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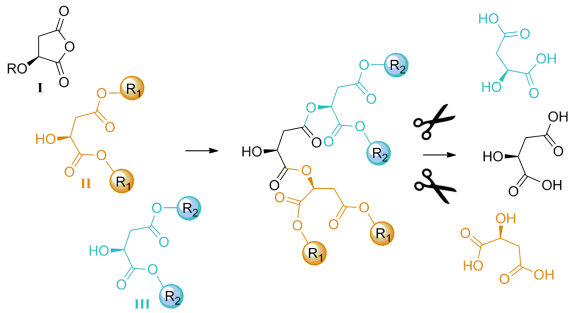
Graphical abstract

Dendron synthesis with malic acid derivatives in a stepwise manner opens for preparation of polyfunctional dendrons, degradable by hydrolysis



Convergent Synthesis of Degradable Dendrons based on L-Malic acid

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Abstract

New degradable polyester dendrons based on the cellular tricarboxylic acid cycle component L-malic acid were synthesized up to the third generation by convergent synthesis. The dendron wedges could be introduced in a stepwise, highly regioselective fashion. HMBC-NMR revealed that the C1-carbonyl on malic acid was exclusively esterified, before the reaction of the second dendron wedge at C4 took place. Degradation studies on a first generation dendron analyzed by HPLC showed that hydrolytic degradation of the dendron most profoundly take place at pH4 and pH 9 with highest degradation rate at alkaline pH. NMR show that the dendron degrades to malic acid - and fumaric acid derivatives. Preliminary studies performed in cell culture shows low toxicity of the dendrons in concentrations up to 50µg/mL.

Introduction

Dendrons and dendrimers are hyperbranched polymers characterized by densely functionalized surfaces and high monodispersity. Therefore, dendrons and dendrimers show promise as potential tools in the design of medically active compounds.[1] Dendrons and dendrimers have been

extensively investigated in biotechnology as e.g. new agents for transfection of DNA[2] or RNA[3,4] into cells,[5] as contrast agents,[6,7] and therapeutics.[8] Dendrimer designs such as poly(propylene)imine (PPI) dendrimers, polyamidoamine (PAMAM), phosphorous, silicon and polyether based dendrimers have been investigated in great detail. These structures show unique properties but are not easily degraded in nature. As a solution to increase degradablity of dendrimers a variety of polyester based dendrimer designs (e.g. Boltorn dendrimers by Perstorp)[9] have been developed and shown to be easily degradable under hydrolytic conditions. [10, 11] Also polyester dendrimers based on metabolizable building blocks such as citric acid, [12, 13] glycerol and succinic acid or adipic acid have been reported [14, 15, 16, 17]. Most of these reports describe the synthesis of dendrimers by divergent strategy using AB_2 building blocks. However, recently synthesis of polyester dendrons has been carried out using AB_3 bulding blocks.[18, 19] In general polyester dendrimers and dendrons have shown to have good properties with respect to biocompatibility.[20, 21]

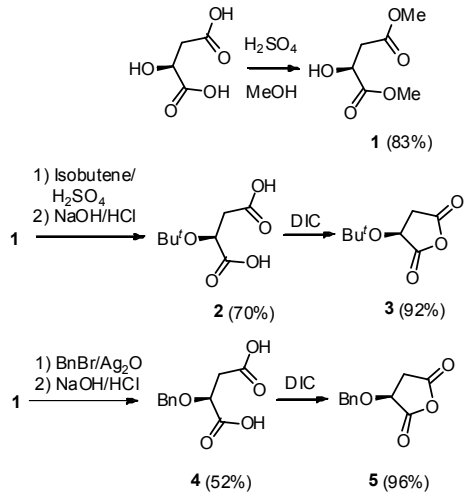
The present work describes the synthesis of new polyester dendrons based on L-malic acid, a tricarboxylate cycle metabolite taking part in the cellular respiration process. It is envisaged that such dendrons in aqueous environment degrade into monomers which can be further broken down to carbon dioxide and water in eukaryotic cells or bacteria.[22]

Results and discussion

For the synthesis of dendrons methyl esters were chosen as surface groups, as they can be used as protective groups for the carboxylic acids during the synthesis and at the same time be selectively converted to the corresponding carboxylic acids in the presence of other alkyl esters. In the synthetic sequence the methyl ester groups were introduced by Fischer esterification which gave the (*S*)-dimethyl malate (**1**) in 83% yield. The hydroxyl group was protected either as the *tert*-butyl ether or benzyl ether (Scheme 1) in the reaction sequence. Benzyl protection of the malic acid hydroxy group can be removed orthogonal to both methyl esters and *tert*-butyl esters.

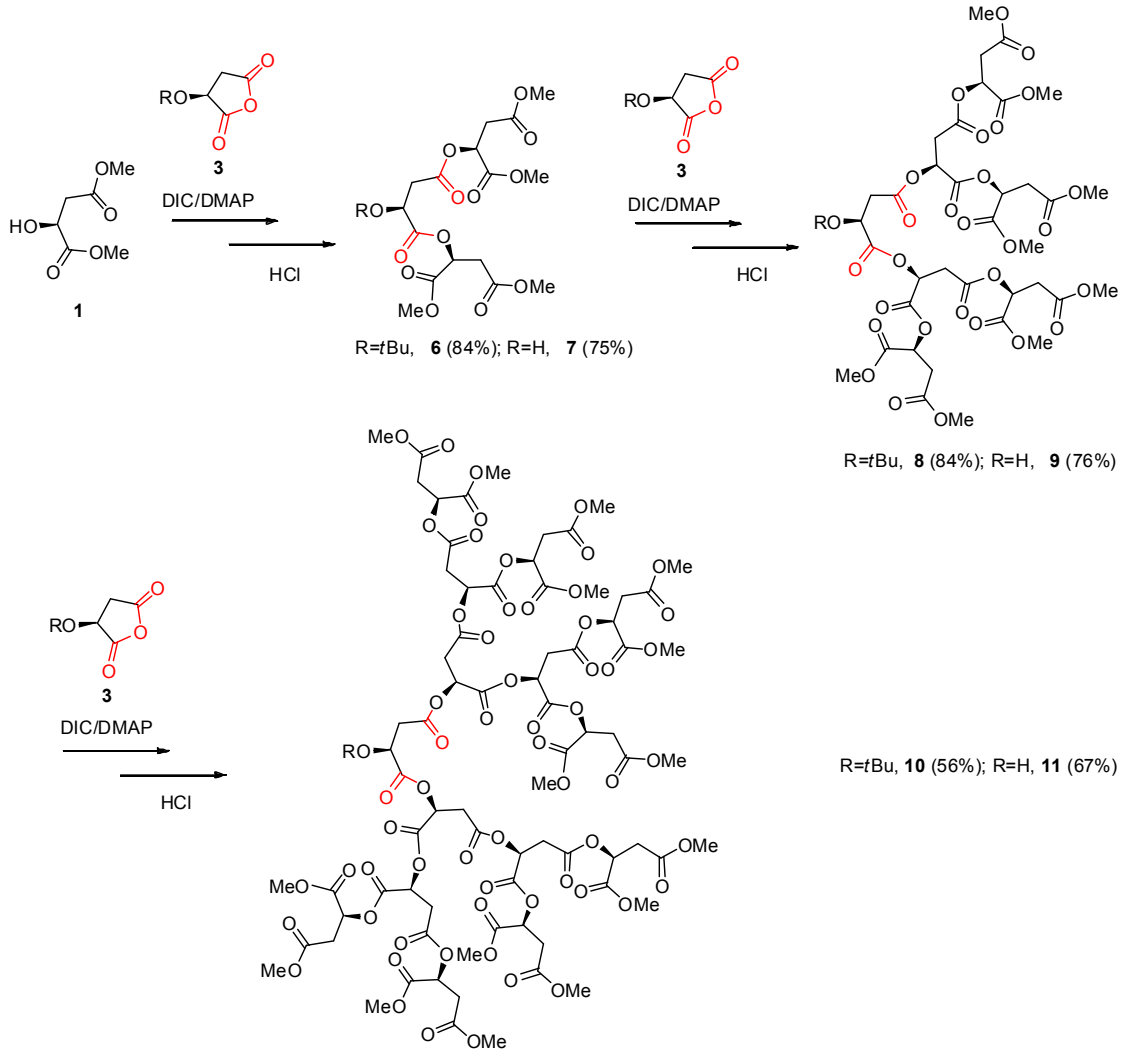
(*S*)-2-(*tert*-Butoxy)-succinic anhydride (**3**) was synthesized in good yields from (*S*)-dimethyl malate (**1**) in a two-step one-pot procedure using isobutene/H_2SO_4 for the introduction of the *tert*-butyl group and subsequent hydrolysis of the methyl esters to yield (*S*)-2-(*tert*-butoxy)-succinic acid (**2**) in 70% yield (Scheme 1). However, compound (**2**) was found not to be stable degrading within a few weeks, presumably due to the cleavage of acid labile *tert*-butoxy group by the adjacent carboxylic acids. Therefore, (**2**) was converted to the corresponding cyclic anhydride (**3**) in 92% yield using *N,N'*-diisopropyl carbodiimide (DIC) as the condensation agent (Scheme 1).

The (*S*)-2-(benzyloxy)succinic anhydride (**5**) was synthesized in a two-step one-pot procedure in which initial treatment of compound **1** with benzyl bromide and silver oxide for 3 days[23] was followed by alkaline ester hydrolysis affording compound **4** in 52% yield. Cyclization using DIC gave compound **5** in over 90% yield (Scheme 1). In the synthesis of benzyl protected dendrons, the cyclic anhydride was in some cases formed *in situ* from (*S*)-2-(benzyloxy)succinic acid (**4**) giving similar yields. In contrast to the (*S*)-2-(*tert*-butoxy)-succinic acid (**2**), the (*S*)-2-(benzyloxy)succinic acid (**4**) showed good shelf stability, however, the solubility of (**4**) in dichloromethane under the DIC mediated esterification was low leading to longer reaction times. Therefore, due to higher stability and solubility, the anhydride was more convenient in use.



Scheme 1. Synthesis of monomer building blocks for convergent dendron synthesis

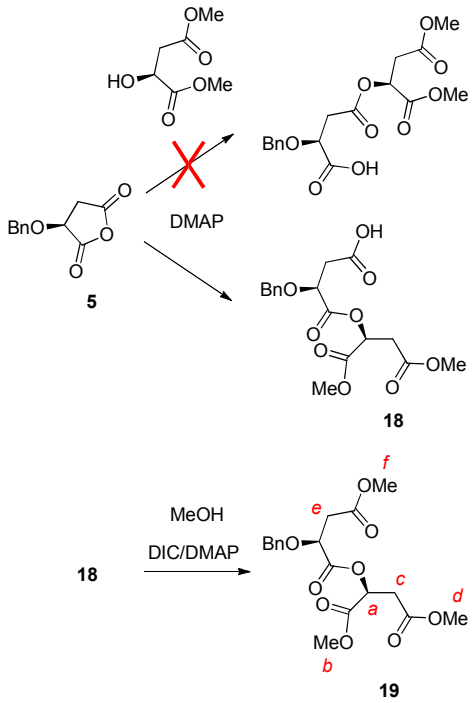
The *tert*-butyl ether and benzyl ether protected cyclic anhydrides (Scheme 1, compound **3** and **5**) were used as stable electrophiles for the subsequent stepwise preparation of the dendrons. The convergent assembly of the dendrons via ester bond formation was carried out in a stepwise coupling sequence which involved initial DMAP catalyzed ring opening of the cyclic anhydride **3** or **5** by the first equivalent of monomer. In the formation of higher generation dendrons, a dendron (with generation G-1) is used for derivatisation of the cyclic anhydride. Subsequently DIC/DMAP mediated esterification with a second equivalent of monomer (or dendron) was carried out in 60-80% yields on the vacant carboxylic group (Scheme 2). DCM was used as solvent to suppress O-N acylurea rearrangement, being favored in polar solvents. NMR investigation on the formation of the G1 dendron indicates that no racemisation/epimerization occurs upon reaction between the formed cyclic anhydride and compound 1. This should otherwise be evident from the diastereotopic methylene signals in the corresponding racemic mixture.[24] The *tert*-Butyl ether hydroxyl protective group could easily be removed under acidic conditions. Hydrogen chloride in dioxane was found to be superior to trifluoroacetic acid in DCM, and gave the deprotected dendrons typically in 70-80% yields (**7**, **9** **11**, Scheme 2). The reaction times for derivatisation of the cyclic anhydrides with the first dendron wedge increased from 30 min (G1 and G2 dendrons) to 2h (G3 dendrons) before derivatisation of the second carboxylic acid was carried out.



