Conjugation of PEG and gold nanoparticles to increase the accessibility and valency of tethered RNA splicing enhancers

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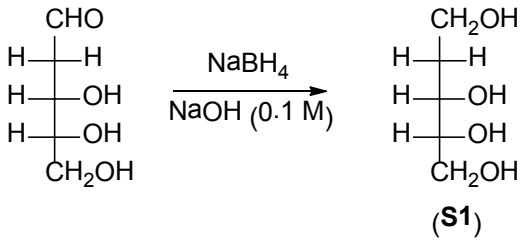
3.0 Synthesis of abasic phosphoramidite (S7)

*3.1 Preparation of (2R,3S)-pentane-1,2,3,5-tetraol (**S1**)*[1]



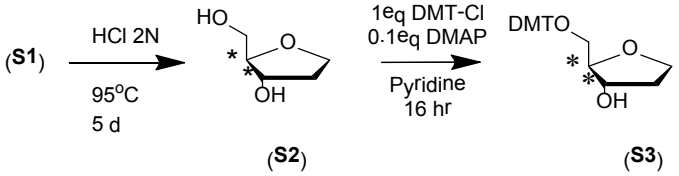
(**S1**)

Compound **S1** was synthesised as previously described by Eritja et al.[1]

2-Deoxy-D-ribose (2.1g, 15mmol) was added to a solution of $NaBH_4$ (215mg, 5.68mmol) in an aqueous solution of sodium hydroxide solution (0.1mM,11mL). The reaction mixture was stirred at ambient overnight. Dowex 50W x 8 (100-200 dry mesh size) was activated by washing the resin five times with sodium hydroxide (1.0 M,200mL) and allowing the resin to settle before removing the supernatant between washings. The resin was washed with water (8 x 300ml washings) until the pH reached 7. The crude reaction mixture was quenched by the addition of 50% acetic acid (pH adjusted to 4) and passed through a column of activated Dowex 50W x 8 resin (25mL resin); products were eluted with five column volumes of water and concentrated *in vacuo* to afford **S1** as a green oil that was used directly in the next reaction.

3.2 Synthesis of

*(2R,3S)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)tetrahydrofuran-3-ol (**S3**)*[1]

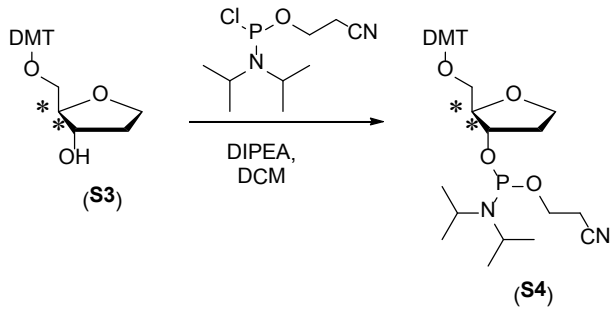


Compound **S3** was prepared as previously described by Eritja et al.[1]

*Preparation of **S2**.* A solution of compound **S1** in 2M HCl (20mL) was heated to 95^{0}C for 5 days. The reaction mixture was then concentrated *in vacuo* to afford a brown oil (**S2**). Anhydrous pyridine (12.5 mL) was added to crude **S2** (2.20g, 18.69x10^{-3}mol) and concentrated *in vacuo*. This process was repeated three times to ensure neutralisation of the HCl.

*Preparation of **S3**.* **S2** (2.20g, 18.69x10^{-3}mol) was dissolved in pyridine (10ml) and molecular sieves (4Å) added. The mixture was stirred under an atmosphere of nitrogen for 7 hrs. A solution of DMAP (15.2mg, 1.25x10^{-3} mol) in anhydrous pyridine (2mL) was added to the reaction mixture followed by a solution of DMT-Cl (4.23g, 12.46x10^{-3}mol) in anhydrous pyridine (7mL). The reaction mixture was stirred at RT for 16 hrs under a nitrogen atmosphere. Column chromatography (SiO_2) using a 20% ethyl acetate/petroleum ether → 50% ethyl acetate gradient afforded compound (**S3**) as a yellow gum (15%, 1.14 g) yield. ES/MS: m/z 443 $[M+Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.76-1.84 (m, 1 H) 2.01-2.12 (m, 1 H) 2.97-3.03 (m, 1 H) 3.13-3.19 (m, 1 H) 3.77-3.83 (m, 1 H), 3.71 (s, 6 H) 3.87-3.92 (m, 2 H) 4.18-4.24 (m, 1 H) 6.72-6.77 (m, 4 H) 7.09-7.15 (m, 1 H) 7.17-7.27 (m, 6 H) 7.33-7.38 (m, 2 H).

*3.3 Synthesis of 2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite (**S4**)*



Compound **S4** was prepared by a literature procedure.[2] Compound **S4** is also commercially available from Berry Associates (Product Number. BA 0033).

A solution of 3-((chloro(diisopropylamino)phosphino)oxy)propanenitrile (238.7µL, 1.07×10^{-3} mol) in dry DCM (2mL) was added to a solution of **S3** (300mg, 7.13×10^{-4} mol) and diisopropylethylamine (161.5µl, 9.27×10^{-4} mol) in degassed anhydrous DCM (2mL). The reaction mixture was stirred at RT for 4 hrs. The product was purified by column chromatography using neutralised SiO_2 (neutralised with pyridine and heated in an oven overnight at 120°C) and eluted with ethyl acetate : *n*-hexane (1:1, with 0.1% triethylamine). Dried 100mL round bottom flasks were used to collect 5 x 50 mL fractions. Direct evaporation of the solvent on a high vacuum line fitted with a cold trap afforded compound **S4** as a colourless oil (74%, 154.3mg); ^{1}H NMR (400 MHz, $CDCl_3$) 0.95-1.19 (m, 13 H) 1.811.93 (m, 1 H) 1.94-2.08 (m, 1 H) 2.26-2.32 (m, 1 H) 2.97-3.12 (m, 2 H) 3.39-3.59 (m, 3 H) 3.65 (s, 7 H) 3.79-3.99 (m, 3 H) 4.26-4.37 (m, 1 H) 6.67-6.76 (m, 4 H) 7.04-7.21 (m, 4 H) 7.22-7.28 (m, 3 H) 7.34-7.39 (m, 2 H). ^{31}P NMR (400 MHz, $CDCl_3$) 148.2 (s, 1 P) 147.9 (s, 1 P).

*3.3.1 ^{1}H NMR spectrum of (**S4**)*

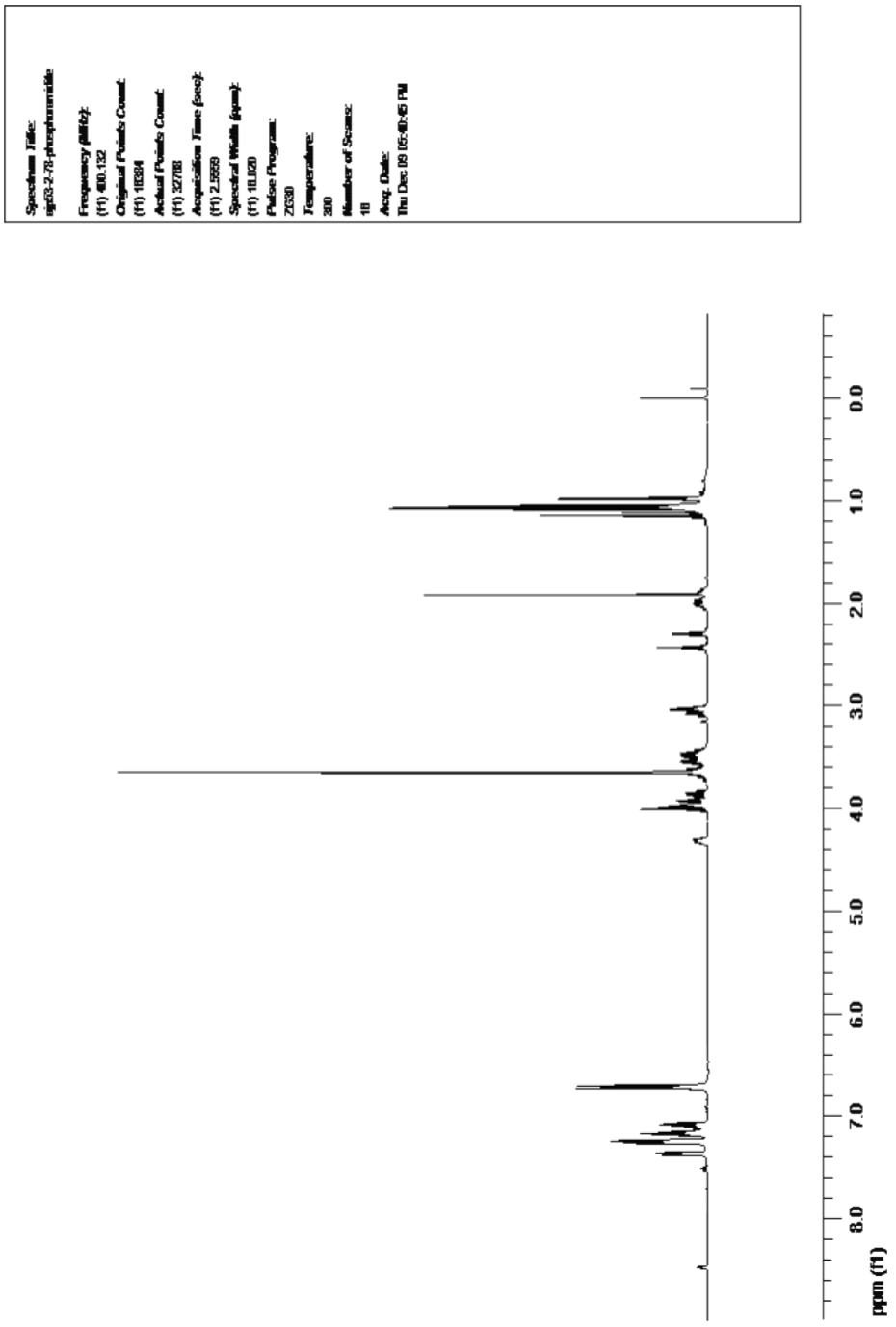


Figure S1: *^{1}H NMR spectrum of compound **S4**.*[1]

