Total syntheses of fully lipidated glycosylphosphatidylinositol anchors of *Toxoplasma gondii*†

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Received (in Cambridge, UK) 27th January 2005, Accepted 21st February 2005
First published as an Advance Article on the web 10th March 2005
DOI: 10.1039/b501373a

Modular syntheses of the glycosylphosphatidylinositol anchors of *Toxoplasma gondii* using a highly convergent strategy are reported.

Toxoplasma gondii is a ubiquitous protozoan parasite that is the origin of congenital infection and severe encephalitis, a major cause of death among AIDS patients.[1] Toxoplasmosis is a benign and asymptomatic infection that can be controlled by the immune system of healthy adults, even though primordial infection during pregnancy may cause congenital toxoplasmosis with the risk of severe infection of the fetus.[2] Glycosylphosphatidylinositols (GPIs) are a class of naturally occurring glycolipids that link proteins and glycoproteins *via* their C-terminus to cell membranes. GPI anchors of *T. gondii* are low molecular weight antigens that elicit an early immune response in humans.[3,4] During toxoplasmal pathogenesis GPIs induce the production of tumor necrosis factor-α (TNFα).[5] Purified *T. gondii* GPIs induce the production of TNFα in macrophages.[5]

Structural analyses of GPI anchors revealed a conserved core structure containing a row of mannoses that is connected to inositol *via* a glucosamine unit. Inositol carries a phospholipid, while the mannoses are decorated by one or more phosphate ethanolamine moieties. Different GPIs have been synthesized using various synthetic strategies and methodologies.[6] Synthetic GPI oligosaccharides are needed to evolve a detailed structure–activity relationship of GPI anchors from different species. Here we report the first total synthesis of a series of fully lipidated *T. gondii* GPI glycans (Fig. 1).

Strategically, complete GPI glycan backbone hexasaccharide **3**, equipped with orthogonal protective groups that mark points to be modified at a later stage, served as our initial key target. Silyl and allyl ethers mask the sites to be phosphorylated, using H-phosphonate chemistry.[7] Hexasaccharide **3** was to be prepared *via* a 4 + 2 coupling between a branched tetrasaccharide and a glucosamine-inositol pseudodisaccharide (Fig. 1). Access to the target structures **1** and **2** would require introduction of the phosphate diester appendages using different H-phosphonate reagents, before hydrogenolysis would result in global deprotection.

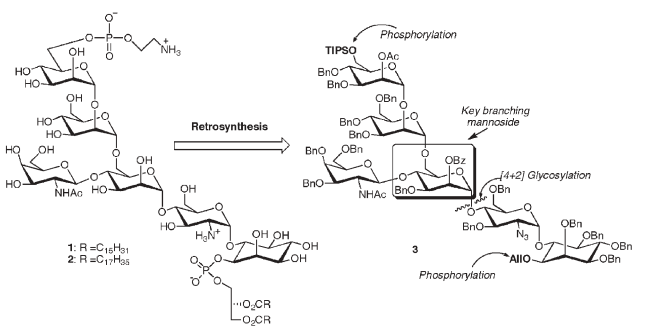
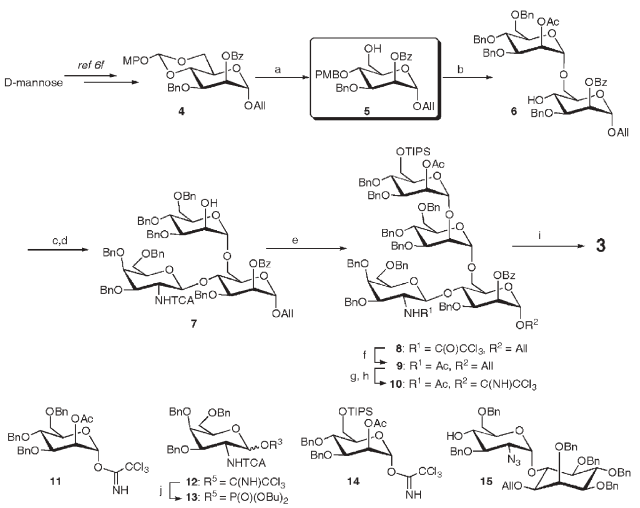


Fig. 1 GPIs of *T. gondii* origin and retrosynthetic plans.

