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Synthesis of peptide-grafted comb polypeptides *via* polymerisation of NCA-peptides†

Hiroshi Enomoto,‡§ Benjamin Nottelet,§ Soultan Al Halifa, Christine Enjalbal, Mathieu Dupré, Julien Tailhades, Jean Coudane, Gilles Subra, Jean Martinez and Muriel Amblard*

A straightforward synthesis of comb-polypeptides of repeated peptide sequences was developed. These polypeptides were obtained by ROP of defined NCA without any post-polymerization grafting. The key to this strategy relies on the preparation of pure NCA bearing a peptide sequence on its side chain, by an original solid supported methodology.

The design of biodegradable and biocompatible polymers is an important field of research that underpins unique molecular architectures that are able to mimic natural compounds with various biological activities or provide unique organizational properties. Over the past decade, biologically compatible materials have been increasingly used in a broad range of medical treatments such as drug delivery, wound healing, ocular treatments, cardiovascular devices, bone repair products as well as cosmetics.^{1–4} Polypeptides and amino acids containing polymers are a class of biomimetic materials that significantly become important, due to their specific properties such as biocompatibility, biodegradability and bioactivity.^{1,5,6} The most common and efficient process for synthesis of long polypeptide chains and related is accomplished by the ring-opening polymerization of amino acid-*N*-carboxyanhydrides (NCAs).^{7–10} Recent efforts have specifically focused on providing well-defined co-polypeptides and block polypeptides.^{11–15} However, NCA polymerization is mostly limited to linear homopolypeptides or copolypeptides. Branched architectures such as star or brush polypeptides can only be obtained by polymerization of NCA from multifunctional initiators or post-grafting on preformed polymer chains.^{14,16} In most cases, the “grafting to”

approach is achieved by polymer post-grafting that requires specific conjugation chemistry where the efficiency of the grafting strongly depends upon the nature of the polymers, the ligation chemistry and the compatibility of the bioactive moiety with the conjugation conditions.^{17–19} An alternative to the post-grafting methodology is the use of monomers bearing the bioactive moiety during the polymerization process.²⁰ This “grafting through” approach allows the direct formation of comb polymers, whose self-assembly behaviours or multivalent properties (RGD-functionalized comb copolymers for example) have attracted much attention in recent years.^{21–23} To our knowledge, two examples of brush polymers that have been synthesized from functionalised NCA amino acids are water-soluble pegylated-peptides and glycopolypeptides obtained *via* living polymerization of pegylated-serine NCA and glycosylated-L-lysine NCA, respectively.^{24–26} However this strategy has not been applied to the production of polymers containing defined peptide sequences. In this study, we report the design and synthesis of comb-shaped peptidic polymers containing repeated peptide sequences appended to the glutamic side chain and generated by one step polymerization. The key to this methodology relies on the preparation and the use of defined monomers, which comprise an NCA bearing the peptide sequence of interest on its side chain (Fig. 1).

Whereas peptide sequences can be easily and conveniently synthesized on a solid support,²⁷ no report on the preparation of *N*-terminally activated species on a solid support has been described in the literature. Consequently, to maintain the advantage of employing solid phase peptide synthesis (SPPS) which avoids potential issues such as solubility, we first developed a convenient and straightforward methodology to generate the NCA activated species directly on the solid support (Scheme 1). In this strategy, the activated peptide construct was released from the solid support ready to be employed in the NCA Ring Opening Polymerisation (ROP) reaction.

To validate this strategy, we prepared the activated monomer model sequence **1** composed of glutamic acid NCA bearing the peptide sequence “Phe-Gly-Ala” on its side chain.

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† Electronic supplementary information (ESI) available: Experimental procedures, HPLC, ¹H/¹³C NMR, LCMS, MS/MS.

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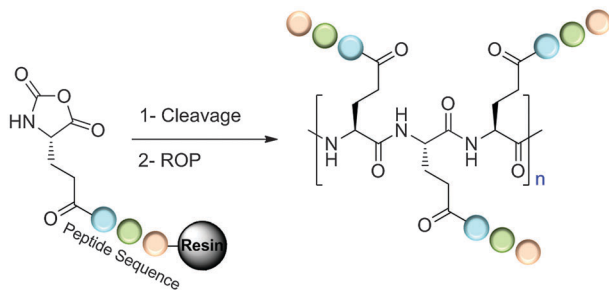


Fig. 1 Polymerization of Glu(peptide)NCA after its cleavage from the resin.

