Synthesis of 12-O-Mono- and Diglycosyl-oxystearates, a New Class of Agonists for the C-type Lectin Receptor Mincle

Le Van Huy,[†] Chiaki Tanaka,[†] Takashi Imai,[‡] Sho Yamasaki,[‡] and Tomofumi Miyamoto^{*,†}

[†]Department of Natural Products Chemistry, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812-8582, Japan

[‡]Department of Molecular Immunology, Immunology Frontier Research Center, Osaka University, Osaka 565-0871, Japan

S Supporting Information

ABSTRACT: Fifteen glycosyl-oxystearates were synthesized by Crich's 4,6-benzylidene and Koenig–Knorr strategies. Assessment of structure–activity relationships using macrophage-inducible C-type lectin (Mincle) receptor cells expressing nuclear factor of activated T cells (NFAT)-green fluorescent protein (GFP) revealed that four dimannopyranosyl-oxystearate analogues were Mincle agonists and that 12-O-(2-O- α -D-mannopyranosyl)- α -D-mannopyranosyl-oxystearate was as an activator of both mouse and human Mincle.

KEYWORDS: Glycosyl-oxystearate, Mincle, β -mannosylation, TDM

The finding that macrophage-inducible C-type lectin (Mincle) was a trehalose dimycolate (TDM) receptor suggested a strategy for developing a potent adjuvant from ligands of Mincle receptors.^{1–3} Mincle binding of glycolipids from pathogenic bacteria and fungi, such as TDM, can initiate a signaling cascade through the immunoreceptor tyrosine-base activation motif (ITAM)-containing adaptor molecule Fc receptor γ -chain (FcR γ). Spleen tyrosine kinase (Syk) recruitment by FcR γ leads to the activation of nuclear factor kappa-light-chain enhancer of activated B cells (NF- κ B) through Card9-Bcl10-Malt1 signalosomes, induces the transcription of genes encoding pro-inflammatory chemokines, growth factors, and cytokines, and consequently elicits responses by Th1/Th17 immune cells. The mechanism by which these effects are induced by interactions between Mincle and its ligand remain unclear.^{4–9} Mincle contains three ligand binding sites, including carbohydrate recognition domain (CRD), cholesterol, and SAP130.^{10,11} CRD have four subsites to recognize glycolipid, which consist of two sugar binding sites and two lipid binding sites. The binding of glycol/glycerolipid ligands to at least three of the four binding subsites of the Mincle CRD is regarded as essential to activate the receptor.^{4,12} Research involving TDM, trehalose-6,6-dibehenate (TDB), and other glycolipid ligands has provided clues about the pure and structurally well-defined potential candidate adjuvants.^{13–16} The effectiveness of this research strategy was the identification of the CRD of Mincle

complexed to trehalose. Indeed, the potential of Mincle ligands as vaccine adjuvants can be illustrated by the use of TDB in combination with a cationic quaternary ammonium in a phase 1 clinical trial against tuberculosis.¹⁷

In 2013, we isolated the novel potent Mincle glycolipid ligand **44–2** from the pathogenic fungus *Malassezia pachydermatis*.¹⁸ The structure of **44–2** was unusual, being composed of β -linked D-mannopyranosyl residues attached to 10-hydroxystearic acid, with the latter esterified onto a L-mannitol core. Testing with mouse Mincle (mMincle) showed that this compound was active as TDM. Moreover, the β -mannosyl oxystearates **44–2–1** and **44–2–2** (Figure 1), generated by the alkaline methanolysis of **44–2**, showed weak Mincle ligand activity. To distinguish between the β -anomeric structures of the active fragments **44–2–1** and **44–2–2** and to gain insight into the relationship between **44–2** structure and activity, we synthesized a series of fifteen 12-O-mono- and diglycosyl analogues of **44–2–1** and **44–2–2**, respectively.

