

The Solid-Phase Synthesis of Amino Acid-Derived Diacetylene Lipids

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Abstract: We prepared amino acid-derived diacetylene monomers using solid-phase organic synthesis. The solid-phase synthetic method allowed for the rapid and efficient preparation of functional diacetylenes. Amino acids having hydrophobic sidechains such as alanine, leucine, and phenylalanine, as well as hydrophilic sidechains such as aspartic acid and lysine, were successfully coupled to the diacetylene lipid. The diacetylene monomers prepared in this way were subjected to routine procedures for the generation of polydiacetylene vesicles. Depending on the nature of the side-chains, pink to blue colored polydiacetylenes were generated.

Keywords: solid-phase synthesis, diacetylene, amino acids.

Introduction

Since the pioneering discovery of blue-to-red color transitions induced by specific ligand-receptor interactions in polymerized diacetylenes,¹ the development of efficient sensory systems based on polydiacetylenes (PDAs) has gained much attention in the context of fundamental and applied research.² Most of the functional diacetylene monomers, reported thus far, have been prepared by using solution-phase synthetic methods, which require multi-step purification procedures.³ A potentially more useful approach for the preparation of functional diacetylenes, which can overcome the limitations involving the solution-phase synthesis, relies on the employment of solid-phase synthetic methods. Recently, solid-phase synthesis has evolved into a key technology for the preparation of a large number of diverse, biologically important ligands.⁴ Thus, we felt that combining the emerging fields of solid-phase synthesis and diacetylene chemistry would lead to new and efficient routes for the preparation of PDA-based chemosensors. As part of our continuing efforts for the development of polydiacetylene-based chemosensors,⁵ we now report the solid-phase synthesis of functional diacetylene lipid monomers. Our initial effort aimed at testing this proposal focused on the solid-phase synthesis of amino acid-derived, diacetylene lipids. Amino acid and peptide-modified diacetylenes were selected for this effort owing to the fact that peptides are attractive

targets for drug discovery. This is a consequence of their high affinities and specificities toward biological receptors and the fact that large peptide libraries can be synthesized by using a combinatorial format.⁶

Experimental

10,12-Pentacosadiynoic acid (PCDA) was purchased from GFS Chemicals. The Rink resin, Fmoc-protected amino acids, and reagents for solid-phase synthesis were purchased from Advanced Chemtech. The solid-phase synthesis of amino acid-modified diacetylene monomers were performed according to the general procedures described in 'Advanced Chemtech Handbook of Combinatorial & Solid Phase Organic Chemistry' (1998 edition). Spectroscopic data for the amino acid-derived diacetylene monomers synthesized on solid support are as follows:

NH₂-Gly-PCDA: M. p. 128-129 °C; ¹H NMR (300 MHz, CDCl₃): δ=0.88 (t, 3H), 1.20-1.80 (m, 32H), 2.24 (m, 6H), 3.96 (d, 2H), 5.40 (s, 1H), 6.00 (s, 1H), 6.20 (s, 1H); ¹³C NMR (100 MHz, CDCl₃): δ=14.6, 18.8, 19.6, 23.1, 25.9, 28.7, 28.8, 29.0, 29.1, 29.3, 29.5, 29.6, 29.7, 29.9, 30.0, 32.3, 36.3, 36.7, 43.2, 65.5, 65.6, 68.4, 77.5, 77.7, 77.9, 173.8, 175.6.

NH₂-Ala-PCDA: M. p. 124-125 °C; ¹H NMR (300 MHz, CDCl₃): δ=0.88 (t, 3H), 1.2-1.8 (m, 35H), 2.19 (t, 2H), 2.25 (t, 4H), 4.54 (q, 1H), 5.52 (s, 1H), 6.2 (d, 1H), 6.44 (s, 1H); ¹³C NMR (100 MHz, CDCl₃): δ=15.0, 19.0, 20.0, 23.5, 26.3, 29.1, 29.5, 29.7, 29.9, 30.1, 30.3, 30.4, 32.7, 37.3, 48.9, 65.9, 66.0, 78.1, 78.3, 173.7, 175.0.

