50.4, 54.8, 60.6, 61.5, 68.1, 68.3, 68.8, 70.3, 74.1, 74.7, 114.6, 114.9, 127.0, 129.1, 136.3, 138.8, 139.1, 168.9, 174.8. LRMS (Electrospray, positive ion mode, *m*/*z*) for $C_{66}H_{88}N_{10}O_{28}$: 1469.3 (MH^+). **22b**: ^{1}H NMR (400 MHz, MeOH–d_4): δ 1.35–2.27 (m, 41H), 2.45–3.00 (m, 4H), 3.20–3.55 (m, 2H), 3.65–4.48 (m, 21H), 5.10–5.55 (m, 8H), 7.15–7.35 (m, 5H), 7.85–8.25 (m, 3H). ^{13}C NMR (100 MHz, MeOH-d_4): δ 19.6, 19.7, 19.8, 21.4, 23.2, 25.0, 29.2, 29.5, 31.0, 32.5, 39.6, 42.7, 42.9, 54.8, 61.2, 61.5, 68.3, 69.2, 72.4, 74.0, 113.6, 114.9, 127.1, 128.4, 136.2, 139.1, 168.4, 174.4. LRMS (Electrospray, positive ion mode, *m*/*z*) for $C_{70}H_{92}N_{10}O_{28}$: 1521.7 (MH^+). **2**: ^{1}H NMR (400 MHz, D_2O): δ 1.45 (br d, $J = 7.2$ Hz, 8H), 1.59–1.67 (m, 2H), 1.72–1.85 (m, 2H), 1.95 (s, 3H), 2.62–2.68 (dd, $J = 14.8$ Hz, 2H), 2.74–2.80 (dd, $J = 14.8$ Hz, 2H), 3.38 (t, $J = 6.8$ Hz, 2H), 3.62–3.79 (m, 6H), 3.88–3.92 (m, 4H), 3.98 (br s, 2H), 4.01–4.04 (m, 2H), 4.05–4.11 (m, 4H), 4.25 (t, $J = 7.1$ Hz, 1H), 4.26–4.39 (m, 4H), 4.47–4.53 (m, 2H), 7.23 (d, $J = 7.4$ Hz, 2H), 7.31 (t, $J = 7.3$ Hz, 1H), 7.37 (t, $J = 7.5$ Hz, 2H), 7.65 (d, $J = 1.7$ Hz, 2H), 7.83–7.89 (m, 1H). ^{13}C NMR (100 MHz, D_2O): δ 17.2, 22.0, 23.1, 28.7, 32.0, 33.0, 40.5, 43.6, 43.9, 51.3, 55.1, 62.1, 73.7, 118.0, 120, 127.9, 128.3, 129.6, 136.0, 141.0, 170.7, 176.8. LRMS (Electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{50}H_{72}N_{10}O_{20}$: 1132.3 (M^+), 1155.5 (MNa^+). **3**: ^{1}H NMR (400 MHz, D_2O): δ 1.25–1.45 (m, 2H), 1.50–1.60 (m, 2H), 1.62–1.80 (m, 2H), 1.84 (s, 3H), 1.85–2.18 (m, 6H), 2.20–2.35 (m, 2H), 2.40–3.05 (m, 4H), 3.25–3.55 (m, 31H), 7.10–7.90 (m, 8H). ^{13}C NMR (100 MHz, D_2O): δ 21.9, 22.6, 24.8, 28.2, 30.0, 30.7, 31.4, 40.0, 43.1, 43.4, 48.7, 61.4, 68.2, 69.1, 70.2, 72.9, 73.6, 117.0, 117.2, 127.3, 127.8, 129.1, 135.9, 138.0, 170.2, 175.7. LRMS (Electrospray, positive ion mode, *m*/*z*) for $C_{54}H_{76}N_{10}O_{20}$: 1185.6 (M^+), 1207.5 (MNa^+).

