Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron Letters
Tetrahedron Letters 47 (2006) 5325–5328

A novel strategy for oligopeptide synthesis using a polymer-supported ammonium fluoride

Michio Kurosu* and Dean C. Crick

Department of Microbiology, Immunology, and Pathology, Colorado State University, 1682 Campus Delivery, Fort Collins, CO 80523-1682, USA

Received 1 March 2006; revised 17 May 2006; accepted 19 May 2006
Available online 12 June 2006

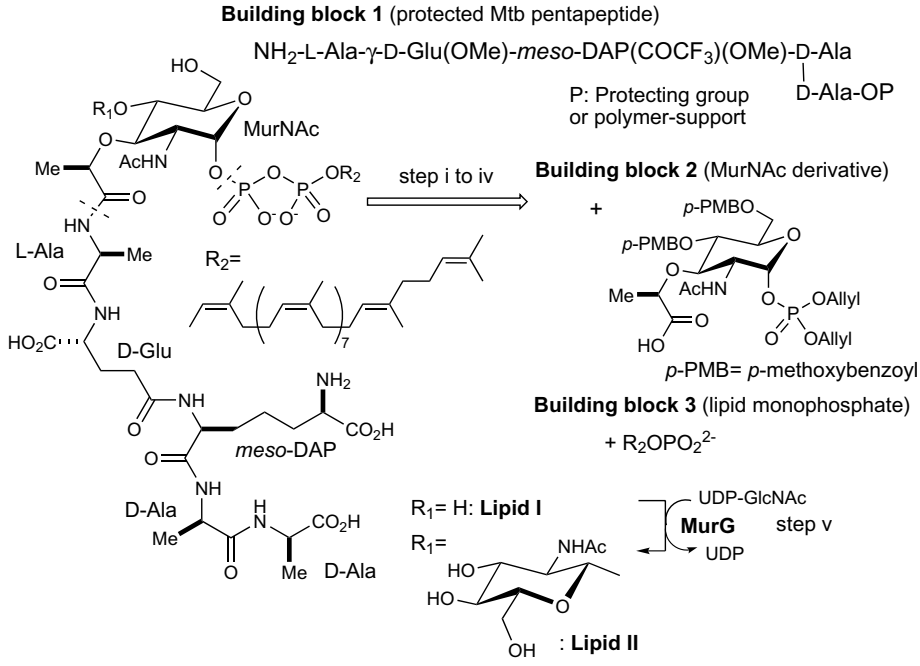
Abstract—A novel method for the preparation of oligopeptides with a PS-ammonium fluoride in the solution phase is reported. The synthesis of lipid II pentapeptide is efficiently synthesized via a PS-ammonium fluoride without chromatographic purifications. The method reported here is very convenient to synthesize a relatively large amount of oligopeptides with abundantly available Fmoc-protected amino acids in a time efficient manner.

In connection with our ongoing efforts to identify and validate new drug targets in *Mycobacterium tuberculosis* (Mtb), we initiated studies on peptidoglycan (PG) biosynthesis in which we demonstrated that mycobacterial lipid II is composed of a complex mixture of modified muramyl and peptide moieties linked to decaprenyl phosphate and that most of these modifications take place on lipid II rather than a precursor such as UDP-MurNAc-pentapeptide (Park's nucleotide).[1] Although the machinery for PG biosynthesis has been considered a crucial target, which step of PG biosynthesis represents the most promising target for binding small molecules that can eventually be developed into a new drug target has not been defined. In order to develop drug target in Mtb, we have been interested in membrane-associated proteins, phospho-*N*-acetylmuramoyl-pentapeptide-transferase (MraY), *N*-acetylglucosamine transferase (MurG), and specialized proteins termed 'flippases' that translocate lipid II from the cytoplasmic side to the external surface of the cell membrane. The development of economical high-throughput screens for Mtb MraY and MurG, and identification of lipid II flippases require significant amounts of Mycobacteria compatible Park's nucleotide, lipid I, and lipid II. The enzymes necessary for enzymatic synthesis of lipid II (i.e. MurA-F, MraY and MurG) are available in our laboratories, however, the specificity of MraY limits the practicality of a completely enzymatic synthesis of appropriate amounts of lipid II analogues.[2] Although elegant chemical and chemoenzymatic synthesis of lipid II have been reported,[3] their syntheses involve a significant number of intermediate purification steps and result in low overall yields. In order to supply gram quantities of (1) Park's nucleotide lipid I for the development of high-throughput screening and (2) lipid II which will be utilized in screening and studying lipid II flippases, it is indispensable to eliminate laborious chromatographic purification steps after coupling of building blocks in rapid and practical synthesis.

