Tetrahedron Letters, Vol.30, No.5, pp 519-522, 1989 0040-4039/89 $3.00 + .00
Printed in Great Britain Pergamon Press plc

SOLID-PHASE SYNTHESIS OF VISCOSIN, A CYCLIC DEPSIPEPTIDE WITH ANTIBACTERIAL AND ANTIVIRAL PROPERTIES

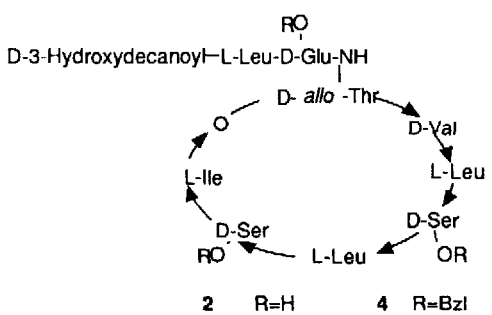
Terrence R. Burke, Jr.,* Martha Knight and Bhaskar Chandrasekhar
Peptide Technologies Corporation, 125 Michigan Ave. N.E., Washington, D.C., 20017, USA
James A. Ferretti
National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892

Abstract: Viscosin, a cyclic depsipeptide (containing a peptide lactone) has been prepared by solid-phase chemistry using an Fmoc-protocol starting with an acid sensitive resin. Cyclization with the activating agent BOP-Cl gave a product which was indistinguishable from natural viscosin thereby supporting the proposed structure.

During the screening of microorganisms for antibiotic substances by Kochi at the Yokohama Medical College, it was found that an isolate from a culture of *Pseudomonas viscosa* had both antiviral[1] and antimicrobial activity against various mycobacteria.[2] The purified active substance was given the name *viscosin*.[1] Structure **1** was proposed from the initial examination of viscosin which showed it to be a monocarboxylic peptide containing the fatty acid (-)-D-3-hydroxydecanoic acid.[3]

D-Hydroxydecanoyl- L-Leu-Gly-L-Ser-D-Val-L-Thr-L-Leu-OH
1

That this structure was incorrect was shown by comparison of natural material with synthetic **1** obtained by solution synthesis.[4] The revised structure **2** was proposed based on further physical characterization which indicated viscosin to be a cyclic depsipeptide (a peptide lactone) having alternating D and L- amino acids with an N-terminal D-3-hydroxydecanoyl group.[5] Ring closure is through an ester linkage between the carboxyl of L-Ile and the hydroxyl of D-*allo*-Thr, leaving the gamma-carboxyl of D-Glu free. To further strengthen the structural assignment, work was undertaken to prepare **2** by a scheme which utilized solid-phase techniques.



2 R=H **4** R=Bzl

The central problem in the synthesis of cyclic depsipeptides is ring closure. It has been found that ring closure is most advantageously effected through amide bond formation rather than through ester formation, and there are numerous examples of such cyclizations.[6] One problem with the use of solid-phase synthesis in the preparation of cyclic depsipeptides has been the difficulty of maintaining full side chain protection in the linear precursor during HF resin cleavage. In an effort to use recent techniques of solid-phase synthesis for the preparation of cyclic depsipeptides, a scheme was developed to employ these techniques in the synthesis of viscosin. Examination of viscosin shows that linear precursor **3** would be a suitable target for preparation by solid-phase synthesis as cyclization to the desired benzyl-protected viscosin **4** would result from amide bond formation between the C-terminal carboxyl of OBzl-D-Ser and the amino group of L-Ile. To prepare **3** by solid-phase synthesis and preserve the benzyl protecting groups during cleavage from the resin, a synthetic scheme was employed using fluorenylmethoxycarbonyl (Fmoc) protected amino acids[7] and the acid sensitive alkoxybenzyl alcohol resin of Wang[8] (which can be cleaved with 50% trifluoroacetic acid (TFA) in CH_2Cl_2). The choice of Boc-amino protection for Ile relies on its stability to the conditions of Fmoc deprotection during peptide synthesis. However, treatment of the resin with TFA would simultaneously remove the Boc group and cleave the peptide from the resin, yielding fragment **3** directly with both amino and carboxyl ends deprotected for cyclization. The value of simultaneous deprotection of amino and carboxyl terminal groups leading directly to material suitable for cyclization has been shown recently in solution synthesis.[9]

D-3-Hydroxydecanoyl-L-Leu-(OBzl)-D-Glu-D-allo-Thr-D-Val-L-Leu-(OBzl)-D-Ser-L-Leu-(OBzl)-D-Ser-OH
H-Ile-O

