Supporting information for

Enzyme transformation to modulate the ligand-receptor interactions between small molecules

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Materials and Methods

Materials. Vancomycin, N,N-diisopropylethylamine (DIPEA), and O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) were purchased from ACROS Organics USA; all amino acid derivatives from GL Biochem (Shanghai) Ltd.

Instrumentation. LC-MS on Waters Acquity Ultra Performance LC with Waters MICROMASS detector, isothermal titration calorimetry on Nano ITC (TA); MTT assay for cell toxicity test on DTX880 Multimode Detector.

Isothermal titration calorimetry. The compounds were dissolved in pH 7.4 PBS buffer under suitable concentrations. Titration experiments were performed on a Nano ITC (Isothermal titration calorimetry, 190 μL) with a gold reaction vessel. The reference cell was filled with 400 μL DI water. Typically, each titration experiment consisted of 25 x 2 μL injections of Van into a solution of **1a** (or **1b**) at 600 s intervals with a stirring speed of 250 rpm. A 300 s baseline was collected before the next injection. The experiments were conducted at 25 ^{o}C.

MTT assays. We seeded 2 x 10^4 (cells/well)[1] of HeLa cells into a 96-well plate (Cell Treat) with 100 μL of MEM medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/ml streptomycin. Incubation at 37 ^{o}C and 5% CO_2 for 12 h allowed HeLa cells to attach to the bottom of the 96-well plate. Then we replaced the medium with another 100 μL of growth medium that contained serial diluents of our compounds and incubated the cells at 37 ^{o}C and 5% CO_2 for an additional 72 h. During the viability measurement of HeLa cells, which were assayed for three days, we added 10 μL of (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT, 0.5 mg/mL) into the assigned wells every 24 h, which was followed by adding 100 μL of 0.1% sodium dodecyl sulfate (SDS) 4 h later. We then collected the assay results after 24 h incubation. Since the mitochondrial reductase in living cells reduced MTT to purple formazan, the absorbance at 595 nm of the whole solution was finally measured with a DTX 880 Multimode Detector. With MEM medium as blank and untreated HeLa cells as control, the absorbance of untreated cells minus that of the blank was defined as 100%,

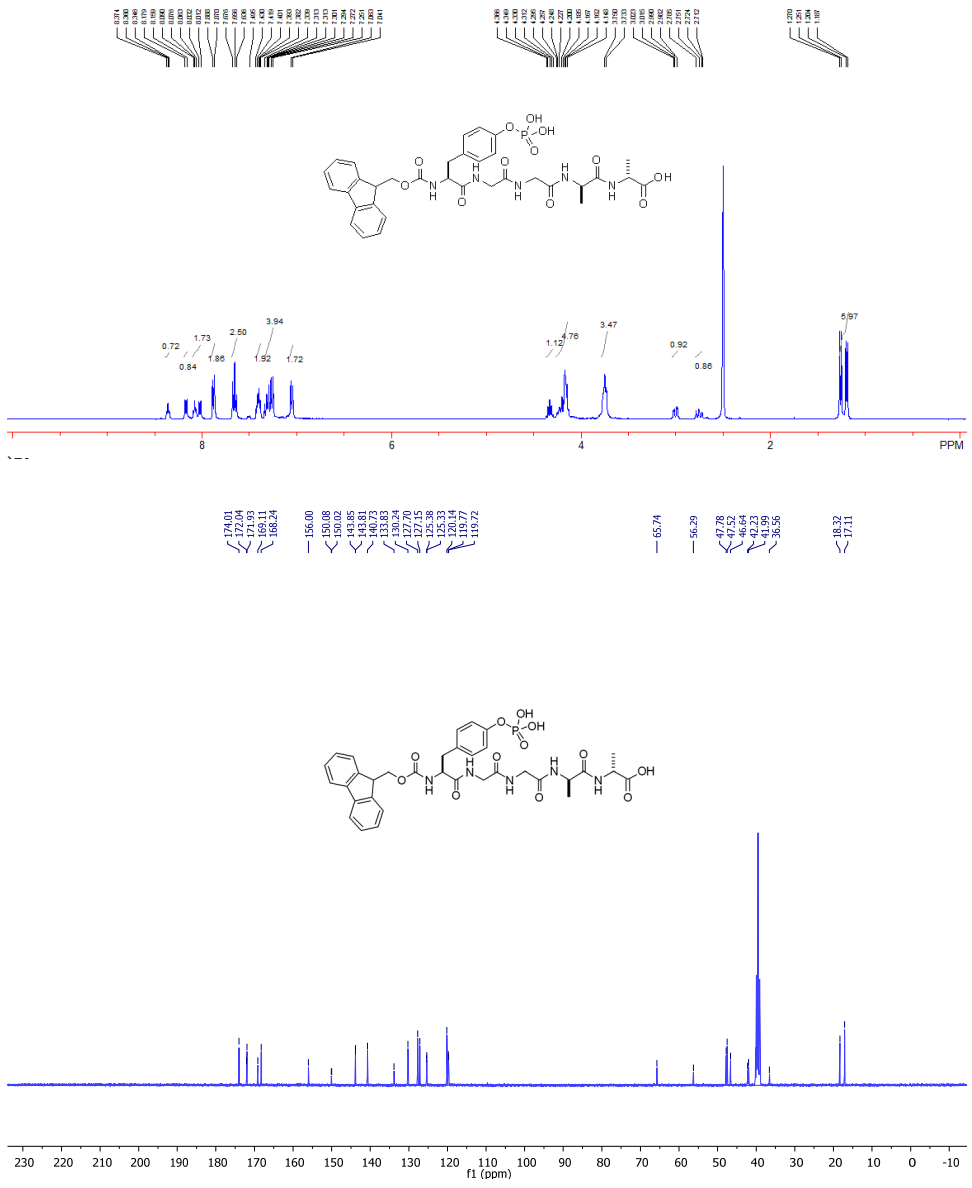
and we measured each concentration of these compounds in triplicate. The IC_{50} values of the compounds were read from their activity curves on day 2.

