

Supplementary Material

Table S1. Chemical shifts and coupling constants of dinucleotides and mononucleotides used as references

| | ATP | Ap ₅ A | Ap ₄ A | Ap ₃ A | Ap ₂ A |
|--------------------|--------|-------------------|-------------------|-------------------|-------------------|
| H2 | 8.24 | 8.12 | 8.10 | 8.06 | 8.00 |
| H8 | 8.51 | 8.40 | 8.34 | 8.25 | 8.14 |
| H1' | 6.12 | 6.02 | 5.99 | 5.97 | 5.92 |
| H2' | 4.78 | ~4.73 | 4.69 | 4.56 | 4.53 |
| H3' | 4.58 | 4.56 | 4.53 | 4.45 | 4.41 |
| H4' | 4.37 | 4.35 | 4.33 | 4.31 | 4.33 |
| H5' | 4.26 | 4.26 | 4.27 | 4.29 | 4.31 |
| H5'' | 4.18 | 4.19 | 4.21 | 4.25 | 4.21 |
| P α | -10.45 | -10.44 | -10.42 | -10.75 | -10.39 |
| P β | -21.86 | -22.19 | -22.20 | -22.20 | / |
| P γ | -8.10 | -22.13 | / | / | / |
| C2 | 153.60 | 153.38 | 153.42 | 153.35 | 153.16 |
| C8 | 140.66 | 140.48 | 140.34 | 139.84 | 139.81 |
| C6 | 156.41 | 155.98 | 155.93 | 155.71 | 155.59 |
| C4 | 149.96 | 149.58 | 149.47 | 148.96 | 148.87 |
| C5 | 119.38 | 118.93 | 118.87 | 118.65 | 118.52 |
| C1' | 87.31 | 87.22 | 87.45 | 87.95 | 87.78 |
| C2' | 74.96 | 75.00 | 75.20 | 75.59 | 75.48 |
| C3' | 71.07 | 71.13 | 71.04 | 70.39 | 70.62 |
| C4' | 84.82 | 84.74 | 84.55 | 83.82 | 84.07 |
| C5' | 65.88 | 65.99 | 65.91 | 65.42 | 65.85 |
| J _{1'2'} | 6.1 | 6.0 | 5.8 | 4.7 | 5.2 |
| J _{2'3'} | 5.2 | 5.0 | 5.1 | 4.8 | 5.0 |
| J _{3'4'} | 3.5 | 3.2 | 3.6 | 4.8 | 4.4±0.5 |
| J _{4'5'} | 2.8 | 2.8 | 2.8 | 2.5±0.4 | 2.1±1 |
| J _{4'5''} | 3.0 | 3.3 | 3.3 | 3.1 | 2.7±1 |
| J _{4'P} | 2.3 | 1.9 | 2.1 | 2.6±0.4 | n.d. |
| J _{5'P} | 6.2 | 6.2 | 6.0 | 6.2±0.4 | 4.2±2 |
| J _{5''P} | 4.5 | 4.2 | 4.6 | 4.3 | 5.4±2 |
| J _{5'5''} | 11.7 | 11.6 | 11.7 | 11.6 | 11.6 |
| J _{C4'P} | 9.2 | 9.0 | 9.2 | 8.5 | 9.0 |
| J _{C5'P} | 5.1 | 5.5 | 4.8 | 4.0 | 4.0 |
| J $\beta\beta$ | / | / | 16.5 | / | / |
| J $\alpha\beta$ | 19.5 | 16.0 | 18.1 | 19.3 | / |
| J $\alpha\beta'$ | / | / | 0.3 | / | / |
| J _{C6H2} | 11.0 | 11.0 | 11.0 | 11.0 | 11.0 |
| J _{C4H2} | 12.0 | 12.3 | 12.3 | 12.0 | 12.0 |
| J _{C4H8} | 5.0 | 5.3 | 4.8 | 5.0 | 5.2 |
| J _{C4H1'} | 1.0 | 1.8 | ≤1 | n.d. | 1.8 |
| J _{C5H8} | 11.0 | 11.0 | 11.0 | 11.0 | 11.5 |
| J _{C2H2} | 202.8 | 202.2 | 203.7 | 202.2 | 202.2 |
| J _{C8H8} | 216.3 | 215.8 | 215.8 | 215.8 | 215.8 |
| J _{C8H1'} | 4.0 | 4.6 | 4.5 | 3.7 | 4.2 |

| | GTP | Gp₅G | Gp₄G | Gp₃G | Gp₂G |
|-----------------------------------|------------|------------------------|------------------------|------------------------|------------------------|
| H8 | 8.10 | 8.04 | 8.02 | 7.96 | 7.90 |
| H1' | 5.90 | 5.84 | 5.82 | 5.79 | 5.75 |
| H2' | 4.80 | 4.79 | 4.75 | 4.63 | 4.59 |
| H3' | 4.57 | 4.55 | 4.52 | 4.44 | 4.41 |
| H4' | 4.33 | 4.32 | 4.29 | 4.26 | 4.25 |
| H5' | 4.24 | 4.25 | 4.24 | 4.23 | 4.23 |
| H5'' | 4.18 | 4.20 | 4.20 | 4.23 | 4.15 |
| Pα | -10.40 | -10.58 | -10.62 | -10.83 | -10.44 |
| Pβ | -21.65 | -22.20 | -22.42 | -22.44 | / |
| Pγ | -7.45 | -22.14 | / | / | / |
| C2 | 154.70 | 153.23 | 153.17 | 153.08 | 154.31 |
| C8 | 138.56 | 137.34 | 137.19 | 136.74 | 137.94 |
| C6 | 159.80 | 158.29 | 158.17 | 157.97 | 159.27 |
| C4 | 152.62 | 151.21 | 151.09 | 150.73 | 151.90 |
| C5 | 117.03 | 115.56 | 115.49 | 115.34 | 116.65 |
| C1' | 87.36 | 86.22 | 86.29 | 86.70 | 87.97 |
| C2' | 74.20 | 72.86 | 72.97 | 73.31 | 74.64 |
| C3' | 71.06 | 69.93 | 69.84 | 69.29 | 70.65 |
| C4' | 84.73 | 83.55 | 83.35 | 82.68 | 83.92 |
| C5' | 65.90 | 64.84 | 64.69 | 64.22 | 65.71 |
| J_{1'2'} | 6.4 | 6.5 | 6.2 | 5.4 | 5.2 |
| J_{2'3'} | 5.2 | 5.2 | 5.0 | 4.8 | 5.1 |
| J_{3'4'} | 3.3 | 3.0 | 3.1 | 4.2 | 4.0±0.5 |
| J_{4'5'} | 3.5 | 3.5 | 3.5 | n.d. | 2.7±1 |
| J_{4'5''} | 3.5 | 3.5 | 3.8 | n.d. | 2.7±1 |
| J_{4'P} | 1.6 | n.d. | n.d. | n.d. | n.d. |
| J_{5'P} | 6.5 | 6.2 | 6.0 | n.d. | 5.4±2 |
| J_{5''P} | 4.5 | 5.0 | 5.0 | n.d. | 5.4±2 |
| J_{5'5''} | 11.5 | 11.5 | 11.5 | n.d. | 10.8 |
| J_{C4'P} | 9.1 | 8.9 | 9.4 | 9.0 | 9.5 |
| J_{C5'P} | 5.5 | 5.4 | 5.3 | 5.3 | 5.5 |
| J$\beta\beta$ | / | n.d. | 16.4 | / | / |
| J$\alpha\beta$ | 19.5 | 15.0 | 18.3 | 19.3 | / |
| J$\alpha\beta'$ | / | n.d. | 0.1 | / | / |
| J_{C4H8} | 5.5 | 5.2 | 4.8 | 5.1 | 5.1 |
| J_{C4H1'} | 2.5 | 2.8 | 2.4 | 2.4 | 2.3 |
| J_{C5H8} | 11.5 | 11.1 | 11.1 | 11.2 | 11.0 |
| J_{C8H8} | 215.8 | 216.1 | 216.1 | 217 | 215.8 |
| J_{C8H1'} | 4.7 | 4.3 | 4.1 | 4.1 | 4.1 |

| | <u>Ap₂U</u> | <u>Ap₃U</u> | <u>Ap₄U</u> | <u>Gp₂C'</u> | <u>Gp₂C</u> | <u>Gp₃C</u> |
|-----------------------------------|------------------------|------------------------|------------------------|-------------------------|------------------------|------------------------|
| H2 | 8.18 | 8.20 | 8.21 | / | / | / |
| H8 | 8.41 | 8.48 | 8.50 | 7.95 | 8.01 | 8.07 |
| H1' | 6.05 | 6.08 | 6.09 | 5.97 | 5.84 | 5.86 |
| H2' | 4.72 | 4.71 | ~4.76 | 4.71 | ~4.78 | ~4.80 |
| H3' | 4.48 | 4.51 | 4.56 | 4.45 | 4.46 | 4.49 |
| H4' | 4.35 | 4.35 | 4.36 | 4.29 | 4.31 | 4.31 |
| H5' | 4.23 | 4.25 | 4.26 | 4.28 | 4.24 | 4.20 |
| H5'' | 4.18 | 4.19 | 4.19 | 4.18 | 4.15 | 4.20 |
| Pα | -10.55or 49 | -10.77 | -10.51or 59 | -10.48 | -10.61 | -10.90 or 78 |
| Pβ | / | -22.27 | -22.27 | / | / | -22.42 |
| Pγ | / | / | / | / | / | / |
| C2 | 153.55 | 153.68 | 153.67 | 154.37 | 153.24 | 154.52 |
| C8 | 140.40 | 140.42 | 140.60 | 138.14 | 137.00 | 138.11 |
| C6 | 156.21 | 156.28 | 156.36 | 159.29 | 158.24 | 159.42 |
| C4 | 149.78 | 149.85 | 149.99 | 152.22 | 151.13 | 152.37 |
| C5 | 119.18 | 119.22 | 119.32 | 116.81 | 115.68 | 116.78 |
| C1' | 87.44 | 87.36 | 87.21 | 87.75 | 86.40 | 87.22 |
| C2' | 74.92 | 75.42 | 75.10 | 74.22 | 72.87 | 74.50 |
| C3' | 71.07 | 71.27 | 71.28 | 70.97 | 69.88 | 71.32 |
| C4' | 84.52 | 84.64 | 84.93 | 84.21 | 83.21 | 84.58 |
| C5' | 65.99 | 65.98 | 66.05 | 66.05 | 64.83 | 65.95 |
| J_{1'2'} | 6.0 | 6.0 | 6.2 | 5.7 | 6.0 | 6.5 |
| J_{2'3'} | 5.3 | 5.0 | 5.2 | 5.3 | 5.3±0.5 | 5.0 |
| J_{3'4'} | 3.5 | 3.4 | 3.1 | 3.7±0.5 | 3.3±0.5 | 3.0 |
| J_{4'5'} | n.d. | 2.6±0.5 | 2.8±0.5 | n.d. | n.d. | n.d. |
| J_{4'5''} | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| J_{4'P} | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| J_{5'P} | n.d. | 6.4±0.5 | 6.3±0.5 | n.d. | n.d. | n.d. |
| J_{5''P} | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| J_{5'5''} | n.d. | 11.3±0.5 | 11.4±0.5 | n.d. | n.d. | n.d. |
| J_{C4'P} | 8.2 | 9.1 | 10.0±1 | 8.7 | 9.0 | 8.7 |
| J_{C5'P} | 4.6 | 5.0 | 5.0±1 | 5.0 | 4.0 | 4.5 |
| J$\beta\beta$ | / | / | ~18 | / | / | / |
| J$\alpha\beta$ | 20.5 | 19.0 | ~18 | / | / | 19.7/18.7 |
| J$\alpha\beta'$ | / | / | / | / | / | / |
| J_{C6H2} | 11.0 | 11.0 | 11.0 | / | / | / |
| J_{C4H2} | 12.2 | 11.6 | 12.0±0.4 | / | / | / |
| J_{C4H8} | 5.3 | 4.8 | 5.2±0.7 | n.d. | 5.0 | 5.0 |
| J_{C4H1'} | 2.3 | 2.3 | 2.3±1 | n.d. | 3.0 | 2.5 |
| J_{C5H8} | 11.5 | 11.5 | 11.5 | 11.0 | 11.0 | 11.0 |
| J_{C2H2} | 202.2 | 202.2 | 202.7 | / | / | / |
| J_{C8H8} | 215.8 | 215.8 | 215.8 | 216.8 | 215.7 | 216.1 |
| J_{C8H1'} | 4.3 | 4.3 | 4.1 | 3.4 | 4.0 | 4.1 |

