Supporting Information
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Progress towards Bioorthogonal Catalysis with Organometallic Compounds**
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1 General Information for the Chemical Experiments

Reactions: All reactions were performed, if not mentioned otherwise, under air at 19–21 °C. Oxygen- or water-sensitive reactions were conducted under a nitrogen atmosphere (Schlenk method) with absolute and degassed solvents. Technical grade solvents were purified by distillation. DMSO and HPLC grade solvents were used without further purification as supplied by ABCR, Carl Roth and VWR. Absolute solvents: THF, toluene and diethyl ether were dried over sodium; DMF and acetonitrile over calcium hydride; acetone over molecular sieve (3 Å). Oxygen-free solvents were degassed through three cycles of the freeze-pump-thaw method. Deionized water was supplied by the department that has passed a cation- and anion-exchanger. All reagents were used without further purification as supplied by Acros Organics, Alfa Aesar, Sigma-Aldrich and TCI. [CpRu(MeCN)$_3$]PF$_6$, [Cp*Ru(MeCN)$_3$]PF$_6$ and [Cp*Ru(COD)Cl] were supplied by Sigma Aldrich. For the synthesis of [Cp*Ru(bipy)(MeCN)]PF$_6$ it was followed the procedure of BRUNEAU et al.\cite{1}, for [CpRu(QA)(allyl)]PF$_6$ (Ru2) the procedure of KITAMURA et al.\cite{2} and for N-(allyloxycarbonyl)-doxorubicin (5) the procedure of Cotterill et al.\cite{3}

Chromatography: TLC was performed on pre-cut aluminum plates coated with silica gel 60 F$_{254}$ (Macherey-Nagel). Spots were visualized by UV light (λ = 254 nm) or by using the appropriate stain followed by heating with a heat gun (300–400 °C). Flash chromatography was performed with silica gel 60 (Pore size: 4.0–6.3 nm; Merck) and compressed air at room temperature. Preparative HPLC was performed on a PLC 2020 Personal Purification System (Gilson) with a Nucleodur C18 HTec-column (5 µm, 250×16 mm; Macherey-Nagel), a flow-rate of 10.0 mL/min and at room temperature. The fractions were detected via UV absorption at λ = 254 nm. Analytical HPLC was performed on an Agilent 1200 Series HPLC-System (Agilent Technologies) with a Purosphere STAR RP-18-column (5 µm, 150×4.6 mm; Merck Millipore), a flow-rate of 1.0 mL/min and at room temperature. For the solvent system was used in the initial 2 min an isocratic run with water followed by a gradual change over 23 min to water/acetonitrile (25:75). The chromatogram was recorded via UV absorption at λ = 254 nm.

Analysis: NMR spectra were recorded in 5 mm tubes at 300 K on the following spectrometers: AV 300 (Bruker), DRX 400 (Bruker), DRX 500 (Bruker), AV 500 (Bruker) and AV 600 (Bruker). The chemical shift δ is given in parts per million (ppm), relatively to tetramethylsilane. The corresponding solvent peaks were used as an internal standard.\cite{4} The information of the signal form is analyzed phenomenologically and does not correspond in some cases with the theoretically expected multiplicity. Multiplicity and coupling constants of the $^1$H spectra were obtained from the Lorentz-to-Gauss transformed FIDs (free induction decay). ESI-HRMS spectra were obtained with a Finnigan LTQ-FT (Thermo Fischer Scientific). FT-IR spectra were recorded on a Alpha-P FT-IR-spectrometer (Bruker). Each spectrum represents the mean value of 20 measurements with a resolution of 2.0 cm$^{-1}$ in the range of 4.000–350 cm$^{-1}$.
2 Experimental Procedure and Analysis

Compound 1

![Chemical Structure of Compound 1]

For the synthesis of 7-aminocoumarin-4-methanesulfonic acid (7) it was followed the procedures of KANAOKA et al.\(^5\) and GRIFFITHS et al.\(^6\) Compound 7 (100 mg, 0.39 mmol, 1.00 eq) was dissolved in triethylammonium bicarbonate buffer (1 M, 4.00 mL, 4.00 mmol, 10.00 eq) and dropwise mixed at 0 °C with allyl chloroformate (97%, 215 µL, 1.96 mmol, 5.00 eq, \(\rho = 1.13\) g/mL). The dispersion was stirred for 1.5 h at 0 °C and for 2 h at room temperature. The mixture was concentrated and purified by preparative HPLC (reversed phase, water/MeCN 1:0 → 0:1). The fractions were concentrated and dried under reduced pressure to give 146 mg (0.33 mmol, 85%) of compound 1 as a colorless solid.

\(R_f = 0.35\) (MeCN/H\(_2\)O/sat. aq. KNO\(_3\), 50:3:1).

**IR** (neat): \(\tilde{\nu} = 3072, 1727, 1712, 1617, 1525, 1462, 1445, 1414, 1394, 1326, 1249, 1222, 1210, 1161, 1098, 1060, 1043, 1029, 988, 930, 885, 875, 856, 816, 770, 746, 735, 702, 656, 617, 583, 552, 526, 497, 473, 460, 448, 397 cm\(^{-1}\).

**\(^1\)H NMR** (300 MHz, DMSO-\(d_6\)): \(\delta = 10.21\) (s, 1H, NH), 8.84 (s (br), 1H, \(HN(CH_2CH_3)_3\)), 7.84 (d, \(J = 8.9\) Hz, 1H, 6-H)), 7.54 (d, \(J = 2.0\) Hz, 1H, 8-H)), 7.34 (dd, \(J = 8.8, 2.2\) Hz, 1H, 5-H)), 6.26 (s, 1H, 3-H)), 6.00 (ddt, \(J = 17.2, 10.4, 5.5\) Hz, 1H, 12-H)), 5.39 (dq, \(J = 17.2, 1.6\) Hz, 1H, 13-H1)), 5.26 (dq, \(J = 10.4, 1.4\) Hz, 1H, 13-H2)), 4.65 (dt, \(J = 5.5, 1.5\) Hz, 2H, 11-H)), 4.03 (s, 2H, 9-H)), 3.07 (q, \(J = 7.4\) Hz, 6H, \(HN(CH_2CH_3)_3\)), 1.15 (t, \(J = 7.3\) Hz, 9H, \(HN(CH_2CH_3)_3\)) ppm.

