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PAPER

Release of bioactive volatiles from supramolecular hydrogels: influence of reversible acylhydrazone formation on gel stability and volatile compound evaporation†

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In the presence of alkali metal cations, guanosine-5′-hydrazide (**1**) forms stable supramolecular hydrogels by selective self-assembly into a G-quartet structure. Besides being physically trapped inside the gel structure, biologically active aldehydes or ketones can also reversibly react with the free hydrazide functions at the periphery of the G-quartet to form acylhydrazones. This particularity makes the hydrogels interesting as delivery systems for the slow release of bioactive carbonyl derivatives. Hydrogels formed from **1** were found to be significantly more stable than those obtained from guanosine. Both physical inclusion of bioactive volatiles and reversible hydrazone formation could be demonstrated by indirect methods. Gel stabilities were measured by oscillating disk rheology measurements, which showed that thermodynamic equilibration of the gel is slow and requires several cooling and heating cycles. Furthermore, combining the rheology data with dynamic headspace analysis of fragrance evaporation suggested that reversible hydrazone formation of some carbonyl compounds influences the release of volatiles, whereas the absolute stability of the gel seemed to have no influence on the evaporation rates.

Introduction

The generation of dynamic combinatorial libraries based on reversible covalent bond formation has recently attracted considerable interest for the development of enzyme inhibitors in drug discovery, the selective preparation of macrocycles by templated synthesis, the development of catalytic ligands, the design of stimuli-responsive polymer materials and other systems which react by adaptation to external influences.[1–4] In particular, the condensation of aldehydes and ketones with amine derivatives to form imines was identified as a versatile reaction for dynamic combinatorial chemistry.[3,4] Similarly, dynamic mixtures composed of carbonyl compounds and amines or specific amine derivatives have been identified as efficient delivery systems for the controlled release of volatile aldehydes and ketones in different practical applications.[5–8]

The concept of using dynamic mixtures for the release of volatiles is based on the fact that the various constituents of the mixture reversibly react to form non-volatile condensation products in equilibrium with the corresponding unreacted species.[5–8] The position of the equilibrium depends on external conditions such as temperature, pH, or the concentration of the different reactants. Once deposited onto a surface, the non-bound volatiles start to evaporate and shift the equilibrium towards the hydrolysis of the condensation products. Particularly interesting in this context is the formation of acylhydrazones, which comprise both a reversibly formed imine unit and a peptide bond.[4] Dynamic mixtures based on reversible acylhydrazone formation were found to be highly efficient to slow down the evaporation of volatile fragrance aldehydes or ketones in functional perfumery applications.[5,6] In the presence of a hydrazide, headspace concentrations of single raw materials released from an equilibrated dynamic mixture from a cotton surface increased by up to two orders of magnitude when compared to a standard reference sample without hydrazide.[6]

As a further step to develop novel delivery systems for bioactive volatiles, we became interested in investigating the release of bioactive volatiles from dynamic mixtures within ordered supramolecular assemblies.[9] Besides reversibly reacting with carbonyl compounds to form acylhydrazones, bifunctional guanosine-5′-hydrazide (**1**)[10–13] also forms stable supramolecular hydrogels in the presence of alkali metal cations by selective self-assembly of a guanosine quartet (G-quartet) structure *via* intermolecular Hoogsten-type hydrogen bonding[14,15] (Scheme 1).

The stability of the G-quartet structure is mainly influenced by the choice of cation; K^+ or Sr^{2+} seem to form particularly

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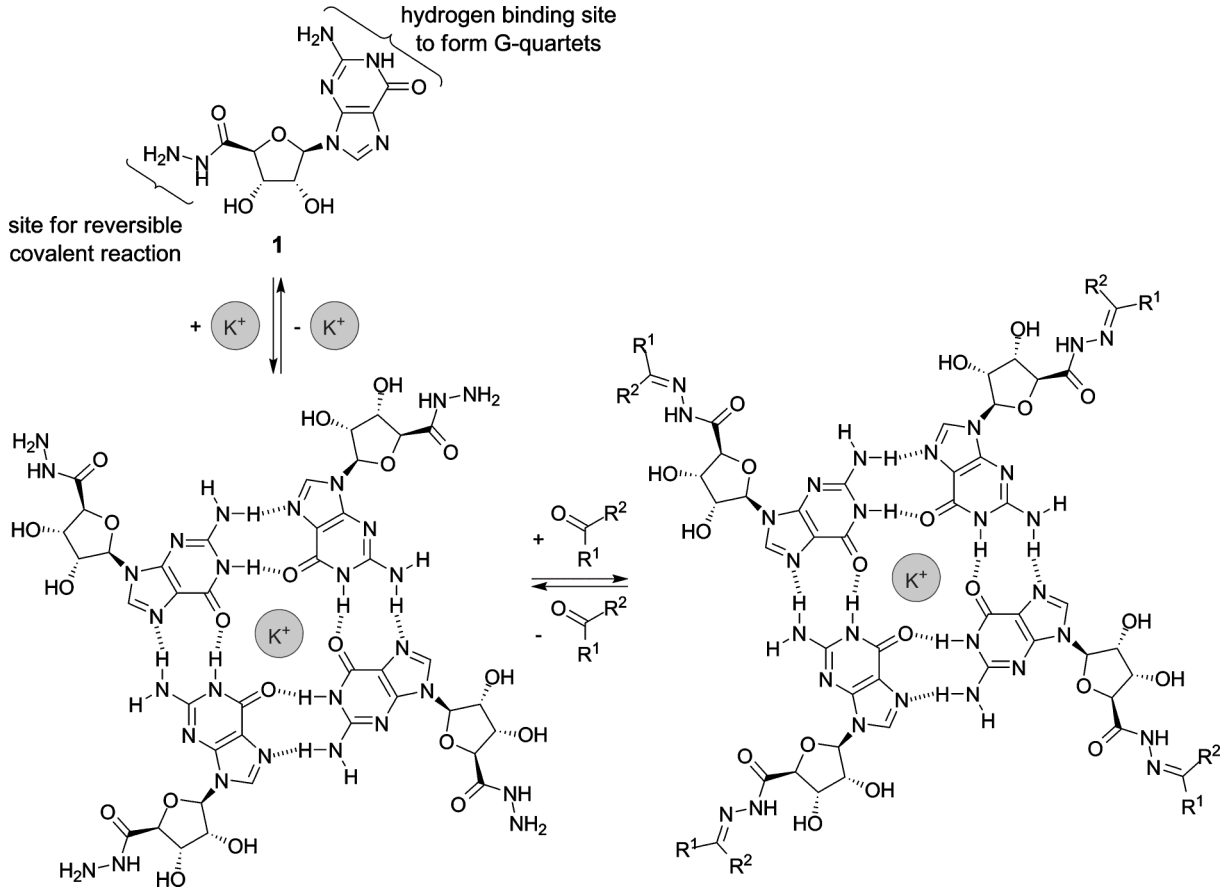
† Electronic supplementary information (ESI) available: synthesis and spectroscopic data of compounds **1**, **4**, **5** and **23** and numerical data for Fig. 8. See DOI: 10.1039/c0ob01139h



Scheme 1 Self-assembly of bifunctional guanosine-5′-hydrazide (**1**) to G-quartets in the presence of cations and reversible acylhydrazone formation between the G-quartet of the hydrogel and an aldehyde or ketone.

stable hydrogels, but $(CH_3)_4N^+$, NH_4^+ or Na^+ are also suitable for gel formation.[14] Besides the nature of the cation, the structure of carbonyl compounds used for reversible hydrazone formation also strongly influences the stability of the hydrogel.[11] It was shown that the addition of stoichiometric amounts of certain aldehydes in a sodium acetate buffer resulted in highly viscous gels, whereas others only gave solutions. Furthermore, if a mixture of aldehydes or ketones was added to a hydrogel of **1** the dynamic mixture selects the compound forming the most stable gel structure in a thermoreversible process.[10]

