Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

Oligonucleotide promoted peptide bond formation using a tRNA mimicking approach

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Methods

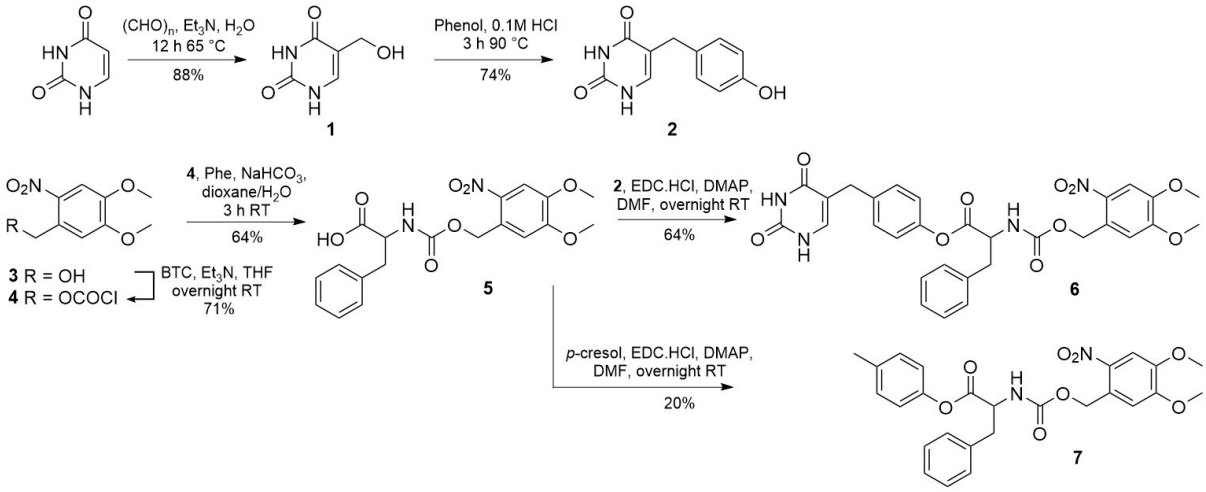
General reaction conditions

For all reactions, chemicals of analytical or synthetic grade were obtained from commercial sources and were used without further purification unless otherwise stated. Oligonucleotides were graciously provided by Guy Schepers (Rega institute, KULeuven). Water was ultrapure (18 MΩcm) and prepared by a Millipore (MilliQ®) purification system. The stationary phase in column chromatography was 70-230 mesh silica 60 (E.M. Merck). Analytical Thin Layer Chromatography was performed on Alugram® silica gel UV254 mesh 60, 0.20 mm (Macherey-Nagel). 1D and 2D NMR were recorded on a Bruker Avance 300 (5mm BBO Probe), Avance 500 (TXI Z gradient probe) or Avance II 600 (5 mm TCI HCN Z gradient cryoprobe). Chemical shifts are expressed as δ units (part per million) down field from TMS for ^{1}H and ^{13}C-spectra. HRMS spectra were acquired on a quadrupole orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Samples were infused at 3 µL/min and spectra were obtained in positive (or negative) ionization mode with a resolution of 15000 FWHM using leucine encephalin as lock mass. LC-MS analysis was conducted on an Agilent 1100 HPLC with quaternary pump, auto sampler, UV-DAD detector and a thermostatic column (25 °C) module coupled to Agilent 6110 single-quadrupole electrospray ionization mass spectrometry (capillary voltage = +3500V or -3000V). The column was a Grace Prevail RP-C18 3 µm 150mm x 2.1mm. Data collection and analysis was done with Agilent LC/MSD Chemstation software. Photolytic cleavage of the NVOC protection group was conducted in a homemade cylindrical reactor, closed from ambient light. Venting holes and fan cooling were present and a cylindrical, polished stainless steel casing provided reflection of UV lamps installed towards the reaction centre. The setup could accommodate 4 circuits of each 4 Sylvania F8W/T5/BL368 UV lamps. Graphs and figures were prepared in GraphPad Prism version 6.01 for Windows (GraphPad Software, La Jolla California USA,

www.graphpad.com). Abbreviations: TMS, tetramethyl silane; NMR, nuclear magnetic resonance; FWHM, full width at half maximum; HRMS, high resolution mass spectrometry; HPLC, high pressure liquid chromatography; UV-DAD, ultra violet-diode array detector; $(CHO)_n$, paraformaldehyde; Et_3N, triethylamine; BTC, bis(trichloromethyl) carbonate; THF, tetrahydrofuran; Phe, phenylalanine; EDC, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride; RT, room temperature; DMAP, 4-dimethylaminopyridine; DMF, dimethylformamide; DMSO, Dimethylsulfoxide; $CDCl_3$, deuterated chloroform; ACN, acetonitrile; LOQ, limit of quantification

Synthesis

Synthetic scheme



5-Hydroxymethyluracil (1) Obtained according to literature (88% yield)[1]

^{1}H NMR (300 MHz, DMSO-d6, δ): 11.05 (s, 1H, CON*H*CO), 10.72 (s, 1H, CON*H*), 7.23 (s, 1H, C*H*), 4.85 (s, 1H, O*H*), 4.10 (s, 2H, CH_2OH); ^{13}C NMR (75MHz, DMSO-d6, δ): 163.80, 151.35, 138.20, 112.72, 55.81; *m/z* = 141.0 [M-H$^+$]; m.p.: >300 °C (lit. 360 °C (dec.))

5-(4-Hydroxybenzyl)uracil (2) Obtained according to literature (74% yield)[2]

^{1}H NMR (300 MHz, DMSO-d6, δ): 11.05 (s, 1H, CON*H*CO), 10.66 (s, 1H, CON*H*), 9.18 (s, 1H, ϕO*H*), 7.12 (s, 1H, C*H*), 7.01 (d, ^3J(H,H) = 8.1 Hz, 2H, ϕC*H*), 6.66 (d, ^3J(H,H) = 8.2 Hz,

2H, ϕC*H*), 3.40 (s, overlap with HOD-peak, CH_2); ^{13}C NMR (75 MHz, DMSO-d6, δ): 164.26, 155.53, 151.31, 138.30, 129.76, 129.43, 114.97, 112.47, 30.81; HRMS (*m/z*): 219.0765 [M+H$^+$]; T_m > 300°C (lit. > 320 °C)

4,5-Dimethoxy-2-nitrobenzyloxycarbonyl chloride (NVOCCl) (4) A solution of triethylamine (3.25 mL, 23.5 mmol, 1 eq) in THF (75 mL) was added dropwise to a stirred solution of BTC (7 g, 23.5 mmol, 1 eq) and **3** (5 g, 23.5 mmol, 1 eq) in THF (100 mL) at 0 °C. Afterwards the mixture was left stirring overnight at room temperature. Any solids were filtered away and the filtrate concentrated *in vacuo*. The residue was recrystallized in toluene to yield 6.48g (71%) as a yellow-orange solid, used without further purification.

