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Solid phase synthesis of oligomannopeptoids that mimic the concanavalin A-binding trimannoside

Hideya Yuasa,[a] Yujiro Kamata,[a] Sadamu Kurono,[b] and Hironobu Hashimoto*,[a]

[a]*Department of Life Science, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology Nagatsuta, Midori-ku, Yokohama 226-8501, Japan*

[b]*Glycobiology Research Group, Frontier Research Program, The Institute of Physical and Chemical Research (RIKEN) 2-1 Hirosawa, Wako-shi, Saitama 351-0106, Japan*

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Abstract

Oligomannopeptoids from the dimer to the hexamer were produced by solid phase synthesis and their abilities to bind to concanavalin A (ConA) were assessed. The assessment indicated similarity between the oligomannopeptoids and the naturally occurring oligomannosides in the enthalpy of the binding and the valence number vs binding strength relationship, encouraging the use of the oligomannopeptoids as oligomannoside mimics. © 1998 Elsevier Science Ltd. All rights reserved.

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Combinatorial chemistry is becoming a powerful tool in pharmaceutical science.[1,2] However, for some groups of compounds, a general route to constructing a library that contains as many kinds of structures as possible has not been found. Complex carbohydrates are among such compounds because of the difficult steps required for their syntheses. Recently, Khane and coworkers have succeeded in the construction of a solid phase carbohydrate library and demonstrated its utility in discovering new drugs.[3] Nonetheless, there still remains a need for preparing a number of suitably protected building blocks. Even if one of the compounds is a "hit," its resynthesis would be expensive and time-consuming. One potential solution to these difficulties is the assemblage of carbohydrates on a simple backbone structure. In this context, Zuckermann and coworkers have developed a solid-phase

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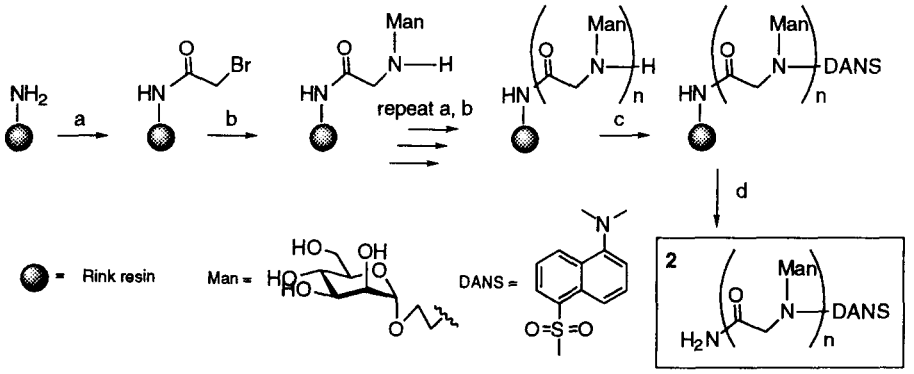
method for the synthesis of oligopeptoids, i.e., oligo(*N*-substituted glycines), which are easy to synthesize and contain no cumbersome asymmetric center.[4] Later, Roy and coworkers achieved solution phase syntheses of a number of glycopeptoids.[5-7] In order for these glycopeptoids to be used in combinatorial chemistry, an array of monosaccharides on the peptoid backbone must be recognized by the subsites of a receptor in the similar way the natural type oligosaccharides are bound. We demonstrate here that oligomannopeptoids are easily obtained by solid phase synthesis and these peptoids resemble naturally occurring oligomannosides in the binding abilities to concanavalin A (ConA).[8] These results encourage us to extend the methodology to the library of glycopeptoids.

The synthetic route was designed as shown in Scheme 1, where [5-(dimethylamino)naphth-1-yl]sulfonyl substituent (DANS) was introduced at the final stage for UV detection in HPLC and for the fluorescence anisotropy binding assay.[9,10] The 2-aminoethyl mannoside (**1**) was chosen as a building block to keep the distance between neighboring mannosides as short as possible. The 2-aminoethyl linkage has been used in *N*-acetylgalactosamine-based oligopeptoids.[5] We decided to synthesize several mannopeptoids, from the monomeric form **2** (n=1) to the hexameric form **2** (n=6), to investigate the saturation point in the binding to ConA. In the case of oligomannose, the binding pocket of ConA is saturated by a trimer residue and additional mannose residues have little effect on the binding strength.[11]



Scheme 1. Synthetic scheme of the oligomannopeptoids **2** (n=1-6) on the solid support. a) Bromoacetic acid, 1,3-diisopropylcarbodiimide, DMF; b) see Table 1; c) DANS-Cl, *N,N*-diisopropylethylamine, DMF; d) 95% TFA

The compound **1** was synthesized by mannosylation of *N*-trifluoroacetyl(TFA)-2-aminoethanol with per-*O*-acetyl-mannopyranosyl bromide, and by the deprotection of the acetates and TFA. It was found that Boc-2-aminoethanol was not useful, as it gave a poor yield. Solid phase synthesis was carried out[1] using Rink resin[12] as a solid support with essentially the same method as that of Zuckermann et al.[4] Since the standard conditions for

