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## Journal Name

## ARTICLE

## A Biocatalytic Approach Towards the Stereoselective Synthesis of Protected Inositols

L. H. Sayer,<sup>a</sup> G. J. Florence<sup>a\*</sup> and T. K. Smith<sup>a\*</sup>

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A biocatalytic flow system utilising *Trypanosoma brucei* L-*myo*-inositol 1-phosphate synthase has been developed to prepare significant quantities of L-*myo*-inositol 1-phosphate. Using this highly valuable biochemical, we have demonstrated its utility as a viable starting material in a drastically improved and shortened synthesis of a key intermediate in the synthesis of the biologically important *myo*-inositol derivatives. This biotransformation allows rapid and stereospecific inositol derivatives to be synthesized, on a larger scale than ever before.

### Introduction

*myo*-Inositol is ubiquitous in nature and can be found as the structural core of a diverse range of biological metabolites, including inositol phosphates, phosphatidylinositols and their phosphates, glycosylphosphatidylinositol (GPI) anchors<sup>1</sup> and mycothiol<sup>2</sup>. Many of these compounds have important and fundamental biological functions, including cellular signalling,<sup>3</sup> protein anchoring<sup>1</sup> and pathogen virulence.<sup>4</sup> Given their essential function in living systems, they have been the focus of significant research in both academia and industry.

The stereo-controlled synthesis of protected *myo*-inositol derivatives presents a number of obstacles and challenges, often requiring lengthy synthetic sequences, which suffer significant material loss as a consequence.<sup>5</sup> *myo*-Inositol chemistry faces the regioselectivity issues associated with classical carbohydrate chemistry, but the situation is further complicated by the inherent symmetry of the *myo*-inositol core and the requirement to break the symmetry plane to access the desired enantiomer. In

order to overcome the complex enantioselectivity issues it is generally necessary to perform a chemical or enzymatic resolution, which further detracts the yield of synthetic routes.

The eukaryotic *de novo* biosynthesis of *myo*-inositol (**3**), shown in Scheme 1, involves the sequential action of two enzymes: L-*myo*-inositol 1-phosphate synthase (INO1, E.C.5.5.1.4) and *myo*-inositol monophosphatase (IMPase, E.C.3.1.3.25).<sup>6</sup> The rate-limiting step of this process is the conversion of D-glucose 6-phosphate (GP, **1**) to L-*myo*-inositol 1-phosphate (IP, **2**) by INO1, which employs nicotinamide adenine dinucleotide (NAD<sup>+</sup>) as a cofactor. A biocatalytic approach using INO1 would facilitate direct access to enantiopure IP (**2**).<sup>7</sup> This is an attractive target due to its high commercial value (€196 mg<sup>-1</sup>, Cayman Chemical), but also due to the potential for its use as a starting point in the syntheses of numerous *myo*-inositol derivatives with significantly reduced step counts. Using selective protection strategies we envisaged the ability to gain access to any protection pattern around the *myo*-inositol core. A clear advantage of the present approach would be the removal of bottleneck resolution step (50% max yield) as IP (**2**) is produced directly and would provide a unique and previously unavailable chiral pool starting material. However, the use of IP for chemical synthesis is not without challenge and will require an extensive study of protecting group

<sup>a</sup> Biomedical Science Research Complex, Schools of Chemistry and Biology, University of St Andrews, North Haugh, St Andrews, KY16 9ST, UK. Email: [gjf1@st-andrews.ac.uk](mailto:gjf1@st-andrews.ac.uk) & [tk1@st-andrews.ac.uk](mailto:tk1@st-andrews.ac.uk)

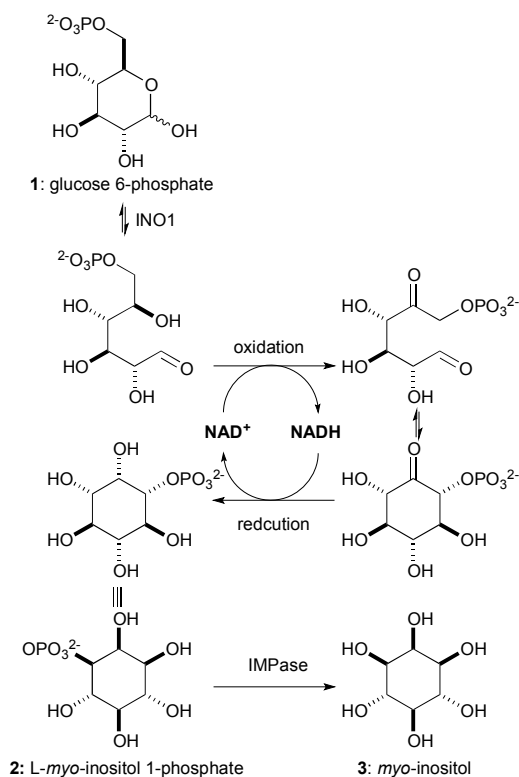
Electronic Supplementary Information (ESI) available: For details of general experimental procedures, synthetic procedures and copies of <sup>1</sup>H and <sup>13</sup>C NMR spectra See DOI: 10.1039/x0xx00000x

## ARTICLE

conditions compatible with the phosphate or its corresponding phosphate ester.

