Electronic Supplementary Information 2

Non-identical Electronic Characters of the Internucleotidic Phosphates in RNA Modulate the Chemical Reactivity of the Phosphodiester Bonds

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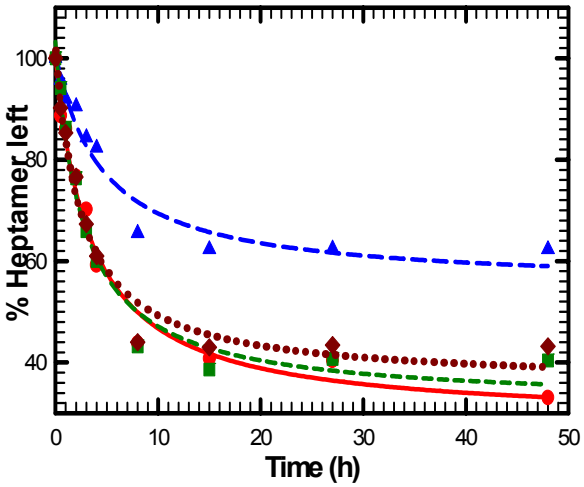


Figure S10A: Time dependent degradation of all four native heptameric ssRNAs [**5b** (●), **6b** (▲), **7b** (■), and **8b** (◆) under alkaline condition: pH 12.5 (aqueous NaOH)/ 20°C. The actual percentage area of the heptamer was calculated from the residual parent heptamer-peak at time points taken at 0 h, ½ h, 1 h, 2 h, 3 h, 4 h, 8 h, 15 h, 27 h and 48 h (experimentals and Figures S12, S15 in Supporting Information 2).

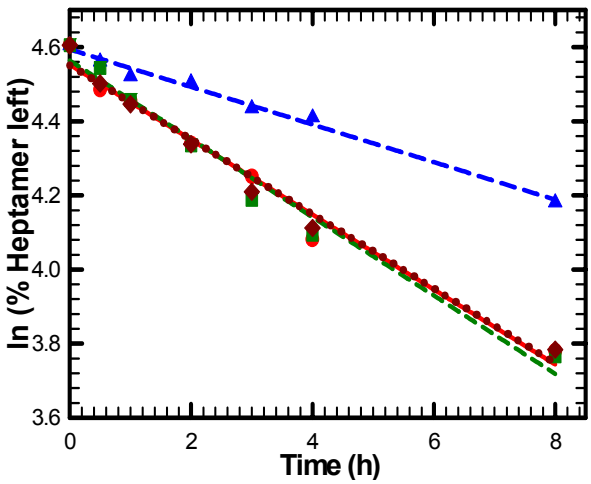


Figure S10B: Plots of ln (% Heptamer left) at various time intervals show the pseudo first-order rate constants (k) for the degradation of all four native heptameric ssRNAs: (●) **5'-r(CAAGAAC)-3' (5b)**, k = 0.1010h^{-1} (R = 0.9903). (▲) **5'-r(CAAGCAC)-3' (6b),** k = 0.0507h^{-1} (R = 0.9969). (■) **5'-r(CACGAAC)-3' (7b),** k = 0.1035 h^{-1} (R = 0.9899). (◆) **5'-r(CACGCAC)-3' (8b),** k = 0.1004 h^{-1} (R = 0.9920). Note that for **5b**, 6b and **8b**, plots are overlapping because they have similar cleavage rates.

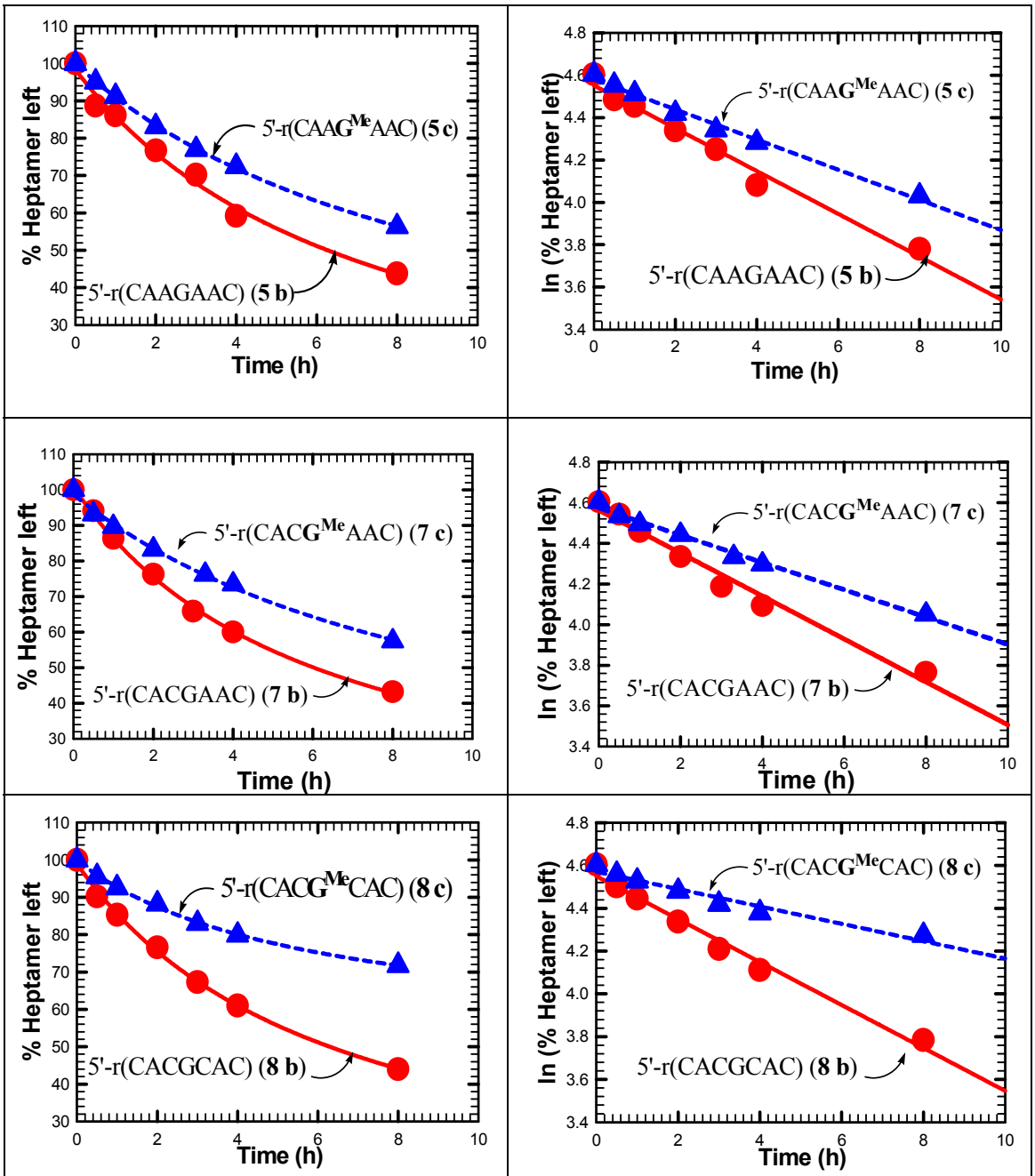
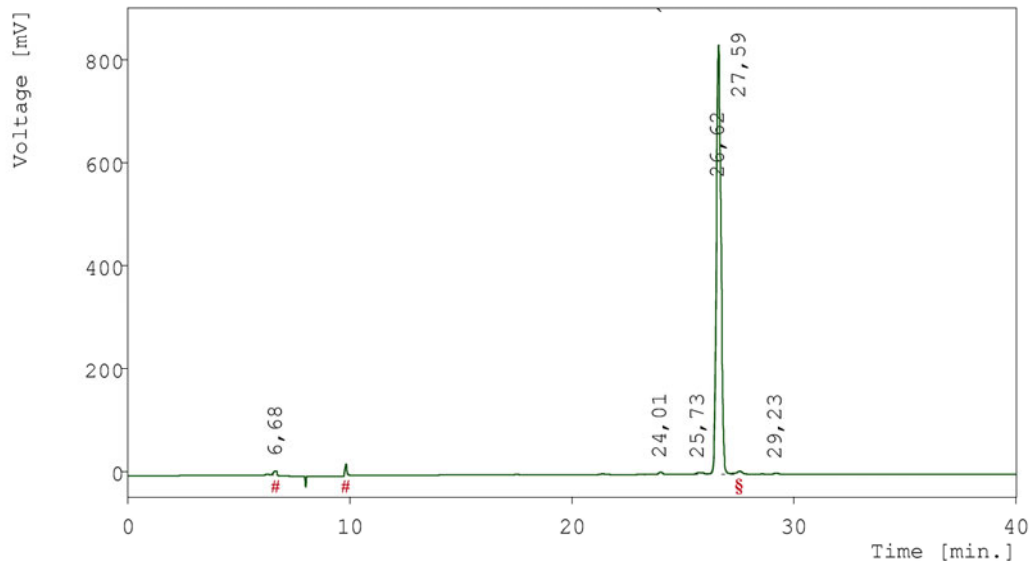


Figure S11A. Pair-wise comparison of the alkaline degradation of the native heptameric ssRNAs (▲) and the $N^{1\text{-Me}}$-G containing heptameric ssRNAs (●) at various time intervals (see Figure 7).

