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Supplemental Information

Thermodynamic, Anticoagulant, and Antiproliferative Properties of Thrombin Binding Aptamer Containing Novel UNA Derivative

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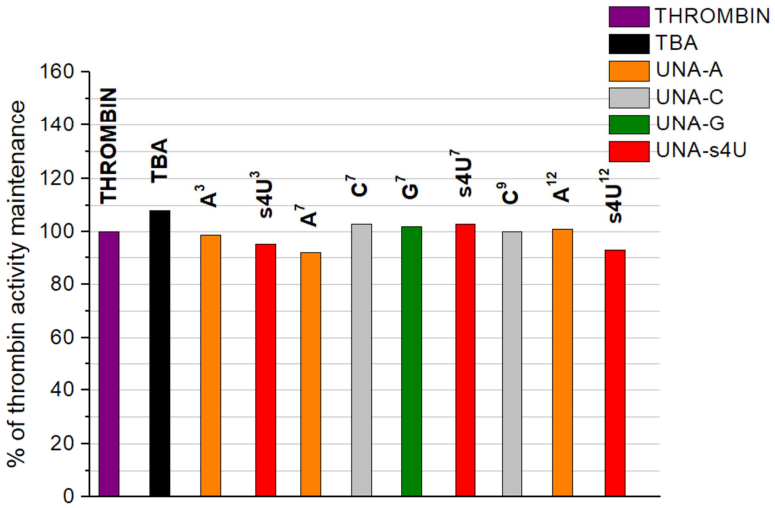


Figure S1. The amidolytic activity of thrombin in the presence of unmodified TBA (black bar) and TBA variants modified with UNA-A (orange bars), UNA-C (grey bars), UNA-G (green bars) and UNA-s4U (red bars).

