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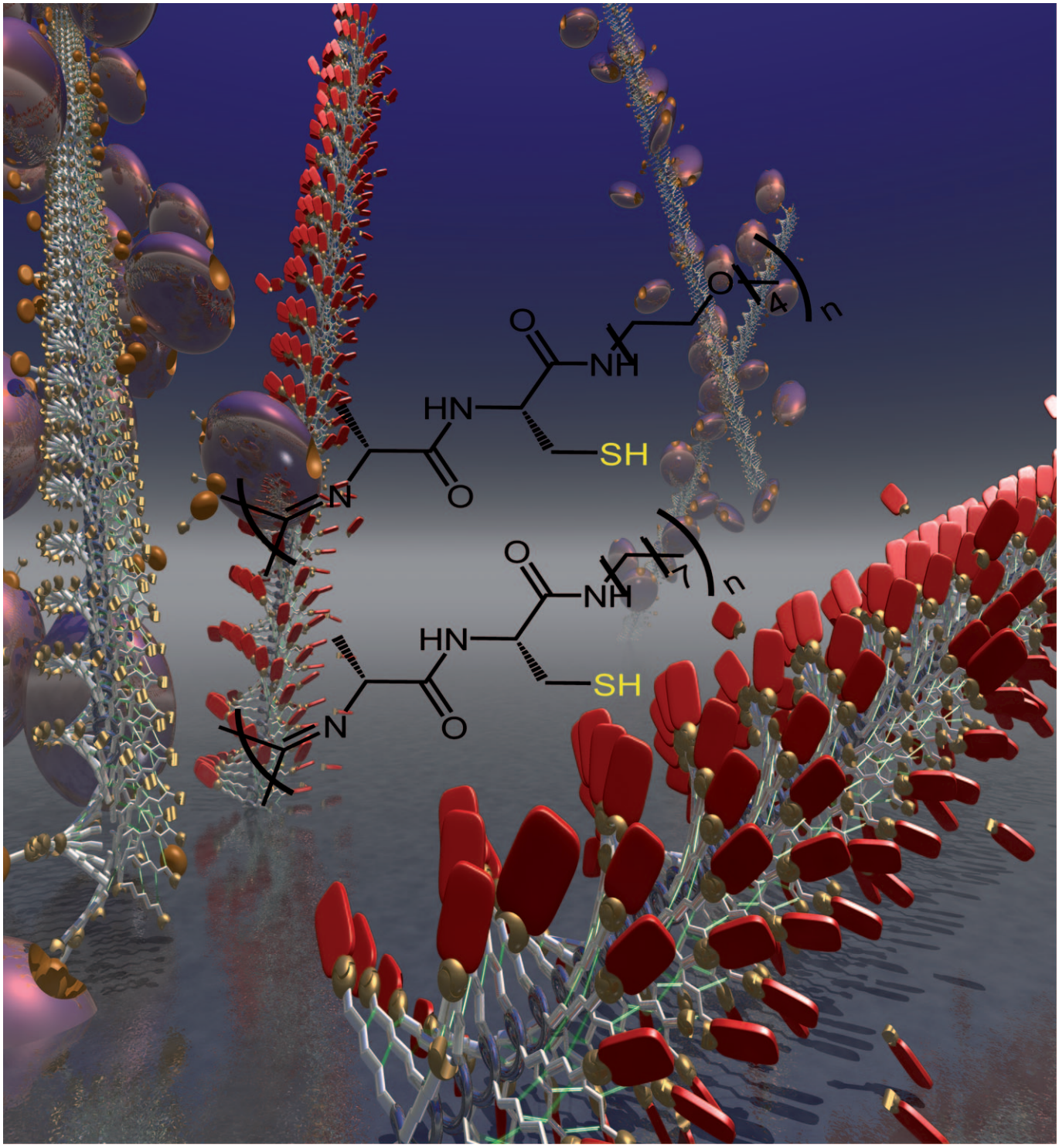
# Cysteine-Containing Polyisocyanides as Versatile Nanoplatforms for Chromophoric and Bioscaffolding

Stéphane Le Gac,*[a, b] Erik Schwartz,[a] Matthieu Koepf,[a] Jeroen J. L. M. Cornelissen,[a, c] Alan E. Rowan,*[a] and Roeland J. M. Nolte*[a]



**Abstract:** The straightforward syntheses of polyisocyanides containing the alanine–cysteine motif in their side chains have been achieved. Detailed characterization of the polymers revealed a well-defined and highly stable helical conformation of the polyimine backbone responsible for the formation of rodlike structures of over one hundred nanometers. The $4_1$ helix is further stabilized by β-sheet-like interactions between the peptide arms. As a result, the cysteine sulfur atoms are regularly aligned along the polymer axis, which provides a unique platform for the scaffolding of various entities by using versatile click-chemistry post-modification approaches. For instance, pyrene derivatives were introduced through thio-specific reactions involving either maleimide, iodoacetamide, or thioester groups, leading to arrays of stacked chromophores with excimer-like emission. A water-soluble cysteine-rich polyisocyanide was successfully biotinylated and coupled to streptavidin.

**Keywords:** bioscaffolding · chromophoric scaffolding · click chemistry · helical structures · polyisocyanides

## Introduction

Molecular platforms can be defined as stable discrete entities, which bear functional groups in well-defined positions to allow efficient modification with a wide range of compounds to give access to novel structures with unique properties. Macrocyclic compounds, such as cyclodextrins,[1] calixarenes,[2] resorcinarenes,[3] and cyclotriveratrylenes,[4] are among the most popular platforms since they possess a rigid bowl-shape structure that can be readily functionalized in a spatially controlled manner. They provide attractive possibilities for the organization of chromophores, for example, for sensor applications, for the binding of substrate molecules, or for assembly with other molecules leading to more complex structures.[5] The organization of functional groups in a precise manner over long-range distances requires, however, the use of well-defined macromolecular platforms.

Recently, natural biomacromolecules, such as DNA[6] and viruses,[7] have been diverted from their natural function to act as macromolecular platforms, especially for chromophoric scaffolding in view of light-harvesting applications. The use of their primary covalent skeleton in combination with secondary noncovalent interactions allows access to programmable assemblies with a high degree of control of their architecture over long distances. New biohybrid materials with astonishing properties are expected to bud in the coming years. To date, only a few synthetic analogues of such well-defined biomacromolecular platforms have been described. We have focused our attention to a particular class of helical, stiff polymers, that is, the polyisocyanides.[8] Isocyanides can be polymerized by the catalytic action of nickel(II) ions (Scheme 1a).[9] The resulting polymers are helical because of restricted rotation around the single bonds connecting the main-chain carbon atoms. The helical conformation can be further stabilized by the introduction of peptide residues, which provide intramolecular hydrogen-bond interactions between the side chains, reminiscent of the interactions in β-sheet structures (Scheme 1b).[10] The so-called polyisocyanopeptides constitute a proteinlike polymer backbone, which can be micrometers long and possess a high persistence length (i.e., $>76$ nm). Among various possible applications, these polymers provide a unique platform for the organization of chromophores. The $n$th and $(n+4)$th positions are regularly positioned in an array along the poly-

[a] Dr. S. Le Gac, Dr. E. Schwartz, Dr. M. Koepf, Prof. Dr. J. J. L. M. Cornelissen, Prof. Dr. A. E. Rowan, Prof. Dr. R. J. M. Nolte
Institute for Molecules and Materials
Radboud University Nijmegen, Toernooiveld 1
6525 ED Nijmegen (The Netherlands)
Fax: (+31) 24-36-53-393
E-mail: stephane.legac@univ-rennes1.fr
a.rowan@science.ru.nl
r.nolte@science.ru.nl

[b] Dr. S. Le Gac
Present address: Sciences Chimiques de Rennes
Université de Rennes1, UMR CNRS 6226
35042 Rennes Cedex (France)

[c] Prof. Dr. J. J. L. M. Cornelissen
Present address: Laboratory for Biomolecular Nanotechnology
MESA+ Institute, Faculty of Science & Technology
University of Twente, P.O. Box 217
7500 AE Enschede (The Netherlands)

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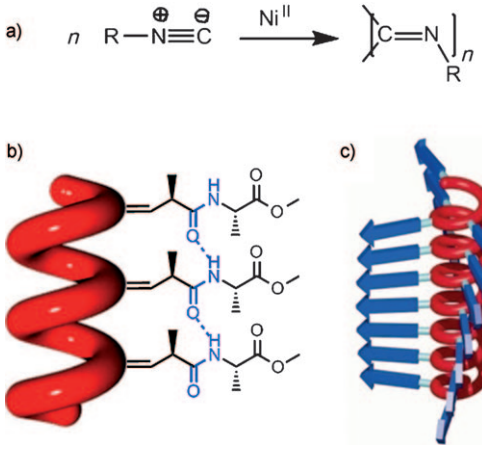



Scheme 1. a) Nickel-catalyzed conversion of isocyanides into polyisocyanides. b) Schematic representation of the hydrogen-bonding network present between the alanine units of the side chains in poly(L-isocyanoalanyl-L-alanine methyl ester) (L,L-PIAA). c) Cartoon of the $4_1$ helical conformation found in polyisocyanopeptides.

mer backbone (Scheme 1c) and attaching porphyrins[11] or perylene-diimides (PDI)[12] leads to well-defined pathways for the fast migration of excitons and electrons, a requirement for optoelectronic applications.

