

Supplementary Materials: Synthesis of Ribavirin, Tecadenoson, and Cladribine by Enzymatic Transglycosylation

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Supplementary Material

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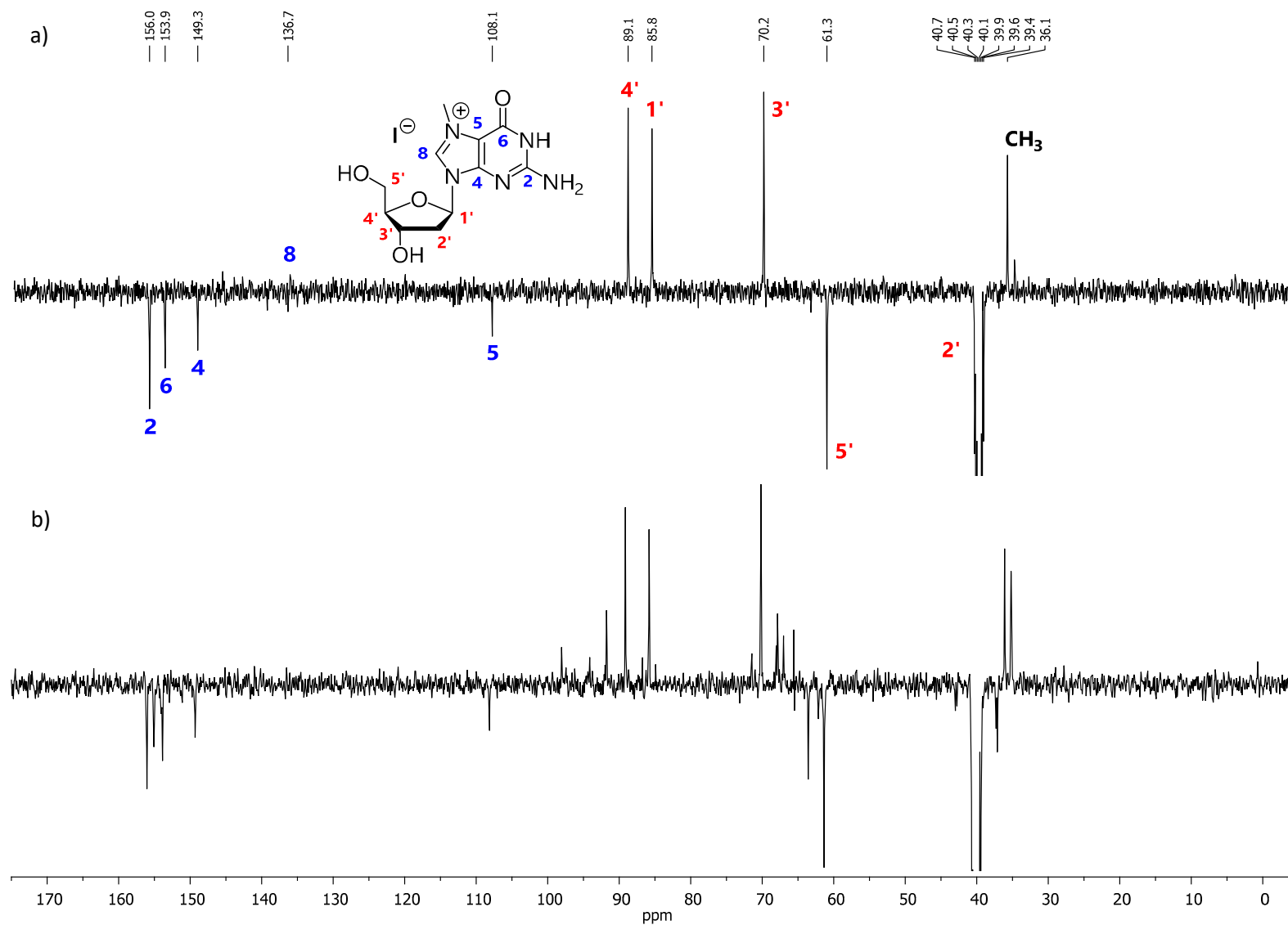
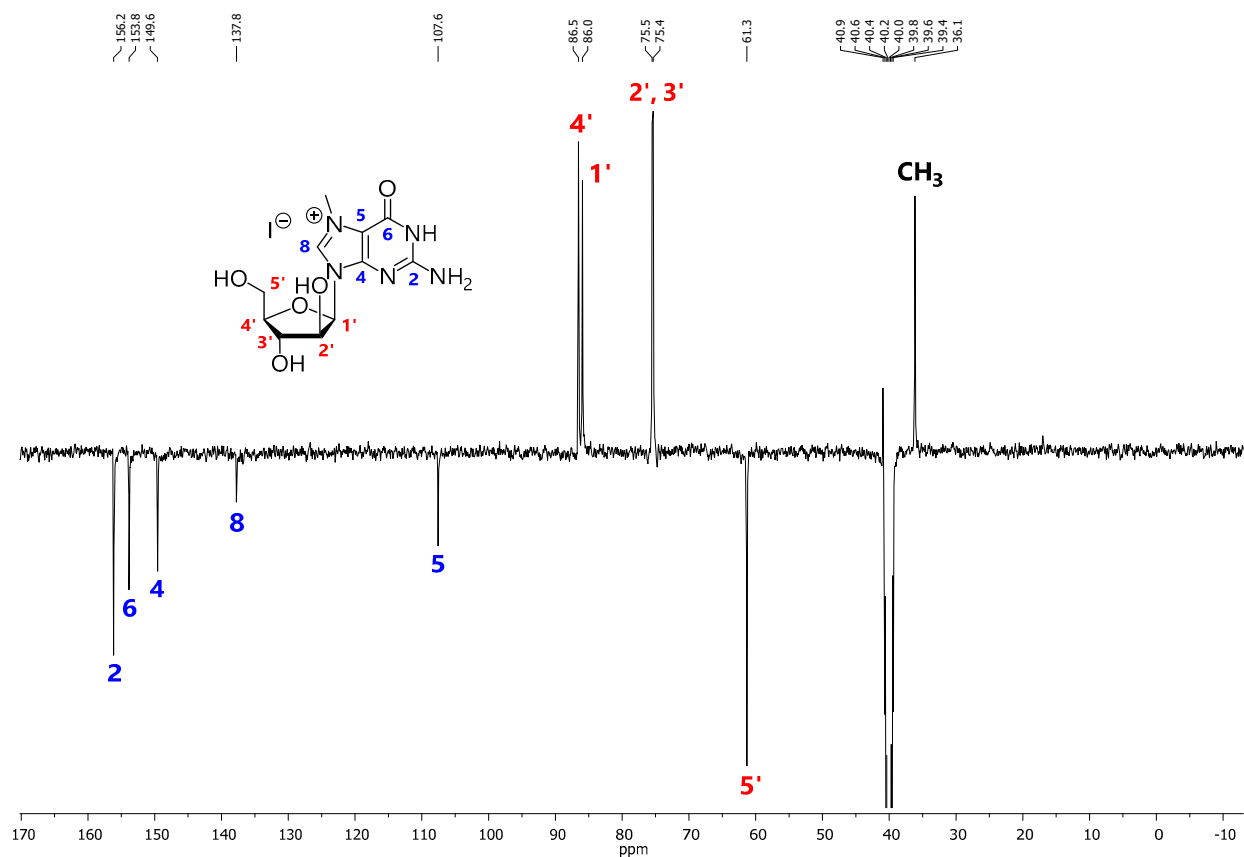
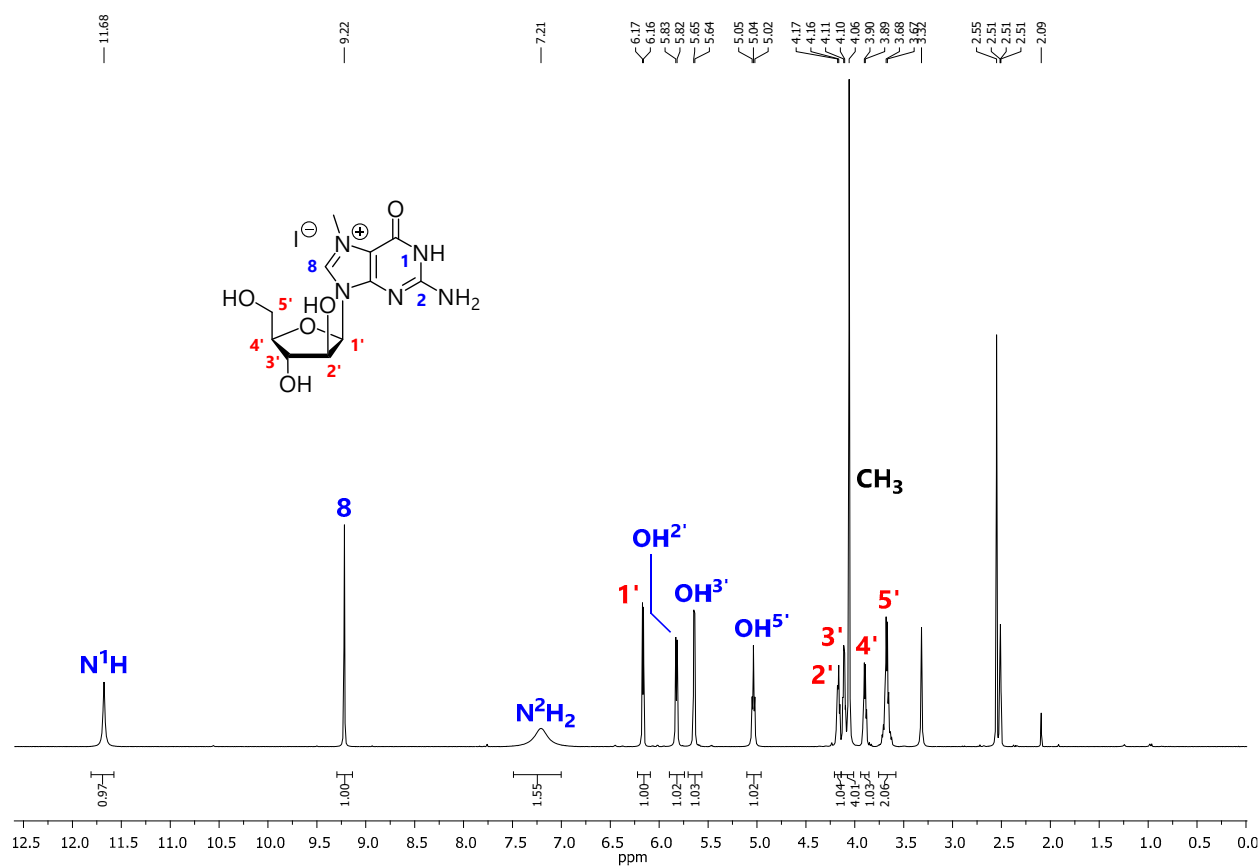
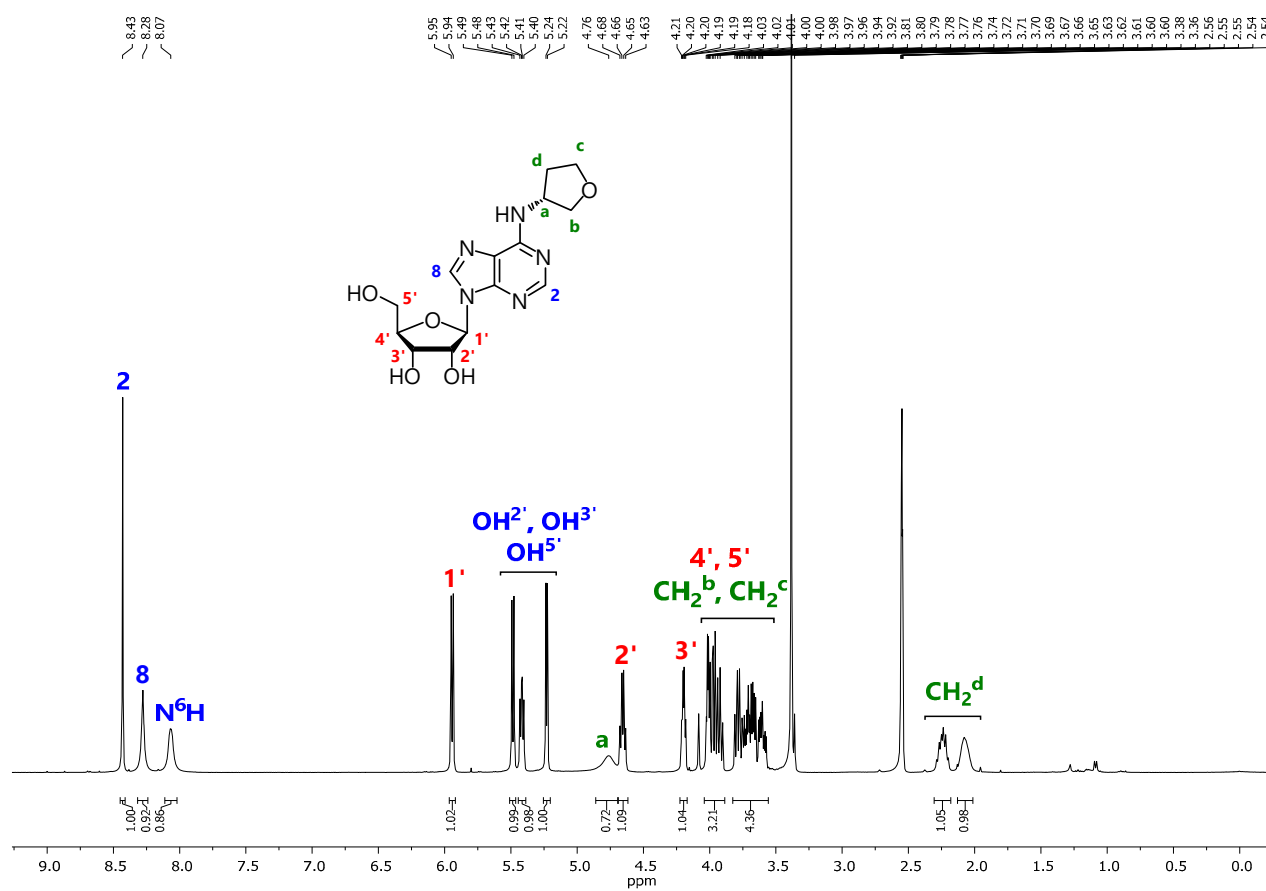
S1. ^{13}C -NMR monitoring of 7-methyl-2'-deoxyguanosine iodide (**8**) stability

