Pergamon

Tetrahedron Letters 39 (1998) 779–782

TETRAHEDRON LETTERS

Towards the Semi-synthesis of Didemnin M. Solution and Solid Phase Syntheses of the Pseudotetrapeptide: pGlu-Glnψ[COO]Ala-Pro-OH

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Received 26 September 1997; revised 5 November 1997; accepted 6 November 1997

Abstract: Two approaches, solution and solid phase syntheses, were developed for the synthesis of the pseudotetrapeptide, pGlu-Glnψ[COO]Ala-Pro-OH. Both approaches gave comparable synthetic yields, but the latter approach provided an expedient entry to the synthesis of the target compound with higher product purity.

The didemnins, a series of cyclic depsipeptides, which share a common 23-membered macrocycle backbone, have been isolated from the Caribbean tunicate *Tridemnum solidum* by Rinehart and colleagues.[1,2] This class of marine natural products possesses potent antiviral, antitumor and immunosuppressive activities, although the biological potencies of different didemnin family members vary widely.[3] Since the major structural difference among the didemnin family is the composition of the linear moiety attached to the macrocycle via N-methyl leucine, it is hypothesized that these linear chains contribute significantly to the didemnin pharmacophore.

The most potent didemnin, didemnin M, has recently been reported to suppress *in vitro* T cell activation in the mixed lymphocyte reaction with an IC_{50} of 0.76 pM.[3] Continuing of our work on the identification of binding proteins for didemnin B [4,5] and as a prerequisite step in the construction of didemnin M-based molecular probes for use in mode of action studies, a semi-synthetic route for didemnin M synthesis was sought:

Didemnin M ⟹ Didemnin A + **1**

The synthesis of compound **1** was initially attempted using a solution phase approach. Retro-analysis of the peptide sequence suggested a stepwise synthetic route:

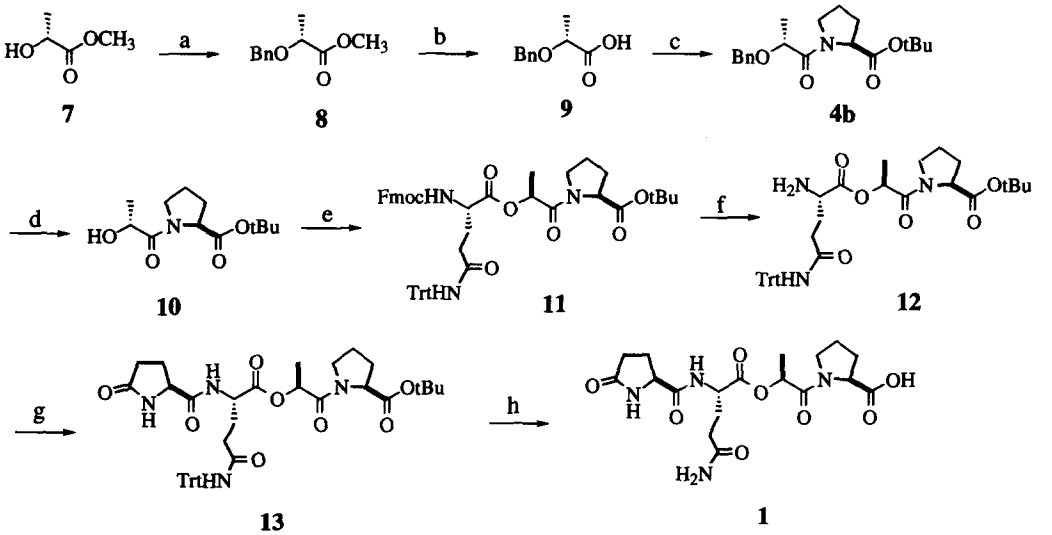
1 ⟹ **2** + **3** + **4a**: R_1=-Fmoc, R_2=-Bn; **4b**: R_1=-Bn, R_2=-tBu

0040-4039/98/$19.00
PII: S0040-4039(97)10609-8

Thus, the dipeptide intermediate **4a** was synthesized via five steps with an overall yield of 45%. However, further treatment of **4a** with 20% piperidine/DMF did not produce the desired HO-Ala-Pro-OBn dipeptide. Instead, a piperidine-adduct **5** was isolated as the major product, along with a cyclic product **6** as the minor product: [6]

4a **5** **6**

Based on this preliminary result, the combination of Fmoc- and Bn- protecting groups proved to be an unproductive strategy. Therefore, a new combination of orthogonal protecting groups was needed to accomplish the synthesis. Subsequently, we explored other combinations of protecting groups and discovered that the combined use of benzyl and *tert*-butyl protecting groups served this purpose well. As illustrated in Scheme 1, the (D)-methyl lactate was initially protected as benzyl ether. After saponification and DIC mediated coupling, the dipeptide intermediate **4b** was obtained. Further elongation of **4b** through hydrogenation, Mitsunobu reaction, and DIC mediated coupling afforded the intermediate **13**. Treatment of **13** with a TFA cocktail yielded a crude product containing the desired product **1** and a *tert*-butyl cation modified side product, which has a molecular weight larger than the desired product as indicated by FAB-MS ($[M+H]^+$=483.3). The ratio of the product versus the side product was about 85:15, as determined by RP-HPLC integration analysis. Various TFA cocktails were thus used in attempts to eliminate or minimize the side product without any significant improvement. The crude product was purified by semi-preparative RP-HPLC, and the desired product **1** was obtained with an overall yield of 19%.[7]

