Post-modification of helical dipeptido polyisocyanides using the 'click' reaction‡†

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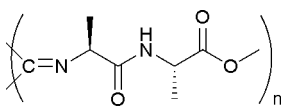
Polyisocyanopeptides have been synthesised containing acetylene groups on the side arms as scaffolds for multifunctional derivatisation by the copper-catalysed click reaction with a variety of azides. By using ethylene glycol azide and perylene azide chromophoric water-soluble polymeric nanowires (M_w 1–2 million Daltons) were formed. The potential to incorporate multiple chromophores was also demonstrated by the reaction of the acetylene-containing polymers with perylene azide and azidocoumarin dyes. In the latter case a blue-shifted emission of the coumarin was observed due to the interaction with the coupled perylene molecules. In particular the ability to form water-soluble dye-containing polymers, which can be modified by the addition of biomolecules, such as antibodies, proteins and peptides, give materials that are very promising as novel biomarker materials.

Introduction

The incorporation of functional dyes into ordered arrays is an area of great interest in the materials sciences, with a number of potential applications, particularly in the fields of molecular photonics and electronics.[1] New materials, which mimic the architecture and function of the systems found in nature, in that they have unique photophysical and electronic properties as a result of excitonic interactions between neighbouring dye units are highly sought after. The photosynthetic light-harvesting antenna used by green plants and purple bacteria employs organised assemblies of bacteriachlorophyll molecules positioned together by protein scaffolds.[2] The precise arrangements of the chromophore pigments results in the efficient absorption and transportation of light energy and its subsequent conversion into chemical energy.[3]

One approach towards the mimicking of these highly efficient natural systems is the utilisation of molecular-recognition and self-assembly of the components within the system; processes driven by π–π stacking, hydrogen bonding, metal–ligand interactions, or electrostatic forces. These interactions allow easy access to well-defined arrays, but the use of this approach allows only limited control over the final structure. An alternative method towards these systems is the use of covalent synthesis.[4] The step-wise construction of such assemblies is a frequent approach, however, the formation of large structures involves multi-step syntheses and the desired products are often obtained in low yields. Work from our group in this area has made use of the well-known polyisocyanides as a means to covalently organise chromophoric units along a protein-like polymer backbone.[5,6]

The formation and study of helical polyisocyanides has been an area of interest for quite some time and is widely discussed in the literature.[7] It is known that polyisocyanides, such as poly (L-isocyanoalanyl-L-alanine methyl ester) (L,L-PIAA, **1**), prepared from amino acid derived monomer units form very stable β-helical architectures due to the presence of a hydrogen-bonding chain parallel to the covalent polymer backbone (Fig. 1a); the secondary network rigidifies the array, resulting in extremely stiff polymers (persistence length 76 nm) and prevents the unwinding of the helix.[8] Polyisocyanides have a 4_1 helical conformation (*i.e.*, four repeat units per helical turn) with an average spacing between the side chains *n* and (*n* + 4) of 4.7 Å (Fig. 1b). In addition, the chiral centre of the peptide unit controls the handedness (left or right) of the helix, allowing the tuning of the architectures; a right-handed (*P*) helix is generated on formation of L,L-PIAA.



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The introduction of chromophoric units into these polymers can be achieved by the synthesis of a chromophore-appended monomer. The porphyrin-appended alanine isocyanide (**2**) gave, on polymerisation with nickel, rod-like structures that were determined by atomic force microscopy (AFM) measurements to have an average mass of 1.1×10^6 Daltons and a polydispersity index of 1.3.[5] Resonance light scattering (RLS) techniques showed the presence of large domains in which energy delocalisation occurs over more than 25 interacting chromophores, and over distances of 100–150 Å. The polymers formed from the perylenediimide containing monomers (**3a** and **3b**) were also

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† Dedicated to Professor Andrew B. Holmes on the occasion of his 65th birthday

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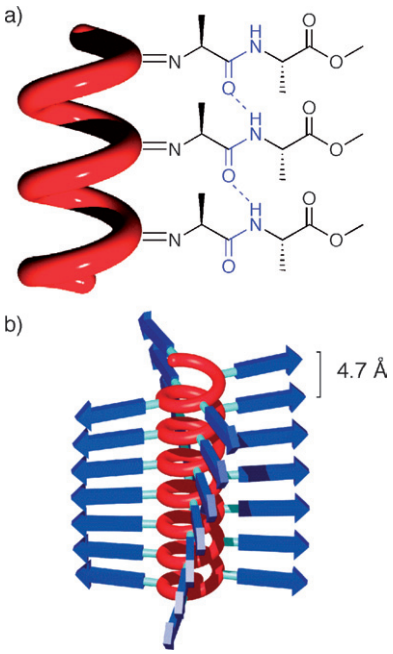
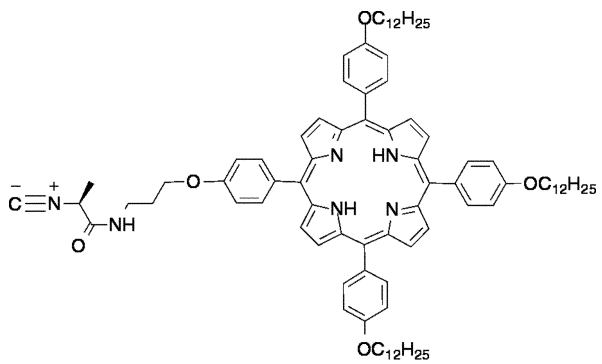


Fig. 1 a) Schematic representation of the hydrogen-bonding network present between the alanine units of the side arms in L,L-PIAA (**1**). b) Representation of the 4_1 helical conformation found in polyisocyanides.

studied.[6] In the case of **3a** the AFM images of the polymer revealed lengths up to 6 μm (the average length is 300 nm) and a polydispersity index of 2.3; the range of molecular masses observed is 1–10 × 10^7 Daltons. The fluorescence decay of the monomer was found to be monoexponential with a characteristic lifetime value (τ) of 3.9 ns, whilst the same measurements for the polymer showed a lifetime value of 19.9 nm, typical for excimer-like species. In addition, measurements of these macromolecules have shown them to have exceptional electron-migration



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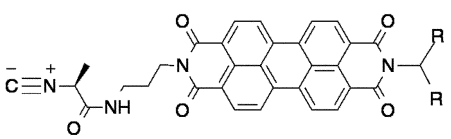
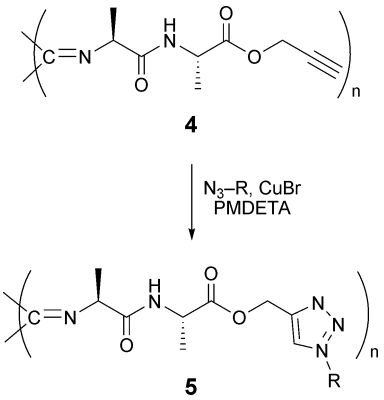