In considering the use of an INO1 enzyme for biocatalysis we identified several key facets. Firstly, the cofactor,  $\text{NAD}^+$ , is both reduced and reoxidised within the reaction, so there is no net change. This allows a much lower amount of  $\text{NAD}^+$  to be used which would be the most expensive component of the system, lowering the potential need for co-factor regeneration *in situ*. The starting material, GP (1), is relatively inexpensive ( $\text{€}29 \text{ g}^{-1}$ , Sigma Aldrich) compared to the IP product. Once established as viable, this system should be able to incorporate a commercial hexokinase upstream to access GP (1) from glucose. Biocatalytic production of IP (2) has previously been accomplished using whole cell fermentation of recombinant *S. cerevisiae* INO1<sup>8</sup> and isolated recombinant INO1 enzyme preparations.<sup>9,10</sup> This work clearly demonstrates the potential for the biocatalytic production of IP, however within an industrial setting these methods may fail to provide an efficient and economical route to IP (2).

The protozoan parasite *Trypanosoma brucei* relies upon the *de novo* synthesis of *myo*-inositol for its survival.<sup>11,12</sup> The *T. brucei* INO1 (*Tb*INO1) has been cloned and overexpressed in *Escherichia coli* using a pBAD TA vector, which encodes a C-terminal hexa-His tag.<sup>11</sup> The recombinant *Tb*INO1 protein was shown to be catalytically active as part of a coupled assay developed for a drug discovery programme, whereby the inhibition of *Tb*INO1 activity was assessed by the phosphate released by produced IP via an IMPase. Saturation kinetic studies showed *Tb*INO1 to have an apparent  $K_m$  of 0.58 mM and  $V_{max}$  of 0.25 mole hour<sup>-1</sup> with respect to [GP (1)]. The specific activity of recombinant *Tb*INO1 was determined to be 756 U mg<sup>-1</sup> at 37 °C where 1 U is the amount of enzyme catalysing the formation of 1 nmole of IP (2) per minute at 37 °C.<sup>11</sup> The specific activity of recombinant *Tb*INO1 is approximately 10-fold higher than that of human recombinant INO1 (70 U mg<sup>-1</sup>). The protein proved relatively stable operating at an optimum temperature



Scheme 1 – Biosynthesis of *myo*-inositol (3).

of 37 °C. The reaction was conducted on a <1 mg scale in tris(hydroxymethyl)aminoethane acetate buffer at pH 8.0 and 37 °C. The reaction solution contained 5 mM GP (1), 1 mM  $\text{NAD}^+$ , 2 mM ammonium acetate, 1 mM dithiothreitol (DTT) and 5  $\mu\text{g}$  of *Tb*INO1 in a total volume of 150  $\mu\text{L}$ . Enzyme activity was dependent upon  $\text{NH}_4^+$  ions, with activity reduced to 10 U mg<sup>-1</sup> in their absence. *Tb*INO1 has been shown to furnish complete conversion to IP (2), which is important when considering the optimization and scale up of this process. It not only increases efficiency, but also avoids potential difficulties in the separation of IP (2) from any remaining GP (1) due to their similarly high polarity. *Tb*INO1, therefore, looks like an ideal candidate for use in the biocatalytic production of IP (2).

## Results and discussion

### *Tb*INO1 Expression, Purification and Assay

Recombinant *Tb*INO1 was expressed as previously detailed<sup>14</sup> and purified by  $\text{Ni}^{2+}$ -affinity chromatography, eluting with an increasing gradient of imidazole in

20 mM ammonium bicarbonate (pH 8.5) and 250 mM NaCl. The protein was dialysed against a solution of 20 mM ammonium bicarbonate (pH 8.5), 50 mM NaCl and 5 mM DTT, and stored with 20 % glycerol at -80 °C. This process yielded the recombinant *TbINO1* enzyme in a yield of 75 mg L<sup>-1</sup> of culture broth. Conversion of *TbINO1* reactions was determined colourimetrically, using the established IMPase assay.<sup>11</sup>