Figure S11B. Pair-wise comparison of the pseudo first order rate constants (k) at 20º C for the alkaline degradation of the native heptameric ssRNAs (▲) and the $N^{1\text{-Me}}$-G containing heptameric ssRNAs (●) at various time intervals (see Figure 7).

Figure S12A.

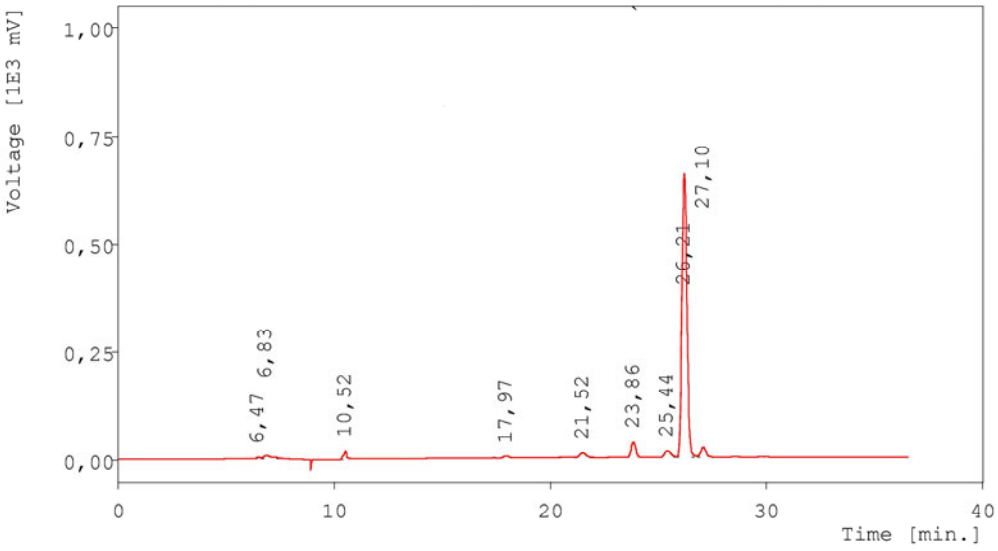


Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	6,680	81,9933	8,361	0,180	0,662	0,971
2	24,013	61,0089	4,669	0,193	0,493	0,542
3	25,727	78,9676	3,309	0,387	0,637	0,385
4	26,620	12015,5213	835,984	0,233	97,000	97,132
5	27,587	119,1940	5,923	0,260	0,962	0,688
6	29,227	30,5076	2,424	0,193	0,246	0,282
-	Total	12387,1927	860,671			

Figure S12(a1): Hplc analysis of alkaline Hydrolysis products of **5′-r(CAAGAAC)-3′** (5b) [after digestion for 0h at pH 12.5 using 0.03N NaOH/ 20°C, followed by quenching with 0.03 N aq. acetic acid]. For Hplc conditions see the experimental section in the text.

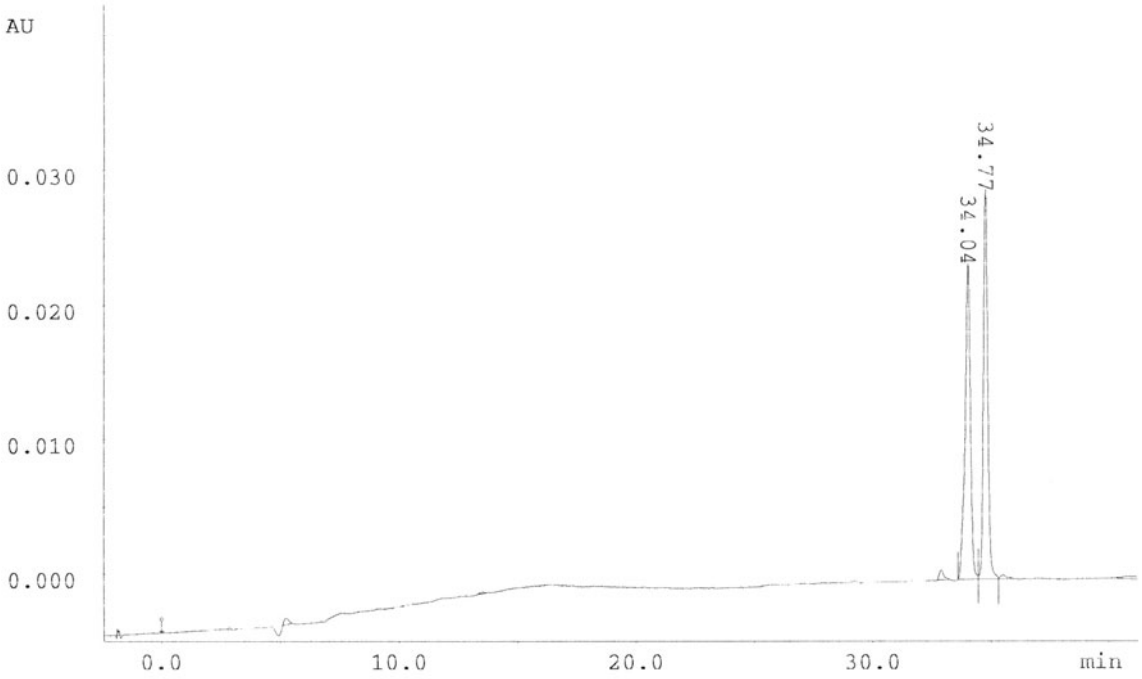
Notes:
Non-nucleot(s)idic impurity
§ 5′-r(AAGAAC)-3′ contamination



Result Table - Calculation Method Uncal

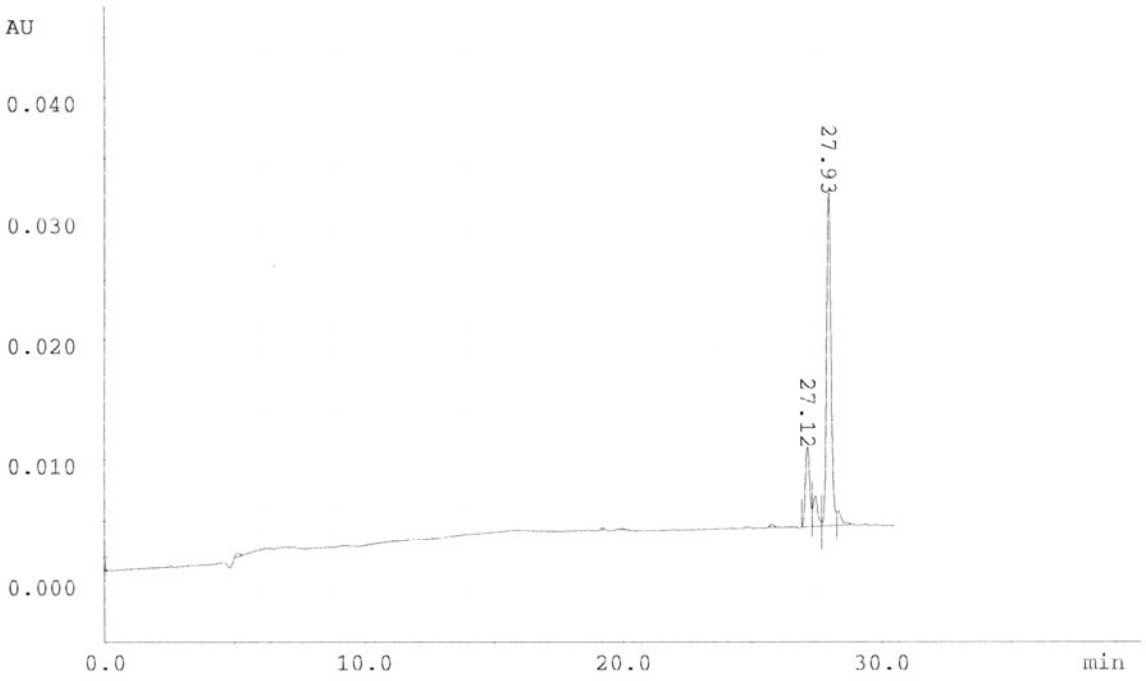
Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	6,473	35,8984	3,353	0,193	0,284	0,429
2	6,833	205,8544	7,305	0,367	1,626	0,935
3	10,520	224,8189	20,507	0,133	1,776	2,626
4	17,967	84,3551	4,634	0,267	0,666	0,593
5	21,520	240,5694	10,983	0,300	1,900	1,406
6	23,860	553,1731	36,321	0,240	4,369	4,651
7	25,440	322,4294	16,316	0,313	2,547	2,089
8	26,213	10565,0110	657,234	0,260	83,447	84,152
9	27,100	428,6745	24,352	0,267	3,385	3,119
-	Total	12660,7842	781,006			

Figure S12(a2): Hplc analysis of alkaline Hydrolysis products of **5′-r(CAAGAAC)-3′ (5b)** [after digestion for **1h** at pH 12.5 using 0.03N NaOH/ 20°C, followed by quenching with 0.03 N aq. acetic acid]. For Hplc conditions see the experimental section in the text. Peaks at R_T= 23.86 and R_T= 25.44 min were further separated by SMART™ Hplc, see below S12(a2i) and (a2j) for the separation profiles.



