Synthesis and the Structure-Activity Relationships of 12-O-Mono-and Di-Glycosyl-Stearate Analogues, New Type of Ligand for the C-Type Lectin Receptor Mincle

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Synthesis and the Structure-Activity Relationships of 12-*O*-Mono-and Di-Glycosyl-Stearate Analogues, New Type of Ligand for the C-Type Lectin Receptor Mincle

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Introduction

One of the noticeable strategies for developing potent adjuvant is the employment of potently immunostimulatory ligands of pattern recognition receptors (PRRs) to initially search potent adjuvant.[1-4] In line of this research theme, the macrophage-inducible C-type lectin (Mincle) was well known as the major trehalose dimycolate (TDM) receptor in 2009,[5, 6] and its interaction with TDM recruited the response of the Th1/Th17 immune cells.[7] Its latter relating researches extremely had set out the fundamental of novel prominent tactic for developing potent adjuvant from ligands of Mincle receptor to fight against the serious tuberculosis (TB) and fungi. This theme of research had recently become more interesting as the discovery the novel potent Mincle ligand **44-2** from pathogenic fungus *M. pachydermatis* in 2013 (Figure 1).[8] Novel glycolipid **44-2** unusually composed of β-linked mannose residues attached to 10-hydroxystearic acid and in turn esterified onto a L-mannitol core, and it particularly displayed potent activity as much as TDM *via* mouse Mincle (mMincle). The β-mannosyl stearates **44-2-1** and **44-2-2** obtained from the alkaline hydrolysis of ligand **44-2** also showed the weaker Mincle ligand activity, and inferred the requirement of the whole structure for the full activity of the ligand **44-2** (Figure 1). In an effort to confirm the distinguish β-anomeric structures of the active fragment **44-2-1** and **44-2-2** of compound **44-2**, and go insight into the concept of structure and activity relationship of the Mincle ligand **44-2**, I had set out to synthesize the first series of eighteen 12-*O*-mono- and di-glycosyl analogues of the active fragment **44-2-1** and **44-2-2** of compound **44-2**, respectively.

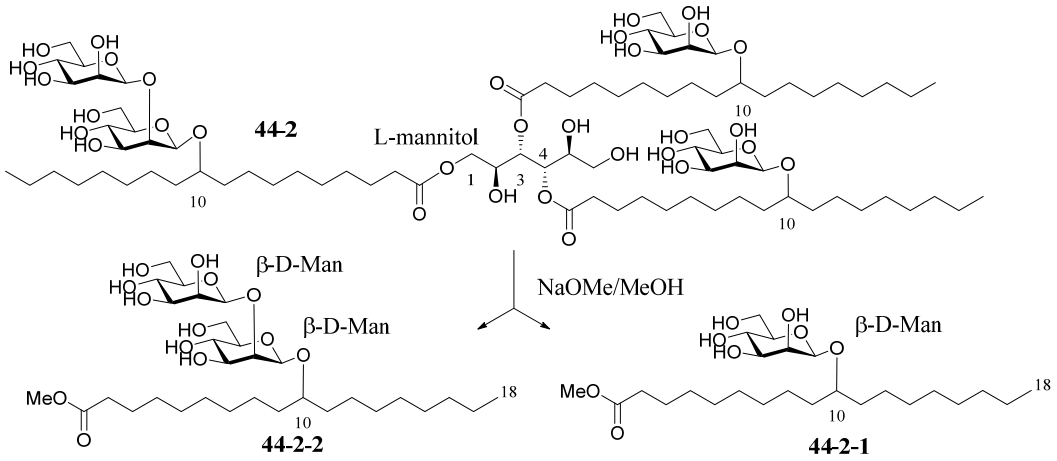
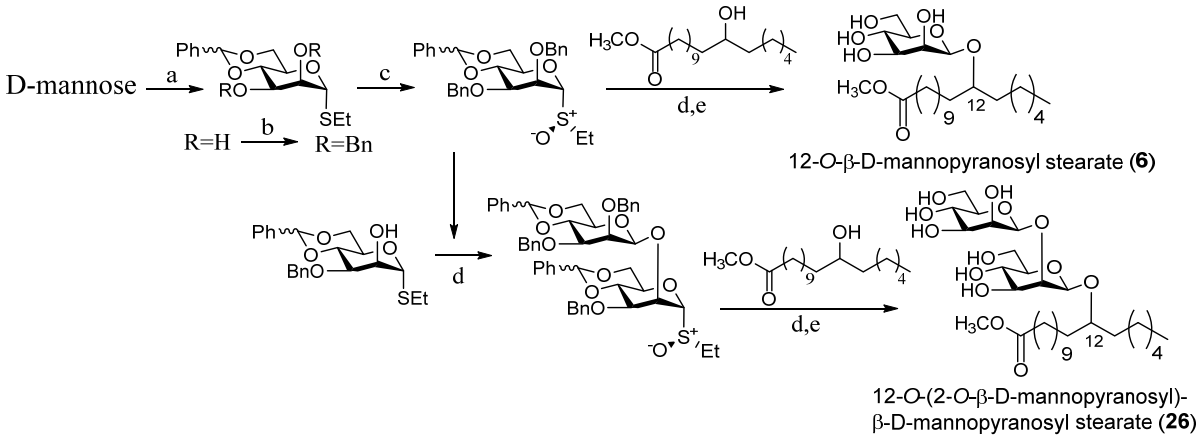


