Supporting Information

for

Triazol-substituted titanocenes by strain-driven 1,3-dipolar cycloadditions

Andreas Gansäuer*¹, Andreas Okkel¹, Lukas Schwach¹, Laura Wagner², Anja Selig², Aram Prokop³

Address: ¹Kekulé-Institut für Organische Chemie und Biochemie der Rheinischen Friedrich-Wilhelms-Universität Bonn, Gerhard-Domagk-Straße 1, D-53121 Bonn, ²Medizinische Klinik für Hämatologie, Onkologie und Tumorimmunologie Campus Vichow Klinikum Charité Berlin, Augustenburger Platz 1, D-13353 Berlin and³Abteilung für Kinderonkologie /-hämatologie Kinderkrankenhaus der Stadt Köln Amsterdamerstrasse 59, D-50735 Köln

Email: Andreas Gansäuer - andreas.gansaeuer@uni-bonn.de

Experimental procedures and compound characterization, cytotoxicity studies
Experimental Section:

All starting materials were purchased from commercial sources and used as received unless stated otherwise. Dichloromethane was dried prior to use over CaH₂. Diazides and amino azides A, B, C, D were synthesised as reported in literature [1-4].

Physical measurements and instrumentation:

¹H NMR and ¹³C NMR spectra were recorded on a DPX 300 and DPX 400 Bruker spectrometer; the chemical shifts (in ppm) are reported relative to nondeuterated solvent residual as reference. EI mass spectra were recorded on a MS 50 spectrometer from Kratos as well as on a MAT 95 spectrometer from Thermoquest. ESI mass spectra were recorded on a micrOTOF-Q spectrometer from Bruker Daltonik and IR spectra were recorded on an ATR Nicolet 380 spectrometer from Thermo Electron. Melting Points were measured on a Büchi 530 Melting Point and are uncorrected.

Determination of cell concentration and cell viability:

In a similar manner as described in [5], cell viability was determined by CASY® Cell Counter + Analyzer System of Schaerfe System GmbH (Reutlingen, Germany). Settings were specifically defined for the requirements of the used cells. With this system the cell concentration is analyzed simultaneously in three different size ranges: cell debris, dead cells, and viable cells were determined in one measurement. BJAB cells were seeded at a density of 1 × 10⁵ cells/mL and treated with different concentrations of a titanocene-derivate, non treated cells served as controls. After 24 h of incubation at 37 °C, 5% CO₂, cells were resuspended properly and 100 μL of each well was diluted in 10 mL CASYton (ready-to-use isotonic saline solution) for an immediate automated count of the cells.

Measurement of DNA fragmentation:

In a similar manner as described in [6], apoptotic cell death was determined by a modified cell cycle analysis, which detects DNA fragmentation on the single cell level. For measurement of DNA fragmentation cells were seeded at a density of 1 × 10⁵ cells/mL and treated with different concentrations of a titanocene-derivate. After 72 h of incubation at 37 °C, 5% CO₂, cells were collected by centrifugation at 1500 rpm for 5 min, washed with PBS at 4 °C, and fixed in PBS/2% (v/v) formaldehyde on ice for 30 min. After fixation, cells were incubated with ethanol/PBS (2:1, v/v) for 15 min, pelleted, and resuspended in PBS containing 50 μg/mL RNase A. After incubation for 30 min at 37 °C, cells were pelleted again and finally resuspended in PBS containing 50 μg/mL propidium iodide. Nuclear DNA fragmentation was then quantified by flow cytometric determination of hypodiploid DNA. Data were collected and analyzed using a FACScan (Becton Dickinson, Heidelberg, Germany) equipped with the CELLQuest software. Data are given in percentage of hypoploidy (subG1), which reflects the number of apoptotic cells.

AnnexinV-propidium iodide binding assay:

In a similar manner as described in [7], early apoptotic rates were assessed with flow
cytometry using the annexin V–fluorescein isothiocyanate/propidium iodide (PI) kit (BD Pharmingen, San Diego, CA, USA), in which annexin V bound to exposed phosphatidylserine of the early apoptotic cells, whereas PI stained the cells that had an increased membrane permeability, i.e., the late apoptotic cells. Samples were prepared according to the manufacturer’s instructions. Flow cytometry analysis was performed using a FACS-Calibur cytometer (Becton Dickinson, Heidelberg, Germany). The annexin-V+/PI- cells were defined as early apoptotic cells.

General procedure for the synthesis of azide-functionalized titanocenes from carboxylates

To a solution of the carboxylate (1 equiv.) in CH₂Cl₂ (3 mL/mmol) was added SOCl₂ (3 mL/mmol). After stirring for 3 h at r.t. excess SOCl₂ and solvent was removed in vacuo for 6 h at 45 °C. The resulting acid chloride was dissolved in CH₂Cl₂ (6 mL/mmol) and transferred via syringe to a suspension of NaH (10 equiv.) and amino azide (2 equiv.) in CH₂Cl₂ (10 mL/mmol). Stirring was continued for 16 h at r.t..

After filtration through Celite the volatiles were removed under reduced pressure and the residue was chromatographed on BioBeads S-X3 to yield the desired product.