In an efficient synthesis of lipid II analogues, we attempted to eliminate laborious purification steps necessary after coupling of each building block. Our synthetic strategy includes (1) a practical synthesis of the pentapeptide (building block 1) in solution phase (step i), (2) transfer the pentapeptide to the polymer-support (step ii), (3) conjugation of appropriately protected MurNAc (building block 2) to the polymer-supported pentapeptide (step iii), (4) introduction of lipid analogues (building block 3) (step iv), and (5) simultaneous deprotection to form lipid I analogues followed by MurG catalyzed GlcNAc addition to lipid I analogues leading to lipid II analogues[3a] (step v) as illustrated in Scheme 1. We have established MurG promoted GlcNAc addition to lipid I analogues with UDP-GlcNAc. In order to synthesize lipid I analogues in a time efficient manner, the laborious synthesis of pentapeptide containing hydrophilic residues needed to be simplified. A

* Corresponding author. Tel.: +1 970 491 7628; fax: +1 970 491 1815; e-mail: michio.kurosu@colostate.edu

0040-4039/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2006.05.129

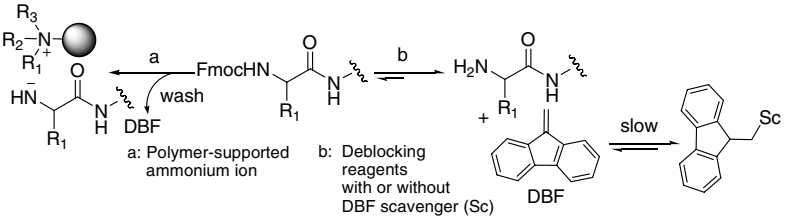


Scheme 1.

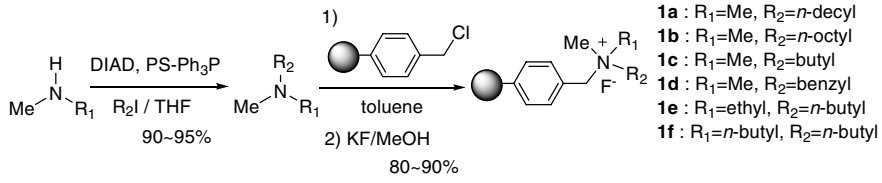
large number of natural and unnatural *N*-9-fluorenylmethoxycarbonyl (Fmoc)-protected amino acids are now commercially available, which have been used in solid phase syntheses. However, depending on physical properties of the amino acid residues, low overall yields caused by incomplete deprotection of Fmoc and by-product formation during the Fmoc deprotection are often observed in the solid phase synthesis of oligo- to long-peptides.[4] Deprotection of Fmoc-protected oligopeptides with a large excess of *secondary* amines in solution causes a significant problem with isolation of the deprotected free amines. In order to be able to utilize the abundantly available Fmoc-protected amino acids as building blocks for solution phase syntheses of oligo- to long peptides, reliable and convenient Fmoc-deprotection conditions needed to be developed. In this letter, we report a novel method for the synthesis of oligopeptides in solution using a polymer-supported ammonium fluoride.

As illustrated in Scheme 2, we envisioned capturing the deprotected amides onto the polymer-support and washing out all by-products derived from Fmoc group. The unfavored equilibrium observed (b in Scheme 2),[5] to which inadequate amounts of *secondary* amines were applied, can be shifted in the desired direction by the formation of an ammonium-amide complex (a in Scheme 2). Such a complex would easily be isolated from the reaction mixtures. Tetrabutylammonium fluoride (TBAF) is known to deprotect Fmoc group.[6] However, the hygroscopic ammonium fluoride is very difficult to apply to the isolation of an amide anion as an ammonium complex. Taking advantage of the hydrophobic nature of polystyrene polymer and by varying the structure of *quaternary* amine, we identified a non-hygroscopic polymer-supported ammonium fluoride possessing excellent F ion donor activity and better cationic properties than the tetrabutylammonium ion.

