# Electronic Supplementary Information 3

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

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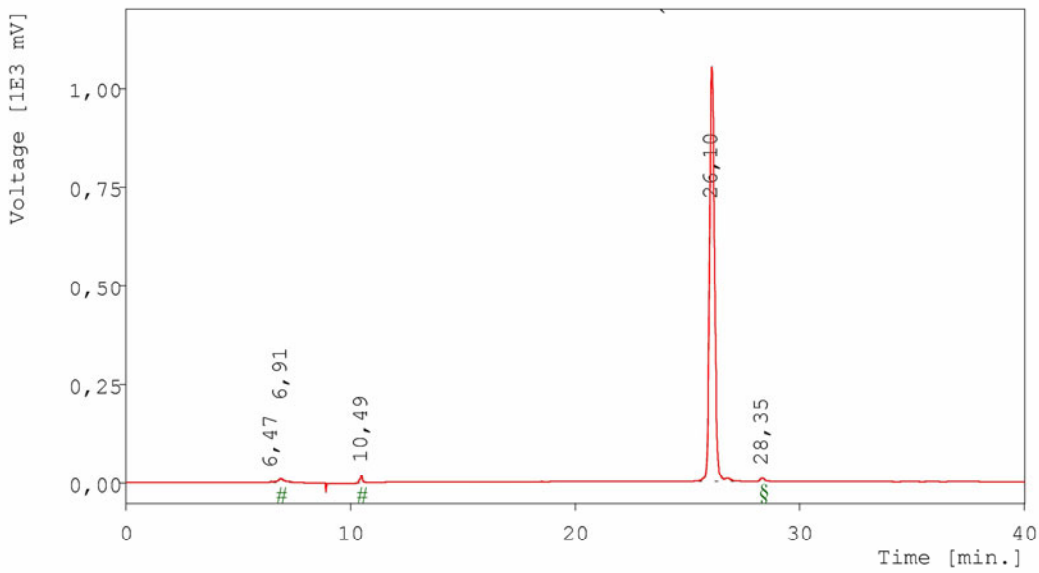
**Content**:

**Figure 12B:**

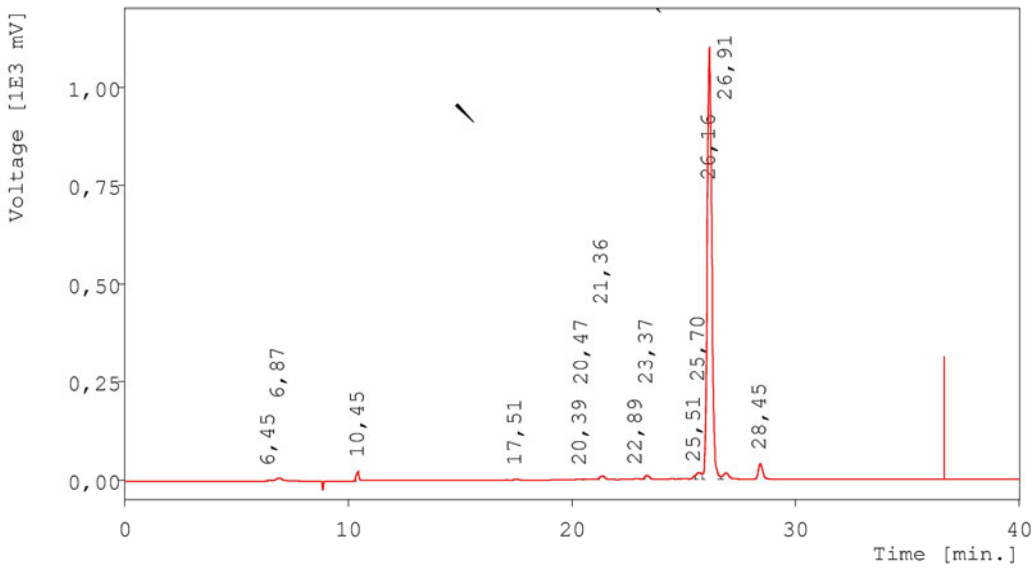



*Result Table - Calculation Method Uncal*

| Peak No. | Reten. time | Area [mV.s] | Height [mV] | W05 [min.] | Area [%] | Height [%] |
|---|---|---|---|---|---|---|
| 1 | 6,467 | 17,3708 | 2,545 | 0,127 | 0,101 | 0,233 |
| 2 | 6,907 | 220,0947 | 10,222 | 0,273 | 1,276 | 0,935 |
| 3 | 10,493 | 182,6978 | 19,034 | 0,120 | 1,059 | 1,741 |
| 4 | 26,100 | 16683,0447 | 1052,671 | 0,247 | 96,703 | 96,286 |
| 5 | 28,353 | 148,6814 | 8,801 | 0,220 | 0,861 | 0,805 |
| - | Total | 17251,8893 | 1093,272 | | | |

**Figure S12(e1):** Hplc analysis of alkaline Hydrolysis products of **5′-r(CAAG$^{Me}$AAC)-3′ (5c)** [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.

