Time course for inhibition of chymotrypsin by 1

Conditions: [ChT] = 4.29×10^{-6} M
[**1**] = 3.09×10^{-5} M
[BTNA] = 9.8×10^{-5} M
pH 7.4, 5 mM sodium phosphate buffer system

Assay method :
Incubation at 25 °C was started by mixing chymotrypsin and **1**. Aliquots were taken at different times and enzymatic activities were determined by hydrolysis rate of the chromogenic substrate (BTNA).

* Kinetic equations

$$\text{ChT} + \mathbf{1} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{ChT-}\mathbf{1} \qquad (1)$$

$$v = k_1 [\text{ChT}][\mathbf{1}] - k_{-1}[\text{ChT-}\mathbf{1}] \qquad (2)$$

At the beginning of such a association reaction the concentration of complex is low as compared to the concentrations of free chymotrypsin and **1**. the second term of equation (2) can vanish. So,

$$v = k_1 [\text{ChT}][\mathbf{1}] \qquad (3)$$

Integration of the equation (3) gives the following equation (4) which describes the time course of most associations nearly to completion (about 90 %)

$$\frac{1}{[\mathbf{1}]_0 - [\text{ChT}]_0} \ln \frac{[\mathbf{1}]_0 - [\text{ChT-}\mathbf{1}]}{[\text{ChT}]_0 - [\text{ChT-}\mathbf{1}]} = k_1 t + \ln \frac{[\mathbf{1}]_0}{[\text{ChT}]_0} \qquad (4)$$

$$P = \frac{1}{[\text{ChT}]_0 - [\mathbf{1}]_0} \ln \frac{[\mathbf{1}]_0 - [\text{ChT-}\mathbf{1}]}{[\text{ChT}]_0 - [\text{ChT-}\mathbf{1}]}$$

$[\mathbf{1}]_0$ = initial concentration of **1**

$[\text{ChT}]_0$ = initial concentration of ChT

[ChT-**1**] = complex concentration

Ref: Vicent, J.-P., Lazdunski, M. *Biochemistry*, 1972, 11,2967.

