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Solid-Phase Synthesis of Muramyl Dipeptide (MDP) Derivatives Using a Multipin Method

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Abstract—Solid-phase synthetic method of muramyl dipeptide derivatives is reported. A diverse library of muramyl dipeptides could be potentially synthesized by acylation, reductive alkylation, sulfonamide formation, urea formation, *N*-alkylation, amine addition, or component Ugi reactions based on this method for drug screening.

Muramyl dipeptide (MDP) is the minimum structure of the cell wall of Gram-positive bacteria that can be recognized by human immunological response.[1] Since it can stimulate non-specifically human macrophage to become active against virus, bacteria, and tumors, it represents a potential therapeutic agent. Drawbacks to its clinical use, however, include poor penetration of macrophages,[2] pyrogenicity,[3] nonspecific induction of autoimmune responses and inflammatory reaction.[4] Many derivatives of MDP have been synthesized by solution-phase methods and these compounds have been further studied by in vitro and in vivo assays.[5] A few of them, including GMDP,[6] threonyl-MDP,[7,8] MTP-PE,[9,10] Murabutide,[11] Romurtide,[12] and B30-MDP[13] are now in clinical trials. In this paper, we are the first to report a solid-phase Multipin parallel synthesis[14] of MDP derivatives method that can potentially be used to make a diverse MDP derivative library with potential application for lead drug screening.

The macro crowns with a loading capacity of 5–8 μmol/pin from Chiron Mimotopes were used for the synthesis of MDP derivatives. In order to explore the solid-phase synthesis on pins, two critical building blocks were synthesized (protected muramic acid[15] and Fmoc-D-isoGln **7** as Scheme 1).

Benzyl ester protected D-glutamic acid **2** was made from D-glutamic acid **1** and benzyl-alcohol in the presence of H_2SO_4 (58.9%). The crude Boc-N_3 that was synthesized from $NaNO_2$, Boc-N_2H_3 in HOAc and H_2O was used to protect amino group of **2** to yield Boc-D-Glu(OBzl)-OH **3**. Compound **3** was further activated by DCC and HOSu to form Boc-D-Glu(OBzl)-OSu **4** active ester in anhydrous THF. Compound **4** was reacted by 25% NH_4.OH to give Boc-D-isoGln(Bzl) **5**. The benzyl group was removed by a 5% Pd/C in HOAc for 3 days to yield Boc-D-isoGln-OH **6**. After removal of Boc by 50% TFA in DCM, Fmoc-OSu was reacted with **6** for an additional 3 days at rt in the presence of 20% $NaHCO_3$ to yield Fmoc-D-isoGln **7** (76.7% totally, mp. 204–205 °C).

The peptide assembly was performed by a standard Fmoc peptide synthetic strategy [16] as shown in Scheme 2. Fmoc protected crowns were treated by 20% piperidine in DMF twice, each for 5 min and 15 min respectively. After thoroughly washing by DMF and methanol separately, the crowns were suspended in a 3-fold-time excess of Fmoc-Lys(Dde)-OH, BOP, HOBt and NMM stock solution for 4 h. These peptide coupling steps were repeated until the protected muramic acid was coupled onto the peptide. The Dde protected group of lysine was then removed by a treatment of 2% NH_2NH_2 in DMF twice, each for 3 min. After repeated washing steps, the exposed free amino group was further acylated with various organic acid building blocks using a BOP as coupling reagent, and HOBt plus NMM as additives at rt overnight. The final products were cleaved off from solid support by a reaction of crowns with 95% TFA/H_2O for 2 h at rt. After removal of TFA by lyophilization, all products were analyzed by HPLC at 214 nm wavelength with a gradient: B from 0–100% over 20 min. A: 0.1% TFA in water, B: 70% ACN in 0.1% TFA water. Sixty compounds were synthesized, all of them have a purity over 75% and correct molecular weight by electrospray ionization (ESI) mass spectrometry without further purification. Table 1 shows