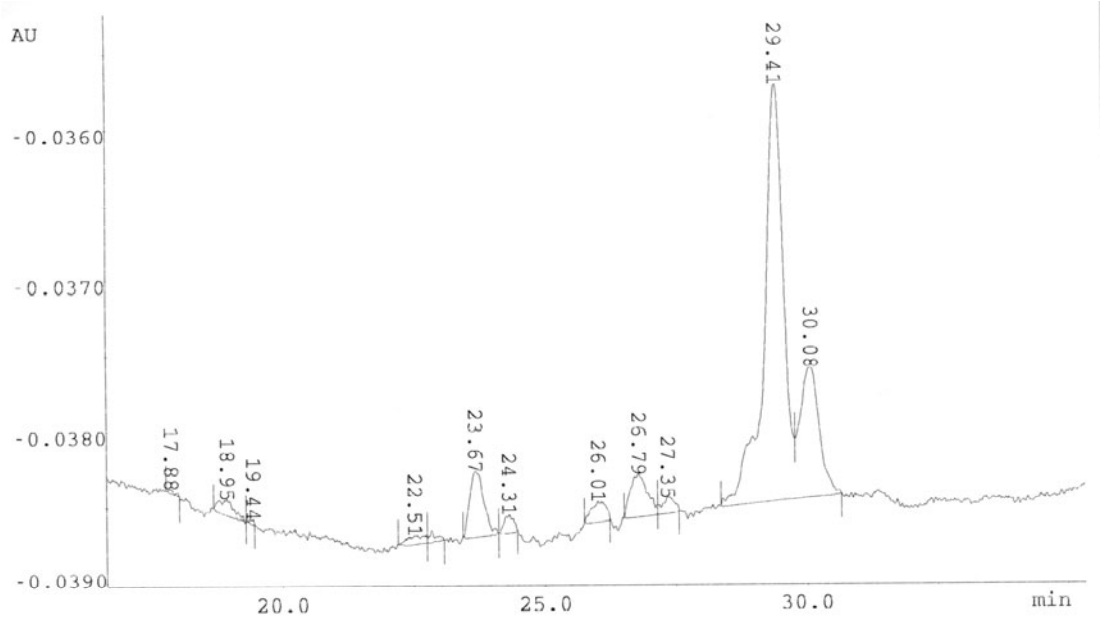
Notes:
# Non-nucleot(s)idic impurity
§ 5′-r(AAG$^{Me}$AAC)-3′ contamination




*Result Table - Calculation Method Uncal*

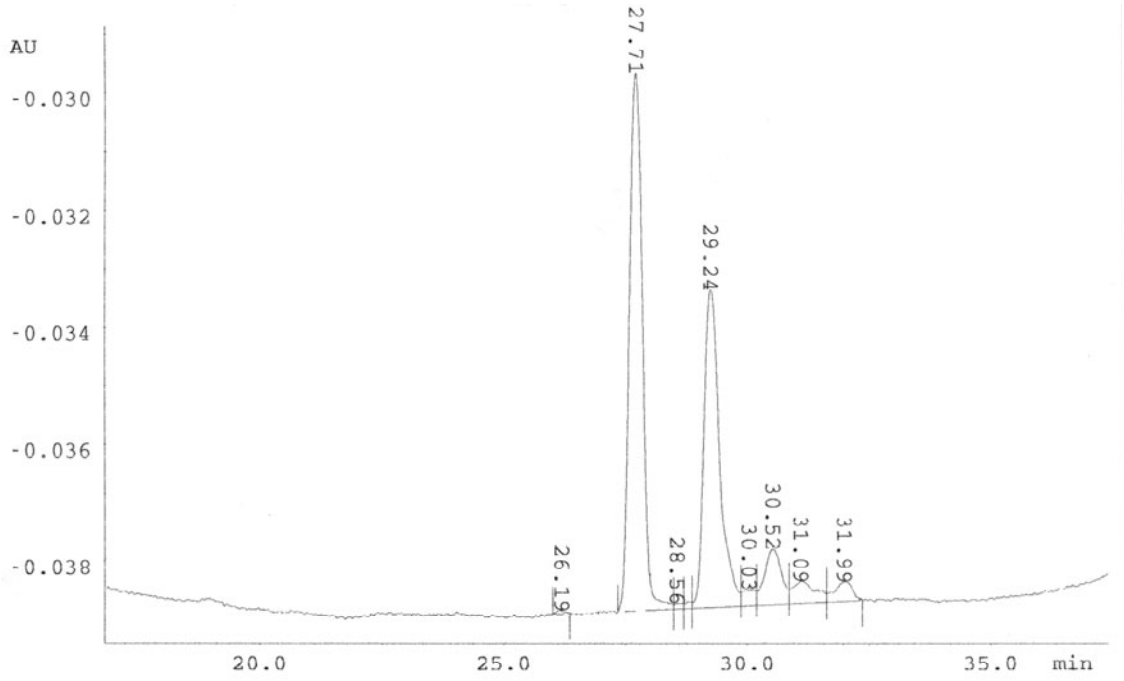
| Peak No. | Reten. time | Area [mV.s] | Height [mV] | W05 [min.] | Area [%] | Height [%] |
|---|---|---|---|---|---|---|
| 1 | 6,447 | 17,5850 | 2,416 | 0,140 | 0,100 | 0,194 |
| 2 | 6,867 | 153,4773 | 7,810 | 0,273 | 0,869 | 0,628 |
| 3 | 10,447 | 244,5266 | 25,133 | 0,147 | 1,385 | 2,019 |
| 4 | 17,507 | 32,0126 | 2,161 | 0,247 | 0,181 | 0,174 |
| 5 | 20,387 | 22,8094 | 1,731 | 0,213 | 0,129 | 0,139 |
| 6 | 20,467 | 47,8386 | 1,574 | 0,660 | 0,271 | 0,126 |
| 7 | 21,360 | 145,8206 | 9,212 | 0,247 | 0,826 | 0,740 |
| 8 | 22,893 | 36,3542 | 2,152 | 0,293 | 0,206 | 0,173 |
| 9 | 23,367 | 141,5256 | 10,232 | 0,220 | 0,802 | 0,822 |
| 10 | 25,507 | 112,3750 | 8,280 | 0,100 | 0,637 | 0,665 |
| 11 | 25,700 | 272,9954 | 16,895 | 0,327 | 1,546 | 1,358 |
| 12 | 26,160 | 15562,4896 | 1101,595 | 0,227 | 88,157 | 88,512 |
| 13 | 26,907 | 327,4041 | 15,847 | 0,307 | 1,855 | 1,273 |
| 14 | 28,447 | 535,9914 | 39,529 | 0,213 | 3,036 | 3,177 |
| - | Total | 17653,2055 | 1244,567 | | | |