^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.77 (s, 1H, 3-C*H*), 7.01 (s, 1H, 6-C*H*), 5.75 – 5.72 (m, 2H, CH_2), 4.02 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 153.75, 150.40, 148.94, 139.88, 124.23, 110.43, 108.40, 69.85, 56.56, 56.51.

N-(4,5-Dimethoxy-2-nitrobenzyloxycarbonyl)-L-phenylalanine (5) *modified protocol*[3]: A solution of **4** (3 g, 10.9 mmol, 1 eq) in dioxane (40 mL) was added dropwise to a stirred suspension of phenylalanine (2.16 g, 13.1 mmol, 1.2 eq) and $NaHCO_3$ (2.29 g, 27.2 mmol, 2.5 eq) in water/dioxane (32 mL, 1:1) at 0 °C. After completion of the addition, the mixture was stirred for 1 h at 0 °C and 3 h at room temperature. The mixture was washed with diethyl ether (3x), acidified with concentrated HCl and extracted with ethyl acetate (5x). The combined organic layers were washed with distilled water (2x) and brine (1x), followed by drying over anhydrous $MgSO_4$. After rotary evaporation of the solvent, the desired product was obtained as an orange-brown solid (2.82 g, 64% yield).

^{1}H NMR (300 MHz, DMSO-d6, δ): 7.94 (d, J = 8.4 Hz, 1H, N*H*), 7.70 (s, 1H, 3-C*H*), 7.29 – 7.25 (m, 3H, ϕC*H*), 7.24 – 7.16 (m, 2H, ϕC*H*), 7.10 (s, 1H, 6-C*H*), 5.42 – 5.18 (m, 2H, CH_2), 4.25 – 4.12 (m, 1H, α-C*H*), 3.86 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.14 – 3.05 (m, 1H, β-CH_2), 2.92 – 2.78 (m, 1H, β-CH_2); ^{13}C NMR (75 MHz, DMSO-d6, δ): 206.50, 173.25, 161.90,

155.59, 153.42, 147.55, 138.89, 137.84, 129.00, 128.14, 126.37, 118.29, 114.69, 109.75, 108.06, 56.11, 56.04, 55.53, 30.66. HRMS (*m/z*): 403.1150 [M-H$^+$]; m.p.: 159-164 °C.

4-((Uracil-5-yl)methyl)phenyl N-(4,5-dimethoxy-2-nitrobenzyloxycarbonyl)-L-phenyl-alaninate (6) A solution of **5** (0.25 g, 0.62 mmol, 1 eq) and DMAP (113.3 mg, 0.93 mmol, 1.5 eq) in DMF (5 mL) was cooled to 0 °C while stirring under N_2 atmosphere. EDC·HCl (177.8 mg, 0.93 mmol, 1.5 eq) and **2** (148.4 mg, 0.68 mmol, 1.1 eq) were subsequently added followed by cooling for another hour. The reaction mixture was allowed to warm up to room temperature and left stirring overnight, followed by pouring the mixture in ethyl acetate/$NaHCO_3$ saturated solution. The organic layer was washed with 1M HCl (3x) and brine (1x), followed by drying over anhydrous $MgSO_4$ and evaporation *in vacuo*. The residue was purified by column chromatography using a gradient of dichloromethane/acetone (9:1 to 5:5) resulting in a pure dark yellow solid identified by 1D and 2D NMR to be the desired compound (373.8 mg, 64%). ^{1}H NMR (300 MHz, DMSO-d6, δ): 11.06 (s, 1H, CON*H*CO), 10.72 (s, 1H, CON*H*), 8.26 (d, ^3J(H,H) = 7.6 Hz, 1H, N*H*), 7.70 (s, 1H, 3-CH), 7.36 – 7.28 (m, 5H, ϕC*H*), 7.27 – 7.20 (m, 3H, ϕC*H*), 7.13 (s, 1H, 6-C*H*), 6.83 (d, ^3J(H,H) = 8.5 Hz, 2H, ϕC*H*), 5.43 – 5.30 (m, 2H, CH_2), 4.55 – 4.47 (m, 1H, α-C*H*), 3.86 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.48 (s, 2H, CH_2), 3.23 – 3.16 (m, 1H, β-CH_2), 3.11 – 3.03 (m, 1H, β-CH_2); ^{13}C NMR (75 MHz, DMSO-d6, δ): 170.88, 164.31, 155.73, 153.42, 151.41, 148.50, 147.82, 139.34, 139.10, 137.91, 137.07, 129.47, 129.32, 128.41, 127.70, 126.78, 121.16, 111.49, 110.51, 108.23, 62.71, 62.57, 56.20, 56.17, 55.74, 36.46, 31.14; HRMS (*m/z*): 603.1724 [M-H$^+$]; m.p.: 147-151 °C

***p*-Cresyl N-(4,5-dimethoxy-2-nitrobenzyloxycarbonyl)-L-phenylalaninate (7)** A solution of **5** (1 g, 3.1 mmol, 1 eq) and DMAP (113.3 mg, 0.93 mmol, 1.5 eq) in DMF (20 mL) was cooled to 0 °C while stirring under N_2 atmosphere. EDC·HCl (0.89 g, 4.65 mmol, 1.5 eq) and *p*-cresol (0.37 g, 3.41 mmol, 1.1 eq) were subsequently added followed by cooling for another hour. The reaction mixture was allowed to warm up to room temperature and left stirring

overnight, followed by pouring the mixture in ethyl acetate/$NaHCO_3$ saturated solution. The organic layer was washed with 1 M HCl (3x) and brine (1x), followed by drying over anhydrous $MgSO_4$ and evaporation *in vacuo*. The residue was purified by column chromatography using a gradient of heptane/ethyl acetate (9:1 to 6:4) resulting in a pure light yellow solid identified by 1D NMR to be the desired compound (306.6 mg, 20%).

