A Selectively Deprotectable 2,6-Diaminogalactose Scaffold for the Solid-Phase Synthesis of Potential RNA Ligands

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Abstract: In the context of aminoglycosides as efficient binders to RNA, a 2,6-diaminogalactose scaffold was developed for combinatorial syntheses of potential RNA ligands in the solid phase. A set of selectively removable, orthogonally stable protecting groups in combination with a linker stable throughout the synthesis, allows for selective deprotection and introduction of a side chain in each position of this scaffold. A few of the synthesized compounds exhibit inhibition of HIV-1 infection in HeLa cells that contain a TAR-controlled reporter gene.

Key words: solid-phase synthesis, RNA ligands, protecting groups, combinatorial chemistry, inhibition of HIV RNA

Small molecules selectively binding to RNA are of particular interest, e.g. as potential antiviral drugs.1 Aminoglycosides, e.g. kanamycin, neomycin B, or tobramycin, were shown to efficiently bind to RNA.2 These molecules are potent inhibitors of the interaction of the HIV-1 TAR RNA with the Tat protein.3 A variety of compounds derived from aminoglycosides as the lead structures have been investigated as ligands of RNA, including spacer-linked dimers,4 heterocyclic derivatives,5 macro cyclic oligo- amino sugars,6 oligomers obtained from diamino sugar carboxylic acids,7 and peptides rich in arginine and lysine.8 To take advantage of a combinatorial search for biologically active molecules, we recently developed selectively deprotectable carbohydrate scaffolds for combinatorial syntheses in solid phase.9 As neamine is an important substructure of aminoglycoside antibiotics, it appeared attractive to apply this scaffold concept to diamino sugars.10 Amino sugar scaffolds should allow not only for a spatial arrangement of amino groups, and therefore positively charged groups as in natural aminoglycosides, but should also offer variable opportunities to install other side chains, e.g. intercalating groups, at the scaffold, provided a sufficiently orthogonal protecting group concept can be developed.

We here report on the elaboration of such a concept for 2,6-diamino-D-galactose as the carbohydrate framework. For selective deprotection of a carbohydrate scaffold in each position, a set of orthogonally stable protecting groups is required.11 In addition, a linker stable throughout all deprotection and substitution reactions is needed if the combinatorial syntheses are performed in solid phase. This linker must be cleavable without the functional side chains introduced into the scaffold during the solid-phase synthesis being affected. To meet these requirements, D-galactose was converted into the O-acetylated 2-azido-galactosyl nitrate 1 (Scheme 1).12 After cleavage of the O-nitrate with hydrazine acetate,13 and subsequent formation of the trichloroacetimidate 2,14 glycosylation of methyl 4-sulfanylbutyrate15 furnished sulfanylglycoside 3 as a mixture of anomers (α/β, 3:1) (Scheme 1).

Removal of the O-acetyl groups with catalytic sodium methoxide in methanol and subsequent formation of the 4,6-(4-methoxybenzylidene) acetal 4 was followed by 3-O-silylation to give 5a (Scheme 1). Reduction of the azido group with benzenethiol and tin(II) chloride16 and subsequent N-acetylation with allyloxycarbonyl chloride (AlocCl) furnished 5b, which was subjected to regioselective reductive acetal opening with triethy silane and p-nitrobenzaldehyde dichloride17 to give 6 (Scheme 1). The free 6-hydroxy group was substituted for the azido group by a Mitsunobu reaction using an acyl azide as the source of the nucleophile.18 The ester group of the thus-obtained sulfanylglycoside 7, fully protected with distinguishable protecting groups, was saponified to furnish the carboxylic acid 8 (Scheme 1), which can be used for immobilization. Carboxylic acid 8 was coupled to Rink amide polystyrene (100–200 mesh, 0.70 mmol/g) or Rink amide Tentagel resin (0.23 mmol/g) by use of 1H-benzotri azol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU)19 and N-hydroxybenzotriazol (HOBt)20 in the presence of N,N-diisopropylethylamine (Scheme 2).

The successful attachment of the scaffold was confirmed by the appearance of a strong IR absorption band of the azido group at 2100 cm⁻¹ and high-resolution magic-angle spinning (HRMAS) NMR spectroscopy.20 In addition, the coupling reactions were monitored by the Kaiser test and by elemental analysis regarding sulfur. Finally, unchanged benzhydryl amino groups were acetylated (capped) with acetic anhydride/pyridine. The selective deprotection reactions on the scaffolds 9 (Scheme 3) were accomplished in analogy to the procedures described for the 2,6-diaminogalactose template.10 The N-Aloc group was removed by palladium(0)-catalyzed allyl transfer to p-toluenesulfonic acid as the trapping nucleophile to af-
ford 10 (Scheme 3). 21 Deprotection of the 3-position to give 11 was achieved with tetrabutylammonium hydrogen difluoride solution (TBAHDF) in acetonitrile (Scheme 3). The p-methoxybenzyl group was removed by selective oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone 22 in dichloromethane and water (10 vol%) to furnish 12 (Scheme 3). In the case of Rink amide polystyrene resin, these cleavage conditions resulted in degradation of the polymer and prevented the isolation of the desired products from 12a. For the reduction of the azido function, a Staudinger protocol23 was applied, for which tri- n-butyolphosphane in a tetrahydrofuran–water–triethylamine mixture was used (Scheme 3). This reaction, resulting in 13, as well as the removal of the N-Aloc group were monitored by the Kaiser test. In addition, all cleavage reactions were controlled once again by FTIR and 1H HRMAS NMR spectroscopy.

For the selective introduction of side chains at the 3- and 4-positions of the monosaccharide scaffold, carbamoylation (and esterification) reactions of 9a and 9b were examined (Scheme 4). Carbamoylation of the resin-linked scaffolds 11 and 12 (obtained from 9a/9b, en route to 14...
and 15, respectively, Scheme 4) was performed with aryl isocyanates in the presence of catalytic amounts of 4-(N,N-dimethylamino)pyridine (Scheme 4). After carbamoylation of the 4-position, it was necessary to remove the silyl ether with tetrabutylammonium hydrogen difluoride prior to the release from the polymer. Otherwise the tert-butyldimethylsilyl group is only incompletely cleaved during the subsequent treatment with trifluoroacetic acid, and mixtures of silylated and desilylated derivatives were obtained. The cleavage from the solid support with 50% trifluoroacetic acid in dichloromethane and sulfanylmethyl polystyrene furnished the desired products 14 and 15 in sufficient purity and satisfying yields (Scheme 4, Table 1).

As an alternative to the formation of urethanes, acylation reactions were performed at scaffolds 10, 11, and 13 to generate a library of amino acid– or peptide–carbohydrate conjugates. Esterification of scaffolds 11 using \( N,N' \)-disopropylcarbodiimide in the presence of 4-(\( N,N' \)-dimethylamino)pyridine according to Steglich gave esters 18 in good yields (Scheme 5). Only during the reaction of 11b (Tentagel) with Fmoc-His(Trt)-OH did some racemization occur. For coupling of the acyl components to the free amino groups in the 2-position (10) or 6-position (13) (to generate 16/17 or 19/20, respectively), \( O-(1H\)-benzotriazol-1-yl)-\( N,N',N' \)-tetramethyluronium tetrafluoroborate (TBTU), \( N \)-hydroxybenzotriazole (HOBt), and \( N,N \)-disopropylethylamine were used (Scheme 5).

