# Synthesis and Biological Evaluation of E-Selectin Antagonists that Present Different Carbohydrate Ligands in a Multivalent Format

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Dedicated to Prof. Bernd Giese on the occasion of his 65th birthday

**Abstract:** The synthesis of the first polylysine conjugates that present different carbohydrate ligands in a multivalent format is reported. Glycopolymers **3c** and **3d** have been prepared with a highly predictable composition as indicated by $^1$H NMR. They function as multivalent E-selectin inhibitors but their potencies are not superior compared to previously-described related compounds.

**Key words:** sialyl Lewis X, slectins, glycopolymers, multivalency, carbohydrate chemistry

The interactions of E-selectin, a vascular endothelial cell surface protein, with its physiological glycoprotein ligand mediates leukocyte rolling on the blood vessel wall.[1] Inhibition of this early stage of the inflammatory response prevents excessive leukocyte recruitment and thus is a potential therapy for acute and chronic inflammatory disorders such as reperfusion injuries, psoriasis, rheumatoid arthritis, or respiratory diseases.[2] The tetrasaccharide sialyl Lewis X (**1**, sLe$^x$; Figure 1) is the minimum epitope recognized by E-selectin.[3] The concentration of sLe$^x$ to achieve 50% inhibition ($IC_{50}$) was determined to be 1000–2000 μM in a static, cell-free E-selectin binding assay[4] which is in line with the observation that monovalent carbohydrate-protein interactions are generally very weak ($K_D = 10^{-3}$–$10^{-4}$ $M^{-1}$).[5] While modifying sLe$^x$ we discovered the simplified analogue **2** (Figure 1) which showed 30-fold improved potency ($IC_{50}$ = 36 μM) compared with sLe$^x$.[6,7]

In some cases high affinity inhibitors can be obtained by multivalent presentation of low affinity ligands.[8] Polymers, liposomes and protein conjugates containing sLe$^x$ or similar carbohydrates have been described.[9] We have prepared multivalent sLe$^x$–polyaspartic acid conjugates[10] and poly-L-lysine–sLe$^x$ conjugates[11] but did not observe improved potencies. However, multivalent polylysine conjugates of antagonist **2**, such as **3a** and **3b** (Scheme 1, Table 1) were highly active E-selectin inhibitors with $IC_{50}$ values as low as 0.04 μM in the binding assay. Furthermore, these glycopolymers reduced neutrophil rolling on activated endothelial cells both in vitro and in vivo.[12,13]

We here report on the synthesis, characterization and biological evaluation of compounds **3c** and **3d**, the first polylysine conjugates with different carbohydrate ligands (Scheme 1).

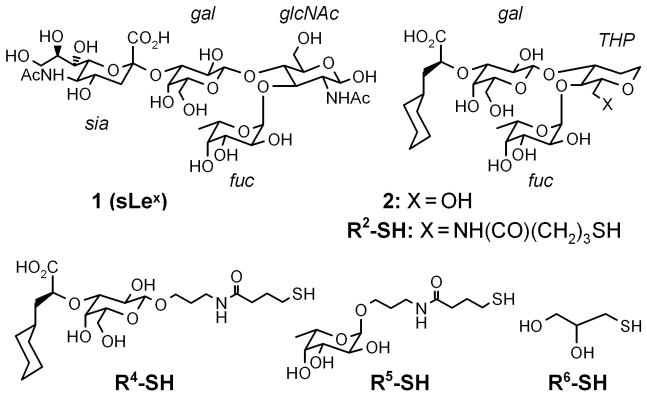
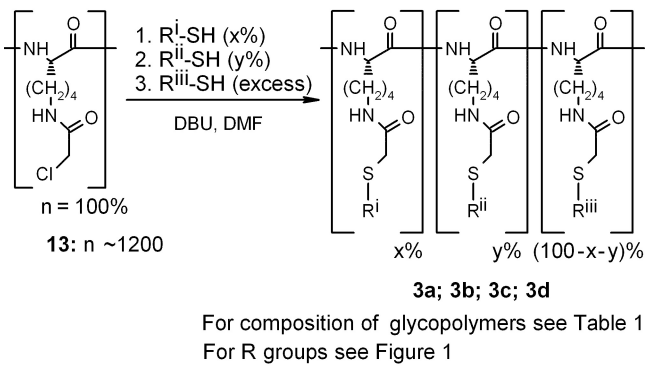