*3.3.2 ^{31}P NMR spectrum of (**S4**)*

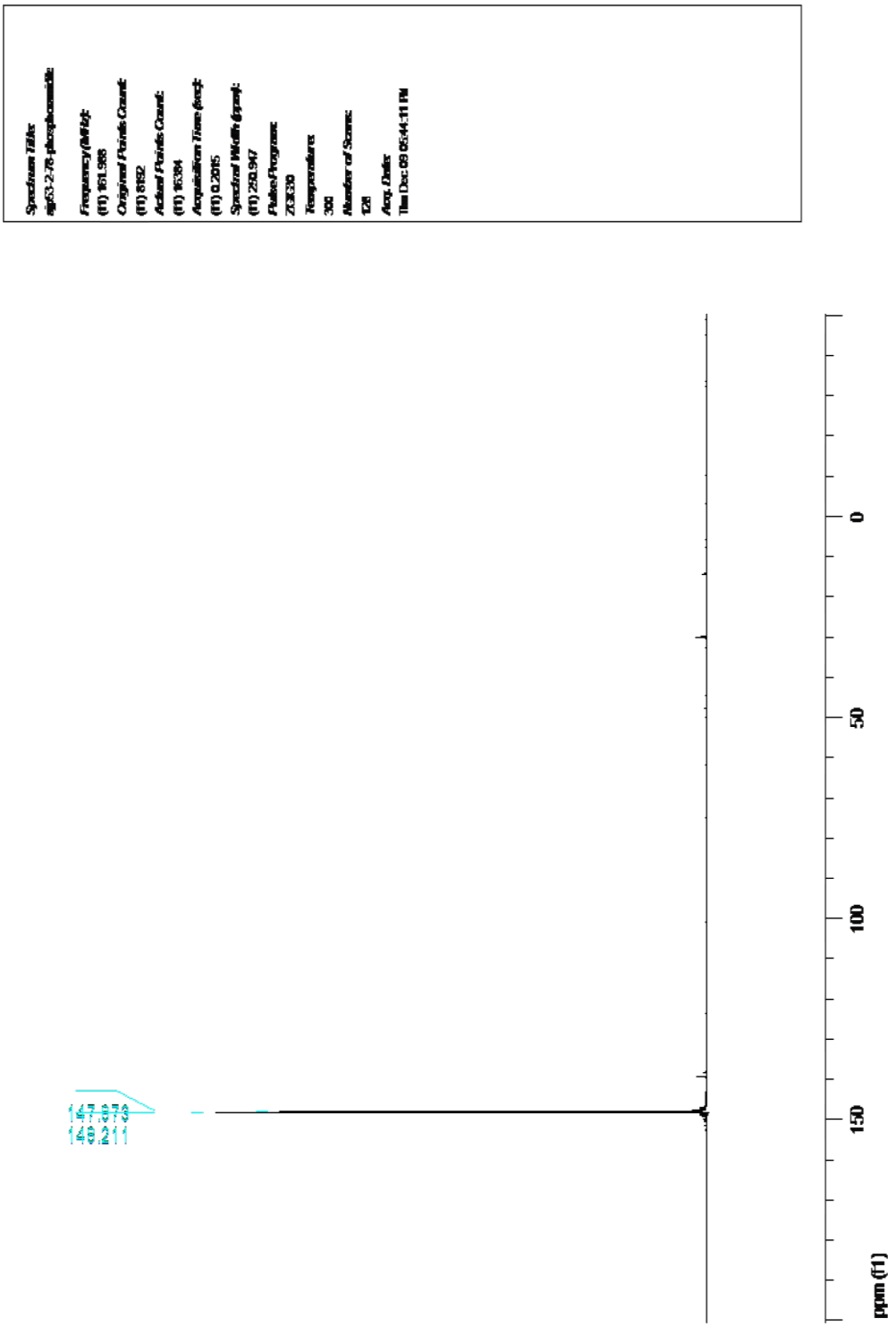
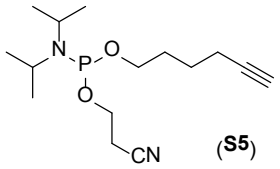


Figure S2. *^{31}P NMR spectrum of compound **S4**.[1]*

*3.4 Synthesis of 2-cyanoethyl hex-5-yn-1-yl dimethylphosphoramidite (**S5**)[3]*



(**S5**)

Compound **S5** was synthesised as previously described by Alvira et al.[3]

A solution of 3-((chloro(diisopropylamino)phosphino)oxy)propanenitrile (683µL, 3.06×10^{-3}mol) in dry DCM (2mL) was added to a solution of hexyn-1-ol (225µl, 2.04×10^{-3}mol), diisopropylethylamine (462µL, 2.65×10^{-3}mol) in degassed anhydrous DCM (2mL). The reaction mixture was stirred at RT for 2 hrs. The crude product was purified by column chromatography using neutralised SiO_2 (neutralised with pyridine and heated in an oven overnight at 120^{o}C) and eluted with 30% dry ethyl acetate/ dry hexane with 1% pyridine. Dried 100mL round bottom flasks were used to collect five 50mL fractions. The solvent was removed by direct evaporation on a high vacuum line fitted with a cold trap to yield **S5** as a colourless oil (83%, 504mg). ^{1}H NMR (300MHz, C_6D_6) 1.03 (t, *J* = 6.45 Hz, 12 H) 1.34-1.54 (m, 4 H) 1.57-1.69 (m, 3 H) 1.85-1.93 (m, 2 H) 3.11-3.28 (m, 2 H) 3.33-3.56 (m, 4 H).^{31}P NMR (300MHz, C_6D_6) 147.96 (s, 1 P).