TEM measurement. Aliquotes (3-5 μL) of sample was added into glow discharge thin carbon-coated copper grid (400 mesh, Pacific Grid-Tech) and incubated for 30 s at room temperature. Excess sample solution was removed by blotting with filter paper touched to the edge of the grid. After removing excess fluid, the grid was washed with three successive drops of deionized water and then exposed to three successive drop 2% (w/v) uranyl acetate. Data were collected at high vacuum on Morgagni 268 transmission electron microscopy.

Peptide synthesis and purification. All compounds were prepared by solid-phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin.[2] Following the removal of an Fmoc group, the first amino acid loaded onto resin at the C-terminal. After the resin was treated with 20% piperidine to remove the protecting group, the next Fmoc-protected amino acid was coupled to the free amino group using N,N-Diisopropylethylamine/O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate(DIPEA/HBTU) as the coupling reagent. The growth of the peptide chain followed the established Fmoc-SPPS protocol. As the final step, the resin-bound peptide was cleaved using a cocktail of TFA/triisopropylsilane/water (95: 2.5: 2.5) for 2h under nitrogen, after which we washed the resin twice using TFA, and collected the filtrate. Crude product was obtained after the addition of cold diethyl ether into concentrated filtrate. The crude product was purified by reverse phase high performance liquid chromatography (HPLC). The resulting peptide solution was frozen by liquid nitrogen and lyophilized to afford purified compounds in about 50% yield for **1a**, 80% yield for **1b** after purification.

