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PAPER

# Synthesis and biological evaluation of potential new inhibitors of the bacterial transferase *MraY* with a $\beta$ -ketophosphonate structure†

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Stable analogs of bacterial transferase *MraY* substrate or product with a pyrophosphate surrogate in their structure are described.  $\beta$ -ketophosphonates were designed as pyrophosphate bioisosteres and were investigated as UDP-GlcNAc mimics. The developed strategy allows introduction of structural diversity at a late stage of the synthesis. The biological activity of the synthesized compounds was evaluated on the *MraY* enzyme.

## Introduction

Enzymes involved in peptidoglycan biosynthesis are key targets for the development of new antibiotics because there are no parallels of this pathway in eukaryotes. Peptidoglycan is a heteropolymer of bacterial cell walls consisting of long glycan chains made of  $\beta$ -1 $\rightarrow$ 4 alternating units of *N*-acetylmuramoyl-peptides (MurNAc-peptides) and *N*-acetylglucosamine (GlcNAc) which are cross-linked together through the short peptide chains.<sup>1</sup> This macromolecule protects the cell from internal osmotic pressure effects and contributes to the cell shape maintenance. Peptidoglycan biosynthesis is a complex process which takes place in various cell compartments: cytoplasm, membrane and periplasm<sup>2</sup> (Fig. 1).

UDP-*N*-acetylglucosamine (UDP-GlcNAc) is first transformed into UDP-*N*-acetylmuramoyl-pentapeptide (UDP-MurNAc-pentapeptide) by the Mur enzymes (MurA, MurB, MurC, MurD, MurE and MurF).<sup>3</sup> Then, at the plasma membrane, two distinctive proteins (*MraY* and MurG) synthesize polyprenyl-linked precursors (lipid I and lipid II) that carry one complete cell-wall subunit (Fig. 2).<sup>4</sup>

Thus, *MraY*<sup>5</sup> transfers the phospho-MurNAc-pentapeptide moiety from the cytoplasmic precursor to the membrane ac-

ceptor undecaprenyl phosphate ( $C_{55}$ -P) yielding undecaprenyl-pyrophosphoryl-MurNAc-pentapeptide (lipid I) and releasing uridine monophosphate (UMP). Thereafter, MurG<sup>6</sup> catalyzes the transfer of the GlcNAc moiety from UDP-GlcNAc to lipid I yielding undecaprenyl-pyrophosphoryl-MurNAc-(pentapeptide)-GlcNAc (lipid II). Lipid II is then translocated through the membrane by a specific flippase. This translocation activity was shown for FtsW<sup>7</sup> from *E. coli*. In the outside of the membrane the lipid II is then used as a substrate for the polymerisation reactions, transglycosylation and transpeptidation, catalyzed by the penicillin-binding proteins (PBPs) and the monofunctional transglycosylases (MGTs).<sup>8</sup>

The enzymes involved in this biosynthetic pathway have been demonstrated as ubiquitous and essential to bacteria<sup>2,3,4a,8,9</sup> and any anomaly in this biosynthesis leads to cell lysis. Therefore, enzymes implicated in this process are ideal targets in the search for new antibacterials.<sup>10</sup> Indeed, several families of widely used antibiotics, such as  $\beta$ -lactams and lipoglycopeptides, are already known to interfere with certain enzymes implicated in peptidoglycan biosynthesis. However, because of the ever-increasing resistance<sup>11</sup> to these antibacterial agents and in the context of a program<sup>12</sup> directed to the synthesis and biological evaluation of new antibacterials, we are focusing on the development of *MraY* inhibitors. Indeed, this enzyme has been little exploited so far as a consequence of its trans-membrane localisation.<sup>13</sup> Therefore, since there is no drug in clinical use targeting this essential enzyme, this can be expected to delay the emergence of resistance. Our goal is to target compounds which are stable analogs of *MraY* substrate or product and displaying a pyrophosphate surrogate in their structure (Fig. 3). Furthermore, some of these compounds can also be considered as potential inhibitors of the MurG enzyme due to their structural analogy with the substrate of this enzyme. Results concerning the synthesis of a first compound have been published in a preliminary form<sup>14</sup> and we are now aiming at reporting our full results dealing with a series of related compounds.

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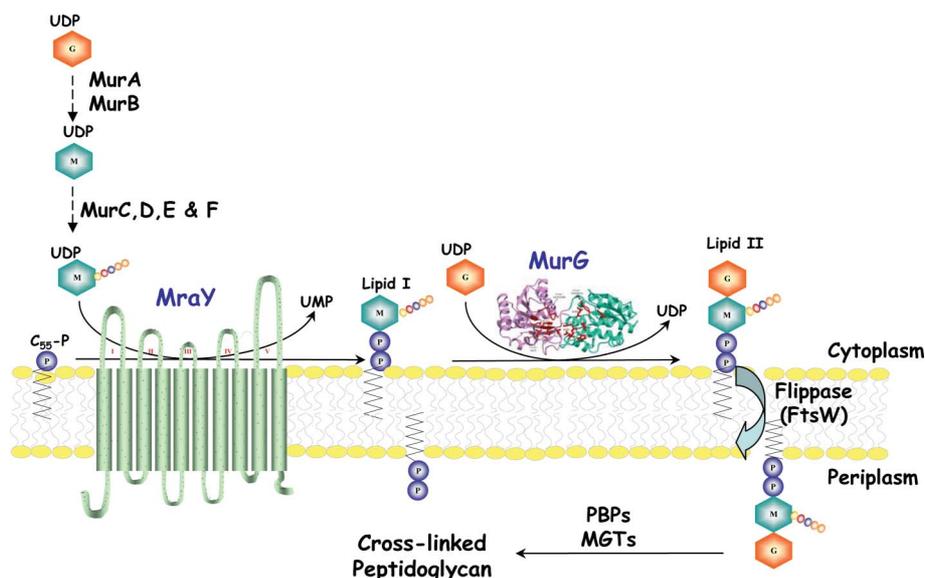


Fig. 1 Peptidoglycan biosynthesis pathway.

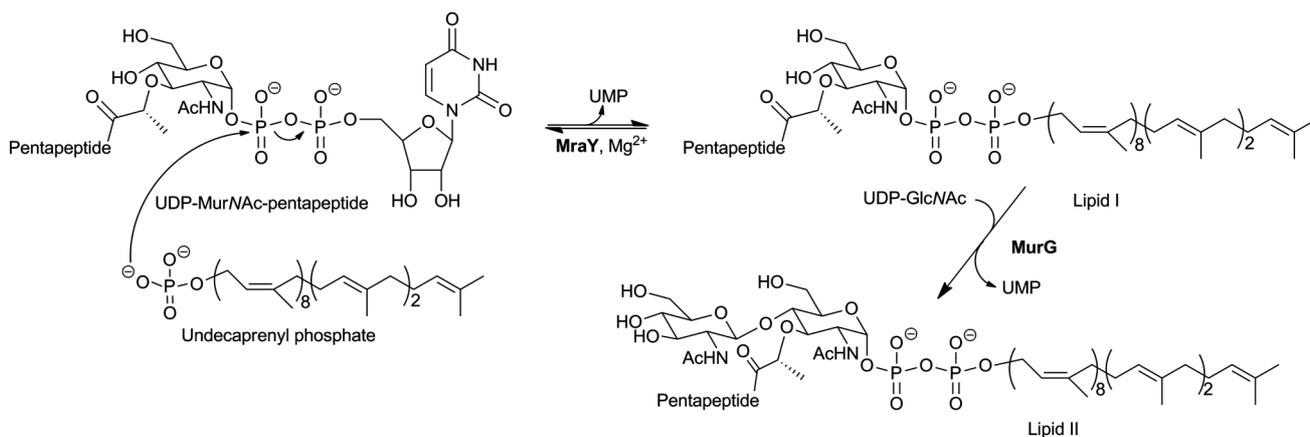


Fig. 2 Enzymatic reactions catalyzed by MraY and MurG enzymes.

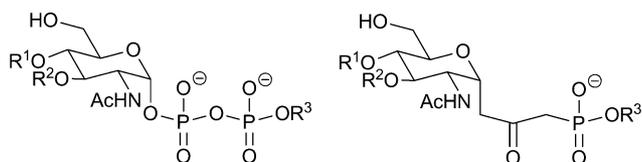


Fig. 3 General structure of MraY and MurG substrates and products and targeted  $\beta$ -ketophosphonate isosteres.

The pyrophosphate present in substrate and product of the reaction catalyzed by MraY is on one hand linked to a GlcNAc derivative and on the other hand to either uridine or undecaprenol. This diphosphate moiety is supposed to interact with the divalent cation  $Mg^{2+}$  which is a co-factor of the enzymatic reaction, suggested to proceed in either one<sup>15</sup> or two steps.<sup>16</sup> Furthermore, it is prone to hydrolysis due to its positioning in the anomeric position of the glycosyl derivative. That is why our strategy has been to replace this pyrophosphate, site of the enzymatic reaction, by a bioisostere displaying a  $\beta$ -ketophosphonate structure. In the resulting compounds, the replacement of the sugar anomeric oxygen atom is expected to enhance the stability of the inhibitors with

regards to hydrolysis. Furthermore, the carbonyl group is proposed as an alternative electrophilic center to the one involved in the enzymatic reaction. The  $\beta$ -ketophosphonate moiety displaying both a phosphonate anion and a polarized carbonyl group should be able to chelate the  $Mg^{2+}$  co-factor. Consequently, the targeted  $\beta$ -ketophosphonate pyrophosphate bioisosteres should be pertinent mimics of the substrate or the product, since they have the required electronic properties to coordinate the metallic co-factor of the reaction as well as being stable to hydrolysis.

## Results and discussion

The design and synthesis of pyrophosphate analogs of glycosyl nucleotides have already been described (Fig. 4). Thus, methylene diphosphonate<sup>17</sup> and 1-C-phosphonophosphate<sup>18</sup> analogs of UDP-Glc or UDP-GlcNAc, respectively, have been reported. Different ways of diphosphate-type connections between GlcNAc and uridine moieties have also been investigated.<sup>19</sup> In some cases, the pyrophosphate has been replaced by a propenyl phosphonate,<sup>20</sup> a hydroxypropyl phosphonate<sup>21</sup> or a propyl phosphate.<sup>22</sup>

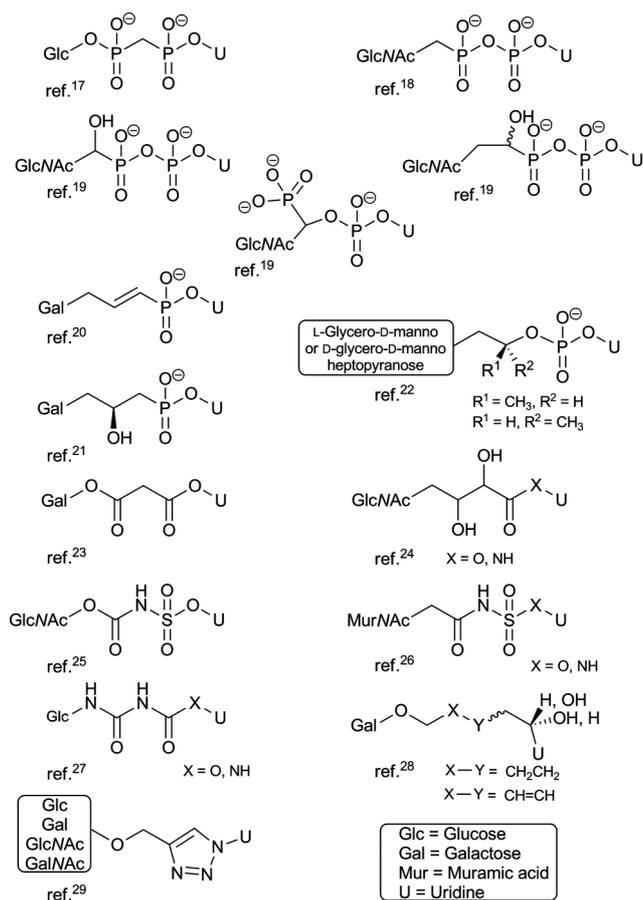


Fig. 4 Structures of pyrophosphate analogs of glycosyl nucleotides previously described.

Other mimics have no phosphorus atoms in their structure, the pyrophosphate moiety being changed to a malonic linkage,<sup>23</sup>  $\alpha,\beta$ -ethylenic ester or amide with or without dihydroxylation of the double bond.<sup>24</sup> In addition, an oxycarbonyl aminosulfonyl linker<sup>25</sup> and a carbonyl linked to *O*- or *N*-sulfamoyl uridine<sup>26</sup> have also been reported as well as allophanates and biuret derivatives.<sup>27</sup> Galactose-linked uridine derivatives without charge or dipole contributions in the linker have been synthesized *via* cross metathesis.<sup>28</sup> Finally, replacement of the pyrophosphate by a triazole moiety has also been recently described.<sup>29</sup> However, none of these pyrophosphate analogs display a glycosyl  $\beta$ -ketophosphonate skeleton. Nevertheless, it should be mentioned that a uridine-containing phosphonate ester analog has previously been synthesized and tested as inhibitor of solubilised *E. coli* transferase *MraY* ( $\text{IC}_{50}$  3.7 mM).<sup>30</sup>

### Chemical synthesis

Two complementary strategies to obtain the targeted compounds have been investigated (Fig. 5). On one hand, the retrosynthesis relies on the condensation of variously substituted lithiomethylenephosphonates on the ester function of a conveniently protected methyl *N*-acetylglucosaminyl acetate (path a) and on the other hand it involves the esterification of a  $\beta$ -ketophosphonate derivative using different alcohol derivatives (path b). The residue  $\text{R}^3$  to be introduced on the phosphonate

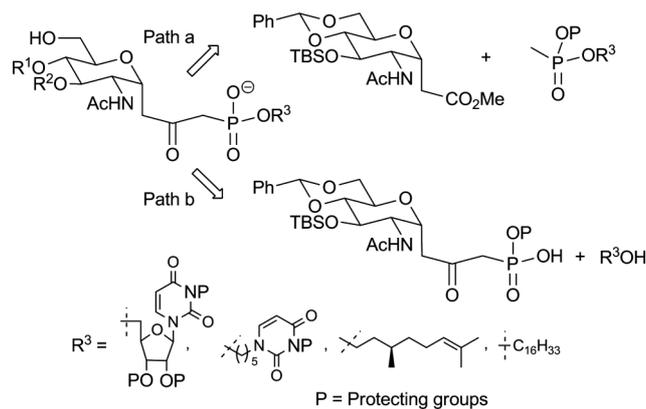
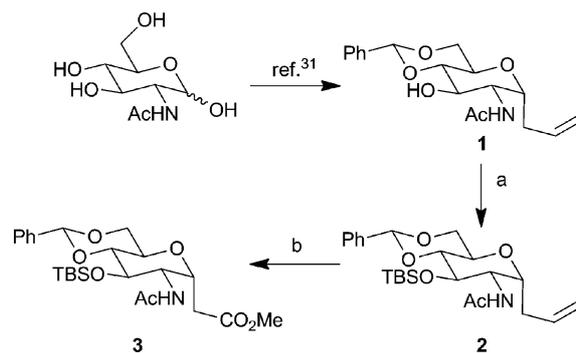


Fig. 5 Retrosynthetic analysis.

has been chosen to mimic either the substrate or the product of the reaction catalyzed by the transferase *MraY*. Accordingly, the uridine moiety present in the substrate has been introduced as it is or as a  $\text{C}_5$ -alkyl uracil analog and the undecaprenyl chain, part of lipid I, has been replaced by a citronellyl or a simple hexadecanyl moiety.