*3.4.1 ^{1}H NMR spectrum of (**S5**)*

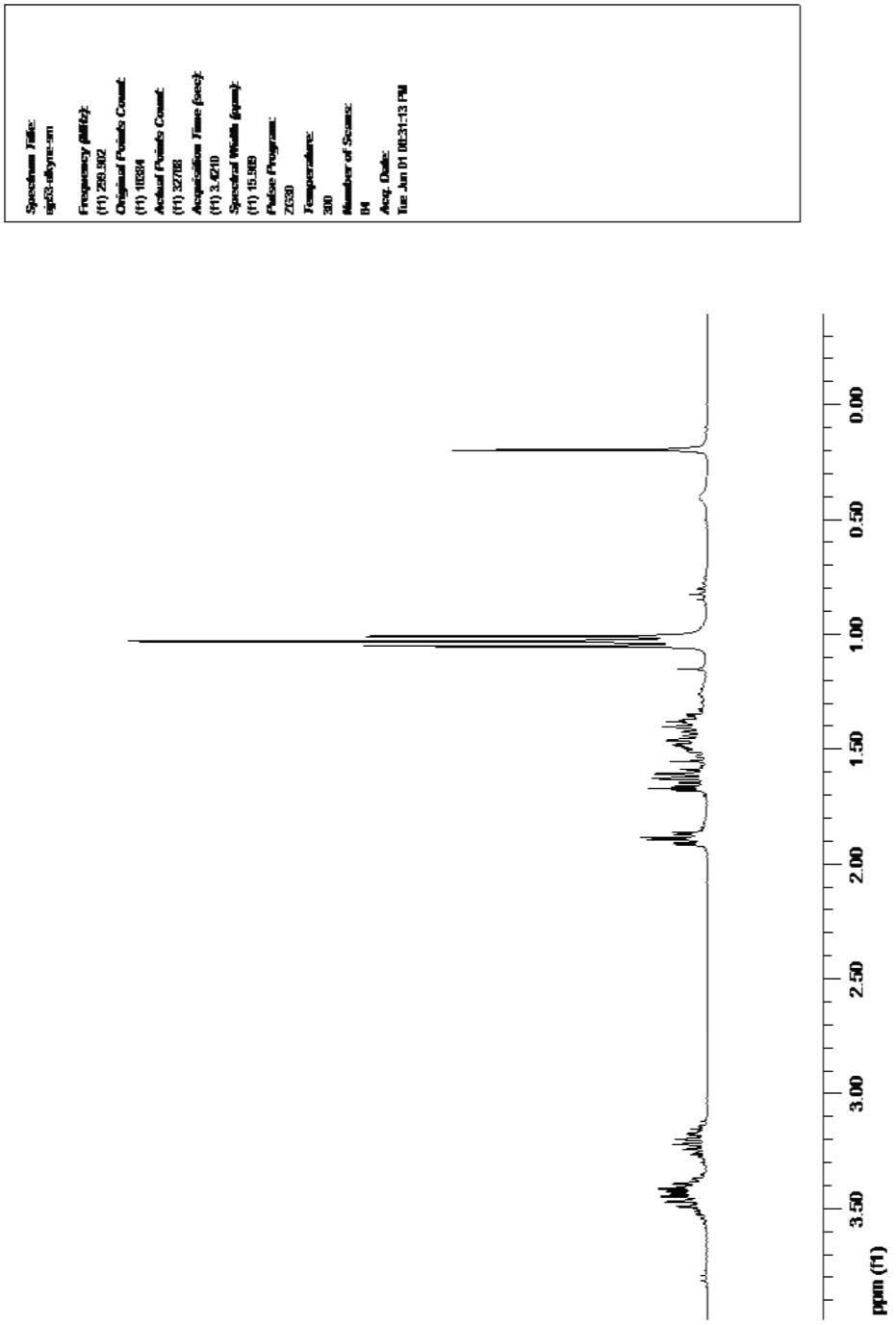


Figure S3. *^{1}H NMR spectrum of compound **S5**.[3]*

*3.4.2 ^{31}P NMR spectrum of (**S5**)*

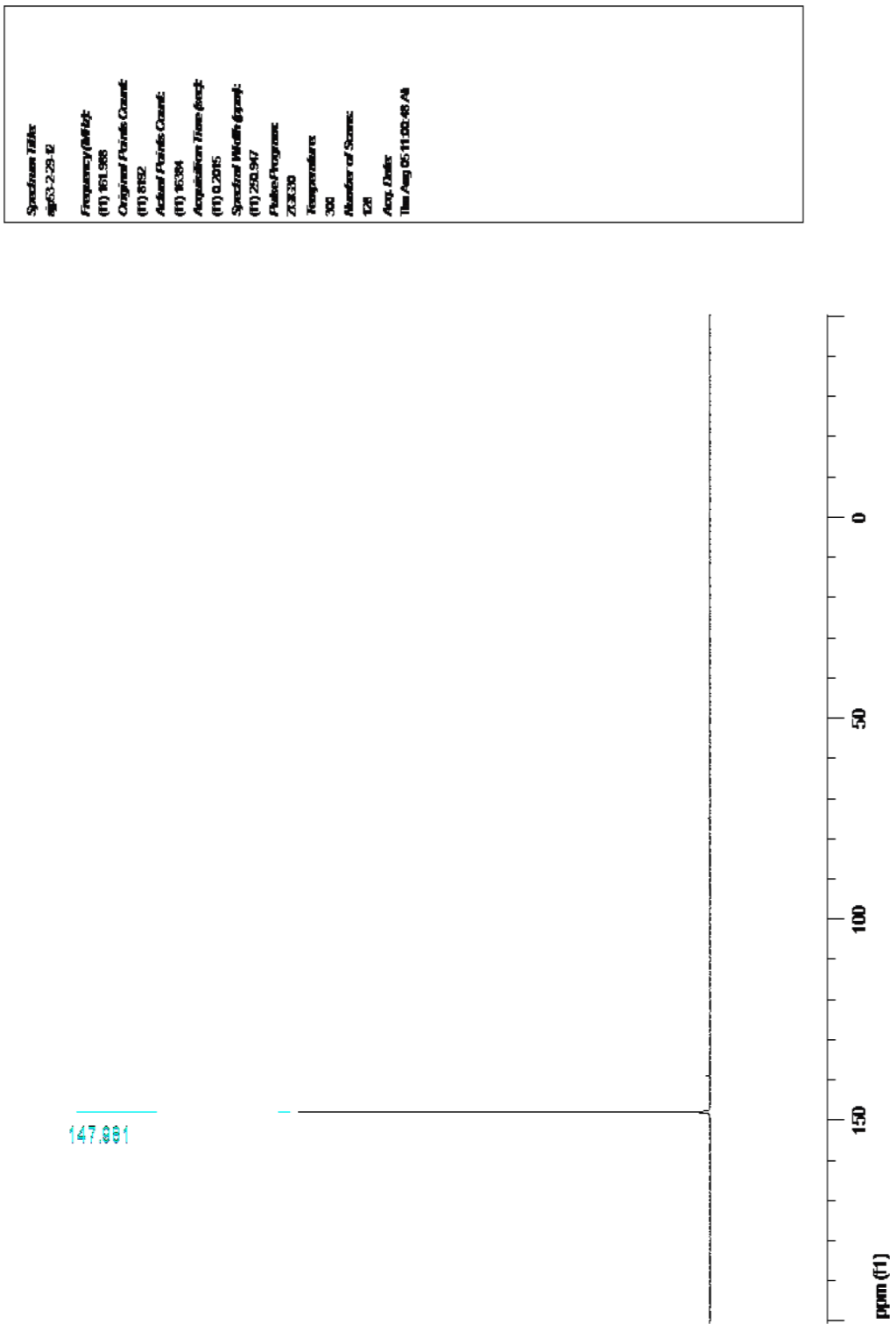


Figure S4. *^{31}P NMR spectrum of compound **S5**.*[3]

3.5 Synthesis of RNA ONs

2'OMe RNA ONs were prepared using commercially available DMT-protected β-(cyanoethyl) phosphoramidites (Link Technologies) on 1 μmol CPG supports using an Applied Biosystems 394 nucleic acid synthesizer. Abasic phosphoramidite (**S4**) and 2-cyanoethyl hex-5-yn-1-yl dimethylphosphoramidite (**S5**) were used in the synthesis of ON strands using protocols previously described.[2a, 3] A double coupling protocol and elongation of coupling times to 18 min was applied for strands RNA1_S2 and RNA1_S3. Coupling times of 11 min were used for the remaining strands. For the preparation of RNA14, commercially available TC-protected phosphoramidites (Link Technologies) were used according to an procedure.[4]

Upon completion of the automated synthesis, the sequences were cleaved from the solid support by aminolysis (35% aqueous ammonia, 4 hours at RT). Deprotection of the amino protecting groups was achieved by heating the ONs in 35% aqueous ammonia solution at 55^{o}C for 16hrs. The aqueous ammonia was removed by concentration *in vacuo*. RNA_S4-S6 were purified by reverse phase HPLC, whereas gel electrophoresis (10% denaturing polyacrylamide) was used to purify RNA_S1-3 and RNA_S7-19 series. Phosphorothioate ONs were prepared according to literature procedures.[5] 10% denaturing polyacrylamide gels were run to confirm purity of RNA_S1-6 (Figure S3). After purification, all RNA samples were desalted using Illustra NAP™ 25 columns, lyophilised and re-dissolved in double distilled H_2O.

3.6 Gel electrophoretic analysis of RNA ONs – abasic and PEG linkers

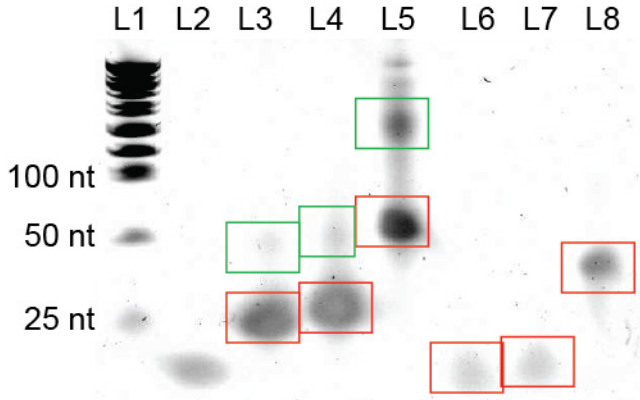


Figure S5. *10% polyacrylamide gel of the purified strands RNA_S1-S6. Lane 1 is a DNA marker. Lane 2 shows a control strand (5'-GAUUUUGUCUAAAAC-3'). Lane 3 RNA_S1. Lane 4 RNA_S2. Lane 5 RNA_S3. Lane 6 RNA_S4. Lane 7 RNA_S5. Lane 8 RNA_S6. Red boxes depict click products whereas the green boxes highlight RNA secondary structures.*

The origins of the shifts in mobility observed in Figure S5 (Lanes 3-5) were confirmed to be secondary structures by heating RNA_S2 and RNA_S3 to 80°C prior to running a gel (Figure S6). Heat treatment prior to gel electrophoretic analysis resulted in a significant reduction of the higher bands (Lanes 3 and 6, Figure 6) as the heat treatment denatured the secondary structure.

