Inositolphosphoglycan Mediators Structurally Related to Glycosyl Phosphatidylinositol Anchors: Synthesis, Structure and Biological Activity

Manuel Martín-Lomas,*[a] Noureddine Khiar,[a] Salud García,[a] Jean-Luc Koessler,[a] Pedro M. Nieto,[a] and Thomas W. Rademacher[b]

Abstract: The preparation of the pseudopentasaccharide **1a**, an inositolphosphoglycan (IPG) that contains the conserved linear structure of glycosyl phosphatidylinositol anchors (GPI anchors), was carried out by using a highly convergent 2+3-block synthesis approach which involves imidate and sulfoxide glycosylation reactions. The preferred solution conformation of this structure was determined by using NMR spectroscopy and molecular dynamics simulations prior to carrying out quantitative structure–activity relationship studies in connection with the insulin signalling process. The ability of **1a** to stimulate lipogenesis in rat adipocites as well as to inhibit cAMP dependent protein kinase and to activate pyruvate dehydrogenase phosphatase was investigated. Compound **1a** did not show any significant activity, which may be taken as a strong indication that the GPI anchors are not the precursors of the IPG mediators.

Keywords: carbohydrates · conformation analysis · inositols · synthesis design · transduction

Introduction

The initial discovery that insulin causes the cleavage of a glycolipid to generate diacylglycerol and modulators of the enzyme cAMP phosphodiesterase[1] has led to the proposal of a new intracellular signalling system which has been postulated to operate for a number of growth factors, classical hormones and cytokines.[2] According to this proposal, the binding of these agonists to their receptors results in the enzymatic cleavage of some inositol-containing glycolipids to produce uncharacterised inositol-containing glycans that modulate the activity of a number of enzymes and mediate a variety of cellular events.[2] These mediators have been called inositolphosphoglycans (IPGs) and their precise chemical structures are still unknown. The chemical composition of these mediators,[3, 4] however, reveals a close structural similarity with the glycan chain of the glycosyl phosphatidylinositols (GPIs) that serve to attach proteins to the outer face of cellular membranes through a covalent linkage (GPI anchors).[5] This glycan chain in all protein GPI anchors presents the conserved linear structure Man$\alpha(1\rightarrow2)$ Man$\alpha(1\rightarrow6)$ Man$\alpha(1\rightarrow4)$ GlcNH$_2\alpha(1\rightarrow6)$ *myo*-Ino (**1**) so that the diversity within the anchors is only reflected in the nature and the location of branching groups.[5] The structural similarities between GPI anchors and IPG mediators is supported by immunological evidence, as antibody probes generated against this GPI glycan chain cross-react with IPG preparations from rat liver and chicken embryo and compounds with IPG-like activities can be removed by immunoprecipitation from the culture medium of insulin stimulated cells.[6–8] These and related data have been taken as an indication that the GPI anchors could be the precursors of the IPG mediators by the sequential action of a lipase and a protease.[2] However, data on the chemical composition of insulin-sensitive glycolipids[9, 3] and radiomethylation studies[11, 12] have been interpreted as indicative of the existence of distinct members of the GPI family, with different structure from the GPI anchors and without anchoring function (free GPIs), as the real precursors of the intracellular IPG mediators.[2] In this context, recent reports by Müller and co-workers have shown that a compound derived from a GPI-anchored ectoprotein from *Saccharomyces cerevisiae*, after protease and phospholipase C cleavage, is able to regulate cellular glucose and lipid metabolism in an insulin-like fashion in insulin-resistant cells and tissues.[13–15] The structure assigned by Müller to this compound is presented as containing the basic features of the glycan chain of a GPI anchor in which the glycosidic linkage between the nonacetylated D-glucos-

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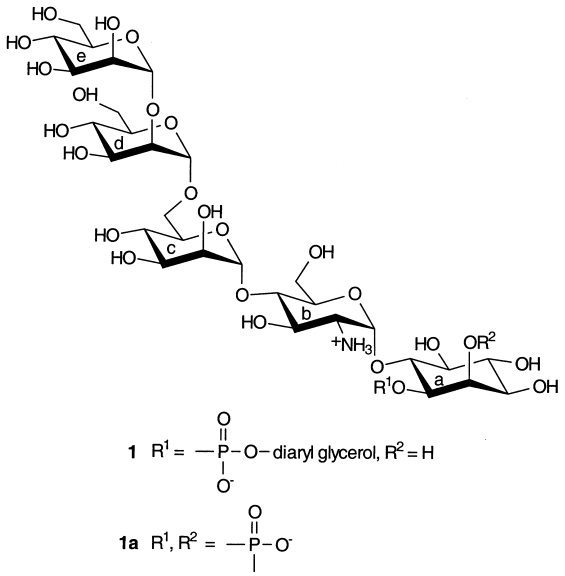
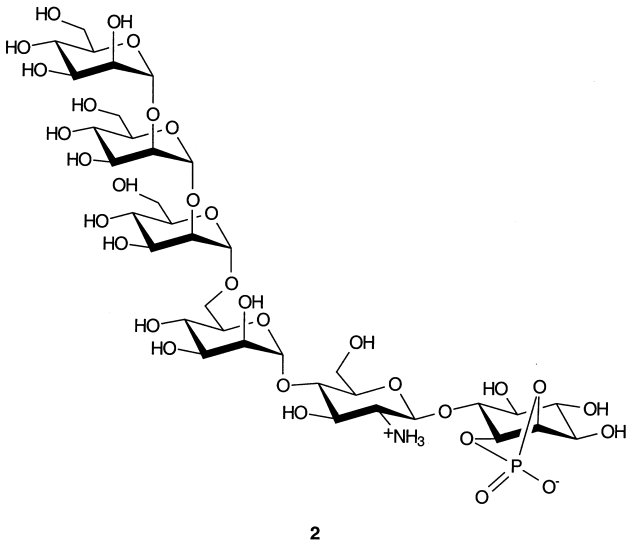




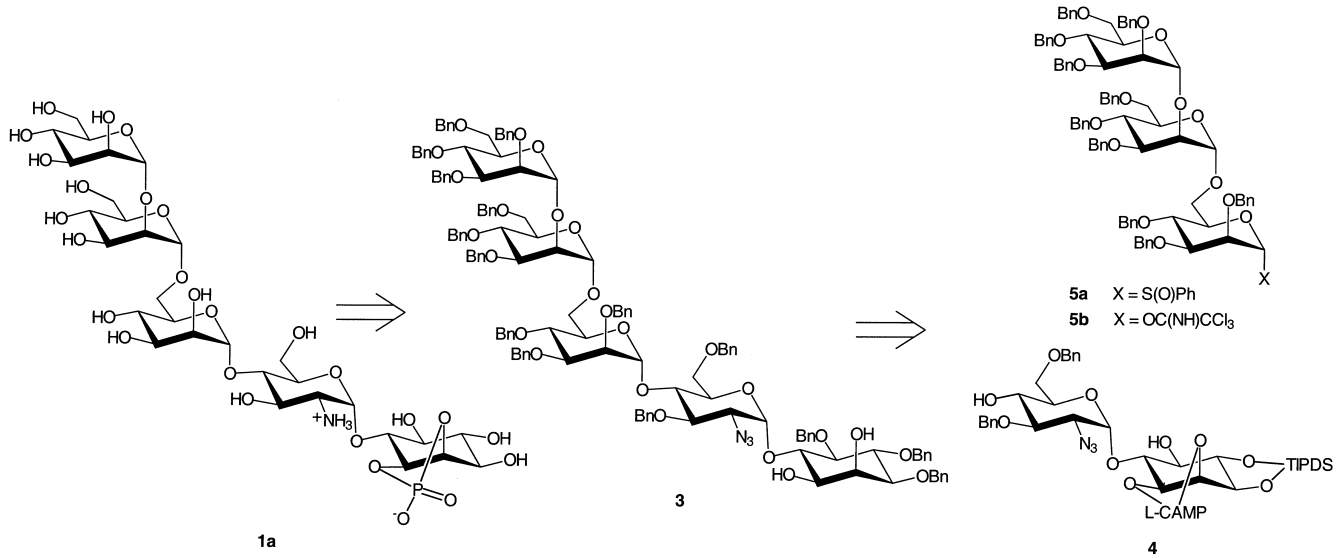
amine unit and the *myo*-inositol moiety posses the β-configuration (**2**).[13] Müller and co-workers have also shown that a number of synthetic compounds, which consist of the complete structure of this glycan chain and variations thereof, stimulate lipogenesis by up to 90% of the maximal insulin response at 20 μM concentration.[16] These results, which may in principle be taken as an indication that the GPI anchors of membrane proteins are the precursors of the intracellular IPG mediators, have nevertheless added further uncertainty in this regard, as the configuration of the glycosidic linkage between the nonacetylated glucosamine unit and the *myo*-inositol ring was shown to be α (**1**) in all the structures of GPI anchors investigated so far.[5]

In the context of our previous studies directed towards establishing the molecular basis of this new intracellular signalling mechanism,[17, 18] it seemed mandatory to unequivocally demonstrate at this stage whether a pure sample of the conserved linear glycan chain of the GPI anchors, prepared by chemical synthesis and fully characterised, shows any appreciable insulin-like activity. This would greatly contribute to the disclosure of the nature of the insulin-sensitive glycolipids either as GPI anchors or as free GPIs. Such an approach involves the development of an effective synthesis of **1** and the investigation of its insulin-like activity. Assuming that a phosphatidylinositol-specific phospholipase C is involved in the first stage of the GPI-IPG signalling mechanism, the 1,2-cyclic phosphate **1a** was the molecule of choice in the present investigation. We have, therefore, carried out an effective synthesis of **1a** and have investigated the capacity of this compound to stimulate lipogenesis in rat adipocites, to inhibit cAMP-dependent protein kinase (PKA) and to activate pyruvate dehydrogenase phosphatase (PDH phosphatase). In addition, we have performed a complete investigation of the conformational and dynamic properties of **1a** in solution as a prerequisite for further structure–activity relationship studies with some key enzymes, such as PKA[19] and glycogen synthase phosphatase 2C—considered to be an excellent model for mitochondrial phosphatases such as the PDH phosphatase[20]—which possesses a well-established binding site geometry determined by X-ray structure analysis.[21–23]

Abstract in Spanish: *Se ha llevado a cabo la síntesis del pseudopentasacárido **1a**—un inositolfosfoglicano (IPG) que contiene la estructura lineal de los glicosil fosfatidilinositoles de anclaje (GPI anchors)—siguiendo un esquema sintético [2+3] altamente convergente que implica reacciones de glicosilación por el método del imidato y por el método del sulfóxido. Se ha determinado también la conformación preferida de esta molécula en solución utilizando métodos de dinámica molecular y espectroscopía de RMN como un paso necesario para futuros estudios de relación estructura-actividad en conexión con el proceso de señalización de la insulina. Por último, se ha estudiado la capacidad del compuesto **1a** para estimular la lipogénesis en adipocitos de rata, inhibir la quinasa dependiente de AMP cíclico y activar la fosfatasa de la piruvato deshidrogenasa. El compuesto **1a** no muestra ninguna actividad significativa en estos sistemas lo que indica que los GPI de anclaje no son los precursores de los segundos menajeros de tipo IPG.*

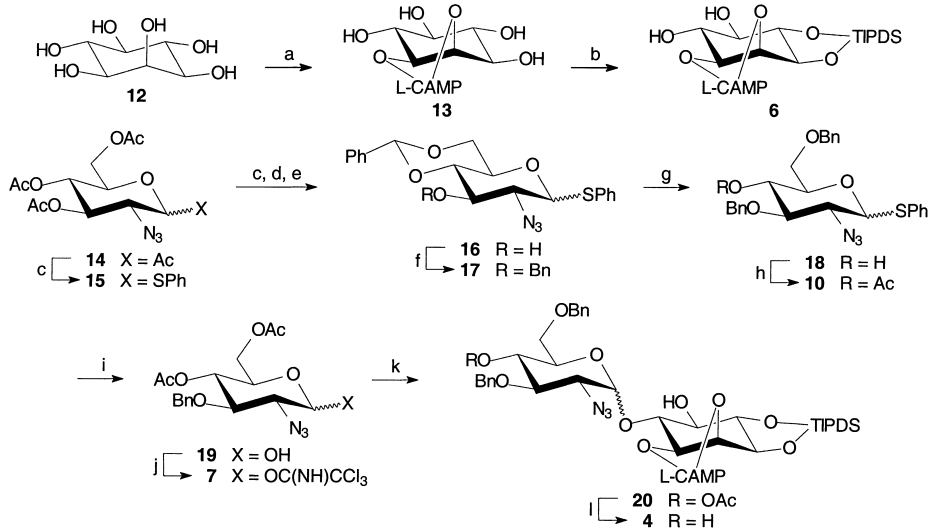
Results and Discussion

Synthesis: The total synthesis of the diacylglycerol-containing GPI anchors of *Trypanosoma brucei*[24, 25] either with the L-[24, 25a] or D-configuration[25b] of the *myo*-inositol and rat brain Thy-1 antigen,[26] and that of the ceramide-containing GPI anchor from yeast[27, 28] have been previously reported by using different synthetic strategies and glycosylation methodologies. A number of partial structures have also been successfully prepared[17, 18, 29–53] and most of this work has been reviewed.[54] Our synthetic approach to structure **1a** is shown in Scheme 1. It was designed as a convergent and versatile route to these inositol-containing glycans that may permit the preparation of other members of the IPG family by using the appropriate substitution pattern in the corresponding building blocks. The retrosynthetic analysis in Scheme 1



Scheme 1. Retrosynthetic steps from **1a**.

shows that the preparation of **1a** was envisaged from diol **3** after phosphorylation and subsequent deprotection. Diol **3** is prepared by using a 2+3-block synthesis approach from the pseudodisaccharide building block **4** and homotrisaccharide building block **5**. The other building blocks involved in the process include the optically pure *myo*-inositol derivative **6** (Scheme 2) and the monosaccharide derivatives **7** (Scheme 2),



Scheme 2. a) L-CAMP$(OMe)_2$, H_2SO_4, DMSO 39%; b) $TIPDSCl_2$, Py, 93%; c) PhSH, $BF_3 \cdot Et_2O$, CH_2Cl_2, 74%; d) NaOMe, MeOH; e) $PhCH(OMe)_2$, TsOH, 92% over the two steps; f) NaH, BnBr, DMF; g) $NaCNBH_3$, HCl, ET_2O; h) Ac_2O, NEt_3, DMAP, CH_2Cl_2, 70% over the three steps; i) NBS, H_2O, Me_2CO, 93%; j) Cl_3CCN, K_2CO_3, CH_2Cl_2, 89%; k) **6**, TMSOTf, Et_2O, 75% (α/β, 3:2); l) NH_3, MeOH, quantitative.

