Thieme Chemistry Journal Awardees – Where are They Now? Synthesis of the Marine Glycolipid Dioctadecanoyl Discoside

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Abstract: The first synthesis of the inositol-containing marine glycolipid dioctadecanoyl discoside is reported. The key glycosylation reaction proceeds with β-selectivity at reduced temperature. The separable anomers could be readily progressed to afford discoside, its peracetate and the unnatural β-derivatives.

Key words: marine sponge, glycolipid, mannose, glycosylation, inositol

The mannose-*myo*-inositol linkage is a ubiquitous structural feature of glycosylphosphatidylinositol (GPI) anchored cell surface proteins essential for cell–cell recognition processes in animals and bacteria.[1] The phosphatidylinositol mannosides (PIMs) and their multiglycosylated lipomannan (LM) forms present in the cell walls of pathogenic *Mycobacterium* are implicated in initial infection and subsequent modulation of the immune response.[2] The resurgence of *Mycobacterium tuberculosis* and the onset of multidrug resistance[3] has prompted significant interest in the evaluation of smaller natural PIMs[4] and synthetic analogues[5] as biological probes and potential new therapeutic agents that act via activation of cytokine production, thus promoting a pro-inflammatory response. Studies by Dunne and co-workers have also shown that carbohydrate fatty acid ester derivatives display inhibitory activity against Gram-positive *Straphylococcus aureus*.[6]

Isolated in 2005 by Fattorusso and co-workers, discoside (**1**; Figure 1) is a unique marine glycolipid, obtained from the Caribbean deep-sea sponge *Discodermia dissoluta,*[7] which has proven to be a rich source of novel bioactive secondary metabolites. Structurally, discoside is the first example to be isolated from either marine or terrestrial sources of a 4,6-*O*-diacylated mannoside α-linked to the 2-hydroxyl of a *myo*-inositol unit; the 4,6-*O*-fatty acyl chains in discoside are present as a mixture of octadecanoate (33% by lipid composition), 10- (51%) and 12-methyloctadecanoate (16%) homologues, which were not separated. The only closely related analogue to **1** is 1-*O*-pentadecanoyl-2-*O*-(6-*O*-heptadecanoyl-α-D-mannopyranosyl)-*myo*-inositol (**2**), isolated in 1968 from strains of terrestrial *Propionibacterium,*[8] which suggests that **1** is produced by a symbiotic cyanobacteria associated with the marine sponge. The unknown biological function and unusual structure of discoside, coupled with our ongoing interest in the synthesis of *myo*-inositol-containing compounds and their value as biological probes,[9] prompted this synthetic venture. Herein, we report the total synthesis of dioctadecanoyl discoside (**3**) and its corresponding peracetate derivative.

As outlined in Scheme 1, our synthetic strategy relied on a challenging glycosylation of the axial C2 alcohol in benzyl-protected inositol **4** with the D-mannose-derived thioglycoside **5**. In order to increase overall convergency, we chose to incorporate the C18 lipid sidechains directly in **5**, thereby minimizing the number of post-coupling transformations and allowing direct access to **3** by global benzyl deprotection, in preference to employing anchimeric assistance and the necessity for further protecting group manipulations.

Dr. Gordon J. Florence was born in Manchester, UK. He gained a BA degree in Natural Sciences from Cambridge University in 1997. He received his PhD from Cambridge University in 2001, under the supervision of Professor Ian Paterson FRS, working on the total synthesis of discodermolide. From 2001 to 2002, he undertook postdoctoral research with Professor Craig J. Forsyth at the University of Minnesota. In 2002, he returned to the group of Professor Paterson in Cambridge to take up a Research Fellowship at Emmanuel College focussing on the synthesis of cytotoxic marine macrolides. In 2005, he was elected to a Royal Society University Research Fellowship and moved to the University of St Andrews, where his research is directed towards the synthesis and structure determination of bioactive natural products and the development of new synthetic methodology. He was awarded the Thieme Chemistry Journal Award in 2006.

