Controlled Stepwise Release of Fragrance Alcohols from Dendrimer-Based 2-Carbamoylbenzoates by Neighbouring Group Participation

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Dedicated to Prof. Daniel Uguen on the occasion of his 60th birthday

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2-Carbamoylbenzoates which are chemically bound to the surface of dendrimers were found to release tertiary fragrance alcohols by neighbouring group assisted alkaline hydrolysis under mild reaction conditions. Owing to the excellent separation of the intermediate reaction products by analytical HPLC, the kinetic rate constants of the first two consecutive reaction steps of fragrance release could be determined for a series of modified dendrimers with increasing size. Because of the intramolecular neighbouring group effect, the kinetic rate constants were found to be independent of the dendrimer generation and are not influenced by steric effects on the surface of the macromolecules.

Introduction

Due to the large variety of possible core structures and the availability of many different branching units and surface groups, parameters such as the size, shape, topology, solubility, and viscosity of dendrimers can be modified to prepare tailor-made macromolecules for a series of practical applications.[1] The encapsulation and release of biologically active materials, either by molecular recognition of a suitably designed central core, by interactions with the branching units or by selective adsorption at the dendrimer surface have been investigated.[1,2] In their pioneering work Meijer and co-workers have reported the release of small molecules from the inside of functionalised poly(propyleneimine) dendrimers by slow diffusion to the exterior after chemical removal of the outer dendrimer shell.[3] However, the possibility to use a chemical reaction to directly release active molecules which are bound to the dendrimer surface has been less investigated.[4,5] Chemical delivery systems based on the release of active compounds by cleavage of a chemical bond of a precursor molecule are an interesting alternative to encapsulation techniques and are successfully used to prolong the effect of odour perception in a broad variety of consumer products in the flavour and fragrance industry.[6] Due to their micellar structures and high molecular weight, dendrimers may be particularly suitable carriers for the transport and deposition of fragrances on different types of surfaces. We now describe the use of dendrimers as chemical delivery systems for the controlled release of fragrance alcohols by neighbouring group assisted[7] alkaline hydrolysis of 2-carbamoybenzoates at the dendrimer surface.[8]

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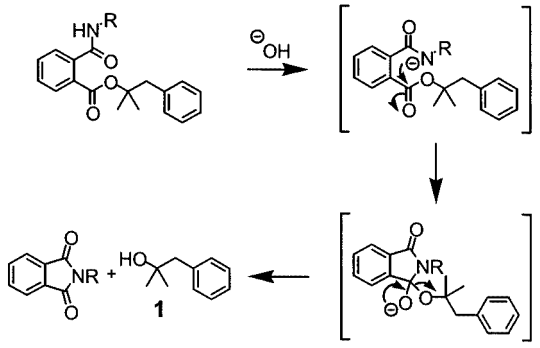
Results and Discussion

In contrast to 2-acylbenzoates, which liberate secondary or tertiary alcohols only very slowly,[9] we found that 2-carbamoylbenzoates release primary, secondary and even tertiary fragrance alcohols in aqueous neutral or alkaline media at rate constants sufficient to allow their practical use in functional perfumery.[10] The release principle is based on proton abstraction from the carbamoyl moiety by the basic buffer solution to form an intermediate nucleophilic species which then intramolecularly attacks the carbonyl group of the neighbouring ester function to release the desired alcohol (Scheme 1).[11] This intramolecular reaction is triggered

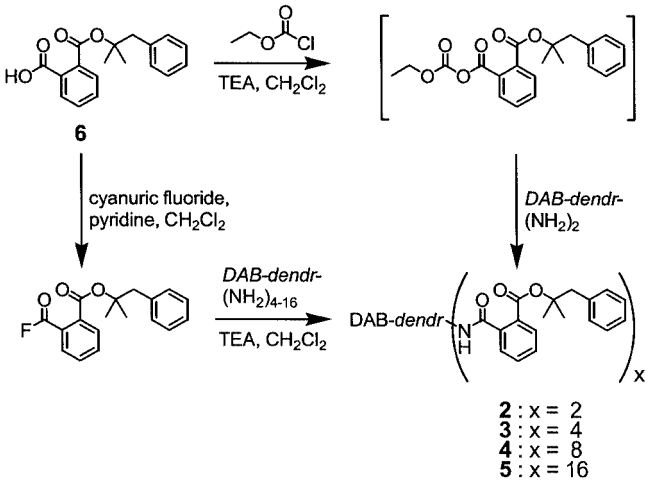


Scheme 1. Neighbouring group assisted cyclisation of 2-carbamoylbenzoates for the release of tertiary fragrance alcohol **1**

by a simple change of pH from acidic to neutral or slightly alkaline conditions and does not require the presence of further reagents that may limit the use of these systems in practical applications. Since the formation of tertiary alcohols in a chemical reaction is generally quite difficult, we decided to investigate the rate constants for the release of 1,1-dimethyl-2-phenylethanol (**1**), an example of a tertiary fragrance alcohol.