**Figure S12(e2):** Hplc analysis of alkaline Hydrolysis products of 5′-r(CAAG$^{Me}$AAC)-3′ (**5c**) [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 $R_T$= 25.51 and $R_T$= 25.70 min were further separated by SMART™ Hplc, see below S12(e2i) and (e2j) for the separation profiles.



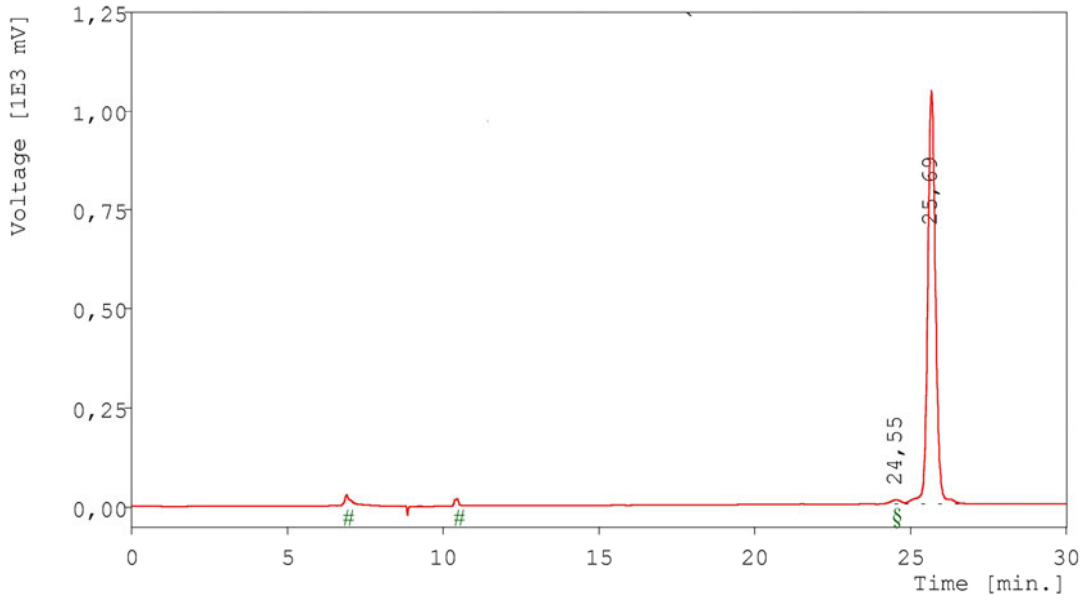

| No | Ret (min) | Peak start (min) | Peak end (min) | Dur (min) | Area (min*mAU) | Height (mAU) |
|---|---|---|---|---|---|---|
| 1 | 29.41 | 28.36 | 29.77 | 1.43 | 1.1857 | 2.761 |
| 2 | 30.08 | 29.77 | 30.65 | 0.89 | 0.3631 | 0.856 |

**Figure S12(e2i):** SMART™ RP-Hplc analysis of the alkaline hydrolysis products co-eluted at **$R_{T=}$ 25.50 min** in **Figure S12(e2)** for **(5c)**. Note the separation of 5'-CAAG$^{Me}$A$_{2'/\ 3'\text{-}P}$ peak at $R_T$ = 29.41 min from 5'-CAAG$^{Me}$AA$_{2'/\ 3'\text{-}P}$ peak at $R_T$ = 30.08 min (See Table S9(E) 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 | 27.71 | 27.35 | 28.49 | 1.16 | 2.8551 | 9.189 |
| 2 | 28.56 | 28.49 | 28.69 | 0.21 | 0.0177 | 0.102 |
| 3 | 28.79 | 28.69 | 28.87 | 0.19 | 0.0165 | 0.120 |
| 4 | 29.24 | 28.87 | 29.87 | 1.01 | 2.0218 | 5.440 |