| | UTP | Up ₂ U | Up ₄ U | Up ₅ U | Ap ₂ U | Ap ₃ U | Ap ₄ U |
|--------------------|--------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| H5 | 5.95 | 5.91 | 5.96 | 5.95 | 5.64 | 5.70 | 5.81 |
| H6 | 7.96 | 7.89 | 7.94 | 7.94 | 7.62 | 7.75 | 7.82 |
| H1' | 5.97 | 5.92 | 5.96 | 5.98 | 5.78 | 5.82 | 5.89 |
| H2' | 4.38 | 4.32 | 4.36 | 4.37 | 4.18 | 4.25 | 4.31 |
| H3' | 4.42 | 4.32 | 4.40 | 4.42 | 4.23 | 4.28 | 4.36 |
| H4' | 4.26 | 4.23 | 4.25 | 4.27 | 4.18 | 4.18 | 4.20 |
| H5' | 4.24 | 4.23 | 4.22 | 4.24 | 4.23 | 4.21 | 4.20 |
| H5'' | 4.21 | 4.15 | 4.22 | 4.21 | 4.12 | 4.21 | 4.20 |
| P α | -10.51 | -10.59 | -10.66 | -10.57 | -10.55or 49 | -10.72 | -10.51or 59 |
| P β | -21.72 | / | -22.40 | -22.09 | / | -22.27 | -22.27 |
| P γ | -7.41 | / | / | -22.09 | / | / | / |
| C2 | 152.62 | 152.44 | 152.61 | 152.68 | 152.06 | 152.17 | 152.51 |
| C6 | 142.46 | 142.32 | 142.41 | 142.44 | 141.62 | 141.77 | 142.12 |
| C4 | 167.00 | 166.84 | 166.93 | 166.98 | 166.36 | 166.42 | 166.76 |
| C5 | 103.40 | 103.36 | 103.49 | 103.51 | 102.91 | 102.97 | 103.27 |
| C1' | 88.85 | 89.04 | 88.70 | 88.69 | 88.91 | 89.03 | 88.77 |
| C2' | 74.45 | 74.49 | 74.47 | 74.41 | 74.68 | 74.72 | 74.51 |
| C3' | 70.28 | 70.32 | 70.49 | 70.53 | 70.19 | 69.97 | 70.35 |
| C4' | 84.12 | 83.86 | 84.28 | 84.38 | 83.74 | 83.60 | 84.07 |
| C5' | 65.56 | 65.60 | 65.79 | 65.89 | 65.56 | 65.34 | 65.71 |
| J _{1'2'} | 5.3 | 4.5±0.5 | 5.4 | 5.6 | 5.0 | 4.5 | 5.1 |
| J _{2'3'} | 5.2 | 5.0±1 | 5.2 | 5.2 | n.d. | 4.8±0.5 | 5.1±0.5 |
| J _{3'4'} | 4.3 | 4.5±1 | 3.8 | 3.8 | n.d. | 4.8±0.5 | n.d. |
| J _{4'5'} | n.d. | 2.5±1 | n.d. | n.d. | n.d. | n.d. | n.d. |
| J _{4'5''} | n.d. | 2.5±1 | n.d. | 2.8±1 | 2.5±1 | n.d. | n.d. |
| J _{4'P} | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| J _{5'P} | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| J _{5''P} | n.d. | 5.0±2 | n.d. | 4.5±1 | 5.0±2 | n.d. | n.d. |
| J _{5'5''} | n.d. | 12.0 | n.d. | 11.1±1 | 11.7 | n.d. | n.d. |
| J _{C4'P} | 9.5 | 9.4 | 9.0 | 9.2 | 7.6 | 9.0 | 10.0±1 |
| J _{C5'P} | 5.0 | 5.2 | 5.0 | 5.2 | 4.3 | 5.0 | 4.2±1 |
| J $\beta\beta$ | / | / | 16.8 | / | / | / | ~18 |
| J $\alpha\beta$ | 20.0 | / | 18.4 | 16.3±1 | 20.5 | 19.0 | ~18 |
| J $\alpha\beta'$ | / | / | 0.15 | / | / | / | / |
| J _{C2H6} | 8.0 | 8.1 | 8.0 | 8.3 | 8.0 | 8.3 | 8.1 |
| J _{C2H1'} | 2.0 | 2.0 | 2.0 | 2.1 | 2.0 | 1.8 | 2.0 |
| J _{C4H5} | 1.6 | 1.6 | 1.4 | 1.7 | 1.5 | 1.8 | 1.3 |
| J _{C4H6} | 11.0 | 10.8 | 11.0 | 10.8 | 10.5 | 11.0 | 10.5 |
| J _{C5H5} | 179.0 | 178.8 | 178.8 | 178.9 | 178.1 | 178.1 | 179.0 |
| J _{C5H6} | 2.0 | 2.3 | 2.0 | 2.1 | 2.3 | 2.1 | 1.8 |
| J _{C6H1'} | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.1 | 4.1 |
| J _{C6H6} | 185.0 | 184.7 | 184.9 | 185.6 | 185.6 | 185.6 | 184.9 |
| J _{C6H5} | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.1 | 4.1 |
| J _{H6-H5} | 8.2 | 8.0 | 8.1 | 8.0 | 8.0 | 8.2 | 8.2 |

| | Gp₂C' | Gp₂C | Gp₃C | CMP |
|-----------------------------------|-------------------------|------------------------|------------------------|------------|
| H5 | 6.92 | 5.88 | 5.91 | 6.11 |
| H6 | 8.08 | 7.72 | 7.80 | 8.07 |
| H1' | 5.82 | 5.82 | 5.87 | 5.98 |
| H2' | 4.21 | 4.16 | 4.21 | 4.31 |
| H3' | 4.24 | 4.22 | 4.25 | 4.31 |
| H4' | 4.24 | 4.18 | 4.18 | 4.22 |
| H5' | 4.34 | 4.23 | 4.24 | 4.03 |
| H5'' | 4.17 | 4.12 | 4.24 | 3.96 |
| Pα | -10.48 | -10.61 | -10.90 or 78 | 4.11 |
| Pβ | / | / | -22.42 | / |
| Pγ | / | / | / | / |
| C2 | 157.24 | 156.81 | 158.00 | 158.55 |
| C6 | 145.07 | 140.27 | 141.51 | 142.57 |
| C4 | 163.53 | 165.27 | 166.46 | 166.92 |
| C5 | 97.37 | 95.55 | 96.79 | 97.33 |
| C1' | 91.15 | 88.81 | 89.91 | 89.79 |
| C2' | 75.50 | 73.99 | 75.08 | 75.08 |
| C3' | 68.98 | 68.31 | 69.33 | 70.33 |
| C4' | 83.01 | 81.74 | 82.82 | 84.21 |
| C5' | 64.76 | 63.84 | 64.87 | 63.64 |
| J_{1'2'} | 2.7 | 4.0 | 3.5 | 3.6±0.5 |
| J_{2'3'} | n.d. | n.d. | n.d. | n.d. |
| J_{3'4'} | n.d. | n.d. | n.d. | n.d. |
| J_{4'5'} | n.d. | n.d. | n.d. | 2.7 |
| J_{4'5''} | n.d. | n.d. | n.d. | 2.9 |
| J_{4'P} | n.d. | n.d. | n.d. | n.d. |
| J_{5'P} | n.d. | n.d. | n.d. | 3.7 |
| J_{5''P} | n.d. | n.d. | n.d. | 5.1 |
| J_{5'5''} | n.d. | n.d. | n.d. | 11.9 |
| J_{C4'P} | 8.7 | 9.0 | 9.3 | 8.5 |
| J_{C5'P} | 3.7 | 4.0 | 4.5 | 4.0 |
| J$\beta\beta$ | / | / | / | / |
| J$\alpha\beta$ | / | / | 19.7/18.7 | / |
| J$\alpha\beta'$ | / | / | / | / |
| J_{C2H6} | n.d. | 6.0 | 6.3 | 6.5 |
| J_{C2H1'} | n.d. | n.d. | 1.3 | 1.8 |
| J_{C4H5} | n.d. | 1.6 | 1.8 | 2.0 |
| J_{C4H6} | 10.0 | 9.0 | 9.0 | 9.0 |
| J_{C5H5} | 182.0 | 177.7 | 173.9 | 175.6 |
| J_{C5H6} | 3.0 | n.d. | 2.2 | 3.0 |
| J_{C6H1'} | 2.5 | 3.6/3.8 | 3.5 | 4.0 |
| J_{C6H6} | 185.5 | 184.5 | 183.8 | 184.9 |
| J_{C6H5} | 5.5±1 | 3.6/3.8 | 3.5 | 4.0 |
| J_{H6-H5} | 7.6 | 7.6 | 7.8 | 7.6 |

n.m. not measured; n.d. not determined

If not mentioned otherwise, J_{HH}, J_{HP}±0.2 Hz, J_{CH}, J_{CP}, J_{PP}±0.5 Hz.

~ H2' overlap with HOD signal. The chemical shift is estimated.

Table S2. Differences in chemical shifts (in ppm) between dinucleoside polyphosphates and the corresponding mononucleotide. ($\Delta\delta = \delta_{\text{mononucleotide}} - \delta_{\text{dinucleotide}}$)

| No. | Dinucleotide | $\Delta\delta_{\text{H2' (pu)}}$ | $\Delta\delta_{\text{H3' (pu)}}$ | $\Delta\delta_{\text{H2' (py)}}$ | $\Delta\delta_{\text{H3' (py)}}$ |
|-----|-------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| 1 | Ap₂A | 0.25 | 0.17 | / | / |
| 2 | Ap₃A | 0.22 | 0.13 | / | / |
| 3 | Ap₄A | 0.09 | 0.05 | / | / |
| 4 | Ap₅A | ~0.05 | 0.02 | / | / |
| 5 | Ap₂U | 0.06 | 0.10 | 0.20 | 0.19 |
| 6 | Ap₃U | 0.07 | 0.07 | 0.13 | 0.14 |
| 7 | Ap₄U | ~0.02 | 0.02 | 0.07 | 0.06 |
| 8 | Up₂U | / | / | 0.06 | 0.10 |
| 9 | Up₄U | / | / | 0.02 | 0.02 |
| 10 | Up₅U | / | / | 0.01 | 0 |
| 11 | Gp₂G | 0.21 | 0.16 | / | / |
| 12 | Gp₃G | 0.17 | 0.13 | / | / |
| 13 | Gp₄G | 0.05 | 0.05 | / | / |
| 14 | Gp₅G | 0.01 | 0.02 | / | / |
| 15 | Gp₂C | ~0.02 | 0.11 | 0.15 | 0.09 |
| 16 | Gp₃C | ~0 | 0.08 | 0.10 | 0.06 |
| 17 | Gp₂C' | 0.09 | 0.12 | 0.10 | 0.07 |

Table S3. Torsion angles of Ap₂A and Ap₄A (including standard deviation)

| | Ap ₂ A (α - α) ^a | Ap ₂ A (β - β) ^a | Ap ₄ A (α - α) ^a | Ap ₄ A (β - β) ^a |
|------------|--|--|--|--|
| α 1 | 188.3 ± 20.7 | 222.2 ± 75.1 | 234.1 ± 87.3 | 229.8 ± 87.6 |
| α 2 | 188.6 ± 21.7 | 195.1 ± 81.0 | -7.9 ± 62.6 | 274.7 ± 61.0 |
| β 1 | 182.4 ± 27.2 | 182.0 ± 30.6 | 180.7 ± 25.8 | 175.6 ± 36.0 |
| β 2 | 184.9 ± 25.9 | 190.2 ± 38.0 | 181.6 ± 81.1 | 130.1 ± 31.2 |
| γ 1 | 62.6 ± 13.9 | 57.4 ± 18.8 | 66.7 ± 46.4 | 50.4 ± 28.0 |
| γ 2 | 62.7 ± 14.3 | 57.3 ± 22.7 | 70.2 ± 19.9 | 43.7 ± 31.6 |
| χ 1 | 227.1 ± 17.0 | 258.7 ± 39.4 | -74.0 ± 36.3 | 239.0 ± 42.5 |
| χ 2 | 227.1 ± 16.6 | 233.5 ± 56.0 | -2.3 ± 47.5 | 241.2 ± 59.5 |

^a The simulations started with a certain stacking mode (β - β or α - α), but this changed during the simulation (to an extended conformation or another mode of stacking), so the data is not an average for only one stacked conformation, but all the stacked forms and the extended form.

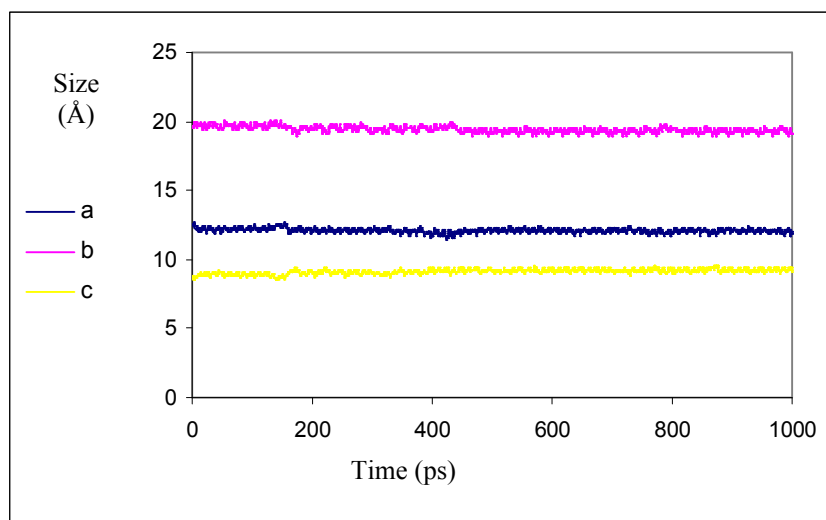


Fig. S1 . The change of the lattice parameters a, b, c of the Ap₄A unit cell during a 1 ns MD simulation.

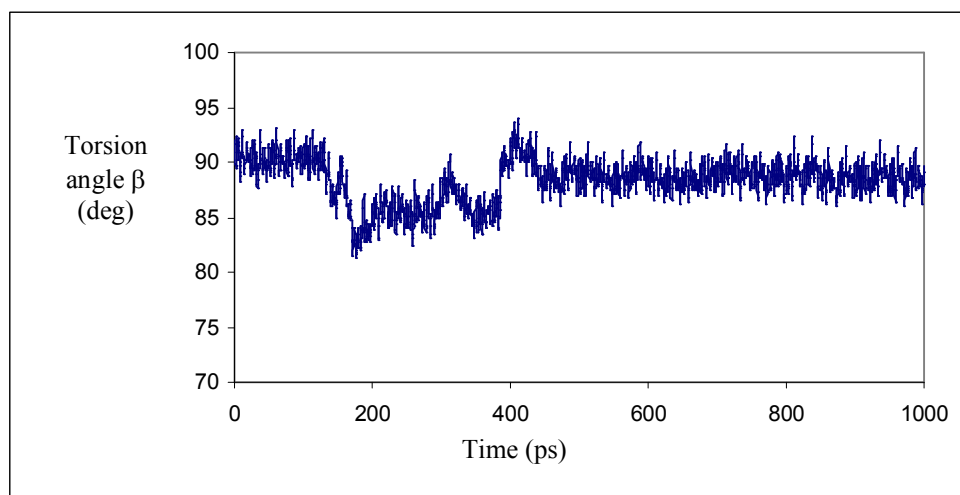


Fig. S2. The change of the angle β of the Ap_4A unit cell during a 1 ns MD simulation.

