

Supplementary Information for: The precise chemical-physical nature of the pharmacore in FK506 binding protein inhibition: ElteX, a new class of nanomolar FKBP12 ligands

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Synthesis of ElteX ligands

General

All the reactions are monitored by TLC on commercially available precoated plates (silica gel 60 F 254) and the products were visualized with acidic vanillin solution. Silica gel 60, 230-400 mesh, is used for column chromatography, unless otherwise stated. EtP refers to light petroleum, bp 40-60 °C and AcOEt to ethylacetate. ¹H and ¹³C NMR spectra were recorded at 400 and 100 MHz on a Varian, Mercury-400 instrument. FTIR spectra are recorded as CDCl₃ solutions. ESI-mass spectra are measured with a LCQ-FLEET, Thermo Scientific instrument. THF was distilled from sodium in the presence of the blue colour of benzophenone kethyl, DCM was distilled from CaH₂.

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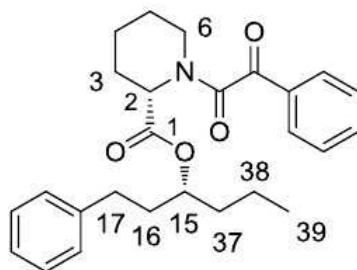


Figure 1

Commercial available reagents, catalysts and ligands are used as obtained, unless otherwise stated, from freshly opened container without further purifications. In this section the name of compounds and intermediates has been done using IUPAC rules, on the other hand the numbering of H and C atoms reported in the description of NMR spectra and indicated in Figure 1 follows that used along the body text of the manuscript.

(3R/S)-1-phenylhexan-3-yl 4-methylbenzenesulfonate¹

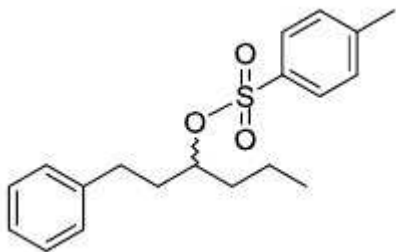


Figure 2: (3R/S)-1

To a solution of (3R/S)-1-phenylhexan-3-ol² (0.43 g, 2.44 mmol) in 2 mL of dry pyridine, p-tosyl chloride (0.93 g, 4.88 mmol) is added, under a N₂ atmosphere. The suspension is stirred at room temperature for 15 hours, then poured onto 15 mL of DCM. The reaction mixture is washed with HCl 10% (3x20 mL), saturated solution of NaHCO₃ (3x20 mL) and Brine (2x50 mL), then dried over Na₂SO₄, filtered and evaporated to obtain a yellowish oil (0.64 g). The ¹H-NMR spectrum of the crude shows the presence of p-tosyl chloride as impurity, not removable

by flash chromatography at this stage. The impurity is easily removed in the next step of the procedure. ^1H NMR, 400MHz, CDCl_3 , δ : 0.84 (t, 3H, $J=7.13$ Hz, CH_3); 0.91 (t, 3H, $J=7.61$ Hz, $\text{CH}_{3\text{tosyl chloride}}$); 1.19-1.38 (m, 4H, 2CH_2); 1.52-1.76 (m, 2H, CH_2); 1.85-1.94 (m, 2H, CH_2); 2.45 (s, 3H, $\text{CH}_{3\text{tosyl}}$); 2.45 (s, 3H, $\text{CH}_{3\text{tosyl chloride}}$); 2.49-2.64 (m, 2H, CH_2); 4.60-4.66 (m, 1H, H-3); 7.16-7.35 (m, 5H, H_{arom}); 7.33 (d, 2H, $J=8.10$, H_{tosyl}); 7.80 (d, 2H, $J=7.13$ Hz, H_{tosyl}). ^{13}C NMR, 100MHz, CDCl_3 , δ : 13.63; 17.88 (2C); 21.69 (1C, CH_3); 31.06; 35.76; 36.20 (3C); 83.35 (1C, C-3); 125.96; 128.23; 126.56; 128.39; 129.68 (5C, C_{arom}); 134.65; 141.02; 144.41 (3C).¹²

(3R/S)-1-(3-azidohexyl)benzene

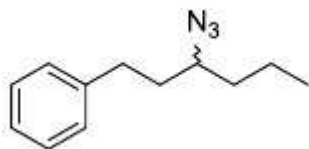


Figure 3: (3R/S)-2

To a solution of (3R/S)-1-phenylhexan-3-yl 4-methylbenzenesulfonate ((**3R/S**)-**1**) (0.25 g, 0.78 mmol) in 3 mL of dry DMF, NaN_3 (0.8 g, 12.4 mmol) is added and the light pink suspension is stirred, under N_2 , for 15 hours, then poured onto 20 mL of AcOEt. The reaction mixture is washed with Brine (4 x 30 mL). The recollected organic layer is then dried over Na_2SO_4 , filtered and evaporated to obtain a yellowish oil (0.32 g). The crude is purified by flash chromatography (eluent EtP:AcOEt/ 10:1) to obtain **2** as a colourless oil (0.15 g, 98% yield). ^1H NMR, 400MHz, CDCl_3 , δ : 0.93 (t, 3H, $J=7.13\text{Hz}$, CH_3); 1.35-1.57 (m, 4H, 2CH_2); 1.78-1.84 (m, 2H, CH_2); 2.63-2.84 (m, 2H); 3.22-3.30 (m, 1H, H-3); 7.18-7.32 (m, 5H, H_{arom}). ^{13}C NMR, 100MHz, CDCl_3 , δ : 13.86 (1C, CH_3); 19.30; 32.40; 36.17; 36.59 (4C, $\text{C}_{\text{aliphatic}}$); 62.03 (1C, C-3); 126.01 (1C, C_{para}); 128.47 (4C, $\text{C}_{\text{orto-meta}}$); 141.30 (1C, C_{quat}).

(3R/S)-1-phenylhexan-3-amine³

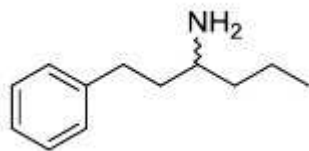


Figure 4: (3R/S)-3

To a solution of (3R/S)-1-(3-azidohexyl)benzene (3R/S)-2 (0.09 g, 0.44 mmol) in 3 mL of MeOH, Pd/C 5% (0.06g, 0.031 mmol) is added in a double neck rounded bottom flask. The suspension is stirred at room temperature in H₂ atmosphere at ordinary pressure for 3 hours then filtered to obtain 3 as a colourless oil (0.03 g, 38% yield). ¹H NMR, 300 MHz, CDCl₃, δ : 0.92 (t, 3H, *J*=6.93Hz, CH₃); 1.22-1.35 (m, 2H, CH₂); 1.39-1.48 (m, 2H, CH₂); 1.52-1.63 (m, 1H, H-8); 1.70-1.80 (m, 1H, H-8); 2.58-2.67 (m, 1H, H-9); 2.70-2.79 (m, 2H, CH₂), 7.15-7.30 (m, 5H, H_{arom}). ¹³C NMR, 100 MHz, CDCl₃, : 14.20 (1C, CH₃); 19.22, 32.59; 39.91; 40.42 (4C, C_{aliphatic}); 50.56 (1C, C-9); 125.65 (1C, C_{para}); 128.31 (4C, C_{orto-meta}); 142.47 (1C, C_{quat}).³

(15R/S,2S)-tert-butyl 2-(1-phenylhexan-3-ylcarbamoyl)piperidine-1-carboxylate

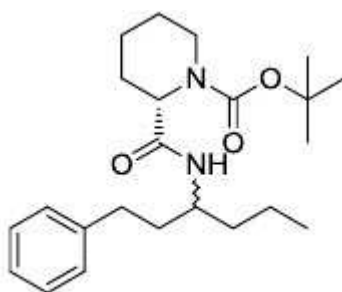


Figure 5: (15R/S,2)S-4

A solution of N-Boc (L)-pipercolinic acid (0.36 g, 1.58 mmol) is dissolved into freshly distilled DCM (5 mL) under a N₂ atmosphere. Then, diisopropylcarbodiimide (0.25 mL, 1.58 mmol), a solution of (R,S)-1- phenylhexan-3-amine 3 (0.14 g, 0.79 mmol) in DCM and DMAP (0.0097 g,

0.079 mmol) are added at 0 °C, under a N₂ atmosphere, while the formation of a white precipitate of diisopropylurea is observed. The reaction is stirred at room temperature for 18 hours, then poured onto diethyl ether (20 mL). The organic layer is alternately washed several times with saturated solutions of NH₄Cl and NaHCO₃, dried over Na₂SO₄, filtered, evaporated and dried under vacuum to obtain 0.21 g of an orange oil. The crude product is purified by flash chromatography using (eluent DCM:AcOEt/ 10:1, followed by 8:1) to obtain **(15R/S,2S)-4** as a colourless oil (0.14 g, 46 % yield). ¹H NMR, 400MHz, CDCl₃, δ: 0.90 (t, *J*= 7.22 Hz, 3H, CH₃), 1.25-1.85 (m, 21H, H_{aliphatic}), 2.24-2.44 (bs, 1H, H-6), 2.55-2.68 (m, 2H, CH₂ H-5), 2.70-2.83 (bs, 1H, H-6), 3.93-4.06 (bs, 1H, H-15), 4.69-4.77 (bs, 1H, H-2), 5.60-6.13 (bs, 1H, NH), 7.15-7.29 (m, 5H, H_{arom}). ¹³C NMR, 100MHz, CDCl₃, : 13.91; 13.94; 19.05; 19.21; 20.56; 24.93; 28.34; 28.37; 32.37; 32.59; 37.39; 37.58 (12C, C_{aliphatic}); 53.40 (1C, C-15); 54.94 (1C, C-2); 80.62 (1C, C-9); 125.83 (1C, C-4); 125.90; 128.29; 128.30; 128.37; 128.43; 141.81 (6C, C_{arom}); 170.74 (1C, C=OBoc); 182.68 (1C, C=O_{amide}). IR (CDCl₃), cm⁻¹: 3422.41 (m, NH stretch.), 3353.44 (m, NH stretch.), 1673.11 (C=O stretch.).

(15R/S,2S)-1-(2-oxo-2-phenylacetyl)-N-(1-phenylhexan-3-yl)piperidine-2-carboxamide

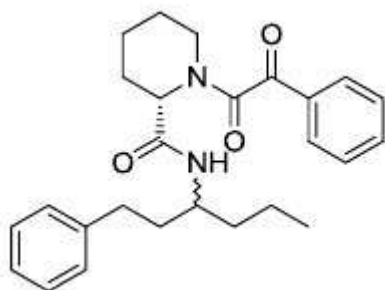


Figure 6: ElteN420

a) Deprotection of N-Boc piperidine: To a solution of (15R/S,2S)-*tert*-butyl 2-(1-phenylhexan-3-ylcarbamoyl)piperidine-1-carboxylate (**(15R/S,2S)-4**) (0.12 g, 0.31 mmol) in 2 mL of DCM, 1 mL of a solution of TFA 99 % (0.4 mL, 5.22 mmol) in 2 mL of DCM is added drop wise at 0 °C. The reaction mixture turns yellow. The solution is stirred at room temperature for 1 hour

and 2 mL of the acid solution are added at 0 °C since residual starting material is monitored by TLC. Then, a saturated solution of NaHCO₃ (25 mL) is added drop wise and the reaction mixture is extracted with DCM (3 x 30 mL). The recollected organic layer is washed with a saturated solution of NaHCO₃ (2x35 mL), dried over Na₂SO₄, filtered, evaporated and dried under vacuum to obtain the NH-piperidine ring as a yellow oil (0.075 g, 76% yield).

b) Derivative 5: To a solution of the previous crude (0.065 g, 0.23 mmol) in freshly distilled DCM (4 mL), phenylglyoxylic acid 98% (0.44 g, 0.29 mmol), DMAP (0.0055 g, 0.045 mmol) and diisopropylcarbodiimide (0.045 mL, 0.29 mmol) are added at 0 °C under a N₂ atmosphere. A white precipitate is observed. The reaction mixture is stirred under a N₂ atmosphere at room temperature for 18 hours. Diethyl ether (20 mL) is added and the organic layer is alternately washed several times with saturated solutions of NH₄Cl and NaHCO₃, dried over Na₂SO₄, filtered and evaporated under vacuum to obtain 0.080 g of a yellow oil. The crude is purified by chromatography (eluent EtP:AcOEt/ 5:1) to obtain a mixture containing three different types of urea as by-products. To get rid of them, the mixture is again purified by flash chromatography using (eluent toluene:Et₂O/ 10:1) to obtain **ElteN420** as a colourless oil (0.011 g, 11 % yield).

The ¹H-NMR spectrum of ElteN420 appears quite complex due to the presence of two rotamers (1:1.3 ratio), each as 1:1 mixture of diastereoisomers.⁴ When the attribution of signals to different rotamers is possible (1) refers to the minor rotamer. ¹H NMR, 400MHz, CDCl₃, : 0.81 (m, 3H, CH₃); 1.1-1.83 (m, 10CH₂, CH₂-16+ CH₂-37 + CH₂-38 + CH₂-3 + CH₂-4); 2.23-2.35 (m, 2H, CH₂-17); 2.50-2.73 (m, 3H, CH₂-5, H-6); 3.15-3.24 (m, 1H, H-2); 3.37-3.44 (m, 1H, H-21); 3.87-4.08 (m, 2H, H-15 + H-151); 4.58-4.66 (m, 1H, H-61); 5.10-5.16 (m, 1H, H-6); 5.72-5.80 (m, 1H, NH); 6.27-6.37 (m, 1H, NH1); 7.06-7.24 (m, 5H, H_{arom}); 7.39-7.50 (m, 2H, H_{meta}); 7.54- 7.63 (m, 1H, H_{para}), 7.88-7.93 (m, 2H, H_{ortho}). IR (CDCl₃), cm⁻¹: 3418.10 (w, NH stretch.), 3021.55 (w, CH_{arom} stretch.), 2926.72 (m, CH aliphatic stretch.), 1679.56 (s, NC=O stretch.), 1640.86 (s, PhC=O stretch.). ESI-MS: *m/z* 862.77 [2M+Na]⁺, 443.31 [M+Na]⁺.

