Supplementary Information

Encoded dendrimers with defined chiral composition via 'click' reaction of enantiopure building blocks

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Experimental procedures

Materials

Commercial reagents were used as received. All the solvents were of technical grade and obtained from Biosolve. Anhydrous tetrahydrofuran was dried on an alumina column. All the chemicals were purchased from Sigma-Aldrich except 4'-ethynylacetophenone which was purchased from Acros. Nicotinamide adenine dinucleotide phosphate (NADPH) and alcohol dehydrogenase (ADH) from *Lactobacillus brevis* (4100 U/mL) (ADH-LB) and *Thermoanaerobacter sp*. (331 U/mL) (ADH-T) were purchased from Julich Chiral Solutions GmbH, a Codexis company, Germany, whilst Novozyme 435 (immobilized Candida Antarctica, Lipase B) was obtained from Novozymes. Azide functional dendrimer containing 24 chain end functional groups was synthesized as described previously.[1] Spectra/Por® 7 dialysis tubing, MWCO 1000 RC, diameter 24 mm was purchased from Serva Electrophoresis.

Methods

All reactions were performed under an argon or nitrogen atmosphere in dried glassware. Reactions were monitored by thin-layer chromatography (TLC) carried out on Sigma Aldrich silica gel 60 F254 plates. Visualization of TLC plates was carried out with 254 nm UV light.

NMR: ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury Vx spectrometer operating at 400 MHz at 25 ºC unless otherwise stated. Chemical shift data are reported in units of δ (ppm) using $CDCl_3$ (δ = 7.26 for ^{1}H NMR) or $(CD_3)_2SO$ (δ = 2.54 for ^{1}H NMR) as the internal standards. Multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad) for ^{1}H spectra. Coupling constants, *J*, are reported in Hz.

FT-IR: Infrared spectra were recorded on a Jasco FT-IR-460 Plus spectrometer equipped with a Specac MKII Golden Gate Single Reflection Diamond ATR System and is reported in wave numbers (cm^{-1}).

GC-MS: The GC-MS mass spectra were recorded on a Varian 450-GC gas chromatograph equipped with an autosampler and a Varian 220-MS mass selective detector on a factor four capillary column VF-5ms 30M×0.25MM with Injector and FID temperatures at 300°C, and a gradient oven temperature programme from 35°C (for 5 min) to 270°C at 10°C/min holding at 280°C for 15 minutes.

Chiral GC: The enantiomeric excess (ee %) were determined using Varian 430-GC on a CP Chiralsil-DEXCB column (25M×0.25MM) with injector at 200°C and FID 250°C, and a gradient temperature programme from 50°C (for 5 min) to 195°C at 15°/min holding at 195°C for 2 minutes.

Optical rotation: Optical rotation was determined by using a JASCO DIP-370 Digital Polarimeter (589 nm, Na D-line, 25 °C) with a cylindrical glass cell (f 3.5 ID X 50 mm) at a concentration of 10 mg mL^{-1} in THF.

SEC: Size exclusion chromatography (SEC) was performed on a Waters Alliance system equipped with a Waters 2695 separation module, a Waters 2414 refractive index detector (40 ºC), a Waters 2486 UV detector, a PSS SDV 5mm guard column followed by 2 PSS SDV 5 mm 500 Å columns in series (8 × 300) at 40°C. Tetrahydrofuran (THF, Biosolve), stabilized with BHT, was used as eluent at a flow rate of 1 ml min^{-1}. The molecular weights were calculated with respect to polystyrene standards (Polymer Laboratories, M_p = 580 g/mol up to M_p = 21000

g/mol). Before SEC analysis was performed, the samples were filtered through a 0.2 µm PTFE filter (13mm, PP housing, Alltech).