Synthesis of 4: Carboxylate (313 mg, 1 mmol), SOCl₂ (3 mL), NaH (240 mg, 10 equiv.) and A (228 mg, 2 equiv.) to yield 4 (347 mg, 78%) over two steps;

M.p.: 72-76 °C (decomposition); ¹H-NMR (400 MHz, CDCl₃): δ = 11.93 (br. s, 1H), 7.21 – 7.13 (m, 1H), 7.00 – 6.86 (m, 1H), 6.68 (s, 5H), 6.60 – 6.53 (m, 1H), 5.95 – 5.89 (m, 1H), 3.30 (t, J = 6.0 Hz, 2H), 3.38 – 3.24 (m, 1H), 3.26 – 3.15 (m, 2H), 2.97 – 2.85 (m, 1H), 1.72 – 1.56 (m, 4H), 1.24 (s, 6H); ¹³C-NMR (100 MHz, CDCl₃): δ = 176.5, 150.2, 125.4, 121.4, 119.5, 117.2, 109.3, 51.0, 46.3, 41.2, 34.6, 30.3, 26.4, 26.4, 26.0; MS: (10.0 eV, ESI): m/z = 405.2 (100); HRMS (10.0 eV, ESI): calcd. for C₂₂H₂₉N₄OTi: 405.1766; found: 405.1765 [M – 2Cl + OCH₃⁺]; IR: ATR, ν [cm⁻¹] = 2935, 2870, 2090, 1610, 1550, 1440, 1370, 1285, 1190, 1000, 825, 730, 415.

Crystallized from CH₂Cl₂, Anal. calcd. for C₁₉H₂₆Cl₂N₄OTi (CH₂Cl₂): C 49.89, H 5.76, N 12.12; found: C 49.69, H 6.02, N 12.04.

Synthesis of 5: Carboxylate (313 mg, 1 mmol), SOCl₂ (3 mL), NaH (240 mg, 10 equiv.) and B (436 mg, 2 equiv.) to yield 5 (280 mg, 51%) over two steps;

M.p.: 155 °C (decomposition); ¹H-NMR (400 MHz, CDCl₃): δ = 11.92 (br. s, 1H), 7.04 (s, 1H), 6.83 – 6.79 (m, 1H), 6.70 (s, 5H), 6.58 (s, 1H), 5.98 (m, 1H), 3.74 – 3.56 (m, 13H), 3.49-3.39 (m, 1H), 3.38 (t, J = 5.1 Hz, 3H), 3.22 (d, J = 14.3 Hz, 1H), 2.91 (d, J = 14.4 Hz, 1H), 1.28 (s, 6H); ¹³C-NMR (100 MHz, CDCl₃): 176.3, 150.2, 124.9, 121.3, 120.0, 116.4, 108.9, 70.7, 70.6, 70.5, 70.4, 70.08, 68.6, 50.8, 46.5, 41.5, 34.4, 29.6, 26.6; MS (2.0 eV, ESI): m/z (%) = 509.2 (100), 475.2 (8); HRMS (2.0 eV, ESI): calcd. for C₂₂H₂₉N₄O₂⁴⁶Ti: 543.1854; found 543.1851 [M – Cl + OCH₃⁺]; IR: ATR, ν [cm⁻¹] = 2922, 2871, 2023, 1615, 1556, 1102, 826, 417; Crystallized from CH₂Cl₂, Anal. calcd. for C₂₃H₃₄Cl₂N₄OTi (CH₂Cl₂): C 45.45, H 5.72, N 8.83; found: C 45.52, H 5.82, N 8.84.
Synthesis of 6: Carboxylate (626 mg, 2 mmol), SOCl₂ (6 mL), NaH (480 mg, 10 equiv.) and C (649 mg, 2 equiv.) to yield 6 (878 mg, 89%) over two steps; M.p.: 185 °C (decomposition); ¹H-NMR (400 MHz, CDCl₃): δ = 12.78 (br. s, 1H), 7.44 (d, J = 8.1 Hz, 2H), 7.30 (d, J = 8.1 Hz, 2H), 6.94 – 6.90 (m, 1H), 6.86 – 6.82 (m, 1H), 6.54 – 6.49 (m, 1H), 6.46 (s, 5H), 6.03 – 5.99 (m, 1H), 4.51 (dd, J = 14.5 Hz, J = 6.4 Hz, 1H), 4.36 (dd, J = 14.5 Hz, J = 5.6 Hz, 1H), 4.30 (s, 2H), 3.33 (d, J = 14.4 Hz, 1H), 2.94 (d, J = 14.4 Hz, 1H), 1.28 (s, 3H), 1.20 (s, 3H); ¹³C-NMR (100 MHz, CDCl₃): δ = 176.6, 150.7, 136.8, 135.1, 128.9, 128.6, 124.7, 121.2, 120.7, 116.2, 109.4, 54.4, 47.1, 44.9, 34.8, 30.1, 26.1; MS (10.0 eV, ESI): m/z (%) = 453.2 (100); HRMS (10.0 eV, ESI): calcd. for C₉₂H₂₄N₂O₄⁴⁶Ti⁺: 453.1767; found: 453.1768 [M – 2Cl + OCH₃⁺]; IR: ATR, ν [cm⁻¹] = 2920, 2095, 1605, 1550, 1445, 1370, 1235, 1205, 1025, 825, 680, 560. Crystallized from CH₂Cl₂, Anal. calcd. for C₂₃H₂₆Cl₂N₄OTi(CH₂Cl₂): C 54.61, H 5.22, N 10.98; found: C 54.61, H 5.30, N 10.99.

