# **SUPPORTING INFORMATION FOR:**

Effective cellular delivery of antisense peptide nucleic acid (PNA) by conjugation to guanidinylated diaminobutanoic acid based peptide dendrons

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# **1.** Luciferase gene carrying an intron (from human β-globin)



**Figure S1.** Luciferase gene carrying an intron (from human  $\beta$ -globin) including an aberrant splice site (705). Untreated cells result in non-expression of Luciferase, whereas blocking the aberrant splice site via PNA conjugation leads to splice correction and restoration of luciferase expression.

| A) Ghx <sub>8</sub> Ghx <sub>8</sub> + CQ Ahx <sub>8</sub> Ahx <sub>8</sub> + CQ (4680) (4653) $\frac{1}{3}$   | Uncorrected<br>Corrected  |
|--|---|
| B)<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$<br>$\overline{P}$ | CQ  |
| C)<br>y Uncorrected<br>% Corrected<br>% Source ted<br>% Corrected<br>91,7<br>91,5<br>8,3<br>8,5<br>16,8<br>25,2<br>18,6<br>91,7<br>91,5<br>8,5<br>16,8<br>25,2<br>18,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>11,8<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,6<br>16,7<br>16,7<br>16,6<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7<br>16,7   | D)       Ghx <sub>8</sub> Ghx <sub>12</sub> Ghx <sub>16</sub> 100       100       100       100       100       100         100       100       100       100       100       100         100       100       100       100       100       100         100       100       100       100       100       100         100       100       100       100       100       100         100       100       100       100       100       100         100       100       100       100       100       100         100       100       100       100       100       100         100       100       100       100       100       100         100       100       100       100       100       100         100       100       100       100       100       100       100         100       100       100       100       100       100       100       100         100       100       100       100       100       100       100       100         100       100       100       100       100 |

# 2. RT-PCR analysis of the corrected luciferase mRNA

| لة<br>E)                     | ntrol              |                    | Ghx<br>(468)                                |              | (                   | Oc <sub>1</sub><br>4934     | 1)           | (•                  | Dec <sub>1</sub><br>4939           | )            | (            | Oc <sub>2</sub><br>4925  | 5)          | (                      | Dec <u>;</u><br>4926                            | 2<br>5)     |  |
|------------------------------|--------------------|--------------------|---|--------------|---------------------|-----------------------------|--------------|---------------------|------------------------------------|--------------|--------------|--|-------------|------------------------|---|-------------|--|
| Wa                           | Cor                | 1uM                | 1.5uM                                       | 3uM          | 1uM                 | 1.5uM                       | 3uM          | 1uM                 | 1.5uM                              | 3uM          | 1uM          | 1.5uM  | 3uM         | 1uM                    | 1.5uM   | 3uM         |  |
|                              |                    |                    |   |              |                     |                             |              |                     |                                    | **           | -            |  |             |                        |   |             |  |
| % uncorrected<br>% corrected | 90,7<br><b>9,3</b> | 91,1<br><b>8,9</b> | 84,9<br>15,1                                | 77,7<br>22,3 | 88,5<br><b>11,5</b> | 57,2<br><b>42,9</b>         | 13,3<br>86,8 | 87,0<br><b>13,1</b> | 40,0<br>60,0                       | 11,4<br>88,6 | 39,2<br>60,8 | 21,0<br><b>79,0</b>  | 2,0<br>98,0 | 39,4<br>60,6           | 6,2<br>93,8                                     | 1,1<br>98,9 |  |
|                              |                    |                    |   |              |                     |                             |              |                     |                                    |              |              |  |             |                        |   |             |  |
| F)                           | rol                | - (                | Ghx <sub>8</sub><br>4680                    | 3<br>))      | C<br>(!             | Quin<br>5171                | в<br>)       | N<br>(5             | aph <sub>8</sub><br>5172)          | 3            | Ph<br>(5     | en A<br>5173)  | -8          | P<br>(4                | he <sub>8</sub><br>965)                         |             |  |
| (A Water                     | Control            | 1uM (              | Ghx <sub>8</sub><br>4680<br>                | 3uM          | 1uM ()              | Quin<br>5171<br><br>        | 3uM (8       | 1uM<br>5, Z         | aph <sub>8</sub><br>5172)<br><br>  | 3uM          | Pho<br>(5    | en A<br>5173)<br>-<br>-<br>-<br>-<br>-   | 3uM 8       | 1 <sub>u</sub> M<br>(4 | he <sub>8</sub><br>965)<br>–<br>M <sub>JZ</sub> | 3uM         |  |
| (H                           | Control            | 1uM )              | Ghx <sub>8</sub><br>4680<br>4 <sub>97</sub> | 3uM          |                     | Quin<br>5171<br>도<br>포<br>전 | 3uM (B       | 1uM<br>C            | aph <sub>8</sub><br>5172)<br><br>¥ | 3 uM         | Pho<br>(5    | en A<br>5173)<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>-<br>- | 3uM 8       | P<br>(4                | 'he <sub>8</sub><br>965)<br>୍ୟୁ                 | 3uM         |  |