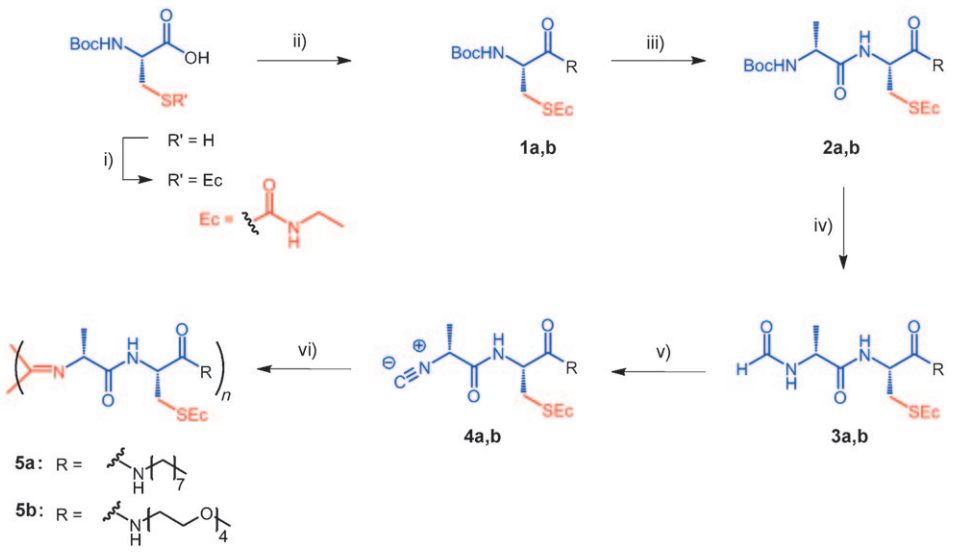
As a more tunable approach to access chemical diversity, we have recently introduced acetylene pendant side chains into the polymers, which allowed their post-modification by using the copper-catalyzed [3+2] Huisgen cycloaddition with azide derivatives.[13] Water-soluble dye-containing polymers, which are promising as novel biomarker materials, have been obtained from these "click"able polyisocyanides.[14] As part of this program, we also became interested in introducing thiol functionalities into the lateral chains to extend the postmodification strategy and to increase the applications of these well-defined macromolecules. Thiol-containing polymers can be readily functionalized by reaction with maleimides,[15] iodoacetamides,[16] other thiols,[17] acrylates,[18] or ene compounds[19] under mild conditions. These reactions form a "click toolbox" that enlarges the possibilities of post-modification. Herein, we present our initial work on the synthesis of cysteine-functionalized polyisocyanides. Our aim is to develop a proteinlike scaffold, rich in precisely positioned thiols, for applications in chromophoric and bio-scaffolding.

## Results and Discussion

For the side chains of the desired helical platforms, the (D,L)-alanine–cysteine dipeptide motif was chosen on the basis of our previous work (Scheme 2). The steric hindrance generated by the alanine methyl group was expected to induce the required helical folding of the polymer, while maintaining a good reactivity of the isocyanides. To obtain polymers soluble in organic and aqueous media, we planned to introduce two different side chains, an octyl and a tetraethyleneglycol chain. The cysteine sulfur atom was protected by converting it into a thioethylcarbamoyl moiety. This protecting group is easily removed under basic conditions and is stable under acidic conditions.

Hence (L)-Boc-cysteine was first reacted with ethyl isocyanate to give the corresponding S-protected cysteine. This compound was subjected to a peptide coupling procedure with either octylamine or 4,7,10,13-tetraoxotridecane amine[20] in the presence of EDC to give compounds **1a** and **1b** in good yields. The Boc group was then removed by treatment with TFA and the corresponding ammonium salts were reacted with (D)-Boc-alanine (EDC coupling) to furnish the dipeptides **2a** and **2b** (88 and 64%, respectively). The corresponding formamides **3a** and **3b** were obtained in good yields upon treatment with TFA followed by refluxing in ethyl formate by using a slight excess of sodium formate. The conversions into the isocyanides were done successfully by using an excess of diphosgene and NMM at −30°C in THF, leading to the desired precursor monomers (86 and 53% for **4a** and **4b**, respectively). Their polymerization was achieved by adding a catalytic amount of $Ni^{II}$ (0.002 equiv) in dichloromethane. IR spectroscopic monitoring indicated a fast polymerization process since no more monomer was present after less than one hour, as attested by the disappearance of the CN vibration at $\nu = 2143\ cm^{-1}$. Polyisocyanides **5a** and **5b** were isolated by precipitation in a mixture of $CHCl_3$/MeOH and $CHCl_3$/$Et_2O$ in yields of 98 and 90%, respectively. As expected with the introduction of the tetraethylene glycol side chains, **5b** was found to be soluble in water ($6\ mg\,mL^{-1}$).

Polyisocyanides **5a** and **5b** were characterized by using different techniques. Their $^1H$ NMR spectra in $CHCl_3$ were extremely broad (see the Supporting Information), in agreement with their polymeric nature, and no interpretable data could be extracted from these spectra. GPC analyses also confirmed the presence of polymeric materials (see the Supporting Information in the case of **5b**) but, because of the rodlike structure and the high persistence length of the polyisocyanides,[21] ill-defined traces were obtained precluding the determination of the molecular weights. More interest-




Scheme 2. Synthesis of polymers **5a** and **5b**: i) EtNCO, TEA, $CH_2Cl_2$, RT, 91%; ii) 1) TFA, $CH_2Cl_2$, RT, 2) $OctNH_2$ or 4,7,10,13-tetraoxotridecane amine, EDC, DIPEA, HOBt, $CH_2Cl_2$, RT, 90 (**1a**), 93% (**1b**); iii) 1) TFA, $CH_2Cl_2$, RT, 2) (D)-Boc-alanine-OH, EDC, DIPEA, HOBt, $CH_2Cl_2$, RT, 88 (**2a**), 64% (**2b**); iv) HCOOEt, HCOONa, reflux, 85 (**3a**), 97% (**3b**); v) diphosgene, NMM, THF, −30°C, 86 (**4a**), 53% (**4b**); vi) $Ni(ClO_4)_2$ cat., $CH_2Cl_2$, RT, 98 (**5a**), 90% (**5b**). Boc = *tert*-butoxycarbonyl; DIPEA = *N*,*N*-diisopropylethylamine; EDC = *N*′-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide; HOBt = 1-hydroxybenzotriazole; NMM = *N*-methylmorpholine; TEA = triethylamine; TFA = trifluoroacetic acid.

ing information came from AFM measurements. Figure 1a and b show representative images of **5a** and **5b**, respectively, obtained from the spin-coating of $CHCl_3$ solutions of the

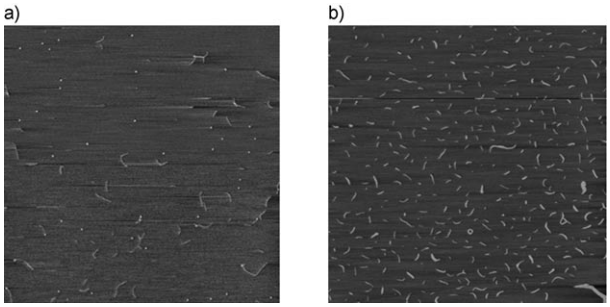



Figure 1. Representative AFM micrographs (5×5 μM) of polyisocyanides **5a** (a) and **5b** (b) spin-coated on mica from $CHCl_3$ solutions ($10^{-6}$ M).

polymers. Single-chain polymers are visible and their rodlike structures are clearly evidenced. Statistical analyses revealed an average length of ≈92 ±14 nm for **5a** (DP = 780) and of ≈116 ± 16 nm for **5b** (DP = 990). These correspond to average molecular weights of ≈300 and ≈450 kDa, respectively.[22]

CD spectra of **5a** and **5b** in $CHCl_3$ showed a pronounced negative Cotton effect at $\lambda = 310$ nm, which is related to the n→π* transition of the imines (Figure 2a) and characteristic for the one-handed helical folding of the polymers responsible for the rodlike structures observed by AFM. In such a conformation, the *n*th and (*n*+4)th side chains are stacked and these polymers are expected to present four rows of well-positioned sulfur atoms (Figure 2a). Interestingly, the CD signal of **5b** was the same in $CHCl_3$ and in water. This is in contrast with previous results in which the water solubility was provided by the ester hydrolysis of the ester functions in the side chains,[14b] leading to charged anionic polymers, which might be partially unfolded due to the harsh hydrolysis conditions. The thermal stability of this helical secondary structure was impressive since, upon warming tetrachloroethane solutions of **5a** and **5b** at 100 °C for one and 1.5 hours and cooling back down, no loss in the CD signal intensity was observed (see the Supporting Information). Polyisocyanide **5b** was also very stable in water since, despite the fact that the polymer precipitated at an elevated temperature, no loss of chirality was found upon warming at 90 °C for 35 min and cooling back to room temperature (see the Supporting Information).[23] This high conformational stability is likely due to interchain hydrogen-bonding interactions between the amide units in the *n*th and (*n*+4)th positions. Indeed, comparison of the IR spectra of the monomers and the polymers in $CHCl_3$ solutions revealed a shift to lower frequencies for the amide I vibrations and for the NH stretching vibrations upon polymerization (Table 1, entries 2 vs. 1 and 4 vs. 3). This shift is characteristic of β-sheet-like interactions between the stacked side chains in the polymer, which strongly reinforces the helical structure.[10] These intrastrand supramolecular interactions were also supported by molecular mechanical calculations upon using the Spartan program (Force Field method). From Figure 2b, it can be seen that both amide units of the side chains are involved in hydrogen-bonding interactions (average *d*[O–N] = 2.7 Å). This allows an accurate positioning of the cysteine sulfur atoms with an interstrand distance of about 4.7 Å, which is similar to what was reported before on the basis of measurements[10,11] and molecular dynamics calculations.[12f] All these data confirmed the presence of polymers with well-defined structures that combined all the required features (good solubility, precise geometry, and high stability) to behave as unique macromolecular scaffolds upon deprotection of the cysteine side chains.