[1]The reaction was carried out in a glass column (12 mm x 100 mm) equipped with a glass filter and a Teflon stop cock at the bottom. The column was agitated on the bottom with IKA Minishaker MS1 at 600 rpm and warmed by circulation of temperature-regulated water around the column

the synthesis of simple oligopeptoids gave poor yields, optimization of the nucleophilic substitution reaction (step b of Scheme 1) was carried out for the monomeric mannopeptoid (Table 1). Though addition of 1,8-diazabicylo[5.4.0]undec-7-ene (DBU) and an elevation of temperature gave slightly better yields, the most significant improvement was achieved by the use of DMF as a solvent instead of DMSO and by increasing the concentration of the sugar amine **1**. The effect of DMF presumably originates from the swelling of the resin, since no such effects were observed in the preliminary solution phase synthesis. Addition of activators other than DBU, e.g., tetrabutylammonium iodide, HMPA, and $AgClO_4$, gave results similar to those obtained with DBU, but showed no additive effects. The concentration of the sugar amine **1** of 1 M was considered optimal because an increase in the concentration with a constant equivalent of **1** ended in poor efficiency of mixing.

Table 1

Reaction conditions of the nucleophilic substitution for the synthesis of **2** (n=1).

[1][a] (M)	Temp (°C)	Solvent	Promoter[b]	Yield(%)[c]
0.5	25	DMSO	none	36
0.5	25	DMSO	DBU	43
0.5	25	DMF	DBU	57
0.5	50	DMF	DBU	66
1.0	50	DMF	DBU	83

[a]40 equivalents with regard to the reactive amine of the solid support. [b]DBU 1 equivalent.

[c]Determined by HPLC.

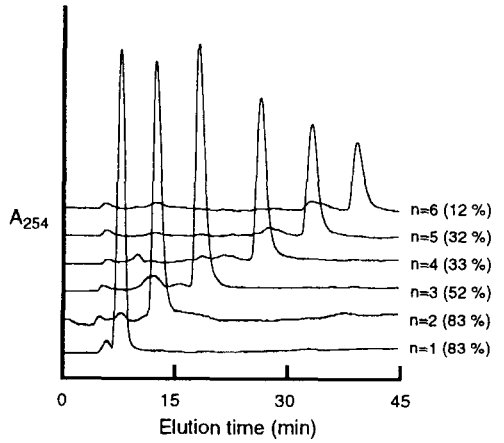


Figure 1. HPLC profile of the oligomannopeptoids **2** (n=1-6). Erma ERC-NH-117 propylamino column; elution, CH_3CN/H_2O 4 to 1.5 with a gradient of 0.33 %/min.

Oligomannopeptoids from the dimer **2** (n=2) to the hexamer **2** (n=6) were synthesized using the above optimized reaction conditions for the nucleophilic substitution reaction and the reported standard conditions[4] for the other reaction steps. After the 2nd cycle of synthesis, the sugar amine **1** recovered in the previous cycle was used without purification. Each oligomannopeptoid was obtained with a satisfactory purity as shown in Figure 1.[2] However, the yields were decreased as the oligomerization proceeded. This result indicates that a hexamer is the upper limit for the application of this method in combinatorial chemistry.

Table 2

Thermodynamic parameters for the binding of oligomannopeptoids **2** and selected mannose derivatives to ConA at 25 °C and pH 7.2.

Sugar	K_d(μmol/L)	ΔG(kcal/mol)	ΔH(kcal/mol)	ΔTS(kcal/mol)
2(n=1)	31	-6.1	-9.5	-3.4
2(n=2)	18	-6.4	-	-
2(n=3)	9	-6.9	-14.1	-7.2
2(n=4)	12	-6.7	-	-
2(n=5)	14	-6.6	-	-
2(n=6)	12	-6.7	-	-
MeMan[a]	122	-5.3	-8.2	-2.9
$(Man)_3$[a]	2	-7.8	-14.4	-6.6

[a]Data from ref. [11].

Dissociation constants for the binding of the oligomannopeptoids **2** (n=1-6) to ConA were determined by fluorescence anisotropy assay.[3][9,10] The plots for the titrated ConA concentration vs the measured anisotropy (r) and the non-linear regression curves are shown in Figure 2A for the monomer **2** (n=1) to the trimer **2** (n=3). For the monomer **2** (n=1) and the trimer**2** (n=3), a van't Hoff plot was also carried out to determine the enthalpy (ΔH) and entropy (ΔS) of the binding (Figure 2B). The resulting thermodynamic parameters including the Gibbs free energy (ΔG) are listed in Table 2 and comparison of $-\Delta G$ between the oligomannopeptoids and oligomannosides is visualized in Figure 3. The binding abilities of the monomer **2** (n=1) and the dimer **2** (n=2) exceed those of the corresponding oligomannosides and the situation is reversed for the larger peptoids. The strong binding between ConA and the monomer **2** (n=1) is reflected in the enthalpy term, probably due to the extra recognition of the peptoid backbone and/or the dansyl group. On the other hand, both the oligomannopeptoids and the oligomannosides show similar tendency in the valence number vs binding strength relationship. Namely, the binding strength reaches plateau at the