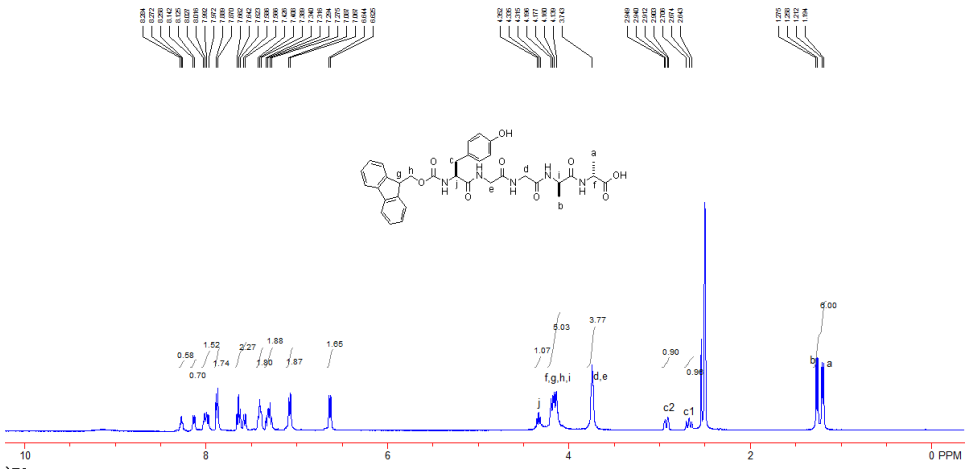
1a: ^{1}H NMR (400 MHz, dmso) δ 8.36 (t, J = 5.4 Hz, 1H), 8.21 – 7.98 (m, 3H), 7.88 (d, J = 7.5 Hz, 2H), 7.66 (t, J = 8.1 Hz, 2H), 7.45 – 7.20 (m, 6H), 7.05 (d, J = 8.4 Hz, 2H), 4.39 – 4.05 (m, 6H), 3.74 (d, J = 6.6 Hz, 4H), 3.06 – 2.93 (m, 1H), 2.84 – 2.68 (m, 1H), 1.23 (dd, J = 26.0, 7.2 Hz, 6H). ^{31}P NMR (400 MHz, dmso) δ -4.98 (s). ^{13}C NMR (101 MHz, dmso) δ 174.01, 172.04, 171.93, 169.11, 168.24, 156.00, 150.05, 143.83, 140.73, 133.83, 130.24, 127.70, 127.15, 125.35, 120.19, 119.72, 65.74, 56.29, 47.78, 47.52, 46.64, 42.23, 41.99, 36.56, 18.32, 17.11. ESI MS (m/z) $C_{34}H_{38}N_5O_{12}P$ $[M]^+$ calcd. 739.23; found 738.17 $[M-H]^-$.

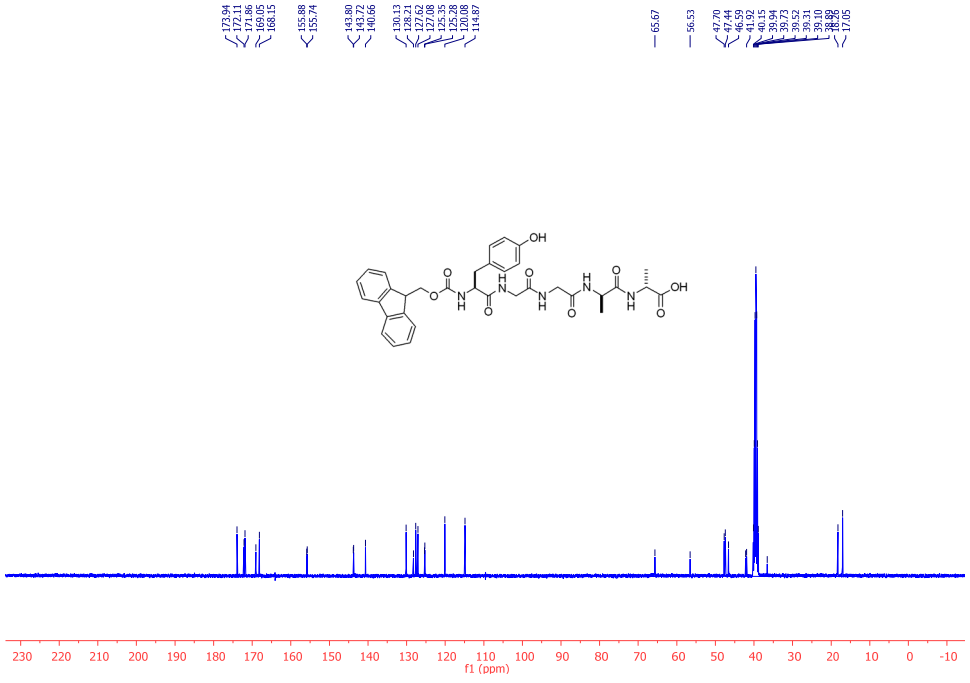
NMR spectra of **1a**



1b: ^{1}H NMR (400 MHz, dmso) δ 8.32 – 8.08 (m, 1H), 8.05 – 7.93 (m, 3H), 7.88 (d, J = 7.4 Hz, 2H), 7.69 – 7.53 (m, 2H), 7.45 – 7.24 (m, 4H), 7.07 (d, J = 7.8 Hz, 2H), 6.63 (d, J = 7.7 Hz, 2H), 4.39 – 4.03 (m, 6H), 3.74 (s, 4H), 2.92 (d, J = 11.0 Hz, 1H), 2.73 – 2.62 (m, 1H), 1.23 (dd, J = 25.3, 7.1 Hz, 6H). ^{13}C NMR (101 MHz, dmso) δ 173.94, 172.11, 171.86, 169.05, 168.15, 155.88, 155.74, 143.76, 140.66, 130.13, 128.21, 127.62, 127.08, 125.31, 120.08, 114.87, 65.67, 56.53, 47.70, 47.44, 46.59, 42.16, 41.92, 40.15, 36.57, 18.26, 17.05. ESI MS (m/z) $C_{34}H_{37}N_5O_9$ $[M]^+$ calcd. 659.26; found 658.29 $[M\text{-}H]^-$.

