



Preparation of tris-based dendrimer-grafted core-shell type resin for solid-phase peptide synthesis

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Abstract—A novel symmetrical dendrimer monomer was synthesized from tris(hydroxymethyl)aminomethane (Tris) and repeatedly coupled to a core-shell type resin. The loading capacity of amino groups was increased up to 4.0 mmol g⁻¹ resin by the dendrimerization process. The high loading dendrimer-grafted resin produced was successfully applied to solid-phase peptide synthesis (SPPS). © 2001 Elsevier Science Ltd. All rights reserved.

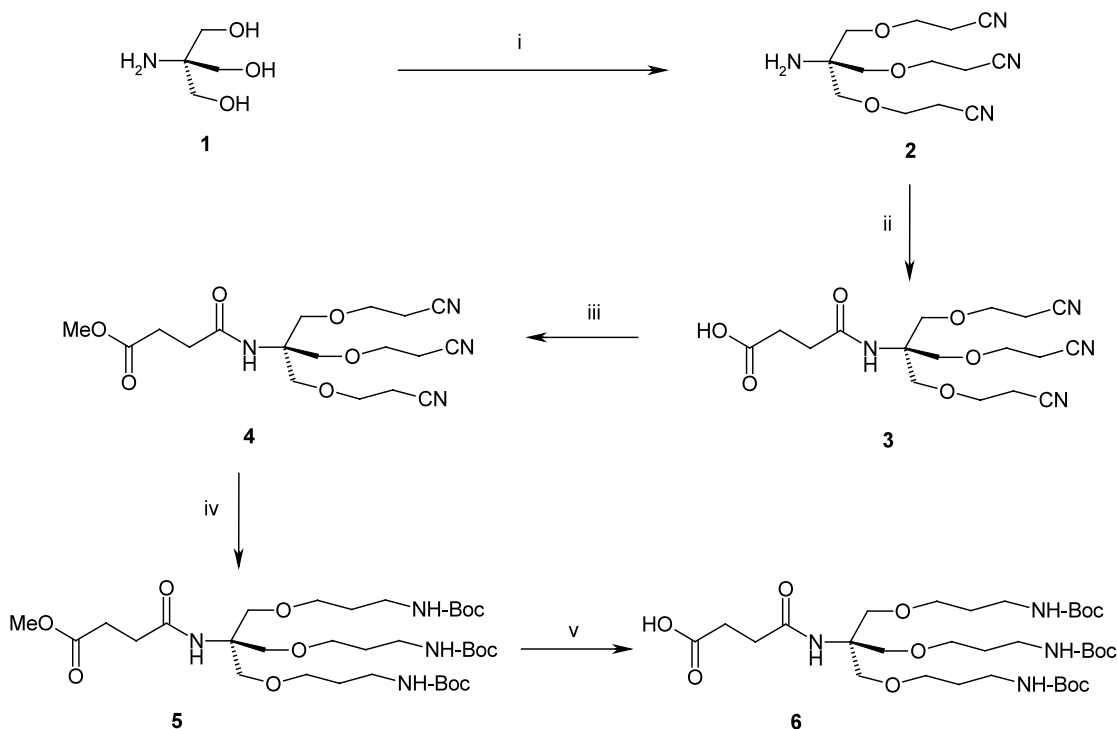
Solid-phase organic synthesis (SPOS) enables us to easily and conveniently prepare a good many organic compounds and hence to build combinatorial libraries for high throughput screening (HTS).¹ In particular, the pool-and-split method is widely used for the construction of very large sets of chemical libraries from resin beads.^{2,3} For the successful assay and structure determination of a compound from a single bead, the resin should have a high loading capacity. Several divergent dendrimerization methods have been applied to the resin bead as a means of amplifying the loading capacity. Protected lysine⁴ and Boc-protected tri-amino acid⁵ have been used as building blocks for the generation of dendrimer on the resin. However, neither compound has structurally equivalent amino groups, which are the starting point of solid-phase synthesis. The solid-phase synthesis of polyamidoamino (PAMAM) dendrimer has been reported as an approach, which does not use the ready-made dendrimer building block.⁶ Although this dendrimerization process yields symmetric amino groups, the resulting dendrimer contains basic tertiary amine, which may induce side effects in certain reactions, i.e. solid-phase peptide synthesis. Recently, the Bradley group reported upon the solid-phase synthesis of tris-based dendrimer on TentaGel resin and achieved an a notable loading capacity enhancement.⁷ However, their method may cause further cross-linking in the

resin matrix because of the use of ethylene diamine during each dendrimerization step. In order to avoid such a possible problem, we synthesized a new tris-based dendrimer monomer with a triple branched symmetrical structure. The dendrimer monomer was synthesized by the following procedure (Scheme 1).