After building up the peptide sequence **2** on the Rink-amide resin *via* HBTU coupling of the N-Fmoc protected amino acids, the N-terminal glutamic acid residue (Boc-Glu-OBzl) was coupled to the growing sequence **2 *via* its side chain yielding **3**. That ensured availability of both α -amino and α -carboxylic acid functions for further NCA elaboration. After lithium hydroxide hydrolysis of peptide resin **3**, the lithiated NCA precursor **4** was obtained. To ensure complete conversion of **4** into the resin-supported N-carboxyanhydride derivative **5**, optimization of NCA formation was required. In addition the stability of **5** during the final cleavage from the solid support also needed to be strictly controlled.**

We first applied the commonly used procedure described in the literature for efficient synthesis of the amino acid NCA moiety. We initially focused on the mild reaction conditions employed for Boc-amino acid conversion using bis(trichloromethyl)carbonate (BTC) in the presence of triethylamine.^{28,29} Unfortunately, on the solid support the best yield obtained by using these conditions was less than 60% providing a peptide of poor quality. We investigated a wide range of other methods (not reported) for NCA formation, the best of which led to an almost total conversion of **4** into **5** when cyanuric chloride was used. This reagent, though widely employed in a variety of synthetic transformations,³⁰ has never been described for the preparation of NCAs. Conversion of **4** into NCA **5** was monitored by TFA cleavage of resin aliquots and HPLC analysis.

The most efficient conversion took 12 h at room temperature. Thus, treatment of resin **5** with neat TFA in anhydrous DCM afforded the peptide-Glu NCA **1** with 90% purity. A rapid purification step was performed by precipitation of the residual hydrolyzed NCA in acetonitrile/4 M hydrogen chloride in dioxane to yield compound **1** in high purity, suitable for polymerization.

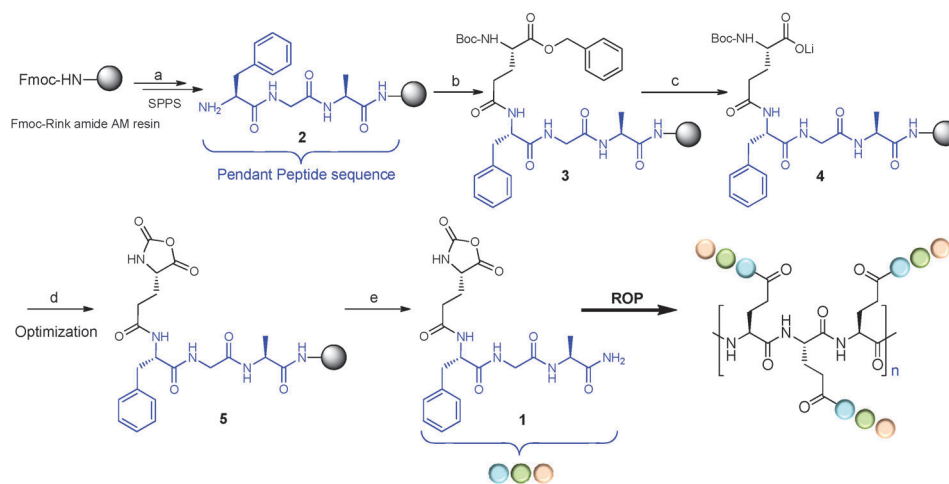
Polymerization was performed under classical NCA ROP conditions (Table 1). To avoid problems of solubility of the starting material **1**, DMF was chosen as the polymerization solvent. Two main mechanisms of NCA ROP could occur, depending on the nucleophile–base initiator balance, namely the normal amine mechanism (NAM) and the activated monomer mechanism (AMM).⁸ In order to introduce functional end groups including potential probes, our first intention was to initiate the polymerization *via* NAM by a primary amine. Hexylamine (HEA), which is known to only promote NAM polymerization, was selected for its fast initiation and more precise molecular weight control. However, in the present study, HEA was found to be ineffective for the ROP of **1**.

Indeed NMR analysis showed only a 13% monomer conversion after 3 days at 30 °C (Polymer **1**, **P1**). This unexpected result suggested that ROP of NCA bearing a peptide sequence *via* NAM was inefficient. As a consequence, diethylamine (DEA) was considered as an alternative initiator. This secondary amine preferably initiates ROP by the normal amine mechanism but can also lead to the activated monomer mechanism.³¹ As such, DEA should allow

Table 1 Polymerization of **1**

Polymer ^a	Initiator	T (°C)	\bar{M}_n (g mol ⁻¹)	<i>D</i>	DP _{the}	DP _{ex}	Conversion (%)
P1	HEA	30	5960	1.15	50	13	13
P2	DEA	30	8470	1.18	50	21	50
P3	DEA	55	11 380	1.16	50	28	75

^a All polymerizations were performed in anhydrous DMF.



