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Facile Detosylation of Cyclic Peptides. An Effective Synthesis of Platelet Glycoprotein IIb/IIIa Inhibitors

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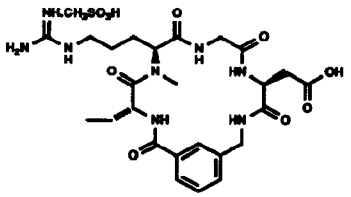
Abstract: A general and effective synthesis of cyclic pentapeptides containing Arg-Gly-Asp sequence, which are potent antithrombotic agents, is reported.

The research of Glycoprotein (GP) IIb/IIIa antagonists as antithrombotic agents has been an active area in recent years.[1-7] A number of cyclic peptides containing Arg-Gly-Asp sequence (RGD) including DMP 728 (**1**) have been discovered as potent IIb/IIIa antagonists. DMP 728 has dissociation constant in the picomolar range when binding to GPIIb/IIIa, and exhibits potent antiplatelet activities both *in vitro* and *in vivo*.[8] As a part of our clinical study program on this antithrombotic agent, an effective large scale preparation of this cyclopeptide is required. This paper reports our effective 10-step, chromatography-free synthesis of DMP 728. The key step in the synthesis is the facile detosylation of RGD containing cyclic peptides leading to DMP 728.



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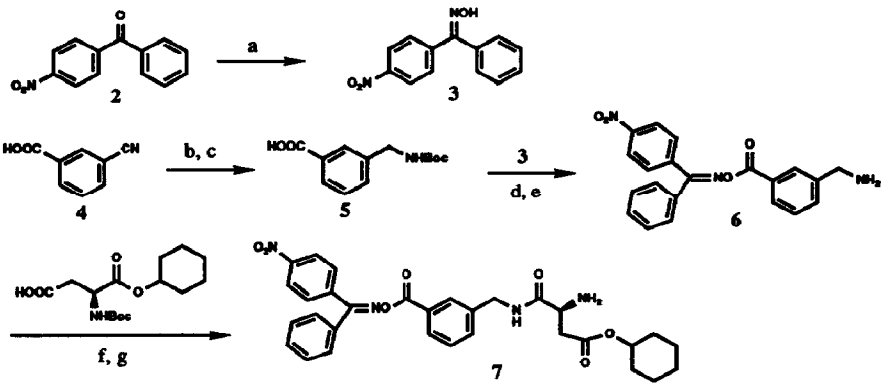
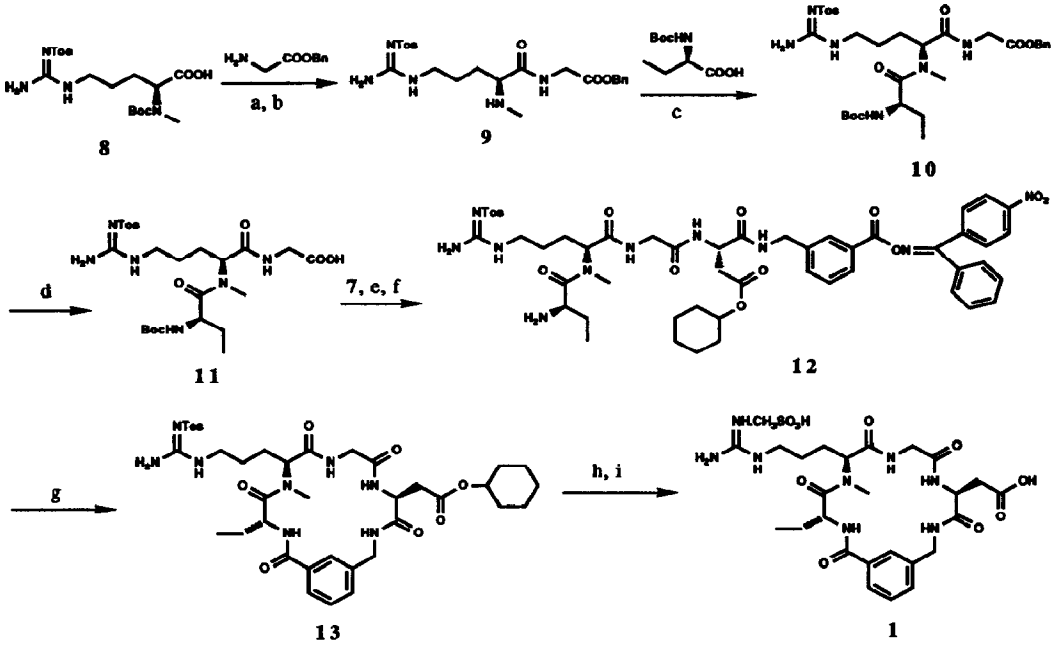
The original 16-step solid phase synthesis was employed to allow quick preparations of many structure similar cyclic peptides.[8] The guanidino group of arginine was protected as tosylate and the β-carboxyl group of aspartic acid was protected as cyclohexyl ester.[8] Both protecting groups were removed by anhydrous HF. The resulting product was purified by HPLC. In our first attempt to produce large quantity of DMP 728, the same 16-step reaction sequence was carried out in solution phase synthesis (Scheme 1 & 2). In this synthesis, the cyclic peptide **13** must be prepared under dilute condition to avoid intermolecular coupling of linear peptide **12**. Compound **13** was directly precipitated out of the reaction solution. The tosyl and cyclohexyl protecting groups were removed by a mixture of trifluoroacetic acid (TFA) and triflic acid. To avoid acid catalyzed ring opening of the cyclic peptide under prolonged reaction time (> 3 hours), the reaction was carried out under carefully monitored temperature (-6 to -8^{0}C). The acids were removed with large volume of anhydrous diethyl ether to provide DMP 728. This synthesis provided enough material for early clinical studies, however, it was not economical and practical as a manufacture process.



