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# The synthesis of a menthol derivative of 2-aminopurine as a fluorescent DNA lesion

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ABSTRACT

An efficient synthetic route to the phosphoramidite of a menthol functionalized guanosine analog is presented. Two procedures were executed for the key introduction of the 6′-allyl menthyl moiety. Stille vinylation on 6-*O*-tosylguanosine followed by cross-metathesis using an excess of allyl menthyl ether proved to be less efficient than a Stille coupling on the same tosylate using an advanced menthyl-allyl stannane derivative. Incorporation of the modified nucleoside using the phosphoramidite method into a DNA 50-mer proceeded uneventfully.



## 1. Introduction

Modified nucleic acids play an important role in biochemistry and medicinal chemistry.[1] Amongst the modifications, fluorescent nucleosides are frequently exploited as reporters in structural studies and for the elucidation of fundamental biological processes, such as DNA repair.[2] In this context we are engaged in a study on nucleotide excision repair (NER), an evolutionary highly conserved DNA repair mechanism, which recognizes a range of structurally unrelated DNA lesions, including artificially made modifications.[3,4] NER is a complicated process, in which recognition of a DNA lesion, and subsequent DNA incision is accomplished by the action of three proteins: UvrA, UvrB, and UvrC. A synthetic oligonucleotide, equipped with a non-natural cholesterol modified deoxyribose as a damage mimic and 2-aminopurine as fluorescence reporter was recently applied to elucidate the DNA repair mechanism.[5] It was demonstrated that upon UvrB binding to the cholesterol lesion both the base adjacent to the 3′-end of the lesion and the base opposite to the lesion in the undamaged strand are flipped out of the DNA helix. To further investigate the function of base flipping in the damage specific binding of UvrB in the NER process, the availability of a fluorescent nucleotide that is recognized as a damage site would be advantageous. Based on the fluorescent properties of 2-aminopurine and our finding that a menthol-thymine adduct is recognized as a damage,[4] nucleoside **1** was designed, in which a menthyl moiety is attached at C-6 of 2-aminopurine via an allyl spacer. Herein we present a synthetic route toward suitably protected phosphoramidite **2** and its incorporation into DNA 50-mer **I** (Fig. 1).

## 2. Results and discussion

Key in the synthetic route of phosphoramidite **2** is an efficient procedure to install the allyl menthyl moiety at C-6 of an easily available nucleoside to obtain a suitably protected fluorescent 2-aminopurine derivative. Deoxyguanosine (dG) was selected as a convenient starting compound as installation of a 6-*O*-tosyl function in dG allows a number of transformations such as palladium catalyzed cross-couplings.[6] In this context, Sasaki and co-workers reported in 1997 that 6-vinyl-2-aminopurine can be prepared via Stille vinylation of 6-*O*-tosyl dG.[6b] It was envisaged that implementation of this procedure would allow elongation of

I = 5′-GGGATTACTTACGGGCACATTACAAA**X**AAACCTCAGAACGACCTCACACG-3′

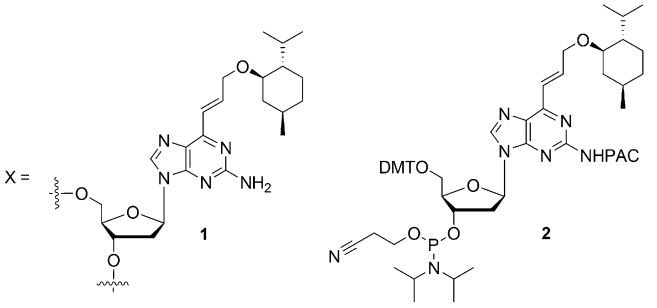


**Figure 1.** Chemical structures of nucleoside **1**, phosphoramidite **2**, and oligonucleotide **I**.

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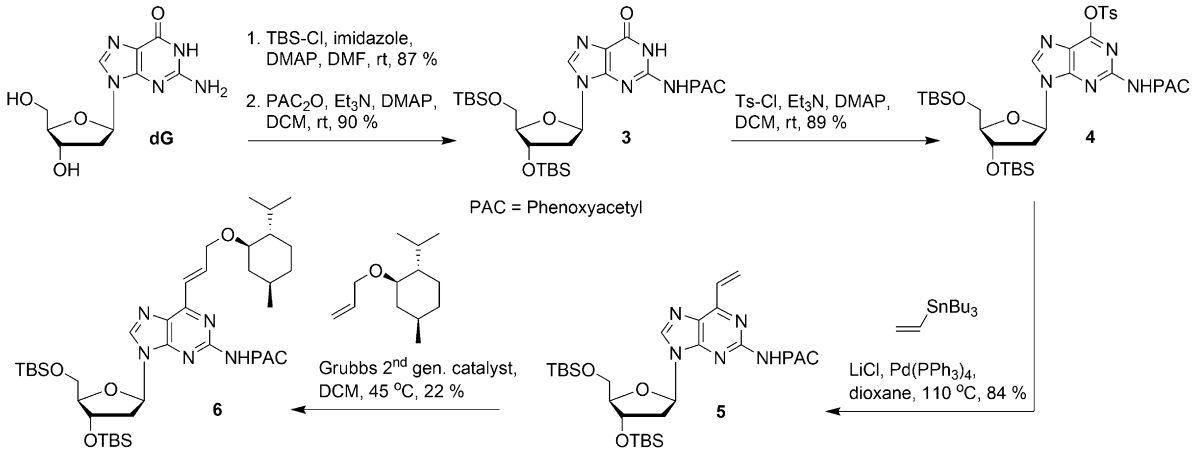
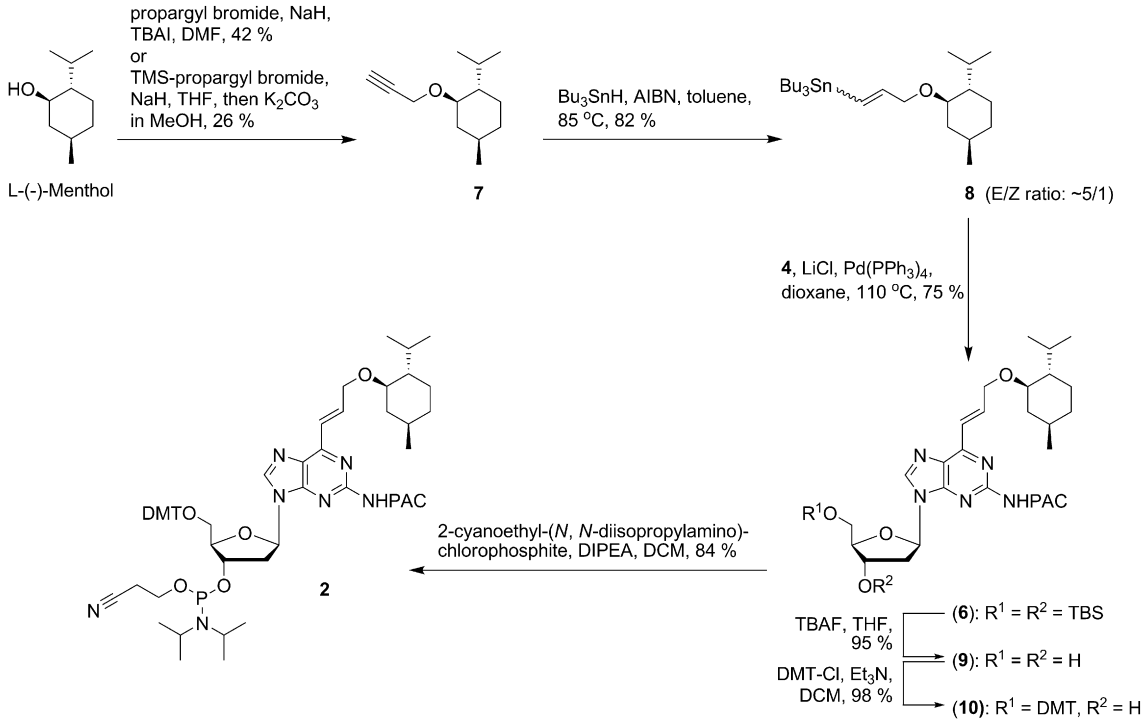