Synthesis of 7: Carboxylate (352 mg, 1 mmol), SOCl₂ (3 mL), NaH (240 mg, 10 equiv.) and A (228 mg, 2 equiv.) to yield 7 (436 mg, 89%) over two steps; M.p.: 108 °C; ¹H-NMR (400 MHz, CDCl₃): δ = 12.30 (br. s, 1H), 6.99 – 6.94 (m, 1H), 6.94 – 6.90 (m, 1H), 6.66 (s, 5H), 6.63 – 6.58 (m, 1H), 6.13 – 6.08 (m, 1H), 3.45 (d, J = 13.8 Hz, 1H), 3.34 (t, J = 6.3 Hz, 2H), 3.38 – 3.29 (m, 1H), 3.28 – 3.19 (m, 1H), 2.78 (d, J = 13.9 Hz, 1H), 1.84 – 1.53 (m, 10H), 1.50 – 1.31 (m, 3H), 1.24 – 1.11 (m, 1H); ¹³C-NMR (75 MHz, CDCl₃): δ = 176.4, 150.7, 125.4, 121.2, 121.1, 116.0, 110.2, 51.0, 46.1, 41.0, 38.8, 38.4, 34.1, 26.4, 26.0, 25.4, 22.2, 21.7; MS (10.0 eV, ESI): m/z (%) = 445.2 (100); HRMS (8.0 eV, ESI): calcd. for C₂₃H₂₈N₂O₂⁴⁶Ti⁺: 445.2080; found: 445.2078 [M – 2Cl + OCH₃⁺]; IR: ATR, ν [cm⁻¹] = 2925, 2850, 2090, 1610, 1550, 1440, 1375, 1000, 825, 730. Crystallized from CH₂Cl₂, Anal. calcd. for C₂₃H₂₆Cl₂N₄OTi(CH₂Cl₂): C 51.81, H 5.98, N 10.79; found: C 52.04, H 6.27, N 10.92.

Synthesis of 8: Carboxylate (352 mg, 1 mmol), SOCl₂ (3 mL), NaH (240 mg, 10 equiv.) and C (324 mg, 2 equiv.) to yield 8 (334 mg, 63%) over two steps; M.p.: 108 °C; ¹H-NMR (400 MHz, CDCl₃): δ = 12.76 (t, J = 5.5 Hz, 1H), 7.44 (d, J = 8.0 Hz, 2H), 7.29 (d, J = 8.0 Hz, 2H), 7.11 – 7.05 (m, 1H), 6.92 – 6.87 (m, 1H), 6.49 (s, 5H), 6.45 – 6.41 (m, 1H), 6.17 – 6.11 (m, 1H), 4.47 (dd, J = 14.2 Hz, J = 6.2 Hz, 1H), 4.32 (dd, J = 13.9 Hz, J = 5.4 Hz, 1H), 4.29 (s, 2H), 3.46 (d, J = 13.3 Hz, 1H), 2.76 (d, J = 13.4 Hz, 1H), 1.86 – 1.05 (m, 10H); ¹³C-NMR (100 MHz, CDCl₃): δ = 176.6, 150.6, 136.7, 135.1, 128.8, 128.8, 124.8, 122.4, 121.1, 115.2, 110.6, 54.5, 46.9, 44.9, 38.7, 38.6, 33.9, 25.3, 22.3, 21.7; MS (8.0 eV, ESI): m/z (%) = 493.2 (100), 527.2 (5); HRMS (8.0 eV, ESI): m/z: calcd. for C₂₃H₃₃N₂O₄⁴⁶Ti⁺: 491.2124; found: 491.2124 [M – 2Cl + OCH₃⁺]; IR: ATR, ν [cm⁻¹] = 2925, 2850, 2090, 1605, 1555, 1435, 1375, 1240, 1020, 825, 730, 685. Crystallized from CH₂Cl₂, Anal. calcd. for C₂₆H₃₀Cl₂N₄OTi(CH₂Cl₂): C 57.18, H 5.57, N 10.18; found: C 57.13, H 5.57, N 10.24.
Synthesis of 9: Carboxylate (530 mg, 1.5 mmol), SOCl₂ (4.5 mL), NaH (360 mg, 10 equiv.) and D (486 mg, 2 equiv.) to yield 9 (635 mg, 71%) over two steps;