**Figure S12(e2j):** SMART™ RP-Hplc analysis of the alkaline hydrolysis products co-eluted at **$R_{T=}$ 25.70 min** in **Figure S12(e2)** for **(5c)**. Note the separation of 5'-G$^{Me}$AAC-3' peak at $R_T$ = 27.71 min from 5'-CAAG$^{Me}$A$_{2',\ 3'\text{-cMP}}$ peak at $R_T$ = 29.24 min (See Table S9(E) 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$^{-1}$.



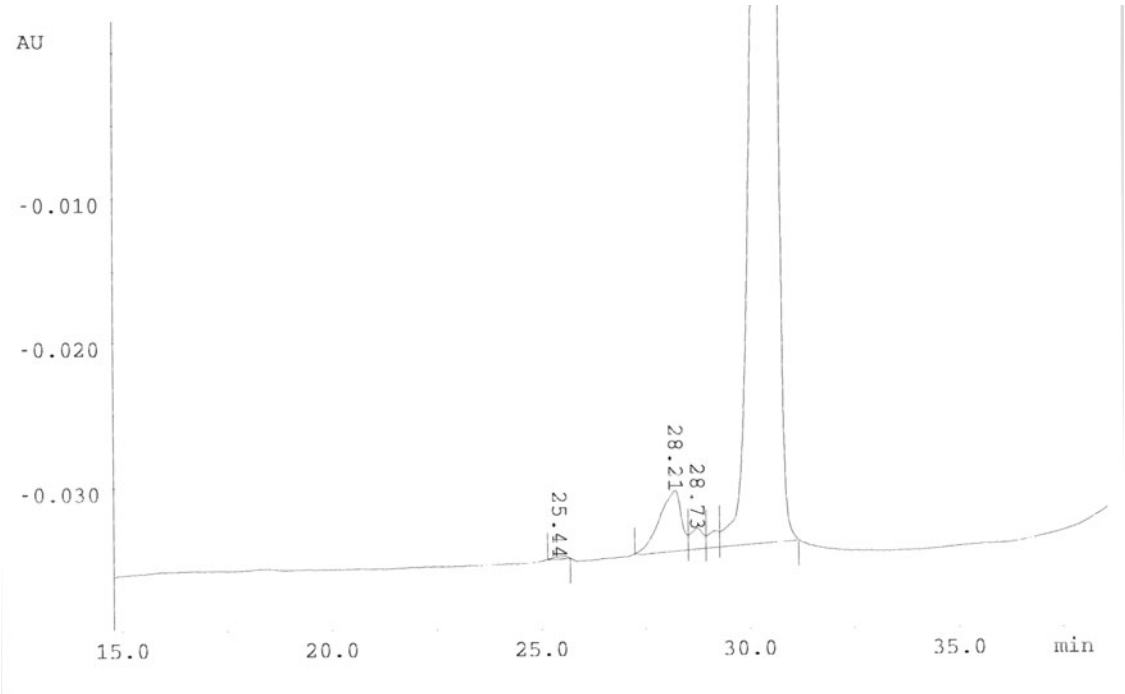

*Result Table - Calculation Method Uncal*

| Peak No. | Reten. time | Area [mV.s] | Height [mV] | W05 [min.] | Area [%] | Height [%] |
|---|---|---|---|---|---|---|
| 1 | 24,553 | 241,2062 | 10,085 | 0,387 | 1,313 | 0,954 |
| 2 | 25,693 | 18133,2053 | 1046,506 | 0,260 | 98,687 | 99,046 |
| - | Total | 18374,4115 | 1056,591 | | | |

**Figure S12(f1):** Hplc analysis of alkaline Hydrolysis products of **5′-r(CACG$^{Me}$AAC)-3′ (7c)** [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.

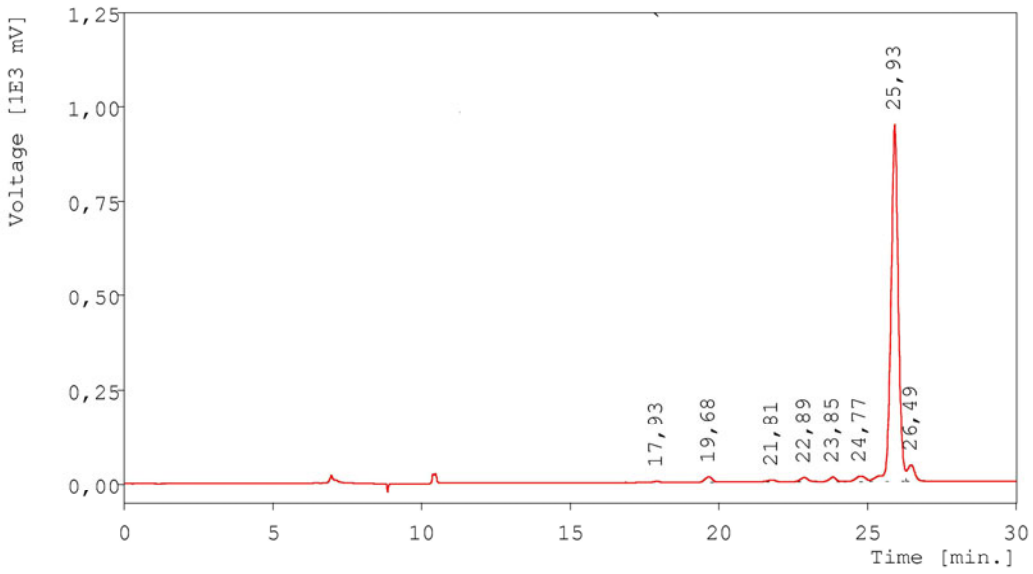
Notes:

# Non-nucleot(s)idic impurity
§ 5′-r(CACG$^{Me}$A$_{2', 3'\text{-cMP}}$) contamination




| No | Ret (min) | Peak start (min) | Peak end (min) | Dur (min) | Area (min*mAU) | Height (mAU) |
|---|---|---|---|---|---|---|
| | 28.21 | 27.24 | 28.52 | 1.29 | 2.5296 | 4.162 |
| | 28.73 | 28.52 | 28.95 | 0.44 | 0.4917 | 1.442 |
| | 29.12 | 28.95 | 29.27 | 0.33 | 0.3220 | 1.153 |
| | 30.55 | 29.27 | 31.16 | 1.91 | 58.1814 | 103.827 |

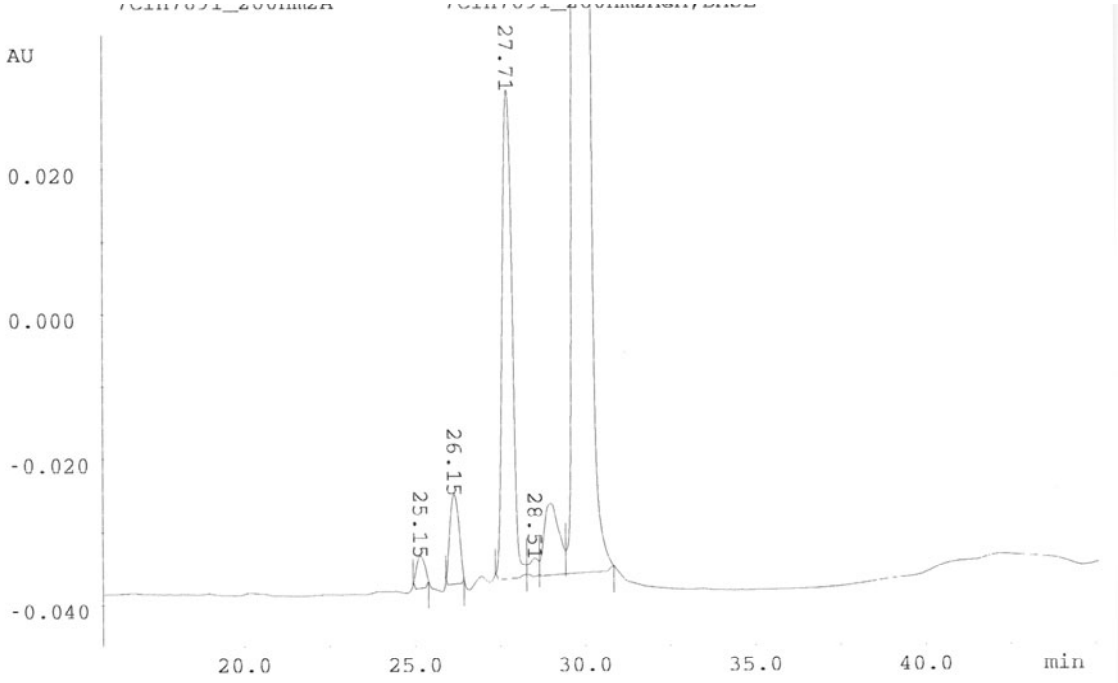
**Figure S12(f1i):** SMART™ RP-Hplc analysis of the alkaline hydrolysis products co-eluted at **$R_{T=}$ 25.69 min** in **Figure S12(f1)** for (**7c**). Note the separation of 5'-GAAC-3' peak at $R_T$ = 28.21 min from the parent heptameric peak. Hplc conditions: Jupiter 5 µm C18 300Å column with 150 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 $min^{-1}$.