MALDI-ToF-MS: Mass spectra were recorded on a Voyager DE-STR from Applied Biosystems equipped with a 337 nm nitrogen laser and an acceleration voltage of 25 kV was used. The dendrimer was dissolved in THF at a concentration of 1 mg/mL. The matrix and ionization agent used were respectively trans-2-[3-(4-tertbutylphenyl)-2-methyl-2-propenylidene] malononitrile (DCTB) (40 mg/mL) and potassium trifluoroacetate (5 mg/mL) dissolved in THF. The matrix, ionization agent and the dendrimer mixture were mixed in a volume ratio of 4:1:4 and spotted (0.3 µL) on a stainless steel target plate. These spectra were recorded in the reflector mode, in positive mode. Spectra were recorded from 500 shots.

Freeze drying: The mixture was frozen in liquid nitrogen for several minutes and the frozen water was removed with a Christ Alpha 2–4 freeze dryer operated at 0.2 mbar and -57°C.

Enantioselective reductions of 4'-ethynylacetophenone using ADH

(R)-1-(4-ethynylphenyl)ethanol (2R): 1g of 4'-ethynylacetophenone was dissolved/suspended in a reaction mixture of *i*PA (40 mL) and PBS buffer solution (pH 7.4, 160 mL) containing 20mM NADPH and 0.5mM $MgCl_2$ and maintained at 37°C with uniform mixing. The enzyme ADH-LB (50 µL, 4100 U/mL) was then added to the reaction mixture and the mixture was allowed to stir for 2 days. The progress of the reaction was monitored by TLC and Chiral GC (Varian 430-GC) measurements and the mixture was treated with excess of water and extracted with methyl t-butyl ether. The organic layer was washed with brine, dried over magnesium sulfate and concentrated under reduced pressure. The product was purified by passing through a silica column (CH_2Cl_2) and was obtained as a white solid.

^{1}H-NMR ($CDCl_3$): δ (ppm) 7.46 (d, J = 7.74 Hz, 2H)), 7.30 (d, J = 7.78, 2H), 4.86 (q, J = 6.46, 6.46, 6.44 Hz, 1H), 3.06 (s, 3H), 1.45 (d, J = 6.46 Hz, 3H); ^{13}C-NMR ($CDCl_3$): δ (ppm) 25.0, 69.9, 77.0, 83.2, 121.1, 125.0, 132.2, 146.4; FTIR (neat) cm^{-1} : 3287, 2973, 1670, 1603, 1500, 1402, 1263, 1081, 1070, 1007, 897, 834, 734; GC-MS (m/z (%)): 144.9 (7.5 %), 130.9 (92 %), 103 (100%), 77 (56%), 50 (20 %), 43 (24%); Chiral GC : retention time, t = 12.95 min; $[\alpha_D]^{25}$ = + 50.91° (c 1.1, THF).

(1S)-1-(4-ethynylphenyl)ethanol (2S): The same procedure as described above was used with the exceptions that the reaction was carried out in the same buffer solution without $MgCl_2$ and that ADH-T (285 µL, 331 U/mL) was used instead of ADH-LB.

^{1}H NMR (400 MHz, $CDCl_3$) d (ppm) 7.47 (d, J = 8.39 Hz, 1H) , 7.32 (d, J = 7.99 Hz, 1H), 4.89 (q, J = 6.47 Hz, 1H), 3.06 (s, 1H), 1.47 (d, J = 6.47 Hz, 1H); ^{13}C-NMR ($CDCl_3$): δ (ppm) 25.1, 70.0, 83.6, 121.1, 125.5, 132.2, 146.6; FTIR (neat) cm^{-1} : 3287, 2973, 1672, 1603, 1502, 1402, 1265, 1085, 1070, 1008, 896, 796, 737; GC-MS (m/z (%)): 144.9 (7.5 %), 130.8 (89.9%), 103 (100%), 77 (51.1%), 51 (22.9 %); Chiral GC : retention time, t = 12.98 min; $[\alpha_D]^{25}$ = - 51.05° (c 1.0, THF).

