

Synthesis, characterization and protein binding properties of supported dendrons†

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Novel benzylether type aldehyde and acetal terminated dendrons were synthesized and attached to a silica gel support; a linear spacer was also introduced as a control material. The supported dendritic compounds were mainly characterized by solid state ¹³C CPMAS NMR, elemental analysis and X-ray photoelectron spectroscopy (XPS) and the presence of free aldehydes was determined by the purpald test. Bovine serum albumin (BSA) protein was coupled to the dendronized support by imine bond formation, followed by irreversible reduction of the carbon–nitrogen double bond. A significant positive dendritic effect was observed on the antibody binding capacity of immobilised BSA as measured by fluorescence immunoassay (FIA).

Introduction

Dendrimers are highly branched monodispersed macromolecules possessing a large number of symmetrical terminal groups. The unique fractal-like dendritic geometry brings about many potential applications.^{1–7} Notably, one of the most exploited properties of dendrimers is their multivalency. Additive effects, an increase in efficiency of binding; as well as dendritic effects, a synergistic increase in affinity, may be observed in dendrimer–substrate associations.^{8,9} Furthermore, the attachment of dendrimers to solid supports,^{10,11} taking advantage of the aforementioned effects and the unique dendritic architecture, has been reported in catalytic reactions,^{12–14} separation processes^{15,16} and biological applications.^{17–22}

Aldehyde terminated dendrimers can be utilized for the conjugation of biologically active molecules.¹⁷ Aldehyde groups are widely used as an immobilization entity due to covalent bond formation with amino side groups, primarily lysines, on the protein surface.^{23–27}

The immobilization of proteins and peptides onto solid supports^{22,28–30} has proved to be important in many areas such as enzymatic catalysis,³¹ biochips³² and biosensors.^{33,34} In recent years, various chemical methods for the immobilization of proteins have been developed, besides the aforementioned aldehyde based covalent linking, ranging from the use of a thiol–ene reaction, through Diels–Alder, Staudinger ligation and ‘click’ methodologies.^{35–41} Two main strategies are widely used: the first based on physical adsorption, while the second employs the formation of covalent bonds.²⁸ In the physical adsorption method, proteins usually retain high activities but leaching may be significant due to weak interactions.⁴² On the other hand, covalent bonds are more difficult to disrupt, but may lead to conformational changes and as a result hinder activity.³¹

In this study, the first results on BSA immobilization on silica gel modified with aldehyde terminal group Fréchet type dendrons are presented. To determine whether dendronized supported proteins could be used for different applications and what effect the dendritic support may have; the immunoactivity of the immobilized protein was evaluated on different dendron generations (*G_n*) and on a linear spacer (*L_n*) that reproduced the length parameters of the second generation dendron.

Results and discussion

Characterization of dendron grafting by elemental analysis

All elemental analyses were repeated in triplicate. The amount (in mmol) of grafted dendron per gram of material (dendron modified silica gel), defined as *n** [mmol g^{−1}], was calculated by the following equations:

$$X_{Gn} = \frac{\%C_{Gn} - \%C_{silica}}{\%C_{(Mw-2)} - \%C_{silica}}$$

$$n^*_{Gn} = \frac{X_{Gn}}{Mw - 2} \times 1000$$

where *X_{Gn}* is the relative mass ratio of the dendron in the dendron modified silica gel, %*C_{Gn}* is the percentage of carbon in the dendron modified silica gel according to elemental (carbon) analysis, %*C_{silica}* is the percentage of carbon in the amine modified silica gel according to elemental (carbon) analysis and %*C_(Mw-2)* is the theoretical percentage of carbon in the dendron's molecular weight minus 2 (*Mw*−2), where 2 is the weight of the two hydrogen atoms lost during the grafting reaction.

