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Table S1. $^1$H-chemical shifts for streptomycin in H$_2$O at 5ºC

| Residue | Atom$^a$ | Chemical Shift (ppm)$^b$ |
|---------|---------|--------------------------|
| S1      | H1      | 3.479                    |
|         | HN1     | 7.676                    |
|         | H11$^*$ | 6.877                    |
|         | H12$^*$ | 6.877                    |
|         | H2      | 3.560                    |
|         | HO2     | 6.932                    |
|         | H3      | 3.626                    |
|         | HN3     | 7.810                    |
|         | H311(pro-Z) | 7.095                |
|         | H312(pro-E) | 6.869                |
|         | H32$^*$ | 6.877                    |
|         | H4      | 3.592                    |
|         | H5      | 3.554                    |
|         | HO5     | 6.127                    |
|         | H6      | 3.474                    |
|         | HO6     | 6.618                    |

| Residue | Atom$^a$ | Chemical Shift (ppm)$^b$ |
|---------|---------|--------------------------|
| R2      | H1      | 5.291                    |
|         | H2      | 4.407                    |
|         | HO3     | 7.195$^c$                |
|         | H3$'$   | 5.053                    |
|         | HO31    | -                        |
|         | HO32    | -                        |
|         | H4      | 4.445                    |
|         | H5$^*$  | 1.243                    |

| G3      | H1      | 5.598                    |
|         | H2      | 3.313                    |
|         | HN21 & HN22 | 8.862 & 8.358$^e$         |
|         | H2$^{*}$ | 2.854                    |
|         | H3      | 3.935                    |
|         | HO3     | -                        |
|         | H4      | 3.513                    |
|         | HO4     | 6.485                    |
|         | H5      | 3.706                    |
|         | H61 (pro-R) | 3.919                |
|         | H62 (pro-S) | 3.830                |

$^a$ Refer to Figure 1 (see text) for atom nomenclature.
$^b$ All chemical shifts were determined at a concentration of 50mM and 278.2 K, pH 6.0 in 10% D$_2$O and referenced directly relative to internal d$_6$-DSS. Std. error: $^1$H $\pm$ 0.001 ppm.
$^c$ Resonance observed for a hydroxyl proton with no COSY correlations and was therefore assigned to R2 HO3; resonance could correspond to R2 HO31 or HO32.
$^d$ Hydroxyl proton was not observed due to fast chemical exchange with solvent.
$^e$ Prochiral stereo-assignment not achieved.
* Chemical shifts labelled with a star indicate nuclei that are degenerate.
Table S2. $^{13}$C and $^{15}$N-chemical shifts for streptomycin in H$_2$O at 5ºC

| Residue | Atom$^a$ | Chemical Shift (ppm)$^b$ |
|---------|---------|-------------------------|
| S1      | C1      | 61.440                  |
|         | N1      | 87.483                  |
|         | C1'     | 160.923                 |
|         | N11     | 73.555                  |
|         | N12     | 73.555                  |
|         | C2      | 73.409                  |
|         | C3      | 60.897                  |
|         | N3      | 86.974                  |
|         | C3'     | 160.489                 |
|         | N31     | 74.378                  |
|         | N32     | 73.555                  |
|         | C4      | 80.224                  |
|         | C5      | 76.007                  |
|         | C6      | 74.136                  |

| Residue | Atom$^a$ | Chemical Shift (ppm)$^b$ |
|---------|---------|-------------------------|
| R2      | C1      | 108.745                 |
|         | C2      | 86.600                  |
|         | C3      | 85.008                  |
|         | C3'     | 91.903                  |
|         | C4      | 80.110                  |
|         | C5      | 15.025                  |

| G3      | C1      | 96.695                  |
|         | C2      | 63.886                  |
|         | N2      | --$^c$                  |
|         | C2'     | 34.504                  |
|         | C3      | 71.860                  |
|         | C4      | 72.095                  |
|         | C5      | 75.841                  |
|         | C6      | 63.031                  |

$^a$ Refer to Figure 1 (see text) for atom nomenclature.

$^b$ All chemical shifts were determined at a concentration of 50mM and 278.2 K, pH 6.0 in 10% D$_2$O and referenced indirectly relative to internal d$_6$-DSS. Std. error: ± 0.020 - 0.050 ppm.

$^c$ Value not determined.

* Chemical shifts labelled with a star indicate nuclei that are degenerate.
Table S3. $^1$H and $^{13}$C-chemical shifts for the aldehyde form of streptomycin in H$_2$O at 5ºC

| Residue | Atom$^a$ | Chemical Shift (ppm)$^b$ |
|---------|---------|-------------------------|
| R2      | H1      | 5.404                   |
|         | H2      | 4.733                   |
|         | H3'     | 9.717                   |
|         | H5*     | 1.173                   |
|         | C1      | 68.889                  |
|         | C2      | 84.678                  |
|         | C3      | 89.805                  |
|         | C3'     | 204.727                 |
|         | C4      | 80.490                  |
|         | C5      | 14.482                  |

$^a$ Refer to Figure 1 (see text) for atom nomenclature.

$^b$ All chemical shifts were determined at a concentration of 50mM and 278.2 K, pH 6.0 in 10% D$_2$O and referenced directly or indirectly relative to internal d$_6$-DSS. Std. errors: $^1$H ± 0.001; $^{13}$C ± 0.020 ppm.

* Chemical shifts labelled with a star indicate nuclei that are degenerate.

_Coupling constants within the aldehyde group and diagnostic for it:_

$^1J_{H3'-C3'} = 172$ Hz

$^2J_{H3'-C3} = 27$ Hz
**Supplementary information**

**Streptomycin solution structure**

Table S4. $^1$H-chemical shifts for streptomycin in D$_2$O at 25°C

| Residue | Atom$^a$ | Chemical Shift (ppm)$^b$ |
|---------|---------|-------------------------|
| S1      | H1      | 3.472                   |
|         | H2      | 3.569                   |
|         | H3      | 3.625                   |
|         | H4      | 3.595                   |
|         | H5      | 3.561                   |
|         | H6      | 3.475                   |

| Residue | Atom$^a$ | Chemical Shift (ppm)$^b$ |
|---------|---------|-------------------------|
| R2      | H1      | 5.292                   |
|         | H2      | 4.392                   |
|         | H3'     | 5.052                   |
|         | H4      | 4.432                   |
|         | H5*     | 1.249                   |

| G3      | H1      | 5.571                   |
|         | H2      | 3.308                   |
|         | H2''    | 2.860                   |
|         | H3      | 3.945                   |
|         | H4      | 3.513                   |
|         | H5      | 3.713                   |
|         | H61 (pro-R) | 3.919                  |
|         | H62 (pro-S) | 3.823                  |

$^a$ Refer to Figure 1 (see text) for atom nomenclature.

$^b$ All chemical shifts were determined at a concentration of 50mM and 298.2 K, pH* 6.0 in 100% D$_2$O and referenced directly relative to internal de-DSS. Std. error: $^1$H ± 0.001 ppm.