Click reactions

Stock solutions of known concentrations of Dendrimer-N_3 (**3**) and 1-(4-ethynylphenyl) ethanol in THF and sodium ascorbate and $CuSO_4$ in deionized water were prepared (Table SI1). A series of reaction mixtures were prepared from these stock solutions with varying amounts of *R*- and *S*-enantiomers of the alkyne (Table SI2). The reactions mixtures were purged with Argon for 15 minutes prior to the addition of $CuSO_4$ solution and then stirred overnight in a vertex rotor at room temperature. Once the reaction was complete, the reaction vessels were set in ice bath and treated with solid NaCl to induce phase separation. The organic layer was separated and loaded to RC dialysis tubing (molecular weight cut off 1000 g/mol) and was dialysed first against DMSO and then against deionised water. During the duration of dialysis, the dialysate was changed for fresh dialysate solution. After dialysis, the samples were transferred to round bottom flasks, frozen in liquid nitrogen and isolated by freeze-drying.

Table SI1. Stock solutions

Compound	M (g/mol)	Weight (mg)	Volume of THF (ml)	Concentration (M)
3	5616.56	427	1260	0.060
2R	146.07	155	187	5.675
2S	146.07	155	187	5.675
Sodium ascorbate	198.11	63.44	500	0.640
$CuSO_4$	249.7	34.35	1000	0.138

Table SI2. Equivalences of each reagent (mol/azide) and amount of each stock solution (μl) used for Huisgen [2+2] cyclo addition reactions

Chiral Dendrimers :	**Equivalence**	**Ratio of R/S**				
		100/0	70/30	50/50	30/70	0/100
		A	**B**	**C**	**D**	**E**
3	1	150	150	150	150	150
2R	1.3	75	52.5	37.5	22.5	0
2S		0	22.5	37.5	52.5	75
Sodium ascorbate	0.3	100	100	100	100	100
$CuSO_4$	0.07	100	100	100	100	100

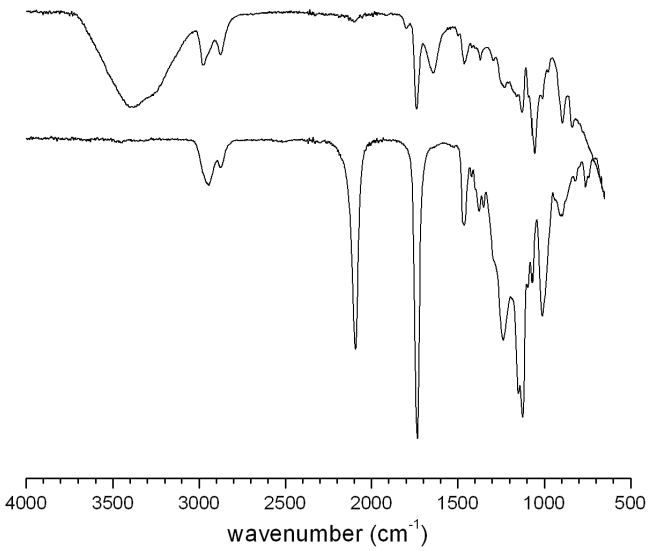

Figure SI1. FT-IR of azide functional dendrimer (**3**) (bottom spectrum) and the dendrimer after Huisgen [2+2] cyclo addition (top spectrum) showing the almost quantitative disappearance of the azide band at 2100 cm^{-1} after the click reaction.

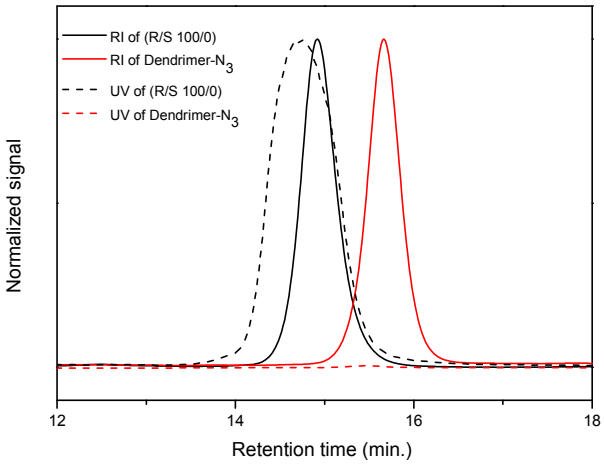

Figure SI2. Size exclusion UV and RI detector signals of azide-functional dendrimer **3** before and after Huisgen [2+2] cyclo addition of **2R**.