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NH₂-Leu-PCDA: M. p. 104-105 °C; ¹H NMR (300 MHz, CDCl₃): δ=0.87 (t, 3H), 0.93 (m, 6H), 1.2-1.8 (m, 34H), 2.20 (m, 6H), 4.54 (m, 1H), 6.03 (d, 1H), 6.54 (s, 1H), 6.95 (s, 1H); ¹³C NMR (100 MHz, CDCl₃): δ=14.9, 20.0, 22.9, 23.5, 23.7, 25.6, 26.4, 29.1, 29.5, 29.6, 29.7, 29.9, 30.1, 30.4, 32.7, 37.1, 41.9, 51.8, 65.9, 66.0, 78.2, 173.9, 175.6.

NH₂-Phe-PCDA: M. p. 94-95 °C; ¹H NMR (300 MHz, CDCl₃): δ=0.85 (t, 3H), 1.2-1.8 (m, 32H), 2.16 (t, 2H), 2.23 (t, 4H), 3.07 (m, 2H), 4.65 (q, 1H), 5.34 (s, 1H), 5.76 (s, 1H), 6.08 (d, 1H), 7.28 (m, 5H); ¹³C NMR (100 MHz, CDCl₃): δ=14.6, 19.6, 23.1, 25.9, 27.4, 28.7, 29.1, 29.3, 29.4, 29.5, 29.7, 29.9, 30.0, 32.3, 36.3, 36.9, 38.5, 54.2, 65.5, 65.6, 77.5, 77.7, 77.9, 127.3, 128.9, 129.4, 136.6, 173.2, 175.4.

NH₂-Asp-PCDA: M. p. 92-93 °C; ¹H NMR (300 MHz, CDCl₃): δ=0.85 (t, 3H), 1.2-1.8 (m, 32H), 2.08 (t, 2H), 2.27 (t, 4H), 2.50 (m, 2H), 4.49 (q, 1H), 7.03 (s, 1H), 7.19 (s, 1H), 7.96 (d, 1H); ¹³C NMR (100 MHz, CDCl₃): δ=15.0, 20.0, 23.5, 26.3, 27.8, 28.8, 29.1, 29.5, 29.7, 29.9, 30.1, 30.3, 30.4, 32.7, 36.7, 49.7, 65.9, 66.0, 78.1, 78.3, 172.0, 173.8, 176.3.

NH₂-Lys-PCDA: M. p. 88-89 °C; ¹H NMR (300 MHz, CDCl₃): δ=0.85 (t, 3H), 1.2-1.8 (m, 38H), 2.01 (t, 2H), 2.26 (t, 4H), 3.75 (m, 2H), 4.16 (m, 1H), 6.97 (s, 1H), 7.29 (s, 1H), 7.81 (d, 1H); ¹³C NMR (100 MHz, CDCl₃): δ=14.6, 28.7, 28.8, 29.0, 29.1, 29.3, 29.4, 29.5, 29.6, 29.7, 29.9, 30.0, 30.1, 49.8, 65.6, 77.5, 77.7, 77.9, 174.6, 175.7, 175.8.

NH₂-Phe-Ala-Ser-PCDA: ¹H NMR (300 MHz, CDCl₃): δ=0.87 (t, 3H), 1.2-1.8 (m, 35H), 2.24 (m, 6H), 3.00-3.06 (dd, 1H), 3.30-3.35 (dd, 1H), 3.45-3.49 (dd, 1H), 3.68-3.71 (dd, 1H), 4.22 (m, 2H), 4.68 (m, 1H), 5.40 (s, 1H), 6.33 (d, 1H), 6.40 (s, 1H), 6.83 (d, 1H), 6.97 (d, 1H), 7.18-7.24 (m, 5H); ¹³C NMR (100 MHz, CDCl₃): δ=14.6, 17.8, 18.8, 19.6, 23.1, 25.9, 27.8, 28.7, 29.0, 29.1, 29.3, 29.5, 29.7, 29.9, 30.0, 32.3, 36.8, 37.6, 50.5, 54.4, 60.9, 65.5, 65.6, 68.5, 74.9, 76.7, 77.9, 126.9, 128.6, 129.2, 137.5, 171.3, 171.7, 173.5, 174.4.