3a R = C_2H_5
3b R = C_6H_{13}



Scheme 1

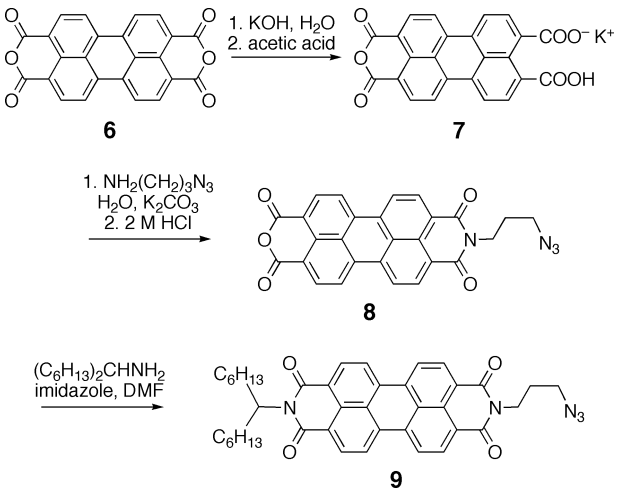
properties that can allow for their utilisation in the construction of working thin-film transistors and photovoltaic devices.

It is evident from above that defined polyisocyanides with chromophoric units have promising characteristics when arranged in a helical environment. One disadvantage, however, is that the synthesis of the chromophore-containing isocyanide monomers is tedious and considerable problems are often encountered in the final dehydration step of the formamide to form the isocyanide. The work in this paper presents an alternative to the synthesis of the costly chromophoric isocyanide monomer and involves the post-modification of a defined polyisocyanide scaffold, which is synthesised from readily available starting materials. The polymer scaffold, poly(L-isocyanoalanyl-L-alanine prop-2-ynol ester) (L,L-PIAAPE, **4**), has side arms containing two alanine groups and an acetylene functionality; post-modification can be achieved using click chemistry to generate a product containing a linking triazole ring (**5**, Scheme 1). The scaffold itself is only mildly soluble in chlorinated solvents, but it was shown that reaction of this polymer with dodecyl azide under click chemistry conditions led to a polymer with greatly increased solubility in organic solvents and modified properties as observed in AFM and CD spectroscopic studies.[9] In this paper we extend the above approach and present our recent studies on the post-modification of acetylene polyisocyanides with both chromophoric and water-soluble azides.

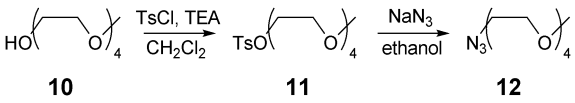
Results and discussion

Synthesis

Two azides, one containing a perylene moiety and the other having ethylene glycol units, were prepared in order to study the scope of the click reaction of the polyisocyanide scaffold. The perylene azide **9** was synthesised in three steps from the commercially available 3,4:9,10-perylenetetracarboxydianhydride (Scheme 2). The first step involved the reaction of the perylene dianhydride **6** with potassium hydroxide in water, followed by addition of acetic acid to generate the potassium salt **7**. The salt was treated with 1-azido-3-aminopropane and after stirring overnight, the addition of potassium carbonate yielded the asymmetric perylene monoimide monoanhydride **8**. The reaction of this imide with 1-hexylheptylamine in the presence of



Scheme 2



Scheme 3

imidazole lead to the isolation of the desired diimide **9**, which was purified by column chromatography.

The second azide contains a tetraethylene glycol unit and was synthesised by the conversion of tetraethylene glycol monomethyl ether (**10**) into the corresponding tosylate **11** under standard tosylation conditions (Scheme 3). The tosylate was then treated with sodium azide in ethanol at reflux to generate the desired azide **12**, which was obtained, after purification by chromatography, as a colourless oil.

The click reaction of the polymer scaffold **4** with the azides was performed under an inert argon environment. The polymer was suspended in dichloromethane and the azide added. The resulting mixture was subsequently treated with PMDETA and copper bromide and stirred overnight. The copper salts were removed from solution by complexation with EDTA and the clicked polymers were subjected to size-exclusion chromatography to remove any unreacted azides. All polymers generated were analysed by circular dichroism (CD), ultraviolet-visible absorption (UV-Vis), infrared (IR), NMR and fluorescence spectroscopies, by gel permeation chromatography (GPC) and in some cases atomic force microscopy (AFM).

Comparison studies

In order to determine whether the polymers obtained from the click reaction approach have the same properties as those obtained from the polymerisation of isocyanide monomers several comparison experiments were performed. In the first instance two perylene-containing polymers were prepared and their properties compared. The first polymer was synthesised from the nickel-induced polymerisation of the perylene isocyanide monomer **3b** in dichloromethane. The second polymer was generated from the click reaction of the scaffold **4** with an excess of the perylene azide **9** to give a polymer containing the