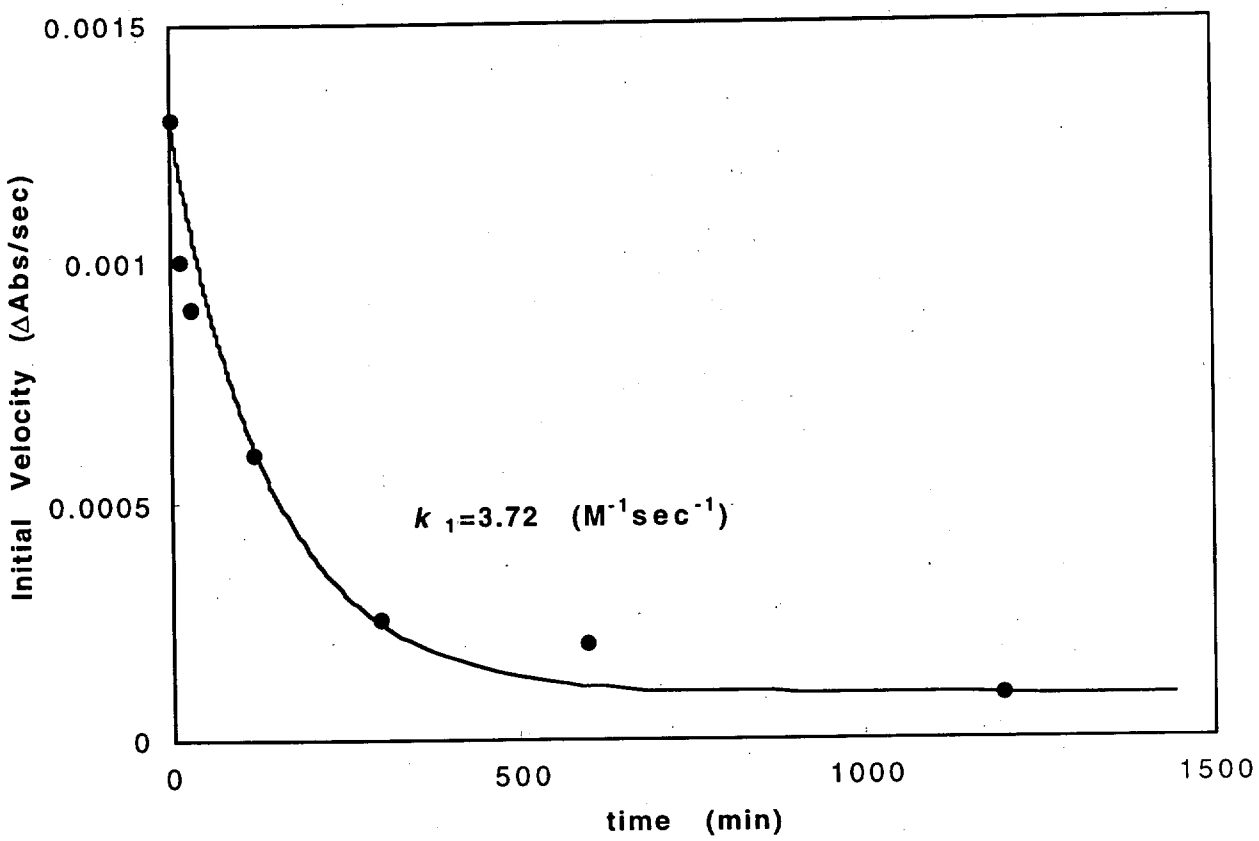

Figure S1. Time course for the inhibition of chymotrypsin by **1**.

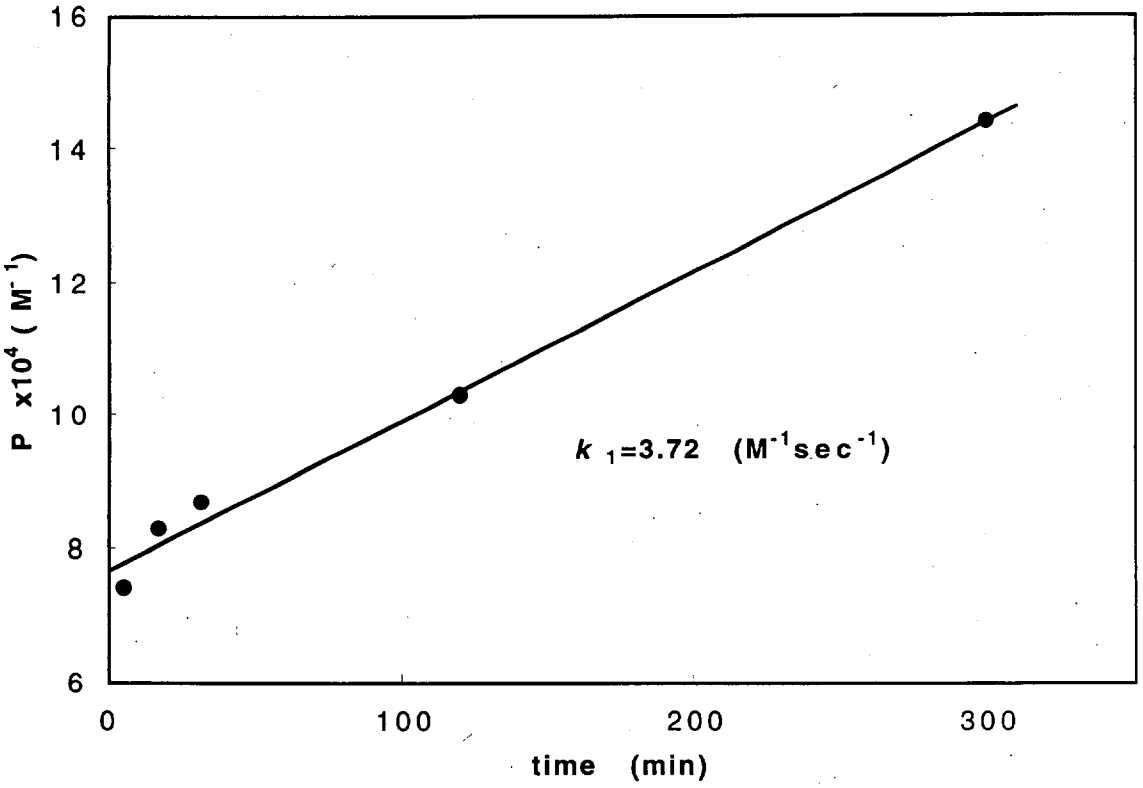

Figure S2. Calculation of association rate constants for the inhibition of chymotrypsin by **1**.

