Supporting Information

Site-specific incorporation of diamondoids on DNA using click chemistry

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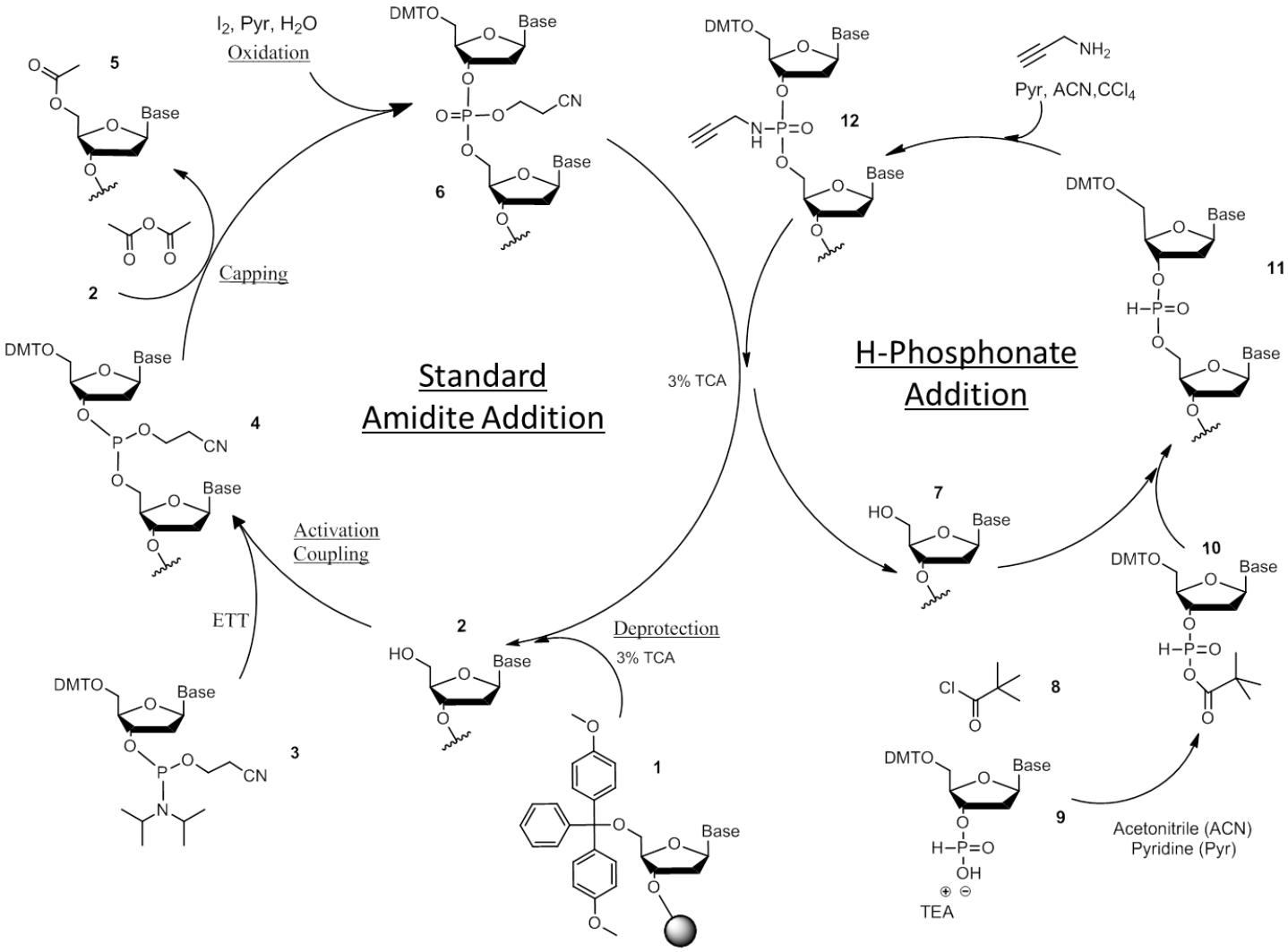
DNA synthesis scheme:

TCA = Trichloroacetic acid

ETT = 5-Ethylthio-1H-tetrazole

TEA = Triethylamine

DMT = Dimethoxy trityl



= Controlled Pore Glass (CPG) solid support

Electronic Supplementary Material (ESI) for Chemical Communications

Phosphoramidite and H-phosphonate synthesis

All DNA sequences were performed on a BioAutomation MerMade synthesizer and the default protocol, which uses standard procedure, were used. The final products were cleaved and deprotected from the CPG using an AMA solution described on page 4. Phosphoramidites and H-phosphonate monomers were purchased from Glen Research:

Base A: 5'-Dimethoxytrityl-N-benzoyl-3'-deoxyAdenosine,2'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

Base T: 5'- Dimethoxytrityl-3'-deoxyThymidine,2'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

Base G: 5'-Dimethoxytrityl-N-dimethylformamidine-3'-deoxyGuanosine,2'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

Base C: 5'-Dimethoxytrityl-N-acetyl-2'-deoxyCytidine,3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite

H-Phosphonate G: 5'-Dimethoxytrityl-N-isobutyryl-2'-deoxyGuanosine,3'-H-phosphonate, TEA salt

The following reagents were prepared immediately before DNA synthesis:

1. H-Phosphonate dG: 52 mg in 1.5 mL of 1:1 ACN:Pyr (0.04 M solution).
2. Pivaloyl chloride *(Piv-Cl)*: 90 μL in 3.0 mL of 1:1 ACN:Pyr (0.24 M solution).
3. Propargyl amine: 48 μL in 3.0mL of 1:1:1 ACN : Pyr : CCl_4 (0.25 M solution)

The following procedures for H-Phosphonate Addition/Oxidation were followed (step 1→7)

1. ***Deblock***: To a dimethoxyl trityl (DMT) protected 1 μmol CPG column, 2 x 200 μL of 3% TCA in DCM solution was injected with 6 x 3.3 sec pulsed incubations to yield a total incubation time of 20 sec.

2. ***Acetonitrile wash***: To the deprotected column, 3 x 250 μL of acetonitrile was injected with a 1 x 1 sec pulsed incubation followed by a 55 x drain pulse to completely remove all acetonitrile from the reaction column.

3. ***Coupling***: To the deprotected column, 2 x 100 μL of H-Phosphonate reagent (0.04 M, 4 μmol) and 2 x 100 μL of Piv-Cl reagent (0.24 M, 24 μmol) were added as a staggered injection with 8 x 12.5 sec pulsed incubations to yield a total incubation time of 100 sec per injection.

4. ***Acetonitrile Wash***: To the column, 3 x 250 μL of acetonitrile was injected with a 1 x 1 sec pulsed incubation followed by a 55 x drain pulse to completely remove all acetonitrile from the reaction column.

5. ***Propargyl addition***: To the column, 5 x 200 μL of propargyl amine (0.25 M, 500 μmol) was injected with a 10 x 24 sec incubation to yield a total incubation time of 240 sec per injection.

6. ***Capping***: To the column, 1 x 125 μL of cap mix A (THF / 2,6-lutidine / acetic anhydride [8:1:1]) and 1 x 125 μL of cap mix B (16 % 1-methyl imidazole in THF) were added as a staggered injection with 9 x 5.5 sec pulsed incubations to yield a total incubation time of 50 sec per injection.

7. ***Acetonitrile Wash***: To the column, 3 x 250 μL of acetonitrile was injected with a 1 x 1 sec pulsed incubation followed by a 55 x drain pulse to completely remove all acetonitrile from the reaction column.

