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Synthesis and immunological evaluation of the 4- β -glucoside of HMBPP

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Abstract

HMBPP ((*E*)-4-hydroxy-3-methyl-2-butenyl pyrophosphate) is a highly potent innate immunogen that stimulates human $\gamma\delta$ T cells expressing the V γ 2V δ 2 T cell antigen receptor. To determine if glycoside conjugates of HMBPP retain activity, the 4- β -glucoside and its acetylated homolog were synthesized and tested for their ability to stimulate $\gamma\delta$ T cells. The glycoside HMBPP conjugate stimulated human $\gamma\delta$ T cells with an EC₅₀ of 78 nM. The tetraacetyl glycoside HMBPP conjugate was also active (EC₅₀ = 360 nM). The two isomeric mono- β -glucosides of the parent (*E*)-2-methylbut-2-ene-1,4-diol, however, were not active. Thus, HMBPP glycosylated at the 4-OH position stimulates $\gamma\delta$ T cells as long as the pyrophosphate moiety is present.

Keywords

Glycosides; Immunology; Nonmevalonate pathway; Phosphoantigens; T cells

HMBPP ((*E*)-4-hydroxy-3-methyl-2-butenyl pyrophosphate, **1**, Fig. 1) is the final unique intermediate in the deoxyxylulose pathway (also called the 2-*C*-methyl-D-erythritol 4-phosphate or MEP pathway) for isoprenoid biosynthesis, a pathway used by many bacteria and most apicomplexan protozoan parasites.¹ This metabolite provides a potent signal for an innate immune response, activating human $\gamma\delta$ T cells expressing V γ 2V δ 2 T cell antigen receptors (also termed V γ 2V δ 2) with an EC₅₀ of 32 pM.² Activation of V γ 2V δ 2 T cells by HMBPP and other phosphorylated metabolites during microbial infections greatly expands V γ 2V δ 2 T cells (to up to 50% of circulating T cells) that then secrete inflammatory cytokines and chemokines and kill infected cells.³ V γ 2V δ 2 T cells also kill a variety of tumor cells in vitro and have been shown to effect partial or even full remissions with a variety of cancers in clinical trials.⁴ While the structural requirements for $\gamma\delta$ T cell activation by HMBPP analogs are incompletely understood, present data suggest that recognition is very stringent.⁵ Recently, an HMBPP analog in which a photoaffinity ligand was conjugated to HMBPP through an ester linkage was shown to retain high potency (EC₅₀ of 80 pM), thereby demonstrating that a free 4-hydroxyl group is not required for high immunostimulatory activity.⁶ Photoaffinity ligand conjugated to HMBPP through highly stable ether linkages were also active albeit less potently (EC₅₀ of 1.8 nM).⁶ To determine if

a glycoside conjugate could retain potency, the 4- β -glucoside of HMBPP (**2**) was synthesized and evaluated.

The synthesis of **2** proceeded from the acetylated glucoside **3** (Scheme 1), which was previously obtained by Königs–Knorr coupling of (*E*)-4-*t*-butyldimethylsilyloxy-2-methyl-2-buten-1-ol and acetobromoglucose, followed by desilylation.⁷ The intermediate alcohol (**3**) was converted into the allylic chloride **4**⁸ using the Corey–Kim NCS-DMS reagent.⁹ Reaction of **4** with tris(tetrabutylammonium) pyrophosphate¹⁰ led to the isolation of the acetylated sugar pyrophosphate **5**¹¹ in 54% yield after silica gel chromatography (*i*-PrOH/MeCN/1% aq NH₄HCO₃ 2:1:1). Deprotection of **5** with aqueous ammonia followed by lyophilization gave the desired product (**2**) in quantitative yield.¹²

Because of the poor yield in the Königs–Knorr reaction, an alternative route to **4** was explored using olefin metathesis. However, although coupling of tetraacetyl methallyl β -glucoside¹³ and (*Z*)-1,4-dichloro-2-butene with 5 mol % of the second generation Grubb's catalyst¹⁴ proceeded in excellent yield, the product was a poorly separable 3:1 mixture of the *E*- and *Z*-isomers of **4**. Therefore, this route was abandoned.

