

Supplementary Materials

FOR THE ARTICLE

Conjugates of Chloramphenicol Amine and Berberine as Antimicrobial Agents

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This file includes:

- I. Supplementary Methods
- II. Supplementary Table S1
- III. Supplementary Figures (S1–S16 with legends)

I. SUPPLEMENTARY METHODS

Detailed Synthesis of CAM-C1-BER

N-[(1*R*,2*R*)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]-2-(2-[(13*RS*,13*aRS*)-9,10-dimethoxy-5,8,13,13*a*-tetrahydro-6*H*-benzo[*g*]-1,3-benzodioxolo[5,6-*a*]quinolizin-13-yl]acetamido)acetamide (CAM-C1-*thBER*)

CAM-C1-*thBER* was obtained as CAM-C8-*thBER* from 75 mg (0.173 mmol) of *thBER*-CH₂-COOH·HCl, 28 mg (0.245 mmol) of HOSu, 50.5 mg (0.245 mmol) of DCC, 66 µL (0.378 mmol) of DIPEA, and 72 mg (0.189 mmol) of CAM-C1-NH₂·TFA. The target product was

isolated on a silica gel column eluting with a solvent system of CH₂Cl₂:MeOH = 9:1. As a result, a light yellow solid was obtained. Yield: 82 mg (73%); TLC: *R_f* (CH₂Cl₂:MeOH, 9:1) 0.45; LC-MS *m/z* calculated for C₃₃H₃₇N₄O₁₀ (M+H)⁺: 649.25, found 648.95; *t_R* = 1.34 min; ¹H NMR (DMSO-*d*₆, 400 MHz) δ (ppm) 8.11 & 8.09 (2H, d, *J* = 8.6 Hz, *o*-H NO₂-Phe), 7.81 & 7.74 (1H, t, *J* = 5.8 Hz, -CH₂-NH-CO-), 7.54 & 7.52 (2H, d, *J* = 8.6 Hz, *m*-H NO₂-Phe), 7.37 & 7.36 (1H, d, *J* = 9.1 Hz, -CH-NH-CO-), 6.87 & 6.80 (1H, d, *J* = 8.5 Hz, H-12), 6.84 (1H, s, H-1), 6.82 & 6.77 (1H, d, *J* = 8.5 Hz, H-11), 6.67 (1H, s, H-4), 5.96 & 5.93 (1H, d, *J* = 1.1 Hz, O-CH₂^a-O), 5.95 & 5.92 (1H, d, *J* = 1.1 Hz, O-CH₂^b-O), 5.86 & 5.83 (1H, d, *J* = 4.5 Hz, -CH-OH), 5.00 & 4.99 (1H, t, *J* = 4.5 Hz, -CH-OH), 4.87 & 4.86 (1H, dd, *J* = 5.9, 2.0 Hz, -CH₂-OH), 4.07 & 4.06 (1H, d, *J* = 16.0 Hz, H-8^a), 3.96–3.84 (1H, m, -CH-NH-CO-), 3.76 & 3.75 (3H, s, C₁₀-OCH₃), 3.72 & 3.71 (3H, s, C₉-OCH₃), 3.66–3.46 (4H, m, H-13, H-13a, -CH₂^a-OH, -CH₂^a-NH-), 3.44–3.38 (1H, m, H-8^b), 3.35–3.23 (2H, m, -CH₂^b-OH, -CH₂^b-NH-), 3.06 (1H, br d, *J* = 10.5 Hz, H-6^a), 2.93–2.82 (1H, m, H-5^a), 2.56 (1H, d, *J* = 15.4 Hz, H-5^b), 2.41 (1H, t, *J* = 11.4 Hz, H-6^b), 2.23–2.12 (1H, m, C₁₃-CH₂^a-CO), 1.94–1.84 (1H, m, C₁₃-CH₂^b-CO); ¹³C NMR (DMSO-*d*₆, 101 MHz) δ (ppm) 172.03 & 171.84 (-CH-NH-CO-), 168.88 & 168.75 (-CH₂-NH-CO-), 151.86 & 151.82 (NO₂-Ph_{para}), 150.10 & 150.06 (C-10), 146.36 & 146.33 (NO₂-Ph_{ipso}), 145.99 & 145.97 (C-2), 145.53 & 145.50 (C-3), 144.33 & 144.30 (C-9), 132.35 & 132.31 (C-12a), 129.25 (C-4a), 128.70 & 128.69 (C-13b), 127.56 & 127.52 (C-8a), 127.33 (2C, NO₂-Ph_{meta}), 124.01 (C-12), 122.85 (2C, NO₂-Ph_{ortho}), 110.97 & 110.89 (C-11), 108.07 (C-4), 105.86 & 105.82 (C-1), 100.65 (O-CH₂-O), 69.07 & 68.99 (-CH-OH), 62.75 (C-13a), 60.40 & 60.36 (-CH₂-OH), 59.46 & 59.45 (C₉-OCH₃), 56.09 & 56.02 (-NH-CH-), 55.64 & 55.61 (C₁₀-OCH₃), 53.85 (C-8), 50.56 (C-6), 42.22 & 41.92 (-CH₂-NH-), 39.24 (C-13), 38.81 & 38.77 (-NH-CO-CH₂-C₁₃), 29.13 (C-5).

13-[[[[(1*R*,2*R*)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]carbamoyl]methyl]carbamoyl]methyl]-9,10-dimethoxy-5,6-dihydrobenzo[*g*]-1,3-benzodioxolo[5,6-*a*]quinolizinium hydroxide (CAM-C1-BER)

CAM-C1-BER was obtained as CAM-C8-BER from 70 mg (0.108 mmol) of CAM-C1-thBER and 38 mg (0.216 mmol) of NBS. The target product was isolated on a silica gel column eluting with a solvent system of CHCl₃:MeOH:NH₄OH = 65:25:4. As a result, a yellow solid was obtained. Yield: 18.8 mg (26%); TLC: *R_f* (CHCl₃:MeOH:NH₄OH, 65:25:4) 0.46; LC-MS *m/z* calculated for C₃₃H₃₃N₄O₁₀ (M)⁺: 645.22, found 645.15; *t_R* = 1.24 min; ¹H NMR (DMSO-*d*₆, 400 MHz) δ (ppm) 9.96 (1H, s, H-8), 8.84 (1H, t, *J* = 5.7 Hz, -CH₂-NH-CO-), 8.12 (1H, d, *J* = 9.3 Hz, H-11), 8.10 (2H, d, *J* = 8.8 Hz, *o*-H NO₂-Phe), 7.96 (1H, d, *J* = 9.3 Hz, H-12), 7.83 (1H, d, *J* = 9.2 Hz, -CH-NH-CO-), 7.61 (2H, d, *J* = 8.8 Hz, *m*-H NO₂-Phe), 7.60 (1H, s, H-1), 7.14 (1H, s, H-4), 6.14 (2H, s, O-CH₂-O), 5.05 (1H, d, *J* = 2.5 Hz, -CH-OH), 4.84 (2H, br s, H-6), 4.23 (2H, s, C₁₃-CH₂-CONH), 4.09 (3H, s, C₉-OCH₃), 4.06 (3H, s, C₁₀-OCH₃), 4.05–3.99 (1H, m, -CH-NH-CO-), 3.86 (1H, dd, *J* = 16.8, 5.7 Hz, -CH₂^a-NH-), 3.73 (1H, dd, *J* = 16.8, 5.7 Hz, -CH₂^b-NH-), 3.59 (1H, dd, *J* = 10.5, 7.9 Hz, -CH₂^a-OH), 3.33 (1H, dd, *J* = 10.5, 5.9 Hz, -CH₂^b-OH), 3.09 (2H, t, *J* = 5.9 Hz, H-5); ¹³C NMR (DMSO-*d*₆, 101 MHz) δ (ppm) 169.82 (-CH-NH-CO-), 168.33 (-CH₂-NH-CO-), 151.92 (NO₂-Ph_{para}), 150.31 (C-10), 149.35 (C-3), 146.71 (C-2), 146.26 (NO₂-Ph_{ipso}), 145.25 (C-8), 144.23 (C-9), 137.44 (C-13a), 134.01 (C-4a), 133.05 (C-12a), 127.79 (C-13), 127.47 (2C, NO₂-Ph_{meta}), 126.15 (C-11), 122.84 (2C, NO₂-Ph_{ortho}), 120.92 (C-8a), 120.91 (C-12), 120.11 (C-13b), 109.27 (C-1), 108.36 (C-4), 102.05 (O-CH₂-O), 69.31 (-CH-OH), 62.08 (C₉-OCH₃), 60.49 (-CH₂-OH), 57.03 (C₁₀-OCH₃), 56.88 (C-6), 56.17 (-NH-CH-), 42.07 (-CH₂-NH-), 37.33 (-NH-CO-CH₂-C₁₃), 27.27 (C-5).

Detailed Synthesis of CAM-C2-BER

N-[[(1*R*,2*R*)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]-3-(2-[(13*RS*,13*aRS*)-9,10-dimethoxy-5,8,13,13*a*-tetrahydro-6*H*-benzo[*g*]-1,3-benzodioxolo[5,6-*a*]quinolizin-13-yl]acetamido)propanamide (CAM-C2-*thBER*)

CAM-C2-*thBER* was obtained as CAM-C8-*thBER* from 75 mg (0.173 mmol) of *thBER*-CH₂-COOH·HCl, 28 mg (0.245 mmol) of HOSu, 50.5 mg (0.245 mmol) of DCC, 66 μ L (0.378 mmol) of DIPEA, and 75 mg (0.189 mmol) of CAM-C2-NH₂·TFA. The target product was isolated on a silica gel column eluting with a solvent system of CH₂Cl₂:MeOH = 9:1. As a result, a light yellow solid was obtained. Yield: 74 mg (65%); TLC: *R*_f (CH₂Cl₂:MeOH, 9:1) 0.36; LC-MS *m/z* calculated for C₃₄H₃₉N₄O₁₀ (M+H)⁺: 663.27, found 662.86; *t*_R = 1.36 min; ¹H NMR (DMSO-*d*₆, 400 MHz) δ (ppm) 8.113 & 8.108 (2H, d, *J* = 8.6 Hz, *o*-H NO₂-Phe), 7.54 (1H, d, *J* = 9.4 Hz, -CH-NH-CO-), 7.53 (2H, d, *J* = 8.6 Hz, *m*-H NO₂-Phe), 7.48 (1H, t, *J* = 5.7 Hz, -CH₂-NH-CO-), 6.88–6.80 (3H, m, H-12, H-11, H-1), 6.66 (1H, s, H-4), 5.95 (1H, d, *J* = 2.4 Hz, O-CH₂^a-O), 5.94 (1H, d, *J* = 2.4 Hz, O-CH₂^b-O), 5.79 & 5.78 (1H, d, *J* = 4.5 Hz, -CH-OH), 4.98 (1H, t, *J* = 3.7 Hz, -CH-OH), 4.83–4.77 (1H, m, -CH₂-OH), 4.06 (1H, d, *J* = 15.9 Hz, H-8^a), 3.95 (1H, q, *J* = 7.3 Hz, -CH-NH-CO-), 3.77 (3H, s, C₁₀-OCH₃), 3.72 (3H, s, C₉-OCH₃), 3.63–3.57 (2H, m, H-13, H-13a), 3.51 (1H, dt, *J* = 9.7, 7.0 Hz, -CH₂^a-OH), 3.40 (1H, d, *J* = 15.9 Hz, H-8^b), 3.25 (1H, tt, *J* = 10.5, 5.3 Hz, -CH₂^b-OH), 3.06 (1H, dd, *J* = 10.9, 4.5 Hz, H-6^a), 3.01–2.81 (3H, m, β -CH₂, H-5^a), 2.57 (1H, d, *J* = 14.4 Hz, H-5^b), 2.41 (1H, td, *J* = 10.9, 2.8 Hz, H-6^b), 2.10 (1H, d, *J* = 14.3 Hz, C₁₃-CH₂^a-CO), 2.08–1.93 (2H, m, α -CH₂), 1.81 (1H, dd, *J* = 14.3, 2.5 Hz, C₁₃-CH₂^b-CO); ¹³C NMR (DMSO-*d*₆, 101 MHz) δ (ppm) 171.47 & 171.44 (-CH-NH-CO-), 170.26 & 170.25 (-CH₂-NH-CO-), 151.95 & 151.93 (NO₂-Ph_{para}), 150.09 (C-10), 146.32 (NO₂-Ph_{ipso}), 145.93 (C-2), 145.46 (C-3), 144.31 (C-9), 132.28 & 132.26 (C-12a), 129.21 (C-4a), 128.71 (C-13b), 127.58 (C-8a), 127.39 & 127.37 (2C, NO₂-Ph_{meta}), 124.05 & 124.04 (C-12), 122.77 (2C, NO₂-Ph_{ortho}), 110.90 (C-11), 108.05 (C-4), 105.84 (C-1), 100.64 (O-CH₂-O), 69.49 & 69.45 (-CH-OH), 62.73 (C-13a), 60.47 & 60.44 (-CH₂-OH), 59.45 (C₉-OCH₃), 55.86 (-NH-CH-), 55.66 (C₁₀-OCH₃), 53.89 (C-8), 50.58 (C-6), 39.46 (C-13), 38.85 (-NH-CO-CH₂-C₁₃), 35.41 (β -CH₂), 35.25 & 35.22 (α -CH₂), 29.13 (C-5).

