A 1 μmole synthesis of compound 7 on a controlled pore glass (CPG) was achieved on an AB394 DNA synthesizer following standard DNA automation protocol (DMT-off mode). The solid-supported dinucleotide 7 was transferred to a 5 mL round bottom flask and a solution of 10% triethylamine in acetonitrile (1 mL) was added. The mixture was stirred at room temperature for 2 h and filtered over a sintered funnel. The solvent was evaporated and compound 8 was cyclized following standard literature procedure. Briefly, each compound was treated with 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MSNT, 0.1M in pyridine) and stirred for 24 h. The solvent was evaporated and aqueous ammonia (1 mL, 28%) was added and the mixture stirred at 55 °C for 8h. Purification was done on a C18 reverse phase column (mobile phase, TEA buffer and acetonitrile or methanol). For the synthesis of c-di-GMP, the TBDMS protecting groups were removed with HF.TEA after the ammonia deprotection. The identities of our compounds were confirmed via ESI MS (see Figures 1-3) and co-elution with enzymatically synthesized c-di-GMP.
ESI (-ve)

Figure 2

ESI (-ve)

Figure 3