Title: Synthesis of analogues of (E)-1-Hydroxy-2-methylbut-2-enyl 4-diphosphate, an isoprenoid precursor and human gamma delta T cell activator

Author(s): Steven Van Hoof, Carl Jeffrey Lacey, RC Röhrich, J Wiesner, H Jomaa and Serge Van Calenbergh

Source: JOURNAL OF ORGANIC CHEMISTRY (2008), 73(4), 1365-1370, **DOI**: 10.1021/jo701873t

Synthesis of analogues of (*E*)-1-hydroxy-2-methyl-but-2-enyl 4-diphosphate, an isoprenoid precursor and human γδ T cell activator

Steven Van Hoof,[†] Carl Jeffrey Lacey,[†] René C. Röhrich,[‡] Jochen Wiesner,[‡] Hassan Jomaa[‡,*] and Serge Van Calenbergh[†,*]

[†] *Laboratory for Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ghent University, Belgium,*

[‡] *Institut für Klinische Immunologie und Transfusionsmedizin, Justus-Liebig-Universität Giessen, Germany*

AUTHOR EMAIL ADDRESS Serge.VanCalenbergh@UGent.be; Hassan.Jomaa@uniklinikum-giessen.de

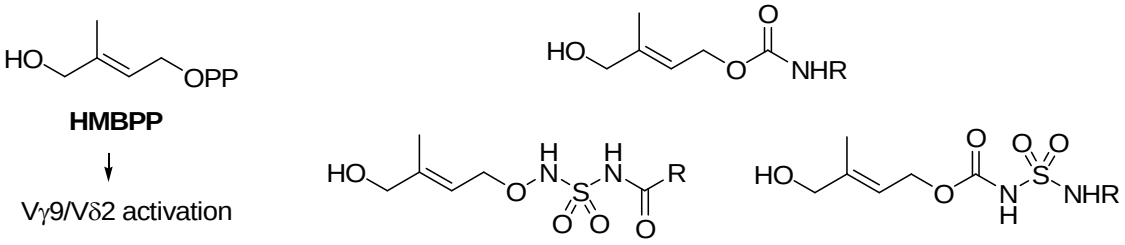
RECEIVED DATE (to be automatically inserted after your manuscript is accepted if required according to the journal that you are submitting your paper to)

CORRESPONDING AUTHOR FOOTNOTE

[†] Harelbekestraat 72, 9000 Gent, Belgium. Phone: +32 9 264 81 24. Fax + 32 9 264 81 46

[‡] Langhansstrasse 7, 35392 Giessen, Germany. Phone: + 49 641 99-41573. Fax + 49 641 99-41589

Table of contents Graphic:



Abstract:

(*E*)-1-hydroxy-2-methyl-but-2-enyl 4-diphosphate (HMBPP) is an intermediate in the non-mevalonate pathway for the biosynthesis of isoprenoids and also serves as a very strong activator of human γδT cells expressing Vγ9/Vδ2 receptors. This paper describes the synthesis of analogues of HMBPP, in which the diphosphate group is replaced by potential isosteric moieties, i.e. carbamate, N-acyl-N'-oxy sulfamate or aminosulfonyl carbamate functionalities. The potential of the synthesized analogues to stimulate Vγ9/Vδ2 T cell response or to inhibit GcpE and LytB, the last enzymes in the non-mevalonate pathway was assessed.

1. Introduction

Various disease-causing organisms as *Plasmodium falciparum* (malaria), *Bacillus anthracis* (anthrax), *Clostridium botulinum* (botulism) and *Mycobacterium tuberculosis* (tuberculosis) use the non-mevalonate biosynthetic pathway to produce isopentenyl diphosphate (IPP) **1** and dimethylallyl diphosphate (DMAPP) **2**.[1,2] These isoprenoids serve as biosynthetic precursors of a wide myriad of terpenes[3], some of them being essential components in life cycles of any cell type. However, in mammals IPP and DMAPP are formed exclusively via the unrelated mevalonate pathway. Selective inhibition of the non-mevalonate pathway would therefore be an interesting approach to the development of new treatments for important infectious diseases.[4]

The non-mevalonate pathway starts with the formation of 1-deoxy-D-xylulose 5-phosphate **5** by condensation of pyruvic acid **3** and D-glyceraldehyde 3-phosphate **4** (Scheme 1). Via 2-*C*-methyl-D-erythritol 5-phosphate **6** the pathway leads to (*E*)-1-hydroxy-2-methyl-but-2-enyl 4-diphosphate (HMBPP) **7**. This intermediate is finally transformed to IPP (**1**) and DMAPP (**2**), in a

reaction catalyzed by the *ispH* (or LytB) enzyme.[5] This enzyme contains an iron-sulfur cluster which suggests a radical mechanism of transformation.[6]

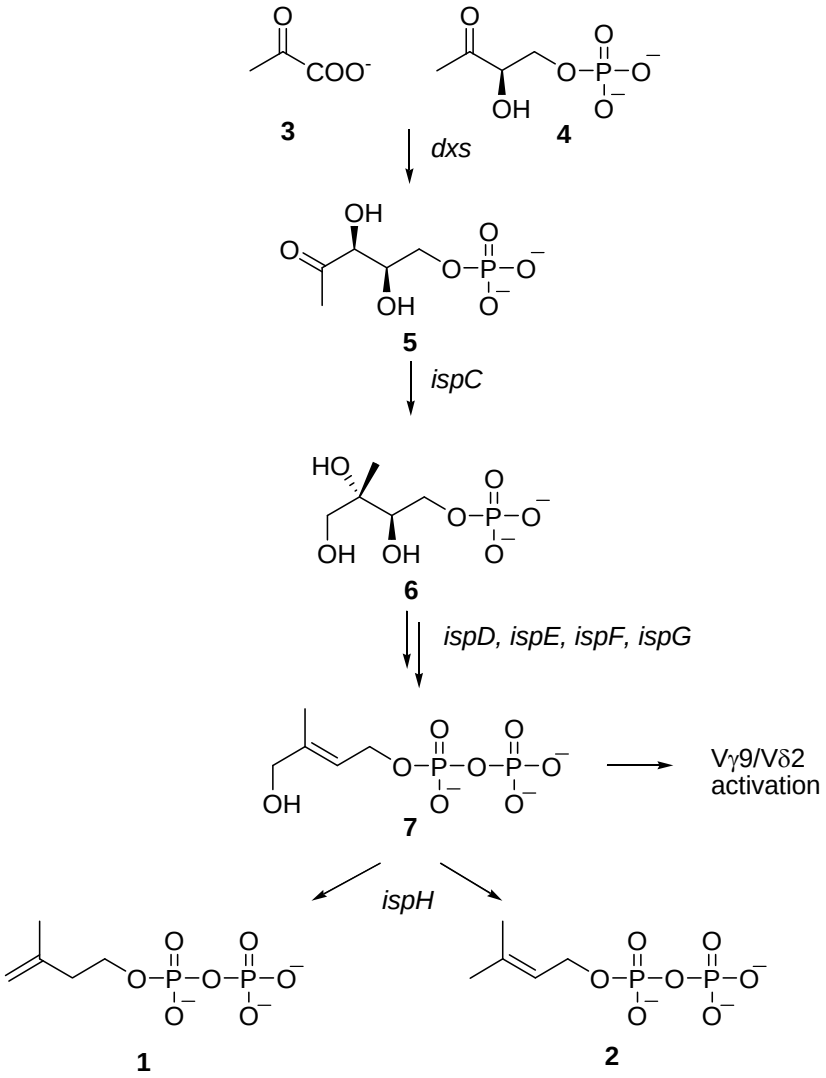
Interestingly, HMBPP has also been identified as a very strong activator of the human γδT cells expressing Vγ9/Vδ2 receptors.[2,7] The γδT cells constitute 0.5-5% of the human peripheral blood T cells, the vast majority of which express the Vγ9/Vδ2 T cell receptor. Activation and proliferation of this subset of T cells is observed in infections with pathogens known to possess the MEP pathway, such as tuberculosis or malaria. Vγ9Vδ2 T cell activation appears to be important in priming and regulating various reactions of the immune system, but its exact function is largely unknown.[8,9] Nevertheless, the evaluation of natural and synthetic activators of Vγ9Vδ2 T cells has new immunomodulatory drugs been repeatedly suggested.[10]

