Applying Molecular Networking for Targeted Isolation of Depsipeptides

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Table S1. HRESIMS data of the compounds 1-10

| Compounds         | formula       | det. m/z  | calc. m/z  | Error (ppm) |
|-------------------|---------------|-----------|------------|-------------|
| Neoantimycin L (1)| $\text{H}^+\text{C}_{37}\text{H}_{46}\text{N}_{2}\text{O}_{12}$ | 711.3106  | 711.3129   | 3.23        |
| Unantimycin B1 (2)| $\text{H}^+\text{C}_{35}\text{H}_{43}\text{NO}_{11}$ | 654.2924  | 654.2914   | 1.53        |
| Unantimycin B2 (3)| $\text{H}^+\text{C}_{35}\text{H}_{43}\text{NO}_{11}$ | 654.2925  | 654.2914   | 1.68        |
| Unantimycin D1 (4)| $\text{H}^+\text{C}_{34}\text{H}_{41}\text{NO}_{11}$ | 640.2747  | 640.2758   | 1.72        |
| Unantimycin D2 (5)| $\text{H}^+\text{C}_{34}\text{H}_{41}\text{NO}_{11}$ | 640.2743  | 640.2758   | 2.34        |
| Neoantimycin G (6)| $\text{H}^+\text{C}_{37}\text{H}_{48}\text{N}_{2}\text{O}_{12}$ | 713.3262  | 713.3286   | 3.36        |
| Unantimycin C1 (7)| $\text{H}^+\text{C}_{35}\text{H}_{45}\text{NO}_{11}$ | 656.3067  | 656.3071   | 0.63        |
| Unantimycin C2 (8)| $\text{H}^+\text{C}_{35}\text{H}_{45}\text{NO}_{11}$ | 656.3069  | 656.3071   | 0.24        |
| Unantimycin E1 (9)| $\text{H}^+\text{C}_{34}\text{H}_{43}\text{NO}_{11}$ | 642.2906  | 642.2914   | 1.25        |
| Unantimycin E2 (10)| $\text{H}^+\text{C}_{34}\text{H}_{43}\text{NO}_{11}$ | 642.2905  | 642.2914   | 1.40        |
Table S2. NMR (600 MHz, DMSO-\textit{d}_6) data for Neoantimycin L (1)

| \( \delta_C \) | \( \delta_H \) | HMBC       | COSY   | ROESY   |
|----------------|----------------|-------------|--------|---------|
| 1              | 202.6          |             |        |         |
| 2              | 76.7           | 5.67, dd (7.6, 5.6) | 1, 3, 12, 13 | 12a, 12b | 14/18, 33 |
| 3              | 167.0          |             |        |         |
| 4              | 75.4           | 5.10, d (5.2) | 3, 5, 19, 20, 21 | 19 | 20, 21 |
| 5              | 168.0          |             |        |         |
| 6              | 55.4           | 5.04, br s  |         | 29      |
| 7              | 70.8           | 5.59, m     | 5, 8   | 6, 29   |
| 8              | 167.7          |             |        |         |
| 9              | 75.6           | 4.83, d (7.6) | 8, 10, 30, 31, 32 | 30 | 31, 32 |
| 10             | 170.9          |             |        |         |
| 11             | 54.1           |             |        |         |
| 12             | 37.1           | 3.16, dd (14.2, 5.6) | 1, 2, 13, 14/18 | 2 | 14/18 |
| 13             | 135.4          |             |        |         |
| 14/18          | 129.6          | 7.20, overlapped | 12, 16 | 15/17   | 2, 12, 34 |
| 15/17          | 128.3          | 7.30, t (7.5) | 13     | 14/18, 16 |
| 16             | 126.9          | 7.25, overlapped |      | 15/17   |
| 19             | 36.4           | 1.79, m     | 3, 4, 20, 21 | 4, 20, 21 |
| 20             | 14.2           | 0.77, overlapped | 4, 19, 21 | 19 | 4 |
| 21             | 23.7           | 1.03, m     | 19, 20 | 19, 35  | 4 |
| 22             | 169.6          |             |        |         |
| 23             | 114.8          |             |        |         |
| 24             | n.o.\(^a\)     |             |        |         |
| 25             | 128.3          |             |        |         |
| 26             | n.o.\(^a\)     | 8.14, m     | 28     | 27      |
| 27             | n.o.\(^a\)     | 6.57, br s  | 26, 28 |         |
| 28             | 123.6          | 7.69, br s  | 27     |         |
| 29             | 15.7           | 1.23, d (6.6) | 6, 7  | 7       | 6, 36 |
| 30             | 35.7           | 1.88, m     | 9, 31, 32 |         |
| 31             | 14.1           | 0.86, d (7.8) | 9, 30, 32 | 30 |
| 32             | 24.0           | 1.43, m     | 9, 30  | 30, 36  |
| 33             | 21.3           | 1.34, s     | 1, 10, 11, 34 | 2 |
| 34             | 21.2           | 1.21, s     | 1, 10, 11, 33 |         |
| 35             | 11.1           | 0.77 overlapped | 19, 21 | 21      |
| 36             | 10.5           | 0.84, t (7.6) | 30, 32 | 32      | 29 |
| 25-NH          |                | 9.70 br s   | CHO    |         |
| CHO            | 159.8          | 8.31, d (2.0) |     | 25-NH   |