Figure 1: Structure of a novel Mincle ligand **44-2** and its alkaline hydrolysis products **44-2-1** and **44-2-2** found from pathogenic fungus *Malassezia*[8]

Results

The result of the synthetic study pointed out that the Crich's 4,6-benzylidene strategy was successfully employed to construct the β-linked mannose residues attached to 12-hydroxystearic acid in moderate yield, while the Köenings-Knorr method was optimized in the preparation of these other glycosyl analogues. Synthesized analogues clarified that both the natural fragments **44-2-1** and **44-2-2** were β-anomers (Scheme 1).



Scheme 1: Synthesis of 12-*O*-β-D-mannosyl-stearate analogues **6** and **26** by Crich's 4,6-benzylidene strategy (a): i) BzCl, Pyridine, ii) EtSH, BF_3, Et_2O, DCE, iii) NaOMe, MeOH (3 steps), (iv) PhCHO/ $ZnCl_2$; (b): NaH, BnBr, DMF; (c): *m*-CPBA, CH_2Cl_2, -78 °C; (d): TTBP, Tf_2O, MS4A, CH_2Cl_2, -78 °C; (e): EtOAc/ *n*-propanol/ THF/ H_2O = 2:1:1:1, 1 atm H_2, Pd-C.

To elucidate the structure-activity relationships of 12-*O*-glycosyl stearate with Mincle, thirteen synthetic analogues have been prepared by Köenings-Knorr method as shown in Figure 2.

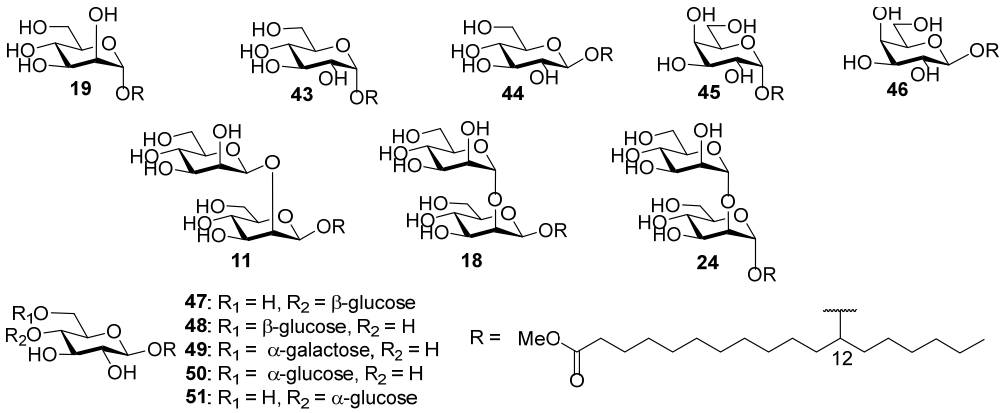


Figure 2: Structures of 13 synthesized compounds
11 [β-D-mannosyl-(1-2)-β-D-mannosyl-stearate]; **18** [α-D-mannosyl-(1-2)-β-D-mannosyl-stearate]; **19** (α-D-mannosyl-stearate); **24** [α-D-mannosyl-(1-2)-α-D-mannosyl-stearate]; **43** (α-D-glucosyl -stearate); **44** (β-D-glucosyl-stearate); **45** (α-D-galactosyl-stearate); **46** (β-D-galactosyl-stearate); **47** (β-cellobiosyl-stearate); **48** (β-gentiobiosyl-stearate); **49** (β-melibiosyl-stearate); **50** (β-isomaltosyl-stearate); **51**(β-maltobiosyl-stearate).