**Figure 1** E-Selectin antagonists and carbohydrate ligands incorporated in polylysine conjugates. *gal:* D-galactose; *glcNAc: N*-acetyl-D-glucosamine; *sia:* sialic acid; *fuc:* L-fucose; THP: tetrahydropyran

The essential pharmacophores of sLe$^x$ and **2** required to bind to E-selectin are the carboxylic acid function, all three OH-groups of the L-fucose as well as the 4-OH and 6-OH groups of the galactose.[14] We have shown that the tetrahydropyran of antagonist **2** mainly functions as spacer to keep the pharmacophores in the appropriate positions with respect to each other.[15] To study if random expression of all important pharmacophores in a multivalent format would lead to a potent antagonist we designed glycoconjugate **3c** containing multiple copies of galactose derivative **R$^4$-S**- and fucose derivative **R$^5$-S**-, which are fragments of antagonist **2**.




**Scheme 1** Synthesis of carbohydrate-polylysine conjugates

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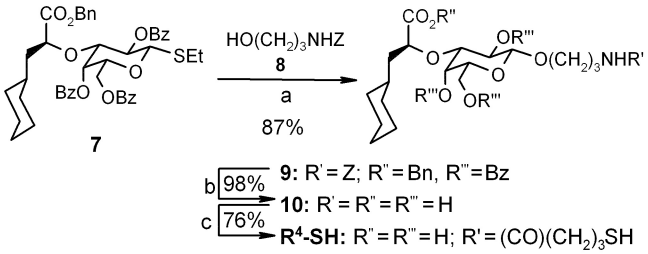
**Table 1** E-Selectin Inhibition[a]

| Compound | $R^{i}$ (% cont.) | $R^{ii}$ (% cont.) | $R^{iii}$ (% cont.) | $IC_{50}$ bind.[b] [μM] |
|---|---|---|---|---|
| **1** | monovalent | | | 1000–2000 |
| **2** | monovalent | | | 36 |
| **3a** | $R^2$ (35) | – | $R^6$ (65) | 0.04 |
| **3b** | $R^2$ (20) | – | $R^6$ (80) | 0.18 |
| **3c** | $R^4$ (30) | $R^5$ (30) | $R^6$ (40) | 1900 |
| **3d** | $R^2$ (20) | – | $R^4$ (80) | 0.40 |

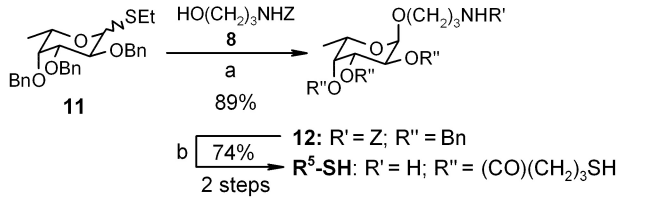
[a] For R groups, see Figure 1.
[b] For multivalent compounds concentrations refer to carbohydrate ligand concentrations but not to macromolecule concentration.




**Scheme 2** *Reagents and conditions*: (a) NIS, $F_3CSO_3H$, $CH_2Cl_2$, –10 °C; (b) 1. Pd/C, $H_2$, dioxane–$H_2O$ 5:1, 20 °C; 2. NaOH, $CH_3OH$, 20 °C; (c) γ-thiobutyrolactone, $Et_3N$, $CH_3OH$, 64 °C; NIS: *N*-iodosuccinimide; Z: benzyl oxycarbonyl; Bn: benzyl; Bz: benzoyl

The structure of the physiological E-selectin ligand has not been fully elucidated[16] but there is evidence that additional fucose residues in the proximity of $sLe^x$ are required for high affinity binding.[17] Glycopolymer **3d** with fucose derivative **R$^5$-S-** in addition to the potent $sLe^x$ mimic **R$^2$-S-** was designed to study the role of additional fucose residues in close proximity to the potent $sLe^x$-like E-selectin inhibitor **2** in a multivalent format.