After three or four steps, the crude amino acid– or peptide–carbohydrate conjugates 16–20 were isolated as mixtures of the 3-hydroxy compound and its silyl ether in satisfying yields in most cases (Scheme 5). After separation by semipreparative HPLC, their structures were confirmed by mass spectrometry. The values given for the purity of products 16–20 were determined by UV absorption, and are too low owing to the presence of impurities with large extinction coefficients. The change of the cation scavenger in the detachment reaction from sulfanyl-
methyl polystyrene to dimethyl sulfide solution appeared advantageous. The yields as well as the purity of the products were improved (Table 2). For the biological evaluation of the synthesized compounds on cells in vivo, a careful purification of products 14–20 was necessary. However, the solubility of compounds 14–20 in solvents needed for purification by semipreparative HPLC is low, and caused problems. Therefore, considerable loss of the synthesized products could not be prevented in the course of purification.

In additional experiments, the extension of the amino acid side chain was explored. To this end, the 9-fluorenylmethoxycarbonyl (Fmoc) group of the carbohydrate-linked amino acid was removed by use of piperidine in N,N-dimethylformamide. Subsequently, a second N-protected amino acid was coupled to give the peptide derivatives 16g and 17g, 19f, and 20f. It is noteworthy that the replacement of the Fmoc group by a less hydrophobic protecting group such as the N-benzoyloxycarbonyl (Z) group afforded products 18e and 18f in comparable yields. The sulfanylglycoside linker introduced as an option for a later activation and glycoside formation also has influence on the solubility of compounds 14–20. Components of a library of analogous glucose derivatives containing a 3-sulfanylpropionamide showed increased solubility in acetonitrile and, logically, these compounds were easier to purify. Despite these problems during the purification, almost all synthesized diaminogalactoside derivatives 14–20, except for the arginine derivatives, were isolated in sufficiently pure form (by analytical HPLC). They were obtained in amounts adequate for a biological evaluation in experiments on cell cultures. It should be noted that the crude products exhibited considerable cytotoxicity; this is obviously due to the toxicity of the accompanying impurities.

Because of these experiences, prior to probing the antiviral effects of these compounds, their cytotoxicity had to be investigated. The vitality of the cultured HeLa P4 cells was monitored in terms of the concentration of ATP in all metabolically active cells, which was measured in a luminometric assay of the ATP-dependent oxidative decarboxylation of luciferin at 37 °C. It was shown that the purified compounds showed no cytotoxicity at a concentration of 100 μM. Some of them, however, exhibited low cytotoxicity at a concentration of 200 μM. The antiviral effects of the synthesized carbohydrate derivatives 14–20 were determined for HeLa P4 cells that had been transfected with the HIV-1 receptors CD4, CCR5, and CXCR4 and a β-galactosidase reporter gene under control of the HIV-LTR promoter. These cells were infected with a solution of the virus strain HIV-1Lai in the absence (control) or presence of synthetic compounds 14–20 in varying concentrations (10, 25, 50, 100, and 200 μM). The virus proliferation was determined by measuring the galactosi-
dase reporter enzyme in a chemiluminescence assay.26 Briefly summarized, the results are the following. The 3-O- and 4-O-carbamates of the diaminogalactose structures 14 and 15 exhibited no cytotoxicity in concentrations of 200 μM, but also showed no inhibitory activity. Likewise, the 3-O-amino acid and peptide conjugates 18 exhibit neither cytotoxicity nor an inhibitory effect, except for 18b, which caused 30% inhibition of virus production at a concentration of 200 μM. Of the 3-O-silyl-2-N-amino acyl derivatives 17, some compounds showed inhibition of virus proliferation even at a concentration of 10 μM: Fmoc-Gly derivative 17a, 30%; Fmoc-Leu 17b, 30%; Fmoc-Glu 17c, 45%; Fmoc-Gln 17f, 25%. The most potent compound, however, belonged to the 6-N-acyl series 19 and 20. The N-(phenylacetyl) derivative 20e caused 60% inhibition of virus production compared to the control at a concentration of 10 μM. However, only one other compound of this series 19 and 20, the Fmoc-Gln derivative 20c, exerted weak inhibition (45% at a concentration of 45 μM) (Figure 1).

Table 2 Synthesis of a Library of O- and N-Acylated Diaminogalactose Derivatives 16–20

<table>
<thead>
<tr>
<th>Products 16–20</th>
<th>Resin 9</th>
<th>R1</th>
<th>R2</th>
<th>R4</th>
<th>Yield (%)</th>
<th>Purity (%)</th>
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<tbody>
<tr>
<td>16a</td>
<td>9a</td>
<td>Fmoc-Gly-</td>
<td>H</td>
<td>N3</td>
<td>60</td>
<td>–</td>
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<tr>
<td>17a</td>
<td>9a</td>
<td>Fmoc-Gly-</td>
<td>TBS</td>
<td>N3</td>
<td>–</td>
<td>40</td>
</tr>
<tr>
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<td>9a</td>
<td>Fmoc-Leu-</td>
<td>H</td>
<td>N3</td>
<td>82</td>
<td>–</td>
</tr>
<tr>
<td>17b</td>
<td>9a</td>
<td>Fmoc-Leu-</td>
<td>TBS</td>
<td>N3</td>
<td>–</td>
<td>33</td>
</tr>
<tr>
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<td>9a</td>
<td>Fmoc-Glu-</td>
<td>H</td>
<td>N3</td>
<td>51</td>
<td>–</td>
</tr>
<tr>
<td>17c</td>
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<tr>
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<td>9a</td>
<td>Fmoc-Arg-</td>
<td>TBS</td>
<td>N3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
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<td>9a</td>
<td>Fmoc-His-</td>
<td>H</td>
<td>N3</td>
<td>56</td>
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<td>17e</td>
<td>9a</td>
<td>Fmoc-His-</td>
<td>TBS</td>
<td>N3</td>
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<td>36</td>
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<tr>
<td>16f</td>
<td>9a</td>
<td>Fmoc-Gln-</td>
<td>H</td>
<td>N3</td>
<td>74</td>
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<td>17f</td>
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<tr>
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<td>9b</td>
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<td>H</td>
<td>N3</td>
<td>–</td>
<td>–100</td>
</tr>
<tr>
<td>17g</td>
<td>9b</td>
<td>Fmoc-Arg-</td>
<td>TBS</td>
<td>N3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>16h</td>
<td>9b</td>
<td>Fmoc-His-</td>
<td>H</td>
<td>N3</td>
<td>–</td>
<td>–100</td>
</tr>
<tr>
<td>17h</td>
<td>9b</td>
<td>Fmoc-His-</td>
<td>TBS</td>
<td>N3</td>
<td>–</td>
<td>24</td>
</tr>
<tr>
<td>16i</td>
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<td>N3</td>
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<td>35</td>
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<tr>
<td>17i</td>
<td>9b</td>
<td>Fmoc-His-</td>
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<td>N3</td>
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<td>–100</td>
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<tr>
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<tr>
<td>16k</td>
<td>9b</td>
<td>Fmoc-Gln-</td>
<td>H</td>
<td>N3</td>
<td>–</td>
<td>53</td>
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<tr>
<td>17k</td>
<td>9b</td>
<td>Fmoc-Gln-</td>
<td>TBS</td>
<td>N3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>16m</td>
<td>9b</td>
<td>Aloc Fmoc-Arg-</td>
<td>N3</td>
<td>51</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>18a</td>
<td>9b</td>
<td>Aloc Fmoc-Gln-</td>
<td>N3</td>
<td>46</td>
<td>43</td>
<td></td>
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<tr>
<td>18b</td>
<td>9b</td>
<td>Aloc Fmoc-His- N3</td>
<td>50</td>
<td>60 (24)</td>
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<tr>
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<td>9b</td>
<td>Aloc Fmoc-His-Gly-</td>
<td>N3</td>
<td>–100</td>
<td>70</td>
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<tr>
<td>18d</td>
<td>9b</td>
<td>Aloc Fmoc-His-Gly-</td>
<td>N3</td>
<td>–100</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>18e</td>
<td>9b</td>
<td>Aloc Z-Gly-Gly-</td>
<td>N3</td>
<td>–100</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>18f</td>
<td>9b</td>
<td>Aloc Z-Gly-His-</td>
<td>N3</td>
<td>–100</td>
<td>37</td>
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<tr>
<td>19a</td>
<td>9b</td>
<td>Aloc H Fmoc-His-</td>
<td>N3</td>
<td>84</td>
<td>54</td>
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<tr>
<td>20a</td>
<td>9b</td>
<td>Aloc TBS Fmoc-His-</td>
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The concept of selectively removable, orthogonally stable protecting groups as given in the 2,6-diaminogalactose scaffolds allows for a selective introduction of side chains in the scaffold in solid-phase syntheses. After three (or four) steps in solid phase and cleavage from the resin, the crude compounds were obtained in satisfying yields. The crude products contain impurities which have high UV extinction coefficients and cause cytotoxicity on the cells used in in vivo inhibition experiments. Sufficient purification of the scaffold derivatives turned out to be the most difficult and time-consuming procedure. Therefore, further syntheses aimed at structure optimization of inhibitors should preferably be performed in solution rather than on solid phase. Among the synthesized compounds, 6-N-acyl and 2-N-aminoacyl derivatives showed the most potent inhibition of HIV-1 infection in HeLa cells determined via a TAR-controlled reporter gene product.