**Scheme 1.** Synthesis of adduct **6**.

the vinyl entity at the C-6 of the purine base by means of cross-metathesis reactions. Up to now, only productive metathesis reactions involving the ribose moiety of purine nucleosides have been reported.[7]

Our route of synthesis started with the protection of the hydroxyls of 2′-deoxyguanosine by *tert*-butyldimethylsilyl (TBS) groups, that were considered to be sufficiently stable to survive upcoming transformations (Scheme 1). The relatively base labile phenoxyacetyl (PAC) protecting group was selected to protect the exocyclic amine function of the purine to avoid prolonged ammonia treatment and, therefore, reduce possible side reactions at the final deprotection stage of the solid-phase synthesis of the oligonucleotide.[8] Introduction of the PAC group with phenoxyacetyl chloride in a mixture of pyridine and DMF proved to be troublesome. Contrary, when performed in DCM at 0 °C using the less reactive phenoxyacetic anhydride, the reaction proceeded uneventfully to give protected guanosine **3**. The 6-*O*-position was subsequently tosylated to provide 6-*O*-tosyl **4** in 70% over three steps.

Stille vinylation of **4** in refluxing dioxane, using 10 mol % of $Pd(PPh_3)_4$ and 5 equiv of tributylvinyl stannane under inert atmosphere gave 6-vinylpurine **5** in 84% yield. The crucial cross-metathesis of 6-vinylpurine **5** with 5 equiv of allyl menthyl ether (prepared from sodium menthoxide and allyl bromide), using Grubbs second generation catalyst[9,10] (10 mol %) gave *E*-adduct **6** in 22% yield while the corresponding *Z*-adduct could not be detected. The low yield of target compound **6** together with the lack of starting alkene **5** and formation of baseline material, as monitored by TLC analysis, suggested substantial dimerization of **5**. Unfortunately, additional treatment of the reaction mixture, after total consumption of starting compound **5**, with both 10 mol % of Grubbs catalyst and 5 equiv of allyl menthyl ether did not improve the outcome.




**Scheme 2.** Improved route to phosphoramidite **2**.

The disappointing outcome of the cross-metathesis reaction and the favorable preceding Stille coupling with tributylvinyl stannane stimulated us to explore the introduction of the allyl menthyl moiety by a Stille coupling of tosylate **4** and the required tributyl stannane derivative of menthol (**8**, Scheme 2). Stannane **8** can be obtained from L-(−)-menthol by propargylation and subsequent hydrostannylation of intermediate alkyne **7**. Propargylation[4] of L-(−)-menthol using sodium hydride and propargylbromide led to the isolation of propargylmenthyl ether **7** in modest yield (42%) and purity (90–95%). Performing this transformation using a twofold excess of commercially available (3-bromoprop-1-ynyl)trimethylsilane and subsequent desilylation of the intermediate with $K_2CO_3$ in MeOH furnished propargyl ether **7** of higher purity (>99%), albeit in lower yield (26%). Hydrostannylation of alkyne **7** (purity >99%), using tributyltin hydride and azobisisobutyronitrile (AIBN) as initiator provided **8** in 82% yield (*E*/*Z* ratio: ~5/1). When the less pure alkyne **7** was used, the stannane product was acquired in a substantially lower yield (50–55%).

Gratifyingly, Stille reaction of stannyl derivative **8** (1.5 equiv) and tosylguanosine **4** for 2.5 h in boiling dioxane gave target *E*-adduct **6** as the sole product in 75% yield. To obtain phosphoramidite **2**, suitable for application in automated solid-phase oligonucleotide synthesis, the following sequence of reaction was executed. Desilylation of **6** with tetrabutylammonium fluoride (TBAF) to diol **9** was followed by regioselective dimethoxytritylation giving **10**, which was subsequently functionalized using 2-cyanoethyl-(*N*,*N*-diisopropylamino)-chlorophosphite in the presence of DIPEA, yielding phosphoramidite **2** in 78% over three steps. For fluorescence studies, a small part of diol **9** was deprotected with aqueous $NH_3$, yielding compound **1**. Nucleoside **1** was compared with 2-amino-9-(2′-deoxyribofuranosyl)-purine (2-AP).[16] The results are summarized in Table 1.

**Table 1**
Fluorescence parameters of **1** compared to 2-AP[a]

| | 2-AP ($H_2O$)[b] | 2-AP (MeOH) | **1** (MeOH) |
|---|---|---|---|
| $\lambda_{max}$ abs. | 305 | 309 | 323 |
| $\lambda_{max}$ exc. | 307 | 312 | 343 |
| $\lambda_{max}$ emi. | 367 | 366 | 439 |
| $\Phi\pm0.01$ | 0.68 | 0.44 | 0.20 |

[a] Compound **1** was prepared by treating **9** with 20% $NH_3$ in dioxane/$H_2O$ (1/4), followed by column chromatography (68% yield).
[b] Results are comparable with literature values.[16]

Phosphoramidite **2** was applied in automated solid-phase DNA synthesis and proved to be as efficient in the phosphitylation step as the commercially available phosphoramidites of the common bases, allowing the assembly of the fully protected and immobilized progenitor of 50-mer **I** by a standard solid-phase protocol.[11,12] Removal of all the protecting groups and concomitant release from the solid support was effected by treatment with aqueous ammonia to give, after purification with anion exchange HPLC and desalting by gel filtration, homogeneous 50-mer **I**. In a first study with 50-mer **I**, it was shown that upon binding of UvrB the modified and fluorescent base of nucleoside **1** is not flipping but retains its original intra-helical position, as reported elsewhere.[13]

## 3. Conclusion

In summary, a novel modified nucleoside has been prepared and incorporated in an oligonucleotide by the application of phosphoramidite **2**. Two routes of synthesis, using deoxyguanosine as starting compound were explored to obtain building-block **2**. The first route entailed eight steps, including a Stille vinylation and a cross-metathesis reaction to give amidite **2** in 10% overall yield. The second route avoided the inefficient metathesis reaction and used advanced stannane **8** in the crucial Stille coupling to afford amidite **2** in 41% overall yield.

