*Glycoconjugate Journal* (1998) **15:** 223–231

# Convergent synthesis of neoglycopeptides by coupling of 2-bromoethyl glycosides to cysteine and homocysteine residues in T cell stimulating peptides

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**The 2-bromoethyl β-glycosides of the disaccharide galabiose [Gal(α1-4)Gal] and the trisaccharides globotriose [Gal(α1-4)Gal(β1-4)Glc] and 3′-sialyllactose [Neu5Ac(α2-3)Gal(β1-4)Glc] have been prepared by improved routes. The 2-bromoethyl glycosides were then used in cesium carbonate promoted alkylations of the sulfhydryl groups of cysteine and homocysteine residues in T cell stimulating peptides. This convergent and general approach was used to prepare 16 neoglycopeptides which were obtained in 52–95% yields after purification by HPLC. $^1$H NMR spectroscopy revealed that β-elimination and epimerization of neoglycopeptide stereocentres did not occur during the synthesis.**

***Keywords*: glycopeptide, synthesis, T cell, immune response**

## Introduction

T cells recognize protein antigens as short processed peptides displayed by MHC molecules on the surface of antigen presenting cells [1–3]. Most proteins are actually glycosylated and the ability of neoglycopeptides and glycopeptide fragments from glycoproteins to bind MHC molecules and elicit specific T cell responses is therefore under investigation in several research groups (reviewed in reference [4]). Within such a research programme we needed a set of specifically glycosylated peptides which were bound by class I MHC D$^b$ and K$^b$ molecules [5–7]. The D$^b$-binding peptides (**16–22**, Table 1) were derived from immunodominant epitopes of influenza A virus (PR8) nucleoprotein (ASNENMETM), adenovirus Ad5EI protein (SGPSNTPPEI) and lymphocytic chloromeningitis virus glycoprotein (SGVENPGGYCLT). K$^b$-binding peptides (**23–27**, Table 1) were in turn derived from vesicular stomatitis virus nucleocapsid protein (RGYVYQGL) and sendai virus nucleoprotein (FAPGNYPAL).

Development of methodology for synthesis of glycopeptides has attracted considerable attention recently and several reviews cover the progress made in this field [4, 8–14]. In general, the use of glycosylated amino acids as building blocks has proved to be a versatile approach to both N- and O-linked glycopeptides. This strategy preferably relies on protection of the α-amino group of the building blocks with a fluoren-9-ylmethoxycarbonyl (Fmoc) group [15], and can be used for synthesis both in solution and on solid phase. A convergent approach whereby a glycosylamine is coupled to an aspartic or glutamic acid residues in a peptide has also been employed for synthesis of *N*-linked glycopeptides [16–18].

In analogy with the convergent approach to N-linked glycopeptides we wanted to use a strategy which allowed different carbohydrates to be linked to specific positions in preformed, unprotected peptides. For that purpose the above T cell peptide epitopes were either extended at the amino terminus by a cysteine residue, or an internal amino acid was replaced by homocysteine. One of the peptides (SGVENPGGYCLT) had a naturally occurring internal cysteine, which was used for conjugation. The nucleophilic properties of the sulfhydryl group were then utilized to create an *S*-linked neoglycopeptide by substitution of the bromine atom in 2-bromoethyl glycosides of galabiose [Gal(α1-4)Gal], globotriose [Gal(α1-4)Gal(β1-4)Glc], and 3′-sialyllactose [Neu5Ac(α2-3)Gal(β1-4)Glc]. The trisaccharides globotriose and 3′-sialyllactose are expressed as

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**Table 1.** Preparation of glycopeptides by coupling of 2-bromoethyl glycosides (**6**, **8** and **15**) with cysteine and homocysteine (**h**) residues in MHC restricted peptides.[a]

| *Peptide*[b] | *2-Bromoethyl glycoside* | *Glycopeptide* | *Yield*[c] | *FAB-MS*[d] *(M+H)* |
|---|---|---|---|---|
| ASNEN**h**ETM (**16**) | **6** (Gal$\alpha$4Gal$\beta$) | **28** | 54 | 1380 |
| | **8** (Gal$\alpha$4Gal$\beta$4Glc$\beta$) | **29** | 74 | 1542 |
| ASN**h**NMETM (**17**) | **6** (Gal$\alpha$4Gal$\beta$) | **30** | 59 | 1382 |
| **C**ASNENMETM (**18**) | **6** (Gal$\alpha$4Gal$\beta$) | **31** | 75 | 1497 |
| | **15** (NeuAc$\alpha$3Gal$\beta$4Glc$\beta$) | **32** | 59 | 1788 |
| SGPSN**h**PPEI **(19)** | **6** (Gal$\alpha$4Gal$\beta$) | **33** | 63 | 1382 |
| SGP**h**NTPPEI (**20**) | **6** (Gal$\alpha$4Gal$\beta$) | **34** | 56 | 1396 |
| **C**SGPSNTPPEI (**21**) | **6** (Gal$\alpha$4Gal$\beta$) | **35** | 70 | 1469 |
| | **15** (NeuAc$\alpha$3Gal$\beta$4Glc) | **36** | 60 | 1761 |
| SGVENPGGY**C**LT (**22**) | **6** (Gal$\alpha$4Gal$\beta$) | **37** | 90 | 1564 |
| | **8** (Gal$\alpha$4Gal$\beta$4Glc$\beta$) | **38** | 88 | 1726 |
| RGYVY**h**GL (**23**) | **6** (Gal$\alpha$4Gal$\beta$) | **39** | 52 | 1312 |
| RGY**h**YQGL (**24**) | **6** (Gal$\alpha$4Gal$\beta$) | **40** | 52 | 1342 |
| **C**RGYVYQGL (**25**) | **6** (Gal$\alpha$4Gal$\beta$) | **41** | 95 | 1426 |
| FAPG**h**YPAL (**26**) | **6** (Gal$\alpha$4Gal$\beta$) | **42** | 86 | 1320 |
| **C**APGNYPAL (**27**) | **6** (Gal$\alpha$4Gal$\beta$) | **43** | 73 | 1273 |

[a] The 2-bromoethyl glycoside and the peptide were coupled to each other in DMF, or DMSO, using $Cs_2CO_3$ as base according to the general procedure given in the experimental section.

[b] Residues used for coupling of the 2-bromoethyl glycoside are in boldface.

[c] Yields were determined after purification by reversed-phase HPLC.

[d] Observed values were in agreement with the calculated values.

glycolipids on Burkitt lymphoma cells [19] and mouse melanoma cells [20], respectively. The use of homocysteine instead of cysteine resulted in a somewhat longer thioether linker arm between the peptide backbone and the carbohydrate part of the resulting neoglycopeptide.