\(^a\)Carbon resonances are not observed due to signal broadening
|    | δC  | δH       | HMBC                 | COSY     | ROESY  |
|----|------|----------|----------------------|----------|--------|
| 1  | 202.4| 5.40, dd | 3, 12, 13            | 12a, 12b | 14/18, 33 |
| 2  | 76.4 | 5.02, d  | 3, 5, 19, 20, 21     | 19       | 20, 21 |
| 3  | 168.09|         |                      |          |        |
| 4  | 75.5 | 5.16, dd | 5, 7, 22             | 7, 6-NH  | 29     |
| 5  | 168.5|         |                      |          |        |
| 6  | 55.4 | 5.16, dd | 5, 8, 29             | 6, 29    | 6-NH   |
| 7  | 167.7|         |                      |          |        |
| 8  | 75.5 | 5.21, d  | 8, 10, 30, 31, 32    | 30       | 31, 32 |
| 9  | 170.8|         |                      |          |        |
| 10 | 170.8|         |                      |          |        |
| 11 | 129.2| 7.28, overlapped | 12, 16 | 15/17 | 2, 12 |
| 12 | 36.6 | 3.16, m | 1, 2, 13, 14/18      | 2        | 33, 14/18 |
| 13 | 136.2|         |                      |          | 14/18  |
| 14/18 | 129.2| 7.28, overlapped | 12, 16 | 15/17 | 2, 12 |
| 15/17 | 128.5| 7.33, overlapped | 13 | 14/18, 16 |        |
| 16 | 126.9| 7.25, m | 14/18 | 15/17 |       |
| 17 | 29.7 | 2.24, m | 3, 4, 20, 21 | 4, 20, 21 |       |
| 18 | 16.1 | 0.79, d | 4, 19, 21 | 19 |       |
| 19 | 18.3 | 0.91, d | 4, 19, 20 | 19 |       |
| 20 | 167.9|         |                      |          |        |
| 21 | 135.2|         |                      |          |        |
| 22 | 114.8| 7.28, overlapped | 26, 28 |       |        |
| 23 | 157.2|         |                      |          |        |
| 24 | 118.5| 6.94, dd | 24, 28 | 27 |       |
| 25 | 129.2| 7.28, overlapped | 23, 25 | 26, 28 |       |
| 26 | 118.5| 7.35, overlapped | 22, 24, 26 | 27 |       |
| 27 | 16.1 | 1.21, d | 6, 7 | 7 | 35 |
| 28 | 36.7 | 1.81, m | 8, 9, 31, 32 | 31, 32 |       |
| 29 | 13.8 | 0.87, d | 9, 30, 32 | 30 |       |
| 30 | 24.0 | 1.45, m | 9, 30, 31 | 30 |       |
| 31 | 1.45, m | 9, 30, 31 | 30 |       | |
| 32 | 21.3 | 1.41, s | 1, 10, 11, 34 | 2, 12a |       |
| 33 | 20.7 | 1.32, s | 1, 10, 11, 33 |       |       |
| 34 | 10.6 | 0.87, t | 30, 32 |       |       |
| 35 | 8.72 | d (9.2) | 5, 7, 22 | 6 |       |
| 36 | 9.66 | s       |                      |          |        |

Table S3. NMR (600 MHz, DMSO-d6) data for Unantimycin B1 (2)
Table S4. NMR (600 MHz, DMSO-\(d_6\)) data for Unantimycin B2 (3)

