Supporting Information

Zinc Complex Mediated Regioselective *O*-Acylation of Therapeutic Agents

Rong Tong and Jianjun Cheng*

Department of Materials Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801

Materials and methods

Materials. BDI ligands and the corresponding metal catalysts **1-3** were prepared by following the published procedure[1] and stored at –30°C in a glovebox. All anhydrous solvents were purified by passing them through an alumina column, kept anhydrous by 4 Å molecular sieves and stored in a glovebox. Paclitaxel (Ptxl), (S)-(+)-camptothecin (Cpt), dasatinib (Dasa) and rapamycin (Rapa) were purchased from the LC Laboratories (Woburn, MA, USA), and used as received. All other chemicals were purchased from Sigma-Aldrich (St Louis, MO, USA) and used as received unless otherwise noted. Triethylamine (TEA) was dried with 4 Å molecular sieves overnight, transferred by a cannula under nitrogen to a clean flask containing CaH_2, refluxed overnight, distilled and collected under nitrogen, and stored in a glovebox with 4 Å molecular sieves. Methacrylic anhydride (MA) was dried with CaH_2 for 24 h, vacuum transferred to a flask containing activated 4 Å molecular sieves, and then fractionally distilled under reduced pressure. The entire process was repeated twice. The freshly purified MA was stored in the freezer of a glovebox at -30°C before use. Succinic anhydride (SA) and chloroacetic anhydride (ClA) were recrystallized from chloroform and stored in glovebox. Pent-4-ynoic anhydride (PA) was synthesized by following literature reported procedures.[2] PA was freshly distilled twice under reduced pressure and stored in the freezer of a glovebox at -30°C before use.

Instruments. The low resolution electrospray ionization mass spectrometry (LR-ESI MS) experiments were conducted on a Waters Quattro II mass spectrometer. The high resolution electrospray ionization mass spectrometry (HR-ESI MS) experiments were performed on a Micromass Q-TOF Ultima system. HPLC analysis was performed on a System Gold system (Beckman Coulter, Fullerton, CA) equipped with a 126P solvent module, a System Gold 128 UV detector and an analytical pentafluorophenyl column (Curosil-PFP, 250 × 4.6 mm, 5 μ, Phenomenex, Torrance, CA) or an analytical C18 column (Luna C18(2), 250 × 4.6 mm, 5 μ, Phenomenex, Torrance, CA). The UV wavelengths for the analysis of Ptxl, Rapa, Cpt,

Dasa, Doxo were 227, 280, 370, 400, 450 nm, respectively. NMR analyses were conducted on a Varian U500, VXR500 or UI500NB (500 MHz). Analytical TLC experiments were performed on 0.25-mm silica gel 60 plates with a 254 nm fluorescent indicator (Merck). Plates were developed in a covered chamber and visualized by UV light and by treatment with anisaldehyde stain followed by heating. Preparative thin layer chromatography (prep TLC) was conducted with silica gel plates (1500 μm thickness, Sigma-Aldrich) with a 254 nm fluorescent indicator.

Cell culture. PC-3 cells (ATCC, Manassas, VA, USA) were cultured in Ham's F12K medium containing 10% FBS (Fetal Bovine Serum), 1000 units/mL aqueous Penicillin G and 100 μg/mL streptomycin. NTera-2 cells ATCC, Manassas, VA, USA) were cultured in Dulbecco's Modified Eagle's Medium (ATCC, Catalogue 30-2002) containing 10% FBS (Fetal Bovine Serum), 1000 units/mL aqueous Penicillin G and 100 μg/mL streptomycin. The media were replaced every day.

***O*-Acylation of Naph.** *General procedures.* In a glovebox, **1** (6.4 mg, 0.01 mmol) was dissolved in anhydrous THF (200 μL). This solution was added to a clean vial containing Naph (1.7 mg, 0.01mmol). The reaction mixture was diluted with THF (300 μL) and stirred for 15 min. SA (1.1 mg, 0.011 mmol) in THF (300 μL) was added to the mixture of Naph and **1** at $[SA]_0$ of 0.01 M. The reaction vial was tightly sealed, immediately moved out of the glovebox, and put in a 40°C oil bath. The reaction mixture was stirred for an additional 4 h and quenched with ice-cold methanol (1 mL). An aliquot of this solution was analyzed by HPLC equipped with an analytical C18 column (Luna C18(2), 250 × 4.6 mm, 5 μ, Phenomenex, Torrance, CA). The mobile phase for the HPLC analysis was a mixed solvent of equal volume of acetonitrile and water with 0.1% TFA. All HPLC spectra were recorded and analyzed with a UV detector at 280 nm. The areas of Naph and Naph-anhydride peaks were integrated and used for their quantification of the yield.

***O*-Acylation of Dasa with MA.** Dasa (4.8 mg, 0.01 mmol) was mixed with **1** at 1:1 molar ratio in a glove box. The mixture was stirred for 20 min. MA was distilled freshly before using and stored in glove box fridge. MA (1.2 equiv) in THF (0.5 mL) was added into the mixture and further stirring over 10 h at room temperature. The solution was quenched by methanol and dried under vacuum. The solid was re-dispersed with 0.5 mL methanol solution. On TLC plates with 1.5 mm thickness, the concentrated solution was applied, and developed by ethyl acetate/methanol (9/1) (R_f = 0.3). The silica gel was scratched from plates and extracted by methanol (2 × 20 mL). The solution was dried under vacuum and used for NMR analysis.

^{1}H NMR (CD_3OD, 500 MHz): δ 8.16 (s, 1H), 7.37 (d, 1H), 7.27 (dd, 1H), 7.25 (d, 1H), 6.17 (s, 1H), 6.14, 5.77 (s, $C(CH_3)=CH_2$), 4.37 (d, 2H), 3.67 (m, 4H), 2.79 (m, 2H), 2.67 (m, 4H), 2.49 (s, 3H), 2.34 (s,

3H), 1.97 (s, C(C*H*$_3$)=CH$_2$). ESI-MS (low resolution, positive mode): calcualted for $C_{26}H_{30}N_7O_3SCl$, *m*/*z* 555.1 $[M+H]^+$, found 555.1 $[M+H]^+$.

***O*-Acylation of Cpt.** *General procedures.* In a glove box, **3** (6.2 mg, 0.01 mmol) was dissolved in anhydrous THF (200 μL). This solution was added to a clean vial containing Cpt (3.5 mg, 0.01mmol). The reaction mixture was diluted with THF (300 μL) and stirred for 15 min until Cpt was completely dissolved. MA (1.70 mg, 0.011 mmol) in THF (300 μL) was dropwise added to the mixture of Cpt and **3** at $[MA]_0$ of 0.01 M. The reaction vial was tightly sealed, immediately moved out of glove box, and put in a 40 ^oC oil bath. The reaction mixture was stirred for an additional 4 h and quenched with ice-cold methanol/acetic acid (1.1 mL, v/v = 10/1). An aliquot of this solution was analyzed by HPLC equipped with an analytical C18 column (Luna C18(2), 250 × 4.6 mm, 5 μ, Phenomenex, Torrance, CA). The mobile phase for the HPLC analysis was a mixed solvent of equal volume of acetonitrile and water with 0.1% TFA. All HPLC spectra were recorded and analyzed with a UV detector at 370 nm. The areas of Cpt and Cpt-MA peaks were integrated and used for their quantification using the corresponding standard curves. An aliquot of the reaction mixture was used for MS analysis.

Pure Cpt-MA used for NMR analysis was separated by preparative thin layer chromatography (prep TLC, silica gel matrix with UV254, 1500 μm thickness, Aldrich) and developed by ethyl acetate/dichloromethane/methanol (1/9/1, v/v/v). The R_f values of Cpt and Cpt-MA were about 0.1 and 0.3, respectively. The silica gels containing Cpt-MA were collected from the glass plate; the Cpt-MA in the gel was extracted with methanol (2 × 30 mL). The methanol solution was then removed under vacuum; the resulting Cpt-MA was analyzed by ^{1}H-NMR.

