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Synthesis of Carbohydrate-Functionalised Sequence-Defined Oligo(amidoamine)s by Photochemical Thiol–Ene Coupling in a Continuous Flow Reactor

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Abstract: Poly/oligo(amidoamine)s (PAAs) have recently been recognised for their potential as well-defined scaffolds for multiple carbohydrate presentation and as multivalent ligands. Herein, we report two complimentary strategies for the preparation of such sequence-defined carbohydrate-functionalised PAAs that use photochemical thiol–ene coupling (TEC) as an alternative to the established azide–alkyne cycloaddition ("click") reaction. In the first approach, PAAs that contained multiple olefins were synthesised on a solid support from a new building block and subsequent conjugation with unprotected thio-carbohydrates. Alternatively, a pre-functionalised building block was prepared by using TEC and assembled on a solid support to provide a carbohydrate-functionalised PAA. Both methods rely on the use of a continuous flow photoreactor for the TEC reactions. This system is highly efficient, owing to its short path length, and requires no additional radical initiator. Performing the reactions at 254 nm in Teflon AF-2400 tubing provides a highly efficient TEC procedure for carbohydrate conjugation, as demonstrated in the reactions of *O*-allyl glycosides with thiols. This method allowed the complete functionalisation of all of the reactive sites on the PAA backbone in a single step, thereby obtaining a defined homogeneous sequence. Furthermore, reaction at 366 nm in FEP tubing in the flow reactor enabled the large-scale synthesis of an fluorenylmethyloxycarbonyl (Fmoc)-protected glycosylated building block, which was shown to be suitable for solid-phase synthesis and will also allow heterogeneous sequence control of different carbohydrates along the oligomeric backbone. These developments enable the synthesis of sequence-defined carbohydrate-functionalised PAAs with potential biological applications.

Keywords: carbohydrates · flow reactors · photochemistry · polymers · solid-phase synthesis

Introduction

Carbohydrate–protein, carbohydrate–DNA, and carbohydrate–carbohydrate interactions underpin many biological processes and are crucial to understanding immunology.[1] The weak nature of these interactions requires multiple presentation of carbohydrate moieties to elicit the desired immune response or receptor interaction.[2] Precise control of the spatial orientation and distance between carbohydrate groups along a scaffold is desirable for promoting multivalent interactions and for controlling the binding affinity and selectivity of the multivalent ligands.[3] As a consequence of efforts to develop such synthetic carbohydrate ligands and to understand the underlying structure–activity relationships (SARs), various strategies for the presentation of carbohydrates have been explored.[4] Recently, we demonstrated the use of solid-phase synthesis for the preparation of monodisperse sequence-defined glycopolymer segments that were based on poly/oligo(amidoamine) (PAA) substructures.[5] These compounds belong to the class of "precision polymers", a new class of macromolecular systems that combine the advantages of biopolymer scaffolds, such as peptides or DNA,[6] with the advantages of synthetic scaffolds, such as polymers[7] or dendrimers,[8] because they are highly defined, versatile in their structure (linear or branched), and biocompatible, with a decreased risk of inherent immunogenicity.[9]

In a previous study, we observed a strong dependence of binding affinity on the number and spacing of the sugar ligands along the oligomeric backbone, thus indicating that monodisperse, sequence-defined scaffolds have a good po-

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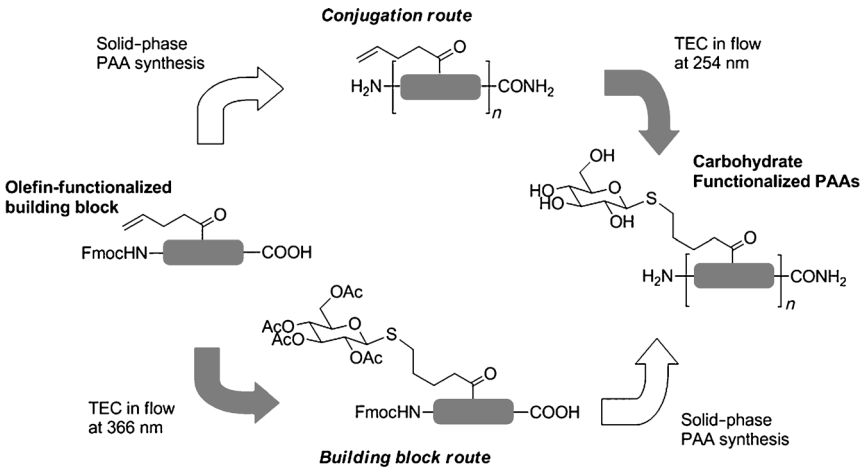
tential for the presentation of new synthetic carbohydrate ligands. Herein, mannose moieties were reacted with alkyne-presenting oligomers through copper-catalysed azide–alkyne cycloaddition.[10] Although this approach is highly suitable for the solid-phase synthesis of glycopolymer segments, the triazole linkage is potentially immunogenic,[11] thus limiting the application of this method in the targeting and stimulation of immune cells.

