

A 1 μ mole 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.¹ 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)

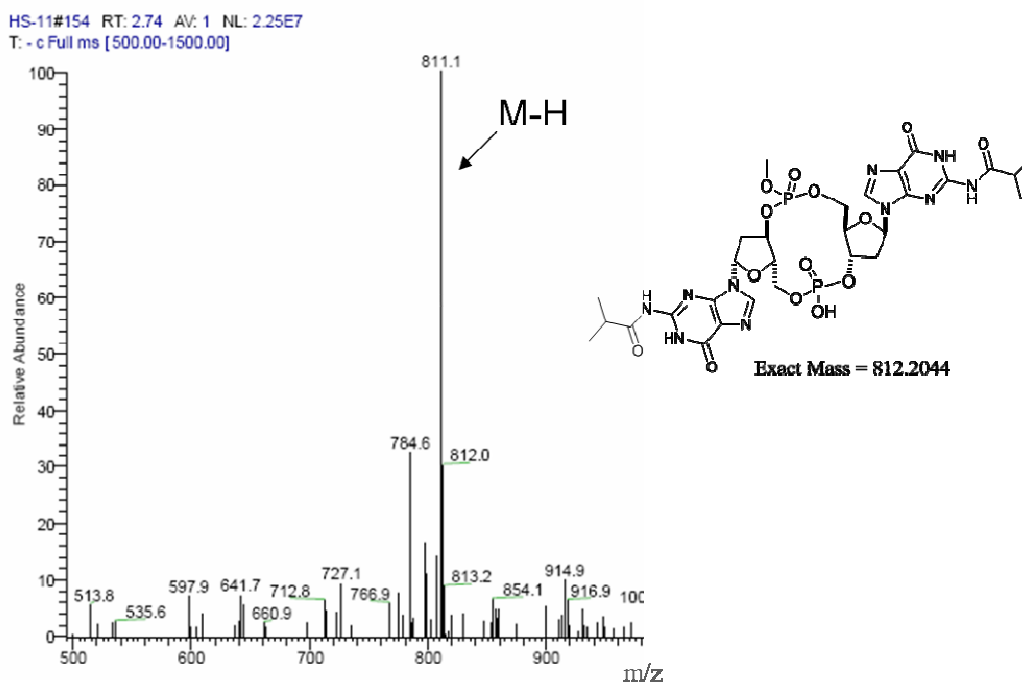


Figure 1

ESI (-ve)

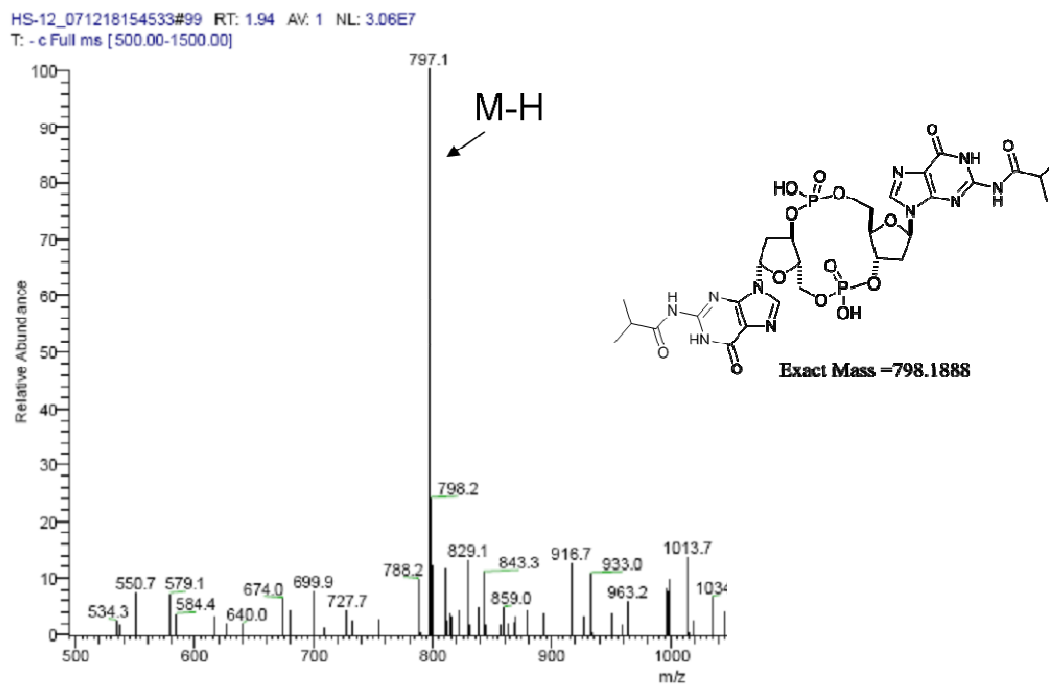


Figure 2

ESI (-ve)

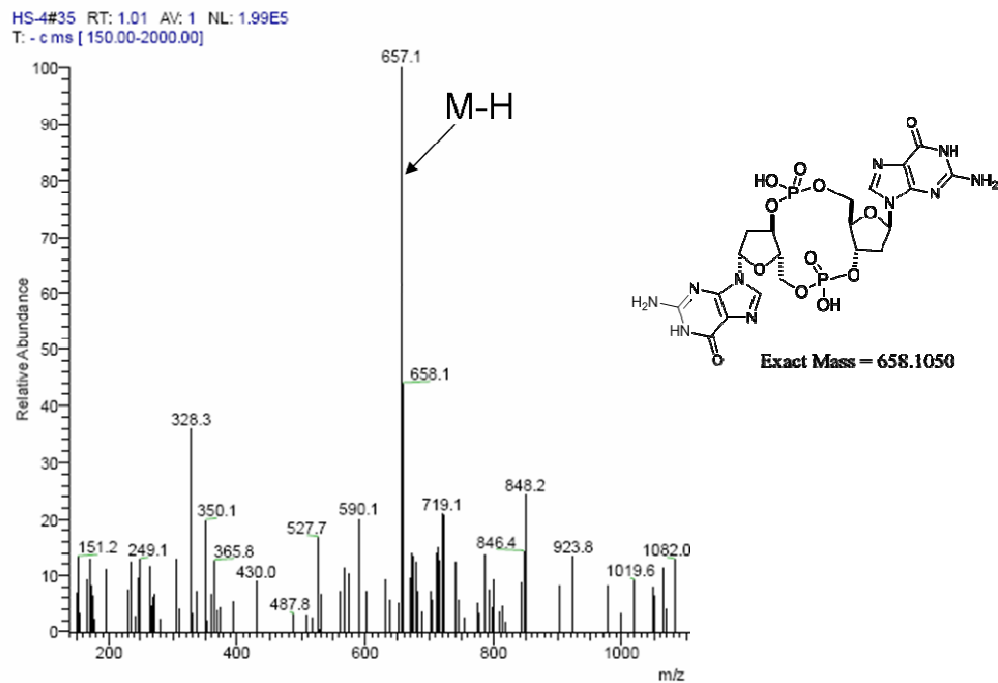


Figure 3

Supplementary Material (ESI) for Molecular BioSystems
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¹ a) Y. Hayakawa, R. Nagata, A. Hirata, M. Hyodo and R. Kawai, *Tetrahedron* 2003, **59**, 6465-6471; b) E. Alazzouzi, N. Escaja, A. Grandas and E. Pedroso, *Angew. Chem. Int. Ed.* 1997, **36**, 1506-1508.