Electronic Supplementary Information (ESI)

Biocatalitic synthesis of unnatural nucleosides possessing a large functional group such as a fluorescent molecule, coumarin by purine nucleoside phosphorylase

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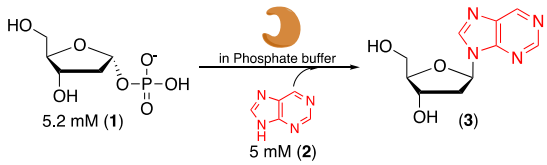
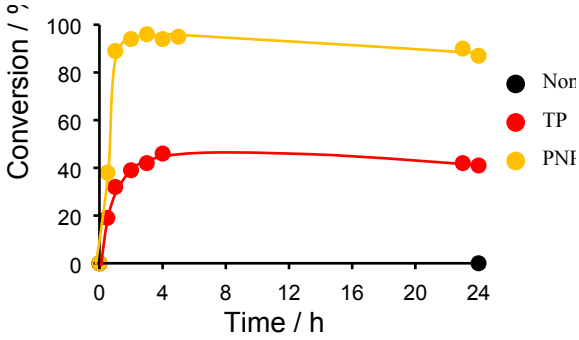
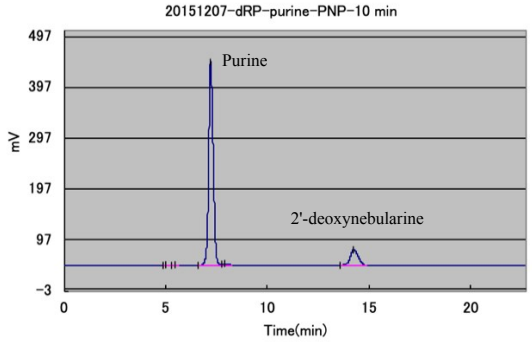
S1 Effect of enzyme on the production of 2'-deoxynebularine from deoxyribose-1α-phosphate and purine. Reaction conditions: 5.2 mM of deoxyribose-1-phosphate and 5.0 mM of purine in 1.0 mM phosphate buffer (pH 6.8) at 40 ˚C. The reaction were carried out under the condition of 5 units/mL of PNP or TP. Product formation was monitored by UV absorption at 254 nm using HPLC (Jasco) with a C18 column (Osaka soda Inc., Capcell pak C18 UG-120, ϕ 4.6–250mm, 2–5 % MeCN in 10 mM phosphate buffer, pH 6.8).



(a) Reaction scheme

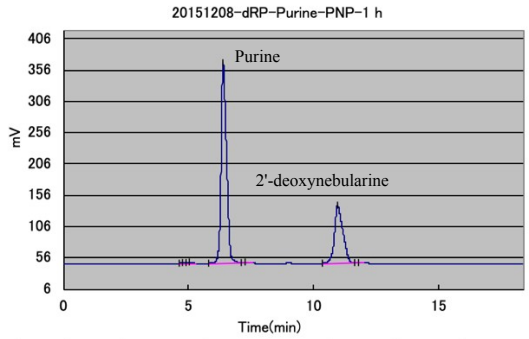


(b) The curves of conversion of 2'-deoxynebularine for time by PNP or TP.



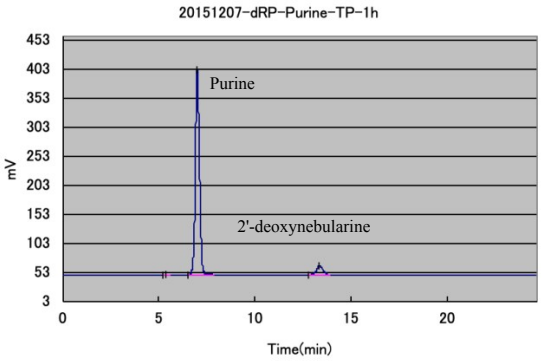
No.	Rt	ﾋﾟｰｸ名	面積	面積(%)	高さ	定量結果
1	5.01		11828.2	0.1679	1089	----
2	5.47		9278	0.1317	959	----
3	7.21		6129750.2	87.0113	399635	----
4	7.92		51509.4	0.7312	3009	----
5	14.24		842411.4	11.958	30541	----
			7044777.2	100	435233	

(c) HPLC chart of using PNP (10 min).