Synthesis of neoglycopeptides 4–7 (Scheme 2). Neoglycopeptide benzyl ester 29. Dipeptide **27** (10.0 mmol), DCC (10.0 mmol), HOBt (10.0 mmol) and DIEA (10.0 mmol) were added to a solution of α-galactosyl carboxyl acid (**18**, 10.0 mmol), in CH_2Cl_2 (100 mL). The solution was stirred at the room temperature for 20 h. The solvent was evaporated in vacuo, and the residue purified over silica gel by flash column chromatography to obtain compound **29** in 87% yield. ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.40–1.90 (m, 6H), 2.04–2.10 (2s, 12H), 2.37–2.60 (ddd, $J = 4.1$, 9.8, 15.3 Hz, 2H), 3.25 (m, 2H), 4.00–4.29 (m, 6H), 4.40 (d, $J = 6.2$ Hz, 2H), 4.70 (m, 1H), 5.00–5.60 (m, 6H), 5.64 (d, $J = 7.4$ Hz, 1H), 6.63 (bs, 1H), 6.68 (bs, 1H), 7.14–7.32 (m, Ar-H, 13H); ^{13}C NMR

($CDCl_3$): δ 172.3, 170.7, 169.9, 169.8, 169.6, 156.3, 143.7, 141.2, 135.0, 129.0, 128.5, 127.7, 127.0, 126.7, 125.2, 125.0, 119.9, 69.2, 69.0, 68.7, 67.8, 67.0, 61.2, 54.3, 47.0, 41.2, 38.8, 31.2, 31.1, 28.6, 22.2, 20.6. LRMS (FAB, TG/Gly 1:1 + 1% HCl, positive ion mode, *m*/*z*) for $C_{46}H_{53}N_3O_{15}$: 910.2 (MNa^+).

Neoglycopeptide building block 30. A solution of benzyl ester **29** in 95% EtOH was hydrogenated at atmospheric pressure in the presence of 10% Pd/C (10 mol%) for 45 min. The catalyst was removed by filtration through a pad of Celite, providing compound **30**, after evaporation of the solvent, in 97% yield. ^1H NMR (400 MHz, D_2O): δ 1.30–1.47 (m, 2H), 1.47–1.57 (m, 2H), 1.65–1.88 (m, 2H), 2.04 (s, 3H), 2.53–2.71 (m, 2H), 3.19 (t, $J = 6.5$ Hz, 2H), 3.64–3.86 (m, 4H), 3.89 (d, $J = 9.2$ Hz, 2H), 3.94–4.06 (m, 2H), 4.19–4.25 (m, 1H), 4.43–4.51 (m, 1H). ^{13}C NMR (100 MHz, D_2O): δ 21.9, 22.7, 28.3, 30.7, 32.7, 39.5, 42.4, 54.6, 61.2, 68.0, 69.1, 70.0, 72.8, 73.3, 173.9, 174.5, 175.1, 175.5. LRMS (Electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{18}H_{32}N_4O_9$: 449.3 (MH^+), 471.3 (MNa^+).

Neoglycopeptide 4. ^1H NMR (400 MHz, D_2O): δ 1.00–1.90 (m, 12H), 1.41 (d, $J = 7$ Hz, 3H), 2.26 (s, 3H), 2.54–2.82 (m, 4H), 3.20 (t, $J = 7$ Hz, 4H), 3.58–3.72 (m, 4H), 3.72–3.78 (m, 2H), 3.78–3.88 (m, 2H), 3.88–3.92 (m, 2H), 3.92–4.06 (m, 8H), 4.22–4.38 (m, 3H), 4.46–4.54 (m, 2H). ^{13}C NMR (100 MHz, D_2O): δ 16.8, 22.0, 22.7, 28.2, 30.7, 32.7, 39.5, 42.5, 42.8, 50.2, 54.4, 61.2, 67.9, 69.1, 70.0, 72.8, 73.3, 171.7, 172.1, 173.8, 174.5, 174.9, 175.1, 175.6, 176.0. LRMS (Electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{39}H_{67}N_9O_{19}$: 966.4 (MH^+), 988.5 (MNa^+), 483.9 ($M^+/2$).

