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Ring-Closing Metathesis Approaches for the Solid-Phase Synthesis of Cyclic Peptoids

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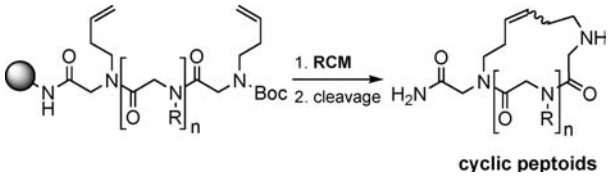
ABSTRACT



Cyclic peptoids were efficiently synthesized on a solid phase in high yields utilizing ring-closing metathesis (RCM). This method should be a valuable tool for easy access to cyclic peptoid libraries and various cyclic compounds.

With the completion of the human genome projects, one of the most important scientific issues is understanding the biological function of each protein including the protein−protein interaction events. In chemical biology studies and drug discovery, various chemical tools with a high affinity and high specificity against proteins must be developed. Although peptides are an attractive class of molecules that contain protein-binding properties, they possess several undesirable drawbacks including a sensitivity to proteases, limited cell permeability, and poor bioavailability. Thus, new peptidomimetics with improved pharmacokinetic characteristics are increasingly needed.[1]

Among them, peptoids, *N*-alkylated glycine oligomers, are easily synthesized on a solid phase with a huge diversity,[2] proteolytically resistant,[3] and much more cell permeable,[4] compared to peptides. In some cases, these oligomers can also adopt stable secondary and even more complex structural features and have proven to possess a variety of interesting bioactivities.[5] Additionally, cyclic peptides and depsipeptides have received a great deal of attention because of their challenging chemical synthesis and numerous interesting bioactivities.[6] Indeed, many naturally occurring bioactive molecules have been found in cyclic forms. Generally, cyclic peptides exhibit an enhanced cell permeability[7] and improved resistance to enzymatic degradation.[8] Moreover, cyclic molecules might

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be presumed to bind more tightly to their protein targets because of their more restricted conformational flexibility.[9] Cyclic peptides have been prepared using various solid-phase synthetic methods to create combinatorial libraries.[10] These peptides have been revealed to possess antibiotic activities,[11] enzymatic inhibitory activities,[12] and therapeutic properties.[13] The limitations of peptides, along with the fascinating properties of cyclic molecules and peptoids, have led to the development of cyclic peptoids. Recently, we have already developed the 'one-bead two-compound' strategy, which is optimized for the construction of cyclic peptoid microarrays.[14] Generally, the macrocyclization of peptoids has been achieved through a head-to-tail cyclization, ligation of the side chains, and polymerization.[15]

In addition to the previous efforts, we also paid attention to olefin metathesis, more exactly, ring-closing metathesis (RCM) on a solid phase as a new macrocyclization method for the construction of cyclic peptoids. Olefin metathesis is a representative carbon−carbon bond formation reaction, which has been applied for many chemical syntheses and industrial uses.[16] Usually, olefin metathesis has been well studied in a solution phase, and even in an aqueous phase. Many metathesis catalysts have been developed for various needs including the improvement of their activity and efficiency. However, this metathesis reaction is still challenging on a solid phase, and only a few examples of solid-phase RCM have been demonstrated.[17] These trials are on a case-by-case basis, and a systematic approach has not yet been developed. Additionally, low yields, ineffective cyclization, and difficult elimination of ruthenium byproducts are common problems for solid-phase RCM. Especially, to the best of our knowledge, the macrocyclization of peptoids using solid-phase RCM has not been reported so far. Thus, we herein describe an efficient approach for the synthesis of cyclic peptoids through solid-phase RCM, and this method can be further applied for the easy generation of cyclic peptoid libraries.