**\(^{13}\)C NMR** (75 MHz, DMSO-\(d_6\)): \(\delta = 160.2\) (C\(_q\)), 154.1 (C\(_q\)), 153.0 (C\(_q\)), 149.9 (C\(_q\)), 142.3 (C\(_q\)), 132.9 (C-12), 127.7 (C-6), 117.9 (C-13), 113.9 (C-5), 113.9 (C-3), 113.8 (C\(_q\)), 104.2 (C-8), 65.1 (C-11), 53.1 (C-9), 45.8 (6C, \(HN(CH_2CH_3)_3\)), 8.6 (9C, \(HN(CH_2CH_3)_3\)) ppm.

**HRMS** (ESI): \(m/z\) calculated for C\(_{14}\)H\(_{12}\)N\(_1\)O\(_7\)S\(_1\), [M]\(^{-}\): 338.0340, found: 338.0340.
For the synthesis of 7-aminocoumarin-4-methanesulfonic acid (7) it was followed the procedures of KANAOKA et al.[5] and GRIFFITHS et al.[6] Compound 7 (50 mg, 0.20 mmol, 1.00 eq) was dispersed in water (3 mL) and mixed with triethylamine (29.9 µL, 0.22 mmol, 1.10 eq, ρ = 0.73 g/mL). After stirring for 30 min at room temperature the clear solution was concentrated, the residue taken up in water and purified by preparative HPLC (reversed phase, water/MeCN 1:0 → 0:1). The fractions were concentrated and dried under reduced pressure to give 70 mg (0.20 mmol, 99%) of compound 2 as a yellow solid.

\[ R_f = 0.25 \text{ (MeCN/H}_2\text{O/sat. aq. KNO}_3, 50:3:1). \]

IR (neat): \( \tilde{\nu} = 3416, 3334, 3174, 3016, 2718, 1689, 1634, 1613, 1546, 1481, 1446, 1401, 1339, 1266, 1222, 1178, 1159, 1140, 1046, 1029, 999, 921, 885, 871, 854, 830, 810, 775, 749, 728, 709, 664, 635, 588, 553, 533, 521, 501, 470, 453, 401 \text{ cm}^{-1}. \]

\(^1\text{H NMR} \text{ (300 MHz, DMSO-d}_6): \delta = 8.84 \text{ (s (br), 1H, } HN(CH}_2CH}_3\text{), 7.55 \text{ (d, } J = 8.7 \text{ Hz, 1H, 6-H), 6.52 \text{ (dd, } J = 8.7, 2.3 \text{ Hz, 1H, 5-H), 6.39 \text{ (d, } J = 2.2 \text{ Hz, 1H, 8-H), 6.06 \text{ (s, 2H, NH}_2\text{), 5.94 \text{ (s, 1H, 3-H), 3.90 \text{ (s, 2H, 9-H), 3.07 \text{ (ddd, } J = 14.5, 7.3, 2.4 \text{ Hz, 6H, } HN(CH}_2CH}_3\text{), 1.15 \text{ (t, } J = 7.3 \text{ Hz, 9H, } HN(CH}_2CH}_3\text{)) ppm.} \]

\(^1\text{C NMR} \text{ (75 MHz, DMSO-d}_6): \delta = 160.9 \text{ (C}_q\text{), 155.7 \text{ (C}_q\text{), 152.7 \text{ (C}_q\text{), 150.3 \text{ (C}_q\text{), 127.8 \text{ (C}-6\text{), 110.9 \text{ (C}-5\text{), 109.3 \text{ (C}-3\text{), 108.5 \text{ (C}_q\text{), 98.3 \text{ (C}-8\text{), 53.3 \text{ (C}-9\text{), 45.8 \text{ (6C, } HN(CH}_2CH}_3\text{), 8.6 \text{ (9C, } HN(CH}_2CH}_3\text{)) ppm.} \]

HRMS (ESI): \( m/z \) calculated for C\(_{10}\)H\(_8\)N\(_1\)O\(_5\)S\(_1\), [M]\(^−\): 254.0129, found: 254.0128.
Compound 3

With some changes it was followed the procedure of MEGGERS et al.\[7\] Rhodamine 110 (4; 100 mg, 0.27 mmol, 1.00 eq) and pyridine (65 µL, 0.80 mmol, 3.00 eq, ρ = 0.98 g/mL) were dissolved in dry DMF (0.50 mL) and dropwise mixed at 0 °C with allyl chloroformate (57 µL, 0.53 mmol, 2.00 eq, ρ = 1.13 g/mL). The solution was allowed to warm to room temperature overnight. After 16 h it was diluted with ethyl acetate (25 mL) and the mixture was washed with aqueous hydrochloric acid (5%, 10 mL) and with aqueous sodium bicarbonate solution (saturated, 2× 10 mL). The organic phase was concentrated, the residue taken up in ethyl acetate and purified by silica gel column (flash chromatography, Hex/EtOAc 3:1 → 1:1). The fractions were concentrated and dried under reduced pressure to give 34 mg (0.07 mmol, 25%) of compound 3 as a colorless solid.