Cpt-MA. ^{1}H-NMR (CD_3OD, 500 MHz): δ 8.63 (s, 1H, 7-H), 8.21 (d, J = 8.5 Hz, 1H, 12-H), 8.07 (d, J = 8.0 Hz, 1H, 9-H), 7.87 (td, J_t = 8.5 Hz, J_d = 1.5 Hz, 1H, 11-H), 7.71 (td, J_t = 8.0 Hz, J_d = 1.0 Hz, 1H, 10-H), 7.43 (s, 1H, 14-H), 6.33, 5.83 (s, C(CH_3)=C*H*$_2$), 5.63, 5.49 (AB, J_{AB} = 17.0 Hz, 2H, 17-H), 5.35 (s, 1H, 5-H), 2.26 (m, 2H, 18-H), 1.98 (s, C(C*H*$_3$)=CH$_2$), 1.04 (t, J = 8.0 Hz, 19-H). MS (LR-ESI, positive mode): calcualted for $C_{24}H_{20}N_2O5$ *m*/*z* 417.1 $[M+H]^+$; found 417.3 $[M+H]^+$.

Cpt-SA. ^{1}H NMR (CD_3OD, 500 MHz): δ 8.63 (s, 1H, 7-H), 8.21 (d, J = 8.5 Hz, 1H, 12-H), 8.07 (d, J = 8.0 Hz, 1H, 9-H), 7.87 (td, J_t = 8.5 Hz, J_d = 1.5 Hz, 1H, 11-H), 7.71 (td, J_t = 8.0 Hz, J_d = 1.0 Hz, 1H, 10-H), 7.43 (s, 1H, 14-H), 5.57, 5.44 (AB, J_{AB} = 17.0 Hz, 2H, 17-H), 5.35 (s, 1H, 5-H), 2.81 (t, J = 7.5 Hz, 2H, -C*H*$_2$-COOH), 2.55 (t, J = 7.5 Hz, 2H, -C*H*$_2$-CH$_2$-COOH), 2.24 (m, 2H, 18-H), 0.99 (t, J = 8.0 Hz, 19-H). MS (LR-ESI, positive mode): calcualted for $C_{24}H_{20}N_2O_7$ *m*/*z* 449.1 $[M+H]^+$; found 449.1. MS (HR-ESI, positive mode): calcualted for $C_{24}H_{20}N_2O_7$, *m*/*z* 449.1349 $[M+H]^+$; found 449.1355 $[M+H]^+$.

***O*-acylation of Rapa with anhydrides**. *General procedures.* In a glove box, Rapa (9.1 mg, 0.01 mmol) was dissolved in anhydrous THF (200 μL). **1** (6.5 mg, 0.01 mmol) was added and allowed to react

with Rapa for 20 min. Freshly distilled MA (1.7 mg, 0.011 mmol) in THF (0.20 mL) was added into the mixture of Rapa/**1** under vigorous stirring. After 6 hours an aliquot of this solution was analyzed by HPLC equipped with an analytical C18 column (Luna C18(2), 250 × 4.6 mm, 5 μ, Phenomenex, Torrance, CA), with a UV detector at 280 nm. The areas of Rapa and Rapa-MA peaks were integrated and used for their quantification of the yield. An aliquot of reaction mixture was used for MS analysis. The reaction was quenched by ice-cold methanol (1 mL), and dried under vacuum. Pure Rapa-40-MA used for NMR analysis was separated by preparative thin layer chromatography (prep TLC, silica gel matrix with UV254, 1500 μm thickness, Aldrich) and developed by acetone/hexane (v/v=2/1). The silica gels containing Rapa-40-MA were collected from the glass plate; the compound in the gel was extracted with methanol (2 × 20 mL). The methanol solution was then removed under vacuum; the resulting Rapa-MA was analyzed by ^{1}H NMR (the summary of ^{1}H NMR assignments of Rapa derivatives is in Table S1).

Rapa-40-MA. ESI-MS (low resolution, positive mode): calcualted for $C_{55}H_{83}NO_{14}Na$, *m*/*z* 1004.9 $[M + Na]^+$; found *m*/*z* 1004.9 $[M + Na]^+$. ^{1}H-NMR in Table S1.

Rapa-40-PA. ESI-MS (low resolution, positive mode): calcualted for $C_{56}H_{83}NO_{14}Na$, *m*/*z* 1016.5 $[M + H]^+$; found *m*/*z* 1016.5. ESI-MS (high resolution, positive mode): calcualted for $C_{56}H_{83}NO_{14}Na$, *m*/*z* 1016.5711 $[M + Na]^+$; found 1016.5722 $[M + Na]^+$. ^{1}H-NMR in Table S1.

Cytotoxicity of Rapa prodrugs for Ntera-2 cells. Ntera-2 cells were placed in a 24-well plate for 24 h (50,000 cells seeded per well) before the addition of Rapa-40-MA and Rapa (as positive control). Cells were then washed with 300 μL, pre-warmed PBS, and followed by the addition of freshly prepared Rapa and Rapa-40-MA in cell medium (300 μL) with selected concentration. The cells for negative control were incubated with 300-μL medium. Cells were incubated at 37^{o}C for a total of 72 h in a 5% CO_2 incubator. Standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay protocols was followed thereafter to assess the toxicity of Rapa and Rapa-40-MA (See Figure S3).

***O*-acylation of Ptxl with anhydrides**. *General procedures*. In a glovebox, Ptxl (8.0 mg, 9.4 μmol) was dissolved in anhydrous THF (0.50 mL). **1** (6.0 mg, 9.4 μmol) was added and allowed to react with Ptxl for 20 min. Freshly distilled MA (1.7 mg, 0.011 mmol) in THF solution (0.20 mL) was added into Ptxl/**1** and further stirred for 3 hours. After 6 hours an aliquot of this solution was analyzed by HPLC equipped with an analytical C18 column (Luna C18(2), 250 × 4.6 mm, 5 μ, Phenomenex, Torrance, CA), with a UV detector at 227 nm. The areas of Ptxl and Ptxl-MA peaks were integrated and used for quantifying the yield. The solution was quenched by ice-cold methanol (1 mL) and dried under vacuum. Pure Ptxl-2'-MA used for NMR analysis was obtained by prep TLC. The concentrated reaction solution was applied to prep-TLC plates with 1.5 mm thickness and developed by ethyl acetate/hexane (v/v=1/3). The product was recovered by extraction using methanol twice (2 × 20 mL). The solution was dried under

vacuum. The resulting Ptxl-2'-MA was analyzed by ^{1}H NMR (See Table S2 for summary of ^{1}H NMR assignments of Ptxl derivatives).

Ptxl-2'-MA. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.13 (d, *J* = 7.57 Hz, 2H), 7.72 (d, *J* = 7.57 Hz, 2H), 7.62 – 7.40 (m, 11H), 6.93 (d, *J* = 9.14 Hz, 1H), 6.29 – 6.23 (m, 2H), 6.01 (d, *J* = 7.14 Hz, 1H), 5.66 (d, *J* = 6.80 Hz, 1H), 5.55 (d, *J* = 2.24 Hz, 1H), 4.96 (d, *J* = 8.79 Hz, 1H), 4.43 (m, 1H), 4.30 (d, *J* = 8.29 Hz, 1H), 4.20 – 4.15 (m, 2H), 3.81 (d, *J* = 6.71 Hz, 1H), 2.56 – 2.34 (m, 3H), 2.45 (s, 3H), 2.21 (s, 3H), 2.19 (m, 1H), 1.95 – 1.82 (m, 3H), 1.92 s, (3H), 1,67 (s, 3H), 1.22 (s, 3H), 1.13 (s, 3H). ESI-MS (low resolution, positive mode): calcualted for $C_{51}H_{55}NO_{15}$, *m/z* 922.5 $[M+H]^+$; found 922.5 $[M+H]^+$.

