

Electronic Supplementary Information 10

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

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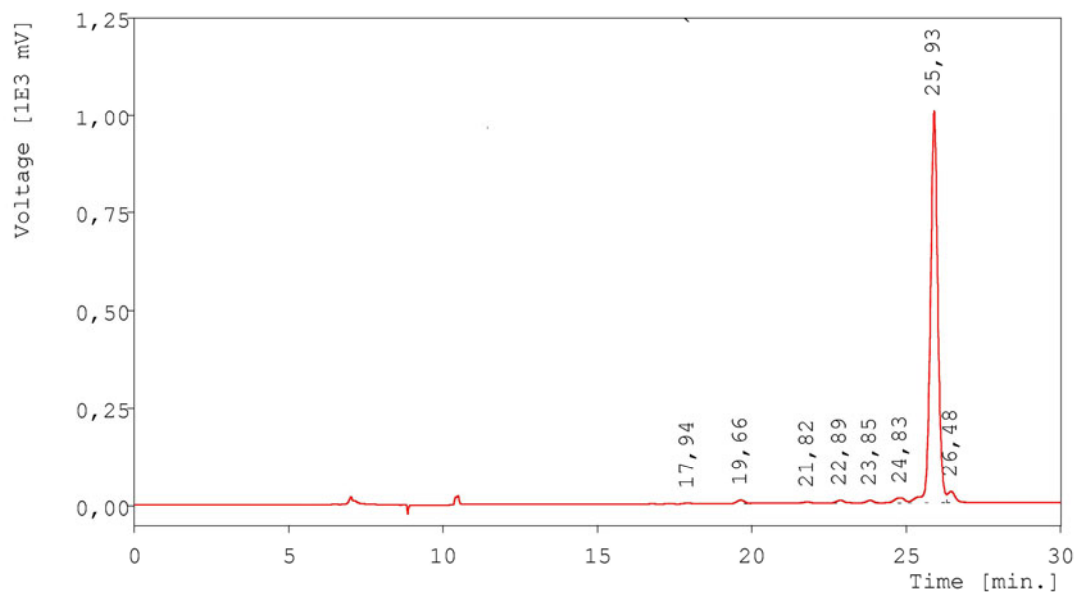
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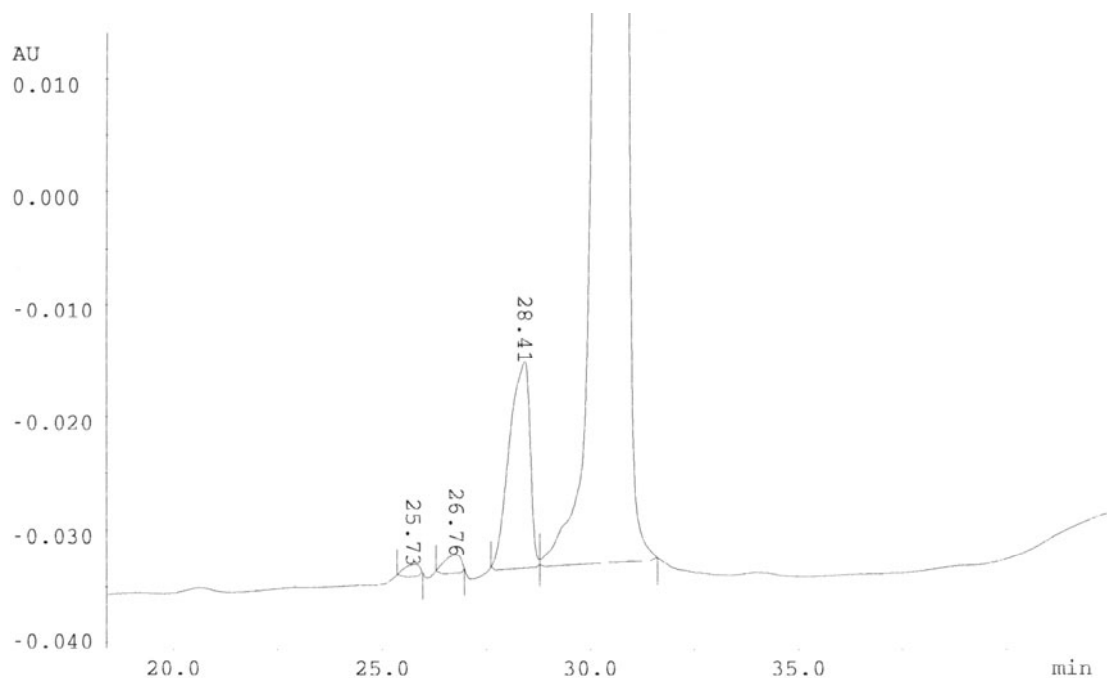
Figure S15F. Panels (f1) – (f7) show the RP-Hplc and SMART™ RP-Hplc profiles at ½, 2, 3, 4, 8, 15, 27 of alkali digestion of N^{1-Me}-G containing heptamer . 5'-r(CACG^{Me}AAC)-3' (7c). **p. S2–S14**