Solvents were distilled and pre-dried according to standard procedures. Reagents were bought in the highest available commercial quality and used without further purification. Rink amide PS was purchased from Novabiochem and Rink amide Tentagel from Rapp Polymere, Tübingen, Germany. Analytical TLC was carried out on silica gel 60 F254 (Merck). Flash chromatography was performed (silica gel, 0.032–1.063 mm, ICN Biomedicals). IR spectra were recorded on a Perkin-Elmer FT-IR 1760X spectrometer. RP-HPLC analyses were carried out on a Knauer Maxi Star HPLC system with a Phenomenex Luna C18(2) column (5 μm, 250 × 4.60 mm), and at a pump rate of 1 mL/min. Semipreparative RP-HPLC separations were performed on a Knauer HPLC system with a Phenomenex Luna C18(2) column (10 μm, 250 × 21 mm), and at a pump rate of 10 mL/min. H2O–MeCN mixtures were used as the solvents. The following gradients were used for analytical HPLC: gradient A: H2O–MeCN, 70:30, 10 min, then 70:30 to 50:50, 2 min, then 50:50 to 30:70, 2

See Scheme 5 for reagents and conditions.

Yield of crude product.

Purities of crude products were determined by RP-HPLC (UV 210–220 nm).

A mixture of epimers was obtained.
min, then 30:00 to 1:00, 2 min, then 0:10, 23 min; gradient B: H2O–MeCN, 70:30 to 0:10, 30 min, then 0:10, 10 min; gradient C: H2O–MeCN, 90:10 to 0:10, 30 min, then 0:10, 10 min; gradient D: H2O–MeCN, 50:50 to 0:10, 30 min, then 0:10, 10 min. The following gradients were used for semipreparative HPLC: gradient E: H2O–MeCN, 70:30, 20 min, then 70:30 to 50:50, 4 min, then 50:50 to 30:70, 6 min, then 30:70 to 0:10, 4 min, then 0:10, 20 min; gradient F: H2O–MeCN, 70:30 to 0:10, 60 min, then 0:10, 15 min; gradient G: H2O–MeCN, 70:30 to 0:10, 90 min, then 0:10, 25 min. 1H and 13C, and 2D NMR spectra were recorded on a Bruker AC-300 or a Bruker AM-400 spectrometer. Centered multiplicities are abbreviated as Ms. Optical rotations [α]D were measured on a Perkin Elmer polarimeter 241. ESI-MS spectra were measured on a ThermoQuest Navigator instrument and FD-MS spectra on a Finnigan-MAT 95 spectrometer.

Methyl 4-[(3,4,6-Tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosylsulfanyl)butyrate (3)

Activated 4 Å MS (10 g) were added to a soln of 2 (19.7 g, 0.041 mol) in Et2O (750 mL). The suspension was stirred at rt. for 1 h and cooled to 0 °C. Methyl 4-sulfanylbutyrate (10.9 g, 0.081 mol) was added. BF3·OEt2 (26.4 mL, 29.8 g, 0.21 mol) was dissolved in Et2O (30 mL) and slowly added to the reaction mixture. Stirring was continued at 0 °C for 1 h and subsequently at rt. for 6 h. After filtration of the mixture, the filtrate was washed with sat. aq NaHCO3 (700 mL). The organic phase was washed with sat. aq NaHCO3 (3 × 500 mL), dried (MgSO4), and concentrated in vacuo. Purification of the residue was performed by flash chromatography (silica gel, cyclohexane–EtOAc, 3:1); this afforded 3.

Yield: 11.0 g (58%); αβ = 3:1; colorless amorphous solid; Rf = 0.12 (cyclohexane–EtOAc, 3:1); this afforded 3.

IR (NaCl): 2113 (N1) cm⁻¹.

1H NMR (400 MHz, CDCl3): δ = 5.54 (d, J1,2 = 5.5 Hz, 0.75 H, H-1α), 5.39 (d, J1,2 = 2.5 Hz, 0.75 H, H-2α), 5.34 (d, J1,2 = 2.6 Hz, 0.25 H, H-4γ), 5.07 (dd, J2,3 = 11.0 Hz, 0.75 H, H-3β), 4.84 (dd, J2,3 = 10.3 Hz, 0.33 H, 25.3 H, 3-β)), 4.52 (t, J2,3 = 6.2 Hz, 0.75 H, 3-Hα), 4.35 (d, J1,2 = 10.2 Hz, 0.25 H, H-1β), 4.20 (dd, J1,2 = 5.5 Hz, J2,3 = 11.0 Hz, 0.75 H, 2-Hα), 3.89–4.01 (m, 2 H, H-6β), 3.71 (t, J1,2 = 7.0 Hz, 0.25 H, H-5β), 2.87–2.89 (m, 2 H, SCH2CH2), 2.45–2.39 (m, 2 H, CH2CH2), 2.11 (s, 3 H, CH3), 2.01, 2.00 (s, 6 H, CH2), 1.96–1.88 (m, 2 H, SCH2CH2).

13C NMR (100.6 MHz, CDCl3): δ = 173.3, 170.4, 170.1, 170.0 (C=O), 85.0 (C1β), 83.5 (C1a), 74.4 (C5β), 73.0 (C5β), 70.2 (C3α), 67.5 (C4α), 67.1 (C5a), 66.6 (C4β), 61.6 (C6), 60.5 (C2β), 57.8 (C2α), 51.6 (CO2CH3), 32.7 (CH2CO2CH3), 32.5 (CH2CO2CH3), 30.4 (SCH2CH2), 29.7 (SCH2CH2), 25.1 (SCH2CH2), 24.6 (SCH2CH2), 20.7 (3 × CH3, OAc).

MS (FD): m/z (%) = 447.7 (100) [M]+.

Methyl 4-[(2-Azido-3-O-(tert-butyldimethylsilyl)-2-deoxy-4-O-(4-methoxybenzylidene)-α-D-galactopyranosylsulfanyl)butyrate (5a)

TSBOTT (3.96 mL, 4.56 g, 17.2 mmol) was added to a soln of 4a (3.6 g, 11.5 mmol) and py (3.96 mL, 9.08 g, 115.0 mmol) in anhyd DMP (100 mL) at 0 °C. After 20 min, the reaction mixture was added to sat. aq NaHCO3 (60 mL) at 0 °C. After addition of CH2Cl2 (100 mL) and H2O (100 mL), the organic layer was extracted with CH2Cl2 (3 × 500 mL), dried (MgSO4), and concentrated in vacuo. Purification by flash chromatography (silica gel) gave 5a.

