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## Solid-Phase Synthesis of Peptide Aminoalkylamides Using an Allyl Linker

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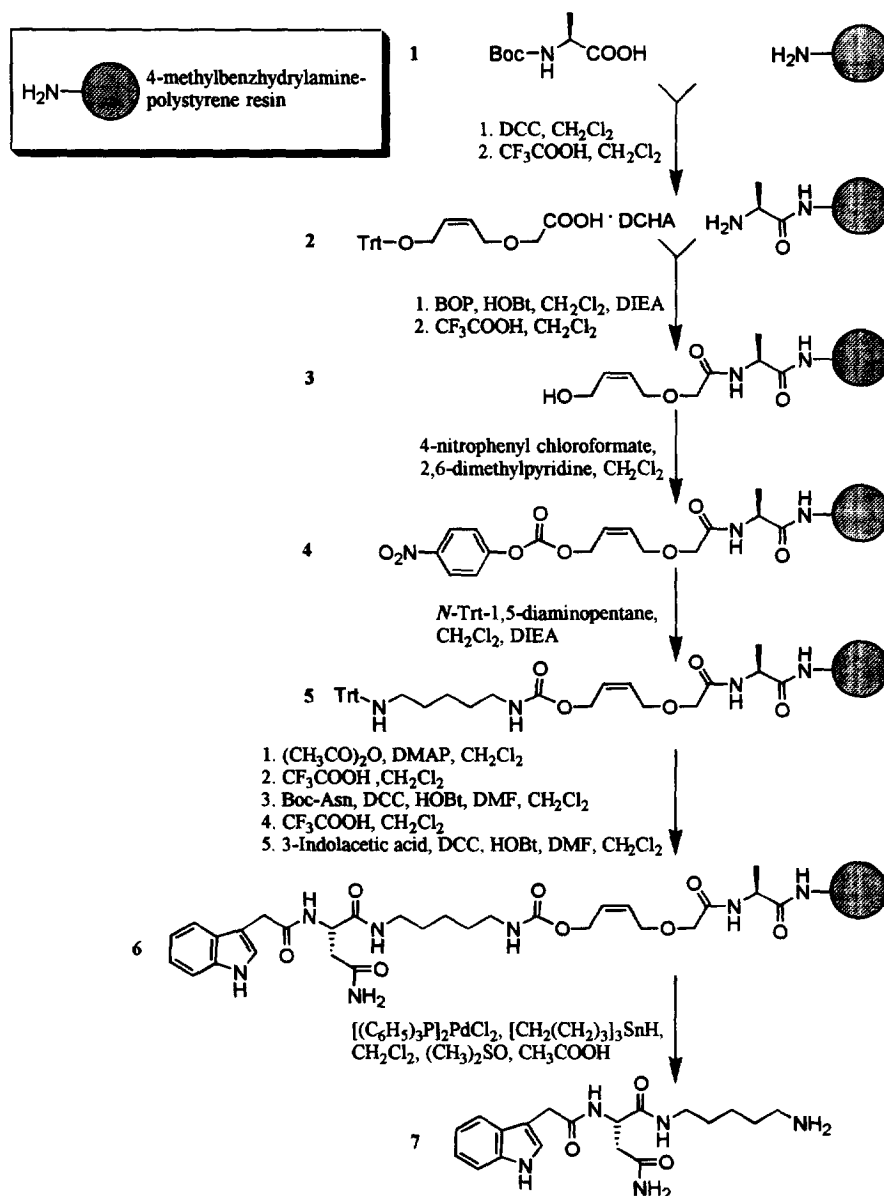
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**Abstract:** The synthesis of an allylic linker is presented to which primary and secondary amino groups can be anchored on solid phase. By acylating MBHA resin with allyl linker HO-CH<sub>2</sub>-CH=CH-CH<sub>2</sub>-O-CH<sub>2</sub>-COOH followed by treatment with 4-nitrophenyl chloroformate a mixed carbonate linker is obtained to which amino groups can be attached. The allyl carbamate linkage is stable to both bases and anhydrous acids and can be cleaved by palladium-catalysed allyl transfer under mild reaction conditions. Copyright © 1996 Elsevier Science Ltd

