

## Deciphering the conformation of C-linked $\alpha$ -D-mannopyranosides and their application toward the synthesis of low nanomolar *E. coli* FimH ligands

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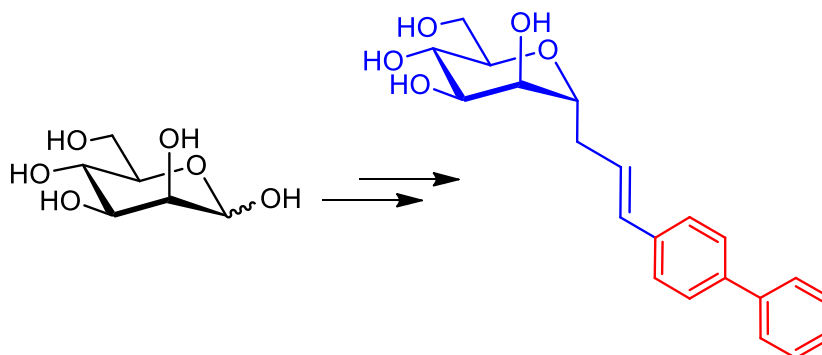
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### Abstract

C-Allyl  $\alpha$ -D-mannopyranosides were prepared via a variety of routes to determine an optimal route to the  $\alpha$ -anomers. The relative conformational energies of the key intermediate was evaluated by molecular modeling which showed the conventional  ${}^4C_1$  chair conformation to be the lowest energy conformer. This finding was also confirmed by NMR and X-ray crystallography. The perbenzoylated C-allyl mannoside was also converted into 1,1'-biphenyl analogues using a palladium-catalyzed Heck reaction. Two of the resulting minor reaction products were co-crystallized with the uropathogenic *E. coli* FimH. Alternatively, the  $K_D$  of the major and expected Heck product was in the low nM range as measured by SPR. Crystal data showed that the C-linked derivatives efficiently bind in the FimH binding cavity near the so-called hydrophobic tyrosine gate.



**Keywords:** *E. coli* FimH, mannoside, Heck reaction, molecular modeling, X-ray

## Introduction

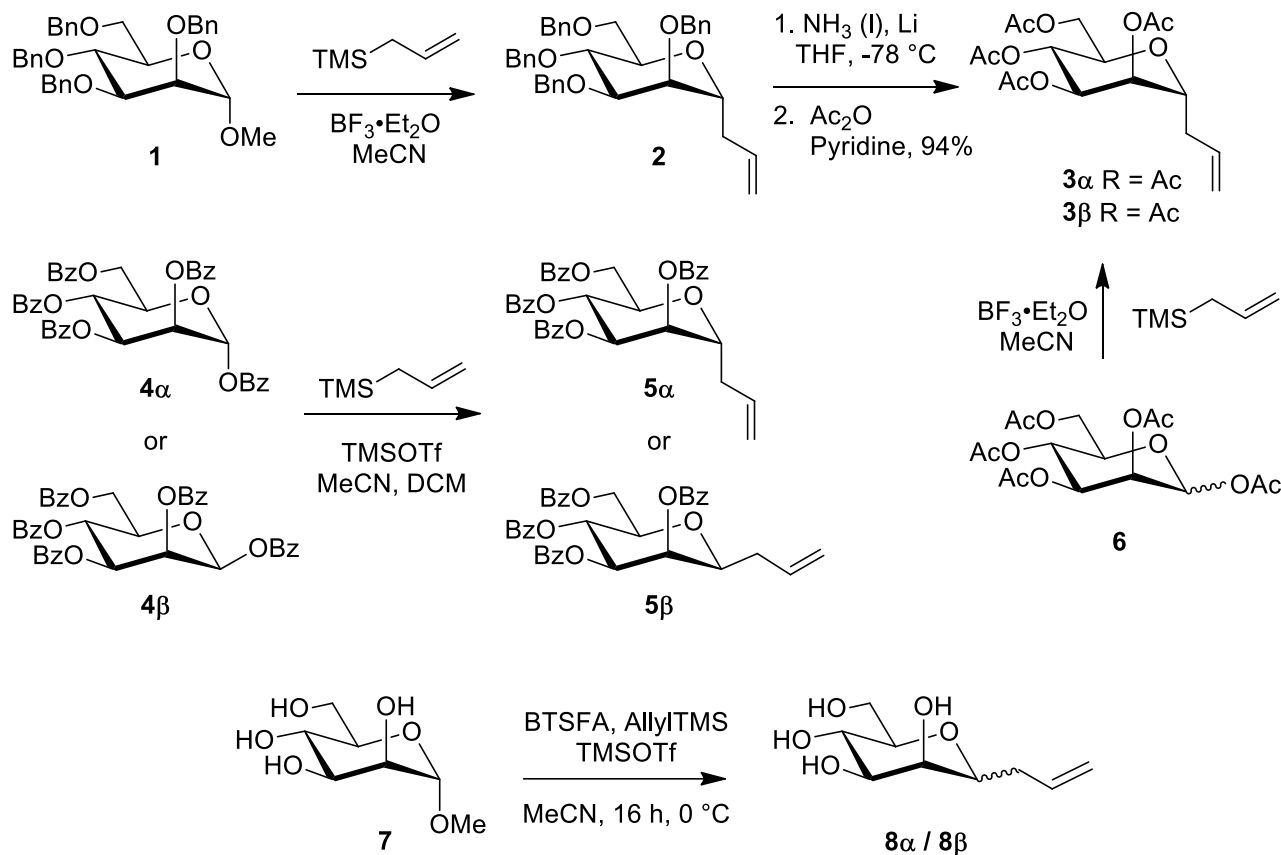
Adherent-invasive *Escherichia coli* (AIEC) infections are a serious health problem for which alternative therapeutic strategies are very timely,<sup>1,2</sup> particularly in light of bacterial resistance against the most recent arsenal of antibacterial agents.<sup>3-5</sup> Amongst these, *Escherichia coli* responsible of urinary tract infections (UTIs), intestinal bowel diseases (IBDs) such as Crohn's disease (CD), and ulcerative colitis constitute major challenges for medicinal chemistry.<sup>6,7</sup> Uropathogenic *E. coli* infections (UPECs) affect 50% of women at least once in their life-times. The premises to infections result from the crucial contacts between the bacterial carbohydrates binding proteins type 1 fimbriae (FimH) and PapG which bind uroplakin Ia glycoprotein and glycolipids, respectively. *E. coli* type FimH has hair-like appendages on the bacterial cell surface which constitute key virulence factors. They are responsible for the initial adhesion to mucosal surfaces via interaction with multiantennary mannosylated receptors.<sup>7</sup> Bacterial adhesion to host cells is a preliminary step toward the release of toxic proteins, therefore the design of *E. coli* FimH antagonists (antiadhesins) has been the target of several efforts from the glycobiology community.<sup>7-25</sup>