13-[(2-[(1*R*,2*R*)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]carbamoyl)ethyl]carbamoyl)methyl]-9,10-dimethoxy-5,6-dihydrobenzo[*g*]-1,3-benzodioxolo[5,6-*a*]quinolizinium hydroxide (CAM-C2-BER)

CAM-C2-BER was obtained as CAM-C8-BER from 45 mg (0.068 mmol) of CAM-C2-*thBER* and 24 mg (0.136 mmol) of NBS. The target product was isolated on a silica gel column eluting with a solvent system of CHCl₃:MeOH:NH₄OH = 65:25:4. As a result, a yellow-brown solid was obtained. Yield: 20 mg (43%); TLC: *R*_f (CHCl₃:MeOH:NH₄OH, 65:25:4) 0.48; LC-MS *m/z* calculated for C₃₄H₃₅N₄O₁₀ (M)⁺: 659.23, found 659.24; *t*_R = 1.31 min; ¹H NMR (DMSO-*d*₆, 400 MHz) δ (ppm) 9.96 (1H, s, H-8), 8.72 (1H, t, *J* = 5.7 Hz, -CH₂-NH-CO-), 8.17 (1H, d, *J* = 9.4 Hz, H-11), 8.10 (2H, d, *J* = 8.5 Hz, *o*-H NO₂-Phe), 7.90 (1H, d, *J* = 9.4 Hz, H-12), 7.77 (1H, d, *J* = 9.1 Hz, -CH-NH-CO-), 7.61 (1H, s, H-1), 7.60 (2H, d, *J* = 8.5 Hz, *m*-H NO₂-Phe), 7.16 (1H, s, H-4), 6.16 (2H, d, *J* = 3.5 Hz, O-CH₂-O), 5.86 (1H, d, *J* = 5.1 Hz, -CH-OH), 5.03 (1H, dd, *J* = 5.1, 2.8 Hz, -CH-OH), 4.91 (1H, t, *J* = 5.6 Hz, -CH₂-OH), 4.85 (2H, br s, H-6), 4.15 (2H, s, C₁₃-CH₂-CONH), 4.09 (3H, s, C₉-OCH₃), 4.07 (3H, s, C₁₀-OCH₃), 4.06–4.01 (1H, m, -CH-NH-CO-), 3.59 (1H, dt, *J* = 10.3, 6.8 Hz, -CH₂^a-OH), 3.33 (1H, dt, *J* = 10.3, 5.2 Hz, -CH₂^b-OH), 3.25 (2H, q, *J* = 6.8 Hz, β -CH₂), 3.10 (2H, t, *J* =

5.9 Hz, H-5), 2.41–2.23 (2H, m, α -CH₂); ¹³C NMR (DMSO-*d*₆, 101 MHz) δ (ppm) 170.26 (-CH-NH-CO-), 169.48 (-CH₂-NH-CO-), 151.98 (NO₂-Ph_{para}), 150.34 (C-10), 149.38 (C-3), 146.71 (C-2), 146.28 (NO₂-Ph_{ipso}), 145.24 (C-8), 144.28 (C-9), 137.42 (C-13a), 134.01 (C-4a), 133.06 (C-12a), 127.96 (C-13), 127.50 (2C, NO₂-Ph_{meta}), 126.30 (C-11), 122.83 (2C, NO₂-Ph_{ortho}), 120.90 (C-8a), 120.80 (C-12), 120.17 (C-13b), 109.21 (C-1), 108.41 (C-4), 102.13 (O-CH₂-O), 69.56 (-CH-OH), 62.14 (C₉-OCH₃), 60.55 (-CH₂-OH), 57.06 (C₁₀-OCH₃), 56.90 (C-6), 56.00 (-NH-CH-), 37.45 (-NH-CO-CH₂-C₁₃), 35.86 (β -CH₂), 35.07 (α -CH₂), 27.27 (C-5).

Detailed Synthesis of CAM-C3-BER

N-[(1*R*,2*R*)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]-4-(2-[(1*3R*,13*aR*)-9,10-dimethoxy-5,8,13,13*a*-tetrahydro-6*H*-benzo[*g*]-1,3-benzodioxolo[5,6-*a*]quinolizin-13-yl]acetamido)butanamide (CAM-C3-*th*BER)

CAM-C3-*th*BER was obtained as CAM-C8-*th*BER from 101 mg (0.232 mmol) of *th*BER-CH₂-COOH·HCl, 37 mg (0.323 mmol) of HOSu, 67 mg (0.323 mmol) of DCC, 88 μ L (0.506 mmol) of DIPEA, and 104 mg (0.253 mmol) of CAM-C3-NH₂·TFA. The target product was isolated on a silica gel column eluting with a solvent system of CHCl₃:MeOH = 9:1. As a result, a light yellow solid was obtained. Yield: 55.4 mg (35%); TLC: *R*_f (CHCl₃:MeOH, 9:1) 0.31; LC-MS *m/z* calculated for C₃₅H₄₁N₄O₁₀ (M+H)⁺: 677.28, found 677.53; *t*_R = 1.42 min; ¹H NMR (DMSO-*d*₆, 400 MHz) δ (ppm) 8.12 (2H, d, *J* = 8.5 Hz, *o*-H NO₂-Phe), 7.60–7.53 (3H, m, *m*-H NO₂-Phe, -CH₂-NH-CO-), 7.42 (1H, d, *J* = 9.3 Hz, -CH-NH-CO-), 6.90–6.73 (3H, m, H-12, H-11, H-1), 6.67 (1H, s, H-4), 5.95 (2H, s, O-CH₂-O), 5.79 (1H, d, *J* = 4.8 Hz, -CH-OH), 5.00 (1H, t, *J* = 3.8 Hz, -CH-OH), 4.81 (1H, t, *J* = 5.6 Hz, -CH₂-OH), 4.06 (1H, d, *J* = 15.7 Hz, H-8^a), 3.96 (1H, tdd, *J* = 8.5, 5.6, 2.7 Hz, -CH-NH-CO-), 3.74 (3H, s, C₁₀-OCH₃), 3.73 (3H, s, C₉-OCH₃), 3.65–3.58 (2H, m, H-13, H-13a), 3.57–3.49 (1H, m, -CH₂^a-OH), 3.41 (1H, d, *J* = 15.7 Hz, H-8^b), 3.27 (1H, dd, *J* = 10.8, 5.6 Hz, -CH₂^b-OH), 3.07 (1H, br d, *J* = 8.4 Hz, H-6^a), 2.93–2.81 (1H, m, H-5^a), 2.78–2.70 (2H, m, γ -CH₂), 2.56 (1H, d, *J* = 15.4 Hz, H-5^b), 2.43 (1H, t, *J* = 11.9 Hz, H-6^b), 2.22–2.13 (1H, m, α -CH₂^a), 2.15–2.06 (1H, m, C₁₃-CH₂^a-CO), 1.93–1.80 (2H, m, C₁₃-CH₂^b-CO, α -CH₂^b), 1.31 (2H, p, *J* = 7.3 Hz, β -CH₂); ¹³C NMR (DMSO-*d*₆, 101 MHz) δ (ppm) 171.31 (-CH-NH-CO-), 170.28 (-CH₂-NH-CO-), 151.93 (NO₂-Ph_{para}), 149.99 (C-10), 146.27 (NO₂-Ph_{ipso}), 145.89 (C-2), 145.42 (C-3), 144.30 (C-9), 132.29 (C-12a), 129.11 (C-4a), 128.67 (C-13b), 127.44 (C-8a), 127.28 (2C, NO₂-Ph_{meta}), 123.94 (C-12), 122.69 (2C, NO₂-Ph_{ortho}), 110.91 (C-11), 107.94 (C-4), 105.74 (C-1), 100.59 (O-CH₂-O), 69.37 (-CH-OH), 62.68 (C-13a), 60.52 (-CH₂-OH), 59.40 (C₉-OCH₃), 55.71 (-NH-CH-), 55.62 (C₁₀-OCH₃), 53.82 (C-8), 50.55 (C-6), 40.20–38.84 (2C, -NH-CO-CH₂-C₁₃, γ -CH₂, overlapped with DMSO), 39.42 (C-13), 32.69 (α -CH₂), 28.96 (C-5), 25.37 (β -CH₂).

13-[(3-[(1*R*,2*R*)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]carbamoyl)propyl]carbamoyl)methyl]-9,10-dimethoxy-5,6-dihydrobenzo[*g*]-1,3-benzodioxolo[5,6-*a*]quinolizinium hydroxide (CAM-C3-BER)

CAM-C3-BER was obtained as CAM-C8-BER from 40 mg (0.059 mmol) of CAM-C3-*th*BER and 21 mg (0.119 mmol) of NBS. The target product was isolated on a silica gel column eluting with a solvent system of CHCl₃:MeOH:NH₄OH = 65:25:4. As a result, a brown solid was obtained. Yield: 14 mg (34%); TLC: *R*_f (CHCl₃:MeOH:NH₄OH, 65:25:4) 0.51; LC-MS *m/z* calculated for C₃₅H₃₇N₄O₁₀ (M)⁺: 673.25, found 673.35; *t*_R = 1.33 min; ¹H NMR (DMSO-*d*₆, 400

MHz) δ (ppm) 9.96 (1H, s, H-8), 8.66 (1H, t, $J = 5.6$ Hz, $-\text{CH}_2\text{-NH-CO-}$), 8.21 (1H, d, $J = 9.3$ Hz, H-11), 8.12 (2H, d, $J = 8.6$ Hz, $o\text{-H NO}_2\text{-Phe}$), 7.95 (1H, d, $J = 9.3$ Hz, H-12), 7.63 (1H, d, $J = 9.2$ Hz, $-\text{CH-NH-CO-}$), 7.60 (1H, s, H-1), 7.59 (2H, d, $J = 8.6$ Hz, $m\text{-H NO}_2\text{-Phe}$), 7.15 (1H, s, H-4), 6.15 (2H, s, $\text{O-CH}_2\text{-O}$), 5.89 (1H, d, $J = 5.0$ Hz, $-\text{CH-OH}$), 5.04 (1H, dd, $J = 5.0, 2.5$ Hz, $-\text{CH-OH}$), 4.91 (1H, t, $J = 5.6$ Hz, $-\text{CH}_2\text{-OH}$), 4.85 (2H, br s, H-6), 4.18 (2H, s, $\text{C}_{13}\text{-CH}_2\text{-CONH}$), 4.10 (3H, s, $\text{C}_9\text{-OCH}_3$), 4.07 (3H, s, $\text{C}_{10}\text{-OCH}_3$), 4.07–3.98 (1H, m, $-\text{CH-NH-CO-}$), 3.57 (1H, dt, $J = 10.4, 7.1$ Hz, $-\text{CH}_2^a\text{-OH}$), 3.32 (1H, dt, $J = 10.4, 5.2$ Hz, $-\text{CH}_2^b\text{-OH}$), 3.10 (2H, t, $J = 6.0$ Hz, H-5), 3.08–2.89 (2H, m, $\gamma\text{-CH}_2$), 2.11 (2H, t, $J = 7.3$ Hz, $\alpha\text{-CH}_2$), 1.66–1.46 (2H, m, $\beta\text{-CH}_2$); ^{13}C NMR (DMSO- d_6 , 101 MHz) δ (ppm) 171.67 ($-\text{CH-NH-CO-}$), 169.35 ($-\text{CH}_2\text{-NH-CO-}$), 152.09 ($\text{NO}_2\text{-Ph}_{para}$), 150.34 (C-10), 149.34 (C-3), 146.69 (C-2), 146.28 ($\text{NO}_2\text{-Ph}_{ipso}$), 145.17 (C-8), 144.23 (C-9), 137.41 (C-13a), 133.99 (C-4a), 133.16 (C-12a), 128.00 (C-13), 127.39 (2C, $\text{NO}_2\text{-Ph}_{meta}$), 126.27 (C-11), 122.81 (2C, $\text{NO}_2\text{-Ph}_{ortho}$), 120.94 (C-8a), 120.78 (C-12), 120.18 (C-13b), 109.24 (C-1), 108.37 (C-4), 102.11 ($\text{O-CH}_2\text{-O}$), 69.41 ($-\text{CH-OH}$), 62.07 ($\text{C}_9\text{-OCH}_3$), 60.62 ($-\text{CH}_2\text{-OH}$), 57.00 ($\text{C}_{10}\text{-OCH}_3$), 56.89 (C-6), 55.90 ($-\text{NH-CH-}$), 38.61 ($\gamma\text{-CH}_2$), 37.43 ($-\text{NH-CO-CH}_2\text{-C}_{13}$), 32.68 ($\alpha\text{-CH}_2$), 27.26 (C-5), 25.34 ($\beta\text{-CH}_2$).

