SURFACE MODIFICATION OF HARD PVC BY MOLECULES WITH ANTIBACTERIAL ACTIVITY

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SURFACE MODIFICATION OF HARD PVC BY MOLECULES WITH ANTIBACTERIAL ACTIVITY

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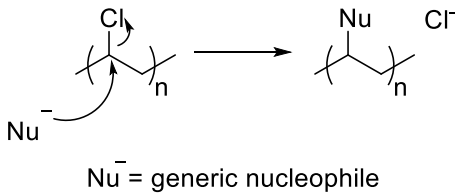
CHAPTER 1. **SUMMARY**

In this work, we present an analysis of different PVC surface modifications, attempted with the intention of attaching antibacterial small molecules, polymers, and oligomers on the plastic. These modifications allowed us to obtain intrinsically antibacterial PVC, which can be potentially applied in healthcare and medical devices. The modification was performed with two procedures, copper-catalyzed azide-alkyne cycloaddition, and nucleophilic substitution. In the first case, the surface of PVC was initially treated with sodium azide to obtain partially azidated PVC, followed by treatment with alkyne-bearing small molecules and polymers. In the second method, the surface was treated with amine-bearing small molecules and polymers, directly substituting the chlorine atoms on PVC. We concluded that the hydrophilicity, the size of the molecule, and the reaction conditions, are the main factors that influence the success of these modifications. Bacteria viability tests were performed on differently-substituted PVC samples, showing good antibacterial activities for PVC surfaces treated with quaternary ammonium salts and acceptable activities for samples modified with polyethyleneimine and oligoethylene glycol.

CHAPTER 2. **INTRODUCTION**

Poly(vinyl chloride) (PVC) is one of the world's most produced and utilized plastics. Its unique chemical structure and tunable macroscopic properties make it an interesting platform with almost endless applicability. In commercial and industrial applications, it has attracted attention in the past 90 years because of its macroscopic physical properties. The conformation of the repeat units in the chains make it a tough and rigid material; however, PVC can be modified by a multitude of plasticizers and other additives, so that its final physical characteristics can be tuned to obtain materials with different features.[1] For example, different modifications can impart softness and flexibility, and hence open the door for an even wider variety of end uses. Thus, poly(vinyl chloride) is a generic name encompassing a multitude of morphologies and molecular masses, depending on the intended end-use [2].

PVC is also highly exploited in the chemistry and materials engineering world, due to the relatively easy chemical modifications that can be performed on its surface. Overall a nonpolar material, PVC retains hydrophobic plasticizers that can diffuse throughout the structure[3], and its C-Cl bonds also provide for good reactivity toward nucleophilic substitution reactions with multiple nucleophiles,[4,5] including cross-linkers.[6]



Scheme 1 Generic substitution reaction of Cl with a nucleophile in PVC

Our purpose is to exploit the chemical modifications that can be performed on the surface of PVC for the addition of antibacterial molecules. Deaths due to bacterial infections are on the rise: every year, 1.7 million hospital-associated infections related to infected or dirty materials, from all types of microorganisms, cause or contribute to 99,000 deaths in the United States.[7] It is reported that one-third of all medical devices, both single-use and multiple-use, are made from PVC, [2] such as flexible tubing, syringes, and containers.[8] Since PVC can be covalently modified, it is plausible to think that attaching antimicrobial scaffolds on its surface might have a positive impact on this infection outbreak.

Many different polymers have been modified in the last few decades to enrich them with antimicrobial properties.[9] Most of these modifications combine features of permanent positive charge with hydrophobic substituents, as in quaternary alkylammonium salts, that have a broad spectrum of antimicrobial activity against both Gram-positive and Gram-negative bacteria.[10] It has also been shown that polymeric antimicrobials are advantageous over their small molecules counterparts because they are nonvolatile, can be rendered chemically stable, have long-term antimicrobial activity, and are hard to permeate through the skin.[11,12]

In the past, PVC has been modified to achieve antibacterial properties by coating with TiO_2 films,[13] zirconium phosphate/silver particles, [14] and covalently linked plasticizers.[15] Notably, two such studies have investigated the use of copper-free or copper-catalyzed azide-alkyne cycloadditions to modify pre-azidated PVC,[15,16] an approach that we also chose to employ.

This work was designed to connect two different interests of our research group. On the one hand, it aimed at expanding the applications of cationic thiabicyclononane-based oligomers which were recently designed, synthesized and tested for their antimicrobial activity, and showed promising properties.[17, 18] In addition, we wanted to continue a project which achieved successful modification of flexible PVC with small molecules and polymers, having a robust synthetic process already available.[19] In this work, we hoped to expand the procedure for this chemical modification to hard PVC, using multiple quaternary ammonium salts and other small molecules or polymers as the reagents, and especially explore if our thiabicyclononane-based oligomers could be effectively attached on PVC, further increasing their promising features.

CHAPTER 3. **RESULTS AND DISCUSSION**

3.1 Chemical modifications

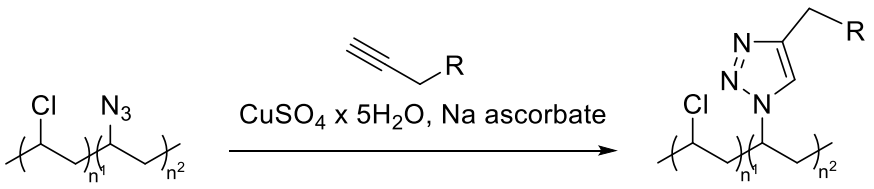
3.1.1 The strategy for the chemical modification of PVC

As mentioned in the introduction, a procedure for the successful modification of flexible PVC was previously designed in our research group.[19] Although this was a good starting point, the conditions had to be slightly modified to be applied to the clear, hard, chemical-resistant PVC used in this study. Our material had a tensile strength of 2,680-3,400 psi and Rockwell R57 hardness, and a K-value of 74; PVC with such hardness is usually employed in containers and rigid materials, while PVC with lower K values, such as the one in the previous study, is usually used in flexible formulations such as sheets and tubing.[2] Differently processed PVC can achieve different final morphologies, and it's natural to expect different chemical reactivities and stabilities as the k-value changes.

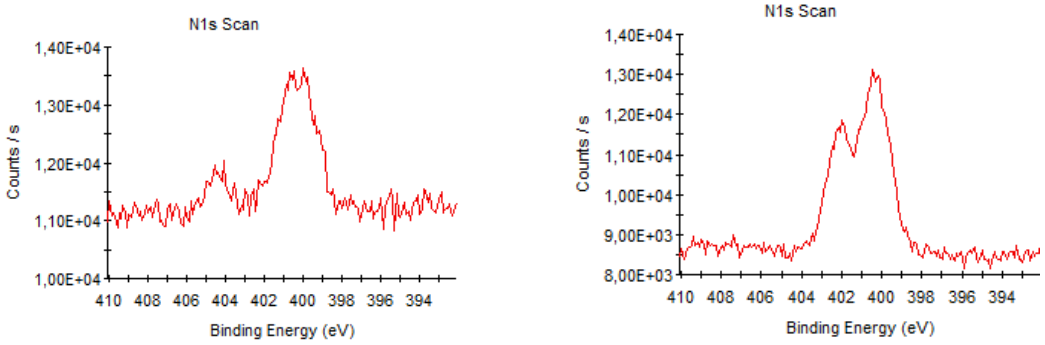
To functionalize the surface, we initially employed a two-step process: azide groups were initially installed on the surface of PVC using NaN_3, followed by copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction between azidated-PVC and alkyne-bearing molecules. Although single-step procedures are available for the modification of this surface, by taking this route we hoped to achieve a modular approach whereby a limited number of azidated precursors could be used to make a larger number of triazole-functionalized surfaces.

Characterization proved to be a challenge. Since PVC is soluble in some organic solvents,[20] samples can be dissolved for analysis by solution-phase ^{1}H NMR. However, it can be hard to distinguish if any modification occurred on the surface due to three main reasons: the difficulty in precisely quantifying proton distributions in polymers, the probable overlap between the signals of protons coming from -CHCl and -CHN_3, and especially the very limited amount of functionalization that can be achieved in the first place, since only the exposed surface of the PVC material undergoes aqueous-phase reaction.

We therefore turned to Fourier transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS) for the surface characterization of PVC. The azide group provides excellent signals in both IR and XPS, the former because of the intense stretching band in an area of the spectrum which is commonly free from other peaks, and the latter because of the characteristic two-peak pattern for the N_3 unit. We therefore used both to determine if -N_3 groups had been installed by reaction with sodium azide, and to what extent they had reacted in CuAAC reaction with alkynes (Figure 1). For reference, a representative PVC disc and the XPS spectrum of unmodified PVC from the manufacturer are shown in Fig. 2. As expected, no nitrogen atoms were detected in the XPS of this material. We were also able to differentiate covalent azide attachment from simple physical adsorption of NaN_3 salt on the surface by virtue of the persistence of these signals after extensive washing. As will be discussed below, an S_N2 reaction was also employed to modify the surface as an alterative to the azidation+CuAAC sequence, and was also followed by FTIR by identifying signature signals of the inserted molecule.



a



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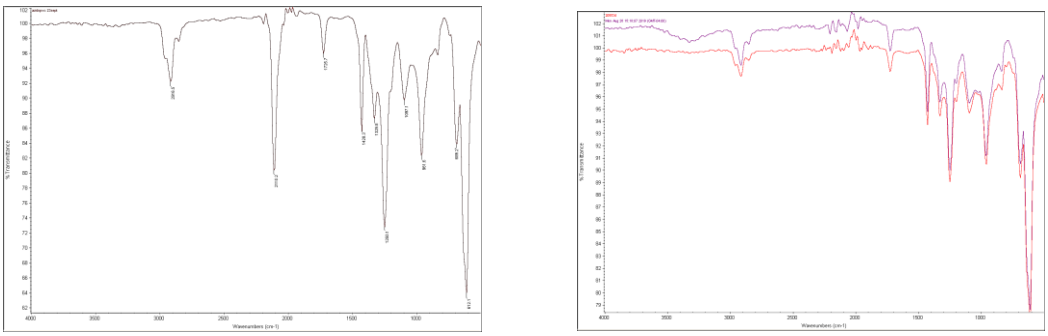


Fig 2 Scheme representing the observable spectral changes (FTIR (a) and XPS (b)) on the surface of PVC discs after different steps of the process. The spectra are used only to demonstrate these modifications, although they were recorded on the samples from this study, as reported below. Blue and red FTIR spectra correspond to the analysis of the two faces of one disc.

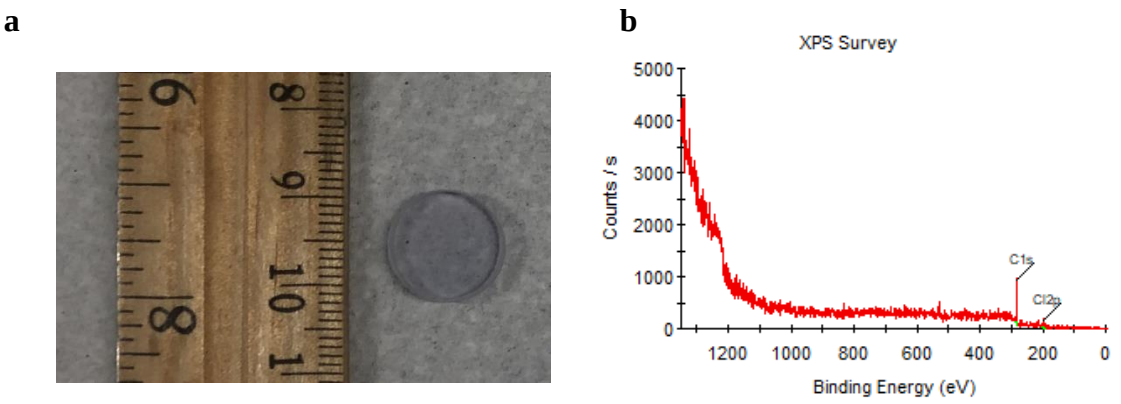


Fig 3 (a) appearance and dimensions and (b) XPS survey spectrum for unmodified PVC as received from the manufacturer. As can be seen, the only elements identified are C(1s) and Cl(2s), as is typical for PVC

3.1.1 Antibacterial molecules and their properties

Variables of reagents, concentration, temperature, and time were explored for the azidation of PVC. Consistently optimal results were obtained at 80°C for 5h in H_2O, using 10 equivalents of NaN_3, and BuN_4Br as a phase transfer catalyst (PTC). Two CuAAC reactions were found to proceed well at 80°C for 2h in H_2O even without PTC, although these conditions could not be applied to all cases, as described below. Using these reactions, we aimed to construct six different types of "antibacterial" PVC, by performing the CuAAC reaction with seven different alkynes: four oligomers, one polymer, and two small molecules, depicted in Fig. 3.

