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Solid-phase synthesis of a new class of oligosaccharide analogues based on azasugars

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Abstract—A strategy for the solid-phase synthesis of a new class of oligosaccharide analogues based on coupling of azasugar building blocks via carbamate bonds is described.

Oligosaccharides play an important role in many biochemical recognition processes.[1] As a consequence, synthetic analogues of these natural biopolymers could be used to study, to influence or even to control these biochemical processes. Although the solid-phase synthesis of peptides and oligonucleotides is already well established, three main problems, caused by the typical properties of the sugar monomers, still complicate the synthesis of oligosaccharides: (i) stereochemical control of the coupling reactions is difficult due to the anomeric centre in the building blocks; (ii) the polyfunctionality of the monomers requires selective protection before coupling and (iii) glycosidation occurs with unpredictable and often very low yields.[2,3] A possible solution for these synthetic problems is based on structural modification of the carbohydrate building blocks in such a way that they allow easy and efficient coupling without however losing their typical properties. An important example of this approach is the use of sugar amino and imino acids for the synthesis of oligosaccharide analogues.[4]

Our approach is based on the use of azasugars as building blocks.[5] The stereochemical diversity, conformational rigidity and numerous possibilities for derivatisation make these monomers ideal tools for the construction of oligomeric combinatorial libraries. In addition, in principle these sugar analogues can be coupled in high yield via carbamate bonds, and the lack of an anomeric centre eliminates the problem of stereocontrol during coupling. Meanwhile one can expect similar properties for the resulting oligomers and natural oligosaccharides due to the resemblance of the monomeric building blocks. Finally, oligosaccharide analogues based on carbamate-linked azasugars should be resistant to proteases and glycosidases.

As an example, we describe the synthesis of three protected derivatives of 1,5-dideoxy-1,5-imino-L-iditol (Scheme 1) starting from commercially available β-D-glucose pentaacetate **1**.[6] Treatment with 4-penten-1-ol under acidic conditions led directly to pentenyl glucoside **2**.[7] Selective protection of the primary and secondary hydroxyl functions as *tert*-butyldiphenylsilyl and benzyl ethers, respectively, gave the fully protected glucoside **3**. Removal of the anomeric pentenyl group according to the method of Fraser–Reid furnished **4**.[8] Reduction of hemiacetal **4** with $NaBH_4$ afforded diol **5** which was converted to dimesylate **6**. Gentle heating of **6** in allylamine induced double nucleophilic S_N2 displacement of both mesylates providing *N*-allyl protected azasugar **7**. Similarly, reaction with neat ethylenediamine led to *N*-(2-aminoethyl) azasugar **12**.[9,10] The S_N2 mechanism of this ring closure causes inversion of the D-configuration in the starting material and leads exclusively to the azasugar analogues with the L-configuration. Removal of the allylic protecting group in compound **7** with 1-chloroethyl chloroformate (ACE-Cl) and subsequent solvolysis with methanol gave azasugar **8**.[11,12] Electrophilic amination of **8** with *N*-fluorenylmethoxycarbonyl-3-(2,4-dichlorophenyl)-oxaziridine furnished *N*-Fmoc protected *N*-amino azasugar **9**.[13,14] Although *N*-amino azasugar **10** could not be isolated due to its instability, the formation of this compound was proven by conversion into its stable dimethyl hydrazone **11**.[15]

