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On-Bead Peptoid Dimerization Induced by Incorporation of Glycosylated Bridging Units in Submonomer Solid-Phase Approach to Glycopeptoids

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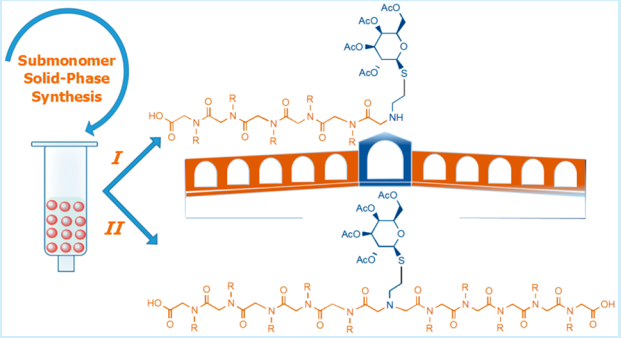
Supporting Information

ABSTRACT: A study on submonomer solid-phase synthesis of *S*-glycopeptoids has been carried out by screening different parameters. Dimeric species, featuring glycosylated bridging amino monomers, were found under suitable conditions. These dimers arise from an on-resin cross-linking reaction occurring with the incorporation of a glycoamino submonomer into the growing chain and subsequent nucleophilic attack of the resulting secondary amine to a still unreacted bromoacetylated unit. The arising byproduct can be regarded as a novel dimeric peptoid type.



Carbohydrate−amino acid bonding entails significant modifications to biological functions of proteins and natural peptides. Indeed, glycosides are known to provide both structural and functional features contributing to protein folding and stability and play pivotal roles in a wide set of recognition events.[1]

Therefore, the introduction of a carbohydrate moiety into a protein or a peptide-like structure is currently a very intriguing topic in organic chemistry. So far, a large effort has been addressed to the elaboration of streamlined synthetic methods for accessing glycosylated proteins, peptides,[2] and peptidomimetics such as peptoids.[3]

Peptoids are based on an oligoglycine scaffold deriving from the shift of the side chain from the α-carbon to the nitrogen atom throughout the peptide backbone. This simple modification leads to important changes to peptide features, mostly in magnification of serum stability and flexibility so that the bioactive action of the molecule can be improved and, more, giving the chance to an easier preparation of a great variety of peptoids adorned with diverse side chains. These advantages make it very attractive molecules suited to different biological applications.[4]

To date, different classes of peptoids have been already reported, such as linear and cyclic α-peptoids,[5] β-peptoids,[6] and α-alkyl β-peptoids.[7] Furthermore, different synthetic strategies to *O*-, *N*-, *C*-, and *S*-linked glycopeptoids have been developed both in solution and on solid phase, by monomer and submonomer strategies, as well as by postchemical glycoconjugation.[3b] Glycopeptidomimetics thus prepared are useful tools in the comprehension of multivalent carbohydrate−protein interactions.

However, only a few examples of glycopeptoid syntheses are strictly based on the solid-phase submonomer approach[8] developed by Zuckermann et al.,[9] which still remains the most straightforward and versatile strategy for oligopeptoid preparation by the use of a wide range of primary amines and monobromoacetic acid (mba) in a two-step iterative procedure (Figure 1).

Toward this goal, we have previously employed readily synthesized 2-aminoethyl peracetyl thioglycosides for the attachment of *S*-linked sugars onto various *N*-phenylmethyl peptoid backbones under submonomer solid-phase conditions.[8b] This approach is the first reported synthetic access to *S*-glycopeptoids; it is founded on the fast preparation of suitable glycosylated building blocks accomplished by a two-step conversion of peracetylated carbohydrates into glycosyl thiolates[10] via glycosyl iodides.[11] Nucleophilic glycosyl thiolates are then entrapped in situ with 2-bromoethylamine to provide the thioglycosyl ethylamines suited to submonomer synthesis and featuring an *N*-homocysteine linkage.

The choice to develop a synthetic method for the access to *S*-glycopeptoids was spurred by the fact that sulfur is isosteric with respect to oxygen and forms an enzymatically more stable glycosidic linkage,[12] as witnessed by the numerous reported methods for the assembly of *S*-glycopeptides.[13]

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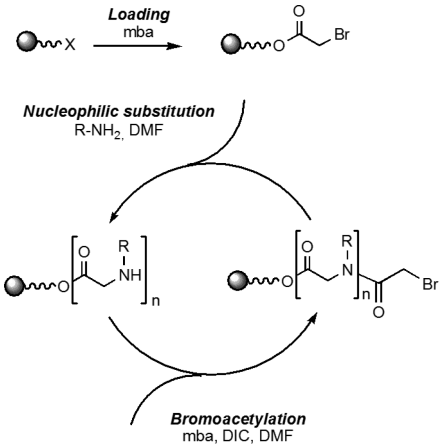


Figure 1. Submonomer solid-phase approach.

