Solid Phase Synthesis of Peptoids

GenScript USA Inc.

Peptide drugs are a rapidly growing class of therapeutics. However, the peptide drug discovery was hampered by its inherent characteristics: low stability to enzymatic digestion, high conformational flexibility resulting in low specificity to target, low hydrophobicity and lack of specific transportation systems.

In order to overcome the above disadvantages of peptides, peptidomimetics are driving extensive research efforts and have shown a promising prospect in peptide drug discovery. A peptidomimetic is a compound designed to mimic the activity of a peptide, such as a peptoid and a beta-peptide. But a peptidomimetic should have structural difference from its parent peptide. This structural difference can bring us greater advantages for its function as a drug. Many classes of peptidomimetics have been reported [for review, see ref. 1, 2].

Among these peptidomimetics, peptoids are completely resistant to proteolysis and easily get a great number of diversity. Peptoids are oligomers N-substituted glycines developed by Zuckerman and colleagues in 1992 [ref.3]. Peptoids represent a new class of synthetic compound. Comparing with a peptide, as we can see in Figure 1, the side chains are connected to the nitrogen of a peptide backbond in a peptoid instead of alpha carbon in a peptide. In native peptides, R represents 20 kinds of substitution, while in peptoids, R is roughly unlimited.

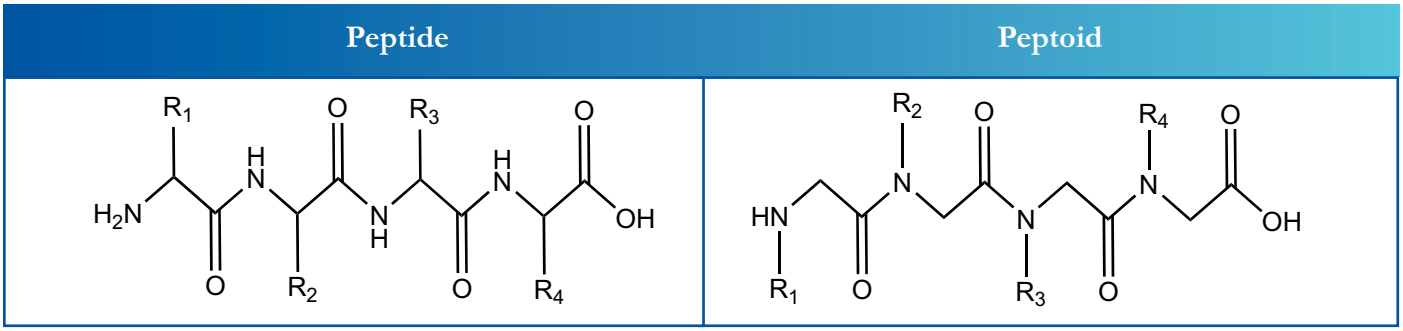


Figure 1: Structural comparison between a peptide and a peptoid

The synthesis of a peptoid can be achieved through monomer and submonomer methods. T monomer method is exactly the same as solid phase peptide synthesis except that all the protected monomers for peptoid synthesis need to be synthesized first. Zuckerman and colleagues provided three routes illustrated in Figure.2 [ref.3] to prepare peptoid monomers. The synthesis of a peptoid oligomer was performed using the standard Fmoc peptide synthesis method with in situ activation by PyBOP or PyBroP. Since all the backbond nitrogens are alkylated, the coupling is more difficult than normal peptide synthesis. That is why PyBOP instead of conventional carbodiimide coupling reagents was used. Using this method, ten peptoid oliogmers were synthesized and showed similar activity with their parent peptides and higher protease resistance [ref.3]. The monomer method is quite straightforward. However, the synthesis of every N^{α}-protected monomer is tedious and time-consuming.