Michael type addition of tris **1** to acrylonitrile gave **2**.⁸ Succinylation reaction of **2** with succinic anhydride in the presence of triethylamine introduced a spacer to afford **3**. To prevent undesirable reduction, the carboxyl acid group of **3** was converted to the methyl ester. The nitrile group was then reduced to the amino group and protected in situ by the Boc group using sodium borohydride, nickel(II) chloride and di-*tert*-butyl dicarbonate.⁹ Finally, the desired compound **6** was obtained by hydrolysis at an overall yield of 51% from **2**.¹⁰ The synthetic dendrimer monomer **6** was coupled to a core-shell type PS-*co*-PEG-NH₂ resin (CutiCore™)¹¹ and a gel type TentaGel resin, using the BOP reagent, HOBt and DIPEA, respectively.¹² After each coupling, the Boc protecting groups were removed by 50% TFA in DCM and neutralized by 10% TEA in DCM. Generations of dendrimer were achieved by repetitive coupling of the synthetic monomer to the resins, followed by deprotection of the Boc group. The average coupling yield in each step with CutiCore resin and TentaGel resin was 89 and 82%, respectively. As expected, we achieved very high loadings, of up to 4.0 mmol g⁻¹, from the core-shell type CutiCore resin (0.10 mmol g⁻¹) after seven generations (7G) of dendrimerization. On the other hand, we could not amplify the loading capacity of TentaGel (0.2 mmol g⁻¹) more than 3.0 mmol g⁻¹ because it reached its dense packing limit after the 5th generation.

Abbreviations: Boc, *tert*-butoxycarbonyl; DIPCDI, *N,N'*-diisopropylcarbo-diimide; DIPEA, diisopropylethylamine; DCM, dichloro-methane; HOBt, 1-hydroxybenzotriazole; TFA, trifluoro-acetic acid; BOP, benzotriazol-1-yloxy-tris(dimethylami-no)phosphonium hexafluorophosphate; TEA, triethylamine; DME, 1,2-dimethoxyethane.

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Scheme 1. (i) Acrylonitrile, 40% KOH, dioxane, 24 h; (ii) succinic anhydride, Et₃N, CHCl₃, 6 h, quantitative; (iii) H₂SO₄, CH₃OH, 88%; (iv) NaBH₄, NiCl₂, (Boc)₂O, CH₃OH, 24 h, 58%; (v) 2N NaOH, DME, H₂O, quantitative.

We tested the swelling properties of the resins in various solvents. CutiCore resin gave 20–30% lower levels of swelling (4.9–2.1 mL g⁻¹) than TentaGel (6.3–3.6 mL g⁻¹) in all of the solvents tested (e.g. DCM, THF, DMF, MeOH, water; listed in decreasing order of swelling), primarily because of its higher degree of cross-linking (more than ca. 4%). After 4G dendrimerization, the swelling volumes of TentaGel were leveled at around 4.6–3.1 mL g⁻¹, while those of CutiCore resin remained almost same.¹⁴

We chose Leu-enkephalinamide (H-Tyr-Gly-Gly-Phe-Leu-NH₂) as a model peptide and tested the usefulness of the dendrimer-grafted CutiCore resin (4G, 1.7 mmol g⁻¹) in solid-phase peptide synthesis, by comparing results with the CutiCore resin (0.10 mmol g⁻¹).¹⁴ After attaching an acid labile Rink amide linker to the amino group of the resins, Leu-enkephalinamide was synthesized in an automatic synthesizer in continuous-flow mode.¹⁵ Peptides were cleaved from each resin with TFA-DCM (9:1) for 3 h and the crude products were characterized by ESI-MS.¹⁶ The results of HPLC analyses showed that the peptides were obtainable at over 96% purity, even though the dendrimer-grafted resin had 17 times the loading capacity of the original (Fig. 1).¹⁷

In summary, we synthesized a novel tris-based triple branched dendrimer monomer, which we believe to be a practically useful monomer for ‘dendrimerization’ on a polymer support. By repetitive coupling of the synthetic dendrimer monomer, the loading capacity of CutiCore resin can be increased dramatically. Furthermore, the high loading dendrimer-grafted resin proved to be useful in solid-phase peptide synthesis.¹⁸