Scheme 2. Condensed reaction scheme for the convergent synthesis of G1-G3 methyl ester terminated malic acid dendrons. Each reaction sequence comprises two reactions 1) esterification and 2) deprotection of the *tert*-Butoxy group by HCl in dioxane. The ester bonds formed in each step are in red color.

Also in the esterification of the second carboxylic acid, the reaction time for increased from ca. 2 h (G1 dendron) to 16 h for the formation of a G3 dendron. According to HPLC-MS a single intermediate product was formed during the stepwise introduction of the dendron wedges onto the cyclic anhydrides. In the formation of G1-dendrons the HPLC peak of the intermediate correlated to the molecular mass of the mono-substituted product. This indicated a high regioselectivity in the ring opening of the cyclic anhydride, as an unselective substitution on the asymmetric cyclic anhydride would otherwise lead to two isomers, which could be distinguishable on HPLC. HPLC-

MS analysis on the synthesis of G2 and G3 dendrons also showed that only a single intermediate product was formed, however, no molecular mass of this intermediate could be detected. To determine which of the carbonyls at the cyclic anhydride was first derivatized we synthesized two small dendrons, in which (*S*)-2-(benzyloxy)succinic anhydride (**5**) was derivatized with only one equivalent (*S*)-dimethyl malate (**1**) to yield the mono-substituted compound leaving the second carboxylic group underivatized. An aliquot of this mono-substituted compound was further esterified on the vacant carboxylic group by methanol and DIC to yield a hetero-bi-substituted dendron. Hereafter, we examined the two compounds by HC-correlated 2D-NMR (HMBC). In contrast to the corresponding *tert*-butoxy-derivatives, the distinct chemical shifts (δ_C, δ_H) of the benzylic methylene can be used as a tool to observe long range coupling to carbons/protons in the dendron structure and thereby give increased ability to map the specific protons/carbons in the dendron structure. By comparing long range HC-correlated couplings for these two compounds the spectrum of the hetero-bi-substituted compound showed a long range coupling between H_f and C_e [^4J H_f-C_e (3.61 ppm, 37.75ppm)], a coupling between C_e (37.75 ppm) and H_g [^2J H_g-C_e (4.43 ppm, 37.75 ppm)] and a long range coupling between C_g (74.16 ppm) and H_h [^4J H_h-C_g (4.72 ppm, 4.46 ppm, 74.16 ppm)], in the mono-substituted compound, the H_f-C_e coupling was absent (Figure 1). This indicates that ring opening of compound **5** proceeded in a highly regioselective manner by exclusive initial reaction at the C1-carbonyl group adjacent to the stereocenter to form (*S*)-3-(benzyloxy)-4-(((*S*)-1,4-dimethoxy-1,4-dioxobutan-2-yl)oxy)-4-oxobutanoic acid **18** (Scheme 3). The subsequent esterification on product **18** takes place at C4 to yield (*S*)-1-((*S*)-1,4-dimethoxy-1,4-dioxobutan-2-yl) 4-methyl 2-(benzyloxy)succinate (**19**) as the hetero-bi-functionalised product (Scheme 3).[25]



Scheme 3. Synthesis of compounds **18** and **19** to determine the selectivity in derivatisation of carbonyl C1 and carbonyl C4. The carbons and hydrogens which couplings are assigned in figure 1 are denoted with red letters

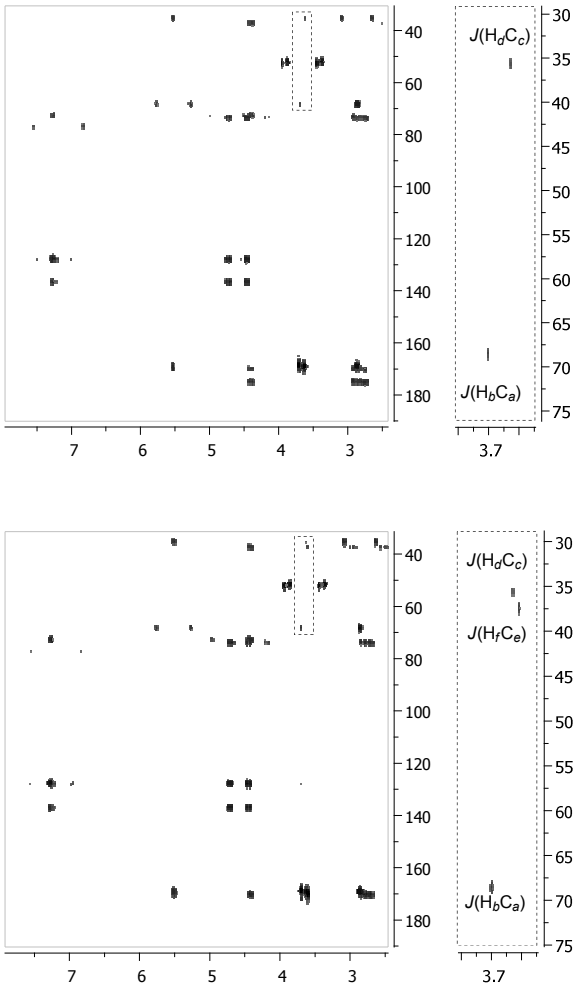
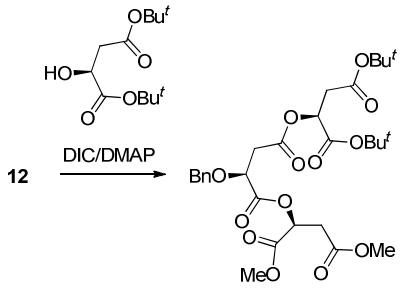


Figure 1. HMBC-NMR spectrum of compound **18** (top) and **19** (bottom). The enlarged area shows the long range coupling between the C4 ester O-methyl group and C2 methylene group.

This study was also carried out on a higher generation hetero-bisubstituted dendron derivative ('semi-G2'), however, interpretation of these HMBC spectra became difficult due to overlapping signals in the diagnostic spectral regions described above.

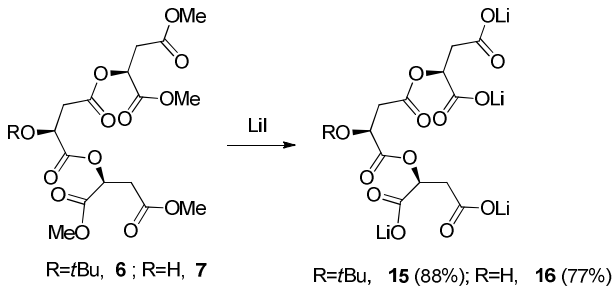
The basis of the regioselectivity towards the carbonyl closest to the stereocenter/oxo group is not evident from sterical reasons, as this position should be more sterical hindred compared to the C4 position. Thus, it may be speculated that the adjacent 2-oxo group results in increased electrophilicty of the adjacent carbonyl due to an inductive effect, or that this group participates during nucleophilic attack from the hydroxyl group on the dendritic wedge e.g. by forming

hydrogen bonds to the incoming nucleophile. Although we only analyzed the derivatization of (*S*)-2-(Benzyloxy)succinic anhydride (**5**) by NMR, a similar selectivity was observed on HPLC in the stepwise derivatization of (*S*)-2-(*tert*-Butoxy)-succinic anhydride (**3**) during the dendron synthesis. This regioselectivity may be used for introduction of diverse dendrons without the need of protective groups. To demonstrate this feature, a hetero-functionalized G1 dendron (**14**) was synthesized, having two methyl ester and two *tert*-butyl ester terminal groups (Scheme 4).



Scheme 4. 'Proof of principle' synthesis of the hetero-functional G1 dendron **14**

The possibility of cleaving the terminal methyl esters selectively without affecting the polyester dendron skeleton was investigated by exposing a G1-dendron either *tert*-butyl protected (compound **6**) or with a free hydroxyl group (compound **7**) to lithium iodide in THF or MeCN (Scheme 5). It was found that the terminal methyl ester groups could, indeed, be selectively transformed to the corresponding lithium carboxylates in good yields. However, a minor extent of elimination resulting in a fumarate byproduct was sometimes observed during methyl ester cleavage on compound (**7**).



Scheme 5. Selective demethylation of terminal methyl esters on compound **6** or **7** by lithium iodide

Hydrolytic degradation of dendrons: To investigate the degradability of the present dendron design, hydrolysis of the G1-dendron (**7**) was investigated by incubation at pH 4, pH 7 and pH 9, respectively, at 37°C over 7 days. It was shown that degradation took place within 1 day at pH 9, and that the dendron was fully degraded after 7 days (Figure 2, D). At acidic pH the degradation was somewhat slower and after 7 days approximately 40% of the intact dendron was still present in the mixture (Figure 2, B).[26] At neutral pH the dendron showed good stability during the period, where only small amounts of degradation products were formed. Upon hydrolysis new products with retention times (t_R) below 10 min emerged, corresponding well with the formation of polar charged malate- and fumarate salts residing from the cleavage of the dendron ester bonds. These observations are in good agreement with the expectation on the stability of ester bonds towards hydrolysis.

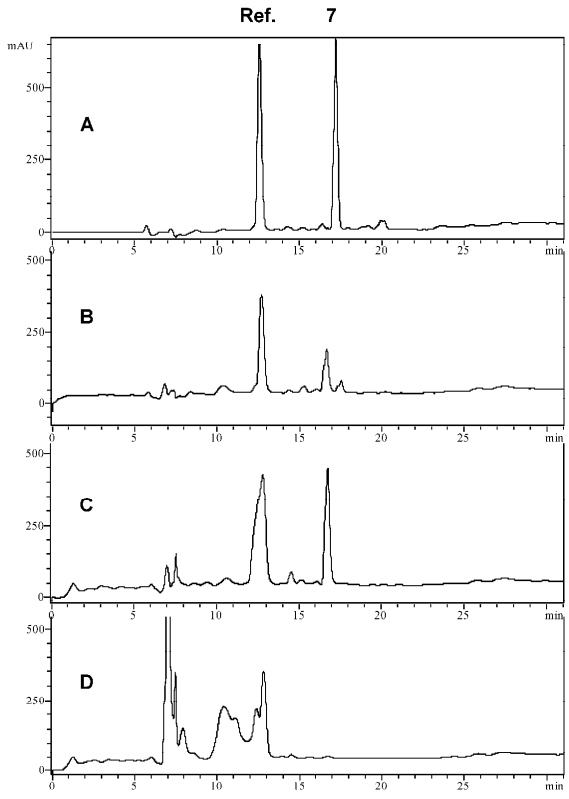


Figure 2. Reversed phase HPLC of A: Stock solution of G1-methyl ester dendron (**7**) mixed with a reference (benzamide); B: Dilute stock solution after 7 days at pH 4; C: Dilute stock solution after 7 days at pH 7, and D: Dilute stock solution after 7 days at pH 9. The broader reference peak observed in 2C and 2D may be due to the formation of decomposition products with similar retention time[27]

The structures of the products formed during the hydrolysis of the dendrons were analyzed by NMR. A G1-methyl ester dendron was chosen as model, as the simple structure of this system would facilitate the interpretation of the spectra upon degradation of the dendron. This study focused on dendron degradation at pH 9 as HPLC had shown the fastest and most complete breakdown of the dendron at this pH, which should also make the interpretation of the spectra more straight forward. NMR (^{1}H, H,H-COSY and HSQC) showed the disappearance of the 'dd' signal at ca. 5.5 ppm upon three days exposure of the G1-methyl ester dendron to aqueous conditions at pH 9 (Figure 3). This signal resides from the chiral Cα proton next to the ester linkage. The appearance of multiplet signals at ca. 4.2-4.3 ppm indicates that the ester linkages of the dendron are

hydrolyzed to afford malic acid derivatives (multiplet, δ ca. 4.3 ppm). The spectrum of (S)-dimethyl malate (**1**) and L-malic acid shows a multiplet signal at ca. 4.3 ppm which correlates to the Cα next to the hydroxyl group. In addition, a singlet at ca. 6.5 ppm was present indicating the formation of fumarate.[28]