Solid-phase synthesis has traditionally been focused on the synthesis of peptides and oligonucleotides. For the generation of such compounds an arsenal of derivatised resins with different stability for the anchoring of carboxylic acids, hydroxyl groups and amides has been developed. More recently, there has been a rapidly growing interest in methods for the synthesis of small organic molecules on solid phase<sup>2</sup>. This requires the development of resins for the anchoring of new sets of functional groups that possess different chemical stability in order to apply various combinations of reaction conditions. For these reasons we developed an acid and base stable linker for the anchoring of primary and secondary amino groups which can be useful in combinatorial organic chemistry. Trifluoroacetic acid cleavable linkers for the anchoring of amino groups to solid phase have been described<sup>3</sup>.

Kunz<sup>4a</sup> has introduced allyl based anchoring strategy for the solid-phase synthesis of peptide acids. Allyl esters and carbamates can be cleaved by Pd complex e.g. [(C<sub>6</sub>H<sub>5</sub>)<sub>3</sub>P]<sub>2</sub>PdCl<sub>2</sub> or [(C<sub>6</sub>H<sub>5</sub>)<sub>3</sub>P]<sub>4</sub>Pd catalysed transfer of allyl moiety to a nucleophile (e.g. dimedone, morpholine, tributyl tin hydride)<sup>5</sup>. This strategy has been used for the synthesis of protected peptide segments and glycopeptides<sup>4,6</sup> and is compatible with Fmoc/tBu orthogonal protection scheme<sup>7</sup>.

The Pseudoargiopinine III<sup>8</sup> is a pharmacologically active venom component from the spider *Argiope lobata* and was chosen as a model compound for evaluation of the resin. The total route of the synthesis is outlined in Figure 1. Boc-Ala 1 was coupled to the 4-methylbenzhydrylamine resin and serves as a spacer arm between the linker and resin and as an internal standard. The allyl linker 2 has been described by Guibé et al<sup>9</sup> for the synthesis of peptide acids. The dicyclohexylamine salt of the linker was coupled to the alanine residue with the BOP reagent and after the removal of trityl protection the hydroxyl groups were reacted with 4-nitrophenyl chloroformate<sup>10</sup>. *N*-Trt-1,5-diaminopentane was added to the obtained mixed carbonate resin 4 resulting in allyl carbamate resin 5<sup>11</sup>. The remaining hydroxyl groups were capped by acetic acid anhydride in the presence of DMAP. Boc-Asn and 3-indolacetic acid were coupled by DCC/HOBt activation. Pseudoargiopinine III attached to allyl carbamate linker 6 was cleaved by [(C<sub>6</sub>H<sub>5</sub>)<sub>3</sub>P]<sub>2</sub>PdCl<sub>2</sub> catalysed allyl

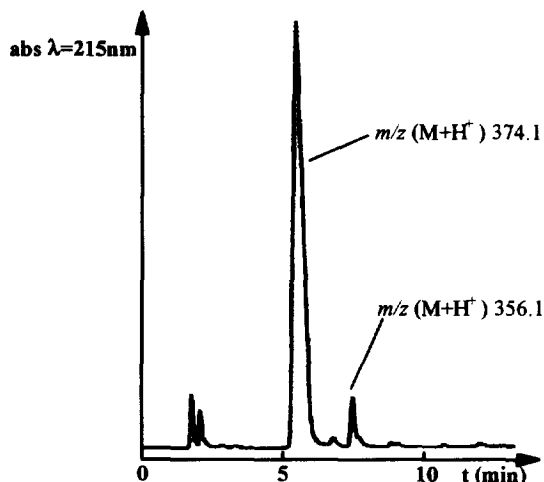


**Scheme 1.** The total route of the solid-phase synthesis of Pseudoargiopinine III (7)

transfer using  $[\text{CH}_3(\text{CH}_2)_3\text{SnH}]$  as nucleophile<sup>12</sup>. It has been shown in previous studies that the cleavage of peptides from allyl ester linker can in some cases proceed with low yields<sup>5</sup>. To evaluate the overall substitution level and cleavage yield for the allyl carbamate linker the samples of the resin before and after the cleavage of Pseudoargiopinine III were analysed by amino acid analysis. The average cleavage yield obtained was 92%<sup>13</sup>.