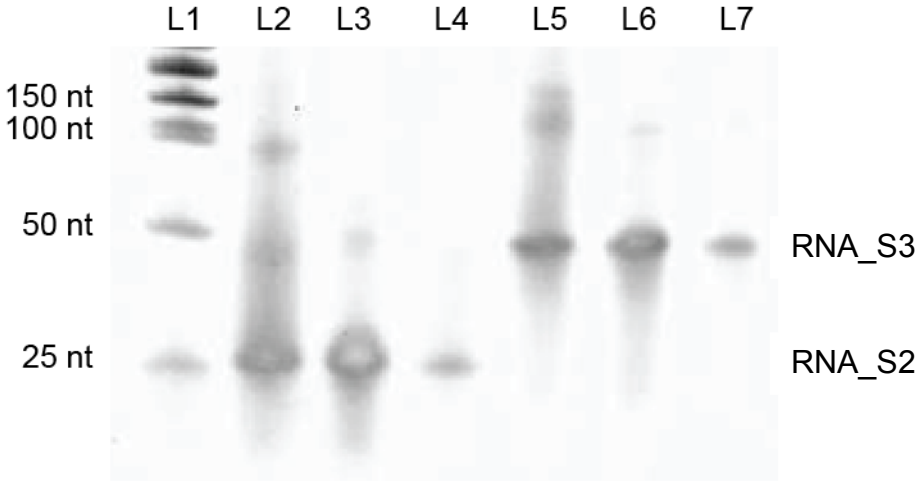


Figure S6. *10% denaturing polyacrylamide gel of RNA_S2 and RNA_S3 samples demonstrating that the higher bands in the purified sample can be attributed to secondary structures. Lane 1. DNA ladder. Lane 2. RNA_S2. Lane 3. RNA_S2 heat treated at 80°C. Lane 4. 1 in 100 dilution of RNA_S2. Lane 5. RNA_S3. Lane 6. RNA_S3 heat treated at 80°C. Lane 7. 1 in 100 dilution of RNA_S3.*

*3.7 Synthesis of 2,5-dioxopyrrolidin-1-yl 4-azidobutanoate (**S7**)[6]*

Compound **S7** was synthesised as previously described by R. Kumar et al.[6]

To a solution of 4-azidobutanoic acid (**S6**, 50mg, 3.87×10^{-4}mol) in anhydrous DCM (2mL) was added solid N-hydroxysuccinimide (66.9mg, 5.81×10^{-4}mol) and HOBt (78.5mg, 5.81×10^{-4}mol) under an argon atmosphere. The reaction mixture was then cooled to 0°C and EDC (111.4mg, 5.81×10^{-4}mol) was added. The reaction was allowed to warm to RT and stirred overnight. The crude product was purified by column chromatography (SiO_2, eluent 1:1 ethyl acetate/petroleum ether) to afford compound (**S7**) as a white solid (34 %, 29.8mg). ^{1}H NMR (300MHz, $CDCl_3$) 1.94 (p, *J* = 6.83 Hz, 2 H) 2.66 (t, *J* = 7.19 Hz, 2 H) 2.77 (s, 4 H) 3.38 (t, *J* = 6.53 Hz, 2 H).

*3.7.1 ^{1}H NMR spectrum of (**S7**)*

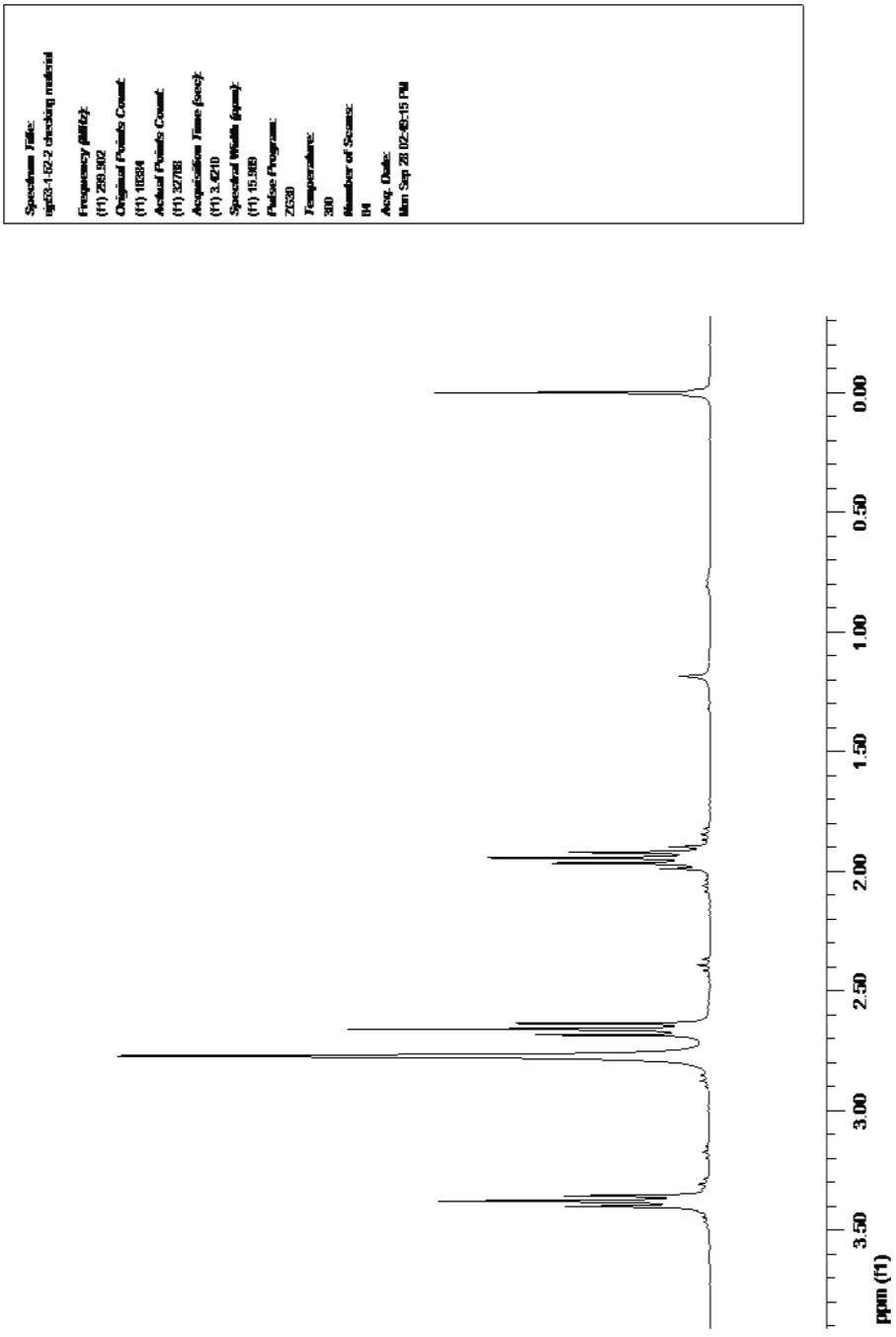


Figure S7. *^{1}H NMR spectrum of compound **S7**.[6]*

4.0 Click chemistry

4.1 2,5-dioxopyrrolidin-1-yl 4-azidobutanoate conjugation

A solution of (**S7**) (2mg, 8.84×10^{-6} mol) in DNA grade acetonitrile (70µL) was added to a solution of an amino-terminated RNA ON (1.4µg) in aqueous sodium hydrogen carbonate (30µL, 0.025M, pH~8). The mixture was vortexed, which resulted in the formation of a precipitate. Sodium hydrogen carbonate (40µL, 0.025M, pH~8) was added drop-wise until the precipitate re-dissolved and the reaction was left to shake at RT overnight. The product was concentrated to remove the acetonitrile and desalted through an Illustra NAP™ 25 column.

4.2 Click Chemistry Ligation

Click chemistry ligation was conducted using a protocol adapted for DNA labelling by click chemistry.[7]

A stock solution of RNA_S11-S15 (60 µL, 17.2 µM) and an RNA_S4-S6 (60 µL, 17.2 µM) were aliquoted into 12 tubes (10 µL per tube). CuBr (3.1 mg, 2.16×10^{-5} mol) was added to a solution of TBTA in DMSO/t-butanol (3:1, 216 µL, 0.1 M), vortexed and diluted to 8.62mM with DMSO. CuTBTA (1 µL, 8.62 mM) was then added to each aliquot, vortexed and then heated to 40 ^{o}C at 750 rpm for 15 min. The samples were desalted (MF-Millipore™ Membrane, 20min) and purified by gel electrophoresis using 10% denaturing polyacrylamide gels at 100 V for 1 hr (four gels used in total). The nucleic acid components were visualised using SYBR™ gold, image-captured on a VersaDoc™ gel imager and the relevant bands isolated by gel excision. Each excised band was placed in separate tubes, treated with RNA elution buffer (350 µL per tube) and left at 5 ^{o}C overnight. The samples were then heated to 30 ^{o}C for 10 min to re-dissolve any precipitated SDS before transferring the supernatants to new tubes. Ethanol (1.05 mL, 3 x vol of RNA elution buffer) was then added to the supernatants, vortexed and finally centrifuged for 30 min at 16,000 g. The supernatants were removed and the resultant RNA

pellet washed with ethanol (250 µL) before being centrifuged again for 15 min. The supernatants were removed and the pellets dried, dissolved in water and combined to afford the final click products (RNA1-13).