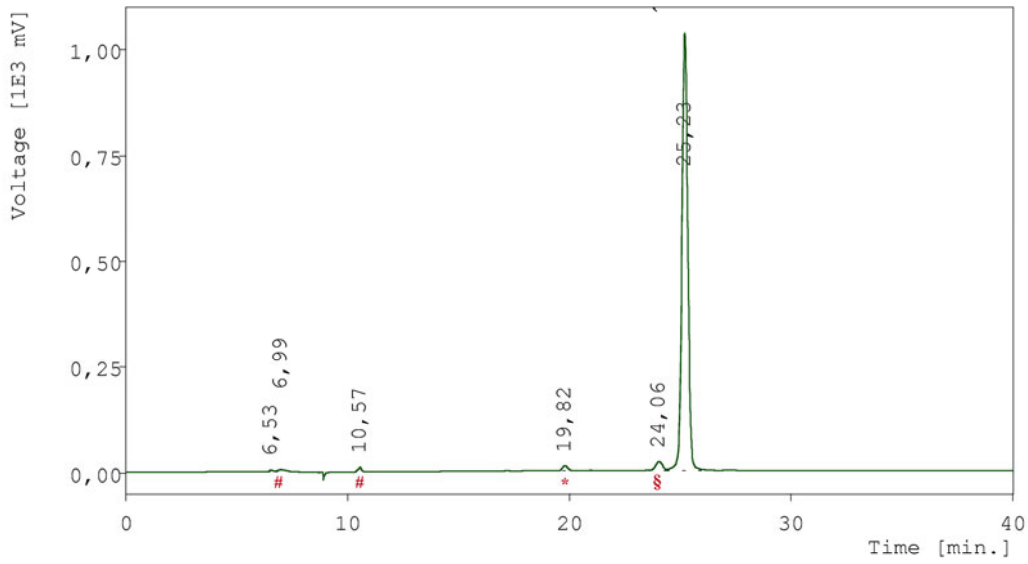
No	Ret (min)	Peak start (min)	Peak end (min)	Dur (min)	Area (min*mAU)	Height (mAU)
1	34.04	33.61	34.45	0.85	6.0568	23.543
2	34.77	34.45	35.32	0.88	6.1302	29.006

Figure S12(a2i): SMART™ RP-Hplc analysis of the alkaline hydrolysis products co-eluted at **$R_{T=}$ 23.86 min** in **Figure S12(a2)** for **(5b)**. Note the separation of 5'-CAAG$_{2', 3'\text{-cMP}}$ peak at R_T = 34.04 min from 5'-GAAC-3' peak at R_T = 34.77 min (See Table S9(A) for Maldi Tof mass-spec characterization). Hplc conditions: Jupiter 5 µm C18 300Å column with 150 x 2 mm dimension. Gradient: linear gradient starting from 0% B Buffer (50% CH_3CN in 0.1M TEAA) + 100% A Buffer (0.1M TEAA) to 20% B Buffer + 80% A Buffer in 45 minutes. Flow rate: 100 µl min^{-1}.



No	Ret (min)	Peak start (min)	Peak end (min)	Dur (min)	Area (min*mAU)	Height (mAU)
1	27.12	26.89	27.29	0.41	1.2619	6.595
2	27.41	27.29	27.67	0.39	0.5119	2.476
3	27.93	27.67	28.24	0.59	5.4434	27.576

Figure S12(a2j): SMART™ RP-Hplc analysis of the alkaline hydrolysis products co-eluted at **$R_{T=}$ 25.44 min** in **Figure S12(a2)** for **(5b)**. Note the separation of 5'-CAAGA$_{2', 3'\text{-cMP}}$ peak at R_T = 27.12 min, 5'-CAAGA$_{2', 3'\text{-cMP}}$ peak at R_T = 27.41 min and 5'-AGAAC-3' peak at R_T = 27.93 min (See Table S9(A) for Maldi Tof mass-spec characterization). Hplc conditions: Jupiter 5 µm C18 300Å column with 150 x 2 mm dimension. Gradient: linear gradient starting from 0% B Buffer (50% CH_3CN in 0.1M TEAA) + 100% A Buffer (0.1M TEAA) to 20% B Buffer + 80% A Buffer in 45 minutes. Flow rate: 100 µl min^{-1}. Temperature: 60°C.

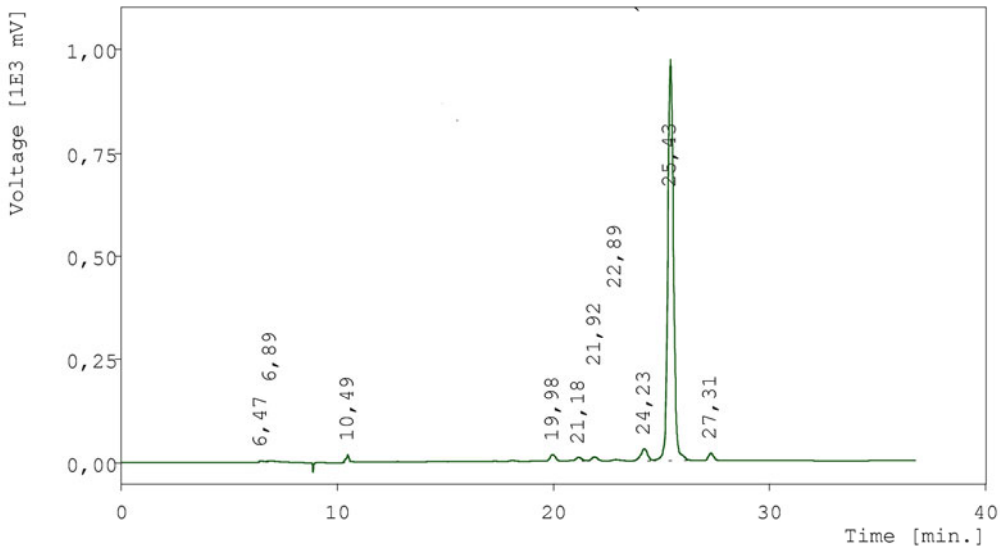


Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	6,533	41,0774	3,459	0,213	0,201	0,317
2	6,993	131,7170	5,099	0,413	0,646	0,467
3	10,567	148,9545	12,563	0,147	0,730	1,151
4	19,820	277,6587	12,402	0,260	1,361	1,136
5	24,060	597,3541	21,698	0,347	2,928	1,988
6	25,227	19205,3614	1036,411	0,287	94,134	94,941
-	Total	20402,1230	1091,633			

Figure S12(b1): Hplc analysis of alkaline Hydrolysis products of **5′-r(CAAGCAC)-3′ (6b)** [after digestion for **0h** at pH 12.5 using 0.03N NaOH/ 20°C, followed by quenching with 0.03 N aq. acetic acid]. For Hplc conditions see the experimental section in the text.

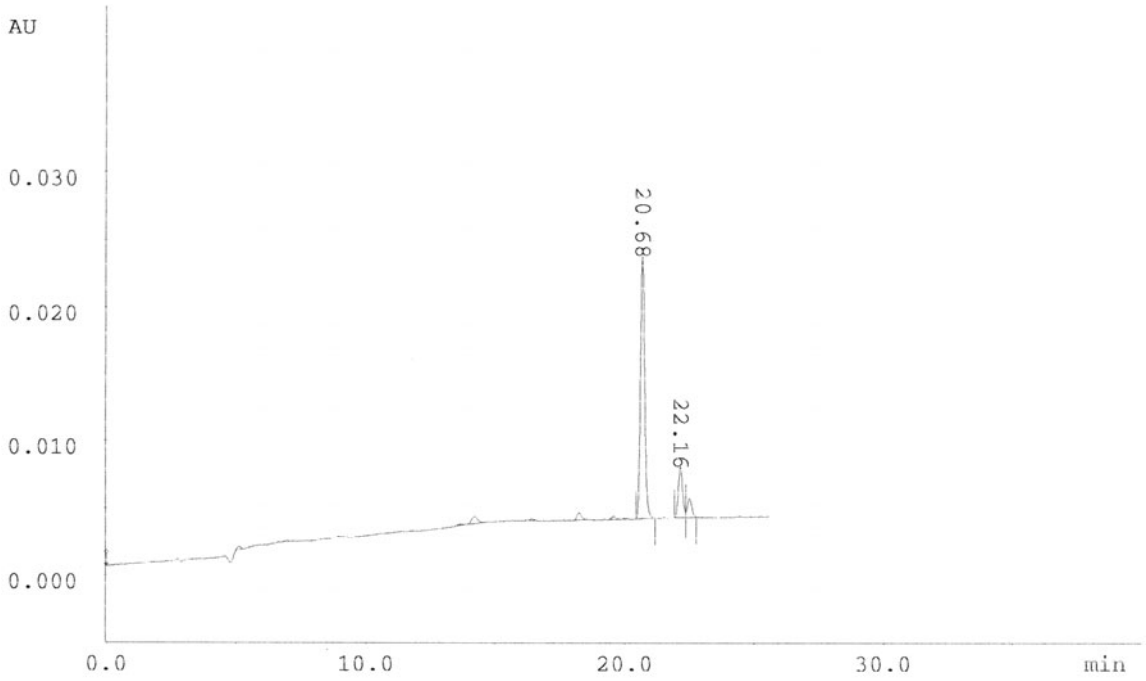
Notes:
Non-nucleot(s)idic impurity
* 5′-r(CAC)-3′ contamination
§ 5′-r(GCAC)-3′ and 5′-r($CAA_{2'/3'-P}$) contamination



