Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Efficient RNA synthesis by *in vitro* transcription of a triazole-modified DNA template

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General method for oligonucleotide synthesis and purification

Standard DNA phosphoramidites, solid supports and additional reagents were purchased from Link Technologies and Applied Biosystems. Oligonucleotides were synthesized on an Applied Biosystems 394 automated DNA/ RNA synthesizer using a standard 0.2 or 1.0 μmole phosphoramidite cycle of acid-catalyzed detritylation, coupling, capping, and iodine oxidation. Stepwise coupling efficiencies and overall yields were determined by the automated trityl cation conductivity monitoring facility and in all cases were >98.0%. All β-cyanoethyl phosphoramidite monomers were dissolved in anhydrous acetonitrile to a concentration of 0.1 M immediately prior to use. The coupling time for normal A, G, C, and T monomers was 35 s, whereas the coupling time for the 5'-iodo dT phosphoramidite monomer was extended to 6 min. Cleavage of oligonucleotides from the solid support and deprotection was achieved by exposure to concentrated aqueous ammonia solution for 60 min at room temperature followed by heating in a sealed tube for 5 hr at 55 °C. The oligonucleotides were purified by reversed-phase HPLC on a Gilson system using an XBridge™ BEH300 Prep C18 10 µM 10x250 mm column (Waters) with a gradient of acetonitrile in ammonium acetate (0% to 50% buffer B over 30 min, flow rate 4 mL/min), buffer A: 0.1 M ammonium acetate, pH 7.0, buffer B: 0.1 M ammonium acetate, pH 7.0, with 50% acetonitrile. Elution was monitored by UV absorption at 305 or 295 nm. After HPLC purification, oligonucleotides were desalted using NAP-10 columns and analyzed by gel electrophoresis.

Synthesis of the 3′-alkyne oligonucleotides

3′-Alkyne oligonucleotides were synthesized on the 1.0 µmole scale by the attachment of 5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-propargyl-5-methyldeoxycytidine to a solid support (33 µmole/g loading, AM polystyrene, Applied Biosystems) according to the published method.[1] The resin was packed into a twist column (Glen Research) then used to assemble the required sequence in the 3′- to 5′-direction by standard phosphoramidite oligonucleotide synthesis. The oligonucleotides were then cleaved and deprotected by exposure to concentrated aqueous ammonia for 60 min at room temperature followed by heating in a sealed tube for 5 h at 55 °C before purification as outlined above in the general method.

ii) Synthesis of the 5′-azide oligonucleotides

Oligonucleotides were assembled on the 1.0 µmole scale (trityl-off) as described above in the general method with 5′-iodo-dT using the commercially available 5′-iodo dT phosphoramidite monomer (Glen Research). To convert the 5′-iodo dT to 5′-azido dT, sodium azide (50 mg) was suspended in dry DMF (1 mL), heated for 10 min at 70 °C then cooled down and the supernatant taken up into a 1 mL syringe, passed back and forth through the column and left for 5 h at 55 °C. The column was then washed with DMF and acetonitrile and dried by the passage of a stream of argon gas. The resultant 5′-azide oligonucleotides were cleaved from the solid support and deprotected by exposure to concentrated aqueous ammonia solution for 60 min at room temperature followed by heating in a sealed tube for 6 h at 55 °C and purified by HPLC as described above in the general method.

Synthesis of the triazole templates (ODN-11) and (ODN-12) (83-mer) for transcription

A solution of Cu^{I} click catalyst was prepared from *tris*-hydroxypropyltriazole ligand[2] (2.8 µmole in 0.2 M NaCl, 88.0 µL), sodium ascorbate (4.0 µmole in 0.2 M NaCl, 8.0 µL) and $CuSO_4.5H_2O$ (0.4 µmole in 0.2 M NaCl, 4.0 µL). The two oligonucleotides (azide and alkyne), and the complementary splint (20.0 nmole of each) in 0.2 M NaCl (150.0 µL) were annealed by heating at 90 °C for 5 min then cooled down slowly to room temperature. The above Cu^{I} click catalyst was added to the annealed oligonucleotides and the mixture was kept at room temperature for 2 hr. Reagents were removed by NAP-10 gel-filtration and the ligated product was analysed and purified by denaturing 12% polyacrylamide gel electrophoresis.

Transcription of the triazole (triazole linkage in the coding region) and unmodified templates with long and short coding strands

The DNA template (triazole ODN-11) or (unmodified ODN-2) and the coding strand (long ODN-1) or (short ODN-3) (39.0 pmole of each in 8.0 µL water) was added to a solution of the buffer* (5X, 4.0 µL) and rNTP (25.0 mM of each rNTP, 6.0 µL). The mixtures were vortexed and the RiboMAX large scale RNA production system-T7 (2.0 µL) was added. The reactions were then heated at 37 °C for the desired time (3

reactions were conducted at 4 hr, 1 hr and 15 min, and timed to finish simultaneously then mixed with formamide and loaded immediately onto 10 % polyacrylamide gel.