Neoglycopeptide 5. ^1H NMR (400 MHz, D_2O): δ 0.80–1.90 (m, 12H), 1.39 (d, $J = 7.3$ Hz, 6H), 2.03 (s, 3H), 2.52–2.72 (m, 4H), 3.19 (t, $J = 6.8$ Hz, 4H), 3.62–3.72 (m, 4H), 3.72–3.78 (m, 2H), 3.78–3.88 (m, 2H), 3.88–3.92 (m, 2H), 3.92–4.04 (m, 10H), 4.22–4.38 (m, 4H), 4.44–4.52 (m, 2H). ^{13}C NMR (100 MHz, D_2O): δ 16.7, 22.0, 22.7, 28.2, 30.9, 32.7, 39.5, 42.6, 42.9, 50.3, 54.4, 61.2, 67.9, 69.1, 70.0, 72.8, 73.3, 171.7, 171.8, 172.1, 173.8, 174.5, 174.9, 175.1, 175.6, 176.0. LRMS (Electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{44}H_{75}N_{11}O_{21}$: 1094.4 (MH^+), 1116.5 (MNa^+).

Neoglycopeptide 6. ^1H NMR (400 MHz, D_2O): δ 0.90–2.44 (m, 16H), 2.03 (s, 3H), 2.52–2.82 (m, 4H), 3.12–3.22 (m, 4H), 3.54–3.70 (m, 8H), 3.70–3.78 (m, 2H), 3.78–3.86 (m, 4H), 3.86–4.06 (m, 6H), 4.06–4.16 (m, 1H), 4.22–4.32 (m, 2H), 4.41–4.52 (m, 2H); ^{13}C NMR (100 MHz, D_2O): δ 22.1, 22.7, 24.8, 28.2, 29.7, 30.6, 31.2, 32.7, 39.5, 42.1, 42.5, 42.8, 47.5, 54.0, 54.2, 61.2, 67.9, 69.1, 70.0, 72.8, 73.4, 169.7, 172.0, 173.8, 174.5, 174.7, 175.0, 175.5. LRMS (Electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{41}H_{69}N_9O_{19}$: 992.3 (MH^+), 1014.4 (MNa^+).

Neoglycopeptide 7. ^1H NMR (400 MHz, D_2O): δ 1.24–1.60 (m, 8H), 1.60–2.44 (m, 12H), 2.03 (s, 3H), 2.50–2.82 (m, 4H), 3.12–3.22 (m, 4H), 3.48–3.76 (m, 12H), 3.76–3.90 (m, 4H), 3.90–4.16 (m, 8H), 4.22–4.32 (m, 2H), 4.40–4.50 (m, 4H). ^{13}C NMR (100 MHz, D_2O): δ 22.1, 22.7, 24.7, 28.2, 29.9, 30.8, 32.7, 39.5, 42.1, 42.2, 42.5, 42.8, 47.4, 54.2, 61.0, 61.2, 67.9, 69.1, 70.0, 72.8, 73.3, 169.6, 169.7, 172.0, 173.8, 174.5, 174.8, 175.0, 175.1, 175.5. LRMS (Electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{48}H_{79}N_{11}O_{21}$: 1146.5 (MH^+), 1168.4 (MNa^+), 574.0 ($M^+/2$).

Synthesis of compound 33 from dipeptide 27 (Scheme 3). Neoglycopeptide benzyl ester 32. α-Galactosyl aldehyde (**28**, 2.2 mmol) was added to a solution of dipeptide **27** (1.0 mmol) in THF (20 mL). The solution was acidified with AcOH to pH 5.0. After stirring at 0°C for 20 min, $NaB(OAc)_3H$ (2.5 mmol) was added to the solution and stirred at room temp for 12 h. Purified compound **32** was obtained in 65% yield. LRMS (Electrospray) for $C_{62}H_{78}N_3O_{23}$: 1232.4 (MH^+). ^1H NMR (600 MHz, $CDCl_3$): δ 1.40–2.00 (m, 12H), 2.00–2.10 (4s, 24H), 2.40–2.53 (m, 6H), 4.00–4.43 (m, 12H), 5.06–5.10 (m, 1H), 5.17 (s, 2H), 5.20–5.28 (m, 4H), 5.40–5.49 (m, 4H), 7.26–7.78 (m, 13H); ^{13}C NMR ($CDCl_3$): δ 172.1, 170.9, 170.3, 169.7, 169.6, 169.1, 156.2, 143.8, 143.6, 141.1, 135.0, 128.4, 128.3, 128.1, 127.5, 126.9, 125.0, 121.9, 119.0, 68.0, 67.9, 67.1, 66.9, 61.1, 60.7, 54.1, 49.7, 49.1, 46.9, 33.5, 32.2, 28.4, 25.3, 22.4, 21.8, 21.2, 21.1, 20.4, 20.1.