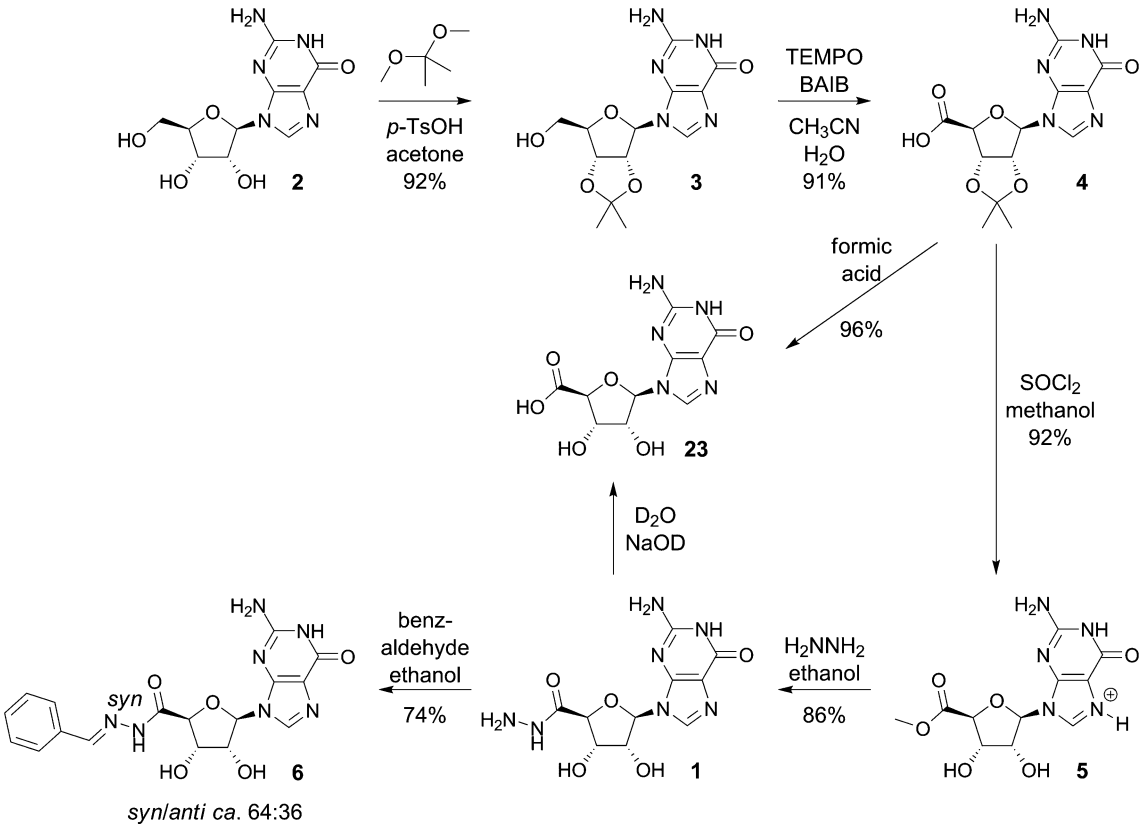
The combination of gelation-driven self assembly[16] with reversible covalent bond formation[1–3] in an aqueous environment makes **1** an interesting compound to control the release of bioactive substances, in particular those with aldehyde or ketone functions.[12,17] In such systems the evaporation of volatile aldehydes and ketones is not only expected to be slowed down by reversible covalent bond formation with the hydrazide function at the periphery of the G-quartet, but also by physical inclusion of the compounds inside the gel structure by non-covalent interactions.

In this work we complete our previous investigations on the stability of the hydrogels[10,12] in the presence of various fragrance aldehydes and ketones by rheology measurements, and compare the release of volatile carbonyl compounds from the gels by dynamic headspace analysis under realistic conditions encountered in practical applications, such as hydrogels for air freshener formulations. In contrast to previous investigations carried out with relatively polar and almost non-volatile carbonyl compounds,[10,12] the inclusion of apolar and highly volatile flavours and fragrances is expected to reduce the water-solubility of the entire system and thus influence the stability of the hydrogels.[18]

Results and discussion

Synthesis and structural characterisation

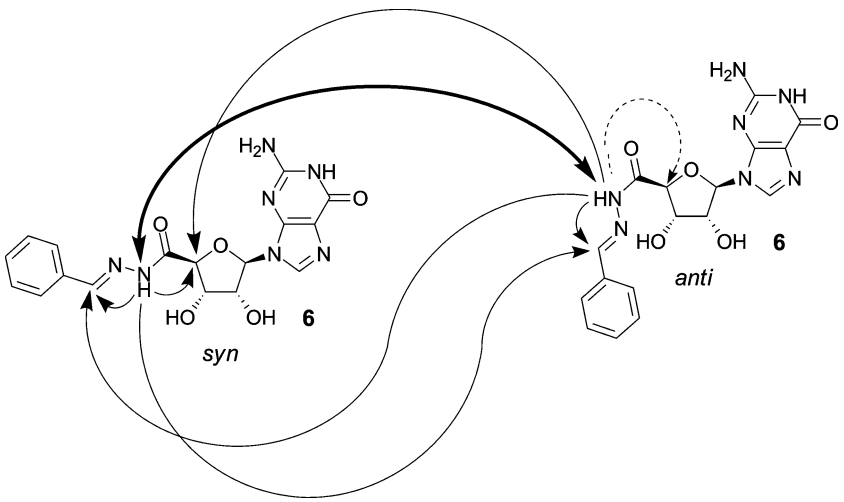
Guanosine-5′-hydrazide (**1**) can be prepared from guanosine (**2**) in a four-step sequence, or from commercially available ketal **3** in three steps[10] (Scheme 2). Starting from less expensive **2**, we protected the free 3′,4′-hydroxyl groups with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid in acetone to give the corresponding isopropylidene derivative **3**.[19] Oxidation of the free hydroxymethyl group to the corresponding carboxylic acid **4** was carried out using the 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO) radical and [bis(acetoxy)iodo]benzene (BAIB) in aqueous acetonitrile.[20] In this step it was important to add the solvent to a mixture of all the other compounds to avoid an excessive formation of CO_2. Carboxylic acid **4** was then transformed to its corresponding methyl ester with $SOCl_2$ in methanol by simultaneous removal of the hydroxyl protecting group.[21] Ester **5** was obtained in its protonated form as shown by NMR analysis. Addition of NaOD to a solution of protonated **5** in DMSO-d_6 shifted the signal of neighbouring C(5) from 110.2



Scheme 2 Preparation of guanosine-5′-hydrazide (**1**) and its corresponding acylhydrazone **6**.

to 116.4 ppm, with the latter value being the one found for the other guanosine derivatives. Finally, treatment of **5** with hydrazine hydrate in ethanol or methanol[10,22] afforded hydrazide **1** in good yield, allowing its preparation on a 10 g scale.

As a reference compound we also prepared acylhydrazone **6** (Scheme 2) which was obtained by heating a suspension of **1** and benzaldehyde in ethanol followed by filtration of the precipitated product as previously described for the preparation of other hydrazones.[6] Benzaldehyde derivative **6** was obtained as a mixture of two isomers (*ca.* 64 : 36) with respect to the amide bond conformation, with the *syn* isomer (Scheme 3) being the major isomer. The structure of the compounds was confirmed by 1D and 2D homonuclear (COSY, NOESY) and ^{1}H-,^{13}C-heteronuclear (HSQC, HMBC) NMR experiments in DMSO which showed that the two species were conformational rather than constitutional isomers.[6,23] In the NOESY experiment crosspeaks between equivalent protons of both isomers were observed that had opposite signs with respect to regular NOE peaks. These are due to a



Scheme 3 Measured NOEs (plain arrows), HMBC (dotted arrow) and chemical exchange (bold arrow) between the *syn* and *anti* isomers of **6** by ^{1}H- and ^{13}C-NMR spectroscopy in DMSO.

conformational exchange between the two molecules that takes place in the mixing period of the NOESY pulse sequence (800 ms). The exchange process was found to be slow on the NMR timescale, as both molecules give rise to separate sets of signals in the NMR spectrum. In addition, NOE crosspeaks were observed between the amide proton of one isomer and protons of the other isomer and *vice versa*, respectively, demonstrating the presence of a conformational exchange. The observed NOEs and chemical exchanges are illustrated in Scheme 3.

Hydrogel formation and visual evaluation of the gel stability

To get a first insight into the influence of the gel stability in the presence of different volatile carbonyl compounds, we prepared a series of hydrogels with guanosine-5′-hydrazide (**1**) (where both physical inclusion and covalent bond formation are possible) and a series of typical fragrance raw materials in a molar ratio of 2 : 1 (15 mM for the hydrogelator and 7.5 mM for the fragrance material) and compared them to hydrogels formed from guanosine (**2**) (where physical inclusion but no covalent bond formation is possible). As more stable G-quartets are usually obtained with K^+ rather than with Na^+ as the cation, we decided to use a potassium acetate buffer (0.5 M, pH 6) for hydrogel preparation.

Hydrogels were formed by dissolving (using sonication!) 0.12 mmol of the hydrogelator (**1** or **2**) in 7 mL of a potassium acetate buffer at pH 6. The mixture was heated on a water bath to *ca*. 70 °C until a homogenous solution was obtained. Then 1 mL of a 0.06 M solution of the volatile carbonyl compound(s) (*e.g.* **7–22**, Fig. 1) in the acetate buffer was added, the sample was left to cool to room temperature (r.t.), and allowed to stand overnight to give a gel containing 15 mM of the hydrogelator and 7.5 mM of the carbonyl derivative. The volatiles were chosen to cover a broad range of vapour pressures (volatilities), ranging from 0.06 Pa for the least volatile of the series (**7**) to 309 Pa for the most volatile one (**15**). Similarly, compounds with different water-solubility (expressed as the octanol/water partition coefficient $\log P_{o/w}$),‡ [24] varying from 0.41 for the most soluble compound (**15**) to 4.36 for the least soluble one (**10**). To see whether a stable gel was obtained or whether the compound precipitated, the samples were inverted and/or analysed visually after cooling to room temperature and, in view of the targeted practical applications, again after storing for 5 days, while allowing evaporation of the volatiles. The data obtained are summarised in Table 1, also showing typical examples for a precipitate, a strong, or a weak gel. Weak gels can be re-dissolved by agitation of the sample.