*Result Table - Calculation Method Uncal*

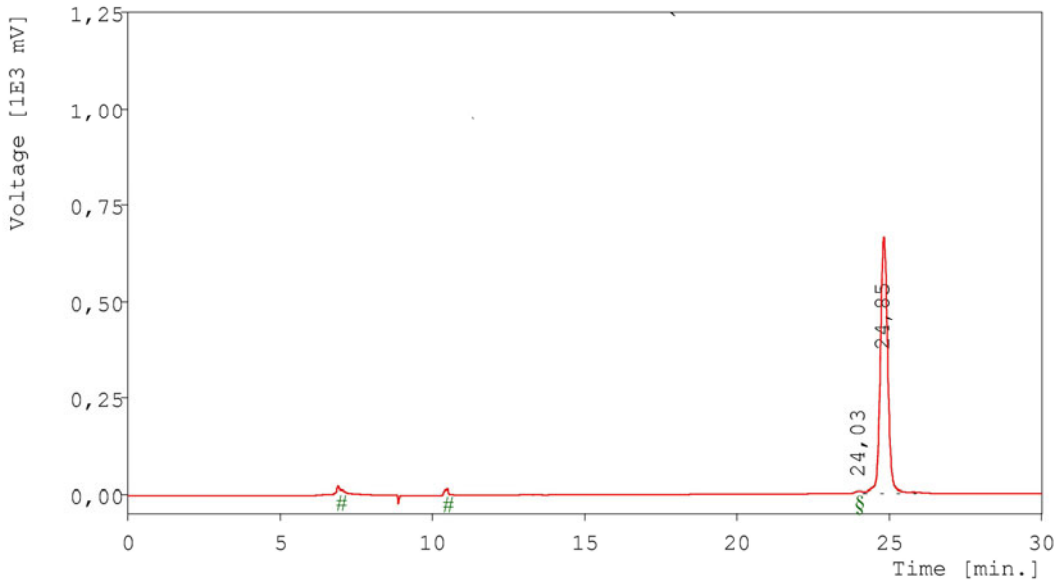
| Peak No. | Reten. time | Area [mV.s] | Height [mV] | W05 [min.] | Area [%] | Height [%] |
|---|---|---|---|---|---|---|
| 1 | 17,927 | 56,3028 | 3,176 | 0,240 | 0,308 | 0,302 |
| 2 | 19,680 | 244,5569 | 14,556 | 0,267 | 1,336 | 1,383 |
| 3 | 21,807 | 223,6669 | 5,586 | 0,320 | 1,222 | 0,531 |
| 4 | 22,893 | 241,4876 | 11,535 | 0,273 | 1,319 | 1,096 |
| 5 | 23,853 | 194,6714 | 12,152 | 0,247 | 1,064 | 1,154 |
| 6 | 24,773 | 335,3467 | 14,013 | 0,387 | 1,832 | 1,331 |
| 7 | 25,933 | 16246,6609 | 947,986 | 0,260 | 88,770 | 90,056 |
| 8 | 26,487 | 759,2864 | 43,659 | 0,300 | 4,149 | 4,147 |
| - | Total | 18301,9797 | 1052,664 | | | |

**Figure S12(f2):** Hplc analysis of alkaline Hydrolysis products of **5′-r(CACG$^{Me}$AAC)-3′ (7c)** [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 $R_T$= 25.93 and $R_T$= 26.49 min were mixed together and further separated by SMART™ Hplc, see below S12(f2i) for the separation profile.




| No | Ret (min) | Peak start (min) | Peak end (min) | Dur (min) | Area (min*mAU) | Height (mAU) |
|---|---|---|---|---|---|---|
| No | Ret (min) | Peak start (min) | Peak end (min) | Dur (min) | Area (min*mAU) | Height (mAU) |
| 1 | 25.15 | 24.93 | 25.39 | 0.47 | 1.2646 | 4.419 |
| 2 | 26.15 | 25.89 | 26.43 | 0.55 | 3.8339 | 12.296 |
| 3 | 27.71 | 27.35 | 28.27 | 0.93 | 22.5661 | 67.279 |
| 4 | 28.51 | 28.27 | 28.64 | 0.39 | 0.7345 | 2.544 |
| 5 | 29.00 | 28.64 | 29.43 | 0.80 | 5.0504 | 9.872 |
| 6 | 29.91 | 29.43 | 30.84 | 1.43 | 317.9507 | 904.903 |

**Figure S12(f2i):** SMART™ RP-Hplc analysis of the alkaline hydrolysis products co-eluted at **$R_{T=}$ 25.93 min** and **$R_{T=}$ 26.49 min** in **Figure S12(f2)** for (**7c**). Note the separation of 5'-AAC-3' peak at $R_T$ = 25.15 and 26.15 min, 5'-G$^{Me}$AAC-3' peak at $R_T$ = 27.71 min, 5'-ACG$^{Me}$AAC-3' peak at $R_T$ = 29.0 min from the parent heptameric peak at $R_T$ = 29.91 min (See Table S9(F) 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 $min^{-1}$.

