Electronic Supplementary Information

Norborn-2-en-7-ones as Physiologically-Triggered Carbon Monoxide-Releasing Prodrugs

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Compound 31. $^{13}$C NMR spectrum (125 MHz, CDCl$_3$).

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BP-CO-23. $^{13}$C NMR spectrum (125 MHz, DMSO-d$_6$).

BP-CO-23. $^{19}$F NMR spectrum (376 MHz, DMSO-d$_6$).
**Experimental**

Experiments requiring anhydrous conditions were performed under a dry nitrogen or argon atmosphere using apparatus heated and dried under vacuum, unless stated otherwise.

Anhydrous dichloromethane (CH$_2$Cl$_2$), tetrahydrofuran (THF), toluene, acetonitrile (CH$_3$CN) and methanol (CH$_3$OH) were dried using the PURE SOLV MD-6 solvent purification system. 6 M HCl in 1,4-dioxane was prepared from 36% aqueous HCl and 1,4-dioxane. All other reagents were purchased as analytical or reagent grade and used without further purification. Aqueous solutions of sodium chloride (NaCl), sodium bicarbonate (NaHCO$_3$) and ammonium chloride (NH$_4$Cl) were saturated. Reactions performed at room temperature (rt) were carried out at approximately 20 °C and reaction temperatures from -78 °C to 0 °C were obtained using the following cooling bath mixtures: acetone/dry ice, -78 °C; acetonitrile/dry ice, -40 °C; NaCl/ice, -15 °C; water/ice, 0 °C.

Reactions were monitored by thin layer chromatography (TLC) carried out on 0.2 mm Kieselgel F254 (Merck) silica gel plates using UV light as a visualising agent and then stained and developed with heat using either vanillin in ethanolic sulfuric acid, ammonium heptamolybdate and cerium sulfate in aqueous sulfuric acid, or potassium permanganate and potassium carbonate in aqueous sodium hydroxide. Separation of mixtures was performed by flash chromatography using 0.063–0.1 mm silica gel with the indicated eluent.

Infrared spectra were recorded on a Bruker Optics Alpha FT-IR spectrometer with a diamond Attenuated Total Reflectance (ATR) top plate. No sample preparation was required. Absorption peaks are reported as wavenumbers (ν, cm$^{-1}$).

NMR spectra were recorded on a Varian 400-MR spectrometer operating at 400 MHz for $^1$H nuclei and 100 MHz for $^{13}$C nuclei at 25 °C, or a Varian 500 MHz AR Premium Shielded Spectrometer at operating at 500 MHz for $^1$H nuclei and 125 MHz for $^{13}$C nuclei at 25 °C.
NMR chemical shifts are reported in parts per million (ppm) relative to the chloroform (CDCl$_3$, δ 7.26), or dimethyl sulfoxide (DMSO-d$_6$, δ 2.50) peak. $^1$H NMR values are reported as chemical shifts δ multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; m, multiplet), coupling constant (J, Hz) and relative integral. Coupling constants were taken directly from the spectra. $^{13}$C NMR chemical shifts are reported in ppm relative to the chloroform (CDCl$_3$, δ 77.0), or dimethyl sulfoxide (DMSO-d$_6$, δ 39.5) peak. $^{13}$C NMR values are reported as chemical shifts δ and assignment. Decoupled $^{19}$F NMR spectra were recorded on a Varian 400-MR spectrometer operating at 376 MHz at 25 °C and data are expressed in ppm. Assignments were made with the aid of DEPT, gCOSY, gHSQC, and gHMBC experiments. The term *inter alia* is used when reporting NMR data for the individual components of mixtures of endo and exo isomers.

Mass spectra were recorded on a Bruker micrOTOF-Q II mass spectrometer by electrospray ionisation in positive and negative mode. High-resolution mass spectra (HRMS) were obtained with a nominal resolution of 5,000 to 10,000.

Microanalyses were performed by Bob McAllister and Pauline Bandeen of the Campbell Microanalytical Laboratory, University of Otago.

**HPLC analysis**

HPLC grade acetonitrile (CH$_3$CN) was purchased from Merck Chemicals. MilliQ grade H$_2$O was obtained from a Millipore purification system. HPLC grade trifluoroacetic acid (TFA) was purchased from Scharlau. HPLC analyses were conducted on an analytical RP-HPLC (Shimadzu LC–20AD equipped with an SPD-20A UV detector [210 and 254 nm]) using a Phenomenex Prodigy column (C–18, 5 μm, 3.00 × 250 mm) at 0.5 mL min$^{-1}$ and heated to 40 °C. Unless otherwise stated, the solvent system for all LC purposes was a mixture of
A (0.05% TFA in H2O) and B (CH3CN). A gradient of 10% to 100% B over 12.5 min, then 100% B for 2.5 min, with a flow rate of 0.5 mL min⁻¹ was used unless otherwise stated.

3a-Bromo-3a,4,7,7a-tetrahydro-4,7-dimethyl-2,5,6-triphenyl-4,7-methano-1H-isoindole-1,3,8(2H)-trione (16)

The 2,5-dimethyl-3,4-diphenylcyclopentadien-1-one dimer (15)¹ (393 mg, 1.51 mmol) and 3-bromo-1-phenyl-1H-pyrrole-2,5-dione (14)²-³ (418 mg, 1.66 mmol) were refluxed in benzene (20 mL) for 6 h. The solution was concentrated in vacuo to a brown solid which was recrystallised from diethyl ether to afford the title compound 16 (551 mg, 71%) as white crystals. m.p. 180 °C. ν_max/cm⁻¹ 1781, 1719, 1367; ¹H NMR (500 MHz, CDCl₃) δ 1.67 (s, 3H), 1.69 (s, 3H), 3.69 (s, 1H), 6.93-7.00 (m, 4H), 7.13-7.28 (m, 8H), 7.40-7.50 (m, 3H). ¹³C NMR (125 MHz, CDCl₃) δ 11.7, 12.5, 56.8, 59.0, 60.3, 60.0, 126.1, 128.2, 128.3, 128.35, 128.41, 129.2, 129.4, 129.5, 129.8, 131.2, 132.5, 132.6, 140.5, 144.6, 171.3, 171.9, 196.8; HRMS-ESI [M + Na]⁺ Calcd for C₂₉H₂₂⁸¹BrNO₃Na⁺ 536.0655, found 536.0673.

4,7-Dimethyl-2,5,6-triphenyl-1H-isoindole-1,3(2H)-dione (17)

NEt₃ (0.33 mL, 2.38 mmol) was added to a solution of 16 (354 mg, 0.691 mmol) in dry THF (10 mL) and stirred for 3 h. The solution was washed with aqueous 1 M HCl, dried over anhydrous magnesium sulfate and concentrated in vacuo to afford a white solid. The crude product was purified by column chromatography (2:1 CH₂Cl₂/Petrol) to afford the title compound 17 (258 mg, 93%) as a white solid. m.p. 260 °C. ν_max/cm⁻¹ 1706, 1375; ¹H NMR (500 MHz, CDCl₃) δ 2.47 (s, 6H), 6.90-6.94 (m, 4H), 7.11-7.21 (m, 6H), 7.38-7.48 (m, 3H), 7.49-7.54 (m, 2H); ¹³C NMR (125 MHz, CDCl₃) δ 15.9, 126.9, 127.0, 127.9, 128.0, 129.1, 129.6, 132.1, 135.0, 138.7, 148.9, 168.2; HRMS-ESI [M + Na]⁺ Calcd for C₂₈H₂₁NO₂Na⁺.
426.1446, found 426.1449; Anal. Calcd for C_{28}H_{21}NO_{2}: C, 83.35; H, 5.25; N, 3.47. Found: C, 83.20; H, 5.30; N, 3.46.

**tert-Butyl (2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)ethyl)carbamate (19)**

A stirred solution of maleimide 18 (1.51 g, 5.67 mmol) in anhydrous dichloromethane (35 mL) was cooled to 0 °C and a solution of tert-butyl (2-aminoethyl)carbamate (1.00 g, 6.24 mmol) in anhydrous dichloromethane (35 mL) added drop-wise. The reaction was stirred under nitrogen for 1 h at 0 °C then stirred overnight at rt. Upon consumption of the succinimidyl ester, the reaction was diluted with dichloromethane, washed with 5% HCl(aq), water and brine, then dried (MgSO_{4}) and filtered. After removal of the solvent the residue was purified by flash column chromatography (EtOAc) to give the **title compound 19** (1.22 g, 69%) as a white solid. Rf 0.4 (EtOAc); m.p. 135–136 °C; IR: ν_{max}/cm^{-1} 3352, 3325, 2976, 2941, 1698, 1679, 1642, 1544, 1525, 1445; ^1H NMR (500 MHz, CDCl_{3}) δ 1.42 (s, 9H), 2.49 (t, J = 7.1 Hz, 2H), 3.22–3.24 (m, 2H), 3.29–3.83 (m, 2H), 3.82 (t, J = 7.1 Hz, 2H), 5.03 (br s, 1H), 6.43 (br s, 1H), 6.69 (s, 2H); ^13C NMR (125 MHz, CDCl_{3}) δ 28.3, 34.3, 34.7, 40.1, 40.7, 79.6, 134.2, 156.9, 170.3, 170.5; HRMS-ESI: [M + Na]^+ Calcd for C_{14}H_{21}N_{3}O_{5}Na 334.1373, found 334.1403; Anal. Calcd for C_{14}H_{21}N_{3}O_{5}: C, 54.01; H, 6.80; N, 13.50; Found: C, 53.83; H, 6.81; N, 13.33.

**tert-Butyl (2-(3-(3-bromo-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)ethyl)carbamate (20)**

Maleimide 19 (1.00 g, 3.21 mmol) was dissolved in carbon tetrachloride (12.5 mL) and treated with bromine (0.18 mL, 3.53 mmol) then heated at reflux for 1 h. Upon cooling, the precipitate was collected by vacuum filtration and re-dissolved in anhydrous THF (15 mL). The solution was cooled to 0 °C and anhydrous NEt_{3} (0.45 mL, 3.21 mmol) added dropwise followed by stirring for 2 h. Volatiles were removed in vacuo and the residue partitioned between ethyl
acetate and water. The organic extracts were washed with brine, dried (MgSO₄) and filtered. Removal of solvent under vacuum gave the crude bromomaleimide which was purified by flash column chromatography (EtOAc) to provide the title compound 20 (708 mg, 57%) as an off-white solid. Rf 0.5 (EtOAc); M.p. 156-158 °C (decomposition); IR: v_max/cm⁻¹ 3343, 3323, 3095, 2984, 2941, 1712, 1678, 1645; ¹H NMR (500 MHz, CDCl₃) δ 1.44 (s, 9H), 2.51 (t, J = 7.1 Hz, 2H), 3.22–3.25 (m, 2H), 3.31–3.34 (m, 2H), 3.87 (t, J = 7.1 Hz, 2H), 4.93 (s, 1H), 6.33 (s, 1H), 6.86 (s, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 28.4, 34.5, 35.2, 40.1, 40.9, 79.8, 131.4, 132.0, 157.0, 165.1, 168.2, 169.9; HRMS-ESI: [M + Na]+ Calcd for C₁₄H₂₀BrN₃O₅Na 412.0479, found 412.0498; HPLC t_R = 9.1 min.

tert-Butyl (2-(3-(3a-bromo-4,7-dimethyl-1,3,8-trioxo-5,6-diphenyl-3a,4,7a-tetrahydro-1H-4,7-methanoisoindol-2(2H)-yl)propanamido)ethyl)carbamate (21)

Bromomaleimide 20 (200 mg, 0.51 mmol) and diene dimer 15 (140 mg, 0.54 mmol) were dissolved in toluene (5 mL) and heated at reflux for 4 h under nitrogen. The solvent was removed under vacuum and the crude residue purified by flash column chromatography (1:1 EtOAc/Petrol) to give the title compound 21 (310 mg, 93%) as a 3:1 mixture of endo- and exo-isomers in the form of a white solid. M.p. 97–100 °C; IR: v_max/cm⁻¹ 3325 (br), 3293 (br), 2978, 2935, 2873, 1782, 1709 (s), 1655, 1514 (br), 1443; HRMS-ESI: [M + Na]+ Calcd for C₃₃H₃₆⁷⁹BrN₃O₅Na 672.1680, found 672.1670; RP-HPLC t_R = 13.5 min.

