

## Electronic Supplementary Information (ESI)

### Nucleotide insertion and bypass synthesis of pyrene- and BODIPY-modified oligonucleotides by DNA polymerases

Claudia Wanninger-Weiß,<sup>a</sup> Francesca Di Pasquale,<sup>b</sup> Thomas Ehenschwender,<sup>a</sup> Andreas Marx<sup>\*b</sup> and Hans-Achim Wagenknecht<sup>\*a</sup>

<sup>a</sup> University of Regensburg, Institute for Organic Chemistry, D-93040 Regensburg, Germany. Fax: +49-941-943-4617; Tel: +49-941-943-4802; E-mail: achim.wagenknecht@chemie.uni-regensburg.de

<sup>b</sup> University of Konstanz, Department of Chemistry, D-78457 Konstanz, Germany. Fax: -49-7531-88-5140; Tel: +49-7531-88-5139; E-mail: andreas.marx@uni-konstanz.de

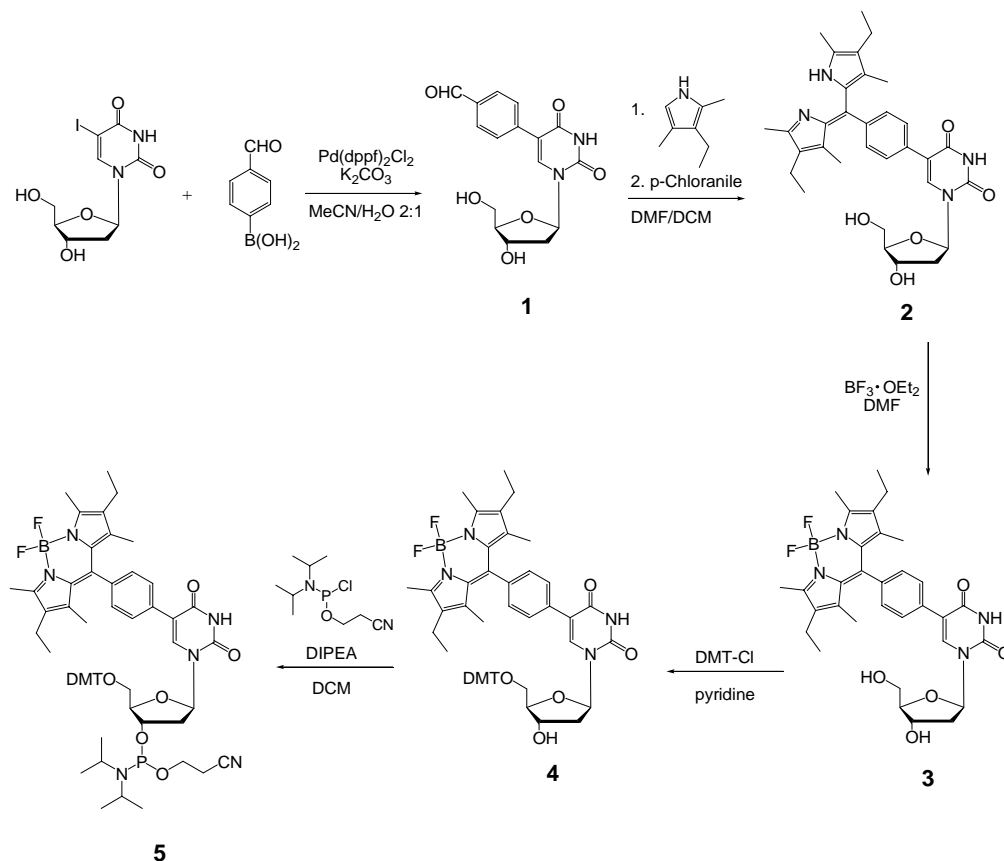
## Experimental Section

**Materials and Methods.** <sup>1</sup>H, <sup>13</sup>C, <sup>31</sup>P and the two dimensional NMR spectra were recorded at 300 K on a Bruker Avanced 300, 400 or 600 MHz spectrometer. NMR signals were assigned on the basis of two dimensional NMR measurements (HSQC, HMBC, NOESY). ESI mass spectra were measured in the analytical facility of the institute. Analytical chromatography was performed on Merck silica gel 60 F254 plates. Flash chromatography was performed on Merck silica gel (40 - 63 μm). C18-RP analytical and semipreparative HPLC columns (300 Å) were purchased from Supelco. Solvents were dried according to standard procedures. All reactions were carried out under a dry nitrogen atmosphere. Commercial chemicals were purchased by Fluka, Sigma-Aldrich and Alpha Aesar and were used without further purification. All spectroscopic measurements were performed in quartz glass cuvettes (1 cm, pump-probe laser spectroscopy: 1 mm) and using Na-P<sub>i</sub>-buffer (10 mM). Absorption spectra (2.5 μM duplex, 250 mM NaCl) were recorded on a Varian Cary 100 spectrometer. The fluorescence spectra (2.5 μM duplex) were recorded on a Fluoromax-3 fluorimeter (Jobin-Yvon) and corrected for Raman emission from the buffer solution. The phosphor-screens of the primer extension experiments were read out by a Molecular Imager FX Pro Plus (BIO-RAD).

**Preparation and characterization of the oligonucleotides.** The DNA building blocks of 1PydU, 2PydU and PydG were synthesized according to previously published procedures.<sup>[1-6]</sup>

The oligonucleotides were prepared on a Expedite 8909 DNA synthesizer from Applied Biosystems by standard phosphoramidite protocols using chemicals and CPG (1  $\mu\text{mol}$ ) from Applied Biosystems and Proligo. Quantitative coupling of the BODIPY-modified building block was achieved using the standard coupling time of 1.6 min in analogy to 1PydU and 2PydU,<sup>[4]</sup> and of the modified guanosine building block (PydG) using a modified protocol with an extended coupling time of 15 min.<sup>[6]</sup> After preparation, the trityl-off oligonucleotide was cleaved off the resin and was deprotected by treatment with conc.  $\text{NH}_4\text{OH}$  at 60 °C for 10 h. The oligonucleotide was dried and purified by HPLC on a semipreparative RP-C18 column (300 Å, Supelco) using the following conditions: (i) unmodified oligonucleotides A =  $\text{NH}_4\text{OAc}$  buffer (50 mM), pH 6.5; B = MeCN; gradient: 0 – 15 % B over 45 min; (ii) pyrene- and BODIPY-modified oligonucleotides A =  $\text{NH}_4\text{OAc}$  buffer (50 mM), pH 6.5; B = MeCN; gradient: 0 – 30 % B over 55 min. The oligonucleotides were lyophilized and quantified by their absorbance at 260 nm and using  $\epsilon_{260}=17000 \text{ M}^{-1} \text{ cm}^{-1}$  (MeOH) for 1PydU,  $\epsilon_{260} = 18600 \text{ M}^{-1} \text{ cm}^{-1}$  (MeOH) for 2PydU,  $\epsilon_{260}=11900 \text{ M}^{-1} \text{ cm}^{-1}$  (MeOH) for BoDU and  $\epsilon_{260} = 17900 \text{ M}^{-1} \text{ cm}^{-1}$  (MeOH) for PydG on a Varian Cary 100 spectrometer.

### Synthesis of the BodU DNA building block.



**Scheme S1:** Synthesis of the BodU DNA building block 5.

**5-(4-Formylphenyl)-2'-desoxyuridine (1).** 5-Iodo-2'-desoxyuridine (600 mg, 1.69 mmol) was dissolved in H<sub>2</sub>O:MeCN=2:1 (22.5 mL) and 4-formylphenylboronic acid (305 mg, 2.05 mmol, 1.21 equiv.), Na<sub>2</sub>CO<sub>3</sub> (365 mg, 3.44 mmol, 2.04 equiv.) and Pd(dppf)<sub>2</sub>Cl<sub>2</sub> (102 mg, 0.11 mmol, 0.067 equiv.) were added. The mixture was stirred for 5 h at 80 °C. After cooling to r.t., H<sub>2</sub>O (20 mL) was added. The solution was washed with NH<sub>4</sub>Cl. After extraction with hot EtOAc (70 °C) the organic phase was evaporated. The residue was washed with cold ethylacetate (0 °C) and dried under vacuum to yield 428 mg (1.29 mmol, 76 %) of a pale brown solid. **T.l.c.** (EtOAc:MeOH:H<sub>2</sub>O=10:1:0.5): R<sub>f</sub> = 0.55. **<sup>1</sup>H-NMR** (600 MHz; [d<sub>6</sub>]-DMSO): δ = 2.22 (m, 2H, 2'-H), 3.62 (m, 2H, 5'-H), 3.82 (m, 1H, 3'-H), 4.30 (m, 1H, 4'-H), 5.18 (s, 1H, 5'-OH), 5.25(s, 1H, 3'-OH), 6.21 (t, 1H, 1'-H), 7.81 (d, 2H, Phenyl-H), 7.88 (d, 2H, Phenyl-H), 8.43 (s, 1H, H-6 (dU)), 9.99 (s, 1H, N-H (dU)), 10.54 (bs, 1H, -CHO). **<sup>13</sup>C-NMR** (150 MHz; [d<sub>6</sub>]-DMSO): δ = 40.3, 60.7, 69.9, 84.8, 87.4, 112.0, 128.1, 129.3, 134.6, 139.5, 139.6, 149.7, 161.8, 192.6. **MS** (ESI): m/z (%) = 331.0 (100) [M].