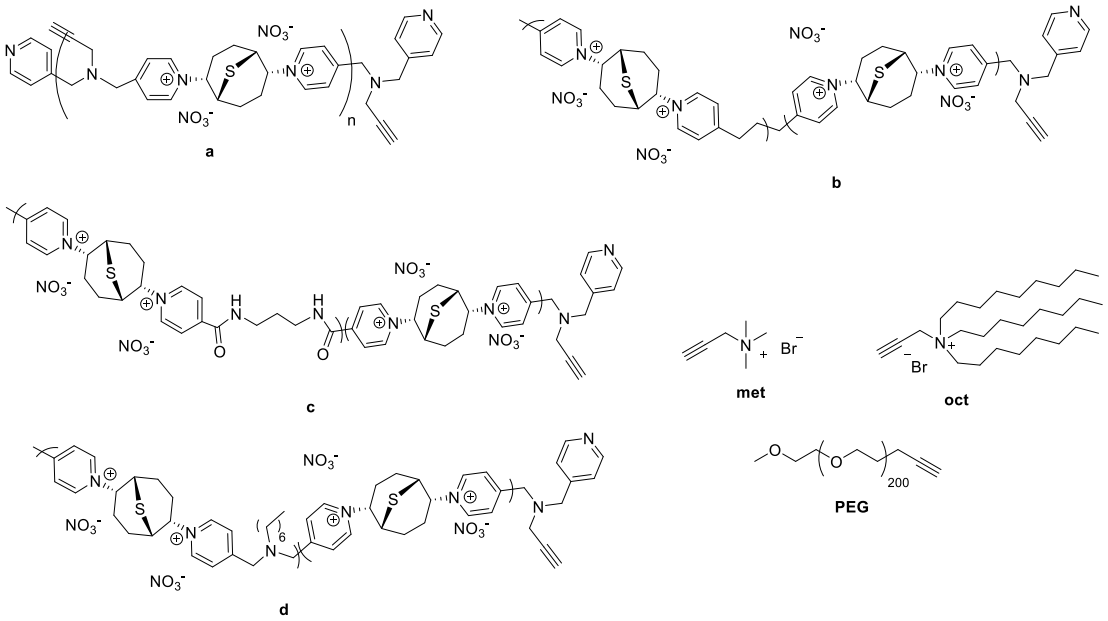


Fig 4 structures of the alkyne-bearing compounds tested for the CuAAC reaction on PVC-N_3: four oligomers (a,b,c,d), one polymer (PEG), and two small molecules (met and oct).

As discussed, oligomers with the same structure as those illustrated in Fig. 3 have been previously designed, synthesized and tested in our laboratory.[17,18] Modifications had to be made to ensure the presence of alkyne groups, either in every repeat unit as in polymer **a**, or only in some of the repeating units, such as in copolymers **b**, **c**, and **d**. Copolymer **b** was already synthesized in a previous work,[18] while the new polymer **c** was formed by the addition of a 4:1 ratio of two dipyridine monomers (N,N'-(propane-1,3-diyl)diisonicotinamide and N,N-bis(pyridin-4-ylmethyl)prop-2-yn-1-amine), and the new polymer **d** was made with a 1:1 ratio of the two monomers N,N-bis(pyridin-4-ylmethyl)prop-2-yn-1-amine and N,N-bis(pyridin-4-ylmethyl)heptylamine. Depending on the nature of the dipyridine linker used, oligomers of different average lengths ranging from 10 to 12 repeating units were obtained, as determined by end-group analysis of the ^{1}H NMR spectra (Experimental section). The PEG was commercially available bearing a terminal alkyne functionality, while *N,N,N*-trialkyl-propargyl ammonium bromides "oct"

and “met” were prepared by reacting propargyl bromide with the respective trialalkylamine (Experimental section). The six structures are considerably different in terms of hydrophilicity, size, and mass-to-charge ratio. Unfortunately, the limited thermal stability of the thiabicyclononane-based oligomers made them unsuitable for use in the current conditions, as these molecules undergo fragmentation at elevated temperatures.[17] Since we found the CuAAC ligation step to be best at 80°C, we tested the stability of polymer **a** to these conditions. The compound was found to completely decompose within two hours at this temperature as shown in Fig. 4, and similar behavior is expected for the other polymers. See below for an account of the attempted surface attachment reactions using these oligomers at lower temperatures.

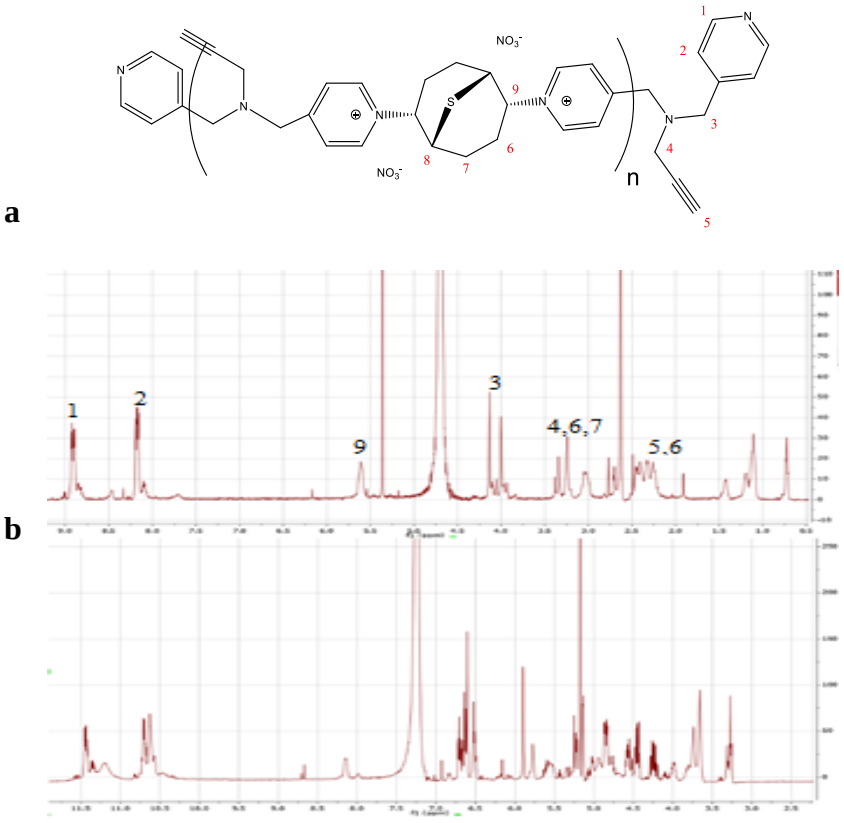


Fig 5 1H NMR spectra of polymer a before (a) and after (b) heating in water at 80°C for 2 h. It can be observed that the polymer decomposed, and no clear structure is visible.

3.1.2 Chemical modification of PVC

Various conditions for the CuAAC reaction of *N*,*N*,*N*-trioctyl-propargyl ammonium bromide with azidated PVC were tested. The best results were obtained with stirring of the alkyne and PVC-N_3 in the presence of 0.05 mol% $CuSO_4$, 0.10 % sodium ascorbate as the reducing agent, and an optional phase-transfer catalyst at 80°C. Under these conditions, the reaction was complete within 2 h, as determined by FTIR and XPS (Fig. 5). When Bu_4N BF_4 was used as the PTC, the XPS survey spectrum showed the presence of residual components adhering to the surface (or incorporated into the bulk) of the PVC discs: F1s (685.87 eV) and some of the N1s (400.71 eV) can be attributed to leftover PTC; Cu $2p^3$ (932.35 eV) results from some leftover catalyst, and O1s (532.71 eV) can be attributed to some adsorbed water on the sample. However, the major peaks correspond to the desired material: C (C1s, 285.34 eV, 74.36%), N (N1s, 400.71 eV, 5.37%), Cl (Cl2p, 200.87 eV, 4.85%), and Br (Br3d5, 68.71 eV) as the counterion of the attached ammonium salt. Later, we found that the reaction can also be successfully run without PTC, so we expect that F won't be present; and we successfully optimized a washing procedure to eliminate leftover Cu.

Besides the XPS measurements, which showed a successful completion of the reaction, a control reaction was also run on a PVC-N_3 sample: the same exact conditions utilized for the CuAAC reaction were adopted, maintaining the same proportion of all the reagents but omitting the alkyne. In this case, no disappearance of the azide peak was observed from

the IR spectrum, confirming that the conditions used do not damage the azide functionality, and that the reaction proceeds as expected (Fig. 5).

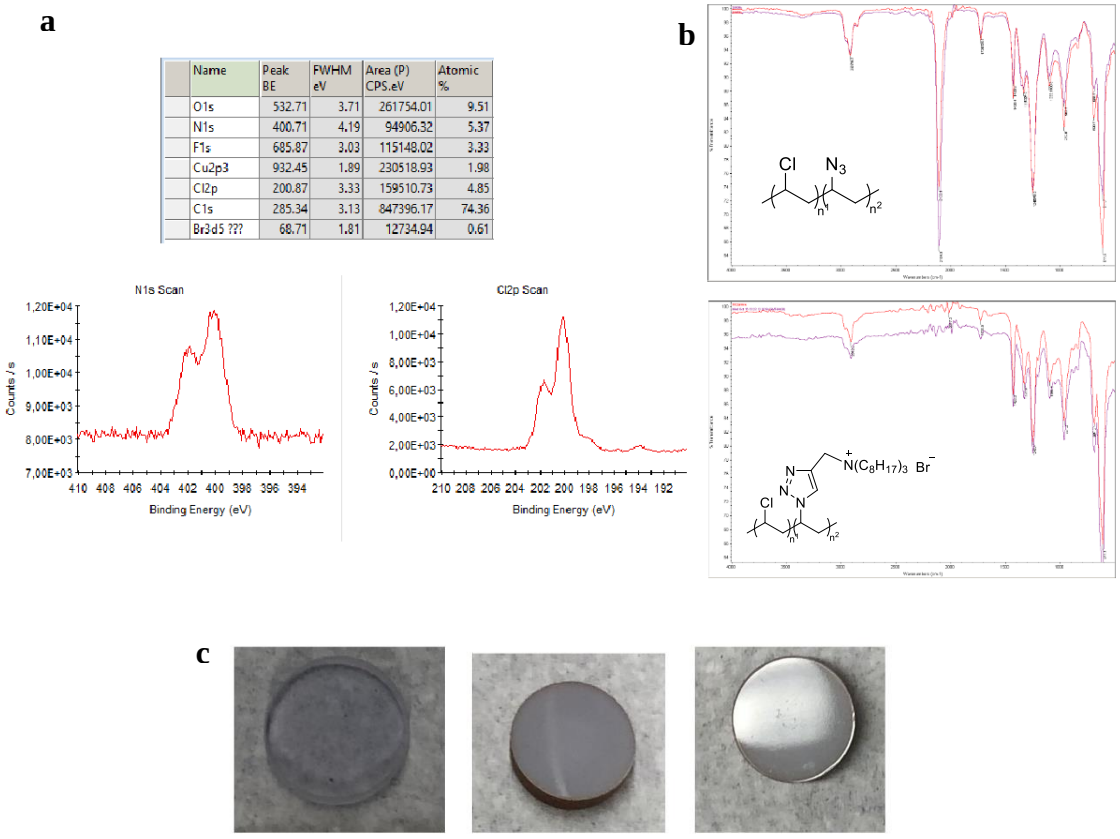


Fig 6 Characterization of *N,N,N*-trioctyl-propargyl ammonium bromide-modified PVC-N_3. (a) represents the results of the XPS analysis, showing the atomic % of different components present on the surface, the signals belong to N1s and Cl2p, and the atomic survey. (b) represents the FTIR spectra of PVC-N_3 at the top, and the same disc after CuAAC reaction at the bottom; red and blue lines represent the two different faces of the same disc. (c) from left to right, unmodified PVC (clear and transparent), PVC-N_3 and oct-PVC (opaque, off-white).