Ptxl-2'-ClA. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.13 (d, *J* = 7.57 Hz, 2H), 7.72 (d, *J* = 7.57 Hz, 2H), 7.62 – 7.40 (m, 11H), 6.93 (d, *J* = 9.14 Hz, 1H), 6.29 – 6.23 (m, 2H), 6.01 (d, *J* = 7.14 Hz, 1H), 5.66 (d, *J* = 6.80 Hz, 1H), 5.55 (d, *J* = 2.24 Hz, 1H), 4.96 (d, *J* = 8.79 Hz, 1H), 4.43 (m, 1H), 4.30 (d, *J* = 8.29 Hz, 1H), 4.20 – 4.15 (m, 2H), 3.81 (d, *J* = 6.71 Hz, 1H), 2.56 – 2.34 (m, 3H), 2.45 (s, 3H), 2.21 (s, 3H), 2.19 (m, 1H), 1.95 – 1.82 (m, 3H), 1.92 s, (3H), 1,67 (s, 3H), 1.22 (s, 3H), 1.13 (s, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 203.6, 171.1, 169.7, 167.3, 167.0, 166.9, 166.3, 142.3, 136.4, 133.6, 133.5, 132.9, 132.0, 130.1, 129.2, 129.1, 128.7, 128.6, 127.0, 126.5, 84.3, 81.0, 79.0, 76.3, 75.4, 75.2, 75.0, 72.2, 72.0, 58.4, 52.7, 45.5, 43.1, 40.1, 35.5, 26.7, 22.6, 22.0, 20.7, 14.7, 9.5. ESI-MS (low resolution, positive mode): calcualted for $C_{49}H_{53}NO_{15}Cl$, *m/z* 930.3 $[M+H]^+$; found 930.3 $[M+H]^+$. ESI-MS (high resolution, positive mode): calcualted for $C_{49}H_{53}NO_{15}Cl$, *m/z* 930.3104 $[M+H]^+$; found 930.3094 $[M+H]^+$.

Ptxl-2'-PA. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.13 (d, *J* = 7.28 Hz, 2H), 7.77 (d, *J* = 7.36 Hz, 2H), 7.61-7.36 (m, 15H), 7.26-7.24 (m, 6H), 6.96 (d, *J* = 9.16 Hz, 1H), 6.30 (s, 1H), 6.26 (d, *J* = 8.9 Hz, 1H), 5.98, 5.97 (dd, *J* = 2.84 Hz, 2.76 Hz, 1H), 5.69 (d, *J* =7.08 Hz, 1H), 5.55 (d, 1H), 4.98 (d, *J* = 9.36 Hz, 1H), 4.98 (m, 3H), 4.65 (d, *J* =11.9 Hz, 1H), 4.32 -4.20 (m, 2H), 3.82 (t, *J* = 9.20 Hz, 1H), 2.72-2.51 (m, 6H), 2.46 (s, 3H), 2.40-2.30 (m, 1H), 2.23 (s, 3H), 1.94 (s, 3H), 1.66 (s, 6H), 1.69 (s, 3H), 1.24 (s, 3H), 1.14 (s, 3H). MS (LR-ESI, positive mode): calcualted for $C_{52}H_{56}NO_{15}$, $[M + H]^+$ *m/z* 934.3; found *m/z* 934.3. MS (HR-ESI, positive mode): calcualted for $C_{52}H_{56}NO_{15}$, *m/z* 934.3650 $[M+H]^+$; found *m/z* 934.3638 $[M+H]^+$.

Ptxl-2'-SA. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.13 (d, *J* = 7.28 Hz, 2H), 7.77 (d, *J* = 7.36 Hz, 2H), 7.61-7.36 (m, 15H), 7.26-7.24 (m, 6H), 7.03 (d, *J* = 9.16 Hz, 1H), 6.25 (s, 1H), 6.22 (d, *J* = 8.9 Hz, 1H), 5.97, 5.95 (dd, *J* = 2.84 Hz, 2.76 Hz, 1H), 5.67 (d, *J* =7.08 Hz, 1H), 5.56 (d, 1H), 5.47 (d, *J* = 2.96 Hz, 1H), 4.96 (d, *J* = 9.36 Hz, 1H), 4.85-4.79 (m, 3H), 4.65(d, *J* =11.9 Hz, 1H), 4.41 (s, 1H), 4.31 -4.27 (m, 2H), 4.19 (d, *J* = 8.44 Hz, 1H), 3.99 (t, *J* = 9.20 Hz, 1H), 3.81-3.73 (m,3H), 3.64 (t, *J* = 9.20 Hz, 1H), 3.31(s, 3H), 2.72-2.51 (m, 6H), 2.42 (s, 3H), 2.40-2.30 (m, 1H), 2.21 (s, 3H), 1.89 (s, 3H), 1.66 (s, 6H), 1.64 (s, 3H), 1.23 (s, 3H), 1.20 (s, 3H), 1.11 (s, 3H) ^{13}C-NMR (125 MHz, $CDCl_3$): δ 203.8, 171.5, 171.2, 171.1,

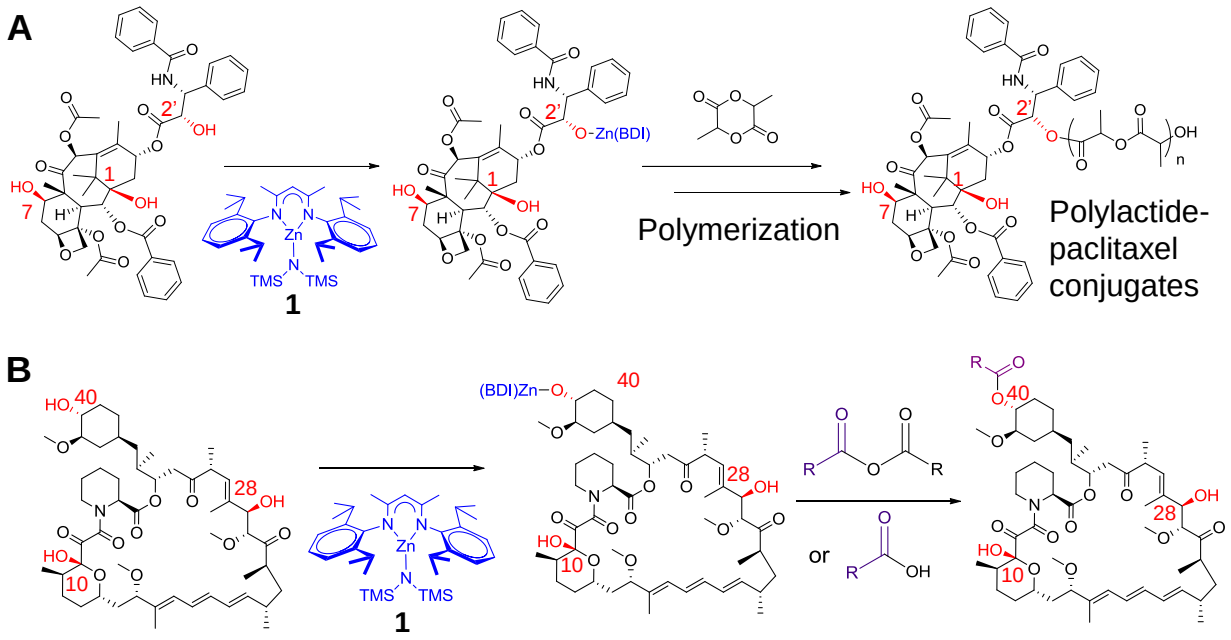
169.8, 167.9, 167.3, 167.0, 142.6, 138.4,137.2, 136.9, 133.7, 133.6, 132.8, 132.0, 130.2, 129.2, 129.1, 129.0, 128.7, 128.5, 128.3, 127.7, 127.6, 127.2, 126.6, 126.0, 101.4, 97.5, 84.4, 82.1, 81.0, 79.0, 76.4, 75.6, 75.1, 74.3, 73.1, 72.1, 71.9, 58.5, 52.8, 49.1, 45.6, 43.2,35.6, 33.9, 29.7, 29.0, 28.9, 26.8, 25.6, 24.9, 22.7, 22.1, 20.8, 14.8, 9.6. ESI-MS (low resolution, positive mode): calculated for $C_{52}H_{57}NO_{16}$, *m/z* 954.3 $[M+H]^+$, found 954.8 $[M+H]^+$.