Detailed Synthesis of CAM-C5-BER

tert-butyl-N-(5-[(1R,2R)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]carbamoyl)pentyl)carbamate (CAM-C5-NH-Boc)

To a cold solution of 1000 mg (4.33 mmol) of *N*-Boc-6-aminocaproic acid and 648 mg (5.63 mmol) of HOSu in 9 mL of DMF, 1161 mg (5.63 mmol) of DCC was added at 0 °C. The mixture was stirred for 2 h at 0 °C and overnight at RT. Then 1077 mg (4.33 mmol) of CAM-HCl in 6 mL of DMF and 981 μL (5.63 mmol) of DIPEA were added, and the resulted mixture was stirred at RT overnight. Then the reaction mixture was diluted with 200 μL of water. The mixture was then extracted with ethyl acetate (3 \times 50 mL), and the combined organic extracts were washed with 0.2 M H_2SO_4 (3 \times 25 mL), H_2O (1 \times 25 mL), 5% NaHCO_3 (3 \times 25 mL), saturated NaCl solution (1 \times 25 mL). The organic layer was dried over anhydrous Na_2SO_4 , and the volatiles were evaporated in vacuo. The target product was isolated on a silica gel column eluting with a solvent system of $\text{CHCl}_3\text{:MeOH} = 9\text{:}1$. The result was a yellowish oily substance. Yield: 942 mg (51%); TLC: R_f ($\text{CHCl}_3\text{:MeOH}, 9\text{:}1$) 0.41; LC-MS m/z calculated for $\text{C}_{20}\text{H}_{32}\text{N}_3\text{O}_7$ ($\text{M}+\text{H}^+$): 426.22, found 426.49; $t_R = 1.77$ min; ^1H NMR (CDCl_3 , 400 MHz) δ (ppm) 8.12 (2H, d, $J = 7.8$ Hz, $o\text{-H NO}_2\text{-Phe}$), 7.56 (2H, d, $J = 8.4$ Hz, $m\text{-H NO}_2\text{-Phe}$), 6.74 (1H, br s, $-\text{CH-NH-CO-}$), 5.19 (1H, d, $J = 2.9$ Hz, $-\text{CH-OH}$), 4.77 (1H, br s, $-\text{CH}_2\text{-NH-Boc}$), 4.19 (1H, dq, $J = 8.9, 4.9$ Hz, $-\text{CH-NH-CO-}$), 3.80 (1H, dd, $J = 11.3, 5.8$ Hz, $-\text{CH}_2^a\text{-OH}$), 3.72 (1H, dd, $J = 11.3, 4.1$ Hz, $-\text{CH}_2^b\text{-OH}$), 2.97 (2H, t, $J = 7.0$ Hz, $\epsilon\text{-CH}_2$), 2.08 (2H, t, $J = 7.3$ Hz, $\alpha\text{-CH}_2$), 1.47–1.30 (4H, m, $\beta\text{-}, \delta\text{-CH}_2$), 1.39 (9H, s, $-\text{CH}_3$), 1.18–1.05 (2H, m, $\gamma\text{-CH}_2$); ^{13}C NMR (CDCl_3 , 101 MHz) δ (ppm) 174.61 ($-\text{CH-NH-CO-}$), 156.50 ($-\text{CH}_2\text{-NH-CO-}$), 149.51 ($\text{NO}_2\text{-Ph}_{para}$), 147.25 ($\text{NO}_2\text{-Ph}_{ipso}$), 127.03 (2C, $\text{NO}_2\text{-Ph}_{meta}$), 123.46 (2C, $\text{NO}_2\text{-Ph}_{ortho}$), 79.67 ($-\text{C}(\text{CH}_3)_3$), 71.94 ($-\text{CH-OH}$), 62.72 ($-\text{CH}_2\text{-OH}$), 56.17 ($-\text{NH-CH-}$), 40.43 ($\epsilon\text{-CH}_2$), 36.17 ($\alpha\text{-CH}_2$), 29.55 ($\delta\text{-CH}_2$), 28.50 (3C, $-\text{CH}_3$), 25.98 ($\gamma\text{-CH}_2$), 25.17 ($\beta\text{-CH}_2$).

6-amino-N-[(1R,2R)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]hexanamide (CAM-C5-NH $_2$)

840 mg (1.97 mmol) of CAM-C5-NH-Boc was dissolved in 10 mL of 50% TFA in CH_2Cl_2 at RT, the obtained mixture was stirred for 30 min. Then the volatiles were evaporated in vacuo,

the residue was precipitated with diethyl ether, the ether was then decanted, and the precipitate was washed with diethyl ether. The target product was isolated on a silica gel column eluting with a solvent system of CHCl₃:MeOH:NH₄OH = 65:25:4. The result was a light yellow oily substance. Yield: 640 mg (100%); TLC: *R_f* (CHCl₃:MeOH:NH₄OH, 65:25:4) 0.38; LC-MS *m/z* calculated for C₁₅H₂₄N₃O₅ (M+H)⁺: 326.17, found 326.34; *t_R* = 0.54 min; ¹H NMR (DMSO-*d*₆, 400 MHz) δ (ppm) 8.15 (2H, d, *J* = 8.4 Hz, *o*-H NO₂-Phe), 7.81 (2H, br s, -NH₂), 7.57 (2H, d, *J* = 8.4 Hz, *m*-H NO₂-Phe), 7.52 (1H, d, *J* = 9.2 Hz, -CH-NH-CO-), 5.88 (1H, d, *J* = 4.9 Hz, -CH-OH), 5.01 (1H, d, *J* = 3.7 Hz, -CH₂-OH), 4.81 (1H, s, -CH₂-OH), 4.00 (1H, ddt, *J* = 10.4, 9.1, 4.5 Hz, -CH-NH-CO-), 3.55 (1H, t, *J* = 9.2 Hz, -CH₂^a-OH), 3.29 (1H, dd, *J* = 10.4, 5.8 Hz, -CH₂^b-OH), 2.68 (2H, t, *J* = 7.6 Hz, ε-CH₂), 1.97 (2H, t, *J* = 7.4 Hz, α-CH₂), 1.42 (2H, p, *J* = 7.6 Hz, δ-CH₂), 1.28 (2H, p, *J* = 7.5 Hz, β-CH₂), 1.13–0.97 (2H, m, γ-CH₂); ¹³C NMR (DMSO-*d*₆, 101 MHz) δ (ppm) 171.98 (-CH-NH-CO-), 152.25 (NO₂-Ph_{para}), 146.32 (NO₂-Ph_{ipso}), 127.43 (2C, NO₂-Ph_{meta}), 122.80 (2C, NO₂-Ph_{ortho}), 69.44 (-CH-OH), 60.64 (-CH₂-OH), 55.82 (-NH-CH-), 38.66 (ε-CH₂), 34.84 (α-CH₂), 26.79 (δ-CH₂), 25.30 (γ-CH₂), 24.80 (β-CH₂).

N-[(1*R*,2*R*)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]-6-(2-[(1*3R*,13*aRS*)-9,10-dimethoxy-5,8,13,13*a*-tetrahydro-6*H*-benzo[*g*]-1,3-benzodioxolo[5,6-*a*]quinolizin-13-yl]acetamido)hexanamide (CAM-C5-thBER)

CAM-C5-thBER was obtained as CAM-C8-thBER from 225 mg (0.518 mmol) of thBER-CH₂-COOH·HCl, 85 mg (0.74 mmol) of HOSu, 152 mg (0.74 mmol) of DCC, 197 μL (1.13 mmol) of DIPEA, and 185 mg (0.57 mmol) of CAM-C5-NH₂. The target product was isolated on a silica gel column eluting with a solvent system of CHCl₃:MeOH = 9:1. As a result, a light yellow solid was obtained. Yield: 168 mg (46%); TLC: *R_f* (CHCl₃:MeOH, 9:1) 0.37; LC-MS *m/z* calculated for C₃₇H₄₅N₄O₁₀ (M+H)⁺: 705.31, found 705.29; *t_R* = 1.50 min; ¹H NMR (DMSO-*d*₆, 400 MHz) δ (ppm) 8.16 & 8.15 (2H, d, *J* = 8.7 Hz, *o*-H NO₂-Phe), 7.570 & 7.567 (2H, d, *J* = 8.7 Hz, *m*-H NO₂-Phe), 7.50 (1H, t, *J* = 5.7 Hz, -CH₂-NH-CO-), 7.44 (1H, d, *J* = 9.2 Hz, -CH-NH-CO-), 6.85 & 6.84 (1H, d, *J* = 8.6 Hz, H-12), 6.83 (1H, s, H-1), 6.80 (1H, d, *J* = 8.6 Hz, H-11), 6.67 (1H, s, H-4), 5.95 (2H, s, O-CH₂-O), 5.80 (1H, d, *J* = 4.5 Hz, -CH-OH), 5.02 (1H, dd, *J* = 4.5, 2.4 Hz, -CH₂-OH), 4.83 (1H, dd, *J* = 6.4, 4.7 Hz, -CH₂-OH), 4.06 (1H, d, *J* = 16.0 Hz, H-8^a), 3.99 (1H, dddd, *J* = 9.2, 8.4, 5.4, 2.4 Hz, -CH-NH-CO-), 3.74 (3H, s, C₁₀-OCH₃), 3.72 (3H, s, C₉-OCH₃), 3.65–3.58 (2H, m, H-13, H-13a), 3.57–3.50 (1H, m, -CH₂^a-OH), 3.40 (1H, d, *J* = 16.0 Hz, H-8^b), 3.28 (1H, dt, *J* = 10.5, 5.4 Hz, -CH₂^b-OH), 3.06 (1H, dd, *J* = 11.0, 4.4 Hz, H-6^a), 2.93–2.70 (3H, m, ε-CH₂, H-5^a), 2.56 (1H, d, *J* = 15.5 Hz, H-5^b), 2.42 (1H, ddd, *J* = 13.9, 11.0, 2.9 Hz, H-6^b), 2.11 (1H, dd, *J* = 14.4, 9.6 Hz, C₁₃-CH₂^a-CO), 1.93 (2H, td, *J* = 7.2, 2.3 Hz, α-CH₂), 1.80 (1H, dd, *J* = 14.4, 2.6 Hz, C₁₃-CH₂^b-CO), 1.28–1.06 (4H, m, β-CH₂, δ-CH₂), 0.95–0.80 (2H, m, γ-CH₂); ¹³C NMR (DMSO-*d*₆, 101 MHz) δ (ppm) 172.03 (-CH-NH-CO-), 171.24 (-CH₂-NH-CO-), 152.14 (NO₂-Ph_{para}), 150.05 (C-10), 146.26 (NO₂-Ph_{ipso}), 145.93 (C-2), 145.44 (C-3), 144.28 (C-9), 132.32 (C-12a), 129.16 (C-4a), 128.75 (C-13b), 127.52 (C-8a), 127.34 (2C, NO₂-Ph_{meta}), 124.04 (C-12), 122.77 (2C, NO₂-Ph_{ortho}), 110.82 (C-11), 108.03 (C-4), 105.81 (C-1), 100.62 (O-CH₂-O), 69.33 (-CH-OH), 62.73 (C-13a), 60.56 (-CH₂-OH), 59.44 (C₉-OCH₃), 55.72 (-NH-CH-), 55.62 (C₁₀-OCH₃), 53.89 (C-8), 50.58 (C-6), 39.48 (C-13), 38.89 (-NH-CO-CH₂-C₁₃), 38.26 (ε-CH₂), 35.02 (α-CH₂), 29.13 (C-5), 28.77 (δ-CH₂), 25.82 (γ-CH₂), 25.00 (β-CH₂).