For example, in the calculation of *G*_{2p}, elemental analysis afforded %*C_{silica}* = 7.78%, %*C_{G2}* = 25.02% and according to calculations %*C_(Mw-2)* = 72.11% and *Mw*−2 = 1015.12 g·mol^{−1}. Thus,

$$X_{G2p} = \frac{25.02 - 7.78}{72.11 - 7.78} = 0.268$$

$$n^*_{G2p} = \frac{0.268}{1015 \text{ g} \cdot \text{mol}^{-1}} \times 1000 = 0.26 \text{ mmol} \cdot \text{g}^{-1}$$

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Table 1 Calculated amount of grafted dendron based on elemental analysis results

Compound	G_n (termini)	$Mw-2$ ($\text{g} \cdot \text{mol}^{-1}$)	$\%C_{Gn}$	$\%C_{(Mw-2)}$	X_{Gn}	n^* ($\text{mmol} \cdot \text{g}^{-1}$)
23	L_{2p} (1)	374.4	21.61	76.91	0.200	0.54
24	L_2 (1)	330.4	20.89	79.91	0.182	0.55
14	G_{0p} (1)	162.2	19.11	73.98	0.171	1.05
15	G_0 (1)	118.2	17.34	81.25	0.130	1.10
17	G_{1p} (2)	446.5	21.18	72.56	0.207	0.46
18	G_1 (2)	358.4	18.95	77.00	0.161	0.45
20	G_{2p} (4)	1015.1	25.02	72.11	0.268	0.26
21	G_2 (4)	838.9	22.46	75.81	0.216	0.26

The parameter n^* allows one to compare the different materials at the same level of binder molecule loading. n^* multiplied by the number of dendron termini provides the number of aldehyde groups (in mmol) per gram of modified silica material. Calculations of grafted dendron after hydrolysis were performed by the same method. According to elemental analysis, the protecting groups were nearly quantitatively removed (Table 1) (NMR evidence supports this observation, *vide infra*). Additionally, elemental analysis indicates that higher generation dendrons afford lower molar loadings on the silica, probably due to steric hindrance effects. This is quite fortuitous since a very similar number of aldehyde groups per gram of dendronized material are obtained in all cases, except for the linear spacer modified silica where about half the number of aldehydes per gram of material were obtained (the molar loading of the L_2 molecules were similar to the G_1 molecules).

Characterization of dendron grafting by NMR

The modified silica gels were characterized by solid state ^{13}C CPMAS NMR. The disappearance of the acetal signals at *ca.* 100 ppm, the ethylene signals at 65 ppm and the appearance of an aldehyde peak around 190 ppm in the spectrum of **21** are indicative of a fruitful deprotection of the acetal groups (Fig. 1). See the ESI† for the other materials.

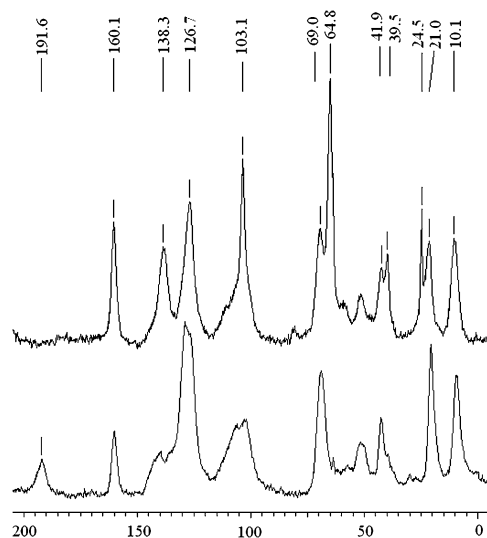


Fig. 1 ^{13}C CPMAS NMR of **20** (top spectrum) and **21** (bottom spectrum).

Characterization of dendron grafting by XPS

Although XPS is considered to be quantitative, it is a surface chemical analysis technique and for correct results it requires samples with a homogeneous surface. Since our dendronized silica gels have a microporous heterogeneous surface, we could only extract qualitative data from the XPS measurements. After the deprotection of the aldehyde by acidic hydrolysis, the amount of carbon atoms per nitrogen and silicon atoms should be reduced and this relative difference may be detected by XPS. As shown in Table 2, XPS analysis indicates a decrease of C/N and C/Si ratios after acetal hydrolysis, as expected.