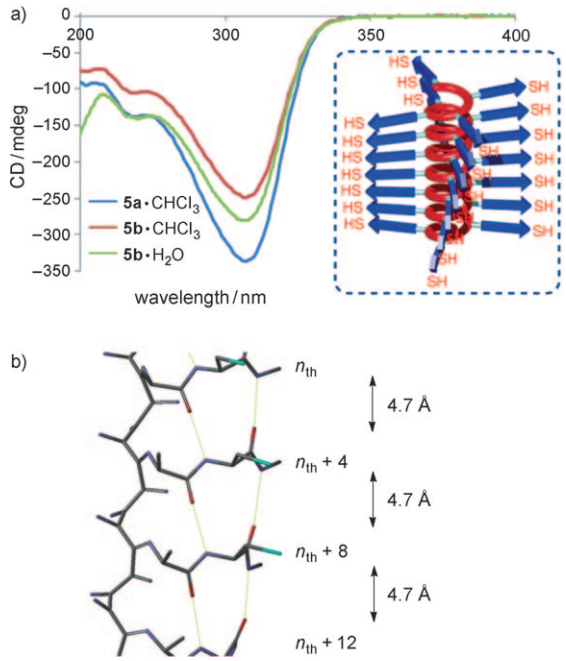



Figure 2. a) CD spectra at room temperature of **5a** in $CHCl_3$ (1.2 mM), of **5b** in $CHCl_3$ (0.9 mM), and of **5b** in water (0.72 mM). Inset: cartoon representation of the cysteine-containing helical platforms. b) MMFF-calculated structure of **6a** (Spartan 04 V1.0.1; 32 repeat units), the dashed lines indicate the hydrogen bonds. Only one strand of side chains is displayed to give a clearer view of the interchain interactions and the octyl end groups have also been omitted for clarity.

Table 1. IR data for the isocyanide monomers and their corresponding polymers ($CHCl_3$ solutions, RT; $\nu$ [$cm^{-1}$]).

| Entry | Compound | $\nu$ NH | $\nu$ amide I |
|---|---|---|---|
| 1 | **4a** | 3429; 3326 | 1678 |
| 2 | **5a** | 3246 | 1645 |
| 3 | **4b** | 3429; 3314 | 1677 |
| 4 | **5b** | 3252 | 1644 |

The removal of the cysteine-protecting ethylcarbamoyl group, which is usually performed in basic conditions, was first tested with the formamide precursor **3a**. This compound was dissolved in a 5:1 $CHCl_3$/MeOH mixture and ammonia was bubbled through this mixture for 15 min. Moni-

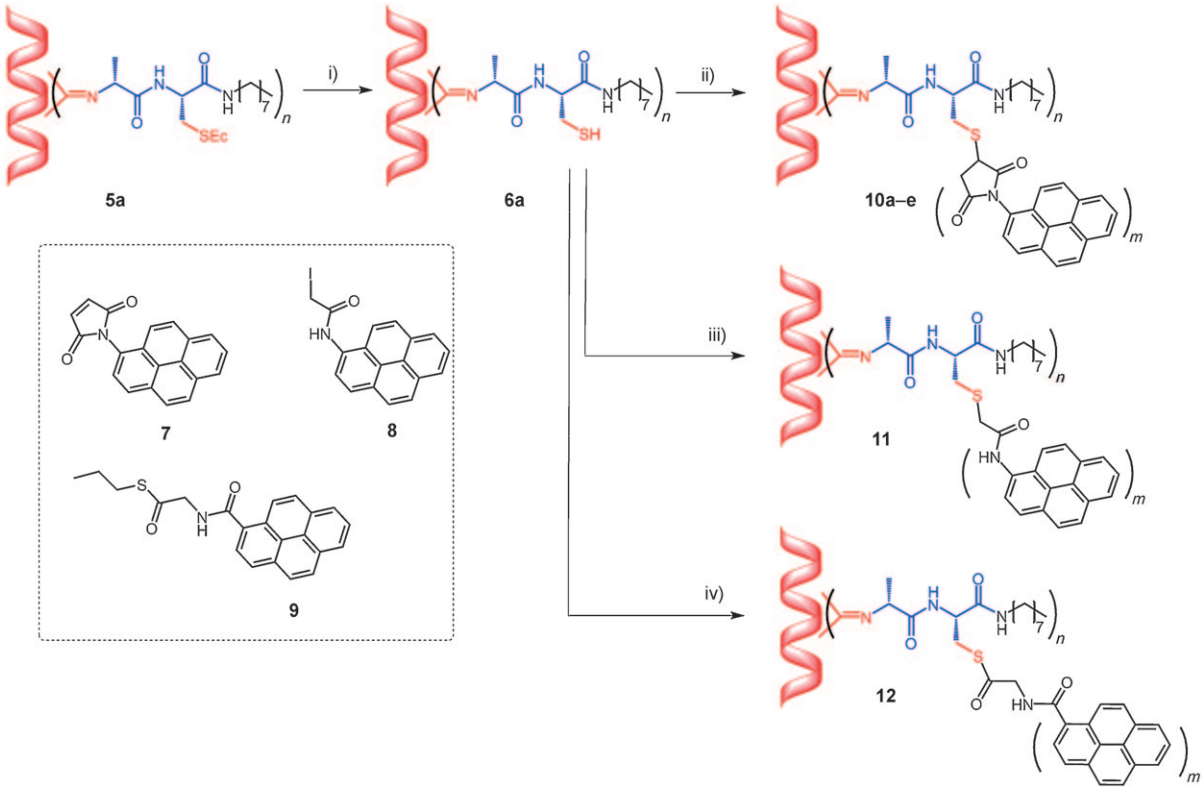
toring of the reaction by $^1$H NMR spectroscopy (see the Supporting Information) revealed complete and clean deprotection after 24 hours stirring at room temperature. For polymer **5a**, monitoring of this reaction with NMR spectroscopy or GPC was impossible because of the broad spectra and ill-defined traces (see above), and also not possible by UV spectroscopy since the released ethylurea absorbed only in the far UV region. We thus turned our attention to a two-step process involving a thiol-specific reagent to probe the efficiency of the deprotection reaction. For this purpose, the profluorescent pyrene maleimide **7** (Scheme 3) was chosen since it reacts readily with thiols generating a blue fluorescence that can be used as a readout. In addition, pyrenes are known to yield excimer emission when they are stacked, which can be also used as a readout to probe the scaffolding efficiency of the polymer. Various conditions were tested for this two-step process and these are summarized in Table 2. All solvents were degassed before use to avoid any side reactions, such as oxidation of thiols to disulfides, and the intermediate polyisocyanide **6a** (Scheme 3) was not isolated. The UV/Vis spectra of the isolated polymers **10a–e** are depicted Figure 3a.

The UV/Vis spectra of polymers **10a–e** showed the characteristic bands of the pyrene chromophores with unshifted maxima at $\lambda = 267$, 278, 314, 327, and 345 nm. At the same weight concentration of the final polymers, polymer sample **10c** displayed the highest pyrene absorbance of all samples. Longer reaction times for the deprotection step (Table 2, entry 4) and higher temperatures for the maleimide coupling step (entry 5) did not lead to an increase in the amount of incorporated pyrene.[24] The different samples of **10** showed blue fluorescence (Figure 3a inset), which reveals the success of the thio-Michael reaction, which is responsible for the covalent grafting of the chromophores.[25] The overall fluorescence spectra of **10a–e** were similar and displayed unshifted maxima at $\lambda = 375$ and 395 nm relative to the emission spectrum of **7** in the presence of a thiol (PrSH) (Figure 3c in the case of polymer **10c**). An extra redshifted emission at $\lambda = 460$ nm was, however, observed with all samples of **10**, which is characteristic of the formation of aggregated pyrenes or excimer-like species. A broadening in the region of $\lambda = 350$–375 nm in the UV spectra of samples **10**, relative to **7**, is also in line with some aggregation of the chromophores (Figure 3a). This data indicates that part of the pyrenes are stacked, which confirms the scaffolding effi-

Table 2. Applied conditions for the deprotection of the cysteine groups of **5a** and subsequent reaction of these groups with **7**.

| Entry | Polymer | Conditions cysteine deprotection[a] | maleimide coupling[b] |
|---|---|---|---|
| 1 | **10a** | 2 d, RT | overnight, RT |
| 2 | **10b** | 4 d, RT | overnight, RT |
| 3 | **10c** | 2 d, 50 °C | overnight, RT |
| 4 | **10d** | 4 d, 50 °C | overnight, RT |
| 5 | **10e** | 2 d, 50 °C | overnight, 50 °C |

[a] $NH_3$, $CHCl_3$/MeOH 9:1. [b] $CHCl_3$, DIPEA (3 equiv), **7** (3 equiv).