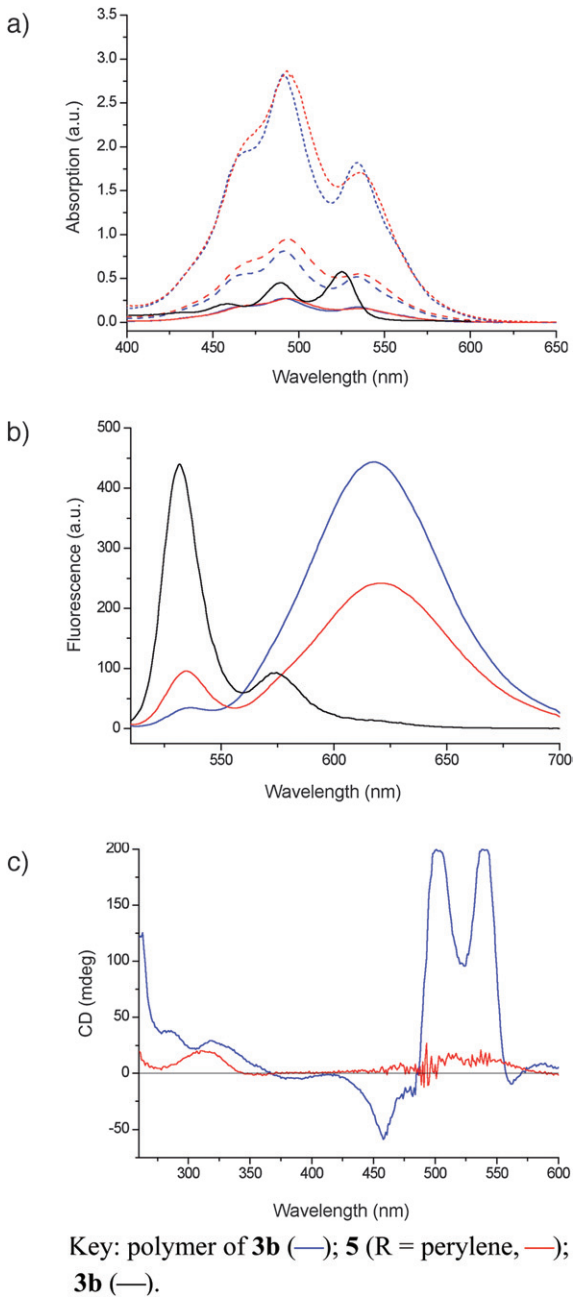


Fig. 2 Comparison studies of the polymer obtained from the nickel-induced polymerisation of **3b** to the clicked polymer **5** (R = perylene) in dichloromethane. a) UV-Vis spectra at various concentrations: 8.7 μM (solid), 31 μM (dashed) and 87 μM (dotted). b) Fluorescence spectra at 0.87 μM. c) CD spectra at 87 μM. Spectra of the monomer **3b** are shown for reference.

triazole ring (**5**, R = perylene). In order to compare these two polymers, solutions were prepared in which the concentration was calculated on the effective monomer unit (in the case of the click reaction this involves the monomer–triazole–perylene unit and assumes that the click reaction is 100% efficient).[10] The absorption spectra (Fig. 2a) of these compounds were measured at three different concentrations. At each concentration, for both polymers, several well-defined peaks were observed in the region of 400–550 nm, with λ_{max} = 469, 492 and 533 nm; the perylene

isocyanide monomer **3b** has peaks within the same region with $\lambda_{max} = 432$, 459, 490 and 527 nm (Fig. 2a), however, these peaks differ in intensity and width to those of the polymer. The red-shifted broadening of the absorption peaks in the region 550–600 nm is evident in the polymer spectra and is indicative of strong Coulombic (exciton) interactions between transition dipole moments of nearby perylene moieties, indicating short inter-chromophoric distances. At each concentration both polymers exhibit these same features indicating that exciton migration is present in both cases and that the presence of the triazole moiety does not significantly disorder the chromophoric array.

The fluorescence emissions of the two polymers and that of the monomer are compared in Fig. 2b. The fluorescence of the perylene monomer **3b** is represented by a strong vibronic band at $\lambda =$ 535 nm and a second weaker band at $\lambda = 578$ nm; the polymer of **3b** has a broad peak centred around $\lambda = 620$ nm. The red-shifted emission observed for the polymer compared to that of the monomer is consistent with the formation of perylenediimide excimer-like species, favoured by exciton interactions and charge transfer, often seen in perylene arrays. The polymer obtained from clicking also has a broad peak centred at $\lambda = 620$ nm of lower intensity to that of the homopolymer (Fig. 2b), however, in this case fluorescence due to monomer emission is also observed. The monomer emission could result from the presence of residual perylene azide, but due to extensive washing and the performance of size-exclusion chromatography it is more likely due to defects in the grafting of the azide onto the polymers. The perylenes situated at the defects will exhibit more monomer-like emission. The difference in the peak size of monomer emission to that of polymer indicates that *ca.* 9% of perylenes on the polymer backbone are in a non-excimer environment.

In contrast to the fluorescence spectra, which are similar, the CD spectra of the two polymers show very different behaviour (Fig. 2c). The polymer of **3b** has strong positive Cotton signals in the region of $\lambda = 450$–550 nm where the absorption bands due to interacting perylenediimides arise. In this polymer, signals having much lower intensity can also be seen at *ca.* $\lambda = 290$ and 330 nm. The CD spectrum of the clicked polymer does not exhibit any effect reminiscent of that observed for the former polymer in the region of $\lambda = 450$–550 nm. In this region a slight increase of the signal from the baseline can be seen, however, no defined peaks are observed; increasing or decreasing the concentration does not result in peaks giving any information. A positive peak is seen at $\lambda = 320$ nm and arises from the imine groups present in the backbone; this spectrum is like that of the polymer scaffold and shows that the inner core of the helix is not influenced by the clicking. The difference in the position of the peaks of the two polymers in the $\lambda = 280$–350 nm region could result from the fact that the homopolymer has only one alanine unit present in the side arm, whereas the clicked polymer has two alanine units and the hydrogen-bonding network is stronger, therefore having an affect on the helical pitch of the polymer. The absence of any signal due to the perylene in the clicked polymer indicates that due to the presence of two alanine units and the triazole moiety, the perylenes are further from the helical backbone and do not lie in a chiral environment with respect to each other. This lack of definition in ordering of the perylenes may also in part account for the slight differences in the fluorescence spectra.

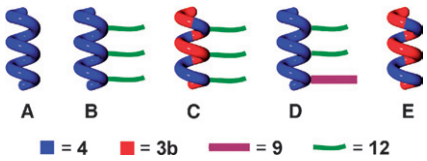


Fig. 3 Schematic representation of the five samples (**A**–**E**) studied in order to determine whether random clicking generates copolymers similar to those obtained by random copolymerisation.

Random copolymer studies

A second set of experiments was carried out to determine whether the formation of random copolymers could also be efficiently achieved using the click chemistry approach. Five samples were prepared and analysed by spectroscopic techniques. The five samples, represented in schematic diagrams in Fig. 3, were as follows: **A**) L,L-PIAAPE (**4**); **B**) L,L-PIAAPE (**4**) clicked to ethylene glycol azide **12**; **C**) a random copolymer formed from L,L-IAAPE (99%) with perylene isocyanide **3b** (1%) followed by the click reaction with an excess of **12**; **D**) L,L-PIAAPE (**4**) randomly clicked to perylene azide **9** (1%) and ethylene glycol azide **12** (99%); **E**) a random copolymer formed from L,L-IAAPE (99%) with perylene isocyanide **3b** (1%). Solutions of these polymers were prepared (*ca.* 0.75 mM) calculated on the molecular weight of the effective monomer unit and neglecting, if necessary, any incorporated perylene. In each case where the click reaction has been used, it is assumed, for calculating concentrations, that the clicking has an efficiency of 100%.

