Synthesis and Catalytic Antibody Functionalization of Dendrimers

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Abstract: Antibody 38C2 catalyzed a *retro*-aldol process upon dendritic modified aliphatic polyesters. This catalytic system was studied in detail and displayed rate enhancements, k_{cat}/k_{uncat}, of greater than 10^6. These antibody-catalyzed reactions took place in a stepwise manner yielding partially modified aldol-dendrimers until a fully substituted aldehyde dendrimer was formed. The catalytic antibody 38C2 only reacted with surface-exposed aldol moieties and did not significantly interact with the core groups for dendrons **4** and **8**. For a higher generation dendron **8** the rate of unmasking slightly decreased presumably due to steric crowding of the aldol functionalities. In addition, catalytic antibody 38C2 was able to selectively differentiate *block*-hybrid dendrons and was regiospecific in the *retro*-aldol reaction of dendron **21**. This is an inaugural report of a catalytic antibody utilizing dendrimers as substrates and suggests that antibodies could be used as selective catalysts for the controlled release and activation of specific molecules attached to biodegradable polymeric materials. Furthermore, this is the first example of catalytic antibody 38C2 displaying regioselectivity on a multifunctional aldol substrate. Important for synthetic applications is the antibody's ability to selectively differentiate regions on dendritic substrates and produce partly aldol functionalized dendrons under conditions mild enough to avoid β-elimination.

Introduction

Dendrimers have attracted much interest due to their unique architectures and properties.[1] Their highly branched and well-defined structure, globular shape, and controlled surface functionality are important characteristics which make them excellent candidates for evaluation as drug carriers. The surface functional groups of dendrimers have been utilized for the conjugation of various biologically active molecules. Examples include antibody[2] and carbohydrate[3] moieties that have been conjugated to poly(amidoamine) dendrimers. Such modifications have afforded dendrimers that may possess site-specific properties through antibody−antigen binding or by interactions between the carbohydrate and its receptor found on the cell surface.

The interior of dendrimers has also been shown to be capable of encapsulating guest molecules. Several host−guest systems have already been developed. Examples include dendritic hosts with unimolecular (inverted) micellar structures,[4] the "dendritic box",[5] crown ether dendrimers,[6] and cyclophane dendrimers.[7] A restricted number of guests, such as rose bengal, can be encapsulated in the "dendritic box" (a fifth generation poly(propylene imine) dendrimer modified with a dense shell of amino acids) and released by simple modification of the shell.[5] Dendritic macromolecules with a hydrophobic interior and hydrophilic chain ends have been prepared. These molecules were shown to behave as unimolecular micelles capable of solubilizing various compounds in aqueous solution.[4a]

Dendritic unimolecular micelles have been used to encapsulate drugs inside their hydrophobic interior and release them slowly into solution.[8] The use of dendrimers in drug delivery has also been broached via attachment of the drug to the chain ends of the dendrimer thus allowing a well-defined molecule to carry a high concentration of drug.[9] Eventually, a targeting feature may be added to these dendrimer−drug conjugates by appending an appropriate ligand to one of the dendritic terminal groups. For these applications hybrid dendrons utilizing different protecting groups that could be selectively removed are of great importance.[10]

The keto functionality is an important moiety in the biomedical arena. Indeed, it can be selectively ligated to hydrazide, hydroxy amino, and thiocarbazide groups under physiological conditions.[11] This process has been shown by Bertozzi et al. to constitute a method to engineer the composition of the cell

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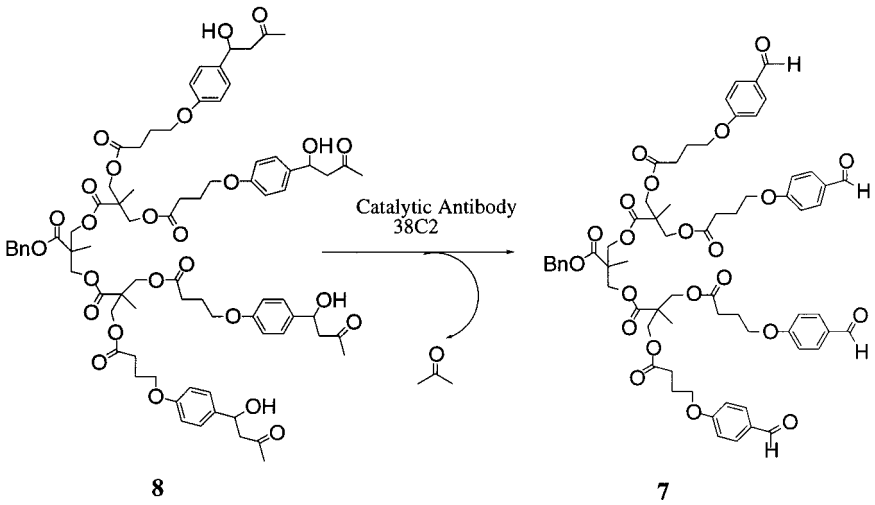
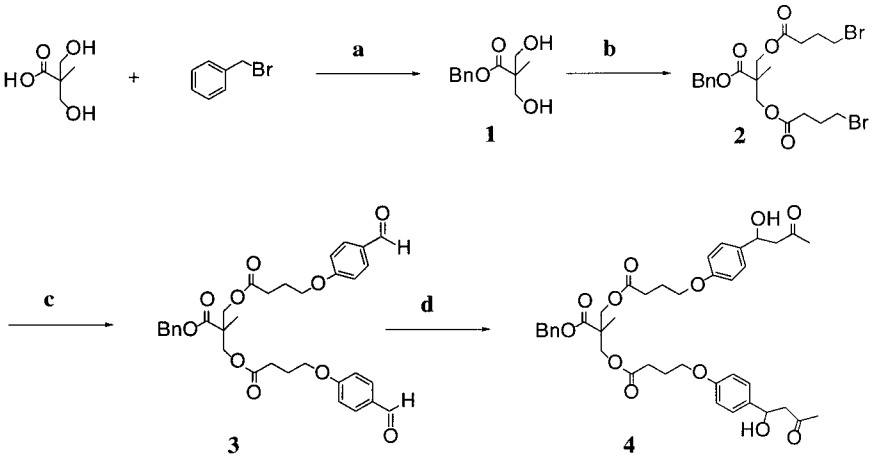
Scheme 1. *retro*-Aldo Reaction of Dendron **8**



Scheme 2[a]



[a] Conditions: (a) KOH, DMF, 100 °C, 60% (b) 4-bromobutyryl chloride, TEA, DMAP, CH_2Cl_2, room temperature, 82%; (c) 4-hydroxybenzaldehyde, K_2CO_3, NaI, DMF, 75 °C, 85%; (d) acetone, LDA, THF, −78 °C, 85%.

surface in vivo.[12] The formation of hydrazones, in vivo, from monosubstituted hydrazines and aldehydes or ketones is a mechanism for cytotoxin self-assembly. For example, Rideout obtained synergistic cytotoxicity against cultured human tumor cells (HeLa) and bacteria (*Escheria coli* J96) by using equal amounts of decanal and *N*-amino-*N*′-1-octylguanidine (AOG).[13] By contrast, no cytotoxicity was obtained with either decanal or AOG alone. These results are explained by invoking a bimolecular reaction in situ between decanal and AOG to form a more cytotoxic hydrazone, *N*-decylidenimino-*N*′-1-octylguanidine.

On the basis of Rideout's findings, dendrimers having an aldehyde functionality at their surface may be candidates for cytotoxin self-assembly and chemoselective modifications in vivo. There have been reports of dendrimers displaying aldehyde moieties at their surface;[14] however, the biocompatability of these has not been evaluated. In contrast, dendrimers with an aliphatic polyester[15] or polyether[16] core would be biodegradable and thus biocompatible.[17]

In this article, we have prepared 4-hydroxybenzaldehyde-terminated polyester dendrons based on 2,2-bis(hydroxymethyl)-propanoic acid (bis-MPA). Since the terminal 4-alkoxybenzaldehyde group can be readily oxidized under physiological conditions, the aldehyde was masked as the corresponding aldol by reaction with acetone. Hybrid dendrons containing different aldol moieties were also prepared. To reveal the aldehyde and to add selectivity to the process an antibody based approach was investigated.

Catalytic antibody 38C2 was generated using the concept of "reactive immunization"[18b] by which the enamine mechanism of natural aldolases was evoked within the antibody-combining

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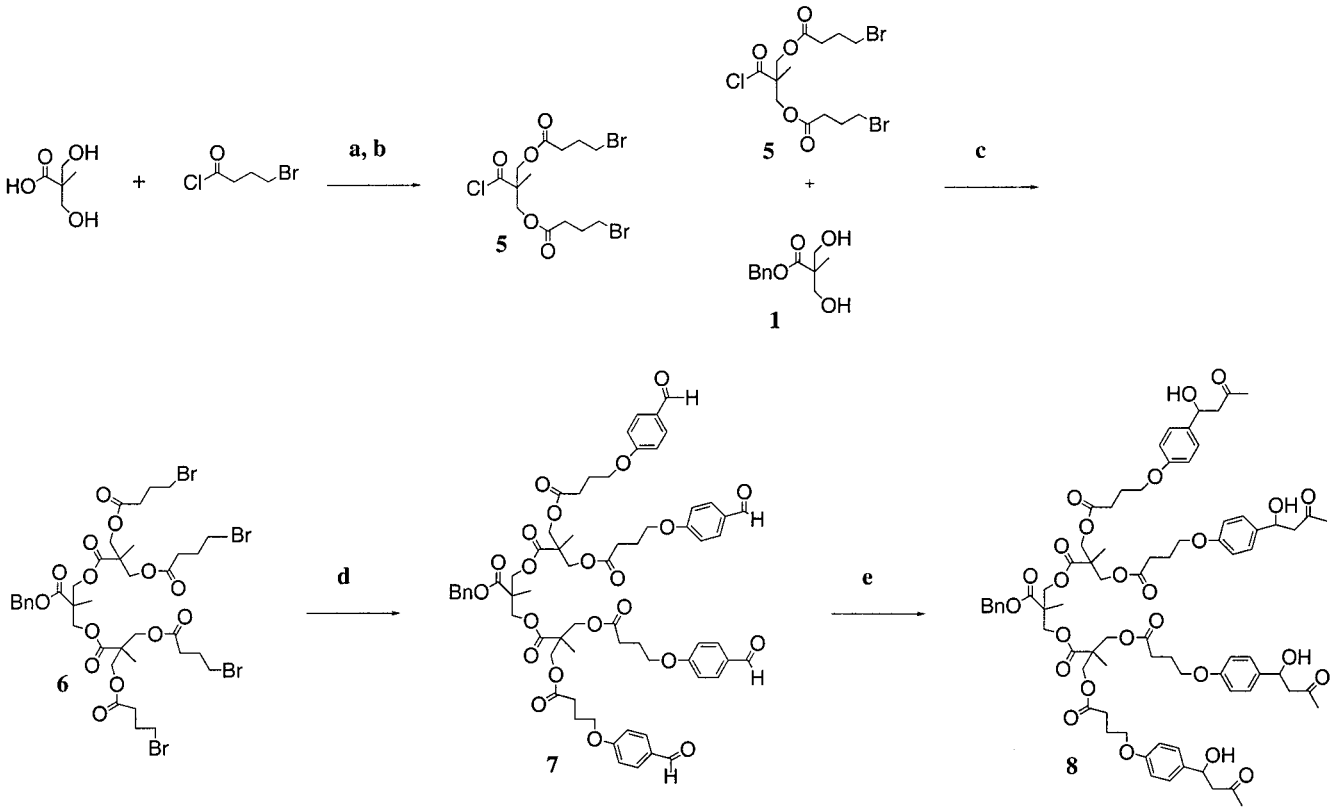
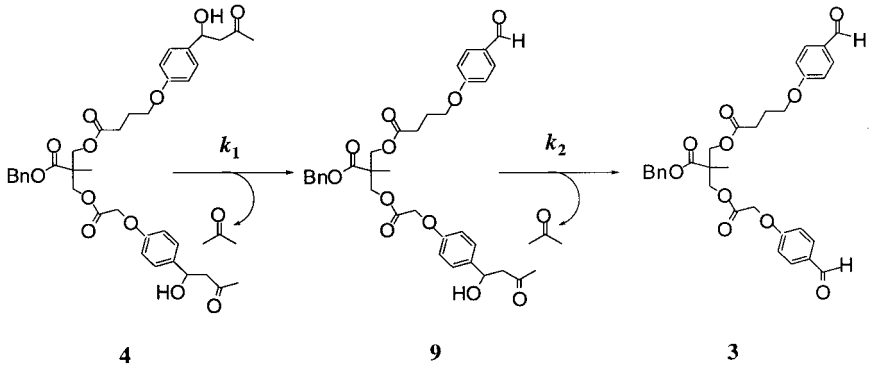
Scheme 3[a]



