Supporting Information

for

Synthesis of antibacterial 1,3-diyne-linked peptoids from an Ugi-4CR/Glaser coupling approach

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Complete experimental procedures, characterization and figures of \( ^1\text{H} \) and \( ^{13}\text{C} \) NMR spectra
Experimental part

General

All commercially available chemicals were used without further purification. \(^1\text{H}\) NMR (CDCl\(_3\), 400 MHz) and \(^{13}\text{C}\) NMR (CDCl\(_3\), 100 MHz) spectra were recorded in CDCl\(_3\) solutions on a Varian Mercury 400 spectrometer at 400 \((^{1}\text{H})\) and 100 MHz \((^{13}\text{C})\), respectively. Chemical shifts (δ) are reported in ppm relative to TMS \((^{1}\text{H}\text{NMR})\) and to residual CDCl\(_3\) signal \((^{13}\text{C}\text{NMR})\). High resolution ESI mass spectra were obtained from a Bruker Apex III Fourier transform ion cyclotron resonance (FT–ICR) mass spectrometer equipped with an Infinity™ cell, a 7.0 Tesla superconducting magnet, an RF-only hexapole ion guide and an external electrospray ion source (Agilent, off axis spray). ESI-MS was recorded on a Finnigan TSQ 7000, LC-Tech Ultra Plus pumps, Linear UV–vis 200 detector, Sepserve Ultrasep ES RP-18 5 μm 1 × 100 mm column, flow 70 μL min\(^{-1}\). Flash column chromatography was carried out using Merck silica gel 60 (0.040–0.063 mm) and analytical thin-layer chromatography (TLC) was performed using Merck silica gel 60 F254 aluminium sheets. HPLC experiments were performed in an Agilent 1100 series equipped with a column SNr. 176: YMC pack 150 × 4.6 LD 102 Å 5 μm ODS-A and UV detector (200–600 nm). The employed gradient was MeOH 0.1% formic acid: H\(_2\)O 0.1% formic acid 1 mL / min (5 μL), MeOH 2%> 20 min> 100% (5 min) at 25 °C.
General procedure for the synthesis of compounds 7a–j

To a stirred solution of aldehyde (2.5 mmol) in methanol (2.5 mL) propargylamine (0.14 g, 0.16 mL, 2.5 mmol) was added. After 30 min carboxylic acid (2.5 mmol) and isocyanide (2.5 mmol) were added. The contents were stirred for 24 h. The solvent was concentrated under reduced pressure in a rotavap. The crude material was purified by isocratic column chromatography to afford the pure product. The same solvent system used for $R_f$ value measurements was applied for performing flash column chromatography.

$N$-tert-Butyl-3-methyl-2-($N$-(prop-2-ynyl)acetamido)butanamide (7a).

Yield: 97%. Purified by column chromatography. $R_f$ 0.59 (EtOAc / hexane 3:7).

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 6.26 (s, 1H), 4.30-4.36 (m, 2H), 3.82 and 3.85 (d, $J = 2.4$ Hz, 1H), 2.14 (t, $J = 2.4$ Hz, 1H), 2.06 (s, 3H), 2.01 (m, $J = 4.4$ Hz, 1H), 1.1 (s, 9H), 0.74 (d, $J = 4.4$ Hz, 3H), 0.69 (d, $J = 4.4$ Hz, 3H). $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) 171.5, 169.1, 79.2, 72.0, 62.1, 50.8, 33.7, 28.1, 26.8, 21.7, 18.9. HRMS (ESI-pos) m/z calcd for C$_{14}$H$_{24}$N$_2$NaO$_2$ (M+Na)$^+$ 275.1735, found 275.1729.
Methyl 2-(3-methyl-2-(N-(prop-2-ynyl)acetamido)butanamido)acetate (7b).

\[
\begin{align*}
\text{O} & \quad \text{N} \\
\text{H} & \quad \text{O} \\
\text{H} & \quad \text{O}
\end{align*}
\]

Yield: 95%. Purified by column chromatography. \( R_f \) 0.15 (EtOAc / hexane 1:1).