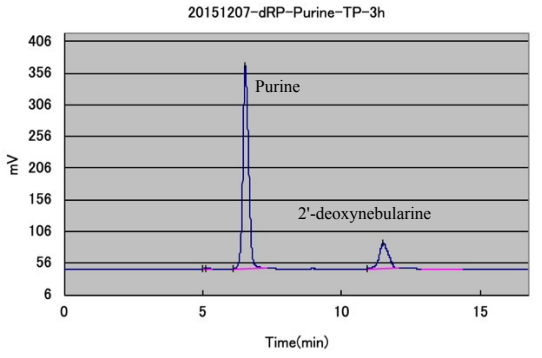
No.	Rt	ﾋﾟｰｸ名	面積	面積(%)	高さ	定量結果
1	4.73		16316.8	0.2312	1491	----
2	5.04		24349.4	0.345	1826	----
3	6.38		4817804.6	68.2534	321271	----
4	7.27		26084.6	0.3695	1684	----
5	10.96		2163569.2	30.6511	92519	----
6	11.81		10577.4	0.1498	732	----
			7058702	100	419523	

(d) HPLC chart of using PNP (1h).



No.	Rt	ﾋﾟｰｸ名	面積	面積(%)	高さ	定量結果
1	5.38		13111.8	0.2291	1346	----
2	7		5316553.2	92.8995	353280	----
3	13.35		393243.2	6.8714	15413	----
			5722908.2	100	370039	

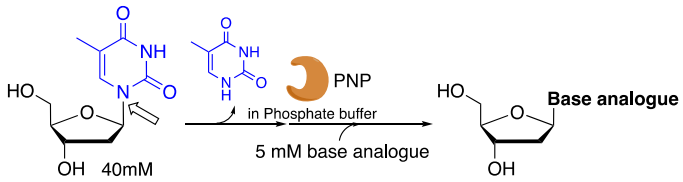
(e) HPLC chart of using TP (1 h).

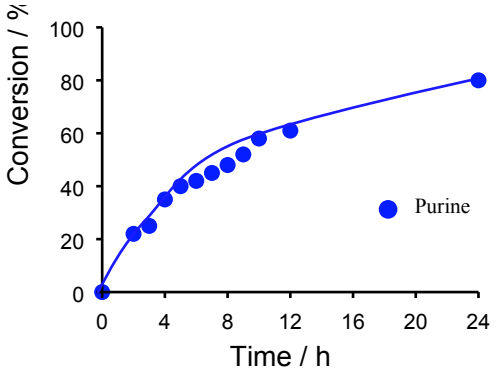


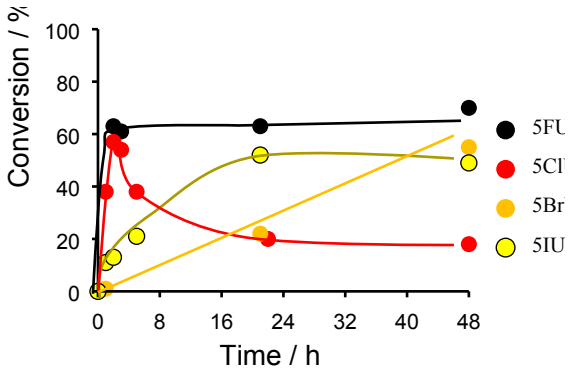
No.	Rt	ﾋﾟｰｸ名	面積	面積(%)	高さ	定量結果
1	5.08		7247.2	0.1313	680	----
2	6.53		4587039.4	83.0781	320393	----
3	11.49		927070	16.7906	40350	----
			5521356.6	100	361423	

(f) HPLC chart of using TP (3 h).

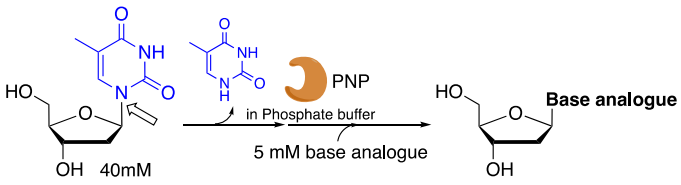
S2-1 PNP-catalysed base-exchange reaction between thymidine and purine or uracil modified with a halogen atom at the 5 position.