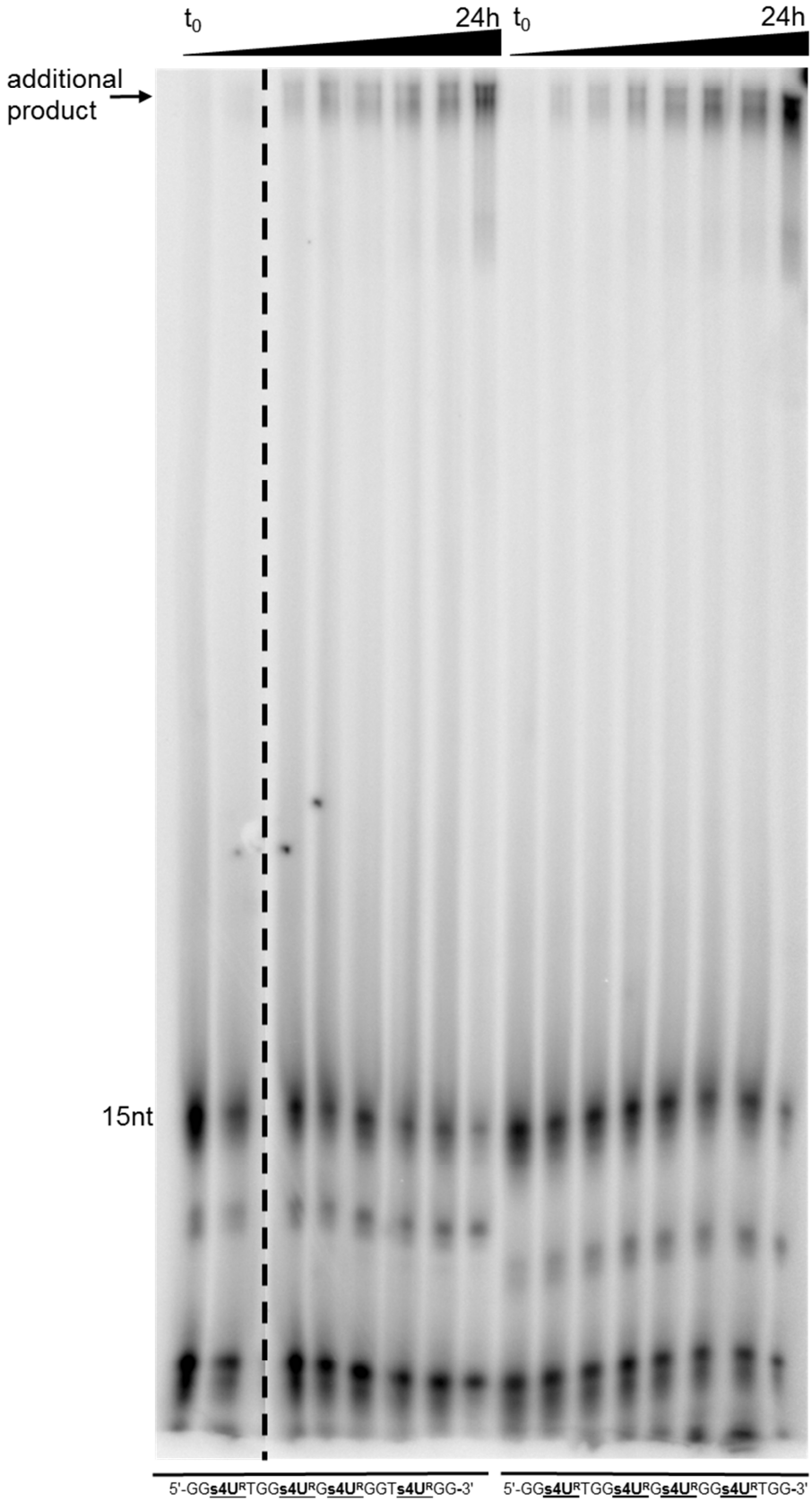


Figure S2. The biostability of TBA variants modified with RNA-s4U at T^3, T^7, T^9, T^{13} or T^3, T^7, T^9, T^{12} in human serum.

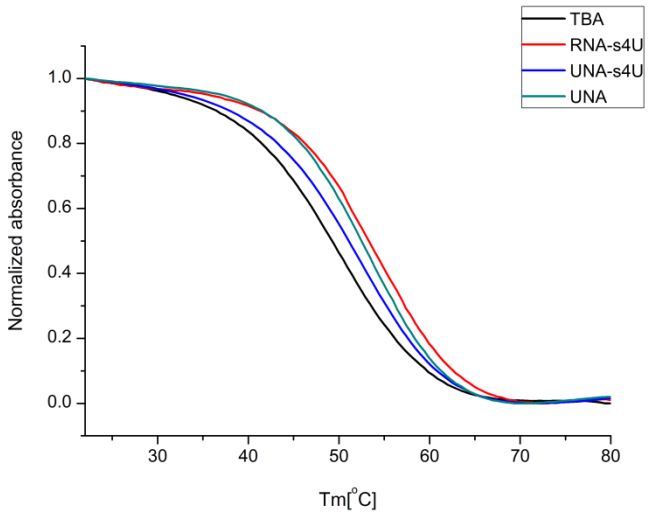


Figure S3. Representative, normalized melting curves of unmodified TBA (black line) and TBA variants modified with canonical UNA (green line, UNA-C at T^7), UNA-s4U (blue line, UNA-s4U at T^7) and RNA-s4U (red line, RNA-s4U at T^{12}).

Table S1. Thermodynamic parameters of G-quadruplex formation with UNA-G (G^U)[a]

Position of modification	Sequence (5′-3′)	Average of curve fits				T_M^{-1} vs log C_T plots				
		-ΔH° (kcal/mol)	-ΔS° (eu)	ΔG°$_{37}$ (kcal/mol)	T_M[b] (°C)	-ΔH° (kcal/mol)	-ΔS° (eu)	ΔG°$_{37}$ (kcal/mol)	T_M[b] (°C)	ΔT_M[b] (°C)
	GGTTGGTGTGGTTGG	41.2±0.9	127.2±2.7	-1.74±0.02	50.7					0
T^4	GGT**G**^{U}GGTGTGGTTGG	72.2±5.4	210.4±17.2	-6.93±0.05	42.5	79.1±3.1	232.4±10.0	-7.01±0.05	42.3	-8.2

[a] buffer: 100 mM KCl, 20 mM sodium cacodylate, 0.5 mM EDTA$(Na)_2$, pH 7.0.
[b] calculated for 10^{-4} M.

