Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 48 (2007) 389–391

A new method for the preparation of 2-chlorotrityl resin and its application to solid-phase peptide synthesis

Tae-Kyung Lee,[a] Sun-Jong Ryoo[b] and Yoon-Sik Lee[a,*]

[a]*School of Chemical and Biological Engineering, Seoul National University, Seoul 151-744, Republic of Korea*
[b]*BeadTech Inc., Institute for Chemical Processes, Seoul National University, Seoul 151-744, Republic of Korea*

Received 19 October 2006; revised 7 November 2006; accepted 13 November 2006
Available online 30 November 2006

Abstract—2-Chlorotritylchloride (2-CTC) resin was prepared efficiently from 1% DVB-crosslinked polystyrene resin and 1-chloro-2-(dichloro(phenyl)methyl)benzene, which was easily obtained from 2-chlorobenzophenone. This 2-CTC resin showed excellent properties as a support for solid-phase peptide synthesis. Four peptide fragments were obtained in high purity using the resin.

Peptides are considered as highly potent drug candidates due to their high specificity and low toxicity. Their importance has further increased as the biological function of proteins and enzymes is intensively studied by the Human Genome Project. Moreover, several hundred peptide-based drugs have entered clinical phase testing or already been commercialized.[1] Accordingly, the demand for the production of peptides in large quantities has also increased.

Such peptides are mostly obtained by biological technology or chemical synthesis. The chemical method, especially solid-phase peptide synthesis (SPPS), is usually adopted for the large-scale production of peptides due to its simplified reaction procedure and easy purification/isolation steps for the target products.[2]

Of the numerous solid supports or linker systems employed in SPPS, 2-chlorotritylchloride (2-CTC) resin is a preferred support because side reactions such as racemization and diketopiperazine formation[3] can be suppressed. This is due to the characteristic hindered structure of 2-chlorotrityl group.[4] Furthermore, 2-CTC resin can be applied to prepare protected peptide acids under mild cleavage conditions, which can be used in the fragment condensation for larger polypeptides. The practical length of the peptide chain which can be obtained directly from the resin is up to 20-mer. For the synthesis of longer peptides (~40-mer), the protected peptide fragments are coupled in solution.

* Corresponding author. Tel.: +82 2 880 7080; fax: +82 2 876 9625; e-mail: yslee@snu.ac.kr

0040-4039/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2006.11.071

Such a solid- and solution-phase hybrid method has been used for the industrial-scale production of peptide drugs, such as T20 and T1249. They are produced by the condensation of three protected peptide fragments which are prepared using 2-CTC resin.[5] Nowadays, 2-CTC resin is no longer a polymer support confined to the laboratory, but can be considered as a bulk chemical. Therefore, an efficient method for the large-scale production of 2-CTC resin is required in response to the increased industrial demand.

Previously, the trityl structure has been introduced onto a resin by one of three methods. The first involves constructing the trityl groups on the resin. The lithio-phenyl polymer derived from the iodophenyl polymer and *n*-butyllithium was reacted with benzophenone to give the tritylated polymer.[6] Inversely, polymeric benzophenone derivatives were prepared from benzoyl chlorides via Friedel–Crafts acylation, and then converted into the trityl structure on the resin with a Grignard reagent or lithium reagent.[7]

The tritylated resin was also prepared by directly attaching a linker with the trityl group to the resin. In this case, handles connecting the linker to the resin were required. Therefore, trityl linkers with a carboxylic acid or a hydroxyl handle were prepared from the corresponding benzophenones with organometallic reagents in solution-phase and coupled to amino resin[8] or Merrifield resin.[9]

In addition to the post-functionalization methods mentioned above, the copolymerization of tritylated

monomers has been used for the preparation of tritylated resins. Thus, the trityl group was introduced into the monomers by the addition of a Grignard reagent or lithium reagent prepared from 4-bromostyrene to benzophenones.[10] Such a tritylated monomer was also prepared by the treatment of 4-vinyl benzophenone with the Grignard reagent.[11] However, these methods for the preparation of tritylated monomers, trityl linkers or trityl groups on the resin, all involved using organometallic reagents, which might pose some problems in scaling-up. In this letter, we report a simple new method for preparing 2-CTC resin, which does not require any organometallic reagents.

