Strained Alkynes Derived from 2,2’-Dihydroxy-1,1’-Biaryls; Synthesis and Copper-Free Cycloaddition with Azides.

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Supporting information:

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Synthetic procedures and NMR spectra.

General experimental.

Solvents and reagents for the synthesis of complexes and catalytic reactions were degassed prior to use and all reactions were carried out under either a nitrogen or argon atmosphere. Reactions were monitored by TLC using aluminum backed silica gel 60 (F254) plates, visualized using UV 254 nm and phosphomolybdic acid, potassium permanganate or vanillin dips as appropriate. Flash column chromatography was carried out routinely on silica gel. Reagents were used as received from commercial sources unless otherwise stated. Dry solvents were purchased and used as received. $^1$H NMR spectra were recorded on a Bruker DPX (400 or 500 MHz) spectrometer. Chemical shifts are reported in $\delta$ units, parts per million relative to the singlet at 7.26 ppm for chloroform and 0.00 ppm for TMS. Mass spectra for analysis of synthetic products were recorded on a Bruker Esquire2000 or a Bruker MicroTOF mass spectrometer. FTICR Mass Spectrometry to measure the binding to peptides and proteins was carried using a Bruker SolariX 12T, Bruker Daltonics (Bremen, Germany) instrument. Coupling constants (J) are measured in Hertz. IR spectra were recorded on a Perkin-Elmer Spectrum One FT-IR Golden Gate. Melting points were recorded on a Stuart Scientific SMP 1 instrument and are uncorrected.

Synthesis of compound 1; 8,13-dioxatricyclo[12.4.0.0$^2$7]octadeca-1(14),2,4,6,15,17-hexaen-10-yne 1.

This is a known compound (U. Koch-Pomeranz, H.-J. Hansen, H. Schmid, *Helv. Chim. Acta.* 1971, 56, 2981-3004). In a round bottom flask under nitrogen atmosphere 2,2’-biphenol (94.4 mg, 0.51 mmol) and but-2-yne-1,4-diyl bis(4-methylbenzenesulfonate) (200 mg, 0.51 mmol) were dissolved in anhydrous acetonitrile (25 mL). K$_2$CO$_3$ (350 mg, 2.53 mmol) was added and the mixture was stirred at room temperature for 12 days. The volatiles were removed and H$_2$O (50 mL) was added. The product was extracted with DCM (3x50 mL). The reunited organic
layers were washed with brine (30 mL) and dried over Na$_2$SO$_4$. The product was purified by flash chromatography on silica gel (eluent: Pentane/DCM = 4:1 to DCM) to give the product 1 (42 mg, 0.18 mmol, 29%) as colourless solid. m.p. 129.7-132.5 °C.

IR (neat) 3053, 3023, 2989, 1952, 2919, 2863, 1595, 1574, 1499, 1470, 1451, 1434, 1348, 1258, 1248 cm$^{-1}$.

$\delta$H (500 MHz, CDCl$_3$) 7.37 (2H, ddd, J = 8.0, 7.0, 2.1 Hz, ArH), 7.11 - 7.27 (6H, m, ArH), 4.47 - 4.58 (2H, m, CHH), 4.27 - 4.39 (2H, m, CHH).

$\delta$c (125 MHz, CDCl$_3$) 154.4 (C), 135.9 (C), 132.0 (CH), 129.0 (CH), 124.2 (CH), 122.5 (CH), 86.6 (C), 63.5 (CH$_2$).

HRMS (ESI-Q-TOF) m/z: [M + Na]$^+$ Calcd for C$_{16}$H$_{12}$O$_2$Na 259.0730; Found 259.0725.

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (125 MHz, CDCl$_3$)
Synthesis of Compound 2 $R$-12,17-dioxapentacyclo[16.8.0.0$^{2,11}$.0$^{3,8}$.0$^{21,26}$]hexacosa-1(26),2,4,6,8,10,18,20,22,24-decaen-14-yne 2.
In a round bottom flask under nitrogen atmosphere (R)-BINOL (726 mg, 2.54 mmol) and but-2-yne-1,4-diyl bis(4-methylbenzenesulfonate) (2.0 g, 5.07 mmol) were dissolved in anhydrous acetonitrile (250 mL). K$_2$CO$_3$ (3.5 g, 25.32 mmol) was added and the mixture was stirred at room temperature for 68 hours. The mixture was filtered through a plug of celite. The volatiles were removed and the product was purified by flash chromatography on silica gel (eluent: Petroleum Ether/EtOAc = 9:1 to EtOAc) to give compound 2 (121 mg, 0.36 mmol, 14%) as colourless solid. Crystals suitable for X-ray spectroscopy were grown by slow evaporation of DCM. [α]$_D$$^{25}$ +263.6 (c 0.505, CHCl$_3$).

m.p. 177.2-178.4 ºC

IR (neat) 3060, 2954, 2913, 2861, 1662, 1619, 1586, 1503, 1472, 1457, 1426, 1405, 1349, 1333, 1268, 1251, 1207, 1194 cm$^{-1}$.

δ$^H$ (500 MHz, CDCl$_3$) 8.01 (2H, d, $J = 8.9$ Hz, Ar$H$), 7.90 (2H, d, $J = 8.2$ Hz, Ar$H$), 7.38 - 7.45 (4H, m, Ar$H$), 7.31 (4H, d, $J = 3.7$ Hz, Ar$H$), 4.51 - 4.58 (2H, m, CH$H$), 4.41 - 4.48 (2H, m, CH$H$).

δ$^C$ (125 MHz, CDCl$_3$) 152.5 (C), 133.7 (C), 131.1 (C), 130.3 (CH), 128.0 (CH), 127.8 (C), 126.6 (CH), 126.1 (CH), 125.2 (CH), 121.1 (CH), 88.0 (C), 62.3 (CH$_2$).

HRMS (ESI-Q-TOF) m/z: [M + H]$^+$ Calcd for C$_{24}$H$_{17}$O$_2$ 337.1223; Found 337.1224.

$^1$H NMR (500 MHz, CDCl$_3$)
$^{13}$C NMR (125 MHz, CDCl$_3$)
Synthesis of Compound 3; 4-17-dibromo-8,13-dioxatricyclo[12.4.0.0²,7]octadeca-1(14),2,4,6,15,17-hexaen-10-yne 3.

\[ \text{O} \quad \text{Br} \quad \text{Br} \]

In a round bottom flask under nitrogen atmosphere 5,5'-dibromo-[1,1'-biphenyl]-2,2'-diol (436 mg, 1.27 mmol) and but-2-yne-1,4-diyl bis(4-methylbenzenesulfonate) (500 mg, 1.27 mmol) were dissolved in anhydrous acetonitrile (63 mL). K₂CO₃ (876 mg, 6.34 mmol) was added and the mixture was stirred at room temperature for 5 days. The volatiles were removed and H₂O (100 mL) was added. The product was extracted with DCM (3 x 100 mL). The reunited organic layers were washed with brine (100 mL) and dried over Na₂SO₄. The product was purified by flash chromatography on silica gel (eluent: Pentane/DCM = 1:1) to give the product 3 (122 mg, 0.31 mmol, 24%) as colourless solid.

m.p. 169.4 °C dec.
IR<sub>(neat)</sub> 2963, 2908, 2858, 1734, 1585, 1566, 1490, 1461, 1406, 1371, 1343, 1248, 1192 cm<sup>-1</sup>.

$\delta_H$ (500 MHz, CDCl<sub>3</sub>) 7.49 (2H, dd, $J = 8.6, 2.4$ Hz, ArH), 7.32 (2H, d, $J = 2.4$ Hz, ArH), 7.04 (2H, d, $J = 8.6$ Hz, ArH), 4.50 - 4.62 (2H, m, CH<sub>2</sub>), 4.25 - 4.35 (2H, m, CH<sub>2</sub>).

$\delta_C$ (125 MHz, CDCl<sub>3</sub>) 153.5 (C), 136.6 (C), 134.6 (CH), 132.5 (CH), 124.4 (CH), 117.3 (C), 86.6 (C), 63.6 (CH<sub>2</sub>).

HRMS (ESI-Q-TOF) m/z: [M + H]<sup>+</sup> Calcd for C<sub>16</sub>H<sub>11</sub>Br<sub>2</sub>O<sub>2</sub> 392.9120; Found 392.9126.

**Alternative procedure using butanone as solvent.**

In a round bottom flask under a nitrogen atmosphere, 5,5'-dibromo-2'-methyl-3',4'-dihydro-[1,1'-biphenyl]-2-ol (689 mg, 2.00 mmol), potassium carbonate (1.38 g, 10.0 mmol) and but-2-yne-1,4-diyl bis(4-methylbenzenesulfonate) (789 mg, 2.00 mmol) were dissolved in butan-2-one (100 mL). The mixture was degassed, stirred at 80 °C for 5 days. The butan-2-one was evaporated under reduced pressure. The organic fractions were redissolved with water (100 mL) and extracted with DCM (3 x 50 mL). The organic extracts were dried over magnesium sulphate, filtered and the DCM was evaporated under reduced pressure. The product was purified by column chromatography (eluent: hexane to hexane/ethyl acetate 88:12) to obtain the product 3 as a white solid (363 mg, 0.92 mmol, 46%). The data for the product matched that reported above.

$^1$H NMR (500 MHz, CDCl<sub>3</sub>)
$^{13}$C NMR (125 MHz, CDCl$_3$)
Synthesis of Compound 4; 4-6-15-17-tetrabromo-8,13-dioxatricyclo[12.4.0.0\(^2,7\)]octadeca-1(14),2,4,6,15,17-hexaen-10-yne 4.

In a round bottom flask under nitrogen atmosphere 3,3',5,5'-dibromo-[1,1'-biphenyl]-2,2'-diol (636 mg, 1.27 mmol) and but-2-yne-1,4-diyl bis(4-methylbenzenesulfonate) (500 mg, 1.27 mmol) were dissolved in anhydrous acetonitrile (63 mL). K\(_2\)CO\(_3\) (876 mg, 6.34 mmol) was added and the mixture was stirred at room temperature for 10 days. The volatiles were removed and H\(_2\)O (100 mL) was added. The product was extracted with DCM (3x150 mL). The reunited organic layers were washed with brine (100 mL) and dried over Na\(_2\)SO\(_4\). The product was purified by flash chromatography on silica gel (eluent: Petroleum ether/EtOAc = 4:1 to 3:1) to give the product 4 (411 mg, 0.74 mmol, 59%) as colourless solid.

m.p. 170.7 °C dec.

IR\(_{(\text{neat})}\) 3094, 3065, 2976, 2961, 2930, 2907, 2854, 1569, 1537, 1461, 1443, 1420, 1369, 1336, 1238, 1207 cm\(^{-1}\).

\(\delta\)_H (500 MHz, CDCl\(_3\)) 7.75 (2H, d, \(J = 2.4\) Hz, Ar\(H\)), 7.28 (2H, d, \(J = 2.4\) Hz, Ar\(H\)), 4.68 - 4.75 (2H, m, CH\(_2\)), 4.47 - 4.54 (2H, m, CHH).

\(\delta\)_C (125 MHz, CDCl\(_3\)) 149.2 (C), 137.8 (C), 135.6 (CH), 134.1 (CH), 119.0 (C), 117.8 (C), 86.7 (C), 60.8 (CH\(_2\)).

HRMS (ESI-Q-TOF) m/z: [M + Na]\(^+\) Calcd for C\(_{16}\)H\(_8\)Br\(_4\)O\(_2\)Na 570.7150; Found 570.7135.

\(^1\)H NMR (500 MHz, CDCl\(_3\)).
$^{13}$C NMR (125 MHz, CDCl$_3$)
Synthesis of Compound 11; Methyl 8,13-dioxatricyclo[12.4.0.0^2,7]octadeca-1(18),2(7),3,5,14,16-hexaen-10-yn-4-carboxylate 11.

In a round bottom flask under nitrogen atmosphere methyl 2',6-dihydroxy-[1,1'-biphenyl]-3-carboxylate (313 mg, 1.28 mmol) 12 and but-2-yne-1,4-diyl bis(4-methylbenzenesulfonate) (500 mg, 1.27 mmol) were dissolved in anhydrous acetonitrile (63 mL). K$_2$CO$_3$ (876 mg, 6.34 mmol) was added and the mixture was stirred at room temperature for 13 days. The volatiles were removed and H$_2$O (100 mL) was added. The product was extracted with DCM (3x150 mL). The reunited organic layers were washed with brine (100 mL) and dried over Na$_2$SO$_4$. The product was purified by flash chromatography on silica gel (eluent: Petroleum ether/EtOAc = 4:1 to 3:1) to give compound 11 (188 mg, 0.64 mmol, 50%) as colourless solid.

m.p. 121.1-122.4 ºC.