Unlike the activation of αβ T cells by small antigenic peptides, the Vγ9/Vδ2 T cells are activated by low molecular weight phosphorylated compounds collectively called phosphoantigens. Specific activation of the T lymphocytes was observed after infection with a broad range of pathogenic organisms using the non-mevalonate pathway. While IPP and DMAPP appeared to be moderate stimulators,[11] HMBPP gave an approximately 10000-fold higher response and probably represents the only phosphoantigen of physiological importance. This stimulatory effect, however, has a high degree of structure specificity.[12] It is thus a challenge to find new immunoregulating compounds via the syntheses of HMBPP analogues.

Various syntheses of HMBPP have been reported,[13] but until now only few reports on HMBPP-analogues have appeared.[12,14] These analogues, but also other Vγ9/Vδ2 T cell activators as the bisphosphonates,[15] all contain a diphoshate or phosphonate function. This has serious drawbacks from therapeutic perspective, as the charged phosphate of phosphonate group imparts poor cellular permeability characteristics. In addition, phosphatases can rapidly cleave the phosphate

groups, resulting in a significant or total loss of activity. This paper describes the synthesis of new analogues of HMBPP with alternative functionalities for the diphosphate group. These analogues potentially constitute valuable tools to antagonize the HMBPP-mediated Vγ9/Vδ2 T cell response or to inhibit LytB activity. By extension, they may be valuable tool compounds to further clarify the role of Vγ9/Vδ2 T cells in host-pathogen interactions in important diseases like tuberculosis.

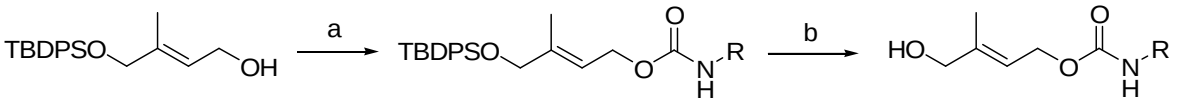
Scheme 1. Biosynthesis of IPP (1) and DMAPP (2) via the non-mevalonate pathway



2. Results and discussion

Starting from the previously described TBDPS-protected compound **8**,[16] several analogues were synthesized in which a carbamate functionality replaces the diphosphate moiety of HMBPP (Scheme 2). Having one partially positive center, this carbamate serves as an isosteric function for a monophosphate. The synthesis was pursued by reaction of **8** in CH_2Cl_2 with different isocyanates in the presence of a catalytic amount of triethylamine to give the corresponding carbamates **9-13** in very good yields (84-98%). Typically, the nitrogen was substituted with a range of aromatic groups possessing different electronic properties as described in the Topliss tree.[17] The formation of benzylcarbamate **14** required a longer reaction time and resulted in a lower yield of 70%. Since it was observed that removal of the TBDPS group using tetrabutylammonium fluoride gave purification problems due to the polarity of the final products, ammonium fluoride was selected as the reagent of choice. The reaction in methanol at room temperature was slow, but reaction rates could be improved by heating up to 50°C. This process yielded the analogues **15-20**.

Scheme 2. Synthesis of carbamate analogues 15-20



8

9: R = Ph
10: R = pMeO-Ph
11: R = pCl-Ph
12: R = pMe-Ph
13: R = 3,4-Cl_2-Ph
14: R = Bn

15: R = Ph
16: R = pMeO-Ph
17: R = pCl-Ph
18: R = pMe-Ph
19: R = 3,4-Cl_2-Ph
20: R = Bn

Reagents and conditions: a) RNCO, NEt_3, CH_2Cl_2, RT, 70-98%; b) NH_4F, MeOH, 50°C, 88-99%