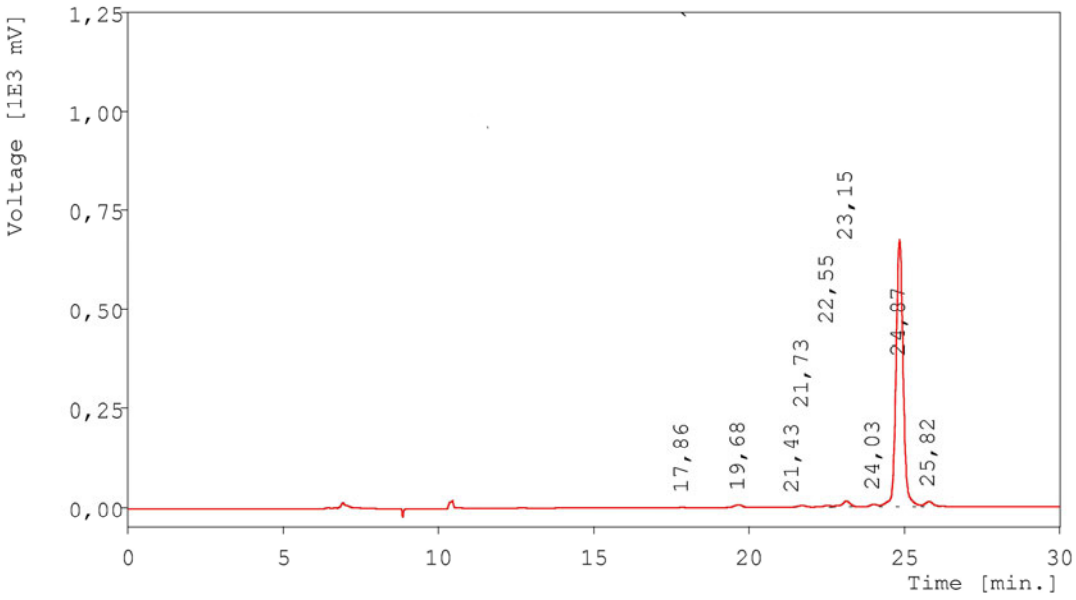



*Result Table - Calculation Method Uncal*

| Peak No. | Reten. time | Area [mV.s] | Height [mV] | W05 [min.] | Area [%] | Height [%] |
|---|---|---|---|---|---|---|
| 1 | 24,033 | 116,8640 | 6,476 | 0,327 | 1,015 | 0,962 |
| 2 | 24,847 | 11396,6271 | 666,671 | 0,260 | 98,985 | 99,038 |
| - | Total | 11513,4910 | 673,147 | | | |

**Figure S12(g1):** RP-Hplc analysis of alkaline Hydrolysis products of **5′-r(CACG$^{Me}$CAC)-3′ (8c)** [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.

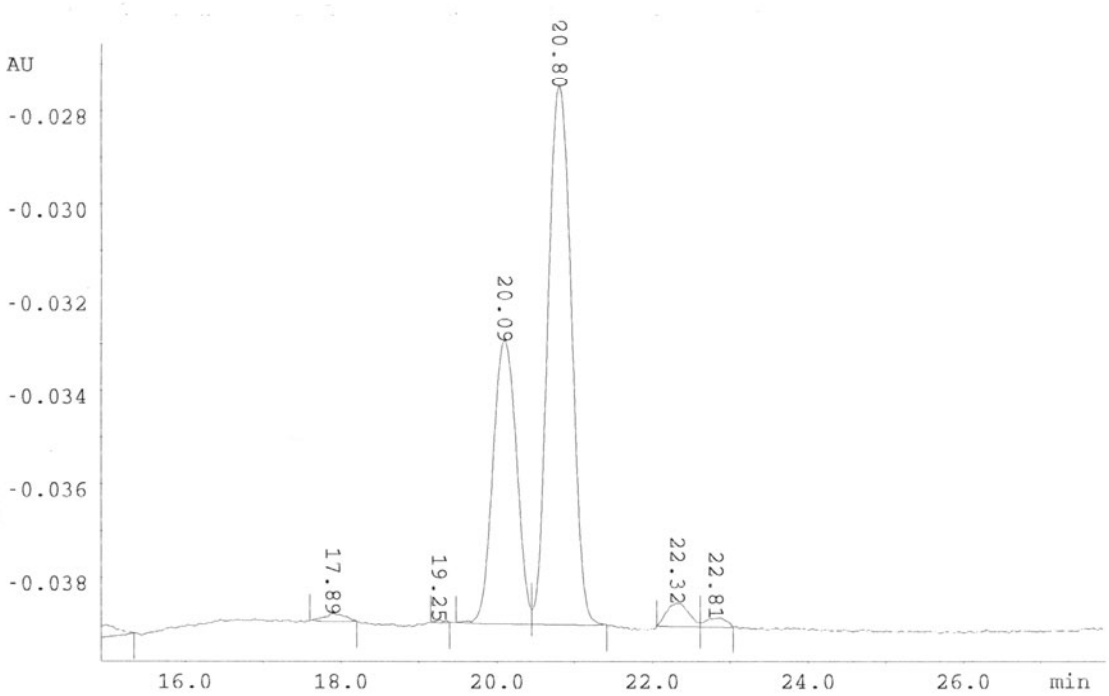
Note:
# and § Non-nucleot(s)idic impurity