## 4. Experimental

### 4.1. General

All chemicals (Acros, Fluka, Merck, Schleicher & Schuell, Sigma–Aldrich) were used as received. Reactions were carried out dry, under an argon atmosphere and at ambient temperature, unless stated otherwise. The commercially available reagents for DNA synthesis were all from Proligo. Column chromatography was performed on Merck silica gel 60 (0.040–0.063 mm). TLC analysis was conducted on DC-fertigfolien (Schleicher & Schuell, F1500, LS254) or HPTLC aluminum sheets (Merck, silica gel 60, F245). Compounds were visualized by UV absorption (245 nm), by spraying with 20% $H_2SO_4$ in ethanol or with a solution of $(NH_4)_6Mo_7O_{24}\cdot4H_2O$ 25 g/l, $(NH_4)_4Ce(SO_4)_4\cdot2H_2O$ 10 g/l, 10% $H_2SO_4$ in $H_2O$ followed by charring at ±140 °C. Some unsaturated compounds were visualized by spraying with a solution of $KMnO_4$ (2%) and $K_2CO_3$ (1%) in water. Optical rotation measurements ($[\alpha]_D^{20}$) were performed on a Propol automated polarimeter (Sodium D-line, $\lambda$=589 nm) in $CHCl_3$ with a concentration of 10 mg/ml ($c$=1), unless stated otherwise. Infrared spectra were recorded on a Shimadzu FTIR 8300 and data are reported in $cm^{-1}$. $^1H$, $^{13}C$ and $^{31}P$ NMR spectra were recorded with a Bruker AC200 (200, 50 and 80.7 MHz, respectively), a Bruker AV 500 (500 and 125 MHz, respectively) or a Bruker DMX 600 (600 and 150 MHz, respectively). NMR spectra were recorded in $CDCl_3$ with chemical shift ($\delta$) relative to tetramethylsilane, unless stated otherwise. High resolution mass spectra were recorded by direct injection (2 µl of a 2 µM solution in water/acetonitrile; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution $R$=60,000 at $m/z$ 400 (mass range $m/z$=150–2000) and dioctylphthalate ($m/z$=391.28428) as a lock mass. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan).

### 4.2. Experimental procedures

*4.2.1. 3′,5′-Di-O-t-butyldimethylsilyl-2′-deoxyguanosine.* 2′-Deoxyguanosine (14.2 g, 53.1 mmol) and imidazole (10.0 g, 149 mmol) were suspended in DMF (450 ml) and cooled to 0 °C. After addition of TBS-Cl (20.0 g, 133 mmol) and a catalytic amount of DMAP, the reaction was allowed to stir for 6 h. The solvents were evaporated and the residue taken up in DCM (400 ml). The solution was washed with aq HCl (1 M), until the pH became slightly acidic. The organic layer was washed once with satd aq $NaHCO_3$ (150 ml), and dried with $MgSO_4$. The solution was concentrated in vacuo and yielded an amber waxy solid, that was washed with $Et_2O$ yielding the title compound (22.9 g, 87%) as a white solid. Mp >294–295 °C (decomp.)[6b]; $^1H$,$^1H$-COSY NMR (500 MHz): $\delta$ 0.07 (s, 6H, 2×$CH_3$ TBS), 0.11 (s, 6H, 2×$CH_3$ TBS), 0.85 (s, 18H, 2×*t*-butyl TBS), 2.34–2.39 (m, 1H, H-2′), 2.43–2.49 (m, 1H, H-2′), 3.74–3.82 (m, 2H, H-5′, H-5′), 3.98 (dd, 1H, $J$=3.5 Hz, 7.0 Hz, H-4′), 4.57 (m, 1H, H-3′), 6.23 (t, 1H, $J$=6.5 Hz, H-1′), 7.84 (s, 1H, H-8).

*4.2.2. 2-N-Phenoxyacetyl-3′,5′-di-O-t-butyldimethylsilyl-2′-deoxyguanosine (**3**).* Silylated dG (2.48 g, 5.00 mmol) was dissolved in DCM (250 ml) and cooled to 0 °C. $Et_3N$ (5.55 ml, 40.0 mmol) and phenoxyacetic acid anhydride ($PAC_2O$, 4.29 g, 15.0 mmol, made from phenoxyacetic acid and DCC) were subsequently added to the reaction mixture. After stirring for 10 min, a catalytic amount of DMAP was added and the reaction was stirred for another 72 h at ambient temperature. The solution was washed with aq HCl (1 M) until the pH was slightly acidic. After washing with satd aq $NaHCO_3$ (100 ml), the organic phase was dried with $MgSO_4$ and

concentrated in vacuo. Silica gel column chromatography (EtOAc/PE) afforded **3** (2.84 g, 90%) as a yellow foam. $[\alpha]_D^{20}$: +1.0; IR: 1096, 1115, 1250, 1607, 1678; $^1$H,$^1$H-COSY NMR (500 MHz): $\delta$ 0.11 (s, 6H, 2×$CH_3$ TBS), 0.13 (s, 6H, 2×$CH_3$ TBS), 0.92 (s, 18H, 2×*t*-butyl TBS), 2.41–2.44 (m, 1H, H-2′), 2.48 (ddd, 1H, *J*=6.5 Hz, 6.5 Hz, 13.0 Hz, H-2′), 3.75–3.81 (m, 2H, H-5′, H-5′), 3.99 (dd, 1H, *J*=3.5 Hz, 6.5 Hz, H-4′), 4.59–4.61 (m, 1H, H-3′), 4.71 (s, 2H, $CH_2$ PAC), 6.29 (t, 1H, *J*=6.5 Hz, H-1′), 7.00–7.12 (m, 3H, $H_{arom}$), 7.36–7.39 (m, 2H, $H_{arom}$), 8.02 (s, 1H, H-8), 9.22 (br s, 1H, NHPAC); $^{13}$C NMR (125 MHz): $\delta$ 17.9 ($C_q$ *t*-butyl TBS), 18.3 ($C_q$ *t*-butyl TBS), 25.7–25.9 (4×$CH_3$ TBS, 2×*t*-butyl $CH_3$ TBS), 41.4 (C-2′), 62.6 (C-5′), 67.0 ($CH_2$ PAC), 71.6 (C-3′), 83.5 (C-1′), 87.9 (C-4′), 114.8, 123.0, 129.2 ($CH_{arom}$), 129.2 (C-8), 146.0, 147.5, 155.3, 156.4 (C-2, C-4, C-5, $C_q$ PAC), 169.5 (C-6); HRMS: $C_{30}H_{47}N_5O_6Si_2$+$H^+$ calculated 630.3138, found 630.3140.