The structure-activity relationship of the synthetic compounds with Mincle was evaluated through the capacity to induce the NFAT-GFP activation through Mincle of the compounds. The result highlighted the extremely influence of the sugar moiety of each analogue on its activity with Mincle. Only four di-mannosyl-stearates **11**, **19**, **24** and **26** signaled through Mincle. They were defined as the new type of ligands for the Mincle. Giving the four active analogues, the 12-*O*-(2-*O*-α-D-mannopyranosyl)-α-D-mannopyranosyl stearate (**24**) incredibly performed the highest activity (Figure 3).

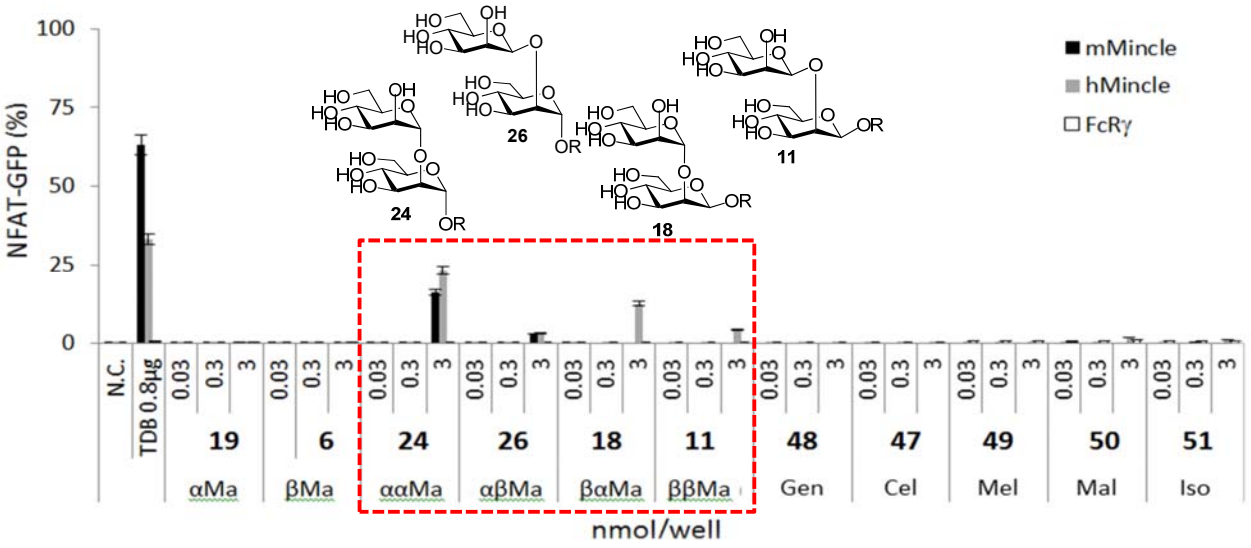


Figure 3: NFAT-GFP reporter cells expressing hMincle/FcRg or mMincle/FcRg or FcRg alone were tested for their reactivity to compounds **6**, **11**, **19**, **24**, **26** and **47**-**51**.

Since the whole ligand **44-2** was as much as potent as TDM, the most active fragment **24** was selective to attach to the D-mannitol core as the mimetic molecule **44-2**. Di- or tri-(di-α-D-mannosyl-stearyl)-D-mannitol (**38** and **40**) together with tri-(β-D-mannosyl-stearyl)-glycerol (**42**) were synthesized and evaluated the Mincle ligand activity (Figure 4).