One of our long-term goals is to apply the solid-phase synthesis of such precision polymers[9] to the synthesis of heteromultivalent ligands that present different sugar ligands at different positions along the backbone. By using a classical polymer approach, until now, heteromultivalent systems have only been obtained in a strongly alternating, block, or random fashion.[7] The synthesis of sequence-controlled heteromultivalent structures is expected to give more-detailed insight into the multivalent binding modes of such artificial ligands, as well as enable the design of ligands with not only increased affinity but also improved selectivity. The most-straightforward synthetic strategy towards such heteromultivalent systems would be the use of glycosylated building blocks during solid-phase synthesis. However, glycosylated building blocks must be obtainable in large quantities and in high quality to be suitable for chain assembly on a solid support. The complicated purification steps that are required to provide Cu-free material after solution-phase conjugation prompted us to explore alternative chemistry to the commonly used azide–alkyne cycloaddition reaction. Thus, we sought to couple carbohydrates to PAAs by using thiol–ene chemistry (TEC), which would result in a non-immunogenic linkage[11a] with a functional group that would remain inert during solid-phase synthesis, thereby allowing the introduction of carbohydrates at the building block level or by functionalisation of the fully assembled oligomer. TEC, that is the anti-Markovnikov addition of a thermally or photolytically generated thiyl radical to an olefin,[12] has been widely used in the development of chemical tools for the study of biological processes,[13] in particular those that involve the presentation of carbohydrates. Precedent for the use of TEC in the synthesis of glycoconjugate vaccines was established by the groups of Kunz, Dondoni, and Davis.[11a,14] The photochemical variant of TEC, which is widely employed for its tolerance of biomolecular functionalities,[15] was chosen to demonstrate this approach.

Therefore, we explored two complementary strategies for the synthesis of carbohydrate-functionalised PAAs by using TEC (Scheme 1): 1) Olefin-presenting oligomers were prepared on a solid phase and subsequently conjugated with thiocarbohydrates, or 2) a pre-functionalised building block was prepared that was then used to construct the same structure but with the potential to access heterogeneous carbohydrate-functionalisation patterns. Herein, we discuss the relative merits of these strategies. We report two new non-natural amino-acid building blocks, based on a diamine and a diacid unit, and their coupling on a solid support by using an automated standard peptide synthesiser and established SPPS procedures. The recently described *tert*-butoxycarbonyl (Boc)-protected building block BDS[16] (Boc-protected diethylenetriamine succinic acid, **2**) was modified for this work to contain the pent-4-enamide moiety, which is highly active towards TEC,[17] thus giving the new DDS (double-bond-presenting diethylenetriamine succinic acid) building block.[18] Our method also required the development of conditions for efficient TEC that would reliably conjugate all of the olefins that were spaced along the scaffold but could also be used to prepare functionalised building blocks on a large scale for solid-supported synthesis. We considered that a continuous flow reactor would be ideal in this case,[19] because such systems allow precise control over the reaction conditions. Efficient irradiation of the sample was achieved by virtue of a sub-millimetre path length and the products were removed as they were formed, thereby minimising unwanted side-reactions. These processes were simple to scale up by driving the reactor for an extended period of time.[20] Thus, we also report the development and testing of two dedicated continuous flow photoreactor systems for performing TEC in a flow and their application in the synthesis of carbohydrate-functionalised PAAs.

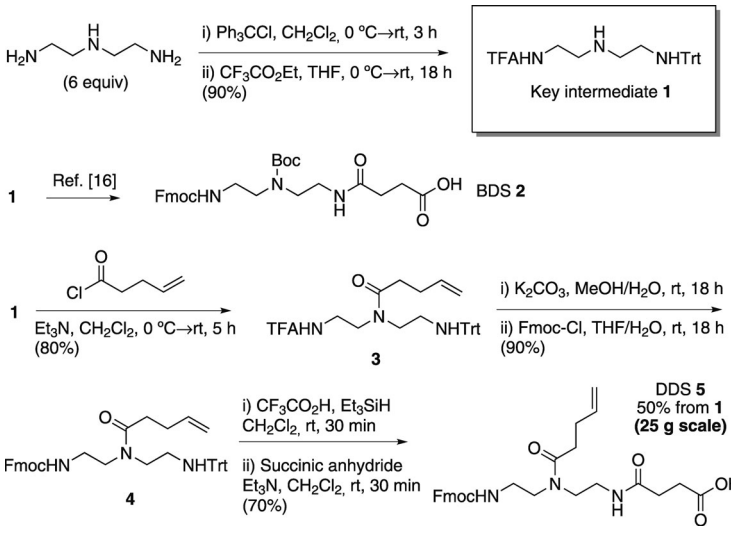
Results and Discussion

Regardless of the approach used, building blocks for the synthesis of carbohydrate-functionalised PAAs have to fulfil certain criteria: They must be soluble in an appropriate coupling solvent, such as DMF or *N*-methyl-2-pyrrolidone (NMP), and must be accessible on a multigram scale with



Scheme 1. Complementary strategies for the preparation of carbohydrate-functionalised PAA.

high purity. Furthermore, decreasing the number of requisite chromatographic purification steps is desirable. Thus, we began by developing large-scale syntheses of olefin-presenting building block DDS (**5**) and spacer building block BDS (**2**) from differentially protected diethylenetriamine (**1**), itself prepared according to our recently published procedure (Scheme 2).[16] Thus, treatment of compound **1** with

