Tetrahedron Letters, Vol.31, No.32, pp 4561-4564, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

IMPROVED COUPLING OF PROTECTED PEPTIDES ON THE KAISER OXIME RESIN USING BOP ACTIVATION

Joseph T. Jarrett and Peter T. Lansbury, Jr.*

Department of Chemistry, Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

Summary: Protected peptides and amino acids can be coupled to resin-bound amine in high yield with low levels of racemization using BOP activation. The synthesis of the 16 amino acid protected peptide VI derived from the ice nucleation protein is described and experimental procedures for these couplings are provided.

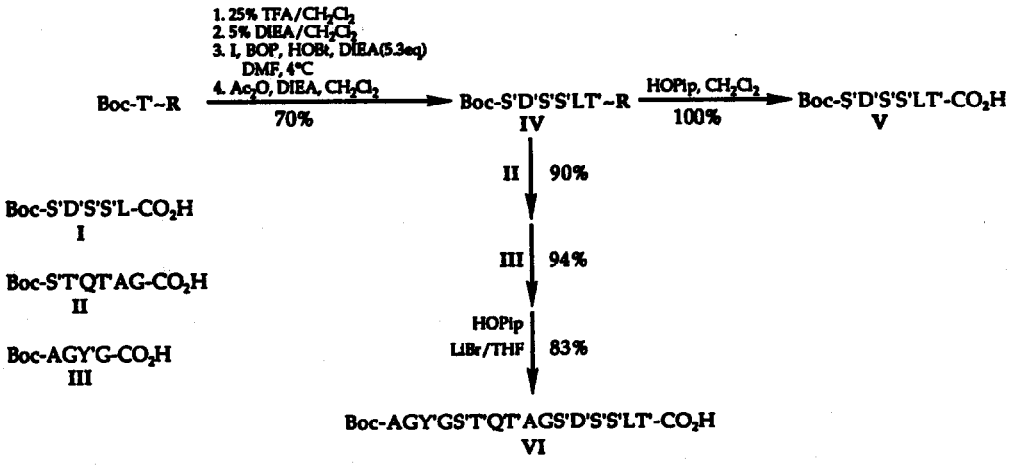
The Kaiser oxime resin[1] is useful for the preparation of pure protected peptides which can be coupled in solution or on the oxime resin.[1,2] The fragment coupling strategy eliminates the possibility of contamination of the target peptide with single residue deletion impurities which unavoidably accumulate in a stepwise synthesis.[1] This strategy is ideally suited for the synthesis of peptides with repeating primary sequences, which can be constructed by sequential couplings of a protected fragment comprising a single sequence repeat.

We report herein the synthesis of a sixteen amino acid protected[8] peptide derived from the conserved repeating sequence found in three bacterial ice nucleation proteins.[3] Using the BOP reagent [benzotriazol-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate[4,5]] for *in situ* activation affords increased yields relative to standard amino acid coupling methods.[6] In addition, we found that coupling of protected peptide fragments on the Kaiser resin using BOP activation proceeds in high yield with low levels of epimerization at the C-terminal residue.

The protected peptides I, II, and III were synthesized on the oxime resin employing BOP activation[6] (see experimental section) rather than the symmetric anhydride coupling procedure.[1] Protected peptides were cleaved from the resin with N-hydroxypiperidine (HOPip) followed by treatment with zinc in acetic acid[1] to afford the free acid which was precipitated and washed with water. Purification by reverse phase HPLC (water/acetonitrile/0.1% acetic acid, Waters Delta Pak C4) provided peptides I, II, and III, which were characterized by amino acid analysis, FAB mass spectroscopy, and ^{1}H NMR[7] The purified yields of protected peptides I, II, and III were 83%, 39%, and 85%, respectively.[9]

Fragments I, II, and III were sequentially coupled to resin-bound O-benzyl threonine.[10] After deprotection and neutralization of resin-bound amine, the protected fragment I [0.3 equiv based on resin-bound Thr(Bzl)] was added to resin in a small amount of dimethyl formamide (DMF), the suspension was cooled to 4°, and BOP, 1-hydroxybenzotriazole (HOBt),[11,12] and diisopropylethylamine (DIEA) were added

to effect coupling in *ca.* 70% yield (see experimental procedure). A small amount of the product resin-peptide (IV) was cleaved[1] to provide the protected six amino acid peptide product (V). Comparison of this product with the diastereomeric peptide Boc-Ser(Bzl)-Asp(Bzl)-Ser(Bzl)-Ser(Bzl)-D-Leu-Thr(Bzl)-CO_2H by HPLC revealed that the fragment coupling product was 97% (±1%) stereochemically pure.[13] The subsequent couplings, which involve glycine at the C-terminus, were effected by a simpler procedure involving *in situ* neutralization with excess DIEA. These couplings were run at room temperature using 1-1.5 equiv of protected fragment (based on resin-peptide). When these conditions were applied to the coupling of I, the product obtained was found to be 92% stereochemically pure. Low temperature and the minimization of base were found to be the critical factors in reducing racemization. The yields[9] determined for the Gly couplings were quite good (≥85%), in fact, we have found that couplings using BOP activation generally proceed in yields which are 10-30% higher than those using carbodiimide activation.[1]