The absorption spectra of the samples containing the chromophore (**C**–**E**) are shown in Fig. 4a. The intensities of the compounds vary, but in each case they exhibit an absorption similar to that seen for perylene monomer absorption. In the case of **D**, however, there is a noticeable broadening and a red shift in the $\lambda = 520$–570 nm region in the UV-Vis spectrum. This shift is seen on polymerisation of the perylene isocyanide and is attributed to the stacking of perylene molecules within the polymer indicating some cooperativity. The cooperativity was further confirmed by close investigation of the excimer region of the fluorescence spectrum (Fig. 4b). Although the three samples **A**–**C** show fluorescence reminiscent to that of monomer emission, as would be expected for such a small quantity of perylene, when studied closely polymer **D** has, in the range of $\lambda = 600$–650 nm, a slight broadening, which is not present in the case of **C**, indicating that interactions exist between neighbouring chromophores (Fig. 4b, inset). It can also be seen that the fluorescence emission of the two polymers containing ethylene glycol units are of a lower intensity to that of the randomly clicked polymer, indicating that the presence of the ethylene glycol units leads to a slight quenching in the fluorescence of the perylene.

The CD spectra of the five samples (Fig. 4c) show interesting trends. In the case of the polymers with no ethylene glycol incorporated (**A** and **E**), a positive CD effect is observed at $\lambda =$ 320 nm arising from the imine moieties. The compounds containing the ethylene glycol units clicked to the polymer exhibit a negative CD effect of a much lower intensity at $\lambda = 290$ nm. The click reaction of the polymer scaffold with dodecyl azide[9] also

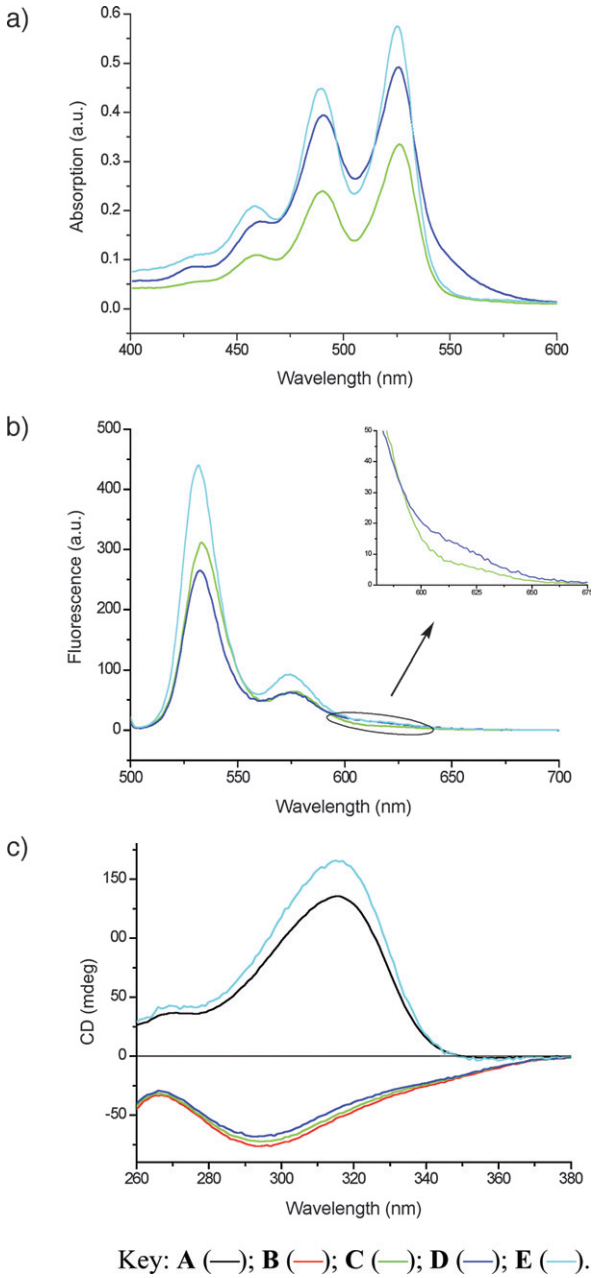


Fig. 4 Measurements of the random copolymers and reference polymers in dichloromethane. a) UV-Vis spectra at 0.75 mM. b) Fluorescence spectra at 5.90 μM with $\lambda_{exc} = 492$ nm (the inset shows the red-shifted broadening of polymer **D**). c) CD spectra at 0.75 mM.

generated a polymer for which the same change in CD signal was observed. The difference was attributed to an induced small change in the helical pitch of the backbone due to the presence of the triazole ring resulting in changes in the hydrogen bonding array. Since no change in the CD signal is observed when perylene azide is clicked to the scaffold, the change in signal seen here must be attributed to properties of the substituent being clicked. It is of interest to note that the three samples with negative CD effects (**B**–**D**) all exhibit the same behaviour when measured in water (Fig. S1, ESI‡).

Table 1 Polymers formed for cooperative studies with varying degrees of perylene azide (**9**) incorporated

Polymer	**P0**	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**
% **9**	0	0.5	1	2	5	7	10	15	20	50	100

Cooperativity studies

In order to study the cooperative effect seen in the UV-Vis and fluorescence spectra of **D** and to analyse these compounds in further detail, a family of polymers were made consisting of different percentages of perylene and ethylene glycol azides randomly clicked onto the polymer scaffold. The samples prepared are given in Table 1.

The UV-Vis spectra obtained of the polymer solutions (1 mg of polymer dissolved in 5 mL of dichloromethane) of samples **P1**–**P8** are shown in Fig. 5a. On increasing the percentage of perylene there is a change in the observed absorption spectrum from that observed for the monomer to that of the polymer. This involves the broadening red-shift and changes in intensity and position of the absorption maxima. The fluorescence spectra of each sample (measured at 1/40th concentration) are shown in Fig. 5b. Sample **P1**, containing 0.5% perylene, is seen to have only monomer-like emission. On increasing to 1% perylene, the intensity of the monomer emission increased, however, the region of the spectrum between $\lambda = 600$–650 nm showed a slight broadening. With greater percentages of perylene the monomer emission can be seen to decrease, whilst that of the excimer emission increases. Separate fluorescence spectroscopic measurements were conducted on **P9** and **P10** at much lower concentrations (Fig. S2, ESI‡) as the intensity of the measurements when the samples were prepared analogous to the other samples were out of the range of the machines used. The absorption of these two polymers is reminiscent of the absorption observed for interacting chromophores and the fluorescence spectra show excimer emission, however, in both cases emission of the monomer can also be seen.