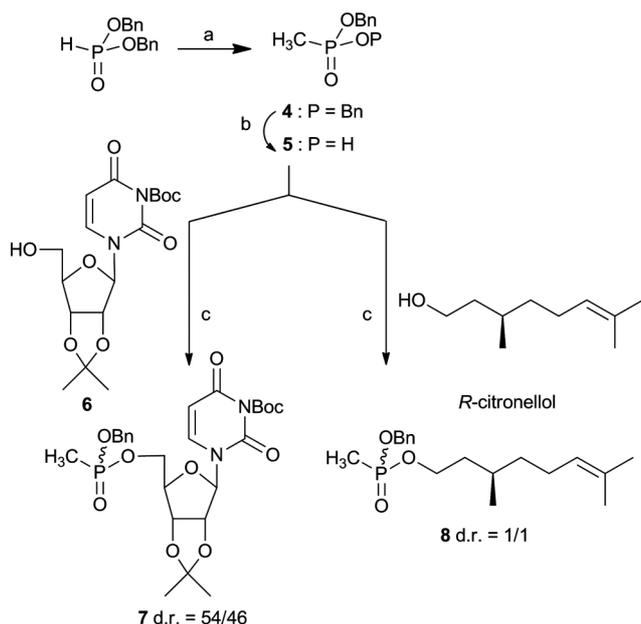
The ester building block **3** (Scheme 1) was synthesized from orthogonally protected  $\alpha$ -1-*C*-allyl-*N*-acetylglucosamine **1** readily obtained in four steps (50% overall yield as an  $\alpha/\beta$  mixture of anomers) from commercial *GlcNAc*.<sup>31</sup> Protection of the secondary alcohol function as its *tert*-butyldimethylsilyl ether derivative **2** allowed isolation of pure  $\alpha$  anomer from the 24/1  $\alpha/\beta$  mixture. Finally, ozonolysis of the alkene under basic conditions in the presence of methanol gave the ester **3**.<sup>32</sup>



Scheme 1 Synthesis of C-glycosyl ester **3**. Reagents and conditions: a) TBSCl, imidazole, DMF, 97%; b)  $\text{O}_3$ ,  $\text{CH}_2\text{Cl}_2$ , MeOH, NaOH, 65%.

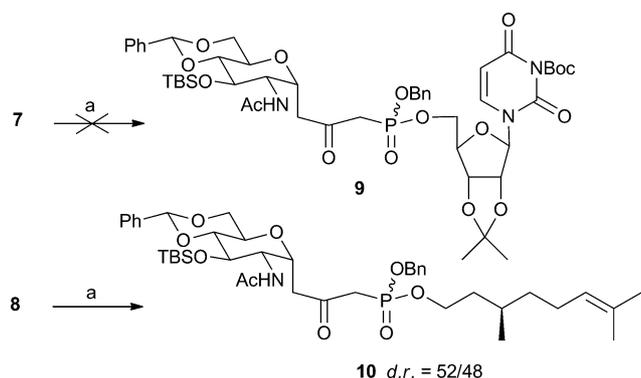
According to path a, we next turned to the synthesis of unsymmetrical methylphosphonates **7** and **8** substituted by an uridinylyl or a citronellyl moiety. Their preparation (Scheme 2) involved NaH treatment, in the presence of methyl iodide, of commercially available dibenzyl *H*-phosphonate, leading to dibenzyl methylphosphonate **4**. It was followed by benzyl mono-deprotection in the presence of DABCO in refluxing toluene<sup>33</sup> to afford monobenzyl methylphosphonate **5**. Esterification with isopropylidene *N*-Boc uridine<sup>34</sup> or citronellol was performed under Mitsunobu conditions in the presence of triphenyl phosphine and diisopropyl azodicarboxylate affording the phosphonate **7** or **8** as a diastereomeric mixture.

Access to the targeted  $\beta$ -ketophosphonates **9** and **10** was then tentatively carried out, by condensation on the ester **3** of lithio



**Scheme 2** Synthesis of unsymmetrical methyl phosphonates **7** and **8**. Reagents and conditions: a) *i.* NaH, THF,  $-15^{\circ}\text{C}$  to r.t.; *ii.* MeI,  $-40^{\circ}\text{C}$  to r.t., 80%; b) *i.* DABCO, toluene, reflux; *ii.*  $\text{H}^+$ ,  $\text{H}_2\text{O}$ , 82%; c) DIAD,  $\text{PPh}_3$ , THF, 83% for **7** and 95% for **8**.

derivatives of phosphonates **7** and **8**, generated in the presence of *n*-butyllithium (Scheme 3). However, even if these conditions proved successful for the synthesis of the  $\beta$ -ketophosphonate **10**, they did not allow that of the uridinyl related compound **9**. It has to be noted that at least 3 equivalents of the nucleophilic entity are required to achieve the reaction due to both the acidity of the acetamide and that of the methylene within the resulting  $\beta$ -ketophosphonate. However, this excess of reagent renders its separation from the formed compound **10** difficult and precludes its use in higher excess in order to improve the yield.



**Scheme 3** Towards targeted  $\beta$ -ketophosphonates **9** and **10** according to path **a**. Reagents and conditions: a) *i.* *n*BuLi, THF,  $-78^{\circ}\text{C}$ , 1 h; *ii.* **3**, THF,  $-78^{\circ}\text{C}$  to r.t., 57% for **10**.

In an alternative manner, the synthesis of the  $\beta$ -ketophosphonate **9** was envisaged according to the path **b** (Scheme 4). It first involved the condensation of the dibenzyl methylenephosphonate anion, generated from **4** in the pres-

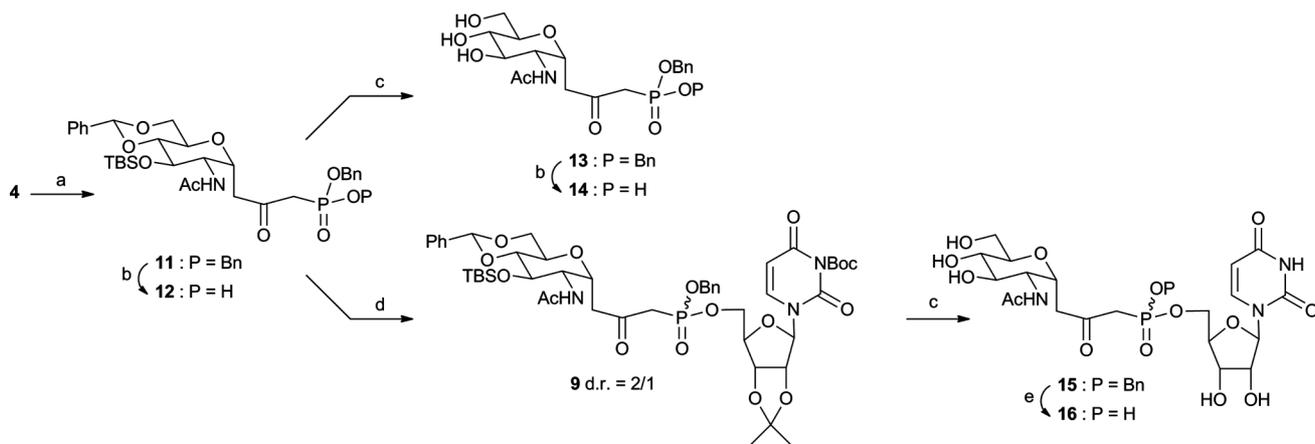
ence of *n*-butyllithium, on the ester **3** giving the dibenzyl  $\beta$ -ketophosphonate **11**. Then, mono-benzyl deprotection in the presence of DABCO led to **12** and was followed by the esterification with isopropylidene *N*-Boc uridine under Mitsunobu conditions in the presence of triphenylphosphine and diisopropyl azodicarboxylate affording the targeted compound **9**. It has to be mentioned that *N*-Boc protection of uridine is necessary to achieve this reaction since the esterification of **12** with isopropylidene uridine under the same conditions was unsuccessful. In order to compare biological activity, compound **11** was also submitted to simultaneous acidic hydrolysis of benzylidene acetal and silyl ether to afford **13** which was then mono-deprotected with DABCO followed by DOWEX  $\text{H}^+$  treatment to provide **14**. The complete deprotection of the phosphonate **9** was then undertaken. Acidic hydrolysis was first performed and allowed the simultaneous removal of benzylidene, isopropylidene, *tert*-butyldimethylsilyl and Boc groups. Finally, cleavage of the benzyl ester was achieved by hydrogenolysis in the presence of palladium on charcoal in methanol leading to **16** as a first analog of the *MraY* substrate.

In a complementary manner, access to other substrate or product analogs of the reaction catalyzed by *MraY* were targeted (Scheme 5). Therefore, on the one hand, a simplified structure of the uridine moiety has been chosen in which the ribose of uridine has been replaced by a  $\text{C}_5$  acyclic alkyl chain,<sup>35</sup> retaining the same five bond linkage between the alcohol function and the uracil residue as in uridine. The imide function present in this 1-(5'-hydroxypentyl)uracil was protected as its *tert*-butyl carbamate **17** to avoid side reactions. On the other hand, the undecaprenyl chain present within the product of the enzymatic reaction was replaced by either citronellyl or hexadecanyl. The citronellyl moiety has been preferred to a terpenic alcohol due to the better stability of homo-allylic phosphonate in acidic conditions compared to allylic phosphonate. Accordingly, phosphonate **12** has been reacted with 1-(5'-hydroxypentyl)-*N*-Boc uracil **17**, citronellol and hexadecanol, under Mitsunobu conditions, affording the protected  $\beta$ -ketophosphonates **18**, **10** and **19**, respectively, as diastereomeric mixtures. Acidic hydrolysis of acid labile protective groups was then carried out in the presence of 1/1 TFA/ $\text{H}_2\text{O}$  which led to **20**, **21** and **23**, respectively. Compound **21** resulted from the addition of water on the citronellyl double bond, catalyzed by the acidic conditions that were used. To limit this side reaction, the acidic hydrolysis of citronellyl  $\beta$ -ketophosphonate **10** was also performed in smoother conditions in the presence of 1/1/1 TFA/ $\text{H}_2\text{O}$ /THF and afforded **22**. Final benzyl ester cleavage of compounds **20**, **21** and **23** was achieved by hydrogenolysis in the presence of palladium on charcoal 10% leading to the final  $\beta$ -ketophosphonates **24**, **25** and **26**, respectively.

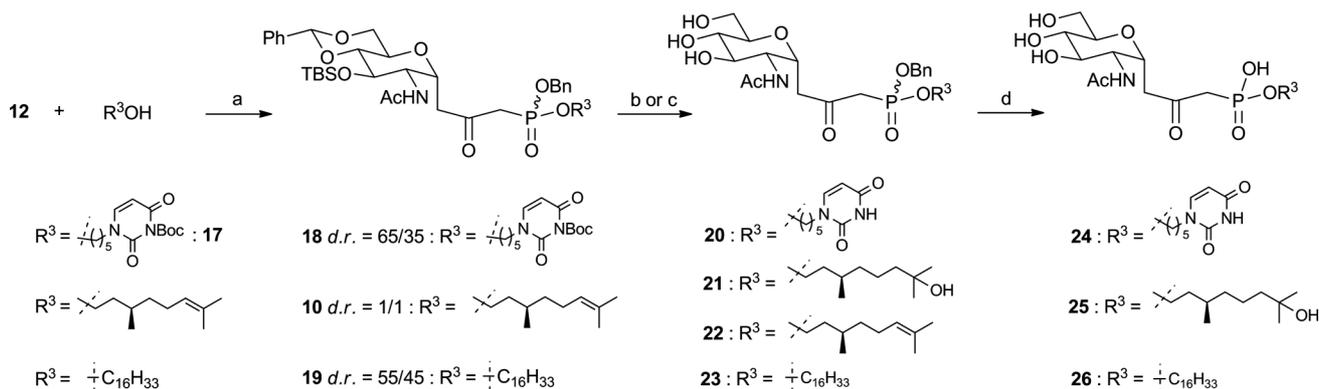
### Biological evaluation

The *in vitro* biological evaluation (Table 1) of compounds **13–16** and **20–26**, on *MraY* purified from *Bacillus subtilis*, was carried out as described in the experimental section. The residual activity of the enzyme was measured in the presence of 1 mM of the tested compounds. For the most active inhibitors, the  $\text{IC}_{50}$  value was determined.

Commercially available tunicamycin from *Streptomyces* sp. was used as a positive control in the tests and resulted in an  $\text{IC}_{50}$  value

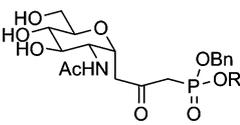
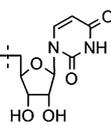
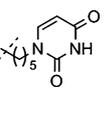
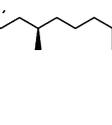
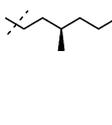
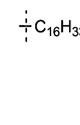
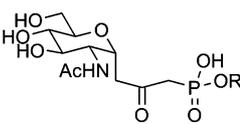
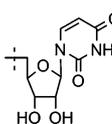
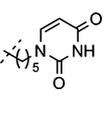
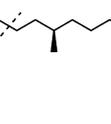
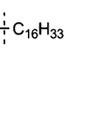


**Scheme 4** Synthesis of substrate analog **16** according to path b. Reagents and conditions: a) *i. n*BuLi,  $-78\text{ }^{\circ}\text{C}$ , 1 h; *ii. 3*,  $-78\text{ }^{\circ}\text{C}$  to r.t., 83%; b) *i. DABCO*, toluene, reflux; *ii. Dowex H<sup>+</sup>*, MeOH, 90% for **12** and 88% for **14**; c) TFA/H<sub>2</sub>O: 1/1, 97% for **13** from **11** and 94% for **15**; d) **6**, DIAD, PPh<sub>3</sub>, THF, 46% or PyBOP, DIEA, DMF, 20%; e) H<sub>2</sub>, Pd/C 10%, MeOH, 85%.



**Scheme 5** Generalisation to the synthesis of  $\beta$ -ketophosphonates **24**, **25** and **26**. Reagents and conditions: a) DIAD, PPh<sub>3</sub>, THF, 27% for **18**, 58% for **10**, 42% for **19**; b) TFA/H<sub>2</sub>O: 1/1, 93% for **20**, 94% for **21**, 90% for **23**; c) TFA/H<sub>2</sub>O/THF: 1/1/1, 84% for **22**; d) H<sub>2</sub>, Pd/C 10%, MeOH, 94% for **24**, 100% for **25**, 87% for **26**.

**Table 1** Inhibitory activity of synthesized compounds on the *MraY* enzyme

Compound	<b>13</b>	<b>14</b>	<b>15</b>	<b>20</b>	<b>21</b>	<b>22</b>	<b>23</b>
	R <sup>1</sup> = Bn	H					
IC <sub>50</sub> (mM)	NI	NI	NI	0.94	0.19	NI	0.82
Compound	<b>16</b>	<b>24</b>	<b>25</b>	<b>26</b>			
	R <sup>1</sup> = 						
IC <sub>50</sub> (mM)	NI	NI	1.3	0.85			

NI: no inhibition at 1 mM concentration.

equal to 0.012 mM. Furthermore, the inhibitors were also tested on the MurG enzyme from *E. coli* which catalyzes the second membrane step of peptidoglycan biosynthesis (Fig. 1), leading to lipid II formation.

The detected inhibitory activities were proven to be rather weak concerning *MraY* inhibition since compounds **20**, **23**, **25** and **26** resulted in  $IC_{50}$  values in the mM range while compounds **13**, **14**, **15**, **16**, **22** and **24** proved not to be inhibitors at 1 mM concentration. Interestingly, compound **21** exhibited significant inhibitory activity with an  $IC_{50}$  value equal to 0.19 mM. Finally, no inhibition of *MurG* activity was observed for the tested compounds at 1 mM concentration. Therefore, the replacement of the phosphorus atom within the pyrophosphate moiety by a carbonyl group leads to low inhibitory activity which could indicate the requirement of a tetrahedral geometry instead of a trigonal one, in the related position, to ensure accurate interaction of the inhibitor with the enzyme. Furthermore, by comparison with compound **22**, the activity observed for compound **21** is encouraging and suggests a possible interaction with the enzyme through a hydrogen bond involving the hydroxyl group present in the inhibitor.

## Conclusion

In summary, we have developed a simple and efficient method for the synthesis of new pyrophosphate analogs of glycosyl nucleotides from *N*-acetylglucosamine. The strategy offers the advantage of allowing introduction of structural diversity at a late stage of the synthesis rendering easy access to a series of compounds. The method has been illustrated by the synthesis of several analogs of *MraY* substrate or product. The activity of the synthesized compounds has been evaluated on purified *MraY* and *MurG*. Unexpectedly, the inhibitory activities were low, except for one compound and the reason for this weak activity could lie in the change in the geometry of the phosphorus atom, site of the enzymatic reaction, probably preventing efficient recognition of the compounds by the enzyme. This study led to reaching some insight into the requirements for inhibition of the trans-membrane protein *MraY*. Taking into account these results, our current work is now focusing on new pyrophosphate mimics with a tetrahedral geometry in the  $\beta$  position of the pyrophosphate. Special attention will also be paid to the introduction of the pentapeptide side chain in  $C_3$  position of *GlcNAc* moiety since it can be important for binding to the enzyme.