|     | \(\delta_C\) | \(\delta_H\)       | HMBC          | COSY    | ROESY   |
|-----|---------------|---------------------|---------------|---------|---------|
| 1   | 202.6         |                     |               |         |         |
| 2   | 76.4          | 5.68, dd (7.7, 5.6) | 1, 3, 12, 13  | 12      | 14/18, 33 |
| 3   | 167.2         |                     |               |         |         |
| 4   | 75.9          | 5.05, d (5.4)       | 3, 19, 20, 21 | 19      | 20, 21  |
| 5   | 168.2         |                     |               |         |         |
| 6   | 55.9          | 4.95, dd (8.4, 3.1) | 5             | 7, 6-NH | 29      |
| 7   | 70.6          | 5.55, m             | 5, 8          | 6, 29   |         |
| 8   | 167.7         |                     |               |         |         |
| 9   | 75.6          | 4.86, d (7.7)       | 10, 30, 31, 32| 30      | 31, 32  |
| 10  | 171.0         |                     |               |         |         |
| 11  | 54.2          |                     |               |         |         |
| 12  | 37.1          | 3.16, dd (14.1, 7.8)| 1, 2, 13, 14/18| 2       | 33, 34  |
|     |               | 3.06, dd (14.1, 7.8)|               |         |         |
| 13  | 135.4         |                     |               |         |         |
| 14/18| 129.6        | 7.20, overlapped    | 12, 16        | 15/17   | 2, 12   |
| 15/17| 128.4        | 7.28, overlapped    | 13            | 14/18, 16|         |
| 16  | 127.0         | 7.25, m             | 14/18         | 15/17   |         |
| 19  | 30.0          | 2.01, m             | 3, 4, 20, 21  | 4, 20, 21|         |
| 20  | 16.8          | 0.69, d (6.8)       | 4, 19, 21     | 19      | 4       |
| 21  | 17.9          | 0.83, overlapped    | 4, 19, 20     | 19      | 4       |
| 22  | 167.8         |                     |               |         |         |
| 23  | 135.0         |                     |               |         |         |
| 24  | 114.7         | 7.28, overlapped    | 26, 28        |         |         |
| 25  | 157.2         |                     |               |         |         |
| 26  | 118.5         | 6.95, dd (7.6, 1.8) | 24, 28        | 9       |         |
| 27  | 129.2         | 7.28, overlapped    | 23, 25        | 26, 28  |         |
| 28  | 118.5         | 7.34, br d (7.8)    | 22, 24, 26    | 27      |         |
| 29  | 15.6          | 1.23, d (6.3)       | 6, 7          | 7       | 6, 31   |
| 30  | 35.7          | 1.88, qd (7.6, 3.6) |               | 9, 31   |         |
| 31  | 14.0          | 0.86, d (6.9)       | 9, 30, 32     | 30      |         |
|     |               | 1.43, m             |               |         |         |
|     |               | 1.13, m             |               |         |         |
| 32  | 24.0          |                     | 9, 30, 31, 35| 30, 35  |         |
| 33  | 21.1          | 1.34, s             | 1, 10, 11, 34|         |         |
| 34  | 21.3          | 1.20, s             | 1, 10, 11, 33|         |         |
| 35  | 10.4          | 0.83, overlapped    | 32, 30        | 32      |         |
| 6-NH| 8.67, d (8.3) |                     | 22            | 6       |         |
| 25-OH|             | 9.68, s             |               |         |         |
|  | δ<sub>C</sub> | δ<sub>H</sub> | HMBC | COSY | ROESY |
|---|---|---|---|---|---|
| 1 | 202.5 |  |  |  |  |
| 2 | 76.4 | 5.37, dd (10.0, 2.6) | 1, 3, 12, 13 | 12a, 12b | 14/18, 33 |
| 3 | 168.1 |  |  |  |  |
| 4 | 75.5 | 5.01, d (2.9) | 3, 5, 19, 20, 21 | 19 | 20, 21 |
| 5 | 168.6 |  |  |  |  |
| 6 | 55.4 | 5.15, overlapped | 5, 7, 22, 29 | 7, 6-NH | 29 |
| 7 | 70.2 | 5.66, qd (6.4, 4.0) | 5, 8, 29 | 6, 29 | 6-NH |
| 8 | 167.7 |  |  |  |  |
| 9 | 76.8 | 5.15, overlapped | 8, 10, 30, 31, 32 | 30 | 31, 32 |
| 10 | 170.9 |  |  |  |  |
| 11 | 55.2 |  |  |  |  |
| 12 | 36.6 | 3.15, dd (14.9, 2.7) | 1, 2, 13, 14/18 | 2 | 33, 14/18 |
|  |  | 2.91, dd (14.9, 2.7) | 14/18 |  | 14/18 |
| 13 | 136.3 |  |  |  |  |
| 14/18 | 129.3 | 7.28, overlapped | 12, 16 | 15/17 | 2, 12 |
| 15/17 | 128.6 | 7.34, overlapped | 13 | 14/18, 16 |  |
| 16 | 127.0 | 7.25, m | 14/18 | 15/17 |  |
| 19 | 29.7 | 2.25, m | 3, 4, 20, 21 | 4, 20, 21 |  |
| 20 | 16.1 | 0.80, d (6.9) | 4, 19, 21 | 19 |  |
| 21 | 18.3<sup>a</sup> | 0.92, d (6.9) | 4, 19, 20 | 19 |  |
| 22 | 168.0 |  |  |  |  |
| 23 | 135.2 |  |  |  |  |
| 24 | 114.8 | 7.28, overlapped | 25, 26, 28 |  |  |
| 25 | 157.2 |  |  |  |  |
| 26 | 118.5 | 6.94, ddd (8.0, 2.5, 1.1) | 24, 25, 28 | 27 |  |
| 27 | 129.3 | 7.28, overlapped | 23, 25 | 26, 28 |  |
| 28 | 118.5 | 7.35, overlapped | 22, 24, 26 | 27 |  |
| 29 | 16.2 | 1.21, d (6.4) | 6, 7 | 7 |  |
| 30 | 30.7 | 1.99, m | 8, 9, 31, 32 | 9, 31, 32 |  |
| 31 | 17.6<sup>a</sup> | 0.88, d (8.4) | 9, 30, 32 | 30 |  |
| 32 | 18.0<sup>a</sup> | 0.90, d (8.4) | 9, 30, 31 | 30 |  |
| 33 | 21.3 | 1.43, s | 1, 10, 11, 34 | 2, 12a |  |
| 34 | 20.6 | 1.33, s | 1, 10, 11, 33 |  |  |
| 6-NH | 8.70, d (9.2) | 5, 7, 22 | 6 |  |  |
| 25-OH | 9.70, s |  |  |  |  |