From XPS measurements, it was also possible to roughly quantify the degree of modification, by comparison of the XPS spectra of unmodified PVC, PVC-N_3, and oct-PVC. The azidation step was found to have substituted approximately 9.6% of all Cl atoms in the outer layer of the material (or in the area that the XPS is able to penetrate, which has a 5 nm depth). Since the CuAAC reaction results in the loss of all of the azide signal in the

FTIR and XPS spectra, and that CuAAC conditions do not decompose the azide in the absence of alkyne, we assume that the loading density of the attached quaternary ammonium salt is also approximately 9.6% of the initial chloride sites.

The same reaction conditions were not appropriate for the attachment of *N*,*N*,*N*-trimethyl-propargyl ammonium bromide to the azidated PVC surface (Fig. 6). Fortunately, switching the PTC from Bu_4NBF_4 to Bu_4NBr, and prolonging the reaction time to 8 hours, proved successful. The reason for this preference for a different PTC is unknown; but our group has reported similar variations in PTC performance in the functionalization of flexible PVC.[21] Further, we discovered that running the reaction in the absence of any PTC, and prolonging the time to 24 h, also generated the desired product.

In contrast to the case of the trioctylammonium salt, the FTIR showed a substantial amount of water (3345.1 cm^{-1}) to be retained in or on the trimethylammonium-functionalized substrates, which does not release even after multiple days of air drying. This is consistent with the expected hydrophilic nature of this surface. Moreover, the samples substantially changed color, turning from transparent (PVC, PVC-N_3, and octyl-PVC are all clear) to dark, indicating that Cl elimination reactions occurred during the click reaction.[22] The appearance of FTIR signals at ~997 cm^{-1}, consistent with the presence of C=C bonds, further supports this hypothesis. We initially assumed that elimination reactions were promoted in this case because of the prolonged reaction times required. However, running the same reaction with *N*,*N*,*N*-trioctyl-propargyl ammonium bromide for 24 h at 80 °C still gave white PVC discs. We suspect that the propargyl-trimethylammonium reactant provides a more polar reaction environment at the surface and thereby favors elimination to a much greater degree than the corresponding trioctylammonium alkyne. We also believe the

CuAAC reaction of the latter species to be accelerated by noncovalent association of the relatively hydrophobic trioctylamonium to the hydrophobic PVC surface (Fig. 7). Surfaces with aqueous contact angles between 90° and 150° are considered to be hydrophobic[23]. As shown in Fig. 7, PVC is in this category.

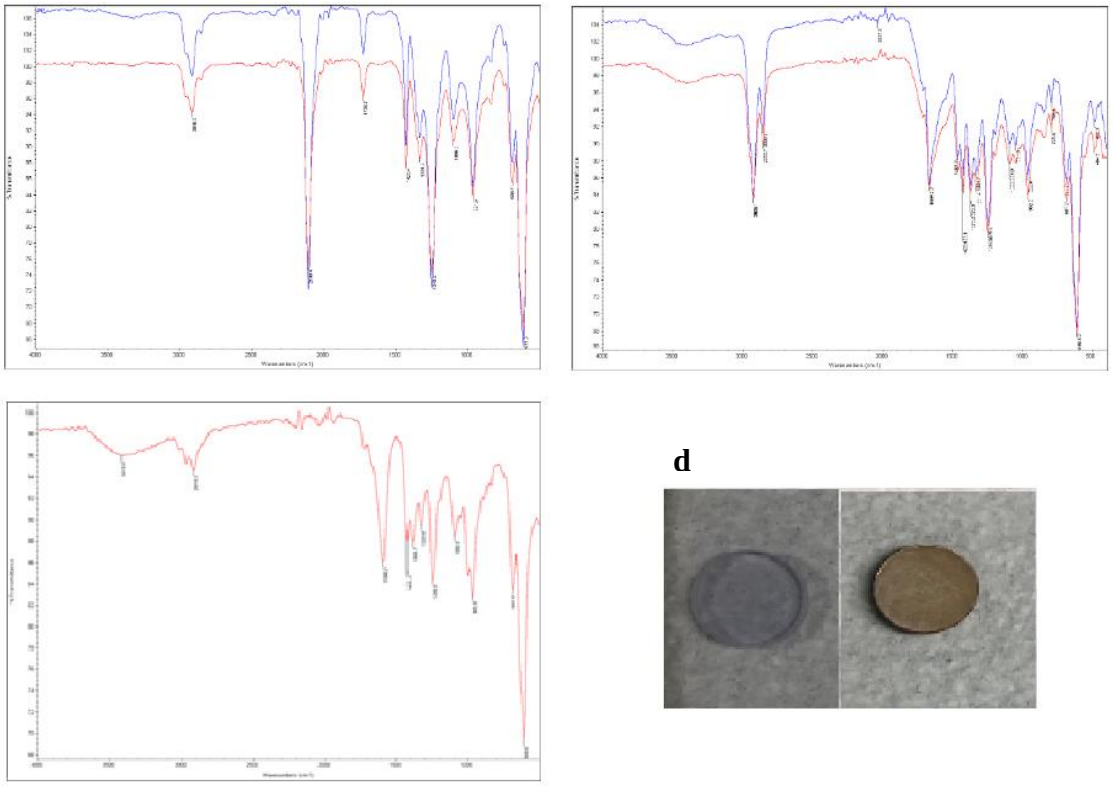


Fig 7 Characterization of *N*,*N*,*N*-trimethyl-propargyl ammonium bromide- modified PVC-N_3: FTIR spectra of samples after CuAAC reaction in different conditions: (a) 2h, 80°C, $Bu_4N\ BF_4$; (b) 8h, 80°C Bu_4NBr; (c) 24h, 80°C. red and blue lines represent the two different faces of the same disc; (d) appearance of unmodified PVC (left) and met-PVC (right).

a

CONTACT ANGLE MEASUREMENT	ANGLE
1	102,3°
2	102,4°
3	102,4°

b

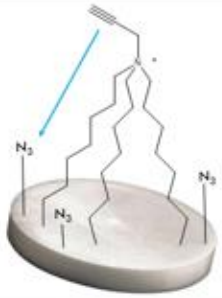
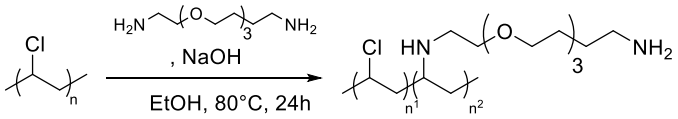


Fig 8 (a) Contact angle measurement (reported measured angles and picture of the experimental conditions) for pure, unmodified PVC. (b) representation of the interaction between alkyne and azide facilitated by the alkyl chains of *N*,*N*,*N*-trioctyl-propargyl ammonium bromide.

We also explored the functionalization of PVC through click reaction with PEG of molecular weight 2000 g/mol bearing an alkyne functional group at one end. PEG chains are commonly used as antibacterial coatings, either because of their relative resistance to adhesion to biological molecules, or because of their reported ability to cause clumping and morphological changes to bacterial cell walls.[24] Reaction of PVC-N_3 with PEG_{2000} alkyne under the propargyl-trioctylammonium optimized conditions (2 h at 80 °C) resulted in little or no reaction (FTIR spectroscopy). Exploration of alternative conditions, including the use of prolonged reaction times (2 to 48 h), change in solvent from water to a 1:1 ethanol/ water mixture, and the use of two different PTCs (Bu_4NBF_4 and Bu_4NBr) did not lead to observable reaction as determined by monitoring of the azide signal in the FTIR spectrum. We therefore concluded that the large nature of the PEG_{2000} reagent made it too difficult to engage azides closely associated with a rigid, flat surface: the kinetics are just too difficult. While it is likely that small amounts of the desired click reaction may have occurred, we could not reliably quantitate such outcomes with FTIR.

We therefore turned to a oligo(glycol) (OEG) reagent with a different attachment strategy, as shown in Scheme 2.



Scheme 2 Reaction scheme of the modification of PVC with the short-chain, di-amine OEG tetraethyleneglycol diamine (Experimental section).

In this case, we rely on direct displacement of chloride by amine on unmodified PVC. Figure 8 shows the FTIR spectra of the material from this reaction compared to a control sample placed under the same conditions but without the oligo(glycol)diamine reagent. Three new peaks were observed for OEG-PVC at 3374.8 cm^{-1} , 1090.5 and 996.7 cm^{-1}. The first of these can be associated with adsorbed ethanol or water, also present in the product of the control reaction (Fig. 8b). The other two peaks are unique to the OEG-containing product and can be assigned to C-O stretching vibrations. Both the control and the OEG materials turned brown (Fig. 8d), presumably due to some degree of base-induced elimination. No signals for N-H stretching modes were observed in these samples, but these are likely to overlap the O-H stretching bands of adsorbed water.

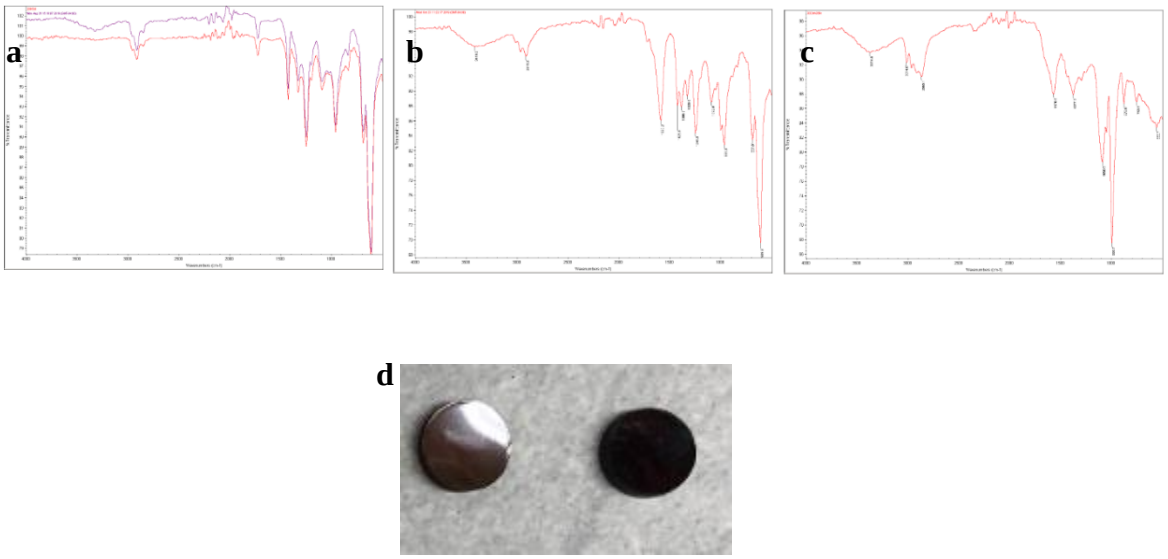
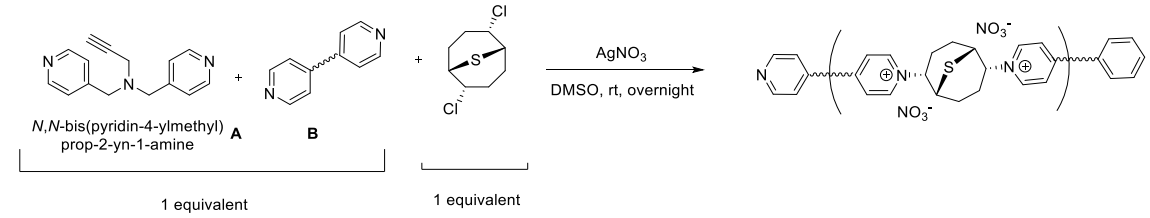


Figure 9 FTIR spectra of (a) unmodified PVC, (b) PVC stirred in a 0.5 M NaOH ethanol solution for 24h at 80°C; (c) PVC modified with the reaction shown in Scheme 1. (d) PVC discs corresponding to the IR spectrum b (right) and c (left).