13-[[[(5-[(1R,2R)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]carbamoyl]pentyl)carbamoyl]methyl]-9,10-dimethoxy-5,6-dihydrobenzo[g]-1,3-benzodioxolo[5,6-a]quinolizinium hydroxide (CAM-C5-BER)

CAM-C5-BER was obtained as CAM-C8-BER from 115 mg (0.163 mmol) of CAM-C5-thBER and 58 mg (0.326 mmol) of NBS. The target product was isolated on a silica gel column eluting with a solvent system of CHCl₃:MeOH:NH₄OH = 65:25:4. As a result, a mustard-colored solid was obtained. Yield: 66 mg (56%); TLC: *R_f* (CHCl₃:MeOH:NH₄OH, 65:25:4) 0.44; LC-MS *m/z* calculated for C₃₇H₄₁N₄O₁₀ (M)⁺: 701.28, found 701.43; *t_R* = 1.43 min; ¹H NMR (DMSO-*d*₆, 400 MHz) δ (ppm) 9.97 (1H, s, H-8), 8.76 (1H, t, *J* = 5.5 Hz, -CH₂-NH-CO-), 8.22 (1H, d, *J* = 9.3 Hz, H-11), 8.14 (2H, d, *J* = 8.6 Hz, *o*-H NO₂-Phe), 7.95 (1H, d, *J* = 9.3 Hz, H-12), 7.61 (1H, s, H-1), 7.59 (2H, d, *J* = 8.6 Hz, *m*-H NO₂-Phe), 7.57 (1H, d, *J* = 9.4 Hz, -CH-NH-CO-), 7.16 (1H, s, H-4), 6.15 (2H, br s, O-CH₂-O), 5.86 (1H, d, *J* = 5.3 Hz, -CH-OH), 5.03 (1H, dd, *J* = 5.3, 2.5 Hz, -CH-OH), 4.90 (1H, t, *J* = 5.6 Hz, -CH₂-OH), 4.85 (2H, br s, H-6), 4.20 (2H, s, C₁₃-CH₂-CONH), 4.10 (3H, s, C₉-OCH₃), 4.06 (3H, s, C₁₀-OCH₃), 4.05–3.96 (1H, m, -CH-NH-CO-), 3.56 (1H, td, *J* = 10.4, 6.5 Hz, -CH₂^a-OH), 3.29 (1H, dt, *J* = 10.4, 5.3 Hz, -CH₂^b-OH), 3.18–3.02 (4H, m, H-5, ε-CH₂), 2.01 (2H, t, *J* = 7.4 Hz, α-CH₂), 1.40 (2H, p, *J* = 7.0 Hz, δ-CH₂), 1.37–1.26 (2H, m, β-CH₂), 1.17–1.05 (2H, m, γ-CH₂); ¹³C NMR (DMSO-*d*₆, 101 MHz) δ (ppm) 172.10 (-CH-NH-CO-), 169.33 (-CH₂-NH-CO-), 152.19 (NO₂-Ph_{para}), 150.36 (C-10), 149.33 (C-3), 146.69 (C-2), 146.22 (NO₂-Ph_{ipso}), 145.18 (C-8), 144.23 (C-9), 137.39 (C-13a), 133.99 (C-4a), 133.14 (C-12a), 128.04 (C-13), 127.40 (2C, NO₂-Ph_{meta}), 126.24 (C-11), 122.76 (2C, NO₂-Ph_{ortho}), 120.94 (C-8a), 120.82 (C-12), 120.18 (C-13b), 109.22 (C-1), 108.38 (C-4), 102.10 (O-CH₂-O), 69.41 (-CH-OH), 62.11 (C₉-OCH₃), 60.56 (-CH₂-OH), 57.07 (C₁₀-OCH₃), 56.88 (C-6), 55.77 (-NH-CH-), 38.89 (ε-CH₂), 37.46 (-NH-CO-CH₂-C₁₃), 35.09 (α-CH₂), 28.77 (δ-CH₂), 27.26 (C-5), 26.00 (γ-CH₂), 25.08 (β-CH₂).

Detailed Synthesis of CAM-C8-NH₂

9-[(*tert*-butoxycarbonyl)amino]nonanoic acid (Boc-NH-(CH₂)₈-COOH)

2 g (11.5 mmol) of 9-aminopelargonic acid, 3 mL (17.3 mmol) of DIPEA, and 3.96 mL (17.3 mmol) of Boc₂O were dissolved in a mixture of 10 mL of anhydrous CH₂Cl₂ and 20 mL of DMF, the obtained mixture was stirred for 3 h at RT. CH₂Cl₂ was then evaporated in vacuo, the mixture was diluted with 200 μL of water and washed with petroleum ether (2×50 mL). 1N aqueous H₂SO₄ was added dropwise to pH 6. The mixture was then extracted with ethyl acetate (3×50 mL), and the combined organic extracts were washed with 1N H₂SO₄ (3×50 mL) and saturated NaCl solution (1×50 mL). The organic layer was dried over anhydrous Na₂SO₄, and the volatiles were evaporated in vacuo. As a result, a white solid was obtained. Yield: 1.62 mg (52%); TLC: *R_f* (CHCl₃:MeOH, 9:1) 0.62; LC-MS *m/z* calculated for C₁₄H₂₇NNaO₄ (M+Na)⁺: 296.18, found 296.33; *t_R* = 2.27 min; ¹H NMR (DMSO-*d*₆, 400 MHz) δ (ppm) 11.90 (1H, s, -COOH), 6.68 (1H, t, *J* = 5.7 Hz, -NH-Boc), 2.89 (2H, q, *J* = 6.8 Hz, θ-CH₂), 2.18 (2H, t, *J* = 7.3 Hz, α-CH₂), 1.48 (2H, p, *J* = 7.3 Hz, η-CH₂), 1.37 (9H, s, -CH₃), 1.37–1.31 (2H, m, β-CH₂), 1.29–1.18 (8H, m, γ-, δ-, ε-, ζ-CH₂); ¹³C NMR (DMSO-*d*₆, 101 MHz) δ (ppm) 174.39 (-COOH), 155.54 (-NH-CO-), 77.21 (-C(CH₃)₃), 39.79 (θ-CH₂), 33.64 (α-CH₂), 29.41 (η-CH₂), 28.66 (δ-CH₂), 28.55 (ε-CH₂), 28.46 (γ-CH₂), 28.23 (3C, -CH₃), 26.18 (ζ-CH₂), 24.45 (β-CH₂).

tert-butyl-N-(8-[(1R,2R)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]carbamoyl)octyl)carbamate (CAM-C8-NH-Boc)

CAM-C8-NH-Boc was obtained as CAM-C5-NH-Boc from 1000 mg (3.663 mmol) of Boc-NH-(CH₂)₈-COOH, 548 mg (4.763 mmol) of HOSu, 982 mg (4.76 mmol) of DCC, 911 mg (3.66 mmol) of CAM·HCl, and 638 µL (3.663 mmol) of DIPEA. The target product was isolated on a silica gel column eluting with a solvent system of CHCl₃:MeOH = 9:1. As a result, a white solid was obtained. Yield: 1.488 mg (87%); TLC: *R_f* (CHCl₃:MeOH, 9:1) 0.47; LC-MS *m/z* calculated for C₂₃H₃₈N₃O₇ (M+H)⁺: 468.27, found 468.54; *t_R* = 2.18 min; ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 8.20–8.12 (2H, m, *o*-H NO₂-Phe), 7.56 (2H, d, *J* = 8.7 Hz, *m*-H NO₂-Phe), 6.44 (1H, d, *J* = 8.8 Hz, -CH-NH-CO-), 5.20 (1H, t, *J* = 3.7 Hz, -CH-OH), 5.01 (1H, br s, -NH-Boc), 4.70 (1H, br s, -CH-OH), 4.19 (1H, dt, *J* = 8.7, 4.3 Hz, -CH-NH-CO-), 4.04 (1H, br s, -CH₂-OH), 3.83–3.75 (2H, m, -CH₂-OH), 3.05 (2H, q, *J* = 6.8 Hz, θ-CH₂), 2.14–2.01 (2H, m, α-CH₂), 1.44–1.38 (11H, m, η-CH₂, -CH₃), 1.31–1.13 (8H, m, β-, δ-, ε-, ζ-CH₂), 1.13–1.03 (2H, m, γ-CH₂); ¹³C NMR (CDCl₃, 101 MHz) δ (ppm) 174.45 (-CH-NH-CO-), 156.48 (-CH₂-NH-CO-), 149.47 (NO₂-Ph_{para}), 147.29 (NO₂-Ph_{ipso}), 126.91 (2C, NO₂-Ph_{meta}), 123.48 (2C, NO₂-Ph_{ortho}), 79.43 (-C(CH₃)₃), 72.43 (-CH-OH), 63.19 (-CH₂-OH), 55.96 (-NH-CH-), 40.63 (θ-CH₂), 36.54 (α-CH₂), 29.96 (η-CH₂), 29.05 (δ-CH₂), 28.92 (ε-CH₂), 28.78 (γ-CH₂), 28.52 (3C, -CH₃), 26.61 (ζ-CH₂), 25.59 (β-CH₂).

9-amino-N-[(1R,2R)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]nonanamide (CAM-C8-NH₂)

CAM-C8-NH₂ was obtained as CAM-C5-NH₂ from 1388 mg (2.97 mmol) of CAM-C8-NH-Boc and 10 mL of 50% TFA in CH₂Cl₂. The target product was isolated on a silica gel column eluting with a solvent system of CHCl₃:MeOH:NH₄OH = 65:25:4. The result was a beige oily substance. Yield: 900 mg (83%); TLC: *R_f* (CHCl₃:MeOH:NH₄OH, 65:25:4) 0.32; LC-MS *m/z* calculated for C₁₈H₃₀N₃O₅ (M+H)⁺: 368.22, found 368.01; *t_R* = 0.92 min; ¹H NMR (DMSO-*d*₆, 400 MHz) δ (ppm) 8.14 (2H, d, *J* = 8.7 Hz, *o*-H NO₂-Phe), 7.58 (2H, d, *J* = 8.7 Hz, *m*-H NO₂-Phe), 7.47 (1H, d, *J* = 9.2 Hz, -CH-NH-CO-), 5.04 (1H, d, *J* = 2.5 Hz, -CH-OH), 4.78 (4H, br s, -NH₂, -CH-OH, -CH₂-OH), 4.01 (1H, tdd, *J* = 8.5, 5.6, 2.5 Hz, -CH-NH-CO-), 3.55 (1H, dd, *J* = 10.3, 8.3 Hz, -CH₂^a-OH), 3.30 (1H, dd, *J* = 10.3, 5.7 Hz, -CH₂^b-OH), 2.64 (2H, t, *J* = 7.3 Hz, θ-CH₂), 2.06–1.87 (2H, m, α-CH₂), 1.41 (2H, p, *J* = 7.4 Hz, η-CH₂), 1.33–1.16 (4H, m, β-CH₂, ζ-CH₂), 1.15–1.04 (4H, m, δ-CH₂, ε-CH₂), 1.00–0.83 (2H, m, γ-CH₂); ¹³C NMR (DMSO-*d*₆, 101 MHz) δ (ppm) 172.09 (-CH-NH-CO-), 152.26 (NO₂-Ph_{para}), 146.21 (NO₂-Ph_{ipso}), 127.34 (2C, NO₂-Ph_{meta}), 122.73 (2C, NO₂-Ph_{ortho}), 69.30 (-CH-OH), 60.60 (-CH₂-OH), 55.71 (-NH-CH-), 39.98 (θ-CH₂), 35.07 (α-CH₂), 29.62 (η-CH₂), 28.79 (δ-CH₂), 28.61 (ε-CH₂), 28.30 (γ-CH₂), 26.12 (ζ-CH₂), 25.30 (β-CH₂).

Detailed Synthesis of CH₃-C5-BER

The scheme for the synthesis of CH₃-Cn-BER is represented in Figure S1.

2-[(13RS,13aRS)-9,10-dimethoxy-5,8,13,13a-tetrahydro-6H-benzol[g]-1,3-benzodioxolo[5,6-a]quinolizin-13-yl]-N-hexylacetamide (CH₃-C5-thBER)

To a cold solution of 200 mg (0.461 mmol) of thBER-CH₂-COOH·HCl in 20 mL of DMF, 124 mg (0.6 mmol) of DCC, 67 µL (0.51 mmol) of hexylamine, and 241 µL (1.383 mmol) of DIPEA were added at 0 °C. The mixture was stirred for 3 h at 0 °C and overnighted at 4 °C. Then the reaction mixture was diluted with 200 µL of water, extracted with ethyl acetate (3×50

mL), and the combined organic extracts were washed with saturated NaCl solution (1×50 mL). The organic layer was dried over anhydrous Na₂SO₄, and the volatiles were evaporated in vacuo. The target product was isolated on a silica gel column eluting with a solvent system of CHCl₃:MeOH = 40:1. As a result, a light yellow solid was obtained. Yield: 115 mg (52%); TLC: *R_f* (CHCl₃:MeOH, 40:1) 0.37; LC-MS *m/z* calculated for C₂₈H₃₇N₂O₅ (M+H)⁺: 481.27, found 481.10; *t_R* = 1.98 min; ¹H NMR (DMSO-*d*₆, COSY, ROESY, 600 MHz) δ (ppm) 7.52 (1H, t, *J* = 5.6 Hz, -NH-), 6.86 (1H, d, *J* = 8.5 Hz, H-12), 6.82 (1H, s, H-1), 6.80 (1H, d, *J* = 8.5 Hz, H-11), 6.67 (1H, s, H-4), 5.95 (2H, d, *J* = 1.3 Hz, O-CH₂-O), 4.06 (1H, d, *J* = 15.9 Hz, H-8^a), 3.75 (3H, s, C₁₀-OCH₃), 3.72 (3H, s, C₉-OCH₃), 3.62 (1H, dt, *J* = 10.0, 2.9 Hz, H-13), 3.59 (1H, d, *J* = 3.4 Hz, H-13a), 3.40 (1H, d, *J* = 15.9 Hz, H-8^b), 3.06 (1H, ddd, *J* = 10.9, 5.0, 1.9 Hz, H-6^a), 2.96 (1H, dq, *J* = 13.1, 6.6 Hz, 1'-CH₂^a), 2.91–2.85 (1H, m, H-5^a), 2.86–2.80 (1H, m, 1'-CH₂^b), 2.56 (1H, d, *J* = 15.9 Hz, H-5^b), 2.42 (1H, td, *J* = 12.0, 2.9 Hz, H-6^b), 2.13 (1H, dd, *J* = 14.5, 10.0 Hz, C₁₃-CH₂^a-CO), 1.81 (1H, dd, *J* = 14.5, 3.0 Hz, C₁₃-CH₂^b-CO), 1.29–1.23 (2H, m, 2'-CH₂), 1.23–1.20 (2H, m, 5'-CH₂), 1.20–1.14 (2H, m, 4'-CH₂), 1.14–1.07 (2H, m, 3'-CH₂), 0.83 (3H, t, *J* = 7.1 Hz, 6'-CH₃); ¹³C NMR (DMSO-*d*₆, HMBC, HSQC, 151 MHz) δ (ppm) 171.76 (-CH₂-NH-CO-), 150.57 (C-10), 146.51 (C-2), 145.85 (C-3), 144.82 (C-9), 132.84 (C-12a), 129.65 (C-4a), 129.28 (C-13b), 128.08 (C-8a), 124.67 (C-12), 111.37 (C-11), 108.66 (C-4), 106.43 (C-1), 101.26 (O-CH₂-O), 63.40 (C-13a), 60.02 (C₉-OCH₃), 56.19 (C₁₀-OCH₃), 54.53 (C-8), 51.21 (C-6), 40.11 (C-13), 39.54 (-NH-CO-CH₂-C₁₃), 39.10 (1'-CH₂), 31.65 (4'-CH₂), 29.73 (C-5), 29.62 (2'-CH₂), 26.72 (3'-CH₂), 22.68 (5'-CH₂), 14.53 (6'-CH₃).

