Electronic Supplementary Material (ESI) for Chemical Communications

Supporting information: Sequence specific macromolecule synthesis using DNA templated chemistry

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1. General methods

Reagents 4-(diphenylphosphino)benzoic acid, 5-(iodoacetamido) fluorescein and azide MegaStokes dye 673 were purchased from Sigma-Aldrich. Succinimidyl 4-formylbenzoate (SFB) and succinimidyl iodoacetate (SIA) were synthesized following literature procedures.[1,2] Remaining Wittig reagents were synthesized as described in Section 10. Streptavidin magnetic beads were purchased from New England Biolabs. All other reagents and solvents (reagent grade) were purchased from commercial suppliers. All amino acids used were of L-configuration. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX400 or a Bruker AV II 700 spectrometer in the solvents indicated at 298 K. Chemical shifts are reported on the δ scale in parts per million and are referenced to residual non-deuterated solvent resonances.

2. PAGE analysis

Denaturing gels were run using a two-layer discontinuous Tris-glycine buffer system with a 5% stacking gel (5% 19:1 acrylamide:bisacrylamide, 7 M urea, 25% formamide, 125 mM Tris, pH 6.8) and a 20% separating gel (20% 19:1 acrylamide:bisacrylamide, 7 M urea, 25% formamide, 400 mM Tris, pH 8.8). Samples (250 nM) were mixed 1:1 with loading buffer and heated to 95°C for 5 minutes before electrophoresis at room temperature and at 300 V in a running buffer containing 2.5 mM Tris, 0.19 M glycine. DNA was visualized using a BioRad Pharos FX Plus Molecular Imager (excitation and emission wavelengths are specified in the manuscript).

3. Mass spectrometry

Liquid chromatography-mass spectrometry (LC-MS) analysis of oligonucleotides was performed on one of two systems.

1) a Bruker UHR-Q-TOF MaXis mass spectrometer using either direct infusion with a syringe pump, with samples diluted in NH_4Ac (25mM)/2-propanol/water (2:1:1), flow rate 2 $\mu L min^{-1}$, or with a coupled Dionex UHPLC 3000RS liquid chromatography system with an Agilent C18RP, column (100×2.1 mm, 1.8 μm particle size), with a mobile phase gradient consisting of (water/ 10% 2-propanol/ NH_4Ac (25 mM)) and (MeCN/ 10% 2-propanol/ NH_4Ac (25mM), flow rate 0.1 ml/min. Analysis and deconvolution were carried out using Bruker software in maximum entropy mode.

2) a LCT Premier reflectron TOF mass spectrometer (Waters) coupled to an HP1050 LC with a Waters XBridge OST C18 column (4.6×50 mm, 2.5 μm particle size) at a flow rate of 0.5 mL/min using a gradient of buffers A and B: buffer A, 400 mM 1,1,1,3,3,3-hexafluoroisopropanol, 16.3 mM triethylamine, 5% methanol); buffer B, 400 mM 1,1,1,3,3,3-hexafluoroisopropanol, 16.3 mM triethylamine, 60 % methanol. The eluent was directly injected into the mass spectrometer, and the data acquired in the negative-ion mode (mass range 505-3500). Analysis and deconvolution were carried out using MassLynx V4.1 by Waters.

Low-resolution mass spectra of small molecules were recorded on a Bruker Esquire2000 electrospray mass spectrometer. High resolution mass spectra were recorded by on a Bruker Electrospray Ultra-High Resolution tandem TOF mass spectrometer.

4. HPLC purification and analysis

HPLC analyses were performed on a Varian 920-LC™ integrated liquid chromatography system or on an Agilent 1200™ system equipped with diode array and fluorescence detectors. Chromatography was performed on a Waters XBridge™ OST C18 2.5 μm 4.6 x 50 mm column heated to 40 ^{o}C. Flow rate was set at 1 mL/min with a linear gradient of the following buffers: Buffer C, 0.1 M triethylammonium acetate, 5% acetonitrile, pH 7.0; buffer D, 0.1 M

triethylammonium acetate, 70% acetonitrile, pH 7.0. Fractions collected were combined and concentrated using a Eppendorf Concentrator Plus or a DNA120 Savant SpeedVac Concentrator. Oligonucleotides were quantified by measuring UV absorbance at 260 nm (A_{260}) using a Perkin Elmer Lambda 35 UV/Vis spectrometer or a Cary 50 Probe UV-Vis Spectrophotometer equipped with a Hellman TrayCell adapter.

5. Oligonucleotide sequences

The following oligonucleotides were purchased from Integrated DNA Technologies (IDT). Amino-modified oligonucleotides were purchased HPLC-purified.

Strand	Sequence	ε_{260} (L* mol^{-1}cm^{-1})
S1	5′/5AmMC6/ **AGG GAT TGT CTT AGT GTG CGA ATA GGT AAC** 3′	303,700
S2	5′**CTG GTA TGA A**CG CAC ACT AAG ACA ATC CCT /3AmMO/3′	290,400
S3	5′/5AmMC6/ **AGG GAT TGT CTT AGT GTG CGG TTA ACA TAT** 3′	297,700
S4	5′**T TCA TCT TTT ATA CCT GAG C**CG CAC ACT AAG ACA ATC CCT /3AmMO/3′	372,000
C1	5′GTT ACC TAT TCG CAC ACT AAG ACA ATC CCT 3′	283,700
C2	5′**AGG GAT TGT CTT AGT GTG CGT TCA TAC CAG** 3′	291,600
C3	5′ATA TGT TAA CCG CAC ACT AAG ACA ATC CCT 3′	293,900
C4	5′**AGG GAT TGT CTT AGT GTG CGG** CTC AGG TAT AAA AGA TGA A /3Biotin/3′	408,200

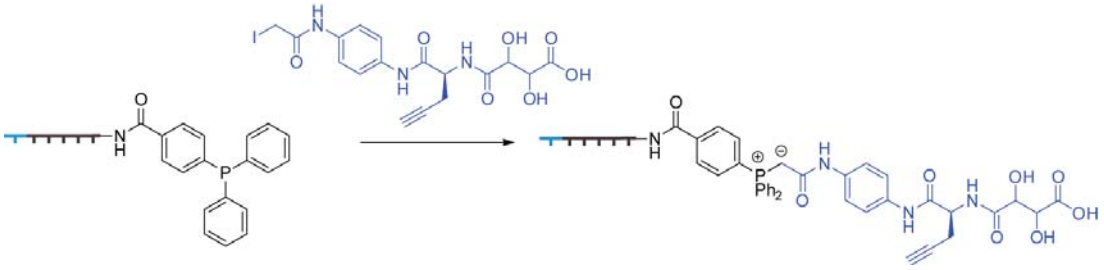
6. Synthesis of DNA adapters

The synthesis of S1-FAM, S2-ALA, S3-PHE and S4-BAL has previously been described.[3] To prevent decomposition of adapters (S2-ALA, S3-PHE and S3-ALK) before use the ylide and aldehyde functional groups were preserved as a phosphonium salt and a diol masked aldehyde respectively, until just before reaction. The precursor forms of the adapters are called S2-ALA-OH, S3-PHE-OH and S3-ALK-OH respectively.