Next, we went on to explore the CuAAC reaction between PVC-N_3 and antimicrobial polycations[18]. These oligomers were constructed as shown in Fig. 9. To make these oligomers reactive for CuAAC ligation, a dipyridine co-monomer was introduced. In oligomer **a**, the only dipyridine monomer used was N,N-bis(pyridin-4-ylmethyl)prop-2-yn-1-amine. Other oligomers were made with mixtures of monomers in different ratios: structures **b** and **c** utilized monomers that were already implemented in previous studies,[17,18] while **d** employed a newly synthesized monomer (Experimental section). The stabilities of these oligomers toward thermal hydrolysis in water at temperatures up to 50°C has been previously reported, showing different rates of decomposition based on the pyridine linker employed.

a



b

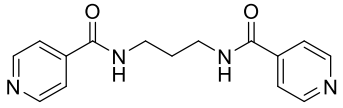
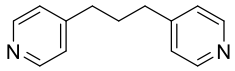
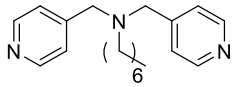
	b	**c**	**d**
B			
A/B ratio	1: 4	1:6	1:1

Fig 10 (a) general mechanism for the formation of the 9-thiabicyclononane-based polycations(from Chem. Mater. 2016, 28, 146−152); (b) table representing the different monomers "b" depicted in the scheme above, and their relative rations to N,N-bis(pyridin-4-ylmethyl)prop-2-yn-1-amine.

Keeping this in mind, our CuAAC reactions were tested at 50°C, 30°C or room temperature, and different reaction times were tried for each temperature. Most tests were performed with oligomer **a** because it contains the most number of alkyne groups, giving the best possible chance for surface attachment. Initially, the CuAAC reaction was run at room temperature for 24 h or 48 h in the presence of $CuSO_4 \times 5H_2O$, sodium ascorbate as the reducing agent, and Bu_4NBF_4 as the PTC. In both cases, the reaction proved unsuccessful. Increasing the reaction temperature to 30°C or 50°C, each for 24 h, also gave

no indication of the desired reaction (by FTIR analysis of the PVC-azide band). In contrast, successful CuAAC reaction (room temperature, 24 h) of polymer **a** was observed with a small-molecule azide (4-methyl benzylazide, Experimental section), highlighting the difficulty of attaching this large molecule to the PVC-azide surface. Changing the phase transfer catalyst[23] to Bu_4NBr or sodium dodecyl sulfate (employed up to a 1:1 molar ratio with respect to alkyne), had no positive effect. Similarly, using CuAAC-accelerating ligands THPTA and TBTA, which stabilize the Cu(I) oxidation state and provide greater amounts of active Cu catalyst,[25] did not result in the attachment of oligomers **a**, **b**, or **c** to PVC-N_3.

Inspired by the success of the propargylated trioctylammonium vs. trimethylammonium salts described above, we designed and prepared a new oligocation (polymer **d**) in which the co-monomer was *N*,*N*-di-4ethylpyridine substituted on N with an *N*-heptyl hydrocarbon chain. We hoped that this alkyl chain would provide some hydrophobic anchoring with the PVC surface. Unfortunately, the reaction of **d** at 50° for 24h, either in the presence or absence of THPTA, proved unsuccessful.

3.1.3 Structural aspects of the alkynes

The above results suggest the existence of two important factors that contribute to the success of the CuAAC procedure on PVC-N_3. The first, highlighted by the difference in CuAAC rate of *N*,*N*,*N*-trimethyl-propargyl ammonium bromide vs. *N*,*N*,*N*-trioctyl-propargyl ammonium bromide, is the favorable presence of hydrophobic moieties on the alkyne reactant. Second, the size of the alkyne and the ratio of alkyne functionalities to other structural components also play a role. For PEG_{2000} and our oligomers, no reaction

was ever observed, which can probably be attributed to their size, making it difficult for alkyne to find surface-bound azide, especially since the azide is not at the end of a flexible tether.

3.1.4 Beyond CuAAC

Polyethyleneimine (PEI) is an inexpensive material that has often been attached to a variety of surfaces, to enable materials to achieve intrinsic antimicrobial and antifungal properties.[26,27,28] We employed a small version of PEI (total average M_n of ~800) containing primary, secondary, and tertiary amine groups. We examined the direct S_N2 reaction of the -NH_2 and -NH groups of PEI with the C-Cl bonds of unmodified PVC. Fig. 10 shows surface FTIR spectra of discs undergoing reaction with PEI compared to a control sample in which PVC discs were stirred under the same conditions (0.5 M NaOH in ethanol at 80 °C for 24 h) with no other reactants The PEI-containing reaction gives distinct peaks at 3860.6 and 3747.3 cm^{-1}, assigned to the N-H stretching of primary and secondary amines. New peaks also appear at 1590.5 cm^{-1}, typical of -NH bending modes, and 1093.5 and 994.6 cm^{-1} , attributed to a combination of C-N stretching signals and C=C stretching signals (due to dichlorination reactions as previously discussed).

From this result, another interesting conclusion can be drawn. Besides the fact that this reaction was not a CuAAC, we could compare it to the previously mentioned reactions of PVC-N_3 and the oligomers or PEG_{2000}. Although PEI is a voluminous molecule (Mn = 800), the reaction with PVC's surface still occurred, highlighting the probability that conducting PVC modifications at 80°C is crucial to overcome the energetic barrier to the reaction, and having an appropriate temperature can ensure successful reactions even with

big molecules. Moreover, PEI has multiple amine groups the structure; hence, once the first reaction has occurred, the following substitutions can be favored by vicinity with the surface.

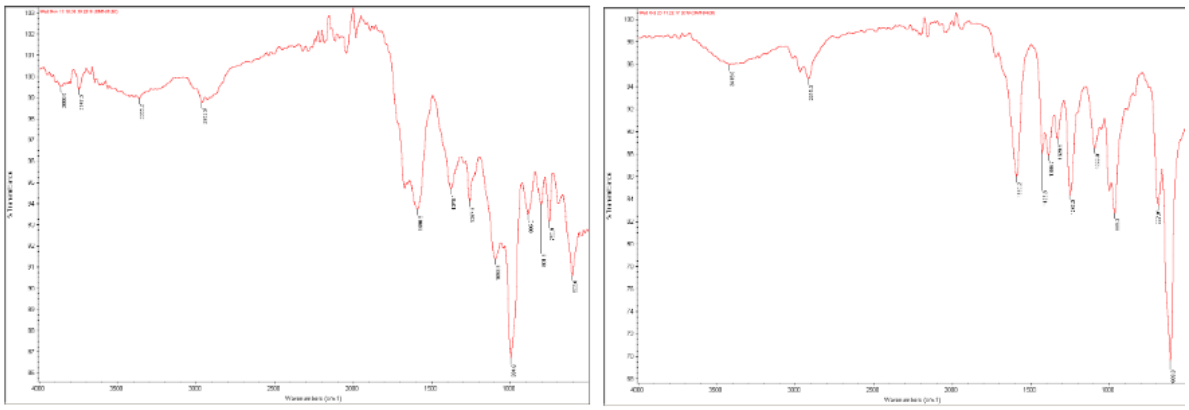


Fig 11 FTIR spectra of (a) PEI-modified PVC and (b) PVC stirred in a 0.5 M NaOH ethanol solution for 24h at 80 °C (also shown in Fig. 8b).

3.2 Bacterial assays

The successfully modified PVC samples are shown in Fig. 11.

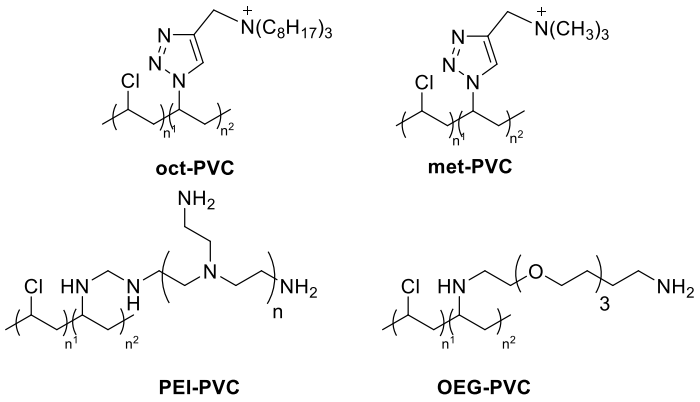


Fig 12 Structures of the available functionalized PVC substrates obtained as discussed above.

These discs were cleaned and purified with the procedure discussed in the Experimental section, and then tested for their antibacterial activity. The method used in these experiments is slightly modified from the standard procedure previously used in our research group.[19] Briefly, a freshly-prepared culture of *E. coli* in standard growth media was diluted in PBS buffer to OD = 0.06. and was incubated for 3 hours in contact with a PVC disc (or, as a control, incubated with no disc). Next, 25 μL of the supernatant was plated on agar plates and incubated at 37° C for 24 h. The plates were observed for bacterial adhesion, and the results are displayed in Table 1.

Table 1 Antibacterial activity of unmodified and modified PVC discs. In the first row, the conditions in which the samples were obtained are reported: with or without Bu_4NBF_4, or with or without $CuSO_4$. The values reported represent the percentage of bacterial colonies eliminated by the discs, relative to the no-disc control (discussed in detail in the Experimental section), while "x" implies that those conditions were not tested.

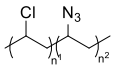
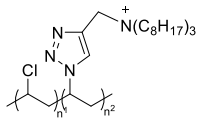
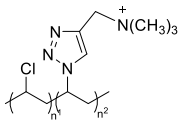
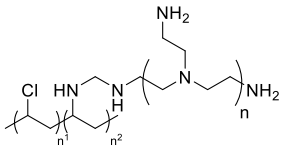
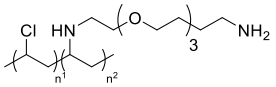
CONDITIONS USED / **SAMPLE**	Bu_4NBF_4 and $CuSO_4$	Bu_4NBF_4, no $CuSO_4$	$CuSO_4$, no Bu_4NBF_4	no $CuSO_4$ and no Bu_4NBF_4
 **PVC**	x	x	x	0%
 **PVC-N_3**	x	100%	x	0%

Table 1 continued

 oct	100%	x	100%	x
 met	100%	x	100%	x
 PEI	x	x	x	37.5%
 PEG	x	x	x	55.1%

All of the chemically modified PVC discs showed some antibacterial activity, whereas PVC and PVC-N_3 had no effect on bacterial cell viability. We initially screened multiple control samples to make sure that the antibacterial agent was, in fact, the covalently bound molecule or polymer, and not any other physically adsorbed component that was used in the CuAAC reaction or the S_N2 reaction. Specifically, two controls for the samples modified by CuAAC reactions were tested: one PVC sample prepared omitting the alkyne species and $CuSO_4$, and one omitting the alkyne species and Bu_4NBF_4. Interestingly, omitting the PTC Bu_4NBF_4, and thus only having $CuSO_4$ as a possible antibacterial agent,[29,30,31] gave no bacterial cell death, showing that the post-reaction aqueous EDTA wash was effective in removing copper.