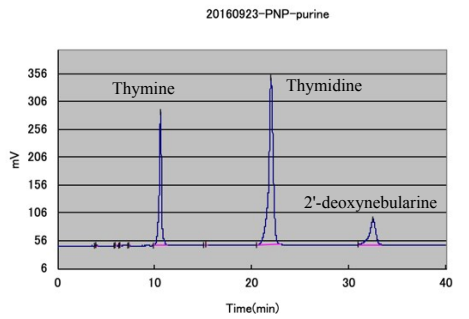






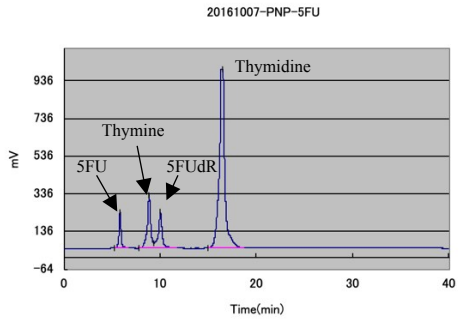
S2-2 HPLC charts of enzymatic reaction between purine and pyrimidine analogue and thymidine using PNP. (a): the base analogue was purine, (b): 5-fluorouracil, (c):5-iodouracil , (d): 5-bromouracil. Reaction conditions: 40 mM of thymidine and 5.0 mM of base analogue in 1.0 mM phosphate buffer (pH 6.8) at 40 ˚C. The reaction volume was 1.0 mL. The reaction was carried out under the condition of 5 units/mL of PNP. Product formation was monitored by UV absorption at 254 nm using HPLC (Jasco) with a C18 column (Osaka soda Inc., Capcell pak C18 UG-120, ϕ 4.6–250mm, 2–5 % MeCN in 10 mM phosphate buffer, pH 6.8).





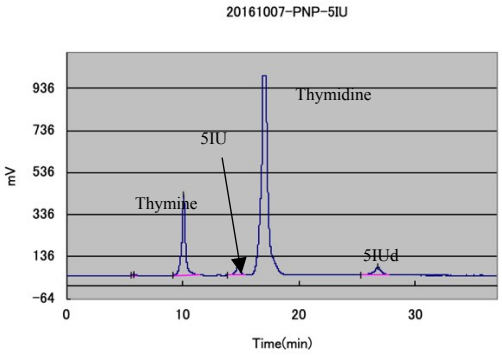
No.	Rt	ピーク名	面積	面積(%)	高さ	定量結果
1	3.95		5432.6	0.0351	755	----
2	5.96		6970	0.045	1002	----
3	6.44		5970.8	0.0385	790	----
4	7.35		6060.4	0.0391	763	----
5	10.6		3977306	25.6676	240023	----
6	15.26		14740.2	0.0951	1058	----
7	21.97		9455951	61.0242	299802	----
8	32.48		2022990.8	13.0554	45724	----
			15495421.8	100	589917	

(a)



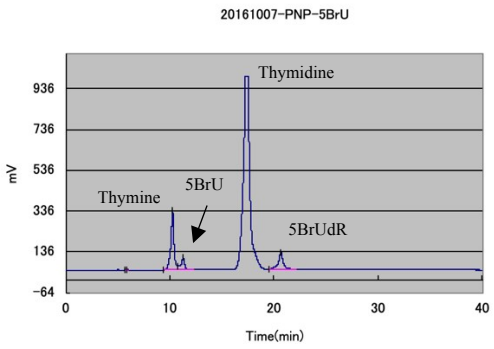
No.	Rt	ピーク名	面積	面積(%)	高さ	定量結果
1	5.85		2816623.4	5.2518	190504	----
2	8.86		6904581.8	12.8741	281196	----
3	10.03		5377429.6	10.0266	191220	----
4	16.52		38533024	71.8475	948684	----
			53631658.8	100	1611604	

(b)



No.	Rt	ピーク名	面積	面積(%)	高さ	定量結果
1	5.83		31897.2	0.2885	2732	----
2	10.1		8227047.2	74.4225	381570	----
3	14.79		1226600	11.0959	49617	----
4	26.78		1568972.4	14.193	38021	----
			11054516.8	100	471940	