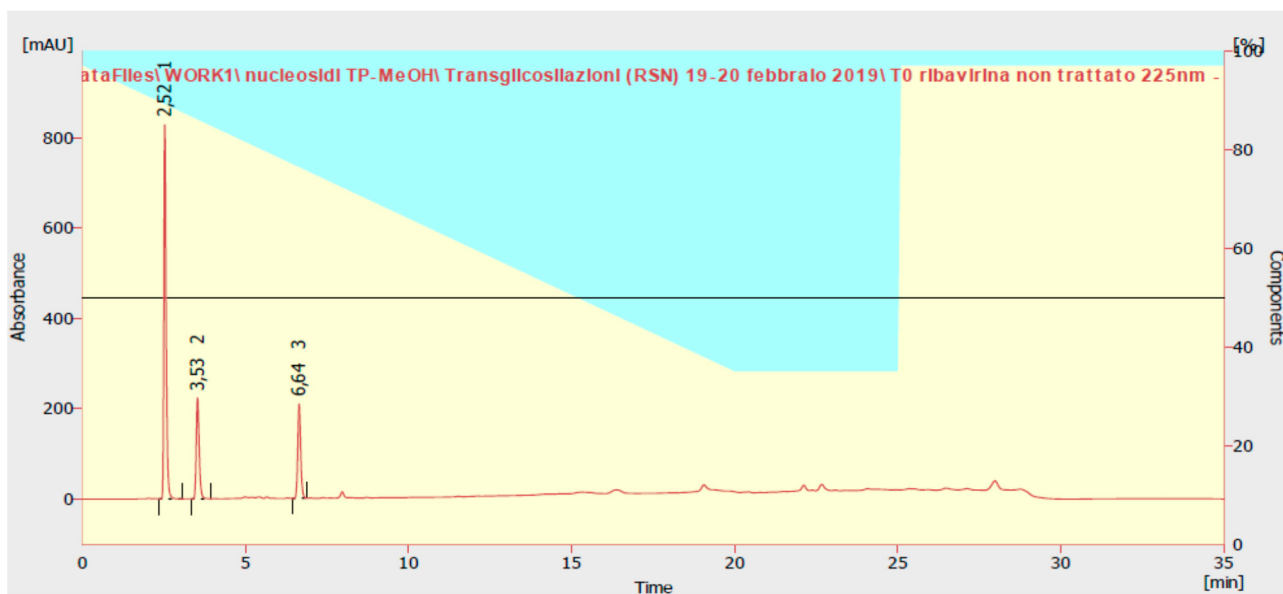
Figure S1. Comparison of ^{13}C -NMR spectra of **8** in $\text{DMSO}-d_6$ (0.05 M): **a**) after 2 h; **b**) after 4 h.