*4.2.3. 2-N-Phenoxyacetyl-6-O-p-toluenesulphonyl-3′,5′-di-O-t-butyldimethylsilyl-2′-deoxyguanosine (**4**).* To a cooled (0 °C) solution of **3** (3.15 g, 5.00 mmol) and $Et_3N$ (1.40 ml, 10.1 mmol) in DCM (100 ml) was added tosylchloride (1.91 g, 10.0 mmol), followed by a catalytic amount of DMAP. After TLC analysis showed conversion to a less polar material, the reaction was quenched with $H_2O$ (10 ml). The organic phase was washed twice with $H_2O$ (30 ml) and once with satd aq NaCl (20 ml) before it was dried with $MgSO_4$. After concentration in vacuo, silica gel column chromatography (EtOAc/PE) yielded **4** (3.50 g, 89%) as a slightly yellow foam. $[\alpha]_D^{20}$: +23.8; IR: 1026, 1177, 1383, 1495, 1587, 1711, 2856, 2928; $^1$H,$^1$H-COSY NMR (500 MHz): $\delta$ 0.10 (s, 6H, 2×$CH_3$ TBS), 0.11 (s, 6H, 2×$CH_3$ TBS), 0.89 (s, 18H, 2×*t*-butyl TBS), 2.42 (s, 3H, $CH_3$ tosyl), 2.43–2.48 (m, 1H, H-2′), 2.66 (ddd, 1H, *J*=6.4 Hz, 6.4 Hz, 12.8 Hz, H-2′), 3.78 (dd, 1H, *J*=3.3 Hz, 11.3 Hz, H-5′), 3.89 (dd, 1H, *J*=4.0 Hz, 11.5 Hz, H-5′), 4.01 (dd, 1H, *J*=3.5 Hz, 7.0 Hz, H-4′), 4.62–4.64 (m, 1H, H-3′), 4.78 (br s, 2H, $CH_2$ PAC), 6.43 (t, 1H, *J*=6.5 Hz, H-1′), 7.02–7.08 (m, 3H, $H_{arom}$), 7.27–7.38 (m, 4H, $H_{arom}$), 8.09 (d, 1H, *J*=8.5 Hz, $H_{arom}$), 8.09 (d, 1H, *J*=8.5 Hz, $H_{arom}$), 8.31 (s, 1H, H-8); $^{13}$C NMR (125 MHz): $\delta$ 17.9 ($C_q$ *t*-butyl TBS), 18.3 ($C_q$ *t*-butyl TBS), 25.7–25.9 (4×$CH_3$ TBS, 2×*t*-butyl $CH_3$ TBS), 41.1 (C-2′), 62.6 (C-5′), 67.9 ($CH_2$ PAC), 71.6 (C-3′), 84.6 (C-1′), 88.1 (C-4′), 114.5 ($CH_{arom}$), 120.2 ($C_q$ tosyl), 122.2, 129.1, 129.6, 129.7 ($CH_{arom}$), 133.4 ($C_q$ tosyl), 143.2 (C-8), 145.8, 150.3, 154.0, 154.5, 157.1 (C-2, C-4, C-5, C-6, $C_q$ PAC); HRMS: $C_{37}H_{53}N_5O_8SSi_2$+$H^+$ calculated 784.3226, found 784.3233.

*4.2.4. 2-N-Phenoxyacetyl-6-vinyl-9-(3′,5′-di-O-t-butyldimethylsilyl-2′-deoxy-β-D-ribofuranosyl) purine (**5**).* A solution of LiCl (165 mg, 3.90 mmol), tri-*n*-butyl-vinylstannane (3.00 ml, 10.2 mmol), and **4** (1.53 g, 1.95 mmol) in dioxane (26 ml) was stirred under argon for 30 min. After the addition of $Pd(PPh_3)_4$ (0.25 g, 0.22 mmol), the mixture was heated under reflux for 1.5 h. After concentration in vacuo, the residue was taken up in EtOAc (100 ml), and washed successively with aqueous $NH_3$ (2.5% v/v, 20 ml) and satd aq NaCl (20 ml). The organic layer was dried with $MgSO_4$ and concentrated to give the crude product, which was purified by silica gel column chromatography (EtOAc/PE) to yield **5** (1.25 g, 84%) as an amorphous transparent yellow solid. $[\alpha]_D^{20}$: +16.6; IR: 1070, 1215, 1252, 1497, 1587, 2361, 2856, 2928; $^1$H,$^1$H-COSY NMR (500 MHz): $\delta$ 0.09 (s, 6H, 2×$CH_3$ TBS), 0.12 (s, 6H, 2×$CH_3$ TBS), 0.92 (s, 18H, 2×*t*-butyl TBS), 2.44–2.49 (m, 1H, H-2′), 2.72 (ddd, 1H, *J*=6.4 Hz, 6.4 Hz, 12.8 Hz, H-2′), 3.79 (dd, 1H, *J*=3.3 Hz, 11.3 Hz, H-5′), 3.89 (dd, 1H, *J*=4.0 Hz, 11.5 Hz, H-5′), 4.02 (dd, 1H, *J*=3.5 Hz, 7.0 Hz, H-4′), 4.65 (ddd, 1H, *J*=3.8 Hz, 3.8 Hz, 6.5 Hz, H-3′), 4.80 (br s, 2H, $CH_2$ PAC), 5.96 (dd, 1H, *J*=1.5 Hz, 11.0 Hz, $CH_2$ vinyl), 6.49 (t, 1H, *J*=6.5 Hz, H-1′), 6.99–7.07 (m, 3H, $H_{arom}$, $CH_2$ vinyl), 7.23–7.37 (m, 4H, $H_{arom}$, CH vinyl), 8.30 (s, 1H, H-8); $^{13}$C NMR (125 MHz): $\delta$ 18.0 ($C_q$ *t*-butyl TBS), 18.4 ($C_q$ *t*-butyl TBS), 25.7–25.9 (4×$CH_3$ TBS, 2×*t*-butyl $CH_3$ TBS), 41.0 (C-2′), 62.8 (C-5′), 68.0 ($CH_2$ PAC), 71.9 (C-3′), 84.2 (C-1′), 88.0 (C-4′), 114.9, 122.2 ($CH_{arom}$), 126.8 ($CH_2$ vinyl), 128.9 ($C_q$ PAC), 129.8 ($CH_{arom}$), 131.4 (CH vinyl), 142.9 (C-8), 151.6, 152.4–152.6, 154.3 (C-2, C-4, C-5, C-6); HRMS: $C_{32}H_{49}N_5O_5Si_2$+$H^+$ calculated 640.3345, found 640.3346.