NMR data for endo-21: ¹H NMR (500 MHz, CDCl₃) δ inter alia 1.42 (s, 9H), 1.59 (s, 3H), 1.61 (s, 3H), 2.51 (td, J = 7.9, 2.2 Hz, 2H), 3.18 – 3.28 (m, 2H), 3.28 – 3.36 (m, 2H), 3.51 (s, 1H), 3.76 – 3.97 (m, 2H), 4.95 (br s, 1H), 6.32 (br s, 1H), 6.80 – 6.90 (m, 4H), 7.12 – 7.29 (m, 6H); ¹³C NMR (125 MHz, CDCl₃) δ inter alia 11.5, 12.3, 28.3, 33.4, 36.1, 40.0, 40.9, 56.4, 57.1, 58.8, 59.0, 60.2, 60.7, 79.8, 126.8, 127.7, 128.0, 128.1, 128.2, 128.3, 128.4, 129.2, 129.4, 129.6, 130.4, 132.3, 132.4, 140.3, 144.6, 156.9, 169.5, 172.1, 172.3, 196.8.
NMR data for exo-21: $^1$H NMR (500 MHz, CDCl$_3$) δ *inter alia* 1.35 (s, 3H), 1.43 (s, 9H), 1.46 (s, 3H), 2.44 (t, $J$ = 6.9 Hz, 2H), 3.37 (s, 1H), 5.06 (br s, 1H), 6.16 (br s, 1H), 7.02 – 7.06 (m, 4H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ *inter alia* 8.1, 9.3, 57.1, 58.8, 61.5, 132.6, 132.8, 144.9, 170.3, 170.6.

*N-(2-Aminoethyl)-3-(3a-bromo-4,7-dimethyl-1,3,8-trioxo-5,6-diphenyl-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindol-2(2H)-yl)propanamide hydrochloride (oCOM-19. HCl)*

Adduct 21 (230 mg, 0.35 mmol) was dissolved in anhydrous 1,4-dioxane (1 mL) and cooled to 0 °C under nitrogen. A solution of 6 M HCl in 1,4-dioxane (3 mL) was added dropwise and the reaction allowed to warm to rt and stirred overnight. Removal of volatiles under vacuum provided the amine hydrochloride salt which was lyophilized to provide the *title compound* oCOM-19.HCl (205 mg, 99%) as a 3:1 mixture of endo- and exo-isomers in the form of a white solid. M.p. 142 – 148 °C (decomposition); IR: $\nu_{\text{max}}$/cm$^{-1}$ 3378 (br), 2979, 2937, 2873, 1781, 1711, 1676, 1520 (br), 1444; HRMS-ESI: [M - Cl]$^+$ Calcd for C$_{28}$H$_{29}$BrN$_3$O$_4$ 550.1336, found 550.1355; Anal. Calcd for C$_{28}$H$_{29}$BrClN$_3$O$_4$H$_2$O: C, 55.59; H, 5.17; N, 6.95; Found: C, 55.61; H, 5.19; N, 6.92; RP-HPLC $t_R$ = 8.8 min.

NMR data for endo- oCOM-19.HCl: $^1$H NMR (500 MHz, DMSO-d$_6$) δ *inter alia* 1.45 (s, 3H), 1.48 (s, 3H), 2.38 – 2.45 (m, 2H), 2.84 (q, $J$ = 6.1 Hz, 2H), 3.28 (q, $J$ = 6.2 Hz, 2H), 3.75 (td, $J$ = 13.5, 6.4 Hz, 2H), 4.00 (s, 1H), 6.91 – 6.82 (m, 4H), 7.19 – 7.26 (m, 6H), 8.03 (br s, 3H), 8.37 (t, $J$ = 5.6 Hz, 1H); $^{13}$C NMR (125 MHz, DMSO-d$_6$) δ *inter alia* 11.2, 11.7, 32.7, 36.0, 36.4, 38.5, 55.7, 58.3, 58.9, 60.1, 128.0, 128.1, 128.3, 129.1, 129.4, 132.3, 133.4, 139.6, 144.6, 169.5, 171.8, 172.3, 196.7.

NMR data for exo- oCOM-19.HCl: $^1$H NMR (500 MHz, DMSO-d$_6$) δ *inter alia* 1.18 (s, 3H), 1.32 (s, 3H), 2.34 (t, $J$ = 7.5 Hz, 2H), 3.91 (s, 1H), 8.26 (t, $J$ = 5.6 Hz,); $^{13}$C NMR (125 MHz, DMSO-d$_6$) δ *inter alia* 169.4, 197.1.
**tert-Butyl (2-(3-(4,7-dimethyl-1,3-dioxo-5,6-diphenylisoindolin-2-yl)propanamido)ethyl)carbamate (22)**

Adduct 21 (200 mg, 0.31 mmol) was dissolved in anhydrous THF (5 mL) and DBU (0.09 mL, 0.62 mmol) was added dropwise. The reaction was stirred and monitored by TLC until no starting material remained (1 h). Volatiles were removed under vacuum and the residue taken up in ethyl acetate and washed with 5\% HCl\_aq. After drying over MgSO\_4 and filtering the organic extracts, removal of volatiles under vacuum gave the crude aromatized material. Purification by flash column chromatography (3:2 EtOAc/Petrol) provided the *title compound* 22 (147 mg, 88\%) as a white solid. Rf 0.2 (1:1 EtOAc/petrol); m.p. 183 – 185 °C; IR: ν\text{max}/cm\text{⁻¹} 3312 (br), 2974, 2932, 1761, 1700, 1646, 1523 (br), 1443; \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) δ 1.43 (s, 9H), 2.39 (s, 6H), 2.63 (t, J = 7.0 Hz, 2H), 3.20 – 3.32 (m, 2H), 3.32 – 3.43 (m, 2H), 4.01 (t, J = 7.0 Hz, 2H), 5.09 (br s, 1H), 6.44 (br s, 1H), 6.82 – 6.95 (m, 4H), 7.05 – 7.22 (m, 6H); \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}) δ 15.6, 28.4, 31.5, 35.2, 40.2, 40.6, 79.6, 126.8, 127.7, 128.0, 129.5, 134.5, 138.6, 148.5, 156.7, 169.0, 170.6; HRMS-ESI: [M + Na]\textsuperscript{⁺} Calcd for C\textsubscript{32}H\textsubscript{35}N\textsubscript{3}O\textsubscript{5}Na 564.2469, found 564.2455.

**N-(2-aminoethyl)-3-(4,7-dimethyl-1,3-dioxo-5,6-diphenylisoindolin-2-yl)propanamide hydrochloride (24) (BP-CO-19)**

6 M HCl in anhydrous 1,4-dioxane (3 mL) was added dropwise to a solution of Boc-protected amine 22 (139 mg, 0.26 mmol) in 1,4-dioxane (1 mL) at 0 °C under nitrogen. The reaction was allowed to warm to rt and stirred overnight. Removal of volatiles under vacuum followed by lyophilization provided the *title compound* BP-CO-19 (117 mg, 95\%) as its HCl salt in the form of a white solid. M.p. 185 – 187 °C; IR: ν\text{max}/cm\text{⁻¹} 3274 (br), 3057, 3024, 2939 (br), 1760, 1695, 1645, 1549, 1495, 1441; \textsuperscript{1}H NMR (500 MHz, DMSO-d\textsubscript{6}) δ 2.30 (s, 6H), 2.49 (t, J = 5.0
Hz, 2H), 2.78 – 2.91 (m, 2H), 3.25 – 3.32 (m, 2H), 3.82 (t, J = 7.3 Hz, 2H), 6.90 – 7.04 (m, 4H), 7.07 – 7.17 (m, 2H), 7.17 – 7.27 (m, 4H), 7.99 (s, 3H), 8.30 (t, J = 5.6 Hz, 1H); \(^{13}\)C NMR (125 MHz, DMSO-\(d_6\)) \(\delta\) 15.2, 34.0, 34.0, 36.4, 38.5, 126.9, 127.7, 127.8, 129.3, 133.2, 138.3, 147.8, 168.1, 170.5; HRMS-ESI: [M - Cl]\(^+\) Calcd for C\(_{27}\)H\(_{28}\)N\(_3\)O\(_3\) 442.2125, found 442.2116.

RP-HPLC: \(t_R = 9.2\) min.

\(N\)-\((2\text{-Aminoethyl})\)-3-\((3\text{-bromo-1H-4,7-dimethyl-1,3,8-trioxo-5,6-diphenyl-isooindolin-2(2H)-yl})\) propanamide 2,2,2-trifluoroacetate (oCOM-19.CF\(_3\)CO\(_2\)H)

6 M HCl in 1,4-dioxane (2 mL) was added to a solution of 21 (305 mg, 0.47 mmol) in 1,4-dioxane (1.4 mL) under a nitrogen atmosphere. The reaction mixture was allowed to warm to rt and monitored by RP-HPLC (10% to 100% B over 12.5 min, then 100% B for 2.5 min, 0.5 mL/min). Upon consumption of the starting material (4-5 h), the solvent was removed \(\text{in vacuo}\). The residue was dissolved in a mixture of approximately 50% H\(_2\)O in 1,4-dioxane (50 mL) and then lyophilised. The off-white solid obtained was dissolved in approximately 20% CH\(_3\)CN in H\(_2\)O (2 x 5 mL) and then loaded onto a C-18 solid phase extraction cartridge (pre-wash procedure100% CH\(_3\)CN [2 x 10 mL], then 90%, then 50% CH\(_3\)CN in H\(_2\)O [1 x 10 mL each], 20% CH\(_3\)CN in H\(_2\)O [2 x 10 mL]). The compound was eluted from the C-18 cartridge (20% to 40% CH\(_3\)CN in H\(_2\)O [10% increments, 2 x 10 mL each], then 50% to 100% CH\(_3\)CN in H\(_2\)O [10% increments, 10 mL each]). The fractions collected were analysed by RP-HPLC (10% to 100% B over 12.5 min, then 100% B for 2.5 min, \(t_R = 8.99\) min) and lyophilised to afford the \(\text{title compound} \ \text{oCOM-19.CF}_3\text{CO}_2\text{H} \) (106.9 mg, 34%, >97% purity, inseparable 10:1 mixture of \(\text{endo:exo} \) isomers) as a white powder. M.p. 142–148 °C; IR (ATR) \(\nu_{\max}/\text{cm}^{-1}\) 3378 (br), 2979, 1937, 2873, 1781, 1711, 1676, 1520 (br), 1444; HRMS (ESI-TOF) \(m/z\): [M – 'O\(_2\)CCF\(_3\)]\(^+\) Calcd for C\(_{28}\)H\(_{28}\)\(^{79}\)BrN\(_3\)O\(_4\)Na\(^+\) 572.1155, found 572.1141; RP-HPLC: \(t_R = 8.99\) min, \(\text{endo-} \) and \(\text{exo-} \)isomers co-elute as a single peak.
Endo- oCOM-19.CF₃CO₂H NMR data: $^1$H NMR (400 MHz, DMSO-d6) δ inter alia 1.45 (s, 3H), 1.49 (s, 3H), 2.41 (t, $J = 8.0$ Hz, 2H), 2.84 (q, $J = 6.0$ Hz, 2H), 3.25 (q, $J = 6.0$ Hz, 2H), 3.71–3.79 (m, 2H), 4.00 (s, 1H), 6.85–6.88 (m, 4H), 7.17–7.29 (m, 6H), 7.68 (br s, 3H), 8.21 (t, $J = 6.0$ Hz, 1H); $^{13}$C NMR (125 MHz, DMSO-d6) δ inter alia 11.2, 11.7, 32.7, 36.0, 36.4, 38.7, 55.7, 58.3, 58.9, 60.0, 128.0, 128.1, 128.3, 129.1, 129.4, 132.3, 132.4, 139.5, 144.6, 169.8, 171.9, 172.3, 196.7; $^{19}$F NMR (376 MHz, DMSO-d6) δ -73.74.

Exo-oCOM-19.CF₃CO₂H NMR data: $^1$H NMR (400 MHz, DMSO-d6) δ inter alia 1.19 (s, 3H), 1.32 (s, 3H), 3.92 (s, 1H).