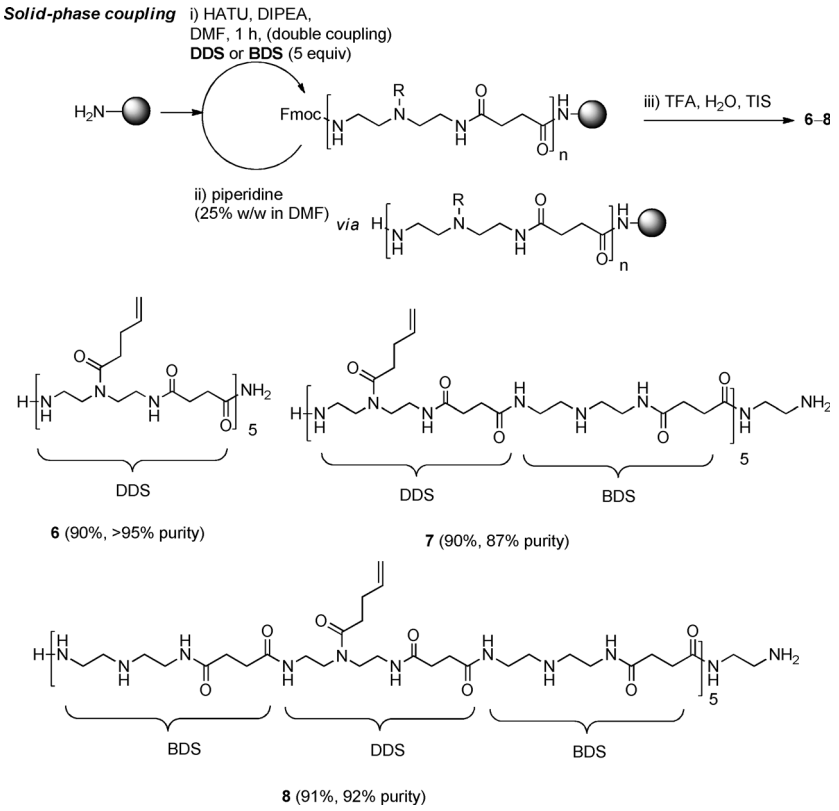


Scheme 2. Synthesis of DDS and BDS building blocks for solid-phase coupling.

4-pentenoyl chloride and TEA gave amide **3**, which contained the required olefin functionality for TEC. Exchange of the trifluoroacetic acid (TFA) protecting group of compound **3** for an Fmoc group afforded carbamate **4**. Acidic trityl deprotection and treatment of the free amine intermediate with succinic anhydride installed the diacid spacer unit to give 25 g of pure DDS (**5**) in 50% overall yield after final recrystallisation (>98% purity by HPLC, integration of the UV-signal at 214 nm). No intermediate chromatographic purification steps were required.

With scalable syntheses of building blocks **2** and **5** established, olefin-bearing PAAs were assembled by automated solid-phase synthesis under *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) activation (Scheme 3). The solid-phase coupling of the DDS (**5**) and BDS (**2**) building blocks allowed a defined distribution of olefins along the backbone, depending on the coupling sequence, to allow a fine tuning of potential multivalent carbohydrate receptor interactions.[5] Firstly, five DDS building blocks were assembled on a solid support, thereby giving pentamer **6** in 90% yield after TFA cleavage from the resin and precipitation with Et_2O. Furthermore, no isomerisation/transacylation was observed, even under prolonged piperidine exposure during the solid-phase synthesis.[21] Next, oligomer **7**, which consisted of five DDS building blocks, each spaced with one BDS building block, was prepared in 90% yield and 87% purity (by HPLC, integration of the UV signal at 214 nm). Finally 15-mer **8**, which consisted of five DDS building blocks, each spaced with two BDS building blocks, was obtained according to the same coupling procedure in similarly high yield and purity (see the Supporting Information).

Next, our attention turned to the functionalisation of oligomers **6**–**8** by photochemical TEC. The design for the flow reactor was based on that of Booker-Milburn[22] and consisted of loops of transparent tubing wrapped around a suitable light source and cooling jacket (see the Supporting Information). The rate of the thiol–ene reaction is wavelength dependent and occurs more rapidly at 254 nm than at 366 nm.[23] Therefore, we envisaged that a reactor that was constructed of a material that was spectroscopically transparent at 254 nm would allow the treatment of water-soluble



Scheme 3. Synthesis of sequence-defined PAAs on a solid support; purity was determined by RP-HPLC (integration of the UV signal at 214 nm).

carbohydrates, PAA scaffolds, and similar compounds without the need for a radical initiator. Because fluorinated ethylene polymer (FEP) tubing decomposes at this wavelength, Teflon AF-2400,[24,25] which is transparent in the deep-UV region, was chosen as the tubing material and was wrapped around a quartz cooling jacket that contained a In/Hg lamp (100 W, Figure 1). Teflon AF-2400 had not previously been

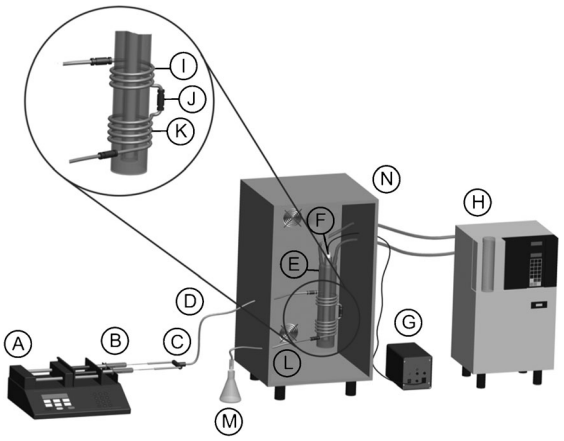


Figure 1. Schematic diagram of the photoreactor for TEC: A) Syringe pump; B) syringes that contain the thiol and olefin reactants; C) ETFE T-union; D) PTFE inlet line (1/16“ OD, 0.3 mm ID); E) quartz cooling jacket; F) 100 W NNIQ In/Hg lamp; G) power supply; H) cryostat filled with ultrapure water; I) loop of Teflon AF-2400 tubing (1700 μL, 0.6 mm ID, 0.8 mm OD); J) PEEK ”Microtight“ union; K) loop of Teflon AF-2400 tubing (566 μL, 0.6 mm ID, 0.8 mm OD); L) PTFE outlet line (1/16” OD, 0.3 mm ID); M) collection vessel; N) safety casing.

used as a material for preparative photochemistry, yet it has been employed as a membrane for the continuous addition of gases in flow reactors.[24b] A temperature of 25 °C was maintained for all experiments with a cryostat by using ultrapure water as a coolant. Separate solutions of the thiol and olefin components were delivered by syringe pump and mixed with a T-union. All optimisation reactions were performed by using a loop of Teflon AF-2400 (566 μL), whilst a 2266 μL loop was used for scale-up. Although the reaction is tolerant towards oxygen,[15a] the solvents were degassed before use.