Attempted transcription of the triazole template (triazole linkage on the promoter region) with long and short coding strands

Attempts to transcribe the triazole template (ODN-12) with the triazole in the promoter region, using both long (ODN-1) and short (ODN-3) coding strands under the previous conditions failed to give any product. The reactions were also attempted in presence of additional $MgCl_2$ (10 mM or 1 mM), spermine tetrachloride (0.5 mM) or a mixture of both, and also failed to give any product.

Transcription of the triazole and unmodified templates with the long coding strand for mass spectrometry

A solution of the DNA template (triazole ODN-11) or (unmodified ODN-2) and the long coding strand (ODN-1) (0.195 nmole, (3.8 μg) of each in 40.0 μL water) was added to a solution of the buffer* (5X, 20.0 μL) and rNTP (25.0 mM of each rNTP, 30.0 μL). The mixtures were vortexed and the RiboMAX large scale RNA production system-T7 (10.0 μL) was added. The reactions were then heated at 37 °C for 4 hrs followed by heating at 65 °C for 10 min to denature the enzyme. 2X NAP-25 gel-filtration columns were used, for each reaction product, to desalt and remove the excess rNTP. After gel-filtration, the absorbance was measured at 260 to quantify the transcription product. 373.0 μg (19.0 nmole) of RNA transcript was formed from the native template (ODN-2) whereas 288.0 μg (14.7 nmole) was formed from the triazole template (ODN-11).

Quantification of the RNA transcribed from the triazole template (ODN-11) using the gel-image analysis (GeneTools, SynGene) was done for 12 different reactions on separate gels and gave 75% ± 5% for 3hr incubation and 81% ± 5% for 4hr incubation relative to the amount of RNA obtained from the unmodified template (ODN-2). A total of 8 reactions were carried out using the long coding strand ODN-1 and 4 reactions were performed using the short coding strand ODN-3.

*5X buffer (was provided with the enzyme): 400 mM HEPES-KOH (pH 7.5), 120 mM $MgCl_2$, 10 mM spermidine and 200 mM DTT.

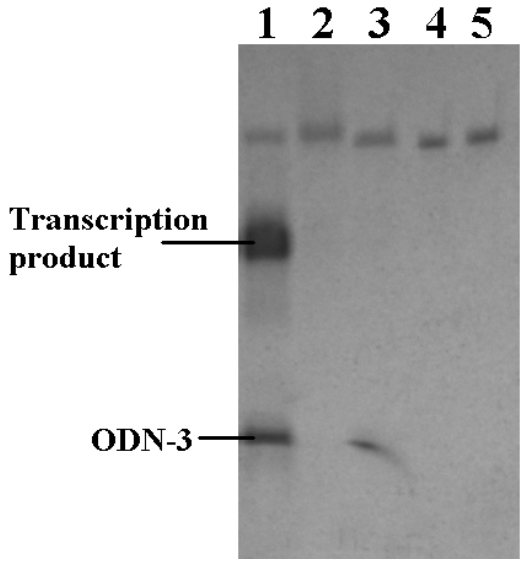

Figure S1. 10% polyacrylamide gel for transcription of 83-mer unmodified and click templates with the modification in the coding region (ODN-11) or in the promoter region (ODN-12). Lane 1: transcription using 83-mer click template (ODN-11) and 35-mer short coding strand (ODN-3); lane 2: attempted transcription using 83-mer click template (ODN-12) and 83-mer long coding strand (ODN-1); lane 3: attempted transcription using click template (ODN-12) and short coding strand (ODN-3); lane 4: control click template (ODN-11); lane 5: control click template (ODN-12).