Yield: 6.22 g (99%); colorless amorphous solid; [α]D = 144.5 (c 1.0, CHCl3); Rf = 0.68 (CH2Cl2–EtOAc, 20:1); Rf = 0.73 (cyclohexane–EtOAc, 1:1).

IR (NaCl): 2100 (N1) cm⁻¹.

1H NMR (400 MHz, CDCl3): δ = 7.44 (d, J2 = 9.0 Hz, 2 H, H3-PMB), 6.89 (d, J2 = 9.0 Hz, 2 H, H5-PMB), 5.51 (s, 1 H, CH, aceatal), 5.46 (d, J2,3 = 5.09 Hz, 1 H, H-1), 4.22–4.20 (m, 2 H, H2–H6a), 4.08 (m, 1 H, H-4), 4.07 (dd, J2 = 12.5 Hz, J1 = 1.6 Hz, 1 H, H-6b), 4.00 (br s, 1 H, H-5), 3.94 (dd, 1 J1 = 10.2 Hz, 3 J1 = 3.5 Hz, 1 H, H-3), 3.80 (s, 3 H, OCH3-PMB), 2.67 (s, 3 H, CO2CH3), 2.64 (m, 2 H, SCH2CH2), 2.43 (t, J = 7.2 Hz, 2 H, CH2CO2CH3), 1.96 (m, 2 H, SCH2CH2), 0.92 (s, 3 H, CH3-1Bu), TBS), 0.17 (s, 3 H, CH3, TBS), 0.13 (s, 3 H, CH3, TBS).
**1**C NMR (100.6 MHz, CDCl₃): δ = 173.4 (C=O), 160.1 (C₆-PMB), 130.0 (C-PMB), 127.4 (C₆-PMB), 113.6 (C₆-PMB), 100.8 (CH₃-PMB), 86.4 (C₁), 76.4 (C₄), 70.9 (C₃), 69.4 (C₆), 63.6 (C₅), 61.1 (C₂), 55.4 (OCH₂-PMB), 51.8 (CO₂CH₃), 32.9 (CH₂CO₂CH₃), 30.3 (SCH₂CH₃), 25.8 (CH₃-Th, TBS), 24.9 (SCH₂CH₃), 18.2 (CH₃-Th), TBS, 4.4 (CH₃-Th), TBS.

MS (FD): m/z (%) = 553.2 (5%) [M⁺], 497.0 (100%) [M+ t-Bu + H⁺], 420.9 (6%) [M − CH₃CH₂CO₂CH₃ + H⁺].

**Methyl 4-(2-[[ Allyloxy]carbonyl][ amino]-3-O-t ( tert- butyldimethylsilyl) -2 deoxy- 4,6- O (4- methoxybenzylidene) -α-D- galactopyranosyl]sulfanyl]butyrate (5b)**

A solvent of 5a ([6.22 g, 11.2 mmol]) was added dropwise to a solution of anhyd SO₃(1.38 g, 16.8 mmol) in Py (400.0 mL). After stirring for 1 h at rt, the mixture was diluted with CH₂Cl₂. The crude residue by flash chromatography (silica gel) afforded pure azide (5b).

**Yield:** 499 mg (62%); colorless oil; [a]₁₀⁺ 82.9 (c 1.0, CHCl₃); Rᵣ = 0.10 (cyclohexane-EToc, 3:1).

**1**H NMR (400 MHz, CDCl₃): δ = 7.30 (d, J = 8.6 Hz, 2 H, CH₂-PMB), 6.88 (d, J = 8.7 Hz, 2 H, CH₂-PMB), 5.91 (m, 1 H, CH₂=CH₂-Ch), 5.38 (d, J = 4.7 Hz, 1 H, H₁, H₁), 5.32 (d, J = 16.7 Hz, 1 H, CH₂=CH-Ch, Alloc, (trans)), 5.22 (dd, J = 10.6 Hz, 1 H, CH₂=CH-Ch, Alloc, (cis)), 4.96 (d, J = 11.1 Hz, 1 H, PMB=CH₂-Ha), 4.70 (d, J = 9.6 Hz, 1 H, NH=CHR), 4.61–4.54 (m, 3 H, H₂=CH₂-Alloc), 4.11 (t, J = 5.3 Hz, 1 H, H₅), 3.80 (s, 4 H, H₆a, OCH₂-PMB), 3.73 (m, 2 H, H₃=CH₃, 3.66 (s, 3 H, CO₂CH₃), 3.55 (dd, J = 11.6 Hz, J = 4.5 Hz, 1 H, H₁-6b, 2.66 (m, 2 H, SCH₂CH₃), 2.42 (m, 2 H, CH₂CO₂CH₃), 1.94 (m, 2 H, SCH₂CH₃), 0.92 (s, 9 H, CH₃-Th, TBS), 0.15 (s, 3 H, CH₃-Th, TBS), 0.12 (s, 3 H, CH₃-Th, TBS).

MS (FD): m/z (%) = 613.5 (100%) [M⁺].

**Methyl 4-(2-[[ Allyloxy]carbonyl][ amino]-6-azido- 3-O-t ( tert- butyldimethylsilyl) - 2,6 die deoxy- 4,6- O (4- methoxybenzylidene) -α-D- galactopyranosyl]sulfanyl]butyrate (7)**

Ph₃P (1.31 g, 5.00 mmol), DIAD (0.99 mL, 1.01 g, 5.00 mmol), and nitromethane azide was added to 6 (770 mg, 1.25 mmol) in anhyd toluene (60 mL) at rt. The mixture was stirred for 2 h. MeOH (20 mL) was added and the solution was concentrated in vacuo. The residue was triturated with Et₂O at 0 °C. The solution was filtered, and the filtrate was concentrated in vacuo. Purification by flash column chromatography (silica gel) gave pure azide 7.

**Yield:** 499 mg (62%); colorless oil; [α]₁₀⁺ 82.9 (c 1.0, CHCl₃); Rᵣ = 0.10 (cyclohexane-EToc, 3:1).

IR (NaCl): 2102 (N₃) cm⁻¹.

**1**H NMR (300 MHz, CDCl₃): δ = 7.28 (d, J = 8.9 Hz, 2 H, PMB), 6.88 (d, J = 8.6 Hz, 2 H, PMB), 5.91 (m, 3 H, CH₃=CH₂-Ch, Alloc), 3.80 (s, 3 H, OCH₂-PMB), 3.75 (m, 1 H, H₃), 3.10 (s, 3 H, CO₂CH₃), 3.18 (m, 2 H, SCH₂CH₃), 2.42 (d, J = 7.0 Hz, J = 2.3 Hz, 2 H, CH₂CO₂CH₃), 1.95 (m, 2 H, SCH₂CH₃), 1.85 (s, 9 H, CH₃-Th, TBS), 0.50 (s, 6 H, 2 × CH₃-Th, TBS).

**MS (FD): m/z (%) = 621.2 (100%) [M + H⁺], 551.5 (62%) [M + t-Bu + H⁺].

**Methyl 4-(2-[[ Alloxy]carbonyl][ amino]-3-O-t ( tert-butylidemethylsilyl)-2-deoxy-4,6-O(4-methoxybenzylidene)-α-D-galactopyranosyl)sulfanyl]butyrate (6)**

A suspension of acetal 5 ([1.03 g, 1.68 mmol]) was activated and 4 Å MS (1.0 g) in CH₂Cl₂ (65 mL) was stirred at rt for 1 h. The suspension was cooled to 78 °C and TESO (0.81 mL, 5.07 g, 5.04 mmol) was added dropwise. Subsequently, Ph₃BCl (0.74 mL, 0.907 g, 5.71 mmol) was added. After 5 min, Et₃N (2 mL) and MeOH were added and the solution was stirred for 5 min. The cold solution was diluted with CH₂Cl₂ (50 mL) and filtered, and the filtrate was washed with sat. NaHCO₃ (3 × 100 mL) and 1 M HCl (100 mL) and finally stirred with 1 M citric acid (100 mL) for 30 min. The organic layer was separated, dried (MgSO₄), and concentrated in vacuo. Purification by flash chromatography (silica gel) afforded pure product 6.