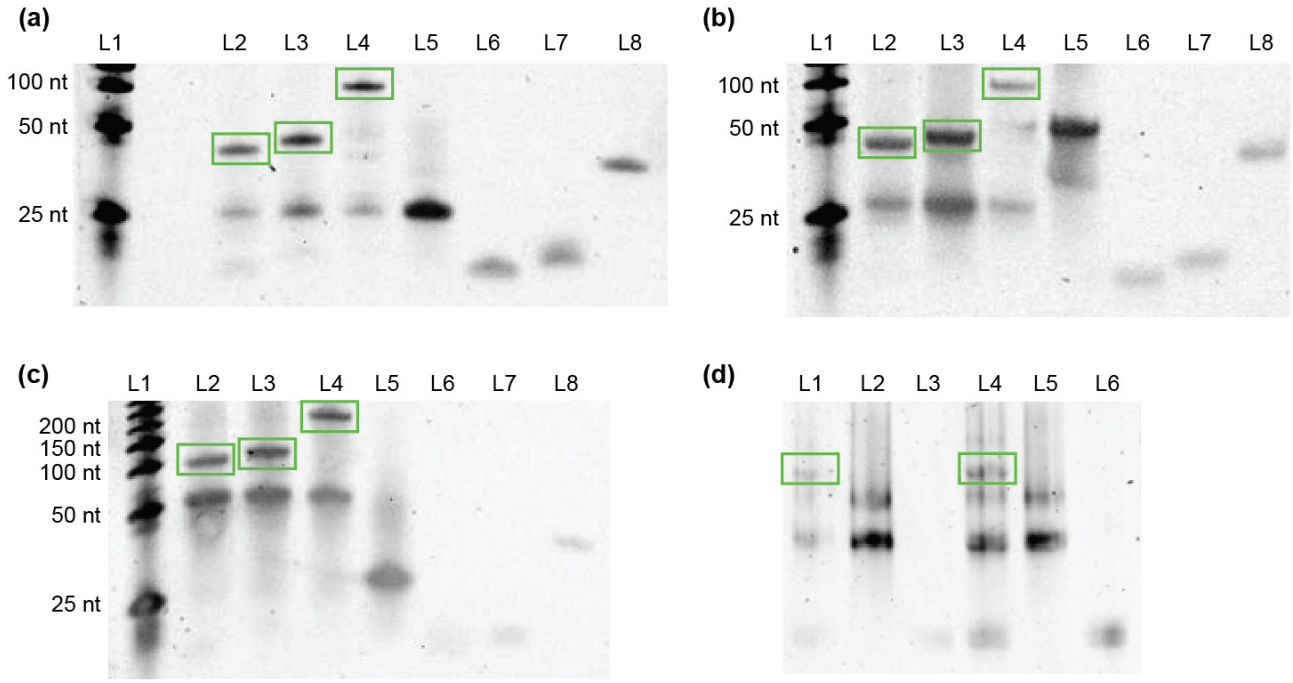


Figure S8. *Crude click chemistry reactions.* ***(a)*** *RNA_S11. Lane 1: DNA ladder. Lane 2: click chemistry reaction between RNA_S11 and RNA_S4. Lane 3: click chemistry reaction between RNA_S11 and RNA_S5. Lane 4: click chemistry reaction between RNA_S11 and RNA_S6. Lane 5-8: controls RNA_S11, RNA_S4, RNA_S5 and RNA_S6 respectively.* ***(b)*** *RNA_S12. Lane 1. DNA ladder. Lane 2: click chemistry reaction between RNA_S12 and RNA_S4. Lane 3: click chemistry reaction between RNA_S12 and RNA_S5. Lane 4: click chemistry reaction between RNA_S12 and RNA_S6. Lane 5-8: controls RNA_S12 (on gel c lane 5), RNA_S4, RNA_S5 and RNA_S6 respectively.****(c)*** *RNA_S13. Lane 1: DNA ladder. Lane 2: click chemistry reaction between RNA_S13 and RNA_S4. Lane 3: click chemistry reaction between RNA_S13 and RNA_S5. Lane 4: click chemistry reaction between RNA_S13 and RNA_S6. Lane 5-8: controls RNA_S13 (on gel b lane 5), RNA_S4, RNA_S5 and RNA_S6 respectively.* ***(d)****RNA_S9, RNA_S10 and RNA_S15.Lane 1: click chemistry reaction between RNA_S9 and RNA_S15. Lane 2: RNA_S15. Lane 3: RNA_S9. Lane 4: click chemistry reaction between RNA_S10 and RNA_S15. Lane 5: RNA_S15. Lane 6: RNA_S10. All the reactions were run on 10% denaturing polyacrylamide gel for 1hr and all the reactions show the 15min time point with green boxes highlighting products. The click products (green boxes) were then excised from the gel.*

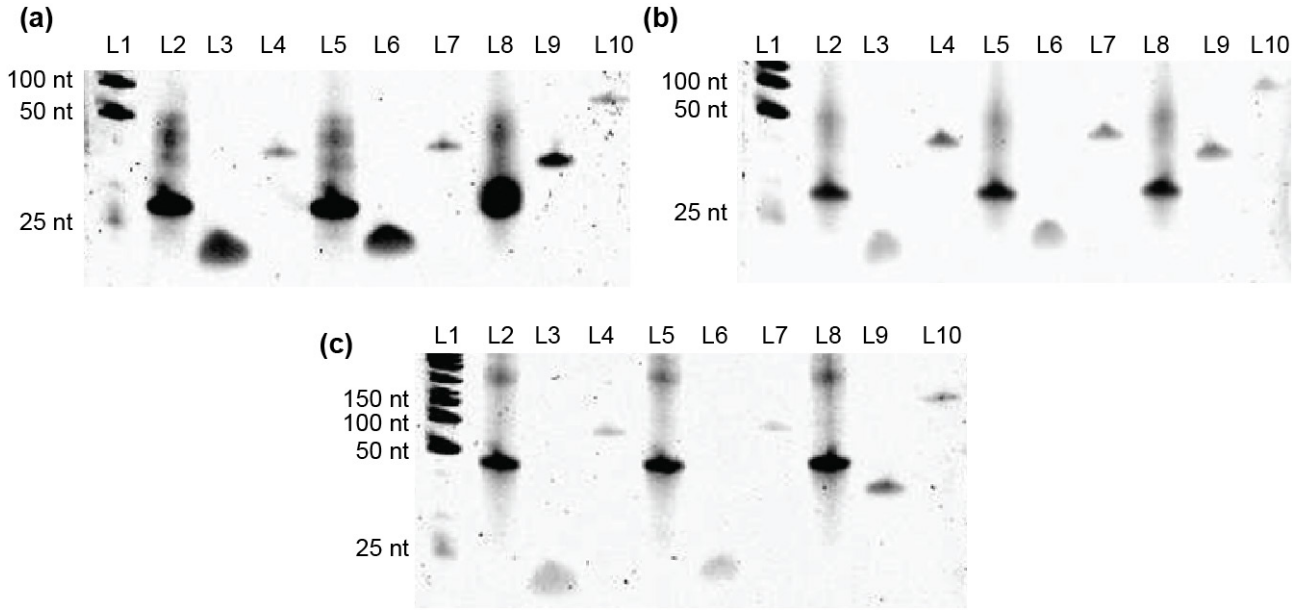


Figure S9.*Gel electrophoresis of purified RNA1-RNA9 (10% denaturing polyacrylamide gels).* ***(a)*** *Lane 1 is a DNA ladder. Lanes 2, 5 & 8 is RNA_S1 (higher molecular weight bands correspond to secondary structures. These bands reduce in intensity when heated to 80 ˚C prior to gel electrophoretic analysis. An exemplar of this is shown in Figure S6). Lane 3 is RNA_S4. Lane 4 is RNA1. Lane 6 is RNA_S5. Lane 7 is RNA2. Lane 9 is RNA_S6. Lanes 10 is RNA3.* ***(b)*** *Lane 1 is a DNA ladder. Lanes 2, 5 & 8 is RNA_S2. Lane 3 is RNA_S4. Lane 4 is RNA4. Lane 6 is RNA_S5. Lane 7 is RNA5. Lane 9 is RNA_S6. Lane 10 is RNA6.* ***(c)*** *Lane 1 is a DNA ladder. Lanes 2, 5 & 8 is RNA_S3. Lane 3 is RNA_S4. Lane 4 is RNA7. Lane 6 is RNA_S5. Lane 7 is RNA8. Lane 9 is RNA_S6. Lane 10 is RNA9.*

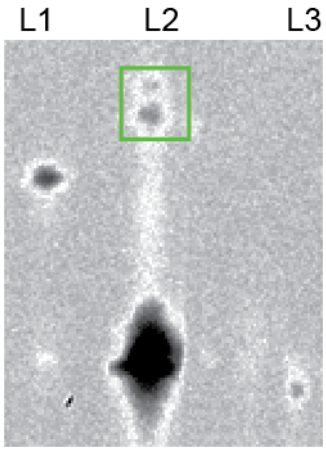


Figure S10. *Gel electrophoresis of purified RNA10 (10% denaturing polyacrylamide gel).Lane 1 shows click chemistry product RNA10. Lane 2 corresponds to RNA_S15. Lane 3corresponds to RNA_S9. Green box depicts higher order structure present in RNA_S15.*

