Rational design of multivalent glycoconjugate ligands. Synthesis of libraries of conformationally flexible rotamers of poly-*N*-linked lactosyl glycines

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A reiterative and convergent oligo(*N*-substituted glycine) strategy is used to construct combinatorial structures of conformationally flexible multivalent *N*-linked lactose-containing glycopeptoids having triethylene glycol spacers between the sugar residues and the peptoid backbones.

The galectins are a family of β-galactoside (Gal)- and *N*-acetylgalactosamine (GalNAc)-binding lectins that are involved in the regulation and trafficking of free or cell-bound glycoproteins bearing clustered Gal–GalNAc (lactose–*N*-acetyllactosamine) motifs.[1] Macrophages[2] and certain metastases[3] have also been found to express galectins. The best characterized representatives of the galectins are the asialoglycoprotein receptors (ASGP-R) on mammalian hepatocytes.[4] In the latter case, multivalent ligands of Gal–GalNAc–lactosides have been synthesized to better define the carbohydrate–protein interactions involved.[4] As part of ongoing activities in the general design and applications of glycoconjugates,[5] we have also described the synthesis of multivalent neoglycoproteins,[6] glycopolymers,[7] telomers[8] and dendrimers[9] containing lactosides.

The new strategy described herein allows the easy scaffolding of multivalent combinatorial oligomers which, by virtue of repeating secondary amide subunits, provides populations of fast equilibrating rotamers in every module. Each of these conformationally flexible and multivalent isomers offers the possibility of exploring complex clustered receptors. The key building *N*-linked lactosyl unit **11** was built as *N*-substituted glycylglycine glycopeptoid[10] having hydrophilic triethylene glycol spacer arm. This strategy also provides the possibility of adjusting both distances between the sugars and the oligomeric backbone and in between each sugar residue.

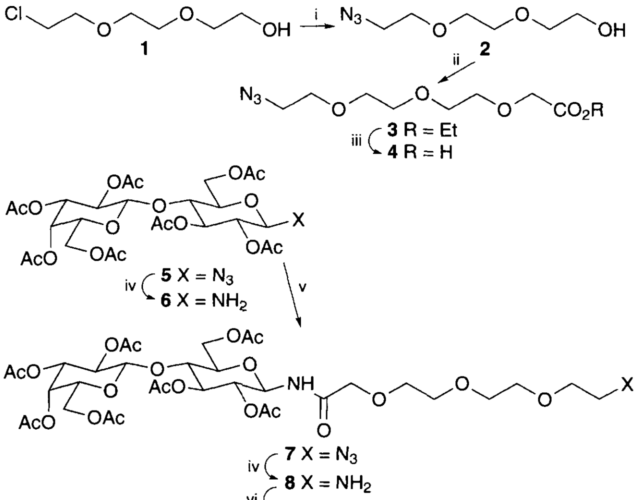
The azido-ester spacer arm was synthesized from 2-[(2-chloroethoxy)ethoxy]ethanol **1** following an adaptation of published procedures[11] (Scheme 1).† Thus, chloride **1** was first transformed into azide **2** in 96% yield by treatment with NaN_3 and NaI in EtOH under reflux. Azide **2** was treated with ethyl diazoacetate in the presence of $BF_3{\cdot}Et_2O$ to give azido ester **3** (80%). Saponification of **3** [KOH (0.5 mol dm^{-3}), EtOH–H_2O] provided acid **4** quantitatively.

β-D-Lactosyl azide **5**, obtained under PTC conditions,[12] was quickly reduced to glycosyl amine **6** in methanol (10% Pd–C, 30 min, quant.). Attachment of amine **6** to acid spacer **4** was effected by DCC coupling in CH_2Cl_2 which afforded azido derivative **7** as an amorphous solid in 90% yield.‡