M.p.: 108 °C (decomposition); ¹H-NMR: (400 MHz, CDCl₃): δ = 12.76 (t, J = 5.5 Hz, 1H), 7.44 (d, J = 8.0 Hz, 2H), 7.29 (d, J = 8.0 Hz, 2H), 7.11 – 7.05 (m, 1H), 6.92 – 6.87 (m, 1H), 6.49 (s, 5H), 6.45 – 6.41 (m, 1H), 6.17 – 6.11 (m, 1H), 4.47 (dd, J = 14.2 Hz, J = 6.2 Hz, 1H), 4.32 (dd, J = 13.9 Hz, J = 5.4 Hz, 1H), 4.29 (s, 2H), 3.46 (d, J = 13.3 Hz, 1H), 2.76 (d, J = 13.4 Hz, 1H), 1.86 – 1.05 (m, 10H); ¹³C-NMR (100 MHz, CDCl₃): δ = 176.6, 150.6, 137.0, 135.1, 128.8, 128.8, 124.8, 122.4, 121.1, 115.2, 110.6, 54.5, 46.9, 44.9, 38.7, 38.6, 33.9, 25.3, 22.3, 21.7; MS: (8.0 eV, ESI): m/z (%) = 493.2 (100), 527.2 (5); HRMS (8.0 eV, ESI): calcd. for C₂₇H₃₃N₂O₂⁴⁶Ti⁺: 491.2124; found 491.2124 [M – 2Cl + OCH₃]⁺; Crystallized from CH₂Cl₂, Anal. calcd. for C₂₆H₃₀Cl₂N₂O₂Ti (CH₂Cl₂): C 57.18, H 5.57, N 10.18; found: C 57.15, H 5.93, N 10.12.

Synthesis of 10: Carboxylate (396 mg, 1 mmol), SOCl₂ (3 mL), NaH (240 mg, 10 equiv.) and B (436 mg, 2 equiv.) to yield 10 (323 mg, 51%) over two steps;

M.P. 165 °C (decomposition); ¹H-NMR (400 MHz; CDCl₃): δ = 11.07 (br. s, 1H), 7.14 (m, 1H), 6.70 (s, 5H), 6.49 – 6.42 (m, 1H), 6.33 (m, 1H), 5.98 (m, 1H), 3.91 – 3.83 (m, 1H), 3.74 – 3.56 (m, 12H), 3.50 – 3.42 (m, 1H), 3.37 (t, J = 4.5 Hz, 2H), 1.79 – 1.44 (m, 4H), 1.70 (s, 3H), 1.39 – 1.17 (m, 3H), 1.17 (s, 3H), 0.89 (t, J = 7.1 Hz, 3H), 0.82 (t, J = 7.1 Hz, 3H), 0.64 (m, 1H); ¹³C-NMR (100 MHz; CDCl₃): δ = 183.1, 149.9, 126.5, 120.8, 119.9, 116.9, 109.3, 70.7, 70.6, 70.4, 70.3, 70.0, 68.9, 50.8, 47.7, 46.6, 42.3, 39.4, 39.1, 23.8, 21.9, 17.8, 17.5, 15.2, 15.03; MS (8.0 eV, ESI): m/z (%) = 593.3 (100), 579.3 (48). HRMS (8.0 eV, ESI): calcd. for C₃₀H₄₈N₄O₅⁴⁶Ti⁺: 593.3182, found: 593.3180 [M – 2Cl + OCH₃]⁺; IR: ATR, ν [cm⁻¹] = 2956, 2869, 2098, 1585, 1525, 1440, 1348, 1282, 1022; Crystallized from CH₂Cl₂, Anal. calcd. for C₂₇H₄₆Cl₂N₄O₄Ti (CH₂Cl₂): C 50.16, H 6.73, N 7.80; found: C 50.19, H 6.77, N 7.83.

Synthesis of 11: Carboxylate (794 mg, 2 mmol), SOCl₂ (6 mL), NaH (480 mg, 10 equiv.) and D (649 mg, 2 equiv.) to yield 11 (357 mg, 31%) over two steps;

M.p.: 94 °C (decomposition); ¹H-NMR (400 MHz; CDCl₃): δ = 11.88 (br. s, 1H), 7.39 – 7.27 (m, 4H), 7.19 – 7.11 (m, 1H), 6.44 – 6.36 (m, 2H), 6.25 (s, 5H), 6.03 – 5.98 (m, 1H), 4.77 (dd, J = 15.1 Hz, J = 6.1 Hz, 1H), 4.68 (d, J = 13.9 Hz, 1H), 4.53 (d, J = 13.9 Hz, 1H), 4.39 (dd, J = 15.1 Hz, J = 4.8 Hz, 1H), 1.82 (s, 3H), 1.78 – 1.14 (m, 7H), 1.11 (s, 3H), 0.89 (t, J = 7.1 Hz, 3H), 0.82 (t, J = 7.1 Hz, 3H), 0.69 – 0.55 (m, 1H); ¹³C-NMR (75 MHz, CDCl₃): δ = 184.4, 150.1, 135.9, 133.2, 129.8, 128.9, 128.0, 127.4, 125.7, 121.1, 120.7, 117.7, 110.7, 52.7, 48.5, 46.9, 42.8, 38.8, 38.8, 23.7, 21.9, 18.0, 17.6, 15.2, 14.9; MS (8.0 eV, ESI): m/z (%) = 541.2 (100), 537.3 (84), 523.3 (5), 445.2 (40), 431.2 (10); HRMS (8.0 eV, ESI): calcd. for C₃₀H₄₁N₂O₂⁴⁶Ti⁺: 535.2750; found 535.2753 C₃₀H₄₁N₂O₂⁴⁶Ti⁺ = [M – 2Cl + OCH₃]⁺; IR: ATR, ν [cm⁻¹] = 2960, 2870, 2095, 1575, 1520, 1360, 1255, 1010, 825, 725; Crystallized from CH₂Cl₂, Anal. calcd. for C₂₉H₃₈Cl₂N₂OTi (CH₂Cl₂): C 59.65, H 6.57, N 13.31; found: C 59.73, H 6.37, N 13.22.
General Procedure for the Synthesis of Triazoles from Azides