Result Table - Calculation Method Uncal

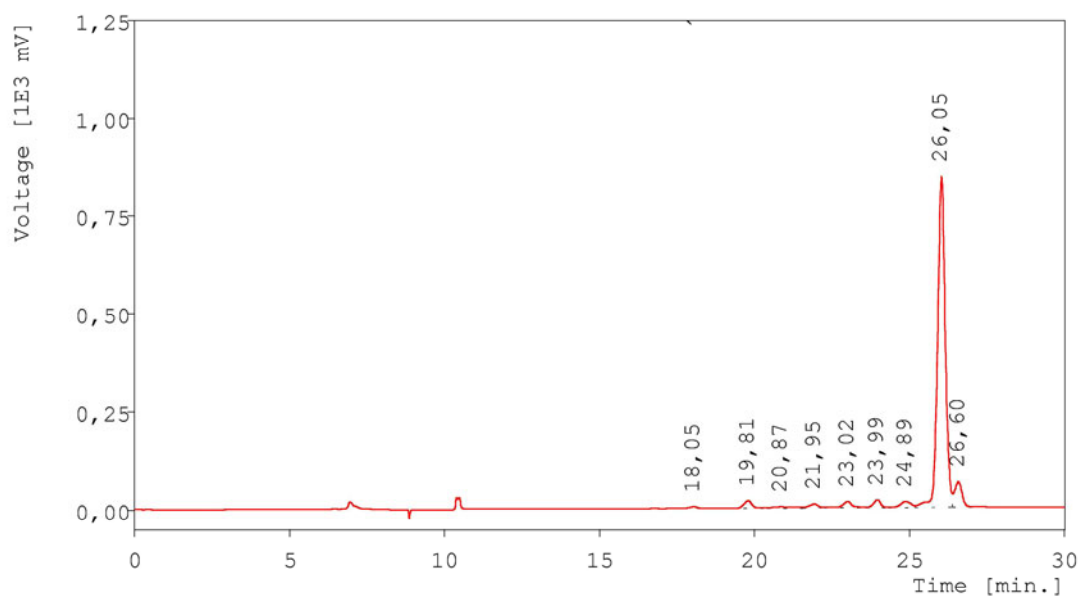
Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	17,940	42,5595	2,437	0,240	0,234	0,226
2	19,660	149,9386	9,174	0,267	0,825	0,852
3	21,820	151,4292	3,335	0,313	0,833	0,310
4	22,887	151,3144	7,325	0,267	0,833	0,680
5	23,853	108,5044	6,725	0,247	0,597	0,624
6	24,833	295,8081	12,950	0,367	1,628	1,202
7	25,927	16768,7814	1006,870	0,253	92,293	93,478
8	26,480	500,6968	28,305	0,280	2,757	2,628
-	Total	18169,0323	1077,121			

Figure S15(f1): RP-Hplc analysis of alkaline Hydrolysis products of **5'-r(CACG^{Me}AAC)-3' (7c)** [after digestion for **0.5h** 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.



No	Ret (min)	Peak start (min)	Peak end (min)	Dur (min)	Area (min*mAU)	Height (mAU)
1	25.73	25.35	25.96	0.63	0.4344	1.073
2	26.76	26.28	26.96	0.69	0.7534	1.624
3	28.41	27.60	28.77	1.19	10.5481	18.307
4	30.69	28.77	31.61	2.85	203.0332	353.293

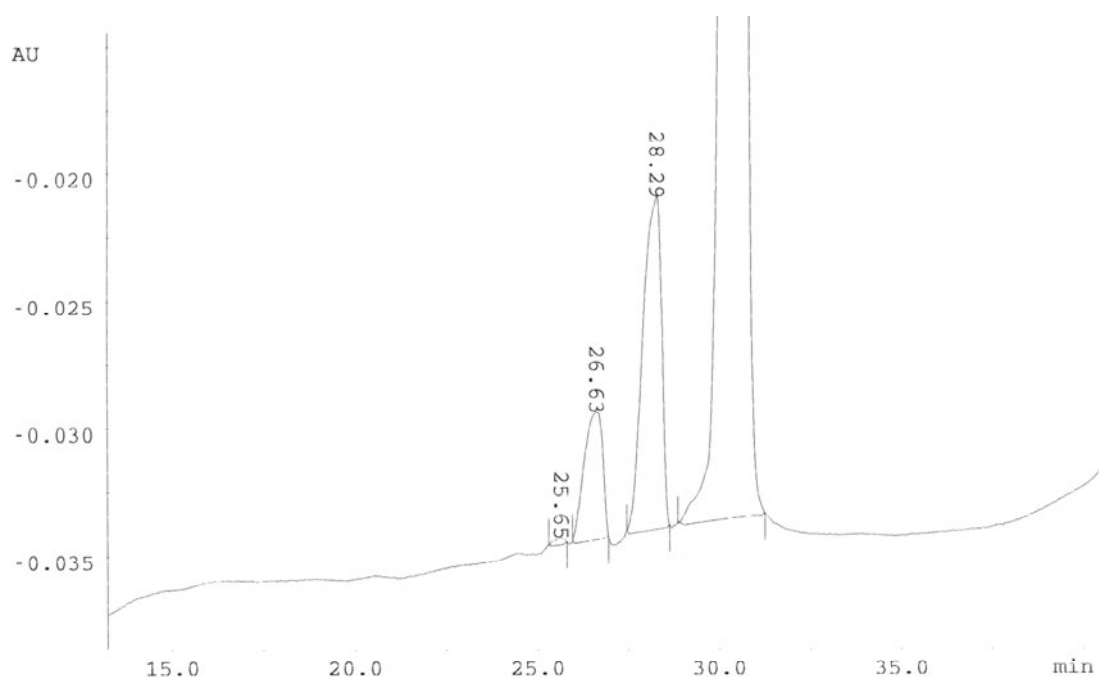
Figure S15(fli): SMART™ RP-Hplc analysis of the alkaline hydrolysis products co-eluted at $R_T = 25.93$ min and $R_T = 26.48$ min in Figure S15(fl) for (7c). Hplc conditions: Jupiter 5 μ m C18 300Å column with 150 x 2 mm dimension. Gradient: linear gradient starting from 0% B Buffer (50% CH₃CN in 0.1M TEAA) + 100% A Buffer (0.1M TEAA) to 20% B Buffer + 80% A Buffer in 45 minutes. Flow rate: 100 μ l min⁻¹.