The E-selectin ligand **R$^2$-SH** was prepared as reported earlier.[11] The modified galactose **R$^4$-SH** was obtained from **7**[18] (Scheme 2). Glycosylation with propanolamine **8** furnished compound **9**, which was deprotected to give **10**. Subsequent reaction with γ-thiobutyrolactone gave thiol




**Scheme 3** *Reagents and conditions*: (a) 1. $Br_2$, $CH_2Cl_2$, 0 °C, 2. $Et_4NBr$, $CH_2Cl_2$–DMF 3:2; (b) 1. $Pd(OH)_2/C$, $H_2$, dioxane/$H_2O$ 4:1, 20 °C; 2. γ-thiobutyrolactone, $Et_3N$, DMF, 90 °C

**R$^4$-SH**. Likewise, fucose **R$^5$-SH** was prepared from **11**[19] which was glycosylated (→ **12**), deprotected and subsequently treated with γ-thiobutyrolactone (Scheme 3). The glycopolymers **3c** and **3b** were prepared from chloroacetylated polylysine **13**.[11] Conjugate **3c** was obtained by reacting **13** with 30 mol% of **R$^4$-SH** and 30 mol% of **R$^5$-SH** in the presence of DBU followed by the addition of an excess of **R$^6$-SH**. Reaction of **13** with 20 mol% of **R$^2$-SH** and subsequent treatment with a small excess of **R$^4$-SH** furnished glycopolymer **3d**. As we have shown that the degree of polymerization of the L-polylysine starting material (n ca. 1200) is not altered under the reaction conditions applied[11] the molecular weight ($M_n$) of **3c** and **3b** can be estimated to ca 400–500 kD. Both composition and integrity of the novel glycopolymers were confirmed by means of $^1$H NMR spectroscopy (Figure 2). Within the experimental error carbohydrate incorporation occurred quantitatively.

The glycoconjugates were evaluated in the binding assay. Compound **3c** was found to inhibit E-selectin with an $IC_{50}$ of 1900 μM. Thus, random expression of all important pharmacophores in a multivalent format resulted in a inhibitory potential similar to monovalent $sLe^x$ (Table 1, Figure 1). Conjugate **3d** was highly active showing an $IC_{50}$ value comparable to **3b** also containing 20% of **R$^2$-S-**

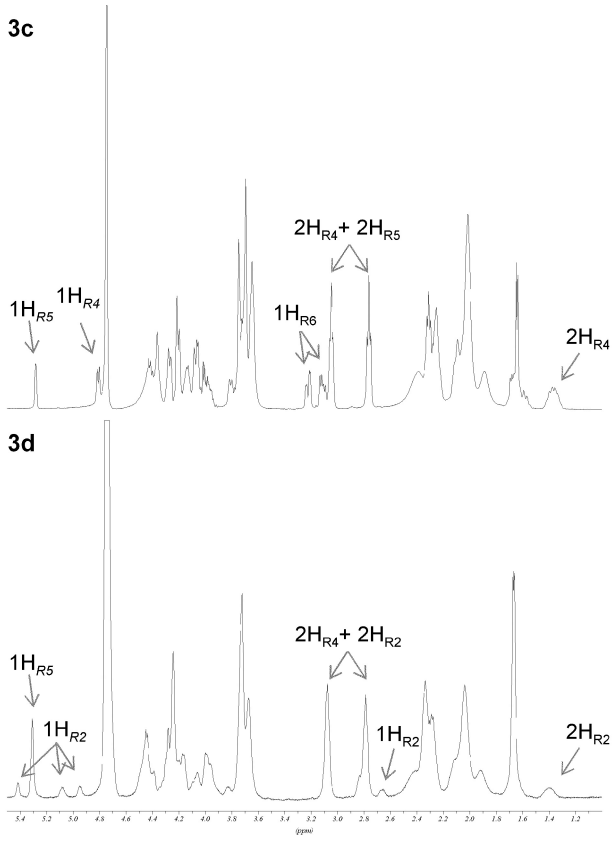



**Figure 2** $^1$H NMR (500 MHz) spectra of glycopolymers **3c** and **3d** in $D_2O$ at 60 °C. Several baseline-separated signals can be assigned to the corresponding R groups. Integration allows the determination of the composition within NMR accuracy.