The resin-bound peptide was cleaved with HOPip (10 equiv.) in 2M LiBr/anhydrous tetrahydrofuran (THF) to provide (after reductive cleavage) the peptide VI. This solvent system has been shown to improve cleavage yields for resin-bound protected peptides which aggregate in most organic solvents.[14] The peptide VI was purified by gel filtration (LH-20, DMSO) and characterized by amino acid analysis, FABMS,[16] and ^1H NMR.[15]

EXPERIMENTAL

Fragment Synthesis

The deprotected resin-bound peptide/amino acid (trifluoroacetate salt) was washed with DMF (20 ml/g resin, contaminating amines were removed by bubbling nitrogen through HPLC-grade DMF and storage over 4 Å molecular sieves) and then gently agitated with a solution of Boc-amino acid (3 equiv. based on resin-bound amine), BOP (3 equiv.), and DIEA (5.3 equiv.) in DMF (10 ml/g resin) for 1 h at 23°. The resin was washed with DMF and the published procedure[1] was followed until the next coupling.

Fragment Coupling

The deprotected resin-bound amino acid (300 mg, 0.3 mmol amine trifluoroacetate salt/ g resin) was washed with 5% DIEA/CH_2Cl_2, then CH_2Cl_2 and DMF. We have found that it is critical to remove excess base in these washes. A solution of peptide I (0.3 equiv., 33 umol) in DMF (5 ml/ g resin) was added and the suspension was cooled to 4°. BOP (50 μmol), HOBt (33 μmol) and DIEA (175 μmol) were added and the suspension was gently agitated for 16h at 4°. The resin was washed extensively and the coupling yield was determined by quantitative amino acid analysis of the resin.[9] Acetylation (10 equiv. acetic anhydride, 5 equiv. DIEA in methylyene chloride, 12 h at 23°) was performed after each coupling.

Fragment Coupling (C-terminal Gly)

The deprotected resin-bound peptide (*ca.* 0.1 mmol amine trifluoroacetate salt/g resin) was washed with methylene chloride, isopropanol, and DMF, and then combined with a solution of the protected fragment (0.3 or 1.5 equiv. based on resin-amine), BOP (1.5 equiv. based on fragment), and HOBt (1.0 equiv.) in DMF (5 ml/g resin). DIEA was added slowly until the solution reached neutrality (4-8 equiv., tested by drop onto moist pH paper). The reaction mixture was gently agitated for 12-24 h at 23°.

ACKNOWLEDGEMENT We are grateful to Merck & Co. (Faculty Development Award) and The Procter and Gamble Co. (University Exploratory Research Program) for support of this research. Joseph Jarrett is a trainee of the NIH Biophysics Training Grant No. 1T32GM08318-01.

REFERENCES

1. a) Kaiser, E.T.; et al., *Science* **1989, 243,** 187.
   b) Kaiser, E.T. *Accts. Chem. Res.* **1989, 22,** 47.
2. Lansbury, P.T.; Hendrix, J.C.; Coffman, A.I. *Tetrahedron Lett.* **1989, 30,** 4915.
3. (a) Warren, G.J.; Corotto, L.; Wolber, P. *Nuc. Acids Res.* **1986, 14,** 8047.

(b) Abe, K.; Watabe, S.; Emori, Y.; Watanabe, M.; Arai, S. *FEBS Letters* **1989**, *258*, 297.

4. Castro, B.; Dormoy, J.R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, *14*, 1219.
5. Coste, J.; Le-Nguyen, D.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 205. The BOP analog reported herein (PyBOP) does not give HMPA as a side-product of coupling. Preliminary results from our laboratory indicate comparable coupling yields.