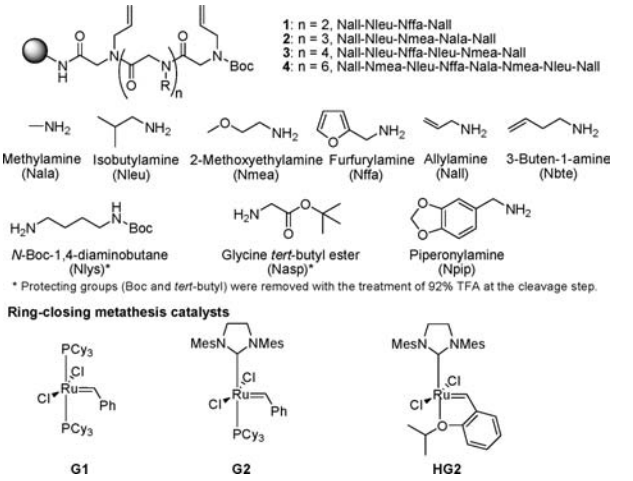

Figure 1. Model peptoids (**1**−**4**) containing allylamines at the first and last monomeric positions, and amines and RCM catalysts G1, G2, and HG2 employed in this study.

In this study, commercially available olefin metathesis catalysts G1, G2, and HG2 were used (Figure 1). In general, the catalysts (G1 and G2) with the phosphine ligand were good for disubstituted cyclic olefins, and the phosphine-free HG2 proved to be more versatile and well tolerant to functional groups. Previous literatures showed that the solid-phase olefin metathesis produced RCM products in poor yields or polymerized compounds of unknown nature. The success of the solid-phase RCM reaction depended on the careful selection of the resins, solvents, olefin metathesis catalysts, and types of alkene fragments. Several linear model peptoids (**1**−**4**) from a tetramer to an octamer, containing allylamines, where the alkene functions were strategically positioned at the first and last monomeric positions, were first synthesized on the Rink amide resin (0.4 mmol/g) in order to test the construction of cyclic peptoids (Figure 1). The terminal amino groups were masked by the Boc group in all peptoids before the RCM in order to prevent the ruthenium complexes from being poisoned by the amino function. The macrocyclization was attempted with the model peptoids (**1**−**4**) using G1, G2, and HG2 under various reaction conditions (see Table S2 in the Supporting Information for details). Common metathesis solvents, such as methylene chloride, 1,2-dichlorobenzene, and 1,2-dichloroethane, were employed. The RCM failed to produce cyclic peptoids at high temperature or in a microwave with G1 and G2. However, the corresponding cyclic peptoids were synthesized from peptoids **3** and **4** with HG2 in very low yields (10−20%), and thus, the RCM could potentially be optimized. In all cases, the linear peptoids were either completely consumed or polymerized. The reaction conditions for the solid-phase RCM were further investigated because of the limited success in producing metathesized products from the allylamine-containing peptoids.

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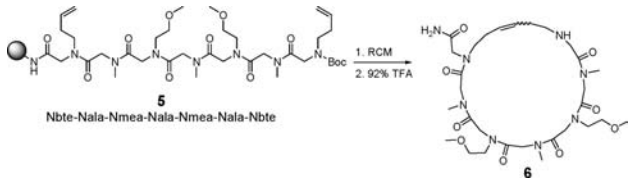
Table 1. RCM of Peptoid **5** Using G1, G2, and HG2



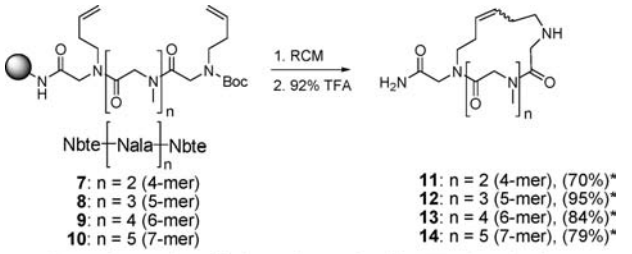
entry	catalyst	conditions[a]	time (min)	yield (%)[b]
1	G1	microwave	2	~10
2	G2	microwave	2	~20
3	HG2	microwave	2	~80

[a] The reactions were carried out under microwave conditions at 300 W in 1,2-dichlorobenzene. [b] Yield was calculated as transformation efficiency determined by HPLC analysis.

