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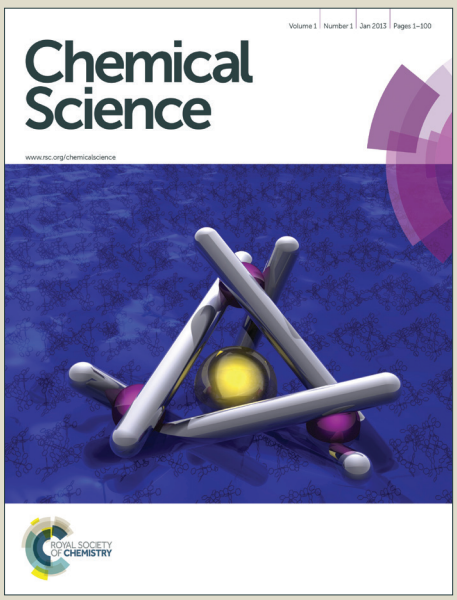

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Supramolecular chemistry with ureido-benzoic acids†

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The controlled self-assembly of multiple molecules into predefined architectures requires highly directional and controllable non-covalent interactions with a high association constant. Here, we introduce the self-complementary ureido-benzoic acid (UBA) quadruple hydrogen-bonding motif. The dimerization constant is in de order of 10^9 M^{-1} in chloroform, which makes it an excellent candidate for supramolecular chemistry in dilute conditions. The self-complementary quadruple hydrogen bonding was confirmed in the solid state by a crystal structure. The applicability of the motif in supramolecular polymers was evaluated by bis-UBA telechelic poly(ethylene-butylene) polymers, which showed a dramatic increase in mechanical properties upon functionalization. The potential of the UBA motif in supramolecular chemistry was further evaluated in solution. One of the synthesized UBA molecules revealed hydrogen bonding to NaPy at high concentrations in chloroform. However, upon dilution the UBA:NaPy hydrogen bonding is disrupted and UBA homodimers are obtained. This shows the potential of NaPy as supramolecular protective group for the UBA molecule, which can be deprotected upon dilution. Furthermore, the dimerization of the UBA motif was reversibly switched between the 'off' and 'on' state using base and acid, demonstrating an alternative method to influence the UBA dimerization. Switching of a UBA molecule in the presence of UPy revealed that UBA dimerization can be selectively switched 'off' and 'on' in the presence of UPy dimers. These results show the applicability and great potential of the self-complementary quadruple hydrogen-bonding UBA motif for supramolecular chemistry.

Introduction

Inspired by the intriguing examples of the complex self-assembled structures created by Nature, developments in the field of supramolecular chemistry pave the way towards supramolecular synthesis; the creation of highly ordered, specific and predetermined structures of multiple molecules based on multiple non-covalent interactions. A key issue for supramolecular synthesis is to gain full control over the assembly behavior, not only in a thermodynamic manner, but also by synthetic design. This will allow the reproducible formation of self-assembled systems, based on multiple non-covalent interactions that are located only at predetermined positions in a molecule. The possibility to weaken or strengthen these non-covalent interactions, for example by temperature or solvent, provides a better control over the aggregation process. In addition, the availability of self-assembling moieties that can assemble in an orthogonal fashion is important as a first step towards realizing the assembly of more complex, multi-molecular systems based on multiple well-defined non-covalent interactions.[1]

While a wide variety of supramolecular interactions have become available, hydrogen bonds are the most versatile and widely applied in supramolecular chemistry due to their high directionality and tunable strength. The directionality of hydrogen bonds allows the molecules to aggregate in an orthogonal fashion, which opens the route towards complex multi-molecular structures.[2] Nowadays, not only highly ordered and complex structures in solution are explored, but also bulk materials with good mechanical properties are developed.[3] In many cases, the self-assembly of hydrogen bonds is isodesmic. A direct consequence hereof is the need for motifs to exhibit high dimerization constants to attain a high degree of polymerization.[4] Our group has gained important insights into the development and characterization of supramolecular systems in the past two decades. Initiated by developments in discotic self-assembled structures,[5] the 2-ureido-4-pyrimidinone (UPy) quadruple hydrogen-bonding motif was developed. This represented a breakthrough because of its high dimerization constant, long life-time and ease of synthesis.[6] The high dimerization constant of the UPy originates from limited repulsive secondary interactions and two attractive secondary

interactions within the motif, and the prearrangement of the motif by an intramolecular hydrogen bond. Intramolecular hydrogen bonding is a powerful tool for creating order within molecules; examples from literature range from the organization of discotic molecules[5] to the creation of foldamers.[7] The intramolecular hydrogen bond is known to preorganise hydrogen-bonding arrays in such a way that their dimerization constants significantly increase.[8]

In recent literature, Zhou and coworkers introduced anthranilic acid (*ortho*-aminobenzoic acid) based molecules. In these systems the intramolecular hydrogen bond between the carbonyl of the benzoic acid and the proton of the alkylated amine was used to planarize the molecule, where after the molecule dimerizes via benzoic acid hydrogen bonding.[9] In the solid state, the moiety shows strong π-π interactions due to the aromatic core of the molecule. The conformational preference of anthranilic acid oligomers due to the intramolecular hydrogen bonding to result in foldamers has also been reported, revealing the potential of this building block.[10] Interestingly, this benzoic acid derivative is fluorescent and is used for fluorescent labeling.[11] Stimulated by our current knowledge of supramolecular chemistry, we became interested in self-complementary hydrogen-bonding motif **1** based on anthranilic acid (Fig. 1). This motif would not only expand the toolbox for supramolecular chemistry, but simultaneously introduces a fluorescent moiety, possibly interesting for imaging purposes or highly sensitive measurements. Furthermore, this motif can be a valuable moiety for use in supramolecular synthesis due to the acid-base type of interaction.

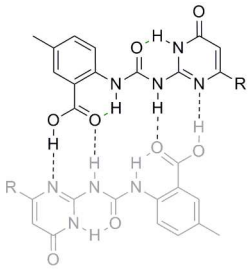


Fig. 1 Proposed dimerization in ureido-benzoic acids **1**.