**Figure S2.** RT-PCR splice correction analysis of the luciferase gene carrying an intron including an aberrant splice site (705). Blocking the aberrant splice site via PNA conjugation leads to splice correction and restoration of luciferase expression. PNA antisense activity when conjugated to: A) 6-aminohexanoic acid (Ahx<sub>8</sub>, 4653) and the guanidino (Ghx<sub>8</sub>, 4680) Dab dendron. B) 6-guanidinohexanoic acid Dab dendron (Ghx<sub>8</sub>, 4680) and an analogous linear (D-Arg)<sub>8</sub>-CPP (2787). C) Dab-dendron conjugates differing at the terminal carbon chain length, guanidinopentanoic acid (Gpn<sub>8</sub>, 4779), guanidinohexanoic acid (Ghx<sub>8</sub>, 4680), guanidinoheptanoic acid (Ghp<sub>8</sub>, 4780) and guanidinooctanoic acid (Goc<sub>8</sub>, 4758). D) Dab-dendron conjugates differing by the number of terminal amino groups: Ghx<sub>8</sub> (4680), Ghx<sub>9</sub> (5066), Ghx<sub>12</sub> (5054) and Ghx<sub>16</sub> (4912). E) Dab dendron conjugates modified with fatty acids of different length and with different ratios such as octanoic acid (Oc1, 4934 and Oc2, 4925) and decanoic acid (Dec1, 4939 and Dec2, 4926). F) Dab-dendron conjugates containing different aromatic moieties such as quinolinecarboxylic acid (Quin<sub>8</sub>, 5171), naphthoic acid (Naph<sub>8</sub>, 5172), phenylacetic acid (Phen A.<sub>8</sub>, 5173) or phenylalanine (Phe<sub>8</sub>, 4965).

# 3. PNA antisense activity when conjugated to dendrons containing 8-amino-3,6dioxaoctanoic acid (eg)



**Figure S3.** Comparison of the PNA antisense activity when bounded to dendrons containing eg in different positions:  $\text{Geg}_8(4936)$ ,  $\text{Ghx}_8\text{-}eg_8(5124)$  and  $\text{Geg}_8\text{-}Phe_8(4963)$ . A) Representation of ligands structure. B) Luciferase assay. C) ATP toxicity assay. Data are expressed as mean ± SD (n=4; Student's t test \**P* < 0.05, \*\**P*< 0.01). *p*-values are indicated as compared to the corresponding Ghx<sub>8</sub> data.

#### 4. Cooperative enhancement effects



**Figure S4.** Study of the mismatch  $MM_2$ -asPNA (4 switched base pairs) antisense activity both when bounded to  $Ghx_8$  and  $Phe_8$  dendrons. Additionally, we study the increasing concentration effect of  $MM_2$ asPNA upon non-mismatch asPNA. A) Luciferase and B) toxicity assay for  $Phe_8$ - $MM_2$  (5501) effect on  $Ghx_8$ (4680). C) Luciferase and D) toxicity assay for  $Phe_8$ - $MM_2$  (5502) effect on  $Phe_8$  (4965). E) Luciferase and F) toxicity assay for  $Ghx_8$ - $MM_2$  (5501) effect on  $Phe_8$  (4965). G) Luciferase and H) toxicity assay for  $Ghx_8$ - $MM_2$  (5502) effect on  $Ghx_8$  (4680). Data are expressed as mean  $\pm$  SD (n=4).

#### 5. Relative Mean Fluorescence Intensity



**Figure S5.** Relative Mean Fluorescence Intensity extracted from confocal microscopy images of Hela-pLuc cells treated with 1  $\mu$ M Ghx<sub>8</sub>-AF568 (5267) after 5, 10, 30 min and 1, 2, 3, 4 and 24h incubation time. Data are expressed as mean ± SD (n=4; Student's t test \**P* < 0.05, \*\**P*< 0.01). *p*-values are indicated as compared to the 24h data.