## Experimental

### Chemical synthesis

$^1H$  NMR (500 MHz),  $^{13}C$  NMR (125 MHz) and  $^{31}P$  NMR (202 MHz) spectra were recorded in  $CDCl_3$  unless indicated on a Bruker Avance or Avance II. Chemical shifts ( $\delta$ ) are reported in ppm and coupling constants are given in Hz. Optical rotations were measured on a Perkin-Elmer 341 polarimeter with a sodium (589 nm) lamp at 20 °C. Mass spectra, electrospray (ESI) and high resolution (HRMS) were recorded by the Service de Spectrométrie de Masse, ICSN Gif sur Yvette. Tunicamycin from *Streptomyces* sp. was purchased from Sigma-Aldrich®. All reactions were carried out under a nitrogen atmosphere, and were

monitored by thin-layer chromatography with Merck 60F-254 pre-coated silica (0.2 mm) on glass. Flash chromatography was performed with Merck Kieselgel 60 (200–500  $\mu m$ ); the solvent systems were given v/v. Spectroscopic  $^1H$ ,  $^{13}C$  and  $^{31}P$  NMR, MS and/or analytical data were obtained using chromatographically homogeneous samples.

### Benzyl (3-*N*-*tert*-butyloxycarbonyl-2',3'-*O*-isopropylidene)-uridin-5'-yl 3-(2-acetamido-4,6-*O*-(*R*)-benzylidene-3-*O*-*tert*-butyldimethylsilyl-2-deoxy- $\alpha$ -D-glucopyranosyl)-2-oxopropylphosphonate **9**

At 0 °C, DIAD (95  $\mu L$ , 0.47 mmol, 1.5 eq.) was added dropwise to a solution of **12** (200 mg, 0.32 mmol, 1 eq.), **6** (121 mg, 0.32 mmol, 1 eq.) and  $PPH_3$  (124 mg, 0.47 mmol, 1.5 eq.) in THF (5 mL). The reaction mixture was stirred at r.t. for 16 h then concentrated. Purification by flash chromatography ( $CH_2Cl_2$ /acetone, 8 : 2 to 7 : 3) afforded **9** (146 mg, 46%, white solid) as a mixture of epimers (d.r. = 2/1):  $R_f$  0.46 ( $CH_2Cl_2$ /acetone = 7 : 3);  $^1H$  NMR  $\delta$  7.50–7.34 (m, 10H,  $H_{ar}$ ), 7.28, 7.27 (2d, 1H,  $J_{H_{6u}-H_{5u}} = 8.1$  Hz,  $H_{6u}^\circ$ ,  $H_{6u}^*$ ), 6.28 (d, 1H,  $J_{NH-H_{2'}} = 8.2$  Hz, NH), 5.71, 5.66 (2d, 1H,  $J_{H_{5u}-H_{6u}} = 8.1$  Hz,  $H_{5u}^\circ$ ,  $H_{5u}^*$ ), 5.66, 5.58 (2d, 1H,  $J_{H_{1'}-H_{2'}} = 2.1$  Hz,  $H_{1'}^*$ ,  $H_{1'}^\circ$ ), 5.50 (s, 1H,  $H_7$ ), 5.18–5.08 (m, 2H,  $CH_2Ph$ ), 4.98, 4.96 (2dd, 1H,  $J_{H_{2'}-H_{3'}} = 6.4$  Hz,  $J_{H_{2'}-H_{1'}} = 2.1$  Hz,  $H_{2'}^*$ ,  $H_{2'}^\circ$ ), 4.81 (dd, 0.7H,  $J_{H_{3'}-H_{2'}} = 6.4$  Hz,  $J_{H_{3'}-H_{4'}} = 3.7$  Hz,  $H_{3'}^*$ ), 4.79–4.73 (m, 1.3H,  $H_{1'}$ ,  $H_{3'}^\circ$ ), 4.37–4.33, 4.33–4.29 (2 m, 1H,  $H_{4'}^\circ$ ,  $H_{4'}^*$ ), 4.29–4.19 (m, 2H,  $H_{2'}$ ,  $H_{5'a}$ ), 4.17–4.09 (m, 2H,  $H_{5'b}$ ,  $H_{6'eq}$ ), 3.85–3.77 (m, 1H,  $H_3$ ), 3.67–3.61 (m, 1H,  $H_{6'ax}$ ), 3.54–3.46 (m, 2H,  $H_4$ ,  $H_5$ ), 3.14, 3.08 (2dd, 1H,  $J_{H_{1a}-P} = 22.3$  Hz,  $J_{H_{1a}-H_{1b}} = 13.9$  Hz,  $H_{1a}^\circ$ ,  $H_{1a}^*$ ), 3.11, 2.99 (2dd, 1H,  $J_{H_{3a}-H_{3b}} = 16.6$  Hz,  $J_{H_{3a}-H_{1'}} = 7.0$  Hz,  $H_{3a}^\circ$ ,  $H_{3a}^*$ ), 3.07, 3.04 (2dd, 1H,  $J_{H_{1b}-P} = 22.9$  Hz,  $J_{H_{1b}-H_{1a}} = 13.9$  Hz,  $H_{1b}^\circ$ ,  $H_{1b}^*$ ), 2.75, 2.70 (2dd, 1H,  $J_{H_{3b}-H_{3a}} = 16.6$  Hz,  $J_{H_{3b}-H_{1'}} = 6.6$  Hz,  $H_{3b}^\circ$ ,  $H_{3b}^*$ ), 1.93, 1.91 (2 s, 3H,  $CH_3CO^*$ ,  $CH_3CO^\circ$ ), 1.59, 1.58 (2 s, 9H,  $CO_2tBu^*$ ,  $CO_2tBu^\circ$ ), 1.55, 1.36, 1.34 (3 s, 6H,  $CMe_2$ ), 0.83 (s, 9H,  $SiRbu$ ), 0.08, –0.01 (2 s, 4H,  $SiMe^*$ ), 0.07, –0.01 (2 s, 2H,  $SiMe^\circ$ );  $^{13}C$  NMR  $\delta$  197.8 (d,  $J_{C_2-P} = 6.5$  Hz,  $C_2$ ), 170.7, 170.7 ( $CH_3CO^\circ$ ,  $CH_3CO^*$ ), 160.4 ( $C_{4u}$ ), 148.3 ( $C_{2u}$ ), 147.6, 147.6 ( $CO_{Boc}^\circ$ ,  $CO_{Boc}^*$ ), 141.7, 141.3 ( $C_{6u}^\circ$ ,  $C_{6u}^*$ ), 137.3 ( $C_{qar}$ ), 135.3 (d,  $J_{Cq-P} = 5.0$  Hz,  $C_{qar}$ ), 129.3, 129.1, 129.0, 128.5, 128.3, 128.2, 126.4, 126.4 ( $CH_{ar}$ ), 114.7, 114.7 ( $CMe_2^*$ ,  $CMe_2^\circ$ ), 102.1, 102.1, 102.0, 101.9 ( $C_{5u}$ ,  $C_7$ ), 95.7, 95.0 ( $C_{1'}^\circ$ ,  $C_{1'}^*$ ), 87.4, 87.3 ( $CMe_3^\circ$ ,  $CMe_3^*$ ), 86.0, 85.8 (2d,  $J_{C_4'-P} = 7.0$  Hz,  $C_{4'}^\circ$ ,  $C_{4'}^*$ ), 84.5, 84.5 ( $C_{2'}^*$ ,  $C_{2'}^\circ$ ), 83.2, 83.2 ( $C_4^\circ$ ,  $C_4^*$ ), 80.5 ( $C_{3'}$ ), 70.7, 70.6 ( $C_{1'}^\circ$ ,  $C_{1'}^*$ ), 70.1, 70.1 ( $C_3^*$ ,  $C_3^\circ$ ), 69.2 ( $C_6'$ ), 69.0, 68.9 (2d,  $J_{CH_2Ph-P} = 6.5$  Hz,  $CH_2Ph^*$ ,  $CH_2Ph^\circ$ ), 65.7, 65.6 (2d,  $J_{C_5'-P} = 6.5$  Hz,  $C_{5'}^\circ$ ,  $C_{5'}^*$ ), 65.3, 65.3 ( $C_5^*$ ,  $C_5^\circ$ ), 54.1, 54.1 ( $C_2^\circ$ ,  $C_2^*$ ), 43.8, 43.7 ( $C_3^*$ ,  $C_3^\circ$ ), 42.0, 41.8 (2d,  $J_{C1-P} = 127.0$  Hz,  $C_1^*$ ,  $C_1^\circ$ ), 27.6 ( $CMe_3$ ), 27.2, 25.4 ( $CMe_2^\circ$ ), 27.1, 25.3 ( $CMe_2^*$ ), 25.8 ( $SiRbu$ ), 23.2, 23.1 ( $CH_3CO^*$ ,  $CH_3CO^\circ$ ), 18.2 ( $SiRbu$ ), –3.9, –4.7 ( $SiMe$ );  $^{31}P$  NMR  $\delta$  21.9 (s, 0.66P,  $P^*$ ), 21.7 (s, 0.33P,  $P^\circ$ ); MS (ESI):  $m/z = 1022$  [ $M + Na$ ] $^+$  100%; HRMS calcd for  $C_{48}H_{66}N_3NaO_{16}PSi^+$  1022.3848, found 1022.3858.

### Benzyl (*R*)-citronellyl 3-(2-acetamido-4,6-*O*-(*R*)-benzylidene-3-*O*-*tert*-butyldimethylsilyl-2-deoxy- $\alpha$ -D-glucopyranosyl)-2-oxopropylphosphonate **10**

**Condensation of 8 on 3.** The reaction was performed as described for **11** with a solution of **8** (446 mg, 1.37 mmol, 3 eq.) in THF (10 mL), *n*BuLi (630  $\mu L$ , 1.42 mmol, 3.1 eq.) and a solution

of **3** (220 mg, 0.46 mmol, 1 eq.) in THF (5 mL). Two successive purifications by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/acetone, 9:1 and cyclohexane/acetone, 2:1) afforded **10** (205 mg, 57%, pale yellow oil) as a mixture of epimers (d.r. = 52/48).

**Mitsunobu with 12 and (R)-citronellol.** At 0 °C, DIAD (70 μL, 0.36 mmol, 1.5 eq.) was added dropwise to a solution of **12** (150 mg, 0.24 mmol, 1 eq.), (R)-citronellol (44 μL, 0.24 mmol, 1 eq.) and PPh<sub>3</sub> (93 mg, 0.36 mmol, 1.5 eq.) in THF (4 mL). The reaction mixture was stirred at r.t. for 16 h then concentrated. Two successive purifications by flash chromatography (cyclohexane/acetone, 2:1 and CH<sub>2</sub>Cl<sub>2</sub>/acetone, 9:1) afforded **10** (107 mg, 58%, pale yellow oil) as a mixture of epimers (d.r. = 1/1): *R<sub>f</sub>* 0.39 (cyclohexane/acetone = 2:1); IR (cm<sup>-1</sup>) 1719 (CO), 1250 (PO); <sup>1</sup>H NMR δ 7.51–7.33 (m, 10H, H<sub>ar</sub>), 6.68, 6.54 (2d, 1H, *J*<sub>NH-H2'</sub> = 8.6 Hz, NH\*, NH°), 5.51, 5.50 (2 s, 1H, H<sub>7'</sub>\*, H<sub>7°</sub>), 5.19–5.02 (m, 3H, CH<sub>2</sub>Ph, H<sub>6°</sub>), 4.81 (ddd, 0.5H, *J*<sub>H1°-H3a°</sub> = 7.9 Hz, *J*<sub>H1°-H2°</sub> = 5.9 Hz, *J*<sub>H1°-H3b°</sub> = 4.9 Hz, H<sub>1°</sub>), 4.77 (ddd, 0.5H, *J*<sub>H1°-H3a\*</sub> = 8.3 Hz, *J*<sub>H1°-H2\*</sub> = 5.9 Hz, *J*<sub>H1°-H3b\*</sub> = 4.4 Hz, H<sub>1\*</sub>), 4.36–4.28 (m, 1H, H<sub>2'</sub>), 4.20–4.13 (m, 1H, H<sub>6°</sub>), 4.11–3.97 (m, 2H, H<sub>1°</sub>), 3.87–3.80 (m, 1H, H<sub>3'</sub>), 3.69–3.62 (m, 1H, H<sub>6°</sub>ax), 3.57–3.43 (m, 2H, H<sub>4'</sub>, H<sub>5'</sub>), 3.30 (dd, 0.5H, *J*<sub>H3a°-H3b°</sub> = 17.0 Hz, *J*<sub>H3a°-H1°</sub> = 7.9 Hz, H<sub>3a°</sub>), 3.22 (dd, 0.5H, *J*<sub>H3a\*-H3b\*</sub> = 17.2 Hz, *J*<sub>H3a\*-H1\*</sub> = 8.3 Hz, H<sub>3a\*</sub>), 3.18 (dd, 0.5H, *J*<sub>H1a°-P°</sub> = 23.1 Hz, *J*<sub>H1a°-H1b°</sub> = 13.5 Hz, H<sub>1a°</sub>), 3.06, 3.06 (AB from ABX, 1H, *J*<sub>AB</sub> = 13.3 Hz, *J*<sub>AX</sub> = 23.3 Hz, *J*<sub>BX</sub> = 21.6 Hz, H<sub>1a\*</sub>, H<sub>1b\*</sub>), 3.01 (dd, 0.5H, *J*<sub>H1b°-P°</sub> = 21.8 Hz, *J*<sub>H1b°-H1a°</sub> = 13.5 Hz, H<sub>1b°</sub>), 2.74 (dd, 0.5H, *J*<sub>H3b°-H3a°</sub> = 17.0 Hz, *J*<sub>H3b°-H1°</sub> = 4.9 Hz, H<sub>3b°</sub>), 2.59 (dd, 0.5H, *J*<sub>H3b\*-H3a\*</sub> = 17.2 Hz, *J*<sub>H3b\*-H1\*</sub> = 4.4 Hz, H<sub>3b\*</sub>), 2.03–1.85 (m, 2H, H<sub>5°</sub>), 1.89, 1.87 (2 s, 3H, CH<sub>3</sub>CO\*, CH<sub>3</sub>CO°), 1.74–1.62 (m, 1H, H<sub>2°</sub>), 1.67 (s, 3H, H<sub>6°</sub>), 1.59 (s, 3H, H<sub>6°</sub>), 1.59–1.38 (m, 2H, H<sub>3°</sub>, H<sub>2°</sub>), 1.35–1.25, 1.21–1.11 (2 m, 2H, H<sub>4°</sub>), 0.89, 0.87 (2d, 3H, *J*<sub>H10°-H3°</sub> = 6.5 Hz, H<sub>10°</sub>, H<sub>10°</sub>\*), 0.83, 0.82 (2 s, 9H, SitBu°, SitBu\*), 0.09, 0.01 (2 s, 3H, SiMe\*), 0.06, -0.01 (2 s, 3H, SiMe°); <sup>13</sup>C NMR δ 197.6, 197.4 (2d, *J*<sub>C2-P</sub> = 5.0 Hz, C<sub>2°</sub>, C<sub>2\*</sub>), 170.7, 170.6 (CH<sub>3</sub>CO\*, CH<sub>3</sub>CO°), 137.5, 137.4 (C<sub>qar\*</sub>, C<sub>qar°</sub>), 135.6, 135.5 (2d, *J*<sub>Cq-P</sub> = 5.5 Hz, C<sub>qar\*</sub>, C<sub>qar°</sub>), 131.6 (C<sub>7°</sub>), 129.2, 129.1, 129.1, 129.0, 128.9, 128.5, 128.3, 128.2, 126.4 (CH<sub>ar</sub>), 124.5 (C<sub>6°</sub>), 102.0 (C<sub>7°</sub>), 83.4, 83.4 (C<sub>4\*</sub>, C<sub>4°</sub>), 70.6, 70.5 (C<sub>1\*</sub>, C<sub>1°</sub>), 70.2, 70.2 (C<sub>3\*</sub>, C<sub>3°</sub>), 69.4 (C<sub>6°</sub>), 68.6, 68.6 (2d, *J*<sub>CH2Ph-P</sub> = 6.0 Hz, CH<sub>2</sub>Ph), 65.8, 65.8 (C<sub>5°</sub>, C<sub>5\*</sub>), 65.7, 65.5 (2d, *J*<sub>C1-P</sub> = 7.0 Hz, C<sub>1°</sub>, C<sub>1\*</sub>), 54.1, 54.0 (C<sub>2°</sub>, C<sub>2\*</sub>), 44.2, 44.0 (C<sub>3°</sub>, C<sub>3\*</sub>), 42.8, 42.5 (2d, *J*<sub>C1-P</sub> = 124.0 Hz, C<sub>1</sub>), 37.4, 37.3 (2d, *J*<sub>C2°-P</sub> = 6.0 Hz, C<sub>2°</sub>, C<sub>2\*</sub>), 37.1, 37.0 (C<sub>4\*</sub>, C<sub>4°</sub>), 29.2, 29.1 (C<sub>3°</sub>, C<sub>3\*</sub>), 25.9 (SitBu, C<sub>8°</sub>), 25.5 (C<sub>5°</sub>), 23.2, 23.1 (CH<sub>3</sub>CO\*, CH<sub>3</sub>CO\*), 19.4, 19.3 (C<sub>10°</sub>, C<sub>10\*</sub>), 18.3 (SitBu), 17.8 (C<sub>9°</sub>), -3.9, -4.7, -4.7 (SiMe); <sup>31</sup>P NMR δ 20.9 (s, 0.52P, P\*), 20.6 (s, 0.48P, P\*); MS (ESI): *m/z* = 772 [M + H]<sup>+</sup> 100%; HRMS calcd for C<sub>41</sub>H<sub>62</sub>NNaO<sub>5</sub>PSi<sup>+</sup> 794.3829, found 794.3831.