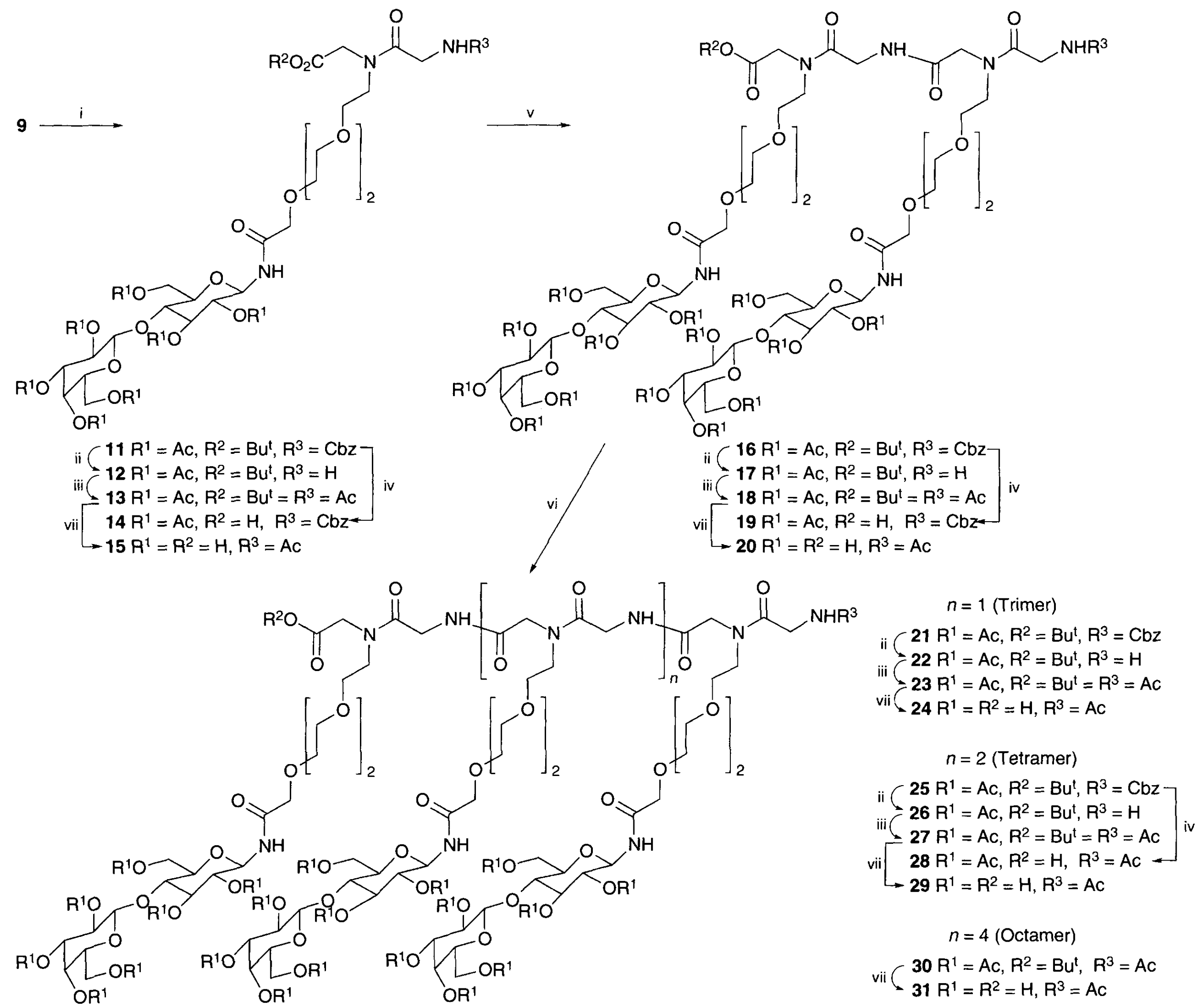
Reduction of the azide group as above gave extended amine **8** also quantitatively. Formation of *N*-substituted glycine derivative **9**‡ was achieved in 65% yield by mono-*N*-alkylation of **8** with *tert*-butyl bromoacetate **10** in acetonitrile containing diisopropylethylamine (DIPEA). The synthesis of the key building block **11** was performed by coupling amine **9** with benzyloxycarbonyl glycine with DCC (85%) (Scheme 2). The Cbz-protected repeating unit **11** was then sequentially transformed into amine ester **12** (10% Pd–C, quant.) or acid **14** (TFA, CH_2Cl_2, 94%). Alternatively, amine **12** was also transformed into monomer **13** having an acetamido end group (AcCl, DIPEA, CH_2Cl_2, 87%). Secondary amide **13** was shown to exist as two rapidly equilibrating rotamers in 1 : 1.5 ratio, as seen from its ^1H and ^{13}C NMR spectra.‡ This particular behaviour represents a key aspect to the present study since the *syn* an *anti* amide rotamers allow the lactosyl residues to occupy two different space *loci*. As the number of secondary amide bonds (n) increases with the size of the glycoconjugates, the number of flexible rotamers increases accordingly as a function of 2^n, thus providing single molecules in which the sugar residues can occupy a large number of loci. Interestingly, the ^1H NMR spectrum of the Cbz-protected monomer **11** was more complex than that of amide-protected **13** (4s, for the *tert*-butyl methyl signals at δ 1.40–1.42 instead of two in **13**). This suggests that the urethane derivatives also exist as *syn* and *anti* rotamers.||

With the orthogonally protected amino acid **11** in hand, the construction of higher oligomers (di-, tri-, tetra- and octa-mers) was successfully accomplished using a reiterative process. Accordingly, protected dimer **16** was obtained in 75% yield using DCC coupling between amine **12** and acid **14**. Reduction of the Cbz-group of **16** and transformation into acetamide **18** was completed by the two step procedures described above (85%). Selective protecting group removal in **16** as above provided dimer amine **17** quantitatively and dimer acid **19** (86%). Similarly, trimer **21** was prepared from **12** and **19** by DCC coupling (81%). Reduction of the CBz-group of **21** and acetylation gave acetamide **23** (84%).