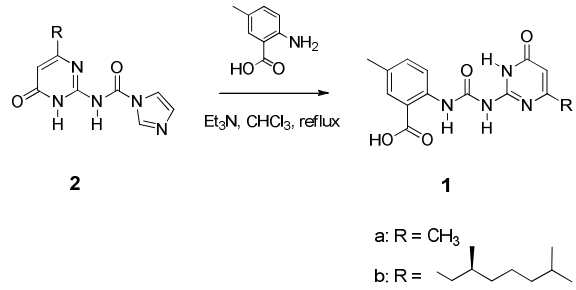
Results and discussion

Design strategy

Self-complementarity is an important prerequisite for a hydrogen-bonding motif; it reduces the amount of synthesis and prevents the need for stoichiometry when creating supramolecular polymers. Carboxylic acid molecules are self-complementary as a result of the Donor-Acceptor (DA) hydrogen-bonding motif and are widely used in bulk materials. However, the double hydrogen-bonding motif does not have a sufficiently high dimerization constant to allow for dimerization at low concentrations. To increase the dimerization constant, the number of hydrogen bonds in the motif should be increased. Keeping in mind the prerequisite for self-complementarity, the motif can be expanded to a DADA array or even longer. After evaluation of different possibilities, we decided to attach the isocytosine to methyl anthranilic acid via a urea linker, creating self-complementary ureido-benzoic acid (UBA) molecule **1** (Fig. 1). While UBA molecules exhibit a self-complementary DADA motif, different tautomers of the isocytosine are possible. In the motif, two intramolecular hydrogen bonds preorganize the molecule into its correct conformation which is anticipated to result in a high dimerization constant.

Synthesis and characterization

The synthesis towards UBA molecules **1** is straightforward. The reaction of 5-methyl anthranilic acid with a 1,1'-carbonyldiimidazole (CDI) activated isocytosine in the presence of base yielded UBA **1** after acidic workup (Scheme 1). The substituent on the isocytosine was varied between methyl and 2(*S*),6-dimethylheptyl. The latter was readily soluble in chloroform and ^{1}H NMR spectroscopy of UBA **1b** in $CDCl_3$ revealed X-H resonances at 17.8, 12.6, 12.2, and 11.0 ppm (Fig. 2). This indicates that all hydrogen-bonding donors are involved in hydrogen bonding. One of the protons shows a surprising downfield shift at 17.8 ppm. In traditional UPy dimers, the NH resonances in $CDCl_3$ appear at 13.2, 11.8, and 10.1 ppm. This indicates that the UBA motif is not dimerized in a fashion similar to UPy dimers. The alkylidene proton at 5.9 ppm is shifted slightly downfield compared to the traditional UPy dimer alkylidene (0.1 ppm). Since the alkylidene proton of the UPy enol tautomer is above 6.2 ppm, this indicates that the motif is in its keto rather than enol tautomeric form.[12] These results all agree with the self-complementary structure as drawn in Fig. 1.



Scheme 1 Synthesis of UBA molecules **1a,b**. Yields: **1a**: 74%; **1b**: 41%.

The NOESY spectrum of **1b** in the hydrogen-bonding region shows clear cross-peaks between the peaks at 17.8, 12.6 and 12.2 ppm (Fig. S1). However, the peak at 11.0 ppm does not show cross-peaks, indicative of a hydrogen bond which is isolated in space from the other hydrogen bonds. Based on this result, the peak at 11.0 ppm is expected to originate from the intramolecular hydrogen bond of the isocytosine moiety to the carbonyl of the urea, since this hydrogen bond is positioned far away from the other hydrogen bonds. Due to its downfield position, the resonance at 17.8 ppm can only originate from the acid proton. Therefore, the peaks at 12.6 and 12.2 ppm originate from the urea NH protons, but no distinction can be made between the intramolecular and intermolecular hydrogen bonded NH.

Additional proof for the proposed hydrogen-bonding array was obtained by X-ray structure analysis. Crystals suitable for single crystal X-ray diffraction could be obtained upon slow diffusion of water vapor into a solution of **1a** in DMF (Fig. 3). The results show that the molecule is indeed the centro-symmetric hydrogen-bonded dimer as proposed in Fig. 1. Two intramolecular hydrogen bonds, which planarize the molecule in a DADA array, can be assigned. Weak hydrogen bonds perpendicular to the dimers connect these units into a one-dimensional chain along the crystallographic *a* axis.

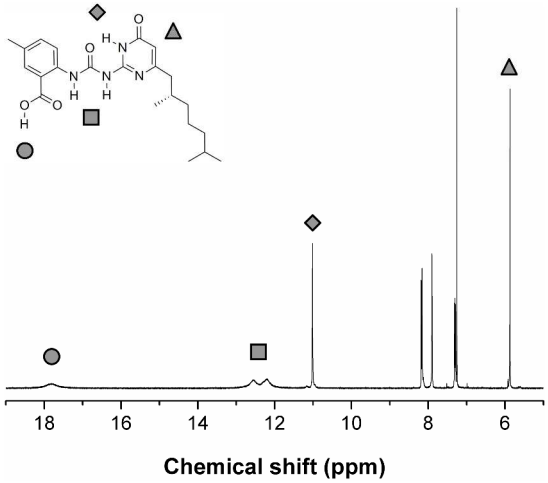


Fig. 2 Partial ^{1}H NMR spectrum of 2(*S*),6-dimethylheptyl UBA **1b** in $CDCl_3$ and assignment of relevant resonances (T = 293 K, c = 1 mM).

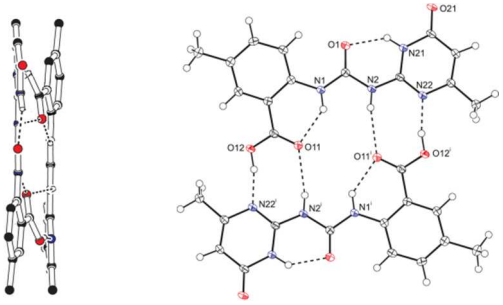
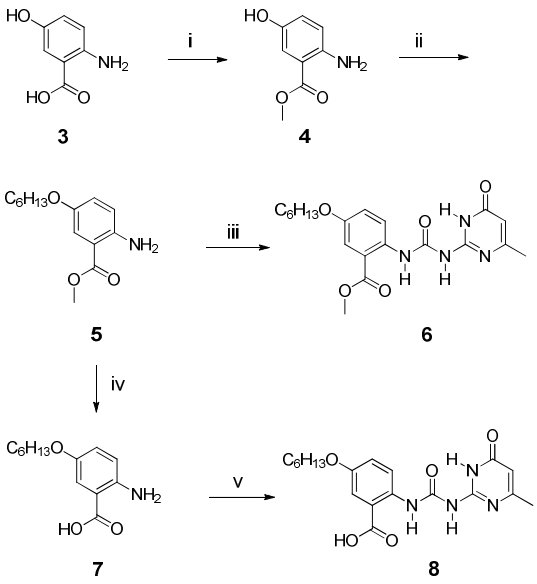


Fig. 3 Side view (left) and top view (right) of the hydrogen-bonded dimer in the crystal structure of UBA molecule **1a**. Displacement ellipsoids are drawn at the 50% probability level. Symmetry code: (i) 1-x, 1-y, 1-z.

