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Tetrahedron Letters
Tetrahedron Letters 46 (2005) 1561–1564

# An efficient strategy for the preparation of one-bead-one-peptide libraries on a new biocompatible solid support

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Received 28 November 2004; revised 19 December 2004; accepted 21 December 2004
Available online 20 January 2005

**Abstract**—A new strategy for the preparation of one-bead-one-peptide libraries compatible with solid-phase screening and subsequent detachment of the peptide from the resin for sequence determination by mass spectrometry is described. The method is based on the use of ChemMatrix, a novel, totally PEG-based resin, together with 4-hydroxymethylbenzoic acid linker. After peptide elongation, which was carried out using the Fmoc/*t*-Bu approach, the side-chain protecting groups were removed with TFA solution. The library was then screened, and peptides were detached from the positive beads with ammonia/THF vapor. Finally, the peptide sequences were determined by MS/MS.


Combinatorial chemistry has brought about a revolution in many fields, as a result medicinal and organic chemistry have themselves undergone an important evolution.[1] While the initial emphasis in synthetic combinatorial chemistry was the simultaneous preparation of thousands or even millions of compounds, the current mindset has been redirected toward the rapid and rational preparation of small to medium-sized libraries of priority compounds.[2] However, the initial concept is still valid when applied to peptides,[3] peptoids,[4] and nucleotides.[5] These biopolymers can be efficiently, and simultaneously, prepared on solid-phase.[6]

Affinity chromatography (AC) is the most powerful method for the direct isolation and purification of biomolecules from complex mixtures.[7] Its high selectivity minimizes contamination and produces samples of high purity in a single step. Antibodies, dyes, metals, and small peptides are ligands frequently utilized in AC. Antibodies are produced by mammalian cells in complex media and must be exhaustively purified before their use as affinity ligands.[8] Moreover, monoclonal antibodies (mAb) are very expensive and can decrease column capacity through time from loss of antibody binding capacity or from leakage. Furthermore, a product will be contaminated with the antibody. Despite the affordability of dyes, their specificity is much lower than that of antibodies and the number of available dyes is rather limited.[9] Metals also show lower affinity and are prone to leak from columns, resulting in contamination of the product.[10]

Short peptides can have advantages as affinity ligands for industrial separation since, unlike antibodies, they do not cause any immune response.[11] In addition, they are more stable than antibodies as they do not require a specific tertiary structure to conserve their biological activity. They can be aseptically manufactured under GMP at a much lower cost than that for antibodies.

In the development of affinity processes, the identification of a peptide structure with sufficient selectivity and interaction strength to perform a separation by AC is essential. The combinatorial synthesis of peptide libraries allows the screening of millions of peptides in an empirical way, thus greatly facilitating the discovery of suitable ligands for any given protein of interest.[12]

*Keywords*: Combinatorial chemistry; Solid-phase; Resin.
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doi:10.1016/j.tetlet.2004.12.105

Among the different solid-phase strategies for library preparation, the divide–couple–recombine (DCR), or mix and split, method is the most convenient.[13] The method entails (i) dividing the solid support into equal portions, (ii) coupling each portion individually with a different building block, and (iii) mixing the portions. This assures a theoretically even representation of the library members and a one-bead-one-compound distribution. This method requires screening on solid-phase, which can be carried out by immunoaffinity.[14] Finally, the structure of peptides contained on positive beads is determined. Although peptides traditionally can be identified by Edman microsequencing, this method is time consuming, expensive, and only convenient for short to medium-length peptides. The latest developments in mass spectrometry have converted it into the technique of choice for the sequencing of peptides and proteins.[15]

The cornerstone of this methodology is the choice of the solid support and handle to be used. The solid support should be water compatible, because screening is usually carried out in aqueous solution. On the other hand, it should allow for the synthesis of peptides in organic solvents. The bond formed between the first amino acid and the linker should be stable to all synthetic elongation reactions, as well as to the conditions required for the removal of the side-chain protecting groups. The removal of protecting groups is required since screening is normally carried out on fully unprotected peptides.[16] Furthermore, the solid-support of choice should be amenable to easy release of the free peptide for MS analysis.