* Chemical shifts labelled with a star indicate nuclei that are degenerate.
Table S5. $^{13}$C and $^{15}$N-chemical shifts for streptomycin in D$_2$O at 25°C

| Residue | Atom$^a$ | Chemical Shift (ppm)$^b$ |
|---------|---------|-------------------------|
| S1      | C1      | 61.482                  |
|         | N1      | 86.743                  |
|         | C1'     | -                       |
|         | N11     | -                       |
|         | N12     | -                       |
|         | C2      | 73.396                  |
|         | C3      | 60.899                  |
|         | N3      | 86.276                  |
|         | C3'     | -                       |
|         | N31     | -                       |
|         | N32     | -                       |
|         | C4      | 80.407                  |
|         | C5      | 75.970                  |
|         | C6      | 74.189                  |

| Residue | Atom$^a$ | Chemical Shift (ppm)$^b$ |
|---------|---------|-------------------------|
| R2      | C1      | 108.631                 |
|         | C2      | 86.899                  |
|         | C3      | 85.008                  |
|         | C3'     | 92.078                  |
|         | C4      | 80.191                  |
|         | C5      | 15.082                  |

| G3      | C1      | 96.955                  |
|         | C2      | 63.946                  |
|         | N2      | 34.567                  |
|         | C2'     | 34.728                  |
|         | C3      | 71.883                  |
|         | C4      | 72.167                  |
|         | C5      | 75.553                  |
|         | C6      | 63.081                  |

$^a$ Refer to Figure 1 (see text) for atom nomenclature.

$^b$ All chemical shifts were determined at a concentration of 50mM and 298.2 K, pH* 6.0 in 100% D$_2$O and referenced indirectly relative to internal d$_6$-DSS. Std. error: ± 0.020 - 0.050 ppm.

$^c$ Value not determined.

* Chemical shifts labelled with a star indicate nuclei that are degenerate.
Table S6. $^1J_{CH}$ coupling constants for streptomycin in D$_2$O at 25°C

| Residue | Coupling constant | Value (Hz) |
|---------|-------------------|------------|
| S1      | $^1J_{C1-H1}$     | 141.1      |
|         | $^1J_{C2-H2}$     | 147.4      |
|         | $^1J_{C3-H3}$     | 141.4      |
|         | $^1J_{C4-H4}$     | 146.4      |
|         | $^1J_{C5-H5}$     | 143.6      |
|         | $^1J_{C6-H6}$     | 145.8      |
| R2      | $^1J_{C1-H1}$     | 178.0      |
|         | $^1J_{C2-H2}$     | 149.5      |
|         | $^1J_{C3'-H3'}$   | 161.7      |
|         | $^1J_{C4-H4}$     | 147.2      |
|         | $^1J_{C5-H5'}$    | 128.1      |
| G3      | $^1J_{C1-H1}$     | 175.3      |
|         | $^1J_{C2-H2}$     | 146.9      |
|         | $^1J_{C2'-H2'}$   | 144.4      |
|         | $^1J_{C3-H3}$     | 146.4      |
|         | $^1J_{C4-H4}$     | 144.8      |
|         | $^1J_{C5-H5}$     | 146.2      |
|         | $^1J_{C6-H61}$    | 144.2      |
|         | $^1J_{C6-H62}$    | 144.1      |

$^a$ Refer to Figure 1 (see text) for atom nomenclature.

$^b$ All coupling constants shifts were determined by direct measurement from the acquisition dimension of a $^{13}$C-HSQC spectrum recorded without broadband $^{13}$C-decoupling during acquisition on a sample of 50mM streptomycin, pH* 6.0, 100% D$_2$O at 298.2 K. Std. error: $^1J_{CH} \pm 0.5$ Hz.

* Chemical shifts labelled with a star indicate nuclei that are degenerate.
### Table S7. $J_{HH}$ coupling constants for streptomycin in aqueous solution

| Residue | Coupling constant<sup>a</sup> | Value (Hz)<sup>b</sup> |
|---------|-------------------------------|----------------------|
| S1      | $^3J_{H1-H2}$                | _<sup>c</sup>        |
|         | $^3J_{H1-HN1}$               | 6.4                  |
|         | $^3J_{H1-H6}$                | _<sup>c</sup>        |
|         | $^3J_{H2-H3}$                | 9.2                  |
|         | $^3J_{H3-HN3}$               | 8.5                  |
|         | $^3J_{H3-H4}$                | 10.2                 |
|         | $^3J_{H4-H5}$                | 8.6                  |
|         | $^3J_{H5-H6}$                | _<sup>c</sup>        |

| R2      | $^3J_{H1-H2}$                | 3.0                  |
|         | $^3J_{H4-H5}^*$              | 6.4                  |

| G3      | $^3J_{H1-H2}$                | 3.2                  |
|         | $^3J_{H2-H3}$                | 11.2                 |
|         | $^3J_{H3-H4}$                | 9.9                  |
|         | $^3J_{H4-H5}$                | 9.9                  |
|         | $^3J_{H5-H6}$                | 2.4                  |
|         | $^3J_{H5-H6}$                | 5.2                  |
|         | $^3J_{H61-H62}$              | -12.3                |

<sup>a</sup> Refer to Figure 1 (see text) for atom nomenclature.

<sup>b</sup> All coupling constants shifts were determined by direct measurement from 1D spectra recorded on a sample of 50mM streptomycin, pH 6.0, 10% (v/v) D<sub>2</sub>O at 278.2 K. Std. errors: $J_{HH} \pm 0.1 - 0.4$ Hz.

<sup>c</sup> Value not determined because of lineshape distortion by strong coupling.

* Chemical shifts labelled with a star indicate nuclei that are degenerate.
## Temperature dependency of chemical shifts