Even though several families of potent  $\alpha$ -D-mannopyranoside-based antiadhesins have been synthesized that included polymers,<sup>26-28</sup> dendrimers,<sup>29-30</sup> gold nanoparticles,<sup>31</sup> and dendrimersomes,<sup>32</sup> small molecule antagonists still represent the foremost choice of the pharmaceutical industry. Glycomimetics are the most promising candidates for the replacement of naturally occurring complex oligomannosides with C-linked glycosyl derivatives being the preferred options.<sup>1,11</sup> However, in the case of C-linked mannopyranosides and those possessing hydrophobic/aryl aglycones in particular, there have been very few studies on their precise conformational analyses.<sup>33</sup> This is particularly important given the propensity of C-mannopyranosides to exist in diverse conformations ( ${}^4C_1$ ,  ${}^1C_4$ ,  ${}^2S_0$ , and  ${}^0S_2$ ).<sup>34-38</sup>

This paper describes practical approaches to the syntheses of C-allyl  $\alpha$ -D-mannopyranoside, which is a versatile building block toward *E. coli* FimH antagonists. Molecular modeling, X-ray crystallography, and binding studies support that C-allyl  $\alpha$ -D-mannopyranosides exist in the required and most stable  ${}^4C_1$  conformation and that the corresponding 1,1'-biphenyl derivatives, obtained through palladium-catalyzed Heck cross-coupling, represent hydrolytically stable and promising leads as *E. coli* FimH antagonists.

## Results and Discussion

In conventional drug design, O-linked glycomimetics should be largely avoided due to their propensity to readily hydrolyze in *in vivo* settings. To avoid this problem, C-linked  $\alpha$ -D-mannopyranosides attached to alkene chains were investigated.<sup>1,11</sup> Diversely protected C-allyl  $\alpha$ -D-mannopyranosides are useful intermediates for the synthesis of this important family of glycomimetics. Conventional routes for their syntheses are illustrated in Scheme 1. So far, in its most  $\alpha$ -stereoselective approach, it was obtained from the known perbenzylated methyl  $\alpha$ -D-mannopyranoside **1**,<sup>39</sup> which under Sakurai reaction<sup>39,40</sup> ( $\text{BF}_3 \cdot \text{OEt}_2$ , allyltrimethylsilane) provided **2** in more than 95% of its  $\alpha$ -anomer (Scheme 1). Unfortunately, benzyl protecting groups have some drawbacks for their forthcoming palladium-catalyzed Heck coupling and hydrogenolysis deprotection.<sup>11</sup> Alternatively, Birch reduction of **2** and acetylation affords the more versatile peracetylated analogue **3 $\alpha$**  in good overall yield, albeit in three consecutive steps. Disappointingly, application of the Sakurai conditions, directly on peracetylated mannose **6 $\alpha,\beta$**  afforded an intractable mixture of **3 $\alpha$**  and **3 $\beta$** .<sup>41</sup> This result prompted us to attempt the reaction on a anomeric mixture of the known perbenzoylated mannopyranose **4 $\alpha$ /4 $\beta$** .<sup>42</sup>

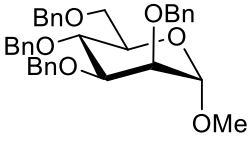
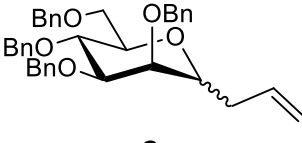
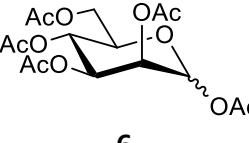
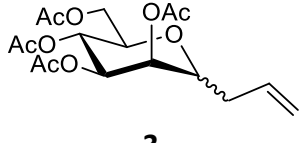
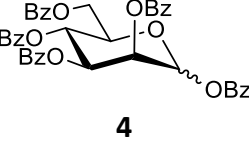
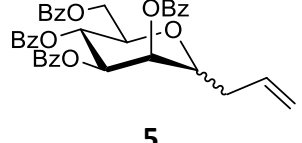
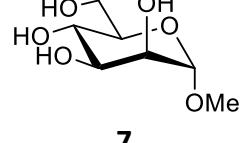
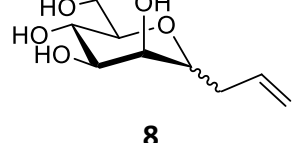


**Scheme 1.** Synthesis of C-allyl  $\alpha$ -D-mannopyranosides under Sakurai condition with different protecting groups (see Table 1).

Delightfully, in contrast to the peracetylated analogue **6**,<sup>41</sup> the Sakurai products **5 $\alpha$**  and **5 $\beta$**  resulting from the perbenzoylated mixture **4 $\alpha$ /4 $\beta$**  were separable, but with reduced  $\alpha$ -stereoselectivity (7:1) when compared to the perbenzylated derivative **1** (15 $\alpha$ :1 $\beta$ ). As anticipated, during the course of this transformation, we also observed that **4 $\alpha$**  reacted faster than its  $\beta$ -anomer **4 $\beta$** . Given that **4 $\alpha$**  and **4 $\beta$**  were also separable,<sup>42</sup> we repeated the reaction on each separated isomer: the **4 $\alpha$**  anomer was completely converted to **5 $\alpha$ /5 $\beta$**  (1.7:1) within 2 h while the **4 $\beta$**  anomer provided **5 $\alpha$ /5 $\beta$**  more slowly but with an improved 4:1 anomeric stereoselectivity. The fact that the above three conditions gave different anomeric stereoselectivities was not surprising given the individual propensity of the precursors ( $\alpha$  vs  $\beta$ ) to react with the Lewis acid competitively.