To assess the reactor prior to its use in the conjugation of PAAs, water-soluble *O*-allyl glycosides **9a–9e** were reacted with L-cysteine in water to give amino acids **10a–10e**.[26] Plots of residence time versus conversion were obtained at various concentrations for the reaction of *O*-allyl galactose (**9a**) with L-cysteine (Figure 2).

Almost-complete conversion was observed within a residence time of 15 min, even at the lowest concentration studied. No thermal background reaction was observed at 0.1 M ($t_{res}=15$ min). To assess the importance of the wavelength (254 nm), the reaction was repeated by using a loop of FEP tubing that was wrapped around a cooled medium-pressure Hg lamp (400 W, $\lambda_{max}=366$ nm) with a Pyrex filter (see

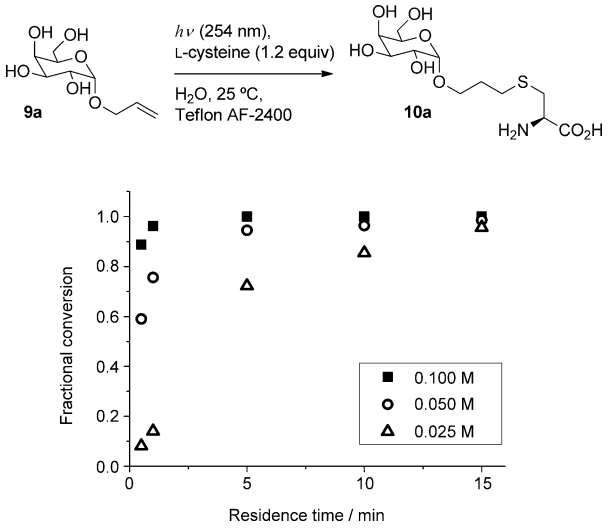
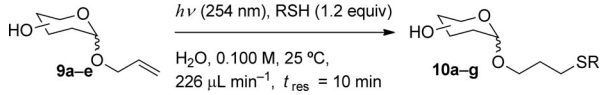


Figure 2. Plot of residence time versus conversion for the TEC of α-*O*-allyl galactose with L-cysteine in water, as determined by RP-HPLC/MS with evaporation light-scattering detection (ELSD).

below). Diminished reactivity was observed (8% conversion by reverse-phase (RP) HPLC, $t_{res}=5$ min).

The reactions of *O*-allyl glycosides **9a–9e** showed little relationship between carbohydrate structure and reactivity[27] under these conditions. The reactions were repeated at 0.1 M to afford coupling products **10a–10e** with complete conversion and in good yields (Table 1). NMR analysis of the

Table 1. Reaction of *O*-allyl glycosides **9a–9e** with thiols at 254 nm.



Entry	Glycoside	Thiol	Product	Yield [%]
1	α-Gal (**9a**)	L-cysteine	**10a**	77[a]
2	β-Gal (**9b**)	L-cysteine	**10b**	70[a]
3	α-Glu (**9c**)	L-cysteine	**10c**	80[a]
4	β-Glu (**9d**)	L-cysteine	**10d**	71[a]
5	α-Man (**9e**)	L-cysteine	**10e**	75[a]
6	α-Gal (**9a**)	$HSCH_2CH_2OH$	**10f**	74

[a] Yield determined by ^{1}H NMR spectroscopy of the crude mixture with maleic acid as an internal standard; analytical samples were obtained as *N*-Fmoc derivatives.

crude mixtures with maleic acid as an internal standard suggested that some decomposition occurred under these conditions. The products were highly water-soluble and, therefore, were purified as their *N*-Fmoc derivatives (**11a–11e**) by preparative HPLC. This process was not limited to cysteine; 2-mercaptoethanol reacted with α-*O*-allyl galactose **9a** in equivalent yield.

Next, continuous-flow TEC conditions were applied directly to the multiple conjugation of oligomers **6–8** with glycosides **12** and **13**. For initial optimisation, the reaction of **6**

(pentamer) with β-thioglucose sodium salt (**12**) under acidic conditions was studied by using the 566 μL loop (see above). The moderate water-solubility of pentamer **6** necessitated a diluting of the reaction concentration to 0.025 M. This was compensated for by use of a larger quantity of the thiol component (2.0 equiv per olefin). Complete conjugation of all five olefin moieties was observed by HPLC-MS within a residence time of 10 min (Figure 3). The entire reactor output was collected, lyophilised, and purified by preparative HPLC. HPLC-MS peaks that corresponded to the addition of between one and five carbohydrate moieties were observed upon shortening the residence time (Figure 3). Pentamer **6** reacted in a similar manner to α-thioethyl-mannose **13**, thereby affording oligomer **15**. Next, these conditions were used for the conjugation of oligomers **7** and **8** with α-thioethyl-mannose **13**. Oxidation of the sulfide product occurred under these conditions. Although the pentameric structure of compound **6** was relatively unaffected (<15% oxidation by HPLC-MS), S oxidation (Figure 3 M+O)[28] was significantly more pronounced during the conjugation of larger structures **7** and **8** to give compounds **16** and **17**, respectively. However, treatment of the crude conjugate with $TMSBr/HSCH_2CH_2SH/DMF$ (TMS = trimethylsilyl) afforded the reduced homogenous product in moderate yield after preparative HPLC (Table 2, PAAs **16** and **17**).[29,30]

By using the previously described conjugation approach in combination with a 254 nm flow reactor (Scheme 1), we observed complete functionalisation whilst consuming 2 equivalents of the thiol-bearing carbohydrate structure per olefin. Nevertheless, this approach only allows the synthesis of a homogenous carbohydrate sequence. To obtain heterogeneous carbohydrate-functionalised PAAs, we explored the alternative approach for the synthesis of functionalised-PAAs by using a DDS building block (**5**) that had been pre-functionalised with protected β-thioglucose by TEC. Large-scale access to this carbohydrate-functionalised building block was required for solid-phase coupling and, therefore, a continuous-flow procedure was sought.