#### 1. Thermal stability (Tm) of the Dab dendron-PNA-DNA duplexes

| No.  | Name                                     | Sequence <sup>c,d</sup>   | Tm (ºC) <sup>e,f</sup> |
|------|--|---|------------------------|
| 2389 | Naked asPNA <sup>a</sup>                 | H-CCT CTT ACC TCA GTT ACA-NH <sub>2</sub>   | 69                     |
| 3380 | MM <sub>1</sub> Naked asPNA <sup>b</sup> | H-CCT CAG ACC TCA TTT ACA-NH <sub>2</sub>   | 44                     |
| 4680 | Ghx <sub>8</sub>                         | H-Ghx <sub>8</sub> -(Dab) <sub>4</sub> -(Dab) <sub>2</sub> -Dab-asPNA   | 76                     |
| 4758 | Goc <sub>8</sub>                         | H-Goc <sub>8</sub> -(Dab) <sub>4</sub> -(Dab) <sub>2</sub> -Dab-asPNA   | 74                     |
| 4965 | Phe <sub>8</sub>                         | H-Ghx <sub>8</sub> -Phe <sub>8</sub> -(Dab) <sub>8</sub> -(Dab) <sub>4</sub> -(Dab) <sub>2</sub> -Dab-asPNA   | 73                     |
| 4926 | Dec <sub>2</sub>                         | H-Ghx <sub>8</sub> -(Dab) <sub>4</sub> -(Dab) <sub>2</sub> /(Dec) <sub>2</sub> -(Dab) <sub>2</sub> -Dab-asPNA | 76                     |
| 5051 | Ghx <sub>8</sub> -MM <sub>1</sub>        | H-Ghx <sub>8</sub> -(Dab) <sub>4</sub> -(Dab) <sub>2</sub> -Dab-MM <sub>1</sub> asPNA                         | 53                     |

**Table S1.** Thermal stability (Tm) of PNA-DNA duplex.

<sup>a</sup>Antisense PNA (asPNA), PNAs targeting splicing correction of mutated luciferase gene in the HeLa pLuc705 cells. <sup>b</sup>Mis-match asPNA (MM asPNA), asPNA with two. <sup>c</sup>The sequences of the PNAs are written from N-terminal to C-terminal end. <sup>d</sup>Abbreviations: Guanidinhexanoic acid (Ghx), guanidinooctanoic acid (Goc), decanoic acid (Dec), Phenylalanine (Phen). <sup>e</sup>Melting temperatures (accuracy +/-1 <sup>o</sup>C) of duplexes were measured in 10mM sodium phosphate (pH 7.0) containing 0.1 mM EDTA, 100m M NaCl. <sup>f</sup>Sense DNA sequence, 5'-TGT AAC TGA GGT AAG AGG-3'.

#### 2. Characterization

Crude product of all different DAB dendron were purified by RP-HPLC. Afterwards, purified fractions were characterized by MALDI-TOF MS and RP-HPLC. MALDI-TOF MS offers information about the mass of the product and RP-HPLC offers information about the purity. SPPS allows accurate control of the synthesis procedure and as consequence of the products structure. Therefore, these two characterization techniques offer enough information even if information on the actual structure is limited.

Analytical HPLC programs:

| 100-60-A |      |          |        |  |  |  |  |
|----------|------|----------|--------|--|--|--|--|
| Time     | Flow | $%A^{1}$ | $%B^2$ |  |  |  |  |
| 0        | 0,8  | 0        | 100    |  |  |  |  |
| 30       | 0,8  | 40       | 0      |  |  |  |  |
| 40       | 0,8  | 100      | 0      |  |  |  |  |
| 45       | 0,8  | 100      | 0      |  |  |  |  |
| 50       | 0,8  | 0        | 100    |  |  |  |  |
| 60       | 0,8  | 0        | 100    |  |  |  |  |

| -           |      |          |        |  |  |  |  |  |
|-------------|------|----------|--------|--|--|--|--|--|
| 100-0-A-400 |      |          |        |  |  |  |  |  |
| Time        | Flow | $%A^{1}$ | $%B^2$ |  |  |  |  |  |
| 0           | 0,8  | 0        | 100    |  |  |  |  |  |
| 2           | 0,8  | 0        | 100    |  |  |  |  |  |
| 32          | 0,8  | 100      | 0      |  |  |  |  |  |
| 35          | 0,8  | 100      | 0      |  |  |  |  |  |
| 40          | 0,8  | 0        | 100    |  |  |  |  |  |
| 55          | 0.8  | 0        | 100    |  |  |  |  |  |