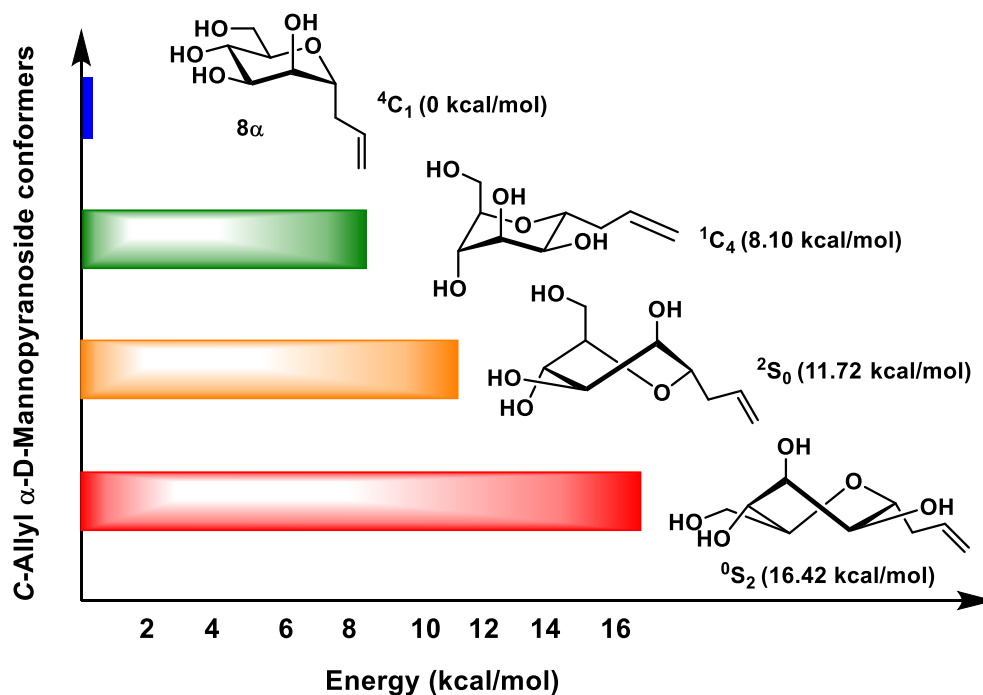
To simplify access to a suitable and versatile starting material, we attempted the Sakurai reaction directly from the commercially available methyl  $\alpha$ -D-mannopyranoside **7** using previously described conditions (BTSFA, AllylTMS, TMSOTf, MeCN,  $0^\circ\text{C}$ , 16 h).<sup>43,44</sup> Although both the yield and the anomeric diastereoselectivity toward unprotected **8 $\alpha$ , $\beta$**  in one single step from **7** was acceptable, the anomeric mixture was not readily separable. The comparative data for the above sets of transformations are illustrated in Table 1. With this information in hand and for practical reasons, it was decided to pursue our goals using the perbenzoylated precursors **4 $\alpha$ , $\beta$** .

**Table 1.** Synthesis and diastereoselectivity of C-allyl  $\alpha$ -D-mannopyranosides using different methods

Starting Material	Reaction Conditions	Product	Yield (%)	Ratio <sup>a</sup> $\alpha$ : $\beta$
 1	AllylTMS, TMSOTf MeCN, 0 °C, 16 h	 2	90	15:1:
 6	AllylTMS, BF <sub>3</sub> ·OEt <sub>2</sub> DCE, 50 °C, 16 h	 3	85	4:1
 4	AllylTMS, BF <sub>3</sub> ·OEt <sub>2</sub> DCE, 50 °C, 16 h	 5	80	7:1
 7	BTSFA, AllylTMS, TMSOTf MeCN, 0 °C, 16 h	 8	85	9:1

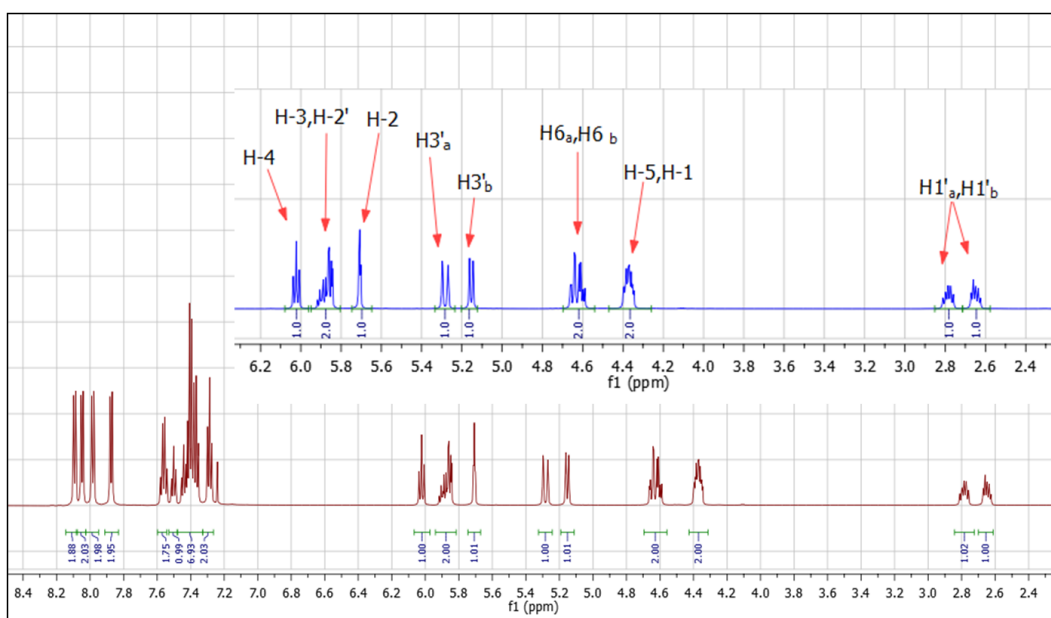
<sup>a</sup> Ratios were obtained from the crude <sup>1</sup>H NMR data.