^{1}H NMR (300 MHz, DMSO-d6, δ): 8.27 (d, J = 7.5 Hz, 1H, N*H*), 7.70 (s, 1H, 3-C*H*), 7.36 – 7.24 (m, 5H, ϕC*H*), 7.18 (d, ^3J(H,H) = 8.3 Hz, 2H, ϕC*H*), 7.13 (s, 1H, 6-C*H*), 6.82 (d, ^3J(H,H) = 8.4 Hz, 2H, ϕC*H*), 5.47 – 5.26 (m, 2H, CH_2), 4.58 – 4.44 (m, 1H, α-C*H*), 3.86 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.25 – 3.15 (m, 1H, β-CH_2), 3.13 – 3.02 (m, 1H, β-CH_2), 2.29 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d6, δ): 170.89, 155.73, 153.42, 148.09, 147.82, 139.32, 137.10, 135.25, 129.92, 129.31, 128.39, 127.72, 126.76, 121.15, 110.47, 108.23, 62.68, 56.16, 55.74, 36.42, 20.43; HRMS (*m/z*): 495.1757 [M+H$^+$]; m.p.: 169-173 °C

4-((Uracil-5-yl)methyl)phenyl N-(benzyloxycarbonyl)-L-phenylalaninate (8) same method as **6** using commercially available N-(benzyloxycarbonyl)-L-phenylalanine. Yield 56%

^{1}H NMR (300 MHz, DMSO-d6, δ): 11.07 (s, 1H, CON*H*CO), 10.75 (s, 1H, CON*H*), 8.05 (d, ^3J(H,H) = 7.5 Hz, 1H, N*H*), 7.43 – 7.14 (m, 12H, ϕC*H*), 6.84 (d, ^3J(H,H) = 8.4 Hz, 2H, ϕC*H*), 5.11 – 4.92 (m, 2H, CH_2), 4.57 – 4.38 (m, 1H, α-C*H*), 3.49 (s, 2H, CH_2), 3.18 – 3.02 (m, 2H, β-CH_2). ^{13}C NMR (75 MHz, DMSO-d6, δ): 170.98, 164.37, 156.20, 151.48, 148.62, 139.13, 137.93, 137.22, 137.00, 129.54, 129.42, 128.47, 127.96, 127.79, 126.81, 121.25, 111.60, 65.70, 55.87, 36.55, 31.22. HRMS (*m/z*): 456.1922 [M-CO_2+H$^+$], 517.2091 [M+NH_4^+], 522.1632 [M+Na$^+$]; m.p.: 137-144 °C

***p*-Cresyl N-(benzyloxycarbonyl)-L-phenylalaninate (9)** same method as **7** using commercially available N-(benzyloxycarbonyl)-L-phenylalanine. Yield 33%

^{1}H NMR (300 MHz, DMSO-d6, δ): 8.02 (d, ^3J(H,H) = 7.4 Hz, 1H, N*H*), 7.39 – 7.23 (m, 10H, ϕC*H*), 7.20 (d, ^3J(H,H) = 8.1 Hz, 2H), 6.84 (d, ^3J(H,H) = 8.1 Hz, 2H, ϕC*H*), 5.04 (s, 2H, CH_2),

4.53 – 4.46 (m, 1H, α-C*H*), 3.22 – 3.14 (m, 1H, β-CH_2), 3.11 – 3.03 (m, 1H, β-CH_2), 2.30 (s, 3H, CH_3).^{13}C NMR (75 MHz, $CDCl_3$, δ): 170.33, 155.63, 148.01, 136.15, 135.85, 135.43, 129.97, 129.47, 128.73, 128.55, 128.23, 128.12, 127.33, 120.88, 67.09, 54.91, 38.29, 20.85; HRMS (*m/z*): 346.1804 [M-CO_2+H^+], 390.1697 [M+H^+], 412.1518 [M+Na^+]; m.p.: 87-90 °C

Photolytic experiments: sample preparation and analysis

Fresh stock solution of compound **6** and **7** ($3.3x10^{-4}$ M in DMSO) were made and kept in the fridge, shielded from light. Buffers stock solution (0.06M) were freshly diluted with MilliQ® water from commercially available 1 M solutions, followed by checking the pH electronically. (pH 6.0 = triethyl ammonium formate (Et_3NH^+ $HCOO^-$) or triethyl ammonium bicarbonate (Et_3NH^+ HCO_3^-), specified in respective figures; pH 8.4 = triethyl ammonium bicarbonate (Et_3NH^+ HCO_3^-)). Samples were prepared by adding 100 µL of compound stock solution in a 1.5 mL clear glass (31x5 mm, 15 mm top) vial with centre hole seal screwcap containing a micro-insert (maximum volume 200 µL). References were prepared in a similar fashion, using dark vials when deemed necessary. Samples were irradiated for 2 h; followed by addition of 100 µL of either water and/or a solution of the investigated product. Final concentrations were: ester = $1.6x10^{-3}$ M, buffer = 0.02 M, oligonucleotide = $8.25x10^{-5}$ M. LC-MS column was stabilized prior to analysis with 2% ACN in MilliQ® water (0.1% formic acid) for 10 min. Samples were analysed after a given amount of incubation time at RT, by injection of a 5 µL aliquot followed by linear gradient (2%-100% ACN).

Kinetic studies

Pseudo first order kinetics of the aminolysis of compound **8** and **9** were obtained by making a 200 µL solution containing $1.6x10^{-3}$ M of respective compound and a given excess of benzylamine in the investigated solvent. The decrease of the area under the curve was quantified by HPLC through fitting on a pre-made calibrating curve. This method was found

to be linear between concentrations 0.16 mM (LOQ) - 20.02 mM for compound **8** and 0.05 mM (LOQ) - 25.68 mM for compound **9** at 254 nm detection. Both calibrating curves included the intercept, allowing the use of one point calibrations before the determinations of each reaction constant. Periodic analysis (5x) per sample was carried out, measurements outside the allotted range were omitted. Kinetic constants were obtained by the linear fit of plotting the natural logarithm of compound concentration versus time. Constants derived from experiments with unsatisfactory fits ($R^2 < 0.9$) were also omitted. Each reported value is the average of at least 3 experiments.