A further fraction containing mixture of oCOM-19 and 22 (112.4 mg, 3:1) was also isolated.

tert-Butyl N-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethylcarbamate (24)

To a solution of tert-butyl (2-ethylamino)carbamate (1.13 g, 7.1 mmol) in saturated NaHCO₃ (35 mL) at 0 ºC was added N-(ethoxycarbonyl)maleimide (23) (1.1 g, 7.1 mmol). The reaction mixture was warmed to rt and stirred for 15 min. THF (55 mL) was then added and the reaction mixture stirred for a further 45 min. H₂O (50 mL) was added and the aqueous phase extracted with EtOAc (3 x 75 mL). The combined organic extracts were washed with saturated NaCl (100 mL) and dried over MgSO₄. Removal of the solvent in vacuo gave an off-white solid that was purified by flash chromatography (0%, then 5%, then 10% EtOAc in CH₂Cl₂) to afford the title compound 24 (1.1 g, 58%) as a white solid. M.p. 126–128 ºC; $R_f$ (10% EtOAc in CH₂Cl₂) 0.3; IR (ATR) $v_{max}$/cm⁻¹ 3350, 3089, 2977, 1701, 1678, 1516, 1434, 1288, 1256, 1167, 944, 844, 692, 623; $^1$H NMR (400 MHz, CDCl₃) δ 1.40 (s, 9H), 3.30–3.35 (m, 2H), 3.66 (t, $J = 6.0$ Hz, 2H), 4.74 (br s, 1H), 6.71 (s, 2H); $^{13}$C NMR (100 MHz, CDCl₃) δ 28.3, 38.0, 39.4, 79.5, 131.1, 155.9, 170.8. The $^1$H and $^{13}$C NMR data obtained were in agreement with those reported from literature.⁶
**tert-Butyl 3-(3-bromo-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethylcarbamate (26)**

A solution of 24 (2.0 g, 8.32 mmol) in CH₂Cl₂ (12 mL) was treated with bromine (470 μL, 9.13 mmol) and heated at reflux for 1 h. The reaction was cooled to rt and concentrated in vacuo to give the dibrominated intermediate as a thick orange oil which was then diluted in anhydrous THF (40 mL) and cooled to 0 °C. Anhydrous NEt₃ (1.16 mL, 8.32 mmol) was added drop-wise and the reaction mixture stirred for 2 h at 0 °C. The resulting thick off-white suspension was diluted in H₂O (20 mL) and the aqueous phase extracted with EtOAc (3 x 20 mL). The combined organic extracts were dried over MgSO₄ and concentrated in vacuo to give a dark brown oil that was purified by flash chromatography (0%, then 5%, then 10% EtOAc in CH₂Cl₂) to afford the title compound 26 (1.944 g, 73%) as a pale yellow solid.

M.p. 90–93 °C; Rₐ (10% EtOAc in CH₂Cl₂) 0.3; IR (ATR) νₓcm⁻¹ 3354, 2970, 2970, 1702, 1681, 1523, 1406, 1284, 1246, 1160; ¹H NMR (400 MHz, CDCl₃) δ 1.40 (s, 9H), 3.32 – 3.36 (m, 2H), 3.70 (t, J = 6.0 Hz, 2H), 4.70 (br s, 1H), 6.88 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 28.3, 39.1, 39.2, 79.7, 131.4, 131.9, 156.0, 165.4, 168.6; HRMS (ESI-TOF) m/z: [M + Na]⁺ Calcd for C₁₁H₁₃BrN₂O₄Na⁺ 341.0107, found 341.0102. Anal. Calcd for C₁₁H₁₃BrN₂O₄: C, 41.40; H, 4.74; N, 8.78; Br, 25.04. Found: C, 41.41; H, 4.51; N, 8.76; Br, 25.23.

**tert-Butyl (2-(3a-bromo-4,7-dimethyl-1,3,8-trioxo-5,6-diphenyl-3a,4,7,7a-tetrahydro-2H-4,7-methanoisoindol-2-yl)ethyl)carbamate (28)**

Boc-bromomaleimide 26 (356 mg, 1.11 mmol) and 15 (305 mg, 0.59 mmol) were dissolved in anhydrous toluene (6.5 mL) and placed under argon. The mixture was then heated at reflux for 4 h. After cooling to rt, the solvent was removed in vacuo to afford a brown oil containing cycloadduct (28) as a 2.5:1 mixture of endo- and exo-isomers. Purification by flash chromatography (0%, then 2%, then 10% EtOAc in CH₂Cl₂) afforded the title compound 28 as a white foamy solid in two fractions: the pure endo-isomer (204 mg, 32%) and a ~1:1 mixture...
of endo- and exo-isomers (333 mg, 52%). Endo-28 data: M.p. 107–109 °C; Rf (2% EtOAc in CH2Cl2) 0.1; IR (ATR) ν max/cm⁻¹ 2984, 2937, 1790, 1713, 1390, 1366, 1248, 1201; ¹H NMR (400 MHz, CDCl3) δ 1.42 (s, 9H), 1.61 (s, 3H), 1.62 (s, 3H), 3.37 – 3.39 (m, 2H), 3.52 (s, 1H), 3.73 (t, J = 5.8 Hz, 2H), 4.72 (br s, 1H), 6.83 – 6.88 (m, 4H), 7.15 – 7.19 (m, 6H); ¹³C NMR (125 MHz, CDCl3) δ 11.5, 12.4, 28.4, 38.7, 40.0, 56.3, 58.7, 60.4, 60.8, 79.8, 128.1, 128.3, 128.3, 129.6, 132.4, 134.0, 144.5, 155.8, 172.4, 172.7, 196.9; HRMS (ESI-TOF) m/z: [M – Boc + H]⁺ Calcd for C25H2479BrN2O3 + 479.0965; Found 479.0949. [M + Na]⁺ Calcd for C30H3179BrN2O5Na + 601.1309 found 601.1303; Anal. Calcd for C30H31BrN2O5: C, 62.18; H, 5.39; N, 4.83; Br, 13.79. Found: C, 62.16; H, 5.39; N, 4.89; Br, 13.89; RP-HPLC: tR = 14.56 min.


2-(3a-Bromo-4,7-dimethyl-1,3,8-trioxo-5,6-diphenyl-2H-4,7-methanoisoindolin-2-yl)ethan-1-aminium 2,2,2-trifluoroacetate (oCOm-21)

6 M HCl in 1,4-dioxane (6 mL) was added to a solution of endo-28 (400 mg, 0.69 mmol) in 1,4-dioxane (2 mL) at 0 °C. The solution was warmed to rt and monitored by RP-HPLC. Upon consumption of the starting material (4-5 h), the solvent was removed in vacuo. The residue was dissolved in a mixture of approximately 75% H2O in 1,4-dioxane (40 mL) and lyophilised. The crude off-white solid obtained was dissolved in approximately 16% CH3CN in H2O (12 mL) and loaded onto a C-18 solid phase extraction cartridge (pre-wash procedure as described for the preparation of oCOM-19.TFA). The product was eluted from the C-18 cartridge (20% to 40% CH3CN in H2O [10% increments, 2 x 10 mL each], then 50% to 100% CH3CN in H2O [10% increments, 10 mL each]). The fractions collected were analysed by RP-HPLC and lyophilised to afford the title compound oCOM-21.TFA (259 mg, 63%) as a white powder. IR (ATR) ν max/cm⁻¹ 2937, 1781, 1711, 1664, 1648, 1443, 1388, 1203, 1180, 1134, 798, 723, 697; ¹H NMR (400 MHz, DMSO-d6) δ 1.46 (s, 3H), 1.52 (s, 3H), 2.95 – 3.07 (m, 2H), 3.77 –
3.81 (m, 2H), 3.93 (s, 1H), 6.83 – 6.87 (m, 4H), 7.20 – 7.24 (m, 6H), 7.90 (br s, 3H); 13C NMR (125 MHz, DMSO-d6) δ 11.3, 11.8, 36.5, 37.0, 55.6, 58.5, 58.7, 60.5, 128.1, 128.2, 128.3, 129.1, 129.3, 132.2, 132.3, 139.5, 144.5, 172.0, 172.6, 196.5; 19F NMR (376 MHz, DMSO-d6) δ -73.97; HRMS (ESI-TOF) m/z: [M – O2CCF3]+ Calcd for C25H2479BrN2O3+ 479.0965, found 479.0978; RP-HPLC: tR = 9.00 min.

2-(3a-Bromo-4,7-dimethyl-1,3,8-trioxo-5,6-diphenyl-2H-4,7-methanoisoindolin-2-yl)ethan-1-aminium chloride (oCOM-21.HCl)

A solution of endo-28 (200 mg, 0.35 mmol) in 1,4-dioxane (1 mL) was mixed with 6 M HCl in 1,4-dioxane (2 mL) at 0 °C. The reaction mixture was allowed to warm to rt and monitored by RP-HPLC. Upon consumption of the starting material (4 h), the solvent was removed in vacuo. The residue was dissolved in a mixture of approximately 75% H2O in 1,4-dioxane (40 mL) and lyophilised to give the title compound oCOM-21.HCl as a pale brown powder (175 mg, 98%). 1H NMR (500 MHz, DMSO-d6) δ 1.45 (3H, s), 1.52 (s, 3H), 2.90 – 3.05 (m, 2H), 3.78 – 3.83 (m, 2H), 3.93 (s, 1H), 6.83 – 6.87 (7.20 – 7.24 (m, 6H), 8.18 (br s, 3H); 13C NMR (125 MHz, DMSO-d6) δ 11.3, 11.8, 36.4, 36.9, 55.5, 58.6, 58.7, 60.5, 128.1, 128.2, 128.3, 129.1, 129.3, 132.2, 132.3, 139.5, 144.5, 172.0, 172.6, 196.5; HRMS (ESI-TOF) m/z: [M – O2CCF3]+ Calcd for C25H2479BrN2O3+ 479.0965, found 479.0932; RP-HPLC: tR = 9.00 min.

tert-Butyl (2-(4,7-dimethyl-1,3-dioxo-5,6-diphenylisoindolin-2-yl)ethyl)carbamate (30)

To a solution of 28 (414 mg, 0.71 mmol, endo:exo 2.5:1) in anhydrous THF (12 mL) at 0 °C was added DBU (210 µL, 1.40 mmol) dropwise. The reaction mixture was allowed to warm to rt. Upon consumption of starting material by TLC (approximately 10 min), saturated NH4Cl
(7 mL) was added and the aqueous phase was extracted with EtOAc (3 x 20 mL). The combined organic extracts were dried over MgSO$_4$ and concentrated in vacuo to afford a brown crystalline solid. Purification by flash chromatography (0%, then 5%, then 10% EtOAc in CH$_2$Cl$_2$) afforded the title compound 30 (205 mg, 61%) as a white solid. M.p. 181–182 °C; $R_f$ (10% EtOAc in CH$_2$Cl$_2$) 0.4; IR (ATR) $\nu_{\text{max}}$/cm$^{-1}$ 3431, 2981, 2944, 1758, 1725, 1698, 1504, 1431, 1401, 1391; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 1.40 (s, 9H), 2.41 (s, 6H), 3.42–3.47 (m, 2H), 3.85 (t, $J$ = 5.6 Hz, 2H), 4.89 (br s, 1H), 6.87–6.89 (m, 4H), 7.10–7.18 (m, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 15.7, 28.3, 37.6, 39.9, 79.4, 126.8, 127.8, 128.1, 129.5, 134.4, 138.7, 148.4, 159.5, 169.3; HRMS (ESI-TOF) $m/z$: [M + Na]$^+$ Calcd for C$_{29}$H$_{30}$N$_2$NaO$_4$: 493.2098, found 493.2097; Anal. Calcd for C$_{29}$H$_{30}$N$_2$O$_4$: C, 74.02; H, 6.43; N, 5.95. Found: C, 74.00; H, 6.52; N, 5.91; RP-HPLC: $t_R$ = 15.21 min.

2-(4,7-Dimethyl-1,3-dioxo-5,6-diphenylisoindolin-2-yl)ethanaminium 2,2,2-trifluoroacetate (BP-CO-21)