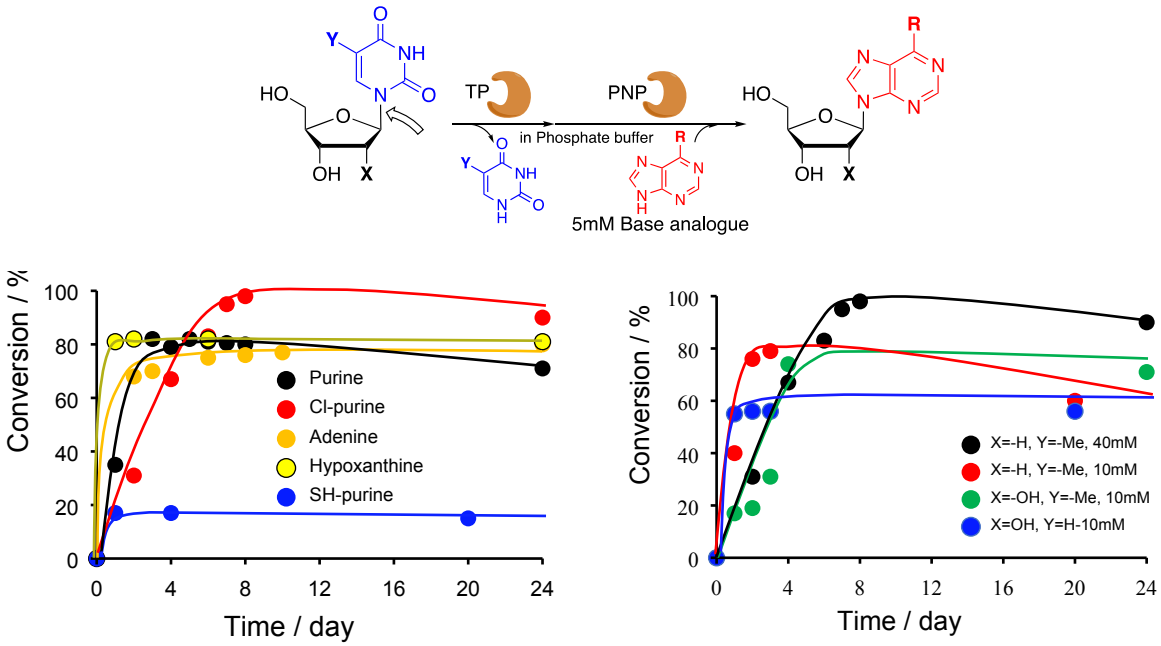
(c)



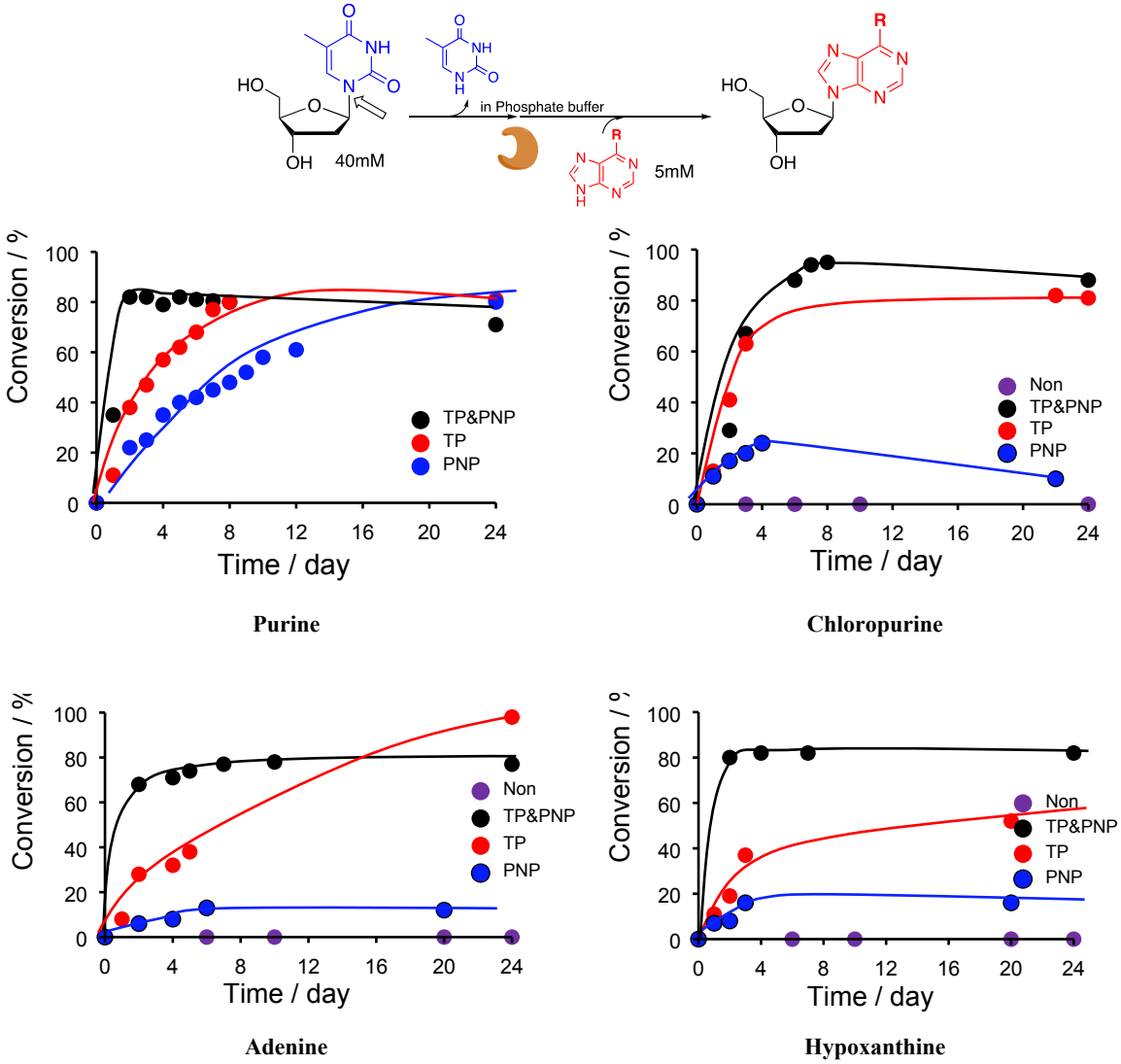
No.	Rt	ピーク名	面積	面積(%)	高さ	定量結果
1	5.87		11460.4	0.1105	1188	----
2	10.25		6155048.6	59.3625	293347	----
3	11.28		1529160	14.748	59660	----
4	20.65		2672912.6	25.779	82938	----
			10368581.6	100	437133	

