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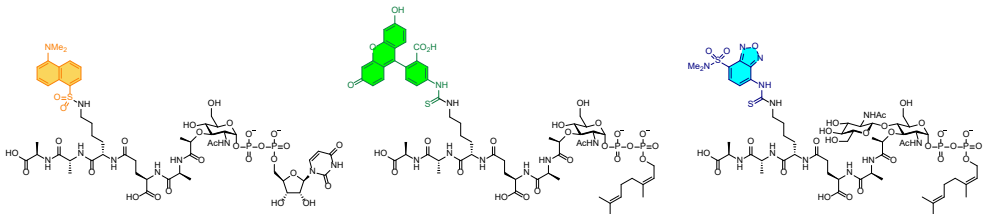

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Solid-phase Synthesis of Fluorescent Analogues of Park's Nucleotide, Lipid I and Lipid II †

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† *Dedicated to Prof. Dale Boger for his contributions to the field of organic&medicinal chemistry and inspiring many in the field.*

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ABSTRACT

Solid-phase modular synthesis of fluorescent analogues of Park's nucleotide, lipid I and II, is described. The Fmoc protected lysine side chain of resin-bound intermediates was selectively modified by various fluorescent groups. This is the first example of solid-phase synthesis of these compounds. This synthetic strategy allows to prepare various fluorescent proves for investigating the mechanisms of the bacterial cell-wall biosynthesis.

Peptidoglycan, which is the main component of bacterial cell walls, consists of repeating β-1-4-*N*-acetylglucosaminyl-*N*-acetylmuraminic acid (β-1-4-GlcNAc-MurNAc) and cross-linked polypeptide chains. Given that peptidoglycan is a characteristic polymer found in bacteria, inhibition of its biosynthetic pathway is an ideal target for the development of antibacterial agents. Indeed, many inhibitors of peptidoglycan biosynthesis, such as β-lactams and vancomycin, are used as antibacterial drugs.[1] Peptidoglycan biosynthesis involves several steps.[2,3] First, Park's nucleotide (Fig. 1, **1**) is synthesized by MurA-F in the cytoplasm. Then, **1** is converted to lipid I (**2**) by a reaction with undecaprenylphosphate catalyzed by phospho-MurNAc-pentapeptide transferase (MraY), and the resulting **2** is further transformed to lipid II (**3**) by *N*-acetylglucosamine transferase (MurG). Lipid II is transported to the periplasm by MurJ, a recently identified flippase.[4,5] Finally, peptidoglycan is produced by the polymerization reaction of lipid II catalyzed by a transpeptidase and a transglycosylase. Of note, **1-3** are used as substrates in studies associated with peptidoglycan biosynthesis, and their probe molecules also have contributed to discovering inhibitors and investigating the mode of action of antibacterial agents. [6-21] For example, a fluorescently modified analogue of **1** is used for screening of MraY inhibitors to develop novel antibacterial agents.[6,7] Kurosu and coworkers reported a MraY/MurX inhibitory assay system using a fluorescently labeled analogue of **2**.[8,9] Cheng's group reported that a fluorescent lipid II analogue can be used as a molecular probe to evaluate inhibitory activity against transglycosylase.[10] Recently, high-throughput screening for inhibitors of peptidoglycan