## Materials and methods

### General methods

TLC was performed on Silica Gel 60 $F_{254}$ (Merck, Germany) with detection by UV light, spraying with 10% aqueous $H_2SO_4$ followed by charring at an elevated temperature, or spraying with a solution of molybdatophosphoric acid and $CeSO_4$ in dilute aqueous $H_2SO_4$ followed by heating [31]. Column chromatography was performed on silica gel (Matrex, 60 Å, 35–70 μm, Grace Amicon, USA) with distilled solvents. $CH_2Cl_2$ and $CH_3CN$ were dried using 4 Å and 3 Å molecular sieves, respectively. Organic solutions were dried over $Na_2SO_4$ before being concentrated.

The $^1H$ and $^{13}C$ NMR spectra of the 2-bromoethylglycosides **6**, **8**, and **15**, and intermediates in the syntheses of these, were recorded for solutions in $CDCl_3$ [residual $CHCl_3$ ($\delta_H$ 7.27 ppm) or $CDCl_3$ ($\delta_C$ 77.0 ppm) as internal standard], $C_6D_6$ [residual $C_6D_5H$ ($\delta_H$ 7.16 ppm) or $C_6D_6$ ($\delta_C$ 128.4 ppm) as internal standard], $CD_3OD$ [residual $CD_2HOD$ ($\delta_H$ 3.33 ppm) or $CD_3OD$ ($\delta_C$ 49.15 ppm) as internal standard] or $D_2O$ [$CH_3COCH_3$ ($\delta_H$ 2.24 and $\delta_C$ 33.19 ppm) as internal standard] at 300 K. For these compounds first-order chemical shifts and coupling constants were obtained from one-dimensional spectra. Resonances for aromatic protons are not reported unless crucial for identification. The $^1H$ NMR spectra of glycopeptides **37**, **39** and **41** were recorded at 500 MHz in a 9 : 1 mixture of $H_2O$ and $D_2O$ [$H_2O$ ($\delta_H$ 4.98 ppm) as internal standard] at 278 K. Chemical shifts and proton resonance assignments for **37**, **39** and **41** were obtained from COSY [32], TOCSY [33], and ROESY [34] experiments. Ions for positive fast atom bombardment mass spectra were produced by a beam of Xenon atoms (6 keV) from a matrix of glycerol and thioglycerol.

Analytical reversed phase HPLC was performed on a Beckman Ultrasphere ODS C-18 column (80 Å, 5 μm, 4.6 × 150 or 250 mm) using a linear gradient of 0 → 80% of *B* in *A* over 60 min, with a flowrate of 1.0 ml $\min^{-1}$ and detection at 214 nm (solvent systems *A* : 0.1% aqueous trifluoroacetic acid and *B* : 0.1% trifluoroacetic acid in $CH_3CN$). Preparative purifications were performed on a Beckman Ultraprep C-18 column (80 Å, 5 μm, 10 × 250 mm) with the same eluant and a flowrate of 5 ml $\min^{-1}$.

Phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-*β*-D-galactopyranoside [35] (**1**), methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside [36] (**2**), 2-bromoethyl 2,3,6-tri-*O*-acetyl-4-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-*β*-D-galactopyranosyl]-*β*-D-glucopyranoside [25] (**7**), 2-bromoethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-*β*-D-glucopyranoside [21] (**9**), and *O*-ethyl

*S*-[methyl(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate] dithiocarbonate [26] (**13**) were prepared as described previously in the cited literature references.

## Methyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-α-D-galactopyranoside (**3**)

Phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside [35] (**1**, 15.6, 24.7 mmol) and methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside [36] (**2**, 10.0 g, 19.7 mmol) were dissolved in a mixture of $CH_2Cl_2$ (70 ml) and diethyl ether (140 ml) containing powdered activated molecular sieves (4 Å, 15 g) under nitrogen. The mixture was cooled to −45 °C. A mixture of *N*-iodosuccinimide (5.55 g, 24.7 mmol) and trifluoromethanesulphonic acid (110 µl, 1.25 mmol) in $CH_2Cl_2$-diethyl ether (1 : 1; 160 ml) was added with stirring for 20 min. TLC (toluene:methyl *tert*-butyl ether; 10 : 1) revealed that the reaction was instantaneous and the mixture was filtered through celite. The filtrate was washed with a mixture of saturated aqueous $Na_2S_2O_3$, saturated aqueous $NaHCO_3$ and water (1 : 1 : 2), and then with water. The organic phase was dried, filtered and concentrated to give a residue that was purified by column chromatography (toluene:methyl *tert*-butyl ether; 40 : 1) to give **3** (18.4 g, 89%); $[\alpha]_D^{22} + 106°$ (*c* 1.0, $CH_2Cl_2$). $^1H$ NMR data (300 MHz, $CDCl_3$): $\delta$ 5.73 (dd, AB-type, J 11 and 3.3 Hz, 1H, H-2), 5.68 (dd, AB-type, J 11 and 3.3 Hz, 1H, H-3), 5.25 (d, J 3.3 Hz, 1H, H-1), 4.95 (d, J 3.5 Hz, 1H, H-1′), 4.20 (dd, J 10.5 and 2.5 Hz, 1H, H-3′), 4.09 (dd, J 10.5 and 3.5 Hz, 1H, H-2′), 3.44 (s, 3H, $CH_3O$). $^{13}C$ NMR data (75 MHz, $CDCl_3$): $\delta$ 166.5, 166.0, 167.0, 138.9–127.0 (aromatic C), 100.8 (C-1 or C-1′), 97.5 (C-1 or C-1′), 79.0, 76.8, 76.1, 75.0, 74.9, 74.2, 72.9, 72.6, 71.0, 69.9, 69.2, 68.6, 67.7, 63.1, 55.4 ($CH_3O$). Anal. Calcd for $C_{62}H_{60}O_{14}$: C, 72.4; H, 5.9. Found: C, 71.7; H, 5.8.

## Phenyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-1-thio-D-galactopyranoside (**4**)

Hydrogenolysis of **3** (9 g, 17.8 mmol) over Pd/C (10%, 3 g) in HOAc (100 ml) for 1 h at 0.22 MPa and room temperature, followed by filtration and concentration gave crude methyl 2,3,6-tri-*O*-benzoyl-4-*O*-α-D-galactopyranosyl-α-D-galactopyranoside. A solution of the crude product in a mixture of pyridine (100 ml) and acetic anhydride (50 ml) was stirred for 16 h at room temperature, then concentrated and co-evaporated several times with toluene to give crude methyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-α-D-galactopyranoside. The crude product was dissolved in acetic anhydride (75 ml) and HOAc (40 ml) and concentrated $H_2SO_4$ (1.15 ml) was added dropwise after cooling to 0 °C. After 2 h ice water was added and the mixture was extracted three times with ethyl acetate, the combined organic phases were washed with water and saturated aqueous $NaHCO_3$, dried, filtered and concentrated to give crude 1-*O*-acetyl-2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-D-galactopyranose which was homogeneous on TLC (toluene:ethyl acetate; 2 : 1). The crude product and thiophenol (2.20 ml, 21.4 mmol) were dissolved in $CH_2Cl_2$ (300 ml) and boron trifluoride etherate (21.5 ml, 171 mmol) was then added at 0 °C over 10 min. The reaction mixture was allowed to attain room temperature and after 18 h it was diluted with $CH_2Cl_2$ (500 ml), washed with cold water, saturated aqueous $NaHCO_3$, brine, and dried. Filtration, concentration and crystallization from methanol gave **4**. Concentration of the mother liquor and column chromatography (toluene : ethyl acetate; 10 : 1) of the residue gave additional **4** (11.1 g in total, 68% overall yield from **3**, $\alpha : \beta = 1 : 9$ at C-1 according to $^1H$ NMR spectroscopy); mp 161–163 °C, $[\alpha]_D^{22} + 101°$ (*c* 1.0, $CHCl_3$). $^1H$ NMR data (300 MHz, $CDCl_3$): $\delta$ 6.21 (d, J 5.5 Hz, 0.1 H, H-1 in 1-α epimer), 5.89 (dd, J 11 and 5.5 Hz, 0.1H, H-2 in 1-α epimer), 5.60 (t, J 10 Hz, 1H, H-2), 5.41 (dd, J 3 and 1 Hz, 1H, H-4′), 5.36 (dd, J 10 and 2.5 Hz), 1H, H-3), 5.31 (dd, J 11 and 3 Hz, 1H, H-3′), 5.20 (dd, J 11 and 3.5 Hz, 1H, H-2′), 5.10 (d, J 3.5 Hz, 1H, H-1′), 4.87 (d, J 9.5 Hz, 1H, H-1), 4.76 (dd, J 8.5 and 7 Hz, 1H), 4.56 (dd, J 11.5 and 5 Hz, 1H), 4.40 (d, J 2.5 Hz, 1H, H-4), 4.19 (dd, J 6.5 and 6 Hz, 1H), 4.09 (bt, J 7 Hz, 1H), 3.93 (dd, J 11 and 8 Hz), 3.75 (dd, J 11 and 6 Hz, 1H), 2.16, 2.09, 2.03, and 1.96 (4 s, each 3H, 4 Ac). $^{13}C$ NMR data (75 MHz, $CDCl_3$): $\delta$ 170.4, 170.3, 170.1, 169.7, 166.1, 166.0, 164.9, 134.1–128.3 (aromatic C), 98.7 (C-1′), 85.5 (C-1), 76.8, 76.5, 74.8, 68.5, 67.7, 67.5, 67.4, 67.2, 63.3, 60.7, 20.8, 20.6, 20.6 and 20.5. Anal. Calcd for $C_{47}H_{46}O_{17}S$: C, 61.7; H, 5.07; S, 3.51. Found: C, 61.1; H, 4.95; S, 3.1.

## 2-Bromoethyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranoside (**5**)

A solution of **4** (5.07 g, 5.54 mmol) and 2-bromoethanol (0.48 ml, 6.65 mmol) in 1,2-dichloroethane (14 ml) containing powdered molecular sieves (4 Å, 4 g) was stirred at room temperature for 15 min and then cooled to 0 °C. A mixture of *N*-iodosuccinimide (1.27 g, 5.54 mmol) and trifluoromethanesulphonic acid (49 µl, 0.55 mmol) in 1,2-dichloroethane-diethyl ether (1 : 1; 50 ml), which had been stirred for 10 min, was then added dropwise and stirring was continued for 30 min, at 0 °C and then for 2 h at room temperature. The mixture was then filtered through celite, washed with 15% aqueous $Na_2S_2O_3$-saturated aqueous $NaHCO_3$ (1 : 1 50 ml), brine (50 ml) and dried. Concentration and crystallization of the residue from methanol gave **5** (4.47 g, 82%); mp 150–151 °C, $[\alpha]_D^{22} + 111°$ (*c* 1.0, $CHCl_3$). $^1H$ NMR data (300 MHz, $C_6D_6$): $\delta$ 6.67 (dd, J 10.6 and 7.9 Hz, 1H, H-2), 5.79 (dd, J 11.0 and 3.3 Hz, 1H, H-3′), 5.58 (dd, J 11.0 and 3.5 Hz, 1H, H-2′), 5.53–5.46 (m, 2H, H-4′ and H-3), 5.23 (d, J 3.5 Hz, 1H, H-1′), 4.37 (d, J 7.7 Hz, 1H, H-1), 4.01 (bd, J 2.2 Hz, 1H, H-4), 3.84–3.75 (m, 1H,

$BrCH_2C\mathbf{H}_2O$), 3.53–3.43 (m, 1H, $BrCH_2C\mathbf{H}_2O$), 2.98–2.93 (m, 2H, $BrCH_2C\mathbf{H}_2O$), 1.96, 1.71, 1.65, and 1.57 (4 s, each 3H, 4 Ac). $^{13}C$ NMR data (75 MHz, $C_6D_6$): δ 168.6, 168.4, 168.3, 167.8, 164.7, 164.7, 163.9, 132.0–126.0 (aromatic C), 100.4 (C-1), 97.8 (C-1′), 75.5, 72.0, 71.7, 68.7, 68.3, 67.7. 67.1, 67.0, 66.4, 62.5, 60.0, 28.4, 19.0, 18.8, 18.8 and 18.5. Anal. Calcd for $C_{43}H_{45}O_{18}Br$: C, 55.5; H, 5.0. Found: C, 55.5; H, 4.9.