**5-[4-(2,2'-Diethyl-1,1',3,3'-tetramethyldipyrromethenyl)-phenyl]-2'-desoxyuridine (2)** **1** (420 mg, 1.26 mmol) was dissolved in abs. CH<sub>2</sub>Cl<sub>2</sub>/DMF 2:1 (15 mL) and 3-ethyl-2,4-dimethylpyrrol 430 μL (3.22mmol, 2.51 equiv.) was added. The mixture was stirred for 15 min at r.t.. After addition of two drops of trifluoroacetic acid, the reaction was stirred for 20 h at r.t. A solution of *p*-chloranil (328 mg, 1.29 mmol, 1 equiv.) in dry CH<sub>2</sub>Cl<sub>2</sub> (80 mL) was added. After 2 h at r.t. the reaction was quenched with H<sub>2</sub>O (100 mL) and the aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic phase was dried with Na<sub>2</sub>SO<sub>4</sub> and the solvent was evaporated under vacuum. The crude product was purified by flash chromatography (SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>:MeOH=100:2 to CH<sub>2</sub>Cl<sub>2</sub>:MeOH=3:1) to yield 599 mg (1.07 mmol, 83 %) of a red solid. **T.l.c.** (CH<sub>2</sub>Cl<sub>2</sub>:MeOH=20:3) R<sub>f</sub> = 0.11, **<sup>1</sup>H-NMR** (300 MHz; [d<sub>4</sub>]-MeOD): δ = 1.13 (t, 6H, Bo-CH<sub>2</sub>-CH<sub>3</sub>), 1.72 (s, 6H, Bo-CH<sub>3</sub>), 2.43 (m, 4H, Bo-CH<sub>2</sub>-CH<sub>3</sub>), 2.53 (m, 2H, 2'-H), 3.82 (m, 2H, 5'-H), 3.98 (m, 1H, 3'-H), 4.47 (m, 1H, 4'-H), 6.36 (t, 1H, 1'-H), 7.39 (d, 2H, Phenyl-H), 7.88 (d, 2H, Phenyl-H), 8.62 (s, 1H, H-6). **<sup>13</sup>C-NMR** (75 MHz; [d<sub>4</sub>]-MeOD): δ = 12.3, 14.8, 18.3, 42.0, 62.5, 72.0, 87.1, 89.3, 114.3, 129.7, 134.6, 136.9, 139.5, 142.3, 147.1, 151.7, 164.3. **MS** (ESI): m/z (%) = 559.4 (100) [M]<sup>+</sup>.

**5-[4-(2,6-Diethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacyl)-phenyl]-2'-desoxyuridine (3)** **2** (1.30 g, 2.33 mmol) was dissolved abs. DMF (15 mL) and dry NEt<sub>3</sub> (3.2 mL, 23.1 mmol, 10 equiv.) was added. The mixture was stirred for 30 min at r.t. While cooling with ice/water, BF<sub>3</sub>·OEt<sub>2</sub> (8.7 mL, 69.5 mmol, 30 equiv.) were added dropwise.

After 5 h stirring at r.t. the reaction was quenched with 2 M aq. NaOH (50 mL). The mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic phase was dried with Na<sub>2</sub>SO<sub>4</sub> and the solvent was removed under vacuum. The crude product was purified by flash chromatography (SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>:acetone:MeOH=4:1:0.1) to yield 760 mg (1.25 mmol, 54%) of a red solid. **T.l.c.** (CH<sub>2</sub>Cl<sub>2</sub>:acetone:MeOH=4:1:0.1) R<sub>f</sub> = 0.23. **<sup>1</sup>H-NMR** (300 MHz; [d<sub>6</sub>]-DMSO): δ = 0.94 (t, 6H, Bo-CH<sub>2</sub>-CH<sub>3</sub>), 1.31 (s, 6H, Bo-CH<sub>3</sub>), 2.25 (m, 2H, H<sub>2</sub>'), 2.29 (q, 4H, Bo-CH<sub>2</sub>-CH<sub>3</sub>), 2.44 (s, 6H, Bo-CH<sub>3</sub>), 3.64 (m, 2H, 5'-H), 3.84 (m, 1H, 3'-H), 4.32 (m, 1H, 4'-H), 5.19 (s, 1H, 5'-OH), 5.29 (s, 1H, 3'-OH), 6.24 (t, 1H, 1'-H), 7.33 (d, 2H, Ph-H), 7.84 (d, 2H, Ph-H), 8.43 (s, 1H, H-6 dU), 11.57 (s, 1H, N-H). **<sup>13</sup>C-NMR** (75 MHz; [d<sub>6</sub>]-DMSO): δ = 11.5, 12.2, 14.6, 16.4, 48.5, 60.7, 69.9, 84.7, 112.0, 127.8, 127.9, 130.0, 132.5, 133.2, 133.9, 138.0, 138.6, 140.4, 149.7, 153.1, 162.0. **MS** (ESI): m/z (%) = 607.3 (100) [M]<sup>+</sup>. **HR-MS** (MALDI-TOF): for C<sub>32</sub>H<sub>37</sub>BF<sub>2</sub>N<sub>4</sub>O<sub>5</sub>: m/z (calcd.) = 607.2903 [M]<sup>+</sup>; m/z (exp.) = 607.2882 [M]<sup>+</sup>.

**5-[4-(2,6-Diethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacyl)-phenyl]-5'-O-dimethoxytrityl-2'-desoxyuridine (4) 3** (224 mg, 0.37 mmol) was dissolved in dry Pyridin (10 mL). 126 mg (0.37 mmol, 1.0 equiv.) 4,4'-dimethoxytritylchloride were added and the solution was stirred for 45 h at r.t.. The reaction was quenched with MeOH (2 mL) and the solvent was removed under vacuum. The crude product was purified by flash chromatography (SiO<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>:acetone=4:1 + 0.1 % pyridine) to yield 74 mg (0.08 mmol, 22 %) of a red solid. **T.l.c.** (EtOAc/petrol ether= 2:1) R<sub>f</sub> = 0.26. **<sup>1</sup>H-NMR** (400 MHz; [d<sub>6</sub>]-DMSO): δ = 0.89 (t, 6H, Bo-CH<sub>2</sub>-CH<sub>3</sub>), 0.97 (s, 6H, Bo-CH<sub>3</sub>), 2.21 (q, 4H, Bo-CH<sub>2</sub>-CH<sub>3</sub>), 2.25 (m, 2H, 2'-H), 2.40 (s, 6H, Bo-CH<sub>3</sub>), 3.10 - 3.30 (m, 2H, 5'-H), 3.62 (s, 6H, DMT-CH<sub>3</sub>), 3.92 (m, 1H, 3'-H), 4.26 (m, 1H, 4'-H), 5.31 (d, 1H, 3'-OH), 6.25 (t, 1H, 1'-H), 7.07 – 7.38 (m, 13H, DMT) 7.33 (d, 2H, Ph-H), 7.63 (d, 2H, Ph-H), 7.90 (s, 1H, H-6), 11.67 (s, 1H, N-H). **<sup>13</sup>C-NMR** (100 MHz; [d<sub>6</sub>]-DMSO): δ = 11.2, 12.1, 14.2, 16.3, 54.8, 63.5, 68.5, 70.5, 84.8, 85.6, 85.8, 112.9, 113.1, 123.9, 126.6, 127.7, 127.8, 128.3, 128.5, 129.6, 129.8, 132.4, 133.3, 133.4, 135.4, 135.5, 137.5, 138.2, 140.1, 144.5, 149.6, 149.7, 153.0, 155.0, 162.0. **MS** (ESI): m/z (%) = 909.5 (100) [M]<sup>+</sup>

**5-[4-(2,6-Diethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacyl)-phenyl]-5'-O-dimethoxytrityl-3'-O-[(2-cyanoethyl)-N,N-diisopropyl]-phosphoramidite]-2'-desoxyuridine (5) 4** (52 mg, 0.057 mmol) was dissolved in abs CH<sub>2</sub>Cl<sub>2</sub> (3 mL). After addition of dry (iPr)<sub>2</sub>NEt (29 μL) and 2-Cyanoethyl-N,N-diisopropylchlorophosphoramidite (13 μL, 0.57 mmol, 1.0 equiv.), the solution was stirred for 30 min at r.t. Again, 2-cyanoethyl-N,N-

*diisopropylchlorophosphoramidite* (10  $\mu\text{L}$ , 0.44 mmol, 0.8 equiv.) was added. After 60 min stirring at r.t. another portion of 2-cyanoethyl-*N,N*-*diisopropyl*-chlorophosphoramidite (7  $\mu\text{L}$ , 0.30 mmol, 0.5 equiv.) was added. After another 60 min stirring at r.t., the reaction was quickly washed with saturated aq.  $\text{NaHCO}_3$ . The organic phase was dried with  $\text{Na}_2\text{SO}_4$  and the solvent was removed under vacuum to yield a red solid. T.l.c. showed quantitative conversion. Due to its hydrolytic instability the product was immediately used for oligonucleotide synthesis. **T.l.c.** (EtOAc:petrol ether=2:1):  $R_f = 0.64$ .  **$^{31}\text{P}$ -NMR** (121 MHz; [d6]-DMSO):  $\delta = 148.22, 148.59$ .