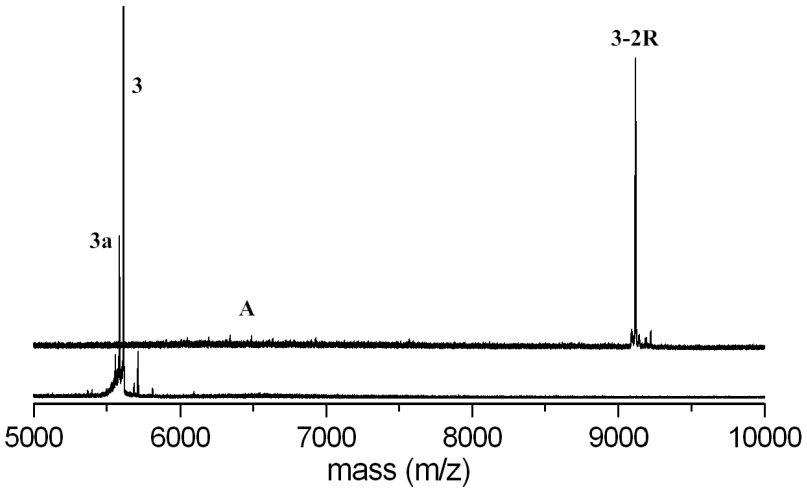

Figure SI3. MALDI-ToF spectrum of **3** showing a fragmentation in the process producing a main peak at m/z 5614 corresponding to **3** with the loss of one azide group (theoretical m/z 5655 (5616 + K)). Further removal of N_2 produces peak **3a**. After functionalisation with 100% **2R** peak 3-2R, a dominant peak at m/z 9122 was obtained, which is close to the theoretical value of 9126). Moreover, small peak of dendrimer with various degrees of functionalisation with 2R (A) can be identified. While the quantification of MALDI spectra is not possible, it is reasonable to assume that these are only present as minor traces. As the ability to desorb from the matrix in the MALDI process is higher for dendrimers with a lower number of OH groups and lower molecular weight the small peaks representing dendrimers with lower degree of functionalisation

are most likely over-emphasized. This is in agreement with SEC and IR results suggesting near quantitative functionalisation in the 'click' reaction.

CALB esterifications

The dendrimers (**A - E**) (11mg) were weighed into different vials. The vials were then charged with Novozyme-435 (5mg) and 3Å molecular sieves and, vacuum dried at 60°C to remove traces of moisture. Then the vials were filled with nitrogen and dry THF (1.3 ml) was transferred via a syringe, followed by vinyl acetate (70 ml). The reaction mixture was stirred at 60°C for 2 days. The samples were filtered and dried before being analyzed by NMR to determine the extent of grafting onto the hydroxyl groups at the periphery of the dendrimer. MALDI-TOF MS was used to determine the molecular weight distribution of these dendrimers (Figure SI3). The same procedure was applied to the enzymatic modification of mixtures of **2S** and **2R**.

