Luminescence method for detection of aflatoxin B1 using ATP-releasing nucleotides

Dongyue Hu, ‡[a] Shusen Xiao, ‡[b] Qiaqia Guo, [b] Rongrong Yue, [b] Demin Geng, [a] Debin Ji*[b]

a. College of Chemistry and Chemical Engineering, Qingdao University, Qingdao, 266071, PR China.
b. Institute of Molecular Sciences and Engineering, Institute of Frontier and Interdisciplinary Science, Shandong University, Qingdao, 266237, PR China.

E-mail: debinji@sdu.edu.cn

The detail of ARNs Synthesis

Preparation of the tributylammonium salts of nucleoside triphosphate.

The free acids or free acid monohydrates of AMP and dTTP was dissolved in distilled deionized water (50 mL) and titrated to pH 7.0 with a dilute solution of tri-n-butylammonium salt.[1] The solutions were concentrated by high vacuum rotary evaporation to approximately one-seventh the original volume and lyophilized. The lyophilized powder of the nucleotide was coevaporated with anhydrous DMF twice and kept under high vacuum for 3 h before the subsequent coupling reaction (below).

Using the above procedure to prepare the tributylamine salt of dGTP from commercially available dGTP disodium salt dihydrate resulted in its decomposition upon conversion to its free acid and titration with tetrabutylammonium hydroxide. Hence the tri-n-butylammonium salt was prepared using a procedure similar to that developed by Gibson and Leonard.[2] An aqueous solution of dGTP disodium salt dihydrate (0.5 g in 20 mL of distilled deionized water) was applied to a Dowex-50-W column (8−10 g) in its pyridinium form. The eluate was collected in a flask containing 20 mL of ethanol and 1.0 mL of tributylamine. The column was washed with water (3 × 30 mL) while collecting the eluate in the above-mentioned flask. The resulting solution was stirred for 10 min and then concentrated by high vacuum rotary evaporation to about one-seventh of the original volume and then lyophilized. The lyophilized powder of the nucleotide was coevaporated with anhydrous DMF twice and kept under high vacuum for 3 h before the subsequent coupling reaction (below).

General method for synthesis of ARNs.

The tributylamine salt of dGTP (35 mg, 30 μmol) or dTTP (32 mg, 30 μmol) was dissolved in 1.5 mL anhydrous DMF and then concentrated under high vacuum for three hours. Add 1.5 mL of anhydrous DMF in an argon atmosphere. To the solution, carbonyldiimidazole (CDI, 25 mg, 160 μmol) was added, and the mixture was stirred at room temperature for 5 h, after which 50 μL MeOH was added to quench the reaction. All solvents were removed under high vacuum for 3 h and the residue redissolved in 1.5 mL anhydrous DMF. The desired AMP tributylamine salt (27 mg, 40 μmol) and anhydrous $MgCl_2$ (3 mg) were added. The mixture was stirred for 72 h at room temperature. After the reaction is over, rinsed the product with an appropriate amount of acetone, collected the precipitate. It was purified by reverse-phase HPLC (RPHPLC) using a preparative C18 column using a gradient of acetonitrile and 50 mM triethylammonium acetate buffer (pH 7). After the separation by HPLC, the product was concentrated and lyophilized, and yielded the final product as a white powder.

Deoxyguanosine-5'-tetraphosphate-P4-5'-Adenosine (dGP_4A).

^{1}H NMR (600 MHz, D_2O) δ 8.25 (s, 1H), 7.96 (s, 1H), 7.78 (s, 1H), 5.95 (t, J = 7.6Hz, 1H), 5.86 (t, J = 7.6Hz, 1H),

4.56–4.54 (m, 1H), 4.51–4.49 (m, 1H), 4.38–4.36 (m, 1H), 4.20–4.19 (m, 1H), 4.13–4.11 (m, 2H), 4.03–3.97 (m, 3H), 2.51–2.46 (m, 1H), 2.28–2.24 (m, 1H). ^{31}P NMR (243 MHz, D_2O) δ -11.21, -11.25, -22.87, -22.99. MALDI–TOF–MS (negative ion): Calculated for $C_{20}H_{28}N_{10}O_{19}P_4$ $[M-H]^-$ 835.37; found m/z 835.86.

Thymidine-5'-tetraphosphate-P4-5'-Adenosine (dTP_4A)

^{1}H NMR (600 MHz, D_2O) δ 8.33 (s, 1H), 8.02 (s, 1H), 7.41 (s, 1H), 6.03 (t, J = 6.9Hz, 1H), 5.91 (d, J = 6.2Hz, 1H), 4.58 (brs, 1H), 4.44–4.39 (m, 2H), 4.22 (brs, 1H), 4.08–3.94 (m, 5H), 2.11–2.05 (m, 2H), 1.66 (s, 3H). ^{31}P NMR (243 MHz, D_2O) δ -11.30, -11.60, -22.94, -23.01. MALDI–TOF–MS (negative ion): Calculated for $C_{20}H_{28}N7O_{20}P_4$ $[M-H]^-$ 810.37; found m/z 810.80.