**Yield:** 480 mg (81%); colorless oil; [α]₁₀⁺ 82.9 (c 1.0, CHCl₃); Rᵣ = 0.10 (cyclohexane-EToc, 3:1).
4-[[Allyloxy]carbonyl]amino]-6-azido-3-O-(tert-butylidemethylmethylidene)-2,6-dideoxy-4-O-(3-methoxybenzyl)-α-D-galactopyranosyl)sulfanyl)butyric Acid (8)

LiOH·H2O (0.55 g, 13.2 mmol) was added in one portion to a soln of ester 7 (2.10 g, 3.3 mmol) in THF–H2O (10:1, 44 mL). The mixture was stirred at r.t. for 16 h and neutralized with an acidic cation exchanger (Amberlite IR120). After filtration of the mixture, the solvent was removed in vacuo and subsequently subjected to lyophilization. The crude carboxylic acid 8 that was obtained was used for coupling to the polymer support.

Yield: 1.96 g (94%); colorless oil; [α]D 213 93.6 (c 1.0, CHCl3); Rf = 0.10 (cyclohexane–EtOAc, 3:1).

1H NMR (300 MHz, CDCl3): δ = 7.26 (m, 2 H, PMB), 6.86 (d, J = 8.6 Hz, 2 H, PMB), 5.89 (m, 1 H, CH2=CH-, Alloc), 5.30 (m, 2 H, H-1, CH2=CH-, Alloc), 5.20 (dd, J = 10.7 Hz, 1 H, PMB-H2A), 4.68 (d, J = 9.6 Hz, 1 H, H-2, Alloc), 4.54 (d, J = 10.4 Hz, 1 H, PMB-CH2B), 4.20–4.16 (m, 1 H, H-5), 3.79 (s, 3 H, OCH3-PMB), 3.72 (m, 1 H, H-3), 3.61 (m, 1 H, H-4), 3.53 (dd, J = 12.8 Hz, 1 H, H-6a), 3.05 (dd, J = 12.7 Hz, 2 H, J = 4.6 Hz, 1 H, H-6b), 2.77–2.58 (m, 2 H, SCH2CH3), 2.46 (t, J = 7.1 Hz, 2 H, SCH2CH2), 1.94 (m, 2 H, SCH2CH2), 0.89 (s, 9 H, CCH3-Bu), TBS), 0.13 (s, 3 H, CH3-TBS), 0.10 (s, 3 H, CH3-TBS).

Polymer-Bound Building Blocks 9a and 9b

Resin 9a

In a solid-phase reaction vessel, Fmoc-protected Rink amide polystyrene (2.63 g, 1.84 mmol, loading 0.70 mmol/g, 100–200 mesh, crosslinked with 1% divinylbenzene) was swollen in DMF (30 mL) for 1 h, before a 50% soln of carboxylic acid 8 (1.149 g, 1.84 mmol) was dissolved in DMF (40 mL) and activated with HBTU (0.698 g, 1.84 mmol), and DIPEA (3.4 mL, 2.38 g, 18.4 mmol). The soln was mixed for 3–5 min and finally added to the prepared Rink amide PS. The mixture was shaken for 30 min at r.t. The polymer was washed with DMF and the removal of the Fmoc group was repeated. Finally, the resin was washed with DMF and dried thoroughly in vacuo.

Resin 9b

Resin 9b was obtained by a reaction between Fmoc-protected Rink amide Tentagel (6.391 g, 1.47 mmol, loading 0.23 mmol/g), carboxylic acid 8 (0.916 g, 1.47 mmol), HBTU (0.472 g, 1.47 mmol), BzOH·H2O (0.225 g, 1.47 mmol), and DIPEA (3.4 mL, 2.38 g, 18.4 mmol). The soln was mixed for 3–5 min and finally added to the prepared Rink amide PS. The mixture was shaken for 17 h at r.t. The polymer was washed with DMF (10 mL), DMF–MeOH (10 mL, 1:1), MeOH–CH2Cl2 (10 mL, 1:1), MeOH–Et0O (10 mL, 1:1), and EtO (10 mL) and dried thoroughly in vacuo; yield: 1.15 mmol/g (determined by elementary analysis of sulfur), corresponding to a coupling yield of 95%. Finally, unchanged benzhydryl alcohol groups were acylated with a soln of Ac2O–py (20 mL, 2:1, v/v) in CH2Cl2 (20 mL) for 45 min. The polymer was washed with CH2Cl2 (10 mL), CH2Cl2–MeOH (10 mL, 1:1), CH2Cl2 (10 mL), and EtO (10 mL), and dried thoroughly in vacuo; this gave resin 9a.

IR (KBr): 2100 (N3) cm–1.

Yield: 31.0 mg (85%, crude product); colorless amorphous solid.

Analytical HPLC: gradient A, tR = 17.60 min (38%).

Semipreparative HPLC: gradient E, tR = 30.00 min; yield: 1.5 mg (25%, based on polymer-bound carbohydrate); colorless amorphous solid.

ESI-MS: m/z (%) = 565.3 (7) [M + K]+, 549.3 (100) [M + Na]+, 527.3 (18) [M + H]+.

4-[[Allyloxy]carbonyl]amino]-6-azido-3-O-(4-fluorophenyl)carbamoyl]-2,6-dideoxy-α-D-galactopyranosyl)sulfanyl)butyramide (14a)

Yield: 9.5 mg (ca. 100%, crude product); yellow amorphone solid.

Analytical HPLC: gradient A, tR = 16.92 min (52%).

Semipreparative HPLC: gradient E, tR = 31.02 min; yield: 3.5 mg (45%, based on polymer-bound carbohydrate); colorless amorphous solid.

ESI-MS: m/z (%) = 565.3 (7) [M + K]+, 549.3 (100) [M + Na]+, 527.3 (18) [M + H]+.

4-[[Allyloxy]carbonyl]amino]-6-azido-3-O-(4-cyanophenyl)carbamoyl]-2,6-dideoxy-α-D-galactopyranosyl)sulfanyl)butyramide (14b)

Yield: 6.5 mg (81%, crude product); yellow oil.

Analytical HPLC: gradient A, tR = 17.60 min (38%).

Semipreparative HPLC: gradient E, tR = 30.00 min; yield: 1.5 mg (19%, based on polymer-bound carbohydrate); colorless amorphous solid.

ESI-MS: m/z (%) = 572.3 (7) [M + K]+, 556.3 (100) [M + Na]+, 543.3 (25) [M + H]+.

4-[[Allyloxy]carbonyl]amino]-6-azido-3-O-(4-chlorophenyl)carbamoyl]-2,6-dideoxy-α-D-galactopyranosyl)sulfanyl)butyramide (14c)

Yield: 31.0 mg (85%, crude product); colorless amorphous solid.

Analytical HPLC: gradient A, tR = 18.28 min (46%).

Semipreparative HPLC: gradient E, tR = 32.57 min; yield: 9.2 mg (25%, based on polymer-bound carbohydrate); colorless amorphous solid.

1H NMR (400 MHz, CDCl3): δ = 7.20 (d, J = 8.2 Hz, 1 H, H5-Me, carbamate), 7.11 (d, J = 8.9 Hz, 2 H, HAry1, carbamate), 5.67 (m, 1 H, CH2=CH-, Alloc), 5.60 (d, J = 9.3 Hz, 1 H, NFF-C2), 5.31 (d, J = 5.4 Hz, 1 H, H-1), 5.08 (dd, J = 17.3 Hz, 1 H, J = 1.3 Hz, 1 H, CH2=CH-, Alloc), 4.69 (dd, J = 11.6 Hz, J = 2.9 Hz, 1 H, H-3), 4.43 (m, 1 H, H-2), 4.37 (m, 2 H, CH2, Alloc), 4.22 (m, 1 H, H-5), 3.89 (m, 1 H, H-4), 3.53 (dd, J = 12.8 Hz, J = 8.2 Hz, 1 H, H6-

H-6a), 3.22 (m, 1 H, H-6b), 2.58 (m, 2 H, SCH₂CH₂), 2.21 (t, J = 7.4 Hz, 2 H, CH₂CO₂CH₂), 1.84 (s, 2 H, SCH₂C₆H₅).