Cytotoxicity of Ptxl prodrugs for PC-3 cells. PC-3 cells were placed in a 96-well plate for 24 h (10,000 cells seeded per well) before the addition of Ptxl-2'-MA, Ptxl-2'-ClA, Ptxl-2'-PA and Ptxl (as the positive control). Cells were then washed with 100-μL, pre-warmed PBS, followed by the addition of freshly prepared Ptxl and Ptxl prodrugs in cell medium (300 μL) under selected concentration. The cells for negative control were incubated with 100-μL medium. Cells were incubated at 37°C for a total of 72 h in a 5% CO_2 incubator. Standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay protocols was followed thereafter to assess the toxicity of Ptxl and its prodrugs (see Table S4).

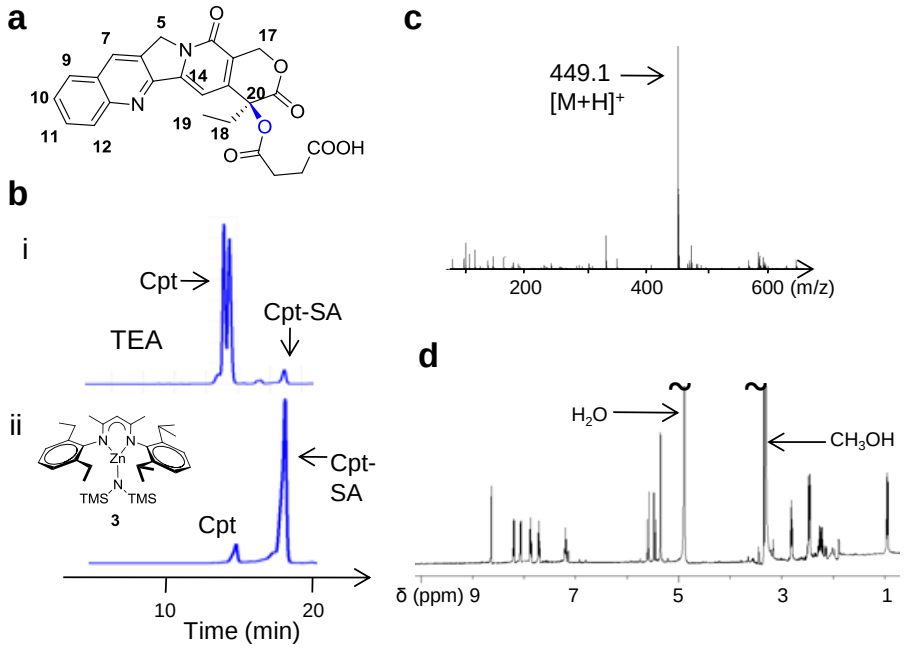
Copper(I)-catalyzed azide–alkyne cycloaddition reaction of Ptxl-2'-PA. Following the published procedures,[3, 4] Cu(I)I was immobilized on the Amberlyst A-21 beads and the loading of Cu(I) on beads was roughly 1.0 mmol/g. QP-TU beads (bearing thiourea functional group, to remove Cu(I)) and PS-PPh_2 beads (to remove azide) were purchased from Sigma-Aldrich.

In a clean vial, 1-azido-1-deoxy-β-D-glucopyranoside tetra-acetate (7.4 mg, 0.02 mmol) and Ptxl-2'-PA (9.3 mg, 0.01 mmol) were dissolved in dichloromethane solution (1.0 mL) and stirred for over 3 days onto Cu(I)I immobilized Amberlyst A-21 beads (1.0 mmol/g, 0.001 mmol, 1 mg). The reaction solution was separated from beads by filtration. The CuI beads were further washed by DCM (2.0 mL) and all filtrate were combined. QP-TU beads (50mg) were added into the solution to scavenge the residue CuI in solution. After 30 minutes, the beads were removed by filtration. The excess azide compounds were removed by passing the reaction solution through a short column packed with phosphine resin (PS–PPh_2, which can capture the azide onto the solid phase *via* Staudinger reaction). The resulted DCM solution was concentrated for MS characterization, to determine whether the starting materials (Ptxl-2'-PA) was left (see Figure S5).

***O*-acylation of Ptxl (or Rapa) using DCC/(BDI)Zn with carboxylic acid.** *General procedures.* In a glove box, PhAc (27.2 mg, 0.2 mmol) in DCM (0.30 mL) was mixed with DCC (20.6 mg, 0.1 mmol) at -20°C. The mixture was stored in the fridge of glove box. After 4 hours, **1** (6.5 mg, 0.01 mmol) was mixed with Ptxl (8.5 mg, 0.01 mmol) in DCM (0.30 mL) for 20 min. The DCC/PhAc solution (30 μL) was added into the **1**/Ptxl solution. The mixture was stirred for 3-4 h and analyzed by HPLC. After reaction, QuadTU beads (Sigma-Aldrich) were added into solution to remove Zn complex. The solution was dried and the final product was purified by prep TLC (EtOAc / hexane, v/v = 2/1) for NMR and MS analysis.



Scheme S1. Regioselective synthesis of polylactide-paclitaxel conjugates (A); and regioselective *O*-acylation of polyol therapeutics, e.g., rapamycin (B).



d

	Cpt (δ, ppm)	Cpt-SA (δ, ppm)	Cpt-MA (δ, ppm)
7-H	8.64	8.63	8.64
12-H	8.21	8.21	8.16
9-H	8.07	8.07	8.07
11-H	7.87	7.87	7.86
10-H	7.72	7.71	7.70
14-H	7.46	7.43	7.34
17-H	5.58, 5.41	5.57, 5.44	5.63, 5.49
5-H	5.36	5.35	5.35
18-H	1.97	2.23	2.26
19-H	1.01	0.99	1.04
SA -CH2COOH		2.81	
SA -OCOCH2		2.55	
MA			5.83
MA			6.33
MA			1.98

Fig. S1 (a) Structure of Cpt-SA. (b) HPLC overlay of Cpt-SA reaction performed with TEA and **3**. The dual peak of Cpt in (i) is due to the existence of both lactone and carboxylate forms of Cpt, which is well known for Cpt.[5] All spectrums were recorded and analyzed at 370 nm. (c) Electron-spraying ionization mass spectrum (ESI-MS, positive mode) of Cpt-SA. $[M+1]^+$: 449.1 (*m*/*z*). High resolution ESI-MS analysis results $[M+1]^+$ (*m*/*z*): 449.1355. Calculated: 449.1349. (d) ^{1}H NMR of Cpt-SA (MeOD-*d*4, 500 MHz). (e) Table of the ^{1}H NMR chemical shifts of Cpt, Cpt-SA and Cpt-MA.