Table S1 Testing chimeric dinucleotides as substrates for luciferase. The ATP (1μM , 10 μL), dGP_4A (1μM , 10 μL), or dTP_4A (1μM , 10 μL) and luciferase reaction buffer (20 μL) were mixed in a 384-well plate. The signals were recorded by multimode reader and shown the 30 minutes values.

	ATP	dGP_4A	dTP_4A
Luminescence (a. u.)	35149	138	78

Values represent the mean of three replicates.

Table S2 Base sequence of oligonucleotides used in this experiment

Name	Sequence (5′ → 3′)
HP1	5′-CCGGGTGTGCGTAACGACAAACATATACCCACACAT GCTGAGGACGCACACCCGCTACCG-3′
HP2	5′-CCCCGCTACCGTAACGACAAACATATACCCACACAT GCTGAGGACGGTAGCGGGTGTGCG-3′
Aptamer	5′-GTTGGGCACGTGTTGTCTCTCTGTGTCTCGTGCCCT TCGCTAGGCCCACAAAAAAAA-biotin-3′
T-DNA	5′-ACAACACGTGCCCAACATGTGTGGGTATATGTTTGT CGTTAAA-3′

Table S3 Comparison with the previous works for AFB1 detection

Detection method	Linear range	Limit of detection (LOD)	Ref.
Fluorescence	10-400 nM	3.4 nM	[3]
Fluorescence	1.6-160 μM	1.4 nM	[4]
Chemiluminescence (CL)	80-270 nM	0.5 nM	[5]
Chemiluminescence (CL)	0.1-10 ng/mL	0.11 ng/mL	[6]
Chemiluminescence (CL)	0.5-40 ng/mL	0.2 ng/mL	[7]
Chemiluminescence (CL)	0.001-30 nM	0.3 pM	This work