6.1. General procedure for synthesis of triphenylphosphino modified oligonucleotides.

Diphenylphosphine benzoic acid in DMF (300 mM, 200 µL), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in DMF (300 mM, 200 µL), *N*-hydroxybenzotriazole in DMF (300 mM, 200 µL) and *N*,*N*-diisopropylethylamine (5.5 µL, 60 µmol) were stirred together under nitrogen atmosphere for 1h to give the in-situ activated ester. Amino modified oligonucleotides **S1**, **S2** or **S3** in water (200 µM, 100 µL, 20 nmol) were diluted with PBS buffer (100 mM sodium phosphate, pH 7.5, 100 mM NaCl). To this was added the in-situ activated ester (200 µL, 100 µM, 20 µmol) and the reaction stirred under argon atmosphere for 18 h, followed by removal of excess small molecules using an Illustra NAP-5 column (GE Healthcare), eluting with PBS (100 mM, pH 7.5, 100 NaCl), to give the triphenylphosphinemodified oligonucleotides **S1-TPP, S2-TPP,** and **S3-TPP** which were used without further purification.

6.2. Synthesis of S3-ALK-OH



Amine **4** (see Section 9.5) in THF (50 µL, 100 mM) was mixed with *N*-succinimidyl iodoacetate [2] (50 µL, 100 mM) at 0 ˚C for 1 h to form the in-situ iodo-compound (shown above). After an aliquot was taken for mass spec analysis [**4**-iodo: **(LR-ESI)**: 501.8 $[M-H]^-$; **(HR-ESI)**: $[M-H]^-$ 502.0117 (Calcd), 502.0117 (Found)] the solution was combined with triphenylphosphino modified oligonucleotide **S3-TPP** in PBS (800 µL, 20nmol) After 3 h incubation under argon atmosphere the samples were concentrated *in vacuo* and excess small molecules were removed using an Illustra NAP-5 column, eluting with water. The samples were then purified by HPLC. Collected fractions were combined to give and concentrated to give **S3-ALK-OH** (calculated: 10148.8). This was quantified by measuring UV absorbance at 260 nm (using extinction coefficients from Section 5).

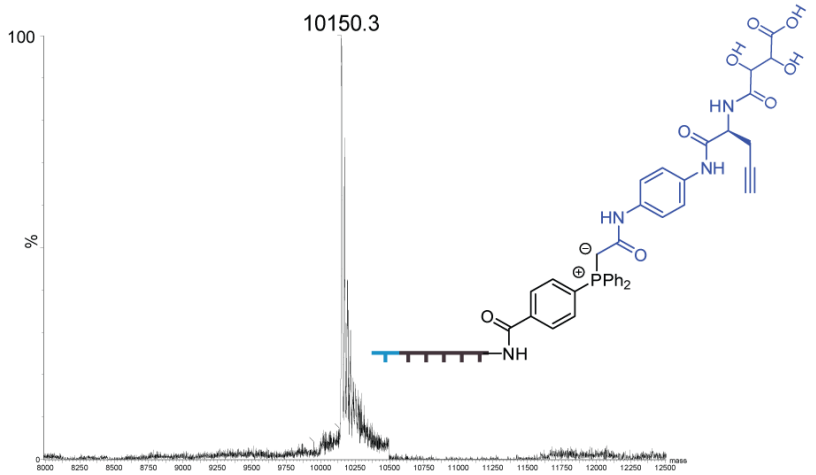
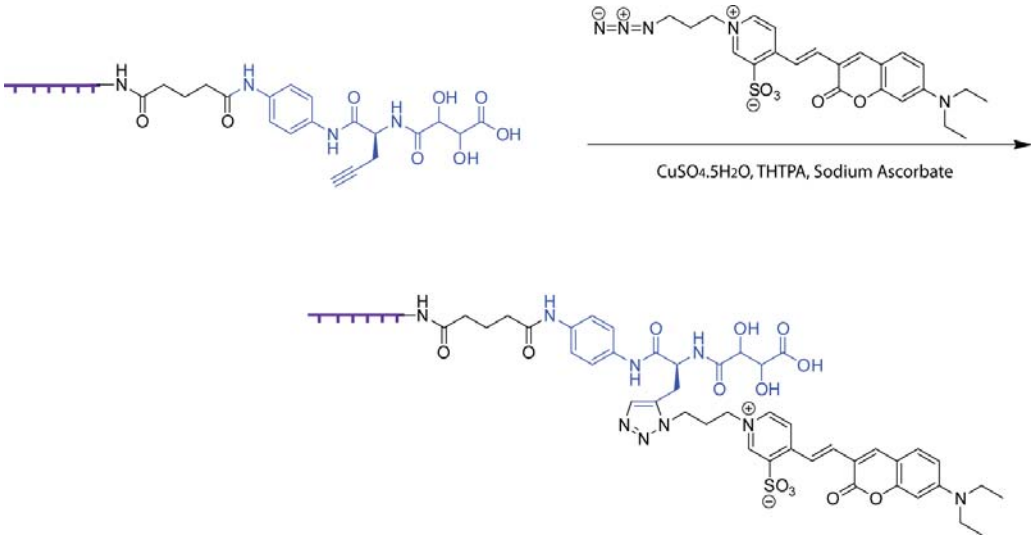


Figure S 1. Mass spec of S3-ALK-OH

7. Derivatization of Alkyne side chain functionality

7.1. General method of CuAAC derviatization



$CuSO_4.5H_2O$ (20 mMM, 2.5 µL in water) was mixed with THPTA (20mM, 12.5 µL in water). To this was added a solution of sodium ascorbate (50 mM, 50 µL in water), followed by addition of phosphate buffer (pH 7.5, 100 mM, 1 M NaCl, 285 µL), DNA-ALK (100 µM, 50 µL in water) and azide MegaStokes dye 673 (Molecular weight: 483.54, 10 mM, 100 µL in water). The reaction was shaken and left at room temperature for 2 h before being buffer exchanged by NAP-5 column into water, reduced in volume *in vacuo*. The product was then characterized by mass spectroscopy (see below).