To a solution of the carboxylate (1 equiv.) in CH₂Cl₂ (10 mL/mmol) was added cyclooctyne (5 equiv.). After stirring for 16 h at r.t. the solvent was removed and the residue washed with cyclohexane and chromatographed on BioBeads S-X3 to yield the desired product.

Synthesis of 12: Azide 4 (99 mg, 0.22 mmol) with cyclooctyne (119 mg) to yield 12 (97 mg, 80%);
M.p.: 82 °C; ¹H-NMR (400 MHz, CDCl₃): δ = 12.07 (br. s, 1H), 7.24 – 7.17 (m, 1H), 6.82 – 6.74 (m, 1H), 6.66 (s, 5H), 6.59 – 6.52 (m, 1H), 5.93 – 5.86 (m, 1H), 4.27 – 4.19 (m, 2H), 3.35 – 3.20 (m, 2H), 3.16 (d, J = 14.4 Hz, 1H), 2.84 (m, 3H), 2.77 - 2.71 (m, 2H), 1.99 – 1.88 (m, 2H), 1.82 – 1.74 (m, 2H), 1.71 – 1.56 (m, 4H), 1.51 – 1.34 (m, 4H), 1.25 (s, 3H), 1.19 (s, 3H); ¹³C-NMR (100 MHz, CDCl₃): δ = 176.5, 149.6, 144.3, 133.7, 125.6, 121.3, 119.2, 117.4, 109.0, 47.2, 45.7, 40.9, 34.4, 29.5, 28.3, 27.3, 26.9, 26.3, 26.0, 25.5, 24.8, 24.4, 21.7; MS (8.0 eV, ESI): m/z (%) = 517.2 (7), 513.3 (100), 499.3 (3), 375.2 (3); HRMS (8.0 eV, ACN, ESI): calcd. for C₂₇H₃₈Cl₄O⁴⁺Ti: 517.2211, found 517.2207 [M – Cl]⁺; IR: ATR, ν [cm⁻¹] = 2925, 2850, 1610, 1550, 1443.651, 1370, 1290, 1200, 825, 725; Crystallized from CH₂Cl₂. Anal. calcd. for C₂₇H₃₈Cl₄N₄OTi (CH₂Cl₂): C 52.68, H 6.32, N 8.78; found: C 52.69, H 6.55, N 8.74.

Synthesis of 13: Azide 4 (220 mg, 0.4 mmol) with cyclooctyne (216 mg) to yield 13 (231 mg, 88%);
M.p.: >200 °C; ¹H-NMR (400 MHz, CDCl₃): 12.00 (br. s, 1H), 7.15 - 7.08 (m, 1H), 7.04 - 6.92 (m, 1H), 6.75 (s, 5H), 6.65 - 6.55 (m, 1H), 6.11 - 5.94 (m, 1H), 4.71 - 4.52 (m, 2H), 4.01 - 3.95 (m, 2H), 3.77 (d, J = 9.8 Hz, 1H), 3.69 - 3.48 (m, 10H), 3.40 - 3.34 (m, 1H), 3.25 - 3.19 (m, 1H), 3.07 - 3.03 (m, 2H), 2.93 - 2.89 (m, 2H), 2.82 - 2.74 (m, 1H), 1.93 - 1.78 (m, 4H), 1.52 (m, 4H), 1.31 (s, 6H), ¹³C-NMR (100 MHz, CDCl₃): δ = 176.2, 150.4, 142.6, 136.5, 124.7, 121.4, 120.4, 116.6, 109.3, 70.7, 70.7, 70.6, 70.5, 69.6, 68.6, 49.3, 46.7, 41.6, 34.4, 29.7, 27.9, 26.5, 26.0, 25.9, 24.6, 23.6, 22.0; MS (7.0 eV, ESI): m/z (%) = 617.3 (100); HRMS (8.0 eV, ESI): calcd. for C₃₂H₄₆Cl₄N₄O⁵⁺Ti: 651.2793 found: 651.2791 [M – Cl + OCH₃]⁺; IR: ATR, ν [cm⁻¹] = 3379, 2023, 1615, 1556, 1102, 826; Crystallized from CH₂Cl₂. Anal. calcd. for C₃₀H₃₆Cl₂N₄O₄Ti (CH₂Cl₂): C 51.77, H 6.52, N 7.55, found: C 51.76, H 6.52, N 7.57.