A solution of 30 (204 mg, 0.43 mmol) in 1,4-dioxane (9 mL) and 6 M HCl in 1,4-dioxane (4 mL) at rt was monitored by RP-HPLC. Upon consumption of the starting material (4-5 h), the solvent was removed in vacuo. The residue was dissolved in a mixture of approximately 60% H$_2$O in 1,4-dioxane (20 mL) and lyophilised. The crude off-white solid obtained was dissolved in approximately 10% CH$_3$CN in H$_2$O (2 x 5 mL) and loaded onto a pre-washed C-18 solid phase extraction cartridge (100% CH$_3$CN [2 x 10 mL]), then 90%, then 50% CH$_3$CN in H$_2$O [1 x 10 mL each], 20% CH$_3$CN in H$_2$O [2 x 10 mL]). The product was eluted from the C-18 cartridge (20% to 40% CH$_3$CN in H$_2$O [10% increments, 2 x 10 mL each], then 50% to 100% CH$_3$CN in H$_2$O [10% increments, 10 mL each]). The fractions collected were analysed by RP-HPLC and lyophilised to afford the title compound BP-CO-21 (163 mg, 78%) as a white powder. M.p. 113 °C (decomposition); IR (ATR) $\nu_{\text{max}}$/cm$^{-1}$ 1763, 1697, 1664, 1430, 1403, 1364;
**1H NMR** (400 MHz, DMSO-$d_6$) $\delta$ 2.32 (s, 6H), 3.11 (q, $J = 6.0$ Hz, 2H), 3.86 (t, $J = 6.0$ Hz, 2H), 6.95 – 6.97 (m, 4H), 7.13 – 7.17 (m, 2H), 7.20 – 7.24 (m, 4H), 7.85 (br s, 3H); **13C NMR** (100 MHz, DMSO-$d_6$) $\delta$ 15.2, 35.1, 37.7, 126.9, 127.8, 128.0, 129.3, 133.3, 138.2, 147.8, 168.4; **19F NMR** (376 MHz, DMSO-$d_6$) $\delta$ -73.78; **HRMS** (ESI-TOF) $m/z$: [M – \text{O}_2\text{CCF}_3]^{+} \text{Calcd} \text{ for } C_{24}H_{23}N_{2}O_{2}^{+} 371.1754, \text{ found } 371.1741; \text{ RP-HPLC: } t_R = 9.22 \text{ min.}

tert-Butyl (2-aminoethyl)(2-((tert-butoxycarbonyl)amino)ethyl)carbamate

A solution of ethyl trifluoroacetate (4 mL, 33.6 mmol) in anhydrous CH$_2$Cl$_2$ (40 mL) was slowly added to a solution of diethyltriamine (3.6 mL, 33.6 mmol) in anhydrous CH$_2$Cl$_2$ (40 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h and then rt for a further 2 h. The solvent was removed in vacuo to obtain a yellow oil that was re-dissolved in anhydrous CH$_2$Cl$_2$ (66 mL) and cooled to 0 °C. A solution of Boc$_2$O (14.8 g, 68 mmol) in anhydrous CH$_2$Cl$_2$ (66 mL) was added over 1 h by dropping funnel and the reaction mixture was allowed to stir at rt for 48 h. Removal of the solvent in vacuo gave a thick yellow oil that was taken up in a solution of 5% H$_2$O in CH$_3$OH (75 mL). K$_2$CO$_3$ (s) (26 g, 188 mmol) was added and the suspension refluxed for 2.5 h. Upon cooling, the CH$_3$OH was removed in vacuo and the residue diluted in distilled H$_2$O (100 mL). The pH was adjusted to ~13 using 15% (w/v) aqueous NaOH and the aqueous phase extracted with CHCl$_3$ (3 x 100 mL). The solvent was removed in vacuo and purified by flash chromatography (0%, then 5% EtOAc in CH$_2$Cl$_2$ with 5% NEt$_3$, then 10% CH$_3$OH in CHCl$_3$ with 5% NEt$_3$) to yield the title compound (4.2 g, 41%) as a yellow oil that solidified upon cooling to form a waxy yellow solid.

M.p. 77–80 °C; $R_f$ (10% EtOAc in CH$_2$Cl$_2$ and 5% NEt$_3$) 0.1; $R_f$ (10% CH$_3$OH in CHCl$_3$ and 5% NEt$_3$) 0.31; **1H NMR** (400 MHz, CDCl$_3$) $\delta$ inter alia 1.41 – 1.44 (m, 18H), 2.90 – 2.93 (m, 2H), 3.23 – 3.26 (m, 4H), 3.29 – 3.33 (m, 4H), 5.28 – 5.45 (m, 1H); **13C NMR** (100 MHz, CDCl$_3$) $\delta$ inter alia 15.2, 35.1, 37.7, 126.9, 127.8, 128.0, 129.3, 133.3, 138.2, 147.8, 168.4.
CDCl₃) δ inter alia 28.3, 28.4, 39.2, 39.6, 40.2, 40.4, 47.6, 48.2, 49.6, 50.3, 79.2, 80.3, 156.2.

The ¹H and ¹³C NMR data obtained were in agreement with those reported in literature.⁷

tert-Butyl (2-((tert-butoxycarbonyl)amino)ethyl)(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)carbamate (25)

To a suspension of crude tert-butyl (2-aminoethyl)-(2-((tert-butoxycarbonyl)amino)ethyl)carbamate (11.1 g, 36.6 mmol) in saturated NaHCO₃ (180 mL) at rt was added powdered N-(ethoxycarbonyl)maleimide (23)⁵ (6.2 g, 36.7 mmol). After stirring the reaction mixture for 15 min, THF was added (280 mL) and the resulting biphasic suspension was stirred vigourously at rt for 2 h. H₂O (100 mL) was then added and the aqueous phase extracted with EtOAc (3 x 100 mL). The combined organic extracts were washed with saturated NaCl (150 mL), dried over MgSO₄ and then concentrated in vacuo to afford an orange oil. Purification by flash chromatography (0%, then 5%, then 10%, then 50% EtOAc in CH₂Cl₂) gave a 1:1 mixture of the title compound 25 and ethyl carbamate (3.7 g) as a yellow oil.

An analytically pure sample of 25 was obtain on re-purification by flash chromatography (0%, then 5%, then 20% EtOAc in CH₂Cl₂, then 100% EtOAc) to afford the title compound 25 as a pale yellow oil that forms colourless crystals upon cooling. M.p. 105–107 °C; Rf (20% EtOAc in CH₂Cl₂) 0.4; IR (ATR) νmax/cm⁻¹ 2976, 2929, 1708, 1508, 1404, 1364; ¹H NMR (500 MHz, CDCl₃) δ inter alia 1.37 – 1.40 (m, 18H), 3.21 – 3.29 (m, 4H), 3.38 – 3.40 (m, 2H), 3.62 – 3.67 (m, 2H), 5.07 (br s, 1H), 6.65 – 6.69 (m, 2H); ¹³C NMR (125 MHz, CDCl₃) δ inter alia 28.2, 28.3, 35.9, 36.2, 39.4, 45.6, 45.9, 46.5, 47.7, 79.1, 80.1, 80.3, 134.1, 134.1, 134.2, 155.6, 155.8, 156.0, 170.3, 170.7; HRMS (ESI-TOF) m/z: [M + Na]⁺ Calcd for C₁₈H₂₉N₅O₆Na⁺ 406.1949 found 406.1968.
**tert-Butyl (3-(4-bromo-1,3-dioxo-1,3-dihydro-1H-pyrrol-1-yl)ethyl)(4-((tert-butoxycarbonyl)amino)ethyl)carbamate (27)**

To a solution containing a 1:1 mixture of maleimide 25 and ethyl carbamate (1.81 g, 4.7 mmol) in CH$_2$Cl$_2$ (25 mL) was added bromine (270 µL, 5.2 mmol) at rt. The dark brown solution was heated to reflux for 1.5 h and then cooled to rt. Removal of the solvent *in vacuo* gave a thick orange gum that was dissolved in anhydrous THF (25 mL) and cooled to 0 °C under argon. Anhydrous NEt$_3$ (730 µL, 5.2 mmol) was added dropwise at 0 °C and the resulting orange suspension was stirred at 0 °C for 4 h. H$_2$O (30 mL) was then added at 0 °C and the aqueous phase was extracted with EtOAc (3 x 40 mL). The combined organic extracts were dried over MgSO$_4$ and concentrated *in vacuo* to afford a brown-yellow oil. Purification by flash chromatography (0%, then 5%, then 10%, then 20% EtOAc in CH$_2$Cl$_2$) gave the *title compound* 27 (550 mg, 25%) as a yellow oil and an approximate 1:1 mixture of 27 and ethyl carbamate (806 mg). $R_f$ (20% EtOAc in CH$_2$Cl$_2$) 0.4; IR (ATR) $\nu_{max}$/cm$^{-1}$ 3370, 2976, 2933, 1716, 1677, 1508; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ *inter alia* 1.39 – 1.46 (m, 18H), 3.24 – 3.32 (m, 4H), 3.40–3.43 (m, 2H), 3.69 – 3.73 (m, 2H), 5.06 – 5.10 (m, 1H), 6.85–6.89 (m, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ *inter alia* 28.2, 28.4, 36.8, 37.3, 39.1, 39.4, 45.4, 45.9, 46.3, 47.8, 79.1, 79.3, 80.3, 80.5, 131.3, 131.5, 131.9, 132.0, 155.8, 155.9, 156.1, 165.0, 165.5, 168.1, 168.5; HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for C$_{18}$H$_{28}$BrN$_3$O$_6$Na$^+$ 484.1054, found 484.1074.

**tert-Butyl (2-(3a-bromo-4,7-dimethyl-1,3,8-trioxo-5,6-diphenyl-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindol-2(2H)-yl)ethyl)(2-((tert-butoxycarbonyl)amino)ethyl)carbamate (29)**
A suspension of bromomaleimide 27 (275 mg, 0.60 mmol) and diene dimer 15 (163 mg, 0.31 mmol) in anhydrous toluene (5 mL) was placed under an argon atmosphere and then heated to reflux for 4 h. The reaction mixture was cooled to rt and the solvent removed in vacuo to afford a brown oil. Purification by flash chromatography (0%, then 5%, then 10% EtOAc in CH₂Cl₂) afforded the endo-isomer of the title compound 29 (261 mg, 60%) as a colourless foam and a 2:1 mixture of the endo:exo-isomers of 29 as a thick yellow oil (115 mg, 27%).

Data for endo-29: M.p. 73–78 °C (decomp.); Rf (10% EtOAc in CH₂Cl₂) 0.5; IR (ATR) νmax/cm⁻¹ 2976, 2922, 1791, 1716, 1685, 1391; ¹H NMR (500 MHz, CDCl₃) δ inter alia 1.41–1.48 (m, 18H), 1.60 (s, 3H), 1.61 (s, 3H), 3.25–3.32 (m, 4H), 3.45–3.51 (m, 3H), 3.74–3.75 (m, 2H), 4.94 (br s, 1H), 6.83–6.86 (m, 4H), 7.16–7.20 (m, 6H); ¹³C NMR (125 MHz, CDCl₃) δ inter alia 11.5, 12.4, 28.4, 37.9, 38.5, 39.4, 44.6, 45.0, 45.0, 46.7, 47.8, 56.2, 56.4, 58.7, 60.4, 61.0, 79.3, 80.6, 80.8, 128.1, 128.1, 128.2, 128.3, 129.3, 129.6, 132.4, 132.4, 132.5, 140.4, 144.6, 155.7, 155.9, 172.2, 172.6, 196.8, 197.0; HRMS (ESI-TOF) m/z: [M + Na]⁺ Calcd for C₃₇H₄₇⁷⁹BrN₀₇Na⁺ 744.2255, found 744.2231; RP-HPLC: t_R = 15.61 min.

Exo-29: Rf (10% EtOAc in CH₂Cl₂) 0.37; RP-HPLC: t_R = 15.61 min.

2-(2-(2-Aminoethyl)aminoethyl)-3a-bromo-3a,4,7,7a-tetrahydro-4,7-dimethyl--5,6-diphenyl-4,7-methano-1H-isouindole-1,3,8(2H)-trione bis-trifluoroacetate salt (oCOM-23)

A solution of endo-29 (261 mg, 0.36 mmol) in 1,4-dioxane (2 mL) and 6 M HCl in 1,4-dioxane (3 mL) at 0 °C was warmed to rt and monitored by RP-HPLC. Upon consumption of the starting material (4 h), the solvent was removed in vacuo to afford an off-white solid that was dissolved in distilled H₂O (40 mL) and then lyophilised to obtain a crude fluffy powder. The crude product was dissolved in 10% CH₃CN in H₂O (2 x 5 mL) and loaded onto a C-18 solid phase extraction cartridge (pre-wash procedure as for the preparation of oCOM-19.TFA).
product was eluted from the C-18 cartridge (20% to 40% CH₃CN in H₂O [10% increments, 2 x 10 mL each], then 50% to 100% CH₃CN in H₂O [10% increments, 10 mL each]). The fractions collected were analysed by RP-HPLC and lyophilised to afford the *endo*-isomer of the *title compound* oCOM-23 (171 mg, 63%) as a white fluffy powder. M.p. 111–114 ºC (decomp.); IR (ATR) ν_max/cm⁻¹ 2965, 1786, 1715, 1670; ¹H NMR (400 MHz, DMSO-d₆) δ 1.46 (s, 3H), 1.52 (s, 3H), 3.12 – 3.20 (m, 6H), 3.79 – 3.90 (m, 2H), 3.99 (s, 1H), 6.84 – 6.88 (m, 4H), 7.21 – 7.24 (m, 6H), 8.11 (br s, 3H), 9.26 – 9.31 (m, 2H); ¹³C NMR (125 MHz, DMSO-d₆) δ 11.3, 11.8, 35.2, 35.7, 43.7, 44.1, 55.6, 58.6, 60.5, 128.1, 128.2, 128.3, 128.3, 129.2, 129.3, 132.2, 132.3, 139.5, 144.6, 171.9, 172.5, 196.5; ¹⁹F NMR (376 MHz, DMSO-d₆) δ −73.67, −73.66; HRMS (ESI-TOF) m/z: [M − [O₂CCF₃]₂ – H]⁺ Calcd for C₂₇H₂₉BrN₅O₃⁺ 522.1387, found 522.1364; RP-HPLC (10% to 100% B over 12.5 min, then 100% B for 7.5 min, with a flow rate of 0.5 mL min⁻¹): t_R = 7.96 min.