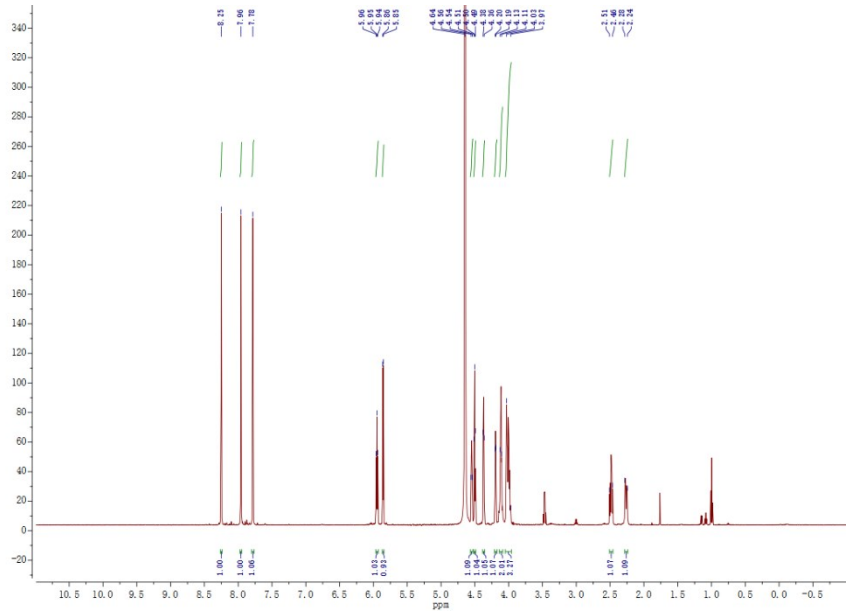

Figure S1. ^{1}H NMR spectrum of dGP$_4$A

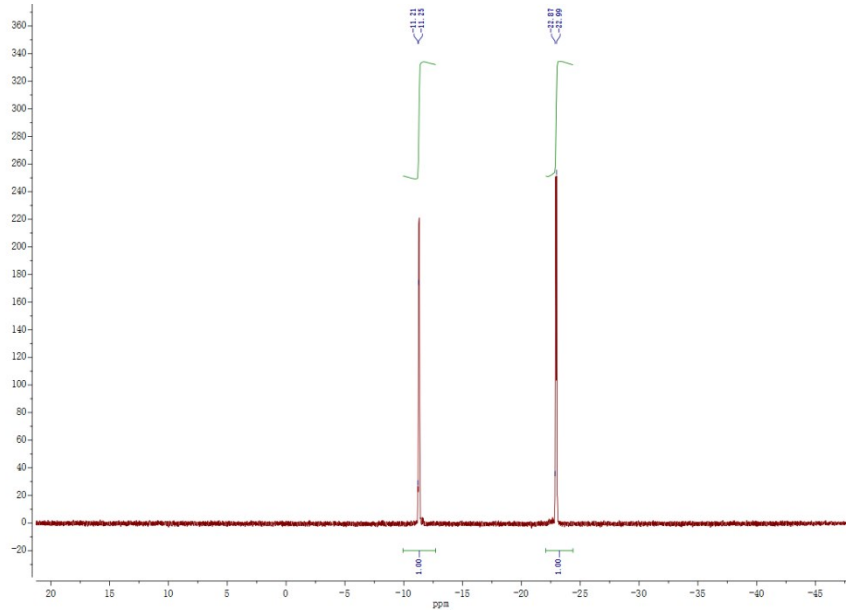

Figure S2. ^{31}P NMR spectrum of dGP$_4$A

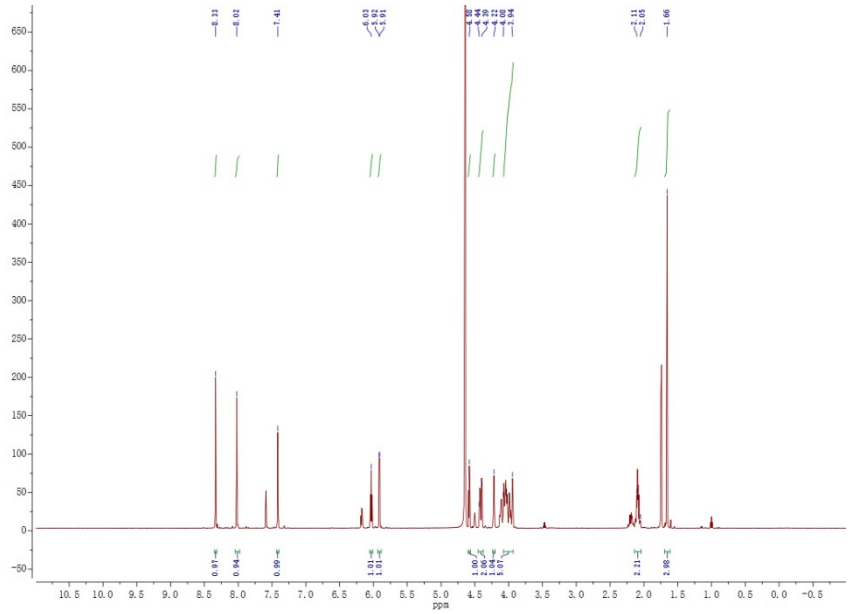

Figure S3. ^{1}H NMR spectrum of dTP_4A

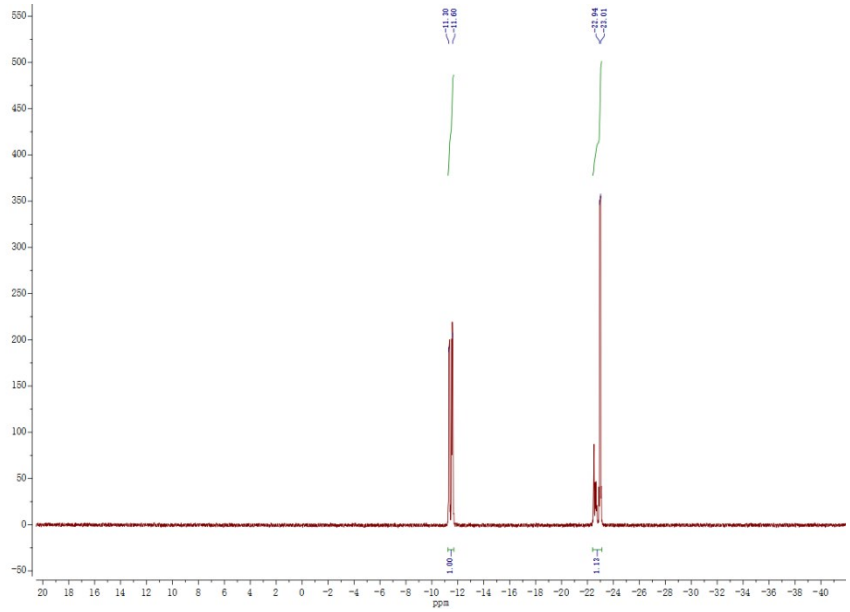

Figure S4. ^{31}P NMR spectrum of dTP_4A

References

1 dTTP was purchased as its sodium salt and was converted into its free acid using a Dowex-50W ion exchange column (H^+ form) column before conversion to its tetrabutylammonium salt. AMP were purchased as their monoacid hydrates.

2 Gibson, K. J.; Leonard, N. J. *Biochemistry*, 1984, **23**, 78-85.

3 F.S. Sabet, M. Hosseini, H. Khabbaz, et al. *Food Chem.* 2017, **220**, 527–532.

4 Z. Lu, X. Chen, Y. Wang, X. Zheng, et al. *Microsc. Acta,* 2015, **182**, 571–578.

5 M. Hosseini, H. Khabbaz, M. Dadmehr, et al. *Acta Chim. Slov.* 2015, **62**, 721–728.

6 W. B. Shim, H. Mun, H. A. Joung, et al. *Food Contr.* 2014, **36**, 30–35.

7 Y. Yao, H. Wang, X. Wang, et al. *Talanta* .2019, **201**, 52–57.