Synthesis of 14: Azide 6 (115 mg, 0.23 mmol) with cyclooctyne (120 mg) to yield 14 (126 mg, 78%);
M.p. 152 °C; ¹H-NMR (400 MHz, CDCl₃): δ = 12.81 – 12.73 (m, 1H), 7.38 (d, J = 8.0 Hz, 2H), 7.13 (d, J = 8.0 Hz, 2H), 7.00 – 6.94 (m, 1H), 6.88 – 6.79 (m, 1H), 6.54 – 6.48 (m, 1H), 6.45 (s, 5H), 6.03 – 5.96 (m, 1H), 5.44 (s, 2H), 4.47 (dd, J = 14.6 Hz, J = 6.2 Hz, 1H), 4.32 (dd, J = 14.6 Hz, J = 5.3 Hz, 1H), 3.27 (d, J = 14.3 Hz, 1H), 2.96
\[2.87 \text{ (m, 3H)}, 2.63 - 2.59 \text{ (m, 2H)}, 1.76 - 1.68 \text{ (m, 2H)}, 1.60 - 1.52 \text{ (m, 2H)}, 1.43 - 1.32 \text{ (m, 4H)}, 1.25 \text{ (s, 3H)}, 1.19 \text{ (s, 3H)}; \]
\[\text{\textsuperscript{13}C-NMR: (100 MHz, CDCl\textsubscript{3})} \delta = 176.6, 150.5, 145.2, 136.6, 135.2, 133.6, 128.6, 127.6, 124.8, 121.2, 120.6, 116.4, 109.4, 51.5, 46.8, 44.7, 34.8, 29.9, 28.2, 26.2, 26.1, 25.9, 24.7, 24.6, 21.9; MS (8.0 eV, ESI): m/z (%) = 561.3 (100); HRMS (8.0 eV, ESI): calcd. for C\textsubscript{32}H\textsubscript{41}N\textsubscript{4}O\textsubscript{2}\textsuperscript{47}Ti\textsuperscript{+}: 560.2742, found 560.2754 [M – 2Cl + OCH\textsubscript{3}]\textsuperscript{+}; IR: ATR, \[\nu \text{ [cm}^{-1}] = 2920, 2850, 1600, 1555, 1435, 1370, 1020, 825, 600, 560;\]
Crystallized from CH\textsubscript{2}Cl\textsubscript{2}, Anal. calcd. for C\textsubscript{31}H\textsubscript{38}Cl\textsubscript{2}N\textsubscript{4}OTi (CH\textsubscript{2}Cl\textsubscript{2}): C 56.00, H 5.87, N 8.16, found: C 56.02, H 5.90, N 8.10.

**Synthesis of 15:** Azide 8 (267 mg, 0.5 mmol) with cyclooctyne (270 mg) to yield 15 (295 mg, 79%).

M.p.: 174 °C; \[\text{\textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3})} \delta = 12.90 \text{ (t, J = 5.8 Hz, 1H)}, 7.39 \text{ (d, J = 8.0 Hz, 2H)}, 7.13 \text{ (d, J = 8.0 Hz, 2H)}, 6.90 \text{ (m, 1H)}, 6.84 \text{ (m, 1H)}, 6.45 \text{ (m, 1H)}, 6.43 \text{ (s, 5H)}, 6.13 \text{ (s, 1H)}, 5.44 – 5.41 \text{ (s, 2H)}, 4.45 \text{ (dd, J = 14.3 Hz, J = 6.0 Hz, 1H)}, 4.29 \text{ (dd, J = 14.4 Hz, J = 5.2 Hz, 1H)}, 3.43 \text{ (d, J = 13.5 Hz, 1H)}, 2.90 – 2.87 \text{ (m, 2H)}, 2.70 \text{ (d, J = 13.6 Hz, 1H)}, 2.63 – 2.57 \text{ (m, 2H)}, 1.77 – 1.49 \text{ (m, 10H)}, 1.45 – 1.26 \text{ (m, 7H)}, 1.17 – 1.06 \text{ (m, 1H)}; \[\text{\textsuperscript{13}C-NMR (100 MHz, CDCl\textsubscript{3})} \delta = 176.6, 150.3, 145.1, 136.8, 135.2, 133.6, 128.7, 127.5, 125.1, 122.0, 121.1, 115.6, 110.7, 51.5, 46.6, 44.7, 38.6, 38.4, 34.1, 28.2, 26.1, 25.8, 25.3, 24.7, 24.5, 22.2, 21.9, 21.7; MS (8.0 eV, ESI): m/z (%) = 635.3 (2), 601.3 (100), 463.2 (2); HRMS (8.0 eV, ESI): calcd. for C\textsubscript{35}H\textsubscript{45}N\textsubscript{4}O\textsubscript{2}\textsuperscript{46}Ti\textsuperscript{+}: 599.3063; found: 599.3063 [M – 2Cl + OCH\textsubscript{3}]\textsuperscript{+}; IR: ATR, \[\nu \text{ [cm}^{-1}] = 2925, 2850, 1605, 1555, 1435, 1375, 1235, 1020, 915, 825, 725;\]
Crystallized from CH\textsubscript{2}Cl\textsubscript{2}, Anal. calcd. for C\textsubscript{34}H\textsubscript{42}Cl\textsubscript{2}N\textsubscript{4}OTi (CH\textsubscript{2}Cl\textsubscript{2}): C 56.63, H 6.00, N 7.49, found: C 56.75, H 5.99, N 7.40.

