Supporting Information

Investigations into chemically stabilized four-letter DNA for DNA-encoded chemistry

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General methods and materials

Unless otherwise noted, chemicals were purchased from abcr, Acros Organics, Alfa Aesar, Fisher Scientific, Merck, Sigma Aldrich, TCI and VWR and were used as provided without further purifications. Dry solvents (ACN, CH₂Cl₂, DCE, DMF, EtOH, MeOH, THF, toluene) were used as commercially available.

5'-Aminolinker-modified DNA oligonucleotides on controlled pore glass solid support (CPG, 1000 Å porosity) were synthesized by IBA (Göttingen, Germany). The 10mer T7De8a-dGC- and 10mer 7De-dAT7De8a-dGC–oligonucleotides as well as the branched 16mer 7De-dAT7De8a-dGC-alkyne conjugates on controlled pore glass solid support (CPG, 1000 Å porosity) were synthesized by Ella Biotech GmBH (Planegg, Germany). DNA hairpin and barcodes for ligation experiments were purchased from Integrated DNA Technologies (IDT).

CPG with oligonucleotide-small molecule conjugates were filtered and washed through synthesis columns using a vacuum manifold (Vac-Man®) from Sigma Aldrich.

**Oligonucleotide concentrations.** Concentrations were determined by UV spectroscopy using a NanoDrop 2000 spectrophotometer from Thermo Fisher Scientific.

**Semi-preparative ion pair RP-HPLC.** Compound purification was performed on a Shimadzu Prominence HPLC System equipped with a C₁₈ stationary phase (Phenomenex, Gemini, 5 µm, C₁₈, 110 Å, 100 x 4.6 mm). A gradient from 100 mM aqueous triethylammonium acetate (pH = 8.0, eluent A) to MeOH (eluent B) was used at a flow rate of 5 mL/min. Fractions containing the desired product were pooled and concentrated.

**Method:** Step gradient of 20% to 70% B within 13 min, then 70% to 100% B within 1 min followed by 100% B for 3 min using 100 mM aqueous triethylammonium acetate (pH = 8.0, eluent A) and MeOH (eluent B) at a flow rate of 5 mL/min.

**Analytical RP-HPLC (I).** HPLC analysis was performed on an Agilent 1100 series chromatograph equipped with 1100 Quaternary Pump (G1311A), a 1100 Multi-Wavelength Detector (G1365B) and an Agilent Eclipse Plus C₁₈ (4.6 x 100 mm, 3.5 µm) column. The conversion and purity of DNA conjugates were determined by integration of peaks recorded at 254 nm wavelength.

**Method:** Step gradient of 10% to 70% B within 10 min, then 70% to 100% B within 2 min followed by 100% B for 2 min using 10 mM aqueous triethylammonium acetate (pH = 8.0, eluent A) and MeOH (eluent B) at a flow rate of 0.6 mL/min.

**Analytical RP-HPLC (II).** HPLC analysis was performed on a Shimadzu Prominence equipped with an Agilent Eclipse Plus C₁₈ (4.6 x 100 mm, 3.5 µm) column. The conversion and purity of DNA conjugates were determined by integration of peaks recorded at 254 nm wavelength.
**Method:** Step gradient of 10% to 60% B within 10 min, then 60% to 100% B within 2 min followed by 100% B for 2 min using 10 mM aqueous triethylammonium acetate (pH = 8.0, eluent A) and MeOH (eluent B) at a flow rate of 0.6 mL/min.

**MALDI-TOF.** Mass analysis was performed on a MALDI TOF/TOF MS from Bruker Daltonics using 2',4',6'-trihydroxyacetophenone (THAP) matrix (Dichrom).

**1H-NMR and 13C-NMR** Bruker AVANCE 700 spectrometer (1H NMR, 700 MHz; 13C NMR, 176 MHz). Data are reported in the following order: chemical shift (δ) values are reported in ppm with the solvent resonance as internal standard (DMSO-D6: δ = 3.33 ppm for 1H, δ = 39.52 ppm for 13C) or relative to TMS (δ = 0 ppm); multiplicities are indicated s (singlet), d (doublet), t (triplet), q (quartet) m (multiplet); coupling constants (J) are given in Hertz (Hz).
Computational details

The general computational approach is nearly identical to the refined procedure applied in Ref.[1] and was outlined and validated in Ref.[2]. The solution-phase structures were generated by manual construction of an exhaustive set of OH rotamers and optimized at the B3LYP/6-311+G(d,p)/IEFPCM level of theory with the default parameters for water as implemented in Gaussian 16 rev. B.01[3] (used for all calculations unless explicitly stated otherwise). These structures were reoptimized in vacuum using B3LYP/6-311+G(d,p), using frequency calculations to confirm the structures as local minima and providing data for thermal corrections to yield gas-phase free energies. The vacuum-optimized structures were employed in MP2/6-311+G(d,p) calculations for determining the gas-phase leg of the solvation free energy and in CCSD(T)/cc-pVQZ calculations using the ORCA software (version 4.0.1) within the R1-F12 approximations for the gas-phase reaction energy baseline.[4,5,6] The PCM-optimized structures were submitted to MP2/6-311+G(d,p)/IEFPCM, MP2/6-311+G(d,p)/EC-RISM[7,8] for computing the solvation free energy relative to MP2/6-311+G(d,p) in the gas phase, and to rigid-body thermodynamic integration (TI) calculations in order to provide an alternative, molecular dynamics-based approach to the solvation free energy. EC-RISM calculations were performed using the computational setup developed during the SAMPL6 blind prediction challenge[9] (140^3 grid points with 0.3 Å spacing, PSE-2 closure,[10] modified SPC/E water model, GAFF force field (version 1.7)[11,12] with Lorentz–Berthelot mixing rules for Lennard-Jones (LJ) interactions, and exact periodicity-corrected solute-solvent electrostatics) on the MP2/6-311+G(d,p) level of theory in Gaussian 09 rev. E.01.[13] For the TI calculations, 4167 SPC/E[14] water molecules were placed in a 50^3 Å cube around the molecule using packmol 1.1.2.023.[15] The NAMD 2.11[16] software was used for the simulations together with AM1-BCC charges, GAFF 1.7[11,12] parameters for LJ interactions, and a timestep of 2.0 fs. Each setup was minimized followed by 0.4 ns equilibration. The TI coupling parameter \( \lambda \) was scaled equidistantly in steps of 0.1 between 0 and 1 first for the LJ terms using soft-core scaling and afterwards linearly, using the same step size, for the electrostatic interactions, followed by a hysteresis estimation in the reverse order. For each \( \lambda \) step the system was equilibrated for 60 ps simulated for 0.4 ns. Langevin temperature and pressure control was used for setting the temperature to 298.15 K and the pressure to 1 bar. A smooth cutoff switching scheme for LJ interactions between 10 and 12 Å and a 4\(^{th}\) order particle mesh Ewald algorithm (1.0 Å grid spacing) for the electrostatic interactions were employed. The water geometry was constrained using the SETTLE algorithm as implemented in NAMD.
Calculation of tautomer populations

The strategy used for the calculation of the tautomer populations of guanine and its derivatives followed closely Refs.\cite{1,2} on the basis of the thermodynamic cycle shown in Figure 4 of Ref.\cite{2}. There are multiple routes to calculate the reaction free energies, two “direct” routes were the free energy differences of the species in solution only are considered (PCM and EC-RISM), and three “indirect” routes were the solvation free energies per species are calculated (PCM, EC-RISM, TI), and the cycle is completed with the gas-phase reaction free energies taken from CCSD(T) calculations including thermal corrections on the B3LYP/6-311+G(d,p) level. The results for the guanine, 7-deazaguanine, 8-aza-7-deazaguanine and 8-azaguanine tautomers are presented in Table S1, all referenced to the Watson-Crick tautomer. Since all five approaches revealed similar trends, the average reaction free energies and resulting tautomer populations over all methods were calculated. The individual free energy components are given in Table S2 and S3; the structures are provided in machine-readable format in the accompanying zip file. As also mentioned in the main text, the uncertainties provided in Refs.\cite{1,2} were erroneously reported to be too small by a factor of $5^{\frac{1}{2}} = 2.236$. Uncertainties are here correct, and the corrected values are also given for reference calculations\cite{2} on canonical guanine I. This correction has no impact on energetic rankings and discussion of tautomer relevance.

Table S1 – Calculated standard reaction free energies $\Delta G$ (kcal mol$^{-1}$) and populations for selected tautomeric forms of guanine derivatives I-IV relative to the Watson-Crick tautomers [I-IVA] from direct MP2/6-311+G(d,p)/PCM calculations (column 2), MP2/6-311+G(d,p)/PCM hydration free energy differences and CCSD(T)/cc-pVTZ gas phase reaction free energies (column 3), direct MP2/6-311+G(d,p)/EC-RISM hydration free energy differences and CCSD(T)/cc-pVTZ gas phase reaction free energies (column 5), and from TI hydration free energy differences and CCSD(T)/cc-pVTZ gas phase reaction free energy (column 6); resulting averaged free energies and populations are shown in columns 7 and 8. The averages from Ref.\cite{2} are presented in columns 9 and 10 and the ones calculated without using the TI results in columns 11 and 12.

| Cmpd. | PCM | PCM | PCM | PCM | Average $\Delta G$ | Population | Average $\Delta G$ | Population | Average $\Delta G$ (without TI) | Population (without TI) |
|-------|-----|-----|-----|-----|---------------------|------------|---------------------|------------|---------------------------------|------------------------|
| Ia    | 0.00 | 0.00 | 0.00 | 0.00 | 0.00                | 0.00       | 0.00                | 0.00       | 0.00                            | 0.00                   |
| Ib    | 4.72 | 4.58 | 6.20 | 6.07 | 5.23 ± 0.74        | 1.45 ± 10^{-4} | 1.81 ± 10^{-4} | 6.6 ± 0.7 | <0.0001 ± <0.0001 | 5.39 ± 0.75             | 0.0001 ± 1.40 ± 10^{-4} |
| Ic    | 9.91 | 8.65 | 6.95 | 5.69 | 15.39              | 9.32 ± 3.36 | 1.48 ± 10^{-7} | 8.39 ± 10^{-7} | 7.5 ± 1.5 | <10^{-5} ± <10^{-5} | 7.80 ± 1.61             | 1.92 ± 10^{-4} ± 5.20 ± 10^{-4} |
| Ila   | 0.00 | 0.00 | 0.00 | 0.00 | 0.00                | 0.00       | 0.00                | 0.00       | 0.00                            | 0.00                   |
| Iib   | 4.52 | 4.50 | 5.85 | 5.75 | 6.24              | 5.37 ± 0.72 | 1.15 ± 10^{-4} | 1.40 ± 10^{-4} | - | -                              | 5.16 ± 0.65             | 0.0002 ± 1.81 ± 10^{-4} |
| IIc   | 9.62 | 8.34 | 6.52 | 5.24 | 13.29              | 8.60 ± 2.78 | 4.95 ± 10^{-9} | 2.33 ± 10^{-9} | - | -                              | 7.43 ± 1.68             | 3.58 ± 10^{-6} ± 1.02 ± 10^{-6} |
| IIla  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00                | 0.00       | >0.9999 ± 1.83 ± 10^{-5} | - | -                              | 0.00                   |
| IIb   | 6.02 | 5.69 | 7.19 | 6.80 | 6.81              | 6.50 ± 0.55 | 1.72 ± 10^{-9} | 1.61 ± 10^{-5} | - | -                              | 6.42 ± 0.60             | 1.95 ± 10^{-6} ± 1.96 ± 10^{-6} |
| IIIC  | 9.44 | 8.36 | 6.72 | 5.84 | 12.51              | 8.33 ± 2.34 | 5.55 ± 10^{-10} | 2.23 ± 10^{-6} | - | -                              | 7.54 ± 1.46             | 2.97 ± 10^{-6} ± 7.32 ± 10^{-4} |
| IVa   | 0.00 | 0.00 | 0.00 | 0.00 | 0.00                | 0.00       | >0.9999 ± 1.51 ± 10^{-5} | - | -                              | 0.00                   |
| IVb   | 6.59 | 5.85 | 7.60 | 6.86 | 6.23              | 6.63 ± 0.59 | 1.39 ± 10^{-9} | 1.38 ± 10^{-5} | - | -                              | 6.73 ± 0.62             | 1.18 ± 10^{-4} ± 1.24 ± 10^{-4} |
| IVc   | 9.65 | 8.52 | 7.15 | 6.01 | 13.23              | 8.91 ± 2.48 | 2.93 ± 10^{-7} | 1.23 ± 10^{-6} | - | -                              | 7.83 ± 1.37             | 1.81 ± 10^{-6} ± 4.21 ± 10^{-4} |