### Table S8. ^1_H-chemical shifts and temperature coefficients

| Residue | Atom^a | Chemical Shift (ppm) at a temperature (ºC) of:^b | Temperature coefficient ppb/K^c |
|---------|-------|-----------------------------------------------|--------------------------------|
|         |       | 3.77  | 5.04  | 9.85  | 14.96 | 19.96 | 25.06 | 35.07 |          |
| S1      | HN1   | 7.691 | 7.686 | 7.667 | 7.646 | 7.627 | 7.608 | 7.570 | -3.9     |
|         | HO2   | 6.951 | 6.948 | 6.941 | -d   | -     | -     | -     | -1.5     |
|         | HN3   | 7.826 | 7.821 | 7.800 | 7.778 | 7.758 | 7.737 | 7.697 | -4.1     |
|         | H311  | 7.110 | 7.106 | 7.090 | 7.070 | -     | -     | -     | -3.6     |
|         | H312*/H32** | 6.884 | 6.880 | 6.873 | 6.864 | 6.852 | 6.839 | 6.815 | -2.2     |
|         | HO5   | 6.132 | 6.117 | 6.061 | 5.999 | 5.942 | 5.877 | -     | -11.9    |
|         | HO6   | 6.664 | 6.657 | 6.602 | -     | -     | -     | -     | -10.6    |
| R2      | H1    | 5.288 | 5.287 | 5.289 | 5.289 | 5.291 | 5.291 | 5.294 | 0.2      |
|         | H2    | 4.401 | 4.400 | 4.398 | 4.395 | 4.393 | 4.390 | 4.386 | -0.5     |
|         | HO3   | 7.211 | 7.197 | 7.167 | -     | -     | -     | -     | -6.9     |
|         | H4    | 4.445 | 4.444 | 4.442 | 4.438 | 4.436 | 4.433 | 4.429 | -0.5     |
|         | H5*   | 1.244 | 1.245 | 1.245 | 1.245 | 1.247 | 1.247 | 1.248 | 0.1      |
| G3      | H1    | 5.593 | 5.590 | 5.585 | 5.579 | 5.575 | 5.569 | 5.560 | -1.0     |
|         | H2    | 3.308 | 3.308 | 3.307 | 3.305 | 3.304 | 3.303 | 3.301 | -0.2     |
|         | HN21^e | 8.868 | 8.860 | 8.838 | 8.811 | 8.794 | 8.757 | -     | -5.0     |
|         | HN22^e | 8.365 | 8.357 | 8.333 | 8.303 | 8.279 | 8.255 | -     | -5.2     |
|         | H2*   | 2.854 | 2.854 | 2.857 | 2.859 | 2.862 | 2.864 | 2.869 | 0.5      |
|         | HO4   | 6.515 | 6.508 | 6.471 | 6.434 | -     | -     | -     | -7.3     |
|         | H5    | 3.701 | 3.701 | 3.705 | 3.707 | 3.711 | 3.713 | 3.717 | 0.5      |

^a Refer to Figure 1 (see text) for atom nomenclature.

^b All chemical shifts were determined at a concentration of 50mM and pH 6.0 in 10% D_2O and referenced directly relative to internal d_6-DSS. Std. error: ^1_H ± 0.001 ppm.

^c Std. error ± 0.1 ppb/K.

^d Proton was no longer observed due to fast chemical exchange with solvent.

^e Prochiral stereoassignment not achieved.

^* Chemical shifts labelled with a star indicate nuclei that are degenerate.
Table S9. Conformational restraints derived from scalar couplings

| Restraint       | Karplus parameters | Observed value (Hz) | Mean predicted value (Hz) | mean $\chi^2$ |
|-----------------|--------------------|---------------------|---------------------------|---------------|
| $^3J_{S1,H1-HN1}$ | 9.4 -1.1 0 0      | 6.4±1.0$^b$         | 6.5                      | 0.84         |
| $^3J_{S1,H3-HN3}$ | 9.4 -1.1 0 0      | 8.5±1.0              | 8.7                      | 0.27         |
| $^3J_{R2,H1-H2}$  | 5.4 -0.9 1 -10    | 3.0±1.0              | 3.2                      | 0.04         |
| $^3J_{G3,H5-H61}$ | 8.31 -0.99 1.37 0 | 2.4±1.0              | 3.8                      | 2.5          |
| $^3J_{G3,H5-H62}$ | 9.58 -0.96 0.74 15 | 5.2±1.0              | 5.6                      | 1.2          |

$^a$Karplus equation in the form $^3J = Acos^2(\theta+\psi) + Bcos(\theta+\psi) + C$, with $\psi$ in degrees.

$^b$Error applied to observed value is that of predictive capability of the Karplus relation, rather than measurement error (see Methods).
Table S10. Conformational restraints derived from 2D-NOESY (D2O) spectrum

| Donor residue | Donor nuclei | Acceptor residue | Acceptor nuclei | Measured height (x10^5) | Mean predicted height (x10^5) | Mean χ² |
|---------------|--------------|------------------|----------------|--------------------------|-------------------------------|---------|
| S1            | H3           | S1               | H1 & H6^a       | 1.10E+03±4.20E+02        | 8.70E+02                     | 0.22    |
| R2            | H1           | S1               | H1 & H6^a       | 1.20E+02±49              | 1.20E+02                     | 0.063   |
| R2            | H2           | S1               | H1 & H6^a       | 29±12                    | 16                            | 1.3     |
| R2            | H4           | S1               | H1 & H6^a       | 35±14                    | 25                            | 0.65    |
| R2            | H5^*         | S1               | H1 & H6^a       | 33±13                    | 20                            | 1.2     |
| G3            | H1           | S1               | H1              | 8.2±12                   | 3.2                           | 0.19    |
| G3            | H61          | S1               | H1              | 25±10                    | 18                            | 0.9     |
| R2            | H1           | S1               | H2              | 2.00E+02±80              | 97                            | 1.6     |
| R2            | H2           | S1               | H2              | 23±9.1                   | 13                            | 1.8     |
| R2            | H31          | S1               | H2              | 6.8±23                   | 1.8                           | 0.049   |
| R2            | H4           | S1               | H2              | 22±8.8                   | 8.9                           | 2.2     |
| R2            | H5^*         | S1               | H2              | 18±7.2                   | 6.2                           | 2.8     |
| G3            | H1           | S1               | H2              | 15±16                    | 8                             | 0.23    |
| G3            | H61          | S1               | H2              | 23±9.1                   | 27                            | 0.51    |
| G3            | H62          | S1               | H2              | 21±8.5                   | 12                            | 1.1     |
| S1            | H1 & H6^a    | S1               | H3              | 1.30E+03±5.30E+02        | 8.70E+02                     | 0.76    |
| S1            | H3           | S1               | H3              | 1.00E+04±4.00E+03        | 8.90E+03                     | 0.087   |
| R2            | H1           | S1               | H3              | 2.60E+02±1.10E+02        | 65                            | 2.9     |
| R2            | H2           | S1               | H3              | 79±37                    | 56                            | 0.48    |
| R2            | H31          | S1               | H3              | 17±17                    | 6.4                           | 0.38    |
| R2            | H4           | S1               | H3              | 58±23                    | 29                            | 1.6     |
| R2            | H5^*         | S1               | H3              | 44±25                    | 10                            | 1.8     |
| G3            | H1           | S1               | H3              | 25±19                    | 31                            | 0.12    |
| G3            | H2^*         | S1               | H3              | 6.7±2.7                  | 5.7                           | 0.31    |
| G3            | H3 & H61^a   | S1               | H3              | 40±16                    | 29                            | 0.86    |
| G3            | H62          | S1               | H3              | 44±25                    | 11                            | 1.8     |
| S1            | H1 & H6^a    | S1               | H4              | 1.20E+03±4.70E+02        | 1.40E+03                     | 0.21    |
| S1            | H4           | S1               | H4              | 9.20E+03±3.70E+03        | 1.30E+04                     | 1.5     |
| R2            | H1           | S1               | H4              | 1.60E+03±6.50E+02        | 1.60E+03                     | 0.052   |
| R2            | H2           | S1               | H4              | 1.20E+02±49              | 74                            | 0.98    |
| R2            | H31          | S1               | H4              | 22±20                    | 9.7                           | 0.38    |
| R2            | H4           | S1               | H4              | 1.10E+02±43              | 65                            | 1.4     |
| R2            | H5^*         | S1               | H4              | 73±29                    | 37                            | 1.8     |
### Supplementary information