8 and **9** (Scheme 4, see below). These last three either have a phenylthio grouping at the anomeric position (**8**) or are obtained from a phenylthioglycoside intermediate (**7** from **10** and **9** from **11**). The synthesis of pseudodisaccharide building block **4** was based on chemistry developed in our laboratory[18] while that of homotrisaccharide building block **5** was performed by using the sulfoxide glycosylation reaction.[55]

The preparation of **4** was carried out following the synthetic strategy previously reported by us[18] as shown in Scheme 2. The *myo*-inositol (**12**) was desymmetrised over the course of a one-pot reaction as originally reported by Bruzik,[56] and the optically pure ketal **13** was treated with the bifunctional protecting agent 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TIPDSCl_2$) to give diol **6** (39% overall yield from commercially available **12**) which can be regioselectively glycosylated at the 6-position.[57] Regio- and stereoselective glycosylation of **6** is most conveniently performed[18] by using the trichloroacetimidate procedure[58] with 2-azido-2-deoxy glycosyl donors that bear protective group patterns compatible with the further transformations required and designed to provide an acceptable reactivity–selectivity balance in the subsequent glycosylation reaction. These glycosyl donors, such as **7**, are easily accessible from commercially available 2-amino-2-deoxy sugar hydrochlorides through a diazo-transfer reaction from triflic azide, previously reported by us,[59] which affords peracetylated derivatives, such as **14**, in a one-pot manner in three steps. The subsequent transformations of these peracetates to give glycosyl donors with an appropriate substitution pattern is most conveniently carried out[18, 60] via a phenylthioglycoside intermediate, such as **15**, owing to the stability and additional versatility of this function.[60–64] Thus trichloroacetimidate **7** was readily prepared from **15** by using the sequence indicated in Scheme 2. Coupling of **6** with **7** in

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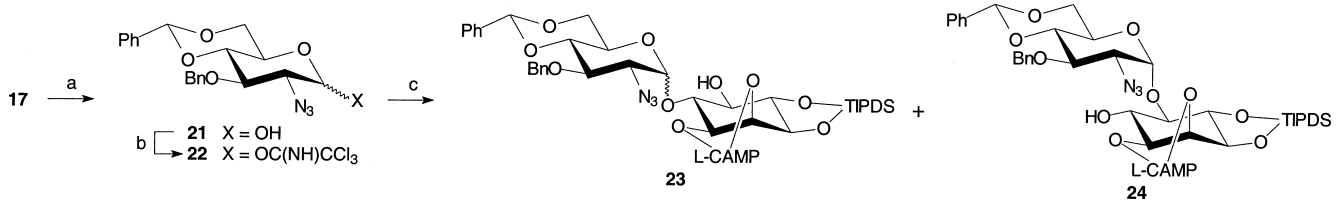
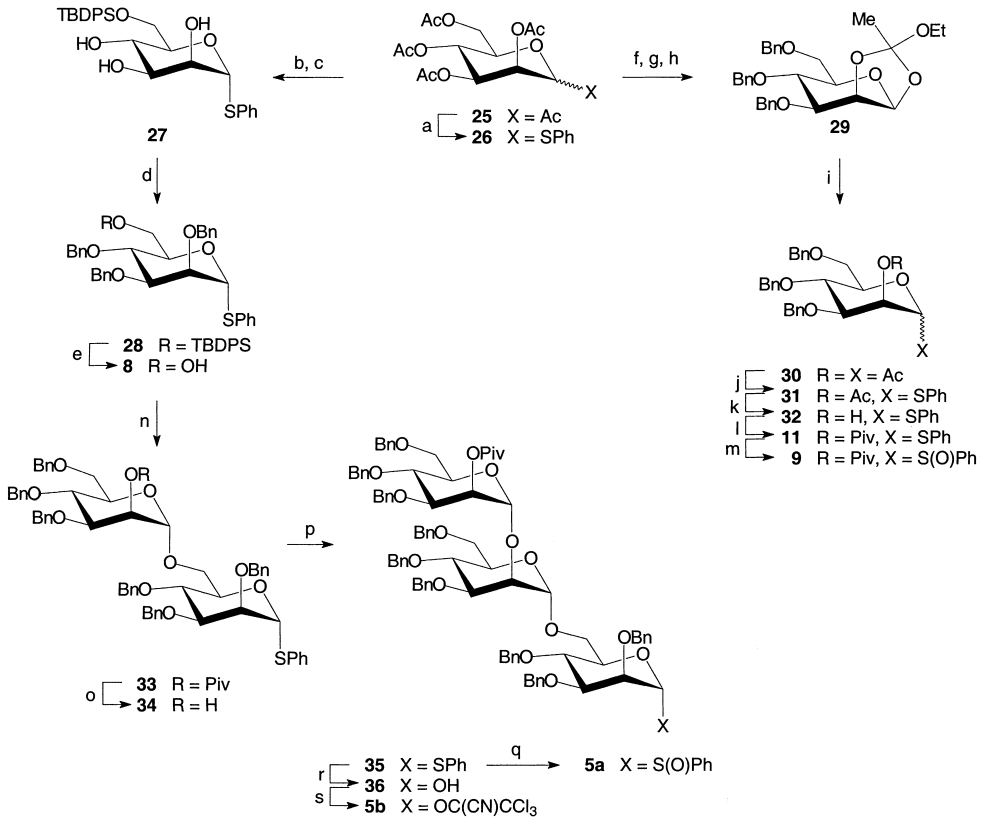
diethyl ether at room temperature with trimethylsilyl triflate (TMSOTf) as a promoter resulted in exclusive 6-*O*-glycosylation with good yield (75%), but poor stereoselectivity (α/β 3:2). A better stereochemical outcome (α/β 5.5:1) was observed when trichloroacetimidate **22** was used as glycosyl donor, but in this case the regioselectivity decreased and an appreciable amount (16%) of 5-*O*-linked pseudodisaccharide (**24**) accompanied the desired 6-*O*-linked regioisomer (**23**) in the reaction mixture (Scheme 3). Since the mixture **20*α***:**20*β*** (Scheme 2) showed a good separation factor and compound **20*β*** is a convenient building block for the construction of substances related to the insulin mimetic structures reported by Müller et al.,[15] the route depicted in Scheme 2 was finally preferred. The key building block **4** was prepared by treatment of **20*α*** with liquid ammonia in methanol, as an attempted Zemplén deacetylation of the latter resulted in simultaneous migration of the silyl protecting group.

The preparation of **5** was carried out as outlined in Scheme 4 by using the sulfoxide glycosylation reaction.[55] This method has been successfully used for the synthesis of glycosides and oligosaccharides both in solution[65] and in the solid phase.[66] It also appears to be particularly attractive with regard to its potential application in new chemoselective glycosylation strategies (such as the "one-pot sequential glycosylation")[67] as previously described for the synthesis of the cyclamicin *O*-trisaccharide.[68] These potential advantages and our previous positive experience with this glycosylation methodology[37, 69] encouraged us to explore its scope and limitations further. The synthesis of **5** was envisaged from mannose pentaacetate (**25**) from which both the thioglycoside glycosyl acceptor **8** and the sulfoxide glycosyl donor **9** were obtained via the key thioglycoside intermediates **27** and **11**, respectively. To secure the 1,2-*trans* stereochemistry of the glycosidic linkage[70] and in order to avoid ortho-ester formation,[71–73] which is particularly likely to occur when



Scheme 3. a) NBS, H_2O, Me_2CO, 90%; b) Cl_3CCN, K_2CO_3, CH_2Cl_2, 89%; c) **6**, TMSOTf, EtO_2. **23**: 64% (α/β, 3:2) **24**: (16%).



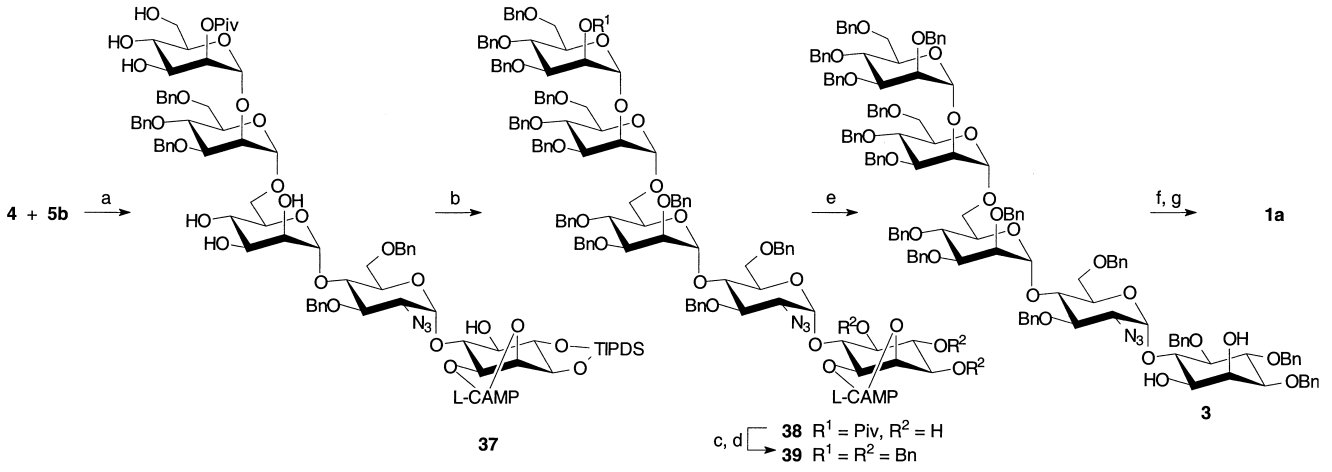
Scheme 4. a) PhSH, $BF_3 \cdot Et_2O$, CH_2Cl_2, 86%; b) MeONa, MeOH, 94%; c) TBDPSCl, imidazole, THF, 87%; d) NaH, BnBr, DMF, 76%; e) TBAF, THF, 90%; f) HBr, AcOH; g) 2,6-lutidine, CH_2Cl_2, EtOH; h) NaOMe, MeOH; i) AcOH, Ac_2O, Py, 80% over the five steps; j) PhSH, $BF_3 \cdot Et_2O$, CH_2Cl_2, 79%; k) NaOMe, MeOH, quantitative; l) PivCl DMAP, Py, 81%; m) mCPBA, CH_2Cl_2, 80%; n) **9**, Tf_2O, DTBMP, Et_2O, $-60\,^{\circ}C$ to rt, 88%; o) MeONa, MeOH, THF, $60\,^{\circ}C$, 97%; p) **9**, Tf_2O, DTBMP, Et_2O, $-60\,^{\circ}C$ to rt, 73%; q) mCPBA, CH_2Cl_2, 90%; r) NBS, H_2O, Me_2CO, 80%; s) Cl_3CCN, DBU, CH_2Cl_2, 70%.

using the sulfoxide glycosylation method,[74] the participating neighbouring group at C2 in **9** was set up as pivaloate. The glycosylation reaction of **8** with **9** under diethyl ether with triflic anhydride as promoter yielded **33** stereoselectively in 88% yield. Removal of the pivaloate group in **33** and subsequent glycosylation of the resulting disaccharide **34** with **9** in the above conditions yielded **35** with excellent stereoselectivity in 73% yield. The thioglycoside group in **35** was then oxidised to give sulfoxide **5a** which can be used directly as a glycosyl donor in the subsequent glycosylation reaction. This oxidation, and that previously performed on thioglycoside **11** to obtain sulfoxide **9**, were carried out with mCPBA in dichloromethane at low temperature and in both cases afforded almost exclusively one diastereomer. As a consequence of the higher accessibility of the pro-*R* lone pair, this will most likely have the *R* absolute configuration at the sulfinyl sulfur, as recently shown by X-ray crystallography.[75] The complete reaction sequence depicted in Scheme 4 constitutes a further example of the potentiality of the sulfoxide glycosylation method, it compares well with other glycosylation procedures and strategies already used to construct the trimannosidic moiety of the conserved linear structure of the GPI anchors.[24–28]

The glycosylation of **4** with **5a** (Scheme 1) was then investigated under different experimental conditions since a stochiometric ratio of 2:1 donor–acceptor, as established for the sulfoxide glycosylation method is not acceptable when elaborate donor structures such as **5a** are involved. Unfortunately the results were discouraging when no excess of the donor was used and we turned to the trichloroacetimidate method for the final glycosylation step (Scheme 5). Thioglycoside **35** was transformed in two high-yielding steps into trichloroacetimidate **5b** (Scheme 4). Glycosylation of **4** with **5b** (Scheme 5) in methylene chloride at room temperature, in the presence of TMSOTf as promoter, afforded pseudopentasaccharide **37** with excellent stereoselectivity in 74% isolated yield. The structure of **37** was confirmed by its ^{13}C NMR spectrum, which showed four signals for anomeric carbons at $\delta = 95.8$, 99.2, 99.4 and 99.6 with $^{1}J_{CH}$ values between 171.4 and 175.9 Hz. These data unequivocally indicate the α-configuration of the newly formed glycosidic linkage. The sequence was then completed by removal of the TIPDS protection, depivaloylation and perbenzylation to give **39**. The L-camphor ketal was then removed and the resulting diol (**3**) reacted with *N*-pyridinium phosphodichloridate.[76] Final hydrogenolysis in the presence of 10% Pd on charcoal in buffered medium gave pseudopentasaccharide **1a** which was purified by ion exchange chromatography.