Results and Discussion

The route for synthesis of amino acid-modified diacetylene monomer on a solid support starts with coupling of a 9-fluorenylmethoxycarbonyl (Fmoc) protected amino acid (0.33 mmol) to the free amine (0.11 mmol) of Rink resin (Figure 1). The reaction is carried out by using the standard condition for a peptide coupling involving 1-hydroxybenzotriazole (HOBt, 0.33 mmol), *o*-benzotriazole-1-yl-tetramethyluronium hexafluorophosphate (HBTU, 0.33 mmol), and diisopropylethylamine (DIEA, 0.33 mmol) in DMF. Removal of the Fmoc group is followed by coupling the free amine with 10,12-pentacosadiynoic acid (PCDA, 0.33 mmol) to give the support linked amino acid-modified diacetylene monomer. Both of the steps in the sequence can be completed within an hour. Cleavage of the H₂N-AA-PCDAs

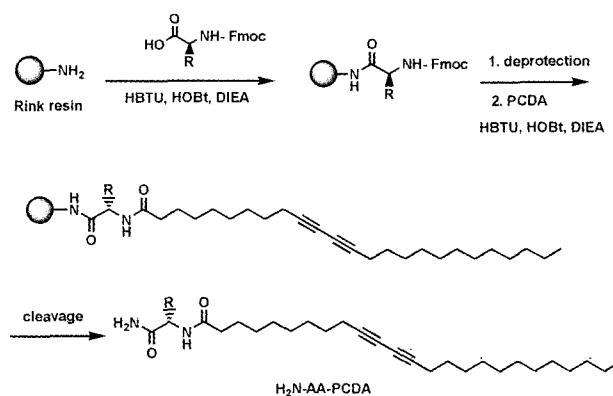


Figure 1. Solid-phase synthesis of amino acid-derived diacetylene lipids.

from the support and complete side-chain deprotection is accomplished by reaction with 10% trifluoroacetic acid (TFA) in CH₂Cl₂. The crude material obtained following concentration *in vacuo* is dissolved in ethyl acetate and precipitated by addition to cold *t*-butylmethyl ether. In general, the precipitated H₂N-AA-PCDAs are pure enough for analytical purposes.

By using the solid-phase synthetic method described above, several representative amino acids and a tripeptide were coupled to 10,12-pentacosadiynoic acid (PCDA) (Figure 2). Amino acids with neutral (Gly), hydrophobic (Ala and Leu), aromatic (Phe) as well as hydrophilic (Asp and Lys) side chains can be incorporated with the diacetylene

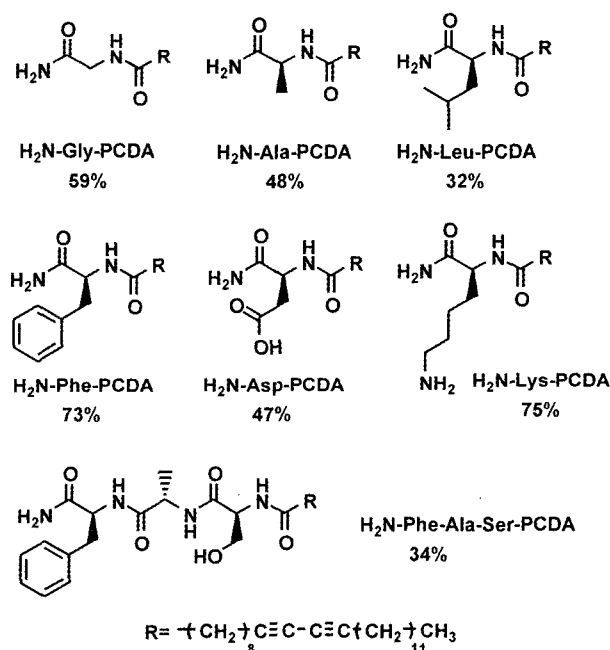


Figure 2. The structures of amino acid-derived diacetylenes synthesized by using the solid state procedure. Yield for each compound is indicated.