We synthesized several polymer-supported ammonium fluorides in over 80% yield, which were determined by elemental analyses of the remaining Cl atoms.[7] As shown in Scheme 3, (chloromethyl)polystyrene (PS) was quaternized with *tertiary* amines. Commercially unavailable *tertiary* amines were synthesized in 70–95% yields from the corresponding secondary amines via a condition of diisopropyl azodicarboxylate (DIAD)/PS-triphenylphosphine/THF. Under these conditions, no overalkylations to form *quaternary* amines were observed.[8] The counter-ions of the quaternized PSs were then exchanged to fluoride ions by the treatment with saturated KF in MeOH.[9]



Scheme 2.

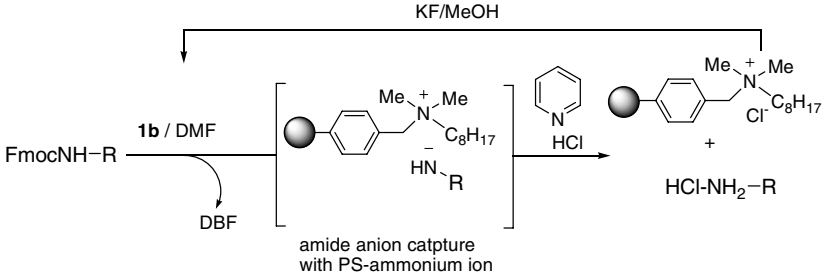


Scheme 3.

The efficiency of the PS-ammonium fluorides (**1a**–**f**) in deprotection of Fmoc-D-Ala-D-Ala-OMe and hydrolytic stability of their ammonium-amide anion complexes was investigated. The rate of the deprotection reaction increased in the order: **1c** < **1b** ≈ **1a** < **1d** ≪ **1e** ⩽ **1f**. The Fmoc-deprotection with **1f** (1.5 equiv at 0.05 M concentrations) was completed within 1 min in DMF. However, its PS-*N*,*N*,*N*-dibutylmethyl-amide complex showed hydrolytic instability; the free amine was leached from the polymer during washing with DMF. On the other hand, Fmoc deprotection of Fmoc-D-Ala-D-Ala-OMe with **1a** required 2–3 h to complete (1.5 equiv at 0.05 M concentrations). The rate of Fmoc-deprotection with **1b** was noticeably slower than that of **1a**, but the deprotection was completed within 4 h. It was confirmed that the D-Ala-D-Ala-OMe amide strongly bound to PS-ammonium ion derived from **1a** and **1b** and was not dissociated in DMF, THF, or the other aprotic solvents. Thus, DBF and its derivatives could easily be removed from the reaction mixtures. The PS-ammonium amide complexes could be efficiently uncomplexed with a stoichiometric amount of pyridine·HCl complex in DMF to provide HCl·H-D-Ala-D-Ala-OMe in 95% yield[10] and PS-ammonium chloride could then be reconverted to the corresponding PS-ammonium fluoride by the treatment with KF in MeOH. Thus, the polymer bound ammonium fluoride can easily be recycled.[9]