Result Table - Calculation Method Uncal

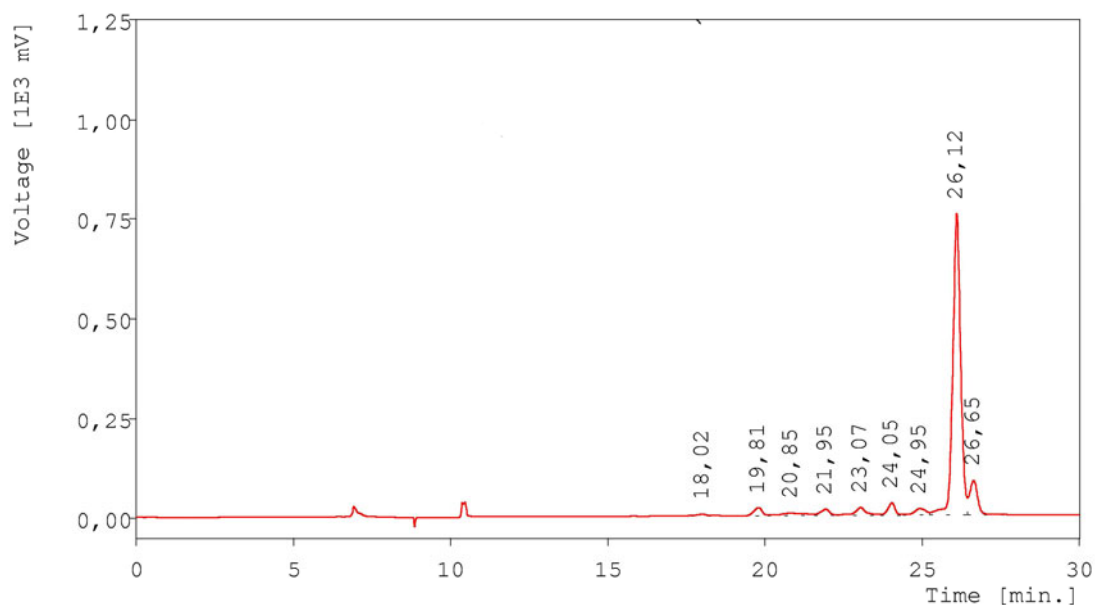
Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	18,053	106,7854	4,380	0,280	0,609	0,437
2	19,813	361,6173	19,212	0,280	2,063	1,919
3	20,873	165,6626	3,254	1,007	0,945	0,325
4	21,953	205,9317	10,153	0,307	1,175	1,014
5	23,020	367,6231	16,207	0,273	2,097	1,619
6	23,987	333,0837	20,545	0,240	1,900	2,052
7	24,887	375,0697	15,403	0,380	2,139	1,538
8	26,053	14461,7201	846,922	0,253	82,484	84,589
9	26,600	1155,2460	65,144	0,313	6,588	6,507
-	Total	17532,7398	1001,222			

Figure S15(f2): RP-Hplc analysis of alkaline Hydrolysis products of **5'-r(CACG^{Me}AAC)-3' (7c)** [after digestion for **2h** 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.



No	Ret (min)	Peak start (min)	Peak end (min)	Dur (min)	Area (min*mAU)	Height (mAU)
1	25.65	25.31	25.81	0.52	0.0905	0.270
2	26.63	25.96	26.96	1.01	2.9335	4.993
3	28.29	27.45	28.63	1.19	7.7175	13.052
4	30.61	28.85	31.25	2.41	68.6909	114.980

Figure S15(f2i): SMART™ RP-Hplc analysis of the alkaline hydrolysis products co-eluted at $R_T = 26.05$ min and $R_T = 26.60$ min in Figure S15(f2) for (7c). Hplc conditions: Jupiter 5 μ m C18 300Å column with 150 x 2 mm dimension. Gradient: linear gradient starting from 0% B Buffer (50% CH₃CN in 0.1M TEAA) + 100% A Buffer (0.1M TEAA) to 20% B Buffer + 80% A Buffer in 45 minutes. Flow rate: 100 μ l min⁻¹.



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	18,020	208,2032	4,629	0,367	1,203	0,483
2	19,807	371,8274	20,313	0,287	2,148	2,121
3	20,847	365,0000	6,458	1,067	2,109	0,674
4	21,953	320,9941	15,617	0,320	1,854	1,630
5	23,067	508,1611	19,201	0,300	2,936	2,004
6	24,053	487,8184	30,492	0,240	2,818	3,183
7	24,947	416,6110	16,221	0,407	2,407	1,693
8	26,120	13065,2153	757,673	0,260	75,481	79,094
9	26,653	1565,5372	87,330	0,307	9,044	9,118
-	Total	17309,3677	957,934			

Figure S15(f3): RP-Hplc analysis of alkaline Hydrolysis products of **5'-r(CACG^{Mc}AAC)-3' (7c)** [after digestion for **3h** 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.