\[R_f = 0.13 \text{ (Hex/EtOAc, 3:1)}.
\]

\[IR \text{ (neat): } \tilde{\nu} = 3291, 2924, 2854, 1733, 1707, 1612, 1465, 1409, 1354, 1323, 1288, 1216, 1166, 1131, 1105, 1086, 1048, 991, 966, 934, 867, 831, 796, 757, 733, 720, 693, 657, 613, 578, 521, 462, 388 \text{ cm}^{-1}.
\]

\[^1H \text{ NMR (300 MHz, acetone-d}_6\]: } \delta = 9.05 (s, 2H, NH), 7.87 (dt, J = 7.6, 1.1 Hz, 1H, 13-H), 7.66 (td, J = 11.1, 1.3 Hz, 1H, 15-H), 7.62 (d, J = 2.1 Hz, 2H, 1-H, 8-H), 7.60 (td, J = 7.5, 1.1 Hz, 1H, 14-H), 7.18 (dt, J = 7.6, 1.1 Hz, 1H, 16-H), 7.08 (dd, J = 8.7, 2.1 Hz, 2H, 4-H, 5-H), 6.64 (d, J = 8.7 Hz, 2H, 3-H, 6-H), 5.86 (ddt, J = 17.3, 10.8, 5.4 Hz, 2H, 20-H), 5.36 (dq, J = 17.2, 1.6 Hz, 2H, 21-H), 5.22 (dq, J = 10.5, 1.4 Hz, 2H, 21-H), 4.66 (t, J = 1.4 Hz, 2H, 19-H), 4.65 (t, 1.5 Hz, 2H, 19-H) ppm.

\[^{13}C \text{ NMR (75 MHz, acetone-d}_6\]: } \delta = 169.5 (C_q), 154.1 (C_q), 154.0 (C_q), 152.7 (C_q), 142.5 (C_q), 142.4 (C_q), 136.2 (C-15), 133.9 (2C, C-20), 130.9 (C-14), 129.4 (2C, C-3, C-6), 127.5 (C_q), 125.5 (C-13), 124.9 (C-16), 117.9 (2C, C-21), 115.3 (2C, C-4, C-5), 115.2 (C_q), 114.3 (C_q), 106.4 (2C, C-1, C-8), 106.4 (C_q), 82.9 (C_q), 66.1 (2C, C-19) ppm.

\[HRMS \text{ (ESI): } m/z \text{ calculated for C}_{28}H_{23}N_{2}O_{7}, [M+H]^+: 499.1500, \text{ found: 499.1497}.
\]
2-Quinolinecarboxylic acid (8; 200 mg, 1.15 mmol, 1.00 eq), sodium bicarbonate (155 mg, 1.84 mmol, 1.60 eq) and allyl bromide (150 µL, 1.73 mmol, 1.50 eq, $\rho = 0.73 \text{ g/mL}$) were dispersed in DMF (6 mL) and stirred for 18 h at 50 °C. The mixture was diluted with water (20 mL) and extracted with DCM (3 × 20 mL). The combined organic phases were concentrated, the residue taken up in ethyl acetate and purified by silica gel column (flash chromatography, Hex/EtOAc 3:1 → 1:1). The fractions were concentrated and dried under reduced pressure to give 242 mg (1.13 mmol, 99%) of compound 1 as a colorless oil.

$R_f = 0.31$ (Hex/EtOAc, 3:1).

**IR (neat):** $\tilde{\nu} = 3063, 2944, 1742, 1716, 1648, 1618, 1593, 1562, 1504, 1461, 1427, 1364, 1342, 1311, 1291, 1271, 1240, 1211, 1132, 1103, 978, 930, 876, 796, 775, 738, 624, 592, 581, 554, 524, 478, 428, 396 \text{ cm}^{-1}$.

**$^1$H NMR** (500 MHz, acetone-d$_6$): $\delta = 8.45$ (dd, $J = 8.5, 1.1 \text{ Hz}, 1\text{H}, 4\text{-H}$), 8.18 (dq, $J = 8.5, 1.0 \text{ Hz}, 1\text{H}, 8\text{-H}$), 8.13 (d, $J = 8.4 \text{ Hz}, 1\text{H}, 3\text{-H}$), 7.99 (dd, $J = 8.2, 1.9 \text{ Hz}, 1\text{H}, 5\text{-H}$), 7.83 (ddd, $J = 8.4, 6.9, 1.5 \text{ Hz}, 1\text{H}, 7\text{-H}$), 7.69 (ddd, $J = 8.2, 6.9, 1.3 \text{ Hz}, 1\text{H}, 6\text{-H}$), 6.14 (dt, $J = 17.2, 10.3, 5.6 \text{ Hz}, 1\text{H}, 11\text{-H}$), 5.50 (dq, $J = 17.2, 1.6 \text{ Hz}, 1\text{H}, 12\text{-H1}$), 5.30 (dq, $J = 10.5, 1.4 \text{ Hz}, 1\text{H}, 12\text{-H2}$), 4.94 (dt, $J = 5.7, 1.5 \text{ Hz}, 2\text{H}, 10\text{-H}$) ppm.

**$^{13}$C NMR** (75 MHz, acetone-d$_6$): $\delta = 165.6$ (C-9), 149.0 (C-2), 148.3 (C-8a), 138.0 (C-4), 133.4 (C-11), 131.1 (C-7), 131.0 (C-8), 130.0 (C-4a), 129.3 (C-6), 128.6 (C-5), 121.6 (C-3), 118.6 (C-12), 66.6 (C-10) ppm.

**HRMS (ESI):** $m/z$ calculated for C$_{13}$H$_{11}$N$_2$O$_2$Na, [M+Na]$^+$: 236.0682, found: 236.0682.
Compound 12

For the synthesis of 4-dimethylamino-2-quinoline carboxylic acid (11) it was followed the procedures of Kato et al.\textsuperscript{[8]} and Serrano-Wu et al.\textsuperscript{[9]} and for the synthesis of compound 12 the same procedure as listed above for compound 9. Conversion of compound 11 (64 mg, 0.30 mmol) gave 64 mg (0.25 mmol, 83\%) of compound 12 as a yellow oil.