[a] Conditions: (a) TEA, DMAP, CH_2Cl_2, room temperature, 80% (b) oxalyl chloride, catalyst DMF, CH_2Cl_2, room temperature, 99%; (c) TEA, DMAP, CH_2Cl_2, room temperature, 65%; (d) 4-hydroxybenzaldehyde, K_2CO_3, NaI, DMF, 75 °C, 85%; (e) acetone, LDA, THF, −78 °C, 65%.

Scheme 4. *retro*-Aldol Reaction of Dendron **4**



site.[18a] Through a reactive lysine buried in a hydrophobic pocket at the base of the substrate-binding site, antibody 38C2 catalyzes aldol and *retro*-aldol reactions at rates approaching those of natural aldolases.[19] However, unlike natural aldolases, antibody 38C2 accepts a wide variety of substrates making it a versatile tool for asymmetric synthesis[20] and antibody-directed enzyme prodrug therapy (ADEPT).[21] Furthermore, the catalytic antibody 38C2 is commercially available[22] and remains catalytically active over weeks after injection into mice.[21] It was also recently humanized by Lerner and co-workers and shows promise in anti-cancer therapy.[23] We therefore sought to use the unique chemistry of this catalyst for the unmasking of a model aldehyde that in theory could be transformed into a cytotoxin. Importantly we also wanted to investigate the use of catalytic antibodies to achieve selective removal of end-groups on dendritic substrates. To our knowledge this is the first example of a catalytic antibody acting on a dendritic substrate (Scheme 1).

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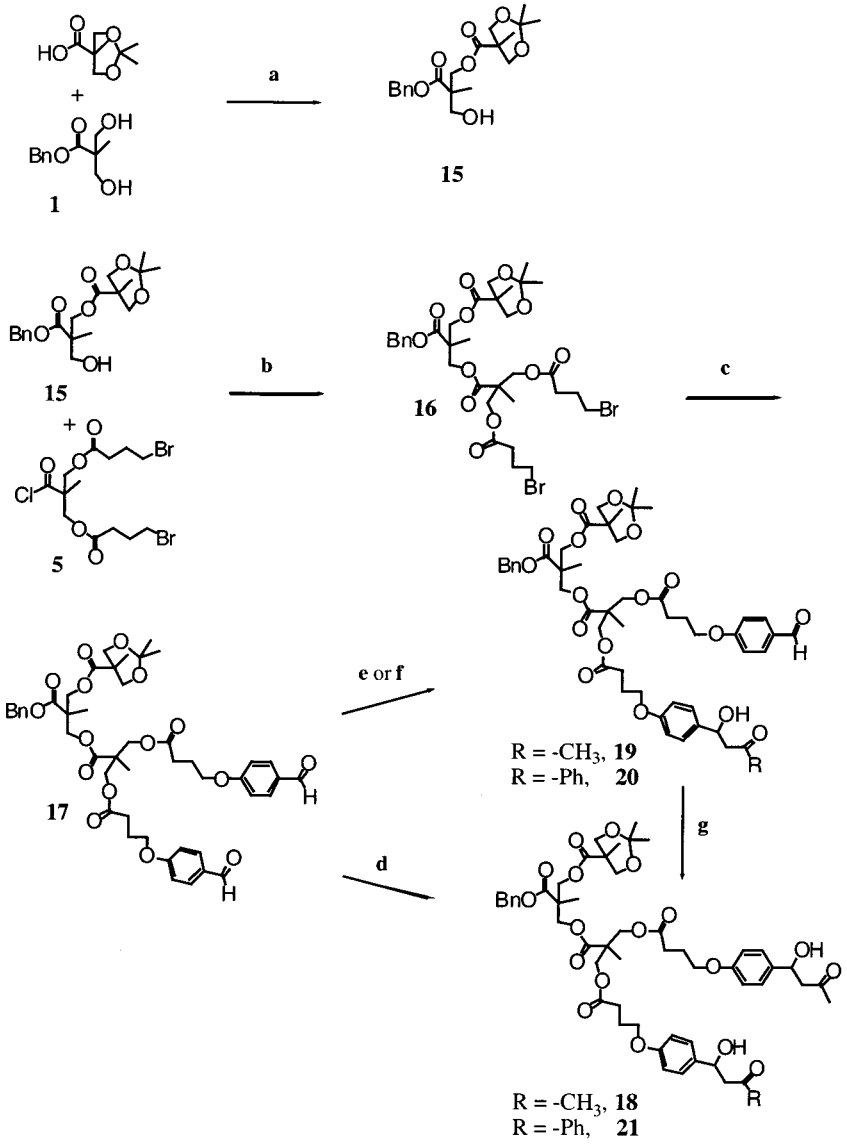
Scheme 5[a]



[a] Conditions: (a) DCC, DMAP, CH_2Cl_2, room temperature, 78%; (b) TEA, DMAP, CH_2Cl_2, room temperature, 65%; (c) 4-hydroxybenzaldehyde, K_2CO_3, NaI, DMF, 70 °C, (85%); (d) acetone (8 equiv), LDA (8 equiv), THF, −78 °C, 65% (**18**); (e) acetone (1.5 equiv), LDA (1.5 equiv), THF, −78 °C, 50% (**19**); (f) acetophenone (1.5 equiv), LDA (1.5 equiv), THF, −78 °C, 55% (**20**); (g) acetone (3 equiv to **20**), LDA (3 equiv to **20**), THF, −78 °C, 45% (**21**).

Results and Discussion

Synthesis of Aldehyde and Aldol Functionalized Dendrons. Bis-MPA was chosen as the AB_2 monomer in the synthesis of dendritic aliphatic polyesters **3**, **4**, **7**, and **8** (Schemes 2 and 3). Hult et al.[15] have developed methodologies for the synthesis of these types of dendrimers based on a convergent growth approach.[1c] Another suitable polyester dendritic core is based on 3-hydroxybutanoic acid and trimesic acid, which Seebach et al. have shown to be biodegradable.[17a]

Dendrons **3** and **4** were prepared as shown in Scheme 2. Bis-MPA was converted to benzyl ester **1** by first forming the potassium salt with KOH, and in a second step reacting the salt with benzyl bromide.[15] Diol **1** was then esterified with 4-bromobutyryl chloride (triethylamine (TEA), a catalytic amount of 4-(dimethylamino)pyridine (DMAP), CH_2Cl_2, 82%) followed by etherification with 4-hydroxybenzaldehyde (K_2CO_3, NaI, DMF, 85%) to form bis-aldehyde **3**. The aldol reaction of **3** with 4 equiv of the lithium enolate of acetone gave dendron **4** in 85% yield. The second generation dendrons **7** and **8** were synthesized as shown in Scheme 3. Starting with bis-MPA, esterification with 4-bromobutyryl chloride (TEA, catalytic DMAP, CH_2Cl_2, 80%) followed by treatment of oxalyl chloride formed acid chloride **5** in 99% yield. Esterification of diol **1** using an excess of **5** (TEA, catalytic DMAP, CH_2Cl_2, 65%) produced the second generation dendron **6**. Using similar procedures as described for **3** and **4**, etherification with 4-hydroxybenzaldehyde gave the tetraaldehyde **7** (85% yield), and reaction of **7** with 8 equiv of the lithium enolate of acetone gave tetraaldol **8** (65% yield). Matrix Assisted-Time-of-Flight MS (MALDI TOF MS) analysis of **7** and **8** showed only single peaks corresponding to **7**+Na^+ (1240 Da) and **8**+Na^+ (1472 Da), respectively. This methodology also allows increasing generations of benzaldehyde-functionalized polyester dendrons to be formed by using benzyl-protected bis-MPA dendrons[15b] as starting materials.

Partially aldol-functionalized dendrons, such as **9** (Scheme 4) and [G#2]-trialdol **10** (Figure 1), for kinetic and HPLC analysis, were synthesized by dissolving **3** or **7**, respectively,

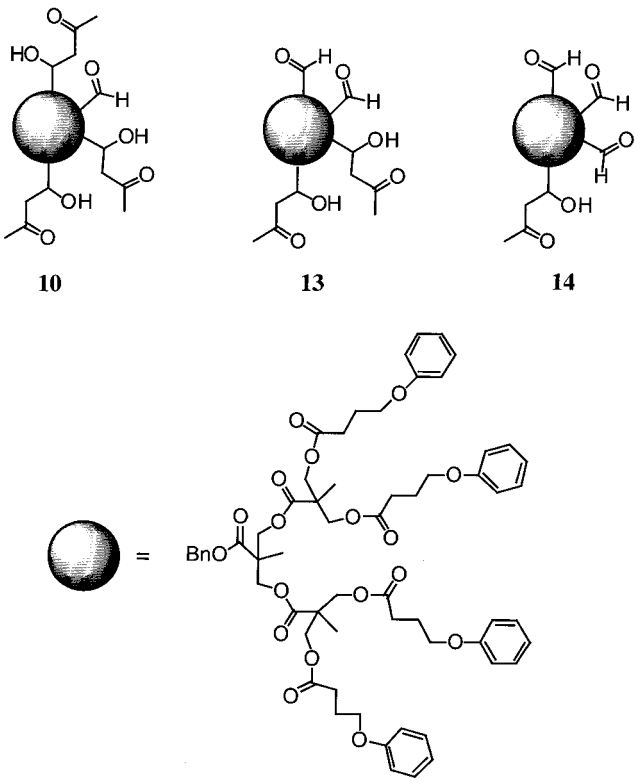


Figure 1. Functionalized dendrons **10**, **13** and **14**.

in acetone and adding 10% (v/v) of 1% (w/v) aqueous NaOH at 0 °C.[24] In contrast to the LDA-promoted aldol formation in THF, β-elimination of the aldol product was a competing reaction.