*13-[(hexylcarbamoyl)methyl]-9,10-dimethoxy-5,6-dihydrobenzo[*g*]-1,3-benzodioxolo[5,6-*a*]quinolizinium hydroxide (CH₃-C5-BER)*

CH₃-C5-BER was obtained as CAM-C8-BER from 71.5 mg (0.149 mmol) of CH₃-C5-thBER and 53 mg (0.298 mmol) of NBS. The target product was isolated on a silica gel column eluting with a solvent system of CHCl₃:MeOH:NH₄OH = 6:1:0.1. As a result, a bright yellow solid was obtained. Yield: 56 mg (76%); TLC: *R_f* (CHCl₃:MeOH:NH₄OH, 6:1:0.1) 0.35; LC-MS *m/z* calculated for C₂₈H₃₃N₂O₅ (M)⁺: 477.24, found 477.24; *t_R* = 1.87 min; ¹H NMR (DMSO-*d*₆, 400 MHz) δ (ppm) 9.98 (1H, s, H-8), 8.82 (1H, t, *J* = 5.6 Hz, -CH₂-NH-CO-), 8.21 (1H, d, *J* = 9.4 Hz, H-11), 7.98 (1H, d, *J* = 9.4 Hz, H-12), 7.62 (1H, s, H-1), 7.17 (1H, s, H-4), 6.16 (2H, s, O-CH₂-O), 4.86 (2H, br s, H-6), 4.22 (2H, s, C₁₃-CH₂-CONH), 4.10 (3H, s, C₉-OCH₃), 4.07 (3H, s, C₁₀-OCH₃), 3.19 (2H, q, *J* = 6.7 Hz, 1'-CH₂), 3.10 (2H, t, *J* = 5.9 Hz, H-5), 1.50 (2H, p, *J* = 7.1 Hz, 2'-CH₂), 1.39–1.20 (6H, m, 3'-, 4'-, 5'-CH₂), 0.87 (3H, t, *J* = 6.9 Hz, 6'-CH₃); ¹³C NMR (DMSO-*d*₆, 101 MHz) δ (ppm) 169.34 (-CH₂-NH-CO-), 150.32 (C-10), 149.31 (C-3), 146.67 (C-2), 145.22 (C-8), 144.25 (C-9), 137.39 (C-13a), 134.02 (C-4a), 133.12 (C-12a), 128.03 (C-13), 126.13 (C-11), 120.93 (C-8a), 120.83 (C-12), 120.17 (C-13b), 109.18 (C-1), 108.39 (C-4), 102.09 (O-CH₂-O), 62.11 (C₉-OCH₃), 57.05 (C₁₀-OCH₃), 56.87 (C-6), 39.00 (1'-CH₂), 37.46 (-NH-CO-CH₂-C₁₃), 31.03 (4'-CH₂), 29.02 (2'-CH₂), 27.23 (C-5), 26.18 (3'-CH₂), 22.12 (5'-CH₂), 13.98 (6'-CH₃).

Detailed Synthesis of CH₃-C8-BER

*2-[(13*RS*,13*aRS*)-9,10-dimethoxy-5,8,13,13*a*-tetrahydro-6*H*-benzo[*g*]-1,3-benzodioxolo[5,6-*a*]quinolizin-13-yl]-*N*-nonylaceta^{amide} (CH₃-C8-thBER)*

CH₃-C8-thBER was obtained as CH₃-C5-thBER from 165 mg (0.380 mmol) of thBER-CH₂-COOH·HCl, 102 mg (0.49 mmol) of DCC, 85 mg (0.38 mmol) of nonylammonium bromide, and 198 µL (1.140 mmol) of DIPEA. The target product was isolated on a silica gel column eluting with a solvent system of CHCl₃:MeOH = 40:1. As a result, a light yellow solid was obtained. Yield: 146 mg (74%); TLC: *R_f* (CHCl₃:MeOH, 40:1) 0.34; LC-MS *m/z* calculated for C₃₁H₄₃N₂O₅ (M+H)⁺: 523.32, found 523.16; *t_R* = 2.40 min; ¹H NMR (DMSO-*d*₆, COSY, ROESY, 600 MHz) δ (ppm) 7.51 (1H, t, *J* = 5.6 Hz, -NH-), 6.85 (1H, d, *J* = 8.5 Hz, H-12), 6.83 (1H, s, H-1), 6.80 (1H, d, *J* = 8.5 Hz, H-11), 6.68 (1H, s, H-4), 5.96 (1H, d, *J* = 1.1 Hz, O-CH₂^a-O), 5.95 (1H, d, *J* = 1.1 Hz, O-CH₂^b-O), 4.06 (1H, d, *J* = 15.9 Hz, H-8^a), 3.75 (3H, s, C₁₀-OCH₃), 3.72 (3H, s, C₉-OCH₃), 3.62 (1H, dt, *J* = 9.5, 2.7 Hz, H-13), 3.61 (1H, d, *J* = 4.4 Hz, H-13a), 3.41 (1H, d, *J* = 15.9 Hz, H-8^b), 3.06 (1H, ddd, *J* = 10.2, 5.0, 1.8 Hz, H-6^a), 2.95 (1H, dq, *J* = 13.0, 6.6 Hz, 1'-CH₂^a), 2.90–2.84 (1H, m, H-5^a), 2.86–2.79 (1H, m, 1'-CH₂^b), 2.57 (1H, d, *J* = 15.5 Hz, H-5^b), 2.42 (1H, td, *J* = 11.5, 2.9 Hz, H-6^b), 2.12 (1H, dd, *J* = 14.4, 9.8 Hz, C₁₃-CH₂^a-CO), 1.80 (1H, dd, *J* = 14.4, 2.9 Hz, C₁₃-CH₂^b-CO), 1.28–1.16 (12H, m, 2'-, 4'-, 5'-, 6'-, 7'-, 8'-CH₂), 1.13–1.07 (2H, m, 3'-CH₂), 0.84 (3H, t, *J* = 7.0 Hz, 9'-CH₃); ¹³C NMR (DMSO-*d*₆, HMBC, HSQC, 151 MHz) δ (ppm) 171.72 (-CH₂-NH-CO-), 150.61 (C-10), 146.23 (C-2), 145.94 (C-3), 144.78 (C-9), 132.84 (C-12a), 129.67 (C-4a), 129.28 (C-13b), 128.11 (C-8a), 124.60 (C-12), 111.30 (C-11), 108.60 (C-4), 106.35 (C-1), 101.20 (O-CH₂-O), 63.25 (C-13a), 60.04 (C₉-OCH₃), 56.21 (C₁₀-OCH₃), 54.47 (C-8), 51.13 (C-6), 40.06 (C-13), 39.45 (-NH-CO-CH₂-C₁₃), 39.02 (1'-CH₂), 31.88 (7'-CH₂), 29.67 (C-5), 29.55 (2'-CH₂), 29.50, 29.44, 29.25 (4'-, 5'-, 6'-CH₂), 26.98 (3'-CH₂), 22.68 (8'-CH₂), 14.51 (9'-CH₃).

13-[(nonylcarbamoyl)methyl]-9,10-dimethoxy-5,6-dihydrobenzo[g]-1,3-benzodioxolo[5,6-a]quinolizinium hydroxide (CH₃-C8-BER)

CH₃-C8-BER was obtained as CAM-C8-BER from 106 mg (0.203 mmol) of CH₃-C8-thBER and 72 mg (0.406 mmol) of NBS. The target product was isolated on a silica gel column eluting with a solvent system of CHCl₃:MeOH:NH₄OH = 6:1:0.1. As a result, a bright yellow solid was obtained. Yield: 73 mg (67%); TLC: *R_f* (CHCl₃:MeOH:NH₄OH, 6:1:0.1) 0.31; LC-MS *m/z* calculated for C₃₁H₃₉N₂O₅ (M)⁺: 519.28, found 519.17; *t_R* = 2.32 min; ¹H NMR (DMSO-*d*₆, 400 MHz) δ (ppm) 9.98 (1H, s, H-8), 8.78 (1H, t, *J* = 5.6 Hz, -CH₂-NH-CO-), 8.21 (1H, d, *J* = 9.4 Hz, H-11), 7.98 (1H, d, *J* = 9.4 Hz, H-12), 7.63 (1H, s, H-1), 7.17 (1H, s, H-4), 6.16 (2H, s, O-CH₂-O), 4.85 (2H, br s, H-6), 4.22 (2H, s, C₁₃-CH₂-CONH), 4.10 (3H, s, C₉-OCH₃), 4.08 (3H, s, C₁₀-OCH₃), 3.18 (2H, q, *J* = 6.6 Hz, 1'-CH₂), 3.10 (2H, t, *J* = 5.9 Hz, H-5), 1.50 (2H, p, *J* = 7.0 Hz, 2'-CH₂), 1.34–1.19 (12H, m, 3'-, 4'-, 5'-, 6'-, 7'-, 8'-CH₂), 0.85 (3H, t, *J* = 6.9 Hz, 9'-CH₂); ¹³C NMR (DMSO-*d*₆, 101 MHz) δ (ppm) 169.33 (-CH₂-NH-CO-), 150.32 (C-10), 149.31 (C-3), 146.67 (C-2), 145.22 (C-8), 144.26 (C-9), 137.40 (C-13a), 134.02 (C-4a), 133.12 (C-12a), 128.04 (C-13), 126.13 (C-11), 120.94 (C-8a), 120.82 (C-12), 120.17 (C-13b), 109.20 (C-1), 108.38 (C-4), 102.08 (O-CH₂-O), 62.11 (C₉-OCH₃), 57.04 (C₁₀-OCH₃), 56.88 (C-6), 38.98 (1'-CH₂), 37.45 (-NH-CO-CH₂-C₁₃), 31.35 (7'-CH₂), 29.06, 29.05, 28.83, 28.74 (2'-, 4'-, 5'-, 6'-CH₂), 27.23 (C-5), 26.52 (3'-CH₂), 22.13 (8'-CH₂), 13.99 (9'-CH₃).

Eukaryotic In Vitro Translation Inhibition Assay

Inhibition of the synthesis of firefly luciferase in the cell-free eukaryotic translation system by the studied compounds was tested in a lysate of HEK293T cells. The reaction was carried out in a total volume of 10 µL with the following reagents: 5 µL of HEK293T cell lysate

(S10 extract); 1 μ L Translation Buffer 10x (200 mM HEPES-KOH pH 7.6, 10 mM dithiothreitol, 5 mM spermidine-HCl, 80 mM creatine phosphate, 10 mM ATP, 2 mM GTP, 0.25 mM of each amino acid); 0.4 μ L of potassium acetate (2 mM); 1 μ L of magnesium acetate (10 mM); 0.5 μ L of D-luciferin (10 mM); 0.1 μ L of RiboLock RNase Inhibitor (40 U/ μ L) (Thermo Fisher Scientific, Waltham, MA, USA); 1 μ L of the tested compound or nuclease-free water; 1 μ L of mRNA *Fluc* (50 ng), capped and with poly-A sequence. Reactions containing all components, except mRNA, were preincubated for 5 min with the tested compounds at a final concentration ranging from 5 to 1000 μ M or with water. Then mRNA was added and the in vitro translation reaction was carried out at 37 °C for 1 h. The activity of in vitro synthesized luciferase was measured by VICTOR X5 Multilabel Plate Reader (PerkinElmer, Waltham, MA, USA) every 30 s.

II. SUPPLEMENTARY TABLE

Table S1. Bacterial strains used in the work.