The HPLC elution profile of the crude Pseudoargiopinine III 7 is shown in Figure 1. The molecular mass of the product was determined by plasma desorption mass spectrometry and was within the error limit of

the instrument ( $\pm 0.1\%$ ). The later eluting peak with 18 daltons lower mass could correspond to a sequence related side reaction where the Asn residue is transformed into aspartimide<sup>14</sup> or an activation related side reaction where the Asn residue is transformed to  $\beta$ -cyanoalanine<sup>15</sup>.



**Figure 1.** The HPLC elution profile of crude Pseudoargiopinine III synthesised on solid phase.

A major advantage of the resin is that the allyl carbamate linkage between resin and peptide is stable both to medium strong bases such as piperidine and shows even good stability against super-strong acids such as liquid hydrogen fluoride. This was investigated by synthesising the model peptide Val-Gly-Phe-(1,5-diaminopentane) on the same resin. No cleavage of the peptide Val-Gly-Phe-(1,5-diaminopentane) was detected by 24 h treatment with 20% piperidine in DMF or 12 h treatment with 50%  $\text{CF}_3\text{COOH}$  in  $\text{CH}_2\text{Cl}_2$ . 45 min treatment with HF at  $0^\circ\text{C}$  caused 10 % cleavage of the carbamate linkage between the peptide Val-Gly-Phe-(1-5-diaminopentane) and the linker<sup>16</sup>. This stability allows a wide range of synthetic procedures to be carried out without cleaving the peptide from the resin. Furthermore, the cleavage conditions are compatible with both the Fmoc and Boc groups and compounds can be prepared which are fully protected on all functional groups except the terminal amino group. This approach can prove to be of value in the solid-phase synthesis of naturally occurring polyamines and also in combinatorial organic chemistry for preparation of libraries of small organic molecules carrying acid sensitive groups.