$R_f = 0.32$ (Hex/EtOAc, 1:1).

**IR** (neat): $\tilde{\nu} = 3082, 2946, 2877, 2846, 2794, 1740, 1715, 1647, 1613, 1576, 1511, 1482, 1456, 1422, 1387, 1362, 1336, 1277, 1236, 1195, 1149, 1117, 1064, 1027, 985, 950, 867, 847, 786, 769, 718, 660, 600, 504, 454, 409 \text{ cm}^{-1}$.

$^1$H NMR (300 MHz, acetone-d\textsubscript{6}): $\delta = 8.17$ (dd, $J = 8.6$ Hz, 1H, 5-H), 8.06 (dd, $J = 8.5$, 2.1 Hz, 1H, 8-H), 7.74 (ddd, $J = 8.4$, 6.9, 1.5 Hz, 1H, 7-H), 7.59 (ddd, $J = 8.4$, 6.9, 1.5 Hz, 1H, 6-H), 7.51 (s, 1H, 3-H), 6.13 (ddt, $J = 17.3$, 10.4, 5.6 Hz, 1H, 11-H), 5.48 (dq, $J = 17.2$, 1.6, 1H, 12-H1), 5.30 (dq, $J = 10.5$, 1.4 Hz, 1H, 12-H2), 4.90 (dt, $J = 5.7$, 1.4 Hz, 2H, 10-H), 3.11 (s, 6H, NMe\textsubscript{2}) ppm.

$^{13}$C NMR (75 MHz, acetone-d\textsubscript{6}): $\delta = 166.4$ (C-9), 159.3 (C-4), 150.2 (C-2), 149.4 (C-8a), 133.6 (C-11), 131.7 (C-8), 130.3 (C-7), 127.1 (C-6), 125.6 (C-5), 124.4 (C-4a), 118.5 (C-12), 107.5 (C-3), 66.5 (C-10), 44.1 (NMe\textsubscript{2}) ppm.

**HRMS** (ESI): $m/z$ calculated for C\textsubscript{15}H\textsubscript{17}N\textsubscript{2}O\textsubscript{2}, [M+H]$^+$: 257.1285, found: 257.1288.
General Procedure for the Complex Synthesis

Method A: Under a nitrogen atmosphere [CpRu(MeCN)$_3$]PF$_6$ or [Cp*Ru(MeCN)$_3$]PF$_6$ (50 mg, 1.00 eq) was dissolved in dry degassed acetone (2.5 mL) and mixed at room temperature with a solution of 2-pyridinecarboxylic acid (13; 1.00 eq) in acetone (1.0 mL). After stirring for 15 min allyl alcohol (1.00 eq, $\rho = 0.85$ g/mL) was added. The reaction mixture was stirred for another 15 min and then concentrated under a nitrogen stream. The resulting precipitate was washed with acetone and dried under reduced pressure to give the desired product.

Method B: Under a nitrogen atmosphere [CpRu(MeCN)$_3$]PF$_6$ or [Cp*Ru(MeCN)$_3$]PF$_6$ (50 mg, 1.00 eq) was dissolved in dry degassed acetone (2.5 mL) and mixed at room temperature with a solution of allyl ester (1.00 eq) in acetone (1.0 mL). After stirring for 15 min the reaction mixture was concentrated under a nitrogen stream. The resulting precipitate was washed with acetone and dried under reduced pressure to give the desired product.

Compound 14

Method A of the general procedure for the complex synthesis. Conversion of 2-pyridinecarboxylic acid (13) gave 41 mg (0.09 mmol, 72%) of compound 14 as a beige solid.

IR (neat): $\tilde{\nu} = 3116, 3045, 1666, 1615, 1573, 1479, 1452, 1425, 1342, 1297, 1257, 1172, 1059, 912, 873, 832, 823, 749, 711, 683, 659, 599, 555, 460, 431, 412, 394$ cm$^{-1}$.

$^1$H NMR (400 MHz, acetone-d$_6$): $\delta = 9.07$ (ddd, $J = 5.6, 1.5, 0.7$ Hz, 1H, 6-H), 8.34 (td, $J = 11.6, 1.5$ Hz, 1H, 4-H), 7.98 (ddd, $J = 7.8, 1.7, 0.7$ Hz, 1H, 3-H), 7.88 (ddd, $J = 7.6, 5.6, 1.7$ Hz, 1H, 5-H), 6.38 (s, 5H, C$_5$H$_5$), 4.92 (tt, $J = 16.5, 6.4$ Hz, 1H, 9-H), 4.66 (dd, $J = 11.0, 1.2$ Hz, 1H, 8-H$_{ax}$), 4.49 (dq, $J = 11.0, 0.8$ Hz, 1H, 10-H$_{ax}$), 4.45 (dd, $J = 6.3, 2.7$ Hz, 1H, 10-H$_{eq}$), 4.27 (dd, $J = 6.4, 3.2$ Hz, 1H, 8-H$_{eq}$) ppm.

$^{13}$C NMR (101 MHz, acetone-d$_6$): $\delta = 171.2$ (C-7), 158.1 (C-6), 150.9 (C-2), 142.8 (C-4), 130.4 (C-5), 128.8 (C-3), 101.8 (C-9), 97.3 (5C, C$_5$H$_5$), 69.2 (C-8), 66.3 (C-10) ppm.

HRMS (ESI): $m/z$ calculated for C$_{14}$H$_{14}$N$_2$O$_2$Ru$_1$, [M]$^+$: 330.0066, found: 330.0064.
Compound 15

Method A of the general procedure for the complex synthesis. Conversion of 2-pyridinecarboxylic acid (13) gave 52 mg (0.10 mmol, 95%) of compound 15 as a brown solid.