S7
Table S2 – Results (in kcal mol\(^{-1}\) for energies) of EC-RISM and vacuum calculations. Electronic solute energy (\(E_{\text{sol}}\), column 2), corrected and uncorrected excess chemical potential (\(\mu^x\), \(\mu^{x,\text{corr}}\), columns 3 and 5), infinite dilution partial molar volume (\(V_m\) in Å\(^3\), column 4), vacuum energies (MP2/6-311+G(d,p) and RI-F12-CCSD(T)/cc-pVTZ results, columns 6 and 7), and thermal corrections (B3LYP/6-311+G(d,p), column 8). “b1” and “b2” denote different OH rotamers, the lines “b” without index show results from a discrete partition function which enter the full reaction free energy. Physically unreasonable partition function estimates are left blank.

| Cmpd. | \(E_{\text{sol}}\) | \(\mu^x\) | \(V_m\) | \(\mu^{x,\text{corr}}\) | \(E_{\text{ex}}(\text{MP2/}6-311+\text{G(d,p)})\) | \(E_{\text{ex}}(\text{CCSD(T)/}\text{cc-pVTZ})\) | TC(\text{B3LYP/}6-311+\text{G(d,p)}) | \(E_{\text{ex}}+\mu^{x,\text{corr}}\) |
|-------|----------------|---------|-------|----------------|-------------------------------|-------------------------------|---------------------------------|----------------|
| la    | -339750.88 | -41.25  | 115.33 | -53.03 | -339781.01 | -340022.99 | 52.22 | -339808.92 |
| Ib,1  | -339766.68 | -23.95  | 117.46 | -35.95 | -339780.89 | -340023.04 | 52.32 | -339802.63 |
| Ib,2  | -339766.24 | -23.31  | 117.47 | -35.31 | -339780.29 | -340022.71 | 52.29 | -339801.55 |
| Ib    | -23.31  | -      | -      | -      | -      | -      | -      | - |
| lc    | -339717.64 | -72.98  | 111.10 | -84.33 | -339762.22 | -340004.61 | 51.38 | -339801.97 |
| lla   | -329703.15 | -25.98  | 130.07 | -39.27 | -329720.81 | -329958.48 | 59.42 | -329742.42 |
| llib,1| -329710.85 | -12.15  | 132.41 | -25.68 | -329719.99 | -329957.67 | 59.46 | -329736.53 |
| llib,2| -329702.80 | -18.68  | 132.10 | -32.17 | -329715.14 | -329953.39 | 59.09 | -329734.98 |
| llib  | -18.68  | -      | -      | -      | -      | -      | -      | - |
| llc   | -329668.10 | -54.90  | 126.26 | -67.80 | -329703.09 | -329941.20 | 58.58 | -329735.90 |
| llia  | -339751.41 | -29.24  | 117.80 | -41.27 | -339770.07 | -340012.90 | 52.28 | -339792.68 |
| llib,1| -339756.84 | -16.36  | 120.00 | -28.62 | -339768.02 | -340011.08 | 52.21 | -339785.47 |
| llib,2| -339748.69 | -22.88  | 118.63 | -35.00 | -339762.97 | -340006.55 | 51.81 | -339783.69 |
| llib  | -22.88  | -      | -      | -      | -      | -      | -      | - |
| llc   | -339719.71 | -54.64  | 113.53 | -66.24 | -339753.11 | -339996.25 | 51.50 | -339785.96 |
| lIa   | -349795.99 | -34.69  | 108.59 | -45.79 | -349814.07 | -350061.10 | 44.41 | -349841.78 |
| lIb,1 | -349803.64 | -19.20  | 110.56 | -30.49 | -349812.35 | -350059.93 | 44.28 | -349834.14 |
| lIb,2 | -349802.29 | -19.12  | 109.91 | -30.35 | -349811.40 | -350059.08 | 43.99 | -349832.64 |
| lIb   | -19.12  | -      | -      | -      | -      | -      | -      | - |
| lIc   | -349760.57 | -63.45  | 103.88 | -74.06 | -349796.28 | -350043.61 | 43.58 | -349834.63 |

S8
Table S3 – Results (in kcal mol$^{-1}$) of PCM (MP2/6-311+G(d,p), column 2) and TI (column 5) calculations. Additionally, the solvation free energies Δ$_{solv}$G calculated using MP2 vacuum energies (columns 3 and 4), the sum of solvation free energies, CCSD(T) and TC (columns 6-8), and the free energies relative to the Watson-Crick tautomer [HIVa] (columns 9-13) are given. “b1” and “b2” denote different OH rotamers, the lines “b” without index show results from a discrete partition function which enter the full reaction free energy. Physically unreasonable partition function estimates are left blank.

| Compd | PCM | Δ$_{solv}$G | Δ$_{solv}$G | Δ$_{solv}$G | Tl | Δ$_{solv}$G | PCM | Δ$_{solv}$G | Δ$_{solv}$G | Δ$_{solv}$G | Δ$_{solv}$G |
|-------|-----|-------------|-------------|-------------|----|-------------|-----|-------------|-------------|-------------|-------------|
|       | PC  | EC-RISM     | CCSD(T)     | CCSD(T)     |    | PC  | EC-RISM     | CCSD(T)     | CCSD(T)     |    | PC  | EC-RISM     |
| la    | -339796.76 | -15.75 | -27.91 | -25.30±0.22 | -339986.53 | -339998.69 | -340048.29 | 0.00 | 0.00 | 0.00 | 0.00 |
| lb,1  | -339791.93 | -11.04 | -21.74 | -20.34±0.22 | -339981.77 | -339992.47 | -340043.38 | - - - - |
| lb,2  | -339791.00 | -10.72 | -21.27 | -20.44±0.23 | -339981.14 | -339991.69 | -340043.15 | - - - - |
| lc    | -339786.85 | -24.64 | -39.75 | -28.29±0.24 | -339977.87 | -339992.99 | -340032.91 | 9.91 | 8.65 | 6.95 | 5.69 | 15.39 |
| llb,1 | -329732.55 | -11.73 | -21.61 | -21.09±0.21 | -329910.80 | -329920.67 | -329979.58 | 0.00 | 0.00 | 0.00 | 0.00 |
| llb,2 | -329728.01 | -8.01 | -16.53 | -15.46±0.22 | -329906.22 | -329914.74 | -329973.12 | - - - - |
| llb   | -329725.89 | -10.76 | -19.84 | -19.25±0.23 | -329905.05 | -329914.13 | -329972.64 | - - - - |
| llb   | -329728.02 | - - - | -329906.30 | -329914.92 | -329973.34 | 4.52 | 4.50 | 5.85 | 5.75 | 6.24 |
| llc   | -329722.92 | -19.84 | -32.82 | -25.09±0.24 | -329902.46 | -329915.44 | -329966.29 | 9.62 | 8.34 | 6.52 | 5.24 | 13.29 |
| llb,1 | -339781.98 | -11.91 | -22.62 | -21.90±0.23 | -339972.54 | -339983.24 | -340034.80 | 0.00 | 0.00 | 0.00 | 0.00 |
| llb,2 | -339775.95 | -7.93 | -17.45 | -16.43±0.23 | -339966.80 | -339976.32 | -340027.51 | - - - - |
| llb   | -339773.49 | -10.52 | -20.72 | -21.09±0.24 | -339965.26 | -339975.46 | -340027.64 | - - - - |
| llb   | -339775.96 | - - - | -339966.85 | -339976.44 | -340027.99 | 6.02 | 5.69 | 7.19 | 6.80 | 6.81 |
| llic  | -339772.54 | -19.43 | -32.85 | -26.04±0.23 | -339964.18 | -339977.60 | -340022.29 | 9.44 | 8.36 | 6.72 | 5.64 | 12.51 |
| lvA   | -349830.00 | -15.93 | -27.70 | -24.29±0.24 | -350032.61 | -350044.38 | -350085.38 | 0.00 | 0.00 | 0.00 | 0.00 |
| lvB,1 | -349823.37 | -11.02 | -21.79 | -18.46±0.24 | -350026.68 | -350037.44 | -350078.39 | - - - - |
| lvB,2 | -349821.84 | -10.44 | -21.24 | -19.88±0.22 | -350025.53 | -350036.32 | -350078.95 | - - - - |
| lvB   | -349823.41 | - - - | -350026.76 | -350037.53 | -350079.15 | 6.59 | 5.85 | 7.60 | 6.86 | 6.23 |
| lvC   | -349820.35 | -24.07 | -38.34 | -28.54±0.21 | -350024.09 | -350038.37 | -350072.15 | 9.65 | 8.52 | 7.15 | 6.01 | 13.23 |
Synthesis of chemically stabilized nucleoside phosphoramidite A-C

The synthesis of nucleoside N⁶-Benzoyl-2⁰-deoxy-5⁰-O-DMT-7-deaza-2⁰-deoxyadenosine 3⁰-CE phosphoramidite A followed the published procedure.¹¹ H, ¹³C and ³¹P NMR spectra are consistent with the published data.¹¹ The synthesis of nucleoside B followed the published procedure.¹³

Synthesis of N⁶-DMF-2⁰-deoxy-5⁰-O-DMT-7-deaza-8-aza-2⁰-deoxyguanosine B

7-deaza-8-aza-2⁰-deoxyguanosine was dried in high vacuum overnight before setting up the reaction. Step 1: The solution of 7-deaza-8-aza-2⁰-deoxyguanosine (300 mg, 1.12 mmol, 1.0 eq.) in dry methanol (6 mL) and DMF-DMA (DMF-dimethyl acetate, 1.2 mL) was stirred at 50°C for 2.5 hours. Then, the reaction mixture was concentrated under vacuo, and the crude material was co-evaporated twice with each 3 mL dry methanol and 3 mL diethyl ether, dried under vacuum, and immediately used in the next step without further purification.

Step 2: To the solution of N⁶-DMF-2⁰-deoxy-7-deaza-8-azaguanosine (355 mg, 1.10 mmol, 1.0 eq.) in dry pyridine (4 mL) at 0°C, DMAP (13 mg, 0.11 mmol, 0.1 eq.) and DMTr-Cl (4,4’-dimethoxytrityl chloride, 411 mg, 1.21 mmol, 1.1 eq.) were added under argon. The reaction mixture was stirred at room temperature overnight. Then, the reaction mixture was concentrated under reduced pressure. To the crude material, CH₂Cl₂ (60 mL) was added and then it was washed with ice-cold brine (3 x 30 mL) and ice-cold water (30 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. The crude material was purified by column chromatography (silica gel, solvent system: CH₂Cl₂/MeOH 100:0 to 90:10) to provide the compound B (550 mg, 80% yield). ¹H NMR (700 MHz, DMSO): δ = 8.71 (s, 1H), 7.84 – 7.81 (m, 1H), 7.34 – 7.29 (m, 2H), 7.23 – 7.14 (m, 7H), 6.81 – 6.73 (m, 4H), 6.51 – 6.47 (m, 1H), 5.28 (d, J = 4.8 Hz, 1H), 4.52 – 4.46 (m, 1H), 3.91 – 3.87
(m, 1H), 3.70 (d, J = 4.8 Hz, 6H), 3.18 (s, 3H), 3.05 (s, 3H), 3.05 – 2.99 (m, 2H), 2.71 – 2.65 (m, 1H), 2.27 – 2.21 ppm (m, 1H).

$^{13}$C NMR (176 MHz, DMSO): $\delta$ = 159.04, 158.59, 158.53, 157.96, 157.91, 154.52, 145.04, 135.68, 135.67, 135.05, 129.70, 129.63, 127.71, 127.64, 126.50, 113.03, 112.99, 102.24, 85.23, 85.21, 82.91, 70.88, 64.51, 54.97, 54.95, 54.91, 40.80, 40.02, 38.26, 34.81 ppm.