### 2-Bromoethyl 4-*O*-α-D-galactopyranosyl-β-D-galactopyranoside (**6**)

Methanolic sodium methoxide (0.2 M, 5 ml) was added to a suspension of **5** (4.47 g, 4.80 mmol) in methanol (100 ml) at room temperature. After 6 h the solution was neutralized with silica, filtered and concentrated. Column chromatography of the residue ($CHCl_3$ : MeOH : water; 65 : 35 : 6) gave **6** (1.93 g, 89%); $[\alpha]_D^{22}$ + 69° (*c* 1.0, MeOH). $^1H$ NMR data (400 MHz, $CD_3OD$): δ 4.97 (d, J 2.8 Hz, 1H, H-1′), 4.35 (d, J 7.2 Hz, 1H, H-1), 4.29 (bt, J 6.2 Hz, 1H, H-5′), 4.12 (dt, J 11.2 and 6.5 Hz, 1H, $BrCH_2C\mathbf{H}_2O$), 4.00 (d, J 2.5 Hz, 1H, H-4), 3.94 (dt, J 11.3 and 6.5 Hz, 1H, $BrCH_2C\mathbf{H}_2O$), 3.84 (dd, J 11.1 and 7.2 Hz, 1H, H-6), 3.77 (dd, J 11.1 and 6.1 Hz, 1H, H-6), 3.73 (dd, J 11.1 and 6.4 Hz, 1H, H-6′), 3.68 (dd, J 11.1 and 5.5 Hz, 1H, H-6′), 3.64 (bt, J 6.6 Hz, 1H, H-5), 3.57 (t, J 6.4 Hz, 1H, $BrCH_2CH_2O$), 3.56 (t, J 6.6 Hz, 1H, $BrCH_2CH_2O$), 3.55 (dd, J 10.0 and 2.2 Hz, 1H, H-3), 3.50 (dd, J 10.1 and 7.2 Hz, 1H, H-2). $^{13}C$ NMR data (75 MHz, $D_2O$): δ 105.9 (C-1), 102.5 (C-1′), 80.0, 78.1, 75.2, 73.8, 73.7, 73.1, 72.1, 72.0, 71.6, 63.5, 63.0, 34.1 ($CH_2Br$). Anal. Calcd for $C_{14}H_{25}BrO_{11}$: C, 37.4; H, 5.6. Found: C, 37.1; H, 5.9.

### 2-Bromoethyl 4-*O*-(4-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**8**)

2-Bromoethyl 2,3,6-tri-*O*-acetyl-4-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside [25] (**7**, 35 mg, 34 μmol) was deacetylated in 0.1 M methanolic sodium methoxide (10 ml) during 16 h. The solution was neutralized with silica gel, filtered, concentrated and the residue was purified by column chromatography ($CHCl_3$ : MeOH : water; 65 : 35 : 6) to give **8** (18.6 mg, 90%); $[\alpha]_D^{22}$ + 51° (*c* 1.0, water). $^1H$ NMR data (400 MHz, $D_2O$): δ 4.96 (d, 1H, J 3.9 Hz, H-1″), 4.57 (d, 1H, J 8.0 Hz, H-1), 4.52 (d, 1H, J 7.8 Hz, H-1′), 4.37 (bt, 1H, J 6.7 Hz, H-5″), 4.23 (dt, 1H, J 11.6 and 5.7 Hz, $BrCH_2C\mathbf{H}_2O$), 4.00 (dd, 1H, J 12.2 and 2.0 Hz, H-6), 3.94 (dd, 1H, J 11.6 and 7.5 Hz, H-6′), 3.92 (dd, 1H, J 10.5 and 3.2 Hz, H-3″), 3.76 (dd, 1H, J 10.3 and 3.1 Hz, H-3′). $^{13}C$ NMR data (75 MHz, $D_2O$): δ 106.2 and 105.1 (C-1, 1′), 103.3 (C-1″), 81.6, 80.3, 78.4, 77.8, 77.3, 75.8, 75.1, 73.9, 73.8, 73.0, 72.1, 71.9, 71.5, 63.5, 63.3, 63.0, 33.9 ($CH_2Br$).

### 2-Bromoethyl 4-*O*-β-D-galactopyranosyl-β-D-glucopyranoside (**10**)

2-Bromoethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside [21] (**9**, 126 g, 0.17 mol) was deacetylated in 0.01 M methanolic sodium methoxide (1.0 l) during 3 h. The solution was neutralized with silica gel, filtered and concentrated. Column chromatography of the residue ($CH_2Cl_2$ : MeOH : water; 65 : 20 : 3) and crystallization from ethanol gave **10** (37 g, 51%); mp 151–152 °C, $[\alpha]_D^{22}$ + 0.6° (*c* 1.0, water). $^1H$-NMR data (300 MHz, $D_2O$): δ 4.57 (d, 1H, J 8.0 Hz, H-1), 4.45 (d, 1H, J 7.7 Hz, H-1′), 4.26–4.19 (m, 1H, $OC\mathbf{H}_2CH_2Br$), 3.98 (dd, 1H, J 12.5 and 1 Hz, H-6/6′), 3.93 (d, 1H, J 3.5 Hz, H-4′), 3.55 (dd, 1H, J 10.0 and 8.0 Hz, H-2′), 3.40–3.30 (m, 1H, H-2). $^{13}C$-NMR data (75 MHz, $D_2O$): δ 105.7 (C-1′), 104.9 (C-1), 81.2, 79.1, 78.1, 77.6, 77.1, 75.5 (C-2′), 75.3, 74.3, 73.7 (C-2), 72.8, 71.3 (C-4′), 63.8 (C-6/6′), 62.9 (C-6/6′), 33.9 ($CH_2Br$).

### 2-Bromoethyl 4-*O*-(3,4-*O*-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (**11**)

A mixture of compound **10** (35 g, 78 mmol) and *p*-toluenesulfonic acid monohydrate (1.5 g, 8.0 mmol) in 2,2-dimethoxypropane (800 ml) was stirred at 50 °C for 1 h and then at room temperature overnight. Triethylamine (12 ml, 80 mmol) was added and the mixture was stirred for 30 min, concentrated and co-concentrated with toluene to remove excess triethylamine. The crude product was dissolved in a mixture of MeOH and water (9 : 1, 900 ml) and heated at reflux for 2 h. Saturated aqueous $NaHCO_3$ (20 ml) was added and the mixture was concentrated. Column chromatography of the residue ($CHCl_3$ : MeOH : $Et_3N$; 10 : 1 : 0.01) and crystallization from ethanol gave **11** (32.7 g, 86%); mp 171–172 °C, $[\alpha]_D^{22}$ + 11.1° (*c* 0.7, MeOH). $^1H$-NMR data (300 MHz, $D_2O$): δ 4.56 (d, 1H, J 7.9 Hz, H-1), 4.49 (d, 1H, J 8.4 Hz, H-1′), 4.37 (dd, 1H, J 5.3 and 1.5 Hz, H-4′), 3.51 (dd, 1H, J 7.9 Hz, H-2′), 3.36 (bt, 1H, J 8.5 Hz, H-2), 1.55 (s, 3H, $CH_3$), 1.40 (s, 3H, $CH_3$). $^{13}C$-NMR data (75 MHz, $D_2O$): δ 113.7 ($(CH_3)_2\mathbf{C}$), 104.9 (C-1 and 1′), 81.4, 81.3, 77.5, 77.0, 76.5, 76.2, 75.5, 72.9, 63.5 (C-6/6′), 62.8 (C-6/6′), 33.8 ($CH_2CH_2Br$), 29.9 ($\mathbf{C}H_3C$), 28.1 ($\mathbf{C}H_3C$). Anal. Calcd for $C_{17}H_{29}BrO_{11}$ : C, 41.7; H, 6.0. Found: C, 41.5; H, 6.0.