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	6,467	42,6980	3,388	0,233	0,216	0,312
2	6,893	99,7021	3,523	0,460	0,504	0,325
3	10,487	220,0198	19,779	0,133	1,112	1,824
4	19,980	266,9162	16,372	0,260	1,350	1,510
5	21,180	172,6643	8,687	0,287	0,873	0,801
6	21,920	178,3442	9,517	0,300	0,902	0,878
7	22,893	64,7540	2,821	0,360	0,327	0,260
8	24,227	649,1966	29,944	0,333	3,282	2,762
9	25,433	17765,7584	971,490	0,280	89,825	89,594
10	27,307	318,1686	18,799	0,267	1,609	1,734
-	Total	19778,2223	1084,322			

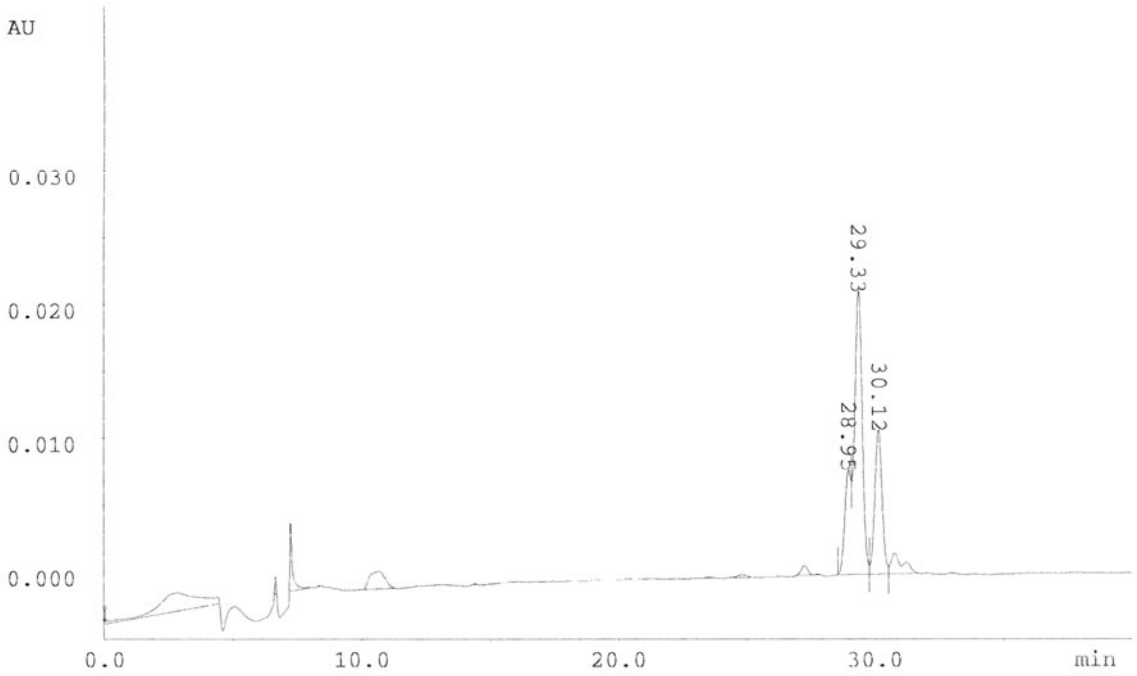
Figure S12(b2): Hplc analysis of alkaline Hydrolysis products of **5′-r(CAAGCAC)-3′ (6b)** [after digestion for **1h** at pH 12.5 using 0.03N NaOH/ 20°C, followed by quenching with 0.03 N aq. acetic acid]. For Hplc conditions see the experimental section in the text. Peaks at R_T= 21.18 and R_T= 24.23 min were further separated by SMART™ Hplc, see below S12(b2i) and (b2j) for the separation profiles.



No	Ret (min)	Peak start (min)	Peak end (min)	Dur (min)	Area (min*mAU)	Height (mAU)
1	20.68	20.44	21.19	0.76	3.7074	19.577
2	22.16	21.93	22.36	0.44	0.7076	3.719
3	22.51	22.36	22.76	0.41	0.2758	1.386

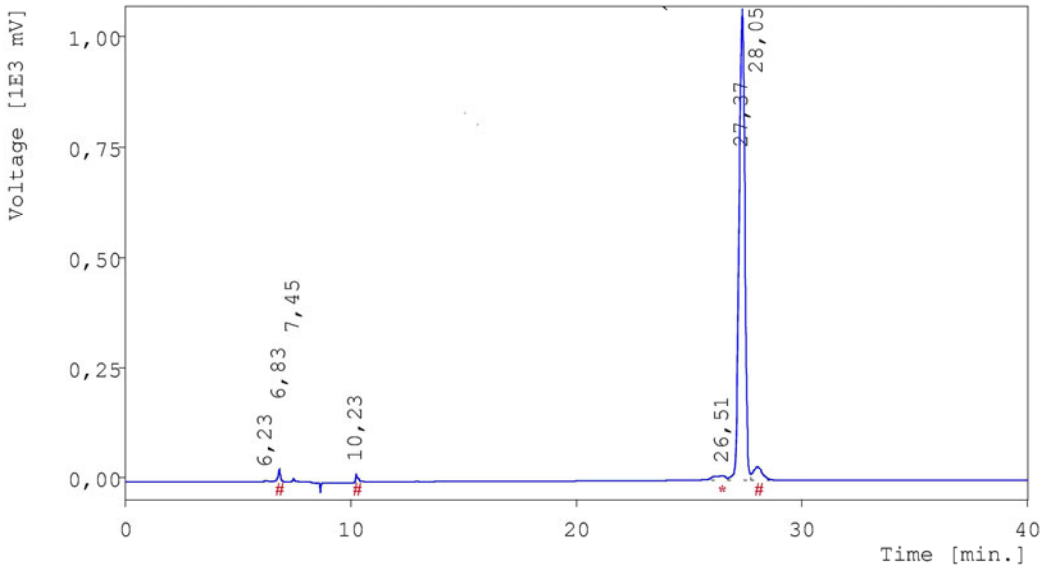
Figure S12(b2i): SMART™ RP-Hplc analysis of the alkaline hydrolysis products co-eluted at **$R_{T=}$ 21.18 min** in **Figure S12(b2)** for **(6b)**. Note the separation of 5'-GCAC-3' peak at R_T = 20.68 min, 5'-$CAA_{2'/3'-P}$ peak at R_T = 22.16 min (See Table S9(B) for Maldi Tof mass-spec characterization) Hplc conditions: Jupiter 5 µm C18 300Å column with 150 x 2 mm dimension. Gradient: linear gradient starting from 0% B Buffer (50% CH_3CN in 0.1M TEAA) + 100% A Buffer

(0.1M TEAA) to 20% B Buffer + 80% A Buffer in 45 minutes. Flow rate: 100 µl min^{-1}. Temperature: 60°C.



No	Ret (min)	Peak start (min)	Peak end (min)	Dur (min)	Area (min*mAU)	Height (mAU)
1	28.95	28.55	29.07	0.53	2.0929	7.818
2	29.33	29.07	29.76	0.71	7.2667	21.176
3	30.12	29.76	30.51	0.76	3.5503	10.717

Figure S12(b2j): SMART™ RP-Hplc analysis of the alkaline hydrolysis products co-eluted at **$R_{T=}$ 24.23 min** in **Figure S12(b2)** for **(6b)**. Note the separation of 5'-CAAG$_{2'/3'\text{-P}}$/5'-CAAG$_{2',\ 3'\text{-cMP}}$ peak at R_T = 28.95 min/ 29.33 min and 5'-CAAGC$_{2',\ 3'\text{-cMP}}$ peak at R_T = 30.12 min (See Table S9(B) for Maldi Tof mass-spec characterization). Hplc conditions: Jupiter 5 µm C18 300Å column with 150 x 2 mm dimension. Gradient: linear gradient starting from 0% B Buffer (50% CH_3CN in 0.1M TEAA) + 100% A Buffer (0.1M TEAA) to 10% B Buffer + 90% A Buffer in 40 minutes. Flow rate: 100 µl min^{-1}.