Conformation: The three-dimensional solution structure of the GPI anchor glycan of *Trypanosoma brucei* VSG was determined ten years ago by using NMR spectroscopy together with molecular orbital calculations and restrained molecular dynamics simulations.[77] From this study it was concluded that the molecule exists in an extended conformation that undergoes large torsional oscillations about $\alpha(1\rightarrow6)$ linkages, with extreme torsional fluctuations of hydroxyl and free hydroxymethyl groups on the picosecond timescale. Advances in NMR instrumentation as well as in computational power, software and protocols during the last ten years have now allowed the conformational study of the linear pseudopentasaccharide **1a** to be approached from a more advantageous position. Furthermore, in the present investigation, the lack of tetragalactopyranosyl branching in structure **1a**, which is present in the glycan chain of the GPI anchor of the VSG of *Trypanosoma brucei*, has simplified the structural problem and permitted experimental evidence to be obtained that defines the Man$\alpha(1\rightarrow6)$Man linkage dynamics in **1a** as a fast 60:40 equilibrium between *gg* and *gt* rotamers. In addition, this reinforces the previous data on the flexibility of the glycan by simultaneous observation of positive and negative NOEs as a function of the position of the different protons along the glycan chain.

The solution structure of **1a** has now been studied by using a combination of NMR spectroscopy and molecular mechanics calculations. As a consequence of the expected flexibility of this structure, the calculation protocol was based on unrestrained molecular dynamics simulations rather than on the study of all four single glycosidic linkages by using adiabatic maps. Molecular mechanics calculations were per-



Scheme 5. a) TMSOTf, CH_2Cl_2, rt, 74%; b) TBAF, THF, 84%; c) CF_3CO_2H, wet CH_2Cl_2, 60%; d) NaOMe, MeOH; e) NaH, BnBr, DMF; f) $MeOP(O)Cl_2$, Py; g) H_2, 10% Pd/C, 60% over the two steps.

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formed by means of the Senderovich-Stille AMBER all atoms reparametrisation for pyranoses[78] optimised for use in combination with the continuum model for solvent generalised Born/solvent accessible surface area (GB/SA).[79] Three different starting structures, corresponding to the three possible Man$\alpha(1\rightarrow6)$Man rotamers, were considered for the dynamics simulation. The *gt* conformer was taken from a Monte Carlo multiple minimum global search,[80, 81] while the *gg* and the *tg* conformers were manually built from the *gt* conformer. A 2.0 ns molecular dynamics simulation was run for each of these starting structures. The *gt* conformer was stable along the whole trajectory, while the *gg* persisted for just over one nanosecond, before becoming *gt* and the *tg* conformer flipped over to the *gt* in a few picoseconds (Figure 1). This unstable *tg* rotamer was therefore not considered in the subsequent investigations. The prediction of two stable conformers (*gt* and *gg*), but not their relative energies, is in agreement with NMR data.[82]

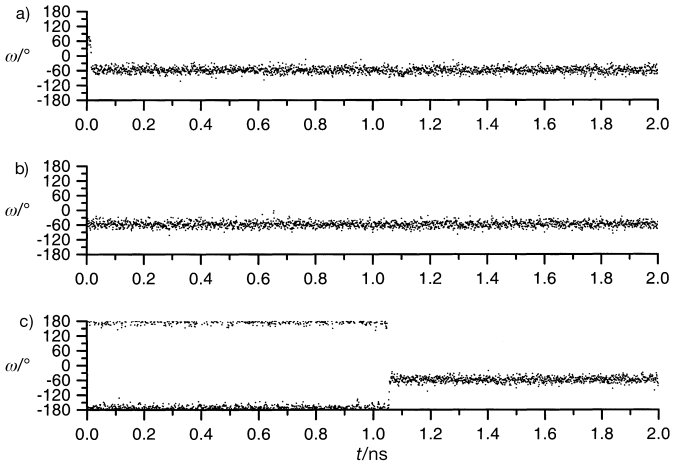

Figure 1. Trajectory plots for 4-Man$\alpha(1\rightarrow6)$3-Man linkage, ω torsion angle of the MD simulations of compound **1a** for a) *tg*, b) *gt* and c) *gg* starting structures.

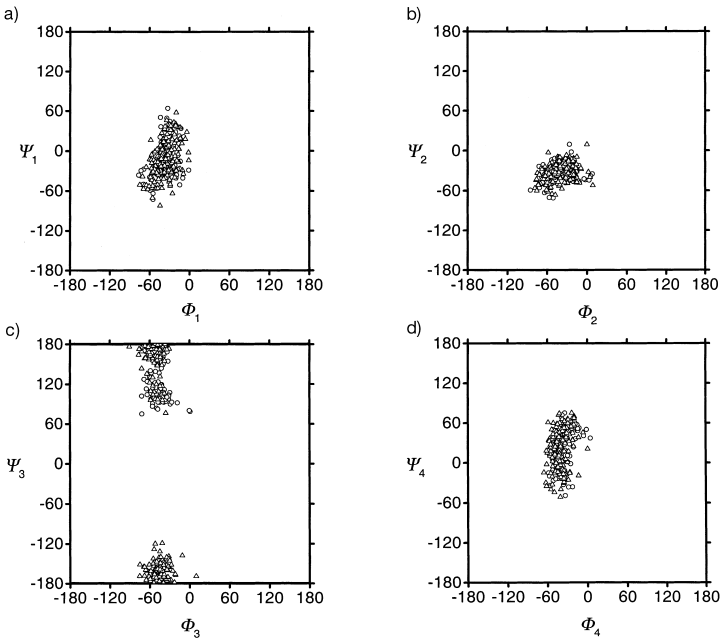

Figure 2. Instantaneous values of Φ versus Ψ during the time course of molecular dynamics runs for: a) 2-GlcN$\alpha(1\rightarrow6)$ 1-Ins; b) 3-Man$\alpha(1\rightarrow4)$2-GlcN; c) 4-Man$\alpha(1\rightarrow6)$3-Man and d) 5-Man$\alpha(1\rightarrow2)$4-Man, for ω 4-Man$\alpha(1\rightarrow6)$3-Man *gg* conformer, triangles and *gt*, circles, values.

Table 1. Φ and Ψ torsional angle mean values and their standard deviation along the MD trajectories for *gg* and *gt* conformers.

Linkage		*gg* [°]	*gt* [°]		*gg* [°]	*gt* [°]
2-GlcN$\alpha(1\rightarrow5)$1-Ins	$\Phi1$	-37 ± 14	-38 ± 16	$\Psi1$	-12 ± 26	-14 ± 29
3-Man$\alpha(1\rightarrow4)$2-GlcN	$\Phi2$	-39 ± 17	-37 ± 20	$\Psi2$	-33 ± 16	-34 ± 15
4-Man$\alpha(1\rightarrow6)$3-Man	$\Phi3$	-147 ± 13	-46 ± 18	$\Psi3$	-143 ± 40	-179 ± 31
5-Man$\alpha(1\rightarrow2)$4-Man	$\Phi4$	-38 ± 11	-38 ± 12	$\Psi4$	-25 ± 28	-23 ± 30

The two molecular dynamics runs were in agreement with an extended structure with the glycosidic linkages fluctuating within a well-defined area of the conformational space (Figure 2). All angles resided in a narrow region along the trajectory, as could be predicted by the *exo*-anomeric effect, and the ψ angles also appeared with a well defined but wider distribution. As expected, the highest conformational flexibility was predicted for the Man$\alpha(1\rightarrow6)$Man connection (Figure 2c). The torsional angle mean values and their standard deviations (Table 1) were in agreement with this extended disposition of the different residues with the exception of the Man$\alpha(1\rightarrow2)$Man structural motif, for which the values were consistent with a stacked orientation of the monosaccharide units; this is a consequence of the axial–axial arrangement of this glycosidic linkage. The 4000 molecular dynamics structures were grouped by using as criterion the root mean square distance (rmsd) between the positions of the heavy atoms. The superposition of representative structures (Figure 3) shows how the overall contribution of the individual local fluctuations of the glycosidic linkages causes the molecule to cover a considerably large conformational space. These calculations predict a hingelike global movement for glycan **1a** which may be considered to be related to the structural function of the GPI anchors that act as a bridge between the anchored protein and the lipid moiety embedded into the cellular membrane.

The ^{1}H and ^{13}C NMR spectra of **1a** were assigned by using a combination of standard correlation spectroscopy, HMQC techniques, and double-pulsed field-gradient spin echo-(dpfgse) one-dimensional TOCSY and one-dimensional NOESY. Chemical shift and coupling constant data are given in the Supporting Information. The observed coupling constants, which indicated a monoconformational behaviour of the pyranose and cyclitol rings, were in agreement with the values calculated, with the Altona equation,[83] for the Monte Carlo minimised minimum-energy conformation. The experimental values for the three-bond ^{31}P-^{1}H couplings were also in agreement with those calculated by using an equation of the Karplus type previously reported.[84]

The inter- and intraresidue NOEs for **1a** were obtained through NOESY by using five mixing times between 100 and 500 ms at 283 K and 500 MHz. Three close key contacts not previously reported[77] have now been detected: 4-Man H1 with 3-Man H6a and H6b, and 4-Man H1 with 5-Man H3.

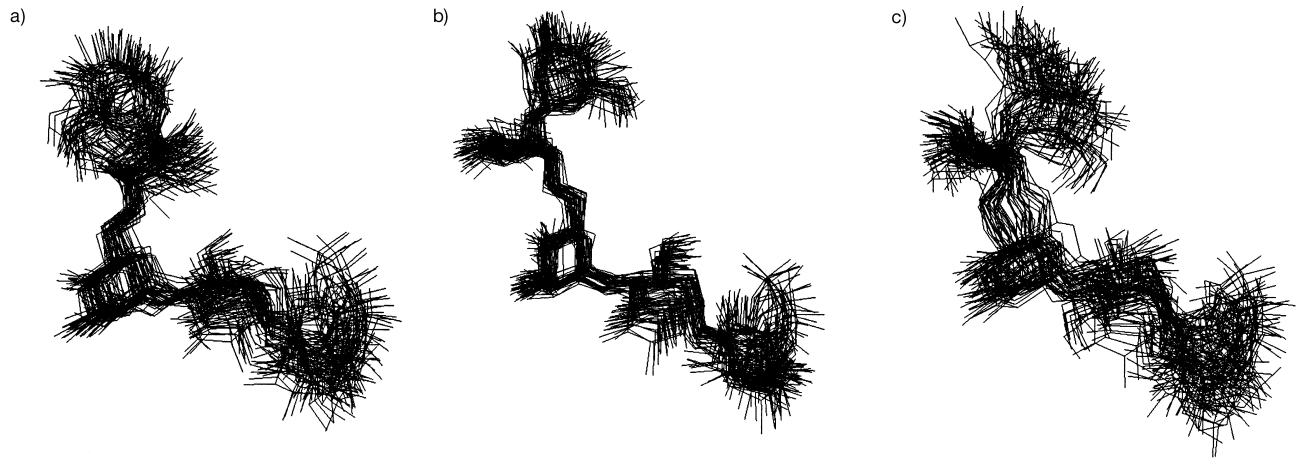

Figure 3. Best fit superimposition of fifty representative structures of the MD runs of a) *gg* conformer, b) *gt* conformer and c) both.