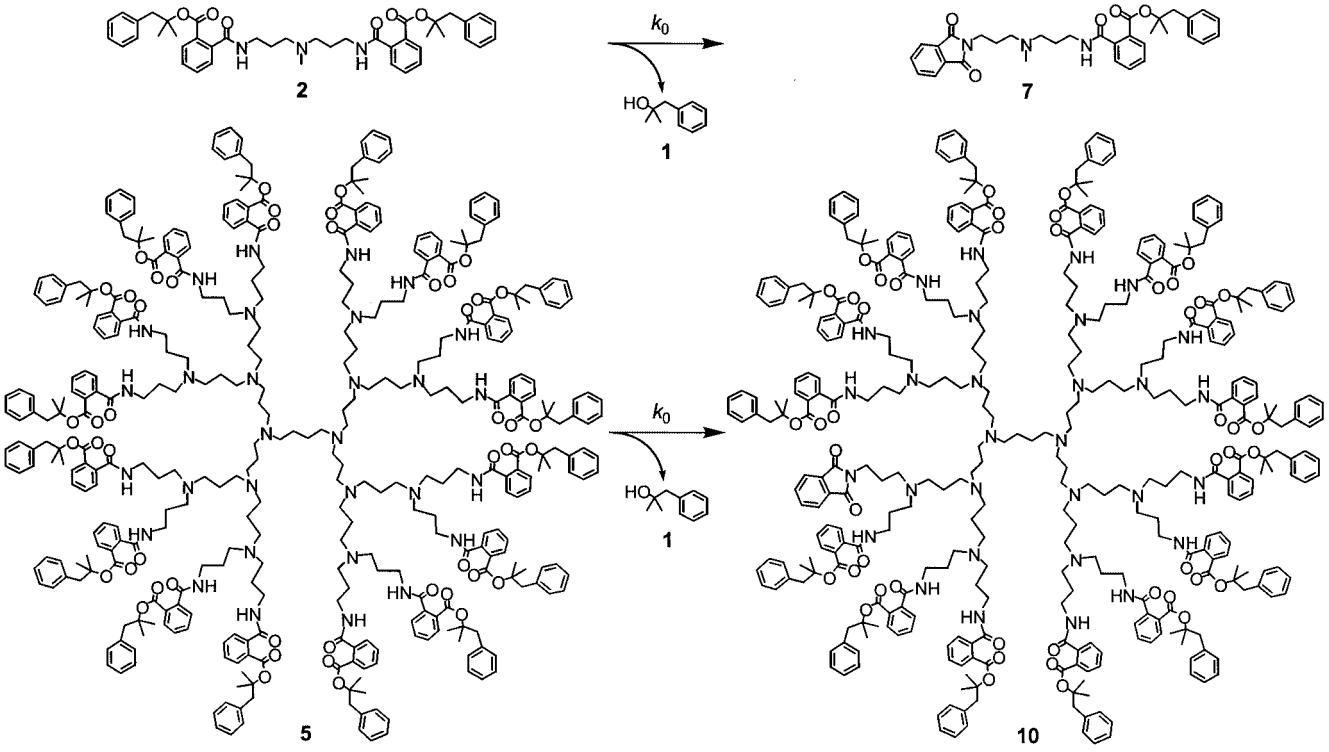
Modified dendrimers **2**−**5** with 2, 4, 8 and 16 2-carbamoylbenzoate end-groups were prepared by reaction of (1,1-dimethyl-2-phenylethyl) hydrogen phthalate (**6**) with the corresponding commercially available poly(propyleneimine) dendrimers [DAB-*dendr*-$(NH_2)_x$, with x = 2, 4, 8 and 16, respectively] in the presence of triethylamine (TEA) as base.

