Exploiting dense shell/packing principles to invoke stereoselectivity in a reaction accelerated by a chiral dendrimer

SUPPORTING INFORMATION

General Experimental Conditions and Equipment

All reagents were supplied from Sigma Aldrich and used without further purification unless stated otherwise. All bulk solvents were supplied by Fischer Scientific. NMR, all samples were prepared in dueterated chloroform supplied by Sigma Aldrich. Both ^{1}H NMR and ^{13}C NMR were performed on a Bruker AC-250 with 5mm CH probe. Chemical shifts are quoted in ppm relative to residual $CHCl_3$ and *J* values are quoted in Hz. UV/Vis was performed on a Hitachi U-2010 spectrophotometer. FT-IR measurements were performed on a Perkin Elmer RX I FT-IR spectophotometer with integrated DuraSample IR-II and values are given in cm^{-1}. Mass Spectroscopy: Electrospray ionization mass spectrometry (ESI-MS) was carried out using a Micromass Prospec spectrometer. Matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry was carried out using dithranol or dihydroxy benzoic acid as the matrices on a Bruker Reflex III mass spectrometer. Flash chromatography was performed using flash silica (Sigma - 35-70μm particles with 60Å pore size) and eluted under an applied pressure supplied from a standard bellows system.

Synthesis

Fmoc-L-phenylalanine N-hydroxysuccinimide. N-Fmoc-L-phenylalanine (10.81 g 27.9 mmol), N-hydroxysuccinimide (3.20 g, 27.8 mmol) and DMAP (0.3 g, 2.46 mmol) were added to 50 mL anhydrous dichloromethane under N_2 and the solution cooled to 0°C. N,N′-Dicyclohexylcarbodiimide (5.75 g, 27.9 mmol) in anhydrous dichloromethane was added over 1 hour. The reaction was allowed to warm to room temperature and left stirring for 24 hours. Solid impurities were removed via vacuum filtration. The product was triturated from dichloromethane into methanol as a white solid, which was collected via vacuum filtration and dried under high vacuum. Yield: 5.45 g, 40% ^{1}H NMR (250 MHz, DMSO), δ ppm: 8.26(d, 1H, J 8.5Hz) 7.75(d, 1H, J 8.5 Hz), 7.86 (d, 2H, J 7.5 Hz), 7.62 (m, 2H), 7.44 (m, 2H), 7.40 (m, 2H), 7.37 (m, 2H), 7.31 (m, 2H), 7.26 (m, 1H), 4.68 (m, 1H), 4.24 (m, 2H), 4.16, (m, 1H), 3.34 (m, 2H), 2.85 (s, 4H); ^{13}C NMR (DMSO), δ ppm): 170.2, 169.0, 156.7, 144.9, 142.0, 137.4, 130.5, 129.6, 128.3, 128.2, 127.6, 126.0, 120.5, 67.4, 59.9, 48.0, 38.2, 26.2; IR (solid, cm-1): 3324, 2928, 2849, 1814, 1788, 1736, 1702, 1622; [αD] = -30.0 (c = 1x 10^{-2} g/ml, T = 22°C, l =1 dm, DMF); MS (ES) m/z: 507 $[M+Na]^{+}$

N-Fmoc-L-phenylalanine DAB-Am-16 Dendrimer (1)