Herein, a strategy based on the ChemMatrix$^{R}$[17] solid support together with 4-hydroxymethylbenzoic acid (HMBA) linker is described.[18]

ChemMatrix$^{R}$ resin is a new high performance, highly crosslinked, amphiphilic solid support composed entirely of polyethyleneglycol (PEG) monomers that contain exclusively primary ether bonds (Fig. 1).[19] These bonds facilitate high resin loading, as compared to polystyrene (PS) resins, and high chemical and mechanical

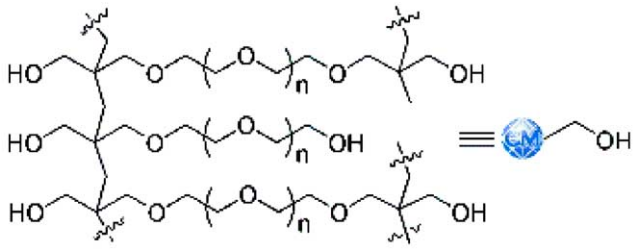


**Figure 1.** Structure of the ChemMatrix$^{R}$ base resin.

stability. It can be stored in dry form, and therefore is easily weighed and transferred with minimum losses. ChemMatrix$^{R}$ resin swells well in $CH_2Cl_2$ (9.5 mL/g), *N*,*N*-dimethylformamide (DMF) (6.5 mL/g), and $H_2O$ (7 mL/g), resulting in 50% more swelling than PS resins in $CH_2Cl_2$ and DMF, and five times more than PS in $H_2O$. Furthermore, it swells extremely well in TFA (16.2 mL/g). All of the aforementioned properties allow chemical elongation of a peptide sequence via fluorenylmethoxycarbonyl (Fmoc) or *t*-butyl (*t*-Bu) strategies as well as screening in aqueous solution.

HMBA linker was first adopted by Atherton et al.[18] for the preparation of peptides avoiding the use of hyperacidic reagents. Release of acidic peptides can be performed with NaOH, whereas methanolic ammonia can be used in the case of amide peptides.[20] Lastly, Maeji and co-workers.[21] have developed a convenient method based on the use of ammonia/THF vapor, which allows simultaneous multiple peptide cleavages and overcomes the formation of methyl esters as side products when methanolic ammonia is used.[20]

HMBA was incorporated into an aminomethyl–ChemMatrix$^{R}$ resin using *N*,*N*′-diisopropylcarbodiimide (DIPCDI) and 1-hydroxybenzotriazole (HOBt), and peptides were synthesized via the Fmoc strategy (Fig. 2). At the C-terminal, four residues of Gly were incorporated with the aim of (i) increasing the molecular weight of the final peptide to facilitate its identification by MS and (ii) overcoming the poor incorporation of

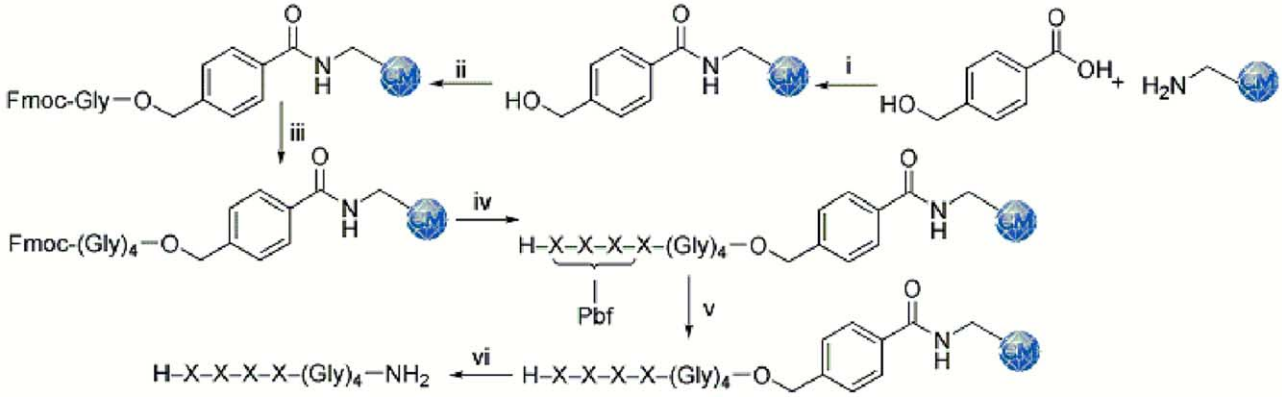


**Figure 2.** Synthetic strategy for the preparation of the library. Reagents and conditions: (i) DIPCDI/HOBt in DMF; (ii) DIPCDI/DMAP (10%) in DMF; (iii) (a) piperidine–DMF (1:4); (b) DMF; (c) Fmoc-Gly-OH, DIPCDI/HOBt in DMF (three times); (iv) (a) piperidine–DMF (1:4); (b) DMF; (c) Fmoc-X-OH, DIPCDI/HOBt in DMF, being X = A, P, or R; reactions are carried out independently in three different reaction vessels; (v) TFA–phenol–$H_2O$ (85:5:10); (vi) ammonia/THF vapor cleavage.

**Figure 3.** Photomicrographs of a positive bead surrounded by negative beads.

very hindered protected amino acids such as Val, Ile, or Arg, onto the HMBA linker. The C-terminal Gly was incorporated with DIPCDI in the presence of *N*,*N*-dimethylaminopyridine (DMAP) (10%) in DMF. The remaining protected amino acids were incorporated with DIPCDI/HOBt.[22]

Once elongation was complete the side-chain protecting groups were removed by treatment with TFA–phenol–$H_2O$ (85:5:10) for 2 h, leaving the unprotected peptide anchored to the resin.