The CD spectra of **P0**–**P10** measured in dichloromethane are shown in Fig. 5c. The samples containing only ethylene glycol units and low percentages of perylene are seen to have negative Cotton effects at $\lambda = 290$ nm. As the percentage of perylene in the samples increases the intensity of the peak at $\lambda = 290$ nm decreases and a positive peak at $\lambda = 320$ nm appears. Samples **9** and **10**, containing 50 and 100% perylene, have only positive peaks at $\lambda = 320$ nm. This leads to the conclusion that the ethylene glycol units affect the arrangement of the inner core of the helix while the perylene units do not. In addition, the perylene units dominate the extent to which the helical pitch is affected. To determine whether the trend observed is dependent on the concentration of the solution, the CD spectra were measured on solutions that were determined to have an absorption of *ca.* 0.8 a.u. at $\lambda = 492$ nm in the UV-Vis spectrum (Fig. S3, ESI‡). The spectra obtained under these conditions generated curves having the same shape as those seen in Fig. 5c, albeit with different intensities. Further dilution or concentration of the samples did not yield spectra with different CD shapes. The stability of the polymers was also determined by variable temperature CD spectroscopy. The polymers **P0** and **P10** in chloroform (1 mg in 5

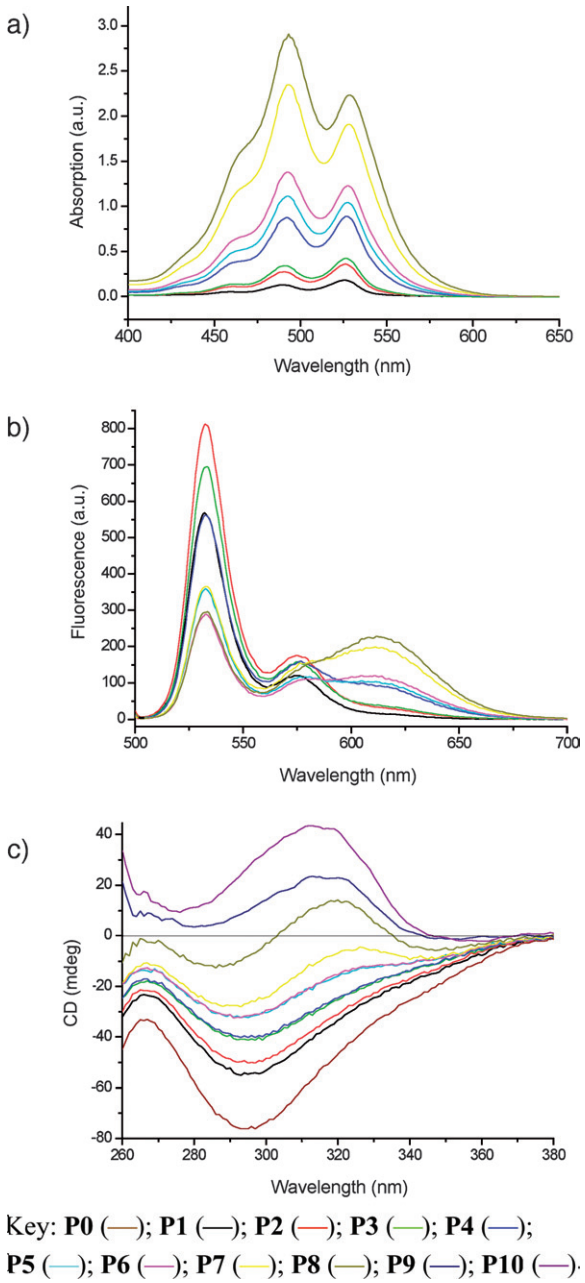


Fig. 5 Measurements of the randomly clicked polymers in dichloromethane. a) UV-Vis spectra (1 mg in 5 mL). b) Fluorescence spectra (25 μg in 5 mL; $\lambda_{exc} = 492$ nm). c) CD spectra (1 mg in 5 mL).

mL) were measured at 10, 20, 30, 40 and 50 °C (Fig. S4, ESI‡). In both polymers no change was observed in the temperature range studied. In order to go to higher temperatures an attempt was made to dissolve the polymers in toluene, however, neither polymer had any solubility in this solvent, which is in stark contrast to the perylene homopolymer obtained from **3b**, which was readily soluble in toluene.[11] The ethylene glycol polymer **P0** was dissolved in water (1 mg in 5 mL) and measured at 10, 20 and 50 °C. No changes were apparent at these temperatures, but on heating the sample to 80 °C the solution became turbid, due to the decreased solubility of ethylene glycol at high temperatures,[12] and a slight decrease of the CD signal was observed (Fig. S5, ESI‡). The original spectrum was, however, restored on cooling of the solution back to 50 °C. The fact that no change in the CD spectrum of each polymer is observed on changing of the concentration, solvent or on heating confirms that the different side arms have different, but stable, interactions with the helical backbone and in the case of the ethylene glycol side arms there is a slight unwinding of the helix occurring.

The GPC traces of **P0**–**P10** after purification on biobeads are shown in Fig. 6. In the case of **P10** a clear single peak is observed, but in all other cases multiple peaks can be seen. This indicates that in most cases there is a distribution of polymer sizes. While the GPC shows that polymers are present, the interaction of these molecules on a mica surface (AFM) is unusual and aggregation appears to be happening (Fig. S6, ESI‡).

Polymers **P0**–**P8** were investigated to see if the ethylene glycol units allowed for the solubility of these polymers in water. Disappointingly it was found that only the polymers with up to 2% of perylene (**P0**–**P3**) were soluble in water.

Towards A–B block formation studies

The cooperative effect seen with the random clicking opens the possibility that instead of random copolymers from clicking, the use of specifically designed azides may allow for the formation of A–B block copolymers. To investigate whether this approach is feasible, the known coumarin dye, 3-azido-7-hydroxycoumarin[13] was used as a second fluorescent marker. The reaction of the polymer scaffold **4** with equimolar equivalents of the perylene azide and the coumarin dye was performed to generate **P11**. The coumarin azide has very little fluorescence, but on formation of the triazole moiety, the intensity of the fluorescence is greatly increased. In order to compare the randomly clicked copolymer (**P11**), the polymer in which the scaffold was reacted with 100% coumarin (**P12**) was also prepared. In the latter sample the absorption spectrum of a dichloromethane solution (0.26 mM based on effective monomer unit) revealed a maximum at $\lambda = 340$ nm as shown in Fig. 7a. The absorption spectrum of **P11** (53 μM, based on half the sum of the two effective monomer units, assuming that clicking of the azide units is equal) shows, in the region of