Preparation of Copper, Adamantane, and AMA solution

Dilution of Adamantane Sources : All adamantane solutions were diluted to a 0.1M concentration using THF. The dilutions were stored in a -78°C freezer between experiments.

Dilution of Copper Sources: Tetrakis(acetonitrile)copper(I)hexafluorophosphate (10 mg) was diluted in anhydrous acetonitrile (268 μL) to yield a 0.1 M solution immediately before each experiment. Copper Sulfate Pentahydrate (10 mg) was diluted in H_2O (400 μL) to yield a 0.1 M solution. Copper bromide dimethyl sulfide, copper bromide, copper cyanide, and copper chloride were all diluted to a 0.1 M solution immediately before each experiment.

Preparation of AMA solution : Ammonium hydroxide and 40% aqueous methylamine were mixed in a 1:1 ratio. http://www.glenresearch.com/GlenReports/GR7-12.html

EMSA Assays

To evenly distributed portions of CPG (~30 nmol) in eppendorf tubes, copper (100 equiv) and adamantane (10 equiv) solutions were added and allowed to react for variable times. The CPG were washed with 2 x 1 mL of DMSO, 1 x 1 mL H_2O, and 1 x 1 mL ACN. The CPG were cleaved with AMA solution (1.0 mL) @65^{o}C for 15 minutes. The supernatant was collected and dried on a GeneVac. The resulting pellet was dissolved in loading buffer, loaded onto a 20% denaturing PAGE gel, and run at 250V for 4 hours. The resulting bands were visualized under a handheld UV lamp.

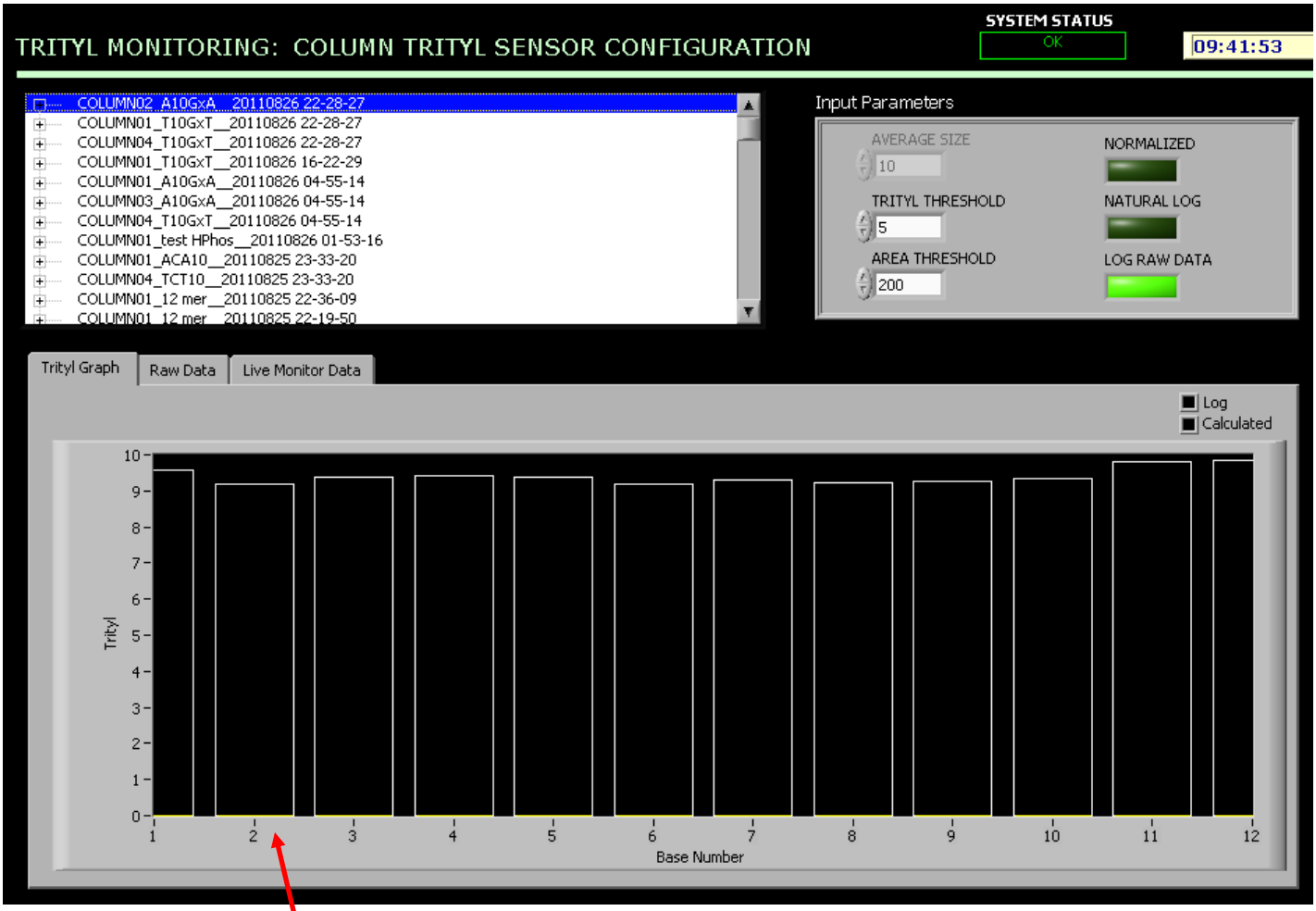
Preparation of MALDI Matrix and DNA sample for MALDI analysis

Zip Tip Desalt : Each DNA sample was desalted using a Harvard C-18 Zip-Tip (Cat # 74-3403)

Preparation of 2',4',6'-Trihydroxyacetophenone monohydrate (THAP) Matrix : In an eppendorf tube, 22mg of Ammonium Citrate Dibasic (AC) was dissolved in 1mL of 1:1 ACN:H_2O. THAP matrix (10 mg) was stirred vigorously with 100uL of the AC solution to prepare a saturated THAP solution. The insoluble particulates were spun down and the supernatant was collected for use as MALDI matrix.