<sup>a</sup> values are interchangeable
### Table S6. NMR (600 MHz, DMSO-\textit{d}_6) data for Unantimycin D2 (5)

|   | δ\textsubscript{C} | δ\textsubscript{H} | HMBC | COSY | ROESY |
|---|------------------|------------------|------|------|--------|
| 1 | 202.6            |                  |      |      |        |
| 2 | 76.4             | 5.69, dd (7.8, 5.6) | 1, 3, 12, 13 | 12a, 12b | 14/18, 33 |
| 3 | 167.2            |                  |      |      |        |
| 4 | 76.0             | 5.05, d (5.3)    | 3, 5, 19, 20, 21 | 19 | 20, 21 |
| 5 | 168.3            |                  |      |      |        |
| 6 | 55.9             | 4.94, dd (8.3, 3.1) | 5, 7, 22 | 7, 6-NH | 29 |
| 7 | 70.6             | 5.54, qd (6.4, 3.0) | 5, 8 | 6, 29 | 6-NH |
| 8 | 167.8            |                  |      |      |        |
| 9 | 76.7             | 4.80, d (7.2)    | 8, 10, 30, 31, 32 | 30 | 31, 32 |
| 10| 171.0            |                  |      |      |        |
| 11| 54.2             |                  |      |      |        |
| 12| 37.2             | 3.15, dd (14.1, 5.6) | 1, 2, 13, 14/18 | 2 | 14/18 |
|   |                  | 3.06, dd (14.0, 7.8) |      |      | 14/18  |
| 13| 135.4            |                  |      |      |        |
| 14/18| 129.6         | 7.20, m         | 12, 16 | 15/17 | 2, 12  |
| 15/17| 128.4         | 7.28, overlapped | 13 | 14/18, 16 |        |
| 16| 127.0            | 7.25, m         | 14/18 | 15/17 |        |
| 19| 30.0             | 2.03, overlapped | 3, 4, 20, 21 | 4, 20, 21 |        |
| 20| 16.8             | 0.69, d (6.8)   | 4, 19, 21 | 19 |        |
| 21| 17.6\textsuperscript{a} | 0.82, d (6.9) | 4, 19, 20 | 19 |        |
| 22| 167.6            |                  |      |      |        |
| 23| 135.0            |                  |      |      |        |
| 24| 114.8            | 7.28, overlapped | 26, 28 |      |        |
| 25| 157.3            |                  |      |      |        |
| 26| 118.6            | 6.95, ddd (8.0, 2.6, 1.1) | 24, 28 | 27 |        |
| 27| 129.3            | 7.28, overlapped | 23, 25 | 26, 28 |        |
| 28| 118.5            | 7.34, m         | 22, 24, 26 | 27 |        |
| 29| 15.7             | 1.23, d (6.5)   | 6, 7 | 7 |        |
| 30| 29.8             | 2.03, overlapped | 8, 9, 31, 32 | 9, 31, 32 |        |
| 31| 17.7\textsuperscript{a} | 0.88, dd (6.8, 2.6) | 9, 30, 32 | 30 |        |
| 32| 17.9\textsuperscript{a} | 0.88, dd (6.8, 2.6) | 9, 30, 31 | 30 |        |
| 33| 21.3             | 1.34, s         | 1, 10, 11, 34 | 2 |        |
| 34| 21.2             | 1.21, s         | 1, 10, 11, 33 | 12 |        |
| 6-NH|               | 8.65, d (8.3)   | 5, 7, 22 | 6 |        |
| 25-OH|              | 9.76, br s      |      |      |        |
|      | δC  | δH          | HMBC         | COSY    | ROESY |
|------|-----|-------------|--------------|---------|-------|
| 1    | 77.8 | 3.29, d (10.5) | 10, 11, 12, 33 | 1-OH    | 2, 12b, 33, 34 |
| 2    | 71.6 | 5.45, dd (10.2, 4.6) | 1, 3, 12, 13 | 12      | 12b, 14/18, 34 |
| 3    | 167.6 |             |             |         |       |
| 4    | 75.7 | 5.34, d (3.7) | 3, 5, 19, 20, 21 | 19      | 20, 21 |
| 5    | 167.6 |             |             |         |       |
| 6    | 55.3 | 5.14, dd (8.7, 3.3) |             | 7       | 29    |
| 7    | 70.7 | 5.56, m    | 5, 22        | 6, 29   |       |
| 8    | 168.1 |            |             |         |       |
| 9    | 74.6 | 4.57, d (8.3) | 8, 10, 30, 31, 32 | 30      | 31, 32 |
| 10   | 174.8 |            |             |         |       |
| 11   | 45.4 |            |             |         |       |
| 12   | 39.2 | 3.04, dd (14.2, 10.2) | 1, 2, 13, 14/18 | 2       | 14/18 |
|      |      | 2.97, dd (14.2, 4.6) |             |         | 1, 14/18 |
| 13   | 137.5 |            |             |         |       |
| 14/18| 129.0 | 7.22, overlapped | 12, 16     | 15/17   | 2, 12 |
| 15/17| 128.3 | 7.28, m     | 13          | 14/18, 16 |       |
| 16   | 126.4 | 7.20, overlapped | 14/18     | 15/17   |       |
| 19   | 36.6 | 1.50, m     | 3, 4, 20, 21 | 4, 20, 21 |       |
| 20   | 14.4 | 0.60, overlapped | 4, 19, 21  |         | 19   |
| 21   | 22.9 | 0.8, m      | 19, 20      |         | 19, 35 |
| 22   | 170.0 |            |             |         |       |
| 23   | 114.7 |            |             |         |       |
| 24   | n.o. |            |             |         |       |
| 25   | 127.4 |            |             |         |       |
| 26   | n.o. | 8.19, d (7.8) | 24, 28      |         | 27    |
| 27   | n.o. | 6.81, br s   | 26, 28      |         |       |
| 28   | 123.6 | 7.87, d (7.2) |             |         | 27    |
| 29   | 15.6 | 1.21, d (6.4) | 6, 7        | 7       | 6, 36 |
| 30   | 35.4 | 1.85, m     |             | 9, 31, 32 |       |
| 31   | 14.0 | 0.87, d (7.1) | 9, 30, 32   |         | 30    |
| 32   | 24.3 | 1.50, m     |             | 30, 36  |       |
| 33   | 26.2 | 1.31, s     | 1, 10, 11, 34 | 1-OH   |       |
| 34   | 22.0 | 1.25, s     | 1, 10, 11, 33 | 1, 2    |       |
| 35   | 11.1 | 0.60 overlapped | 19, 21      |         | 21    |
| 36   | 10.3 | 0.84, d (7.6) | 30, 32      |         | 32    |
| CHO  | 159.8 | 8.32, d (1.7) |             | 25-NH   |       |
| 1-OH | 4.39 | 4.39, d (10.4) |             |         | 1     |
|      | \( \delta_C \) | \( \delta_H \) | HMBC | COSY | ROESY |
|------|----------------|----------------|------|------|-------|
| 24-OH |                | 12.7, s        |      |      |       |
| 25-NH |                | 9.78 br s      |      |      | CHO   |