**Streptomycin solution structure**

| G3  | H1 | S1   | H4  | 43±26 | 33  | 0.24 |
|-----|----|------|-----|-------|-----|------|
| G3  | H2 | S1   | H4  | 22±8.9| 5.6 | 3.5  |
| G3  | H2*| S1   | H4  | 15±6  | 6.2 | 2.2  |
| G3  | H3 | S1   | H4  | 68±27 | 85  | 1.2  |
| G3  | H5 | S1   | H4  | 1.30E+02±51 | 83 | 0.9  |
| G3  | H62| S1   | H4  | 37±15 | 35  | 0.48 |
| R2  | H1 | S1   | H5  | 2.60E+02±1.00E+02 | 84 | 2.9  |
| R2  | H2 | S1   | H5  | 42±17 | 37  | 0.21 |
| R2  | H31| S1   | H5  | 20±24 | 11  | 0.15 |
| R2  | H4 | S1   | H5  | 83±33 | 1.20E+02 | 2.2 |
| R2  | H5*| S1   | H5  | 42±17 | 49  | 0.75 |
| G3  | H1 | S1   | H5  | 12±23 | 10  | 0.016|
| G3  | H1 | S1   | H6  | 7.6±12 | 4.2 | 0.091|
| S1  | H1 & H6* | R2 | H1 | 1.60E+02±64 | 1.20E+02 | 0.43 |
| S1  | H2 & H5* | R2 | H1 | 4.70E+02±1.90E+02 | 1.80E+02 | 2.4  |
| S1  | H4  | R2   | H1  | 1.40E+03±5.50E+02 | 1.60E+03 | 0.14 |
| R2  | H1  | R2   | H1  | 1.60E+04±6.40E+03 | 1.60E+04 | 0.02 |
| R2  | H31 | R2   | H1  | 66±27 | 40  | 0.94 |
| R2  | H4  | R2   | H1  | 1.70E+02±69 | 1.40E+02 | 0.23 |
| R2  | H5* | R2   | H1  | 1.40E+02±57 | 86  | 0.96 |
| G3  | H1  | R2   | H1  | 1.80E+02±72 | 1.60E+02 | 0.24 |
| G3  | H2  | R2   | H1  | 35±16 | 23  | 0.58 |
| G3  | H2* | R2   | H1  | 60±24 | 28  | 2.2  |
| G3  | H3  | R2   | H1  | 2.10E+02±84 | 2.10E+02 | 0.12 |
| G3  | H5  | R2   | H1  | 8.50E+02±3.40E+02 | 7.30E+02 | 0.32 |
| G3  | H62 | R2   | H1  | 1.10E+02±44 | 80  | 0.53 |
| S1  | H1 & H6* | R2 | H2  | 27±11 | 16  | 1.1  |
| S1  | H2 & H5* | R2 | H2  | 68±27 | 50  | 0.47 |
| S1  | H3  | R2   | H2  | 61±25 | 56  | 0.28 |
| S1  | H4  | R2   | H2  | 1.20E+02±48 | 74  | 0.86 |
| R2  | H2  | R2   | H2  | 1.70E+04±6.90E+03 | 1.30E+04 | 0.47 |
| R2  | H31 | R2   | H2  | 6.00E+02±2.40E+02 | 8.80E+02 | 1.6  |
| R2  | H5* | R2   | H2  | 1.50E+02±58 | 1.40E+02 | 0.021|
| G3  | H1  | R2   | H2  | 2.10E+03±8.40E+02 | 2.20E+03 | 0.096|
| G3  | H2  | R2   | H2  | 1.40E+02±57 | 1.40E+02 | 0.045|
| G3  | H2* | R2   | H2  | 3.30E+02±1.30E+02 | 2.20E+02 | 0.79 |
| G3  | H3  | R2   | H2  | 1.10E+02±45 | 89  | 0.37 |
|     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|
| G3  | H4  | R2  | H2  | 27±13 | 25 | 0.071 |
| G3  | H5  | R2  | H2  | 4.20E+02±1.70E+02 | 2.00E+02 | 2.1 |
| G3  | H62 | R2  | H2  | 60±25 | 23 | 2.4 |
| S1  | H2 & H5a | R2 | H31 | 20±8 | 12 | 0.93 |
| R2  | H1  | R2  | H31 | 46±19 | 40 | 0.12 |
| R2  | H2  | R2  | H31 | 6.10E+02±2.40E+02 | 2.00E+02 | 2.1 |
| G3  | H1  | R2  | H31 | 3.20E+02±1.30E+02 | 2.90E+02 | 0.34 |
| G3  | H5  | R2  | H31 | 21±10 | 19 | 0.33 |
| R2  | H1  | R2  | H31 | 4.60E+02±1.90E+02 | 8.80E+02 | 1.5 |
| S1  | H2 & H5a | R2 | H31 | 1.00E+02±40 | 1.30E+02 | 1.1 |
| S1  | H2 & H5a | R2 | H31 | 20±8 | 12 | 0.21 |
| S1  | H3  | R2  | H4  | 48±31 | 29 | 0.4 |
| S1  | H4  | R2  | H4  | 91±47 | 65 | 0.71 |
| R2  | H1  | R2  | H4  | 1.40E+02±63 | 1.40E+02 | 0.014 |
| R2  | H2  | R2  | H4  | 4.90E+02±2.00E+02 | 7.50E+02 | 1.7 |
| R2  | H31 | R2  | H4  | 7.50E+02±3.00E+02 | 8.00E+02 | 0.12 |
| R2  | H4  | R2  | H4  | 1.60E+04±6.30E+03 | 1.40E+04 | 0.057 |
| G3  | H1  | R2  | H4  | 74±40 | 96 | 0.36 |
| G3  | H2  | R2  | H4  | 12±4.8 | 7.6 | 0.84 |
| G3  | H2* | R2  | H4  | 33±13 | 18 | 1.3 |
| G3  | H5  | R2  | H4  | 23±23 | 18 | 0.083 |
| R2  | H1  | R2  | H5* | 70±30 | 86 | 0.3 |
| R2  | H1  | R2  | H5* | 93±39 | 1.40E+02 | 1.7 |
| R2  | H31 | R2  | H5* | 3.90E+02±1.60E+02 | 7.80E+02 | 5.9 |
| G3  | H1  | R2  | H5* | 33±17 | 34 | 0.079 |
| G3  | H2* | R2  | H5* | 49±20 | 18 | 2.5 |
| S1  | H4  | G3  | H1  | 36±17 | 33 | 0.28 |
| R2  | H1  | G3  | H1  | 1.60E+02±63 | 1.60E+02 | 0.18 |
| R2  | H2  | G3  | H1  | 1.90E+03±7.50E+02 | 2.20E+03 | 0.29 |
| R2  | H31 | G3  | H1  | 2.90E+02±1.20E+02 | 2.90E+02 | 0.34 |
| R2  | H4  | G3  | H1  | 1.20E+02±49 | 96 | 0.25 |
| R2  | H5*  | G3   | H1   | 47±21 | 34   | 0.44 |
|-----|------|------|------|-------|------|------|
| G3  | H1   | G3   | H1   | 1.60E+04±6.60E+03 | 1.10E+04 | 0.6  |
| G3  | H2*  | G3   | H1   | 1.50E+03±5.80E+02 | 9.60E+02 | 0.76 |
| G3  | H3   | G3   | H1   | 1.10E+02±45 | 1.80E+02 | 2    |
| G3  | H4   | G3   | H1   | 1.10E+02±43 | 1.20E+02 | 0.19 |
| G3  | H5   | G3   | H1   | 1.50E+02±59 | 1.50E+02 | 0.019|
| G3  | H62  | G3   | H1   | 43±19 | 32   | 0.41 |
| R2  | H1   | G3   | H2   | 37±22 | 23   | 0.38 |
| R2  | H2   | G3   | H2   | 1.50E+02±60 | 1.40E+02 | 0.046|
| R2  | H31  | G3   | H2   | 30±20 | 24   | 0.12 |
| R2  | H4   | G3   | H2   | 14±17 | 7.6  | 0.14 |
| R2  | H5*  | G3   | H2   | 12±17 | 5.1  | 0.17 |
| G3  | H2   | G3   | H2   | 1.50E+04±6.00E+03 | 1.30E+04 | 0.16 |
| G3  | H4   | G3   | H2   | 8.90E+02±3.60E+02 | 1.30E+03 | 1.1  |
| G3  | H5   | G3   | H2   | 1.20E+02±50 | 1.10E+02 | 0.038|
| G3  | H62  | G3   | H2   | 74±30 | 43   | 1.2  |
| R2  | H2   | G3   | H2*  | 1.80E+02±74 | 2.20E+02 | 0.56 |
| R2  | H31  | G3   | H2*  | 59±24 | 97   | 2.9  |
| G3  | H1   | G3   | H2*  | 9.50E+02±3.80E+02 | 9.60E+02 | 0.16 |
| R2  | H1   | G3   | H3   | 1.40E+02±60 | 98   | 0.65 |
| R2  | H2   | G3   | H3   | 71±33 | 57   | 0.2  |
| R2  | H31  | G3   | H3   | 12±4.9 | 16   | 0.85 |
| R2  | H5*  | G3   | H3   | 23±18 | 8.5  | 0.71 |
| G3  | H1   | G3   | H3   | 1.20E+02±52 | 1.30E+02 | 0.016|
| G3  | H2*  | G3   | H3   | 1.50E+02±61 | 1.30E+02 | 0.2  |
| G3  | H3   | G3   | H3   | 1.20E+04±4.80E+03 | 1.80E+04 | 1.7  |
| G3  | H5   | G3   | H3   | 1.00E+03±4.20E+02 | 1.20E+03 | 0.2  |
| R2  | H1   | G3   | H4   | 45±26 | 25   | 0.57 |
| R2  | H2   | G3   | H4   | 29±11 | 25   | 0.16 |
| R2  | H31  | G3   | H4   | 7.5±17 | 4.9  | 0.025|
| R2  | H5*  | G3   | H4   | 9.7±3.9 | 2.2  | 3.8  |
| G3  | H1   | G3   | H4   | 1.40E+02±59 | 1.20E+02 | 0.093|
| G3  | H2   | G3   | H4   | 9.80E+02±3.90E+02 | 1.30E+03 | 0.5  |
| G3  | H2*  | G3   | H4   | 1.00E+02±40 | 1.40E+02 | 1.2  |
| G3  | H4   | G3   | H4   | 1.40E+04±5.80E+03 | 1.80E+04 | 0.35 |
| G3  | H62  | G3   | H4   | 8.00E+02±3.20E+02 | 3.50E+02 | 2.1  |
| S1  | H4   | G3   | H5   | 1.80E+02±73 | 83   | 1.9  |
### Supplementary information