\(^1\)H NMR (CDCl\(_3\), 400 MHz): \( \delta \) (ppm) 7.37 (bs, 1H), 4.60 and 4.58 (s, 1H), 4.31 and 3.89 (d, \( J = 2.4 \) Hz, 2H), 3.73-3.88 (m, 2H), 3.57 (s, 3H), 2.24 (t, \( J = 2.4 \) Hz, 1H), 2.14 (m, 4H), 0.80-0.84 (m, 6H). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \( \delta \) (ppm) 172.1, 170.5, 169.7, 79.9, 72.3, 61.7, 51.8, 40.5, 34.1, 26.6, 21.7, 19.1. HRMS (ESI-pos) m/z calcd for C\(_{13}\)H\(_{20}\)N\(_2\)O\(_4\) (M+Na)\(^+\) 291.1321, found 291.1315.

\[\text{N-tert-Butyl-2-(4-methoxyphenyl)-2-(N-(prop-2-ynyl)acetamido)acetamide (7c).}\]

\[
\begin{align*}
\text{OMe} & \quad \text{N} \\
\text{O} & \quad \text{N} \\
\text{H} & \quad \text{O} \\
\text{H} & \quad \text{O}
\end{align*}
\]

Yield: 99%. Purified by column chromatography. \( R_f \) 0.23 (EtOAc / hexane 1:1).

\(^1\)H NMR (CDCl\(_3\), 400 MHz): \( \delta \) (ppm) 7.29 (d, \( J = 8.4 \) Hz, 2H), 6.88 (d, \( J = 8.4 \) Hz, 2H), 6.15 (s, 1H), 6.00 (bs, 1H), 4.08 (d, \( J = 2.4 \) Hz, 2H), 3.81 (s, 3H), 2.26 (s, 3H), 2.02 (t, \( J = 2.4 \) Hz, 1H), 1.36 (s, 9H). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \( \delta \) (ppm) 171.5, 169.1, 159.4, 130.7, 127.0, 113.9, 79.5, 71.2, 59.7, 55.1, 51.5, 35.5, 28.5, 22.0. HRMS (ESI-pos) m/z calcd for C\(_{18}\)H\(_{24}\)N\(_2\)O\(_3\) (M+Na)\(^+\) 339.1685, found 339.1679.
\[ N-(1-(\text{tert-Butylamino})-3\text{-methyl-1-oxobutan-2-yl})-N-(\text{prop-2-ynyl})\text{benzamide} \ (7d). \]

Yield: 70%. Purified by column chromatography. \( R_f \) 0.56 (EtOAc / hexane 1:4).

\(^1\)H NMR (CDCl\(_3\), 400 MHz): \( \delta \) (ppm) 7.54 (d, \( J = 6.4 \) Hz, 2H), 7.37-7.45 (m, 3H), 6.58 (bs, 1H), 4.21-4.30 (m, 2H), 3.87 and 3.92 (s, 1H), 2.55 (m, \( J = 6.0 \) Hz, 1H), 2.23 (s,1H), 1.31 (s, 9H), 0.99-1.04 (m, 6H). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \( \delta \) (ppm) 173.1, 169.0, 135.4, 130.1, 128.2, 126.8, 79.6, 72.5, 66.2, 50.9, 37.3, 28.4, 26.5, 19.5. HRMS (ESI-pos) m/z calcd for C\(_{19}\)H\(_{26}\)N\(_2\)O\(_2\) (M+Na\(^+\)) 337.1892, found 337.1886.

\[ N-(2-(\text{tert-Butylamino})-2\text{-oxo-1-\(p\)tolylethyl})-N-(\text{prop-2-ynyl})\text{butyramide} \ (7e). \]

Yield: 91%. Purified by column chromatography. \( R_f \) 0.16 (EtOAc / hexane 1:4).

\(^1\)H NMR (CDCl\(_3\), 400 MHz): \( \delta \) (ppm) 7.17 (d, \( J = 8.0 \) Hz, 2H), 7.07 (d, \( J = 8.0 \) Hz, 2H), 6.07 (s, 1H), 6.06 (s, 1H), 3.98 (s, 2H), 2.41 (t, \( J = 6.4 \) Hz, 2H), 2.26 (s, 3H), 1.96 (s, 1H), 1.59 (m, \( J = 6.4 \) Hz, 2H), 1.27 (s, 9H), 0.86 (t, \( J = 6.4 \) Hz, 3H).