Characterization of dendron grafting by the purpald test

The reaction of purpald⁴³ (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole) in 1 N NaOH with aldehydes has been used in solution to detect and quantify aldehyde presence taking advantage of the deeply coloured reaction product. This test has also been found to be suitable for the qualitative determination of aldehyde groups grafted on solid supports.^{43b} However, specific conditions should be employed and calibrated according to the type of support materials.

Dendron/linear spacer modified silica gels before and after hydrolysis were analyzed for the presence of aldehyde groups using purpald. Indeed, orange/brown colours were developed in the cases of the unprotected dendron/linear spacer modified silica gel (Table 3) exclusively.

Protein binding

Once aldehyde terminated dendronized silica materials of three generations and the related supported linear spacer were secured and characterized, (corresponding to 1, 2 and 4 free aldehyde groups per dendron for the different generations and one aldehyde per linear spacer molecule) BSA was immobilized on the

Table 2 C/N and C/Si ratios obtained from XPS

Compound	G_n	C/N	C/Si
23	L_{2p}	20.3	1.7
24	L_2	15.0	1.3
14	G_{0p}	11.1	1.5
15	G_0	9.9	1.1
17	G_{1p}	19.9	1.8
18	G_1	11.8	1.3
20	G_{2p}	17.0	2.3
21	G_2	13.4	2.2

Table 3 Purpald test

Compound	G_n	Colour change
23	L_{2p}	Not observed
24	L_2	Observed
14	G_{0p}	Not observed
15	G_0	Observed
17	G_{1p}	Not observed
18	G_1	Observed
20	G_{2p}	Not observed
21	G_2	Observed

silica support by incubation of a solution of protein (10% THF in PBS) with the supported dendrons. The imines obtained were then reduced with NaCNBH_3 to irreversibly immobilize the protein on the support.

The antibody binding capacity of the BSA bound on different generations of dendronized silica was measured by a fluorescence immunoassay (FIA), with fluorescein isothiocyanate (FITC) conjugated chicken anti-BSA antibody (Fig. 2).

To our satisfaction, a more than three fold increase in immunoactivity was observed for the higher generation dendrons. Even though the calculated number of aldehyde groups according to elemental analyses in **15**, **18** and **21** was approximately the same; the higher generation supported dendron scaffold **21** afforded a significantly larger fluorescence signal when the protein was bound. The results suggest that either more protein is bound with the same amount of aldehyde in the higher generation dendrons, or that the protein bound by higher generation dendrons is more efficient at binding its antibody. The introduction of a non-dendritic spacer also elicited more efficient binding when compared to the G_0 support; however, the use of a dendritic scaffold of the same spacer length provided a significantly superior binding (even when the four fold ratio of aldehydes per molecule of dendron to linear spacer is taken into account).

The enhancement of binding capacity with the generation increase might be explained by superior protein binding through a multivalency effect. The results by Kim and coworkers on

lysine cross-linking in BSA with small molecule cross-linkers support the assumption that a multivalent dendrimer may bind multiple lysines on the BSA surface.⁴⁴ It is also possible that the observed improvement may be due to reduced antibody access in proteins bound to lower generation dendrons due to protein conformational changes. Further experiments are ongoing to try to elucidate these assumptions.

Conclusions

In conclusion, three generations of acetal terminated benzylether dendrons were synthesized and characterized, together with a linear spacer that emulates the distance of the termini to the focal group in the second generation dendron. These dendrons were efficiently attached to a silica gel support and deprotected. The new materials were characterized by NMR, XPS and elemental analysis. BSA protein could be covalently bound to the dendronized silica. A noticeable dendritic effect on protein binding could be assessed by FIA analyses; higher generation dendrimers led to more efficient protein binding on the silica surface. Distancing the protein from the silica surface using a linear spacer also showed activity enhancement. However, the use of a multivalent molecule, able to simultaneously bind several neighbouring lysine groups, was more than two times more efficient than the monovalent analogue evidencing a significant positive dendritic effect. Additionally, the ability to judiciously deprotect supported dendrons (by acidic hydrolysis) may allow for future site specific protein binding for advanced materials.

