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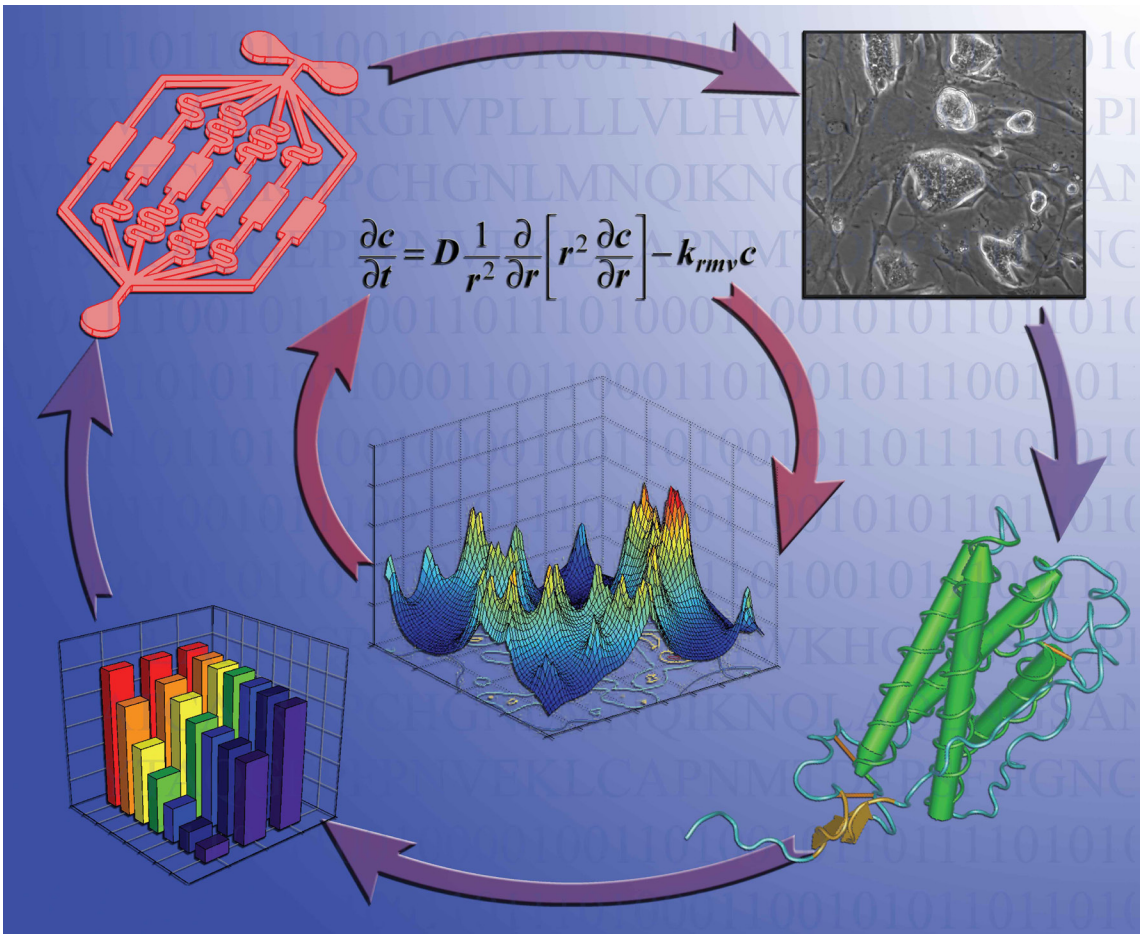

Design, synthesis and biological evaluation of sugar-derived esters, α-ketoesters and α-ketoamides as inhibitors for *Mycobacterium tuberculosis* antigen 85C†‡

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Peptide-based 1,2-dicarbonyl compounds have emerged as potent inhibitors for serine proteases. Herein, we have designed and synthesized D-arabinose and D-trehalose-based esters, α-ketoesters and α-ketoamides, and evaluated their inhibitory activity against *Mycobacterium tuberculosis* (*Mtb*) antigen 85C (ag85C), an acyltransferase in the serine hydrolase superfamily. In addition the compounds were evaluated for the ability to inhibit the growth of *Mycobacterium smegmatis* ATCC 14468, a non-pathogenic surrogate for *Mtb*. Among the synthetic analogs evaluated only the methyl ester **1** derived from D-arabinose was found to inhibit the acyltransferase activity of ag85C (IC_{50} = 25 mM). Based on this weak inhibitory activity it was not surprising that none of the compounds inhibits the growth of *M. smegmatis*. In spite of the weak inhibitory activity of **1**, X-ray crystallography on crystals of ag85C soaked with **1** suggested the formation of a covalent ester adduct between **1** and the Ser124 side chain hydroxyl moiety found within the catalytic site of ag85C; however, some of the active site electron density appears to result from bound glycerol. The lack of activity associated with the α-ketoester and α-ketoamide derivatives of D-trehalose may be the result of intramolecular cyclization of the α-keto moiety with the nearby C-4/4′ hydroxyls leading to the formation of stable bicyclo-ester and amide derivatives.

Introduction

An estimated 30% of the world's population is infected with tuberculosis (TB) and 1.7 million people die of the disease annually.[1,2] *Mycobacterium tuberculosis* (*Mtb*) is the main causative pathogen for this infection. The emergence of multidrug resistant (MDR) and extremely drug resistant (XDR) TB as well as co-infection with HIV poses new challenges for the treatment of this disease once thought to be under control.[2] Therefore, an urgent need for new classes of anti-tubercular agents is obvious. One reason among several that *Mtb* is difficult to treat is the presence of a thick hydrophobic cell wall[3–8] responsible for protecting the organism from foreign substances. The antigen 85 complex (ag85A-C)[9] is believed to be responsible for the attachment of the hydrophobic mycolyl groups onto the cell wall of *Mtb*. In this work we explore potential inhibitors against the ag85s which represent potential targets for the development of new anti-tubercular agents.

Ag85A-C are acyltransferases belonging to the α,β-hydrolase superfamily and convert trehalose 6-monomycolate (TMM) to trehalose 6,6′-dimycolate (TDM) and mycolate the peptidoglycan bound D-arabino-D-galactan (AGP) to form mycolated AGP (mAGP), the major hydrophobic structure of the cell wall of *Mtb*.[10–14] mAGP and other non-covalently bound glycolipids like TMM and TDM form an impermeable barrier to many hydrophilic drugs.[15] Ag85C is the most active component of the ag85 complex largely responsible for cell wall mycolation.[16–18] In our efforts[19–21] to identify potential inhibitors of ag85s, we speculated that 1,2-dicarbonyl compounds such as α-ketoamides and α-ketoesters, known transition state inhibitors of related serine proteases, may have inhibitory activity against ag85s.[22,23] The catalytic triad of serine proteases such as chymotrypsin and subtilisin contain a Ser-His-Asp[24,25] charge-relay system similar to that of Ser-His-Glu system found in ag85s.[26] A putative tetrahedral intermediate in the transfer of the mycolyl moiety from ag85s to the D-arabino-D-galactan (AG) or TMM (not shown) is illustrated in Fig. 1A (Ser124 corresponds to *Mtb* ag85C numbering). At the onset of our study we proposed that the Ser124 nucleophile would be trapped by esters and α-keto compounds according to models shown in Fig. 1B and C, respectively. In the case of the α-keto compounds the enzyme-bound hemiketal could be stabilized through hydrogen bonding with the nearby amides known to stabilize the oxyanion present during acyltransfer (Fig. 1C).

With these design features in mind, three different classes of carbonyl compounds consisting of methyl esters (**1** and **4**), α-ketoesters (**2** and **5**), and α-ketoamides (**3** and **6**) derived from β-D-arabinose and D-trehalose, respectively, were designed (Fig. 2). D-Arabinose and D-trehalose were chosen as templates

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‡ Electronic supplementary information (ESI) available: Copies of ^{1}H, and ^{13}C NMR for compounds **1–4**, **8**, **10–15**, **18**, **20–21** and **24–30**; ^{31}P NMR for compounds **13** and **26**. See DOI: 10.1039/b902284h

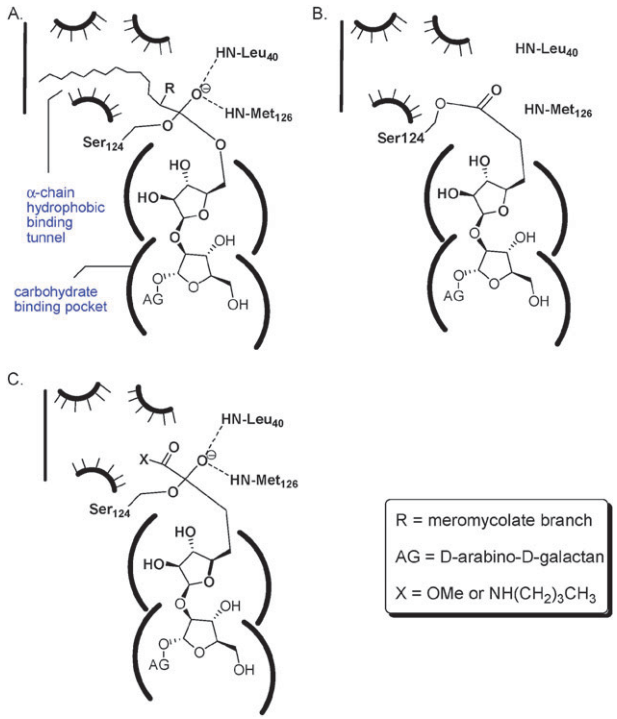


Fig. 1 A. Tetrahedral intermediate formed during the transfer of an acyl group bound to Ser124 of ag85C and the AG; B. A covalent adduct formed between ag85C and a proposed AG-based methyl ester; C. A tetrahedral intermediate formed between ag85C and an AG-based α-ketoester or α-ketoamide.

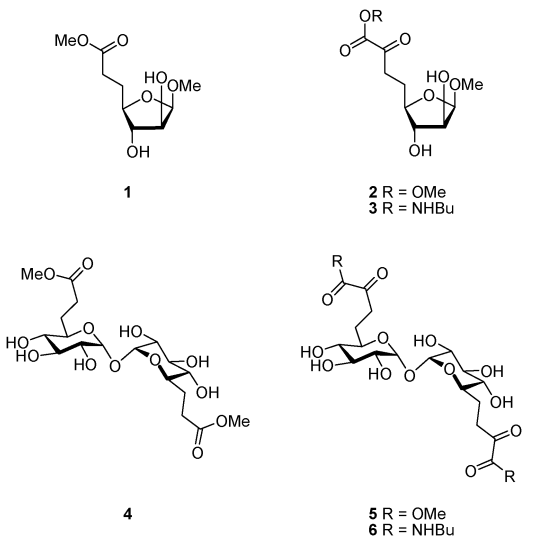


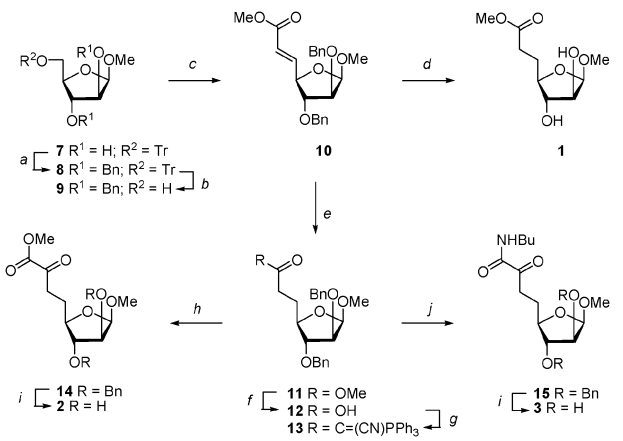
Fig. 2 Proposed esters, α-ketoesters and α-ketoamides.

for the inhibitor design since both are substrates for ag85. The target compounds were thought to be obtainable starting from an arabinose methyl glycoside or trehalose followed by modification at the hydroxymethyl moiety with either a methyl ester, α-ketoester, or an α-ketoamide. The methyl esters were expected to act as potential suicide substrates[27] capable of forming a covalent adduct with serine at the Ser124 of ag85C while the 1,2-dicarbonyl compounds were expected to act as transition state inhibitors.[28] Herein, we describe the synthesis of the arabinose and trehalose-based carbonyl compounds and evaluate their ability to inhibit ag85C.