As stated above,<sup>33-38</sup> there are several papers indicating that C-linked  $\alpha$ -D-mannopyranosides can exist in conformations other than those observed for the corresponding O-linked derivatives, normally seen as <sup>4</sup>C<sub>1</sub> conformers. Obviously, changing bond lengths, bond angles, torsion angles, and conformations would have a detrimental effect upon binding of antagonists to *E. coli* FimH. Given the importance of our key precursor, C-allyl  $\alpha$ -D-mannopyranoside **8 $\alpha$** , we measured the energy levels of its various conformations in the gas phase. The geometry optimizations of the <sup>4</sup>C<sub>1</sub>, <sup>1</sup>C<sub>4</sub>, <sup>2</sup>S<sub>0</sub> and <sup>0</sup>S<sub>2</sub> conformers were performed using density functional theory (DFT) with the hybrid functional B3LYP and 6-31G\* basis set. For that purpose, the Firefly<sup>45</sup> and GaussSum<sup>46</sup> computer programs were used. Figure 1 clearly showed that the <sup>4</sup>C<sub>1</sub> conformer was more stable than its <sup>1</sup>C<sub>4</sub> conformational isomer by at least 8.10 kcal/mol. In addition, the respective skew boats <sup>2</sup>S<sub>0</sub> and <sup>0</sup>S<sub>2</sub> conformers were 11.72 and 16.42 kcal/mol, respectively higher than that of the <sup>4</sup>C<sub>1</sub> conformer. These results agreed with the solution phase data obtained from high field <sup>1</sup>H NMR (900 MHz)<sup>11</sup> coupling constants and nOe experiments (see below). Solid phase X-ray data also strongly supported these results.

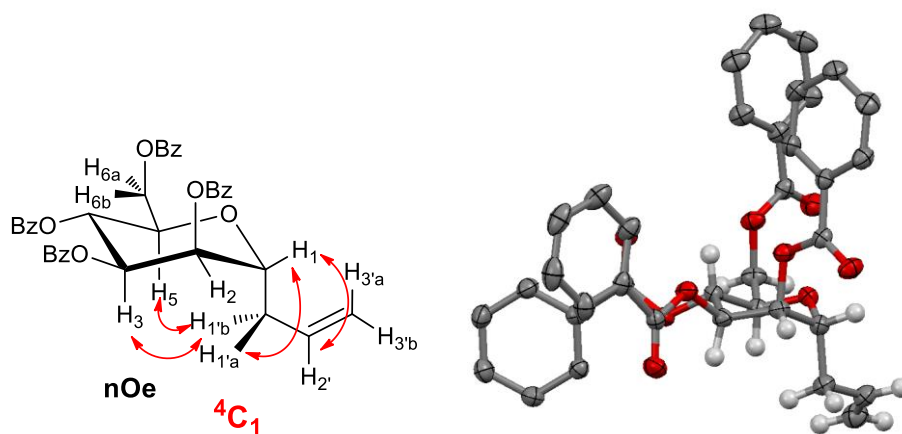


**Figure 1.** Relative energy level of C-allyl  $\alpha$ -D-mannopyranoside (**8 $\alpha$** ) conformers.

NOESY NMR at 600 MHz helped us to demonstrate the solution conformation of C-allyl 2,3,4,6-tetra-O-benzoyl- $\alpha$ -D-mannopyranoside (**5 $\alpha$** ) (Figure 2). In addition to the expected correlation between H<sub>1</sub> and H<sub>1'a</sub> and H<sub>1'b</sub>, we observed a strong nOe between H<sub>1'b</sub> and H<sub>3</sub> and H<sub>5</sub>, further confirming that even in the fully protected forms the C-linked mannopyranoside existed in the suitable <sup>4</sup>C<sub>1</sub> conformation. Importantly, the coupling constants for the H<sub>4</sub> signal at  $\delta$ <sub>H</sub> 6.02 (dd, 1H,  $J_{3,4} = J_{4,5} = 9$  Hz, H-4) clearly indicated a *trans*-diaxial relationship between H<sub>4</sub> and both H<sub>3</sub> and H<sub>5</sub>. Moreover, a crystal structure of **5 $\alpha$**  showed it existed in the same <sup>4</sup>C<sub>1</sub> conformation. Overall, the data on both fully protected as well as unprotected analogues supported that this family of C-linked mannopyranosides existed in the desired <sup>4</sup>C<sub>1</sub> conformation (Figure 3).



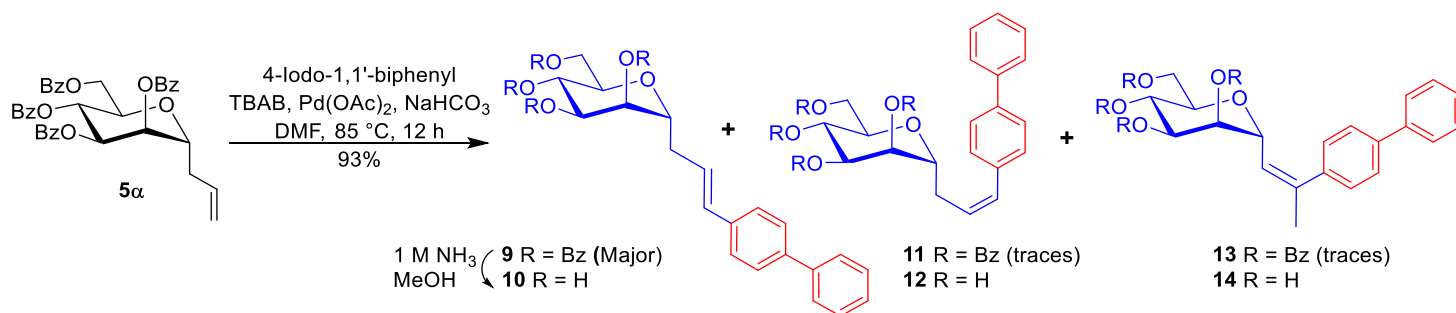
**Figure 2.** <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz) of perbenzoylated C-allyl  $\alpha$ -D-mannopyranoside **5 $\alpha$** .



**Figure 3.** Left panel: conformational studies of *C*-allyl 2,3,4,6-tetra-*O*-benzoyl- $\alpha$ -D-mannopyranoside (**5 $\alpha$** ) in solution by *n*Oe  $^1\text{H}$  NMR; right panel: ORTEP diagram for the X-ray structure of **5 $\alpha$** .