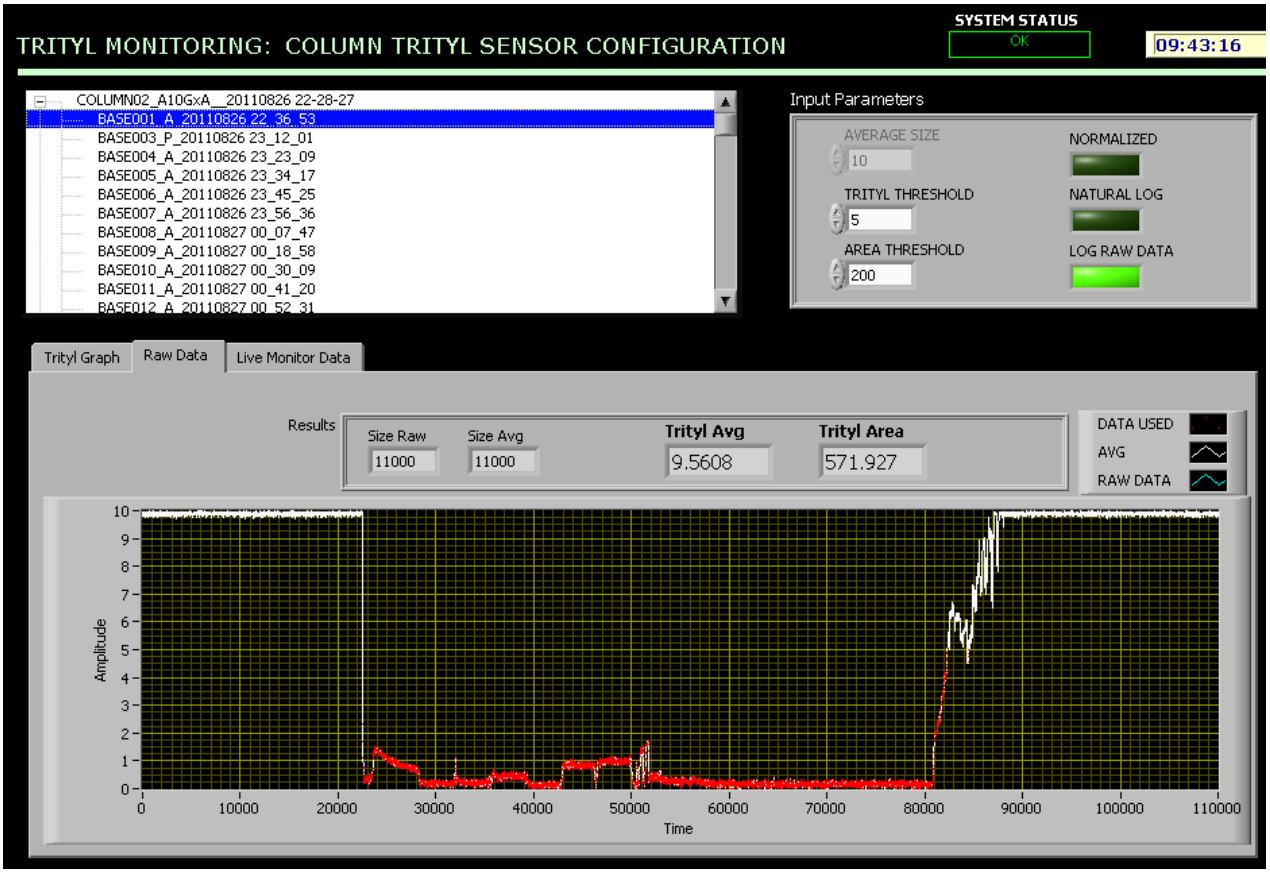
Trityl Graphs

Trityl graphs for the synthesis of AAA AAA AAA AG**x**A x=propargyl.

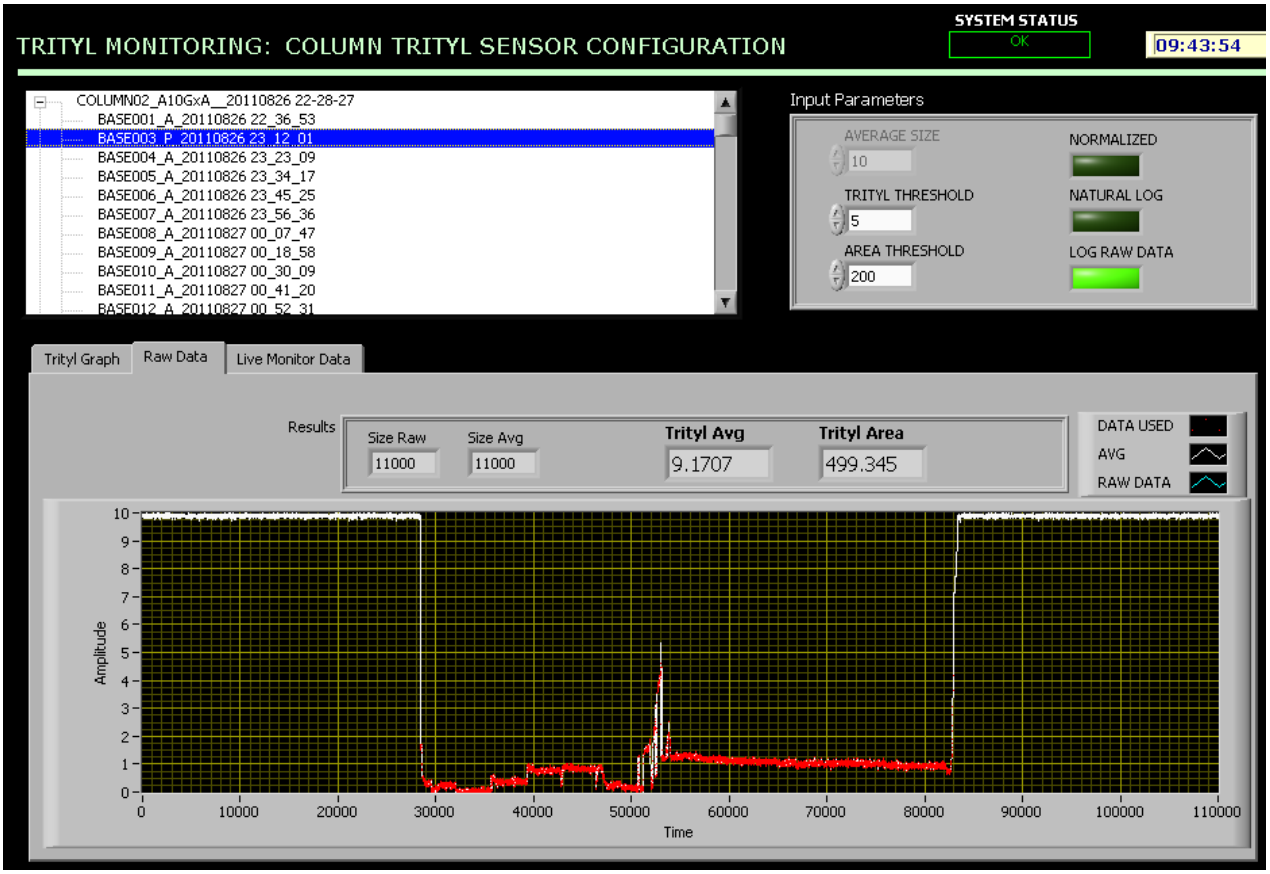


This is the detritylation step after the H-phosphonate chemistry and subsequent oxidation with CCl_4/propargyl amine.

Raw trityl data for Base 1: Data is representative of conventional phosphoramidite coupling efficiencies

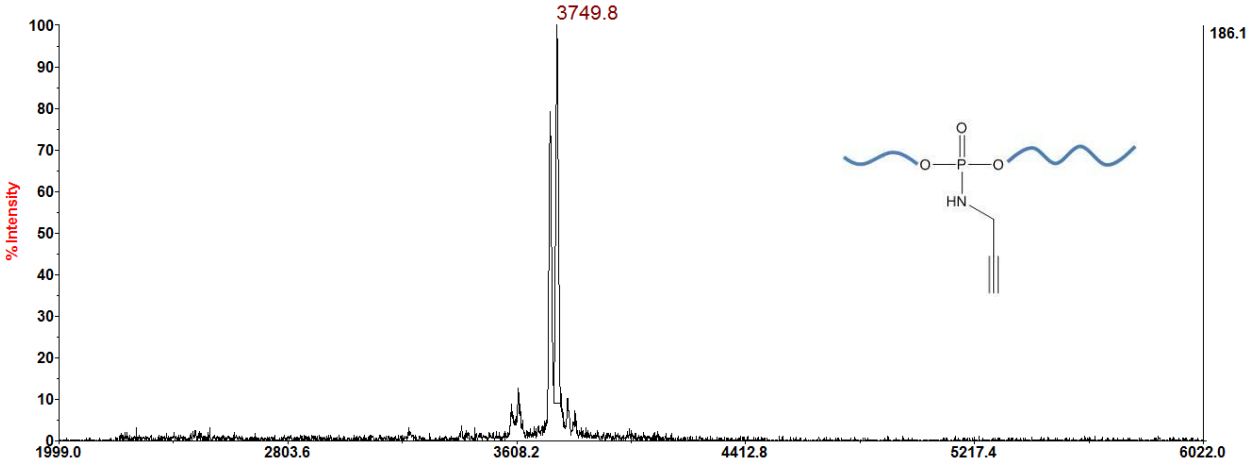


Raw trityl data for Base 2: Data is representative of H-phosphonate coupling efficiencies