**Dibenzyl 3-(2-acetamido-4,6-O-(R)-benzylidene-3-O-tert-butylidimethylsilyl-2-deoxy-α-D-glucopyranosyl)-2-oxopropylphosphonate 11**

To a solution of **17** (890 mg, 3.2 mmol, 3.25 eq.) in THF (15 mL) was added dropwise *n*BuLi (1.5 mL, 3.43 mmol, 3.5 eq.) at -78 °C. After 1 h stirring at -78 °C, the mixture was added to a cold solution of **3** (475 mg, 0.99 mmol, 1 eq.) in THF (3 mL). The reaction mixture was slowly warmed to r.t. overnight and quenched with saturated aqueous NH<sub>4</sub>Cl solution. The aqueous phase was extracted with EtOAc and the combined

organic layers were dried (MgSO<sub>4</sub>) and concentrated. Purification by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/acetone, 8:2) afforded **11** (600 mg, 83%, white solid): *R<sub>f</sub>* 0.47 (CH<sub>2</sub>Cl<sub>2</sub>/acetone = 8:2); [α]<sub>D</sub><sup>20</sup> +26 (*c* 1.0, CH<sub>2</sub>Cl<sub>2</sub>); IR (cm<sup>-1</sup>) 1718 (CO), 1250 (PO); <sup>1</sup>H NMR δ 7.51–7.47 (m, 2H, H<sub>ar</sub>), 7.42–7.30 (m, 13H, H<sub>ar</sub>), 6.62 (d, 1H, *J*<sub>NH-H2'</sub> = 8.7 Hz, NH), 5.50 (s, 1H, H<sub>7'</sub>), 5.12, 5.02 (AB from ABX, 2H, *J*<sub>AB</sub> = 11.6 Hz, *J*<sub>A-P</sub> = 9.5 Hz, *J*<sub>B-P</sub> = 10.0 Hz, CH<sub>2</sub>Ph), 5.01 (d, 2H, *J*<sub>H-P</sub> = 8.4 Hz, CH<sub>2</sub>Ph), 4.78 (ddd, 1H, *J*<sub>H1°-H3a°</sub> = 8.0 Hz, *J*<sub>H1°-H2°</sub> = 5.7 Hz, *J*<sub>H1°-H3b°</sub> = 5.1 Hz, H<sub>1°</sub>), 4.31 (ddd, 1H, *J*<sub>H2°-H3°</sub> = 9.8 Hz, *J*<sub>H2°-NH</sub> = 8.7 Hz, *J*<sub>H2°-H1°</sub> = 5.7 Hz, H<sub>2°</sub>), 4.15 (dd, 1H, *J*<sub>H6°eq-H6°ax</sub> = 10.3 Hz, *J*<sub>H6°eq-H5°</sub> = 4.3 Hz, H<sub>6°eq</sub>), 3.83 (dd, 1H, *J*<sub>H3°-H2°</sub> = 9.8 Hz, *J*<sub>H3°-H4°</sub> = 8.3 Hz, H<sub>3°</sub>), 3.65 (dd, 1H, *J*<sub>H6°ax-H6°eq</sub> = 10.3 Hz, *J*<sub>H6°ax-H5°</sub> = 9.2 Hz, H<sub>6°ax</sub>), 3.51 (dd, 1H, *J*<sub>H4°-H5°</sub> = 9.6 Hz, *J*<sub>H4°-H3°</sub> = 8.3 Hz, H<sub>4°</sub>), 3.47 (ddd, 1H, *J*<sub>H5°-H4°</sub> = 9.6 Hz, *J*<sub>H5°-H6°ax</sub> = 9.2 Hz, *J*<sub>H5°-H6°eq</sub> = 4.3 Hz, H<sub>5°</sub>), 3.19 (dd, 1H, *J*<sub>H3a-H3b</sub> = 17.1 Hz, *J*<sub>H3a-H1°</sub> = 8.0 Hz, H<sub>3a</sub>), 3.10, 3.05 (AB from ABX, 2H, *J*<sub>AB</sub> = 13.5 Hz, *J*<sub>A-P</sub> = 23.2 Hz, *J*<sub>B-P</sub> = 21.9 Hz, H<sub>1a</sub>, H<sub>1b</sub>), 2.63 (dd, 1H, *J*<sub>H3b-H3a</sub> = 17.1 Hz, *J*<sub>H3b-H1°</sub> = 5.1 Hz, H<sub>3b</sub>), 1.85 (s, 3H, CH<sub>3</sub>CO), 0.83 (s, 9H, SitBu), 0.10, 0.01 (2 s, 6H, SiMe); <sup>13</sup>C NMR δ 197.4 (d, *J*<sub>C2-P</sub> = 4.7 Hz, C<sub>2</sub>), 170.7 (CH<sub>3</sub>CO), 137.4 (C<sub>qar</sub>), 135.4, 135.3 (2d, *J*<sub>Cq-P</sub> = 6.0 Hz, C<sub>qar</sub>), 129.2, 129.1, 129.0, 129.0, 128.9, 128.5, 128.2, 128.2, 126.4 (CH<sub>ar</sub>), 102.0 (C<sub>7°</sub>), 83.4 (C<sub>4°</sub>), 70.5 (C<sub>1°</sub>), 70.1 (C<sub>3°</sub>), 69.3 (C<sub>6°</sub>), 68.6, 68.5 (2d, *J*<sub>CH2Ph-P</sub> = 6.5 Hz, CH<sub>2</sub>Ph), 65.7 (C<sub>5°</sub>), 54.0 (C<sub>2°</sub>), 43.9 (C<sub>3°</sub>), 42.9 (d, *J*<sub>C1-P</sub> = 124.8 Hz, C<sub>1</sub>), 25.8 (SitBu), 23.0 (CH<sub>3</sub>CO), 18.2 (SitBu), -3.9, -4.7 (SiMe); <sup>31</sup>P NMR δ 21.4 (s); MS (ESI): *m/z* = 1469 [2M + Na]<sup>+</sup> 100%; HRMS calcd for C<sub>38</sub>H<sub>50</sub>NNaO<sub>5</sub>PSi<sup>+</sup> 746.2890, found 746.2898.

**Benzyl 3-(2-acetamido-4,6-O-(R)-benzylidene-3-O-tert-butylidimethylsilyl-2-deoxy-α-D-glucopyranosyl)-2-oxopropylphosphonate 12**

To a solution of **11** (600 mg, 0.83 mmol) in toluene (8 mL) was added DABCO (110 mg, 0.99 mmol, 1.2 eq.). The reaction mixture was then refluxed for 7 h and concentrated. The residue was taken up in methanol, acidified with DOWEX H<sup>+</sup> (50WX8-100) ion exchange resin. Methanol was removed *in vacuo* to afford **12** (475 mg, 90%, white solid): [α]<sub>D</sub><sup>20</sup> +18 (*c* 1.0, CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR (acetone-d<sub>6</sub>) δ 7.54–7.32 (m, 11H, NH, H<sub>ar</sub>), 5.62 (s, 1H, H<sub>7'</sub>), 5.18–5.08 (m, 2H, CH<sub>2</sub>Ph), 4.66 (ddd, 1H, *J*<sub>H1°-H3a°</sub> = 6.8 Hz, *J*<sub>H1°-H3b°</sub> = 6.3 Hz, *J*<sub>H1°-H2°</sub> = 5.9 Hz, H<sub>1°</sub>), 4.28 (ddd, 1H, *J*<sub>H2°-H3°</sub> = 10.0 Hz, *J*<sub>H2°-NH</sub> = 9.4 Hz, *J*<sub>H2°-H1°</sub> = 5.9 Hz, H<sub>2°</sub>), 4.09 (dd, 1H, *J*<sub>H6°eq-H6°ax</sub> = 9.7 Hz, *J*<sub>H6°eq-H5°</sub> = 4.5 Hz, H<sub>6°eq</sub>), 3.99 (dd, 1H, *J*<sub>H3°-H2°</sub> = 10.0 Hz, *J*<sub>H3°-H4°</sub> = 8.9 Hz, H<sub>3°</sub>), 3.68 (dd, 1H, *J*<sub>H6°ax-H5°</sub> = *J*<sub>H6°ax-H6°eq</sub> = 9.7 Hz, H<sub>6°ax</sub>), 3.62 (ddd, 1H, *J*<sub>H5°-H6°ax</sub> = 9.7 Hz, *J*<sub>H5°-H4°</sub> = 9.2 Hz, *J*<sub>H5°-H6°eq</sub> = 4.5 Hz, H<sub>5°</sub>), 3.52 (dd, 1H, *J*<sub>H4°-H5°</sub> = 9.2 Hz, *J*<sub>H4°-H3°</sub> = 8.9 Hz, H<sub>4°</sub>), 3.37 (dd, 1H, *J*<sub>H1a-P</sub> = 22.8 Hz, *J*<sub>H1a-H1b</sub> = 13.4 Hz, H<sub>1a</sub>), 3.35 (dd, 1H, *J*<sub>H3a-H3b</sub> = 17.1 Hz, *J*<sub>H3a-H1°</sub> = 6.8 Hz, H<sub>3a</sub>), 3.24 (dd, 1H, *J*<sub>H1b-P</sub> = 21.9 Hz, *J*<sub>H1b-H1a</sub> = 13.4 Hz, H<sub>1b</sub>), 3.00 (dd, 1H, *J*<sub>H3b-H3a</sub> = 17.1 Hz, *J*<sub>H3b-H1°</sub> = 6.3 Hz, H<sub>3b</sub>), 1.84 (s, 3H, CH<sub>3</sub>CO), 0.82 (s, 9H, SitBu), 0.08, 0.00 (2 s, 6H, SiMe); <sup>13</sup>C NMR (acetone-d<sub>6</sub>) δ 199.9 (d, *J*<sub>C2-P</sub> = 5.0 Hz, C<sub>2</sub>), 170.5 (CH<sub>3</sub>CO), 139.0 (C<sub>qar</sub>), 137.6 (d, *J*<sub>Cq-P</sub> = 6.0 Hz, C<sub>qar</sub>), 129.6, 129.4, 129.3, 129.1, 128.7, 128.7, 127.3 (CH<sub>ar</sub>), 102.5 (C<sub>7°</sub>), 84.4 (C<sub>4°</sub>), 72.0 (C<sub>1°</sub>), 71.0 (C<sub>3°</sub>), 69.7 (C<sub>6°</sub>), 68.0 (d, *J*<sub>CH2Ph-P</sub> = 5.0 Hz, CH<sub>2</sub>Ph), 65.9 (C<sub>5°</sub>), 54.6 (C<sub>2°</sub>), 43.8 (d, *J*<sub>C1-P</sub> = 124.0 Hz, C<sub>1</sub>), 43.5 (C<sub>3°</sub>), 26.3 (SitBu), 23.1 (CH<sub>3</sub>CO), 18.8 (SitBu), -3.8, -4.5 (SiMe); <sup>31</sup>P NMR (acetone-d<sub>6</sub>) δ 18.8 (s); MS (ESI): *m/z* = 632 [M - H]<sup>-</sup> 100%; HRMS calcd for C<sub>31</sub>H<sub>43</sub>NO<sub>5</sub>PSi<sup>-</sup> 632.2445, found 632.2460.

### Dibenzyl 3-(2-acetamido-2-deoxy- $\alpha$ -D-glucopyranosyl)-2-oxopropylphosphonate **13**

To a suspension of **11** (100 mg, 0.14 mmol) in water (5 mL) was added trifluoroacetic acid (5 mL) at 0 °C. The reaction mixture was stirred at r.t. for 1 h and concentrated. Purification by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 85 : 15) and lyophilization afforded **13** (70 mg, 97%, white solid): *R*<sub>f</sub> 0.48 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 85 : 15); [ $\alpha$ ]<sub>D</sub><sup>20</sup> +38 (*c* 1.0, CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  7.67 (d, 1H, *J*<sub>NH-H2'</sub> = 7.8 Hz, NH), 7.41–7.31 (m, 10H, H<sub>ar</sub>), 5.07–4.98 (m, 5H, OH<sub>4'</sub>, CH<sub>2</sub>Ph), 4.88 (d, 1H, *J*<sub>OH3'-H3'</sub> = 5.3 Hz, OH<sub>3'</sub>), 4.44 (dd, 1H, *J*<sub>OH6'-H6'b</sub> = 6.0 Hz, *J*<sub>OH6'-H6'a</sub> = 5.8 Hz, OH<sub>6'</sub>), 4.40 (ddd, 1H, *J*<sub>H1'-H3a</sub> = 9.1 Hz, *J*<sub>H1'-H2'</sub> = 5.6 Hz, *J*<sub>H1'-H3b</sub> = 4.6 Hz, H<sub>1'</sub>), 3.71 (ddd, 1H, *J*<sub>H2'-H3'</sub> = 10.0 Hz, *J*<sub>H2'-NH</sub> = 7.8 Hz, *J*<sub>H2'-H1'</sub> = 5.6 Hz, H<sub>2'</sub>), 3.56 (ddd, 1H, *J*<sub>H6'a-H6'b</sub> = 11.6 Hz, *J*<sub>H6'a-OH6'</sub> = 5.8 Hz, *J*<sub>H6'a-H5'</sub> = 2.5 Hz, H<sub>6'a</sub>), 3.50 (dd, 1H, *J*<sub>H1a-P</sub> = 21.8 Hz, *J*<sub>H1a-H1b</sub> = 14.4 Hz, H<sub>1a</sub>), 3.47 (dd, 1H, (ddd, 1H, *J*<sub>H6'b-H6'a</sub> = 11.6 Hz, *J*<sub>H6'b-OH6'</sub> = 6.0 Hz, *J*<sub>H6'b-H5'</sub> = 5.7 Hz, H<sub>6'b</sub>), 3.41 (dd, 1H, *J*<sub>H1b-P</sub> = 21.5 Hz, *J*<sub>H1b-H1a</sub> = 14.4 Hz, H<sub>1b</sub>), 3.40 (ddd, 1H, *J*<sub>H3'-H2'</sub> = 10.0 Hz, *J*<sub>H3'-H4'</sub> = 8.0 Hz, *J*<sub>H3'-OH3'</sub> = 5.3 Hz, H<sub>3'</sub>), 3.35 (ddd, 1H, *J*<sub>H5'-H4'</sub> = 8.8 Hz, *J*<sub>H5'-H6'b</sub> = 5.7 Hz, *J*<sub>H5'-H6'a</sub> = 2.5 Hz, H<sub>5'</sub>), 3.15 (ddd, 1H, *J*<sub>H4'-H5'</sub> = 8.8 Hz, *J*<sub>H4'-H3'</sub> = 8.0 Hz, *J*<sub>H4'-OH4'</sub> = 5.3 Hz, H<sub>4'</sub>), 2.90 (dd, 1H, *J*<sub>H3a-H3b</sub> = 16.1 Hz, *J*<sub>H3a-H1'</sub> = 9.1 Hz, H<sub>3a</sub>), 2.66 (dd, 1H, *J*<sub>H3b-H3a</sub> = 16.1 Hz, *J*<sub>H3b-H1'</sub> = 4.6 Hz, H<sub>3b</sub>), 1.77 (s, 3H, CH<sub>3</sub>CO); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  200.5 (d, *J*<sub>C2-P</sub> = 6.0 Hz, C<sub>2</sub>), 169.3 (CH<sub>3</sub>CO), 136.2 (d, *J*<sub>Cq-P</sub> = 6.0 Hz, C<sub>qar</sub>), 128.4, 128.2, 127.7 (CH<sub>ar</sub>), 74.9 (C<sub>5'</sub>), 70.6 (C<sub>4'</sub>), 70.3 (C<sub>3'</sub>), 69.3 (C<sub>1'</sub>), 67.0, 66.9 (2d, *J*<sub>CH2Ph-P</sub> = 6.0 Hz, CH<sub>2</sub>Ph), 60.9 (C<sub>6'</sub>), 52.6 (C<sub>2'</sub>), 41.8 (d, *J*<sub>Cl-P</sub> = 126.0 Hz, C<sub>1</sub>), 41.4 (C<sub>3</sub>), 22.6 (CH<sub>3</sub>CO); <sup>31</sup>P NMR (DMSO-*d*<sub>6</sub>)  $\delta$  21.8 (s); MS (ESI): *m/z* = 544 [M + Na]<sup>+</sup> 100%; HRMS calcd for C<sub>25</sub>H<sub>32</sub>NNaO<sub>9</sub>P<sup>+</sup> 544.1712, found 544.1707.