(3S)-1-phenylhex-5-en-3-ol^{5,6}

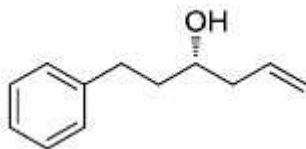


Figure 7: (3S)-6

To a solution of (-)- β -allyldiisopinocampheylborane 1M in dioxane (5.00 mL, 5.00 mmol) in freshly distilled diethyl ether (2.6 mL), 3-phenylpropionaldehyde (0.66 mL, 5.00 mmol) is added at -100 °C under a N₂ atmosphere and then allowed to reach 0 °C. The reaction is stirred under a N₂ atmosphere at 0 °C for 1 hour and then allowed to reach room temperature. NaOH 3M (3.50 mL) and H₂O₂ 30 % (1 mL) are added and the mixture is stirred at reflux at for 1 hour. A white precipitate is observed. Distilled water (20 mL) is added and the aqueous layer is extracted with diethyl ether (3x50 mL). The organic layer is washed with Brine (3x60 mL), dried over MgSO₄, filtered and evaporated under vacuum to obtain 1.91 g of a pale yellow oil. The crude is purified by flash chromatography (eluent toluene:AcOEt/ 5:1) to obtain **(3S)-6** as a colourless oil (0.48 g, 55 % yield). ¹H NMR, 400MHz, CDCl₃, δ : 1.73 (br, 1H, OH), 1.72-1.82 (m, 2H, CH₂, H-2), 2.20 (dtt, $J_1 = 14.0$ Hz, $J_2 = 8.0$ Hz, $J_3 = 0.8$ Hz, 1H, H-4), 2.33 (dtt, $J_1 = 14.0$ Hz, $J_2 = 5.2$ Hz, $J_3 = 1.2$ Hz, 1H, H-4), 2.66 (dt, $J_1 = 13.6$ Hz, $J_2 = 8.4$ Hz, 1H, H-1), 2.83 (dt, $J_1 = 13.6$ Hz, $J_2 = 7.6$ Hz, 1H, H-1), 3.71-3.65 (m, 1H, H-3), 5.13-5.18 (m, 2H, CH₂-6), 5.83 (dddd, $J_1 = 16.0$ Hz, $J_2 = 10.0$ Hz, $J_3 = 7.6$ Hz, $J_4 = 6.0$ Hz, 1H, H-5), 7.18-7.32 (m, 5H, H_{arom}). ¹³C NMR, 100MHz, CDCl₃, δ : 32.00 (1C, C-2); 38.40 (1C, C-1); 42.00 (1C, C-4); 69.80 (1C, C-3); 118.30 (1C, C-6); 125.80 (1C, C_{para}); 128.30 (2C, C_{ortho}); 128.40 (2C, C_{meta}); 134.00 (1C, C-5); 144.00 (1C, C_{quat}). IR (CDCl₃), cm⁻¹: 3390 (s, OH stretch. free), 3062, 3026 (s, CH stretch. arom), 2860 (m, CH stretch. aliph), 1640 (m, C=C stretch.), 1494 (s, C=C stretch.), 1454 (s, CH bend.), 1047 (s, C-OH stretch.).

(R)-1-phenylhexan-3-ol^{7,8}

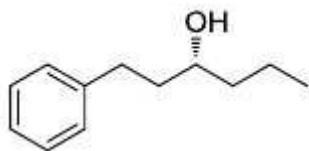


Figure 8: (3R)-7

To a solution of (S)-1-phenylhex-5-en-3-ol (3S)-6 (0.33 g, 1.88 mmol) in dry MeOH (26 mL), Pd/C 5% (0.032 g) is added in a double neck rounded bottom flask. After two nitrogen-vacuum cycles two hydrogen-vacuum cycles are performed. The reaction mixture is stirred in a H₂ atmosphere at ordinary pressure at room temperature for 2.5 hours. The reaction mixture is filtered, the solid fraction is washed with 10 mL of DCM and the collected liquid layer is evaporated under vacuum to obtain 0.26 g of a colourless oil. The crude is purified by flash chromatography (eluent EtP:AcOEt/ 5:1) to obtain **(3R)-7** as a colourless oil (0.24 g, 71 % yield). ¹H NMR, 400MHz, CDCl₃, δ : 0.95 (t, J = 7.1 Hz, 3H, CH₃), 1.33-1.51 (m, 5H, 2CH₂ + OH), 1.70- 1.86 (m, 2H, CH₂), 2.19-2.72 (m, 1H, H-1), 2.73-2.86 (m, 1H, H-1), 3.66-3.67 (m, 1H, H-3), 7.19-7.23 (m, 3H, H_{ortho-para}), 7.27-7.32 (m, 2H, H_{meta}). ¹³C NMR, 100MHz, CDCl₃, δ : 14.33; 19.02; 32.23; 39.33; 39.98 (5C, C_{aliphatic}); 71.34 (1C, C-3); 126.00 (1C, C_{para}); 128.61(2C, C_{ortho}); 128.62 (1C, C_{meta}); 142.43 (1C, C_{quat}). $[\alpha]_D^{25}$: -11.24, (c = 1.69, EtOH), (Lit.**: -4.05 (c = 1.85, EtOH)).

(15R,2S)-tert-butyl-1-phenylhexan-3-yl piperidine-1,2-dicarboxylate

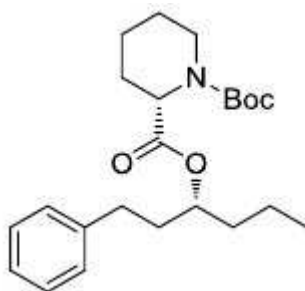


Figure 9: (15R,2S)-8

To a solution of (R)-1-phenylhexan-3-ol (3R)-7 (0.28 g, 1.53 mmol) in 10 mL of dry DCM, N-Boc-L- pipercolinic acid (0.42 g, 1.84 mmol), DMAP (0.018 g, 0.15 mmol) and diisopropylcarbodiimide (0.29 mL, 1.84 mmol) are added at 0 °C, under a N₂ atmosphere. A white precipitate indicates the formation of diisopropylurea. The reaction is stirred under a N₂ atmosphere at room temperature for 5 hours, then poured onto 20 mL of diethyl ether. The organic layer is filtered and alternately washed several times with saturated solutions of NH₄Cl and NaHCO₃, dried over Na₂SO₄, filtered and evaporated under vacuum to obtain **(15R,2S)-8** as a yellow oil (0.28 g, 47 % yield). ¹H NMR, 400MHz, CDCl₃, δ: 0.91 (t, *J* = 7.90 Hz, 3H, CH₃), 1.24-1.39 (m, 2H, 2H-38), 1.45 (s, 9H, C(CH₃)₃), 1.46-1.63 (m, 6H, 3CH₂), 1.78-1.93 (m, 4H, CH₂-17 + CH₂-37), 2.54-2.71 (m, 2H, CH₂-4), 2.87-3.08 (m, 1H, H-5), 3.88-4.12 (m, 1H, H-5), 4.72-4.83 (m, 1H, H-2), 4.87-5.05 (m, 1H, H-15), 7.14-7.31 (m, 5H, H_{arom}). ¹³C NMR, 100MHz, CDCl₃, δ: 13.89; 18.52; 20.76; 22.47; 24.34; 24.67; 24.86; 26.86 (5C, C_{aliphatic}); 28.33 (3C, C_{t-butyl}); 31.86; 36.25; 36.40; 41.08; 42.16; 53.89; 54.87 (4C, C_{aliphatic} + rotamer); 74.44 (1C, C-15); 79.85; 80.33 (1C, C_{t-butyl}-9 + rotamer); 125.97; 128.32; 128.41; 141.45 (6C, C_{arom}); 155.40; 155.93 (1C, C=O_{carb}); 171.81 (1C, C=O_{ester}). IR (CDCl₃), cm⁻¹: 1726.88 (m, C=O_{ester} stretch.), 1683.87 (s, C=O_{carb} stretch.), 1365.59 (m, C-O stretch.), 1275.26 (m, N-CO-O stretch. as), 1043.01 (w, N-CO-O stretch. sy). [α]_D²³: -0.47 (c = 1.80, CHCl₃).

(15R,2S)-1-phenylhexan-3-yl piperidine-2-carboxylate

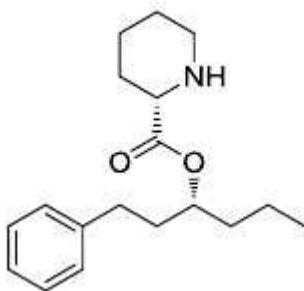


Figure 10: (15R,2S)-9

To a solution of (15R,2s)-*tert*-butyl-1-phenylhexan-3-yl piperidine-1,2-dicarboxylate (15R,2S)-8 (0.19 g, 0.48 mmol) in DCM (2 mL) 1 mL of a solution of TFA 99% (0.20 mL, 2.58 mmol) in 2 mL of DCM is added drop wise at 0 °C. The reaction mixture turns yellow. The reaction mixture is stirred at room temperature for 4 hours during which 1.5 mL of the acid solution is added at 0 °C since residual starting material is monitored by TLC. A saturated solution of NaHCO₃ (20 mL) is added drop wise and the aqueous layer is extracted with DCM (3 x 25 mL). The organic layer is washed with a saturated solution of NaHCO₃ (4x80 mL), dried over Na₂SO₄, filtered and evaporated under vacuum to obtain **(15R,2S)-9** as a yellow oil (0.12 g, 86 % yield) used in the next step without further purification. In the ¹H NMR spectrum when the attribution of signals to different rotamers is possible (1) refers to the minor rotamer. ¹H NMR, 400MHz, CDCl₃, δ: 0.82 (t, *J*=7.2 Hz, 3H, CH₃1), 0.83 (t, *J*= 7.2 Hz, 3H, CH₃), 1.24-1.39 (m, 2H, 2H-38), 1.42-1.63 (m, 6H, 2CH₂), 1.78-1.93 (m, 5H, CH₂-17 + CH₂-37 + NH), 2.54-2.71 (m, 3H, H-5 + CH₂-4), 3.01-3.13 (m, 1H, H-5), 3.29-3.36 (m, 1H, H-2), 4.97-5.05 (m, 1H, H-15), 6.50 (d, *J*= 9.5 Hz, 1H, NH1), 6.63 (d, *J*= 9.01 Hz, 1H, NH), 7.14-7.30 (m, 5H, H_{arom}). ¹³C NMR, 100MHz, CDCl₃, δ: 13.94 (1C, CH₃); 18.48; 24.14; 25.88; 29.39; 31.74; 35.83; 36.30; 45.68 (8C, C_{aliphatic}); 58.72 (1C, C-2); 74.01 (1C, C-15); 125.86; 128.28; 128.36; 141.61 (6C, C_{arom}); 173.33 (1C, C=O_{ester}). IR (CDCl₃), cm⁻¹: 1722.58 (vs, C=O stretch.), 1602.15 (m, N-H bend.), 1197.84, 1124.73 (s, 2 C-O stretch.). $[\alpha]_D^{23}$ (CHCl₃): -1.53, (c = 1.50 mg/mL). $[\alpha]_D^{25}$: -6.43, (c = 1.85, EtOH).

(15R,2S)-1-phenylhexan-3-yl 1-(2-oxo-2-phenylacetyl)piperidine-2-carboxylate

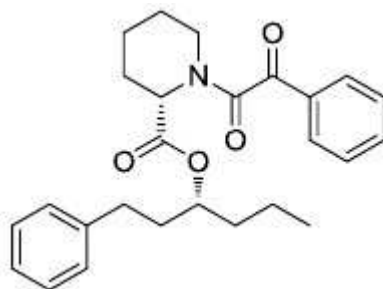


Figure 11: R-Elte421

To a solution of (15R,2S)-1-phenylhexan-3-yl piperidine-2-carboxylate (15R,2S)-9 (0.12 g, 0.41 mmol) in freshly distilled DCM (4 mL), TEA (0.11 mL, 0.82 mmol) is added under a N₂ atmosphere. The reaction mixture is cooled at 0 °C and a solution of 2-oxo-2-phenylacetyl chloride (0.070 g, 0.41 mmol) in 0.39 mL of freshly distilled DCM is added drop wise. The solution turns pale yellow. The reaction mixture is stirred under a N₂ atmosphere at room temperature for 4 hours. DCM (35 mL) is added and the reaction mixture is washed with Brine (3 x 30 mL), dried over Na₂SO₄, filtered and evaporated under vacuum to obtain 0.14 g of an orange oil. The crude is purified by flash column chromatography (eluent EtP:AcOEt/ 7:1) to obtain **R-Elte421** as a colourless oil (0.060 g, 34 % yield). In the ¹H NMR spectrum when the attribution of signals to different rotamers is possible (*) refers to the minor rotamer. ¹H NMR, 400MHz, C₆D₆, δ: 0.76 (t, *J*= 7.03 Hz, 3H, CH₃); 0.84 (t, *J*= 7.12 Hz, 3H, CH₃*); 0.9-1.82 (m, 10H, CH₂-37 + CH₂-38 + CH₂-3 + CH₂-4 + CH₂- 5); 2.06-2.12 (m, 2H, CH₂-16); 2.25-2.43 (m, 2H, CH₂-171); 2.44-2.70 (m, 2H, CH₂-17);3.01-3.12 (m, 1H, H-6); 3.31-3.45 (m, 1H, H-6); 4.52-4.54 (m, 2H, CH₂-161); 4.80-4.87 (m, 1H, H-21); 4.93-5.00 (m, 1H, H- 151); 5.09-5.17 (m, 1H, H-2); 5.54-5.58 (m, 1H, H-15); 6.94-7.15 (m, 8H, H_{arom}); 8.18-8.21 (m, 2H, H_{ortho}*); 8.30-8.34 (m, 2H, H_{ortho}). ¹³C NMR, 100MHz, C₆D₆, δ: 13.66; 18.40; 20.97; 24.45; 25.81; 31.70; 35.91; 36.25; 43.72; 51.60 (10C, C_{aliphatic}); 74.62 (1C, C-15); 125.99; 128.21; 128.37; 129.03; 129.75; 133.19; 134.74; 141.16 (10C, C_{arom}); 167.75 (1C, C=O_{carb}); 170.23 (1C, C=O_{ester}); 191.68 (1C, C=O_{ketone}). IR (CDCl₃), cm⁻¹:1726.88 (s, OC=O stretch.); 1679.56 (m, NC=O stretch.); 1640.86 (s, PhC=O

stretch.), 1447.31 (m, Car=Car stretch.). $[\alpha]_D^{25}$: -31.42, (c = 1.85, EtOH). ESI-MS: m/z 865 $[2M+Na]^+$, 444 $[M+Na]^+$.