We then applied PS-ammonium fluoride **1b**[11] to the deprotection of Fmoc-protected amines, mono- and oligopeptides. Representative results are summarized in Table 1. Regardless of the nature of amino acid residue, deprotection of the Fmoc group was completed within 4 h with 1.5 equiv of **1b**. Because PS-ammonium fluoride reacts with Fmoc-protected amino acids selectively to capture the amides, unreacted free amines (coupling counter parts) and excess reagents are removed by a simple filtration. Therefore, it is possible to synthesize oligopeptides without chromatographic purification after each peptide coupling by using the PS-ammonium fluoride. We demonstrate the Fmoc/PS-ammonium fluoride strategy of synthesizing bacterial lipid II pentapeptide as a model study in which L-Lys($COCF_3$) was utilized instead of *meso*-DAP($COCF_3$)(OMe) in Scheme 1. HCl·H-D-Ala-D-Ala-OAllyl was coupled with 0.9 equiv of Fmoc-L-Lys($COCF_3$)-OH via PyBOP[12] as a coupling reagent to provide Fmoc-L-Lys($COCF_3$)-D-Ala-D-Ala-OAllyl, which was then deprotected with **1b**. The generated complex was then decomplexed with pyridine·HCl, after removing DBF derivatives, affording HCl·H-Lys($COCF_3$)-D-Ala-D-Ala-OAllyl in 95% yield. This was subjected to the coupling reaction with the *N*-hydroxysuccinimide (NHS) ester of Fmoc-γ-D-Glu(OMe)-OH followed by (1) scavenging the liberated NHS[13] with Si-carbonate,[14] (2) Fmoc-deprotection with

Table 1.



Entry	Fmoc-protected amine or peptides (FmocNH-R)	PS-ammonium F	Reaction time (h)	Yield (%)[a,b]
1	Fmoc-NHC_6H_4Cl-*p*	**1b**	0.5	98
2	Fmoc-L-Phe	**1b**	2	99
3	Fmoc-L-Lys($COCF_3$)-OMe	**1b**	4	95
4	Fmoc-D-Ala-D-Ala-OAllyl	**1b**	4	96
5	Fmoc-D-Ala-D-Ala-OMe	**1b**	4	95
6	Fmoc-L-Ala-γ-D-Glu$(OMe)_2$	**1b**	4	94
7	Fmoc-L-Lys($COCF_3$)-D-Ala-D-Ala-OAllyl	**1b**	4	93
8	Fmoc-L-Ala-L-Phe-L-Gly-L-Gly-OMe	**1b**	4	92

All reactions were carried out with 1.5 equiv of PS-ammonium F (1.5–1.7 mmol/g).
All products were isolated as their HCl salts.
[a] Yield was established by ^1H NMR analysis.
[b] No Fmoc-derived by-products were observed in ^1H NMR.

1b, (3) washing the complex, and (4) decomplexation with pyridine·HCl to provide the desired HCl·H-γ-D-Glu(OMe)-L-Lys($COCF_3$)-D-Ala-D-Ala-OAllyl in 90% yield. In a similar manner, the protected lipid II pentapeptide, HCl·H-L-Ala-γ-D-Glu(OMe)-L-Lys($COCF_3$)-D-Ala-D-Ala-OAllyl, was synthesized by coupling with Fmoc-L-Ala-OH. Overall yield of the synthesis of the pentapeptide from H-D-Ala-OAllyl was 65–70%.[15] Thus, the lipid II pentapeptide was readily synthesized without chromatographic purification.

In conclusion, we have developed a novel Fmoc-deprotection method with a polymer-supported ammonium fluoride for synthesizing oligopeptides in a time efficient manner. This method is very practical for the synthesis of relatively large amounts of the target oligopeptides without chromatographic purification. Moreover, the polymer-supported ammonium fluoride utilized in the reactions can be recovered as its ammonium chloride and regenerated to the ammonium fluoride without loss of reactivity.

Acknowledgements

We thank the National Institutes of Health (NIAID grant AI049151) and Colorado State University for generous financial support.