\(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \( \delta \) (ppm) 173.4, 169.0, 137.4, 131.9, 128.9, 128.8, 79.6, 70.9, 60.1, 50.9, 34.9, 34.6, 28.1, 29.7, 17.9, 13.3. HRMS (ESI-pos) m/z calcd for C\(_{20}\)H\(_{28}\)N\(_2\)O\(_2\) (M+Na\(^+\)) 351.2048, found 351.2042.
N-(2-(tert-Butylamino)-2-oxoethyl)-N-(prop-2-ynyl)butyramide (7f).

Yield: 70%. Purified by column chromatography. 

\[ R_f 0.34 (\text{EtOAc} / \text{hexane } 1:1). \]

\[ \text{^1H NMR (CDCl}_3, 400 \text{ MHz): } \delta (\text{ppm}) 6.10 \text{ and } 6.06 (s, 1H), 4.29 \text{ and } 4.19 (s, 2H), 3.98 (s, 2H), 2.45 \text{ and } 2.25 (t, J = 6.4 \text{ Hz, } 2H), 2.43 \text{ and } 2.31 (t, J = 2.4 \text{ Hz, 1H}), 1.68 (m, 2H), 1.37 \text{ and } 1.33 (s, 9H), 0.99 (m, 3H). \]

\[ \text{^13C NMR (CDCl}_3, 100 \text{ MHz): } \delta (\text{ppm}) 173.3, 173.1, 167.7, 166.9, 78.8, 77.8, 73.0, 72.6, 51.4, 50.9, 50.4, 38.6, 36.0, 34.7, 34.6, 28.4, 18.2, 18.0, 13.6, 13.5. \]

HRMS (ESI-pos) m/z calcd for C_{13}H_{22}N_{2}O_2 (M+Na)^+ 261.1579, found 261.1573.

N-tert-Butyl-2-(4-fluorophenyl)-2-(2-methoxy-N-(prop-2-ynyl) acetamido)acetamide (7g).

Yield: 98%. Purified by column chromatography. 

\[ R_f 0.25 (\text{EtOAc} / \text{hexane } 1:1). \]

\[ \text{^1H NMR (CDCl}_3, 400 \text{ MHz): } \delta (\text{ppm}) 7.36 (dd, J_{H-H} = 6.8 \text{ Hz, } J_{H-F} = 5.2 \text{ Hz, } 2H), 7.05 (m, 2H), 6.20 (m, 2H), 4.30 (s, 2H), 4.19-4.06 (m, 2H), 3.41 (s, 3H), 2.07 (t, J = 2.4 \text{ Hz, 1H}), 1.36 (s, 9H). \]

\[ \text{^13C NMR (CDCl}_3, 100 \text{ MHz): } \delta (\text{ppm}) 169.9, 168.1, 164.0, 160.8, 131.2, 131.0, 130.4, 130.3, 115.6, 115.3, 78.6, 71.8, 70.8, 59.0, 58.9, 51.5, 33.7, 28.3. \]

HRMS (ESI-pos) m/z calcd for C_{18}H_{23}F_{2}N_{2}O_3 (M+Na)^+ 357.1590, found 357.1585.
(S)-tert-Butyl 1-((2-(tert-butilamino)-2-oxoethyl)(prop-2-ynyl)amino)-1-oxo-3-phenylpropan-2-ylcarbamate (7h).

Yield: 82%. Purified by column chromatography. $R_f$ 0.34 (EtOAc / hexane 3:7).

$^1$H NMR (CDCl$_3$, 400 MHz): δ (ppm) 7.09-7.18 (m, 5H), 6.28 and 6.87 (s, 1H), 5.55 and 5.80 (d, $J = 8.0$ Hz, 1H), 4.47 and 4.72 (q, $J = 6.4$ Hz, 1H), 3.58-4.35 (m, 4H), 2.77-3.07 (m, 2H), 2.17 and 2.31 (s, 1H), 1.21-1.26 (m, 18H). $^{13}$C NMR (CDCl$_3$, 100 MHz): δ (ppm) 172.1, 171.9, 166.9, 166.4, 155.2, 154.9, 135.9, 135.6, 129.0, 128.1, 126.6, 126.5, 79.3, 79.2, 77.7, 77.4, 73.7, 72.5, 51.7, 51.5, 51.1, 50.8, 50.2, 49.9, 38.1, 37.8, 35.5, 28.2, 27.8. HRMS (ESI-pos) m/z calcd for C$_{23}$H$_{33}$N$_3$O$_4$(M+Na)$^+$ 438.2369, found 438.2363.