[2].^{1}H NMR spectra of **2** (n=1) and **2** (n=2) were in accord with those synthesized in solution phase. FAB Mass (m/z): **2**(n=1), 514.5 (M+H); **2**(n=2), 777.6 (M+H), 799.4 (M+Na); **2**(n=3), 1040.7 (M+H), 1062.7 (M+Na); **2**(n=4), 1303.9 (M+H), 1326.0 (M+Na); **2**(n=5), 1567.2 (M+H), 1589.1 (M+Na); **2**(n=6), 1830.1 (M+H), 1852.2 (M+Na).

[3].To a temperature-regulated cell containing an oligomannopeptoid (9.14 μM) in 0.1 M HEPES 500 μL(pH 7.2, NaCl 0.9 M, $CaCl_2$ 1 mM, $MnCl_2$ 1 mM), was added a solution of ConA (1.4 mM) and the peptoid (9.14 μM) in amounts of 5-50 μL. Measurement of the anisotropy (r) was carried out 1 minute after each addition of the ConA solution.

trimer. Similarity is further exemplified by the small difference in enthalpy of the binding between the trimannopeptoid **2** (n=3) and the trimannoside (0.3 kcal/mol). The larger negative values of entropy for the oligomannopeptoids reflect the flexibility of these molecules.

In conclusion, we demonstrated that oligomannopeptoids could be synthesized on a solid support and these might be useful as inhibitors of oligomannoside-binding receptors.

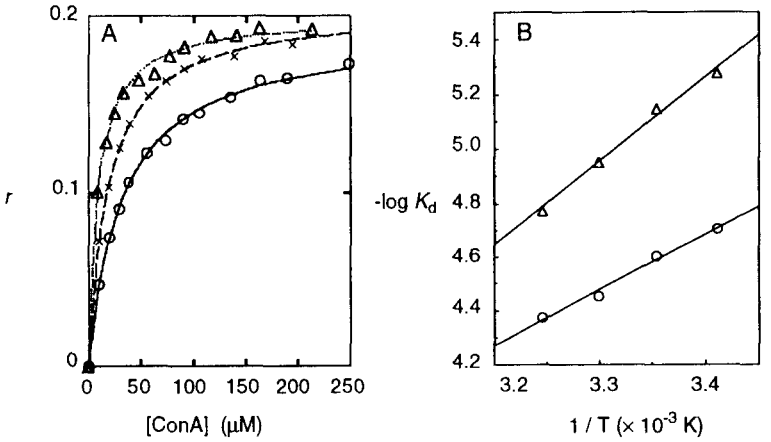


Figure 2. A) Titration curves of the fluorescence anisotropy assay for the binding of the oligomannopeptoids **2** (n=1; O), (n=2; ×), (n=3; Δ) to ConA and B) van't Hoff plots for the compounds **2** (n=1; O), (n=3; Δ).

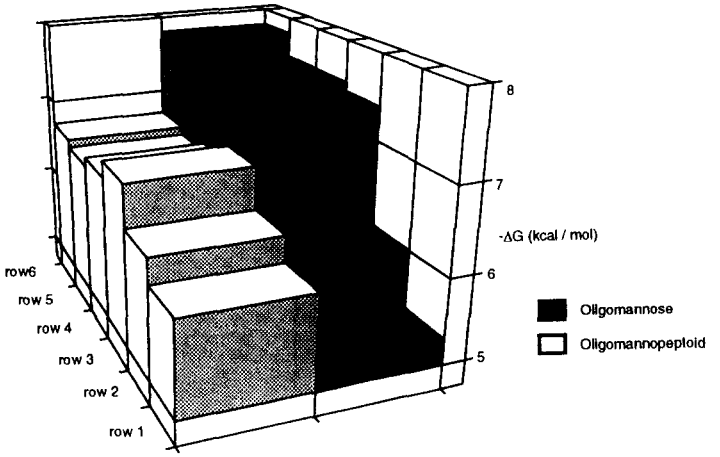


Figure 3. Comparison of binding ability to ConA between oligomannopeptoids **2** and oligomannoses. All of oligomannoses shown have the core mannose structure of N-linked oligomannose-type carbohydrates and the data are from ref. [8]. Row 1: **2** (n=1), MeMan; row 2: **2** (n=2), Manα(1,3)Man; row 3: **2** (n=3), $(Man)_3$; row 4: **2** (n=4), $(Man)_5$; row 5: **2** (n=5), $(Man)_{7/8}$; row 6: **2** (n=6), $(Man)_9$.

Acknowledgments

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