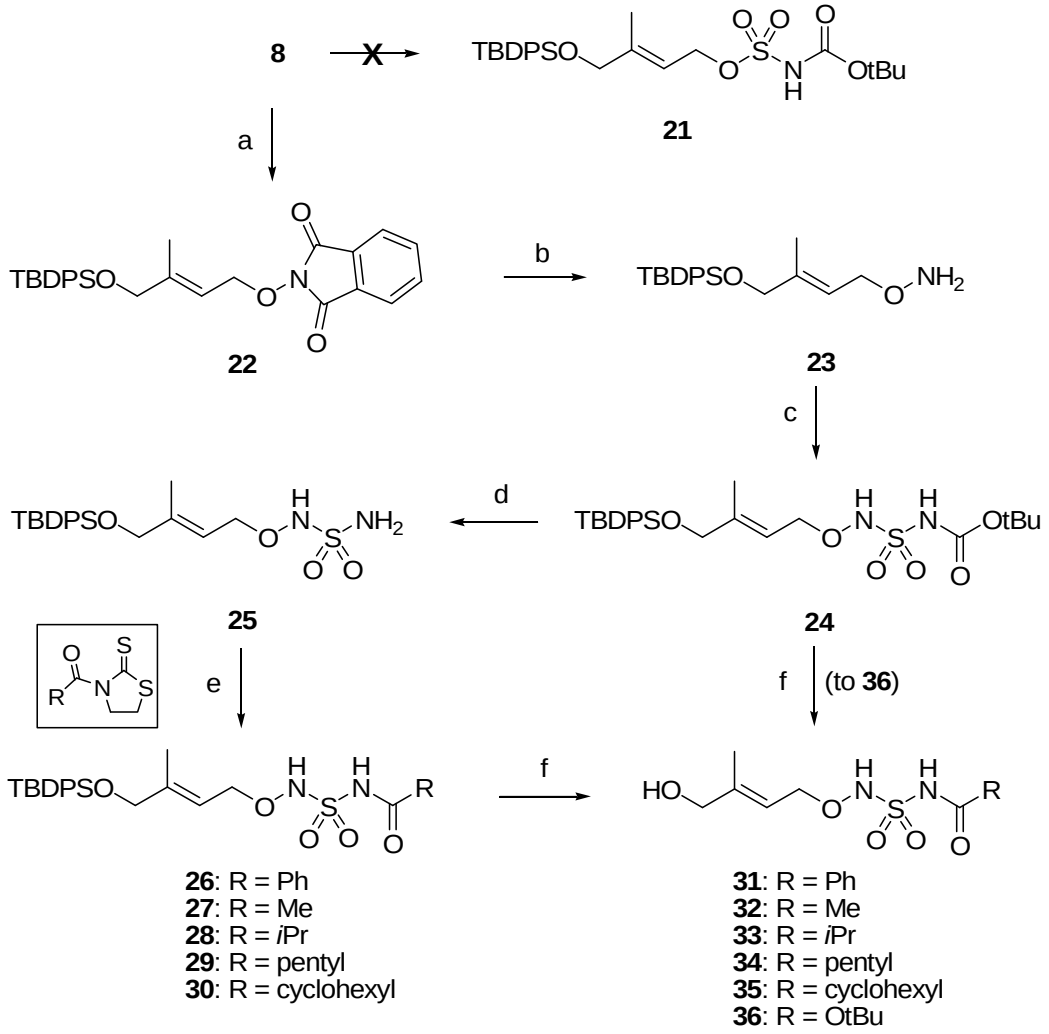
Initially an N-acyl sulfamate group was selected as an alternative isostere of the diphosphate group, since it also contains two partially positive charged centers. Introduction of this group can be performed with the chlorosulfonyl isocyanate reagent. By primary addition of an appropriate alcohol, the reagent is transformed into a carbamate protected chlorosulfonamide. In our case, best results were obtained by using tert-butanol,[18] thus generating a Boc-protecting group, but other alcohols like benzylalcohol have been used by others in the past.[19] Next the *in situ*-prepared chlorosulfonamide was reacted with alcohol **8**, but unfortunately the formed product **21** appeared to be unstable. Therefore, we envisaged the synthesis of a series of N-acyl-N'-oxy sulfamide analogues. This class of compounds was anticipated to be more stable then the N-acyl sulfamate group and can still serve as an isosteric moiety for the diphosphate.

Thus, alcohol **8** was transformed into a hydroxylamine via a Mitsunobu reaction using N-hydroxy phthalimide as the acid in the presence of diisopropylazodicarboxylate and triphenylphosphine. This reaction rendered product **22** in a good yield. Deprotection of the phthaloyl group with hydrazine hydrate in ethanol proceeded smoothly to give hydroxylamine **23** with 94% yield. This product was treated with *in situ*-prepared N-Boc-chlorosulfonamide as described above to give the N-Boc-N'-oxy product **24**. In contrast to compound **21**, product **24** is very stable. Removal of the Boc group was realized by the careful addition of a solution of trifluoroacetic acid in dichloromethane at 0°C and maintaining the resulting solution at 0°C for 24 hours.[20]

For the acylation of compound **25**, literature precedence supported the use of acid chlorides in the presence of triethylamine and 4-dimethylaminopyridine,[21] but in our case these conditions did not render any product at all. Another literature procedure, the direct coupling of the carboxylic acid and the amine with a carbodiimide reagent,[22] was also completely ineffective. When using carboxylic acids activated as the N-hydroxysuccinimide ester and DBU as the base,[23] fairly good results could be obtained. The best results, however, were generated by performing a

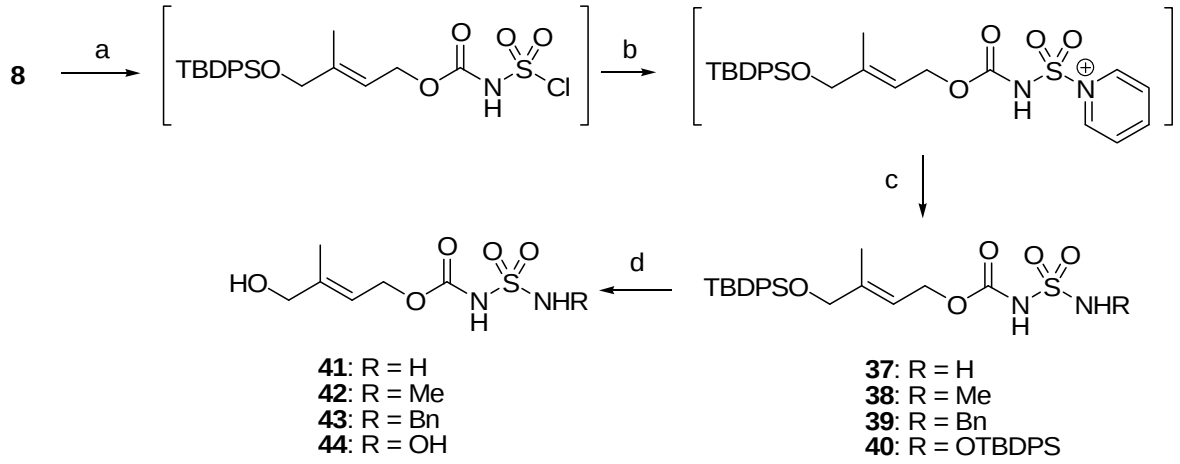
transamidation reaction, using thiazolidinethiones as the activated form of the chosen acids[24] in the presence of DBU. To our knowledge this reagent has never been used before for the acylation of sulfamates. This approach gave compounds **26-30** in fair to good yields. Finally, the products **31-35** were formed by the removal of the protecting group using ammonium fluoride, giving high yields (80-97%), except for the acetyl derivative (43%). The intermediate **24** was deprotected by the same method to yield product **36** in moderate yield (63%).

Scheme 3. Synthesis of N-acyl-N'-oxy sulfamate analogues 30-35



Reagents and conditions: a) DIAD, PPh_3, N-hydroxyphthalimide, THF, 5°C to RT, 4.5h, 85%; b) $H_2NNH_2.H_2O$, THF, EtOH, RT, 4h, 94%; c) $ClSO_2NHBoc$, CH_2Cl_2, pyridine, 0°C, 84%; d) TFA, CH_2Cl_2, 0°C, 20h, 81%; e) appropriate N-acylthiazolidinethione, DBU, THF, RT, 40-81%; f) NH_4F, MeOH, RT, 43-97%

Schema 4. Synthesis of aminosulfonyl carbamate analogues 41-44



Reagents and conditions: a) $ClSO_2NCO$, toluene, 0°C, 30 min; b) pyridine, toluene, 0°C, 30 min; c) RNH_2, 0°C to RT, 76-97%; d) NH_4F, MeOH, RT, 14-92%

An unusual reverse approach delivers the possibility of coupling two entities via an aminosulfonyl carbamate group which represents an isosteric group of the diphosphate moiety.[25] Chlorosulfonyl isocyanate first reacts with the alcohol **8** in toluene at 0°C to form the carbamate function. The resulting chlorosulfonyl carbamate could serve as the electrophile for a substitution with any amine, but since this intermediate is very unstable and easily hydrolyses, these amines should be very pure. The impractical use of, for example, liquid ammonia could be circumvented by the addition of 2.2 equivalents of pyridine. The Burgess type salt that is formed is stable to water, so even aqueous solutions of an amine can be used to perform the substitution reaction.[26]

This was exemplified by the use of aqueous ammonia in a one-pot reaction to give compound **37** in 97% yield. Other amines that were used are methylamine, benzylamine and O-TBDPS-protected hydroxylamine. Deprotection of the hydroxyl function(s) using ammonium fluoride in methanol at room temperature finally yielded the compounds **41-44**. The yield for hydroxylamine analogue **44** was significantly lower (14%) than for the others (88-92%), a result which may be explained by stability issues.