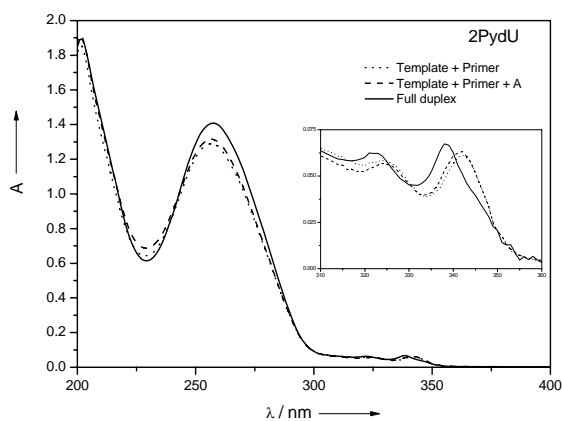
**Primer Extension Experiments.** The reaction mixture (20  $\mu\text{L}$ ) contained the corresponding polymerase, dNTPs (200  $\mu\text{M}$ ), primer (150 nM 5'-CGT TGG TCC TGA AGG AGG ATA GG-3'), and template (225 nM, 3'-CAT GCA ACC AGG ACT TCC TCC TAT AAX ACT AAA-5', X = modified nucleotide) in the corresponding reaction buffer. The primer was labeled by use of [ $\gamma$  $^{32}\text{P}$ ]-ATP according to standard techniques. Reaction mixtures were incubated for 30 min at 37  $^\circ\text{C}$  and were stopped by addition of stop solution (45  $\mu\text{L}$ , 80% [v/v] formamide, 20 mM EDTA, 0.025% [w/v] bromophenol blue, 0.025% [w/v] xylene cyanol). Reaction mixtures were separated by use of a 12% denaturing PAGE. Visualization was performed by phosphoimaging.

Reaction promoted by KF- comprised 50 mM Tris-HCl pH 7.3, 10 mM  $\text{MgCl}_2$ , 1 mM DTT and 2 nM enzyme; For Pol  $\beta$  and Dpo4 DNA polymerase: 50mM Tris-HCl pH 8.0, 10mM  $\text{MgCl}_2$ , 2mM DTT, 20mM NaCl, 20mM KCl, 1% glycerol, 200 $\mu\text{g}/\text{ml}$  BSA and 50 nM enzyme.

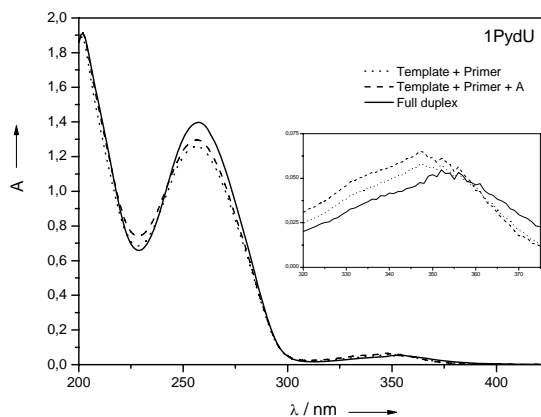
**DNA Polymerase activity determination on unmodified and modified templates.** For activity determination primer extension reactions were carried out with varying concentrations of DNA polymerase. 20  $\mu\text{l}$  reactions contained 150 nM primer, 225 nM of the respective template, 200  $\mu\text{M}$  dNTPs in 1xKF reaction buffer. For reactions comprising non-modified templates we employed a DNA oligonucleotide that harbours a dA instead of the modified nucleotide. Reactions were performed at 37 $^\circ\text{C}$  for 2 min, subsequently quenched with stop solution and products were separated on a 12 % denaturing PAGE. Phosphoimaging was carried out and the obtained intensities for each band were transformed into dNTP conversion. The total amount of incorporated nucleotides for each reaction equals the sum of incorporated nucleotide of each band. Enzyme concentration was plotted against dNTP

conversion and the resulting slopes were compared. The results derive from measurements that were at least two times independently repeated.

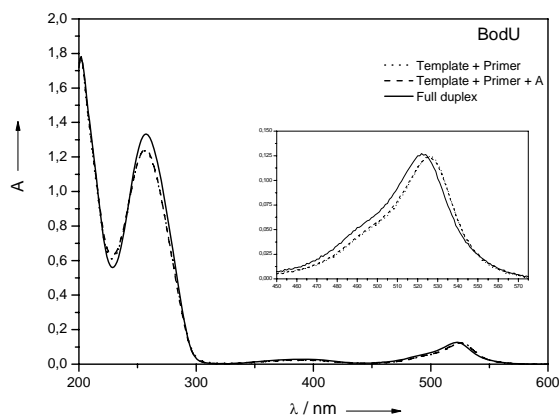
**Figure S1.** UV/Vis absorption spectra of 2PydU-modified template (2.5  $\mu\text{M}$ ) in the presence of the primer, the primer elongated with A, and the full-length counterstrand, in 10 mM Na-Pi-buffer (pH 7.0), 250 mM NaCl, 20  $^{\circ}\text{C}$ .



**Figure S2.** UV/Vis absorption spectrum of 1PydU-modified template (2.5  $\mu\text{M}$ ) in the presence of the primer, the primer elongated with A, and the full-length counterstrand, in 10 mM Na-Pi-buffer (pH 7.0), 250 mM NaCl, 20  $^{\circ}\text{C}$ .



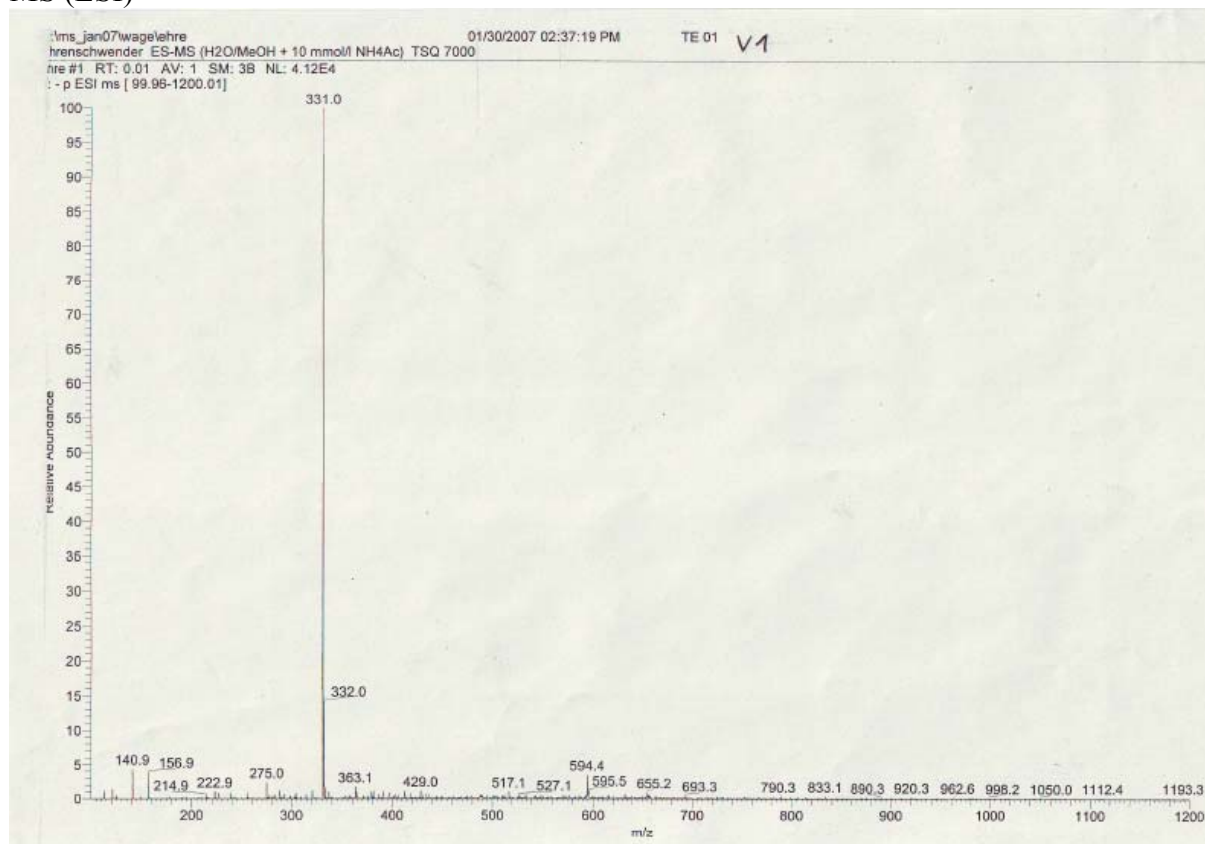
**Figure S3.** UV/Vis absorption spectra of BodU-modified template (2.5  $\mu\text{M}$ ) in the presence of the primer, the primer elongated with A, and the full-length counterstrand, in 10 mM Na-Pi-buffer (pH 7.0), 250 mM NaCl, 20  $^{\circ}\text{C}$ .