*Result Table - Calculation Method Uncal*

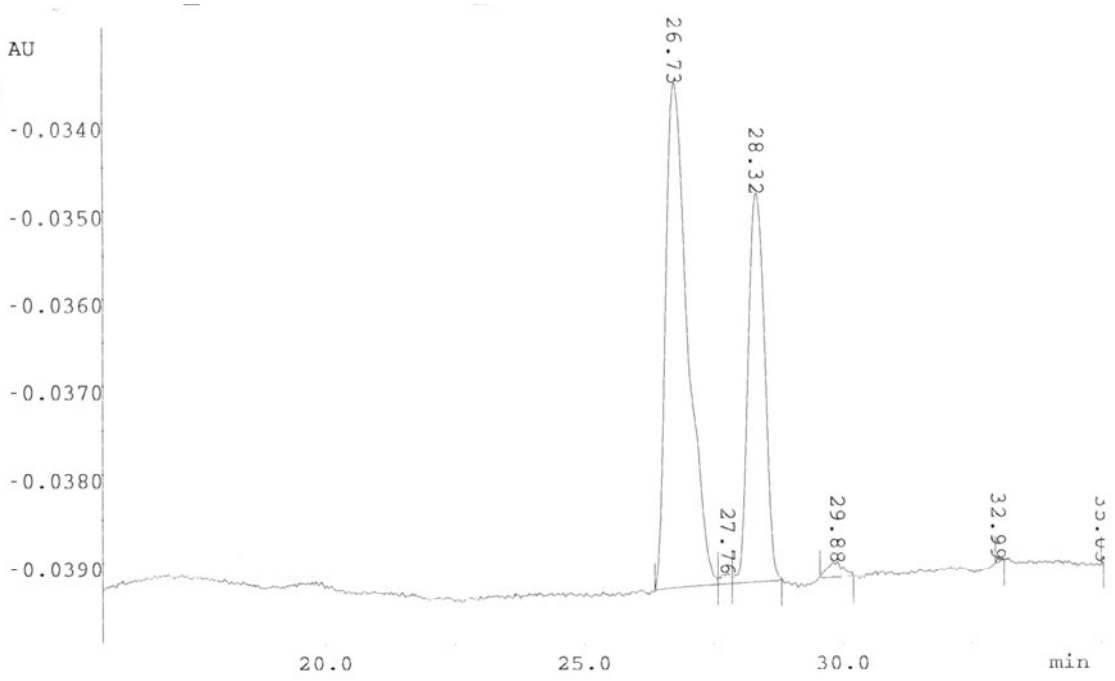
| Peak No. | Reten. time | Area [mV.s] | Height [mV] | W05 [min.] | Area [%] | Height [%] |
|---|---|---|---|---|---|---|
| 1 | 17,860 | 11,7804 | 0,731 | 0,260 | 0,100 | 0,101 |
| 2 | 19,680 | 113,2888 | 6,279 | 0,287 | 0,962 | 0,865 |
| 3 | 21,433 | 44,9898 | 0,333 | 0,520 | 0,382 | 0,046 |
| 4 | 21,727 | 62,3310 | 3,862 | 0,260 | 0,529 | 0,532 |
| 5 | 22,547 | 61,4830 | 4,169 | 0,273 | 0,522 | 0,575 |
| 6 | 23,153 | 288,4676 | 14,749 | 0,260 | 2,450 | 2,033 |
| 7 | 24,033 | 108,5148 | 6,449 | 0,320 | 0,922 | 0,889 |
| 8 | 24,867 | 10786,8465 | 675,370 | 0,247 | 91,622 | 93,084 |
| 9 | 25,820 | 295,5221 | 13,610 | 0,293 | 2,511 | 1,875 |
| - | Total | 11773,2240 | 725,551 | | | |

**Figure S12(g2):** RP-Hplc analysis of alkaline Hydrolysis products of **5′-r(CACG$^{Me}$CAC)-3′ (8c)** [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 $R_T$= 19.68 min and $R_T$= 23.15 min were further separated by SMART™ Hplc, see below S12(g2i), (g2j) for the separation profiles.




| No | Ret (min) | Peak start (min) | Peak end (min) | Dur (min) | Area (min*mAU) | Height (mAU) |
|---|---|---|---|---|---|---|
| | 20.09 | 19.47 | 20.44 | 0.99 | 2.1382 | 6.085 |
| | 20.80 | 20.44 | 21.41 | 0.99 | 4.0624 | 11.551 |

**Figure S12(g2i):** SMART™ RP-Hplc analysis of the alkaline hydrolysis products co-eluted at **$R_{T=}$ 23.86 min** in **Figure S12(g2)** for **(8c)**. Note the separation of 5'-CAC-3' peak at $R_T$ = 20.09 min from 5'-$CAC_{2'}$, $_{3'\text{-cMP}}$ peak at $R_T$ = 20.80 min (See Table S9(G) 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) |
|---|---|---|---|---|---|---|
| | 26.73 | 26.36 | 27.57 | 1.23 | 2.8502 | 5.749 |
| | 27.76 | 27.57 | 27.85 | 0.29 | 0.0250 | 0.118 |
| | 28.32 | 27.85 | 28.80 | 0.96 | 1.6463 | 4.427 |

**Figure S12(g2j):** SMART™ RP-Hplc analysis of the alkaline hydrolysis products co-eluted at **$R_{T=}$ 23.15 min** in **Figure S12(g2)** for **(8c)**. (See Table S9(G) 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 $min^{-1}$.