In contrast, the discs that were reacted in the absence of copper and in the presence of Bu_4NBF_4, after sonication in H_2O and ethanol only (Experimental section), did show antibacterial activity, presumably due to the presence of the PTC, a quaternary ammonium salt,[32, 33, 34] still adsorbed on the surface of PVC-N_3. For this reason, a more vigorous procedure previously developed in our group[19] was used, involving successive washes with different solvents at high temperatures. After the adsorbed Bu_4NBF_4- PVC-N_3 was washed using this method, no antibacterial activity was observed, confirming that the washing was effective. Importantly, oct-PVC and met-PVC showed antibacterial activity both before and after this washing procedure, proving that they are effective due to their covalently bound group.

Interestingly, and in contrast to PVC-N_3, the treatment of unmodified PVC with Bu_4NBF_4 under the conditions discussed above gave rise to no antibacterial activity by the resulting material. This suggests that either the azide group itself plays a role in retaining Bu_4NBF_4, or that modification of the surface morphology that may occur during azidation promotes the subsequent adsorbance of the phase transfer agent (given the close resemblance between the dipole moments of the C-Cl and C-N_3 bonds, the latter explanation seems more likely). We observed that oct-PVC and met-PVC still retain their antibacterial activity even when synthesized without using the PTC. Nonetheless, the physisorbed Bu_4NBF_4- PVC-N_3 remains an interesting material for antimicrobial use, since it is easily made and the quaternary salt remains bound to the surface under washing conditions less severe than 80 °C.

In the case of OEG-PVC and PEI-PVC, antibacterial properties are still observed, although with a lower efficacy (only 37,5 and 55,1 % of bacteria dead after 2 h contact, respectively)

if compared to the samples prepared via CuAAC. A negative control, a PVC disc only stirred in a 0.5 M NaOH ethanol solution for 24 h at 80°C, had no antimicrobial activity. This confirmed that the efficacy, although limited, derives from the attached molecule.

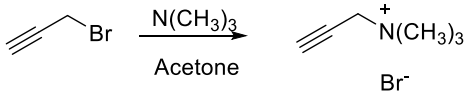
CHAPTER 4. **EXPERIMENTAL SECTION**

4.1 Material and instrumentation

All solvents and reagents were purchased from Sigma Aldrich, TCI, Acros Organics, Alfa Aesar, Oakwood Chemical, and EMD Millipore, and used as received. Water was purified via a Milli-Q® Integral 3 Water Purification System. Clear Chemical-Resistant PVC Sheets (6" x 6" x 1/8") were purchased from McMaster Carr, and laser-cut into 0.5" diameter discs at the Montgomery Machinery Mall at the George W. Woodruff School of Mechanical Engineering at Georgia Institute of Technology. $mPEG_{2000}$–alkyne was purchased from Advanced BioChemicals. *E. coli* DH5α was purchased from New England Biolabs.

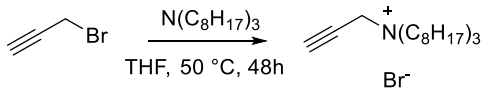
^1H NMR spectra were recorded in deuterated solvents on a 500 MHz Bruker Advance IIIHD Spectrometer. IR spectra were recorded on a Nicolet 6700 FTIR spectrophotometer with a Smart Performer single-bounce ATR module on a diamond crystal. High-resolution mass spectrometry was performed on an Agilent 6230 ESI-TOF LC/MS instrument (G6230B) operating at 4 GHz with internal reference. X-ray photoelectron spectroscopy (XPS) was performed using a Thermo Scientific K-α XPS with a monochromated Al Kα source, hemispherical analyzer, and multichannel detector. Contact angle was measured on a Rame-Hart Inc model 300 goniometer. Bacterial CFU were determined using the Fiji software.

4.2 Experimental procedures

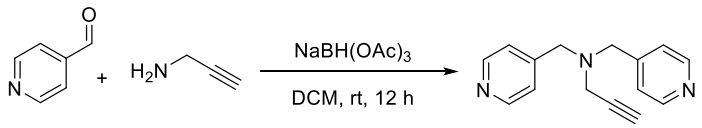


Procedure from: *Org. Lett. 2005, 7, 6, 1035-1037*: 0.8 mL propargyl bromide (in toluene, 80%) and 2,0 mL triethylamine (in water, 50%) were dissolved in 5 mL acetone, and stirred for 5 seconds. Two layers separated and the top acetone layer was removed with a pipette.

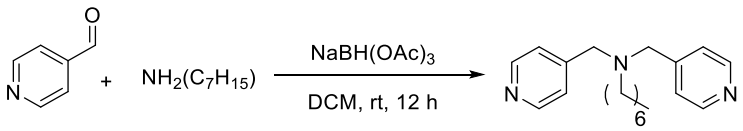
The bottom layer was washed with acetone twice , then 15 mL acetonitrile was added to the water solution. 100 mL acetone was added and a white precipitate was observed. The powder was filtered under vacuum and dried to obtain light yellow crystals (0,9870 g, 76,9%). 1H NMR (500 MHz, Deuterium Oxide) δ 4.16 (s, 2H), 3.13 (d, J = 2.6 Hz, 9H).



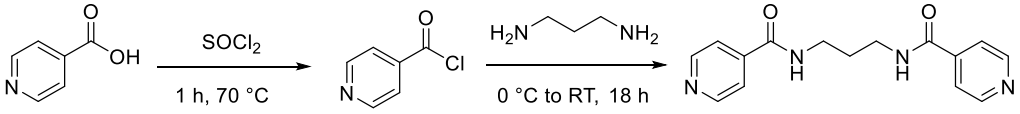
0,36 g propargyl bromide (in toluene, 80%) is added to a solution of 0,54 g trioctylamine in 2 mL THF. The mixture is heated at 50 °C for 48 h. Then the sovent is evaporated to obtain a viscous dark yellow liquid (0,90 g, >99%). 1H NMR (500 MHz, Chloroform-d) δ 4.75 (d, J = 2.8 Hz, 2H), 3.47 (dd, J = 10.5, 6.0 Hz, 6H), 2.98 (dt, J = 13.5, 4.9 Hz, 2H), 1.78 (h, J = 9.4, 7.8 Hz, 6H), 1.46 – 1.17 (m, 30 H), 0.89 (t, J = 6.7 Hz, 9H).



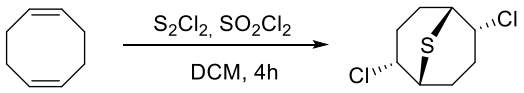
1,41 g 4-pyridinecarboxaldehyde and 0,33 g propargylamine are mixed in 50 mL dichloromethane. 4,24 g sodium triacetoxy borohydride is added and the reaction is stirres at room temperature for overnight. The reaction is quenched with 20 mL sat. NaHCO3 (aq), the product is extracted with dichloromethane washing with water (3x30 mL), dried over NaSO4 and filtered, then dried under vacuum. Column cromatography (EtOAc, 15% MeOH, 2% TEA) allows to obtain a brown liquid product (2,66 g, 61%). 1H NMR (500 MHz, Chloroform-d) δ 8.73 – 8.60 (m, 4H), 7.56 (p, J = 6.0 Hz, 4H), 3.83 (q, J = 3.7 Hz, 4H), 3.33 (t, J = 2.3 Hz, 2H), 2.38 (t, J = 2.6 Hz, 1H).



1,41 g 4-pyridinecarboxaldehyde and 0,92 g propargylamine are mixed in 50 mL dichloromethane. 4,24 g sodium triacetoxy borohydride is added and the reaction is stirres at room temperature overnight. The reaction is quenched with 20 mL sat. NaHCO3 (aq), the product is extracted with dichloromethane, washing with water (3x30 mL), dried over NaSO4 and filtered, then dried under vacuum. Column chromatography (gradient, 100% EtOAc to EtOAc, 10% MeOH) allows to obtain a brown liquid product (3,91 g, 56%). 1H NMR (500 MHz, Chloroform-d) δ 8.76 (t, J = 5.1 Hz, 4H), 7.74 – 7.63 (m, 4H), 3.87 (d, J = 17.1 Hz, 4H), 2.61 (t, J = 7.3 Hz, 2H), 1.67 (p, J = 7.1 Hz, 2H), 1.37 (d, J = 10.1 Hz, 9H), 0.99 (td, J = 7.0, 1.8 Hz, 3H), MS (ESI^+) m/z 298,23567.



1,27 g isonicotinic acid is stirred in 30 mL $SOCl_2$ for 1h at 70 °C. Excess $SOCl_2$ is distilled for 1h, and the product is dried under vacuum for 2 h. Then the product is dissolved in 30 mL dichloromethane and cooled to 0°C. 8,2 dry triethylamine and 0,340 mL 1,3-diaminopropane are added, and the reaction is stirred at rt for overnight. The solvent is then extracted with dichloromethane washing with water (3x30 mL) and dried under vacuum. Column chromatography (DCM, 3% MeOH, 3% TEA) allows to obtain a light yellow liquid product (1,31 g, 41%). 1H NMR (500 MHz, Chloroform-d) δ 8.84 – 8.78 (m, 4H), 7.78 – 7.72 (m, 4H), 3.61 (q, J = 6.3 Hz, 4H), 1.95 – 1.81 (m, 3H).

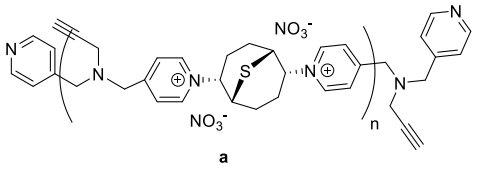


Procedure from: *Molecules 2006, 11(4), 212-218*: A 4-L, round-bottomed flask equipped with a magnetic stirrer, dropping funnel, and capped with a drying tube was charged with a solution of 122 mL 1,5-cyclo-octadiene in 2.5 L dichloromethane. The solution was cooled with an ice/water bath and 43 mL sulfur monochloride was slowly added in dropwise fashion with vigorous stirring. The reaction mixture was allowed to stir for an additional 2.5 hours at 0–4°C and was then treated with 48 mL sulfuryl chloride added through the dropping funnel over a period of 20 minutes. The cooling bath was removed and the reaction mixture was allowed to stir for 90 minutes while warming to room temperature. TLC analysis showed complete consumption of the starting diolefin. The mixture was washed with a saturated aqueous solution of Na2S2O5 (2 x 200 mL), 0.5 N NaOH (2 x 200 mL), brine (1 x 200 mL), and the organic phase was dried over MgSO4. Removal of the solvent by rotary evaporation gave a yellow solid (200.6 g, 95% crude yield), which was dissolved in the minimum amount of chloroform (400 mL). Twice the volume of hexane was added and the mixture was stirred with decolorizing charcoal. Following filtration through a pad of silica and removal of the solvent, an off-white solid was obtained (1,43 g, 52%). 1H NMR (500 MHz, Chloroform-d) δ 4.73 (dddd, J = 11.1, 7.4, 4.2, 1.2 Hz, 2H), 2.87 (q, J = 3.8 Hz, 2H), 2.74 – 2.62 (m, 2H), 2.41 – 2.16 (m, 6H).

General procedure for the synthesis of polymers:

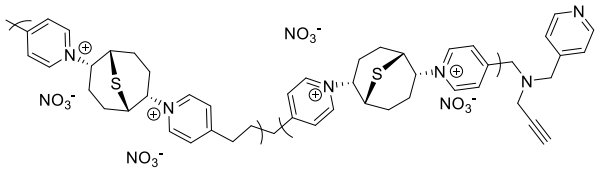
Procedure from J. *Am. Chem. Soc. 2017, 139, 15401-15406*: 0,2 mmol 2,6-dichloro-9-thiabicyclo[3.3.1]nonane and a total 0,2 mmol dipyridine of choice (or a total of 0,2 mmol

combined dipyridines) are added in 1 mL dry DMSO and mixed with 0,4 mmol of AgNO3, and the solution is stirred under inhert atmosphere at rt for 24 h. The solution is transfered in a centrifugation vial and centrifuged for 5 min at 2000 rpm. The precipitate is discarded and the supernatant is added to 15 mL dichloromethane and centrifuged 3 times at 2000 rpm, collecting the precipitate every time. The precipitate fractions are collected and dried in air, and then stored at -20 °C until used. The 1H NMR integration results are presented below. (1H NMR integrations are reported integrating the terminal pyridine protons to 4.)