Based on the RCM efficiency of different lengths of the carbamate-protected acyclic amines,[18] 3-buten-1-amine (abbreviated as Nbte) was used instead of allylamine. The formation of the metallocyclobutane intermediates could potentially be facilitated by increasing the length of the hydrocarbon by one methylene unit in 3-buten-1-amine. Thus, the model peptoid **5**, which contained 3-buten-1-amine at the first and last positions, was synthesized. This heptamer was reacted with G1, G2, and HG2 in 1,2-dichlorobenzene under microwave conditions for 2 min. After the compounds were released from the resin by 92% TFA, the HPLC analysis showed that the cyclic peptoid **6** was produced at yields of about 10% and 20% with G1 and G2, respectively, while the yield was about 80% with HG2 (Table 1). The formation of homodimerized compounds was also observed in these cases.

With this information in hand, the HG2 catalyst was preferentially selected for the remainder of the RCM reactions. Tetramer to heptamer (**7**−**10**) peptoids in different sizes with methylamines as the side chains were prepared in order to systematically elaborate on the viability of the RCM (Scheme 1). The reaction conditions including the microwave conditions, the temperature, and the reaction time, as well as the effects of the ring size, were examined with respect to the cyclization. The macrocyclization of peptoids (**7**−**10**) was carried out using HG2 under microwave conditions. The yields were very high (70−85%), but the small amounts of dimers or metathesis products between the cyclic and linear peptoids were also observed in the MALDI-TOF analysis. Then the same RCM reactions were also carried out in methylene chloride at 40 °C for 2 h. After the peptoids were cleaved from the solid support, the cyclic peptoids were produced in much higher yields (70−95%) along with < 5% of the dimeric products. These results revealed that the solid-phase RCM of peptoids worked well with HG2 under both conditions, even though the reflux conditions in methylene chloride seemed slightly better.

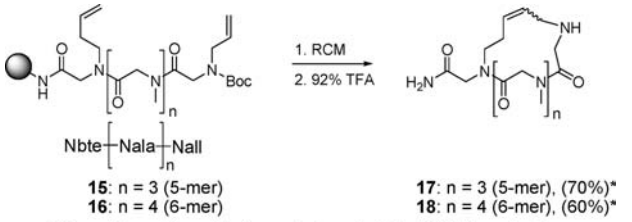
Scheme 1. RCM of Peptoids Containing 3-Buten-1-amines



*Transformation efficiency determined by HPLC analysis.

Moreover, pentamer and hexamer (**15**−**16**) peptoids with both 3-buten-1-amine at the first position and allylamine at the terminal end were also synthesized on beads (Scheme 2). The RCM of these two peptoids (**15**−**16**) was performed to produce the cyclized peptoids at a modest yield of 60−70%, emphasizing the importance of the length of the alkenyl moiety for the favorable formation of the metallocyclobutane intermediates.

Scheme 2. RCM of Peptoids Containing Both 3-Buten-1-amine and Allylamine



*Transformation efficiency determined by HPLC analysis.

Based upon these excellent findings, tetramers to heptamers (**19**−**29**) with various side chains containing hydrophobic and protected hydrophilic groups were synthesized using both Rink amide and TentaGel resins in order to prove whether solid-phase RCM could generally work well in most of peptoids (Table 2). The RCM of these peptoids in the presence of 2 mol % HG2 catalyst was carried out at 40 °C. The HPLC analysis showed that the solid-phase RCM produced cyclic peptoids in excellent yields (up to almost the quantitative yield), regardless of their ring sizes and the identity of side chains (Figure 2 and Figures S11−S21 in the Supporting Information). Especially, it would be valuable that the RCM also worked very well on the hydrophilic TentaGel resin which is usually used in on-bead screenings against biological targets. In addition, the RCM approach for the solid-phase synthesis of cyclic peptoids has the distinct advantage that the alkene