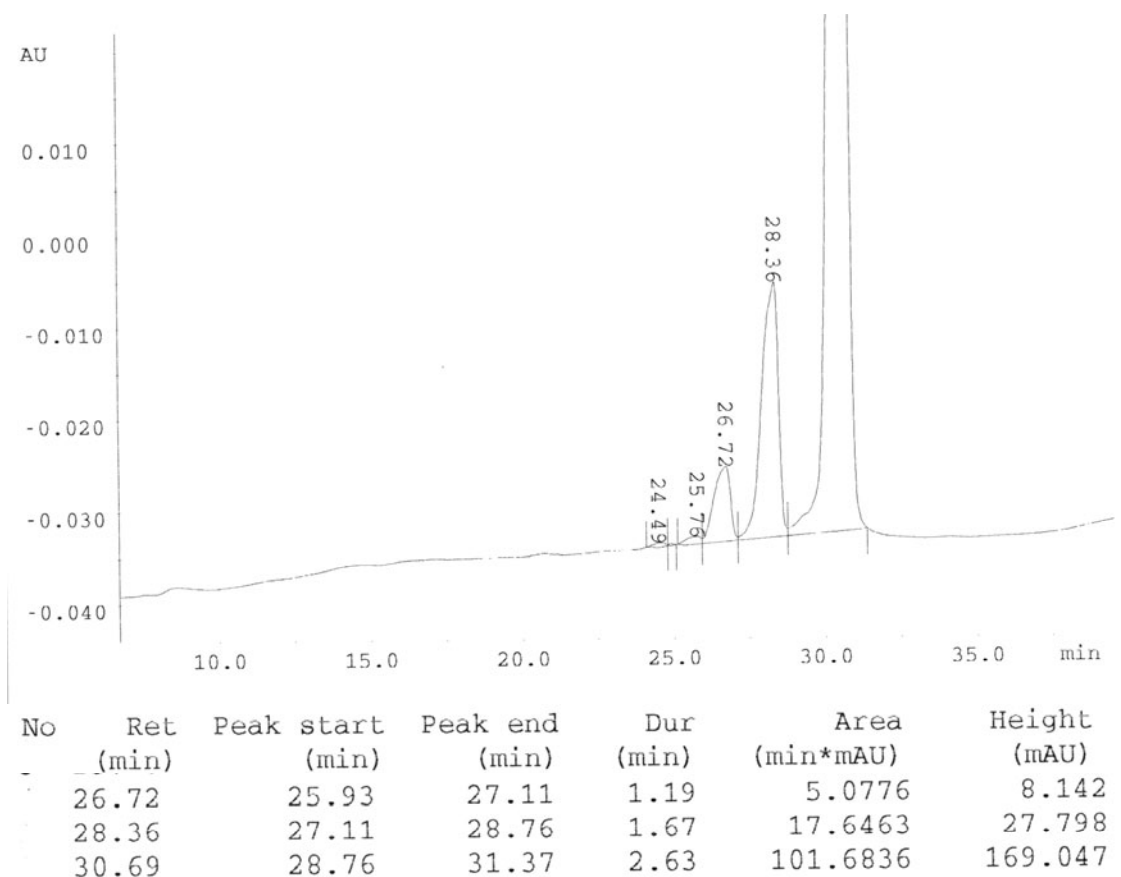
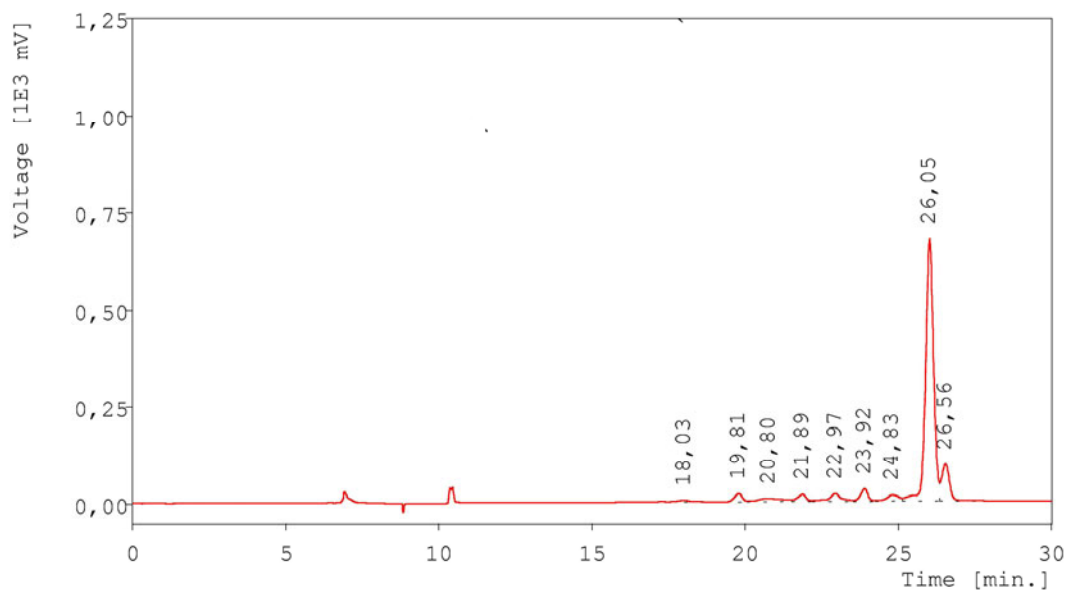


Figure S15(f3i): SMART™ RP-Hplc analysis of the alkaline hydrolysis products co-eluted at $R_T = 26.12$ min and $R_T = 26.65$ min in Figure S15(f3) for (7c). Hplc conditions: Jupiter 5 μ m C18 300Å column with 150 x 2 mm dimension. Gradient: linear gradient starting from 0% B Buffer (50% CH₃CN in 0.1M TEAA) + 100% A Buffer (0.1M TEAA) to 20% B Buffer + 80% A Buffer in 45 minutes. Flow rate: 100 μ l min⁻¹.