Cross-relaxation rates (σ^{NOE}) were obtained from these values by extrapolation of $[I_{ij}(t_m)/I_{ii}(t_m)]/t_m$ versus t_m to zero mixing time.[85] The interprotonic distances were evaluated by taking the nearest H1-H2 distance as a reference to make the evaluation less sensitive to deviations in the local correlation times due to segmental motions. These interprotonic distances were in good agreement with the $\langle r^{-6}\rangle$ average calculated over the 4000 structures collected during the molecular dynamics runs (Table 2), thus validating the performed simulations. It

Table 2. Experimental and calculated interprotonic distances [Å] for **1**.

Proton pair	Exptl	Calcd
H1 GlcN-2/H6 Ins-1	2.36	2.33
H1 Man-3/H4 GlnN-2	2.23	2.46
H1 Man-4/H6b Man-3	2.67	2.83
H1 Man-4/H6a Man-3	2.20	2.37
H1 Man-4/H5 Man-5	2.60	2.67
H1 Man-4/H3 Man-5	3.03	4.59
H1 Man-5/H2 Man-4	2.30	2.30

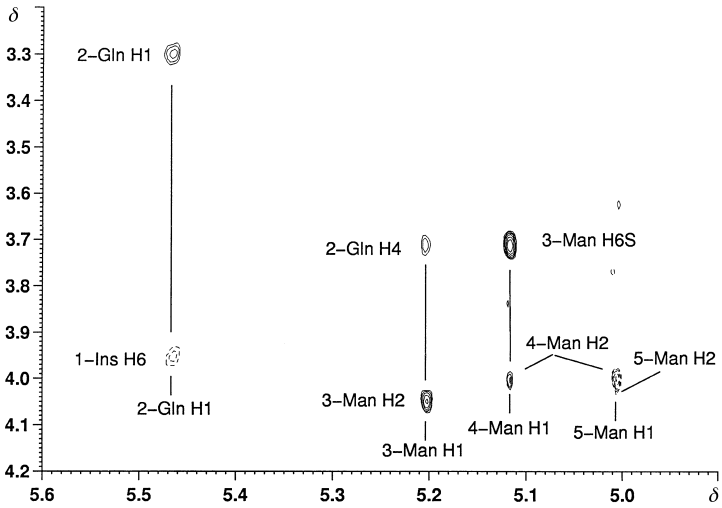

Figure 4. Expansion of NOESY spectra of **1a** (500 ms) at 298 K showing the key intraresidue NOE: Dashed lines, negative contours and positive NOE; filled lines, positive contours and negative NOE.

can therefore be concluded that this unrestrained molecular dynamics simulation provides a fair description of the solution structure of glycan **1a**.

The local conformation around the ω torsion angle of the $\alpha(1\rightarrow6)$ glycosidic linkage could be determined from the coupling constant values ($J_{H5\cdot H6S} = 1.8$ Hz and $J_{H5\cdot H6R} = 5.49$ Hz) and NOE experimental data (4-Man H1 3-Man H6*R*/H6*S*). The stereochemical assignment of the methylenic protons was carried out after comparison of the $\langle r^{-6}\rangle$ molecular dynamics averaged distances with the experimental distances. A ratio of *gg*/*gt*/*tg* of 60:40:0 was obtained by using three different sets of equations from the experimental data.[84, 86, 87]

An interesting spectral feature is that a higher temperature NOESY (298 K) is close to the zero crossing point of the NOE and weak positive and negative cross peaks were observed (Figure 4). This behaviour reflects a dispersion of the correlation times of interprotonic vectors faster and slower than the NOE-narrowing limit condition ($\omega\tau_c = 1.2$). With regard to the structure, the negative NOEs are related to the inner protons and the positive ones to the external residues. Although anisotropy can obscure the interpretation of segmental mobility influence on NOE, in this case, in spite of the apparent cylindrical shape of the molecules, no evidence of this behaviour was found as the distances calculated, by assuming isotropic motion, fit with the predicted ones. This observation provides experimental evidence that the faster local movements correspond to the extremes of the molecule, as could be expected from the predicted hingelike behaviour of the molecule in the molecular dynamics simulation.

In summary, the structure **1a** may be described as an extended, relatively flexible structure. This relative flexibility is the result of the contribution of its four glycosidic linkages, all of which, with the exception of the ω torsion of the $1\rightarrow6$ link, reside in one single global minimum but oscillate within the potential-energy well. Although in some previous studies which used more simple but structurally related models, we characterised an additional local low populated minimum for the glucosamine-*myo* inositol $\alpha(1\rightarrow6)$ glycosidic linkage, this minimum could not be observed for **1a**. This fact, however, cannot be considered as definitive evidence of a single

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minimum situation for this glycosidic linkage in the case of **1a**, since the exclusive NOE for this conformation may become undetectable as a result of either an unfavourable correlation time or a smaller conformational population at the lower temperature used for the NOESY experiments.

In conclusion, the pioneer work by Homans on the structure of the GPI anchor glycan of *Trypanosoma brucei* VSG[77] clearly established that, as a result of torsional oscillations around the glycosidic linkages, it was not possible to define a unique conformation for this molecule and proposed the use of molecular dynamics as a suitable tool for the prediction of the conformational behaviour of the glycan. These have since become generally accepted concepts in structural carbohydrate chemistry. Our present study on the conformation of **1a** has arrived at the same general conclusions, although the major simplicity of this structure and the high sophistication of the instrumentation presently available have now permitted the disclosure of some more detailed structural features. These include the characterisation of the dynamics of the $\alpha(1\rightarrow6)$ glycosidic linkage as a 60:40 fast equilibrium between *gg* and *gt* rotamers and the experimental evidence of the flexibility of the core GPI glycan structure on the basis of the simultaneous observation of positive and negative NOE values.

The above structural data on compound **1a** and those on other IPG-like molecules previously carried out in our laboratory[18, 91] are of major importance in structure–activity relationship studies involving these molecules and enzymes with well-defined binding-site geometries such as PKA[19] and glycogen synthase phosphatase 2C.[20]

Biological activity: The IPG-like activity of compound **1a** was studied. Since naturally occurring IPGs have been shown to mimic the lipogenic activity of insulin in adipocites and to be inhibitors of the enzyme PKA (type A IPGs)[3] or activators of PDH phosphatase (type P IPGs),[4] the capacity of **1a** to produce these biological effects was investigated.

The assay of the activation of PDH phosphatase by **1a** was performed by using the spectrophotometric variant of the two-stage system described by Lilley et al.[92] Compound **1a** was examined at concentrations of 10^{-4} and 10^{-6} M. This activity was also investigated in the presence of Zn^{2+} (5×10^{-5} M) and Mn^{2+} (10^{-5} M) as it has been established that the presence of these metallic cations influences the activity of natural IPGs.[10] There was no stimulation of PDH phosphatase above the base line found in compound **1a** (data not shown). Type P IPG from rat liver was simultaneously assayed as a positive control. (Six microliters of this liver IPG, which is equivalent to 100 mg liver extracted, gave a value of 103% above the base line.) It can therefore be concluded that structure **1a** does not behave as an IPG P-type mimetic.

Similar negative results were obtained when the ability of **1a** to inhibit the enzyme PKA was determined by using a colorimetric assay kit and a standard PKA preparation. Structure **1a** was found to be inactive in inhibiting this enzyme, which is inhibited by A type IPGs, and this unambiguously establishes that structure **1a** is not an IPG A-type mimetic (Figure 5).

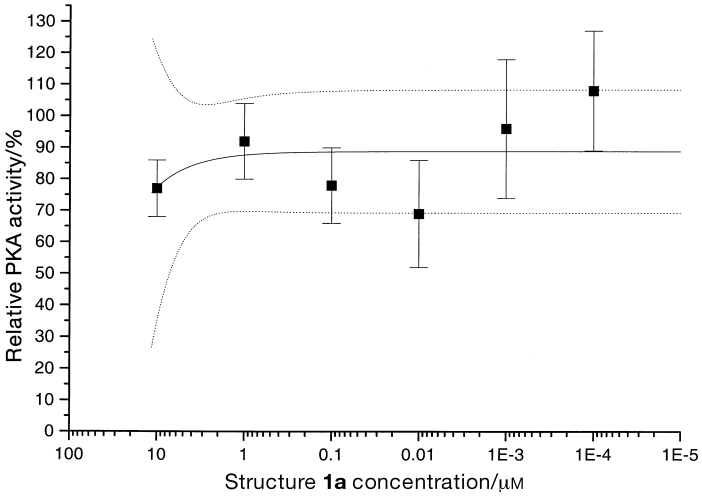

Figure 5. Effects of structure **1a** on the enzyme activity of PKA. No statistically significant effect of **1a** was found over the concentration ranges tested ($R=0.58$, $p=0.22$).

Finally, structure **1a** was not found to have any stimulatory lipogenic activity in the absence of insulin, whereas natural IPG A-type substances are able to stimulate lipogenesis under these conditions (Figure 6). However, a small synergistic

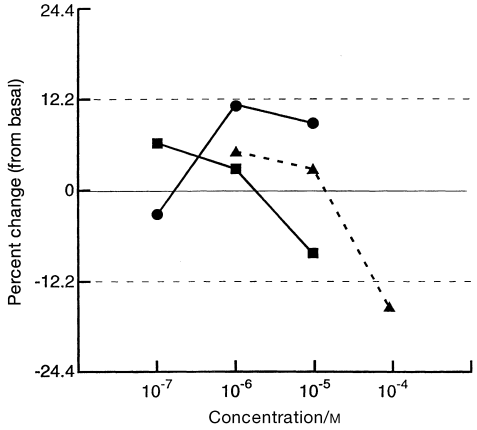

Figure 6. Effects of structure **1a** on basal lipogenesis. The parallel dashed lines indicate the percentage change from basal +/− 25 D. No statistically significant values were found for compound **1a** alone (●). Zinc (▲) on its own inhibited basal lipogenesis (15%, 10^{-4} M). This effect was reversed by addition of **1a** (■) which suggests that **1a** was able to chelate the zinc and prevent the zinc induced inhibition. The concentrations in the figure indicate the following: (●) concentration of **1a** (■) concentration of **1a** in the presence of a tenfold molar excess of zinc and (▲) concentration of zinc.

stimulation was observed in the presence of insulin (Figure 7). This synergistic activity of structure **1a** could be effected by a variety of mechanisms that may involve binding to the surface of the adipocites, but not to stimulation of key metabolic control enzymes within the cytoplasm, as proposed for naturally occurring IPGs.

In summary, synthetic structure **1a**, which contains the basic structural features that may be expected for IPGs derived from GPI anchors, does not show any significant IPG-like activity. This may be taken as a strong indication that the protein GPI anchors are not the precursors of the IPG mediators.

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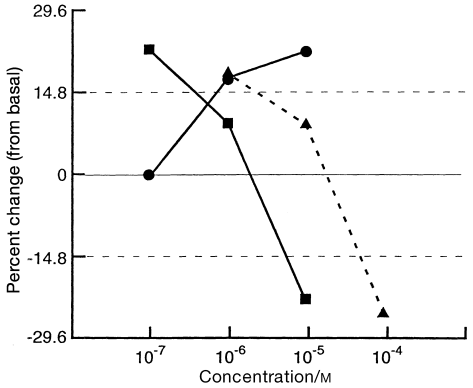

Figure 7. Effects of structure **1a** (●) alone and zinc (▲) alone on insulin stimulated lipogenesis. At concentrations of 10^{-6} and 10^{-5} M of compound **1a**, a synergistic effect was seen with insulin. The stimulatory effect was antagonised by the addition of tenfold molar excess of zinc (■), this suggests that compound **1a** may be able to complex with zinc. This complex, however, appears to have no activity and further inhibits the activity of **1a**. The concentrations in the figure indicate the following: (●) concentration of compound **1a**, (■) concentration of **1a** in the presence of a tenfold molar excess of zinc and (▲) concentration of zinc.

Experimental Section

General methods: All reactions were run under an atmosphere of dry argon with oven-dried glassware and freshly distilled and dried solvents. THF and diethyl ethr were distilled from sodium and benzophenone. Dichloromethane and acetonitrile were distilled from calcium hydride. Thin layer chromatography (TLC) was performed on silica gel GF_{254} (Merck) with detection by charring with phosphomolibdic acid/EtOH. For flash chromatography, silica gel (Merck 230–400 mesh) was used. Columns were eluted with positive air pressure. Chromatographic eluents are given as volume to volume ratios (v/v). Routine NMR spectra were recorded with Bruker Avance DPX300 (^{1}H, 300 MHz), Bruker Avance DRX400 (^{1}H, 400 MHz) and Bruker Avance DRX500 (^{1}H, 500 MHz) spectrometers. Chemical shifts are reported in ppm and coupling constants are reported in Hz. Spectra were referenced to the residual proton or carbon signals of the solvent. High-resolution mass spectra were recorded on a Kratos MS-80RFA 241-MC apparatus. Optical rotations were determined with a Perkin-Elmer 341 polarimeter. Elemental analyses were performed by using a Leco CHNS-932 apparatus. The organic extracts were dried over anhydrous sodium sulfate and concentrated in vacuo.

o*-(4-*O*-Acetyl-2-azido-3,6-di-*O*-benzyl-2-deoxy-*α* and β-D-glucopyranosyl)-(1→6)-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-1,2-*O*-(L-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol (20*α* and 20β)**: A mixture of **6**[18, 56] (471 mg, 0.84 mmol) and **7** (352 mg, 0.61 mmol) in diethyl ether (20 mL), which contained freshly activated 4 Å molecular sieves (400 mg), was stirred for 1 h at room temperature. The mixture was then cooled to −40 °C and treated with TMSOTf in diethyl ether [185 μL of a 0.1 M solution (18.3 μM)]. After 30 min, solid sodium carbonate was added and the mixture was filtered over Celite and concentrated. Flash chromatography of the residue (hexane/EtOAc 9:1) gave **20*α (285 mg, 48%) and **20β** (152 mg, 26%).