but with **R⁶-S**- instead of additional fucose (Table 1, Figure 1). Thus, incorporation of fucose in a multivalent format does not lead to improved potency.

In conclusion we have prepared the first polylysine conjugates, which present different carbohydrate ligands in a multivalent format with a highly predictable composition. These compounds inhibit E-selectin but are not superior compared to previously-described related analogues.

All reactions were carried out under an atmosphere of anhyd Ar. Commercially available absolute solvents were used. The NMR spectra were recorded on a Bruker Avance DPX 400 spectrometer. Chemical shifts of the $^1$H NMR signals of water-soluble compounds are reported relative to the shift of the DHO peak (4.75 ppm). The signal assignments are based on 2D $^1$H–$^1$H-correlation (COSY) and $^1$H–$^{13}$C-correlation spectroscopy (HSQC). The MS spectra were obtained on a Finnigan MAT 90 mass spectrometer, the HRMS spectra on a Bruker Daltonics 9.4T APEX-III FT-MS mass spectrometer. Ultrafiltrations were performed using Amicon stirred cells 8010 (volume: 10 mL; diameter: 25 mm) and Amicon disc membranes YM3 (molecular weight cut-off: 3000).

**Compound 3c**

DBU (91 mg, 0.60 mmol) was added at 20 °C to a solution of **13** (50 mg, 0.25 mmol) and **R²-SH** (37 mg; 0.05 mmol) in a mixture of DMF (4 mL) and water (0.1 mL). The mixture was stirred for 15 min. Then, **R⁵-SH** (120 mg, 0.37 mmol) and DBU (73 mg, 0.49 mmol) were added and stirring continued for 2 h. The solution was added dropwise to a mixture of EtOH–$Et_2O$ (30 mL, 1:1). The formed precipitate was filtered off and washed with EtOH. The crude product was dissolved in water and further purified by means of ultrafiltration (5 × 10 down to 2 mL, with the volume being made up with distilled water on each occasion). Following lyophilization, the product **3c** was isolated as a colorless powder (128 mg, 91%).

$^1$H NMR: see Figure 2.

**Compound 3d**

DBU (55 mg, 0.37 mmol) was added at 20 °C to a solution of **13** (50 mg, 0.25 mmol), **R⁴-SH** (39 mg, 0.08 mmol) and **R⁵-SH** (26 mg; 0.08 mmol) in a mixture of DMF (4 mL) and water (0.2 mL). The mixture was stirred for 15 min. Then, **R⁶-SH** (79 mg, 0.73 mmol) and $Et_3N$ (0.2 mL) were added and stirring continued for 2 h. The solution was added dropwise to a mixture of EtOH–$Et_2O$ (30 mL, 1:1). The formed precipitate was filtered off and washed with EtOH. The crude product was dissolved in water and further purified by means of ultrafiltration (5 × 10 down to 2 mL, with the volume being made up with distilled water on each occasion). Following lyophilization, the product **3d** was isolated as a colorless powder (107 mg, 100%).

$^1$H NMR: see Figure 2.

**Compound R⁴-SH**

Under rigorous exclusion of oxygen a solution of **10** (1000 mg, 2.56 mmol), γ-thiobutyrolactone (2600 mg, 25.6 mmol) and $Et_3N$ (2600 mg, 25.6 mmol) in degassed $CH_3OH$ (30 mL) was heated under reflux for 16 h. The solvent and volatile side products were removed in vacuo and the residue subjected to chromatography on silica gel (EtOAc–*i*-PrOH–water, 1:1:0.25). Lyophilization afforded the product **R⁴-SH** as a colorless powder (956 mg, 76%); $[\alpha]_D^{20}$ –33.5 (*c* = 0.76, $CH_3OH$).