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	18,027	119,5016	4,356	0,340	0,712	0,488
2	19,807	368,4282	20,733	0,280	2,194	2,324
3	20,800	466,8229	8,181	1,107	2,780	0,917
4	21,893	367,6722	18,451	0,307	2,190	2,068
5	22,967	567,6920	20,202	0,313	3,381	2,264
6	23,920	540,5829	32,628	0,247	3,220	3,657
7	24,827	441,2782	15,932	0,447	2,628	1,786
8	26,047	12218,7127	677,242	0,273	72,773	75,908
9	26,560	1699,5816	94,464	0,313	10,122	10,588
-	Total	16790,2724	892,189			

Figure S15(f4): RP-Hplc analysis of alkaline Hydrolysis products of **5'-r(CACG^{Mc}AAC)-3' (7c)** [after digestion for **4h** 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.

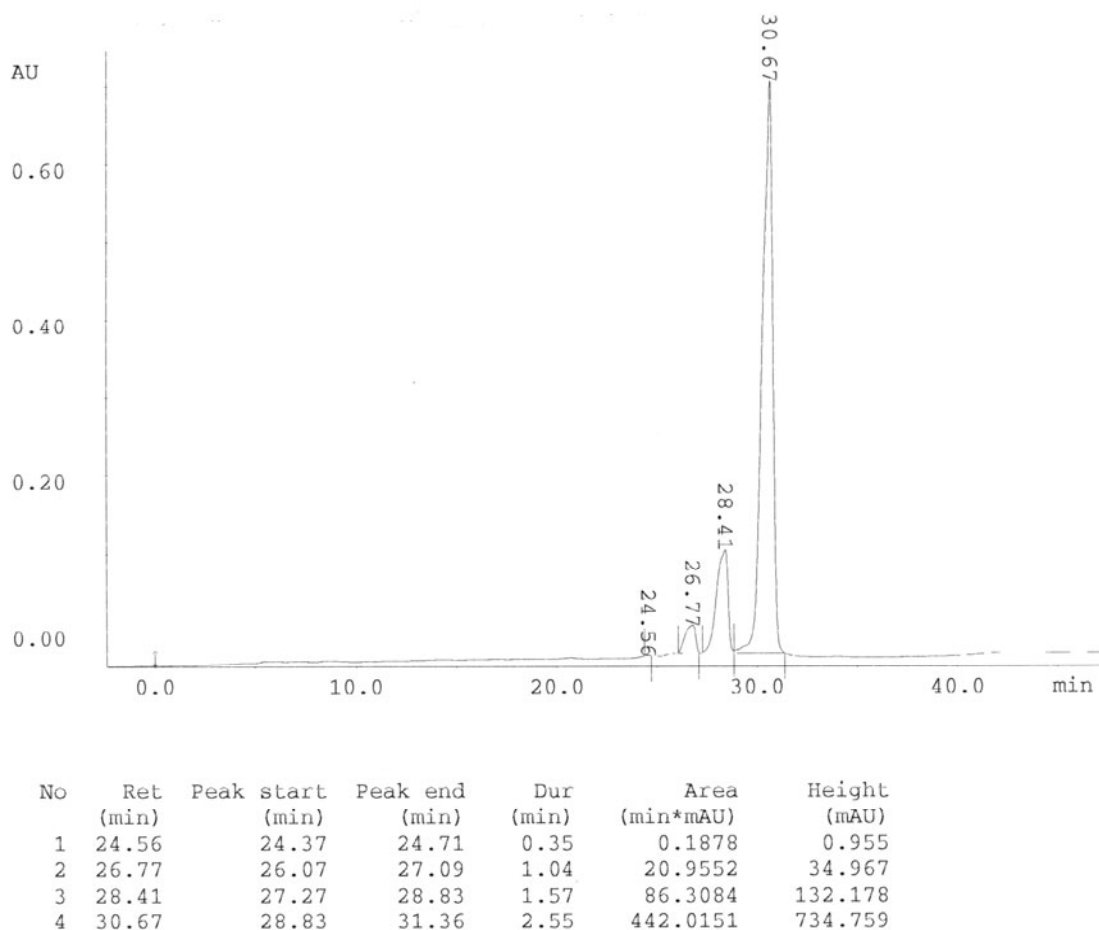
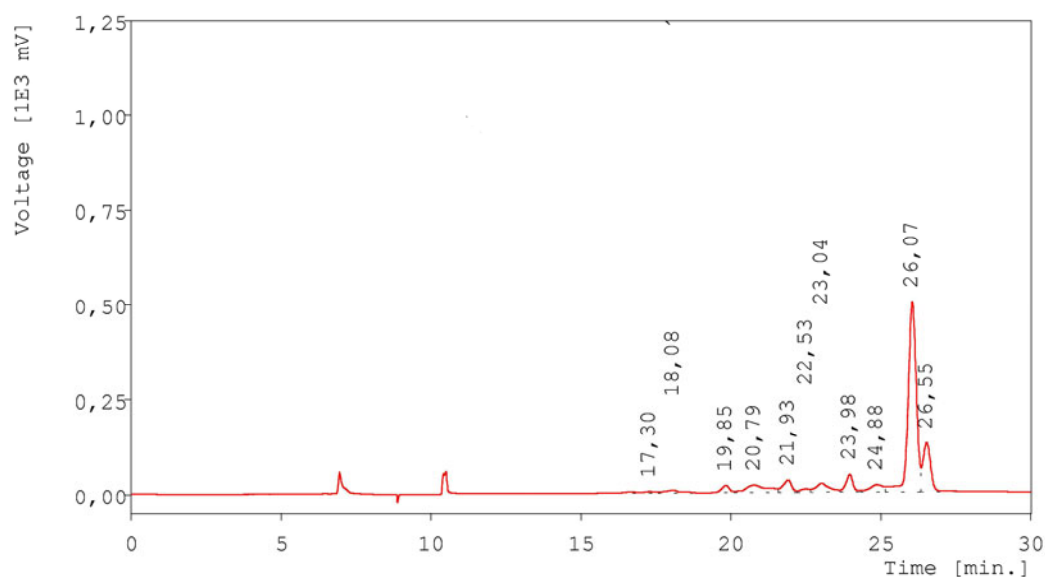


Figure S15(f4i): SMART™ RP-Hplc analysis of the alkaline hydrolysis products co-eluted at $R_T = 26.05$ min and $R_T = 26.56$ min in Figure S15(f4) for (7c). Hplc conditions: Jupiter 5 μ m C18 300Å column with 150 x 2 mm dimension. Gradient: linear gradient starting from 0% B Buffer (50% CH₃CN in 0.1M TEAA) + 100% A Buffer (0.1M TEAA) to 20% B Buffer + 80% A Buffer in 45 minutes. Flow rate: 100 μ l min⁻¹.