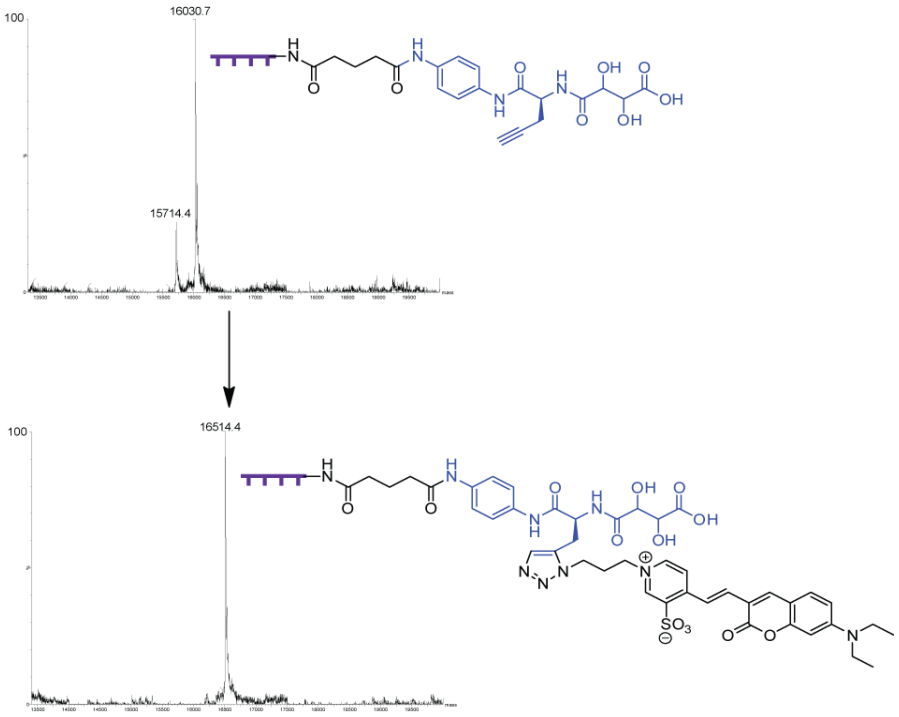


Figure S 2. Mass spec of DNA-ALK before (above) and after (below) click derivatization with Azide MegaStokes.

*DNA sequence = 5'-AGG GAT TGT CTT AGT GTG CGG CTC AGG TAT GCC ATT TCT CGG ACT TCA TT-3'

7.2. Deriviatization before Wittig transfer reaction

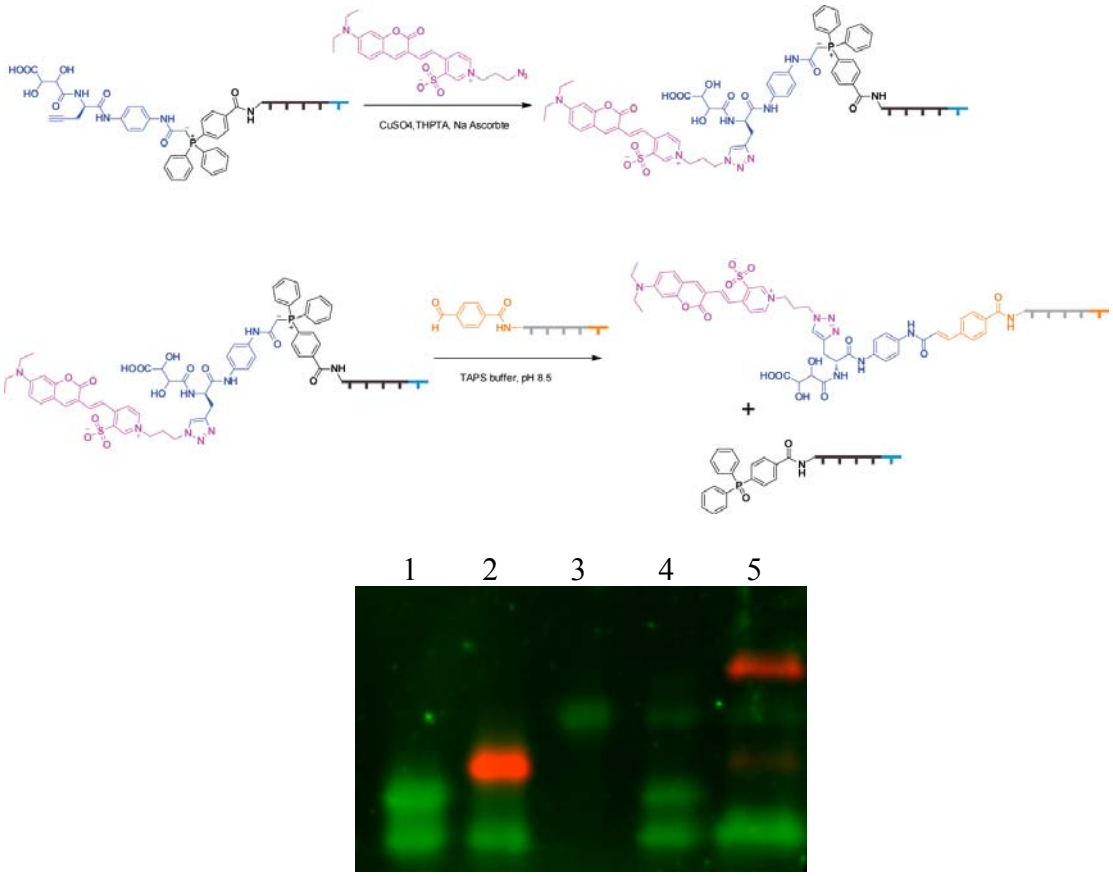


Figure S 3. Fluorescent azide MEGA stokes dye was attached *via* click chemistry directly to the S3-ALK-OH adapter. 20% denaturing gel scanned at Ex488/Em530 (SybrGold stain, green) and Ex532/Em695 (MEGA label, red). Lane 1: S3-ALK-OH, Lane 2: S3-ALK-MEGA, Lane 3: S4-BAL, Lane 4: S4-BAL+S3-ALK, Lane 5: S4-BAL+S3-ALK-(OH)-MEGA. The fluorescent band (red) in lane 5 was observed corresponding to the product S4-BAL-ALK-(OH)-MEGA. *Note: the gel procedure sometimes causes partial oxidation of the DNA-ylides (such as S3-ALK).