(S)-1-phenylhexan-3-ol

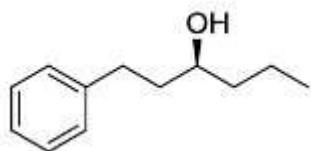


Figure 12: (3S)-7

For the synthesis of this compound, we exploit the procedure described above for the synthesis of (R)-1-phenylhexan-3-ol **R-7** except that we use the (+)- β -allyldiisopinocampheylborane instead of the (-)- β -allyldiisopinocampheylborane, to have the starting material (R)-1-phenylhex-5-en-3-ol. We obtain the final product as a colourless oil in 65 % yield. 1H NMR, ^{13}C NMR and IR spectra have been recorded and are comparable to the ones of (S)-1-phenylhex-5-en-3-ol described above. $[\alpha]_D^{24}$: +11.02, (c = 1.85, EtOH).

(15S,2S)-1-phenylhexan-3-yl 1-(2-oxo-2-phenylacetyl)piperidine-2-carboxylate

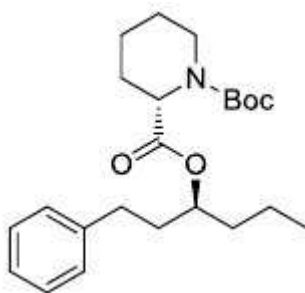


Figure 13: (15S,2S)-8

a) Initial amidation: For the synthesis of this compound, we exploit the procedure described above for the synthesis of (15R,2S)-*tert*-butyl-1-phenylhexan-3-yl piperidine-1,2-dicarboxylate

(15R,2S)-8 except that we use the (S)-1-phenylhexan-3-ol (3S)-7 instead of the (R)-1-phenylhexan-3-ol (3R)-7. We obtain the final product as a colourless oil in 42% yield [α_D^{25} : -11,50, (c =1.85, EtOH).

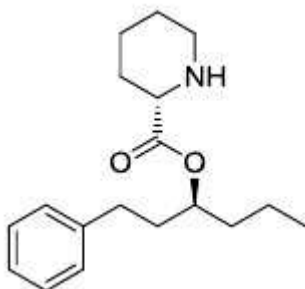


Figure 14: (15S,2S)-9

b) Deprotection: To a solution of (15S,2S)-*tert*-butyl-1-phenylhexan-3-yl piperidine-1,2-dicarboxylate (15S,2S)-8 (0.20 g, 0.52 mmol) in DCM (3 mL) 1 mL of a solution of TFA 99% (0.20 mL in 2 mL of DCM, 2.58 mmol) is added drop wise at 0 °C. The reaction mixture turns yellow and is stirred at room temperature for 4 hours during which 0.5 mL of TFA in 1.5 mL of DCM are added at 0 °C. The mixture is poured onto 20 mL of a saturated solution of NaHCO₃ and extracted with DCM (3 x 25 mL). The recollected organic layer is washed with a saturated solution of NaHCO₃ (4x80 mL),

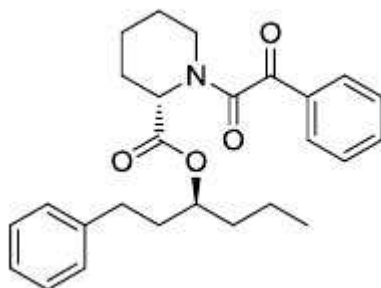


Figure 15: S-Elte421

dried over Na₂SO₄, filtered and evaporated under vacuum to obtain **(15S,2S)-9** as a pale yellow oil (0.128 g, 86 % yield). ¹H NMR, 400MHz, CDCl₃, : 0.88 (t, *J*= 7.42 Hz, 3H, CH₃), 1.24-1.39 (m, 2H, CH₂), 1.42-1.61 (m, 6H, 3 CH₂), 1.78-1.92 (m, 5H, 2 CH₂ + NH), 2.54-2.70 (m, 3H, H + CH₂), 3.00-3.11 (m, 1H), 3.29-3.34 (m, 1H), 4.97-5.03 (m, 1H, H-15), 7.12- 7.29 (m, 5H, H_{arom}).

^{13}C NMR, 100MHz, CDCl_3 , δ : 13.94 (1C, CH_3), 18.44, 24.12, 25.82, 29.37, 31.72, 35.84, 36.28, 45.65 (8C, $\text{C}_{\text{aliphatic}}$), 58.72 (1C, C-2), 74.03 (1C, C-15), 125.87, 128.27, 128.28, 128.35, 128.38, 141.61 (6C, C_{arom}), 173.30 (1C, $\text{C}=\text{O}_{\text{ester}}$). $[\alpha]_D^{25}$: -8.91, (c = 1.85, EtOH).

(2S)-1-(2-oxo-2-phenylacetyl)-N-(3-phenylpropyl)piperidine-2-carboxamide

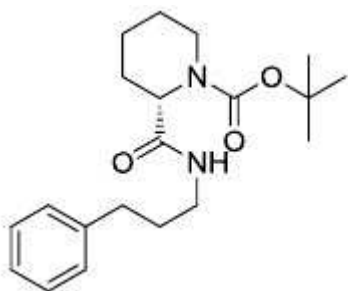


Figure 16: (2S)-10

a) Initial amidation: To a solution of 3-phenylpropan-1-amine (0.74 g, 5.45 mmol) in 6 mL of dry DCM, N- Boc-L- pipecolinic acid (2.5 g, 10.90 mmol), DMAP (0.066 g, 0.54 mmol) and diisopropylcarbodiimide (1.70 mL, 10.90 mmol) are added at 0 °C, under a N_2 atmosphere. A white precipitate indicates the formation of diisopropylurea. The reaction is stirred under a N_2 atmosphere at room temperature overnight, then poured onto 30 mL of diethyl ether. The organic layer is filtered and alternately washed several times with saturated solutions of NH_4Cl and NaHCO_3 , dried over Na_2SO_4 , filtered and evaporated under vacuum to obtain 2.8 g of a yellowish oil. The crude is purified by flash column chromatography (eluent DCM:AcOEt/ 8:1) to obtain **(2S)-10** as a colourless oil (0.50 g, 26% yield).

b) Deprotection: To a solution of (2S)-tert-butyl 2-(3-phenylpropylcarbamoyl)piperidine-1-carboxylate (2S)- 10 (0.36 g, 1.04 mmol) in DCM (6 mL) 1 mL of a solution of TFA 99% in 2 mL of DCM (0.70 mL, 9.03 mmol) is added drop wise at 0 °C. The reaction mixture turns yellow and is stirred at room temperature for 4 hours during which 1.22 mL of TFA in 3.0 mL of DCM are added at 0 °C. The mixture is poured onto 30 mL of a saturated solution of NaHCO_3 sat.sol. and extracted with DCM (3 x 55 mL). The recollected organic layer is washed with a saturated

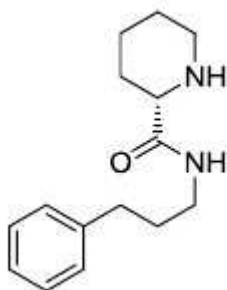


Figure 17: (2S)-11

solution of NaHCO_3 (4x80 mL), dried over Na_2SO_4 , filtered and evaporated under vacuum to obtain **(2S)-11** as a pale yellow powder (0.184 g, 72% yield), used directly in the next step.

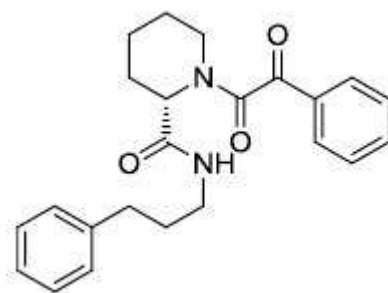


Figure 18: ElteN378

c) Final amidation: To a solution of (2S)-N-(3-phenylpropyl)piperidine-2-carboxamide (2S)-11 (0.132 g, 0.54 mmol) in freshly distilled DCM (7 mL), phenylglyoxylic acid (0.106 g, 0.70 mmol), DMAP (0.013 g, 0.11 mmol) and diisopropylcarbodiimide (0.11 mL, 0.70 mmol) are added at 0 °C under a N_2 atmosphere. A white precipitate is observed. The reaction mixture is stirred under a N_2 atmosphere at room temperature for 18 hours. Diethyl ether (30 mL) was added and the organic layer was alternately washed several times with saturated solutions of NH_4Cl and NaHCO_3 , dried over Na_2SO_4 , filtered and evaporated under vacuum to obtain 0.230 g of a yellow oil. The crude is initially purified by chromatography using DCM : AcOEt 8 : 1. Eventually, to get rid of ureas byproducts still accompanying the target compounds the mixture is purified again by flash chromatography using (eluent toluene:Et₂O/ 1:2) to obtain ElteN378 as a colourless oil (0.065 g, 32% yield). The ^1H -NMR showed the presence of a 1.6:2.4 mixture of rotamers.

When the attribution of signals to different rotamers is possible (*) refers to the minor rotamer.

^1H NMR, 400MHz, CDCl_3 , δ : 1.27- 1.84 (m, 8H, CH_2 -5 + CH_2 -16+ CH_2 -3 + CH_2 -3*); 2.19-2.30 (m, 2H, CH_2 -4); 2.60 (at, J =7.38 Hz, 2H, CH_2 -17); 2.62-2.70 (m, 2H, CH_2 -17*); 3.15-3.33 (m, 4H, CH_2 -15, H-6, H-15*); 3.35-3.41 (m, 1H, H-6); 4.00-4.03 (m, 1H, H-2*); 4.53-4.60 (m, 1H, H-6*); 5.11-5.15 (m, 1H, H-2); 6.16 (bt, 1H, J =6.12 Hz, 1H, NH); 6.68 (bt, 1H, J =6.28 Hz, NH1); 7.10-7.23 (m, 5H, H_{arom}); 7.41-7.48 (m, 2H, H_{meta}); 7.55-7.62 (m, 1H, H_{para}), 7.89-7.91 (m, 2H, H_{ortho}). ^{13}C NMR, 100MHz, CDCl_3 , δ : 20.41; 20.70; 25.05; 25.20; 25.83; 26.61; 31.14; 31.28; 33.14; 33.19 (5C, C_{aliph}); 39.25 (1C, C-6); 39.41 (1C, C-15); 39.52 (1C, C-15*); 44.55 (1C, C-6*); 51.79 (1C, C-2); 57.06 (1C, C-2*); 125.99; 126.01; 128.38; 128.41; 128.46; 128.48; 129.18; 129.22; 129.63; 129.87; 132.71; 132.93; 135.03; 135.40; 141.28; 141.33(20 C, 10 C_{arom} + 10 C_{arom}^*); 166.29; 167.58; 168.33; 169.50 (4C, 2 $\text{C}=\text{O}_{\text{amide}}$ + 2 $\text{C}=\text{O}_{\text{amide}}^*$); 191.58; 192.43 (2C, $\text{C}=\text{O}_{\text{ket}}$ + $\text{C}=\text{O}_{\text{ket1}}$). ESI-MS: m/z 778.92 [$2\text{M}+\text{Na}$] $^+$, 401.33 [$\text{M}+\text{Na}$] $^+$, 377.17 [$\text{M}-\text{H}$] $^-$. IR (CDCl_3), cm^{-1} : 3435.34 (w, NH free stretch.), 3366.37 (w, NH free stretch.), 3030.17 (w, CH_{arom} stretch.), 2943.96 (m, $\text{CH}_{\text{aliphatic}}$ stretch.), 1679.56 (s, $\text{NC}=\text{O}$ stretch.), 1640.86 (s, $\text{PhC}=\text{O}$ stretch.). $[\alpha]_D^{24}$: -23.28, (c = 1.80, EtOH).