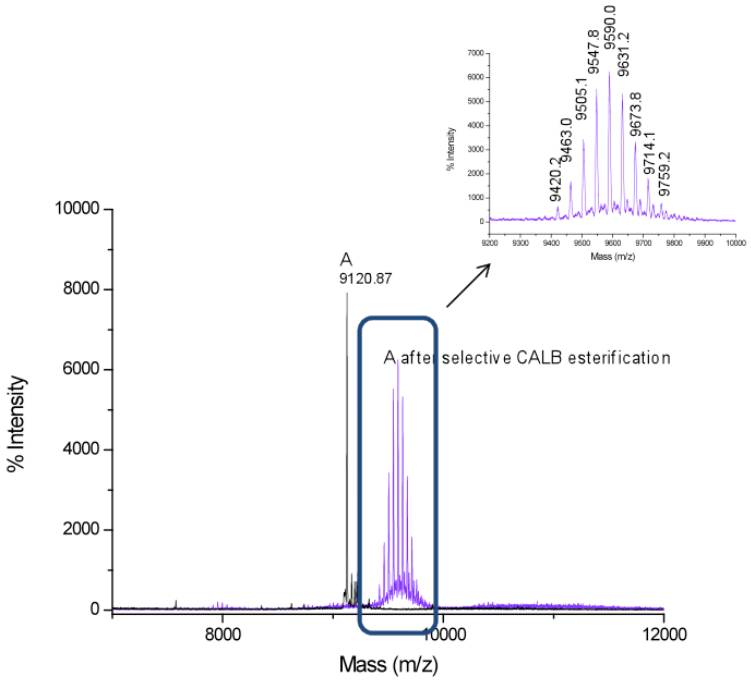

Figure SI4. MALDI-ToF MS of azide-functional dendrimer **A** before (black) and after selective CALB esterification (purple).

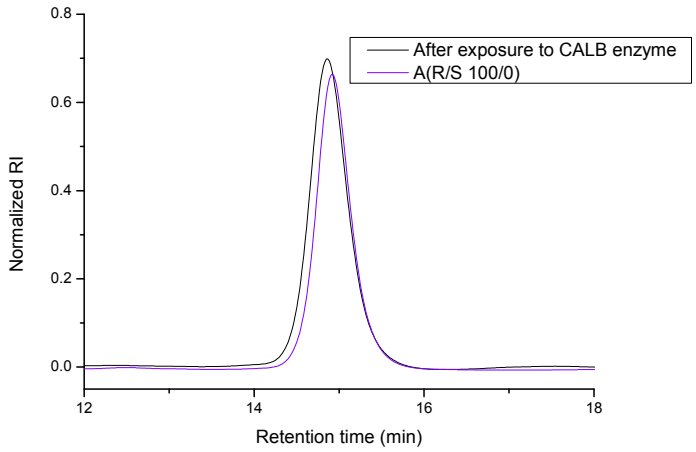

Figure SI5. SEC traces of A (chiral dendrimer which was decorated with 100 % (*R*)-building blocks) before (purple) and after (black) CALB selective esterification.

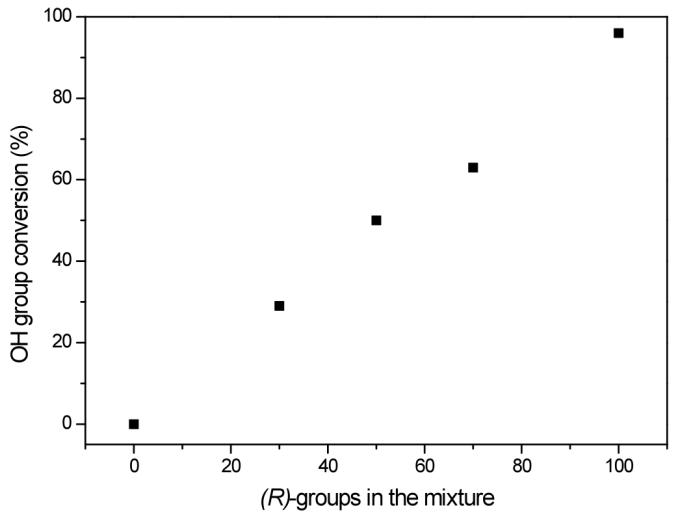

Figure SI6. Control experiment showing the CALB SEC traces of A (chiral dendrimer which was decorated with 100 % (*R*)-building blocks) before (purple) and after (black) CALB selective esterification.

[1] Vestberg, R.; Malkoch, M.; Kade, M.; Wu, P.; Fokin, V. V.; Sharpless, K. B.; Drockenmuller, E.; Hawker, C. J. *J Polymer Chem Part A: Polym Chem* , **2007**, 45, 2835-2846