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Chemoselective and Microwave-Assisted Synthesis of Glycopeptoids

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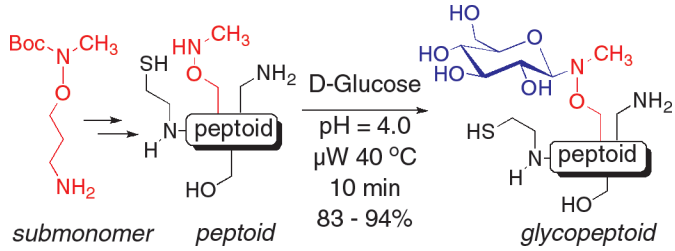
ABSTRACT



The chemoselective glycosylation of *N*-alkylaminooxy side chains with unprotected reducing sugars has proven useful for the synthesis of glycopeptides. Herein, we extend the *N*-alkylaminooxy strategy to the synthesis of glycopeptoids. A *N*-methylaminooxy submonomer was efficiently synthesized and incorporated into peptoids. Glycosylation of the peptoids proceeded chemoselectively and site-specifically at the *N*-methylaminooxy moieties. Employing microwave irradiation significantly increased the degree of glycosylation and shortened the reaction times.

Glycosylation, a ubiquitous post-translational modification in proteins, plays critical roles in protein folding,[1] stabilization,[2] trafficking, and recognition.[3] Owing to the inherent complexity of carbohydrates, glycosylation can produce enormous structural diversity in proteins and induce a variety of functional changes. In an attempt to decipher these structure−function relationships, protein and peptide chemists have developed various chemical and enzymatic methods for the synthesis of homogeneous and well-defined glycoproteins.[4]

Chemoselective glycosylation methods have enabled the convergent and site-specific modification of unprotected peptides. Orthogonal ligation techniques employing aminooxy,[5] hydrazine,[6] hydrazide,[7] and chemoenzymatic[8] strategies have been utilized. Among these ligation methods, the *N*-alkylaminooxy strategy first demonstrated by Peri and co-

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workers,[5c] wherein peptides containing *N*-alkylaminooxy groups are glycosylated in mildly acidic aqueous solutions with native, completely unprotected reducing sugars, is particularly attractive. The two key advantages are that attached sugars maintain cyclic conformations and that no synthetic sugar chemistry is required. Previously, we have synthesized *N*-methylaminooxy-containing amino acids suitable for Boc or Fmoc solid-phase peptide synthesis.[9] These amino acids were successfully incorporated into peptides, and their chemoselective glycosylation yielded neoglycopeptides. We now extend this *N*-alkylaminooxy strategy to the synthesis of glycopeptoids.

Peptoids are a unique class of peptide mimics based on oligoglycine scaffolding.[10] While their sequence of backbone atoms is identical to that of a peptide, non-natural *N*-substituents render them protease-stable.[11] Using submonomer protocols developed by Zuckermann et al.,[12] diverse oligopeptoids are readily synthesized with a variety of different primary amines and bromoacetic acid (Figure 1);

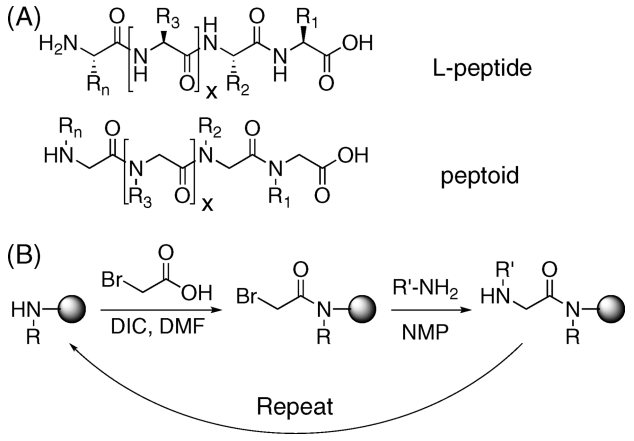


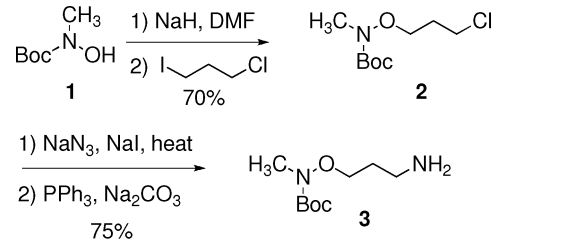
Figure 1. (A) Structures of an α-peptide and a peptoid, and (B) peptoid submonomer synthesis protocol (DIC = *N*,*N*′-diisopropylcarbodiimide).