3

Coupling of amino acids through D-Val followed a standard Fmoc-based protocol. In summary, Fmoc-OBzl-D-Ser (all Fmoc-amino acids were either purchased or synthesized as crystalline solids using Fmoc-N-hydroxysuccinimide ester[10]) was coupled to alkoxybenzyl alcohol resin (1.0 mmol -OH g^{-1} resin) using dicyclohexylcarbodiimide (DCC) activation in the presence of 0.1 eq. of dimethylaminopyridine (DMAP)[11], to yield 0.4 to 0.6 mmol/g resin. After capping the resin with benzoyl chloride[8], the synthesis was continued through Fmoc-D-Val using the following repetitive protocol for sequential coupling of Fmoc-amino acids to the growing peptide chain: 1) 3 x DMF washes. 2) 3 x CH_2Cl_2 washes. 3) amine deprotection using piperidine: DMF 1:1, 20 min. 5) coupling amino acid using 2.5 eq. of Fmoc amino acid, hydroxybenzotriazole (HOBT)[12] and DCC in DMF, 2-3 h. 6) monitoring of coupling using the Kaiser ninhydrin test[13], repeating the coupling if not completed. Single couplings were adequate for Fmoc-OBzl-D-Ser and Fmoc-D-Val, with multiple couplings being required for Fmoc-L-Leu approximately 50% of the time.

One important aspect of the use of solid-phase chemistry in the synthsis of cyclic depsipeptides is the preparation of the ester branch point while the peptide is still attached to the resin. In this regard,the strategy for building the Boc-L-Ile ester branch point called for the addition of Fmoc-D-*allo*-Thr with its side chain hydroxyl unprotected. Although such coupling of an unprotected Thr is known in solution synthesis, it has been assumed that this was incompatible with solid-phase synthesis due to self acylation resulting from the large excess of amino acid needed to achieve high coupling effeciency. Since direct DCC mediated coupling could result in significant acylation of the unprotected hydroxyl,[14] coupling was achieved using the pentafluorophenyl ester (Pfp)[15], which reacts rapidly with amines, but sluggishly with alcohols. Although D-*allo*-Thr is not readily commercially available,epimerization of D-Thr gave an inexpensive source of D-*allo*-Thr.[16] This was first protected as the Fmoc derivative, then esterified with

pentafluorophenol / DCC to yield Fmoc-D-*allo*-Thr-Pfp before coupling cleanly and quantitatively to the resin in a single coupling reaction using 2.5 eq. of amino acid, 5 h in DMF. As anticipated,examination of a sample of TFA-cleaved resin by HPLC gave no indication of significant acylation of the D-*allo*-Thr hydroxyl.

It was anticipated that esterification of the Fmoc-D-*allo*-Thr hydroxyl with Boc-L-Ile directly could lead to side product formation due to O ->N shift of Boc-Ile under the conditions which would subsequently be necessary to deblock the Fmoc-D-*allo*-Thr.[17,18] Since the reverse, N ->O shift would not be expected to occur under the non-acidic conditions of the synthesis, the resin was first deblocked with piperidine in the usual manner and then coupled with Fmoc-OBzl-D-Glu-Pfp. Again, the use of Pfp activation allowed amino coupling to occur without acylation of the unprotected hydroxyl. The free hydroxyl of D-*allo*-Thr was then esterified with Boc-L-Ile (DCC activation with 0.1 eq. of DMAP[19]) to give the desired Fmoc-OBzl-D-Glu-(O-Boc-L-Ile)-D-*allo*-Thr- branch point as a single major product on HPLC.

Having successfully prepared the branch point, it was then found that piperidine deblock of the Fmoc-OBzl-D-Glu followed by coupling with Fmoc-L-Leu (DCC / HOBT) gave a major side product which was at times equal to 40% of the desired product. This chain-terminated side product gave amino acid analysis and fast atom bombardment (FAB) mass spectral data consistent with the cyclization[20] of the OBzl-D-Glu to pyroglutamic acid. This was readily removed chromatographically and in subsequent synthesis was essentially eliminated by decreasing the piperidine deblock time to 5 min.

Linear peptide **3** was completed by acylating the peptide resin with the Pfp-ester of D-3-hydroxydecanoic acid in DMF. Again, the use of Pfp ester activation allowed the acid to be coupled with its hyrdroxyl unprotected. The synthesis of 3-hydroxydecanoic acid (mp 55-56° C; lit.[21] 57° C) was from *n* -octyl aldehyde by reaction with lithium *tert* -butyl acetate[22] followed by TFA hydrolysis and resolution as the (–)-cinchonidine salt (mp 119-120° C; lit.[19] 119-120° C) which upon neutralization gave (-)-D-3-hydroxydecanoic acid ($[a]_D$= -21.4°(c 1.0,$CHCl_3$); lit.[19] $[a]_D$= −17.5°).The final peptide was cleaved from the resin with TFA (50% in CH_2Cl_2 , 30 min.) and lyophilized from dioxane to yield the crude peptide **3**. This was readily purified by reverse phase HPLC using a C_{18} column and an H_2O - acetonitrile gradient system containing 0.1 % TFA, with the resulting peptide being relyophilized from dilute anhydrous dioxane - HCl to yield **3**•HCl as a white solid in 25% overall yield based on resin substitution.