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	17,300	154,3016	3,331	0,473	0,927	0,409
2	18,080	246,5649	7,136	0,427	1,481	0,877
3	19,847	352,5298	19,791	0,280	2,117	2,432
4	20,793	1025,9538	19,805	1,093	6,161	2,434
5	21,927	681,0300	33,265	0,320	4,090	4,088
6	22,527	194,6638	9,008	0,380	1,169	1,107
7	23,040	768,3319	24,246	0,480	4,614	2,980
8	23,980	791,5051	46,775	0,253	4,753	5,749
9	24,880	610,2955	19,447	0,533	3,665	2,390
10	26,067	9475,0996	501,702	0,280	56,900	61,661
11	26,547	2352,0467	129,145	0,313	14,123	15,873
-	Total	16652,3226	813,650			

Figure S15(f5): RP-Hplc analysis of alkaline Hydrolysis products of **5'-r(CACG^{Me}AAC)-3' (7b)** [after digestion for **8h** 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.

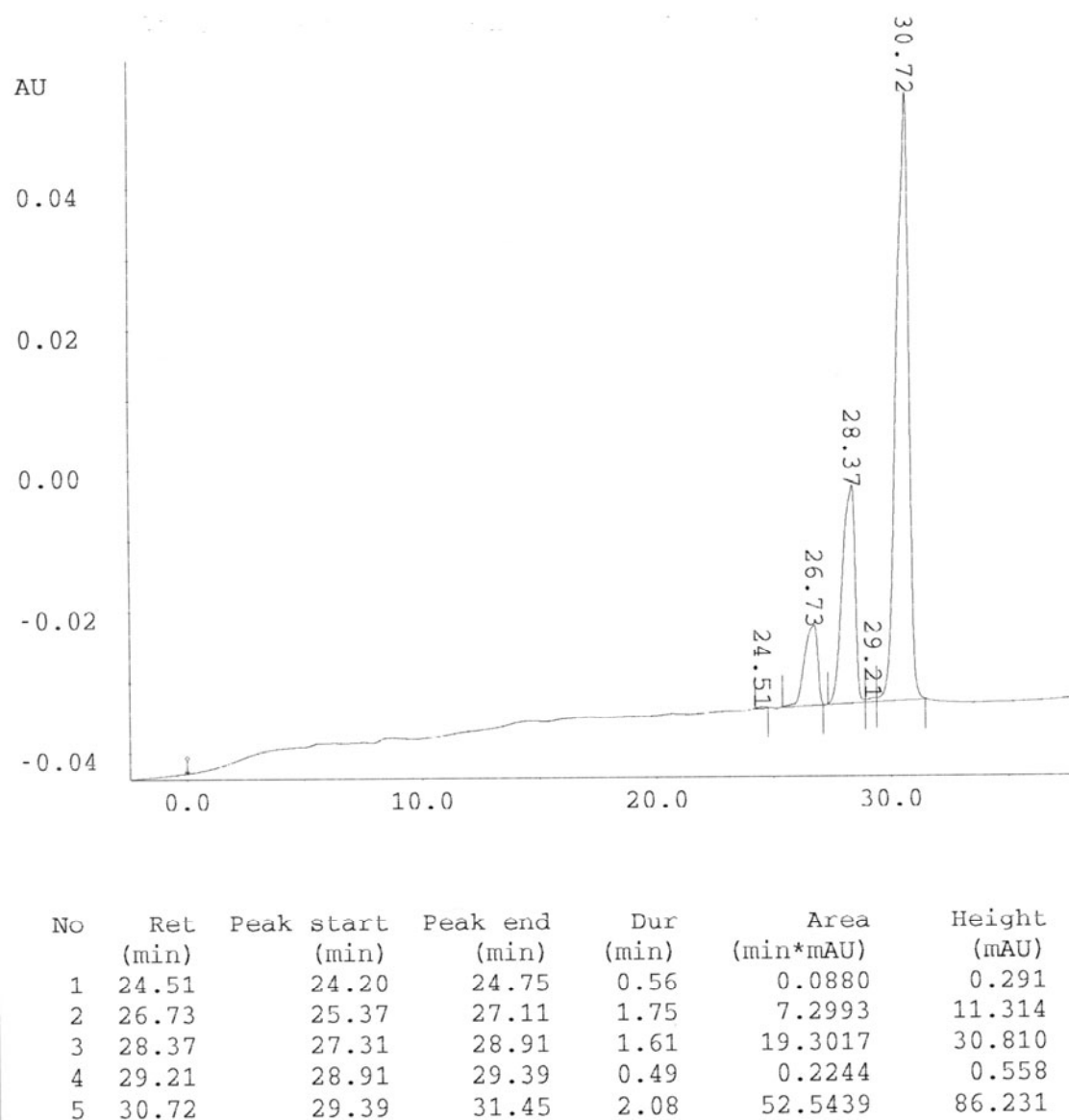
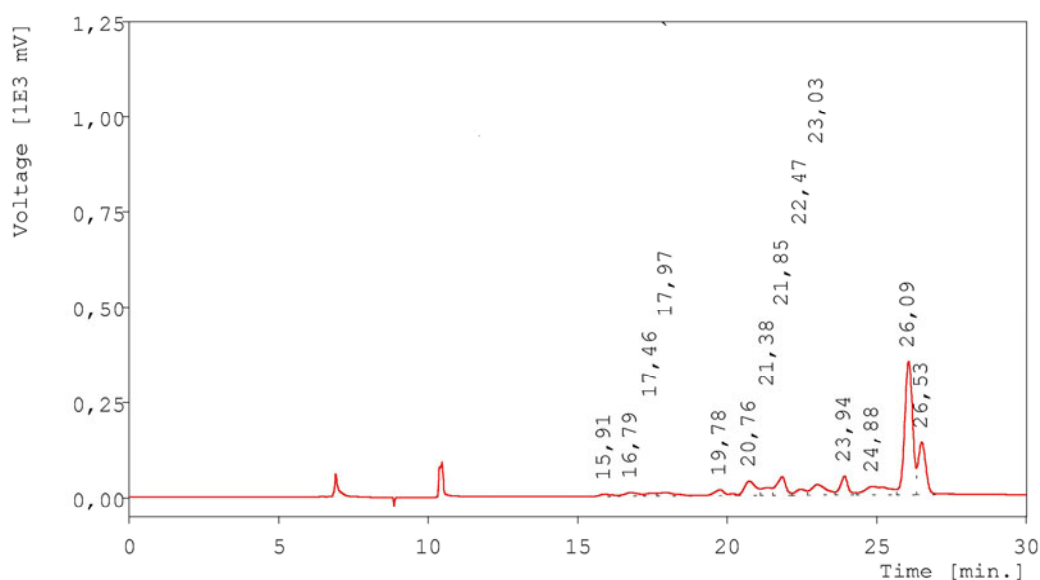