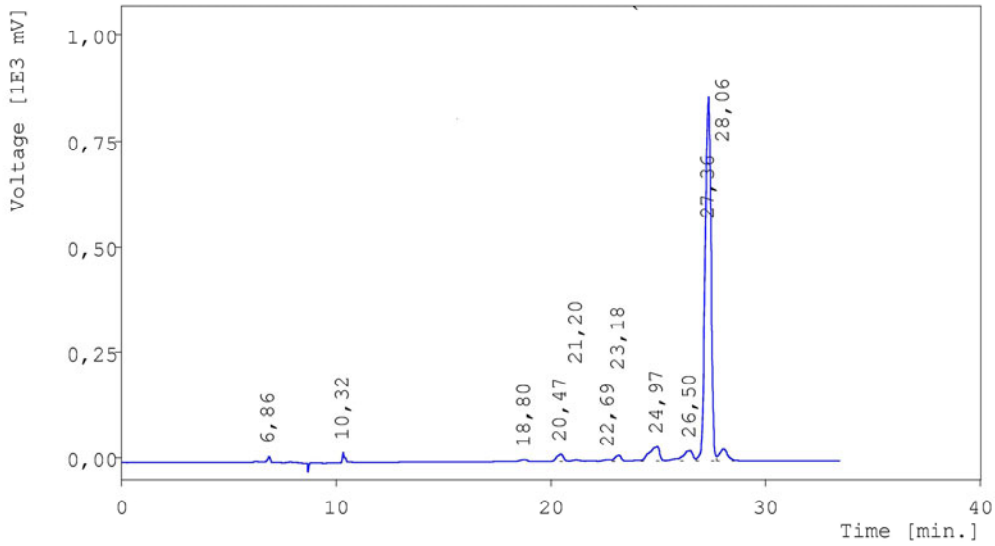


Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	6,227	26,9699	2,312	0,193	0,128	0,197
2	6,827	245,2731	31,666	0,100	1,168	2,694
3	7,453	49,6972	7,136	0,087	0,237	0,607
4	10,233	190,5694	19,119	0,120	0,907	1,627
5	26,507	430,2516	10,491	0,700	2,048	0,892
6	27,373	19161,7639	1072,245	0,293	91,232	91,218
7	28,047	898,9227	32,510	0,440	4,280	2,765
–	Total	21003,4478	1175,480			

Figure S12(c1): RP-Hplc analysis of alkaline Hydrolysis products of **5′-r(CACGAAC)-3′ (7b)** [after digestion for **0h** at pH 12.5 using 0.03N NaOH/ 20°C, followed by quenching with 0.03 N aq. acetic acid]. For Hplc conditions see the experimental section in the text.

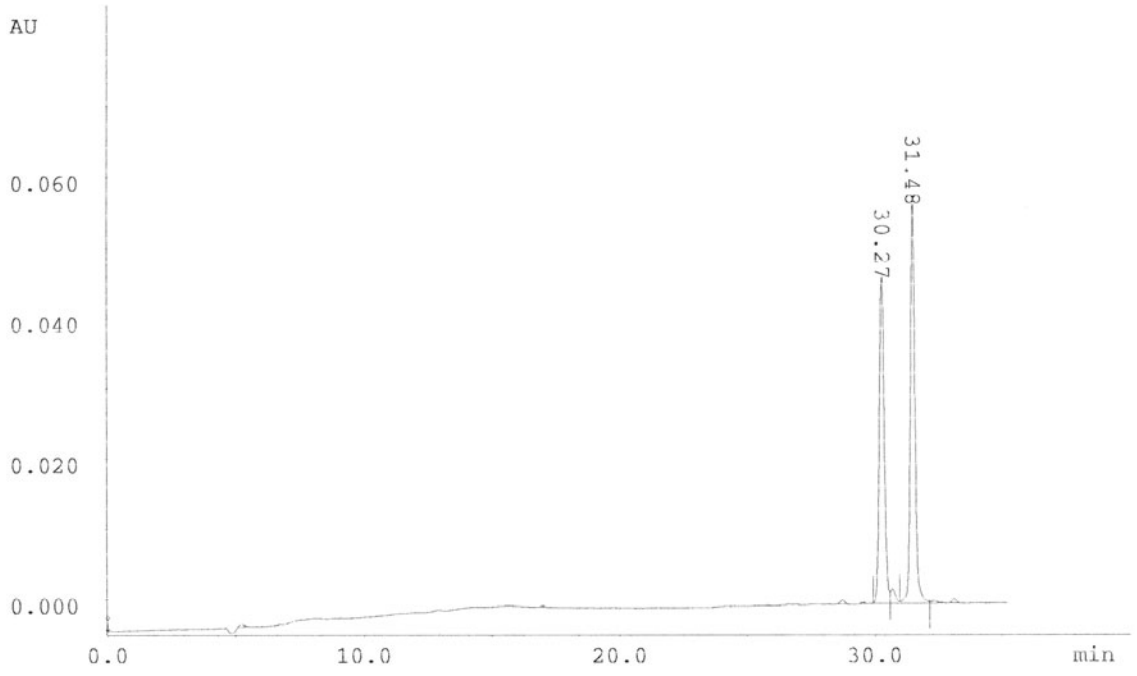
Notes:
Non-nucleot(s)idic impurity
* 5′-r(CACGA$_{2'/3'\text{-P}}$) and 5′-r(ACGAAC)-3′ contamination



Result Table - Calculation Method Uncal

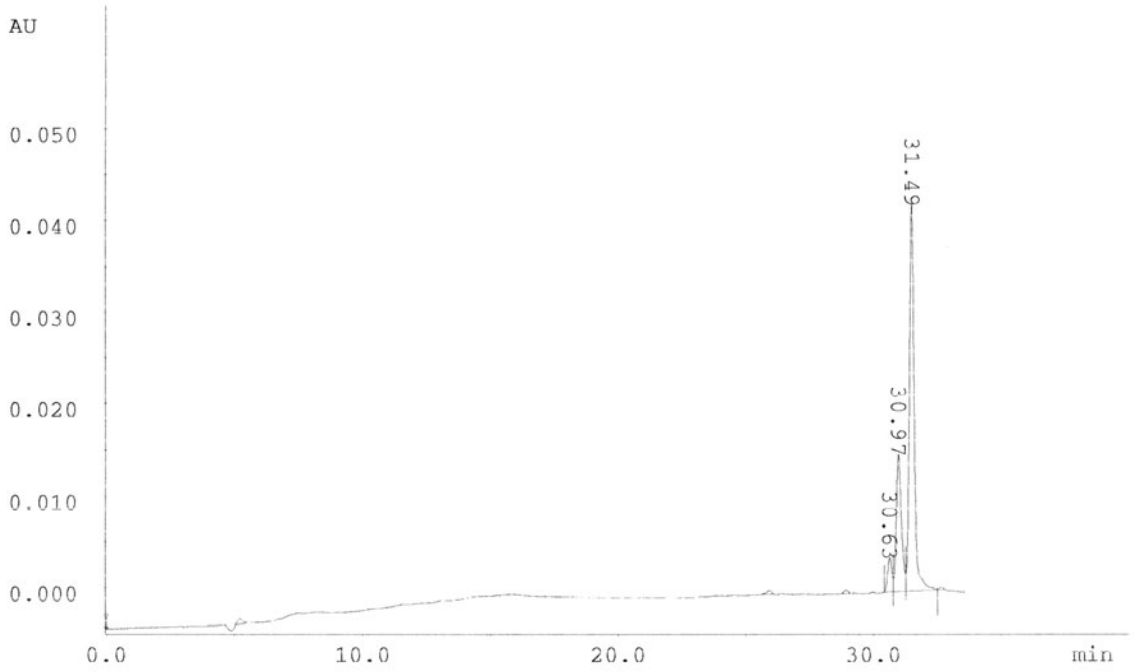
Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	6,860	102,9652	13,313	0,113	0,501	1,293
2	10,320	239,9007	24,700	0,120	1,168	2,400
3	18,800	83,7326	4,009	0,367	0,408	0,389
4	20,467	356,3526	16,718	0,360	1,734	1,624
5	21,200	97,8667	3,003	0,407	0,476	0,292
6	22,693	84,1912	3,755	0,413	0,410	0,365
7	23,180	271,0981	13,928	0,320	1,319	1,353
8	24,973	1173,6147	34,679	0,567	5,712	3,369
9	26,500	831,4738	24,559	0,453	4,047	2,386
10	27,360	16554,3554	862,865	0,313	80,570	83,825
11	28,060	751,1158	27,831	0,420	3,655	2,704
-	Total	20546,6669	1029,359			

Figure S12(c2): RP-Hplc analysis of alkaline Hydrolysis products of **5′-r(CACGAAC)-3′ (7b)** [after digestion for **1h** at pH 12.5 using 0.03N NaOH/ 20°C, followed by quenching with 0.03 N aq. acetic acid]. For Hplc conditions see the experimental section in the text. Peaks at R_T= 24.97 and R_T= 26.50 min were further separated by SMART™ Hplc, see below S12(c2i) and (c2j) for the separation profiles.