Simulation Results on Elte-X ligands

Force field parameterization

In Figure 19 the AMBER03⁹ atomic types assignment is shown for ElteN378 (top), for (R,S)Elte421 and (R,S)ElteN420 (middle) and for V10,367 (bottom). In the Figures 20 and 21 we report the

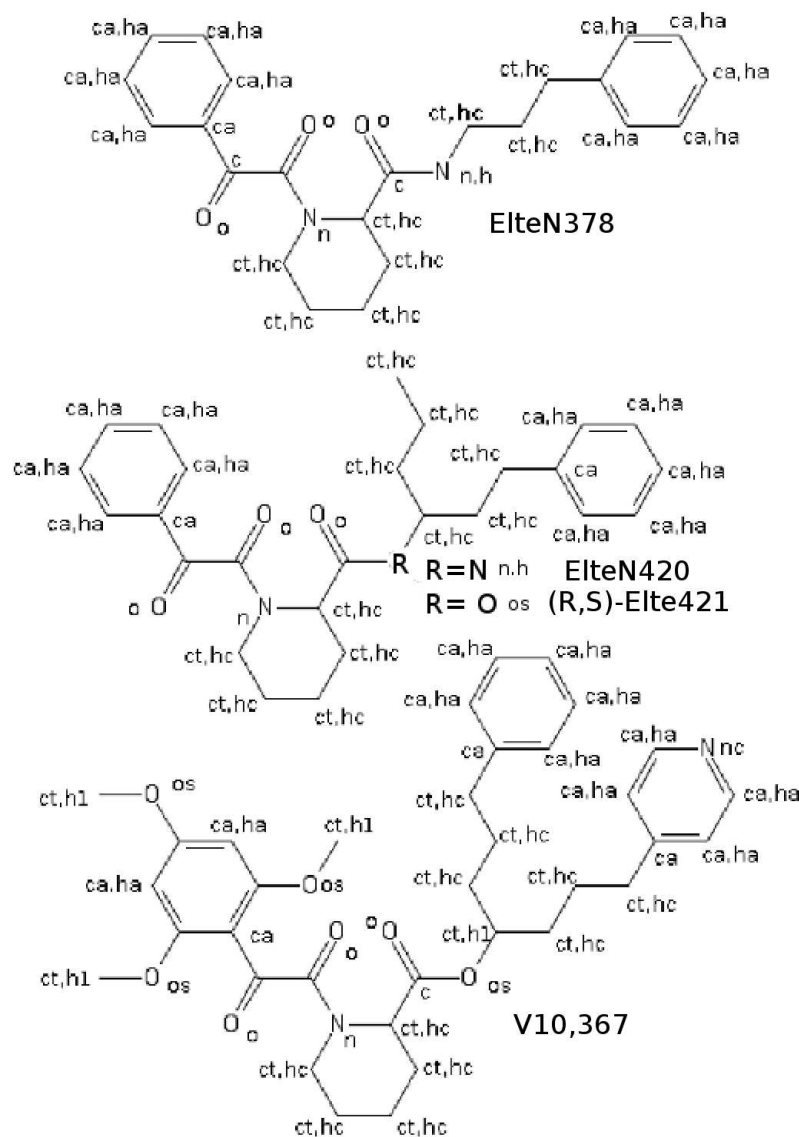


Figure 19: Atomic types assignment for ElteX ligands

atomic charges in electrons.

ATOM	TYPE	X/A	Y/A	Z/A	q/e
C10	ca	9.370	-2.187	-5.367	-0.079729
C35	ca	8.314	-1.950	-4.416	-0.152282
H40	ha	8.461	-1.422	-3.485	0.148420
C34	ca	7.018	-2.310	-4.697	-0.075099
C33	ca	6.712	-2.898	-5.948	-0.093880
H38	ha	5.681	-3.136	-6.162	0.120388
H39	ha	6.234	-2.254	-3.957	0.111298
C32	ca	7.719	-3.152	-6.911	-0.144106
C31	ca	9.028	-2.795	-6.604	0.080447
H37	ha	7.516	-3.549	-7.895	0.125822
H36	ha	9.783	-2.929	-7.364	0.086070
C9	c	10.774	-1.879	-5.185	0.390808
O4	o	11.579	-2.138	-6.130	-0.419553
C8	c	11.412	-1.288	-3.847	0.546292
O3	o	10.738	-1.282	-2.868	-0.566465
N7	n	12.742	-0.935	-3.701	-0.347359
C6	ct	13.201	-0.550	-2.322	0.006826
H12	hc	12.516	0.148	-1.842	0.062302
H13	hc	13.295	-1.469	-1.744	0.080220
C5	ct	14.535	0.172	-2.220	-0.056321
H14	hc	14.946	0.282	-1.216	0.039820
H15	hc	14.414	1.204	-2.548	0.020276
C4	ct	15.611	-0.464	-3.081	0.049036
H16	hc	16.546	0.096	-3.070	0.015620
H17	hc	15.877	-1.450	-2.699	0.011831
C3	ct	15.158	-0.478	-4.519	-0.200627
H18	hc	15.026	0.536	-4.898	0.085159
H19	hc	15.834	-1.072	-5.134	0.064159
C2	ct	13.832	-1.253	-4.684	0.050676
H20	hc	13.507	-1.009	-5.695	0.082562
C1	c	14.150	-2.796	-4.581	0.594049
O2	o	14.148	-3.346	-3.482	-0.453283
O1	os	14.312	-3.479	-5.756	-0.540534
C15	ct	14.600	-4.871	-5.919	0.224090
H21	hc	14.704	-4.995	-6.997	0.081001
C39	ct	15.950	-5.224	-5.212	-0.248591
H48	hc	15.821	-5.232	-4.130	0.035397
H49	hc	16.150	-6.245	-5.536	0.028438
C38	ct	17.173	-4.450	-5.612	0.185992
H43	hc	16.995	-3.380	-5.502	-0.016547
H47	hc	17.925	-4.642	-4.846	-0.035010
C37	ct	17.754	-4.744	-7.017	-0.285626
H44	hc	18.600	-4.070	-7.150	0.064037
H45	hc	17.055	-4.513	-7.820	0.064037
H46	hc	18.024	-5.800	-7.011	0.064037
C16	ct	13.552	-5.944	-5.529	0.101753
H27	hc	13.290	-5.937	-4.471	-0.068558
H28	hc	13.865	-6.964	-5.750	0.023706
C17	ct	12.236	-5.653	-6.264	-0.526375
H29	hc	11.467	-6.392	-6.040	0.130749
H30	hc	11.935	-4.645	-5.978	0.129434
C18	ca	12.364	-5.661	-7.795	0.373349
C19	ca	12.173	-6.880	-8.525	-0.274436
H31	ha	11.868	-7.737	-7.943	0.131586
C20	ca	12.301	-6.849	-9.962	-0.066777
C21	ca	12.581	-5.625	-10.636	-0.191501
H32	ha	12.185	-7.726	-10.582	0.109288
H33	ha	12.600	-5.665	-11.715	0.124333
C22	ca	12.724	-4.425	-9.912	-0.054748
C23	ca	12.550	-4.430	-8.490	-0.313000
H34	ha	13.045	-3.557	-10.470	0.108915
H35	ha	12.756	-3.504	-7.974	0.165192

ATOM	TYPE	X/A	Y/A	Z/A	q/e
C10	ca	9.992	7.431	8.794	-0.044880
C35	ca	10.946	6.462	9.167	-0.209315
H40	ha	11.424	5.814	8.447	0.171422
C34	ca	11.336	6.262	10.454	-0.043999
C33	ca	10.822	6.961	11.498	-0.093108
H39	ha	12.185	5.644	10.704	0.099969
H38	ha	10.995	6.783	12.549	0.116417
C32	ca	9.838	7.970	11.231	-0.141239
C31	ca	9.480	8.197	9.879	-0.036706
H37	ha	9.419	8.535	12.050	0.124480
H36	ha	8.798	8.997	9.629	0.107154
C9	c	9.712	7.899	7.461	0.453082
O4	o	9.223	8.980	7.303	-0.478256
C8	c	10.208	7.088	6.176	0.515781
O3	o	10.693	5.942	6.335	-0.598315
N7	n	10.193	7.566	4.873	-0.381733
C6	ct	9.743	8.900	4.423	-0.053774
H12	hc	8.822	9.171	4.941	0.092644
H13	hc	10.525	9.555	4.806	0.095716
C5	ct	9.517	8.994	2.869	-0.022630
H14	hc	9.315	10.051	2.695	0.031938
H15	hc	8.679	8.359	2.582	0.021735
C4	ct	10.707	8.512	2.074	0.024889
H16	hc	10.596	8.703	1.007	0.013929
H17	hc	11.662	8.976	2.321	0.020775
C3	ct	10.802	7.019	2.384	-0.147039
H18	hc	9.840	6.577	2.125	0.056030
H19	hc	11.602	6.688	1.723	0.056131
C2	ct	11.186	6.961	3.845	0.128569
H20	hc	11.258	5.882	3.984	0.053802
C1	c	12.686	7.269	4.087	0.703090
O2	o	12.950	8.317	4.641	-0.560719
N1	n	13.583	6.394	3.573	-0.831550
H41	h	13.408	5.656	2.905	0.419295
C15	ct	14.969	6.525	4.032	0.424483
H21	hc	15.131	7.592	4.187	0.004943
C39	ct	15.231	5.738	5.330	-0.326109
H48	hc	15.170	4.668	5.133	0.065572
H49	hc	16.259	5.974	5.602	0.064749
C38	ct	14.258	6.136	6.466	0.267358
H43	hc	14.196	7.222	6.536	-0.029850
H47	hc	13.294	5.832	6.059	-0.048693
C37	ct	14.550	5.476	7.710	-0.280577
H44	hc	13.824	5.844	8.434	0.065902
H45	hc	15.525	5.848	8.027	0.065902
H46	hc	14.462	4.397	7.588	0.065902
C16	ct	15.976	6.147	2.900	0.072910
H27	hc	15.837	5.149	2.485	0.007618
H28	hc	17.011	6.112	3.240	0.036420
C17	ct	16.087	7.181	1.751	-0.385533
H29	hc	15.806	8.195	2.035	0.083065
H30	hc	17.116	7.212	1.392	0.109069
C18	ca	15.348	6.944	0.432	0.259402
C19	ca	14.068	7.506	0.203	-0.236234
H31	ha	13.523	8.085	0.933	0.133580
C20	ca	13.442	7.336	-1.069	-0.088439
C21	ca	14.093	6.571	-2.071	-0.145822
H32	ha	12.504	7.806	-1.326	0.112316
H33	ha	13.607	6.340	-3.008	0.116237
C22	ca	15.301	5.876	-1.816	-0.095205
C23	ca	15.903	6.091	-0.551	-0.231265
H34	ha	15.708	5.304	-2.637	0.113991
H35	ha	16.841	5.635	-0.270	0.134725

Figure 20: Right: atomic charges and coordinates for Elte421. Left atomic charges and coordinates for ElteN420