Alkoxy derivatives of UBAs

Since the UBA motif shows the desired self-complementary hydrogen-bonding motif both in solution and in the solid state, it is crucial that the motif can be readily attached to other molecules or prepolymers to make it broadly applicable for supramolecular chemistry. Therefore, the synthesis towards ureidobenzoic acid molecule **8** was performed. In this approach we used the commercially available 5-hydroxy-anthranilic acid **3** as a starting material since the additional phenol introduces a handle for further modifications (Scheme 2). First, 5-hydroxy-anthranilic acid (**3**) was esterified to methyl ester **4**, after which a Williamson ether synthesis with hexylbromide was performed. The methyl ester **5** was hydrolyzed to the free acid **7**, and reaction with a CDI activated isocytosine yielded ureidobenzoic acid molecule **8**. As a reference compound, methyl ester **6** was synthesized as well.



Scheme 2 Synthesis route towards molecules **6** and **8**. Reagents and conditions: (i) MeOH, H_2SO_4, reflux, 83%; (ii) NaH, $C_6H_{13}Br$, DMF, 70°C, 45%; (iii) **2a**, Et_3N, dry $CHCl_3$, reflux, 60%; (iv) $LiOH.H_2O$, $MeOH/H_2O$ 9/1 v/v, reflux, 78%; (v) **2a**, Et_3N, dry $CHCl_3$, reflux, 89%.

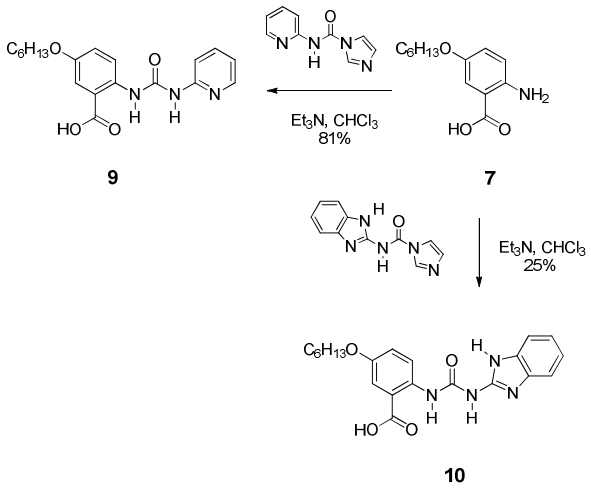
Gratifyingly, the attachment of the hexyl chain significantly increased the solubility of **8** compared to UBA **1a**. Compound **8** is soluble in tetrachloroethane (TCE) and, to a lesser extent, in chloroform. The ^{1}H NMR spectrum of UBA **8** shows the X-H resonances at 18.0, 12.8, 12.2, and 10.9 ppm (Fig. 4). While slightly different compared to the values of **1b** due to the electronic effect of the ether, these positions indicate strong dimerization via hydrogen bonding. The CH_2 next to the oxygen, which originates from the Williamson ether synthesis, is clearly present at 4.0 ppm. Apparently, substituents at the 5-position of the benzene do not interfere with the quadruple hydrogen bonding. This allows the attachment of other groups to the UBA motif via an ether synthesis.

In contrast, the ^{1}H NMR spectrum of methyl ester **6**, which is anticipated to lack the ability of strong dimerization via quadruple hydrogen bonding, shows two broad resonances at 12.5 and 11.0 ppm in $CDCl_3$ (Fig. S2). Most likely, these resonances originate from two intramolecular hydrogen bonds, which corresponds to the interpretation of the NOESY spectrum of UBA **1b**.

Synthesis of UBA derivatives

To investigate whether this novel quadruple hydrogen-bonding motif is broadly applicable, pyridine derivative **9** and benzimidazole derivative **10** were synthesized by reacting the aminobenzoic acid with the corresponding CDI activated amines (Scheme 3).[13] Both **9** and **10** show the same DADA intermolecular hydrogen-bonding motif as found for UBA **8** and are therefore expected to form dimers in solution. UBA **9** lacks the second intramolecular hydrogen bond, which is expected to lower the dimerization constant and could result in the loss of intermolecular hydrogen bonding in dilute solutions. However, ^{1}H NMR reveals X-H resonances at 18.6, 11.6, and 11.0 ppm, indicative of the formation of hydrogen-bonded dimers (Fig. 4). Also UBA **10** shows X-H resonances at 19.1, 14.5, 12.1, and 11.6 ppm, indicative for the formation of hydrogen-bonded dimers. The X-H resonance at 14.5 ppm is significantly different compared to the resonances observed for

UBA molecules **8** and **9** and can be a result of the benzimidazole moiety. All three ureido-benzoic acid molecules show resonances typical for hydrogen-bonding dimers in ^{1}H NMR, revealing the general applicability of the ureido-benzoic acid motif. Dilution to 0.1 mM concentrations did not result in a shift of the X-H resonances or in the appearance of new signals, suggesting that the dimerization constant of UBAs **8-10** is $\geq 10^5$ M^{-1}.[14]



Scheme 3 Synthesis route towards ureido-benzoic acids **9** and **10**.

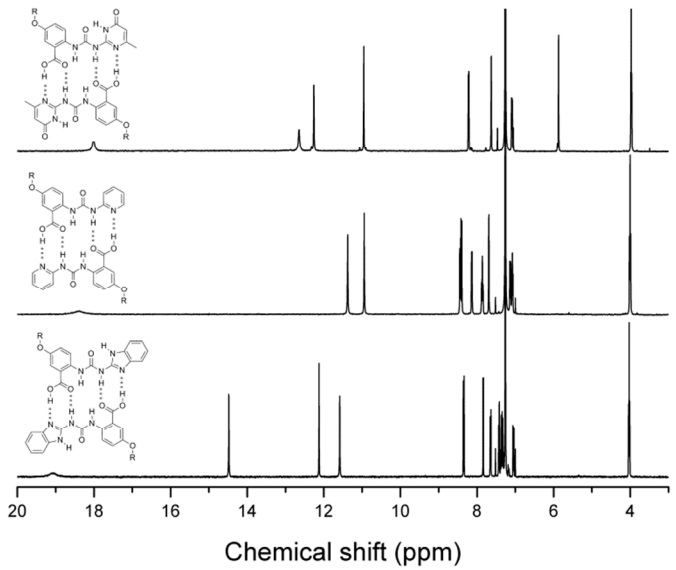


Fig. 4 Partial ^{1}H NMR spectra of UBA molecules **8** (top), **9** (middle) and **10** (bottom), all three displaying strong self-complementary hydrogen bonding in $CDCl_3$ (c = 1 mM, T = 293 K). R = C_6H_{13}.

Determination of the dimerization constant

In the past, strong quadruple hydrogen-bonding motifs via homo- and heterodimerization have been developed and their association constants have been quantified.[15] The potential of UBAs was therefore further explored by assessing their association constant. ^{1}H NMR studies of UBA molecules **8-10** showed the presence of only dimers upon dilution to 100 µM in $CDCl_3$ or TCE-d_2, suggesting that their dimerization constant is $\geq 10^5$ M^{-1}.[14b] All attempts to accurately determine the dimerization constant, however, failed. Therefore, we used the approach of heterocomplexation with NaPy, which was previously successfully applied to determine the dimerization constants of several UPy derivatives.[14] Since UBA **8** comprises an isocytosine moiety similar to UPys, we anticipated that it forms a heterodimer with NaPy (Fig. 5, top).[16] Similar to the UPy:NaPy heterocomplex, the UBA:NaPy heterodimer is expected to give a new absorbance at 355 nm in UV. This permits the determination of the binding constant by competitive binding, in which the amount of UBA:NaPy heterocomplex is dependent on the association constant of the UBA:NaPy complex and the dimerization constant of the UBA homodimers.