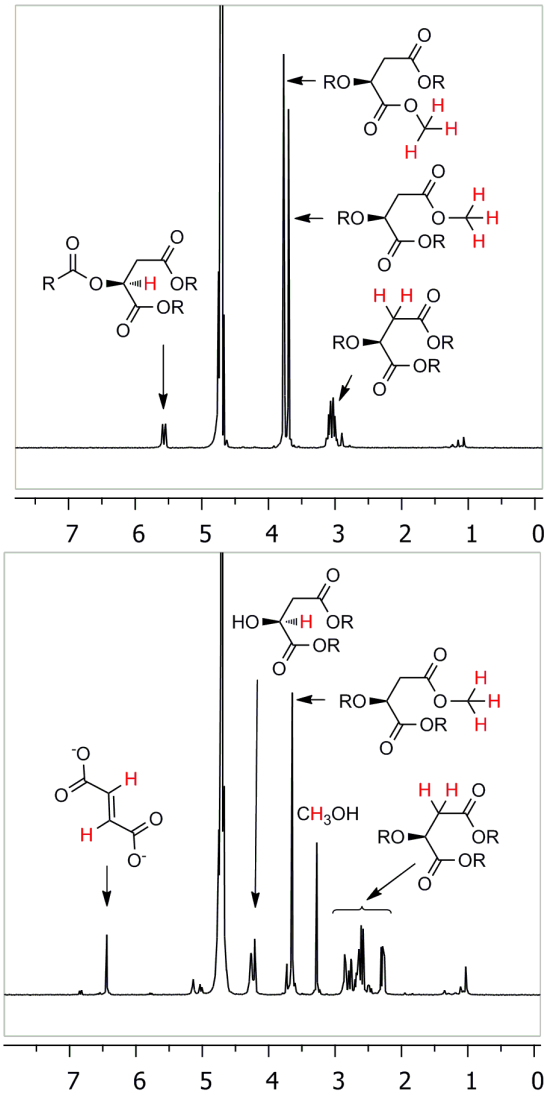


Figure 3. ^{1}H-NMR shows degradation in 1 M $NaHCO_3$ (aq) at 37 ^{o}C of a first generation dendron with methyl ester terminal groups (compound 7) at t = 0 (top) and t = 3 d (bottom)

Furthermore, NMR showed disappearance of the singlet at ca. 3.70 ppm indicating complete hydrolysis of the C1 methyl ester. In contrast, the singlet at ca. 3.69 ppm was still present indicating a higher stability of the C4 methyl ester towards hydrolysis (Figure 3). This correlates well with the earlier findings in this paper regarding the regioselectivity for nucleophilic attack on the C1 carbonyl group. Degradation studies were also carried out on a G2-dendron and analogous to the G1 dendron, where a breakdown to malic- and fumaric acid derivatives was observed, albeit, the mixture of degradation products was more complex in this higher generation system (data not shown).

The acute cytotoxicity of the polyester dendrons was tested in peripheral blood mononuclear cells (PBMCs). These experiments have shown that the dendrons possess low acute toxicity, albeit, with a reduced viability of the cells at high concentrations when exposing the cells to methyl ester surfaced G1 and G2 dendrons. The G1-carboxylate dendron did not show significant toxicity even at the highest concentration. The somewhat higher toxicity of the methyl ester dendrons compared to the anionic carboxylate surfaced dendron is in agreement with the earlier observations showing that anionic dendrons have lower cytoxicity compared to dendrons carrying more hydrophobic surface groups. As the G-2 methylester dendron was not water soluble, ethanol was added as co-solvent and may therefore have an added toxic effect on the cells (Figure 4). However, more elaborate studies on various cell lines have to be performed to thoroughly characterize the cytotoxicity of the dendrons.[29]

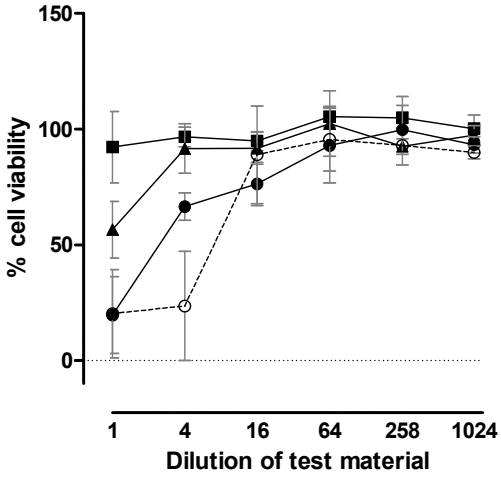


Figure 4. Viability assay (MTT) of dendrons in PBMC culture analysed in 4-fold dilutions in PBS (final concentrations: 200 µg/ml – 0.2 µg/ml; ethanol reference concentrations 19.2 % - 0.02%). Figure shows mean +/- SD of 48 hour-analysis performed with cells from two animals (pig), ▲(G1-methyl ester), ■ (G1-carboxylate dendron),● (G2-methyl ester), ○ (ethanol).

Conclusion

Malic acid based dendrons up to third generation were synthesized by a convergent strategy. The intermediate cyclic anhydride can be functionalized in a regioselective manner, with initial introduction of functional groups/dendritic wedges on C1. This selectivity can be utilized to introduce poly-functionality without the need for protective groups and was demonstrated in the synthesis of a G1-dendron with mixed methyl-and *tert*-butyl ester terminal groups. The dendrons show high degradability at pH 9, which is in good agreement with the high lability of ester groups at alkaline pH. Significant break down (approximately 60%) of the dendrons was also observed at pH 4, whereas the dendrons appeared to have the highest stability at neutral pH. Preliminary studies on the dendrons show that they generally have low acute toxicity in cell culture. Further synthetic development and applications of the dendrons will be investigated in the future. The present dendron design may be useful for biomedical applications were the ability of a drug to be broken down into harmless metabolites after use is highly warranted.

Experimental section

General methods

Chemicals were purchased from Sigma-Aldrich, Fisher Scientific and IRIS Biotech. Solvents were used as received or dried over molecular sieves (4 Å) when necessary. Silica gel 60 Å, 130-170 mesh was used for column chromatography. HPLC-MS analysis was performed on a Shimadzu LCMS 2010, using a Phenomenex Jupiter C5 column (5 μm, 300 Å) and a 1 mL/min linear gradient from 3 to 95% buffer B over 18 min (buffer A, 0.025% TFA in 10% aq MeCN buffer B, 0.025% TFA in 90% aq MeCN) with a quadrupole mass spectrometer in ESI^+ mode. MS (MALDI-TOF) was carried out on a Bruker Autoflex using trihydroxyacetophenone (THAP) as matrix. HRMS (ESI^+) was performed on a Dionex UltiMate 3000 system with a Bruker MicroTOF-QII-System with ESI-source nebulizer 1.2 bar, 8.0 dry gas L/min, 200°C, HRMS samples were calibrated by an automated pre-run internal mass scale calibration of the individual samples by injecting a sodium formate calibration solution prior to the measurement. NMR (^1H, ^{13}C, APT, H,H-COSY, HSQC, HMBC) were performed on a 300 MHz Bruker Avance 300 equipped with a BBO probe and autosampler and a 500 MHz Bruker Ultrashield 500 plus. IR was performed on a Shimadzu IRAffinity-1 instrument using attenuated reflection technique (ATR) performed either on the crystalline compounds or on evaporated droplets from DCM solutions. Elemental analysis (C, H) was performed on a FlashEA 1112.

Synthetic procedures

(*S*)-Dimethyl malate[30] (1): L-Malic acid (33 g, 0.25 mol) was dissolved in methanol (150 mL). Conc. H_2SO_4 (3 mL, 0.06 mol) was added and the mixture was stirred 16 h at r.t. The methanol was evaporated in vacuo (40°C) yielding a clear oil. $NaHCO_3$ (17 g, 0.20 mol) and water (75 mL) was added and the mixture was stirred for ca. 15 min (CO_2 evolution) until the pH of the solution was

neutral. Ethyl acetate (3 x 200 mL) was used to extract the product and the combined organic phases was backwashed with 1 M $NaHCO_3$ (2 x 10 mL) and brine (10 mL), dried (Na_2SO_4) and the solvent was removed at reduced pressure to yield (33 g, 83%) of the desired product as a viscous colorless oil. HPLC-MS: One peak t_R 10.5 min, MS (ESI^+) m/z 163 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 4.32 (t, J = 5.3 Hz, 1H), 3.74 (broad, 1H), 3.55 (s, 3H), 3.47 (d, 3H), 2.63 (dd, J = 16.3 Hz, 4.6 Hz, 1H), 2.54 (dd, J = 16.3 Hz, 6.5 Hz, 1H). ^{13}C NMR (75 MHz, CDCl3) δ 173.54, 170.88, 67.07, 52.29, 51.64, 38.29. ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.54, 170.88, 67.07, 52.29, 51.64, 38.29. IR (ATR) ν (cm^{-1}): 3481 (broad, OH stretch), 1724 (C=O, ester), 1375 (CH_3). Analysis calcd for $C_6H_{10}O_5$ (162.16) C 44.45, H 6.22, found C 44.20 H 6.25.

(*S*)-2-(*tert*-Butoxy)succinic acid[31] **(2)**: Liquid 2-methylpropene (75 mL, 0.80 mol) was added to a solution of compound **1** (40 g, 0.25 mol) in dry DCM (150 mL) in a 250 mL round-bottom flask. Conc. H_2SO_4 (3 mL, 0.06 mol) was added drop-wise and the reaction vessel was firmly sealed with a plastic stopper to safely contain a slight overpressure. The mixture was slowly stirred for 2 d, HPLC showed no peak relating to starting material and a new peak appeared. The mixture was transferred to a separation funnel and left to allow some of the isobutene to evaporate. The solution was washed with water (200 mL) and the solvent and residual isobutene were evaporated at reduced pressure to give (*S*)-dimethyl-2-(*tert*-butoxy) succinate as a colorless oil (ca. 50 g). HPLC-MS: One peak t_R 19 min, MS (ESI^+) m/z 236 $[M+NH_4]^+$, 277 $[M+MeCN+NH_4]^+$, 282 $[M+MeCN+Na]^+$. ^1H NMR (300 MHz, CDCl3, δ in ppm) 4.40 (dd, J = 7.4 Hz, 5.7 Hz, 1H), 3.67 (s, 3H), 3.62 (s, 3H), 2.61 (d, J = 5.7 Hz, 1H), 2.61 (d, J = 7.4 Hz, 1H), 1.13 (s, 9H). The oil was directly dissolved in methanol (75 mL), cooled on ice and treated with a solution of NaOH (33 g, 0.83 mol) in water (75 mL) by vigorous stirring of the mixture over night gradually heating up to r.t. The methanol was removed at reduced pressure and the aqueous residue was washed with diethyl ether (200 mL) and then kept under 15°C on ice whilst slowly adjusted to pH 2 dropwise with conc. hydrochloric acid

(ca. 70 mL, 0.84 mol). The product was extracted from the aqueous solution with ethyl acetate (3 x 300 mL). The combined organic layer was backwashed with water (25 mL), dried (Na_2SO_4) and reduced in vacuo to afford 32.7 g (70%) of the product as a white solid which should be stored in the freezer to prolong lifetime. HPLC-MS: One peak t_R 13 min, MS (ESI^+) m/z 208 $[M+NH_4]^+$, 213 $[M+Na]^+$, 249 $[M+MeCN+NH_4]^+$, 254 $[M+MeCN+Na]^+$; MS (TOF ESI^+) m/z 213 $[M+Na]^+$. ^1H NMR (300 MHz, D_2O, δ in ppm) 4.41 (t, J = 6.0 Hz, 1H), 2.79 (dd, J = 15.5 Hz, 6.0 Hz, 1H), 2.71 (dd, J = 15.5 Hz, 6.0 Hz, 1H), 1.26 (s, 9H). ^{13}C NMR (75 MHz, D_2O, δ in ppm) 176.92, 174.03, 76.98, 67.97, 39.11, 26.64. IR (ATR) ν (cm^{-1}): 3500-2300 (broad, OH stretch), 2977(C-H, Bu^t), 2939 (C-H, Bu^t), 2876 (C-H, Bu^t), 1710 (C=O, carboxylic acid), 1369 (CH_3)

(*S*)-2-(*tert*-Butoxy)succinic anhydride[31] **(3):** Compound **2** (10 g, 53 mmol) was dissolved in dry diethyl ether (50 mL) under nitrogen atmosphere. The solution was stirred on ice bath and DIC (8.2 mL, 53 mmol) was added slowly through the septum over 10 min and the reaction mixture was left stirred for 1 h then sealed and placed in a refrigerator overnight. The reaction was monitored with IR-spectroscopy (C=O stretch shifted from 1710 cm^{-1} in the carboxylic acid to 1784 cm^{-1} in the cyclic anhydride). The supernatant was filtered under nitrogen atmosphere and the solvent was removed under reduced pressure to yield white solid which was recrystallized from cyclohexane (30 mL) to yield 8.3 g (92%) of the product as a white crystalline. MS (TOF ESI^+) m/z (dissolved in MeOH) 195 $[M+Na]^+$, 227 $[M+MeOH+Na]^+$. ^1H NMR (300 MHz, $CDCl_3$, δ in ppm) 4.65 (dd, J = 8.9 Hz, 6.7 Hz, 1H), 3.14 (dd, J = 18.4 Hz, 8.9 Hz, 1H), 2.83 (dd, J = 18.4 Hz, 6.7 Hz, 1H), 1.22 (s, 9H). ^{13}C NMR (75 MHz, CDCl3, δ in ppm) 168.48, 164.93, 74.58, 65.12, 35.42, 25.36. IR (ATR) ν (cm^{-1}): 2981 (Bu^t), 2962 (Bu^t), 2936 (Bu^t), 1863 (C=O, anhydride), 1784 (C=O, anhydride), 1368 (CH_3), 1230 (cyclic anhydride).

