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Synthesis of cyclic peptides through hydroxyl side-chain anchoring

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Abstract—A general method was developed for the synthesis of serine or threonine containing cyclic peptides utilizing the β-hydroxyl side-chain of these residues as an anchor point to Wang resin. The peptide chain was assembled by conventional Fmoc/tBu solid-phase chemistry followed by palladium catalyzed exposure of the allyl protected C-terminus and on-resin cyclization. The cyclic heptapeptide stylostatin 1 was prepared to demonstrate the utility of this technique.

The on-resin cyclization of peptides is a particularly advantageous approach in that it avoids the isolation and purification of the linear intermediate, minimizes dimerization side-reactions and results in increased overall yield of the final product. Practical application of this method to homodetic 'head-to-tail' cyclic peptides, however, requires a specialized solid-phase strategy, including orthogonal protection for the C-terminus as well as solid support linkage to either an amino acid side-chain or peptide backbone.[1,2] To date, side-chain anchoring strategies have been devised for a number of amino acid side-chain functionalities. Merrifield and co-workers first reported the synthesis of a hexapeptide through dinitrophenyl linkage to a histidine imidazole.[3] Subsequent application of this concept utilized the side-chains of Asp/Asn and Glu/Gln in conjunction with temporary allyl protection for the C-terminal α-carboxyl group.[4,5] This strategy was further extended to other side-chains using active carbonate linker for hydroxyl side-chains of Ser, Thr,[6] and Lys,[7] as well as the recently reported chroman linkage for Arg.[8] Additionally, the phenol group of Tyr has been successfully linked to solid support through Mitsunobu chemistry.[9]

We recently reported a procedure to link amino acid alcohols to activated Wang resin as an initial step in the synthesis of C-terminal peptide alcohols.[10] We reasoned that the β-hydroxyl group of serine and threonine would function equally well as substrates in the BF_3 etherate catalyzed loading of trichloroacetimidate activated Wang resin. Indeed, Fmoc-Ser-OAllyl[11] was loaded with a substitution level of 0.44 mmol/g, and similarly Fmoc-Thr-OAllyl achieved a loading of 0.35 mmol/g using previously reported conditions.[10,12] Briefly, commercially available trichloroacetimidate Wang resin (1.0 mmol, 0.77 mmol/g) was swollen in DCM for 30 min and then washed several times with dry THF. Fmoc-Ser-OAllyl (4.0 mmol) was dissolved in dry THF and transferred to the resin bed. Following the addition of BF_3 etherate (1.0 mmol) via syringe, the mixture was gently agitated for 1 h at room temperature. Methanol (2.0 mL) was then added and the reaction was allowed to proceed for another 5 min. The substitution level was determined by the method of Meienhofer.[13]

To demonstrate the utility of this technique, we carried out the synthesis of stylostatin 1, a cycloheptapeptide originally isolated from the South Pacific Ocean sponge[14] and synthesized previously by several groups.[15] The side-chain immobilized Fmoc-Ser-(Wang resin)-OAllyl prepared above was used in the Fmoc/tBu-based synthesis. The primary sequence was assembled using an ABI 433A synthesizer utilizing a single coupling, DCC/HOBt activation protocol, a four-fold excess of each amino acid residue and a conventional protecting group scheme. The removal of allyl protection was carried out according to the method by Albericio and co-workers.[16] Two 30 min treatments at room temperature with a catalytic amount of $Pd(PPh_3)_4$ (0.1 equiv) and an excess of allyl acceptor phenylsilane (24 equiv) in DCM resulted

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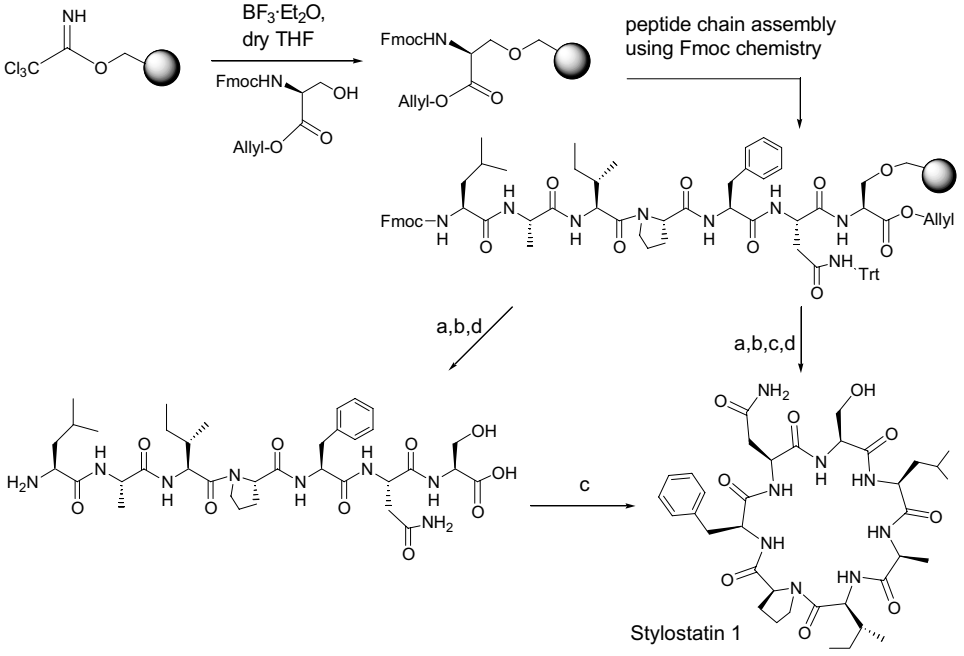