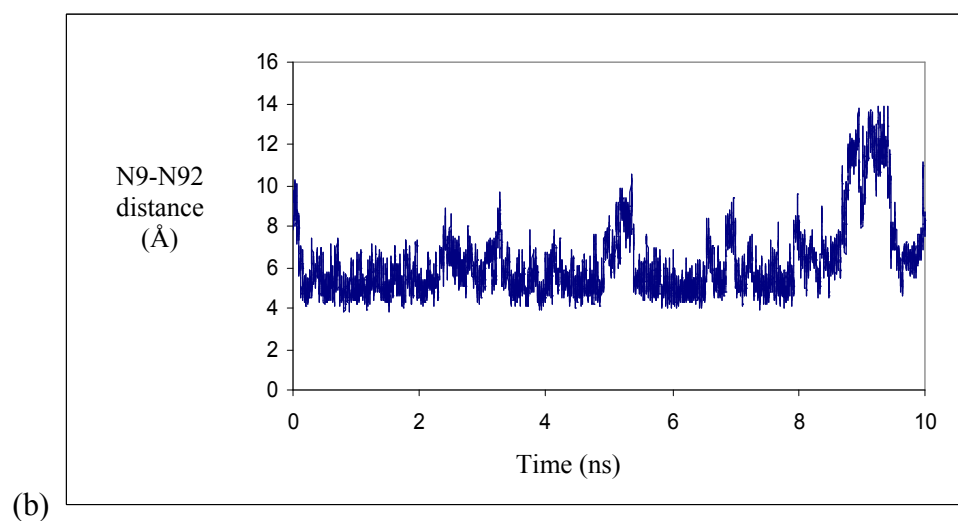
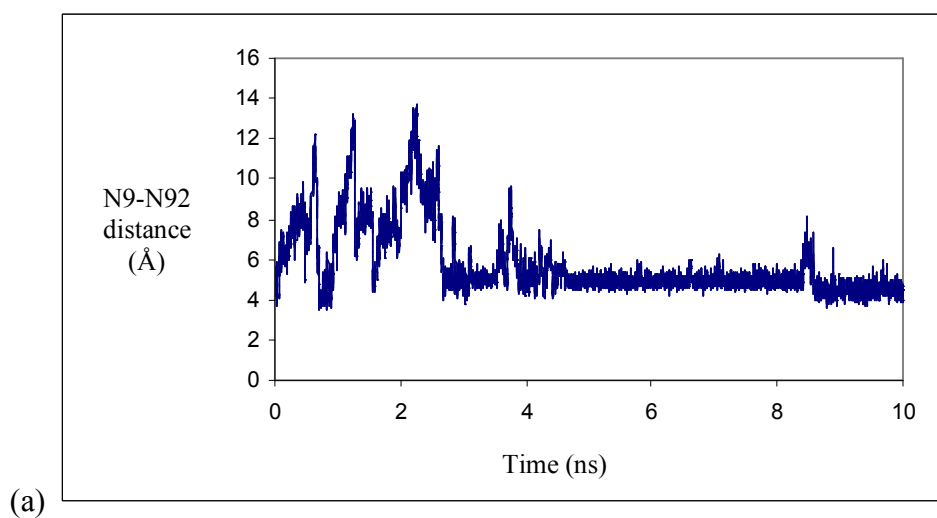


Fig. S3. The inter-base distance (N9-N92) (Fig. 9) as a function of time during 10 ns simulations for (a) Ap₂A (b) Ap₄A

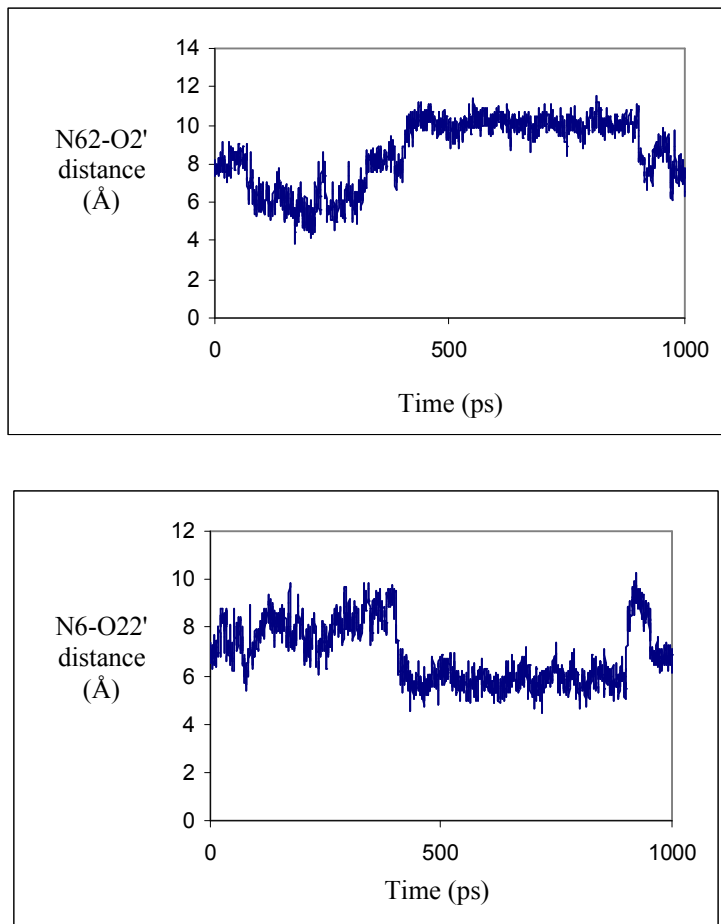


Fig. S4 . Distances N62-O2' and N6-O22' (Fig. 11) for Ap₂A in the β - β stacking form, during 1 ns simulation.

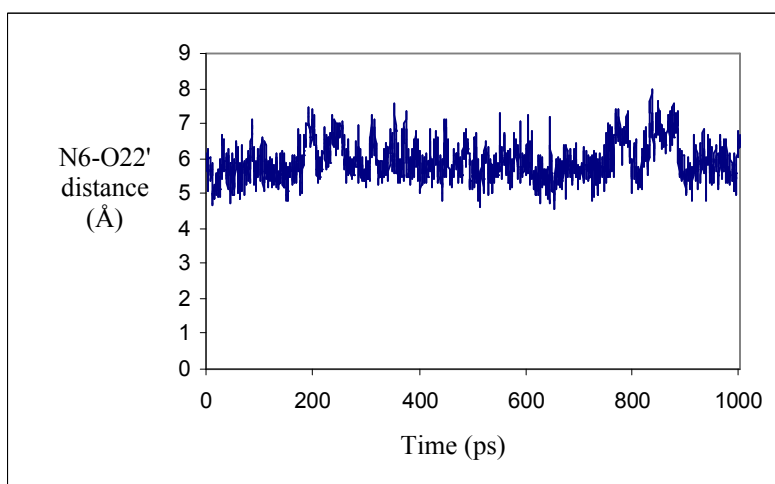
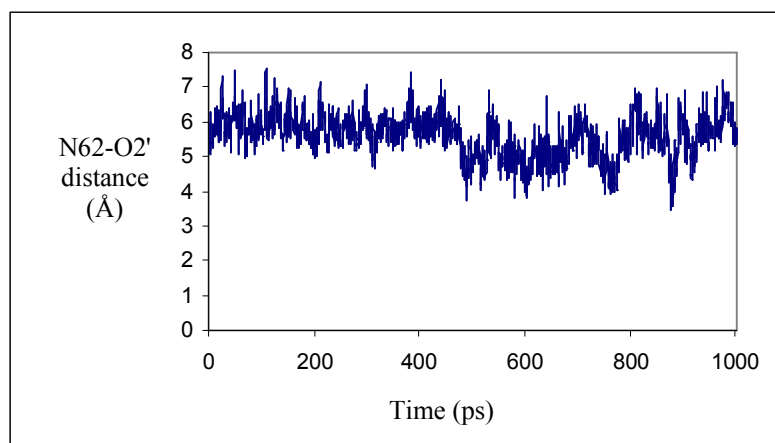
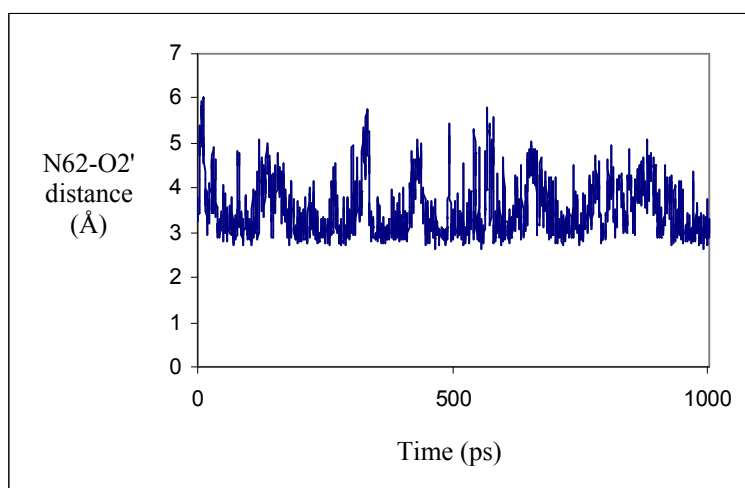


Fig. S5 . Distances N62-O2' and N6-O22' (Fig. 11) for Ap₄A in the β - β stacking form, during 1 ns simulation.



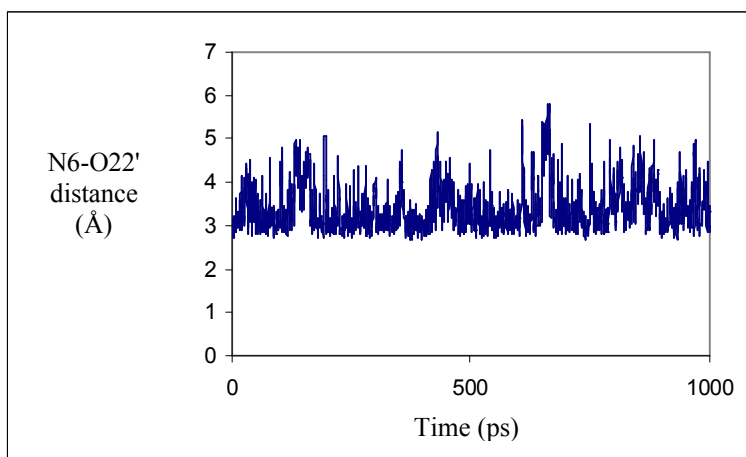


Fig. S6 . Distances N62-O2' and N6-O22' (Fig. 11) for Ap₂A in the α - α stacking form, during 1 ns simulation.

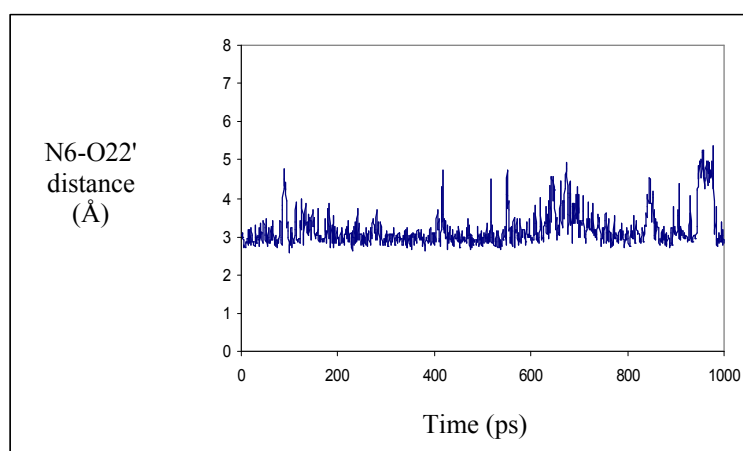
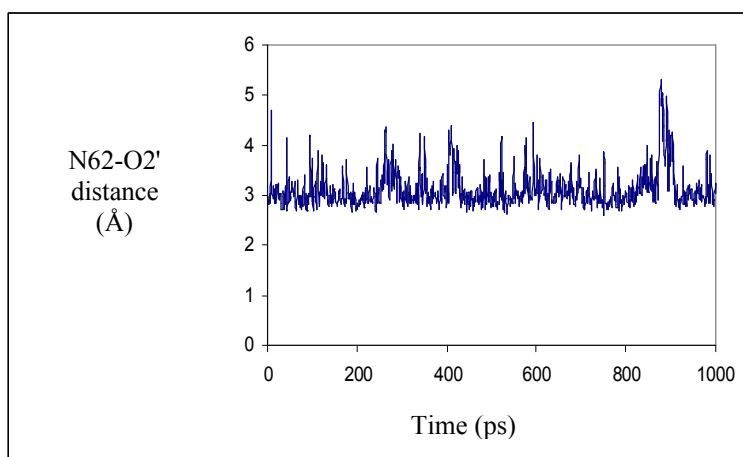


Fig. S7. Distances N62-O2' and N6-O22' (Fig. 11) for Ap₄A in the α - α stacking form, during 1 ns simulation.