Scheme 3. Postmodification of polymer **5a**: i) $NH_3$, $CHCl_3$/MeOH, 50 °C; ii) **7**, DIPEA, $CHCl_3$, RT; iii) **8**, DIPEA, $CHCl_3$, RT; iv) **9**, DIPEA, $CHCl_3$, RT.

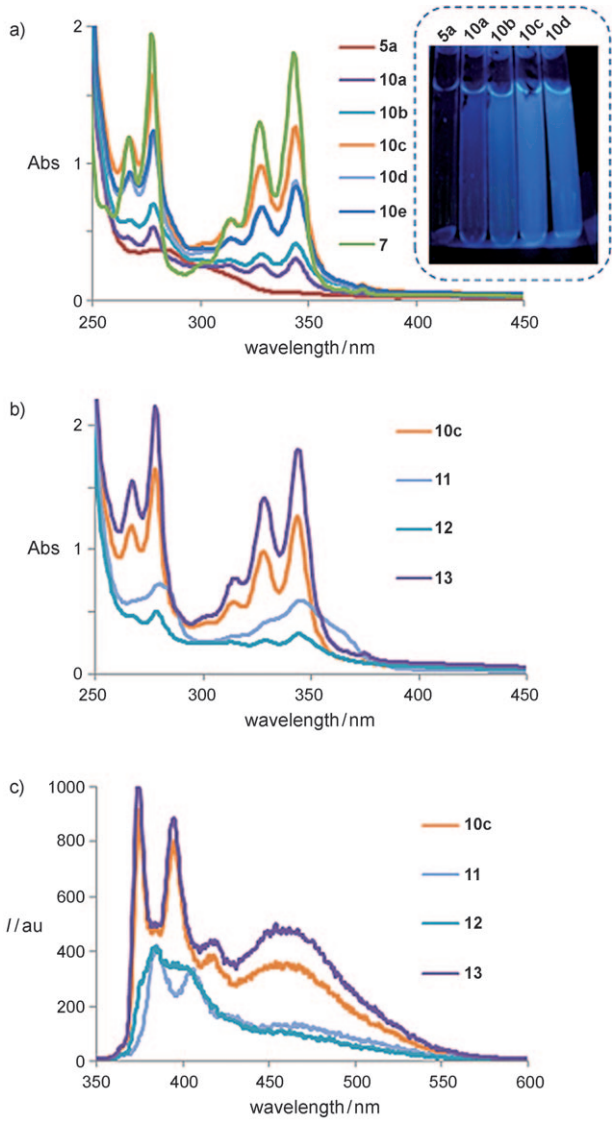



Figure 3. a) Comparison of the UV spectra of polymers **10a–e** ($c=0.5\,mg\,mL^{-1}$ in $CHCl_3$, RT), prepared according to different reaction conditions, and of **7** ($5\times10^{-5}$ M); inset: fluorescence of the different polymers as seen with a UV lamp ($\lambda_{ex}=365$ nm). b) Comparison of the UV spectra of polymers **10c**, **11**, **12**, and **13** ($c=0.5\,mg\,mL^{-1}$ in $CHCl_3$, RT). c) Comparison of the fluorescence spectra of the same polymers after 1:50 dilution (for **10c**, **11**, and **12**) or 1:92 dilution (for **13**) ($\lambda_{ex}=329$ nm).

ciency of the highly structured cysteine-rich polymer forcing the chromophores into a well-defined arrangement.

The stability and chiroptical properties of **10c** were investigated (see the Supporting Information for selected data). No change in the CD spectrum of **10c** was observed relative to the parent polymer **5a**, which shows that the helical conformation is not affected by the treatment with base. No CD signal was observed in the pyrene absorption region. The fact that excimer-like emission is observed in the fluorescence spectra but no CD activity is observed for the attached chromophores indicates that, while there is a certain degree of organization of the chromophores, well-defined stacking of the attached molecules may not be present. Similar observations were made in the case of the closely related clickable polyisocyanides, probably because the chromophores are too far away from the chiral polyimine backbone.[14a] The thermal stability of **10c** also remained unchanged, that is, no loss of chirality was observed after this polymer was heated for one hour at 100 °C. In addition, AFM measurements showed that the polymer length distribution was not affected by this two-step process, and only single-chain polymers were observed, which tends to indicate that side reactions, such as cross-linking due to intermolecular disulfide-bridge formation, are unlikely to occur under the utilized conditions (i.e., degassed solvents).[26]

To test the versatility of the thio-click strategy, particularly with regard to chromophoric scaffolding, we decided to attach other pyrene derivatives to the polymer by using different thio-specific click reactions. For this purpose, pyrene-iodoacetamide **8** and pyrene-thioester **9**[27] (Scheme 3) were chosen. The reaction of thiols with iodoacetamides also belongs to the click toolbox since it occurs readily under mild conditions (room temperature);[16] it is widely used, for instance, in the field of molecular biology for protein labeling. Thiols also react with thioesters under basic conditions through a thioester exchange mechanism.[28] This process is dynamic, which is of particular interest for the construction of adaptive materials, such as dynamic polymers (i.e., dynamers)[29] or the preparation of supported dynamic libraries.[30] Polyisocyanide **5a** was deprotected under the same conditions as used for **10c** (Table 2, entry 3) and reacted with pyrenes **8** or **9** in $CHCl_3$ in the presence of DIPEA. The corresponding polymers **11** and **12** were isolated by precipitation and their UV spectra (Figure 3b) showed the characteristic bands of the chromophores, which demonstrated the success of the coupling reaction. Similar to **10c**, fluorescence spectra of **11** and **12** showed both monomer and excimer-like emission in agreement with the stacking of the pyrenes (Figure 3c). The amount of incorporated pyrenes in the case of **9** seemed to be lower, which can be due to the dynamic nature of the thioester linkage.

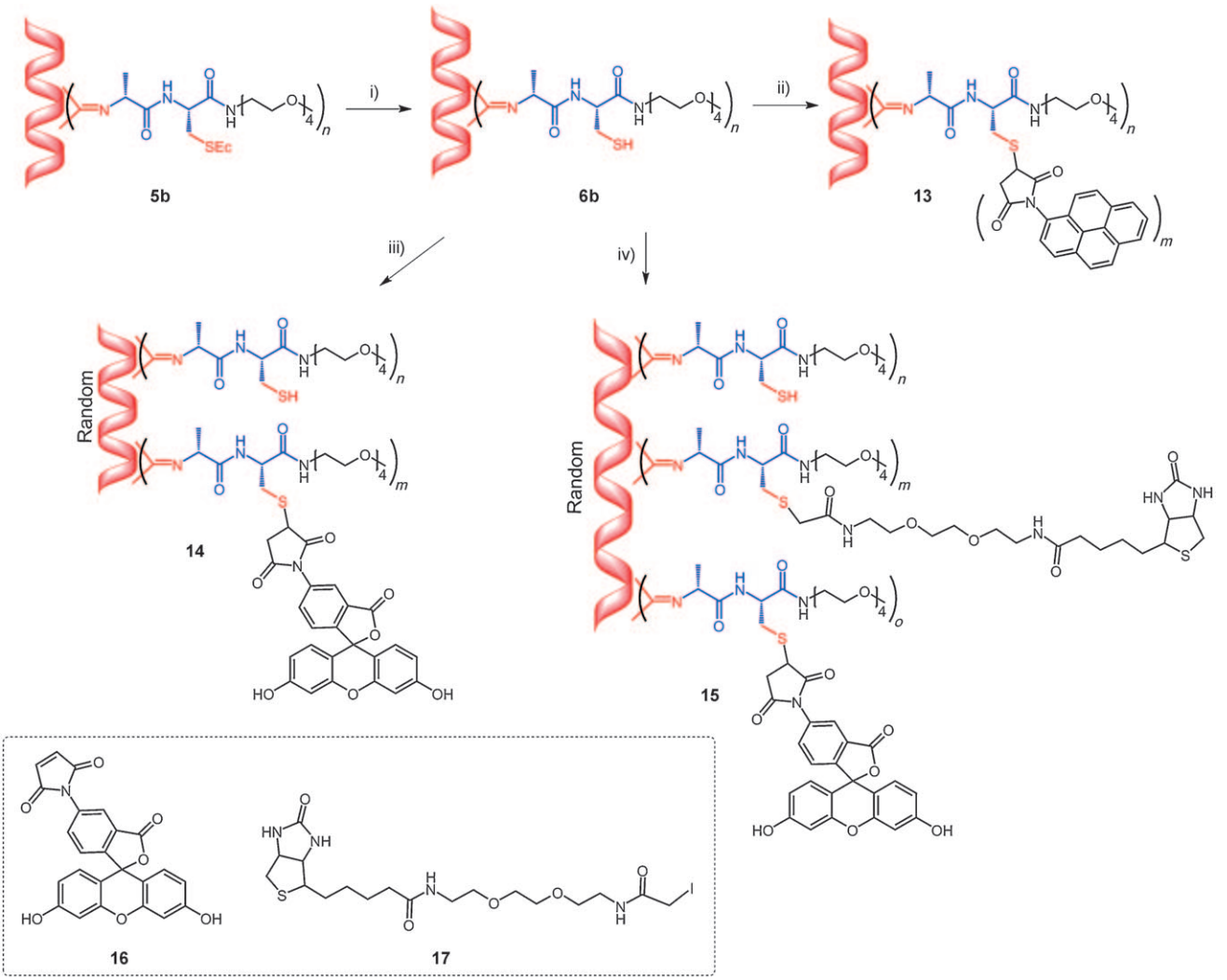
This first set of data clearly shows the potential of the cysteine-containing polyisocyanides with regard to chromophoric scaffolding. The ordering of biomolecules, such as proteins, onto this scaffold is of great interest and should also be possible. Indeed, due to their high persistence length, peptide-based polyisocyanides possess a rigid rodlike structure of over one-hundred nanometers and the grafting of enzymes or virus coat-proteins could lead to new biohybrid materials with unique properties, for example, for cascade catalysis or tissue engineering. To increase the scope of these cysteine-rich platforms, especially in the field of bioscaffolding, we decided to introduce biotins into the polymer backbone and to investigate whether or not the resulting biotinylated polymer would interact with streptavidin. Biotin is known to form a very stable complex with streptavidin ($Ka=10^{13}\,M^{-1}$) and this system has been widely used in molecular biology for a wide range of applications including the construction of sophisticated biohybrid materials.[31]