Pseudo first order kinetics

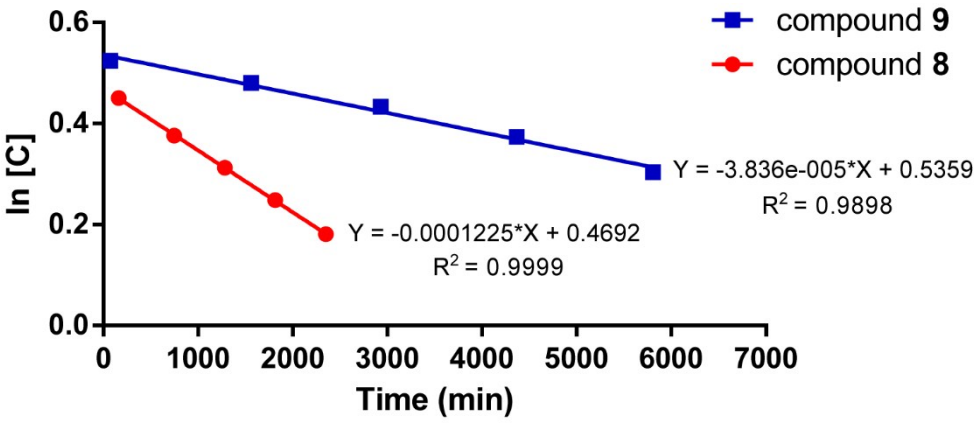


Figure S1. Example of 1 experiment of pseudo first order kinetics determination for compound 8 (red) and compound 9 (blue). Solvent ACN

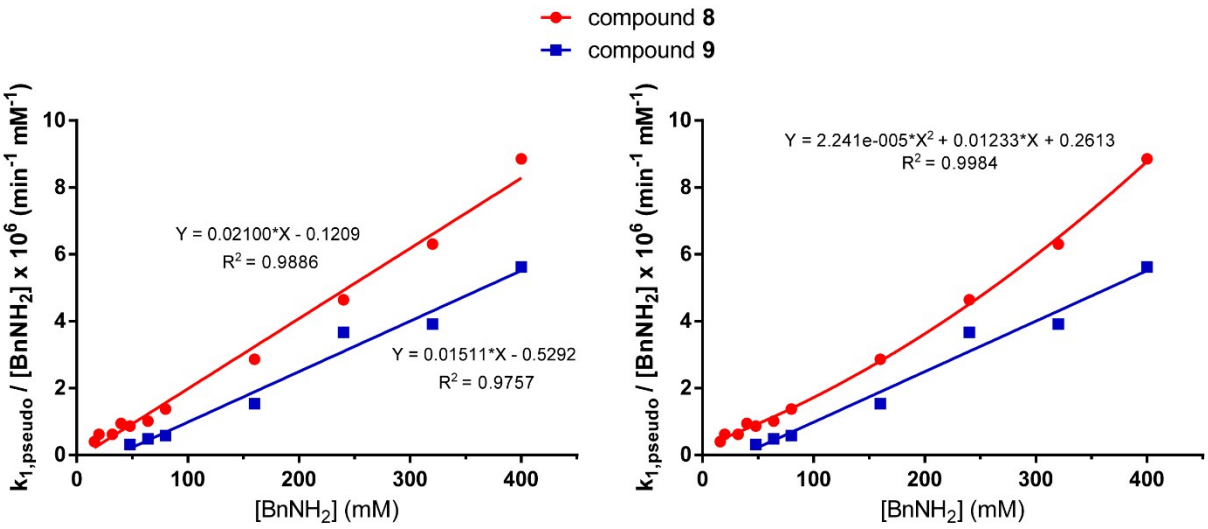


Figure S2. Plot of pseudo first order kinetics in function of benzylamine concentration for compound 8 (red) and compound 9 (blue): $K_{1\ pseudo}$/[benzylamine] in function of [benzylamine] with linear fit (left), same figure with quadratic fit for compound **8** (right). All values are averages of 3 experiments. Solvent ACN

Derivation of rate equation for fitting kinetic experiments

$$v = k_2[Ester][Amine] + k_3[Ester][Amine]^2$$

$$= [Ester][Amine]\,(k_2 + k_3[Amine]) \qquad (1)$$

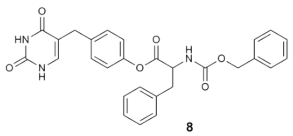
$$k_{pseudo} = [Amine]\,(k_2 + k_3[Amine]) \quad (2)$$

$$\frac{k_{pseudo}}{[Amine]} = k_2 + k_3[Amine] \quad (3)$$

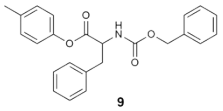
Indications for a small catalytic effect in acetonitrile was found (SI Table 1, first entry and SI Figure 1-3), however adding one equivalent of **2** to a solution of **9** did not increase the rate more than the residual amount of DMSO from the stock solution of **2**. (Table 1, second and third entry). Plotting k_1 pseudo/[amine] versus [amine] for **8** and **9**, showed a linear fit as expected from previous literature regarding amide bond formation (SI Figure 2).[4] However in the case of compound **8**, with the LG of interest **2**, a quadratic fit is better (SI Figure 3) indicating the possible presence of an additional term in the rate equation (SI Eq 1-3) and thus an influence of the uracil moiety on the reaction rate. Looking at the values for DMSO (Table 1, entry four), compound **8** is still more reactive towards aminolysis than compound **9**, but the ratio is smaller (1.6x versus 2.5x). This time, spiking **9** with **2** does give a rate increase, resulting in a value similar to the one derived from the aminolysis of **8**. (Table 1, entry five). Adding water results in more than twenty-fold rate increase, reaching the limits of the experimental set-up (Table 1, entry six), which is reason to believe that the potential influence of LG is minimal in comparison to solvent effects on peptide bond formation.