$N$-tert-Butyl-3-methyl-2-(2-(2-phenylacetamido)-N-(prop-2-ynyl) acetamido) butanamide (7i).

Yield: 82%. Purified by column chromatography. $R_f$ 0.30 (EtOAc / hexane 2:3).

$^1$H NMR (CDCl$_3$, 400 MHz): δ (ppm) 7.77 and 6.77 (t, $J = 4.4$ Hz,1H), 7.23-7.36 (m, 5H), 6.01 (s, 1H), 4.53 and 4.05 (d, $J = 2.4$ Hz, 2H), 4.49-3.76 (m, 3H), 3.61 and 3.55 (s, 2H), 2.35 and 2.14 (t, $J = 2.4$ Hz, 1H), 2.27 (m, 1H), 1.29 and 1.27 (s, 9H), 0.94 (d, $J = 6.8$ Hz, 3H), 0.86 (d, $J = 6.8$ Hz, 3H). $^{13}$C NMR (CDCl$_3$, 100 MHz): δ (ppm) 171.4, 170.6, 169.6, 168.7, 168.3, 167.8, 134.4, 134.2, 129.0, 128.9, 128.4, 126.8, 79.5, 78.2, 73.1, 70.3, 65.9, 62.9, 51.0, 42.9, 42.6, 41.0,
41.1, 32.4, 31.9, 28.0, 26.7, 19.2, 18.9. HRMS (ESI-pos) m/z calcd for C_{22}H_{31}N_{3}NaO_{3} (M+Na)^+ 408.2263, found 408.2258.

Benzyl 2-((2-(tert-butylamino)-2-oxoethyl)(prop-2-ynyl)amino)-2-oxoethylcarbamate (7j)

\[
\text{\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{image.png}
\caption{Benzyl 2-((2-(tert-butylamino)-2-oxoethyl)(prop-2-ynyl)amino)-2-oxoethylcarbamate (7j)}
\end{figure}}
\]

Yield: 80%. Purified by column chromatography. R_f 0.19 (EtOAc / hexane 1:1).

^1H NMR (CDCl$_3$, 400 MHz): δ (ppm) 7.28-7.25 (m, 5H), 6.36 and 6.16 (s, 1H), 5.95 (s, 1H), 5.04 (d, J = 8 Hz, 2H), 4.05 and 4.03 (s, 2H), 4.23 and 3.92 (s, 2H), 4.10 and 3.97 (s, 2H), 2.36 and 2.26 (s, 1H), 1.30 and 1.27 (s, 9H). ^13C NMR (CDCl$_3$, 100 MHz): δ (ppm) 169.1, 167.1, 166.2, 156.4, 136.3, 128.5, 128.1, 127.9, 78.2, 74.1, 73.3, 66.8, 51.8, 51.5, 50.3, 49.7, 42.6, 37.9, 36.4, 28.6. HRMS (ESI-pos) m/z calcd for C$_{19}$H$_{25}$N$_{3}$NaO$_{4}$ (M+Na)$^+$ 382.1743, found 382.1737.

**General procedure for the synthesis of compounds 8a–j**

In a 10 mL round bottom flask, to stirred solution of a suitable alkyne 7a–j (0.25 mmol) in dry DMSO (0.5 mL), CuCl (1.3 mg, 0.013 mmol/5 mol %) was added. The contents were stirred at 90 °C under air atmosphere. After 24 h the reaction mixture was diluted with ethyl acetate (10 mL) and filtered through a Celite plug. The solvent was removed under reduced pressure in a rotavap. The crude material was purified by column chromatography to afford the pure product. The
same solvent system for the $R_f$ values measurements was employed for column chromatography.

2,2’-(Hexa-2,4-diyne-1,6-diylbis(acetylazanediyl))bis(N-tert-butyl-3-methylbutanamide) (8a, mixture of diastereoisomers).

Yield: 88%. Purified by column chromatography. $R_f$ 0.49 (EtOAc). $^1$H NMR (CDCl$_3$, 400 MHz): δ (ppm) 5.91 (s, 4H), 4.54-4.41 (m, 8H), 4.14 and 4.09 (s, 4H), 2.24 (s, 12H), 2.18 (m, 4H), 2.18 (m, 4H), 1.31 (s, 36H), 0.94 (d, $J = 6.8$ Hz, 12H), 0.86 (d, $J = 6.8$ Hz, 12H). $^{13}$C NMR (CDCl$_3$, 100 MHz): δ (ppm) 171.9, 169.1, 74.4, 68.0, 51.4, 42.6, 34.7, 28.5, 26.9, 22.0, 19.3, 19.0. HRMS (ESI-pos) m/z calcd for C$_{28}$H$_{46}$N$_4$NaO$_4$ (M+Na)$^+$ 525.3417, found 525.3411.