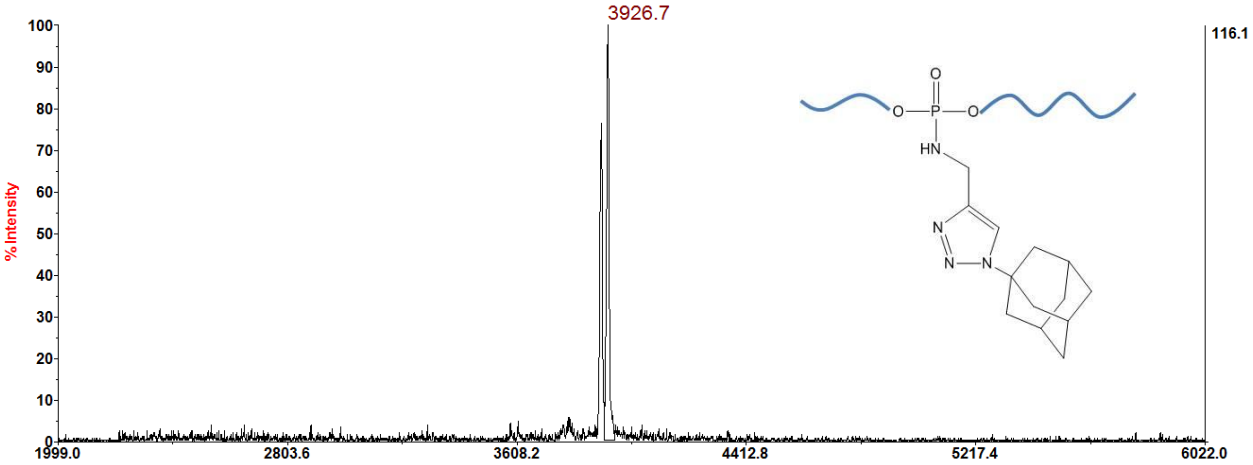


Spectra of EMSA Diamondoid DNAs

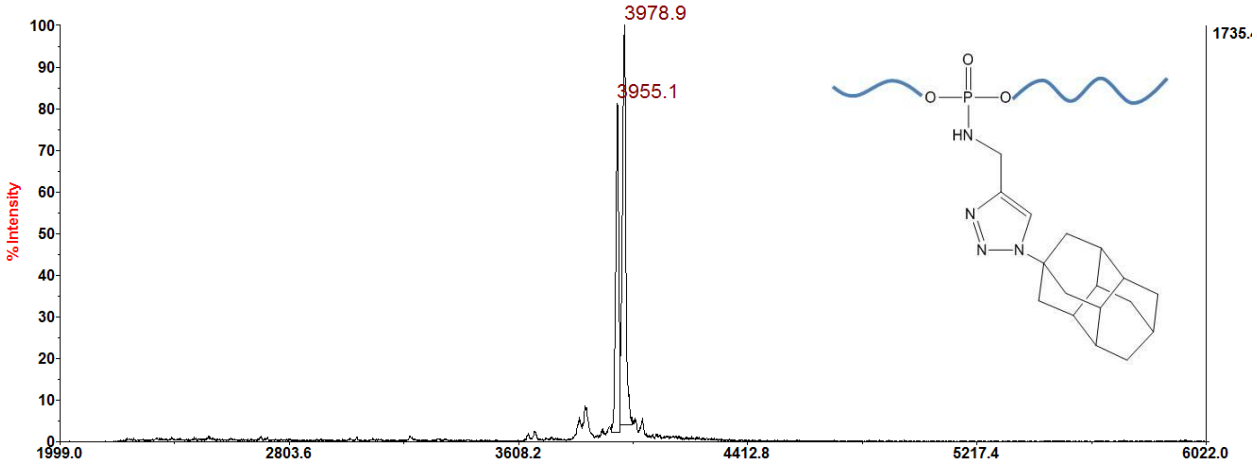
Oligonucleotide 1, Sequence: AAA AAA AAA AG**x**A x=propargyl; Predicted: 3748.7, Detected: 3749.8



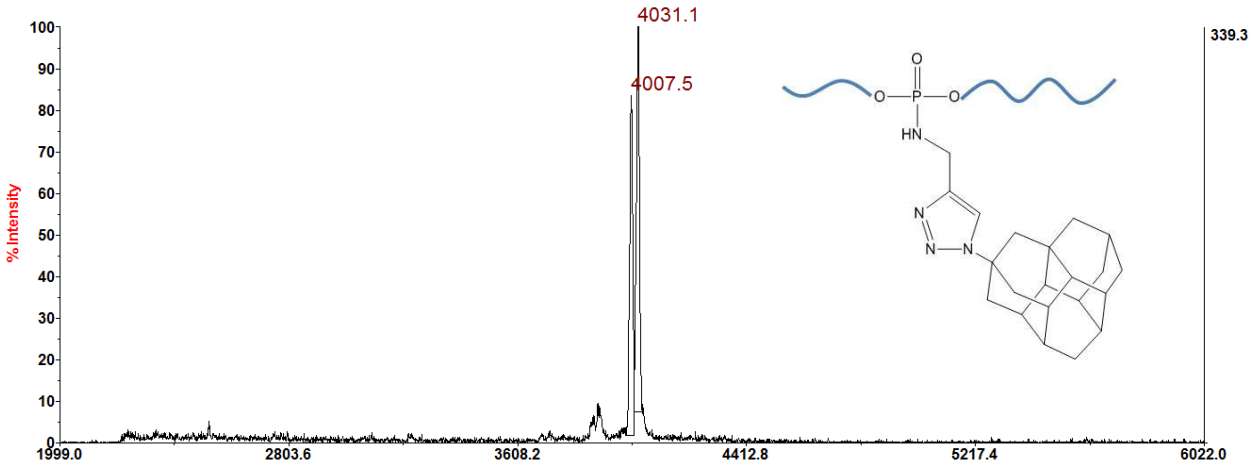
Oligonucleotide 2, Sequence: AAA AAA AAA AG**x**A x=AA1; Predicted: 3925.9, Detected: 3926.7



Oligonucleotide 3, Sequence: AAA AAA AAA AG**x**A x=AA2; Predicted: 3977.5 , Detected: 3978.9