*4.2.5. 2-N-Phenoxyacetyl-6-(3-O-L-menthyl-propenyl)-9-(3′,5′-di-O-t-butyldimethylsilyl-2′-deoxy-β-D-ribofuranosyl)purine (**6**).* A solution of **5** (115 mg, 0.180 mmol) and allyl menthyl ether (177 mg, 0.902 mmol) in DCM (6.0 ml) was stirred under argon for 30 min. Grubbs second generation catalyst (30 mg, 0.035 mmol) was added and the resulting mixture was gently refluxed (~45 °C) for 1 h. After the addition of a second portion of catalyst (30 mg, 0.035 mmol), the mixture was refluxed for another hour. Evaporation of the solvent and purification by silica gel column chromatography (EtOAc/PE) yielded **6** (32 mg, 22%) as a colorless oil. $[\alpha]_D^{20}$: −12.3; IR: 777, 833, 1068, 1213, 1495, 1587, 1709, 2928; $^1$H,$^1$H-COSY NMR (600 MHz): $\delta$ 0.08 (s, 6H, 2×$CH_3$ TBS), 0.12 (s, 6H, 2×$CH_3$ TBS), 0.78 (d, 3H, *J*=7.2 Hz, $CH_3$ menthol), 0.89–1.00 (m, 27H, 2×*t*-butyl TBS, 2×$CH_3$ menthol, 3×C*H*H menthol), 1.28–1.36 (m, 2H, 2×CH menthol), 1.62–1.66 (m, 2H, 2×CH*H* menthol), 2.14 (m, 1H, CH*H* menthol), 2.30–2.33 (m, 1H, CH menthol), 2.44–2.48 (m, 1H, H-2′), 2.71–2.73 (m, 1H, H-2′), 3.17 (ddd, 1H, *J*=3.9 Hz, 10.7 Hz, 14.7 Hz, CH menthol), 3.79 (dd, 1H, *J*=3.3 Hz, 11.1 Hz, H-5′), 3.88 (dd, 1H, *J*=4.2 Hz, 11.4 Hz, H-5′), 4.02 (dd, 1H, *J*=3.3 Hz, 6.9 Hz, H-4′), 4.23 (ddd, 1H, *J*=1.5 Hz, 4.3 Hz, 15.3 Hz, $CH_2$ propenyl), 4.45 (ddd, 1H, *J*=1.8 Hz, 4.8 Hz, 15.0 Hz, $CH_2$ propenyl), 4.64–4.65 (m, 1H, H-3′), 4.80 (br s, 2H, $CH_2$ PAC), 6.48 (t, 1H, *J*=6.3 Hz, H-1′), 7.02–7.06 (m, 3H, $H_{arom}$), 7.18 (d, 1H, *J*=16.2 Hz, CH-2″ propenyl), 7.33–7.36 (m, 2H, $H_{arom}$), 7.64 (ddd, 1H, *J*=4.8 Hz, 4.8 Hz, 15.6 Hz, CH-1″ propenyl), 8.28 (s, 1H, H-8); $^{13}$C NMR (150 MHz): $\delta$ 16.2 ($CH_3$ menthol), 18.0 ($C_q$ *t*-butyl TBS), 18.4 ($C_q$ *t*-butyl TBS), 21.0 ($CH_3$ menthol), 22.3 ($CH_3$ menthol), 23.3 ($CH_2$ menthol), 25.5 (CH menthol), 25.8–25.9 (4×$CH_3$ TBS, 2×*t*-butyl $CH_3$ TBS), 31.5 (CH menthol), 34.5 ($CH_2$ menthol), 40.3 ($CH_2$ menthol), 41.0 (C-2′), 48.2 (CH menthol), 62.8 (C-5′), 68.0 ($CH_2$ propenyl), 68.3 ($CH_2$ PAC), 71.9 (C-3′), 79.2 (CH menthol), 84.2 (C-1′), 88.0 (C-4′), 114.9 ($CH_{arom}$), 122.2 ($CH_{arom}$), 124.6 (CH-1′ propenyl), 128.7 ($C_q$ PAC), 129.8 ($CH_{arom}$), 141.5 (CH-2′ propenyl), 142.6 (C-8), 151.3, 152.3, 154.4, 157.2 (C-2, C-4, C-5, C-6); HRMS: $C_{43}H_{69}N_5O_6Si_2$+$H^+$ calculated 808.4859, found 808.4865.

*4.2.6. L-Menthyl propargyl ether (**7**)[4]. Method a*: L-Menthol (7.81 g, 50.0 mmol) and tetra-*n*-butyl ammonium iodide (1.40 g, 3.79 mmol) were dissolved in DMF (300 ml) and cooled to 0 °C. NaH (60% dispersion in mineral oil, 2.4 g, 60 mmol) and propargylbromide (80% solution in toluene, 21.5 ml, 200 mmol) were slowly added. The mixture was allowed to stir at room temperature for 96 h. The reaction was quenched with MeOH (20 ml) and concentrated in vacuo. The oily residue was taken up in $Et_2O$ (400 ml) and washed twice with $H_2O$ (200 ml) and once with satd aq NaCl (100 ml), before it was dried over $MgSO_4$. After evaporation of the solvent, silica gel column chromatography (toluene/PE) gave **7** (4.06 g, 42%) as a slightly yellow oil. *Method b*: To a flask equipped with L-menthol (0.312 g, 2.00 mmol) in THF (10 ml) was added NaH (60% dispersion in mineral oil, 0.12 g, 3.0 mmol). After stirring at room temperature for 30 min TMS-propargylbromide (0.627 ml, 4.00 mmol) was added. After 96 h $K_2CO_3$ (5.0 g, 36 mmol) and MeOH (5.0 ml) were added and the mixture stirred for another 3 h. The mixture was then diluted with $Et_2O$ (40 ml) and washed with $H_2O$ (20 ml) and satd aq NaCl (20 ml) before it was dried with $MgSO_4$. The organic layer was concentrated under reduced pressure and the resulting oil purified by silica gel column chromatography ($Et_2O$/pentane). Menthyl propargyl ether **7** (99 mg, 26%) was obtained as an colorless oil. $[\alpha]_D^{20}$: −116.6; IR: 1072, 1084, 1454, 2920, 3310; $^1$H,$^1$H-COSY NMR (200 MHz): $\delta$ 0.78–0.97 (m, 12H, 3×$CH_3$ menthol, 3×C*H*H menthol), 1.10–1.42 (m, 2H, 2×CH menthol), 1.57–1.69 (m, 2H, 2×CH*H* menthol), 2.07–2.30 (m, 2H, CH

menthol, CH*H* menthol), 2.36–2.42 (m, 1H, CH propyn), 3.27 (ddd, 1H, *J*=4.4 Hz, 11.0 Hz, 14.6 Hz, CH menthol), 4.09–4.28 (m, 2H, $CH_2$ propyn).