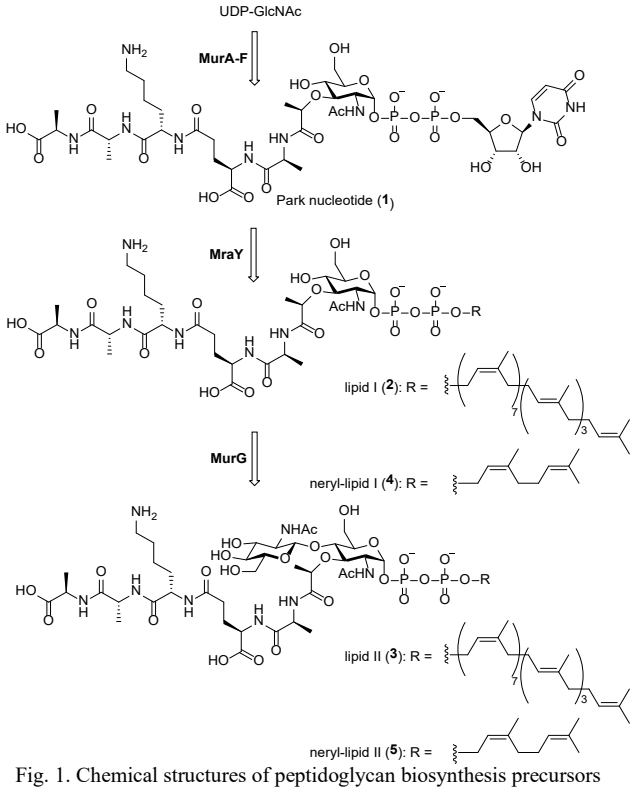
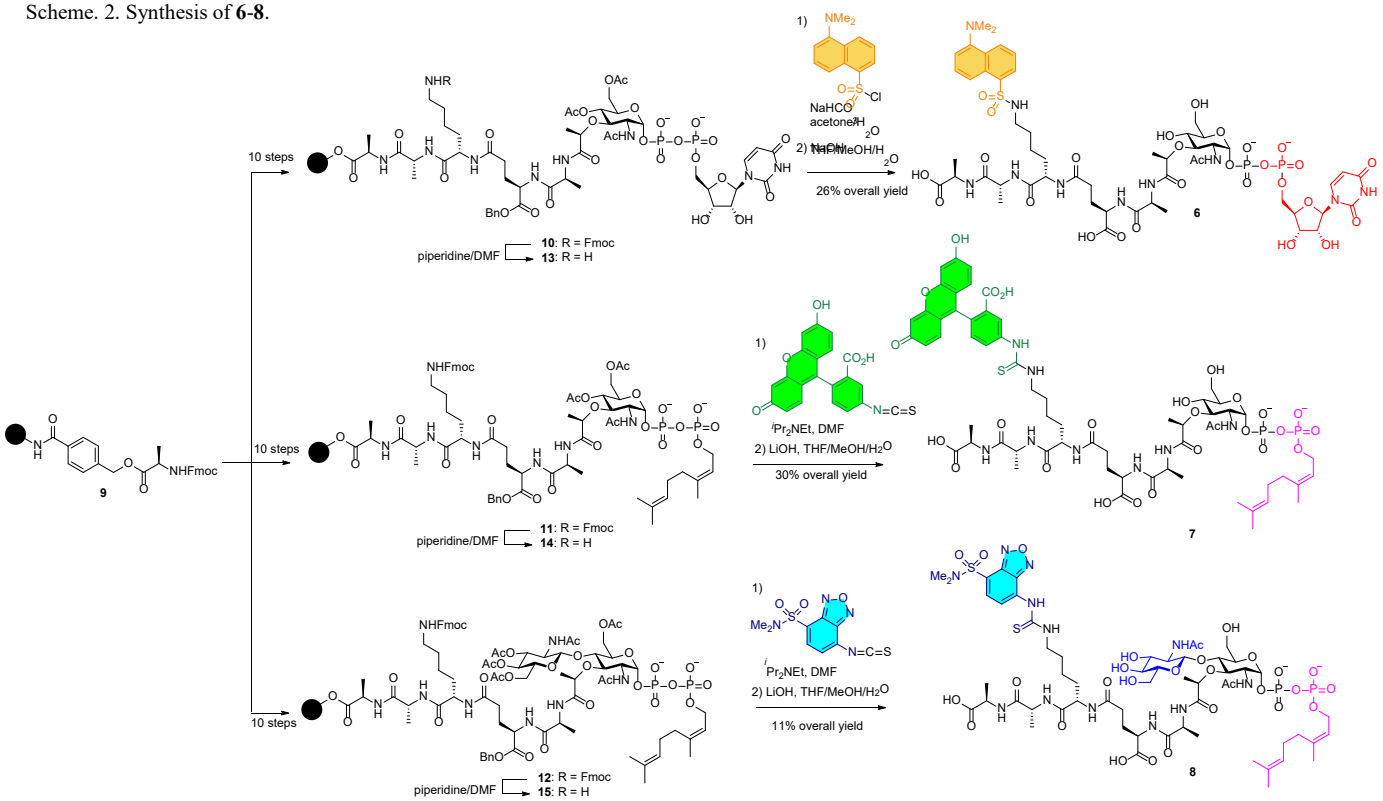


Fig. 1. Chemical structures of peptidoglycan biosynthesis precursors

Scheme. 2. Synthesis of **6**-**8**.



biosynthesis[22] and elucidation of the mode of action of teixobactin, which is a recently isolated natural product exhibiting strong antibacterial activity, were reported using fluorescently modified **3**.[23] Although these fluorescent probes are useful, there is no unified synthetic method applicable to prepare analogues of Park's nucleotide, lipid I and lipid II. The complex chemical structures of **1**-**3**, which consist of sugar(s), a peptide, and phosphate(s), require multistep transformations such as protection and deprotection of functional groups to obtain the desired probes. In addition, their amphiphilic properties as well as their molecular size occasionally represent difficulties in purification during synthesis. Therefore, we have previously developed a unified solid-phase synthesis of **1**, neryl lipid I (**4**) and neryl lipid II (**5**), which are derivatives of **2** and **3**.[24] This strategy allows us to provide highly functionalized macromolecules in a short time with a single purification step. Herein, we report the application of this method for synthesizing fluorescent probes of **1**-**3**. The synthetic method allows the modular introduction of various fluorescent groups into **1**-**3**.