Results and discussion

To explore our hypothesis we synthesized esters **1** and **4**, α-ketoesters **2** and **5**, and α-ketoamides **3** and **6** (Fig. 2) starting from methyl β-D-arabinofuranoside and D-trehalose, respectively. The tritylated derivative **7**[29] was first benzylated with benzyl bromide and sodium hydride in DMF to produce **8** in 81% yield and the product **8** was detritylated[30] with 80% acetic acid at 70 °C to generate alcohol **9**[31] in 83% yield. The hydroxymethyl moiety of **9** was oxidized quantitatively to an aldehyde by Swern conditions[32] and the crude aldehyde[33] was used directly in a Horner–Wadsworth–Emmons condensation[32] in the presence of methyl diethylphosphonoacetate and sodium hydride in THF to produce α,β-unsaturated ester **10** (61% yield in two steps). Reduction of **10** with Pearlman's catalyst $Pd(OH)_2/C$[32] under H_2 (1 atm) afforded the target ester **1** in 54% yield (Scheme 1). The α,β-unsaturated double bond of **10** was then selectively reduced with Wilkinson's catalyst $(PPh_3)_3RhCl$[34] at 50 psi of H_2 in THF to furnish **11** in 83% yield. Carboxylic acid **12** was generated in 73% yield when **11** was exposed to LiOH in THF–H_2O.

Subsequent coupling[35] of **12** with (cyanomethylene)triphenylphosphorane[36] in the presence of 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) and 4-dimethylaminopyridine (DMAP) in CH_2Cl_2 produced α-keto-cyanophosphorane derivative **13** in 89% yield. DMDO oxidation[37] of **13** in MeOH yielded **14** in high yield. Finally, the target α-ketoester **2** was obtained in 56% yield by hydrogenolysis of **14** using catalytic hydrogenation (10% Pd/C–H_2 in THF) in good yield. Similarly, **13** was converted



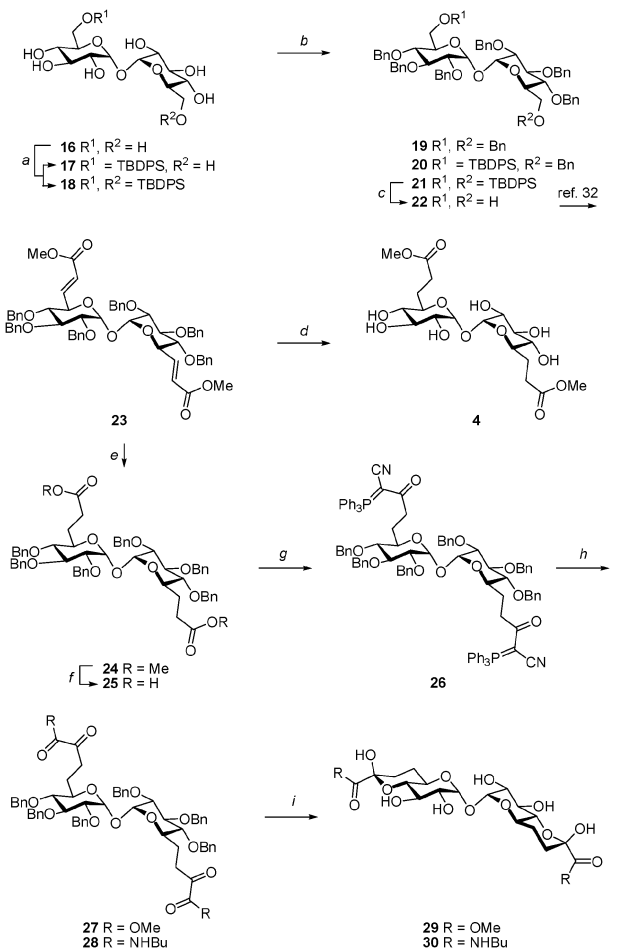
Scheme 1 Arabinofuranoside-derived ester, α-ketoester and α-ketoamide. *Reagents and conditions*: a. BnBr, NaH, DMF, rt, 7 h, 81%; b. 80% AcOH–H_2O, 70 °C, 1.5 h, 83%; c. (i) $(COCl)_2$, DMSO, TEA, CH_2Cl_2, −78 °C to −10 °C, 2.0 h; (ii) Crude aldehyde, methyl diethylphosphonoacetate, NaH, THF, −20 °C, 3 h, 61%; d. $Pd(OH)_2/C$, H_2, MeOH, 4.5 h, 54%; e. $(PPh_3)_3RhCl$, THF, H_2 (50 psi), rt, 24 h, 83%; f. LiOH·H_2O, THF–H_2O (3 : 1), rt, 24 h, 90%; g. Ph_3PCHCN, EDCI, DMAP, CH_2Cl_2, rt, 4 h, 89%; h. DMDO (2 equiv.), MeOH, rt, 1 h, 85%; i. Pd/C, H_2, THF, rt, 24 h, **2** = 56% and **3** = 73%; j. DMDO (excess), CH_2Cl_2, −65 °C, 20 min; and butylamine, −65 °C, 0.5 h, 24%.

to **15** in 24% yield by DMDO oxidation in CH_2Cl_2 followed by amidation[37] with butylamine at −78 °C. The target α-ketoamide **3** was accessed in 73% yield from **15** by Pd/C–H_2 reduction[32] (Scheme 1).

α-D-Trehalose ester **4** was synthesized starting from D-trehalose (**16**). The first step of the synthesis was a regioselective silylation of C-6 and C-6′ positions with *tert*-butyldiphenylsilyl chloride (TBDPSCl) in the presence of imidazole in DMF using standard conditions. The reaction at room temperature generated both mono- and di-silylated products **17** and **18**, which were isolated as their benzylated derivatives **20** (24% yield) and **21** (31% yield) in the next step (Scheme 2). During benzylation of the mixture (**17** and **18**) with benzyl bromide and sodium hydride in DMF in the presence of tetrabutylammonium iodide, along with desired products **20** and **21**, a minor amount of octa-*O*-benzyl-D-trehalose **19**[32] (8% yield) was isolated as the side product. However, compound **18** was isolated in 84% yield when the silylation on **16** was performed at 35 °C under similar reaction conditions. Further, benzylation of the pure **18** produced the desired product **21** in 45% yield accompanied by **19** and **20** in 8% and 24% yields, respectively, as the undesired products. Next, the silyl protective groups of **21** were removed with tetrabutylammonium fluoride trihydrate (TBAF·3H_2O) in THF to produce **22**[32] in 84% yield. Compound **22** was converted to **23**[32] in high yield by oxidation followed by Horner–Wadsworth–Emmons condensation using the previously described conditions. Methyl ester **4** was finally obtained in 79% yield by catalytic reduction[32] with Pearlman's catalyst, $Pd(OH)_2/C$, in anhydrous THF under H_2 atmosphere. Regioselective reduction of α,β-unsaturated double bonds in **23** was accomplished with Wilkinson's catalyst, $(PPh_3)_3RhCl$,[34] under 50 psi of H_2 to afford **24** in 67% yield.

An increased yield of **24** (84%) was obtained from **19** when flash column chromatography was carried out at the final step. Carboxylic acid **25** was generated in 95% yield when **24** was treated with LiOH·H_2O in THF–H_2O (3 : 1). Compound **25** was coupled with (cyanomethylene)triphenylphosphorane[36] by literature procedure[35] in the presence of EDCI and DMAP in CH_2Cl_2 to generate **26** in 78% yield. Compound **26** when oxidized with DMDO[37] in MeOH yielded α-ketoester **27** in 64% yield. Finally catalytic reduction on **27** using 10% Pd/C under H_2 (1 atm) rendered **29** in 62% yield. Compound **30** was similarly obtained from **26**, which was first oxidized to **28** in 46% yield with DMDO[37] followed by treatment of butylamine in CH_2Cl_2 at −78 °C, and finally, catalytic reduction by 10% Pd/C under H_2 on **28** produced desired **30** in 65% yield (Scheme 2).

It appeared that under standard benzylation conditions 6,6′-di-*O*-silylated trehalose derivative **18** was not fully stable and underwent desilylation leading to the formation of fully benzylated trehalose derivative **19** and mono-silylated trehalose derivative **20** as the undesired products. We found that methyl esters **1** and **4** formed smoothly under the reaction conditions described above in both the arabinose and trehalose substrates with the ester carbonyl carbon appearing at δ = 174.4 and 176.2 ppm in their respective ^{13}C NMR. Characteristic carbonyl peaks of α-ketoester **2** (δ = 161.4 ppm for ester carbonyl, and 193.9 ppm for α-keto carbonyl) and its amide congener **3** (δ = 160.28 ppm for amide carbonyl, and 199.03 ppm for α-keto carbonyl) in their ^{13}C NMR confirmed the presence of these two functional groups as expected. We employed the similar reaction conditions for accessing trehalose-derived α-ketoester **5** and its amide congener **6** from their precursors **27** and **28**, respectively. To our surprise, due to proximity of hydroxyl groups at C-4/C-4′, bicyclo-ester **29** and its amide congener **30** formed readily during the palladium-charcoal reduction at the final step. As expected, no α-keto carbonyl was found around 200 ppm in either of **29** or **30** in the ^{13}C NMR spectra of bicyclo-products **29** or **30**. The carbonyl peak for α-keto-functionality of compound **29** was absent (see ESI‡), rather an additional characteristic carbon peak at δ = 95.4 ppm for C-8/C-8′ appeared in the vicinity of the anomeric carbon, δ = 95.9 (C-1/C-1′). Similarly, a set of two adjacent carbon peaks at δ = 94.8 and 94.9 ppm was found in ^{13}C NMR of **30**. A literature search



Scheme 2 Trehalose-derived ester, α-ketoester and α-ketoamide. *Reagents and conditions*: a. TBDPSCl, imidazole, DMF, rt → 35 °C, 16 h, 84%; b. BnBr, NaH, TBAI, DMF, rt, 24 h: **20** → 24%, **19** → 8% and **21** → 45%; c. TBAF·3H_2O, THF, rt, 24 h, 84%; d. $Pd(OH)_2/C$, H_2, THF, rt, 16 h, 79%; e. $(PPh_3)_3RhCl$, H_2 (50 psi), rt, 48 h, 84%; f. LiOH·H_2O, THF–H_2O (3 : 1), rt, 12 h, 95%; g. PPh_3CHCN, EDCI·HCl, DMAP, CH_2Cl_2, rt, 2 h, 78%; h. (i) for **27**; DMDO, MeOH, rt, 0.5 h, 64%; (ii) for **28**; DMDO, CH_2Cl_2, −78 °C, 15 min; then $BuNH_2$, CH_2Cl_2, −78 °C, 0.5 h, 46%; i. (i) for **29**; Pd/C (10%), H_2, THF, rt, 24 h, 62%; (ii) for **30**; Pd/C (10%), H_2, THF, rt, 24 h, 65%.

shows that the configuration at C-8/C-8′ resembles the C-2 carbon of *N*-acetyl neuraminic acid, which appears at δ = 96.4 ppm value.[38] By comparing the δ-value of C-8/C-8′ with that for anomeric carbon of the acid, we concluded C-8/C-8′ as having the similar configuration in both **29** and **30**. Compounds **1–4**, **8**, **10–15**, **18**, **20**, **21**, **24–30** were further characterized by ^{1}H, ^{1}H–^{1}H gCOSY, ^{13}C NMR and HRMS.