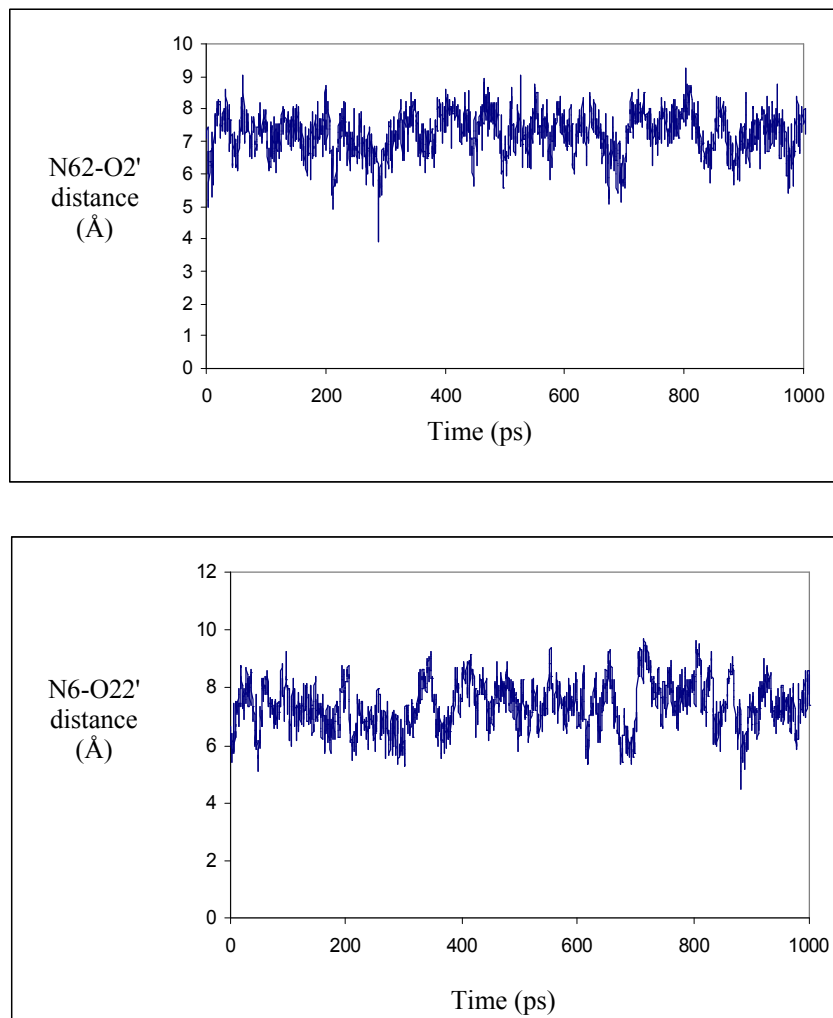


Fig. S8. Distances N62-O2' and N6-O22' (Fig. 11) for Ap₂A in the α - β stacking form, during 1 ns simulation.

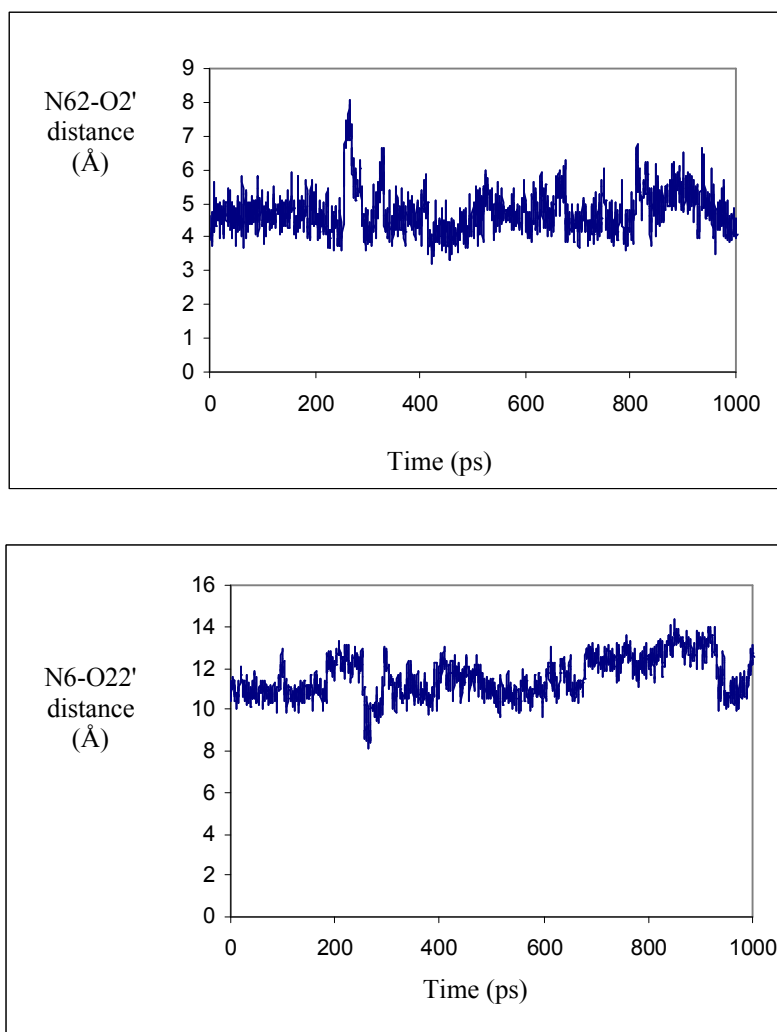


Fig. S9. Distances N62-O2' and N6-O22' (Fig. 11) for Ap₄A in the α - β stacking form, during 1 ns simulation.

Assignment of $A_p_nA_s$ coupling constants as compared to literature

The conformation of $A_p_nA_s$ $n = 2-6$ in solution was investigated by Mayo et al before.¹ We confirmed that there are upfield shifts ($\Delta\delta$) of H8, H2, H1', H2' and H3' chemical shifts compared to the corresponding mononucleotides. These $\Delta\delta$ values become smaller for longer phosphate chains. We also confirmed that $A_{p2}A$ and $A_{p3}A$ have smaller $J_{1'2'}$ and larger $J_{3'4'}$ values as compared to longer $A_p_nA_s$, *i.e.*, $n = 4, 5$. However, we found differences in the measured coupling constants of up to *ca.* 5 Hz as compared to literature values (Table S4). We disagree with the reported assignment of the coupling constants ${}^3J_{4'5'}$, ${}^3J_{4'5''}$ and ${}^3J_{P5'}$, ${}^3J_{P5''}$.¹ The coupling constants ${}^3J_{P5'}$ and ${}^3J_{P5''}$ are actually larger than ${}^3J_{4'5'}$, ${}^3J_{4'5''}$ based on $A_{p4}A$ J-resolved spectrum (Fig. S10) and 1H -coupled ${}^{31}P$ NMR spectrum for $A_{p3}A$ (Fig. S11). This finding is consistent with literature assignment for mononucleotides.²

Table S4. Comparison of the J values (Hz) determined in this study (in bold) with literature data

| | $J_{4'5'}$ | | $J_{4'5''}$ | | $J_{5'P}$ | | $J_{5''P}$ | |
|-----------|------------|------------|-------------|------------|-----------|------------|------------|------------|
| $A_{p2}A$ | 2.5 | 2.1 | 2.5 | 2.7 | 1.3 | 4.2 | 1.9 | 5.4 |
| $A_{p3}A$ | 7.5 | 2.5 | 3.8 | 3.1 | 2.5 | 6.2 | 4.4 | 4.3 |
| $A_{p4}A$ | 6.9 | 2.8 | 5.0 | 3.3 | 3.1 | 6.0 | 3.8 | 4.6 |
| $A_{p5}A$ | 6.3 | 2.8 | 3.8 | 3.3 | 3.1 | 6.2 | 3.8 | 4.2 |

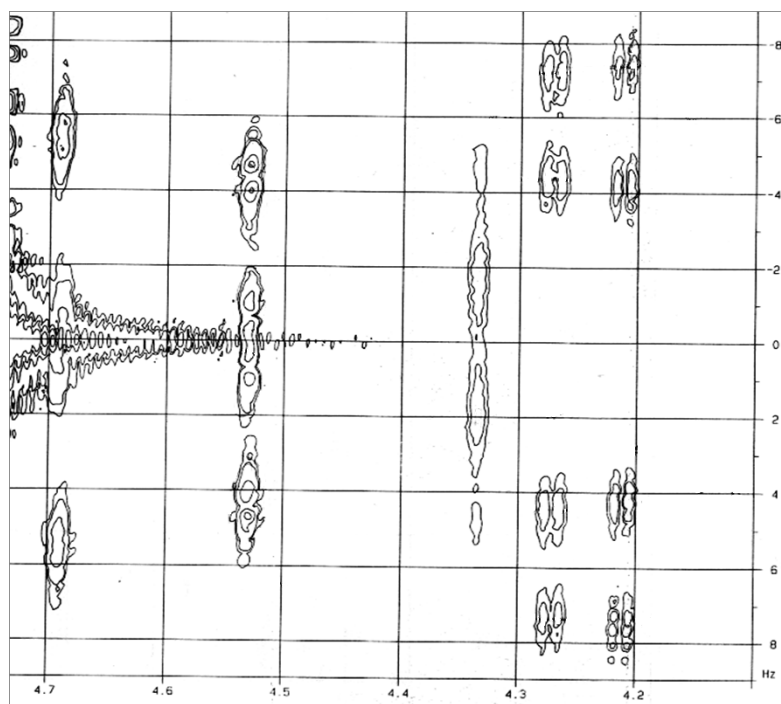


Fig. S10. 600 MHz J-resolved spectrum of Ap₄A. Sample concentration 7.4 mM in D₂O, pD 7.5, 300 K.

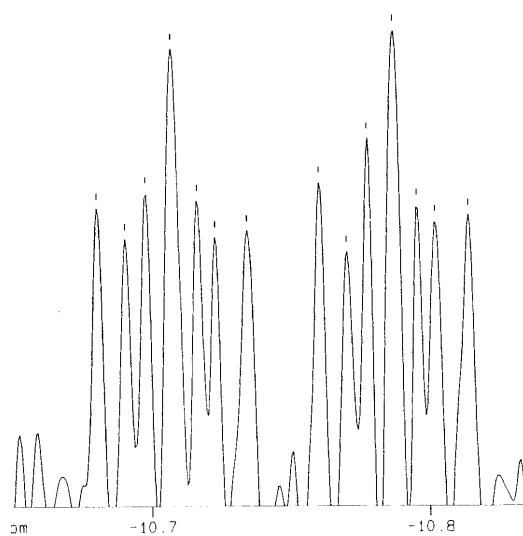


Fig. S11. 243 MHz ^{31}P NMR spectrum without decoupling of Ap_3A . Sample concentration 7.3 mM in D_2O , pD 7.48, 300 K. constants that were obtained: J_{PP} , J_{P5} , $J_{\text{P5}''}$, J_{P4} .

- .1 K. H. Mayo, O. M. Mvele and R. N. Puri, FEBS Lett., 1990, 265, 97-100.
- .2 C. R. Cremo, Methods Enzymol., 2003, 360, 128-177.

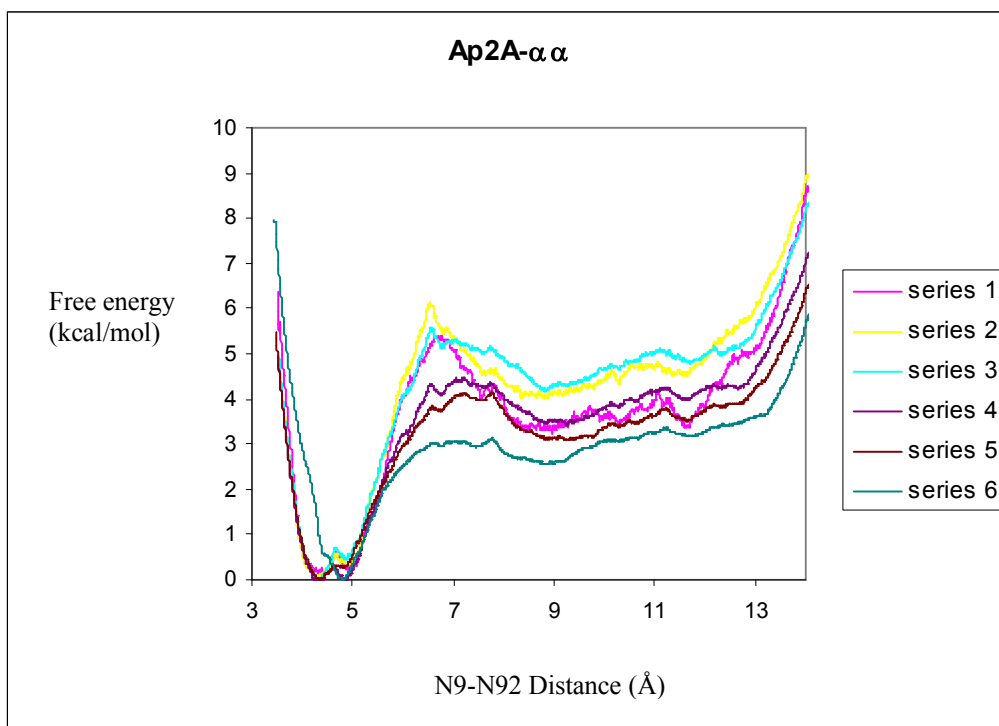


Fig. S12. Progressive PMF profiles of $\text{Ap}_2\text{A-}\alpha\alpha$.

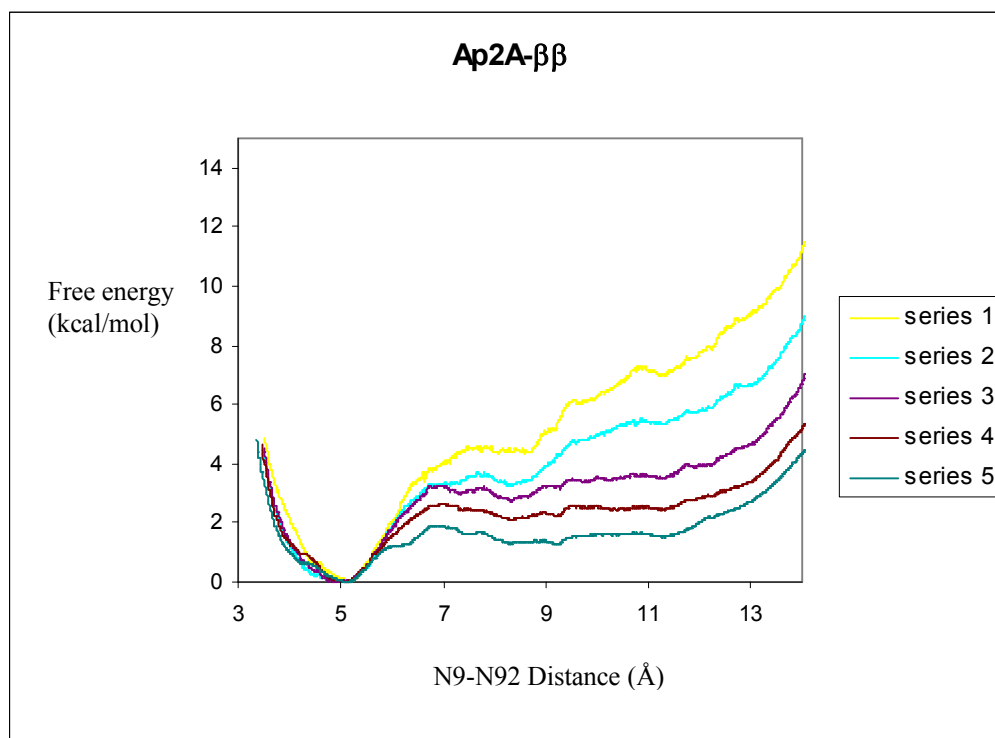


Fig. S13. Progressive PMF profiles of Ap₂A-ββ.