### 2-Bromoethyl 2,6-di-*O*-benzoyl-4-*O*-(6-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**12**)

Compound **11** (9.0 g, 18.4 mmol) was dissolved in a mixture of dry pyridine (60 ml) and $CH_2Cl_2$ (150 ml) and cooled to − 50 °C. Benzoylchloride (10.5 ml, 90.6 mmol) in dry $CH_2Cl_2$ (150 ml) was added dropwise during 3 h and the mixture was stirred for an additional 1 h. MeOH was then added and the solution was concentrated. The residue was dissolved in $CH_2Cl_2$ (500 ml) and washed with aqueous HCl (2 M, 250 ml), saturated aqueous $NaHCO_3$ (250 ml), saturated aqueous NaCl (250 ml), dried and concentrated. The crude product was dissolved in 80% aqueous HOAc (250 ml), stirred at 80 °C for 30 min and then concentrated. Column chromatography of the residue ($CH_2Cl_2$ : MeOH;

30 : 1) gave amorphous **12** (4.3 g, 31%); $[\alpha]_D^{22} + 15.8°$ (*c* 1.0, $CHCl_3$). $^1H$-NMR data (300 MHz, $CDCl_3$): $\delta$ 8.03–7.23 (m, 15H, 3 Ph), 5.19 (dd, 1H, J 9.6 and 8.2 Hz, H-2), 4.84 (dd, 1H, J 12.0 and 1.5 Hz, H-6/6′), 4.66 (dd, 1H, J 11.9 and 3.5 Hz, H-6/6′), 4.64 (d, 1H, J 8.1 Hz, H-1), 4.48 (dd, 1H, J 12.0 and 5.8 Hz, H-6/6′), 4.37 (dd, 1H, J 11.9 and 8.8 Hz, H-6/6′), 4.35 (d, 1H, J 7.9 Hz, H-1′). $^{13}C$-NMR data (75 MHz, $CDCl_3$): $\delta$ 166.8, 166.6, 165.5, 104.1 (C-1′), 101.0 (C-1), 81.7, 73.5, 73.2, 73.1, 73.0, 72.9, 70.8, 69.6, 68.5, 64.0 (C-6/6′), 63.9 (C-6/6′), 29.6 ($OCH_2\mathbf{C}H_2Br$). Anal. Calcd for $C_{35}H_{37}BrO_{14}$: C, 55.2; H, 4.9. Found: C, 55.2; H, 4.9.

## 2-Bromoethyl 2,6-di-*O*-benzoyl-4-*O*-{6-*O*-benzoyl-3-*O*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)onate]-*β*-D-galactopyranosyl}-*β*-D-glucopyranoside (**14**)

Compound **12** (200 mg, 0.26 mmol), *O*-ethyl *S*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate] dithiocarbonate [26] (**13**, 263 mg, 0.44 mmol) and powdered molecular sieves (3 Å, 300 mg) were stirred in a mixture of $CH_3CN$ and $CH_2Cl_2$ (9 : 4, 8 ml) for 1 h under argon. Silver trifluoromethanesulfonate (118 mg, 0.45 mmol) was added and the reaction mixture was cooled to − 60 °C. Methylsulfenyl bromide [27] in $CH_2Cl_2$ (3.48 M, 126 μl, 0.44 mmol) was added dropwise during 5 min and the mixture was then stirred for 1 h, Diisopropylamine (0.7 ml, 2.6 mmol) was added and stirring was continued for 0.5 h. The mixture was allowed to attain room temperature and was then filtered and concentrated. Column chromatography of the residue ($CHCl_3$ : MeOH; 50 : 1) gave amorphous **14** (244 mg, 75%); $[\alpha]_D^{22} + 14.9°$ (*c* 1.0, $CHCl_3$). $^1H$-NMR data (300 MHz, $CDCl_3$): $\delta$ 5.33 (m, 1H, H-8″), 5.28 (dd, 1H, J 8.0 Hz, H-7″), 5.26 (dd, 1H, J 9.6 Hz, H-2), 5.03–4.93 (m, 2H, H-4″, H-6/6′), 4.74 (dd, 1H, J 11.9 and 3.4 Hz, H-6/6′), 4.70 (d, 1H, J 8.1 Hz, H-1), 4.59 (d, 1H, J 7.9 Hz, H-1′), 4.50 (dd, 1H, J 12.1 and 5.9 Hz, H-6/6′), 2.70 (dd, 1H, J 13.0 and 4.5 Hz, H-$3''_{eq}$). H-3, H-2′ and H-4′ were shifted downfield after acetylation of **14**. $^{13}C$-NMR data (75 MHz, $CDCl_3$): $\delta$ 170.7, 170.4, 170.3, 170.1, 170.1, 168.1 (C-1″, $J_{C\text{-}1'', H\text{-}3''ax}$ 6.1 Hz), 166.6, 166.1, 165.4, 104.4 (C-1′), 101.1 (C-1), 97.6 (C-2″), 82.2, 76.4, 73.4, 73.0, 72.9 (2C), 69.5, 68.9 (2C), 68.3, 68.0, 67.0, 63.7, 63.4, 62.4, 53.2 ($CH_3O$), 49.6 (C-5″), 37.6 (C-3″), 29.6 ($OCH_2\mathbf{C}H_2Br$), 23.1 ($NCO\mathbf{C}H_3$), 21.0, 20.7, 20.6 and 20.5. Anal. Calcd for $C_{55}H_{64}BrNO_{26}$: C, 53.5; H, 5.2. Found: C, 53.5; H, 5.5.

## 2-Bromoethyl 4-*O*-{3-*O*-sodium (5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl) onate]-*β*-D-galactopyranosyl}-*β*-D-glucopyranoside (**15**)

Compound **14** (200 mg, 0.16 mmol) was stirred in methanolic sodium methoxide (0.03 M, 50 ml) for 6 h. Water (50 μl) was added and after 5 min the solution was neutralized with silica gel, filtered and concentrated. Column chromatography of the residue ($CHCl_3$ : MeOH : water; 6 : 4 : 1) gave amorphous **15** (118 mg, 96%); $[\alpha]_D^{22}$-0.9° (*c* 1.0, water). $^1H$-NMR data (300 MHz, $D_2O$): $\delta$ 4.57 (d, 1H, J 8.1 Hz, H-1/1′), 4.54 (d, 1H, J 8.5 Hz, H-1/1′), 2.77 (dd, 1H J 12.2 and 4.3 Hz, H-$3''_{eq}$), 2.03 (s, 3H, $NCOCH_3$), 1.81 (bt, 1H, H-$3''_{ax}$). $^{13}C$-NMR data (75 MHz, $D_2O$): $\delta$ 177.0, 176.7, 105.6 (C-1′), 105.1 (C-1), 102.8 (C-2″), 81.3, 78.4, 78.1, 77.8, 77.2, 75.8, 75.7, 74.7, 73.0, 72.3, 71.2, 71.1, 70.4, 65.6, 63.9, 63.0, 54.7 (C-5″), 42.7 (C-3″), 34.0 ($OCH_2\mathbf{C}H_2Br$), 25.0 ($NCO\mathbf{C}H_3$).