### Benzyl 3-(2-acetamido-2-deoxy- $\alpha$ -D-glucopyranosyl)-2-oxopropylphosphonate **14**

To a solution of **13** (34 mg, 65  $\mu$ mol) in toluene (1 mL) was added DABCO (9 mg, 78  $\mu$ mol, 1.2 eq.). The reaction mixture was then refluxed for 4 h and concentrated. The residue was taken up in water, acidified with DOWEX H<sup>+</sup> (50WX8-100) ion exchange resin. Water was removed *in vacuo* to afford **14** (25 mg, 88%, white solid): <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  7.73 (d, 1H, *J*<sub>NH-H2'</sub> = 8.0 Hz, NH), 7.42–7.28 (m, 5H, H<sub>ar</sub>), 4.94 (d, 2H, *J*<sub>CH2Ph-P</sub> = 7.5 Hz, CH<sub>2</sub>Ph), 4.66 (ddd, 1H, *J*<sub>H1'-H3a</sub> = 8.3 Hz, *J*<sub>H1'-H3b</sub> = *J*<sub>H1'-H2'</sub> = 5.2 Hz, H<sub>1'</sub>), 3.72 (ddd, 1H, *J*<sub>H2'-H3'</sub> = 10.3 Hz, *J*<sub>H2'-NH</sub> = 8.0 Hz, *J*<sub>H2'-H1'</sub> = 5.2 Hz, H<sub>2'</sub>), 3.56 (dd, 1H, *J*<sub>H6'a-H6'b</sub> = 11.5 Hz, *J*<sub>H6'a-H5'</sub> = 2.2 Hz, H<sub>6'a</sub>), 3.46 (dd, 1H, *J*<sub>H6'b-H6'a</sub> = 11.5 Hz, *J*<sub>H6'b-H5'</sub> = 5.7 Hz, H<sub>6'b</sub>), 3.41 (dd, 1H, *J*<sub>H3'-H2'</sub> = 10.3 Hz, *J*<sub>H3'-H4'</sub> = 8.1 Hz, H<sub>3'</sub>), 3.35 (ddd, 1H, *J*<sub>H5'-H4'</sub> = 8.8 Hz, *J*<sub>H5'-H6'b</sub> = 5.7 Hz, *J*<sub>H5'-H6'a</sub> = 2.2 Hz, H<sub>5'</sub>), 3.17, 3.12 (AB from ABX, 2H, *J*<sub>AB</sub> = 13.3 Hz, *J*<sub>A-P</sub> = 21.7 Hz, *J*<sub>B-P</sub> = 22.0 Hz, H<sub>1</sub>), 3.12 (dd, 1H, *J*<sub>H4'-H5'</sub> = 8.8 Hz, *J*<sub>H4'-H3'</sub> = 8.1 Hz, H<sub>4'</sub>), 2.88 (dd, 1H, *J*<sub>H3a-H3b</sub> = 16.2 Hz, *J*<sub>H3a-H1'</sub> = 8.3 Hz, H<sub>3a</sub>), 2.75 (dd, 1H, *J*<sub>H3b-H3a</sub> = 16.2 Hz, *J*<sub>H3b-H1'</sub> = 5.2 Hz, H<sub>3b</sub>), 1.76 (s, 3H, CH<sub>3</sub>CO); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  201.5 (d, *J*<sub>C2-P</sub> = 5.5 Hz, C<sub>2</sub>), 169.3 (CH<sub>3</sub>CO), 137.2 (d, *J*<sub>Cq-P</sub> = 7.0 Hz, C<sub>qar</sub>), 128.3, 127.8, 127.4 (CH<sub>ar</sub>), 74.8 (C<sub>5'</sub>), 70.8 (C<sub>4'</sub>), 70.4 (C<sub>3'</sub>), 69.5 (C<sub>1'</sub>), 66.1 (d, *J*<sub>CH2Ph-P</sub> = 5.0 Hz, CH<sub>2</sub>Ph), 61.0 (C<sub>6'</sub>), 52.7 (C<sub>2'</sub>), 43.9 (d, *J*<sub>Cl-P</sub> = 120.0 Hz, C<sub>1</sub>), 41.1 (C<sub>3</sub>), 22.6 (CH<sub>3</sub>CO); <sup>31</sup>P NMR (DMSO-*d*<sub>6</sub>)  $\delta$  16.2 (s); MS (ESI): *m/z* = 430 [M – H]<sup>–</sup> 100%.

### Benzyl uridin-5'-yl 3-(2-acetamido-2-deoxy- $\alpha$ -D-glucopyranosyl)-2-oxopropylphosphonate **15**

To a suspension of **9** (146 mg, 0.15 mmol) in water (5 mL) was added trifluoroacetic acid (5 mL) at 0 °C. The reaction mixture was stirred at r.t. for 2 h and concentrated. Purification by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 3 : 1) and lyophilization afforded **15** (91 mg, 94%, white solid) as a mixture of epimers (d.r. = 2/1): *R*<sub>f</sub> 0.10 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 3 : 1); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  11.33 (s, 1H, H<sub>3u</sub>), 7.70 (d, 1H, *J*<sub>NH-H2'</sub> = 7.7 Hz, NH), 7.62, 7.58 (2d, 1H, *J*<sub>H6u-H5u</sub> = 8.0 Hz, H<sub>6u</sub><sup>o</sup>, H<sub>6u</sub><sup>\*</sup>), 7.42–7.31 (m, 5H, H<sub>ar</sub>), 5.78 (d, 1H, *J*<sub>H1''-H2''</sub> = 5.5 Hz, H<sub>1''</sub>), 5.61, 5.57 (2d, 1H, *J*<sub>H5u-H6u</sub> = 8.0 Hz, H<sub>5u</sub><sup>o</sup>, H<sub>5u</sub><sup>\*</sup>), 5.48, 5.47 (2d, 1H, *J*<sub>OH2''-H2''</sub> = 5.5 Hz, OH<sub>2''</sub><sup>o</sup>, OH<sub>2''</sub><sup>\*</sup>), 5.29 (d, 1H, *J*<sub>OH3''-H3''</sub> = 4.8 Hz, OH<sub>3''</sub>), 5.10–5.01 (m, 3H, CH<sub>2</sub>Ph, OH<sub>4'</sub>), 4.91 (d, 1H, *J*<sub>OH3''-H3''</sub> = 5.1 Hz, OH<sub>3''</sub>), 4.47–4.40 (m, 1H, OH<sub>6'</sub>), 4.40 (ddd, 1H, *J*<sub>H1'-H3a</sub> = 9.0 Hz, *J*<sub>H1'-H2'</sub> = 5.5 Hz, *J*<sub>H1'-H3b</sub> = 4.2 Hz, H<sub>1'</sub>), 4.21–4.10 (m, 2H, H<sub>5''</sub>), 4.04 (ddd, 1H, *J*<sub>H2''-H1''</sub> = 5.5 Hz, *J*<sub>H2''-OH2''</sub> = 5.5 Hz, *J*<sub>H2''-H3''</sub> = 5.5 Hz, H<sub>2''</sub>), 4.02–3.91 (m, 2H, H<sub>3''</sub>, H<sub>4''</sub>), 3.71 (ddd, 1H, *J*<sub>H2'-H3'</sub> = 9.9 Hz, *J*<sub>H2'-NH</sub> = 7.7 Hz, *J*<sub>H2'-H1'</sub> = 5.5 Hz, H<sub>2'</sub>), 3.60–3.28 (m, 6H, H<sub>1</sub>, H<sub>3'</sub>, H<sub>5'</sub>, H<sub>6'</sub>), 3.14 (ddd, 1H, *J*<sub>H4'-H3'</sub> = 8.4 Hz, *J*<sub>H4'-H5'</sub> = 8.4 Hz, *J*<sub>H4'-OH4'</sub> = 5.4 Hz, H<sub>4'</sub>), 2.89 (dd, 1H, *J*<sub>H3a-H3b</sub> = 16.0 Hz, *J*<sub>H3a-H1'</sub> = 9.0 Hz, H<sub>3a</sub>), 2.65, 2.65 (2dd, 1H, *J*<sub>H3b-H3a</sub> = 16.0 Hz, *J*<sub>H3b-H1'</sub> = 4.2 Hz, H<sub>3b</sub><sup>o</sup>, H<sub>3b</sub><sup>\*</sup>), 1.78, 1.78 (2s, 3H, CH<sub>3</sub>CO<sup>o</sup>, CH<sub>3</sub>CO<sup>\*</sup>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  200.7, 200.7 (2d, *J*<sub>C2-P</sub> = 6.0 Hz, C<sub>2</sub>), 169.4 (CH<sub>3</sub>CO), 163.0 (C<sub>4u</sub>), 150.6 (C<sub>2u</sub>), 140.7, 140.6 (C<sub>6u</sub><sup>o</sup>, C<sub>6u</sub><sup>\*</sup>), 136.2 (d, *J*<sub>Cq-P</sub> = 6.5 Hz, C<sub>qar</sub>), 128.5, 128.3, 127.7 (CH<sub>ar</sub>), 102.0 (C<sub>5u</sub>), 88.2, 88.2 (C<sub>1''</sub><sup>o</sup>, C<sub>1''</sub><sup>\*</sup>), 82.1 (d, *J*<sub>C4''-P</sub> = 6.0 Hz, C<sub>4''</sub>), 74.9 (C<sub>5'</sub>), 72.6 (C<sub>2''</sub>), 70.6 (C<sub>4'</sub>), 70.2 (C<sub>3'</sub>), 69.5 (C<sub>3''</sub>), 69.3 (C<sub>1'</sub>), 67.1 (d, *J*<sub>CH2Ph-P</sub> = 5.5 Hz, CH<sub>2</sub>Ph), 65.5, 65.3 (2d, *J*<sub>C5''-P</sub> = 5.5 Hz, C<sub>5''</sub><sup>o</sup>, C<sub>5''</sub><sup>\*</sup>), 60.9 (C<sub>6'</sub>), 52.7 (C<sub>2'</sub>), 41.5 (C<sub>3</sub>), 41.4, 41.4 (2d, *J*<sub>Cl-P</sub> = 127.0 Hz, C<sub>1</sub><sup>o</sup>, C<sub>1</sub><sup>\*</sup>), 22.6 (CH<sub>3</sub>CO); <sup>31</sup>P NMR (DMSO-*d*<sub>6</sub>)  $\delta$  22.2 (s, 0.66P, P<sup>\*</sup>), 22.0 (s, 0.33P, P<sup>o</sup>); MS (ESI): *m/z* = 1337 [2M + Na]<sup>+</sup> 100%; HRMS calcd for C<sub>27</sub>H<sub>36</sub>N<sub>3</sub>NaO<sub>14</sub>P<sup>+</sup> 680.1833, found 680.1832.

### Uridin-5'-yl 3-(2-acetamido-2-deoxy- $\alpha$ -D-glucopyranosyl)-2-oxopropylphosphonate **16**

A suspension of **15** (22 mg, 33  $\mu$ mol) and Pd/C 10% (22 mg) in methanol (2 mL) was purged from air and filled with H<sub>2</sub>. The reaction was stirred at r.t. for 3 h then diluted with methanol, filtered through a Celite pad and concentrated. Lyophilization afforded **16** (16 mg, 84%, white solid): [ $\alpha$ ]<sub>D</sub><sup>20</sup> +34 (*c* 1.0, H<sub>2</sub>O); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  11.27 (s, 1H, H<sub>3u</sub>), 8.22 (d, 1H, *J*<sub>NH-H2'</sub> = 6.0 Hz, NH), 7.85 (d, 1H, *J*<sub>H6u-H5u</sub> = 8.0 Hz, H<sub>6u</sub>), 5.80 (d, 1H, *J*<sub>H1''-H2''</sub> = 5.5 Hz, H<sub>1''</sub>), 5.62 (2d, 1H, *J*<sub>H5u-H6u</sub> = 8.0 Hz, H<sub>5u</sub>), 4.40–4.32 (m, 1H, H<sub>1'</sub>), 4.11–4.05 (m, 1H, H<sub>2''</sub>), 4.05–3.99 (m, 1H, H<sub>3''</sub>), 3.97–3.91 (m, 1H, H<sub>4''</sub>), 3.91–3.83 (m, 2H, H<sub>5''</sub>), 3.77–3.70 (m, 1H, H<sub>2'</sub>), 3.61–3.54 (m, 1H, H<sub>6'a</sub>), 3.47–3.25 (m, 3H, H<sub>3'</sub>, H<sub>5'</sub>, H<sub>6'b</sub>), 3.09–2.96 (m, 2H, H<sub>4'</sub>, H<sub>3a</sub>), 2.90 (dd, 1H, *J*<sub>H1a-P</sub> = 21.5 Hz, *J*<sub>H1a-H1b</sub> = 11.5 Hz, H<sub>1a</sub>), 2.81–2.67 (m, 2H, H<sub>1b</sub>, H<sub>3b</sub>), 1.76 (s, 3H, CH<sub>3</sub>CO); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  203.7 (d, *J*<sub>C2-P</sub> = 5.0 Hz, C<sub>2</sub>), 169.6 (CH<sub>3</sub>CO), 163.1 (C<sub>4u</sub>), 150.8 (C<sub>2u</sub>), 140.9 (C<sub>6u</sub>), 101.9 (C<sub>5u</sub>), 87.4 (C<sub>1''</sub>), 83.5 (d, *J*<sub>C4''-P</sub> = 6.5 Hz, C<sub>4''</sub>), 74.8 (C<sub>5'</sub>), 73.2 (C<sub>2''</sub>), 71.3 (C<sub>4'</sub>), 70.7 (C<sub>3'</sub>), 70.3 (C<sub>3''</sub>), 69.5 (C<sub>1'</sub>), 63.5 (d, *J*<sub>C5''-P</sub> = 5.0 Hz, C<sub>5''</sub>), 61.4 (C<sub>6'</sub>), 52.7 (C<sub>2'</sub>), 45.9 (d, *J*<sub>Cl-P</sub> = 107.0 Hz, C<sub>1</sub>), 41.3 (C<sub>3</sub>), 22.7 (CH<sub>3</sub>CO); <sup>31</sup>P NMR (DMSO-*d*<sub>6</sub>)  $\delta$  8.9 (s); MS (ESI): *m/z* = 566 [M – H]<sup>–</sup> 100%; HRMS calcd for C<sub>20</sub>H<sub>29</sub>N<sub>3</sub>O<sub>14</sub>P<sup>–</sup> 566.1387, found 566.1384.