Antibody Catalysis with Dendrimer Substrates. We first investigated the specificity of catalytic antibody 38C2 with monomer substrates (4*R*,4*S*)-hydroxy-4-(4′-methoxyphenyl)-butan-2-one (**11**) and 4-methoxybenzaldehyde (**12**), respectively (Scheme 8). The Michaelis−Menten and Lineweaver−Burke plots of the *retro*-aldol reaction at 25 °C are depicted in Figure 2, parts a and b, respectively. Antibody 38C2 catalyzed the *retro*-aldol of **11** following Michaelis−Menten kinetics ($k_{cat} = 0.29$ min^{-1} and $K_M = 1.1$ mM). The uncatalyzed rate of this *retro*-aldol reaction was determined to be 2.6×10^{-7} min^{-1} (100 mM PBS buffer at pH 7.4). The relative rate enhancement over background provided by the antibody for this reaction (k_{cat}/k_{uncat}) was 1.1×10^6 and the specificity constant (k_{cat}/K_M) was 264 min^{-1} M^{-1}. Comparing the specificity of antibody 38C2 for **11** with that of (4*S*)-hydroxy-4-(4′-isobutylamidophenyl)butan-2-one ($k_{cat}/K_M = 5.2 \times 10^3$ min^{-1} M^{-1})[20d] implies that the change from a 4-amidophenyl group to a 4-methoxyphenyl group lowers the specificity of the antibody by a factor of ~20. Importantly, catalytic antibody 38C2 also displayed stereoselectivity for **11**. At 80% conversion of the *retro*-aldol reaction, the unconverted (*R*)-**11** was obtained in 88% ee as determined by chiral HPLC.[20b] Interestingly, the aldol reaction forming **11** was ~145 times slower compared to the *retro*-aldol reaction of **11**. The kinetic parameters for the aldol reaction of **12** were $k_{cat} = 0.002$ min^{-1} and $K_M = 1.2$ mM at an acetone concentration of 1 M. The specificity constant (k_{cat}/K_M) was 1.7 min^{-1} M^{-1} and the rate enhancement over background (k_{cat}/k_{uncat}) was 4×10^5. Both reactions were completely inhibited by the addition of 2 equiv of butan-2,4-dione or 6-(4′-acetamidophenyl)hexan-2,4-dione.[19,20d]

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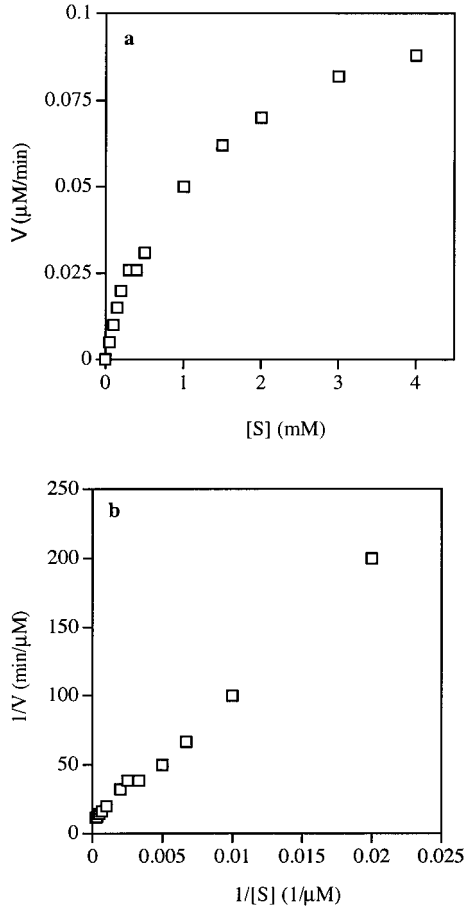

Figure 2. (a) Michaelis−Menten diagram for the *retro*-aldol reaction of **11** (Scheme 8) at 25 °C in PBS buffer at pH 7.4 ([IgG] = 0.2 μM). The initial rates were determined by HPLC (VYDAC reverse phase C_{18}-column, 20:80 acetonitrile:H_2O (0.1% TFA), $\upsilon = 1.5$ mL/min, $\lambda_{abs} = 276$ nm) with 4-methoxyacetophenone as standard. (b) The Lineweaver−Burke plot of the above reaction ($K_M = 1.1(\pm 0.01)$ mM and $k_{cat} = 0.29(\pm 0.01)$ min^{-1}).

Over-oxidation of **11** to the corresponding benzoic acid was not observed.

The time progress for the antibody 38C2-catalyzed *retro*-aldol reaction of **4** at 25 and 37 °C is shown in Figure 3, parts a and b, respectively. The product formation follows the pattern of a consecutive reaction, where **9** (Scheme 4) is a transient intermediate that appears and is then consumed.[25] Antibody 38C2 also displayed a higher activity at 37 °C compared to 25 °C. The rate constant, k_1, for reaction of **4** to **9** was derived from eq I by plotting ln[A] against time, the slope of the straight line being equal to k_1. Solving eq II gives the rate constant, k_2, for the transformation of **9** to **3.** This was performed by plotting ln[B] against time where the slope of the linear region after the faster process has died out gives the rate constant of the slower process (Figure S1, Supporting Information).[26]

$$[A] = [A]_0 \times \exp(-k_1 t) \quad \text{(I)}$$

$$[B] = [A]_0 \times k_1/(k_2 - k_1) \times (\exp(-k_1 t) - \exp(-k_2 t)) \quad \text{(II)}$$

The values for k_1 and k_2 at 37 °C were 0.69 and 0.03 h^{-1}, respectively, hence intermediate **9** was formed 22 times faster than it was converted to **3**. The half-life ($\tau_{1/2}$) of **4** was 1 h.

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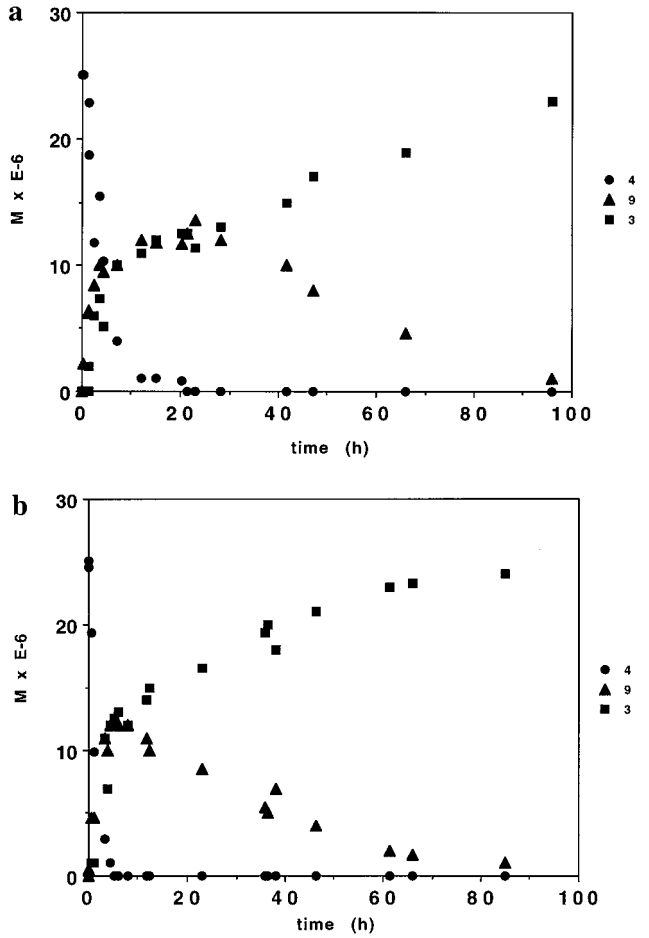


Figure 3. (a) The time progress of the antibody 38C2-catalyzed ([IgG] = 0.4 μM) *retro*-aldol reaction of **4** (25 μM) in PBS buffer at 25 °C. (b) The time progress of the antibody 38C2-catalyzed ([IgG] = 0.2 μM) *retro*-aldol reaction of **4** (25 μM) in PBS buffer at 37 °C. The consumption of **4** and production of **9** and **3** were measured by HPLC (VYDAC reverse phase C_{18}-column, 52:48 acetonitrile:H_2O (0.1% TFA), v = 1.5 mL/min, λ_{abs} = 276 nm) with naphthalene as standard.

Table 1. Kinetic Parameters at [S] = 25 μM and [IgG] = 0.2 μM for the Retroaldol Reaction in PBS Buffer at pH 7.4

substrate	temp (°C)	v_i[a] (μM/ min)	$k_{cat,\,app}$[b] (min^{-1})	k_{uncat} (min^{-1})	$k_{cat,\,app}/k_{uncat}$
9	25	0.08	0.20	n.d.[c]	n.d.[c]
9	37	0.22	0.55	n.d.[c]	n.d.[c]
4	25	0.13	0.35	n.d.[d]	n.d.[c]
4	37	0.40	1.00	0.8×10^{-6}	1.3×10^{6}
8	25	0.10	0.25	n.d.[d]	n.d.[c]
8	37	0.28	0.70	0.6×10^{-6}	1.1×10^{6}

[a] Determined by HPLC. [b] Described in terms of apparent rate due to substrate solubility limits. [c] Not determined. [d] No product detected after 7 days reaction time.

Antibody 38C2 showed multiple turnovers (63 per active site) and high conversion, 98% of **4** to **3** within 67 h.

The relevant kinetic parameters of antibody 38C2 are presented in Table 1. The initial rates are calculated for the first step in the sequential reactions, for example, the conversion of **8** to [G#2]-trialdol (**10**) in the overall reaction of **8** to **7**. It should be noted that the solubility limit (25 μM) of the apolar dendritic substrates created a limitation with regards to our kinetic

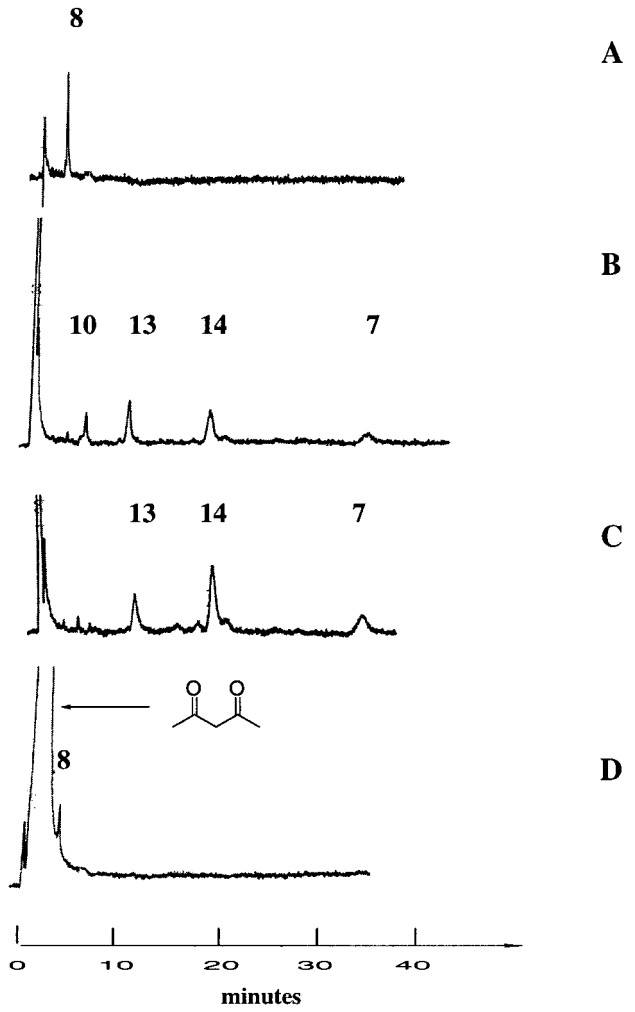


Figure 4. The product formation of the *retro*-aldol reaction of **8** (25 μM) in PBS buffer at 37 °C after 24 h reaction time: (A) the uncatalyzed reaction; (B) antibody 38C2-catalyzed reaction ([IgG] = 0.4 μM); (C) antibody 38C2-catalyzed reaction ([IgG] = 5 μM); and (D) complete inhibition of the antibody 38C2-catalyzed reaction in part C ([IgG] = 5 μM) by addition of 3 equiv of pentan-2,4-dione. The analysis was performed by HPLC (VYDAC reverse phase C_{18}-column, 55:45 acetonitrile/H_2O (0.1% TFA), v = 1.5 mL/min, λ_{abs} = 276 nm).