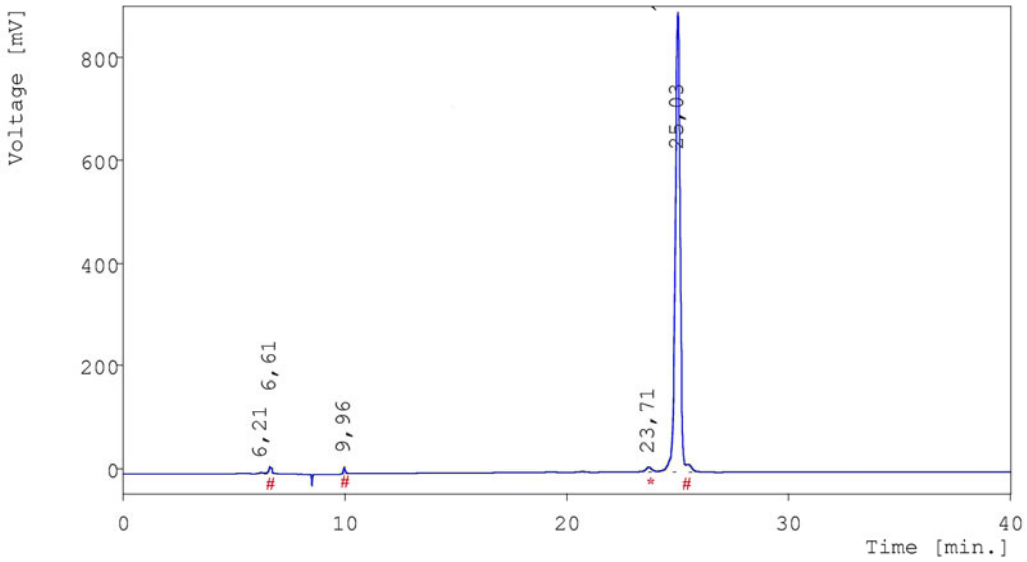
No	Ret (min)	Peak start (min)	Peak end (min)	Dur (min)	Area (min*mAU)	Height (mAU)
1	30.27	29.93	30.59	0.67	10.3322	46.539
2	31.48	30.96	32.13	1.19	11.7966	56.805

Figure S12(c2i): SMART™ RP-Hplc analysis of the alkaline hydrolysis products co-eluted at **$R_{T=}$ 24.97 min** in **Figure S12(c2)** for (**7b**). Note the separation of 5'-CACG$_{2',\ 3'\text{-cMP}}$ peak at R_T = 30.27 min and 5'-GAAC-3' peak at R_T = 31.48 min (See Table S9(C) for Maldi Tof mass-spec characterization). Hplc conditions: Jupiter 5 µm C18 300Å column with 150 x 2 mm dimension. Gradient: linear gradient starting from 0% B Buffer (50% CH_3CN in 0.1M TEAA) + 100% A Buffer (0.1M TEAA) to 20% B Buffer + 80% A Buffer in 40 minutes. Flow rate: 100 µl min^{-1}.



No	Ret (min)	Peak start (min)	Peak end (min)	Dur (min)	Area (min*mAU)	Height (mAU)
1	30.63	30.41	30.77	0.37	0.7146	3.739
2	30.97	30.77	31.25	0.49	3.4687	14.993
3	31.49	31.25	32.49	1.25	9.1518	42.097

Figure S12(c2j): SMART™ RP-Hplc analysis of the alkaline hydrolysis products co-eluted at **$R_{T=}$ 26.50 min** in **Figure S12(c2)** for **(7b)**. Note the separation of 5'-CACGA$_{2'/3'\text{-P}}$ peak at R_T = 30.63 min, 5'-CACGA$_{2',\ 3'\text{-cMP}}$ peak at R_T = 30.97 min and 5'-ACGAAC-3' peak at R_T = 31.49 min (See Table S9(C) for Maldi Tof mass-spec characterization). Hplc conditions: Jupiter 5 µm C18 300Å column with 150 x 2 mm dimension. Gradient: linear gradient starting from 0% B Buffer (50% CH_3CN in 0.1M TEAA) + 100% A Buffer (0.1M TEAA) to 25% B Buffer + 75% A Buffer in 45 minutes. Flow rate: 100 µl min^{-1}.



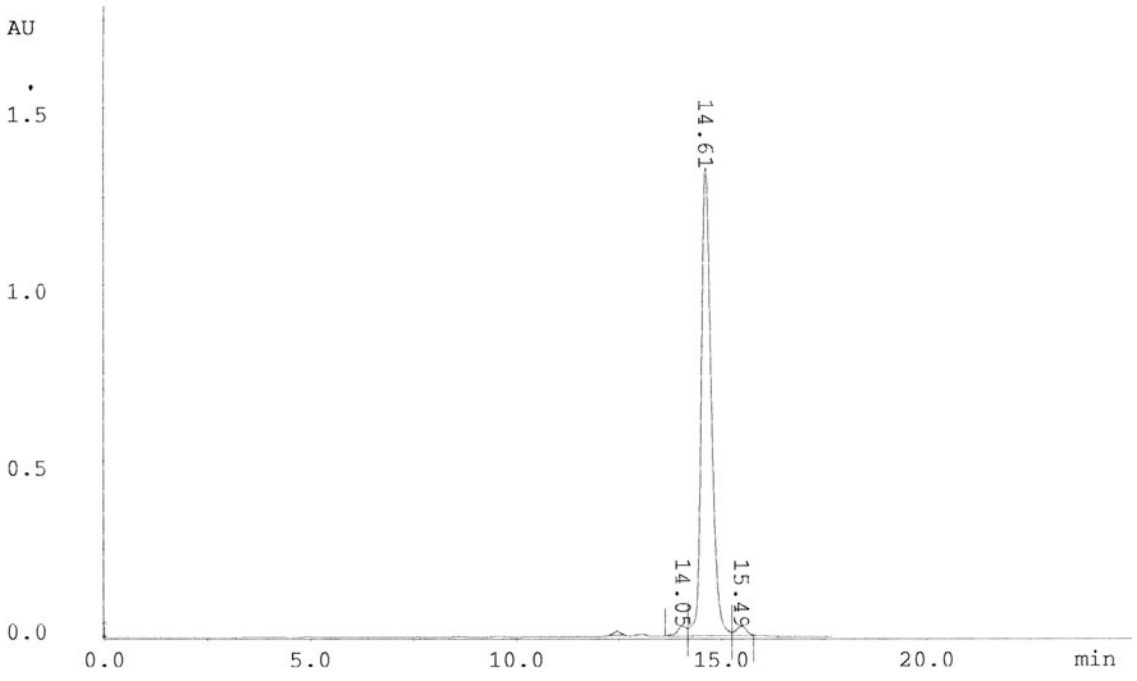
Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	6,213	25,0344	2,492	0,200	0,172	0,266
2	6,607	149,7529	14,236	0,160	1,030	1,521
3	9,960	103,1474	13,518	0,107	0,710	1,444
4	23,707	202,4217	9,486	0,293	1,393	1,013
5	25,027	14055,6415	896,403	0,240	96,695	95,756
-	Total	14535,9979	936,136			

Figure S12(d1): RP-Hplc analysis of alkaline Hydrolysis products of **5′-r(CACGCAC)-3′ (8b)** [after digestion for **0h** at pH 12.5 using 0.03N NaOH/ 20°C,

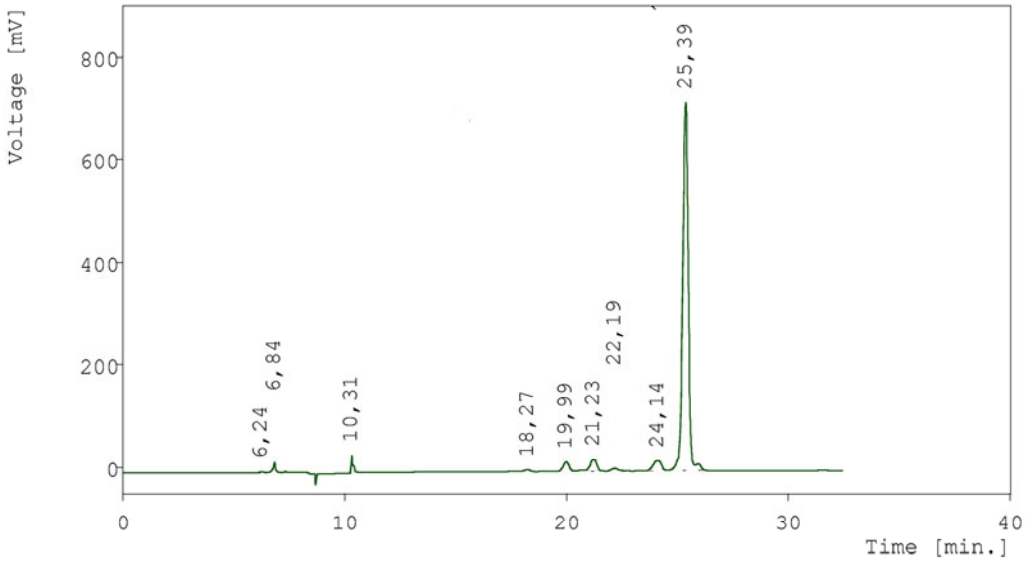
followed by quenching with 0.03 N aq. acetic acid]. For Hplc conditions see the experimental section in the text.