atom	type	X/A	Y/A	Z/A	q/e	atom	type	X/A	Y/A	Z/A	ElteN378 q/e	ElteN378 ⁺ q/e
C10	ca	-8.726	-11.516	-12.107	-0.097552	C10	ca	2.484	-75.924	-20.327	-0.044880	-0.046155
C35	ca	-7.698	-10.693	-12.588	-0.468058	C35	ca	3.270	-76.855	-19.591	-0.209315	-0.206168
H40	ha	-6.862	-11.104	-13.135	0.221147	H40	ha	3.933	-77.552	-20.083	0.171422	0.155276
C31	ca	-9.762	-10.839	-11.449	-0.237599	C34	ca	3.046	-77.164	-18.231	-0.043999	-0.058482
H36	ha	-10.486	-11.457	-10.939	0.141841	C33	ca	2.148	-76.372	-17.492	-0.093108	-0.058392
C34	ca	-7.808	-9.259	-12.537	0.458982	H39	ha	3.728	-77.856	-17.758	0.099969	0.122625
O39	os	-6.806	-8.507	-12.990	-0.366014	H38	ha	1.920	-76.585	-16.458	0.116417	0.128795
C93	ct	-5.610	-9.134	-13.513	-0.067709	C32	ca	1.563	-75.253	-18.120	-0.141239	-0.124840
H07	hl	-4.842	-8.452	-13.878	0.090258	C31	ca	1.680	-75.028	-19.555	-0.036706	-0.025188
H08	hl	-6.027	-9.670	-14.365	0.090258	H37	ha	0.956	-74.576	-17.536	0.124480	0.137653
H09	hl	-5.150	-9.871	-12.854	0.090258	H36	ha	1.242	-74.167	-20.036	0.107154	0.115852
C33	ca	-8.933	-8.636	-11.958	-0.123656	C9	c	2.739	-75.798	-21.724	0.453082	0.414333
O38	os	-8.936	-7.294	-12.100	-0.262270	O4	o	3.331	-76.768	-22.186	-0.478256	-0.447621
C92	ct	-9.996	-6.493	-11.652	-0.137257	C8	c	2.513	-74.540	-22.687	0.515781	0.510956
H04	hl	-10.986	-6.787	-12.000	0.100796	O3	o	2.283	-73.395	-22.172	-0.598315	-0.610038
H05	hl	-9.813	-5.439	-11.864	0.110337	N7	n	2.519	-74.651	-24.017	-0.381733	-0.386470
H06	hl	-10.050	-6.580	-10.567	0.117932	C6	ct	2.593	-75.901	-24.727	-0.053774	0.145445
C32	ca	-9.971	-9.463	-11.421	0.458982	H12	hc	3.632	-76.216	-24.625	0.092644	0.060613
O37	os	-11.011	-8.850	-10.906	-0.366014	H13	hc	1.912	-76.650	-24.323	0.095716	0.038854
C91	ct	-12.147	-9.531	-10.303	-0.067709	C5	ct	2.217	-75.728	-26.207	-0.022630	-0.091781
H01	hl	-11.893	-10.236	-9.512	0.090258	H14	hc	1.184	-75.386	-26.138	0.031938	0.049297
H02	hl	-12.784	-10.014	-11.044	0.090258	H15	hc	2.144	-76.737	-26.611	0.021735	0.068542
H03	hl	-12.857	-8.840	-9.848	0.090258	C4	ct	3.036	-74.649	-26.970	0.024889	-0.005265
C9	c	-8.630	-12.960	-12.062	0.550520	H16	hc	3.803	-75.177	-27.537	0.013929	0.033792
O4	o	-9.456	-13.581	-11.388	-0.493239	H17	hc	2.402	-74.301	-27.786	0.020775	0.059026
C8	c	-7.625	-13.854	-12.911	0.476004	C3	ct	3.565	-73.566	-26.022	-0.147039	-0.083380
O3	o	-7.028	-13.265	-13.852	-0.548380	H18	hc	4.454	-74.025	-25.590	0.056030	0.047213
N7	n	-7.370	-15.185	-12.741	-0.428275	H19	hc	3.837	-72.612	-26.473	0.056131	0.061429
C6	ct	-7.746	-15.913	-11.491	-0.009491	C2	ct	2.624	-73.395	-24.841	0.128569	0.050185
H12	hc	-7.744	-15.219	-10.650	0.056950	H20	hc	3.205	-72.642	-24.308	0.053802	0.120273
H13	hc	-8.784	-16.230	-11.584	0.088902	C1	c	1.251	-72.765	-25.197	0.703090	0.666531
C5	ct	-6.833	-17.060	-11.137	-0.060387	O2	o	0.183	-73.322	-25.045	-0.560719	-0.394023
H14	hc	-5.869	-16.856	-10.672	0.029455	N1	n	1.320	-71.520	-25.603	-0.831550	-0.557529
H15	hc	-7.310	-17.577	-10.304	0.051809	H41	h	2.219	-71.064	-25.538	0.419295	0.405027 (+ h 0.389266)
C4	ct	-6.562	-18.034	-12.306	0.103723	C15	ct	0.199	-70.587	-25.438	0.33958	-0.087222
H16	hc	-5.757	-18.728	-12.068	0.005672	H21	hc	-0.702	-71.040	-25.026	0.00	0.151811
H17	hc	-7.515	-18.462	-12.617	-0.033669	H49	hc	0.452	-69.908	-24.623	0.00	0.103024
C3	ct	-5.987	-17.149	-13.421	-0.217674	C16	ct	-0.057	-69.707	-26.642	0.072910	-0.074185
H18	hc	-5.011	-16.760	-13.130	0.080452	H27	hc	0.923	-69.434	-27.035	0.007618	0.056599
H19	hc	-5.870	-17.800	-14.287	0.064799	H28	hc	-0.581	-68.784	-26.395	0.036420	0.100071
C2	ct	-6.936	-16.023	-13.871	0.214390	C17	ct	-0.839	-70.553	-27.650	-0.385533	-0.103026
H20	hc	-6.353	-15.388	-14.539	0.054466	H29	hc	-1.168	-69.939	-28.488	0.083065	0.036335
C1	c	-8.183	-16.548	-14.680	0.834491	H30	hc	-0.188	-71.378	-27.937	0.109069	0.066874
O2	o	-8.803	-17.554	-14.387	-0.556085	C18	ca	-2.092	-71.226	-27.167	0.259402	0.116885
O1	os	-8.531	-15.759	-15.653	-0.719996	C19	ca	-3.094	-70.351	-26.631	-0.236234	-0.230450
C15	ct	-9.639	-16.042	-16.489	0.685245	H31	ha	-3.005	-69.275	-26.674	0.133580	0.140827
H21	hc	-10.284	-16.813	-16.069	-0.049434	C20	ca	-4.279	-70.893	-26.083	-0.088439	-0.083328
C16	ct	-9.113	-16.490	-17.832	-0.350976	C21	ca	-4.329	-72.253	-25.814	-0.145822	-0.110612
H27	hc	-8.303	-15.796	-18.056	0.100011	H32	ha	-5.154	-70.275	-25.941	0.112316	0.130750
H28	hc	-9.881	-16.315	-18.586	0.095889	H33	ha	-5.253	-72.625	-25.395	0.116237	0.130113
C17X	ct	-8.717	-18.001	-17.846	0.219843	C22	ca	-3.417	-73.132	-26.441	-0.095205	-0.120157
H29X	hc	-9.447	-18.600	-17.302	-0.012383	C23	ca	-2.349	-72.597	-27.222	-0.231265	-0.174325
H30X	hc	-7.717	-18.032	-17.412	-0.010661	H34	ha	-3.574	-74.197	-26.356	0.113991	0.136467
C17Y	ct	-8.688	-18.609	-19.262	-0.399838	H35	ha	-1.664	-73.250	-27.742	0.134725	0.127898
H29Y	hc	-9.711	-18.567	-19.638	0.104125							
H30Y	hc	-8.494	-19.680	-19.221	0.112299							
C18	ca	-7.631	-18.028	-20.179	0.594462							
C19	ca	-6.259	-18.239	-20.077	-0.649068							
H31	ha	-5.950	-19.024	-19.403	0.201797							
C20	ca	-5.371	-17.541	-20.826	0.556289							
N00	nc	-5.791	-16.623	-21.699	-0.761067							
H32	ha	-4.307	-17.641	-20.669	0.004396							
C22	ca	-7.079	-16.339	-21.774	0.596233							
C23	ca	-8.045	-17.050	-21.062	-0.658642							
H34	ha	-7.397	-15.510	-22.388	-0.012889							
H35	ha	-9.083	-16.809	-21.236	0.195795							
C39	ct	-10.407	-14.704	-16.665	-0.240075							
H48	hc	-9.692	-13.967	-17.031	0.096383							
H49	hc	-11.104	-14.782	-17.500	0.042747							
C38	ct	-11.086	-14.162	-15.351	0.072260							
H47	hc	-10.333	-14.027	-14.574	0.030375							
H46	hc	-11.682	-14.971	-14.928	-0.012545							
C37	ct	-12.115	-12.998	-15.482	-0.373743							
H45	hc	-12.834	-13.057	-14.665	0.116317							
H44	hc	-12.683	-13.086	-16.407	0.110747							
C18B	ca	-11.489	-11.616	-15.421	0.221065							
C19B	ca	-11.890	-10.710	-14.463	-0.211341							
H31B	ha	-12.717	-10.882	-13.790	0.138912							
C20B	ca	-11.273	-9.469	-14.371	-0.055074							
C21B	ca	-10.166	-9.152	-15.139	-0.180942							
H32B	ha	-11.658	-8.761	-13.652	0.098622							
H33B	ha	-9.714	-8.177	-15.025	0.124874							
C22B	ca	-9.767	-10.028	-16.107	-0.081246							

Figure 21: Right: atomic charges and coordinates for V10,367. Left atomic charges and coordinates for ElteN378 and ElteN378⁺

Solute Tempering-Hamiltonian Replica Exchange (ST-HREM)

In our ST-HREM simulations only the intraligand potential (torsional and non bonded) was scaled throughout the replica progression with a maximum and minimum scaling factors of 1.0 and 0.20 corresponding to a solute temperature ranging from 300 K to 1500 K. The potential energy for the k -th replica is then given by $V_k = V + (c_k - 1)V_T^{[\text{ligand}]}$ where V is the full potential energy of the system, $V_T^{[\text{ligand}]}$ is the intraligand potential and c_k is the corresponding scaling factor. Such a solute tempering approach,¹⁰ by reducing the degrees of freedom that are involved in the scaling in the generalized ensemble (i.e. only those affected by the proper, improper torsions and non bonded interactions *within* the ligand), allows to minimize, for a given replica exchange rate, the number of replicas needed in the given scaling range (300-1500 K) while maintaining at same time a high flexibility of the ligand in the “hot” replicas. Eight replicas were distributed in the given 300-1500 K range according to scheme given in Ref.¹¹ The starting REM configuration in bulk water was prepared in a cubic box in the NPT ensemble as described in Ref.¹² The samples contained from 499 to 502 TIP3 water molecules with a mean box length ranging from 25.16 Å and 25.21 Å in the target replica at T=300 K and P=1 Atm. The electrostatic interaction were treated using the Smooth Particle Mesh Ewald method,¹³ with 20 grid points in each directions and a 4-th order B-spline interpolation. The equations of motion were integrated using a multiple time step r-RESPA algorithm¹⁴ with a potential subdivision specifically tuned for bio-molecular systems.^{15,16} For each ligand, the (Hamiltonian) REM simulations were conducted in the NPT ensemble with isotropic stress tensor,¹⁵ and lasted in all cases 12 ns for a total simulation of 96 ns in the generalized ensemble. The mean acceptance exchange ratio in both simulations resulted in the range 0.35-0.55.

The ST-HREM simulation of ElteN378 in DMSO were performed using an intrasolute scaling range of 1.0-0.2 (300-1500 K). The force field for DMSO was taken from ref.¹⁷ The cubic box contained in both cases 112 DMSO molecules with mean side length of 23.97 for (15S)-Elte and (15R)-Elte. The 12 ns REM simulations in DMSO (total time in the generalized ensemble of 96ns) had a mean acceptance exchange ratio in the range 0.35-0.55.

A discussion of the errors in the simulations of the FKBP12 ligands is provided in Ref.¹²

Simulation of Elte421 in bulk water

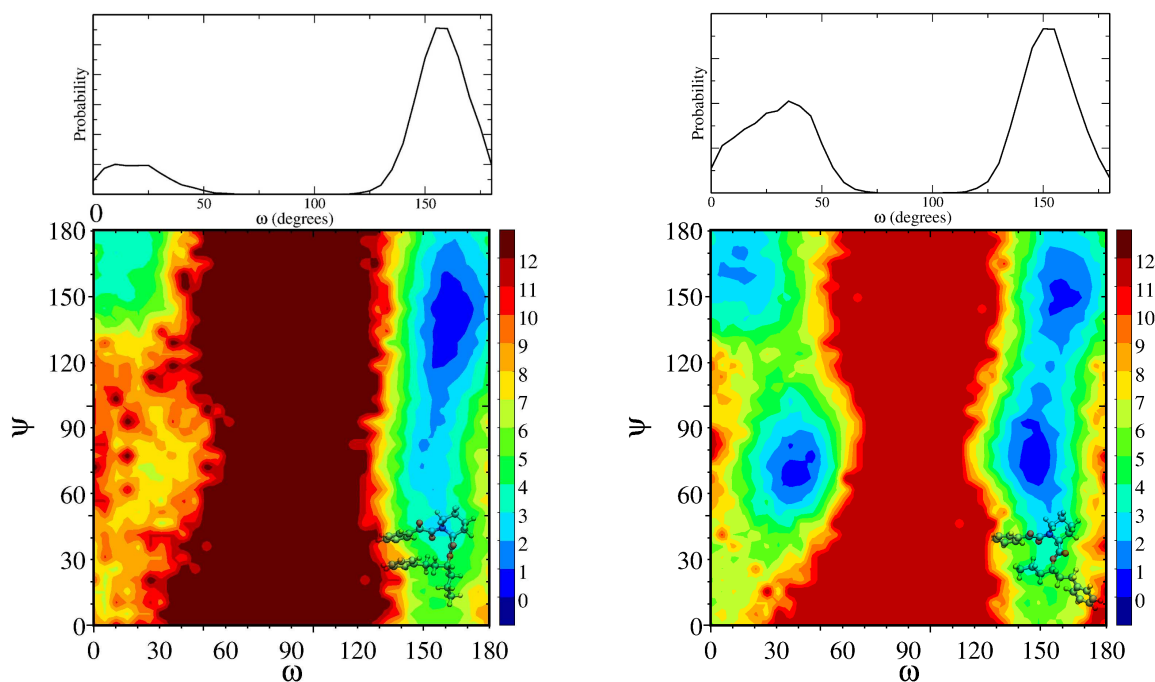


Figure 22: Free energy surface (FES) with respect to the dihedral angles ω and ψ (in degrees) of R-Elte421 (left) and S-Elte421 (right) calculated via solute tempering REM in water bulk solution. The energy scale (labels on the color coded bar on the right of each diagram) is in kJ mol^{-1} . The most populated clusters for R-Elte421 and S-Elte421 (populations 26.6% and 15,8% respectively) are reported in the 2D diagrams in the ω, ψ FKBP12 binding region. In the top figures we report the ω probability distribution for R-Elte421 (left) and S-Elte421(right)

In Figures 22 and 23 we collected the results obtained in the ST-HREM simulation in water bulk solution at $T=300$ K and $P=1$ Atm for R-Elte421 and S-Elte421. For each C15 diastereoisomer we calculated the FES as a function of the ω, ψ angles (see Figure 1 of the main paper) for 20000 configurations taken from the target (0) replica spanning 12 ns using previously generated trajectory data.² The FES is calculated as $G(\omega, \psi) = -RT \ln P(\omega, \psi)$, with $P(\omega, \psi)$ being the corresponding distribution probability. The maximum of the 2D function $P(\omega, \theta)$ defines the zero in

the 2D FES. The 1D probability distribution as a function of ω alone (also reported in Figure 22) shows that in the S diastereoisomer the ω -*cis* rotamer is significantly more populated with respect to the R epimer.

