Supplementary Figure 1  Sigma A weighted 2Fo-Fc electron density map for 3-(p-tolyl)allyl-Neu5Ac2en bound to different subtype NAs. The following color scheme is used: A, 09N1 (green), B, 09N1-I149V mutant (orange), C, N8 (blue), D, N5 (magenta) and E, N3 (cyan). The maps are contoured at 1.5 sigma, or only 1 sigma for N8 (C) and N3 (E).

Supplementary Table 1  Data collection and refinement statistics

|                      | 09N1-I149V | 09N1_IG173 | 09N1-I149V_IG173 | N3_IG173 | N5_IG173 |
|----------------------|------------|------------|------------------|----------|----------|
| **Data collection**  |            |            |                  |          |          |
| Spacegroup           | C222₁      | C222₁      | C222₁            | I4       | P4       |
| Cell dimensions      |            |            |                  |          |          |
| a,b,c(Å)             | 118.17,136.54,117.89 | 118.93,138.39,118.88 | 117.59,136.29,117.65 | 106.05,106-05,66.41 | 112.66,112.66, 90.90,90 |
| α, β, γ (°)          | 90,90,90   | 90,90,90   | 90,90,90         | 90,90,90 | 90,90,90 |
| Resolution(Å)        | 50-1.60(1.66-1.60) | 50-1.70(1.76-1.70) | 50-2.00(2.07-2.00) | 0.127(0.552) | 24.7(11.4) |
| Rmerge               | 0.086(0.395) | 0.162(0.467) | 0.127(0.552)     | 0.082(0.135) | 18.7(5.0) |
| Completeness (%)     | 97.9(96.4) | 97.5(86.4) | 99.9(99.9)       | 100.0(99.7) | 100.0(100.0) |
| Redundancy           | 6.6(6.5)   | 6.8(4.6)   | 6.3(6.1)         | 7.2(4.7)  | 6.9(7.1) |
| **Refinement**       |            |            |                  |          |          |
| Resolution(Å)        | 32.79-1.60 | 36.29-1.70 | 49.08-2.00       | 33.54-1.60 | 43.03-1.80 |
| No.reflections       | 121574     | 104138     | 60452            | 48212    | 75083    |
| Rwork/Rfree          | 0.1429/0.1612 | 0.1560/0.1817 | 0.1556/0.1875 | 0.1313/0.1529 | 0.1492/0.1795 |
| Protein              | 6090       | 6046       | 6044             | 3101     | 6158     |
| Ligand/ion           | 5          | 65         | 65               | 32       | 64       |
| Water                | 9514       | 943        | 556              | 538      | 845      |
| B-factors            |            |            |                  |          |          |
| Protein              | 11.3       | 10.0       | 20.3             | 7.9      | 14.3     |
| Ligand/ion           | 10.1       | 13.9       | 23.5             | 10.9     | 12.7     |
| Water                | 30.3       | 26.5       | 30.5             | 23.8     | 28.9     |
| R.m.s.deviation s    |            |            |                  |          |          |
| Bondlengths(Å)       | 0.007      | 0.009      | 0.009            | 0.008    | 0.006    |
| Bondangles(°)        | 1.176      | 1.243      | 1.230            | 1.244    | 1.062    |

*Values in parentheses are for highest-resolution shell. Here 3-(p-tolyl)allyl-Neu5Ac2en is referred to as iG173.*