Herein, we present a new synthetic opportunity offered by the reported method of glycopeptoid assembly that was unveiled upon varying different reaction parameters such as the peptoid sequence, the nature of the resin, and its loading.

Typically, peptoids were synthesized on medium-loaded resins (0.4−0.9 mmol/g) such as polystyrene-bound Rink amide or PEG-based Tentagel resins. In order to obtain free C-terminal glycopeptoids, we used 2-chlorotrityl resin, particularly useful also to suppress diketopiperazine formation. This polystyrene-based resin is characterized by high-loading functionalization, up to 1.6 mmol/g.

In attempting the preparation of an alternated *N*-(2-methoxyethyl)glycine (*N*me) and *N*-(galactosylthio)ethyl glycine (*N*galte) peptoid sequence on highly loaded 2-chlorotrityl resin (1.6 mmol/g), we had to stop the synthesis at the dipeptoid stage. In fact, surprisingly, only traces of the expected dipeptoid **1** (***I***) (Figure 2), with 581 *m*/*z* value was found (peak **1** (***I***), Table 1, entry a), and instead, a major product with a higher mass of 754 *m*/*z* was observed (peak **2** (***II***), Table 1, entry a).

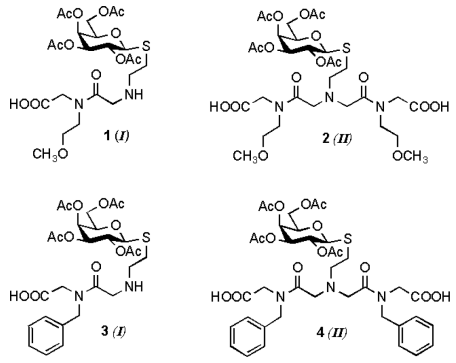


Figure 2. Expected products of the submonomer synthesis (**1** and **3**) and their correlated dimeric byproducts (**2** and **4**).

Intrigued by this observation, we repeated the same procedure for the preparation of a dipeptoid composed of *N*-phenylmethyl glycine (*N*pm) and *N*galte, since it was already successfully accomplished in previous work.[8b] As a matter of fact, the expected dipeptoid **3** (***I***), with 613 *m*/*z* value, was formed in good yield, although a byproduct with a higher mass (m/z = 818) was again found (peak **4** (***II***), Table 2, entry a).

Table 1. Stack Chromatograms of Crudes from *N*me-*N*galte Dipeptoid Submonomer Synthesis at Different Loadings of 2-Chlorotrityl Resin (TRT) (a−d) and of Other Resin Types (e, f)

entry	resin	loading (mmol/g)	product ratio (%) (**I**/**II**)[a]
a	TRT	1.6	25/75
b	TRT	1.0	40/60
c	TRT	0.6	60/40
d	TRT	0.1	84/16
e	TGR	0.25	88/12
f	TGT	0.16	97/3

[a]Product ratio in a percentage calculated by integration of the each HPLC at 210 nm and normalized by the titration curve.

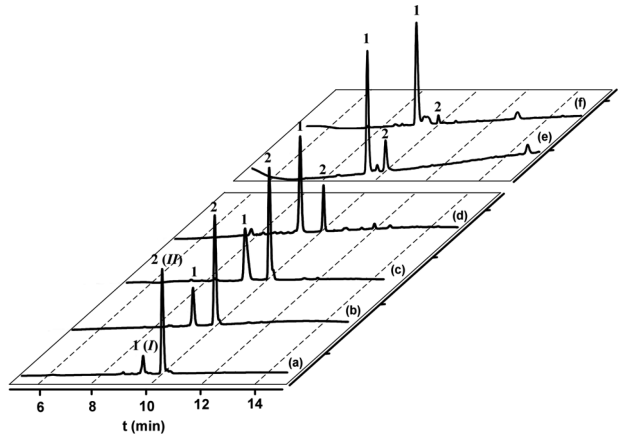
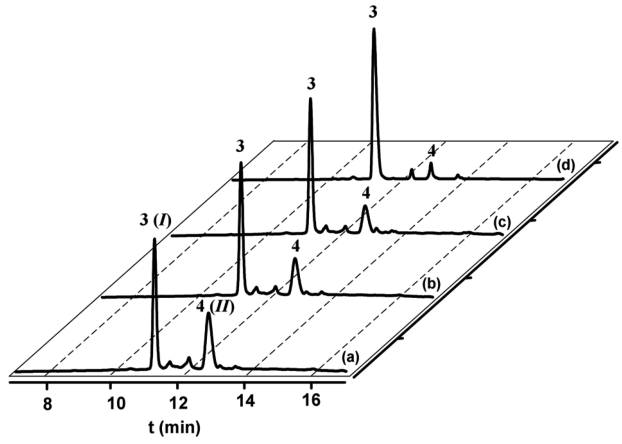