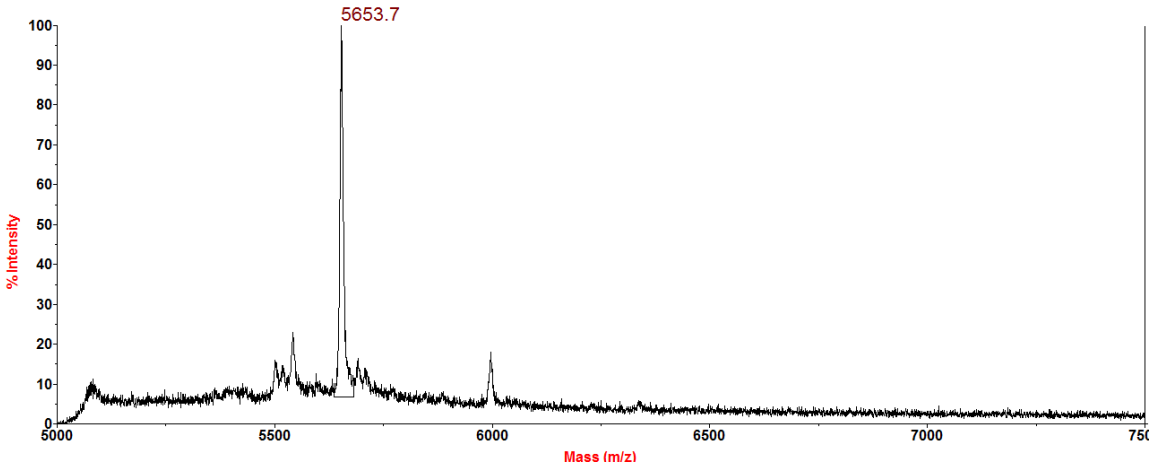


Oligonucleotide 4, Sequence: AAA AAA AAA AG**x**A x=AA3; Predicted: 4029.9, Detected: 4031.1

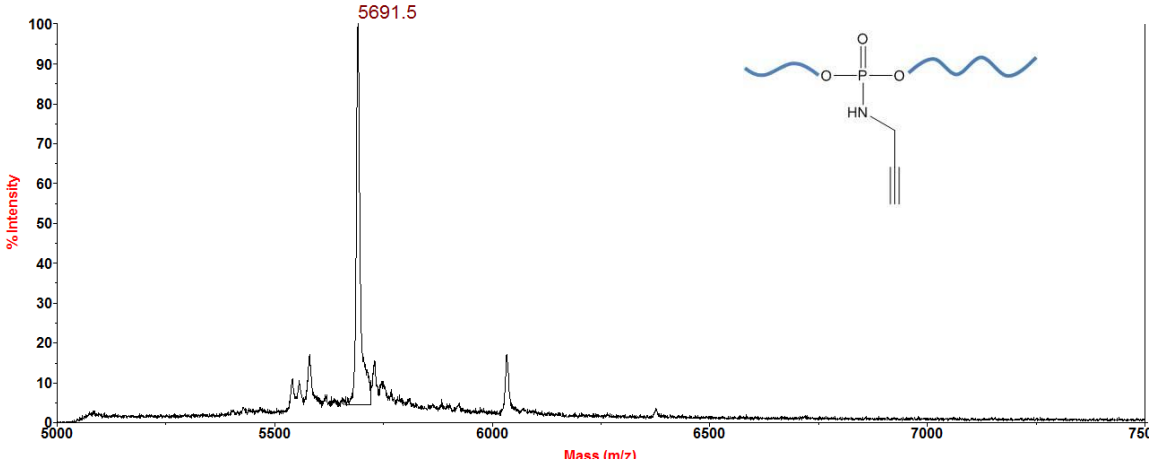


Spectra of FAM labelled DNAs

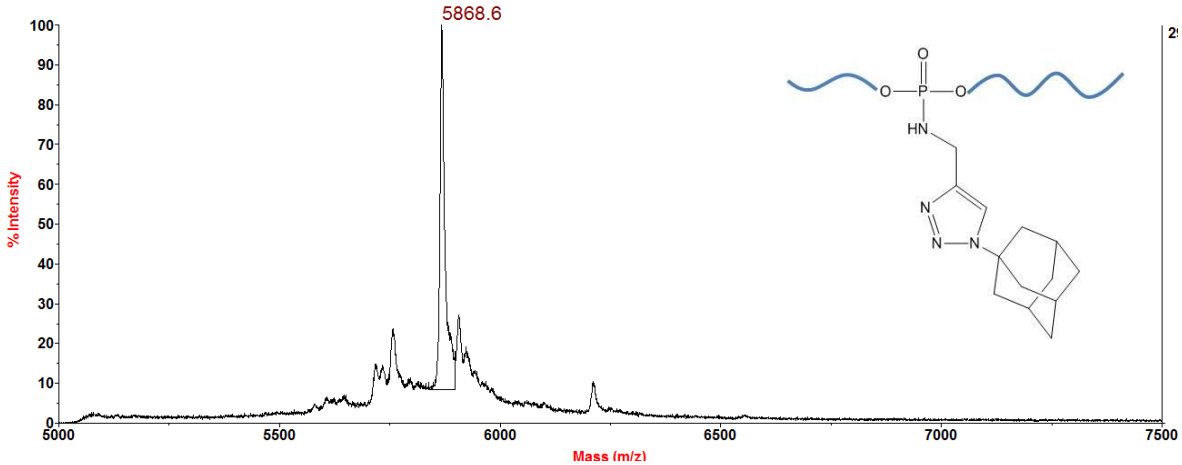
Unmodified FAM: Sequence: 5′ – ACC CAA CAC TAC TCG GC – FAM – 3′; Predicted: 5653.8, Detected: 5653.7



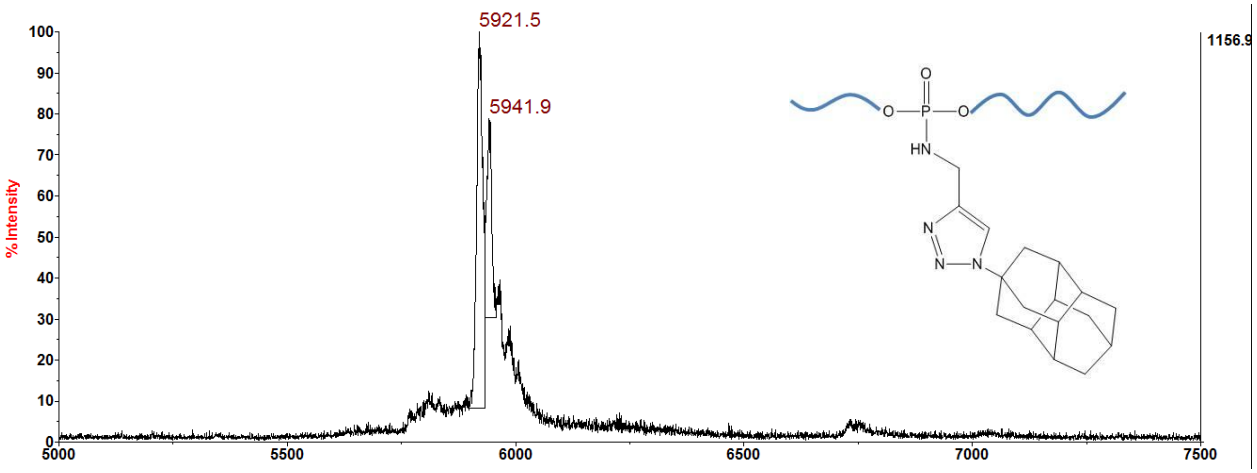
Oligonucleotide 5-Propargyl: Sequence: 5′ – A**x**CC CAA CAC TAC TCG GC – FAM – 3′; x=Propargyl; Predicted: 5690.8, Detected: 5691.5