Immunoaffinity screening of the solid supported library using anti-Granulocyte-Macrophage-Colony-Stimulating Factor (GM-CSF) mAb was performed as described by Hirabayashi et al.[14]

Beads showing a positive reaction to *anti* GM-CSF mAb were mechanically isolated and subjected to ammonia/THF vapor cleavage. Free peptides, which remained absorbed on the solid support, were eluted with a mixture of acetic acid (HOAc)–acetonitrile (ACN)–$H_2O$ (3:4:3).[21]

As proof of concept, a combinatorial library of the octapeptide XXXXGGGG, where X = A, P, or R, was synthesized on the HMBA–ChemMatrix$^R$ resin using the strategy outlined above: DCR method with Fmoc chemistry and TFA to remove the Pbf, which was used to protect the Arg side-chain. The combinatorial mixture of those amino acids contained the sequence Ala-Pro-Ala-Arg, which was previously selected from the screening of a 130,321 tetrapeptide library previously prepared and analyzed.[23]

Electrospray-ionization MS/MS analysis of the positive beads to the anti GM-CSF mAb assay (Fig. 3) indicated the sequence APARGGGG as previously obtained.

In conclusion, a new and efficient strategy for the preparation of one-bead-one-peptide libraries based on a HMBA–ChemMatrix$^R$ resin is described. The immunoaffinity screening was greatly facilitated by the amphiphilicity of the ChemMatrix$^R$ beads. The use of MS/MS assay to identify the positive peptides reduced the cost and time of the assay. By generating optimized affinity ligands, this strategy should facilitate the development of chromatographic matrices for the purification of biomolecules.

## Acknowledgements

This work was partially supported by grants from the Agencia Nacional de Promoción Científica y Tecnológica de la República Argentina and the University of Buenos Aires, and from CICYT (PBQU2004-0089).

## References and notes

1. *Handbook of Combinatorial Chemistry*; (a) Nicolaou, K. C., Hanko, R., Hartwig, W., Eds.; Wiley-VCH, Verlag GmbH & Co. KGaA: Weinheim, Germany, 2002; (b) Lee, A.; Breitenbucher, J. *Curr. Opin. Drug Discov. Develop.* **2003**, *6*, 494–508; (c) Lombardino, J. G.; Lowe, J. A. *Nat. Rev. Drug Discov.* **2004**, *3*, 853–862; (d) Sanchez-Martin, R. M.; Mittoo, S.; Bradley. *Curr. Topics Med. Chem.* **2004**, *4*, 653–669; (e) Reetz, M. T. *Compreh. Coord. Chem. II* **2004**, *9*, 509–548.
2. Cano, M.; Balasubramanian, S. *Drugs Future* **2003**, *28*, 659–678.
3. Pastor, M. T.; Perez-Paya, E. *Mol. Div.* **2003**, *6*, 149–155.
4. Garcia-Martinez, C.; Humet, M.; Planells-Cases, R.; Gomis, A.; Caprini, M.; Viana, F.; De la Pena, E.; Sanchez-Baeza, F.; Carbonell, T.; De Felipe, C.; Perez-Paya, E.; Belmonte, C.; Messeguer, A.; Ferrer-Montiel, A. *PNAS USA* **2002**, *99*, 2374–2379.
5. Strobel, H.; Dugue, L.; Marliere, P.; Pochet, S. *ChemBioChem* **2002**, *3*, 1251–1256.
6. *Solid-Phase Synthesis. A Practical Guide*; Kates, S. A., Albericio, F., Eds.; Marcel Dekker: New York, 2000.
7. (a) Yang, C. M.; Tsao, G. T. *Adv. Biochem. Eng.* **1982**, *26*, 19–42; (b) Lowe, C. R.; Lowe, A. R.; Gupta, G. *J. Biochem. Biophys. Methods* **2001**, *49*, 561–574.
8. Brassfield, A. L. In *Methods in Molecular Biology (Monoclonal Antibody Protocols)*; Humana: Totowa, NJ, 1995; Vol. 45, pp 195–203.
9. Scopes, R. K. *J. Chromatogr.* **1986**, *376*, 131–140.
10. Porath, J. *Protein Exp. Purif.* **1992**, *3*, 263–281.
11. (a) Kaufman, D. B.; Hentsch, M. E.; Baumbach, G. A.; Buettner, J. A.; Dadd, C. A. *Biotechnol. Bioprocess.* **2002**, *77*, 278–289; (b) Huang, P. Y.; Hammond, D. J.; Carbonell, R. G. *Biotechnol. Bioprocess.* **2002**, *77*, 278–289.
12. (a) Fassina, G.; Palomba, D. *Chim. Oggi.* **2003**, *21*(10/11), 38–41; (b) Labrou, N. E. *J. Chromatogr. B.* **2003**, *790*, 67–78.
13. (a) Lam, K. S.; Salmon, S. E.; Hersh, E. M.; Hruby, V. J.; Kazmierski, W. M.; Knapp, R. J. *Nature* **1991**, *354*, 82–84; (b) Houghten, R. A.; Pinilla, C.; Blondelle, S. E.; Appel, J. R.; Dooley, C. T.; Cuervo, J. H. *Nature* **1991**, *354*, 84–86; (c) Furka, A.; Sebestyen, F.; Asgedom, M.; Dibo, G. *Int. J. Peptide Protein Res.* **1991**, *37*, 487–493.
14. Hirabayashi, Y.; Fukuda, H.; Kimura, J.; Miyamoto, M.; Yasui, K. *J. Virol. Methods* **1996**, *61*, 23–36.
15. Spengler, B. *J. Mass Spectrom.* **1997**, *32*, 1019–1036.
16. Unprotected solid supported peptides have been synthesized on PEGA resin, containing a fluorenylmethyl-based handle, following a Boc/Bzl strategy, and an HF treatment for removal of the side-chain protecting groups. Peptide detachment can be done with morpholine, thereby allowing direct sequencing via MALDI-TOF MS: Pastor, J. J.; Fernandez, I.; Rabanal, F.; Giralt, E. *Org. Lett.* **2002**, *4*, 3831–3833; A similar approach using a photolabile linker and Fmoc/*t*-Bu strategy was also reported: Halkes, K. M.; Gotfredsen, C. H.; Grötli, M.; Miranda, L. P.; Duus, J. O.; Meldal, M. *Chem. Eur. J.* **2001**, *7*, 3584–3591.
17. Cote, S. PCT/cd 2004/001461, patent pending Int.