The entire set of reactions was repeated to obtain tetramer **25** (82%) and its derivatives (Scheme 2). The final octameric unit was prepared from tetrameric amine **26** and acetamide **28** (75%). Fully deprotected glycoconjugates **15**, **20**, **24**, **29** and **31** were all obtained in essentially quantitative yields by the following sequence of reactions. Zemplén removal of the



Scheme 1 *Reagents and conditions*: i, NaI, NaN_3, EtOH, reflux, 24 h, 96%; ii, $N_2CH_2CO_2Et$, $BF_3{\cdot}Et_2O$, CH_2Cl_2, room temp., 6 h, 80%; iii, KOH (0.5 mol dm^{-3}), EtOH, H_2O, reflux, 6 h, then H^+ resin, quant.; iv, H_2, 10% Pd–C, MeOH, 30 min. for **5**, 1 h for **7**, quant.; v, DCC, **4**, CH_2Cl_2, room temp., 1 h, 90%; vi, $BrCH_2CO_2Bu^t$ **10**, DIPEA, MeCN, room temp., 3 h, 65%



Scheme 2 *Reagents and conditions*: i, CbzGlyOH, DCC, CH_2Cl_2, room temp., 3 h, 85%; ii, 10% Pd–C, MeOH, 1 h, quant.; iii, AcCl, DIPEA, CH_2Cl_2, room temp., 30 min., 87% (**13**), 85% (**18**), 84% (**23**), 87% (**27**); iv, TFA, CH_2Cl_2 (1 : 4, *v*/*v*), room temp., 3 h, 94% (**14**), 86% (**19**), 86% (**28**); v, **12** and **14**, DCC, MeCN, CH_2Cl_2 (1 : 1, *v*/*v*), room temp., 2 h, 75%, vi, **12** and **19**, DCC, MeCN, CH_2Cl_2 (1 : 1, *v*/*v*), room temp., 3 h, 81% (trimer **21**), for **17** and **19**, 82% (tetramer **25**), for **26** and **28**, 75% (octamer **30**); vii, NaOMe, MeOH, room temp., 1 h, quant., TFA, CH_2Cl_2 (1 : 2, *v*/*v*), room temp., 6 h, quant., fully deprotected **15** (monomer), **20** (dimer), **24** (trimer), **29** (tetramer), **31** (octamer)

O-acetyl group of the lactose residues (NaOMe, MeOH, room temp., 1 h) followed by acid deprotection of the terminal *tert*-butyl esters (TFA, CH_2Cl_2, 1 : 2, *v*/*v*, room temp., 6 h) provided the target family of multivalent lactosylated conjugates almost quantitatively.

Footnotes

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† All new compounds exhibited consistent spectral ^1H and ^{13}C NMR and MS data.

‡ *Selected data* for **7**: $[\alpha]_D$ + 2.4 (*c* 1.36, $CHCl_3$); $\delta_{^1H}$ ($CDCl_3$) 3.36 (m, 2 H, CH_2N_3), 4.44 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1′), 5.20 (dd, 1 H, $J_{1,NH}$ 9.5, $J_{1,2}$ 7.8 Hz, H-1); δ_{13C} 50.6 (CH_2N_3), 77.6 (C1), 100.9 (C1′); For **9**: $[\alpha]_D$ +2.66 (*c* 0.9, $CHCl_3$), FAB MS 935.5 (M^+ +1, 1.4%); $\delta_{^1H}$ ($CDCl_3$, 500 MHz) δ 1.42 (s, 9 H, CMe_3), 1.91–2.10 7s, 21 H, OAc), 2.57 (b, 1 H, N*H*CH_2CO), 2.78 (t, 2 H, *J* 4.2 Hz, C*H*$_2$NHCO), 3.30 (s, 2 H, C*H*$_2CO_2Bu^t$), 3.56–3.65 (m, 10 H, PEG-CH_2), 3.70 (m, 1 H, H5), 3.76 (dd, 1 H, $J_{3,4}$ 9.2, $J_{4,5}$ 9.8 Hz, H4). For **13**: $\delta_{^1H}$ ($CDCl_3$, 500 MHz) 1.43 and 1.46 (C*Me*$_3$), 2.12 and 2.16 (NAc), δ_c 28.0, 28.1 (C*Me*$_3$), 81.9, 82.9 (*C*Me_3); FABMS 1038.5 (M^+, 16.4%).

‖ The structural integrity of the higher oligomers was better ascribed on the basis of the integration of the *N*-acetyl/*tert*-butyl/anomeric signals in their ^1H NMR spectra.

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