Experimental

Materials and reagents

All reactions were run under a dry nitrogen atmosphere using standard Schlenck techniques. All solvents and reagents were of reagent grade quality, purchased commercially, and used without further purification, except for THF and toluene which were freshly distilled from sodium benzophenone, acetone which was dried under CaSO_4 and freshly distilled, and dichloromethane which was dried under activated molecular sieves 4A (pore size 4Å) prior to use. Flash chromatography was performed on Fluka 0.05–0.15 mm basic alumina ($\text{pH} = 9.5 \pm 0.5$). Silica gel with a 0.04–0.063 mm particle size and 60 Å pore diameter was obtained from Merck; and was dried *in vacuo* at 110 °C overnight before use. Antibodies were purchased from ICN biomedical, Inc. (#654381 chicken anti-BSA affinity purified FITC conjugated).

General

Nuclear magnetic resonance (NMR) spectra were acquired on a Bruker DMX 500 spectrometer; coupling constants are reported in Hertz (Hz). The ^1H NMR chemical shifts were referenced to the residual protio solvent peak at 7.26 ppm in neutralized chloroform-d (CDCl_3). Solid-state ^{13}C CPMAS NMR was acquired on a Bruker DMX 500 spectrometer and chemical shifts were referenced to a glycine external standard at 176.0 ppm. LC-MS data was acquired with a Bruker esquire 3000 (Bruker Daltonics). GC-MS data was acquired with an Agilent 6850 GC-MS apparatus. Determination of C and N by elemental

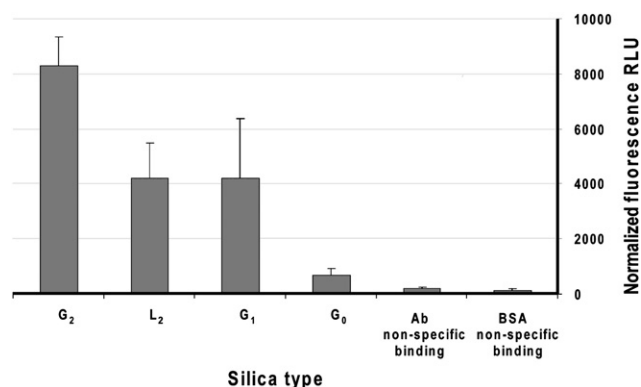


Fig. 2 Function experiment of the post immobilization of BSA. Fluorescence measurements determined the amount of fluorescein isothiocyanate conjugated antibody to BSA, immobilized on the dendronized silica, per one gram of silica, normalized by the number of aldehyde groups obtained from elemental analysis results.

analysis was obtained with a Perkin-Elmer 2400 series II Analyzer. XPS surface analysis was performed with a Thermo-Electron Escalab-250 instrument including a hemispherical analyzer (energy resolution 0.45 eV or better) and microfocused monochromatic X-ray excitation (Al source). General survey spectra were recorded for quantitative and qualitative analysis. Fluorescence in the FIA was read using Synergy™ HT Multi-Mode Microplate Reader (Biotek) in wavelengths of 528/485 nm.

Synthesis

Acetal protected compounds **1–7** (Scheme 1) were prepared by a convergent repetitive methodology according to literature procedures.⁴⁵

Methyl 4-(4-(1,3-dioxolan-2-yl)benzyloxy)benzoate (**8**)