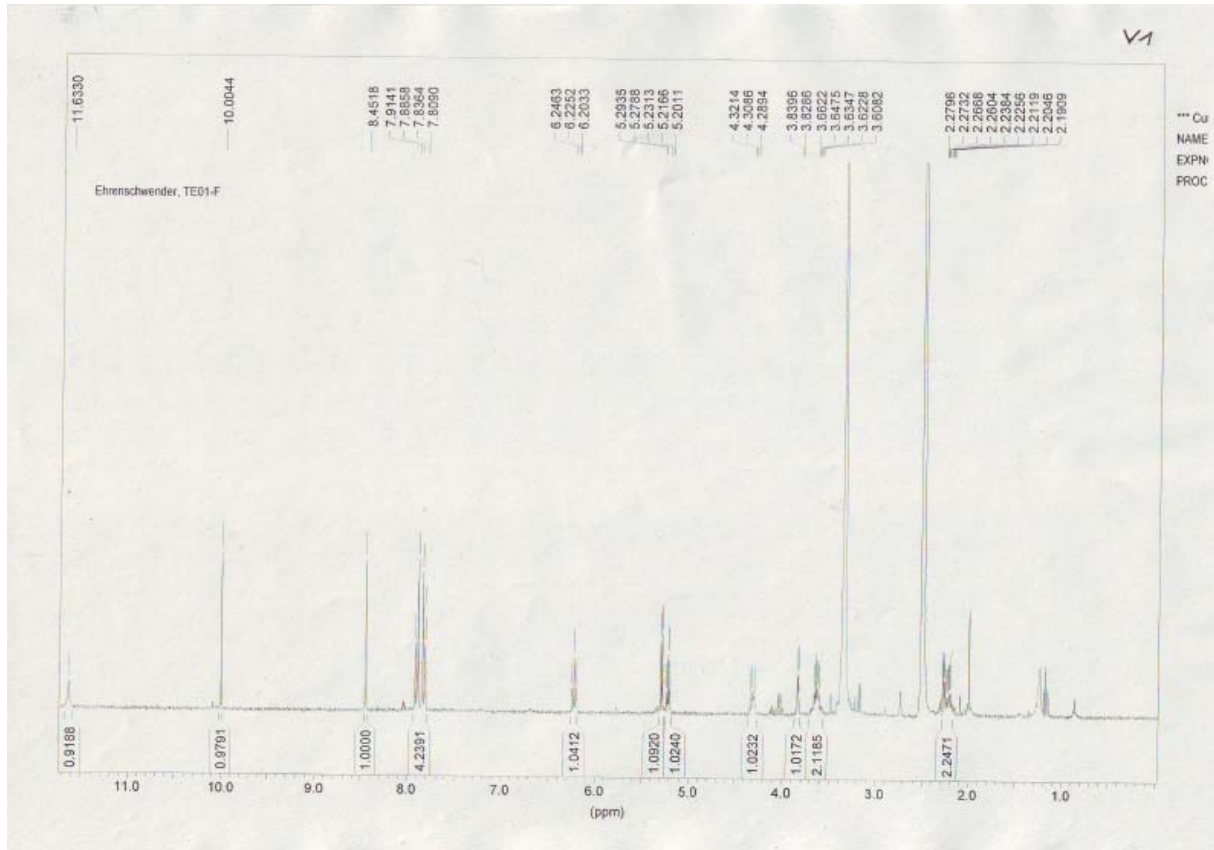
## Spectra

### Compound 1

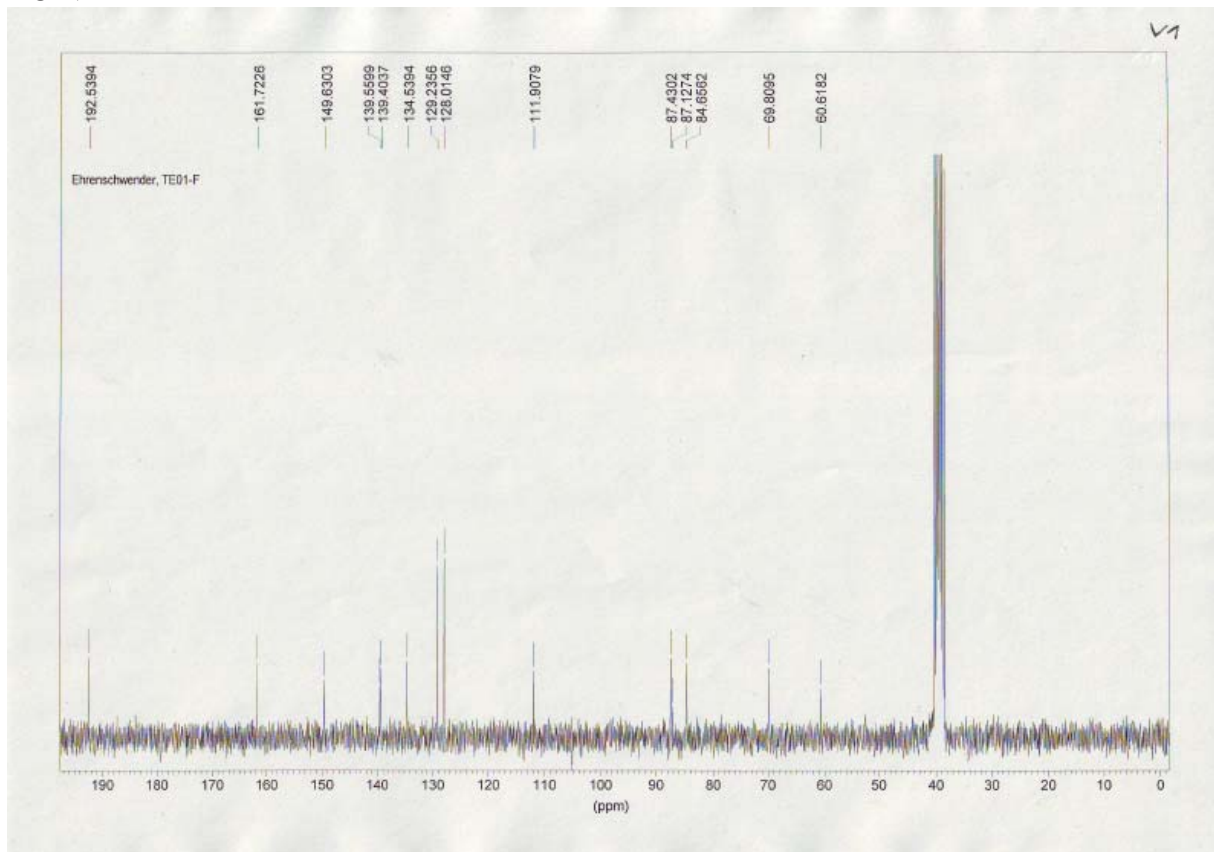
### MS (ESI)



