Supplementary informations

A microbiological system for screening the interference of XNA monomers with DNA and RNA metabolism

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Material

Chemical synthesis of ribo- and deoxynucleoside triphosphate analogues

The synthesis of the libraries of hexitol TPs[1,2], cyclohexenyl TPs[3–5], araTPs[3,6], morpholino TPs[7], altritol TPs[8], pyrimidine and purine base modified deoxyribo TPs[9–11] was carried out using the Ludwig method[12] with a few modifications, as described below. For the synthesis of P-α-methyl-h-HxTP we used methylphosphonic dichloride as the phosphitylating reagent. Dideoxycytidine triphosphate (ddCTP) was obtained from Trilink, 5-fluoro dCTP, 5-BrdCTP and 5-Me dCTP were obtained from Jena Bioscience. dZTP was synthesized as previously described[13] and obtained from Trilink for replicate.

Methods

General procedure for synthesis of nucleoside triphosphates

To an ice-cold solution or suspension of corresponding nucleoside (0.1 mmol) in trimethyl phosphate (TMP) (1.0 mL) was added phosphoryl oxychloride (20 uL, 0.22 mmol) or methylphosphonyl dichloride (30 uL, 0.25 mmol) and the solution was stirred at 0°C for 5 hours. Tributylamine (300 uL, 1.6 mmol) and tetrabutylammonium pyrophosphate solution (0.5 M in DMF, 1.1 mmol) were added simultaneously, and the solution was stirred for a further 30 minutes. The reaction was then quenched by the addition of 0.5 M triethylammonium bicarbonate (TEAB) buffer (10 mL), and stored at 4°C overnight. The solvent was evaporated and the residue was treated with 25% ammonia (4 mL). The solution was evaporated to dryness and re-dissolved in water (5 mL) and applied to a Sephadex A25 column in 0.1 M TEAB buffer. The column was eluted with a linear gradient of 0.1-1.0 M TEAB. Appropriate fractions were pooled and evaporated to dryness to give desired product. HPLC (Alltima 5µ C-18 reverse phase column 10x250 mm, buffer A, 0.1 M TEAB; buffer B, 0.1 M TEAB, 25% MeCN. 0% to 100% buffer B over 60 minutes at 3 mL/min.) showed the product to be pure.

1',5'-Anhydro-2',3'-dideoxy-2'-(hypoxanthin-9-yl)-D-*arabino*-hexitol 6'-triphosphate (1A3) was prepared from corresponding nucleoside[14]. 31P NMR (D2O): δ -7.89 (1P, d), -10.66 (1P,d), -22.33 (1P,t). ESIHRMS found: m/z 504.9944. calcd for C11H17N4O13P3: (M-H)– 504.9932.

4(R)-Hydroxy-2(S)-oxymethyl-N-(adenin-9-y1)acetyl-pyrrolidine triphosphate (1F1) was prepared from corresponding nucleoside[15]. 31P NMR (D2O): δ -10.72 (2P, m), -23.41 (1P,t). ESIHRMS found: m/z 531.0205. calcd for C10H15N4O14P3: (M-H)– 531.0196.

1',5'-Anhydro-2',3'-dideoxy-2'-(guanin-9-yl)-D-*arabino*-hexitol 6'-(α-methylphosphonyl)diphosphate (1F4) was prepared from corresponding nucleoside [1,2] as a racemic mix of (R)p and (S)p diastereoisomers. 31P NMR (D2O): δ 27.85 (1P, m), -6.11 (1P,d), -22.90 (1P,t). ESIHRMS found: m/z 518.0245. calcd for C12H20N5O12P3: (M-H)– 518.0248.

Bacterial Strains and Growth Conditions

All strains used in this study are derived from the wild-type *Escherichia coli* K12 strain MG1655 and cultured in LB medium. The detailed procedure for the construction of the XE763 and XE858 with their respective genotype Δtdk ΔphoA ΔompT::aac and Δtdk ΔphoA ∇ompT::aad ntt2:M46V, has been previously reported[16].