A solution of *p*-hydroxybenzoic acid methyl ester (6.8 g, 28 mmol), **1** (4.2 g, 28 mmol), K₂CO₃ (4.4 g, 33 mmol) and 18-crown-6 (0.37 g, 1.40 mmol) in acetone (150 cm³) was refluxed for 24 h. Then, acetone was removed under reduced pressure. To the resultant solid, water was added and the aqueous layer was extracted twice with ethyl acetate. The organic layer was dried over magnesium sulfate, filtered, and evaporated. The resultant solid was washed with methanol and filtered to afford the desired product **8** (7.6 g, 86%) as a white powder: δ_{H} (500 MHz; CDCl₃) 3.88 (3 H, s, CO₂CH₃), 4.14–4.03 (4 H, m, 2 × CHOCH₂), 5.13 (2 H, s, PhOCH₂), 5.82 (1 H, s, CHOCH₂), 6.97 (2 H, d, *J* 9.0, 2 × *H*-Ph), 7.44 (2 H, d, *J* 7.9, 2 × *H*-Ph), 7.51 (2 H, d, *J* 7.9, 2 × *H*-Ph), 7.99 (2 H, d, *J* 9.0, 2 × *H*-Ph); δ_{C} (125 MHz; CDCl₃) 51.9, 65.3, 69.7, 103.4, 114.5, 122.9, 126.8, 127.3, 131.6, 137.3, 137.9, 162.3, 166.8; *m/z* (EI) 314 (M⁺, 20%), 283 (11, M-CH₃O), 163 (100, M-C₈H₇O₃), 91 (59).

(4-(4-(1,3-Dioxolan-2-yl)benzyloxy)phenyl)methanol (**9**)

To a suspension of lithium aluminium hydride (LAH) (2.7 g, 72 mmol) in THF (100 cm³) cooled to 0 °C was added dropwise a solution of **8** (7.6 g, 24 mmol) in THF (100 cm³). Then, the solution was allowed to warm to room temperature and stirred for 24 h. Excess LAH was quenched by slow addition of a semi-saturated solution of sodium sulfate. THF was removed under reduced pressure. The resultant aqueous solution was extracted twice with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and evaporated to acquire the desired product **9** (6.4 g, 93%) as a white powder: δ_{H} (500 MHz; CDCl₃) 4.14–4.02 (4 H, m, 2 × CHOCH₂), 4.59 (2 H, s, OHCH₂), 5.08 (2 H, s, PhOCH₂), 5.82 (1 H, s, CHOCH₂), 6.94 (2 H, d, *J* 8.3, 2 × *H*-Ph), 7.27 (2 H, d, *J* 8.3, 2 × *H*-Ph), 7.43 (2 H, d, *J* 8.3, 2 × *H*-Ph), 7.49 (2 H, d, *J* 8.3, 2 × *H*-Ph); δ_{C} (125 MHz; CDCl₃) 64.9, 65.3, 69.7, 103.4, 114.9, 126.7, 127.3, 128.6, 133.4, 137.6, 138.0, 158.2; *m/z* (EI) 286 (M⁺, 21%), 163 (100, M-C₇H₇O₂), 91 (88).

Methyl 3-(4-(4-(1,3-dioxolan-2-yl)benzyloxy)benzyloxy)benzoate (**10**)

To a mixture of **9** (0.93 g, 3.25 mmol), *m*-hydroxybenzoic acid methyl ester (0.55 g, 3.62 mmol) and triphenylphosphine (PPh₃) (0.95 g, 3.62 mmol) in THF (20 cm³) at 0 °C was added dropwise