*Carbon resonances are not observed due to signal broadening*
|   | Experimental δ<sub>C</sub> (125 MHz) | Literature<sup>a</sup> δ<sub>C</sub> (100 MHz) | Experimental δ<sub>H</sub> (600 MHz) | Literature<sup>a</sup> δ<sub>H</sub> (400 MHz) |
|---|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| 1 | 77.8                             | 77.9                             | 3.29, d (10.5)                   | 3.28, d (10.4)                   |
| 2 | 71.6                             | 71.6                             | 5.45, dd (10.2, 4.6)             | 5.44, dd (10.2, 4.5)             |
| 3 | 167.6                            | 167.6                            | 5.34, d (3.7)                    | 5.33, d (3.6)                    |
| 4 | 75.7                             | 75.8                             | 5.14, dd (8.7, 3.3)              | 5.15, dd (8.7, 3.1)              |
| 5 | 70.7                             | 70.6                             | 5.56, m                         | 5.55, qd (6.5, 3.1)              |
| 6 | 168.1                            | 168.1                            | 4.57, d (8.3)                    | 4.56, d (8.3)                    |
| 7 | 174.8                            | 174.8                            |                                  |                                  |
| 8 | 45.4                             | 45.4                             | 3.04, dd (14.2, 10.2)            | 3.03, dd (14.2, 10.2)            |
| 9 |                                  |                                  | 2.97, dd (14.2, 4.6)             | 2.95, dd (14.2, 4.5)             |
| 10|                                 |                                  |                                  |                                  |
| 11| 39.2                             | 39.2                             |                                  |                                  |
| 12| 137.5                            | 137.6                            | 7.22, overlapped                 | 7.22, d (7.5)                    |
| 13| 128.3                            | 128.3                            | 7.28, m                         | 7.27, dd (7.3, 7.5)              |
| 14/18| 126.4                           | 126.4                            | 7.20, overlapped                 | 7.19, t (7.3)                    |
| 15/17| 36.6                             | 36.6                             | 1.50, m                         | 1.50, m                         |
| 16| 14.4                             | 14.3                             | 0.60, overlapped                 | 0.58, d (6.8)                    |
| 17| 22.9                             | 22.9                             | 0.8, m                          | 0.80, m                         |
| 18| 127.4                            | 127.1                            |                                  |                                  |
| 19| n.o.<sup>b</sup>                 | 150.7                            |                                  |                                  |
| 20| 125.0                            | 125.0                            | 8.19, d (7.8)                    | 8.20, d (7.7)                    |
| 21| n.o.<sup>b</sup>                 | n.o.<sup>b</sup>                 | 6.81, br s                      | 6.90, br s                      |
| 22| 123.6                            | 123.6                            | 7.87, d (7.2)                    | 7.92, br s                      |
| 23| 15.6                             | 15.5                             | 1.21, d (6.4)                    | 1.20, d (6.5)                    |
| 24| 35.4                             | 35.4                             | 1.85, m                         | 1.84, dqd (8.3, 7.0, 3.6)        |
| 25| 14.0                             | 14.0                             | 0.87, d (7.1)                    | 0.86, d (7.0)                    |
| 26| 24.3                             | 24.3                             | 1.50, m                         | 1.49, m                         |
| 27| 26.2                             | 26.2                             | 1.31, s                         | 1.30, s                         |
| 28| 22.0                             | 22.0                             | 1.25, s                         | 1.25, s                         |
| 29| 11.1                             | 11.1                             | 0.60 overlapped                 | 0.59, t (6.8)                    |
| 30| 10.3                             | 10.2                             | 0.84, d (7.6)                    | 0.84, dd (7.5, 7.5)             |
| 31| 159.8                            | 160.4                            | 8.32, d (1.7)                    | 8.31, d (1.8)                    |