S2. ^1H -NMR and ^{13}C -NMR spectra of 7-methylguanine arabinoside iodide (9)

S3. ^1H -NMR spectrum of Tecadenoson (2)

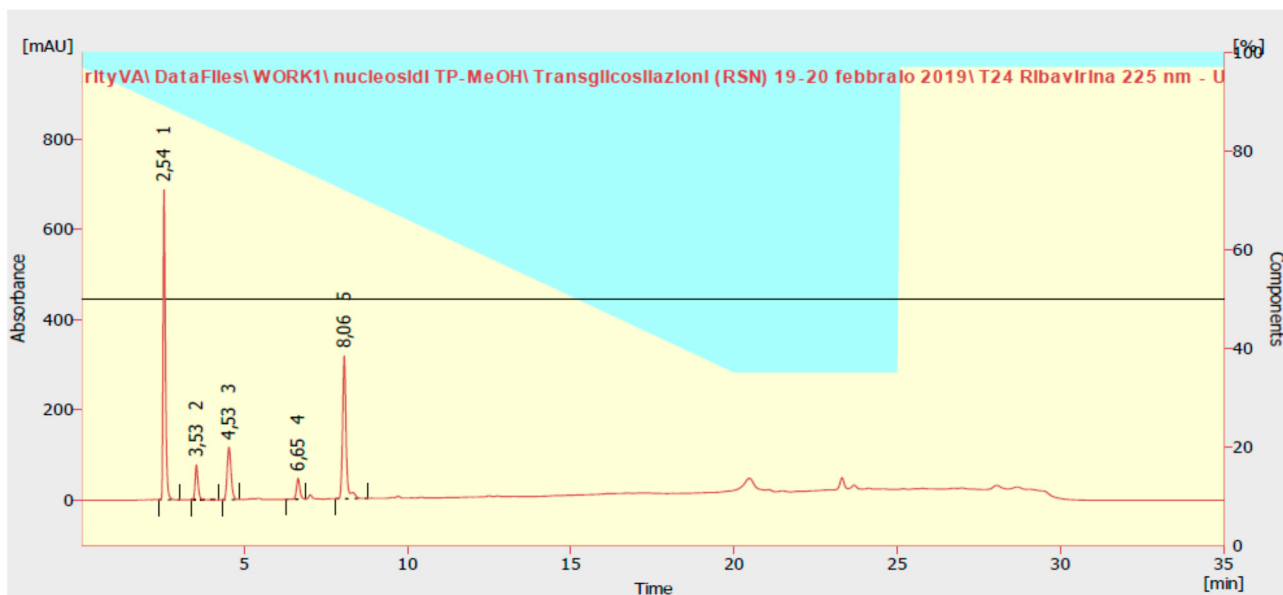
S4. HPLC monitoring of the enzymatic synthesis of Ribavirin (**1**)

a) t=0

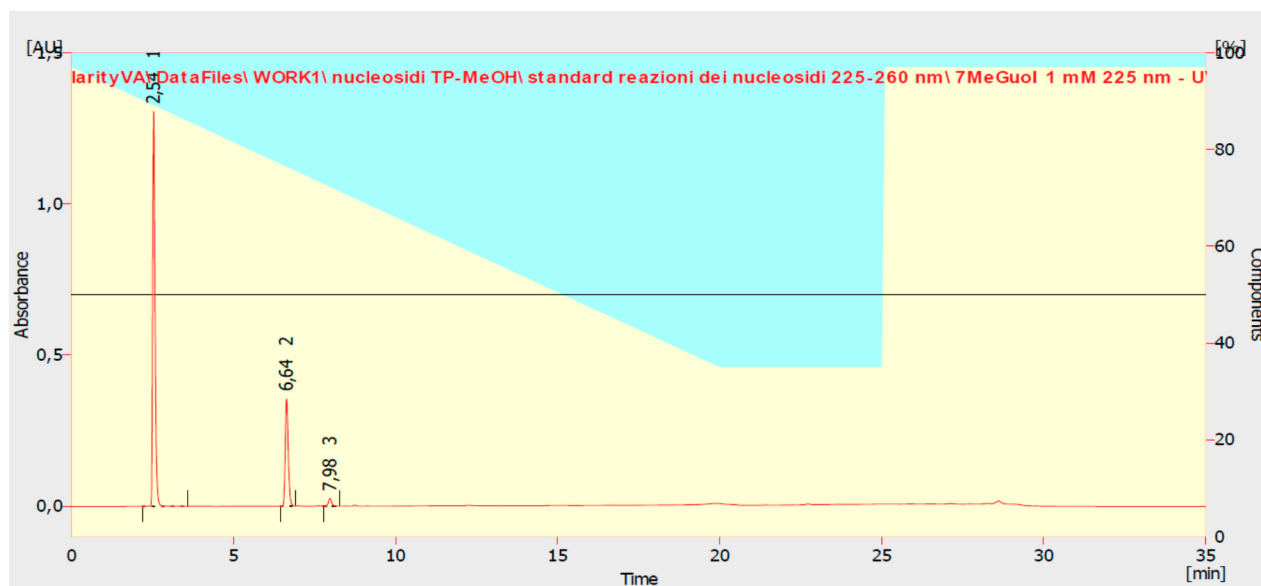


tr: 2.52 min (solvent); 3.53 min (1,2,4-triazole-3-carboxamide, **15**); 6.64 min (7-methylguanosine iodide, **7**)

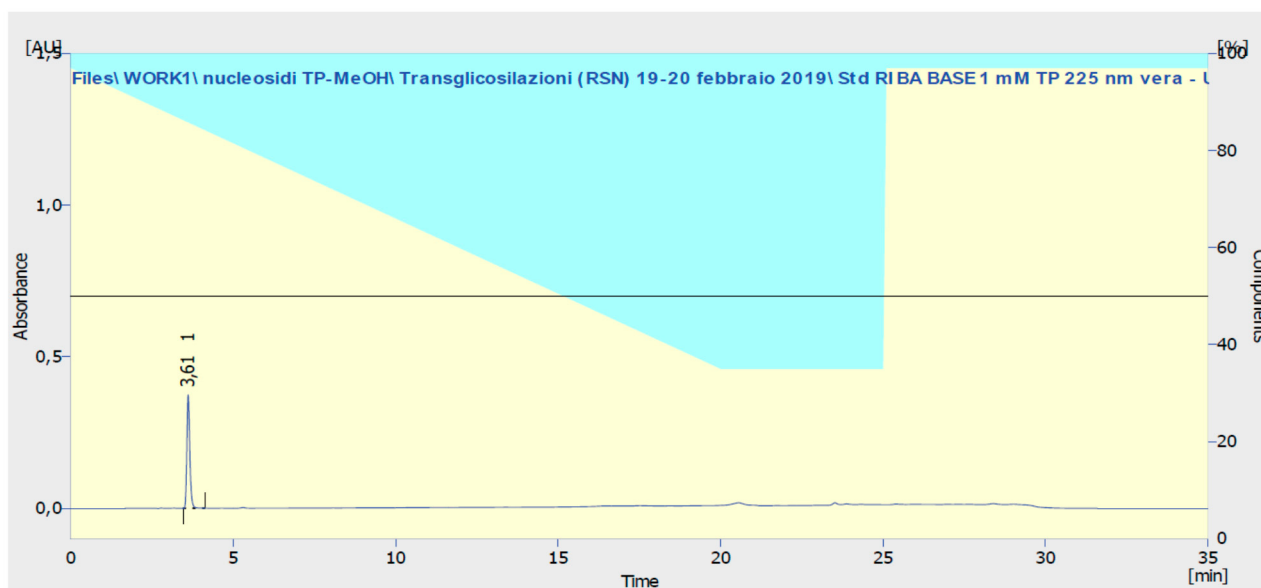
b) t=24 h



tr: 2.54 min (solvent); 3.53 min (1,2,4-triazole-3-carboxamide, **15**); 4.53 min (Ribavirin, **1**); 6.65 min (7-methylguanosine iodide, **7**); 8.06 min (tentatively attributed to 7-methylguanine, **19**)