Table S2. Concentration dependence of thrombin time values[a]

Position of modification	Sequence (5′-3′)	Concentration [μM]						
		0.0825	**0.165**	**0.33**	**0.495**	**0.66**	**0.825**	**1**
		Thrombin time [s]						
	GGTTGGTGTGGTTGG	28.3 ± 1.4	38.3 ± 0.5	60.0 ± 1.7	95.2 ± 3.8	139.9 ± 7.7	195.9 ± 16.8	300.0 ± 0.0*
T^{3}	GG**A**^{U}TGGTGTGGTTGG	23.7 ± 1.5	27.7 ± 2.4	39.2 ± 2.2	41.0 ± 9.8	51.4 ± 10.9	57.0 ± 4.4	102.0 ± 4.4
T^{7}	GGTTGG**A**^{U}GTGGTTGG	26.5 ± 0.4	36.1 ± 1.1	59.3 ± 2.3	97.7 ± 2.5	153.0 ± 22.3	300.0 ± 0.0*	300.0 ± 0.0*
T^{7}	GGTTGG**C**^{U}GTGGTTGG	28.0 ± 1.0	39.4 ± 1.8	68.4 ± 1.6	116.3 ± 5.6	164.3 ± 8.0	300.0 ± 0.0*	300.0 ± 0.0*
T^{7}	GGTTGG**G**^{U}GTGGTTGG	25.5 ± 1.5	30.8 ± 1.6	45.3 ± 1.8	64.6 ± 3.1	89.7 ± 5.4	111.5 ± 5.0	162.4 ± 15.5
T^{9}	GGTTGGTG**C**^{U}GGTTGG	25.5 ± 1.5	27.6 ± 1.2	35.2 ± 1.5	41.6 ± 1.0	49.6 ± 1.0	56.4 ± 1.0	64.3 ± 2.6
T^{12}	GGTTGGTGTGG**A**^{U}TGG	23.2 ± 0.7	26.9 ± 1.0	37.4 ± 0.8	49.7 ± 1.0	57.6 ± 2.3	76.3 ± 3.5	92.9 ± 6.5
T^{3}	GG**s4U**^{U}TGGTGTGGTTGG	21.6 ± 0.8	25.9 ± 1.3	27.3 ± 1.3	34.5 ± 0.3	46.5 ± 1.3	63.9 ± 1.6	66.6 ± 2.1
T^{7}	GGTTGG**s4U**^{U}GTGGTTGG	25.9 ± 1.1	34.1 ± 1.4	60.5 ± 2.0	83.9 ± 5.4	119.9 ± 8.5	156.9 ± 11.3	300.0 ± 0.0*
T^{12}	GGTTGGTGTGG**s4U**^{U}TGG	21.8 ± 0.8	26.5 ± 1.4	36.2 ± 1.3	33.0 ± 1.2	37.8 ± 2.7	45.5 ± 1.1	50.6 ± 3.3

[a] Thrombin time value for plasma without aptamers was in the range of 16.9 – 19.8 s.
* The coagulometer limit of the TT measurement reliability was up to 300 seconds.

Table S3. Concentration dependence of anticoagulant effect measured *via* thrombin time assay

Position of modification	Sequence (5′-3′)	Concentration [µM]						
		0.0825	**0.165**	**0.33**	**0.495**	**0.66**	**0.825**	**1**
		Anticoagulant effect [s]						
	GGTTGGTGTGGTTGG	8.8	18.7	40.4	74.8	119.6	175.5	279.7
T^{3}	GG**A**^{U}TGGTGTGGTTGG	4.9	8.9	20.4	22.2	32.6	38.3	82.3
T^{7}	GGTTGG**A**^{U}GTGGTTGG	6.2	16.4	39.7	77.7	132.6	279.7	280.1
T^{7}	GGTTGG**C**^{U}GTGGTTGG	8.4	19.8	48.8	95.9	143.9	279.7	279.7
T^{7}	GGTTGG**G**^{U}GTGGTTGG	5.9	11.9	26.4	46.6	71.6	92.6	144.4
T^{9}	GGTTGGTG**C**^{U}GGTTGG	5.9	8.6	16.2	22.6	30.5	37.4	45.2
T^{12}	GGTTGGTGTGG**A**^{U}TGG	4.1	7.8	19.1	30.7	39.2	57.9	73.9
T^{3}	GG**s4U**^{U}TGGTGTGGTTGG	3.5	6.9	9.2	16.3	26.8	44.2	46.9
T^{7}	GGTTGG**s4U**^{U}GTGGTTGG	6.8	15.0	41.1	64.7	100.8	137.8	280.9
T^{12}	GGTTGGTGTGG**s4U**^{U}TGG	3.9	7.7	16.5	14.3	19.1	25.8	31.8