Compound 20*α*: ^{1}H NMR ($CDCl_3$, 500 MHz) $\delta = 7.50-7.20$ (m, 10H, arom.), 5.50 (d, $J = 3.4$ Hz, 1H, H-1b), 5.17 (t, $J = 9.7$ Hz, 1H, H-4b), 4.70 (d, $J_{CH2Ph} = 11.0$ Hz, 2H, CH_2Ph), 4.60 (d, $J = 12.1$ Hz, 2H), 4.42 (m, 1H, H-5b), 4.21(t, $J = 4.5$ Hz, 1H, H-2a), 4.06 (t, $J = 5.9$ Hz, 1H, H-3a), 4.01 (t, $J = 9.7$ Hz, H-3b), 3.95 (dd, $J = 3.95$, 9.28 Hz, 1H, H-1a), 3.87 (t, $J = 9.2$ Hz, 1H, H-6a), 3.80 (dd, $J = 6.5$, 10.1 Hz, 1H, H-4a), 3.53–3.42 (m, 4H), 2.60 (s, 1H, OH), 1.85 (s, 3H, Ac), 1.97–0.88 (m, 38H), 0.84 (s, 3H), 0.83 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 169.5$, 138.1, 137.9, 128.4, 128.2, 127.9, 127.8, 127.5, 117.9, 95.9, 79.7, 78.0, 76.3, 75.8, 74.6, 73.3, 72.9, 72.4, 71.1, 68.7, 68.6, 63.0, 51.5, 48.0, 45.2, 45.1, 36.6, 29.6, 27.1, 24.7, 23.3, 20.9, 20.5, 20.2, 17.5, 17.4, 17.3, 17.3, 17.2, 17.1, 17.0, 13.0, 12.7, 12.2, 12.0, 9.7; $[\alpha]_D^{23} + 53$ ($c = 1$, in $CHCl_3$); elemental analysis calcd (%) for $C_{50}H_{75}N_3O_{12}Si_2$: C 62.15, H 7.82, N 4.35; found: C 62.04, H 8.15, N 4.27.

Compound 20β: ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta = 7.33-7.25$ (m, 10H, arom.), 5.05 (t, $J = 9.1$ Hz, 1H, H-4b), 4.81 (d, $J = 8.1$ Hz, 1H, H-1b), 4.70 (d, $J_{CH2Ph} = 11.4$ Hz, 2H, CH_2Ph), 4.40 (d, $J = 11.9$ Hz, 2H,), 4.20 (t, $J = 4.6$ Hz, 1H, H-2a), 3.90 (m, 2H, H-1a, H-3a), 3.82 (t, $J = 9.2$ Hz, 1H, H-6a or H-4a), 3.71 (dd, $J = 6.8$, 10.1 Hz, 1H, H-4a or H-6a), 3.50 (m, 5H), 3.40 (t, $J = 9.5$ Hz, 1H, H-3b), 2.77 (s, 1H, OH), 1.56 (s, 3H, Ac), 1.56–0.92 (m, 38H), 0.79 (s, 3H), 0.74 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 169.6$, 137.9, 137.7, 128.3, 128.2, 127.9, 127.7, 127.6, 117.5, 102.0, 82.0, 80.7, 80.3, 76.6, 75.5, 74.7, 74.3, 74.3, 73.5, 72.9, 72.6, 71.7, 70.2, 67.9, 65.8, 51.5, 45.0, 29.4, 27.0, 25.6, 20.7, 20.5, 20.2, 17.5, 17.4, 17.4, 17.3, 17.2, 17.1, 17.0, 13.0, 12.7, 12.3, 12.0, 9.8. $[\alpha]_D^{23} - 16$ ($c = 1$ in $CHCl_3$); elemental analysis calcd for $C_{50}H_{75}N_3O_{12}Si_2$: C 62.15, H 7.82, N 4.35; found: C 61.87, H 7.85, N 4.63.

O*-(2-Azido-3,6-di-*O*-benzyl-2-deoxy-*α*-D-glucopyranosyl)-(1→6)-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-1,2-*O*-(L-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene-D-*myo*-inositol (4)**: Compound **20*α (0.62 g, 6.1 mol) was conventionally deacylated by treatment with liquid ammonia in methanol to give compound **4**. ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta = 7.42-7.25$ (m, 10H, arom.), 5.45 (d, $J = 3.5$ Hz, 1H, H-1b), 4.90 (AB system, $J_{AB} = 11.2$ Hz, $\Delta v = 26.4$ Hz, 2H, Bn), 4.57 (AB system, $J_{AB} = 12.2$ Hz, $\Delta v = 31.6$ Hz, 2H, Bn), 4.25 (m, 1H, H-5b), 4.20 (t, $J = 5.1$ Hz, 1H, H-2a), 4.04 (t, $J = 5.2$ Hz, 1H, H-1a), 3.90 (m, 3H, H-3a, H-4b, H-6b), 3.65 (dd, $J = 4.3$, 10.5 Hz, 1H, H-6′b), 3.40 (t, $J = 9.6$ Hz, H-5a), 3.33 (dd, $J = 3.5$, 10.2 Hz, 1H, H-2b), 2.50 (s, 1H, OH), 1.96–0.97 (m, 38H), 0.84 (s, 3H), 0.82 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 138.3$, 138.0, 128.6, 128.4, 128.0, 127.9, 127.7, 117.8, 96.1, 79.9, 79.4, 77.3, 77.0, 76.8, 76.3, 75.8, 75.0, 73.5, 72.8, 72.6, 72.3, 69.7, 69.5, 63.0, 51.5, 48.0, 45.2, 45.1, 29.6, 27.1, 20.5, 20.2, 17.6, 17.4, 17.3, 17.2, 17.1, 17.0, 13.0, 12.7, 12.3, 12.0, 9.7. $[\alpha]_D^{23} + 45$ ($c = 1.0$ in $CHCl_3$); elemental analysis calcd (%) for $C_{48}H_{73}N_3O_{11}Si_2$: C 62.36, H 7.98, N 4.50; found: C 62.70, H 7.53, N 4.03.

o*-(2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-*α*-D-glucopyranosyl)-(1→6)-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-1,2-*O*-(L-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol (23*α*), *o*-(2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→6)-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-1,2-*O*-(L-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol (23β) and *o*-(2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-*α*-D-glucopyranosyl)-(1→5)-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-1,2-*O*-(L-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol (24)**: TMSOTf (29 μL, 0.16 mmol) at −20 °C was added to a solution of **22** (1.698 g, 3.22 mmol) and **6** (2.51 g, 4.51 mmol) in dry diethyl ether (50 mL). The mixture was slowly warmed to 0 °C (4.30 h), then neutralized with solid sodium bicarbonate, stirred for an additional 30 min and filtered over Celite. After concentration under vacuo, the mixture was purified by flash column chromatography (hexane/EtOAc 95:5→75:25), which afforded three pseudodisaccharides **23*α (1.642 g, 1.78 mmol, 55%), **23*β*** (0.287 g, 0.31 mmol, 9%) and **24** (0.473 g, 0.51 mmol, 16%).

Phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-*α*-D-mannopyranoside (26): Thiophenol (5.3 mL), followed by boron trifluoride diethyl ether (2.5 mL), was added to a solution of D-mannose pentaacetate **25** (20 g) in dry dichloromethane (60 mL). After 20 h at room temperature, the solution was diluted with dichloromethane and was washed twice with saturated aqueous sodium bicarbonate solution and then with water. The organic layer was dried over sodium sulfate and concentrated under vacuum. The crude mixture obtained was purified by column chromatography (EtOAc/Hexane 7:3) to give **26** as a colourless oil (18.9 g, 83%). $[\alpha]_D^{23} + 107$ ($c = 1.07$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$), 500 MHz): $\delta = 7.50-7.20$ (m, 5H, arom.), 5.50 (m, 2H, H-1, H-2), 5.35 (m, 2H, H-3, H-4), 4.55 (m, 1H, H-5), 4.30 (dd, $J = 5.8$, 12.2 Hz, H-6), 4.00 (dd, $J = 2.4$, 12.2 Hz, 1H, H-6′), 2.15, 2.08, 2.05, 2.02 (4s, 4Ac); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 170.3$, 169.7, 169.6, 169.5, 132.4, 131.9, 129.0, 127.9, 85.5, 70.7, 69.3, 69.2, 66.1, 62.2, 20.7, 20.5, 20.6; elemental analysis calcd (%) for $C_{20}H_{24}O_9S$: C 54.54, H 5.49; found: C 54.23, H 5.41.

Phenyl 2,3,4-tri-*O*-benzyl-1-thio-*α*-D-mannopyranoside (8): A solution of **26** (18.9 g, 42.8 mmol) in dry methanol was treated with a catalytic amount of MeONa at room temperature. After 1 h the reaction mixture was neutralised with Amberlite IR-120 (H^+), filtered and evaporated to give the corresponding tetrol (10.9 g, 94%). A solution of this crude product (7.9 g, 29 mmol) and imidazol (5.96 g) in THF (60 mL) was treated with TBDPS (9 mL) at 0 °C. After 20 h saturated aqueous NH_4Cl was added and the mixture was extracted with CH_2Cl_2, washed with brine, dried over sodium sulfate and concentrated to dryness. The residue was purified by flash chromatography (MeOH/CH_2Cl_2 5:95) to give **27** (13.4 g, 87%).

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^{1}H NMR ($CDCl_3$, 500 MHz): $\delta = 7.67-7.16$ (m, 5H, arom.), 5.45 (br s, H-1), 4.20–3.80 (m, 6H, H-2, H-3, H-4, H-5, H-6, 6′) 1.05 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 135.7$, 135.6, 131.5, 129.9, 129.0, 127.8, 127.4, 88.0, 72.7, 72.2, 72.1, 70.1, 64.9, 27.0.

A solution of the above product (**27**, 6.7 g, 13.1 mmol.) in THF (30 mL) was added through a canula to a stirred suspension of sodium hydride (1.9 g) and TBAI (catalytic amount) in THF (10 mL) at 0 °C. After 15 min, benzyl bromide (5.6 mL) was added and stirring was continued at room temperature for 20 h. The mixture was then diluted with CH_2Cl_2 and washed successively with saturated aqueous NH_4Cl and brine, dried over sodium sulfate and evaporated to dryness. The residue was purified by column chromatography (Et_2O/hexane 10:90) to give pure **28** (7.70 g, 76%) as a colourless oil. ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta = 7.70$ (m, 4H, arom.), 7.40–7.20 (m, 26H, arom.), 5.59 (s, 1H, H-1), 4.94–4.45 (m, 6H, Bn), 4.20–3.80 (m, 6H), 1.03 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 138.9$, 138.7, 138.6, 138.5, 136.4, 136.0, 135.5, 134.3, 133.7, 131.3, 130.0, 129.9, 129.3, 128.8, 128.7, 128.4, 128.3, 128.1, 128.0, 127.9, 127.4, 86.0, 80.7, 77.6, 77.3, 77.0, 75.8, 75.1, 74.4, 72.6, 72.4, 63.4, 27.1, 19.7, 16.0.

A 1M solution of TBAF in THF (11.5 mL) was added dropwise to a solution of **28** (7.0 g, 8.96 mmol) in THF (70 mL) at room temperature. After 7 h, the mixture was treated with a saturated solution of NH_4Cl in water and then extracted with CH_2Cl_2. The organic layer was dried over sodium sulfate and concentrated to dryness, and the residue purified by column chromatography (EtOAc/hexane, 1:4) to afford **8** (4.37 g, 90%) as a solid. $[\alpha]_D^{20} = +73.3$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta = 7.39-7.27$ (m, 20H, arom.) 5.50 (s, 1H, H-1) 4.95 (d, $J = 10.9$ Hz, 1H, CH_2Ph), 4.50 (m, 1H, H-5), 4.03 (t, $J = 9.4$ Hz, 1H, H-4), 3.99 (dd, $J = 1.6$, 1.1 Hz, 1H, H-2), 3.88 (dd, $J = 9.2$, 2.9 Hz, 1H, H-3), 1.88 (dd, $J = 6.7$, 1.0 Hz, 1H, OH); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 138.5$, 138.3, 137.9, 134.1, 131.9, 129.2, 129.1, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 86.1, 80.2, 76.5, 75.4, 74.9, 74.7, 73.5, 72.7, 72.5, 72.3, 62.2; elemental analyis calcd (%) for $C_{33}H_{34}SO_5$: C 73.04, H 6.31; found: C 72.75, H 6.20.