Table 2. Stack Chromatograms of Crudes from the *N*pm-*N*galte Dipeptoid Submonomer Synthesis at Different Loadings of 2-Chlorotrityl Resin (TRT)

entry	resin	loading (mmol/g)	product ratio (%) (**I**/**II**)[a]
a	TRT	1.6	73/27
b	TRT	1.0	83/17
c	TRT	0.6	85/15
d	TRT	0.1	96/4

[a]Product ratio in a percentage calculated by integration of the each HPLC at 210 nm and normalized by the titration curve.



Isolated byproducts **2** and **4** were characterized by NMR and mass analysis. Integration of proton signals in the ^{1}H NMR spectra indicated that the species with a higher mass are bearing a single sugar unit, and the corresponding mass values were perfectly coherent with the dimeric structures reported in Figure 2.

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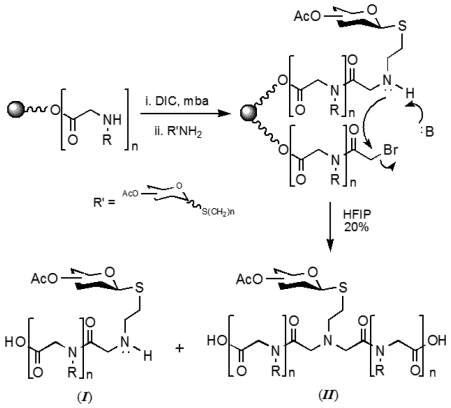
With this evidence in hand, we put forward a possible mechanism accounting for the dimer formation: during the preparation of the dipeptoid oligomer, a nucleophilic substitution by the newly formed glycosyl secondary amine can occur for the still unreacted bromoacylated precursor with formation of a dimeric species featuring the glycosidic moiety in the middle of the molecule (Scheme 1).

Scheme 1. Reaction Mechanism of the Dimer Formation in the Submonomer Approach



This intermolecular side reaction leads to chain termination and can heavily affect the efficiency of the normal oligomerization. Thus, in order to prevent dimerization, we first tested different resin loadings. Checking the outcome of the solid-phase procedure at dipeptoid stage, we found out that, in the case of *N*pm-*N*galte peptoid synthesis, the monomeric species (**I**) was formed in a larger amount than the dimeric species (**II**) even with a very high loading rate (1.6 mmol/g); expectedly, by using lower loading rates the dimer was observed in trace amounts (Table 2, entries a–d). Considering that the dimer absorbs twice respect with the monomer, as measured by a titration curve (see Supporting Information), we determined the overall yield normalizing the integration peak ratios. In this way we found that the yield of the monomeric species could range from good (73%) to excellent (96%), in accordance with the results already reported for the synthesis of this sequence.[8b]

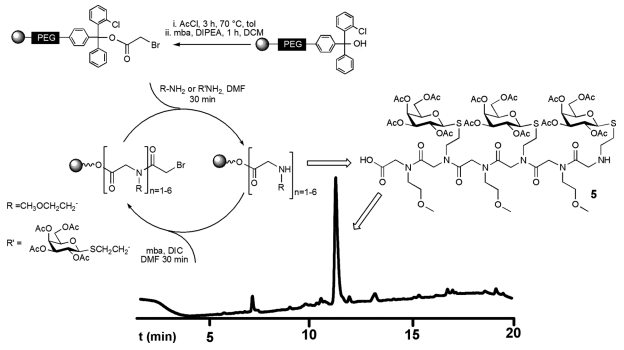
Conversely, the formation of the dimer was markedly pronounced in the presence of the methoxyethyl side chain, much less hindered than the benzyl group. As a matter of fact, the yield of *N*me-*N*galte peptoid synthesis was drastically reduced by the use of high to medium loaded 2-chorotrytil resins (Table 1, entries a–c).

To afford a very good yield for the *N*me-*N*galte peptoid sequence we had to lower the loading of 2-chlorotrityl resin down to 0.1 mmol/g, eventually observing the full inversion in the abundance of the two peaks (Table 1, entry d). Thus, the higher the loading, the lower the efficiency of the oligomerization, and this trend confirmed the hypothesis that interchain reactions occurred on the same resin bead.