Arabinofuranoside analogs **1–3** and trehalose congeners **4**, **29** and **30** were screened for their ability to inhibit *M. smegmatis* ATCC 14 468 using Kirby–Bauer disk diffusion assay.[39] Isoniazid (INH) was used as a positive control and DMSO, the diluent, as a negative control. *M. smegmatis* ATCC 14 468 was used as a fast growing non-pathogenic surrogate for *M. tuberculosis*.[40] The strain was previously used for screening antimycobacterial compounds.[32,41] Unfortunately **1–4** and **29–30** showed no inhibitory activity against this organism.

The *in vitro* activity of the synthetic compounds was evaluated using a recently developed ag85C acyltransferase assay.[21] The trehalose-derived analogs (**4** and **29–30**) did not produce any inhibitory activity (Fig. 3; panel (a)). Among the arabinose-derived substrates, only methyl ester **1** showed inhibition of the enzymatic activity of ag85C at millimolar concentration (Fig. 3; panel (b)). When the enzyme was preincubated with **1** prior to the experiment, 50% inhibition of enzymatic activity was observed at 25 mM concentration of **1**.

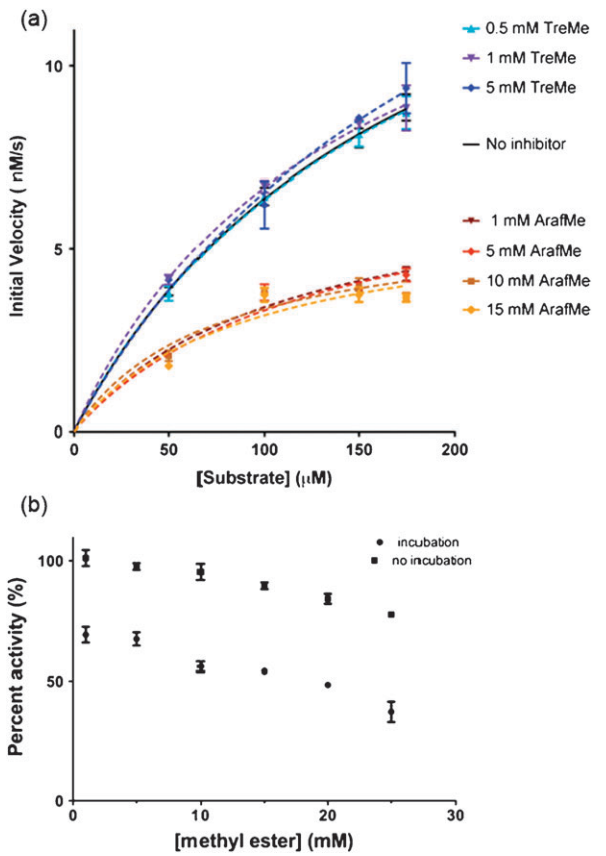


Fig. 3 A. Michaelis–Menten fit of initial velocity *versus* substrate concentration curves obtained for different concentrations of inhibitors (**1** and **4**). B. Percent initial velocity (reaction with inhibitor compared to control without inhibitor) *versus* concentration of **1**.

By comparison, 20% inhibition of the enzymatic activity was observed without incubation at the same inhibitor concentration. The time dependent nature of the results suggests weak binding between the enzyme and **1** leading to the formation of a covalent bond between the enzyme and the inhibitor. Because the low inhibitory activity of **1** required the use of relatively high concentrations to observe any inhibition, insufficient amounts were available for performing a thorough kinetic analysis. From the current data, it is not possible to ascertain the type of inhibition imparted by **1** on ag85C. Considering that **1** is structurally analogous to the true substrate, our continuing hypothesis is that it is a competitive inhibitor.

Crystallographic studies of ag85C using the inhibitor to confirm this hypothesis are inconclusive (Table 1, PDB: 3HRH). Strong electron density is observed in the carbohydrate-binding pocket of the enzyme, however, the identity of the compound or compounds producing this density is not known at this time. Two different compounds were added to the protein crystals and each of three different scenarios could represent the observed electron density. First, compound **1** could be binding within the active site and forming a covalent intermediate with the nucleophile Ser124. The density between Ser124 and the density within the carbohydrate-binding pocket is contiguous, indicating that a bond has been formed. Surprisingly, the positions of the catalytic triad residues do not deviate between this structure and the native form. If a bond is being formed, this result seemingly contradicts our previous contention that the conformational change seen in the ag85C-DEP (diethylphosphate) structure is induced upon formation of the covalent bond between the Ser124 nucleophile and the substrate/inhibitor. However, it is possible that within the context of the protein crystal the propensity of the enzyme to undergo a conformational change is not sufficient to overcome the lattice energy of the crystal.

The second scenario is that the electron density corresponds to glycerol. In the previous structure of ag85C, no electron density resembling glycerol was observed in the carbohydrate-binding pocket. However, in those previous studies, the crystal was exposed to glycerol for less than 10 seconds before flash cooling to 100 K. In preparing the sample for this diffraction study, the crystal was incubated in a solution containing both glycerol at a final concentration of 20% v/v and **1** for an extended period of time. It is likely that the significantly higher

Table 1 Crystallographic statistical data (data set *vs.* refined structure)

Parameters for ag85C soaked with **1**	
Resolution/Å	50–2.2
Completeness (total/highest)	99.8 (99.6)
Number of reflections (total/unique)	267 697/38 641
$I/\sigma I$	7.6
R_{sym} (overall/highest)	13.8/69.4
R-factor (%) (50–2.3 Å)	19.2
R_{free} (%) (50–2.3 Å)	22.3
rmsd for bond lengths/Å	0.006
rmsd for bond angles/°	1.22
Average *B* factor/Å^2	26.9

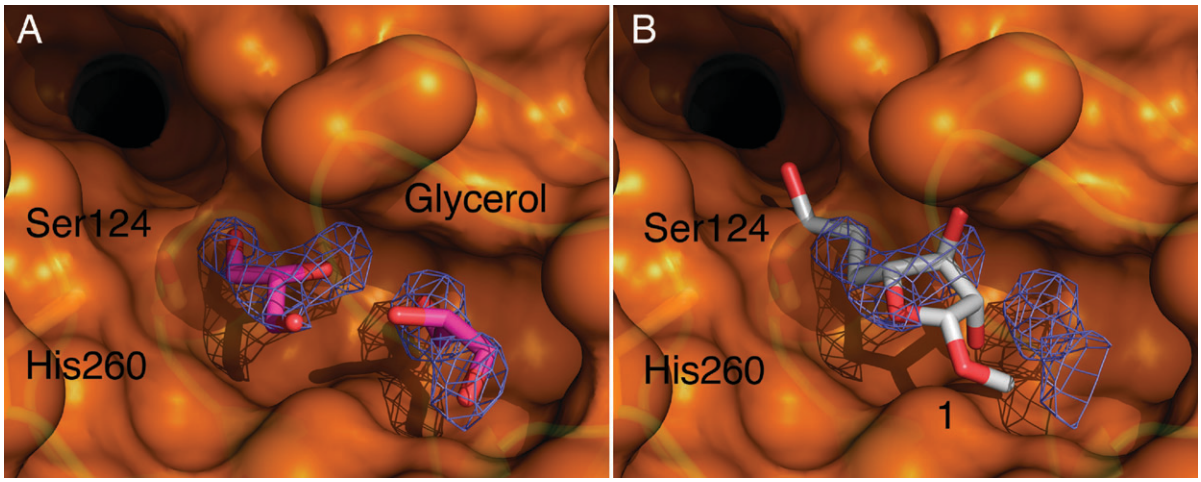


Fig. 4 Molecular surfaces representing the carbohydrate-binding pocket of ag85C. (A) Two glycerol molecules are shown fit in the Fo–Fc map of the current structure. The map is contoured at 3σ. (B) Methyl β-D-arabinofuranoside (**1**) modeled within the same density and forming a covalent bond with Ser124.

concentration of glycerol in the cryo-protecting solution promoted the displacement of **1** from the active site, leaving the glycerol bound within that same site. This presumes that no covalent bond is formed between the inhibitor and the enzyme. This scenario is strengthened by the fact that the current structure exhibits no change in the active site. The third explanation is that the electron density represents a combination of both previously mentioned states, where glycerol and **1** are bound at less than 100% occupancy. Considering all of the available information, multiple glycerol molecules were placed within the enzyme active site (Fig. 4A).

While the structure lacking **1** is disappointing, the information garnered from that structure still offers important insights to how ag85C can interact with various carbohydrates. To better understand how **1** may inhibit ag85C activity, the available electron density from the current crystal structure was used as a basis for modeling **1** within the carbohydrate-binding pocket (Fig. 4B). The most reasonable explanation is that the binding energy between protein molecules required for crystal packing cannot be overcome by formation of the covalent bond in the active site. This lack of an observed conformational change offers the advantage of understanding the interactions between the carbohydrate-binding site of ag85C and potential substrates and inhibitors.

Conclusions

In summary, we have synthesized an arabinose-based ester, α-ketoester and α-ketoamide, as designed. Under the similar reaction conditions, a trehalose-based ester was prepared smoothly. Due to proximity of the hydroxyl functionality close to the α-keto moiety in the trehalose series the α-ketoester and α-ketoamide cyclized at the final step, and bicyclo-products were isolated in each case. To the best of our knowledge, the observation is the first to show the formation of a bicyclo-derivative in trehalose-based compounds, which might open up an avenue for accessing synthetically challenging analogs of the compound class. Based on this result it appears difficult to install α-ketoester and α-ketoamide functionalities in trehalose substrates due to this substrate-controlled intramolecular cyclization phenomenon. In an ag85C acyltransferase assay among the synthetic analogs **1–4**, **29** and **30**, only methyl ester derivative of D-arabinofuranoside **1** has emerged with moderate inhibitory activity against ag85C at 25 mM concentration under preincubation conditions. Unfortunately, but not unexpectedly, compounds **1–4**, **29** and **30** showed no antibacterial activity against *M. smegmatis* ATCC 14468. Finally, the results from co-crystallization and X-ray diffraction studies of ag85C with **1**, while ambiguous, offer hints for designing more potent inhibitors possessing improved binding affinity and specificity for the antigen 85 active site.

Experimental section

General method

Starting materials such as D-arabinose and D-trehalose, and all fine chemicals were purchased from commercial suppliers and were used without further purification. All solvents were obtained from Fisher and used as received. Silica (230–400 mesh) for flash column chromatography was obtained from Sorbent Technologies; thin-layer chromatography (TLC) precoated plates were from EMD. TLCs (silica gel 60, f_{254}) were visualized under UV light or by charring (5% H_2SO_4–MeOH). Flash column chromatography was performed on silica gel (230–400 mesh) using solvents as received. ^{1}H NMR were recorded either on a Varian VXRS 400 MHz or an INOVA 600 MHz spectrometer in $CDCl_3$, CD_3OD or DMSO-d_6 using residual $CHCl_3$, CH_3OH and DMSO as internal references, respectively. ^{13}C NMR were recorded on a Varian VXRS 100 MHz or Varian INOVA 150 MHz in $CDCl_3$ using the triplet centered at δ 77.3, in CD_3OD, using the septet centered at δ 49.0 or in DMSO-d_6 using septet as internal references, respectively. High resolution mass spectrometry (HRMS) was performed on a Micromass Q-TOF2 instrument.