therefore, rapid and convenient access to a wide array of peptoid sequences is possible.[13] The combination of ease of synthesis, ability to display diverse functionality, and favorable pharmacological properties has fueled interest in peptoids, and many examples of biologically active peptoids have been reported recently.[14]

The advantages of peptoids can be carried to their glycosylated counterparts, glycopeptoids, which have great potential both as glycopeptide mimics[15] and as novel carbohydrate-presenting materials. Glycopeptoids have been synthesized by different strategies.[16] Herein, we report the synthesis of glycopeptoids by the chemoselective ligation of *N*-methylaminooxy-containing peptoid oligomers with unprotected reducing sugars. In addition, we report that microwave irradiation greatly enhances *N*-alkylaminooxy glycosylation reactions. Thus, we present a simple and practical strategy for the rapid generation of a wide range of glycopeptoids.

The synthesis of *N*-methylaminooxy-containing peptoid oligomers was enabled by designing and making the *N*-methylaminooxy submonomer **3** (Scheme 1). Its synthesis

Scheme 1. Synthesis of *N*-Methylaminooxy Submonomer **3**



was accomplished in two steps and 53% overall yield. *N*-Boc,*N*-methylhydroxylamine, **1**,[17] was deprotonated with sodium hydride and used to monoalkylate 3-iodo-1-bromopropane to produce **2**. Conversion of the chloride to an azide followed by reduction with triphenylphosphine then afforded the desired amine **3**.

The protected *N*-methylaminooxy submonomer **3** was readily incorporated into oligopeptoids using standard solid-phase peptoid synthesis procedures, and no distinct side products were observed. In this study we synthesized four different model peptoids (**4** − **7**, Figure 2; see Supporting Information for synthetic details).

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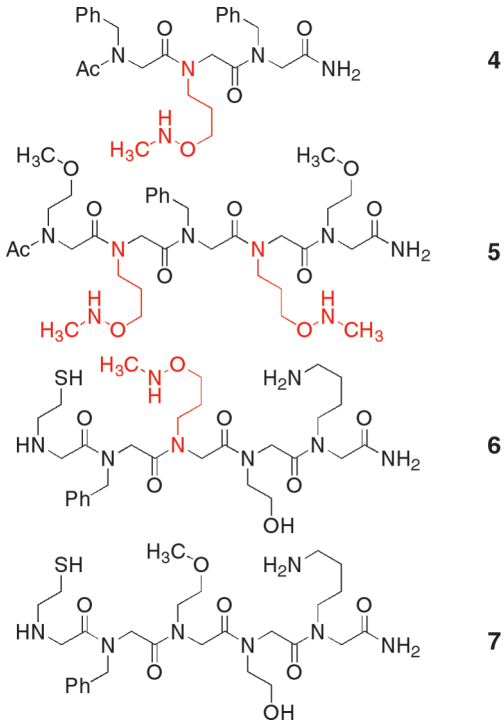

Figure 2. Peptoids synthesized in this study.

Oligopeptoid **4** was used to optimize the glycosylation conditions and to investigate the range of sugars that could be incorporated (Figure 3). We first used our previously described conditions (0.1 M NaOAc, pH = 4.0, 40 °C).

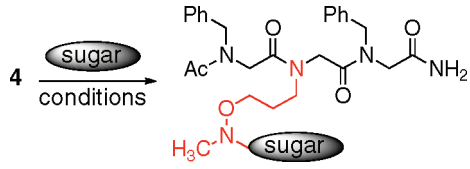

Figure 3. Glycosylation of **4**. The range of sugars and conditions investigated is detailed in Table 1.