investigation of this reaction. Consequently, reactions above this concentration could not be performed. Despite this, catalytic antibody 38C2 displayed good catalytic proficiency for substrates **9**, **4**, and **8** ($k_{cat,app}/k_{uncat} = 1 \times 10^6$). Control experiments demonstrated that nonspecific antibody or bovine serum albumin did not catalyze any of the reactions described. However, increasing the temperature from 25 to 37 °C increased the rates by a factor of ~3 for all substrates. Since the K_M for acetone is approximately 1 M in the aldol reaction, the equilibrium between the aldol and its precursors favors *retro*-aldolization. The uncatalyzed reaction was nonexistent at 25 μM dendrimer concentration and no product formation could be observed even after 7 days of incubation at 25 °C. The initial rate for **4** as compared to **9** was ~2 times greater and the specific concentration of aldol adduct was also 2 times greater for **4** (50 μM) than for **9** (25 μM).

$$S = v_A/v_B = (k_{cat}/K_M)_{\mathbf{A}}/(k_{cat}/K_M)_{\mathbf{B}} \times [A]/[B] \qquad \text{(III)}$$

Thus, the specificity ratio between two competing substrates can be expressed as the ratio of their specificity constants.[27] The specificity ratio has here been derived as the ratio of the

(27) Fersht, A. In *Enzyme Structure and Mechanism*, 2nd ed.; Freeman and Company: New York, 1992.

(28) The enantioselectiveity was not determined for the dendritic substrates.

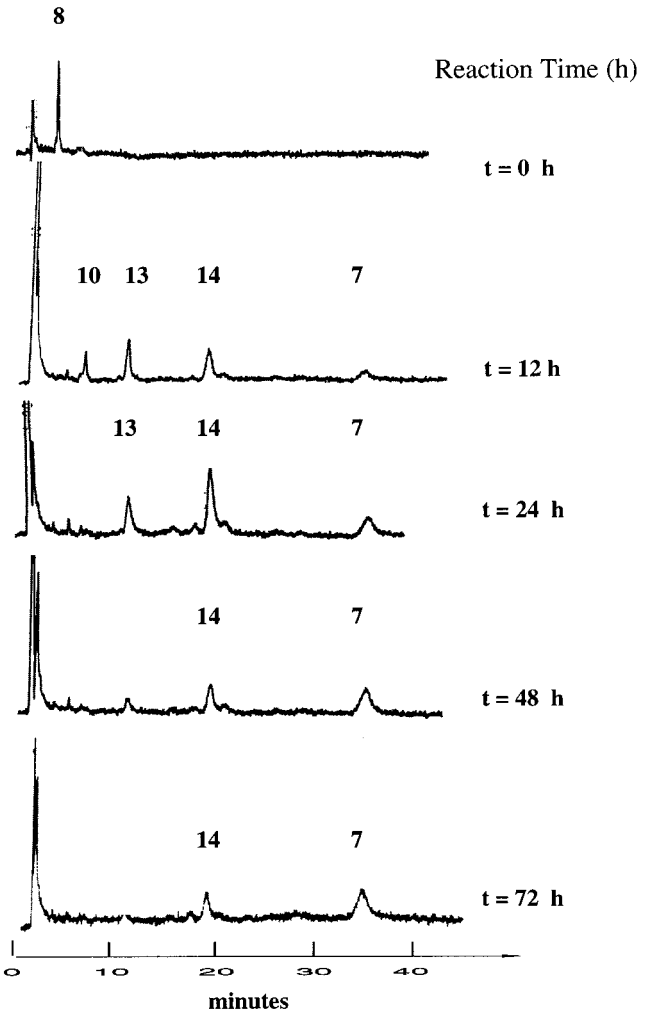


Figure 5. The time progress of the antibody 38C2-catalyzed ([IgG] = 5 μM) *retro*-aldol reaction of **8** (25 μM) in PBS buffer at 37 °C. The analysis was performed by HPLC (VYDAC reverse phase C_{18}-column, 55:45 acetonitrile/water (0.1% TFA), $\upsilon = 1.5$ mL/min, $\lambda_{abs} =$ 276 nm).

initial rates in a combined incubation ($S = \upsilon_A/\upsilon_B$) eq III. If [A] is the specific concentration of **4** and [B] is the specific concentration of **9**, their specificity ratio can be calculated. Using this equation a specificity ratio of $(k_{cat,app}/K_M)_{\mathbf{4}}/(k_{cat,\ app}/k_M)_{\mathbf{9}} =$ 1 between **4** and **9** was found.[27] Hence, antibody 38C2 did not show any significant discrimination within the same generation of substrates. However, antibody 38C2 may show a high stereoselectivity for the surface exposed aldols.[20b,28] The initial rate for **8** compared to the initial rate of **4** was 1.4 times lower and compared to the initial rate of **9** was 1.3 times higher. Inserting these initial rate ratios and the specific aldol concentrations of the respective compounds into eq III gives $(k_{cat,app}/K_M)_{\mathbf{4}}/(k_{cat,app}/K_M)_{\mathbf{8}} = 3$ and $(k_{cat,app}/K_M)_{\mathbf{9}}/(k_{cat,app}/K_M)_{\mathbf{8}} = 3$, which corresponds to a difference in activation energy ($\Delta\Delta G = RT \times \ln S$) of 0.7 kcal/mol between the two dendrimer generations at 37 °C.[27] Therefore, second-generation dendrimer **8** was a poorer substrate for antibody 38C2 than the first generation **4**. Taken as a whole these results suggest the following: (1) The specific concentration of surface aldol functionalities was important for the rate of reaction within the same dendrimer generation. (2) A decrease in specificity and rate for the higher dendrimer generation compared to the lower dendrimer generation was caused by steric crowding of surface aldol moieties.

An overall visualization of antibody catalysis with dendrimer

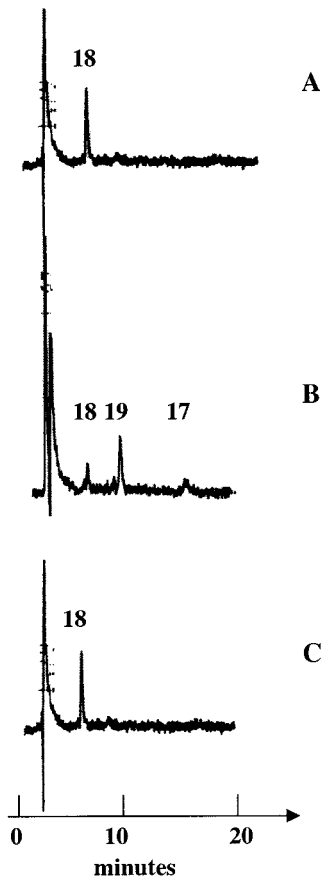
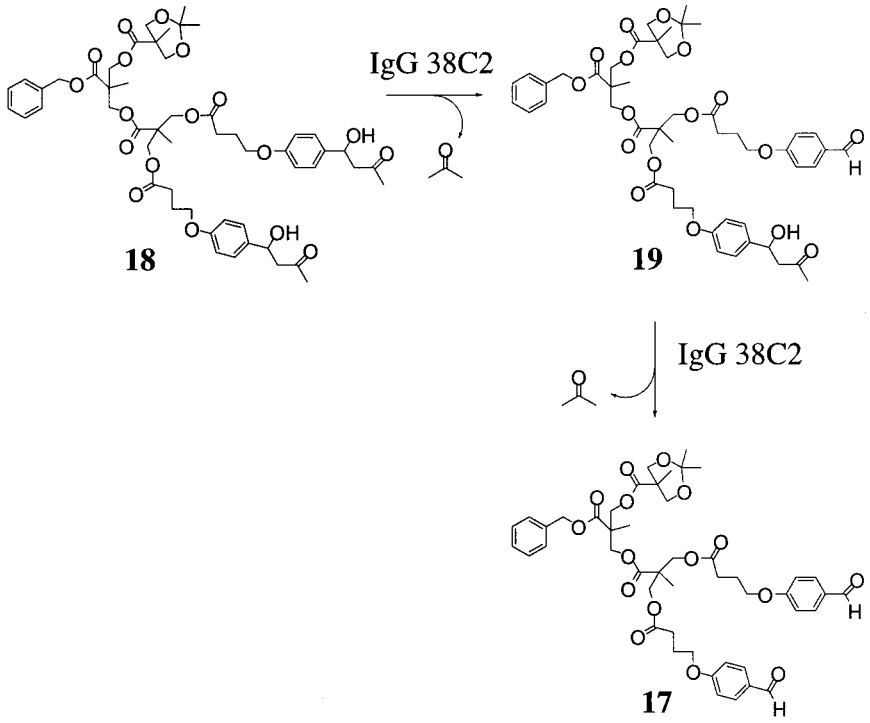


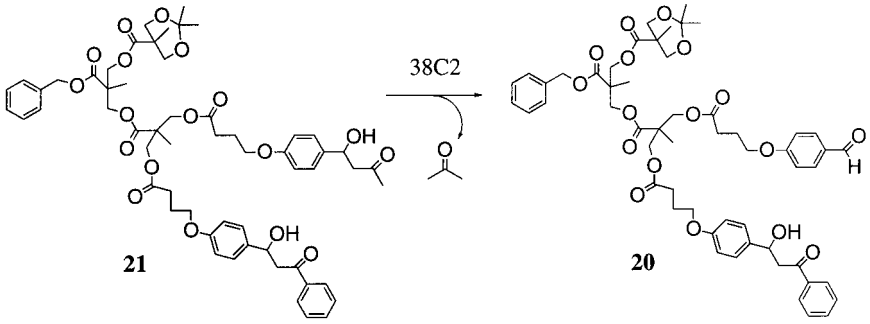
Figure 6. The product formation of the *retro*-aldol reaction of **18** (25 μM) in PBS buffer at 37 °C: (A) the starting point ($t = 0$ h); (B) antibody 38C2-catalyzed reaction ([IgG] = 1.0 μM) after 24 h; and (C) the uncatalyzed reaction after 24 h. The analysis was performed by HPLC (VYDAC reverse phase C_{18}-column, 55:45 acetonitrile/H_2O (0.1% TFA), $\upsilon = 1.5$ mL/min, $\lambda_{abs} = 276$ nm).