These results prompted us to compare the outcome of analogous syntheses also on alternative resins. Commonly used SPPS supports,[14] such as TGT alcohol and TGR PEG-polystyrene based resins, were therefore tested. Fortunately, with pegylated resins dimerization was mostly suppressed and the yield of the monomeric species was excellent and even better than that obtained on TRT resin at a lower loading (Table 1, entries e and f).

To confirm this result, we performed the synthesis of a longer *S*-glycopeptoid containing *N*-methoxyethyl glycines on a TGT resin. The introduction of three sugar moieties was successfully accomplished, the triglycosylated hexapeptoid being obtained in very good yield (Scheme 2, **5**).

Scheme 2. Submonomer Synthesis of Peptoid 5 on Novasyn TGT Resin



With the aim of deeply investigating the cross-linking reaction, we performed the synthesis of a commonly prepared homopentapeptoid $(Nme)_5$ on highly loaded 2-chlorotrityl resin (1.6 mmol/g). As expected, the synthesis was smoothly accomplished and afforded the pentapeptoid in a very high yield (Figure 3, **7**). However, the LC–MS spectra of the crude

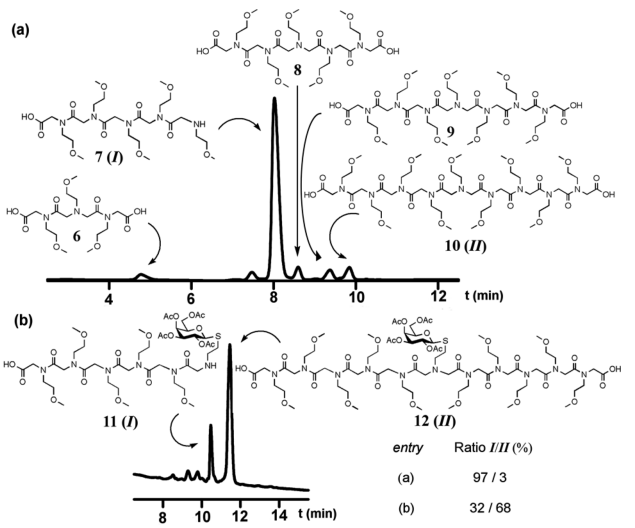


Figure 3. Distribution of products in the submonomer synthesis of the peptoid **7** (entry a) and the glycopeptoid **11** (entry b) using 2-chlorotrytil resin (loading 1.6 mmol/g). Product ratio in a percentage calculated by integration of the each HPLC at 210 nm and normalized by the titration curve.

product disclosed the formation of dimers at each nucleophilic substitution step (Figure 3, entry a, compounds **6**, **8**, **9** and **10**), even if in a very tiny amount. Instead, on attempting the synthesis of hexapeptoid **11** (Figure 3) from pentapeptoid **7** by introducing the thiogalactosylethyl unit by the same submonomer pathway and reaction conditions, the prevalent formation of the dimer **12** (Figure 3) was found.

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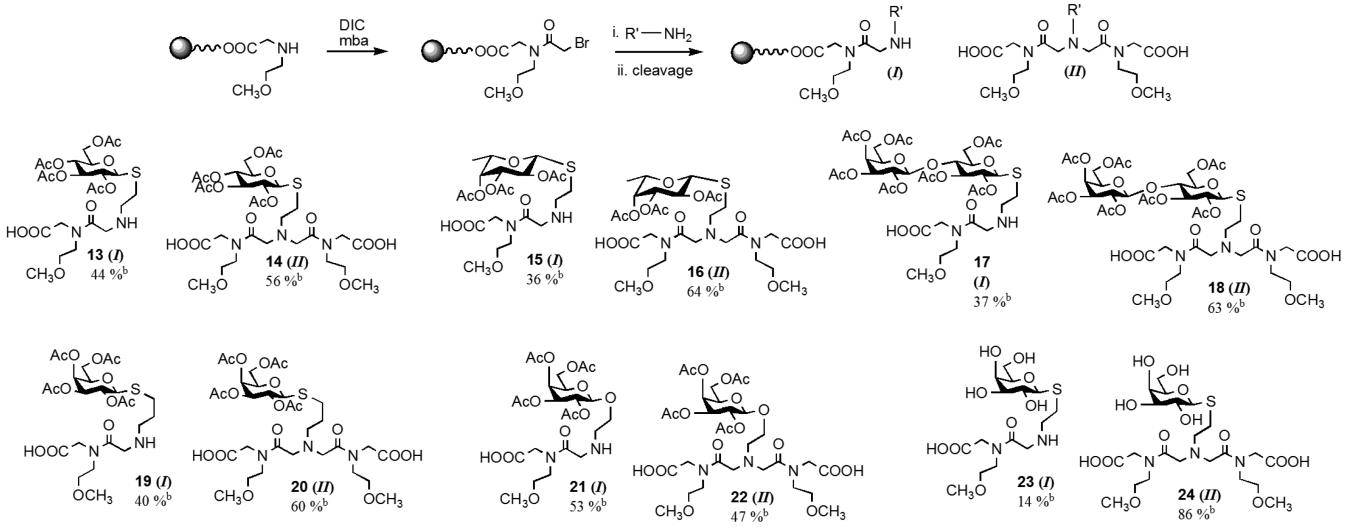
Scheme 3. Differently Glycosylated Dipeptoids and Their Correlated Dimers Obtained on 2-Chlorotrityl Resin[a]