<sup>a</sup> Literature values are provided for comparison.
|          |   |          |
|----------|---|----------|
| 1-OH     | 4.39, d (10.4) | 4.39, d (10.4) |
| 24-OH    | 12.7, s         | 12.8, br s    |
| 25-NH    | 9.78 br s       | 9.81, br s    |

\(^a\) Salim, A. A. *et al.* *Org. Lett.* 2014, 16 (19), 5036–5039.
|   | δ_C     | δ_H     | HMBC             | COSY   | ROESY   |
|---|---------|---------|------------------|--------|---------|
| 1 | 75.8    | 3.48, overlapped | 10, 11, 12, 33, 34 | 1-OH   | 33, 34  |
| 2 | 76.7    | 5.42, t (7.0)  | 3, 12, 13        | 12a, 12b | 14/18, 33, 34 |
| 3 | 168.4^a | 5.17, d (4.6)  | 3, 5, 19, 20, 21 | 19     | 20, 21  |
| 4 | 76.9    | 5.80, qd (6.4, 2.9) | 8       | 6, 29  | 6-NH    |
| 5 | 170.4   | 7.29, overlapped | 8, 10, 30, 31, 32 | 30     | 31, 32  |
| 6 | 174.2   | 7.29, overlapped | 1, 2, 13, 14/18 | 2      | 33, 14/18 |
| 10 | 129.3  | 7.29, overlapped | 12, 16 | 15/17  | 2, 12   |
| 11 | 128.4  | 7.29, overlapped | 13     | 14/18, 16 |
| 12 | 126.4  | 7.20, overlapped | 14/18  | 15/17  |
| 13 | 137.4  | 7.29, overlapped | 4, 20, 21 | 19     |
| 14/18 | 126.4  | 7.20, overlapped | 4, 19, 21 | 19     |
| 15/17 | 137.4  | 7.29, overlapped | 4, 19, 20 | 19     |
| 15/17 | 135.1  | 7.29, overlapped | 22, 26, 28 |
| 16 | 128.4  | 7.29, overlapped | 22, 26, 28 |
| 17 | 15.9   | 1.28, d (6.4)  | 6, 7   | 35, 6-NH |
| 18 | 15.9   | 1.28, d (6.4)  | 6, 7   | 35, 6-NH |
| 19 | 15.9   | 1.28, d (6.4)  | 6, 7   | 35, 6-NH |
| 20 | 15.9   | 1.28, d (6.4)  | 6, 7   | 35, 6-NH |
| 21 | 15.9   | 1.28, d (6.4)  | 6, 7   | 35, 6-NH |
| 22 | 15.9   | 1.28, d (6.4)  | 6, 7   | 35, 6-NH |
| 23 | 15.9   | 1.28, d (6.4)  | 6, 7   | 35, 6-NH |
| 24 | 15.9   | 1.28, d (6.4)  | 6, 7   | 35, 6-NH |
| 25 | 15.9   | 1.28, d (6.4)  | 6, 7   | 35, 6-NH |
| 26 | 15.9   | 1.28, d (6.4)  | 6, 7   | 35, 6-NH |
| 27 | 15.9   | 1.28, d (6.4)  | 6, 7   | 35, 6-NH |
| 28 | 15.9   | 1.28, d (6.4)  | 6, 7   | 35, 6-NH |
| 29 | 15.9   | 1.28, d (6.4)  | 6, 7   | 35, 6-NH |
| 30 | 15.9   | 1.28, d (6.4)  | 6, 7   | 35, 6-NH |
| 31 | 15.9   | 1.28, d (6.4)  | 6, 7   | 35, 6-NH |
| 32 | 15.9   | 1.28, d (6.4)  | 6, 7   | 35, 6-NH |
| 33 | 15.9   | 1.28, d (6.4)  | 6, 7   | 35, 6-NH |
| 34 | 15.9   | 1.28, d (6.4)  | 6, 7   | 35, 6-NH |
| 35 | 15.9   | 1.28, d (6.4)  | 6, 7   | 35, 6-NH |
| 1-OH| 4.85, br d (10.3) | 1, 10, 11, 34 | 2, 12  |
| 6-NH| 8.77, d (8.9)  | 22, 6   | 28, 29  |
a values are interchangeable
|   | δ_C | δ_H         | HMBC                  | COSY                   | ROESY            |
|---|-----|-------------|-----------------------|------------------------|------------------|
| 1 | 77.9| 3.29, d (10.6)| 10, 11, 12, 33, 34   | 1-OH                  | 12b, 34         |
| 2 | 71.8| 5.38, dd (10.4, 4.4)| 3, 12                | 12                    | 14/18, 34       |
| 3 | 168.1|                       |                       |                        |                 |
| 4 | 75.4| 5.29, d (3.4)       | 3, 5, 19, 20, 21     | 19                    | 20, 21          |
| 5 | 167.8|                       |                       |                        |                 |
| 6 | 55.5| 5.05, dd (9.1, 3.4)  | 5, 7, 22              | 7, 6-NH               | 29              |
| 7 | 71.0| 5.50, qd (6.4, 3.4)  | 8                     | 6, 29                 |                 |
| 8 | 168.1|                       |                       |                        |                 |
| 9 | 74.4| 4.57, d (8.6)       | 8, 10, 30, 31, 32    | 30                    | 31, 32          |
| 10| 174.9|                       |                       |                        |                 |
| 11| 45.3|                       |                       |                        |                 |
| 12| 39.2| 3.04, dd (14.0, 10.4)| 1, 2, 13, 29, dd (14.0, 4.4)| 14/18            | 33, 14/18       |
| 13| 137.6|                       |                       |                        |                 |
| 14/18| 129.1| 7.23, overlapped| 12, 16               | 15/17                 | 2, 12           |
| 15/17| 128.3| 7.28, overlapped| 13                   | 14/18, 16            |                 |
| 16| 126.4| 7.20, overlapped| 14/18                | 15/17                 |                 |
| 19| 30.3| 1.70, m          | 4                     | 19, 21                | 19              |
| 20| 15.9| 0.30, d (6.9)    | 4, 19, 20             | 19                    |                 |
| 21| 18.4| 0.67, d (6.9)    | 4, 19, 20             | 19                    |                 |
| 22| 167.8|                       |                       |                        |                 |
| 23| 135.2|                       |                       |                        |                 |
| 24| 114.8| 7.28, overlapped| 22, 26, 28           |                       |                 |
| 25| 157.2|                       |                       |                        |                 |
| 26| 118.5| 6.94, ddd (8.0, 2.5, 1.0)| 24, 25, 28          | 27                    |                 |
| 27| 129.2| 7.28, overlapped| 25                   | 26, 28                |                 |
| 28| 118.5| 7.34, br d (7.7)  | 22, 24, 26            | 27                    |                 |
| 29| 15.4| 1.20, d (6.4)    | 6, 7                 | 7                     | 35, 6-NH        |
| 30| 35.4| 1.84, m         | 9, 31                | 9, 31, 32             |                 |
| 31| 13.9| 0.86, d (6.9)   | 9, 30, 32             |                       | 30              |
| 32| 24.2| 1.49, m         | 31                   | 30, 35                |                 |
| 33| 26.3| 1.30, s         | 1, 10, 11, 34        |                       | 1, 1-OH        |
| 34| 21.9| 1.26, s         | 1, 10, 11, 33        |                       | 1, 2           |
| 35| 10.2| 0.83, t (7.5)   | 30, 32               | 32                    | 29, 32         |
|   | δ<sub>C</sub> | δ<sub>H</sub>     | HMBC | COSY | ROESY |
|---|-------------|----------------|------|------|-------|
| 1-OH | 4.38, d (10.5) | 1          | 1           | 33    |
| 6-NH | 8.65, d (9.1)  | 5, 22      | 6           | 28, 29|
| 25-OH | 9.67 br, s      |            |             |       |
Table S10. $^1$H NMR (600 MHz CDCl$_3$) Data for the MTPA Esters of Bhdo