Butoxycalix[4]arene tetracyclo-(GDGD) (1)

To a solution of butoxycalix[4]arene tetraacid (86 mg, 0.10 mmol) and oxalyl chloride (254 mg, 2.0 mmol) in dry CH_2Cl_2 (10 mL) was added DMF (0.025 mL) through a silica gel filter and the mixture was stirred at room temperature for 8 h. The reaction mixture was evaporated in vacuo to obtain the acid chloride (102 mg). A solution of cyclo-GDGDSp-NH_2 (266 mg, 0.44 mmol) and DIEA (80 mg, 0.60 mmol) in dry CH_2Cl_2 (10 mL) was added to the evaporated residue. The mixture was stirred at room temperature for 14 h. The reaction mixture was purified by preparative TLC (SiO_2, first 1/9 = MeOH/CH_2Cl_2, later 1/4 = MeOH/CH_2Cl_2) to obtain the desired compound as a yellow powder (281 mg, 89 %): mp > 350 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.10 (broad s, 4H), 8.95 (broad, 4H), 8.49 (broad, 4H), 8.25 (d, J = 8.7 Hz, 4H), 8.09 (broad, 4H), 7.99 (m, 8H), 7.67 (s, 4H), 7.60 (s, 8H), 7.37 (s, 4H), 4.81 (m, 8H), 4.51 (m, 8H), 3.99 (m, 20H), 3.63 (m, 8H), 3.46 (m, 4H), 2.79 (m, 4H), 2.62 (m, 4H), 2.31 (m, 8H), 1.97 (m, 8H), 1.47 (m, 8H), 1.35 (m, 72H), 1.02 (t, J = 8.4 Hz, 12H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 120.1, 117.8, 80.0 (2C), 75.0, 49.1, 48.8, 44.2, 42.3, 41.4, 37.5, 36.9, 31.9, 30.5, 27.6, 18.9, 14.0; To above compound (298 mg, 0.093 mmol) was added TFA (3 mL) and dry CH_2Cl_2 (3 mL) and the mixture was stirred at room temperature for 1 hr. The mixture was evaporated under reduced pressure. The crude product was passed through anion exchange resin (Amberlite IRA-400(OH), water) and cation exchange resin (Amberlite IR 120 (plus), water) to remove ions. Water was lyophilized to give the titled compound (229 mg, 90%): mp > 350 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.02 (s, 4H), 8.81 (s, 4H), 8.48 (s, 4H), 8.31 (d, J = 8.7 Hz, 4H), 7.97 (m, 12H), 7.64 (s, 4H), 7.55 (s, 8H), 7.30 (s, 4H), 4.76 (m, 8H), 4.52 (m, 8H), 4.0-3.4 (m, 32H), 2.8-2.6 (m, 8H), 2.5-2.3 (m, 8H), 1.97 (m, 8H), 1.50 (m, 8H), 1.01 (t, 8.1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.2, 171.7, 170.9, 170.4, 169.3, 168.5, 165.1, 159.0, 140.2, 138.8, 134.4, 134.2, 128.7, 128.3, 121.4, 119.9, 117.7, 75.0, 49.1, 44.3, 42.2, 41.3, 36.1, 31.9, 30.5, 18.9, 14.0; HR FAB-MS *m/e* calcd for $C_{128}H_{145}N_{24}O_{44}$ (M + H^+) 2721.9767, found 2721.985.