Whereas the activation of the acid functionality of **6** with ethyl chloroformate and addition of *N*,*N*-bis(3-aminopropyl)methylamine gave precursor **2** in 97% yield, this reaction was less successful for the preparation of the larger structures. Crude precursors **3**−**5** were thus prepared from **6** via the corresponding 2-(fluorocarbonyl)benzoate according to the procedure of Carpino et al.,[12] followed by addition of the dendrimers (Scheme 2). Pure modified dendrimers **3**−**5** were finally obtained by reversed-phase medium-pressure liquid chromatography (MPLC). Reversed-phase chromatography was found to be an extremely powerful tool for the separation of the pure dendrimers **2**−**5** from monocyclised intermediates **7**−**10**. Figure 1 shows the high performance liquid chromatography (HPLC) baseline separation of monocyclised dendrimer **10** (M_w = 6020 Da) together with its non-cyclised precursor **5** (M_w = 6170 Da), as shown by ESI-MS analysis. With the mass difference of the two compounds corresponding to 150 g mol^{-1}, **10** was assigned to be the result of the release of one molecule of **1** from **5** (Scheme 3).



Scheme 2. Preparation of dendritic 2-carbamoylbenzoates **2**−**5** from (1,1-dimethyl-2-phenylethyl) hydrogen phthalate (**6**)

This efficient separation allowed the precise determination of the individual rate constants for consecutive cyclisation steps by repetitive analytical HPLC. Due to solubility reasons, the release experiments were carried out in buffered solutions of water/acetonitrile (2:1) at 20 °C and, in view of the targeted perfumery applications, 1% by weight of a non-



Scheme 3. Schematic representation of the first step in the neighbouring group assisted release of alcohol **1** from precursors **2** and **5**

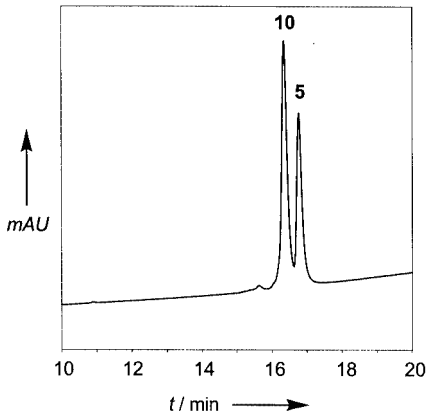


Figure 1. Analytical HPLC trace (conditions see Exp. Sect.) obtained for the co-injection of two pure preparative MPLC fractions, containing monocylised dendrimer **10** and its noncyclised precursor **5**, respectively

ionic surfactant was added.[13] The reaction progress was monitored by HPLC at constant time intervals. The formation of alcohol **1** was verified by LC/MS analysis.[14] Since the hydroxide concentration was held constant by the buffer, the second-order rate expression $r = k_2$ [OH^-] [precursor] can be reduced to the first order expression $r = k_0$ [precursor].[9] Plotting the logarithm of the decreasing peak-area quotients (A_t/A_0) of the precursors against time gave k_0 as the slope of the straight lines with good correlation coefficients ($r^2 > 0.99$) for all the measurements, thus justifying the general assumption of first order kinetics.

The rate constants k_0' for the second cyclisation step were determined by correlating the experimental peak-areas for the monocyclised species (B) with those calculated for consecutive first-order reactions by iteration of k_0' in Equation (1) (with A_0 and B_0 being the peak areas measured at $t = 0$).[15] For **3**−**5** further cyclisation results in the formation of isomers, and the quantitative determination of rate constants for consecutive parallel reactions is more complicated. The rate constants k_0 and k_0' obtained for the first and second cyclisation steps of modified dendrimers **2**−**5** and their monocyclised analogues **7**−**10**, respectively, at equimolar precursor concentration or at constant 2-carbamoylbenzoate group concentration are summarised in Table 1.

$$\mathrm{A} \xrightarrow{k_0} \mathrm{B} \xrightarrow{k_0'} \mathrm{C}$$

2–5 **7–10**

1st consecutive order reaction

$$\mathrm{B} = \frac{k_0\,\mathrm{A}_0}{k_0' - k_0}\,e^{-k_0\,\Delta t} + \frac{k_0'\,\mathrm{B}_0 - k_0\,(\mathrm{A}_0 + \mathrm{B}_0)}{k_0' - k_0}\,e^{-k_0'\,\Delta t} \quad (1)$$