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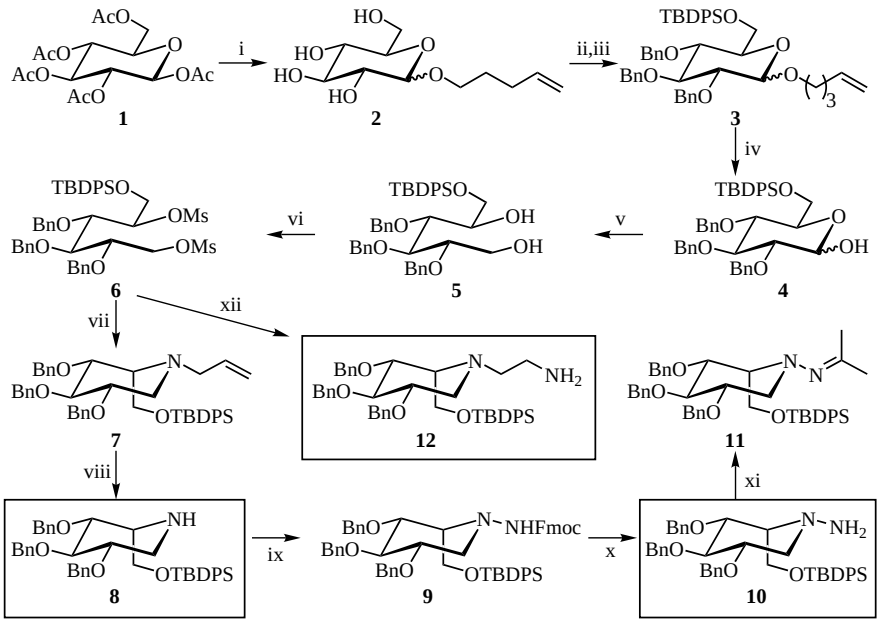
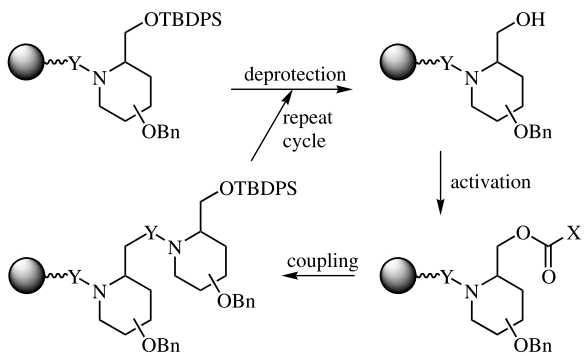


Scheme 1. Synthesis of the building blocks. *Reagents and conditions*: (i) 4-penten-1-ol, PTSA, $CHCl_3$, 90°C, 70 h (85%); (ii) DBU, TBDPSCl, THF, 22 h (94%); (iii) NaH, BnBr, 18-crown-6, THF, 70°C, 19 h (85%); (iv) NBS, 1% aq. CH_3CN, 2 h (70%); (v) $NaBH_4$, EtOH, 20 h (92%); (vi) MsCl, Et_3N, DMAP, CH_2Cl_2, −15°C, 3 h (80%); (vii) allylamine, 70°C, 39 h (73%); (viii) (1) ACE-Cl, $ClCH_2CH_2Cl$, 90°C, 24 h, (2) MeOH, 80°C, 21 h (82%); (ix) *N*-Fmoc-3-(2,4-dichlorophenyl)oxaziridine, $CHCl_3$, 0°C, 3 h (60%); (x) piperidine, DMF, 16 h; (xi) acetone, 40°C (70% for two steps); (xii) ethylenediamine, 70°C, 46 h (72%).

Our strategy for the solid-phase synthesis of oligosaccharide analogues is based on a three-step coupling procedure (Scheme 2). In the first step the primary hydroxyl function of a resin-bound azasugar is released after selective cleavage of the *tert*-butyldiphenylsilyl ether with TBAF. The second step consists of activation of this free hydroxyl group as a reactive carbonate or carbamate. In the final step the resulting activated intermediate is treated with an azasugar. Nucleophilic attack of this building block at the activated carbonyl group leads to the formation of the carbamate bond between the monomers. Repetition of this convenient coupling cycle offers the possibility to construct oligomeric carbohydrate analogues.