Scheme 1 Synthesis of the Glu(Phe-Gly-Ala-NH₂)-NCA monomer on resin. *Reagents and conditions:* (a) SPPS: deprotection: piperidine–DMF (20/80), rt, 30 min; coupling: Fmoc-AA-OH (3 eq.), HBTU (3 eq.), DIEA (3 eq.), DMF, rt, 2 h; (b) (1) deprotection: piperidine–DMF (20/80), rt, 30 min; (2) Boc-Glu-OBzl (3 eq.), HBTU (3 eq.), DIEA (3 eq.), DMF, rt, 2 h; (c) 2 M LiOH aq.–THF (30/70), rt, 3 h; (d) cyanuric chloride (5 eq.), anhydrous DCM, rt, overnight; (e) TFA–anh. DCM (50/50), rt, 1.5 h.

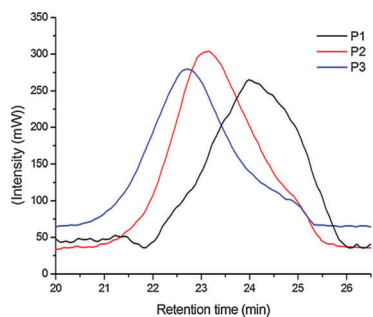


Fig. 2 SEC chromatograms of polymers P1 to P3.

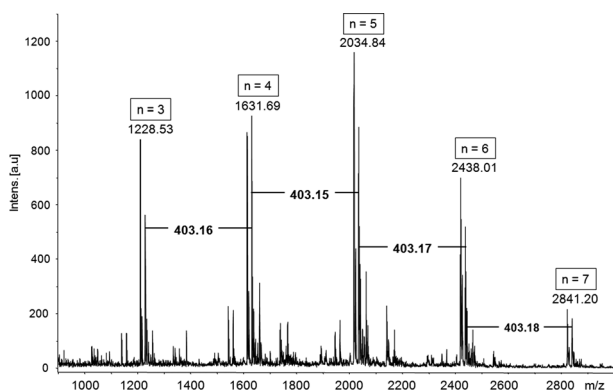


Fig. 3 MALDI-TOF mass spectrometry of P2.

discrimination between these two polymerization pathways. Under the same ROP experimental conditions, DEA led to 50% conversion and Size Exclusion Chromatography (SEC) analysis confirmed the presence of polymer chains with a polymerization degree (DP) of 21 instead of 50 (Polymer 2, P2 Fig. 2 and Table 1).

Although an improvement, conversion remained too low. To overcome this limitation the polymerization temperature was increased to 55 °C. Under these conditions, conversion increased up to 75% and polymer 3 (P3) was obtained with an average molecular weight of 11 400 g mol⁻¹, which corresponds to a DP of 28. It was interesting to notice that monomodal distributions and low dispersities \bar{D} (1.15–1.18) were observed in all cases, demonstrating the ability of NCA building blocks to polymerize under classical ROP conditions.

P2 was further analysed by MALDI-TOF mass spectrometry (Fig. 3). This study, which allowed qualitative determination of the monomeric increment, also helped in the partial elucidation of the polymerisation mechanism.

The expected polymer fingerprint with ions separated by the mass of the monomer (403 Da) was clearly detected (Fig. 3). The major ion distribution was assigned to the carboxylic acid-terminated polymer arising from the AMM mechanism. MS/MS experiments confirmed the structure of the polymeric compounds (Fig. SI-4, ESI⁺). No compound incorporating the DEA moiety could be detected supporting an absence of NAM mechanism. This finding confirmed that the unexpected AMM mechanism was preferred for the ROP of compound 1 under the conditions studied.

In this study, we have developed a straightforward synthesis of comb-polypeptides that bear pendant peptide sequences. This new class of polypeptides was obtained without any post-polymerization grafting by ROP of NCA substituted with a model peptide sequence. The prerequisite for this methodology is the preparation of highly pure reactive complex monomers. For that purpose, we developed an original and efficient solid support synthesis of NCA activated species. The ROP study performed in the presence of a primary amine (HEA) and a secondary amine (DEA) demonstrated an unexpected preference for AMM polymerization of these novel NCA derivatives. Future studies will be dedicated to the enlargement of this innovative approach to comb-polypeptides substituted with peptide sequences of biological interest and comb copolymers containing different peptide sequences.