To complete the synthesis, cyclization reactions were carried out with a peptide concentration of 1 mM. Using DCC / HOBT in the presence of triethylamine, a single main product was obtained having a FAB mass spectrum consistent with the N-acylurea resulting from the O<–>N peptide rearrangement[23] of the intermediate O-acylisourea[24] formed by reaction with DCC. Using bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl)[25] and triethylamine in dioxane, the desired cyclic **4** (amino acid analysis, FAB mass spectrum) was obtained in 24% yield from **3** after HPLC purification. Debenzylation was carried out in MeOH using ammonium formate and 10% Pd•C[26]. Purification of the crude reaction product by HPLC yielded a white solid in 78% yield which had the expected amino acid analysis and FAB mass spectrum consistent with **2**. Synthetic **2** was shown to be identical to natural viscosin both chromatographically (HPLC using a reverse phase C_{18} column with an aqueous acetonitrile system containing 0.1% TFA) and by NMR (300 MHz proton spectrum) thereby supporting **2** as the correct structural assignment for viscosin.

In summary, the successful synthesis of cyclic depsipeptides by solid phase techniques is possible through the preparation of suitable branched fragments. Important aspects of this approach are the use of orthogonal Fmoc/Boc amino protection. Elaboration of the crucial ester branch point using unprotected hydroxyl-bearing amino acids is possible through the use of Pfp activation and a coupling order which minimizes O <-> N acyl migration.

Acknowledgements

Appreciation is expressed to Sumitomo Chemical Company, Osaka, Japan for providing a reference sample of viscosin and to Dr. Anthony Mauger for helpful discussions and Sara Gluch for amino acid analysis. Some of the FAB mass spectral determinations were carried out at the Middle Atlantic Mass Spectrometry Laboratory, a National Science Foundation Shared Instrumentation Facility. Funding for this work was provided by National Institutes of Health grant no. AI 23571.

References

1. V. Groupe, L.H. Pugh , D. Weiss and M. Kochi. *Proc. Soc. Exptl. Biol. Med.*, **1951**,*78* , 354-358 .

2. M. Kochi, D.W.Weiss, L.H. Pugh and V. Groupe. *Bact. Proc.* , **1951**, 29-30.

3. T. Ohno, S.Tajima and K. Toki. *J. Agr. Chem. Soc. Japan* , **1953**,*27*, 665-669. [CA **49**, 3012d (1955)].

4. M. Hiramoto, K. Okada, S. Nagai and H. Kawamoto. *Biochem. Biophys. Res. Commun.*,**1969**,*35*, 702-706.

5.M.Hiramoto, K. Okada and S. Nagai. *Tetrahedron Lett.*, **1970**,*13* , 1087-1090 .

6. K.L. Rinehart, V.Kishore, S. Nagarajan, R.J. Lake, J.B. Gloer, F.A. Bozich, K.-M. Li, R.E. Maleczka, Jr., W.L. Todsen, M.H. G. Munro, D.W. Sullins and R. Sakai. *J. Amer. Chem. Soc.* ,**1987**,*109* , 6846-6848.

7.L.A. Carpino and G.Y. Han. *.J. Amer. Chem. Soc.*,**1970**,*92* , 5748-5749.

8.S.S. Wang. . *J. Amer. Chem. Soc.* ,**1973**,*95* , 1328-1333.

9. R. E. Shute and D.H. Rich. *Tetrahedron Lett.*,**1987**,*28*, 3419-3422.

10.A. Paguet. *Can. J. Chem.*, **1982**,*60* , 976-980.

11. S.S. Wang. *J. Org. Chem.*, **1975**,*40* , 1235-1239.

12. W. Konig and R. Geiger, *Chem. Ber.*, **1970**,*103* , 788-798.

13. E. Kaiser, R.L. Colescott, C.D. Bossinger and P.I. Cook.*Anal. Biochem.*,**1970**, *34* , 595-598.

14. J.M. Stewart, T. Mizoguchi and D.W. Wooley, Abstract 0206, 153rd Annual Meeting of the American Chemical Society, April, 1967.

15. L. Kisfaludy and I. Schon. *Synthesis*, **1983** , 325-327.

16. D.F. Elliott. *J. Chem. Soc.*, **1950**, 62-68.

17. D. Levy and F.H. Carpenter. *Biochemistry*, **1970**,*9* , 3215-3222.

18. A.B. Mauger and O.A. Stuart. *Int. J. Peptide Protein Res.*, **1987**,*30* , 481-488.

19. C. Gilon and Y. Klausner. *Tetrahedron Lett.*, **1979**, *40* , 3811-3814.

20. A.J. Hubert, R. Buyle and B. Hargitay. *Helv. Chim .Acta.*, **1963**,*46* , 1429-1445.

21. N.J. Cartwright. *Biochem. J.* ,**1957**,*67* , 663-669.

22. M.W. Rathke and D.F. Sullivan. . *J. Amer. Chem. Soc.* ,**1973**,*95* , 3050-3051.

23. D.F. DeTar and R. Silverstein. *J. Amer. Chem. Soc.* ,**1966**,*88*, 1013-1019.

24. H.G. Khorana. . *Chem Rev.*, **1953**,*53* ,145-166.

25. R.E. Shute, B. Dunlap and D.H. Rich. *J. Med. Chem.* ,**1987**,*30* , 71-78.

26. M.K. Anwer and A.F. Spatola. *Synthesis* , **1980**, 929-932.

(Received in USA 9 August 1988)