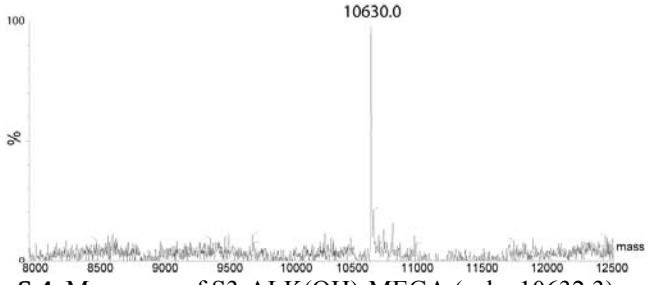


Figure S 4. Mass spec of S3-ALK(OH)-MEGA (calc. 10632.3).

7.3. Deriviatization after Wittig transfer reaction

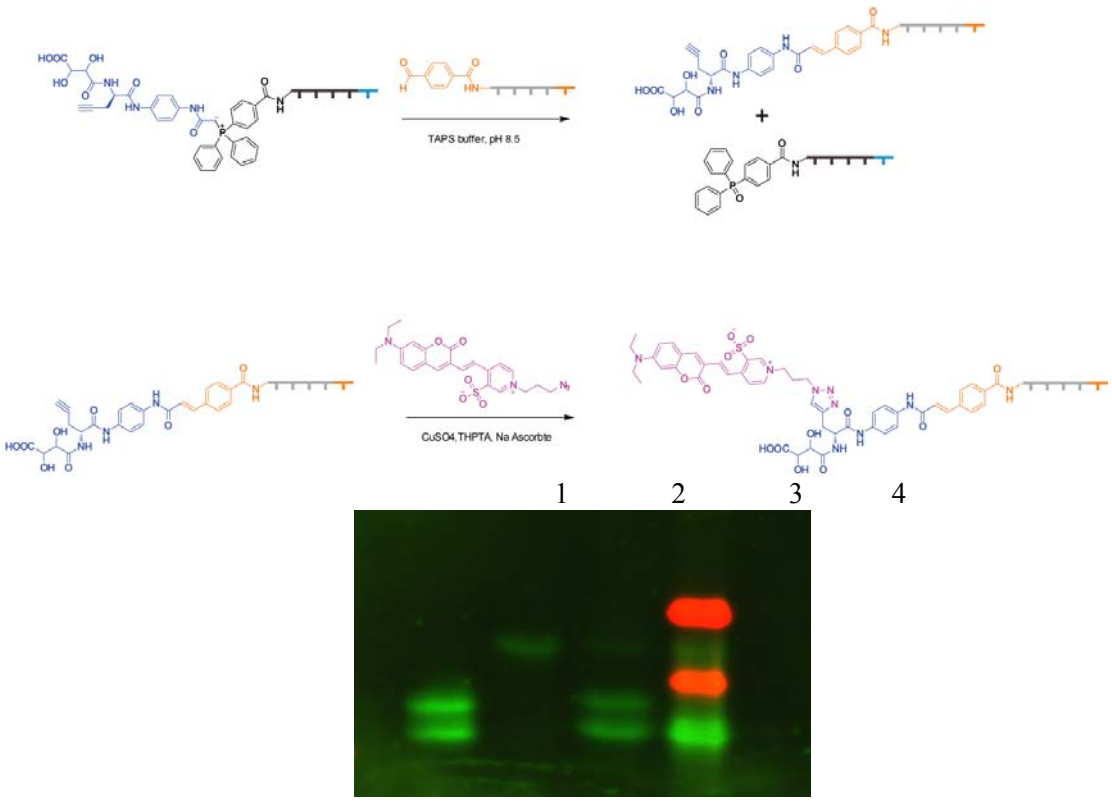
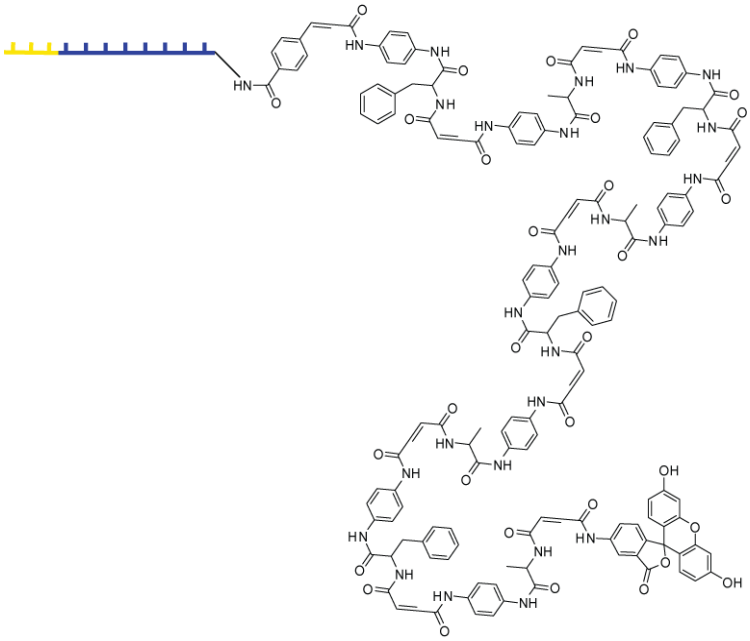


Figure S 5 Fluorescent azide MEGA stokes dye was attached *via* click chemistry after olefin product formation. 20% denaturing gel scanned at Ex488/Em530 (SybrGold stain, green) and Ex532/Em695 (MEGA label, red) Lane 1: S3-ALK, Lane 2: S4-BAL, Lane 3: S4-BAL+S3-ALK, Lane 4: S4-BAL+S3-ALK + MEGA azide under click conditions. The higher fluorescent band (red) was observed corresponding to the product S4-BAL-ALK-(OH)-MEGA, and the lower red band corresponds to S3-ALK(OH)-MEGA from untransferred S3-ALK. *Note: the gel procedure sometimes causes partial oxidation of the DNA-ylides (such as S3-ALK).