### Batch Scale and Flow Optimisation

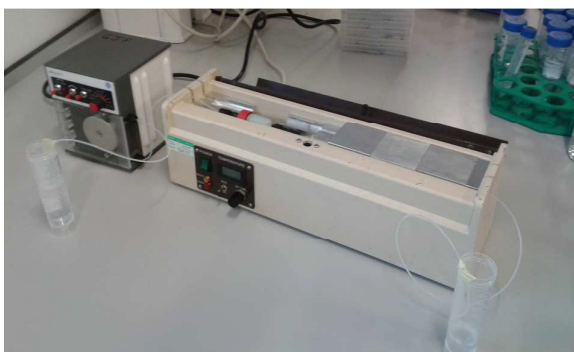
In assessing the original *TbINO1* assay conditions for batch reactions,<sup>14</sup> we sought to exchange the buffer from tris(hydroxymethyl)aminoethane acetate to ammonium bicarbonate. This change facilitates the direct isolation of IP due to the volatility of ammonium bicarbonate, while also reducing the number of components in the reaction solution but maintaining ammonium ion concentration. Initial experiments were run in the ammonium bicarbonate buffer on a 0.4 mg scale in a total volume of 150 µL. Optimisation to a conversion of >95 % was achieved with the reaction containing 10 mM GP (**1**), 2 mM NAD<sup>+</sup>, 1 mM DTT, 50 mM ammonium bicarbonate (pH 8.5) and 87 µg *TbINO1* at 37 °C overnight. Conversion was determined by an IMPase assay using the malachite green method of Itaya and Ui to determine free phosphate released.<sup>15</sup> Scaling up to 10 mg scale was accomplished by increasing *TbINO1* to 300 µg and the total reaction volume to 600 µL and provided quantitative conversions. The concentrations of NAD<sup>+</sup>, DTT and ammonium bicarbonate (pH 8.5) were maintained at the original levels.

Attempts to further increase the scale of the batch reaction beyond a 10 mg scale proved less satisfactory. On 100 mg GP (**1**) scale a maximum conversion of 76% was achieved after six days using a reaction system containing 4 mM NAD<sup>+</sup>, 1 mM DTT, 50 mM ammonium bicarbonate (pH 8.5) and 300 µg *TbINO1* in a total volume of 1 mL. In assessing the various factors that prevented full conversion, we observed the yellowing of the reaction mixture to be consistent with the breakdown of NAD<sup>+</sup> over extended time periods in

basic reaction medium.<sup>16</sup> Furthermore, additional NAD<sup>+</sup> dosing did not restore enzyme activity and no further GP conversion was observed. Furthermore, the abrupt halt to reaction progress after six days coincided with precipitation of denatured *TbINO1*. Thus for the batch reaction to be viable full conversion must be achieved within this time frame. Efforts to improve upon the 76 % conversion including enzyme redosing, GP concentration variation and dosing, failed to deliver and the lack of full conversion on this larger batch scale was attributed to an equilibrium affect between GP and IP. Thus, it was concluded that the batch reaction would not prove viable for scale up of the process given the requirements defined at the outset.

Conducting the biocatalysis in-flow presents many advantages over an in-batch system.<sup>17</sup> In general, flow systems are more readily amenable to scale up than batch reaction processes. Immobilisation of enzymes tends to improve their stability and aids enzyme recycling. Additionally, product purification is further simplified with high conversion rates. The use of a flow system for *TbINO1* is particularly attractive because it would resolve many of the issues encountered within batch process, in other words an equilibrium between substrate and product that would be detrimental would not be encountered. An immobilized *TbINO1* system set up that passes a low concentration of GP (**1**) at an optimal flow rate would ensure full conversion to IP (**2**) and overcome any issues associated with unfavourable product inhibition at equilibrium. Once protein activity drops the reaction can be stopped and fractions containing only fully converted product can be collated for isolation.

In these proof of concept studies, our recombinant *TbINO1* was immobilized directly on a Ni<sup>2+</sup>-charged IMAC Sepharose<sup>TM</sup> Fast Flow 6 column taking advantage of the C-terminal hexa His-tag. This resin, designed for protein purification though not optimal, has a loading capacity of up to 40 mg mL<sup>-1</sup> of resin and provided a suitable enzyme support to perform preliminary studies. Initially a 1 mL column was loaded with purified *TbINO1* by passing a solution at 25 mL h<sup>-1</sup>.



**Figure 1** – Reaction set up. The *TbINO1* loaded  $\text{Ni}^{2+}$ -charged column was placed within a column oven to enable the reaction to be conducted at a constant  $37\text{ }^\circ\text{C}$ , and a peristaltic pump was used to control the movement of liquid through the system.