Strain	Abbreviation
Strains of <i>Escherichia coli</i>	
<i>Escherichia coli</i> JW5503 (Δ <i>tolC</i>) (KanS) pDualrep2	<i>E. coli</i> Δ <i>tolC</i> pDualrep2
<i>Escherichia coli</i> JW5503 (Δ <i>tolC</i>) (KanS)	<i>E. coli</i> Δ <i>tolC</i>
<i>Escherichia coli</i> JW5503 (Δ <i>tolC</i>) (KanS) pCA24N- <i>LacZ</i>	<i>E. coli</i> Δ <i>tolC</i> -CAT
<i>Escherichia coli</i> SQ110 (Δ <i>tolC</i>) (A2058G)	<i>E. coli</i> SQ110 Δ <i>tolC</i> (A2058G)
<i>Escherichia coli</i> ATCC 25922	<i>E. coli</i> ATCC
<i>Escherichia coli</i> K-12	<i>E. coli</i> K-12
Strains of <i>Bacillus subtilis</i>	
<i>Bacillus subtilis</i> 168	<i>B. subtilis</i>
<i>Bacillus subtilis</i> ATCC 6633	<i>B. subtilis</i> ATCC
<i>Bacillus subtilis</i> 168 pHT01	<i>B. subtilis</i> -CAT
Other strains	
<i>Leuconostoc mesenteroides</i> VKPM B-4177	<i>L. mesenteroides</i>
<i>Staphylococcus aureus</i> INA 00761 (MRSA)	<i>St. aureus</i>
<i>Mycobacterium smegmatis</i> VKPM Ac 1339	<i>Myc. smegmatis</i> VKPM Ac 1339
<i>Mycobacterium smegmatis</i> mc ² 155	<i>Myc. smegmatis</i> mc ² 155
Fungi	
<i>Aspergillus niger</i> INA 00760	<i>A. niger</i>
<i>Saccharomyces cerevisiae</i> RIA 259	<i>S. cerevisiae</i>

III. SUPPLEMENTARY FIGURES

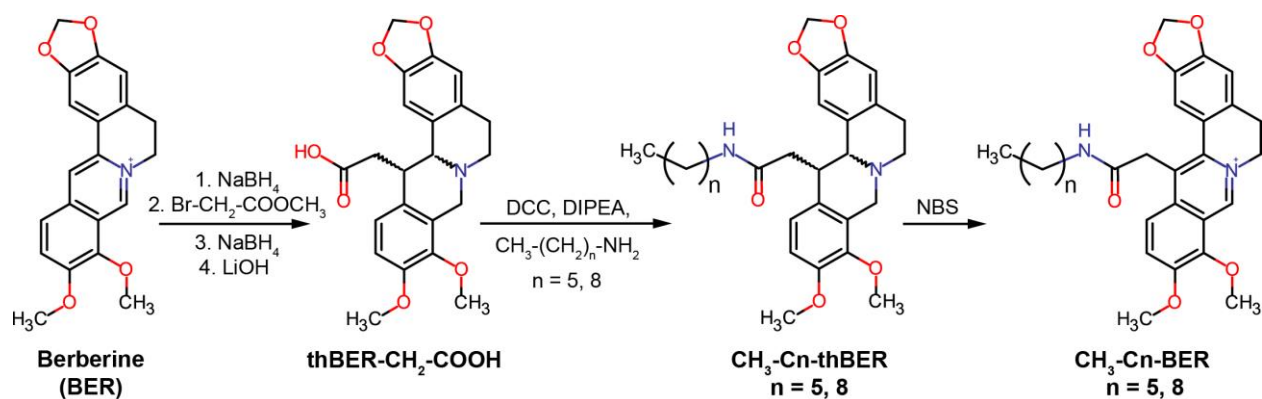


Figure S1. Scheme of the chemical synthesis of 13-alkyl-derivatives of berberine – CH₃-Cn-BER, $n = 5, 8$.

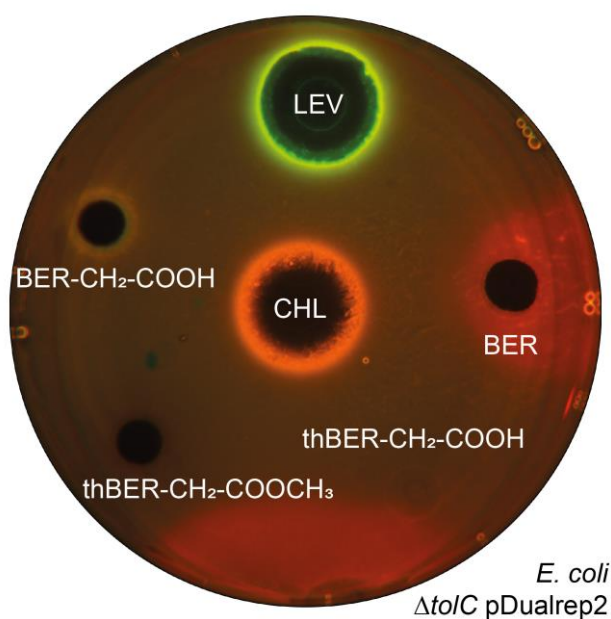


Figure S2. Testing of the BER, BER-CH₂-COOH, thBER-CH₂-COOCH₃, and thBER-CH₂-COOH antibacterial activity using *E. coli* ΔtolC pDualrep2 reporter strain. Levofloxacin (LEV) and chloramphenicol (CHL) are used as controls. The induction of the red fluorescent protein expression (green halo around the inhibition zone, pseudocolor) is triggered by DNA-damage, while the induction of Katushka2S protein (red halo, pseudocolor) occurs in response to ribosome stalling.

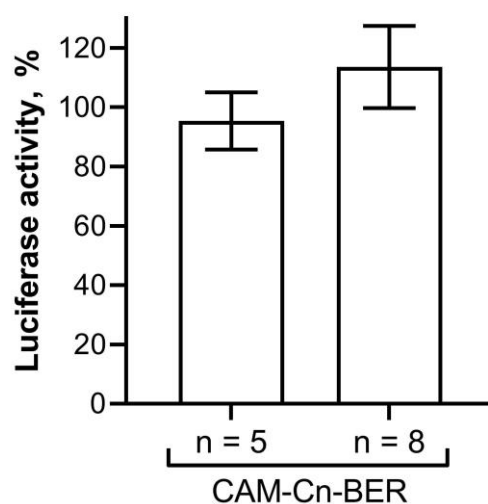


Figure S3. Eukaryotic translation. The inhibition of protein synthesis in vitro by 30 μ M of CAM-C5-BER and CAM-C8-BER in the cell-free eukaryotic transcription-translation coupled system. The relative enzymatic activity of in vitro synthesized firefly luciferase is shown. The error-bars represent the standard deviations.

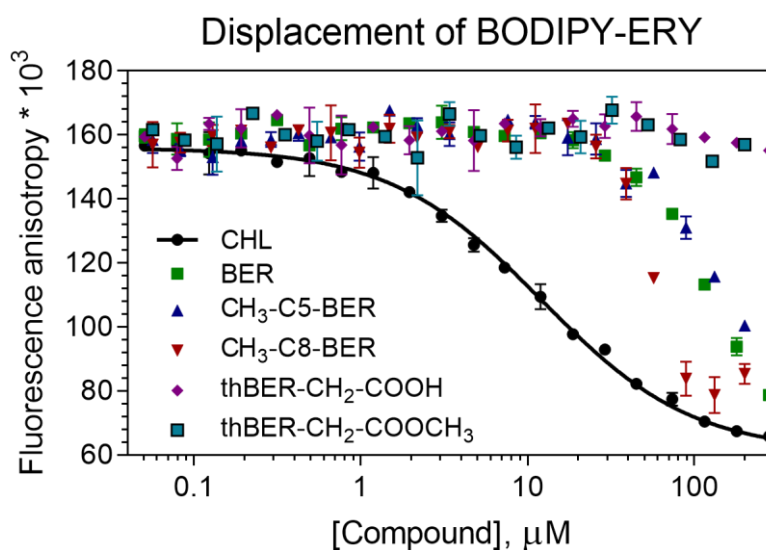


Figure S4. A competitive binding assay to test the affinity of CHL, BER, CH₃-Cn-BER (n = 5, 8), thBER-CH₂-COOH, and thBER-CH₂-COOCH₃ to *E. coli* 70S ribosomes measured by fluorescence anisotropy of fluorescently labeled analog of the erythromycin, BODIPY-ERY. All reactions were repeated at least two times. Error bars represent the standard deviation.

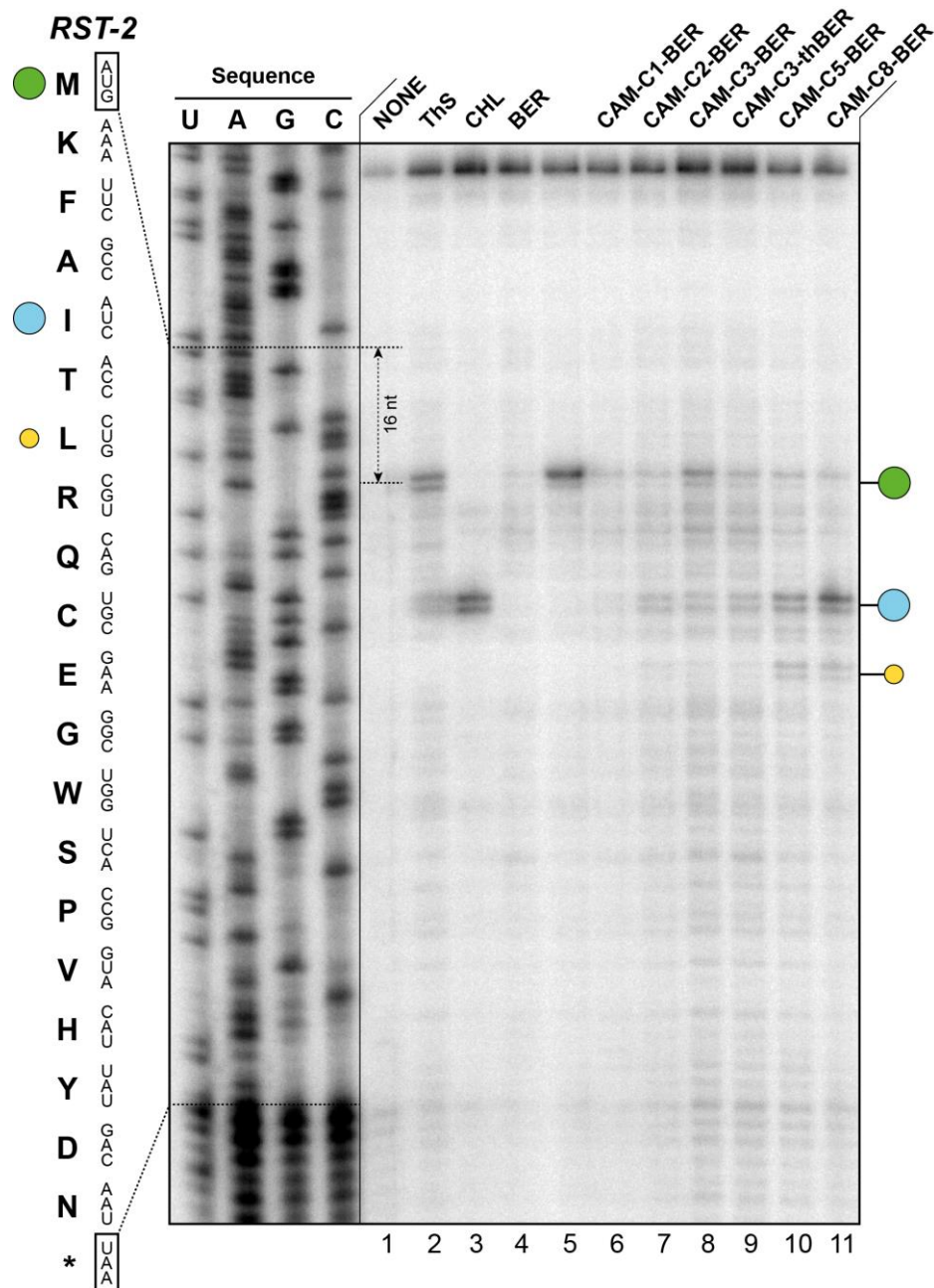


Figure S5. Original version of the Figure 3C. Ribosome stalling by CAM-Cn-BER (n = 1, 2, 3, 5, 8, lanes 6, 7, 8, 10, 11) and CAM-C3-thBER (lane 9) on *RST-2* mRNA as detected by a reverse-transcription primer-extension inhibition (toeprinting) assay in a cell-free translation system. DMSO, 0.5% (NONE, lane 1), ThS (inhibits translation at the start codon, lane 2), CHL (lane 3), and BER (lane 4) were used as controls. The nucleotide sequence of *RST-2* mRNA and its corresponding amino acid sequence are shown on the left. The green circle marks the translation arrest at the start codon, while the blue and yellow circles denote drug-induced arrest sites within the coding sequence of the mRNA used. Note that due to the large size of the ribosome, the reverse transcriptase used in the toeprinting assay stops 16 nucleotides downstream of the codon located in the P-site. The asterisk indicates a stop codon.