#### Streptomycin solution structure

|    |     |     |       |       |       |     |
|----|-----|-----|-------|-------|-------|-----|
| R2 | H1  | G3  | H5    | 1.10E+03±4.30E+02 | 7.30E+02 | 0.76 |
| R2 | H2  | G3  | H5    | 5.60E+02±2.30E+02 | 2.00E+02 | 2.8  |
| R2 | H31 | G3  | H5    | 33±29          | 19      | 0.27 |
| R2 | H5* | G3  | H5    | 44±32          | 12      | 0.99 |
| G3 | H1  | G3  | H5    | 2.20E+02±2×93  | 1.50E+02 | 0.67 |
| G3 | H2  | G3  | H5    | 1.70E+02±74    | 1.10E+02 | 0.76 |
| G3 | H2* | G3  | H5    | 75±30          | 35      | 1.8  |
| G3 | H5  | G3  | H5    | 1.90E+04±7.50E+03 | 1.30E+04 | 0.68 |
| R2 | H1  | G3  | H61   | 1.10E+02±45    | 1.10E+02 | 0.17 |
| R2 | H2  | G3  | H61   | 76±33          | 32      | 1.9  |
| R2 | H31 | G3  | H61   | 8.1±14         | 5       | 0.077|
| R2 | H5* | G3  | H61   | 9.8±3.9        | 2.9     | 3.1  |
| G3 | H1  | G3  | H61   | 43±22          | 48      | 0.27 |
| G3 | H2  | G3  | H61   | 36±20          | 39      | 0.34 |
| G3 | H2* | G3  | H61   | 21±8.5         | 14      | 0.92 |
| G3 | H4  | G3  | H61   | 3.50E+02±1.40E+02 | 1.70E+02 | 1.8  |
| R2 | H1  | G3  | H62   | 80±40          | 80      | 0.099|
| R2 | H2  | G3  | H62   | 43±29          | 23      | 0.6  |
| R2 | H5* | G3  | H62   | 9.2±23         | 2.5     | 0.087|
| G3 | H1  | G3  | H62   | 33±26          | 32      | 0.036|
| G3 | H2  | G3  | H62   | 55±31          | 43      | 0.21 |
| G3 | H2* | G3  | H62   | 16±6.4         | 11      | 0.62 |
| G3 | H4  | G3  | H62   | 5.10E+02±2.00E+02 | 3.50E+02 | 0.79 |