A reaction solution containing  $5\text{ mM}$  GP (**1**),  $2\text{ mM}$   $\text{NAD}^+$ ,  $1\text{ mM}$   $\beta$ -mercaptoethanol and  $50\text{ mM}$  ammonium bicarbonate ( $\text{pH } 8.5$ ) could then be passed through the system (Figure 1). The immobilized *TbINO1* was able to achieve an  $81\%$  conversion in one pass at a flow rate of  $2.5\text{ mL hr}^{-1}$  at  $37\text{ }^\circ\text{C}$ . The product was collected and acidified to  $\text{pH} < 2$  using concentrated hydrochloric acid, and following lyophilisation furnished IP, in its free acid form, as a white solid containing traces of  $\text{NAD}^+/\text{NADH}$ . Dissolution in MeOH and filtration to remove the sparingly soluble  $\text{NAD}^+/\text{NADH}$ , followed by concentration gave the final product. However initial attempts to chemically modify the IP (**2**) were hampered by  $\text{Ni}^{2+}$ -ions that had leached from the immobilised *TbINO1* column. This issue was resolved by the incorporation of a  $\text{Ni}^{2+}$ -free column to scavenge leached  $\text{Ni}^{2+}$  ions.

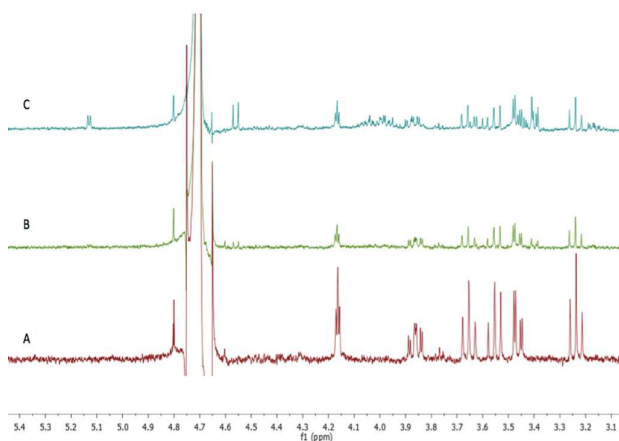
Following this initial success the stationary phase volume was increased to a  $5\text{ mL}$  column to accommodate additional *TbINO1* (up to  $200\text{ mg}$  enzyme) to allow for a higher concentration of GP (**1**) to be processed under flow conditions and enable reaction monitoring via  $^1\text{H}$  NMR analysis. A reaction solution containing  $18\text{ mM}$  GP (**1**),  $1\text{ mM}$   $\text{NAD}^+$ ,  $1\text{ mM}$   $\beta$ -mercaptoethanol and  $50\text{ mM}$  ammonium bicarbonate ( $\text{pH } 8.5$ ) can be passed through the system and achieve full conversion to IP (**2**) in one pass. After  $36\text{ hours}$  the *TbINO1* begins to significantly lose activity and was unable to fully convert the substrate (Figure 2). At this flow rate, and amount of enzyme ( $\sim 200\text{ mg}$ ),

the system produced  $420\text{ mg}$  of IP (**2**) in  $36\text{ hours}$ . The increased level of conversion in the flow system can be attributed to the increased amount of enzyme immobilized on the Sephadex support avoiding the problems of protein denaturation and co-factor decomposition which plagued the homogeneous batch reaction variant. Furthermore the lack of newly formed product (IP) interacting with the enzyme allowing an unfavourable equilibrium between GP and IP is significantly reduced, thus, near full conversion is achieved under flow conditions.

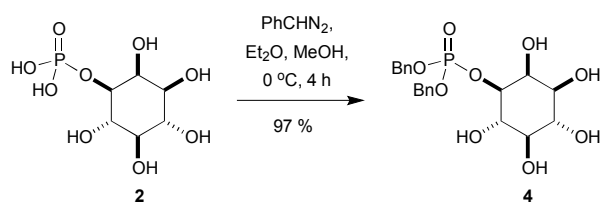
#### Further processing and chemical derivatisation of L-*myo*-inositol-1-phosphate (**2**)

Having established access to IP (**2**) we sought to explore its utility as a potential starting material in chemical synthesis. Our first priority was to effectively cap the sensitive phosphate moiety in **2**. Not unsurprisingly there is little precedent for this transformation, however Griffin *et al.* used diazomethane for the selective methylation of simpler phosphates over alcohols.<sup>18</sup> Following this precedent we opted for benzylation of the phosphate with phenyldiazomethane (Scheme 2). Thus, treatment of a high dilution solution of IP (**2**) in MeOH with freshly prepared ethereal phenyldiazomethane provide the bis-benzyl phosphate ester derivative **4** in  $97\%$  yield as a colourless solid after lyophilisation.





**Figure 2** – Reaction progress monitored by  $^1\text{H}$  NMR spectroscopy. Reaction was conducted at  $37^\circ\text{C}$  with a flow rate of  $2.5\text{ mL h}^{-1}$  and the solution contained  $18\text{ mM GP (1)}$ ,  $1\text{ mM NAD}^+$ ,  $1\text{ mM } \beta\text{-mercaptoethanol}$  and  $50\text{ mM ammonium bicarbonate (pH 8.5)}$ . A: 0 to 20 h fractions, B: 20 to 30 h fraction, C: 30 to 40 h fraction.