4-[[2-[(Allyloxy)carbonyl]amino]-6-azido-2,6-dideoxy-3-O-[[4-methyl-3-nitrophenyl]carbamoyl]-2-O-[(3-fluorophenyl)carbamoyl]-2-O-sulfanyl]butyramide (14f)

Yield: 31.8 mg (82%, crude product); colorless amorphous solid.

ESI-MS: m/z (%) = 1129.4 (44) [2M + Na]+, 1107.4 (11) [2M + H]+, 576.2 (100) [M + Na]+, 554.2 (19) [M + H]+.

4-[[2-[(Allyloxy)carbonyl]amino]-6-azido-2,6-dideoxy-3-O-[[4-ethoxyphenyl]carbamoyl]-2-O-[(2,4-difluorophenyl)carbamoyl]-2-O-sulfanyl]butyramide (14j)

Yield: 23.4 mg (60%, crude product); pale yellow amorphous solid.

Analytical HPLC: gradient A, tₚ = 17.0 min (32%).

Semipreparative HPLC: gradient E, tₚ = 30.77 min; yield: 11.6 mg (32%, based on polymer-bound carbohydrate); colorless amorphous solid.

ESI-MS: m/z (%) = 1113.1 (100) [2M + Na]+, 567.1 (40) [M + Na]+, 545.2 (5) [M + H]+.

4-[[2-[(Allyloxy)carbonyl]amino]-6-azido-2,6-dideoxy-3-O-[[2,4-difluorophenyl]carbamoyl]-2-O-[(allyloxy)carbonyl]-2-O-sulfanyl]butyramide (14k)

Yield: 11.6 mg (31%, crude product); pale yellow amorphous solid.

The desired product could not be detected.

ESI-MS: m/z (%) = 606.2 (19) [M + K]+, 590.2 (100) [M + Na]+, 568.2 (5) [M + H]+.

4-[[2-[(Allyloxy)carbonyl]amino]-6-azido-2,6-dideoxy-3-O-[[4-nitrophenyl]carbamoyl]-2-O-[(allyloxy)carbonyl]-2-O-sulfanyl]butyramide (14n)

Yield: 28.0 mg (79%, crude product); pale yellow amorphous solid.

Analytical HPLC: gradient A, tₚ = 18.58 min (36%).

Semipreparative HPLC: gradient E, tₚ = 31.87 min; yield: 9.8 mg (27%, based on polymer-bound carbohydrate); colorless amorphous solid.

ESI-MS: m/z (%) = 1144.4 (7) [2M + K]+, 1127.4 (78) [2M + Na]+, 1105.4 (100) [2M + H]+, 575.2 (62) [M + Na]+, 553.2 (2) [M + H]+.

Carbamates 15

One portion of resin 9a (100 mg, loading 0.67 mmol/g) and two portions of resin 9b (100 mg each, loading 0.15 mmol/g) were filled in 5-mL syringes. To each of the syringes CH₂Cl₂ (2.5 mL) was added, and the polymers were swelled for 1 h. A mixture of DDQ (158.9 mg, 0.70 mmol for 9a or 52.2 mg, 0.23 mmol for 9b) in CH₂Cl₂ (2.5 mL) and 10 vol% H₂O were added to the polymers. The syringes were shaken for 30 min. The resins were washed thoroughly with CH₂Cl₂ (10 mL) and an additional portion of DDQ was added. After 30 min the polymer support was washed with DMF (10 mL), DMF–MeOH (10 mL, 1:1), MeOH–CH₂Cl₂ (10 mL, 1:1), MeOH–Et₂O (10 mL, 1:1), and Et₂O (10 mL) and directly used for carbamoylation. The preparation of the carbamates was carried out according to the protocol for the preparation of derivatives 14. The procedures for the removal of the TBS protecting group and the cleavage of the

H NMR (400 MHz, CDCl₃): Δ = 8.27 (d, J = 8.6 Hz, 1 H, H₅), 8.08 (d, J = 8.6 Hz, 1 H, H₆), 7.76 (m, 1 H, H₃), 7.09 (m, 1 H, H₄), 5.72 (m, 1 H, CH2=CH-, Aloc), 5.49 (d, J = 9.5 Hz, 1 H, NH-C₂H₅), 5.38 (d, J = 5.3 Hz, 1 H, H-1), 5.13 (d, J = 17.3 Hz, 1 H, CH2=CH-, Aloc, (trans)), 5.00 (d, J = 10.4 Hz, 1 H, CH2=CH-, Aloc, (cis)), 4.79 (dd, J = 11.5 Hz, J = 2.9 Hz, 1 H, H-3), 4.52 (m, 1 H, H-2), 4.43 (m, 2 H, CH₂=CH2), 4.27 (m, 1 H, H-4), 3.59 (dd, J = 12.5 Hz, J = 1.6 Hz, 1 H, 6-H₆a), 3.27 (dd, J = 12.9 Hz, J = 4.4 Hz, 1 H, H-6b), 2.62 (m, 2 H, SCH₂CH₂), 2.26 (t, J = 7.4 Hz, 2 H, CH2CO₂CH₂), 1.89 (s, 2 H, SCH₂C₆H₅).
desired products from polymer support were performed as described for the isolation of carbamates 14. The products 15 thus obtained were weighed and analyzed by RP-HPLC and ESI-MS and purified by semipreparative RP-HPLC.

4-[[{Allyloxy}carbonyl]-amino]-6-azido-2,6-dideoxy-4-O-[[4-fluorophenyl]carbamoyle-α-D-galactopyranosyl]sulfanyl]butyramide (15a)
Yield: 4.6 mg (58%, crude product); colorless amorphous solid.
Analitical HPLC: gradient A, tR = 16.25 min (91%).

4-[[{Allyloxy}carbonyl]-amino]-6-azido-4-O-[[4-cyanophenyl]carbamoyle]-2,6-dideoxy-α-D-galactopyranosyl)sulfanyl]butyramide (15b)
Yield: 6.2 mg (78%, crude product); colorless amorphous solid.
Analytical HPLC: gradient A, tR = 17.60 min (85%).

4-[[{Allyloxy}carbonyl]-amino]-6-azido-4-O-[[4-chlorophenyl]carbamoyle]-2,6-dideoxy-α-D-galactopyranosyl)sulfanyl]butyramide (15c)
Yield: 13.3 mg (37%, crude product); pale brown amorphous solid.
Analytical HPLC: gradient A, tR = 18.07 min (33%).

N-Acylated Derivatives 16 and 17
In a solid-phase reaction vessel, resin 9a (1.3 g, 1.84 mmol) was swollen for 1 h in anhyd dioxane (20 mL). The mixture was passivated using ultrasonication under an argon atmosphere, before Toluene (0.781 g, 4.58 mmol) was added. TBTU (73.8 mg, 0.23 mmol), HOBt·H2O (35.2 mg, 0.23 mmol), DIPEA (78.7 μL, 0.46 mmol), and Fmoc-Ar-Pmc-OH, Fmoc-His(Trt)-OH, and Fmoc-Glu(Trt)-OH (0.23 mmol, respectively). For the synthesis of products 17a and 17b, the procedure required additional steps. Before the product was cleaved from the resin, the Fmoc protecting group was removed as described for the preparation of building blocks 9, and the coupling of Fmoc-His(Trt)-OH (0.23 mmol) followed according to the peptide-coupling protocol given above.