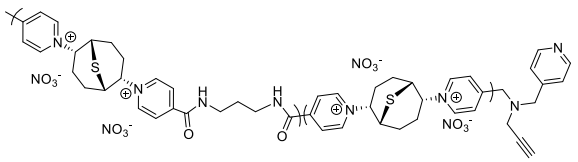
a

1H NMR (500 MHz, Deuterium Oxide) δ 8.92 (d, J = 6.4 Hz, 4H), 8.18 (d, J = 6.2 Hz, 4H), 5.61 (d, J = 12.6 Hz, 2H), 4.11 (d, J = 16.5 Hz, 4H), 3.32 (d, J = 6.2 Hz, 2H), 3.24 (s, 2H), 3.04 (s, 2H), 2.45 – 2.18 (m, 7H).



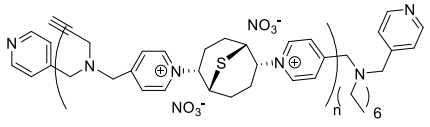
b

1H NMR (500 MHz, Deuterium Oxide) δ 8.92 (d, J = 6.3 Hz, 1H), 8.83 (d, J = 6.6 Hz, 4H), 8.76 (d, J = 6.2 Hz, 1H), 8.18 (d, J = 6.3 Hz, 2H), 7.94 (d, J = 6.2 Hz, 4H), 5.57 (d, J = 11.1 Hz, 2H), 4.13 (s, 2H), 3.36 (d, J = 34.6 Hz, 2H), 3.20 (d, J = 19.4 Hz, 4H), 3.11 – 2.90 (m, 8H), 2.45 – 2.05 (m, 11H).



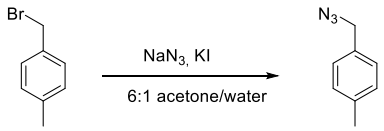
c

1H NMR (500 MHz, Deuterium Oxide) δ 9.16 (d, J = 6.1 Hz, 4H), 9.08 (d, J = 6.3 Hz, 1H), 8.92 (s, 3H), 8.36 (d, J = 6.2 Hz, 4H), 8.18 (d, J = 6.4 Hz, 3H), 5.73 (s, 2H), 5.59 (d, J = 30.8 Hz, 2H), 4.11 (d, J = 17.9 Hz, 3H), 3.49 (t, J = 6.6 Hz, 4H), 3.38 – 3.20 (m, 5H), 3.04 (s, 3H), 2.49 – 2.21 (m, 13H), 1.95 (p, J = 7.3 Hz, 4H).



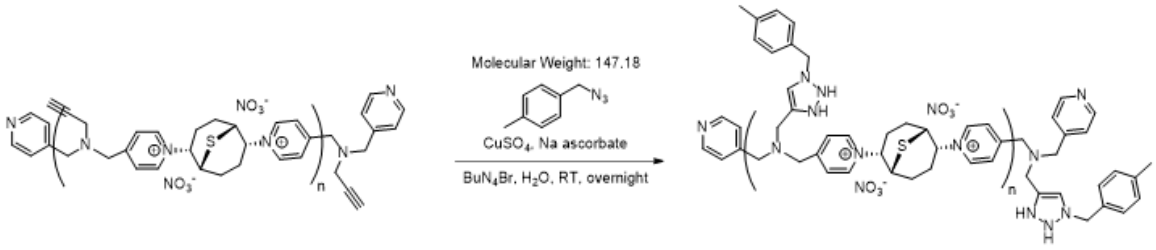
d

1H NMR (500 MHz, Deuterium Oxide) δ 8.93 (d, J = 6.3 Hz, 4H), 8.90 (d, J = 6.6 Hz, 4H), 8.84 (d, J = 8.7 Hz, 1H), 8.18 (dd, J = 11.0, 6.4 Hz, 9H), 5.58 (d, J = 35.2 Hz, 5H), 4.13 (s, 4H), 4.03 (d, J = 28.2 Hz, 5H), 3.36 (d, J = 21.3 Hz, 3H), 3.24 (s, 5H), 3.04 (d, J = 13.2 Hz, 5H), 2.50 – 2.10 (m, 17H), 1.43 (s, 2H), 1.15 (d, J = 42.9 Hz, 10H), 0.72 (t, J = 6.7 Hz, 3H).



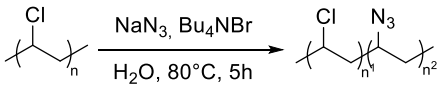
0.200 g 4-methylpropargyl bromide is dissolved in 5 mL of a 6:1 mixture of acetone/water and NaN3 is added, stirring vigorously. KI is added and the reaction is stirred at rt for overnight. The product is extracted with dichloromethane washing with water and dried

under vacuum to obtain a light yellow liquid (0,1590 g, >99%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.32 – 7.30 (m, 2H), 7.19 – 7.16 (m, 2H), 4.51 (s, 2H), 2.37 (s, 3H).



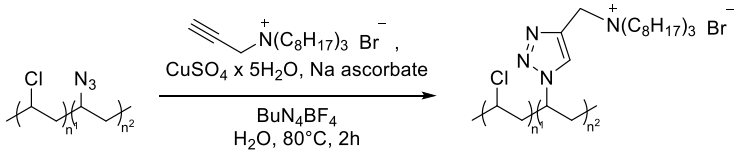
10 mg polymer a and 1 mg $CuSO_4$ x $5H_2O$ are stirred in 1 mL of a 4:1 water/tert-butanol solution. 2 mg sodium ascorbate are added and the reaction is stirred for 5 min, then 25,9 mg 4-methyl benzylazide is added and the reaction is stirred at rt for overnight. The product is washed throughly with dichloromethane to eliminate all non-reacted 4-methyl benzylazide, and extracted with water, then liofilized. 1H NMR in D2O was taken, but due to the difficulty in purifying the water soluble polymer from the other water soluble components, only the aromatic region of the 1H NMR spectrum is analyzed, to look for the new peak coming from 4-methyl benzylazide. If the peak is retained even after intensive washing with DCM, the reaction is considered successful.

General method for the synthesis of azidated PVC (PVC-N_3):

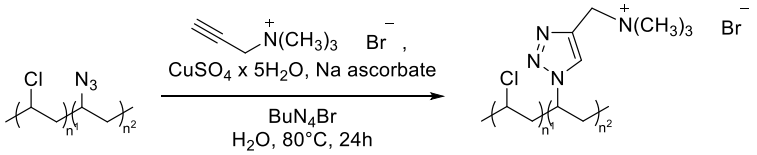


5 PVC discs (0,5’ diameter, ~350 mg weight each) are added to a solution of 0.615 g sodium azide and 0,495 g tetrabutylammonium bromide in 10 mL H2O in a 100 mL conical flask. The vessel is capped, and the reaction is stirred at 80°C for 5 h. The reaction is let cool down to room temperature, then the supernatant is discarded, and the discs are added

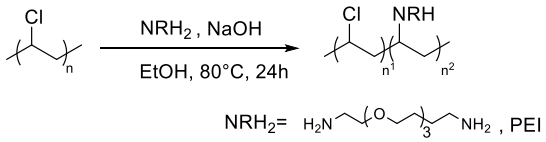
to 10 mL fresh DI water and sonicated for 3 times at 19°C for 3 minutes, changing the water each time. The same is repeated in 3x10 mL ethanol. The discs are gently wiped with Kimwipes and ethanol, then let air dry. ATR-FTIR cm-1 2913.9 (C-H, stretching), 2109.4 (-N≡N, stretching), 1144.5 (-CH2, bending), 611.7 (C-Cl, stretching).



33.0 mg $CuSO_4$ x $5H_2O$ (0.131 mmol) and 31.1 mg N,N,N-triocty-propargylammonium bromide (0,066 mmol) are stirred in 5 mL H_2O in a screw-top sealed glass vial (20 mL), and then 660.0 mg (3.333 mmol) is added and the reaction is stirred at room temperature for 5 min. The vial is opened quickly and 170.2 mg Bu_4NBF_4 (0.517 mmol, optional) and one PVC-N_3 are added and stirred at 80°C for 2 h. The discs are removed and placed in 10 mL of an EDTA(aq) sat. solution and sonicated at 19°C for 3 minutes, repeating 3 times changing the solution every time. If Bu_4NBF_4 was used, the discs were heated in 10 mL of a 10% DMF(aq) solution at 80°C for 24 h. After this time, the discs were heated at 80°C for 1 h three times, with 10 mL fresh DI water every time, and eventually wiped with a Kimwipe and ethanol, and let air dry. The same sonication is performed 1x with clean water and 1x with ethanol, then the discs are let air dry. ATR-FTIR cm-1 2913.9 (C-H, stretching), 1144.5 (-CH2, bending), 611.7 (C-Cl, stretching).

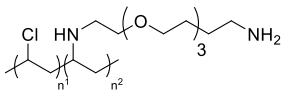


33.0 mg $CuSO_4$ x $5H_2O$ (0.131 mmol) and 31.1 mg N,N,N-triocty-propargylammonium bromide (0,066 mmol) are stirred in 5 mL H_2O in a screw-top sealed glass vial (20 mL), and then 660.0 mg (3.333 mmol) is added and the reaction is stirred at room temperature for 5 min. The vial is opened quickly and 170.2 mg Bu_4NBr (0.517 mmol, optional) and one PVC-N_3 are added, and stirred at 80°C for 24 h. The discs are removed and placed in 10 mL of an EDTA(aq) sat. solution and sonicated at 19°C for 3 minutes, repeating 3 times changing the solution every time. The same sonication is performed 1x with clean water and 1x with ethanol, then the discs are let air dry. If Bu_4NBr was used, the discs were heated in 10 mL of a 10% DMF(aq) solution at 80°C for 24 h. After this time, the discs were heated at 80°C for 1 h three times, with 10 mL fresh DI water every time, and eventually wiped with a Kimwipe and ethanol, and let air dry. ATR-FTIR cm-1 2913.9 (C-H, stretching), 1144.5 (-CH2, bending), 611.7 (C-Cl, stretching).

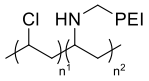


10 mL EtOH and 200.0 mg NaOH are stirred at room temperature in a round bottom flask. The amine of choice (10 mM final concentration) is added and stirred for 5 minutes. One PVC disc (0,5’ diameter, ~350 mg weight) is added and the reaction is stirred at 80°C for 24 h. The reaction is let cool down to room temperature, then the supernatant is discarded and the discs are added to 10 mL fresh DI water and sonicated for 3 times at 19°C for 3

minutes, changing the water each time. The same is repeated in 3x10 mL ethanol. The discs are gently wiped with Kimwipes and ethanol, then let air dry.