All final compounds were tested in the Vγ9Vδ2 T cell activation assay in parallel with HMBPP as positive control. As expected, significant outgrowth of Vγ9Vδ2 T cells was observed with HMBPP at concentrations down to 0.1 nM. However, there was no activity with the test compounds up to 100 μM. This result supports previous findings that the diphosphate group of HMBPP is essential for potent Vγ9Vδ2 T cell activation. It was demonstrated that only very minor modifications of this group such as in (*E*)-4-hydroxy-3-methyl-but-2-enyl-methylenediphosphonate (HMB-PCP), which represents the bis-phosphonate analogue of HMBPP, results in 10,000-fold reduced activity in the Vγ9Vδ2 T cell activation assay.[27] A QSAR study by Gossman and Oldfield revealed four essential components arranged in the appropriate relative geometry are critical for γδ T cell activation: an H-bond donor (e.g., the OH group of HMBPP (**7**)), a hydrophobic feature (e.g., the methyl group of HMBPP) and two negative ionisable groups (e.g., the two pyrophosphate phosphate groups of HMBPP).[28] While all analogues investigated contain the former two pharmacophore features, our data suggest that the polar diphosphate mimetics investigated are unable to bioisosterically replace the pyrophosphate moiety of HMBPP, despite the fact that a NH group flanked by a carbonyl and a sulphonamide as in **31**-**36** and **41**-**44** will be deprotonated at physiological pH.

The potential of compounds **15**-**20** and **31**-**36** to inhibit GcpE and LytB was also assessed (Table 1). At 1mM concentration, carbamate **18** and analogues **32** and **36**, both featuring a N-acyl-N'-oxy sulfamate moiety, were found to marginally inhibit GcpE or LytB.

Table 1. Inhibition of GcpE and LytB activity by the target compounds at 1 mM.

Compound	GcpE	LytB
	% residual activity at 1 mM	
15	98	90
16	96	85
17	94	89
18	90	62
19	81	95
20	89	92
31	94	79
32	66	87
33	95	98
34	95	98
35	99	102
36	99	61

In conclusion, we have reported the syntheses of three types of HMBPP analogues with some structurally diverse diphosphate mimicking groups. Incorporation of a carbamate function mimics a phosphate group. Also, two isosteres of the diphosphate group were introduced. The N-acyl-N'-oxy sulfamate function was formed via coupling of the isoprenoid alcohol and an *in situ*-protected chlorosulfamide, followed by deprotection and acylation using thiazolidinethione reagents. The aminosulfonyl carbamate moiety was prepared via a three-step one-pot reaction, with a Burgess-type salt as an intermediate. The isoprenoid part of HMBPP was left unchanged.

All compounds investigated failed to show significant biological activity.

4. Experimental section

General procedure for the synthesis of compounds 9-14. To a 0.1M solution of compound **8** in dry dichloromethane were added the appropriate isocyanate (1.1 eq) and triethylamine (0.1 eq). The reaction mixture was stirred at room temperature until completion was determined via TLC-analyses. The reaction was quenched by the addition of methanol, and the mixture was concentrated under reduced pressure. The resulting residue was purified by column chromatography to yield compounds **9-14**.

(2*E*)-4-(*tert*-butyldiphenylsilyloxy)-3-methylbut-2-en-1-yl phenylcarbamate 9

Preparation of the title compound according to general procedure described above gave 127 mg colourless oil (90%). R_f 0.23 (hexane/ethylacetate 9/1); ^{1}H-NMR (300.01 MHz, aceton-d_6) δ 8.65 (1H, br s), 7.74-7.69 (4H, m), 7.60-7.57 (2H, m), 7.49-7.39 (6H, m), 7.33-7.26 (2H, m), 7.01 (1H, ddt, J = 7.6, 7.0, 1.2 Hz), 5.81 (1H, tqt, app. t sext, J = 7.0, 1.5 Hz), 4.73 (2H, dq, app. br dd, J = 7.0, 0.6 Hz), 4.15 (2H, q, J = 0.6 Hz), 1.72 (3H, m), 1.06 (9H, s); ^{13}C-NMR (75.00 MHz, aceton-d_6) δ 153.8 (C), 139.8 (C), 139.7 (C), 135.6 (CH), 133.6 (C), 130.1 (CH), 128.9 (CH), 128.0 (CH), 122.7 (CH), 118.5 (CH), 67.9 (CH_2), 60.8 (CH_2), 26.5 (CH_3), 19.2 (C), 13.0 (CH_3); Exact mass (ESI-MS): calculated for $C_{28}H_{33}NO_3Si$ [M+Na^+] 482.2128; found 482.2121

General procedure for the synthesis of compounds 15-20. To a 0.2M solution of the carbamates **9-14** in methanol was added ammonium fluoride. The reaction mixture was heated to 50°C and stirred as such until complete reaction was visible by TLC-analyses. Concentrating under reduced pressure gave a residue which was purified by column chromatography, to afford the desired unprotected carbamates **15-20**.

(2*E*)-4-hydroxy-3-methylbut-2-en-1-yl phenylcarbamate 15

Preparation of the title compound according to general procedure described above gave 47 mg colourless oil (94%). R_f 0.23 (hexane/ethylacetate 1/1); ^{1}H-NMR (300.01 MHz, aceton-d_6) δ 8.60 (1H, br s), 7.58-7.54 (2H, m), 7.32-7.25 (2H, m), 7.03-6.98 (1H, m), 5.65 (1H, tqt, app. t sext, J = 7.0, 1.5 Hz), 4.68 (2H, br dd, J = 7.0, 0.6 Hz), 3.97-3.90 (3H, m); ^{13}C-NMR (75.00 MHz, aceton-d_6) δ 153.8 (C), 141.5 (C), 139.7 (C), 128.9 (CH), 122.7 (CH), 118.4 (CH), 118.0 (CH), 66.5 (CH_2), 60.9 (CH_2), 13.1 (CH_3); Exact mass (ESI-MS): calculated for $C_{12}H_{15}NO_3$ [M+Na^+] 244.0950; found 244.0942

N-{[(2*E*)-4-(*tert*-butyldiphenylsilyloxy)-3-methylbut-2-en-1-yl]oxy} phtalimide 22