(*S*)-2-(Benzyloxy)succinic acid[32] **(4):** Silver oxide (25 g, 108 mmol) was suspended in dry DCM (150 mL) while stirring with a heavy stirring bar. Compound **1** (25 g, 156 mmol) and benzyl bromide (25 mL, 208 mmol) were added and the suspension was stirred for 3 d at r.t. During this time the flask was occasionally shaken to loosen the precipitate. The mixture was filtered and transferred to a separation funnel and the residual precipitate washed with additional DCM (2 x 100 mL). The organic layer was washed with water (3 x 100 mL). The solvent was removed in vacuo to give clear oil (ca. 40 g). To the residue was added NaOH (40 g, 1 mol) dissolved in water (150 mL) and the mixture was stirred overnight at r.t. The mixture was washed with diethyl ether (3 x 100 mL), cooled on an ice bath and adjusted to pH 2 with conc. HCl (ca. 84 mL, 1 mol). Residual methanol was removed by mild evaporation in vacuo (40°C, 5 min). The residual mixture was freeze dried and the solid residue was suspended in ethyl acetate (300 mL) under vigorous stirring overnight. NaCl was filtered off and the organic solution was washed with 1 M HCl (3 x 100 mL), dried (Na_2SO_4) and evaporated in vacuo and gave the product as a semi solid. Suspension in water followed by freeze drying gave the product as a white solid (18 g, 52%). HPLC: One peak t_R 15.5 min. MS (TOF ESI$^+$) m/z 247 $[M+Na]^+$. ^{1}H NMR (300 MHz, D_2O, δ in ppm) 7.38 – 7.30 (m, 5H), 4.67 (d, J = 11.5 Hz, 1H), 4.50 (d, J = 11.5 Hz, 1H), 4.35 (dd, J = 7.6 Hz, 4.5 Hz, 1H), 2.83 (dd, J = 16.3 Hz, 4.5 Hz, 1H), 2.72 (dd, J = 16.3 Hz, 7.7 Hz, 1H); ^{13}C NMR (75 MHz, D_2O, δ in ppm) 175.18, 174.02, 136.50, 128.74, 128.69, 128.53, 74.14, 72.82, 37.38. IR (ATR) ν (cm^{-1}): 3600-2400 (broad, OH stretch), 3031 (C-H, aromate), 1698 (C=O, carboxylic acid). Analysis calcd for $C_{11}H_{12}O_5$ (224.21) C 58.93, H 5.39, found C 58.73 H 5.37.

(*S*)-2-(Benzyloxy)succinic anhydride (5): Compound **4** (5 g, 22 mmol) was dissolved in dry diethyl ether (50 mL) and kept under a stream of nitrogen atmosphere during all subsequent steps except when otherwise noted. The solution was stirred and cooled on ice. DIC (3.5 mL, 23 mmol) was added over 15 min then left stirring on ice for 1 h. The reaction vessel was sealed and placed at

in a refrigerator at 5°C overnight. The diisopropylurea precipitate was filtered off, and the precipitate was washed with dry diethyl ether (10 mL). The combined filtrate was stirred vigorously at r.t. and petrolether was slowly added until the solution became permanently turbid. An oily substance containing residual diisopropylurea and colored byproducts was left to settle on the walls of the reaction vessel and the clear supernatant was decanted off, transferred to another flask and stirred vigorously for 10 min on a dry ice/acetone bath. The resulting precipitate was left to settle and the supernatant was removed by decantation. The solid white residue was dissolved in dry boiling diethyl ether (20 mL) then stirred on ice for 20 min while petrol ether (20 mL) was slowly added during the first 10 min causing precipitation. The supernatant was removed by filtration and the white crystalline product dried in vacuo (4.36 g, 96%). MS (TOF ESI^+) m/z (dissolved in MeOH) 229 $[M+Na]^+$, 261 $[M+MeOH+Na]^+$. ^1H NMR (300 MHz, $CDCl_3$, δ in ppm) 7.36 – 7.24 (m, 5H), 4.91 (d, *J* = 11.7 Hz, 1H), 4.69 (d, *J* = 11.7 Hz, 1H), 4.48 (dd, *J* = 8.6 Hz, 5.7 Hz, 1H), 3.10 (dd, *J* = 18.7 Hz, 8.6 Hz, 1H), 2.88 (dd, *J* = 18.7 Hz, 5.7 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm) 169.76, 167.26, 135.72, 128.79, 128.69, 128.40, 73.24, 72.23, 36.14. IR (ATR) ν (cm^{-1}): 3034 (C-H, aromate), 1871 (C=O, anhydride), 1778 (C=O, anhydride), 1227 (cyclic anhydride). Analysis calcd for $C_{11}H_{12}O_5$ (206.06) C 64.07, H 4.89, found C 63.70 H 4.76.

O-*tert*-Butyl-G1-methyl ester dendron (6): Compound **1** (10 g, 61 mmol) was dissolved in dry DCM (50 mL) under nitrogen atmosphere and **3** (5 g, 29 mmol) was added. The mixture was cooled on an ice bath and DMAP (0.36 g, 2.9 mmol) was added. After 30 min DIC (4.7 mL, 31 mmol) was added slowly to the cold solution in intervals during a period of 30 min then stirred for another 60 min. The solvent was removed at reduced pressure and the remaining white-yellow paste residue was stirred for 10 min with diethyl ether (50 mL). The mixture was filtrated and the filter washed with diethyl ether (10 mL). The combined organic fraction was washed with water (5 x 50 mL), 1 M $NaHCO_3$ (50 mL), brine (10 mL) and dried (Na_2SO_4). In some cases discoloration was removed

by passing the solution through a silica plug (0.5 cm) without suction. The solvent was removed at reduced pressure. Yield: Colorless oil (11.6 g, 84%). HPLC-MS: One peak t_R 21.8 min, MS (ESI^+) m/z 496 $[M+NH_4]^+$, (weak) 501 $[M+Na]^+$, 423 $[M+H\text{-isobutene}]^+$, 441 $[M+NH\text{-isobutene}]^+$. ^1H NMR (300 MHz, $CDCl_3$, δ in ppm) 5.48 (dd, J = 7.5 Hz, 5.0 Hz, 1H), 5.44 (t, J = 6.1 Hz, 1H), 4.49 (dd, J = 9.2 Hz, 3.6 Hz, 1H), 3.70 (s, 3H), 3.69 (s, 3H), 3.65 (s, 6H), 2.90 (dd, J = 16.0 Hz, 5.0 Hz, 1H), 2.83 (dd, J = 16.0 Hz, 7.5 Hz, 14H) 2.83 (d, J = 6.1 Hz, 2H), 2.83 (dd, J = 16.0 Hz, 3.6 Hz, 1H), 2.68 (dd, J = 16.0 Hz, 9.2 Hz, 1H), 1.12 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.14, 169.38, 169.34, 169.19, 169.10, 168.82, 75.85, 68.59, 68.52, 67.77, 52.70, 52.65, 52.14, 38.61, 35.87, 35.67, 27.62. IR (ATR) ν (cm^{-1}): 2956 (broad, aliphatic C-H), 1737 (C=O, ester), 1369 (CH_3).

G1-methyl ester dendron (7): Compound **6** (10 g, 21 mmol) was dissolved in 4 M HCl in dioxane (25 mL) and stirred under nitrogen atmosphere at 40°C for 2 h. The solution was reduced at reduced pressure at 40°C. The remaining oil was dissolved in acetone (50 mL) and treated with $NaHCO_3$ (5 g) by stirring at 40°C for 15 min. The solids were removed by filtration and solvent in vacuo. The residue was dissolved in ethyl acetate (75 mL) and washed with 1 M $NaHCO_3$ (10 mL) and brine (10 mL) and dried (Na_2SO_4). The solvent was removed at reduced pressure to yield a clear to slightly yellow oil (6.7 g, 75%)[33]. HPLC-MS: One peak t_R 17.2 min, MS (ESI^+) m/z 423 $[M+H]^+$, 440 $[M+NH_4]^+$, 445 $[M+Na]^+$. MS (MALDI-TOF) m/z 445 $[M+Na]^+$. ^1H NMR (300 MHz, $CDCl_3$, δ in ppm) 5.53 (dd, J = 6.4 Hz, 5.6 Hz, 1H), 5.47 (dd, J = 6.3 Hz, 5.6 Hz, 1H), 4.60 (dd, J = 7.5 Hz, 3.9 Hz, 1H), 3.71 (s, 3H), 3.71 (s, 3H), 3.66 (s, 6H), 2.99 (dd, J = 16.5 Hz, 4.0 Hz, 1H), 2.87 (t, J = 6.8 Hz, 4H), 2.78 (dd, J = 16.5 Hz, 7.6 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm) 171.84, 169.53, 169.35, 169.31, 169.10, 168.58, 69.06, 68.50, 67.07, 52.86, 52.81, 52.25, 52.22, 38.68, 35.72, 35.64. IR (ATR) ν (cm^{-1}): 3501 (broad, OH stretch), 1736 (C=O, ester), 1375 (CH_3). Analysis calcd for $C_{16}H_{22}O_{13}$ (422.34) C 45.50, H 5.25, found C 45.66 H 5.09.

O-*tert*-Butyl-G2-methyl ester dendron (8): Compound **7** (6.79 g, 16 mmol) was suspended in dry DCM (50 mL) and compound **3** (1.38 g, 8 mmol) was added and the mixture stirred under nitrogen until a clear solution was obtained. DMAP (98 mg, 0.80 mmol) was added and the mixture was cooled on an ice bath. After 10 min DIC (1.25 mL, 8 mmol) was added through a septum in intervals over 30 min. The reaction mixture was stirred for an additional 2 h. The solvent was removed at reduced pressure. The residue was extracted with diethyl ether (3 x 50 mL) and filtered. The combined filtrate was treated with acetone (50 mL) and water (50 mL). After vigorous stirring and subsequent phase separation most solids were dissolved leaving mainly a top organic phase and a heavier water/acetone phase which was separated off. Any foamy solid was kept along with the top phase during the separations. The upper layer was washed with water (3 x 100 mL), 1 M $NaHCO_3$ (100 mL), brine (20 mL) and dried (Na_2SO_4). The solvent was removed in vacuo to afford the product as a slightly yellow clear oil[34] (6.74 g, 84%). HPLC: One peak t_R 23.6 min. MS (MALDI-TOF) m/z 999 $[M+H]^+$, 1021 $[M+Na]^+$. ^1H NMR (300 MHz, $CDCl_3$, δ in ppm) 5.64 – 5.42 (m, 6H), 4.62 (dd, *J* = 9.5 Hz, 3.3 Hz, 1H), 3.71 (s, 6H), 3.70 (s, 6H), 3.66 (s, 3H), 3.66 (s, 9H), 3.10 (dd, *J* = 17.1 Hz, 3.3 Hz, 1H), 3.06 (dd, *J* = 17.0 Hz, 3.6 Hz, 1H), 3.01 – 2.80 (m, 10H), 2.81 (dd, *J* = 16.2 Hz, 3.3 Hz, 1H), 2.66 (dd, *J* = 16.2 Hz, 9.5 Hz, 1H), 1.19 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.69, 169.52, 169.48, 169.46, 169.45, 169.23, 169.19, 169.16, 168.90, 168.81, 168.51, 167.89, 167.66, 167.07, 77.21, 69.18, 69.14, 69.08, 68.76, 68.74, 68.54, 67.12, 52.91, 52.88, 52.87, 52.83, 52.31, 52.28, 52.27, 52.25, 38.15, 35.75, 35.71, 35.67, 35.61, 35.52, 35.36, 29.69. IR (ATR) ν (cm^{-1}): 2958 (broad, aliphatic C-H), 1738 (C=O, ester), 1369 (CH_3).