1-(Phenylsulfinyl)-3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-1-deoxy-α-D-mannopyranoside (9): Thiophenol (6.3 mL, 61.7 mmol) and $BF_3 \cdot Et_2O$ (5.21 mL) were added to a solution of **30**[92] (22 g, 41.15 mmol) in CH_2Cl_2 (60 mL) at room temperature. The mixture was stirred for 2 h, then diluted with CH_2Cl_2 (400 mL) and poured into water 8500 mL). The organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (2 × 200 mL) and water (300 mL), dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography (EtOAc/hexane, 1:4) to afford **32** (18 g, 79% α/β, 35:1) as a colourless oil. ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta =$ 7.60–7.15 (m, 20H, arom.), 5.80 (dd, $J = 3.2$, 1.1 Hz, 1H, H-2β), 5.65 (dd, $J = 2.8$, 1.6 Hz, 1H, H-2α), 5.55 (d, $J = 1.6$ Hz, 1H, H-1α), 4.88 (d, $J =$ 1.1 Hz, H-1β), 4.48–4.42 (m, 8H, CH_2Ph), 4.35 (m, 1H, H-5α), 4.30–3.65 (m, 5H), 3.55 (m, 1H, H-5β), 2.55 (s, 3H, Ac), 2.15 (s, 3H, Ac); ^{13}C NMR α anomer ($CDCl_3$, 125 MHz): $\delta = 170.2$, 138.2, 138.1, 137.5, 133.6, 131.7, 128.9, 128.4, 128.3, 128.2, 128.1, 127.8, 127.7, 127.6, 127.5, 86.1, 78.4, 75.2, 74.4, 73.3, 72.4, 71.8, 70.3, 68.7, 21.0.

A 1M solution of NaOMe in MeOH (4 mL) was added to a solution of **32** (18 g, 32.57 mmol) in MeOH (50 mL). After stirring for 1 h at room temperature, the reaction mixture was neutralised with Amberlite IR-120 (H^+). The resin was then filtered off and the solution concentrated to give crude **32** as a colourless oil that was dissolved in pyridine (75 mL). A catalytic amount of DMAP and pivaloyl chloride (12 mL) were added to this solution with stirring. Stirring was continued for 60 h at room temperature, then MeOH (50 mL) was added and the solution concentrated to dryness. The residue was dissolved in CH_2Cl_2 (400 mL) and washed successively with 10% HCl (50 mL), saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, concentrated and purified by column chromatography (EtOAc/hexane, 1:15) to give **11** (16.5 g, 81%). Data for the α-anomer: ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta =$ 7.70–7.30 (m, 20H, arom.), 5.75 (s, 1H, H-2), 5.60 (s, 1H, H-1), 4.52 (AB system, $J = 10.8$ Hz, 2H, CH_2Ph), 4.75 (AB system, $J = 11.1$ Hz, CH_2Ph), 4.70 (AB system, $J = 12.0$ Hz, CH_2Ph), 4.50 (m, 1H, H-5), 4.10 (m, 3H, H-2, H-3, H-4), 4.00 (dd, 1H, $J = 10.8$, 4.5 Hz, H-6), 3.90 (dd, 1H, $J = 10.8$, 1.26 Hz, H-6′), 1.35 (s, 9H, Piv); ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 177.6$, 138.5, 138.4, 133.8, 132.3, 129.2, 128.5, 128.4, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.5, 86.5, 78.9, 75.4, 74.5, 73.3, 72.5, 71.6, 69.9, 69.1, 39.1, 27.3.

A solution of *m*-chlorobenzoic acid (*m*-CPBA) (60%) in CH_2Cl_2 (30 mL) was added through a canula to a solution of **33α** (9.4 g, 15 mmol) in CH_2Cl_2 (30 mL) at −78 °C. After addition of saturated aqueous $NaHCO_3$ (50 mL), the organic layer was washed with brine, dried and evaporated to dryness. The residue was purified by column chromatography (EtOAc/hexane, 1:5 → 1:2) to give 5.76 g of the less polar isomer, 0.63 g of the more polar isomer and 1.38 g of the mixture of both sulfoxides (total yield 81%). Data for the major isomer: ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta = 7.65-7.10$ (m, 20H, arom.), 6.00 (dd, $J = 4.6$, 2.4 Hz, 1H, H-2), 4.86 (d, $J = 10.8$ Hz, 1H, CH_2Ph), 4.77 (d, $J = 11.1$ Hz, 1H, CH_2Ph), 4.57 (d, $J = 11.6$ Hz, 1H, CH_2Ph), 4.54 (d, $J = 1.7$ Hz, 1H, H-2), 4.52–4.42 (m, 4H, CH_2Ph), 4.28 (dd, $J = 9.3$, 3.2 Hz, 1H, H-3), 4.18 (m, 1H, H-5), 3.84 (t, $J = 9.7$ Hz, 1H, H-4), 3.73 (dd, $J = 10.7$, 1.9 Hz, 1H, H-6), 3.65 (dd, $J = 10.7$, 5.4 Hz, 1H, H-6′), 1.15 (s, 9H, Piv); elemental analysis calcd (%) for $C_{38}H_{42}SO_7$: C, 71.00; H, 6.58; *S*, 4.99. Found: C, 71.05; H, 6.38; S, 4.66.

Phenyl 2,3,4-tri-*O*-benzyl-6-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-α-D-mannopyranosyl)-1-thio-α-D-mannopyranoside (33): A solution of **9** (1.03 g, 1.6 mmol) in diethyl ether (40 mL) at −78 °C was treated with triflic anhydride (0.135 mL, 0.8 mmol). After 5 min, a mixture of **8** (0.43 g, 0.8 mmol) and DTBMP (0.98 g, 4.8 mmol) in diethyl ether (10 mL) was added dropwise. The reaction mixture was stirred while the temperature was allowed to rise from −60 to −10 °C, then hydrolysed by addition of saturated aqueous $NaHCO_3$, extracted with CH_2Cl_2 (4 × 100 mL), dried over sodium sulfate and concentrated. The crude mixture was purified by flash column chromatography (Hexanes/EtOAc, 10:1) to give **33** (601 mg, 71%, 88% on 100 mg scale) $[\alpha]_D^{23} = +44.68$ ($c = 1.09$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.40-7.00$ (m, 30H, arom.), 5.55 (d, $J = 1.2$ Hz, H-1c), 5.41 (t, $J = 2.4$ Hz, H-2d), 4.88 (d, $J = 1.5$, 1H, H-1c), 4.73 (d, $J =$ 11.1 Hz, CH_2Ph), 4.65 (d, $J = 10.8$ Hz, CH_2Ph), 4.56 (d, $J = 12.3$ Hz, CH_2Ph), 4.50 (d, $J = 12.1$ Hz, CH_2Ph), 4.60 (m, 4H, CH_2Ph), 4.22 (dd, $J = 4.3$, 9.7 Hz, H-5d), 3.99 (t, $J = 2.6$ Hz, 1H, H-2c), 3.93 (m, 3H, H-3d, H-4c, H-6c), 3.86 (t, $J = 9.6$ Hz, 1H, H-4c), 3.83 (dd, $J = 9.2$, 2.9 Hz, H-3c), 3.79 (ddd, $J = 3.7$, 9.7, 1.5 Hz, H-5c), 3.92 (m, 2H), 3.62 (dd, $J = 1.5$, 10.8, Hz, H-6d), 1.19 (s, 9H, Piv); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 177.5$, 138.6, 138.5, 138.5, 138.4, 138.3, 138.1, 138.0, 134.8, 131.0, 129.2, 128.8, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6, 127.5, 98.1, 85.5, 80.3, 76.3, 75.2, 75.1, 74.8, 74.2, 73.1, 72.2, 72.0, 71.4, 71.1, 68.9, 68.0, 66.8, 27.2; elemental analysis calcd (%) for $C_{65}H_{70}O_{11}S$: C 73.70, H 6.66; found: C 73.73, H 6.73.

Phenyl 2,3,4-tri-*O*-benzyl-6-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-1-thio-α-D-mannopyranoside (34): A solution of **33** (1.26 g, 1.19 mmol) in dry methanol (15 mL) and THF (5 mL) was treated with a 1M solution of MeONa in MeOH (2.5 mL). The reaction mixture was heated to 65 °C overnight, then neutralised with Amberlite IR-120 (H^+), the resin filtered off and the solution evaporated. The crude mixture was purified by flash column chromatography (EtOAc/hexane, 1:1.5) to give pure **34** (1.12 g, 97%) as a colourless oil. $[\alpha]_D^{20} = +60°$ ($c = 0.7$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.44-7.13$ (m, 30H, arom.), 5.57 (d, $J = 1.5$ Hz, 1H, H-1c), 5.04 (d, $J = 1.5$ Hz, H-1d), 4.75 (d, $J = 11.0$ Hz, 2H, CH_2Ph), 4.69 (d, $J = 10.8$ Hz, 2H, CH_2Ph), 4.68 (d, $J = 12.2$ Hz, 2H, CH_2Ph), 4.58 (d, $J = 10.8$ Hz, 2H, CH_2Ph), 4.60 (m, 4H, CH_2Ph), 4.24 (ddd, $J = 4.9$, 1.5, 9.7 Hz, 1H, H-5c), 4.08 (s, 1H, H-2d), 4.04 (dd, $J = 2.9$, 1.5 Hz, 1H, H-2c), 3.98 (t, $J = 9.7$ Hz, 1H, H-4c), 3.96 (dd, $J = 11.4$, 4.9 Hz, 1H, H-6c), 3.85 (dd, $J = 8.9$, 3.1 Hz, H-3d), 3.90 (t, $J = 9.4$ Hz, H-4d), 3.81 (ddd, $J = 4.1$, 1.8, 9.4 Hz, H-4d), 3.76 (dd, $J = 1.5$ Hz, H-6′c), 3.71 (dd, $J = 10.7$, 4.1 Hz, H-6′d), 3.64 (dd, $J =$ 1.8 Hz, H-6d), 2.39 (s, 1H, OH); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 138.6$, 138.4, 138.3, 138.1, 137.9, 137.9, 134.7, 131.0, 129.2, 128.5, 128.5, 128.5, 128.4, 128.3, 128.3, 128.2, 128.0, 127.8, 127.7, 127.6, 127.3, 99.6, 85.9, 80.3, 79.7, 76.8, 75.2, 75.1, 74.7, 74.2, 73.4, 72.5, 72.1, 71.6, 71.0, 68.8, 66.5; elemental analysis calcd (%) for $C_{65}H_{70}O_{11}S$: C 73.90, H 6.20; found: C 74.19, H 6.25.

Phenyl 2,3,4-tri-*O*-benzyl-6-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-1-thio-α-D-mannopyranoside (35): A mixture of **9** (189 mg, 0.29 mmol) and DTBMP (8230 mg, 1.1 mmol) in CH_2Cl_2 (12 mL) was treated with triflic anhydride (24.7 μL) at −78 °C. The reaction was stirred for 5 min, then acceptor **34** (319 mg, 0.5 mmol) in CH_2Cl_2 (4 mL) was added dropwise at −60 °C. Stirring was continued for 1 h while the temperature was allowed to rise to 0 °C. The reaction mixture was hydrolysed with saturated aqueous $NaHCO_3$ (30 mL), extracted with CH_2Cl_2 (3 × 20 mL), dried over sodium sulfate and concentrated. The crude mixture was purified by flash column chromatography (hexanes/EtOAc, 3:1) to give pure **35** (161 mg, 73%). $[\alpha]_D^{23} = +37$ ($c = 1.16$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.45-7.00$ (m, 45H, arom.), 5.54 (d, $J =$ 1.2 Hz, 1H, H-1c), 5.50 (t, $J = 2$ Hz, 1H, H-2e), 5.02 (d, $J = 1.5$ Hz, 1H,

H-1e), 4.96 (d, $J = 1.4$ Hz, 1H, H-1d), 4.70–4.30 (m, 18H, CH_2Ph), 4.20 (m, 1H, H-5c), 4.02 (s, 1H, H-2d), 3.97 (m, 2H, H-2c, H-3d), 3.95–3.55 (m, 13H), 1.11 (s, 9H, Piv); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 177.3$, 138.7, 138.6 (×2), 138.4 (×2), 138.3, 138.1, 137.9, 99.5, 99.1, 84.4, 128.6, 128.5, 128.3, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 127.6, 127.5, 127.4, 127.3, 127.2, 79.6, 79.1, 78.3, 76.8, 75.1, 75.1, 75.0, 74.8, 74.7, 74.5, 74.2, 73.2, 73.1, 72.3, 72.0, 71.9, 71.8, 71.7, 71.4, 69.0, 68.2, 67.3, 27.2; elemental analysis calcd (%) for $C_{92}H_{98}O_{16}S$: C 74.07, H 6.62; found: C 74.17, H 6.42.