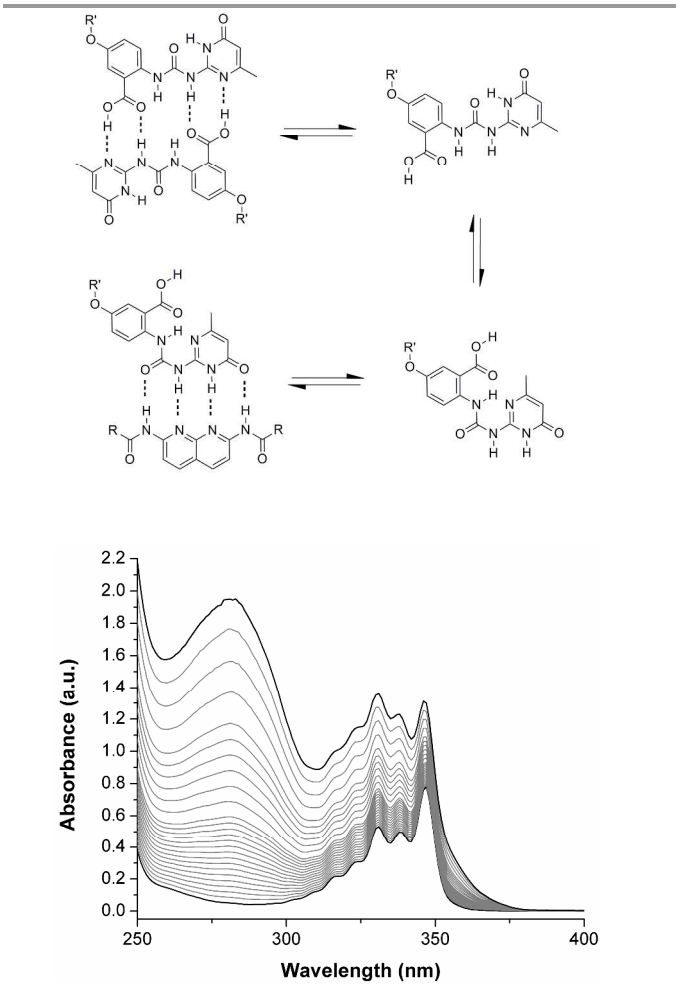


Fig. 5 (Top) Equilibrium of UBA **8** with NaPy (R = $C_{11}H_{23}$, R' = C_6H_{13}); (Bottom) UV-Vis spectra of the titration of up to 5 equivalents of UBA **8** to a 20 µM solution of NaPy in chloroform.

As shown in Fig. 5 (bottom), titration of up to 5 equivalents of UBA **8** to a 20 µM NaPy in chloroform results in the appearance of only a small shoulder above 350 nm, which originates from the increased amount of UBA added during the titration. The absence of UBA:NaPy heterocomplex can be either caused by a high dimerization constant of the UBA homodimer, or by a conformational restraint which does not allow the molecule to adapt the conformation that is necessary to bind to NaPy. Another possibility is a low association constant of UBA molecule **8** to NaPy, resulting in no heterodimerization at 20 µM concentration.

A control experiment was performed with methyl ester **6**, which lacks the ability of strong dimerization by strong quadruple hydrogen bonding but is similar to UBA **8** and therefore will give important information on whether the motif is able to bind to the NaPy. When adding molecule **6** to a 18 μM solution of NaPy in chloroform, a distinct absorbance at 355 nm is observed, indicating heterodimerization (Fig. S3). This indicates that the UBA motif is capable of changing its conformation and binding to a NaPy molecule. As a result, the heterodimerization with NaPy is suppressed at the concentrations for the UV-vis titration experiments because of a very high dimerization constant of UBA **8**.

For the UPy:NaPy heterodimerization, it is known that the equilibrium between homo- and heterodimerization is shifted to heterodimerization when the concentration is increased.[16a] Therefore, the UBA:NaPy titration was repeated at 2 mM concentration and followed by ^{1}H NMR. To ensure sufficient solubility of UBA **8** at these high concentrations, the titration was performed in deuterated tetrachloroethane (TCE-d_2). Upon addition of NaPy, the peaks originating from the UBA homodimers (circles, Fig. 6) decrease and an additional set of peaks at 13.9, 12.8, 11.3, and 10.9 ppm is observed (squares, Fig. 6), which are attributed to the UBA:NaPy heterodimer. The signals of the NaPy amides are positioned between 8.2 and 8.6 ppm and shift downfield with increasing NaPy concentration.

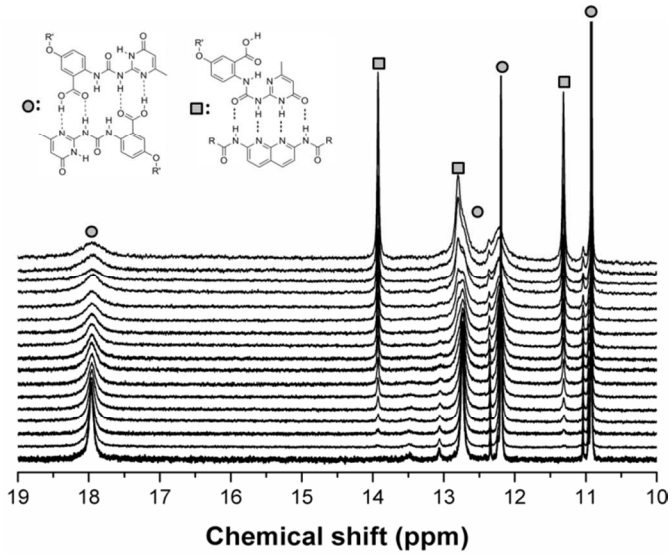


Fig. 6 Result of the NMR titration upon addition of up to 8 equivalents of NaPy in TCE-d_2 to a 2 mM solution of molecule **8**. R = $C_{11}H_{23}$, R′ = C_6H_{13}. Circles correspond to UBA dimers while the squares correspond to the UBA:NaPy complex.

Remarkably, adding even up to 8 equivalents of NaPy does not result in complete heterodimerization. This demonstrates that the dimerization of the UBA is very strong. Fitting of the data obtained from this titration suggest a dimerization constant of the UBA in the order of 10^9 M^{-1} and an association constant of the UBA with the NaPy of approximately $3x10^4$ M^{-1} (Figure S4).[14a] This indicates that the dimerization constant of the DADA array in UBAs is more than 10 times higher compared to the DDAA self-complementary UPy[6b] and ureidoazapterin motif developed by Zimmerman[15a] but still 1000 times lower than the DDDD-AAAA complexes developed by Leigh.[15c] At the same time, the association constant for the UBA:NaPy heterodimer is slightly lower, resulting in less heterodimerization.