Synthesis of neoglycopeptides 8a–c to 10a–c from 18, 30 and 33 (Scheme 3). General procedure. To a solution of the carboxylic acid (1.0 mmol) in CH_2Cl_2 was added DCC (1.0 mmol) and HOBt (1.0 mmol). The reaction mixture was then left to stir at room temperature for 15 min, at which time the amine (2.0 mmol) component was then added and the reaction mixture stirred a further 4–6 h. In the case of $NHEt_2$, 1.0 equiv of DIEA was added to the reaction mixture. The solvent was evaporated in vacuo and the product purified by flash chromatography over silica gel. The amine derivative was treated with 20% piperidine in DMF (30 mL) for 1 h, at which point TLC indicated complete removal of the Fmoc protecting group. Acetic anhydride and a catalytic amount of DMAP were then added to the reaction mixture and stirred for a further 4–5 h. After removal of DMF, the reaction mixture was purified by flash chromatography over silica gel. The fully acetylated product was dissolved in MeOH, 0.5 M NaOMe solution was added until the pH of the solution was 10–11, and stirred at room temperature for 3–4 h. The solution was neutralized with solid CO_2, generating neoglycopeptides **8a–c** to **10a–c** in 60–75% yields after purification by reverse phase HPLC. **8a**: ^1H NMR (400 MHz, D_2O): δ 0.98 (t, $J = 7.2$ Hz, 3H), 1.09 (t, $J = 7.2$ Hz, 3H), 2.62 (a of ABX, $J = 9.3$ Hz, $J = -15.9$ Hz, 1H), 2.71 (b of ABX, $J = 4.4$ Hz, $J = -15.9$ Hz, 1H), 3.26 (q, $J = 7.2$ Hz, 2H), 3.34 (q, $J = 7.2$ Hz, 2H), 3.56 (A of ABX, $J = 7.6$ Hz, $J = -11.6$ Hz, 1H), 3.65 (B of ABX, $J = 4.4$ Hz, $J = -11.6$ Hz, 1H), 3.69 (dd, $J = 3.2$ Hz, 1H), 3.72–3.74 (m, 1H), 3.85–3.90 (m, 1H), 3.90 (dd, $J = 9.6$ Hz, 1H), 4.46 (dt, $J = 5.6$ Hz, 1H); ^{13}C NMR (100 MHz, D_2O): δ 12.4, 13.7, 29.6, 41.4, 43.4, 61.0, 68.3, 68.9, 70.1, 72.7, 73.4, 172.8; LRMS (Electrospray, H_2O, positive ion mode, *m*/*z*) for $C_{12}H_{23}NO_6$: 278.1 (MH^+), 300.1 (MNa^+). **8c**: ^1H NMR (400 MHz, CD_3OD): δ 0.92 (t,