Table S4. Concentration dependence of antiproliferative effect – preliminary MTT experiments

Position of modification	Sequence (5′-3′)	Concentration [μM]	
		0.10	**5.0**
		***HeLa* cell viability [%]**	
	GGTTGGTGTGGTTGG	99.3	72.4
T^3	GG**G**^{U}TGGTGTGGTTGG	96.8	86.1
T^4	GGT**A**^{U}GGTGTGGTTGG	85.6	92.2
T^4	GGT**C**^{U}GGTGTGGTTGG	84.1	89.3
T^4	GGT**G**^{U}GGTGTGGTTGG	82.6	82.2
T^7	GGTTGG**A**^{U}GTGGTTGG	90.9	97.3
T^7	GGTTGG**C**^{U}GTGGTTGG	90.5	82.1
T^7	GGTTGG**G**^{U}GTGGTTGG	90.4	82.1
T^8	GGTTGGT**A**^{U}TGGTTGG	103.9	84.7
T^8	GGTTGGT**C**^{U}TGGTTGG	89.1	89.8
T^9	GGTTGGTG**A**^{U}GGTTGG	90.7	84.4
T^9	GGTTGGTG**C**^{U}GGTTGG	85.7	82.0
T^{12}	GGTTGGTGTGG**A**^{U}TGG	83.6	97.8
T^{12}	GGTTGGTGTGG**C**^{U}TGG	103.0	89.4
T^{12}	GGTTGGTGTGG**G**^{U}TGG	94.3	86.7
T^{13}	GGTTGGTGTGGT**A**^{U}GG	93.6	80.4
T^{13}	GGTTGGTGTGGT**C**^{U}GG	93.5	86.3
T^{13}	GGTTGGTGTGGT**G**^{U}GG	82.8	91.8
T^3	GG**s4U**^{R}TGGTGTGGTTGG	102.1	90.3
T^3	GG**s4U**^{U}TGGTGTGGTTGG	92.6	81.5
T^4	GGT**s4U**^{R}GGTGTGGTTGG	94.6	83.5
T^4	GGT**s4U**^{U}GGTGTGGTTGG	99.6	96.4
T^7	GGTTGG**s4U**^{R}GTGGTTGG	104.8	94.3
T^7	GGTTGG**s4U**^{U}GTGGTTGG	92.9	76.5
T^8	GGTTGGT **s4U**^{R}TGGTTGG	97.9	75.6
T^8	GGTTGGT**s4U**^{U}TGGTTGG	92.6	81.5
T^9	GGTTGGTG**s4U**^{R}GGTTGG	93.9	75.8
T^9	GGTTGGTG**s4U**^{U}GGTTGG	93.8	103.4
T^{12}	GGTTGGTGTGG**s4U**^{R}TGG	101.7	89.4
T^{12}	GGTTGGTGTGG**s4U**^{U}TGG	88.4	94.5
T^{13}	GGTTGGTGTGGT**s4U**^{R}GG	93.4	66.5
T^{13}	GGTTGGTGTGGT**s4U**^{U}GG	75.2	89.5
T^3, T^7, T^9, T^{12}	GG**s4U**^{R}TGG**s4U**^{R}G**s4U**^{R}GG**s4U**^{R}TGG	93.0	45.0
T^3, T^7, T^9, T^{12}	GG**s4U**^{U}TGG**s4U**^{U}G**s4U**^{U}GG**s4U**^{U}TGG	84.1	105.6
T^3, T^7, T^9, T^{13}	GG**s4U**^{R}TGG**s4U**^{R}G**s4U**^{R}GGT**s4U**^{R}GG	99.5	9.2
T^3, T^7, T^9, T^{13}	GG**s4U**^{U}TGG**s4U**^{U}G**s4U**^{U}GGT**s4U**^{U}GG	83.7	93.7