IR$_{\text{neat}}$ 2952, 2921, 2854, 1713, 1608, 1598, 1577, 1499, 1475, 1434, 1405, 1346, 1307, 1288, 1266, 1232, 1190 cm$^{-1}$.

$\delta$H (500 MHz, CDCl$_3$) 8.07 (1H, dd, $J$ = 8.5, 2.2 Hz, ArH), 7.93 (1H, d, $J$ = 2.2 Hz, ArH), 7.37 - 7.44 (1H, m, ArH), 7.16 - 7.25 (4H, m, ArH), 4.49 - 4.62 (2H, m, CHH), 4.30 - 4.41 (2H, m, CHH), 3.88 (3H, s, CH$_3$).

$\delta$c (125 MHz, CDCl$_3$) 166.6 (C), 158.5 (C), 154.4 (C), 136.0 (C), 135.0 (C), 133.7 (CH), 131.8 (CH), 130.7 (CH), 129.5 (CH), 126.2 (C), 124.3 (CH), 122.8 (CH), 122.6 (CH), 87.2 (C), 86.1 (C), 63.7 (CH$_2$), 63.5 (CH$_2$), 52.1 (CH$_3$).

m/z (ESI) [M + Na]$^+$, 317.1

HRMS (ESI-Q-TOF) m/z: [M + Na]$^+$ Calcd for C$_{18}$H$_{14}$O$_4$Na 317.0784; Found 317.0789.

$^1$H NMR (500 MHz, CDCl$_3$)
$^{13}$C NMR (125 MHz, CDCl$_3$)
From this procedure, some 2+2 (54 mg, 15%) and some 3+3 (36 mg, 10%) product were also isolated.

The 2+2 product NMRs are below (298b). The HRMS is also shown, to confirm the structure of the macrocycle:

\[ \text{[2+2]} \quad \text{[3+3]} \]

(regiosomeric mixtures)

\(^1\text{H NMR (500 MHz, CDCl}_3\).\]
$^{13}$C NMR (125 MHz, CDCl$_3$).
HRMS:
The 3+3 product NMRs are below (298c). The HRMS is also shown, to confirm the structure of the macrocycle:
$^1$H NMR (500 MHz, CDCl$_3$).

$^{13}$C NMR (125 MHz, CDCl$_3$).
HRMS:
Alternative procedure using butanone as solvent;

In a round bottom flask under nitrogen atmosphere, methyl 2',6-dihydroxy-[1,1'-biphenyl]-3-carboxylate (500 mg, 2.05 mmol) and but-2-yne-1,4-diyl bis(4-methylbenzenesulfonate) (808 mg, 2.05 mmol) were dissolved in butan-2-one (102 mL). The mixture was degassed and heated to 80 °C for 5 days. After this time the solvent was removed under reduced pressure. The resulting oil was dissolved in DCM
(50 mL) and washed with water (100 mL). The aqueous phase was extracted with DCM (2 x 50 mL). The combined organic extracts were dried over magnesium sulphate, filtered and the DCM was evaporated under reduced pressure. The product was purified by column chromatography, (elucent hexane to hexane/ethyl acetate 88:12) to obtain the product as a white solid (193 mg, 0.656 mmol, 32 %). The data for the product matched that reported above.


In a round bottom flask, methyl 8,13-dioxatricyclo[12.4.0.0²,⁷]octadeca-1(18),2(7),3,5,14,16-hexaen-10-yne-4-carboxylate 11 (100 mg, 0.325 mmol) was dissolved in 1:1 MeOH/H₂O (3 mL). NaOH (26 mg, 0.65 mmol) was added. The mixture was refluxed at 100 °C overnight. The mixture was acidified at pH ≈5 by adding 0.5 M HCl dropwise. The organic fractions were extracted with ethyl acetate (4 x 20 mL). The combined organic extracts were washed with saturated sodium chloride solution and then were dried over sodium sulphate. The solution was filtered and the solvent removed under reduced pressure to yield the product 13 as a white solid (88 mg, 0.315 mmol, 97 %).

m.p. 176.0 °C dec.

IR

| 2955, 2921, 2852, 1681, 1607,1597, 1579, 1499, 1476, 1446, 1423, 1399, 1348, 1310, 1272, 1249, 1191 cm⁻¹ |

m/z (ESI) [M + H]⁺, 428.1; [M + Na]⁺, 303.0

δH (500 MHz, d⁶-Acetone) δ = 8.07 (1H, dd, J = 8.5, 2.0 Hz, ArH), 7.86 (1H, d, J = 2.1 Hz, ArH), 7.39 - 7.46 (1H, m, ArH), 7.37 (1H, d, J = 8.4 Hz, ArH), 7.28 (1H, d, J
δ (125 MHz, d6-Acetone) δ = 168.1 (C), 159.7 (C), 155.9 (C), 137.0 (C), 136.2 (C), 134.5 (CH), 132.7 (CH), 131.6 (CH), 130.4 (CH), 128.1 (C), 124.9 (CH), 123.9 (CH), 123.8 (CH), 88.1(C), 87.3(C), 64.2(CH2), 64.0 (CH2).

m/z (ESI) 303.0 ([M + Na]+, 100%).

HRMS (ESI-Q-TOF) m/z: [M + Na]+ Calcd for C17H12NaO4 303.0628; Found 303.0627.

1H NMR (500 MHz, d6-Acetone)

13C NMR (125 MHz, d6-Acetone)
**Synthesis of Activated ester 14:** 5-dioxopyrrolidin-1-yl 8,13-dioxatricyclo[12.4.0.0²,7]octadeca-1(14),2(7),3,5,15,17-hexaen-10-yne-4-carboxylate 14.

In a round bottom flask, a solution of compound 13 (82 mg, 0.29 mmol) in dry DCM (1.2 mL) N-hydroxysuccinimide (54 mg, 0.464 mmol) and EDC.HCl (67 mg, 0.348 mmol) were added and the mixture was stirred overnight at room temperature. The reaction mixture was extracted with diethyl ether (2 x 20 mL), washed twice with water (2 x 20 mL) and saturated sodium chloride solution (20 mL). After drying of the organic phase over sodium sulphate and filtration, the solvents were evaporated under reduced pressure. The product was purified by column chromatography (eluent: Hexane to Hexane/Ethyl acetate 80:20) to obtain the product 14 as a white solid (63.1 mg, 0.167 mmol, 55 %).

Mp: 220-223 °C.

(found (ESI): M⁺ + Na, \( C_{21}H_{15}NO_6Na \) requires M, 400.0752 (0.5 ppm error)).

IR\(_{\text{neat}}\) 1734, 1189, 1066, 963, 729 cm\(^{-1}\).

δ\(_H\) (CDCl₃, 500 MHz) 8.08 (1H, dd, \( J \) 9.0, 2.0, Ar\( H \)), 7.96 (1H, d, \( J \) 2.0, Ar\( H \)), 7.33 (1H, dt, \( J \) 8.0, 2.0, Ar\( H \)), 7.22 (1H, d, \( J \) 8.5, Ar\( H \)), 7.15-7.08 (3H, m, Ar\( H \)), 4.57-5.46 (2H, m, OC\( H_2 \)), 4.34-4.24 (1H, m, OCH\(_2\)), 2.82 (4H, brs, CH\(_2\)CH\(_2\)).

δ\(_C\) (CDCl₃, 125 MHz) 168.3 (C), 160.3 (C), 159.4 (C), 153.4 (C), 135.9 (C), 133.9 (CH), 133.3 (C), 130.7 (CH), 130.6 (CH), 128.7 (CH), 123.3 (CH), 122.5 (CH), 121.7 (CH), 120.0 (C), 86.5 (C triple), 84.8 (C triple), 62.9 (OCH\(_2\)), 62.4 (OCH\(_2\)), 24.6 (CH\(_2\)CH\(_2\)).

\( m/z \) (ESI) 400.1 (M⁺ + Na, 100%).

HRMS (ESI-Q-TOF) \( m/z \): \([M + Na]^+\) Calcd for \( C_{21}H_{15}NO_6Na \) 400.0752; Found 400.0790.
$^1$H NMR (CDCl$_3$, 500 MHz)

$^{13}$C NMR (CDCl$_3$, 125 MHz).
Synthesis of compound 9; 13-phenyl-8,11,16-trioxa-12-azatetracyclo[15.4.0.0²,⁷.0⁰¹,⁴]henicosa-1(17),2,4,6,10(14),12,18,20-octaene 9.

A mixture of strained alkyne 1 (50 mg, 0.21 mmol) and PhC(Cl)NOH (39 mg, 0.25 mmol) was stirred in DMF (0.5 mL) in the presence of 4Å MS (350 mg) for 3 days at rt. At the end of this time water (10 mL) and EtOAc (10 ml) were added and the organic layer was separated. The water layer was extracted with EtOAc (2 x 20 mL) and the combined organic layers were washed with water (3 x 20 mL). The solvent was removed to leave a crude product which was purified by chromatography on silica gel (hexane/EtOAC gradient) to give the product 9 as a clear oil (33 mg, 0.842 mmol, 40%).

The Product was below the strained alkyne by TLC; 4/1 hexane/EtOAc, silica gel, product Rf 0.30, alkyne Rf 0.60, visualised by uv and KMnO₄.
IR (neat) 1460, 1440, 1257, 1188, 1104, 837, 751, 725, 696 cm\(^{-1}\);
\(\delta_H\) (500 MHz, CDCl\(_3\)) 7.65-7.58 (2H, m, ArH), 7.54-6.45 (3H, m, ArH), 7.35-7.24
(4H, m, ArH), 7.18-7.07 (3H, m, ArH), 6.93 (1H, d, \(J = 8.5\), ArH), 5.52 (1H, d, \(J = 13.5\), CHH), 5.35 (1H, d, \(J = 13.5\), CHH), 5.23 (1H, d, \(J = 13.5\), CHH), 5.07 (1H, d, \(J = 13.5\), CHH). COSY, HSQC,
\(\delta_C\) (120 MHz, CDCl\(_3\)) 168.9 (C), 162.7 (C), 157.0 (C), 155.5 (C), 149.6 (CH), 130.66
(CH), 130.5 (C), 130.1 (C), 130.0 (CH), 129.0 (CH), 128.9 (CH), 128.55
(CH), 128.2 (C), 123.0 (CH), 116.0 (CH), 113.7 (CH), 113.6 (C), 62.0
(CH\(_2\)), 61.8 (CH\(_2\)).

\(m/z\) (ESI+) 356.1 (M\(^+\) + H, 45%), 378.1 (M\(^+\) + Na, 100%).

HRMS (ESI-Q-TOF) \(m/z\): [M + Na]\(^+\) Calcd for C\(_{23}\)H\(_{17}\)NO\(_3\)Na 378.1101; Found
378.1105.

\(^1^H\) NMR (500 MHz, CDCl\(_3\))

\(^{13}\)C NMR (125 MHz, CDCl\(_3\))
**Compound 5a;** 1-Benzyl-4,15-dihydro-1H-dibenzo[7,8:9,10][1,6]dioxecino[3,4-d][1,2,3]triazole 5a.
In a round bottom flask, compound 1 (35.4 mg, 0.15 mmol) was dissolved in acetonitrile (0.6 mL). Benzyl azide (19.9 mg, 0.15 mmol) was added and the mixture was heated at 60 ºC. After 20 hours the mixture was cooled to room temperature and the volatiles were removed. The product was purified by flash chromatography on silica gel (eluent: Petroleum ether/EtOAc = 3:2 to 2:3) to give compound 5a (52 mg, 0.14 mmol, 93%) as colourless solid.

m.p. 207.6-208.8 ºC.

IR (neat) 3059, 3016, 2969, 2945, 1603, 1593, 1571, 1497, 1478, 1453, 1440, 1391, 1368, 1353, 1339, 1303, 1285, 1259, 1244, 1214, 1204 cm⁻¹.