Comparable k_0 and k_0' values were measured for the first and second cyclisation steps (Table 1). Please note that the kinetic values k_0, k_0' and $t_{1/2}$ reported in Table 1 reflect the disappearance of the dendritic precursors resulting from one cyclisation step and not the appearance of the released fragrance alcohol **1**. At equimolar precursor concentrations, the rate constants for each cyclisation step increase (and the related $t_{1/2}$ values decrease) exponentially with increasing dendrimer size, and plotting the logarithm of $t_{1/2}$ (or log k_0) against the number of end-groups gave a straight line. At constant 2-carbamoylbenzoate group concentration, a linear relationship was obtained by correlating $t_{1/2}$ (or k_0^{-1}) with the number of end-groups (Figure 2). The slope of the line in Figure 2 being close to −1 (−1.19) therefore indicates that, in contrast to previous reports,[5] the rate of alcohol release is almost independent of the size of the dendrimer. Assuming that all consecutive cyclisation steps occur at comparable rate constants, a horizontal line would be expected by plotting $t_{1/2}$ for the alcohol formation against the number of dendrimer end-groups. This means that the same amount of released alcohol would be obtained at a given time from dendrimers **2**−**5** at constant end-group concentration.

The non-dependence of the alcohol release on the dendrimer size may be explained by the fact that the present

Table 1. Rate constants k_0, k_0' and half-life times $t_{1/2}$ for the first two steps of the alkaline hydrolysis of dendrimer based 2-carbamoylbenzoates **2**−**5** in a buffered solution of water/acetonitrile 2:1 (pH 7.62) in the presence of 1% by weight of non-ionic surfactant at 20 °C; average values of 2−4 measurements

Structure No.	No. of end-groups	Constant molecular concentration $c_{mol} = 4.3 \times 10^{-4}$ mol L^{-1}		Constant end-group concentration $c_{eg} = 8.6 \times 10^{-4}$ mol L^1	
1st step		k_0 [s^{-1}]	$t_{1/2}$ [h]	k_0 [s^{-1}]	$t_{1/2}$ [h]
2	2	1.05×10^{-5}	18.3	1.05×10^{-5}	18.3
3	4	1.11×10^{-5}	17.3	1.20×10^{-5}	16.1
4	8	3.49×10^{-5}	5.5	1.71×10^{-5}	11.1
5	16	1.72×10^{-4}	1.1	1.23×10^{-4}	1.6
2nd step		k_0' [s^{-1}]	$t_{1/2}$ [h]	k_0' [s^{-1}]	$t_{1/2}$ [h]
7	1	n.d.[a]		n.d.[a]	
8	3	1.22×10^{-5}	15.8	1.22×10^{-5}	15.8
9	7	2.79×10^{-5}	6.9	1.70×10^{-5}	11.4
10	15	2.35×10^{-4}	0.9	1.09×10^{-4}	1.8

[a] Not determined due to co-elution of **7** with the surfactant.

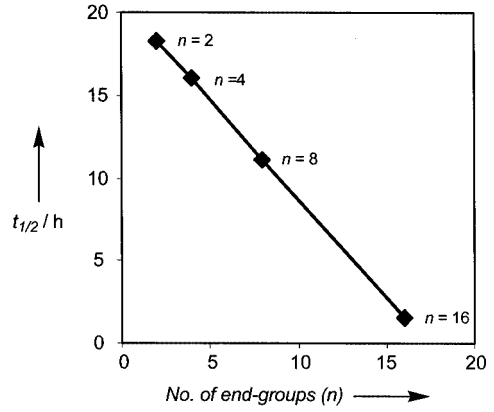


Figure 2. Half-life times at constant end-group concentration determined for the monocyclisation of dendritic 2-carbamoylbenzoates **2**−**5** with 2, 4, 8 and 16 end-groups, respectively

delivery system is based on an intramolecular reaction involving two groups which are located on the same branch of the dendrimer. Each dendrimer branch may thus be considered as reacting individually rather than would be the case for species attached to different dendrimer branches. The latter situation, as well as intermolecular reactions, would give rise to steric effects at the dendrimer surface and thus result in an increase of the rate constants.