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Table 2. RCM of Peptoids Containing Arbitrary Sequences[a]

entry[b]	L-PO	C-PO	sequence	yield (%)[c]
1	**19**	**30**	c(Nbte-Nffa-Nmea-Nbte)	78
2	**20**	**31**	c(Nbte-Nmea-Nleu-Nffa-Nbte)	>95
3	**21**	**32**	c(Nbte-Nmea-Nleu-Nffa-Nmea-Nbte)	80
4	**22**	**33**	c(Nbte-Nlys-Nffa-Nasp-Nmea-Nbte)	82
5	**23**	**34**	c(Nbte-Nmea-Nleu-Nffa-Nmea-Nleu-Nbte)	81
6	**24**	**35**	c(Nbte-Nffa-Nlys-Nbte)	88
7	**25**	**36**	c(Nbte-Npip-Nmea-Nffa-Nbte)	91
8	**26**	**37**	c(Nbte-Nffa-Nasp-Npip-Nbte)	92
9	**27**	**38**	c(Nbte-Nmea-Npip-Nffa-Nleu-Nbte)	89
10	**28**	**39**	c(Nbte-Nlys-Nffa-Nasp-Nmea-Nbte)	87
11	**29**	**40**	c(Nbte-Nmea-Nlys-Nffa-Nasp-Nleu- Nbte)	94

[a] The reactions were typically carried out by using 2 mol % HG2 in methylene chloride at 40 °C for 2 h. [b] Rink Amide AM resin and TentaGel MB RAM resin were used in entries 1−5 and entries 6−11, respectively. [c] Yield was calculated as transformation efficiency determined by HPLC analysis.

functional group of cyclic peptoid can be oxidatively cleaved to provide a sequenceable linear molecule. Thus, we simply tested the oxidative cleavage reaction of a cyclic peptoid. Ozonolysis of a cyclic peptoid successfully resulted in the formation of the corresponding linear compound (see the Supporting Information for details).

In summary, we have successfully developed the efficient synthesis of cyclic peptoids using solid-phase RCM, regardless of the ring sizes for 16- to 25-membered cyclic peptoids. The phosphine-free catalyst HG2 was a better catalyst than G1 and G2 for the solid-phase RCM. The solid-phase RCM could be carried out both under microwave conditions and at 40 °C, even though the conditions in methylene chloride at 40 °C might be slightly better for minimizing the formation of the dimerized products or

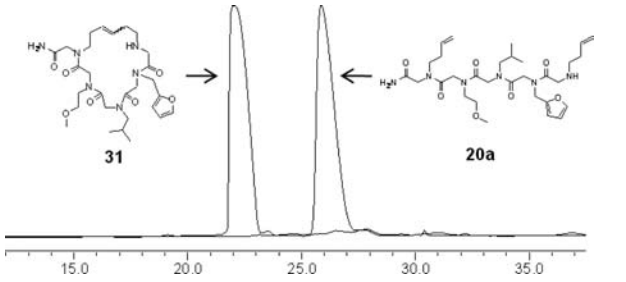

Figure 2. RP-HPLC chromatograms of the crude linear peptoid (**20a**) before the RCM and the crude cyclic peptoid (**31**) after the solid-phase RCM.

other byproducts. The advantages of 3-buten-1-amine over allylamine were clearly evident. This RCM-based macrocyclization is a valuable addition to the synthetic methods that have been used for the synthesis of cyclic peptoids. Additionally, alkene function in the cycles could be oxidatively cleaved by ozonolysis for the purpose of sequence determination and can also be used for further postchemical modifications. This systematic study into the use of solid-phase RCM for the synthesis of cyclic peptoids provides a particularly valuable tool for easy access to the molecular sources of cyclic compounds and the rapid generation of cyclic peptoid libraries. The biological application studies including binding assays of cyclic peptoids against proteins of interest are currently underway.

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Supporting Information Available. Experimental details for peptoids synthesis, solid-phase RCM and ozonolysis, MALDI-TOF spectra, and RP-HPLC chromatograms. This material is available free of charge via the Internet at http://pubs.acs.org.