Simulation of ElteN420 diastereoisomers in bulk water

In Figure 23 we collected the results obtained in the ST-HREM simulation in water bulk solution at T=300 K and P=1 Atm for R-ElteN420 and S-ElteN420. For each epimer, all data are calculated on 24000 configurations taken from the target (0) replica spanning 12 ns. For each C15 diastereoisomer we report i) in the top part of the diagram: the O2-O3 distance involving the carbonyl of the binding domain as a function of the ω, ψ angles ($D_{OO}(\omega, \psi)$) and ii) in the bottom part: the FES with respect to the O2-O3 and H(N1)-O3 distances $G(r_{O3-O2}, r_{HN1-O3})$. The latter function is calculated as $G(r_{O3-O2}, r_{HN1-O3}) = -RT \ln P(r_{O3-O2}, r_{HN1-O3})$, with $P(r_{O3-O2}, r_{HN1-O3})$ being the corresponding distribution probability. The maximum of the function $P(r_{O3-O2}, r_{HN1-O3})$ defines the zero in $G(r_{O3-O2}, r_{HN1-O3})$.

Concerning the $D_{OO}(\omega, \psi)$ function (top diagrams), we note that $\omega \psi$ *cis/trans* and *trans/cis* regions, in both epimers with minor differences, are characterized by distances in the range 4.2-5.0 Å, corresponding to the O2-O3 distance range found in the PDB structures of the Ligand-FKBP complexes.¹⁸⁻²² The binding state is the ω -*trans* and ψ -*cis* state. The symmetrical ω -*cis* and ψ -*trans* states have also optimal O2-O3 distances, but in this case, at variance with the ω -*trans* and ψ -*cis* configuration, the spatial arrangements of the hydrophobic moieties in the ligand interfere with binding.

In the bottom part of the Figure we show the color coded contour plot of the function $G(r_{O3-O2}, r_{HN1-O3})$ for the R and S diastereoisomers. The diamond bluish shape (regions of high probability, low free energy) seen in the FES reported in Figure 23 is again a manifestation of the stability of the *cis/cis*, *cis/trans*, *trans/cis* and *trans/trans* rotameric states in the ω, ψ conformational space (see also Figure 3 of the main paper). In the FES reported in Figure 23, the minimum at O2-O3 distances below 4.0 has no H(N1)-O3 hydrogen bond and corresponds to a ω, ψ *trans-trans* rotameric state (see

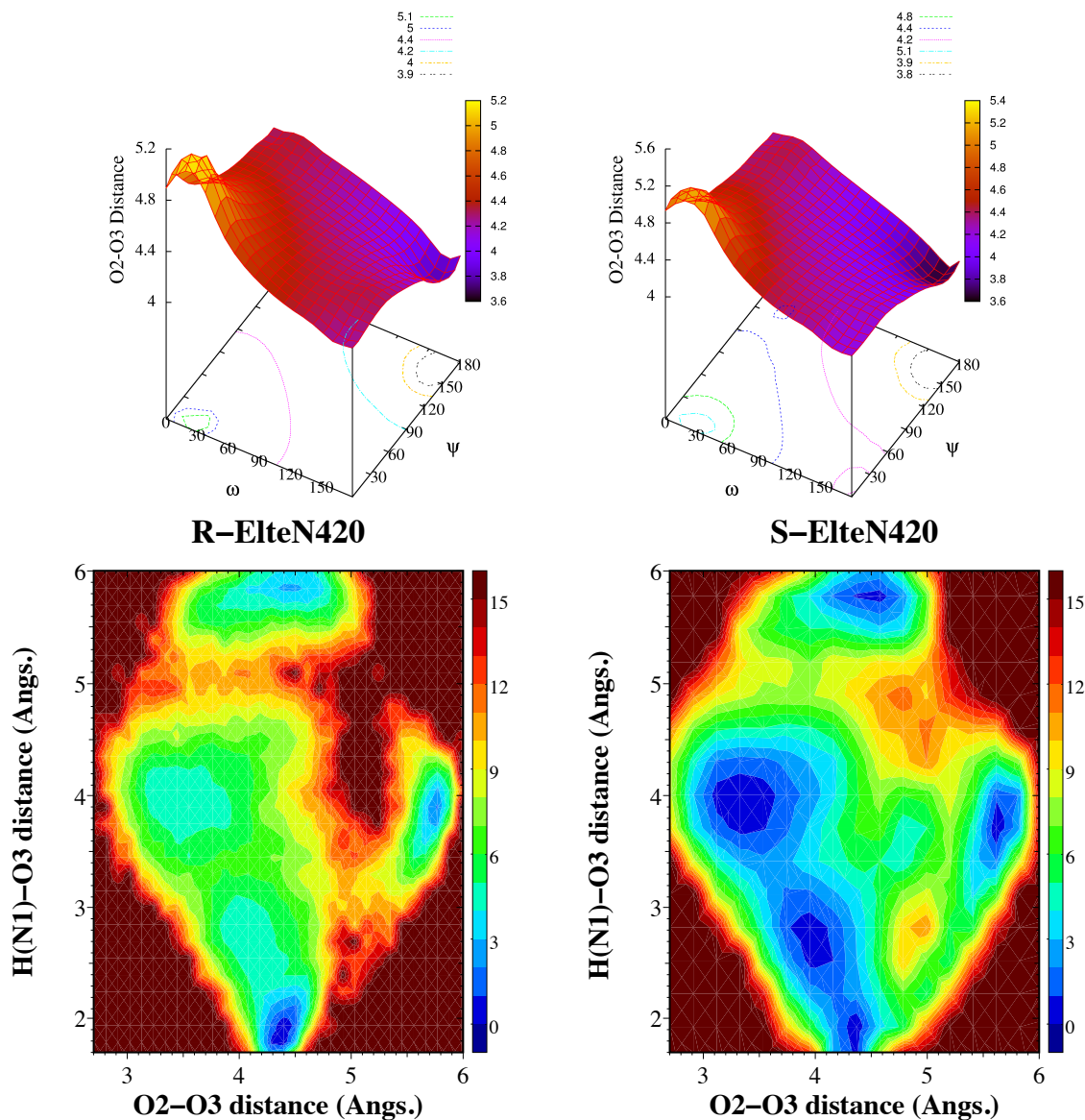


Figure 23: Top: O2-O3 distance (in Å) as a function of ω and ψ (in degrees) in R-ElteN420 (left) and S-ElteN420 (right). Bottom: Free Energy Surface (FES) with respect to the O2-O3 distance and the H(N1)-O3 distance. The energy scale (labels on the color coded bar on the right of each diagram) is expressed in kJ mol⁻¹.

top Figures). The minimum seen at $r_{\text{O3-O2}} \simeq 4.4$ Å and $r_{\text{HN1-O3}} \simeq 2$ Å corresponds to the binding state stabilized by the H(N1)-O3 H-bond. In the $G(r_{\text{O3-O2}}, r_{\text{HN1-O3}})$ function, the differences between the two epimer are significant: while in the R epimer the minimum at $r_{\text{O3-O2}} \simeq 4.4$ Å and $r_{\text{HN1-O3}} \simeq 2$ Å is the deepest minimum, in the S epimer such basin competes with other strong

minima. The symmetrical counterpart of the binding minimum (at the same O3-O3 distance but with large H(N1)-O3 distances) corresponds the ω, ψ *cis-trans* conformational state that appears more stable in the R epimer. The minimum at larger O2-O3 distances ($r_{\text{O3-O2}} \simeq 4.4$) is a manifestation of the ω, ψ *trans-trans* state, a non FKBP binding conformation and again is deeper in the S epimer, suggesting that, in the case of the ElteN420 mixture, S-ElteN420 is a less effective FKBP binder with respect to the R diastereoisomer.

Simulation of V10,367 in bulk water

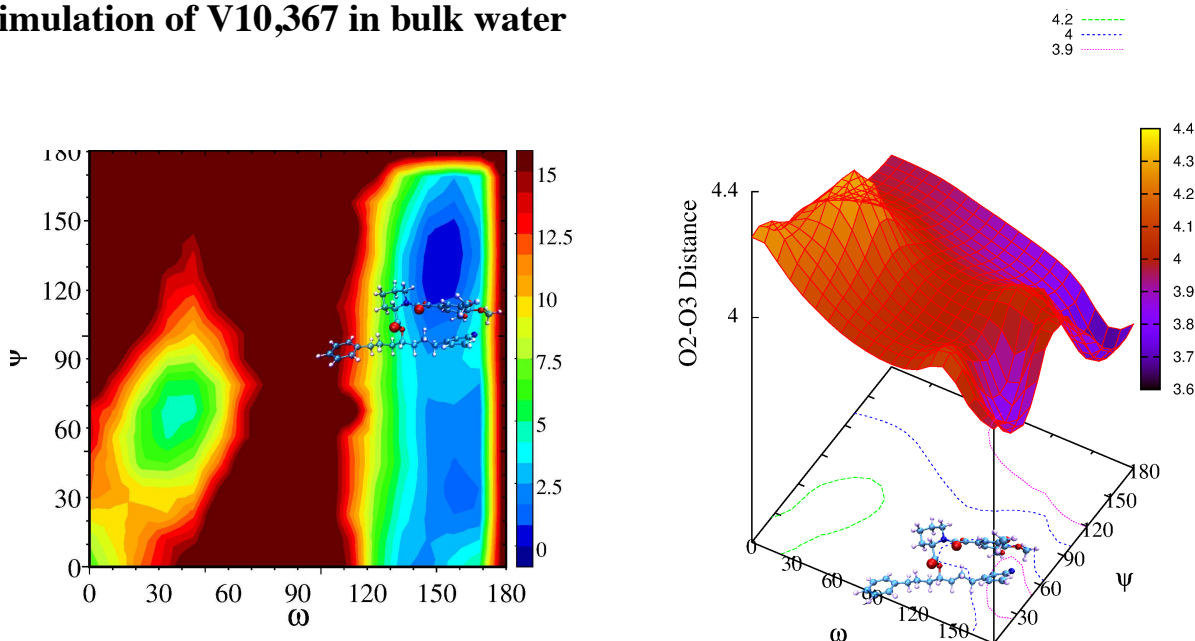


Figure 24: Left: Free Energy Surface (FES) with respect to the dihedral angles ω and ψ (in degrees) of V10,367 calculated via solute tempering REM in water bulk solution. The energy scale (labels on the color coded bar on the right of each diagram) is in kJ mol^{-1} . A representative structure of one of the most populated cluster (population $\simeq 10\%$) is reported in the corresponding ω, ψ FKBP12 region. The O2, O3 atoms are highlighted with an enhanced sphere radius. Right: Contour map of the O2-O3 distance (in Å) as a function of ω and ψ (in degrees) in V10,367.

The simulation of V10,367 at $T=300\text{K}$ and $P=1\text{ Atm}$ reveals that such compound behaves in water bulk solution essentially as an Elte-like ligand (see Figure 6 of the main paper) with frequent stacking (70% of the 20000 sampled conformations are stacked in the target replica) between

phenyl-1 and either the phenyl-2 (as in cluster 1, population $\simeq 12\%$) or the pyridyl moiety (as in cluster 2, population $\simeq 11\%$). The ω, ψ FES and the O2-O3 distance as a function of ω, ψ (see Figure 24) follows the pattern of, e.g., Elte421. Inspection of the ω, ψ FES reveals also that the most populated PKA conformer in V10,367 is the ω, ψ *trans-trans* rotamer and not the Tyr82 and Ile56 binding *trans-cis* state as in ElteN378. As there is no significant barrier between the ψ -*trans*- ψ -*cis* states conversion between these two rotamers is rapid and hence (as in R-ELte421) such feature does not reduce the binding affinity *vs* FKBP12. It must be also noted that the configurational entropy of V10,367 in bulk water is sensibly larger than that of ElteN378 (see table 1 of the main paper). This is expected since TS is an extensive quantity and V10,367 has 1.6 times the atoms ElteN378 has. Probably a large contribution to TS in V10,367 is coming from the methoxy groups on the phenyl-1. However, what counts in the binding process is the $T\Delta S$ and it can be reasonably assumed that the conformational contribution to the entropy arising from the methoxy groups in V10,367 does not change significantly upon binding to FKBP.

ElteN378: conformation of the protonated form in water

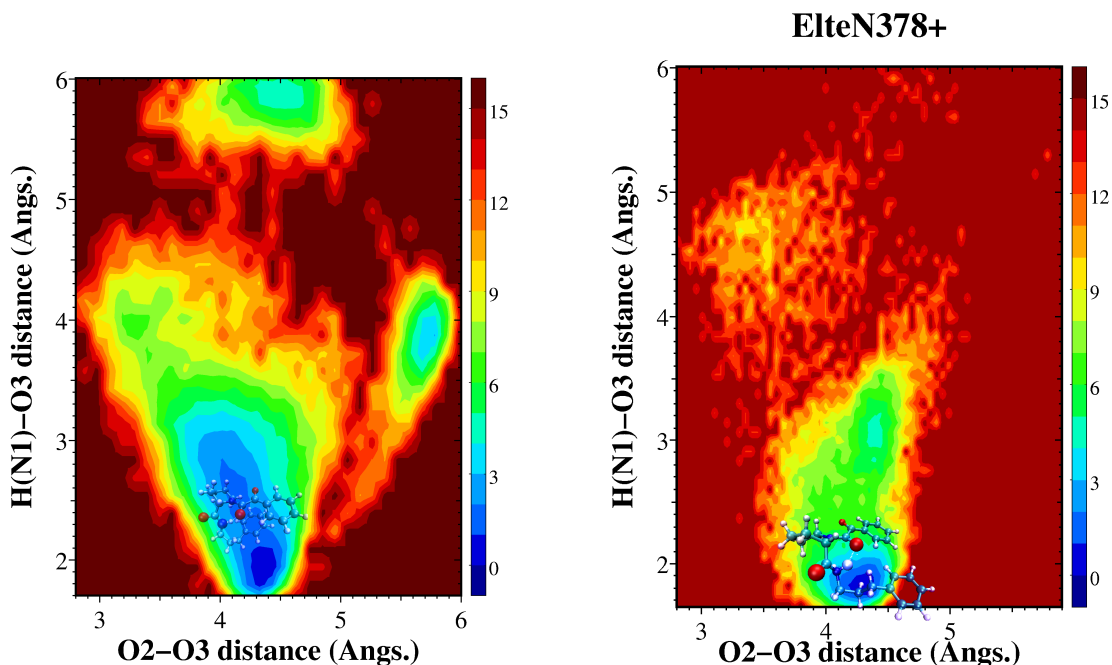


Figure 25: Free Energy Surface (FES) with respect to the O2-O3 distance and the H(N1)-O3 distance in ElteN378(left) and in ElteN378⁺ (right) in water bulk solution at 300=K and P=1 Atm. The energy scale (labels on the color coded bar on the right of each diagram) is expressed in kJ mol⁻¹. Data calculated on 20000 configurations of the target replica.