8 can be seen in Figure 4. Here the *retro*-aldolization of **8** is shown after 24 h of reaction time (PBS buffer, 37 °C). The HPLC trace A represents the uncatalyzed reaction. Clearly evident is that no significant amounts of product could be observed. In trace B, the antibody concentration was 0.4 μM and products **10**, [G#2]-dialdol (**13**), [G#2]-monoaldol (**14**) (see Figure 1), and **7** could be observed. When the antibody concentration was increased to 5 μM the *retro*-aldolization rate increased proportionally (trace C). However, increasing the antibody concentration to 10 μM did not significantly change the product distribution after 24 h. In diagram **D**, 3 equiv of pentan-2,4-dione were added and the catalytic activity of 38C2 was completely inhibited. An HPLC time progress of the *retro*-aldolization of **8** (25 μM), catalyzed by antibody 38C2 (5μM), at 37 °C is shown in Figure 5. Almost 90% of compound **7** was formed, based on starting compound **8**, within 80 h and the mechanism was sequential. However, complete conversion of **8** to **7** was never achieved and ~10% of **14** (Figure 1), was also present even at longer reaction times. Based on these findings we suggest that as the unmasking process progressed the acetone concentration in the reaction mixture increased and consequently shifted the equilibrium toward a favored aldol reaction. This change in equilibrium will be more important for higher dendrimer generations and reaching complete unmasking could be more difficult to achieve. However, a decrease in reaction rate could be turned into an advantage, especially since it allows the preparation of partially modified dendrons under milder and more selective conditions than chemical aldol

Scheme 6. *retro*-Aldol Reaction of Dendron **18**



Scheme 7. *retro*-Aldol Reaction of Dendron **21**



transformations. For example, in the case of the chemical synthesis of **10**, **13**, and **14** competing side reactions such as β-elimination and hydrolysis of the polyester core occurred. Furthermore, from the standpoint of synthetic applications the antibody-catalyzed *retro*-aldol reaction could be performed under biphasic conditions, thus avoiding solubility problems.[29]

Synthesis of Hybrid Peripheral Copolymer Dendrons. Making use of the versatility of convergent synthesis, hybrid dendrons displaying both the ketal and aldol functionalities were synthesized. With these hybrids, we wished to investigate a catalytic antibody's ability to selectively perform *retro*-aldol reactions on dendrimers containing a periphery of aldol product moieties that were not orthogonal to each other and thus more difficult to remove without a highly selective catalyst.

To examine such scenarios second-generation *block*-hybrid dendrons were synthesized as shown in Scheme 5. The monoesterification of diol **1** with acetonide protected bis-MPA (DCC, catalytic DMAP, CH_2Cl_2) produced the monocoupled product **15** (78%). Esterification of **15** using a slight excess of **5** (TEA, catalytic DMAP, CH_2Cl_2) gave the di(4-bromobutoroyl) derivatized dendron **16** (65%). Etherification of **16** with 4-hydroxybenzoic acid gave dialdehyde *block*-hybrid dendrimer **17** in 85% yield, and reaction of **17** with 8 equiv of the lithium enolate of acetone produced dialdol **18** (65%). Dialdehyde **17** was also reacted with 1.5 equiv of either the lithium enolates of acetone or acetophenone to produce **19** (50%) and **20** (55%), respectively. Monoaldol **20** was also reacted with the lithium enolate of acetone to produce monoacetophenone aldol-monoacetone aldol-*block*-hybrid dendron **21** in 45% yield. MALDI TOF MS analysis of **21** showed only a single peak corresponding to **21**+Na^+ (1077 Da). We observed that the hydrophobicity of the formed aldol adduct was an important factor. If the aldol moiety had a similar polarity to the benzaldehyde moiety on the hybrid dendrons separation problems would occur between starting dialdehyde dendron **17** and the aldol substituted products. In fact, this was the case with the *tert*-butyl group of the pinacolone aldol adduct and it was therefore not used in our studies.

Antibody Catalysis with *block*-Hybrid Dendrimer Substrates. Initial investigations were performed on the antibody-catalyzed *retro*-aldolization of dialdol **18** to determine if we could produce monoaldol **19** in higher conversion than the chemical aldol reaction route (Scheme 6). Dendron **19** could be important for the synthesis of a variety aldol-substituted dendrimers. In Figure 6, it is clearly seen that 38C2 efficiently converted **18** to **19** and a small amount of **17** within 24 h (trace

(29) Turner, J. M.; Bui, T.; Lerner, R. A.; Barbas, C. F., III; List, B. *Chem. Eur. J.* **2000**, *6*, 2772.

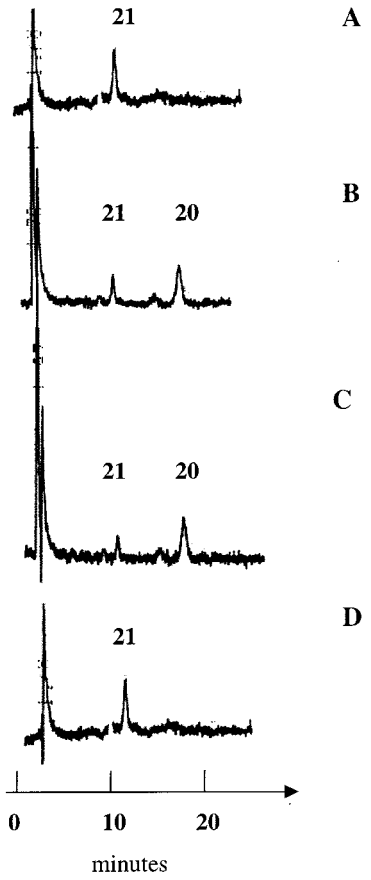
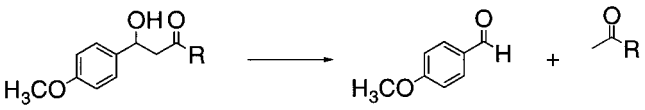


Figure 7. The product formation of the *retro*-aldol reaction of **21** (25 μM) in PBS buffer at 37 °C: (A) the starting point (t = 0 h); (B) antibody 38C2-catalyzed reaction ([IgG] = 1.0 μM) after 17 h; (C) antibody 38C2-catalyzed reaction ([IgG] = 1.0 μM) after 40 h; and (D) the uncatalyzed reaction after 40 h. The analysis was performed by HPLC (VYDAC reverse phase C_{18}-column, 55:45 acetonitrile/H_2O (0.1% TFA), v = 1.5 mL/min, λ_{abs} = 276 nm).

B). The substrate distribution was 6, 92, and 2% between **18**, **19**, and **17**, respectively. These results demonstrate that the antibody-catalyzed route was superior in producing **19** than the chemical reaction route, which gave **19** in 50% yield. The reaction without catalytic antibody 38C2 did not show any product formation during this time frame (trace C). Thereafter we wanted to explore whether catalytic antibody 38C2 displayed selectivity between the different aldol moieties as found on **21** (Scheme 7). In fact, the antibody was regiospecific in the removal of the acetone aldol adduct of **21** to exclusively produce **20** within 25 h (Figure 7, trace C) and no peaks corresponding to **19** could be observed. Noteworthy was the fact that the reaction mixture without 38C2 did not give any visible amounts of **20** within 40 h of reaction time (trace D). This then is the first demonstration where catalytic antibody 38C2 has been shown to display regioselectivity on a multifunctional aldol substrate. This is certainly an attractive feature and suggests that catalytic antibodies could be used as catalysts for regio- and chemoselective differentiation of dendrimers. These results can be rationalized based on the following monomer results. Thus, the uncatalyzed rate for the *retro*-aldolization of the monomer 1-phenyl-3-(4-methoxyphenyl)-3-hydroxypropan-2-one (**22**) was 10^2 times faster than the same transformation of **11** in PBS buffer (pH 7.4, [S] = 1 mM, 37 °C) (Scheme 8). Catalytic antibody 38C2 did not catalyze the conversion of **22** to **12**. Hence the results in Figure 7, clearly display the efficiency

Scheme 8. *retro*-Aldol Reaction of Monomer **11** and **22**



22 = Ph
11 = CH_3

12

of aldolase antibody 38C2 to catalyze the regioselective *retro*-aldol transformation.

Conclusion

A convenient methodology for synthesis of aldehyde-functionalized polyester dendrons was developed based on the monomer bis-MPA. Utilizing these molecules the first investigation in which a catalytic antibody catalyzes end-group functionalization of a dendrimer was accomplished. This antibody was able to catalyze the *retro*-aldolization of aldol modified polyester dendrons efficiently ($k_{cat}/k_{uncat} = 1 \times 10^6$) and in high conversion (90%) at 37 °C. While the dendrons employed in this study were limited in their solubility, changing the dendritic core to a more water-soluble one, would presumably allow the exploration of these processes under physiological conditions.

Methodologies were also developed for the synthesis of *block*-hybrid polyester dendrons with acetonide and different aldol peripheral end-groups. The antibody catalyzed the *retro*-aldol reactions regiospecifically and produced differentiated dendrons in high conversion (92%). This was also the first example where catalytic antibody 38C2 has been shown to display regioselectivity on a multifunctional aldol substrate. On the basis of these results, we envision antibodies catalyzing highly selective and mild surface modifications of biodegradable and biocompatible multifunctional polymeric substrates.

Experimental Section

General. Catalytic antibody 38C2 (M_w = 150 000 Da, 14.5 mg/mL in PBS buffer at pH 7.4) was obtained as previously described in ref 17a. All chemicals were purchased from Aldrich or Sigma and used without any further purification. All reactions requiring anhydrous conditions were performed in oven-dried glassware under Ar or N_2 atmosphere. Solvents were either used as supplied or purified by standard techniques. THF was distilled from sodium−benzophenone. Thin-layer chromatography (TLC) was performed on Merck 60 F_{254} silica gel plates, and compounds were visualized by irradiation with UV light and/or by treatment with a solution of 25 g of phosphomolybdic acid, 10 g of $Ce(SO_4)_2 \cdot H_2O$, 60 mL of concentrated H_2SO_4, and 940 mL of H_2O, followed by heating and/or staining with a solution of 12 g of 2,4-dinitrophenyl hydrazine in 60 mL of concentrated H_2SO_4, 80 mL of H_2O, and 200 mL of 95% EtOH. For flash chromatography (FC), Merck 60 silica gel (particle size 0.040−0.063 mm) was used; the eluent is given in parentheses. For ^{1}H NMR, Bruker AMX 500, AM 300, and AC 250 instruments were used. ^{13}NMR experiments were run with a Bruker AMX 500 (125 MHz) instrument. The chemical shifts are given in δ relative to TMS (δ = 0 ppm). The spectra were recorded in $CDCl_3$ at room temperature. High-resolution mass spectra (HRMS) were recorded on an Ion Spec Fourier Transform Mass Spectrometer using dihydroxybenzoic acid (DHB) as the matrix. Matrix Assisted Laser Desorption-Time-of-Flight mass spectra (MALDI-TOF MS) were recorded on a Voyager-DE Perspective Biosystems using DHB as the matrix.

Antibody-Catalyzed Reactions. All antibody-catalyzed reactions were performed in PBS (0.01 M phosphate buffer, 2.7 mM KCl, 137 mM NaCl, pH 7.4) and 10% DMSO. Antibody-catalyzed reactions and background reactions were monitored by high-pressure liquid chromatography (HPLC, Hitachi L-500 HPLC System) using a VYDAC reverse-phase C_{18} column and acetonitrile/water mixtures (containing

0.1% trifluoroacetic acid) as eluents at a flow rate of 1.5 mL/min and $\lambda_{abs} = 276$ nm. The enantioselectivity of the antibody 38C2-catalyzed *retro*-aldolization of **11** was determined by chiral HPLC (Diacel Chiral Pak AS column, 10:90 2-propanol:hexane, $\upsilon = 1.0$ mL/min, $\lambda_{abs} = 230$ nm) according to ref 20c.