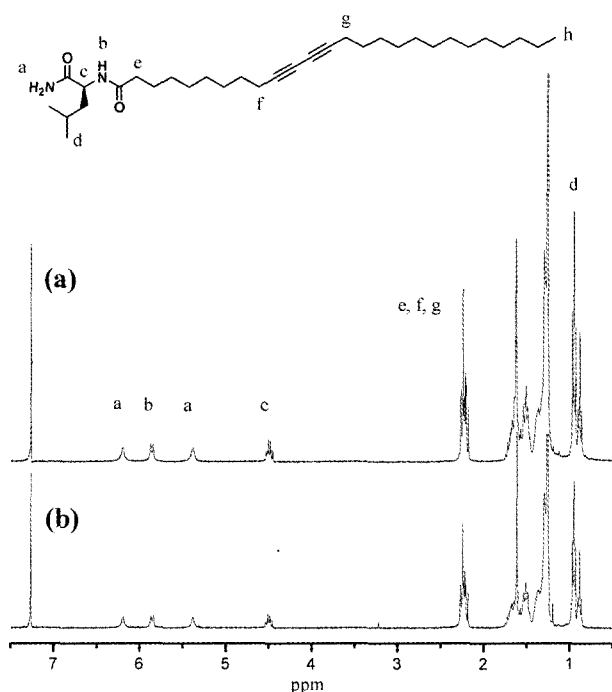


Figure 3. $^1\text{H-NMR}$ (300 MHz, CDCl_3) of $\text{NH}_2\text{-Leu-PCDA}$ prepared on support (a) and in solution (b).

monomer without difficulty. A tripeptide-modified diacetylene, $\text{H}_2\text{N-Phe-Ala-Ser-PCDA}$, was also prepared in moderate yield by using the solid-phase synthesis approach. In this case, construction of the Phe-Ala-Ser sequence via standard solid-phase peptide synthesis procedures is followed by coupling with PCDA before cleavage from the support.

The purity of the lipid monomers prepared from the solid-phase method was compared with that obtained by the solution phase method (Figure 3). As can be seen from the inspection of ^1H NMR spectra, the purity of the leucine-modified diacetylene lipid, prepared on support, is almost identical with that obtained from leucinamide and PCDA through a solution-phase synthesis.

The next phase of the current investigation was aimed at elucidating the feasibility of photopolymerization reactions of the amino acid-modified diacetylene monomers. For this purpose, a thin film of the amino acid-derived diacetylene monomer was formed by in a test tube by evaporation of a chloroform solution with a stream of N_2 . [4-(2-Hydroxyethyl)-1-piperazineethane-sulfonic acid] (HEPES) buffer (pH 7.8) was added to yield a total lipid concentration of 1 mM. The sample was heated at 80°C for 15 min and then sonicated for 15 min. The resulting solution was filtered through a $0.8\ \mu\text{m}$ filter and the filtrate was cooled at 4°C for 12 h. Photopolymerization was performed at room temperature by irradiation with 254 nm light ($1\ \text{mW}/\text{cm}^2$) for 5–60 s. As can be seen by viewing the data in Table I, irradiation of the amino acid-modified diacetylenes in aqueous solution gives rise to pink to blue colored polymers. In general, poly-

Table I. Properties of the Polydiacetylene Liposomes Prepared from $\text{H}_2\text{N-AA-PCDA}$ in HEPES Buffer

Entry	AA	Polymn.	λ_{max} (nm)	Color
1	Gly	Y	640	Blue
2	Ala	Y	580	Purple
3	Leu	Y	536	Pink-Red
4	Phe	Y	533	Pink
5	Asp	Y	519 (630) ^a	Pink-Red
6	Lys	Y	620	Blue
7	Phe-Ala-Ser	Y	629	Blue

^aShoulder.

merized liposomes obtained with diacetylene monomers, having hydrophobic side chains (Ala, Leu, Phe), have lower absorption wavelength maxima than those prepared from diacetylenes with hydrophilic head groups (Asp, Lys, Gly). Interestingly, the polymerized liposome solution derived from the relatively hydrophobic side chain rich tripeptide-modified diacetylene, $\text{H}_2\text{N-Phe-Ala-Ser-PCDA}$, was found to have a wavelength maximum of 629 nm. The detailed studies regarding the effect of the amino acids on the colors of the resulting liposomes are under current investigation.

In summary, we have prepared amino acid-derived diacetylene lipid monomers using a new solid-phase synthetic protocol. This method allows rapid and efficient preparation of functionalized diacetylene monomers. We believe that, by utilizing the strategies described above, a variety of diacetylene monomers having biologically important ligand head groups can be prepared readily. Since the solid-phase synthesis method can be applied to combinatorial chemistry, the technique should enable ready generation of functional diacetylene monomer libraries. Thus, the observations made in this effort should serve as an important foundation for the development of polydiacetylene-based chemosensors.

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