Methyl 2,3-di-*O*-benzyl-5-*O*-trityl-β-D-arabinofuranoside (8)

To a well-stirred suspension of sodium hydride (142.32 mg, 5.93 mmol; obtained by washing 237.21 mg of 60% w/w dispersion in mineral oil with dry *n*-hexane) in anhydrous DMF (5 mL) was added methyl 5-*O*-trityl-β-D-arabinofuranoside **7**[26] (0.86 g, 2.11 mmol) in DMF (5 mL) dropwise under N_2 atmosphere. Benzyl bromide (0.7 mL, 5.93 mmol) was added dropwise and the resulting solution was stirred at ambient temperature. The reaction was monitored by TLC and appeared complete after 7 h. Two to three ice-cubes were added into the reaction mixture and the solution was stirred for 15 min to hydrolyze the excess reagents. The reaction mixture was diluted with 50 mL water and aqueous layer was extracted with ether (3 × 40 mL). Combined organic layer was dried (anhydrous Na_2SO_4) and filtered. The filtrate was concentrated under reduced pressure to get a crude material, which was purified by silica gel flash column chromatography (230–400 mesh; 10 × 5.5 cm) using 1 : 9 (250 mL) and then 1 : 4 (750 mL) EtOAc–hexanes to produce **8** as a yellow gum: yield 1.01 g (81%); R_f = 0.46 (1 : 4 EtOAc–hexanes); ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.18–3.24 (m, 2 H, H-5/H-5′), 3.22 (s, 3 H, OMe), 4.03 (dd, 1 H, J = 4.2, 6.6 Hz, H-2), 4.07 (dd, 1 H, J = 6.0, 10.8 Hz, H-4), 4.12 (t, 1 H, J = 7.2 Hz, H-3), 4.55 (d, 1 H, J = 11.4 Hz, benzylic), 4.61 (m, 3 H, benzylic), 4.69 (d, 1 H, J = 4.2 Hz, H-1), 7.21 (m, 5 H, aromatic), 7.27 (m, 10 H, aromatic), 7.34 (m, 4 H, aromatic), 7.46 (m, 6 H, aromatic); ^{13}C NMR (150 MHz, $CDCl_3$): δ 55.1 (OMe), 66.0 (C-5), 72.6 (CH_2), 72.7 (CH_2), 80.6 (C-4), 82.8 (C-3), 84.2 (C-2), 101.5 (C-1), 127.2, 127.7, 127.8, 128.0, 128.1, 128.3, 128.5, 128.6, 128.9, 137.8, 138.24, 144.1; mass spectrum (ESI-MS), m/z = 367.1493 $(M + Na - Tr)^+$ ($C_{20}H_{24}NaO_5$ requires 367.1521).

Methyl 2,3-di-*O*-benzyl-β-D-arabinofuranoside (9)[31]

Compound **8** (1.04 g, 1.77 mmol) was dissolved in 80% AcOH–H_2O and the resulting mixture was stirred at 70 °C following a literature procedure.[27] TLC showed no starting material after 1.5 h. The reaction mixture was diluted with 100 mL water and aqueous layer was extracted with ether (4 × 40 mL). The organic phase was washed with saturated aqueous $NaHCO_3$ until pH = neutral (checked by pH paper) followed by brine. The ether layer was dried (anhydrous Na_2SO_4) and filtered. The filtrate was concentrated under reduced pressure to get a crude material. Purification of the crude material by silica gel flash column chromatography (230–400 mesh; 8 × 3.1 cm) using 1 : 4 (250 mL) and then 2 : 3 (250 mL) EtOAc–hexanes generated **9** as a colorless gum: yield 0.51 g (83%); R_f = 0.11 (3 : 7 EtOAc–hexanes).

Methyl (5*E*)-2,3-di-*O*-benzyl-5,6-dideoxy-β-D-arabino-hept-5-enofuranosiduronic acid methyl ester (10)

To a cooled solution of oxalyl chloride (0.57 mL, 6.67 mmol) in CH_2Cl_2 (5 mL) at −78 °C was added DMSO (0.95 mL, 13.35 mmol) in CH_2Cl_2 (3 mL) dropwise under N_2 atmosphere over a period of 5 min following a literature method.[32] After stirring the reaction for 10 min at −78 °C, the reaction mixture was allowed to attain −60 °C and compound **9** (1.53 g, 4.45 mmol) in CH_2Cl_2 (25 mL) was added dropwise for 30 min. The resulting reaction mixture was stirred for 45 min and then temperature was again raised to −45 °C and anhydrous TEA (3.75 mL, 26.7 mmol) was added dropwise. After the reaction was stirred for 0.5 h, temperature was raised to −10 °C. Cold CH_2Cl_2 (80 mL) was added at 0 °C followed by cold water (100 mL). Organic layer was separated by a separatory funnel and the aqueous phase was extracted with CH_2Cl_2 (2 × 50 mL). Combined organic phase was dried (anhydrous Na_2SO_4) and filtered. The filtrate was concentrated under reduced pressure to get the crude aldehyde.

Methyl diethylphosphonoacetate (1.4 mL, 8.01 mmol) was dissolved in THF (10 mL) and NaH (213.64 mg, 8.9 mmol; obtained by washing 356.08 mg of 60% w/w dispersion in mineral oil with dry *n*-hexane) in THF (5 mL) was added to the flask dropwise at −20 °C. The resulting solution was stirred for 0.5 h and then crude aldehyde without further purification was dissolved in anhydrous THF (10 mL) and added dropwise under N_2 atmosphere. The reaction was stirred for 3 h at −20 °C. After completion of the reaction (monitored by TLC), ether (60 mL) was added and the organic phase was washed with saturated aqueous NH_4Cl and brine, and dried (anhydrous Na_2SO_4). The filtrate was concentrated under reduced pressure to get a crude material, which was purified by silica gel flash column chromatography (230–400 mesh; 10 × 5.5 cm) using 1 : 9 (1 L) and then 3 : 17 (1 L) EtOAc–hexanes to produce **10** as a colorless oil: yield 1.08 g (61%); R_f = 0.32 (1 : 4 EtOAc–hexanes); ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.41 (s, 3 H, OMe), 3.75 (s, 3 H, COOMe), 4.06 (dd, 1 H, J = 4.2, 7.8 Hz, H-2), 4.17 (t, 1 H, J = 6.6 Hz, H-3), 4.42 (ddd, 1 H, J = 1.4, 6.3, 6.3 Hz, H-4), 4.60 (d, 1 H, J = 12.0 Hz, benzylic), 4.65 (s, 2 H, benzylic), 4.73 (m, 2 H, benzylic and H-1), 5.99 (dd, 1 H, J = 1.2, 15.6 Hz, H-6), 6.90 (dd, 1 H, J = 6.0, 15.6 Hz, H-5), 7.29–7.39 (m, 10 Hz, aromatic); ^{13}C NMR (150 MHz, $CDCl_3$): δ 51.6 (ester Me), 55.4 (OMe), 72.7 (CH_2), 72.9 (CH_2), 79.9 (C-4), 83.6 (C-3), 85.0 (C-2), 101.8 (C-1), 127.9, 127.93, 128.1, 128.2, 128.5, 137.6, 137.8, 146.9, 166.5 (C═O); mass spectrum (HRMS), m/z = 421.1603 $(M + Na)^+$ ($C_{23}H_{26}NaO_6$ requires 421.1627).

Methyl β-D-arabino-heptafuranosiduronic acid methyl ester (1)

Compound **10** (0.34 g, 0.86 mmol) was dissolved in anhydrous MeOH (10 mL) and to the flask was added Pearlman's catalyst, $Pd(OH)_2/C$ (85 mg). The suspension was degassed carefully using low vacuum and a balloon of H_2 was connected to the flask *via* a needle. The reaction mixture was stirred at room temperature and monitored by TLC. After completion of the reaction (4.5 h), the catalyst was filtered through filter paper and the filtrate was concentrated under reduced pressure to provide a crude material. Purification by silica gel flash chromatography (230–400 mesh; 8 × 4.5 cm) with 3 : 2 EtOAc–hexanes (1 L) afforded **1** as a colorless gum: yield 0.103 g (54%); R_f = 0.19 (4 : 1 EtOAc–hexanes); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.87 (m, 1 H, H-5), 2.0 (m, 1 H, H-5′), 2.42 (ddd, 1 H, J = 6.6, 8.4, 16.2 Hz, H-6), 2.48 (ddd, 1 H, J = 6.0, 9.0, 15.0 Hz, H-6′), 3.36 (br s, 1 H, OH-2), 3.39 (s, 3 H, OMe), 3.66 (s, 3 H, COOMe), 3.75 (m, 1 H, H-4), 3.90 (t, 1 H, J = 7.2 Hz, H-3), 4.03 (m, 2 H, H-2 and OH-3), 4.74 (d, 1 H, J = 4.8 Hz, H-1); ^{13}C NMR (150 MHz, $CDCl_3$): δ

30.2 (C-5), 30.4 (C-6), 52.0 (ester Me), 55.3 (OMe), 78.2 (C-2), 79.4 (C-3), 80.8 (C-4), 102.0 (C-1), 174.4 (C═O); mass spectrum (HRMS), m/z = 243.0844 $(M + Na)^+$ ($C_9H_{16}NaO_6$ requires 243.0845).

Methyl 2,3-di-*O*-benzyl-β-D-arabino-heptafuranosiduronic acid methyl ester (11)

Compound **10** (0.4 g, 1.00 mmol) was dissolved in distilled THF (4 mL) and Wilkinson's catalyst (0.28 g, 0.3 mmol) was added to the flask. H_2 gas was flashed two times; finally the reaction was stirred at 50 psi pressure of H_2. The reaction was monitored by TLC and appeared complete after 24 h. Excess solvent was removed under reduced pressure and the crude material thus obtained was purified by silica gel column (230–400 mesh; 10 × 4.5 cm) using 3 : 17 EtOAc–hexanes (1 L) to yield **11** as a yellow oil: yield 0.33 g (83%); R_f = 0.22 (1 : 4 EtOAc–hexanes); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.6–2.0 (m, 2 H, H-5 and H-5′), 2.38 (ddd, 1 H, J = 7.2, 9.0, 16.2 Hz, H-6), 2.47 (ddd, 1 H, J = 6.0, 9.0, 15.0 Hz, H-6′), 3.36 (s, 3 H, OMe), 3.67 (s, 3 H, COOMe), 3.88 (m, 1 H, H-4), 4.03 (m, 2 H, H-2 and H-3), 4.62 (dd, 2 H, J = 12.0, 21.6 Hz, benzylic), 4.67 (dd, 2 H, J = 12.0, 60.6 Hz, benzylic), 4.68 (d, 1 H, J = 3.5 Hz, H-1), 7.28–7.39 (m, 10 H, aromatic); ^{13}C NMR (150 MHz, $CDCl_3$): δ 30.5 (C-5), 31.2 (C-6), 51.7 (ester Me), 55.1 (OMe), 72.5 (CH_2), 72.6 (CH_2), 80.1, 84.4, 85.2, 101.6 (C-1), 127.9, 127.9, 128.1, 128.4, 128.5, 128.6, 137.7, 138.1, 173.8 (C═O); mass spectrum (HRMS), m/z = 423.1778 $(M + Na)^+$ ($C_{23}H_{28}NaO_6$) requires 423.1784).