$J = 6.8$ Hz, 3H), 1.31 (br s, 22H), 1.49–1.55 (m, 2H), 2.49 (A of ABX, $J = 3.7$ Hz, $J = -15.2$ Hz, 1H), 2.63 (B of ABX, $J = 10.5$ Hz, $J = -15.2$ Hz, 1H), 3.19 (t, $J = 7.1$ Hz, 2H), 3.64–3.70 (m, 2H), 3.79–3.83 (m, 1H), 3.86–3.92 (m, 2H), 3.97 (t, $J = 3.0$ Hz, 1H), 4.43 (dt, $J = 3.4$ Hz, $J = 3.7$ Hz, $J = 10.4$ Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 13.4, 22.7, 27.0, 29.4, 29.5, 29.7, 29.8, 32.1, 33.5, 39.5, 60.6, 68.6, 69.2, 71.1, 71.4, 73.9, 173.1; LRMS (FAB, TG/Gly 1:1 + 1% HCl, positive ion mode, m/z) for $C_{22}H_{43}NO_6$: 418.2 (MH^+). **9c**: ^{1}H NMR (400 MHz, CD_3OD): δ 0.92 (t, $J = 6.7$ Hz, 3H), 1.25–1.64 (m, 29H), 1.64–1.76 (m, 1H), 1.76–1.88 (m, 1H), 2.03 (s, 3H), 2.50 (A of ABX, $J = 3.5$, 15.1 Hz, 1H), 2.64 (B of ABX, $J = 10.7$, 15.1 Hz, 1H), 3.16–3.29 (m, 4H), 3.63–3.98 (m, 7H), 4.12–4.19 (m, 1H), 4.40 (dt, $J = 3.5$, 10.7 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 13.4, 21.5, 22.7, 23.1, 26.9, 29.0, 29.3, 29.5, 29.7, 29.8, 30.9, 32.1, 33.4, 39.0, 39.5, 42.5, 54.8, 60.8, 68.7, 69.1, 71.1, 71.4, 73.9, 170.4, 173.0, 173.3, 174.2; LRMS (Electrospray, H_2O, positive ion mode, m/z) for $C_{32}H_{60}N_4O_9$: 645.5 (MH^+), 323.4 ($MH^+/2$). **10c**: ^{1}H NMR (400 MHz, D_2O): δ 0.76 (t, $J = 6.6$ Hz, 3H), 1.17 (bs, 17H), 1.38–1.45 (m, 4H), 1.61–1.81 (m, 4H), 1.92–2.22 (m, 4H), 1.95 (s, 3H), 3.03–3.18 (m, 4H), 3.50–3.72 (m, 11H), 3.75 (s, 2H), 3.84–3.94 (m, 4H), 4.00–4.08 (m, 2H), 4.10–4.15 (m, 1H); ^{13}C NMR (100 MHz, D_2O): δ 13.8, 19.7, 22.0, 22.4, 22.5, 23.3, 26.3, 28.6, 28.7, 28.9, 29.0, 29.1, 29.2, 30.5, 31.6, 39.7, 42.9, 51.1, 53.4, 54.4, 61.4, 68.1, 69.1, 70.0, 73.1, 73.2, 171.2, 175.0; LRMS (Electrospray, H_2O, positive ion mode, m/z) for $C_{37}H_{70}N_4O_{13}$: 779.6 (MH^+), 390.5 ($MH^+/2$).

Synthesis of 11a–c and 12a,c (Scheme 4). Compound 36. α-Galactosyl aldehyde (**28**, 0.9 mmol) was added to a solution of glycine benzyl ester **35** (1.0 mmol) in THF (20 mL). The solution was acidified with AcOH to pH 5.0. After stirring at 0°C for 20 min, $NaB(OAc)_3H$ (1.2 mmol) was added to the solution and stirred at room temp for 12 h. Purified compound **36** was obtained in 50% isolated yield.

Compound 37. To a solution of α-galactosyl acid **18** (5.0 mmol) in CH_2Cl_2 (40 mL) was added DCC (5.0 mmol) and HOBt (5.0 mmol). A solution of the glycine benzyl ester derivative **36** (5.0 mmol) and DIEA (5.0 mmol) in CH_2Cl_2 (10 mL) was then added and the reaction mixture stirred at room temp for 16 h. DCU produced was then filtered off and the solution evaporated to dryness. Further purification was effected by flash chromatography, giving compound **37** in 80% yield. As in previous cases, compound **37** was subjected to hydrogenation to obtain the carboxylic derivative **38**.

Compounds 12a and 12c from 38. The synthesis of **12a** and **12c** was accomplished from compound **38** in a series of steps as discussed earlier.