c) 7-Methylguanosine iodide (**7**) (standard)

tr: 2.54 min (solvent); 6.64 min (7-methylguanosine iodide, **7**); 7.98 min (unknown)

d) 1,2,4-Triazole-3-carboxamide (**15**) (standard)

tr: 3.61 min

S5. HPLC monitoring of the enzymatic synthesis of CCPA (**17**)

This reaction was performed according to a “fed batch” mode as reported in the Table below:

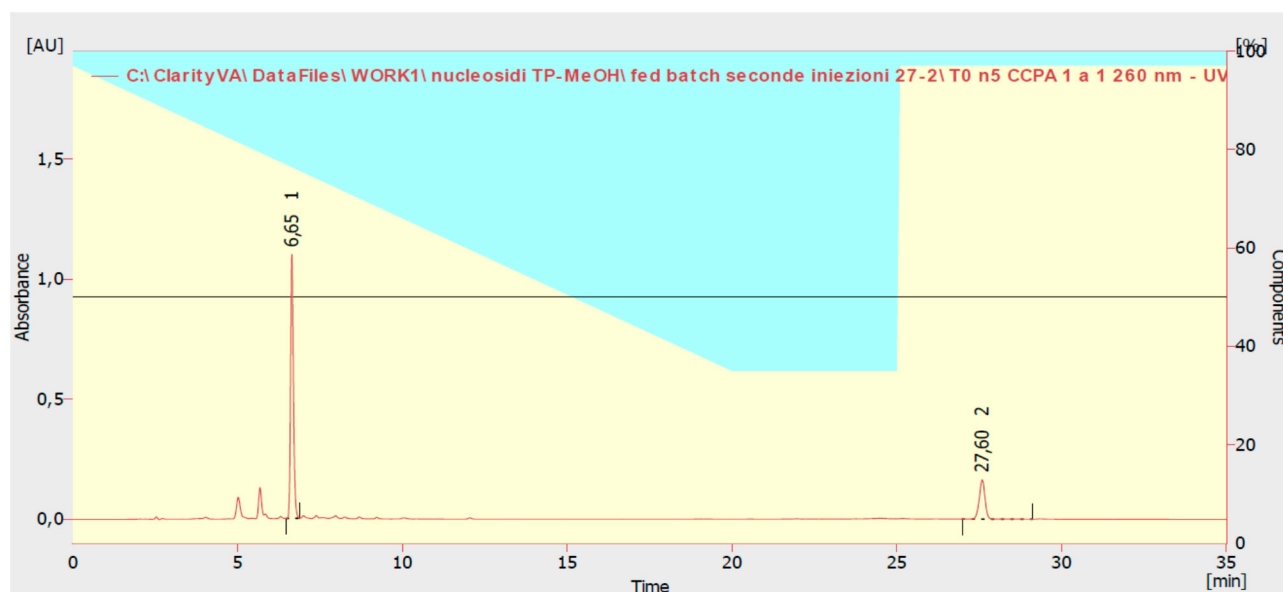
Table. Enzymatic synthesis of CCPA (**17**): reaction set-up and monitoring

Reaction time (h)	Reaction volume (mL)	[Donor] (mM)	Overall added volume of 12 (mL) ¹	[12] (mM)	DMSO (% v/v)	Conversion (%; mM)
0	4.625	1.08	0.125	0.27	2.5	0
2	4.750	1.05	0.250	0.52	5	71% (0.19 mM) ²
4	4.875	1.03	0.375	0.77	7.5	64% (0.49 mM)
6	5.000	1.00	0.500	1.00	10	54% (0.54 mM)

¹A 10 mM stock solution of **12** in DMSO was used. Time monitoring (HPLC): 0.5, 1, 2, 3, 4, 5, 6, 7, 24 h. The supernatant was diluted 1:1 with the mobile phase and analyzed by HPLC as reported in the main text (Materials and Methods, paragraph 3.3). ²Conversion was calculated before the addition of the second portion of **12**