δH (500 MHz, CDCl₃) 7.32 - 7.39 (3H, m, ArH), 7.22 - 7.31 (2H, m, ArH), 7.12 - 7.21 (5H, m, ArH), 7.07 (1H, t, J = 7.3 Hz, ArH), 6.99 - 7.05 (1H, m, ArH), 6.65 (1H, d, J = 7.9 Hz, ArH), 5.73 (1H, d, J = 15.7 Hz, CHH), 5.48 (1H, d, J = 13.4 Hz, CHH), 5.28 - 5.42 (2H, m, 2xCHH), 5.18 (1H, d, J = 13.2 Hz, CHH), 5.07 (1H, d, J = 13.2 Hz, CHH).

δC (125 MHz, CDCl₃) 156.5 (C), 155.5 (C), 144.8 (C), 134.6 (C), 132.3 (C), 130.7 (CH), 130.6 (C), 130.4 (CH), 129.4 (C), 129.2 (CH), 128.8 (CH), 128.7 (CH), 128.6 (CH), 127.1 (CH), 123.2 (CH), 122.1 (CH), 115.3 (CH), 114.2 (CH), 62.9 (CH₂), 60.5 (CH₂), 52.3 (CH₂).

m/z (ESI) [M + H]⁺, 370.2; [M + Na]⁺, 392.1.

HRMS (ESI-Q-TOF) m/z: [M + Na]⁺ Calcd for C₂₃H₁₉N₃O₂Na 392.1369; Found 392.1369.

¹H NMR (500 MHz, CDCl₃)
$^{13}$C NMR (125 MHz, CDCl$_3$)
Compound 6; 1-Benzyl-4,19-dihydro-1H-dinaphtho[2',1':7,8;1'',2'':9,10][1,6]dioexcino[3,4-d][1,2,3]triazole 6.

In a round bottom flask, compound 2 (50 mg, 0.15 mmol) was dissolved in DCM (1 mL). Benzyl azide (19.9 mg, 0.15 mmol) was added. After 4 days the volatiles were removed and the product was purified by flash chromatography on silica gel (eluent: Pentane/EtOAc = 4:1 to 1:3) to give compound 6 (64 mg, 0.14 mmol, 92%) as colourless solid. 

$\alpha_d^{25} - 152.7$ ($\epsilon$ 0.53, CHCl$_3$).

m.p. 114 °C dec.
IR (neat) 3055, 3034, 3009, 2944, 2892, 1619, 1590, 1471, 1455, 1431, 1355, 1326, 1267, 1218 cm\(^{-1}\).

\(\delta_h\) (500 MHz, CDCl\(_3\)) 7.77 - 7.94 (4H, m, ArH), 7.55 (1H, d, \(J = 9.0\) Hz, ArH), 7.28 - 7.43 (2H, m, ArH), 7.15 - 7.28 (5H, m, ArH), 7.11 (2H, t, \(J = 7.7\) Hz, ArH), 7.05 (1H, d, \(J = 8.5\) Hz, ArH), 6.94 (2H, d, \(J = 7.5\) Hz, ArH), 5.57 - 5.73 (2H, m, C\(HH\)), 5.38 (1H, d, \(J = 15.7\) Hz, C\(HH\)), 5.26 (1H, d, \(J = 14.0\) Hz, C\(HH\)), 5.18 (1H, d, \(J = 13.1\) Hz, C\(HH\)), 4.96 (1H, d, \(J = 13.1\) Hz, C\(HH\)).

\(\delta_c\) (125MHz, CDCl\(_3\)) 155.1 (C), 152.8 (C), 144.5 (C), 134.7 (C), 133.6 (C), 132.1 (C), 130.6 (C), 130.0 (CH), 129.6 (C), 129.5 (CH), 128.9 (CH), 128.3 (CH), 128.06 (CH), 128.05 (CH), 126.8 (CH), 126.7 (CH), 126.4 (CH), 126.1 (CH), 125.5 (CH), 124.8 (CH), 124.0 (CH), 123.4 (C), 121.4 (C), 118.8 (CH), 116.6 (CH), 63.3 (CH\(_2\)), 61.9 (CH\(_2\)), 52.1 (CH\(_2\)).

\(m/z\) (ESI) 470.2 ([M + H]\(^+\), 100%).

HRMS (ESI-Q-TOF) \(m/z\): [M + Na]\(^+\) Calcd for C\(_{31}\)H\(_{23}\)N\(_3\)O\(_2\)Na 492.1682; Found 492.1686.

\(^1\)H NMR (500 MHz, CDCl\(_3\)).

\(^{13}\)C NMR (125 MHz, CDCl\(_3\)).
**Compound 5b;** 1-Benzyl-8,11-dibromo-4,15-dihydro-1H-dibeno[7,8:9,10][1,6]dioxecino[3,4-d][1,2,3]triazole **5b.**
In a round bottom flask, compound 3 (59 mg, 0.15 mmol) was dissolved in acetonitrile (1.5 mL). Benzyl azide (19.9 mg, 0.15 mmol) was added and the mixture was stirred at room temperature for 5 days. The volatiles were removed and the product was purified by flash chromatography on silica gel (eluent: Petroleum ether/EtOAc = 3:2 to 2:3) to give compound 5b (73 mg, 0.14 mmol, 93%) as a colourless solid.

m.p. 101.2-102.6 ºC.

IR (neat) 3062, 3032, 1721, 1486, 1455, 1397, 1332, 1270, 1252, 1232, 1207 cm⁻¹.

δH (500MHz, CDCl₃) 7.33 - 7.43 (5H, m, ArH), 7.29 (1H, d, J = 2.4 Hz, ArH), 7.24 - 7.28 (1H, m, ArH), 7.14 - 7.21 (2H, m, ArH), 7.03 (1H, d, J = 8.7 Hz, ArH), 6.49 (1H, d, J = 8.7 Hz, ArH), 5.80 (1H, d, J = 15.7 Hz, CHH), 5.30 - 5.48 (3H, m, CHH), 5.20 (1H, d, J = 13.3 Hz, CHH), 5.00 (1H, d, J = 13.3 Hz, CHH).

δC (125 MHz, CDCl₃) 155.6 (C), 154.6 (C), 144.4 (C), 134.4 (C), 133.2 (CH), 133.0 (CH), 131.96 (CH), 131.94 (C), 131.92(CH), 131.4 (C), 130.2 (C), 129.3 (CH), 128.8 (CH), 127.1 (CH), 117.2 (CH), 116.1 (CH), 115.7 (C), 114.4 (C), 63.2 (CH₂), 60.9 (CH₂), 52.6 (CH₂).

m/z (ESI) [M + H]⁺, 525.9; [M + Na]⁺, 547.9.

HRMS (ESI-Q-TOF) m/z: [M + Na]⁺ Calcd for C₂₅H₁₇⁷⁹Br₂N₃O₂Na 547.9580; Found 547.9577.

¹H NMR (500 MHz, CDCl₃).
$^{13}$C NMR (125 MHz, CDCl$_3$).
Compound 5c; 1-Benzyl-6,8,11,13-tetrabromo-4,15-dihydro-1H-dibenzo[7,8:9,10][1,6]dioxecino[3,4-d][1,2,3]triazole 5c.

In a J. Young NMR tube compound 4 (50 mg, 0.09 mmol) was dissolved in CDCl₃ (0.9 mL). Benzyl azide (11.9 mg, 0.15 mmol) was added. After 14 days the volatiles were removed and the products was purified by flash chromatography on silica gel (eluent: Hexane/EtOAc = 4:1 to 7:3) to give compound 5c (57 mg, 0.08 mmol, 92%) as colourless solid.

m.p. 203.1-204.5 °C.

IR (neat) 3068, 3060, 3032, 2920, 2850, 1742, 1729, 1550, 1463, 1437, 1399, 1372, 1330, 1282, 1256, 1222 cm⁻¹.

δH (500 MHz, CDCl₃) 7.70 (1H, d, J = 2.1 Hz, ArH), 7.65 (1H, d, J = 2.1 Hz, ArH), 7.26 - 7.38 (3H, m, ArH), 7.15 (1H, d, J = 2.1 Hz, ArH), 7.07 (1H, d, J = 2.1 Hz,
ArH), 6.97 (2H, d, J = 7.0 Hz, ArH), 5.69 (1H, d, J = 15.7 Hz, CHH), 5.55 - 5.65 (2H, m, CHH), 5.12 (1H, d, J = 13.3 Hz, CHH), 4.91 (1H, d, J = 14.2 Hz, CHH), 4.78 (1H, d, J = 14.2 Hz, CHH).

δc (125 MHz, CDCl₃) 152.0 (C), 151.7 (C), 143.3 (C), 136.3 (CH), 135.8 (CH), 134.3 (C), 134.2 (C), 133.8 (CH), 133.2 (C), 132.4 (CH), 131.6 (C), 129.1 (CH), 128.6 (CH), 126.9 (CH), 118.5 (C), 117.5 (C), 117.2 (C), 116.6 (C), 65.8 (CH₂), 62.6 (CH₂), 52.6 (CH₂).

m/z (ESI) 707.8 ([M + Na]⁺, 100%).

HRMS (ESI-Q-TOF) m/z: [M + Na]⁺ Calcd for C₂₃H₁₅Br₄N₃O₂Na 703.7790; Found 703.7787.

¹H NMR (500 MHz, CDCl₃)

¹³C NMR (125 MHz, CDCl₃)
Compound 15: Methyl 1-benzyl-4,15-dihydro-1H-
dibenzo[7,8:9,10][1,6]dioxecino[3,4-d][1,2,3]triazole-8-carboxylate and methyl 1-
benzyl-4,15-dihydro-1H-dibenzo[7,8:9,10][1,6]dioxecino[3,4-d][1,2,3]triazole-11-
carboxylate 15.

In a round bottom flask compound 11 (44.1 mg, 0.15 mmol) was dissolved in \( d_3 \)-
acetonitrile (0.6 mL). Benzyl azide (19.9 mg, 0.15 mmol) was added. After 4 days the
volatiles were removed and the products was purified by flash chromatography on
silica gel (eluent: Hexane/EtOAc = 1:1) to give a ~ 1:1 ratio of isomers of compound
15 (59 mg, 0.14 mmol, 92%) as colourless solid.

m.p. 138.5 °C dec.
IR (neat) 3062, 3031, 2950, 1709, 1597, 1575, 1496, 1434, 1412, 1372, 1311, 1239,
1210, 1140 cm\(^{-1}\).

\( \delta \)H (500 MHz, CDCl\(_3\)) 7.97 (1H, dd, \( J = 8.5, 2.1 \) Hz, ArH), 7.89 (1H, d, \( J = 2.1 \) Hz,
ArH), 7.93 (1H, d, \( J = 2.1 \) Hz, ArH), 7.77 (1H, dd, \( J = 8.5, 2.0 \) Hz, ArH), 7.25 - 7.42
(8H, m, ArH), 7.09 - 7.24 (9H, m, ArH), 7.06 (1H, t, \( J = 7.5 \) Hz, ArH), 6.73 (1H, d, \( J = 8.1 \) Hz, ArH), 6.47 (1H, dd, \( J = 8.5, 1.7 \) Hz, ArH), 5.68 - 5.83 (2H, m, CH\(_2\)), 5.56
(1H, d, \( J = 13.7 \) Hz, CH\(_2\)), 5.30 - 5.44 (5H, m, CH\(_2\)), 5.12 - 5.26 (3H, m, CH\(_2\)), 5.03
(1H, d, \( J = 13.1 \) Hz, CH\(_2\)), 3.85 (3H, s, CH\(_3\)), 3.86 (3H, s, CH\(_3\)).

\( \delta \)C (CDCl\(_3\), 125 MHz) 166.6 (C), 166.4 (C), 159.8 (C), 159.2 (C), 156.8 (C), 156.0
(C), 144.7 (C), 144.4 (C), 134.6 (C), 134.4 (C), 132.4 (CH), 132.3 (C), 132.2 (CH),
132.0 (C), 130.8 (CH), 130.7 (CH), 130.6 (CH), 130.5 (C), 130.3 (CH), 129.8 (C),
129.5 (C), 129.4 (CH), 129.3 (CH), 129.2 (CH), 128.8 (CH), 128.7 (CH), 128.6 (C),
127.2 (CH), 127.1 (CH), 124.9 (C), 123.9 (C), 123.5 (CH), 122.4 (CH), 115.9 (CH),
114.8 (CH), 114.1 (CH), 113.5 (CH), 63.7 (CH\(_2\)), 62.9 (CH\(_2\)), 60.9 (CH\(_2\)), 60.2 (CH\(_2\)),
52.6 (CH\(_2\)), 52.4 (CH\(_2\)), 52.0 (CH\(_3\)), 51.9 (CH\(_3\)).

m/z (ESI) [M + H]\(^+\), 428.1; [M + Na]\(^+\), 450.1.
HRMS (ESI-Q-TOF) m/z: [M + Na]^+ Calcd for C_{25}H_{21}N_{3}O_{4}Na 450.1424; Found 450.1427.