8. DNA-templated sequential Wittig transfer reactions

In the following reactions buffer exchange and desalting were carried out by passing the samples through an Illustra NAP-5 or NAP-10 column pre-equilibrated with water or the indicated buffers. TAPS buffer concentrated solution refers to 300 mM TAPS (N-Tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid), 3M NaCl, pH 8.5.

8.1. Synthesis of Decamer 1, S4-BAL-(PHE-ALA-)$_4$-FAM



Step 1. To **S2-ALA-OH** in water (400 µL, 11.4 nmol) was added $NaIO_4$ (400 µL, 50 mM) and sodium acetate buffer (100 mM, pH 3.5) and left to react for 30 min at room temperature. The products were buffer exchanged by NAP-10 column into water and reduced in volume *in vacuo* to give **S2-ALA**. The concentration of the adapter was calculated by UV absorbance. An aliquot of this solution (1.00 nmol) was mixed with **S1-FAM** in water (1.00 nmol) and sufficient TAPS buffer concentrated solution to bring the overall solution to TAPS (50 mM, 500 mM NaCl, pH 8.5). The solution was briefly shaken, then centrifuged and left to hybridize and react over 30 min. Then remover strand **C1** was added in water (1.00 nmol) and the solution was briefly shaken, then centrifuged and left to hybridize for 30 min to give the product of **step 1**. At this stage an aliquot was taken for PAGE analysis (0.25 % of overall = 2.5 pmol).

Step 2. **S3-PHE-OH** in water (190 µL, 9.9 nmol) was mixed with $NaIO_4$ (190 µL, 50 mM, 100 mM sodium acetate buffer, pH 3.5 for 30 min at room temperature, before being buffer exchanged by NAP-10 column into water, reduced in volume *in vacuo* to give **S3-PHE**. The concentration of the adapter was calculated by UV absorbance. Equimolar **S3-PHE** was then combined with **step 1** and sufficient TAPS buffer concentrated solution to bring the overall solution to TAPS (50 mM, 500 mM NaCl, pH 8.5). The solution was briefly shaken, then centrifuged and left to hybridize and react over 30 min. Then an equimolar amount of remover strand **C2** was added in water and the solution was briefly shaken, then centrifuged and left to hybridize 30 min to give the product of **step 2**. An aliquot was taken for PAGE analysis (0.25 % of overall = 2.5 pmol).

Step 3. Equimolar **S2-ALA** combined with **step 2** and sufficient TAPS buffer concentrated solution to bring the overall solution to TAPS (50 mM, 500 mM NaCl, pH 8.5). The solution was briefly shaken, then centrifuged and left to hybridize and react over 30 min. Then an equimolar amount of remover strand **C2** was added in water and the solution was briefly shaken, then centrifuged and left to hybridize 30 min to give the product of **step 3**. An aliquot was taken for PAGE analysis (0.25 % of overall = 2.5 pmol).

Step 4. Equimolar **S3-PHE** combined with **step 3** and sufficient TAPS buffer concentrated solution to bring the overall solution to TAPS (50 mM, 500 mM NaCl, pH 8.5). The solution was briefly shaken, then centrifuged and left to hybridize and react over 30 min. Then an equimolar amount of remover strand **C3** was added in water and the solution was briefly shaken, then centrifuged and left to hybridize 30 min to give the product of **step 4**. An aliquot was taken for PAGE analysis (0.25 % of overall = 2.5 pmol).

Step 5 and Step 7. Identical to Step 3, except the quantities are reduced after taking into account the removal of PAGE aliquots.

Step 6 and Step 8. Identical to Step 4, except the quantities are reduced after taking into account the removal of PAGE aliquots.

Step 9. Equimolar **S4-BAL** was added to **step 8** and sufficient TAPS buffer concentrated solution to bring the overall solution to TAPS (50 mM, 500 mM NaCl, pH 8.5). The solution was briefly shaken, then centrifuged and left to hybridize and react over 30 min. Then an equimolar amount of remover strand **C3** was added in water and the solution was briefly shaken, then centrifuged and left to hybridize 30 min to give the product of **step 9**. An aliquot was taken for PAGE analysis (0.25 % of overall = 2.5 pmol). Then an equimolar amount of Biotinylated strand **C4** was added in water, followed by capture using streptavidin-coated magnetic beads. Analysis of the product by UV absorbance gave a recovery yield of 0.42 nmol. An aliquot was taken for PAGE analysis (0.25 % of overall = 2.5 pmol) which gave 73% purity for an overall product yield of 31%.

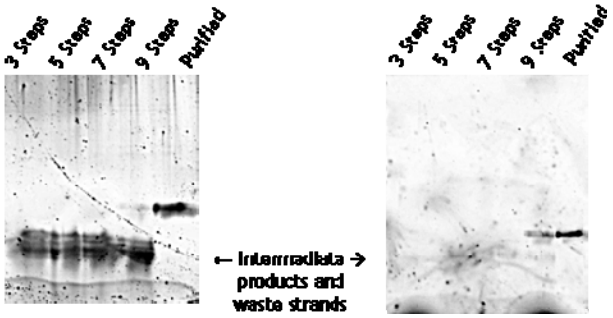


Figure S6. 20% denaturing PAGE analysis of products of the multi-step synthesis of the DNA-macromolecule product 1. Left: Visualised with SYBR gold staining. Right: Fluorescence scan using Ex488/Em530 nm wavelengths. Densitometric analysis of the purified product lane (corrected for background) gave a ratio of 0.73:0.27 for the desired product relative to a faster migrating band (tentatively identified as unreacted S4-BAL).