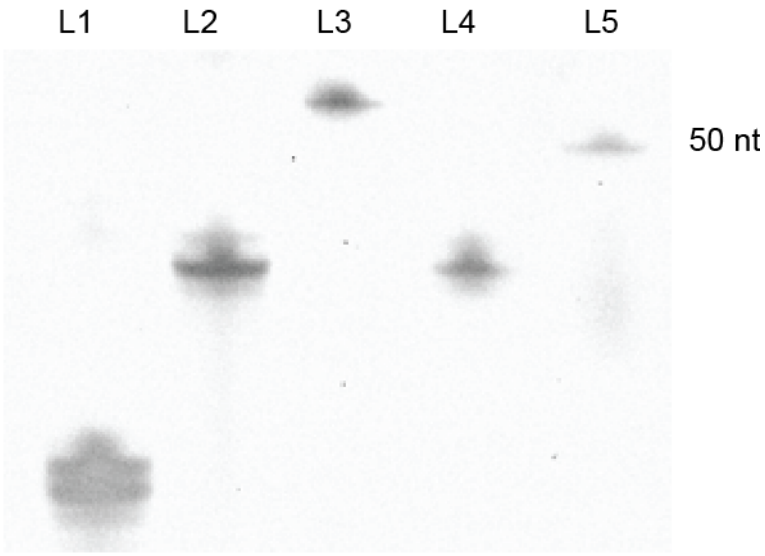


Figure S11. *Gel electrophoresis of purified RNA11 (20% denaturing polyacrylamide gel, heating samples to 80°C before loading). Lane 1 is RNA_10; Lane 2 is RNA_S8; Lane 3 is purified RNA11. Lane 4 is the recovered RNA_S8. Lane 5 is the DNA ladder.*

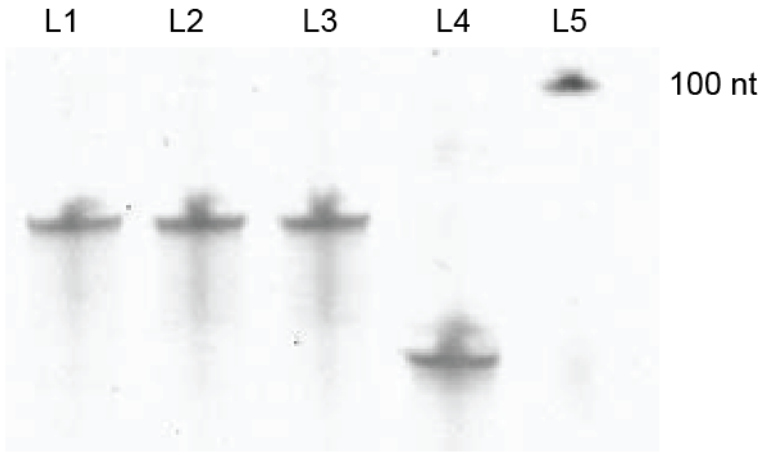


Figure S12. *Gel electrophoresis of purified RNA_S7, RNA13 and RNA14 (20% denaturing polyacrylamide gel). Lane 1 is RNA13; Lane 2 is RNA14; Lane 3 is purified RNA12. Lane 4 is RNA_S7. Lane 5 is the DNA ladder.*

5.0 Lipoic acid ligation

RNA_S16 (500 µL, 414 µM), RNA_S17 (500 µL, 587 µM), RNA_S18 (500 µL, 540 µM) and RNA_S19 (500 µL, 560 µM) were dissolved in sodium hydrogen carbonate (180µL, 0.025M, pH 8). A solution of lipoic acid-NHS [40 mg, 1.32 x 10^{-4} mol dissolved in 1.68 mL DNA grade acetonitrile. 420 µL was used][8] was then added to each 2'OMe RNA ON, vortexed and then left shaking overnight at 25 ^{o}C at 300 rpm. The products were concentrated *in vacuo*, desalted (GE healthcare NAP 25 column) and lyophilised.

6.0 Preparation of GNP-BSPP polyconjugates

GNP-BSPP conjugates were prepared according to a procedure described previously by Kumar et al.[9]

Bis (p-sulfonatophenyl)phenyl phosphine (BSPP, 6 mg, 1.12 x 10^{-5} mol) was added to an aqueous solution of citrate GNPs (20nm, 18 nm, 10 nm and 5 nm) purchased from BBInternational (10 ml), covered with foil and left to shake overnight at 200 rpm (25^{o}C). Sodium Chloride (2 mg, 3.41 x 10^{-5} mol) was then added to the BSPP coated GNPs. After each 2 mg addition of NaCl, the sample was vortexed until the solution turned purple. The GNP solution was split into 1 mL aliquots and centrifuged at 12,000 rpm for 10 min at RT. The supernatant was removed from each aliquot and the GNP aliquots combined in a BSPP/water solution (200 µL, 2.5 mM). Methanol (200 µL) was then added ,vortexed and centrifuged again at 12,000 rpm for 10min at RT. The supernatant was removed again and the GNP-BSPP conjugates were re-suspended in an aqueous solution of BSPP (200 µL, 2.5mM) and stored at 5^{o}C.

7.0 Preparation of GNP-RNA polyconjugates

The protocol used for the preparation of GNP-RNA polyconjugates was based on a related protocol for the preparation of GNP-DNA conjugates reported by Hurst et al.[10]

TCEP (3.6 mg, $1.26x10^{-5}$ mol) was dissolved in 0.5 x TBE (48.30 µl, pH = 8.3) to a final concentration of 260 mM. A TCEP (5 µl, 260 mM, $1.3x10^{-6}$ mol) solution was then added to an aqueous solution of 2'OMe RNA14 (60 µl, 700 µM), RNA17 (60 µL, 700 µM) and peg-SH (60 µl, 700 µM), vortexed and left at 25 ^{o}C for 1hr mixing at 750 rpm. GNP-BSPP conjugates (50 µl, 1.74 µM) were aliquoted into three tubes. A solution of reduced 2'OMe RNA ONs (Table 3c) in 0.5 x TBE, 0.015% SDS (300 µl) was added to the GNP-BSPP solutions, vortexed and then left at 25 ^{o}C, 750 rpm for 20 min. A solution of 2 M NaCl in 0.5 x TBE, 0.015% SDS (10 µl to 30 µl) was then added every 20 min to gradually increase the salt concentration from 50 mM to 700 mM. After each salt addition the samples were vortexed, sonicated for 10 seconds, vortexed again and then returned to the shaker for 20 min, 750 rpm at 25 ^{o}C. If the samples turned purple/blue (i.e. a sign of aggregation) before the 700 mM NaCl addition, then 0.5 x TBE, 0.015% SDS was added until the solution turned red again and left overnight at 25 ^{o}C, 750 rpm. For samples that didn't aggregate after the 700 mM addition of 2 M NaCl, these samples were left for a further 20 min to check for aggregation (turn purple/blue). Samples that retained their red colour were left overnight at 25 ^{o}C, 750 rpm. After overnight incubation, the reactions were vortexed and then centrifuged for 10 min at 10 ^{o}C, 13,200 rpm. The supernatants were removed and the GNP-RNA conjugates were re-suspended in 0.02% SDS in ddH_2O (300 µl). The samples were vortexed again followed by centrifugation at 10 ^{o}C for 10 min at 13,200 rpm. This process was repeated four times; re-suspending each time in 0.02% SDS in ddH_2O (300 µl). After washing four times, the GNP-RNA conjugates were centrifuged again at 13,200 rpm, 10 ^{o}C for 10min. The supernatants were discarded and the samples re-dissolved in 0.02% SDS in ddH_2O (50 µL). Polyconjugated particles were examined by 2% agarose gel (gels ran at 100 V for 1 hour, Figure S9). For the preparation of GNP9, GNP12, GNP15 and GNP18, a commercially available thiol (*O*-(2-

Mercaptoethyl)-*O*′-methyl-hexa(ethylene glycol. Sigma Aldrich catalogue number 672572) was used to passivate the surface.

8.0 Colloidal stability of RNA-GNP conjugates (GNP1-18)

8.1 *18 nm GNP.*

GNP1-2 and GNP4-7 were stable up to 700 mM NaCl. GNP3, GNP8 and GNP9 were stable up to 400 mM NaCl.

8.2 *20 nm GNP.*

GNP10-11 were stable at 700 mM NaCl. GNP12 was stable up to 100 mM NaCl.

8.3 *10 nm GNP.*

GNP13-15 were stable at 700 mM NaCl.

8.4 5 nm GNP.

GNP16-18 also showed no aggregation at NaCl concentrations as high as 700 mM even after overnight incubation.