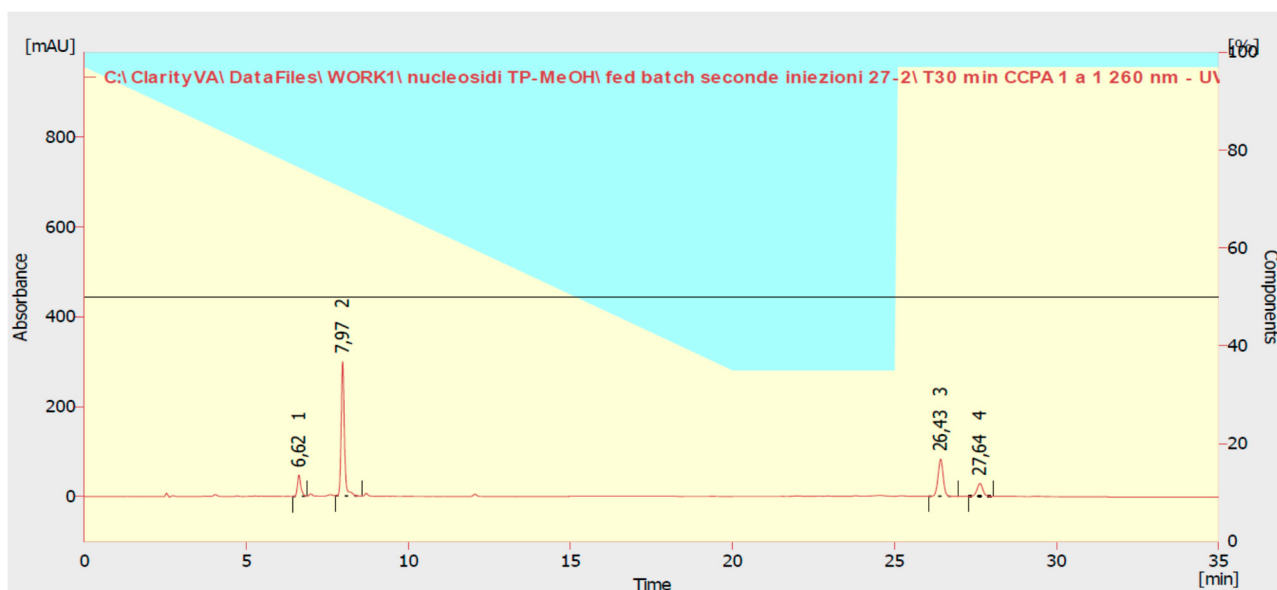
Chromatograms at t=0, 0.5 h, 6 h, 24 h

a) t=0

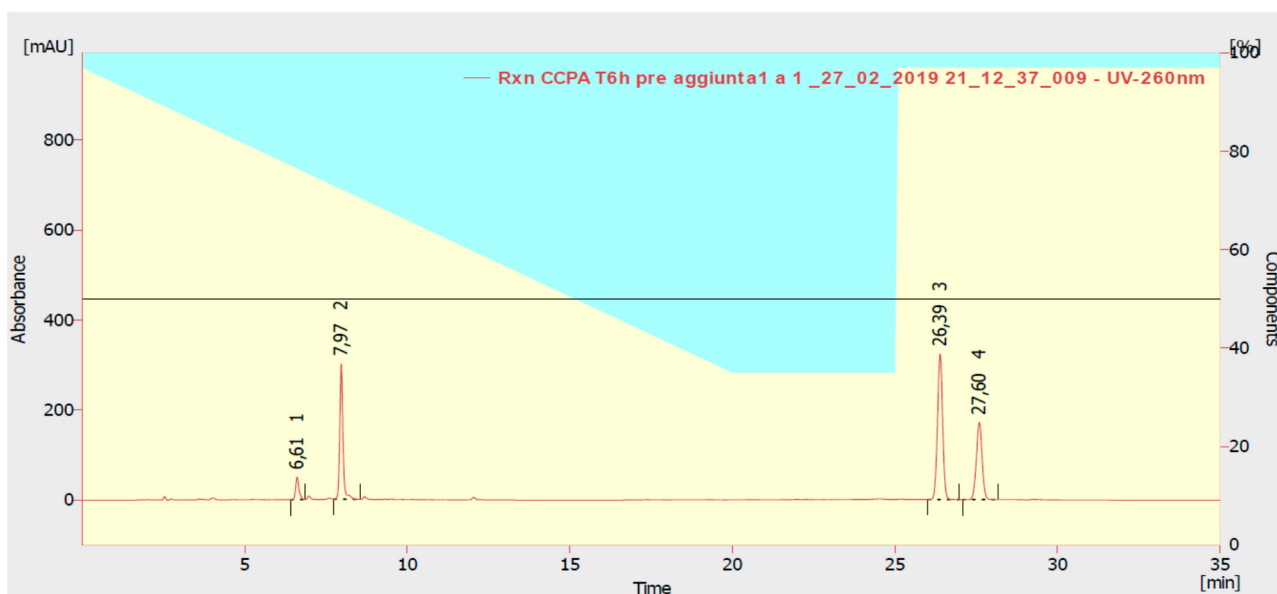


tr: 6.65 min (7-methylguanosine iodide, **7**); 27.60 min (2-chloro-*N*⁶-cyclopentyladenine, **12**)

b) t=0.5 h

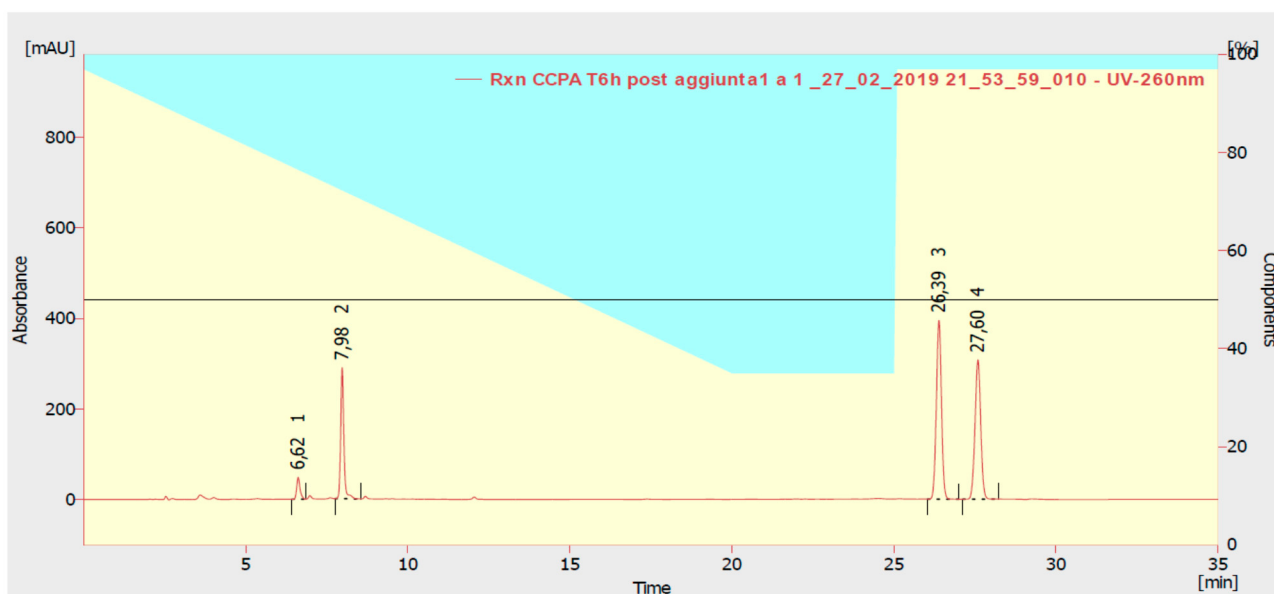


tr: 6.62 min (7-methylguanosine iodide, **7**); 7.97 min (tentatively attributed to 7-methylguanine, **19**); 26.43 min (CCPA, **17**); 27.64 min (2-chloro-*N*⁶-cyclopentyladenine, **12**)

c) t=6 h (before adding the last portion of **12**)

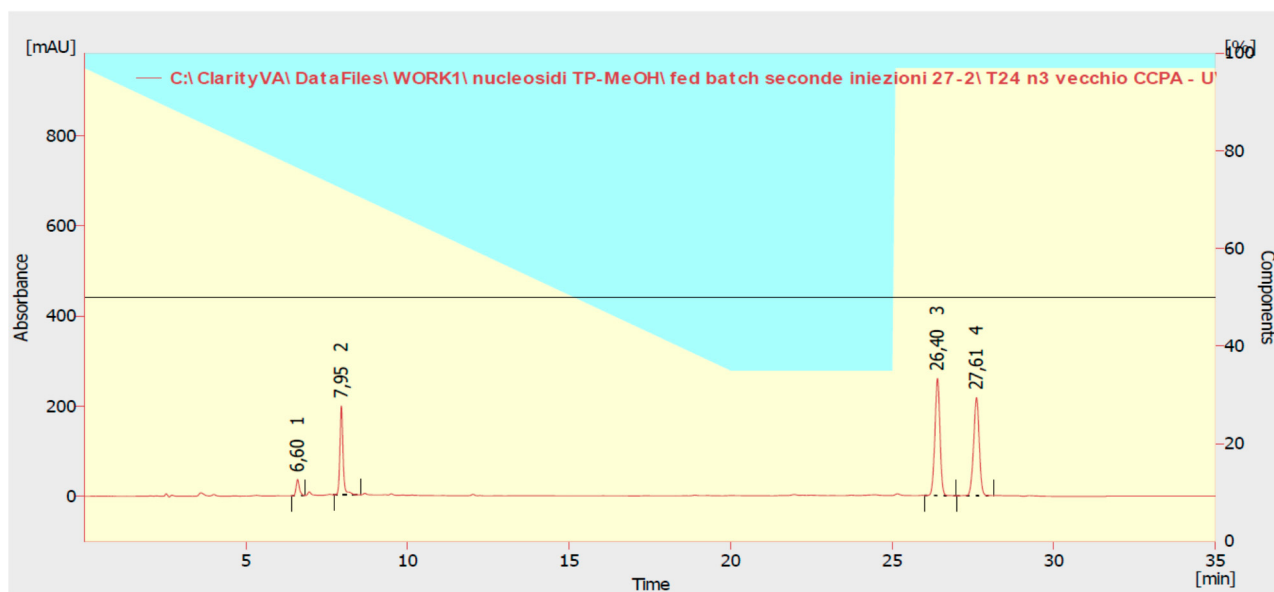
tr: 6.61 min (7-methylguanosine iodide, **7**); 7.97 min (tentatively attributed to 7-methylguanine, **19**); 26.39 min (CCPA, **17**); 27.60 min (2-chloro-*N*⁶-cyclopentyladenine, **12**)

d) t=6 h (after adding the last portion of **12**)