SI Table 1. Pseudo-first order reaction constants (k (min^{-1}))[a]

Solvent	Additive	Compound **8**	Compound **9**
ACN	None	$1.14x10^{-4}$	$4.63x10^{-5}$
	None [b]	-	$6.37x10^{-5}$
	2[b,c]	-	$6.63x10^{-5}$
DMSO	None	$1.65x10^{-4}$	$1.04x10^{-4}$
	2[c]	-	$1.47x10^{-4}$
DMSO: H2O (1:1)[d]	None	$>10^{-3}$	$>10^{-3}$



8



9

[a] 50eq benzylamine; [b] 4.2% DMSO; [c] 1eq; [d] reaction rate near limit of measurable range

Photolysis experiments

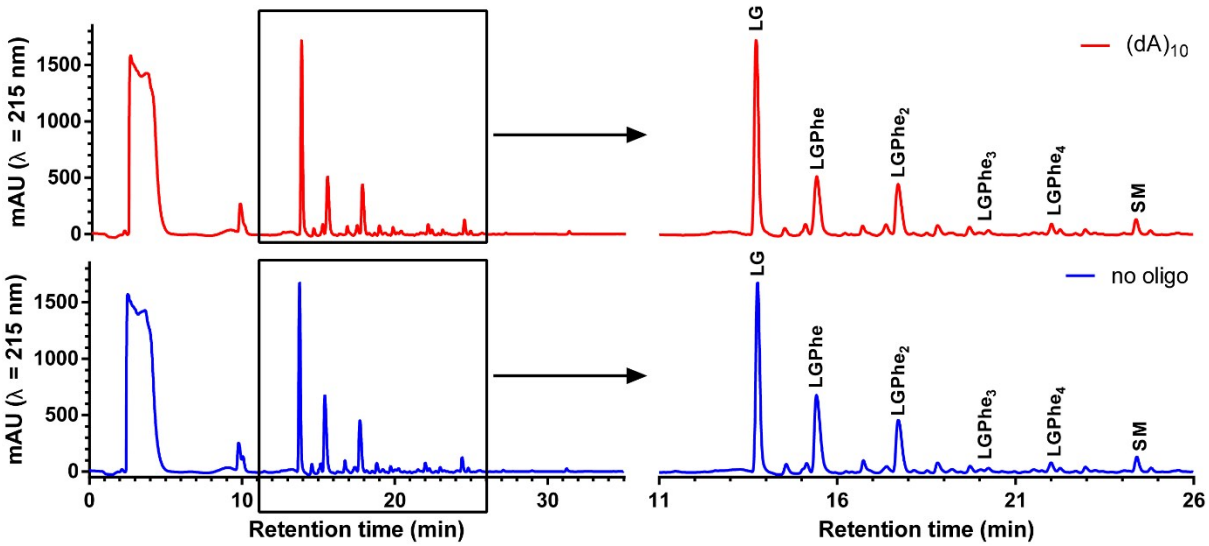


Figure S3. Photolytic reaction of 6, result with (red) or without (blue) prior addition of oligonucleotide: Reaction conditions: compound **6** in DMSO and equal volume of water (blue) or oligonucleotide solution $(dA)_{10}$, final concentration = 5 mol%, followed by 2h 368nm. Incubation time 1h RT before analysis. This experiment indicated possible degradation of the template when adding the oligonucleotide before photolysis, probably due to imperfect lamps emitting also at lower wavelength.[5]

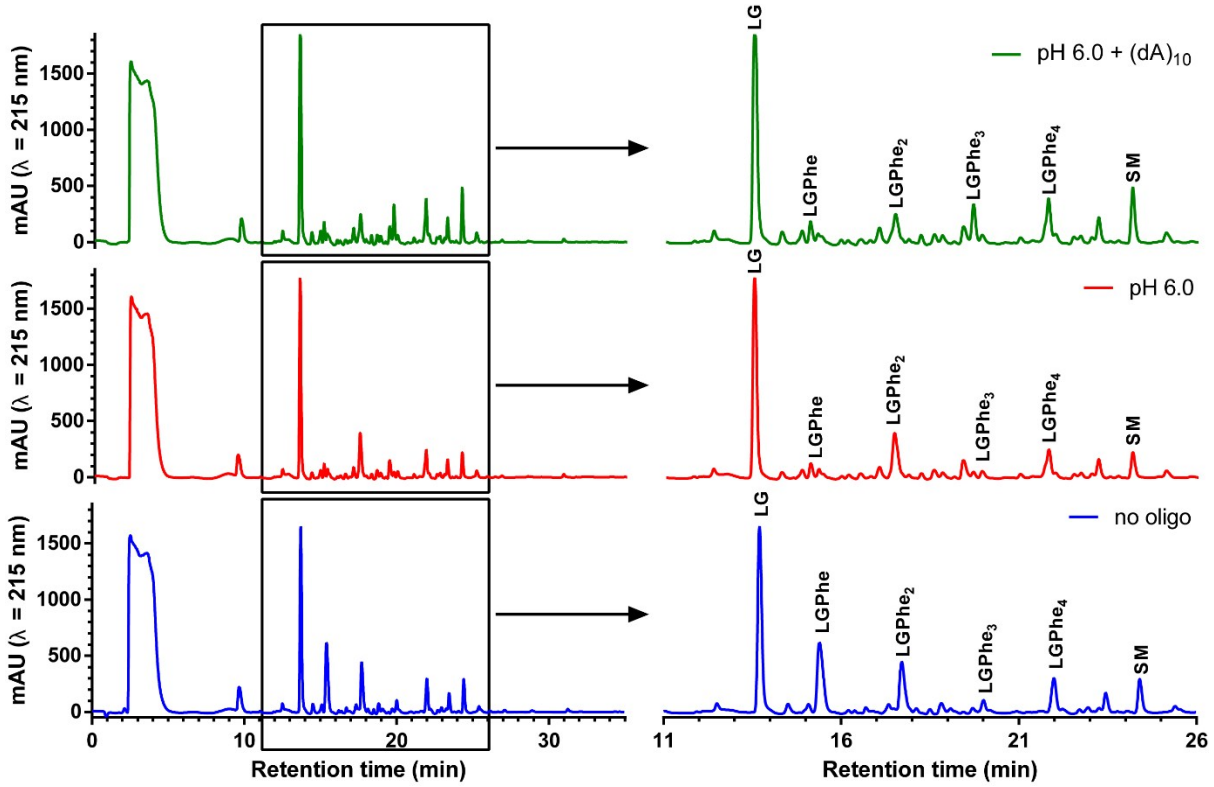


Figure S4. Photolytic reaction of 6, effect of acidic pH (Et_3NH^+ $HCOO^-$): (blue) no oligonucleotide, no buffering; (red) no oligonucleotide, pH 6.0 (Et_3NH^+ $HCOO^-$); (green) with oligonucleotide and pH 6.0.[1] Reaction conditions: compound **6** in DMSO 2h 368nm, followed by addition of an equal volume of water, buffer or oligonucleotide solution $(dA)_{10}$, final concentration = 5 mol%. Incubation time 1h before analysis.