Butoxycalix[4]arene tetracyclo-(GDGY) (2)

To a solution of butoxycalix[4]arene tetraacid (22.5 mg, 0.027 mmol) in 5 mL dry CH_2Cl_2 was added oxalyl chloride (146 mg, 1.1 mmol) and catalytic amount of DMF (0.2 μL), and the mixture was stirred at r.t. overnight. The reaction mixture was evaporated in vacuo to give the acid chloride. A solution of cyclo-(Gly-Asp(O^tBu)-Gly-Tyr(O^tBu)-Amab) (78.0 mg, 0.12 mmol) and DIEA (15 mg, 0.12 mmol) in dry CH_2Cl_2 (5 mL) was added to the acid chloride and the mixture was stirred at r.t. for 2 days. The solvent and excess reagent were evaporated and the residue was applied to a Sephadex LH-20 gel filtration column eluted with CH_2Cl_2. The appropriate portions were collected and evaporated to give the fully protected product. Further treatment with 25% TFA/CH_2Cl_2 (5 mL) at r.t. for 2 hr afforded the final product as a light-yellow powder (52.6 mg, 66%): mp > 350 °C (dec.); ^1H NMR (500 MHz, DMSO-d_6) δ 9.99 (s, 4H), 9.15 (s, b, 4H), 8.74 (s, 4H), 8.42 (d, J = 8.4 Hz, 4H), 8.41 (s, 4H), 8.19 (d, J = 7.2 Hz, 4H), 7.96 (s, 4H), 7.84 (s, 8H), 7.63 (s, 4H), 7.55 (s, 4H), 7.31 (s, 4H), 6.99 (d, J = 8.4 Hz, 8H), 6.64 (d, J = 8.3 Hz, 8H), 4.75 (m, 4H), 4.58 (m, 8H), 4.43 (m, 4H), 4.01 (s, b, 8H), 3.94 (d, J = 14.7 Hz, 4H), 3.74 (m, 12H), 3.57 (m, 8H), 2.90 (dd, J = 13.8 Hz, 5.3 Hz, 4H), 2.80 (dd, J = 16.4 Hz, 5.9 Hz, 4H), 2.63 (dd, J = 13.6 Hz, 9.0 Hz, 4H), 2.48 (m, 4H), 1.96 (m, 8H), 1.49 (m, 8H), 1.03 (t, J = 7.3 Hz, 12H); ES-MS calcd for $C_{148}H_{161}N_{24}O_{40}$ 2916.90 ($M+H^+$), found 2914.74±0.38.

Butoxycalix[4]arene tetracyclo-(GKGK) (3)

To a solution of butoxycalix[4]arene tetraacid (9.2 mg, 0.011 mmol) in 2 mL dry CH_2Cl_2 was added oxalyl chloride (29 mg, 0.23 mmol) and a catalytic amount of DMF (0.1 μL), and the mixture was stirred at r.t. overnight. The reaction mixture was evaporated in vacuo to give the acid chloride. A solution of cyclo-(Gly-Lys(BOC)-Gly-Lys(BOC)-Amab) (32.0 mg, 0.044 mmol) and DIEA (6 mg, 0.048 mmol) in dry CH_2Cl_2 (2 mL) was added to the acid chloride and the mixture was stirred at r.t. for 2 days. The solvent and excess reagent were evaporated and the residue was

applied to a Sephadex LH-20 gel filtration column eluted with CH_2Cl_2. The appropriate portions were collected and evaporated to give the fully protected product. Further treatment with 25% TFA/CH_2Cl_2 (3 mL) at r.t. for 2 hr afforded the final product as its TFA salt (18 mg, 57%): mp > 290 °C (dec.); ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.97 (s, 4H), 8.71 (s, 4H), 8.42 (s, 4H), 8.33 (d, J = 9.2 Hz, 4H), 8.23 (d, J = 6.5 Hz, 4H), 7.95 (s, 4H), 7.85 (s, 8H), 7.67 (s, 4H), 7.49 (d, J = 13.8 Hz, 4H), 7.29 (s, 4H), 4.50 (m, 8H), 4.22 (m, 8H), 3.94 (m, 24H), 3.75 (m, 4H), 3.59 (m, 4H), 2.76 (s, 16H), 1.93 (m, 12H), 1.68 (m, 4H), 1.51 (m, 28H), 1.30 (m, 20H), 1.02 (t, J = 7.3 Hz, 12H); FAB-MS calcd for $C_{144}H_{201}N_{32}O_{28}$ 2827.28 ($M+H^+$), found 2827.4.