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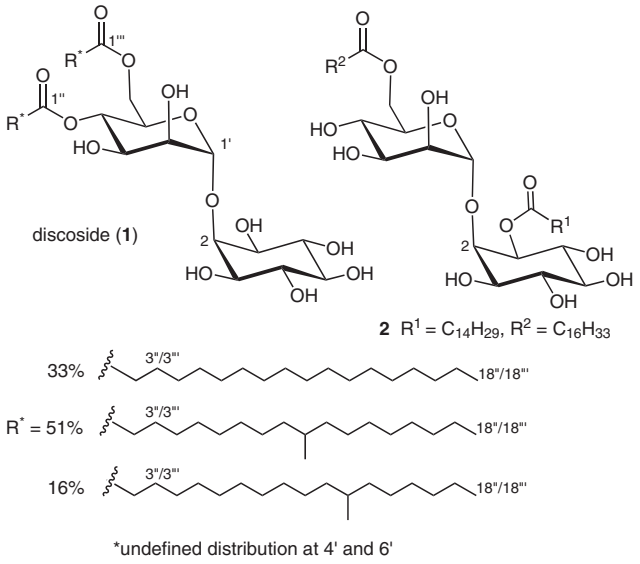
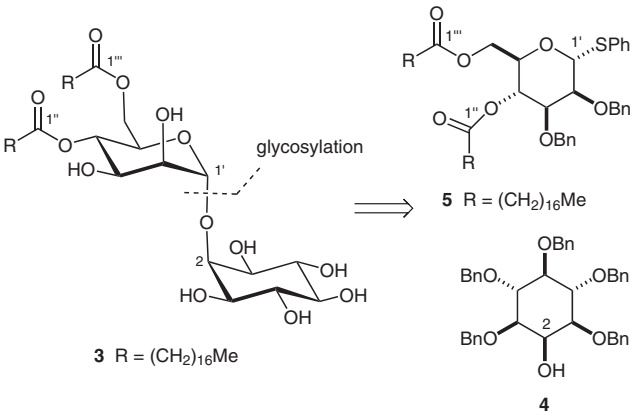


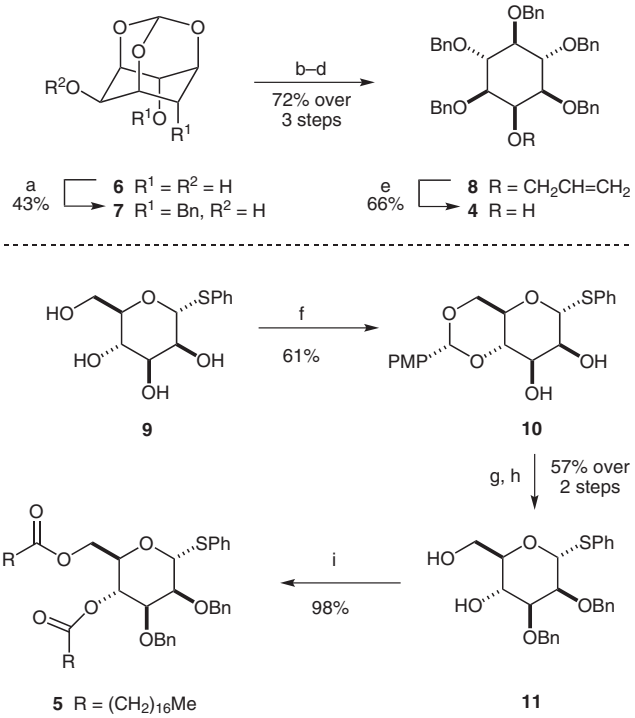
Figure 1 Structure of discoside (**1**) and structurally related 1-*O*-pentadecanoyl-2-*O*-(6-*O*-heptadecanoyl-α-D-mannopyranosyl)-*myo*-inositol (**2**)



Scheme 1 Retrosynthetic strategy for dioctadecanoyl discoside (**3**)

As shown in Scheme 2, the synthesis of the inositol subunit **4** started with benzylation of the axial hydroxyls of the orthoformate **6**.[10–12] Allylation of the remaining hydroxyl in **7** was followed by cleavage of the orthoformate (3M HCl/MeOH, reflux) and benzylation (NaH/BnBr/DMF) to afford **8** in 72% yield over three steps. Boon's modified Rh-mediated isomerisation of allyl ether **8** [$(Ph_3P)_3RhCl$, *n*-BuLi],[13] and acidic methanolysis (AcCl/MeOH), completed the inositol subunit **4** in 66% yield.