The simulation of ElteN378⁺ in water shows that the H(N1)-O3 hydrogen bond further stabilizes, with respect to ElteN378, the *ω-trans* rotamer. This is clearly seen in Figure 25 (left) where we compare the FES with respect to the O2-O3 distance and the H(N1)-O3 distance of the neutral and protonated species. In the Figure we also shown the most populated clusters found for the two species in aqueous environment. The O2 O3 and N1 atoms are highlighted with a larger sphere radius. The H-bond between H(N1) and O3 is also shown. The FES with respect to *ω, ψ* of Elte378⁺ (data not shown) closely follows the pattern of the neutral counterpart with the further weakening of the *ω, ψ cis-cis* and *cis-trans* minima (see Figure 5 of the main paper). Remarkably, at variance with neutral ElteN378 where approximately 50% of the conformations populate a parallel displaced stacked structure, in the most populated cluster of ElteN378⁺ (population % 32)

the two phenyl ring are edge-to-edge or in T-shaped configuration (see next section in the SI and discussion of fluorescence spectra of ElteN378 compounds in various solvents in the main paper).

Cluster analysis of ElteX compounds.

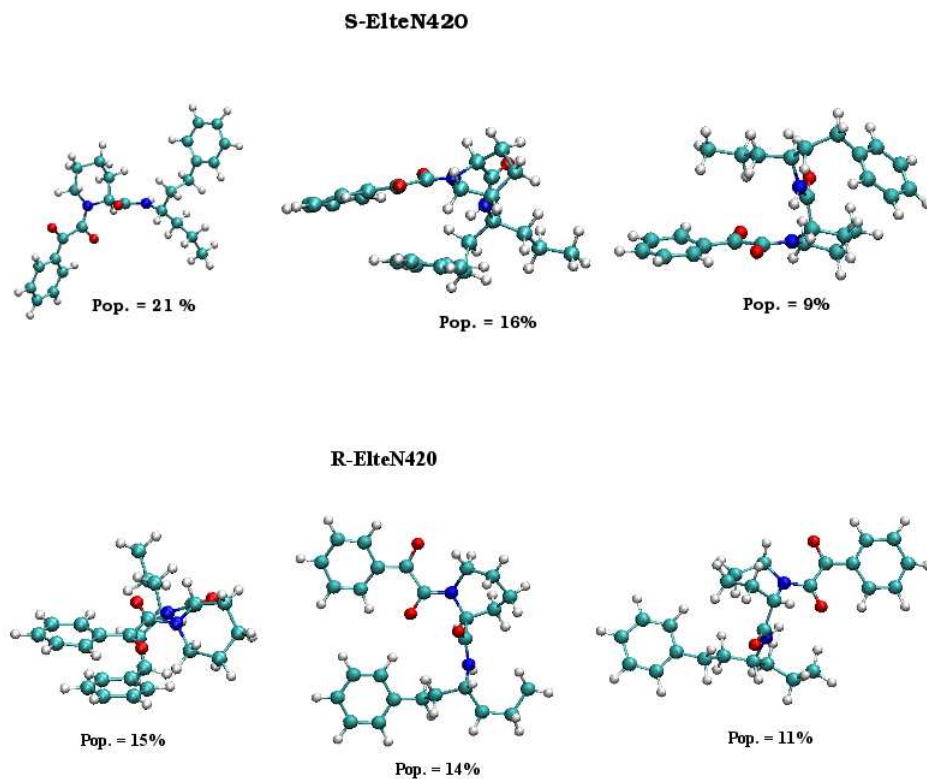


Figure 26: Cluster analysis computed on 24000 sampled configurations of the target (unscaled) replica at T=300 K and P=1 Atm in water solution for S-ElteN420 and R-ElteN420. Data were computed only on non hydrogen atoms with maximum intraligand threshold distance of 3.0 Å.

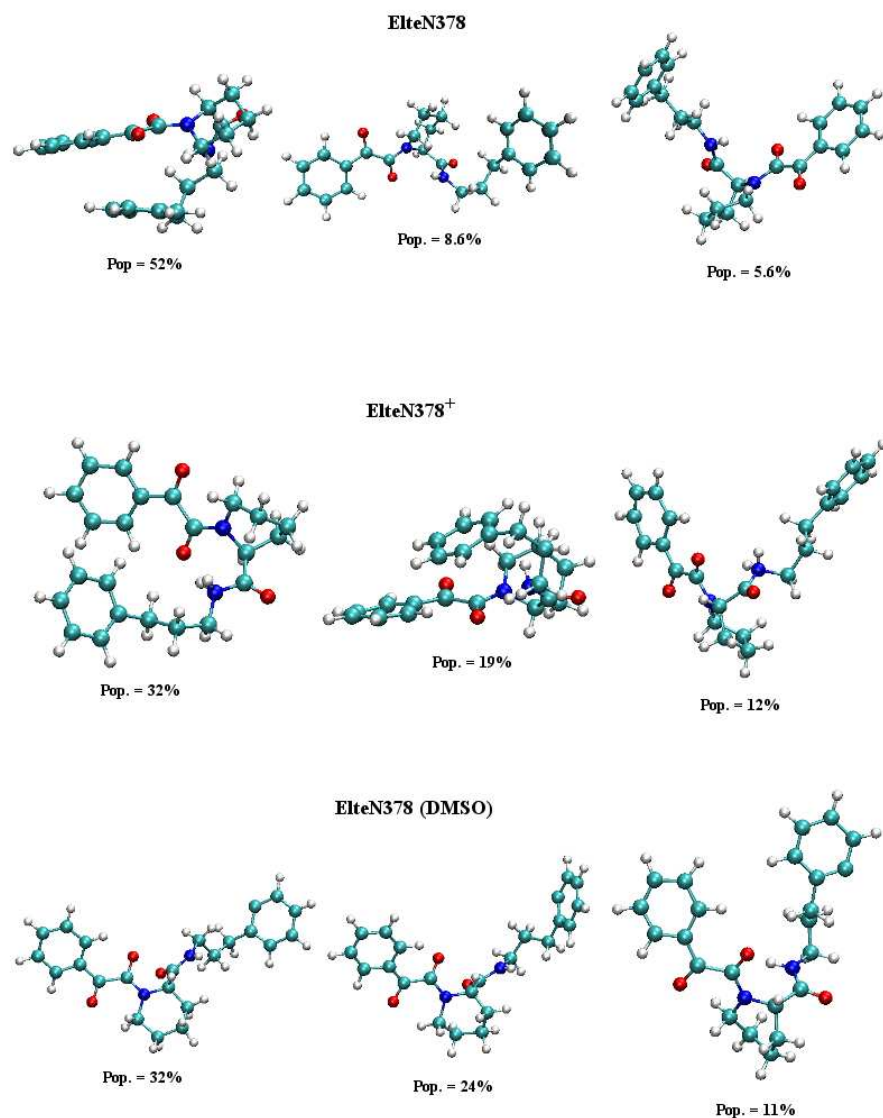


Figure 27: Cluster analysis computed on 20000 sampled configurations of the target (unscaled) replica at T=300 K and P=1 Atm for ElteN378 in water and DMSO and for ElteN378⁺ in water. Data were computed only on non hydrogen atoms with maximum intraligand threshold distance of 3.0 Å.

PPI mechanism and inhibition

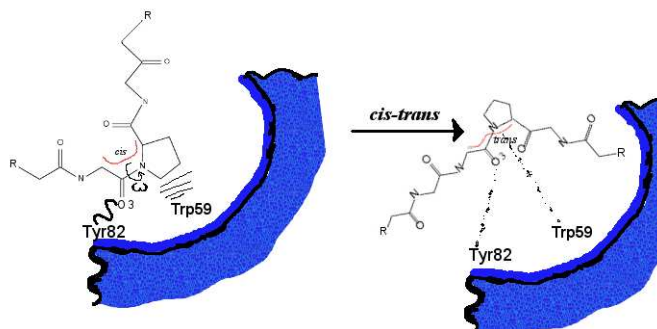


Figure 28: *cis*-proline $\rightarrow$ *trans*-proline isomerization mechanism in the PPI domain of FKBP proteins. The binding pocket of the PPI domain is drawn in blue. The bend substrate anchors the concave binding pocket at Tyr82 (via CO of the flanking Pro residue) and through proline-Trp59 stacking: the peptide bond involving *cis*proline is weakened and the ω -torsional barrier is lowered. When the proline *cis-trans* isomerization occurs, the substrate is rectified, no longer fitting in the concave binding pocket so that it must release the O3-HO(Tyr82) H-bond and the Trp59-pro stacking, leaving the FKBP active site

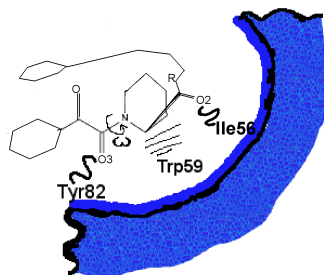


Figure 29: Inhibition mechanism in the PPI domain of FKBP proteins. The concave binding pocket of the PPI domain is drawn in blue. The ligand anchors to the binding pocket at Tyr82 (via CO of the flanking the piperidinic ring) and at Ile56 (via the following carbonyl in the peptidomimetic chain). The two anchoring points prevent the *trans-cis* isomerization locking the in the binding pocket.

Photo-physical data

Binding Affinity measurement

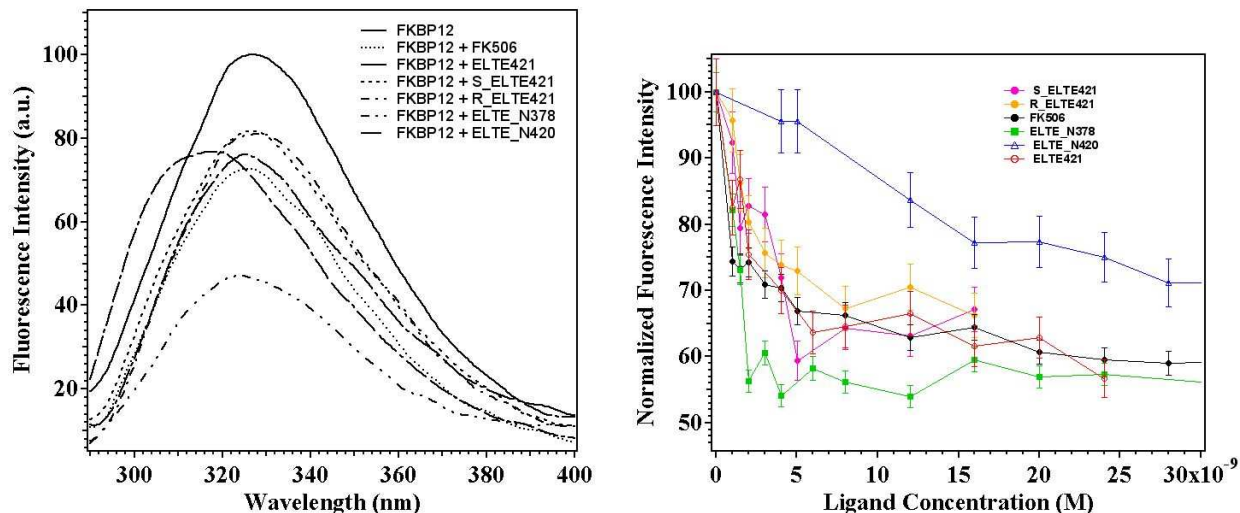


Figure 30: Left: Fluorescence emission spectra of FKBP12 in PBS buffer solution compared to spectra obtained when a 4nM solution of the ligand is added to the protein. The protein concentration was 1.05 μ M in all samples. The excitation wavelength was $\lambda = 265$ nm and the temperature was kept constant 288 K in all experiments. Background emission of protein free-samples was always subtracted from the experimental spectra. Right: Relative fluorescence intensity decrease of FKBP12 fluorescence at 325 nm (excitation wavelength $\lambda_{\text{exc}} = 265$ nm) as a function of ligand concentration for the series of ElteX compounds and for FK506.

Table 1: Comparison of dissociation constant ratios $R = K_{\text{Elte421}} / K_{\text{FK506}}$ obtained for ELte421 1:1 diastereoisomeric mixture using FKBP12 provided by Sigma and FKBP12 provided by CIRMMP. R^1 : fitting of the linear portion of the F_0/F plots. R^2 : fitting in the entire quenching range.