Our previous synthesis of **1**, **4** and **5** utilized intermediates containing an Fmoc-protected L-lysine side chain, and the amino group of the L-Lys side chain could be selectively labeled by various fluorophores in a late-stage modification. To demonstrate the versatility of this synthetic strategy, we planned to synthesize three fluorescent analogues **6**-**8**; dansylated Park's nucleotide **6**, neryl lipid I analogue with fluorescein **7**, and neryl lipid II analogue possessing 4-(*N*,*N*-dimethylaminosulfonyl)-2,1,3-benzoxadiazole (DBD) **8** (Scheme 1). Synthesis of the fluorescent analogues was undertaken with 4-(hydroxymethyl)bezoylamidyl polyethyleneglycol (HMBA-PEG) resin-bound Fmoc-D-Ala **9**. Given that the HMBA-PEG resin swells extensively in various solvents, including water, both organic solvent and water can be used in the synthesis.[25] The resin-bound Fmoc-L-Ala was converted into protected Park's nucleotide **10** over 10 steps as previously reported (for details, see the ESI).[24] The resin was further treated with piperidine to remove the Fmoc group to yield **13**. Then, the conventional method using dansyl chloride and $^{i}Pr_2NEt$ in DMF was applied for the dansylation of the liberated amine of **13**. However, no reaction occurred to provide the corresponding dansylated product. After extensive investigation, we found that the conditions using dansyl chloride and $NaHCO_3$ in aqueous acetone cleanly afforded the desired dansylated product. The completion of the reaction on the resin was confirmed by Kaiser's test. Considering the instability of diphosphate under acidic conditions, cleavage from the resin should be conducted under basic conditions. Thus, cleavage from the resin and global deprotection were performed using NaOH in H_2O/MeOH/THF, and the resulting material was purified by reverse-phase HPLC to afford **6** possessing the dansyl group as a fluorophore. The analytical data of synthetic **6** agreed well with those reported.[6] For the synthesis of **7**, the Fmoc group of protected neryl lipid I **11**, which was bound to the resin, was removed to give **14**. Then, the sequential two step reactions: (1) labeling with fluorescein isothiocyanate (FITC) in the presence of $^{i}Pr_2NEt$ in DMF; (2) cleavage from the resin and simultaneous global deprotection, afforded **7** in 30% yield from **9**. Similar to the synthesis of **7**, **8** was synthesized from **12** using 4-(*N*,*N*-dimethylaminosulfonyl)-7-isothiocyanato-2,1,3-benzoxadiazole (DBD-NCS) for labeling the amino group of the L-lysine residue.

We also briefly examined an enzymatic transformation of **6** to dansylated lipid I. Namely, **6** and undecaprenylphosphate (C_{55}-P) were treated with MraY from *S. aureus* ($MraY_{SA}$). and the reaction progress was monitored by TLC. As a result, the formation of a new spot was observed, suggesting the formation of dansylated lipid I (for details, see the ESI).

In summary, a solid-phase method was developed for the synthesis of fluorescent probes of **1**-**3**. Many fluorescent probes derived from Park's nucleotide, lipid I and lipid II have been prepared by liquid-phase synthetic methods. This study is the first example to synthesize these probes by a solid-phase strategy. This method enables the preparation of a range of analogues of Park's nucleotide, lipid I and lipid II possessing not only fluorophores but also other functional groups, which is useful for investigating the mechanism of peptidoglycan biosynthesis and developing novel antibacterial agents.

