Synthesis and NMR studies of malonyl-linked glycoconjugates of N-(2-aminoethyl)glycine. Building blocks for the construction of combinatorial glycopeptide libraries

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Abstract
Four glycoconjugate building blocks for the construction of combinatorial PNA like glycopeptide libraries were prepared in 75–79% yield by condensing tert-butyl N-[2-(N-9-fluorenylmethoxycarbonylamino)ethyl]glycinate (AEG) 5 with 3-oxo-3-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosylamino) (6a), 3-oxo-3-(β-D-galactopyranosylamino) (6b), 3-oxo-3-(2-acetamido-2-deoxy-3,4,6-tetra-O-acetyl-β-D-galactopyranosylamino)-propanoic acid (6d), respectively. The resulting AEG glycoconjugates 1a–d were converted into the corresponding free acids 2a–d in 97–98% yield by treatment with aqueous formic acid. The Fmoc group of compound 1c was removed and the intermediate amine 9 was condensed with 2a to afford the corresponding glycosylated AEG dipeptide 4 in 58% yield. All glycoconjugate building blocks showed the presence of cis and trans rotamers. Compounds 1a, 1b and 4 were subjected to temperature dependent 1H NMR spectroscopy in order to determine the coalescence temperature which resulted in calculated rotation barriers of 17.9–18.3 kcal/mol for the rotamers.

Introduction
The glycocalyx is a fringy or fuzzy polysaccharide layer coating most animal and many bacterial cells. It is covalently bound to the surface of the cell membrane through glycoproteins and plays a major role in numerous biologically important recognition mechanisms like cell–cell recognition, signal transduction and immunological processes [1-4]. Therefore, investigating the delicate carbohydrate–protein interactions on a molecular level is an inalienable prerequisite for a deep understanding of the fundamental cellular recognition processes involving the complex saccharides of the glycocalyx [5-8]. Unfortunately, isolation of pure carbohydrate material or specific glycoconjugates from natural sources remains a difficult, sometimes even an
unrealizable task, for naturally occurring saccharides exhibit micro-heterogenity which makes it nearly impossible to obtain pure material from such sources. Chemical or chemoenzymatic syntheses of complex oligosaccharides, on the other hand, may provide sufficient amounts of pure material for such studies. Despite the great achievements in oligosaccharide synthesis during the past decades, the preparation of complex oligosaccharides can be tedious, lengthy or circuitous, and the often intrinsic intricacy of a chemical saccharide synthesis makes it sometimes impossible to prepare a certain saccharide or glycoconjugate [9]. Therefore, gaining access to new glycoconjugates which are easily accessible by chemical synthesis and which are able to mimic the interaction between a specific protein and its natural oligosaccharide ligand are highly desirable [10-13]. In our previous work we introduced various trifunctional glycopeptide building blocks derived from aspartic acid, 3-aminoethyl-5-aminobenzoic acid [14] and from the PNA-like N-(2-aminoethyl)glycine (AEG) backbone to which sugar moieties were linked through either simple alkyl chains [15,16], amino alcohols [17,18] or 1,2,3-triazoles [19-21]. These building blocks were used for automated SPOT synthesis on a cellulose surface in order to construct complex glycoconjugates which are able to specifically bind to lectins [15,18,20].

In continuation of these studies, we now describe the preparation of PNA-based glycoconjugate building blocks 1–3 as well as a dimeric glycoconjugate 4 in which the sugar moieties are attached through a malonyl linker (Figures 1–3). For these compounds we studied the cis/trans-rotamer structures via temperature-dependent $^1$H NMR spectroscopy. Unfortunately, the amide protons of the rotamers of the unprotected glycoconjugates could not be observed in the $^1$H NMR spectra in D$_2$O due to the fast H/D exchange with the solvent (Figure 2). Nevertheless, we could identify two rotameric forms exhibiting a cis/trans ratio of 2:1 which was in accordance with similar rotamers described in literature [22]. Hereupon, we

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**Figure 1:** cis- and trans rotamer of protected PNA building blocks 1a–d and 2a–d.

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**Figure 2:** cis- and trans rotamer of unprotected PNA building block 3.
report on our investigations concerning the structures of the fully protected conjugates 1 and 4 for the rotamers of which around the C–N bond we calculated the respective $\Delta G^\ddagger_r$-values.