**tert-Butyl** (3-((tert-butoxycarbonyl)amino)ethyl)(2-(4,7-dimethyl-1,3-dioxo-5,6-diphenylisoindolin-2-yl)ethyl)carbamate (31)

To a solution of adduct 29 (220 mg, 0.31 mmol, *endo*:exo ~1:1) in anhydrous THF (5 mL) at 0 ºC was added DBU (90 µL, 0.60 mmol) dropwise. The brown reaction mixture was then allowed to warm to rt. Upon consumption of the starting material by TLC (approximately 30 min), a brown precipitate had formed and saturated NH₄Cl (20 mL) was then added. The aqueous phase was extracted with EtOAc (3 x 10 mL). The combined organic extracts were dried over MgSO₄ and concentrated in vacuo to afford a brown crystalline solid. Purification by flash chromatography (0%, then 5%, then 10% EtOAc in CH₂Cl₂) furnished the *title compound* 31 (142 mg, 76%) as a white solid. M.p. 202–205 ºC (decomp.); R_f (5% EtOAc in CH₂Cl₂) 0.17, R_f (10% EtOAc in CH₂Cl₂) 0.5; IR (ATR) ν_max/cm⁻¹ 3345, 2976, 2922, 1702, 1677, 1518, 1402, 1381; ¹H NMR (500 MHz, CDCl₃) δ 1.32 – 1.35 (m, 9H), 1.44 (s, 9H), 2.39
(s, 6H), 3.31 – 3.41 (m, 4H), 3.51 – 3.55 (m, 2H), 3.83 – 3.86 (m, 2H), 5.17 – 5.30 (m, 1H), 6.86 – 6.88 (m, 4H), 7.09 – 7.17 (m, 6H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 15.6, 15.6, 28.0, 28.0, 28.1, 28.4, 35.8, 36.2, 39.4, 39.5, 45.8, 46.3, 46.6, 48.1, 79.1, 79.9, 80.1, 126.7, 126.8, 127.7, 128.1, 128.2, 129.4, 134.1, 134.4, 138.6, 138.8, 148.1, 148.5, 155.7, 156.0, 168.9, 169.2; HRMS (ESI-TOF) $m/z$: [M + Na]$^+$ Calcd for C$_{36}$H$_{43}$N$_3$O$_6$Na$^+$ 636.3044, found 636.3071; RP-HPLC (10% to 100% B over 12.5 min, then 100% B for 7.5 min, with a flow rate of 0.5 mL min$^{-1}$); $t_R$ = 16.23 min.

2-(2-(2-Aminoethyl)aminoethyl)-4,7-dimethyl-5,6-diphenyl-1H-isindole-1,3,8(2H)-dione bis-trifluoroacetate salt (BP-CO-23)

6 M HCl in 1,4-dioxane (1.7 mL) was added dropwise to a suspension of 31 (120 mg, 0.20 mmol) in 1,4-dioxane (2 mL) at rt. Additional quantities of 1,4-dioxane (7 mL) and distilled H$_2$O (5 mL) were added. The reaction mixture was monitored by RP-HPLC. After stirring for 6 h, the reaction mixture was heated to 30 °C for 14.5 h and then 50 °C for 7 h. The solvent was removed in vacuo to afford a white precipitate that was dissolved in 10% 1,4-dioxane in H$_2$O (22 mL) and lyophilised. The resulting white solid was then dissolved in 10% CH$_3$CN in H$_2$O (2 x 5 mL) and loaded onto a C-18 solid phase extraction cartridge (pre-wash procedure as for the preparation of oCOM-19.TFA). The product was eluted from the C-18 cartridge (20% to 40% CH$_3$CN in H$_2$O [10% increments, 2 x 10 mL each], then 50% to 100% CH$_3$CN in H$_2$O [10% increments, 10 mL each]). The fractions collected were analysed by RP-HPLC and lyophilised to afford the title compound BP-CO-23 (102 mg, 82%) as a white powder. IR (ATR) $\nu_{\text{max}}$/cm$^{-1}$ 2976, 1765, 1700, 1671, 1429, 1404; $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.32 (s, 6H), 3.13 (t, $J = 7.5$ Hz, 2H), 3.24 (t, $J = 7.5$ Hz, 2H), 3.33 – 3.34 (m, 2H), 3.92 (t, $J = 5.0$ Hz, 2H), 6.94 – 6.96 (m, 4H), 7.13 – 7.17 (m, 2H), 7.20 – 7.24 (m, 4H), 8.09 (br s, 3H), 9.07 (br s, 2H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 15.2, 34.0, 35.1), 44.1, 45.4, 117.1 (q, $^1$JC-
**tert-Butyl (2-(3-(4,7-dimethyl-1,3,8-trioxo-5,6-diphenyl-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindol-2(3H)-yl)propanamido)ethyl)carbamate (32)**

Maleimide (19) (90 mg, 0.29 mmol) and diene dimer (15) (75 mg, 0.29 mmol) were dissolved in toluene (3 mL) and heated at reflux for 4 h under nitrogen. Solvent was removed under vacuum and the crude residue purified by flash column chromatography (1:1 EtOAc / Petrol) to provide the *title compound 32* as a white solid (163 mg, 99%) as an 11:1 mixture of *endo-* and *exo*-cycloadducts. Rf 0.1 (3:2 EtOAc / Petrol). IR (ATR) νmax/cm⁻¹ 3317 (br), 2973, 2930, 2869, 1787, 1772, 1699, 1652, 1516, 1443; HRMS (ESI-TOF) m/z: [M + Na]^+ Calcd for C33H37N3O6Na 594.2575, found 594.2604.

NMR data for *endo* 32: ¹H NMR (500 MHz, CDCl₃) δ *inter alia* 1.42 (s, 9H), 1.56 (s, 6H), 2.45 (t, J = 7.3 Hz, 2H), 3.18 – 3.25 (m, 2H), 3.24 (s, 2H), 3.26 – 3.32 (m, 2H), 3.81 (t, J = 7.3 Hz, 2H), 4.96 (br s, 1H), 6.35 (br s, 1H) 6.82 – 6.95 (m, 4H), 7.11 – 7.22 (m, 6H); ¹³C NMR (125 MHz, CDCl₃) δ *inter alia* 12.2, 28.3, 33.8, 35.5, 40.1, 40.9, 47.9, 56.5, 79.8, 127.7, 128.0, 128.2, 128.3, 129.0, 129.5, 133.0, 141.6, 169.9, 175.4, 199.5.

NMR data for *exo* 32: ¹H NMR (500 MHz, CDCl₃) δ *inter alia* 1.15 (s, 3H), 1.20 (s, 3H), 1.38 (s, 9H), 2.40 (t, J = 6.8 Hz, 2H), 3.32 – 3.38 (m, 2H), 5.10 (br s, 1H), 6.18 (br s, 1H), 6.96 – 7.08 (m, 4H), 7.22 – 7.25 (m, 6H); ¹³C NMR (126 MHz, CDCl₃) δ *inter alia* 9.1, 49.7, 55.5, 173.3, 197.1.
$N$-(2-Aminoethyl)-3-(4,7-dimethyl-1,3,8-trioxo-5,6-diphenyl-3a,4,7,7a-tetrahydro-$1H$-4,7-methanoisoindol-2(3H)-yl)propanamide hydrochloride (14) (DB-CO-19)

Boc-protected amine (32) (100 mg, 0.35 mmol) was dissolved in 1,4-dioxane (0.5 mL) with cooling to 0 °C under nitrogen and 6M HCl in 1,4-dioxane (2.5 mL) added dropwise. The reaction allowed to warm to rt and stirred overnight. Removal of volatiles under vacuum followed by lyophilization gave the amine HCl salt (DB-CO-19) as a white solid (53 mg, 85%) as a 13:1 mixture of endo- and exo-isomers. IR (ATR) $\nu_{\text{max}}$/cm$^{-1}$ 3317 (br), 2973, 2930, 2869, 1787, 1772, 1699, 1652, 1516, 1443 cm$^{-1}$; HRMS (ESI-TOF) $m/z$: [M – Cl]$^+$ Calcd for C$_{28}$H$_{30}$N$_3$O$_4$ 472.2231, found 472.2252. RP-HPLC: $t_R$ = 8.4 min (100%).

NMR data for endo-DB-CO-19: $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ inter alia 1.41 (s, 6H), 2.31 - 2.36 (m, 2H), 2.83 (t, $J = 6.1$ Hz, 2H), 3.25 (q, $J = 5.9$ Hz, 2H), 3.47 (s, 2H), 3.60 – 3.65 (m, 2H), 6.86 – 6.88 (m, 4H), 7.14 – 7.30 (m, 6H), 7.77 (br s, 3H), 8.21 (t, $J = 5.7$ Hz, 1H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ inter alia 11.9, 30.7, 32.9, 35.1, 36.3, 38.6, 38.6, 47.4, 55.7, 127.6, 128.1, 129.2, 133.1, 141.3, 170.0, 175.8, 199.1, 206.5.

**tert-Butyl** (2-(4,7-dimethyl-1,3,8-trioxo-5,6-diphenyl-3a,4,7,7a-tetrahydro-$1H$-4,7-methanoisoindol-2(2H)-yl)ethyl)carbamate (33)

Maleimide 24 (150 mg, 0.62 mmol) and diene dimer 15 (171 mg, 0.66 mmol) were dissolved in anhydrous toluene (3.6 mL) and placed under argon. The mixture was then heated to reflux for 4 h. After cooling to rt, the solvent was removed in vacuo to afford an orange oil that was purified by flash chromatography (0%, then 5%, then 7%, then 20% EtOAc in CH$_2$Cl$_2$) to afford an inseparable ~4.2:1 mixture (endo:exo) of the title compound 33 (300 mg, 52%) as a white foam. M.p. 81 °C (decomp.); IR (ATR) $\nu_{\text{max}}$/cm$^{-1}$ 3411, 2977, 2933, 1788, 1770, 1704, 1698, 1693, 1509, 1392; HRMS (ESI-TOF) $m/z$: [M + Na]$^+$ Calcd for C$_{30}$H$_{32}$N$_2$NaO$_5^+$
523.2203, found 523.2173; RP-HPLC: \( t_R = 14.56 \) min, endo- and exo-isomers co-elute as a single peak.

Endo-33 NMR data: \(^1\)H NMR (500 MHz, CDCl\(_3\)) \( \delta \) inter alia 1.40 (s, 9H), 1.57 (s, 6H), 3.23 (s, 2H), 3.32 – 3.35 (m, 2H), 3.64 (t, \( J = 5.0 \) Hz, 2H), 4.78 (br s, 1H), 6.87 – 6.91 (m, 4H), 7.15 – 7.17 (m, 6H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \( \delta \) inter alia 12.2, 28.3, 38.9, 39.6, 48.0, 56.5, 79.5, 127.7, 128.2, 129.5, 133.0, 141.6, 156.1, 175.7, 199.7.

Exo-33 NMR data: \(^1\)H NMR (500 MHz, CDCl\(_3\)) \( \delta \) inter alia 1.40 (s, 6H), 1.45 (s, 9H), 3.20 – 3.22 (m, 2H), 3.23 (s, 2H), 3.68 (t, \( J = 5.0 \) Hz, 2H), 4.78 (br s, 1H), 7.03 – 7.05 (m, 4H), 7.23 – 7.25 (m, 6H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \( \delta \) inter alia 9.1, 28.3, 38.1, 38.7, 49.8, 55.7, 79.4, 128.0, 128.3, 129.0, 133.0, 144.4, 155.8, 173.4, 204.7.

2-(4,7-Dimethyl-1,3,8-trioxo-5,6-diphenyl-3a,4,7a-tetrahydro-1H-4,7-methanoisoindol-2(2H)-yl)ethanaminium 2,2,2-trifluoroacetate (DB-CO-21)

6 M HCl in 1,4-dioxane (9 mL) was added to a solution of 33 (513 mg, 1.02 mmol) in 1,4-dioxane (3 mL) at 0 °C. The reaction mixture was allowed to warm to rt and monitored by RP-HPLC. Upon consumption of the starting material (2-3 h) as indicated by the formation of a white precipitate, the solvent was removed \textit{in vacuo} to afford an off-white solid that was dissolved in 16% CH\(_3\)CN in H\(_2\)O (15 mL) and loaded onto a C-18 solid phase extraction cartridge (pre-wash procedure as for the preparation of oCOM-19.TFA). The product was eluted from the C-18 cartridge (20% to 40% CH\(_3\)CN in H\(_2\)O [10% increments, 2 x 10 mL each], then 50% to 100% CH\(_3\)CN in H\(_2\)O [10% increments, 10 mL each]). The fractions collected were analysed by RP-HPLC and lyophilised to afford an inseparable ~4.1:1 mixture (endo:exo) of the title compound DB-CO-21 (407 mg, 73%) as a pale yellow oil. IR (ATR) \( \nu_{\text{max}}/\text{cm}^{-1} \) 2984, 2933, 1790, 1774, 1736, 1702, 1697, 1670, 1394; HRMS (ESI-TOF) \( m/z: [M – \)
O$_2$CCF$_3$]$^+ \text{Calcd for } C_{25}H_{25}N_2O_3^+ \ 401.1860, \text{ found } 401.1840; \ \text{RP-HPLC: } t_R = 8.60 \text{ min, endo- and exo-isomers co-elute as a single peak.}

\textit{Endo-DB-CO-21} NMR data: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ \textit{inter alia} 1.43 (s, 6H), 2.93–2.97 (m, 2H), 3.51 (s, 2H), 3.67 (t, $J = 8.0$ Hz, 2H), 6.84 – 6.87 (m, 4H), 7.17 – 7.28 (m, 6H), 7.81 (br s, 3H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ \textit{inter alia} 11.8, 36.1, 36.7, 47.7, 55.8, 127.6, 128.1, 129.2, 131.1, 141.3, 176.1, 199.0; $^{19}$F NMR (376 MHz, DMSO-$d_6$) $\delta$ -73.77.