#### ACKNOWLEDGEMENT

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#### REFERENCES AND NOTES

- Abbreviations used in this paper: Boc, *tert*-butoxycarbonyl; BOP, benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphoniumhexafluorophosphate; DCC, dicyclohexylcarbodiimide; DCHA, dicyclohexylamine; DIEA, diisopropylethylamine; DMAP, 4,4-dimethylaminopyridine; DMF, *N,N*-dimethylformamide; Fmoc, 9-Fluorenylmethoxycarbonyl; HOBt, 1-hydroxybenzotriazole; *t*Bu, *tert*-butyl; Trt, triphenylmethyl.
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10. 60 mg (0.3 mmol) of 4-nitrophenyl chloroformate dissolved in 1.6 ml of a mixture of CH<sub>2</sub>Cl<sub>2</sub>/2,6-dimethylpyridine 75/25 v/v were added to 100 mg of resin (~ 0.1 mmol hydroxyl groups). The suspension was shaken on ice for 5 h and the resin washed with 2,6-dimethylpyridine and CH<sub>2</sub>Cl<sub>2</sub>. The substitution level obtained was 61%. It was determined by collecting the washing solvents after the coupling of *N*-Trt-1,5-diaminopentane, diluting the sample in DMF containing 3% DIEA and determining the concentration of 4-nitrophenol spectrophotometrically at 435 nm.
11. 0.5 mmol (170 mg) of *N*-Trt-1,5-diaminopentane and 0.1 mmol (20 µl) diisopropylethylamine was added to 100 mg of resin. The suspension was shaken for 6 h and the resin washed with (CH<sub>3</sub>)<sub>2</sub>SO and CH<sub>2</sub>Cl<sub>2</sub>.
12. The cleavage reaction was performed essentially as described in Loffet, A.; Zhang, H., X. *Int. J. Peptide Protein Res.* **1993**, *42*, 346-351 for allyl-based protecting groups. 125 mg (0.1 mmol) of resin was suspended in 1.5 ml mixture of CH<sub>2</sub>Cl<sub>2</sub>/(CH<sub>3</sub>)<sub>2</sub>SO (50/50 v/v) and 5 µmol (3.5 mg) of [(C<sub>6</sub>H<sub>5</sub>)<sub>3</sub>P]<sub>2</sub>PdCl<sub>2</sub> and 0.3 mmol (17 µl) of CH<sub>3</sub>COOH were added. The suspension was agitated by N<sub>2</sub> stream and 0.2 mmol (55 µl) of [CH<sub>3</sub>(CH<sub>2</sub>)<sub>3</sub>]<sub>3</sub>SnH was added in 3 portions over 20 min after that the resin was mixed additional 10 min. The solvent was collected, the resin was washed twice with 2 ml 5% CH<sub>3</sub>COOH in DMF and the cleavage process was repeated. Pooled fractions were diluted with 50 ml 5% CH<sub>3</sub>COOH solution in water, extracted twice with 5 ml CH<sub>2</sub>Cl<sub>2</sub> and the water phase was freeze-dried leaving crude Pseudoargiopinine III (7).  
7 Plasma Desorption Mass Spectrometry *m/z* 374.1 (M+H<sup>+</sup>), calculated for C<sub>19</sub>H<sub>28</sub>N<sub>5</sub>O<sub>3</sub> 374.2. <sup>1</sup>H NMR (D<sub>2</sub>O, 500 MHz) Indolacetic acid residue: δ 2, 7.021, s; 4, 7.295 dt, *J*=7.9, 2x0.8; 5, 6.878, ddd, *J*=7.9, 7.1, 0.8; 6, 6.970, ddt, *J*=8.2, 7.1, 1.0; 7, 7.226, dt, *J*=8.2, 2x0.8; C-CH<sub>2</sub>-CO, 3.482, s. Asparagine residue: δ CH, 4.314, dd, *J*=5.9, 7.9; CH<sub>2</sub>, 2.406, dd, *J*=5.9, 15.5 & 2.354, dd, *J*=7.9, 15.5. Diaminopentane moiety (numbered from NH end): δ C-1, 2.518, t, *J*=7.3; C-2, 1.216, p, *J*=7.4; C-3, 0.856, p, *J*=7.3; C-4, 1.037, p, *J*=7.2; C-5, 2.794, t, *J*=6.9. <sup>13</sup>C NMR (D<sub>2</sub>O, 125 MHz) Indolacetic acid residue: δ 2, 125.7; 3, 108.1; 3a, 127.3; 4, 119.0; 5, 120.3; 6, 122.9; 7, 112.7; 7a, 137.0; C-CH<sub>2</sub>-CO, 33.0; CO, 175.2. Asparagine residue: δ CH, 51.5; CH<sub>2</sub>, 36.8; CO(NH<sub>2</sub>), 172.9; CO(NH), 175.8. Diaminopentane moiety (numbered from NH end): δ C-1, 40.0; C-2, 26.9; C-3, 23.3; C-4, 28.3; C-5, 39.6.
13. Approximately 1 mg of resin was mixed with 100 µl of propionic acid/HCl mixture (50/50 v/v) and samples were heated 20 h at 105°C. Amino acid analysis was performed by Fmoc method as described in: *Handbook of Amino Acid Analysis. Theory & Laboratory Techniques*, Pharmacia LKB Biotechnology AB, 1988. The overall substitution level and cleavage yield was determined by comparing the relative ratio of Asp/Ala before and after the cleavage. The overall substitution level obtained was 52%.
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16. The data for stability towards piperidine and CF<sub>3</sub>COOH was obtained by amino acid analysis of resin samples before and after the treatment of the peptide-resin. The relative ratios between the Val, Gly, & Phe/Ala were determined. The value for HF stability was obtained comparing the ratios of peptides Val-Gly-Phe-(1,5-diaminopentane)-linker-Ala-NH<sub>2</sub> and Val-Gly-Phe-(1,5-diaminopentane) in cleavage products analysed by HPLC at 215 nm.