Organic LETTERS

Letter

pubs.acs.org/OrgLett

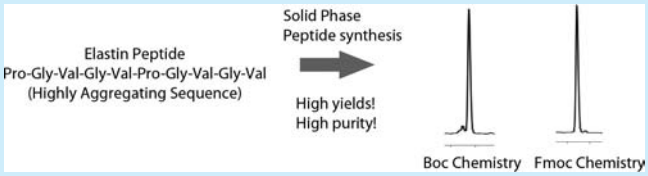
Enhanced Solvation of Peptides Attached to "Solid-Phase" Resins: Straightforward Syntheses of the Elastin Sequence Pro-Gly-Val-Gly-Val-Pro-Gly-Val-Gly-Val

Bobo Dang,[†] Balamurugan Dhayalan,[†] and Stephen B. H. Kent*

Department of Chemistry, University of Chicago, Chicago, Illinois 60637, United States

Supporting Information

ABSTRACT: The solubility-enhancing power of covalent attachment to solvent-swollen cross-linked resin supports was illustrated by syntheses of the highly aggregating elastin-derived 10-residue peptide sequence Pro-Gly-Val-Gly-Val-Pro-Gly-Val-Gly-Val using standard protocols for both Boc and Fmoc chemistry SPPS.



Solvation of a protected peptide chain is *enhanced* by its covalent attachment to the solvent-swollen "solid" resin support used in solid phase peptide synthesis (SPPS).[1] This physicochemical phenomenon is the most fundamental aspect of SPPS and underlies its near-universal applicability. The solvent-swollen peptide-resin behaves as a solid in macroscopic terms and can thus be collected and washed by conventional filtration, yet at the molecular level the branched interpenetrating polymer network that is the cross-linked resin is highly solvated and the covalently attached protected peptide is effectively in solution.[2] Counterintuitively, it is the cross-linked nature of the resin support that maximizes the solvation of the covalently attached peptide chains. Phase separation is minimized because of the cross-links, even relative to peptides attached to soluble linear polymers: in SPPS the incipient oligomeric state of aggregation-prone peptides is disfavored by the unavoidable incorporation, because of the cross-links, of resin polymer chains into nascent peptide aggregates. The unfavorable energetics of mixing dissimilar less-polar resin polymer chains with more-polar peptide chains disfavors the aggregated state and thus enhances the free energy of solvation of the growing peptide chains that are covalently attached to the swollen resin matrix.[1] For that reason, growing peptide chains in SPPS are more highly solvated than the same peptide chains in free solution and are more available for reaction.

This fundamental consequence of covalent attachment of the protected peptide chain to the resin support is the key to the broad utility of SPPS, but is under-appreciated and often ignored. Here we report peptide syntheses that dramatically illustrate this enhanced solvation effect in SPPS.

A recent paper in this journal described the solution synthesis of what was described as a highly aggregating elastin-derived 10-residue peptide sequence: Pro-Gly-Val-Gly-Val-Pro-Gly-Val-Gly-Val.[3] In that work, the use of high pressure was needed to overcome the excessive/disabling viscosity of solutions of the protected peptide intermediates in the synthesis that resulted from their intermolecular aggregation in organic solvents, and to thus facilitate the synthetic reactions.[3] We set out to explore the utility of standard SPPS for the synthesis of this well-documented example of a highly aggregation-prone peptide sequence. Syntheses were undertaken using both Boc and Fmoc chemistry SPPS.

Manual Boc chemistry SPPS using "in situ neutralization" protocols[4] was carried out on a 0.2 mmol scale using 1.0 mmol/g Val-OCH_2phenylCH_2CO-$NHCH_2$-copoly(styrene −1% DVB) resin.[5] Standard HBTU-mediated single couplings were performed for 12 min at room temperature. The final cleavage using anhydrous HF gave a 61% isolated yield of high purity crude product. Full details of the synthesis are given in the Supporting Information.

Manual Fmoc chemistry SPPS was carried out on a 0.1 mmol scale using 0.45 mmol/g Rink amide-linker ChemMatrix resin.[6] Standard HBTU/HOBT mediated single couplings were performed for 30 min at room temperature. The final cleavage of the peptide resin by TFA/TIPS/water (95:2.5:2.5) and subsequent precipitation with ice-cold diethyl ether gave a 62% isolated yield of the crude product. Full details of the synthesis are given in the Supporting Information.

LCMS analyses of the crude products from both Boc and Fmoc chemistries SPPS of the elastion sequence Pro-Gly-Val-Gly-Val-Pro-Gly-Val-Gly-Val are shown in Figure 1.