4-[[{6-Azido-3-(ter-butyldimethylsilyl)-2,6-dideoxy-2-[[N-(9-fluorenylmethoxy carbonyl)]-glycyl]-amino]-α-D-galactopyranosyl+sulfanyl]butyramide (17a)
Yield: 26.6 mg (60%, crude product); brown amorphous solid.
Analytical HPLC: gradient A, tR = 25.72 min (40%).

Analytical HPLC: gradient F, tR = 31.45 min; yield: 5.3 mg (12% based on polymer-bound carbohydrate); colorless amorphous solid.

ESI-MS: m/z (%) = 1435.7 (13) [2M + K] +, 1419.8 (87) [2M + Na] +, 721.3 (100) [M + Na] +.

4-[[{6-Azido-3-(ter-butyldimethylsilyl)-2,6-dideoxy-2-[[N-(9-fluorenylmethoxy carbonyl)]-l-leucyl]-amino]-α-D-galactopyranosyl+sulfanyl]butyramide (17b)
Yield: 39.2 mg (82%, crude product); brown amorphous solid.
Analytical HPLC: gradient A, tR = 27.85 min (33%), not isolated by semipreparative HPLC.
ESI-MS (crude product): m/z (%) = 793.11 (10) [M + K] +, 777.18 (100) [M + Na] +, 663.21 (25) [3-OH, M + Na] +.

4-[[{6-Azido-3-(ter-butyldimethylsilyl)-2,6-dideoxy-2-[[N-(9-fluorenylmethoxy carbonyl)]-l-glutamyl]-amino]-α-D-galactopyranosyl+sulfanyl]butyramide (17c)
Yield: 24.9 mg (51%, crude product); brown amorphous solid.
Analytical HPLC: gradient A, tR = 22.72 min (36%).

Analytical HPLC: gradient F, tR = 30.10 min; yield: 4.8 mg (10% based on polymer-bound carbohydrate); pale yellow amorphous solid.
ESI-MS: m/z (%) = 809.26 (100) [M + K] +, 793.31 (40) [M + Na] +.

4-[[{6-Azido-2,6-dideoxy-2-[[N-(9-fluorenylmethoxy carbonyl)]-l-arginyl]-amino]-α-D-galactopyranosyl+sulfanyl]butyramide (16d) and Its 3-OTBS Derivative 17d
From reaction on aminomethyl-PS: Yield: 31.5 mg (64%, crude product); brown amorphous solid; desired product not detected.
From reaction on Tentagel resin: Yield: 23.9 mg (ca. 100%).
Analytical HPLC: gradient B, tR = 15.37 min (12%), 15.42 min (17%), 16.68 min (29%), 17.13 min (7%). This gave mixture of 16d and 17d; separation of products and isolation failed.

ESI-MS (crude product): m/z (%) = 798.59 (30) [M + H] +, 684.52 (20) [M + H] +, 576.50 (15) [M – Fmoc + H] +, 420.20 (5) [2-NH2, M – Fmoc-Arg] +, 195.04 (100%), 179.05 (65) [DBF + H] +.
4-[[6-Azido-2,6-dideoxy-2-[[N-(9-fluorenylethoxycarbonyl)-L-histidyld]amino]-α-D-galactopyranosyl]sulfanyl]butyramide (16e)

From reaction on Tentagel resin: Yield: 19.8 mg (ca. 100%, crude product), yellow oil.

Analytical HPLC: gradient B, \( t_R = 10.62 \text{ min} \) (24%).

Semipreparative HPLC: gradient G, \( t_R = 20.32 \text{ min} \); yield: 3.9 mg (31% based on polymer-bound carbohydrate); colorless amorphous solid.

ESI-MS: \( m/z \) (%): 687.49 (5) \([M + Na]^+\), 665.49 (100) \([M + H]^+\), 179.05 (5) \([DBF + H]^+\).

O-Acylated Derivatives 18

Six portions of resin 9b (100 mg each, loading 0.19 mmol/g) were placed in six 5-mL syringes, and the above-described procedure for the removal of the TBS protecting group (preparation of carbamates 14) was applied. The appropriate Fmoc- and Z-terminated amino acids (0.23 mmol each) were activated with DIC (47.4 µL, 37.9 mg, 0.23 mmol) and DMAP (2.6 mg, 0.21 mmol) in DMF (2.5 mL) and then added to the polymers. After shaking of the mixtures for 16 h at r.t., the polymer supports were washed with DMF (10 mL), DMF–MeOH (10 mL, 1:1), MeOH–CH₂Cl₂ (10 mL, 1:1), MeOH–Et₂O (10 mL, 1:1), and Et₂O (10 mL) and dried thoroughly in vacuo. The cleavage of the products from the polymer support was carried out according to the procedure described for preparation of derivatives 16 and 17. The synthesis of derivatives 18d–f contained additional steps, which are described in the protocol for the preparation of compounds 16g and 17g. Products 18 thus obtained were analyzed by RP-HPLC and ESI-MS and purified by semipreparative RP-HPLC.

4-[[6-Azido-2,6-dideoxy-2-[[N-(9-fluorenylethoxycarbonyl)-L-glutaminyl]amino]-α-D-galactopyranosyl]sulfanyl]butyramide (17e)

Yield: 24.9 mg (~100%, crude product); colorless amorphous solid.

Analytical HPLC: gradient B, \( t_R = 17.08 \text{ min} \) (35%).

Semipreparative HPLC: gradient G, \( t_R = 50.73 \text{ min} \); yield: 1.0 mg (7% based on polymer-bound carbohydrate); pale yellow amorphous solid.

ESI-MS: \( m/z \) (%): 779.57 (100) \([M + Na]^+\), 557.47 (5) \([M – Fmoc + H]^+\).

4-[[6-Azido-2,6-dideoxy-2-[[N-(9-fluorenylethoxycarbonyl)-L-histidyld]amino]-α-D-galactopyranosyl]sulfanyl]butyramide (17f)

Yield: 19.8 mg (ca. 100%, crude product); colorless amorphous solid.

Analytical HPLC: gradient B, \( t_R = 11.37 \text{ min} \) (25%), 24.47 min (26%).

Semipreparative HPLC: gradient G, \( t_R = 18.67 \text{ min} \); yield: 1.0 mg (7% based on polymer-bound carbohydrate); pale yellow amorphous solid.

ESI-MS: \( m/z \) (%): 678.48 (8) \([M + Na]^+\), 472.32 (15) \([M – Fmoc + K]^+\), 456.31 (40) \([M – Fmoc + Na]^+\), 434.26 (100) \([M – Fmoc + H]^+\), 195.05 (35%), 179.05 (60) \([DBF + H]^+\).

4-[[6-Azido-2,6-dideoxy-2-[[N-(9-fluorenylethoxycarbonyl)-L-arginyl]amino]-α-D-galactopyranosyl]sulfanyl]butyramide (18a)

Yield: 7.4 mg (51%, crude product); colorless amorphous solid; the isolation failed.

ESI-MS (crude product): \( m/z \) (%): 768.38 (100) \([M + H]^+\).

4-[[6-Azido-2,6-dideoxy-2-[[N-(9-fluorenylethoxycarbonyl)-L-glutaminyl]amino]-α-D-galactopyranosyl]sulfanyl]butyramide (18b)

Yield: 6.5 mg (46%, crude product); colorless amorphous solid.

Analytical HPLC: gradient B, \( t_R = 15.92 \text{ min} \) (43%).

Semipreparative HPLC: gradient G, \( t_R = 25.77 \text{ min} \); yield: 0.8 mg (6%, based on polymer-bound carbohydrate); colorless amorphous solid.

