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PEPTIDE CYCLIZATION ON TFA LABILE RESIN USING THE TRIMETHYLSILYL (TMSE) ESTER AS AN ORTHOGONAL PROTECTING GROUP

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Abstract: *The trimethylsilylethyl ester group was used as an orthogonal protecting group to perform an on-resin cyclization of a peptide prepared by conventional Fmoc protection strategy. Fmoc-Asp(OTMSE)OH was prepared, incorporated into a peptide, deprotected in the presence of fluoride, cyclized in the presence of BOP, and cleaved from the resin to afford directly a head-to-sidechain cyclic peptide*

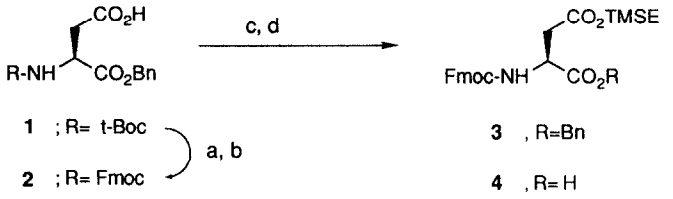
Our group and others have been involved in generating and screening chemical diversity by a variety of peptide synthesis methodologies to accelerate drug discovery. We have developed automated methods to produce multiple peptides and equimolar mixtures of solution-phase peptides.[1] Related approaches have been the multi-pin method,[2] the tea-bag method,[3] and the use of cellulose discs.[4] Solid-phase bound peptide methods include light-directed spatially addressable parallel chemical synthesis[5] and resin-bound synthetic libraries.[6]

Although addition of structural constraints to linear peptide sequences[7,8] by cyclization has been reported as an optimization technique,[7,9] this methodology has not been applied generally to the synthesis of diverse chemical libraries. These libraries could be generated using our recently reported automated equimolar peptide mixture synthesizer, which utilizes Fmoc chemistry and a TFA cleavage station. In this format, amide cyclizations while resin-bound on TFA labile resin using the appropriate orthogonal deprotection/cyclization scheme would be most efficient for producing large numbers of cyclic peptides. Furthermore, amide cyclizations benefit from being much more solution stable and less reactive than disulfide cyclic peptides.

Most current methodologies for resin-bound amide cyclization utilize t-Boc chemistry with base-labile Fmoc and OFm[10] groups for amino and carboxylate, respectively, or Fmoc chemistry with TFA-labile t-Boc and Ot-Bu. Either system can be orthogonally deprotected and cyclized on the resin, however, both methods rely on corrosive HF for cleavage from the resin. Recently, two methods, which utilize TFA-labile resin, namely allyl based protection[11a] and super acid-labile dimethoxybenzyl (Dmb) ester,[12] have been reported. However the former system involves the use of air sensitive $Pd(Ph_3P)_4$ and non-standard TFA-labile Pal-PEG-PS[11b] resin to increase deprotection efficiency. The Dmb method requires balancing cleavage with 1% TFA with avoiding general sidechain deprotection and cleavage from the resin. In contrast, trimethylsilylethyl (TMSE) esters are cleaved quickly and efficiently by solution-stable fluoride. The first example of the application of this functionality as an orthogonal protecting group to make cyclic peptides is reported herein.

The trimethylsilylethyl ester as a carboxylate protecting group was originally reported by Sieber,[13] as an alpha-carboxylate protecting group for solution synthesis, but not as orthogonal sidechain protection for use in solid phase chemistry. Therefore, Fmoc-Asp(OTMSE)OH (**4**) was prepared in four steps from commercially