Synthesis of the 12-O-mono- and diglycosyl analogues of **44–2–1** and **44–2–2** required the synthesis of a β -mannosidic linkage, a synthesis that may be inhibited due to steric hindrance by secondary glycosyl acceptors.¹⁹ Use of Crich's 4,6-benzylidene,^{20–24} however, enabled the construc-

Received: September 7, 2018

Accepted: December 13, 2018

Published: December 13, 2018

Figure 1. Structures of a novel Mincle ligand **44-2** found from pathogenic fungus *M. pachydermatis* and its alkaline methanolysis products **44-2-1** and **44-2-2**.

Figure 2. ¹H NMR spectra of the natural ligand **44-2-1**, the synthetic analogue **1**, and α-mannosyl-oxystearate **6** (600 MHz, py-d₅, 303 K).

Figure 3. ¹H NMR spectra of the natural ligand **44-2-2** and the synthetic analogue **2** (600 MHz, py-d₅, 303 K).

tion of a β-mannosidic linkage, as well as the successful construction of a β-mannosidic linkage with less active secondary glycosyl acceptors.^{25,26} This method was therefore selected to construct the β-mannosidic linkage of 12-*O*-mono-(**1**) and di(**2**) β-mannosyl-oxystearates.

Briefly, the hydroxyl groups of D-mannose were protected with benzoyl groups, followed by conversion to the thioethyl mannoside. Debenzoylation, and then benzylidenation afforded *S*-ethyl 4,6-*O*-benzylidene-α-mannoside^{14,21} The hydroxyl groups at C-2 and C-3 were protected with benzyl groups to yield the dibenzyl ether **15**. The dibenzyl ether **15** was oxidized to a mannosyl sulfoxide **16**. Using Crich's 4,6-benzylidene, the glycosyl donor **16** (1 equiv) was treated with a glycosyl acceptor (2 equiv of methyl 12-hydroxystearate) to

form the β-mannosylated derivative **17**, in a yield of 44%. The β-mannosidic linkage of intermediate **17** was characterized by a consistent upfield shift at H-5 [δ_{H} 3.33 (m, 1H)].²⁵ Finally, the benzylidene and benzoyl groups of **17** were deprotected, generating the 12-*O*-β-mannosyl oxystearate analogue **1** in a yield of 91%. The upfield chemical shifts of H-1 and H-5 [δ_{H} 4.92 (s, 1H), 3.83 (m, 1H) in py-d₅] and the ¹J_{CH} (155.4 Hz) of **1**²⁷ were not only similar to those of typical β-mannoside but differed from those of its α-mannoside **6** (Table S1 and Scheme 3). The ¹H NMR spectra of synthetic analogue **1** was similar to that of the natural ligand **44-2-1** (Figure 2).

Crich's method was also used to synthesize the 12-*O*-β-dimannosyl oxystearate analogue **2**. Using this method, the prototypical glycosyl donor **16** was modified to yield compound **20** (Scheme 1). Briefly, the hydroxyl group at C-3 of compound **14** was selectively benzylated to yield the glycosyl acceptor **18**, which was treated with the glycosyl donor **16** to form *S*-ethyl β-dimannoside **19**, in a yield of 51%. Compound **19** was converted to the glycosyl donor **20**. The reaction between the glycosyl donor **20** and the glycosyl acceptor methyl 12-hydroxystearate resulted in the β-dimannosylated intermediate **21**, in a yield of 38%. Finally, deprotection of compound **21** resulted in 12-*O*-(2-*O*-β-D-mannopyranosyl)-β-D-mannopyranosyl-oxystearate (analogue **2**), in a yield of 80%. The ¹J_{CH} values of β-dimannosyl oxystearate **2** were estimated to be 158.8 Hz (C1–H1) and 156.3 Hz (C1'–H1'), and the ¹H NMR spectrum was almost identical to that of the natural ligand **44-2-2** (Figure 3).