As a precursor to the trityl group, we selected 1-chloro-2-(dichloro(phenyl)methyl)benzene (DCPB) as a pseudo-activated linker. It has two phenyl rings which can be converted into trityl groups after the reaction with another phenyl group in polystyrene (PS) resin. Moreover, DCPB can be easily obtained from 2-chlorobenzophenone. Thus, DCPB (**2**) was obtained in 90% yield by reacting 2-chlorobenzophenone (**1**) with phosphorus pentachloride (Scheme 1), according to the method described in previous reports.[12] Even though the resulting DCPB contained a small amount of 2-chlorobenzophenone, it was used in the next step without further purification.

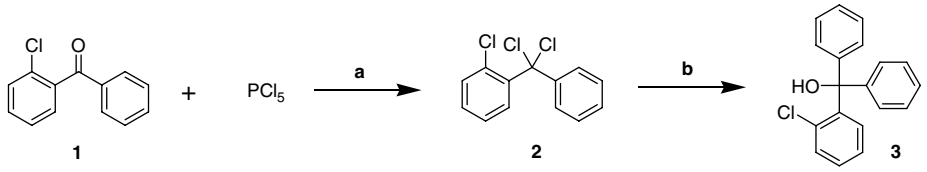
As a model reaction in solution, DCPB was first reacted with benzene in the presence of aluminum chloride and (2-chlorophenyl)diphenylmethanol (**3**) was obtained in 78% yield after the work-up. Based on this result, we confirmed that the trityl structure could be obtained from our new linker without using any organometallic reagents (Scheme 1). Then, we tried to load this linker onto polystyrene (PS) resin. To accomplish this, DCPB was reacted with 1% divinylbenzene (DVB)-crosslinked PS resin in the same way. After quenching and washing, 2-chlorotritylalcohol (2-CTA) resin (**4**) was obtained. After chlorination of the resulting 2-CTA resin (**4**) with thionyl chloride, 2-CTC resin (**5**) was obtained (Scheme 2).[13]

The substitution level of the 2-CTC resin turned out to be 1.3 mmol/g resin by Fmoc-titration after the coupling of Fmoc-Leu-OH. The 2-CTA resin and 2-CTC resin had the same chemical structure as the resins prepared using organometallic reagents, as proven by FT-IR. These resins also had a clean surface and good swelling properties (see Supplementary data).

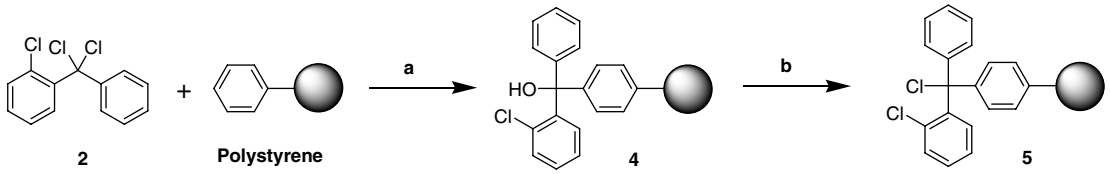
In addition to the chemical and physical properties of the resins, the distribution of the functional groups within the resin beads was reported to be important in SPPS, because of the accessibility of the reaction sites within the resin beads.[14] Therefore, the distribution of the CTC groups within the 2-CTC resin beads was analyzed by physically slicing the resin beads after loading the first amino acid, Fmoc-Trp, and fluorescence staining. In the case of the 2-CTC resin prepared by our method, the trityl groups were more populated in the surface layer than in the inner layer. This is probably caused by the bulkiness of the pseudo-activated linker during Friedel–Crafts alkylation on the PS resin. As the PS resin is alkylated from the surface, the trityl groups are considered to exert greater steric hindrance on the incoming activated pseudo-linkers and therefore, resulted in such an asymmetric structure. In contrast, the 2-CTC resin by the conventional organometallic method[7] showed a relatively even distribution of the functional groups within the resin beads (Fig. 1).

Next, we examined the applicability of our 2-CTC resin to SPPS. For the efficient peptide synthesis on the resin, the first amino acids should be loaded easily and the peptides elongated efficiently with high purity on the resin. First, three amino acids (Fmoc-Gln-OH, Fmoc-Leu-OH and Fmoc-Trp(Boc)-OH) were loaded onto the 2-CTC resin. In the presence of a base, these Fmoc-amino acids were loaded efficiently with a high loading level (Table 1).