$^1$H NMR ($D_2O$): δ = 0.90–1.75 (m, 13 H, C$H_2$-*c*C$_6H_{11}$), 1.79 (quint, *J* = 6.5 Hz, 2 H, OCH$_2$C$H_2$CH$_2$NH), 1.83 (quint, *J* = 7.5 Hz, 2 H, COCH$_2$C$H_2$CH$_2$SH), 2.30 (t, *J* = 7.5 Hz, 2 H, COC$H_2$CH$_2$CH$_2$SH), 2.49 (t, *J* = 7.5 Hz, 2 H, COCH$_2$CH$_2$C$H_2$SH), 3.25 (m, 2 H, OCH$_2$CH$_2$C$H_2$NH), 3.36 (dd, *J* = 9.5, 3.0 Hz, 1 H, H3), 3.54 (dd, *J* = 9.5, 8.0 Hz, 1 H, H2), 3.63 (m, 1 H, OC*Ha*HbCH$_2$), 3.66–3.77 (m, 3 H, H5, H6, H6′), 3.87 (br d, *J* = 3.0 Hz, 1 H, H4), 3.93 (m, 2 H, OCHa*Hb*CH$_2$, OC*H*CO$_2$H), 4.35 (d, *J* = 8.0 Hz, 1 H, H1).

$^{13}$C NMR ($D_2O$): δ = 22.6, 25.3, 25.5, 25.7, 27.8, 29.0, 31.3, 32.8, 33.1, 33.9, 35.9, 40.7, 60.6, 65.7, 67.3, 69.5, 74.0, 78.8, 82.4, 102.2, 175.6, 182.2.

HRMS: *m*/*z* [M + Na]$^+$ calcd for $C_{22}H_{39}NO_9S$: 516.2238; found: 516.2240.

**Compound R⁵-SH**

A mixture of **12** (1000 mg, 1.60 mmol), $Pd(OH)_2$ (500 mg, 10% on charcoal), dioxane (16 mL) and $H_2O$ (4 mL) was hydrogenated by means of a balloon at 20 °C for 16 h. Following filtration the solvent was removed to give a colorless oil which was dissolved in degassed DMF (20 mL). γ-Thiobutyrolactone (1630 mg, 16.0 mmol) and $Et_3N$ (1620 mg, 16.0 mmol) were added and the mixture was stirred for 16 h at 90 °C. The solvent and volatile side products were removed in vacuo and the residue subjected to chromatography on silica gel ($CHCl_3$–MeOH–$H_2O$, 3:1:0 → 3:1:0.3). The product **R⁵–SH** was isolated as a colorless oil (383 mg, 74%); $[\alpha]_D^{20}$ –112.2 (*c* = 0.76, $CH_3OH$).

$^1$H NMR ($D_2O$): δ = 1.15 (d, *J* = 6.5 Hz, 3 H, H6), 1.79 (m, 2 H, OCH$_2$C$H_2$CH$_2$NH), 1.83 (quint, *J* = 7.5 Hz, 2 H, COCH$_2$C$H_2$-CH$_2$SH), 2.31 (t, *J* = 7.5 Hz, 2 H, COC$H_2$CH$_2$CH$_2$SH), 2.49 (t, *J* = 7.5 Hz, 2 H, COCH$_2$CH$_2$C$H_2$SH), 3.17–3.33 (m, 2 H, OCH$_2$CH$_2$C$H_2$NH), 3.45 (m, 1 H, OC*Ha*HbCH$_2$), 3.68 (m, 1 H, OCHa*Hb*CH$_2$), 3.71 (dd, *J* = 10.5, 4.0 Hz, 1 H, H2), 3.74 (br d, *J* = 3.5 Hz, 1 H, H4), 3.81 (dd, *J* = 10.5, 3.5 Hz, 1 H, H3), 3.98 (br q, *J* = 6.5 Hz, 1 H, H5), 4.81 (d, *J* = 4.0 Hz, 1 H, H1).