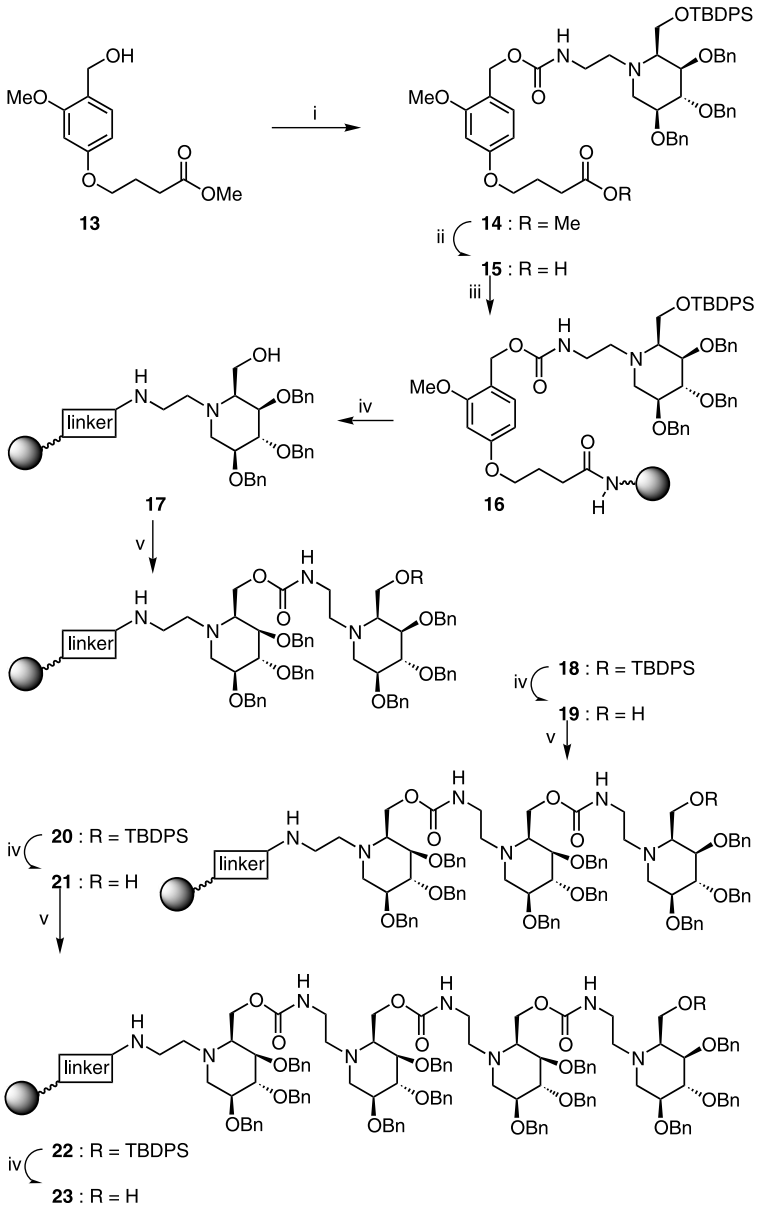
In order to demonstrate the viability of this solid-phase coupling strategy we envisaged the synthesis of a repetitive tetrasaccharide analogue on Tentagel™ R NH_2 resin. This resin features a better swelling and a lower loading than the standard Tentagel™ resins and was especially developed for difficult syntheses and the synthesis of oligomers. The acid-labile HMPB linker **13** offers perfect compatibility with the basic reaction conditions during the coupling cycle and the protecting groups in the building blocks.[16] This enables monitoring of each reaction step via TLC and ES-MS after cleavage of a small amount of product from the resin.

Whereas preloading of the HMPB linker **13** (after activation as a reactive carbonate) with azasugar **8** proved to be impossible due to steric hindrance of the secondary amino function in this building block and the instability of the activated linker,[17] coupling of **13** with azasugar **12** (after activation as the isocyanate) afforded preloaded linker **14** in good yield (Scheme 3). Indeed, conversion of the primary amine of **12** into a highly reactive isocyanate with di-*tert*-butyltricarbonate offers the possibility for an alternative preloading procedure where the use of the unstable activated linker is avoided.[18] Saponification of methyl ester **14** led to carboxylic acid **15** which was linked to the resin via an amide bond using standard peptide coupling conditions. The 2,4,6-trinitrobenzenesulfonic acid test (TNBS test) indicated quantitative reaction after 24 h.[19] All further solid-phase reactions were monitored via TLC and ES-MS after release of a small amount of product from the resin by treatment with 1% TFA in dichloromethane.