Decomposition of DDS building block **5** under 254 nm irradiation, presumably owing to the presence of the Fmoc protecting group, necessitated a change in the reactor design and the use of a longer wavelength. This effect could also be expected for peptide sequences that contain aromatic groups, such tryptophan or tyrosine; thus, for conjugation in the presence of such peptide segments, reaction at 366 nm would also be advisable. The modified photoreactor consisted of loops of FEP tubing wrapped around a Pyrex-filtered, medium-pressure Hg lamp (400 W, λ_{max} = 366 nm) that was cooled to 25 °C by using a cryostat (see the Supporting Information). Continuous delivery of the reagents was enabled by replacement of the syringe pump with a Vapourtec R2 two-channel pump module. TEC of DDS (**5**), which was stable at 366 nm, with acetyl protected β-thioglucose (**18**)[14a]

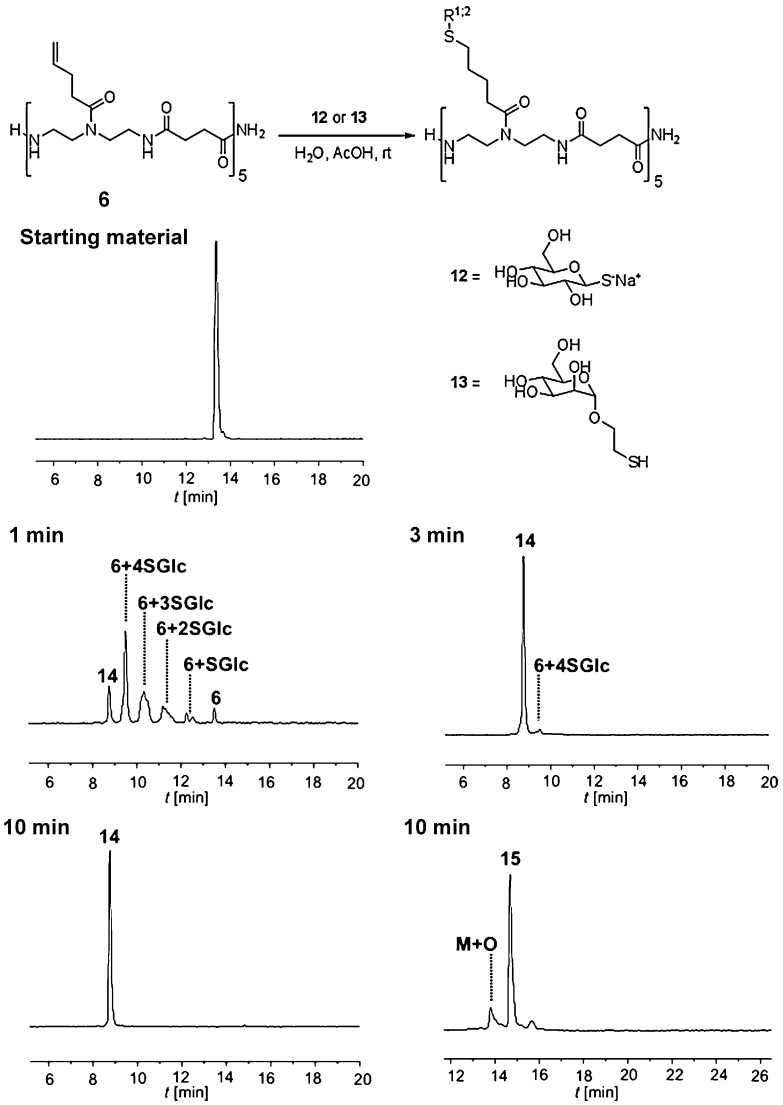


Figure 3. RP-HPLC analysis of oligomer **6** (MeCN/water, 5–95%, within 30 min) and (crude) multiple TEC in flow with β-GlcSNa (**12**, MeCN/water, 5–95%, within 30 min) and α-HSEt-Man (**13**, MeCN/water, 5–50%, within 30 min), thereby giving carbohydrate-functionalised PAAs **14** and **15**, respectively.

Table 2. Overview of the carbohydrate-functionalised oligo(amidoamine)s; complete conversion was obtained in all cases.

Product	PAA	Thiol component	MS (MALDI) *m/z*		Yield after RP-HPLC [%]
			calcd[a]	found	
14	**6**	β-GlcSNa (**12**)	2356.01 $[M+Na]^+$	2356.13	61
15	**6**	α-HSEt-Man (**13**)	2576.14 $[M+Na]^+$	2576.26	45
16	**7**	α-HSEt-Man (**13**)	3544.77 $[M+Na]^+$	3544.62	33
17	**8**	α-HSEt-Man (**13**)	4448.37 $[M+H]^+$	4447.98	30