[a]All reactions were carried out by using 2-chlorotrityl resin loaded 1.0 mmol/g. [b]Product ratio in a percentage calculated by integration of each HPLC at 210 nm and normalized by the titration curve.

These results highlighted the leading role of the thioglycosyl amine that works, regardless of the oligomer length, to favor significantly the dimer formation. In contrast, when common unglycosylated primary amines were used, the dimer formation was a totally negligible byreaction, as witnessed by the fact that no cross-reaction was mentioned so far, in spite of the countless examples of submonomer solid-phase synthesis reported.

A systematic study was thus carried out to decipher the peculiar behavior of the thiogalactosyl ethylamine on the solid phase.

First, we prepared different *S*-glycosyl amines to be employed in the submonomer solid-phase route to *N*-methoxyethyl peptoids on a medium-functionalized 2-chlorotrityl resin. Collected data revealed a similar behavior for glucosyl, fucosyl, and lactosyl analogues, showing that the cross-reaction is not dependent on the sugar configuration (Scheme 3, **13**−**18**) or on the length of the linker bearing the sugar (Scheme 3, **19** and **20**). Suspecting a role of the sulfur atom in a possible anchimeric assistance of the cross-reaction, we also tested the *O*-analogue, employing 2-aminoethyl peracetyl-*O*-galactose in the dipeptoid route. In this case, we found a slight reduction of the cross-reaction obtaining a similar amount of the monomer and the dimer (Scheme 3, **21** and **22**). Even if a small effect of the sulfur cannot be ruled out, it is evident that other factors are responsible for the cross-reaction. Interestingly, upon use of a free thiogalactosyl ethylamine the dimer formation was even higher than with its peracetylated analogue (Scheme 3, **23** and **24**).

On these bases, the reactivity of the thioglycosyl ethylamines on the solid phase seems to be mainly correlated to the nature of the solid support and the polarity of the sugar functionalities. Actually, it is known that many carbohydrate-binding proteins can interact with carbohydrate ligands with aromatic amino acid residues in their binding sites via CH/π interactions arising from a stacking geometry.[15] Likewise, the hydrophobic face of the sugars could tender to adhere to the flat faces of polystyrene support by interacting via CH/π interaction and promoting the interchain nucleophilic substitution. Thus, with polystyrene-supported 2-chlorotrityl resin the dimer formation is highly promoted contrary to what happens on pegylated solid supports.

In perspective, the on-resin interchain reaction can be exploited as a route for the access to peptoid dimers comprising a sugar moiety. High-yielding dimerization can be contemplated for less sterically hindered peptoid sequences prepared on high-loaded polystyrene resins. This method is clearly more straightforward with respect to classical dimerization methods on the solid phase which typically entail the construction of the product step by step. Thus, with the use of the described strategy the dimer preparation takes half the time and requires a lower reagent amount. In addition, it returns dicarboxylated compounds ready for further functionalizations.

In conclusion, we have detected and characterized the byproduct featuring a bridge-sugar moiety formed during the submonomer solid-phase route to *S*-glycopeptoids and resulting from a chain termination reaction. Different reaction conditions to modulate or totally avoid the formation of the dimeric product have been investigated. It was found that dimerization takes place only when the glycosyl amine is added and occurs at every position of the growing chain during the submonomer synthesis. The bridge-sugar dimer is obtained in good yield on highly loaded polystyrene-supported resin, and it is completely suppressed on medium-loaded PEG-based solid supports.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b01242.

Full experimental details, characterization of all compounds, ^{1}H and ^{13}C NMR spectra, and LC−MS profiles of all compounds (PDF)

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Notes

The authors declare no competing financial interest.

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