Polyisocyanide **5b** was thought to be particularly suited for this study since the tetraethylene glycol side chains provide good solubility in aqueous media. The deprotection of the cysteines was first probed with the profluorescent pyrene maleimide **7**. Treatment of **5b** with ammonia, under the same conditions as applied for **10c**, followed by reaction with **7**, led to the blue fluorescent polymer **13** (Scheme 4). Its UV, CD, and fluorescence spectra in $CHCl_3$ were very similar to those of the closely related polymer **10c** (Figure 3b and c), showing that the deprotection step works well and that the sulfur atoms are available for reaction. Also, the scaffolding propensity of the system is high.

To investigate the potential of **5b** to bind proteins, we planned to label the polymer with a water-soluble fluorescent dye in addition to labeling with biotin. Polymer **14** was thus designed as a reference compound, whereas **15** was designed to be the "active" polymer (Scheme 4). Both polymers were prepared from **6b** by using a degassed PBS buffer at pH 8, the former by reacting **6b** with the fluorescein maleimide derivative **16** (0.05 equiv), the latter by a one-pot procedure involving first a reaction of **6b** with the biotin derivative **17** (0.05 equiv), which contains an iodoacetamide reactive group, and then a reaction with the fluorescein maleimide derivative **16** (0.05 equiv). Polymers **14** and **15** were dialyzed against water to remove any unreacted material. Their UV and fluorescent spectra (Figure 4) showed the characteristic absorption and emission bands of the fluorescein dye.

Polymers **14** and **15** were subsequently brought into contact with rhodamine-labeled streptavidin (1:30 and 1:70 streptavidin/monomer ratio, respectively) in PBS buffer (0.02 M, pH 7.5, NaCl 150 mM). After one hour at 4 °C, the unpurified solutions were subjected to SDS-PAGE experiments and analyzed with a fluorescence imager. Fluorescein and rhodamine can be detected separately allowing the visualization of both the polymer and the streptavidin. Figure 5 shows an overlaid image with excitation at $\lambda = 473$ (fluorescein visualization in green) and $\lambda = 532$ nm (rhodamine visu-




Scheme 4. Postmodification of polymer **5b**: i) $NH_3$, $CHCl_3$/MeOH, 50 °C; ii) **7**, DIPEA, $CHCl_3$, RT; iii) **16** (0.05 equiv), 0.1 M PBS buffer, pH 8, RT; iv) 1) **17** (0.05 equiv), 0.1 M PBS buffer, pH 8, RT, 2) **16** (0.05 equiv), 0.1 M PBS buffer, pH 8, RT.

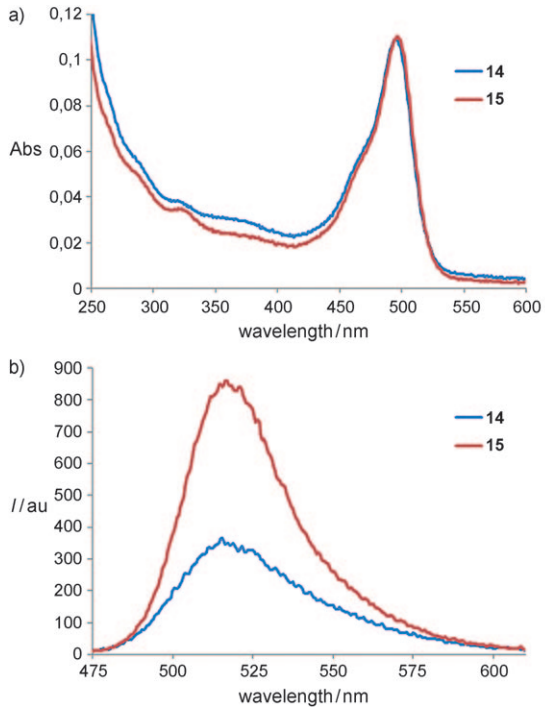



Figure 4. a) UV/Vis spectra in water at room temperature of polyisocyanides **14** (24 μg mL$^{-1}$) and **15** (56 μg mL$^{-1}$). b) Fluorescence spectra in water at 1:50 dilution ($\lambda_{ex}$ = 465 nm).

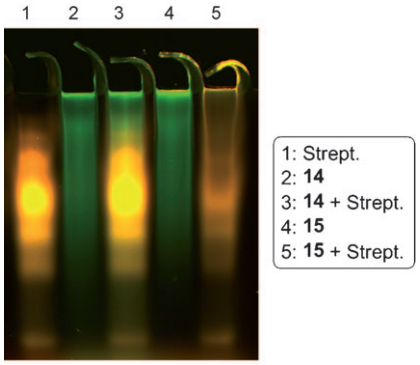



Figure 5. SDS-PAGE experiment, scanned with a fluorescence imager, carried out to study the binding of streptavidin to polyisocyanides **14** and **15**; an overlay is depicted from two images obtained at $\lambda_{ex}$ = 473 (selective fluorescein excitation, green fluorescence) and 532 nm (selective rhodamine excitation, red fluorescence). 1: streptavidin; 2: **14**; 3: **14**+ streptavidin; 4: **15**; 5: **15**+streptavidin.

alization in yellow; see the Supporting Information for the separated images). Because of their rodlike character and since they are not charged, polyisocyanides **14** and **15** are mostly retained on the gel (see the green smear in lanes 2 and 4). When **14** was mixed with streptavidin, no change in the streptavidin running behavior or in its fluorescence intensity was observed (yellow bands, lane 3 vs. 1). Also the fluorescence of the polymer did not change (lane 3 vs. 2). In contrast, with polymer **15**, which is expected to contain ≈5 mol% of biotin, a clear change was observed for the streptavidin running behavior and for the polymer fluorescence (compare lane 5 with 4 and 1). Furthermore, in lane 5 the fluorescence of rhodamine is overlapping with the characteristic polymer smear as is indicated by the presence of a yellow smear. This clearly points to the attachment of rhodamine-labeled streptavidin to the polymer scaffold. By comparing the fluorescence intensity of the rhodamine/streptavidin derivative in lane 5 with the fluorescence intensity in lane 1, one can deduce that ≈60% of the introduced protein is bound to the polymer. One should note, however, that the fluorescence intensity upon excitation at both $\lambda$ = 473 and 532 nm is reduced when compared to the other samples (see also Figure S8 in the Supporting Information). Quenching due to the close proximity of the different dyes is one explanation, but also gelation or precipation of the polymer materials upon complexing the tetravalent streptavidin, leading to a smaller amount of materials on the gel, can be a possible reason for this behavior. The suggestion of polymer cross-linking by streptavidin resulting in networks with reduced solubility is strengthened by the observation that the biohybrid material precipitated after standing overnight at 4 °C.[32] More detailed experiments are needed to substantiate the protein binding to the rigid polyisocyanide scaffold and characterize the biohybrid materials formed.

## Conclusion

In the present paper, we describe the successful synthesis of two new polyisocyanides, which have cysteine residues in their side chains. One polymer is soluble in organic solvents and the other in water. Due to the combination of the helical folding of the polyimine backbone and the supramolecular interactions between the side chains, these polymers possess a very stable and highly defined rodlike structure over a length of almost one-hundred nanometers. The sulfur atoms of the cysteines are aligned in four rows along the axis of the polymer, which make these polyisocyanides very attractive as scaffolding platforms. By using thio-specific "click" reactions, they could be efficiently functionalized with pyrene derivatives, yielding excimer-like fluorescence as a result of the scaffolding in a constrained environment. Biotinylation of the water-soluble polymer could also be successfully carried out, as demonstrated by PAGE experiments, which revealed the binding of streptavidin to the polymer. These results open interesting perspectives for both the fields of material science and biohybrid systems. Selective multichromophoric scaffolding by means of dynamic processes, protein assembly by using the polymers as nucleation sites, or metal-ion binding are among the foreseen applications of these cysteine-rich platforms.