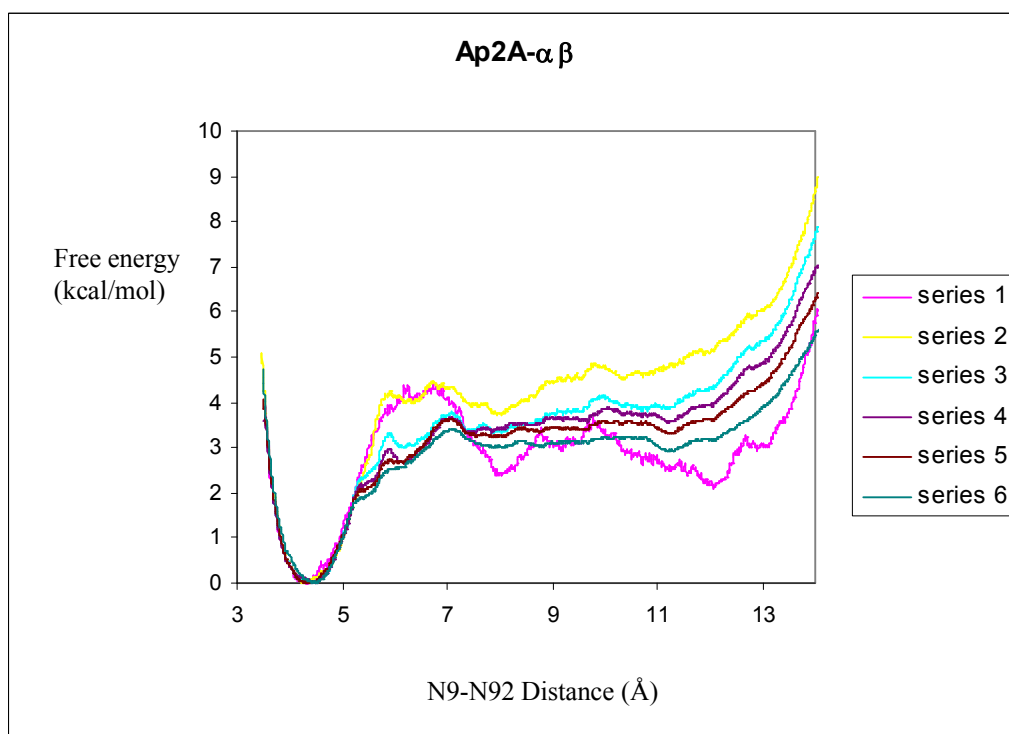


Fig. S14. Progressive PMF profiles of Ap₂A-αβ.

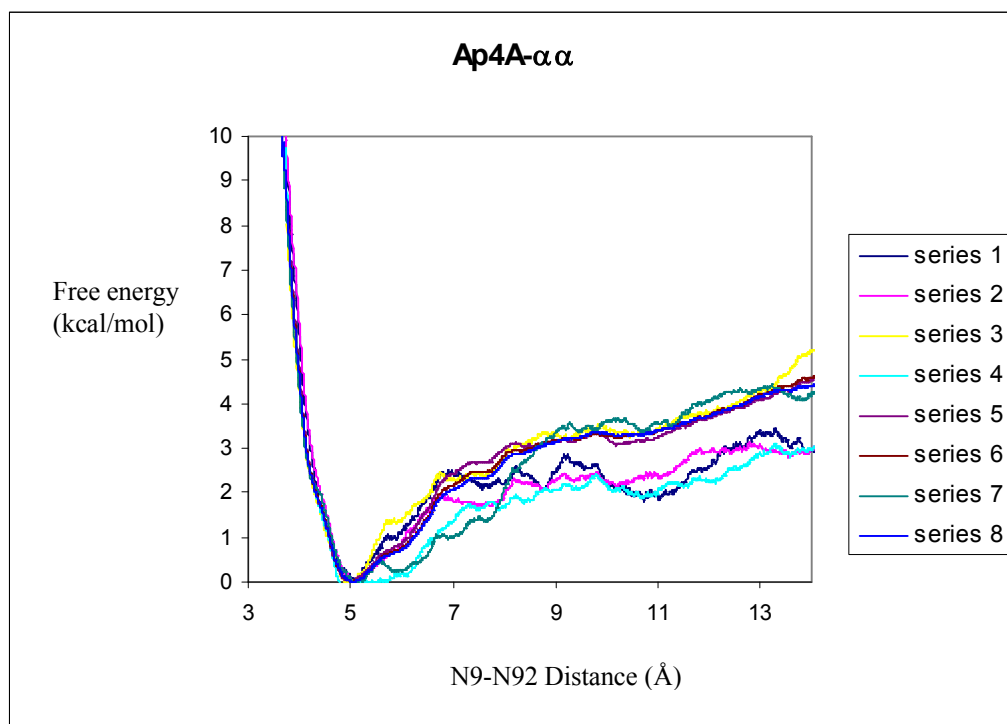


Fig. S15. Progressive PMF profiles of Ap₄A- $\alpha\alpha$.

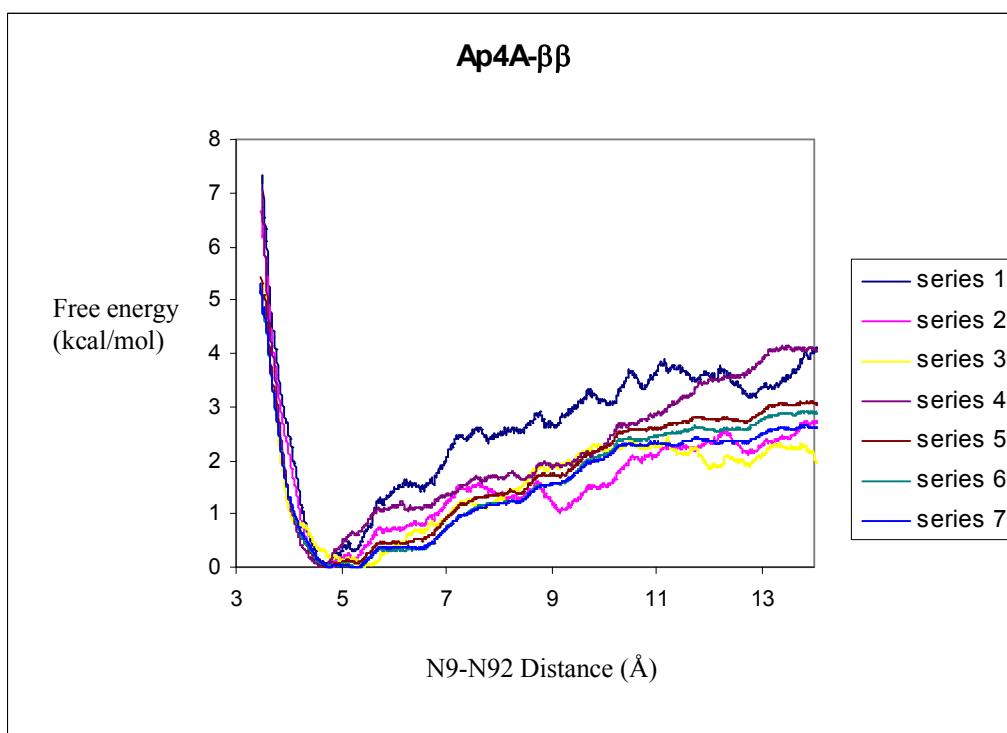


Fig. S16. Progressive PMF profiles of Ap₄A- $\beta\beta$.

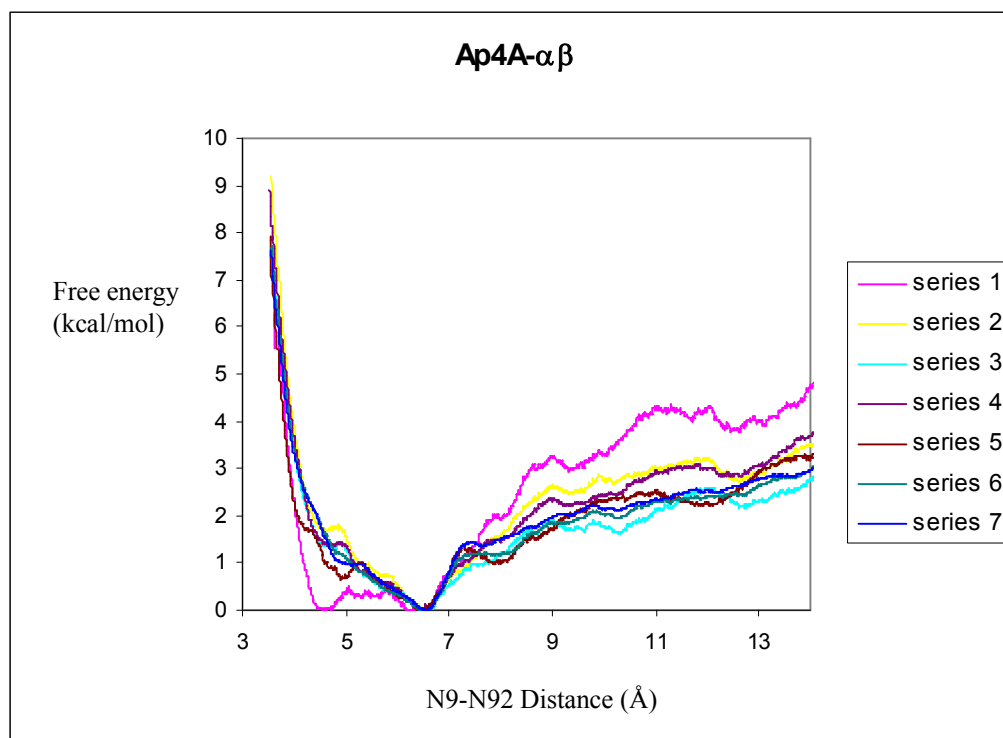


Fig. S17. Progressive PMF profiles of Ap₄A- $\alpha\beta$.