<sup>1</sup>H NMR

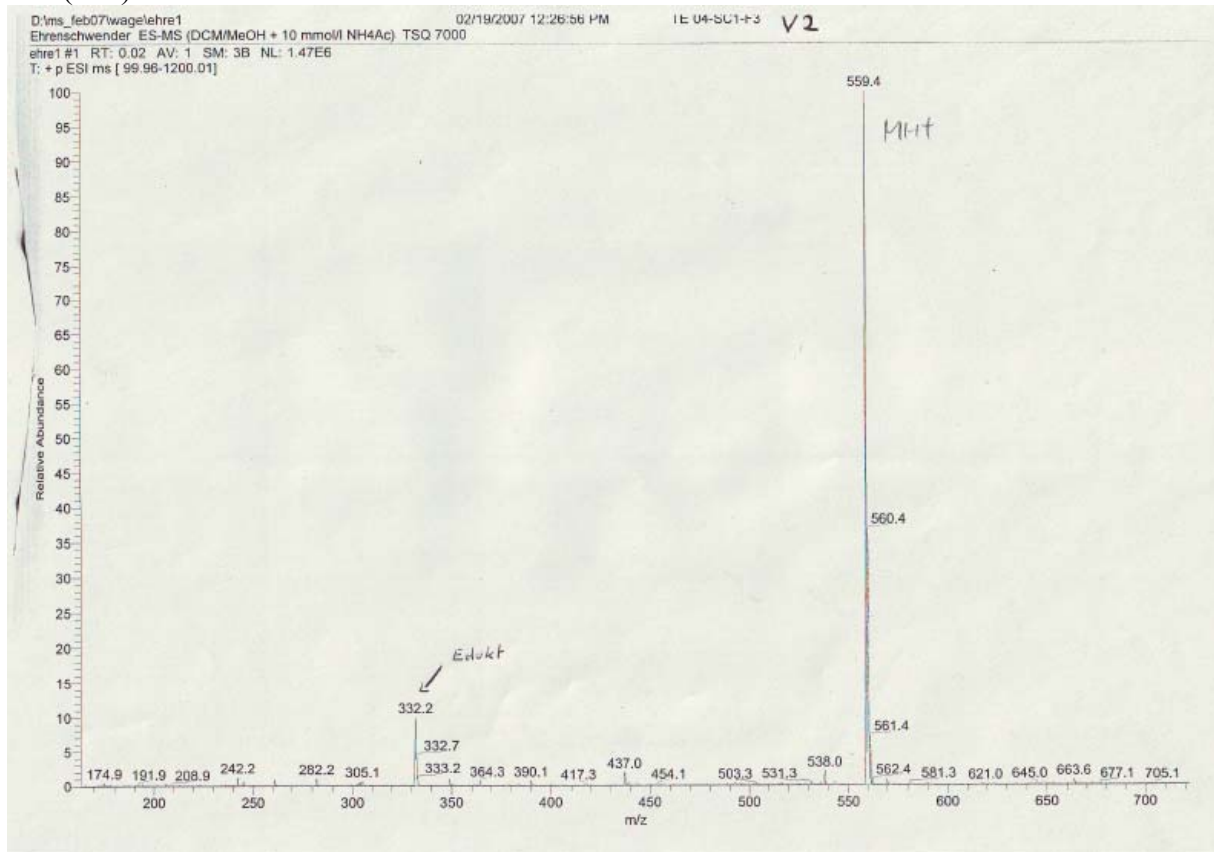


<sup>13</sup>C NMR

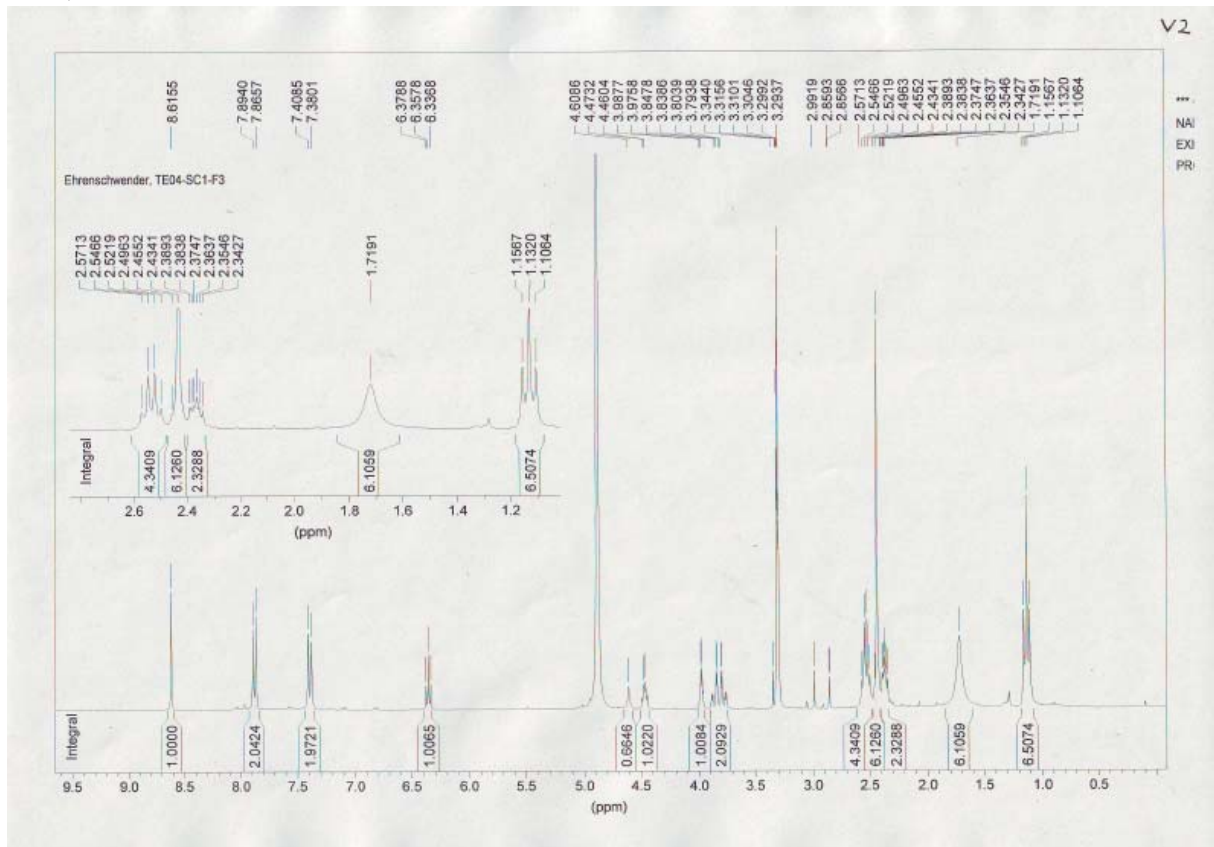




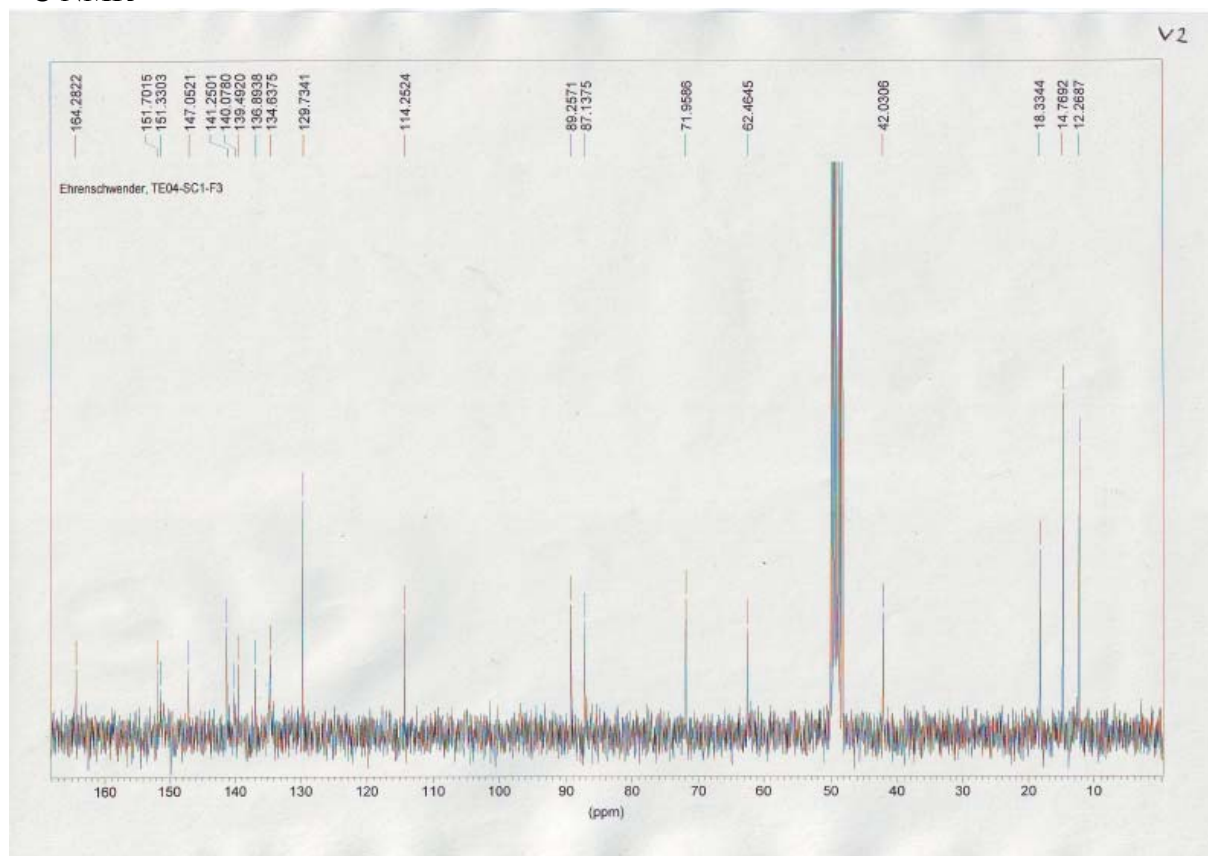
Compound 2:  
MS (ESI)