*These restraints were included as overlaps between the NOE cross-peaks indicated.*
### noNOEs

| Donor residue | Donor nuclei | Acceptor residue | Acceptor nuclei | Measured height (x10^5) | Mean predicted height (x10^5) | Mean $\chi^2$ |
|---------------|--------------|------------------|----------------|------------------------|-------------------------------|--------------|
| R2            | H31          | S1               | H1             | 0±2.8                  | 1.5                           | 0.3          |
| G3            | H2           | S1               | H1             | 0±1.50E+02             | 0.65                          | 1.90E-05     |
| G3            | H2*          | S1               | H1             | 0±13                   | 0.95                          | 0.0059       |
| G3            | H3           | S1               | H1             | 0±99                   | 0.8                           | 6.70E-05     |
| G3            | H5           | S1               | H1             | 0±57                   | 2.5                           | 0.002        |
| G3            | H2           | S1               | H2             | 0±6.3                  | 1.8                           | 0.087        |
| G3            | H2*          | S1               | H2             | 0±2.9                  | 2                             | 0.49         |
| G3            | H3           | S1               | H2             | 0±17                   | 2.8                           | 0.029        |
| G3            | H2           | S1               | H3             | 0±4.4                  | 4.1                           | 0.92         |
| G3            | H2           | S1               | H5             | 0±36                   | 1.6                           | 0.002        |
| G3            | H2*          | S1               | H5             | 0±2.9                  | 2.6                           | 0.84         |
| G3            | H3           | S1               | H5             | 0±29                   | 2                             | 0.0048       |
| G3            | H62          | S1               | H5             | 0±33                   | 3                             | 0.0083       |
| R2            | H31          | S1               | H6             | 0±2.8                  | 2.2                           | 0.63         |
| G3            | H2           | S1               | H6             | 0±58                   | 0.99                          | 0.00029      |
| G3            | H2*          | S1               | H6             | 0±3.3                  | 1.2                           | 0.14         |
| G3            | H3           | S1               | H6             | 0±40                   | 1.9                           | 0.0022       |
| G3            | H5           | S1               | H6             | 0±25                   | 8.5                           | 0.13         |
| G3            | H62          | S1               | H6             | 0±47                   | 7                             | 0.026        |
| S1            | H1           | R2               | H31            | 0±2.8                  | 1.5                           | 0.29         |
| S1            | H6           | R2               | H31            | 0±2.9                  | 2.2                           | 0.57         |
| G3            | H4           | R2               | H31            | 0±5.4                  | 4.9                           | 0.83         |
| G3            | H62          | R2               | H31            | 0±3.3                  | 3.5                           | 1.3          |
| G3            | H3           | R2               | H4             | 0±6.5                  | 7.4                           | 1.3          |
| G3            | H4           | R2               | H4             | 0±16                   | 2.4                           | 0.023        |
| G3            | H61          | R2               | H4             | 0±6.5                  | 4.2                           | 0.42         |
| G3            | H62          | R2               | H4             | 0±4.6                  | 2.9                           | 0.42         |
| S1            | H1           | R2               | H5*            | 0±11                   | 5.6                           | 0.27         |
| G3            | H2           | R2               | H5*            | 0±5.5                  | 5.1                           | 0.86         |
| G3            | H3           | R2               | H5*            | 0±11                   | 8.5                           | 0.57         |
| G3            | H4           | R2               | H5*            | 0±11                   | 2.2                           | 0.04         |
| G3            | H5           | R2               | H5*            | 0±15                   | 12                            | 0.67         |
| G3            | H61          | R2               | H5*            | 0±11                   | 2.9                           | 0.068        |
| G3            | H62          | R2               | H5*            | 0±4.3                  | 2.5                           | 0.35         |
| S1            | H1           | G3               | H1             | 0±25                   | 3.2                           | 0.016        |
|      | H6  | G3  | H1      | 0±18 | 4.2  | 0.053 |
|------|-----|-----|---------|------|------|-------|
| S1   | H2  | G3  | H2      | 0±97 | 1.8  | 0.00037 |
| S1   | H3  | G3  | H2      | 0±13 | 4.1  | 0.11  |
| S1   | H4  | G3  | H2      | 0±13 | 5.6  | 0.19  |
| S1   | H1  | G3  | H2*     | 0±19 | 0.95 | 0.0025 |
| S1   | H2  | G3  | H2*     | 0±19 | 2    | 0.012 |
| S1   | H3  | G3  | H2*     | 0±16 | 5.7  | 0.14  |
| S1   | H4  | G3  | H2*     | 0±19 | 6.2  | 0.12  |
| S1   | H5  | G3  | H2*     | 0±17 | 2.6  | 0.025 |
| S1   | H6  | G3  | H2*     | 0±14 | 1.2  | 0.0083 |
| R2   | H4  | G3  | H2*     | 0±26 | 18   | 0.51  |
| G3   | H62 | G3  | H2*     | 0±9.1 | 11 | 1.5  |
| S1   | H3  | G3  | H3      | 0±21 | 4.6  | 0.06  |
| S1   | H3  | G3  | H4      | 0±81 | 2.6  | 0.0011 |
| R2   | H4  | G3  | H4      | 0±5.7 | 2.4 | 0.18  |
| S1   | H3  | G3  | H61     | 0±26 | 25   | 1     |
| R2   | H4  | G3  | H61     | 0±9.3 | 4.2 | 0.21  |
| R2   | H31 | G3  | H62     | 0±2  | 3.5  | 3.3   |
| R2   | H4  | G3  | H62     | 0±4.8 | 2.9 | 0.37  |
Table S11. Conformational restraints derived from 2D-NOESY (H2O) spectrum

### NOEs

| Donor residue | Donor nuclei | Acceptor residue | Acceptor nuclei | Measured height ($\times 10^5$) | Mean predicted height ($\times 10^5$) | Mean $\chi^2$ |
|---------------|--------------|------------------|-----------------|----------------------------------|-------------------------------------|--------------|
| S1            | H3 & H4<sup>a</sup> | S1               | H311            | 5.4±2.2                           | 2.1                                 | 2.5          |
| R2            | H1           | S1               | H311            | 0.63±0.46                         | 0.16                                | 1.1          |
| R2            | H2           | S1               | H311            | 1±0.62                            | 1.1                                 | 1.5          |
| G3            | H1           | S1               | H311            | 1.2±0.68                          | 1.4                                 | 0.94         |
| G3            | H3 & H61<sup>a</sup> | S1            | H311            | 0.96±0.38                         | 0.83                                | 0.72         |
| S1            | H1 & H6<sup>a</sup> | S1               | HN1             | 9±3.6                             | 6.1                                 | 0.78         |
| S1            | H2 & H5<sup>a</sup> | S1               | HN1             | 6.1±2.4                           | 4.5                                 | 0.65         |
| S1            | H2 & H5<sup>a</sup> | S1               | HN3             | 12±4.8                            | 4.8                                 | 2.5          |
| R2            | H1           | S1               | HN3             | 3.7±1.9                           | 3.2                                 | 2.5          |

<sup>a</sup>These restraints were included as overlaps between the NOE cross-peaks indicated.