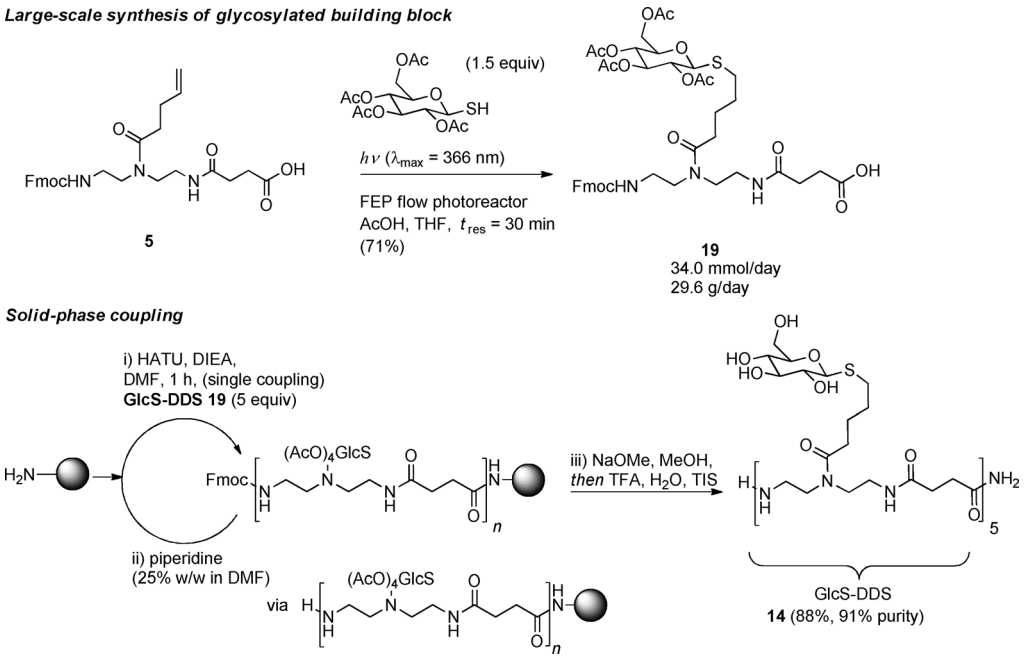
[a] The most-abundant ion adduct is shown here; detailed MS (MALDI) analysis and spectra can be found in the Experimental Section and the Supporting Information.

was optimised by using a 2.14 mL reactor loop. The reactants were poorly soluble in MeOH and in MeOH/water mixtures, thereby limiting the reaction concentration; both components were highly soluble in either THF or AcOH/THF (10% v/v). AcOH increased the solubility of DDS (**5**) in THF and ensured Fmoc stability during the TEC. Although an increased residence time (30 min) was required compared to experiments at 254 nm, high conversion (98%) was obtained without the need for an additional photoinitiator, by using 0.2 M solutions of DDS (**5**) and acetyl-protected β-thioglucose (**18**). Mixing of the solutions at an equal rate gave an overall 0.1 M reaction concentration. To increase productivity, a 10 mL loop was fitted; however, a lower conversion was observed (81%), which was attributed to uneven irradiation of the increased area of tubing along the arc of the lamp. Nevertheless, under these conditions, GlcS–DDS (**19**) was obtained in 71% yield after column chromatography, thus corresponding to a throughput of the functionalised building block of 29.6 g (in 24 h). Subsequently, oligomer **14** was prepared from GlcS–DDS **19** on a solid support (Scheme 4) with HATU activation. Although it possesses a higher molecular weight than the BDS and DDS building blocks, glycosylated building block **19** retained a high coupling efficiency. Pentamer **14** was prepared with HATU activation by using a single coupling procedure. Deacetylation with NaOMe/MeOH, cleavage from the resin with TFA/triisopropylsilyl (TIS)/water, and precipitation from the cleavage solution with Et_2O afforded oligomer **14** in high yield and purity (as determined by RP-HPLC, see the Supporting Information).

Evaluation of the benefits and drawbacks of these strategies for the synthesis of carbohydrate-functionalised PAAs must take into account the yield-limiting nature of the solid-phase coupling step. Functionalisation of an olefin-presenting scaffold only requires a small excess of the thiol-bearing carbohydrate and, therefore, is preferable when only low quantities of the complex carbohydrates are available. Homogenously glycosylated scaffolds are exclusively obtained. Conversely, the solid-phase assembly of a carbohydrate-functionalised building block allows a defined sequence of carbohydrates to be constructed along the PAA scaffold. Although a far greater excess of the carbohydrate is consumed during the solid-phase synthesis, this approach is appropriate for simpler carbohydrates that are available on a larger scale and, in particular, if the presentation of different carbohydrates is required.

Conclusion

In summary, we have demonstrated two complementary routes for the synthesis of carbohydrate-functionalised PAAs *via* solid phase synthesis and TEC. Scalable syntheses



Scheme 4. Synthesis of carbohydrate-functionalised PAAs by using glycosylated building blocks for chain assembly on a solid support.

of olefin-presenting or carbohydrate-functionalised building blocks were developed that provide large quantities of material required for solid phase synthesis. An efficient continuous flow photoreactor was developed for the photochemical TEC that can be performed in a variety of solvents at either 254 or 366 nm without an additional radical initiator. After evaluation of this system using the reaction of *O*-allyl glycosides with thiols, PAA scaffolds of varying length, each presenting five olefins were conjugated in flow at 254 nm (Teflon AF-2400 tubing) with β-thioglucose and α-thioethyl-mannose. Partial *S*-oxidation during conjugation of larger scaffolds **7** and **8** was overcome by reductive work up of the crude mixture. Reconfiguration of the flow reactor allowed the scalable functionalisation of DDS building block **5** with β-thioglucose **18** at 366 nm (FEP tubing), which was coupled on solid phase to give carbohydrate-functionalised PAA **14**. Presented together, these techniques allow the synthesis of monodisperse, sequence-defined carbohydrate functionalised PAAs with a non-immunogenic linkage between the scaffold and the carbohydrate as well as the potential for the solid phase assembly of heteromultivalent systems. The choice of technique depends on UV absorptions properties of the reactants, the amount of carbohydrate available and the desired application. Studies into the biological applications of these functionalised sequence-defined scaffolds as an antigen presenting platform and investigations of carbohydrate-protein interactions by using variations of the carbohydrate functionalisation patterns are ongoing.