***S* and *R* (phenylsulfinyl) 2,3,4-tri-*O*-benzyl-6-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-α-D-mannopyranosyl)-α-D-mannopyrnosyl]-1-deoxy-α-D-mannopyranoside (5a)**: A solution of *m*-CPBA (60%) in CH_2Cl_2 (0.30 mL) was added by syringe to a solution of **35** (36.9 mg, 0.0247 mmol) in CH_2Cl_2 (1.5 mL) at −78 °C. After 1.5 h, the reaction was stopped by addition of saturated aqueous $NaHCO_3$, the organic layer was washed with brine, dried over sodium sulfate and evaporated under vacuo. The residue was purified by column chromatography (EtOAc/hexanes, 1:3) to give 30.8 mg (83%) of the less polar (major) isomer. ^1H NMR ($CDCl_3$, 500 MHz): $\delta = 7.50$ (d, $J = 7.6$ Hz, 2H, arom.), 7.40 (t, $J = 7.6$ Hz, 2H, arom.), 7.30–7.11 (m, 46H, arom.), 5.49 (t, $J = 2.2$ Hz, 1H, H-2e), 5.03 (s, 1H, H-1e), 4.90 (s, 1H, H-1d), 4.86 (d, $J = 10.96$ Hz, 1H, CH_2Ph), 4.85 (d, $J = 10.7$ Hz, 1H, CH_2Ph), 4.80 (d, $J = 11.0$ Hz, 1H, CH_2Ph), 4.63–4.34 (m, 16H), 4.16 (dd, $J = 3.1$, 9.1 Hz, H-3c), 4.05 (m, 2H), 3.97 (dd, $J = 3.0$, 9.1 Hz, 1H, H-3e), 3.85 (m, 7H), 3.72 (dd, 1H), 3.65 (m, 3H), 3.55 (m, 2H), 1.17 (s, 9H, Piv); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 177.3$, 142.2, 138.6, 138.5, 138.3, 138.2, 138.1, 138.0, 137.5, 131.2, 129.4, 128.5, 128.4, 128.4, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 124.0, 99.5, 99.3, 95.9, 79.6, 79.1, 78.3, 77.1, 75.1, 75.0, 74.5, 74.4, 74.1, 73.7, 73.2, 73.1, 72.6, 72.1, 71.9, 71.8, 71.7, 71.4, 69.0, 68.2, 67.3, 60.4, 27.2.

2,3,4-Tri-*O*-benzyl-6-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-α-D-mannopyranosyl)-α-D-mannopyranosyl]-D-mannopyranoside (36): A solution of **35** (381 mg, 0.255 mmol) in 99% wet acetone (20 mL) was treated portionwise with NBS (60 mg, 0.322 mmol) at −15 °C. The reaction mixture was stirred for 30 min with exclusion of light, then neutralised with saturated aqueous $NaHCO_3$, extracted with CH_2Cl_2 (3 × 20 mL), dried over sodium sulfate and concentrated under vacuo. The crude mixture was purified by flash column chromatography (EtOAc/hexanes, 10:90) which gave **36** (348 mg, 98%) as a colourless oil in a 4:1 α/β mixture. ^1H NMR (500 MHz, $CDCl_3$): $\delta = 5.00$ (s, H-2eβ), 5.47 (d, $J =$ 1.4 Hz, H-2eα), 5.05 (s, H-1eβ), 5.02 (s, H-1dβ), 4.96 (d, $J = 2.2$ Hz, H-1dβ), 4.90 (s, H-1cα), 4.90–4.20 (m, 18H, CH_2Ph), 4.09 (t, $J = 2.6$ Hz, H-2dα), 4.20 (s, H-2dβ), 3.98 (dd, $J = 10.4$ Hz H-3eα), 3.89 (m, 1H, H-3dα), 3.80–3.50 (m, 14H), 1.18(s, 9H, Piv); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta =$ 177.3, 138.5, 138.5, 138.4, 138.3, 138.2, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 127.6, 127.5, 127.4, 98.6, 92.1, 79.9, 78.2, 75.3, 75.2, 75.0, 74.9, 74.4, 73.7, 73.3, 73.2, 72.5, 72.0, 71.8, 71.7, 71.5, 70.8, 69.7, 69.3, 68.1, 68.0, 27.7, 27.2; HRFABMS calcd for $C_{86}H_{94}O_{17}NA$: 1421.638872; found: 1421.665 0540.

2,3,4-Tri-*O*-benzyl-6-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-α-D-mannopyranosyl)-α-D-mannopyranoside-trichloroacetimidate (5b): NaH (60%, 15 mg), previously washed with hexanes, was added to a solution of **36** (334 mg, 0.239 mmol) and trichloroacetonitrile (0.359 mL) in CH_2Cl_2 (12 mL). After 2 h, the solvent was evaporated and the mixture purified by column chromatography by using an Et_3N pretreated silica gel to give **5b** (314 mg, 85%) as a colourless oil. $R_f =$ 0.86; ^1H NMR (400 MHz, C_6D_6): $\delta = 8.65$ (s, 1H, NH), 7.50–7.00 (m, 45H, arom.), 6.72 (d, $J = 1.8$ Hz, 1H, H-1c), 5.95 (dd, $J = 3.0$ Hz, 1H, H-1e), 5.10 (d, $J = 11.1$ Hz, 1H, CH_2Ph), 5.08 (d, $J = 10.8$ Hz, 2H, CH_2Ph), 4.77–4.60 (m, 10H), 4.50 (m, 6H), 4.43 (t, 1H), 4.35 (m, 2H), 4.2 (m, 7H), 4.1 (dd, 1H), 4.03 (t, 1H), 4.0 (m, 3H), 3.86 (d, 1H), 3.76 (d, 1H), 1.2 (s, 9H, Piv); elemental analysis calcd (%) for $C_{88}H_{94}NO_{17}Cl_3$: C 68.45, H 6.14, N 0.9; found: C 68.55, H 6.38, N 1.02.

***O*-(3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-*O*-(2-azido-3,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxanedi-1,3-yl)-1,2-*O*-(L-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-myo-inositol (37)**: TMSOTf (1.6 μL, 0.008 mmol) was added to a mixture of **4** (114 mg, 1 equiv), **5b** (210 mg) and 4 Å molecular sieves in CH_2Cl_2 (200 mg) at room temperature. After 1 h, the reaction mixture was neutralised with solid $NaHCO_3$, filtered over Celite and evaporated. The crude mixture was purified by flash column chromatography (EtOAc/hexanes, 1:5) to give **37** (210 mg, 74%) as a colourless oil. ^1H NMR (500 MHz, $CDCl_3$): $\delta = 7.30$–7.05 (m, 55H, arom), 5.49 (t, $J = 2.9$ Hz, 1H, H-2e), 5.47 (d, $J = 3.5$ Hz, 1H, H-1a), 5.31 (d, $J = 1.6$ Hz, 1H, H-1c), 4.99 (d, $J = 1.4$ Hz, 1H, H-1d), 4.90–4.75 (m, 3H, CH_2Ph), 4.60–4.17 (m, 21H), 4.05 (m, 2H, H-1a, H-2d), 3.96 (dd, $J = 8.7$ Hz, 2.9 Hz, 1H, H-3e), 3.90 (t, 1H, $J = 10.0$, H-3b), 3.77 (m, 2H, H-4b, H-6b), 3.72 (t, $J = 2.2$, 1H, H-2c), 3.66 (m, 1H, H-6′b), 3.27 (dd, $J =$ 10.3, 3.5 Hz, 1H, H-2b), 2.61 (s, 1H, OH), 1.98–0.90 (m, 38H), 0.8 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 177.2$, 138.7, 138.6, 138.6, 138.5, 138.4, 138.3, 128.2, 137.9, 135.2, 129.7, 128.7, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.6, 127.6, 127.5, 127.4, 127.4, 127.3, 127.3, 127.2, 127.0, 117.8, 99.9, 99.4, 99.2, 95.8, 80.2, 80.0, 79.4, 78.3, 76.3, 75.9, 75.8, 75.0, 74.8, 74.1, 73.9, 73.2, 73.1, 72.9, 72.3, 72.2, 72.1, 71.9, 71.9, 71.5, 71.4, 69.7, 69.1, 69.0, 68.2, 66.7, 63.2, 51.6, 48.0, 45.3, 45.1, 38.9, 29.7, 29.5, 29.4, 27.2, 27.1, 20.5, 20.2, 17.6, 17.5, 17.4, 17.3, 17.2, 17.1, 17.0, 13.0, 12.7, 12.2, 12.0, 9.7; elemental analysis calcd (%) for $C_{134}H_{165}N_3O_{27}Si_2$: C 69.80, H 7.21, N 1.82; found: C 69.66, H 7.22, N 1.99.

***O*-(3,4,6-Tri-*O*-benzyl-2-*O*-pivaloyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-*O*-(2-azido-3,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-1,2-*O*-(L-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-myo-inositol (38)**: A solution of **37** (232 mg, 0.1 mmol) in THF (12 mL) was treated with TBAF (1M solution in THF, 173 μL, 0.173 mmol) at −15 °C. After 20 min, the reaction was hydrolysed by addition of saturated aqueous NH_4Cl (50 mL), extracted with EtOAc (5 × 20 mL), dried over sodium sulfate and evaporated. The mixture was purified by column chromatography (EtOAc/hexanes, 1:1) to give pure **38** (8180 mg, 87%) as a colourless oil. ^1H NMR (500 MHz, $CDCl_3$): $\delta = 7.30$–7.10 (m, 55H, arom), 5.50 (t, $J = 2.9$ Hz, 1H, H-2e), 5.24 (d, $J = 3.7$ Hz, 1H, H-1b), 5.18 (d, $J =$ 1.5 Hz, 1H, H-1c), 5.09 (d, $J = 1.5$ Hz, 1H, H-1e), 4.91 (d, $J = 1.3$ Hz, 1H, H-1d), 4.82 (d, $J = 10.9$ Hz, 2H, CH_2Ph), 4.76 (d, $J = 11.0$ Hz, 1H, CH_2Ph), 4.74 (d, $J = 11.7$ Hz, 1H, CH_2Ph), 4.63–4.27 (m, 19H), 4.26 (m, 1H, H-2a), 4.08 (t, $J = 2.83$ Hz, 1H, H-2d), 3.97 (dd, $J = 9.5$, 2.9 Hz, H-3e), 3.88 (t, $J =$ 9.5 Hz, 1H, H-4d), 3.8 (dd, $J = 9.5$, 2.8 Hz, H-3d), 3.90 (m, 2H), 3.77 (m, 1H, H-3b), 3.77–3.58 (m, 17H), 3.75 (m, 1H, H-5d), 3.43 (t, $J = 9.3$ Hz, 1H, H-5a), 3.27 (dd, $J = 9.9$, 3.7 Hz, 1H, H-2b), 3.22 (td, $J = 9.2$, 3.6 Hz, 1H, H-4a), 3.10 (s, 1H, OH), 2.10 (s, 1H, OH), 1.90 (m, 2H), 1.15 (s, 9H, Piv), 0.65 (s, 3H), 1.70–0.70 (m, 29H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 177.2$, 138.7, 138.5, 138.3, 138.2, 138.1, 137.9, 137.9, 137.8, 128.2, 128.1, 127.9, 127.7, 127.6, 126.8, 118.4, 100.2, 99.4, 99.0, 97.1, 82.4, 80.1, 80.0, 79.6, 78.4, 78.2, 76.0, 75.6, 75.5, 75.1, 75.0, 74.9, 74.4, 74.3, 74.2, 73.4, 73.3, 73.2, 73.1, 73.0, 72.8, 72.7, 72.5, 72.0, 71.8, 71.6, 71.5, 71.3, 70.4, 70.2, 69.1, 69.0, 68.8, 67.9, 67.4, 63.0, 51.6, 47.9, 45.1, 45.0, 38.9, 29.7, 29.6, 29.4, 29.1, 27.0, 20.5, 20.4, 14.1, 9.8; FABMS calcd for $C_{122}H_{128}N_3O_{26}Na$ $[M+Na]^+$: 2086; found 2086.

***O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→4)-*O*-(2-azido-3,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→6)-3,4,5-tri-*O*-benzyl-D-myo-inositol (3)**: A solution of **38** (150 mg, 0.007 mmol) in MeOH/THF (1:1, 2 mL) was treated with a 1M solution of MeONa in MeOH (150 μL). The mixture was stirred at 60 °C for 24 h, cooled to room temperature, neutralised with Amberlite IR-120 (H^+) and evaporated. The residue was dissolved in DMF (2.5 mL) and NaH (17.5 mg, 0.43 mmol) was added to this solution. The mixture was stirred for 30 min at room temperature and then benzyl bromide (70 μL, 0.584 mmol) was added dropwise. After 1 h, the mixture was cooled to 0 °C, MeOH (5 mL) was added followed by CH_2Cl_2 (100 mL). The organic layer was washed with saturated aqueous NH_4Cl, dried and concentrated. The residue was purified by column chromatography (EtOAc/hexane, 1:4.5) to give **39**. ^1H NMR (500 MHz, $CDCl_3$): $\delta = 7.03$–7.34 (m, 75H, arom), 5.57 (d, $J = 3.5$ Hz, 1H, H-1b), 5.25 (s, 1H, H-1c), 5.11 (s, 1H, H-1e), 4.88 (s, 1H, H-1d), 4.51–4.88 (m, 13H, CH_2PH), 4.32–4.47 (m, 15H, CH_2Ph), 4.46 (dd, 1H), 4.20 (dd, 2H, CH_2Ph), 4.06–4.10 (m, 2H), 3.98–4.04 (m, 3H), 3.91 (t, 1H), 3.59–3.86 (m, 16H), 3.48–3.54 (m, 3H), 3.38–3.42 (m, 3H), 3.26 (dd, $J = 9.7$, 3.5 Hz, 1H, H-2b), 185–1.90 (m, 2H), 1.67–1.72 (m, 2H), 1.43 (d, 1H), 1.16–1.35 (m, 2H), 1.03 (s, 3H), 0.82 (s, 3h), 0.80 (s, 3H); ^{13}C NMR: $\delta = 138.9$, 138.8, 138.6, 138.5, 138.4, 138.2, 138.1, 137.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.4, 127.3, 127.2, 127.0, 118.2, 100.3, 99.4, 95.3, 80.9, 80.7, 80.0, 79.9, 79.7, 77.9, 77.2, 77.0, 76.2, 75.2, 75.0, 74.9, 74.8, 74.7, 74.2, 73.9, 73.8, 73.3, 73.2, 73.1, 72.6, 72.3, 72.2, 72.1, 72.0, 71.9, 69.9, 69.2, 68.8, 66.7, 63.0, 51.6, 48.0, 45.2, 44.9, 29.9, 29.7, 27.0, 20.6, 20.5, 20.4, 9.7.