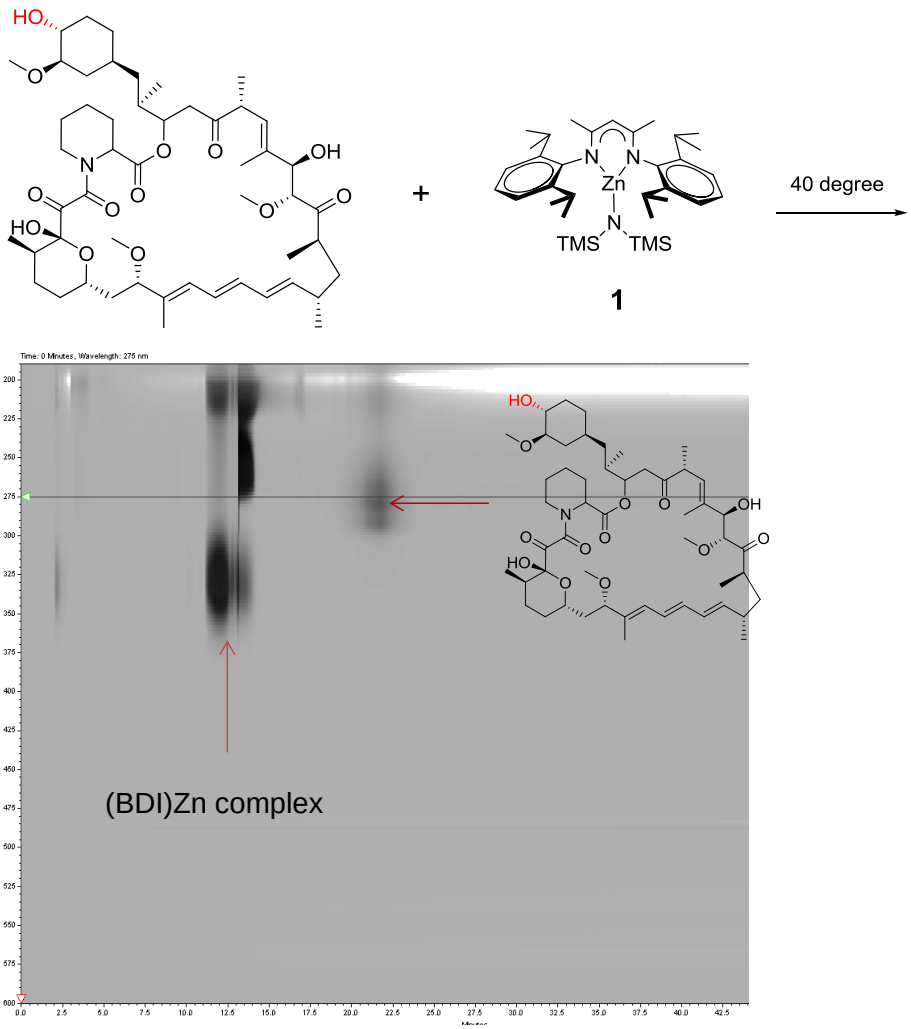


Fig.S2 HPLC spectrum of Rapa mixed with **1** and heated at 40°C for 30 minutes. No degradation species of Rapa were found in the HPLC spectrum. The peak at 22.5 min with highest UV absorbance at 280 nm was Rapa. The peaks around 12-14 min were BDI-Zn complex species (degraded by the elution solvent of HPLC (water/ACN/TFA).

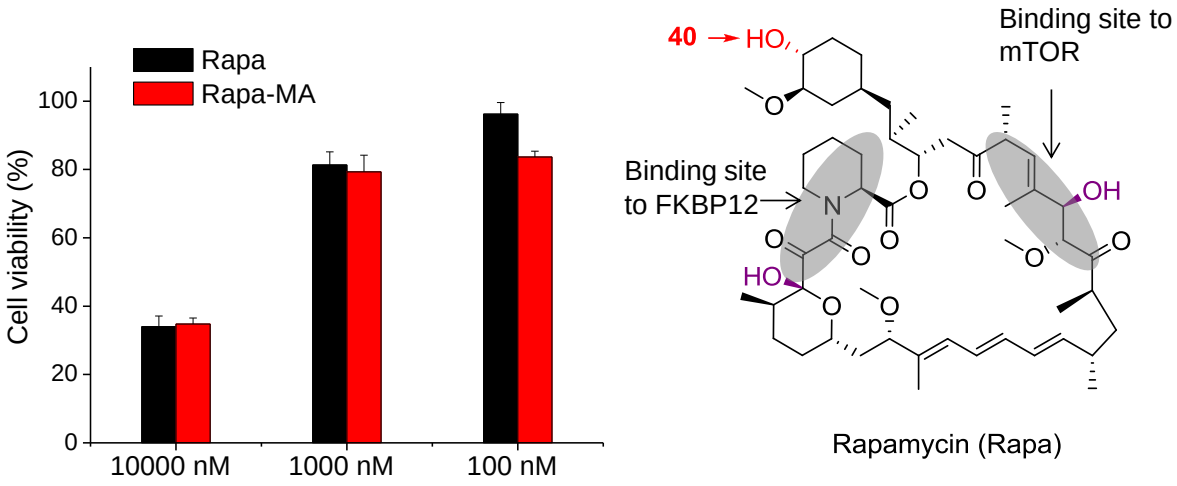


Fig. S3 Anti-proliferation of Rapamycin and Rapa-MA to NTera-2 cells.