**Synthesis of N$^6$-DMF-2'-deoxy-5'-O-DMT-2'-7-deaza-8-aza-2'-deoxyadenosine 3'-CE phosphoramidite C**

$^{10}$-DMF-2'-deoxy-5'-O-DMT-2'-7-deaza-8-aza-2'-deoxyguanosine **B** was dried in high vacuum overnight before setting up the reaction. To the solution of compound **B** (350 mg, 0.56 mmol, 1.0 eq.) in dry CH$_2$Cl$_2$ (5.5 mL) at 0°C, DIPEA (390 µL, 2.24 mmol, 4.0 eq.) and CEP-Cl (150 µL, 0.67 mmol, 1.2 eq.) were added under argon. The cooling bath was removed after 10 minutes, and the solution was stirred at room temperature for 3.5 hours. The solution was filtered through a stringf filter and diluted with CH$_2$Cl$_2$ (10 mL). The organic phase was washed with saturated aq. NaHCO$_3$ (2 x 20 mL) and brine (20 mL), then dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated *in vacuo*. The product **C** was obtained as a colorless foam and as a diastereoisomeric mixture (448 mg, 97% yield). It was used without further purification for solid-phase oligonucleotide synthesis. $^1$H NMR (700 MHz, DMSO): $\delta$ = 8.72 – 8.68 (m, 2H), 7.84 – 7.81 (m, 2H), 7.34 – 7.28 (m, 4H), 7.22 – 7.13 (m, 14H), 6.81 – 6.72 (m, 8H), 6.54 – 6.46 (m, 2H), 4.84 – 4.75 (m, 2H), 4.05 – 3.98 (m, 2H), 3.76 – 3.72 (m, 1H), 3.72 – 3.67 (m, 12H), 3.61 – 3.44 (m, 6H), 3.40 – 3.35 (m, 2H), 3.19 – 3.16 (m, 6H), 3.16 – 3.07 (m, 2H), 3.05 (s, 6H), 3.04 – 2.98 (m, 2H), 2.89 – 2.78 (m, 2H), 2.76 – 2.73 (m, 2H), 2.64 – 2.60 (m, 2H), 2.47 – 2.36 (m, 2H), 1.22 – 1.13 (m, 6H), 1.13 – 1.06 (m, 18H), 0.99 – 0.95 ppm (m, 6H). $^{13}$C NMR (176 MHz, DMSO): $\delta$ = 159.12, 159.10, 158.59, 158.53, 158.00, 157.96, 154.52, 145.04, 135.68, 135.67, 135.05, 129.70, 129.63, 127.71, 127.64, 126.50, 113.03, 112.99, 102.24, 85.23, 85.21, 82.91, 70.88, 64.51, 54.97, 54.95, 54.91, 40.80, 40.02, 38.26, 34.81 ppm. $^{31}$P NMR (283 MHz, DMSO): $\delta$ = 147.97, 147.23 ppm.
NMR spectra

^1H NMR

^13C NMR
$^{31}\text{P NMR}$
Chemical stability screening of DNA barcodes

Representative Procedures

Treatment of solid support-bound stabilized oligonucleotide with aqueous acids (RP-01)

DMT-cleavage: The DMT-protecting group of DNA strand bound to 1000 Å controlled pore glass (CPG) solid support (1 µmol, ~40 mg of 10mer T7De8a-dGC- and 10mer 7De-dAT7De8a-dGC -sequence 5 and 6) was cleaved by addition of 200 µL 3% trichloroacetic acid in CH₂Cl₂ for 1 min. Orange coloring of the solution indicated successful removal of protecting group. The deprotection was repeated 3-5 times until no further coloring of the solution was observed. CPG-bound deprotected DNA was washed three times with each 200 µL of 1% TEA in ACN, DMF, MeOH, ACN and CH₂Cl₂ and dried in vacuo.

Investigation of stability: 20 nmol of CPG-bound stabilized oligonucleotide (0.75 mg) was treated with 50 µL aqueous acid. The suspension was shaken at ambient temperature for 22 h. Afterwards solution was removed under vacuum filtration, CPG was washed with excess of 1% TEA and three times with each 200 µL of 0.1 M MgCl₂ solution, water, DMF, MeOH, ACN and CH₂Cl₂ and dried in vacuo.

Cleavage and Analysis: DNA was deprotected and cleaved from CPG by shaking in 500 µL of an AMA solution (AMA = aqueous ammonia (30%)/ aqueous methylamine (40%), 1:1, vol/vol) for 4 h at room temperature. Afterwards 20 µL of 1 M Tris buffer (pH = 7.5) were added, the mixture was dried under reduced pressure (SpeedVac) and dissolved in 200 µL of distilled water. The product was analyzed by analytical RP-HPLC and MALDI-TOF-MS.

Treatment of solid support-bound stabilized oligonucleotides with metal salts or organic reagents (RP-02)

DMT-cleavage: DMT-protecting group of DNA strand bound to 1000 Å controlled pore glass (CPG) solid support (1 µmol, ~40 mg of 10mer T7De8a-dGC- and 10mer 7De-dAT7De8a-dGC-sequence) was cleaved by addition of 200 µL 3% trichloroacetic acid in CH₂Cl₂ for 1 min. Orange coloring of the solution indicated successful removal of protecting group. The deprotection was repeated 3-5 times until no further coloring of the solution was observed. CPG-bound deprotected DNA was washed three times with each 200 µL of 1% TEA in ACN, DMF, MeOH, ACN and CH₂Cl₂ and dried in vacuo.

Investigation of stability: 20 nmol of CPG-bound stabilized oligonucleotide (0.75 mg) were treated with 200 equiv. of metal salt/organic reagent (4 µmol) solved in 50 µL dry solvent. The suspension was shaken at ambient temperature for 22 h. Afterwards the solvent was removed under vacuum filtration, CPG was washed three times with each 200 µL of 0.1 M
EDTA solution, 0.1 M MgCl₂ solution, water, DMF, MeOH, ACN and CH₂Cl₂ and dried *in vacuo*.

**Cleavage and Analysis:** DNA was deprotected and cleaved from CPG by shaking in 500 µL of an AMA solution (AMA = aqueous ammonia (30%)/ aqueous methylamine (40%), 1:1, vol/vol) for 4 h at room temperature. Afterwards 20 µL of 1 M Tris buffer (pH = 7.5) were added, the mixture was dried under reduced pressure (SpeedVac) and DNA was dissolved in 200 µL distilled water. The product was analyzed by analytical RP-HPLC and MALDI-TOF-MS.

**Table S4** – Stability of chemically modified oligonucleotides 5 and 6 in the presence of different metal salts, organocatalysts and acids

| Entry | Reagent            | Solvent | T7De8a-dGC 5 | 7De-dAT7De8a-dGC 6 |
|-------|--------------------|---------|--------------|-------------------|
| 1     | 3.7% HCl           | H₂O     |              |                   |
| 2     | Ce(NH₄)₂(NO₃)₆     | MeOH    |              |                   |
| 3     | Co(acac)₃          | ACN     |              |                   |
| 4     | Cu(MeCN)₄PF₆      | ACN     |              |                   |
| 5     | FeCl₂·4 H₂O        | ACN     |              |                   |
| 6ᵇ    | La(Oi-Pr)₃        | THF     |              |                   |
| 7     | Ni(acac)₂          | ACN     |              |                   |
| 8     | Pd(OAc)₂          | ACN     |              |                   |
| 9     | RuCl₃             | ACN     |              |                   |
| 10    | Grubbs 1ˢᵗ Gen.    | CH₂Cl₂  |              |                   |
| 11    | Sc(OTf)₃          | ACN     |              |                   |
| 12ᶜ   | Sc(OTf)₃          | ACN     |              |                   |
| 13    | SeO₂              | MeOH    |              |                   |
| 14    | VO(acac)₂         | MeOH    |              |                   |
| 15    | ZnCl₂             | ACN     |              |                   |
| 16    | DDQ 7             | EtOH    |              |                   |
| 17    | PIDA 8            | ACN     |              |                   |
| 18    | TEMPO 9           | ACN     |              |                   |

ᵃ For each: 20 nmol DNA, aqueous acids or 200 equiv. transition metal salt or 200 equiv. organic reagent, 50 µL solvent, rt, 22 h. ᵇ Poor solubility, added as suspension. ᶜ Experiment was performed at 40 °C. ACN = acetonitrile, MeOH = methanol.

5 = 5'-TT7De8a-dG CT7De8a-dG CC7De8a-dG T-3'-CPG  
6 = 5'-7De8a-dGTC 7De-dAT7De8a-dG 7De-dATC T-3'-CPG
HPLC traces and MALDI-MS spectra of metal ion screens

**CPG-oligonucleotide + 3.7% HCl**

According to the representative procedure RP-01 solid support-coupled oligonucleotide (20 nmol) was treated with 3.7% HCl.

| CPG-oligonucleotide | Analytical data |
|---------------------|-----------------|
| 10mer T7De8a-dGC    |                 |
| Analytical RP-HPLC (I) trace |                 |
| MALDI-MS spectrum | mass\(_{calc}\) = 3007.5 |
|                     | mass\(_{found}\) = 3010.3 |
| 10mer 7De-dAT7De8a-dGC |                 |
| Analytical RP-HPLC (I) trace |                 |
| MALDI-MS spectrum | mass\(_{calc}\) = 3016.0 |
|                     | mass\(_{found}\) = 3016.5 |
**CPG-oligonucleotide + Ce(NH$_4$)$_2$(NO$_3$)$_6$**

According to the representative procedure RP-02 solid support-coupled oligonucleotide (20 nmol) was treated with Ce(NH$_4$)$_2$(NO$_3$)$_6$.

| CPG-oligonucleotide | Analytical data |
|---------------------|-----------------|
| 10mer T7De8a-dGC   | Analytical RP-HPLC (I) trace |
|                     | MALDI-MS spectrum |
| mass$_{calc}$ = 3007.5 |
| mass$_{found}$ = 3010.7 |
| 10mer 7De-dAT7De8a-dGC | Analytical RP-HPLC (I) trace |
|                     | MALDI-MS spectrum |
| mass$_{calc}$ = 3016.0 |
| mass$_{found}$ = 3016.5 |
CPG-oligonucleotide + Co(acac)$_3$

According to the representative procedure RP-02 solid support-coupled oligonucleotide (20 nmol) was treated with Co(acac)$_3$.

| CPG-oligonucleotide | Analytical data |
|----------------------|-----------------|
| 10mer T7De8a-dGC    |                 |
| Analytical RP-HPLC (I) trace |                 |
| MALDI-MS spectrum | mass$_{calc}$ = 3007.5  
mass$_{found}$ = 3010.2 |
| 10mer 7De-dAT7De8a-dGC |                 |
| Analytical RP-HPLC (I) trace |                 |
| MALDI-MS spectrum | mass$_{calc}$ = 3016.0  
mass$_{found}$ = 3013.8 |
CPG-oligonucleotide + Cu(CH$_3$CN)$_4$PF$_6$

According to the representative procedure RP-02 solid support-coupled oligonucleotide (20 nmol) was treated with Cu(CH$_3$CN)$_4$PF$_6$.

| CPG-oligonucleotide | Analytical data |
|----------------------|-----------------|
| 10mer T7De8a-dGC    | Analytical RP-HPLC (I) trace |
| MALDI-MS spectrum   |                 |
| mass$_{calc}$ = 3007.5 |
| mass$_{found}$ = 3009.3 |
| 10mer 7De-dAT7De8a-dGC | Analytical RP-HPLC (I) trace |
| MALDI-MS spectrum   |                 |
| mass$_{calc}$ = 3016.0 |
| mass$_{found}$ = 3013.7 |
CPG-oligonucleotide + FeCl$_2 \cdot 4$ H$_2$O

According to the representative procedure RP-02 solid support-coupled oligonucleotide (20 nmol) was treated with FeCl$_2 \cdot 4$ H$_2$O.

| CPG-oligonucleotide | Analytical data |
|---------------------|-----------------|
| 10mer T7De8a-dGC    |                 |
| Analytical RP-HPLC (I) trace | mass$_{calc}$ = 3007.5 |
| MALDI-MS spectrum   | mass$_{found}$ = 3009.1 |
| 10mer 7De-dAT7De8a-dGC | mass$_{calc}$ = 3016.0 |
| Analytical RP-HPLC (I) trace | mass$_{found}$ = 3014.0 |
| MALDI-MS spectrum   |                 |
**CPG-oligonucleotide + La(Oi-Pr)$_3$**

According to the representative procedure RP-02 solid support-coupled oligonucleotide (20 nmol) was treated with La(Oi-Pr)$_3$.

| CPG-oligonucleotide | Analytical data |
|----------------------|-----------------|
| 10mer T7De8a-dGC     | **Analytical RP-HPLC (I) trace** |
|                      | ![MALDI-MS spectrum](image) |
|                      | mass$\text{calc} = 3007.5$ |
|                      | mass$\text{found} = 3008.7$ |
| 10mer 7De-dAT7De8a-dGC | **Analytical RP-HPLC (I) trace** |
|                      | ![MALDI-MS spectrum](image) |
|                      | mass$\text{calc} = 3016.0$ |
|                      | mass$\text{found} = 3014.8$ |
**CPG-oligonucleotide + Ni(acac)$_2$**

According to the representative procedure RP-02 solid support-coupled oligonucleotide (20 nmol) was treated with Ni(acac)$_2$.

| CPG-oligonucleotide | Analytical data |
|----------------------|-----------------|
| 10mer T7De8a-dGC     |                 |
| Analytical RP-HPLC (I) trace |                 |
| MALDI-MS spectrum    | mass$\text{calc} = 3007.5$ mass$\text{found} = 3009.8$ |
| 10mer 7De-dAT7De8a-dGC |                 |
| Analytical RP-HPLC (I) trace |                 |
| MALDI-MS spectrum    | mass$\text{calc} = 3016.0$ mass$\text{found} = 3014.7$ |
CPG-oligonucleotide + PdOAc$_2$