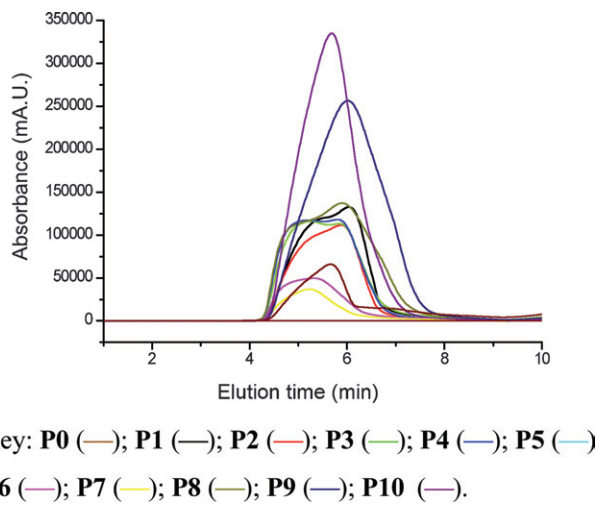
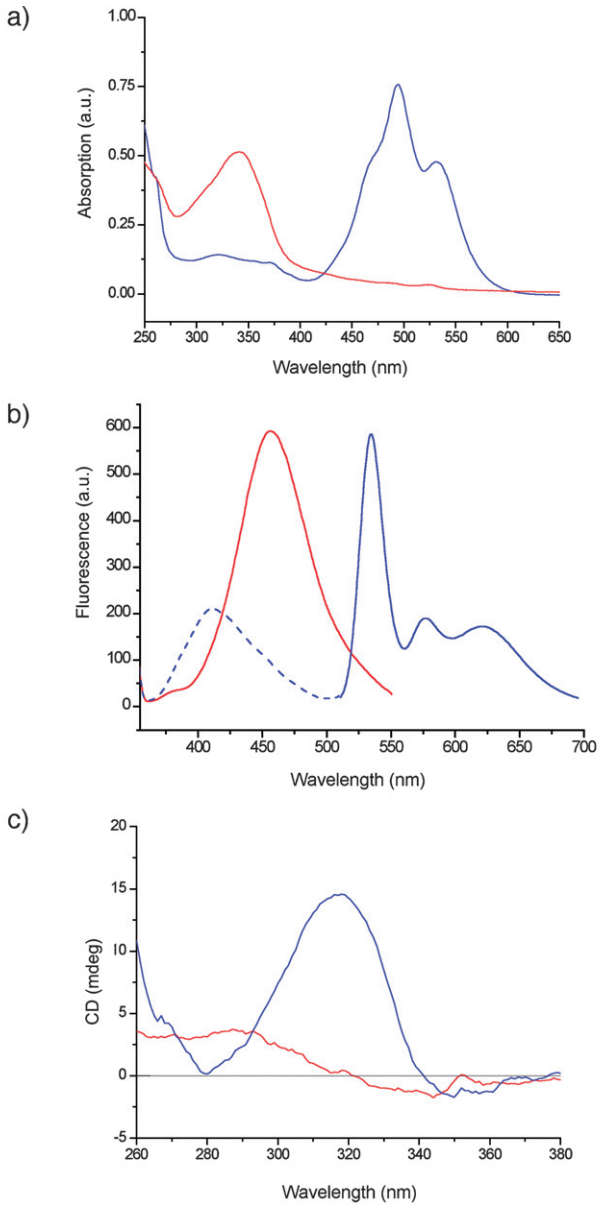


Fig. 6 GPC traces of the randomly clicked polymers in chloroform.



Key:
a) **P11** (53 μM, —), **P12** (0.26 mM, —).
b) **P11** (54 μM, λ_{exc} = 340 nm, ----; 2 μM, λ_{exc} = 492 nm, —), **P12** (0.64 μM, λ_{exc} = 340 nm, —).
c) **P11** (0.32 mM, —), **P12** (0.51 mM, —).

Fig. 7 Measurements of the block formation studies of polymers **P11** and **P12** in dichloromethane. a) UV-Vis spectra. b) Fluorescence spectra. c) CD spectra.

absorption for the perylene, peaks reminiscent to those of the homopolymer. In addition, two broad and overlapping peaks centred at λ = 322 and 370 nm corresponding to the coumarin unit were also seen. The extinction coefficient for 7-hydroxycoumarin is known to be 40 000 L mol^{-1} cm $^{-1}$ at 409 nm,[14] while that of perylene is 385 000 L mol^{-1} cm $^{-1}$ at 435.75 nm.[15] The differences in these coefficient values were reflected in the concentration differences of the solutions studied by

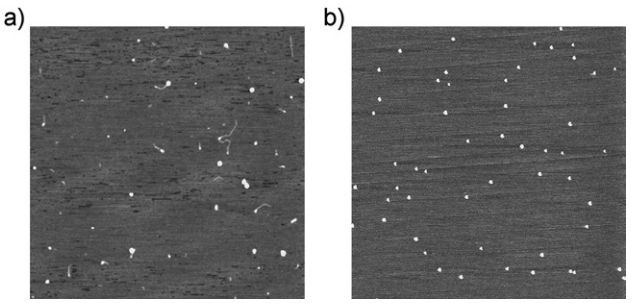


Fig. 8 AFM micrographs of **P12** spin-coated on mica from chloroform. a) Before size-exclusion chromatography (3 × 3 μm). b) After size-exclusion chromatography (5 × 5 μm).

fluorescence (Fig. 7b). The fluorescence spectrum of a solution of **P11** (53 μM), when excited at 340 nm, exhibits broad overlapping peaks between the region of λ = 375–500 nm from the coumarin; the polymer **P12** containing only coumarin (0.64 μM) has a fluorescence emission at λ = 462 nm. When a solution of **P11** (2 μM) is excited at 492 nm the fluorescence corresponding to the perylene is observed. In this case, as would be expected, there is fluorescence corresponding to both monomer and excimer emission. The existence of multiple peaks for **P11** in both the UV-Vis and fluorescence spectra indicates that there is indeed communication between the coumarin and the perylene; the coumarin molecules in close proximity of a perylene have a largely quenched and blue-shifted emission.

The CD spectrum (Fig. 7c) obtained for copolymer **P11** shows a positive Cotton effect at λ = 320 nm, however, that of the **P12** is very different. In this case there is a slight negative peak at λ = 340 nm and a positive peak of twice the intensity at 290 nm. It is clear from the CD spectra obtained from these and earlier experiments that the perylene molecules dominate the arrangement of the helical backbone in cases where clicking involves more than one azide.