**Synthesis of 16:** Azide 9 (267 mg, 0.5 mmol) with cyclooctyne (270 mg) to yield 16 (279 mg, 79%).

M.p.: 178 °C; \[\text{\textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3})} \delta = 12.74 \text{ (br. s, 1H)}, 7.49 \text{ (d, J = 7.4 Hz, 1H)}, 7.36 \text{ (dd, J = 7.5 Hz, J = 7.5 Hz, 1H)}, 7.26 \text{ (dd, J = 7.5 Hz, J = 7.5 Hz, 1H)}, 7.09 – 6.99 \text{ (m, 1H)}, 6.91 \text{ (d, J = 7.5 Hz, 1H)}, 6.92 – 6.86 \text{ (m, 1H)}, 6.50 \text{ (s, 5H)}, 6.41 \text{ (m, 1H)}, 6.15 \text{ (m, 1H)}, 5.74 \text{ (d, J = 15.7 Hz, 1H)}, 5.61 \text{ (d, J = 15.7 Hz, 1H)}, 4.66 \text{ (dd, J = 14.8 Hz, J = 6.4 Hz, 1H)}, 4.32 \text{ (dd, J = 14.8 Hz, J = 4.6 Hz, 1H)}, 3.30 \text{ (d, J = 13.5 Hz, 1H)}, 2.93 – 2.89 \text{ (m, 2H)}, 2.92 – 2.83 \text{ (m, 1H)}, 2.78 - 2.69 \text{ (m, 1H)}, 2.47 \text{ (d, J = 13.5 Hz, 1H)}, 1.83 – 1.58 \text{ (m, 8H)}, 1.56 – 1.27 \text{ (m, 9H)}, 1.15 – 1.01 \text{ (m, 1H)}; \[\text{\textsuperscript{13}C-NMR (100 MHz, CDCl\textsubscript{3})} \delta = 176.8, 150.7, 144.7, 135.3, 134.8, 133.3, 130.7, 129.3, 128.8, 124.2, 123.2, 121.4, 114.8, 110.2, 50.2, 47.6, 42.9, 38.9, 38.5, 33.8, 28.4, 26.3, 25.7, 25.3, 24.7, 24.6, 22.3, 22.2, 21.8; MS: (8.0 eV, ESI): m/z (%) = 601.3 (100), 450.2 (2); HRMS (8.0 eV, ESI): calcd. for C\textsubscript{35}H\textsubscript{45}N\textsubscript{4}O\textsubscript{2}\textsuperscript{46}Ti\textsuperscript{+}: 599.3063; found: 599.3067 [M – 2Cl + OCH\textsubscript{3}]\textsuperscript{+}; IR: ATR, \[\nu \text{ [cm}^{-1}] = 2925, 2850, 1600, 1555, 1440, 1375, 1235, 1015, 825, 730, 685;\]
Crystallized from CH\textsubscript{2}Cl\textsubscript{2}, Anal. calcd. for C\textsubscript{34}H\textsubscript{42}Cl\textsubscript{2}N\textsubscript{4}OTi (CH\textsubscript{2}Cl\textsubscript{2}): C 59.46, H 6.24, N 7.99; found: C 59.41, H 6.43, N 7.94.
Synthesis of 17: Azide 10 (267 mg, 0.5 mmol) with cyclooctyne (270 mg) to yield 17 (341 mg, 92%);

M.p.: >200 °C; $^1$H-NMR (400 MHz; CDCl$_3$): $\delta = 11.00$ (br. s, 1H), 7.35 - 7.30 (m, 1H), 6.71 (s, 5H), 6.56 - 6.50 (m, 1H), 6.44 - 6.40 (m, 1H), 5.96 - 5.86 (m, 1H), 4.43 - 4.33 (m, 2H), 3.90 - 3.77 (m, 3H), 3.73 - 3.60 (m, 3H), 3.60 - 3.53 (m, 2H), 3.53 - 3.38 (m, 6H), 2.95 - 2.87 (m, 2H), 2.82 - 2.77 (m, 2H), 1.79 (m, 2H), 1.76 - 1.69 (m, 4H), 1.66 (s, 3H), 1.62 - 1.38 (m, 8H), 1.31 - 1.18 (m, 2H), 1.12 (s, 3H), 0.86 (t, $J = 7.1$ Hz, 3H), 0.80 (t, $J = 7.1$ Hz, 3H), 0.66 - 0.56 (m, 1H), $^{13}$C-NMR (400 MHz; CDCl$_3$): $\delta = 183.1$, 149.7, 143.9, 134.9, 126.5, 120.9, 119.8, 117.6, 110.0, 70.7, 70.5, 70.3, 70.2, 69.8, 68.9, 48.1, 47.6, 46.5, 42.2, 39.4, 39.1, 28.4, 26.3, 26.0, 24.8, 24.4, 23.9, 21.8, 21.7, 17.7, 17.5, 15.2, 14.9; MS (8.0 eV, ESI): m/z (%) = 701.4 (100), 687.4 (44); HRMS (8.0 eV, ESI): calcd. for C$_{39}$H$_{61}$N$_4$O$_5$Ti: 701.4121; found: 701.4120 [M - 2Cl + OCH$_3$]; IR: ATR, $\nu$ [cm$^{-1}$] = 2923, 2850, 1599, 1555, 1443, 1378, 1235, 1015; Crystallized from CH$_2$Cl$_2$, Anal. calcd. for C$_{37}$H$_{58}$Cl$_2$N$_4$O$_4$Ti (CH$_2$Cl$_2$): C 55.22, H 7.36, N 6.78; found: C 55.20, H 7.33, N 7.76.