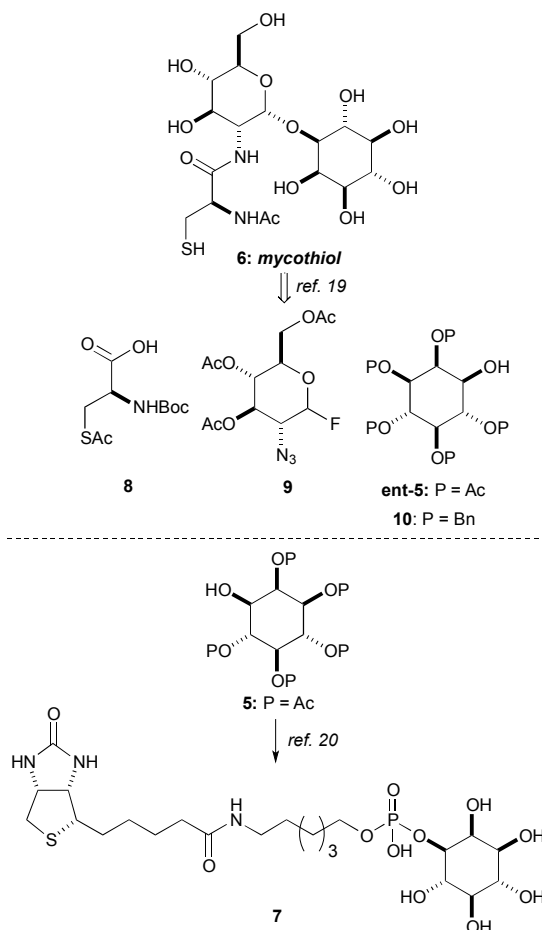


**Scheme 2** – Selective phosphate benzylation using phenyldiazomethane.

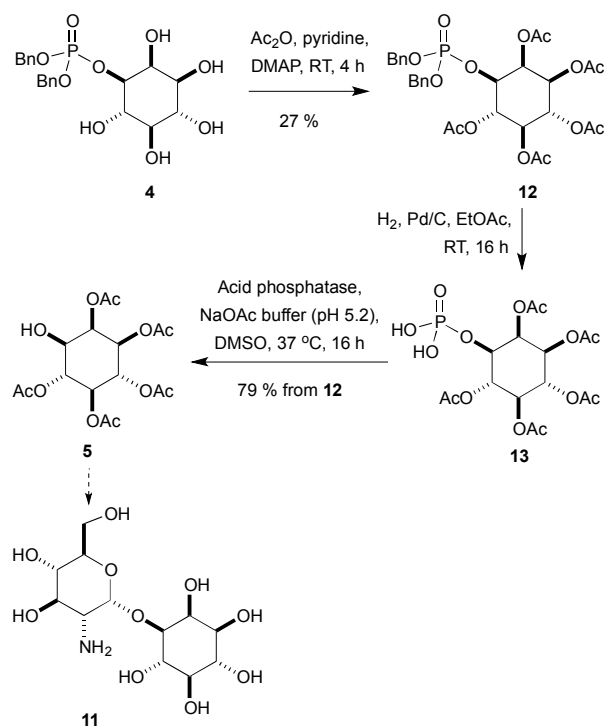
In order to demonstrate the viability of **4** as a building block for the synthesis of inositol derivatives we identified the pentaacetate **5** as suitable protected inositol intermediate, as shown in Scheme 3. This intermediate in either of its enantiomeric forms has been used in the synthesis of mycothiol **6** by Steenkamp *et al.*,<sup>19</sup> and in the preparation of biotinylated chemical probes **7** by Otsuka *et al.*<sup>20</sup> Mycothiol is the major low molecular weight thiol used in most actinomycetes cells to reduce toxic oxidants and maintain an intracellular reducing environment.<sup>21-24</sup> *Mycobacterium tuberculosis*, contains some of the highest levels of mycothiol,<sup>19</sup> and it is hypothesized that this organism could become vulnerable to therapeutics and other stress factors if the biosynthesis or functions of mycothiol are interrupted.<sup>25</sup> Therefore, mycothiol and analogues thereof are important targets that could

lead to urgently required novel therapeutic treatments for tuberculosis.<sup>26</sup>

Synthetic routes to mycothiol (**6**) and derivatives adhere to similar synthetic strategies (Scheme 3) involving a protected cysteine (**8**), a glucosamine donor (**9**) and an enantiopure *myo*-inositol acceptor, such as ent-**5** or **10**. Synthetic routes have relied upon classical manipulations of *myo*-inositol with the shortest route to date of ent-**5** requiring seven steps proceeding with a 7% overall yield.<sup>19</sup> From our dibenzylated phosphate derivative **4** we sought to rapidly access the pentacetate **5** (Scheme 4),<sup>27</sup> which could be used in the synthesis of an unnatural analogue (**11**) and that has previously proven to be valuable in the study of mycothiol biosynthesis.<sup>19</sup>