^{1}H NMR (500 MHz, CDCl₃)

^{13}C NMR (125 MHz, CDCl₃)
1-Benzyl-6,8,11,13-tetrabromo-4,15-dihydro-1H-dibenzo[7,8;9,10][1,6]dioxecino[3,4-d][1,2,3]triazole 7.

In a round bottom flask 8,13-dioxatricyclo[12.4.0.027,7]octadeca-1(14),2,4,6,15,17-hexaen-10-yne (30 mg, 0.127 mmol) 1 was dissolved in DCM (0.3 mL). o-Tolyl azide (~0.5 M in tert-butyl methyl ether, 0.5 mL, 0.25 mmol) was added. After 10 days the volatiles were removed and the products was purified by flash chromatography on silica gel (eluent: Hexane/EtOAc = 4:1 to 3:2) to give 1-(o-tolyl)-4,15-dihydro-1H-dibenzo[7,8;9,10][1,6]dioxecino[3,4-d][1,2,3]triazole 7 (45 mg, 0.122 mmol, 96%) as a colourless solid.

m.p. 76.2 °C dec.

IR (neat) 3059, 3026, 2928, 2891, 1594, 1573, 1499, 1479, 1440, 1382, 1372, 1336, 1260, 1242, 1212 cm⁻¹.

δH (500 MHz, CDCl₃) 7.45 - 7.51 (1H, m, ArH), 7.23 - 7.44 (6H, m, ArH), 7.18 - 7.23 (2H, m, ArH), 7.12 (1H, td, J = 7.4, 0.9 Hz, ArH), 7.04 (1H, td, J = 7.4, 0.8 Hz, ArH), 6.81 (1H, d, J = 7.8 Hz, ArH), 5.61 (1H, d, J = 13.4 Hz, CH), 5.45 (1H, d, J = 13.4 Hz, CH), 5.07 (2H, d, J = 6.7 Hz, CH₂), 1.97 (3H, br. s., CH₃).

δC (125 MHz, CDCl₃) 156.7 (C), 155.6 (C), 143.8 (C), 135.8 (C), 134.4 (C), 133.6 (C), 131.5 (CH), 130.9 (C), 130.8 (CH), 130.7 (CH), 130.6 (CH), 129.6 (C), 128.8 (CH), 128.7 (CH), 127.2 (CH), 128.6 (CH), 123.5 (CH), 122.1 (CH), 115.5 (CH), 114.2 (CH), 62.9 (CH₂), 61.0 (CH₂), 17.1 (CH₃).

m/z (ESI) [M + Na]⁺, 392.1.

HRMS (ESI-Q-TOF) m/z: [M + Na]⁺ Calcd for C₂₃H₁₉N₃O₂Na 392.1369; Found 392.1368.

ⁱH NMR (500 MHz, CDCl₃)
$^{13}$C NMR (126 MHz, CDCl$_3$)
1-(p-Tolyl)-4,15-dihydro-1H-dibenzo[7,8:9,10][1,6]dioxecino[3,4-d][1,2,3]triazole 8.

In a J. Young NMR tube, 8,13-dioxatricyclo[12.4.0.0²,⁷]octadeca-1(14),2,4,6,15,17-hexaen-10-yne 1 (23.6 mg, 0.10 mmol) was dissolved in CDCl₃ (1 mL). Benzyl azide (13.3 mg, 0.10 mmol) was added and the mixture was heated to 60 °C. After 24 hours the volatiles were removed and the products was purified by flash chromatography on silica gel (eluent: Hexane/Et₂O = 4:1 to 8:2) to give 1-(p-tolyl)-4,15-dihydro-1H-dibenzo[7,8:9,10][1,6]dioxecino[3,4-d][1,2,3]triazole 8 (34 mg, 0.92 mmol, 92%) as a colourless solid. m.p. 194.5-195.6 °C. IR (neat) 3054, 3034, 2954, 2880, 1606, 1593, 1572, 1517, 1499, 1480, 1455, 1442, 1390, 1365, 1340, 1304, 1285, 1264, 1240, 1212 cm⁻¹.
$\delta_H$ (500 MHz, CDCl$_3$) 7.36 - 7.44 (2H, m, ArH), 7.26 - 7.36 (5H, m, ArH), 7.18 - 7.23 (2H, m, ArH), 7.15 (1H, t, $J = 7.5$ Hz, ArH), 7.03 (1H, d, $J = 7.3$ Hz, ArH), 6.90 - 6.96 (1H, m, ArH), 5.67 (1H, d, $J = 13.9$ Hz, CHH), 5.37 (1H, d, $J = 13.9$ Hz, CHH), 5.30 (1H, d, $J = 12.4$ Hz, CHH), 5.12 (1H, d, $J = 12.5$ Hz, CHH), 2.45 (3H, s, CH$_3$).

$\delta_C$ (125 MHz, CDCl$_3$) 157.3 (C), 155.0 (C), 144.7 (C), 140.1 (C), 133.0 (C), 132.3 (C), 131.1 (C), 130.7 (CH), 130.2 (CH), 129.4 (C), 128.8 (CH), 124.7 (CH), 123.6 (CH), 122.0 (CH), 116.7 (CH), 113.5 (CH), 62.1 (CH$_2$), 61.9 (CH$_2$), 21.2 (CH$_3$).

m/z (ESI) [M + H]$^+$: 369.9; [M + Na]$^+$, 392.0.

HRMS (ESI-Q-TOF) m/z: [M + Na]$^+$ Calcd for C$_{23}$H$_{19}$N$_3$O$_2$Na 392.1369; Found 392.1364.

$^1$H NMR (500 MHz, CDCl$_3$)

$^{13}$C NMR (126 MHz, CDCl$_3$)
Synthesis of compound 10.

This is a known compound (a) Allen, D. W.; Braunton, P. N.; Millar, I. T.; Tebby, J. C. J. Chem. Soc. C 1971, 3454-3468. B) Braunton, P. N.; Millar, I. T.; Tebby, J. C.; J. Chem. Soc. Perkin II 1972, 138-412. c) Simpson, J. E.; Daub, G. H.; Hayes, F. N. J. Org. Chem. 1973, 38, 1771. d) Surry, D. S.; Fox, D. J.; Macdonald, S. J. F.; Spring, D. R. Chem. Commun. 2005, 2589-2590). The procedure used below was adapted from: Benniston, A. C.; Harriman, A.; Li, P.; Patel, P. V.; Sams, C. A. J. Org. Chem. 2006, 71, 3481-3493. 1,4-Dibromobutane (484 mg, 2.20 mmol) was added dropwise to a stirred solution of 2,2'-biphenol (376 mg, 2.0 mmol) and K$_2$CO$_3$ (560 mg, 4.00 mmol) in DMF (10 mL) at room temperature. The mixture was stirred overnight. At the end of this time, water (50 mL) was added followed by EtOAc (50 mL). The water phase was extracted with EtOAc (2 x 40 mL) and the combined organic extracts were then washed with water (3 x 40 mL). The solvent was removed using a rotary evaporator to yield a crude product which was purified by chromatography on silica gel (hexane/EtOAC, gradient from 100:1-75:25-50:50 in increments). This yielded product 10 as a white crystalline solid (398 mg, 1.64 mmol, 82%).

TLC: Silica gel, 4:1 hexane:EtOAc, Rf 0.85, uv nd KMnO$_4$ to visualise.

Mp 109-110 °C.

IR (neat) 1434 1261, 1228, 939, 756 cm$^{-1}$.

$\delta$H (400 MHz, CDCl$_3$) 7.45-7.40 (4H, m, ArH), 7.10-7.00 (4H, m, ArH), 4.55-4.50 (4H, m, OCH$_2$), 4.25-4.20 (4H, m, OCH$_2$), 2.00-1.85 (4H, m, CH2CH2).

$\delta$C (100 MHz, CDCl$_3$) 157.06 (C), 131.30 (CH), 129.61 (C), 128.53 (CH), 121.83 (CH), 115.79 (CH), 70.96 (CH$_2$), 27.15 (CH$_2$).

m/z (CI) 241.1 (M+ + H, 65%), 263 (M+ + Na, 95%).

HRMS (found (EI+): M+ + Na, 263.1038. C$_{16}$H$_{16}$O$_2$Na requires M+Na, 263.1043, 1.5 ppm error).

$^1$H NMR (400 MHz, CDCl$_3$).
$^{13}$C NMR (100 MHz, CDCl$_3$)
Synthesis of compound 16; 8,13-dioxatricyclo[12.4.0.0²,⁷]octadeca-
1(18),2(7),3,5,14,16-hexaen-10-yn-4-ylmethanol 16.

In a Schlenk tube under a nitrogen atmosphere, compound 11 (50 mg, 0.17 mmol) was dissolved in THF (1 mL). The solution was cooled to -78 °C and DiBAL-H (0.17 mmol, 2 M solution in hexane) was added dropwise. The mixture was degassed, stirred at -78 °C for 1 hour. The mixture was warmed to room temperature. After 2 hours, water (5 mL) and HCl (2-3 mL) were added and the product was extracted with ethyl acetate (3 x 15 mL). The organic extracts were dried over magnesium sulphate. The solvent was removed under reduced pressure to give the product 16 (31 mg, 0.117 mmol, 69%) as a brown solid.

TLC: Silica gel, 1:4 Hexane: EtOAc, Rf 0.18.

IR (neat) 3391, 2913, 2863, 1449, 1189, 1105, 966 cm⁻¹.

δH (CDCl₃, 500 MHz) 7.40-7.35 (2H, m, ArH), 7.20-7.10 (5H, m, ArH), 4.66 (2H, s, HOCH₂), 4.54-4.47 (2H, m, OCH₂), 4.31 (2H, brd, J = 14.0, OCH₂), 2.05 (1H, d, J = 12.1, OH)

δc (CDCl₃, 125 MHz) 154.4 (C), 153.9 (C), 136.8 (C), 135.9 (C), 135.8 (C), 132.0 (CH), 130.7 (CH), 129.2 (CH), 127.9 (CH), 124.3 (CH), 122.7 (CH), 122.7 (CH), 86.7 (C triple), 86.7 (C triple), 64.8 (OCH₂), 63.6 (HOCH₂). Note; two CH₂ groups are overlapped.

m/z (ESI) 289 (M⁺ + Na, 100).

HRMS (ESI-Q-TOF) m/z: [M + Na]⁺ Calcd for C₁7H₁₄O₃Na 289.0835; Found 289.0837.

¹H NMR (CDCl₃, 500 MHz)
$^{13}$C NMR (CDCl$_3$, 125 MHz).
Reactions of activated ester 14 with peptide and proteins, followed by addition of benzyl azide.

FT-ICR MS experiments were run to test if compound 14 could react with and conjugate to the peptides; substance P, Lys 8 vasopressin and Lys 3 bombesin. This was achieved by combining a 1 mM solution of each peptide with 1, 2 and 3 equivalents respectively of 14. The final concentrations of the peptide and the binding agent are summarised in Table 1. However due to competing hydrolysis of the activated ester, full functionalisation was not anticipated.