DAB-Am-16 dendrimer (96.7 mg, $5.73x10^{-5}$ mol) was dissolved in anhydrous dichloromethane (20 mL) under N_2 and cooled to 0°C. before adding the succinimide active ester of N-Fmoc-L-phenylalanine (0.5 g, 1.03 mmol) in dichloromethane (25 mL) was added over 20 minutes. The reaction mixture was then allowed to warm to room temperature and left stirring under N_2 for 48 hours. The resulting solution was washed with H_2O (40 mL), then washed with saturated sodium hydrogen carbonate (40 mL) and dried over $MgSO_4$. The pale yellow solution was then concentrated under reduced pressure and dried under high vacuum. Yield: 0.34 g, 78 %; IR (solid, cm^{-1}): 3296, 3062, 2941, 1648; ^{1}H NMR (250 MHz, $CDCl_3$, δ ppm): 7.80-6.80(m, br, 208H, Ar), 4.10-4.50 (m, br, 32H, COOC$\underline{H}_2$), 4.10-3.85(m, br, 32H, C$\underline{H}$ + COC$\underline{H}$NH), 3.40-2.05(m, br, 180H, C$\underline{H}_2$Ar + $CH_2C\underline{H}_2N$,+ $CH_2N\underline{H}CO$ + $CH_2N\underline{H}COO$), 2.00-1.00(m, br, 60H, C$\underline{H}_2CH_2N$); ^{13}C NMR (125.8 MHz, $CDCl_3$, δ ppm): 176.8, 156.0, 143.9, 141.2, 138.0, 129.6, 128.7, 128.2, 127.0, 125.1, 121.0, 119.8, 66.6, 57.1, 54.0, 52.3, 50.8, 47.1, 44.0, 38.4, 30.9, 25.0 $[\alpha]_D$ -34.0 (c = $1x10^{-2}$ g/mL, T=22°C, l=10 cm, DMF); $C_{23}2H_{352}N_{46}O_{16}$:$6H_2O$ requires; C, 67.15; H, 8.84; N, 15.53; O, 8.48: found; C, 66.72; H, 9.09; N, 15.37; O, 9.15; MS: (MALDI-TOF) m/z: 7598 $[M]^+$, 7619 $[M + Na]^+$.

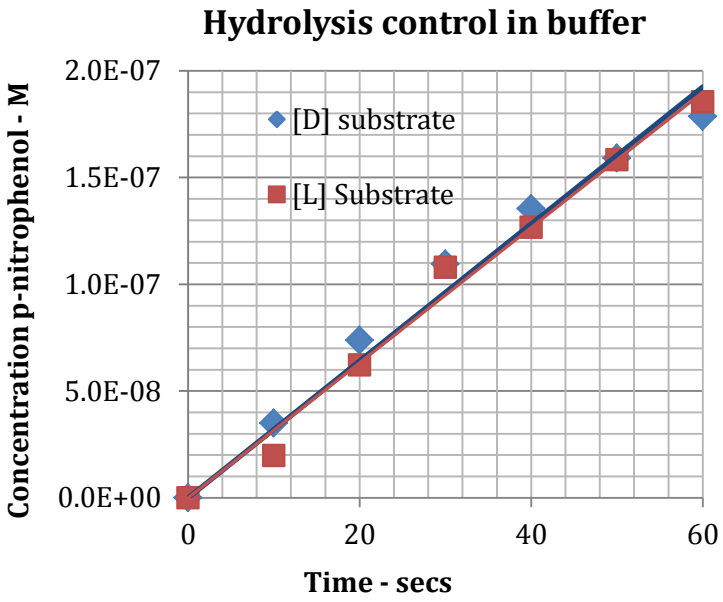
L-phenylalanine terminated DAB-Am-16 (2)