To investigate the structure–activity relationship of Mincle ligands, we synthesized three dimannosyl isomers **3–5** of **2** as shown in Scheme 2, together with ten 12-*O*-mono or diglycosyl oxystearate **6–13** as shown in Scheme 3. The dimannosyl isomers **3–5** were synthesized using a combination of Koenings–Knorr and Crich's 4,6-benzylidene method. The compound 12-*O*-(2-*O*-α-D-mannopyranosyl)-β-D-mannopyranosyl-oxystearate **3** was synthesized using 2-*O*-*p*-methoxybenzyl-*S*-ethyl sulfoxide **22** as a glycosyl donor, whereas 12-*O*-(2-*O*-α-D-mannopyranosyl)-α-D-mannopyranosyl-oxystearate **4** and 12-*O*-(2-*O*-β-D-mannopyranosyl)-α-D-mannopyranosyl-oxystearate **5** were synthesized using 12-*O*-α-D-mannopyranosyl-oxystearate **6** as a starting material. The ¹J_{CH} value of each mannosidic linkage was estimated by the nondecoupled HSQC method, resulting in ¹J_{CH} value for α-anomers of 168.2–177.5 Hz and for β-anomers of 154.0–158.7 Hz (Figure 4).

The other 12-*O*-mono- and diglycosyl-oxystearates **6–13** were synthesized using the Koenings–Knorr method (Scheme 3).

To assess the structure–activity relationships of these synthesized compounds, we evaluated their ability to activate a nuclear factor of activated T cells (NFAT)-green fluorescent protein (GFP) through Mincle.²⁸ NFAT-GFP reporter cells expressing human Mincle (hMincle)/FcRγ, mouse Mincle (mMincle)/FcRγ, or FcRγ alone were cultured for 16 h on plates that were pre-coated with the indicated amounts of synthetic mono- or diglycosyl oxystearates or TDB as a positive control. The NFAT-dependent activation of cells was monitored by the detection of GFP-positive cells by flow cytometry.

The compound β,β-mannosyl-oxystearate **2**, an analogue of the natural ligand **44-2-2**, showed the ability to signal through both hMincle and mMincle, whereas the compound mono-β-mannosyl-oxystearate **1**, an analogue of the natural ligand **44-2-1**, was unable to signal through either Mincle at

Scheme 1. Synthesis of 12-*O*-Mono- and Di- β -mannosyl-oxystearate Analogues 1 and 2^a

^aReagents and conditions: (a) (i) BzCl, Py, (ii) EtSH, BF₃, Et₂O, 1,2-dichloroethane (DCE), (iii) NaOMe, MeOH (81% over 3 steps), (iv) PhCHO/ZnCl₂ 53%; (b) NaH, BnBr, DMF 91%; (c) *meta*-chloroperoxybenzoic acid (*m*-CPBA), CH₂Cl₂, -78 °C 83% for 16, 78% for 20; (d) 2,4,6-tri-*tert*-butylpyrimidine (TTBP), Tf₂O, molecular sieve 4A (MS4A), CH₂Cl₂, -78 °C, 44% for 17, 51% for 19, 38% for 21; (e) EtOAc/*n*-propanol/THF/H₂O = 2:1:1:1, 1 atm H₂, Pd-C, 1% for 1, 80% for 2; (f) *n*-Bu₂SnO, PhMe, reflux, CsF, BnBr, DMF, 97%.

Scheme 2. Synthesis of 12-*O*-Di-mannosyl-oxystearate Isomers 3, 4, and 5^a

^aReagents and conditions: (a) (i) Bu₄NI, NaH, *para*-methoxybenzyl chloride (PMBCl), DMF, 81.0%, (ii) *m*-CPBA, CH₂Cl₂, -78 °C, -20 °C, 90%; (b) (i) TTBP, Tf₂O, MS4A, CH₂Cl₂, -78 °C, 25%, (ii) 2,3-dichloro-5,6-dicyano-*para*-benzoquinone (DDQ), CH₂Cl₂/H₂O, 80%; (c) AgClO₄, Ag₂CO₃, MS4A, CH₂Cl₂, rt., 5 h, 89%; (d) (i) NaOMe/MeOH, 90%, (ii) EtOAc/*n*-propanol/THF/H₂O = 2:1:1:1, 1 atm H₂, Pd-C, 84%; (e) PhCHO/ZnCl₂, 31%; (f) *n*-Bu₂SnO, PhMe, reflux, CsF, BnBr, DMF, 84%; (g) AgClO₄, Ag₂CO₃, MS4A, CH₂Cl₂, rt., 5 h, 99%; (h) NaOMe/MeOH, 61%, (ii) EtOAc/*n*-propanol/THF/H₂O = 2:1:1:1, 1 atm H₂, Pd-C, 72%; (i) TTBP, Tf₂O, MS4A, CH₂Cl₂, 73%, -78 °C; (j) EtOAc/*n*-propanol/THF/H₂O = 2:1:1:1, 1 atm H₂, Pd-C, 75%.