The experiments showed that in all cases investigated so far the hydrogels formed from guanosine-5′-hydrazide (**1**) were significantly more stable than those obtained from guanosine (**2**), where precipitation occurred after storing the samples for 5 days. Furthermore, according to the choice of the carbonyl compound added to the hydrogelator, gels of different stability were obtained. It is interesting to note that the evaporation of the fragrances does not seem to generally influence the stability of hydrogels formed from **1** (only in the presence of Lilial® (**10**) and Trifernal® (**11**) the gel stability decreased; in the case of benzylacetone (**18**) an increase

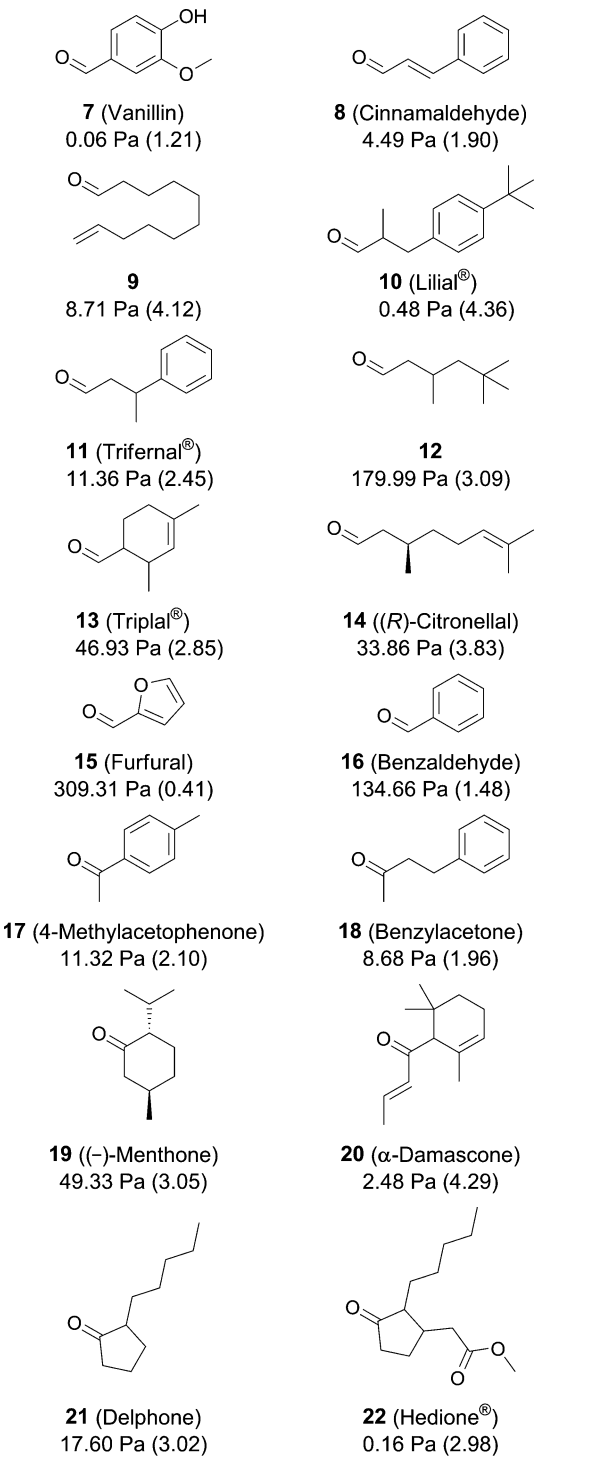


Fig. 1 Structures and trivial names of typical volatile carbonyl compounds used as fragrances with their calculated vapour pressures (in Pa) and $\log P_{o/w}$ values (in brackets).‡

was observed) whereas the weak hydrogels of **2** all precipitated after evaporation of the fragrance for 5 days (Table 1).

In some cases, gels were prepared in duplicate or triplicate and, based on the visual analysis of the gels after cooling to room temperature and allowing them to stand for 5 days, a good reproducibility was achieved, with the exception of the gels prepared in the presence of cinnamaldehyde (**8**) and (*R*)-citronellal

‡ Vapour pressures and octanol/water partition coefficients ($\log P_{o/w}$) were calculated with the PBT Calculator (v. 1.0.0, EPA's Office of Pollution Prevention and Toxics and Syracuse Research Corporation) based on the EPIwin program (US Environmental Protection Agency).

Table 1 Visual evaluation of hydrogels formed in the presence of fragrance aldehydes or ketones (**7**–**22**, Fig. 1) using a molar ratio between the hydrogelator and the fragrance of 2 : 1. The photographs show the formation of a precipitate of **2** in the presence of citronellal (**14**, left), of a strong gel of **1** in the presence of (–)-menthone (**19**, right) and of a weak gel of **1** in the presence of Trifernal® (**11**, middle) (after 5 days)

2 + 14 **1 + 11** **1 + 19**

	Guanosine-5′-hydrazide (**1**)		Guanosine (**2**)	
Aldehyde or ketone	after cooling	after 5 d	after cooling	after 5 d
—	weak gel	weak gel	precipitate	precipitate
Vanillin (**7**)	strong gel	strong gel	weak gel	precipitate
Cinnamaldehyde (**8**)	strong gel	strong gel	weak gel	precipitate
10-Undecanal (**9**)	strong gel	strong gel	weak gel	precipitate
Lilial® (**10**)	strong gel	weak gel	weak gel	precipitate
Trifernal® (**11**)	strong gel	weak gel	weak gel	precipitate
3,5,5-Trimethylhexanal (**12**)	weak gel	weak gel	precipitate	precipitate
Triplal® (**13**)	weak gel	weak gel	weak gel	precipitate
(*R*)-Citronellal (**14**)	strong gel	strong gel	weak gel	precipitate
Furfural (**15**)	strong gel	strong gel	weak gel	precipitate
Benzaldehyde (**16**)	strong gel	strong gel	precipitate	precipitate
4-Methylacetophenone (**17**)	strong gel	strong gel	weak gel	precipitate
Benzylacetone (**18**)	weak gel	strong gel	precipitate	precipitate
(–)-Menthone (**19**)	strong gel	strong gel	precipitate	precipitate
α-Damascone (**20**)	strong gel	strong gel	precipitate	precipitate
Delphone (**21**)	strong gel	strong gel	precipitate	precipitate
Hedione® (**22**)	strong gel	strong gel	precipitate	precipitate

(**14**), where either strong or weak gels were obtained for different samples. At the given concentrations, neither the vapour pressure of the fragrance, nor its hydrophilicity seems to influence the stability of the hydrogels obtained.

In the case of **1** the carbonyl compounds can react with the hydrogelator, being physically trapped inside the gel structure and/or remaining as free molecules in the solvent. However, in the case of **2**, carbonyl compounds can only be physically trapped inside the gel structure or remain in the solvent. As a consequence of the reduced mobility of the gel with respect to the NMR timescale, the amount of hydrazide **1** or aldehydes and ketones which are trapped in the hydrogel structure give rise to broad signals in the ^{1}H-NMR spectrum which cannot be quantified.[10,12] This amount can thus only be estimated indirectly by integrating the signals of the compounds remaining in the water phase (= "free" compounds), giving rise to sharp peaks which can be integrated with respect to an internal standard that does not interact with the hydrogel. In our experiments we chose dioxane as the internal standard, which was added to the buffer solution to correspond to 0.5 equivalents with respect to the total amount of **1** used to form the gel. Hydrogels were prepared in an NMR tube by adding 100 μL of a 0.5 M deuterated potassium acetate buffer containing dioxane and different amounts of benzaldehyde (**16**) to 700 μL of the hydrogelator in a deuterated potassium acetate buffer without dioxane, to give 15 mM solutions of **1** with 0.5, 1.0 or 1.5 molar equivalents of **16**, respectively, and 0.5 molar equivalents of dioxane. The tubes were heated and cooled to room temperature overnight to form the gels. ^{1}H-NMR spectra were recorded on the fully solidified samples and the percentage of free hydrogelator (in mol%) was determined by integrating the proton signal of H(8) on the guanine group with respect to the internal dioxane reference, and that of free **16** by integrating the H(3) proton signals of the aromatic ring. Table 2 lists the amounts of free **1** and free **16** determined in the water phase of the different samples.

Table 2 Amounts of guanosine-5′-hydrazide (**1**) and benzaldehyde (**16**) determined in the water phase (in mol%) by ^{1}H-NMR spectroscopy with respect to the total amount of compounds in the NMR tube using dioxane as the internal standard

Total amount of **1**	Total amount of dioxane	Total amount of **16**	Amount of free **1** [mol-%]	Amount of free **16** [mol-%]
1.0 eq.	0.5 eq.	0.5 eq.	4.7	13.7
1.0 eq.	0.5 eq.	1.0 eq.	4.9	8.9
1.0 eq.	0.5 eq.	1.5 eq.	4.3	5.6

After 5 days at room temperature, the NMR samples were re-heated until the gel dissolved, and then cooled again to room temperature. Re-measuring the ^{1}H-NMR spectra indicated 13.9%, 8.3% and 5.6% of free **16**, and 4.2%, 3.2% and 3.2% of free **1**, respectively, thus showing good reproducibility of the gel formation.

In agreement with previous measurements,[10] the data show that more than 95% of the hydrogelator were incorporated in the gel structure. The fact that only 5–14% of free **16** remained in the trapped liquid phase, although it was used in excess with respect to the hydrogelator in one case, indicates that most of the active aldehyde or ketone is in fact incorporated into the supramolecular hydrogel structure. It can either be physically trapped inside the gel and/or be covalently linked to **1**.

It is interesting to note that an increasing amount of **16** added to the samples results in a lower percentage of the respective compounds outside the gel, whereas a more or less constant amount of hydrazide **1** was detected in the three samples. The observed decrease of the amount of **16** outside the gel structure might be the result of its limited solubility in the aqueous buffer. Nevertheless, due to the fact that an excess of **16** with respect to hydrogelator **1** was used, the data in Table 2 show that at least a part of the aldehyde is physically trapped within the gel structure. However, the measurements did not prove the formation of the corresponding hydrazone.