ESI-MS: \( m/z \) (%): 1501.58 (64) \([2M + Na]^+\), 778.27 (3) \([M + K]^+\), 762.28 (100) \([M + Na]^+\).

4-[[6-Azido-2,6-dideoxy-2-[[N-(9-fluorenylethoxycarbonyl)-L-histidyld]glycyl]amino]-α-D-galactopyranosyl]sulfanyl]butyramide (18c)

Yield: 7.1 mg (50%, crude product); colorless amorphous solid.

Analytical HPLC: gradient B, \( t_R = 17.27 \text{ min} \) (60%, major epimer 18c), \( t_R = 18.30 \text{ min} \) (24%, minor epimer 18c*).

Semipreparative HPLC: gradient A, \( t_R = 29.00 \text{ min} \) (46%); yield: 1.8 mg (13%, based on polymer-bound carbohydrate); colorless amorphous solid.

ESI-MS: \( m/z \) (%): 1519.59 (39) \([2M + Na]^+\), 1497.61 (6) \([2M + Na]^+\), 787.27 (3) \([M + K]^+\), 771.29 (100) \([M + Na]^+\), 749.31 (16) \([M + H]^+\).

Semipreparative HPLC: gradient A, \( t_R = 31.13 \text{ min} \) (18c*); yield: 0.4 mg (3%, based on polymer-bound carbohydrate); colorless amorphous solid.

ESI-MS: \( m/z \) (%): 1519.60 (10) \([2M + Na]^+\), 787.28 (6) \([M + K]^+\), 771.29 (61) \([M + Na]^+\), 749.31 (100) \([M + H]^+\), 412.15 (3) \([M – Fmoc – His – 3-TBSO + Na]^+\).

4-[2-[[[(Allyloxy)carbonyl]amino]-6-azido-2,6-dideoxy-3-O-[N-(9-fluorenylmethoxy carbonyl)-L-histidy]glycyl]-a-D-galactopyranosyl]sulfanyl]butyramide (18d)

Yield: 14.1 mg (ca. 100%, crude product); colorless amorphous solid.

ESI-MS: m/z (%) = 745.32 (15) [M + Na]+, 723.36 (20) [M – H]+, 604.35 (50), 179.05 (100) [DBF + H]+.

4-[2-[[[(Allyloxy)carbonyl]amino]-3-O-[(tert-butyldimethylsilyl)-2,6-dideoxy-6-[N-(9-fluorenylmethoxy carbonyl)-L-histidy]amino]-a-D-galactopyranosyl]sulfanyl]butyramide (20a)

Analytical HPLC: gradient B, tR = 22.67 min (54%).

Semipreparative HPLC: gradient G, tR = 28.65 min; yield: 2.5 mg (16% based on polymer-bound carbohydrate); colorless amorphous solid.

ESI-MS: m/z (%) = 856.46 (5) [M + Ca]2+, 742.36 (65) [3-OH, M + Ca]2+, 604.35 (40), 179.04 (100) [DBF + H]+.

4-[2-[[[(Allyloxy)carbonyl]amino]-2,6-dideoxy-6-[N-(9-fluorenylmethoxy carbonyl)-L-arginyl]amino]-a-D-galactopyranosyl]sulfanyl]butyramide (19b)

Yield: 15.6 mg (96%); yellow amorphous solid.

Analytical HPLC: gradient B, tR = 7.53 min (44%), 15.00 min (14%), 17.17 min (42%); the isolation failed.

ESI-MS (crude product): m/z (%) = 764.32 (5) [M + Na]+, 742.34 (10) [M + H]+, 500.15 (5) [6-NH2, M – Fmoc-Arg + Na]+, 179.03 (100) [DBF + H]+.

4-[2-[[[(Allyloxy)carbonyl]amino]-3-O-[(tert-butyldimethylsilyl)-2,6-dideoxy-6-[N-(9-fluorenylmethoxy carbonyl)-L-arginyl]amino]-a-D-galactopyranosyl]sulfanyl]butyramide (20b)

The desired product could not be detected.

4-[2-[[[(Allyloxy)carbonyl]amino]-2,6-dideoxy-6-[N-(9-fluorenylmethoxy carbonyl)-L-glutamyl]amino]-a-D-galactopyranosyl]sulfanyl]butyramide (19c)

Yield: 16.2 mg (ca. 100%, crude product); colorless amorphous solid.

Analytical HPLC: gradient B, tR = 12.90 min (21%).

Semipreparative HPLC: gradient G, tR = 19.08 min; yield: 0.9 mg (7% based on polymer-bound carbohydrate); colorless amorphous solid.

ESI-MS: m/z (%) = 736.43 (10) [M + Na]+, 697.86 (20) [M – NH2]+, 641.74 (50), 401.38 (85), 360.41 (100).

4-[2-[[[(Allyloxy)carbonyl]amino]-3-O-[(tert-butyldimethylsilyl)-2,6-dideoxy-6-[N-(9-fluorenylmethoxy carbonyl)-L-glutamyl]amino]-a-D-galactopyranosyl]sulfanyl]butyramide (20c)

Analytical HPLC: gradient B, tR = 21.72 min (79%).

Semipreparative HPLC: gradient G, tR = 46.70 min; yield: 1.7 mg (11% based on polymer-bound carbohydrate); colorless amorphous solid.

ESI-MS: m/z (%) = 850.49 (45) [M + Na]+, 828.58 (5) [M + H]+, 628.41 (80) [M – Fmoc + Na]+, 606.48 (90) [M – Fmoc + H]+, 469.41 (95), 179.07 (100) [DBF + H]+.

4-[2-[[[(Allyloxy)carbonyl]amino]-2,6-dideoxy-6-[[[(1-phenyl-1H-1,2,3-triazol-4-yl)carbonyl]amino]-a-D-galactopyranosyl]sulfanyl]butyramide (19d)

Yield: 14.4 mg (ca. 100%, crude product); colorless oil.

Analytical HPLC: gradient B, tR = 10.78 min (30%).
Semipreparative HPLC: gradient G, \( t_k = 11.65 \) min; yield: 1.9 mg (19% based on polymer-bound carbohydrate); colorless amorphous solid.

ESI-MS: \( m/z \% = 557.37 (100) [M + Na]^+ \), 406.25 (45).

4-[2-[[Allyloxy]carbonyl]amino]-3-O-[( tert-butylmethylylimidazolyl)-2,6-dideoxy-6-[(1-phenyl-1H-1,2,3-triazol-4-yl)carbonyl]amino]-a-D-galactopyranosyl]sulfanyl]butyramide (20e)

Analytical HPLC: gradient A, \( R = 24.93 \) min; yield: 1.9 mg (5% based on polymer-bound carbohydrate); colorless amorphous solid.

ESI-MS: \( m/z \% = 618.17 (100) [M + Na]^+ \), 596.13 (<5) [3-TBSO, M – Fmoc + H]^+.


Yield: 13.0 mg (35%, crude product); brown amorphous solid. The desired product could not be detected.


Analytical HPLC: gradient A, \( t_k = 25.98 \) min (35%).

Semipreparative HPLC: gradient E, \( t_k = 24.93 \) min; yield: 1.9 mg (5% based on polymer-bound carbohydrate); colorless amorphous solid.

ESI-MS: \( m/z \% = 681.17 (100) [M + Na]^+ \), 596.13 (<5) [3-TBSO, M + H]^+.


Yield: 18.8 mg (~100%, crude product); colorless amorphous solid. The desired product could not be detected.


Analytical HPLC: gradient B, \( t_k = 12.42 \) min (34%).

Semipreparative HPLC: gradient G, \( t_k = 19.15 \) min; yield: 2.9 mg (20% based on polymer-bound carbohydrate); colorless amorphous solid.

ESI-MS: \( m/z \% = 802.4 (15) [M + Na]^+ \), 780.42 (100) [M + H]^+.

References


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