G2-methyl ester dendron (9): Compound **8** (9.1 g, 9.1 mmol) was dissolved and stirred in 4 M HCl in dioxane (25 mL) under nitrogen for 2 h at 40°C. The mixture was evaporated in vacuo at 40°C to a clear yellowish oil which was dissolved in acetone (50 mL). $NaHCO_3$ (5 g, 60 mmol) was added and the suspension was stirred at 40°C until pH reached neutrality (ca. 15 min). The

suspension was filtered and reduced in vacuum. The residue was dissolved in ethyl acetate (50 mL) and washed with water (10 mL), 1 M $NaHCO_3$ (10 mL), brine (10 mL) and dried (Na_2SO_4). The solvent was removed in vacuo by membrane pump. The oily residue was stirred vigorously in diethyl ether (100 mL) and the supernatant was removed by decantation. Residual diethyl ether was removed in vacuum (oil pump) overnight yielding the oily product (6.5 g, 76%). HPLC: One peak t_R 21.4 min. MS (MALDI-TOF) m/z 965 $[M+Na]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 5.57 (dd, J = 9.5 Hz, 3.5 Hz, 1H), 5.51 (dd, J = 9.3 Hz, 3.5 Hz, 1H), 5.50 – 5.41 (m, 4H), 4.69 – 4.61 (m, 1H), 3.70 (s, 12H), 3.66 (s, 12H), 3.11 (dd, J = 16.8 Hz, 3.6 Hz, 1H), 3.06 (dd, J = 16.6 Hz, 3.7 Hz, 1H), 3.02 – 2.90 (m, 3H), 2.86 (dd, J = 10.2 Hz, 5.9 Hz, 8H), 2.80 (dd, J = 16.4 Hz, 8.2 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm) 171.64, 169.44, 169.44, 169.16, 169.15, 169.14, 168.83, 168.76, 168.45, 168.35, 167.84, 167.63, 167.48, 167.04, 69.13, 69.02, 68.69, 68.68, 68.46, 68.10, 67.01, 52.82, 52.80, 52.78, 52.74, 52.22, 52.22, 52.19, 52.19, 38.44, 35.61, 35.56, 35.56, 35.53, 35.42, 35.42. IR (ATR) ν (cm^{-1}): 3524 (broad, OH stretch), 1740 (C=O, ester), 1373 (CH_3). Analysis calcd for $C_{36}H_{46}O_{29}$ (942) C 45.87, H 4.92, found C 46.25 H 4.77.

O-*tert*-Butyl-G3-methyl ester dendron (10): Compound **9** (1.0 g, 0.95 mmol), and **3** (91 mg, 0.5 mmol) were dissolved in dry DCM (10 mL) and DMAP (7 mg, 0.05 mmol) was added. The mixture stirred for 2 h then cooled on an ice bath. A solution of DIC (82 µL, 0.5 mmol) in dry DCM (0.5 mL) in a syringe was added in intervals over a period of 30 min. After 1 h additional stirring on ice bath the reaction mixture was placed in the refrigerator for 16 h. The mixture was transferred to a separation funnel and acetone (20 mL), water (20 mL) and diethylether (20 mL) was added. The bottom water/acetone layer was removed and the organic layer was washed with 1 M $NaHCO_3$ (20 mL), water (3 x 20 mL), brine (20 mL) and dried (Na_2SO_4). The solvent was removed in vacuo. The oily residue was dissolved in diethyl ether (20 mL) and washed with water (3 x 20 mL). The solution was filtered through a silica plug (0.5 g) and dried (Na_2SO_4). The oily product was

separated from the solution by slow addition of petrol ether (20 mL). The supernatant was decanted off and discarded. Solvent residues were removed in vacuo to afford a clear oil (0.596 g, 56%). HPLC: One peak t_R 24.9 min. MS (MALDI-TOF) m/z 2061 $[M+Na]^+$. ^1H NMR (300 MHz, $CDCl_3$, δ in ppm) 5.58 (dd, J = 9.6 Hz, 3.5 Hz, 1H), 5.54 (dd, J = 7.7 Hz, 3.2 Hz, 2H), 5.51 – 5.43 (m, 11H), 4.52 (dd, J = 10.0 Hz, 2.7 Hz, 1H), 3.70 (s, 24H), 3.65 (s, 24H), 3.15 (dd, J = 10.3 Hz, 3.2 Hz, 1H), 3.11 – 3.02 (m, 5H), 3.00 – 2.91 (m, 5H), 2.91 – 2.82 (m, 17H), 2.75 – 2.81 (m, 1H), 2.64 (dd, J = 16.1 Hz, 10.0 Hz, 1H), 1.08 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm) 171.40, 169.47, 169.47, 169.44, 169.43, 169.23, 169.17, 169.14, 169.14, 169.14, 168.86, 168.84, 168.80, 168.52, 168.48, 168.41, 168.39, 167.99, 167.97, 167.81, 167.68, 167.58, 167.51, 167.50, 167.34, 167.31, 167.31, 167.08, 167.01, 166.76, 75.71, 69.25, 69.22, 69.02, 69.02, 68.86, 68.86, 68.77, 68.76, 68.74, 68.70, 68.62, 68.35, 68.32, 67.88, 52.90, 52.89, 52.86, 52.86, 52.83, 52.80, 52.80, 52.78, 52.31, 52.29, 52.29, 52.27, 52.24, 52.21, 52.21, 52.21, 35.70, 35.67, 35.67, 35.67, 35.62, 35.60, 35.46, 35.37, 35.33, 35.29, 35.25, 35.20, 35.12, 35.12, 27.57. IR (ATR) ν (cm^{-1}): 2958 (broad, aliphatic C-H), 1734 (C=O, ester), 1369 (CH_3).

G3-methyl ester dendron (11): Compound **10** (0.58 g, 0.29 mmol) was dissolved in 4 M HCl in dioxane (2 mL) and stirred under nitrogen for 2 h at 40°C. The mixture was evaporated in vacuo and the residual oil was dissolved in ethyl acetate (5 mL) and washed with 1 M $NaHCO_3$ (5 mL), brine (5 mL) and dried (Na_2SO_4). The solvent was removed in vacuo to give the product as a clear oil (0.38 g, 67%). If desired, further purification could be carried out by suspending the oil in dry diethylether (8 mL) and stirring vigorously for 1 h followed by decantation of the diethyl ether supernatant and drying in vacuo. HPLC: One peak t_R 24.1 min. MS (MALDI-TOF) m/z found 2006 $[M+Na]^+$. ^1H NMR (500 MHz, $CDCl_3$, δ in ppm) 5.62 (dd, J = 9.9 Hz, 3.3 Hz, 1H), 5.57 (dd, J = 9.8 Hz, 3.7 Hz, 1H), 5.53 (dd, J = 9.0 Hz, 3.8 Hz, 2H), 5.46 (dd, J = 8.9 Hz, 4.6 Hz, 10H), 4.64 (dd, J = 9.0 Hz, 3.0 Hz, 1H), 3.70 (s, 24H), 3.65 (s, 15H), 3.65 (s, 6H), 3.63 (s, 3H), 3.15 (dd, J = 17.1

Hz, 3.5 Hz, 2H), 3.07 (m, 4H), 3.01 – 2.92 (m, 6H), 2.87 (t, J = 6.4 Hz, 18H),2.75 (dd, J = 16.3 Hz, 9.2 Hz, 1H), 2.69 (dd, J = 17.8 Hz, 9.1 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$, δ in ppm) 171.67, 169.51, 169.47, 169.46, 169.46, 169.46, 169.33, 169.30, 169.19, 169.18, 169.15, 169.15, 169.09, 168.86, 168.85, 168.78, 168.78, 168.58, 168.47, 168.47, 168.37, 168.36, 167.87, 167.87, 167.65, 167.65, 167.51, 167.50, 167.06, 167.06, 69.14, 69.13, 69.04, 69.03, 69.01, 68.71, 68.70, 68.69, 68.50, 68.49, 68.47, 68.14, 68.12, 67.04, 67.03, 52.87, 52.86, 52.84, 52.84, 52.83, 52.82, 52.78, 52.78, 52.26, 52.26, 52.26, 52.24, 52.24, 52.23, 52.22, 52.19, 38.47, 35.77, 35.69, 35.68, 35.64, 35.63, 35.60, 35.59, 35.59, 35.58, 35.56, 35.55, 35.46, 35.44, 35.43. IR (ATR) ν (cm^{-1}): 3500 (broad, low intensity, OH stretch), 1738 (C=O, ester), 1373 (CH_3). Analysis calcd for $C_{76}H_{94}O_{61}$ (1982) C 46.02 H 4.78, found C 46.25 H 4.94.

(*S*)-3-(benzyloxy)-4-(((*S*)-1,4-dimethoxy-1,4-dioxobutan-2-yl)oxy)-4-oxobutanoic acid (12): Compound **5** (1.62 g, 7.8 mmol), **1** (1.62 g, 10 mmol) and DMAP (96 mg, 0.78 mmol) were dissolved in dry DCM (10 mL) and stirred for 15 min, then put in the fridge overnight. The mixture was washed with $KHSO_4$ (2 x 10 mL) and dried (Na_2SO_4). The solvent was evaporated in vacuo to give a clear oil (2.3 g, 79%). HPLC-MS: One peak t_R 20.0 min. MS (ESI^+) m/z 369 $[M+H]^+$, 386 $[M+NH_4]^+$, 391 $[M+Na]^+$. ^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm) 7.31 – 7.20 (m, 5H), 5.53 (t, J = 6.0 Hz, 1H), 4.74 (d, J = 11.2 Hz, 1H), 4.48 (d, J = 11.2 Hz, 1H), 4.41 (dd, J = 8.5 Hz, 4.2 Hz, 1H), 3.71 (s, 3H), 3.63 (s, 3H), 2.89 (dd, J = 16.4 Hz, 3.9 Hz, 1H), 2.87 (d, J = 6.2 Hz, 2H), 2.78 (dd, J = 16.7 Hz, 8.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm) 175.05, 170.28, 169.37, 168.76, 136.89, 128.40, 128.25, 128.06, 73.81, 73.06, 68.69, 52.88, 52.25, 37.60, 35.71.

(*S*)-1-((*S*)-1,4-dimethoxy-1,4-dioxobutan-2-yl) 4-methyl 2-(benzyloxy)succinate (13): Compound **12** (102 mg, 0.28 mmol), methanol (22 mg, 0.69 mmol) and DMAP (5 mg, 0.04 mmol) were dissolved in dry DCM (1 mL). DIC (42µL, 0.28 mmol) was added and the mixture was stirred

30 min at r.t. Diethyl ether (5 mL) was added and the organic layer was washed with 1 M $NaHCO_3$ (2 x 5 mL), water (5 x 5 mL), brine (5 mL), dried (Na_2SO_4) and evaporated to give the product (50 mg, 47%) as a clear oil. HPLC-MS: One peak t_R 22.0 min, MS (ESI^+) m/z 383 $[M+H]^+$, 405 $[M+Na]^+$. ^1H NMR (300 MHz, $CDCl_3$, δ in ppm) 7.54 – 7.02 (m, 5H), 5.52 (t, J = 6.1 Hz, 1H), 4.72 (d, J = 11.3 Hz, 1H), 4.46 (d, J = 11.2 Hz, 1H), 4.43 (dd, J = 8.6 Hz, 4.3 Hz, 1H), 3.70 (s, 3H), 3.63 (s, 3H), 3.61 (s, 3H), 2.86 (d, J = 6.1 Hz, 2H), 2.83 (dd, J = 16.3 Hz, 4.2 Hz, 1H), 2.73 (dd, J = 16.3 Hz, 8.7 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ in ppm) 169.49, 169.30, 168.32, 167.76, 136.10, 127.35, 127.20, 126.96, 73.16, 71.95, 67.59, 51.80, 51.18, 50.95, 36.74, 34.72.