Experimental Section

General procedures for the reactions of *O*-allyl glycosides with thiols at 254 nm: *General procedure A, for the optimisation of the reaction*: A photoreactor was set up according to the diagram in the Supporting Information, Figure S1, with a loop of Teflon AF-2400 polymer tubing (566 μL, 0.8 mm outer diameter (OD), 0.6 mm internal diameter (ID)) wrapped around a quartz cooling jacket that contained a NNIQ low-pressure In/Hg lamp. A recirculating chiller (Julabo FL-460, filled with spectroscopically pure water as a coolant) was used to maintain a reactor temperature of 25 °C. By using a syringe pump (Harvard PHD2000), separate solutions of the thiol (1.2 equiv) and the allyl glycoside in degassed water (1.0 equiv) were mixed through a T-mixer and passed into the photoreactor at the specified rate. After two reactor volumes of product had eluted from the reactor, an aliquot of the output solution was collected and analysed directly by HPLC/MS to assess the fractional conversion of the allyl glycoside (**9**) into glycoamino acid **10**.

General procedure B: A photoreactor was set up according to the diagram in the Supporting Information, Figure S1, with a loop of Teflon AF-2400 polymer tubing (2266 μL, 0.8 mm OD, 0.6 mm ID) wrapped around a quartz cooling jacket that contained a NNIQ low-pressure In/Hg lamp. A recirculating chiller (Julabo FL-460, filled with spectroscopically pure water as a coolant) was used to maintain a reactor temperature of 25 °C. By using a syringe pump (Harvard PHD2000), separate solutions of the thiol (0.545 mmol, 1.2 equiv) and the allyl glycoside (100 mg, 0.454 mmol, 1.0 equiv) in degassed water (2.27 mL) were mixed through a T-mixer at a rate of 113 μL min^{-1} (per syringe, combined flow rate: 226 μL min^{-1}, residence time: 10 min) and passed into the photoreactor. After discarding the first reactor volume of output, the entire product solution was collected and freeze-dried under reduced pressure to afford the crude products. The yields of the glycoamino acids were determined by ^{1}H NMR spectroscopy in D_2O (relaxation delay: 5 s) by using maleic acid as an internal standard.

General procedure C: To the freeze-dried crude material from general procedure B (30 mg, 0.088 mmol, 1.0 equiv) were added acetone (0.5 mL), (9*H*-fluoren-9-yl)methyl-(2,5-dioxopyrrolidin-1-yl)carbonate (36 mg, 0.105 mmol, 1.2 equiv), and sodium bicarbonate (11 mg, 0.132 mmol, 1.5 equiv). After stirring at RT for 12 h, the reaction mixture was taken up in water (10 mL), washed with CH_2Cl_2 (3×10 mL), and the aqueous phase was concentrated by freeze-drying under reduced pressure. Purification by preparative HPLC (Nucleodur C18 Pyramid, MeCN/water/TFA, 10:90:0.1–50:50:0.1, 30 min) gave glycoamino acids **11** as white solids.

Selected procedure for the reaction of *O*-allyl glycosides with thiols at 254 nm: A solution of α-*O*-allyl galactose **9a** (100 mg, 0.454 mmol, 1.0 equiv) in water (2.27 mL) was reacted with a solution of L-cysteine (66 mg, 0.545 mmol, 1.2 equiv) in water (2.24 mL) according to general procedure B and concentrated by freeze-drying under reduced pressure to give compound **10a** (168 mg, 71% purity, 77% yield) as an off-white solid. A sample of the crude mixture (30 mg) was reacted according to general procedure C. Purification by preparative HPLC (Nucleodur C18 Pyramid column, MeCN/water 20–50%, within 30 min, retention time: 26.1 min) afforded an analytical sample of compound **11a** as a white solid. M.p. 69–71 °C; $[\alpha]_D^{20} = +64.4$ ($c = 0.25$ in MeOH); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.82$ (d, $J = 7.5$ Hz, 2H), 7.71 (d, $J = 7.5$ Hz, 2H), 7.41 (t, $J = 7.5$ Hz, 2H), 7.41 (t, $J = 7.5$ Hz, 2H), 7.33 (t, $J = 7.5$ Hz, 2H), 4.82 (d, $J = 3.0$ Hz, 1H), 4.42–4.33 (m, 3H), 4.27 (t, $J = 7.0$ Hz, 1H), 3.90 (br s, 1H), 3.86–3.80 (m, 2H), 3.78–3.76 (m, 2H), 3.72 (dd, $J = 6.5$, 3.0 Hz, 2H), 3.54 (dt, $J = 10.0$, 6.0 Hz, 1H), 3.07 (dd, $J = 14.0$, 4.0 Hz, 1H), 2.88 (dd, $J = 14.0$, 8.0 Hz, 1H), 2.73 (t, $J = 7.5$ Hz, 2H), 1.98–1.87 ppm (m, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 171.9$, 155.3, 143.9, 143.9, 140.7, 127.6, 127.1, 125.3, 120.1, 98.8, 71.2, 69.6, 68.7, 68.5, 65.50, 65.45, 60.36, 55.0, 46.7, 34.2, 29.4, 28.3 ppm; IR (film): $\tilde{\nu} = 3221$, 2926, 1694, 1586, 1145, 1031, 972, 738 cm^{-1}; MS (ESI): *m/z*: 1149 [M_2+Na]$^+$, 586 [*M*+Na]$^+$, 440, 179; HRMS (ESI): *m/z* calcd for $C_{27}H_{33}NO_{10}S$: 586.1723 [*M*+Na]$^+$; found: 586.1716.