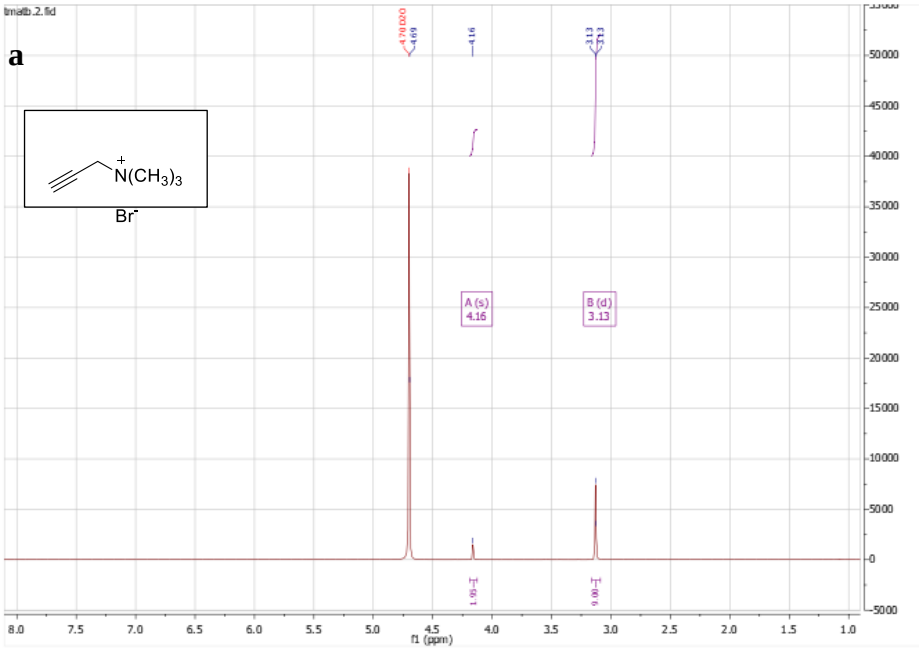


ATR-FTIR cm-1 2913.9 (C-H, stretching), 1144.5 (-CH2, bending), 611.7 (C-Cl, stretching). 3014.0 (C=C, stretching), 1377.0 (C-C, stretching), 1090.5, 996.7 (C-O, stretching and C=C, bending).



ATR-FTIR cm-1 3860.6, 3747.3 (-NH, stretching), 2962.9 (C-H, stretching), 1590.5 (-NH, bending), 1371.5, 1257.9 (-CH, bending) 994.6 (C=C, bending).

4.2.1 Spectra



b

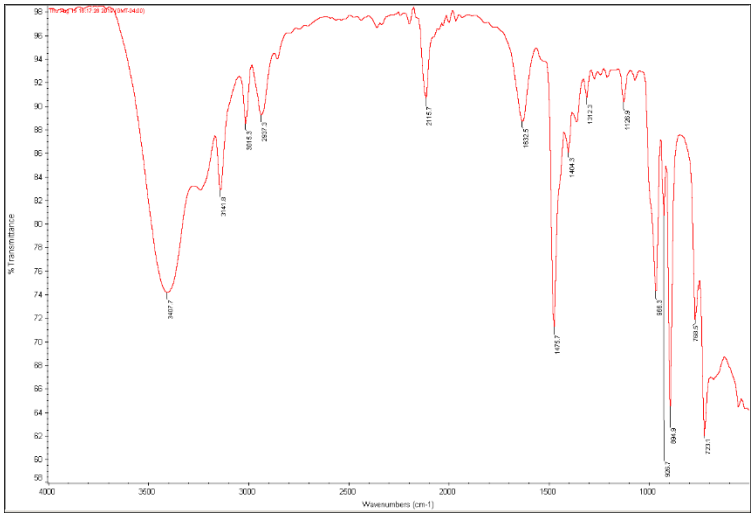


Fig 13 Characterization of $N(CH_3)_3Br$ (a) 1H NMR spectrum ; (b) IR spectrum.

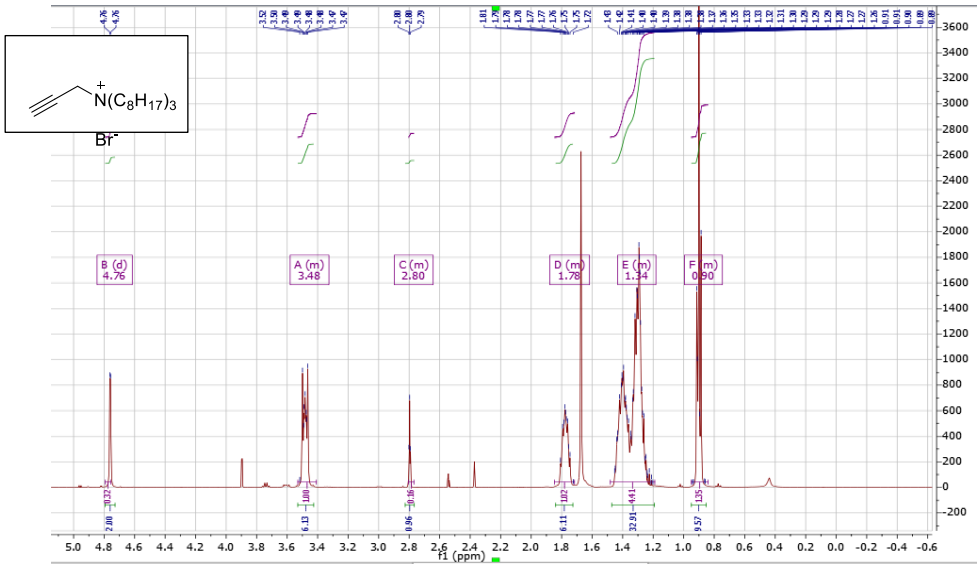


Fig 14 1H NMR spectrum of $N(C_7H_{18})_3Br$.

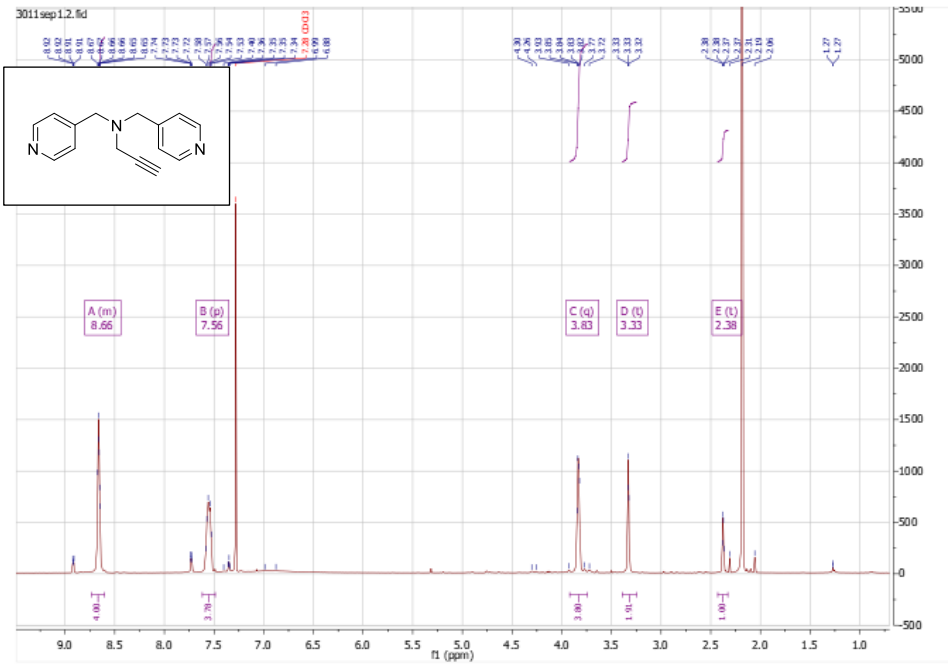


Fig 15 1H NMR spectrum of N,N-bis(pyridin-4-ylmethyl)prop-2-yn-1-amine.

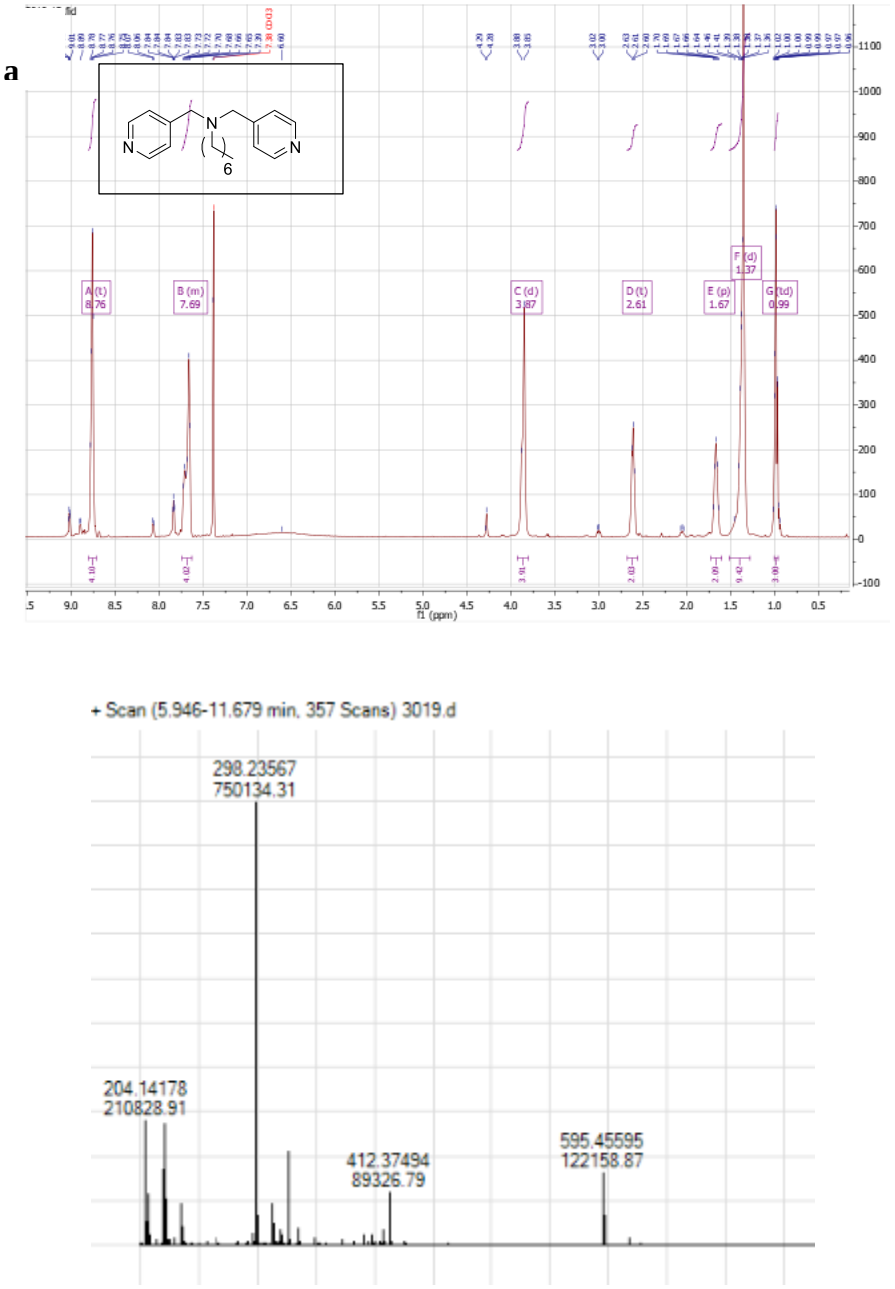


Fig 16 Characterization of N,N-bis(pyridin-4-ylmethyl)heptan-1-amine (a) 1H NMR spectrum ; (b) MS (positive mode) spectrum.

