***Synthesis of hydrophobic 3TC derivative***

3TC (4 mmol) was dissolved in dimethylformamide (DMF, 6 mL) in an ice bath. Imidazole (6 mmol) and tert-butyldimethylsilyl chloride (4.8 mmol) were then added separately and the reaction mixture stirred at room temperature (25 °C) overnight. After completion of the reaction, the mixture was concentrated at reduced pressure and purified on a silica gel column using dichloromethane and methanol (2-10%) as eluents. The silylated compound was dissolved in dry pyridine (6 mL), followed by dropwise addition of a solution of 4,4’-dimethoxytrityl chloride (DMTr-Cl, 8 mmol) at 0 °C. The reaction mixture was gradually warmed to room temperature and stirring under argon was continued until all the starting material had been converted into the product according to analysis by thin layer chromatography. The mixture was concentrated, diluted with dichloromethane (200 mL), and washed with saturated sodium bicarbonate solution (200 mL). The aqueous layer was extracted further three times with dichloromethane (200 mL). The organic extracts were combined, dried over sodium sulfate and concentrated *in vacuo*. The desired compound was isolated by silica gel column chromatography as previously described. The amine and alcohol protected compound was dissolved in anhydrous tetrahydrofuran (6 mL) and treated with tetrabutylammonium fluoride solution (6.2 mL, 1.5 molar ratio) to cleave the silyl protecting group. The reaction mixture was concentrated and purified by silica column chromatography. The free alcohol compound was then dissolved in DMF (10 mL) and reacted with myristic acid (945 mg, 2 molar ratio) in the presence of 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluoro-phosphate (1.72 g, 2.2 molar ratio) and N,N-diisopropylethylamine (1.5 mL, 3.4 molar ratio). The myristoylated compound was purified by silica gel chromatography and subjected to amine deprotection using a solution of trifluroacetic acid (80%) in dichloromethane. The final purified product was characterized by nuclear magnetic resonance spectroscopy (1H-NMR) on a Varian Inova 500 MHz. Chemical shifts are reported in parts per million (ppm). The structure of the final compound (MTC) was further characterized by Fourier transform infrared spectroscopy (FTIR) using a Spectrum Two FTIR spectrometer (PerkinElmer, Waltham, MA, USA). To evaluate the solubility of MTC, 10 mg MTC was added to 1 mL distilled water in a 1.7 mL microcentrifuge tube. The sample was shaken at 25 °C for 1 h. The solution was centrifuged at 16,000 × g for 5 min and drug concentration in the supernatant was determined by high-performance liquid chromatography (HPLC) using a Phenomenex Synergi™ 4 µm Hydro-RP 80Å column (Phenomenex Inc., Torrance, CA, USA). Triplicate 20 μL samples were assayed using a mobile phase of 80% acetonitrile/20% 5 mM Na2HPO4, pH 9.0, pumped at 1.0 mL/min with UV/Vis detection at 272 nm. Drug was quantitated by comparison of peak areas to a standard curve of MTC (0.025–50 μg/mL) in methanol.

***Plasma and tissue preparation and UPLC-MS/MS analysis***

Plasma was prepared by centrifugation of blood collected in heparinized tubes at 2000 x g for 5 min. Tissues (liver, spleen, and lymph nodes) were collected after sacrifice on day 14. 3TC and MTC from plasma and tissues were extracted using acetonitrile and assayed by UPLC-MS/MS. In preparation for drug analysis, 3TC and MTC were extracted from plasma (20 μL) using 1 mL of acetonitrile. Internal standard (IS; 10 μL) was added to each sample and consisted of 1.33 µg/mL 15N213C-3TC and 0.5 µg/mL lopinavir. Samples were dried using a ThermoScientific Savant Speed Vacuum (ThermoScientific, Waltham, MA, USA), reconstituted in 100 l of 80% v/v methanol in Optima grade water, and 10 l was injected directly for MTC UPLC-MS/MS analysis. Thirty l of the above reconstituted samples were diluted with 50 l water for 3TC UPLC-MS/MS analysis. Final concentration for both 15N213C-3TC (IS for 3TC) and lopinavir (IS for MTC) were 50 ng/mL. Standard curves of 3TC or MTC were prepared similarly, in blank mouse plasma (BioreclamationIVT, Hicksville, NY, USA) in the range of 0.7-7000 ng/mL and 0.25-2500 ng/mL, respectively.

For tissue preparation, 50-100 mg of liver, spleen and lymph nodes were spiked with protease inhibitor (10 mM PMSF in HPLC grade isopropanol) and homogenized using a TissueLyzer II (Qiagen, Valencia, CA, USA) in 4 volumes of an esterase inhibitor mixture (12.5 mg/mL sodium fluoride and 3.75 mg/mL EDTA solution in Optima grade water). Tissue homogenate (100 μL) was mixed with 10 μL of IS (5 µg/mL 15N213C-3TC and 2 µg/mL lopinavir), extracted in 300 μL methanol, and 10 l was injected directly for MTC UPLC-MS/MS analysis. A 100 l of the above reconstituted samples were diluted with 150 l water for 3TC UPLC-MS/MS analysis. Final concentration for both 15N213C-3TC (IS for 3TC) and lopinavir (IS for MTC) were 50 ng/mL. Standard curves of 3TC or MTC were prepared similarly in blank tissues obtained from untreated mice in the range of 2.5-25,000 and 1-10,000 ng/g tissue, respectively. A Waters ACQUITY H-class UPLC system (Waters, Milford, MA, USA) connected to a Waters Xevo TQS-micro mass spectrometer with an electrospray ionization source was used for UPLC-MS/MS analysis.

Chromatographic separation of 10 μL 3TC sample injections was achieved on an ACQUITY UPLC CSH C18 column (1.7 μm, 2.1 mm x 100 mm) using a 13 min gradient of mobile phase A (7.5 mM ammonium bicarbonate in Optima grade water and adjusted to pH 7 using glacial acetic acid) and mobile phase B (100% Optima grade methanol) at a flow rate of 0.25 mL/min. The initial mobile phase composition was 10% B for the first 2.5 min and was gradually increased to 95% B in 6.5 min and held constant for 1.5 min. Mobile phase B was then reset to 10% in 0.45 min and the column was equilibrated for 2.25 min before the next injection. MTC chromatographic separation was achieved using the same CSH C18 column and mobile phases, but with a 16 min isocratic method using 81% mobile phase B and flow rate of 0.3 mL/min. 3TC and MTC were detected at a cone voltage of 4 V, and 10 V, respectively, and a collision energy of 10 V, and 12 V, respectively, in the positive ionization mode. Multiple reaction monitoring (MRM) transitions used for 3TC, MTC, 15N213C -3TC, and lopinavir were 230.23 > 111.97,440.10 > 111.97, 233.23 > 114.97, and 629.18 > 447.20 m/z, respectively. Spectra were analyzed and quantified by MassLynx software version 4.1. All calculations were made using analyte peak area to internal standard peak area ratios.