Table S5. Concentration dependence of antiproliferative effect

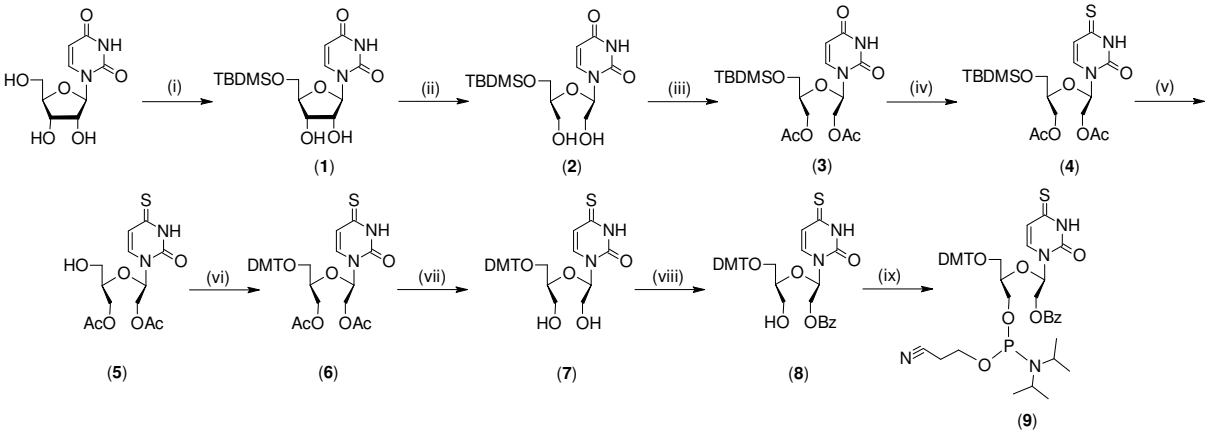
Position of modification	Sequence (5′-3′)	Concentration [μM]		
		0.10	**5.0**	**10.0**
			***HeLa* cell viability [%]**	
	GGTTGGTGTGGTTGG	99.0	82.8	33.9
T^3, T^7, T^9, T^{12}	GG**s4U^R**TGG**s4U^R**G**s4U^R**GG**s4U^R**TGG	89.4	24.3	14.1
T^3, T^7, T^9, T^{13}	GG**s4U^R**TGG**s4U^R**G**s4U^R**GGT**s4U^R**GG	86.3	29.7	5.5

Table S6. Thrombin time (TT) values of TBA and TBA variants modified with canonical UNAs (A^U, C^U, G^U, U^U), UNA-s4U ($s4U^U$) and RNA-s4U ($s4U^R$)[a]