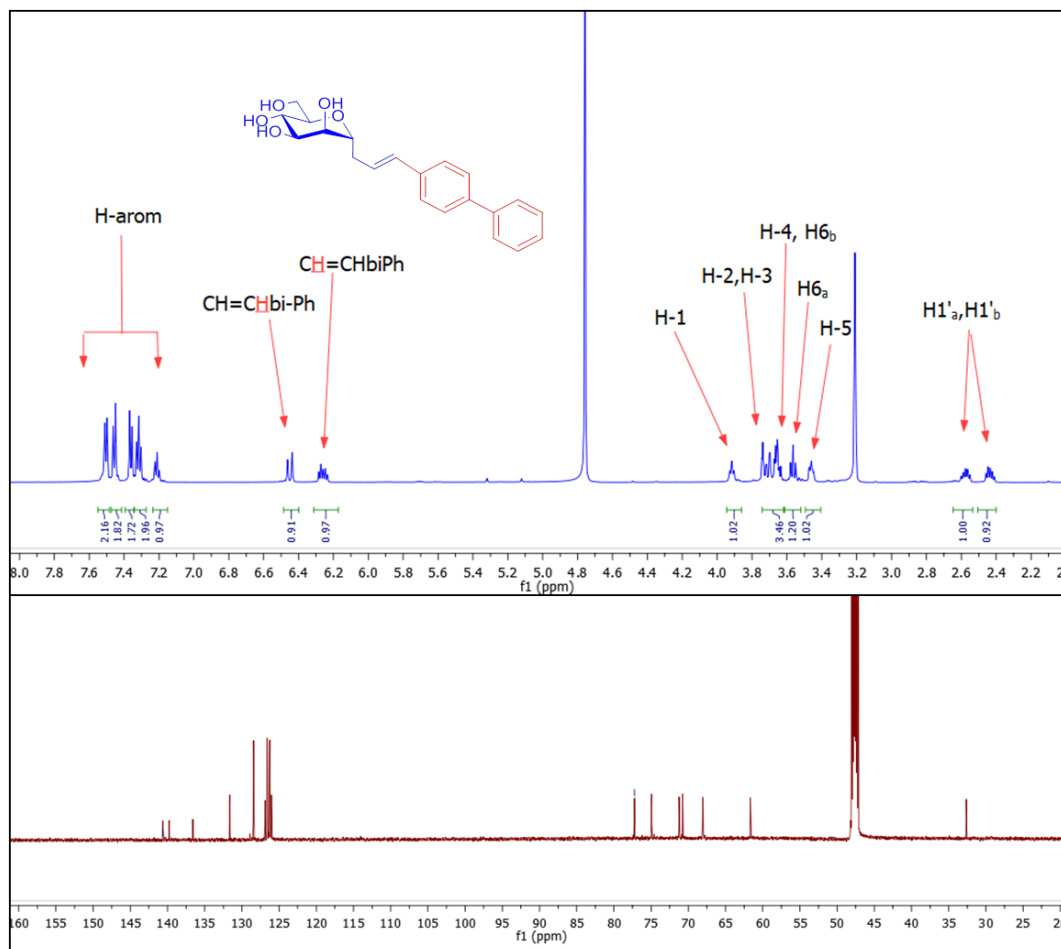
Having secured the accurate  $^4\text{C}_1$  conformation of these valuable anomeric precursors (**4 $\alpha$**  and **5 $\alpha$** ) in the gas phase, solution, and solid-state (X-ray) (Figure 3), we pursued our goals toward potent *E. coli* FimH antagonists capable of adequately placing hydrophobic pharmacophores within the FimH tyrosine gate (Tyr48/Tyr137). Our hope was to bring anomeric aromatic substituents properly oriented and at proper distances from these two hydrophobic aromatic amino acids for appropriate  $\pi$ - $\pi$  stacking (or CH- $\pi$  stacking), known to greatly improve the binding affinities of FimH ligands.<sup>10-15</sup> To this end, we opted for an elongation of the aglycone side chain using palladium-catalyzed Heck cross coupling between allylic **5 $\alpha$**  and aryl iodide such as 4-iodo-1,1'-biphenyl.

Somewhat surprisingly, when allylic  $\alpha$ -D-mannopyranoside **5 $\alpha$**  was treated under our optimized conditions (4-iodo-1,1'-biphenyl, Pd(OAc)<sub>2</sub>, TBAB, NaHCO<sub>3</sub>, DMF, 85 °C, 12 h),<sup>1,11</sup> compound **9** was obtained as a major product (93%) together with traces amount of stereo- and regio-isomers **11** and **13** (Scheme 2). The mixture of side products (**11** and **13**) was clearly visible from the  $^1\text{H}$  NMR spectra of the crude reaction mixture. Their formation has been previously explained when considering the reaction mechanism of the Heck reaction.<sup>1</sup> After isolation of pure **9** (Scheme 2) and **11/13** mixture, they were separately submitted to de-*O*-benzoylation (1 M NH<sub>3</sub>, MeOH, r.t., 36 h) to afford pure **10** (Figure 4) and an intractable mixture of **12/14**. These two side products could not be readily purified and fully characterized. Nevertheless, their mixture was submitted to co-crystallization with *E. coli* FimH together with the major product. Even though we could not obtain X-ray data from the complex of FimH and major product **10**, both side products were individually and successfully co-crystallized with the FimH<sup>47</sup> in the open and closed tyrosine gate, respectively, thus establishing their exact structures (Figure 5).

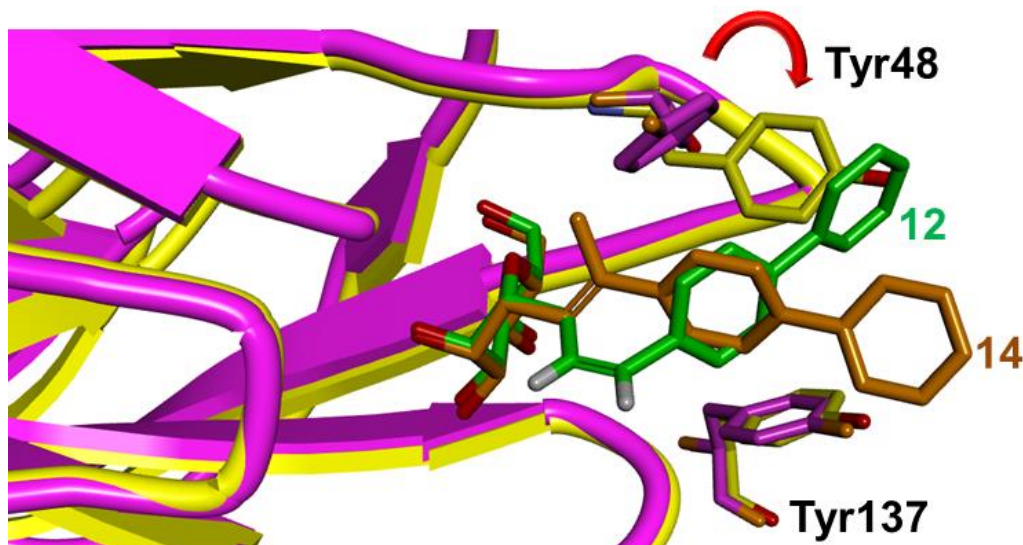


**Scheme 2.** Heck cross-coupling reactions between **5 $\alpha$**  and 4-iodo-1,1'-biphenyl that afforded three regio/stereoisomers **9**, **11** and **13**.