Table 1.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Peptide</th>
<th>Concentration of peptide</th>
<th>Concentration of 14</th>
<th>Ratio of peptide to 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Substance P</td>
<td>0.67 mM</td>
<td>0.67 mM</td>
<td>1:1</td>
</tr>
<tr>
<td>2</td>
<td>Substance P</td>
<td>0.50 mM</td>
<td>1.00 mM</td>
<td>1:2</td>
</tr>
<tr>
<td>3</td>
<td>Substance P</td>
<td>0.40 mM</td>
<td>1.20 mM</td>
<td>1:3</td>
</tr>
<tr>
<td>4</td>
<td>vasopressin</td>
<td>0.67 mM</td>
<td>0.67 mM</td>
<td>1:1</td>
</tr>
<tr>
<td>5</td>
<td>vasopressin</td>
<td>0.50 mM</td>
<td>1.00 mM</td>
<td>1:2</td>
</tr>
<tr>
<td>6</td>
<td>vasopressin</td>
<td>0.40 mM</td>
<td>1.20 mM</td>
<td>1:3</td>
</tr>
<tr>
<td>7</td>
<td>bombesin</td>
<td>0.67 mM</td>
<td>0.67 mM</td>
<td>1:1</td>
</tr>
<tr>
<td>8</td>
<td>bombesin</td>
<td>0.50 mM</td>
<td>1.00 mM</td>
<td>1:2</td>
</tr>
<tr>
<td>9</td>
<td>bombesin</td>
<td>0.40 mM</td>
<td>1.20 mM</td>
<td>1:3</td>
</tr>
</tbody>
</table>

Reaction of activated ester 14 with peptides:
In a round bottom flask, compound 14 (7.5 mg, 0.019 mmol) was dissolved in MeOH/H$_2$O (50%-50%, 10 mL) to create a 2 mM stock solution. Solutions of three peptides (1 mM solutions of Substance P, Lys 8 Vasopressin, Lys 3 Bombesin) were prepared. To portions of 100 μL of each peptide solution was added respectively 50 μL, 100 μL and 150 μL of the solution of compound 7 to create the following solutions respectively; i) 0.67 mM peptide + 0.67 mM ester (1:1), ii) 0.50 mM peptide + 1 mM ester (1:2) and iii) 0.40 mM peptide + 1.2 mM ester (1:3) respectively. The solutions were allowed to stand at room temperature for 1 day and were then analysed by FT-ICR MS.

**Reaction of peptide from reaction above with benzyl azide.**

![Reaction Diagram]

In a round bottom flask, PhCH$_2$N$_3$ (250 μL, 2 mmol) was dissolved in MeOH/H$_2$O (50%-50%, 10 mL) to create a 0.2 M stock solution. Volumes of 0.25 mL, 0.50 mL and 0.75 mL of this solution were added to the previously prepared 1:1, 1:2 and 1:3 solutions of each peptide respectively. The solutions were allowed to stand at room temperature for 7 days and were then analysed by FT-ICR MS.

**Reaction between compound 14 and myoglobin.**

![Reaction Diagram]

In a round bottom flask, compound 14 (7.5 mg, 0.019 mmol) was dissolved in MeOH/H$_2$O (50%-50%, 10 mL) to create a 2 mM stock solution. Myoglobin (8.45 mg) was dissolved in 0.5 mL ultra-pure water. The stock myoglobin solution was left in the vortexer for 5 min and afterwards put in the centrifuge for stirring at 14,000 rpm for 5 min. To portions of 100 μL of myoglobin solution was added respectively
50 μL, 100 μL and 150 μL of the solution of compound 14 to create the following solutions respectively; i) 0.67 mM protein + 0.67 mM ester (1:1), ii) 0.50 mM protein + 1 mM ester (1:2) and iii) 0.40 mM protein + 1.2 mM ester (1:3) respectively. The solutions were allowed to stand at room temperature for 1 day and were then analysed by FT-ICR MS.

**Reaction of functionalised myoglobin with benzyl azide.**

In a round bottom flask, PhCH$_2$N$_3$ (250 μL, 2 mmol) was dissolved in MeOH/H$_2$O (50%-50%, 10 mL) to create a 0.2 M stock solution. Volumes of 0.25 mL, 0.50 mL and 0.75 mL of this solution were added to the 1:1, 1:2 and 1:3 solutions of each peptide respectively. The solutions were allowed to stand at room temperature for 7 day and were then analysed by FT-ICR MS.

**Mass spectra of products from peptide and protein conjugation studies.**

**Substance P.**

The mass spectra (MS) of substance P with and without the addition of compound 14 are presented in Figures 1 and 2 respectively. The data was obtained with the 1:2 ratio mixture (Table 1 entry 2) is illustrated. The comparison of the shapes illustrates the form of a new peak at the latter MS spectrum. This peak at 805.40393 is corresponds to the peptide bound with the conjugation agent 14 [Substance P + allyne + 2H]$^{2+}$. Furthermore, this was verified by creating a mathematical simulation and comparing it with the experimental isotope pattern.
Figure 1. The mass spectrum of Substance P (Sub P) without adding compound 14.

Figure 2: The mass spectrum of Substance P (Sub P) with compound 14 (‘alkyne’).
On the top right, the comparison of the experimental result with the simulation is shown.
After verifying that the conjugation agent 14 was attached to the peptide, the position which is bonded the molecule was examined, to determine, the amino acid which is attached to the agent. Therefore, a MS/MS experiment was run. The MS/MS spectrum revealed a b₃, b₆, b₇, b₈, b₁₀ and an y₁₀ ion of substance P attached to 14. Based on this outcome, a fragmentation map was created (Figure 3). As indicated in Figure 3, lysine and proline are the potential bindings sites of the peptide. This goes to pinpoint that the primary assumption was valid and the molecule actually binds to the lysine.

<table>
<thead>
<tr>
<th>Assignment</th>
<th>Theoretical m/z</th>
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**Figure 3:** The fragmentation map of Substance P with compound 14 based on the MS/MS spectrum (above), and the MS/MS mass error listing.

Excess azide was added to the sample (ca. 0.1 M solution), and it was left for one week at room temperature. The mass spectrum (Figure 4) with the addition of the azide showed the absence of the peak at 805.40393 and the formation of a peak at 871.93351. This peak indicates to \([\text{Substance P + 14} + \text{C}_7\text{H}_7\text{N}_3 + 2\text{H}]^2+\) and it is confirmed by the comparison of the mathematical simulation with the experimental isotope pattern. By the absence of the peak 805.39641 that all of the substance P-14 adduct had reacted with the azide.
Figure 4: The mass spectrum of Substance P (Sub P) with compound 14 (‘alkyne’) and azide (C₇H₇N₃). The comparison of the experimental result with the simulation is shown.

An illustration with the three spectra is shown in Figure 5. A peak at 805.39641 appeared after the addition of compound 14. In addition, a new peak at 871.93351 was formed as a result of the addition of the azide.
Figure 5: The three mass spectra of Substance P (Sub P). The first one is without compound 14, the second with the addition of compound 14 (‘alkyne’) and the last with the addition of both compound 14 and the azide (C₇H₇N₃).

Lys 8 Vasopressin.

The mass spectra of Lys 8 Vasopressin were obtained with and without the addition of compound 14 (Figures 6 and 7 respectively). The result obtained with the 1:1 ratio mixture (Table 1, entry 4) is illustrated. In Figure 7, a peak at 670.73929 has appeared which indicates the binding of 14 with the peptide [K8Vaso + ‘alkyne’ + H + Na]²⁺. The experimental isotope pattern matched the mathematical simulation.
Figure 6: The mass spectrum of Lys 8 Vasopressin (K8Vaso) without adding compound 14.

Figure 7: The mass spectrum of Lys 8 Vasopressin (K8Vaso) with compound 14 (‘alkyne’). On the top right, the comparison of the experimental result with the simulation is shown.
As before, it was assumed that 14 is bound to the lysine. An MS/MS experiment was run to investigate this assumption. The MS/MS revealed a y₃ ion of the protein attached to 14. A fragmentation map was created (Figure 8) based on this outcome. As is illustrated, there are only one fragment with the conjugation agent attached. This is due to the cysteine bond; it is difficult to break a cysteine bond by collisionally activated dissociation (CAD). The possible binding sites are the lysine, proline and glutamine. It can be assumed based on proline's and glutamine's structure that the binding couldn't occur to these residues. Therefore, based on the above there are one possible binding site, i.e. the lysine residue.

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<th>Assignment</th>
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<th>Error/ ppm</th>
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<td>528.72316</td>
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</table>
Figure 8: The fragmentation map of the Lys 8 Vasopressin (K8Vaso) with compound 14 based on the MS/MS spectra.

Benzyl azide was added in excess (0.2 M) to the sample and the solution was left for one week at room temperature. The MS spectrum (Figure 9) displayed the absence of the peak at 670.73929 and the formation of a peak at 737.27534. This peak confirms the attachment of the peptide to the azide \([\text{K8Vaso + C}_7\text{H}_7\text{N}_3 + \text{H + Na}}]^2+\). This is verified by the comparison of the mathematical simulation with the experimental isotope pattern. The absence of the 670.73929 peak indicates that all of the functionalised peptide reacted with the azide.
Figure 9: The mass spectrum of the Lys 8 Vasopressin (K8Vaso) with compound 14 (‘alkyne’) and the benzyl azide (C7H7N3). The comparison of the experimental result with the simulation is shown.

The three spectra illustrated in Figure 10. These three spectra are without any addition, with addition of compound 14 and the last with adding both compound 14 and the azide.
Figure 10: The three mass spectra of Lys 8 Vasopressin (K8Vaso); without compound 14, with the addition of compound 14 ('alkyne') and with the addition of both compound 14 and the azide (C7H7N3).

Lys 3 Bombesin.

The mass spectra of Lys 3 Bombesin with and without the addition of compound 14 are shown in Figures 11 and 12 respectively. The result was obtained with the 1:3 ratio mixture (Table 1, entry 9) is shown. The comparison showed the appearance of a new peak at the latter MS spectrum. This peak at 938.41993 indicates the peptide bound with the conjugation agent [K3BBs + 14 + H + Na]^{2+}. Furthermore, this was verified by creating a mathematical simulation and comparing it with the experimental isotope pattern.

Figure 11: The MS spectra of Lys 3 Bombesin (K3BBS) without adding compound 14.
After verifying that the conjugation agent was conjugated to the peptide, it was necessary to determine which amino acid is bound to the molecule. The MS/MS spectrum revealed a $b_5$, $b_6$, $b_7$, $b_8$, $b_{10}$ and a $y_{10}$ ion of the protein attached to the molecule. Based on this outcome, a fragmentation map was created (Figure 13). It can be concluded that the 14 binds to the lysine residue.
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</table>

**Figure 13:** The fragmentation map of the Lys 3 Bombesin (K3BBS) with compound 14 (‘alkyne’) based on the MS/MS spectra.

The addition of excess azide was undertaken, following the procedure previously described. The reaction mixture was left for one week at room temperature. MS spectrum (Figure 14) with the addition of the azide was obtained. The absence of the peak at 938.41993 and the formation of a novel peak at 1004.96868 was observed. This peak indicates the attachment of the peptide to compound 14 and to the azide [K3BBs + 14 + C₇H₁₁N₃ + H + Na]²⁺. This is supported by the comparison of the mathematical simulation with the experimental isotope pattern.
Figure 14: The mass spectrum of the Lys 3 Bombesin (K3BBS) with compound 14 (‘alkyne’) and the azide (C7H7N3). The comparison of the experimental result with the simulation is shown.

All these changes are summarised in Figure 15.
Myoglobin.

After verifying that compound 14 attached to several peptides, a protein (myoglobin) was tested. The mass spectra of myoglobin with and without the addition of compound 14 are shown in Figures 16 and 17 respectively. The appearance of new peaks at the latter mass spectrum were observed. These peaks correspond to the peptide bound with the conjugation agent in various charge states. This was confirmed using a mathematical simulation and comparing it with the experimental isotope pattern (Figure 18).

Figure 15: The mass spectra of the Lys 3 Bombesin (K3BBS) with compound 14 (‘alkyne’) and the azide (C7H7N3).
Figure 16: The mass spectrum of Myoglobin without adding compound 14.

Figure 17: The mass spectrum of Myoglobin following reaction with compound 14 (‘alkyne’).
Figure 18: The specified region MS spectra of Myoglobin with compound 14 (identified here as ‘LG009’). On the centre, the comparison of the experimental result with the simulation is shown.

Benzyl azide was added in excess and the sample was left for one week at room temperature. The mass spectrum (Figure 19) with the addition of the azide was obtained. The peaks which indicate the binding of 14 with the myoglobin were replaced by novel peaks which indicate the attachment of the myoglobin to compound 14 and to the azide which is confirmed by the comparison of the mathematical simulation with the experimental isotope pattern (Figure 20).
Figure 19: The mass spectrum of Myoglobin with compound 14 and the azide \((\text{C}_7\text{H}_7\text{N}_3)\).