Dimethyl 6,13-diacetyl-4,15-dioxo-5,14-di(propan-2-yl)-3,6,13,16-tetraazaoctadeca-8,10-diyne-1,18-dioate (8b, mixture of diastereoisomers).

Yield: 80%. Purified by column chromatography. $R_f$ 0.29 (EtOAc / MeOH 19:1). $^1$H NMR (CDCl$_3$, 400 MHz): δ (ppm) 6.79 (t, $J = 5.2$ Hz, 4H), 4.65-4.06 (m, 12H), 3.95 (d, $J = 5.2$ Hz, 8H), 3.73 (s, 12H), 2.28 (m, 16H), 0.99-0.95 (m, 24H). $^{13}$C NMR (CDCl$_3$, 100 MHz): δ (ppm) 172.4, 170.6, 169.8, 74.3, 68.2, 62.3,
58.3, 52.3, 41.1, 40.9, 35.1, 31.0, 26.7, 22.1, 19.4, 19.3. HRMS (ESI-pos) m/z calcd for \( C_{26}H_{38}N_4NaO_8 \) (M+Na)\(^+\) 557.2587, found 557.2581.

2,2’-(Hexa-2,4-diyn-1,6-diylibis(acetylanediyi))bis(\( N \)-\( tert \)-butyl-2-(4-methoxyphenyl)acetamide) (8c, mixture of diastereoisomers)

Yield: 99%. Purified by column chromatography. \( R_f \) 0.28 (EtOAc). \( ^1H \) NMR (CDCl\(_3\), 400 MHz): \( \delta \) (ppm) 7.28-7.20 (m, 8H), 6.85-6.81 (m, 8H), 6.08 (s, 4H), 5.83 (bs, 4H), 4.09 (m, 8H), 3.77 (s, 12H), 2.19 (s, 12H), 1.31 (m, 36H). \( ^{13}C \) NMR (CDCl\(_3\), 100 MHz): \( \delta \) (ppm) 171.4, 169.5, 169.3, 169.1, 159.7, 159.3, 130.7, 130.6, 128.4, 126.7, 114.2, 74.2, 67.1, 67.0, 59.6, 56.3, 55.3, 55.2, 51.7, 51.6, 42.6, 36.3, 28.6, 28.5, 23.2, 22.1. HRMS (ESI-pos) m/z calcd for \( C_{36}H_{46}N_4NaO_6 \) (M+Na)\(^+\) 653.3315, found 653.3309.

\( N,N'-(\text{Hexa-2,4-diyn-1,6-diyl})\text{bis}(\( N \)-(\( 1 \)-\( \text{tert} \)-butylamino)-3-methyl-1-oxobutan-2-yl)benzamide) \) (8d, mixture of diastereoisomers).

Yield: 91%. Purified by column chromatography. \( R_f \) 0.34 (EtOAc / hexane 3:7). \( ^1H \) NMR (CDCl\(_3\), 400 MHz): \( \delta \) (ppm) 7.42-7.53 (m, 20H), 6.47 (bs, 4H), 4.45-4.32 (m, 8H), 4.01 (m, 4H), 2.51 (m, 4H), 1.35 (s, 36H), 1.08-1.04 (m, 24H). \( ^{13}C \) NMR
(CDCl₃, 100 MHz): δ (ppm) 173.4, 169.1, 135.4, 130.5, 128.6, 126.9, 74.9, 68.4, 65.7, 51.3, 37.8, 28.6, 26.8, 19.7, 19.5. HRMS (ESI-pos) m/z calcd for C₃₈H₅₀N₄NaO₄ (M+Na)⁺ 649.3730, found 649.3724.

N,N'-((Hexa-2,4-diyne-1,6-diyl)bis(N-((2-(tert-butylamino)-2-oxo-1-p-tolylethyl)-butyramide) (8e, mixture of diastereoisomers).