The fluorescent properties of benzoic acid moieties make them ideal candidates for sensitive fluorescence spectroscopy measurements. Indeed, ureido-benzoic acids **6**,**8-10** show fluorescent emission upon excitation between 250 and 350 nm with emission maxima around 400 nm. Remarkably, the emission intensity of methyl ester **6** is considerably higher than that of acid **8** (Fig. S4). The normalized emission intensity of UBA **8** increases at concentrations below the nanomolar regime (Fig. S5). It is not uncommon for dye molecules to show quenching at high concentrations, which is attributed to internal conversion and intersystem crossing in the aggregates of the dye molecules.[17] Since the ureido-benzoic acid molecule forms dimers via hydrogen bonding, quenching of the fluorescence emission upon dimerization is therefore plausible. Moreover, benzoic acid hydrogen bonding influences the energy levels of the transition states, revealing more quenching of the fluorescence emission upon hydrogen bonding of the benzoic acids.[18] At the same time, molecule **6** does not dimerize in the concentration regime of the fluorescence experiments. This results in monomers only and thus a stronger fluorescence intensity due to the absence of quenching.

Although fluorescence experiments are highly sensitive, the accuracy of the measurements below the nanomolar regime was insufficient to reliably determine the dimerization constant of **8**. Nevertheless, the fluorescent properties of the ureido-benzoic acids open up the possibility for optical analyses at low concentrations.

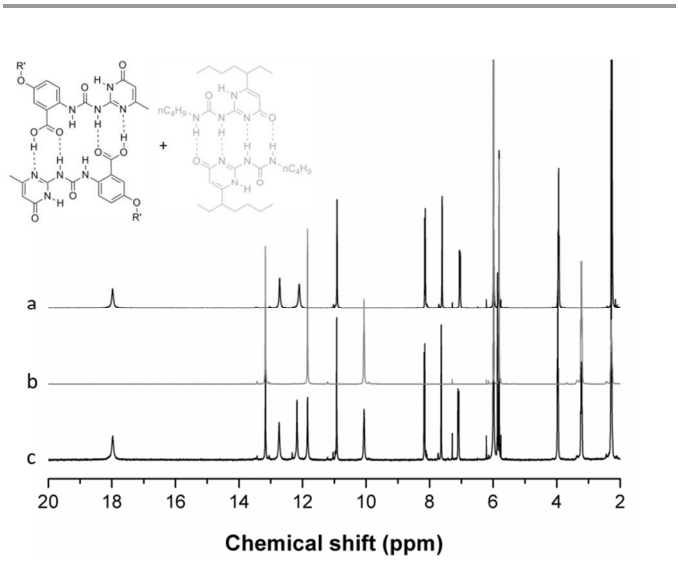


Fig. 7 Partial ^{1}H NMR spectra showing a 3 mM solution of (a) UBA molecule **8** (b) ethylpentyl-UPy and (c) a 1:1 mixture of UBA **8** and UPy in TCE-d_2. R′ = hexyl.

Orthogonal self-assembly of UBA and UPy molecules in solution

Orthogonal self-assembly processes pave the way towards the supramolecular synthesis of highly ordered and complex structures based on multiple non-covalent interactions. One of the advantages of quadruple hydrogen-bonding arrays is their high directionality and high dimerization constant. The high dimerization constant of the UBA and the UPy motif make them interesting candidates for the use in orthogonal self-assembly. Since UBA molecule **8** closely resembles UPy molecules, we evaluated the ^{1}H NMR spectra of a 1:1 mixture of UBA and UPy at 3 mM with the spectra comprising

only UBA or UPy dimers (Fig. 7). No additional peaks could be observed in the mixture, indicating that either UBA or UPy homodimers form in the 1:1 mixture. ^{1}H NMR experiments with UBA molecules **9** and **10** showed similar results. Thus, the self-assembly of the UPy and UBA motifs is fully orthogonal, despite their structural similarities. The high selectivity found for these two hydrogen-bonding motifs highlight the potential of the UBA motif in the supramolecular synthesis of macromolecules.

Reversible off/on switching of the UBA motif

The presence of acidic protons in hydrogen-bonding motifs is a convenient tool to turn "off" or "on" their hydrogen-bond-based dimerization by adding base and acid, respectively.[15d] Also in UBAs, the acid group is essential for the formation of dimers, but at the same time highly susceptible to the presence of a base. Upon addition of a base, the benzoic acid can be deprotonated, which will disrupt the UBA dimers. Since the deprotonation is reversible, acidification of the solution should restore the UBA dimerization.

When 1.5 equivalents of triethylamine are added to a 5 mM solution of molecule **8** in TCE-d_2, the resonances characteristic for quadruple hydrogen bonding disappear. New peaks appear at 13.5 and 12.6 ppm and a broad peak at 11.9 ppm, coinciding with a shift of two of the aryl protons from 8.1 and 7.1 ppm to 8.2 and 7.0 ppm, respectively (Fig. 8). The addition of a stoichiometric amount of trifluoroacetic acid (with respect to triethylamine) to the solution results in an immediate reappearance of the X-H protons at 17.8, 12.7, 12.2, and 10.9 ppm. Next to the disappearance of the resonances characteristic for the quadruple hydrogen bonding upon the addition of triethylamine, the fluorescence emission maximum of a 10 μM solution in chloroform shifts from 406 nm to 452 nm and increases in intensity. This is in agreement with the fluorescence dilution experiments in which the monomer showed higher fluorescence compared to the corresponding dimers, although the charge that is created upon the addition of base may result in different fluorescent properties of the molecule. These results suggest the formation of UBA monomers, possibly triethylamine salts, upon the addition of base. Upon acidification, a shift of the emission maximum from 452 nm back to 406 nm is observed.

The ^{1}H NMR and fluorescence spectra after the addition of base and subsequent acid correspond well to the values found for the starting spectra of dimers of molecule **8**, indicative for full restoration of the UBA dimers. Repetition of the addition of base and subsequent addition of trifluoroacetic acid results in identical behavior, revealing the possibility to repetitively switch 'off' and 'on' the UBA hydrogen bonding within one experiment.[19] The peak at 11.4 ppm increases in intensity after the second addition of trifluoroacetic acid and corresponds to the value found for triethylamine salts that are produced during the switching experiment.