Compound 12c. To a solution of acid **38** (0.062 mmol) in CH_2Cl_2 (2 mL) was added DCC (0.093 mmol) and HOBT (0.093 mmol). After stirring for 0.5 h, 1-tetradecylamine (0.093 mmol) was added and the reaction stirred overnight. The reaction mixture was filtered, concentrated and purified by flash chromatography (CH_2Cl_2 to 5% MeOH in CH_2Cl_2) to give compound **39** in 85% yield. This was subjected to deacetylation reaction conditions followed by purification using reverse phase HPLC to obtain pure compound **12c** in 86% yield. **12c**: ^{1}H NMR (400 MHz, D_2O): δ 0.91 (t, $J = 6.8$ Hz, 3H), 1.29 (bs, 22H), 1.42–1.67 (m, 2H), 1.69–2.06 (m, 2H), 2.53–2.98 (m, 2H), 3.16–3.35 (m, 2H), 3.36–3.55 (m, 2H), 3.56–4.27 (m, 14H); LRMS (Electrospray, H_2O, positive ion mode, m/z) for $C_{32}H_{60}N_3O_{12}$: 665.3 (MH^+).

Compound 40. To a solution of compound **38** (1.0 mmol) in CH_2Cl_2 (10 mL) was added DCC (1.0 mmol) and HOBt (1.0 mmol) and stirred for 15 min. A solution of the dipeptide (1.0 mmol) and DIEA (2.0 mmol) in CH_2Cl_2 (2 mL) was then added. Neoglycopeptide derivative **40** was obtained in 83% yield after purification. LRMS (Electrospray) for $C_{64}H_{79}N_4O_{25}$: 1303.4 (MH^+); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.04–1.76 (m, 10H), 1.80–1.99 (m, 4H), 2.0, 2.02, 2.03, 2.06, 2.08, 2.10, 2.12 (7s, 24H), 2.92–3.61 (m, 7H), 3.14–3.72 (m, 14H), 5.13–5.17 (m, 3H), 5.22–5.28 (m, 1H), 5.30–5.35 (m, 1H), 5.39–5.41 (m, 2H), 7.24–7.76 (m, 13H); ^{13}C NMR ($CDCl_3$): δ 172.4, 170.4, 169.9, 169.6, 169.5, 168.9, 156.1, 143.6, 141.1, 135.1, 128.4, 128.1, 127.5, 126.9, 126.2, 125.0, 119.8, 70.7, 69.1, 68.6, 68.3, 68.2, 67.7, 66.3, 66.2, 62.3, 61.3, 60.4, 54.2, 51.2, 48.9, 46.9, 45.2, 41.1, 33.6, 32.0, 31.7, 28.3, 24.7, 23.5, 22.1, 20.5. Synthesis of **11a–c** was achieved from compound **40** in a number of steps. **11a**: ^{1}H NMR (400 MHz, D_2O): δ 0.97 (t, $J = 7.1$ Hz, 3H), 1.08 (t, $J = 7.1$ Hz, 3H), 1.24–1.30 (m, 2H), 1.37–1.43 (m, 2H), 1.58–1.76 (m, 2H), 1.83–1.93 (m, 2H), 1.93 (s, 3H), 2.58–2.80 (m, 2H), 3.07–3.14 (m, 2H), 3.24 (q, $J = 7.1$ Hz, 2H), 3.26 (q, $J = 7.1$ Hz, 2H), 3.42–3.52 (m, 2H), 3.54–3.74 (m, 9H), 3.83–3.98 (m, 4H), 3.94 (s, 2H), 3.98 (s, 2H), 4.18 (dd, $J = 5.2$ and 8.8 Hz, 1H), 4.47 (t, $J = 6.1$ Hz, 1H); ^{13}C NMR (100 MHz, D_2O): δ 12.4, 13.2, 22.0, 22.7, 23.2, 28.3, 30.5, 31.0, 39.4, 41.1, 41.4, 42.2, 47.1, 50.2, 54.1, 61.0, 61.7, 68.1, 68.8, 69.5, 70.0, 70.2, 72.4, 72.6, 73.2, 73.4, 73.8, 169.3, 171.1, 174.3, 174.8, 174.9; LRMS (Electrospray, H_2O, positive ion mode, m/z) for $C_{32}H_{57}N_5O_{15}$: 752.3 (MH^+), 774.2 (MNa^+), 376.8 ($MH^+/2$). **11b**: ^{1}H NMR (400 MHz, D_2O): δ 0.74 (t, $J = 7.1$ Hz, 3H), 1.15 (br s, 16H), 1.29–1.39 (m, 6H), 1.62–1.80 (m, 2H), 1.87–1.92 (m, 5H), 2.61–2.86 (m, 2H), 3.07–3.16 (m, 4H), 3.42–3.79 (m, 12H), 3.83–4.02 (m, 7H), 4.07 (br s, 1H), 4.45–4.52 (m, 1H); ^{13}C NMR (100 MHz, D_2O): δ 14.2, 22.3, 23.0, 27.3, 28.6, 29.2, 29.8, 30.1, 30.8, 32.3, 39.9, 42.9, 45.4, 47.0, 50.1, 54.4, 61.0, 61.7, 68.2, 68.5, 68.7, 69.5, 70.1, 70.3, 72.6, 73.1, 73.9, 78.9, 170.8, 170.9, 173.9, 174.2, 174.9; LRMS (Electrospray, H_2O, positive ion mode, m/z) for $C_{39}H_{71}N_5O_{15}$: 850.4 (MH^+), 426.0 ($MH^+/2$). **11c**: ^{1}H NMR (400 MHz, D_2O): δ 0.74 (t, $J = 6.5$ Hz, 3H), 1.14–1.19 (m, 24H), 1.27–1.44 (m, 4H), 1.51–1.79 (m, 4H), 1.90 (s, 3H), 2.61–2.88 (m, 2H), 3.03–3.09 (m, 4H), 3.33–3.73 (m, 12H), 3.86–3.96 (m, 7H), 4.06 (br s, 1H), 4.45–4.52 (m, 1H); ^{13}C NMR (100 MHz, D_2O): δ 14.2, 21.8, 22.4, 22.6, 23.0, 27.4, 29.3, 30.0, 30.3, 30.5, 32.4, 39.9, 42.9, 44.9, 47.0, 50.1, 55.0, 61.0, 61.8, 68.2, 68.6, 68.7, 69.5, 70.2, 70.3, 72.6, 73.1, 73.6, 78.9, 170.7, 170.9,

