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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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論文名：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

（新規C型レクチン受容体Mincleリガンド，12-*O*-モノ、ジグリコシルステアレートアナログの合成と構造活性相関）

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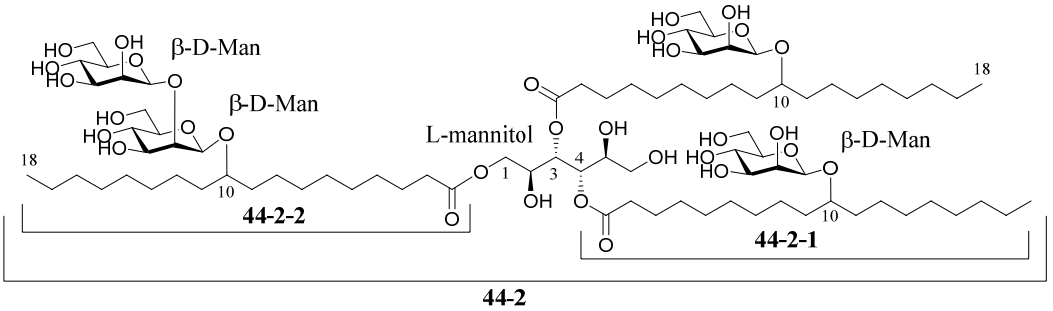
論文審査結果の要旨

下記に示すように、論文提出者は病原性真菌マラセチアより単離・構造決定された、新規ミンクルリガンド活性を有する糖脂質マンノシルステアリルマンニトールアナログ体3種の合成を達成するとともに、その合成過程において、13種の12-*O*-グリコシルステアレート誘導体を合成し、ミンクルリガンド活性を評価し、β-マンノシルステアレートのみならず、α－マンノシルステアレートにもミンクルリガンド活性を有することを明らかにした。以上の研究成果は、マンノシルステアレートアルコール型糖脂質の機能解析および新しいワクチンアジュバント開発に重要な知見を与えるものであり、本学位論文は博士（創薬科学）の学位に値すると判断した。

Introduction

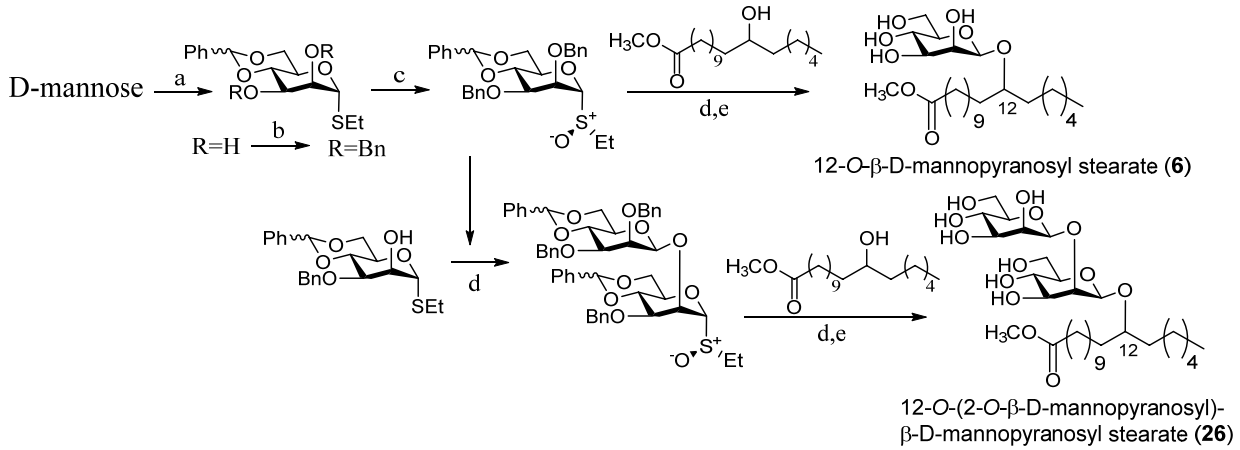
A novel glycolipid **44-2** unusually composed of β-linked mannose residues attached to 10-hydroxystearic acid and in turn esterified onto a L-mannitol core, and it particularly displayed potent activity as much as TDM *via* mouse Mincle (mMincle) was found from pathogenic fungus *M. pachydermatis* in 2013 (Figure 1).[1] 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**. In an effort to confirm the distinguish β-anomeric structures of the active fragment **44-2-1** and **44-2-2**, 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**, respectively.



Results

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*

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 help to confirm that both the natural fragments **44-2-1** and **44-2-2** were β-anomers (Scheme 1). To elucidate the structure-activity relationships of 12-*O*-glycosyl stearate, thirteen synthetic analogues (**11** [β-D-mannosyl-(1-2)-β-D-mannosyl-st]; **18** [α-D-mannosyl-(1-2)-β-D-mannosyl-st]; **19** (α-D-mannosyl-st); 24 [α-D-mannosyl-(1-2)-α-D-mannosyl-st]; **43** (α-D-glucosyl -st); **44** (β-D-glucosyl-st); **45** (α-D-galactosyl-st); **46** (β-D-galactosyl-st); **47** (β-cellobiosyl-st); **48** (β-gentiobiosyl-st); **49** (β-melibiosyl-st); **50** (β-isomaltosyl-st); **51**(β-maltobiosyl-ste) have been prepared by Köenings-Knorr method.



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 **℃**,; (d): TTBP, Tf_2O, MS4A, CH_2Cl_2, -78 **℃**; (e): EtOAC/ *n*-propanol/ THF/ H_2O = 2:1:1:1, 1 atm H_2, Pd-C.

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 and 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. 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**) have been synthesized and evaluated the Mincle ligand activity. 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.

I progress the synthesis of di-β-D-mannosyl stearates on the D-mannitol or glycerol to develop potent adjuvant candidates.

1. Tetsuaki Ishikawa, Tomofumi Miyamoto, and Sho Yamasaki *et al.*, *Cell Host & Microbe*, 2013, **13**, 477-488.