Synthesis of 18: Azide 11 (289 mg, 0.5 mmol) with cyclooctyne (270 mg) to yield 18 (257 mg, 75%);

M.p.: 181 °C; $^1$H-NMR (400 MHz, CDCl$_3$): $\delta = 11.95$ (br. s, 1H), 7.44 (d, $J = 7.3$ Hz, 1H), 7.32 (dd, $J = 7.2$ Hz, $J = 7.2$ Hz, 1H), 7.22 (dd, $J = 7.2$ Hz, $J = 7.2$ Hz, 1H), 7.11 - 7.06 (m, 1H), 6.72 (d, $J = 7.5$ Hz, 1H), 6.43 - 6.40 (m, 2H), 6.39 (s, 5H), 6.06 - 6.02 (m, 1H), 5.89 (d, $J = 16.2$ Hz, 1H), 5.55 (d, $J = 16.0$ Hz, 1H), 4.93 (dd, $J = 14.7$ Hz, $J = 6.4$ Hz, 1H), 4.44 (dd, $J = 14.8$ Hz, $J = 4.0$ Hz, 1H), 2.98 - 2.87 (m, 2H), 2.84 - 2.70 (m, 2H), 1.79 (s, 3H), 1.76 - 1.41 (m, 11H), 1.33 - 1.13 (m, 4H), 1.10 (s, 3H), 0.86 (t, $J = 7.1$ Hz, 3H), 0.83 (t, $J = 7.1$ Hz, 3H), 0.62 (m, 1H); $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta = 184.4$, 150.3, 145.1, 134.4, 134.3, 133.7, 128.6, 128.5, 128.5, 127.8, 125.7, 121.2, 120.9, 117.5, 110.5, 49.2, 48.6, 47.0, 43.2, 38.9, 38.8, 28.4, 26.2, 26.1, 24.8, 24.7, 23.8, 21.9, 21.9, 18.2, 17.7, 15.1, 15.0; MS (8.0 eV, ESI): m/z (%) = 645.4 (100), 631.3 (3); HRMS (8.0 eV, ESI): calcd. for C$_{38}$H$_{53}$N$_4$O$_2$Ti: 645.3646; found: 645.3646 [M - 2Cl + OCH$_3$]; IR: ATR, $\nu$ [cm$^{-1}$] = 2930, 2870, 1570, 1520, 1440, 1400, 1360, 1235, 1195, 1050, 1010, 825, 730; Crystallized from CH$_2$Cl$_2$, Anal. calcd. for C$_{37}$H$_{50}$Cl$_2$N$_4$OTi (CH$_2$Cl$_2$): C 59.23, H 6.80, N 7.27; found: C 59.16, H 6.88, N 7.28.
Figure S1: Apoptosis-induction by 15

Figure S2: Viability of 15
Figure S3: Apoptosis-induction by 16

Figure S4: Viability of 16
Figure S5: Apoptosis-induction by 18

Figure S6: Viability of 18
References:

[1] Thomas, J. R.; Liu, X.; Hergenrother, P. J. *J. Am. Chem. Soc.* **2005**, *127*, 12434–12435.

[2] Hou, Z.-S.; Tan, Y.-B.; Kim, K.; Zhou, Q.-F. *Polymer* **2006**, *47*, 742–750.

[3] Lau, K.-N.; Chow, H.-F.; Chan, M.-C.; Wong, K.-W. *Angew. Chem. Int. Ed.* **2008**, *47*, 6912–6916.

[4] Risseeuw, M. D. P.; De Clercq, D. J. H.; Lievens, S.; Hillaert, U.; Sinnaeve, D.; Van den Broeck, F.; Martins, J. C.; Tavernier, J.; Van Calenbergh, S. *ChemMedChem* **2013**, *8*, 521–526.

[5] Shults, E. E.; Velder, J.; Schmalz, H.-G.; Chernov, S. V.; Rubalova, T. V.; Gatilov, Y. V.; Henze, G.; Tolstikov, G. A.; Prokop, A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4228-4232.

[6] Essmann, F.; Wieder, T.; Otto, A.; Müller, E.-C.; Dörken, B.; Daniel, P. T. *Biochem. J.* **2000**, *346*, 777-783.

[7] Yang, J.; Li, H.; Chen, Y.-Y; Wang, X.-J.; Shi, G.-Y.; Hu, Q.-S.; Kang, X.-L.; Lu, Y.; Tang, X.-M.; Guo, Q.-S.; Yi, J. *Free Radical Biol. Med.* **2004**, *37*, 2027-2041.