(d)

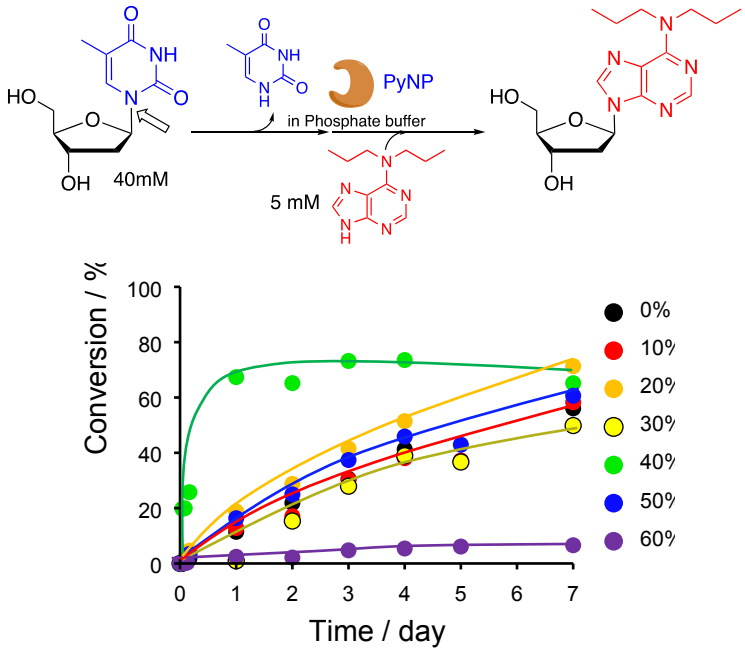
S3 Effect of a two-enzyme system, TP and PNP, on the base-exchange reaction of pyrimidine nucleoside with various purines. Left: 5 mM of thymidine and 40 mM of purine modified at 6 position. Right: 10 mM of 6-chloropurine and 5 mM of four kinds of ribosyl donor.