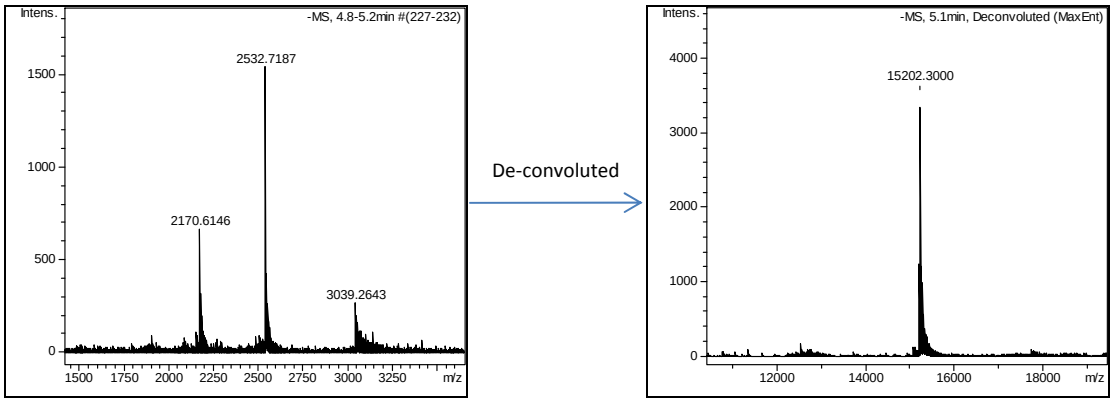
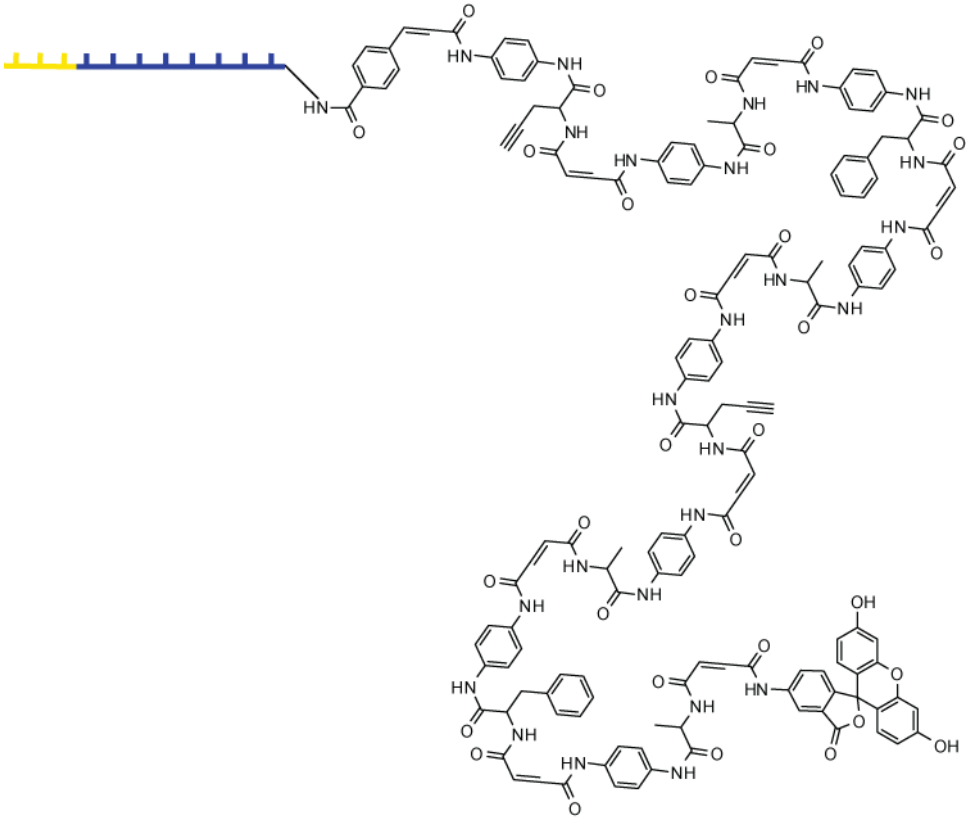


Figure S7. Mass spectrometry analysis of macromolecule product 1.

8.2. Synthesis of Decamer 2, S4-BAL-(ALK-ALA-PHE-ALA-)$_2$FAM



Step 1. **S2-ALA** (from the batch described above for decamer **1**) (1.00 nmol) was mixed with **S1-FAM** in water (1.00 nmol) and sufficient TAPS buffer concentrated solution to bring the overall solution to TAPS (50 mM, 500 mM NaCl, pH 8.5). The solution was briefly shaken, then centrifuged and left to hybridize and react over 30 min. Then remover strand **C1** was added in water (1.00 nmol) and the solution was briefly shaken, then centrifuged and left to hybridize 30 min to give the product of **step 1**. At this stage an aliquot was taken for PAGE analysis (0.25 % of overall = 2.5 pmol).

Steps 2 and 3. These steps were carried out identically to those described in the synthesis of decamer **X**.

Step 4. **S3-ALK-OH** in water (190 µL, 7.1 nmol) was added $NaIO_4$ (190 µL, 50 mM, 100 mM sodium acetate buffer, pH 3.5) for 30 min at room temperature, before being buffer exchanged by NAP-10 column into water, reduced in volume under vacuum to give **S3-ALK**. The concentration of the adapter was calculated by UV absorbance. An equimolar aliquot of this solution was mixed with **step 3** and sufficient TAPS buffer concentrated solution to bring the overall solution to TAPS (50 mM, 500 mM NaCl, pH 8.5). The solution was briefly shaken, then centrifuged and left to hybridize and react over 30 min. Then an equimolar amount of remover strand **C2** was added in water and the solution was briefly shaken, then centrifuged and left to hybridize 30 min to give the product of **step 4**. At this stage an aliquot was taken for PAGE analysis (0.25 % of overall = 2.5 pmol).

Steps 5, 6, and 7. These steps were carried out identically to those described in the synthesis of decamer **1**.

Step 8. Identical to Step 4, except the quantities are reduced after taking into account the removed PAGE aliquots.

Step 9. This step was carried out identically to those described in the synthesis of decamer **1**. Analysis of the product by UV absorbance gave a recovery yield of 0.36 nmol. An aliquot was taken for PAGE analysis (2.5 pmol) which gave 63% purity for an overall product yield of 23%.