### noNOEs

| Donor residue | Donor nuclei | Acceptor residue | Acceptor nuclei | Measured height ($\times 10^5$) | Mean predicted height ($\times 10^5$) | Mean $\chi^2$ |
|---------------|--------------|------------------|-----------------|----------------------------------|-------------------------------------|--------------|
| S1            | H1           | S1               | H311            | 0±0.26                           | 0.088                               | 0.12         |
| S1            | H6           | S1               | H311            | 0±0.26                           | 0.022                               | 0.0077       |
| R2            | H31          | S1               | H311            | 0±0.16                           | 0.068                               | 0.27         |
| R2            | H4           | S1               | H311            | 0±0.3                            | 0.095                               | 0.17         |
| R2            | H5<sup>*</sup> | S1              | H311            | 0±0.68                           | 0.015                               | 0.00064      |
| G3            | H2           | S1               | H311            | 0±0.43                           | 0.11                                | 0.07         |
| G3            | H2<sup>*</sup> | S1              | H311            | 0±13                             | 0.14                                | 0.0002       |
| G3            | H4           | S1               | H311            | 0±0.36                           | 0.043                               | 0.015        |
| G3            | H62          | S1               | H311            | 0±0.27                           | 0.24                                | 0.96         |
| R2            | H31          | S1               | H312            | 0±0.13                           | 0.039                               | 0.15         |
| R2            | H5<sup>*</sup> | S1              | H312            | 0±1.3                            | 0.017                               | 0.00025      |
| G3            | H2           | S1               | H312            | 0±0.39                           | 0.024                               | 0.0051       |
| G3            | H2<sup>*</sup> | S1             | H312            | 0±0.58                           | 0.031                               | 0.005        |
| S1            | H3           | S1               | HN1             | 0±0.5                            | 0.18                                | 0.14         |
| R2            | H1           | S1               | HN1             | 0±0.39                           | 0.028                               | 0.0055       |
| R2            | H2           | S1               | HN1             | 0±0.52                           | 0.0066                               | 0.00017      |
| R2            | H31          | S1               | HN1             | 0±0.19                           | 0.002                               | 0.00012      |
| R2            | H4           | S1               | HN1             | 0±0.52                           | 0.0095                               | 0.00037      |
| R2            | H5<sup>*</sup> | S1             | HN1             | 0±1.9                            | 0.0072                               | 1.50E-05     |
| G3            | H1           | S1               | HN1             | 0±0.52                           | 0.0041                               | 7.10E-05     |
|     |     |     |     |     |
|-----|-----|-----|-----|-----|
| G3  | H2  | S1  | HN1 | 0±1 | 0.0014 | 2.10E-06 |
| G3  | H2* | S1  | HN1 | 0±0.92 | 0.0014 | 2.40E-06 |
| G3  | H3  | S1  | HN1 | 0±0.59 | 0.002 | 1.20E-05 |
| G3  | H5  | S1  | HN1 | 0±0.6 | 0.0058 | 0.0001 |
| G3  | H61 | S1  | HN1 | 0±0.59 | 0.011 | 0.0004 |
| G3  | H62 | S1  | HN1 | 0±0.32 | 0.0052 | 0.00033 |
| S1  | H1  | S1  | HN3 | 0±1.8 | 0.18 | 0.012 |
| S1  | H6  | S1  | HN3 | 0±1.8 | 0.35 | 0.041 |
| R2  | H2  | S1  | HN3 | 0±1.1 | 0.25 | 0.053 |
| R2  | H31 | S1  | HN3 | 0±0.3 | 0.022 | 0.0053 |
| R2  | H4  | S1  | HN3 | 0±1.1 | 0.056 | 0.0026 |
| R2  | H5* | S1  | HN3 | 0±5 | 0.025 | 2.70E-05 |
| G3  | H1  | S1  | HN3 | 0±0.8 | 0.44 | 0.36 |
| G3  | H2  | S1  | HN3 | 0±0.97 | 0.069 | 0.0053 |
| G3  | H2* | S1  | HN3 | 0±2.1 | 0.038 | 0.00037 |
| G3  | H3  | S1  | HN3 | 0±1.1 | 0.1 | 0.011 |
| G3  | H5  | S1  | HN3 | 0±0.94 | 0.57 | 0.46 |
| G3  | H61 | S1  | HN3 | 0±1.1 | 1.7 | 2.6 |
| G3  | H62 | S1  | HN3 | 0±0.67 | 0.48 | 0.63 |
Table S12. Conformational restraints derived from residual dipolar couplings

*Alignment with 4.5% polyacrylamide gel*

| First residue | First nucleus | First residue | First nucleus | Measured RDC (Hz) | Mean predicted RDC (Hz) | Mean $\chi^2$ |
|---------------|---------------|---------------|---------------|-------------------|------------------------|--------------|
| S1            | C1            | S1            | H1            | 2.3±1             | 2.4                    | 0.02         |
| S1            | C2            | S1            | H2            | 3.1±1.5           | 2.4                    | 0.042        |
| S1            | C3            | S1            | H3            | 2.7±1             | 2.4                    | 0.031        |
| S1            | C4            | S1            | H4            | 2.4±1             | 2.4                    | 0.019        |
| S1            | C5            | S1            | H5            | 3.9±1.5           | 2.4                    | 0.16         |
| S1            | C6            | S1            | H6            | 2.8±1             | 2.4                    | 0.04         |
| R2            | C1            | R2            | H1            | 0.92±1            | 2                      | 0.1          |
| R2            | C2            | R2            | H2            | 4.6±1             | 2.8                    | 0.34         |
| R2            | C31           | R2            | H31           | -0.51±1           | 0.5                    | 0.32         |
| R2            | C4            | R2            | H4            | 2±1               | 1.6                    | 0.15         |
| G3            | C1            | G3            | H1            | 0.67±1            | 1.6                    | 0.2          |
| G3            | C2            | G3            | H2            | 0.02±1            | 0.5                    | 0.046        |
| G3            | C3            | G3            | H3            | 0.84±1            | 0.52                   | 0.03         |
| G3            | C4            | G3            | H4            | 1.1±1             | 0.47                   | 0.072        |
| G3            | C6            | G3            | H61           | -2±1              | -1.7                   | 0.13         |
| G3            | CN2           | G3            | H2*           | -0.32±1           | -0.97                  | 0.079        |
| R2            | H4            | R2            | H5*           | 0.55±1            | 1.1                    | 0.019        |
| G3            | H2            | G3            | H3            | 0.55±1            | 0.25                   | 0.02         |
| G3            | H61           | G3            | H62           | 2.5±1             | -0.2                   | 3.5          |
| G3            | H5            | G3            | H62           | 0.48±1            | 0.19                   | 0.021        |
**Supplementary information**