N-Fmoc-L-phenylalanine terminated DAb-Am-16 dendrimer **1** (100 mg, $1.45x10^{-5}$ mol) was dissolved in a solution of DMF and Piperidine (5:1, 2 mL) and stirred for 12 hours. Water (10 mL) and dichloromethane (5 mL) were added and the aqueous layer extracted 3 times with dichloromethane. The aqueous layer was freeze dried to yield a cream coloured solid. Yield: 20 mg, 34%; ^{1}H NMR (500 MHz, D_2O), δ ppm): 7.35-7.05 (m, 80H, Ar), 5.75-4.95(br, 32H, N$\underline{H}_2$), 3.68(t, 16H, J = 6.0 Hz, COC$\underline{H}$$NH_2$), 3.03(m, 32H, COCHC$\underline{H}_2$-Ar]), 2.95-2.05(m, br, 116H, $CH_2C\underline{H}_2N$), 1.66(m, 16H, $CH_2N\underline{H}CO$), 1.60-1.20(m, 60H, C$\underline{H}_2CH_2N$); ^{13}C NMR (125.8 MHz, D_2O, δ ppm): 176.2, 135.9, 129.2, 128.8, 127.2, 56.3, 50.9, 50.0, 44.4, 38.0, 37.4, 24.7, 24.1, 22.1, 21.3; IR (solid, cm^{-1}): 300 (br), 2944 (br), 2359, 1585; $[\alpha]_D$ = -20.0 (c = $0.25x10^{-2}$ g/mL, T = 22°C, l = 10 cm, H_2O). (MALDI-TOF) m/z: 4042 $[M]^+$, 4065 $[M + Na]^+$.

Hydrolysis Reaction (control)

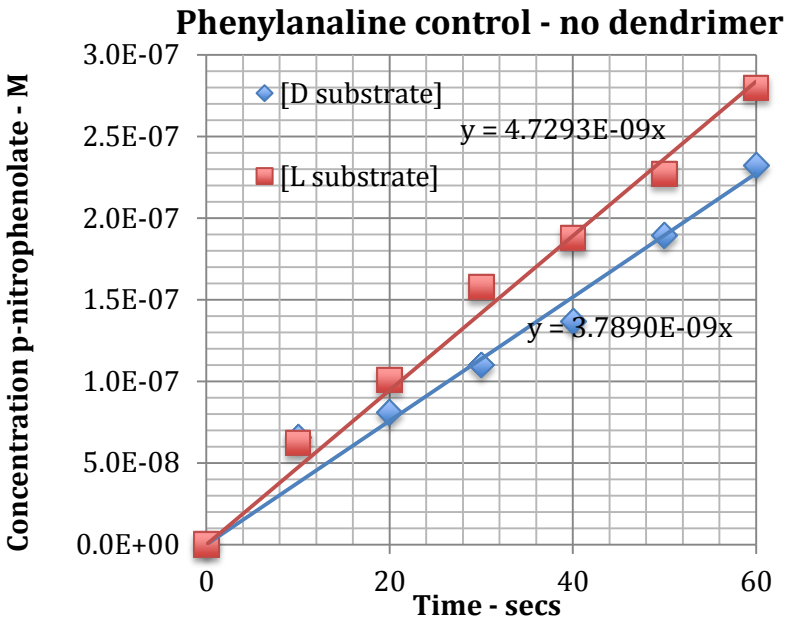
0,2 mL of a 0.45mM acetonitrile solution of the D or L substrate was added to a cuvette containing 1.5 mL of tris buffer (0.1M, pH 8.5). Absorptions at 410 nM were recorded every 10 seconds. Values were converted to concentration and plotted against time. The gradient was

recorded over the linear region during the first 60 seconds and used to determine the initial rates ($3.11x10^{-9}Ms^{-1}$ for the L and D substrates) – see below.