As already mentioned above, molecules that are engaged in the gel formation are not visible in the NMR spectrum, due to reduced mobility. A possible reason for this might be incomplete averaging of dipolar couplings or magnetic susceptibility gradients. In order to elucidate this further we carried out High Resolution Magic Angle Spinning (HRMAS) NMR spectroscopy, where such unfavourable interactions can by eliminated by rotation of the sample at the magic angle. However, with regard to the relative peak intensities, the spectra are identical to the ones obtained with standard liquid state NMR. We therefore assume that the extensive line broadening is caused by slow to intermediate motion on the NMR time scale of the complex and a subsequent decrease of the transverse relaxation time T_2.

HRMAS ^{1}H-NMR spectra of the guanosine-5′-hydrazide gels show, besides the large peaks corresponding to the residual solvent signal, dioxane, and the acetate buffer, the expected guanosine peaks H(8) (8.03 ppm), H(*R1*) (6.01 ppm), and two other protons of the sugar ring (4.68 ppm, 4.61 ppm). Our measurements showed that, according to the visible peaks in the NMR spectrum, the amount of free guanosine-5′-hydrazide (**1**) in a 30 mM sample is 10.3%. This value increased when the concentration of **1** in the gel decreased. In a 15 mM sample the amount of free **1** in the gel structure was found to be 24.9%. Increasing the temperature to 60 °C gave 88.1% of the free hydrogelator, and at 70 °C 94.6% of free **1** were measured, corresponding to the physical melting and disruption of the gel structure.

Incorporation experiments were carried out with **1** (15 mM) and bioactive volatiles (0.5 eq.) undecenal (**9**) and (*R*)-citronellal (**14**), respectively, in 0.5 M potassium acetate buffer. The NMR spectrum of these gels displayed weak signal intensities corresponding to the free fraction of acylhydrazones.

Decreasing the concentration of **1** to 7.5 mM (and those of the fragrances to 3.75 mM) generally resulted in considerably less stable hydrogels. Thus the concentration of the hydrogelators was maintained at 15 mM in the following experiments.

As a further option to get insight into the composition of the gel structure, we envisioned dissolving the hydrogel after its equilibration by the addition of base. This was expected to "freeze" equilibration by slowing down the possible hydrolysis of hydrazones and thus allow NMR analysis of the gel composition. To investigate this option and, in particular, to verify the stability of the hydrazide in basic media, we prepared an aqueous solution of guanosine-5′-hydrazide (**1**) in D_2O. Addition of two drops of NaOD to the solution of **1** resulted in rapid degradation and formation of carboxylic acid **23**.[21] The structure of **23** was confirmed by reaction of previously prepared guanosine derivative **4** with formic acid (Scheme 2).[25]

Under the same conditions (D_2O and a few drops of NaOD), hydrazone **6** was found to be at least temporarily stable. In the presence of NaOD the conformational exchange between the *syn* and *anti* isomer was accelerated giving rise to a single set of peaks for the two structures. We thus prepared a gel by dissolving guanosine-5′-hydrazide (**1**) by sonication in a 0.5 M potassium acetate buffer (pH 6). The sample was heated to 80 °C prior to the addition of a solution containing equimolar amounts of carbonyl compounds **14–17**, dissolved in the buffer, yielding a 15 mM solution of **1** and a 3.3 mM solution for each aldehyde or ketone. The glass container was closed, left cooling to r.t. overnight to form the gel which then submitted to four heating and cooling cycles and left standing at r.t. for 60 h. The gel was dissoved by addition of a few drops of NaOD and the resulting solution analysed by high-resolution electrospray mass spectrometry (in the positive and negative ion mode). No mass peaks corresponding to either one of the hydrazones possibly formed and no peaks corresponding to **1** or its degradation product **23** could be detected. Hydrazone formation in the gels could thus not be proved with this method. Attempts to follow the hydrazone formation by IR spectroscopy were also not successful.

Mixtures containing various fragrance aldehydes or ketones were prepared by adding a total of 1.5 equivalents of three compounds (0.06 M for each component) to the hydrogelator. A solution of the carbonyl compound mixture (8 mL) was added to hydrogelator (**1**). The sample was heated to 70 °C on a water bath and cooled to room temperature. Individual experiments with furfural (**15**), benzaldehyde (**16**) and 4-methylacetophenone (**17**) all gave stable gels. However, addition of a mixture of the three compounds to **1** gave a precipitate, even at 70 °C (presumably due to reasons of solubility). Filtration of the precipitate, drying under vacuum, and NMR analysis in DMSO showed that a complex mixture of compounds was obtained. Comparison of the ^{13}C-NMR spectrum of the precipitate with those of **1** and **6** (see Fig. 2) indicated that both compounds were part of the product mixture. In particular, the formation of acylhydrazone **6** (*syn* and *anti* isomers) shows that the expected reversible reaction with the hydrogelator takes place spontaneously after only a short period of heating.

Precipitation was also observed from mixtures of **1** with (*R*)-citronellal (**14**), benzaldehyde (**16**), and 4-methylacetophenone (**17**), or Triplal® (**13**), (*R*)-citronellal (**14**) and benzaldehyde (**16**). Analysis of the precipitates of these mixtures was found to be complicated and was thus not further pursued. Since the preparation of these mixtures was also complicated by solubility problems of the fragrance molecules in the buffer solution, we decided to reduce their amount by a factor of two with respect to the hydrogelator.

New gels were thus prepared and analysed visually after cooling to room temperature, and after storing at room temperature for 5 days as described above. The data obtained are summarised in Table 3. Once again, the hydrogels formed from

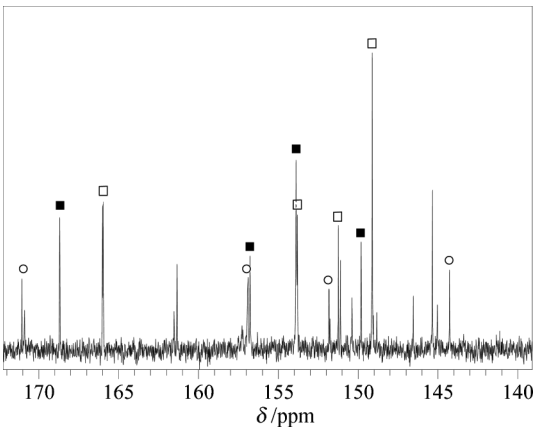
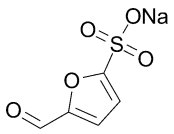


Fig. 2 Expansion of the ^{13}C-NMR spectrum of the precipitate isolated from a mixture of furfural (**15**), benzaldehyde (**16**), 4-methylacetophenone (**17**) and hydrogelator **1**. Assigned peaks correspond to **1** (■) and **6** (*syn*-isomer (□) and *anti*-isomer (○)).

guanosine-5′-hydrazide (**1**) were significantly more stable than those obtained from guanosine (**2**), where precipitation occurred after storing the samples for 5 days.

To see whether the precipitation observed at higher fragrance concentration is due to the formation of the relatively hydrophobic hydrazone **6**, we prepared a mixed G-quartet gel[12] containing **1** and **6** in a molar ratio of *ca.* 3 : 1. The hydrogel formed in the acetate buffer at pH 6 after cooling to room temperature was found to be more or less stable, and stabilised even further after storing for 5 days. This phenomenon may be due to the hydrolysis of the hydrazone followed by the slow evaporation of benzaldehyde, which then increased the water-solubility of the hydrogelator. Preparation of a gel composed of **2** and benzaldehyde (at 0.2 molar equivalents) gave a precipitate after cooling to room temperature. No gel was formed from a mixture consisting of **1** and **6** (*ca.* 3 : 1) in the presence of citronellal and acetophenone (each at 0.2 molar equivalents with respect to **1**).

Previous studies have shown that 2-formylbenzenesulfonic acid sodium salt (**24**, Fig. 3) stabilises the formation of the hydrogels of **1**.[10] As aldehyde **24** is highly water-soluble and not very volatile,



24
0.24 (-1.14)

Fig. 3 Structure, vapour pressure in Pa and log$P_{o/w}$ value (in brackets)‡ of 2-formylbenzenesulfonic acid sodium salt (**24**).

we used it as a reference for the preparation of mixtures with a variety of more volatile and less water soluble fragrance molecules. Hydrogels of **1** and **2** were thus prepared in the presence of an equimolar amount of a fragrance aldehyde (15 mM for both the hydrogelator and the fragrance) and varying amounts of **24** (0, 0.125, 0.250 and 0.500 eq., respectively). The gels were prepared as described above by dissolving **1** or **2** in a 0.5 M potassium acetate buffer (pH 6) and heating to 70 °C. A solution of the fragrance aldehyde and a given amount of **24**, both dissolved in the buffer, was added and the mixture allowed cooling to room temperature. The samples were inverted and/or analysed visually after cooling to room temperature and after storing for 5 days while allowing the fragrance to evaporate. The observations are summarised in Table 4.

From this data it is very difficult to estimate the influence of **24** on the stability of the different gels. In the case of Trifernal® (**11**) as the fragrance aldehyde, an increasing amount of **24** seemed to give rise to more stable hydrogels. On the other hand, in the case of Lilial® (**10**) it seems that the slow evaporation of the aldehyde also influenced the stability of the gel, as higher gel stability was observed after 5 days. Under the same conditions guanosine (**2**) did not form stable gels at all.