Position of modification	Sequence (5′-3′)	TT [s]	Position of modification	Sequence (5′-3′)	TT [s]
	GGTTGGTGTGGTTGG	35.4 ± 1.7	T^{13}	GGTTGGTGTGGT**C^U**GG	19.3 ± 0.6
T^3	GG**A^U**TGGTGTGGTTGG	25.8 ± 0.8	T^{13}	GGTTGGTGTGGT**G^U**GG	19.2 ± 0.8
T^3	GG**C^U**TGGTGTGGTTGG	22.7 ±0.9	T^3	GG**s4U^R**TGGTGTGGTTGG	22.1 ± 0.6
T^3	GG**G^U**TGGTGTGGTTGG	23.8 ± 0.6	T^3	GG**s4U^U**TGGTGTGGTTGG	26.8 ± 1.6
T^4	GGT**A^U**GGTGTGGTTGG	18.7 ± 0.8	T^4	GGT**s4U^R**GGTGTGGTTGG	19.2 ± 0.6
T^4	GGT**C^U**GGTGTGGTTGG	18.6 ± 0.4	T^4	GGT**s4U^U**GGTGTGGTTGG	18.5 ± 0.6
T^4	GGT**G^U**GGTGTGGTTGG	18.8 ± 0.4	T^7	GGTTGG**s4U^R**GTGGTTGG	22.9 ± 0.8
T^7	GGTTGG**A^U**GTGGTTGG	33.3 ± 1.2	T^7	GGTTGG**s4U^U**GTGGTTGG	33.6 ± 2.3
T^7	GGTTGG**C^U**GTGGTTGG	34.2 ± 1.2	G^8	GGTTGGT**s4U^R**TGGTTGG	23.7 ± 0.8
T^7	GGTTGG**G^U**GTGGTTGG	28.2 ± 0.9	G^8	GGTTGGT**s4U^U**TGGTTGG	21.8 ± 0.3
G^8	GGTTGGT**A^U**TGGTTGG	22.2 ± 0.4	T^9	GGTTGGTG**s4U^R**GGTTGG	19.5 ± 0.5
G^8	GGTTGGT**C^U**TGGTTGG	21.1 ± 0.4	T^9	GGTTGGTG**s4U^U**GGTTGG	25.0 ± 0.8
G^8	GGTTGGT**U^U**TGGTTGG	22.6 ± 0.4	T^{12}	GGTTGGTGTGG**s4U^R**TGG	23.1 ± 0.8
T^9	GGTTGGTG**A^U**GGTTGG	23.4 ± 0.6	T^{12}	GGTTGGTGTGG**s4U^U**TGG	26.4 ± 1.9
T^9	GGTTGGTG**C^U**GGTTGG	24.9 ± 1.0	T^{13}	GGTTGGTGTGGT**s4U^R**GG	20.5 ± 0.7
T^9	GGTTGGTG**G^U**GGTTGG	23.5 ± 0.8	T^{13}	GGTTGGTGTGGT**s4U^U**GG	18.2 ± 0.4
T^{12}	GGTTGGTGTGG**A^U**TGG	24.9 ± 0.6	T^3, T^7, T^9, T^{12}	GG**s4U^R**TGG**s4U^R**G**s4U^R**GG**s4U^R**TGG	18.9 ± 0.7
T^{12}	GGTTGGTGTGG**C^U**TGG	23.0 ± 0.5	T^3, T^7, T^9, T^{12}	GG**s4U^U**TGG**s4U^U**G**s4U^U**GG**s4U^U**TGG	18.7 ± 0.4
T^{12}	GGTTGGTGTGG**G^U**TGG	24.3 ± 0.4	T^3, T^7, T^9, T^{13}	GG**s4U^R**TGG**s4U^R**G**s4U^R**GGT**s4U^R**GG	18.0 ± 0.7
T^{13}	GGTTGGTGTGGT**A^U**GG	18.8 ± 0.4	T^3, T^7, T^9, T^{13}	GG**s4U^U**TGG**s4U^U**G**s4U^U**GGT**s4U^U**GG	18.7 ± 0.9

[a] Thrombin time value for plasma without aptamers was in the range of 16.3 – 19.3 s.

Chemical synthesis of UNA 4-thiouridine phosphoramidite



Scheme S1. Chemical synthesis of UNA 4-thiouridine phosphoramidite. Reagents: (i) TBDMSCl, imidazole, DCM; (ii) $NaIO_4$, 1,4-dioxane/water; $NaBH_4$, 1,4-dioxane/water; (iii) Ac_2O, Py; (iv) Lawesson reagent, Py_{anh}; (v) TEA·3HF, DCM; (vi) DMTCl, Py; (vii) Et_3N/MeOH (1:10, v/v); (viii) BzCl, DCM, -70 °C; (ix) 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite, tetrazole, MeCN.

***Synthesis of 5′-O-(tert-butyldimethylsilyl)-uridine* (1)**

Uridine (12.2 g, 50 mmol) was dissolved in 200 ml of *N,N*′-dimethylformamide. To this solution was added tert-butyldimethylsillyl chloride (8.44 g, 60 mmol) and imidazole (8.17 g, 120 mmol). The mixture was stirred at rt. After 24 h the reaction mixture was condensed, poured into dichloromethane and washed with saturated aqueous solution of sodium dihydrogen phosphate. The organic phase was separated, dried over Na_2SO_4 and evaporated to dryness affording an oil product (**1**). ^{1}H NMR (DMSO-d_6): δ 11.31 (s, NH), 7.76 (d, H6), 5.86 (d, H1′), 5.55 (d, 2′OH), 5.08 (d, 3′OH), 3.98 (q, H2′), 3.91 (q, H3′), 3.87 (m, H4′), 3.76 (d, H5′), 0.87 (s, CH_3-C), 0.07 (s, CH_3-Si).