(a) $NH_2OH.HCl$, >95%. (b) H_2, Pd/C, 95%. (c) BOC-ON, TEA, 75%. (d) DCC, DMAP, 52%. (e) TFA, 87%. (f) HBTU, DIEA, 99%. (g) TFA, >95%.

Scheme 1

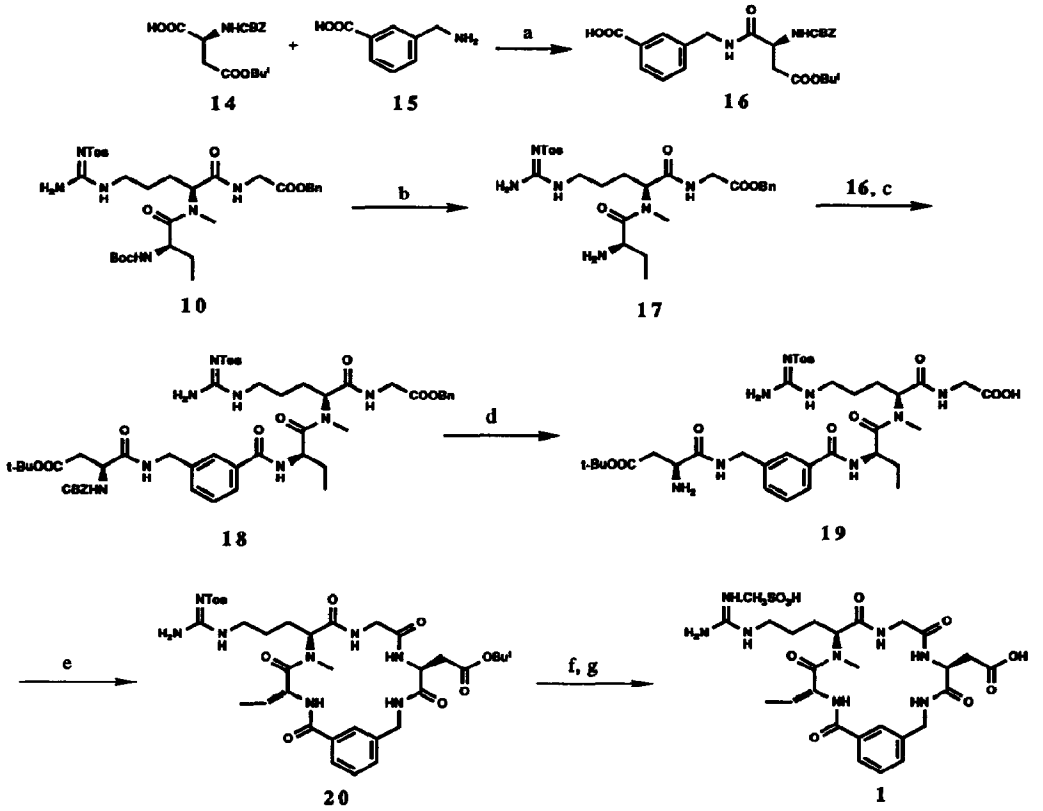


(a) HBTU, DIEA. (b) TFA. (c) HBTU, DIEA. (d) H_2, Pd/C. (e) HBTU, DIEA. (f) TFA. (g) DIEA, HOAc, 40% from 8. (h) TFA, triflic acid, anisole, -6 - -8^{0}C, >3h; ether, 90%. (i) CH_3SO_3H, 99%.

Scheme 2

Our effective 10-step synthesis of DMP 728 was developed as outlined in Scheme 3. We protected the aspartic acid 14 as β–t-butyl ester which could be easily removed with trifluoroacetic acid. We envisioned that the resulting carboxylic acid would facilitate the detosylation of the cyclopeptide by an intramolecular migration.[9]

Reaction of N-CBZ-β-t-butyl-L-aspartic acid **14** with 1,1-carbonyldiimidazole, followed by addition of *m*-aminomethylbenzoic acid **15** provided dipeptide **16** in 73% yield. Tripeptide **10** was synthesized in three steps as shown in Scheme 2 with some modification. Coupling of Nα-t-BOC, Nα-methyl-Nω-Tosyl-L-arginine **8** with glycine benzyl ester in the presence of HBTU generated the dipeptide which was converted into **9** in quantitative yield. The coupling reaction of Nα-BOC-D-α-aminobutyric acid with peptide **9** was troublesome due to the steric hindrance of the secondary amine of peptide **9**. The best result was achieved by using HBTU as coupling agent in acetonitrile to produce tripeptide **10** (85% yield). As shown in Scheme 3, the N-Boc protecting group of tripeptide **10** was removed by trifluoroacetic acid to afford peptide **17** (98% yield). Coupling of peptides **16** and **17** gave pentapeptide **18**. Removal of both benzyl and CBZ groups of **18** in a single step provided peptide **19**. Cyclization of **19** was carried out by adding linear pentapeptide **18** into a solution of HBTU in acetonitrile at 5 - 10^{0}C to produce the desired peptide **20** in excellent yield without cross coupling by-product.