Oscillating disk rheology measurements

Our previous experiments have shown that the structure of the carbonyl compound added to hydrogelator **1** influences the stability of the gel. Furthermore, we could demonstrate that carbonyl compounds are incorporated into the gel structure by

Table 3 Formation of hydrogels in the presence of three fragrance aldehydes or ketones using a molar ratio between the hydrogelator and each fragrance of 4 : 1

	guanosine-5′-hydrazide (**1**)		guanosine (**2**)	
Aldehydes or ketones	after 1 d	after 5 d	after 1 d	after 5 d
Furfural (**15**) Benzaldehyde (**16**) 4-Methylacetophenone (**17**)	weak gel	stable gel	weak gel	precipitate
(*R*)-Citronellal (**14**) Benzaldehyde (**16**) 4-Methylacetophenone (**17**)	stable gel	stable gel	precipitate	precipitate
Triplal® (**13**) (*R*)-Citronellal (**14**) Benzaldehyde (**16**)	stable gel	stable gel	weak gel	precipitate
Triplal® (**13**) (*R*)-Citronellal (**14**) 4-Methylacetophenone (**17**)	stable gel	stable gel	weak gel	precipitate

Table 4 Visual evaluation of hydrogels formed from **1** or **2** in the presence of fragrance aldehydes **10**, **11**, **12** or **13** and a different molar ratio of 2-formylbenzenesulfonic acid sodium salt (**24**)

	eq. of	guanosine-5′-hydrazide (**1**)		guanosine (**2**)	
Aldehyde	**24**	after cooling	after 5 d	after cooling	after 5 d
Lilial® (**10**)	—	stable gel	stable gel	weak gel	precipitate
	0.125	weak gel	stable gel	precipitate	precipitate
	0.250	weak gel	stable gel	precipitate	precipitate
	0.500	stable gel	stable gel	weak gel	precipitate
Trifernal® (**11**)	—	weak gel	weak gel	weak gel/precipitate	precipitate
	0.125	weak gel	weak gel	weak gel/precipitate	precipitate
	0.250	no gel	weak gel	precipitate	precipitate
	0.500	stable gel	stable gel	precipitate	precipitate
3,5,5-Trimethylhexanal (**12**)	—	stable gel	stable gel	precipitate	precipitate
	0.125	stable gel	stable gel	weak gel/precipitate	precipitate
	0.250	stable gel	stable gel	weak gel/precipitate	precipitate
	0.500	stable gel	stable gel	weak gel/precipitate	precipitate
Triplal® (**13**)	—	stable gel	stable gel	precipitate	precipitate
	0.125	stable gel	stable gel	weak gel	weak gel/precipitate
	0.250	stable gel	stable gel	weak gel/precipitate	precipitate
	0.500	stable gel	stable gel	weak gel/precipitate	precipitate

physical inclusion as well as by reversible reaction with the hydrazide function of **1**, as suggested by indirect evidence (see above).

In order to get more reliable, quantitative data for the stability of the gels, and to see whether particular fragrance aldehydes or ketones have a stabilising or de-stabilising effect, we decided to determine the viscosity of the gels by rheology measurements, in analogy to similar experiments carried out previously.[10] Despite the fact that rotating disk measurements (performed with a stress controlled rotating cone plate (35 mm, 4°) at shear rates varying between 0.38 and 40 s^{-1})[10] were quite reproducible, the interpretation of the results was found to be difficult, due to the fact that the gels were easily destroyed when starting the measurements with the rotating disk. Rheology measurements using a rotating disk are thus not suitable for characterisation of the stability of the different hydrogels. We therefore decided to analyse the stability of the hydrogels using an oscillating disk.

In a typical measurement, the solution of the hydrogelator (0.12 mmol) was heated to 80 °C, prior to the addition of the carbonyl compounds (0.06 mmol) dissolved in the buffer (or an equivalent of pure buffer solution), to give a final solution of 15 mM of **1** and 7.5 mM (or 0 mM) of carbonyl compound(s). Measurements were carried out with a cone plate (40 mm, 4°). The sample was placed onto the rheometer ground plate at 80 °C. To avoid evaporation of the fragrance during the measurement, a film of Neobee® was placed at the border of the rheometer plate. The gel was then rapidly cooled to 20 °C and left equilibrating at 20 °C for 5 min. A sweep of strain from 0.1 to 10% was carried out with a logarithmic variation of deformation and the storage modulus G' (measure of a sample's ability to store energy, also called the elastic modulus) and the loss modulus G'' (measure of the sample's ability to dissipate energy) were determined. G' and G'' were measured for 30 min at 1 Hz. To operate at a deformation which does not disrupt the gel structure (and thus not influence the measurement of G' and G''), we first determined the domain of linearity of a hydrogel prepared from **1** (in a 0.5 M potassium acetate buffer) in the dynamic oscillating mode (Fig. 4).

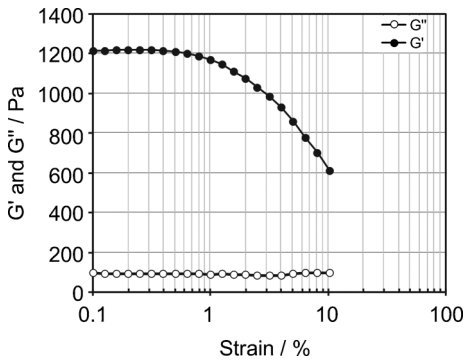


Fig. 4 Determination of the domain of linearity for a hydrogel prepared of pure **1** (15 mM in a potassium acetate buffer) by rheology measurements in the dynamic oscillating mode.

Fig. 4 shows that the linearity domain was not followed when strains above *ca.* 0.5% were applied. In the following measurements the strain applied to the gels was fixed at 0.4%.

To determine the gelation temperature T_{gel}, measurements were carried out while cooling the sample from 80 °C to 20 °C (at 1 °C min^{-1}). From the interception of the curves of G' and G'', the gelation temperature of **1** was found to be about 68 °C (15 mM in a potassium acetate buffer), thus confirming the previously determined value.[10] The presence of benzaldehyde (**16**, 7.5 mM, 0.5 eq.) considerably decreased T_{gel} to about 50 °C as shown in Fig. 5. Furthermore, the presence of **16** results in less stable hydrogels as compared to the reference without fragrance (lower G' and G'' values).

With the possibility to control the temperature during the rheology measurement, we also investigated the reproducibility of the gelation by repeating the cooling and heating cycles. With each cycle, G' and G'' increased, until stable values were obtained (usually after 3 to 5 cycles). Fig. 6 shows the evolution of G' and G'' obtained for hydrogels formed from **1**, and Fig. 7 those measured for hydrogels of **2**, after three to five consecutive cooling and heating cycles in the presence and absence of different carbonyl

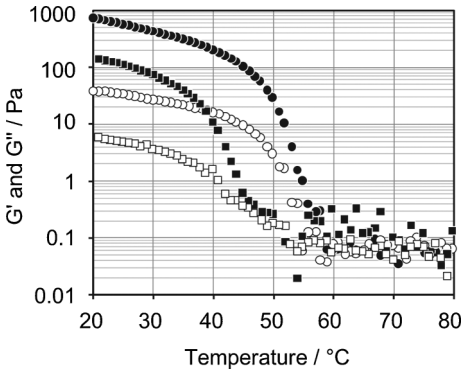


Fig. 5 Temperature dependent variation of G' and G'' for a hydrogel of **1** in the presence (■, □) and absence (●, ○) of benzaldehyde (**16**, 0.5 eq.).

compounds. An increase of the stability of the gel was observed for the hydrogel of **1** alone, as well as for **1** in the presence of volatile or non-volatile carbonyl compounds. This illustrates that the stabilisation of the gel is a general observation,§ and that, in these cases, the thermodynamic equilibration process within the gel structure is relatively slow.

The values for G' and G'' listed in Table 5 were recorded after several cooling and heating cycles. The data show that the stability of the hydrogels varies over a broad range of values. In the case of hydrazide **1**, the most stable gel was obtained in the absence of any carbonyl compound (G' = 3126 Pa), the least stable gel in the presence of furfural (**15**, G' = 761 Pa) if the addition of a single carbonyl compound is considered. It is interesting to note that, in the present case, ketones on average give rise to more stable hydrogels than aldehydes.

§ Please note that the data listed in Table 1, 3 and 4 were recorded without repetitive heating and cooling cycles, which might explain the differences of gel stabilities observed in some cases.

In the case of carbonyl compound mixtures, the most stable gel was observed after addition of benzaldehyde (**16**) and 4-methylacetophenone (**17**) (G' = 1726 Pa), a mixture of an aldehyde and a ketone, the least stable for a mixture of Triplal® (**13**) and furfural (**15**) (G' = 652 Pa), a mixture of two aldehydes. Comparison of the storage modules G' of single compounds and compound mixtures shows that there must be, at least in some cases, a cooperative effect of gel stabilisation or destabilisation. (*R*)-Citronellal (**14**) and furfural (**15**) gave rise to relatively weak hydrogels with **1** (G' = 869 and 761 Pa, respectively), whereas the presence of **13** resulted in relatively stable gels. Equimolar amounts of **13** and **14** and **1** afforded a considerably more stable hydrogel (G' = 1210 Pa) than the corresponding mixture of **13** and **15** (G' = 652 Pa). The data given in Table 5 suggest that furfural (**15**) has a tendency to destabilise hydrogels of **1**, whereas Triplal® (**13**) or 4-methylacetophenone (**17**) seem to have a stabilising effect. This is interesting, as **15** is the most water-soluble compound of the fragrances tested in the present work. Nevertheless, based on the given data, the effect is not readily predictable, as a mixture of **13** and **16** affords a relatively weak gel (G' = 681 Pa) as compared to the respective individual carbonyl compounds (G' = 1365 and 2051 Pa, respectively).