The activities of **2** and **5** were assessed in a V γ 2V δ 2 T cell activation bioassay.¹⁵ Glucoside **2** had an EC₅₀ of 78 nM; 2,400 times less potent than HMBPP itself, but 12 times more potent than isopentenyl pyrophosphate (IPP) (Fig. 2). The acetylated homolog **5** also stimulated V γ 2V δ 2 T cells although it was 4.6-fold less active (EC₅₀ = 360 nM) (Fig. 2). The dephosphorylated analog of **2** (**6**) and its positional isomer (**8**) have been isolated from plants.¹⁶ These compounds were synthesized (Scheme 1)¹⁷ and showed a complete lack of activity for $\gamma\delta$ T cells at concentrations up to 300 μ M.

While the $\gamma\delta$ bioactivity of the HMBPP glucoside conjugate **2** could possibly be due to enzymatic hydrolysis to give HMBPP, this seems less likely in light of the similar activity observed with the tetraacetylated glucoside **5** (only 4.6-fold less active). In experiments with monoacetyl glucosides, it has been shown that acetylated glucosides are not substrates for β -glucosidases.¹⁸ Moreover, photoaffinity compounds linked to HMBPP at the 4-position by ether linkages are also active but would not be susceptible to esterases or glycosidases.⁶ We conclude that the HMBPP glucoside conjugates are most likely directly recognized. However, it cannot be excluded that the acetyl groups are removed by highly efficient nonspecific esterases prior to the weak action of a glucosidase to generate a small amount of HMBPP.¹⁹ In the absence of the pyrophosphate moiety, a complete lack of $\gamma\delta$ bioactivity for **6** and **8** confirms the requirement for the pyrophosphate moiety.⁵

To summarize, the presence of the monosaccharide moiety (with or without acetylation) is compatible with immunostimulatory activity. Given that bioactivity of HMBPP is retained when the hydroxyl group at position-4 is replaced with an ester or ether,⁶ perhaps a H-bond acceptor is all that is required at this position. We speculate that HMBPP will retain activity for $\gamma\delta$ T cells when other moieties are linked through the 4-position. It may be possible to conjugate HMBPP to complex carbohydrates, other chemical structures, or proteins that could serve to specifically target this molecule to tumor cells or antigen presenting cells for localized stimulation of V γ 2V δ 2 T cells.

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- Compound 4**: TLC R_f = 0.82 (hexane/ethyl acetate 1:2). (^1H NMR: see Table 1). ^{13}C NMR (CDCl_3 , 75 MHz) 170.6, 170.2, 169.3, 169.2, 136.8, 123.5, 99.3, 73.5, 72.8, 71.8, 71.2, 68.4, 61.8, 39.7, 20.6, 20.6, 20.5, 20.5, 13.4.
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- Compound 5**: TLC R_f = 0.67 (*i*-PrOH/MeCN/1% aq NH_4HCO_3 2:1:1). (^1H NMR: see Table 1). ^{13}C NMR (D_2O , 151 MHz) 174.1 (6'-Ac), 173.5 (3'-Ac), 173.1 (4'-Ac), 173.1 (2'-Ac), 135.6 (C-2), 126.0 (C-3, J = 7 Hz), 99.1 (C-1'), 75.4 (C-1), 73.5 (C-3'), 72.0 (C-2'), 71.5 (C-5'), 68.8 (C-4'), 62.5 (C-4, J = 5 Hz), 62.2 (C-6'), 20.6 (Ac), 20.6 (Ac), 20.5 (Ac), 20.5 (Ac), 13.7 (2-Me). ^{31}P NMR (D_2O , 243 MHz) -6.5 (br), -9.2 (br).
- Compound 2**: (^1H NMR: see Table 1). ^{13}C NMR (D_2O , 151 MHz) 136.6 (C-2), 124.8 (C-3, J = 6 Hz), 101.3 (C-1'), 76.3 (C-3' or 5'), 76.2 (C-3' or 5'), 74.7 (C-1), 73.6 (C-2'), 70.2 (C-4'), 62.9 (C-4, J = 3 Hz), 61.2 (C-6'), 13.9 (2-Me). ^{31}P NMR (D_2O , 243 MHz) -8.3 (br), -9.2 (br).
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- Compounds 6–8**: ^1H NMR: see Table 1.
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Figure 1.
Structures of $\gamma\delta$ T cell activators.