a concentration of 3 nmol/well (Figure 5). Interestingly, the α -mannosyl-oxystearate 6 showed a weak ability to signal through mMinle. The other monoglycosyl-oxystearates, 7 α / β and 8 α / β , did not signal through Minle at concentrations of 3 nmol/well (data not shown). Surprisingly, the α / α and β / α -dimannosyl isomers 3–5 showed a greater ability to signal through hMinle than β / β -mannosyl-oxystearate 2. In contrast, gentiobiosyl-oxystearate 10 signaled only through mMinle, whereas the other biosyl-oxystearates did not signal through Minle. These results indicated that these dimannosyl-oxystearates 2–5 acted as the agonist of Minle ligands.

Pathogen-derived Minle ligands, including trehalose, glucose, glycerol mycolates, corynomycolates, and various β -gentiobiosyl diacylglycerides, have the ability to signal through Minle. Gentiobiosyl diacylglycerides were isolated from *Mycobacterium tuberculosis* and *M. pachydermatis*, together with the novel potent ligand 44–2. Recently, α -glucosyl diacylglycerides were identified from the pathogenic bacterium *Streptococcus pneumoniae* and the other monoglycosyldiacylglycerol (MGDG) produced from Group A *Streptococcus* and induced proinflammatory cytokines in a Minle-dependent manner.^{9,29}

Figure 4. ^1H NMR spectra of the synthetic isomers **3** and **4** (CD_3OD) and **5** [$\text{CD}_3\text{OD}/\text{CDCl}_3$ (9/1)] (600 MHz, 298 K).

Scheme 3. Synthesis of 12-*O*-Mono- and Diglycosyloxystearates 6–13^a

^aReagents and conditions: (a) BzCl, pydine; (b) 30% HBr/AcOH, CH₂Cl₂; (c) (i) AgClO₄, Ag₂CO₃, MS4A, CH₂Cl₂, (ii) α/β separation by silica gel column chromatography, (iii) NaOMe/MeOH.

A study of the structure–activity relationships of gentiobiosyl diacyl glycerides with various alkyl chains showed that signaling through both human and mouse Mincle was

dependent on acyl chain length, as had been previously determined for the activation of mMincle by trehalose glycolipids of different length.¹⁶ Although the gentiobiosyl-oxystearate **10** showed weak signaling through mMincle, the dimannosyl-oxystearates **2–5** signaled through both human and mouse Mincle. The amino acid structures of hMincle and mMincle are 85% identical,³⁰ with both sharing the conservative binding motif responsible for the interaction between mMincle and the sugar headgroup of TDM.^{31–33} The carbohydrate moieties of gentiobiosyl diacylglycerides have been reported to bind to the same region of Mincle as trehalose, in the trehalose-Mincle-carbohydrate recognition domain (CRD) complex. Both human and mouse Mincle can bind to mannose monocorynomycolate (ManMCM), and mannose 2-tetradecyloctadecanoate (ManC₁₄C₁₈), as well as to their corresponding glucose derivatives.⁴ However, the monomannosyl-oxystearates **1** and **6** and the monoglycosyl-oxystearates **7** and **8** did not signal through Mincle. Additional research is required to decipher the interaction of the CRD of human and mouse Mincle with dimannosyl-oxystearates.

In conclusion, the results of this study indicated that dimannosyl-oxystearates can act as novel agonists of Mincle ligands, and the compound that consists of dimannosyl oxystearates esterified to mannitol may act as a Mincle ligand. These results show that **44-2-2** and analogues, in which the position of sugar modification on the lipid is varied as well as when the nature of the sugar group itself is changed, may have potential as adjuvants.