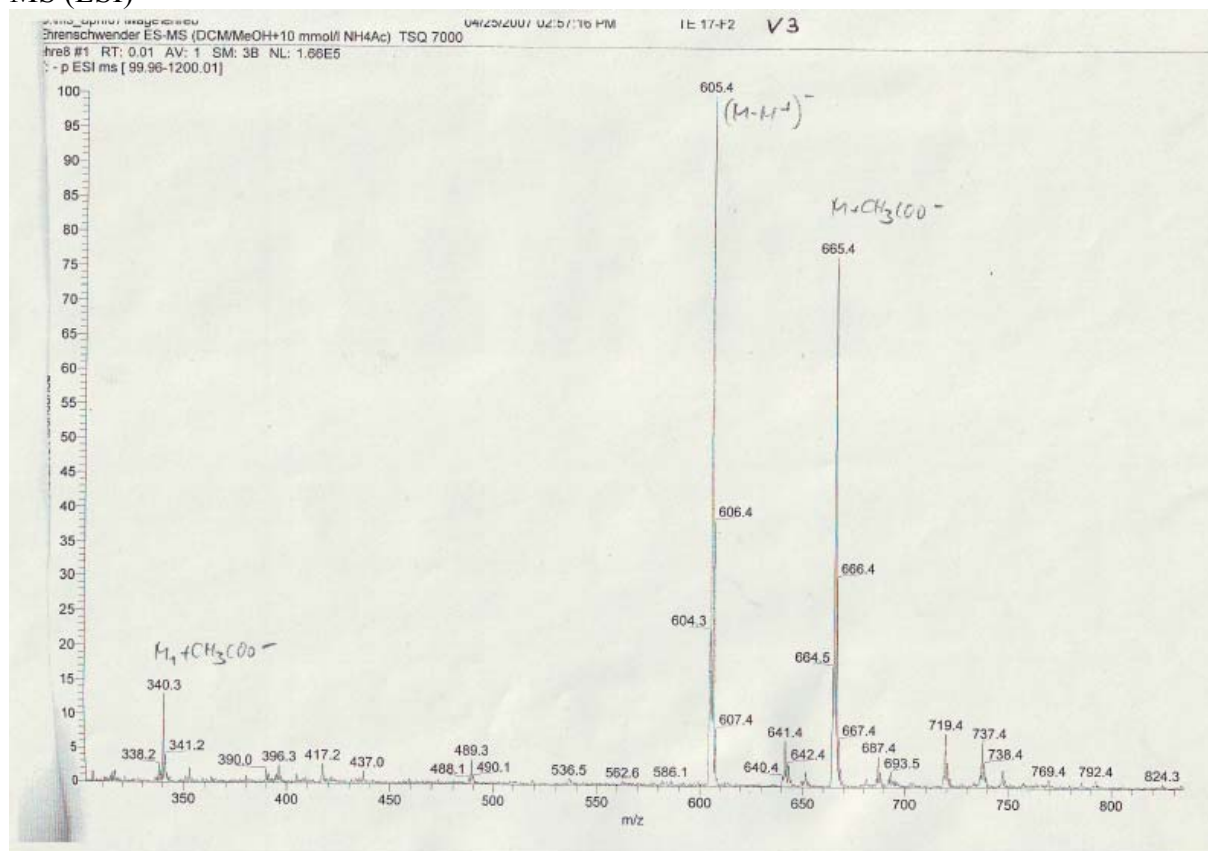
<sup>1</sup>H NMR



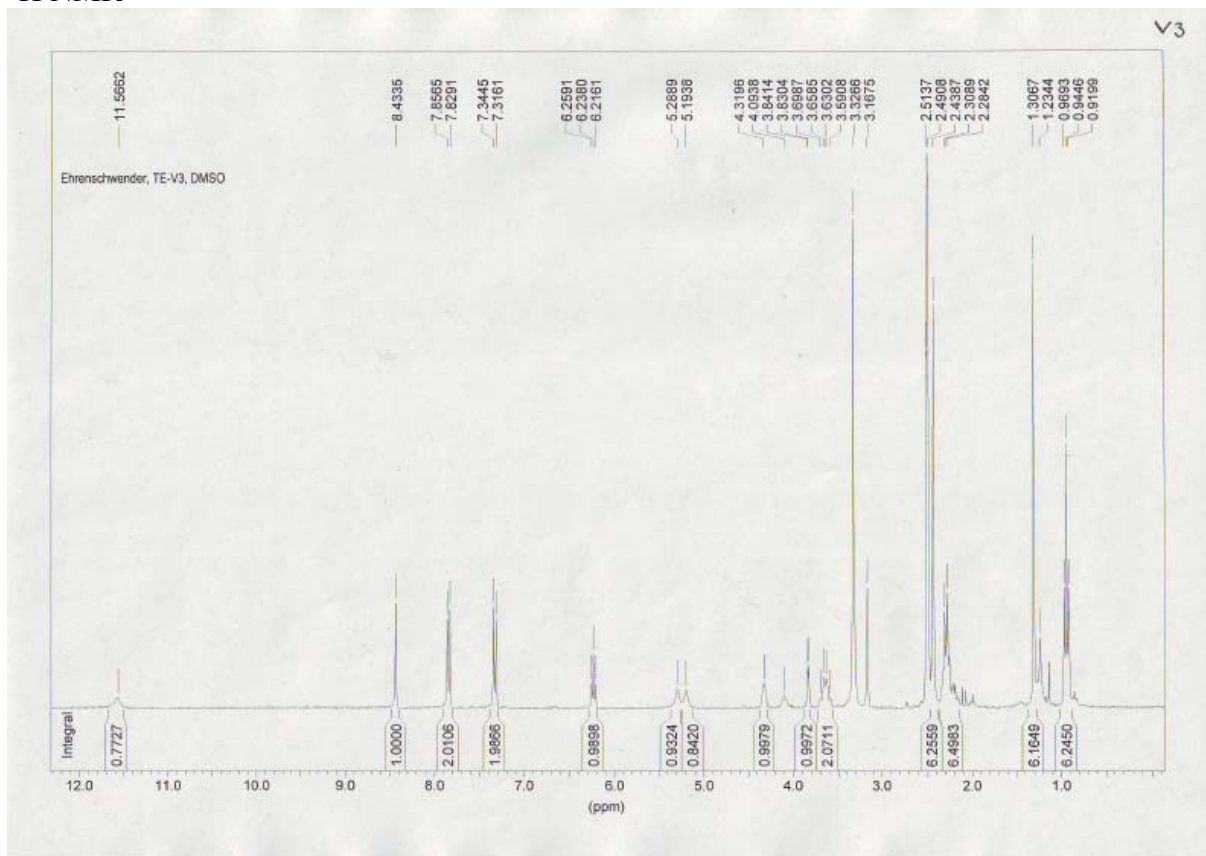
$^{13}\text{C}$  NMR



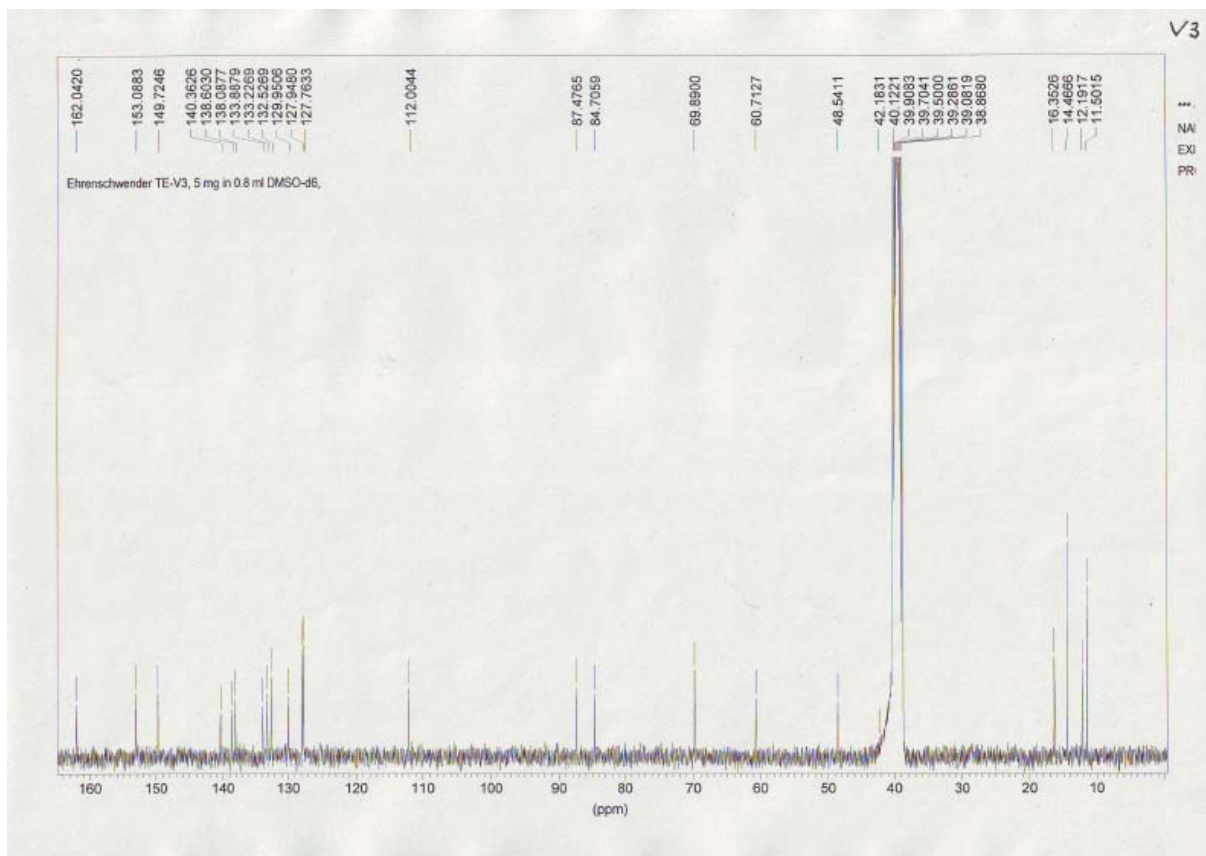
Compound 3  
MS (ESI)