[1] Buffering at pH 6 was difficult in our LC-MS set-up, the chosen buffer has a buffering rang of 3.8-5.8. This was utilized as an acidifying additive. The pH did not fluctuate significantly during the reaction.

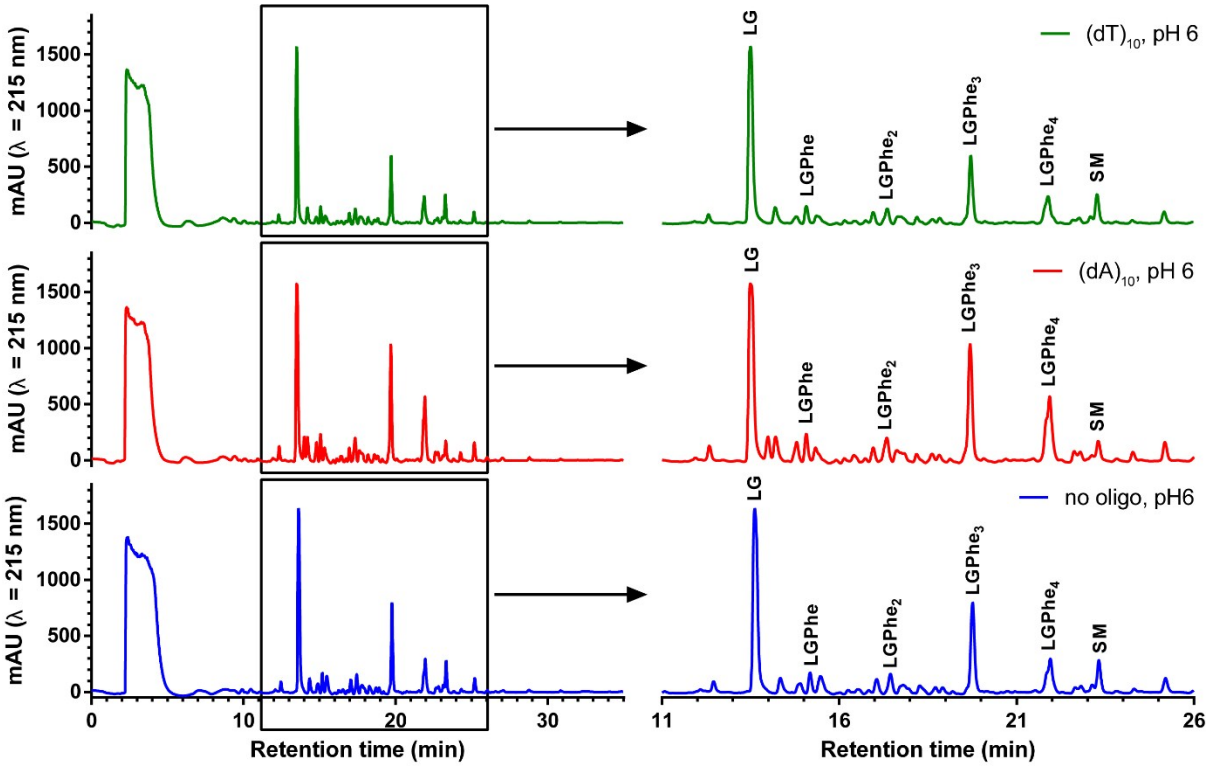


Figure S5. Photolytic reaction of 6, effect of acidic pH (Et_3NH^+ HCO_3^-): (blue) no oligonucleotide, pH6.0; (red) no oligonucleotide, pH 6.0 (Et_3NH^+ $HCOO^-$); (green) with oligonucleotide and pH 6.0.[2] Reaction conditions: compound **6** in DMSO 2h 368nm, followed by addition of an equal volume of water, buffer or oligonucleotide solution, final concentration = 5 mol%. Incubation time 1h before analysis.

[2] Buffering at pH 6 was difficult in our LC-MS set-up. This was utilized as an acidifying additive to compare with Figure S4. The pH did not fluctuate significantly during the reaction.