■ ASSOCIATED CONTENT

S Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acsmedchemlett.8b00413](https://doi.org/10.1021/acsmedchemlett.8b00413).

Figure 5. NFAT-GFP reporter cells expressing human Mincle (hMincle)/FcR γ , mouse Mincle (mMincle)/FcR γ , or FcR γ alone were tested for their reactivity to trehalose dibehenate (TDB; 0.8 nmol/well) and 12-*O*-mono- and diglycosyl-oxystearates **1–6** and **9–13**.

General experimental procedures, characterization data, ^1H and ^{13}C NMR spectra, and ESI-MS of new compounds (PDF)

AUTHOR INFORMATION

Corresponding Author

*Tel: +81-92-642-6636. E-mail: miyamoto@phar.kyushu-u.ac.jp.

ORCID

Tomofumi Miyamoto: 0000-0003-1589-5807

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

L.V.H. would like to thank the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan for financial support (scholarship). This research was supported by a Grant-in-Aid for Research on Innovative Areas (No. 26100).

REFERENCES

- (1) Schoenen, H.; Bodendorfer, B.; Hitchens, K.; Manzanero, S.; Werninghaus, K.; Nimmerjahn, F.; Agger, E. M.; Stenger, S.; Andersen, P.; Ruland, J.; Brown, G. D.; Wells, C.; Lang, R. Mincle Is Essential for Recognition and Adjuvanticity of the Mycobacterial Cord Factor and its Synthetic Analog Trehalose-Dibehenate. *J. Immunol.* **2010**, *184*, 2756–2760.
- (2) Ishikawa, E.; Ishikawa, T.; Morita, Y. S.; Toyonaga, K.; Yamada, H.; Takeuchi, O.; Kinoshita, T.; Akira, S.; Yoshikai, Y.; Yamasaki, S. Direct Recognition of the Mycobacterial Glycolipid, Trehalose Dimycolate, by C-type Lectin Mincle. *J. Exp. Med.* **2009**, *206*, 2879–2888.
- (3) Huber, A.; Kallerup, R. S.; Korsholm, K. S.; Franzyk, H.; Lepenies, B.; Christensen, D.; Foged, C.; Lang, R. Trehalose Diester Glycolipids Are Superior to the Monoesters in Binding to Mincle, Activation of Macrophages In Vitro and Adjuvant Activity in Vivo. *Innate Immun.* **2016**, *22*, 405–418.
- (4) Decout, A.; Silva-Gomes, S.; Drocourt, D.; Barbe, S.; André, I.; Cueto, F. J.; Lioux, T.; Sancho, D.; Pérouzel, E.; Vercellone, A.; Prandi, J.; Gilleron, M.; Tiraby, G.; Nigou, J. Rational Design of Adjuvants Targeting the C-type Lectin Mincle. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, 2675–2680.
- (5) Williams, S. J. Sensing Lipids with Mincle: Structure and Function. *Front. Immunol.* **2017**, *8*, 1662.
- (6) Braganza, C. D.; Teunissen, T.; Timmer, M. S. M.; Stocker, B. L. Identification and Biological Activity of Synthetic Macrophage Inducible C-Type Lectin Ligands. *Front. Immunol.* **2018**, *8*, 1940.
- (7) Foster, A. J.; Nagata, M.; Lu, X.; Lynch, A. T.; Omahdi, Z.; Ishikawa, E.; Yamasaki, S.; Timmer, M. S. M.; Stocker, B. L. Lipidated Bratemicin Analogues Are Potent TH1-Stimulating Vaccine Adjuvants. *J. Med. Chem.* **2018**, *61*, 1045–1060.
- (8) Nagata, M.; Omahdi, Z.; Yamasaki, S. Pathogen-Sensing by Mincle: Function and Molecular Aspects. In *C-Type Lectin Receptors in Immunity*; Yamasaki, S., Ed.; Springer: Japan, 2016; pp 15–34.
- (9) Imai, T.; Matsumura, T.; Mayer-Lambertz, S.; Wells, C. A.; Ishikawa, E.; Butcher, S. K.; Barnett, T. C.; Walker, M. J.; Imamura, A.; Ishida, H.; Ikebe, T.; Miyamoto, T.; Ato, M.; Ohga, S.; Lepenies, B.; van Sorge, N. M.; Yamasaki, S. Lipoteichoic Acid Anchor Triggers Mincle to Drive Protective Immunity against Invasive Group A *Streptococcus* Infection. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115*, E10662–E10671.
- (10) Kiyotake, R.; Oh-hora, M.; Ishikawa, E.; Miyamoto, T.; Ishibashi, T.; Yamasaki, S. Human Mincle Binds to Cholesterol Crystals and Triggers Innate Immune Responses. *J. Biol. Chem.* **2015**, *290*, 25322–25332.
- (11) Yamasaki, S.; Ishikawa, E.; Sakuma, M.; Hara, H.; Ogata, K.; Saito, T. Mincle Is an ITAM-coupled Activating Receptor that Senses Damaged Cells. *Nat. Immunol.* **2008**, *9*, 1179–1188.