**Scheme 3** – Applications of inositol pentaacetate **5** in synthesis.



**Scheme 4** – Synthesis of inositol pentaacetate derivative **5**.

Starting with peracetylation with acetic anhydride, pyridine and DMAP at room temperature provided **12** in a modest 27% yield (Scheme 4).<sup>28</sup> The removal of the phosphate was now required and a variety of direct acid hydrolysis conditions were screened without success including aqueous HF and HCl/MeOH. To overcome this problem, the benzyl groups were removed by hydrogenolysis and the crude intermediate **13** was treated directly with an acid phosphatase to produce desired alcohol **5**. This route produced the desired peracetylated inositol intermediate **5** in four steps and 21 % yield from L-myoinositol 1-phosphate (**2**), an improvement over those present within the literature.<sup>19,20,27</sup>

## Conclusions

We have developed a novel biocatalytic flow process that can produce L-myoinositol 1-phosphate (**2**) at a rate of roughly 12 mg h<sup>-1</sup>. The recombinant *TbINO1* is stable under the reaction conditions for roughly 36 hours, allowing access to 430 mg of the highly desirable product in one preparation. We have demonstrated the convenience of using enantiopure L-myoinositol 1-

phosphate (**2**) as a starting point in the synthesis of *myo*-inositol derivatives, furnishing enantiomerically pure pentaacetate **5** in four steps, a vast improvement over literature methods. We aim to achieve different protection patterns about the *myo*-inositol core, in order to gain access to and drastically reduce routes to other important *myo*-inositol derivatives. The biocatalytic *TbINO1* flow system is not fully optimised at this stage requiring further investigation to determine the best method to immobilise *TbINO1* and will be reported on in due course. However these studies clearly demonstrate the potential of a biocatalytic approach in addressing the complexities of inositol synthesis.

## Experimental

### Materials and methods

**Trypanosoma brucei L-myoinositol 1-phosphate Synthase (*TbINO1*) Expression and Purification**<sup>11,14</sup> Recombinant expression of *TbINO1* was conducted using the pET15b-*TbINO1* construct within BL21 Rosetta (DE3) cells (Invitrogen). *TbINO1* was purified by Ni<sup>2+</sup>-affinity chromatography, eluted with an increasing step gradient of imidazole (10, 20, 50, 100, 250 and 400 mM) in 20 mM ammonium bicarbonate (pH 8.5) and 250 mM NaCl. The His-tagged protein was dialysed against 20 mM ammonium bicarbonate (pH 8.5), 50 mM NaCl and 5 mM DTT. The resulting solution could be stored with 10 % glycerol at -80 °C for up to 6 months. In a typical expression, 600 mg of *TbINO1* was collected from 8 L of cell culture (75 mg L<sup>-1</sup>).

***TbINO1* Immobilisation:** Frozen pellets from 4 L of growth media were suspended in a lysis buffer of 20 mM ammonium bicarbonate (pH 8.5), 200 mM NaCl and 3 mM 2-mercaptoethanol. Cells were lysed by sonication (7 x 20 second bursts, 40 second rests) and the supernatant cleared by centrifugation (40,000 rcf, 20 minutes, 4 °C). The supernatant was filtered (0.45 μm) prior to loading onto a freshly charged, new 5 mL Ni<sup>2+</sup>-Sephacrose column. The column was washed with 25 mL of a solution containing 10 mM imidazole, 50 mM ammonium bicarbonate (pH 8.5) and 1 mM 2-mercaptoethanol to elute the unwanted cell extracts. The column was finally washed with a solution containing 50 mM ammonium bicarbonate (pH 8.5) and 1 mM 2-mercaptoethanol, before fitting a Sepharose column in series that had been previously stripped of any Ni<sup>2+</sup> ions by washing with 50 mM EDTA solution.

**L-myoinositol 1-phosphate (**2**).** A solution containing 18 mM D-glucose 6-phosphate (**1**), 1 mM NAD<sup>+</sup>, 50 mM ammonium bicarbonate (pH 8.5) and 1 mM 2-mercaptoethanol was passed through the previously prepared flow system at a rate of 2.5 mL h<sup>-1</sup>. 25 mL fractions were collected and each fraction was separately acidified to pH <2 using concentrated HCl, before being lyophilised to produce crude L-myoinositol 1-phosphate (**2**). <sup>1</sup>H NMR was used to determine which fractions contained no D-glucose 6-