Scheme 1

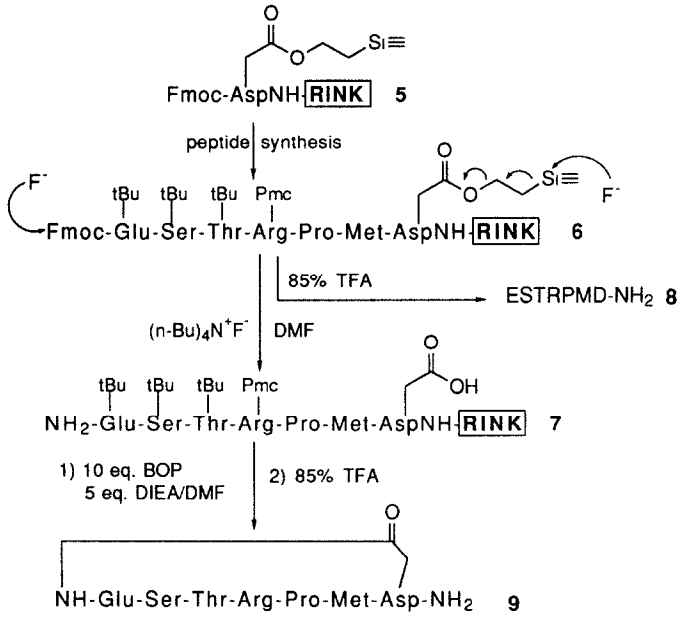


a: 50% TFA/CH_2Cl_2; b: FmocOSu, Na_2CO_3(aq), dioxane; c: 1.1 eq trimethylsilylethanol, 1.1 eq DCC, 1M 50% pyridine/CH_2Cl_2; d: H_2, 10% Pd/C, EtOAc.

available tBoc-AspOBn (**1**) by deprotection with 50%TFA/CH_2Cl_2 and standard introduction of the Fmoc group[15] (Scheme 1). Esterification[13] with trimethylsilylethanol[14] was smoothly accomplished in 50% pyridine/CH_2Cl_2 by activation with DCC. Catalytic hydrogenolysis removes the benzyl ester to afford the desired TMSE protected Fmoc-Asp residue **4**[16] after chromatographic purification, in overall yield of 63%.

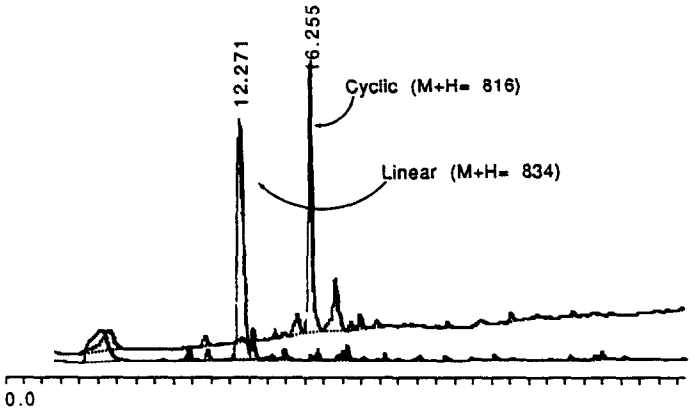
Fmoc-Asp(OTMSE)OH was loaded onto Rink amine resin[14] using standard DCC/HOBt peptide coupling conditions, followed by acetic anhydride to block any free amines (Scheme 2). The sequence ESTRPM was elaborated onto the loaded resin **5**

Scheme 2:



using an ABI peptide synthesizer,[14] giving resin **6**. Treatment with 1.0 M tetrabutlyammonium fluoride in DMF for 20 min. deprotected the TMSE and Fmoc[17] functionalities simultaneously, giving the resin-bound peptide **7**. Alternatively, resin **6** was fully deprotected, including TMSE[13], and cleaved from the resin with reagent K to give the linear peptide **8**.[18,19] Cyclization of **7** using BOP/DIEA in DMF was >99% complete in 4 hr as judged by quantitative ninhydrin analysis.[20] Side-chain deprotection and cleavage, as before, afforded cyclic peptide **9**.[19] HPLC analysis[21] of crude cyclic **9** showed no linear peptide **8** present (Figure 1). This confirmed that deprotection was complete and that the cyclization was efficient.

Figure 1: HPLC trace of linear **8** and cyclic **9**.



Application of the TMSE group to head-to-tail cyclizations of a set of peptides varying in length and in sequence will be reported in due course. In addition, application of the corresponding amine protection, the trimethylsilyloxycarbonyl (TEOC) group,[22] allows sidechain-to-sidechain cyclizations on TFA-labile resin.

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14. Abbreviations: ACN, acetonitrile; tBoc, tert-butoxycarbonyl; BOP, benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate, DCC, N, N'-dicyclohexylcarbodiimide; DIEA, diisopropylethylamine, DMF, N,N-dimethyl-formamide; Fmoc, 9-fluorenylmethylcarbonyl; OFm, 9-fluorenylmethylester; HOBt, 1-hydroxybenzotriazole; ABI, Applied Biosystems, Inc.; 95% TFA, 95% trifluoroacetic acid/5% water. Amino acids were purchased from Bachem, chemicals from Aldrich, and Rink resin from Nova Biochem.
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