Yield: 99%. Purified by column chromatography. Rₚ 0.52 (EtOAc / hexane 1:1). ¹H NMR (CDCl₃, 400 MHz): δ (ppm) 7.28-7.16 (m, 16H), 6.09 (s, 4H), 5.79 (s, 4H), 4.12 (s, 8H), 2.43 (t, J = 6.4 Hz, 8H), 2.34 (s, 12H), 1.71 (m, 8H), 1.34 (s, 36H), 0.98 (t, J = 6.4 Hz, 12H). ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) 173.9, 172.2, 169.1, 138.3, 135.8, 131.8, 129.5, 129.4, 129.3, 127.1, 74.4, 67.1, 60.3, 51.7, 35.7, 35.5, 28.6, 28.5, 21.2, 21.1, 19.0, 18.5, 13.9. HRMS (ESI-pos) m/z calcd for C₄₀H₅₄N₄NaO₄ (M+Na)⁺ 677.4043, found 677.4037.
N,N’-(Hexa-2,4-diyne-1,6-diyl)bis(N-(2-(tert-butylamino)-2-oxoethyl)butyramide) (8f)

Yield: 99%. Purified by column chromatography. $R_f$ 0.38 (EtOAc / hexane 4:1). 

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 6.82 and 6.10 (s, 2H), 4.28 (m, 4H), 3.96 (m, 4H), 2.41 and 1.66 (m, 4H), 2.31 and 2.25 (m, 4H), 1.37-1.32 (m, 18H), 0.97 (m, 6H). $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) 173.5, 173.4, 167.8, 166.8, 166.6, 74.5, 73.7, 73.5, 72.9, 68.8, 68.5, 68.2, 67.8, 51.9, 51.7, 51.6, 51.3, 51.3, 50.6, 50.5, 43.7, 39.4, 38.2, 36.9, 36.1, 34.8, 28.6, 18.4, 18.3, 13.9, 13.8, 13.7. 

HRMS (ESI-pos) m/z calcd for $C_{26}H_{42}N_4O_4$ (M+Na)$^+$ 497.3104, found 497.3098.

2,2’-(4,13-Dioxo-2,15-dioxa-5,12-diazahexadeca-7,9-diyne-5,12-diyl)bis(N-tert-butyl-2-(4-fluorophenyl)acetamide) (8g, mixture of diastereoisomers)

Yield: 97%. Purified by column chromatography. $R_f$ 0.42 (EtOAc). $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 7.32 (m, 8H), 7.06 (m, 8H), 6.11 (s, 4H), 5.83 (s, 4H), 4.22-4.18 (m, 16H), 3.44 (s, 12H), 1.34 (m, 36H). $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm)
MHz): δ (ppm) 170.0, 168.2, 164.0, 161.5, 131.3, 130.2, 115.9, 115.7, 71.2, 59.3, 51.8, 42.6, 34.6, 28.5. HRMS (ESI-pos) m/z calcd for C_{36}H_{44}F_{2}N_{4}NaO_{6} (M+Na)^+ 689.3127, found 689.3121.

**Di-tert-butyl(2R,2'S)-1,1''-(2,2,17,17-tetramethyl-4,15-dioxo-3,6,13,16-tetraaza-octadeca-8,10-diyn-6,13-diyl)bis(1-oxo-3-phenylpropane-2,1-diyl) dicarbamate (8h)**

Yield: 99%. Purified by column chromatography. $R_f$ 0.34 (EtOAc). $^1$H NMR (CDCl$_3$, 400 MHz): δ (ppm) 7.32-7.15 (m, 10H), 6.63 and 6.05 (s, 2H), 5.21 and 5.38 (d, $J = 7.6$ Hz, 2H), 4.73 (m, 1H), 4.47 (m, 4H), 4.07 (m, 2H), 3.67 (m, 2H), 3.41 (m, 1H), 2.98 (m, 4H), 1.38-1.32 (m, 36H). $^{13}$C NMR (CDCl$_3$, 100 MHz): δ (ppm) 172.4, 172.2, 167.0, 166.9, 166.4, 166.3, 155.5, 155.3, 135.8, 135.5, 129.4, 128.8, 128.7, 127.4, 127.2, 80.2, 80.0, 74.1, 72.9, 720, 69.7, 68.5, 68.0, 52.0, 51.8, 51.4, 50.8, 50.6, 39.0, 38.7, 38.5, 36.4, 28.7, 28.6, 28.2. HRMS (ESI-pos) m/z calcd for C_{46}H_{64}N_{6}NaO_{8} (M+Na)^+ 851.4683, found 851.4634.
2,2’-(2,5,14,17-Tetraoxo-1,18-diphenyl-3,6,13,16-tetraazaoctadeca-8,10-diyn-6,13-diyl)bis(N-tert-butyl-3-methylbutanamide) (8i, mixture of diastereoisomers)

Yield: 96%. Purified by column chromatography. $R_f$ 0.41 (EtOAc / hexane 4:1).