Protein	R^1	R^2
FKBP12 (Sigma)	2.3 ± 0.1	1.3 ± 0.1
FKBP12 (CIRMMP)	2.1 ± 0.1	1.2 ± 0.1

From the Tables 1 and 2, it is indeed worth noticing that, while the *relative* measurements of the affinity constants of ElteX ligands are reasonably independent on either the selected model or the experimental conditions (see, e.g., the comparison of Table 1 done on two set of independent measurements using FKBP12 from different sources), the absolute values of the binding affinities

Table 2: K_i for FKBP12 binding to ligands of the ElteX series and to FK506. Comparison among different fitting procedures.

Ligand	K_i/nM (static quenching ²³)	K_i/nM ²⁴	K_i/nM ²⁵
FK506	6.2 ± 2.3	1.2 ± 0.2	1.3 ± 0.3
Elte421	7.9 ± 1.2	1.5 ± 0.3	1.9 ± 0.4
S-Elte421	7.1 ± 0.7	2.4 ± 0.4	1.5 ± 0.3
R-Elte421	9.9 ± 1.7	4.7 ± 0.3	3.6 ± 0.3
ElteN420	38.8 ± 5.8	27.5 ± 0.4	21.1 ± 0.4
ElteN378	3.0 ± 0.5	1.1 ± 0.3	0.7 ± 0.2

are not. Indeed, the measurement of the absolute binding constant are extremely sensitive to both model representation (see Table 2) and, above all, to the experimental conditions. In the framework of fluorescence based K_i determination, factors like temperature, impurities in the expressed protein, protein concentration, DMSO percentage, excitation wavelength, buffer preparation and composition, may have a significant impact on the absolute value of the binding constant. All these factors are obviously normalized away by measuring the affinity of a FKBP ligand with respect to a reference system (in our case FK506) *in the same experimental conditions*. With this regard, we point out that the above factors, along with other variables that are not included in fluorescence experiments such as substrate concentration of initial reaction velocity, are particularly crucial in the K_i measurements via IC_{50} determination in enzymology assays. In fact, while IC_{50} values can be reliably calculated in dose-response curves, extrapolation of the binding affinity from IC_{50} values in presence of contaminants and/or when the inhibitor is of the tight-binding type (the so-called Cha equation regime²⁶) can be affected by a large error.²⁷ Most importantly, in the tight binding regime (nanomolar or picomolar activities), when the enzyme concentration is significantly larger than the K_i of the inhibitors, the determination of the K_i via IC_{50} and the Cha or Morrison equations becomes notoriously^{27,28} inaccurate.

Excitation and emission spectra of ElteN378 in various solvents

In Figure 31 we report the intensity ratio between the neutral and protonated peaks for ElteN378 in water and PBS (see discussion near Figure 7 of the main paper). In the case of ElteN378 in PBS,

we can see that for $C \leq 7 \times 10^{-7}$ the ratio is constant indicating that below such concentration these peaks are originated from *intraligand* long lived structures of the monomer. Intermolecular aggregation phenomena are negligible in the case of ElteN378 in pure water, where the protonated species is prevalent (See discussion near Figure 7 of the main paper).

Figure 31: Intensities ratio I_{310}/I_{380} obtained from the emission spectra of ElteN378 in PBS buffer (triangles) and in water (circles) as a function of ElteN378 concentration ($\lambda_{exc} = 250$ nm)

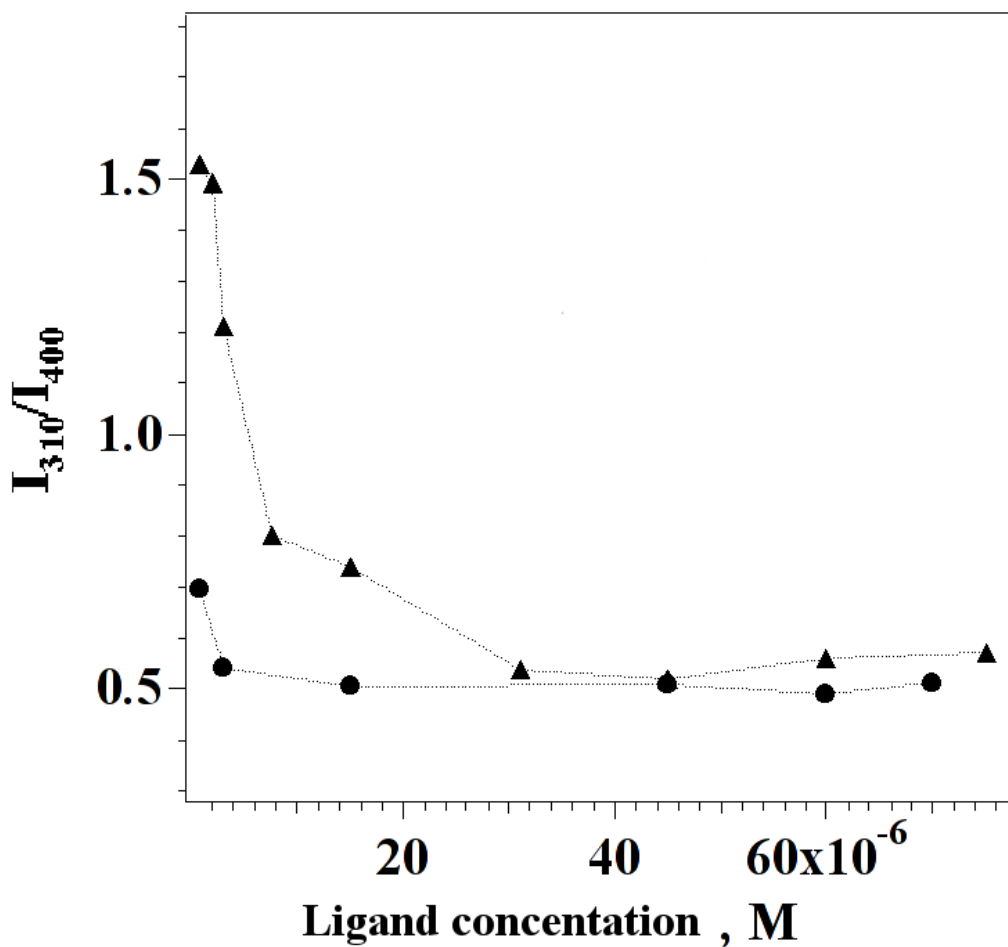


Figure 32: Excitation spectra of ElteN378 at $\lambda_{\text{em}} = 310$ nm in different solvents: PBS buffer (solid line), water (dashed line) and DMSO (dotted line). The y-scale on the left refers to the intensity in DMSO.

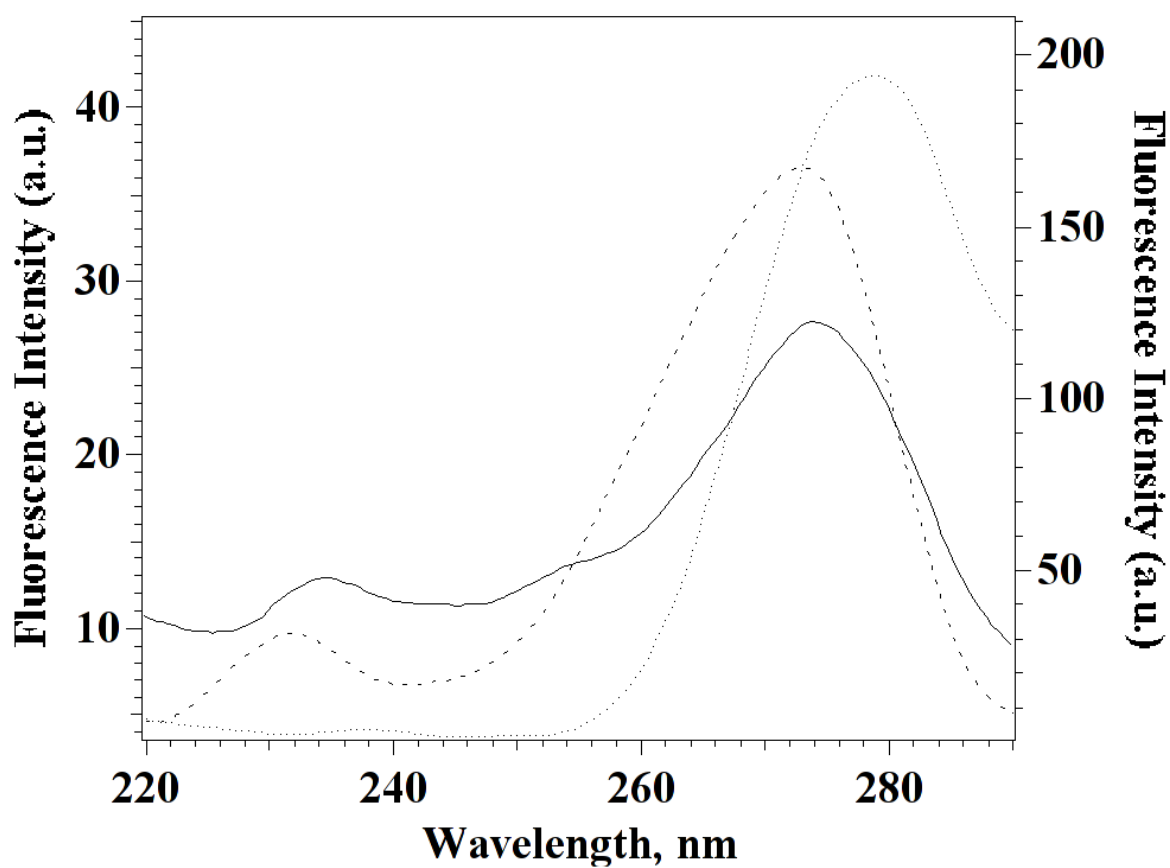
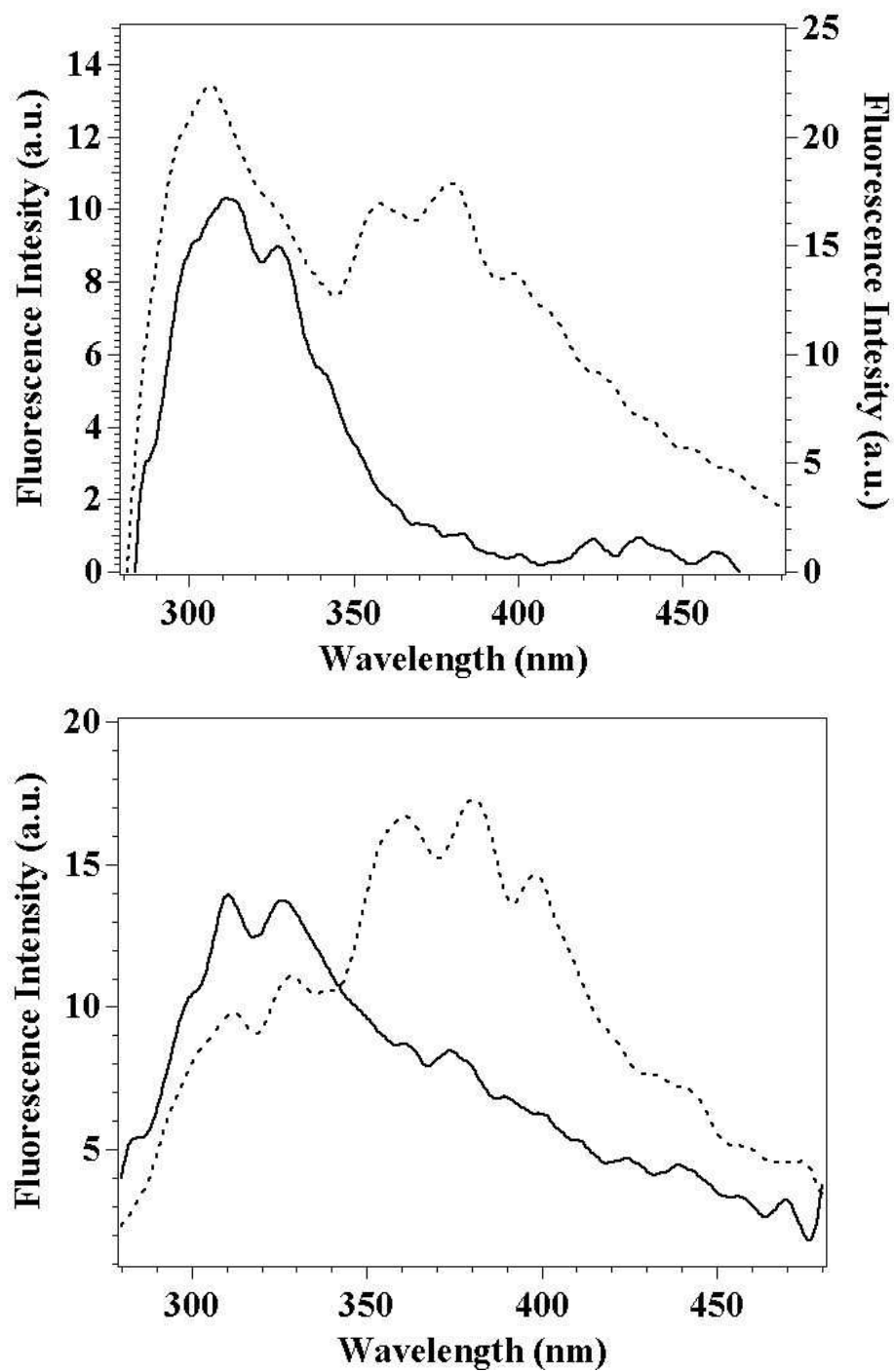


Figure 33: Excitation dependency of the emission spectra of ElteN378 at $\lambda_{em} = 260$ nm and $\lambda_{em} = 250$ nm in different solvents: PBS buffer (solid line), water (dashed line) and DMSO (dotted line).



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