AFM micrographs of polymers **P11** and **P12** were collected. It was noted that the micrographs of a solution of **P12** before size-exclusion chromatography (Fig. 8a) contained polymers of much greater lengths than those observed after the chromatography (Fig. 8b). In fact, the latter micrographs do not show polymer strands clearly, more aggregation of very short polymers. This leads to the conclusion that the click reaction conditions do not destroy the polymer as previously hypothesised,[9] but merely that the long polymers are sticking to the biobeads and are being removed from the shorter polymers.

Conclusions

The post-modification of the polymer scaffold L,L-PIAAPE has been effectively achieved using a variety of azide moieties. Although the fluorescence intensity of the polymer obtained from clicking a perylene azide onto the scaffold is not as great as that of the perylene homopolymer from an isocyanide monomer, the clicking method was found to be effective for making random copolymers and there are indications that in this process cooperative interactions lead to blocks of perylene units along the polymer backbone. Use of an ethylene glycol azide lead to the

formation of water-soluble polymers, and further incorporation of the perylene azide gave chromophoric water-soluble polymers in which the CD spectrum is the same in water as in dichloromethane. By use of a second chromophore, a coumarin dye, random polymers were formed by the click reaction in which absorption and emission from both polymers could be observed. In this case, interaction between the chromophores was observed, evidenced by a quenched and blue-shifted emission of the coumarin molecules in close proximity to a perylene molecule. The clicking method has been found to be an effective method for forming polymers from compounds which are costly and for which the synthesis of the isocyanide may not be straightforward. In particular the ability to form water-soluble dye-containing polymers, which can be modified by the addition of biomolecules, such as antibodies, proteins and peptides, give materials that are very promising as novel biomarker materials.

Experimental

General

All click reactions were performed under Schlenk conditions using distilled solvents. Tetraethylene glycol monomethyl ether, sodium azide and PMDETA were purchased from ACROS chemicals. Copper bromide and 3,4:9,10-perylenetetracarboxydianhydride sodium azide were purchased from Sigma Aldrich. All purchased chemicals were used as received. 1-Azido-3-aminopropane,[16] 1-hexylheptylamine,[17] 3-azido-7-hydroxycoumarin[13] and L,L-PIAAPE[9] were prepared by literature procedures. Column chromatography was performed using silica gel (40–60 μm) purchased from Merck. TLC analyses were carried out using silica 60 F_{254} coated glass obtained from Merck and the compounds visualised with Ninhydrine, iodine or $Ni(ClO_4)\cdot 6H_2O$ in ethanol. Size-exclusion chromatography was performed using Bio Bead S-X1 using $CHCl_3$ as eluent. ^1H NMR spectra were recorded, at 20 °C, on a Varian Inova400 or Bruker AC-300 machines operating at 400 and 300 MHz, respectively. ^{13}C NMR spectra were recorded on a Bruker AC-300 machine operating at 75 MHz. FT-IR spectra were recorded on a ThermoMattson IR300 spectrometer equipped with a Harrick ATR unit; compounds were measured as an oil or solid. Melting points were measured on a Buchi B-545 and are reported uncorrected. Mass spectrometry measurements were performed on a VG 7070E instrument (EI/CI) or on a JEOL Accutof instrument (ESI). CD spectra were recorded on a Jasco 810 instrument equipped with a Peltier temperature control unit and were measured at 20 °C unless otherwise stated. AFM experiments were performed using a Nanoscope IV instrument from Digital Instruments. Solutions of the samples were spin-coated onto freshly cleaved Muscovite mica. All images were recorded with the AFM operating in tapping mode in air at room temperature with a resolution of 512 × 512 pixels using moderate scan rates (1–2 lines per second). Commercial tapping-mode tips (NT-MDT) were used with a typical resonance frequency around 300 kHz. 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 using chloroform as an eluent (1 mL min^{-1} at 35 °C).

3,4,9,10-Perylenetetracarboxy-3,4-anhydride potassium salt 7

3,4:9,10-Perylenetetracarboxydianhydride (10.0 g, 25.5 mmol) was placed in a 3-necked round bottom flask and water (800 mL) added. Potassium hydroxide (40.0 g, 0.71 mol) was added and the resulting mixture was stirred at 90 °C for 2 h, over which time the reaction mixture changed from red to green. Addition of acetic acid (50 mL) resulted in a colour change to brown and after stirring at 90 °C for 40 min the purple solid (11.0 g, 96%) was removed by filtration and washed thoroughly with methanol before being dried at 120 °C. Due to the insolubility of this compound in all solvents, characterisation could not be performed.

N^1-(3-Azidopropyl)-3,4,9,10-perylenetetracarboxy-3,4-anhydride-9,10-imide 8

3,4,9,10-Perylenetetracarboxy-3,4-anhydride potassium salt (1.92 g, 4.30 mmol) and 1-azido-3-aminopropane (2.15 g, 21.50 mmol) were placed in a round bottom flask and water (60 mL) added. The solution was stirred at 90 °C for 48 h before the addition of aqueous potassium carbonate (25%, 200 mL). The solution was heated at 90 °C for 3 h, over which time the colour changed from purple to green. The solid was filtered off and washed from the filter with a water (300 mL) and triethylamine (10 mL) mixture. The filtrate was diluted with HCl (2 M, 500 mL) and after sitting for one day the precipitated solid was filtered off and washed with methanol. The product was obtained as a purple solid (2.0 g, 98%). Due to the insolubility of this compound in all solvents, characterisation could not be performed.