[10] The cyclic peptide was again precipitated out of the reaction mixture as a pure product. This synthetic sequence provided analytically pure cyclic peptide **20** in 50% overall yield from commercial available starting material **8**.



(a) CDI, 73%. (b) TFA, 98%. (c) HBTU, DIEA, 90%. (d) H_2, Pd/C, 98%. (e) HBTU, 50% from **8**. (f) TFA, 5^{0}C; triflic acid, anisole, -20 - -8^{0}C, 20 min, 99% yield. (g) CH_3SO_3H, 92%.

Scheme 3

With large quantity of cyclic peptide **20** in hand, the stage was set to remove the remaining two protecting groups to form DMP 728. There is a number of reports for the detosylation of arginine,[10a,11] and arginine containing peptides.[10a,12] However, these methods did not produce satisfactory results in our case. Finally, this was achieved by an one pot two step process. The β-t-butyl group of aspartic acid was removed by trifluoroacetic acid at 5^{0}C. Then the tosyl group of arginine was removed by triflic acid in presence of anisole at the temperature range of -20 to -8^{0}C. The reaction was completed in 20 minutes. The product was precipitated out from the reaction medium by addition of n-butyl ether, neutralized, and converted into its methanesulfonic acid salt (92% from **20**). In contrast with the previous slow detosylation of cyclic peptide **13** (Scheme 2), we believe that the facile detosylation of peptide **20** is due to the carboxylic function participation in the deprotection step.[9]

In conclusion, we have developed an efficient synthesis of cyclic peptide DMP 728. No chromatography separation is needed in the synthesis. The desired product is isolated in high yield and high purity. The reactions are readily to scale up, and amenable to commercial manufacture.

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