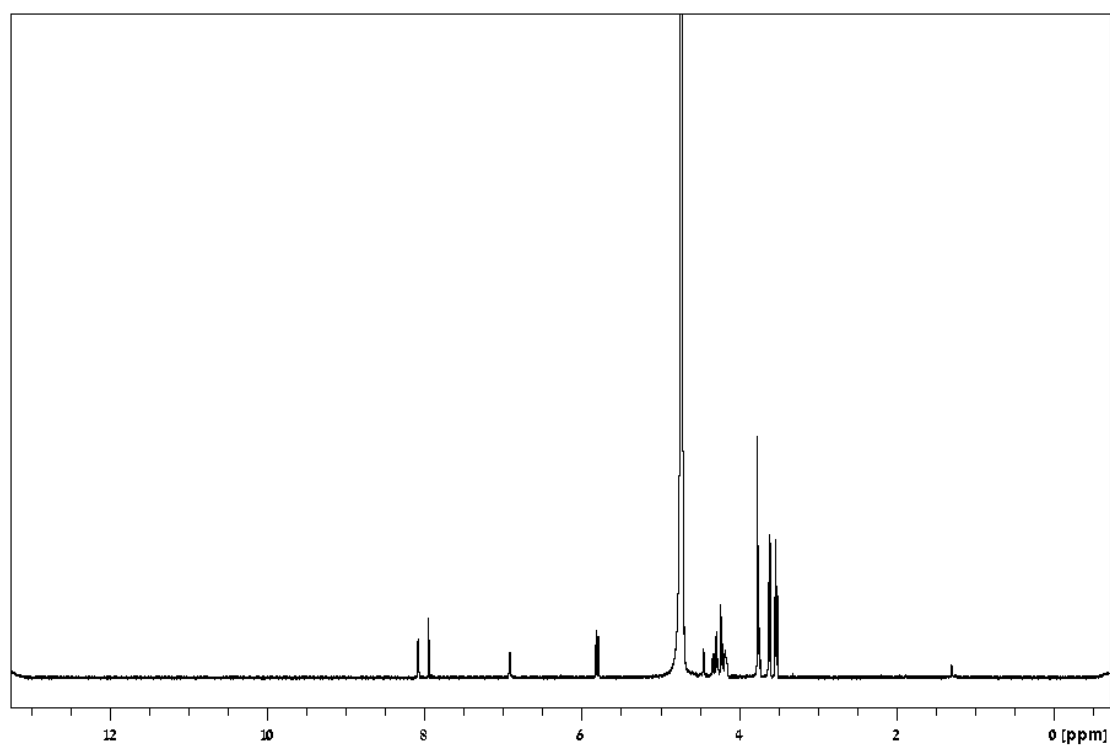
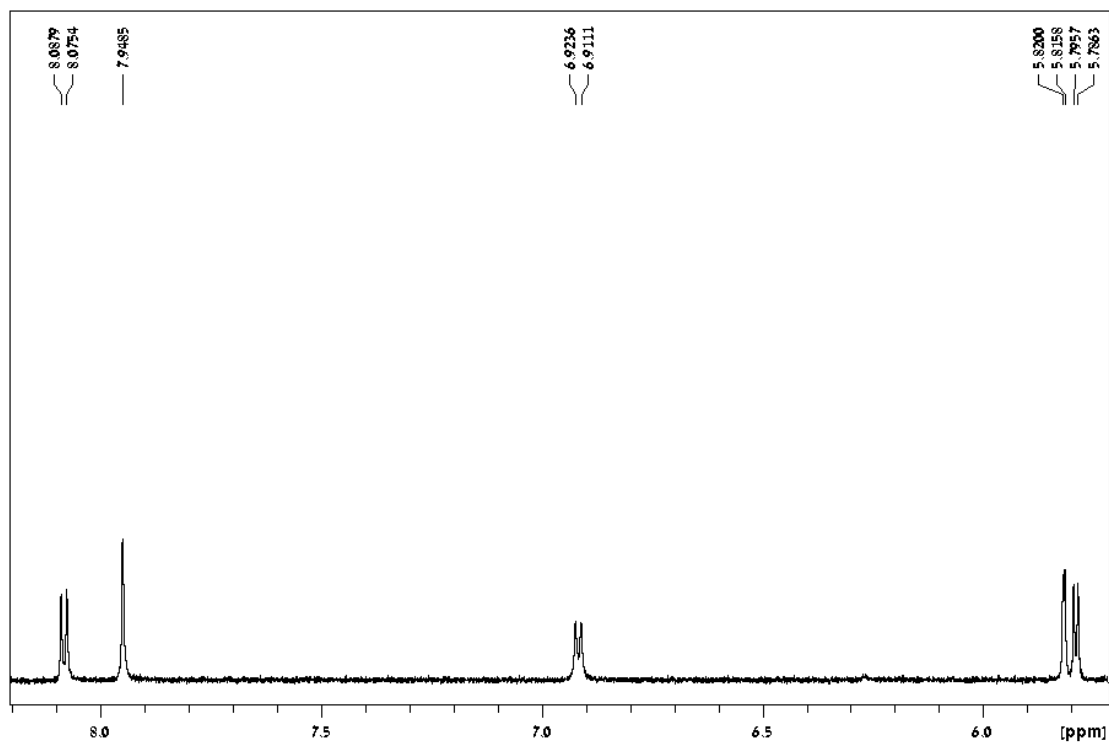
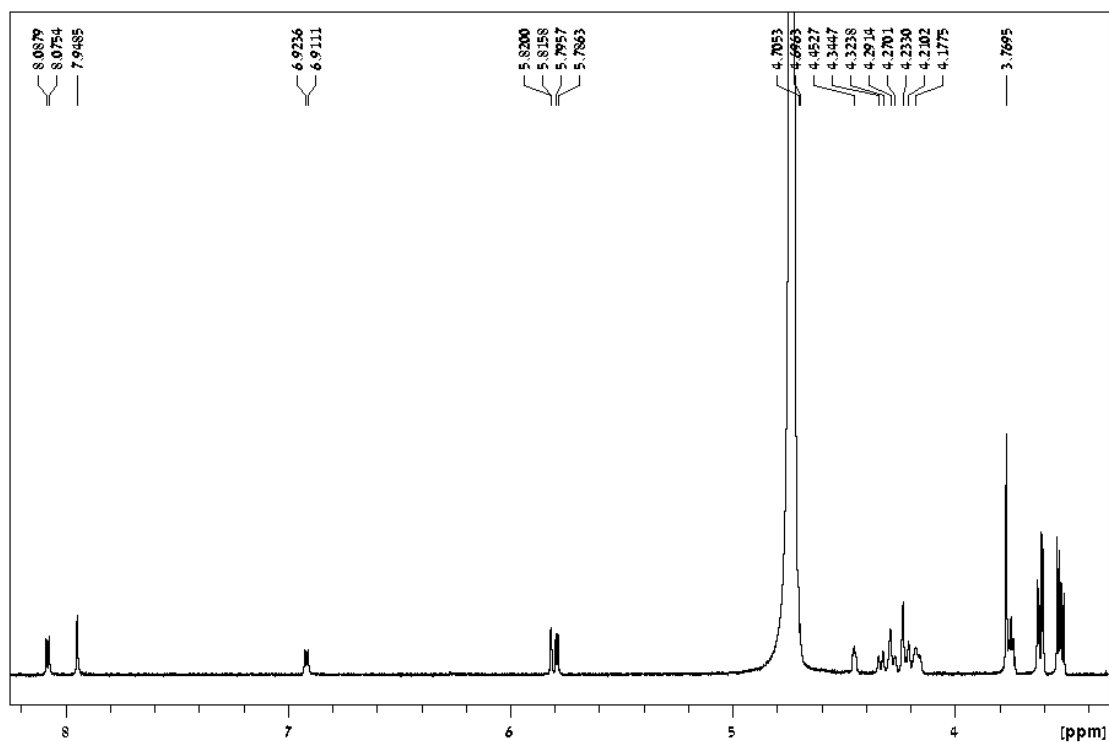


Fig. S18. ¹H NMR spectra of Gp₂C' (17) in D₂O at 600 MHz with glycerol as reference (3.5-3.8 ppm).



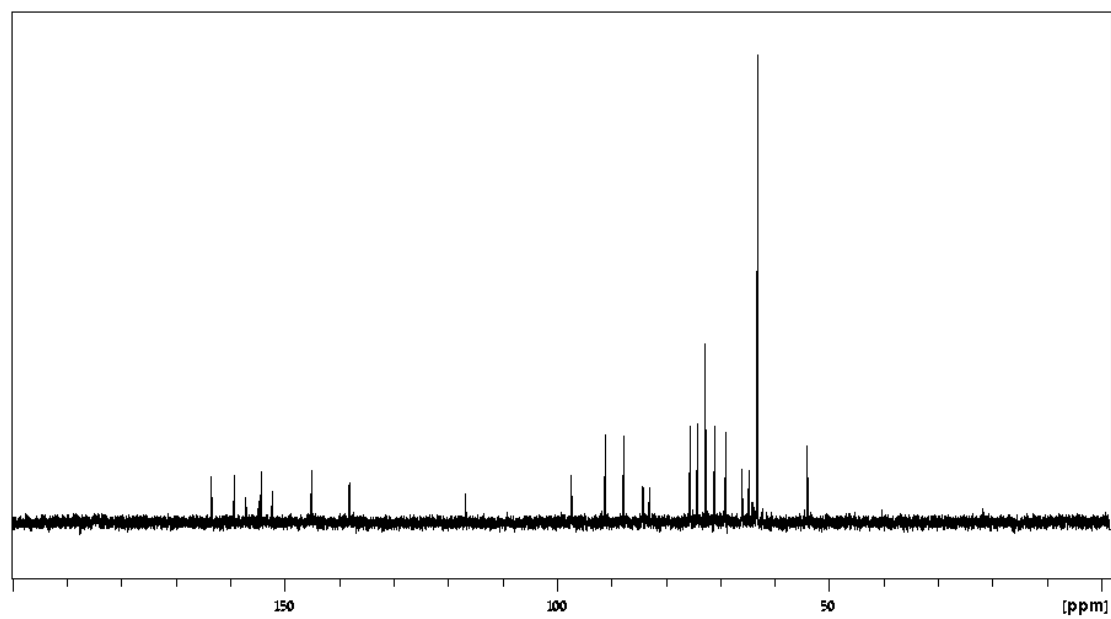
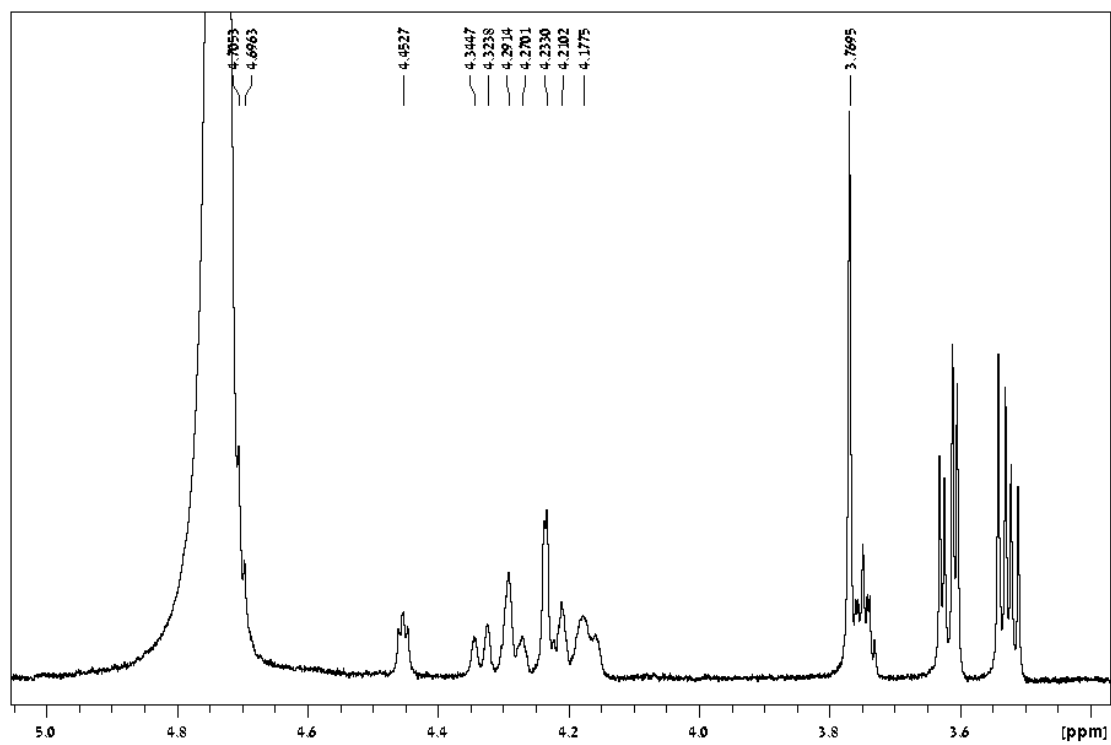
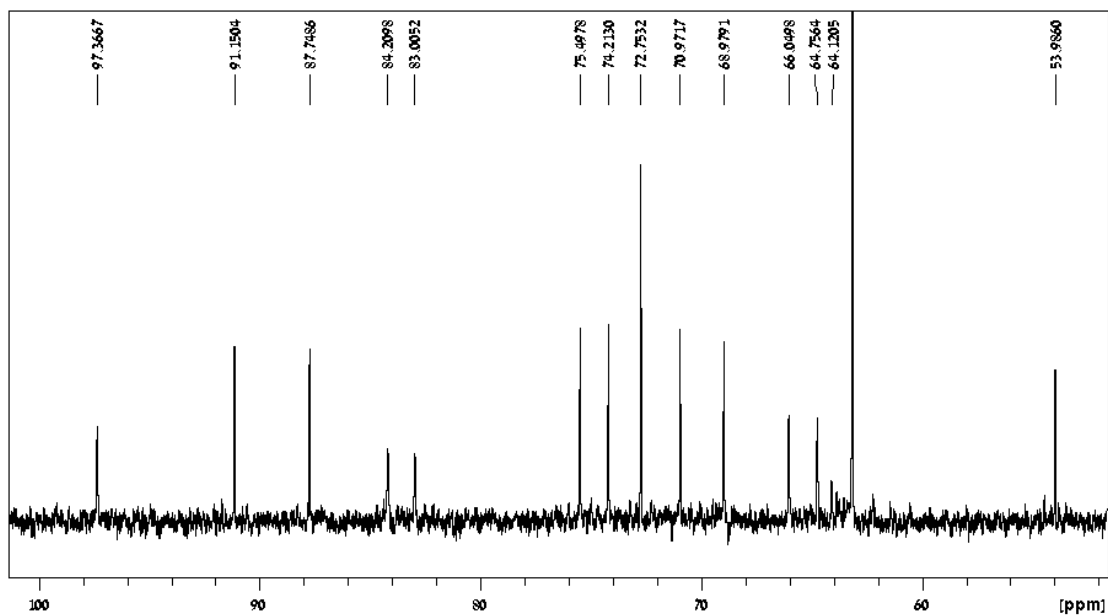
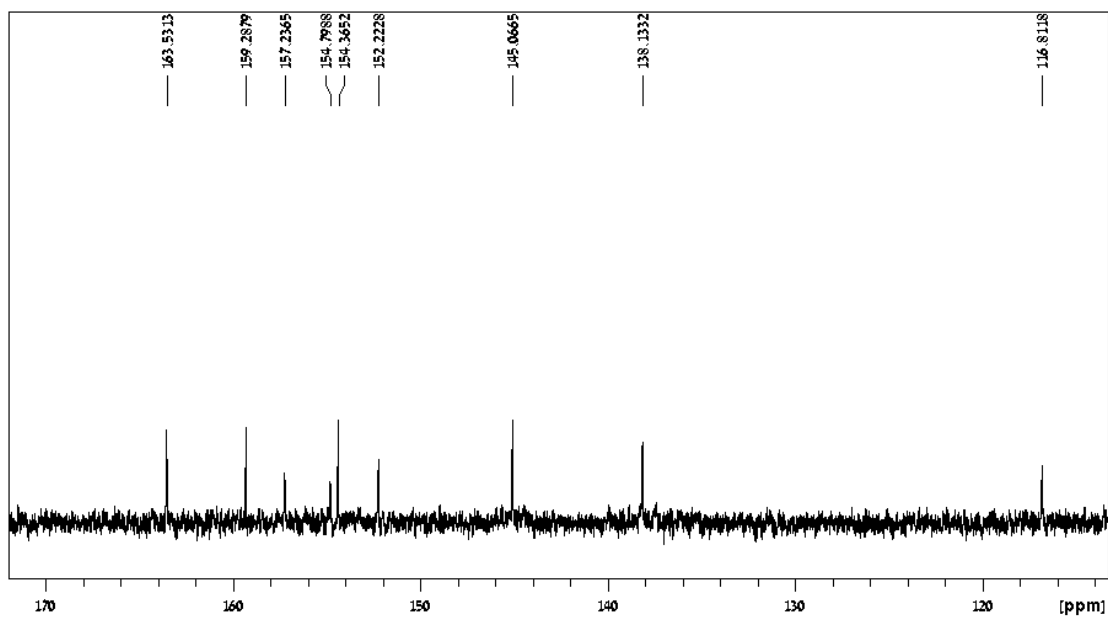


Fig. S19. ¹³C NMR spectra of Gp₂C' (17) in D₂O at 151 MHz.