*4.2.7. 3-Tri-n-butyltin-2-propenyl menthyl ether (**8**).* To a flask, charged with **7** (486 mg, 2.50 mmol) in toluene (7.5 ml), were added tri-*n*-butyltin hydride (800 mg, 2.75 mmol) and 2,2′-azobis(2-methylpropionitrile) (50 mg, 0.30 mmol). The mixture was stirred overnight at 85 °C before it was allowed to cool to room temperature. The reaction mixture was diluted with $Et_2O$ (40 ml) and washed with $H_2O$ (20 ml) and satd aq NaCl (20 ml). The organic layer was dried with $MgSO_4$ and concentrated in vacuo. The crude product was then obtained as a mixture of regioisomers (~9/1, 3-stannyl (**8**)/2-stannyl) that could be separated by silica gel column chromatography ($Et_2O$/PE/pentane). **8** (996 mg, 82%, ~5/1 *E*/*Z*) was acquired as a colorless oil. $[\alpha]_D^{20}$: −41.6; IR: 758, 1084, 1456, 2920, 2955; $^1$H,$^1$H-COSY NMR (500 MHz): *E-product*: δ 0.77–0.98 (m, 27H, 3×$CH_3$ menthol, 3×C*H*H menthol, 3×$CH_3$ $Bu_3Sn$, 3×$CH_2$ $Bu_3Sn$), 1.22–1.34 (m, 8H, 2×CH menthol, 3×$CH_2$ $Bu_3Sn$), 1.43–1.52 (m, 6H, 3×$CH_2$ $Bu_3Sn$), 1.59–1.65 (m, 2H, 2×CH*H* menthol), 2.08–2.10 (m, 1H, CH*H* menthol), 2.23–2.27 (m, 1H, CH menthol), 3.08 (ddd, 1H, *J*=4.3 Hz, 10.8 Hz, 14.8 Hz, CH menthol), 3.91–3.95 (m, 1H, $CH_2$ allyl), 4.12–4.15 (m, 1H, $CH_2$ allyl), 6.06–6.09 (m, 1H, CH allyl), 6.16 (d, 1H, *J*=19.0 Hz, CH allyl terminus); *Z-product*: δ 0.77–0.98 (m, 27H, 3×$CH_3$ menthol, 3×C*H*H menthol, 3×$CH_3$ $Bu_3Sn$, 3×$CH_2$ $Bu_3Sn$), 1.22–1.34 (m, 8H, 2×CH menthol, 3×$CH_2$ $Bu_3Sn$), 1.43–1.52 (m, 6H, 3×$CH_2$ $Bu_3Sn$), 1.59–1.65 (m, 2H, 2×CH*H* menthol), 2.08–2.10 (m, 1H, CH*H* menthol), 2.23–2.27 (m, 1H, CH menthol), 3.06–3.11 (m, 1H, CH menthol), 3.84–3.88 (m, 1H, $CH_2$ allyl), 4.06–4.09 (m, 1H, $CH_2$ allyl), 6.02–6.05 (d, 1H, *J*=11.5 Hz, CH allyl terminus), 6.60–6.64 (m, 1H, CH allyl); $^{13}$C NMR (125 MHz): *E-product*: δ 9.5 (3×$CH_2$ $Bu_3Sn$), 13.7 (3×$CH_2$ $Bu_3Sn$), 16.2 (3×$CH_3$ menthol), 21.0 ($CH_3$ menthol), 22.4 ($CH_3$ menthol), 23.4 ($CH_2$ menthol), 25.5 (CH menthol), 27.3 (3×$CH_2$ $Bu_3Sn$), 29.1 (3×$CH_2$ $Bu_3Sn$), 31.6 (CH menthol), 34.6 ($CH_2$ menthol), 40.6 ($CH_2$ menthol), 48.3 (CH menthol), 72.5 ($CH_2$ allyl), 78.3 (CH menthol), 130.4 (CH allyl terminus), 145.8 (CH allyl); *Z-product*: δ 9.5 (3×$CH_2$ $Bu_3Sn$), 13.7 (3×$CH_2$ $Bu_3Sn$), 16.2 (3×$CH_3$ menthol), 21.0 ($CH_3$ menthol), 22.4 ($CH_3$ menthol), 23.4 ($CH_2$ menthol), 25.5 (CH menthol), 27.3 (3×$CH_2$ $Bu_3Sn$), 29.1 (3×$CH_2$ $Bu_3Sn$), 31.6 (CH menthol), 34.6 ($CH_2$ menthol), 40.6 ($CH_2$ menthol), 48.3 (CH menthol), 71.9 ($CH_2$ allyl), 78.7 (CH menthol), 131.1 (CH allyl terminus), 145.6 (CH allyl); HRMS: $C_{25}H_{50}OSn+H^+$ calculated 487.2961, found 487.2953.

*4.2.8. 2-N-Phenoxyacetyl-6-(3-O-L-menthyl-propenyl)-9-(3′,5′-di-O-t-butyldimethylsilyl-2′-deoxy-β-D-ribofuranosyl)purine (**6**).* A mixture of tosylate **4** (430 mg, 0.886 mmol), stannyl derivative **8** (688 mg, 1.42 mmol), and LiCl (82.6 mg, 1.95 mmol) in dioxane (8.0 ml) was stirred under argon for 30 min. After addition of $Pd(PPh_3)_4$ (102 mg, 0.0886 mmol), the mixture was heated under reflux for 2.5 h. The mixture was then diluted with EtOAc (40 ml) and washed successively with aqueous $NH_3$ (2.0% v/v, 20 ml), $H_2O$ (20 ml), and satd aq NaCl (20 ml). The organic layer was dried with $Na_2SO_4$ and concentrated in vacuo. Silica gel column chromatography (EtOAc/PE) delivered **6** (334 mg, 75%, pure *E*) as a lime-colored transparent oil. $[\alpha]_D^{20}$: −12.3; IR: 777, 833, 1068, 1213, 1495, 1587, 1709, 2928; $^1$H,$^1$H-COSY NMR (600 MHz): δ 0.08 (s, 6H, 2×$CH_3$ TBS), 0.12 (s, 6H, 2×$CH_3$ TBS), 0.78 (d, 3H, *J*=7.2 Hz, $CH_3$ menthol), 0.89–1.00 (m, 27H, 2×*t*-butyl TBS, 2×$CH_3$ menthol, 3×C*H*H menthol), 1.28–1.36 (m, 2H, 2×CH menthol), 1.62–1.66 (m, 2H, 2×CH*H* menthol), 2.14 (m, 1H, CH*H* menthol), 2.30–2.33 (m, 1H, CH menthol), 2.44–2.48 (m, 1H, H-2′), 2.71–2.73 (m, 1H, H-2′), 3.17 (ddd, 1H, *J*=3.9 Hz, 10.7 Hz, 14.7 Hz, CH menthol), 3.79 (dd, 1H, *J*=3.3 Hz, 11.1 Hz, H-5′), 3.88 (dd, 1H, *J*=4.2 Hz, 11.4 Hz, H-5′), 4.02 (dd, 1H, *J*=3.3 Hz, 6.9 Hz, H-4′), 4.23 (ddd, 1H, *J*=1.5 Hz, 4.3 Hz, 15.3 Hz, $CH_2$ propenyl), 4.45 (ddd, 1H, *J*=1.8 Hz, 4.8 Hz, 15.0 Hz, $CH_2$ propenyl), 4.64–4.65 (m, 1H, H-3′), 4.80 (br s, 2H, $CH_2$ PAC), 6.48 (t, 1H, *J*=6.3 Hz, H-1′), 7.02–7.06 (m, 3H, $H_{arom}$), 7.18 (d, 1H, *J*=16.2 Hz, CH-2″ propenyl), 7.33–7.36 (m, 2H, $H_{arom}$), 7.64 (ddd, 1H, *J*=4.8 Hz, 4.8 Hz, 15.6 Hz, CH-1″ propenyl), 8.28 (s, 1H, H-8); $^{13}$C NMR (150 MHz): δ 16.2 ($CH_3$ menthol), 18.0 ($C_q$ *t*-butyl TBS), 18.4 ($C_q$ *t*-butyl TBS), 21.0 ($CH_3$ menthol), 22.3 ($CH_3$ menthol), 23.3 ($CH_2$ menthol), 25.5 (CH menthol), 25.8–25.9 (4×$CH_3$ TBS, 2×*t*-butyl $CH_3$ TBS), 31.5 (CH menthol), 34.5 ($CH_2$ menthol), 40.3 ($CH_2$ menthol), 41.0 (C-2′), 48.2 (CH menthol), 62.8 (C-5′), 68.0 ($CH_2$ propenyl), 68.3 ($CH_2$ PAC), 71.9 (C-3′), 79.2 (CH menthol), 84.2 (C-1′), 88.0 (C-4′), 114.9 ($CH_{arom}$), 122.2 ($CH_{arom}$), 124.6 (CH-1′ propenyl), 128.7 ($C_q$ PAC), 129.8 ($CH_{arom}$), 141.5 (CH-2′ propenyl), 142.6 (C-8), 151.3, 152.3, 154.4, 157.2 (C-2, C-4, C-5, C-6); HRMS: $C_{43}H_{69}N_5O_6Si_2+H^+$ calculated 808.4859, found 808.4865.