O-Benzyl-G1-hemi (*tert*-butyl)-hemi methyl dendron (14): Compound **12** (380 mg, 1.03 mmol), di-*tert*-butyl malate[35] (256 mg, 1.03 mmol) and DMAP (16 mg, 0.103 mmol) were dissolved in dry DCM (5 mL) under a nitrogen atmosphere and cooled on an ice bath. After 5 min DIC (160 µL, 1.03 mmol) in dry DCM (0.8 mL) was added over 5 min. The mixture was put in the fridge overnight. The solvent was evaporated and the residue was stirred in diethyl ether (10 mL) and filtered. The filtrate was washed with 1 M $NaHCO_3$ (20 mL), water (3 x 20 mL), eluted through a silica gel plug (1 cm) without suction, dried over (Na_2SO_4) and evaporated in vacuo to give the product as a clear oil (350 mg, 57%). HPLC: One peak t_R 27.4 min. HRMS (ESI^+) m/z $[M+Na]^+$ calcd for $[C_{29}H_{40}NaO_{13}]^+$ 619.2361, found 619.2361. ^1H NMR (500 MHz, $CDCl_3$) δ 7.41 – 7.29 (m, 5H), 5.61 (dd, J = 6.8 Hz, 5.4 Hz, 1H), 5.37 (dd, J = 7.3 Hz, 5.1 Hz, 1H), 4.79 (d, J = 10.9 Hz, 1H), 4.58 (d, J = 11.2 Hz, 1H), 4.60 – 4.56 (m, 1H), 3.79 (s, 3H), 3.73 (s, 3H), 3.03 (dd, J = 16.7 Hz, 3.2 Hz, 1H), 3.00 – 2.93 (m, 2H), 2.88 (dd, J = 16.8 Hz, 9.7 Hz, 1H), 2.78 – 2.72 (m, 2H), 1.48 (s, 9H), 1.43 (s, 9H).^{13}C NMR (126 MHz, $CDCl_3$, δ in ppm) 170.46, 169.36, 169.13, 168.79, 168.19, 167.69, 137.18, 128.30, 128.29, 127.88, 82.69, 81.57, 74.16, 73.19, 69.45, 68.65, 52.84, 52.22, 37.49, 37.35, 35.71, 27.98, 27.92. IR (ATR) ν (cm^{-1}): 1740 (C=O, ester), 1368 (CH_3).

O-*tert*-Butyl-G1-lithium carboxylate dendron (15): In a three-necked 100 mL reaction flask O-tert-butyl-G1-methyl ester dendron 6 (0.69 g, 1.43 mmol) and LiI (1.54 g, 11.5 mmol) were dissolved in dry THF (25 mL). A steady flow (1 bubble/s) of nitrogen gas was bubbled through the yellow solution via a long needle inserted through a septum in the side neck. An addition funnel with dry THF was applied to the third neck of the flask and THF was slowly added to the reaction mixture with a frequency of 1 drop per 10 s. The mixture was gently refluxed overnight (oil bath temperature ca. 75^{o}C), while bubbling nitrogen gas through the solution to remove methyl iodide formed in the reaction. During the night the solvent evaporated leaving an orange crystalline residue which was triturated twice in acetone (25 mL) for 30 min to give the product as white crystalline (0.565 g, 88%). HPLC-MS: One peak tR 13.7 min (Li-salt protonated with TFA), MS (ESI+) m/z 440 $[M+NH4]^+$, 445 $[M+Na]^+$. HRMS (ESI+) m/z $[M+Li]^+$ calcd for $[C_{16}H_{22}LiO_{13}]^+$ 429.1215, found 429.1212. 1H NMR (500 MHz, D2O, δ in ppm) 5.07 (dd, *J* = 11.5 Hz, 2.9 Hz, 1H), 5.04 (dd, *J* = 10.9 Hz, 2.9 Hz, 1H), 4.62 (dd, *J* = 10.1 Hz, 2.5 Hz, 1H), 2.93 (dd, *J* = 15.7 Hz, 2.6 Hz, 1H), 2.70 (dd, *J* = 14.4 Hz, 2.8 Hz, 1H), 2.68 (dd, *J* = 15.6, 9.6 Hz, 1H), 2.67 (dd, *J* = 15.3, 2.9 Hz, 1H), 2.51 (dd, *J* = 15.0, 11.4 Hz, 1H), 2.51 (dd, *J* = 15.1, 11.5 Hz, 1H), 1.14 (s, 9H). ^{13}C NMR (126 MHz, D_2O, δ in ppm) 177.96, 177.85, 176.84, 176.48, 174.53, 171.73, 77.32, 74.64, 74.57, 68.01, 39.68, 39.48, 38.27, 26.80. IR (ATR) ν (cm-1): 1584 (C=O, carboxylate), 1732 (C=O, ester), 1369 (CH_3).

G1-lithium carboxylate dendron (16): In a two necked flask equipped with a reflux condenser on one neck and a septum on the other, the G1-methyl ester dendron (100 mg, 0.24 mmol) and LiI (254 mg, 1.9 mmol) were dissolved in dry acetonitrile (20 mL) and refluxed with an oil bath temperature of 95^{o}C. A long needle was internalized through the septum and allowed the mixture to be gently flushed with nitrogen while refluxing in order to ensure complete removal of methyl iodide formed during the reaction. The mixture was refluxed for 16 h evaporating off most of the solvent. The

yellow residue was triturated twice in acetone for 30 min followed by filtration to yield the product as a white crystalline (71 mg, 77%). HPLC: One peak tR 7 min. HRMS (ESI+), (Li-salt protonated with TFA) $[M+Li]^+$ calcd for $[C_{12}H_{14}LiO_{13}]^+$ 373.0589, found 373.0586. ^{1}H NMR (300 MHz, D_2O, δ in ppm) 5.05 (dd, *J* = 11.3 Hz, 3.3 Hz, 1H), 5.04 (dd, *J* = 11.4 Hz, 3.4 Hz, 1H), 4.66 (dd, *J* = 10.2 Hz, 4.8 Hz, 1H), 3.06 (dd, *J* = 16.7 Hz, 2.9 Hz, 1H). 2.75 (dd, *J* = 16.6 Hz, 10.1 Hz, 1H), 2.68 (dd, *J* = 15.5 Hz, 2.9 Hz, 1H), 2.66 (dd, *J* = 15.5 Hz, 3.0 Hz, 1H), 2.50 (dd, *J* = 16.2 Hz, 10.5 Hz, 1H), 2.48 (dd, *J* = 16.0 Hz, 10.6 Hz, 1H). ^{13}C NMR (75 MHz, D_2O, δ in ppm) 178.29, 178.19, 176.99, 176.56, 173.61, 172.02, 74.74, 74.35, 67.04, 39.59, 39.45, 38.38. IR (ATR) ν (cm-1): 1582 (C=O, carboxylate), 1728 (C=O, ester)

Chemical and biological assays

Degradation studies of G1-methyl ester dendron at pH 4, pH 7 and pH 9 analyzed by HPLC: G1-methyl ester dendron (500 mg, 1.2 mmol) was dissolved in MilliQ water (10 mL). Benzylamide (100 mg, 0.83 mmol) was dissolved in MilliQ water (10 mL). The two solutions were mixed to form a stock solution with a G1-methyl ester dendron/benzylamide ratio of 20:1. In this ratio the compounds gave the same absorbance at 220 nm and with HPLC peaks at t_R 12.6 min (benzylamide) and 17.2 min (G1-methyl ester dendron). The stock solution was kept in the freezer (-20 ^{o}C) when not in use. Three buffers were prepared, Buffer (pH 4): 1 M phosphoric acid (100 mL) and 1 M NaOH (100 mL); pH 7: 1 M phosphoric acid (157 mL) and 1 M NaOH (100 mL); pH 9: 1 M $NaHCO_3$. Four aliquots of stock solution (500 µL) were mixed with each of the buffers (500 µL) or water (500 µL) in case of the reference. The solutions were sealed with a stopper and shaken at 37 ^{o}C and after 7 and 14 d small samples (50 µL) was taken out, diluted in water/acetonitrile (250 µL, 1:5 mixture) and analyzed by HPLC (30 µL injection volume).

Degradation studies of G1-methyl ester dendron at pH 9 analyzed by NMR: G1-methyl ester dendron (50 mg, 0.12 mmol) was dissolved in 1 M $NaHCO_3$ (2 mL, pH 9) and the mixture was shaken for 48 h at 37 ^oC. The mixture was freeze-dried and the residue was dissolved in D_2O and analyzed by HSQC NMR and H,H COSY-NMR.[28]

MTT assay (peripheral blood mononuclear cells)[36] **:** G1-dendrons were dissolved in MilliQ water (1 mg/mL), whereas G2-methyl ester terminated dendron was dissolved in ethanol (1 mg/mL) and these solutions were used as stock solutions. Porcine peripheral blood mononuclear cells (PBMCs) from healthy animals were used. Cells were suspended in RPMI 1640 supplemented with 10% (v/v) heat-inactivated fetal calf serum, and 80 µl (2 x 10^5 cells) were incubated at 37 °C, in 5% CO_2, with 20 µl of PBS or dendron solutions (final concentration 200 mg/mL- 0.2 mg/mL, dilution in PBS). Incubation was performed in 96-well flat bottom cell culture plates for 24 h or 48 h, and 10 µL **3-(**4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) dissolved in PBS (10 µg/mL) was added for the last 4-5h of incubation. Then 0.04 M HCl in isopropanol (150 µL) was added to the solutions and mixed thoroughly to dissolve formazan.[37] The absorbance was measured at 550 nm using micro plate reader with subtraction of background absorbance at 650 nm. Analysis was performed in duplicate and effect of test material on cell viability calculated as % cell activity compared to control wells without test material (100% cell activity).

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Supporting Information Available: Analytical data (NMR and MS spectra, and HPLC chromatograms) are provided on selected products. NMR assignment of diastereotopic proton signals in compound 6 and compound 13. Assigned HMBC-NMR spectrum for compound **19**.

Assigned H, H-COSY and HSQC NMR spectra on the degradation experiments. This material is available free of charge via the internet at http://

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24) See assigned diasterotopic CH2 protons for compound 6 and 13 in supporting information

25) See fully assigned HMBC spectra of compound **19** in supporting information.

26) The degradation was determined by comparing HPLC peak area (at 220 nm) of the dendron after degradation with the peak area of the reference

27) When subjecting the dendron to buffer solution a small difference in retention time was immediately observed (16.7 min in buffer versus 17.2 min in Milli Q water). As this shift happened immediately we have not attributed this to a commencing break down of the dendron

28) Interpreted HSQC, H,H-COSY NMR spectra and HPLC chromatograms in relation to the degradation studies is available in supporting information

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33) G1-methyl ester dendron (5 grams) may be purified from traces of apolar impurities by dissolving in 100 mL water and stirring vigorously with diethyl ether (20 mL) for 30 min. The organic layer was removed by separation and the water was evaporated at reduced pressure (< 0.1 bar at 45^{o}C). The residing oil was dissolved in ethyl acetate (75 mL), dried (Na_2SO_4) and the solvent was removed in vacuo.

34) In some cases the product was dissolved in diethyl ether, washed with water, dried (Na_2SO_4) and eluted through a short silica plug by gravity to remove discoloration.