N^1-(3-Azidopropyl)-N^2-(1-hexylheptyl)-3,4:9,10-perylenetetracarboxydiimide 9

N^1-(3-Azidopropyl)-3,4,9,10-perylenetetracarboxy-3,4-anhydride-9,10-imide (1.10 g, 2.31 mmol) and 1-hexylheptylamine (0.92 g, 4.63 mmol) were suspended in a solution of imidazole (49 g) and DMF (60 mL). The mixture was heated overnight at 120 °C before being cooled to room temperature. The addition of ethanol (200 mL) followed by the addition of aqueous citric acid (10%, 200 mL) generated a solid, which was removed by filtration. The crude solid was subjected to column chromatography (eluent: $CHCl_3$) to give the product as a dark red solid (0.82 g, 54%) having mp > 218 °C (dec.). Anal. Calcd for $C_{40}H_{41}N_5O_4$: C, 73.26; H, 6.30; N, 10.68. Found: C, 73.51; H, 6.30; N, 10.48. ^1H NMR (300 MHz, $CDCl_3$, 20 °C): $\delta = 0.83$ (t, 6 H, $^3J_{HH} = 6.90$ Hz, CH_3), 1.20–1.41 (overlapping multiplets, 16 H, $(CH_2)_4CH_3$), 1.92 (m, 2 H, $NCHCH_2$), 2.06 (p, 2 H, $^3J_{HH} = 6.90$ Hz, $CH_2CH_2N_3$), 2.26 (m, 2 H, $NCHCH_2$), 3.46 (t, 2 H, $^3J_{HH} = 6.90$ Hz, CH_2N_3), 4.24 (t, 2 H, $^3J_{HH} = 6.90$ Hz, $CH_2CH_2CH_2N_3$), 5.16 (m, 1 H, NCH), 8.21 (d, 2 H, $^3J_{HH} = 8.43$ Hz, perylene), 8.28 (d, 2 H, $^3J_{HH} = 8.10$ Hz, perylene), 8.36 (d, 2 H, $^3J_{HH} = 7.80$ Hz, perylene), 8.49 (d, 2 H, $^3J_{HH} = 6.60$ Hz, perylene). ^{13}C NMR (75 MHz, $CDCl_3$, 20 °C): $\delta = 14.0$ (CH_3), 22.5 (CH_2CH_3), 27.0, 27.6 (CH_2), 29.2 ($CH_2CH_2N_3$), 31.8 (CH_2), 32.3 ($NCHCH_2$), 38.0 (NCH_2), 49.4 (CH_2N_3), 54.9 (NCH), 122.6, 122.8, 125.8, 125.8, 128.9, 129.2, 130.9, 133.6, 134.2 (perylene C), 162.9 (C = O). FT-IR (cm^{-1}, ATR) 2954, 2919, 2850 (C–H stretch), 2094 (N_3), 1692,

1640 (amide) 1588, 1573 (C=C aromatic), 1333 (C–N stretch), 806, 741 (CH aromatic). MS-ESI m/z = 1333 $[2M + Na]^+$.

11-Methoxy-3,6,9-trioxaundecyl *p*-toluenesulfonate 11

Tetraethylene glycol monomethyl ether (2.20 g, 10.57 mmol) and triethylamine (1.62 mL, 11.62 mmol) were placed in a Schlenk flask under nitrogen and dissolved in dichloromethane (50 mL). The solution was cooled to 0 °C and tosyl chloride (2.22 g, 11.62 mmol) in dichloromethane (60 mL) was added dropwise over 1 hour. The solution was left stirring overnight at room temperature after which time TLC showed residual tosyl chloride and the desired product. The crude mixture was subject to column chromatography; dichloromethane was used to elute the excess tosyl chloride and 2% MeOH in CH_2Cl_2 to elute the desired compound. The product was obtained as a colourless oil (2.55 g, 67%). Anal. Calcd for $C_{16}H_{26}O_7S$: C, 53.02; H, 7.23. Found: C, 53.01; H, 7.42. ^{1}H NMR (400 MHz, $CDCl_3$, 20 °C): δ = 2.44 (s, 3 H, CH_3), 3.37 (s, 3 H, OCH_3), 3.53–3.70 (overlapping multiplets, 14 H, CH_2CH_2OMe), 4.16 (t, 2 H, $^3J_{HH}$ = 4.84 Hz, CH_2OTS), 7.34 (d, 2 H, $^3J_{HH}$ = 8.08 Hz, ArH *ortho* to OMe), 7.79 (d, 2 H, $^3J_{HH}$ = 8.32 Hz, ArH *ortho* to OTs). ^{13}C NMR (300 MHz, $CDCl_3$, 20 °C): δ = 21.6 (CH_3), 59.0 (OCH3), 68.7, 69.2, 70.5, 70.5, 70.6, 70.6, 71.9 (CH_2CH_2), 128.0, 129.0 (ArC), 133.0 (CSO_2), 144.8 (CCH_3). FT-IR (cm^{-1}, ATR): 2870 (br, CH_2/CH_3), 1175 (S=O).

1-Azido-11-methoxy-3,6,9-trioxaundecane 12

Tosylate **11** (0.68 g, 1.88 mmol) was dissolved in ethanol (50 mL) and sodium azide (0.25 g, 3.77 mmol) added. The solution was heated under reflux for 2.5 h, after which time TLC (10% MeOH in $CHCl_3$) showed no starting material present. The solvent was removed under vacuum and CH_2Cl_2 added to the residue. The organic layer was extracted with water (3 times) and dried ($MgSO_4$). The solvent was removed and the crude product was purified on silica (2% MeOH in CH_2Cl_2) to give a colourless oil (0.43 g, 99%). Anal. Calcd for $C_9H_{14}O_4N_3$: oil to volatile for measurements. ^{1}H NMR (400 MHz, $CDCl_3$, 20 °C): δ = 3.38 (s, 3 H, OCH_3), 3.40 (br s, 2 H, CH_2N_3) 3.53–3.56 (m, 2 H, $CH_2CH_2N_3$), 3.64–3.69 (overlapping multiplets, 12 H, $-CH_2CH_2OMe$). ^{13}C NMR (300 MHz, $CDCl_3$, 20 °C): δ = 50.7 (CN_3), 59.0 (OCH_3), 70.0, 70.5, 70.6, 70.6, 70.7, 70.7 72.9 ($-CH_2CH_2O$). FT-IR (cm^{-1}, ATR): 2872 (br, CH_2/CH_3), 2101 (N_3), 1105 (C–O–C).

Typical procedure for random click reactions

The polymer scaffold was suspended in a solution of CH_2Cl_2 (10 mL) under an inert atmosphere and the azides added (THF was added when coumarin was used to avoid precipitation). The addition of PMDETA and CuBr was carried out and the solutions were stirred overnight. The precipitated copper salts were removed from the solution by the repeated addition of a saturated aqueous EDTA solution and removal of the water layer from the top. The organic layer was dried over $MgSO_4$ and the solvent removed to give the polymer as a solid. The polymers were subjected to size-exclusion chromatography to remove any unreacted azides.

^{1}H NMR Spectroscopy

In each case of clicking the ^{1}H NMR spectrum was measured. The spectra are broadened as expected for a polymer. The acetylene peak seen at 2.51 ppm in the scaffold is no longer present and there is an additional peak at 7.60 ppm corresponding to the proton of the triazole ring. This peak is very evident in the samples in which the percentage of perylene is low, however, as the quantity of perylene in the samples increases, the aromatic region of the spectrum becomes broad and the triazole peak is incorporated into the broad resonances for the perylene protons. ^{1}H NMR spectra of **P1**, **P4** and **P8** (in $CDCl_3$) are shown in Fig. S7, ESI‡.

IR spectroscopy

In each case of clicking the IR spectrum was measured. The peak corresponding to the acetylene stretching frequency at 2129 cm^{-1} is no longer evident and in addition no azide peaks can be seen that would indicate unreacted azide in the sample.

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