*4.2.9. 2-N-Phenoxyacetyl-6-(3-O-L-menthyl-propenyl)-9-(2′-deoxy-β-D-ribofuranosyl)purine (**9**).* A solution of **6** (1.00 g, 1.24 mmol) in THF (50 ml) was cooled to 0 °C. After adding TBAF (1 M solution in THF, 3.7 ml) the mixture was stirred for 2 h at room temperature. After evaporation of the solvent, silica gel column chromatography (MeOH/DCM) afforded **9** (681 mg, 95%) as an amorphous green solid. $[\alpha]_D^{20}$: −21.6; IR: 748, 1088, 1227, 1350, 1497, 1589, 1666, 2924; $^1$H,$^1$H-COSY NMR (600 MHz): δ 0.78–0.99 (m, 12H, 3×$CH_3$ menthol, 3×C*H*H menthol), 1.26–1.36 (m, 2H, 2×CH menthol), 1.62–1.67 (m, 2H, 2×CH*H* menthol), 2.12–2.14 (m, 1H, CH*H* menthol), 2.28–2.30 (m, 1H, CH menthol), 2.42–2.45 (m, 1H, H-2′), 3.07 (ddd, 1H, *J*=6.9 Hz, 6.9 Hz, 13.5 Hz, H-2′), 3.17 (ddd, 1H, *J*=4.2 Hz, 10.8 Hz, 14.4 Hz, CH menthol), 3.86–3.88 (m, 1H, H-5′), 3.96–3.98 (m, 1H, H-5′), 4.17 (m, 1H, H-4′), 4.22 (dd, 1H, *J*=3.6 Hz, 15.6 Hz, $CH_2$ propenyl), 4.45 (dd, 1H, *J*=3.0 Hz, 15.6 Hz, $CH_2$ propenyl), 4.73 (br s, 2H, $CH_2$ PAC), 5.02 (br s, 1H, H-3′), 6.38 (t, 1H, *J*=6.9 Hz, H-1′), 7.03–7.08 (m, 3H, $H_{arom}$), 7.16 (d, 1H, *J*=15.6 Hz, CH-1″ propenyl), 7.34–7.37 (m, 2H, $H_{arom}$), 7.63 (ddd, 1H, *J*=4.8 Hz, 4.8 Hz, 15.6 Hz, CH-2″ propenyl), 8.11 (s, 1H, H-8); $^{13}$C NMR (150 MHz): δ 16.2 ($CH_3$ menthol), 21.0 ($CH_3$ menthol), 22.3 ($CH_3$ menthol), 23.3 ($CH_2$ menthol), 25.5 (CH menthol), 31.5 (CH menthol), 34.5 ($CH_2$ menthol), 40.3 ($CH_2$ menthol), 40.8 (C-2′), 48.2 (CH menthol), 62.7 (C-5′), 67.5 ($CH_2$ PAC), 68.2 ($CH_2$ propenyl) 72.1 (C-3′), 79.4 (CH menthol), 86.3 (C-1′), 88.4 (C-4′), 114.7 ($CH_{arom}$), 122.4 ($CH_{arom}$), 124.2 (CH-1′ propenyl), 129.7 ($C_q$ PAC), 129.9 ($CH_{arom}$), 142.1 (CH-2′ propenyl), 145.0 (C-8), 151.0, 151.8, 155.0, 157.0 (C-2, C-4, C-5, C-6); HRMS: $C_{31}H_{41}N_5O_6+H^+$ calculated 580.3130, found 580.3128.

*4.2.10. 2-N-Phenoxyacetyl-6-(3-O-L-menthyl-propenyl)-9-(5′-O-[4,4′-dimethoxytrityl]-2′-deoxy-β-D-ribofuranosyl)purine (**10**).* Purine **9** (406 mg, 0.700 mmol) was dissolved in DCM (25 ml) and cooled to 0 °C. Subsequently, $Et_3N$ (0.20 ml, 1.4 mmol) and DMT-Cl (356 mg, 1.05 mmol) were added and the reaction was stirred for 3 h. After quenching with MeOH (5 ml) the mixture was concentrated in vacuo. Purification of the crude product by silica gel column chromatography (MeOH/DCM/$Et_3N$) gave **10** (603 mg, 98%) as an amorphous off-white solid. $[\alpha]_D^{20}$: −6.4; IR: 756, 826, 1034, 1173, 1242, 1504, 1589, 1697; $^1$H,$^1$H-COSY NMR (600 MHz): δ 0.78–1.00 (m, 12H, 3×$CH_3$ menthol, 3×C*H*H menthol), 1.23–1.31 (m, 2H, 2×CH menthol), 1.63–1.67 (m, 2H, 2×CH*H* menthol), 2.13–2.15 (m, 1H, CH*H* menthol), 2.30–2.32 (m, 1H, CH menthol), 2.63–2.65 (m, 1H, H-2′), 2.82 (ddd, 1H, *J*=6.6 Hz, 6.6 Hz, 13.2 Hz, H-2′), 3.17 (ddd, 1H, *J*=4.2 Hz, 10.5 Hz, 14.7 Hz, CH menthol), 3.37 (dd, 1H, *J*=4.2 Hz, 10.2 Hz, H-5′), 3.45 (dd, 1H, *J*=4.8 Hz, 10.2 Hz, H-5′), 3.75 (s, 6H, 2×$CH_3$ DMT), 4.19–4.24 (m, 2H, H-4′, $CH_2$ propenyl), 4.45 (dd, 1H, *J*=3.0 Hz, 16.2 Hz, $CH_2$ propenyl), 4.67 (br s, 2H, $CH_2$ PAC), 4.86 (br s, 1H, H-3′), 6.61 (m, 1H, H-1′), 6.76–6.78 (m, 4H, $H_{arom}$), 7.00–7.08 (m, 3H, $H_{arom}$), 7.14–7.24 (m, 4H, CH-1′ propenyl, $H_{arom}$), 7.26–7.29 (m, 4H, $H_{arom}$), 7.34–7.39 (m, 3H, $H_{arom}$), 7.63 (ddd, 1H, *J*=4.8 Hz, 4.8 Hz, 15.6 Hz, CH-2′ propenyl), 8.14 (s, 1H, H-8); $^{13}$C NMR (150 MHz): δ 16.3 ($CH_3$ menthol), 21.0 ($CH_3$ menthol), 22.3 ($CH_3$ menthol), 23.3 ($CH_2$ menthol), 25.5 (CH menthol), 31.5 (CH menthol), 34.5 ($CH_2$