X = leaving group, Y = OCO, OCONH, $OCONHCH_2CH_2$

Scheme 2. Three-step coupling cycle.



Scheme 3. Solid-phase synthesis. *Reagents and conditions*: (i) (1) azasugar **12**, di-*tert*-butyltricarbonate, $CHCl_3$, rt, 15 min, (2) add **13**, 70°C, 24 h (84%); (ii) NaOH, MeOH, 16 h (88%); (iii) Tentagel™ R NH_2, DIC, HOBt, DMAP, CH_2Cl_2, 24 h; (iv) TBAF, THF, 24 h; (v) (1) bis(4-nitrophenyl) carbonate, DMAP, DMF, 24 h, (2) azasugar **12**, DIPEA, DMF, 24 h.

Table 1. Coupling reagents and conditions for the synthesis of **18** on solid phase (Scheme 3)

Entry	Reaction conditions	Yield (%)[a]	Yield (%)[b]
1	(i) *N*,*N*′-Disuccinimidyl carbonate (10 equiv.), DMAP (1 equiv.), DMF, 2 h; (ii) **12** (5 equiv.), DIPEA (10 equiv.), DMF, 24 h	72	89
2	(i) 1,1′-Carbonyldiimidazole (10 equiv.), DMAP (1 equiv.), DMF, 2 h; (ii) **12** (5 equiv.), DIPEA (10 equiv.), DMF, 24 h	33	–
3	(i) 4-Nitrophenyl chloroformate (10 equiv.), pyridine, CH_2Cl_2, 0°C, 2 h; (ii) **12** (5 equiv.), DIPEA (10 equiv.), DMF, 24 h	41	–
4	(i) Bis(4-nitrophenyl) carbonate (10 equiv.), DMAP (1 equiv.), DMF, 24 h; (ii) **12** (5 equiv.), DIPEA (10 equiv.), DMF, 24 h	100	–
5	(i) **12** (5 equiv.), Di-*tert*-butyltricarbonate (5 equiv.), 30 min; (ii) add resin **17**, $CHCl_3$, 70°C, 24 h	45	65

[a] After a single coupling.
[b] After repetition of the coupling sequence.

Treatment of resin **16** with TBAF resulted in deprotection of the silyl ether and afforded primary alcohol **17**. In Table 1, the efficiency of four different reagents for the activation of the primary hydroxyl group of **17** (entries 1–4) and one for activation of the amino group of **12** (entry 5) was compared by measuring the yield of the dimer after treatment of the activated intermediate with building block **12** (entries 1–4) or with resin **17** (entry 5). Somewhat surprisingly, the best results were obtained with bis(4-nitrophenyl) carbonate (entry 4).[20] All other reagents led to lower yields (entries 1 and 5) or to formation of side products (entries 2 and 3). Hence, starting from resin **17**, deprotection of the *tert*-butyldiphenylsilyl ether with TBAF, followed by activation of the primary hydroxyl function with bis(4-nitrophenyl) carbonate and treatment of the activated 4-nitrophenyl carbonate with the next building block gave dimer **18**.[21] Repetition of this coupling cycle led to trimer **20** and tetramer **22**. ES-MS and TLC analysis indicated nearly quantitative yields for each reaction step and purities of >95%, impurities mainly resulting from incomplete coupling. Compounds **19**, **21** and **23** were cleaved from the resin under mild acidic conditions and, after purification, debenzylated in solution using transfer hydrogenation on Pd/C with cyclohexene as hydrogen donor (Scheme 4 and Fig. 1).[22] Purification of the fully deprotected dimer **25**, trimer **26** and tetramer **27** was performed on silicagel using eluent mixtures of iso-propanol and 25% aqueous ammonia.