Inhibition Assays on Plates

For testing the toxicity of the nucleosides triphosphates molecules in vivo, the *E. coli* XE858 strain was cultivated overnight in LB medium in the presence of spectinomycin, then diluted to an OD of 0.04 in 5 mL of fresh medium and spread on MH agar plates supplemented with phosphates (KPi 10mM). After 30 minutes, the excess liquid (around 4 ml) was removed by pipetting, tilting the dish slightly, a central well was dug and 35 µl of 100 mM of the compound to be tested were placed in the central well. ddCTP, 5-Aza-dCTP and 5-Br-dCTP were used at a concentration of 10 mM. Plates were incubated overnight at 30°C.

Mutagenic effect

An overnight culture of XE858 in MH spectinomycin was diluted 1:100 in the same fresh medium containing 30, 100 or 300 µM of the nucleoside triphosphate and incubated for 16 hours at 37°C before plating. Viable cell counts (cfu) were done on LB plates after serial dilutions. Rifampicin-resistant clones were selected on LB agar supplemented with 50 mg/L rifampicin. The mutation frequency was calculated as the number of mutant cells divided by the total number of viable cells. The controls without nucleoside triphosphate (negative control) and in the presence of 5Aza dCTP (positive control) were carried out for each series of tests. The number of clones obtained on Rifampicin was between 1 and 12 clones without nucleoside triphosphate and between 122 and 350 clones for 5 Aza dCTP giving an average mutation frequency of $2.5\ 10^{-8}$ (standard deviation : +/- $2,2\ 10^{-8}$) and $3.8\ 10^{-6}$ (standard deviation : +/- $2,8\ 10^{-6}$) respectively. Experiments were done twice when possible due to compounds availability and the corresponding uncorrected standard deviation was calculated.

Compound	Concen-tration (μM)	Mutation frequency	Mutation frequency	Average	Standard deviation (uncorrected)
h-TTP	30	1,3E-08	2,9E-09	8,0E-09	5,1E-09
	100	0,0E+00	5,3E-09	2,7E-09	2,7E-09
	300	4,9E-09	0,0E+00	2,4E-09	2,4E-09
h-CTP	30	4,5E-09	2,2E-08	1,3E-08	8,7E-09
	100	0,0E+00	0,0E+00	0,0E+00	0,0E+00
	300	1,3E-08	5,3E-09	9,3E-09	4,0E-09
h-HxTP	30	0,0E+00	5,0E-09	2,5E-09	2,5E-09
	100	1,1E-08	6,6E-09	9,1E-09	2,4E-09
	300	1,2E-08	5,1E-09	8,6E-09	3,4E-09
h-isoGTP	30	1,2E-07			
	100	1,3E-07			
	300	1,0E-07			
h-GTPαmet	30	8,2E-08			
	100	1,5E-07			
	300	8,7E-08			
ce-TTP	30	7,1E-09	4,4E-09	5,7E-09	1,3E-09
	100	0,0E+00	0,0E+00	0,0E+00	0,0E+00
	300	2,0E-08	1,6E-08	1,8E-08	2,1E-09
ce-CTP	30	1,5E-08	1,4E-08	1,5E-08	7,8E-10
	100	7,3E-09	6,8E-09	7,1E-09	2,1E-10
	300	0,0E+00	0,0E+00	0,0E+00	0,0E+00
ce-ATP	30	2,3E-08	1,8E-08	2,1E-08	2,6E-09
	100	1,3E-08	4,5E-08	2,9E-08	1,6E-08
	300	3,4E-08	5,4E-08	4,4E-08	9,6E-09
ce-GTP	30	6,2E-08	0,0E+00	3,1E-08	3,1E-08
	100	5,0E-09	4,1E-09	4,6E-09	4,7E-10
	300	0,0E+00	0,0E+00	0,0E+00	0,0E+00
ara-TTP	30	1,5E-08	1,5E-08	1,5E-08	5,5E-10
	100	1,2E-08	6,9E-09	9,3E-09	2,4E-09
	300	1,3E-07	3,5E-08	8,4E-08	4,9E-08
ara-GTP	30	1,9E-08	1,4E-08	2,0E-08	2,1E-10
	100	8,5E-08	7,5E-08	8,0E-08	5,0E-09
	300	9,0E-08	6,2E-08	7,6E-08	1,4E-08
mo-ATP	30	1,0E-07			
	100	9,3E-08			
	300	1,6E-08			
mo-CTP-N-Benzoyl	30	8,0E-09	4,3E-09	6,1E-09	1,9E-09