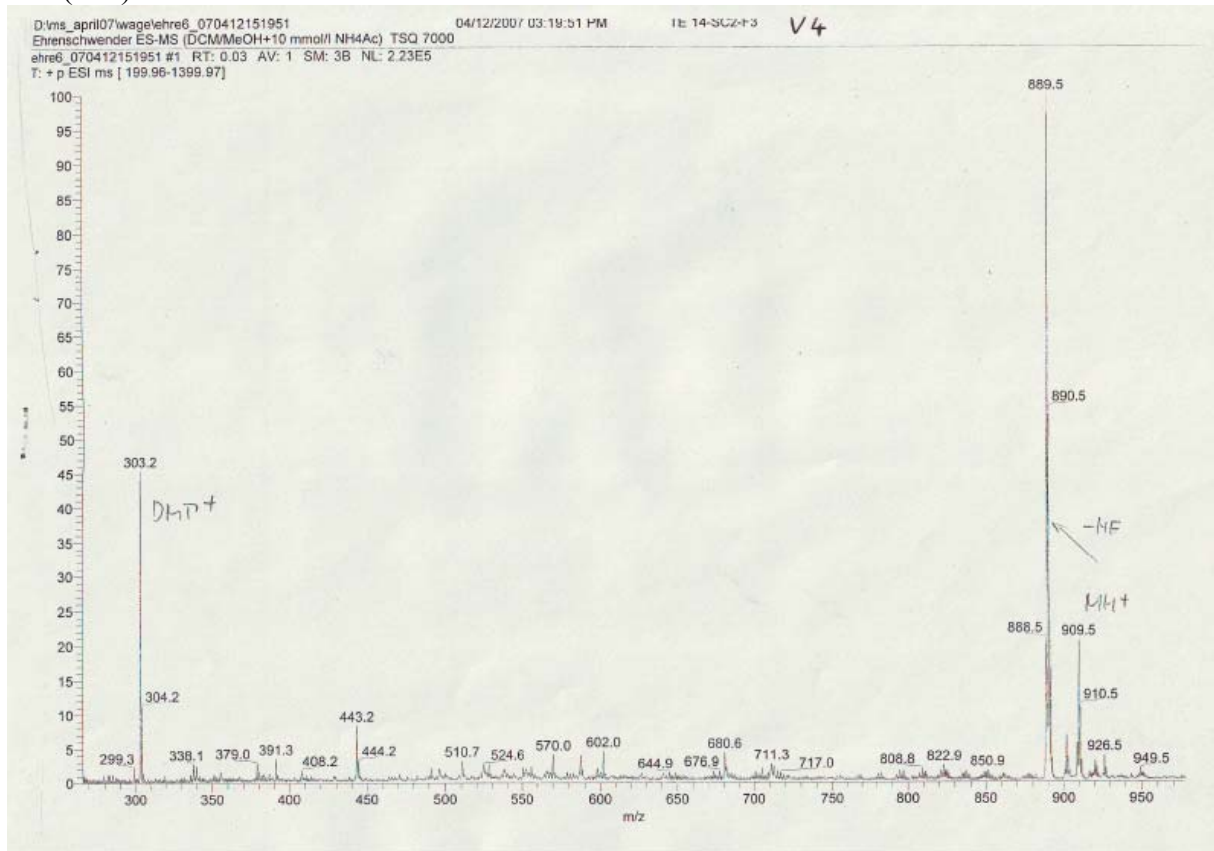
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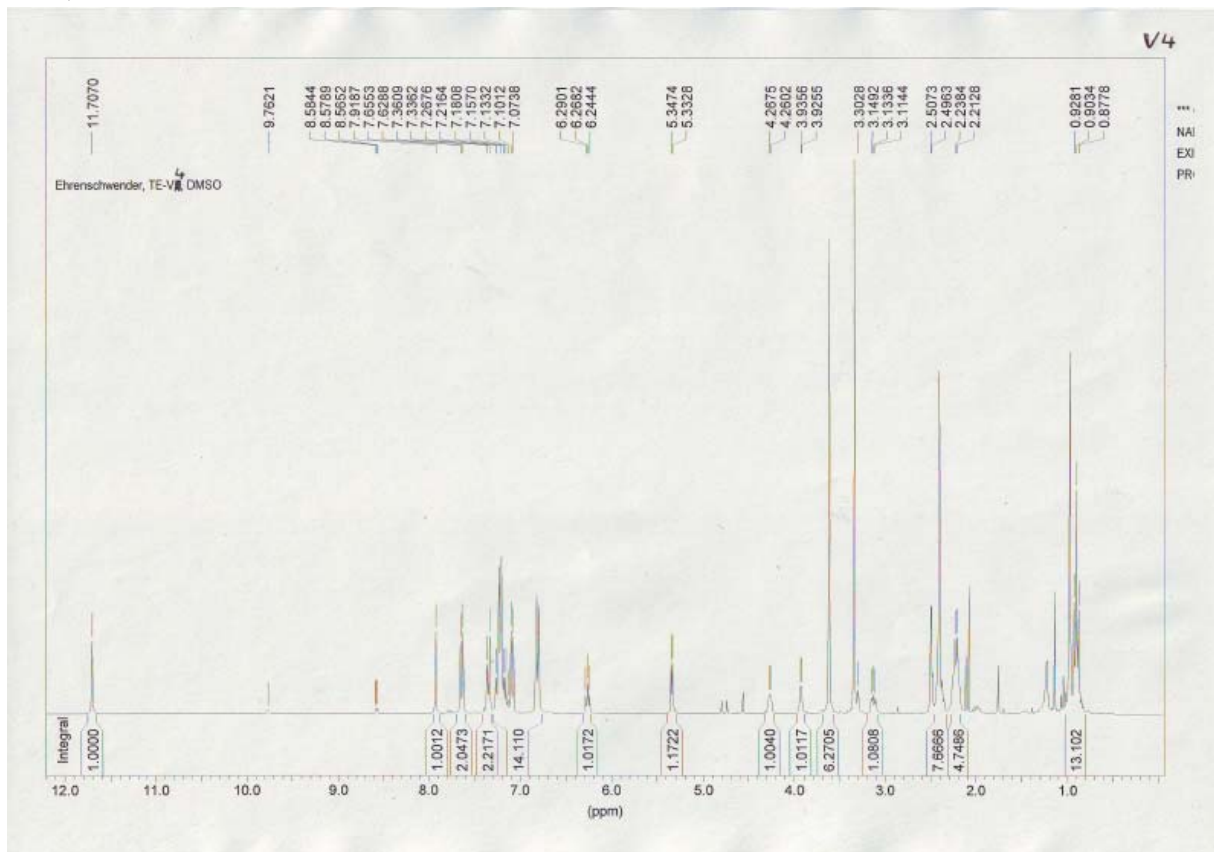
<sup>13</sup>C NMR