It should be noticed that this type of azasugar oligomers is quite remarkable, as the iminosugar units are coupled via the 5a-nitrogen atom (carbohydrate numbering), which in this case corresponds to the pyranose ring oxygen in the real sugar, and not to the anomeric carbon atom. Examples of biologically active oligosaccharides incorporating a similar 5a-azasugar unit coupled via the nitrogen atom are very scarce.[23]

In conclusion, we have developed a strategy for the solid-phase synthesis of a new class of azasugar-based oligosaccharide analogues. Our strategy relies on a highly efficient three-step (deprotection–activation–coupling) formation of a carbamate bond between azasugar building blocks, and leads to a new class of highly functionalised oligosaccharide mimics with potential bioactivity. The viability of our approach was illustrated for a derivative of 1,5-dideoxy-1,5-imino-L-iditol, for which a synthesis was developed. Our approach should be easily adaptable to the solid-phase coupling of other types of aza-sugars, e.g. isofagomine and its stereoisomers.

Acknowledgements

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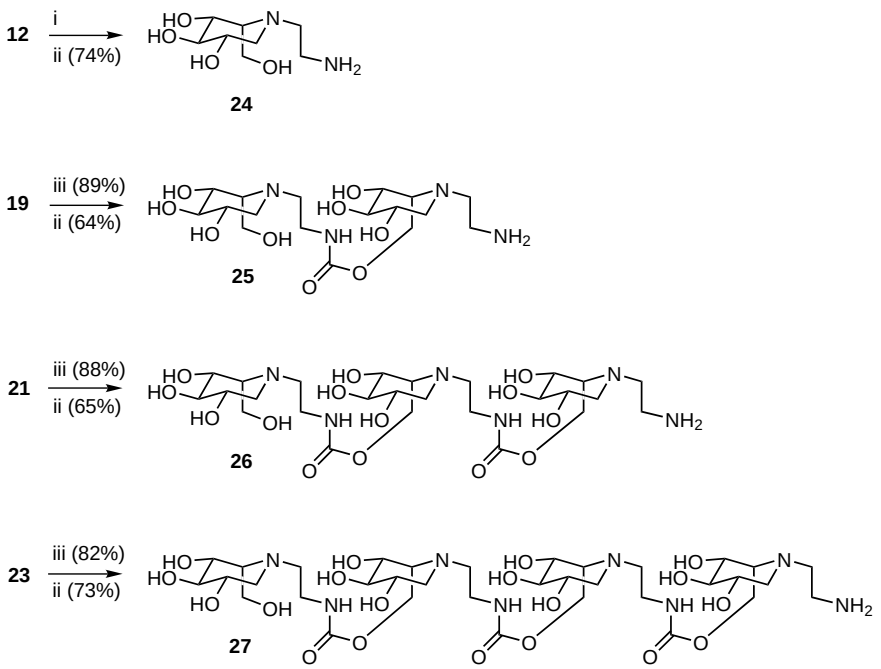
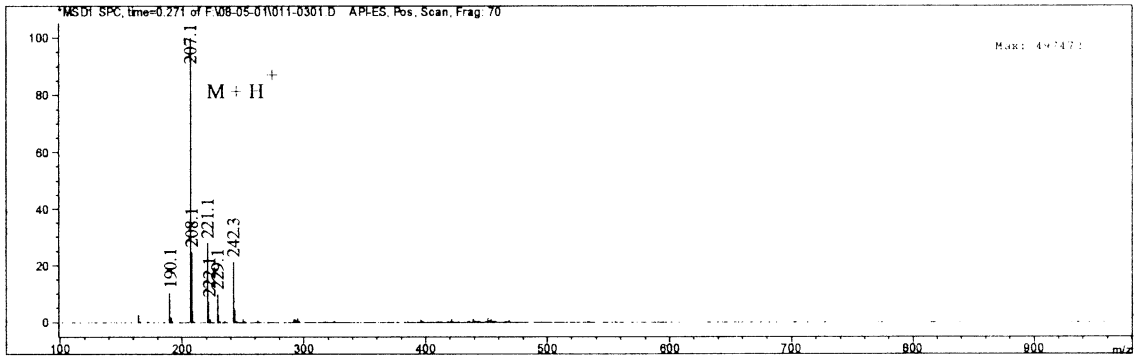
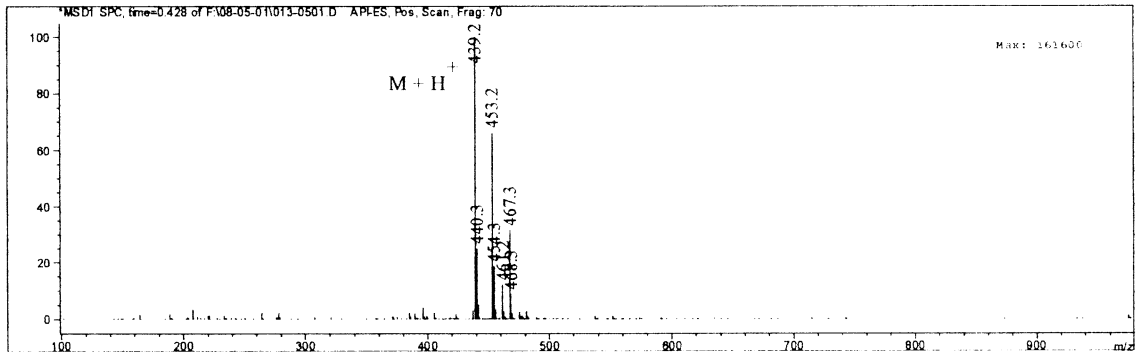