Figure 2. V γ 2V δ 2 T cell activation by glucoside conjugates of HMBPP. The 12G12 V γ 2V δ 2 T cell clone was cultured with various compounds and mitomycin C-treated Va2 tumor cells as antigen presenting cells. On day 1, 1 μ Ci of 3 H-thymidine (2 Ci/mmol) was added to each well and the cells harvested 16–24 h later. Mean proliferation and SEM of triplicate cultures is shown.

Scheme 1.

Synthesis. Reagents and conditions: (a) Acetobromoglucose (1.5 equiv), Ag_2CO_3 (1.1 equiv), 4 Å mol sieves, DCM, rt, 16 h, 38%. (b) TBAF (1 equiv), THF, rt, 0.5 h, quant. (c) 4 equiv NCS plus 4.2 equiv DMS, DCM, $-30\text{ }^\circ\text{C}$ to $>0\text{ }^\circ\text{C}$, 5 min, then add **3** at $-45\text{ }^\circ\text{C}$ and warm to rt over 2.5 h, 84%. (d) $(\text{Bu}_4\text{N}^+)_3\text{HOPO}_3\text{PO}_3^{3-}$ (2 equiv), MeCN, rt, 8 h, 54%. (e) 0.6 M NH_4OH , $30\text{ }^\circ\text{C}$, 8 h, quant.

Table 1

600 MHz ^1H NMR assignments^a

Position	2	4 ^b	5	6	7 ^b	8
1	4.35 d 12.4 4.24 d 12.2	4.22 d 13.3 4.02 d 12.8	4.32 d 12.2 4.23 d 12.2	4.34 d 12.1 4.21–4.26 m	4.21–4.27 m (2H)	4.50 dd 6.7, 12.2 4.44 dd 7.9, 12.2
2					5.56 br t 6.3	5.68 br t 7.2
2-Me	1.80 s (3H)	1.70 s (3H)	1.76 s (3H)	1.78 s (3H)		
3	5.82 br t 6.2	5.67 br t 7.8	5.78 br t 6.3	5.77 br t 6.8		
3-Me					1.68 s (3H)	1.78 s (3H)
4	4.61 br t 5.8 (2H)	4.09 d 8.0 (2H)	4.61 br t 6.4 (2H)	4.21–4.26m (2H)	4.48 s (2H)	4.11 s (2H)
1'	4.52 d 8.0	4.49 d 8.0	4.93 d 8.1	4.51 d 8.0	4.53 d 8.0	4.54 d 8.0
2'	3.34 dd 8.2, 9.2	5.02 dd 8.4, 9.2	5.04 dd 8.2, 9.5	3.36 dd 8.2, 9.2	4.98 dd 8.3, 9.3	3.34 dd 8.1, 9.3
3'	3.55 t 9.2	5.20 t 9.5	5.42 t 9.4	3.54 t 9.1	5.20 t 9.5	3.55 t 9.1
4'	3.44 t 9.5	5.08 t 9.7	5.17 t 9.7	3.44 dd 9.1, 9.8	5.08 t 9.7	3.46 dd 9.0, 9.6
5'	3.50 ddd 2.2, 5.9, 9.7	3.67 ddd 2.3, 4.5, 9.9	4.12 ddd 2.5, 3.6, 10.2	3.49 ddd 2.1, 5.9, 9.8	3.68 ddd 2.3, 4.6, 9.9	3.51 ddd 2.3, 5.9, 9.9
6'	3.97 dd 2.1, 12.5 3.77 dd 5.9, 12.4	4.24 dd 4.7, 12.2 4.15 dd 2.2, 12.4	4.46 dd 3.8, 12.6 4.28 dd 2.1, 12.6	3.97 dd 2.1, 12.3 3.78 dd 5.9, 12.4	4.33 dd 6.0, 12.3 4.15 dd 2.0, 12.2	3.99 dd 2.2, 12.4 3.80 dd 5.9, 12.4
Ac		2.09 s (3H)	2.19 s (3H)		2.08 s (3H)	
Ac		2.05 s (3H)	2.19 s (3H)		2.08 s (3H)	
Ac		2.02 s (3H)	2.15 s (3H)		2.04 s (3H)	
Ac		2.00 s (3H)	2.11 s (3H)		2.02 s (3H)	
Ac					2.00 s (3H)	

^a All ^1H signals are 1H unless specified otherwise; the coupling constants are given in Hz. The assignments were determined by HSQC and HMBC 2-D MR experiments. Solvent = D₂O unless specified otherwise.

^b Solvent = CDCl₃.