|  | $S$-MTPA ester $\delta_H$ (multiplicity, $J$ Hz) | $R$-MTPA ester $\delta_H$ (multiplicity, $J$ Hz) | $\Delta \delta$ ($\delta_S$ - $\delta_R$) values |
|---|---|---|---|
| 2-Me | 1.34, s | 1.33, s | +0.01 |
| | 1.12, s | 1.06, s | +0.06 |
| 3 | 5.47, d (3.8) | 5.51, d (3.7) | -0.04 |
| 4 | 4.83, dt (9.4, 3.8) | 4.89, dt (9.4, 3.8) | -0.06 |
| 5 | 2.78, dd (14.7, 9.4) | 2.82, dd (14.7, 9.4) | -0.04 |
| | 2.69, dd (14.9, 4.0) | 2.76, dd (14.7, 3.8) | -0.07 |
| 7/11 | 7.13, d (6.8) | 7.18, d (7.4) | -0.05 |
| 8/10 | 7.48, m | 7.48, m | |
| 9 | 7.53, m | 7.53, m | |
Figure S1 MS/MS spectrum of NAT-H (A), NAT-L (B), UAT-B1&B2 (C), and UAT-D1&D2 (D)
Figure S2a $^1$H NMR spectrum of 1 in DMSO-$d_6$
Figure S2b $^{13}$C NMR spectrum of 1 in DMSO-$d_6$
Figure S2c HSQC spectrum of 1 in DMSO-$d_6$
Figure S2d HMBC spectrum of 1 in DMSO-$_d_6$
Figure S2e $^1$H-$^1$H COSY spectrum of 1 in DMSO-$d_6$
Figure S2f ROESY spectrum of 1 in DMSO-$d_6$
Figure S3a $^1$H NMR spectrum of 2 in DMSO-$d_6$.
Figure S3b $^{13}$C NMR spectrum of 2 in DMSO-$d_6$
Figure S3c HSQC spectrum of 2 in DMSO-$d_6$
Figure S3d HMBC spectrum of 2 in DMSO-$d_6$
Figure S3e $^1$H-$^1$H COSY spectrum of 2 in DMSO-$d_6$. 
Figure S3f ROESY spectrum of 2 in DMSO-$d_6$
Figure S4a $^1$H NMR spectrum of 3 in DMSO-$d_6$
Figure S4b $^{13}$C NMR spectrum of 3 in DMSO-$d_6$
Figure S4c HSQC spectrum of 3 in DMSO-$d_6$
Figure S4d HMBC spectrum of 3 in DMSO-\textsubscript{d\textsubscript{6}}}
Figure S4e $^1$H-$^1$H COSY spectrum of 3 in DMSO-$d_6$
Figure S4f ROESY spectrum of 3 in DMSO-$d_6$
Figure S5a $^1$H NMR spectrum of 4 in DMSO-$d_6$
Figure S5b $^{13}$C NMR spectrum of 4 in DMSO-$d_6$
Figure S5c HSQC spectrum of 4 in DMSO-$d_6$
Figure S5d HMBC spectrum of 4 in DMSO-$d_6$
Figure S5e $^1$H-$^1$H COSY spectrum of 4 in DMSO-$d_6$
Figure S5f ROESY spectrum of 4 in DMSO-$d_6$
Figure S6a $^1$H NMR spectrum of 5 in DMSO-$d_6$
Figure S6b $^{13}$C NMR spectrum of 5 in DMSO-$d_6$
Figure S6c HSQC spectrum of 5 in DMSO-$d_6$
Figure S6d HMBC spectrum of 5 in DMSO-$d_6$
Figure S6e $^1$H-$^1$H COSY spectrum of 5 in DMSO-$d_6$
Figure S6f ROESY spectrum of 5 in DMSO-d$_6$
Figure S7a $^1$H NMR spectrum of 6 in DMSO-$d_6$
Figure S7b $^{13}$C NMR spectrum of 6 in DMSO-$d$
Figure S7c HSQC spectrum of 6 in DMSO-$d_6$
Figure S7d HMBC spectrum of 6 in DMSO-d$_6$
Figure S7e $^1$H-$^1$H COSY spectrum of 6 in DMSO-$d_6$
Figure S7f ROESY spectrum of 6 in DMSO-$d_6$
Figure S7g HETLOC spectrum of 6 in DMSO-$d_6$
Figure S8a $^1$H NMR spectrum of 7 in DMSO-$d_6$
Figure S8b $^{13}$C NMR spectrum of 7 in DMSO-$d_6$
Figure S8c HSQC spectrum of 7 in DMSO-$d_6$
Figure S8d HMBC spectrum of 7 in DMSO-$d_6$
Figure S8e $^1$H-$^1$H COSY spectrum of 7 in DMSO-$d_6$
Figure S8f ROESY spectrum of 7 in DMSO-$d_6$
Figure S8g HETLOC spectrum of 7 in DMSO-$d_6$
Figure S9a $^1$H NMR spectrum of 8 in DMSO-$d_6$
Figure S9b $^{13}$C NMR spectrum of 8 in DMSO-$d_6$
Figure S9c HSQC spectrum of 8 in DMSO-$d_6$
Figure S9d HMBC spectrum of 8 in DMSO-$d_6$
Figure S9e $^1$H-$^1$H COSY spectrum of 8 in DMSO-$d_6$
Figure S9f ROESY spectrum of 8 in DMSO-$d_6$
Figure S9g HETLOC spectrum of 8 in DMSO-$d_6$
Figure S10 $^1$H NMR spectrum of 9 in DMSO-$d_6$
Figure S11 $^1$H NMR spectrum of 10 in DMSO-$d_6$
Figure S12 Relative Stereochemical Analysis of NATs