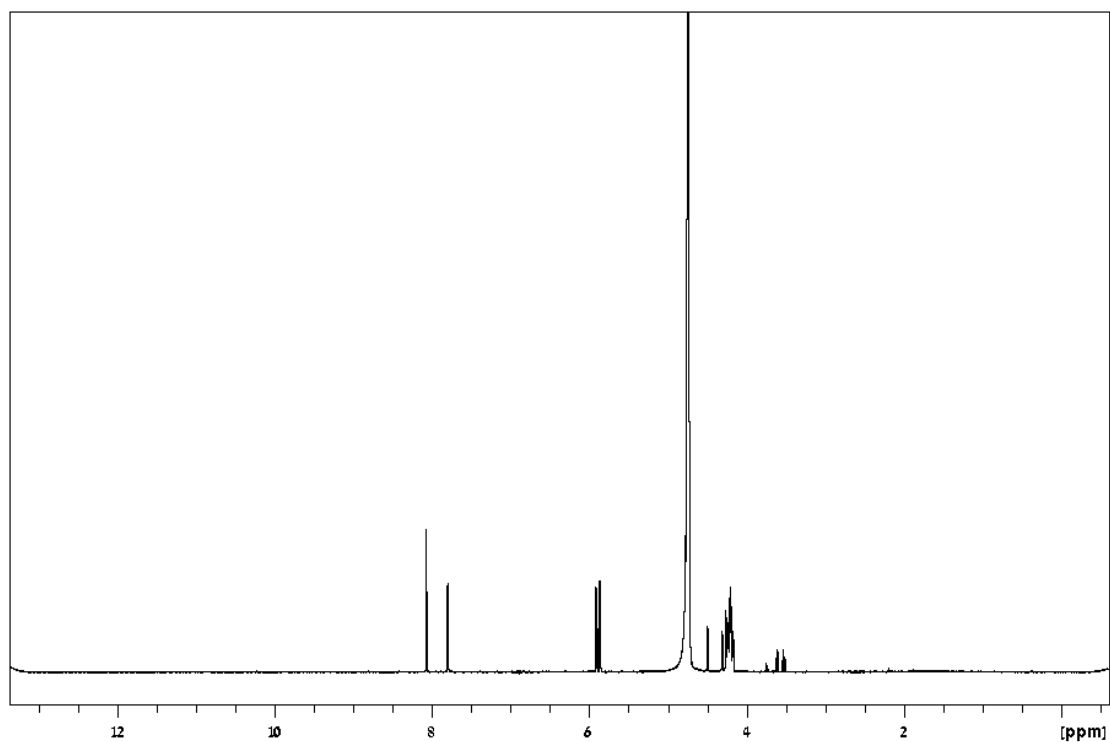
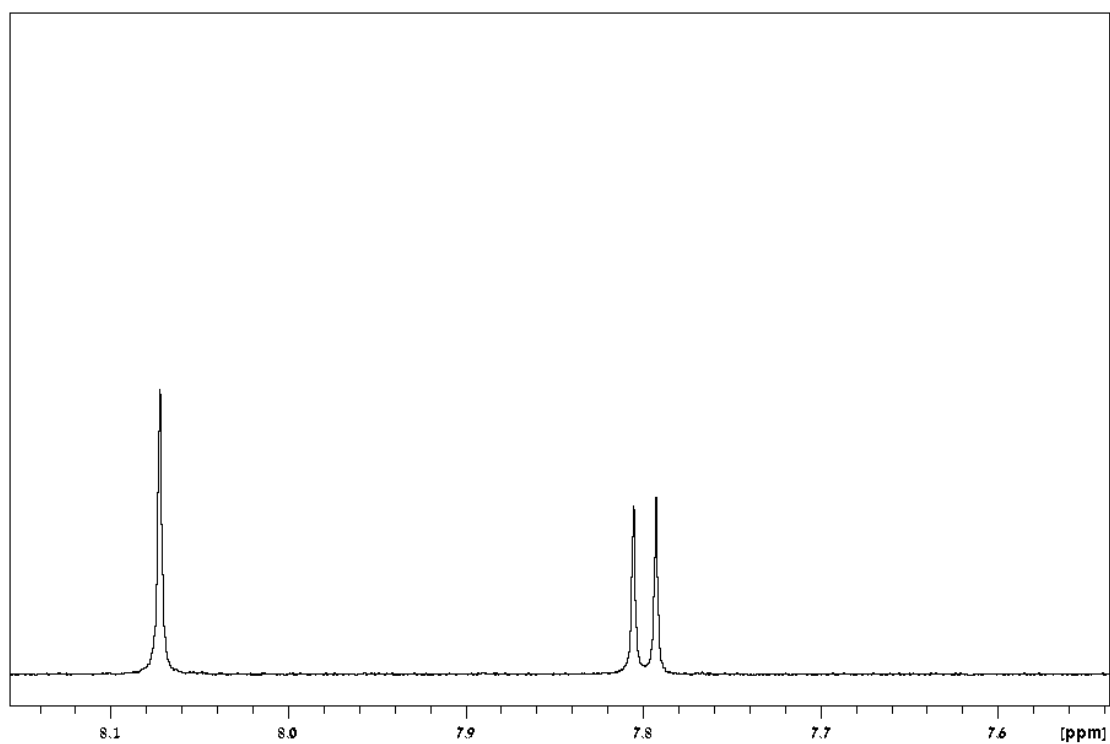
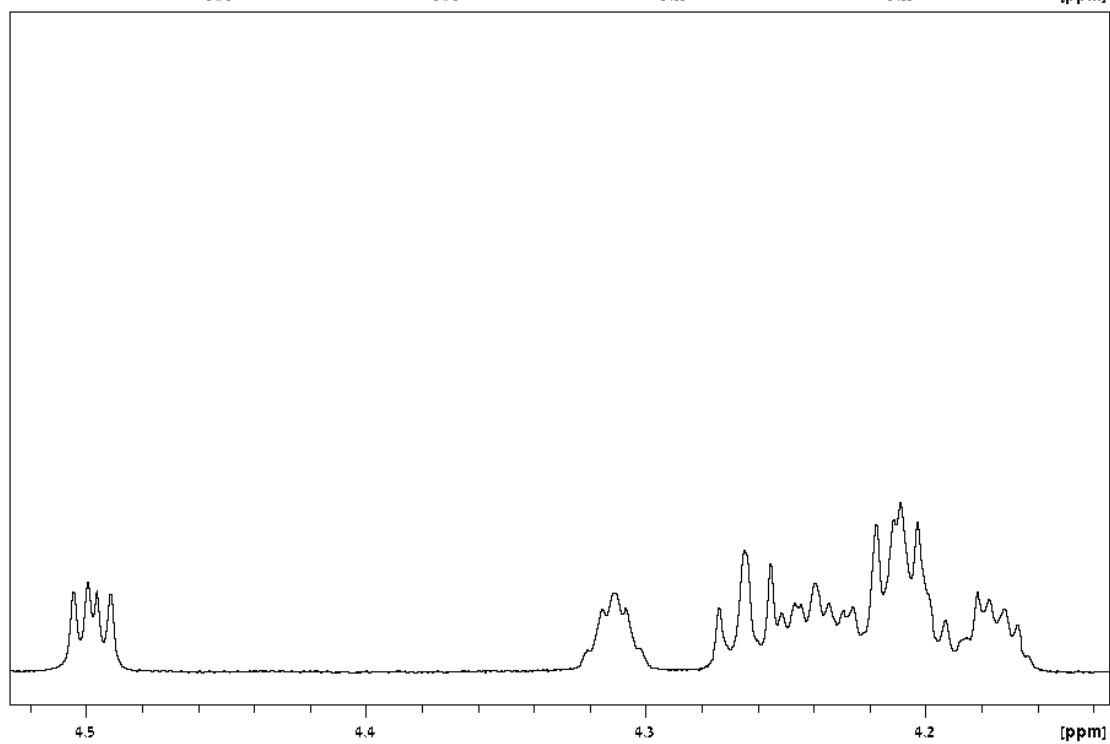
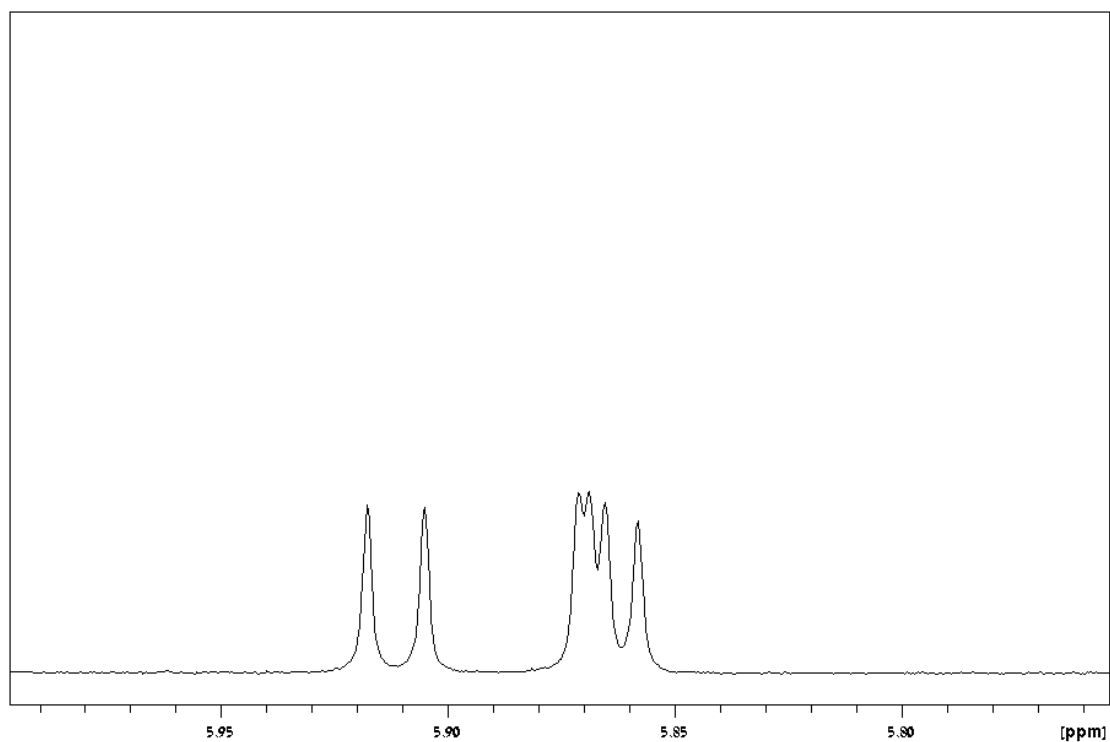


Fig. S20. ^1H NMR spectra of Gp_3C (**16**) in D_2O at 600 MHz with glycerol as reference (3.5-3.8 ppm).





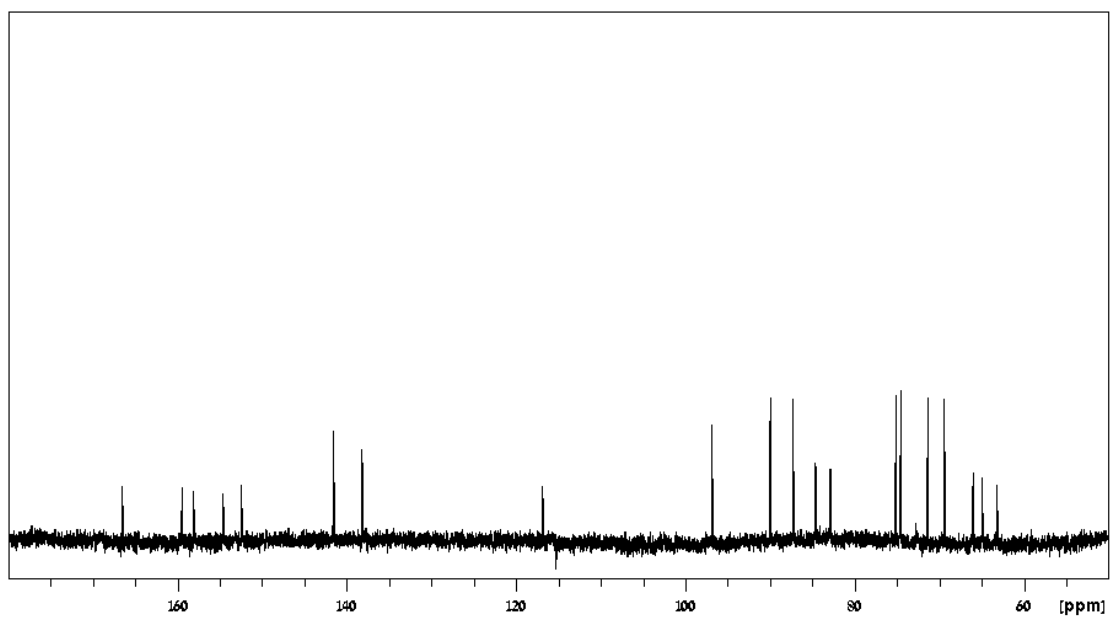
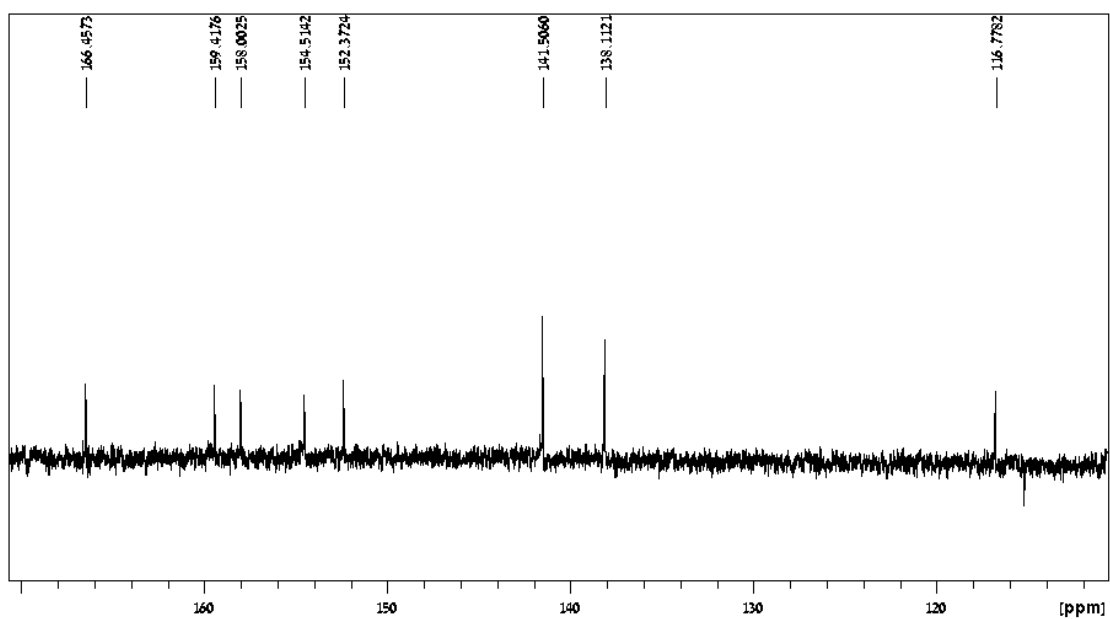


Fig. S21. ^{13}C NMR spectra of Gp₃C (**16**) in D₂O at 151 MHz.