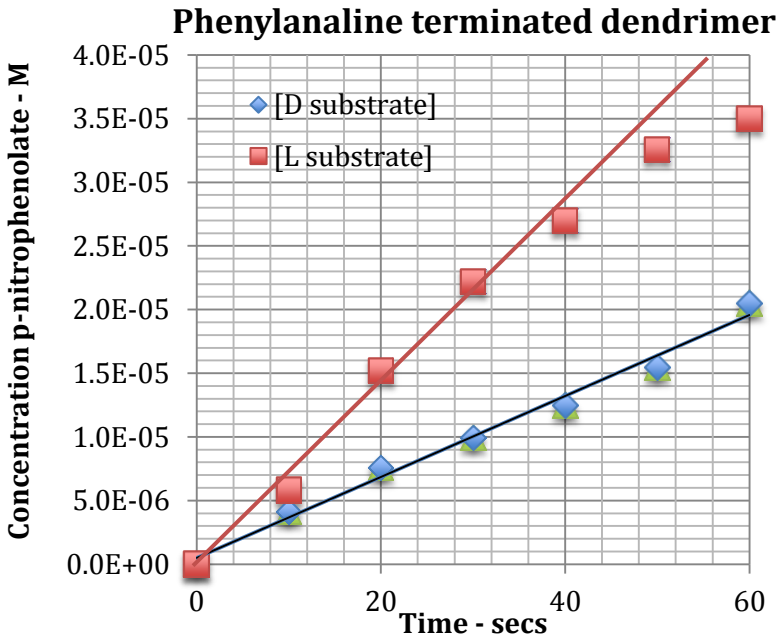
Aminolysis reaction (control)

0.2 mL of a 0.45mM acetonitrile solution of the D or L substrate was added to a cuvette containing 1.5 mL of a 6.4 mM solution of the control amine (L-phenylalanine methylester **3**) in tris buffer (0.1M, pH 8.5). Absorptions at 410 nM were recorded every 10 seconds. Values were converted to concentration and plotted against time. The gradient was recorded over the linear region during the first 60 seconds and used to determine the initial rates ($4.41x10^{-9}Ms^{-1}$ and $3.71x10^{-9}Ms^{-1}$ for the L and D substrate respectively) – see below.



Aminolysis reaction (dendrimer catalysis)

0.2 mL of a 0.45mM acetonitrile solution of the D or L substrate was added to a cuvette containing 1.5 mL of a 0.4 mM solution of dendrimer **2** (6.4 mM in amine) in tris buffer (0.1M, pH 8.5). Absorptions at 410 nM were recorded every 10 seconds. Values were converted to concentration and plotted against time. The gradient was recorded over the linear region during the first 40-60 seconds and used to determine the initial rates ($6.06 \times 10^{-7} Ms^{-1}$ and $3.77 \times 10^{-7} Ms^{-1}$ for the L and D substrate respectively) – see blow.



HPLC-MS Control Experiment (to confirm that the dendrimer's terminal amine groups were reacting with the substrates and that the dendrimer was ***not*** simply catalysing their hydrolysis). The analysis was performed using exactly the same general conditions used for the rate experiments. For the control experiment, 0,2 mL of a 0.45mM acetonitrile solution of the hydroylsis product (Boc-Phe-OH) was added to a sample vial containing 1.5 mL of tris buffer (0.1M, pH 8.5) and dendrimer (64mM). LC-MS analysis in negative ionization mode revealed a single peak at 1.82 minutes with a molecular weight of 264 (M^-). Conformation that hydrolysis was not taking place in the dendrimer accelerated reactions was demonstrated by an absence of this peak when the *p*-nitrophenyl active ester of Boc-protected-phenylalanine **4** and dendrimer **2** were studied. The LC-MS concentrations/conditions were exactly the same as those used in the rate experiments. That is, 0,2 mL of a 0.45mM acetonitrile solution of the L substrate was

added to a sample vial containing 1.5 mL of tris buffer (0.1M, pH 8.5) This solution was left for 5 minutes before a sample was injected into a Waters LCT Premier Mass Spectrometer. The mass spec/ionisation technique was electrospray (negative mode). The LC component was a Waters Acquity LC System with an Acquity UPLC BEH C18 1.7um, 1.0 x 50mm column. LC Conditions; Solvent A: water + 0.1% formic acid. Solvent B: acetonitrile + 0.1% formic acid.

Time(min)	Flow Rate(ml/min)	%A	%B
1. Initial	0.400	95.0	5.0
2. 2.50	0.400	5.0	95.0
3. 3.50	0.400	5.0	95.0
4. 3.60	0.400	95.0	5.0
5. 5.00	0.400	95.0	5.0.