Then, three peptide fragments of T20 were prepared using the Fmoc-amino acid loaded 2-CTC resin with Fmoc/*t*Bu strategy.[5] After their elongation, the peptide fragments in their protected forms were cleaved from the resins with weak acid (1% TFA in dichloromethane), under which conditions the protecting groups at the side chains remained intact. As expected, the cleaved



Scheme 1. Preparation of 1-chloro-2-(dichloro(phenyl)methyl)benzene (**2**) and (2-chlorophenyl)diphenylmethanol (**3**). Reagents and conditions: (a) 130–140 °C, 2 h; (b) benzene, $AlCl_3$, CH_2Cl_2, 25 °C, 12 h, and then 3 N HCl.



Scheme 2. Preparation of 2-chlorotritylchloride resin (**5**). Reagents and conditions: (a) $AlCl_3$, CH_2Cl_2/nitrobenzene, 25 °C, 4 h, and then 3 N HCl; (b) $SOCl_2$, CH_2Cl_2, 25 °C, 2 h.

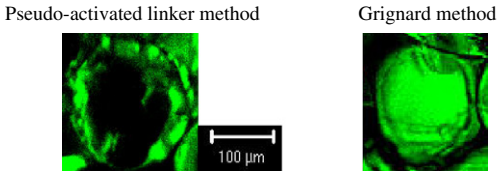


Figure 1. Confocal microscopic images of cross-sectioned resin beads: fluorescein isothiocyanate-coupled Trp(Boc)-CTC resin prepared by (a) pseudo-activated linker method and (b) Grignard method.

Table 1. Loading levels of Fmoc-amino acid loaded 2-CTC resins and purities of the peptide fragments synthesized using the corresponding resins

Fmoc-amino acid loaded 2-CTC resin	Loading level (mmol/g resin)	Peptide purity (%) (sequence)
Fmoc-Gln-resin	0.54	84 (T20 1–16)
Fmoc-Leu-resin	0.74	95 (T20 17–26)
Fmoc-Trp(Boc)-resin	0.69	91 (T20 27–35)

peptides were found to be very pure by HPLC and ESI-Mass analyses (Table 1) (see also Supplementary data).

We also compared our 2-CTC resin with the conventional 2-CTC resin (prepared using the Grignard method). A well-known difficult sequence, fragment 65–74 of the acyl carrier protein (ACP 65–74),[15] was also synthesized on both of the 2-CTC resins with Fmoc/*t*Bu strategy. The 2-CTC resin obtained using the pseudo-activated linker method gave ACP 65–74 in higher purity (76%) than the conventional 2-CTC resin (41%). It is considered that such a difference in the synthetic efficiency originates from the differences in the distributions of the functional groups. As in the case of the 2-CTC resin obtained by the pseudo-activated linker method, the functional groups in the core–shell type structure were believed to be more accessible to the incoming reagents.

In summary, we developed a simple and efficient method for the preparation of 2-CTC resin using 1-chloro-2-(dichloro(phenyl)methyl)benzene as a pseudo-activated linker. This 2-CTC resin has a good physical appearance and efficient loading/cleavage properties. Moreover, it also has a core–shell type structure, wherein the functional groups are more concentrated at the surface layer of the resin beads. With this new 2-CTC resin, four peptide fragments (T20 1–16, T20 17–26, T20 27–35 and ACP 65–74) were prepared in high purity.

Acknowledgments

This study was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (A050432).

Supplementary data

The analytical data and experimental procedures for the compounds and peptide fragments. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.11.071.