It was already shown by visual analysis of the gels that guanosine (**2**) forms less stable hydrogels than the corresponding hydrazide and that the stability of the gel can be influenced by addition of different carbonyl compounds or compound mixtures. In contrast to hydrazide **1**, guanosine (**2**) itself did not form the most stable hydrogel of the series.

The fact that the stability of the hydrogels prepared from **1** and **2** can be influenced by addition of one or several other compounds indicates that the gel stability is at least in part influenced by the more or less favourable inclusion/reaction of these compounds into/with the gel structure. However, based on the present data, the effect of reversible covalent bond formation on the gel stability cannot readily be estimated.

Table 5 Data of G' and G'' measured for hydrogels prepared from **1** and **2** in the presence or absence of different fragrance aldehydes or ketones after 3–5 cooling and heating cycles by rheology in the dynamic oscillating mode (values taken after *ca.* 1200 s, n.d. = not determined)

	Guanosine-5′-hydrazide (**1**) 15 mM		Guanosine (**2**) 15 mM	
Aldehyde or ketone 7.5 mM	G' [Pa]	G'' [Pa]	G' [Pa]	G'' [Pa]
—	3126	145.1	36	1.1
Trifernal® (**11**)	945	50.1	97	2.6
3,5,5-Trimethylhexanal (**12**)	1342	53.6	136	3.7
Triplal® (**13**)	2051	97.8	22	1.0
(*R*)-Citronellal (**14**)	869	48.1	234	5.7
Furfural (**15**)	761	32.9	173	3.7
Benzaldehyde (**16**)	1365	67.2	68	2.1
4-Methylacetophenone (**17**)	1713	81.2	70	2.2
Benzylacetone (**18**)	1433	60.0	45	1.6
(–)-Menthone (**19**)	1541	64.3	9	0.6
13 + **14**	1210	65.3	310	5.9
13 + **15**	652	29.0	34	1.1
13 + **16**	681	29.6	20	1.5
14 + **15**	1363	74.7	116	2.5
14 + **17**	947	40.3	327	11.0
15 + **16**	690	45.8	106	2.7
15 + **17**	1053	55.8	136	2.5
16 + **17**	1726	73.8	n.d.	n.d.
24	1715	61.0	n.d.	n.d.

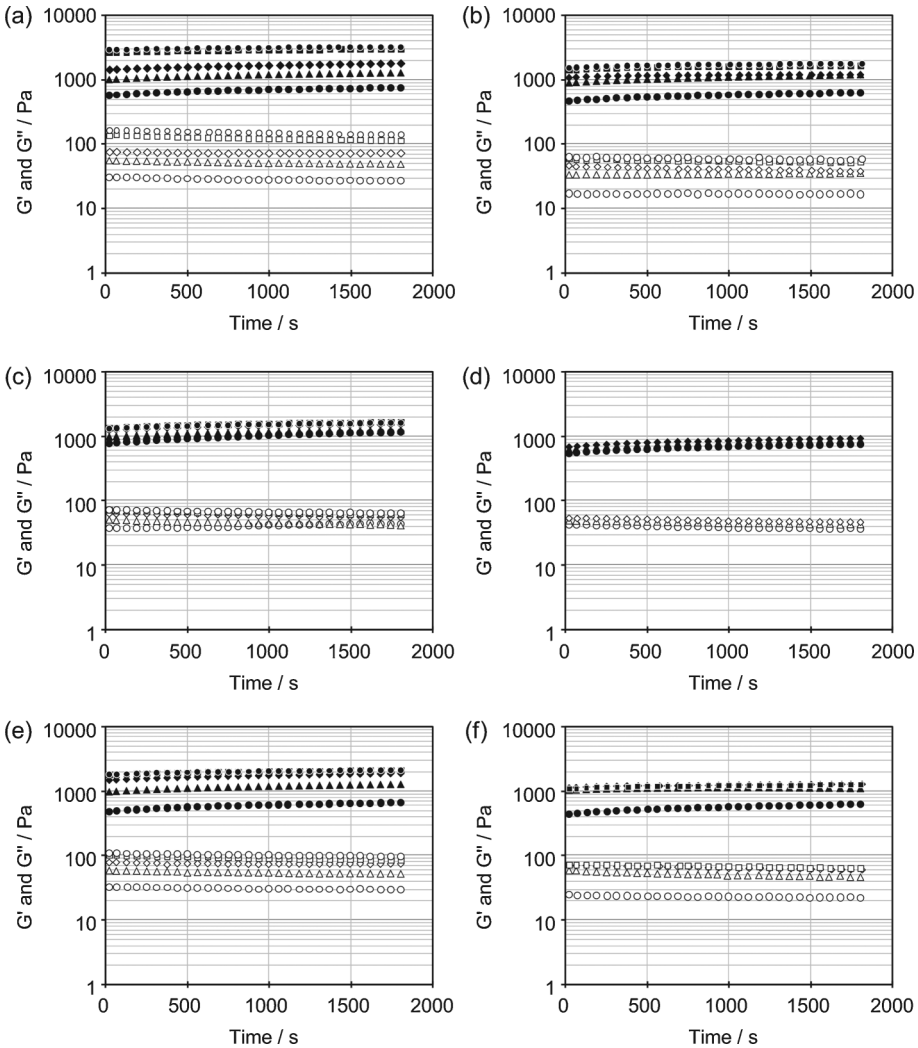


Fig. 6 Increase of G' (full symbols) and G'' (empty symbols) for hydrogels of **1** (a) and **1** in the presence of 0.5 eq. of **24** (b), menthone (**19**, c), (*R*)-citronellal (**14**, d), Triplal® (**13**, e) or an equimolar mixture of **13** and **14** (0.25 eq. each, f) after several consecutive cooling and heating cycles.

Dynamic headspace analysis

As a further step, and in view of practical applications of the hydrogels as air freshener formulations, we investigated the release of an equimolar mixture of two volatile aldehydes and ketones from the hydrogels by dynamic headspace analysis,[26] which allows monitoring the evaporation of the volatile carbonyl compounds without destroying the hydrogel. Headspace analysis is a fast and convenient tool for the analysis of mixtures of volatiles as long as the individual compounds can be separated by GC. In view of possible practical applications as dynamic fragrance delivery systems, we were essentially interested to follow the evaporation of several fragrances at the same time and in particular to see whether or not the presence of other carbonyl compounds influences the evaporation of a given aldehyde or ketone from a hydrogel formed from **1**. Four carbonyl compounds ((*R*)-citronellal (**14**), furfural (**15**), benzaldehyde (**16**), and 4-methylacetophenone (**17**)) spanning a wide range of different vapour pressures (from 309 to 11 Pa, see Fig. 1) were thus chosen to be investigated pairwise. The headspace concentrations measured for the evaporation of a given carbonyl compound from the gel in the presence of one of the other three volatiles are illustrated in Fig. 8 (numerical data to Fig. 8, including standard deviations are given in the ESI†).

The hydrogels were prepared as described above. The hydrogelator (**1**) was mixed with the carbonyl compounds at 80 °C, then the glass vials were closed and left to cool to room temperature overnight. The samples were then submitted to four heating and cooling cycles and left standing at room temperature for 60 h before being opened, to allow evaporation of the fragrance. Headspace samples were taken after 1, 2, 4, 7, 9 and 15 days and the experiment was repeated three times. The data points of the measured headspace concentrations (in Fig. 8) were connected with a line to illustrate the continuity of fragrance evaporation over the entire time frame.

In view of the targeted practical application in perfumery, the headspace measurements were carried out under ambient conditions without controlling the temperature (room temperature) or ambient humidity during fragrance evaporation. The lack of control of these external parameters might explain the relatively large standard deviations observed in some cases. Especially in the

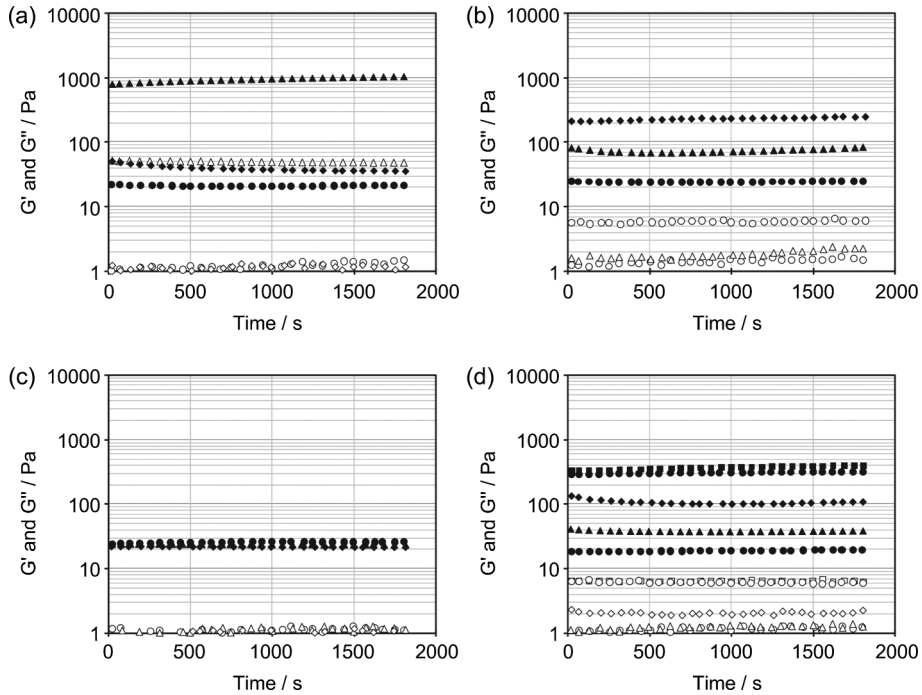


Fig. 7 Increase of G' (full symbols) and G'' (empty symbols) for hydrogels of **2** (a) and **2** in the presence of 0.5 eq. of (*R*)-citronellal (**14**, b), Triplal® (**13**, c), or an equimolar mixture of **13** and **14** (0.25 eq. each, d) after several consecutive cooling and heating cycles.