6. Fournier, A.; Wang, C.; Felix, A.M. *Int. J. Pep. Prot. Res.* **1988**, *31*, 86.

7. **Boc-AGY(Cl_2Bzl)G (III):** aa anal(uncorrected) A 1.1(1 expected) G 2.5(2) Y 0.7(1) mass spec MW=624.2 $[M+H^+]$=625.2 ^1H NMR (DMSO, 300 MHz): δ 8.4(t, 1H, J=8 Hz, G NH), 8.1(d, 1H, J=9, A NH) 7.5(m, 3H, Y CBz H), 7.2(d, 2H, J=10, Y 2.6H), 7.0(d, 1H, Y NH), 6.8(d, 2H, J=10, Y 3.5H), 5.2(s, 2H, Y CBz H), 4.5(m, 1H, Y αH), 4.0(t, 1H, J=7, A αH), 3.77, 3.71, 3.63, 3.58(4d, 4H, J=6, G αH), 3.0(q, 2H, J=6, Y βH), 1.4(s, 9H, Boc), 1.1(d, 3H, J=7, A βH). **Boc-S(Bzl)T(Bzl)QT(Bzl)AG (II):** aa anal S 0.6(1) T 1.8(2) Q 1.2(1) A 1.1(1) G 1.2(1) mass spec MW=933.5 $[M+H^+]$=934.2 ^1H NMR (DMSO, 300 MHz): δ 8.23(t, 1H, J=6 Hz, G NH), 8.1(d, 1H, J=7.5), 8.0(d, 1H, J=9), 7.9(d, 1H, J=8), 7.75(d, 1H, J=9), 7.3(m, 16H, Bz+Q NH_2), 7.14(d, 1H, J=8), 6.8(s, 1H, Q NH_2), 4.4(m, 10H), 4.3(m, 1H), 3.9(m, 2H, T βH), 3.8-3.5(m, 4H), 2.14(m, 2H, Q γH), 1.8(m, 2H, Qβ H), 1.35(s, 9H, Boc), 1.2(d,3H, J=7, A βH), 1.0(t, 6H, T γH). **Boc-S(Bzl)D(Bzl)S(Bzl)S(Bzl)L (I):** aa anal S 1.8(3) D 0.8(1) L 1.2(1) mass spec MW=967.5 $[M+H^+]$=968.2 ^1H NMR (DMSO, 300 MHz): δ 8.36(d, 1H, J=8 Hz), 8.19(d, 1H, J=8), 8.05(t, 2H, J=7.5), 7.3(m, 20H, Bz), 6.9(d, 1H, J=8), 5.05(s, 2H, D Bz), 4.77(m, 1H), 4.6(m, 2H), 4.45(m, 6H), 4.23(m, 2H), 3.6(m, 6H), 2.87(d, 1H, J=7), 2.82(d, 1H, J=7), 2.67(d, 1H, J=9), 2.63(d, 1H, J=9), 1.6(m, 1H, L γH), 1.45(t, 2H, L βH), 0.81(d, 3H, J=7, L δH), 0.77(d, 3H, J=7, L δH).

8. Reactive side chains were protected as follows: Ser (Bzl), Thr (Bzl), Tyr (2,6-Cl_2Bzl), Asp (Bzl).

9. Cleavage yields are based on average substitution on the resin before and after cleavage. Stepwise synthesis yields are from the first amino acid on the resin to the purified peptide. Fragment coupling yields are based on the ratio of coupled peptide to total peptide used (for I) or on the ratio of fragments in the peptide-resin product. Quantitation was determined by amino acid analysis using the Waters Pico Tag® method.

10. The first amino acid was attached to the Kaiser oxime resin by DCC activation.[1]

11. Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927.

12. Steinauer, R.; Chen, F.M.F.; Benoitin, N.L. *Int. J. Pep. Prot. Res.* **1989**, *34*, 295.

13. Resin-peptide IV from the coupling of I was cleaved by the standard procedure[1] to afford the protected peptide Boc-Ser(Bzl)-D(Bzl)-S(Bzl)-S(Bzl)-L-T(Bzl)-CO_2H (V). The product was analyzed by reverse-phase HPLC (C8 Lichrosorb, Hibar® (Merck), 42% [water-0.1% trifluoroacetic acid-(TFA)]/58% {95:5:0.1 acetonitrile-trifluoroethanol-TFA], isocratic elution). Under these conditions, the L-Leu (ret. vol.=31.2ml) and D-Leu (ret. vol.= 34.3 ml) containing diastereomers of this peptide were resolved and their ratio was measured by integration. The D-Leu containing diastereomer standard was synthesized by stepwise synthesis on the oxime resin.

14. Hendrix, J.C.; Halverson, K.; Jarrett, J.; Lansbury, P.T.; submitted for publication.

15. **Peptide VI:** aa anal D 1.1(1 expected) Q 1.1(1) S 1.7(4) G 3.2(3) T 2.5(3) A 2.1(2) Y 0.8 (1) L 1.0(1) ;^1H NMR (DMSO, 300 MHz): δ 8.3(m, 2H), 8.2-7.8(m), 7.5(d, 3H, J=9), 7.42(d, 1H, J=7), 7.38(d, 1H, J=7), 7.2(m), 7.12(d, J=9), 6.9(s), 6.85(d, 3H, J=9), 6.7(m), 5.1(s, 2H, Y CBz), 5.0(s, 2H, D Bz), 4.78(m, 1H), 4.65(m, 2H), 4.5-4.2(m), 3.87(m), 3.7(m), 3.63(d, J=6), 3.55(m), 2.1(m, Q γH), 1.8(m), 1.5(m, L γH), 1.4(m, L βH), 1.37(s, 9H, Boc), 1.22(s), 1.17(d, J=7, A βH), 1.09(d, J=7, A βH), 0.98(m, T γH), 0.7(m, L δH), 0.6(s).

16. Peptide VI was deprotected (triflic acid/TFA, 23°, 1 h) and purified by RPHPLC (C4) to provide a product with the calculated MW (plasma desorption MS).

(Received in USA 15 June 1990)