Executing this plan, the assembly of tetrasaccharide trichloroacetimidate **10** commenced with the preparation of key mannoside **5** for the installation of the branching galactosamine moiety. Differentially protected mannose **4**[6f] was readily available from D-mannose. The regioselective opening of the *p*-methoxybenzylidene group of mannose **4** by exposure to sodium cyanoborohydride under trimethylsilyl chloride activation furnished **5**,[8] where the 6-hydroxyl group was available for elongation of the mannose chain (Scheme 1). Coupling of mannosyl trichloroacetimidate **11**[9] and 4-*O*-PMB-protected mannoside **5** provided disaccharide **6** with concomitant removal of the PMB ether protective group thus readying the nucleophile for the next glycosidation. The acid lability of the PMB group can be exploited to access branched or unbranched GPI structures by varying the temperature of the coupling reaction. At low temperatures the PMB group is retained



Scheme 1 Assembly of key hexasaccharide **3**. a) $NaCNBH_3$, TMSCl, CH_3CN, 67%; b) **11**, TMSOTf, CH_2Cl_2, 92%; c) **13**, TMSOTf, CH_2Cl_2, 93%; d) HCl (AcCl, CH_3OH), 70%; e) **14**, TMSOTf, CH_2Cl_2, 98%; f) AIBN, Bu_3SnH, toluene, 94%; g) $PdCl_2$, NaOAc, AcOH, H_2O, 83%; h) Cl_3CCN, DBU, CH_2Cl_2, 86%; i) **15**, TMSOTf, CH_2Cl_2, 91%; j) $HOP(O)(OBu)_2$, toluene, 58%.

† Electronic supplementary information (ESI) available: copies of ^1H, ^{13}C and ^{31}P NMR spectra for all new compounds. See http://www.rsc.org/suppdata/cc/b5/b501373a/

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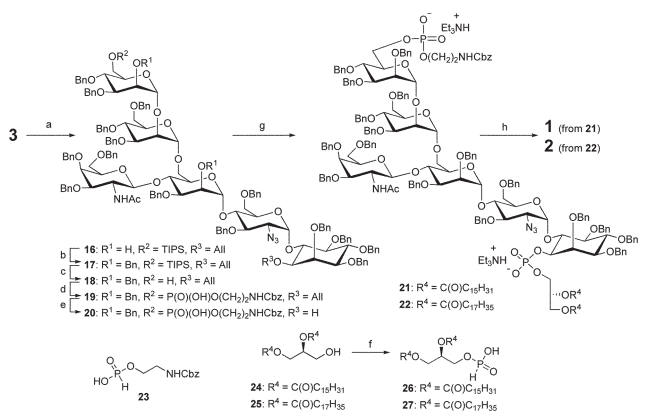
and thus access to unbranched glycans can be achieved, while at higher temperatures the PMB group is lost and branched structures are obtained. A host of reaction conditions failed to induce the coupling of disaccharide **6** and galactosamine trichloroacetimidate **12**.[6f] However, galactosamine dibutylphosphate **13**, derived from **12**, readily reacted with **6** to give trisaccharide in 93% yield. At the stage of the trisaccharide, an acetate ester had to be cleaved while leaving the benzoate unscathed. Treatment with methanolic hydrogen chloride cleanly lived up to the task. Glycosylation of trisaccharide **7** by activation of differentially protected mannose **14**[10] yielded tetrasaccharide **8**. The *N*-trichloroacetyl group present in **8** was transformed into the resident *N*-acetate by treatment with AIBN and Bu_3SnH furnishing **9**. Preparation of tetrasaccharide unit **10** proceeded *via* removal of the anomeric allyl group with palladium(II) chloride in acetate buffer,[11] followed by installation of the trichloroacetimidate leaving group with trichloroacetonitrile and DBU. Coupling of tetrasaccharide **10** with glucosamine-inositol pseudodisaccharide **15**[6d,7a] afforded key hexasaccharide **3** in 91% yield.

The protecting groups present in **3** were readied for further functionalization by replacing all esters by benzyl ethers to avoid base treatment at the final stages, when lipid ester groups have been placed. Ester cleavage by sodium methoxide was followed by benzylation with benzyl bromide (2.2 molar eq.) and sodium hydride to give **17**. The undesired *N*-benzylation was not observed (Scheme 2). Desilylation by exposure to scandium triflate freed the hydroxyl group that served as the site of phosphorylation using H-phosphonate **23**[6a] before oxidation with wet iodine provided **19**. Now, in anticipation of a further phosphorylation and thereby introduction of different phospholipids, treatment with palladium(II) chloride in acetate buffer[11] removed the allyl ether to give **20**. Two H-phosphonates (**26** and **27**) were derived from commercially available glycerols **24** and **25** following established procedures.[12] Installation of the phospholipids **26** and **27** afforded fully protected hexasaccharides **21** and **22** respectively. Only global deprotection remained and was accomplished in a single step by hydrogenolysis over Pearlman's catalyst.[6c] The synthetic target GPIs **1** and **2** were obtained in excellent yield.