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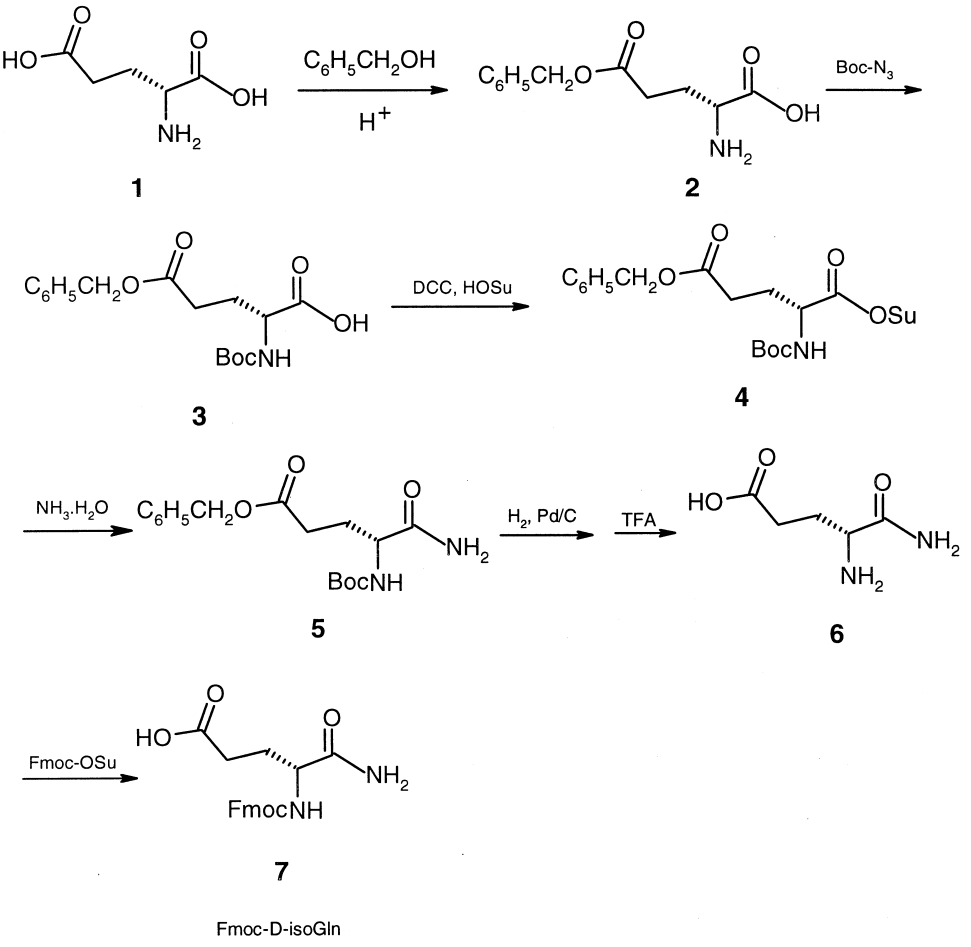
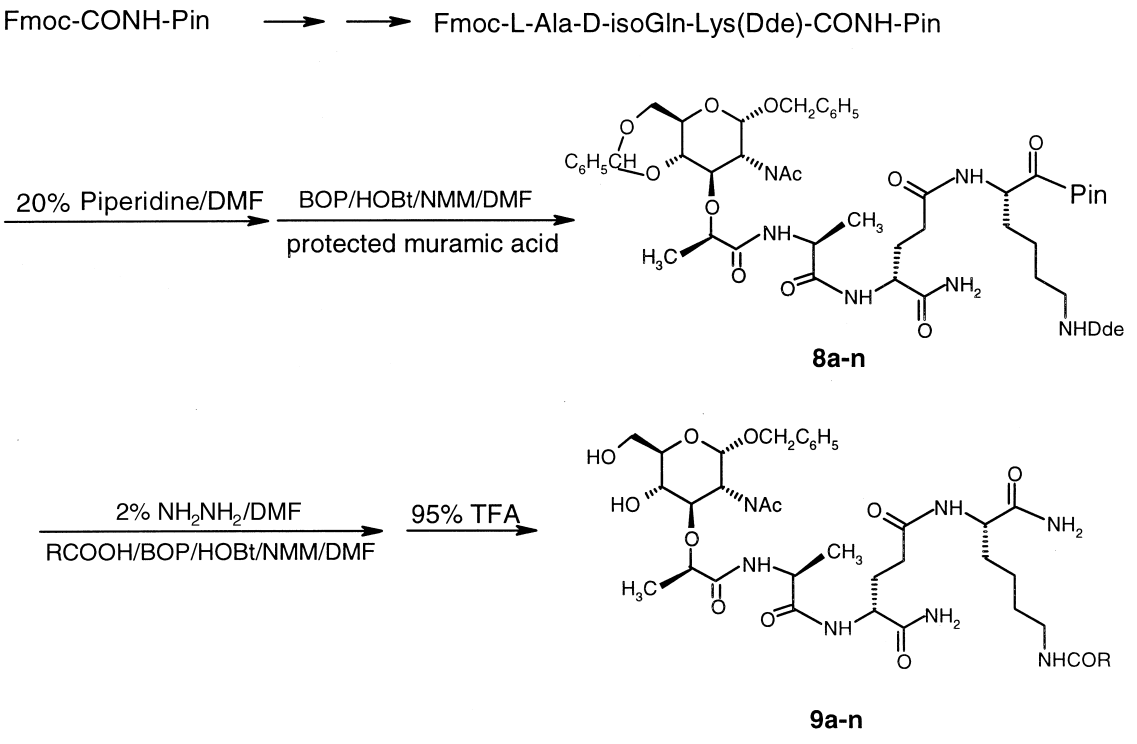
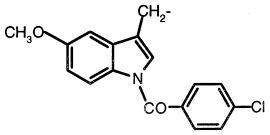
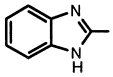
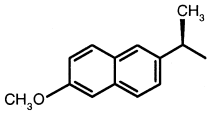
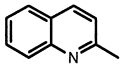
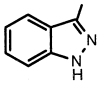
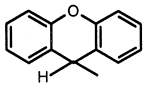
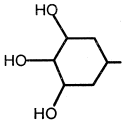
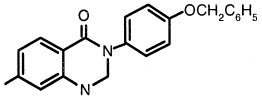
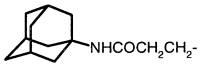
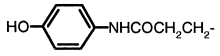
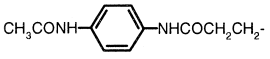
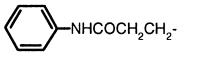


Scheme 1. Synthesis of Fmoc-D-isoGln.



Scheme 2. Synthetic muramyl dipeptide derivatives on the crown.

Table 1. The synthetic muramyl dipeptide derivatices

	R	MWt (Calcd/found)		R	MWt (Calcd/found)
9a		1049.7/1049.6	**9h**		854.1/854.2
9b		922.2/922.4	**9i**		865.1/865.4
9c		854.1/8554.4	**9j**		917.9/918.3
9d		862.1/862.2	**9k**		1063.9/1064.1
9e	$CH_3(CH_2)_{14}$–	948.4/948.5	**9l**	Murml acyl	1075.4/1075.3
9f		943.3/943.6	**9m**		901.1/901.4
9g		942.2/942.2	**9n**		899.2/899.3

examples of 14 typical compounds with correct molecular weight.

In conclusion, we have developed a solid-phase synthetic method of muramyl dipeptide derivatives using the Multipin approach. Further more, a diversity library synthesis is under way by acylation, reductive alkylation, sulfonamide formation, urea formation, *N*-alkylation, amine addition, or component Ugi reactions based on this solid-phase synthetic method.

Acknowledgements

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