All the modifications are summarised in Figure 20, for one charge state of the myoglobin derivatives.

Figure 20: The specified region MS spectra of Myoglobin (MG). The first one is without compound 14, the second with the addition of compound 14 (‘alkyne’) and the last with the addition of both compound 14 and the azide \((\text{C}_7\text{H}_7\text{N}_3)\).
X-Ray Crystallographic data:

Table 2. Summary of X-ray crystallographic data.

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<td>8</td>
<td>4</td>
</tr>
<tr>
<td>F(000)</td>
<td>3168.0</td>
<td>1536.0</td>
<td>1040.0</td>
<td>1168.0</td>
<td>1232.0</td>
<td>1552.0</td>
<td>512.0</td>
</tr>
<tr>
<td>Goodness-of-fit on F$^2$</td>
<td>1.041</td>
<td>1.083</td>
<td>1.173</td>
<td>1.054</td>
<td>1.131</td>
<td>1.012</td>
<td>1.109</td>
</tr>
<tr>
<td>Final R indexes [I&gt;2σ(I)]</td>
<td>R$_1$ = 0.0315, wR$_2$ = 0.0829</td>
<td>R$_1$ = 0.0304, wR$_2$ = 0.0799</td>
<td>R$_1$ = 0.0251, wR$_2$ = 0.0636</td>
<td>R$_1$ = 0.0378, wR$_2$ = 0.1028</td>
<td>R$_1$ = 0.0672, wR$_2$ = 0.1835</td>
<td>R$_1$ = 0.0369, wR$_2$ = 0.0911</td>
<td>R$_1$ = 0.0370, wR$_2$ = 0.1057</td>
</tr>
<tr>
<td>Final R indexes [all data]</td>
<td>R$_1$ = 0.0318, wR$_2$ = 0.0830</td>
<td>R$_1$ = 0.0306, wR$_2$ = 0.0801</td>
<td>R$_1$ = 0.0257, wR$_2$ = 0.0640</td>
<td>R$_1$ = 0.0419, wR$_2$ = 0.1072</td>
<td>R$_1$ = 0.0699, wR$_2$ = 0.1850</td>
<td>R$_1$ = 0.0407, wR$_2$ = 0.0939</td>
<td>R$_1$ = 0.0380, wR$_2$ = 0.1068</td>
</tr>
<tr>
<td>Flack parameter</td>
<td>0.07(5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Compound 2 (BINOL-derived, local code adg1) CCDC1515151:

The three crystallographically independent but chemically equivalent macrocycles in the asymmetric unit of adg1.

One of the crystallographically independent macrocycles in the asymmetric unit of adg1 with atom labeling and thermal ellipsoids drawn at 50% probability level.
looking down the naphthyl-naphthyl bond of the macrocycle above showing the bend in the alkyne bridge.

**Crystal structure determination of [adg1]**

The asymmetric unit contains three crystallographically independent but chemically identical molecules. There are 18 in the unit cell.

**Angle between mean planes through the naphthalene groups on each macrocycle**

Naphthalene ring C107 C108 C109 C110 C111 C112 C113 C114 C115 C116 to naphthalene ring C117 C118 C119 C120 C121 C122 C123 C124 C125 C126 is 70.633 (0.043) degrees.

Naphthalene ring C207 C208 C209 C210 C211 C212 C213 C214 C215 C216 to naphthalene ring C217 C218 C219 C220 C221 C222 C223 C224 C225 C226 is 78.518 (0.041) degrees.

Naphthalene ring C307 C308 C309 C310 C311 C312 C313 C314 C315 C316 to naphthalene ring C317 C318 C319 C320 C321 C322 C323 C324 C325 C326 is 74.258 (0.043) degrees.

The bridging butyne chain is characterized by a torsion angle between the CH$_2$s of each butyne chain and the angle between each CH$_2$ and the alkyne bond.

**Torsion angles**

Torsion angle C102 C103 C104 C105 -10.85 (2.44) degrees (the minus charge is just by definition, torsion angle C105 C104 C103 C102 is +10.85)
Torsion angle C202 C203 C204 C205 -21.37 (2.14) degrees
Torsion angle C302 C303 C304 C305 -20.50 (2.12) degrees

Each CH2 alkyne angle
Angle C102 C103 C104 166.32 (0.29) degrees
Angle C103 C104 C105 163.82 (0.30) degrees
Angle C202 C203 C204 166.88 (0.29) degrees
Angle C203 C204 C205 162.98 (0.29) degrees
Angle C302 C303 C304 163.98 (0.28) degrees
Angle C303 C304 C305 165.91 (0.28) degrees

Hooft y: 0.11(6) Olex2
Flack x: 0.07(5) Shelxl 2014

The Flack parameter is a little high 0.07(5) (should preferably be close to zero with a low esd) but there are no heavy atoms and it is from a known chiral starting material.
All crystals examined were twinned to some extent. The best crystal was measured and the data is presented here.
The crystal is a merohedral twin and the twin component ratio refined to BASF 0.6670(14).

Experimental
Single crystals of C_{24}H_{16}O_{2} [adg1] were grown from slow evaporation of DCM. A suitable crystal was selected and mounted on a glass fibre with Fromblin oil and placed on an Oxford Diffraction diffractometer with a Ruby CCD area detector. The crystal was kept at 150(2) K during data collection. Using Olex2 [1], the structure was solved with the XS [2] structure solution program using Direct Methods and refined with the ShelXL [3] refinement package using Least Squares minimisation.

Crystal Data for C_{24}H_{16}O_{2} (M =336.37 g/mol): hexagonal, space group P6_{1} (no. 169),
a = 13.54378(8) Å, c = 48.3660(3) Å, V = 7683.33(10) Å³, Z = 18, T = 150(2) K,
Crystal data and structure refinement for adg1.

Identification code  
adg1

Empirical formula  
C_{24}H_{16}O_{2}

Formula weight  
336.37

Temperature/K  
150(2)

Crystal system  
Hexagonal

Space group  
P6_{1}

a/Å  
13.54378(8)

b/Å  
13.54378(8)

c/Å  
48.3660(3)

α/°  
90

β/°  
90

γ/°  
120

Volume/Å³  
7683.33(10)

Z  
18

ρ_{calc}/g/cm³  
1.309

μ/mm⁻¹  
0.650

F(000)  
3168.0

Crystal size/mm³  
0.5 × 0.2 × 0.14 colourless block

Radiation  
CuKα (λ = 1.54178)

2Θ range for data collection/°  
7.31 to 155.924

Index ranges  
-17 ≤ h ≤ 14, -17 ≤ k ≤ 15, -59 ≤ l ≤ 60

Reflections collected  
42875

Independent reflections  
10592 [R_{int} = 0.0357, R_{sigma} = 0.0233]

Data/restraints/parameters  
10592/1/704

Goodness-of-fit on F²  
1.041

Final R indexes [I > 2σ (I)]  
R₁ = 0.0315, wR₂ = 0.0829

Final R indexes [all data]  
R₁ = 0.0318, wR₂ = 0.0830

Largest diff. peak/hole / e Å⁻³  
0.19/-0.21
Flack parameter 0.07(5)

**Compound 3 (DiBr, local code adg4) CCDC 1515152:**

The solid state structure of the macrocycle in adg4 with atom labeling and thermal ellipsoids drawn at 50% probability level.

**Crystal structure determination of [adg4]**
The asymmetric unit contains the macrocycle, there are eight in the unit cell.
The angle between mean planes through the two aromatic rings of the biphenyl are C7 C8 C9 C10 C11 C12 to C13 C14 C15 C16 C17 C18 is 67.140 (0.065) degrees

There is a close contact (halogen bonding anyone) that shows up as a B alert in the cif checker; O1 - Br10$_1$ 3.0467 (0.0015) Angstroms.

Symmetry operator used to generate symmetry equivalent atoms in above contact were $1 1.5-X, 1.5-Y, 1.5-Z$

**Experimental**
Single crystals of C\textsubscript{16}H\textsubscript{10}Br\textsubscript{2}O\textsubscript{2} [adg4] were grown from slow evaporation of DCM. A suitable crystal was selected and mounted on a glass fibre with Fromblin oil and placed on an Xcalibur Gemini diffractometer with a Ruby CCD area detector. The crystal was kept at 150(2) K during data collection. Using Olex2 [1], the structure was solved with the ShelXS [2] structure solution program using Direct Methods and refined with the XL [3] refinement package using Least Squares minimisation.


**Crystal Data** for C\textsubscript{16}H\textsubscript{10}Br\textsubscript{2}O\textsubscript{2} (\(M = 394.06 \text{ g/mol}\)): monoclinic, space group I2/a (no. 15), \(a = 14.16761(6) \text{ Å}, b = 7.46338(4) \text{ Å}, c = 28.10737(12) \text{ Å}, \beta = 103.6158(4)^\circ, V = 2888.50(2) \text{ Å}^3\). \(Z = 8\), \(T = 150(2) \text{ K}\), \(\mu(\text{CuK}\alpha) = 7.105 \text{ mm}^{-1}\), \(D_{\text{calc}} = 1.812 \text{ g/cm}^3\), 27581 reflections measured \((6.472^\circ \leq 2\Theta \leq 156.314^\circ)\), 3075 unique \((R_{\text{int}} = 0.0448, R_{\text{sigma}} = 0.0177)\) which were used in all calculations. The final \(R_1\) was 0.0304 \((I > 2\sigma(I))\) and \(wR_2\) was 0.0801 \(\text{(all data)}\).

**Crystal data and structure refinement for adg4.**

<table>
<thead>
<tr>
<th>Identification code</th>
<th>adg4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C\textsubscript{16}H\textsubscript{10}Br\textsubscript{2}O\textsubscript{2}</td>
</tr>
<tr>
<td>Formula weight</td>
<td>394.06</td>
</tr>
<tr>
<td>Temperature/K</td>
<td>150(2)</td>
</tr>
<tr>
<td>Crystal system</td>
<td>monoclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>I2/a</td>
</tr>
<tr>
<td>(a/\text{Å})</td>
<td>14.16761(6)</td>
</tr>
<tr>
<td>(b/\text{Å})</td>
<td>7.46338(4)</td>
</tr>
<tr>
<td>(c/\text{Å})</td>
<td>28.10737(12)</td>
</tr>
<tr>
<td>(\alpha/^{\circ})</td>
<td>90</td>
</tr>
<tr>
<td>(\beta/^{\circ})</td>
<td>103.6158(4)</td>
</tr>
<tr>
<td>(\gamma/^{\circ})</td>
<td>90</td>
</tr>
<tr>
<td>Volume/\text{Å}^3</td>
<td>2888.50(2)</td>
</tr>
</tbody>
</table>
Z
ρ_{calc}: g/cm\(^3\)
μ:mm\(^{-1}\)
F(000)
Crystal size/mm\(^3\)
Radiation
2θ range for data collection/°
Index ranges
Reflections collected
Independent reflections
Data/restraints/parameters
Goodness-of-fit on F\(^2\)
Final R indexes [I>=2σ (I)]
Final R indexes [all data]
Largest diff. peak/hole / e Å\(^{-3}\)

8
1.812
7.105
1536.0
0.3 × 0.2 × 0.1 colourless block
CuKα (λ = 1.54184)
6.472 to 156.314
-17 ≤ h ≤ 17, -9 ≤ k ≤ 7, -35 ≤ l ≤ 35
27581
3075 [R_{int} = 0.0448, R_{sigma} = 0.0177]
3075/0/181
1.083
R_1 = 0.0304, wR_2 = 0.0799
R_1 = 0.0306, wR_2 = 0.0801
0.68/-0.68

Compound 4 (tetraBr, local code adg5) CCDC1515153:

[Image of molecular structure of the tetrabromomacrocycle in adg5 with atom labels and thermal ellipsoids at 50% probability level]
Crystal structure determination of [adg5]
The asymmetric unit contains the tetrabromomacrocycle, there are 4 in the unit cell.
No problems to report in the refinement.