**IR** (neat): $\tilde{\nu} = 1666, 1608, 1474, 1381, 1328, 1291, 1161, 1023, 876, 830, 762, 707, 691, 555, 454, 383 \text{ cm}^{-1}$.

$^1\text{H NMR}$ (300 MHz, acetone-d$_6$): $\delta = 8.70$ (dd, $J = 5.7, 2.1$ Hz, 1H, 6-H), 8.36 (td, $J = 11.5, 1.3$ Hz, 1H, 4-H), 8.03 (ddd, $J = 7.7, 1.7, 0.6$ Hz, 1H, 3-H), 7.95 (ddd, $J = 7.6, 5.7, 1.7$ Hz, 1H, 5-H), 5.11 (tt, $J = 15.7, 6.2$ Hz, 1H, 9-H), 4.26 (ddd, $J = 6.2, 2.8, 0.9$ Hz, 1H, 10-H$_{ax}$), 4.09 (dd, $J = 6.4, 2.9$ Hz, 1H, 8-H$_{ax}$), 3.38 (dd, $J = 10.6, 0.8$ Hz, 1H, 8-H$_{eq}$), 2.99 (dt, $J = 10.6, 1.0$ Hz, 1H, 10-H$_{eq}$), 1.74 (s, 15H, C$_5$Me$_5$) ppm.

$^{13}$C NMR (75 MHz, acetone-d$_6$): $\delta = 170.8$ (C-7), 155.0 (C-6), 151.0 (C-2), 142.9 (C-4), 131.3 (C-5), 128.8 (C-3), 108.8 (5C, C$_5$Me$_5$), 100.3 (C-9), 74.5 (C-8), 67.8 (C-10), 9.2 (5C, C$_5$Me$_5$) ppm.

**HRMS** (ESI): $m/z$ calculated for C$_{19}$H$_{24}$N$_1$O$_2$Ru$_1$, [M]$^+$: 400.0850, found: 400.0844.

Compound 16

Method B of the general procedure for the complex synthesis. Conversion of 2-quinolinecarboxylic acid allyl ester (9) gave 56 mg (0.09 mmol, 94%) of compound 16 as a beige solid.

**IR** (neat): $\tilde{\nu} = 1664, 1459, 1385, 1356, 1337, 1181, 1021, 883, 833, 801, 778, 741, 556, 511, 413, 380 \text{ cm}^{-1}$.

$^1\text{H NMR}$ (500 MHz, acetone-d$_6$): $\delta = 8.91$ (dd, $J = 8.4, 1.1$ Hz, 1H, 4-H), 8.34 (dd, $J = 8.2, 1.7$ Hz, 1H, 5-H), 8.22 (ddd, $J = 8.8, 7.0, 1.6$ Hz, 1H, 7-H), 8.18 (d, $J = 8.4$ Hz, 1H, 3-H), 7.99 (ddd, $J = 8.2, 7.1, 1.1$ Hz, 1H, 6-H), 7.75 (dq, $J = 8.9, 0.9$ Hz, 1H, 8-H), 5.19 (tt, $J = 15.9, 6.4$ Hz, 1H, 11-H), 4.45 (dd, $J = 6.4, 2.9$ Hz, 1H, 12-H$_{ax}$), 4.23 (dd, $J = 6.4, 2.9$ Hz, 1H, 10-H$_{ax}$), 3.66 (dd, $J = 10.5, 0.9$ Hz, 1H, 10-H$_{eq}$), 3.26 (dd, $J = 10.8, 0.9$ Hz, 1H, 10-H$_{eq}$), 1.79 (s, 15H, C$_5$Me$_5$) ppm.

$^{13}$C NMR (126 MHz, acetone-d$_6$): $\delta = 171.9$ (C-7), 153.5 (C-2), 146.1 (C-8a), 144.0 (C-4), 133.4 (C-7), 133.1 (C-4a), 131.5 (C-5), 130.7 (C-6), 129.4 (C-8), 124.9 (C-3), 109.3 (5C, C$_5$Me$_5$), 103.1 (C-11), 78.4 (C-10), 67.0 (C-12), 9.7 (5C, C$_5$Me$_5$) ppm.

**HRMS** (ESI): $m/z$ calculated for C$_{23}$H$_{26}$N$_1$O$_2$Ru$_1$, [M]$^+$: 450.1008, found: 450.1007.
Compound Ru3

Method B of the general procedure for the complex synthesis. For the synthesis of 4-methoxy-2-quinolinecarboxylic acid allyl ester (10) it was followed the procedure of Waymouth et al.\textsuperscript{[10]}

Conversion of compound 10 gave 43 mg (0.08 mmol, 65\%) of compound Ru3 as a beige solid.

\textbf{IR} (neat): $\tilde{\nu} = 3118, 1674, 1590, 1569, 1463, 1430, 1398, 1370, 1338, 1188, 1169, 1156, 1133, 1119, 1020, 995, 915, 831, 794, 774, 758, 741, 653, 640, 617, 603, 556, 521, 494, 463, 415, 397, 387 cm$^{-1}$.

$^1$H NMR (400 MHz, acetone-d$_6$): $\delta = 8.43$ (ddd, $J = 8.3, 1.5, 0.7$ Hz, 1H, 5-H), 8.13 (ddd, $J = 8.8, 2.0, 0.7$ Hz, 1H, 8-H), 8.10 (ddd, $J = 8.8, 6.1, 1.5$ Hz, 1H, 7-H), 7.91 (ddd, $J = 8.3, 6.1, 2.0$ Hz, 1H, 6-H), 7.59 (s, 5H, C$_5$H$_5$), 4.90 (m, 1H, 10-H$_{ax}$), 4.87 (m, 1H, 11-H), 4.69 (m, 1H, 12-H$_{ax}$), 4.40 (m, 1H, 10-H$_{eq}$), 4.34 (m, 1H, 12-H$_{eq}$), 4.33 (s, 3H, OMe) ppm.