According to the representative procedure RP-02 solid support-coupled oligonucleotide (20 nmol) was treated with PdOAc$_2$.

| CPG-oligonucleotide | Analytical data |
|---------------------|-----------------|
| 10mer T7De8a-dGC    | mass$_{calc}$ = 3007.5  |
|                     | mass$_{found}$ = 3008.9 |
| MALDI-MS spectrum   |                 |
| 10mer 7De-dAT7De8a-dGC | mass$_{calc}$ = 3016.0  |
|                     | mass$_{found}$ = 3014.6 |
| MALDI-MS spectrum   |                 |
CPG-oligonucleotide + RuCl₃

According to the representative procedure RP-02 solid support-coupled oligonucleotide (20 nmol) was treated with RuCl₃.

| CPG-oligonucleotide                  | Analytical data                                      |
|--------------------------------------|------------------------------------------------------|
| 10mer T7De8a-dGC                     | Analytical RP-HPLC (I) trace                         |
|                                      | ![MALDI-MS spectrum](image1)                         |
|                                      | mass<sub>calc</sub> = 3007.5                         |
|                                      | mass<sub>found</sub> = 3009.5                        |
| 10mer 7De-dAT7De8a-dGC               | Analytical RP-HPLC (I) trace                         |
|                                      | ![MALDI-MS spectrum](image2)                         |
|                                      | mass<sub>calc</sub> = 3016.0                         |
|                                      | mass<sub>found</sub> = 3014.8                        |
**CPG-oligonucleotide + Grubbs 1\textsuperscript{st} Gen.**

According to the representative procedure RP-02 solid support-coupled oligonucleotide (20 nmol) was treated with Grubbs 1\textsuperscript{st} Gen..

| CPG-oligonucleotide | Analytical data |
|----------------------|-----------------|
| 10mer T7De8a-dGC     |                 |
| Analytical RP-HPLC (I) trace |                 |
| MALDI-MS spectrum    |                 |
| mass\textsubscript{calc} = 3007.5 | mass\textsubscript{found} = 3010.3 |
| 10mer 7De-dAT7De8a-dGC |                 |
| Analytical RP-HPLC (I) trace |                 |
| MALDI-MS spectrum    |                 |
| mass\textsubscript{calc} = 3016.0 | mass\textsubscript{found} = 3016.5 |
CPG-oligonucleotide + Sc(OTf)$_3$

According to the representative procedure RP-02 solid support-coupled oligonucleotide (20 nmol) was treated with Sc(OTf)$_3$.

| CPG-oligonucleotide         | Analytical data                      |
|-----------------------------|--------------------------------------|
| 10mer T7De8a-dGC            | Analytical RP-HPLC (I) trace          |
|                             | MALDI-MS spectrum                     |
|                             | mass$_{\text{calc}}$ = 3007.5         |
|                             | mass$_{\text{found}}$ = 3008.7        |
| 10mer 7De-dAT7De8a-dGC      | Analytical RP-HPLC (I) trace          |
|                             | MALDI-MS spectrum                     |
|                             | mass$_{\text{calc}}$ = 3016.0         |
|                             | mass$_{\text{found}}$ = 3014.7        |
**CPG-oligonucleotide + Sc(OTf)$_3$**

According to the representative procedure RP-02 solid support-coupled oligonucleotide (20 nmol) was treated with Sc(OTf)$_3$ at 40 °C.

| CPG-oligonucleotide          | Analytical data                          |
|------------------------------|-------------------------------------------|
| 10mer T7De8a-dGC             | Analytical RP-HPLC (I) trace              |
|                              | MALDI-MS spectrum                         |
|                              | mass$_{calc}$ = 3007.5                    |
|                              | mass$_{found}$ = 3008.6                   |
| 10mer 7De-dAT7De8a-dGC       | Analytical RP-HPLC (I) trace              |
|                              | MALDI-MS spectrum                         |
|                              | mass$_{calc}$ = 3016.0                    |
|                              | mass$_{found}$ = 3014.5                   |
CPG-oligonucleotide + SeO$_2$

According to the representative procedure RP-02 solid support-coupled oligonucleotide (20 nmol) was treated with SeO$_2$.

| CPG-oligonucleotide | Analytical data |
|----------------------|-----------------|
| 10mer T7De8a-dGC | **Analytical data** |
|                      | **Analytical RP-HPLC (I)** |
|                      | trace |
| MALDI-MS spectrum | mass$_{calc}$ = 3007.5 |
|                     | mass$_{found}$ = 3008.6 |
| 10mer 7De-dAT7De8a-dGC | **Analytical data** |
|                      | **Analytical RP-HPLC (I)** |
|                      | trace |
| MALDI-MS spectrum | mass$_{calc}$ = 3016.0 |
|                     | mass$_{found}$ = 3014.8 |
CPG-oligonucleotide + VO(acac)$_2$

According to the representative procedure RP-02 solid support-coupled oligonucleotide (20 nmol) was treated with VO(acac)$_2$.

| CPG-oligonucleotide | Analytical data |
|----------------------|-----------------|
| 10mer T7De8a-dGC     |                 |
| Analytical RP-HPLC (I) trace |                 |
| MALDI-MS spectrum    |                 |
| mass$_{calc}$ = 3007.5 mass$_{found}$ = 3008.6 |

| 10mer 7De-dAT7De8a-dGC |                 |
| Analytical RP-HPLC (I) trace |                 |
| MALDI-MS spectrum    |                 |
| mass$_{calc}$ = 3016.0 mass$_{found}$ = 3013.7 |
CPG-oligonucleotide + ZnCl₂

According to the representative procedure RP-02 solid support-coupled oligonucleotide (20 nmol) was treated with ZnCl₂.

| CPG-oligonucleotide | Analytical data |
|----------------------|-----------------|
| 10mer T7De8a-dGCT7De8a-dGC | Analytical RP-HPLC (I) trace |
|                       | MALDI-MS spectrum |
|                       | mass\text{calc} = 3007.5 |
|                       | mass\text{found} = 3010.4 |
| 10mer 7De-dAT7De8a-dGC | Analytical RP-HPLC (I) trace |
|                       | MALDI-MS spectrum |
|                       | mass\text{calc} = 3016.0 |
|                       | mass\text{found} = 3014.9 |
CPG-oligonucleotide + DDQ

According to the representative procedure RP-02 solid support-coupled oligonucleotide (20 nmol) was treated with DDQ 7.

| CPG-oligonucleotide | Analytical data |
|----------------------|-----------------|
| 10mer T7De8a-dGC     |                 |
| Analytical RP-HPLC (I) trace |                 |
| MALDI-MS spectrum | mass<sub>calc</sub> = 3007.5  
mass<sub>found</sub> = 3009.3 |
| 10mer 7De-dAT7De8a-dGC | mass<sub>calc</sub> = 3016.0  
mass<sub>found</sub> = 3013.5 |
CPG-oligonucleotide + PIDA

According to the representative procedure RP-02 solid support-coupled oligonucleotide (20 nmol) was treated with PIDA 8.

| CPG-oligonucleotide | Analytical data |
|---------------------|-----------------|
| 10mer T7De8a-dGC    |                 |
| Analytical RP-HPLC (I) trace | mass\(_{calc}\) = 3007.5 |
|                     | mass\(_{found}\) = 3008.8 |
| MALDI-MS spectrum   |                 |
| 10mer 7De-dAT7De8a-dGC | mass\(_{calc}\) = 3016.0 |
| Analytical RP-HPLC (I) trace | mass\(_{found}\) = 3014.8 |
| MALDI-MS spectrum   |                 |
CPG-oligonucleotide + TEMPO

According to the representative procedure RP-02 solid support-coupled oligonucleotide (20 nmol) was treated with TEMPO 9.

| CPG-oligonucleotide | Analytical data |
|---------------------|----------------|
| 10mer T7De8a-dGC    | Analytical RP-HPLC (I) trace |
|                     | MALDI-MS spectrum |
|                     | mass\text{calc} = 3007.5 \text{ mass}\text{found} = 3008.6 |
| 10mer 7De-dAT7De8a-dGC | Analytical RP-HPLC (I) trace |
|                     | MALDI-MS spectrum |
|                     | mass\text{calc} = 3016.0 \text{ mass}\text{found} = 3013.7 |
Biological experiments

T4 ligation and amplification by PCR

Table S5 – Sequences of DNA oligonucleotides I–III′/III′ and used primer sequences.

| DNA   | Sequence (5'-3')                                                                 |
|-------|-------------------------------------------------------------------------------|
| HP    | CAA ATC CGT TCA SAG GTC GGT GTG AAC GGA TTT GAG TC                             |
|       | CT*T CT 7De8a-dGTC 7De8a-dGT 7De-dACC T                                        |
| I (a-d)| CT*T CTC7De8a-dG 7De-dATT C7De8a-dGC 7De-dACC T                               |
|       | CT*T CTC7De-dA 7De8a-dG7De-dAT TTC 7De-dACC T                                 |
|       | CT*T CTC7De8a-dG 7De-dA7De8a-dGC CTC 7De-dACCT                               |
| I'    | TAG G AG GT*i aai iaa iAG AGG ACT                                             |
| II    | GTA TCA AGC AG G                                                              |
| II'   | TAG GCC TGC TTG                                                              |
| III   | CCT ACT CTC GTA TGA CCT CAA CTA CAT GGT CTA CA                               |
| III'  | TGT AGA CCA TGT AGT TGA GGT CAT ACG AGA G                                     |
| forward primer | TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG AGG TCG GTG          |
| reverse primer  | GTC TCG TGG CTA GAT GTG TAT AAG AGA CAG AGG TCG GTG                              |
| forward primer (qPCR) | AGG TCG GTG TGA ACG GAT TTG AG                                          |
| reverse primer (qPCR) | GTA GAC CAT GTA GTT GAG GTC A                                                  |

*S = C*-Spacer, T* = Ethynyl-dU, i = inosine; a = abasic site.

5'-phosphorylation of DNA

For 5'-phosphorylation of 280 pmol DNA in a total reaction volume of 20 μL, 10 units of T4 polynucleotide kinase (T4 PNK, *Thermo Fisher Scientific*), 1x PNK Buffer A (50 mM Tris-HCl, 10 mM MgCl₂, 5 mM DTT, 0.1 mM spermidine, pH = 7.6 at 25 °C, *Thermo Fisher Scientific*) and 1 mM ATP (*Thermo Fisher Scientific*) were used. Reaction mixtures were incubated at 37 °C for 20 min, then heat-inactivated at 75 °C for 15 min and slowly cooled down to 4 °C.

Ligation of DNA

Prior to enzymatic ligation of DNA, the oligonucleotides were annealed by incubation at 85 °C for 10 min and cooling down to 4 °C. For ligation (40 μL scale), 100 pmol of each oligonucleotide, 600 units of T4 DNA Ligase (T4 DNA ligase, *New England Biolabs*) and 1x T4 DNA Ligase Buffer (50 mM Tris-HCl, 10 mM MgCl₂, 10 mM DTT, 1 mM ATP, pH = 7.5 at 25 °C, *New England Biolabs*) were mixed. Ligation reactions were performed at 25 °C for 16 h, then stopped by heat inactivation at 75 °C for 15 min and cooled down to 4 °C.
Analysis of DNA ligation
For analysis of DNA ligation reactions, agarose gel electrophoresis was performed using a 3% or 4% agarose gel. Electrophoresis was carried out in TBE buffer (89 mM Tris-borate, 2 mM EDTA, pH = 8.3) at 100 V constant voltage for 15 min and then 150 V constant voltage for about 45 min. For staining of the DNA, Midori Green Direct (NIPPON Genetics) and as a reference, GeneRuler Ultra Low Range DNA Ladder (Thermo Fisher Scientific) was used. Imaging of the gels was performed using the Bio-Rad Gel Doc™ XR system.

Purification of DNA by ethanol precipitation
After the first and second ligation, the DNA was precipitated by adding 1/10 volume of 3 M aq. sodium acetate (pH = 5.2) and 3 volumes of 100% ethanol and incubating this solution for about 4 h or overnight at -80 °C. Afterwards the samples were centrifuged at 4 °C for 30 min (13200 rpm; Centrifuge 5415 R, Eppendorf), the supernatant was taken off, additional 100 µL of 100% ethanol were added and the solution was incubated for 1 h at -80 °C. Afterwards the samples were centrifuged at 4 °C for 30 min (13200 rpm; Centrifuge 5415R, Eppendorf), the supernatant was taken off, and the DNA pellets were dried at 37 °C. The DNA samples were dissolved in ddH₂O.

Purification of DNA by gel extraction
After the third ligation, the DNA samples were gel extracted using the “QIAquick Gel Extraction Kit” (Qiagen) according to the manufacturer protocol.