¹H CAM-C8-BER

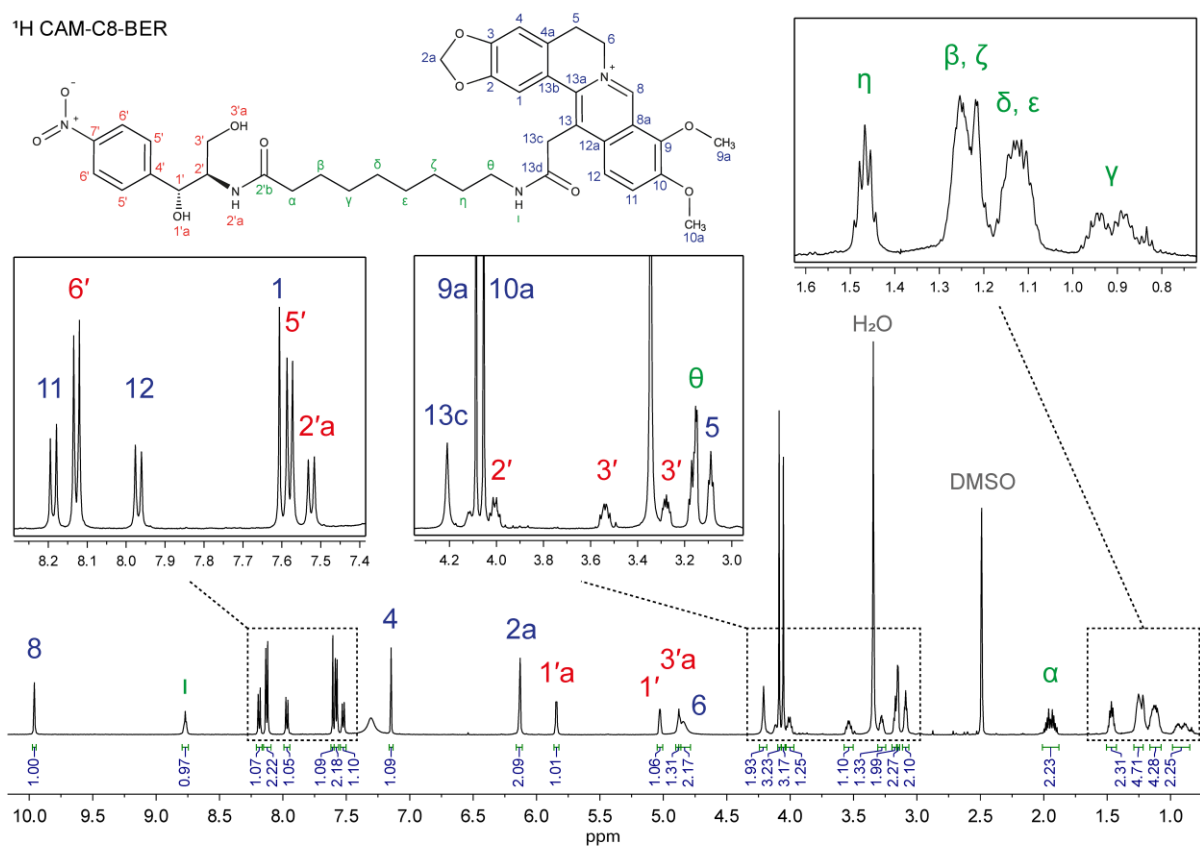


Figure S6. ¹H NMR spectrum of CAM-C8-BER.

¹³C CAM-C8-BER

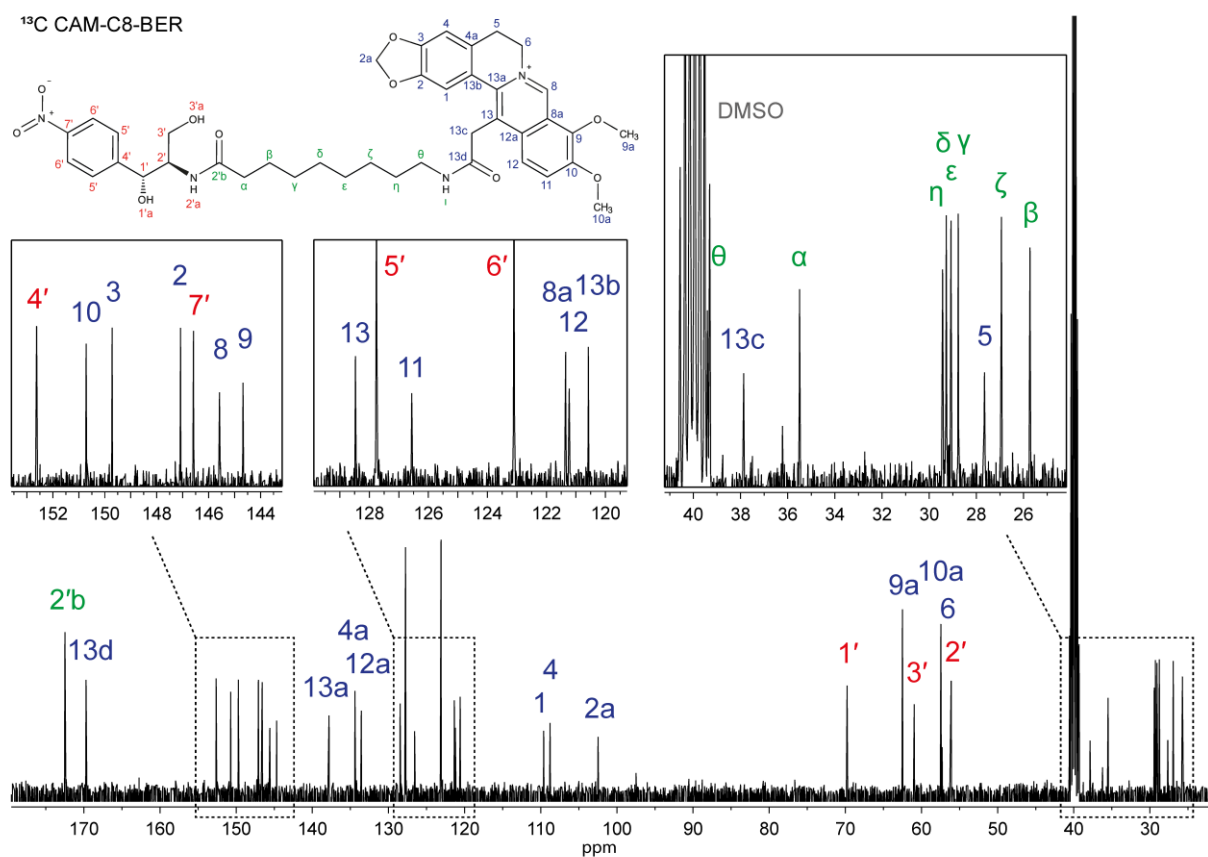


Figure S7. ¹³C NMR spectrum of CAM-C8-BER.

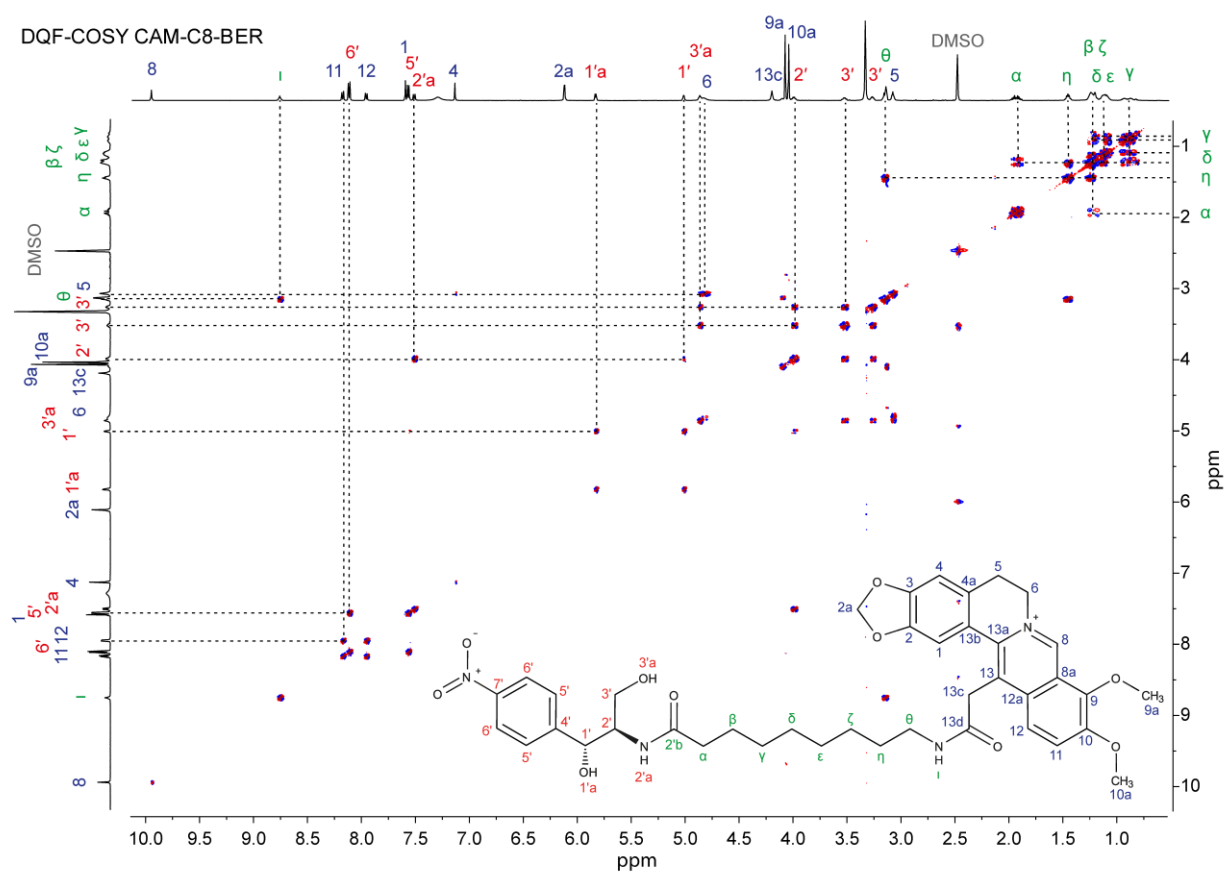


Figure S8. DQF-COSY NMR spectrum of CAM-C8-BER.

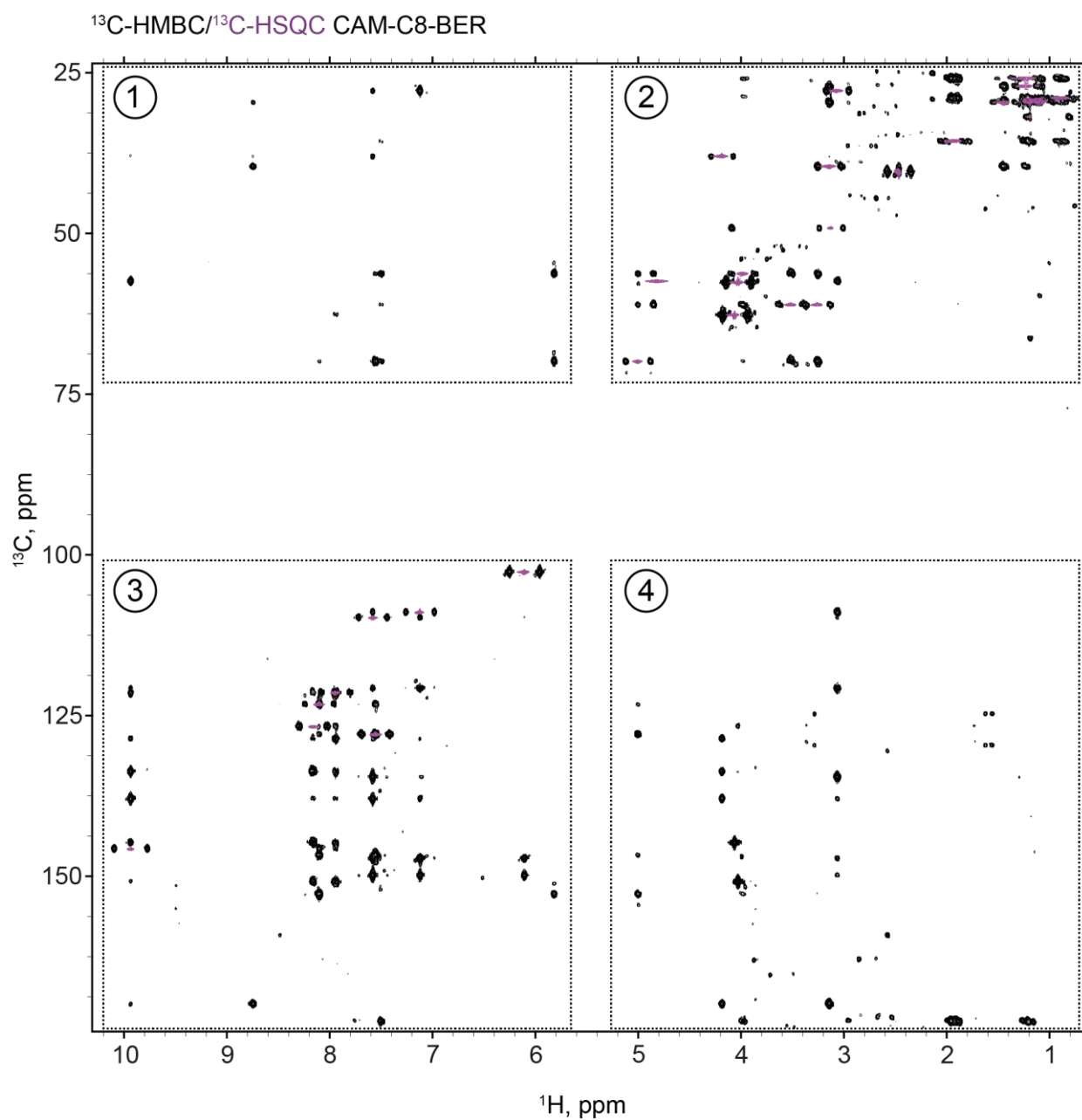


Figure S9. Overlay of ^1H - ^{13}C HMBC (black) and ^1H - ^{13}C HSQC (magenta) NMR spectra of CAM-C8-BER. The general view of spectra is shown.