\textit{Exo-DB-CO-21} NMR data: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ \textit{inter alia} 1.19 (s, 6H).

tert-Butyl (2-((tert-butoxycarbonyl)amino)ethyl)(2-(4,7-dimethyl-1,3,8-trioxo-5,6-diphenyl-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindol-2(2H)-yl)ethyl)carbamate (34)

A suspension of maleimide 25 (109 mg, 0.28 mmol) and diene dimer 15 (111 mg, 0.29 mmol) in anhydrous toluene (2 mL) was placed under an argon atmosphere. The mixture was heated to reflux for 4 h and then allowed to cool to rt. The solvent was removed under reduced pressure to afford a brown oil. Purification by flash chromatography (0%, then 10%, then 20% EtOAc in CH$_2$Cl$_2$) furnished the \textit{title compound 34} (80 mg, 43%) as an inseparable mixture of endo- and exo-isomers (11:1) as a colourless foam. $R_f$ (20% EtOAc in CH$_2$Cl$_2$) 0.3; IR (ATR) $\nu_{\text{max}}$/cm$^{-1}$ 2980, 2936, 1790, 1702, 1677, 1392; HRMS (ESI-TOF) $m/z$: [M + Na]$^+$ Calcd for C$_{37}$H$_{45}$N$_3$O$_7$Na$^+$ 666.3150, found 666.3169.

\textit{Endo-34} NMR data: $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ \textit{inter alia} 1.42–1.46 (18H, m), 1.56 (s, 6H), 3.18 – 3.24 (m, 4H), 3.27 – 3.31 (m, 2H), 3.37 – 3.45 (m, 2H), 3.64 – 3.68 (m, 2H), 4.96–5.17 (m, 1H), 6.87 – 6.89 (m, 4H), 7.15 – 7.16 (m, 6H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ \textit{inter alia} 12.2, 28.3, 28.4, 37.3, 37.9, 39.6, 44.8, 45.4, 48.1, 48.2, 56.4, 79.3, 79.4, 80.2, 127.6, 128.1, 129.6, 133.1, 141.7, 156.0, 156., 175.2, 175.9, 199.5, 200.0; RP-HPLC (10% to 100% B over 12.5 min, then 100% B for 7.5 min, with a flow rate of 0.5 mL min$^{-1}$): $t_R = 14.97$ min.
Exo-34 NMR data: $^1$H NMR (500 MHz, CDCl$_3$) δ inter alia 1.38 (s, 6H), 7.01-7.05 (m, 4H), 7.23–7.24 (m, 6H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ inter alia 9.1, 46.6, 128.3, 129.0; RP-HPLC: $t_R = 15.19$ min.

2-(2-(2-Aminoethyl)aminoethyl)-3a,4,7,7a-tetrahydro-4,7-dimethyl-5,6-diphenyl-4,7-methano-1H-isoindole-1,3,8(2H)-trione bis-trifluoroacetate salt (DB-CO-23)

6 M HCl in dioxane (1 mL) was added dropwise to a suspension of 34 (80 mg, 0.12 mmol) in 1,4-dioxane (1 mL) at rt. Additional 1,4-dioxane (1 mL) was added to allow for dissolution of the precipitate. The reaction mixture was stirred at rt and monitored by RP-HPLC. After stirring for 4 h the solvent was removed in vacuo. The resulting yellow precipitate was then dissolved in 10% CH$_3$CN in H$_2$O (2 x 5 mL) and loaded onto a pre-washed C-18 solid phase extraction cartridge (100% CH$_3$CN [2 x 10 mL]), then 90%, then 50% CH$_3$CN in H$_2$O [1 x 10 mL each], 10% CH$_3$CN in H$_2$O [2 x 10 mL]). The product was eluted from the C-18 cartridge (20% to 40% CH$_3$CN in H$_2$O [10% increments, 2 x 10 mL each], then 50% to 100% CH$_3$CN in H$_2$O [10% increments, 10 mL each]). The fractions collected were analysed by RP-HPLC. The initial fractions (20% CH$_3$CN in H$_2$O and the first 30% CH$_3$CN in H$_2$O collection) were found to contain unknown impurities that co-eluted with DB-CO-23 and were kept separate. Pending RP-HPLC analysis, the later fractions were pooled and lyophilised to afford the title compound DB-CO-23 (68 mg, 82%) as an approximate 15:1 mixture of endo- and exo-isomers in the form of a white powder). IR (ATR) $v_{max}$/cm$^{-1}$ 1773, 1707, 1670, 1396, 1199, 1132, 837, 799, 722, 698; HRMS (ESI-TOF) m/z: [M – [O$_2$CCF$_3$]$_2$ – H]$^+$ Calcd for C$_{27}$H$_{30}$N$_3$O$_3$+ 444.2282, found 444.2291; RP-HPLC (10% to 100% B over 12.5 min, then 100% B for 7.5 min, 0.5 mL/min): $t_R = 7.47$ min.

Endo-DB-oCOm-23 NMR data: $^1$H NMR (400 MHz, DMSO-d$_6$) δ inter alia 1.43 (s, 6H), 3.04–3.21 (m, 6H), 3.52 (s, 2H), 3.71 (t, $J = 5.9$ Hz, 2H), 6.85–6.88 (m, 4H), 7.18–7.22 (m, 6H),
7.93 (br s, 3H), 8.88 (br s, 2H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ inter alia 11.8, 34.9, 35.1, 43.8, 44.3, 47.7, 55.8, 127.6, 128.2, 129.3, 133.0, 141.3, 158.5 (CF$_3$, q, $^2$J$_{CF}$ 125.0, O$_2$CF$_3$), 176.0, 198.9; $^{19}$F NMR (376 MHz, DMSO-$d_6$) $-$73.69, $-$73.66 (6F, O$_2$CF$_3$).

Exo-DB-oCOM-23 NMR data: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ inter alia 1.20 (s, 6H), 3.44 (s, 2H), 7.13 – 7.16 (m, 4H), 7.27 – 7.32 (m, 6H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ inter alia 9.0.
General procedure for HPLC CO-release study

The CO compound was weighed out into a sample tube (oCOM-21 [~0.5 mg], and for oCOM-23 [~0.7-0.75 mg]). In separate vessels prior to mixing, the CO compound (oCOM-21 or oCOM-23) and the TRIS-sucrose buffer (pH 7.4) were both allowed to equilibrate to the specified temperature (thermostat waterbath = 37 °C or cold room = 4-5 °C). TRIS-sucrose buffer (1 mL) was added to the CO compound at the specified temperature and mixed by gently swirling the HPLC vial (final concentration of oCOM-21, ~843 μM, final concentration of oCOM-23, ~930-999 μM). Prior to removing an aliquot for analysis, the sample was gently mixed by hand and then an aliquot was sampled (30 μL) and immediately added to a solution of 0.1 M aq. HCl (170 μL). The acidified aliquot was sonicated for approximately 10 seconds to ensure the entire sample was homogeneous. These aliquots were immediately analysed by RP-HPLC (3 replicate injections for each aliquot taken) using the specified HPLC method.

RP-HPLC method for oCOM-21 CO release study: (30% to 60% B over 12.5 min, then 60% to 100% B over 2.5 min, 100% B for 2.0 min, 100% to 30% B over 2.0 min, 0.5 mL min⁻¹).

RP-HPLC method for oCOM-23 CO release study: (35% to 50% B over 15.0 min, then 50% to 100% B over 1.0 min, 100% B for 2.0 min, 100% to 35% B over 2.0 min, 0.5 mL min⁻¹).

The area under the CO compound and the BP compound was integrated in the 254 nm spectrum and used to calculate the % conversion using the following formula:

\[
\% \text{ conversion of CO compound} = \frac{\text{peak area of CO compound}}{\text{peak area of CO compound} + \text{peak area of BP compound}} \times 100
\]
Table S1: CO-release study for oCom-21 in TRIS-sucrose buffer (pH 7.4) at 37 °C

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Concentration (mg/mL)</th>
<th>Concentration (µM)</th>
<th>Approx. time for 50% conversion of oCom-21 to BP-CO-21 (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>0.5</td>
<td>843</td>
<td>23</td>
</tr>
<tr>
<td>02</td>
<td>0.5</td>
<td>843</td>
<td>18</td>
</tr>
<tr>
<td>03</td>
<td>0.5</td>
<td>843</td>
<td>19</td>
</tr>
<tr>
<td>04</td>
<td>0.5</td>
<td>843</td>
<td>16</td>
</tr>
</tbody>
</table>

Average time at 37 °C 19

a Samples were hand mixed prior to taking an aliquot. Aliquots were mixed with 0.1 M HCl by sonication (~10 secs.)

Table S2: CO-release study for oCom-21 in TRIS-sucrose buffer (pH 7.4) at 4-5 °C

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Concentration (mg/mL)</th>
<th>Concentration (µM)</th>
<th>Approx. time for 50% conversion of oCom-21 to BP-CO-21 (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>0.5</td>
<td>843</td>
<td>1433 (~23.8 h)</td>
</tr>
<tr>
<td>02</td>
<td>0.5</td>
<td>843</td>
<td>2082 (~34.7 h)</td>
</tr>
<tr>
<td>03</td>
<td>0.5</td>
<td>843</td>
<td>1159 (~19.3 h)</td>
</tr>
<tr>
<td>04</td>
<td>0.5</td>
<td>843</td>
<td>2275 (~37.9 h)</td>
</tr>
<tr>
<td>05</td>
<td>0.5</td>
<td>843</td>
<td>2264 (~37.7 h)</td>
</tr>
</tbody>
</table>

Combined average time at 4-5 °C 1843 (30.7 h)

a Samples were hand mixed prior to taking an aliquot. Aliquots were mixed with 0.1 M HCl by sonication (~10 seconds).
Table S3: CO-release study for oCOM-23 in TRIS-sucrose buffer (pH 7.4) at 37 °C

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Concentration (mg/mL)</th>
<th>Concentration (µM)</th>
<th>Approx. time for 50% conversion of oCOM-23 to BP-CO-23 (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>0.7</td>
<td>930</td>
<td>95</td>
</tr>
<tr>
<td>02</td>
<td>0.7</td>
<td>930</td>
<td>63</td>
</tr>
<tr>
<td>03</td>
<td>0.7</td>
<td>930</td>
<td>65</td>
</tr>
<tr>
<td>04</td>
<td>0.74</td>
<td>986</td>
<td>75</td>
</tr>
<tr>
<td>05</td>
<td>0.74</td>
<td>986</td>
<td>77</td>
</tr>
</tbody>
</table>

Average time at 37 °C 75

* Samples were hand mixed prior to taking an aliquot. Aliquots were mixed with 0.1 M HCl by sonication (10 secs.).

Table S4: CO-release study for oCOM-23 in TRIS-sucrose buffer (pH 7.4) at 4-5 °C

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Concentration (mg/mL)</th>
<th>Concentration (µM)</th>
<th>Approx. time for 50% conversion of oCOM-23 to BP-CO-23 (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>0.7</td>
<td>930</td>
<td>1570 (26.2 h)</td>
</tr>
<tr>
<td>02</td>
<td>0.75</td>
<td>999</td>
<td>2300 (38.3 h)</td>
</tr>
<tr>
<td>03</td>
<td>0.7</td>
<td>930</td>
<td>1920 (32 h)</td>
</tr>
<tr>
<td>04b</td>
<td>0.7</td>
<td>930</td>
<td>1960 (32.7 h)</td>
</tr>
</tbody>
</table>

Average time at 4-5 °C ~1940 (~32.3 h)

* Samples were hand mixed prior to taking an aliquot. Aliquots were mixed with 0.1 M HCl by sonication (10 secs.).
Detection of Carbon Monoxide Release by amperiometric CO selective electrode

The rate of CO release from oCom-21 and oCom-23 was measured in Tris-sucrose buffer pH 7.4, 37°C using an amperiometric CO selective electrode (ISO-COP-2, World Precision Instruments (WPI), Inc, Sarasota, USA). The amount of CO released was recorded as the magnitude of electrode current generated on CO diffusion through a selective gas permeable membrane and oxidised to CO₂ on the electrode. Current changes were recorded using a WPI TRB4100 4-channel free radical analyser with the poise potential set to 950mV. Solutions of oCom-21 and oCom-23 were freshly prepared in ultrapure water (nominally pH 6.9) and 10µL immediately injected into a water jacketed chamber containing 500µL of Tris-sucrose solutions at a physiological pH to give a final CO donor concentration of 75µM.