## Experimental Section

**General**: All solvents were distilled over $CaCl_2$ or sodium prior to use. All other chemicals were commercial products and used as received.

Column chromatography was performed by using silica gel (40–60 μm) purchased from Merck. TLC analyses were carried out on silica 60 $F_{254}$ coated glass from Merck and the compounds were visualized by using ninhydrine, $KMnO_4$, or $Ni(ClO_4)_2{\cdot}6H_2O$ in EtOH. $^1H$ and $^{13}C$ NMR spectra were recorded on a Bruker AC-300 MHz instrument operating at 300 and 75 MHz, respectively. FTIR spectra were recorded on a Thermo-Mattson IR300 spectrometer equipped with a Harrick ATR unit and the compounds were measured as a solid or an oil. Liquid IR spectra were recorded on a Bruker Tensor 27. Melting points were measured on a Buchi B-545 and are reported uncorrected. Mass spectrometry measurements were performed on a JEOL Accutof instrument (ESI) or Thermo LCQ. Optical rotations were measured on a Perkin–Elmer 241 polarimeter at room temperature and are reported in $10^{-1}\,deg\,cm^2\,g^{-1}$. UV/Vis spectra were recorded on a Varian Cary 50 spectrometer and fluorescence spectra on a Perkin–Elmer Luminescence spectrometer LS50B. For AFM measurements, solutions of the samples were spincoated onto freshly cleaved Muscovite Mica. All AFM images were recorded on a Dimension 3100 microscope controlled with a NanoscopeIII controller (Digital Instruments, Santa Barbara, CA) operating in TappingMode in air at room temperature with a resolution of 512×512 pixels by using moderate scan rates (1—2 lines $s^{-1}$). Commercial gold-coated silicon tips (NSG10, NT-MDT) were used with a typical resonance frequency of around 300 kHz. All values derived from AFM data presented in this paper carry an error noted as the standard deviation of the values from different measurement sessions. Gel-permeation chromatography (GPC) was performed on a Shimadzu size-exclusion chromatographer (SEC) equipped with a guard column and a styragel HT-6E (7.8×300 mm, Waters) column with differential refractive index and UV/Vis detection by using THF as an eluent (1 mL $min^{-1}$ at 35 °C). CD spectra were recorded on a Jasco 810 instrument equipped with a Peltier temperature control unit. Dialysis tubing (regenerated cellulose membrane) with a molecular cut-off (MWCO) of 3500 was obtained from Spectra/Por. SDS-PAGE was performed by using 10% polyacrylamide gel on a mini*Protean Tetra system (BioRad) according to standard procedures. The composition of the running buffer was 25 mM Tris, 192 mM glycine, 0.1% (w/v) SDS, pH 8.3. The final gel was visualized with a fluorescence gel scanner (Fujifilm FLA-5100), working at two different excitation wavelengths ($\lambda$=473 and 532 nm). Streptavidin-Rhodamine Red-X conjugate was purchased from Molecular Probes (Leiden, The Netherlands). The Biotin Polyethyleneoxide Acetamide **17** was purchased from Sigma.

***N*-Boc-*S*-(ethylcarbamoyl)-L-cysteine**: TEA (2.08 mL, 14.96 mmol) and EtNCO (1.18 mL, 14.90 mmol) were added to a solution of (L)-Boc-Cys (3.00 g, 13.55 mmol) in distilled dichloromethane. After the mixture had been stirred overnight under an inert atmosphere, the solution was diluted with dichloromethane (250 mL), washed with an aqueous HCl solution (1 M, 100 mL), water (50 mL), and brine (50 mL), and dried with $Na_2SO_4$. The *N*-Boc-*S*-(ethylcarbamoyl)-L-cysteine compound (3.59 g, 91%) was obtained as a white solid without further purification. M.p. 139 °C; $[\alpha]_D=-17$ ($c$=0.43 in $CHCl_3$); FTIR (ATR): $\tilde{\nu}$=3314, 1693, 1655, 1512 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$=1.17 (t, $J$=7 Hz, 3H; $CH_3$), 1.45 (s, 9H; *t*Bu), 3.20–3.45 (m, 4H; $2CH_2$), 4.43 (q, $J$=6 Hz, 1H; CH), 5.50 (brs, 1H; NH), 5.94 ppm (brs, 1H; NH); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$=14.8, 28.3, 31.6, 36.7, 54.5, 80.6, 156.2, 166.9, 173.2 ppm; MS (ESI): $m/z$: 315 $[M+Na]^+$; HRMS (ESI-TOF): $m/z$: calcd for $C_{11}H_{20}N_2NaO_5S$: 315.0990 $[M+Na]^+$; found: 315.0973.

***N*-Boc-*S*-(ethylcarbamoyl)-L-cysteine octylamide (1a)**: Octylamine (850 μL, 5.13 mmol), DIPEA (931 μL, 6.63 mmol), HOBt (762 mg, 5.64 mmol), and EDC (1.08 g, 5.63 mmol) were successively added to a solution of *N*-Boc-*S*-(ethylcarbamoyl)-L-cysteine (1.50 g, 5.13 mmol) in distilled dichloromethane. The solution was stirred overnight then diluted with dichloromethane (150 mL), washed with an aqueous HCl solution (1 M, 50 mL), water (50 mL), and brine (20 mL), dried over $Na_2SO_4$, concentrated under vacuum, and the residue was subjected to flash chromatography ($CH_2Cl_2$/AcOEt 3:2). Compound **1a** was obtained as a colorless oil (1.87 g, 90%). $[\alpha]_D=-127$ ($c$=0.42 in $CHCl_3$); FTIR (ATR): $\tilde{\nu}$= 3298, 1649, 1518 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$=0.87 (t, $J$=7 Hz, 3H; $CH_3$), 1.18 (t, $J$=7 Hz, 3H; $CH_3$), 1.27 (m, 10H; $5CH_2$), 1.44 (s, 9H; *t*Bu), 1.51 (m, 2H; $CH_2$), 3.10–3.35 (m, 6H; $3CH_2$), 4.24 (brq, $J$=5 Hz, 1H; CH), 5.55 (brs, 1H; NH), 5.77 (d, $J$=7 Hz, 1H; NH), 6.46 ppm (brs, 1H; NH); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$=14.0, 14.8, 22.6, 26.8, 28.3, 29.1(5), 29.1(9), 29.4, 31.8, 32.1, 36.7, 39.6, 55.9, 80.2, 156.1, 167.4, 170.2 ppm; MS (ESI): $m/z$: 426 $[M+Na]^+$; HRMS (ESI-TOF): $m/z$: calcd for $C_{19}H_{37}N_3NaO_4S$: 426.2403 $[M+Na]^+$; found: 426.2405.

***N*-Boc-*S*-(ethylcarbamoyl)-L-cysteine 4,7,10,13-tetraoxatridecanamide (1b)**: Compound **1b** (5.10 g, 93%, yellow oil) was prepared by following the procedure described for **1a**, by starting from *N*-Boc-*S*-(ethylcarbamoyl)-L-cysteine (3.50 g, 11.97 mmol) and 4,7,10,13-tetraoxotridecane amine, except that the flash chromatography eluent was a $CH_2Cl_2$/MeOH 95:5 mixture. $[\alpha]_D=-2$ ($c$=0.52 in $CHCl_3$); FTIR (ATR): $\tilde{\nu}$=3525, 3297, 1654, 1520 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$=1.17 (t, $J$=7 Hz, 3H; $CH_3$), 1.44 (s, 9H; *t*Bu), 3.20–3.40 (m, 4H; $2CH_2$), 3.37 (s, 3H; $OCH_3$), 3.44 (brq, $J$=5 Hz; $CH_2$), 3.50–3.70 (m, 14H; $7CH_2$), 4.27 (brm, 1H; CH), 5.78 (brm, 2H; 2NH), 6.93 ppm (brs, 1H; NH); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$=14.8, 28.3, 32.2, 36.6, 39.4, 55.6, 58.9, 69.6, 70.2(5), 70.4, 70.5(0), 70.5(1), 71.9, 80.0, 155.8, 166.9, 170.3 ppm; MS (ESI): $m/z$: 504 $[M+Na]^+$; HRMS (ESI-TOF): $m/z$: calcd for $C_{20}H_{39}N_3NaO_8S$: 504.2355 $[M+Na]^+$; found: 504.2369.