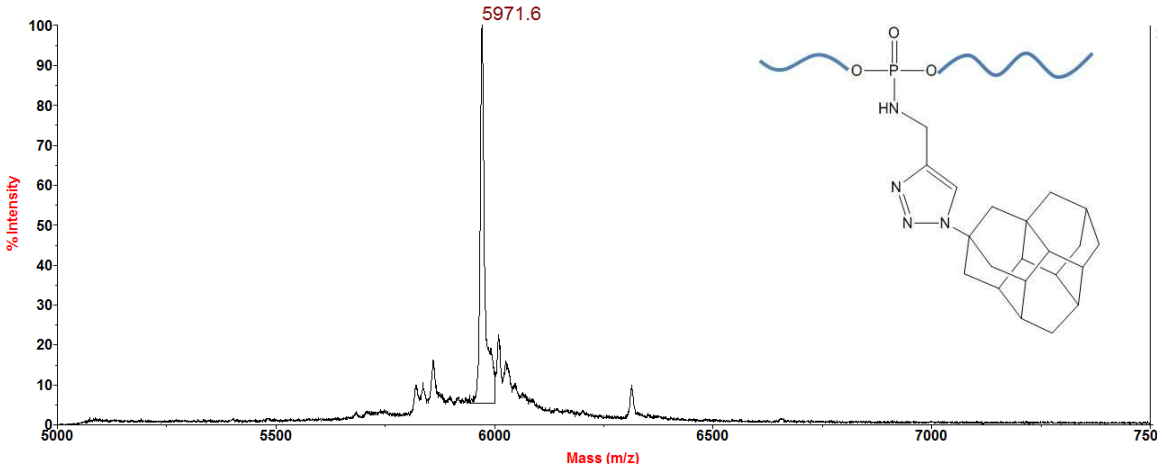
Oligonucleotide 5: Sequence: 5′ – A**x**CC CAA CAC TAC TCG GC – FAM – 3′; x=AA1; Predicted: 5867.9, Detected: 5868.6



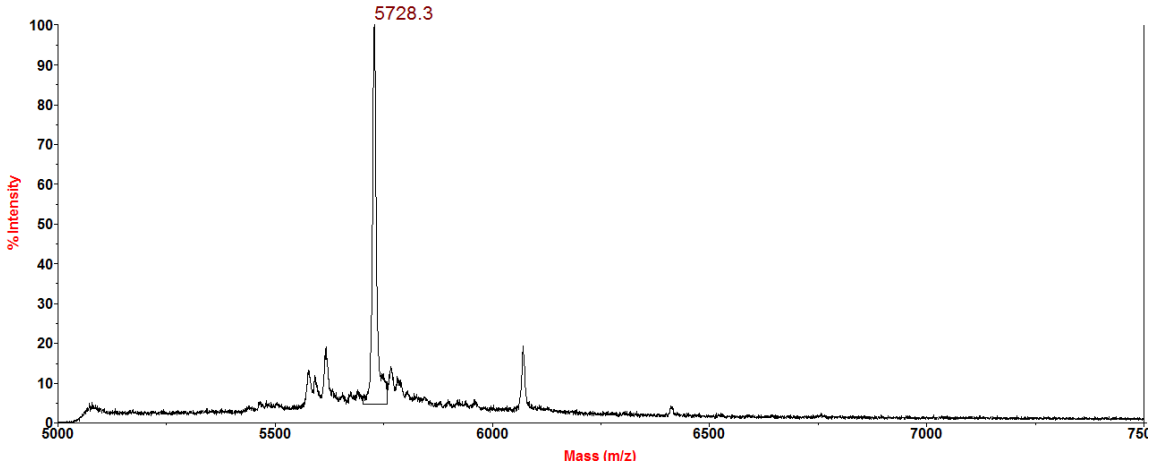
Oligonucleotide 6: Sequence: 5′ – A**x**CC CAA CAC TAC TCG GC – FAM – 3′; x=AA2; Predicted: 5920.0, Detected: 5921.5



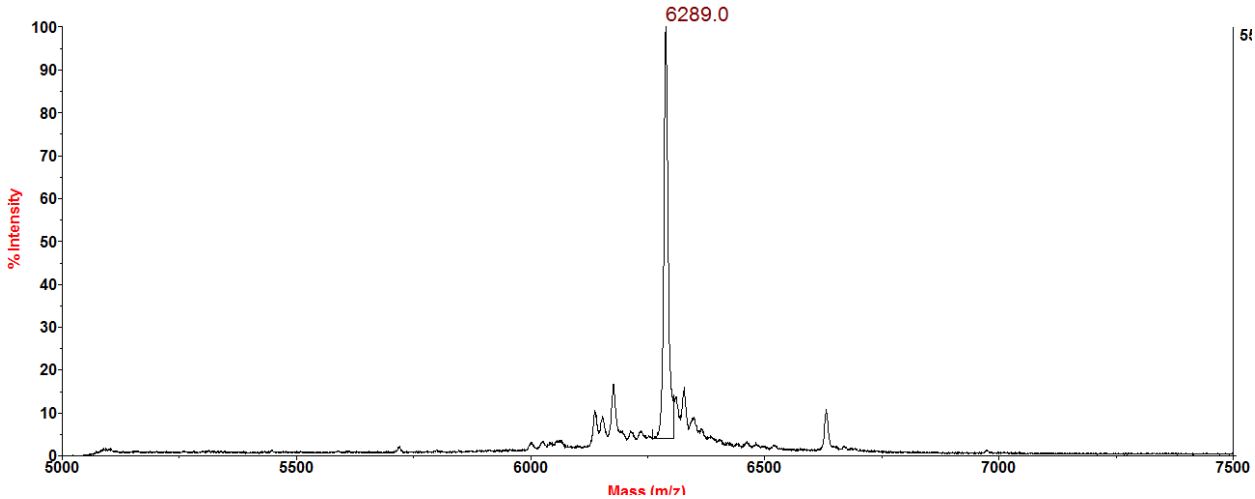
Oligonucleotide 7: Sequence: 5′ – A**x**CC CAA CAC TAC TCG GC – FAM – 3′; x=AA3; Predicted: 5972.0, Detected: 5971.6



Oligonucleotide 8-Propargyl: Sequence: 5′ – A**x**CC CAA**x** CAC TAC TCG GC – FAM – 3′; x=Propargyl; Predicted: 5727.9, Detected: 5728.3



Oligonucleotide 8: Sequence: 5′ – A**x**CC CAA**x** CAC TAC TCG GC – FAM – 3′; x=AA3; Predicted: 6290.3, Detected: 6289.0