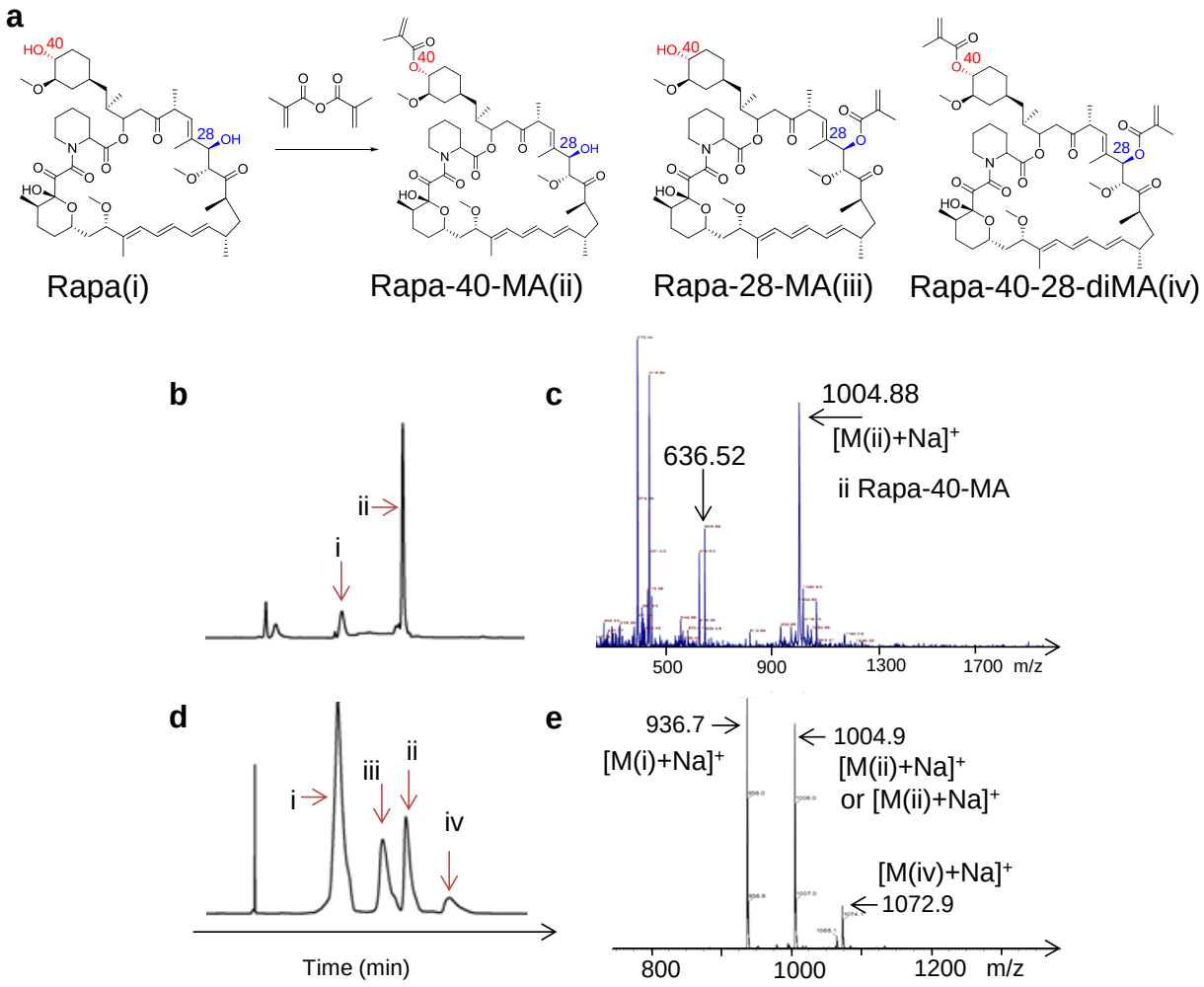


Fig. S4 *O*-acylation reaction of Rapa with MA. (a) Scheme of potential Rapa/MA reaction products. (b) HPLC and (c) MALDI-MS of the reaction combination of Rapa/MA/ **1** (Table 2, entry 1). (d) HPLC and (e) MALDI-MS analysis of the reaction mixture of Rapa/MA/TEA/NMI (Table 2, entry 5).

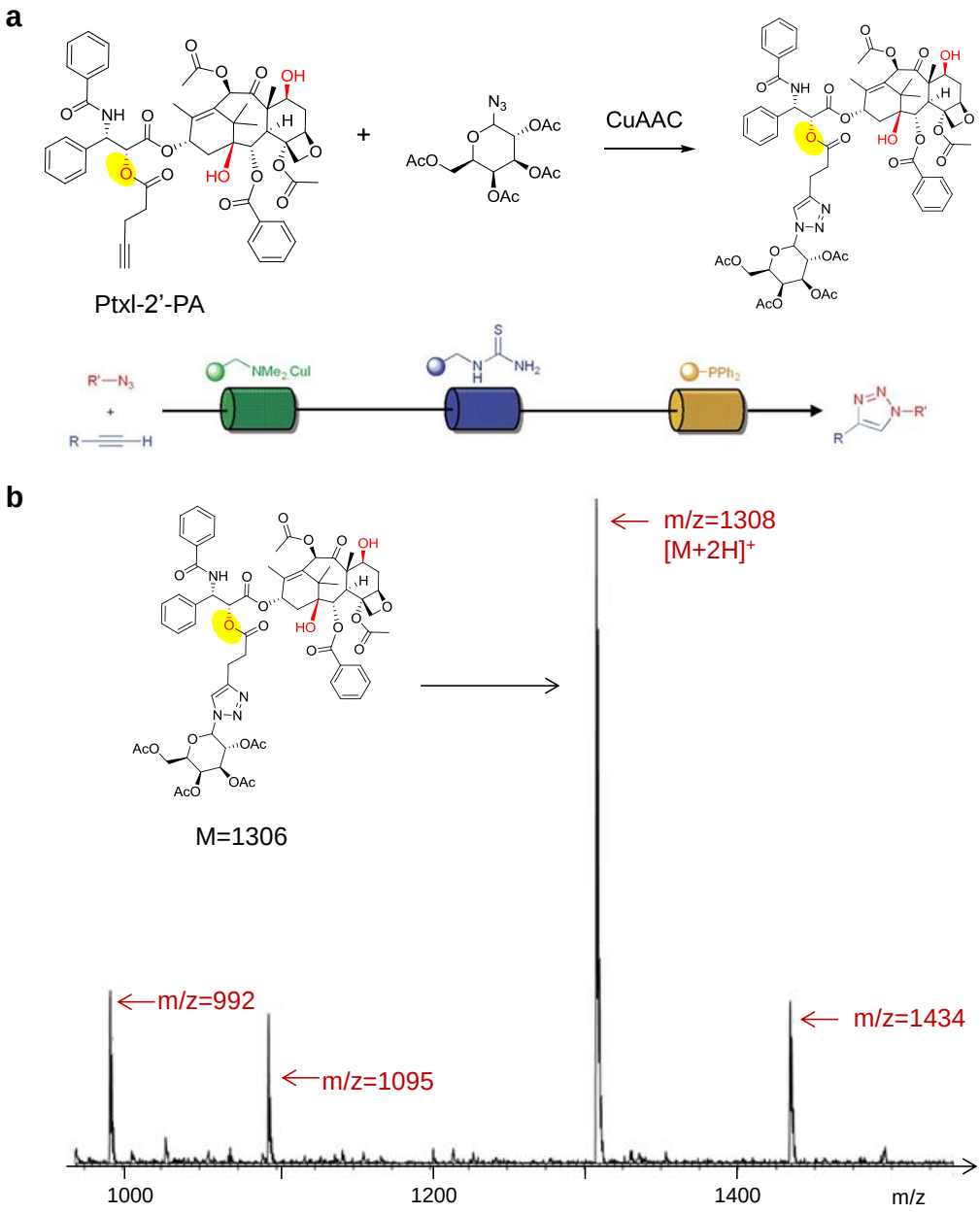
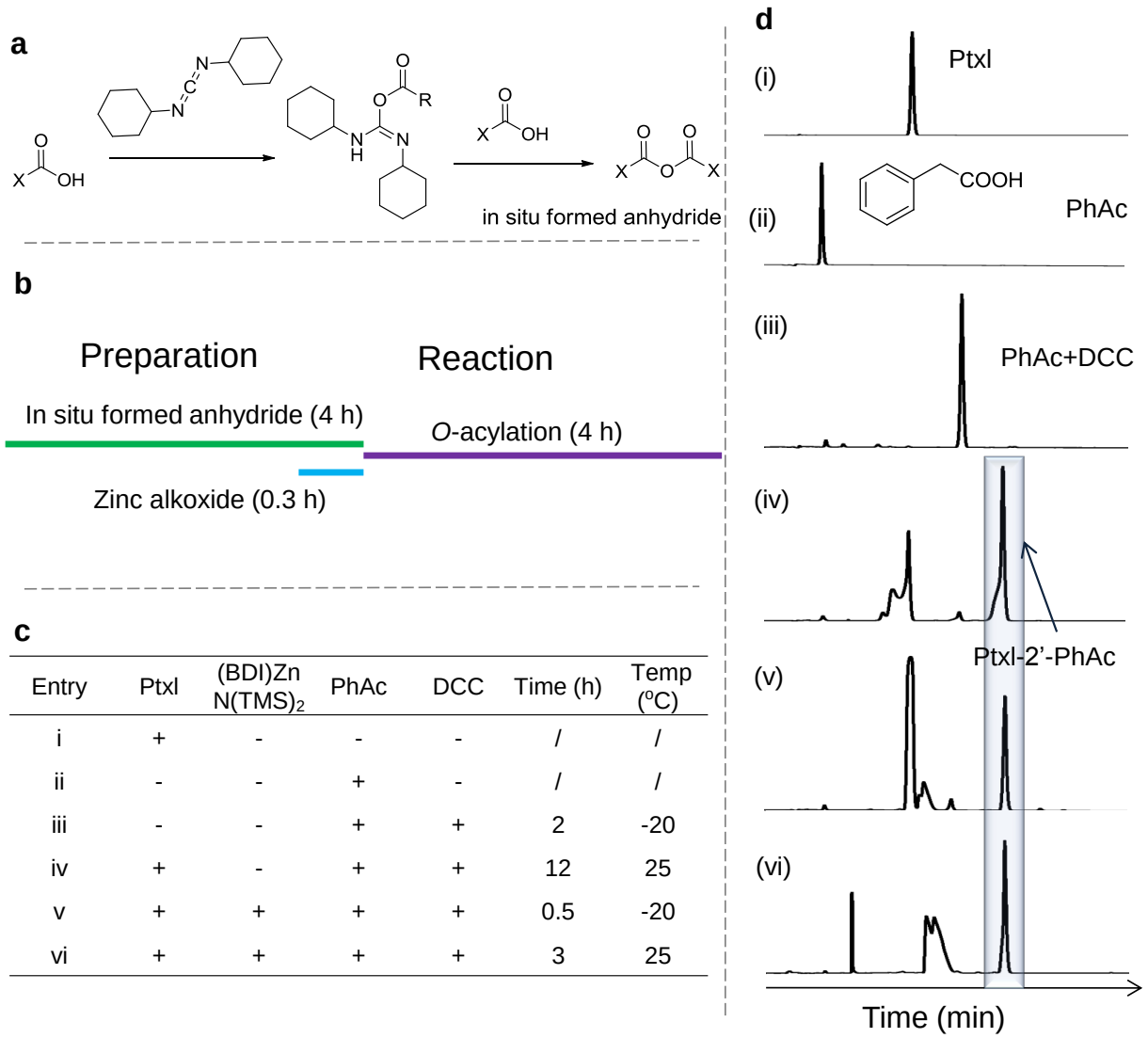