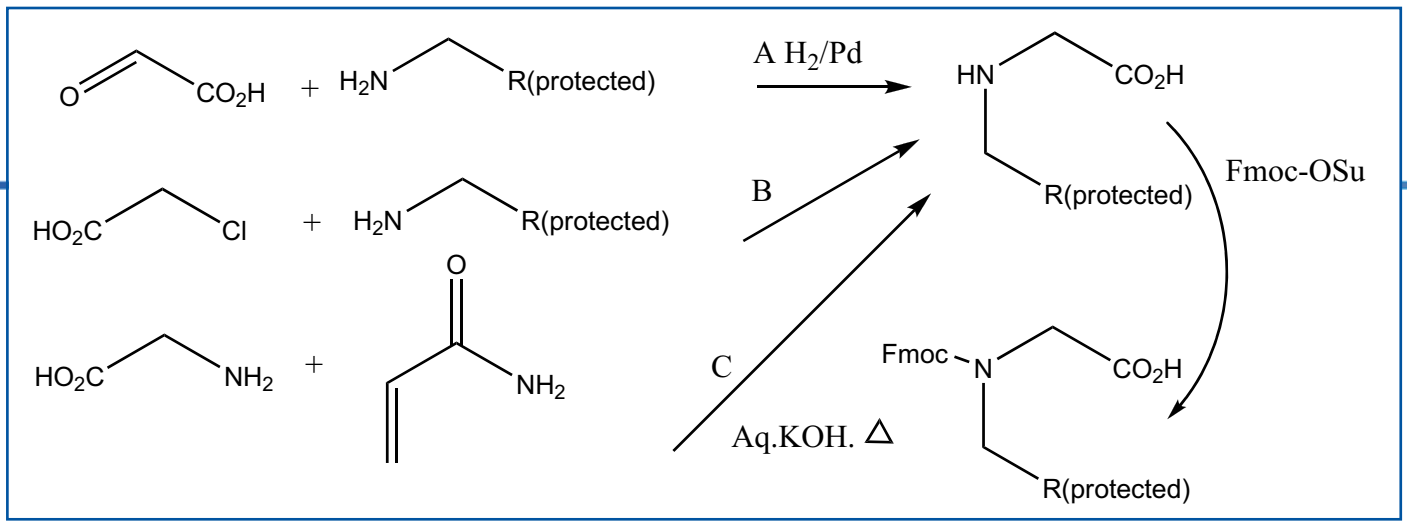


Figure 2: General route for synthesis of peptoid monomers

The submonomer method [ref.4] eliminates the need of N^{α}-protected monomers. It consists of two step simple reactions: an acylation step performed by the addition of bromoacetic acid and DIC and a nucleophilic displacement of bromide with a primary amine as can be seen in Figure 3. Bromoacetic acid and primary amines are readily available submonomers. Because this approach shows greater advantages than the monomer approach, it has been widely applied to peptoid library construction [ref. 5, 6].

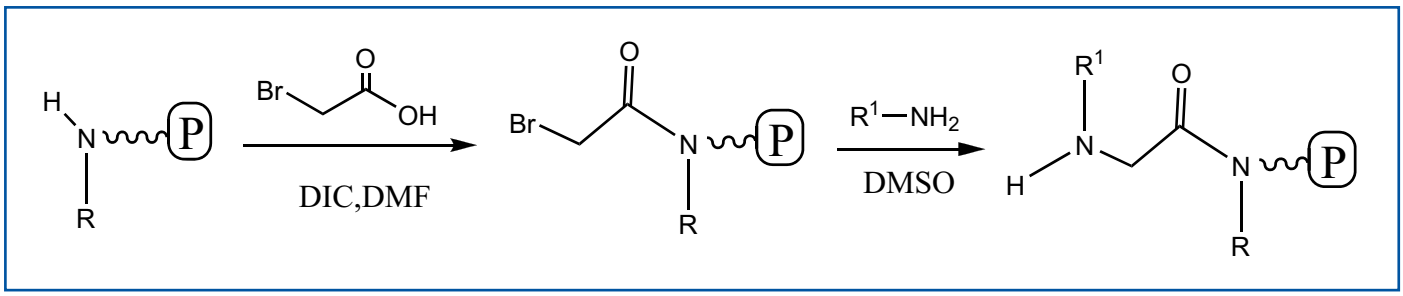


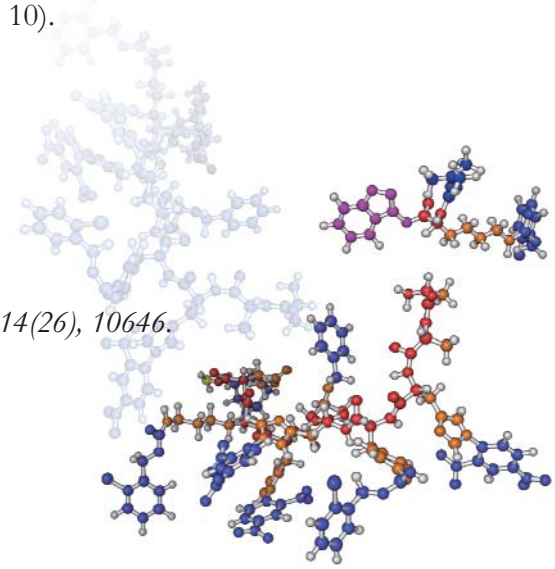
Figure 3: Submonomer route to synthesize peptoids

Both of the above methods are quite straightforward and easily adapted to automated synthesis. However, the diketopiperizine formation and reaction of stero-hindered monomers or submonomers are still problematic. Microwave technology was applied to accelerate the reaction especially for the hindered amines [ref. 7, 8]. Selection of a stero-hindered resin such as a 2-chloro-trityl resin can effectively avoid the formation of diketopiperizine during peptoid synthesis. Microwave technology combined with a multi-channel peptide synthesizer enables us get hundreds of peptoids within hours. The "Split-mix" method (for review, see ref. 9) further generates large combinatorial libraries of peptoids.

As mentioned above, peptoids show enhanced proteolytic stabilities and increased cellular permeabilities relative to their parent peptides. With the progress of peptoid high-throughput synthesis and screening, peptoids hold significant promises for therapeutic applications (for review, see ref. 10).