PCR amplification
Following the third ligation, fully encoded DNA was amplified by PCR. Thereby, 5 µL of gel extracted DNA, 5 U of Taq DNA polymerase (Thermo Fisher Scientific), 1x Taq Buffer (10 mM Tris-HCl, 50 mM KCl, 0.08% (v/v) Nonidet P40, pH = 8.8 at 25 °C, Thermo Fisher Scientific), 3 mM MgCl₂, 0.625 mM of each dNTP (dATP, dGTP, dTTP, and dCTP, corresponding to 2.5 mM of the mixture of dNTPs) and 1 µM of the reverse primer in a reaction volume of 39 µL. The PCR program started with pre-denaturation at 95 °C for 3 min, followed by denaturation for 30 s at 95 °C, annealing for 30 s at 55 °C, and elongation for 30 s at 72 °C. After 10 cycles, 1 µM of the forward primer was added and additional 20 cycles were performed. After PCR, the time for elongation was prolonged to 5 min. The PCR products were analyzed by agarose gel electrophoresis.
Silica-membrane-based purification of DNA

PCR products were purified using the “QIAquick PCR Purification Kit” (Qiagen) according to the manufacturer’s protocol.

Figure S1 – Encoding scheme for test ligations with chemically stabilized barcodes I.

Figure S2 – Agarose gel (4%) of hairpin-based encoding strategy and PCR amplification of fully encoded DNA using stabilized DNA in barcode I. Stabilized DNA barcode I was ligated to hairpin HP, DNA I’ and to DNA duplexes II/II’ in one pot to encode the first building block and the heterocyclic scaffold yielding the duplex HP-I-II/’-II’. Then, DNA duplex HP-I-II/’-II’ was ligated to DNA duplexes III/III’ to encode acid building blocks. Lane 1: hairpin HP, lane 2: ligation of stabilized DNA barcode I to hairpin HP and DNA duplexes II/II’, lane 3: ligation of DNA duplex HP-I-II/’-II’ to DNA duplexes III/III’, lane 4: PCR amplification of DNA duplex HP-I-II-III/’-II’-III’.

Sanger sequencing

Sanger sequencing of purified PCR products was performed by Microsynth Seqlab GmbH (Göttingen, Germany). The sequencing data was analyzed with Benchling [Biology Software] (2021). Retrieved from https://benchling.com.
Table S6 – Sanger sequencing results of the PCR products of encoded sequences containing five different stabilized codes Ia-e (T* = Ethynyl-dU)

| Code | 5'-CT'C TCT 7De8a-dG TC T7De8a-dG T 7De-dACC T-3' |
|------|---------------------------------------------------|
|      | forward primer                                    |
| Ia   | GAGTCTCTCTGACGCTACGCTGATAGCTACAGCTACGTTCACCCTCT |
|      | reverse primer                                    |
| Ia   | TGTGTAAGCAGAGGGCTGCTGAGATGCTACGCTACGTTCACCCTCT |

| Code | 5'-CT'C TC7De8a-dG 7De-dATT C7De8a-dG C 7De-dACC T-3' |
|------|------------------------------------------------------|
|      | forward primer                                      |
| Ib   | GAGTCTCTGATCGACGCTACGCTGATAGCTACAGCTACGTTCACCCTCT |
|      | reverse primer                                      |
| Ib   | AGGTGCAAATCGAGAGCTCAATATCCGGTTCACGCGACCTCTCTTTTACAC |


| Code | 5'-CT'C TC7De-dA 7De8a-dG 7De-dAT TTC 7De-dACC T-3' |
|------|---------------------------------------------------|
|      | forward primer                                    |
|      | GAGTCTCTCAGATTTCACCTGATCAAGCAGGCTACTCTCAGCTATGACCTCAACTACATGGT |
|      | aligned sequence MV26                              |
|      | reverse primer                                    |
|      | CTTGATAAGGTTGAAGCTGAGGGACCTCAATCCGTTACACCGACCTCTGTC |
|      | aligned sequence MV26                              |

| Code | 5'-CT'C TC7De8a-dG 7De-dAT7De8a-dG C CTC 7De-dACCCT-3' |
|------|------------------------------------------------------|
|      | forward primer                                       |
|      | TTGACTCTCTCGAGGCCTCCAACCTCTATCAAGCAGGCTACTCTCAGCTATGACCTCAACT |
|      | aligned sequence MV27                                |
|      | reverse primer                                       |
|      | AGGTAGGCTCGAGACGACTCAAATCCGTTACACCGACCTCTGCTT       |
|      | aligned sequence MV27                                |
qPCR

For qPCR experiments the following were combined in PCR plate wells (GK480K-50, Kisker) in a total volume of 20 μL: DNA template (5 μL, ligation product 3), 200 nM forward primer (0.8 μL, 5 μM stock), 200 nM reverse primer (0.8 μL, 5 μM stock), SsoAdvanced universal SYBR® Green supermix (10 μL, Bio-Rad) and H₂O (3.4 μL).

For all qPCR experiments the following amplification method using the LightCycler® 480 II system from Roche was performed: hot start at 95 °C for 30 s, then 35 cycles of 95 °C for 15 s (denaturation), 60 °C for 30 s (annealing) and 72 °C for 30 s (elongation).

The specificity of the PCR amplification was analyzed by melting curve measurements. Analysis was done with the LightCycler® 480 – software version 1.5.

![amplification curves native DNA](image1)

![amplification curves stabilized DNA](image2)

Figure S3 – Amplification curves (qPCR) using different concentrations of DNA sequences containing native or stabilized DNA barcodes Ia (5’- CT°C TCT 7De8a-dGT C T7De8a-dGT T7De-dACC T-3’).

![standard curve native DNA](image3)

![standard curve stabilized DNA](image4)

Figure S4 – Standard curve (qPCR) using different concentrations of DNA sequences containing native or stabilized DNA barcodes Ia (5’- CT°C TCT 7De8a-dGT C T7De8a-dGT T7De-dACC T-3’).
**Figure S5** – Melting curves of the PCR products after qPCR of different concentrations of DNA sequences containing native or stabilized DNA barcodes Ia (5'-CT*TCT 7De8a-dGT 7De-dACC T-3').

**Figure S6** – Amplification and melting curves (qPCR) using different concentrations of DNA sequence containing stabilized DNA barcode Ib (5'-CT*C T7De8a-dG T7De-7De-7De8a-dG C7De8a-dGC 7De-dACC T-3').

**Figure S7** – Amplification and melting curves (qPCR) using different concentrations of DNA sequences containing stabilized DNA barcode Ic (5'-CT*C TC7De8a-dA 7De8a-dG7De-dAT TTC 7De-dACC T-3').
Figure S8 – Amplification and melting curves (qPCR) using different concentrations of DNA sequences containing stabilized DNA barcode Id (5’-CT*C TC7De8a-dG 7De-dA7De8a-dGC CTC 7De-dACCT-3’).

Figure S9 – Differences in the amplification rate of different DNA sequences containing stabilized DNA barcodes (Ia-d) and native DNA (Ia) [250 pg/µL].
DNA-encoded chemistries
Representative Procedures

The syntheses followed published procedures.[1,18-24]

**Copper(I)-promoted alkyne-azide cycloaddition (RP-03)**

The CPG-bound oligonucleotide-alkyne conjugate (400 nmol) was suspended in 280 µL of H₂O/MeOH (1:1). Subsequently, the azide (33.60 µmol, 84 equiv.) dissolved in 100 µL of DMF, TBTA (16.80 µmol, 42 equiv.) dissolved in 120 µL of DMF, Na-ascorbate (16.80 µmol, 42 equiv.) dissolved in 10 µL of H₂O, and CuSO₄·5H₂O (1.68 µmol, 4.2 equiv.) dissolved in 10 µL of H₂O were added to the suspension in this order. Stock solutions of all reactants were prepared before the reaction was started. The reaction mixtures were shaken at 50 °C overnight. Then the CPG-bound conjugate was filtered over a filter column and washed three times with each 200 µL of 0.1 M EDTA solution, 0.1 M MgCl₂ solution, water, DMF, MeOH, ACN and CH₂Cl₂ and dried in vacuo.

The completeness of the reaction was controlled by cleavage of a small portion (~20 nmol) of CPG-bound oligonucleotide conjugate with 500 µL AMA (AMA = aqueous ammonia (30%)/aqueous methylamine (40%), 1:1, vol/vol) for 4 h at ambient temperature. To this solution 20 µL of 1 M Tris buffer (pH = 7.5) were added, the mixture was dried in a SpeedVac, and dissolved in 200 µL of distilled water. The crude was analyzed by analytical RP-HPLC and MALDI-MS.

**Amide coupling (RP-04)**
**Step 1:** The Fmoc-protecting group of the CPG-bound oligonucleotide (250 nmol, 9-10 mg) was cleaved off by addition of 200 µL 20% piperidine in dry DMF and shaking for 5 min. Afterwards, the CPG-bound deprotected oligonucleotide was washed three times with each 200 µL of DMF, MeOH, ACN and CH₂Cl₂ and then dried *in vacuo*.

**Step 2:** CPG-bound oligonucleotide, carboxylic acid and HATU were dried *in vacuo* for 15 min. Stock solutions of all reactants in dry DMF were prepared before the reaction was started. To the solution of carboxylic acid (25 µmol, 100 equiv.) in 75 µL dry DMF, HATU (25 µmol, 100 equiv.) dissolved in 75 µL dry DMF and DIPEA (62.5 µmol, 250 equiv.) were added. The mixture was shaken for 5 min and added to CPG-bound DNA suspended in 75 µL dry DMF (250 nmol, 1 equiv.). The amide coupling reaction was shaken at ambient temperature for 2 hours. Next, CPG-bound conjugate was filtered over a filter column, washed three times with each 200 µL of DMF, MeOH, ACN and CH₂Cl₂ and dried *in vacuo*. Amide coupling was repeated two times.

Completeness of amide coupling was controlled by cleaving off a small portion of CPG-bound oligonucleotide conjugate (0.7–0.9 mg, ~20 nmol) with 500 µL AMA (AMA = aqueous ammonia (30%)/ aqueous methylamine (40%), 1:1, vol/vol) 1 h (TC-sequences) or 4 h (ATGC- and 7De-dATC-sequences) at ambient temperature. Afterwards 20 µL of 1 M Tris buffer (pH = 7.5) were added, the mixture was dried under reduced pressure (SpeedVac) and DNA was dissolved in 200 µL distilled water. Crude reaction mixture was analyzed by analytical RP-HPLC and MALDI-MS. In case of uncompleted coupling (<90%) the reaction was repeated a third time.

Unreacted amines were capped with acetic anhydride (three times 200 µL, 30 s, 1:1 mixture of THF/methylimidazole, 9:1, vol/vol, and THF/pyridine/acetic anhydride 8:1:1, vol/vol). Capped CPG-bound oligonucleotide conjugate was washed three times with each 200 µL of DMF, MeOH, ACN and CH₂Cl₂ and dried *in vacuo*.

**Step 3:** DMT-protecting group of CPG-bound oligonucleotide (250 nmol, 9-10 mg of solid phase material) was removed by addition of 200 µL 3% trichloroacetic acid in CH₂Cl₂ for 1 min. Orange coloring of the solution indicated successful removal of protecting group. The deprotection was repeated 3-5 times until no further coloring of the solution was observed. CPG-bound deprotected DNA was washed three times with each 200 µL of 1% TEA in ACN, DMF, MeOH, ACN and CH₂Cl₂ and dried *in vacuo*. 
Prior to use, CPG-bound oligonucleotide aldehyde conjugate was dried in vacuo for 15 min. A solution of propylamine (1000 equiv., 20 µmol) in 50 µL MeOH was added to the CPG-bound DNA-aldehyde conjugate. The reaction mixture was shaken at ambient temperature for 3 h to effect imine formation. Afterwards, acetic acid (1000 equiv., 20 µmol, solid acids were dissolved in 15 µL MeOH) was pipetted to the reaction mixture, followed by the addition of tert-butylisocyanide (1000 equiv., 20 µmol). The reaction mixture was shaken for 16 h at 50 °C. The CPG-bound conjugate was filtered over a filter column, washed three times with each 200 µL of DMF, MeOH, ACN and CH₂Cl₂ and dried in vacuo. The CPG-bound DNA conjugate was cleaved from the solid phase and deprotected with 500 µL AMA solution for 4 h at ambient temperature. Afterwards the mixture was dried in a SpeedVac and the remaining DNA pellet was dissolved in 200 µL of distilled water. The crude was analyzed by analytical RP-HPLC and MALDI-MS. The product was isolated by preparative RP-HPLC.
reaction mixture was shaken for 16 h at 50 °C. The CPG-bound conjugate was filtered over a filter column, washed three times with each 200 µL of DMF, MeOH, ACN and CH₂Cl₂ and dried in vacuo. The CPG-bound DNA conjugate 19 was cleaved from the solid phase and deprotected with 500 µL AMA solution for 4 h at ambient temperature. Afterwards the mixture was dried in a SpeedVac and the remaining DNA pellet was dissolved in 200 µL of distilled water. The crude was analyzed by analytical RP-HPLC and MALDI-MS. The product was isolated by preparative RP-HPLC.