## General procedure for solid-phase synthesis of peptides **16–27**

Peptides **16–27** were synthesized on PAM-resins prefunctionalized with the C-terminal amino acid using an ABI 438 peptide synthesizer. t-Boc amino acids carrying standard side-chain protective groups were activated in the peptide synthesizer using dicyclohexylcarbodiimide and then coupled to peptide resin. The t-Boc group was deprotected with trifluoroacetic acid in $CH_2Cl_2$. Cleavage of the peptide from the resin and deprotection of the side chains was performed with HF containing anisole (10%, by vol) at 0 °C for 30–40 min. The crude peptide was extracted from the resin with 30% aqueous acetic acid, or with DMF-water (1 : 1), for 1–2 h and the solution was freeze dried. Purification was then achieved with preparative reversed-phase HPLC and the peptides were characterized by amino acid composition and sequence analyses.

## General procedure for synthesis of neoglycopeptides **28–43**

Peptide (10 μmol) was added to a mixture of a 2-bromoethyl glycoside (40 μmol) and cesium carbonate 100 μmol) in dry DMF (1.0 ml) under argon. The mixture was sonicated for 5 min and then stirred at room temperature until analytical reversed phase HPLC indicated that all peptide was consumed. Then 0.1% aqueous trifluoroacetic acid (20 ml) was added and the mixture was freeze-dried. Purification of the residue by preparative reversed phase HPLC gave the target glycopeptide. Glycopeptides **28–43** were obtained in 52–95% yields and were characterized by FAB MS (Table 1). In addition glycopeptides **37**, **39** and **41** were characterized by $^1H$ NMR spectroscopy (Tables 2–4).

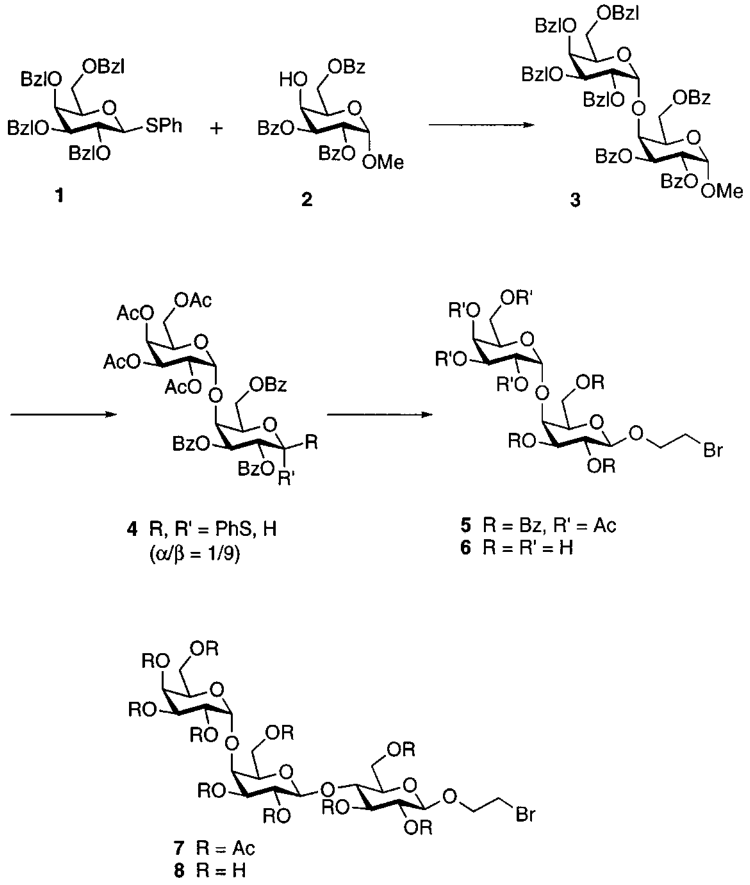
## Results and discussion

Preliminary attempts to couple the 2-bromoethyl galabioside **6**, prepared by deacetylation of the corresponding heptaacetate [21], to peptide sulfhydryl groups were promising. Therefore, an improved route to **6** and routes to the 2-bromoethyl glycosides of globotriose (**8**) and 3′-sialyllactose (**15**) were developed to allow preparation of large amounts of these spacer glycosides required for preparation of neoglycopeptides **28–43** (Table 1).

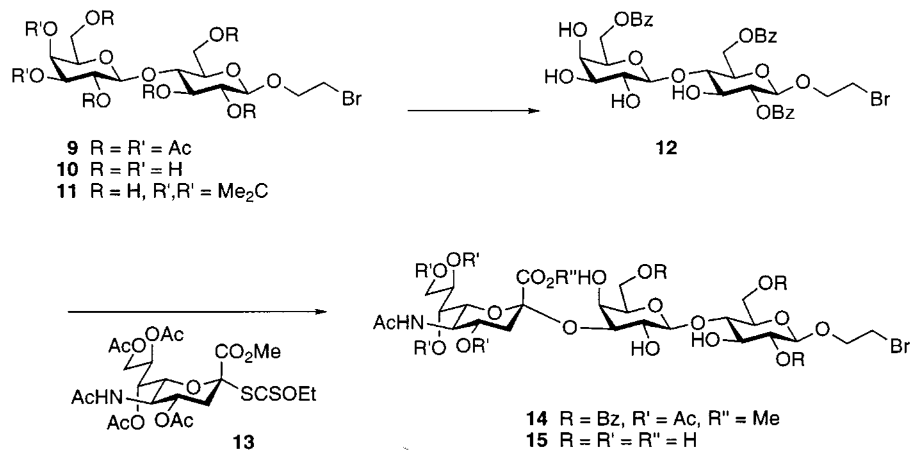
Condensation of the galactose derivatives **1** and **2** in a mixture of dichloromethane and diethyl ether under promotion by *N*-iodosuccinimide and a catalytic amount of trifluoromethanesulphonic acid [22, 23] gave the galabiose derivative **3** in 89% yield after purification by chromatography. Hydrogenolytic cleavage of the benzyl protective groups of **3**, followed by *O*-acetylation and acetolysis of the methyl glycoside, gave a crude galabiose 1-*O*-acetate. This was converted to the thiophenyl glycoside **4** by reaction with thiophenol in methylene chloride, using boron trifluoride etherate [24] as a promoter. Compound **4** was obtained as an anomeric mixture ($\alpha/\beta$: 1/9) in 68% overall yield from **3**, after purification by crystallization followed by chromatography of the mother liquor. It should be noted that both anomers of **4** were active as glycosyl donors in the subsequent glycosylation step. The thioglycoside **4** was thus reacted with 2-bromoethanol under promotion by *N*-iodosuccinimide and trifluoromethanesulphonic acid in 1,2-dichloroethane to give the corresponding 2-bromoethyl galabioside **5** in 82% yield after purification by crystallization. Finally, deprotection of **5** in methanolic sodium methoxide, followed by chromatography, gave the target, unprotected 2-bromoethyl galabioside **6** in 89% yield.