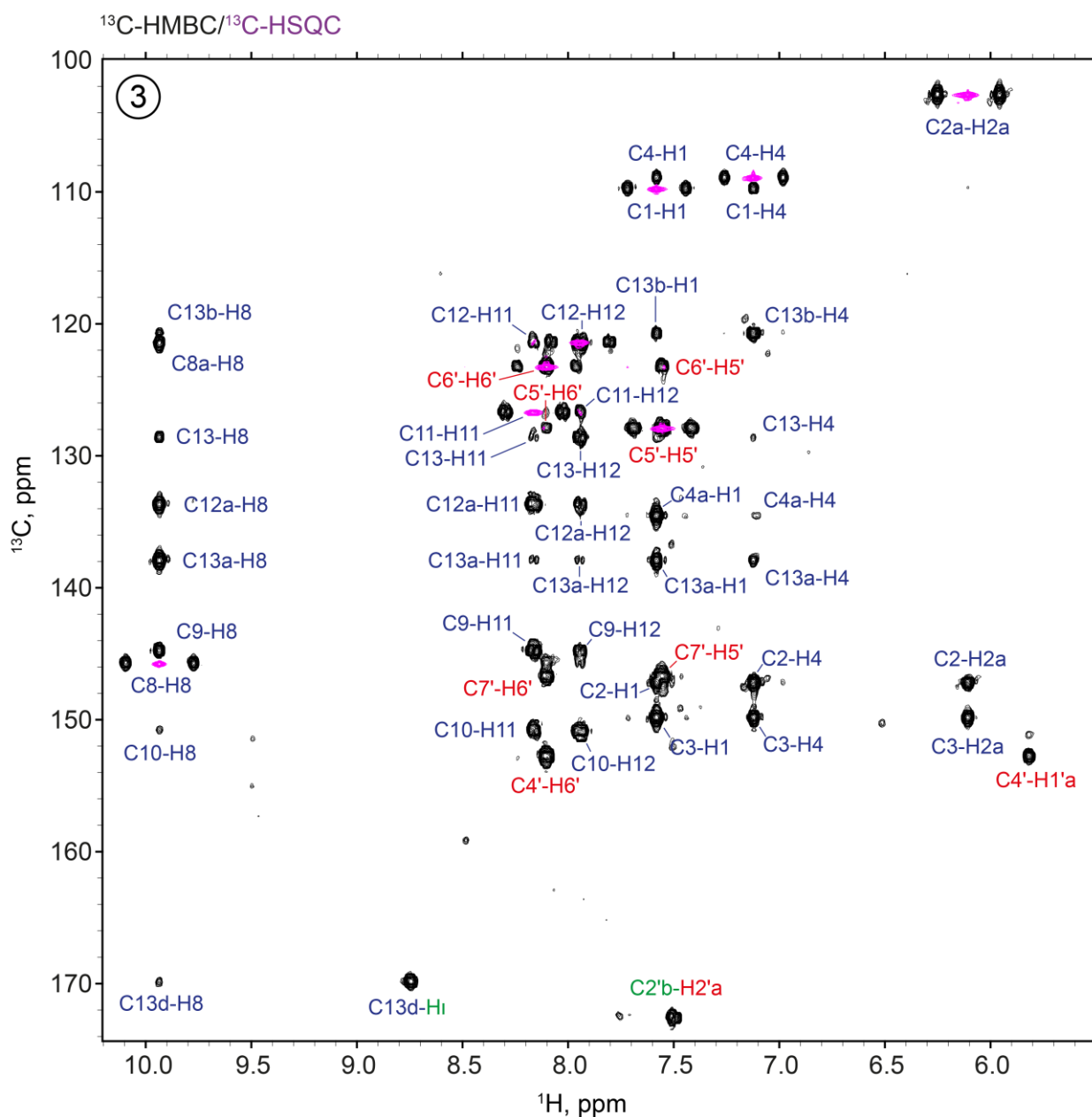


Figure S12. Overlay of ¹H-¹³C HMBC (black) and ¹H-¹³C HSQC (magenta) NMR spectra of CAM-C8-BER (area 3).

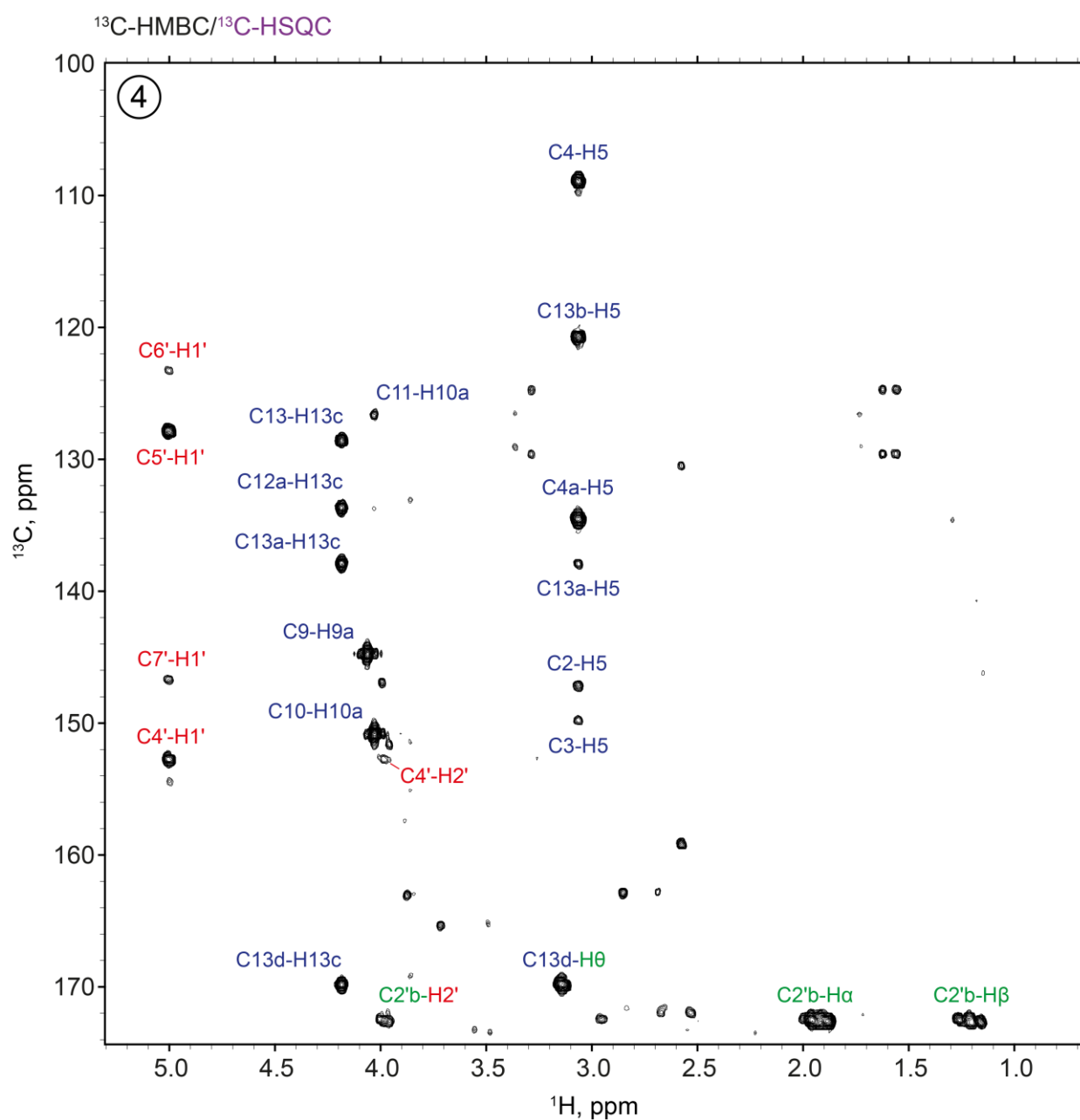


Figure S13. Overlay of ¹H-¹³C HMBC (black) and ¹H-¹³C HSQC (magenta) NMR spectra of CAM-C8-BER (area 4).

ROESY CAM-C8-BER

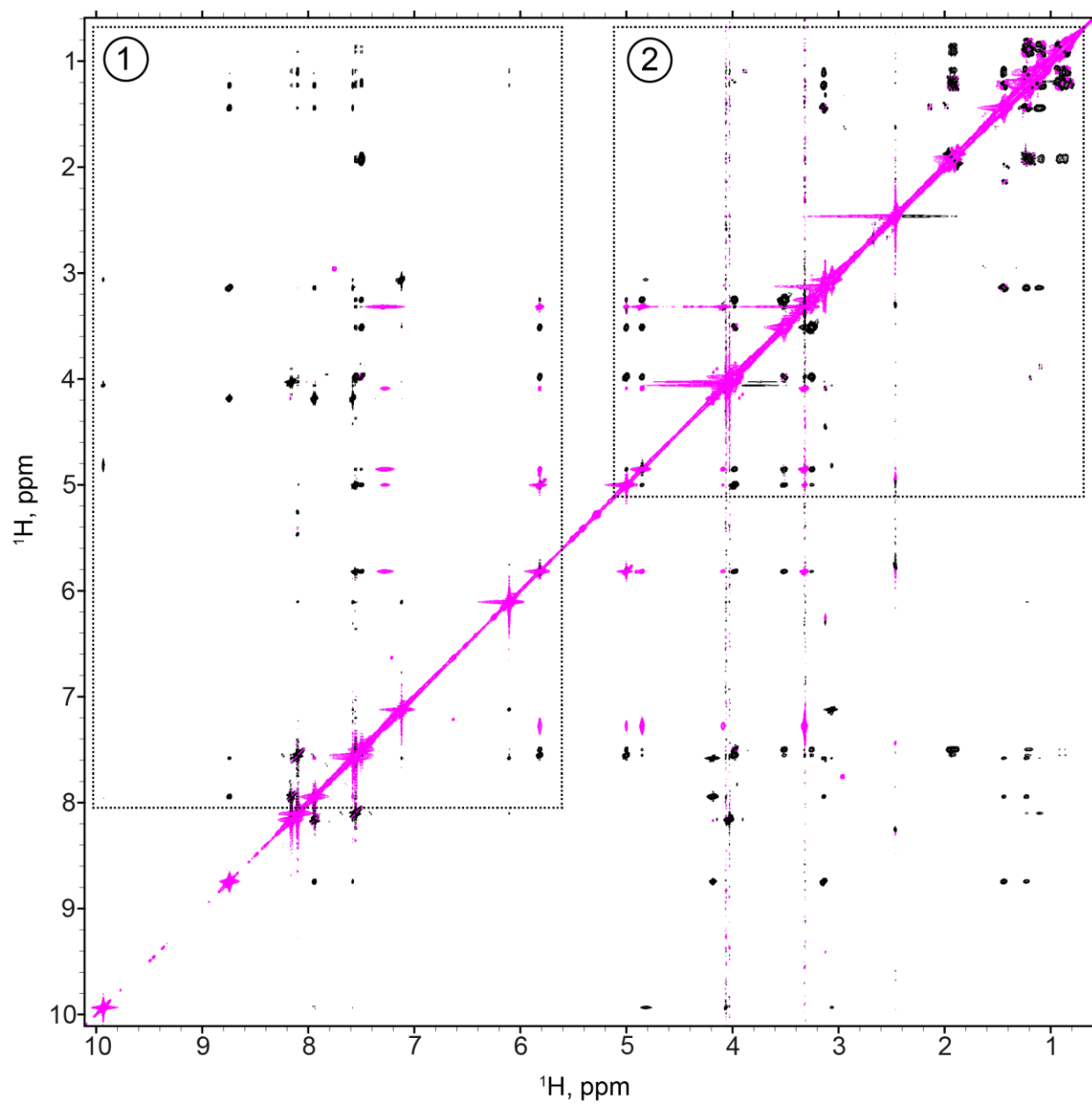


Figure S14. ^1H - ^1H ROESY NMR spectrum of CAM-C8-BER. The general view of the spectrum is shown.