**Benzyl 5-(3-*N*-*tert*-butyloxycarbonyl-uracil-1-yl)pentyl 3-(2-acetamido-4,6-*O*-(*R*)-benzylidene-3-*O*-*tert*-butyldimethylsilyl-2-deoxy- $\alpha$ -D-glucopyranosyl)-2-oxopropylphosphonate 18**

At 0 °C, DIAD (47  $\mu$ L, 0.24 mmol, 1.5 eq.) was added dropwise to a solution of **12** (100 mg, 0.16 mmol, 1 eq.), **17** (47 mg, 0.16 mmol, 1 eq.) and PPh<sub>3</sub> (62 mg, 0.24 mmol, 1.5 eq.) in THF (2.5 mL). The reaction mixture was stirred at r.t. for 16 h then concentrated. Purification by preparative chromatography (CHCl<sub>3</sub>/MeOH, 95 : 5) afforded **18** (39 mg, 27%, white solid) as a mixture of epimers (d.r. = 65/35): *R*<sub>f</sub> 0.35 (cyclohexane/acetone = 1 : 1); <sup>1</sup>H NMR  $\delta$  7.49–7.32 (m, 10H, H<sub>ar</sub>), 7.15, 6.99 (2d, 1H, *J*<sub>H6u-H5u</sub> = 8.0 Hz, H<sub>6u</sub>\*, H<sub>6u</sub>°), 6.57 (2d, 1H, *J*<sub>NH-H2'</sub> = 8.5 Hz, NH\*, NH°), 5.69, 5.64 (2d, 1H, *J*<sub>H5u-H6u</sub> = 8.0 Hz, H<sub>5u</sub>\*, H<sub>5u</sub>°), 5.50, 5.49 (s, 1H, H<sub>7'</sub>), 5.16–5.02 (m, 2H, CH<sub>2</sub>Ph), 4.79 (ddd, 0.35H, *J*<sub>H1°-H3a°</sub> = 7.6 Hz, *J*<sub>H1°-H3b°</sub> = 6.2 Hz, *J*<sub>H1°-H2°</sub> = 4.8 Hz, H<sub>1°</sub>°), 4.76 (ddd, 0.65H, *J*<sub>H1°-H3a\*</sub> = 7.3 Hz, *J*<sub>H1°-H2\*</sub> = 5.7 Hz, *J*<sub>H1°-H3b\*</sub> = 5.4 Hz, H<sub>1°</sub>\*), 4.32–4.24 (m, 1H, H<sub>2'</sub>), 4.17–4.12 (m, 1H, H<sub>6'eq</sub>), 4.08–3.90 (m, 2H, H<sub>1''</sub>), 3.85–3.78 (m, 1H, H<sub>3'</sub>), 3.73–3.61 (m, 3H, H<sub>5''</sub>, H<sub>6'ax</sub>), 3.54–3.45 (m, 2H, H<sub>4'</sub>, H<sub>5'</sub>), 3.17 (dd, 0.35H, *J*<sub>H3a°-H3b°</sub> = 16.8 Hz, *J*<sub>H3a°-H1°</sub> = 7.6 Hz, H<sub>3a°</sub>°), 3.17 (dd, 0.35H, *J*<sub>H1a°-P°</sub> = 22.6 Hz, *J*<sub>H1a°-H1b°</sub> = 14.6 Hz, H<sub>1a°</sub>°), 3.14 (dd, 0.65H, *J*<sub>H3a\*-H3b\*</sub> = 17.2 Hz, *J*<sub>H3a\*-H1\*</sub> = 7.3 Hz, H<sub>3a\*</sub>\*), 3.07 (dd, 0.65H, *J*<sub>H1b°-P°</sub> = 21.7 Hz, *J*<sub>H1b°-H1a°</sub> = 14.6 Hz, H<sub>1b°</sub>°), 3.06, 3.02 (AB from ABX, 1.3H, *J*<sub>AB</sub> = 13.5 Hz, *J*<sub>AX</sub> = 23.0 Hz, *J*<sub>BX</sub> = 22.3 Hz, H<sub>1a\*</sub>, H<sub>1b\*</sub>), 2.81 (dd, 0.35H, *J*<sub>H3b°-H3a°</sub> = 16.8 Hz, *J*<sub>H3b°-H1°</sub> = 6.2 Hz, H<sub>3b°</sub>°), 2.66 (dd, 0.65H, *J*<sub>H3b\*-H3a\*</sub> = 17.2 Hz, *J*<sub>H3b\*-H1\*</sub> = 5.4 Hz, H<sub>3b\*</sub>\*), 1.89 (2 s, 3H, CH<sub>3</sub>CO\*, CH<sub>3</sub>CO°), 1.72–1.58 (m, 4H, H<sub>2''</sub>, H<sub>4''</sub>), 1.59 (s, 9H, CO<sub>2</sub>tBu), 1.41–1.30 (m, 2H, H<sub>3''</sub>), 0.82 (s, 9H, Si*t*Bu), 0.08, 0.00 (2 s, 1.3H, SiMe\*), 0.06, –0.01 (2 s, 0.7H, SiMe°); <sup>13</sup>C NMR  $\delta$  197.9, 197.8 (2d, *J*<sub>C2-P</sub> = 5.5 Hz, C<sub>2</sub>°, C<sub>2</sub>\*), 170.7, 170.6 (CH<sub>3</sub>CO°, CH<sub>3</sub>CO\*), 160.9, 160.9 (C<sub>4u</sub>\*, C<sub>4u</sub>°), 149.1 (C<sub>2u</sub>), 148.0 (CO<sub>Boc</sub>), 143.9, 143.7 (C<sub>6u</sub>\*, C<sub>6u</sub>°), 137.4 (C<sub>qar</sub>), 135.5 (d, *J*<sub>Cq-P</sub> = 5.0 Hz, C<sub>qar</sub>), 129.2, 129.1, 129.0, 128.9, 128.5, 128.2, 126.4, 126.3 (CH<sub>ar</sub>), 102.0, 101.9, 101.9 (C<sub>5u</sub>°, C<sub>7</sub>°), 86.9, 86.9 (CMe<sub>3</sub>°, CMe<sub>3</sub>\*), 83.3 (C<sub>4'</sub>), 70.7, 70.5 (C<sub>1'</sub>°, C<sub>1'</sub>\*), 70.2 (C<sub>3'</sub>), 69.3 (C<sub>6'</sub>), 68.7 (d, *J*<sub>CH2Ph-P</sub> = 6.5 Hz, CH<sub>2</sub>Ph), 66.4, 66.2 (2d, *J*<sub>C1'-P</sub> = 7.0 Hz, C<sub>1'</sub>°, C<sub>1'</sub>\*), 65.6, 65.5 (C<sub>5'</sub>°, C<sub>5'</sub>\*), 54.1, 54.0 (C<sub>2'</sub>°, C<sub>2'</sub>\*), 49.2, 49.1 (C<sub>5'</sub>°, C<sub>5'</sub>\*), 43.8, 43.3 (C<sub>3'</sub>°, C<sub>3'</sub>\*), 42.5, 42.4 (2d, *J*<sub>C1-P</sub> = 125.0 Hz, C<sub>1</sub>°, C<sub>1</sub>\*), 29.7, 29.6 (2d, *J*<sub>C2'-P</sub> = 6.5 Hz, C<sub>2'</sub>°, C<sub>2'</sub>\*), 28.4, 28.3 (C<sub>4''</sub>°, C<sub>4''</sub>\*), 27.6 (CMe<sub>3</sub>), 25.8 (Si*t*Bu), 23.1 (CH<sub>3</sub>CO), 22.5, 22.5 (C<sub>3''</sub>°, C<sub>3''</sub>\*), 18.2 (Si*t*Bu), –3.9, –4.7 (SiMe); <sup>31</sup>P NMR  $\delta$  21.1 (s, 0.35P, P°), 20.9 (s, 0.65P, P\*); MS (ESI): *m/z* = 936 [M + Na]<sup>+</sup> 100%; HRMS calcd for C<sub>45</sub>H<sub>63</sub>N<sub>3</sub>O<sub>13</sub>PSi<sup>+</sup> 912.3868, found 912.3856.

**Benzyl hexadecan-1-yl 3-(2-acetamido-4,6-*O*-(*R*)-benzylidene-3-*O*-*tert*-butyldimethylsilyl-2-deoxy- $\alpha$ -D-glucopyranosyl)-2-oxopropylphosphonate 19**

At 0 °C, DIAD (54  $\mu$ L, 0.27 mmol, 1.5 eq.) was added dropwise to a solution of **12** (114 mg, 0.18 mmol, 1 eq.), hexadecan-1-ol (44 mg, 0.18 mmol, 1 eq.) and PPh<sub>3</sub> (71 mg, 0.27 mmol, 1.5 eq.) in THF (3 mL). The reaction mixture was stirred at r.t. for 16 h then concentrated. Two successive purifications by flash chromatography (cyclohexane/acetone, 2 : 1 and CH<sub>2</sub>Cl<sub>2</sub>/acetone, 9 : 1) afforded **19** (66 mg, 42%, colorless oil) as a mixture of epimers (d.r. = 55/45): *R*<sub>f</sub> 0.35, 0.25 (CH<sub>2</sub>Cl<sub>2</sub>/acetone = 9 : 1); <sup>1</sup>H NMR  $\delta$  7.52–7.31 (m, 10H, H<sub>ar</sub>), 6.68, 6.53 (2d, 1H, *J*<sub>NH-H2'</sub> = 8.6 Hz, NH\*,

NH°), 5.51, 5.50 (2 s, 1H, H<sub>7'</sub>\*, H<sub>7'</sub>°), 5.19–5.02 (m, 2H, CH<sub>2</sub>Ph), 4.81 (ddd, 0.45H, *J*<sub>H1'-H3a</sub> = 7.6 Hz, *J*<sub>H1'-H2'</sub> = 5.7 Hz, *J*<sub>H1'-H3b</sub> = 5.0 Hz, H<sub>1'</sub>°), 4.77 (ddd, 0.55H, *J*<sub>H1'-H3a</sub> = 8.3 Hz, *J*<sub>H1'-H2'</sub> = 5.5 Hz, *J*<sub>H1'-H3b</sub> = 4.4 Hz, H<sub>1'</sub>\*), 4.36–4.28 (m, 1H, H<sub>2'</sub>), 4.20–4.13 (m, 1H, H<sub>6'eq</sub>), 4.09–3.94 (m, 2H, H<sub>1''</sub>), 3.84 (dd, 1H, *J* = 9.6 Hz, *J* = 8.1 Hz, H<sub>3'</sub>), 3.65 (dd, 1H, *J*<sub>H6'ax-H5'</sub> = *J*<sub>H6'ax-H6'eq</sub> = 9.6 Hz, H<sub>6'ax</sub>), 3.56–3.43 (m, 2H, H<sub>4'</sub>, H<sub>5'</sub>), 3.29 (dd, 0.45H, *J*<sub>H3a-H3b</sub> = 17.0 Hz, *J*<sub>H3a-H1'</sub> = 7.6 Hz, H<sub>3a</sub>°), 3.22 (dd, 0.55H, *J*<sub>H3a-H3b</sub> = 17.2 Hz, *J*<sub>H3a-H1'</sub> = 8.3 Hz, H<sub>3a</sub>\*), 3.18 (dd, 0.45H, *J*<sub>H1a-P</sub> = 23.0 Hz, *J*<sub>H1a-H1b</sub> = 13.4 Hz, H<sub>1a</sub>°), 3.06, 3.04 (AB from ABX, 1.1H, *J*<sub>AB</sub> = 12.7 Hz, *J*<sub>A-P</sub> = 22.3 Hz, *J*<sub>B-P</sub> = 22.7 Hz, H<sub>1a\*</sub>, H<sub>1b\*</sub>), 3.01 (dd, 0.45H, *J*<sub>H1b-P</sub> = 22.0 Hz, *J*<sub>H1b-H1a</sub> = 13.4 Hz, H<sub>1b</sub>°), 2.75 (dd, 0.45H, *J*<sub>H3b-H3a</sub> = 17.0 Hz, *J*<sub>H3b-H1'</sub> = 5.0 Hz, H<sub>3b</sub>°), 2.59 (dd, 0.55H, *J*<sub>H3b-H3a</sub> = 17.2 Hz, *J*<sub>H3b-H1'</sub> = 4.4 Hz, H<sub>3b</sub>\*), 1.89, 1.87 (2 s, 3H, CH<sub>3</sub>CO\*, CH<sub>3</sub>CO°), 1.69–1.57 (m, 2H, H<sub>2''</sub>), 1.35–1.22 (m, 26H, H<sub>3''</sub> to H<sub>15''</sub>), 0.88 (t, 3H, *J*<sub>H16''-H15''</sub> = 6.7 Hz, H<sub>16''</sub>), 0.83, 0.82 (2 s, 9H, Si*t*Bu\*, Si*t*Bu°), 0.09, 0.01 (2 s, 3.3H, SiMe\*), 0.06, –0.01 (2 s, 2.7H, SiMe°); <sup>13</sup>C NMR  $\delta$  197.7, 197.4 (2d, *J*<sub>C2-P</sub> = 5.0 Hz, C<sub>2</sub>°, C<sub>2</sub>\*), 170.7, 170.6 (CH<sub>3</sub>CO\*, CH<sub>3</sub>CO°), 137.4, 137.4 (C<sub>qar</sub>\*, C<sub>qar</sub>°), 135.6, 135.5 (2d, *J*<sub>Cq-P</sub> = 6.0 Hz, C<sub>qar</sub>\*, C<sub>qar</sub>°), 129.1, 129.1, 129.0, 128.9, 128.9, 128.5, 128.2, 128.1, 126.4 (CH<sub>ar</sub>), 102.0 (C<sub>7</sub>), 83.4, 83.4 (C<sub>4'</sub>\*, C<sub>4'</sub>°), 70.6, 70.5 (C<sub>1'</sub>°, C<sub>1'</sub>\*), 70.2 (C<sub>3'</sub>), 69.4 (C<sub>6'</sub>), 68.6 (d, *J*<sub>CH2Ph-P</sub> = 6.5 Hz, CH<sub>2</sub>Ph), 67.3, 67.1 (2d, *J*<sub>C1'-P</sub> = 6.5 Hz, C<sub>1'</sub>°, C<sub>1'</sub>\*), 65.7, 65.7 (C<sub>5'</sub>°, C<sub>5'</sub>\*), 54.0, 53.9 (C<sub>2'</sub>°, C<sub>2'</sub>\*), 44.1, 43.9 (C<sub>3'</sub>°, C<sub>3'</sub>\*), 42.7, 42.5 (2d, *J*<sub>C1-P</sub> = 124.0 Hz, C<sub>1</sub>°, C<sub>1</sub>\*), 32.0, 29.8, 29.8, 29.7, 29.6, 29.5, 29.2, 25.6, 25.5, 23.1, 23.1 (C<sub>3''</sub> to C<sub>15''</sub>), 30.5, 30.3 (2d, *J*<sub>C2'-P</sub> = 6.0 Hz, C<sub>2'</sub>°, C<sub>2'</sub>\*), 25.8 (Si*t*Bu), 22.8 (CH<sub>3</sub>CO), 18.2 (Si*t*Bu), 14.2 (C<sub>16''</sub>), –3.9, –4.7, –4.7 (SiMe); <sup>31</sup>P NMR  $\delta$  20.9 (s, 0.55P, P\*), 20.6 (s, 0.45P, P°); MS (ESI): *m/z* = 880 [M + Na]<sup>+</sup> 100%; HRMS calcd for C<sub>47</sub>H<sub>75</sub>NO<sub>9</sub>PSi<sup>+</sup> 856.4949, found 856.4951.