Methyl 2,3-di-*O*-benzyl-β-D-arabino-heptafuranosiduronic acid (12)

A solution of **11** (0.68 g, 1.69 mmol) and $LiOH{\cdot}H_2O$ (0.25 g, 5.94 mmol) in THF–H_2O (3 : 1, 11 mL) was stirred at room temperature. The reaction was monitored by TLC and appeared complete after 24 h. Excess base was neutralized with Ambertite 120 H resin (requisite quantity) (checked by pH paper). Resin was filtered off through filter paper. The filtrate was concentrated under reduced pressure to obtain crude residue, which was purified by silica gel flash column (230–400 mesh; 5.5 × 4.5 cm) with 3 : 7 (500 mL) and then 2 : 3 (500 mL) EtOAc–hexanes to furnish **12** as a colorless thick gum: yield 0.59 g (90%); R_f = 0.22 (2 : 3 EtOAc–hexanes); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.87–1.99 (m, 2 H, H-5 and H-5′), 2.42 (ddd, 1 H, J = 6.6, 8.4, 16.2 Hz, H-6), 2.50 (ddd, 1 H, J = 6.0, 9.0, 16.8 Hz, H-6′), 3.36 (s, 3 H, OMe), 3.89 (m, 1 H, H-4), 4.04 (m, 2 H, H-2 and H-3), 4.62 (dd, 2 H, J = 12.0, 22.2 Hz, benzylic), 4.67 (dd, 2 H, J = 12.0, 64.2 Hz, benzylic), 4.69 (d, 1 H, J = 3.6 Hz, H-1), 7.28–7.39 (m, 10 H, aromatic); ^{13}C NMR (150 MHz, $CDCl_3$): δ 30.5 (C-5), 31.1 (C-6), 55.2 (OMe), 72.5 (CH_2), 72.7 (CH_2), 80.0, 84.4, 85.1, 101.7 (C-1), 128.0, 128.0, 128.2, 128.4, 128.60, 137.7, 138.1, 179.4 (C═O); mass spectrum (HRMS), m/z = 409.1612 $(M + Na)^+$ ($C_{22}H_{26}NaO_6$ requires 409.1627).

Methyl 2,3-di-*O*-benzyl-7-keto-8-(triphenylphosphanylidene)-β-D-arabino-nonafuranosiduronitrile (13)

To a well-stirred solution of **12** (0.59 g, 1.53 mmol), EDCI (0.44 g, 2.3 mmol), DMAP (0.018 g, 0.15 mmol) in CH_2Cl_2 (15 mL) was added dropwise a solution of (cyanomethylene)triphenylphosphorane (0.57 g, 1.69 mmol) in CH_2Cl_2 (10 mL) under N_2 atmosphere. The resulting solution was stirred at ambient temperature. The reaction was monitored by TLC and appeared complete after 4.5 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and water (100 mL) was added. The organic phase was pooled and aqueous layer was extracted with CH_2Cl_2 (2 × 40 mL). Combined organic phase was washed with saturated aqueous $NaHCO_3$ and brine, and then dried (anhydrous Na_2SO_4) and filtered. The filtrate was concentrated under reduced pressure to render the crude mass, which was purified by silica gel flash column (230–400 mesh; 6 × 4 cm). Elution with 2 : 3 (750 mL) and then 1 : 1 (750 mL) EtOAc–hexanes produced **13** as a white fluffy hygroscopic material: yield 0.96 g (89%); R_f = 0.29 (3 : 2 EtOAc–hexanes); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.93–2.05 (m, 2 H, H-5 and H-5′), 2.79 (m, 1 H, H-6), 2.91 (m, 1 H, H-6′), 3.38 (s, 3 H, OMe), 3.91 (m, 1 H, H-4), 4.04 (dd, 1 H, J = 4.2, 6.6 Hz, H-2), 4.08 (t, 1 H, J = 6.0 Hz, H-3), 4.60–4.69 (m, 4 H, benzylic), 4.71 (d, 1 H, J = 4.2 Hz, H-1), 7.25 (t, 1 H, J = 7.2 Hz, aromatic), 7.29–7.38 (m, 9 H, aromatic), 7.49 (m, 6 H, aromatic), 7.56–7.63 (m, 9 H, aromatic); ^{13}C NMR (150 MHz, $CDCl_3$): δ 32.1, 35.9, 35.9, 48.1, 48.9, 55.2 (OMe), 72.1 (CH_2), 72.5 (CH_2), 80.85, 84.6, 85.8, 101.5 (C-1), 122.6, 122.7, 123.1, 123.7, 127.7, 128.0, 128.1, 128.4, 128.5, 128.5, 128.6, 129.2, 129.3, 132.2, 132.25, 133.2, 133.2, 133.2, 133.7, 133.7, 137.8, 138.4, 196.3, 196.3; ^{31}P NMR (80.95 MHz, $CDCl_3$): δ 20.88 (PPh_3); mass spectrum (HRMS), m/z = 692.2545 $(M + Na)^+$ ($C_{42}H_{40}NNaO_5P$ requires 692.2542).

Methyl 2,3-di-*O*-benzyl-7-keto-β-D-arabino-octafuranosiduronic acid methyl ester (14)

Compound **13** (0.1 g, 0.153 mmol) was dissolved in anhydrous MeOH (5 mL) and freshly prepared DMDO in excess of 2 equiv. was added dropwise at room temperature under N_2 atmosphere. The resulting mixture was stirred for 10 min and monitored by TLC. After 1 h, reaction was complete. Excess solvent was evaporated under reduced pressure and crude material thus obtained was purified by silica gel flash column (230–400 mesh; 6.5 × 4.2 cm). Elution with 1 : 3 EtOAc–hexanes (1 L) afforded **14** as a colorless thick gum: yield 0.056 g (85%); R_f = 0.78 (3 : 2 EtOAc–hexanes); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.88–2.00 (m, 2 H, H-5 and H-5′), 2.89 (ddd, 1 H, J = 6.0, 7.8, 18.6 Hz, H-6), 3.01 (ddd, 1 H, J = 6.0, 7.8, 18.0 Hz, H-6′), 3.34 (s, 3 H, OMe), 3.85 (s, 3 H, COOMe), 3.88 (m, 1 H, H-4), 4.03 (m, 2 H, H-1 and H-3), 4.62 (dd, 2 H, J = 12.0, 19.2 Hz, benzylic), 4.66 (d, 1 H, J = 3.6 Hz, H-1), 4.68 (dd, 2 H, J = 12.0, 82.8 Hz, benzylic), 7.28–7.38 (m, 10 H, aromatic); ^{13}C NMR (150 MHz, $CDCl_3$): δ 29.6 (C-5), 35.9 (C-6), 53.2 (ester Me), 55.3 (OMe), 72.6 (CH_2), 72.8 (CH_2), 79.9 (C-4), 84.3 (C-3), 85.0 (C-2), 101.7 (C-1), 128.0, 128.1, 128.2, 128.4, 128.6, 128.7, 137.7, 138.1, 161.3 (ester C═O), 193.7 (C═O); mass spectrum (HRMS), m/z = 451.1715 $(M + Na)^+$ ($C_{24}H_{28}NaO_7$ requires 451.1733).

Methyl 7-keto-β-D-arabino-octafuranosiduronic acid methyl ester (2)

Compound **14** (0.072 g, 0.168 mmol) and Pd/C (35 mg; 10% Pd on activated carbon, anhydrous version) were taken together and placed in vacuum for 15 min before addition of

anhydrous THF (4 mL). The resulting suspension was stirred at room temperature under a balloon pressure of H_2. The reaction was monitored by TLC and appeared complete after 24 h. Suspended solid was filtered off through a pad of Celite®. The Celite® bed was washed with two bed volumes of anhydrous THF and the combined filtrate was concentrated under reduced pressure. The crude product thus obtained was purified by a silica gel flash column (230–400 mesh; 9.5 × 2 cm). Elution with 1 : 19 MeOH–$CHCl_3$ (0.5 L) generated **2** as a yellow gum: yield 0.023 g (56%); R_f = 0.26 (1 : 9 MeOH–$CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.92 (m, 1 H, H-5), 2.03 (m, 1 H, H-5′), 2.15 (br hump, 1 H, OH-3), 2.95 (ddd, 1 H, J = 6.6, 7.8, 18.6 Hz, H-6), 2.98 (br hump, 1 H, OH-2), 3.05 (ddd, 1 H, J = 6.6, 7.8, 18.6 Hz, H-6′), 3.40 (s, 3 H, OMe), 3.79 (m, 1 H, H-4), 3.87 (s, 3 H, COOMe), 3.90 (m, 1 H, H-3), 4.01 (t, 1 H, J = 6.6 Hz, H-2), 4.75 (d, 1 H, J = 4.2 Hz, H-1); ^{13}C NMR (150 MHz, $CDCl_3$): δ 28.7 (C-5), 35.6 (C-6), 53.3 (ester Me), 55.6 (OMe), 78.5 (C-4), 79.9 (C-3), 80.6 (C-2), 102.0 (C-1), 161.5 (ester C═O), 193.9 (C═O); mass spectrum (HRMS), m/z = 271.0793 $(M + Na)^+$ ($C_{10}H_{16}NaO_7$ requires 271.0794).

Methyl *N*-butyl-2,3-di-*O*-benzyl-7-keto-β-D-arabino-octafuranosiduronamide (15)

To a well-stirred solution of **13** (0.18 g, 0.262 mmol) in CH_2Cl_2 (3 mL) was added anhydrous freshly prepared DMDO in excess of two equivalents at −78 °C. The reaction was monitored by TLC for 0.5 h when maximum starting material converted to the product ketonitrile (based on TLC). Butylamine (30 μL, 0.288 mmol) in CH_2Cl_2 (1 mL) was added dropwise to the flask and stirring was continued. TLC showed maximum conversion of the starting ketonitrile after 30 min. Excess solvent was removed under reduced pressure at low temperature and crude material was dried in high vacuum pump for 1 h before being loaded on the silica gel flash column (230–400 mesh; 10 × 3.1 cm). Elution with 1 : 3 (0.5 L) and then 1 : 1 (0.5 L) EtOAc–hexanes yielded **15** as a yellow gum: yield 0.03 g (24%); R_f = 0.7 (1 : 1 EtOAc–hexanes); ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.93 (t, 3 H, CH_3), 1.35 (m, 2 H, CH_2), 1.52 (m, 2 H, CH_2), 1.91 (m, 2 H, H-5 and H-5′), 2.99 (ddd, 1 H, J = 6.0, 7.8, 18.6 Hz, H-6), 3.12 (ddd, 1 H, J = 6.0, 8.4, 19.2 Hz, H-6′), 3.28 (dd, 2 H, J = 7.2 Hz, NHCH_2), 3.34 (s, 3 H, OMe), 3.89 (m, 1 H, H-4), 4.04 (m, 2 H, H-2 and H-3), 4.62 (m, 3 H, benzylic), 4.67 (d, 1 H, J = 3.6 Hz, H-1), 4.73 (d, 1 H, J = 12.0 Hz, benzylic), 6.92 (br s, 1 H, NH), 7.28–7.38 (m, 10 H, aromatic); ^{13}C NMR (150.83 MHz, $CDCl_3$): δ 13.9 (CH_3), 20.3 (CH_2), 30.0 (C-5), 31.5 (CH_2), 33.5 (C-6), 39.3 (NHCH_2), 55.3 (OMe), 72.6 (benzylic CH_2), 72.8 (benzylic CH_2), 80.1 (C-4), 84.5 (C-3), 85.4 (C-2), 101.7 (C-1), 128.0, 128.1, 128.2, 128.4, 128.5, 128.6, 128.7, 128.7, 137.8, 138.3, 160.1 (amide C═O), 199.0 (C═O); mass spectrum (HRMS), m/z = 492.2247 $(M + Na)^+$ ($C_{27}H_{35}NNaO_6$ requires 492.2362).