Scheme 4. Cleavage and deprotection. *Reagents and conditions*. (i) TBAF, THF, 20 h (95%); (ii) Pd/C, cyclohexene, MeOH, reflux, 24 h; (iii) 1% TFA in CH_2Cl_2, kt, 5×10 min.

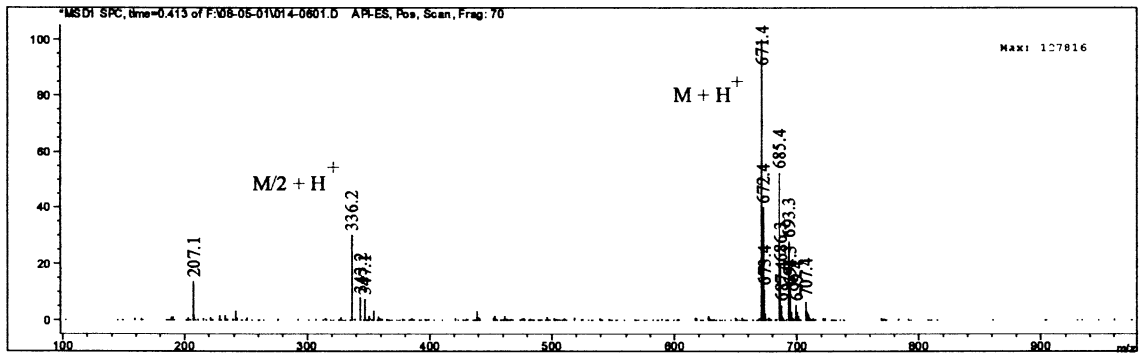
Monomer **24** :



Dimer **25** :



Trimer **26** :



Tetramer **27** :