Fig. S5 (a) CuAAC reaction scheme mediated by solid bead for reaction catalyst and purification scavenger.[4] (b) LR-ESI-MS of the solution of Ptxl-2'-PA CuAAC reaction with 1-azido-1-deoxy-β-D-glucopyranoside tetraacetate. The calculated MS of final compounds: $[M + 2H]^+$ *m/z*=1308, found: 1308. The Ptxl-2'-PA peak ($[M+H]^+$ m/z=934) was not found in the spectrum, indicating all the starting materials used up in the reaction.



c

Entry	Ptxl	(BDI)Zn N(TMS)$_2$	PhAc	DCC	Time (h)	Temp (^{o}C)
i	+	-	-	-	/	/
ii	-	-	+	-	/	/
iii	-	-	+	+	2	-20
iv	+	-	+	+	12	25
v	+	+	+	+	0.5	-20
vi	+	+	+	+	3	25

Fig. S6 (a) Carboxylic acid reacted with DCC to *in situ* prepare corresponding anhydride. (b) Time line of DCC/BDI-Zn acylation strategy. (c) PhAc/Ptxl reactions setup condition. HPLC traces of the corresponding entry was shown in (d).

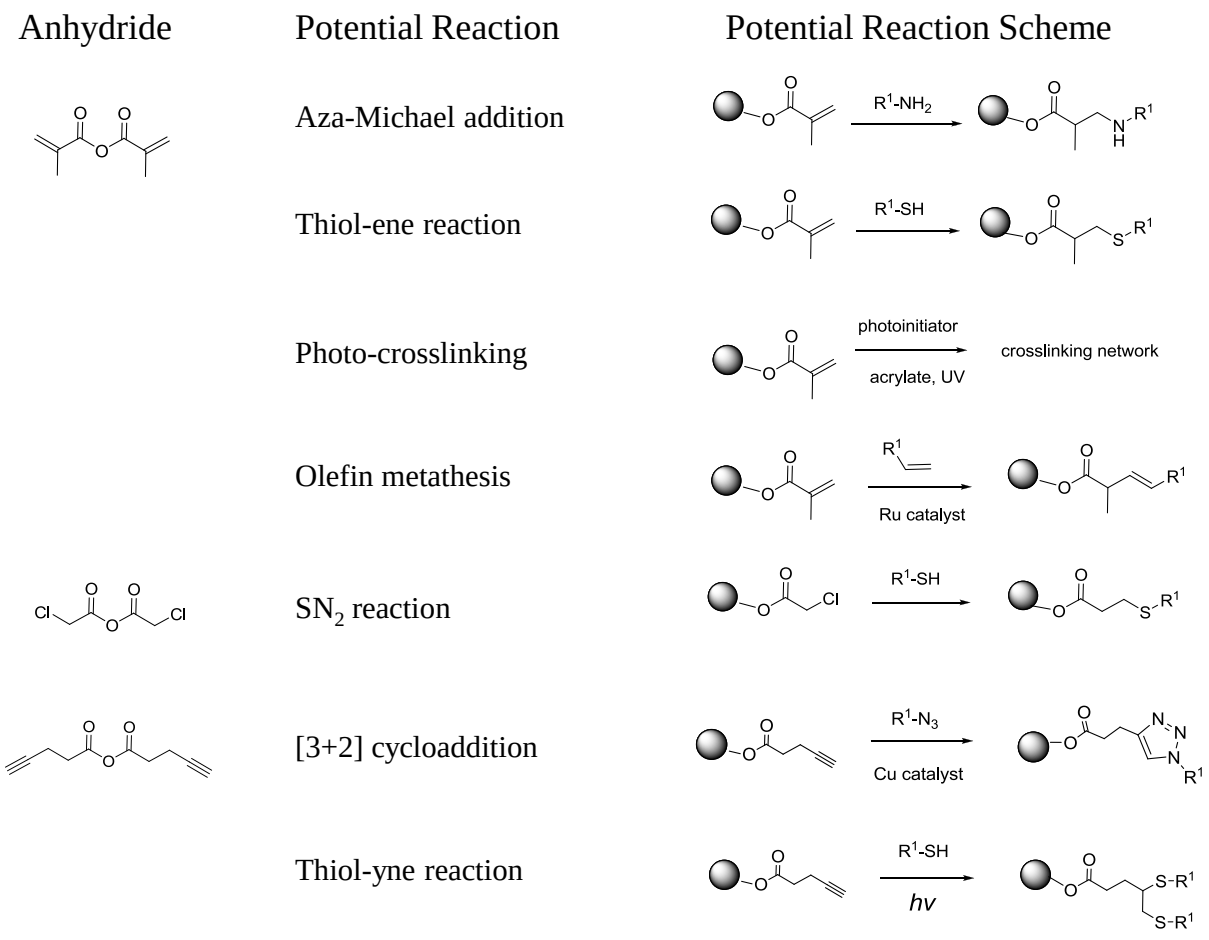
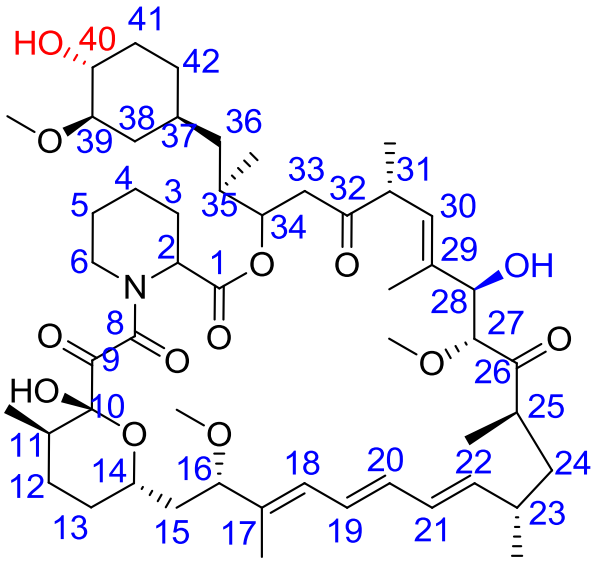