Methyl *N*-butyl-7-keto-β-D-arabino-octafuranosiduronamide (3)

Compound **15** (0.033 g, 0.071 mmol) and Pd/C (50 mg; 10% Pd on activated carbon, anhydrous version) were taken together and placed in vacuum for 15 min before addition of anhydrous THF (3 mL). The resulting suspension was stirred at room temperature under a balloon pressure of H_2. The reaction was monitored by TLC and appeared complete after 24 h. Suspended solid was filtered off through a pad of Celite®. The Celite® bed was washed with two bed volumes of anhydrous THF and the combined filtrate was concentrated under reduced pressure. The crude product thus obtained was purified by a silica gel flash column (230–400 mesh; 9 × 1.5 cm). Elution with 1 : 19 MeOH–$CHCl_3$ (0.3 L) generated **3** as a yellow glassy hygroscopic solid: yield 0.015 g (73%); R_f = 0.42 (1 : 9 MeOH–$CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.93 (t, 1 H, CH_3), 1.35 (m, 2 H, CH_2), 1.53 (m, 2 H, CH_2), 189–2.02 (m, 2 H, H-5 and H-5′), 3.02 (dt, 1 H, J = 7.2, 7.2, 18.6 Hz, H-6), 3.11 (dt, 1 H, J = 7.2, 7.2, 18.6 Hz, H-6′), 3.29 (dd, 2 H, J = 6.6 Hz, NHCH_2), 3.41 (s, 3 H, OMe), 3.81 (dd, 1 H, J = 6.6, 13.2 Hz, H-4), 3.93 (t, 1 H, J = 7.2 Hz, H-3), 4.03 (t, 1 H, J = 6.6 Hz, H-2), 4.76 (d, 1 H, J = 4.2 Hz, H-1), 7.03 (br s, 1 H, NH); ^{13}C NMR (150.83 MHz, $CDCl_3$): δ 13.9 (CH_3), 20.2 (CH_2), 28.9 (C-5), 32.5 (CH_2), 33.0 (C-6), 39.4 (NHCH_2), 55.5 (OMe), 78.6 (C-4), 79.9 (C-3), 80.8 (C-2), 102.0 (C-1), 160.3 (amide C═O), 199.0 (C═O); mass spectrum (HRMS), m/z = 312.1375 $(M + Na)^+$ ($C_{13}H_{23}NNaO_5$ requires 312.3185).

6,6′-Di-*O*-*tert*-butyldiphenylsilyl-α-D-trehalose (18)

To a well-stirred solution of trehalose **16** (2.0 g, 5.84 mmol) and imidazole (0.99 g, 14.6 mmol) in anhydrous DMF (15 mL) was added TBDPSCl (3.8 mL, 14.6 mmol) dropwise at room temperature under N_2 atmosphere. The resulting solution was stirred at 35 °C for 16 h. After completion of the reaction (TLC), 2 mL MeOH were added to the reaction flask and the mixture was stirred for 0.5 h. Excess solvent was removed under reduced pressure to get a crude material. Purification of crude material by silica gel flash chromatography (230–400 mesh; 10 × 6.1 cm) using 3 : 17 MeOH–EtOAc (2 L) afforded **18** as a white amorphous solid: yield 4.05 g (84%); R_f = 0.6 (1 : 3 MeOH–EtOAc); ^{1}H NMR (600 MHz, DMSO-d_6): δ 0.99 (s, 18 H, 2 × ^{t}Bu), 3.24 (m, 2 H, H-4/H-4′), 3.27 (m, 2 H, H-2/H-2′), 3.65 (m, 2 H, H-3/H-3′), 3.78 (m, 2 H, H-6/H-6′), 3.83 (m, 4 H, H-5/H-5′ and H-6/H-6′), 7.38–7.44 (m, 12 H, aromatic), 7.68 (m, 8 H, aromatic); ^{13}C NMR (150 MHz, DMSO-d_6): δ 19.0, 26.6, 63.4, 69.9, 71.7, 72.3, 73.2, 92.8 (C-1/C-1′), 127.7, 129.6, 129.7, 133.3, 133.5, 135.2, 135.3 (aromatic); mass spectrum (HRMS), m/z = 841.3419 $(M + Na)^+$ ($C_{44}H_{58}NaO_{11}Si_2$ 841.3415). When the reaction was carried out at room temperature, both **17** and **18** were formed and the mixture was separated after benzylation in the next step.

2,2′,3,3′,4,4′,6′-Hepta-*O*-benzyl-6,6′-di-*O*-*tert*-butyldiphenylsilyl-α-D-trehalose (20) and 2,2′,3,3′,4,4′-hexa-*O*-benzyl-6,6′-di-*O*-*tert*-butyldiphenylsilyl-α-D-trehalose (21)

To the suspension of NaH (3.93 g, 98.4 mmol-60% label washed thoroughly with dry hexanes; three times) in anhydrous DMF (30 mL) was added **18** (4.03 g, 4.92 mmol) in DMF dropwise. The resulting solution was stirred under N_2 atmosphere for 0.5 h and then benzyl bromide (11.77 mL, 98.41 mmol) and tetrabutylammonium iodide (TBAI; 0.54 g,

1.47 mmol) were added. The reaction mixture was stirred at room temperature. The reaction was monitored by TLC and complete to a major extent within 3 h but stirring was continued for 24 h. MeOH (2 mL) was added to the reaction flask and the mixture was stirred for 0.5 h. Water (60 mL) was added to the flask and the aqueous phase was extracted with ether (4 × 60 mL). Combined ether layers were dried (anhydrous Na_2SO_4) and filtered. The filtrate was concentrated to dryness under reduced pressure and crude material thus obtained was purified by flash chromatography (15 × 6.5) using hexanes (1.5 L) and EtOAc–hexanes—1 : 19 (2 L), 1 : 9 (1 L) and 1 : 4 (1.5) to generate **19**[32] (8% yield), **20** and **21**; **20**: yield 1.44 g (24%); R_f = 0.49 (1 : 4 EtOAc–hexanes); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.09 (s, 9 H, ^{t}Bu), 3.42 (dd, 1 H, J = 3.0, 10.8 Hz, H-6‴), 3.55 (dd, 1 H, J = 3.0, 12.0 Hz, H-6″), 3.60 (dd, 1 H, J = 3.6, 9.6 Hz, H-2′), 3.67 (m, 2 H, H-2/H-6′), 3.71 (t, 1 H, J = 9.6 Hz, H-4′), 3.82 (dd, 1 H, J = 2.4, 11.4 Hz, H-6), 3.92 (t, 1 H, J = 9.6 Hz, H-4), 4.06 (t, 1 H, J = 9.6 Hz, H-3′), 4.09 (m, 1 H, H-5), 4.12 (t, 1 H, J = 9.6 Hz, H-3), 4.21 (m, 1 H, H-5′), 4.44 (d, 1 H, J = 12.0 Hz, benzylic), 4.49 (d, 1 H, J = 10.8 Hz, benzylic), 4.54 (d, 1 H, J = 12.0 Hz, benzylic), 4.61 (dd, 2 H, J = 4.2, 12.0 Hz, benzylic), 4.77–5.05 (m, 9 H, benzylic), 5.23 (d, 1 H, J = 3.6 Hz, H-1′), 5.32 (d, 1 H, J = 4.2 Hz, H-1), 7.01 (t, 2 H, J = 7.8 Hz, aromatic), 7.10 (m, 3 H, aromatic), 7.16 (m, 2 H, aromatic), 7.28–7.44 (m, 32 H, aromatic), 7.69 (d, 2 H, J = 6.6 Hz, aromatic), 7.75 (d, 2 H, J = 6.6 Hz, aromatic); ^{13}C NMR (150 MHz, $CDCl_3$): δ 19.5, 27.0, 62.3, 68.3, 70.7, 71.8, 72.8, 73.0, 73.7, 75.3, 75.5, 75.8, 76.1, 77.8, 77.84, 79.6, 80.2, 82.0, 82.1, 94.3, 127.4, 127.5, 127.55, 127.6, 127.7, 127.73, 127.85, 127.87, 127.88, 127.9, 128.1, 128.16, 128.2, 128.4, 128.5, 128.6, 128.65, 129.78, 129.8, 133.4, 133.8, 135.8, 136.1, 137.9, 138.1, 138.5, 138.6, 138.7, 139.0, 139.1; mass spectrum (HRMS), m/z = 1233.5527 $(M + Na)^+$ ($C_{77}H_{82}NaO_{11}Si$ requires 1233.5524); compound **21**: yield 3.02 g (45%); R_f = 0.49 (1 : 4 EtOAc–hexanes); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.05 (s, 18 H, 2 × ^{t}Bu), 3.56 (dd, 2 H, J = 3.6, 9.6 Hz, H-2/H-2′), 3.58 (m, 2 H, H-6/H-6′), 3.72 (dd, 2 H, J = 1.8, 11.4 Hz, H-6″/H-6‴), 3.84 (t, 2 H, J = 9.6 Hz, H-4/H-4′), 4.02 (m, 2 H, H-5/H-5′), 4.03 (t, 2 H, J = 9.6 Hz, H-3/H-3′), 4.55 (d, 2 H, J = 11.4 Hz, benzylic), 4.63 (d, 2 H, J = 12.0 Hz, benzylic), 4.70 (d, 2 H, J = 10.8 Hz, benzylic), 4.86 (d, 2 H, J = 10.8 Hz, benzylic), 4.91 (d, 2 H, J = 10.2 Hz, benzylic), 4.97 (d, 2 H, J = 10.8 Hz, benzylic), 5.19 (d, 2 H, J = 3.6 Hz, H-1/H-1′), 6.99 (t, 4 H, J = 7.8 Hz, aromatic), 7.07 (t, 5 H, J = 6.6 Hz, aromatic), 7.28–7.40 (m, 25 H, aromatic), 7.45 (m, 4 H, aromatic), 7.65 (d, 4 H, J = 7.2 Hz, aromatic), 7.72 (d, 4 H, J = 6.6 Hz, aromatic); ^{13}C NMR (150 MHz, $CDCl_3$): δ 19.6, 27.1, 62.4, 71.7, 73.0, 75.4, 76.1, 77.8, 80.3, 82.1, 94.4, 127.3, 127.5, 127.7, 127.86, 127.87, 127.9, 128.1, 128.4, 128.4, 128.6, 128.7, 129.8, 129.84, 133.6, 133.8, 135.9, 136.2, 138.3, 138.7, 139.0; mass spectrum (HRMS), m/z = 1381.6218 $(M + Na)^+$ ($C_{86}H_{94}NaO_{11}Si_2$ requires 1381.6232).

2,2′,3,3′,4,4′-Hexa-*O*-benzyl-α-D-trehalose (22)[32]

Compound **21** (2.98 g, 2.2 mmol) and TBAF·3H_2O (4.15 g, 13.15 mmol) were taken together and dried in high vacuum pump for 15 min before addition of anhydrous THF (30 mL). The resulting yellow solution was stirred at ambient temperature. The reaction was monitored by TLC and disappearance of starting material was complete after 36 h. Excess solvent was removed and crude material thus obtained was purified by silica gel flash column (230–400 mesh; 10 × 6.5 cm) with 3 : 7 (1 L), 2 : 3 (1 L) and 3 : 2 (2.5 L) EtOAc–hexanes to produce **22** as a colorless foamy glassy mass: yield 1.63 g (84%); R_f = 0.1 (1 : 1 EtOAc–hexanes).