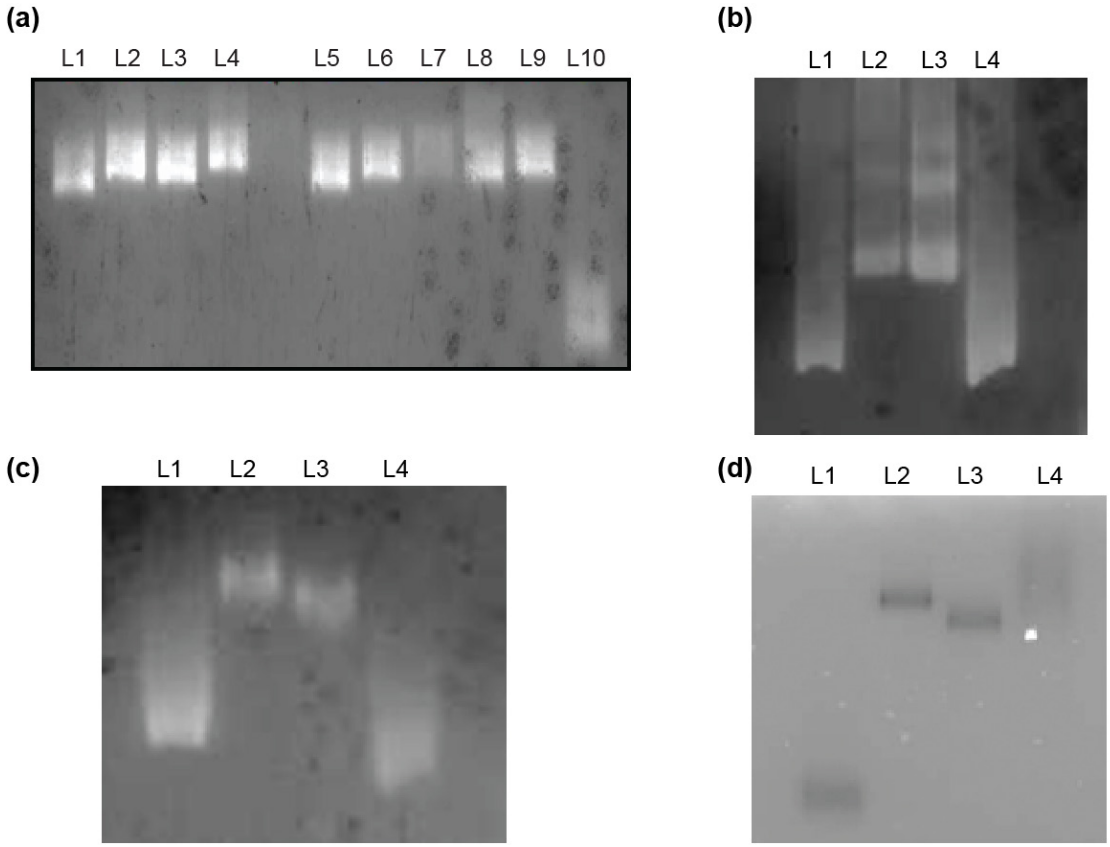


Figure S13. *Agarose (2 %) gel electrophoresis of* ***(a)*** *RNA-GNP (18nm) conjugates. Lane 1: GNP1. Lane 2: GNP2. Lane 3: GNP3. Lane 4: GNP4. Lane 5: GNP5. Lane 6: GNP6. Lane 7: GNP6. Lane 8: GNP7. Lane 9: GNP8. Lane 10: GNP9.* ***(b)*** *RNA-GNP (20 nm) conjugates. Lane 1 is BSPP coated 20 nm Au NPs. Lane 2: GNP10. Lane 3: GNP11. Lane 4: GNP12.* ***(c)*** *RNA-GNP (10 nm). Lane 1: BSPP-GNP (10 nm). Lane 2: GNP13. Lane 3: GNP14. Lane 4:GNP15.* ***(d)*** *RNA-GNP (5nm) conjugates. Lane 1: BSPP coated 5nm GNPs. Lane 2: GNP16. Lane 3: GNP17. Lane 4:GNP18.*

9.0 Determination of surface coverage of RNA ONs on 5 nm GNPs

Gold Particle Characterization:

9.1 UV-vis Spectroscopy

UV-vis spectra were recorded on a Thermo Spectronic spectrometer, Genesys 20-S using quartz cuvettes with 1 cm path length, recording across a 400-800 nm wavelength range. Surface plasmon resonance (SPR) peak profiles showed no aggregation of the particles after fictionalisation with RNA ON ligands. (λ_{max}= 514 – 516 nm). Concentrations of GNP dispersions were estimated according to a previously reported literature procedure.[11]

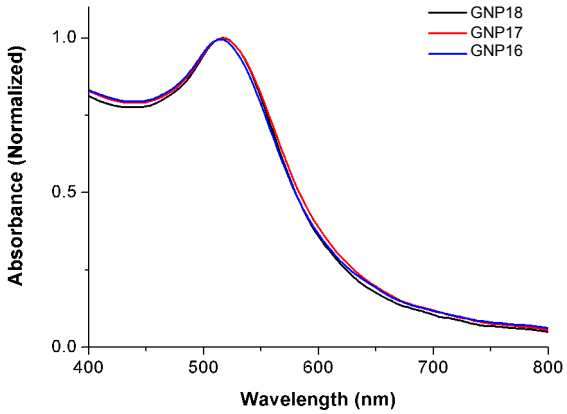


Figure S14. *UV-vis spectra of GNP16, GNP17 and GNP18 colloidal dispersions in milli-Q water (λ_{max} =514 – 516 nm).*

9.2 ICP-AES Analysis: Determination of Au and S content

ICP-AES analysis was performed on a Side-on-Plasma (SOP) ICP-OES Spectro Ciros spectrometer. For calibration, Au and S standards (100 ppm) were purchased from Sigma Aldrich, and dissolved in *aqua regia* matrix. For the analysis of Au and S content in the samples, 90 µL of GNP dispersions (GNP16, GNP17, GNP18 of the following nanoparticle concentrations: 1.95, 2.02 and 2.17 µM respectively) were dissolved in 5 mL freshly prepared *aqua regia* (caution: corrosive!!) and digested for 72 h. The Au and S content found were as follows: GNP18- 18.73 ppm Au and 0.68 ppm S; GNP17-19.16 ppm Au and 0.76 ppm S; GNP16-10.28 ppm Au and 0.34 ppm S.

9.3 Differential Centrifugal Sedimentation (DCS) Analysis of GNPs[12]

GNP size and their distribution were analyzed using Differential Centrifugal Sedimentation (DCS). A CPS disc centrifuge DC24000 (CPS Instruments Inc.) was used. As a gradient fluid, 8–24 wt % sucrose solution in milli-Q water was used and filled successively in nine steps into the disc, starting with the dilution of highest density. For analysis, the disc

rotation speed was set to 24 000 rpm. Calibration was performed with polyvinylchloride (PVC) particles (0.263 µm) used as calibration standard (Analytik Ltd.) and injected before each analysis. All samples were sonicated for 15 s before injection into the disc centrifuge. DCS measurements showed monodisperse distributions of functionalized GNPs (in a measured size range between 2-50 nm), resulting in 3.5 nm (GNP16) and 3.8 nm (GNP17) particle diameter, referred to the particle density of gold (19.3 gcm^{-1}). The analysis showed a significant shift in the particle size (1.9 and 1.6 nm, respectively) indicating successful conjugation for GNP16 and GNP17 with respect to a PEGylated-GNP (GNP18, d_{DCS}=5.4 nm).[12]

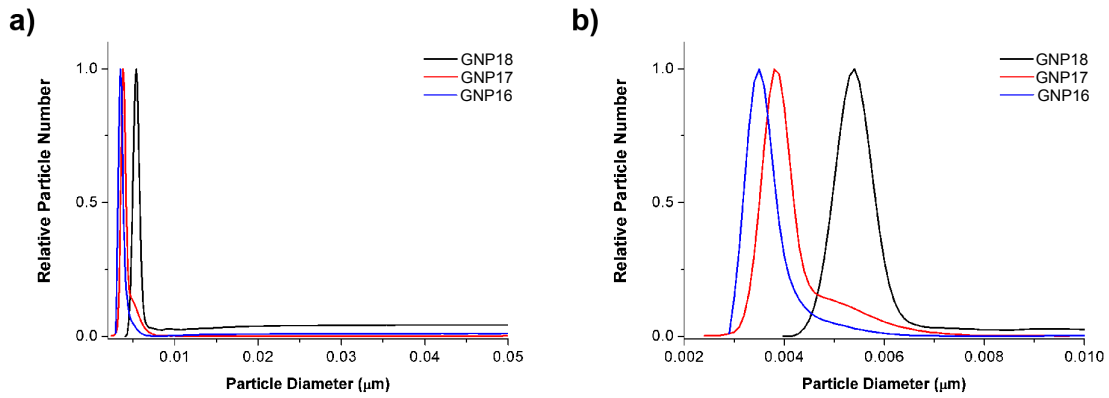


Figure S15. *Differential centrifugal sedimentation (DCS) particle size measurements showing distributions of GNPs (samples GNP16, GNP17 and GNP18) measured in a) 2-50 nm and b) 2-10nm size range.*

9.4 Transmission Electron Microscopy (TEM)

AFEI Tecnai Spirit TEM (operating at 120 kV) was utilized for imaging of the GNPs. For the determination of the particle mean diameter, images were processed using AnalySIS software (Soft Imaging Systems). Samples for TEM imaging were prepared by evaporating ca. 10 µL of the colloidal dispersion onto carbon coated copper grids (Agar Scientific), 400 mesh. Particle size distribution charts were obtained by counting a population of 150 nanoparticles.

