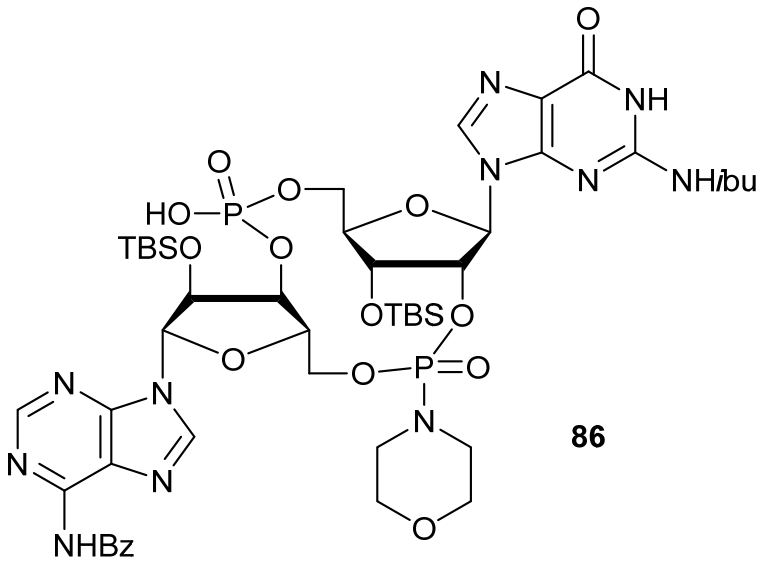
Step 1: phosphoramidate 44



86

[0326] To a solution of **C3** (16 mg, 0.014 mmol, co-evaporation with Py 1 mL × 3) in Py (0.5 mL) is added DMOCP (10.4 mg, 0.056 mmol). After stirring for 15 minutes, NIS (4.1 mg, 0.0183 mmol) and morpholine (0.012 mL, 0.141 mmol) are added and the mixture is stirred for 1 hour before sodium bisulfite aqueous solution (0.14%, 1 mL) and sodium bicarbonate (80 mg) is added. The mixture is then extracted with DCM (5 mL × 3), dried over anhydrous sodium sulfate, and concentrated. The residue is then stirred in MeCN (0.5 mL) and *t*-butylamine (0.5 mL) at room temperature for 15 minutes before concentrated. The resulting residue is then co-evaporated with MeCN (1 mL × 3) and purified by HPLC to give **86** as a white solid (2.4 mg, 15%). (MS: $[M+H]^+$ 1046.2)

Step 2: E24

[0339] 2’3’-cGAMP can be degraded by the enzyme ecto-nucleotide pyrophosphatase/phosphodiesterase (ENPP1) which is present in fetal bovine serum (FBS) (Li et al., 2015, Nat Chem Biol, 11, 235). To test if cGAMP analogues have improved stability, 5 μL of of synthetic cGAMP analogues (100 μM stock) were incubated with 45 μL of FBS in a final volume of 50 μL at 37 °C for 1, 2 and 4 hours. At the indicated time, 10 μL of the reaction mixture was taken out and mixed with 10 μL phosphate buffered saline (PBS), then heated at 95 °C to denature proteins, which were removed by centrifugation at 13000 *g* for 5 minutes. The supernatants were delivered to THP1-ISG-luciferase cell line in the presence of PFO to measure the activity of remaining cGAMP analogues, as described above. Category A indicates less than 10% decrease of activity after 4-hour incubation, B indicates 10–75% decrease of activity after 4-hour incubation, and C indicates more than 75% loss of activity after 4-hour incubation.

Table 7. Stability of cyclic dinucleotides and analogues in fetal bovine serum

Example	Reduction of activity after FBS incubation
E1	C
E2	C
E3	A
E6	C
E7	C
E8	A
E9	A
E16	B
E18	B
E20	A
E21	B
E22	A
E23	B
E25	A
EA1	A
EA4	A
EA6	A

Example	Reduction of activity after FBS incubation
EA7	A
EA8	A
EA10	C
EA11	A
EB1	A
EB2	B
EB3	A
EB4	A
EB5	A
EC1	A
EC2	A
EC3	B
EC5	A
EC13	A
EC15	A
EC24	A
EC26	A

Activity Code	A <10%
	B >10%

Activity Code	A <10%
	B >10%