Scheme 2 Synthesis of GPI **1** and **2**. a) NaOMe, MeOH–CH_2Cl_2, 82%; b) BnBr, NaH, TBAI, 4Å MS, DMF, 88%; c) $Sc(OTf)_3$, H_2O, CH_3CN, 97%; d) **23**, PivCl, pyridine, then I_2, H_2O, 95%; e) $PdCl_2$, NaOAc, AcOH, H_2O, 87%; f) PCl_3, imidazole, Et_3N, toluene, 90–95%; g) **26** (or **27**), PivCl, Py, then I_2, H_2O, 65–75%; h) $Pd(OH)_2$, H_2, AcOH, $CHCl_3$–CH_3OH–H_2O (10:10:3), 90–99%.

In conclusion, we have developed a convergent synthetic route to fully lipidated GPI glycans of *T. gondii*. Our synthetic strategy provides the flexibility to vary the GPI core structure to access other GPI structures for biological investigations. The new synthetic GPIs **1** and **2** found on *T. gondii* serve as molecular probes to assay the effect of defined GPIs on macrophages and to establish a structure–activity relationship for GPI-receptor interactions.‡

This research was supported by the ETH Zürich. Y.-U. Kwon thanks Korea Science and Engineering Foundation (KOSEF) for financial support (Postdoctoral Fellowship).

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Notes and references

‡ Compounds **21** and **22**: a mixture of **20** (0.01 mmol) and H-phosphonate **26** or **27** (0.05 mmol) was co-evaporated with pyridine (3 × 3 mL) and dried *in vacuo*. To a solution of the residue in pyridine (2 mL) was added pivaloyl chloride (0.1 mmol) at room temperature. After the mixture had been stirred for 5 h, water (0.05 mL) and iodine (0.06 mmol) were added. After 1 h, the mixture was diluted with CH_2Cl_2 and washed with 1 M aqueous $Na_2S_2O_3$ solution and water. The organic phase was dried over $MgSO_4$, filtered and concentrated. Chromatography (MeOH–CH_2Cl_2 gradient with 1% Et_3N) afforded fully protected hexasaccharides **21** and **22** (65–75%), respectively. Compound **21**: $[\alpha]_D^{25}$ + 19.0 (c = 1.65 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85–0.89 (m, 6H), 1.19–1.37 (m, 48H), 1.48–1.58 (m, 4H), 1.94 (s, 3H), 2.19–2.24 (m, 4H), 3.10–5.02 (m, 85H), 5.21–5.24 (m, 1H), 5.91 (d, J = 3.3 Hz, 1H), 6.37 (m, 1H), 7.05–7.35 (m, 90H); ^{31}P NMR (121 MHz, $CDCl_3$): δ −1.31, 0.32; HRMS-ESI (*m*/*z*): calcd for $C_{202}H_{243}N_5O_{42}P_2$ $[M]^{2-}$, 1736.8265; found 1736.8240. Compound **22**: $[\alpha]_D^{25}$ + 23.1 (c = 1.0 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85–0.89 (m, 6H), 1.13–1.36 (m, 56H), 1.47–1.59 (m, 4H), 1.94 (s, 3H), 2.17–2.26 (m, 4H), 3.20–5.04 (m, 85H), 5.21–5.24 (m, 1H), 5.92 (d, J = 3.6 Hz, 1H), 6.38 (m, 1H), 7.05–7.33 (m, 90H); ^{31}P NMR (121 MHz, $CDCl_3$): δ −1.17, 0.54; HRMS-ESI (*m*/*z*): calcd for $C_{206}H_{251}N_5O_{42}P_2$ $[M]^{2-}$, 1764.8578; found 1764.8606.