$^{13}$C NMR (101 MHz, acetone-d$_6$): $\delta = 172.4$ (C-9), 168.2 (C-4), 154.8 (C-2), 149.0 (C-8a), 134.3 (C-7), 129.6 (C-6), 129.4 (C-8), 124.8 (C-4a), 124.4 (C-5), 104.7 (C-3), 104.1 (C-11), 97.3 (5C, C$_5$H$_5$), 71.3 (C-10), 65.3 (C-12), 58.3 (OMe) ppm.

\textbf{HRMS} (ESI): $m/z$ calculated for C$_{19}$H$_{18}$N$_1$O$_3$Ru$_1$, [M]$^+$: 410.0326, found: 410.0326.

Compound Ru4

Method B of the general procedure for the complex synthesis. Conversion of 4-dimethylamino-2-quinolinecarboxylic acid allyl ester (12) gave 26 mg (0.05 mmol, 76\%) of compound Ru4 as a yellow solid.

\textbf{IR} (neat): $\tilde{\nu} = 3116, 3071, 1706, 1660, 1586, 1545, 1528, 1508, 1453, 1413, 1349, 1333, 1310, 1272, 1226, 1209, 1198, 1163, 1135, 1084, 1061, 1047, 1008, 993, 977, 903, 876, 828, 800, 787, 766, 740, 660, 637, 616, 597, 555, 531, 499, 455, 387 cm$^{-1}$.

$^1$H NMR (500 MHz, acetone-d$_6$): $\delta = 8.35$ (m, 1H, 5-H), 7.95 (m, 2H, 7-H, 8-H), 7.72 (ddd, $J = 8.4, 6.2, 2.1$ Hz, 1H, 6-H), 7.32 (s, 1H, 3-H), 6.45 (s, 5H, C$_5$H$_5$), 4.83 (d, $J = 11.0$ Hz, 1H, 10-H$_{ax}$), 4.72 (dt, $J = 16.3, 6.3$ Hz, 1H, 11-H), 4.60 (d, $J = 11.0$ Hz, 1H, 12-H$_{ax}$), 4.32 (dd, $J = 6.2, 2.9$ Hz, 1H, 10-H$_{eq}$), 4.23 (dd, $J = 6.3, 2.8$ Hz, 1H, 12-H$_{eq}$), 3.41 (s, 6H, NMe$_2$) ppm.

$^{13}$C NMR (126 MHz, acetone-d$_6$): $\delta = 173.0$ (C-9), 161.7 (C-4), 151.1 (C-2), 149.6 (C-8a), 133.0 (C-7), 129.3 (C-8), 128.1 (C-5), 126.6 (C-6), 124.3 (C-4a), 108.6 (C-3), 103.8 (C-11), 97.1 (5C, C$_5$H$_5$), 70.5 (C-10), 65.0 (C-12), 44.2 (2C, NMe$_2$) ppm.

\textbf{HRMS} (ESI): $m/z$ calculated for C$_{20}$H$_{21}$N$_2$O$_2$Ru$_1$, [M]$^+$: 423.0647, found: 423.0642.
Figure S1. Absorption spectra of N-(allyloxycarbonyl)-aminocoumarin 1 (λ\text{max} 331 nm) and amino-coumarin 2 (λ\text{max} 353 nm) and the fluorescence spectrum of 2 (λ\text{max} 460 nm). The absorption spectra were recorded in translucent 96-well plates at 5 mM in 250 µL water and the fluorescence spectrum (λ\text{ex} 353 nm, λ\text{cut} 420 nm) in black 96-well plates at 10 µM in 250 µL water.

Figure S2. Standard curve of aminocoumarin 2. Fluorescence intensity (λ\text{ex} 353 nm, λ\text{em} 460 nm, λ\text{cut} 420 nm) at different concentrations in 250 µL water. Linear regression curve for 0–25 µM is shown.
Table S1. Fluorescence intensity of aminocoumarin 2 in relation to the background fluorescence of N-(allyloxycarbonyl)-aminocoumarin 1 at different excitation wavelengths. Excitation at 395 nm shows lowest signal-to-noise ratio (entry 3).†

<table>
<thead>
<tr>
<th>Entry</th>
<th>λ / nm</th>
<th>Fluorescence Intensity</th>
<th>Ratio (1:2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>coumarin 1</td>
<td>coumarin 2</td>
</tr>
<tr>
<td>1</td>
<td>385</td>
<td>703</td>
<td>44498</td>
</tr>
<tr>
<td>2</td>
<td>390</td>
<td>438</td>
<td>36398</td>
</tr>
<tr>
<td>3</td>
<td>395</td>
<td>306</td>
<td>28827</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>213</td>
<td>20011</td>
</tr>
<tr>
<td>5</td>
<td>405</td>
<td>155</td>
<td>12285</td>
</tr>
</tbody>
</table>

† Fluorescence intensity (λ_{ex} 385–405 nm, λ_{em} 460 nm, λ_{cut} 420 nm) of 1 and 2 was measured at a concentration of 10 µM in 250 µL water.

Figure S3. Linear correlation between fluorescence intensity (λ_{ex} 395 nm, λ_{em} 460 nm, λ_{cut} 420 nm) and yield of aminocoumarin 2. Solutions for defined yields consist of 10 µM 2:1 mixtures in 250 µL water. For example: 0% yield (10 µM 1), 10% yield (1 µM 2, 9 µM 1), 50% yield (5 µM 2, 5 µM 1) and 100% yield (10 µM 2). Linear regression curve is shown.
4 Catalytic Deprotection of Caged Aminocoumarin (Biol. Relev. Cond.)