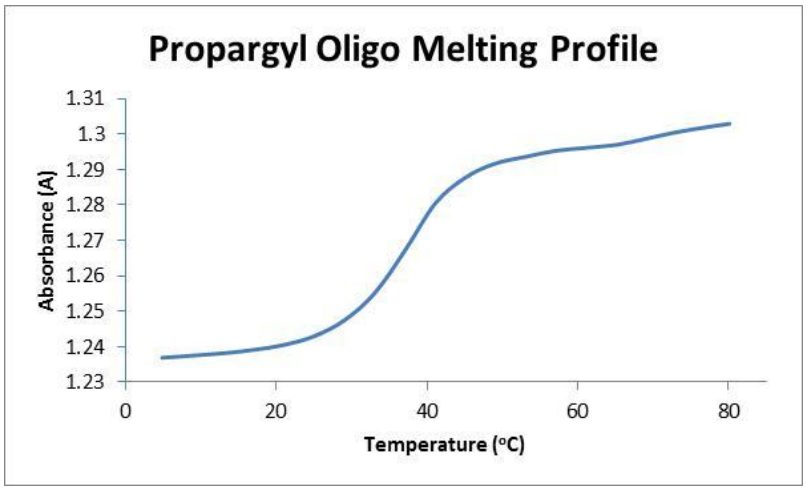


Electronic Supplementary Material (ESI) for Chemical Communications

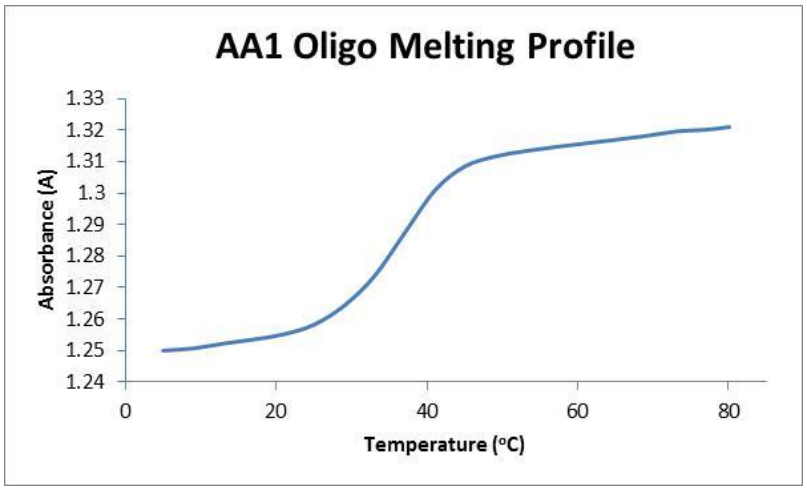
Melting Profiles of DNA Duplexes

All melting temperatures were determined by averaging 4 independent melting profiles consisting of 2 heating and 2 cooling thermal ramps.The following graphs are representative melting curves for each oligonucleotide sample.