(A) The Relative stereochemistry of C-1 and C-2; (B) The Relative stereochemistry of C-9 and C-30
Figure S13 LC-MS chromatogram of extracted ion at 333 [M - H]^− and 347 [M - H]^− from Mosher’s esterification reaction of 6-10

(i) L-Ila reacted with R-MTPA-Cl, (ii) D-Ila reacted with R-MTPA-Cl, (iii) (2R)-Hia reacted with R-MTPA-Cl, (iv) (2S)-Hia reacted with R-MTPA-Cl, (v) Hydrolysate of 6 reacted with R-MTPA-Cl, (vi) Hydrolysate of 7 reacted with R-MTPA-Cl, (vii) Hydrolysate of 8 reacted with R-MTPA-Cl, (viii) Hydrolysate of 9 reacted with R-MTPA-Cl, (ix) Hydrolysate of 10 reacted with R-MTPA-Cl
Figure S14 LC-MS chromatogram of extracted ion at 437 [M + H]+ from Mosher’s esterification reaction of 6-10

(i) (4R, 5R)-Bhdo reacted with R-MTPA-Cl, (ii) (4R, 5R)-Bhdo reacted with S-MTPA-Cl, (iii) Hydrolysed sample of 6 reacted with R-MTPA-Cl, (iv) Hydrolysed sample of 7 reacted with R-MTPA-Cl, (v) Hydrolysed sample of 8 reacted with R-MTPA-Cl, (vi) Hydrolysed sample of 9 reacted with R-MTPA-Cl, (vii) Hydrolysed sample of 10 reacted with R-MTPA-Cl
Figure S15 LC-MS chromatogram of extracted ion at 412 [M - H]^+ from FDLA 6-10

(i) Standard L-threonine reacted with L-FDLA or D-FDLA to give (a) L-FDAA-L-Thr, (b) D-FDAA- L-Thr
(ii) Standard L-allo-Thr reacted with L-FDLA or D-FDLA to give (a) L-FDAA-L-allo-Thr, (b) D-FDAA- L-allo-Thr
(iii) Hydrolysate of 6 reacted with D-FDLA
(iv) Hydrolysate of 7 reacted with D-FDLA
(v) Hydrolysate of 8 reacted with D-FDLA
(vi) Hydrolysate of 9 reacted with D-FDLA
(vii) Hydrolysate of 10 reacted with D-FDLA