***N*-Boc-D-alanyl-*S*-(ethylcarbamoyl)-L-cysteine octylamide (2a)**: Under an inert atmosphere, TFA (5 mL, 67.3 mmol) was added to a solution of **1a** (1.85 g, 4.58 mmol) in distilled dichloromethane (125 mL). The mixture was stirred overnight and subsequently concentrated under reduced pressure. The oily residue was dissolved in $CHCl_3$ and evaporated to dryness (this operation was repeated twice). The dry residue was dissolved in distilled dichloromethane (125 mL) and (D)-BocAla (867 mg, 4.58 mmol), DIPEA (1.67 mL, 10.08 mmol), HOBt (681 mg, 5.04 mmol), and EDC (966 mg, 5.04 mmol) were successively added. The solution was stirred overnight and then diluted with dichloromethane (150 mL), washed with an aqueous HCl solution (1 M, 80 mL), water (50 mL), and brine (30 mL), dried over $Na_2SO_4$, and concentrated under vacuum. The residue was subjected to flash chromatography ($CH_2Cl_2$/AcOEt 1:1). Compound **2a** was obtained as a white solid (1.92 g, 88%). M.p. 107 °C; $[\alpha]_D=-38$ ($c$= 0.32 in $CHCl_3$); FTIR (ATR): $\tilde{\nu}$=3297, 1648, 1524 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$=0.87 (t, $J$=7 Hz, 3H; $CH_3$), 1.17 (t, $J$=7 Hz, 3H; $CH_3$), 1.26 (m, 10H; $5CH_2$), 1.34 (d, $J$=7 Hz, 3H; $CH_3$), 1.43 (s, 9H; *t*Bu), 1.51 (m, 2H; $CH_2$), 3.05–3.40 (m, 6H; $3CH_2$), 3.98 (quint., $J$=7 Hz, 1H; $CH_{ala}$), 4.52 (q, $J$=7 Hz, 1H; $CH_{cyst}$), 5.30 (brd, $J$=6 Hz, 1H; NH), 5.70 (brt, 1H; NH), 7.00 (brs, 1H; NH), 7.49 ppm (d, $J$=7 Hz, 1H; NH); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$=14.0, 14.7, 17.7, 22.6, 26.8, 28.2, 29.1(2), 29.1(8), 29.3, 31.3, 31.7, 36.6, 39.7, 50.9, 55.0, 80.0, 155.7, 167.9, 169.4, 173.5 ppm; MS (ESI): $m/z$: 497 $[M+Na]^+$; HRMS (ESI-TOF): $m/z$: calcd for $C_{22}H_{42}N_4NaO_5S$: 497.2774 $[M+Na]^+$; found: 497.2765.

***N*-Boc-D-alanyl-*S*-(ethylcarbamoyl)-L-cysteine 4,7,10,13-tetraoxatridecanamide (2b)**: Compound **2b** (590 mg, 64%, yellow oil) was prepared by following the procedure described for **2a**, by starting from **1b** (800 mg, 1.66 mmol), except that the flash chromatography eluent was a $CH_2Cl_2$/acetone 70:30 mixture. $[\alpha]_D=-19$ ($c$=0.52 in $CHCl_3$); FTIR (ATR): $\tilde{\nu}$= 3498, 3297, 1653, 1523 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$=1.17 (t, $J$= 7 Hz, 3H; $CH_3$), 1.35 (d, $J$=7 Hz, 3H; $CH_3$), 1.44 (s, 9H; *t*Bu), 3.20–3.40 (m, 4H; $2CH_2$), 3.33 (s, 3H; $OCH_3$), 3.45–3.70 (m, 16H; $8CH_2$), 4.10 (quint., $J$=7 Hz, 1H; CH), 4.55 (q, $J$=7 Hz, 1H; CH), 5.16 (brs, 1H; NH), 5.83 (brs, 1H; NH), 7.48 ppm (brd, $J$=7 Hz, 1H; NH); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$=14.8, 18.2, 28.3, 31.5, 36.7, 39.5, 50.6, 54.7, 58.9, 69.5, 70.2, 70.4(0), 70.4(6), 70.5(2), 70.5(4), 71.9, 79.9, 155.5, 167.5, 169.7, 173.3 ppm; MS (ESI): $m/z$: 575 $[M+Na]^+$; HRMS (ESI-TOF): $m/z$: calcd for $C_{23}H_{44}N_4NaO_9S$: 575.2726 $[M+Na]^+$; found: 575.2731.

***N*-Formyl-D-alanyl-*S*-(ethylcarbamoyl)-L-cysteine octylamide (3a)**: Under an inert atmosphere, TFA (5 mL, 67.3 mmol) was added to a solution of **2a** (1.87 g, 3.94 mmol) in distilled dichloromethane (150 mL). The mixture was stirred overnight after which it was concentrated under reduced pressure. The oily residue was dissolved in $CHCl_3$ and evaporated to dryness (this operation was repeated twice). The dry residue was dissolved in ethylformate (150 mL) and the solution was refluxed in the presence of HCOONa (295 mg, 4.33 mmol) for 3 d. A white precipitate formed upon cooling to room temperature. The mixture was concentrated under vacuum to about one third of its volume, cooled to 0 °C, and the precipitate was isolated by filtration. After washing with cold ethylformate, compound **3a** was obtained as a white powder (1.35 g, 85%). M.p. 164–

165 °C; $[\alpha]_D = -11$ ($c = 0.28$ in DMSO); FTIR (ATR): $\tilde{\nu} = 3324$, 3296, 1636, 1545 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta = 0.87$ (t, $J = 7$ Hz, 3H; $CH_3$), 1.17 (t, $J = 7$ Hz, 3H; $CH_3$), 1.27 (m, 10H; $5CH_2$), 1.41 (d, $J = 7$ Hz, 3H; $CH_3$), 1.49 (m, 2H; $CH_2$), 3.10–3.45 (m, 6H; $3CH_2$), 4.42 (quint., $J = 7$ Hz, 1H; CH), 4.49 (m, 1H; CH), 5.67 (brs, 1H; NH), 6.33 (brd, $J = 5$ Hz, 1H; NH), 6.81 (brs, 1H; NH), 7.66 (brd, $J = 7$ Hz, 1H; NH), 8.15 ppm (s, 1H; CHO); $^{13}C$ NMR (75 MHz, [D$_6$]DMSO): $\delta = 13.9$, 14.7, 18.3, 22.1, 26.3, 28.6(4), 28.6(8), 28.9, 31.0, 31.2, 35.6, 47.1, 53.1, 160.8, 164.9, 169.3, 171.8 ppm; MS (ESI): $m/z$: 425 $[M+Na]^+$; HRMS (ESI-TOF): $m/z$: calcd for $C_{18}H_{34}N_4NaO_4S$: 425.2198 $[M+Na]^+$; found: 425.2194.

***N*-Formyl-D-alanyl-*S*-(ethylcarbamoyl)-L-cysteine 4,7,10,13-tetraoxatridecanamide (3b)**: Compound **3b** (998 mg, 97%, yellow oil) was prepared by following the procedure described for **3a**, by starting from 1.18 g (0.416 mmol) of **2b**, except that 2.2 equiv of HCOONa were used and the final purification was done by flash chromatography ($CH_2Cl_2$/MeOH 95:5). $[\alpha]_D = +3$ ($c = 0.48$ in $CHCl_3$); FTIR (ATR): $\tilde{\nu} = 3502$, 3278, 1653, 1538 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta = 1.16$ (t, $J = 7$ Hz, 3H; $CH_3$), 1.40 (d, $J = 7$ Hz, 3H; $CH_3$), 3.20–3.70 (m, 20H; $10CH_2$), 3.39 (s, 3H; $OCH_3$), 4.54 (m, 2H; 2CH), 5.91 (brs, 1H; NH), 6.95 (brd, $J = 5$ Hz, 1H; NH), 7.36 (brs, 1H; NH), 7.77 (brd, $J = 5$ Hz, 1H; NH), 8.16 ppm (s, 1H; CHO); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta = 14.7$, 17.9, 31.2, 36.6, 39.4, 48.0, 54.8, 59.0, 69.8, 70.0, 70.1, 70.2(0), 70.2(5), 70.3, 71.7, 161.6, 167.3, 170.1, 172.7 ppm; MS (ESI): $m/z$: 503 $[M+Na]^+$; HRMS (ESI-TOF): $m/z$: calcd for $C_{19}H_{36}N_4NaO_8S$: 503.2151 $[M+Na]^+$; found: 503.2144.

**D-Isocyanoalanyl-*S*-(ethylcarbamoyl)-L-cysteine octylamide (4a)**: Under an inert atmosphere, a solution of **3a** (500 mg, 1.24 mmol) and NMM (545 μL, 4.96 mmol) in anhydrous THF (250 mL) was cooled to −30 °C. A solution of diphosgene (107 μL, 0.89 mmol) in anhydrous dichloromethane (40 mL) was then quickly added. The mixture was stirred for 30 min at −30 °C (a pale-yellow color appeared) and the reaction was quenched by the addition of a saturated aqueous solution of $Na_2CO_3$ (40 mL). The mixture was stirred vigorously while warming to room temperature and stirred for a further 20 min. The THF layer was separated from the aqueous mixture and the latter was extracted with dichloromethane (30 mL). The combined organic layers were concentrated under reduced pressure and the aqueous residue was dissolved in dichloromethane (100 mL). The aqueous layer was discarded and the organic solution was evaporated and subjected to flash chromatography ($CH_2Cl_2$/AcOEt 1:1), yielding 412 mg of **4a** as a pale-yellow solid (86%). M.p. 147–148 °C (dec.); $[\alpha]_D = -38$ ($c = 0.28$ in $CHCl_3$); FTIR ($CHCl_3$): $\tilde{\nu} = 3429$, 3326, 1678, 1525 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta = 0.87$ (t, $J = 7$ Hz, 3H; $CH_3$), 1.17 (t, $J = 7$ Hz, 3H; $CH_3$), 1.27 (m, 10H; $5CH_2$), 1.49 (m, 2H; $CH_2$), 1.64 (d, $J = 7$ Hz, 3H; $CH_3$), 3.15–3.45 (m, 6H; $3CH_2$), 4.27 (q, $J = 7$ Hz, 1H; CH), 4.50 (q, $J = 7$ Hz, 1H; CH), 5.60 (brs, 1H; NH), 6.43 (brs, 1H; NH), 7.79 ppm (brd, $J = 5$ Hz, 1H; NH); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta = 14.1$, 14.8, 19.7, 22.6, 26.8, 29.2, 29.3, 31.5, 31.8, 36.8, 39.8, 53.2, 54.9, 161.2, 167.2(7), 167.3(2), 168.8 ppm; MS (ESI): $m/z$: 407 $[M+Na]^+$; HRMS (ESI-TOF): $m/z$: calcd for $C_{18}H_{32}N_4NaO_3S$: $[M+Na]^+$: 407.2093; found: 407.2087.