The 2-bromoethyl globotrioside **8** was obtained in 90% yield simply by deacetylation of the corresponding deca-*O*-acetate **7** [25] in methanolic sodium methoxide followed by purification by chromatography.

The 2-bromoethyl glycoside of 3′-sialyllactose was synthesized via the following route. Deacetylation of the 2-bromoethyl lactoside heptaacetate **9** [21] with methanolic sodium methoxide, followed by chromatography and crystallization, gave **10** in 51% yield. Reaction of **10** with 2,2-dimethoxypropane, using *p*-toluenesulfonic acid as a catalyst, gave the 3′,4′-*O*-isopropylidene derivative **11** which was contaminated by side-products presumed to carry 2-methoxyprop-2-yl groups at different hydroxyl groups in **11**. After work up, these acetals were hydrolysed by boiling the crude product in a mixture of methanol and water, and **11** was then obtained in 86% yield after purification by chromatography and crystallization. Regioselective benzoylation of the 2-, 6- and 6′-positions in **11**, followed by hydrolysis of the 3′,4′-isopropylidene acetal with aqueous acetic acid and chromatographic purification of the product gave the partially protected glycoside **12** in 31% yield. Sialylation at the 3′-position of **12** was then performed by reaction with the dithiocarbonate derivative **13** of



Scheme 1.



**Scheme 2.**

*N*-acetylneuraminic acid [26], promoted by methylsulfenyl bromide and silver trifluoromethanesulfonate [27] in a mixture of dichloromethane and acetonitrile at low temperature [28]. After purification by chromatography, the partially protected trisaccharide **14** was obtained in 75% yield. Deacetylation of **14** in methanolic sodium methoxide, followed by hydrolysis of the sialic acid methyl ester, gave the target 2-bromoethyl glycoside **15** of 3′-sialyllactose in 96% yield.

Peptides **16–27** (Table 1) were synthesized on solid phase according to the t-Boc strategy using an ABI 438 peptide synthesizer. The peptides were cleaved from the resin with hydrogen fluoride, purified by reversed-phase HPLC and their structures were confirmed by amino acid composition and sequence analyses.

Alkylation of the sulfhydryl group in peptides **16–27** with an unprotected 2-bromoethyl glycoside (**6**, **8** or **15**) was achieved in dry *N*,*N*-dimethylformamide at ambient temperature, using cesium carbonate as base. Formation of the *S*-linked neoglycopeptides was monitored by analytical reversed-phase HPLC and conversion of a peptide into the corresponding neoglycopeptide was in general achieved within an hour (Figure 1). The reactions were then quenched with aqueous trifluoroacetic acid and the target neoglycopeptides **28–43** (Table 1) were obtained in 52–95% yields after purification by reversed-phase HPLC. The homogeneity and identity of the neoglycopeptides was confirmed by HPLC and FAB-MS, respectively. During the preparation of this manuscript a similar approach for attachment of simple alkyl groups to cysteine containing peptides was reported [29].

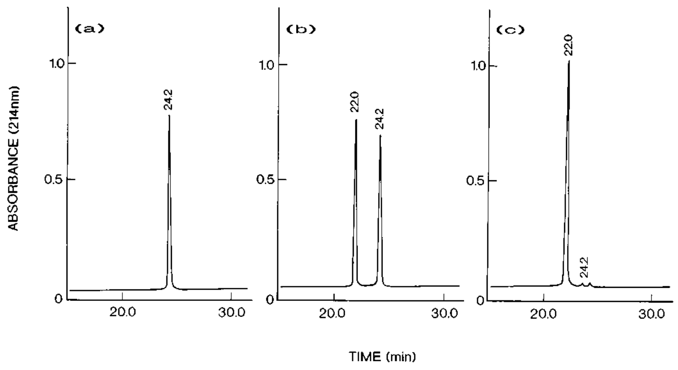


**Figure 1.** Analytical reversed-phase HPLC chromatogram of (**a**) peptide **22**, (**b**) the conversion of **22** to give neoglycopeptide **37** after 10 min reaction time, and (**c**) after 45 min reaction. HPLC conditions are described under General methods in the Material and methods section.

Base catalysed side reactions such as $\beta$-elimination and epimerization of peptide stereocentres have in some cases been encountered during removal of acyl protective groups from the carbohydrate moieties of O-linked glycopeptides (summarized in reference [30]). In a recent systematic investigation it was found that conditions in common use for removal of *O*-acetyl protective groups from glycopeptides did not cause base-catalysed side-reactors [30]. However, the more severe conditions required for removal of benzoyl groups were accompanied both by $\beta$-elimination and epimerization. Furthermore, $^1$H NMR spectroscopy was found to be a sensitive tool for detection of such side-products in glycopeptides. Epimerization of stereocentres and $\beta$-elimination of alkylated cysteine, could not *a priori* be excluded from occurring during the basic reaction conditions (cesium carbonate in DMF) used for preparation of neoglycopeptides **28–43**. We therefore analysed the chemical as well as the stereochemical integrity of three representative neoglycopeptides (**37**, **39** and **41**, Tables 2–4) by 2D $^1$H NMR spectroscopy. The glycopeptides were chosen so as to be alkylated on an internal cysteine, internal homocysteine or an *N*-terminally placed cysteine moiety, respectively. The NMR spectra of the three glycopeptides were all in agreement with the postulated structures. Furthermore, no minor peaks could be detected on careful inspection of the NMR spectra indicating that epimerization and $\beta$-elimination do not occur to any significant extent during the conditions described here for conjugation.