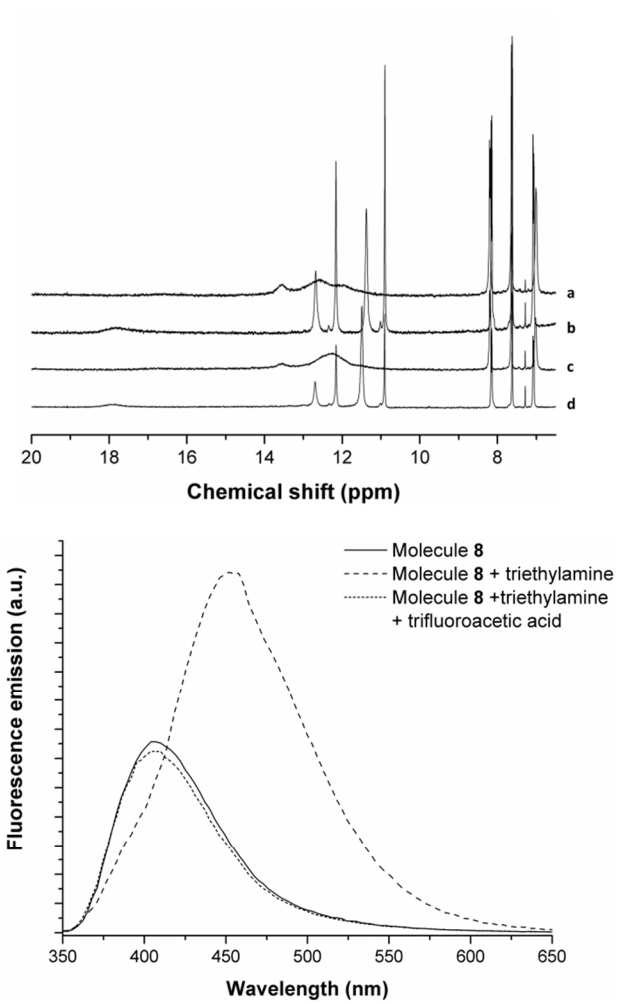


Fig. 8 Top: ^{1}H NMR data of a 5 mM solution of molecule **8** in TCE-d_2 upon addition of 1.5 equivalents triethylamine (a,c) and subsequent addition of 1.5 equivalents trifluoroacetic acid (b,d). Bottom: Fluorescence spectra of a 10 μM solution of molecule **8** (solid line) in chloroform upon addition of 10 equivalents triethylamine (dashed) and subsequent addition of 10 equivalents acid (dotted).

Orthogonal off/on switching of the UBA motif in the presence of UPy molecules

The self-assembly of the UBA motif is orthogonal with that of the UPy motif and, in addition, can be switched off and on by the addition base and acid, respectively. The selective on/off switching of the UBA motif in the presence of UPy dimers would therefore be highly interesting. To evaluate this possibility, on/off switching of a 1:1 mixture of UBA molecule **8** and UPy was investigated. Although 'off' switching with triethylamine is indeed possible, the acidification with trifluoroacetic acid resulted in loss of the UPy dimerization. It is possible that a small excess of trifluoroacetic acid was added, resulting in disruption of the UPy dimers. Therefore, a biphasic system with NaOH and HCl in H_2O was used. Upon the addition of 1.5 equivalents of NaOH, the UBA is deprotonated. Due to the charge created on the molecule, the majority of molecule **8** is transferred to the aqueous droplets in the emulsion, resulting in disappearance of the UBA signals in ^{1}H NMR (Fig. 9). However, the signals of the UPy dimers remain unchanged, indicating that the UPy dimerization is unaffected by the basic conditions. Upon acidification with a stoichiometric amount of HCl, the acid is protonated and

molecule **8** dissolves in the organic layer. The signals of the UBA dimers reappear in the ^{1}H NMR spectrum, although the peaks at 18.0 and 12.7 ppm are broadened, possibly due to exchange with water protons. These results demonstrate the possibility to selectively switch 'on' or 'off' the UBA dimerization in the presence of UPy dimers.

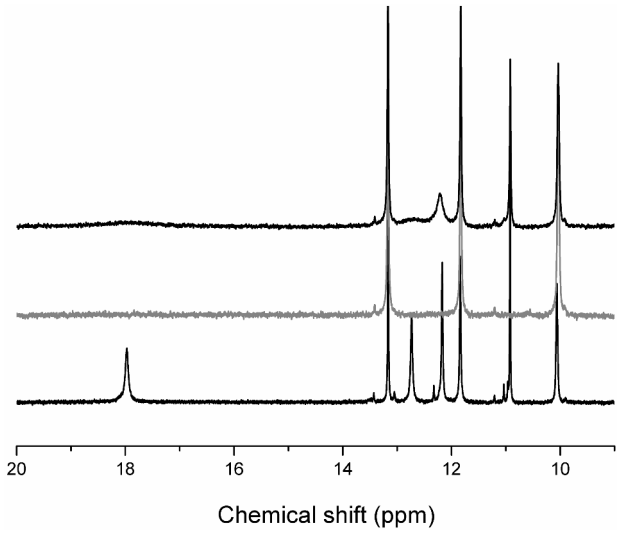


Fig. 9 ^{1}H NMR spectra of the effect of the addition of base (middle) and subsequent addition of acid (top) to a 1:1 mixture of UPy and UBA dimers in TCE-d_2 (bottom, 3 mM each).