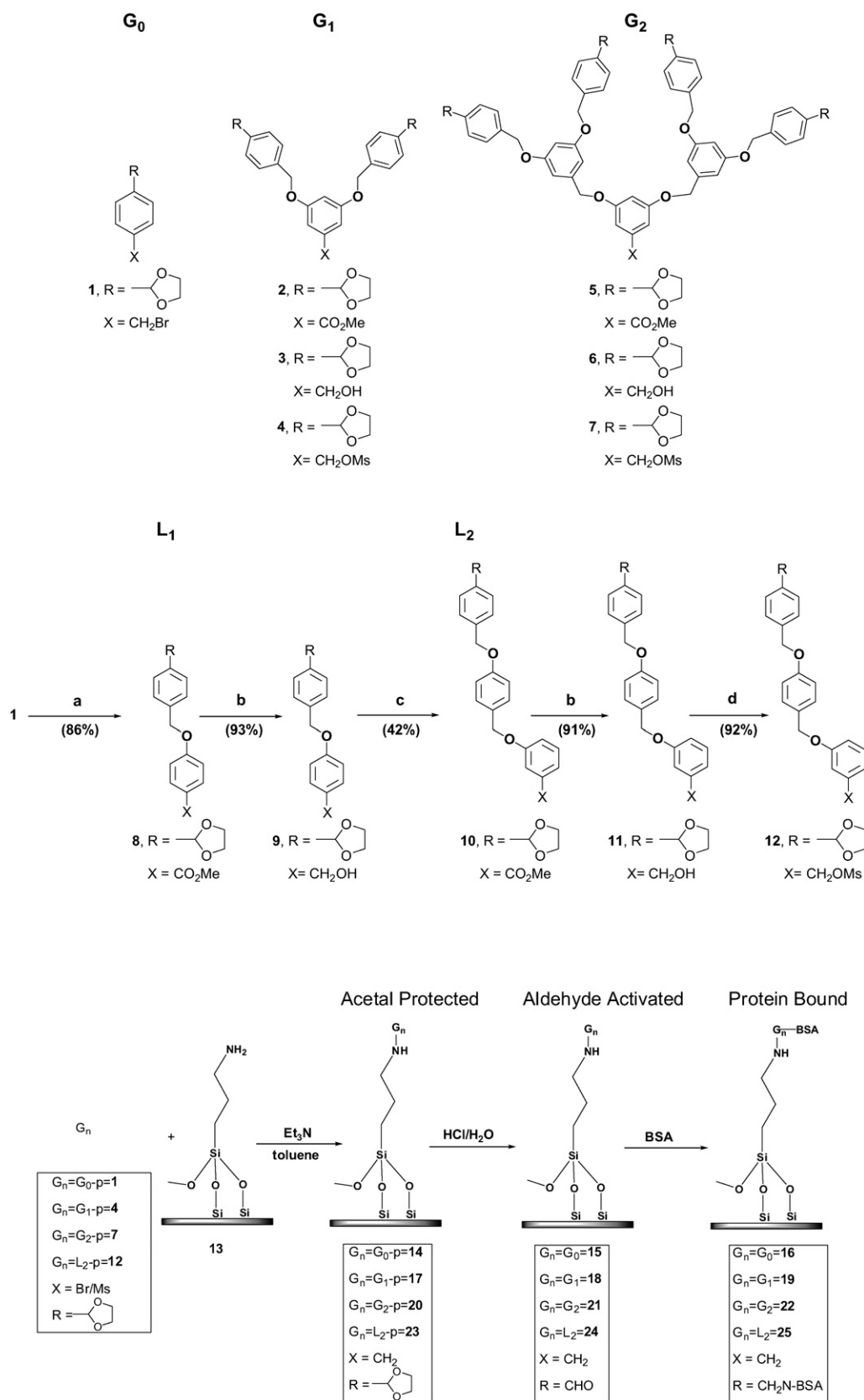
a solution of diisopropyl azodicarboxylate (DIAD) (0.75 cm³, 3.8 mmol) in THF (50 cm³). The reaction was warmed to room temperature and stirred for 24 h. The reaction was stopped by adding water and evaporated to remove THF. The aqueous layer was extracted twice with ethyl acetate, each organic layer was washed with 1 M aqueous KOH and an equal volume of water. The combined organic layers were dried over sodium sulfate, filtered and evaporated. The resultant solid was washed with methanol, filtered and then was further purified by column chromatography on basic alumina using (20/80 petroleum ether/ethyl acetate) as eluent to give the desired product **10** (0.56 g, 42%) as a white powder: δ_{H} (500 MHz; CDCl₃) 3.91 (3 H, s, CO₂CH₃), 4.14–4.03 (4 H, m, 2 × CHOCH₂), 5.02 (2 H, s, PhOCH₂), 5.10 (2 H, s, PhOCH₂), 5.83 (1 H, s, CHOCH₂), 6.98 (2 H, d, *J* 8.7, 2 × *H*-Ph), 7.16 (1 H, br d, *H*-Ph), 7.34 (1 H, t, *J* 7.8, *H*-Ph), 7.36 (2 H, d, *J* 8.7, 2 × *H*-Ph), 7.45 (2 H, d, *J* 8.3, 2 × *H*-Ph), 7.50 (2 H, d, *J* 8.3, 2 × *H*-Ph), 7.65–7.64 (2 H, m, *H*-Ph); δ_{C} (125 MHz; CDCl₃) 52.2, 65.3, 69.7, 69.9, 103.5, 115.0, 120.4, 122.1, 126.7, 127.3, 128.9, 129.3, 129.3, 131.4, 137.7, 137.9, 158.6, 158.7, 166.9; *m/z* (EI) 269 (51, M-C₁₅H₁₃O₄), 163 (100, M-C₈H₇O₃), 91 (47).