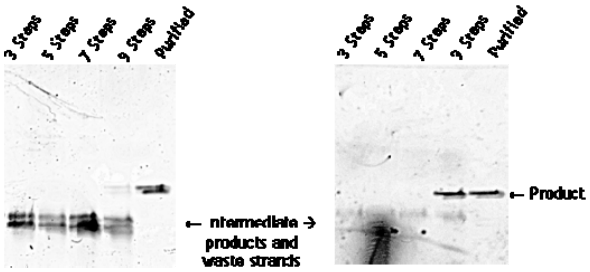


Figure S8. 20% denaturing PAGE analysis of products of the multi-step synthesis of DNA-macromolecule product 2. Left: Visualised with SYBR gold staining. Right: Fluorescence scan using Ex488/Em530 nm wavelengths. Densitometric analysis of the purified product lane (corrected for background) gave a ratio of 0.63:0.37 for the desired product relative to a faster migrating band (tentatively identified as unreacted S4-Bal).

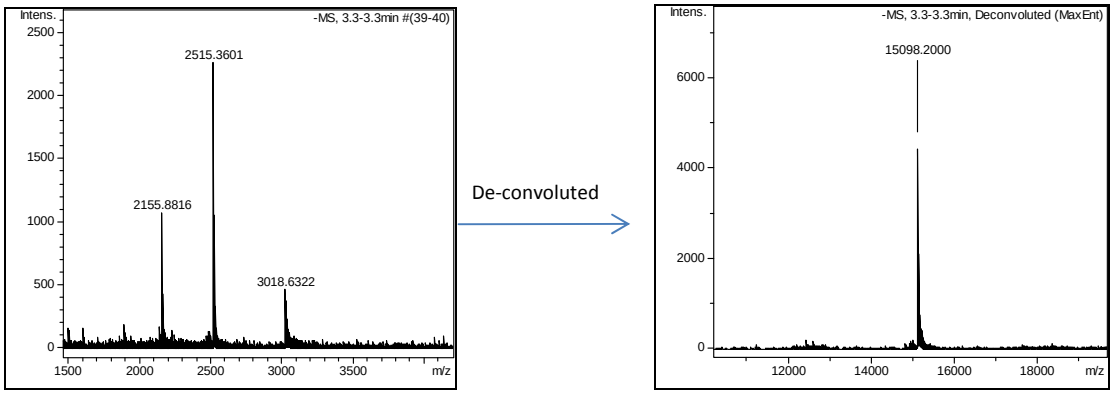
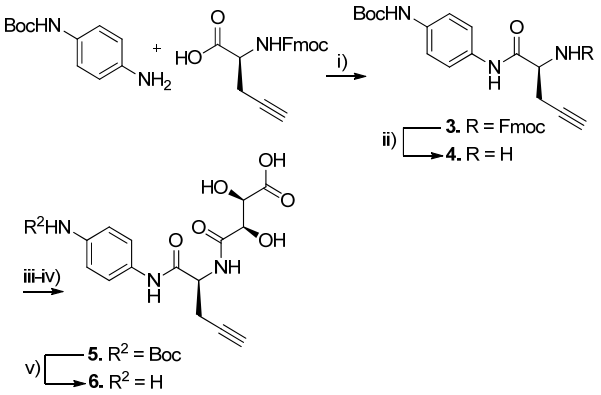


Figure S9. Mass spectrometry analysis of macromolecule product 2.

9. Synthesis of small molecule compounds

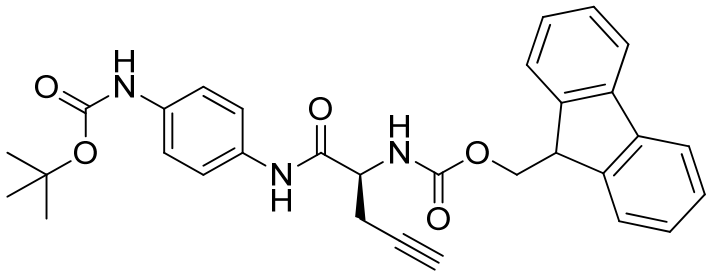
Synthesis of small molecules used in the synthesis of **ALA** and **PHE** has been previously described.[3]

9.1. Synthesis of ALK.



i) HBTU, DMF, 96%; ii) Cyclohexylamine, CH_2Cl_2, DMF, 83%; iii) (+)-*O,O*-Diacetyl-L-tartaric anhydride, CH_2Cl_2, 0 ˚C; iv) NaOH, MeOH, 92% over 2 steps; v) TFA, CH_2Cl_2, 54%.

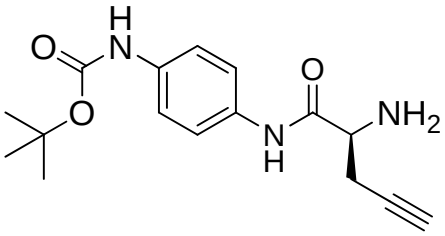
9.2. Compound 3.



3

Fmoc-Pra-OH (1000 mg, 2.982 mmol), HBTU (1130 mg, 2.982 mmol) and *N*-Boc-*p*-phenylenediamine (621 mg, 2.982 mmol) dissolved in DMF (15 mL). To the reaction was added DIPEA (571 µL, 3.280 mmol) and the reaction stirred for 24 h. The reaction was diluted with ethyl acetate (100 mL) and water (100 mL). The organic layer was removed and the aqueous layer washed with ethyl acetate (2 x 50 mL). The combined organic layers washed with water (2 x 50 mL), 1 M HCl (50 mL) and saturated $NaHCO_3$ (50 mL), then dried with $MgSO_4$, filtered and concentrated *in vacuo* to give a grey solid, 1510 mg (96%): **^{1}H NMR** (d_6 -DMSO, 400 MHz): 9.29 (s, 1H, 1 x ArNH), 7.90 (d, 2H, 2 x ArH, J = 8 Hz), 7.83 (d, 1H, 1 x ArNH, J = 8 Hz), 7.76 (d, 2H, 2 x ArH, J = 8 Hz), 7.52 (d, 2H, 2 x ArH, J = 8 Hz), 7.46 – 7.30 (m, 7H, 6 x ArH, 1 x NH), 4.38-4.21 (m, 4H, 1 x CH_2, 2 x CH), 2.91 (s, 1H, 1 x CCH), 2.70-2.52 (m, 2H, 1 x CH_2), 1.47 (s, 9H, 1 x C(CH_3)$_3$); **^{13}C NMR** (d_6 -DMSO, 175 MHz): 169.0 (C), 156.3 (C), 153.3 (C), 144.2 (C), 141.1 (C), 135.7 (C), 133.7 (C), 128.1 (CH), 127.6 (CH), 125.9 (CH), 125.8 (CH), 120.5 (CH), 120.3 (CH), 81.1 (C), 79.3 (C), 73.5 (C), 66.3 (CH_2), 54.8 (CH), 47.1 (CH), 28.6 (CH_3), 22.4 (CH_3); **(LR-ESI)**: 548.2 $[M+Na]^+$; **(HR-ESI)**: $[M+Na]^+$ 548.2156 (Calcd), 548.2149 (Found).