C18 column

%A<sup>1</sup>: 95% milliQ water, 5% Acetonitrile, 0,01% TFA

%B<sup>2</sup>: 95% Acetonitrile, 5% milliQ water, 0,01% TFA

# - (D-Arg)<sub>8</sub> (2787)



MALDI-TOF MS spectrum of 2787. Mass calc./found 6072/6084



Analytical **RP-HPLC** trace of **2787**. T<sub>R</sub> = 17.7 min (program: 100\_60\_A, 40 min,  $\lambda$  = 260 nm)

# Ahx<sub>8</sub> (4653)

PNA4653 fr 3 Mw=6371.65

Shimadzu Biotech Axima Assurance 2.8.5.20090305: Mode Linear, Power: 110, Blanked, P.Ext. @ 4000 (bin 85)



MALDI-TOF MS spectrum of 4653. Mass calc./found 6372/6373



Analytical **RP-HPLC** trace of **4653**.  $T_R$  = 18.0 min (program: 100\_60\_A, 40 min,  $\lambda$  = 260 nm)

# - Ghx<sub>8</sub> (4680)

#### PNA 4680 G2 fr3/2 Mw=6711.21

Shimadzu Biotech Axima Assurance 2.8.5.20090305: Mode Linear, Power: 110, Blanked, P.Ext. @ 4000 (bin 85)



MALDI-TOF MS spectrum of 4680. Mass calc./found 6711/6709



Analytical **RP-HPLC** trace of **4680**. T<sub>R</sub> = 19.9 min (program: 100\_60\_A, 40 min,  $\lambda$  = 260 nm)

# - Goc<sub>8</sub> (4758)

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MALDI-TOF MS spectrum of 4758. Mass calc./found 6934/6932

Analytical **RP-HPLC** trace of **4758**.  $T_R$  = 30.7 min (program: 100\_60\_A, 40 min,  $\lambda$  = 260 nm)



# - Gpn<sub>8</sub> (4779)



MALDI-TOF MS spectrum of 4779. Mass calc./found 6599/6604



Analytical **RP-HPLC** trace of **4779**.  $T_R$  = 18.8 min (program: 100\_60\_A, 40 min,  $\lambda$  = 260 nm)



MALDI-TOF MS spectrum of 4780. Mass calc./found 6823/6828

PNA 4780 fr3/3 Mw=6823,21



Analytical **RP-HPLC** trace of **4780**.  $T_R$  = 21.4 min (program: 100\_60\_A, 40 min,  $\lambda$  = 260 nm)

# - Ghx<sub>16</sub> (4912)



MALDI-TOF MS spectrum of 4912. Mass calc./found 8757/8760



Analytical **RP-HPLC** trace of **4912**.  $T_R$  = 20.9 min (program: 100\_60\_A, 40 min,  $\lambda$  = 260 nm)

# - Oc<sub>1</sub> (4934)

PNA4934 fr=6/2 Mw=6955





MALDI-TOF MS spectrum of 4934. Mass calc./found 6955/6951



Analytical **RP-HPLC** trace of **4934**.  $T_R$  = 22.8 min (program: 100\_60\_A, 40 min,  $\lambda$  = 260 nm)

# - Dec<sub>1</sub> (4939)

PNA4939 fr=3 Mw=6983

Shimadzu Biotech Axima Assurance 2.8.5.20090305: Mode Linear, Power: 140, Blanked, P.Ext. @ 4000 (bin 85)



MALDI-TOF MS spectrum of 4939. Mass calc./found 6983/6961



Analytical **RP-HPLC** trace of **4939**.  $T_R$  = 24.2 min (program: 100\_60\_A, 40 min,  $\lambda$  = 260 nm)

# - Oc<sub>2</sub> (4925)

PNA4925 fr=2 Mw=7178

Shimadzu Biotech Axima Assurance 2.8.5.20090305: Mode Linear, Power: 140, Blanked, P.Ext. @ 4000 (bin 85)



MALDI-TOF MS spectrum of 4925. Mass calc./found 7178/7161

![](_page_17_Figure_5.jpeg)

Analytical **RP-HPLC** trace of **4925**.  $T_R$  = 26.00 min (program: 100\_60\_A, 40 min,  $\lambda$  = 260 nm)

#### - Dec<sub>2</sub> (4926)

![](_page_18_Figure_1.jpeg)

![](_page_18_Figure_2.jpeg)