menthol), 40.3 ($CH_2$ menthol), 40.7 (C-2′), 48.2 (CH menthol), 55.2 (2×$CH_3$ DMT), 64.1 (C-5′), 67.8 ($CH_2$ PAC), 68.3 ($CH_2$ propenyl) 72.5 (C-3′), 79.3 (CH menthol), 84.1 (C-1′), 86.6 (C-4′), 113.1 ($CH_{arom}$), 114.9 ($CH_{arom}$), 122.3 ($CH_{arom}$), 123.4 (CH-1′ propenyl), 124.5, 126.9, 127.8, 128.1 (4×$CH_{arom}$), 128.8 ($C_q$ PAC), 129.8 ($CH_{arom}$), 130.0 ($CH_{arom}$), 135.7 (2×$C_q$ DMT), 141.5 (CH-2′ propenyl+C-8), 144.6 ($C_q$ DMT), 151.0, 151.8, 154.5, 158.5 (C-2, C-4, C-5, C-6); HRMS: $C_{52}H_{59}N_5O_8+H^+$ calculated 882.4436, found 882.4447.

*4.2.11. 2-N-Phenoxyacetyl-6-(3-O-L-menthyl-propenyl)-9-(5′-O-[4,4′-dimethoxytrityl]-3′-O-[{N,N-diisopropylamino}-2-cyanoethoxy-phosphite]-2′-deoxy-β-D-ribofuranosyl)purine (**2**).* A mixture of **10** (450 mg, 0.510 mmol) and DIPEA (0.135 ml, 0.815 mmol) in DCM (7.0 ml) was cooled to 0 °C. 2-Cyanoethoxy-(*N*,*N*-diisopropylamino)-chlorophosphine (0.148 ml, 0.663 mmol) was slowly added and the mixture was allowed to stir for 3 h. After quenching with $H_2O$ (1.0 ml), the mixture was diluted with DCM (20 ml) and washed twice with satd aq $NaHCO_3$ (15 ml), and once with satd aq NaCl (15 ml). The organic phase was dried ($Na_2SO_4$) and concentrated under reduced pressure. After silica gel column chromatography (EtOAc/PE/$Et_3N$), **2** (462 mg, 84%) was afforded as a colorless oil. $^{31}P$ NMR (80.7 MHz, $CD_3CN$): $\delta$ 150.1; $^1H$ NMR (200 MHz, $CD_3CN$): $\delta$ 0.72–0.88 (m, 12H, 3×$CH_3$ menthol, 3×C*H*H menthol), 1.00–1.31 (m, 14H, 2×CH menthol, 4×$CH_3$ isopropylamino), 1.52–1.65 (m, 2H, 2×CH*H* menthol), 2.06–2.25 (m, 2H, CH*H* menthol, H-2′), 2.43–2.60 (m, 3H, CH menthol, $CH_2$ cyanoethoxy), 2.99–3.81 (m, 14H, CH menthol, H-2′, 2×H-5′, $CH_2$ cyanoethoxy, 2×CH isopropylamino, 2×$CH_3$ DMT), 4.08–4.47 (m, 3H, H-4′, $CH_2$ propenyl), 4.79–4.89 (m, 3H, H-3′, $CH_2$ PAC), 6.35 (t, 1H, *J*=6.2 Hz, H-1′), 6.62–6.71 (m, 4H, $H_{arom}$), 6.93–6.97 (m, 3H, $H_{arom}$), 7.06–7.32 (m, 11H, CH-1′ propenyl, $H_{arom}$), 7.56 (ddd, 1H, *J*=5.1 Hz, 5.1 Hz, 16.0 Hz, CH-2′ propenyl), 8.17 (s, 1H, H-8), 8.87 (br s, 1H, NHPAC).

*4.2.12. Synthesis of oligonucleotide **I** (5′-GGGATTACTTACGGG-CACATTACAAAXAAACCTCAGAACGACCTCACACG-3′).* The solid-phase synthesis of the oligonucleotide was performed on a fully automated Expedite instrument (PerSeptive Biosystems) starting from controlled pore glass functionalized with the appropriate nucleoside. The synthesis was performed on a 1-μmol scale via phosphoramidite methodology[11,12] but using mildly removable *N*-*t*-butylphenoxyacetyl (tac) protection for nucleobases.[14] Elongation was performed by coupling of the 3′-phosphoramidite derivatives of DMT-protected nucleosides (5′-DMT-$dA^{tac}$, 5′-DMT-$dC^{tac}$, 5′-DMT-$dG^{tac}$, and 5′-DMT-T, 10 equiv, 10 μmol, 0.1 M stock) with 4,5-dicyanoimidazole[15] as the activator (50 equiv, 50 μmol 0.25 M stock), for 3 min. The phosphoramidites of the fluorescent nucleoside analogs (15 equiv) were coupled for 5 min with 75 equiv of 4,5-dicyanoimidazole (75 μmol). The 5′-DMT group was removed using 3% TCA in DCM. After each coupling, remaining free 5′-hydroxyls were blocked using a mixture of cap A (*t*-butylphenoxyacetic anhydride, 0.2 M in THF) and cap B (1-methylimidazole in THF/pyridine) followed by oxidation of the phosphite linkage to the phosphate using 0.05 M of $I_2$ in THF/pyridine/water (1 min). After final DMT removal the modified oligonucleotide was cleaved from the resin by 25% ammonium hydroxide solution at room temperature (2 h). The resulting oligonucleotide was purified on a Q-Sepharose column at pH 12 applying a gradient of buffer B (0.01 M NaOH+2 M NaCl) in buffer A (0.01 M NaOH). Fractions containing the pure product were combined, desalted on Sephadex G-25 column (0.15 M ammonium bicarbonate), and lyophilized. The identity and purity of the product were confirmed by MALDI TOF mass spectrometry (Voyager DE PRO, PerSeptive Biosystems, positive ionization mode, matrix 3-hydroxypicolinic acid/ammonium hydrogen citrate), and IE HPLC (DNA-Pac PA200 analytical column, Dionex). For MALDI TOF analysis calcd 15,528 (average); found 15,578 (approx.) broad signal because of sodium clusters.

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