Figure S15(f5i): SMART™ RP-Hplc analysis of the alkaline hydrolysis products co-eluted at $R_T = 26.07$ min and $R_T = 26.55$ min in Figure S15(f5) for (7c). Hplc conditions: Jupiter 5 μ m C18 300Å column with 150 x 2 mm dimension. Gradient: linear gradient starting from 0% B Buffer (50% CH₃CN in 0.1M TEAA) + 100% A Buffer (0.1M TEAA) to 20% B Buffer + 80% A Buffer in 45 minutes. Flow rate: 100 μ l min⁻¹.



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	15,907	147,9257	5,133	0,607	0,909	0,683
2	16,787	306,8358	9,154	0,640	1,886	1,218
3	17,460	178,1433	7,332	0,467	1,095	0,976
4	17,973	370,7055	9,072	0,633	2,278	1,207
5	19,780	421,6520	15,398	0,353	2,591	2,049
6	20,760	994,5395	36,810	0,487	6,112	4,899
7	21,380	453,6381	19,817	0,400	2,788	2,637
8	21,853	1041,2004	48,394	0,353	6,399	6,441
9	22,473	360,1731	15,362	0,420	2,214	2,044
10	23,027	950,3813	27,463	0,560	5,841	3,655
11	23,940	886,3158	49,286	0,253	5,447	6,559
12	24,880	1267,7827	22,056	1,087	7,792	2,935
13	26,087	6396,2901	349,816	0,293	39,312	46,556
14	26,527	2495,1108	136,294	0,327	15,336	18,141
-	Total	16270,6939	751,386			

Figure S15(f6): RP-Hplc analysis of alkaline Hydrolysis products of **5'-r(CACG^{Me}AAC)-3' (7c)** [after digestion for **15h** 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.