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ (ppm) 8.68 and 8.42 (s, 4H), 7.39-7.20 (m, 20H), 6.45 and 5.66 (s, 4H), 4.72-4.01 (m, 14H), 3.75 (m, 2H), 3.59 (m, 8H), 2.98 (s, 4H), 2.18 (m, 4H), 1.25-1.30 (m, 36H), 0.96-0.80 (m, 24H).

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ (ppm) 171.9, 171.7, 170.1, 169.9, 169.6, 168.6, 168.5, 168.4, 168.1, 168.0, 135.2, 135.1, 134.5, 134.4, 129.5, 129.3, 128.9, 128.6, 128.5, 127.4, 126.9, 75.7, 75.1, 73.8, 72.1, 69.4, 68.6, 66.5, 66.0, 63.3, 51.7, 51.6, 51.5, 51.4, 43.5, 43.2, 43.1, 42.9, 41.9, 41.8, 41.7, 41.7, 33.2, 33.1, 33.0, 32.7, 28.7, 28.6, 28.5, 27.2, 20.0, 19.3, 19.2, 19.1. HRMS (ESI-pos) m/z calcd for C$_{44}$H$_{60}$N$_6$NaO$_6$ (M+Na)$^+$ 791.4472, found 791.4466.

Dibenzyl (2,2,17,17-tetramethyl-4,15-dioxo-3,6,13,16-tetraazaoctadeca-8,10-diyn-6,13-diyl)bis(2-oxoethane-2,1-diyl)dicarbamate (8j)
Yield: 99%. Purified by column chromatography. \( R_f \) 0.43 (EtOAc). \(^1\)H NMR (CDCl\(_3\), 400 MHz): \( \delta \) (ppm) 7.33 (m, 10H), 6.36 and 6.11 (s, 2H), 6.19 and 5.95 (s, 2H), 5.83 (s, 2H), 5.09 (m, 2H), 4.35- 3.89 (m, 12H), 1.34 and 1.31 (m, 18H).

\(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \( \delta \) (ppm) 169.3, 169.1, 168.9, 166.9, 165.9, 165.8, 156.3, 136.3, 128.5, 128.2, 127.0, 74.4, 73.5, 72.9, 72.0, 69.5, 69.1, 68.4, 67.0, 52.0, 51.2, 50.1, 49.6, 42.6, 38.4, 36.8, 28.6. HRMS (ESI-pos) m/z calcd for C\(_{38}\)H\(_{48}\)N\(_6\)NaO\(_8\) (M+Na)\(^+\) 739.3431, found 739.3425.

**Combinatorial approach to dimers**

In a 10 mL round bottom flask, to stirred solution of the alkynes 7f, 7j and 7h (0.25 mmol each), CuCl (0.07 mmol / 5 mol%) was added. The contents were stirred at 90 °C under air atmosphere. After 24 h the reaction mixture was diluted with ethyl acetate (10 mL) and filtered through a Celite plug. The solvent was removed under reduced pressure. The crude material analyzed by HPLC.

**Biological activity assay**

The antibacterial activity against *Bacillus subtilis* was determined with a fluorescence based antibacterial growth inhibition assay. The fluorescence was measured on a microtiter plate reader GENios Pro (Fa. Tecan, excitation, 510 nm; emission, 535 nm). The *Bacillus subtilis* strain 168 (P\(_{AbrB}\)-IYFP) was maintained on TY (tryptone-yeast extract) medium supplemented with 1 % Bacto-tryptone, 0.5 % Bacto-yeast extract, 1 % NaCl and chloramphenicol (5 µg ml\(^{-1}\)). Details of the assay are published:

Katharina Michels, Ramona Heinke, Oscar P. Kuipers, Norbert Arnold and
Ludger A. Wessjohann “A Fluorescence-based Bioassay for Antibacterials and its Application in Screening Natural Product Extracts”, *J. Antibiot.* submitted.