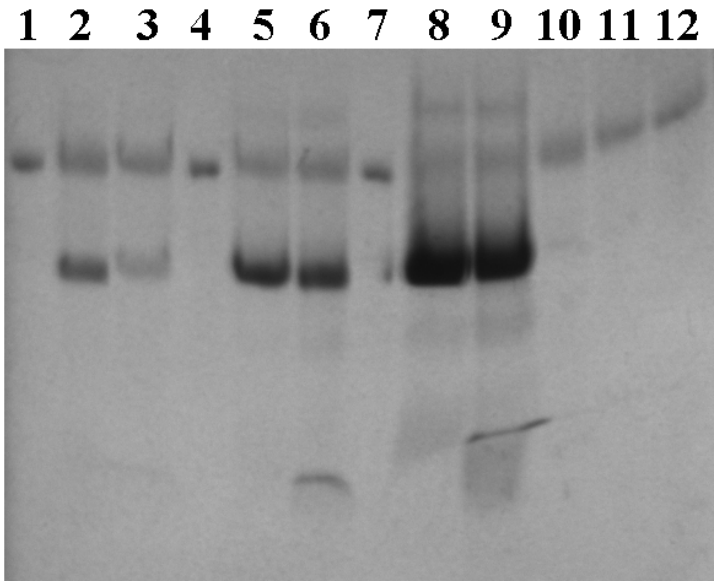

Figure S2. 10% polyacrylamide gel for transcription of 83-mer unmodified and click templates with coding strand (ODN-1). Lane 1: control coding strand (ODN-1); lane 2: transcription using unmodified template (ODN-2) 15 min; lane 3: transcription using click template (ODN-11) 15 min; lane 4: control coding strand (ODN-1); lane 5: transcription using unmodified template (ODN-2) 1 hr; lane 6: transcription using click template (ODN-11) 1hr; lane 7: control coding strand (ODN-1); lane 8: transcription using unmodified template (ODN-2) 4 hr, lane 9; transcription using click template (ODN-11) 4 hr; lane 10: attempted transcription using click template (ODN-12) and long coding strand (ODN-1) in presence of $MgCl_2$ (10 mM) 4 hr; lane 11: attempted transcription using click template (ODN-12) and long coding strand (ODN-1) in presence of spermine tetrahydrochloride (0.5 mM) 4 hr; lane 12: attempted transcription using click template (ODN-12) and long coding strand (ODN-1) in presence of $MgCl_2$ (10 mM) and spermine tetrahydrochloride (0.5 mM) 4 hr.

Table S1: Oligonucleotides used in this study

Code	Oligonucleotide sequences (5'-3')
ODN-01	GCAACCTAATACGACTCACTATAgGGAGAATTTCTGGTGACGTTTGGCGGT ATCAGTTTTACTCCGTGACTGCTCTGCCGCCC native coding strand Transcription start (+1) lower case, promoter region underlined
ODN-02	GGGCGGCAGAGCAGTCACGGAGTAAAACTGATACCGCCAAACGTCACCAG AAA*TTCTCCCTATAGTGAGTCGTATTA*GGTTGC native template in which underlined region is transcribed, promoter in italics
ODN-03	GCAACCTAATACGACTCACTATAgGGAGAATTTCT native short coding strand Transcription start (+1) in lower case, promoter region underlined
ODN-04	pppGGGAGAAUUUCUGGUGACGUUUGGCGGUAUCAGUUUUACUCCGUGA CUGCUCUGCCGCCCc expected transcribed RNA
ODN-05	GGGCGGCAGAGCAGTCACGGAGTAAAA$^{Me}C^{k}$
ODN-06	GGGCGGCAGAGCAGTCACGGAGTAAAACTGATACCGCCAAACGTCACCAG AAATT$^{Me}C^{k}$
ODN-07	^{z}TGATACCGCCAAACGTCACCAGAAATTCTCCCTATAGTGAGTCGTATTAG GTTGC
ODN-08	^{z}TCCCTATAGTGAGTCGTATTAGGTTGC
ODN-09	GGTGACGTTTGGCGGTATCAGTTTTACTCCGTGACTGCT splint
ODN-10	ATACGACTCACTATAGGGAGAATTTCTGGTGACGTTTGG splint
ODN-11	GGGCGGCAGAGCAGTCACGGAGTAAAA$^{Me}C_t$TGATACCGCCAAACGTCACC AGAAA*TTCTCCCTATAGTGAGTCGTATTA*GGTTGC template with triazole downstream in which underlined region is transcribed, promoter in italics
ODN-12	GGGCGGCAGAGCAGTCACGGAGTAAAACTGATACCGCCAAACGTCACCAG AAA*TT$^{Me}C_t$TCCCTATAGTGAGTCGTATTA*GGTTGC template with triazole in promoter, in which underlined region should be transcribed, promoter in italics

^{z}= 5'-azide, ^{k} = 3'-propargyl, t = -triazole linkage, p = phosphate.

Table S2: Mass spectrometry of oligonucleotides used in this study

Code	Calc. Mass	Found. Mass
ODN-01	25509	25508
ODN-02	25652	25650
ODN-03	10723	10723
ODN-04	19726	19724 (N), 19726 (T)
ODN-05	8774	8771
ODN-06	17347	17344
ODN-07	16894	16891
ODN-08	8321	8319
ODN-09	12026	12025
ODN-10	12076	12074
ODN-11	25668	25666
ODN-12	25668	25665

N = mass of the transcribed RNA formed from the unmodified template (ODN-2). T = mass of transcribed RNA formed from the triazole template (ODN-11),
Mass spectra were recorded on a Bruker micrOTOF™ II focus ESI-TOF MS instrument in ES^{-} mode
A molecular weight calculator can be found at:
http://www.ambion.com/techlib/misc/oligo_calculator.html

1. A. H. El-Sagheer, A. P. Sanzone, R. Gao, A. Tavassoli and T. Brown, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, 108, 11338–11343.
2. T. R. Chan, R. Hilgraf, K. B. Sharpless and V. V. Fokin, *Org. Lett.*, 2004, 6, 2853-2855.