Bis(methyl-α-D-*gluco*-octopyranosyluronate) ether (4)

A suspension of **23** (0.21 g, 0.21 mmol) and $Pd(OH)_2$/C (100 mg) in a 2.5 : 1 mixture of anhydrous MeOH–EtOAc was degassed under vacuum and a balloon of H_2 was connected to the reaction vessel. The resulting suspension was stirred at ambient temperature under H_2 atmosphere. The reaction was monitored by TLC and appeared complete after 16 h. The suspension was filtered through a pad of Celite® and Celite® bed was washed with MeOH and combined filtrate was concentrated under reduced pressure to obtain a crude residue, which was precipitated from 1 : 1 MeOH–EtOAc to get **4** as a white amorphous solid: yield 0.076 g (79%); R_f = 0.21 (1 : 3 MeOH–EtOAc); ^{1}H NMR (600 MHz, CD_3OD): δ 1.62 (m, 2 H, H-6/H-6″), 2.13 (m, 2 H, H-6′/H-6‴), 2.36 (m, 2 H, H-7/H-7″), 2.43 (m, 2 H, H-7′/H-7‴), 3.01 (t, 2 H, J = 9.0 Hz, H-4/H-4′), 3.40 (dd, 2 H, J = 4.2, 10.2 Hz, H-2/H-2″), 3.61 (s, 6 H, 2 × COOMe), 3.66 (t, 2 H, J = 9.6 Hz, H-3/H-3′), 3.71 (ddd, 2 H, J = 2.4, 9.6 Hz, H-5/H-5′), 4.92 (d, 2 H, J = 3.6 Hz, H-1/H-1′); ^{13}C NMR (100 MHz, CD_3OD): δ 28.4, 31.5, 52.3 (COOMe), 72.1, 73.4, 74.6, 75.9, 95.2 (C-1/C-1′), 176.2 (ester carbonyl); mass spectrum (HRMS), m/z = 477.1590 $(M + Na)^+$ ($C_{18}H_{30}NaO_{13}$ requires 477.1584).

Bis(methyl-2,3,4-tri-*O*-benzyl-α-D-*gluco*-octopyranosyluronate) ether (24)

To a solution of **23** (0.56 g, 0.57 mmol) in THF (7 mL) was added Wilkinson's catalyst (0.47 mg, 513.4 mmol) and the solution was purged with H_2 thrice before a constant pressure of 50 psi of H_2 is maintained. The reaction was stirred at ambient temperature and monitored by TLC. After completion of the reaction (36–48 h), excess solvent was removed under reduced pressure to get the crude material. The crude material was purified by silica gel flash chromatography (230–400 mesh; 8 × 4.5 cm). Elution with 1 : 9 EtOAc–hexanes generated **24** as a colorless amorphous solid: yield 0.38 g (67%); R_f = 0.37 (3 : 7 EtOAc–hexanes); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.74 (m, 2 H, H-6/H-6″), 2.01 (m, 2 H, H-6′/H-6″), 2.1 (m, 2 H, H-7/H-7″), 2.26 (m, 2 H, H-7′/H-7″), 3.25 (t, 2 H, J = 9.0 Hz, H-4/H-4′), 3.57 (dd, 2 H, J = 3.6, 9.6 Hz, H-2/H-2′), 3.62 (s, 6 H, 2 × COOMe), 4.01 (ddd, 2 H, J = 3.0, 9.6, 9.6 Hz, H-5/H-5′), 4.10 (t, 2 H, J = 9.6 Hz, H-3/H-3′), 4.65 (d, 2 H, J = 11.4 Hz, benzylic), 4.75 (d, 2 H, J = 12.6 Hz, benzylic), 4.81 (d, 2 H, J = 12.6 Hz, benzylic), 4.88 (d, 2 H, J = 10.8 Hz, benzylic), 4.93 (d, 2 H, J = 10.8 Hz, benzylic), 4.98 (d, 2 H, J = 10.8 Hz, benzylic), 5.09 (d, 2 H, J = 3.6 Hz, H-1/H-1′), 7.24–7.38 (m, 30 H, aromatic); ^{13}C NMR (150 MHz, $CDCl_3$): δ 27.0 (C-6/C-6′), 30.1 (C-7/C-7′), 51.6 (COO*Me*), 70.2, 73.3, 75.3, 75.7, 79.6, 81.8, 81.9, 92.7 (C-1/C-1′), 127.5, 127.7, 127.8, 127.9, 128.07, 128.1, 128.5;

mass spectrum (HRMS), m/z = 1017.4395 $(M + Na)^+$ ($C_{60}H_{66}NaO_{13}$ requires 1017.4401).

Bis(methyl-2,3,4-tri-*O*-benzyl-α-D-*gluco*-octopyranosyluronic acid) ether (25)

To a well-stirred solution of **24** (0.77 g, 0.78 mmol) in 3 : 1 THF–H_2O (10 mL) was added $LiOH·H_2O$ (0.21 g, 5.06 mmol) at room temperature. The resulting suspension was stirred at room temperature for 12 h and monitored by TLC. The reaction appeared complete after 12 h. The reaction mixture was neutralized by Amberlite 120 H^+ resin (checked by pH paper).

The resin was filtered off through a cotton plug and excess solvent was concentrated under reduced pressure. The crude material thus obtained was purified by silica gel flash column (230–400 mesh; 10 × 3.1 cm). Elution with 1 : 19 and then 1 : 9 MeOH–$CHCl_3$ afforded **25** as an yellow fluffy mass: yield 0.71 g (95%); R_f = 0.5 (1:9 MeOH–$CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.75 (m, 2 H, H-6/H-6″), 1.95 (m, 2 H, H-6′/H-6‴), 2.12 (t, 4 H, J = 7.2 Hz, H-7/H-7′/H-7″/H-7‴), 3.23 (t, 2 H, J = 9.0 Hz, H-4/H-4′), 3.57 (dd, 2 H, J = 3.6, 9.6 Hz, H-2/H-2′), 3.91 (ddd, 2 H, J = 2.4, 9.6, 9.6 Hz, H-5/H-5′), 4.09 (t, 2 H, J = 9.0 Hz, H-3/H-3′), 4.61 (d, 2 H, J = 11.4 Hz, benzylic), 4.74 (d, 2 H, J = 12.0 Hz, benzylic), 4.81 (d, 2 H, J = 12.0 Hz, benzylic), 4.86 (d, 2 H, J = 10.8 Hz, benzylic), 4.91 (d, 2 H, J = 10.8 Hz, benzylic), 4.96 (d, 2 H, J = 10.8 Hz, benzylic), 5.11 (d, 2 H, J = 3.0 Hz, H-1/H-1′), 7.23 (m, 6 H, aromatic), 7.28–7.37 (m, 24 H, aromatic); ^{13}C NMR (150 MHz, $CDCl_3$): δ 26.7 (C-6/C-6′), 29.9 (C-7/C-7′), 70.4, 73.5, 75.3, 75.7, 79.7, 81.8, 81.9, 92.5 (C-1/C-1′), 127.5, 127.7, 127.8, 128.0, 128.1, 128.15, 128.6, 128.62, 128.63, 138.3, 138.4, 138.8, 180.2 (COOH); mass spectrum (HRMS), m/z = 989.4086 $(M + Na)^+$ ($C_{58}H_{62}NaO_{13}$ requires 989.4088).

Bis(2,3,4-tri-*O*-benzyl-9-cyano-8-oxo-9-(triphenylphosphanylidene)-α-D-*gluco*-nonapyranosyluronononitrile) ether (26)

Compound **25** (0.71 g, 0.74 mmol), EDCI (0.42 g, 2.22 mmol) and DMAP (9 mg, 0.07 mmol) were taken together and dried under vacuum for 15 min before CH_2Cl_2 (5 mL) was added. Cyanophosphorane derivative (0.28 g, 0.84 mmol) dissolved in CH_2Cl_2 (5 mL) was added dropwise to the flask under N_2 atmosphere at ambient temperature. The reaction was monitored by TLC and appeared complete in 2 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with water, saturated aqueous $NaHCO_3$, and brine. Aqueous phases were back extracted with CH_2Cl_2 (2 × 40 mL). Combined organic phases were dried (anhydrous Na_2SO_4), filtered and the filtrate was concentrated under reduced pressure to obtain the crude residue. The crude material was purified by silica gel flash chromatography (230–400 mesh; 10 × 3.5 cm). Elution with 3 : 2 EtOAc–hexanes yielded **26** as a white fluffy mass: yield 0.89 g (78%); R_f = 0.96 (1 : 9 MeOH–$CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.88 (m, 2 H, H-6/H-6″), 1.99 (m, 2 H, H-6′/H-6‴), 2.53 (m, 2 H, H-7/H-7″), 2.78 (m, 2 H, H-7′/H-7‴), 3.29 (t, 2 H, J = 9.6 Hz, H-4/H-4′), 3.63 (dd, 2 H, J = 3.6, 9.6 Hz, H-2/H-2′), 4.11 (m, 4 H, H-3/H-3′/H-5/H-5′), 4.63 (d, 2 H, J = 10.8 Hz, benzylic), 4.76 (d, 2 H, J = 12.0 Hz, benzylic), 4.83 (d, 2 H, J = 10.8 Hz, benzylic), 4.87 (d, 2 H, J = 10.8 Hz, benzylic), 4.96 (d, 2 H, J = 10.8 Hz, benzylic), 5.32 (d, 2 H, J = 3.0 Hz, H-1/H-1′), 7.19 (m, 6 H, aromatic), 7.24–7.32 (m, 20 H, aromatic), 7.39 (m, 4 H, aromatic), 7.47 (m, 12 H, aromatic), 7.55–7.61 (m, 18 H, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.6, 35.12, 35.2, 47.3, 48.5, 70.6, 72.9, 75.0, 75.5, 79.7, 81.8, 82.1, 92.0 (C-1/C-1′), 122.5, 122.6, 123.1, 124.0, 127.2, 127.4, 127.5, 127.7, 127.9, 128.0, 128.3, 128.36, 129.1, 129.2, 133.1, 133.2, 133.6, 133.7, 138.7, 138.8, 139.1, 196.5, 196.53; ^{31}P (80.95 MHz, $CDCl_3$): 20.8 (PPh_3); mass spectrum (HRMS), m/z = 1555.5864 $(M + Na)^+$ ($C_{98}H_{90}N_2NaO_{11}P_2$ requires 1555.5918).

Bis(methyl-2,3,4-tri-*O*-benzyl-8-oxo-α-D-*gluco*-nonapyranosyluronate) ether (27)

To a well-stirred solution of **26** (0.14 g, 0.092 mmol) in MeOH (1 mL) was added DMDO in acetone (in excess of 2 equiv.) and the resulting solution was stirred at ambient temperature. The reaction was monitored by TLC and appeared complete in 0.5 h. Excess solvent and reagent were removed under reduced pressure to get a gummy material. Purification of the crude material by silica gel flash column (230–400 mesh; 9 × 3.1 cm) with 1 : 3 EtOAc–hexanes produced **27** as a colorless thick gum: yield 0.062 g (64%); R_f = 0.6 (1 : 1 EtOAc–hexanes); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.73 (m, 2 H, H-6/H-6″), 1.93 (m, 2 H, H-6′/H-6‴), 2.45 (m, 2 H, H-7/H-7″), 2.55 (m, 2 H, H-7′/H-7‴), 3.23 (t, 2 H, J = 6.0 Hz, H-4), 3.51 (dd, 2 H, J = 3.6, 9.6 Hz, H-2/H-2′), 3.79 (s, 6 H, 2 × COOMe), 4.01 (m, 2 H, H-5/H-5′), 4.05 (t, 2 H, J = 9.6 Hz, H-3/H-3′), 4.62 (d, 2 H, J = 11.4 Hz, benzylic), 4.74 (d, 2 H, J = 11.4 Hz, benzylic), 4.82 (d, 2 H, J = 12.0 Hz, benzylic), 4.87 (d, 2 H, J = 12.0 Hz, benzylic), 4.91 (d, 2 H, J = 11.4 Hz, benzylic), 4.96 (d, 2 H, J = 3.6 Hz, H-1/H-1′), 4.97 (d, 2 H, J = 14.4 Hz, benzylic), 7.22 (m, 6 H, aromatic), 7.26–7.36 (m, 24 H, aromatic); ^{13}C NMR (150 MHz, $CDCl_3$): δ 25.9, 35.2, 53.0 (COO*Me*), 70.0, 73.4, 75.3, 75.8, 79.7, 81.5, 81.9, 93.3 (C-1/C-1′), 127.4, 127.7, 127.8, 128.1, 128.14, 128.2, 128.3, 128.6, 128.64, 128.7, 138.4, 138.41, 138.8, 161.4 (ester keto), 193.8 (α-keto); mass spectrum (HRMS), m/z = 1073.4303 $(M + Na)^+$ ($C_{62}H_{66}NaO_{15}$ requires 1073.4299).