(3-(4-(4-(1,3-dioxolan-2-yl)benzyloxy)benzyloxy)phenyl)methanol (**11**)

To a suspension of LAH (0.90 g, 24 mmol) in THF (50 cm³) cooled to 0 °C was added dropwise a solution of **10** (3.3 g, 7.9 mmol) in THF (50 cm³). Then, the solution was allowed to warm to room temperature and stirred for 24 h. Excess LAH was quenched by slow addition of a semi-saturated solution of sodium sulfate. THF was removed under reduced pressure. The resultant aqueous solution was extracted twice with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and evaporated to afford the desired product **11** (2.8 g, 91%) as a white powder: δ_{H} (500 MHz; CDCl₃) 4.14–4.03 (4 H, m, 2 × CHOCH₂), 4.64 (2 H, s, OHCH₂), 4.99 (2 H, s, PhOCH₂), 5.09 (2 H, s, PhOCH₂), 5.83 (1 H, s, CHOCH₂), 6.93 (1 H, br d, *H*-Ph), 6.94 (1 H, br d, *H*-Ph), 6.96 (2 H, d, *J* 8.7, 2 × *H*-Ph), 7.00 (1 H, br s, *H*-Ph), 7.27 (1 H, t, *J* 7.0, *H*-Ph), 7.35 (2 H, d, *J* 8.7, 2 × *H*-Ph), 7.44 (2 H, d, *J* 8.1, 2 × *H*-Ph), 7.50 (2 H, d, *J* 8.1, 2 × *H*-Ph); δ_{C} (125 MHz; CDCl₃) 65.1, 65.2, 69.6, 103.4, 113.1, 114.1, 114.9, 119.2, 126.7, 127.2, 129.3, 129.1, 129.5, 137.6, 138.0, 142.5, 158.5, 159.0; *m/z* (EI) 269 (48, M-C₇H₇O₂), 163 (100, M-C₁₄H₁₃O₃), 91 (57).

3-(4-(4-(1,3-dioxolan-2-yl)benzyloxy)benzyloxy)benzyl methanesulfonate (**12**)

To a stirred solution of **11** (0.98 g, 2.5 mmol) in dichloromethane (15 cm³), were added triethylamine (0.53 cm³, 3.8 mmol) and methanesulfonyl chloride (0.23 cm³, 3.0 mmol) at 0 °C. The reaction was stirred for 2 h. Then, the solution was washed with water and the organic layer was dried under sodium sulfate, filtered and evaporated to afford the desired product **12** (1.2 g, 92%) as a white powder: δ_{H} (500 MHz; CDCl₃) 2.87 (3 H, s, SO₃CH₃), 4.14–4.024 (4 H, m, 2 × CHOCH₂), 4.99 (2 H, s, SO₃CH₂), 5.09 (2 H, s, PhOCH₂), 5.20 (2 H, s, PhOCH₂), 5.83 (1 H, s, CHOCH₂), 6.98 (2 H, d, *J* 8.6, 2 × *H*-Ph), 6.99 (2 H, m, 2 × *H*-Ph), 7.01 (1 H, br d, *H*-Ph), 7.30 (1 H, t, *J* 7.8, *H*-Ph), 7.34



(2 H, d, J 8.6, $2 \times H$ -Ph), 7.45 (2 H, d, J 8.3, $2 \times H$ -Ph), 7.50 (2 H, d, J 8.3, $2 \times H$ -Ph); δ_{C} (125 MHz; CDCl_3) 38.3, 65.2, 69.6, 69.7, 71.3, 103.4, 115.0, 114.9, 115.0, 115.9, 121.1, 126.7, 127.2, 128.9, 129.2, 130.0, 134.7, 137.7, 137.9, 158.5, 159.0; m/z (EI) 269 (43, $\text{M}-\text{C}_8\text{H}_9\text{O}_4\text{S}$), 163 (100, $\text{M}-\text{C}_{15}\text{H}_{15}\text{O}_5\text{S}$), 91 (61).