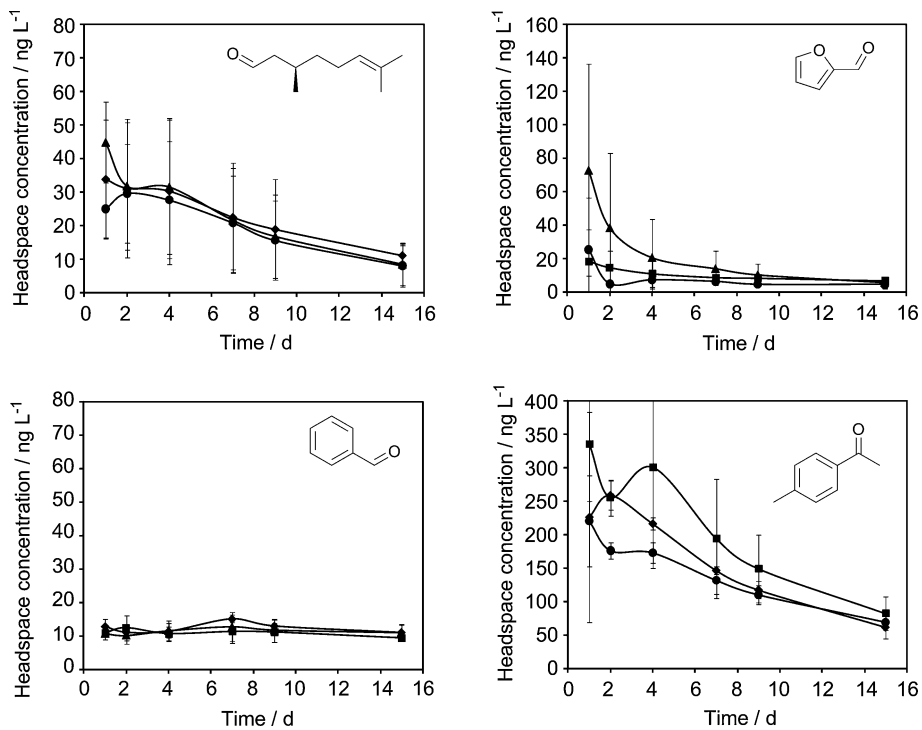


Fig. 8 Headspace concentrations of a given carbonyl compound in the presence of (*R*)-citronellal (**14**, ■), furfural (**15**, ◆), benzaldehyde (**16**, ●) or 4-methylacetophenone (**17**, ▲) evaporated from a hydrogel of **1** (average values of three measurements, numerical data are given in the ESI†).

case of the highly volatile furfural large standard deviations were measured, in particular at the beginning of the measurements.

Considering the three individual measurements for each mixture, it was found that the headspace concentrations for benzaldehyde and citronellal are almost identical within the same measurement, independent of the nature of the second carbonyl compound in the mixture. However, in the case of furfural and 4-methylacetophenone, significantly different headspace concentrations were recorded in the presence of another volatile carbonyl compound in at least one of the three measurements.

The data illustrated in Fig. 8 allow several conclusions. First of all, the evaporation of a given volatile carbonyl compound from the hydrogel is not always influenced by the presence of another compound. Whereas in the case of furfural and 4-methylacetophenone, different headspace concentrations were measured in the presence of different other carbonyl derivatives, the evaporation of benzaldehyde and citronellal were found to be almost unaffected by the presence of other compounds. Furthermore, when considering the rheology measurements summarised in Table 5, it seems that the stability of the gel alone (as expressed by G') does not significantly influence the rates of fragrance evaporation. If the stability of the gel influenced fragrance evaporation, an increased slow release effect would be expected with increasing gel stability. Benzaldehyde (**16**) was released from **1** at similar rates in the presence of either 4-methylacetophenone (**17**) (stable gel, G' = 1762 Pa) or furfural (**15**) (less stable gel, G' = 690 Pa). This suggests that the lower headspace concentrations measured, *e.g.* for the release of **17** in the presence of **16** as compared to those determined in the presence of **14**, might be due to different compositions of the equilibria set-up between the different carbonyl compounds and the hydrogelator (**1**). This may be due to a reversible reaction of the ketone with the hydrogelator upon (partial) formation of the corresponding hydrazone, thus supporting previous results suggesting reversible hydrazone formation within the hydrogel structure.

Conclusions

Reversible covalent bond formation combined with the generation of supramolecular assemblies, in which the active molecules also get physically trapped inside the supramolecular structure, are interesting as delivery systems for bioactive compounds. Following the successful release of fragrance aldehydes and ketones by reversible acylhydrazone formation in aqueous media, we have now investigated the potential of bifunctional guanosine-5′-hydrazide (**1**) as a delivery system for volatile carbonyl compounds from hydrogels.

Previous and present work has shown that bioactive volatile carbonyl derivatives are both physically trapped in the hydrogel structure and, at least partially, covalently linked to the hydrogelator by reversible hydrazone formation. Hydrogels of **1** were found to be generally much more stable than hydrogels of guanosine (**2**), where bioactive compounds can only be physically enclosed in the gel structure. Stable hydrogels of **1** are formed with fragrances having different vapour pressures and water solubilities. Both parameters do not seem to directly influence the gel stability, but of course limited water solubility also limits the amount of fragrance that can be added to the gel.

Oscillating disk rheology measurements after several cooling and heating cycles showed that the gel stability increases after several cycles, indicating that the thermodynamic equilibration of the gel is a rather slow process. Dynamic headspace analysis of mixtures showed that the absolute stability of the hydrogel is not decisive for the evaporation of the fragrances, which means that the physical inclusion is not sufficient to achieve an increased long-lastingness of fragrance evaporation. However, the measurements suggest that equilibria resulting from reversible hydrazone formation are responsible for the different evaporation profiles of some of the compounds.

The fact that a detailed analysis of the gel composition was not possible, limits the current understanding of the different phenomena involved in the inclusion and release process. The effects resulting from a combination of physical inclusion and reversible covalent bonding of bioactive compounds is thus based on indirect evidence only. This is particularly true in the case of more complex mixtures, where a full understanding of the different phenomena is so far not possible.

Experimental

General

General aspects and the instrumentation used are described in the ESI.† Compounds **1**, **4**, **5** and **23** were prepared as previously described in the literature (see ESI†).[10,20,21,25]

Synthesis of (2S,3S,4R,5R)-5-(2-amino-6-oxo-1,6-dihydro-9H-purin-9-yl)-3,4-dihydroxytetrahydro-2-furancarboxylic acid benzylidene hydrazide (6)

A suspension of **1** (300 mg, 0.96 mmol) and benzaldehyde (153 mg, 1.45 mmol) in ethanol (10 mL) was refluxed for 6 h. After cooling to room temperature (r.t.) the mixture was filtered to give 250 mg (74%) of a grey solid as a mixture of two isomers with respect to the amide bond conformation (*syn*/*anti ca.* 64 : 36). UV/Vis (ethanol): λ (ε) 302 (sh, 9800), 275 (23000), 258 (22300), 224 (sh, 12600), 218 (sh, 16800), 203 nm (28400). IR (neat): $\tilde{\nu}_{max}$ 3310 m (br.), 3189 m (br.), 3102 m, 2927 m (br.), 2760 w, 1731 w, 1672 s, 1650 m, 1635 s, 1507 s, 1571 s, 1545 s, 1489 m (br.), 1448 w, 1406 m, 1386 w, 1365 m (br.), 1311 m, 1287 w, 1266 w, 1229 w, 1204 w, 1171 m, 1112 m, 1089 m, 1081 m, 1058 s, 1023 m, 979 w, 956 m, 910 w, 891 w, 876 w, 866 w, 832 m, 792 m, 776 m, 753 m, 733 w, 689 s, 682 s, 635 s, 622 w, 607 s cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-d$_6$, *syn*): δ 11.69 (s, 1 H); 10.88 (s, 1 H); 8.35 (s, 1 H); 8.28 (s, 1 H); 7.77–7.66 (m, 2 H); 7.54–7.39 (m, 3 H); 6.65 (br. s, 2 H); 5.88 (d, J = 6.1, 1 H); 5.67 (br. s, 2 H); 4.61 (dd, J = 6.1, 1.0, 1 H); 4.47 (d, J = 3.1, 1 H); 4.31 ppm (dd, J = 3.1, 1.0, 1 H). ^{1}H-NMR (400 MHz, DMSO-d$_6$, *anti*): δ 11.72 (s, 1 H); 10.81 (s, 1 H); 8.39 (s, 1 H); 8.05 (s, 1 H); 7.77–7.66 (m, 2 H); 7.54–7.39 (m, 3 H); 6.65 (br. s, 2 H); 5.96 (d, J = 7.2, 1 H); 5.67 (br. s, 2 H); 5.34 (m, 1 H); 4.43 (dd, J = 4.1, 3.1, 1 H); 4.26 ppm (m, 1 H). ^{13}C-NMR (100.6 MHz, DMSO-d$_6$, *syn*): δ 166.45 (s); 156.69 (s); 154.42 (s); 151.50 (s); 149.50 (d); 136.24 (d); 134.38 (s); 130.70 (d); 129.20 (d); 127.60 (d); 116.15 (s); 87.34 (d); 83.24 (d); 73.73 (d); 73.41 ppm (d). ^{13}C-NMR (100.6 MHz, DMSO-d$_6$, *anti*): δ 171.51 (s); 156.72 (s); 154.42 (s); 152.03 (s); 144.82 (d); 135.74 (d); 134.26 (s); 130.45 (d); 129.17 (d); 127.41 (d); 115.58 (s); 86.22 (d); 81.40 (d); 75.21 (d); 73.65 ppm (d). MS (ESI): m/z 401 [M+2]$^+$, 400 [M+H]$^+$. HRMS (ESI): m/z calc. for $C_{17}H_{18}N_7O_5^+$: 400.1364 ([M+H]$^+$), found: 400.1258 ([M+H]$^+$).