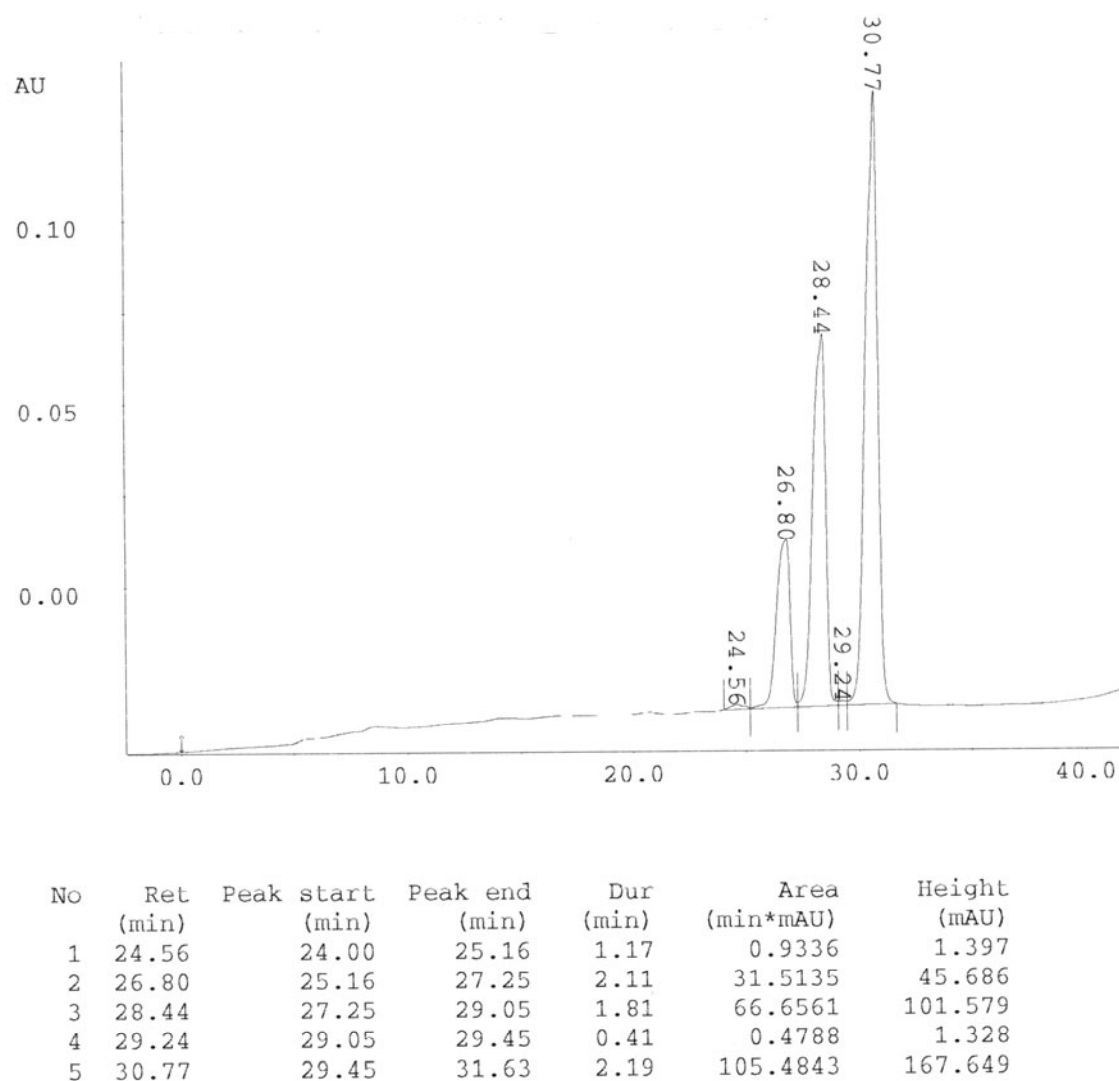
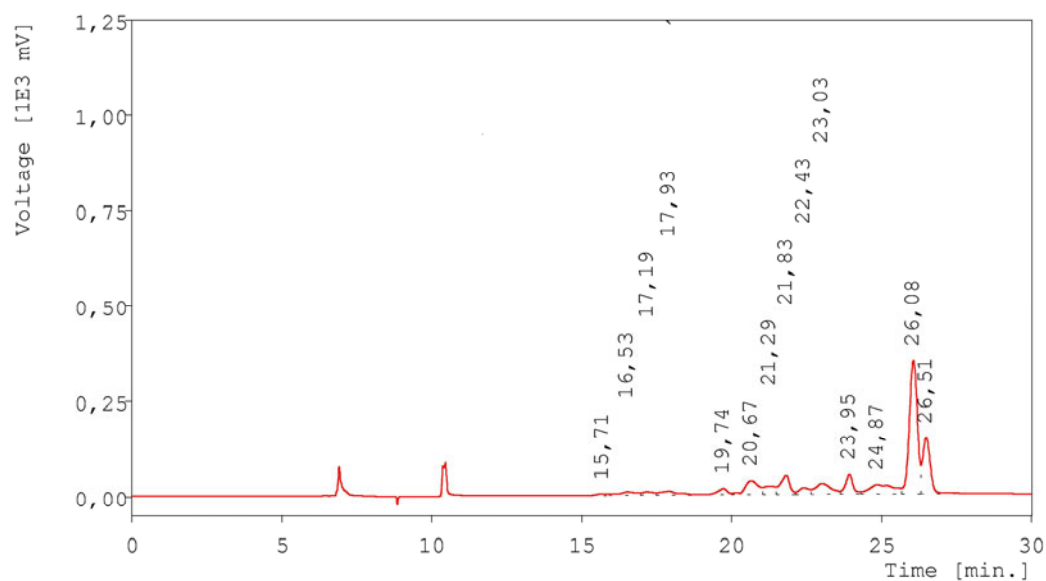


Figure S15(f6i): SMART™ RP-Hplc analysis of the alkaline hydrolysis products co-eluted at $R_T = 26.09$ min and $R_T = 26.53$ min in Figure S15(f6) for (7c). Hplc conditions: Jupiter 5 μ m C18 300Å column with 150 x 2 mm dimension. Gradient: linear gradient starting from 0% B Buffer (50% CH₃CN in 0.1M TEAA) + 100% A Buffer (0.1M TEAA) to 20% B Buffer + 80% A Buffer in 45 minutes. Flow rate: 100 μ l min⁻¹.



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	15,713	97,0941	4,021	0,413	0,572	0,520
2	16,527	357,1674	8,609	0,647	2,105	1,114
3	17,193	226,9261	8,371	0,533	1,337	1,083
4	17,927	417,3770	9,581	0,660	2,460	1,239
5	19,740	356,1370	15,820	0,333	2,099	2,046
6	20,667	1133,4546	35,909	0,593	6,679	4,645
7	21,287	575,9778	22,013	0,467	3,394	2,847
8	21,833	1071,9678	49,958	0,353	6,317	6,462
9	22,427	395,5028	17,038	0,420	2,331	2,204
10	23,033	1033,4179	28,220	0,600	6,090	3,650
11	23,947	905,6909	52,020	0,253	5,337	6,729
12	24,873	1384,0643	24,078	1,107	8,156	3,115
13	26,080	6415,8005	350,609	0,293	37,807	45,352
14	26,507	2599,2764	146,832	0,313	15,316	18,994
-	Total	16969,8546	773,077			

Figure S15(f7): RP-Hplc analysis of alkaline Hydrolysis products of **5'-r(CACG^{Me}AAC)-3' (7c)** [after digestion for **27h** 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.