Kinetics. Reaction 11→ 12: Substrate **11** (50 mM, in DMSO) was added to capped vials containing PBS buffer and DMSO. The reactions were then initialized by addition of the antibody 38C2 stock solution to obtain a final volume of 1 mL (10% (v/v) DMSO) and the desired substrate concentration at constant IgG concentration ([IgG] = 0.2 μM). The vials were left for different periods of time at 25 °C and samples were withdrawn from the reaction mixture and immediately analyzed by HPLC (20:80 acetonitrile:water (0.1% TFA), $\upsilon = 1.5$ mL/min) using 4-nitroacetophenone as standard. The uncatalyzed reaction rate was measured under the same conditions. The Michaelis−Menten parameters were determined by a Lineweaver−Burke plot of $1/V$ vs $1/[S]$ where the y-intercept is $1/V_{max}$ and the x-intercept is $-1/K_M$. k_{cat} was determined by dividing V_{max} with the two active sites of the IgG ($2 \times$ [IgG]).

Solubility limitations of the dendritic substrates and products precluded the use of a wide range of substrate concentrations to satisfy first-order kinetics, therefore the k_{cat} value was derived from the maximal velocity at the solubility limits of the dendrons (25 μM). Two independent active sites were assumed per IgG and the antibody concentration was kept constant at 0.2 μM. The buffer system and sample preparation were as described vide supra at 25 and 37 °C, respectively. HPLC assay conditions for compounds **3**, **4**, and **9**: 52:48 acetonitrile:water (0.1% TFA), $\upsilon = 1.5$ mL/min, using naphthalene as standard. HPLC assay conditions for compounds **8** and **10**: 53:47 acetonitrile:water (0.1% TFA), $\upsilon = 1.5$ mL/min, using triphenylene as standard.

Benzyl-2,2-bis(methylol)propionate (1). Bis-MPA (9.00 g, 67.1 mmol) and KOH (4.3 g, 76.8 mmol) were dissolved in DMF (50 mL). After the mixture was stirred at 100 °C for 1 h benzylbromide (13.8 g, 80.7 mmol) was added, and the reaction was stirred for an additional 15 h at 100 °C. The DMF was evaporated and the residue was dissolved in EtOAc (200 mL) and washed with water and brine. The organic phase was dried ($MgSO_4$), concentrated, and purified by column chromatography (50:40 hexane:EtOAc) to give **1** as white crystals: 9.0 g (60%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.08 (s, 3H, $-CH_3$), 3.74 (d, 2H, $-CH_2OR$), 3.95 (d, 2H, $-CH_2OR$), 5.22 (s, 2H, ArCH_2OR), 7.36 (m, 5H, ArH);^{13}C NMR ($CDCl_3$) δ 17.16, 49.35, 66.60, 66.93, 128.25, 128.57, 128.82, 135.81, 174.33. The NMR spectra are in accord with those in the literature.[14]

General Esterification Procedure: Preparation of [G#1]-Br$_2$ (2). 4-Bromobutyryl chloride (3.4 g, 18.3 mmol) was added dropwise to a solution of **1** (1.92 g, 8.6 mmol), TEA (2.22 g, 21.9 mmol), and DMAP (0.9 mmol) at 0 °C. The reaction was then warmed to room temperature and stirred for 24 h. The CH_2Cl_2 was evaporated and the dark brown residue was purified by liquid chromatography on silica gel (30:10 hexane:EtOAc) to give **2** as a colorless oil: 3.5 g (82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (s, 3H, $-CH_3$), 2.17 (m, 4H, $CH_2CH_2CH_2$), 2.51 (t, 4H, ROOCCH_2CH_2), 3.45 (t, 4H, CH_2CH_2Br), 4.25 (s, 4H, $-CH_2OR$), 5.16 (s, 2H, ArCH_2OR), 7.35 (s, 5H, ArH); ^{13}C NMR ($CDCl_3$) δ 18.11, 27.80, 32.49, 32.76, 46.65, 65.78, 67.19, 128.50, 128.70, 128.87, 135.80, 172.22, 172.73. HRMS calcd for $C_{20}H_{26}O_6Br_2$ (M + Na^+) 542.9988, found 542.9980.

General Alkylation Procedure: Preparation of [G#1]-Dialdehyde (3). 4-Hydroxybenzaldehyde (1.71 g, 14 mmol), K_2CO_3 (3.8 g, 21 mmol), NaI (0.5 g, 3.5 mmol), and **2** (3.42 g, 6.98 mmol) were dissolved in DMF (40 mL). The reaction mixture was heated to 75 °C and stirred for 24 h. The DMF was then removed under reduced pressure and the residue dissolved in EtOAc (50 mL) and extracted with water and brine. The organic phase was dried ($MgSO_4$), filtered, and purified by column chromatography (50:50 hexane: EtOAc) to give **3** as a clear viscous oil: 5.5 g (85%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.23 (s, 3H, $-CH_3$), 2.10 (m, 4H, $CH_2CH_2CH_2$), 2.51 (t, 4H, ROOCCH_2CH_2), 4.05 (t, 4H, CH_2CH_2OAr), 4.20 (s, 4H, $-CH_2OR$), 5.12 (s, 2H, ArCH_2OR), 6.96 (d, 4H, ArH), 7.33 (s, 5H, ArH), 7.81 (d, 4H, ArH), 9.87 (s, 4H, ArCHO); ^{13}C NMR ($CDCl_3$) δ 18.07, 24.54, 30.62, 46.68, 65.75, 67.11, 67.21, 115.01, 128.38, 128.65, 128.86, 130.31, 132.27, 135.79, 164.05, 172.66, 172.73, 191.05. HRMS calcd for $C_{34}H_{36}O_{10}$ (M + Na^+) 627.2201, found 627.2191.

General Aldol Formation Procedure: [G#1]-Dialdol (4). LDA solution (Aldrich, 2 M, heptane/THF/ethylbenzene, 4 mL, 8 mmol) was added dropwise to a stirred solution of acetone (0.6 mL, 8 mmol) in THF (15 mL) at −78 °C. After 30 min **3** (0.8 g, 1.3 mmol) in THF (5 mL) was added, and the reaction mixture was stirred for an additional 35 min at −78 °C. The reaction was then quenched with saturated ammonium chloride solution (3 mL) and extracted with EtOAc ($3 \times$ 20 mL). The organic layer was washed with water and brine, dried ($MgSO_4$), filtered, concentrated, and purified by column chromatography on silica gel (40:10 hexane:EtOAc) to give **4** as a colorless oil: 0.62 (65%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.25 (s, 3H, $-CH_3$), 2.01 (m, 4H, $CH_2CH_2CH_2$), 2.18 (s, 6H, $COCH_3$), 2.44 (t, 4H, ROOCCH_2-CH_2), 2.79 (m, 4H, CHOHCH_2CO), 3.93 (t, 4H, CH_2CH_2OAr), 4.25 (s, 4H, $-CH_2OR$), 5.06 (m, 2H, ArCHOHCH_2), 5.13 (s, 2H, ArCH_2-OR), 6.84 (d, 4H, ArH), 7.25 (d, 4H, ArH), 7.32 (s, 5H, ArH);^{13}C NMR ($CDCl_3$) δ 18.11, 24.78, 31.06, 46.66, 52.22, 65.75, 66.99. 67.10, 69.79, 114.79, 127.22, 128.44, 128.64, 128.87, 135.39, 135.86, 158.62, 172.82, 172.89, 209.36. HRMS calcd for $C_{40}H_{48}O_{12}$ (M + Na^+) 743.308, found 743.3037.

2,2-Bis(4-bromobutyroxymethyl)propionic Acid. 4-Bromobutyryl chloride (7 g, 39.4 mmol) was added dropwise to a mixture of Bis-MPA (2.5 g, 18.6 mmol), TEA (4.7 g, 46.5 mmol), and DMAP (0.12 g, 0.94 mmol) in CH_2Cl_2 (75 mL) at room temperature. After 40 min of stirring the reaction mixture was concentrated and EtOAc (50 mL) was added. The organic phase was washed with 1 M HCl, water, and brine, dried ($MgSO_4$), concentrated, and purified by column chromatography on silica gel (20:30 hexane:EtOAc) to give 2,2-bis(4-bromobutyroxymethyl)propionic acid as a colorless oil: 5.9 g (80%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.29 (s, 3H, $-CH_3$), 2.11 (m, 4H, $CH_2CH_2CH_2$Br), 2.52 (t, 4H, ROOC$H_2CH_2CH_2$), 3.45 (t, 4H, CH_2CH_2-Br), 4.25 (bs, 4H, $-CH_2OR$); ^{13}C NMR ($CDCl_3$) δ 18.07, 27.84, 32.53, 32.76, 46.41, 65.44, 172.30, 178.66. HRMS calcd for $C_{13}H_{20}Br_2O_6$ (M + Na^+) 452.9519, found 452.9525.

2,2-Bis(4-bromobutyroxymethyl)propionic Acid Chloride (5). To a solution of the above acid (3.78 g, 9.45 mmol) and 3 drops of DMF in CH_2Cl_2 (40 mL) was added oxalyl chloride (2.4 g, 18.9 mmol) dropwise with stirring for 2 h at 25 °C. The excess oxalyl chloride was removed on the rotary evaporator by a striping procedure with several portions of chloroform to give **5** as a yellow crude oil and was used without further purification: 3.93 g (99%).^{13}C NMR ($CDCl_3$) δ 18.11, 27.74, 32.43, 32.63, 44.10, 65.29, 172.14, 175.38.

[G#2]-Br$_4$ (6). 2,2-Bis(4-butyroxymethyl)propionic acid chloride **5** (2.27 g, 5.4 mmol, diluted in a small amount of CH_2Cl_2), benzyl-2,2-bis(methylol)propionate **2** (0.56 g, 2.5 mmol), DMAP (30 mg 0.13 mmol), and TEA (0.66 g, 6.5 mmol) were reacted according to the general esterification procedure for 48 h to give a slightly yellow product that was purified by column chromatography on silica gel (30:20 hexane/EtOAc) to give **6** as a colorless oil: 3.7 g (65%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.18 (s, 6H, $-CH_3$), 1.28 (s, 3H, $-CH_3$), 2.17 (m, 8H, $CH_2CH_2CH_2$), 2.51 (t, 8H, ROOCCH_2CH_2), 3.45 (t, 8H, CH_2-CH_2Br), 4.17 (bs, 8H, $-CH_2OR$), 4.28 (m, 4H, $-CH_2OR$), 5.16 (s, 2H, ArCH_2OR), 7.36 (s, 5H, ArH);^{13}C NMR ($CDCl_3$) δ 17.91, 18.04, 27.86, 32.48, 32.83, 46.68, 47.00, 65.51, 66.04, 67.46, 128.65, 128.85, 129.00, 135.62, 172.23, 172.37. HRMS calcd for $C_{38}H_{52}O_{14}Br_4$ (M + Na^+) 1049.0163, found 1049.0155.