**Streptomycin solution structure**

**Alignment with 6% polyacrylamide gel**

| First residue | First nucleus | First residue | First nucleus | Measured RDC (Hz) | Mean predicted RDC (Hz) | Mean $\chi^2$ |
|---------------|---------------|---------------|---------------|-------------------|-------------------------|---------------|
| S1            | C1            | S1            | H1            | 5.2±1.5           | 5.4                     | 0.031         |
| S1            | C3            | S1            | H3            | 5.8±2             | 5.3                     | 0.11          |
| S1            | C4            | S1            | H4            | 4.1±1             | 5.3                     | 0.18          |
| S1            | C5            | S1            | H5            | 5.1±2             | 5.2                     | 0.028         |
| S1            | C6            | S1            | H6            | 4.6±1.5           | 5.2                     | 0.035         |
| R2            | C1            | R2            | H1            | 5.1±1             | 4.3                     | 0.084         |
| R2            | C2            | R2            | H2            | 11±1              | 6.1                     | 2.1           |
| R2            | C31           | R2            | H31           | -1.2±1            | 1.1                     | 1.7           |
| R2            | C4            | R2            | H4            | 3.7±1             | 3.4                     | 0.083         |
| G3            | C1            | G3            | H1            | 4.3±1             | 3.4                     | 0.33          |
| G3            | C2            | G3            | H2            | 2.2±1             | 1.1                     | 0.27          |
| G3            | CN2           | G3            | H2*           | -0.16±1           | -2.2                    | 0.62          |
| G3            | C3            | G3            | H3            | -0.38±1           | 1.1                     | 0.35          |
| G3            | C4            | G3            | H4            | 1.2±1             | 1                       | 0.067         |
| G3            | C6            | G3            | H61           | -3.1±1            | -3.8                    | 0.94          |
Density analysis of structural restraints

Table S13. Number of restraints involving each proton used in the 4D-structure determination

| Residue | Atom a,b | No. of restraints |
|---------|----------|------------------|
| S1      | H1       | 27               |
|         | HN1      | 16               |
|         | H2       | 25               |
|         | H3       | 22               |
|         | HN3      | 16               |
|         | H311(pro-Z) | 14          |
|         | H312(pro-E) | 4             |
|         | H4       | 22               |
|         | H5       | 13               |
|         | H6       | 26               |
| R2      | H1       | 34               |
|         | H2       | 36               |
|         | H3’      | 35               |
|         | H4       | 40               |
|         | H5*      | 32               |
| G3      | H1       | 36               |
|         | H2       | 34               |
|         | H2*      | 34               |
|         | H3       | 27               |
|         | H4       | 22               |
|         | H5       | 25               |
|         | H61 (pro-R) | 21          |
|         | H62 (pro-S) | 29          |

a Refer to Figure 1 (see text) for atom nomenclature.

b Hydrogen atoms in streptomycin not included in this list did not have any structural restraints involving them.

* Chemical shifts labelled with a star indicate nuclei that are degenerate.
Supplementary information

Streptomycin solution structure

Figure S1. Temperature coefficient fits

1H (ppm) vs Temperature (K)

- H2O
- S1 HN1
- S1 HO2
- S1 HN3
- S1 H311
- S1 H32*
Figure S2. [1H, 15N]-HSQC spectrum of streptomycin in H2O at 5°C (50mM, pH 6.0) showing resonance assignments for nuclei within the streptidine residue (S1) guanidinium sidechains. One of the four terminal amino groups is clearly in a unique environment. See inset for nomenclature.
Figure S3. [1H]-1D spectrum of solvent-exchangeable nuclei

Figure S3. [1H]-1D spectrum of streptomycin in H_2O at 5°C (50mM, pH 6.0) showing resonance assignments for nuclei that can exchange with the solvent. See inset for nomenclature.
Figure S4. Comparison of the first order libration model (top) with a VDW steric limitation included for the second order of the libration model (bottom).
Figure S5. Likely transient intramolecular hydrogen bonds (dotted lines) present in streptomycin in aqueous solution. Potential hydrogen bonds in both the primary (left) and secondary (right) R2-S3 linkage two conformational families are shown.
**Figure S6.** Comparison of the streptose ring (R2) pucker parameters (Kremer & Pople, JACS, 97, 1354-8) for the aqueous solution 4D-structure of streptomycin determined in this work (grey) with published crystal conformations (colours). Values from 1614 low-energy structures predicted using molecular dynamics simulation are shown in light blue. The distance from the centre of the circle represents the $q$ value (Å) while the polar coordinate represents the $\phi$ angle (°). The $\phi$ angle corresponding to each canonical envelope and twist conformation is given. The bioactive conformation (i.e., when streptomycin is bound to the ribosome, PDB code 1FJG) is shown in blue and the free crystal structure of the streptomycin oxime salt (Neidle et al., 1978) is in green. The conformations of streptomycin bound to artificially-selected RNA aptamers (1NTA, 1NTB) are given in yellow and the three conformations measured from the off-target low affinity co-complex with aminoglycoside-6-adenyl-transferase are given in red (3HAV).
For residual dipolar couplings (RDCs) the dependence on angle is highly non-linear and thus an extra error correction has to be applied. Correction of the error to take this into can be achieved by applying a scaling the error. The scaling (to produce an effective error \( \varepsilon_{\text{exp}}' \)) can be derived in the following way. If \( \theta \) is the angle between the major axis of alignment in the molecular frame, then starting from the equation defining residual dipolar couplings, equation (1) is obtained, which allows the calculation error to be obtained by differentiations, equation (2). Suitable approximations result in equation (3).

\[
\text{RDC} \propto \cos^2 \theta - 1 \quad (1)
\]

\[
\text{Calculation error} = \left| \frac{d}{d\theta} (\cos^2 \theta - 1) \right| \quad (2)
\]

\[
= |2 \sin \theta \cos \theta|
\]

\[
= |\sin 2\theta|
\]

\[
= \frac{1}{2} (1 - \cos 4\theta) \quad (3)
\]

Substituting the identity: 
\[
\cos 4\theta = 8\cos^4 \theta - 8\cos^2 \theta + 1
\]

into (3) and dividing this into the experimental error, results in equations (4) and (5), the latter of which is almost identical to equation (4), but avoids division by zero by having a minimum value of \( \frac{1}{4} \) in the denominator and is therefore used in practice.

\[
\Rightarrow \varepsilon_{\text{exp}}' = \frac{\varepsilon_{\text{exp}}}{4(\cos^2 \theta - \cos^4 \theta)} \quad (4)
\]

\[
\Rightarrow \varepsilon_{\text{exp}}' = \frac{\varepsilon_{\text{exp}}}{0.25 + 3(\cos^4 \theta - \cos^2 \theta)} \quad (5)
\]

Using equation (5), it is possible to increase the total experimental error estimate (\( \varepsilon_{\text{exp}} \)) to take into account errors associated with predictions of residual dipolar couplings, which can then be used to more-accurately assess the degree of fit with the experimental data.