References

(1) Vagner, J. et al, *Curr. Opin. Chem. Biol.* **2008**, *12(3), 292.*
(2) Soth, M. J.and Nowick, J. S. *Curr. Opin. Chem. Biol.* **1997**, *1, 120.*
(3) Simon R. J.; et al, *Proc. Natl. Acad. Sci. U.S.A.* **1992**,*89,9367.*
(4) Zuckermann, R.. N.et al, *Journal of the American Chemical Society, 114(26), 10646.*
(5) Zuckermann, R.. N., et al, *J. Med. Chem.* ***1994****, 37, 2678.*
(6) Martin, E.J., et al, *J. Med. Chem.* ***1995****, 38, 1431.*
(7) Hernando J. et al, *Org. Lett.*, ***2002****, 4 (23), 4057.*
(8) Benjamin C. et al, *Org. Lett.*, ***2005****, 7 (8), 1521*
(9) Lam, K. et al, *Chem. Rev.*, ***1997****, 97 (2), 411*
(10) Sarah, A.et al, *Org. Biomol. Chem.*, ***2009****, 7, 1508-1524*



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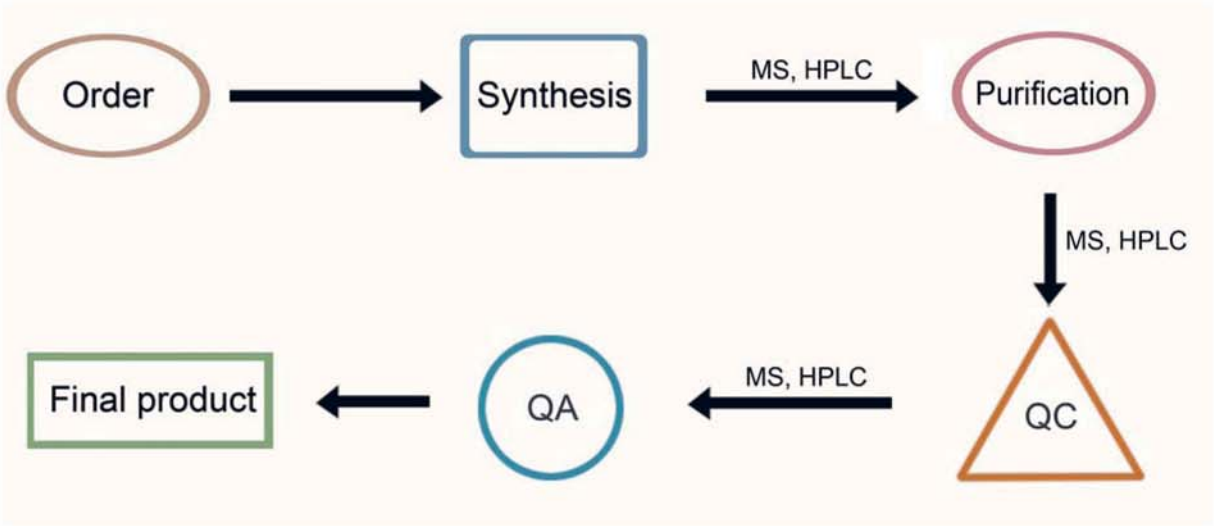

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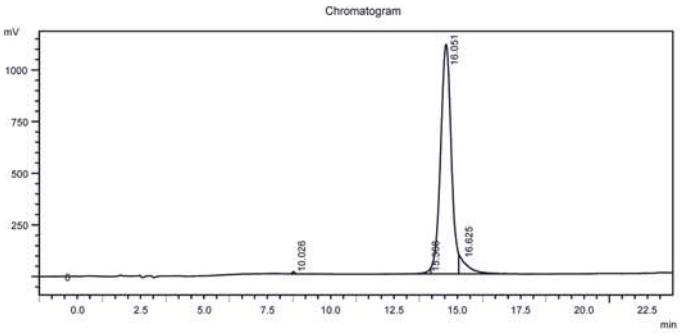
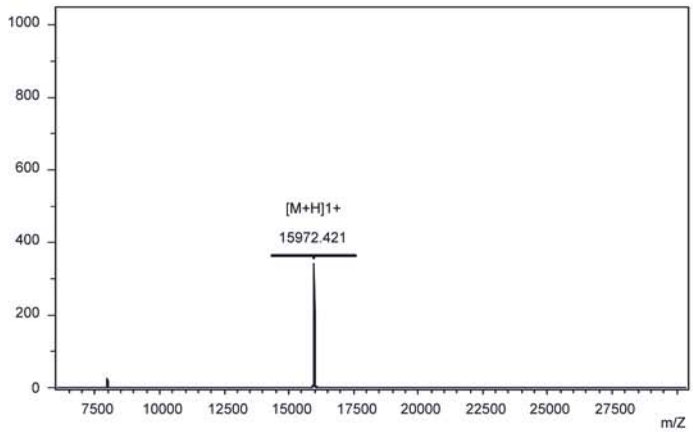


MS results:



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