A high field  $^1\text{H}$  NMR spectrum of deprotected **10** (major product) was fully assigned (Figure 4). The chemical shifts and the coupling constants of the sugar ring protons, together with those of the aglycone were in agreement with derivative **10** being in its expected  $^4\text{C}_1$  chair conformation.

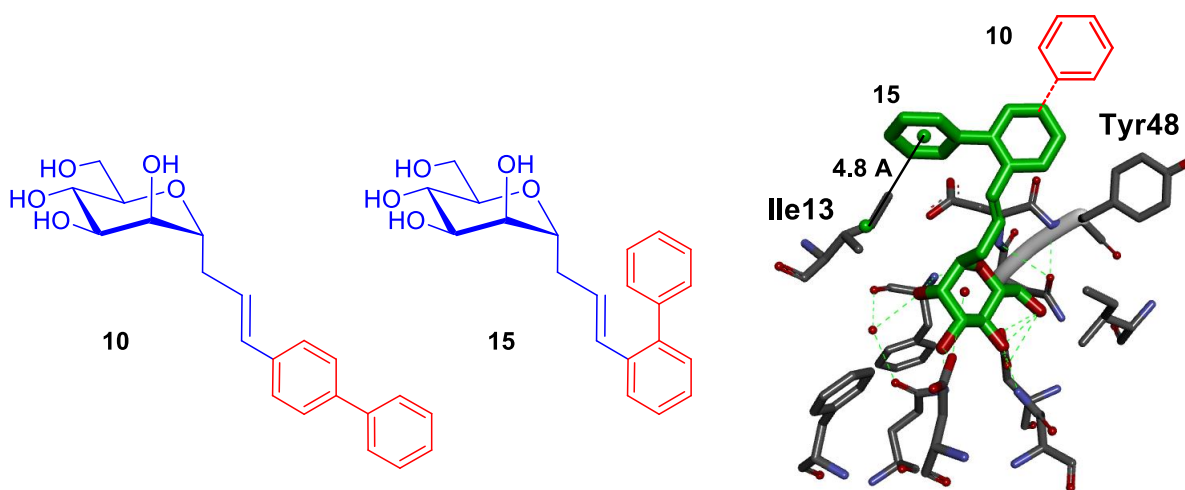


**Figure 4.**  $^1\text{H}$  NMR (MeOH, 600 MHz) and  $^{13}\text{C}$  NMR of compound **10**.



**Figure 5.** *E. coli* FimH complexed with side products **12** (green) bound in the open Tyr gate (Tyr48-Tyr137, protein pink, PDB: 5aal) and **14** (brown) in the closed tyrosine gate (protein yellow, PDB:5aap).<sup>47</sup>

We next undertook affinity measurements between FimH and compound **10** by surface plasmon resonance (SPR).<sup>11</sup> Compound **10**, having its second phenyl group in the *para*-position, was a potent ligand ( $K_d$  17 nM) but it was less potent than the recently identified *ortho*-substituted analogue **15**<sup>11</sup> having an almost 3-fold affinity enhancement ( $K_d$  6.9 nM). As opposed to the two side products **12** and **14**, both compounds could not be co-crystallized with FimH. However, based on previous work,<sup>11</sup> docking of its lowest energy conformer, obtained through modeling and high field NMR spectra, with the FimH adhesin indicated that the *ortho*-substituted phenyl ring of **15** was able to interact with an additional amino acid isoleucine-13 (Ile13), located in the clamp loop, thus opening the route for further lead improvement.



**Figure 6.** Comparison between *para*-substituted biphenyl **10** ( $K_d$  17 nM) and *ortho*-substituted biphenyl **15** ( $K_d$  6.9 nM); docking of the lowest energy conformer of **15** in the active site of FimH indicated additional hydrophobic interactions with Ile13 (centroid with the *ortho*-phenyl at 4.8 Å).



## Conclusions

Several approaches to an important key intermediate for the synthesis of families of C-linked  $\alpha$ -D-mannopyranosides have been presented. Although some were more  $\alpha$ -stereoselective than others, we opted for the use of perbenzoylated precursors for practical reasons. Using optimized Sakurai allylation, good yields of the C-allylated glycosyl derivatives were obtained. We demonstrated that these existed in the required  ${}^4C_1$  conformation, in the gas phase, solutions, and in the solid-state, as requested for efficient binding interactions with one of the essential *E.coli* virulence factor FimH. We then explored the formation of the typical, as well as of side products, obtained during the course of our palladium-catalyzed Heck cross-coupling reaction with a key aryl iodide (4-iodo-1,1'-biphenyl). Even though, an X-ray complex between the FimH and the major and expected Heck product could not be acquired, data were obtained from the two minor side products. The findings reveal new opportunities for the design of novel drug candidates against FimH and offer previously unexplored binding interactions within the active site of the protein.