With the inositol subunit **4** in hand, attention was then directed towards the preparation of the thiomannoside donor **5**, as shown in Scheme 2. Starting from α-D-phenylthiomannoside **9**, which was readily available in three steps from D-mannose,[14] temporary protection of the 4- and 6-position hydroxyl groups was achieved by treatment with *p*-$MeOC_6H_4CH(OMe)_2$ and CSA (20 mol%) to give **10** in 61% yield.[14a] The remaining two hydroxyl groups were then benzylated under standard conditions (BnBr, NaH, TBAI), prior to acidic methanolysis (CSA, $MeOH/CH_2Cl_2$) of the anisylidene acetal to provide diol

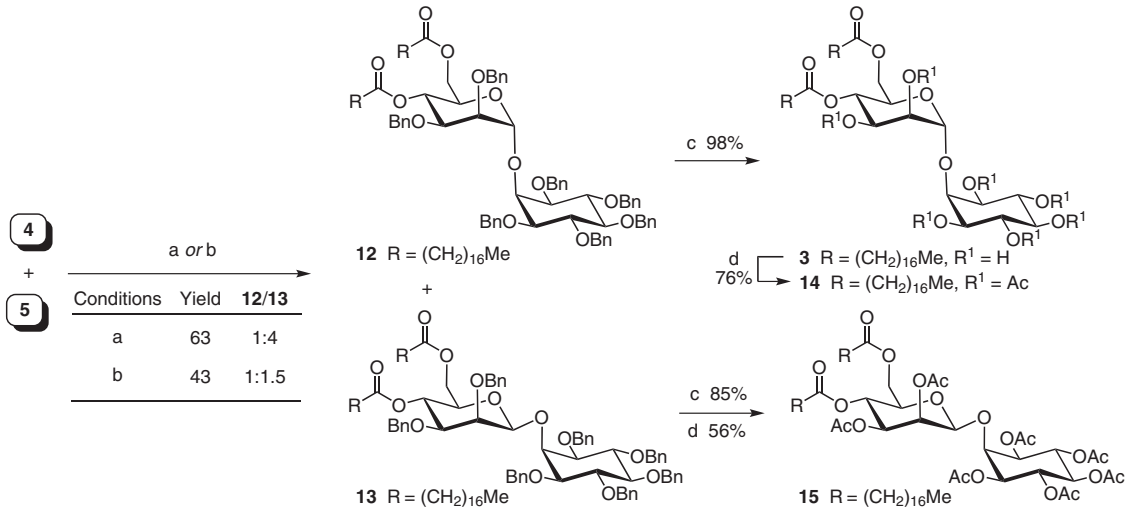


Scheme 2 *Reagents and conditions*: (a) NaH, BnBr, DMF, 0 °C→r.t., 18 h; (b) AllBr, NaH, imidazole, DMF, 0 °C→r.t., 22 h; (c) HCl, MeOH, 20 min, reflux; (d) NaH, BnBr, DMF, 0 °C→r.t., 5 h; (e) (i) $(Ph_3P)_3RhCl$, *n*-BuLi, THF, reflux, 5 h; (ii) HCl, MeOH, CH_2Cl_2, r.t., 3 h; (f) *p*-$MeOC_6H_4CH(OMe)_2$, *p*-$TsOH{\cdot}H_2O$ (15 mol%), DMF, 50 °C, 130 mmHg, 3 h; (g) NaH, BnBr, TBAI, DMF, 0 °C→r.t., 3 h; (h) CSA (20 mol%), MeOH–CH_2Cl_2 (3:1), 75 °C, 1 h; (i) octadecanoic acid, DCC, DMAP, CH_2Cl_2, 16 h.

11 in 57% yield over two steps. The required introduction of the two octadecanoate ester sidechains proceeded smoothly under Steglich conditions (DCC, DMAP, CH_2Cl_2) to provide the α-thiomannoside donor **5** in excellent yield.[15]

With key subunits **4** and **5** in hand, we then focused on the key glycosylation reaction (Scheme 3). Thus, activation of **5** [NIS (2.5 equiv), TESOTf (1.9 equiv), CH_2Cl_2, –40 to –20 °C] and reaction with **6** afforded a mixture of anomers **12** and **13** in 63% yield with an α:β ratio of 1:4,[16,17] which were separable by column chromatography. In an effort to improve the α-selectivity of the process, we found that performing the reaction at 0 °C led to a combined 43% yield of **12** and **13** with an α:β ratio of 1:1.5. The observed β-selectivity can be rationalised by the formation of a low-temperature stable α-triflate intermediate following the activation of **5**,[14a,17] which, in combination with the preferential equatorial addition of the axial 2-hydroxyl of the inositol acceptor, leads to the β-linkage.[4a,18] Completion of the total synthesis by global debenzylation via hydrogenolysis of **12** with catalytic Pd black at atmospheric pressure provided 4,6-octadecanoyl discoside **3** in excellent yield, as an amorphous solid.