Bis(*N*-butyl-2,3,4-tri-*O*-benzyl-8-oxo-α-D-*gluco*-nonapyranosyluronamide) ether (28)

Compound **26** (0.1 g, 0.065 mmol) was dissolved in CH_2Cl_2 (4 mL) and freshly prepared DMDO in acetone (in excess of 4 equiv.) at −78 °C was cannulated into the reaction mixture at −78 °C. The resulting solution was stirred for 15 min under N_2 atmosphere when TLC showed disappearance of starting material. Excess DMDO was removed by applying vacuum for 15 min. Butylamine (16 μL, 0.16 mL) in CH_2Cl_2 (2 mL) was added dropwise at −78 °C and the reaction mixture was stirred for 0.5 h. After disappearance of starting material (TLC), the reaction was allowed to attain room temperature. Excess solvent was removed under reduced pressure. The crude material was purified by silica gel flash column (230–400 mesh; 4 × 3.5 cm) using 1 : 3 EtOAc–hexanes to afford **28** as a colorless gum: yield 0.034 g (46%); R_f = 0.63

(1 : 1 EtOAc–hexanes); ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.92 (t, 6 H, J = 7.2 Hz, 2 × Me), 1.35 (m, 4 H, 2 × CH_2), 1.51 (m, 4 H, 2 × CH_2), 1.78 (m, 2 H, H-6/H-6″), 1.96 (m, 2 H, H-6′/H-6‴), 2.62 (m, 2 H, H-7/H-7″), 2.73 (m, 2 H, H-7′/H-7‴), 3.27 (m, 6 H, H-4/H-4′ and CH_2), 3.54 (dd, 2 H, J = 3.6, 9.6 Hz, H-2/H-2′), 3.99 (m, 2 H, H-5/H-5′), 4.07 (t, 2 H, J = 9.6 Hz, H-3/H-3′), 4.63 (d, 2 H, J = 10.8 Hz, benzylic), 4.80 (dd, 4 H, J = 12.0, 17.4 Hz, benzylic), 4.86 (d, 2 H, J = 9.6 Hz, benzylic), 4.91 (d, 2 H, J = 9.6 Hz, benzylic), 4.96 (d, 2 H, J = 11.4 Hz, benzylic), 5.03 (d, 2 H, J = 3.6 Hz, H-1/H-1′), 6.96 (t, 2 H, J = 5.4 Hz, NH), 7.21 (m, 6 H, aromatic), 7.28–7.37 (m, 24 H, aromatic); ^{13}C NMR (150 MHz, $CDCl_3$): δ 13.9 (Me), 20.2, 26.3, 29.9, 31.5, 33.3, 39.2, 70.2, 73.3, 75.3, 75.8, 79.8, 81.8, 82.0, 92.6 (C-1), 127.5, 127.6, 127.8, 127.9, 128.2, 128.6, 128.61, 138.5, 138.6, 138.9, 160.5 (amide C═O), 199.5 (α-keto); mass spectrum (HRMS), m/z = 1155.5566 ($C_{68}H_{80}N_2NaO_{13}$ requires 1155.5558).

Synthesis of trehalose-derived bicyclo-methyl ester (29)

To a well-stirred solution of **27** (0.05 g, 0.0475 mmol) in THF (4 mL) was added Pd/C (70 mg) and a balloon of H_2 was connected to the reaction flask. The resulting suspension was stirred at room temperature. The reaction was monitored by TLC and appeared complete after 24 h. The catalyst was filtered through a pad of Celite® and excess solvent was removed under reduced pressure. The crude material thus obtained was purified by silica gel flash column (230–400 mesh; 6 × 3.5 cm) using 3 : 3 : 19 MeOH–acetone–$CHCl_3$ to generate **29** as a yellow glassy solid: yield 0.015 g (62%); R_f = 0.26 (1 : 3 MeOH–EtOAc); ^{1}H NMR (600 MHz, CD_3OD): δ 1.77 (m, 4 H, H-6/H-6′/H-6″/H-6‴), 1.89 (m, 4 H, H-7/C-7′/C-7″/C-7‴), 3.48 (dd, 2 H, J = 4.2, 9.6 Hz, H-2/H-2′), 3.51 (t, 2 H, J = 9.6 Hz, H-4/H-4′), 3.71 (s, 6 H, 2 × COOMe), 3.81 (m, 2 H, H-5/H-5′), 3.83 (t, 2 H, J = 9.6 Hz, H-3/H-3′), 4.98 (d, 2 H, J = 4.2 Hz, H-1/H-1′); ^{13}C NMR (150 MHz, CD_3OD): δ 25.3, 32.3, 53.2, 67.9, 71.7, 73.9, 76.0, 95.4, 95.9, 172.7 (*C*OOMe); mass spectrum (HRMS), m/z = 533.1489 $(M + Na)^+$ ($C_{20}H_{30}NaO_{15}$ requires 533.1482).

Synthesis of trehalose-derived bicyclo-*N*-butylamide (30)

To a well-stirred solution of **28** (0.05 g, 0.044 mmol) in THF (4 mL) was added Pd/C (54 mg) and a balloon of H_2 was connected to the reaction flask. The resulting suspension was stirred at room temperature. The reaction was monitored by TLC and appeared complete after 24 h. The catalyst was filtered through a pad of Celite® and excess solvent was removed under reduced pressure. The crude material thus obtained was purified by silica gel flash column (230–400 mesh; 7.5 × 2.1 cm) using 3 : 3 : 19 MeOH–acetone–$CHCl_3$ to generate **30** as a yellow glassy solid: yield 0.017 g (65%); R_f = 0.42 (1 : 1 MeOH–EtOAc); ^{1}H NMR (600 MHz, 1 : 1 CD_3OD–$CDCl_3$): δ 1.08 (t, 6 H, J = 7.2 Hz, 2 × Me), 1.51 (m, 4 H, 2 × CH_2), 1.66 (m, 4 H, 2 × CH_2), 1.83 (m, 2 H, H-6′/H-6‴), 2.0 (m, 4 H, H-6/H-6″/H-7′/H-7‴), 2.24 (m, 2 H, H-7/H-7″), 3.32 (m, 2 H, NHCH_2), 3.40 (m, 2 H, N′HCH_2), 3.74 (m, 4 H, H-2/H-2′/H-4/H-4′), 3.92 (m, 2 H, H-5/H-5′), 4.05 (t, 2 H, J = 9.6 Hz, H-3/H-3′), 5.23 (d, 2 H, J = 3.6 Hz, H-1/H-1′); ^{13}C NMR (100 MHz, 1 : 1 CD_3OD–$CDCl_3$): δ 14.1, 20.7, 24.7, 30.3, 32.1, 39.7, 67.4, 71.3, 73.1, 75.0, 94.8, 94.9, 173.1 (amide C═O); mass spectrum (HRMS), m/z = 615.2744 ($C_{26}H_{44}N_2NaO_{13}$ requires 615.2741).

Kirby–Bauer disk diffusion assay

Disk diffusion assays were adopted from methods described by Wang *et al*. and the NCCLS.[32,40] *M. smegmatis* ATCC 14 468 was inoculated into Middlebrook 7H9 containing 0.2% glycerol and ADC enrichment, respectively. The inocula were incubated at 37.5 °C for approximately 24 h and 160 rpm to yield OD600 = 0.27. The bacteria were plated on agar (Middlebrook 7H10 containing 0.5% glycerol and OADC enrichment) using a cotton-tipped applicator. Concentration of 20 mg mL^{-1} of each synthetic analog and 500 μg mL^{-1} of INH were prepared in DMSO and 10 μL of each solution was applied to 6 mm sterile paper disks. The plates were incubated for 24 h at 37 °C. The diameter of zone of inhibition (DZI) was measured using a ruler.

Antigen 85C acyltransferase assay

The components of the assay were prepared as described previously.[21] The acyl donor solution in DMSO (50–175 μM) was added to the well followed by buffer and the tested compound. A master mix was prepared with 2 U of beta-glucosidase, 3 mM of D-glucose, 150 nM of antigen 85C for each reaction and buffer for a total volume of 100 μL. To start the reaction, the master mix was added to the well. The reaction was followed by UV-vis spectrophotometry. The release of the signaling molecule, *p*-nitrophenolate, was monitored by measurement of the absorbance at 405 nm every 30 s for 30 min. The data from three independent experiments were exported from SoftMax® Pro5 and analyzed in GraphPad Prism 5®.

For the arabinosylfuranoside methyl ester, the enzyme was incubated with the compound prior to the experiment. In that particular case, the experimental procedure was modified as follows. In each well, 175 μM of the acyl donor in solution in DMSO were added, followed by buffer. A master mix was prepared with, for each reaction, 2 U of beta-glucosidase, 3 mM of D-glucose, 150 nM of antigen 85C preincubated, if necessary, with the desired concentration of inhibitor and buffer for a total volume of 100 μL. To start the reaction, the master mix was added to the well. The percent initial velocity was calculated by dividing the value obtained in the presence of inhibitor (with or without preincubation) by the value obtained in the absence of inhibitor.

Method of crystallization

The crystal used to solve the structure was obtained by soaking experiment. Antigen 85C was crystallized in a solution of 0.3 M ammonium sulfate and 0.1 M sodium acetate (pH = 4.5). A solution of 0.05 M of methyl ester **1** was prepared and 1 μL was added to the crystallization drop (4 μL) containing the crystals for a final concentration of 10 mM of **1**. A solution identical to the crystallization solution was prepared with 20% (w/v) of glycerol. The crystals were briefly soaked into this solution before being flash-frozen in the liquid nitrogen cryo stream.

The data were collected at beamline 23 GM-CA/CAT at the Advanced Photon Source at Argonne National Laboratory. The data were collected at a wavelength of 1 Å and was integrated using the HKL2000 program.[42] The crystals were isomorphous with the crystal leading to the native structure (accession code 1dqz).[26,43] Using this structure, a difference Fourier map was calculated in CNS version 1.2.[44,45]

Simulated annealing, energy minimization, and *B*-factor refinement in CNS were used to refine the structure. Fo–Fc maps calculated using this partially refined model exhibited additional electron density contiguous with residue S124 and extending into the known carbohydrate-binding pocket of the ag85C active site.[43] The methyl ester arabinofuranoside model was built in Pymol and incorporated into the model using Coot and CNS.[46] Energy minimization and *B*-factor refinement, followed by the addition of solvent, produced the final model.

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