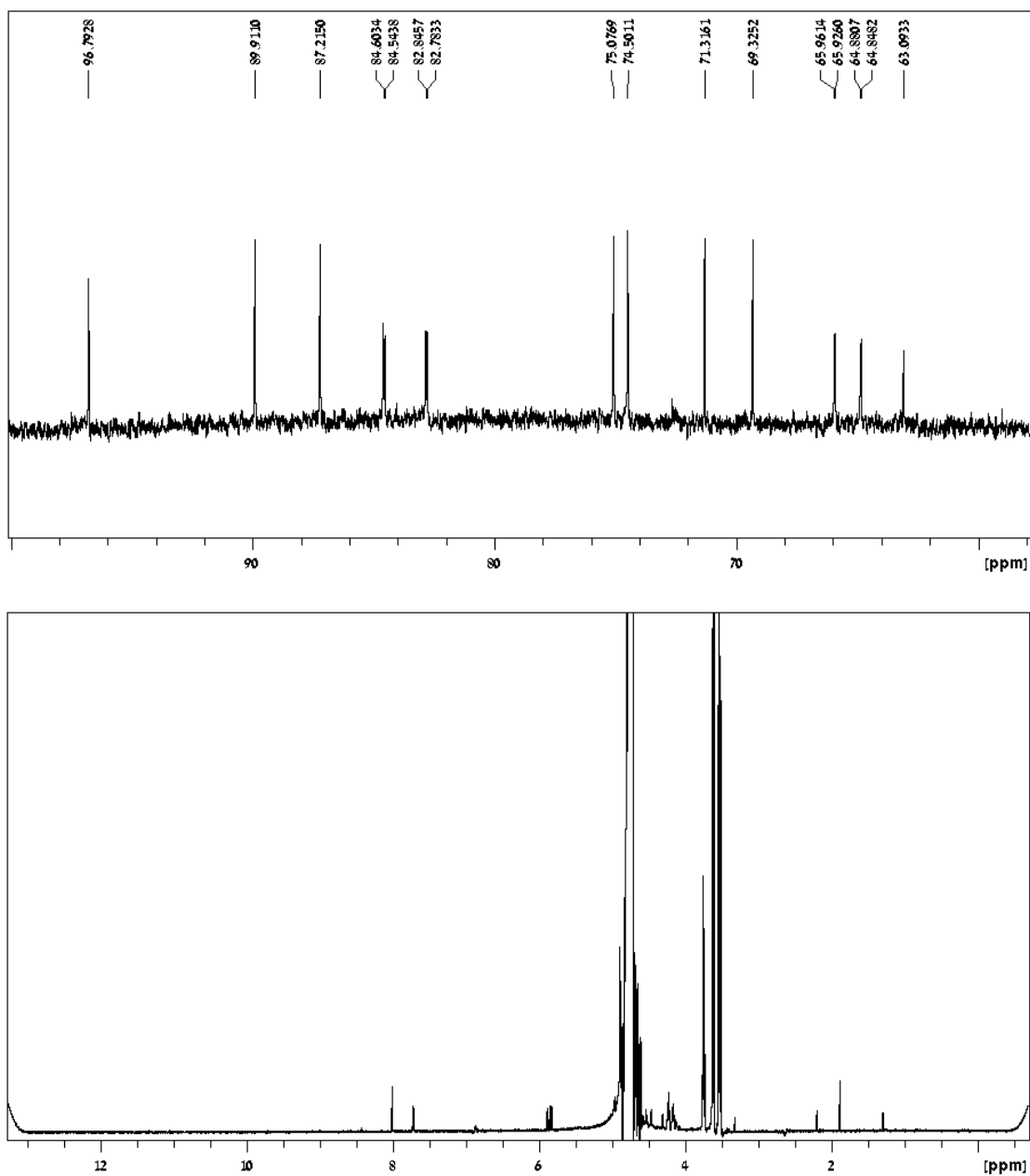
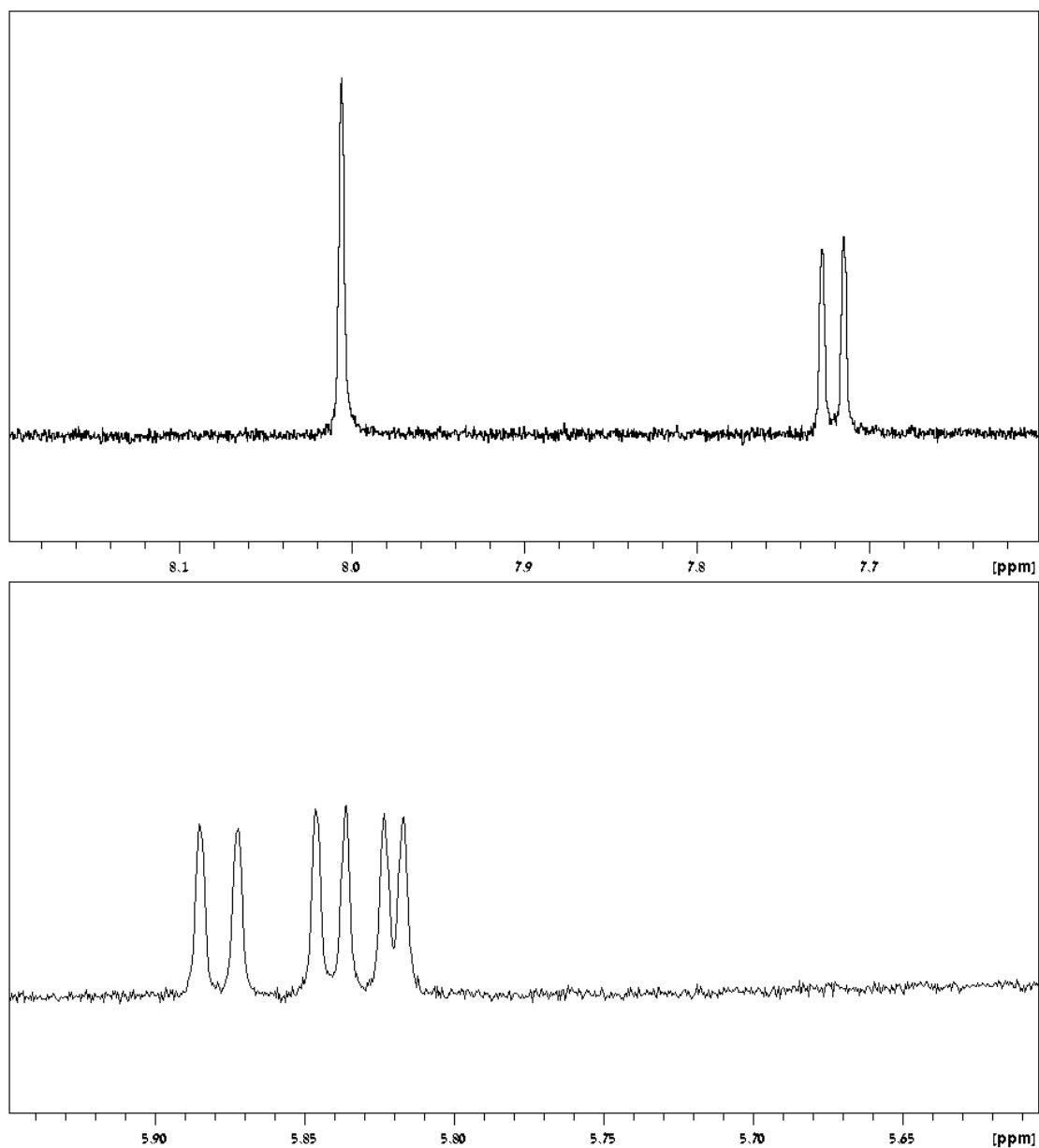


Fig. S22. ¹H NMR spectra of Gp₂C (**15**) in D₂O at 600 MHz with glycerol as reference (3.5-3.8 ppm).



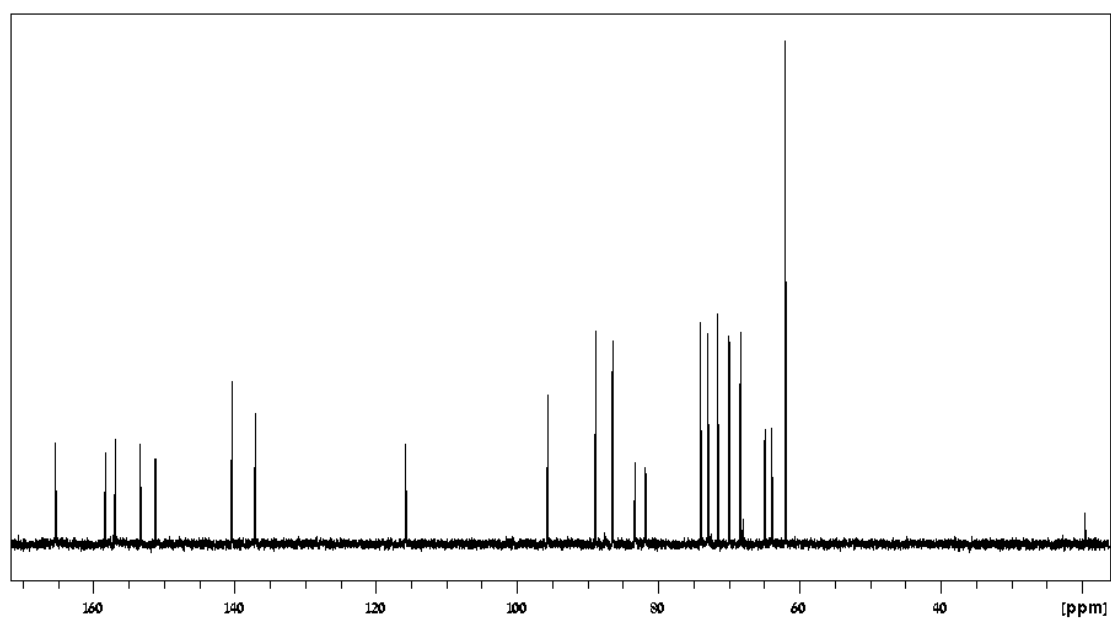
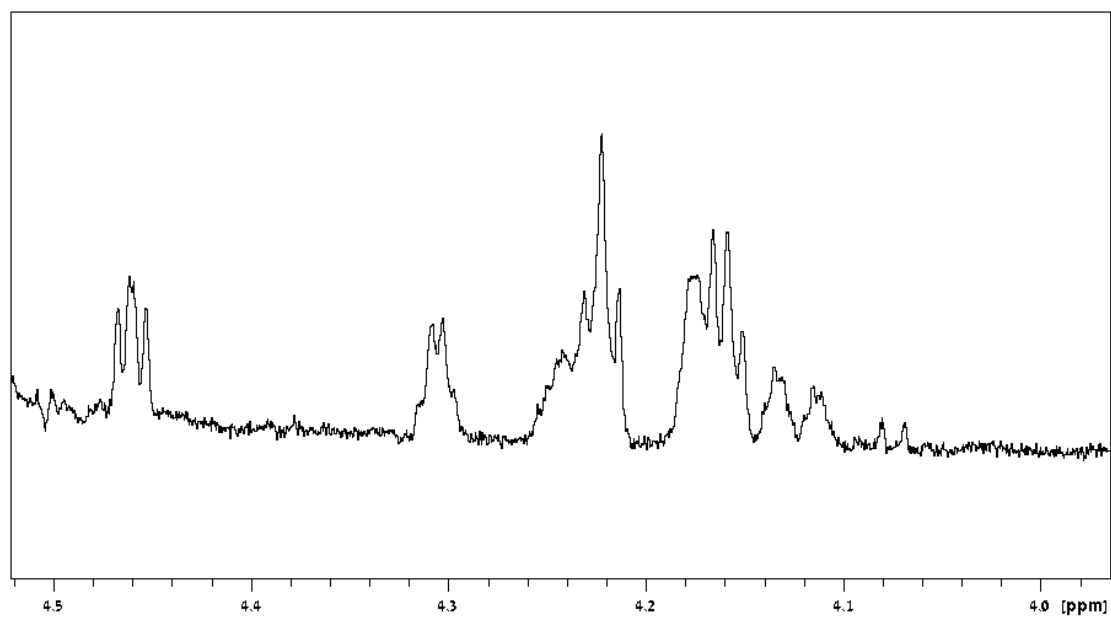


Fig. S23. ^{13}C NMR spectra of Gp₂C (**15**) in D₂O at 151 MHz.