Spectroscopic characterization of **3** proved challenging due to its insolubility across a range of solvents (DMSO-d_6, CD_3OD, $CDCl_3$, D_2O, $CD_3OD/CDCl_3$).[19] In order to



Scheme 3 *Reagents and conditions*: (a) TESOTf (1.9 equiv), NIS (2.5 equiv), CH_2Cl_2, 4 Å MS, –40 to –20 °C, 2 h; (b) TESOTf (1.9 equiv), NIS (2.5 equiv), CH_2Cl_2, 4 Å MS, 0 °C, 1.5 h; (c) Pd black, EtOH, H_2 (1 atm), r.t., 1 h; (d) Ac_2O, pyridine, r.t., 16 h.

overcome this problem, synthetic **3** was treated with Ac_2O in pyridine to give the peracetate **14** in 76% yield (Scheme 3), according to the original isolation by Fattorusso.[7] In this case, the spectroscopic data (^1H and ^{13}C NMR, IR, MS) and specific rotation {synthetic $[\alpha]_D^{20}$ +11.3 (*c* 0.37, $CHCl_3$) cf. natural $[\alpha]_D^{25}$ +12 (*c* 0.5, $CHCl_3$)}[7] for the synthetic material were in excellent agreement with those reported for natural discoside peracetate.[20] In an analogous manner, the β-anomer **13** was readily transformed into its respective β-peracetate **15**.[21] This allowed detailed NOE and HSQC analysis of the synthetic α- and β-anomers. Characteristic $^1J_{C1'\text{-}H1'}$ anomeric coupling constants of 175 and 162 Hz for **14** and **15** were observed in the respective HSQC experiments, thus providing unambiguous confirmation of the C1′-configuration in **1**.[7]

In conclusion, we have completed the first synthesis of dioctadecanoyl discoside (**3**) and its peraceatate derivative via a convergent route that proceeds in eight steps from *myo*-inositol. The key glycosylation reaction proceeds with high levels of β-selectivity at low temperature, and further studies of 4,6-*O*-acylated phenylthiomannosides are underway to examine the utility of this effect for the synthesis of β-glycosides. With access to both anomers, deprotection directly afforded both the dioctadecanoyl discoside (**3**) and the unnatural β-analogue, and the respective peracetate derivatives enabled direct spectroscopic comparison. Assessment of both the natural and unnatural anomers for biological function and antimicrobial activity is currently being undertaken and will be reported in due course.