- (12) Stocker, B. L.; Khan, A. A.; Chee, S. H.; Kamena, F.; Timmer, M. S. M. On One Leg: Trehalose Monoesters Activate Macro in a Mincle-Dependent Manner. *ChemBioChem* **2014**, *10*, 382–388.
- (13) Geijtenbeek, T. B.; Gringhuis, S. I. C-type Lectin Receptors in the Control of T Helper Cell Differentiation. *Nat. Rev. Immunol.* **2016**, *16*, 433–448.
- (14) van der Peet, P. L.; Gunawan, C.; Torigoe, S.; Yamasaki, S.; Williams, S. J. Corynomycolic Acid-containing Glycolipids Signal Through the Pattern Recognition Receptor Mincle. *Chem. Commun.* **2015**, *51*, 5100–5103.
- (15) Smith, D. G.; Williams, S. J. Immune Sensing of Microbial Glycolipids and Related Conjugates by T Cells and the Pattern Recognition Receptors MCL and Mincle. *Carbohydr. Res.* **2016**, *420*, 32–45.
- (16) Richardson, M. B.; Torigoe, S.; Yamasaki, S.; Williams, S. J. Mycobacterium Tuberculosis β -gentiobiosyl Diacylglycerides Signal Through the Pattern Recognition Receptor Mincle: Total Synthesis and Structure Activity Relationships. *Chem. Commun.* **2015**, *51*, 15027–15030.
- (17) van Dissel, J. T.; Joosten, S. A.; Hoff, S. T.; Soonawala, D.; Prins, C.; Hokey, D. A.; O'Dee, D. M.; Graves, A.; Thierry-Carstensen, B.; Andreasen, L. V. A Novel Liposomal Adjuvant System, CAF01, Promotes Long-lived Mycobacterium Tuberculosis-specific T-cell Responses in Human. *Vaccine* **2014**, *32*, 7098–7107.
- (18) Ishikawa, T.; Itoh, F.; Yoshida, S.; Saijo, S.; Matsuzawa, T.; Gono, T.; Saito, T.; Okawa, Y.; Shibata, N.; Miyamoto, T.; Yamasaki, S. Identification of Distinct Ligands for the C-type Lectin Receptors Mincle and Dectin-2 in the Pathogenic Fungus *Malassezia*. *Cell Host Microbe* **2013**, *13*, 477–488.
- (19) Crich, D.; Sun, S. Direct Synthesis of α -Mannopyranosides by the Sulfoxide Method. *J. Org. Chem.* **1997**, *62*, 1198–1199.
- (20) Stick, R. V.; Williams, S. J. *Carbohydrates: The Essential Molecules of Life*, 2nd ed.; Elsevier: Great Britain, 2010.
- (21) Frihed, T. G.; Bols, M.; Pedersen, C. M. Mechanisms of Glycosylation Reactions Studied by Low-Temperature Nuclear Magnetic Resonance. *Chem. Rev.* **2015**, *115*, 4963–5013.
- (22) Crich, D.; Chandrasekera, N. S. Mechanism of 4,6-O-Benzylidene-Directed β -Mannosylation as Determined by α -Deuterium Kinetic Isotope Effects. *Angew. Chem., Int. Ed.* **2004**, *43*, 5386–5389.
- (23) Crich, D.; Hu, T.; Cai, F. Does Neighboring Group Participation by Non-Vicinal Esters Play a Role in Glycosylation Reactions? Effective Probes for the Detection of Bridging Intermediates. *J. Org. Chem.* **2008**, *73*, 8942–8953.
- (24) Crich, D. Mechanism of a Chemical Glycosylation Reaction. *Acc. Chem. Res.* **2010**, *43*, 1144–1153.
- (25) Crich, D.; Banerjee, A.; Yao, Q. Direct Chemical Synthesis of the α -D-Mannans: The α -(1–2) and α -(1–4) Series. *J. Am. Chem. Soc.* **2004**, *126*, 14930–14934.
- (26) Dromer, F.; Chevalier, R.; Sendid, B.; Improvisi, L.; Jouault, T.; Robert, R.; Mallet, J. M.; Poulain, D. Synthetic Analogues of β -1,2-Oligomannosides Prevent Intestinal Colonization by the Pathogenic Yeast *Candida albicans*. *Antimicrob. Agents Chemother.* **2002**, *46*, 3869–3876.
- (27) Bock, K.; Pederson, C. J. A Study of ^{13}C Coupling Constants in Hexopyranoses. *J. Chem. Soc., Perkin Trans. 2* **1974**, *2*, 293–299.
- (28) Ishikawa, E.; Ishikawa, T.; Morita, Y. S.; Toyonaga, K.; Yamada, H.; Takeuchi, O.; Kinoshita, T.; Akira, S.; Yoshikai, Y.; Yamasaki, S. Direct Recognition of the Mycobacterial Glycolipid, Trehalose Dimycolate, by C-type Lectin Mincle. *J. Exp. Med.* **2009**, *206*, 2879–2888.
- (29) Behler-Janbeck, F.; Takano, T.; Maus, R.; Stolper, J.; Jonigk, D.; Tarrés, M.-T.; Fuehner, T.; Prasse, A.; Welte, T.; Timmer, M. S. M.; Stocker, B. L.; Nakanishi, Y.; Miyamoto, T.; Yamasaki, S.; Maus, U. A. C-type Lectin Mincle Recognizes Glucosyldiacylglycerol of *Strepto-*