173.8, 174.2, 174.8; LRMS (Electrospray, H_2O, positive ion mode, m/z) for $C_{42}H_{77}N_5O_{15}$: 892.4 (MH^+), 446.9 ($MH^+/2$).

Synthesis of 13a,c from 38 (Scheme 4). Compound 43. Diisopropylethylamine (0.38 mmol) was added to a solution of the benzyl ester (0.19 mmol), acid **38** (0.19 mmol), and HATU (72.3 mg, 0.19 mmol) in dry CH_2Cl_2 (4 mL). After stirring overnight, the reaction mixture was purified by flash chromatography (CH_2Cl_2 to 5% MeOH in CH_2Cl_2) to give compound **43** (97% yield). **43**: ^{1}H NMR (400 MHz, $CDCL_3$): δ 1.23 (s, 1H), 1.61 (s, 3H), 1.85–2.12 (m, 24H), 2.52–2.85 (m, 2H), 3.38-3.61 (m, 2H), 3.86–4.34 (m, 9H), 7.28–8.06 (m, 9H); LRMS (Electrospray, H_2O, positive ion mode, m/z) for $C_{48}H_{58}N_3O_{22}$: 1015.4 (MH^+), 527.3 ($MH^+/2$). **13a**: ^{1}H NMR (400 MHz, D_2O): δ 1.00 (t, $J=7.1$ Hz, 3H), 1.13 (t, $J=7.1$ Hz, 3H), 1.79–2.03 (m, 2H), r_1 2.68 and r_2 2.85 (d's, $J=6.0$ Hz, $J=10.0$ Hz for two rotamers, r_1 and r_2, 2H), 3.21 (q, $J=7.1$ Hz, 2H), 3.42 (q, $J=7.1$ Hz, 2H), 3.47–3.82 (m, 10H), 3.83–4.08 (m, 5H), r_1 4.17 and r_2 4.37 (bs's, 2H), 4.46–4.57 (m, 1H), 7.28–7.36 (m, 2H), 7.42–7.51 (m, 2H); ^{13}C NMR (100 MHz, D_2O): δ 12.3, 13.5, 22.2, 23.3, 29.9, 40.5, 44.8, 45.7, 47.2, 50.8, 52.4, 61.0, 61.5, 61.7, 68.1, 68.2, 68.4, 68.7, 68.8, 69.4, 69.5, 70.1, 70.2, 72.2, 72.4, 72.6, 73.2, 73.3, 73.8, 77.2, 78.9, 121.9, 122.0, 127.4, 127.5, 132.8, 133.0, 138.1, 138.3, 169.7, 170.0, 173.2, 173.3, 174.4, 174.5; LRMS (Electrospray, H_2O, positive ion mode, m/z) for $C_{29}H_{45}N_3O_{13}$: 644.3 (MH^+). **13c**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.84 (t, $J=6.8$ Hz, 3H), 1.24 (bs, 22 H), 1.45–1.55 (m, 2H), 1.66–1.94 (m, 2H), 3.17–3.30 (m, 2H), 3.40–3.86 (m, 13H), 4.02–4.98 (m, 8H), 7.64 (t, $J=9.0$, 2H), 7.80 (t, $J=8.6$, 2H); LRMS (Electrospray, H_2O, positive ion mode, m/z) for $C_{39}H_{65}N_3O_{13}$: 784.4 (MH^+), 392.9 ($MH+/2$).