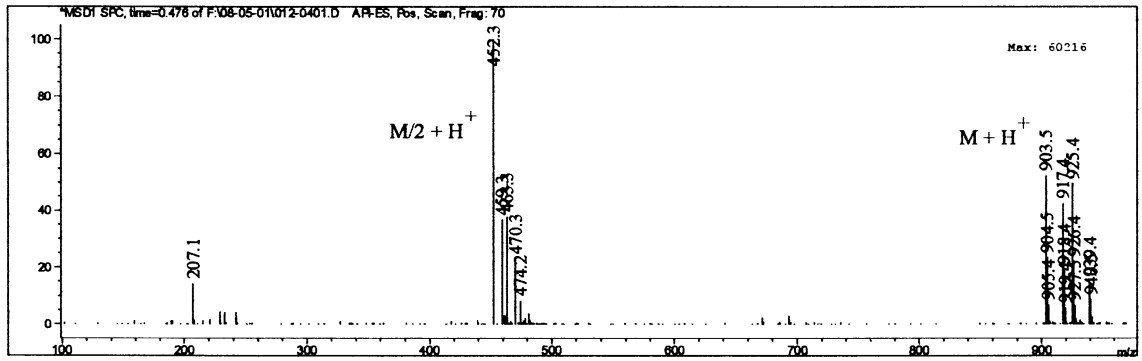


Figure 1. ESMS spectra for compounds **24**, **25**, **26** and **27** (API-ES, Positive mode, Flow Injection Analysis).

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10. Selected data for **12**: δ_H (500 MHz, $CDCl_3$) 1.06 (9H, s), 1.50 (2H, br s), 2.74 (4H, m), 2.91 (2H, m), 3.27 (1H, m), 3.56 (1H, m), 3.62 (1H, t, $J=9.4$ Hz), 3.68 (1H, dd, $J=5.7$ Hz, $J=9.4$ Hz), 3.93 (1H, dd, $J=2.4$ Hz, $J=11.2$ Hz), 4.08 (1H, dd, $J=7.2$ Hz, $J=11.2$ Hz), 4.56 (1H, d, $J=11.6$ Hz), 4.58 (1H, d, $J=11.6$ Hz), 4.65 (1H, d, $J=11.6$ Hz), 4.73 (1H, d, $J=11.6$ Hz), 4.76 (1H, d, $J=10.9$ Hz), 4.80 (1H, d, $J=10.9$ Hz), 7.22 (2H, m), 7.32 (17H, m), 7.42 (2H, m), 7.72 (4H, m) ppm. δ_C (125 MHz, $CDCl_3$) 18.9, 26.7, 39.4, 48.9, 57.5, 58.3, 61.9, 72.5, 72.8, 75.3, 79.0, 80.2, 82.9, 127.2, 127.3, 127.4, 127.5, 127.5, 127.8, 128.1, 128.2, 129.5, 129.5, 133.0, 133.1, 135.5, 138.3, 138.5, 138.9 ppm.
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12. Selected data for **8**: δ_H (500 MHz, $CDCl_3$) 1.07 (9H, s), 2.84 (1H, dd, $J=7.1$ Hz, $J=12.5$ Hz), 2.99 (1H, dd, $J=4.1$ Hz, $J=12.5$ Hz), 3.29 (1H, ddd, $J=4.5$ Hz, $J=6.0$ Hz, $J=9.1$ Hz), 3.47 (1H, dd, $J=6.7$ Hz, $J=10.7$ Hz), 3.62 (1H, t, $J=6.7$ Hz), 3.72 (1H, dd, $J=4.5$ Hz, $J=6.7$ Hz), 3.76 (1H, dd, $J=6.0$ Hz, $J=10.3$ Hz), 3.89 (1H, dd, $J=9.1$ Hz, $J=10.3$ Hz), 4.45 (1H, d, $J=11.4$ Hz), 4.52 (1H, d, $J=11.4$ Hz), 4.58 (1H, d, $J=11.8$ Hz), 4.62 (1H, d, $J=11.8$ Hz), 4.65 (2H, s), 7.18 (2H, m), 7.34 (19H, m), 7.66 (4H, m) ppm. δ_C (125 MHz, $CDCl_3$) 19.2, 26.9, 44.0, 56.3, 60.1, 72.1, 72.5, 74.2, 77.0, 77.3, 78.5, 127.4, 127.5, 127.5, 127.6, 127.7, 127.7, 127.8, 128.2, 128.3, 129.7, 133.4, 133.5, 135.5, 138.4, 138.5, 138.6 ppm.
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15. Selected data for **11**: δ_H (500 MHz, $CDCl_3$) 1.07 (9H, s), 1.86 (3H, s), 1.88 (3H, s), 2.94 (1H, dd, $J=4.6$ Hz, $J=13.2$ Hz), 3.18 (2H, m), 3.90 (2H, m), 4.01 (2H, m), 4.12 (1H, dd, $J=3.4$ Hz, $J=10.7$ Hz), 4.63 (1H, d, $J=11.7$ Hz), 4.65 (1H, d, $J=11.8$ Hz), 4.72 (1H, d, $J=11.8$ Hz), 4.75 (1H, d, $J=11.7$ Hz), 4.78 (1H, d, $J=11.0$ Hz), 4.85 (1H, d, $J=11.0$ Hz), 7.35 (21H, m), 7.77 (4H, br d, $J=7.9$ Hz) ppm. δ_C (125 MHz, $CDCl_3$) 18.3, 19.2, 25.2, 26.9, 52.5, 60.8, 64.9, 72.9, 73.4, 75.1, 77.5, 78.2, 82.7, 127.5, 127.5, 127.7, 127.9, 128.0, 128.0, 128.3, 129.5, 129.6, 133.5, 133.6, 135.7, 135.8, 138.9, 139.1, 139.2, 164.4 ppm.
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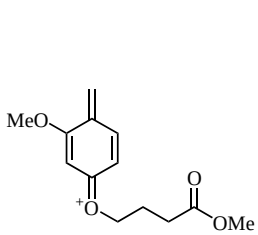
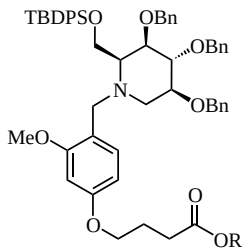
17. The slow nucleophilic attack of the sterically hindered building block **8** allows mesomerically induced decomposition of the activated linker. Conjugate addition of **8** to the highly reactive decomposition product ***i*** leads to formation of a preloaded compound ***ii*** with a benzylic C–N bond between the azasugar and the HMPB linker in 14% yield.