Glycosylation proceeded well with D-glucose (Glc), and 68−78% conversion was observed after 12 h (Table 1, entries 1 and 2). We were pleased to find that the reactivity was greatly enhanced with microwave irradiation: 83% conversion was observed in 10 min with 300 W microwave irradiation at 40 °C in a CEM MARS multimodal microwave reactor (Table 1, entry 4; see Supporting Information for detailed microwave conditions). The same conditions without microwave irradiation only resulted in 35% conversion (Table 1, entry 3). The best conversion that we observed under microwave conditions was 94% in 10 min (Table 1, entry 6); only approximately 5% of the starting peptoid **4** remained unreacted. In general, conversion yields were greater when longer microwave irradiation and higher reagent concentrations were used.

Table 1. Glycosylation of **4** at 40 °C with Microwave Irradiation[a]

entry	sugar	peptoid concn (mM)	sugar excess (molar)	reaction time	conv[c] (%)
1[b]	D-glucose	3.8	50	12 h	68
2[b]	D-glucose	7.6	50	12 h	78
3[b]	D-glucose	6.3	100	10 min	35
4	D-glucose	6.3	100	10 min	83
5	D-glucose	6.3	100	30 min	88
6	D-glucose	6.3	200	10 min	94
7	D-maltose	6.3	100	10 min	87
8	D-melibiose	6.3	100	10 min	81
9	maltotriose	6.3	100	10 min	85
10	D-lactose	8.0	100	5 h	89
11	GlcNAc[d]	6.3	100	5 h	87

[a] Reaction corresponds to Figure 3; 0.1 M sodium acetate buffer/methanol = 6:1, pH = 4.0. [b] Reaction performed at 40 °C *without* microwave irradiation. [c] Conversion based on HPLC integrations at 220 nm. [d] *N*-Acetyl-D-glucosamine.

Encouraged by these results, we next sought additional carbohydrates that could be efficiently incorporated. D-Maltose (D-Glc-α(1→4)-D-Glc), D-melibiose (D-Gal-β(1→4)-D-Glc), and maltotriose (D-Glc-α(1→4)-D-Glc-α(1→4)-D-Glc), where the hydroxyl groups of the first sugar are all equatorial, ligated smoothly to the peptoid (Table 1, entries 7−9). Reaction proceeded much more slowly with D-lactose (D-Gal-α(1→6)-D-Glc) and *N*-acetyl-D-glucosamine (Table 1, entries 10 and 11). D-Galactose and D-mannose, which contain axial hydroxyl groups at their C4 and C2 positions, respectively, yielded mixtures of glycosylation products that we attribute to the presence of α and β stereoisomers or pyranose and furanose sugar forms as reported for related reactions with these sugars.[5c,18] *N*-Acetyl-D-galactosamine behaved similarly to D-galactose. Fructose, a monosaccharide with a ketone rather than an aldehyde functional group, was also tried, and virtually no reaction occurred.

Overall, the reactivity differences seen between various sugars mirrored those reported for other *N*-alkylaminooxy glycosylations, but the advantage provided by microwave irradiation proved dramatic. As an example, Peri et al. found the reaction of an *N*-alkylaminooxy-containing peptide to be much slower with lactose than with glucose, and heating the lactose reaction for 144 h at 60 °C was required to achieve a 57% yield.[5c] Reaction with lactose was also slower in our case, but only 5 h at 40 °C were required to achieve an 89% conversion when microwave irradiation was employed (Table 1, entry 10).

Divalent glycosylation was attempted next with model peptoid **5**. Using the microwave-assisted reaction conditions, **5** was diglucosylated nearly completely (Figure 4).