## Experimental Section

**General.** Reactions were carried out under Nitrogen using commercially available ACS grade solvents which were stored over 4 Å molecular sieves. Solutions in organic solvents were dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated under reduced pressure. Reagents were obtained from Sigma Aldrich Canada LTD and used without further purification. Compounds **1** and **6** are commercially available, **2**,<sup>39</sup> and **8**<sup>43</sup> were prepared according to published data and their physical data were in agreement with the proposed structures. Melting points were measured on a Fisher Jones apparatus and are uncorrected. Optical rotations were measured with a JASCO P-1010 polarimeter. Reactions were monitored by thin-layer chromatography using silica gel 60 F<sub>254</sub> coated plates (E. Merck).  ${}^1\text{H}$  and  ${}^{13}\text{C}$  NMR spectra were recorded at 300 or 600 MHz and 75 or 150 MHz, respectively, with Varian Gemini 2000 (300 MHz) and Varian Inova (600 MHz) spectrometers. All NMR spectra were measured at 25 °C in indicated deuterated solvents. Proton and carbon chemical shifts ( $\delta$ ) are reported in ppm relative to the chemical shift of residual  $\text{CHCl}_3$ , which was set at 7.28 ppm ( ${}^1\text{H}$ ) and 77.16 ppm ( ${}^{13}\text{C}$ ). Coupling constants ( $J$ ) are reported in Hertz (Hz), and the following abbreviations are used for peak multiplicities: singlet (s), doublet (d), doublet of doublets (dd), doublet of doublet with equal coupling constants ( $t_{\text{ap}}$ ), triplet (t), multiplet (m). Assignments were made using COSY (CORrelated Spectroscopy) and HSQC (Heteronuclear Single Quantum Coherence) experiments. High-resolution mass spectra (HRMS) were measured with a LC-MS-TOF (Liquid Chromatography Mass Spectrometry Time of Flight) instrument (Agilent Technologies) in positive and/or negative electrospray mode by the analytical platform of UQAM. Compounds **11-14** were only obtained in trace amounts that could not be fully characterized except through the X-ray data of their unprotected FimH complexes (**12**, **14**) (Figure 5). The X-ray data were deposited as PDB accession no. 5AAL and 5AAP, respectively.

**1,2,3,4,6-Penta-O-benzoyl-D-mannopyranose (4 $\alpha$ , 4 $\beta$ ).**<sup>42</sup> D-Mannose (3.00 g, 16.7 mmol) was dissolved in pyridine to which was added benzoyl chloride (1:5 v/v). After stirring for 16 h at room temperature, the excess benzoyl chloride was quenched with methanol and the reaction mixture was concentrated under vacuum. The crude residue was dissolved in dichloromethane, washed successively with  $\text{NaHCO}_3$ , brine, and dried ( $\text{Na}_2\text{SO}_4$ ). A crude mixture of **4 $\alpha$ /4 $\beta$**  (10.6 g, 14.27 mmol) was obtained. The anomeric ratio ( ${}^1\text{H}$  NMR) of the two anomers was (75 $\alpha$ :25 $\beta$ ) similar to that previously obtained from the literature.<sup>42</sup> The residue was purified by

flash silica gel column chromatography using (PhMe/EtOAc, 9.8:0.2) to provide **4 $\alpha$**  (40%),  $R_f\alpha = 0.33$  and **4 $\beta$**  (48%),  $R_f\beta = 0.21$ . Physical data agreed with those present in the literature.<sup>42</sup>

**2,3,4,6-(Tetra-O-benzoyl- $\alpha$ -D-mannopyranosyl)-1-propene (5 $\alpha$ )**. To an ice-cold solution of 1,2,3,4,6-tetra-O-benzoyl- $\alpha$ -D-mannopyranose (**4**) (3.46 g, 4.94 mmol) in dry acetonitrile (13 mL) and CH<sub>2</sub>Cl<sub>2</sub> (7 mL) was added allylTMS (1.17 mL, 1.5 equiv) followed by BF<sub>3</sub>·Et<sub>2</sub>O (1.08 mL, 1.9 equiv) and TMSOTf (0.98 mL, 1.1 equiv) added dropwise during 20 min. The reaction mixture was heated at 50 °C under N<sub>2</sub> for 48 h. The course of the reaction was monitored by TLC. The solution was evaporated under reduced pressure then diluted with dichloromethane which was washed with NaHCO<sub>3</sub>. The organic layer was separated and dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated to provide an  $\alpha/\beta$  anomeric mixture in 7:1 ratio:  $R_f\alpha = 0.36$ ,  $R_f\beta = 0.31$ . Purification and separation of  $\alpha$  anomer was done by silica gel column chromatography (Hex/EtOAc, 4:1) to afford the title compound **5 $\alpha$**  as a colorless powder (1.2 g, 40%): mp 112-114 °C (EtOH/Et<sub>2</sub>O);  $[\alpha]_{20}^D = -62.6$  (c = 0.18, CHCl<sub>3</sub>). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>):  $\delta_H$  7.24-8.21 (m, 20H, H-arom), 6.02 (t, 1H,  $J_{3,4} = J_{4,5} = 9.0$ , H-4), 5.91-5.84 (m, 2 H, CH=CH<sub>2</sub> and H-3), 5.71 (dd, 1H,  $J_{1,2} = J_{2,3} = 2.9$ , H-2), 5.28 (dd, 1H,  $J_{trans} = 17.1$ ,  $J_{ge} < 1.0$ , CH=CHH), 5.15 (dd 1H,  $J_{cis} = 10.2$ ,  $J_{gem} < 1.0$ , CH=CHH), 4.66 (dd, 1H,  $J_{6a,6b} = 12.0$ ,  $J_{5,6a} = 5.4$ , H-6a), 4.60 (dd, 1H,  $J_{6a,6b} = 12.0$ ,  $J_{5,6b} = 2.9$ , H-6b), 4.45-4.25 (m, 2H, H-1,H-5), 2.77 (ddd, 1H,  $J_{1'a,1'b} = 8.5$ ,  $J_{1'a,2'} = 7.9$ ,  $J_{1'a,1} = 7.2$ ), 2.65 (ddd, 1H,  $J_{1'b,1'a} = 7.7$ ,  $J_{1'b,2} = 7.2$ ,  $J_{1'b,1} = 6.4$ ); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta_C$  166.6, 165.6, 165.5, 165.4 (4 CO), 133.4 (C-2'), 133.3, 133.3, 133.0 (C arom-q), 129.7, 129.5, 128.9, 128.1, (C arom), 118.4 (C-3'), 74.6 (C-1), 71.2 (C-5), 70.6 (C-4), 69.9 (C-3), 67.4 (C-2), 62.8 (C-6), 33.5 (C-1'). ESI<sup>+</sup>-HRMS:  $[M+H]^+$  calcd for C<sub>37</sub>H<sub>32</sub>O<sub>9</sub> + H<sup>+</sup>: 621.2119, found, 621.2143.