Purification of verotoxin (VT) and isolation and purification of Gb3.[23] Verotoxin was isolated and purified from the high expression recombinant *E. coli* pJB28 strain. The glycosphingolipid Gb3 was isolated from the neutral glycolipid fraction from human renal tissue as described.

Immobilization of Gb3 onto 96-well plates. A stock solution of 1.2 μg Gb3/mL in ethanol was prepared and 50 μL/well was aliquoted into 96-well assay plates. The plates were then left on the bench at room temperature all day or overnight to allow the MeOH to evaporate. Once dry, plates were stored desiccated for up to a week.

Preparation of inhibitors. Soluble inhibitors of VT binding were prepared in phosphate buffered saline (PBS) and then mixed 1:1 with a 2× solution of VT (8 ng per 50 μL in 0.4% BSA–PBS) and incubated for 120 min at room temperature. The VT–inhibitor mixture was added to the wells in triplicate at 50 μL per well. 11 Serial dilutions of the inhibitors were made to give a final concentration range of 30 nM to 3 mM.

Incubation. 96-Well plates were pre-incubated with 150 μL 0.2% BSA in PBS per well for 60 min. Before adding the inhibitor and verotoxin mixture, wells were washed twice with 150 μL/well of 0.2% BSA–PBS. 50 μL/well of the pre-incubated inhibitor–VT dilutions were added in triplicates, plates covered with parafilm and left on the bench for 120 min.

Detection and analysis of VT bound. After incubation with the inhibitor–VT mixture the solution was shaken out of the wells into the sink and the wells washed three times with 150 μL of 0.2% BSA–PBS. Following the last wash step, 50 μL/well of a 1 μg/ml solution of the monoclonal antibody PH1 in 0.2% BSA–PBS was added. Plates were covered with parafilm and left on the bench for 60 min. Again, after the incubation, wells were washed three times with 150 μL of 0.2% BSA–PBS. After the last wash, 50 μL of a 1:2000 dilution of GAM–HRP in 0.2% BSA–PBS was added to each well. Plates were covered with parafilm and left on the bench for 60 min. After this incubation, wells were washed three times with 150 μL of 0.2% BSA–PBS leaving the third wash in the wells for 3 min before a final quick wash with PBS only. 100 μL/Well of ABTS solution (2,2′-azino-bis[3-ethylbenzthiazoline-6-sulfonic acid]; 0.5 mg/mL containing 3 μL of 30% H_2O_2 per 10 mL) was added and the plates covered with tin-foil. For color development, plates were agitated gently on a shaking table. After sufficient color had developed, plates were read using an ELISA plate reader at 410 nm wavelength at intervals until the desired absorption value was reached (O.D. <2.0). The percentage of specific bound VT was calculated using the value for total bound VT (i.e.: without any inhibitors present) after subtracting background activity.

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