**General Procedure:** The catalytic deprotection of \( \text{N-(allyloxycarbonyl)-aminocoumarin} \) 1 to \( \text{aminocoumarin} \) 2 under biologically relevant conditions was performed in an open 2 mL Eppendorf tube. For this a solution of 1 (20 µL, 20 mM in water, 1.0 eq) and a solution of glutathione (40 µL, 100 mM in water, 10 eq) were added to water (736 µL). After mixing, a solution of the catalyst (4 µL, 0.1–5.0 mM in DMSO, \( 10^{-3} \)–0.05 eq) was added. The reaction mixture was mixed again and incubated for 24 h at room temperature and in the presence of air. At regular intervals reaction aliquots (5 µL) were diluted with water (245 µL) - to reduce the fluorophore concentration to 10 mM - in black 96-well plates (F96 Microwell, Nunc) and then analyzed in a microplate reader (Spectramax M5, Molecular Devices). Fluorescence was exited at 395 nm and recorded at 460 nm. The cut-off filter was set to 420 nm. Every measured value is the mean value of three aliquots. Standard deviation bar was omitted due to legibility reasons when the value of standard deviation is smaller than the size of the data point.

![Graph](https://via.placeholder.com/150)

**Figure S4.** Catalytic deprotection of \( \text{N-(allyloxycarbonyl)-aminocoumarin} \) 1 with different organoruthenium complexes at a loading of 5.0 mol% under biologically relevant conditions. Reaction conditions: caged amino-coumarin (1; 1.0 eq, 500 µM), catalyst (0.05 eq, 25 µM) and glutathione (10 eq, 5 mM) in water/DMSO (200:1) at room temperature and in the presence of air. Yields were determined by fluorescence measurements (\( \lambda_{ex} \) 395 nm, \( \lambda_{em} \) 460 nm, \( \lambda_{cut} \) 420 nm). The mean values of three independent measurements are shown.
**Figure S5.** Catalytic deprotection of $N$-(allyloxy carbonyl)-aminocoumarin 1 with different organoruthenium complexes at a loading of 1.0 mol% under biologically relevant conditions. Reaction conditions: caged aminocoumarin ($1$; 1.0 eq, 500 µM), catalyst (0.01 eq, 5 µM) and glutathione (10 eq, 5 mM) in water/DMSO (200:1) at room temperature and in the presence of air. Yields were determined by fluorescence measurements ($\lambda_{\text{ex}}$ 395 nm, $\lambda_{\text{em}}$ 460 nm, $\lambda_{\text{cut}}$ 420 nm). The mean values of three independent measurements are shown.

**Figure S6.** Catalytic deprotection of $N$-(allyloxy carbonyl)-aminocoumarin 1 with different organoruthenium complexes at a loading of 0.1 mol% under biologically relevant conditions. After 48 h the following TON were measured: Ru2 = 94, Ru3 = 146 and Ru4 = 267. Reaction conditions: caged aminocoumarin ($1$; 1.0 eq, 500 µM), catalyst ($10^{-3}$ eq, 0.5 µM) and glutathione (10 eq, 5 mM) in water/DMSO (200:1) at room temperature and in the presence of air. Yields were determined by fluorescence measurements ($\lambda_{\text{ex}}$ 395 nm, $\lambda_{\text{em}}$ 460 nm, $\lambda_{\text{cut}}$ 420 nm). The mean values of three independent measurements are shown.
Figure S7. Catalytic deprotection of N-(allyloxycarbonyl)-aminocoumarin 1 at different temperatures under biologically relevant conditions. Reaction conditions: see Figure S4. Yields were determined by fluorescence measurements ($\lambda_{ex}$ 395 nm, $\lambda_{em}$ 460 nm, $\lambda_{cut}$ 420 nm). The mean values of three independent measurements are shown.

<table>
<thead>
<tr>
<th>para substituent</th>
<th>Hammett constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>R phenyl</td>
<td>0.00</td>
</tr>
<tr>
<td>R phenyl-OME</td>
<td>-0.27</td>
</tr>
<tr>
<td>R phenyl-NMe2</td>
<td>-0.83</td>
</tr>
</tbody>
</table>

Figure S8. Hammett plot for the organoruthenium complexes Ru2, Ru3 and Ru4 shows a correlation between π-backbonding (Hammett constant\(^{(11)}\)) and TON. Reaction conditions: see Figure S6.
5 Catalytic Deprotection of Caged Doxorubicin (Biol. Relev. Cond.)

**General Procedure:** The catalytic deprotection of 5-(allyloxycarbonyl)-doxorubicin 5 to doxorubicin 6 under biologically relevant conditions was performed in an open 2 mL Eppendorf tube. For this a solution of 5 (10 µL, 10 mM in DMSO, 1.0 eq) and a solution of glutathione (50 µL, 100 mM in water, 50 eq) were added to water (940 µL). After mixing, a solution of the catalyst (1 µL, 5.0 mM in DMSO, 0.05 eq) was added. The reaction mixture was mixed again and incubated for 24 h at room temperature and in the presence of air. The reaction mixture was analyzed by analytic HPLC (for details, see General Information for the Chemical Experiments). The positive control reaction was performed with free doxorubicin 6 instead of caged doxorubicin 5 and the negative control reaction was performed without a catalyst.

![Figure S9. Catalytic deprotection of N-(allyloxycarbonyl)-doxorubicin 5 with Ru4 at a loading of 5.0 mol% under biologically relevant conditions. Reaction conditions: caged doxorubicin (5; 1.0 eq, 100 µM), catalyst (0.05 eq, 5 µM) and glutathione (50 eq, 5 mM) in water/DMSO (200:1) at room temperature and in the presence of air. Yield was determined by analytical HPLC. A) Negative Control; B) Reaction mixture after 24 h with yield ≥95%; C) Positive control.](image-url)
6 General Information for the Biological Experiments

**General Executions and Substances:** All steps were performed on a sterile clean bench (MSC-Advantage, Thermo Scientific) at room temperature. Solutions stored in a fridge were warmed beforehand in a water bath (37 °C). All substances were supplied by Sigma-Aldrich.