Modification of the silica gel surface

Modification of the silica gel surface was carried out by standard silanization procedures using 3-aminopropyltrimethoxysilane.

To a stirred suspension of silica gel (10 g) in dry toluene (120 cm^3) under nitrogen was added a solution of 3-aminopropyltrimethoxysilane (3.6 cm^3) and the mixture was refluxed for 80 h. After cooling to room temperature it was filtered by suction and washed with toluene, dichloromethane, and methanol and dried *in vacuo* to afford **13**.

The amount of amino groups according to elemental (nitrogen) analysis introduced onto silica gel was 1.6 mmol g^{-1} (amino functionalized silica gel).

Dendron immobilization on 3-aminopropyltrimethoxysilane modified silica **13**

Modified silica gels **14**, **17**, **20**, **23** (Scheme 1) were prepared by post-synthetic immobilization of protected compounds **1**, **4**, **7** and **12** through the reactive functional group (either the bromide or mesylate) at the focal point. Aldehyde terminated materials were obtained by acetal deprotection by acidic hydrolysis. The modified silica gels were characterized by solid state CPMAS ^{13}C NMR, Purpald aldehyde test, elemental analysis and X-ray photoelectron spectroscopy (XPS).

General procedure. To a suspension of **13** (1 eq.) in toluene under nitrogen were added dendron/linear spacer (1.5 eq.) and triethylamine (3 eq.). The mixture was refluxed for 48 h. Then the solvent was decanted; the silica residue was triturated using a sonicator twice with methanol, twice with distilled water, five times with methanol, five times with dichloromethane, three times with diethyl ether and dried over vacuum overnight.

Hydrolysis of acetal groups on dendron modified silica

General procedure. A suspension of dendron/linear spacer modified silica in a solution of acetone and HCl 1.5 M (acetone:HCl 2 : 1) was sonicated and stirred for 4 h. Then the solvent was decanted; the silica residue was washed with distilled water, twice with a saturated solution of NaHCO_3 , twice with distilled water, five times with methanol, five times with dichloromethane, three times with diethyl ether and dried under vacuum overnight.

Purpald test⁴³

General procedure. To a solution of purpald (50 mg) in 1 M NaOH (1 cm^3) was added 5 mg of modified silica and the suspension was stirred for 10 min. During this time orange/brown colors developed exclusively in the cases of unprotected dendron/linear spacer modified silica gel.

Protein immobilization and measurement of the protein binding capacity by FIA

Five milligrams of dendronized silica were poured into 6 tubes, and then incubated for 7 h. with $200 \mu\text{l}$ of $400 \mu\text{g}/\text{cm}^3$ BSA solution in 10% THF in PBS. The imines were reduced by a solution of 0.02 M NaCNBH_3 . The tubes were centrifuged, and the precipitate was washed 6 times with 10% THF in PBS. Non-dendronized silica amine was used as the control. Blocking of non-specific binding sites was done by incubating the samples with $200 \mu\text{l}$ of 1 M glycine in 10% THF in PBS for 2 h. The tubes were centrifuged and the supernatant removed and washed twice with 10% THF in PBS. The antibody binding reaction was performed by incubation of the samples with FITC conjugated anti BSA, $100 \mu\text{l}$ of 1/100 diluted commercial solution to each tube for 8 h. Four washing steps followed.

The modified silica materials were mixed with $150 \mu\text{l}$ of 10% THF in PBS and moved to a fluorescence 96-well black plate. The fluorescence was read in wavelengths of 528/485 nm.

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