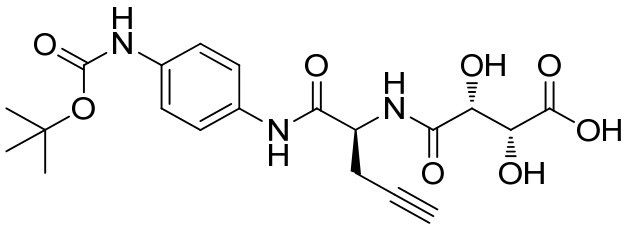
9.3. Compound 4.



4

Compound **3** (1100 mg, 2.094 mmol) dissolved in DCM (15 mL) and DMF (15 mL). To this was added cyclohexylamine (15 mL) and the reaction stirred under nitrogen atmosphere for 3 h. Solvents removed *in vacuo*. Purification achieved by flash chromatography (silica; ethyl acetate/methanol (100:0 → 80:20)) to give a yellow oil, 487 mg (83%): **^{1}H NMR** (CD_3OD, 400 MHz): 7.37 (d, 2H, 2x ArH, J = 9 Hz), 7.25 (d, 2H, 2 x ArH, J = 9 Hz), 3.49 (t, 1H, 1 x CH, J = 6 Hz), 2.58-2.45 (m, 2H, 2 x CH_2,), 2.30 (t, 1H, 1 x CCH, J = 3 Hz), 1.42 (s, 9H, 3 x CH_3); **^{13}C NMR** (d_6 -DMSO, 100 MHz): 173.8 (C), 155.4 (C), 137.2 (C), 134.2 (C), 122.0 (CH), 120.2 (CH), 80.7 (C), 80.3 (C), 72.6 (C), 55.5 (CH), 28.8 (CH_3), 26.0 (CH_3); **(LR-ESI)**: 304.1 $[M+H]^+$; **(HR-ESI)**: $[M+H]^+$ 304.1656 (Calcd), 304.1653 (Found).

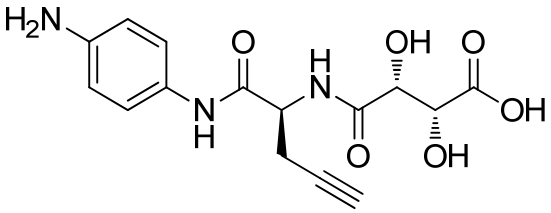
9.4. Compound 5.



5

Compound **4** (500 mg, 1.649 mmol) dissolved in DCM (50 mL) and cooled to 0 ˚C. To this was added a solution of (+)-O,O-diacetyl-L-tartaric anhydride (374 mg, 1.732 mmol) in DCM (30 mL) dropwise over 1 h and the reaction left to stir under nitrogen atmosphere whilst warming to room temperature for a further 18 h. DCM removed *in vacuo* and the residue redissolved in NaOH in methanol (0.1 M, 50 mL) and the reaction stirred for 3 h. Solvents removed *in vacuo* and the residue diluted with ethyl acetate and water. Solution acidified with the addition of HCl (0.1 M) and the organic layer extracted with ethyl acetate (3 x 50 mL). Combined organic layers washed with brine (50 mL) and concentrated *in vacuo* to give a yellow oil, 409 mg (92%): **^{1}H NMR** (CD_3OD, 400 MHz): 7.38 (d, 2H, 2 x ArH, J = 9 Hz), 7.24 (d, 2H, 2 x ArH, J = 9 Hz), 4.61 (t, 1H, 1 x $CHCH_2$), J = 6 Hz), 4.45 (s, 2H, 2 x CHOH), 2.75 (dd, 2H, 2 x $CHCH_2CCH$, J = 6 Hz, 3 Hz), 2.34 (t, 1H, 1 x CCH, J = 3 Hz), 1.40 (s, 9H, 3 x CH_3); **(LR-ESI)**: 434.0 $[M-H]^-$; **(HR-ESI)**: $[M-H]^-$ 434.1569 (Calcd), 434.1565 (Found).

9.5. Compound 6.



5

Compound **5** (409 mg, 0.940 mmol) dissolved in DCM (16 mL), to this was added TFA (4 mL). The reaction left to stir under nitrogen atmosphere for 3 h, and then concentrated under vacuum. Residual acid removed by azeotropic distillation with toluene (twice) and residue dried *in vacuo* to give a glassy brown oil. This was dissolved in a small quantity of MeOH and precipated with the addition of EtOAc dropwise. The precipitate was collect and dried to give a grey solid. 170 mg (54%): **^{1}H NMR** (CD_3OD, 400 MHz): 7.46 (d, 2H, 2 x ArH, J = 9 Hz), 6.94 (d, 2H, 2 x ArH, J = 9 Hz), 4.61 (t, 1H, 1 x CHCH$_2$, J = 6 Hz), 2.74 (dd, 2H, 2 x CHCH$_2$CCH, J = 6 Hz, 3 Hz), 2.34 (t, 1H, 1 x CCH, J = 3 Hz); **(LR-ESI)**: 358.1 $[M+Na]^+$, 334.0 $[M-H]^-$; **(HR-ESI)**: $[M+H]^+$ 336.1190 (Calcd), 336.1195 (Found).

10. References

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(2) Morimoto, H.; Saljoughian, M.; Than, C.; Williams, P. G. *US Patent 6160128.*
(3) McKee, M. L.; Milnes, P. J.; Bath, J.; Stulz, E.; Turberfield, A. J.; O'Reilly, R. K. *Angew. Chem., Int. Ed.* 2010, 49, 7948–7951.