Notes:
Non-nucleot(s)idic impurity
* 5′-r(CACG$_{2', 3'\text{-cMP}}$) and 5′-r(CACGC$_{2', 3'\text{-cMP}}$) contamination



No	Ret (min)	Peak start (min)	Peak end (min)	Dur (min)	Area (min*mAU)	Height (mAU)
1	14.05	13.64	14.18	0.55	6.8711	26.476
2	14.61	14.18	15.26	1.09	375.1071	1327.177
3	15.49	15.26	15.78	0.53	7.3552	25.103

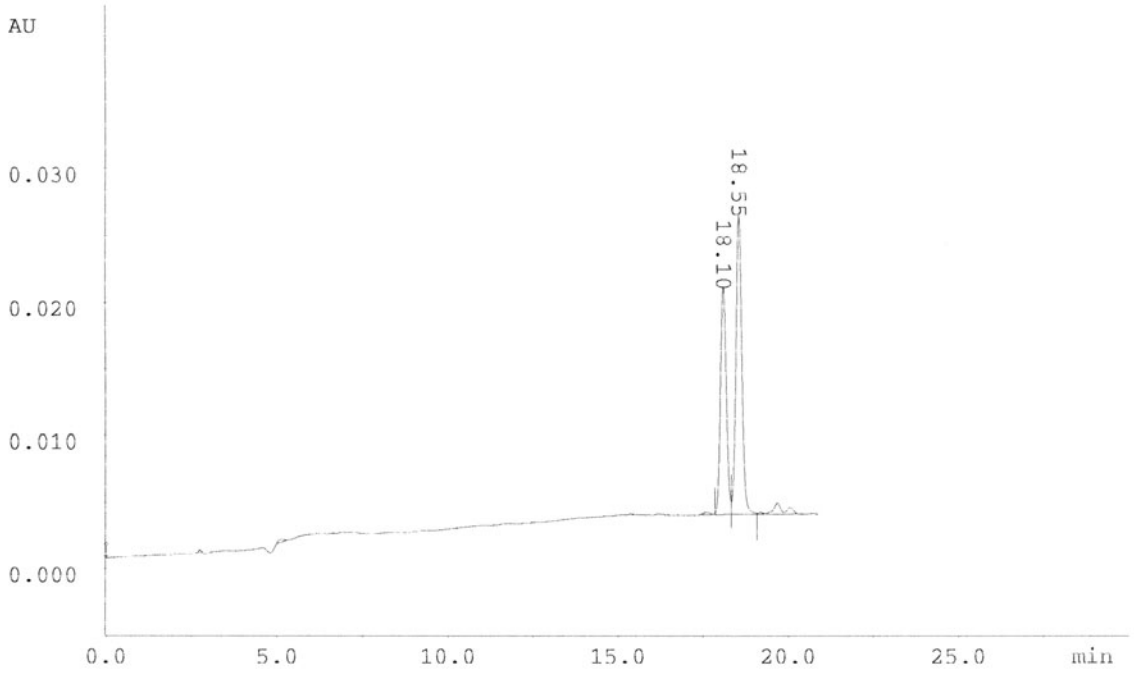
Figure S12(d1i): SMART™ RP-Hplc analysis of the heptameric peak at **$R_{T=}$ 25.03 min** in **Figure S12(d1)** for **(8b)**. Hplc conditions: Jupiter 5µm C18 300Å column with 150 x 2 mm dimension. Gradient: linear gradient, starting from 0% B Buffer (50% CH_3CN in 0.1M TEAA) + 100% A Buffer (5% CH_3CN in 0.1M TEAA) to 20% B Buffer + 80% A Buffer in 45 minutes with a constant flow rate of 100 µl min^{-1}.



Result Table - Calculation Method Uncal

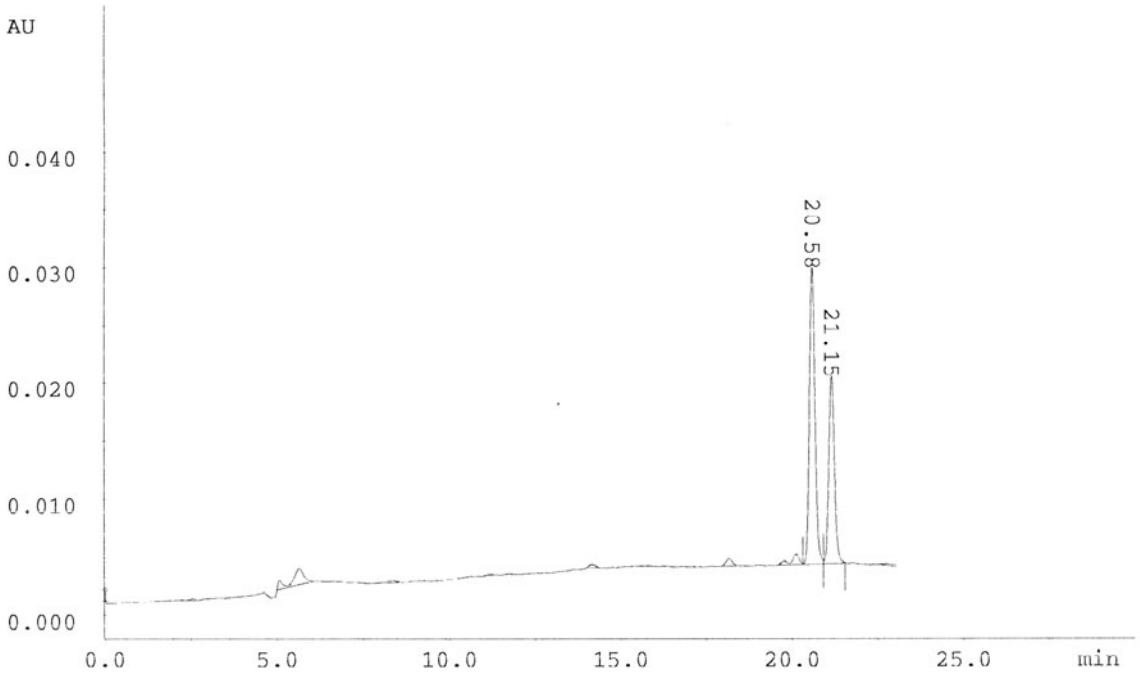
Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	6,240	22,6147	2,243	0,180	0,154	0,265
2	6,840	176,6760	20,126	0,100	1,205	2,376
3	10,313	255,3706	36,065	0,073	1,741	4,257
4	18,267	63,4813	3,684	0,287	0,433	0,435
5	19,993	333,3590	18,278	0,293	2,273	2,158
6	21,227	509,9925	23,349	0,320	3,477	2,756
7	22,193	118,7029	5,266	0,333	0,809	0,622
8	24,140	528,2745	19,810	0,427	3,602	2,338
9	25,393	12658,9642	718,357	0,273	86,306	84,793
-	Total	14667,4358	847,180			

Figure S12(d2): RP-Hplc analysis of alkaline Hydrolysis products of **5′-r(CACGCAC)-3′ (8b)** [after digestion for **1h** at pH 12.5 using 0.03N NaOH/ 20°C, followed by quenching with 0.03 N aq. acetic acid]. For Hplc conditions see the experimental section in the text. Peaks at R_T= 19.99, R_T= 21.23, R_T= 24.14 and R_T= 25.39 min were further separated by SMART™ Hplc, see below S12(b2i), (b2j), (b2k) and (b2l) for the separation profiles.