**Table 2.** $^1$H NMR chemical shifts ($\delta$, ppm) for glycopeptide **37** in water containing 10% $D_2O^a$.

| *Residue* | *NH* | *Hα* | *Hβ* | *Hγ* | *Others* |
|---|---|---|---|---|---|
| Ser$^1$ | – | 4.11 | 3.90$^b$ | | |
| Gly$^2$ | 8.77 | 3.95$^b$ | | | |
| Val$^3$ | 8.28 | 4.01 | 1.98 | 0.83$^b$ | |
| Glu$^4$ | 8.61 | 4.16 | 1.83, 1.90 | 2.19$^b$ | |
| Asn$^5$ | 8.60 | 4.87 | 2.56, 2.71 | | 6.95 and 7.64 ($\beta$-CONH$_2$) |
| Pro$^6$ | – | 4.30 | 1.89, 2.19 | 1.95$^b$ | 3.65$^b$ (H$\delta,\delta'$) |
| Gly$^7$ | 8.55 | 3.82$^b$ | | | |
| Gly$^8$ | 8.11 | 3.80$^b$ | | | |
| Tyr$^9$ | 8.02 | 4.48 | 2.84, 2.96 | | 6.74 (3,5-H), 7.03 (2,6-H) |
| Cys$^{10}$ | 8.44 | 4.40 | 2.76, 2.90 | | 2.71$^b$ (SC**H**$_2$CH$_2$O), 3.74 and 3.97 (SCH$_2$C**H**$_2$O), Gal$\alpha$4Gal$\beta^c$ |
| Leu$^{11}$ | 8.50 | 4.34 | 1.57$^b$ | 1.55 | 0.79 and 0.86 ($\gamma,\gamma'$-Me) |
| Thr$^{12}$ | 7.79 | 4.06 | 4.16 | 1.06 | |

[a] Obtained at 500.14 MHz, 278 K, and pH 5.4 with H$_2$O ($\delta_H$ 4.98 ppm) as internal standard.
[b] Degeneracy has been assumed.
[c] Chemical shifts ($\delta$, ppm) for the galabiose moiety in **37**: 4.83 (H-1′), 3.70 (H-2′), 3.80 (H-3′), 3.90 (H-4′), 4.28 (H-5′), 3.59$^b$ (H-6′), 4.39 (H-1), 3.47 (H-2), 3.64 (H-3), 3.92 (H-4), 3.66 (H-5), 3.74 and 3.80 (H-6).

**Table 3.** $^1$H NMR chemical shifts ($\delta$, ppm) for glycopeptide **39** in water containing 10% $D_2O^a$.

| *Residue* | *NH* | *Hα* | *Hβ* | *Hγ* | *Others* |
|---|---|---|---|---|---|
| Arg$^1$ | – | 3.98 | 1.85$^b$ | 1.57$^b$ | 3.12$^b$ (H$\delta,\delta'$);<br>6.46, 6.90 and 7.16 (NH) |
| Gly$^2$ | 8.78 | 3.85, 4.00 | | | |
| Tyr$^3$ | 8.42 | 4.43 | 2.80, 2.90 | | 6.73 (3,5-H), 6.99 (2,6-H) |
| Val$^4$ | 7.96 | 3.90 | 1.79 | 0.75, 0.79 | |
| Tyr$^5$ | 8.47 | 4.35 | 2.83, 2.99 | | 6.76 (3,5-H), 7.12 (2,6-H) |
| Hcy$^6$ | 8.36 | 4.37 | 1.77, 2.00 | 2.34, 2.50 | 2.67$^b$ (SC**H**$_2$CH$_2$O), 3.73 and 3.95 (SCH$_2$C**H**$_2$O), Gal$\alpha$4Gal$\beta^c$ |
| Gly$^7$ | 7.11 | 3.75$^b$ | | | |
| Leu$^8$ | 7.91 | 4.15 | 1.54$^b$ | 1.53 | 0.80 and 0.85 ($\gamma,\gamma'$-Me) |

[a] Obtained at 500.14 MHz, 278 K, and pH 5.2 with H$_2$O ($\delta_H$ 4.98 ppm) as internal standard.
[b] Degeneracy has been assumed.
[c] Chemical shifts ($\delta$, ppm) for the galabiose moiety in **39**: 4.87 (H-1′), 3.73 (H-2′), 3.83 (H-3′), 3.94 (H-4′), 4.31 (H-5′), 3.62$^b$ (H-6′), 4.38 (H-1), 3.47 (H-2), 3.65 (H-3), 3.94 (H-4), 3.65 (H-5), 3.76 and 3.81 (H-6).

**Table 4.** $^1$H NMR chemical shifts ($\delta$, ppm) for glycopeptide **41** in water containing 10% $D_2O^a$.

| *Residue* | *NH* | *Hα* | *Hβ* | *Hγ* | *Others* |
|---|---|---|---|---|---|
| Cys$^1$ | – | 4.05 | 2.93, 3.05 | | 2.77$^b$ (SCH$_2$C**H**$_2$O), 3.78 and 3.99 (SCH$_2$C**H**$_2$O), Gal$\alpha$4Gal$\beta^c$ |
| Arg$^2$ | – | 4.26 | 1.73$^b$ | 1.57$^b$ | 3.11$^b$ (H$\delta$), 7.16 (NH) |
| Gly$^3$ | 8.57 | 3.86 | | | |
| Tyr$^4$ | 8.18 | 4.45 | 2.84, 2.90 | | 6.73 (3,5-H), 6.95 (2,6-H) |
| Val$^5$ | 8.02 | 3.92 | 1.81 | 0.75, 0.78 | |
| Tyr$^6$ | 8.44 | 4.36 | 2.84, 2.97 | | 6.75 (3,5-H), 7.10 (2,6-H) |
| Gln$^7$ | 8.33 | 4.19 | 1.77, 1.99 | 2.18$^b$ | 6.89 and 7.53 ($\gamma$-CONH$_2$) |
| Gly$^8$ | 7.35 | 3.74, 3.81 | | | |
| Leu$^9$ | 7.94 | 4.15 | 1.54$^b$ | 1.54 | 0.81 and 0.85 ($\gamma,\gamma'$-Me) |

[a] Obtained at 500.14 MHz, 278 K, and pH 5.8 with H$_2$O ($\delta_H$ 4.98 ppm) as internal standard.
[b] Degeneracy has been assumed.
[c] Chemical shifts ($\delta$, ppm) for the galabiose moiety in **41**: 4.85 (H-1′), 3.76 (H-2′), 3.82 (H-3′), 3.93 (H-4′), 4.32 (H-5′), 3.62$^b$ (H-6′), 4.40 (H-1), 3.49 (H-2), 3.66 (H-3), 3.95 (H-4), 3.66 (H-5), 3.82$^b$ (H-6).

In summary, routes to 2-bromoethyl glycosides of the disaccharide galabiose and the trisaccharides globotriose and 3′-sialyllactose have been developed. The 2-bromoethyl glycosides were then employed for preparation of a series of neoglycopeptides by base-promoted alkylation of the sulfhydryl groups of cysteine and homocysteine residues in T cell stimulating peptides. This convergent method appears to be general and proceeds without detectable $\beta$-elimination of alkylated cysteine residues or epimerization of glycopeptide stereocentres.

## Acknowledgements

We are grateful to Karl-Erik Bergquist for recording NMR spectra of the glycopeptides. This work was funded by Astra AB, Södertälje, Sweden and by grants from the Swedish Natural Science Research Council.

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Received 14 February 1997, accepted 3 April 1997