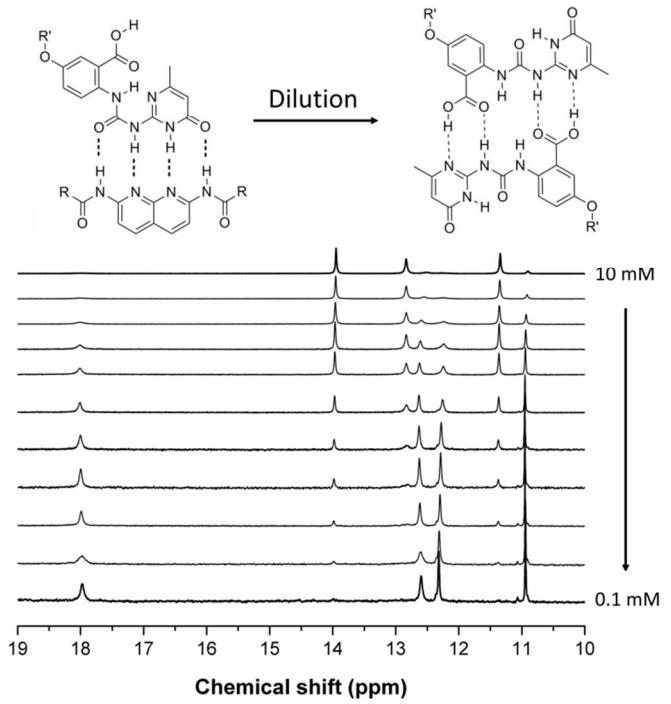


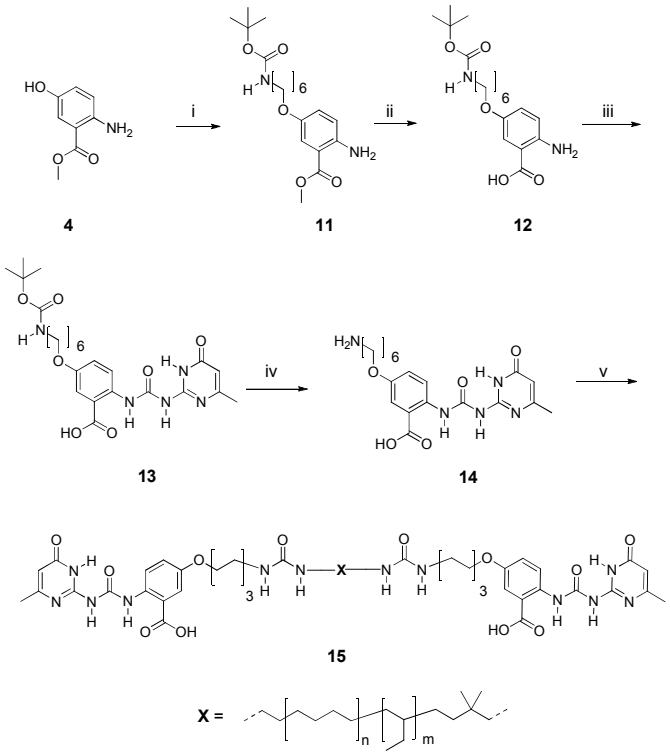
Fig. 10 ^{1}H NMR experiment in $CDCl_3$ showing switching from the UBA-NaPy (top) to the UBA-UBA (bottom) dimer of a solution containing 6 equivalents of NaPy upon dilution. R = $C_{11}H_{23}$, R' = C_6H_{13}.

Dilution-induced deprotection of the UBA motif

The competitive binding experiments of UBA molecule **8** with NaPy at high concentrations show the formation of heterodimers according to ^{1}H NMR, whereas at low concentrations no heterodimers were observed using UV-vis spectroscopy. This suggests that the NaPy molecule can be used as a supramolecular protective group, which can be deprotected by dilution. In other words, the UBA homodimerization can be induced by diluting the system, which is strikingly different to normal aggregation processes.[20] This prompted us to study the dilution induced homodimerization of such a UBA-NaPy system. However, extremely high concentrations are necessary to obtain heterodimerization for a 1:1 mixture of UBA and NaPy. Since the solubility of UBA molecule **8** is limited, 6 equivalents of NaPy were used. This drastically decreases the concentration necessary to obtain UBA:NaPy heterodimers. At a 10 mM concentration of UBA molecule **8** in the presence of 6 equivalents of NaPy a small fraction (9%) of resonances characteristic for the UBA homodimers is present (Fig. 10).[21] A new set of resonances is observed at 13.9, 12.8 and 11.3 ppm, and attributed to the UBA:NaPy heterodimer. Upon dilution the equilibrium between UBA:NaPy heterodimers and UBA homodimers is shifted towards the UBA homodimers: at a 0.1 mM concentration the solution only consists of UBA homodimers and NaPy monomers. It is important to note that at this concentration the association constant of the NaPy should theoretically still be large enough to bind to the UBA, although this is not observed anymore.

Supramolecular UBA polymers

Our approach to functionalize telechelic polymers with the newly developed UBA moiety is depicted in Scheme 4. Molecule **4** was alkylated with *N*-BOC protected 6-aminohexyl bromide. The BOC group was removed by deprotection with TFA and the amine was coupled to CDI activated α,ω-bisamino-poly(ethylene-butylene) to afford UBA-U supramolecular polymer **15**. The supramolecular polymer comprises UBA groups at each end of the oligomer, which are connected to the polymer backbone via urea linkers. The urea linker is expected to impart supramolecular crosslinks via lateral urea hydrogen bonding as is observed for UPy supramolecular polymers.[22] The change in material properties is apparent, since the starting poly(ethylene-butylene) oligomer is a viscous liquid, while after attachment of the UBA motif the material is isolated as a white, fibrous and elastic solid.



Scheme 4 Synthetic scheme towards α,ω-UBA-U polymer **15**. Reagents and conditions: (i) NaH, 6-(Boc-amino)hexyl bromide, DMF, 75°C, 68%; (ii) $LiOH.H_2O$, $MeOH/H_2O$ 9/1 v/v, reflux, 96%; (iii) **2a**, Et_3N, dry $CHCl_3$, reflux, 71%; (iv) TFA, DCM, ambient temperature; (v) *N,N'*-bis-imidazolylcarbonylamino-poly(ethylene-butylene), Et_3N, dry $CHCl_3$, reflux, 44%.

The ^{1}H NMR spectrum of polymer **15** in TCE-d_2 shows the X-H resonances at 10.9, 12.7 and 18.0 ppm (Fig. 11). The positions correspond well to those observed for molecule **8**, but all signals are significantly broadened. Broadening of the resonances corresponding to the aromatic protons and the isocytosine alkylidene proton (5.9 ppm) is also observed. This is accompanied by a downfield shift of the urea protons between 4 and 5 ppm, indicative of hydrogen bonding and lateral aggregation into stacks. This is rather remarkable, since at this concentration (2 mM) no lateral aggregation is observed for the UPy-urea PEB polymers,[23] demonstrating that the additional benzene moiety can stabilize lateral aggregation via π-π interactions.

Variable temperature ^{1}H NMR of polymer **15** shows a sharpening of the aromatic signals and the isocytosine alkylidene proton upon heating. In addition, a downfield shift of the urea protons from 4.2 to 4.7 ppm is observed (Fig. 11). This suggests that the disassembly of the urea stacks occurs at elevated temperatures. The signals corresponding to the intermolecular hydrogen bonds of the ureido-benzoic acid motif disappear upon heating. This can be caused by exchange of the protons with water present in the solvent, which results in disappearance of the signals due to broadening.

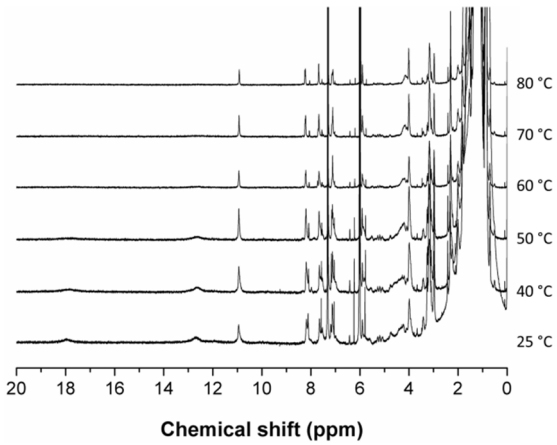


Fig. 11 Temperature-dependent ^{1}H NMR spectra of supramolecular polymer **15** in TCE-d_2 (c = 2 mM).