NMR spectra of **1b**





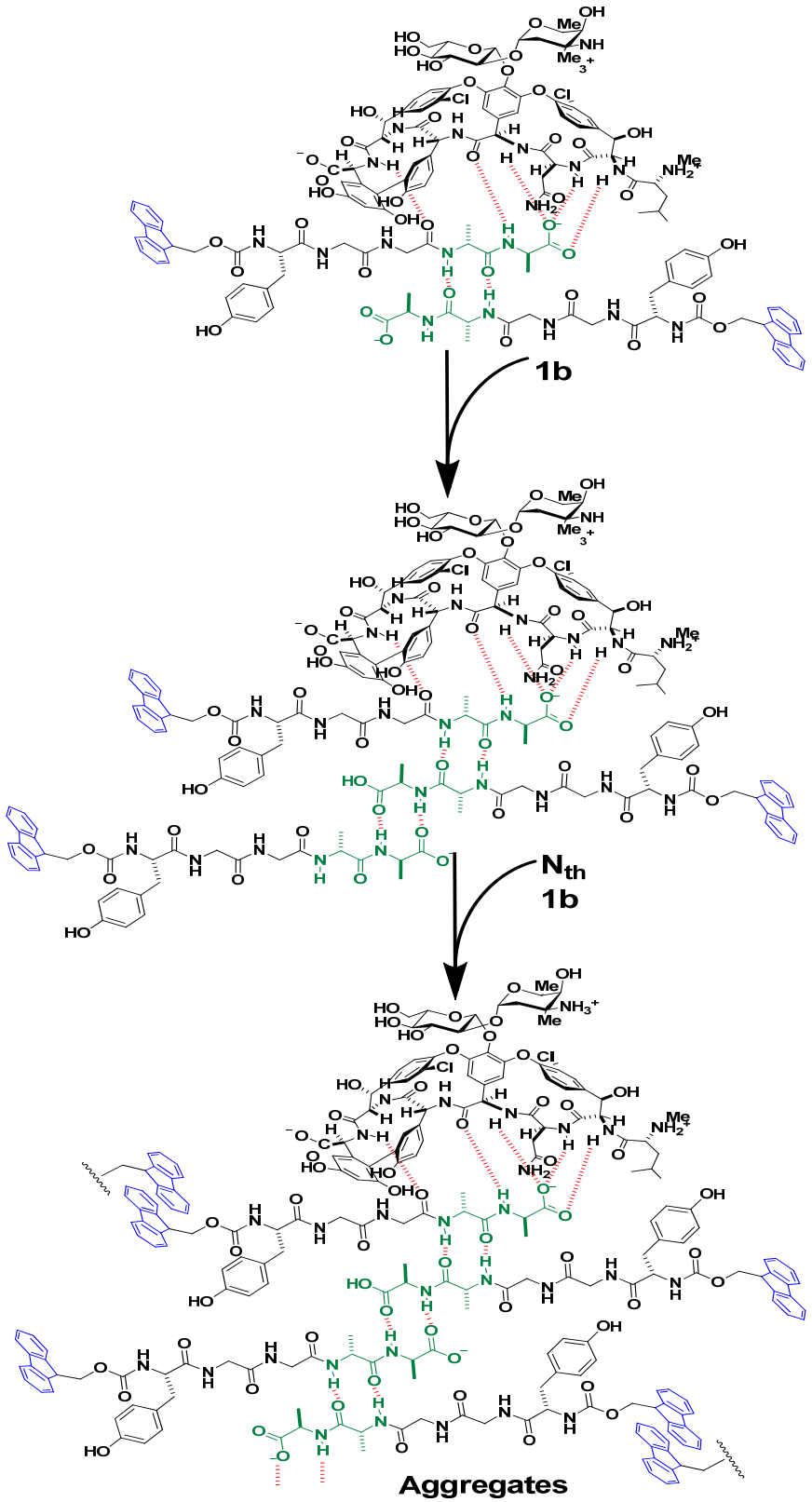


Figure S1. Plausible supramolecular interactions of two molecules of **1b** binding with one Van, thus induced the aggregation of **1b**.