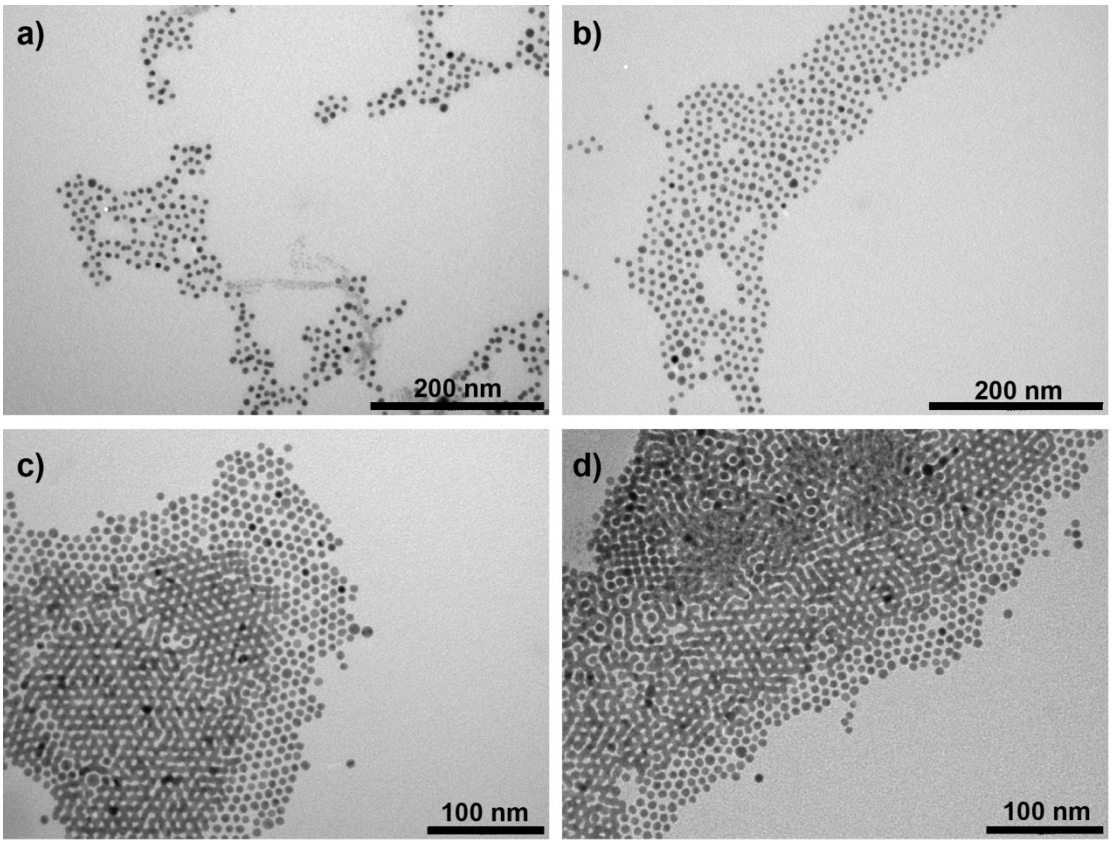


Figure S16.Transmission electron micrographs of (a) PEGylated GNPs (GNP18) and RNA-functionalized GNPs (b) GNP17; (c, d) GNP16.

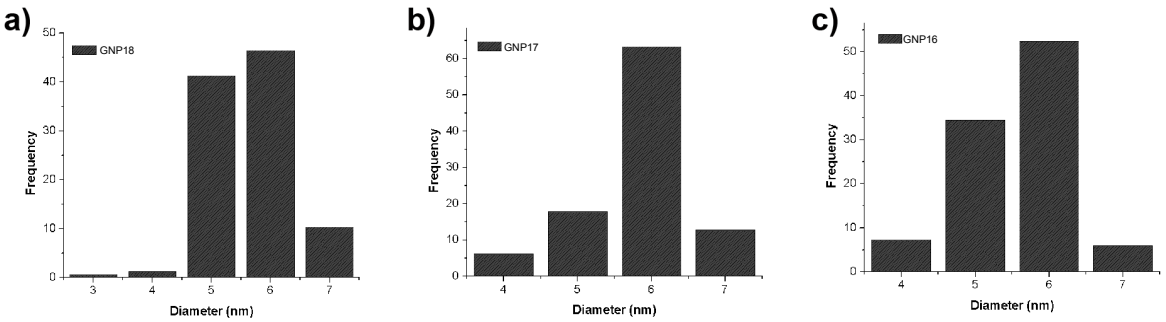


Figure S17.Particle size distribution (PSD) charts obtained counting a population of 150 nanoparticles from TEM micrographs of samples (a) GNP18, (b) GNP17 and (c) GNP16.

10.0 Transcription and splicing assays

10.1 RNA splicing assays using tripartite RNA ONs.

RNA tripartite structures (2 μM) were treated with ^{32}P labelled RNA (1 μl) and Kglu (2 μl, 250 mM) before adding water to make up to a total volume of 4.9 μl. The reactions were heated to 80 ^{o}C followed by slow cooling to RT over 35 minutes. After incubation the samples were placed on ice and then transferred onto 1.5 mL Eppendorf tubes. The master mix of ATP, $MgCl_2$ and creatine phosphate (CrPi) was combined with nuclear extract (4 μl, 40% nuclear extract in the final splicing mixture) and then 5.1 μL was added to each reaction. The reactions were vortexed before being placed in a 30^{o}C water bath for 2 hours. Samples taken during the time course were placed on dry ice to stop the reaction. Once the splicing reaction was complete, proteinase K (10 μl, 1 in 25 dilutions in PK buffer) was added to each of the reactions and heated at 37 ^{o}C for 30 mins. After 30 mins the transcripts were precipitated with ethanol. The precipitates were dissolved in 10 uL formamide and dyes prior to gel electrophoretic analysis.

The products were analysed by running a 6% denaturing polyacrylamide gel at 30 W for $1^3/_4$ hours. The plates were then separated and the gel transferred to 3M^{TM} paper. The open side of the gel was covered in saran wrap before the gel was dried for one hour. Once dried the gel was exposed to a phosphor screen overnight and then imaged.

(a)

exon 7 in/(exon 7 in + exon 7 ex)		
	50nM	100nM
control	0.09	0.11
RNA1	0.17	0.20
RNA2	0.20	0.21
RNA3	0.23	0.21
RNA4	0.35	0.40
RNA5	0.20	0.35
RNA6	0.21	0.19
RNA7	0.23	0.25
RNA8	0.21	0.19
RNA9	0.20	0.16
GGA-O	0.20	0.26

(b)

exon 7 in/(exon 7 in + exon 7 ex)	
	100nM
control	0.05
RNA4	0.15
RNA5	0.13
RNA10	0.08
RNA11	0.07
GGA-O	0.09

(c)

exon 7 in/(exon 7 in + exon 7 ex)	
	mean
Control	0.14
RNA4	0.55
RNA12	0.36
RNA13	0.44
GGA-O	0.37

(d)

Exon 7 in/(exon 7 in + exon 7 ex)			
	200nM	100nM	50nM
Control	0.11		
GGA	0.88	0.79	0.67
RNA14	0.86	0.81	0.64
GGA-O	0.30	0.17	0.10
RNA13	0.31	0.32	0.15

Table S1. *Proportions of exon 7 inclusion in the presence of RNA tripartite structures.* ***(a)*** *SMN2 splicing in the presence of RNA1-9 against GGA-O (a 2'OMe control).* ***(b)*** *SMN2 splicing in the presence of RNA4,5,10 and 11 tripartite sequences against GGA-O (a 2'OMe control).* ***(c)*** *SMN2 splicing in the presence of RNA4, 12 and 13.tripartite sequences against GGA-O (a 2'OMe control).* ***(d)****SMN2 splicing in the presence of RNA13 and RNA14 tripartite sequences against GGA and GGA-O.*

10.2 RNA splicing assays using GNP-RNA conjugates

Nuclear Extract was dialysed for 2 hr in the cold room with DTT free buffer D. DTT free buffer D was made up from:-

800 µl 1M HEPES buffer pH 8 (20mM final concentration)

5 ml 80% Glycerol (10% final concentration)

16 µl 0.5M EDTA (0.2mM final concentration)

4 ml 1M KCl (100mM final concentration)

30.19 ml water

During the dialysis, relevant GNP stocks were prepared from 1 µM of a GNP-RNA conjugate. 1 µl aliquots of these stocks were then treated with ^{32}P-labelled RNA (0.5 µl) and heated in a 30 ^{o}C oven for 10 min. After incubation the samples were placed on ice for 10 min. While the samples were on ice a master mix was prepared. The master mix consisted of:

6.1 µL 100 mM ATP

15 µL 0.5 M CrPi

9.5 µL 80 mM $MgCl_2$

3.8 µL 1M HEPES KOH pH 7.5

3.8 µL 1% Nonyl phenoxypolyethoxylethanol (NP40)

75 µL Potassium Glutamate (Kglu)

After dialysis the dialysed nuclear extract (150 µL) was added to the master mix, vortexed and then 3.5 µL added to each reaction. The reactions were vortexed before being placed in a 30 ^{o}C oven for 2 hours. Once the splicing reaction was complete proteinase K [10 µL, 1 in 25 dilutions in proteinase K(PK) buffer] was added to each of the reactions and heated to 37 ^{o}C for 30 min. After 30 minutes the samples were precipitated with ethanol. The samples were then treated with 10 µL formamide dyes prior to gel electrophoretic analysis.

The products were analysed by running a 6% denaturing polyacrylamide gel at 30 W for $1^3/_4$ hr. The plates were then separated and the gel transferred to 3M™ paper. The open side of the gel was covered in saran wrap before the gel was dried on a gel drier for approximately an hour. The gel was then exposed to a phosphorus screen overnight in and imaged using a Packard Cyclone™ imager.

11.0 References

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