**Benzyl 5-uracil-1-yl-pentyl 3-(2-acetamido-2-deoxy- $\alpha$ -D-glucopyranosyl)-2-oxopropylphosphonate 20**

To a suspension of **18** (37 mg, 40  $\mu$ mol) in water (2 mL) was added trifluoroacetic acid (2 mL) at 0 °C. The reaction mixture was stirred at r.t. for 3 h and concentrated. Purification by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 8 : 2) afforded **20** (23 mg, 93%, white solid) as a mixture of epimers: *R*<sub>f</sub> 0.50 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 8 : 2); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  11.19 (s, 1H, H<sub>3u</sub>), 7.72–7.67 (m, 1H, NH), 7.65, 7.62 (2d, 1H, *J*<sub>H6u-H5u</sub> = 7.8 Hz, H<sub>6u</sub>°, H<sub>6u</sub>\*), 7.43–7.30 (m, 5H, H<sub>ar</sub>), 5.54, 5.53 (2d, 1H, *J*<sub>H6u-H5u</sub> = 7.8 Hz, H<sub>5u</sub>°, H<sub>5u</sub>\*), 5.10–4.98 (m, 2H, CH<sub>2</sub>Ph), 4.39 (ddd, 1H, *J*<sub>H1'-H3a</sub> = 9.2 Hz, *J*<sub>H1'-H2'</sub> = 5.3 Hz, *J*<sub>H1'-H3b</sub> = 4.5 Hz, H<sub>1'</sub>°), 3.99–3.88 (m, 2H, H<sub>1''</sub>), 3.71 (ddd, 1H, *J*<sub>H2'-H3'</sub> = 9.4 Hz, *J*<sub>H2'-NH</sub> = 7.3 Hz, *J*<sub>H2'-H1'</sub> = 5.3 Hz, H<sub>2'</sub>), 3.62 (t, 2H, *J*<sub>H5''-H4''</sub> = 7.2 Hz, H<sub>5''</sub>), 3.56 (ddd, 1H, *J*<sub>H6'a-H6'b</sub> = 11.6 Hz, *J* = 5.8 Hz, *J* = 1.9 Hz, H<sub>6'a</sub>), 3.47 (ddd, 1H, *J*<sub>H6'b-H6'a</sub> = 11.6 Hz, *J* = 5.8 Hz, *J* = 5.5 Hz, H<sub>6'b</sub>), 3.43–3.26 (m, 4H, H<sub>1</sub>, H<sub>3'</sub>, H<sub>5'</sub>), 3.18–3.10 (m, 1H, H<sub>4'</sub>), 2.90 (dd, 1H, *J*<sub>H3a-H3b</sub> = 16.2 Hz, *J*<sub>H3a-H1'</sub> = 9.2 Hz, H<sub>3a</sub>), 2.64 (dd, 1H, *J*<sub>H3b-H3a</sub> = 16.2 Hz, *J*<sub>H3b-H1'</sub> = 4.7 Hz, H<sub>3b</sub>), 1.78, 1.76 (2 s, 3H, CH<sub>3</sub>CO\*, CH<sub>3</sub>CO°), 1.62–1.50 (m, 4H, H<sub>2''</sub>, H<sub>4''</sub>), 1.33–1.21 (m, 2H, H<sub>3''</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  200.6 (C<sub>2</sub>), 169.3 (CH<sub>3</sub>CO), 163.7 (C<sub>4u</sub>), 150.9 (C<sub>2u</sub>), 145.6 (C<sub>6u</sub>), 136.3 (d, *J*<sub>Cq-P</sub> = 6.3 Hz, C<sub>qar</sub>), 128.4, 128.2, 127.7 (CH<sub>ar</sub>), 100.8 (C<sub>5u</sub>), 74.9 (C<sub>3'</sub>), 70.6 (C<sub>4'</sub>), 70.3 (C<sub>5'</sub>), 69.4 (C<sub>1'</sub>), 66.9 (2d, *J*<sub>CH2Ph-P</sub> = 5.8 Hz, CH<sub>2</sub>Ph\*, CH<sub>2</sub>Ph°), 65.6 (2d, *J*<sub>C1'-P</sub> = 5.5 Hz, C<sub>1'</sub>°, C<sub>1'</sub>\*), 60.9 (C<sub>6'</sub>), 52.6 (C<sub>2'</sub>), 47.2 (C<sub>5''</sub>), 42.3 (C<sub>1</sub>), 41.3 (C<sub>3</sub>), 29.3 (d, *J*<sub>C2'-P</sub> = 5.5 Hz, C<sub>2''</sub>), 27.9 (C<sub>4''</sub>), 22.6 (CH<sub>3</sub>CO), 21.8 (C<sub>3''</sub>); <sup>31</sup>P NMR (DMSO-*d*<sub>6</sub>)

$\delta$  21.16 (m); MS (ESI):  $m/z = 634$  [M + Na]<sup>+</sup> 100%; HRMS calcd for C<sub>27</sub>H<sub>37</sub>N<sub>3</sub>O<sub>11</sub>P<sup>-</sup> 610.2166, found 610.2194.

### Benzyl (*R*)-7-hydroxy-3,7-dimethyloctyl 3-(2-acetamido-2-deoxy- $\alpha$ -D-glucopyranosyl)-2-oxopropylphosphonate 21

To a suspension of **10** (205 mg, 0.27 mmol) in water (5 mL) was added trifluoroacetic acid (5 mL) at 0 °C. The reaction mixture was stirred at r.t. for 2 h and concentrated. Purification by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 85 : 15) afforded **21** (147 mg, 94%, white solid) as a mixture of epimers (d.r. = 52/48):  $R_f$  0.36 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 85 : 15); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  7.69 (d, 1H,  $J_{\text{NH-H2}'} = 7.7$  Hz, NH), 7.41–7.32 (m, 5H, H<sub>ar</sub>), 5.07–4.99 (m, 3H, CH<sub>2</sub>Ph, OH<sub>4'</sub>), 4.42 (ddd, 1H,  $J = 6.0$  Hz,  $J_{\text{OH6'-H6'a}} = 5.8$  Hz,  $J = 3.1$  Hz, OH<sub>6'</sub>), 4.39 (ddd, 1H,  $J_{\text{H1'-H3a}} = 9.1$  Hz,  $J_{\text{H1'-H2}'} = 5.6$  Hz,  $J_{\text{H1'-H3b}} = 4.6$  Hz, H<sub>1'</sub>), 4.01–3.90 (m, 2H, H<sub>1''</sub>), 3.71 (ddd, 1H,  $J_{\text{H2'-H3}'} = 9.9$  Hz,  $J_{\text{H2'-NH}} = 7.7$  Hz,  $J_{\text{H2'-H1}'} = 5.6$  Hz, H<sub>2'</sub>), 3.56 (ddd, 1H,  $J_{\text{H6'a-H6'b}} = 11.6$  Hz,  $J_{\text{H6'a-OH6'}}$  = 5.8 Hz,  $J_{\text{H6'a-H5'}}$  = 2.5 Hz, H<sub>6'a</sub>), 3.50–3.44 (m, 1H, H<sub>6'b</sub>), 3.43–3.27 (m, 4H, H<sub>1</sub>, H<sub>3</sub>, H<sub>5</sub>), 3.17–3.11 (m, 1H, H<sub>4</sub>), 2.90, 2.89 (2dd, 1H,  $J_{\text{H3a-H3b}} = 16.1$  Hz,  $J_{\text{H3a-H1'}}$  = 9.1 Hz, H<sub>3a</sub>), 2.65, 2.64 (2dd, 1H,  $J_{\text{H3b-H3a}} = 16.1$  Hz,  $J_{\text{H3b-H1'}}$  = 4.6 Hz, H<sub>3b</sub>), 1.78, 1.78 (2 s, 3H, CH<sub>3</sub>CO\*, CH<sub>3</sub>CO°), 1.62–1.54 (m, 1H, H<sub>2'a</sub>), 1.52–1.43 (m, 1H, H<sub>3''</sub>), 1.39–1.16 (m, 6H, H<sub>2''b</sub>, H<sub>4'a</sub>, H<sub>5''</sub>, H<sub>6''</sub>), 1.10–1.01 (m, 1H, H<sub>4''b</sub>), 1.05, 1.05 (2 s, 6H, H<sub>8''</sub>, H<sub>9''</sub>), 0.82, 0.83 (2d, 3H,  $J_{\text{H10''-H3''}} = 6.6$  Hz, H<sub>10''</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  200.6 (d,  $J_{\text{C2-P}} = 5.0$  Hz, C<sub>2</sub>), 169.2 (CH<sub>3</sub>CO), 136.4 (d,  $J_{\text{Cq-P}} = 6.0$  Hz, C<sub>qar</sub>), 128.4, 128.2, 127.7 (CH<sub>ar</sub>), 74.8 (C<sub>5'</sub>), 70.6 (C<sub>4'</sub>), 70.3 (C<sub>3'</sub>), 69.4 (C<sub>1'</sub>), 68.7 (C<sub>7''</sub>), 66.9, 66.8 (2d,  $J_{\text{CH2Ph-P}} = 6.0$  Hz, CH<sub>2</sub>Ph), 64.2 (d,  $J_{\text{C1'-P}} = 6.5$  Hz, C<sub>1''</sub>), 60.9 (C<sub>6'</sub>), 52.6 (C<sub>2'</sub>), 43.8 (C<sub>6''</sub>), 41.8 (d,  $J_{\text{C1-P}} = 120.0$  Hz, C<sub>1</sub>), 41.3 (C<sub>3</sub>), 37.0, 36.9 (C<sub>4''\*</sub>, C<sub>4''°</sub>), 36.8, 36.7 (2d,  $J_{\text{C2'-P}} = 5.0$  Hz, C<sub>2''\*</sub>, C<sub>2''°</sub>), 29.3, 29.2 (C<sub>8''</sub>, C<sub>9''</sub>), 28.7 (C<sub>3''</sub>), 22.6 (CH<sub>3</sub>CO), 21.1 (C<sub>5''</sub>), 19.1, 19.1 (C<sub>10''°</sub>, C<sub>10''\*</sub>); <sup>31</sup>P NMR (DMSO-*d*<sub>6</sub>)  $\delta$  21.2 (s, 0.48P, P°), 21.1 (s, 0.52P, P\*); MS (ESI):  $m/z = 610$  [M + Na]<sup>+</sup> 100%; HRMS calcd for C<sub>28</sub>H<sub>46</sub>NNaO<sub>10</sub>P<sup>+</sup> 610.2757, found 610.2779.

### Benzyl (*R*)-citronellyl 3-(2-acetamido-2-deoxy- $\alpha$ -D-glucopyranosyl)-2-oxopropylphosphonate 22

To a solution of **10** (80 mg, 0.10 mmol) in THF (2 mL) was added water (2 mL) and trifluoroacetic acid (2 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h and at r.t. for 4 h. The mixture was then neutralized with aqueous NH<sub>4</sub>OH solution. The aqueous phase was extracted with Et<sub>2</sub>O and the combined organic layers were dried (MgSO<sub>4</sub>) and concentrated. Purification by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 85 : 15) and lyophilization afforded **22** (50 mg, 84%, white solid):  $R_f$  0.40 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 85 : 15); <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  7.38–7.20 (m, 5H, H<sub>ar</sub>), 5.10–4.95 (m, 3H, H<sub>6'</sub>, CH<sub>2</sub>Ph), 4.59–4.49 (m, 1H, H<sub>1'</sub>), 4.03–3.88 (m, 2H, H<sub>1''</sub>), 3.86 (dd, 1H,  $J_{\text{H2'-H3}'} = 9.5$  Hz,  $J_{\text{H2'-H1}'} = 5.4$  Hz, H<sub>2'</sub>), 3.68–3.58 (m, 2H, H<sub>6'</sub>), 3.49 (dd, 1H,  $J_{\text{H3'-H2}'} = 9.5$  Hz,  $J_{\text{H3'-H4'}}$  = 8.3 Hz, H<sub>3'</sub>), 3.45–3.38 (m, 1H, H<sub>5'</sub>), 3.30–3.18 (m, 3H, H<sub>4'</sub>, H<sub>1</sub>), 3.00–2.90 (m, 0.6H, H<sub>3a</sub>), 2.78–2.72 (m, 0.4H, H<sub>3b</sub>), 1.95–1.78 (m, 2H, H<sub>5''</sub>), 1.85 (s, 3H, CH<sub>3</sub>CO), 1.62–1.50 (m, 1H, H<sub>2'a</sub>), 1.56 (s, 3H, H<sub>8''</sub>), 1.50 (s, 3H, H<sub>9''</sub>), 1.47–1.38 (m, 1H, H<sub>3''</sub>), 1.37–1.27 (m, 1H, H<sub>2''b</sub>), 1.26–1.15 (m, 1H, H<sub>4''b</sub>), 1.09–0.98 (m, 1H, H<sub>4'a</sub>), 0.78, 0.77 (2d,  $J_{\text{H10''-H3''}} = 6.4$  Hz, 3H, H<sub>10''</sub>); <sup>13</sup>C NMR (CD<sub>3</sub>OD)  $\delta$  201.8 (C<sub>2</sub>), 174.0 (CH<sub>3</sub>CO), 137.5 (d,  $J_{\text{Cq-P}} = 5.5$  Hz, C<sub>qar</sub>), 132.3 (C<sub>7''</sub>), 129.9, 129.4 (CH<sub>ar</sub>), 125.8 (C<sub>6''</sub>), 76.5 (C<sub>5'</sub>), 72.1,

72.1 (C<sub>3'</sub>, C<sub>4'</sub>), 70.2 (C<sub>1'</sub>), 69.7 (d,  $J_{\text{CH2Ph-P}} = 6.7$  Hz, CH<sub>2</sub>Ph), 66.7 (C<sub>1''</sub>), 62.7 (C<sub>6'</sub>), 54.4 (C<sub>2'</sub>), 45.0 (C<sub>1</sub>), 42.8 (C<sub>3</sub>), 38.4 (2d,  $J_{\text{C2'-P}} = 6.8$  Hz, C<sub>2''</sub>), 38.1 (C<sub>4''</sub>), 30.2 (C<sub>3''</sub>), 26.5, 26.0 (C<sub>8''</sub>, C<sub>5''</sub>), 22.9 (CH<sub>3</sub>CO), 19.7 (C<sub>10''°</sub>, C<sub>10''\*</sub>), 17.9 (C<sub>9''</sub>); <sup>31</sup>P NMR (CD<sub>3</sub>OD)  $\delta$  22.3 (m); MS (ESI):  $m/z = 570$  [M + H]<sup>+</sup> 50, 592 [M + Na]<sup>+</sup> 100%; HRMS calcd for C<sub>28</sub>H<sub>45</sub>NO<sub>9</sub>P<sup>+</sup> 570.2832, found 570.2806.

### Benzyl hexadecanyl 3-(2-acetamido-2-deoxy- $\alpha$ -D-glucopyranosyl)-2-oxopropylphosphonate 23

To a suspension of **19** (65 mg, 76  $\mu$ mol) in water (2 mL) was added trifluoroacetic acid (2 mL) at 0 °C. The reaction mixture was stirred at r.t. for 3 h and concentrated. Purification by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 9 : 1) afforded **23** (45 mg, 90%, white solid) as a mixture of epimers (d.r. = 55/45):  $R_f$  0.32 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 9 : 1); <sup>1</sup>H NMR  $\delta$  7.45–7.39 (m, 1H, NH), 7.38–7.28 (m, 5H, H<sub>ar</sub>), 5.12–5.00 (m, 2H, CH<sub>2</sub>Ph), 4.76–4.69 (m, 1H, H<sub>1'</sub>), 4.09–4.01 (m, 1H, H<sub>2'</sub>), 3.97–3.88 (m, 2H, H<sub>1''</sub>), 3.90–3.82 (m, 1H, H<sub>6'a</sub>), 3.77–3.66 (m, 2H, H<sub>3'</sub>, H<sub>6'b</sub>), 3.63–3.52 (m, 2H, H<sub>4'</sub>, H<sub>5'</sub>), 3.26–3.12 (m, 2H, H<sub>1</sub>), 3.04–2.82 (m, 2H, H<sub>3</sub>), 1.94, 1.93 (2 s, 3H, CH<sub>3</sub>CO\*, CH<sub>3</sub>CO°), 1.58–1.50 (m, 2H, H<sub>2''</sub>), 1.33–1.18 (m, 26H, H<sub>3''</sub> to H<sub>15''</sub>), 0.87 (t, 3H,  $J_{\text{H16''-H15''}} = 7.0$  Hz, H<sub>16''</sub>); <sup>13</sup>C NMR  $\delta$  200.1, 200.0 (2d,  $J_{\text{C2-P}} = 5.5$  Hz, C<sub>2'</sub>, C<sub>2''\*</sub>), 172.2 (CH<sub>3</sub>CO), 135.9 (d,  $J_{\text{Cq-P}} = 5.5$  Hz, C<sub>qar</sub>), 128.8, 128.8, 128.8, 128.2, 128.1 (CH<sub>ar</sub>), 75.1 (C<sub>5'</sub>), 71.2 (C<sub>3'</sub>), 70.7 (C<sub>4'</sub>), 68.6 (C<sub>1'</sub>), 68.5, 68.4 (2d,  $J_{\text{CH2Ph-P}} = 6.5$  Hz, CH<sub>2</sub>Ph\*, CH<sub>2</sub>Ph°), 67.3 (d,  $J_{\text{C1'-P}} = 6.5$  Hz, C<sub>1''</sub>), 61.6 (C<sub>6'</sub>), 52.9 (C<sub>2'</sub>), 42.9 (C<sub>3</sub>), 42.2, 42.1 (2d,  $J_{\text{C1-P}} = 127.0$  Hz, C<sub>1''\*</sub>, C<sub>1''°</sub>), 32.1, 29.9, 29.8, 29.8, 29.7, 29.5, 29.3, 25.5, 23.1 (C<sub>3''</sub> to C<sub>15''</sub>), 30.5, 30.4 (2d,  $J_{\text{C2'-P}} = 5.0$  Hz, C<sub>2''°</sub>, C<sub>2''\*</sub>), 22.8 (CH<sub>3</sub>CO), 14.2 (C<sub>16''</sub>); <sup>31</sup>P NMR  $\delta$  21.1 (s); MS (ESI):  $m/z = 1333$  [2M + Na]<sup>+</sup> 100%; HRMS calcd for C<sub>34</sub>H<sub>58</sub>NNaO<sub>9</sub>P<sup>+</sup> 678.3747, found 678.3765.