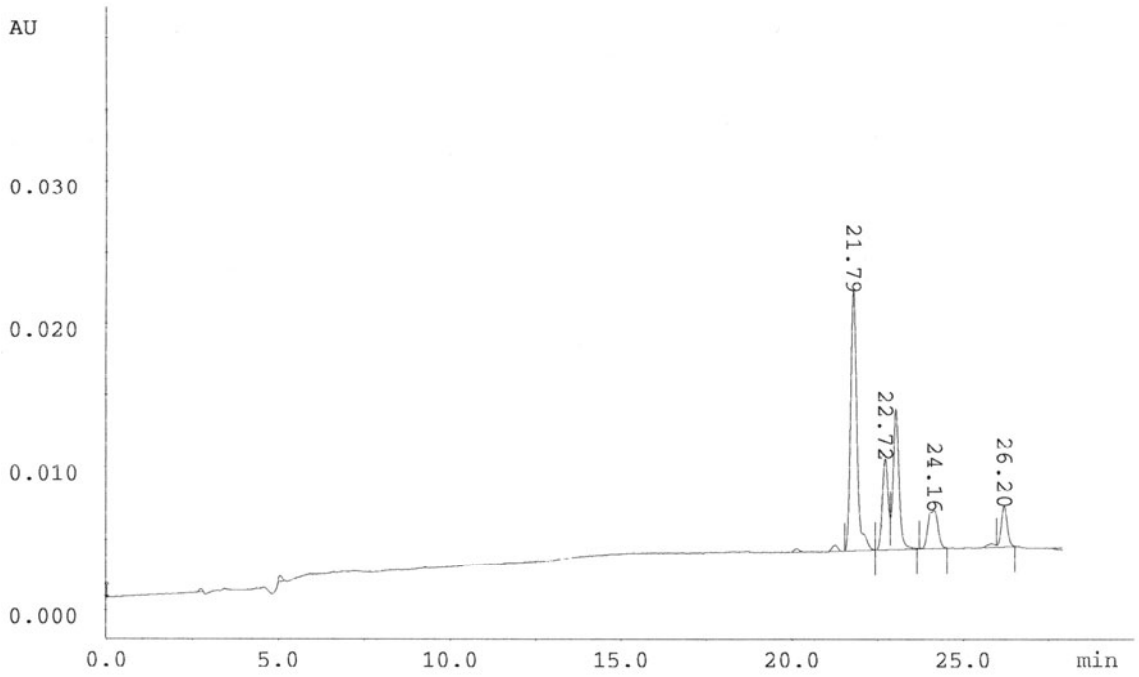
No	Ret (min)	Peak start (min)	Peak end (min)	Dur (min)	Area (min*mAU)	Height (mAU)
1	18.10	17.85	18.34	0.49	3.3881	17.039
2	18.55	18.34	19.09	0.76	4.5203	22.437

Figure S12(d2i): SMART™ RP-Hplc analysis of the alkaline hydrolysis products co-eluted at **$R_{T=}$ 19.99 min** in **Figure S12(d2)** for **(8b)**. Note the separation of 5'-CAC-3' peak at R_T = 18.10 min and 5'-$CAC_{2',\ 3'\text{-cMP}}$ peak at R_T = 18.55 min (See Table S9(D) for Maldi Tof mass-spec characterization). Hplc conditions: Jupiter 5 µm C18 300Å column with 150 x 2 mm dimension. Gradient: linear gradient starting from 0% B Buffer (50% CH_3CN in 0.1M TEAA) + 100% A Buffer (0.1M TEAA) to 20% B Buffer + 80% A Buffer in 40 minutes. Flow rate: 100 µl min^{-1}. Temperate: 60°C.



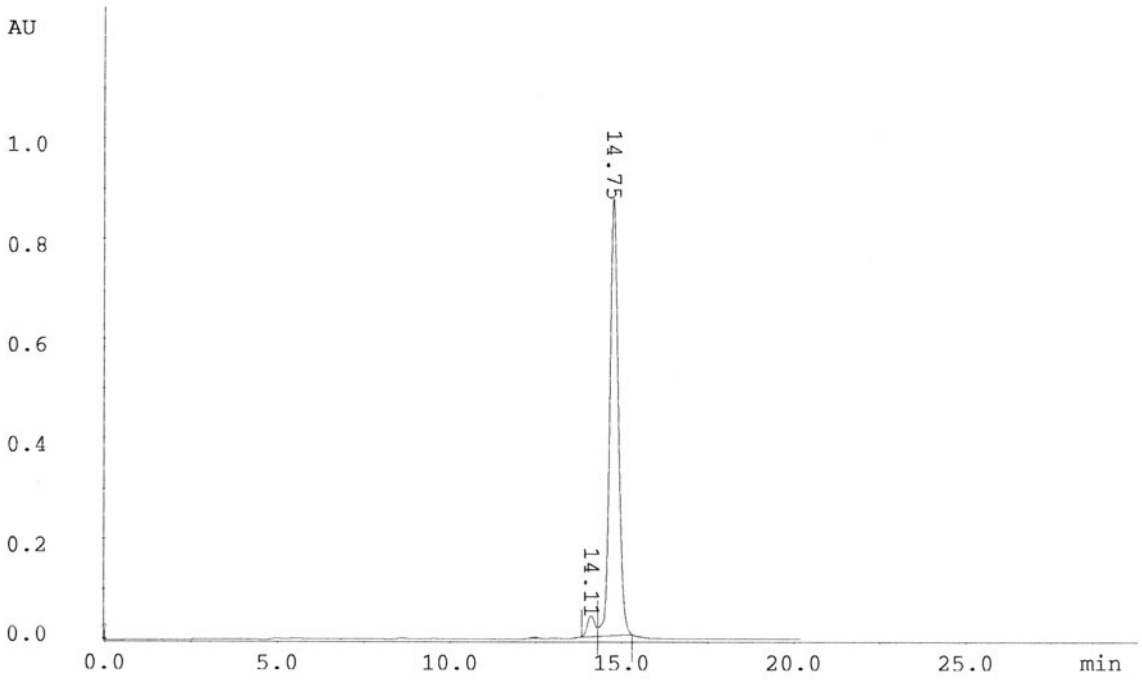
No	Ret (min)	Peak start (min)	Peak end (min)	Dur (min)	Area (min*mAU)	Height (mAU)
1	20.58	20.31	20.91	0.61	4.7899	25.691
2	21.15	20.91	21.55	0.64	3.0660	16.193

Figure S12(d2j): SMART™ RP-Hplc analysis of the alkaline hydrolysis products co-eluted at **$R_{T=}$ 21.23 min** in **Figure S12(d2)** for **(8b)**. Note the separation of 5'-GCAC-3' peak at R_T = 20.58 min and 5'-CGCAC-3' peak at R_T = 21.15 min (See Table S9(D) for Maldi Tof mass-spec characterization). Hplc conditions: Jupiter 5 µm C18 300Å column with 150 x 2 mm dimension. Gradient: linear gradient starting from 0% B Buffer (50% CH_3CN in 0.1M TEAA) + 100% A Buffer (0.1M TEAA) to 20% B Buffer + 80% A Buffer in 40 minutes. Flow rate: 100 µl min^{-1}. Temperate: 60°C.



No	Ret (min)	Peak start (min)	Peak end (min)	Dur (min)	Area (min*mAU)	Height (mAU)
1	21.79	21.55	22.43	0.89	3.7711	18.070
2	22.72	22.43	22.87	0.45	1.1884	6.295
3	23.04	22.87	23.65	0.80	2.0104	9.687
4	24.16	23.72	24.53	0.83	0.8884	2.637
5	26.20	25.97	26.52	0.56	0.5542	2.839

Figure S12(d2k): SMART™ RP-Hplc analysis of the alkaline hydrolysis products co-eluted at **$R_{T=}$ 24.14 min** in **Figure S12(d2)** **(8b)**. Note the separation of 5'-CACG$_{2', 3'\text{-cMP}}$ peak at R_T = 21.79 min, 5'-CACG$_{2'/3'\text{-P}}$ peak at R_T = 22.72 min, 5'-CACGC$_{2', 3'\text{-cMP}}$ peak at R_T = 23.04 min, 5'-CACGC$_{2'/3'\text{-P}}$ peak at R_T = 24.16 min and parent heptameric peak at R_T = 26.20 min (See Table S9(D) for Maldi Tof mass-spec characterization). Hplc conditions: Jupiter 5 µm C18 300Å column with 150 x 2 mm dimension. Gradient: linear gradient starting from 0% B Buffer (50% CH_3CN in 0.1M TEAA) + 100% A Buffer (0.1M TEAA) to 20% B Buffer + 80% A Buffer in 40 minutes. Flow rate: 100 µl min^{-1}. Temperate: 60°C.



No	Ret (min)	Peak start (min)	Peak end (min)	Dur (min)	Area (min*mAU)	Height (mAU)
1	14.11	13.84	14.31	0.48	10.6750	41.504
2	14.75	14.31	15.31	1.00	250.6014	874.346

Figure S12(d2$_l$): SMART™ RP-Hplc analysis of the heptameric peak at **$R_{T=}$ 25.39 min** in **Figure S12(d2)** for **(8b)**. Note the separation of the hexamer (5'-ACGCAC-3') peak at R_T = 14.11 min from the heptamer peak eluting at R_T = 14.75 min. Hplc conditions: Jupiter 5µm C18 300Å column with 150 x 2 mm dimension. Gradient: linear gradient starting from 0% B Buffer (50% CH_3CN in 0.1M TEAA) + 100% A Buffer (5% CH_3CN in 0.1M TEAA) to 20% B Buffer + 80% A Buffer in 45 minutes with a constant flow rate of 100 µl min^{-1}.