Results and Discussion

Synthesis of building blocks

The preparation of building blocks 1a–d and 2a–d started from tert-butyl $N$-(2-((N-9-fluorenylmethoxycarbonylamino)ethyl))glycinate hydrochloride (5) which was synthesized in 39% yield from tert-butyl bromoacetate according to the procedure published by Thomson et al. [23]. Acetyl protected glycosylaminomalonic acids 6a–d were prepared from the corresponding tert-butyl esters as previously described [14]. Coupling of 5 with acids 6a–d was achieved with either $N,N',N''$-tetramethyl-$O$-(1H-benzo[triazol-1-yl]uronium hexafluorophosphate (HBTU) or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (Scheme 1, Table 1). In general, HBTU gave higher yields of malonamides 1a–d than EDCI. Previously, we used
Table 1: Synthesis of building blocks 1a–d.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting material</th>
<th>Coupling method</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6a</td>
<td>A) HBTU</td>
<td>1a</td>
<td>A) 79%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B) EDCI</td>
<td></td>
<td>B) 40%</td>
</tr>
<tr>
<td>2</td>
<td>6b</td>
<td>A) HBTU</td>
<td>1b</td>
<td>A) 79%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B) EDCI</td>
<td></td>
<td>B) 47%</td>
</tr>
<tr>
<td>3</td>
<td>6c</td>
<td>A) HBTU</td>
<td>1c</td>
<td>A) 75%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B) EDCI</td>
<td></td>
<td>B) 57%</td>
</tr>
<tr>
<td>4</td>
<td>6d</td>
<td>A) HBTU</td>
<td>1d</td>
<td>A) 77%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B) EDCI</td>
<td></td>
<td>B) 42%</td>
</tr>
</tbody>
</table>

EDCI for coupling acids 6a–d to aniline derivatives because HBTU resulted in byproducts which were difficult to be removed [14]. Such byproducts were not observed here though.

Next, the tert-butyl ester groups of building blocks 1a–d were removed under acid conditions with a 2:1 mixture of formic acid and dichloromethane at room temperature to give the corresponding free acids 2a–d in 97–98% yield (Scheme 1, Table 2).

Partially deprotected building block 3 was prepared from 1a as follows. First, removal of the Fmoc group in 1a under basic conditions with triethylamine in DMF gave the crude amino derivate which was acetylated with Ac₂O to give building block...
Table 2: Synthesis of building blocks 2a–d.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting material</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1a</td>
<td>2a</td>
<td>98%</td>
</tr>
<tr>
<td>2</td>
<td>1b</td>
<td>2b</td>
<td>97%</td>
</tr>
<tr>
<td>3</td>
<td>1c</td>
<td>2c</td>
<td>97%</td>
</tr>
<tr>
<td>4</td>
<td>1d</td>
<td>2d</td>
<td>97%</td>
</tr>
</tbody>
</table>

7 in 60% yield. Removal of the acetyl groups of the sugar moiety in 7 to afford compound 3 could be achieved in a virtually quantitative yield by subjection 7 to a saturated solution of NH$_3$ in MeOH (7 N) (Scheme 2).

In addition to the monomeric glycoconjugates 1a–d and 2a–d we also prepared dimer 4 from the glucose containing conjugate 2a and the N-acetylglucosamine containing conjugate 1c. Treatment of 1e with 20% piperidine in DMF at room temperature for 3.5 h gave partially protected compound 8 which was used for the next step without further purification. Coupling of 2a with crude 8 using HBTU, 1-hydroxytriazole (HOBt) and diisopropylethylamine (DIPEA) in DMF gave dipeptide 4 in 58% yield (Scheme 3).
**1D-NMR investigation**

Glycoconjugates \( 1a, b \) and \( 4 \) were submitted to temperature-dependent \(^1\text{H}\) NMR spectroscopy in order to reveal, verify and determine their cis- and trans-rotameric structures (see also Figures 1–3). The \(^1\text{H}\) NMR spectra of \( 1a \) and \( 1b \) in CDCl\(_3\) at room temperature revealed two separate doublets at 8.19 and 7.99 ppm for \( 1a \) and 8.26 and 7.99 ppm for \( 1b \), respectively for the anomeric amide proton indicating the presence of two rotamers (Figure 4 and Figure 5). The signals at lower field were assigned to the respective trans rotamers whereas the signals at higher field were assigned to the corresponding cis rotamers of \( 1a \) and \( 1b \) (see 2D NMR investigations below).

The \(^1\text{H}\) NMR spectrum of the dimeric PNA glycoconjugate \( 4 \) in CDCl\(_3\) showed the presence of four different rotameric structures (see also Figure 3). This was evident from eight distinct doublet signals for the anomeric amide protons (Figure 6). Here, no unambiguous assignment of the observed doublets to respective cis/trans rotameric forms could be achieved by 2D NMR spectroscopy.

Since deuterochloroform is not suitable for temperature-dependent \(^1\text{H}\) NMR experiments at higher temperatures which, in turn, are necessary for determining the coalescence temperature of both rotamers we measured the \(^1\text{H}\) NMR of \( 1a \) in DMSO-\( d_6 \), DMF-\( d_7 \), and chlorobenzene-\( d_5 \) as well (Figure 7). In DMSO-\( d_6 \) and DMF-\( d_7 \) which both are common solvents for temperature-dependent NMR spectroscopy [24] the anomeric amide protons of the two rotamers of \( 1a \) were not sufficiently separated. Furthermore, Fmoc groups are known to be unstable in DMSO and DMF at higher temperatures [24,25]. Indeed, when \( 1a \) was heated in DMSO-\( d_6 \) above 60 °C a signal of dibenzofulvene at 6.21 ppm appeared, indicating the cleavage of the Fmoc group.
in 1a (Figure 8). In chlorobenzene-d5 (bp 131 °C), however, sufficient separation of the two anomic amide protons of the rotamers of 1a were observed and no cleavage of the Fmoc group occurred. Höck et al. [24] could also show that chlorobenzene-d5 does not cause cleavage of Fmoc groups in peptides up to 120 °C.