### 5-Uracil-1-yl-pentyl 3-(2-acetamido-2-deoxy- $\alpha$ -D-glucopyranosyl)-2-oxopropylphosphonate 24

A suspension of **20** (15 mg, 24  $\mu$ mol) and Pd/C 10% (15 mg) in methanol (2 mL) was purged from air and filled with H<sub>2</sub>. The reaction was stirred at r.t. for 4 h then diluted with methanol, filtered through a Celite pad and concentrated. Lyophilization afforded **24** (12 mg, 94%, white solid);  $[\alpha]_{\text{D}}^{20} +26$  (c 1.0, DMSO); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  11.19 (s, 0.8H, H<sub>3u</sub>), 9.99 (s, 0.2H, H<sub>3u</sub>), 7.65 (d, 1H,  $J_{\text{H6u-H5u}} = 7.6$  Hz, H<sub>6u</sub>), 7.65 (d, 1H,  $J_{\text{H5u-H6u}} = 7.6$  Hz, H<sub>5u</sub>), 7.28 (bs, 1H, NH), 5.01 (s, 1H, OH<sub>4'</sub>), 4.80 (s, 1H, OH<sub>3'</sub>), 4.50 (m, 1H, OH<sub>6'</sub>), 4.36 (ddd, 1H,  $J_{\text{H1'-H3a}} = 7.3$  Hz,  $J_{\text{H1'-H3b}} = 6.0$  Hz,  $J_{\text{H1'-H2}'} = 5.7$  Hz, H<sub>1'</sub>), 3.75 (ddd, 1H,  $J_{\text{H2'-H3}'} = 9.8$  Hz,  $J_{\text{H2'-NH}} = 7.8$  Hz,  $J_{\text{H2'-H1}'} = 5.7$  Hz, H<sub>2'</sub>), 3.73–3.67 (m, 2H, H<sub>1''</sub>), 3.65 (t, 2H,  $J_{\text{H5''-H4''}} = 7.0$  Hz, H<sub>5''</sub>), 3.58 (ddd, 1H,  $J_{\text{H6'a-H6'b}} = 11.4$  Hz,  $J_{\text{H6'a-OH6'}}$  = 5.7 Hz,  $J_{\text{H6'a-H5'}}$  = 1.5 Hz, H<sub>6'a</sub>), 3.47–3.35 (m, 2H, H<sub>6'b</sub>, H<sub>3'</sub>), 3.34–3.25 (m, 1H, H<sub>3'</sub>), 3.16–3.00 (m, 2H, H<sub>4'</sub>, H<sub>3a</sub>), 2.86 (dd, 1H,  $J_{\text{H1a-P}} = 19.7$  Hz,  $J_{\text{H1a-H1b}} = 11.6$  Hz, H<sub>1a</sub>), 2.71 (dd, 1H,  $J_{\text{H1b-P}} = 19.7$  Hz,  $J_{\text{H1b-H1a}} = 11.6$  Hz, H<sub>1b</sub>), 2.61 (dd, 1H,  $J_{\text{H3b-H3a}} = 16.3$  Hz,  $J_{\text{H3b-H1'}}$  = 6.0 Hz, H<sub>3b</sub>), 1.76 (s, 3H, CH<sub>3</sub>CO), 1.65–1.45 (m, 4H, H<sub>2''</sub>, H<sub>4''</sub>), 1.36–1.20 (m, 2H, H<sub>3''</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  203.5 (C<sub>2</sub>), 169.8 (CH<sub>3</sub>CO), 163.8 (C<sub>4u</sub>), 151.0 (C<sub>2u</sub>), 145.8 (C<sub>6u</sub>), 100.8 (C<sub>5u</sub>), 75.0 (C<sub>3'</sub>), 71.3 (C<sub>4'</sub>), 70.7 (C<sub>5'</sub>), 69.4 (C<sub>1'</sub>), 63.4 (d,  $J_{\text{C-P}} = 4.1$  Hz, C<sub>1''</sub>), 61.4 (C<sub>6'</sub>), 52.7 (C<sub>2'</sub>), 47.5 (C<sub>5''</sub>), 45.7 (C<sub>1</sub>), 41.4 (C<sub>3</sub>), 29.3 (d, C<sub>2''</sub>,  $J_{\text{C-P}} = 6.6$  Hz), 28.2 (C<sub>4''</sub>), 22.7 (CH<sub>3</sub>CO), 21.4 (C<sub>3''</sub>); <sup>31</sup>P NMR (DMSO-*d*<sub>6</sub>)  $\delta$  7.91 (m); MS (ESI):  $m/z = 520$  [M – H]<sup>-</sup> 100%; HRMS calcd for C<sub>20</sub>H<sub>31</sub>N<sub>3</sub>O<sub>11</sub>P<sup>-</sup> 520.1696, found 520.1722.

**(R)-7-Hydroxy-3,7-dimethyloctyl 3-(2-acetamido-2-deoxy- $\alpha$ -D-glucopyranosyl)-2-oxopropylphosphonate 25**

A suspension of **21** (75 mg, 0.13 mmol) and Pd/C 10% (75 mg) in methanol (4 mL) was purged from air and filled with H<sub>2</sub>. The reaction was stirred at r.t. for 3 h then diluted with methanol, filtered through a Celite pad and concentrated. Lyophilization afforded **25** (63 mg, 100%, white solid): [ $\alpha$ ]<sub>D</sub><sup>20</sup> +51 (*c* 1.0, MeOH); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  7.88 (d, 1H, *J*<sub>NH-H2'</sub> = 8.0 Hz, NH), 4.35 (ddd, 1H, *J*<sub>H1'-H3b</sub> = 7.3 Hz, *J*<sub>H1'-H2'</sub> = 6.0 Hz, *J*<sub>H1'-H3a</sub> = 5.5 Hz, H<sub>1'</sub>), 3.91–3.78 (m, 2H, H<sub>1''</sub>), 3.71 (ddd, 1H, *J*<sub>H2'-H3'</sub> = 10.0 Hz, *J*<sub>H2'-NH</sub> = 8.0 Hz, *J*<sub>H2'-H1'</sub> = 6.0 Hz, H<sub>2'</sub>), 3.56 (bd, 1H, *J*<sub>H6'a-H6'b</sub> = 11.6 Hz, H<sub>6'a</sub>), 3.48–3.37 (m, 2H, H<sub>3'</sub>, H<sub>6'b</sub>), 3.36–3.29 (m, 1H, H<sub>5'</sub>), 3.09 (dd, 1H, *J* = 8.9 Hz, *J* = 8.3 Hz, H<sub>4'</sub>), 3.01, 2.97 (AB from ABX, 1H, *J*<sub>AB</sub> = 13.1 Hz, *J*<sub>AX</sub> = 21.6 Hz, *J*<sub>BX</sub> = 20.5 Hz, H<sub>1a</sub>, H<sub>1b</sub>), 2.84, 2.78 (AB from ABX, 1H, *J*<sub>AB</sub> = 16.3 Hz, *J*<sub>AX</sub> = 5.5 Hz, *J*<sub>BX</sub> = 7.3 Hz, H<sub>3a</sub>, H<sub>3b</sub>), 1.77 (s, 3H, CH<sub>3</sub>CO), 1.64–1.47 (m, 2H, H<sub>2''a</sub>, H<sub>3''</sub>), 1.40–1.15 (m, 6H, H<sub>2''b</sub>, H<sub>4''a</sub>, H<sub>5''</sub>, H<sub>6''</sub>), 1.12–1.04 (m, 1H, H<sub>4''b</sub>), 1.06 (s, 6H, H<sub>8''</sub>, H<sub>9''</sub>), 0.85 (d, 3H, *J*<sub>H10''-H3''</sub> = 6.4 Hz, H<sub>10''</sub>); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>)  $\delta$  201.9 (d, *J*<sub>C2-P</sub> = 5.0 Hz, C<sub>2</sub>), 169.4 (CH<sub>3</sub>CO), 74.8 (C<sub>5'</sub>), 71.0 (C<sub>4'</sub>), 70.4 (C<sub>3'</sub>), 69.5 (C<sub>1'</sub>), 68.7 (C<sub>7''</sub>), 62.7 (d, *J*<sub>C1'-P</sub> = 6.0 Hz, C<sub>1'</sub>), 61.1 (C<sub>6'</sub>), 52.7 (C<sub>2'</sub>), 44.3 (d, *J*<sub>C1-P</sub> = 121.0 Hz, C<sub>1</sub>), 43.9 (C<sub>6''</sub>), 41.3 (C<sub>3</sub>), 37.2, 37.1 (C<sub>2''</sub>, C<sub>4''</sub>), 29.3, 29.2 (C<sub>8''</sub>, C<sub>9''</sub>), 28.9 (C<sub>3''</sub>), 22.6 (CH<sub>3</sub>CO), 21.1 (C<sub>5''</sub>), 19.2 (C<sub>10''</sub>); <sup>31</sup>P NMR (DMSO-d<sub>6</sub>)  $\delta$  14.1 (s); MS (ESI): *m/z* = 993 [2M – H]<sup>+</sup> 100%; HRMS calcd for C<sub>21</sub>H<sub>39</sub>NO<sub>10</sub>P<sup>−</sup> 496.2312, found 496.2314.

**Hexadecanyl 3-(2-acetamido-2-deoxy- $\alpha$ -D-glucopyranosyl)-2-oxopropylphosphonate 26**

A suspension of **23** (40 mg, 61  $\mu$ mol) and Pd/C 10% (40 mg) in ethanol (10 mL) was purged from air and filled with H<sub>2</sub>. The reaction was stirred at r.t. for 3 h then diluted with methanol. Filtration through a Celite pad and concentration afforded **26** (30 mg, 86%, white solid): [ $\alpha$ ]<sub>D</sub><sup>20</sup> +41 (*c* 1.0, MeOH); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  8.64 (d, 1H, *J*<sub>NH-H2'</sub> = 9.0 Hz, NH), 4.34–4.26 (m, 1H, H<sub>1'</sub>), 3.81–3.70 (m, 1H, H<sub>2'</sub>), 3.68–3.52 (m, 2H, H<sub>1''</sub>), 3.50–3.38 (m, 1H, H<sub>3'</sub>), 3.38–3.22 (m, 4H, H<sub>3a</sub>, H<sub>5'</sub>, H<sub>6'</sub>), 3.04–2.96 (m, 1H, H<sub>4'</sub>), 2.81 (dd, 1H, *J*<sub>H1a-P</sub> = 21.5 Hz, *J*<sub>H1a-H1b</sub> = 11.5 Hz, H<sub>1a</sub>), 2.60–2.35 (m, 2H, H<sub>1b</sub>, H<sub>3b</sub>), 1.73 (s, 3H, CH<sub>3</sub>CO), 1.50–1.40 (m, 2H, H<sub>2''</sub>), 1.32–1.18 (m, 26H, H<sub>3''</sub> to H<sub>15''</sub>), 0.85 (t, 3H, *J*<sub>H16''-H15''</sub> = 7.0 Hz, H<sub>16''</sub>); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>)  $\delta$  203.3 (d, *J*<sub>C2-P</sub> = 6.0 Hz, C<sub>2</sub>), 169.6 (CH<sub>3</sub>CO), 75.0 (C<sub>5'</sub>), 71.7 (C<sub>4'</sub>), 70.8 (C<sub>3'</sub>), 69.3 (C<sub>1'</sub>), 63.1 (d, *J*<sub>C1'-P</sub> = 6.0 Hz, C<sub>1'</sub>), 61.6 (C<sub>6'</sub>), 52.5 (C<sub>2'</sub>), 46.3 (d, *J*<sub>C1-P</sub> = 104.0 Hz, C<sub>1</sub>), 41.8 (C<sub>3</sub>), 31.2, 29.0, 28.9, 28.8, 28.6, 25.4, 22.6 (C<sub>3''</sub> to C<sub>15''</sub>), 30.6 (C<sub>2''</sub>), 22.0 (CH<sub>3</sub>CO), 13.9 (C<sub>16''</sub>); <sup>31</sup>P NMR (DMSO-d<sub>6</sub>)  $\delta$  7.9 (s); MS (ESI): *m/z* = 564 [M – H]<sup>−</sup> 100%; HRMS calcd for C<sub>27</sub>H<sub>51</sub>NO<sub>9</sub>P<sup>−</sup> 564.3301, found 564.3304.

**Enzymatic assays**

The activities of the compounds against *MraY* transferase were tested as previously described.<sup>3b,16</sup> The assay was performed in a reaction mixture of 10  $\mu$ L containing, in final concentrations, 100 mM Tris-HCl, pH 7.5, 40 mM MgCl<sub>2</sub>, 1.1 mM C<sub>55</sub>-P, 250 mM NaCl, 0.25 mM UDP-MurNAc-[<sup>14</sup>C]pentapeptide (337 Bq), and 8.4 mM *N*-lauroyl sarcosine. The reaction was initiated by the addition of *MraY* enzyme purified from *Bacillus subtilis* (50 ng), and the mixture was incubated for 30 min at 37 °C under shaking with a thermomixer (Eppendorf). The reaction was stopped by

heating at 100 °C for 1 min. The compounds were also tested against MurG purified from *E. coli* as previously described.<sup>36,37</sup> Reaction mixtures contained, in a final volume of 12.5  $\mu$ L, 200 mM Tris-HCl, pH 7.5, 10 mM MgCl<sub>2</sub>, 16  $\mu$ M UDP-[<sup>14</sup>C]GlcNAc (1.7 kBq), 16  $\mu$ M lipid I analogue, 30% (v/v) dimethyl sulfoxide and MurG (7 ng). After 30 min at 37 °C, it was stopped by boiling for 3 min. Then, the mixture was lyophilized and taken up in 10  $\mu$ L of 2-propanol/ammonium hydroxide/water (6 : 3 : 1; v/v/v). In both cases, the radiolabeled substrate (UDP-MurNAc-pentapeptide in the case of *MraY*, UDP-GlcNAc in the case of MurG) and reaction product (lipid I, product of *MraY* and lipid II, product of MurG) were separated by TLC on silica gel plates LK6D (Whatman) using 2-propanol/ammonium hydroxide/water (6 : 3 : 1; v/v/v) as the mobile phase. The radioactive spots were located and quantified with a radioactivity scanner (model Multi-Tracemaster LB285; EG&G Wallac/Berthold). Residual activities were calculated with respect to a control assay without inhibitors. IC<sub>50</sub> values were determined with 7 inhibitor concentrations. Data represent the mean of independent triplicate determinations, and the standard deviations were less than 10%.

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