phosphate (**1**), which were combined and dissolved in methanol, filtered and concentrated under reduced pressure to yield *L*-myo-inositol 1-phosphate (**2**, 341 mg).  $[\alpha]_{\text{D}}^{20}$  -4.60 (*c* 1.00, H<sub>2</sub>O at pH 9); IR (ATR) 3262, 3041, 2965, 2936, 1634, 1033, 999; <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O)  $\delta$  4.24 (1H, t, *J* = 2.8 Hz, H-2), 3.94 (1H, ddd, *J* = 9.8, 8.8, 2.8 Hz, H-1), 3.73 (1H, t, *J* = 9.7 Hz, H-6), 3.63 (1H, t, *J* = 9.7 Hz, H-4), 3.54 (1H, dd, *J* = 10.0, 2.9 Hz, H-3) and 3.31 (1H, t, *J* = 9.4 Hz, H-5); <sup>13</sup>C NMR (126 MHz, D<sub>2</sub>O)  $\delta$  75.7, 73.8, 72.2, 71.4 (*d*, *J* = 6.1 Hz), 71.1 and 70.7; <sup>31</sup>P NMR (202 MHz, D<sub>2</sub>O)  $\delta$  -0.19; HRMS (ES<sup>+</sup>) Calc. for C<sub>6</sub>H<sub>12</sub>O<sub>5</sub>P [M-H]<sup>-</sup> 259.0224, found 259.0222.

**1-Bis(benzyloxy)phosphoryl-L-myoinositol (4).** To a stirred solution of *L*-myo-inositol 1-phosphate (**2**, 200 mg, 0.77 mmol) in methanol (600 mL) at 0 °C was added an excess of ethereal phenyldiazomethane solution (freshly prepared, 6 equivalents of hydrazide S2 used in preparation). The reaction was stirred for 4 h at 0 °C before being quenched with acetic acid (0.50 mL) and stirred for a further 10 minutes. The reaction was concentrated before being dissolved in water (100 mL) and washed with diethyl ether (100 mL x 3). The water was removed by lyophilisation to yield 1-bis(benzyloxy)phosphoryl-*L*-myo-inositol (**4**, 329 mg, 0.75 mmol, 97 %) as a pale yellow solid.  $[\alpha]_{\text{D}}^{20}$  +6.50 (*c* 0.10, H<sub>2</sub>O); IR (ATR) 3323, 3134, 3035, 2808, 1402, 1010, 999; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  7.48-7.34 (m, 10H, H-Ar), 5.12-5.06 (m, 4H, H-7), 4.16-4.10 (m, 1H, H-1), 4.07 (t, *J* = 2.9 Hz, 1H, H-2), 3.76 (t, *J* = 9.7 Hz, 1H, H-6), 3.60 (t, *J* = 9.6 Hz, 1H, H-4), 3.40 (dd, *J* = 10.0, 2.8 Hz, 1H, H-3) and 3.25 (t, *J* = 9.5 Hz, 1H, H-5); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  135.1, 129.1, 128.8, 128.4, 78.7, 73.6, 71.9, 70.8, 70.8, 70.7, 70.7, 70.6, 70.5, 70.4, 70.3; <sup>31</sup>P NMR (202 MHz, CDCl<sub>3</sub>)  $\delta$  -1.95; HRMS (ES<sup>+</sup>) Calc. for C<sub>20</sub>H<sub>26</sub>O<sub>9</sub>P [M+H]<sup>+</sup> 441.1309, found 441.1308

**2,3,4,5,6-Penta-O-acetyl 1-bis(benzyloxy)phosphoryl L-myoinositol (12).** To a stirred suspension of 1-bis(benzyloxy)phosphoryl *L*-myo-inositol (**4**, 100 mg, 0.23 mmol, 1 equiv.) in pyridine (5 mL) was slowly added acetic anhydride (1.06 mL, 11.21 mmol, 50 equiv.) and 4-dimethylaminopyridine (3 mg, 0.02 mmol, 0.1 equiv.) at 0 °C. The reaction was stirred at this temperature for 10 minutes before being stirred at room temperature for 5 h. The reaction was concentrated under reduced pressure and the residue dissolved in dichloromethane (10 mL). The solution was washed with water (10 mL), HCl solution (1 M, 2 x 10 mL), water (10 mL) and brine (10 mL) before being dried over magnesium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, 50 % ethyl acetate in hexane) to yield **12** as a colourless oil (40 mg, 0.06 mmol, 27 %). Rf 0.35 (50 % ethyl acetate in hexane);  $[\alpha]_{\text{D}}^{20}$  +13.0 (*c* 1.00, CHCl<sub>3</sub>); IR (ATR) 3036, 2965, 1749, 1210, 1009, 999; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  7.34-7.18 (10H, m, Ar), 5.66 (1H, t, *J* = 3.0 Hz, H-2), 5.46-5.36 (2H, m, H-6 and H-4), 5.03 (1H, t, *J* = 9.9 Hz, H-5), 5.98-4.83 (5H, m, H-3 and 2 x CH<sub>2</sub>), 4.50 (1H, dt, *J* = 3.0, 9.8 Hz, H-1), 2.07 (3H, s, Ac-2), 1.94 (3H, s, Ac-4), 1.93 (6H, s, Ac-3 and Ac-5) and 1.79 (3H, s, Ac-6); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  169.9, 169.7, 169.7, 169.7, 169.5,