***Synthesis of 5′-O-(tert-butyldimethylsilyl)-2′,3′-secouridine* (2)**

Nucleoside (**1**) (17.4 g, 50 mmol) was dissolved in a mixture of 1,4-dioxane (400 ml) and water (80 ml). To a mixed solution was added $NaIO_4$ (11.8 g, 55 mmol) dissolved in water (80 ml). The reaction mixture was stirred at rt. During 1 h of the reaction the white precipitate was formed. Additional 1,4-dioxane (100 ml) was added and after 15 min of stirring the suspension was filtered and the filter cake was washed with dioxane. The filtrates were combined, $NaBH_4$ (2.1 g, 55.5 mmol) was added, and the mixture was stirred for 30 min at rt. The reaction mixture was neutralized by the addition of pyridine/acetic acid mixture (1:1, v/v, approx. 25 ml). The mixture was concentrated, dichloromethane was added and washed with saturated aqueous solution of sodium bicarbonate. The organic phase was separated, dried over Na_2SO_4 and evaporated to dryness affording a white powder of compound (**2**). ^{1}H NMR (DMSO-d_6): δ 11.31 (s, NH), 7.56 (d, H6), 5.66 (t, H1′), 5.55 (d, H5), 5.01 (t, 2′OH), 4.77 (t, 3′OH), 3.54 (m, H2′, H4′), 3.43 (m, H3′, H5′), 0.79 (s, CH_3-C), -0.04 (s, CH_3-Si).

***Synthesis of 2',3'-O-diacetyl-5'-O-(tert-butyldimethylsilyl)-2',3'-secouridine* (3)**

Nucleoside (**2**) (17.52 g, 50 mmol) was dissolved in anhydrous pyridine (200 ml) and acetic anhydride was added (13.2 ml, 120 mmol). Reaction mixture was stirred for 2 h at rt. Next, the solution was concentrated, dichloromethane was added and all was washed with saturated aqueous solution of sodium bicarbonate. The organic phase was separated, dried over Na_2SO_4 and evaporated to dryness affording compound (**3**). ^{1}H NMR (DMSO-d_6): δ 11.41 (s, NH), 7.66 (d, H6), 5.75 (t, H1′), 5.62 (d, H5), 4.31 (dd, H2′), 4.23 (dd, H3′), 4.14 (dd, H2′), 4.03 (dd, H3′), 3.73 (m, H4′), 3.57 (m, H5′), 2.05 (s, OAc), 1.99 (s, OAc), 0.81 (s, CH_3-C), -0.01 (s, CH_3-Si).

***Synthesis of 2',3'-O-diacetyl-5'-O-(tert-butyldimethylsilyl)-2',3'-seco-4-thiouridine* (4)**

Nucleoside (**3**) (21.72 g, 50 mmol) was dissolved in anhydrous pyridine (200 ml). The reaction solution was heated to 95°C and the Lawesson reagent was added (30.34 g, 75 mmol). After 24 h the reaction mixture was concentrated, dichloromethane was added and all was washed with saturated aqueous solution of sodium bicarbonate. The organic phase was separated, dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by column chromatography (0-4% methanol in dichloromethane) affording nucleoside (**4**) as a yellow foam (12.91 g, 28.1 mmol). ^{1}H NMR (DMSO-d_6): δ 12.71 (s, NH), 7.56 (d, H6), 6.29 (d, H5), 5.98 (t, H1′), 4.32 (dd, H2′), 4.23 (dd, H3′), 4.16 (dd, H2′), 4.04 (dd, H3′), 3.79 (m, H4′), 3.59 (m, H5′), 2.04 (s, OAc), 1.99 (s, OAc), 0.82 (s, CH_3-C), -0.01 (s, CH_3-Si).

***Synthesis of 2',3'-O-diacetyl-2',3'-seco-4-thiouridine* (5)**

Nucleoside (**4**) (12.91 g, 28.1 mmol) was dissolved in dichloromethane (200 ml) and triethylamine trihydrofluoride (6.87 ml, 42.15 mmol) was added. The reaction mixture was stirred at rt. After 24 h the reaction mixture was concentrated, dichloromethane was added and all washed with saturated aqueous solution of sodium bicarbonate. The organic phase was separated, dried over Na_2SO_4 and evaporated to dryness affording compound (**5**) as a yellow foam. ^{1}H NMR (DMSO-d_6): δ 7.59 (d, H6), 6.03 (d, H5), 5.96 (t, H1′), 4.30 (dd, H2′), 4.21 (dd, H3′), 4.13 (dd, H2′), 4.03 (dd, H3′), 3.70 (m, H4′), 3.37 (m, H5′), 2.05 (s, OAc), 1.99 (s, OAc).