**Groebke-Blackburn-Bienaymé three-component reaction on CPG-bound oligonucleotides (RP-07)**[18]

Prior to use, CPG-bound oligonucleotide aldehyde conjugate and 2-aminopyridine 20 were dried in vacuo for 15 min.[26] 2-aminopyridine (1000 equiv., 20 µmol) was added to the CPG-bound DNA-aldehyde conjugate 13 in 50 µL MeOH. The reaction mixture was shaken at ambient temperature for 6 h to effect imine formation. Afterwards, tert-butylisocyanide 16 (1000 equiv., 20 µmol) was pipetted to the reaction mixture, followed by the addition of acetic acid as Brønsted acid (final volume: 80 µL, acid concentration: 1%). The reaction mixture was shaken for 16 h at ambient temperature. The CPG-bound conjugate was filtered over a filter column, washed three times with each 200 µL of DMF, MeOH, ACN and CH₂Cl₂ and dried in vacuo. The CPG-bound DNA conjugate 21 was cleaved from the solid phase and deprotected with 500 µL AMA solution for 4 h at ambient temperature. Afterwards the mixture was dried in a SpeedVac and the remaining DNA pellet was dissolved in 200 µL of distilled water. The crude was analyzed by analytical RP-HPLC and MALDI-MS. The product was isolated by preparative RP-HPLC.
Ugi four-component/aza-Wittig reaction on CPG-bound oligonucleotides (RP-08)\textsuperscript{[18]}

CPG-bound oligonucleotide, \textit{N}-Boc-piperazine 22, (isocynoimino)triphenylphosphorane 24 and solid acids were dried in vacuo for 15 min.\textsuperscript{[26]} \textit{N}-Boc-piperazine 232 (1000 equiv., 20 \textmu mol) was added to the CPG-bound DNA-aldehyde conjugate 13 in 30 \textmu L 1,2-dichloroethane. The reaction mixture was shaken at ambient temperature for 3 h to effect imine formation. Then, benzoic acid 23 (1000 equiv., 20 \textmu mol) was dissolved in 80 \textmu L 1,2-dichloroethane, transferred to (isocynoimino)triphenylphosphorane 4 (1000 equiv., 20 \textmu mol) and this mixture was added to the CPG-bound conjugate. The reaction mixture was shaken for 16 h at 50 °C. The CPG-bound conjugate was filtered over a filter column, washed three times with each 200 \textmu L of DMF, MeOH, ACN and CH\textsubscript{2}Cl\textsubscript{2} and dried in vacuo. The CPG-bound DNA conjugate 25 was cleaved from the solid phase and deprotected with 500 \textmu L AMA solution for 4 h at ambient temperature. Afterwards the mixture was dried in a SpeedVac and the remaining DNA pellet was dissolved in 200 \textmu L of distilled water. The crude was analyzed by analytical RP-HPLC and MALDI-MS. The product was isolated by preparative RP-HPLC.

\[(R)(\cdot)-\text{BNDHP}\text{-mediated Biginelli reaction on CPG-bound oligonucleotides (RP-09)}\textsuperscript{[19]}\]

The CPG-bound oligonucleotide 13, urea 26 and \textit{(R)(\cdot)-BNDHP} were dried in vacuo for 15 min.\textsuperscript{[27]} Urea 6 (10 \textmu mol, 500 equiv.) and \textit{(R)(\cdot)-BNDHP} (1 \textmu mol, 50 equiv.) were
dissolved both in 30 μL ethanol. The solutions were added to CPG-coupled oligonucleotide-aldehyde conjugate 13 (20 nmol) followed by ethyl acetoacetate 27 (10 μmol, 500 equiv.). The reaction mixture was shaken at 50 °C for 20 h. Then the CPG-bound oligonucleotide conjugate 28 was filtered over a filter column, washed three times with each DMF, MeOH, ACN and CH₂Cl₂ and dried in vacuo. CPG-bound oligonucleotide conjugate 28 was cleaved from solid support and deprotected with 500 μL AMA at ambient temperature for 4 h. Afterwards 20 μL of 1 M Tris buffer (pH = 7.5) were added, the mixture was dried under reduced pressure (SpeedVac) and DNA was dissolved in 200 μL distilled water. The crude reaction mixture was analyzed by analytical RP-HPLC and MALDI-TOF-MS. The product was purified by preparative RP-HPLC.

(R)-(-)-BNDHP-mediated Povarov reaction on CPG-bound oligonucleotides (RP-10)[19]

Prior to use, CPG-bound oligonucleotide, solid anilines and (R)-(-)-BNDHP were dried in vacuo for 15 min.[27] Aniline 29 (10 μmol, 500 equiv.) was dissolved in 24 μL ethanol. The solution was added to CPG-bound oligonucleotide-aldehyde conjugate 13 (20 nmol) suspended in 12 μL triethyl orthoformate. The suspension was shaken at ambient temperature for 4 h. Afterwards 30 μL of (R)-(-)-BNDHP (2 μmol, 100 equiv.) in ethanol followed by N-Boc-2,3-dihydro-1H-pyrrole 30 (10 μmol, 500 equiv.) was added. The reaction mixture was shaken at 50 °C for 16 h. Then the CPG-bound oligonucleotide conjugate 31 was filtered over a filter column, washed three times with each DMF, MeOH, ACN and CH₂Cl₂ and dried in vacuo. CPG-bound oligonucleotide conjugate 31 was cleaved from solid support and deprotected with 500 μL AMA at ambient temperature for 4 h. Afterwards 20 μL of 1 M Tris buffer (pH = 7.5) were added, the mixture was dried under reduced pressure (SpeedVac) and DNA was dissolved in 200 μL distilled water. The crude reaction mixture was analyzed by analytical RP-HPLC and MALDI-TOF-MS. The product was purified by preparative RP-HPLC.
TFA-mediated Pictet-Spenger reaction on CPG-bound oligonucleotides (RP-11)\textsuperscript{[20,21]}

Prior to use, CPG-bound oligonucleotide 32 was dried in vacuo for 15 min.\textsuperscript{[28,29]} Benzaldehyde 33 (30 µmol, 1500 equiv.) was dissolved in 50 µL of a 5% trifluoroacetic acid in CH\textsubscript{2}Cl\textsubscript{2} solution. This solution was added to CPG-bound oligonucleotide-tryptophan conjugate 32 (20 nmol) and the reaction mixture was shaken at ambient temperature for 20 h. Afterwards CPG bound DNA was filtered over a filter column, washed with excess of 1% TEA and three times with each 200 µL of DMF, MeOH, ACN and CH\textsubscript{2}Cl\textsubscript{2} and dried in vacuo. CPG-bound oligonucleotide conjugate 34 was cleaved from solid support and deprotected with 500 µL AMA at ambient temperature for 4 h. Afterwards 20 µL of 1 M Tris buffer (pH = 7.5) were added, the mixture was dried under reduced pressure (SpeedVac) and DNA was dissolved in 200 µL distilled water. The crude reaction mixture was analyzed by analytical RP-HPLC and MALDI-TOF-MS. The product was purified by preparative RP-HPLC.

Cu(I)/bpy-mediated Petasis reaction on CPG-bound oligonucleotides (RP-12)\textsuperscript{[22]}

Prior to use all solid materials were dried in vacuo for 30 min.\textsuperscript{[30]} CuCl (4.0 µmol, 200 equiv., 40 mM calculated for the final volume of 100 µL) and 2,2’-bipyridine (bpy, 4.0 µmol, 200 equiv., 40 mM calculated for the final volume of 100 µL) were dissolved in 48 µL DMF. The solution was shaken at 50 °C for 1 h. Phenylboronic acid 37 (50 µmol, 2500 equiv., 500 mM calculated for the final volume of 100 µL) were dissolved in the CuCl/bpy solution in DMF. 12 µL triethyl orthoformate and glyoxylic acid 36 (40 µmol, 2000 equiv., 400 mM
calculated for the final volume of 100 μL) dissolved in 40 μL DMF were added. The solution was added to CPG-coupled-DNA-secondary amine conjugate 35 (20 nmol, 1 equiv.) and the suspension was shaken at 50 °C for 20 h. Then the CPG-bound DNA conjugate was filtered over a filter column, washed three times with each 200 μL of 0.1 M EDTA solution, 0.1 M MgCl₂ solution, water, DMF, MeOH, ACN and CH₂Cl₂ and dried in vacuo. CPG-bound oligonucleotide conjugated α-aryl glycine 38 were cleaved from solid support and deprotected with 500 μL AMA for 4 h at ambient temperature. Afterwards 20 μL of 1 M Tris buffer (pH = 7.5) were added, the mixture was dried under reduced pressure (SpeedVac) and the DNA was dissolved in 200 μL distilled water. The crude reaction mixture was analyzed by analytical RP-HPLC and MALDI-TOF-MS. The product was purified by preparative RP-HPLC.

**Zn(II)-mediated aza-Diels-Alder reaction on CPG-bound oligonucleotides (RP-13)[19]**

CPG-bound oligonucleotide 13, and ZnCl₂ were dried in vacuo for 15 min.[27] Aniline 30 (10 μmol, 500 equiv.) was dissolved in 24 μL acetonitrile. The solution was added to CPG-coupled oligonucleotide-aldehyde conjugate 13 (20 nmol) suspended in 12 μL triethyl orthoformate. The suspension was shaken at ambient temperature for 4 h. Afterwards 30 μL of ZnCl₂ (2 μmol, 100 equiv.) in ACN followed by Danisheskys’s diene 40 (20 μmol, 1000 equiv.) was added. The reaction mixture was shaken for 1 h at ambient temperature. Then the CPG-coupled oligonucleotide conjugate 41 was filtered over a filter column, washed three times with each 200 μL of 0.1 M EDTA solution, 0.1 M MgCl₂ solution, water, DMF, MeOH, ACN and CH₂Cl₂ and dried in vacuo. CPG-coupled oligonucleotide conjugate 38 was cleaved from solid support and deprotected with 200 μL aqueous ammonia (30%) at 50 °C for 6 h. Afterwards 20 μL of 1 M Tris buffer (pH = 7.5) were added, the mixture was dried under reduced pressure (SpeedVac) and DNA was dissolved in 200 μL distilled water. The crude reaction mixture was analyzed by analytical RP-HPLC and MALDI-TOF-MS. The product was purified by preparative RP-HPLC.
Yb(III)-mediated Castagnoli-Cushman reaction on CPG-bound oligonucleotides (RP-14)[21]

CPG-bound oligonucleotide 13, homophtalic anhydride 42 and Yb(OTf)$_3$ were dried *in vacuo* for 15 min. Aniline 30 (10 μmol, 500 equiv.) was dissolved in 24 μL CH$_2$Cl$_2$. The solution was added to the CPG-bound oligonucleotide-aldehyde conjugate 13 (20 nmol) suspended in 12 μL triethyl orthoformate. The suspension was shaken at ambient temperature for 4 h. Afterwards 30 μL of a suspension of Yb(OTf)$_3$ (1 μmol, 50 equiv.) in CH$_2$Cl$_2$ was added, followed by 30 μL of a suspension of homophtalic anhydride 42 (10 μmol, 500 equiv.) in CH$_2$Cl$_2$. Prior addition to the reaction vessel both suspensions were vortexed and pipetted up and down to obtain homogeneous suspensions. The reaction mixture was shaken for 1 h at ambient temperature. Then the CPG-bound conjugate was filtered over a filter column and washed three times with each 200 μL of 0.1 M EDTA solution, 0.1 M MgCl$_2$ solution, water, DMF, MeOH, ACN and CH$_2$Cl$_2$ and dried *in vacuo*. CPG-bound oligonucleotide conjugate 43 was then cleaved from the solid support and deprotected with 500 μL AMA solution for 4 h at ambient temperature. To this solution 20 μL of 1 M Tris buffer (pH = 7.5) were added, the mixture was dried in a SpeedVac and afterwards dissolved in 200 μL of distilled water. The crude was analyzed by analytical RP-HPLC and MALDI-MS. The product was purified by preparative RP-HPLC.
**Yb(PFO)$_3$-mediated three-component synthesis of pyrazoles on CPG-bound oligonucleotides (RP-15)**