Scheme 1. Synthesis of stylostatin 1 (all reactions were carried out at room temperature unless otherwise specified): (a) 20% piperidine in DMF; (b) $Pd(Ph_3P)_4/PhSiH_3$ in DCM, 30 min twice; (c) PyBOP/HOBt/DIEA in DMF; (d) TFA.

in clean removal of the allyl protecting group. The resin-bound peptide was then used in the on-resin cyclization, while a portion was subjected to acidolytic cleavage to obtain the linear precursor (Scheme 1).

The crude linear peptide showed more than 70% purity on reverse-phase HPLC (Fig. 1A) and the desired mass (theoretical mass 760.9; found MH^+ 761.6, $(M+Na)^+$ 783.4). This further demonstrated that the loading of Fmoc-Ser-OAllyl was successful and the peptide chain assembly on this preloaded resin was efficient. Cyclization of the crude linear peptide in solution (4 equiv PyBOP/HOBt and 10 equiv of DIEA) at highly diluted conditions[17] was completed within 4 h at room temperature and afforded the reference compound stylostatin 1. The product was purified to homogeneity on reverse phase HPLC (Fig. 1B) and showed the expected mass (theoretical mass 742.8; mass observed MH^+ 743.7, $(M+Na)^+$ 765.8) with a yield of 59.9%.

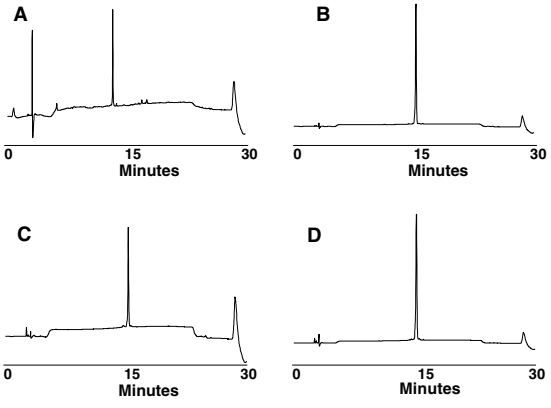

Figure 1. HPLC traces of specific compounds: (A) Crude linear stylostatin 1; (B) purified stylostatin 1 cyclized in solution; (C) purified stylostatin 1 cyclized on-resin; (D) mixture (1:1) of stylostatin 1 from cyclizations on-resin and in solution. HPLC conditions: Solvent A: water with 0.1% TFA; solvent B: acetonitrile with 0.1% TFA; C18 Vydac reverse-phase, 4.6×250 mm, 5 μM, 300 Å pore size; gradient: linear from 0% B to 65% B over 20 min, then 90% B for 5 min, and 0% B for another 5 min. Wavelength used for detection: 214 nm.

On-resin cyclization was accomplished with 4 equiv of PyBOP/HOBt and 10 equiv of DIEA in DMF overnight at room temperature. The cyclic product was then deprotected and cleaved from the resin support using a mixture of TFA/Tis/H_2O/thioanisole (92.5/2.5/2.5/2.5, v/v) for 2 h at room temperature. The final product stylostatin 1 was purified to homogeneity (Fig. 1C) and showed the expected mass (theoretical mass 742.8; observed MH^+ 743.7, $(M+Na)^+$ 765.4). Based on the initial loading, overall yield of the on-resin stylostatin synthesis was calculated at 13.9%.

A mixture (1:1) of stylostatin 1 obtained by on-resin cyclization and by the solution method was homogenous and showed a single peak on reverse phase HPLC (Fig. 1D), which suggests that the two methods afford the same product.

In summary, we have demonstrated that the β-hydroxyl function of serine and threonine could be conveniently used as a tether point for solid-phase linkage. With appropriate temporary, orthogonal protection for the C-terminal α-carboxyl group this resin construct can then function in an on-resin protocol for cyclic peptide synthesis. This technique effectively expands the scope of on-resin Fmoc/tBu-based cyclic peptide synthesis to include serine and threonine containing peptides as synthetic targets.

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17. Linear stylostatin 1 (15.3 mg, 0.02 mmol) in 3 mL of dry DMF was added continuously to a solution of PyBOP/HOBt/DIEA in DMF (3 mL)/DCM (7 mL) over a period of 2 h through a syringe pump. The reaction was allowed to proceed for another 2 h at RT and LCMS confirmed that there was no detectable linear starting material. Solvent removal was followed by RP-HPLC purification to give the final product stylostatin 1 (8.9 mg, a yield of 59.9%).