	100	6,6E-08	4,8E-09	3,5E-08	3,1E-08
	300	6,3E-09	1,3E-07	7,0E-08	6,4E-08
al-CTP	30	3,3E-09	0,0E+00	1,7E-09	1,7E-09
	100	0,0E+00	1,0E-08	5,2E-09	5,2E-09
	300	2,7E-08	2,2E-08	2,4E-08	2,3E-09
al-UTP	30	2,2E-07			
	100	8,4E-09			
	300	4,1E-07			
al-ATP	30	1,3E-07			
	100	1,8E-07			
	300	6,9E-08			
pro-ATP	30	1,0E-07			
	100	7,6E-08			
	300	3,1E-07			
7-deaza-dGTP	30	4,4E-07			
	100	1,4E-08			
	300	1,2E-07			
8-aza-dGTP	30	9,0E-09			
	100	6,4E-08			
	300	8,2E-08			
8-methyl-dGTP	30	1,0E-07			
	100	1,6E-08			
	300	5,6E-07			
7-fluoro-7-deaza-dGTP	30	4,1E-08			
	100	4,4E-08			
	300	3,5E-08			
5-methyl-dCTP	30	6,7E-08	5,4E-08	6,0E-08	6,5E-09
	100	7,9E-09	0,0E+00	3,9E-09	3,9E-09
	300	0,0E+00	0,0E+00	0,0E+00	0,0E+00
5-fluoro-dCTP	30	0,0E+00	0,0E+00	0,0E+00	0,0E+00
	100	1,3E-08	5,7E-09	9,3E-09	3,6E-09
	300	1,2E-08	1,7E-08	1,5E-08	2,7E-09
5-bromo-dCTP	30	8,7E-08	0,0E+00	4,3E-08	1,7E-08
	100	0,0E+00	1,1E-08	5,7E-09	5,7E-09
	300	0,0E+00	7,2E-09	3,6E-09	4,4E-09
7-deaza-dATP	30	2,9E-07	1,3E-07	2,1E-07	8,2E-08
	100	6,8E-08	4,2E-08	5,5E-08	1,3E-08
	300	1,4E-07	1,9E-07	1,6E-07	2,4E-08
7-deaza-8aza-dATP	30	6,5E-09	5,2E-09	5,8E-09	6,7E-10

	100	3,2E-08	0,0E+00	1,6E-08	1,6E-08
	300	4,8E-09	3,4E-08	1,9E-08	1,4E-08
8-aza-dATP	30	6,9E-09			
	100	2,4E-08			
	300	1,0E-08			
dZTP	30	5,3E-07	6,7E-07	6,0E-07	7,4E-08
	100	1,2E-06	2,2E-06	1,7E-06	5,1E-07
	300	3,1E-06	4,4E-06	3,8E-06	6,5E-07
5-chloro-dUTP	30	2,3E-08	3,7E-09	1,3E-08	9,7E-09
	100	7,4E-09	1,6E-08	1,2E-08	4,4E-09
	300	2,5E-08	1,9E-08	2,2E-08	2,8E-09
8-aza-8-d-isodGTP	30	0,0E+00			
	100	4,8E-08			
	300	0,0E+00			
8-OH-dHxTP	30	3,5E-07	5,6E-08	2,0E-07	1,5E-07
	100	8,4E-07	3,4E-07	5,9E-07	2,5E-07
	300	2,0E-06	9,7E-07	1,5E-06	5,1E-07
					Standard deviation
no triP				2,5E-08	2,3E-08
5-aza-dCTP				3,80E-06	2,80E-06

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