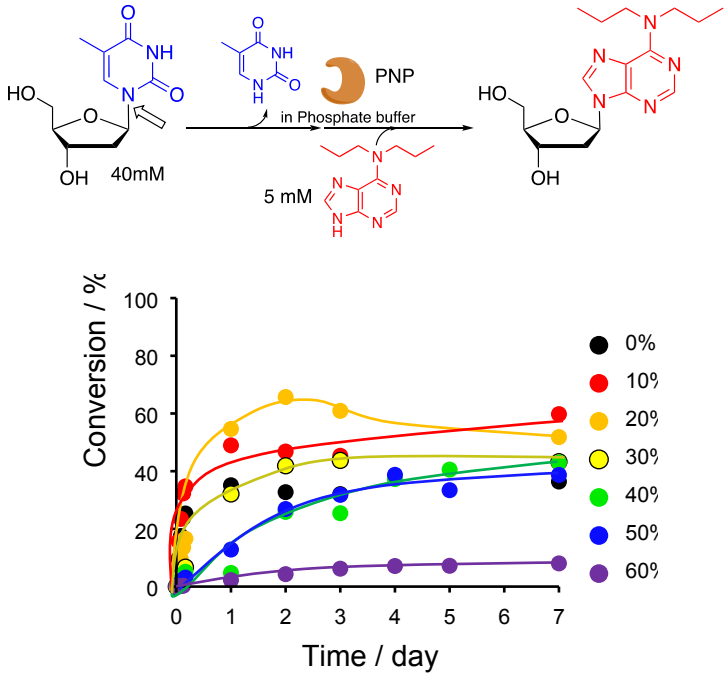
S4 Effect of TP, PNP, and TP+PNP on the base-exchange conversion of thymidine to a modified purine nucleoside.



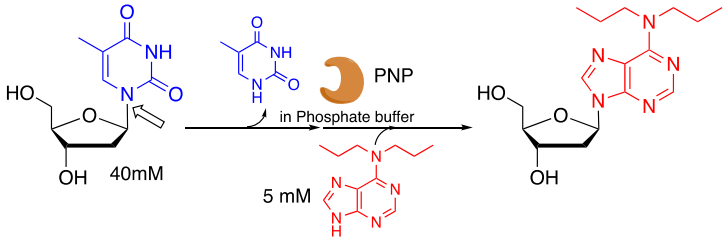
S5-1 Effect of addition of DMSO and hydrophobicity of alkyl amino purine as substrate for base-exchange reaction using PyNP.

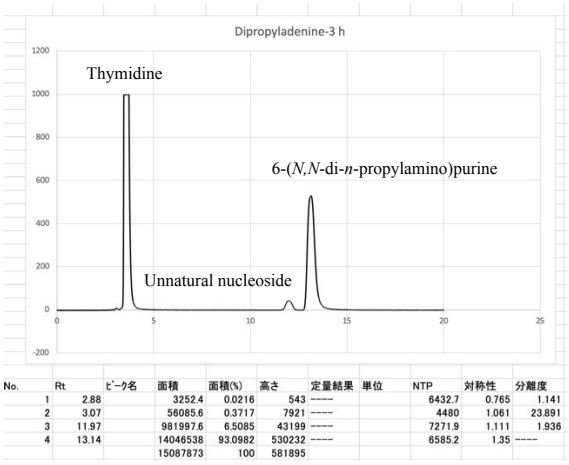


S5-2 Effect of addition of DMSO and hydrophobicity of alkyl amino purine as substrate for base-exchange reaction using PNP.



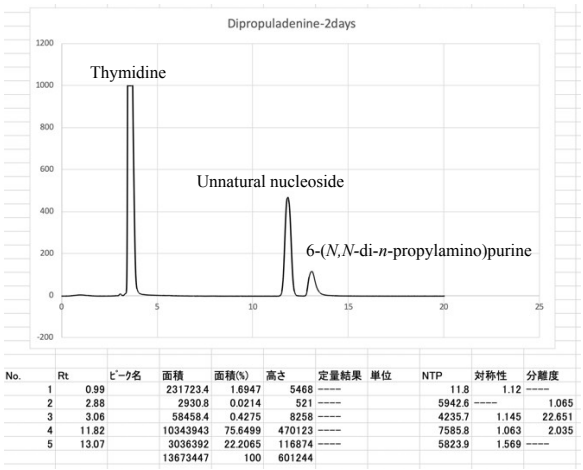
S5-3 HPLC charts of enzymatic reaction between 6-(*N,N*-di-*n*-propylamino) purine and thymidine in 40 % DMSO/buffer using PNP. Reaction conditions: 40 mM of thymidine and 5.0 mM of 6-propylaminopurine in 1.0 mM phosphate buffer (pH 6.8) and the addition of DMSO (40%, v/v) at 40 ˚C. The reaction volume was 1.0 mL. The reaction were carried out under the condition of 5 units/mL of PNP. Product formation was monitored by UV absorption at 254 nm using HPLC (Jasco) with a C18 column (Osaka soda Inc., Capcell pak C18 UG-120, ϕ 4.6–250mm, 10 % MeCN in 10 mM phosphate buffer; pH 6.8).