### Compound 4 MS (ESI)

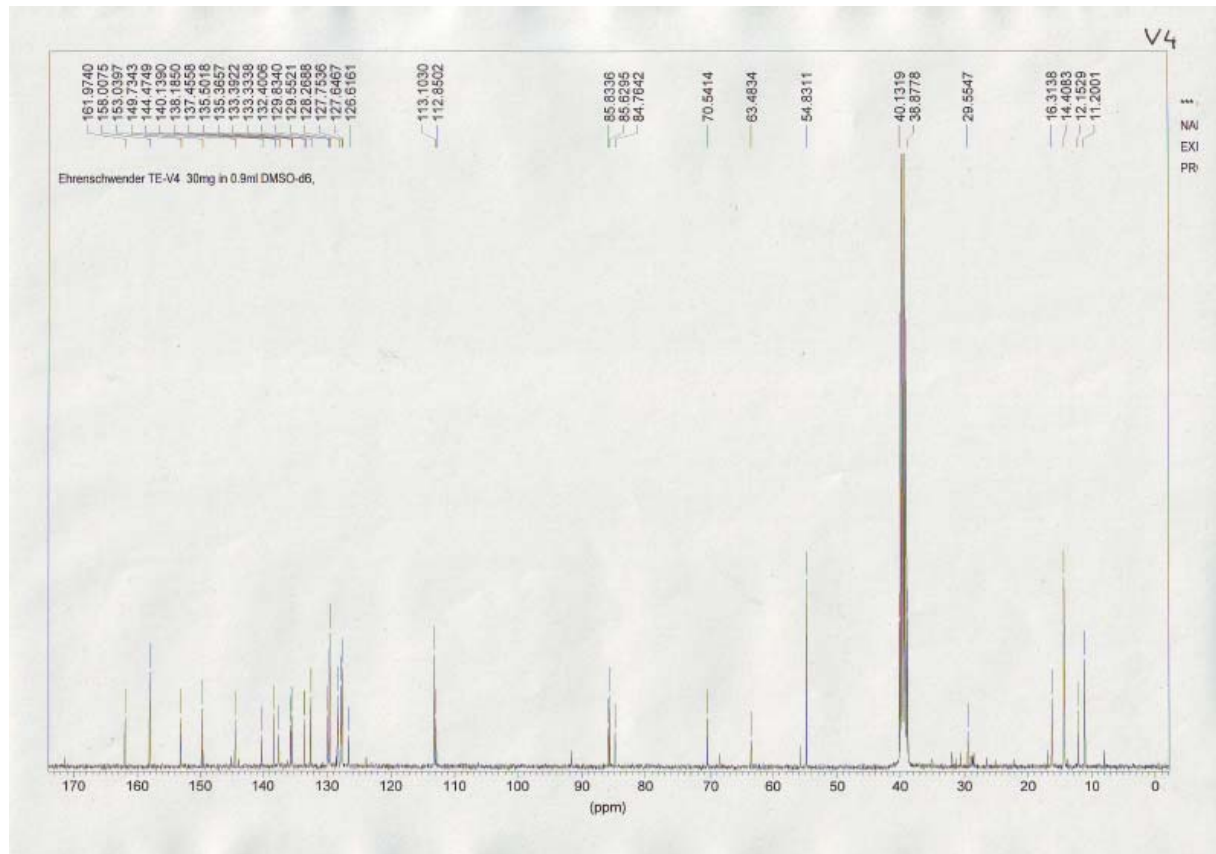


### <sup>1</sup>H NMR

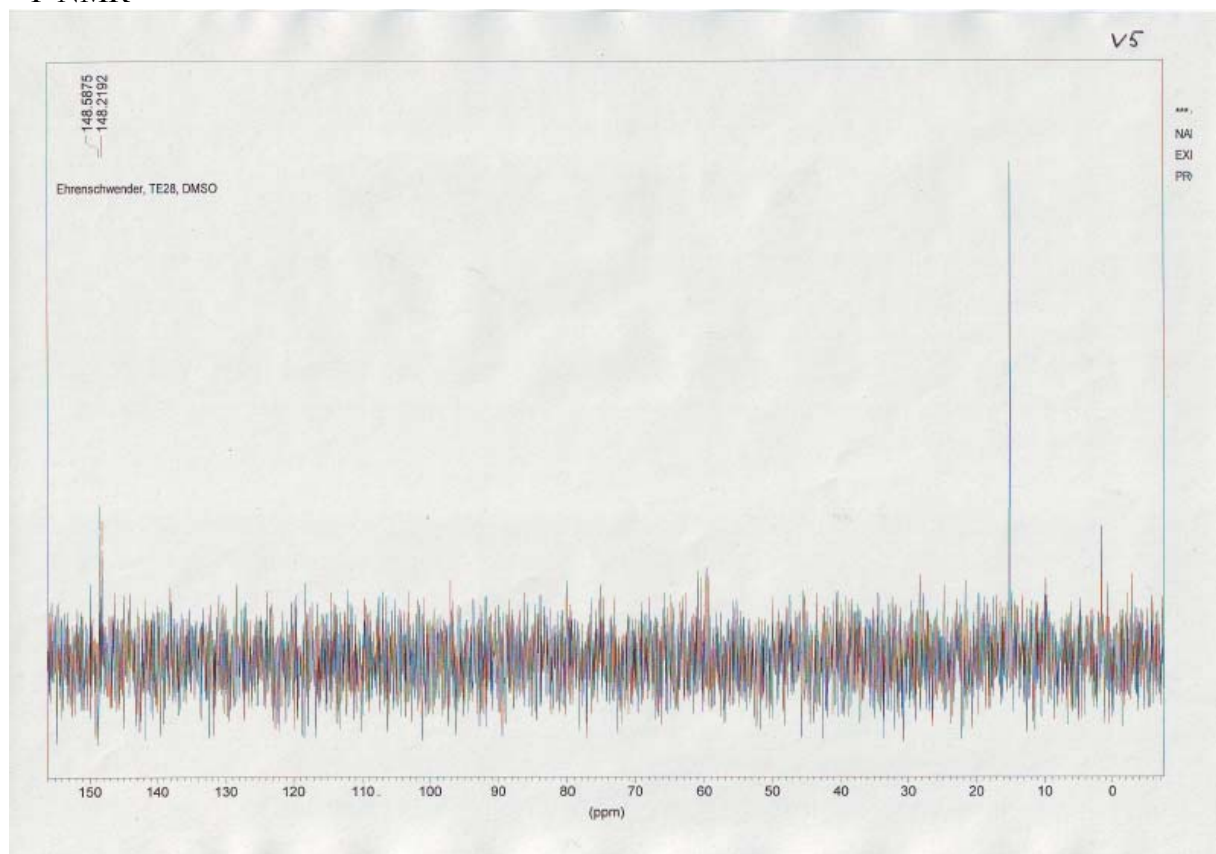




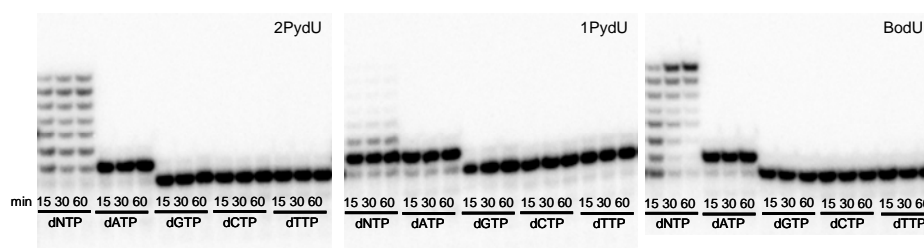
$^{13}\text{C}$  NMR



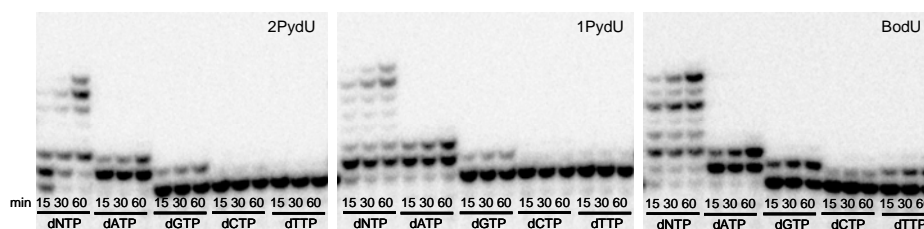
Compound 5  
 $^{31}\text{P}$  NMR



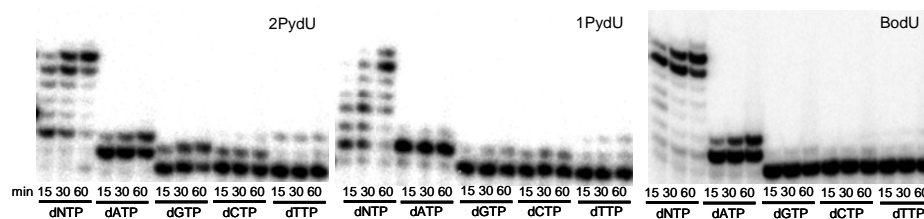
**Figure S4.** Primer extension by Pol  $\beta$  with three different incubation times: 15 min, 30 min, 60 min, (37 °C), c (Pol  $\beta$ ) = 100 nM, c (dNTPs) = 200  $\mu$ M.



**Figure S5.** Primer extension by Dpo4 with three different incubation times: 15 min, 30 min, 60 min (37 °C), c (Dpo4) = 100 nM, c (dNTPs) = 200  $\mu$ M.



**Figure S6.** Primer extension by KF(exo-) with three different incubation times: 15 min, 30 min, 60 min (37 °C), c (Dpo4) = 100 nM, c (dNTPs) = 200  $\mu$ M.



**References:**

- [1] N. Amann, E. Pandurski, T. Fiebig, H.-A. Wagenknecht, *Chem. Eur. J.* **2002**, 8, 4877-4883.
- [2] E. Mayer, L. Valis, R. Huber, N. Amann, H.-A. Wagenknecht, *Synthesis* **2003**, 2335-2340.
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- [4] C. Wanninger-Weiß, H.-A. Wagenknecht, *Eur. J. Org. Chem.* **2008**, 64-71.
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- [6] C. Wanninger-Weiß, L. Valis, H.-A. Wagenknecht, *Bioorg. Med. Chem.* **2008**, 16, 100-106.