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Trifluoroacetic acid (0.4 mL) was added to a solution of **39** (149 mg, 0.06 mmol) in wet chloroform (3 mL) and the mixture kept for 18 h at room temperature. Saturated aqueous $NaHCO_3$ was then added at 0 °C, the aqueous layer extracted with CH_2Cl_2 (3 × 10 mL) and the combined organic extracts were dried and concentrated. The residue was purified by column chromatography (EtOAc/hexane, 1:25) to afford pure **3** (85 mg, 60 %) as a colourless oil. ^{1}H NMR (500 MHz, C_6D_6): $\delta = 7.50-6.96$ (m, 75 H, arom), 5.66 (d, $J = 3.5$ Hz, 1 H, H-1b), 5.59 (d, $J = 1.6$ Hz, 1 H, H-1c), 5.49 (d, $J = 1.3$ Hz, 1 H, H-1e), 5.33 (d, $J = 1.5$ Hz, 1 H, H-1d), 5.11–5.04 (m, 3 H, CH_2Ph), 4.98–4.94 (m, 2 H, CH_2Ph), 4.91–4.87 (m, 2 H, CH_2Ph), 4.79–4.65 (m, 5 H, CH_2Ph), 4.61–4.18 (m, 29 H), 4.13–4.00 (m, 7 H), 3.90 (t, $J = 2.4$ Hz, 1 H, H-2c), 3.89–3.65 (m, 8 H), 3.55–3.51 (m, 1 H, H-1a), 3.37 (t, $J = 9.5$ Hz, 1 H, H-5a), 3.12 (dd, $J = 9.6$, 2.6 Hz, 1 H, H-3a), 3.08 (dd, $J = 10.2$, 3.5 Hz, 1 H, H-2b), 2.97 (d, $J = 7.3$ Hz, 1 H, OH); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 138.8$, 138.7, 138.6, 138.5, 138.4, 138.2, 138.1, 137.7, 137.6, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 127.0, 100.2, 99.4, 98.0, 81.6, 81.0, 80.6, 79.9, 79.8, 79.7, 76.9, 76.1, 75.8, 75.2, 74.9, 74.6, 74.4, 74.1, 73.3, 73.2, 72.8, 72.3, 72.1, 72.0, 71.9, 70.8, 69.6, 69.2, 68.9, 66.6, 64.3; FABMS calcd for $C_{135}H_{141}N_3O_{25}Na[M+Na]^+$: 2228; found: 2228.

***O*-α-D-Mannopyranosyl-(1 → 2)-*O*-α-D-mannopyranosyl-(1 → 6)-*O*-α-D-mannopyranosyl-(1 → 4)-*O*-2-ammonio-2-deoxy-α-D-glucopyranosyl-(1 → 6)-D-*myo*-inositol-1,2-cyclic phosphate (1a)**: A solution of dichloromethylphosphate (10 μL, 19.9 μmol) in pyridine (180 μL) was stirred at room temperature until formation of a white precipitate occured(30 min). A solution of **3** in pyridine (120 μL) was added dropwise. The mixture was stirred for 30 min and then saturated aqueous $NaHCO_3$ (0.7 mL) was added. The whole mixture was then evaporated and the residue dissolved in water, acidified with aqueous HCl (2 M) until the solution reached pH 1, extracted with EtOAc and the extract was dried over sodium sulfate and concentrated under vacuo. The residue was dissolved in THF/EtOH/H_2O, 1:11:2 (1.8 mL) that contained NH_4OAc (7 mg) and 10 % Pd/C. The reaction mixture was stirred for 18 h under atmospheric pressure of H_2, filtered over Celite and concentrated. The crude mixture was passed through Sephadex C-18, loaded on Sephadex G-25 and eluted with 10 % H_2O/EtOH. Liophylisation gave **1a** as a white powder. ^{1}H NMR (500 MHz, D_2O): $\delta = 5.45$ (d, $J = 3.6$ Hz, H-1b), 5.18 (s, H-1c), 5.10 (s, H-1d), 4.97 (s, H-1e), 4.65 (t, $J = 4.2$ Hz, H-1a), 4.52 (ddd, $J = 4.5$, 8.0, 20.4 Hz, H-2a), 4.08–3.61 (m, 25 H), 3.5 (t, $J = 9.8$ Hz, 1 H, H-4e), 3.36 (t, $J = 9.9$ Hz, H-5a), 3.31 (dd, $J = 3.4$, 10.8 Hz, H-2b).

NMR measurements: NMR experiments were recorded on a DRX500 Bruker spectrometer with standard or 200 μL microsample NMR tubes. DQF-COSY, HSQC, HMQC and TOCSY measurements used for the full assignment of **1a** were recorded by using the standard *z*-pulsed-field gradient-enhanced or selected-pulse sequences when possible. NOESY measurements were recorded with mixing times of 100, 200, 300 and 400 ms. Selective one-dimensional NOESY and TOCSY experiments were recorded with the dpfgse technique.[88] Due to severe overlapping, ^{1}H-^{1}H and ^{31}P-^{1}H coupling constants were extracted from the dqf-COSY by deconvolution of the two-dimensional antiphase peaks. Calculation of the ω rotamer populations were done with a program written in-house.[89]

Molecular modelling and molecular dynamics: Glycosidic torsion angles were defined as Φ H-1-C-1-O-1-C-X, Ψ C-1-O-1-C-X-H-X and ω H-5-C-5-C-6-O-6. All simulations: single minimisation, Monte Carlo (MC) multiple minimum conformational search or molecular dynamics (MD) were performed by using AMBER* with force-field parameters for pyranose oligosaccharides. Solvent effects were included by using the GB/SA continuum model for water.

All minimisations were run by using up to 9999 Polak Ribiere conjugate gradient iterations until the convergence criterion (rmsd derivative lower than 0.001 kJ Å^{-1} mol^{-1}) was achieved. A multiple minimum conformational search, which varied all the torsional angles involved in the glycosidic linkages simultaneously, was performed with 1000 Monte Carlo steps, with a previously accepted structure used as the starting structure in the next step. After every MC step, the resulting geometry was minimised with 3000 iterations of steepest descent. The structure was checked against duplication and accepted within a window of 50 kJ mol^{-1} from the minima. The minimum energy structure was a Manα(1 → 6)3-Man *gt* rotamer; *gg* and *tg* starting structures used in MD were manually built from the first one and minimised. Molecular dynamics simulations for the above mentioned structures were run for 2.0 ns, with an integration step of 1.5 fs, at a constant temperature of 300 K with a thermal-bath coupling constant of 25 ps, and SHAKE was used for hydrogen bonds; the structures were saved each picosecond. The conditions were optimised by running previous 500–1000 ps dynamics and by varying the integration step values (1, 1.5 and 2 fs) and thermal bath coupling constants (5, 10, 25 and 50 ps), and by using a constant dielectric electrostatic treatment ($\varepsilon = 80$). Trajectories were analysed with software written in-house.[90] The molecular dynamic structures, excluding the first 20 ps, were grouped into two sets. The first one included the first 1020 ps structures of the *gg* starting conformation simulation, for which this rotamer is stable, and the second one corresponded to the structures from the whole *gt* simulation. Both sets were clustered in fifty groups according their heavy atom rmsd and then the representative structure of each cluster was extracted.

Biological assays: Coenzyme A, lithium salt, sodium pyruvate, thiamine pyrophosphate and dithiothreitol were obtained from Sigma (Poole, Dorset, UK). ATP disodium salt, NAD and EGTA were obtained from Boehringer Mannheim (Germany); pyruvate dehydrogenase and pyruvate dehydrogenase phosphatase assays were carried out with a Jasco v-560 UV/Vis spectrophotometer, the circulating temperature in the cell housing was 30 °C. D-[U-^{14}C] glucose and D-[3-^{3}H] glucose were purchased from ICN Biomedicals (Ohio). Collagenase D was from Boehringer and Insulin was purchased from Sigma. Flaco Primaria microtest tissue culture plates were purchased from Becton Dickinson. All reagents were of analytical grade or better. Male Wistar rats (120–140 g) were purchased from Harlan Olac (Bicester, UK).

Activation of PDH phosphatase: The biological activity of **1a** was determined by using the activation of PDH phosphatase.[92] The assay of the activation of the phosphatase was performed by the spectrophotometric variant of the two-stage system described. The pyruvate dehydrogenase complex (PDC) and the PDH phosphatase were prepared from bovine heart and stored at −80 °C until use. The assay for PDH phosphatase, in the presence or the absence of **1a** and liver IPG, was based upon the initial rate of the activation of the inactivated phosphorylated PDH complex. After inactivation with ATP, the activity of the PDC was reduced to less than 1 % of the original value. A two-stage assay was used to quantify the phosphatase activity. A sample of inactivated PDC (50 μL) was preincubated at 30 °, for 3 min C in a solution that contained 1 mg mL^{-1} fat-free bovine serum albumin, 10 mM $MgCl_2$, 0.1 mM $CaCl_2$, 1 mM dithiothreitol in 20 M potassium phosphate buffer, pH 7.0 (total volume 250 μL). After this time, 5 μL of metal, 10 μL of analogue or IPG and 10 μL of the PDH phosphatase were added and the incubation was continued for a further 3 min. At the end of this time, NaF (300 mM, 135 μL)was added. The activated PDH was determined at the second stage spectrophotometrically by measuring the rate of production of the reduced form of NADH. Two hundred microliters of the stopped reaction were added to 1 mL of reaction mixture that contained 50 mM potassium phosphate buffer at pH 8.0, 2.5 mM coenzyme A, 0.32 mM dithiothreitol and 2 mM sodium pyruvate. The production of NADH was followed by using an absorbance at 340 nm for 5 min.

Inbition of cAMP-dependent protein kinase (PKA): The ability of **1a** to inhibit PKA activity was determined by using a colorimetric assay kit and a standard PKA preparation (Pierce, Rockford, IL, USA). A PKA inhibitory peptide was used as a positive control.

Isolation of adipocytes: Adipocytes were isolated from epididymal fat pads of male rats by collagenase digestion as described by Rodbell with minor modifications.[93] Briefly, fat pads from two rats were finely minced with scissors and incubated in 10 mL Krebs Ringer Hepes (KRH) buffer that contained Hepes (9.2 mM), $NaH_2PO_4 \cdot 2H_2O$ (2.2 mM), $NaHCO_3$ (10 mM), NaCl (132 mM), KCl (4.7 mM), $MgSO_4 \cdot 7H_2O$ (1.2 mM), $CaCl_2 \cdot 6H_2O$ (2.5 mM), 2 % BSA and glucose (5 mM), pH 7.4, and collagenase (20 mg) for 20–30 min at 37 °C in a shaking water bath with continuous gassing with 95 % O_2 and 5 % CO_2.

Multiwell plate lipogenesis: Lipogenesis was determined as the incorporation of [3-^{3}H] glucose into toluene-extractable lipids. Briefly, 100 μL of adipocyte suspension (3.5×10^5 mL^{-1} KRH, with or without 10^{-8} M insulin) was incubated in a 96-multiwell plate for 30 minutes at 37 °C in a CO_2 incubator with 2 μL of various concentrations of **1a**, with or without a tenfold excess of Zn^{2+}. Lipogenesis was initiated by the addition of 100 μL KRH containing 0.2 μCi D-[3-^{3}H] glucose and the incubation continued for 2 hours. Adipocytes were harvested onto glassfibre filter mats by using a

cell harvester and rinsed with 5 mM glucose in 0.154 M NaCl. A toluene-based scintillation cocktail (3 ml) was added to each filter disc for counting the radioactivity incorporated into lipids.

Statistical analysis: Data are presented as the mean standard error of the percentage changes from basal and statistical significance was tested by Student's two-tailed t-test for unpaired observations. For the multiwell plate lipogenesis, statistical significance of changes, the mean and the standard deviation of the natural logarithms of the net counts per minute were calculated. The standard deviations for insulin-treated and untreated cells were separately pooled by averaging their squares, calculating the square root of the average and plotting the difference of the mean of each treatment from the basal on a log scale. The error lines above and below the zero are given by $0 + 2d$, in which $d = s\,(1/n + 1/m)$, s is the pooled standard deviation, n is the number of replicates in each treatment mean and m is the number of basal replicates subtracted from the mean. They represent the range outside which a change is significantly different from the basal. The percent change from the basal was obtained as $(e^x - 1)$, in which x is the difference from the basal and multiplied by 100.

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