The catalyst Yb(PFO)$_3$ was prepared according to a published procedure. Prior to the reaction, the hydrazine was extracted with diluted NH$_3$ solution and CH$_2$Cl$_2$, dried over MgSO$_4$ and finally dried *in vacuo* if the hydrazine was present as a hydrochloride salt. The hydrazine 44 (250 equiv., 5 µmol), dissolved in 30 µL toluene was added to the CPG-bound DNA-aldehyde conjugate 13 and the reaction mixture was shaken at ambient temperature for 0.5 h. Afterwards, ethyl acetoacetate 28 (3000 equiv., 60 µmol) and 50 µL of a suspension of Yb(PFO)$_3$ (250 equiv., 5 µmol) in toluene was added. Prior addition to the reaction vessel the Yb(PFO)$_3$ suspension was vortexed and pipetted up and down to obtain a homogeneous suspension. The reaction mixture was shaken at 50 °C for 16 h. The CPG-bound conjugate was filtered over a filter column and washed with each 3x 200 µL of 0.1 M EDTA solution, 0.1 M MgCl$_2$ solution, water, DMF, MeOH, ACN and CH$_2$Cl$_2$ and then dried *in vacuo*. The CPG-bound DNA conjugate 45 was cleaved from the solid phase and deprotected by adding 500 µL AMA solution and shaking for 1 h (TC-sequences) or 4 hours (ATCG- and 7De-dATC-sequences) at ambient temperature. Afterwards the mixture was dried in a SpeedVac and the remaining DNA pellet was dissolved in 200 µL of distilled water.
Au(I)/Ag(I)-promoted pyrazoline-containing spiroheterocycle synthesis on CPG-bound oligonucleotides (RP-16)[23]

CPG-bound oligonucleotide 13, tert-butyl 2-benzylhydrazinecarboxylate 47, [Tris(2,4-di-tert-butylphenyl)phosphite]gold chloride and AgSbF₆ were dried in vacuo for 15 min.[32] The solution of tert-butyl 2-benzylhydrazine-carboxylate 47 (500 equiv., 15 µmol) in 20 µL THF and pent-4-yne-1-ol 46 (1000 equiv., 30 µmol) were added to CPG-bound DNA-aldehyde conjugate 13 (30 nmol) followed by equimolar mixture of Au(I)/AgSbF₆ (250 equiv., 7.5 µmol) suspended in 30 µL THF. Prior addition to the reaction vessel the mixture was vortexed and pipetted up and down. The reaction mixture was shaken at room temperature for 20 h. Then the CPG-bound conjugate was filtered over a filter column and washed three times with each 200 µL of 0.1 M EDTA solution, 0.1 M MgCl₂ solution, water, DMF, MeOH, ACN and CH₂Cl₂ and dried in vacuo. CPG-bound oligonucleotide conjugate 48 was then cleaved from the solid support and deprotected with 500 µL AMA solution for 4 h at ambient temperature. To this solution 20 µL of 1 M Tris buffer (pH = 7.5) were added, the mixture was dried in a SpeedVac and afterwards dissolved in 45 µL of distilled water. 5 µL of 1,3,5-triazine-2,4,6-trithiol trisodium salt solution (15% in H₂O) were added and the solution was shaken for 30 min at ambient temperature. Afterwards the sample was centrifuged at 4 °C for 30 min (13200 rpm; Centrifuge 5415 R, Eppendorf), the supernatant was taken off and diluted with 5 µL of a 3 M sodium acetate (pH = 5.2) and 200 µL 100% ethanol. The solution was incubated overnight at -80 °C. Afterwards the samples were centrifuged at 4 °C for 30 min (13200 rpm; Centrifuge 5415 R, Eppendorf), the supernatant was taken off, additional 100 µL of 100% ethanol were added to the pellet and the solution was incubated again for 1 h at -80 °C. Afterwards the sample was centrifuged at 4 °C for 30 min (13200 rpm; Centrifuge 5415 R, Eppendorf), the supernatant was taken off, and the DNA pellets were dried at 37 °C. The DNA samples were dissolved in 100 µL ddH₂O. The crude was analyzed by analytical RP-HPLC and MALDI-MS. The product was purified by preparative RP-HPLC.
Au(I)/Ag(I)-promoted pyrazoline synthesis on CPG-bound oligonucleotides (RP-17)\[20\]

CPG-bound oligonucleotide 49, tert-butyl 2-benzylhydrazinecarboxylate 47, chloro[1,3-bis(2,6-diisopropylphenyl)imidazol-2-ylidene] gold(I) and AgOTf were dried in vacuo for 15 min.\[29\] The solution of tert-butyl 2-benzylhydrazinecarboxylate 47 (1000 equiv., 20 µmol) in 20 µL dry acetonitrile and aliphatic aldehyde 50 (1000 equiv., 20 µmol) were added to CPG-bound DNA-alkyne conjugate 49 (20 nmol) followed by equimolar mixture of Au(I)/AgOTf (250 equiv., 5 µmol) suspended in dry 30 µL acetonitrile. Prior addition to the reaction vessel the mixture was vortexed and pipetted up and down. The reaction mixture was shaken at room temperature for 20 h. Then the CPG-bound conjugate was filtered over a filter column and washed three times with each 200 µL of 0.1 M EDTA solution, 0.1 M MgCl₂ solution, water, DMF, MeOH, ACN and CH₂Cl₂ and dried in vacuo. CPG-bound oligonucleotide conjugate 51 was then cleaved from the solid support and deprotected with 500 µL AMA solution for 4 h at 50 °C. To this solution 20 µL of 1 M Tris buffer (pH = 7.5) were added, the mixture was dried in a SpeedVac and afterwards dissolved in 45 µL of distilled water. 5 µL of 1,3,5-triazine-2,4,6-trithiol trisodium salt solution (15% in H₂O) were added and the solution was shaken for 30 min at ambient temperature. Afterwards the sample was centrifuged at 4 °C for 30 min (13200 rpm; Centrifuge 5415 R, Eppendorf), the supernatant was taken off and diluted with 5 µL of a 3 M sodium acetate (pH = 5.2) and 200 µL 100% ethanol. The solution was incubated overnight at -80 °C. Afterwards the samples were centrifuged at 4 °C for 30 min (13200 rpm; Centrifuge 5415 R, Eppendorf), the supernatant was taken off, additional 100 µL of 100% ethanol were added to the pellet and the solution was incubated again for 1 h at -80 °C. Afterwards the sample was centrifuged at 4 °C for 30 min (13200 rpm; Centrifuge 5415 R, Eppendorf), the supernatant was taken off, and the DNA pellets were dried at 37 °C. The DNA samples were dissolved in 100 µL ddH₂O. The crude was analyzed by analytical RP-HPLC and MALDI-MS. The product was purified by preparative RP-HPLC.
CPG-bound oligonucleotide 49, tert-butyl 2-benzylhydrazinecarboxylate 47, [tris(2,4-di-tert-butylphenyl)phosphite]gold chloride and AgOTf were dried *in vacuo* for 15 min.\[29\] The solution of tert-butyl 2-benzylhydrazine-carboxylate 47 (1000 equiv., 20 µmol) in 20 µL glacial acetic acid and benzaldehyde 34 (1000 equiv., 20 µmol) were added to CPG- bound DNA-alkyne conjugate 49 (20 nmol) followed by equimolar mixture of Au(I)/AgOTf (250 equiv., 5 µmol) suspended in dry 30 µL glacial acetic acid. Prior addition to the reaction vessel the mixture was vortexed and pipetted up and down. The reaction mixture was shaken at 60 °C for 20 h. Then the CPG-bound conjugate was filtered over a filter column and washed three times with each 200 µL of 0.1 M EDTA solution, 0.1 M MgCl₂ solution, water, DMF, MeOH, ACN and CH₂Cl₂ and dried in *vacuo*. CPG-bound oligonucleotide conjugate 52 was then cleaved from the solid support and deprotected with 500 µL AMA solution for 4 h at ambient temperature. To this solution 20 µL of 1 M Tris buffer (pH = 7.5) were added, the mixture was dried in a SpeedVac and afterwards dissolved in 45 µL of distilled water. 5 µL of 1,3,5-triazine-2,4,6-trithiol trisodium salt solution (15% in H₂O) were added and the solution was shaken for 30 min at ambient temperature. Afterwards the sample was centrifuged at 4 °C for 30 min (13200 rpm; Centrifuge 5415 R, *Eppendorf*), the supernatant was taken off and diluted with 5 µL of a 3 M sodium acetate (pH = 5.2) and 200 µL 100% ethanol. The solution was incubated overnight at -80 °C. Afterwards the samples were centrifuged at 4 °C for 30 min (13200 rpm; Centrifuge 5415 R, *Eppendorf*), the supernatant was taken off, additional 100 µL of 100% ethanol were added to the pellet and the solution was incubated again for 1 h at -80 °C. Afterwards the sample was centrifuged at 4 °C for 30 min (13200 rpm; Centrifuge 5415 R, *Eppendorf*), the supernatant was taken off, and the DNA pellets were dried at 37 °C. The DNA samples were dissolved in 100 µL ddH₂O. The crude was analyzed by analytical RP-HPLC and MALDI-MS. The product was purified by preparative RP-HPLC.

**Trifluoroacetic acid-mediated Boc cleavage on oligonucleotide scaffold conjugates in solution (RP-19)**
An isolated pellet of Boc-protected oligonucleotide conjugate 32 was dissolved in 20 µL of a 10% trifluoroacetic acid in H₂O solution. The solution was shaken at ambient temperature for 4 h. The Boc deprotected oligonucleotide conjugate 53 was precipitated by adding 2 µL of a 3 M sodium acetate (pH = 5.2) and 80 µL of 100% ethanol and storing this solution for overnight at -80 °C. Afterwards, the samples were centrifuged at 4 °C for 30 min (13200 rpm; Centrifuge 5415 R, Eppendorf), the supernatant was taken off and the DNA pellets were dried. Oligonucleotide conjugate was dissolved in ddH₂O and analyzed by analytical RP-HPLC and MALDI-TOF-MS.
Table S7 – Overview of diverse chemical reactions on CPG-bound stabilized barcode.

| Entry | Reaction | Conditions |
|-------|----------|------------|
| 1     | Ugi four-component reaction (RP-05)\(^{[18]}\) | 1. MeOH, 50 °C  
2. AMA, rt |
| 2     | Ugi-azide four-component reaction (RP-06)\(^{[18]}\) | 1. MeOH, 50 °C  
2. AMA, rt |
| 3     | Groebke-Blackburn-Bienaymé reaction (RP-07)\(^{[18]}\) | 1. 1% acetic acid in MeOH, rt  
2. AMA, rt |
| 4     | Ugi/aza-Wittig reaction (RP-08)\(^{[18]}\) | 1. MeOH, 50 °C  
2. AMA, rt |
| 5     | Biginelli reaction (RP-09)\(^{[19]}\) | 1. (R)-(-)BNDHP  
EtOH 50 °C  
2. AMA, rt |
6 Povarov reaction (RP-10$^{[19]}$)

1. (R)-(-)-BNDHP
   EtOH/TEOF, 50 °C
2. AMA, rt

6 Pictet-Spengler reaction (RP-11$^{[20,21]}$)

1. TFA
   CH₂Cl₂, rt
2. AMA, rt

7 Petasis reaction (RP-12$^{[22]}$)

1. CuCl/bpy
   DMF/TEOF, 50 °C
2. AMA, rt

8 aza-Diels-Alder reaction (RP-13$^{[19]}$)

1. ZnCl₂
   ACN/TEOF, rt
2. aq. NH₃, 50 °C

9 Castagnoli-Cushman reaction (RP-14$^{[21]}$)

1. Yb(OTf)₃
   CH₂Cl₂/TEOF,
   rt
2. AMA, rt
10 Three-component pyrazole synthesis (RP-15)[1]

11 Pyrazoline-containing spiroheterocycle synthesis (RP-16)[23]

12 Pyrazoline synthesis (RP-17)[20]

13 Pyrazole synthesis (RP-18)[20]
HPLC traces and MALDI-MS spectrum

CPG-bound DNA-starting material conjugates

CPG-bound 16mer 7De-dAT7De8a-dGC-alkyne conjugate was reacted with Boc-N-amido-PEG(4)-azide according to RP-03.

HPLC trace of crude reaction mixture (analytical RP-HPLC (II))

MALDI-MS spectrum of crude reaction mixture

$\text{mass}_{\text{calc.}} = 5038.5$ (DMT$_{\text{off}}$)

$\text{mass}_{\text{found}} = 5039.9$ (DMT$_{\text{off}}$)
DNA conjugate 13: CPG-bound 16mer 7De-dAT7De8a-dGC-PEG(4)-NH₂ conjugate was reacted with 4-formyl-phenoxyacetic acid according to RP-04.

HPLC trace of crude reaction mixture 13 (analytical RP-HPLC (I))

MALDI-MS spectrum of crude reaction mixture 13

mass_{calc} = 5200.6
mass_{found} = 5202.9
DNA conjugate 33: CPG-bound 16mer 7De-dAT7De8a-dGC-PEG(4)-NH₂ conjugate was reacted with N-Boc-tryptophan according to RP-04.