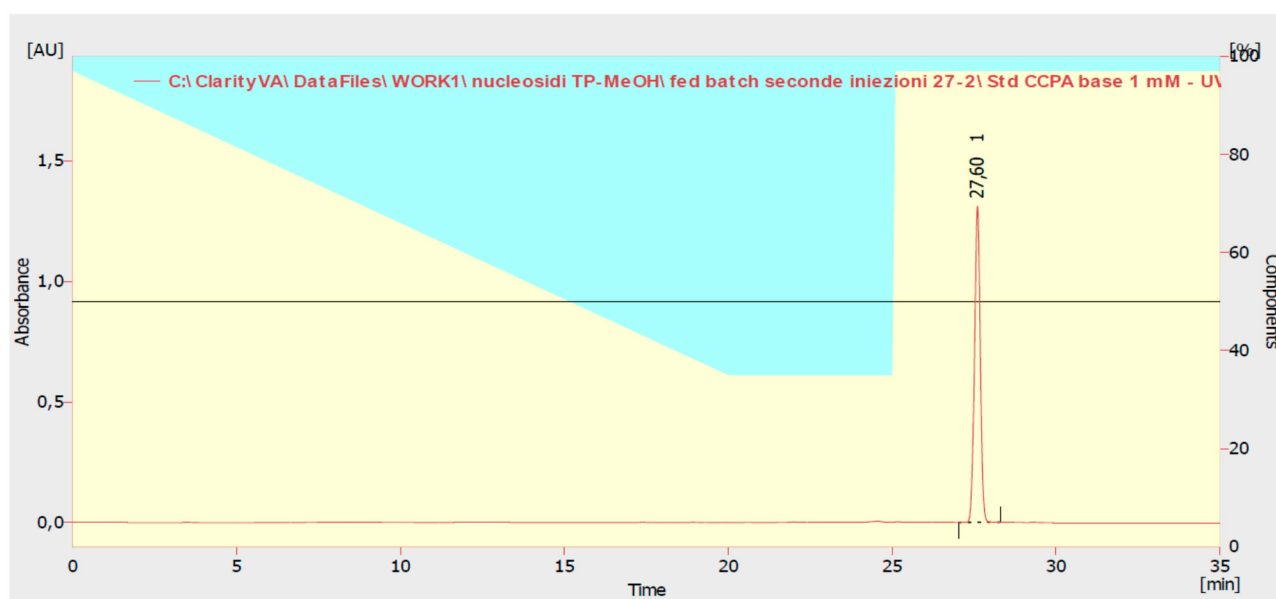


tr: 6.62 min (7-methylguanosine iodide, **7**); 7.98 min (tentatively attributed to 7-methylguanine, **19**); 26.39 min (CCPA, **17**); 27.60 min (2-chloro-*N*⁶-cyclopentyladenine, **12**)

e) t=24 h



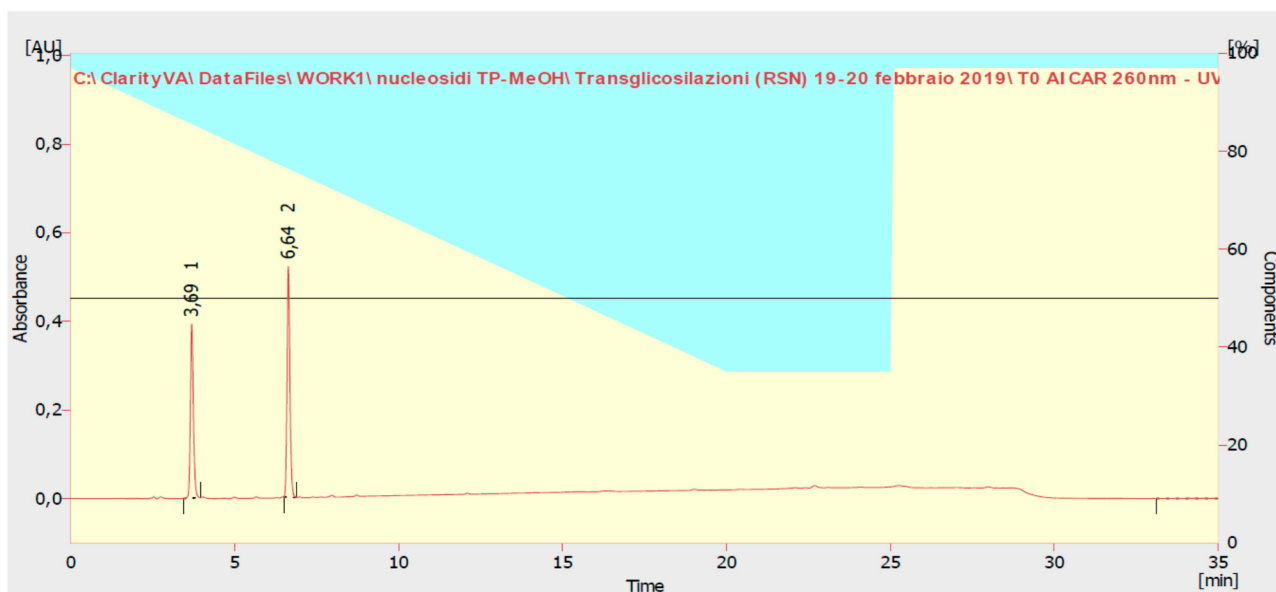
tr: 6.60 min (7-methylguanosine iodide, **7**); 7.95 min (tentatively attributed to 7-methylguanine, **19**); 26.40 min (CCPA, **17**); 27.61 min (2-chloro-*N*⁶-cyclopentyladenine, **12**)

f) 2-Chloro-*N*⁶-cyclopentyladenine (**12**) (standard)

t_R : 27.60 min

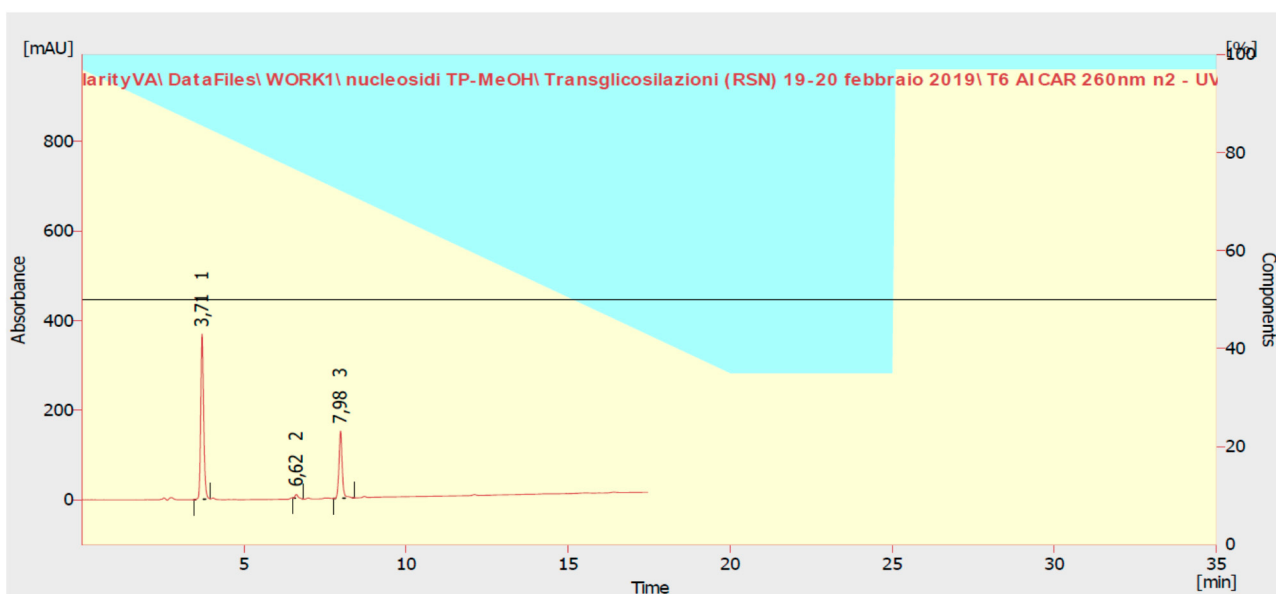
S6. HPLC monitoring of the enzymatic synthesis of Acadesine (18)