The angle between mean planes through the two aromatic rings define by the following atoms C7 C8 C9 C10 C11 C12 to was C13 C14 C15 C16 C17 C18 was 57.881 ( 0.066 ) degrees. Closest atomic contact C9 - C11$_1$ 3.4789 (0.0030) Angstroms
Symmetry operator used to generate symmetry related atoms discussed in above contacts was $1$ -X,1-Y,1-Z

Experimental
Single crystals of C$_{16}$H$_{8}$Br$_{4}$O$_{2}$ [adg5] were grown from slow evaporation of DCM. A suitable crystal was selected and mounted on a glass fibre with Fromblin oil and placed on an Xcalibur Gemini diffractometer with a Ruby CCD area detector. The crystal was kept at 175(2) K during data collection. Using Olex2 [1], the structure was solved with the ShelXT [2] structure solution program using Direct Methods and refined with the ShelXL [3] refinement package using Least Squares minimisation.

Crystal Data for C$_{16}$H$_{8}$Br$_{4}$O$_{2}$ ($M$ =551.86 g/mol): monoclinic, space group P2$_1$/n (no. 14), $a$ = 7.84858(5) Å, $b$ = 15.11182(7) Å, $c$ = 13.51546(9) Å, $\beta$ = 101.0480(6)$^\circ$, $V$ = 1573.310(16) Å$^3$, $Z$ = 4, $T$ = 175(2) K, $\mu$(CuKα) = 12.522 mm$^{-1}$, $D_{\text{calc}}$ = 2.330 g/cm$^3$, 21338 reflections measured ($8.87^\circ \leq \Theta \leq 155.778^\circ$), 3343 unique ($R_{\text{int}}$ = 0.0383, $R_{\text{sigma}}$ = 0.0218) which were used in all calculations. The final $R_1$ was 0.0251 ($I > 2\sigma(I)$) and $wR_2$ was 0.0640 (all data).

Crystal data and structure refinement for adg5.
Identification code: adg5
Empirical formula: C_{16}H_{8}Br_{4}O_{2}
Formula weight: 551.86
Temperature/K: 175(2)
Crystal system: monoclinic
Space group: P2_1/n
a/Å: 7.84858(5)
b/Å: 15.11182(7)
c/Å: 13.51546(9)
α/°: 90
β/°: 101.0480(6)
γ/°: 90
Volume/Å³: 1573.310(16)
Z: 4
ρ_{calc}/g/cm³: 2.330
μ/mm⁻¹: 12.522
F(000): 1040.0
Crystal size/mm³: 0.2 × 0.2 × 0.2 colourless block
Radiation: CuKα (λ = 1.54184)
2Θ range for data collection/°: 8.87 to 155.778
Index ranges: -9 ≤ h ≤ 9, -19 ≤ k ≤ 19, -17 ≤ l ≤ 17
Reflections collected: 21338
Independent reflections: 3343 [R_{int} = 0.0383, R_{sigma} = 0.0218]
Data/restraints/parameters: 3343/0/199
Goodness-of-fit on F²: 1.173
Final R indexes [Iσ(I)]: R₁ = 0.0251, wR₂ = 0.0636
Final R indexes [all data]: R₁ = 0.0257, wR₂ = 0.0640
Largest diff. peak/hole / e Å⁻³: 0.45/-0.90

**Compound 13 (acid, local code adg9) CCDC 1515154:**
solid sate structure of adg9 with atom labeling and thermal ellipsoids drawn at 50% probability level

Crystal structure determination of [local code adg9]

The asymmetric unit contains the alkyne carboxylic acid. There are eight times of these in the unit cell.

The OH of the carboxylic acid was located in a difference map. It was allowed to refine freely but given thermal parameters Uiso 1.5 times the Uequiv of the parent oxygen. It forms the classic carboxylic acid H-bonded dimer with a symmetry related carboxylic acid.

Specified hydrogen bonds (with esds except fixed and riding H)

\[
\begin{array}{cccc}
D-H & H\cdots A & D\cdots A & \angle(DHA) \\
0.89(2) & 1.74(2) & 2.6307(12) & 178(2) \\
\end{array}
\]

O1-H1...O2$_1$
The atoms used to define mean planes between the two aromatic rings and the angle between these mean planes is C3 C4 C5 C6 C19 C20 to C13 C14 C15 C16 C17 C18 is 65.968 (0.037) degrees

**Experimental**

Single crystals of C_{17}H_{12}O_{4} [adg9] were grown from acetone. A suitable crystal was selected and mounted on a Mitegen head with Fromblin oil and placed on an Xcalibur Gemini diffractometer with a Ruby CCD area detector. The crystal was kept at 150(2) K during data collection. Using Olex2 [1], the structure was solved with the ShelXT [2] structure solution program using Direct Methods and refined with the ShelXL [3] refinement package using Least Squares minimisation.


**Crystal Data** for C_{17}H_{12}O_{4} (M = 280.27 g/mol): monoclinic, space group C2/c (no. 15), \(a = 23.6448(5)\) Å, \(b = 7.08950(10)\) Å, \(c = 18.3229(4)\) Å, \(\beta = 119.266(2)\)°, \(V = 2679.42(10)\) Å\(^3\), \(Z = 8\), \(T = 150(2)\) K, \(\mu(\text{CuK}\alpha) = 0.822\) mm\(^{-1}\), \(D_{\text{calc}} = 1.390\) g/cm\(^3\), 7620 reflections measured (8.574° \(\leq\) 2\(\Theta\) \(\leq\) 155.73°), 2804 unique \((R_{\text{int}} = 0.0245, R_{\text{sigma}} = 0.0274)\) which were used in all calculations. The final \(R_1\) was 0.0378 (I > 2\(\sigma(I)\)) and \(wR_2\) was 0.1072 (all data).

**Crystal data and structure refinement for adg9.**

- **Identification code**: adg9
- **Empirical formula**: C_{17}H_{12}O_{4}
- **Formula weight**: 280.27
- **Temperature/K**: 150(2)
- **Crystal system**: monoclinic
- **Space group**: C2/c
- **a/Å**: 23.6448(5)
- **b/Å**: 7.08950(10)
- **c/Å**: 18.3229(4)
$\alpha/^{\circ}$ 90
$\beta/^{\circ}$ 119.266(2)
$\gamma/^{\circ}$ 90
Volume/$\AA^3$ 2679.42(10)
$Z$ 8
$\rho_{\text{calc}}$/g/cm$^3$ 1.390
$\mu$/mm$^{-1}$ 0.822
$F(000)$ 1168.0
Crystal size/mm$^3$ 0.2 $\times$ 0.16 $\times$ 0.08 colourless
Radiation CuK$\alpha$ ($\lambda = 1.54178$)
2$\Theta$ range for data collection/$^{\circ}$ 8.574 to 155.73
Index ranges -29 $\leq$ h $\leq$ 29, -8 $\leq$ k $\leq$ 8, -23 $\leq$ l $\leq$ 19
Reflections collected 7620
Independent reflections 2804 [R$_{\text{int}}$ = 0.0245, R$_{\text{sigma}}$ = 0.0274]
Data/restraints/parameters 2804/0/193
Goodness-of-fit on $F^2$ 1.054
Final R indexes [$I\geq2\sigma (I)$] $R_1 = 0.0378$, w$R_2 = 0.1028$
Final R indexes [all data] $R_1 = 0.0419$, w$R_2 = 0.1072$
Largest diff. peak/hole / e $\AA^{-3}$ 0.31/-0.19

Compound 11 (Ester, local code mw10) CCDC 1515156:
Solid state structure of one of the crystallographically independent molecules in mw10 with atom labelling and thermal ellipsoids drawn at 50% probability level.

Crystal structure determination of [local code mw10]

The asymmetric unit contains two crystallographically independent but chemically identical molecules, eight molecules in the unit cell.

The angle between the phenyl groups is defined by mean planes through the aromatic systems of each ester

Plane C204 C221 C220 C207 C206 C205 to C219 C218 C217 C216 C215 C214 is 63.401 (0.089) degrees

Plane C104 C121 C120 C107 C106 C105 to plane C119 C118 C117 C116 C115 C114 is 62.773 (0.085) degrees

The angle between a mean plane through the biphenyl unit and the alkyne handle for each compound is;
Mean plane C104 C106 C120 C114 C116 C118 to a mean plane through the alkyne handle O108 C109 C110 C111 C112 O113 is 88.597 (0.125) degrees.

Mean plane C204 C206 C220 C214 C216 C218 to a mean plane through the alkyne handle O208 C209 C210 C211 C212 O213 is 88.717 (0.115) degrees.

**Experimental**

Single crystals of $\text{C}_{18}\text{H}_{14}\text{O}_4$ [mw10] were grown from slow evaporation of EtOAc. A suitable crystal was selected and mounted on a glass fibre with Fromblin oil and placed on a Rigaku Oxford Diffraction diffractometer with a Dual source (Cu at zero) with an AtlasS2 CCD area detector. The crystal was kept at 150(2) K during data collection. Using Olex2 [1], the structure was solved with the ShelXT [2] structure solution program using Direct Methods and refined with the ShelXL [3] refinement package using Least Squares minimisation.


**Crystal Data** for $\text{C}_{18}\text{H}_{14}\text{O}_4$ ($M = 294.29$ g/mol): monoclinic, space group $\text{P2}_1/\text{c}$ (no. 14), $a = 19.9896(4)$ Å, $b = 7.31234(10)$ Å, $c = 20.2663(4)$ Å, $\beta = 106.1392(19)^\circ$, $V = 2845.58(9)$ Å$^3$, $Z = 8$, $T = 150(2)$ K, $\mu(\text{CuK}\alpha) = 0.799$ mm$^{-1}$, $D_{\text{calc}} = 1.374$ g/cm$^3$, 51652 reflections measured ($8.972^\circ \leq 2\Theta \leq 147.348^\circ$), 5692 unique ($R_{\text{int}} = 0.1165$, $R_{\text{sigma}} = 0.0414$) which were used in all calculations. The final $R_1$ was 0.0672 ($I > 2\sigma(I)$) and $wR_2$ was 0.1850 (all data).

**Crystal data and structure refinement for mw10.**

<table>
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<tr>
<th>Identification code</th>
<th>mw10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>$\text{C}<em>{18}\text{H}</em>{14}\text{O}_4$</td>
</tr>
<tr>
<td>Formula weight</td>
<td>294.29</td>
</tr>
<tr>
<td>Temperature/K</td>
<td>150(2)</td>
</tr>
<tr>
<td>Crystal system</td>
<td>monoclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>$\text{P2}_1/\text{c}$</td>
</tr>
<tr>
<td>Parameter</td>
<td>Value</td>
</tr>
<tr>
<td>-----------</td>
<td>-------</td>
</tr>
<tr>
<td>a/Å</td>
<td>19.9896(4)</td>
</tr>
<tr>
<td>b/Å</td>
<td>7.31234(10)</td>
</tr>
<tr>
<td>c/Å</td>
<td>20.2663(4)</td>
</tr>
<tr>
<td>α/°</td>
<td>90</td>
</tr>
<tr>
<td>β/°</td>
<td>106.1392(19)</td>
</tr>
<tr>
<td>γ/°</td>
<td>90</td>
</tr>
<tr>
<td>Volume/Å³</td>
<td>2845.58(9)</td>
</tr>
<tr>
<td>Z</td>
<td>8</td>
</tr>
<tr>
<td>ρ calc/g/cm³</td>
<td>1.374</td>
</tr>
<tr>
<td>μ/mm⁻¹</td>
<td>0.799</td>
</tr>
<tr>
<td>F(000)</td>
<td>1232.0</td>
</tr>
<tr>
<td>Crystal size/mm³</td>
<td>0.2 × 0.14 × 0.1 colourless block</td>
</tr>
<tr>
<td>Radiation</td>
<td>CuKα (λ = 1.54184)</td>
</tr>
<tr>
<td>2Θ range for data collection/°</td>
<td>8.972 to 147.348</td>
</tr>
<tr>
<td>Index ranges</td>
<td>-24 ≤ h ≤ 24, -9 ≤ k ≤ 9, -24 ≤ l ≤ 24</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>51652</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>5692 [R int = 0.1165, R sigma = 0.0414]</td>
</tr>
<tr>
<td>Data/restraints/parameters</td>
<td>5692/0/399</td>
</tr>
<tr>
<td>Goodness-of-fit on F²</td>
<td>1.131</td>
</tr>
<tr>
<td>Final R indexes [I&gt;=2σ (I)]</td>
<td>R₁ = 0.0672, wR₂ = 0.1835</td>
</tr>
<tr>
<td>Final R indexes [all data]</td>
<td>R₁ = 0.0699, wR₂ = 0.1850</td>
</tr>
<tr>
<td>Largest diff. peak/ hole / e Å⁻³</td>
<td>0.35/-0.37</td>
</tr>
</tbody>
</table>

**Compound 5a (Cycloadduct, local code adg) CCDC 1515150:**
Solid state structure of one of the crystallographically independent but chemically identical triazines in adg with atom labeling and thermal ellipsoids drawn at 50% probability level

Crystal structure determination of [local code adg]

The asymmetric unit contains two crystallographically independent but chemically identical triazines in the asymmetric unit. Four times this in the unit cell. This was not a chiral synthesis, however the molecules have crystallised in an achiral space group. The correct assignment of the handedness of the crystal chosen is measured by the Flack parameter

\[
\text{Flack x: } -0.05(8) \text{ Shelx 2014}
\]
\[
\text{Hooft y: } -0.03(8) \text{ Olex2}
\]

As this number is relatively small (-0.05(8)) with a error that takes it to zero you can have confidence in the assignment of the handedness of the crystal chosen.