MALDI-TOF MS spectrum of 4926. Mass calc./found 7220/7212

![](_page_18_Figure_4.jpeg)

Analytical **RP-HPLC** trace of **4926**.  $T_R$  = 29.4 min (program: 100\_60\_A, 40 min,  $\lambda$  = 260 nm)

# - Geg<sub>8</sub> (4936)

![](_page_19_Figure_1.jpeg)

MALDI-TOF MS spectrum of 4936. Mass calc./found 6968/6974

![](_page_19_Figure_3.jpeg)

Analytical RP-HPLC trace of 4936. T<sub>R</sub> = 19.5 min (Program 100\_60\_A, 40 min,  $\lambda$  = 260 nm)

# - Geg<sub>8</sub>-Phe<sub>8</sub> (4963)

![](_page_20_Figure_1.jpeg)

MALDI-TOF MS spectrum of 4963. Mass calc./found 8148/8155

![](_page_20_Figure_3.jpeg)

Analytical **RP-HPLC** trace of **4963**. T<sub>R</sub> = 27.1 min (Program 100\_60\_A, 40 min,  $\lambda$  = 260 nm)

# - Phen<sub>8</sub> (4965)

![](_page_21_Figure_1.jpeg)

Shimadzu Biotech Axima Assurance 2.8.5.20090305: Mode Linear, Power: 120, Blanked, P.Ext. @ 4000 (bin 85) %Int. 1.3 mV[sum= 128 mV] Profiles 1-100 Smooth Av 5 -Baseline 150

![](_page_21_Figure_3.jpeg)

MALDI-TOF MS spectrum of 4965. Mass calc./found 7889/7893

![](_page_21_Figure_5.jpeg)

Analytical **RP-HPLC** trace of **4965**. T<sub>R</sub> = 31.0 min (Program 100\_60\_A, 40 min,  $\lambda$  = 260 nm)

#### - Ghx<sub>8</sub>-MM<sub>1</sub> (5051)

![](_page_22_Figure_1.jpeg)

Shimadzu Biotech Axima Assurance 2.8.5.20090305: Mode Linear, Power: 140, Blanked, P.Ext. @ 4000 (bin 85) %Int. 15 mV[sum= 1535 mV] Profiles 1-100 Smooth Av 5 -Baseline 150

![](_page_22_Figure_3.jpeg)

MALDI-TOF MS spectrum of 5051. Mass calc./found 6711/6725

![](_page_22_Figure_5.jpeg)

Analytical **RP-HPLC** trace of **5051** T<sub>R</sub> = 19.8 min (Program 100\_60\_A, 40 min,  $\lambda$  = 260 nm)

# - Ghx<sub>12</sub> (5054)

![](_page_23_Figure_1.jpeg)

MALDI-TOF MS spectrum of 5054. Mass calc./found 7847/7845

![](_page_23_Figure_3.jpeg)

Analytical **RP-HPLC** trace of **5054**. T<sub>R</sub> = 20.3 min (program: 100\_60\_A, 40 min,  $\lambda$  = 260 nm)

#### - Phe<sub>8</sub>-MM<sub>1</sub> (5055)

![](_page_24_Figure_1.jpeg)

MALDI-TOF MS spectrum of 5055. Mass calc./found 7889/7891

![](_page_24_Figure_3.jpeg)

Analytical **RP-HPLC** trace of **5055** T<sub>R</sub> = 28.9 min (Program 100\_60\_A, 40 min,  $\lambda$  = 260 nm)

#### - Ghx<sub>9</sub> (5066)

![](_page_25_Figure_1.jpeg)

MALDI-TOF MS spectrum of 5066. Mass calc./found 7080/7082

![](_page_25_Figure_3.jpeg)

Analytical **RP-HPLC** trace of **5066**. T<sub>R</sub> = 20.0 min (program: 100\_60\_A, 40 min,  $\lambda$  = 260 nm)

#### - Ghx<sub>8</sub>- eg<sub>8</sub> (5124)

PNA5124 fr3 Mw=7876

![](_page_26_Figure_2.jpeg)

![](_page_26_Figure_3.jpeg)

MALDI-TOF MS spectrum of 5124. Mass calc./found 7876/7889

![](_page_26_Figure_5.jpeg)

Analytical **RP-HPLC** trace of **5124**.  $T_R$  = 21.0 min (Program 100\_60\_A, 40 min,  $\lambda$  = 260 nm)

# - Quin<sub>8</sub> (5171)

![](_page_27_Figure_1.jpeg)