Notes and references

- 1 T. J. Deming, *Prog. Polym. Sci.*, 2007, **32**, 858–875.
- 2 L. S. Nair and C. T. Laurencin, *Prog. Polym. Sci.*, 2007, **32**, 762–798.
- 3 J. S. Boateng, K. H. Matthews, H. N. E. Stevens and G. M. Eccleston, *J. Pharm. Sci.*, 2008, **97**, 2892–2923.
- 4 R. Wessely, *Nat. Rev. Cardiol.*, 2010, **7**, 194–203.
- 5 M. Barz, R. Luxenhofer, R. Zentel and M. J. Vicent, *Polym. Chem.*, 2011, **2**, 1900–1918.
- 6 B. Apostolovic, S. P. E. Deacon, R. Duncan and H. A. Klok, *Biomacromolecules*, 2010, **11**, 1187–1195.
- 7 H. R. Kricheldorf, *Angew. Chem., Int. Ed.*, 2006, **45**, 5752–5784.
- 8 J. Cheng and T. J. Deming, *Top. Curr. Chem.*, 2012, **310**, 1–26.
- 9 G. J. M. Habraken, A. Heise and P. D. Thornton, *Macromol. Rapid Commun.*, 2012, **33**, 272–286.
- 10 J. W. Robinson and H. Schlaad, *Chem. Commun.*, 2012, **48**, 7835–7837.
- 11 T. J. Deming, *Nature*, 1997, **390**, 386–389.
- 12 G. J. M. Habraken, K. H. R. M. Wilsens, C. E. Koning and A. Heise, *Polym. Chem.*, 2011, **2**, 1322–1330.
- 13 T. J. Deming, *Macromolecules*, 1999, **32**, 4500–4502.
- 14 N. Hadjichristidis, H. Iatrou, M. Pitsikalis and G. Sakellariou, *Chem. Rev.*, 2009, **109**, 5528–5578.
- 15 C. Fetsch and R. Luxenhofer, *Macromol. Rapid Commun.*, 2012, **33**, 1708–1713.
- 16 J. Rodriguez-Hernandez, M. Gatti and H. A. Klok, *Biomacromolecules*, 2003, **4**, 249–258.
- 17 H. G. Borner, *Prog. Polym. Sci.*, 2009, **34**, 811–851.
- 18 H. G. Borner, *Macromol. Rapid Commun.*, 2011, **32**, 115–126.
- 19 B. Le Droumaguet and J. Nicolas, *Polym. Chem.*, 2010, **1**, 563–598.
- 20 S. S. Sheiko, B. S. Sumerlin and K. Matyjaszewski, *Prog. Polym. Sci.*, 2008, **33**, 759–785.
- 21 M. W. Neiser, S. Muth, U. Kolb, J. R. Harris, J. Okuda and M. Schmidt, *Angew. Chem., Int. Ed.*, 2004, **43**, 3192–3195.
- 22 J. Wang, H. Lu, R. Kamat, S. V. Pingali, V. S. Urban, J. J. Cheng and Y. Lin, *J. Am. Chem. Soc.*, 2011, **133**, 12906–12909.
- 23 U. Hersel, C. Dahmen and H. Kessler, *Biomaterials*, 2003, **24**, 4385–4415.
- 24 J. Y. Hwang and T. J. Deming, *Biomacromolecules*, 2001, **2**, 17–21.
- 25 J. R. Kramer and T. J. Deming, *J. Am. Chem. Soc.*, 2010, **132**, 15068–15071.
- 26 D. Pati, A. Y. Shaikh, S. Hotha and S. Sen Gupta, *Polym. Chem.*, 2011, **2**, 805–811.
- 27 M. Amblard, J. A. Fehrentz, J. Martinez and G. Subra, *Mol. Biotechnol.*, 2006, **33**, 239–254.
- 28 W. H. Daly and D. Poche, *Tetrahedron Lett.*, 1988, **29**, 5859–5862.
- 29 R. Wilder, *J. Org. Chem.*, 1992, **57**, 2755–2756.
- 30 G. Blotny, *Tetrahedron*, 2006, **62**, 9507–9522.
- 31 W. N. E. van Dijk-Wolthuis, L. van de Water, P. van de Wetering, M. J. van Steenbergen, J. J. Kettenes-van den Bosch, W. J. W. Schuyl and W. E. Hennink, *Macromol. Chem. Phys.*, 1997, **198**, 3893–3906.