$^{13}$C NMR ($CDCl_3$): δ = 15.8, 23.7, 28.6, 29.1, 34.3, 36.7, 65.6, 65.8, 68.8, 70.8, 71.4, 98.1, 172.1.

HRMS: *m*/*z* [M + Na]$^+$ calcd for $C_{13}H_{25}NO_6S$: 346.1295; found: 346.1295.

**Compound 9**

Under rigorous exclusion of moisture $CF_3SO_3H$ (70 μL) was added at –10 °C to a solution of **7** (1070 mg, 5.13 mmol), **8** (2000 mg, 2.56 mmol) and NIS (866 mg, 3.85 mmol) in $CH_2Cl_2$ and the mixture stirred for 30 min. EtOAc was added and the mixture extracted with $Na_2S_2O_3$, $NaHCO_3$ and brine. The solvent was removed and the residue subjected to flash chromatography on silica gel (hexane–EtOAc, 3:1 → 1:1). Compound **9** was isolated as a colorless oil (2070 mg, 87%); $[\alpha]_D^{20}$ +17.3 (*c* = 0.74, $CH_3OH$).

$^1$H NMR ($CDCl_3$): δ = 0.44–1.79 (m, 15 H, C$H_2$*c*-C$_6H_{11}$, OCH$_2$C$H_2$CH$_2$NH), 3.14 (m, 2 H, OCH$_2$CH$_2$C$H_2$NH), 3.55 (m, 1 H, OC*Ha*HbCH$_2$), 3.90 (dd, *J* = 9.5, 3.0 Hz, 1 H, H3), 3.94 (m, 1 H, OCHa*Hb*CH$_2$), 3.97 (br t, *J* = 6.0 Hz, 1 H, H5), 4.19 (dd, *J* = 8.0, 4.5 Hz, 1 H, OC*H*CO$_2$H), 4.43 (dd, *J* = 11.5, 5.0 Hz, 1 H, H6), 4.49 (dd, *J* = 11.5, 7.0 Hz, 1 H, H6′), 4.55 (d, *J* = 8.0 Hz, 1 H, Hvv1), 4.99 (br t, *J* = 6.0 Hz, 1 H, NH), 5.03 (m, 2 H, OBn), 5.08 (d, *J* = 12 Hz, 1 H, CO$_2$Bn), 5.19 (d, *J* = 12 Hz, 1 H, CO$_2$Bn), 5.63 (dd, *J* = 10.0, 8.0 Hz, 1 H, H2), 5.94 (br d, *J* = 3.0 Hz, 1 H, H4), 7.29–8.16 (m, 25 H, ArH).

$^{13}$C NMR ($CDCl_3$): δ = 16.2, 28.6, 39.7, 66.0, 66.1, 67.6, 72.9, 73.0, 74.3, 75.7, 77.3, 79.1, 97.8, 127.0–138.4 (signals of aromatic C-atoms), 158.2.

HRMS: *m*/*z* [M + Na]$^+$ calcd for $C_{54}H_{57}NO_{13}$: 950.3722; found: 950.3724.

**Compound 10**

A mixture of **9** (2.07 g, 2.23 mmol), $Pd(OH)_2$ (1.00 g, 10% on charcoal), dioxane (40 mL), $H_2O$ (10 mL) and HOAc (0.34 mL) was hydrogenated by means of a balloon at 20 °C for 16 h. Following

filtration the solvent was removed and the residue dissolved in a mixture of MeOH (50 mL) and NaOH (2 N, 11 mL). The solution was stirred at 60 °C for 4 h. The solvents were removed and the residue subjected to flash chromatography on silica gel (EtOAc–*i*-PrOH–$H_2O$, 1:1:0.5). Compound **10** was isolated as a colorless oil (0.855 g, 98%).