a) t=0



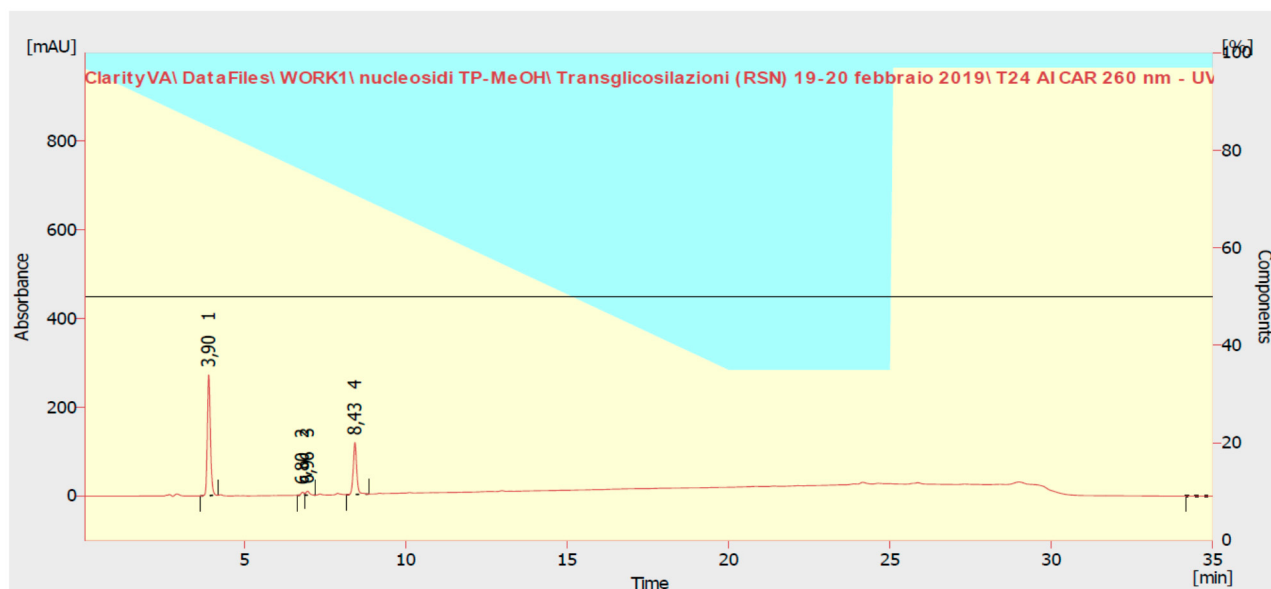
tr: 3.69 min (5-amino-1*H*-imidazole-4-carboxamide, **16**); 6.64 min (7-methylguanosine iodide, **7**)

b) t=6 h



tr: 3.71 min (5-amino-1*H*-imidazole-4-carboxamide, **16**); 6.62 min (7-methylguanosine iodide, **7**); 7.98 min (tentatively attributed to 7-methylguanine, **19**)

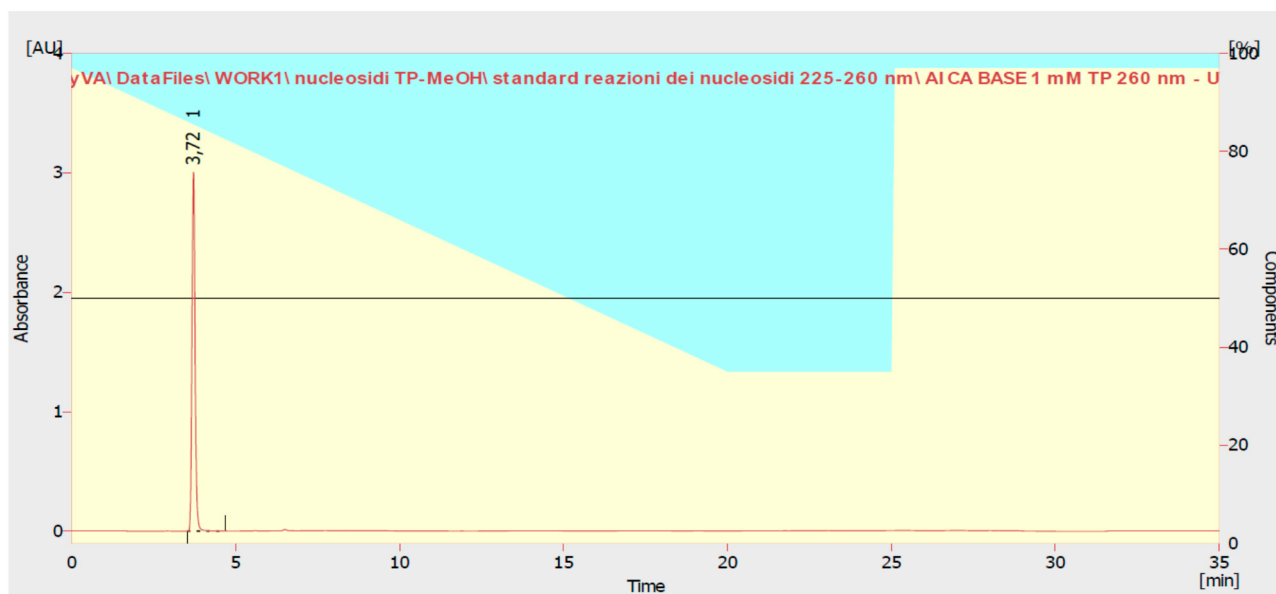
c) t=24 h



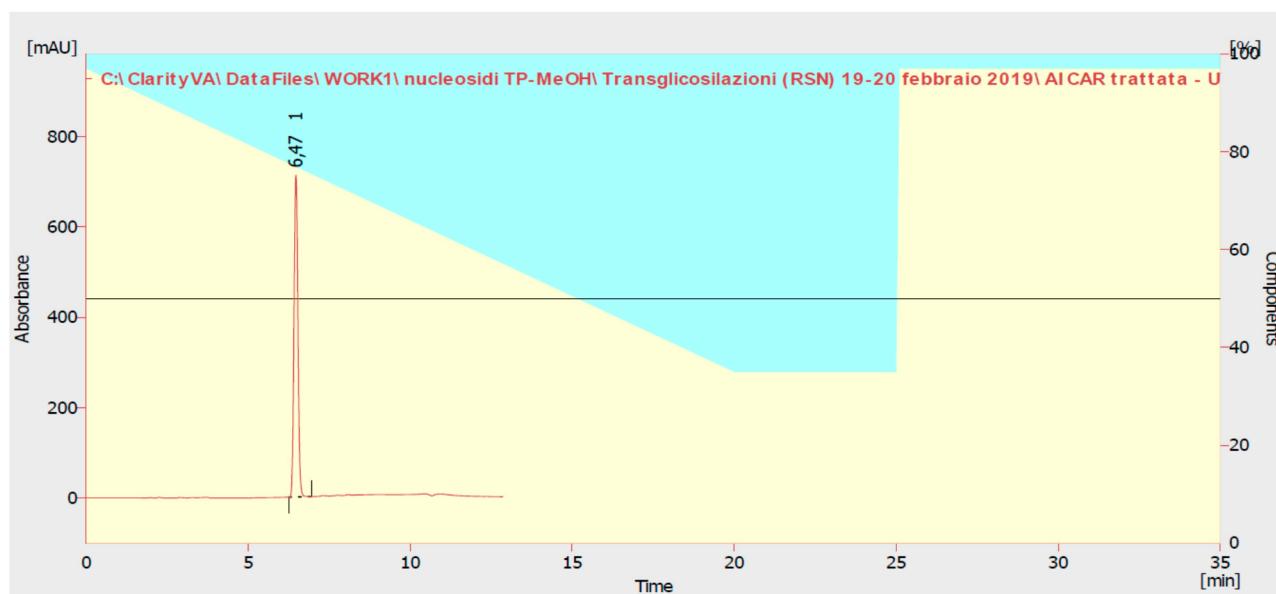
t_R : 3.90 min (5-amino-1*H*-imidazole-4-carboxamide, **16**); 6.96 (7-methylguanosine iodide, **7**); 8.43 min (tentatively attributed to 7-methylguanine, **19**)