[G#2]-Tetraaldehyde (7). 4-Hydroxybenzaldehyde (2.45 g, 20.1 mmol), [G#2]-Br$_4$ **6** (4.8 g, 4.6 mmol), K_2CO_3 (8.3 g, 60.3 mmol), and NaI (1.1 g, 7.3 mmol) were reacted according to the general alkylation procedure for 48 h to give a crude product that was purified by column chromatography (20:30 hexane:EtOAc) to give **7** as a colorless viscous oil: 4.8 g (85%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.15 (s, 6H, $-CH_3$), 1.23 (s, 3H, $-CH_3$), 2.10 (m, 8H, $CH_2CH_2CH_2$), 2.51 (t, 8H, ROOCCH_2-CH_2), 4.05 (t, 8H, CH_2CH_2OAr), 4.07 (bs, 8H, $-CH_2OR$), 4.20 (m, 4H, $-CH_3OR$), 5.12 (s, 2H, ArCH_2OR), 6.96 (d, 8H, ArH), 7.33 (s, 5H, ArH), 7.81 (d, 8H, ArH), 9.87 (s, 4H, ArCHO); ^{13}C NMR ($CDCl_3$) δ 17.82, 17.95, 24.54, 46.65, 46.91, 65.38, 65.91, 67.20, 67.39, 115.00, 128.56, 128.82, 128.96, 130.31, 132.24, 135.53, 164.03, 172.14, 172.22, 172.62, 191.00. HRMS calcd for $C_{66}H_{72}O_{22}$ (M + Na^+) 1239.4407, found 1239.4455.

[G#2]-Tetraaldol (8). Acetone (99 mg, 1.71 mmol), LDA solution (Aldrich, 2 M, heptane/THF/ethylbenzene, 0.86 mL, 1.71 mmol) and [G#2]-tetraaldehyde **7** (0.26 g 0.2 mmol) were reacted according to the general aldol formation procedure to give a crude product that was purified by column chromatography (10:80:5 hexane:EtOAc: MeOH) to give pure **8** as a colorless viscous oil: 0.2 g (65%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.14 (s, 6H, $-CH_3$), 1.22 (s, 3H, $-CH_3$), 2.10 (m, 8H, $CH_2CH_2CH_2$), 2.19 (s, 12H, $COCH_3$), 2.49 (t, 8H, $ROOCCH_2CH_2$), 2.79 (m, 8H, $CHOHCH_2CO$), 3.95 (t, 8H, CH_2CH_2OAr), 4.15 (bs, 8H, $-CH_2OR$), 4.21 (m, 4H, $-CH_2OR$), 5.06 (m, 4H, $ArCHOHCH_2$), 5.13 (s, 2H, $ArCH_2OR$), 6.83 (d, 8H, ArH), 7.24 (d, 8H, ArH), 7.33 (s, 5H, ArH);^{13}C NMR ($CDCl_3$) δ 17.73, 17.90, 24.70, 30.17, 30.98, 46.84, 52.19, 65.37, 65.88, 66.81, 67.32, 69.69, 114.68, 127.15, 128.54, 128.72, 128.89, 135.46, 135.53, 158.48, 172.17, 172.22, 172.80, 209.21. HRMS calcd for $C_{78}H_{96}O_{26}$ ($M + Na^+$) 1471.6082, found 1471.6098.

[G#1]-Monoaldol (9). To a solution of **3** (1.5 g, 1.23 mmol) in acetone (60 mL) was added 1% aqueous NaOH solution (6 mL) at 0 °C. After stirring at 0 °C for 10 min the solution was neutralized with 3 drops of 1 N HCl, and concentrated under reduced pressure. The residue was dissolved in H_2O (10 mL) and extracted with EtOAc (2 × 30 mL). The organic phase was washed with citric acid and brine, dried ($MgSO_4$), concentrated, and purified by column chromatography (10:20 hexane:EtOAc) to give **9** as a colorless viscous oil: 0.4 g (50%).^{1}H NMR (250 MHz, $CDCl_3$) δ 1.22 (s, 3H, $-CH_3$), 2.10 (m, 4H, $CH_2CH_2CH_2$), 2.19 (s, 3H, $COCH_3$), 2.49 (m, 4H, $ROOCCH_2CH_2$), 2.79 (m, 2H, $CHOHCH_2CO$), 3.94 (t, 2H, CH_2CH_2OAr), 4.05 (t, 2H, CH_2CH_2OAr), 4.21 (bs, 4H, $-CH_2OR$), 5.06 (m, 1H, $ArCHOHCH_2$), 5.13 (s, 2H, $ArCH_2OR$), 6.83 (d, 2H, ArH), 6.96 (d, 2H, ArH), 7.24 (d, 2H, ArH), 7.33 (s, 5H, ArH), 7.81 (d, 2H, ArH), 9.87 (s, 1H, ArCHO);^{13}C NMR ($CDCl_3$) δ 18.12, 24.57, 24.79, 30.65, 30.85, 31.08, 46.68, 52.23, 65.72, 66.90, 67.13, 67.25, 69.81, 114.80, 115.04, 127.25, 128.44, 128.67, 128.88, 130.31, 132.30, 135.42, 135.85, 158.60, 164.09, 172.70, 172.79, 172.91, 191.09, 209.36. HRMS calcd for $C_{37}H_{42}O_{11}$ ($M + Na^+$) 685.2619, found 685.2634.

[G#2]-Trialdol (10). To a solution of **7** (0.1 g, 82 μmol) in acetone (60 mL) was added 1% aqueous NaOH solution (6 mL) at 0 °C. After stirring at 0 °C for 10 min the solution was neutralized with 3 drops of 1 N HCl and concentrated under reduced pressure. The residue was dissolved in H_2O (5 mL) and extracted with EtOAc (2 × 30 mL). The organic phase was washed with citric acid and brine, dried ($MgSO_4$), concentrated, and purified by column chromatography (10:30 hexane: EtOAc) to give **9** as a colorless viscous oil: 34 mg (30%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.14 (s, 6H, $-CH_3$), 1.22 (s, 3H, $-CH_3$), 2.10 (m, 8H, $CH_2CH_2CH_2$), 2.19 (s, 9H, $COCH_3$), 2.49 (m, 8H, $ROOCCH_2CH_2$), 2.79 (m, 6H, $CHOHCH_2CO$), 3.94 (t, 6H, CH_2CH_2OAr), 4.05 (t, 2H, CH_2CH_2OAr), 4.15 (bs, 8H, $-CH_2OR$), 4.21 (m, 4H, $-CH_2OR$), 5.06 (m, 3H, $ArCHOHCH_2$), 5.13 (s, 2H, $ArCH_2OR$), 6.83 (d, 6H, ArH), 6.96 (d, 2H, ArH), 7.24 (d, 6H, ArH), 7.33 (s, 5H, ArH), 7.81 (d, 2H, ArH), 9.87 (s, 1H, ArCHO);^{13}C NMR ($CDCl_3$) δ 17.83, 18.00, 24.57, 24.79, 30.59, 30.79, 46.67, 46.93, 52.22, 65.43, 65.50, 65.99, 66.90, 67.27, 67.42, 69.80, 114.78, 115.04, 127.25, 128.61, 128.82, 128.98, 130.30, 132.28, 135.47, 135.60, 158.59, 164.09, 172.23, 172.26, 172.30, 172.67, 172.86, 191.08, 209.33. HRMS calcd for $C_{75}H_{90}O_{25}$ ($M + Na^+$) 1413.5663, found 1413.5608.

(4*R*,4*S*)-Hydroxy-4-(4′-methoxyphenyl)butan-2-one (11). Acetone (0.64 g, 11 mmol), LDA solution (Aldrich, 2 M, heptane/THF/ethylbenzene, 5.5 mL, 11 mmol), and 4-methoxybenzaldehyde **12** (0.5 g, 3.7 mmol) were reacted according to the general aldol formation procedure to give a crude product that was purified by column chromatography (55:45 hexane:EtOAc) to give pure **11** as a colorless oil: 0.5 g (70%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.19 (s, 3H), 2.86 (m, 2H), 3.81 (s, 3H), 5.10 (m, 1H), 6.88 (d, J = 8.8 Hz, 2H), 7.27 (d, J = 8.8 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 31.06, 52.24, 55.59, 69.81, 114.23, 127.21, 135.20, 159.45, 209.41. HRMS calcd for $C_{11}H_{14}O_3$ ($M + Na^+$) 217.0835, found 217.0844.

4-Methoxybenzaldehyde (12). 4-Hydroxybenzaldehyde (1.0 g, 8.2 mmol), K_2CO_3 (3.14 g, 12.3 mmol), and iodomethane (1.2 g, 8.2 mmol) were reacted according to the general etherification procedure to give a crude product that was purified by column chromatography (30:20 hexane:EtOAc) to give pure **12** as a white solid: 1.1 g (97%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.9 (s, 3H), 7.01 (d, J = 8.8 Hz, 2H), 7.85 (d, J = 8.8 Hz, 2H), 9.90 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 55.85, 114.59, 130.24, 132.27, 164.91, 191.10.

[G#1.5]-Monoacetonide (15). DCC was added to a solution of **1** (2.62 g, 11.6 mmol), acetonide protected bis-MPA (2.85 g, 16 mmol), and DMAP (0.5 g, 4.1 mmol) in CH_2Cl_2 at room temperature and the reaction mixture was stirred for 24 h under argon atmosphere. The DCC-urea was filtered off and the crude product was purified by column chromatography (50:50 hexane:EtOAc) to give **15** as a clear oil: (78%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.07 (s, 3H, $-CH_3$), 1.24 (s, 3H, $-CH_3$), 1.36 (s, 3H, $-CH_3$), 1.42 (s, 3H, $-CH_3$), 2.75 (t, H, $-OH$), 3.56 (d, 2H, $-CH_2OR$), 3.64 (d, 2H, $-CH_2OH$), 4.41 (dd, 2H, $-CH_2OR$), 5.17 (s, 2H, $ArCH_2OR$), 7.35 (s, 5H, ArH).^{13}C NMR ($CDCl_3$) δ 10.5, 11.2, 14.3, 18.8, 35.1, 41.4, 58.1, 59.0, 59.1, 59.2, 59.6, 91.1, 120.9, 121.3, 121.5, 128.5, 166.9, 167.3. HRMS calcd for $C_{20}H_{28}O_7$ ($M + Na^+$) 403.1727, found 403.1738.

[G#2]-Monoacetonide-Br$_2$ (16). 2,2-Bis(4-butyroxymethyl)propionic acid chloride **5** (2.94 g, 7.0 mmol, diluted in a small amount of CH_2Cl_2), acetonide protected **15** (2.48 g, 6.54 mmol), DMAP (30 mg 0.13 mmol), and TEA (0.79 g, 7.8 mmol) were reacted according to the general esterification procedure for 24 h to give a slightly yellow product that was purified by column chromatography on silica gel (40: 20 hexane/EtOAc) to give **16** as a colorless oil: 3.7 g (65%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.08 (s, 3H, $-CH_3$), 1.18 (s, 3H, $-CH_3$), 1.30 (s, 3H, $-CH_3$), 1.34 (s, 3H, $-CH_3$), 1.41 (s, 3H, $-CH_3$), 2.15 (m, 4H, $CH_2CH_2CH_2$), 2.50 (t, 4H, $ROOCCH_2CH_2$), 3.45 (t, 6H, CH_2CH_2Br), 3.56 (d, 2H, $-CH_2OR$), 4.08 (d, 2H, $-CH_2OR$), 4.13 (s, 4H, $-CH_2OR$), 4.31 (d, 4H, $-CH_2OR$), 5.16 (s, 2H, $ArCH_2OR$), 7.35 (s, 5H, ArH). ^{13}C NMR ($CDCl_3$) δ 18.07, 18.1, 18.8, 22.3, 25.7, 28.0, 32.6, 32.9, 42.4, 46.8, 47.2, 65.5, 65.6, 66.3, 67.4, 98.5, 128.7, 128.9, 129.0, 135.8, 167.8, 172.3, 172.5, 173.9. HRMS calcd for $C_{33}H_{46}O_{12}Br_2$ ($M + Na^+$) 815.1248, found 815.1233.