$^1$H NMR ($D_2O$): δ = 0.83–1.73 (m, 13 H, $CH_2c$-$C_6H_{11}$), 1.98 (m, 2 H, $OCH_2CH_2CH_2NH$), 3.14 (t, *J* = 7.0 Hz, 2 H, $OCH_2CH_2CH_2NH$), 3.39 (dd, *J* = 10.0, 3.0 Hz, 1 H, H3), 3.58 (br t, *J* = 9.0 Hz, 1 H, H2), 3.68 (m, 1 H, H5), 3.74 (m, 2 H, H6, H6′), 3.79 (m, 1 H, OC*Ha*Hb), 3.89 (d, *J* = 3.0 Hz, 1 H, H4), 3.94 (dd, *J* = 8.0, 3.0 Hz, 1 H, $OCHCO_2H$), 4.03 (m, 1 H, OCHa*Hb*v), 4.39 (d, *J* = 8.0 Hz, 1 H, H1).

MS: *m*/*z* = 392 $[M + H]^+$.

**Compound 12**

A solution of $Br_2$ (2.21 g, 13.8 mmol) in $CH_2Cl_2$ (20 mL) was added dropwise at 0 °C to a solution of **11** (6.0 g, 12.6 mmol) in $CH_2Cl_2$ (20 mL). After stirring for 30 min at 0 °C cyclohexene (2.5 mL) was added to consume excessive $Br_2$. The solution was added within 10 min to a mixture of **8** (5.25 g, 25.1 mmol) and $Et_4NBr$ (6.30 g, 30.1 mmol; dried for 2 h at 200 °C) in DMF–$CH_2Cl_2$ (100 mL, 1:1). The mixture was stirred for 90 h at 20 °C, diluted with EtOAc, washed with $NaHCO_3$, $H_2O$, HCl (0.5 M) and brine and dried with $Na_2SO_4$. The solvent was removed and the residue subjected to flash–chromatography on silica gel (hexane–acetone, 6:1). Compound **12** was isolated as a colorless oil (7.0 g, 89%); $[\alpha]_D^{20}$ –74.5 (*c* = 1.2, $CH_3OH$).

$^1$H NMR ($CDCl_3$): δ = 1.09 (d, *J* = 6.5 Hz, 3 H, H6), 1.80 (quint, *J* = 6.0 Hz, 2 H, $OCH_2CH_2CH_2NH$), 3.25 (m, 1 H, $OCH_2CH_2$-C*Ha*HbNH), 3.39–3.53 (m, 2 H, OC*Ha*HbCH$_2$CHa*Hb*NH), 3.59 (m, 1 H, H4), 3.78–3.86 (m, 2 H, H5, OC*Ha*Hb), 3.88 (dd, *J* = 10.0, 2.0 Hz, 1 H, H3), 4.01 (dd, *J* = 10.0, 4.0 Hz, 1 H, H2), 4.65 (m, 3 H, OBn), 4.71 (d, *J* = 4.0 Hz, 1 H, H1), 4.78 (d, *J* = 11.5 Hz, 1 H, OBn), 4.80 (d, *J* = 11.5 Hz, 1 H, OBn), 4.96 (d, *J* = 11.5 Hz, 1 H, OBn), 5.03 (d, *J* = 12.0 Hz, 1 H, OBn), 5.10 (d, *J* = 12.0 Hz, 1 H, OBn), 5.90 (m, 1 H, NH), 7.24–7.36 (m, 20 H, ArH).

$^{13}$C NMR ($CDCl_3$): δ = 25.1, 25.4, 25.7, 29.1, 32.2, 32.9, 33.0, 37.6, 40.0, 62.4, 65.9, 66.2, 67.0, 69.4, 71.5, 72.2, 76.7, 77.8, 101.1, 127.5–135.1 (signals of aromatic C-atoms), 156.2, 164.8, 165.5, 165.9, 172.1.

HRMS: *m*/*z* $[M + Na]^+$ calcd for $C_{38}H_{43}NO_7$: 648.2932; found: 648.2931.

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