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(19) The ^{1}H NMR of **3** in 1% $CD_3OD/CDCl_3$ gave a broad, unresolved spectrum. Selected data for **3**: Amorphous colourless solid; mp 110–115 °C; ^{1}H NMR (400 MHz, 1% $CD_3OD/CDCl_3$): δ = 5.40–4.90 (m, 4 H), 4.50–3.30 (m, 8 H), 2.40–2.10 (m, 4 H, H-2″ and H-2‴), 1.65–1.40 (m, 4 H, H-3″ and H-3‴), 1.20 (s, 56 H, 28 × CH_2-lipid), 0.85–0.75 (m, 6 H, H-18″ and H-18‴); LRMS (MALDI-TOF): *m/z* (%) = 897.9 (20) $[M + Na]^+$, 453.5 (100).
(20) Data for α-peracetate **14**: Oil; R_f = 0.46 (PE–EtOAc, 6:4); $[\alpha]_D^{20}$ +11.3 (*c* 0.73 $CHCl_3$) (Lit.[3] +12.0, *c* 0.5 $CHCl_3$); IR (neat): 2918.2, 2850.3, 1753.2, 1369.3, 1225.2, 1043.9 cm^{-1}; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 5.53 (dd, *J* = 10.3, 10.2 Hz, 1 H, H-4), 5.50 (dd, *J* = 10.4, 9.6 Hz, 1 H, H-6), 5.43–5.40 (m, 2 H, H-3′ and H-4′), 5.38–5.36 (m, 1 H, H-2′), 5.19 (t, *J* = 9.6 Hz, 1 H, H-5), 5.09 (dd, *J* = 10.4, 2.4 Hz, 1 H, H-3), 5.00 (dd, *J* = 10.8, 2.8 Hz, 1 H, H-1), 4.95 (d, *J* = 1.6 Hz, 1 H, H-1′), 4.30 (t, *J* = 2.8 Hz, 1 H, H-2), 4.26 (dd, *J* = 12.3, 4.2 Hz, 1 H, H-6′a), 4.20–4.15 (m, 1 H, H-5′), 4.09 (dd, *J* = 12.3, 2.4 Hz, 1 H, H-6′b), 2.36–2.27 (m, 4 H, H-2″ and H-2‴), 2.14 (s, 3 H), 2.09 (s, 3 H), 2.07 (s, 3 H), 2.03 (s, 3 H), 2.02 (s, 3 H), 2.01 (s, 3 H), 2.00 (s, 3 H), 1.65–1.56 (m, 4 H, H-3″ and H-3‴), 1.33–1.22 (m, 56 H, 28 × CH_2-lipid), 0.89–0.83 (m, 6 H, H-18″ and H-18‴); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 173.4, 172.5, 169.84, 169.78, 169.76, 169.6, 169.5, 169.4, 169.3, 99.5, 76.4, 70.6, 70.4, 69.6 (3 × C), 69.5, 69.3, 68.7, 65.2, 61.7, 34.1, 34.0, 31.9, 29.7, 29.7, 29.6, 29.5, 29.5, 29.4, 29.3, 29.3, 29.2, 29.1, 24.9, 24.7, 22.7, 20.8, 20.7, 20.7, 20.6 (2 × C), 20.5, 20.5, 14.1; LRMS (ES$^+$): *m/z* (%) = 1191.7 (100) $[M + Na]^+$; HRMS (ES$^+$): *m/z* $[M + Na]^+$ calcd for $C_{62}H_{104}O_{20}Na$: 1191.7019; found: 1191.7043.
(21) Data for β-peracetate **15**: Oil; R_f = 0.67 (PE–EtOAc, 6:4); $[\alpha]_D^{20}$ –20.0 (*c* 0.73, $CHCl_3$); IR (neat): 2923.1, 2853.6, 1753.3, 1369.4, 1225.5, 1043.8 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.58 (dd, *J* = 3.2, 0.8 Hz, 1 H, H-2′), 5.41 (t, *J* = 10.4 Hz, 1 H, H-4), 5.39 (t, *J* = 10.0 Hz, 1 H, H-6), 5.23 (t, *J* = 10.0 Hz, 1 H, H-4′), 5.11 (t, *J* = 9.6 Hz, 1 H, H-5), 5.10–5.05 (m, 2 H, H-3 and H-3′), 4.70 (dd, *J* = 10.4, 2.4 Hz, 1 H, H-1), 4.67 (d, *J* = 0.8 Hz, 1 H, H-1′), 4.51 (t, *J* = 2.8 Hz, 1 H, H-2), 4.23 (dd, *J* = 12.4, 6.0 Hz, 1 H, H-6′a), 4.05 (dd, *J* = 12.4, 2.4 Hz, 1 H, H-6′b), 3.57 (ddd, *J* = 10.0, 6.4, 1.6 Hz, 1 H, H-5′), 2.40–2.30 (m, 2 H, H-2″), 2.29–2.23 (m, 2 H, H-2‴), 2.27 (s, 3 H), 2.09 (s, 3 H), 2.03 (s, 3 H), 2.01 (s, 3 H), 2.00 (s, 3 H), 2.00 (s, 3 H), 1.97 (s, 3 H), 1.63–1.54 (m, 4 H, H-3″ and H-3‴), 1.33–1.22 (m, 56 H), 0.88 (t, *J* = 6.8 Hz, 6 H, H-18″ and H-18‴); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 173.4, 172.5, 169.84, 169.78, 169.76, 169.6, 169.5, 169.4, 169.3, 99.5, 76.4, 70.6, 70.4, 69.6 (3 × C), 69.5, 69.3, 68.7, 65.2, 61.7, 34.1, 34.0, 31.9, 29.7, 29.7, 29.6, 29.5, 29.5, 29.4, 29.3, 29.3, 29.2, 29.1, 24.9, 24.7, 22.7, 20.8, 20.7, 20.7, 20.6 (2 × C), 20.5, 20.5, 14.1; LRMS (ES$^+$): *m/z* (%) = 1191.7 (100) $[M + Na]^+$; HRMS (ES$^+$): *m/z* $[M + Na]^+$ calcd for $C_{62}H_{104}O_{20}Na$: 1191.7019; found: 1191.7015.