Compound **8** (5.00 g, 14.68 mmol), triphenylphosphine (4.62 g, 17.62 mmol, 1.2 eq) and N-hydroxyphthalimide (2.87 g, 17.62 mmol, 1.2 eq) were dissolved in dry THF (58.72 mL) and the solution was cooled to 5°C. Diisopropyl azodicarboxylate (3.47 mL, 17.62 mmol, 1.2 eq) was added dropwise. The reaction was stirred below 10°C for 3 hours, and then allowed to warm up to room temperature. After 1.5 hours the reaction mixture was concentrated under reduced pressure. The resulting residue was purified via column chromatography (hexane/ethyl acetate

8/2) to give pure **22** (6.07 g, 85%) as an oil. ^{1}H-NMR (300.01 MHz, DMSO-d_6) δ 7.87 (4H, m), 7.61-7.58 (4H, m), 7.47-7.39 (6H, m), 5.87 (1H, tqt, app. t sext, J = 7.5, 1.5 Hz), 4.80 (2H, d, J = 7.8 Hz), 4.05 (2H, s), 1.60 (3H, s), 0.98 (9H, s); ^{13}C-NMR (75.00 MHz, DMSO-d_6) δ 164.3 (C), 144.4 (C), 135.9 (CH), 135.8 (CH), 133.7 (C), 130.9 (CH), 129.4 (C), 128.9 (CH), 124.2 (CH), 116.3 (CH), 73.6 (CH_2), 67.9 (CH_2), 27.5 (CH_3), 19.7 (C), 14.3 (CH_3); Exact mass (ESI-MS): calculated for $C_{29}H_{31}NO_4Si$ [M+K^+] 524.1659; found 524.1655

(2*E*)-4-(aminooxy)-1-(*tert*-butyldiphenylsilyloxy)-2-methyl-but-2-ene 23

To a solution of **22** (11.14 g, 22.94 mmol) in THF (57.35 mL) and ethanol (57.35 mL) was added hydrazine hydrate (3.345 mL, 68.82 mmol). The reaction mixture was stirred at room temperature for 4 hours. It was diluted with ether (500 mL) and washed with water (2 x 200 mL) and brine (150 mL). Drying over Na_2SO_4, filtration and concentration under reduced pressure gave a residue that was purified by column chromatography (dichloromethane/methanol 99/1) to yield pure **23** (7.64 g, 94%) as an oil. ^{1}H-NMR (300.01 MHz, DMSO-d_6) δ 7.65-7.60 (4H, m), 7.50-7.40 (6H, m), 5.89 (2H, br s), 5.65 (1H, tqt, app. t sext, J = 6.6, 1.5 Hz), 4.12 (2H, dd, J = 6.6 Hz, 0.9 Hz), 4.07 (2H, s), 1.58 (3H, m), 1.01 (9H, s); ^{13}C-NMR (75.00 MHz, DMSO-d_6) δ 137.4 (C), 134.8 (CH), 132.9 (C), 129.8 (CH), 127.8 (CH), 119.4 (CH), 71.0 (CH_2), 67.5 (CH_2), 26.5 (CH_3), 18.8 (C), 13.4 (CH_3); Exact mass (ESI-MS): calculated for $C_{21}H_{29}NO_2Si$ [M+H^+] 356.2045; found 356.2036

***tert*-butyl [({[(2*E*)-4-(*tert*-butyldiphenylsilyloxy)-3-methylbut-2-en-1-yl]oxy}amino)sulfonyl] carbamate 24**

To a solution of chlorosulfonyl isocyanate (1.13 mL, 12.95 mmol, 5 eq) in dry dichloromethane (20 mL), cooled to 0°C, was added dropwise a solution of tert-butanol (1.30 mL, 13.73 mmol, 5.3 eq) in dry dichloromethane (4 mL). The mixture was stirred at 0°C for 1.5 hours and then added dropwise to a solution of **23** (920 mg, 2.59 mmol) in dry pyridine (10.36 mL), cooled to 0°C. The reaction mixture was allowed to warm up to room temperature slowly, and stirred for 17 hours. Reaction work-up was performed by diluting with ethyl acetate (150 mL), washing with 5% citric acid solution (2 x 150 mL), water (150 mL) and brine (150 mL). Drying over $MgSO_4$, filtration and concentration under reduced pressure gave a residue that was purified by column chromatography (dichloromethane/ethanol 99/1) to yield pure **24** (1.16 g, 84%) as an oil. ^{1}H-NMR (300.01 MHz, aceton-d_6) δ 10.08 (1H, br s), 8.86 (1H, br s), 7.74-7.69 (4H, m), 7.50-7.40 (6H, m), 5.78 (1H, tqt, app. t sext, J = 6.9, 1.5 Hz), 4.57 (2H, dd, J = 6.9, 0.6 Hz), 4.15 (2H, m), 1.68 (3H, m), 1.47 (9H, s), 1.07 (9H, s); Exact mass (ESI-MS): calculated for $C_{26}H_{38}N_2O_6SSi$ [M+Na^+] 557.2117; found 557.2106

_N_-{[(2_E_)-4-(_tert_-butyldiphenylsilyloxy)-3-methylbut-2-en-1-yl]oxy}sulfamide 25

To a solution of **24** (2.760 g, 5.16 mmol) in dry dichloromethane (10.3 mL) was added at 0°C a 50% solution of trifluoroacetic acid in dichloromethane (9 mL) in a dropwise manner over 10 hours. The reaction was then stirred at 0°C for another 10 hours. The reaction mixture was concentrated and the remaining residue was co-evaporated with dichloromethane (20 mL) two times. Purification by column chromatography (dichloromethane/ethanol 98/2) gave pure **25** (1.806 g, 81%) as an oil. R_f 0.24 (dichloromethane/ethanol 98/2); ^{1}H-NMR (300.01 MHz, aceton-d_6) δ 8.42 (1H, br s), 7.73-7.70 (4H, m), 7.47-7.40 (6H, m), 6.31 (2H, m), 5.79 (1J, br t, J = 6.9 Hz), 4.55 (2H, d, J = 6.9 Hz), 4.14 (2H, s), 1.67 (3H, s), 1.07 (9H, s); ^{13}C-NMR (75.00 MHz,

aceton-d_6) δ 140.9 (C), 136.2 (CH), 134.3 (C), 130.7 (CH), 128.6 (CH), 119.2 (CH), 72.7 (CH_2), 68.8 (CH_2), 27.2 (CH_3), 19.8 (C), 13.9 (CH_3); Exact mass (ESI-MS): calculated for $C_{21}H_{30}N_2O_4SSi$ [$M+H^+$] 435.1773; found 435.1770

General procedure for the synthesis of the compounds 26-29

To a 0.2 M solution of **25** in dry THF were added the appropriate thiazolidinethione reagent (1 eq) and DBU (1 eq). The reaction was stirred at room temperature until completion. Tetramethylguanidine (0.2 eq) was added and stirring was continued for 1 hour. Next the reaction mixture was diluted with dichloromethane and washed with 1N solution of HCl and brine. Drying over $MgSO_4$, filtration and concentration under reduced pressure gave a residue that was purified by column chromatography to give the respective compounds **26-29**.