***Synthesis of 5'-O-(4,4'-dimethoxytrityl)-2',3'-O-diacetyl-2',3'-seco-4-thiouridine* (6)**

Nucleoside (**5**) (9.73 g, 28.1 mmol) was dissolved in anhydrous pyridine (200 ml) and 4,4′-dimethoxytrityl chloride (11.43 g, 33.72 mmol) was added. Reaction mixture was stirred for 1.5 h at rt. Next, the solution was concentrated, dichloromethane was added and all washed with saturated aqueous solution of sodium bicarbonate. The organic phase was separated, dried over Na_2SO_4 and evaporated to dryness affording compound (**6**) as yellow foam. ^{1}H NMR (DMSO-d_6): δ 12.83 (s, NH), 7.58 (d, H6), 7.07 (m, DMT), 6.22 (d, H5), 5.97 (t, H1′), 4.36 (dd, H2′), 4.21 (ss, H3′), 4.15 (dd, H2′), 4.05 (dd, H3′), 3.82 (m, H4′), 3.74 (s, OMe), 3.72 (s, OMe), 3.10 (m, H5′), 1.99 (s, OAc), 1.98 (s, OAc).

***Synthesis of 5'-O-(4,4'-dimethoxytrityl)-2',3'-seco-4-thiouridine* (7)**

Nucleoside (**6**) (18.22 g, 28.1 mmol) was dissolved in trimethylamine/methanol mixture (110 ml, 1:10, v/v). The solution was stirred for 120 h at rt. Next, the solution was evaporated, the oil product was purified by column chromatography (0-10% methanol in dichloromethane) affording nucleoside (**7**) as yellow foam (14.11 g, 25 mmol). ^{1}H NMR (DMSO-d_6): δ 12.77 (s,

NH), 7.57 (d, H6), 7.30 (m, DMT), 6.68 (m, DMT), 6.23 (d, H5), 5.81 (t, H1′), 5.17 (t, 2′OH), 4.77 (t, 3′OH), 3.67 (m, H4′), 3.40 (m, H3′), 2.98 (m, H5’).

***Synthesis of 2'-O-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-2',3'-seco-4-thiouridine* (8)**

Nucleoside (**7**) (14.11 g, 25 mmol) was dissolved in anhydrous pyridine (22 ml) along with anhydrous dichloromethane (500 ml). The reaction mixture was stirred, cooled to -70°C and benzoyl chloride (3.69 g, 26.25 mmol) was added over 20 min. The reaction was continued for 1 h at -70°C, next it was warmed to rt and ethanol (10 ml) was added. The solution was concentrated, dichloromethane was added and all was washed with saturated aqueous solution of sodium bicarbonate. The organic phase was separated, dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by column chromatography (0-6% methanol in dichloromethane) affording nucleoside (**8**) as yellow foam (10.72 g, 15.86 mmol). ^{1}H NMR (DMSO-d_6): δ 12.85 (s, NH), 7.71 (d, H6), 7.67 (m, DMT), 7.34 (m, DMT, OBz), 6.68 (m, DMT), 6.25 (d, H5), 6.20 (t, H1′), 4.77 (t, 3′OH), 4.63 (dd, H2′), 4.54 (dd, H2′), 3.73 (s, OMe), 3.71 (s, OMe), 3.69 (m, H4′), 3.45 (m, H3′), 3.08 (m, H5′), 3.04 (m, H5′).

***Synthesis of 2'-O-Benzoyl-3'-O-(2-cyanoethoxy(diisopropylamino)-phosphino)-5'-O-(4,4'-dimethoxytrityl)-2',3'-seco-4-thiouridine* (9)**

Nucleoside (**8**) (668 mg, 1 mmol) along with tetrazole (70 mg, 1 mmol) was dissolved in anhydrous acetonitrile and 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite (362 mg, 1.2 mmol) was added. The reaction mixture was stirred overnight at rt. The reaction mixture was extracted with dichloromethane containing 3% trimethylamine and with saturated aqueous solution of sodium bicarbonate. The organic phase was separated, dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by column chromatography (0 - 100% ethyl acetate/hexane with addition of 3% of trimethylamine) affording amidite (**9**) as a yellow foam (604 mg, 0,695 mmol). ^{32}P NMR (DMSO-d_6): δ 147.65.