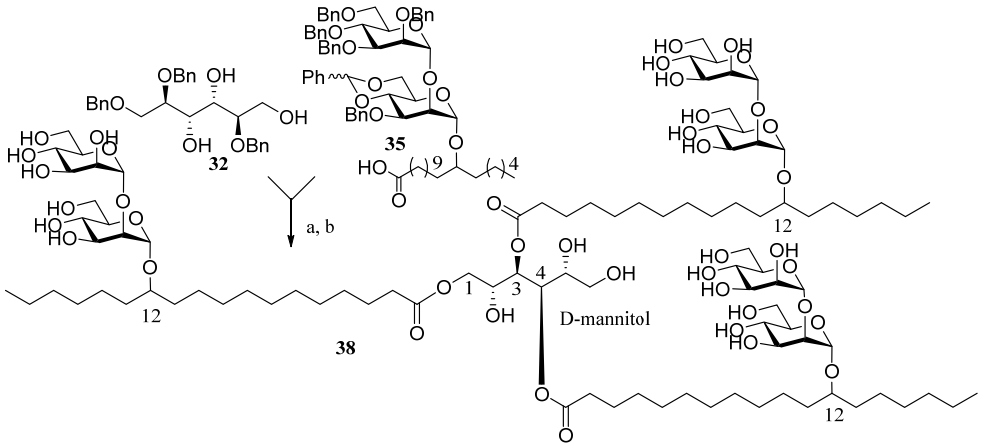


Figure 4: Synthesis of 1,3,4-tri-(di-α-D-mannosyl-stearyl)-D-mannitol **38**
(a) DCC, DMAP, CH_2Cl_2, 61.3%; (b) EtOAc/ *n*-propanol/ THF/ H_2O = 2:1:1:1, 1 atm H_2, Pd-C, 55.8%.

Compound **38** was the potent Mincle ligand, signaled through Mincle receptor at the level of only 0.03 nmol/well. Interestingly, the initially esterification 12-*O*-β-D-mannopyranosyl stearate on glycerol core (**42**) also provided a dramatic enhancement to the activity through Mincle (Figure 5).

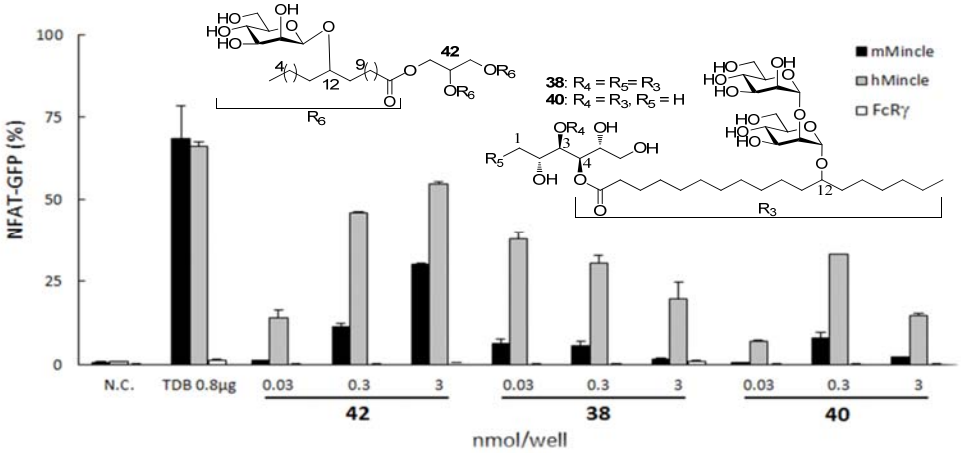


Figure 5: NFAT-GFP reporter cells expressing hMincle/FcRγ or mMincle/FcRγ or FcRγ alone were tested for their reactivity to compounds **38**, **40** and **42**.

Conclusion

In this study, I confirmed that the both the natural fragments **44-2-1** and **44-2-2** were β-anomers. I also found four 12-*O*-di-D-mannopyranosyl stearates as the new type of ligands for the Mincle. Through the esterification of the most active di-α-D-mannopyranosyl stearate **24** on the D-mannitol, I achieved the molecule 1,3,4-tri-(di-α-D-mannosyl-stearyl)-D-mannitol **38** which was the potent Mincle ligand, signaled through Mincle receptor at the level of only 0.03 nmol/well. I progress the synthesis of di-β-D-mannosyl stearates on the D-mannitol or glycerol to develop potent adjuvant candidates.

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