coccus Pneumoniae and Plays a Protective Role in Pneumococcal Pneumonia. *PLoS Pathog.* **2016**, *12*, PPAT1006038.

(30) Matsumoto, M.; Tanaka, T.; Kaisho, T.; Sanjo, H.; Copeland, N. G.; Gilbert, D. J.; Jenkins, N. A.; Akira, S. A Novel LPS-Inducible C-Type Lectin Is a Transcriptional Target of NF-IL6 in Macrophages. *J. Immunol.* **1999**, *163*, 5039–5048.

(31) Rambaruth, N. D.; Jégouzo, S. A.; Marlor, H.; Taylor, M. E.; Drickamer, K. Mouse Mincle: Characterization as a Model for Human Mincle and Evolutionary Implications. *Molecules* **2015**, *20*, 6670–6682.

(32) Jégouzo, S. A.; Harding, E. C.; Acton, O.; Rex, M. J.; Fadden, A. J.; Taylor, M. E.; Drickamer, K. Defining the Conformation of Human Mincle that Interacts with Mycobacterial Trehalose Dimycolate. *Glycobiology* **2014**, *24*, 1291–1300.

(33) Feinberg, H.; Jégouzo, S. A.; Rowntree, T. J.; Guan, Y.; Brash, M. A.; Taylor, M. E.; Weis, W. I.; Drickamer, K. Mechanism for Recognition of an Unusual Mycobacterial Glycolipid by the Macrophage Receptor Mincle. *J. Biol. Chem.* **2013**, *288*, 28457–28465.