***N*-[({[(2*E*)-4-(*tert*-butyldiphenylsilyloxy)-3-methylbut-2-en-1-yl]oxy}amino)sulfonyl] benzamide 26**

Preparation of the title compound according to general procedure described above gave 100 mg as a colourless oil (62%). ^{1}H-NMR (300.01 MHz, aceton-d_6) δ 11.00 (1H, br s), 9.03 (1H, s), 8.06-8.03 (2H, m), 7.71-7.65 (5H, m), 7.57-7.52 (2H, m), 7.45-7.36 (6H, m), 5.76 (1H, tqt, app. t sext, J = 6.9, 1.5 Hz), 4.58 (2H, dd, J = 6.9, 0.6 Hz), 4.13 (2H, m), 1.67 (3H, m), 1.05 (9H, s); ^{13}C-NMR (75.00 MHz, aceton-d_6) δ 166.7 (C), 142.2 (C), 136.3 (CH), 134.3 (C), 132.5 (C), 130.7 (CH), 129.7 (CH), 129.2 (CH), 128.7 (CH), 118.2 (CH), 73.4 (CH_2), 68.8 (CH_2), 27.2 (CH_3), 19.8 (C), 13.9 (CH_3); Exact mass (ESI-MS): calculated for $C_{28}H_{34}N_2O_5SSi$ [$M+K^+$] 577.1594; found 577.1582

***N*-[({[(2*E*)-4-(*tert*-butyldiphenylsilyloxy)-3-methylbut-2-en-1-yl]oxy}amino)sulfonyl] cyclohexane-carboxamide 30**

Preparation of the title compound according to general procedure described above gave 186 mg white crystals (81%). mp 133-134°C; ^{1}H-NMR (300.01 MHz, aceton-d_6) δ 10.43 (1H, br s), 8.75 (1H, s), 7.73-7.69 (4H, m), 7.48-7.40 (6H, m), 5.77 (1H, tqt, app. t sext, J = 6.9, 1.5 Hz), 4.56 (2H, dd, J = 6.9, 0.6 Hz), 4.14 (2H, br d, J = 0.9 Hz), 2.42 (1H, tt, J = 11.2, 3.2 Hz), 1.90-1.85 (2H, m), 1.78-1.72 (2H, m), 1.67 (3H, m), 1.65-1.60 (1H, m), 1.50-1.19 (5H, m), 1.07 (9H, s); ^{13}C-NMR (75.00 MHz, aceton-d_6) δ 175.7 (C), 141.9 (C), 136.3 (CH), 134.3 (C), 130.7 (CH), 128.7 (CH), 118.2 (CH), 73.3 (CH_2), 68.7 (CH_2), 45.3 (CH), 29.7 (CH_2), 27.2 (CH_3), 26.3 (CH_2), 26.0 (CH_2), 19.9 (C), 13.9 (CH_3); Exact mass (ESI-MS): calculated for $C_{28}H_{40}N_2O_5SSi$ [M+Na^+] 567.2325; found 567.2319

General procedure for the synthesis of compounds 31-36

To a 0.2M solution of compounds **26-30** in methanol was added ammonium fluoride (2 eq). The mixture was stirred at room temperature until the reaction was complete as indicated by TLC-analysis. Removal of solvent under reduced pressure gave a residue that was purified by column chromatography, to yield compounds **31-36**.

***N*-[({[(2*E*)-4-hydroxy-3-methylbut-2-en-1-yl]oxy}amino)sulfonyl]benzamide 31**

Preparation of the title compound according to general procedure described above gave 91 mg as a gum (91%). ^{1}H-NMR (300.01 MHz, aceton-d_6) δ 8.95 (1H, br s), 8.06-8.02 (2H, m), 7.67-7.61 (1H, m), 7.55-7.48 (2H, m), 5.59 (1H, tqt, app. t sext, J = 6.9, 1.5 Hz), 4.53 (2H, dd, J = 6.9, 0.6 Hz), 3.93 (2H, s), 1.65 (3H, m); ^{13}C-NMR (75.00 MHz, aceton-d_6) δ 143.5 (C), 134.0 (CH),

133.2 (C), 129.5 (CH), 129.3 (CH), 117.9 (CH), 73.5 (CH_2), 67.2 (CH_2), 13.9 (CH_3); Exact mass (ESI-MS): calculated for $C_{12}H_{16}N_2O_5S$ [M+Na^+] 323.0678; found 323.0669

General procedure for the synthesis of compounds 37-40

To a 0.75M solution of **8** in dry toluene, cooled to 0°C, was added dropwise chlorosulfonyl isocyanate (1 eq). After stirring for 30 minutes, the reaction is triturated to a concentration of 0.1 M by the addition of dry toluene. Pyridine (2.2 eq) was added and stirring was continued for 30 minutes at 0°C. Subsequently, a solution of the appropriate amine (6 eq) in water or THF was added. The reaction was stirred at 0°C for 2 hours and than at room temperature until completion was noted. The reaction mixture was poured out into a mixture of water and EtOAc. The pH was lowered to 1 by adding concentrated sulfuric acid. The aqueous layer was extracted with EtOAc and the combined organic layers were washed with brine. After drying over $MgSO_4$, filtering and concentrating of the solution, a residue was obtained that was purified by column chromatography to yield compounds **37-40**.

(2*E*)-4-(*tert*-butyldiphenylsilyloxy)-3-methylbut-2-en-1-yl (aminosulfonyl)carbamate 37

Preparation of the title compound according to general procedure described above gave 136 mg white crystals (97%). R_f 0.22 (hexane/ethylacetate 65/35); mp 78-79°C; ^{1}H-NMR (300.01 MHz, aceton-d_6) δ 9.92 (1H, br s), 7.73-7.69 (4H, m), 7.50-7.41 (6H, m), 6.66 (2H, br s), 5.78 (1H, tqt, t sext, J = 7.0, 1.5 Hz), 4.75 (2H, dd, J = 7.0, 0.6 Hz), 4.15 (2H, br s), 3.41 (3H, m), 1.06 (9H, s); ^{13}C-NMR (75.00 MHz, aceton-d_6) δ 152.3 (C), 140.8 (C), 135.6 (CH), 133.5 (C), 130.1 (CH), 128.1 (CH), 117.6 (CH), 67.9 (CH_2), 62.1 (CH_2), 26.6 (CH_3), 19.2 (C), 13.1 (CH_3); Exact mass (ESI-MS): calculated for $C_{22}H_{30}N_2O_5SSi$ [M+Na^+] 485.1543; found 485.1537

General procedure for the synthesis of compounds 41-44

To a 0.2M solution of compounds **36-40** in methanol was added ammonium fluoride. The reaction was stirred at room temperature until completion was determined by TLC-analysis. Removal of the solvent under reduced pressure gave a residue that was purified by column chromatography to yield compounds **41-44**.