i



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21. **Typical experimental coupling procedure**:
To resin **18** (0.3 mmol) were added dry THF (10 ml) and a 1 M solution of TBAF in THF (3.0 ml, 3.0 mmol). After shaking the resin for 20 h under inert atmosphere the resin was washed with DMF (10 ml), EtOH (10 ml) and CH_2Cl_2 (10 ml). This washing procedure was repeated five times. After thorough drying of the resin, DMAP (37 mg; 0.3 mmol) and a solution of bis(4-nitrophenyl) carbonate (915 mg; 3.0 mmol) in dry DMF (15 ml) were added. After shaking for 24 h under inert atmosphere the resin was washed quickly five times with dry DMF (10 ml), followed by addition of a solution of building block **12** (1.07 g; 1.5 mmol) and DIPEA (0.51 ml; 3.0 mmol) in dry DMF (15 ml). The reaction was

shaken for another 24 h under inert conditions affording resin **20**. Finally the resin was washed consecutively with CH_2Cl_2 (5×10 ml) and MeOH (5×10 ml).

22. **Typical experimental cleavage and deprotection procedure**: A portion of resin **19** (600 mg) was treated with a freshly prepared 1% solution of TFA in dry CH_2Cl_2 (10 ml). After shaking the resin for 10 min the resin was filtered and the filtrate was collected. This procedure was repeated five times and the combined filtrates were neutralised with a resin-bound proton scavenger (piperidinomethyl polystyrene HL resin, 2% DVB, 200–400 mesh, loading 3.57 mmol/g, Novabiochem). The residue obtained after filtration of the scavenger and concentration of the filtrate in vacuo was purified via column chromatography (CH_2Cl_2/MeOH: 95/5). The resulting pure product was dissolved in MeOH, and cyclohexene (20% v/v) and 10% Pd/C (100% w/w) were added. After refluxing for 24 h the catalyst was removed via filtration over Celite, and the residue obtained after concentration of the filtrate in vacuo was purified via column chromatography on silica gel (isopropanol/NH_3 (25% in H_2O):55/45) affording **25** (57% overall yield).
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