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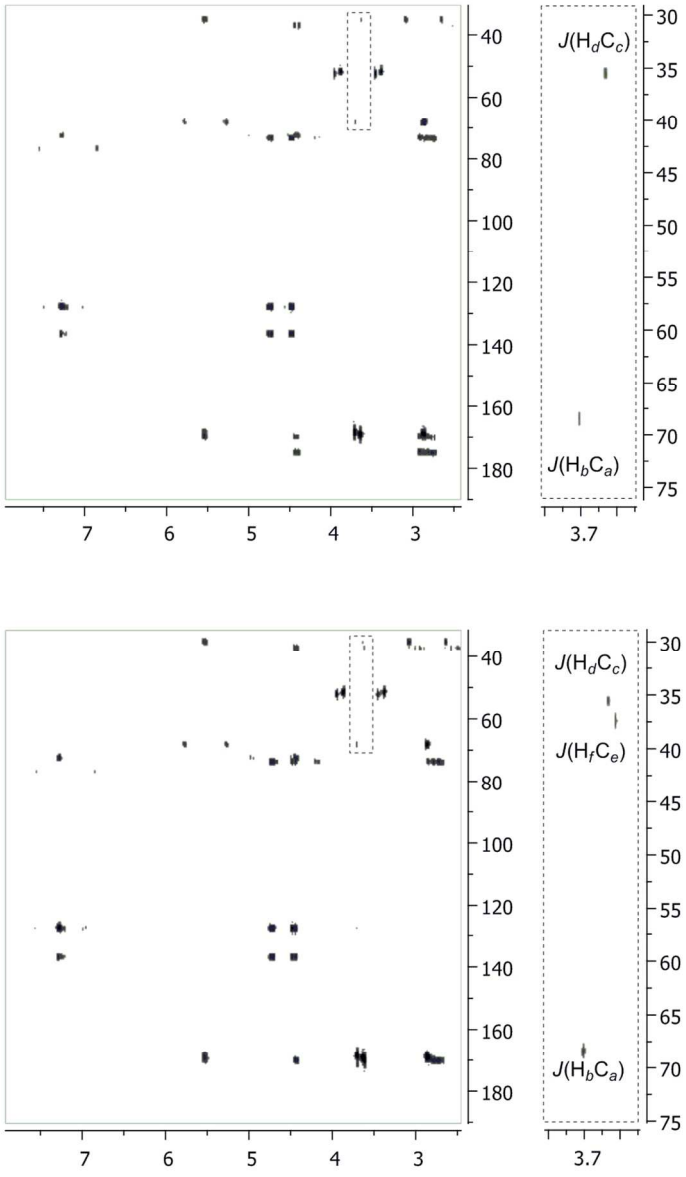
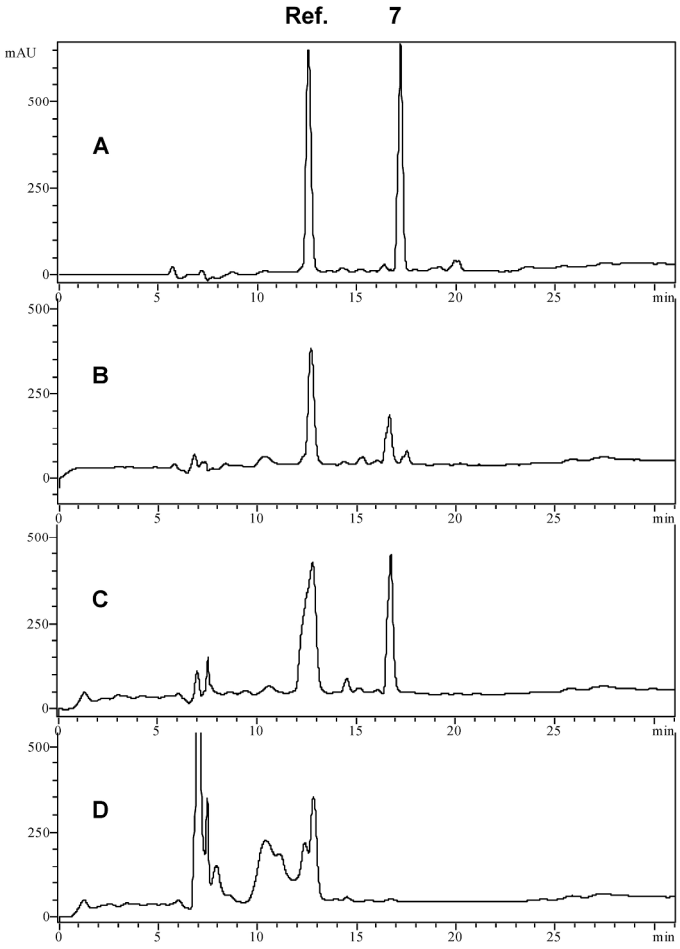
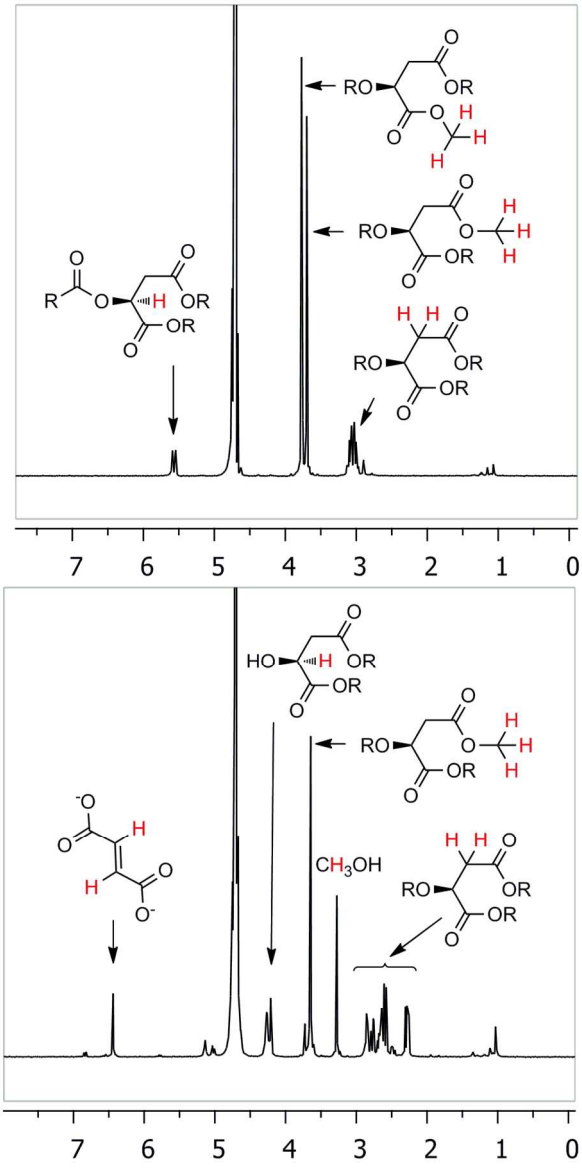


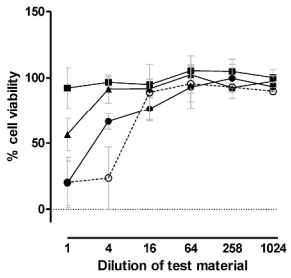
HMBC-NMR spectrum of compound 18 (top) and 19 (bottom). The enlarged area shows the long range coupling between the C4 ester O-methyl group and C2 methylene group.
89x142mm (300 x 300 DPI)



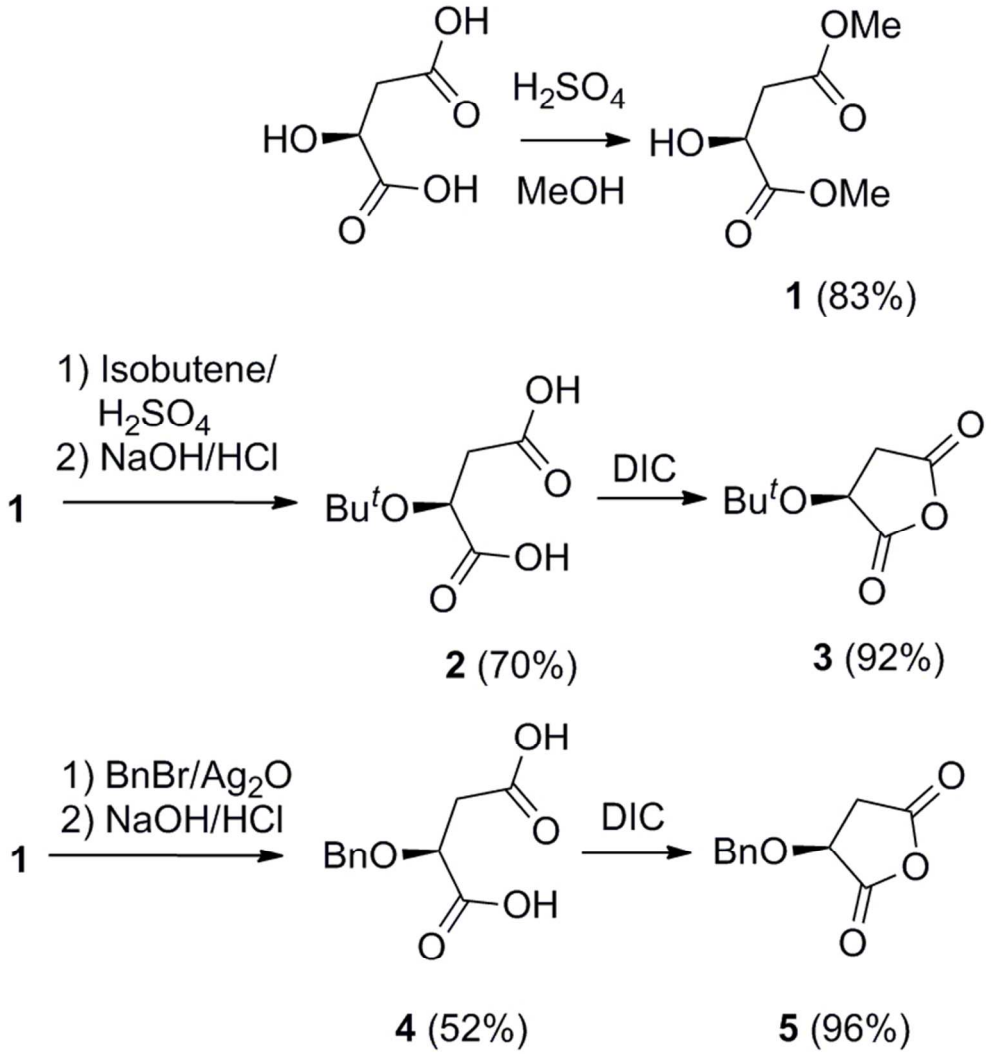
Reversed phase HPLC of A: Stock solution of G1-methyl ester dendron (7) mixed with a reference (benzamide); B: Dilute stock solution after 7 days at pH 4; C: Dilute stock solution after 7 days at pH 7, and D: Dilute stock solution after 7 days at pH 9. The broader reference peak observed in 2C and 2D may be due to the formation of decomposition products with similar retention time
254x338mm (300 x 300 DPI)