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References and notes

1. Sarkar, P.; Yarlagadda, V.; Ghosh, C.; Haldar, J. *MedChemComm* **2017**, *8*, 516-533.
2. Bouhss, A.; Trunkfield, A. E.; Bugg, T. D. H.; Mengin-Lecreulx, D. *FEMS Microbiol. Rev.* **2008**, *32*, 208-233.
3. Bugg, T. D. H.; Walsh, C. T. *Nat. Prod. Rep.* **1992**, *9*, 199-215.
4. Sham, L.-T.; Butler, E. K.; Lebar, M. D.; Kahne, D.; Bernhardt, T. G.; Ruiz, N. *Science* **2014**, *345*, 220-222.
5. Ruiz, N. *Proc. Natl. Acad. Sci. USA.* **2008**, *105*, 15553-15557.
6. Wohnig, S.; Spork, A. P.; Koppermann, S.; Mieskes, G.; Gisch, N.; Jahn, R.; Ducho, C. *Chem. Eur. J.* **2016**, *22*, 17813-17819.
7. Brandish, P. E.; Burnham, M. K.; Lonsdale, J. T.; Southgate, R.; Inukai, M.; Bugg, T. D. H. *J. Biol. Chem.* **1996**, 271, 7609-7614.
8. Mitachi, K.; Siricilla, S.; Klaić, L.; Clemons, W. M.; Kurosu, M. *Tetrahedron Lett.* **2015**, *56*, 3441-3446.
9. Siricilla, S.; Mitachi, K.; Skorupinska-Tudek, K.; Swiezewska, E.; Kurosu, M. *Anal. Biochem.* **2014**, *461*, 36-45.
10. Liu, C.-Y.; Guo, C.-W.; Chang, Y.-F.; Wang, J.-T.; Shih, H.-W.; Hsu, Y.-F.; Chen, C.-W.; Chen, S.-K.; Wang, Y.-C.; Cheng, T.-J. R.; Ma, C.; Wong, C.-H.; Fang, J.-M.; Cheng, W.-C. *Org. Lett.* **2010**, *12*, 1608-1611.
11. Chen, K.-T.; Chen, P.-T.; Lin, C.-K.; Huang, L.-Y.; Hu, C.-M.; Chang, Y.-F.; Hsu, H.-T.; Cheng, T.-J. R.; Wo, Y.-T.; Cheng, W.-C. *Sci. Rep.* **2016**, *6*, 31579.
12. 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*, 3656-3660.
13. Hitchcock, S. A.; Eid, C. N.; Aikins, J. A.; Zia-Ebrahimi, M.; Blaszczak, L. C. *J. Am. Chem. Soc.* **1998**, *120*, 1916-1917.
14. Shih, H.-W.; Chen, K.-T.; Cheng, T.-J. R.; Wong, C.-H.; Cheng, W.-C. *Org. Lett.* **2011**, *13*, 4600-4603.
15. Mitachi, K.; Mohan, P.; Siricilla, S.; Kurosu, M. *Chem. Eur. J.* **2014**, *20*, 4554-4558.
16. VanNieuwenhze, M. S.; Mauldin, S. C.; Zia-Ebrahimi, M.; Aikins, J. A; Blaszczak, L. C. *J. Am. Chem. Soc.* **2001**, *123*, 6983-6988.
17. Schwartz, B.; Markwalder, J. A.; Wang, Y. *J. Am. Chem. Soc.* **2001**, *123*, 11638-11643.
18. Eid, C. N.; Nesler, M. J.; Zia-Ebrahimi, M.; Wu, C.-Y. E.; Yao, R.; Cox, K.; Richardson, J. *J. Labelled Compd. Rad.* **1998**, *41*, 705-716.
19. Meng, F.-C.; Chen, K.-T.; Huang, L.-Y.; Shih, H.-W.; Chang, H.-H.; Nien, F.-Y.; Liang, P.-H.; Cheng, T.-J. R.; Wong, C.-H.; Cheng, W.-C. *Org. Lett.* **2011**, *13*, 5306-5309.
20. Ueda, T.; Feng, F.; Sadamoto, R.; Niikura, K.; Monde, K.; Nishimura, S. *Org. Lett.* **2004**, *6*, 1753-1756.
21. Li, K.; Kurosu, M. *Heterocycles* **2008,** *76*, 455-469.
22. Boes, A.; Olatunji, S.; Mohammadi, T.; Breukink, E.; Terrak, M. *Sci. Rep.* **2020**, *10*, 8280.
23. Shukla, R.; Medeiros-Silva, J.; Parmar, A.; Vermeulen, B. J. A.; Das, S.; Paioni, A. L.; Jekhmane, S.; Lorent, J.; Bonvin. A. M. J. J. Baldus. M.; Lelli, M.; Veldhuizen, E. J. A.; Breukink, E.; Singh, I.; Weingarth, M. *Nature Commun.* **2020**, *11*, 2848.
24. Katsuyama, A.; Sato, K.; Yakushiji, F.; Matsumaru, T.; Ichikawa, S. *Chem. Pharm. Bull.* **2018**, *66*, 84-95.
25. Grøtli, M.; Gotfredsen, C. H.; Rademann, J.; Buchardt, J.; Clark, A. J.; Duus, J. Ø.; Meldal, M. *J. Comb. Chem.* **2000**, *2*, 108-119.

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