**D-Isocyanoalanyl-*S*-(ethylcarbamoyl)-L-cysteine 4,7,10,13-tetraoxatridecanamide (4b)**: Compound **4b** (102 mg, 53%, pale-yellow oil) was prepared by following the procedure described for **4a**, by starting from **3b** (200 mg, 0.416 mmol), except that for the flash chromatography eluent a $CH_2Cl_2$/MeOH 95:5 mixture was used. $[\alpha]_D = -20$ ($c = 0.53$ in $CHCl_3$); FTIR ($CHCl_3$): $\tilde{\nu} = 3429$, 3314, 2143, 1677, 1523 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta = 1.17$ (t, $J = 7$ Hz, 3H; $CH_3$), 1.64 (d, $J = 7$ Hz, 3H; $CH_3$), 3.25–3.70 (m, 20H; $10CH_2$), 3.37 (s, 3H; $OCH_3$), 4.28 (q, $J = 7$ Hz, 1H; CH), 4.53 (dt, $J_1 = 5$, $J_2 = 6$ Hz, 1H; CH), 5.72 (brs, 1H; NH), 6.84 (brs, 1H; NH), 7.73 ppm (brd, $J = 7$ Hz, 1H; NH); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta = 14.7$, 19.7, 36.7, 39.5, 54.5, 58.9, 69.5, 70.2, 70.3, 70.4(2), 70.4(4), 70.5, 71.8, 160.8, 166.7, 166.8, 169.0 ppm; MS-ESI: $m/z$: 485 $[M+Na]^+$; HRMS (ESI-TOF): $m/z$: calcd for $C_{19}H_{34}N_4NaO_7S$: 485.2045 $[M+Na]^+$; found: 485.2027.

**Poly[D-isocyanoalanyl-*S*-(ethylcarbamoyl)-L-cysteine octylamide] (5a)**: A solution of $Ni(ClO_4)_2 \cdot 6H_2O$ (1 mL, 0.7 mM) in MeOH was added to a solution of **4a** (150 mg, 0.39 mmol) in distilled dichloromethane (100 mL). The solution quickly became turbid and was stirred for 1 h under ambient conditions. MeOH (15 mL) was then added resulting in a white creamy precipitate, which was isolated by centrifugation. The solid was dissolved in a minimal amount of $CHCl_3$ and precipitated by the addition of MeOH, isolated by centrifugation, and washed with MeOH to give 148 mg of **5a** as a pale-yellow solid (98%). FTIR ($CHCl_3$): $\tilde{\nu} = 3246$, 1645, 1541 $cm^{-1}$.

**Poly[D-isocyanoalanyl-*S*-(ethylcarbamoyl)-L-cysteine 4,7,10,13-tetraoxatridecanamide] (5b)**: Polymer **5b** (18 mg, 90%, pale-yellow solid) was prepared by following the procedure described for **5a**, by starting from **4b** (20 mg, 0.043 mmol), except that the final precipitation was done in a $CHCl_3/Et_2O$ mixture. FTIR ($CHCl_3$): $\tilde{\nu} = 3252$, 1644, 1523 $cm^{-1}$.

**Polymer 10c**: In a sealed reactor and under an inert atmosphere, polymer **5a** (30 mg, 0.078 mmol) was dissolved in a 9:1 $CHCl_3$/MeOH mixture (45 mL, solvents were degassed by prolonged argon bubbling before use) and ammonia was bubbled through the solution for 20 min. The reactor was sealed and the solution was heated at 50 °C for 48 h. After the mixture had been cooled to room temperature, the solvent was removed by bubbling argon through the solution. The residue was dissolved in degassed $CHCl_3$ (10 mL) and pyrene maleimide **7** (70 mg, 0.234 mmol) and DIPEA (35 μL, 0.229 mmol) were successively added. The solution was stirred overnight at room temperature under an inert atmosphere. Polymer **10c** was isolated as a brown solid (25 mg) by precipitation (×3) in a 1:1 $CHCl_3$/MeOH mixture.

**Polymer 11**: Polymer **5a** (22 mg, 0.057 mmol) was deprotected by following the procedure described for the preparation of **10c**. The residue was dissolved in degassed $CHCl_3$ (10 mL) and pyrene iodoacetamide **8** (66 mg, 0.171 mmol) and DIPEA (26 μL, 0.170 mmol) were successively added. The solution was stirred overnight at room temperature under inert atmosphere. Polymer **11** was isolated as a brown solid (20 mg) by precipitation (×3) in a 1:1:1 $CH_2Cl_2$/THF/MeOH mixture.

**Polymer 12**: Polymer **5a** (20 mg, 0.052 mmol) was deprotected by following the procedure described for the preparation of **10c**. The residue was dissolved in degassed $CHCl_3$ (20 mL) and pyrene thioester **9** (112 mg, 0.31 mmol) and DIPEA (48 μL, 0.31 mmol) were successively added. The solution was stirred for 9 d at room temperature under an inert atmosphere. Polymer **12** was isolated as a brown solid (18 mg) by precipitation (×3) in a 1:1 $CHCl_3$/MeOH mixture.

**Polymer 13**: Polymer **5b** (15 mg, 0.031 mmol) was deprotected by following the procedure described for the preparation of **10c**, except that a 2:1 $CHCl_3$/MeOH ratio was used. The residue was dissolved in degassed $CHCl_3$ (10 mL) and pyrene maleimide **7** (27 mg, 0.091 mmol) and DIPEA (15 μL, 0.098 mmol) were successively added. The solution was stirred overnight at room temperature under inert atmosphere. Polymer **13** was isolated as a brown solid (12 mg) by precipitation (×3) in a 1:1 $CHCl_3/Et_2O$ mixture.

**Polymer 14**: Polymer **5b** (15 mg, 0.031 mmol) was deprotected by following the procedure described for the preparation of **13**. Under an inert atmosphere, the residue was dissolved in degassed PBS buffer (0.1 M, pH 8, 5 mL) and fluoresceine maleimide **16** (0.7 mg, 1.63 μmol) was added. The solution was stirred overnight at room temperature and dialyzed extensively in the dark against water by using a cellulose membrane of MWCO 3500. Polymer **14** was kept in solution under inert atmosphere at a concentration of 1.2 mg $mL^{-1}$.

**Polymer 15**: Polymer **5b** (15 mg, 0.031 mmol) was deprotected by following the procedure described for the preparation of **13**. Under an inert atmosphere, the residue was dissolved in degassed PBS buffer (0.1 M, pH 8, 5 mL) and the biotin derivative **17** (0.9 mg, 1.66 μmol) was added. The solution was stirred 2 h at room temperature and fluoresceine maleimide **16** (0.7 mg, 1.63 μmol) was added. The solution was stirred overnight at room temperature and dialyzed extensively in the dark against water by using a cellulose membrane of MWCO 3500. Polymer **15** was kept in solution under inert atmosphere at a concentration of 2.8 mg $mL^{-1}$.

**Complexation of streptavidin**: The stock solutions of polyisocyanides **14** and **15** (30 μL, which corresponds to 36 and 84 μg of the polymers, respectively) were added to a PBS buffer solution (270 μL, 0.02 M, pH 7.5, NaCl 150 mM) containing the streptavidin–rhodamine derivative (120 μg).

The mixtures were gently stirred at 4 °C for 1 h and then subjected to SDS-PAGE analysis.

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[22] Based on an average spacing between the side chains *n*th and (*n*+4)th of 4.7 Å, we assume that a polyisocyanide of 100 nm in length contains 850 monomeric units.
[23] The polymer was found to be soluble again after the heating cycle.
[24] The fact that more harsh conditions are required for the deprotection step relative to those used for the formamide precursor **3a** might be attributed to the fact that the ethylcarbamoyl protecting groups of **5a** are involved in interchain hydrogen-bonding interactions, which make them more stable towards nucleophilic attack.
[25] The released ethylurea byproduct was also observed by $^1$H NMR spectroscopy after workup.
[26] In contrast, when polyisocyanide **5a** was deprotected by following the procedure described for **10c**, then stirred in chloroform solution for three weeks at ambient atmosphere, AFM analysis (see the Supporting Information) revealed fiberlike structures up to μm length. This might suggest the formation of cross-linked polymers; however, more detailed studies are needed to substantiate this.
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[32] In the case of polyisocyanide **14**, no precipitation of the mixture was observed after one week at 4 °C.

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