References and notes

1. Andersson, L.; Blomberg, L.; Flegel, M.; Lepsa, L.; Nilsson, B.; Verlander, M. *Biopolymers* **2000**, *55*, 227–250; Bruckdorfer, T.; Marder, O.; Albericio, F. *Curr. Pharm. Biotech.* **2004**, *5*, 29–43.
2. Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149–2153.
3. Atherton, E.; Benoiton, N. L.; Brown, E.; Sheppard, R. C.; Williams, B. J. *J. Chem. Soc., Chem. Commun.* **1981**, 336–337; Sieber, P. *Tetrahedron Lett.* **1987**, *28*, 6147–6150; Pedroso, E.; Grandas, A.; De Las Heras, X.; Eritja, R.; Giralt, E. *Tetrahedron Lett.* **1986**, *27*, 743–746.
4. Barlos, K.; Chatzi, O.; Gatos, D.; Stavropoulos, G. *Int. J. Pept. Protein Res.* **1991**, *37*, 513–520; Anne, C.; Fournie-Zaluski, M.-C.; Roques, B. P.; Cornille, F. *Tetrahedron Lett.* **1998**, *39*, 8973–8974; Zikos, C.; Livaniou, E.; Leondiadis, L.; Ferderigos, N.; Ithakissios, D. S.; Evangelatos, G. P. *J. Pept. Sci.* **2003**, *9*, 419–429.
5. Kang, M. C.; Bray, B.; Lichty, M.; Mader, C.; Merutka, G. U.S. Patent 6 015 881, 2000; Schneider, S. E.; Bray, B. L.; Mader, C. J.; Friedrich, P. E.; Anderson, M. W.; Taylor, T. S.; Boschernitzan, N.; Niemi, T. E.; Fulcher, B. C.; Whight, S. R.; White, J. M.; Greene, R. J.; Stoltenberg, L. E.; Lichty, M. *J. Pept. Sci.* **2005**, *11*, 744–753.
6. Melby, L. R.; Strobach, D. R. *J. Am. Chem. Soc.* **1967**, *89*, 450–453.
7. Hayatsu, H.; Khorana, H. G. *J. Am. Chem. Soc.* **1967**, *89*, 3880–3887; Cramer, F.; Köster, H. *Angew. Chem., Int. Ed. Engl.* **1968**, *7*, 473–474; Fréchet, J. M. J.; Haque, K. E. *Tetrahedron Lett.* **1975**, *16*, 3055–3056; Orosz, G.; Kiss, L. P. *Tetrahedron Lett.* **1998**, *39*, 3241–3242.
8. Zikos, C. C.; Ferderigos, N. G. *Tetrahedron Lett.* **1994**, *35*, 1767–1768; Quibell, M.; Packman, L. C.; Johnson, T. *J. Am. Chem. Soc.* **1995**, *117*, 11656–11668; Leondiadis, L.; Vassiliadou, I.; Zikos, C.; Ferderigos, N.; Livaniou, E.; Ithakissios, D. S.; Evangelatos, G. P. *J. Chem. Soc., Perkin Trans. 1* **1996**, 971–975; Blelcher, K. U.S. Patent 5 922 890, 1999.
9. Hidai, Y.; Kan, T.; Fukuyama, T. *Tetrahedron Lett.* **1999**, *40*, 4711–4714.
10. Borhan, B.; Wilson, J. A.; Gasch, M. J.; Ko, Y.; Kurth, D. M.; Kurth, M. J. *J. Org. Chem.* **1995**, *60*, 7375–7378; Nzeru, A.; Ebdon, J. R.; Rimmer, S. *J. Org. Chem.* **1997**, *62*, 8928–8929; Choi, M. K. W.; Toy, P. H. *Tetrahedron* **2004**, *60*, 2903–2907.
11. Manzotti, R.; Reger, T. S.; Janda, K. D. *Tetrahedron Lett.* **2000**, *41*, 8417–8420.
12. Cognacq, J.-C.; Chodkiewicz, W. *Bull. Soc. Chim. Fr.* **1965**, 2183–2187; Toldy, L.; Toth, I.; Balogh, T.; Pataki, S.; Koncz, L.; Pribek, F.; Simonyi, I.; Ladanyi, L.; Illes Alfoldi, I. HU Patent 25247, 1983; Laube, T.; Bannwart, E.; Hollenstein, S. *J. Am. Chem. Soc.* **1993**, *115*, 1731–1733.
13. Harre, M.; Nickisch, K.; Tilstam, U. *React. Funct. Polym.* **1999**, *41*, 111–114.
14. Cho, J. K.; Park, B. D.; Lee, Y. S. *Tetrahedron Lett.* **2000**, *41*, 7481–7485; Cho, J. K.; Park, B. D.; Park, K. B.; Lee, Y. S. *Macromol. Chem. Phys.* **2002**, *203*, 2211–2217; Kim, H. Y.; Cho, J. K.; Chung, W. J.; Lee, Y. S. *Org. Lett.* **2004**, *6*, 3273–3276; Lee, T. K.; Ryoo, S. J.; Byun, J. W.; Lee, S. M.; Lee, Y. S. *J. Comb. Chem.* **2005**, *7*, 170–173; Lee, T. K.; Lee, S. M.; Ryoo, S. J.; Byun, J. W.; Lee, Y. S. *Tetrahedron Lett.* **2005**, *46*, 7135–7138.
15. Atherton, E.; Clive, D. L. J.; Sheppard, R. C. *J. Am. Chem. Soc.* **1975**, *97*, 6584–6585.