**(E)-4-[3-(2,3,4,6-Tetra-O-benzoyl- $\alpha$ -D-mannopyranosyl)prop-1-en-1-yl]-1,1'-biphenyl (9)**. To a solution of mannoside **5 $\alpha$**  (50 mg, 0.08 mmol) in DMF were added 4-iodo-1,1'-biphenyl (45 mg, 2 equiv), 15% palladium(II) acetate, tetrabutylammonium bromide (25 mg, 1 equiv), and sodium bicarbonate (20 mg, 3 equiv). The reaction mixture was heated at 85 °C under N<sub>2</sub> for 24 h. The solution was concentrated under reduced pressure and the residue was purified by flash column chromatography using silica gel column chromatography (PhMe/EtOAc, 3:1) to afford the title compound **9** as a colorless oil (57.2 mg, 93%).  $[\alpha]_{20}^D = -11.05$  (c = 0.7, CHCl<sub>3</sub>). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>)  $\delta_H$  8.10-7.25 (m, 29H, H-arom), 6.65 (d, 1H,  $J_{2',3'} = 15.8$ , CH=CH-biPh), 6.36-6.23 (m, 1 H, CH=CH-biPh), 5.96 (dd, 1H,  $J_{3,4} = 8.9$ ,  $J_{4,5} = 2.0$ , H-4), 5.91 (dd, 1H,  $J_{2,3} = 3.0$ ,  $J_{3,4} = 8.9$ , H-3), 5.20 (dd, 1H,  $J_{1,2} = 1.5$ ,  $J_{2,3} = 3.3$ , H-2), 4.58 (dd, 1H,  $J_{6a,6b} = 12.0$ ,  $J_{5,6a} = 6.1$ , H-6a), 4.50 (dd, 1H,  $J_{6a,6b} = 12.1$ ,  $J_{5,6b} = 2.8$ , H-6b), 4.57-4.33 (m, 2H, H-5, H-1), 3.10-2.90 (m, 1H, H1'a), 2.90-2.70 (m, 1H, H1'b); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta_C$  171.7, 166.3, 165.6, 165.5 (4CO), 140.8, 140.0, 136.9 (C arom-q), 136.0, 133.7, 133.2, 129.9, 128.5, 126.9, (C arom, C-3'), 124.2 (C-2'), 74.6 (C-1), 71.3 (C-5), 70.8 (C-4), 69.8 (C-3), 67.4 (C-2), 63.1 (C-6), 33.0 (C-1'). ESI<sup>+</sup>-HRMS:  $[M+H]^+$  calcd for C<sub>49</sub>H<sub>40</sub>O<sub>9</sub> + H<sup>+</sup>: 773.2745; found, 773.2745.

**(E)-4-[3-( $\alpha$ -D-Mannopyranosyl)prop-1-en-1-yl]-1,1'-biphenyl (10)**. Compound **9** (50 mg, 0.064 mmol) was deprotected by treatment with 1 M ammonia in methanol (0.1 M) at room temperature for 36 h. Removal of solvent under vacuum afforded the crude residue which was purified by semi-preparative HPLC (**A**: H<sub>2</sub>O + 0.1% trifluoroacetic acid, **B**: MeCN + 0.1% trifluoroacetic acid, 5 mL/min) to afford the title compound **10** (24.9 mg, 96%).  $[\alpha]_{20}^D = 30.2$  (c = 1.0, MeOH). <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD):  $\delta_H$  7.50 (d, 2H,  $J_{HH} = 7.5$ , H-arom), 7.45 (t, 2H,  $J_{HH} = 8.2$ , H-arom), 7.36 (d, 2H,  $J_{HH} = 8.2$ , H-arom), 7.31 (dd, 2H,  $J_{HH} = 8.0$ , 15.8, H-arom), 7.20 (dd, 1H,  $J_{HH} = 8.1$ , 15.4, H-arom), 6.45 (d, 1H,  $J_{2',3'} = 15.6$ , CH=CHPh), 6.29-6.22 (m, 1H, CH=CHPh), 3.93-3.90 (m, 1H, H-1), 3.74 (dd, 1H  $J_{1,2} = J_{2,3} = 2.8$ , H-2), 3.71 (dd, 1H,  $J_{2,3} = 2.6$ ,  $J_{3,4} = 11.7$ , H-3), 3.68-3.62 (m, 2H, H-4, H-6b), 3.56 (dd, 1H,  $J_{5,6a} = 9.0$ ,  $J_{6a,6b} = 8.4$ , H-6a), 3.48-3.41 (m, 1H, H-5), 2.67-2.51 (m, 1H, Ha'), 2.49-2.40 (m, 1H, Ha). <sup>13</sup>C NMR (600 MHz, CD<sub>3</sub>OD):  $\delta_C$  140.6, 140.1, 136.9 (C arom-q), 131.6 (C-3'), 128.4, 126.8, 126.6, 126.3, 126.2, 126.0 (C arom, C-2'), 77.2 (C-1), 74.9 (C-5), 71.2 (C-4), 70.7 (C-3), 68.0 (C-2), 61.6 (C-6), 32.5 (C-1'). ESI<sup>+</sup>-HRMS:  $[M+NH_4]^+$  calcd for C<sub>21</sub>H<sub>28</sub>NO<sub>5</sub>, 374.1962; found, 374.1960.

**X-Ray crystal-structure of 5a** and refinement data: formula, (C<sub>37</sub>H<sub>32</sub>O<sub>9</sub>), orthorhombic, space group P212121, a 9.4849(3) Å, b 12.6698(4) Å, c 26.5906(8) Å,  $\alpha$  90°,  $\beta$  90°,  $\gamma$  90°, V 3195.44(17) Å<sup>3</sup>, D<sub>calcd</sub> 1.290 g/cm<sup>3</sup>, crystallize: 0.3 × 0.28 × 0.18 mm<sup>3</sup>. Index ranges -12 ≤ h ≤ 12, -16 ≤ k ≤ 16, -34 ≤ l ≤ 34. Crystallographic data for the structure reported in this paper has been deposited at the Cambridge Crystallographic Data Centre (CCDC) with deposition no: 1560370 for C<sub>37</sub>H<sub>32</sub>O<sub>9</sub>. Supplementary data can be obtained free of charge from CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk, [website http://www.ccdc.cam.ac.uk](http://www.ccdc.cam.ac.uk)).

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