Figure 9 shows the temperature-dependent ¹H NMR spectra (7.65–8.30 ppm area only) of 1a in the temperature range between 25 and 100 °C. Heating the sample caused a downfield shift of the anomic amide protons with coalescence (T_c) at 90–95 °C (363–368 K). Unfortunately, the amide signals partially overlapped with the proton signals of the Fmoc group at the coalescence temperature so that no exact coalescence temperature could be determined.
Figure 10 shows the temperature-dependent $^1$H NMR spectra (7.65–8.50 ppm area only) of $\text{1b}$ in the range between 25 and 100 °C. In this case the corresponding coalescence temperature ($T_c$) could be determined at 90–95 °C (363–368 K).

Nevertheless, crosspeaks between the amidic protons and the malonyl protons could be observed, and were used for an indirect assignment of the amidic doublets. Stronger NOE crosspeaks between the methylene protons of the side chain (3.20 ppm) and the methylene protons of the PNA backbone (3.93 ppm) indicated that the distance between these protons should be shorter in comparison to the distance of the protons with a chemical shift at 3.36 and 3.93 ppm. Therefore, the signal of the protons at 3.20 ppm and the doublet at 8.18 ppm belong to the $\text{trans}$ rotamer whereas the doublet at 8.01 ppm should belong to the $\text{cis}$ rotameric structure.

**Calculation of $\Delta G^r$-values**

In order to evaluate the rotation barrier around the tertiary peptide bond (C–N bond) we calculated the corresponding $\Delta G^r$ values for building blocks $\text{1a}, \text{b}$ from the measured coalescence temperatures ($T_c$) by using the Eyring model [26]. The $\Delta G^r$ values of the dimeric glycoconjugate $\text{4}$ could not be determined though because no unambiguous assignment of the rotameric structures to the corresponding specific proton signals could be made. Nevertheless, the rotation barriers of glycoconjugate $\text{4}$ should be rather similar to the barriers of building blocks $\text{1a}, \text{b}$ due to the almost identical coalescence temperatures ($T_c$).

$$k_e = \frac{kT_c}{h} e^{-\frac{\Delta G^r}{kT_c}}$$

$$\Delta G^r = 4.56 \cdot T_e \left( \frac{T_c}{k_c} \right) \cdot 10^{-3} \text{ kcal/mol}$$

$$k_e = 2.22 \Delta \nu \text{ (in ppm)} \cdot 600 \text{ MHz}$$

The respective $\Delta \nu$ values ($\text{NH}_{\text{trans}} - \text{NH}_{\text{cis}}$) were extracted from the corresponding $^1$H NMR spectra of the specific building blocks $\text{1a}, \text{b}$ in chlorobenzene-d$_5$ at 25 °C (298 K). Table 3 summarizes the calculated $\Delta G^r$ values and illustrates that there is only a small difference between the calculated $\Delta G^r$ values of building blocks $\text{1a}$ and $\text{1b}$ in chlorobenzene-d$_5$. It is obvious that the different saccharide moieties do not significantly influence the rotation barrier around the tertiary peptide bond. Furthermore, the calculated $\Delta G^r$ values are in good accordance with those of other PNA derivates (17.9–19 kcal/mol) [27,28].

**Conclusion**

We have described the efficient chemical synthesis of a series of novel PNA-based glycopeptoids. We also studied the $\text{cis}/\text{trans}$ rotamers of these glycopeptoids via temperature-depend-
Figure 12: A) Missing correlation between the amidic proton and the methylene or 2-aminoethyl protons of the PNA backbone; B) Observed NOE-correlations of PNA building block 1a.

Table 3: $\Delta G^\ddagger_r$ values of building blocks 1a,b.

<table>
<thead>
<tr>
<th>Entry</th>
<th>building block</th>
<th>$T_c$ in °C (K)</th>
<th>$\Delta\nu$ in ppm</th>
<th>$k_r$ in Hz</th>
<th>$\Delta G^\ddagger_r$ in kcal/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1a</td>
<td>90–95 (363–368)</td>
<td>0.07</td>
<td>93.2</td>
<td>18.1–18.3</td>
</tr>
<tr>
<td>2</td>
<td>1b</td>
<td>90–95 (363–368)</td>
<td>0.09</td>
<td>119.9</td>
<td>17.9–18.1</td>
</tr>
</tbody>
</table>

Acknowledgements

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Supporting Information

Supporting Information File 1
Experimental data.
[http://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-12-183-S1.pdf]

Supporting Information File 2
NMR spectra of building blocks 1a–d, 2a–d, 3, 4 and 7; 2D NMR spectra of building block 1a.
[http://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-12-183-S2.pdf]
performing the elemental analyses. We also thank Dr. Gregor Lemanski for numerous discussions on the topic.

References