128.7, 128.7, 128.7, 128.6, 128.0, 128.0, 73.0 (*d*, *J* = 5.3 Hz), 70.7, 70.0 (*d*, *J* = 5.0 Hz), 69.9 (*d*, *J* = 5.9 Hz), 69.6 (*d*, *J* = 5.7 Hz), 69.3, 68.9 (*d*, *J* = 2.81 Hz), 68.5, 20.7, 20.6 and 20.5; <sup>31</sup>P NMR (202 MHz, CDCl<sub>3</sub>)  $\delta$  -1.76; HRMS (ES<sup>+</sup>) Calc. for C<sub>30</sub>H<sub>36</sub>O<sub>14</sub>P [M+H]<sup>+</sup> 651.1837, found 651.1823.

**2,3,4,5,6-penta-O-acetyl L-myoinositol (5).** To a stirred solution of pentaacetate **12** (6.3 mg, 9.73  $\mu$ mol) in ethyl acetate (3 mL) was added palladium on carbon (10 wt % loading, 10 mg, 0.09 mmol). The reaction was purged with hydrogen and left under a positive pressure of hydrogen with stirring overnight. The reaction was filtered through Celite and washed through with ethyl acetate, before being concentrated under reduced pressure to provide intermediate **13** (4 mg) which was used directly. The crude phosphate **13** (4 mg, 8.9  $\mu$ mol) was taken up in a mixture of sodium acetate buffer (50 mM, pH 5.2, 860  $\mu$ L) and DMSO (40  $\mu$ L) before lyophilised acid phosphatase (from potato, 3.3 Umg<sup>-1</sup>, 1 mg) was added and the reaction incubated with shaking at 37 °C overnight. The product was extracted into ethyl acetate (1 mL x 5) before being concentrated under reduced pressure to yield **5** (3 mg, 7.7  $\mu$ mol, 79 % over 2 steps). Rf 0.56 (10 % methanol in DCM);  $[\alpha]_{\text{D}}^{20}$  +3.0 (*c* 0.2, CHCl<sub>3</sub>) [lit<sup>27</sup> +8.7 (*c* 1.6, CHCl<sub>3</sub>)]; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  5.62 (1H, t, *J* = 3.0 Hz, H-2), 5.48 (1H, t, *J* = 10.2 Hz, H-4), 5.33 (1H, t, *J* = 10.0 Hz, H-6), 5.17 (1H, t, *J* = 9.8 Hz, H-5), 4.99 (1H, dd, *J* = 10.6, 2.8 Hz, H-3), 3.91 (1H, dd, *J* = 10.1, 3.0 Hz, H-1), 2.24 (3H, s, Ac), 2.12 (3H, s, Ac), 2.05 (3H, s, Ac), 2.04 (3H, s, Ac) and 2.02 (3H, s, Ac); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  171.1, 170.3, 169.9, 169.8, 169.8, 72.5, 70.7, 70.5, 69.4, 69.1, 69.0, 20.9, 20.8, 20.6, 20.6 and 20.6.

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## Notes and references

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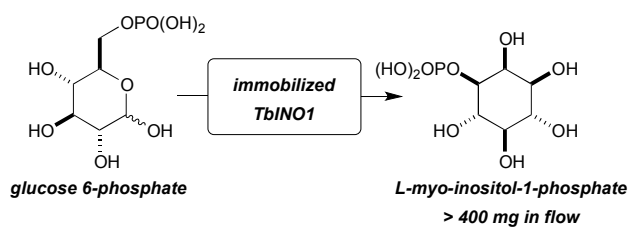
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- 28 The derivatisation of **5** under a variety of peracetylation conditions was studied ( $\text{Ac}_2\text{O}/\text{pyr}$ ;  $\text{Ac}_2\text{O}/\text{l}_2$ ;  $\text{Ac}_2\text{O}$ , pH 10.5, DMF or DMSO). In all cases the C1-phosphate ester was prone to migration resulting in low conversions and isolated yields. Further studies are now focussed on the assessment of alternative protecting group manipulations compatible with the presence of the phosphate ester in **4**.

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*Table of Contents Graphic and Caption*

A Biocatalytic Approach Towards the Stereoselective Synthesis of Protected Inositols

L.H. Sayer, G. J. Florence and Terry K. Smith

*Table of Contents Graphic and Caption*

Immobilized TbINO1 produces >400 mg of chiral inositol 1-phosphate in biocatalytic flow process.