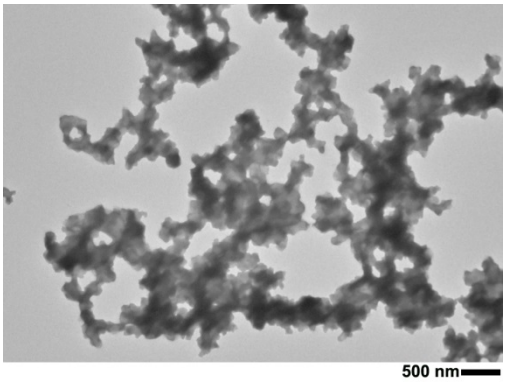


Figure S2. TEM images of the mixture of **1a** and Van upon the addition of ALP (0.5 U/mL) for 6 hours. [**1a**]$_0$= [Van]$_0$ = 300 μM.

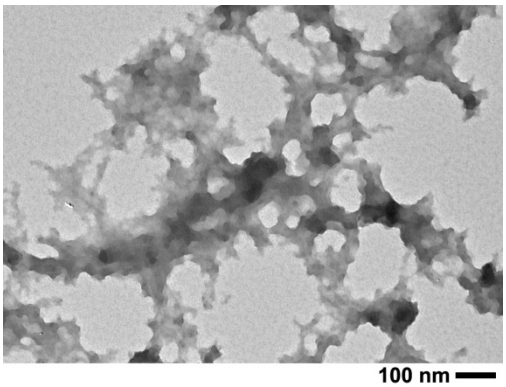


Figure S3. TEM images of the mixture of **1b** and Van ([**1b**]$_0$= [Van]$_0$ = 300 μM).

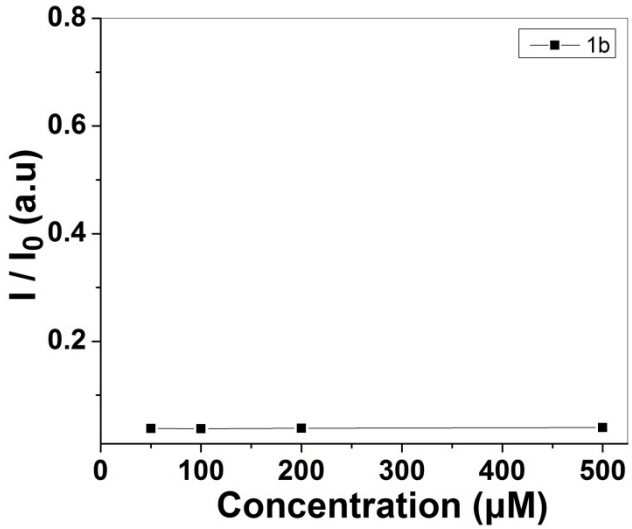


Figure S4. Light scattering intensity (I/I$_0$) as a function of concentration of **1b** after 24h.

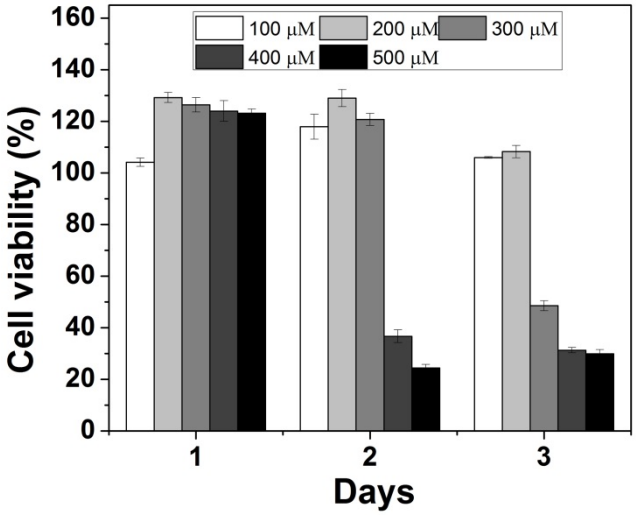


Figure S5. Cell viability of HeLa cells incubated with **1a** for 12h, and then adds the Van at the same molar ratio.

References

(1) Sadeghi-Aliabadi, H.; Minaiyan, M.; Dabestan, A. *Research in pharmaceutical sciences* **2010**, *5*, 127.
(2) *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*; Chan, W. C.; white, P. D., Eds.; Oxford University Press Inc.: New York, 2000.