18. Atherton, E.; Logan, C. J.; Sheppard, R. C. *J. Chem. Soc., Perkin Trans. 1* **1981**, 538–546.
19. A ChemMatrix$^{R}$ similar resin is SPOCC: Rademann, J.; Grötli, M.; Meldal, M.; Block, K. *J. Am. Chem. Soc.* **1999**, *121*, 5459–5466. Other PEG-based resin, but containing primary and secondary ethers and hydroxy functions, is POEPOP: Renil, M.; Meldal, M. *Tetrahedron Lett.* **1996**, *37*, 6185–6188. Co-polymers of PEG and other polymers are: PEG-PS: Zalipsky, S.; Chang, J. L.; Albericio, F.; Barany, G. *React. Polym.* **1994**, *22*, 243–258; , Tenta-Gel: Rapp, W. In *Combinatorial Peptide and Non-peptide Libraries*; Jung, G., Ed.; VCH: Weinheim, Germany, 1996; pp 425–464; PEGA:Meldal, M. *Tetrahedron Lett.* **1992**, *33*, 3077–3080; CLEAR: Kempe, M.; Barany, G. *J. Am. Chem. Soc.* **1996**, *118*, 7083–7093; NovaGel (Champion): Adams, J. H.; Cook, R. M.; Hudson, D.; Jammalamadaka, V.; Lyttle, M. H.; Songster, M. F. A. *J. Org. Chem.* **1998**, *63*, 3706–3716; and ArgoGel: Gooding, O. W.; Baudart, S.; Deegan, T. L.; Heisler, K.; Labadie, J. *J. Comb. Chem.* **1999**, *1*, 113–122.
20. Atherton, E.; Sheppard, R. C. *Solid Phase Peptide Synthesis a Practical Approach*; IRL at Oxford University Press: Oxford, UK, 1989; pp 152–153.
21. Bray, A. M.; Valerio, R. M.; Maeji, N. J. *Tetrahedron Lett.* **1993**, *34*, 4411–4414.
22. Removal of the Fmoc group from the second residue of Gly should be carried out quickly in order to avoid the formation of diketopiperazine ($3 \times 1$ min). Alternatively, the second residue of Gly can be incorporated with Boc as the Gly protecting group, whereby removal is achieved with TFA–$CH_2Cl_2$ (4:6). Incorporation of the third Gly residue would then use Fmoc protected Gly and an in situ neutralization/coupling protocol Gairi, M.; Lloyd-Williams, P.; Albericio, F.; Giralt, E. *Tetrahedron Lett.* **1990**, *31*, 7363–7366.
23. Camperi, S. A.; Iannucci, N. B.; Albanesi, G. J.; Oggero Eberhardt, M.; Etcheverrigaray, M.; Messeguer, A.; Albericio, F.; Cascone, O. *Biotechnol. Lett.* **2003**, *25*, 1545–1548.