Measurement of CO binding to Myoglobin

CO released from oCom-19 and oCom-21 was measured using an established carboxymyoglobin spectrophotometric assay, adapted to a 96 well plate format to enable the simultaneous correction at all absorbance wavelengths arising from any potential interference from the donor compounds. Freshly prepared horse heart myoglobin (66µM) in a PBS solution (pH 7.4) was reduced with 10% w/v sodium dithionite and added to a 96 well plate. The CO donors, oCom-19 and oCom-21, as well as the commercially available CO donor, CORM-2 (Sigma-Aldrich, Castle Hill, NSW, Australia), were added to each well in triplicate to produce a final concentration range of 100-800µM and 50µL paraffin oil added to each well to prevent the diffusion of gases. Absorption spectra (λ 500 - 600nm) were run at ten minute intervals using a Spectramax 96-well spectrophotometer (Molecular Devices, Crawley, UK) at 37°C. CO gas saturated PBS (714µM), was employed as a positive control.
Cell Toxicity

The loss of cell adhesion for anchorage-dependent cell lines, such as Madin Darby Canine Kidney (MDCK) cells, represents a surrogate marker of cell death which can be measured by the crystal violet cell adhesion (CVCA) assay. MDCK cells plated at $1 \times 10^6$ cells/mL in 24 well plates were grown to 60% confluency in DMEM supplemented with 4% donor calf serum and 1% antibiotic (Gibco Life Technologies, Auckland NZ). CO donor compounds, BP-compounds or non CO releasing, DB-derivatives were dissolved in either ultrapure water or for non-water soluble compounds, in DMSO and diluted to 20-100μM (to a final concentration of 1% DMSO where appropriate). oCOm-19 was initially dissolved in 0.1% DMSO while oCom-21, -23 and -24 were dissolved directly in deionised, distilled water. Cells were exposed to CO donor compounds or the respective vehicle control solution for 1 hour at 37°C prior to washing and incubating for a further 23 hours in fresh media. Cells were fixed in 96% ethanol and stained in 0.05% crystal violet solution in 20% ethanol, prior to washing and solubilising (24 hours) in 2mL 0.1% acetic acid in 50% ethanol. Sample solutes (100μL) from each well were transferred into a 96 well plate and absorbance measured at 585nm. Cell survival was reported as a percentage of the value obtained for cells exposed to vehicle only and used to plot a concentration-response curve for each compound from which an EC$_{50}$ value was obtained.
General procedure for determining solubility of CO compounds by HPLC

The CO compound (4.2 mg, or 6.0 mg) was dissolved in CH$_3$CN (1 mL) and then passed through a syringe filter (0.45 µM, nylon membrane) to create stock solution A and stock solution B respectively. A series of solutions were created by dilution of the stock solution (Table 6 for stock solution A, Table 5 for stock solution B)

Table 5: Solutions created from stock solution A (4.2 mg/mL)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Concentration (mg.mL$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>2</td>
<td>2.1</td>
</tr>
<tr>
<td>3</td>
<td>1.03</td>
</tr>
<tr>
<td>4</td>
<td>0.51</td>
</tr>
<tr>
<td>5</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Table 6: Solutions created from stock solution B (6.0 mg/mL)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Concentration (mg.mL$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.0</td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
</tr>
<tr>
<td>3</td>
<td>1.49</td>
</tr>
<tr>
<td>4</td>
<td>0.50</td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Each solution was analysed by three replicate injections using RP-HPLC. HPLC analyses were conducted on an analytical RP-HPLC using the method specified in the general experimental section for HPLC analysis. The average peak integration values obtained from the three replicate injections monitored at $\lambda$ 254 nm were used to generate a standard curve of integrated peak area (y-axis) against concentration in mg.mL$^{-1}$ (x-axis).

In separate vessels, the CO compound to be investigated and tissue culture H$_2$O (Sigma Aldrich, W3500, Lot RNBB4164) was first allowed to equilibrate to the specified temperature (20 °C or 8 °C). Tissue culture H$_2$O was then added in small aliquots until most of the solid had dissolved to form a saturated solution of CO compound, which was then passed through a syringe filter (0.45 µM, nylon membrane) at the specified temperature (20 °C or 8 °C). The filtered solution of CO compound was diluted as necessary with tissue culture H$_2$O and then
analysed by RP-HPLC (three replicate injections). The solubility of the CO compound was calculated using formula (2) derived from the standard curve for that particular CO compound:

\[
[y = mx]
\]

formula (1)

Where \( y \) = integrate area under peak (254 nm), \( m \) = gradient of standard curve, \( x \) = concentration in mg.mL\(^{-1}\).

Rearrangement of formula (1) to determine \( x \) = concentration in mg.mL\(^{-1}\)

\[
x = \left( \frac{y}{m} \right) \times \text{dilution factor}
\]

formula (2)

Formulas for calculating water solubility of CO compounds

For oCOM-19

\[
x = \left( \frac{y}{2226006} \right) \times \text{dilution factor}
\]

formula (3)

For oCOM-21

\[
x = \left( \frac{y}{4444262} \right) \times \text{dilution factor}
\]

formula (4)

For DB-CO-21

\[
(x = \frac{y}{4261592}) \times \text{dilution factor}
\]

formula (5)

For oCOM-23

\[
x = \left( \frac{y}{4491248} \right) \times \text{dilution factor}
\]

formula (6)

For DB-CO-23

\[
x = \left( \frac{y}{4051904} \right) \times \text{dilution factor}
\]

formula (7)
Table S7: Solubility of oCOm-19 in tissue culture water at 20 °C

<table>
<thead>
<tr>
<th>Integration area (254 nm)</th>
<th>1211597</th>
<th>1563066</th>
<th>1440397</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average area under peak</td>
<td></td>
<td></td>
<td>1405020</td>
</tr>
<tr>
<td>Dilution factor</td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Concentration (mg.mL⁻¹)</td>
<td></td>
<td></td>
<td>3.2</td>
</tr>
</tbody>
</table>

Table S8: Solubility of oCOm-21 in tissue culture water at 20 °C

<table>
<thead>
<tr>
<th>Integration area (254 nm)</th>
<th>9589947</th>
<th>9592734</th>
<th>9600962</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average area under peak</td>
<td></td>
<td></td>
<td>9594548</td>
</tr>
<tr>
<td>Dilution factor</td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Concentration (mg.mL⁻¹)</td>
<td></td>
<td></td>
<td>10.8</td>
</tr>
</tbody>
</table>

Table S9: Solubility of oCOm-21 in tissue culture water at 8 °C

<table>
<thead>
<tr>
<th>Integration area (254 nm)</th>
<th>8625496</th>
<th>8645923</th>
<th>8648255</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average area under peak</td>
<td></td>
<td></td>
<td>8639891</td>
</tr>
<tr>
<td>Dilution factor</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Concentration (mg.mL⁻¹)</td>
<td></td>
<td></td>
<td>3.9</td>
</tr>
</tbody>
</table>

Table S10: Solubility of DB-CO-21 in tissue culture water at 20 °C

<table>
<thead>
<tr>
<th>Integration area (254 nm)</th>
<th>15874422</th>
<th>15916820</th>
<th>15896945</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average area under peak</td>
<td></td>
<td></td>
<td>15896062</td>
</tr>
<tr>
<td>Dilution factor</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Concentration (mg.mL$^{-1}$)</td>
<td>7.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table S11: Solubility of oCOM-23 in tissue culture water at 20 °C

<table>
<thead>
<tr>
<th>Integration area (254 nm)</th>
<th>16348778</th>
<th>16365832</th>
<th>16348778</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average area under peak</td>
<td></td>
<td></td>
<td>16354463</td>
</tr>
<tr>
<td>Dilution factor</td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Concentration (mg.mL(^{-1}))</td>
<td></td>
<td></td>
<td>18.2</td>
</tr>
</tbody>
</table>

### Table S12: Solubility of oCOM-23 in tissue culture water at 8 °C

<table>
<thead>
<tr>
<th>Integration area (254 nm)</th>
<th>9858537</th>
<th>9887512</th>
<th>9907150</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average area under peak</td>
<td></td>
<td></td>
<td>9884400</td>
</tr>
<tr>
<td>Dilution factor</td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Concentration (mg.mL(^{-1}))</td>
<td></td>
<td></td>
<td>17.6</td>
</tr>
</tbody>
</table>

### Table S13: Solubility of DB-CO-23 in tissue culture water at 20 °C

<table>
<thead>
<tr>
<th>Integration area (254 nm)</th>
<th>10171940</th>
<th>10181776</th>
<th>10186914</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average area under peak</td>
<td></td>
<td></td>
<td>10180210</td>
</tr>
<tr>
<td>Dilution factor</td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Concentration (mg.mL(^{-1}))</td>
<td></td>
<td></td>
<td>20.1</td>
</tr>
</tbody>
</table>
Standard curve for oCOM-19

**Peak area (254 nm) vs. concentration (mg.mL$^{-1}$) for oCOM-19**

$y = 2,226,006x$

$R^2 = 0.9924$

Standard curve for oCOM-21

**Peak area (254 nm) vs. concentration (mg.mL$^{-1}$) for oCOM-21**

$y = 4,444,262x$

$R^2 = 0.9996$
Standard curve for DB-CO-21

![Graph showing peak area vs. concentration for DB-CO-21](image)

\[ y = 4,261,592x \]

\[ R^2 = 0.9999 \]

Standard curve for oCOM-23

![Graph showing peak area vs. concentration for oCOM-23](image)

\[ y = 4,491,248x \]

\[ R^2 = 0.9998 \]
Standard curve for DB-CO-23

Peak area (254 nm) vs. concentration (mg.mL\(^{-1}\)) for DB-CO-23

\[ y = 4,051,904x \]

\[ R^2 = 0.9996 \]
Imaging of Carbon Monoxide in Living Cells

A palladium CO-responsive small-molecule fluorescent probe (COP-1)\textsuperscript{10} was synthesised and carbonylation of the probe by CO confirmed as previously described.\textsuperscript{10} The ability of the CO donors to increase intracellular levels of CO was tested in MDCK cells. 96 well black-walled tissue culture plates were seeded with MDCK cells at $1 \times 10^6$ cells/mL in advanced Glutamax® DMEM containing 1% glutamine, and supplemented with 5% foetal bovine serum and 1% antibiotic (Gibco Life Technologies, Auckland NZ) and incubated at 37°C, 65% relative humidity for 48 hours. The media was replaced with fresh media and oCOM-21, oCOM-23, BP-CO-21 and BP-CO-23 dissolved in sterile DPS were added in triplicate at 5 to 50µM final concentration. Cells were incubated for 1 hour at either 8 or 37°C then 1µM COP-1 in DPS added per well and cells incubated for a further 15 minutes. Cells were imaged on a fluorescent inverted microscope (Nikon Eclipse Ti-E).

Animals

All procedures were approved and conducted in accordance with the guidelines of the Animal Ethics Committee at the University of Otago and complied with the University of Otago “Code of Ethical Conduct for the Manipulation of Animals”. Male Sprague Dawley rats (300 - 320 g) were obtained from the University of Otago Animal Resource Unit. Animals were housed on a 12 hour light/dark cycle at 22 °C with food and water \textit{ad libitum} and left to acclimate for 5 days prior to experimentation.

Oral Bioavailability

Male Sprague Dawley rats (300 - 320g) were orally dosed with either oCOM-19 (133.33 µmol/kg) or oCOM-21 (33.33 µmol/kg) in ultra-pure water by oral gavage. Animals were observed every 15 minutes for any changes in behaviour or signs of distress. Tail vein
blood samples were collected in heparinised capillary tubes immediately prior to and at 1 - 3 hours post CO-donor administration. Blood gas analysis on each sample was performed using a Radiometer ABL800 Flex analyser.