An important advantage of the chemical release of active material from the dendrimer surface as compared to encapsulation techniques is that a higher loading of active compound per precursor molecule can be achieved in the former case. The ratio of releasable active compound to total mass of the delivery system for precursors **2**−**5** is about 0.4, independent of the generation number. Encapsulating systems, such as the dendritic box of Meijer and coworkers,[3] require higher molecular weight structures so as to obtain a dense shell at the dendrimer surface. Even a large dendrimer with 64 end-groups can only take up about 16 molecules of **1**, which would correspond to a mass ratio of 0.1. This favours the use of the chemical delivery system in all cases in which the delivered material is less expensive than the precursor support. Due to their different physicochemical properties at constant alcohol release, delivery systems **2**−**5** may thus be chosen according to the specific requirements of different applications.

Conclusion

We have shown that different generation dendritic 2-carbamoylbenzoate derivatives are useful delivery systems for the controlled release of tertiary fragrance alcohols by neighbouring group assisted alkaline hydrolysis. Due to the excellent separation of the products of hydrolysis by HPLC, the kinetic rate constants for the individual reaction steps can be measured separately. Comparable rate constants were found for the consecutive reaction steps of a given dendrimer and, due to intramolecular reaction at the same dendrimer branch, alcohol formation is almost independent of the dendrimer generation. The principle of neighbouring group assisted release of fragrance alcohols from the dendrimer surface may be applied generally for the release of biologically active compounds and thus find various applications in the pharmaceutical or agrochemical industry.

Experimental Section

Typical Procedure for the Preparation of Modified Dendrimers: A solution of DAB-*dendr*-$(NH_2)_{16}$ (0.59 g, 0.35 mmol) in CH_2Cl_2 (10 mL) was added dropwise at room temperature to a solution of 1,1-dimethyl-2-phenylethyl 2-(fluorocarbonyl)benzoate (2.00 g, 6.66 mmol) and TEA (1.35 g, 13.32 mmol) in CH_2Cl_2 (20 mL). After stirring for 30 min, the reaction mixture was poured into aq. $KHSO_4$ (5%, 50 mL), extracted, dried (Na_2SO_4) and concentrated to give 3.11 g of the crude compound. MPLC of 1.50 g [RP-C4 (Vydac 214TP C4), water/acetonitrile 1:1, containing 0.1% of trifluoroacetic acid (TFA)] and addition of a trace of $KHSO_4$ to the product fraction gave, after concentration and drying at 0.2 mbar, 0.79 g (ca. 76%) of **5** as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.60−7.51 (m, 16 H), 7.46−7.34 (m, 16 H), 7.36−7.26 (m, 48 H), 7.25−7.08 (m, 80 H), 3.45−3.31 (m, 32 H), 3.31−3.18 (m, 32 H), 3.16−2.88 (m, 52 H), 3.06 (s, 32 H), 2.36−2.14 (m, 24 H), 2.06−1.88 (m, 32 H), 1.75−1.63 (m, 4 H), 1.45 (s, 96 H) ppm. ^{13}C NMR (125.8 MHz, $CDCl_3$): δ = 170.70 (s), 166.46 (s), 137.02 (s), 136.89 (s), 131.41 (d), 130.63 (d, s), 129.72 (d), 129.53 (d), 128.05 (d), 127.52 (d), 126.62 (d), 83.99 (s), 52.14 (t, br.), 50.65 (t), 49.23 (t, br.), 48.62 (t, br.), 46.50 (t), 36.50 (t), 25.71 (q), 23.63 (t), 20.32 (t, br.), 18.04 (t, br.) ppm. ESI MS: *m*/*z* = 2058.0 $[M + 3H]^{3+}$, 1543.8 $[M + 4H]^{4+}$, 1235.3 $[M + 5H]^{5+}$.

Procedure for the Hydrolysis Experiments: Thermostatted solutions (at, 20 °C) of compounds **2**−**5** with given concentration in a phosphate buffer solution (water/acetonitrile 2:1, containing 1% by weight of Triton® X100, Union Carbide) at pH = 7.62 ± 0.02 were injected immediately after preparation into an HPLC apparatus (t = 0) and then re-analysed every 53 min (20 times). The samples were eluted at 1 mL min^{-1} on a reversed-phase column (Macherey−Nagel, Nucleosil 100−7 C2, 250 × 4 mm i.d.) using a water/acetonitrile gradient (50:50 to 5:95 during 20 min) containing 0.1% of TFA, and detected at λ = 254 nm.

Acknowledgments

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[14] On-going work involves the preparation of analogues of **2** which release other secondary or tertiary fragrance alcohols such as 1-phenyl-2-pentanol or linalool, thus demonstrating the general use of the delivery systems described in this work.

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