HPLC trace of crude reaction mixture 33 (analytical RP-HPLC (I))

MALDI-MS spectrum of crude reaction mixture 33

mass\textsubscript{calc.} = 5224.7
mass\textsubscript{found} = 5225.9
DNA conjugate 36: CPG-bound 16mer 7De-dAT7De8a-dGC-PEG(4)-NH₂ conjugate was reacted with N-Fmoc-piperidine-4-carboxylic acid according to RP-04.

HPLC trace of crude reaction mixture 36 (analytical RP-HPLC(I))

MALDI-MS spectrum of crude reaction mixture 36

mass \text{calc.} = 5149.6
mass \text{found} = 5150.8
DNA conjugate 49: CPG-bound 16mer 7De-dAT7De8a-dGC-PEG(4)-NH₂ conjugate was reacted with 4-ethynylbenzoic acid according to RP-04.

HPLC trace of crude reaction mixture 49 (analytical RP-HPLC (I))

MALDI-MS spectrum of crude reaction mixture 49

mass\(_{\text{calc}} = \) 5166.6
mass\(_{\text{found}} = \) 5167.9
Isocyanide multicomponent reactions

Ugi four-component reaction

DNA conjugate 17: CPG-bound 16mer 7De-dAT7De8a-dGC-aldehyde conjugate 13 was reacted with acetic acid 15, propylamine 14 and tert-butylisocyanide 16 according to RP-05.

HPLC trace of crude reaction mixture 17 (analytical RP-HPLC (I))

HPLC trace of isolated product 17 (analytical RP-HPLC (I))

MALDI-MS spectrum of isolated product 17

mass\_calc. = 5384.9
mass\_found = 5385.2
Ugi-azide three-component reaction

**DNA conjugate 20**: CPG-bound 16mer 7De-dAT7De8a-dGC-aldehyde conjugate 13 was reacted with piperidine 18, tert-butylisocyanide 16 and trimethylsilyl azide 19 according to RP-06.

HPLC trace of crude reaction mixture 20 (analytical RP-HPLC (I))

| Ret. Time | Width min | Height | Area | Area % |
|-----------|-----------|--------|------|--------|
| 5.365     | 0.900     | 21.935 | 1281.375 | 28.367 |
| 6.635     | 0.171     | 11.319 | 115.857  | 2.749  |
| 7.940     | 0.227     | 191.513| 2611.569 | 61.959 |
| 8.407     | 0.121     | 35.317 | 285.950  | 6.785  |

HPLC trace of isolated product 20 (analytical RP-HPLC (I))

MALDI-MS spectrum of isolated product 20

mass_{calc} = 5393.9
mass_{found} = 5395.6
**Groebke-Blackburn-Bienyamé three-component reaction**

DNA conjugate 22: CPG-bound 16mer 7De-dAT7De8a-dGC-aldehyde conjugate 13 was reacted with 2-aminopyridine 21 and tert-butylisocyanide 16 according to RP-07.

**HPLC trace of crude reaction mixture 22 (analytical RP-HPLC (I))**

**HPLC trace of isolated product 22 (analytical RP-HPLC (I))**

**MALDI-MS spectrum of isolated product 22**

mass\(_{\text{calc.}} = 5359.8\)
mass\(_{\text{found}} = 5361.3\)
DNA conjugate 26: CPG-bound 16mer 7De-dAT7De8a-dGC-aldehyde conjugate 13 was reacted with benzoic acid 24, N-Boc-piperazine 23 and (isocyanimino)triphenylphosphorane 25 according to RP-08.

HPLC trace of crude reaction mixture 26 (analytical RP-HPLC (I))

HPLC trace of isolated product 26 (analytical RP-HPLC (II))

MALDI-MS spectrum of isolated product 26

mass$\text{calc.} = 5515.0$
mass$\text{found} = 5515.6$
Brønsted acid-mediated reactions
Biginelli reaction

DNA conjugate 29: CPG-bound 16mer 7De-dAT7De8a-dGC-aldehyde conjugate 13 was reacted with urea 27, and ethyl acetoacetate 28 according to RP-09.

HPLC trace of crude reaction mixture 29 (analytical RP-HPLC (I))

| Ret. Time | Width min | Height | Area   | Area % |
|-----------|-----------|--------|--------|--------|
| 6.915     | 0.204     | 112.473| 1378.739| 13.908 |
| 6.924     | 0.262     | 543.819| 8534.291| 86.092 |

HPLC trace of isolated product 29 (analytical RP-HPLC (II))

MALDI-MS spectrum of isolated product 29

mass\text{calc.} = 5354.8
mass\text{found} = 5355.1
DNA conjugate 29a: CPG-bound 16mer 7De-dAT7De8a-dGC-aldehyde conjugate 13 was reacted with phenylurea, and ethyl acetoacetate 28 according to RP-09 with 200 equivalents of (R)-BNDHP.

HPLC trace of crude reaction mixture 29a (analytical RP-HPLC (I))

| Ret. Time | Width (min) | Height (arb. units) | Area (arb. units) | Area % |
|-----------|-------------|---------------------|-------------------|--------|
| 6.574     | 0.241       | 115.177             | 1665.489          | 32.653 |
| 7.953     | 0.226       | 253.170             | 3435.080          | 67.347 |

HPLC trace of isolated product 29a (analytical RP-HPLC (II))

MALDI-MS spectrum of isolated product 29a

mass_{calc.} = 5430.9
mass_{found} = 5430.9
Povarov reaction

DNA conjugate 32: CPG-bound 16mer 7De-dAT7De8a-dGC-aldehyde conjugate 13 was reacted with aniline 30, and N-Boc-2,3-dihydro-1H-pyrrole 31 according to RP-10.

HPLC trace of crude reaction mixture 32 (analytical RP-HPLC (II))

HPLC trace of isolated product 32 (analytical RP-HPLC (II))

MALDI-MS spectrum of isolated product 32

mass \text{calc.} = 5444.9
mass \text{found} = 5445.0
**Pictet-Spengler reaction**

DNA conjugate 35: CPG-bound 16mer 7De-dAT7De8a-dGC-tryptophan conjugate 33 was reacted with benzaldehyde 34 according to RP-11.

HPLC trace of crude reaction mixture 35 (analytical RP-HPLC (I))

| Ret. Time | Width min | Height   | Area     | Area % |
|-----------|-----------|----------|----------|--------|
| 6.437     | 0.220     | 32,258   | 425,207  | 13.027 |
| 7.361     | 0.265     | 178,428  | 2838,747 | 86.973 |

HPLC trace of isolated product 35 (analytical RP-HPLC (II))

MALDI-MS spectrum of isolated product 35

mass_{calc} = 5312.8
mass_{found} = 5313.1
Lewis acid-promoted reactions
Petasis reaction

**DNA conjugate 39**: CPG-bound 16mer 7De-dAT7De8a-dGC-piperidine conjugate 36 was reacted with phenylboronic acid 38 and glyoxylic acid monohydrate 37 according to RP-12.

HPLC trace of crude reaction mixture 39 (analytical RP-HPLC (I))

| Ret. Time | Width (min) | Height  | Area       | Area %  |
|-----------|-------------|---------|------------|---------|
| 6.200     | 0.293       | 101.582 | 1788.189   | 31.347  |
| 6.410     | 0.286       | 255.414 | 3916.385   | 68.653  |

HPLC trace of isolated product 39 (analytical RP-HPLC (II))

MALDI-MS spectrum of isolated product 39

mass<sub>calc.</sub> = 5283.7
mass<sub>found</sub> = 5283.8
aza-Diels-Alder reaction
DNA conjugate 41: CPG-bound 16mer 7De-dAT7De8a-dGC-aldehyde conjugate 13 was reacted with aniline 30 and danishefsky's diene 40 according to RP-13.

HPLC trace of crude reaction mixture 41 (analytical RP-HPLC (I))

HPLC trace of isolated product 41 (analytical RP-HPLC (II))

MALDI-MS spectrum of isolated product 41
mass_{calc} = 5343.8
mass_{found} = 5343.8
Castagnoli-Cushman reaction

**DNA conjugate 43:** CPG-bound 16mer 7De-dAT7De8a-dGC-aldehyde conjugate 13 was reacted with aniline 30 and homophthalic anhydride 42 according to RP-14.

HPLC trace of crude reaction mixture 43 (analytical RP-HPLC (I))

| Ret. Time | Width min | Height   | Area     | Area % |
|-----------|-----------|----------|----------|--------|
| 6.594     | 0.273     | 260.515  | 4274.831 | 56.510 |
| 6.525     | 0.236     | 232.599  | 3289.860 | 43.490 |

HPLC trace of isolated product 43 (analytical RP-HPLC (II))

MALDI-MS spectrum of isolated product 43

mass\text{calc} = 5437.9
mass\text{found} = 5437.5
Yb(PFO)$_3$-mediated three-component synthesis of pyrazoles

DNA conjugate 45: CPG-bound 16mer 7De-dAT7De8a-dGC-aldehyde conjugate 13 was reacted with phenylhydrazine 44 and ethyl acetoacetate 28 according to RP-15.

HPLC trace of crude reaction mixture 45 (analytical RP-HPLC (I))

| Ret. Time | Height | Area | Area % |
|-----------|--------|------|--------|
| 5.732     | 0.816  | 34.005 | 52.992 |
| 6.713     | 0.210  | 64.148 | 25.741 |
| 7.544     | 0.146  | 10.303 | 2.874  |
| 7.718     | 0.204  | 10.423 | 4.069  |
| 8.401     | 0.111  | 27.510 | 5.633  |
| 8.555     | 0.154  | 17.360 | 5.105  |
| 8.691     | 0.136  | 13.061 | 3.385  |

HPLC trace of isolated product 45 (analytical RP-HPLC (II))

MALDI-MS spectrum of isolated product 45

mass$_{calc} = 5400.9$

mass$_{found} = 5402.2$
Au(I)/Ag(I)-promoted pyrazoline-containing spiroheterocycle synthesis
DNA conjugate 48: CPG-bound 16mer 7De-dAT7De8a-dGC-aldehyde conjugate 13 was reacted with pent-4-yn-1-ol 46 and tert-butyl 2-benzylhydrazine-carboxylate 47 according to RP-16.

HPLC trace of crude reaction mixture 48 (analytical RP-HPLC (I))

| Ret. Time | Width min | Height | Area   | Area % |
|-----------|-----------|--------|--------|--------|
| 6.691     | 0.191     | 132.812| 1518.079| 26.172 |
| 9.052     | 0.372     | 37.819 | 845.369 | 14.574 |
| 5.401     | 0.348     | 164.433| 3436.979| 59.254 |

HPLC trace of isolated product 48 (analytical RP-HPLC (II))

MALDI-MS spectrum of isolated product 48
mass_{calc} = 5489.0
mass_{found} = 5490.2
Au(I)/Ag(I)-promoted pyrazoline synthesis

DNA conjugate 51: CPG-bound 16mer 7De-dAT7De8a-dGC-alkyne conjugate 49 was reacted with isobutyraldehyde 50 and tert-butyl 2-benzylhydrazine-carboxylate 47 according to RP-17.

HPLC trace of crude reaction mixture 51 (analytical RP-HPLC (I))

HPLC trace of isolated product 51 (analytical RP-HPLC (II))

MALDI-MS spectrum of isolated product 51

mass\text{calc.} = 5443.0
mass\text{found} = 5443.8
**Au(I)/Ag(I)-promoted pyrazole synthesis**

**DNA conjugate 52:** CPG-bound 16mer 7De-dAT7De8a-dGC-alkyne conjugate 49 was reacted with benzaldehyde 34 and tert-butyl 2-benzylhydrazine-carboxylate 37 according to RP-18.

**HPLC trace of crude reaction mixture 52 (analytical RP-HPLC (I))**

*Peak list:*

| Ret. Time | Width (min) | Height | Area     | Area % |
|-----------|-------------|--------|----------|--------|
| 6.504     | 0.206       | 32.204 | 397.243  | 15.121 |
| 9.581     | 0.362       | 78.979 | 1713.328 | 65.218 |
| 10.507    | 0.432       | 19.921 | 816.818  | 19.661 |

**HPLC trace of isolated product 52 (analytical RP-HPLC (II))**

**MALDI-MS spectrum of isolated product 52**

mass\(_{calc.}\) = 5375.9  
mass\(_{found}\) = 5377.0
Boc cleavage in aqueous solution
DNA conjugate 53: CPG-bound 16mer 7De-dAT7De8a-dGC-Povarov conjugate 32 was Boc-deprotected according to RP-19.

HPLC trace of purified 16mer 7De-dAT7De8a-dGC-Povarov conjugate 32 (analytical RP-HPLC (II))

MALDI-MS spectrum of isolated product 53
mass\textsubscript{calc} = 5344.8
mass\textsubscript{found} = 5343.7
5210.8 -1G
5076.9 -2G
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