MALDI-TOF MS spectrum of 5171. Mass calc./found 8756/8761

![](_page_27_Figure_3.jpeg)

Analytical **RP-HPLC** trace of **5171**. T<sub>R</sub> = 25.9 min (program: 100\_60\_A, 40 min,  $\lambda$  = 260 nm)

# - Naph<sub>8</sub> (5172)

![](_page_28_Figure_1.jpeg)

MALDI-TOF MS spectrum of 5172. Mass calc./found 8748/8751

![](_page_28_Figure_3.jpeg)

Analytical **RP-HPLC** trace of **5172**.  $T_R$  = 31.0 min (Program 100\_60\_A, 40 min  $\lambda$  = 260 nm)

# - Phen A.<sub>8</sub> (5173)

![](_page_29_Figure_1.jpeg)

MALDI-TOF MS spectrum of 5173. Mass calc./found 8460/8504

![](_page_29_Figure_3.jpeg)

Analytical **RP-HPLC** trace of **5173**. T<sub>R</sub> = 26.9 min (Program 100\_60\_A, 40 min,  $\lambda$  = 260 nm)

#### Ghx<sub>8</sub>-AF568 (5267) -

PNA5267 fr5 Mv=7694 Shimadzu Biotech Axima Assurance 2.8.5.20090305: Mode Linear, Power: 140, Blanked, P.Ext. @ 4000 (bin 85)

![](_page_30_Figure_3.jpeg)

1.1 mV[sum= 113 mV] Profiles 1-100 Smooth Av 5 -Baseline 150 %Int.

![](_page_30_Figure_5.jpeg)

![](_page_30_Figure_6.jpeg)

Analytical **RP-HPLC** trace of **5267** T<sub>R</sub> = 17.4 min (Program 100\_0\_A\_400, 40 min,  $\lambda$  = 260 nm)

#### Ghx<sub>8</sub>-MM<sub>2</sub> (5501) -

PNA5501 fr6 Mw=6710

Shimadzu Biotech Axima Assurance 2.8.5.20090305: Mode Linear, Power: 140, Blanked, P.Ext. @ 4000 (bin 85) %Int. 4.8 mV[sum= 478 mV] Profiles 1-100 Smooth Av 5 -Baseline 150

![](_page_31_Figure_3.jpeg)

MALDI-TOF MS spectrum of 5501. Mass calc./found 6710/6715

![](_page_31_Figure_5.jpeg)

Analytical **RP-HPLC** trace of **5501** T<sub>R</sub> = 19.5 min (Program 100\_60\_A, 40 min  $\lambda$  = 260 nm)

#### - Phe<sub>8</sub>-MM<sub>2</sub> (5502)

![](_page_32_Figure_1.jpeg)

MALDI-TOF MS spectrum of 5502. Mass calc./found 7889/7888

![](_page_32_Figure_3.jpeg)

Analytical **RP-HPLC** trace of **5502** T<sub>R</sub> = 28.0 min (Program 100\_60\_A, 40 min,  $\lambda$  = 260 nm)

# - Ghx<sub>16</sub>-MM<sub>1</sub> (5534)

![](_page_33_Figure_1.jpeg)

MALDI-TOF MS spectrum of 5534. Mass calc./found 8757/8776

![](_page_33_Figure_3.jpeg)

Analytical **RP-HPLC** trace of **5534** T<sub>R</sub> = 20.0 min (Program 100\_60\_A, 40 min,  $\lambda$  = 260 nm)

#### - Goc<sub>8</sub>-MM<sub>1</sub> (5538)

PNA5538 fr7 Mw=6934

![](_page_34_Figure_2.jpeg)

![](_page_34_Figure_3.jpeg)

MALDI-TOF MS spectrum of 5538. Mass calc./found 6934/6933

![](_page_34_Figure_5.jpeg)

Analytical **RP-HPLC** trace of **5538** T<sub>R</sub> = 29.7 min (Program 100\_60\_A, 40 min,  $\lambda$  = 260 nm)

#### - Dec<sub>2</sub>-MM<sub>1</sub> (5539)

![](_page_35_Figure_1.jpeg)

![](_page_35_Figure_2.jpeg)

%Int. 12 mV[sum= 1229 mV] Profiles 1-100 Smooth Av 5 -Baseline 150

![](_page_35_Figure_4.jpeg)

![](_page_35_Figure_5.jpeg)

Analytical **RP-HPLC** trace of **5539** T<sub>R</sub> = 31.6 min (Program 100\_60\_A, 40 min,  $\lambda$  = 260 nm)