**Table S1**

| Compound | Growth inhibition\(^a\) in % at 1 µM\(^d\) | Standard deviation\(^d\) | Growth inhibition in % at 10 µM\(^d\) | Standard deviation\(^d\) |
|----------|---------------------------------|-----------------|---------------------|-----------------|
| 8a       | 26.4                            | 18.9            | 40.9                | 24.5            |
| 8b       | 44.0                            | 26.7            | 52.3                | 27.8            |
| 8c       | 1.3                             | 5.1             | 23.7                | 12.7            |
| 8d       | 44.0                            | 21.8            | 54.9                | 19.1            |
| 8e       | 29.3                            | 11.4            | 34.1                | 16.2            |
| 8f       | 2.3                             | 13.5            | 30.1                | 21.4            |
| 8g       | 36.2                            | 15.5            | 41.2                | 17.1            |
| 8h       | 43.9                            | 23.0            | 49.9                | 23.5            |
| 8i       | 39.2                            | 12.6            | 44.3                | 10.4            |
| 8j       | 23.2                            | 17.0            | 57.6                | 26.5            |
| Std.\(^b\) | 70.8                          | 4.5             | NP\(^c\)            | NP\(^c\)       |

\(^a\) Measured after 15 h  
\(^b\) Erythromycin  
\(^c\) Not performed.  
\(^d\) Mean values of two trials involving 3 replicates

**Figures of \(^1\)H and \(^13\)C NMR spectra**

Please note that spectra of *N*-alkyl-amides (peptoids) like Ugi products display double signal sets in NMR due to interconvertible isomers with *s-cis* and *s-trans* amide bonds. Depending on substitution pattern, solvent and temperature, the equilibrium between these forms is shifted and may lead to broadened or doubled peaks of varied intensity.
Figure S1: $^1$H NMR spectrum of compound 7a
Figure S2: $^{13}$C NMR spectrum of compound 7a
Figure S3: $^1$H NMR spectrum of compound 7b
Figure S4: $^{13}$C NMR spectrum of compound 7b
Figure S5: $^1$H NMR spectrum of compound 7c
Figure S6: $^{13}$C NMR spectrum of compound 7c
Figure S7: $^1$H NMR spectrum of compound 7d
Figure S8: $^{13}$C NMR spectrum of compound 7d
Figure S9: $^1$H NMR spectrum of compound 7e
Figure S10: $^{13}$C NMR spectrum of compound 7e
Figure S11: $^1$H NMR spectrum of compound 7f
Figure S12: $^{13}$C NMR spectrum of compound 7f
Figure S13: $^1$H NMR spectrum of compound 7g
Figure S14: $^{13}$C NMR spectrum of compound 7g
Figure S15: $^1$H NMR spectrum of compound 7h
Figure S16: $^{13}$C NMR spectrum of compound 7h
Figure S17: $^1$H NMR spectrum of compound 7i
Figure S18: $^{13}$C NMR spectrum of compound 7i
Figure S19: $^1$H NMR spectrum of compound 7j
Figure S20: $^{13}$C NMR spectrum of compound 7j
Figure S21: $^1$H NMR spectrum of compound 8a
Figure S22: $^{13}$C NMR spectrum of compound 8a
Figure S23: $^1$H NMR spectrum of compound 8b
Figure S24: $^{13}$C NMR spectrum of compound 8b
Figure S25: $^1$H NMR spectrum of compound 8c
Figure S26: $^{13}$C NMR spectrum of compound 8c
Figure S27: $^1$H NMR spectrum of compound 8d
Figure S28: $^{13}$C NMR spectrum of compound 8d
Figure S29: $^1$H NMR spectrum of compound 8e
Figure S30: $^{13}$C NMR spectrum of compound 8e
Figure S31: $^1$H NMR spectrum of compound 8f
Figure S32: $^{13}$C NMR spectrum of compound 8f
Figure S33: $^1$H NMR spectrum of compound 8g
Figure S34: $^{13}$C NMR spectrum of compound 8g
Figure S35: $^1$H NMR spectrum of compound 8h
Figure S36: $^{13}$C NMR spectrum of compound 8h
Figure S37: $^1$H NMR spectrum of compound 8i
Figure S38: $^{13}$C NMR spectrum of compound 8i
Figure S39: $^1$H NMR spectrum of compound 8j
Figure S40: $^{13}$C NMR spectrum of compound 8j