References and notes

1. (a) Mahapatra, S.; Yagi, T.; Belisle, J. T.; Espinosa, B. J.; Hill, P. J.; McNeil, M. R.; Brennan, P. J.; Crick, D. C. *J. Bact.* **2005**, *187*, 2747; (b) Mahapatra, S.; Scherman, H.; Brennan, P. J.; Crick, D. C. *J. Bact.* **2005**, *187*, 2341.
2. Experiments with Mtb MraY indicated that this enzyme has a high degree of specificity for endogenous prenylphosphate and doses not accept exogenously supplied prenylphosphate.
3. (a) Ha, Sha.; Chang, E.; Lo, M.-C.; Men, H.; Park, P.; Ge, M.; Walker, S. *J. Am. Chem. Soc.* **1999**, *121*, 8416; (b) VanNieuwenhze, M. S.; Mauldin, S. C.; Zia-Ebrahimi, M.; Winger, B. E.; Hornback, W. J.; Saha, S. L.; Aikins, J. A.; Blaszczak, L. C. *J. Am. Chem. Soc.* **2002**, *124*, 3656.
4. (a) We encountered difficulty in purifying the desired protected pentapeptide (L-Ala-γ-D-Glu(OMe)-L-Lys-($COCF_3$)-D-Ala-D-Ala-OMe) from by-products generated on the polymer-support by using a standard Fmoc strategy (piperidine as a deblocking reagent); (b) Zhang, A. J.; Russell, D. H.; Zhu, J.; Burgess, K. *Tetrahedron Lett.* **1998**, *39*, 7439.
5. (a) Carpino, L. A.; Mansour, E. M. E.; Knapczyk, J. *J. Org. Chem.* **1983**, *48*, 666; (b) Sheppeck, J. E., II; Kar, H.; Hong, H. *Tetrahedron Lett.* **2000**, *41*, 5324.
6. Ueki, M.; Tsurusaki, T.; Okumura, J. *Pept. Chem.* **1994**, 213.
7. *A general procedure for the synthesis of the polymer-supported ammonium fluoride*. (Chloromethyl)polystyrene (~1.7 mmol/g, crosslinked with 1% DVB, 10 g) and the *tertiary* amine (3–5 equiv) in toluene (50 mL) were heated at 90 °C for 3 h. The resins were washed with MeOH, THF-water(3/1), THF, and EtOAc. The quaternized PS-ammonium chlorides were soaked in sat. KF in MeOH (40 mL) for 20 min and the supernatant was removed. This process was repeated thrice. The resins were washed with MeOH-water (3/1), MeOH, and THF and dried under high vacuum.
8. Kurosu, M.; Dey, S. S.; Crick, D. C. *Tetrahedron Lett.* **2006**, *47*, 4871–4875.
9. Microscopic analyses of surface of these polymer-supported ammonium fluorides (**1a**–**f**) revealed that neither section nor fragmentation was observed on the spheres of polymers.
10. No by-products were detected in ^{1}H NMR of HCl·NH_2-D-Ala-D-Ala-OMe.
11. Because *N*,*N*-dimethyloctylamine is an inexpensive building block, **1b** was utilized for further studies.
12. (a) Chen, S.; Xu, J. *Tetrahedron Lett.* **1992**, *33*, 647; (b) Carpino, L. A.; Imazumi, H.; El-Faham, A.; Ferrer, F. J.; Zhang, C.; Lee, Y.; Foxman, B. M.; Henklein, P.; Hanay, C.; Mügge, C.; Wenschuh, H.; Klose, J.; Beyermann, M.; Bienert, M. *Angew. Chem. Int. Ed.* **2002**, *41*, 442.
13. A large excess of NHS cause decomplexation to leach the free amine.
14. Sauer, D. R.; Kalvin, D.; Phelan, K. M. *Organic Lett.* **5**, 2721.
15. *A general procedure for the synthesis of oligopeptide with the polymer-supported ammonium fluoride* **1b**. Fmoc-protected oligopeptides were synthesized using PyBOP (1.1 equiv) and *N*-methylmorpholine (2.0 equiv) in DMF. After standard water work-up, the crude product was dried under high vacuum. Into the solution of crude Fmoc-protected oligopeptide in DMF (0.1–0.05 M) was added **1b** (1.5 equiv), and the reaction mixture was gently stirred for 1 h. The reaction mixture was filtered through a glass-filter and the remaining PS-ammonium amide anions were washed with DMF (thrice). The PS-ammonium amide anions were dried under high vacuum and swelled in DMF (0.1 M). Into the reaction mixture pyridine·HCl (1.0 equiv) was added. After 10 min, the reaction mixture was filtered through a glass-filter and the solvent was removed under high vacuum to give the oligopeptide HCl salt. The recovered PS-ammonium chloride was subjected to the ion exchange reaction with KF in MeOH to afford **1b**.