(2*E*)-4-hydroxy-3-methylbut-2-en-1-yl (aminosulfonyl)carbamate 41

Preparation of the title compound according to general procedure described above gave 35 mg as a sticky oil (92%). R_f 0.15 (dichloromethane/methanol 9/1); ^{1}H-NMR (300.01 MHz, aceton-d_6) δ 11.08 (1H, br s), 7.39 (2H, br s), 5.53 (1H, tqt, app. t sext, J = 7.3, 1.5 Hz), 4.90 (1H, t, J = 5.6 Hz), 4.64 (2H, d, J = 7.0 Hz), 3.82 (2H, br d, J = 5.3 Hz), 1.62 (3H, br s); ^{13}C-NMR (75.00 MHz, aceton-d_6) δ 152.4 (C), 142.4 (C), 117.0 (CH), 66.3 (CH_2), 62.1 (CH_2), 13.1 (CH_3); Exact mass (ESI-MS): calculated for $C_6H_{12}N_2O_5S$ [M+Na^+] 247.0365; found 247.0359

GcpE and LytB inhibition assay

Recombinant GcpE and LytB of *Thermus thermophilus* and *Aquifex aeolicus*, respectively, were produced as described.[29,30] Protein purification and conduct of the enzyme assays was carried out under anaerobic conditions in a tent (Coy Laboratory Products, Inc., Grass Lake, USA) floated with a gas mixture consisting of 95% N_2 and 5% H_2. Residual O_2 was removed with palladium catalysts. Buffers were degassed in an ultrasound bath by bubbling a stream of helium through the liquid. Before use, the buffers were equilibrated overnight in the tent under stirring. For the enzyme activity assays a spectrophotometer (DU 530 with a Peltier temperature control module,

Beckman Coulter) was installed inside the tent. The activity of GcpE and LytB was determined by monitoring the oxidation of dithionite-reduced methyl viologen at 732 nm (ε_{732} = 2200 $M^{-1}cm^{-1}$). For the GcpE assay, the reaction mixture consisted of 150 mM NaCl, 30 mM Tris-HCl (pH 7.5), 0.2% bovine serum albumin (BSA), 2 mM methyl viologen, 1 mM MEcPP and 2.5 μM GcpE in a total volume of 0.8 ml. For the LytB assay, the reaction mixture consisted of 150 mM NaCl, 30 mM Tris-HCl (pH 7.5), 0.2% BSA, 2 mM methyl viologen, 1 mM HMBPP and 0.1 μM LytB in a total volume of 0.8 ml. The methyl viologen was partly reduced by the addition of sodium dithionite until an extinction between 1.3 and 1.4 at 732 nm was reached, corresponding to 0.59 to 0.64 mM reduced methyl viologen. Typically, 25 μl of a 10 mM sodium dithionite stock solution were added to the reaction mixture. The solid sodium dithionite was stored in the oxygen-free tent and the stock solution freshly prepared under anaerobic conditions. For the inhibition assays, the test compounds were dissolved in DMSO at 100 mM and added to the reaction mixture at a final concentration of 1 mM. The activity was recorded in comparison to a mock control with DMSO.

Vγ9Vδ2 T cell activation assay

Flow cytometric analysis of human Vγ9Vδ2 T cells was basically performed as described.[31] 2 x 10^5 peripheral blood mononuclear cells (PBMC) were seeded in 200 ml RPMI 1640 medium supplemented with 25 mM HEPES, 2 mM L-glutamine, 25 μg/ml gentamycin, 10 U/ml recombinant human interleukin-2 and 10% human AB serum. The test samples were dissolved in DMSO at 10 mM and added to the assay in 3-fold serial dilutions ranging from 0.14 μM to 100 μM. As negative control, DMSO was used at the same dilutions. As positive control, HMBPP in a 10-fold serial dilution ranging form 0.01 nM and 10 μM was used. All compounds were tested

in duplicates with PBMCs from 3 different donors. After incubation for 6 days at 37°C and 5 % CO_2, the cells were analysed on an Epics XL flow cytometer supported with Expo32 software (Beckman Coulter), using CD3-FITC and TCRVgamma9-PC5 antibodies (Beckman Coulter).

5. Acknowledgment

This study was supported by grants from the European Commission (STREP, LSH-2003-2.3.0-1), INTAS (03-51-4077) and by the Deutsche Forschungsgemeinschaft Grant JO565/1-1 (to H. J.).

6. Supporting information available:

General experimental procedures, analytical data on compounds **10-14**, **16-20**, **27-29**, **32-36**, **38-40**, **42-44** and ^{13}C-NMR spectra of compounds **9-20**, **22-23** and **25-44** and ^{1}H-NMR spectrum of compound **24**. This material is available free of charge via the Internet at http://pubs.acs.org.

7. References

[1] Rohdich, F.; Kis, K.; Bacher, A.; Eisenreich, W. *Curr. Opin. Chem. Biol.* **2001**, *5*, 535-540

[2] Eberl, M.; Hintz, M.; Reichenberg, A.; Kollas, A.-K.; Wiesner, J.; Jomaa, H. *FEBS Lett.* **2003**, *544*, 4-10

[3] Eisenreich, W.; Bacher, A.; Arigoni, D.; Rohdich, F. *Cell. Mol. Life Sci.* **2004**, *61*, 1401-1426

[4] Pink, R.; Hudson, A.; Mouriès, M.-A.; Bendig, M. *Nat. Rev. Drug Discov.* **2005**, *4*, 727-740

[5] (a) Cunningham Jr.; F. X.; Lafond, T. P.; Gantt, E. *J. Bacteriol.* **2000**, *182*, 5841-5848; (b) Altincicek, B.; Duin, E. C.; Reichenberg, A.; Hedderich, R.; Kollas, A.-K.; Hintz, M.; Wagner, S.; Wiesner, J.; Beck, E.; Jomaa, H. *FEBS Lett.* **2002**, *532*, 437-440; (c) Rohdich, F.; Hecht, S.; Gärtner, K.; Adam, P.; Krieger, C.; Amslinger, S.; Arigoni, D.; Bacher, A.; Eisenreich, W. *Proc. Nat. Acad. Sci. USA* **2002**, *99*, 1158-1163

[6] Gräwert, T.; Kaiser, J.; Zepeck, F.; Laupitz, R.; Hecht, S.; Amslinger, S.; Schramek, N.; Schleicher, E.; Weber, S.; Haslbeck, M.; Buchner, J.; Rieder, C.; Arigoni, D.; Bacher, A.; Eisenreich, W.; Rohdich, F. *J. Am. Chem. Soc.* **2004**, *126*, 12847-12855

[7] Hintz, M.; Reichenberg, A.; Altincicek, B.; Bahr, U.; Gschwind, R. M.; Kollas, A.-K.; Beck, E.; Wiesner, J.; Eberl, M.; Jomaa, H. *FEBS Lett.* **2001**, *509*, 317-322

[8] Vermijlen, D.; Ellis, P.; Langford, C.; Klein, A.; Engel, R.; Willimann, K.; Jomaa H.; Hayday, A.C.; Eberl, M. *J. Immunol.* **2007**,*178*, 4304-14