**Cell Culture:** HeLa cells were cultured in 75 cm² cell culture flasks (BD Falcon) with DMEM supplemented with fetal bovine serum (FBS; 10 vol%), L-glutamine (2 mM), penicillin (100 U/mL) and streptomycin (0.1 mg/mL). The cells were incubated at 37 °C in a 5% CO₂ atmosphere (Galaxy 170S, New Brunswick Scientific) and were passaged after 2–3 days before they reached confluence.

**Fluorescence microscopy:** All images were recorded on a TCS SP8X (Leica) confocal laser scanning microscope with a 100× objective lens (NA = 1.40). Excitation of HeLa cells containing deprotected rhodamine 110 (4) was carried out at 488 nm and emission was collected at 500–555 nm.
The standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to assess the cytotoxicity of Ru2. For this HeLa cells were adjusted to a concentration of 9,000 cells/well, seeded in translucent 96-well microplates (Microtest 96, BD Falcon) with DMEM (100 µL) and incubated for 24 h. Serial dilutions of Ru2 with DMSO (2%) in DMEM (100 µL) were added to each well and incubated for additional 24 h. The negative control cells were treated with DMSO (1%) in DMEM. Wells containing only culture medium without cells were used as blanks for the formazan absorption. On completion of the incubation, DMEM was removed and MTT solution (200 µL, 5 mg MTT in 1 mL PBS buffer and 10 mL DMEM) added. After 3 h of incubation MTT solution (155 µL) was removed, DMSO (90 µL) added and the plate incubated in a shaker (37 °C, 150 U, 10 min; Multitron II, Infors HT). The absorbance of each well was measured at 535 nm using a microplate reader (Spectramax M5, Molecular Devices). The half-maximal inhibitory concentration (IC50 = 100 µM) was determined by a plot of the viability versus the Ru2 concentration.

Figure S10. Cell viability in correlation to concentrations of the quinoline complex Ru2 after an incubation time of 24 h (IC50 = 100 µM). Data points were fitted to a sigmoidal equation.
General Procedure for the Deprotection of Caged Rhodamine: The catalytic deprotection of bis-N-(allyloxycarbonyl)-rhodamine 110 (3) to rhodamine 110 (4) inside HeLa cells was analyzed by live-cell imaging with a confocal fluorescence microscope (TCS SP8X, Leica). For this HeLa cells were adjusted to a concentration of 350,000 cells/dish, seeded on imaging dishes (35 mm/12 mm/180 µm, ibiTreat standard bottom, ibidi) with DMEM (2.0 mL) and incubated for 24 h. Then a solution of caged fluorophore 3 (10 µL, 100 mM in DMSO) and DMSO (10 µL) were added to the dish. After careful agitation the dish was incubated for 30 min. Subsequently the medium was removed, the cells washed with PBS buffer (2 × 1.0 mL) and then a solution of fresh DMEM (1 mL) with a solution of catalyst (4 µL, 5 mM in DMSO) and DMSO (6 µL) were added. For the reaction with [Cp*Ru(COD)Cl], catalyst solution (4 µL, 5 mM in DMSO), thiophenol solution (1.25 µL, 250 mM in DMSO) and DMSO (5 µL) in DMEM (1 mL) was added and for the negative control reaction, DMSO (10 µL) in DMEM (1 mL). The imaging dish was placed in the fluorescence microscope and incubated at 37 °C. The increase of green fluorescence (λ<sub>ex</sub> 488 nm, λ<sub>em</sub> 500–555 nm) was measured in intervals of 30 s. The intensity increase was evaluated with ImageJ 1.46r (Wayne Rasband) and is given as a mean value of 8 cells. Data points were fitted to a sigmoidal equation.

General Procedure for the Deprotection of Caged Doxorubicin: For the catalytic deprotection of N-(allyloxycarbonyl)-doxorubicin (5) to doxorubicin (6) it was followed a similar procedure as described for the cell viability assay. For this HeLa cells were adjusted to a concentration of 9.000 cells/well, seeded in translucent 96-well microplates (Microtest 96, BD Falcon) with DMEM (100 µL) and incubated for 24 h. Serial dilutions of caged doxorubicin 5 with DMSO (2%) in DMEM (100 µL) were added to each well and incubated for 3 h. Subsequently the medium was removed, the cells washed with PBS buffer (200 µL) and then a solution of fresh DMEM (100 µL) with catalyst (20 µM) and DMSO (1%) was added. To determine the toxicity of the catalyst, the cells were incubated with DMSO (1%) instead of caged doxorubicin 5. For the toxicity of caged doxorubicin 5, the cells were incubated with DMSO (1%) instead of the catalyst and for the toxicity of uncaged doxorubicin (6) the cells were incubated with 6 instead of 5 and with DMSO (1%) instead of the catalyst. After a final incubation time of 24 h the cell survival rate was determined with the MTT method as described above.
Deprotection of Caged Doxorubicin with Ru1:

Figure S11. Catalytic deprotection of N-(allyloxycarbonyl)-doxorubicin (5) with Ru1 inside HeLa cells. Reaction conditions: HeLa cells were grown in 96-well plates (9,000 cells/well); blank bar: negative control; cells were incubated with 1% DMSO for 3 h, washed with PBS buffer and incubated in fresh medium with Ru1 (20 µM) or PhSH (250 µM) for 24 h; black bar: catalytic in-cell activation; cells were incubated with 5 (50 µM) for 3 h, washed with PBS buffer and incubated in fresh medium with Ru1 (20 µM) for 24 h. Survival rates were determined through MTT method. The mean values of two independent experiments (9 wells each) are shown.
9 References