Preparation of buffer solutions

A 0.5 M potassium acetate buffer (pH 6) was prepared from potassium acetate (46.34 g), glacial acetic acid (1.65 g) and demineralised water (969.55 g, filled up to 1000 mL). For a buffer of a similar composition, a pH value of 5.95 (± 0.045) was measured at 25.0 °C (± 0.21) (Mettler-Toledo MP220 with an InLab 410-Ag/AgCl glass electrode) after two-point calibration.

Similarly, 0.5 M buffer solutions in D_2O (pD 6) were obtained by dissolving potassium acetate (1.1544 g, 11.77 mmol) and glacial acetic acid (0.0397 g, 0.66 mmol) in D_2O (26.79 g, 25 mL), or by dissolving potassium acetate (2.3056 g), glacial acetic acid (0.0794 g), and dioxane (0.266 g, serving as internal standard for NMR measurements) in D_2O (53.49 g, 50 mL).

General procedure for the preparation of hydrogels for visual evaluation

In a glass vial, guanosine-5′-hydrazide (**1**, 37.3 mg, 0.12 mmol) was dissolved using sonication in 7 mL of the above described 0.5 M potassium acetate buffer solution at pH 6. The vial was heated on a water bath (at *ca*. 70 °C) until the hydrazide completely dissolved, then 1 mL of the corresponding aldehyde or ketone (0.06 M) dissolved in the potassium acetate buffer was added, yielding a 15 mM solution of **1** and a 7.5 mM solution of aldehyde or ketone. The sample was left cooling to r.t. In a comparison experiment guanosine (**2**, 33.8 mg, 0.12 mmol) was used instead of **1**. To see whether a gel was obtained or whether the compound precipitated, the samples were inverted and/or analysed visually after cooling to r.t. and after storing at r.t. for 5 days.

General procedure for the determination of the amounts of free fragrances and of fragrances included into the hydrogel

To determine the amount of free hydrogelator and free benzaldehyde within the gel structures, guanosine-5′-hydrazide (**1**, 37.1 mg, 0.12 mmol) was dissolved in 7 mL of a 0.5 M potassium acetate buffer in D_2O (pD 6, see above). Then different amounts of benzaldehyde (**16**, 31.6 mg, 0.30 mmol; 62.9 mg, 0.59 mmol or 94.7 mg, 0.89 mmol) were dissolved in 5 mL of a 0.5 M potassium acetate buffer in D_2O containing dioxane (60.4 mM) as internal standard. For the measurement, 700 μL of the buffer containing **1** and 100 μL of either one of the buffer solutions containing **16**, respectively, were added to a NMR tube together with some sodium 3-trimethylsilyl-tetradeuteriopropionate (as internal lock), yielding a 15 mM solution of **1** with 0.5, 1.0 or 1.5 molar equivalents of **16**, and 0.5 molar equivalents of dioxane. The tubes were heated on a water bath (*ca*. 70 °C) until a clear solution was obtained and then left cooling to room temperature overnight. The ^{1}H-NMR spectra (500 MHz) were recorded on the fully solidified samples. The percentage of free hydrogelator (in mol-%) was determined by integrating the proton signal of H(8) on the guanine group (8.0 ppm, 1 H) with respect to the internal dioxane reference (3.8 ppm, 8 H), and that of free benzaldehyde by integrating the H(3) proton signals of the aromatic ring (7.9 ppm, 2 H).

To investigate the formation of gels with mixtures of fragrance aldehydes and/or ketones 0.075 mol of three different aldehydes or ketones, respectively, were dissolved in 20 mL of the above described 0.5 M potassium acetate buffer (pH 6). In a glass vial, **1** (37.1 mg, 0.12 mmol) was dissolved using sonication in 8 mL of the 0.5 M potassium acetate buffer solution containing the aldehydes, yielding a 15 mM solution of **1** and a 3.75 mM solution for each aldehyde or ketone. To see whether a gel was obtained or whether the compound precipitated, the sample was heated and cooled to r.t. as described above and a comparison experiment using **2** (33.8 mg, 0.12 mmol) instead of **1** was carried out under the same conditions.

At higher fragrance concentrations the mixtures precipitated. A total of 8 mL of a solution containing 0.075 mol of furfural (**15**), benzaldehyde (**16**), and 4-methylacetophenone (**17**) in 10 mL of the above described 0.5 M potassium acetate buffer were added to 0.0374 g of **1** at 70 °C. Filtration of the precipitate, and drying under vacuum afforded 0.04 g of a grey solid containing compounds **1** and **6** (*syn* and *anti* isomers).

Mixtures containing a fragrance aldehyde and a variable amount of **24** were prepared by dissolving guanosine-5′-hydrazide (**1**, 37.3 mg, 0.12 mmol) using sonication in 7 mL of the above described 0.5 M potassium acetate buffer solution. The vial was heated on a water bath (at *ca*. 70 °C) until the hydrazide completely dissolved, then 1 mL of a fragrance aldehyde (0.12 M) and **24** (0 mg, 29.6 mg = 0.015 M, 59.2 mg = 0.030 M or 118.8 mg = 0.06 M) dissolved in the potassium acetate buffer was added, yielding a concentration of **1** and the fragrance aldehyde at 15 mM each and variable concentrations of **24**. The samples were left cooling to r.t. and analysed visually.

Oscillating disk rheology measurements

As described above, guanosine-5′-hydrazide (**1**, 37.3 mg, 0.12 mmol) was dissolved using sonication in 7 mL of a 0.5 M potassium acetate buffer solution at pH 6. The sample was heated on a water bath (at *ca*. 80 °C) until the hydrazide completely dissolved, then 1 mL of a solution of fragrance aldehyde or ketone (0.06 M) in the potassium acetate buffer (or 1 mL of the buffer solution as a reference) was added, yielding a concentration of **1** at 15 mM and the (total) fragrance aldehyde or ketone at 7.5 mM (or 0 mM, reference) each. Alternatively, if two different fragrance compounds were added, 0.5 mL of each of the 0.06 M solutions were used. The sample was placed on the pre-heated rheometer (at 80 °C) and cooled to 20 °C for the measurements. In a comparison experiment guanosine (**2**, 33.9 mg, 0.12 mmol) was used instead of **1**.

Rheology measurements were carried out in the dynamic oscillation mode on a TA instruments AR 1500 rheometer equipped with a Peltier element and a cone plate (40 mm, 4°). The sample was placed on the rheometer at 80 °C, rapidly cooled to 20 °C and left equilibrating at 20 °C for 5 min. To avoid evaporation of the volatiles during the measurements, a film of Neobee® was placed between the border of the rheometer ground plate and the cone plate. G' and G'' were measured over 30 min at 1 Hz and a strain of 0.4%. The heating and cooling cycles were repeated (3 to 5 times) until stable values were obtained for G' and G''. Values for G' and G'' were taken after 1200 s.

Dynamic headspace sampling

As described above, guanosine-5′-hydrazide (**1**, 37.3 mg, 0.12 mmol) was dissolved by sonication in 7 mL of a 0.5 M potassium acetate buffer (pH 6). The sample was heated to 80 °C prior to the addition of 0.5 mL of two of the fragrance solutions (0.60 mmol) containing (*R*)-citronellal (**14**, 92.1 mg), furfural (**15**, 57.8 mg), benzaldehyde (**16**, 63.6 mg) or 4-methylacetophenone (**17**, 80.5 mg) dissolved in 10 mL of buffer, yielding a 15 mM solution of **1** and a 3.3 mM solution for each aldehyde or ketone

(corresponding to a total fragrance content of 7.5 mM). The glass container was closed (to avoid evaporation of the fragrances) and left cooling to r.t. overnight to form the gels. The gels were then submitted to four heating and cooling cycles and left standing at r.t. for 60 h. The glass containers were then opened, and the headspace measured after 1, 2, 4, 7, 9 and 15 d, by placing the samples inside a headspace sampling cell (*ca*. 650 mL), respectively, and exposed to a constant air flow of 200 mL min^{-1}. The air was filtered through active charcoal and aspirated through a saturated solution of NaCl. After equilibration for 15 min, the volatiles were adsorbed during 15 min (after 1 and 2 d), 30 min (after 5, 7 and 9 d) or 40 min (after 15 d) on a clean Tenax® cartridge, respectively. The cartridges were desorbed and analysed as described in the ESI† (General).[6] Headspace concentrations were obtained by external standard calibrations of the corresponding fragrance aldehydes and ketones using ethanol solutions of five different concentrations. 0.1 μL of each calibration solution was injected onto Tenax® cartridges, which were immediately desorbed under the same conditions as those resulting from the headspace sampling. All samples were prepared and analysed in triplicate.

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