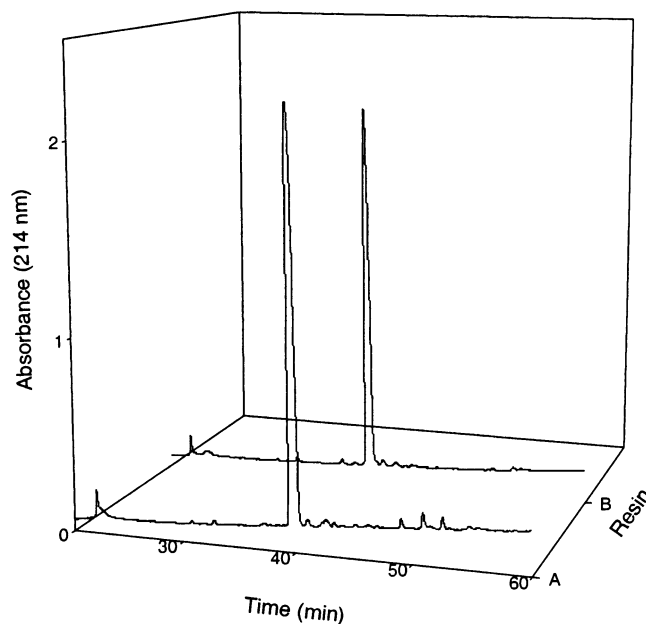


Figure 1. HPLC analyses of the crude product (H-Tyr-Gly-Gly-Phe-Leu-NH₂) cleaved from (A) CutiCore resin (0.1 mmol g⁻¹) and (B) 4G dendrimer-grafted CutiCore resin (1.7 mmol g⁻¹).

Acknowledgements

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10. δ_{H} (300 MHz, CDCl_3) δ 1.44 (27H, s, Boc), δ 1.70 (6H, qn, $-\text{C}(\text{CH}_2\text{OCH}_2\text{CH}_2\text{CH}_2-)_3$), δ 2.44–2.57 (4H, m, $-\text{COCH}_2\text{CH}_2\text{CONH}-$), δ 3.21 (6H, t, $-\text{C}(\text{CH}_2\text{OCH}_2\text{CH}_2\text{CH}_2-)_3$), δ 3.47 (t, 6H, $-\text{C}(\text{CH}_2\text{OCH}_2\text{CH}_2\text{CH}_2-)_3$), δ 3.70 (6H, s, $\text{C}(\text{CH}_2\text{OCH}_2\text{CH}_2\text{CH}_2-)_3$), δ 4.85 (3H, br, $-\text{C}(\text{CH}_2\text{OCH}_2\text{CH}_2\text{CH}_2\text{NH}-\text{Boc})$).
11. (a) Cho, J. K.; Lee, Y. S. *Tetrahedron Lett.* **2000**, *41*, 7481–7485; (b) Papers for detailed synthetic procedures are submitted to other journals.
12. The end point of each coupling step was determined by ninhydrin color test.¹³
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14. We considered 4G CutiCore resin to be optimal for comparison because CutiCore resin reached dense packing limit after 7th generation. The swelling volumes (mL g^{-1}) of CutiCore resin and 4G CutiCore resin were as follows: 4.9, 4.3 (DCM); 3.5, 3.9 (THF); 3.6, 4.0 (DMF); 2.4, 2.6 (MeOH); 2.1, 1.8 (water). The resins are physically and chemically stable under various SPPS conditions (SEM, IR analysis).
15. After coupling of Fmoc-Rink-OH, each resin was then transferred to an Applied Biosystems 431A peptide synthesizer. Chain assembly was carried out in C \rightarrow N direction by repetitive cycles for ‘Fmoc chemistry’. In this protocol, couplings were mediated by DCC/HOBt in NMP.
16. Leu-enkephalinamide has a calculated exact mass of 554.28. ESI/MS, m/z positive $[\text{M}+\text{H}]^+$: 554.81.
17. A flow rate of 1 mL min^{-1} and a 10 min constant flow of 0.1% TFA/water followed by a 50 min gradient of 0–50% CH_3CN in 0.1% TFA/water was used. Absorbance was measured at 214 nm. A Vydac C_{18} column (4.6 \times 250 mm) of which temperature was controlled at 45°C using MetaChem MetaTherm HPLC Column Temperature Control was used.
18. A decapeptide, acyl carrier protein (74–65), was also successfully synthesized from 4G CutiCore resin by the same method as Ref. 15 with 78% purity (HPLC). ESI/MS, m/z positive $[\text{M}+\text{H}]^+$: 1061.81. The detailed results will appear elsewhere.