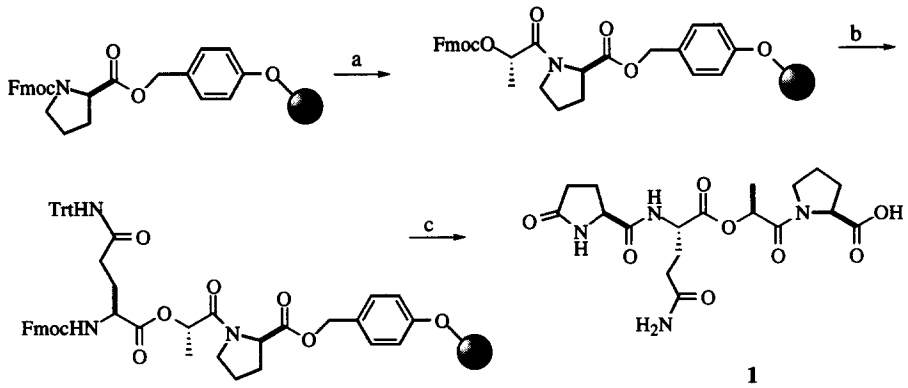


7 **8** **9** **4b** **10** **11** **12** **13** **1**

Scheme 1

Reagents and conditions: a: $PhCH_2Br$, NaH, DME, 80%; b: 2N NaOH, MeOH, 89%; c: HCl·Pro-OtBu, DIC, HOBt, THF, 96%; d: Pd/C, H_2, THF, 79%; e: Fmoc-Gln(Trt)-OH, DEAD, Ph_3P, benzene, 66%; f: 20% piperidine/DMF, 76%; g: pGlu-OH, DIC, HOBt, CH_2Cl_2, 80%; h: 50% TFA/40% CH_2Cl_2/5% Anisole/5% H_2O, 87%.

This solution phase synthesis strategy provides a practical approach for synthesizing a large quantity of product **1**, but it involves several steps and the last TFA cleavage step was problematic. In order to expedite the synthesis and to improve product purity, a solid phase approach was therefore developed. As shown in Scheme 2, the synthesis was initiated using Fmoc-Pro-Wang resin. After removal of the Fmoc group with 20% piperidine in DMF, Fmoc-protected lactate [8] was coupled to the solid support using HBTU as coupling reagent. Again, the Fmoc-group was removed, and the ester bond formation between the carboxylate of Fmoc-Gln(Trt) and the resin bound hydroxy group was originally attempted using Mitsunobu reaction conditions as described by Krchnak.[9] Subsequent deprotection of Fmoc group and photometric assay of the released dibenzofulvene or its piperidine-adduct revealed a disappointing low esterification yield of 32%. However, the esterification yield could be improved to about 60% when the reaction was mediated by DIC as coupling reagent in the presence of a catalytic amount of DMAP. The remaining unreactive hydroxy groups on the resin



Scheme 2

Reagents and conditions: a: (i) 20% Piperidine/DMF; (ii) Fmoc-(L)-O-CH(CH_3)-COOH/HBTU/HOBt/DIEA; b: (i) 20% Piperidine/DMF; (ii) Fmoc-Gln(Trt)-OH/DIC/DMAP; (iii) Ac_2O/DMF; c: (i) 20% Piperidine/DMF; (ii) pGlu-OH/HBTU/HOBt /DIEA; (iii) 90% TFA/5% anisole/5% H_2O

were capped with acetic anhydride, followed by the deprotection of Fmoc and coupling of pGlu to the solid support. The peptide resin thus obtained was treated with a TFA cleavage cocktail at room temperature for 1.0 hour. The cleaved crude product was purified using preparative RP-HPLC to afford the pure desired pseudopeptide **1**.[10] The overall synthetic yield was approximately 24%, based on the initial loading level of the resin support.

In conclusion, we have successfully synthesized the target pseudopeptide using the solution phase and solid phase synthesis approaches. The total synthesis of didemnin M and its application for identifying its binding protein are currently in progress and will be reported in due course.

Acknowledgment: We gratefully acknowledge the financial support provided by PharmaMar, s.a., The Tobacco Research Council (grant #4499) and a Burroughs Wellcome New Investigator Award in the Basic Pharmacological Sciences to C.M.C.

References and Notes

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6. **5**: ^{1}H-NMR ($CDCl_3$): δ 4.87 (m, 1H); 4.39 (m, 1H); 3.73 (m, 1H); 3.59 (m, 1H); 3.46-3.55 (m, 4H); 2.16 (m, 2H); 1.98 (m, 1H); 1.90 (m, 1H); 1.73 (m, 1H); 1.52-1.67 (m, 5H); 1.34 (d, 3H, J=6.7 Hz). FAB MS: calc for [$C_{13}H_{22}O_3N_2$, H^+] 255.3, found 255.2.
   6: FAB MS: calc for [$C_8H_{11}O_3N$, H^+] 170.2, found 170.1.
7. **1**: ^{1}H-NMR (DMSO-d_6): δ 8.38 (d, 1H, J=7.2 Hz); 7.75 (s, 1H); 7.23 (s, 1H); 6.77 (s, 1H); 5.18 (m, 1H); 4.23 (m, 2H); 4.03 (m, 1H); 3.60 (m, 1H); 3.50 (m, 1H); 1.80-2.23 (m, 13 H); 1.32 (d, 3H, J=6.7 Hz); $[\alpha]^{22}_D$ = -0.227 (c= 0.5, MeOH); FAB MS: calc for [$C_{18}H_{26}O_8N_4$, H^+] 427.4, found 427.2.
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10. The product from the solid phase synthesis has identical ^{1}H-NMR, RP-HPLC and FAB MS data as that of the product from the solution phase synthesis and $[\alpha]^{22}_D$ = -0.236 (c= 0.5, MeOH).