Lastly, the chemoselectivity of the glycosylation was investigated with peptoid **6**, which contains hydroxyl, amino,

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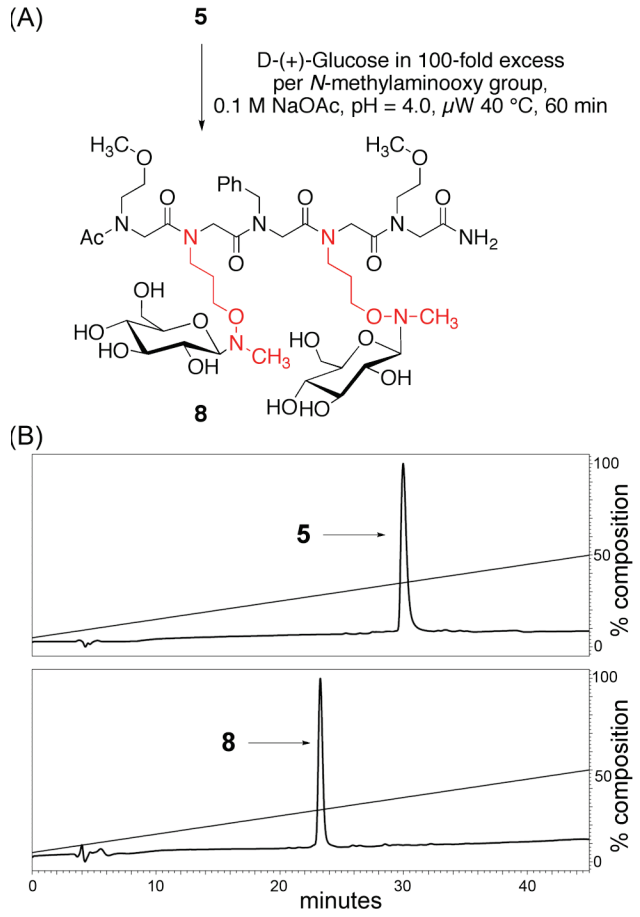

Figure 4. (A) Synthesis of divalent glycopeptoid **8**, and (B) HPLC analysis of the synthesis (UV detection at 220 nm). See Supporting Information for detailed HPLC conditions.

sulfhydryl, and carboxamido functionalities in addition to the *N*-methylaminooxy group. After 10 min of microwave reaction with a large excess of D-glucose, the HPLC chromatogram and ESI-MS analysis indicated an 88% conversion of **6** to a monoglucosylated peptoid. No additional glucosylation was observed. To confirm that glucosylation occurred solely at the *N*-alkylaminooxy site, we synthesized the control peptoid **7** where the *N*-methylaminooxy group of **6** was replaced by methoxy. Treatment of **7** under the same glycosylation conditions yielded only unreacted starting material.

As seen with other *N*-alkylaminooxy glycosylation products,[9a,18c] the glycopeptoids were pH responsive. They remained stable in solutions at pH > 6; however, at pH = 2 they rapidly returned to the parent peptoid and sugar. We are investigating how we might take advantage of the pH-induced reversability of the peptoid-sugar linkage for biomaterial applications.

Although our strategy is not particularly amenable to the inclusion of different sugars within a single peptoid molecule, it is ideal for providing differently glycosylated versions of a single peptoid. One may synthesize a single *N*-alkylaminooxy-containing peptoid oligomer and subsequently glycosylate it with a variety of sugars to provide a panel of glycopeptoids. The glycosylation reactions are simple, fast, and efficient, and most importantly they do not require sugar synthesis or any expensive commercial sugar derivatives. Furthermore, the strategy requires the synthesis of only a single submonomer rather than a different submonomer for each desired sugar.

Thus we have developed an efficient, new strategy to access glycopeptoids by the use of a *N*-alkylaminooxy-containing submonomer. Microwave irradiation enabled rapid reactions with high conversion as well as divalent glycosylation reactions with maintainence of chemoselectivity. We note that the advantage provided by microwave irradiation will also benefit those using similar strategies for the glycosylation of peptides, proteins, and small molecule drugs. We envision the use of this method to generate an extensive range of biologically functional glycopeptoids. Our current focus is on the synthesis of multivalent glycopeptoids to investigate the influence of dense glycosylation on peptoid secondary structure.

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Supporting Information Available: Detailed procedures for the synthesis, characterization, and purification of all new compounds and oligopeptoids. This material is available free of charge via the Internet at http://pubs.acs.org.

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