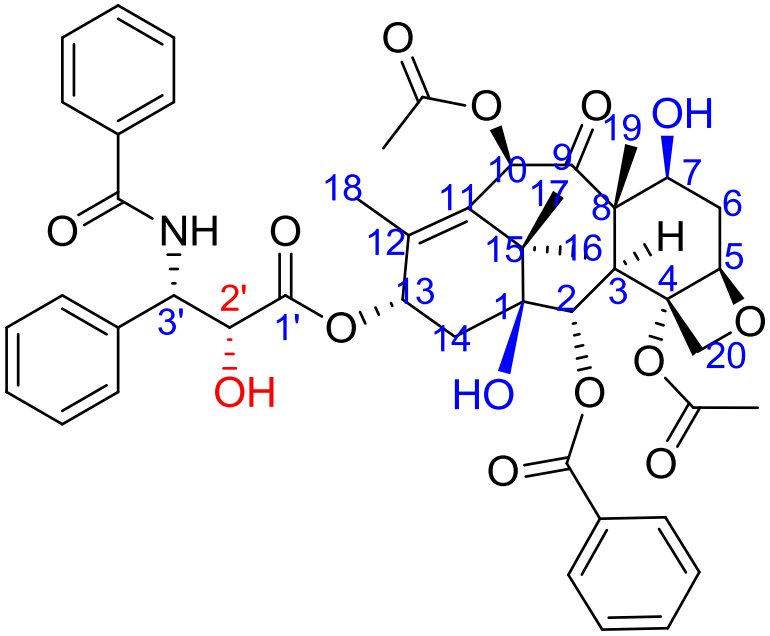
Fig. S7 Potential bioconjugation reactions derived from drug-*O*-acylation conjugates

Table S1. Table of chemical shift comparison in ^{1}H-NMR spectrum among Rapa and its derivatives.[6]



	Rapa (δ, ppm)	Rapa-40-MA (δ, ppm)	Rapa-40-PA (δ, ppm)		Rapa (δ, ppm)	Rapa-40-MA (δ, ppm)	Rapa-40-PA (δ, ppm)
-OH	4.83	4.80	4.77	12	1.6	1.61	1.61
19	6.39	6.39	6.38	23	2.33	2.33	2.33
20	6.32	6.32	6.29	38	2.10	2.10	2.12
21	6.15	6.16	6.14	11	1.98	1.95	1.96
18	5.97	5.97	5.98	3	1.92, 1.60	1.92, 1.60	1.92, 1.60
22	5.57	5.53	5.52	24	1.83, 1.68	1.83, 1.68	1.83, 1.68
30	5.42	5.43	5.32	4	1.78, 1.47	1.78, 1.47	1.78, 1.47
2	5.29	5.29	5.30	5	1.75, 1.48	1.75, 1.48	1.75, 1.48
34	5.17	5.18	5.16	15	1.85, 1.52	1.86, 1.52	1.86, 1.52
28	4.17	4.19	4.14	47, 29-CH_3	1.74	1.76	1.76
14	3.86	3.87	3.92	44, 17-CH_3	1.65	1.66	1.66
27	3.71	3.74	3.75	13	1.62, 1.33	1.62, 1.33	1.62, 1.33
16	3.66	3.67	3.68	37	1.39	1.37	1.37
6	3.59, 3.44	3.58, 3.44	3.57, 3.47	43, 11-CH_3	0.95	0.96	0.96
51, 27-OCH_3	3.40	3.40	3.40	45, 23-CH_3	1.05	1.06	1.06
40	3.35	4.73	4.69	46, 25-CH_3	1.00	1.00	1.01
50, 16-OCH_3	3.32	3.34	3.37	48, 31-CH_3	1.11	1.11	1.11
31	3.30	3.31	3.27	49, 35-CH_3	0.92	0.92	0.92
52, 39-OCH_3	3.14	3.14	3.13	41	1.99, 1.33	2.04, 1.34	1.98, 1.32
39	2.91	3.22	3.16	42	1.70, 1.00	1.74, 0.92	1.74, 0.92
25	2.72	2.74	2.73	36	1.22, 1.12	1.21, 1.16	1.21, 1.16
33	2.72, 2.56	2.72, 2.57	2.72, 2.57				

Table S2. Table of chemical shift comparison in ^{1}H-NMR spectrum among Ptxl and its derivatives.[7]



	Ptxl	Ptxl-2'-SA (δ, ppm)	Ptxl-2'-ClAA (δ, ppm)	Ptxl-2'-MAA (δ, ppm)	Ptxl-2'-PA (δ, ppm)	Ptxl-2'-AzPhAc (δ, ppm)	Ptxl-2'-PhAc (δ, ppm)
3'-NH	7.01	7.01	6.93	6.95	6.96	6.68	6.69
10	6.27	6.29	6.29	6.31	6.30	6.29	6.29
13	6.23	6.25	6.25	6.28	6.26	6.27	6.26
3'	5.78	6.00, 5.98	6.01	5.98, 5.96	5.98, 5.97	5.93, 5.91	5.90, 5.87
2	5.67	5.69	5.66	5.7	5.69	5.69	5.68
5	4.94	4.98	4.96	5.00	4.98	4.98	4.98
2'	4.78	5.55	5.55	5.53	5.55	5.47	5.43
7	4.40	4.44	4.43	4.47	4.45	4.45	4.44
20	4.30, 4.19	4.31, 4.20	4.30	4.34, 4.20	4.32, 4.20	4.32, 4.20	4.31, 4.20
3	3.79	3.81	3.81	3.83	3.82	3.82	3.80
6	2.54, 1.88	2.54, 1.89	2.54, 1.86	2.58, 1.90	2.54, 1.85	2.54, 1.86	2.54, 1.87
4-OAc	2.38	2.45	2.45	2.46	2.46	2.46	2.46
14	2.35, 2.28	2.37, 2.20	2.35, 2.19	2.36, 2.17	2.34, 2.17	2.34, 2.17	2.34, 2.17
10-OAc	2.23	2.23	2.21	2.25	2.23	2.23	2.22
18-CH3	1.79	1.92	1.92	1.96	1.94	1.94	1.94
19-CH3	1.68	1.69	1.67	1.70	1.69	1.70	1.69
17-CH3	1.24	1.23	1.22	1.25	1.24	1.24	1.24
16-CH3	1.14	1.14	1.13	1.16	1.14	1.14	1.14

Table S3. Results of regioselective *O*-acylation by *in situ* forming anhydrides from carboxylic acid.

Entry	R-OH	Acid	Catalyst	Temp. (^{o}C)	Time (h)	Yield (%)*	Regioselectivity %
1	Naph	PhAc	**1**/DCC	25	0.5	96	/
2	Naph	AzPhAc	**1**/DCC	25	0.5	94	/
3	Ptxl	PhAc	**1**/DCC	25	4	99	2'-OH (>99)
4	Ptxl	PhAc	DCC	25	12	61	2'-OH (>99)
5	Ptxl	AzPhAc	**1**/DCC	25	4	>98	2'-OH (>99)
6	Ptxl	AzPhAc	DCC	25	12	48	2'-OH (>99)
7	Rapa	PhAc	**1**/DCC	25	4	<15	40-OH (>99)
8	Rapa	PhAc	**1**/DCC	40	4	51	40-OH (>99)
9	Rapa	PhAc	DCC	40	12	<10	/
10	Rapa	PhAc	DCC/DMAP/TEA	40	12	<10	/
11	Rapa	PAc	**2**/DCC	40	4	82	40-OH (>99)

Reaction conditions: R-OH/acid/DCC/(BDI)Zn = 0.01/0.02/0.01/0.01 mmol. Abbreviation: Temp.: temperature. The regioselectivity was determined by HPLC using analytical reverse-phase C18 column. The acylation reagents were first generated 4 hours before the acylation reaction.

Table S4. Evaluation of Ptxl and its derivatives anticancer effect using cytotoxicity assay (MTT assay)

Drug	Ptxl	Ptxl-2'-ClA	Ptxl-2'-MA	Ptxl-2'-PA
IC_{50} (nM)	87 ± 10	553 ± 12	3477 ± 83	397 ± 11

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