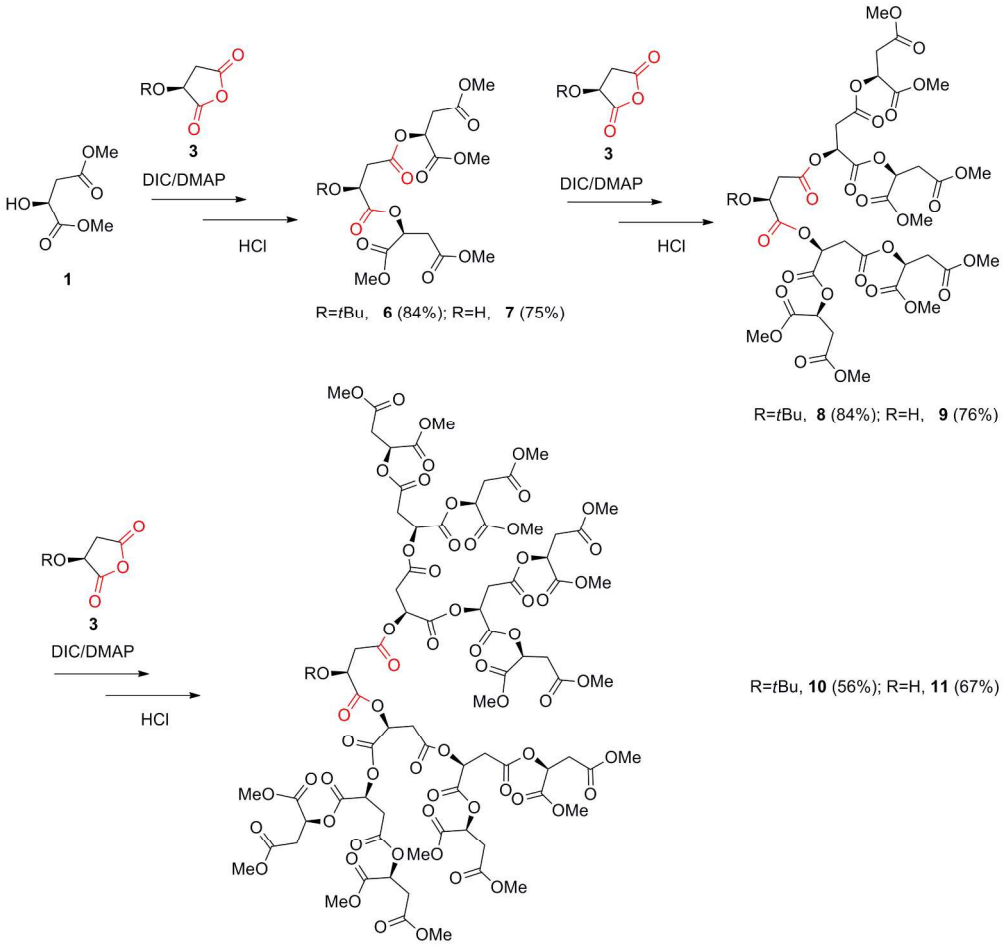
1H-NMR shows degradation in 1 M NaHCO3 (aq) at 37 oC of a first generation dendron with methyl ester terminal groups (compound 7) at t = 0 (top) and t = 3 d (bottom)
82x158mm (300 x 300 DPI)



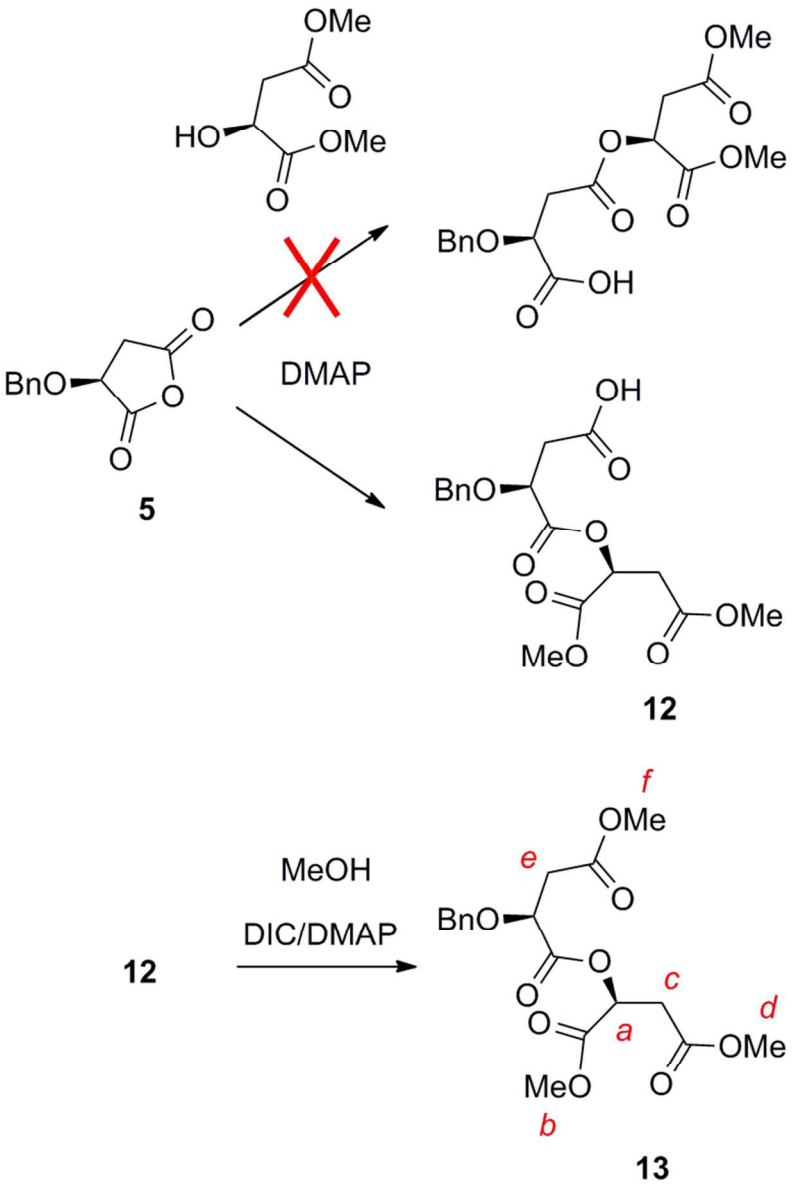
Viability assay (MTT) of dendrons in PBMC culture analysed in 4-fold dilutions in PBS (final concentrations: 200 µg/ml – 0.2 µg/ml; ethanol reference concentrations 19.2 % - 0.02%). Figure shows mean +/- SD of 48 hour-analysis performed with cells from two animals (pig), ▲(G1-methyl ester), ■ (G1-carboxylate dendron),● (G2-methyl ester), ○ (ethanol).
297x420mm (300 x 300 DPI)



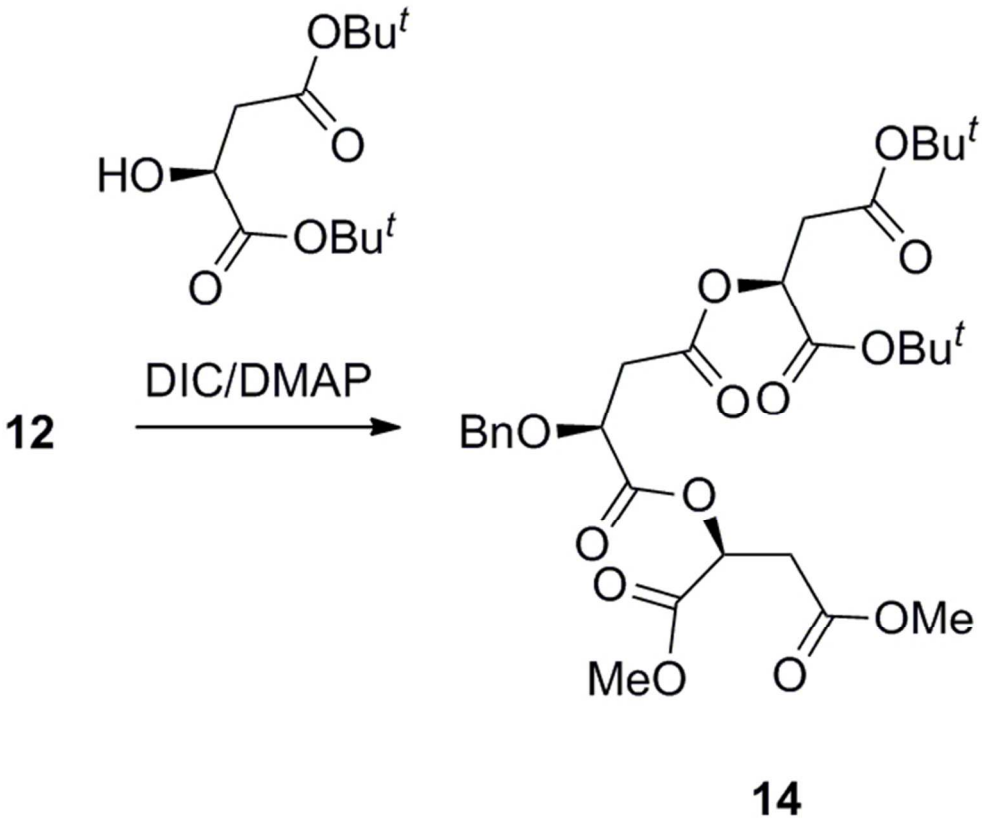
Synthesis of monomer building blocks for convergent dendron synthesis
69x73mm (300 x 300 DPI)



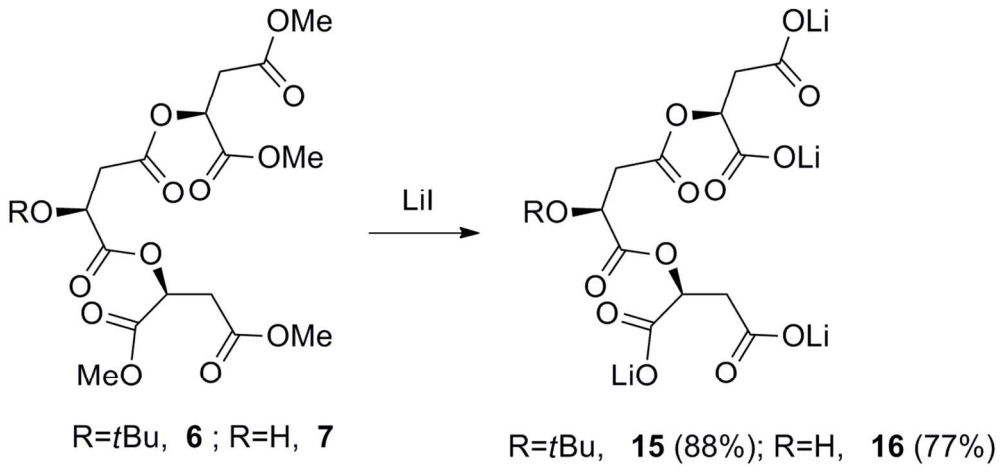
Condensed reaction scheme for the convergent synthesis of G1-G3 methyl ester terminated malic acid dendrons. Each reaction sequence comprises two reactions 1) esterification and 2) deprotection of the tert-Butoxy group by HCl in dioxane. The ester bonds formed in each step are in red color.
206x194mm (300 x 300 DPI)



Synthesis of compounds 18 and 19 to determine the selectivity in derivatisation of carbonyl C1 and carbonyl C4. The carbons and hydrogens which couplings are assigned in figure 1 are denoted with red letters
66x99mm (300 x 300 DPI)



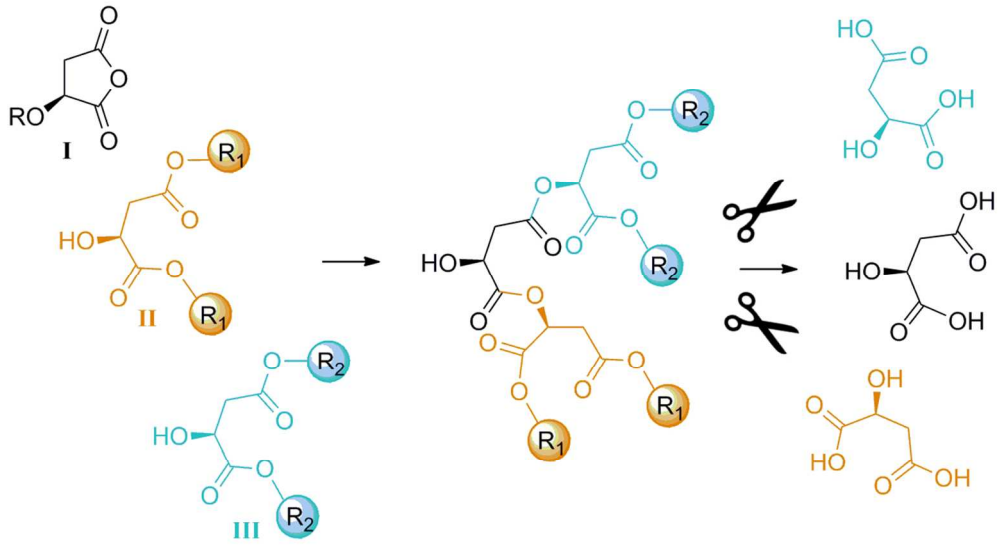
'Proof of principle' synthesis of the hetero-functional G1 dendron 14
56x48mm (300 x 300 DPI)



R=*t*Bu, **6** ; R=H, **7**

R=*t*Bu, **15** (88%); R=H, **16** (77%)

Selective demethylation of terminal methyl esters on compound 6 or 7 by lithium iodide
113x53mm (300 x 300 DPI)



Dendron synthesis with malic acid derivatives in a stepwise manner opens for preparation of polyfunctional dendrons, degradable by hydrolysis
106x59mm (300 x 300 DPI)