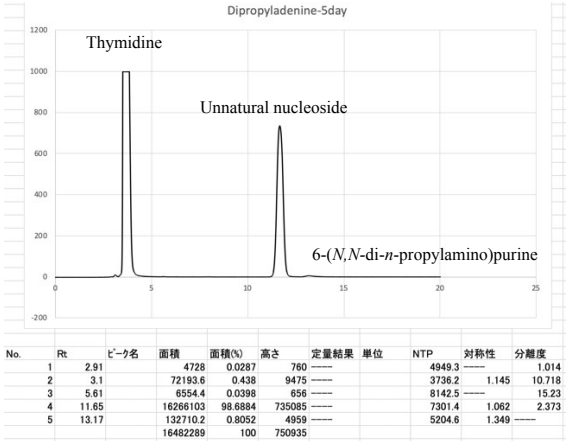
No.	Rt	ピーク名	面積	面積(%)	高さ	定量結果	単位	NTP	対称性	分離度
1	2.88		3252.4	0.0216	543	-----		6432.7	0.765	1.141
2	3.07		56085.6	0.3717	7921	-----		4480	1.061	23.891
3	11.97		981997.6	6.5085	43199	-----		7271.9	1.111	1.936
4	13.14		14046538	93.0982	530232	-----		6585.2	1.35	-----
			15087873	100	581895					

(a) 3h



No.	Rt	ピーク名	面積	面積(%)	高さ	定量結果	単位	NTP	対称性	分離度
1	0.99		231723.4	1.6947	5468	-----		11.8	1.12	-----
2	2.88		2930.8	0.0214	521	-----		5942.6	-----	1.065
3	3.06		58458.4	0.4275	8258	-----		4235.7	1.145	22.651
4	11.82		10343943	75.6499	470123	-----		7585.8	1.063	2.035
5	13.07		3036392	22.2065	116874	-----		5823.9	1.569	-----
			13673447	100	601244					

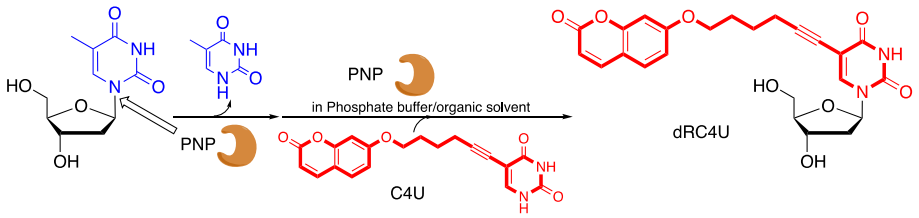
(b) 2days



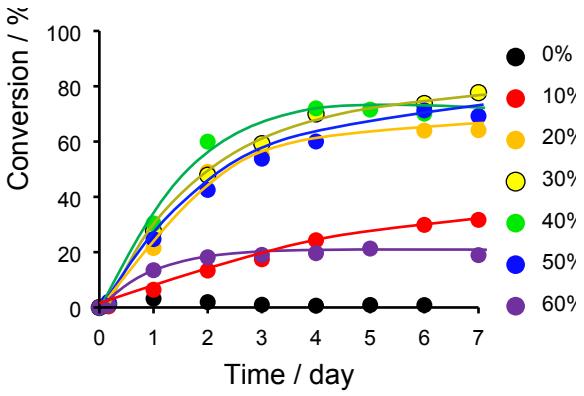
No.	Rt	ピーク名	面積	面積(%)	高さ	定量結果	単位	NTP	対称性	分離度
1	2.91		4728	0.0287	760	-----		4949.3	-----	1.014
2	3.1		72193.6	0.438	9475	-----		3736.2	1.145	10.718
3	5.61		6554.4	0.0398	656	-----		8142.5	-----	15.23
4	11.65		16266103	98.6884	735085	-----		7301.4	1.062	2.373
5	13.17		132710.2	0.8052	4959	-----		5204.6	1.349	-----
			16482289	100	750935					