Compounds **1** and **2**: a mixture of **21** or **22** (5.0 μmol), $Pd(OH)_2$/C (30 mg) and acetic acid (25 mg) in a mixed solvent (CH_3OH:$CHCl_3$ = 1:1, 2 mL) was stirred under H_2 (1 atm). After 5 h, water (0.3 mL) was added. After stirring for 30 h, the catalyst was filtered off through a pad of Celite and the solvent was evaporated under reduced pressure. The crude products were triturated with CH_2Cl_2 to give the GPI glycans **1** and **2** (90–99%), respectively. Compound **1**: ^{1}H NMR (300 MHz, DMSO-d_6:D_2O = 40:1, selected peaks only): δ 0.80–0.84 (m, 6H), 1.17–1.30 (m, 48H), 1.37–1.46 (m, 4H), 1.81 (s, 3H), 2.23–2.26 (m, 4H), 4.26 (d, J = 4.5 Hz, 1H), 4.76 (s, 1H), 4.89 (s, 1H), 5.06 (s, 1H); ^{31}P NMR (121 MHz, DMSO-d_6:D_2O = 40:1): δ −1.22, −0.28; MALDI-TOF-MS (*m*/*z*): calcd for $C_{75}H_{140}N_3O_{40}P_2$ $[M + Na]^+$, 1806.8; found 1807.2. Compound **2**: ^{1}H NMR (300 MHz, DMSO-d_6:D_2O = 40:1, selected peaks only): δ 0.81–0.88 (m, 6H), 1.16–1.24 (m, 48H), 1.40–1.48 (m, 4H), 1.82 (s, 3H), 2.23–2.28 (m, 4H), 4.26 (d, J = 4.2 Hz, 1H), 4.81 (s, 1H), 4.85 (s, 1H), 5.05 (s, 1H); ^{31}P NMR (121 MHz, DMSO-d_6:D_2O = 40:1) δ −1.98, −0.83; MALDI-TOF-MS (*m*/*z*): calcd for $C_{79}H_{148}N_3O_{40}P_2$ $[M + H]^+$, 1840.9; found 1841.0.

1 B. J. Luft and J. S. Remington, *Clin. Infect. Dis.*, 1992, **15**, 211–222.
2 J. S. Remington and G. Desmonts, in *Infectious Diseases of the Fetus and Newborn Infant*, ed. J. S. Remington and J. O. Klein, W. B. Saunders Co., Philadelphia, 1990, pp. 89–195.
3 B. Striepen, C. F. Zinecker, J. B. L. Damm, P. A. T. Melgers, G. J. Gerwig, M. Koolen, J. F. G. Vliegenthart, J. F. Dubremetz and R. T. Schwarz, *J. Mol. Biol.*, 1997, **266**, 797–813.
4 B. Striepen, J. F. Dubremetz and R. T. Schwarz, *Biochemistry*, 1999, **38**, 1478–1487.
5 F. Debierre-Grockiego, N. Azzouz, J. Schmidt, J. F. Dubremetz, H. Geyer, R. Geyer, R. Weingart, R. R. Schmidt and R. T. Schwarz, *J. Biol. Chem.*, 2003, **278**, 32987–32993.

6 (*a*) C. Murakata and T. Ogawa, *Carbohydr. Res.*, 1992, **235**, 95–114; (*b*) T. G. Mayer, B. Kratzer and R. R. Schmidt, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 2177–2181; (*c*) A. S. Campbell and B. Fraser-Reid, *J. Am. Chem. Soc.*, 1995, **117**, 10387–10388; (*d*) D. K. Baeschlin, A. R. Chaperon, L. G. Green, M. G. Hahn, S. J. Ince and S. V. Ley, *Chem. Eur. J.*, 2000, **6**, 172–186; (*e*) M. Martín-Lomas, N. Khiar, S. García, J. L. Koessler, P. M. Nieto and T. W. Rademacher, *Chem. Eur. J.*, 2000, **6**, 3608–3621; (*f*) K. Pekari, D. Tailler, R. Weingart and R. R. Schmidt, *J. Org. Chem.*, 2001, **66**, 7432–7442; (*g*) K. Pekari and R. R. Schmidt, *J. Org. Chem.*, 2003, **68**, 1295–1308; (*h*) J. Xue and Z. Guo, *J. Am. Chem. Soc.*, 2003, **125**, 16334–16339; (*i*) J. Lu, K. N. Jayaprakash, U. Schlueter and B. Fraser-Reid, *J. Am. Chem. Soc.*, 2004, **126**, 7540–7547.

7 (*a*) P. H. Seeberger, R. R. Soucy, Y. U. Kwon, D. A. Snyder and T. Kanemitsu, *Chem. Commun.*, 2004, 1706–1707; (*b*) X. Liu and P. H. Seeberger, *Chem. Commun.*, 2004, 1708–1709.

8 R. Johansson and B. Samuelsson, *J. Chem. Soc., Perkin Trans. 1*, 1984, 2371–2374.

9 F. Yamazaki, S. Sato, T. Nukada, Y. Ito and T. Ogawa, *Carbohydr. Res.*, 1990, **201**, 31–50.

10 M. C. Hewitt, D. A. Snyder and P. H. Seeberger, *J. Am. Chem. Soc.*, 2002, **124**, 13434–13436.

11 T. Ogawa, S. Nakabayashi and T. Kitajima, *Carbohydr. Res.*, 1983, **114**, 225–236.

12 I. Lindh and J. Stawinski, *J. Org. Chem.*, 1989, **54**, 1338–1342.