Selected procedures for oligomer functionalisation by TEC under a continuous flow: *H_2N-[GlcS DDS]$_5$-CONH$_2$ (**14**)*: By using a photochemical flow reactor (set up according to the Supporting Information, Figures S1 and S3) that was fitted with a loop of Teflon AF2400 tubing (566 μL), a solution of 1-thio-β-D-glucose sodium salt hydrate (**12**, 81 mg, 345 μmol, 10.0 equiv) in water (900 μL) was reacted with a solution of oligomer **6** (50 mg, 34 μmol, 1.0 equiv) in water (900 μL) and AcOH (20 μL; residence time: 10 min, flow rate: 28.3 μL min^{-1} per syringe). The reactor output was lyophilised and purified by preparative HPLC (Waters XBridge Prep C18, MeCN/water, 5–50%, within 30 min) to give glucose-functionalised oligomer **14** (49 mg, 61% yield) as a hygroscopic white powder. ^{1}H NMR (400 MHz, D_2O): $\delta = 4.52$ (d, $J = 10.0$ Hz, 5H), 3.89 (d, $J = 11.5$ Hz, 5H), 3.73–3.16 (m, 65H), 2.88–2.65 (m, 10H), 2.55–2.37 (m, 30H), 1.66 ppm (br s, 20H); MS (MALDI TOF): *m/z* calcd for $C_{95}H_{168}N_{16}O_{40}S_5Na$: 2356.01 [*M*+Na]$^+$; found: 2356.13 (monoisotopic); RP-HPLC: MeCN/water 5–25%, 60 min, $t_r = 16.9$ min.

*H_2N-[ManEtS DDS-BDS]$_5$-CONH$_2$ (**16**)*: By using a photochemical flow reactor (set up according to the Supporting Information, Figure S1) that was fitted with a loop of Teflon AF2400 tubing (566 μL), a solution of α-thioethyl-mannose (**13**, 33 mg, 137 μmol, 10.0 equiv) in water (300 μL) was reacted with oligomer **7** (32 mg, 14 μmol, 1.0 equiv) in water (300 μL) and AcOH (8 μL; residence time: 10 min, flow rate: 28.3 μL min^{-1} per syringe). The reactor output was lyophilised and dissolved in a solution of DMF (4 mL), TMSBr (200 μL), and ethanedithiol (120 μL). After shaking for 35 min, the crude product was precipitated with ice-cold Et_2O (20 mL) and washed once with Et_2O (20 mL). The crude product was dissolved in water (1 mL) and purified by preparative HPLC (Waters XBridge Prep C18, MeCN/water, 5–30%, within 30 min) to give mannose-functionalised PAA **16** (16 mg, 33% yield) as a white, hygroscopic powder. ^{1}H NMR (400 MHz, D_2O): $\delta = 4.91$ (s, 5H), 3.98–3.63 (m, 40H), 3.60–3.09 (m, 84H), 2.88–2.77 (m, 10H), 2.71–2.37 (m, 60H), 1.65 ppm (br s, 20H); MS (MALDI TOF): *m/z* calcd for

$C_{147}H_{268}N_{32}O_{55}S_5Na$: 3544.77 $[M+Na]^+$; found: 3544.62 (monoisotopic); RP-HPLC: MeCN/water 5–30 %, 60 min, $t_r = 26.7$ min.

Synthesis of GlcS−DDS (19) referring to a 366 nm flow reactor: By using a photochemical flow reactor (set up according to the Supporting Information, Figures S2 and S4) that was fitted with a loop of FEP tubing (10 mL), a solution of acetyl-protected β-thioglucose **18** (3.45 g, 9.46 mmol, 1.5 equiv) in THF (31 mL) was allowed to react with DDS **5** (3.2 g, 6.3 mmol, 1.0 equiv) in THF (27.9 mL) and AcOH (3.1 mL; residence time: 30 min, flow rate: 167 μL min^{-1} per syringe). The reactor output was concentrated under reduced pressure and purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 15:1,+1 % AcOH) to give GlcS−DDS (**19**, 3.9 g, 71 %) as a white foam. $[\alpha]_D^{20} = -10.33$ ($c = 0.25$ in MeOH); ^{1}H NMR (600 MHz, [D$_6$]DMSO): $\delta = 12.03$ (br s, 1H), 7.87 (d, $J = 7.5$ Hz, 2H), 7.68–7.60 (m, 2H), 7.41–7.38 (m, 2H), 7.33–7.29 (m, 2H), 5.25 (dt, $J = 9.5$, 5.0 Hz, 1H), 4.91–4.84 (m, 2H), 4.80 (dt, $J = 9.5$, 3.5 Hz, 1H), 4.28 (dd, $J = 18.0$, 7.0 Hz, 2H), 4.18 (t, $J = 6.8$ Hz, 1H), 4.14–4.08 (m, 1H), 4.01–3.91 (m, 2H), 3.32–3.05 (m, 8H), 2.66–2.53 (m, 2H), 2.42–2.37 (m, 2H), 2.30–2.22 (m, 4H), 2.00–1.90 (m, 12H), 1.52 ppm (br s, 4H); ^{13}C NMR (150 MHz, [D$_6$]DMSO, mixture of rotamers): $\delta =$ 173.8, 173.7, 172.1, 172.0, 171.3, 171.0, 170.0, 170.0, 169.5, 169.2, 169.0, 169.0, 156.2, 156.1, 143.9, 143.8, 140.7, 127.6, 127.0, 125.1, 125.0, 120.1, 82.0, 74.3, 73.0, 69.8, 68.2, 65.4, 65.3, 61.9, 47.1, 47.0, 46.7, 46.7, 45.3, 45.0, 38.2, 37.5, 36.6, 31.5, 31.4, 30.0, 30.0, 29.3, 29.3, 29.1, 29.0, 29.0, 28.9, 23.9, 20.5, 20.4, 20.4, 20.4, 20.3, 20.3 ppm; IR (film): $\tilde{\nu} = 2944$, 1749, 1631, 1037 cm^{-1}; HRMS (ESI): *m/z*: calcd for $C_{42}H_{53}N_3O_{15}SNa$: 894.3095 $[M+Na]^+$; found: 894.3103; RP-HPLC: MeCN/water 5–95 %, 10 min, $t_r =$ 8.0 min.

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