**Isolated Rat Aortic Ring Preparations**

Transverse ring sections (2mm) of aorta were isolated from naïve untreated animals \( (n=5\) per group) under halothane anaesthesia and suspended under a 1g tension in an organ bath containing 10mL of oxygenated (95% O\(_2\)/5% CO\(_2\)) Krebs Henseleit (KH) buffer containing 118mM NaCl, 4.7mM KCl, 1.2mM KH\(_2\)PO\(_4\), 1.2mM MgSO\(_4\).7H\(_2\)O, 22mM NaHCO\(_3\), 11mM glucose, 0.03mM K\(^+\)EDTA, and 2.5mM CaCl\(_2\) (37°C) as previously described.\(^{11}\) Indomethacin (10µM; Sigma-Aldrich, Auckland, NZ) was present in the buffer to exclude endogenous prostaglandins as potential modulators of vascular tone. Relaxation responses to oCOM-19 and oCOM-21 (0.01 – 300µM) were assessed using individual dosing in tissues pre-contracted with a submaximal concentration (0.1µM) of phenylephrine (Sigma-Aldrich, Auckland, NZ). All results were subsequently expressed as a percentage of the maximal phenylephrine-induced contraction. Following repeat wash steps (x4) with Krebs Henseleit buffer to remove the pressor agent, smooth muscle function and viability was confirmed by repeating the contractile response to phenylephrine. Aortic responses to individual EC\(_{50}\) concentrations of oCOM-19 in phenylephrine vasoconstricted rings were repeated separately, in the presence of the selective, irreversible inhibitor of sGC, 1H-(1,2,4)oxadiazolo[4,3-a]quinoxalin-1-one, (ODQ, 10µM), as previously described.\(^{12}\)
References

Figures for Supporting Information
RP-HPLC chromatograms showing the conversion of oCOM-21 to BP-CO-21 in TRIS-sucrose buffer (pH 7.4) at 37 °C at specified time points: (a) 0 minutes (b) 20 minutes (c) 60 minutes. Key ♦ = oCOM-21 * = BP-CO-21. RP-HPLC method: 30-60% B over 12.5 mins, 60%-100% B over 2.5 mins, 100% B for 2.5 mins, flowrate 0.5 mL.min⁻¹. Injection volume = 3 µL. Column: Phenomenex, C-18 Prodigy, 250 mm x 3 mm, 5 µM.

RP-HPLC chromatograms showing the conversion of oCOM-23 to BP-CO-23 in TRIS-sucrose buffer (pH 7.4) at 37 °C at specified time points: (a) 0 minutes (b) 90 minutes (c) 180 minutes. Key ▼ = oCOM-23 ♦ = BP-CO-23. RP-HPLC method: 35-50% B over 15.0 mins, 50%-100% B over 1.0 min, 100% B for 1.0 min, flowrate 0.5 mL.min⁻¹. Injection volume = 3 µL. Column: Phenomenex, C-18 Prodigy, 250 mm x 3 mm, 5 µM.
Figure S2a: Comparison of CO release from oCOM-21 (75uM) and oCOM-23 (75uM) at 37°C in Tris sucrose buffer (pH7.4) measured using a CO selective electrode.
Figure S2b: The effect of pH on CO release from oCOM-19 in tris-sucrose buffer at 37 °C measured using a CO selective electrode.
Figure S2c: The generation of physiologically active CO by the CO donor compounds was demonstrated by myoglobin binding studies, measured as a shift in the absorbance spectra peaks to 540nm.
Figure S3a: TGA analysis of oCOM-21 using Method A (heating rate of 2.00 °C/min from 20 °C to 300 °C).

A gradual loss in mass (~8% loss in mass) was observed up to the temperature of 125 °C. Between the temperatures of 153–183 °C, a sudden loss in mass (approximately 15% loss in mass) was detected indicating thermal instability at this temperature range. A second and much larger loss in mass (67% loss) was observed between 211–291 °C.
Figure S3b: TGA analysis of oCOM-21 using Method B (heating rate of 2.00 °C/min from 20 °C to 90 °C and then isothermal at 90 °C for 30 mins).
**Figure S3c**: HPLC chromatogram of oCOM-21 sample heat treated at 90 °C for 30 mins (a), reference samples of oCOM-21 and BP-CO-21 included in entries (b) and (c).
Figure S3c: Cell viability as a % of control 23 hrs following 1hr exposure to the CO donors oCOM-19, -21 and -23 or their inactive debromo (DB-CO-23) or CO expired (BP-CO-19, -21) analogues as assessed at 37°C in MDCK cells using the crystal violet assay as a marker of loss of cellular adhesion.
Table S1: Plasma carboxyhaemoglobin recorded at 1-3 hours following CO donor administration.

<table>
<thead>
<tr>
<th></th>
<th>% Carboxyhaemoglobin in blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-dosing</td>
</tr>
<tr>
<td>oCOM -19 133.3 µmol/kg</td>
<td>0.7 ± 0.28</td>
</tr>
<tr>
<td>oCOM -21 33.3 µmol/kg</td>
<td>0.54 ± 0.36</td>
</tr>
<tr>
<td>BP-CO-19 133.3 µmol/kg</td>
<td>0.57 ± 0.25</td>
</tr>
</tbody>
</table>

Effects on circulating carboxyhaemoglobin levels of oCOM-19 and oCOM-21 administered by oral gavage in Sprague Dawley rats (n=3). Data expressed as mean ± SEM. * indicates P<0.05 compared to the pre-dosing level as determined by Student t test.
Scheme S1: Synthesis of DB-CO-19, -21 and -23

19 $R = \text{CONH}(\text{CH}_2)_2\text{NHBoc}$
24 $R = \text{NHBoc}$
25 $R = \text{N(Boc)CH}_2\text{CH}_2\text{NHBoc}$
32 $R = \text{CONH}(\text{CH}_2)_2\text{NHBoc}$
33 $R = \text{NHBoc}$
34 $R = \text{N(Boc)CH}_2\text{CH}_2\text{NHBoc}$

DB-CO-19 $R = \text{CONH}(\text{CH}_2)_2\text{NH}_2\cdot\text{TFA}$
DB-CO-21 $R = \text{NH}_2\cdot\text{TFA}$
DB-CO-23 $R = \text{NHOCH}_2\text{CH}_2\text{NH}_2\cdot\text{2TFA}$
NMR Spectra
Compound 16. $^1$H NMR spectrum (500 MHz, CDCl$_3$).
Compound 16 $^{13}$C NMR spectrum (500 MHz, CDCl$_3$).
Compound 17. $^1$H NMR spectrum (500 MHz, CDCl$_3$).
Compound 17 $^{13}$C NMR spectrum (500 MHz, CDCl$_3$).
Compound 19. $^1$H NMR spectrum (500 MHz, CDCl$_3$).
Compound 19 $^{13}$C NMR spectrum (500 MHz, CDCl$_3$).
Compound 20 $^1$H NMR spectrum (500 MHz, CDCl$_3$).
Compound 20 $^{13}$C NMR spectrum (500 MHz, CDCl$_3$).
Compound 21. $^1$H NMR spectrum (500 MHz, CDCl$_3$).
Compound 21 $^{13}$C NMR spectrum (500 MHz, CDCl$_3$).
Compound 22. $^1$H NMR spectrum (500 MHz, CDCl$_3$).
Compound 22 $^{13}\text{C}$ NMR spectrum (500 MHz, CDCl$_3$).
oCOME-19. HCl salt (3:1 mixture of *endo*- and *exo*-isomers). $^1$H NMR spectrum (400 MHz, DMSO-$d_6$).
oCOM-19. HCl salt (3:1 mixture of endo- and exo-isomers) $^{13}$C NMR spectrum (125 MHz, DMSO-d6)
oCOM-19. TFA salt (10:1 mixture of *endo*- and *exo*-isomers). $^1$H NMR spectrum (400 MHz, DMSO-$d_6$).
oCOM-19. TFA salt (10:1 mixture of endo- and exo-isomers). $^{13}$C NMR spectrum (125 MHz, DMSO-$_d$6).
oCOM-19. TFA salt (10:1 mixture of endo- and exo-isomers). 19F NMR spectrum (376 MHz, DMSO-d6).
BP-CO-19. HCl salt (10:1 mixture of *endo-* and *exo-*isomers). $^1$H NMR spectrum (500 MHz, DMSO-d6).
BP-CO-19. HCl salt (10:1 mixture of endo- and exo-isomers). 1H NMR spectrum (500 MHz, DMSO-d6).
Compound 25. $^1$H NMR spectrum (500 MHz, CDCl$_3$).
Compound 25. $^{13}$C NMR spectrum (125 MHz, CDCl$_3$).
Compound 26. $^1$H NMR spectrum (400 MHz, CDCl$_3$).
Compound 26. $^{13}$C NMR spectrum (100 MHz, CDCl$_3$).
Compound 27. $^1$H NMR spectrum (500 MHz, CDCl$_3$).
Compound 27. $^{13}$C NMR spectrum (125 MHz, CDCl$_3$).
Compound 28 (endo-isomer). $^1$H NMR spectrum (400 MHz, CDCl$_3$).
Compound 28 (endo-isomer). $^{13}$C NMR spectrum (125 MHz, CDCl$_3$).
oCOM-21 (endo-isomer). $^1$H NMR spectrum (400 MHz, DMSO-$d_6$).
oCOM-21 (endo-isomer). $^{13}$C NMR spectrum (125 MHz, DMSO-$d_6$).
oCOM-21 (endo-isomer). $^{19}$F NMR spectrum (376 MHz, DMSO-$d_6$).
Compound 30. $^1$H NMR spectrum (400 MHz, CDCl$_3$).
Compound 30. $^{13}$C NMR spectrum (100 MHz, CDCl$_3$).
BP-CO-21. $^1$H NMR spectrum (400 MHz, DMSO-$d_6$).
BP-CO-21. $^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$).
BP-CO-21. $^{19}$F NMR spectrum (376 MHz, DMSO-$d_6$).
Compound 32 (11:1 mixture of endo- and exo-isomers). $^1$H NMR spectrum (500 MHz, CDCl$_3$).
Compound 32 (11:1 mixture of endo- and exo-isomers). $^{13}$C NMR spectrum (125 MHz, CDCl$_3$).
DB-CO-19 (13:1 mixture of endo- and exo-isomers). $^1$H NMR spectrum (500 MHz, DMSO-$d_6$).
DB-CO-19 (13:1 mixture of endo- and exo-isomers). $^{13}$C NMR spectrum (125 MHz, DMSO-$d_6$).
Compound 33 (4.2:1 mixture of endo- and exo-isomers). $^1$H NMR spectrum (500 MHz, CDCl$_3$).
Compound 33 (4.2:1 mixture of endo- and exo-isomers). $^{13}$C NMR spectrum (100 MHz, CDCl$_3$).
**DB-CO-21** (4.1:1 mixture of *endo*- and *exo*-isomers). $^1$H NMR spectrum (400 MHz, DMSO-$d_6$).
DB-CO-21 (4.1:1 mixture of endo- and exo-isomers). $^{13}$C NMR spectrum (125 MHz, DMSO-$d_6$).
DB-CO-21 (4.1:1 mixture of endo- and exo-isomers). $^{19}$F NMR spectrum (376 MHz, DMSO-$d_6$).
Compound 34 (11:1 mixture of *endo-* and *exo-*isomers). $^1$H NMR spectrum (500 MHz, CDCl$_3$).
Compound 34 (11:1 mixture of endo- and exo-isomers). $^{13}$C NMR spectrum (125 MHz, CDCl$_3$).
DB-CO-23 (15.7:1 mixture of endo- and exo-isomers). $^1$H NMR spectrum (400 MHz, DMSO-$d_6$).
DB-CO-23 (15.7:1 mixture of *endo*- and *exo*-isomers). $^{13}$C NMR spectrum (125 MHz, DMSO-$d_6$).
DB-CO-23 (15.7:1 mixture of *endo*- and *exo*-isomers). $^{19}$F NMR spectrum (376 MHz, DMSO-$d_6$).
Compound 29 (endo-isomer). $^1$H NMR spectrum (500 MHz, CDCl$_3$).
Compound 29 (endo-isomer). $^{13}$C NMR spectrum (125 MHz, CDCl$_3$).
oCOM-23 (endo-isomer). $^1$H NMR spectrum (400 MHz, DMSO-d$_6$).
**oCOM-23** (*endo*-isomer). $^{13}$C NMR spectrum (125 MHz, DMSO-$d_6$).
**oCOM-23** (*endo*-isomer). $^{19}$F NMR spectrum (376 MHz, DMSO-$d_6$). 

![NMR spectrum](image_url)
Compound 31. $^1$H NMR spectrum (500 MHz, CDCl$_3$).
Compound 31. $^{13}$C NMR spectrum (125 MHz, CDCl$_3$).
BP-CO-23. $^1$H NMR spectrum (500 MHz, DMSO-$d_6$).
BP-CO-23. $^{13}$C NMR spectrum (125 MHz, DMSO-$d_6$).
BP-CO-23. $^{19}$F NMR spectrum (376 MHz, DMSO-$d_6$).