A peak at t_R 6.80 min was detected (<2%).

d) 5-Amino-1*H*-imidazole-4-carboxamide (**16**) (standard)



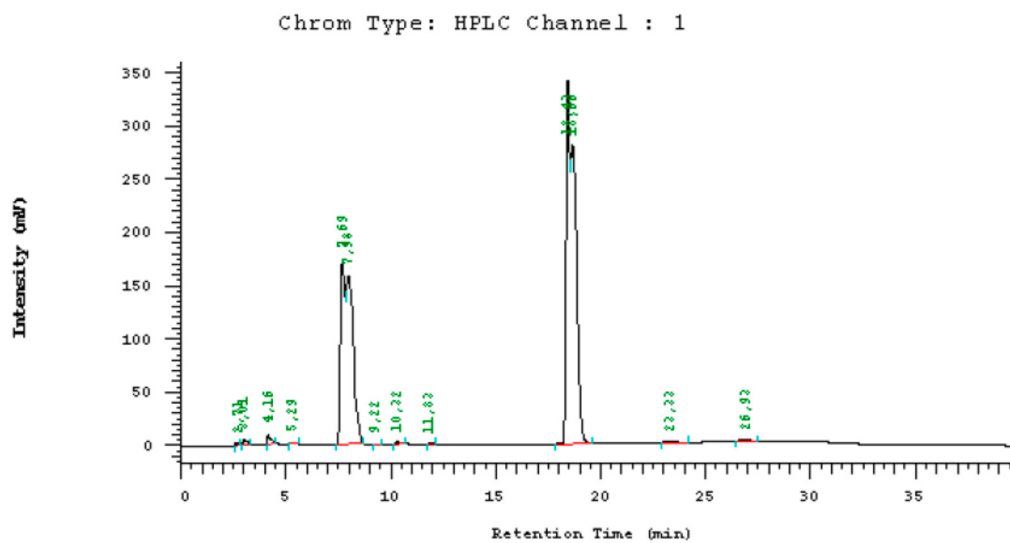
t_R : 3.72 min

e) Acadesine or AICAR (**18**) (standard)

tr: 6.47 min

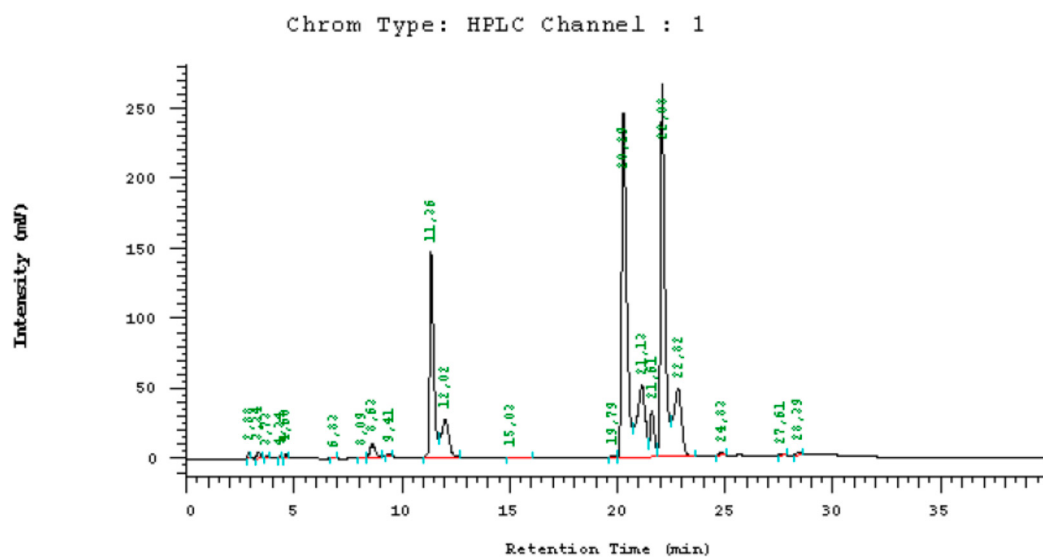
S7. HPLC monitoring of the enzymatic synthesis of Tecadenoson (2)

a) t=0

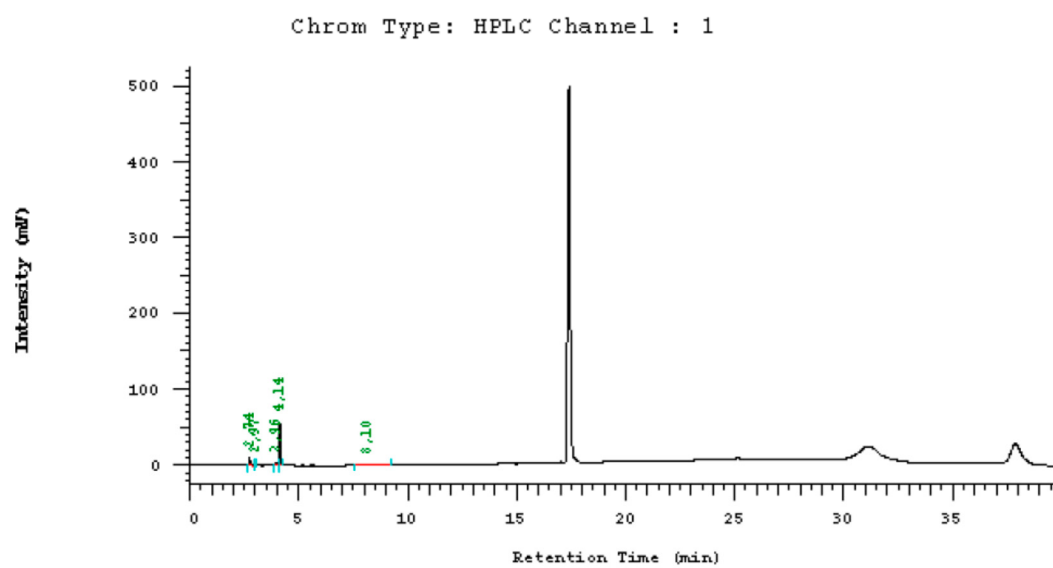


tr: 7.69 min (7-methylguanosine iodide, **7**); 18.48 min (6-(3-aminotetrahydrofuryl)purine (**14**))

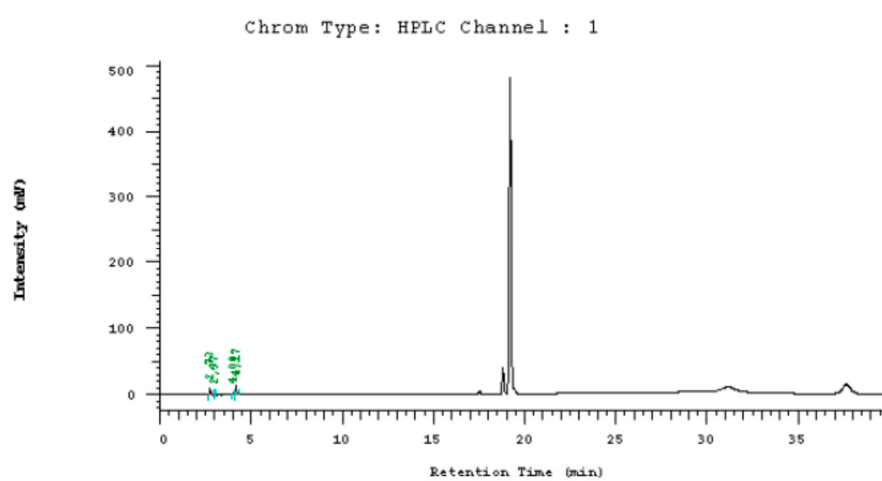
b) t=24 h



tr: 8.62 min (7-methylguanosine iodide, **7**); 11.26 min (tentatively attributed to 7-methylguanine, **19**); 20.20 min (6-(3-aminotetrahydrofuryl)purine (**14**)); 22.08 (Tecadenoson, **2**)

c) 6-(3-Aminotetrahydrofuranyl)purine (**14**) (standard)

tr: 17.46 min

d) Tecadenoson (**2**) (standard)

tr: 19.32 min