[G#2]-Monoacetonide-Dialdehyde (17). 4-Hydroxybenzaldehyde (0.5 g, 4.2 mmol), [G#2]-monoacetonide-Br$_2$ **16** (1.7 g, 2.1 mmol), K_2CO_3 (0.9 g, 6.3 mmol), and NaI (0.2 g, 1.1 mmol) were reacted according to the general alkylation procedure for 28 h to give a crude product that was purified by column chromatography (30:20 hexane: EtOAc) to give **17** as a colorless viscous oil: 1.6 g (85%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.06 (s, 3H, $-CH_3$), 1.17 (s, 3H, $-CH_3$), 1.27 (s, 3H, $-CH_3$), 1.33 (s, 3H, $-CH_3$), 1.40 (s, 3H, $-CH_3$), 2.07 (m, 4H, $CH_2CH_2CH_2$), 2.50 (t, 4H, $ROOCCH_2CH_2$), 3.56 (d, 2H, $-CH_2OR$), 4.07 (m, 6H), 4.19 (s, 4H, $-CH_2OR$), 4.31 (bs, 4H, $-CH_2OR$), 5.15 (s, 2H, $ArCH_2OR$), 6.96 (d, 4H, ArH), 7.34 (s, 5H, ArH), 7.81 (d, 4H, ArH), 9.87, (s, 2H, ArCHO).^{13}C NMR ($CDCl_3$) δ 17.9, 18.0, 18.7, 22.1, 24.6, 25.6, 30.6, 42.3, 46.7, 47.0, 65.4, 65.5, 66.1, 66.2, 67.2, 67.3, 98.4, 115.0, 128.5, 128.8, 128.9, 130.3, 132.3, 135.7, 163.9, 172.2, 172.7, 191.0. HRMS calcd for $C_{47}H_{56}O_{16}$ ($M + Na^+$) 899.3461, found 899.3471.

[G#2]-Monoacetonide-Diacetonealdol (18). Acetone (87 mg, 1.5 mmol), LDA solution (Aldrich, 2 M, heptane/THF/ethylbenzene, 0.75 mL, 1.5 mmol), and [G#2]-monoacetonide-dialdehyde **17** (0.3 g 0.3 mmol) were reacted according to the general aldol formation procedure to give a crude product that was purified by column chromatography (10:30 hexane:EtOAc) to give pure **18** as a colorless viscous oil: 0.2 g (65%).^{1}H NMR (250 MHz, $CDCl_3$) δ 1.06 (s, 3H, $-CH_3$), 1.15 (s, 3H, $-CH_3$), 1.26 (s, 3H, $-CH_3$), 1.33 (s, 3H, $-CH_3$), 1.39 (s, 3H, $-CH_3$), 2.07 (m, 4H, $CH_2CH_2CH_2$), 2.18 (s, 6H, $COCH_3$), 2.50 (t, 4H, $ROOCCH_2CH_2$), 2.79 (m, 4H, $CHOHCH_2CO$), 3.21 (s, 2H, $-OH$) 3.56 (d, 2H, $-CH_2OR$), 3.95 (t, 4H, CH_2CH_2OAr), 4.07 (d, 2H, $-CH_2OR$), 4.19 (s, 4H, $-CH_2OR$), 4.31 (s, 4H, $-CH_2OR$), 5.06 (m, 2H, $ArCHOHCH_2$), 5.14 (s, 2H, $ArCH_2OR$), 6.83 (d, 4H, ArH), 7.24 (d, 2H, ArH), 7.33 (s, 5H, ArH). ^{13}C NMR ($CDCl_3$) δ 17.9, 18.0, 18.7, 22.3, 24.8, 25.4, 30.7, 31.0, 42.3, 46.6, 47.0, 52.2, 65.5, 66.1, 66.2, 66.9, 67.3, 69.8, 98.4, 114.8, 127.2, 128.5, 128.7, 128.9, 135.4, 135.7, 158.6, 172.3, 172.4, 172.8, 173.8, 209.2. HRMS calcd for $C_{53}H_{68}O_{18}$ ($M + Na^+$) 1015.4298, found 1015.4316.

[G#2]-Monoacetonide-Monoacetone Aldol (19). Acetone (35 mg, 0.6 mmol), LDA solution (Aldrich, 2 M, heptane/THF/ethylbenzene, 0.30 mL, 0.6 mmol), and [G#2]-monoacetonide-dialdehyde **17** (106 mg, 0.12 mmol) were reacted according to the general aldol formation procedure to give a crude product that was purified by column chromatography (20:30 hexane:EtOAc) to give pure **19** as a colorless

viscous oil: 56 mg (50%).^{1}H NMR (250 MHz, $CDCl_3$) δ 1.06 (s, 3H, $-CH_3$), 1.16 (s, 3H, $-CH_3$), 1.27 (s, 3H, $-CH_3$), 1.33 (s, 3H, $-CH_3$), 1.40 (s, 3H, $-CH_3$), 2.07 (m, 4H, $CH_2CH_2CH_2$), 2.18 (s, 3H, $COCH_3$), 2.50 (m, 4H, $ROOCCH_2CH_2$), 2.79 (m, 2H, $CHOHCH_2CO$), 3.21 (s, 1H, $-OH$), 3.56 (d, 2H, $-CH_2OR$), 3.95 (t, 2H, CH_2CH_2OAr), 4.07 (m, 4H), 4.19 (s, 4H, $-CH_2OR$), 4.31 (bs, 4H, $-CH_2OR$), 5.06 (m, 1H, $ArCHOHCH_2$), 5.15 (s, 2H, $ArCH_2OR$), 6.83 (d, 2H, ArH), 6.96 (d, 2H, ArH), 7.24 (d, 2H, ArH), 7.33 (s, 5H, ArH), 7.81 (d, 2H, 2H), 9.87 (s, 1H, ArCHO).^{13}C NMR ($CDCl_3$) δ 17.9, 18.0, 18.7, 22.3, 24.8, 25.6, 30.7, 31.1, 42.9, 46.7, 47.0, 52.2, 65.5, 66.1, 66.9, 67.3, 69.8, 98.4, 114.8, 115.0, 127.2, 128.5, 128.7, 128.8, 128.9, 132.3, 158.6, 172.3, 172.4, 172.7, 172.8, 191.1, 209.2. HRMS calcd for $C_{50}H_{62}O_{17}$ ($M + Na^+$) 957.3879, found 957.3873.

[G#2]-Monoacetonide-Monoacetophenone Aldol (20). Acetophenone (33.8 mg, 0.3 mmol), LDA solution (Aldrich, 2 M, heptane/THF/ethylbenzene, 0.19 mL, 0.3 mmol), and [G#2]-monoacetonide-dialdehyde **17** (164 mg 0.2 mmol) were reacted according to the general aldol formation procedure to give a crude product that was purified by column chromatography (20:30 hexane:EtOAc) to give pure **20** as a colorless viscous oil: 108 mg (55%).^{1}H NMR (250 MHz, $CDCl_3$) δ 1.06 (s, 3H, $-CH_3$), 1.17 (s, 3H, $-CH_3$), 1.27 (s, 3H, $-CH_3$), 1.33 (s, 3H, $-CH_3$), 1.40 (s, 3H, $-CH_3$), 2.07 (m, 4H, $CH_2CH_2CH_2$), 2.50 (t, 4H, $ROOCCH_2CH_2$), 3.34 (m, 2H, $CHOHCH_2CO$), 3.56 (d, 3H, $-CH_2OR$ and $-OH$), 3.96 (t, 2H, CH_2CH_2OAr), 4.07 (t, 2H, CH_2CH_2OAr), 4.08 (d, 2H, $-CH_2OR$), 4.18 (s, 4H, $-CH_2OR$), 4.30 (s, 4H, $-CH_2OR$), 5.14 (s, 2H, $ArCH_2OR$), 5.29 (m, 1H, $ArCHOHCH_2$), 6.83 (d, 2H, ArH), 6.98 (d, 2H, ArH), 7.34 (m, 7H, ArH), 7.44 (m, 2H, ArH), 7.50 (m, 1H, ArH), 7.91 (d, 2H, ArH), 7.95 (d, 2H, ArH), 9.86 (s, 1H, ArH). HRMS calcd for $C_{55}H_{64}O_{17}$ ($M + Na^+$) 1019.4035, found 1019.4072.

[G#2]-Monoacetonide-Monoacetone Aldol-Monoacetophenone Aldol (21). Acetone (15.6 mg, 0.03 mmol), LDA solution (Aldrich, 2 M, heptane/THF/ethylbenzene, 0.13 mL, 0.03 mmol), and [G#2]-monoacetonide-monoacetophenonealdol **20** (13 mg, 0.01 mmol) were reacted according to the general aldol formation procedure to give a crude product that was purified by column chromatography (10:20 hexane:EtOAc) to give pure **21** as a colorless viscous oil: 6.3 mg (45%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.07 (s, 3H, $-CH_3$), 1.16 (s, 3H, $-CH_3$), 1.27 (s, 3H, $-CH_3$), 1.33 (s, 3H, $-CH_3$), 1.40 (s, 3H, $-CH_3$), 2.07 (m, 4H, $CH_2CH_2CH_2$), 2.19 (s, 3H, $COCH_3$), 2.50 (t, 4H, $ROOCCH_2CH_2$), 2.79 (m, 2H, $CHOHCH_2CO$), 3.21 (s, 1H, $-OH$), 3.34 (m, 2H, $CHOHCH_2CO$), 3.56 (d, 3H, $-CH_2OR$ and $-OH$), 3.96 (m, 2H, CH_2CH_2OAr), 4.08 (d, 2H, $-CH_2OR$), 4.18 (s, 4H, $-CH_2OR$), 4.30 (s, 4H, $-CH_2OR$), 5.06 (m, 1H, $ArCHOHCH_2$), 5.15 (s, 2H, $ArCH_2OR$), 5.29 (m, 1H, $ArCHOHCH_2$), 6.83 (d, 4H, ArH), 6.84 (d, 4H, ArH), 7.24 (d, 2H, ArH), 7.34 (m, 7H, ArH), 7.44 (m, 2H, ArH), 7.50 (m, 1H, ArH), 7.91 (d, 2H, ArH). ^{13}C NMR ($CDCl_3$) δ 17.9, 18.0, 18.7, 22.3, 24.8, 25.5, 26.4, 30.8, 31.8, 42.3, 46.7, 47.1, 47.6, 52.2, 65.5, 66.1, 66.2, 66.9, 67.3, 69.8, 70.0, 98.4, 114.8, 127.2, 127.3, 128.5, 128.6, 128.8, 128.9, 129.0, 133.9, 135.4, 135.6, 135.7, 137.0, 158.6, 172.3, 172.5, 172.9, 173.4, 200.5, 209.3. HRMS calcd for $C_{58}H_{70}O_{18}$ ($M + Na^+$) 1077.4454, found 1077.4489.

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Supporting Information Available: Tabulated data for Figures 2 and 3b; Figure S1 showing the proton/carbon NMR spectra of compound **7**, **8**, **17**, and **21** and MALDI TOF MS spectra of **7**, **8**, and **21**, respectively (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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