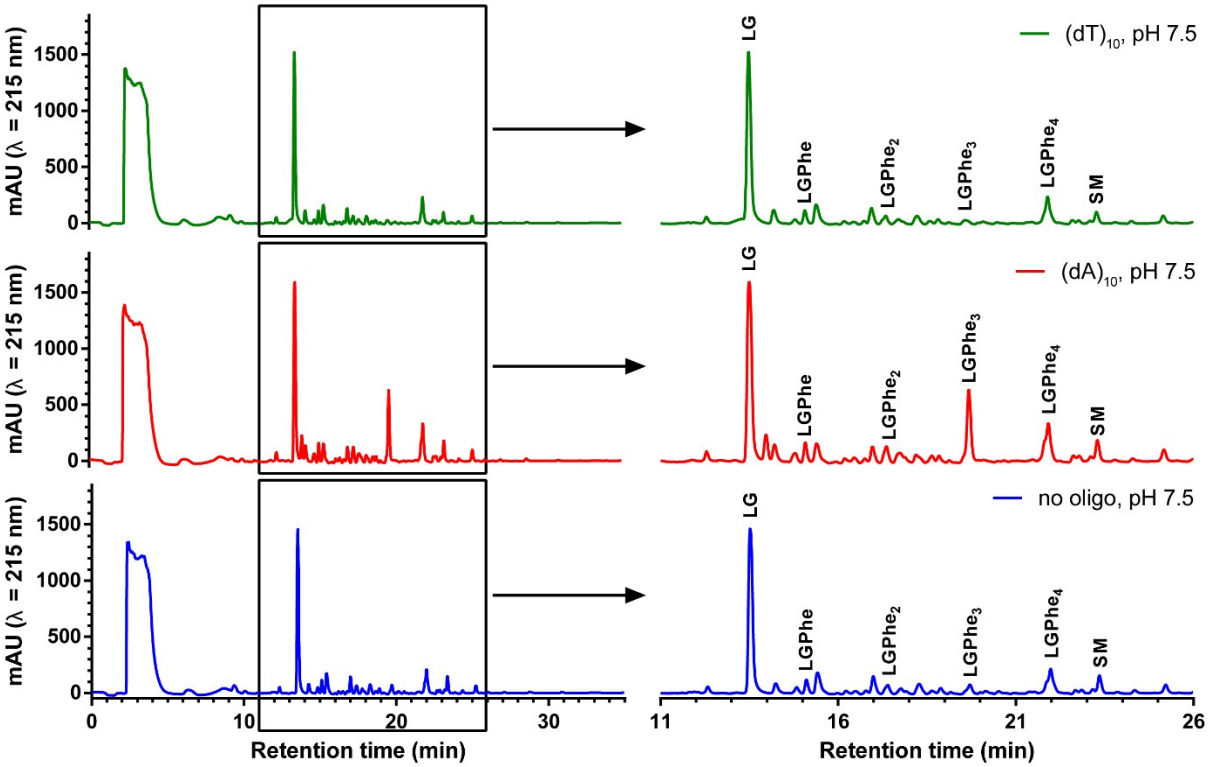


Figure S6. Photolytic reaction of 6, effect of near neutral pH: (blue) no oligonucleotide, pH 7.5 (Et_3NH^+ $HCOO^-$); (red) no oligonucleotide, pH 7.5; (green) with oligonucleotide and pH 7.5. Reaction conditions: compound **6** in DMSO 2h 368nm, followed by addition of an equal volume of water, buffer or oligonucleotide solution, final concentration = 5 mol%. Incubation time 1h before analysis.

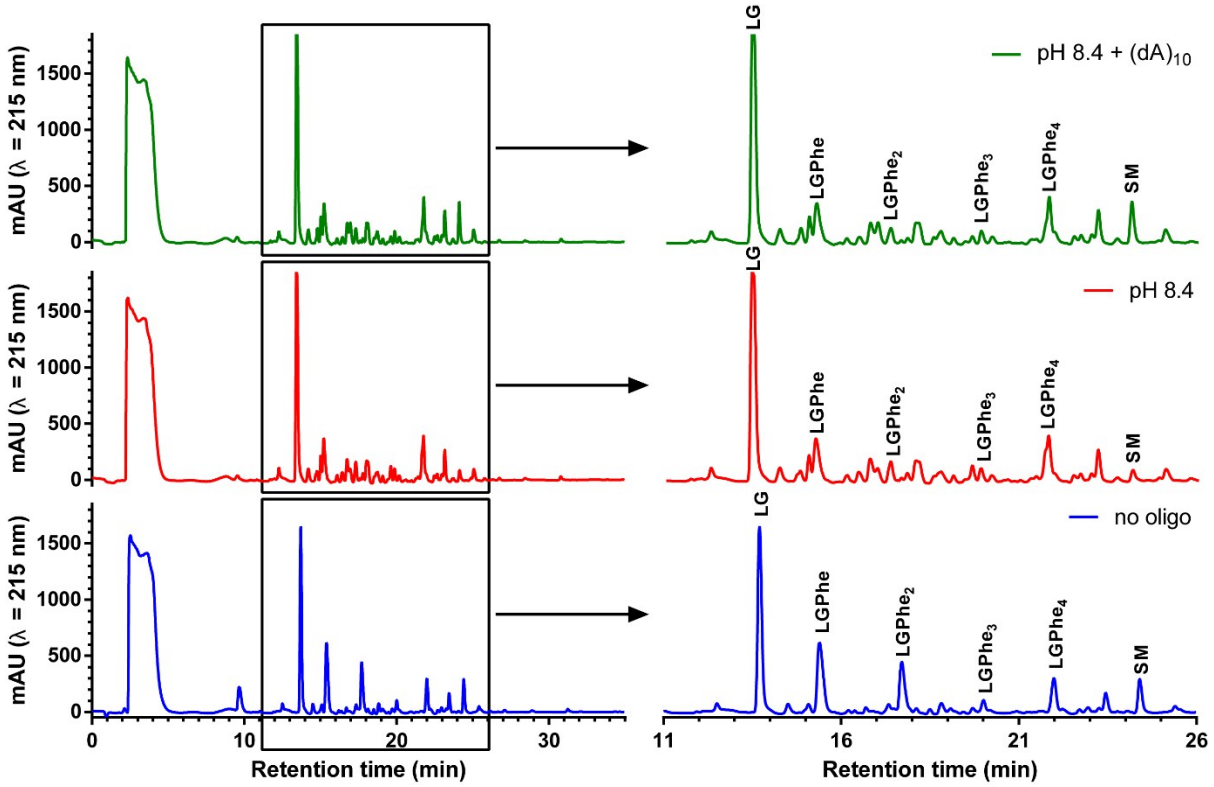


Figure S7. Photolytic reaction of 6, effect of alkaline pH: (blue) no oligonucleotide, no buffering; (red) no oligonucleotide, pH 8.4; (green) with oligonucleotide and buffering at pH 8.4. Reaction conditions: compound **6** in DMSO 2 h 368 nm, followed by addition of an equal volume of water, buffer or oligonucleotide solution $(dA)_{10}$, final concentration = 5 mol%. Incubation time 1h before analysis.