SIMU restraints were used to give the nitrogens and benzylic carbons of one of the triazines (C107 N108 N109 N110 C111 C128) similar thermal parameters to chemically equivalent atoms.
Experimental

Single crystals of C_{23}H_{19}N_{3}O_{2} [adg] were grown from slow evaporation of DCM. A suitable crystal was selected and mounted on a glass fibre with Fromblin oil and placed on an Xcalibur Gemini diffractometer with a Ruby CCD area detector. The crystal was kept at 150(2) K during data collection. Using Olex2 [1], the structure was solved with the ShelXT [2] structure solution program using Direct Methods and refined with the ShelXL [3] refinement package using Least Squares minimisation.


Crystal Data for C_{23}H_{19}N_{3}O_{2} (M =369.41 g/mol): orthorhombic, space group P2_{1}2_{1}2_{1} (no. 19), a = 9.54430(10) Å, b = 19.2006(2) Å, c = 20.0739(2) Å, V = 3678.66(7) Å³, Z = 8, T = 150(2) K, μ(CuKα) = 0.697 mm⁻¹, D_{calc} = 1.334 g/cm³, 22984 reflections measured (8.81° ≤ 2Θ ≤ 156.046°), 7685 unique (R_{int} = 0.0306, R_{sigma} = 0.0298) which were used in all calculations. The final R_{1} was 0.0369 (I > 2σ(I)) and wR_{2} was 0.0939 (all data).

Crystal data and structure refinement for adg.

Identification code adg
Empirical formula C_{23}H_{19}N_{3}O_{2}
Formula weight 369.41
Temperature/K 150(2)
Crystal system orthorhombic
Space group P2_{1}2_{1}2_{1}
a/Å 9.54430(10)
b/Å 19.2006(2)
c/Å 20.0739(2)
α/° 90
β/° 90
γ/° 90
Volume/Å³ 3678.66(7)
Z 8
ρ<sub>calc</sub> g/cm³ 1.334
μ/mm⁻¹ 0.697
F(000) 1552.0
Crystal size/mm³ 0.3 × 0.1 × 0.1 colorless
Radiation CuKα (λ = 1.54184)
2Θ range for data collection/° 8.81 to 156.046
Index ranges -8 ≤ h ≤ 11, -21 ≤ k ≤ 24, -25 ≤ l ≤ 25
Reflections collected 22984
Independent reflections 7685 [R<sub>int</sub> = 0.0306, R<sub>sigma</sub> = 0.0298]
Data/restraints/parameters 7685/54/505
Goodness-of-fit on F² 1.012
Final R indexes [I>=2σ (I)] R<sub>1</sub> = 0.0369, wR<sub>2</sub> = 0.0911
Final R indexes [all data] R<sub>1</sub> = 0.0407, wR<sub>2</sub> = 0.0939
Largest diff. peak/hole / e Å⁻³ 0.27/-0.29
Flack parameter -0.05(8)

**Compound 10 ((CH₂)₄, (local code MW9) CCDC 1515155.**
solid state structure of mw9 with only the asymmetric unit labelled and thermal ellipsoids drawn at 50% probability level

Crystal structure determination of [local code mw9]

The asymmetric unit contains half the macrocycle which lies on a two fold axis. Four macrocycles in the unit cell (two R and two S, related by inversion centres).

The angle between mean planes through ring C1 C2 C3 C4 C5 C6 and symmetry related ring C1$_1$ C2$_1$ C3$_1$ C4$_1$ C5$_1$ C6$_1$ is 47.844 (0.033) degrees. Distance between oxygens O7 - O7$_1$ in the macrocycle is 2.6562 (0.0015) Angstroms. There is a stack of macrocycles that alter S to R traveling along the ‘c’ axis of the cell and are connected by short C-H… O contacts and C-H- pi contacts.

Short contact tabulated below

Specified hydrogen bonds (with esds except fixed and riding H)

D-H   H...A   D...A   <(DHA)
CH-pi contact between both C-Hs of C9 and the benzene ring

C-H pi bond C9-H9B to centroid of C1 C2 C3 C4 C5 C6
H9B to Centroid distance: 3.17375(4) Å (Olex2)

C-H pi bond C9-H9A to centroid of C1 C2 C3 C4 C5 C6
H9A to Centroid distance: 3.23698(4) Å (Olex2)

(Olex2 generates an esd on this measurement by just using the cell e.s.d as a matrix for the calculation and not by refinement)

Symmetry operators used to define symmetry related atoms in above discussions were
$1 1-X,+Y,0.5-Z$
$2 1-X,1-Y,-Z$

Experimental

Single crystals of C$_{16}$H$_{16}$O$_2$ [mw9] were grown from DCM/hexane. A suitable crystal was selected and mounted on a Mitegen head and placed on an Xcalibur Gemini diffractometer with aRuby CCD area detector. The crystal was kept at 150(2) K during data collection. Using Olex2 [1], the structure was solved with the ShelXT [2] structure solution program using Direct Methods and refined with the ShelXL [3] refinement package using Least Squares minimisation.


Crystal Data for C$_{16}$H$_{16}$O$_2$ (M =240.29 g/mol): orthorhombic, space group Pbcn (no. 60), $a = 15.81826(15)$ Å, $b = 9.23296(10)$ Å, $c = 8.32936(9)$ Å, $V = 1216.50(2)$ Å$^3$, $Z = 4$, $T = 150(2)$ K, μ(CuKα) = 0.676 mm$^{-1}$, $D_{calc} = 1.312$ g/cm$^3$, 9414 reflections measured (11.096° ≤ 2Θ ≤ 156.52°), 1299 unique ($R_{int} = 0.0305$, $R_{sigma} = 0.0147$) which were used in all calculations. The final $R_1$ was 0.0370 (I > 2σ(I)) and $wR_2$ was 0.1068 (all data).
Crystal data and structure refinement for mw9.

Identification code       mw9
Empirical formula       C₁₆H₁₆O₂
Formula weight          240.29
Temperature/K           150(2)
Crystal system           orthorhombic
Space group             Pbcn

a/Å                      15.81826(15)
b/Å                      9.23296(10)
c/Å                      8.32936(9)
α/°                      90
β/°                      90
γ/°                      90
Volume/Å³                1216.50(2)
Z                        4
ρcalc/g/cm³              1.312
μ/mm⁻¹                   0.676
F(000)                   512.0
Crystal size/mm³         0.6 × 0.4 × 0.2 colourless block
Radiation               CuKα (λ = 1.54184)
2Θ range for data collection/°       11.096 to 156.52
Index ranges            -19 ≤ h ≤ 19, -10 ≤ k ≤ 11, -9 ≤ l ≤ 10
Reflections collected   9414
Independent reflections 1299 [Rint = 0.0305, Rsigma = 0.0147]
Data/restraints/parameters 1299/0/83
Goodness-of-fit on F²    1.109
Final R indexes [I>=2σ (I)] R₁ = 0.0370, wR₂ = 0.1057
Final R indexes [all data] R₁ = 0.0380, wR₂ = 0.1068
Largest diff. peak/hole / e Å⁻³ 0.24/-0.20
**Graphs of Kinetic Data:** *General procedure:* A solution of strained alkyne, azide (1:1) ratio and anisole as internal standard, in the solvent indicated, were held at the indicated temperature in a J. Young’s NMR tube. The initial concentrations in each case are given in Table 1 of the main paper. At the time intervals indicated on the graph, NMR spectra were recorded. The second order rate constants were calculated by plotting $1/[\text{alkyne}]$ against time.

![Chemical structure diagram](image)

![Graph of kinetetic data](image)
CD$_3$CN 25 °C

\[ y = 1.85 \times 10^{-04}x + 3.96 \times 10^{00} \]

\[ R^2 = 1.00 \times 10^{00} \]

Time (sec)

CD$_3$CN 40 °C

\[ y = 7.56 \times 10^{-04}x + 3.46 \times 10^{00} \]

\[ R^2 = 9.99 \times 10^{-01} \]

Time (sec)

CD$_3$CN 60 °C

\[ y = 3.12 \times 10^{-03}x + 2.70 \times 10^{00} \]

\[ R^2 = 9.99 \times 10^{-01} \]

Time (sec)
CDCl₃ 25 °C

\[ y = 1.41E-04x + 1.00E+01 \]
\[ R^2 = 1.00E+00 \]
\[
\begin{align*}
\text{CDCl}_3 & 25 \, ^\circ \text{C} \\
1 / [\text{Alkyne}] & = 3.64 \times 10^{-5} x + 1.02 \times 10^1 \\
R^2 & = 9.95 \times 10^{-1}
\end{align*}
\]
\[ \Delta E^\ddagger = 14.6 \text{ kcal/mol} \]
\[ \Delta H^\ddagger = 14.0 \text{ kcal/mol} \]
\[ \Delta S^\ddagger = -29.2 \text{ cal/mol*K} \]
Arrhenius and Eyring plots for reactions with benzyl azide and with p-tolylazide.

\[
\begin{align*}
\text{Arrhenius plot} & : \quad y = -1.59E+04x + 1.83E+01 \\
R^2 & = 9.98E-01
\end{align*}
\]

\[
\begin{align*}
\text{Eyring plot} & : \quad y = -1.52E+04x - 2.43E+01 \\
R^2 & = 9.97E-01
\end{align*}
\]

\[\Delta E^\ddagger = 15.9 \text{ kcal/mol}\]
\[\Delta H^\ddagger = 15.2 \text{ kcal/mol}\]
\[\Delta S^\ddagger = -24.3 \text{ cal/mol*K}\]
Arrhenius plot

Eyring plot

\[ \Delta E^\ddagger = 14.6 \text{ kcal/mol} \]
\[ \Delta H^\ddagger = 14.0 \text{ kcal/mol} \]
\[ \Delta S^\ddagger = -29.2 \text{ cal/mol} \cdot K \]
Variable Temperature NMR studies:

The Gibbs free energy $\Delta G^\#$ required for atropisomer interconversion has been calculated by variable temperature NMR experiments to be 16.7 kcal mol$^{-1}$ (coalescence temperature for the OCH$_2$ peaks is 74 °C) for 10 and 16.3 kcal mol$^{-1}$ (coalescence temperature for the OCH$_2$ peaks is 56 °C) for 5a.$^1$ In contrast, the Gibbs free energy $\Delta G^\#$ of atropisomer interconversion for 1 cannot be calculated by variable temperature NMR experiments since no broadening has been observed even at 100 °C, which was the highest temperature permitted by the instrument.

$^{1} \Delta G^\# = 4.58 T_c[9.97+\log(T_c/\delta\nu)]$ where $T_c =$ Temperature of coalescence and $\delta\nu =$ the chemical shift difference in Hz between the two resonance at low temperature; J. A. Pople, W. G. Schneider, and H. J. Bernstein, *High Resolution Nuclear Magnetic Resonance*, McGraw-Hill Book Company, New York, 1959.
Variable Temperature NMR spectra for 10:

![Variable Temperature NMR spectra for 10, in d6-DMSO](image)
Note a VT-NMR study has been reported on an analogous bipyridyl compound; Durand, J.; Zangrando, E.; Carfagna, C.; Milani, B. *Dalton Trans.* **2008**, 2171-2182.

**Variable Temperature NMR spectra for 5a:**
in CD$_3$CN