The mechanical properties of supramolecular polymer **15** were investigated by performing tensile tests on films cast from chloroform. The Young's modulus of the material is 1.18 MPa with an ultimate tensile strength of 0.43 MPa (Fig. 12). For UPy-urea supramolecular polymers, the end groups aggregate into long nano-fibers, creating crystalline crosslinks thereby reinforcing the material.[22] Investigation of the surface morphology of UBA polymer **15** cast from chloroform with atomic force microscopy (AFM) revealed the presence of short nano-fibers on the surface. DSC analysis of the polymer showed a T_g at -54.7 °C characteristic for the glass transition temperature of poly(ethylene-butylene) polymers. The first run showed a melt transition at 169 °C with a melt enthalpy of 2.53 J/g, which could not be observed in the subsequent runs. The low melt enthalpy is in agreement with the short and less densely packed nano-fibers as visualized by AFM and can be the reason for the low toughness of the material. Nevertheless, supramolecular polymer **15** exhibits a remarkable enhancement of its macroscopic properties compared to the unfunctionalized oligomer. This clearly shows the potential of the UBA motif in the field of supramolecular polymer chemistry.

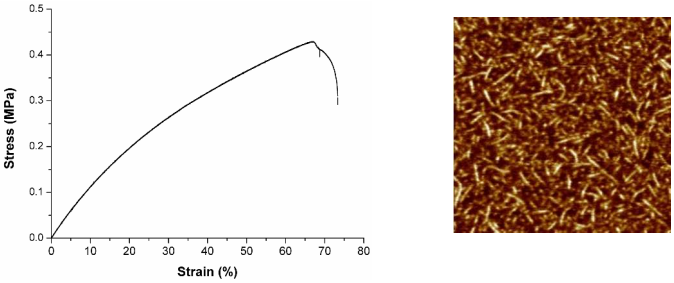


Fig. 12 Mechanical properties of polymer **15** and corresponding tapping mode AFM phase image (500x500 nm)

Conclusions

Here we introduced a novel quadruple hydrogen-bonding motif based on ureido-benzoic acid. Using an easy, straightforward synthesis, a library of different ureido-benzoic acid molecules was synthesized and characterized. ^{1}H NMR revealed strong and self-complementary quadruple hydrogen bonding in organic solutions, while single crystal X-ray diffraction

confirmed the quadruple hydrogen bonding stabilized by two intramolecular hydrogen bonds in the solid state. Characterization of ureido-benzoic acid molecule **8** in dilute solution revealed an extremely high dimerization constant in the order of 10^9 M^{-1} in chloroform. The molecules are fluorescent, which make them attractive for use in highly sensitive or highly diluted measurements. Fluorescent dilution experiments revealed an increase in fluorescence emission intensity upon dissociation of the dimers into monomers. Although fluorescence spectrometry is a highly sensitive technique, the fluorescence could only be measured reliably down to 10^{-10} M^{-1}, which is insufficient to determine the association constant via this technique. The functionalization of poly(ethylene-butylene) telechelic oligomers with an UBA-U motif resulted in a dramatic increase of the macroscopic properties of the polymer and revealed the possibility to create supramolecular polymers based on the ureido-benzoic acid motif. AFM showed the formation of short nano-fibers. Possibly the crystallization into the nano-fibers can be improved by changing the spacer between the ureido-benzoic acid and the urea moiety.

The aggregation of the ureido-benzoic acid motif with UPy revealed orthogonal self-assembly of UBA and UPy molecules. Even though molecule **8** exhibits a molecular structure that shows similarity to UPy molecules, the presence of UBA does not influence the UPy hydrogen bonding significantly and vice versa. Due to the high dimerization constants of both hydrogen-bonding motifs, this allows for the orthogonal self-assembly in dilute conditions.

Interestingly, the strong quadruple hydrogen bonding can be switched 'off' and 'on' by the use of base and acid, respectively. Upon the addition of base, the benzoic acid group is deprotonated, resulting in disruption of the UBA dimers according to ^{1}H NMR and fluorescence spectroscopy. When acidifying the solution, the characteristic peaks of the UBA dimers return. The switching behavior is feasible at room temperature using a small excess of base, and the switching can be repeated. To combine the orthogonal self-assembly of UBA and UPy molecules with the reversible 'on' or 'off' switching of the UBA molecules, switching experiments were performed in a 1:1 mixture of UBA and UPy. When introducing an aqueous layer containing a base or acid to the organic layer containing UBA and UPy dimers allowed for the selective 'off' and subsequent 'on' switching of the UBA dimerization in the presence of UPy dimers. The switching of the biphasic system proved to be orthogonal with UPy aggregation. This method of influencing the dimerization of the UBA hydrogen bonding could therefore be a valuable tool in supramolecular synthesis.

Molecule **8** displayed UBA:NaPy heterodimerization only at high concentrations due to the high dimerization constant of the UBA. Simple dilution of a mixture containing UBA:NaPy heterodimers resulted in the quantitative conversion into UBA homodimers and NaPy monomers. This UBA:NaPy heterodimerization could have a potential application as supramolecular protective group in supramolecular synthesis, where deprotection can be obtained by simple dilution of the system.

The experiments shown in this paper exemplify the potential of the ureido-benzoic acid quadruple hydrogen-bonding motif in supramolecular chemistry. Due to the high dimerization constant of the UBA motif, a wide concentration regime is available for self-assembly in dilute conditions. In combination with the switching capabilities of the UBA motif, this motif shows promising properties for potential use in the supramolecular synthesis of macromolecules. Using the orthogonal UBA and UPy self-assembly and the dilution induced deprotection of the UBA:NaPy heterodimers, this may be a first step towards the controlled self-assembly of complex macromolecular structures based on the ureido-benzoic acid hydrogen-bonding motif. Currently we are evaluating the applicability of the UBA motif in the supramolecular synthesis of supramolecular polymers.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Full experimental details, spectroscopic data and selected spectra. See DOI: 10.1039/b000000x/

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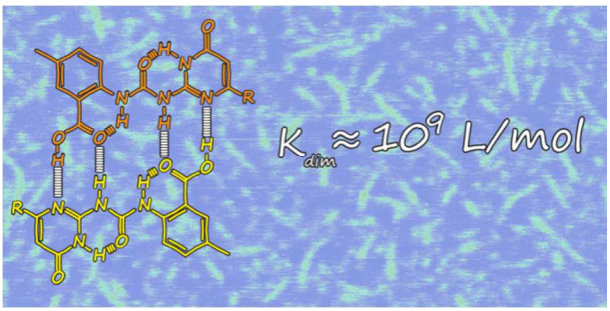
TOC:
Supramolecular chemistry with ureido-benzoic acids

Wilco P.J. Appel, Marko M.L. Nieuwenhuizen, Martin Lutz, Bas F.M. de Waal, Anja R. A. Palmans and E.W. Meijer*



A novel, complementary quadruple hydrogen-bonding motif is presented that shows very strong dimerization and is switchable with pH.