(c) 5days

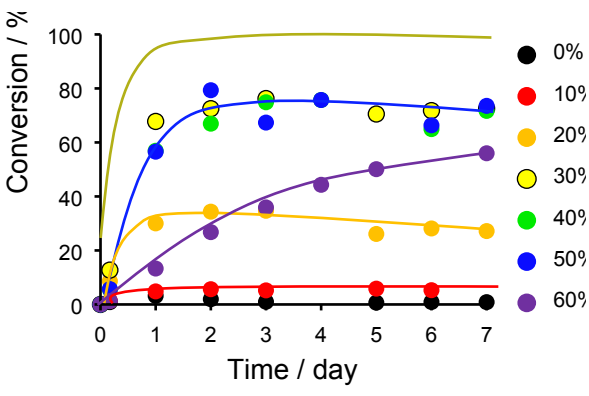
S6 Effect of organic solvent and enzyme on synthesis of dRC4U.



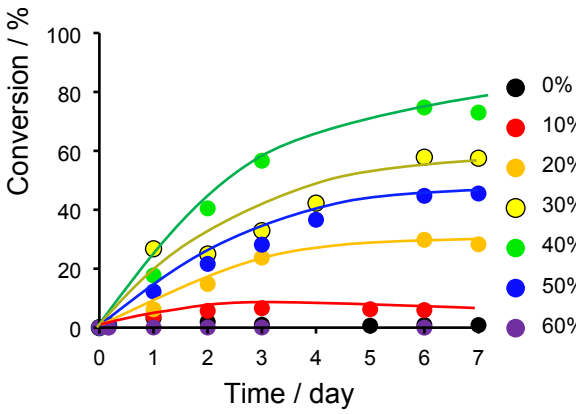
DMF (by PNP)



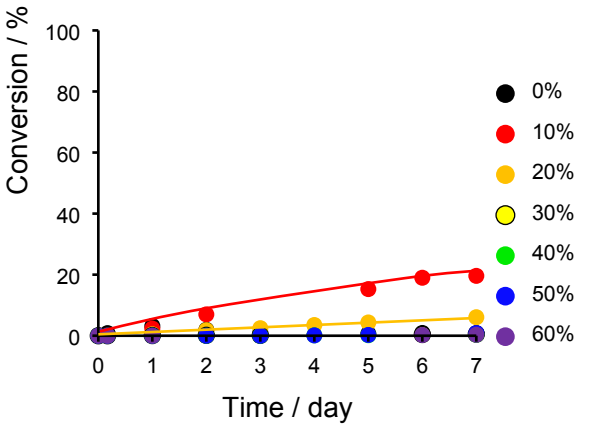
DMSO (by PNP)



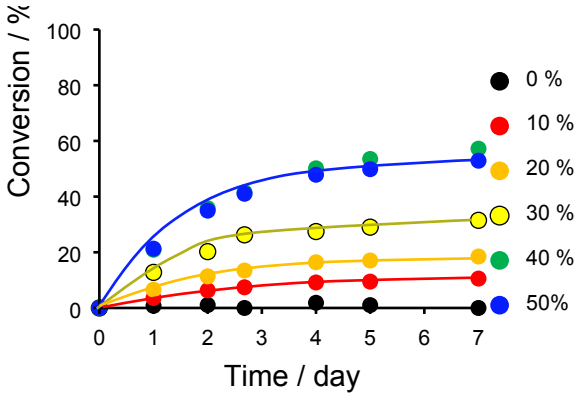
MeOH (by PNP)



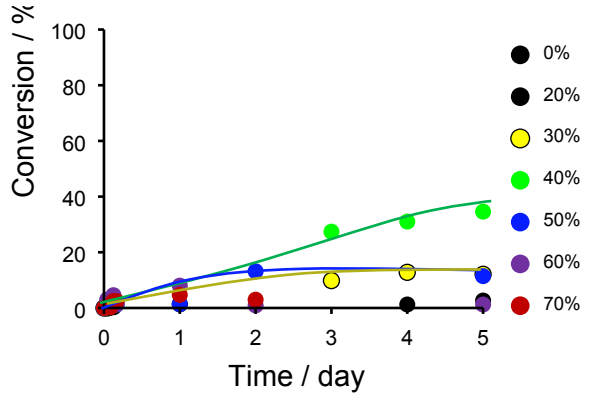
THF (by PNP)



DMSO (by TP)



DMSO (by PyNP)



S7 Effect of chain length of the alkyl linker between coumarin and the 5 position of uracil on the production of an unnatural nucleoside. Time courses of dRC4U (**a**) and dRC6U (**b**) conversion at varying DMSO concentrations.