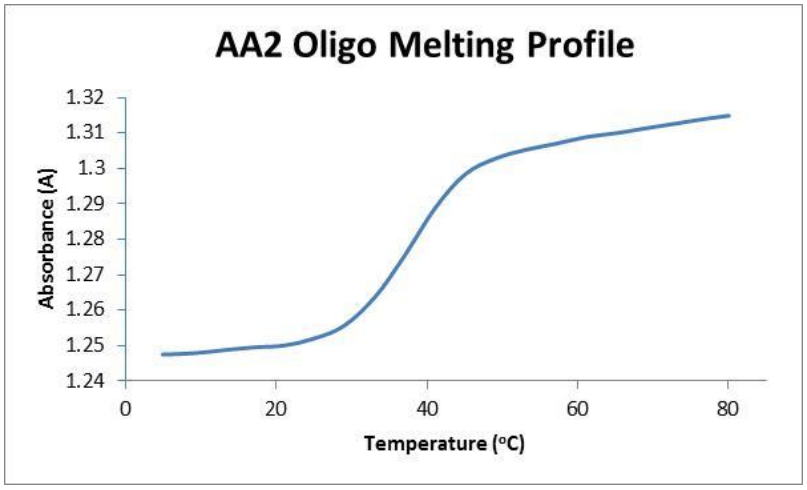
Oligonucleotide 1



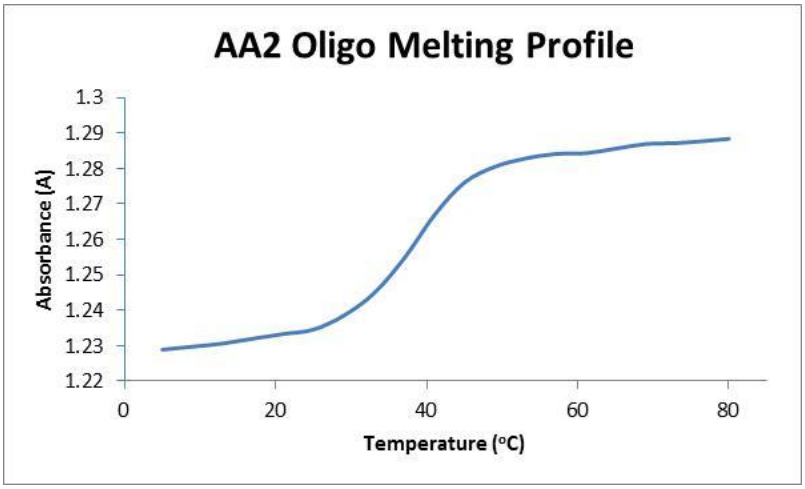
Oligonucleotide 2



Oligonucleotide 3

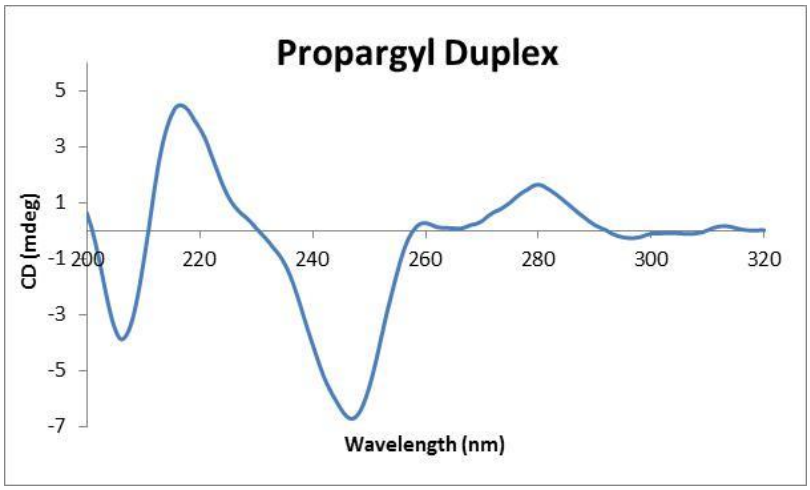


Oligonucleotide 4

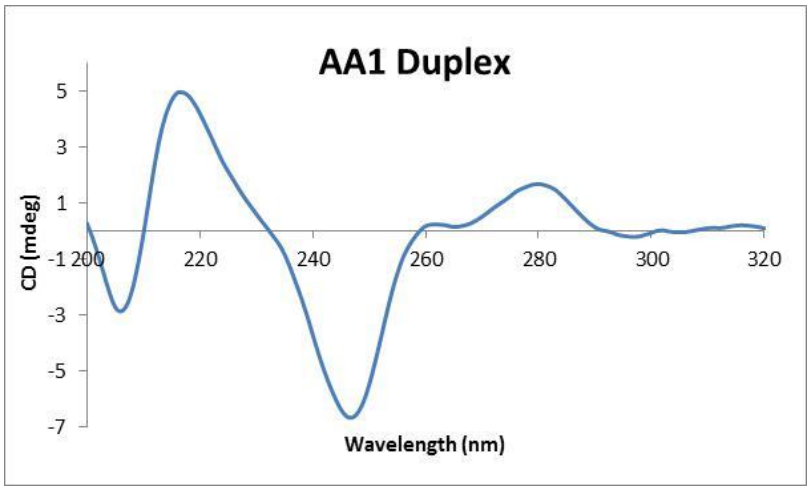


CD Profiles of DNA Duplexes

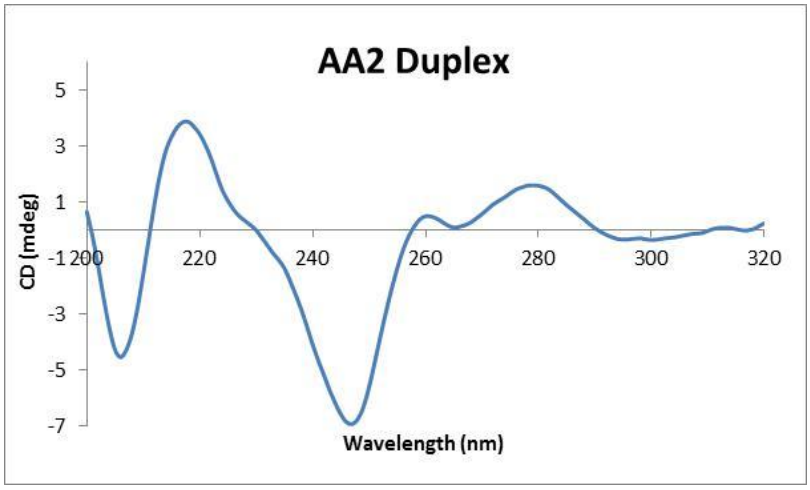
Oligonucleotide 5



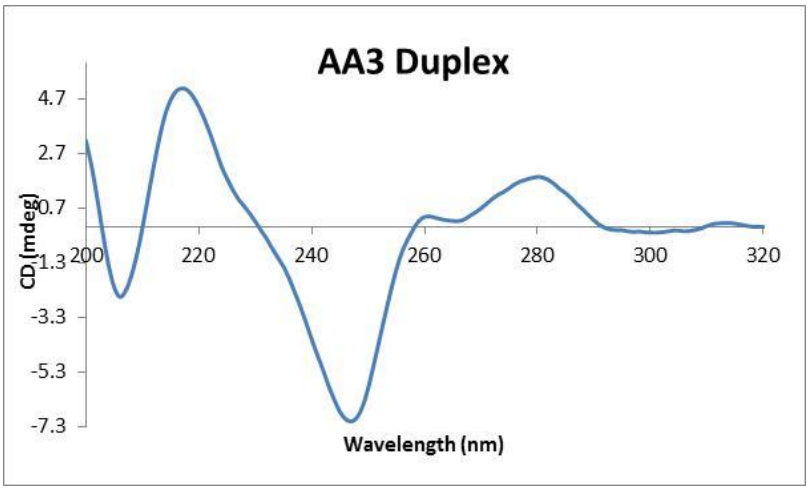
Oligonucleotide 6



Oligonucleotide 7

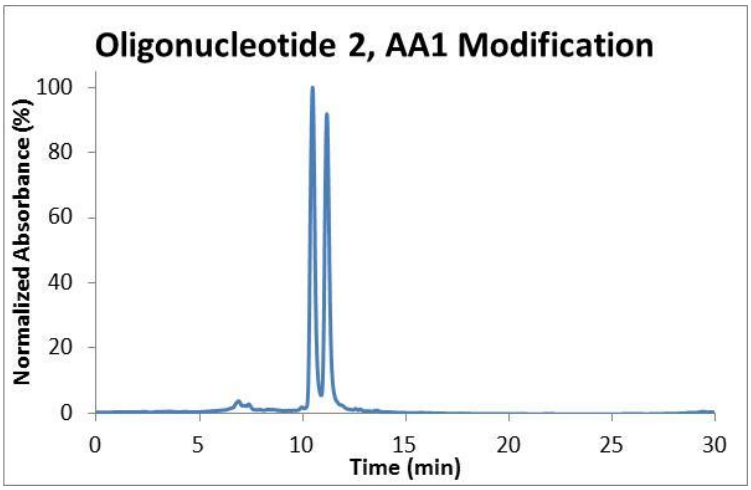


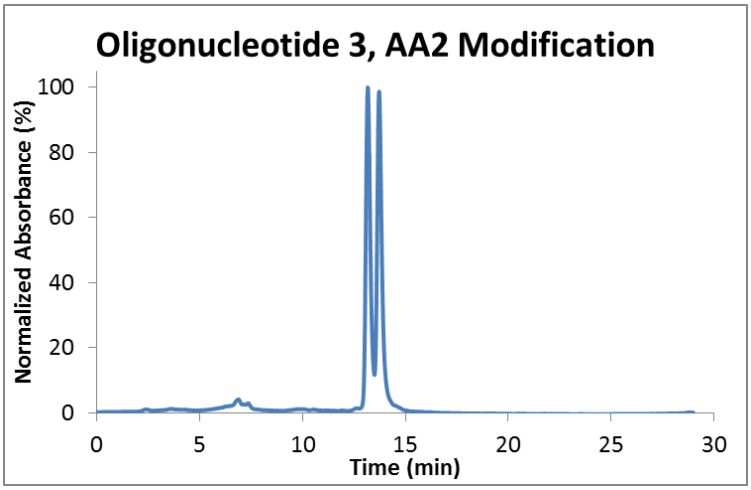
Oligonucleotide 8

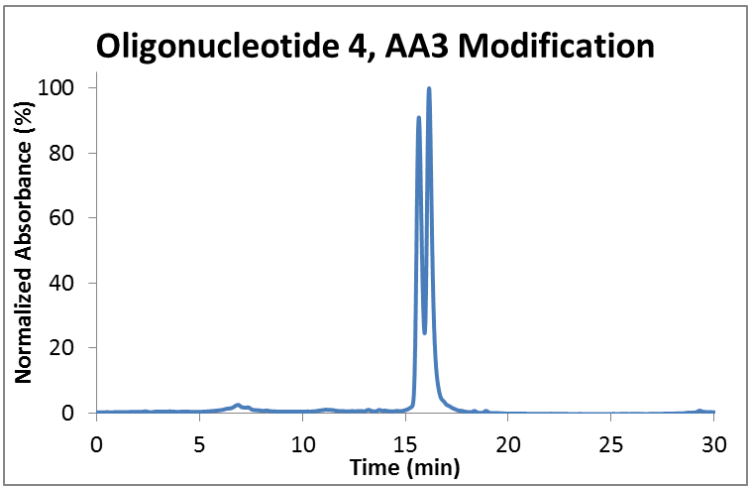


Purity analysis of 'clicked' products using HPLC

The results of the HPLC separation of the 'clicked' products are shown below. For each oligonucleotide-diamondoid conjugate, two peaks appear as a result of the formation of 2 diastereomers. All HPLCs were performed on an Agilent Zorbax SB-C18 column (2.1 x 150 mm). A gradient elution was run from 0-20 min (10% ACN/0.1M TEAA→40% ACN/0.1M TEAA).







Digestion and HPLC/MS analysis of Oligonucleotide 3

S1 nuclease buffer = 30 mM sodium acetate (pH 4.6 at 25 °C), 50 mM NaCl, 1 mM $ZnCl_2$, 0.5 mg/ml denatured calf thymus DNA and 5 % glycerol.

Digestion: 50 μL of oligonucleotide 2 (121.6 μM) was diluted with 9 μL of 10x S1 nuclease buffer followed by 1 μL of S1 nuclease (98 units/μL). The mixture was incubated @ 37 ^{o}C overnight. The next day, 40 μL of 0.1 M Tris Base (pH = 8.5) was added to the reaction buffer and 2 units (2 μL) of calf intestinal alkaline phosphatase was added and incubated @ 37 ^{o}C for 2 hours. The solution was filtered and analyzed by HPLC using an Agilent Zorbax SB-C18 column (2.1 x 150 mm). Mobile phase A = 20 mM KH_2PO_4 buffer (pH = 5.5) and mobile phase B = 30% (v/v) buffer A in methanol. A gradient elution was run from 0-60 min (0 % B → 100% B).

dA peak (rt = 27.6 min) : ESI-MS m/z calcd for $C_{10}H_{13}N_5O_3$ $[M+H]^+$ 252.10, found 252.10

dA/dG dimer peak (rt = 31.6 min): MALDI-MS m/z calcd for $C_{37}H_{47}N_{14}O_8P$ $[M+H]^+$ 847.34, found 847.15