[9] Ali, Z.; Shao, L.; Halliday, L.; Reichenberg, A.; Hintz, M.; Jomaa, H.; Chen, Z.W. *J. Immunol.* **2007**, *179*, 8287-96

[10] Sicard, H.; Ingoure, S.; Luciani, B.; Serraz, C.; Fournié, J.J.; Bonneville, M.; Tiollier, J.; Romagné, F. *J. Immunol.* **2005**, *175*, 5471-80

[11] Tanaka, Y.; Morita, C. T.; Tanaka, Y.; Nieves, E.; Brenner, M. B.; Bloom, B. R. *Nature* **1995**, *375*, 155-158

[12] Amslinger, S.; Hecht, S.; Rohdich, F.; Eisenreich, W.; Adam, P.; Bacher, A.; Bauer, S. *Immunobiology* **2007**, *212*, 47-55

[13] (a) Wolff, M.; Seemann, M.; Grosdemange-Billiard, C.; Tritsch, D.; Campos, N.; Rodríguez-Concepción, M.; Boronat, A.; Rohmer, M. *Tetrahedron Lett.* **2002**, *43*, 2555-2559; (b) Amslinger, S.; Kis, K.; Hecht, S.; Adam, P.; Rohdich, F.; Arigoni, D.; Bacher, A.; Eisenreich, W. *J. Org. Chem.* **2002**, *67*, 4590-4594; (c) Fox, D. T.; Poulter, C. D. *J. Org. Chem.* **2002**, *67*, 5009-5010; (d) Gao, W.; Loeser, R.; Raschke, M.; Dessoy, M. A.; Fulhorst, M.; Alpermann, H.; Wessjohann, L. A.; Zenk, M. H. *Angew. Chem. Int. Ed.* **2002**, *41*, 2604-2607; (e) Giner, J.-L. *Tetrahedron Lett.* **2002**, *43*, 5457-5459

[14] (a) Espinosa, E.; Belmant, C.; Pont, F.; Luciani, B.; Poupot, R.; Romagné, F.; Brailly, H.; Bonneville, M.; Fournié, J.-J. *J. Biol. Chem.* **2001**, *276*, 18337-18344; (b) Reichenberg, A.; Hintz, M.; Kletschek, Y.; Kuhl, T.; Haug, C.; Engel, R.; Moll, J.; Ostrovsky, D. N.; Jomaa, H.; Eberl, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1257-1260; (c) Song, Y.; Zhang, Y.; Wang, H.; Raker, A. M.; Sanders, J. M.; Broderick, E.; Clark, A.; Morita, C. T.; Oldfield, E. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4471-4477

[15] Sanders, J. M.; Ghosh, S.; Chan, J. M. W.; Meints, G.; Wang, H.; Raker, A. M.; Song, Y.; Colantino, A.; Burzynska, A.; Kafarski, P.; Morita, C. T.; Oldfield, E. *J. Med. Chem.* **2004**, *47*, 375-384

[16] Ikeda, S.; Shibuya, M.; Iwabuchi, Y. *Chem. Commun.* **2007**, 504-506

[17] Topliss, J. G. *J. Med. Chem.* **1972**, *15*, 1006-1011

[18] (a) Ragaïna, Z.; Abdaoui, M.; Aouf, N.-E.; Dewynter, G.; Montero, J.-L. *Tetrahedron* **2000**, *56*, 381-387; (b) Zhong, J.; Lai, Z.; Groutas, C. S.; Wong, T.; Gan, X.; Alliston, K. R.; Eichhorn, D.; Hoidal, J. R.; Groutas, W. C. *Bioorg. Med. Chem.* **2004**, *12*, 6249-6254; (c) Salim, S. S.; Bellingham, R. K.; Brown, R. C. D. *Eur. J. Org. Chem.* **2004**, 800-806

[19] Park, J. D.; Kim, D. H.; Kim, S.-J.; Woo, J.-R.; Ryu, S. E. *J. Med. Chem.* **2002**, *45*, 5295-5302

[20] Dewynter, G.; Aouf, N.; Criton, M.; Montero, J.-L. *Tetrahedron* **1993**, *49*, 65-76

[21] Hamprecht, G.; Puhl, M.; Reinhard, R.; Sagasser, I.; Schmidt, T.; Goetz, N.; Zierke, T. PCT Int. Appl. WO 2003097589, 2003

[22] Lee, J.; Kim, S. E.; Lee, J. Y.; Kim, S. Y.; Kang, S. U.; Seo, S. H.; Chun, M. W.; Kang, T.; Choi, S. Y.; Kim, H. O. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1087-1092

[23] Somu, R. V.; Boshoff, H.; Qiao, C.; Bennett, E. M.; Barry, C. E.; Aldrich, C. C. *J. Med. Chem.* **2006**, *49*, 31-34

[24] Nagao, Y.; Seno, K.; Kawabuta, K.; Miyasaka, T.; Takao, S.; Fujita, E. *Tetrahedron Lett.* **1980**, *21*, 841

[25] (a) Camarasa, M. J.; Fernandez-Resa, P.; Garcia-Lopez, M. T.; De las Heras, F. G.; Mendez-Castrillon, P. P.; Alarcon, B.; Carrasco, L. *J. Med. Chem.* **1985**, *28*, 40-46; (b) Castro, A.; Erickson, S. E.; Shechter, I.; Spencer, T. A. *Bioorg. Chem.* **1996**, *24*, 242-250; (c) Nguyen, C.; Kasinathan, G.; Leal-Cortijo, I.; Musso-Buendia, A.; Kaiser, M.; Brun, R.; Ruiz-Pérez, L. M.; Johansson, N. G.; González-Pacanowska, D.; Gilbert, I. H. *J. Med. Chem.* **2005**, *48*, 5942-5954

[26] Masui, Y.; Watanabe, H.; Masui, T. *Tetrahedron Lett.* **2004**, *45*, 1853-1856

[27] Reichenberg, A.; Hintz, M.; Kletschek, Y.; Kuhl, T.; Haug, C.; Engel, R.; Moll, J.; Ostrovsky, D.N.; Jomaa, H.; Eberl, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1257-60

[28] Gossman, W.; Oldfield, E. *J. Med. Chem.* **2002**, *45*, 4868-4874.

[29] Kollas, A.K.; Duin, E.C.; Eberl, M.; Altincicek, B.; Hintz, M.; Reichenberg, A.; Henschker, D.; Henne, A.; Steinbrecher, I.; Ostrovsky, D.N.; Hedderich, R.; Beck, E.; Jomaa, H.; Wiesner, J. *FEBS Lett.* **2002**, *532*, 432-436

[30] Altincicek, B.; Duin, E.C.; Reichenberg, A.; Hedderich, R.; Kollas, A.K.; Hintz, M.; Wagner, S.; Wiesner, J.; Beck, E.; Jomaa, H. *FEBS Lett.* **2002**, 532, 437-40

[31] Constant, P. ; Davodeau, F. ; Peyrat, M.A. ; Poquet, Y.; Puzo, G. ; Bonneville, M. ; Fournié, J.J. *Science* **1994**, 264, 267-70