Synthesis of the elastin-derived peptide Pro-Gly-Val-Gly-Val-Pro-Gly-Val-Gly-Val was straightforward by SPPS, using either Boc chemistry or Fmoc chemistry standard protocols. Boc chemistry SPPS was performed manually using "in situ neutralization" protocols.[4] In Fmoc chemistry SPPS, removal of the Fmoc group at each stage of the synthesis was by treatment with 20% v/v 4-methylpiperidine in DMF. Only single couplings for standard times were used during the synthesis for both Boc- and Fmoc-chemistry SPPS protocols. Note that "capping", typically carried out by treatment with a high concentration of a powerful acylating agent such as acetic

Received: June 3, 2015
Published: June 25, 2015

DOI: 10.1021/acs.orglett.5b01632
Org. Lett. 2015, 17, 3521−3523

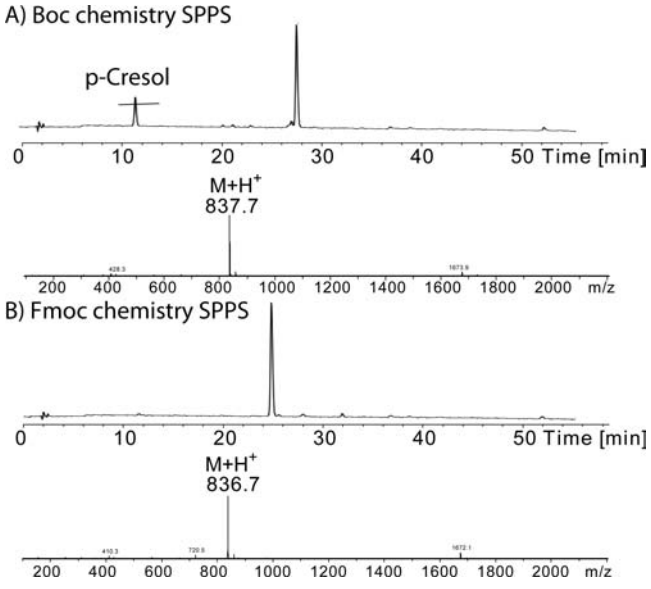


Figure 1. Crude products from standard SPPS of the elastin-derived peptide Pro-Gly-Val-Gly-Val-Pro-Gly-Val-Gly-Val. (A) LCMS analysis of peptide·COOH from Boc chemistry SPPS using $-OCH_2Pam-$ linker S-DVB resin. MS data acquired across the entire main UV peak. Mass calcd 836.5 Da (monoisotopic), obsd 836.7 ± 0.2 Da. (B) LCMS analysis of peptide·$CONH_2$ from Fmoc chemistry SPPS using Rink amide linker ChemMatrix resin. MS data acquired across the entire main UV peak. Mass calcd 835.5 Da (monoisotopic), obsd 835.7 ± 0.2 Da.

anhydride in an attempt to block unreacted peptide chains,[7] was not used in either synthesis.

So-called "difficult sequences" in SPPS are aggregation-prone protected peptide intermediates that cause slow reactions even when the peptide is attached to the solvated resin support.[2b,8] In Boc chemistry SPPS, this residual tendency to aggregate is revealed as sequence-dependent slow/incomplete coupling reactions. Difficulties arising from aggregation-prone peptide sequences are exaggerated in Fmoc chemistry SPPS, where both slow couplings and slow N^{α}-Fmoc deprotections are frequently observed,[9] because of the absence of the highly solvating TFA treatments used at each amino acid in Boc chemistry SPPS. The increased problems with aggregation-prone sequences in Fmoc chemistry SPPS have necessitated the use of dramatic measures, such as microwave heating that has been introduced in recent years.[10] Such measures are not without their own shortcomings (e.g., increased racemization[10,11]). Because of the foregoing considerations, we also explored the use of Fmoc chemistry SPPS to make the target elastin peptide sequence. That crude product is shown in Figure 1B.

Both Boc and Fmoc chemistries using standard SPPS protocols gave crude Pro-Gly-Val-Gly-Val-Pro-Gly-Val-Gly-Val products of purity comparable to that reported for the solution synthesis of the same sequence, facilitated by the use of high pressure.[3] Only very minor amounts of deletion peptide byproducts were observed, despite the use of single couplings for a standard time at room temperature. Our yields were also comparable to that obtained for the synthesis in solution, despite the small scale of these exploratory SPPS syntheses. Relatively high loadings (i.e., millimole peptide per gram of resin) were used, especially for the Boc chemistry SPPS where ~500 mg of product were obtained per gram of S-DVB resin used.