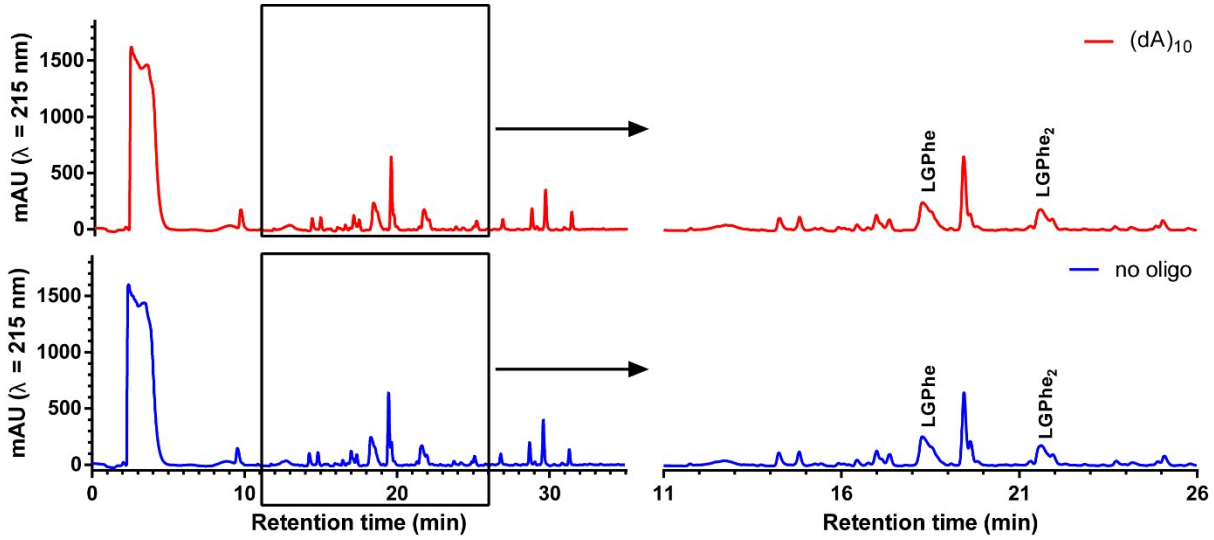


Figure S8. Photolytic reaction of 7, result with (red) or without (blue) addition of oligonucleotide: Reaction conditions: compound **7** in DMSO 2 h 368 nm, followed by addition of an equal volume of water or oligonucleotide solution $(dA)_{10}$, final concentration = 5 mol%. Incubation time 1 h RT before analysis. LG = *p*-cresol

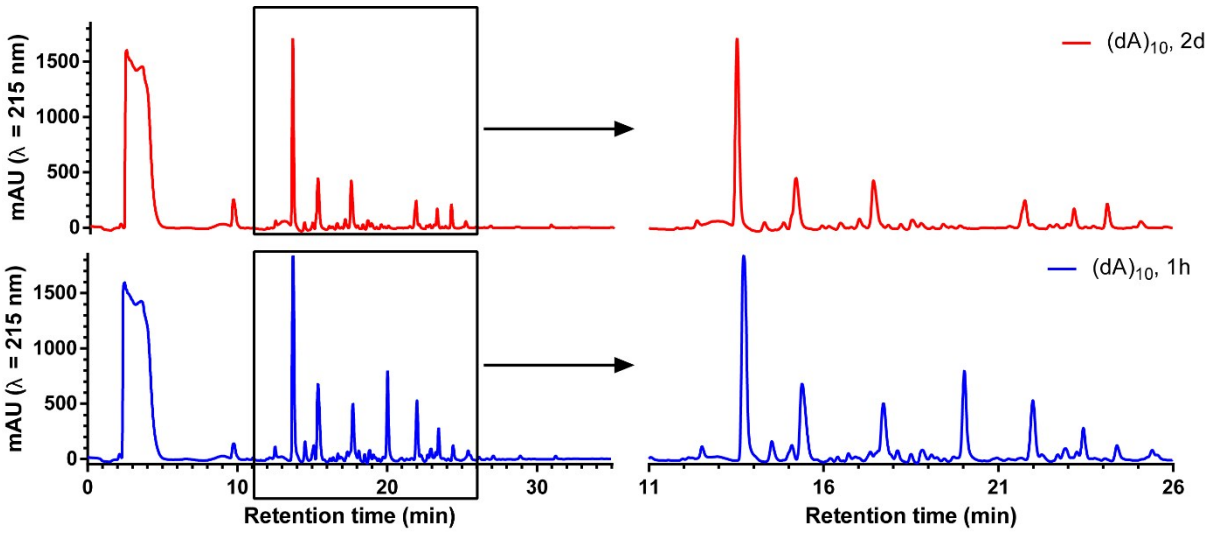


Figure S9. Photolytic reaction of 6, result after 1 h (blue) and 2 d (red) of incubation with oligonucleotide: Reaction conditions: compound **6** in DMSO 2 h 368 nm, followed by addition of an equal volume of water or oligonucleotide solution $(dA)_{10}$, final concentration = 5 mol%. Incubation time 1 h RT (blue) or 2 d RT (red) before analysis of the same sample.

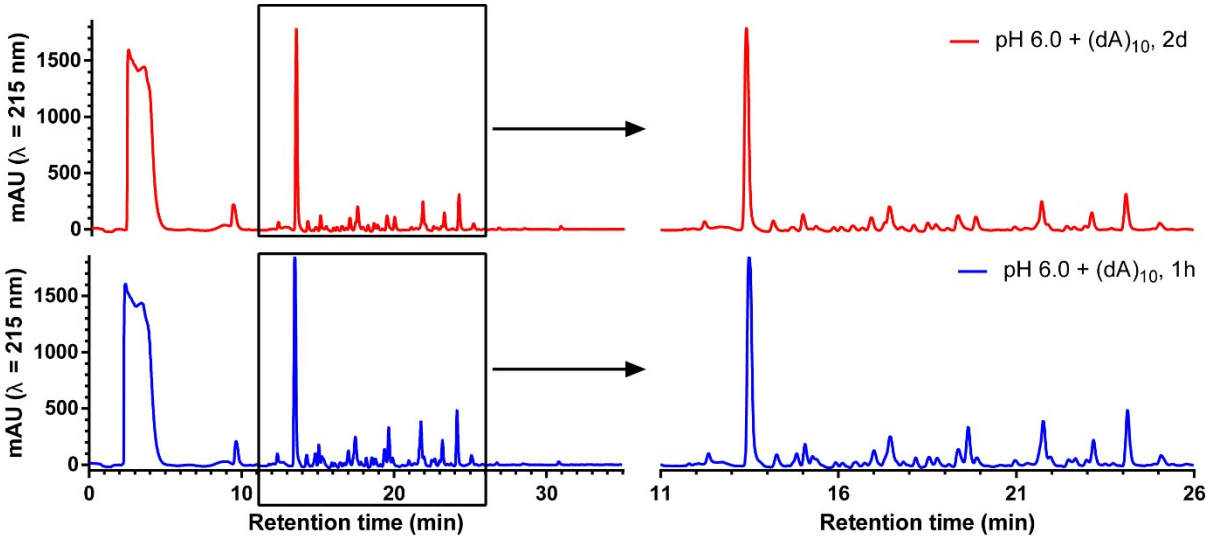


Figure S10. Photolytic reaction of 6, result after 1 h (blue) and 2 d (red) of incubation with oligonucleotide at pH 6.0: Reaction conditions: compound **6** in DMSO 2 h 368 nm,

followed by addition of an equal volume of oligonucleotide solution $(dA)_{10}$, final concentration = 5 mol% and/or buffer at pH 6.0 . Incubation time 1 h RT (blue) or 2 d RT (red) before analysis of the same sample.

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