These results are a striking refutation of statements in the reported solution synthesis of the elastin peptide Pro-Gly-Val-Gly-Val-Pro-Gly-Val-Gly-Val. There it was said that *"peptide aggregation can occur even on solid phase resin"*, and that *"intermolecular hydrophobic interactions are minimized in less polar organic solvents compared with the polar organic solvents (typically N,N-dimethylformamide or N-methylpyrrolidone) commonly used in solid phase peptide synthesis"*.[3] Our data would suggest that such statements are overly broad. While it is true that so-called "difficult sequences" observed in SPPS can arise from (residual) intermolecular H-bonded interaction of especially prone-to-aggregation peptide sequences,[8] such was clearly not the case for the current elastin peptide sequence Pro-Gly-Val-Gly-Val-Pro-Gly-Val-Gly-Val during its assembly by SPPS. Furthermore, the current work demonstrates that use of the polar organic solvent DMF is compatible with efficient synthesis of the elastin peptide, as would be expected for SPPS of a proline-containing, even if hydrophobic, peptide sequence.[12]

The results reported here for the SPPS of the elastin peptide Pro-Gly-Val-Gly-Val-Pro-Gly-Val-Gly-Val dramatically illustrate the solubility-enhancing power of covalent attachment to the solvent-swollen "solid" resin support. It is the solubility-enhancing aspect of SPPS that gives rise to its near-universal applicability for the synthesis of peptides regardless of sequence.[13] This fundamental aspect of solid phase synthesis that we have illustrated here is not widely appreciated, and has yet to be taken advantage of in nonpeptide polymer-supported organic synthesis. Facile synthetic transformations of normally insoluble synthetic intermediates by covalent attachment to appropriate swelling resin supports have applications beyond peptide synthesis and would enable transformations that will greatly expand organic synthesis reaction space.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and analytical data for the peptide syntheses. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01632.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: skent@uchicago.edu.

Author Contributions

†B. Dang and B. Dhayalan contributed equally.

Notes

The authors declare the following competing financial interest(s): Stephen Kent is a consultant for Bachem AG.

■ REFERENCES

(1) Sarin, V. K.; Kent, S. B. H.; Merrifield, R. B. *J. Am. Chem. Soc.* **1980**, *102*, 5463−5470.

(2) (a) Live, D.; Kent, S. B. H. *ACS Symp. Ser.* **1983**, *193*, 501−515. (b) Kent, S. B. H. *Annu. Rev. Biochem.* **1988**, *57*, 957−989. (c) Giralt, E.; Rizo, J.; Pedroso, E. *Tetrahedron* **1984**, *40*, 4141−4152. (d) Espinosa, J. F. *Curr. Top. Med. Chem.* **2011**, *11*, 74−92.

(3) Okada, Y.; Hosoya, S.; Suzuki, H.; Chiba, K. *Org. Lett.* **2014**, *16*, 6448−6451.

DOI: 10.1021/acs.orglett.5b01632
Org. Lett. 2015, 17, 3521−3523

(4) Schnölzer, M.; Alewood, P.; Alewood, D.; Kent, S. B. H. *Int. J. Pept. Res. Ther.* **2007**, *13*, 31−44.
(5) Mitchell, A. R.; Kent, S. B. H.; Engelhard, M.; Merrifield, R. B. *J. Org. Chem.* **1978**, *43*, 2845−2852.
(6) (a) Frutos, S.; Tulla-Pucha, J.; Albericio, F.; Giralt, E. *Int. J. Pept. Res. Ther.* **2007**, *13*, 221−227. (b) García-Ramos, Y.; Paradís-Bas, M.; Tulla-Puchea, J.; Albericio, F. *J. Pept. Sci.* **2010**, *16*, 675−678.
(7) Amblard, M.; Fehrentz, J.-A.; Martinez, J.; Subra, G. *Mol. Biotechnol.* **2006**, *33*, 239−254.
(8) (a) Warrass, R.; Wieruszeski, J.-M.; Boutillon, C.; Lippens, G. *J. Am. Chem. Soc.* **2000**, *122*, 1789−1795. (b) Wang, S.; Ishii, Y. *J. Am. Chem. Soc.* **2012**, *134*, 2848−2851.
(9) Abdel-Aal, A.-B. M.; Papageorgiou, G.; Quibell, M.; Offer, J. *Chem. Commun.* **2014**, *50*, 8316−8319.
(10) Pedersen, S. L.; Tofteng, A. P.; Malik, L.; Jensen, K. J. *Chem. Soc. Rev.* **2012**, *41*, 1826−1844.
(11) Palasek, S. A.; Cox, Z. J.; Collins, J. M. *J. Pept. Sci.* **2007**, *13*, 143−148.
(12) Johnson, E. C. B.; Kent, S. B. H. *J. Am. Chem. Soc.* **2006**, *128*, 7140−7141.
(13) (a) Vaino, A. R.; Janda, K. D. *J. Comb. Chem.* **2000**, *2*, 579−596. (b) Cherkupally, P.; Acosta, G. A.; Ramesh, S.; De la Torre, B. G.; Govender, T.; Kruger, H. G.; Albericio, F. *Amino Acids* **2014**, *46*, 1827−1838.

DOI: 10.1021/acs.orglett.5b01632
Org. Lett. 2015, 17, 3521−3523