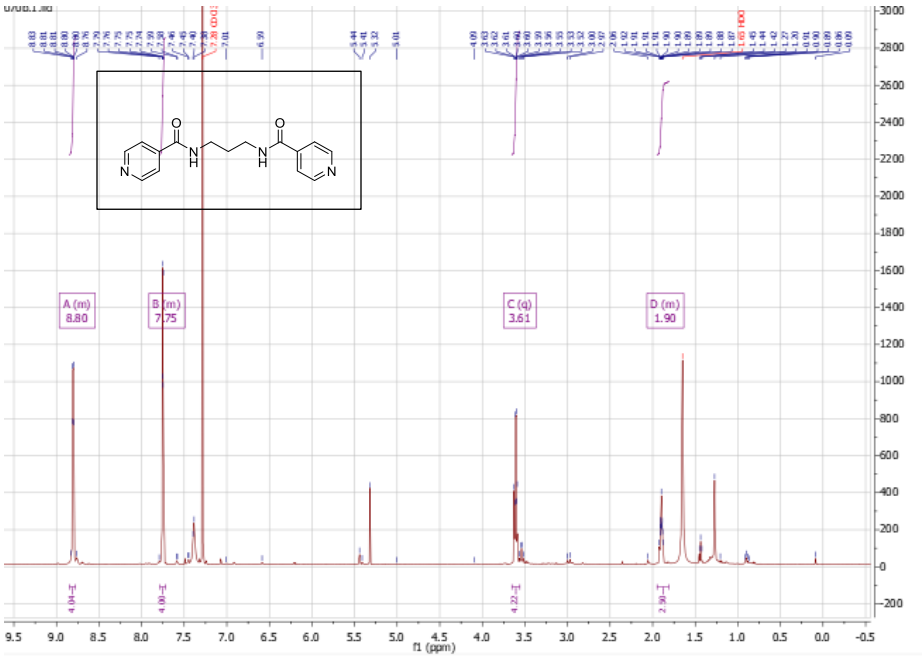


Fig 17 1H NMR spectrum of N,N'-(propane-1,3-diyl)diisonicotinamide.

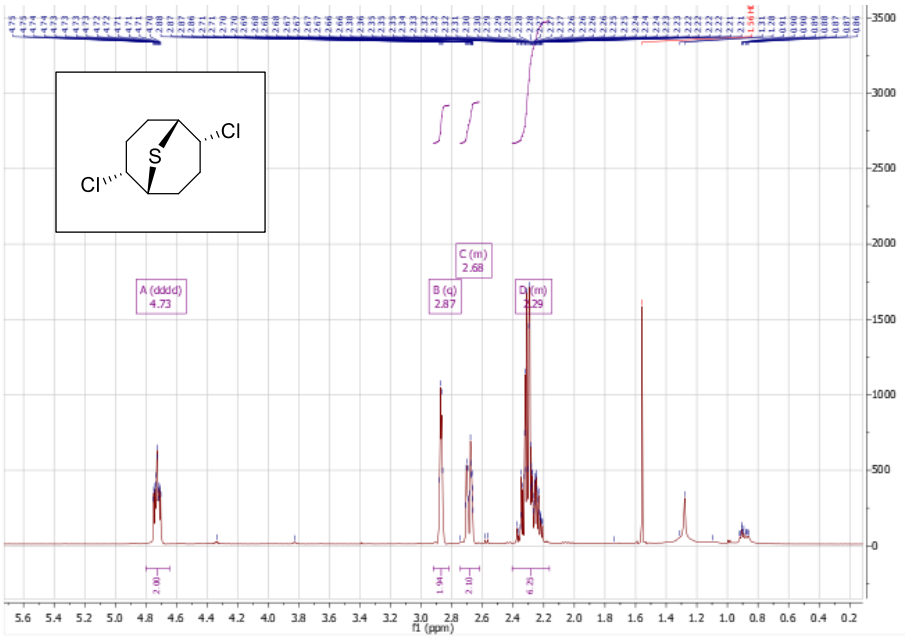


Fig 18 1H NMR spectrum of 2,6-dichloro-9-thiabicyclo[3.3.1]nonane.

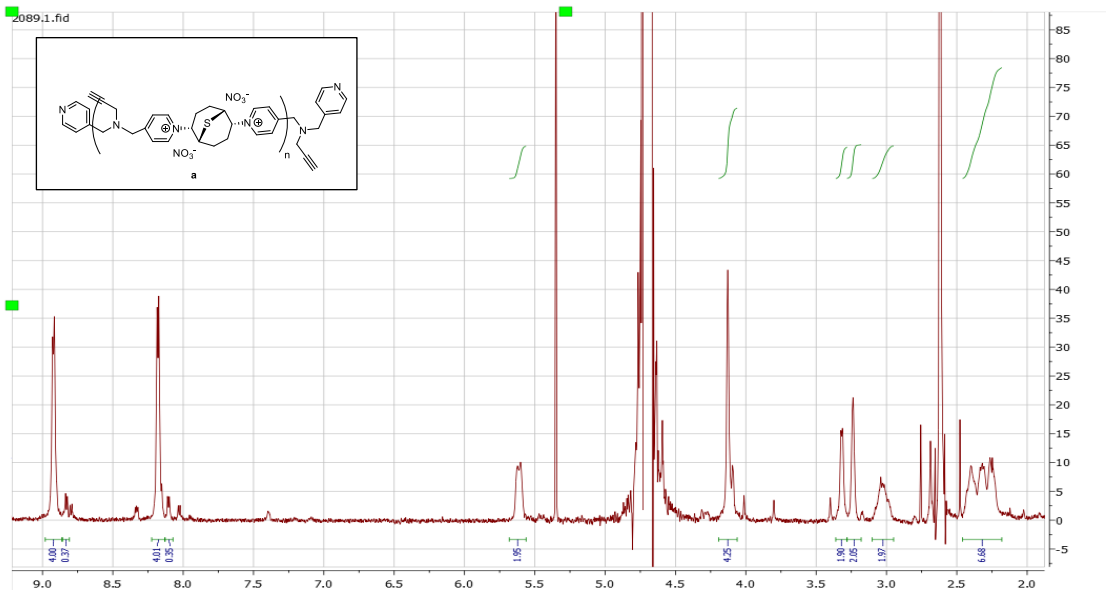


Fig 19 1H NMR spectrum of polymer a.

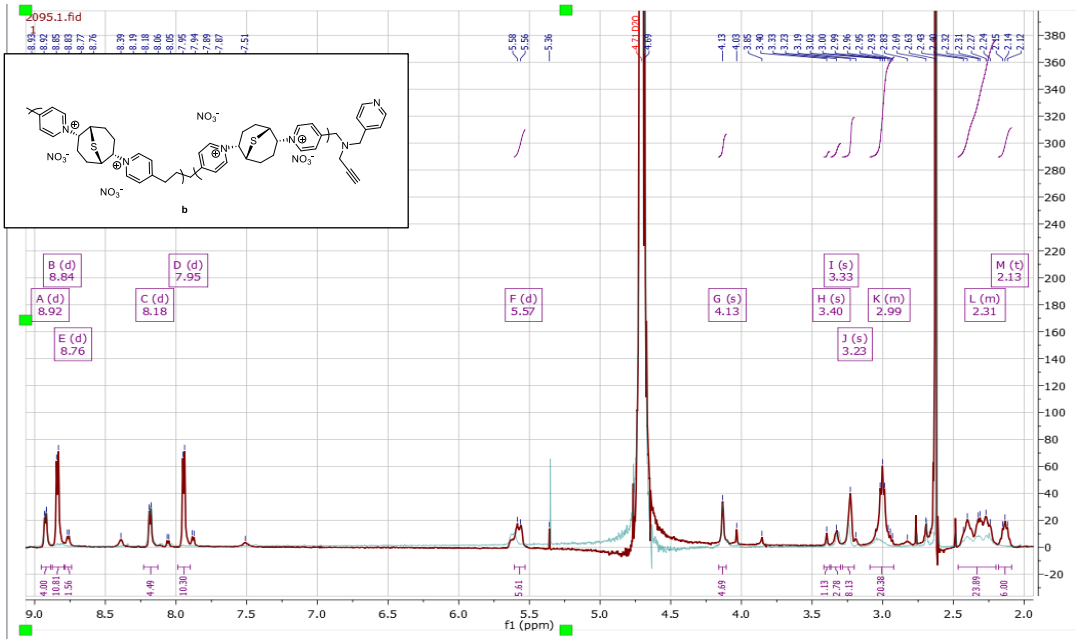


Fig 20 1H NMR spectrum of polymer b (red) and as a reference, the spectrum of polymer a (light blue)

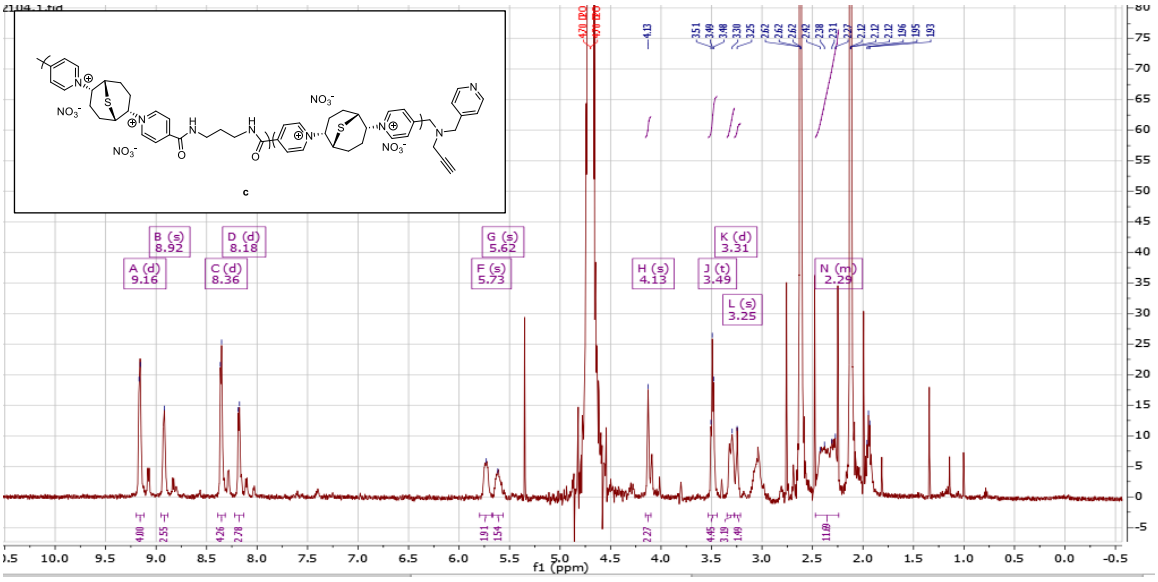


Fig 21 1H NMR spectrum of polymer c.

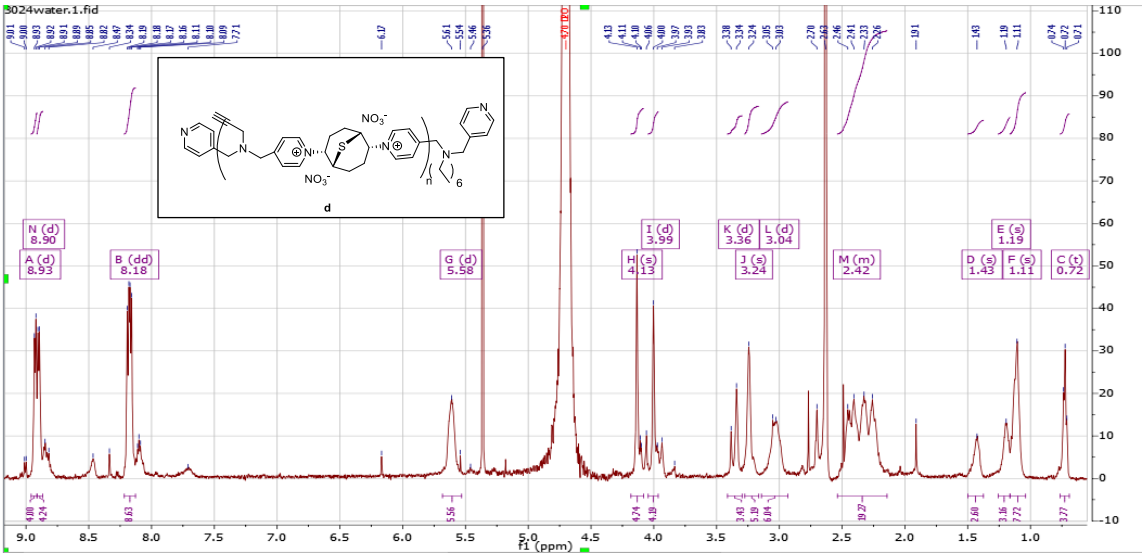


Fig 22 1H NMR spectrum of polymer d.

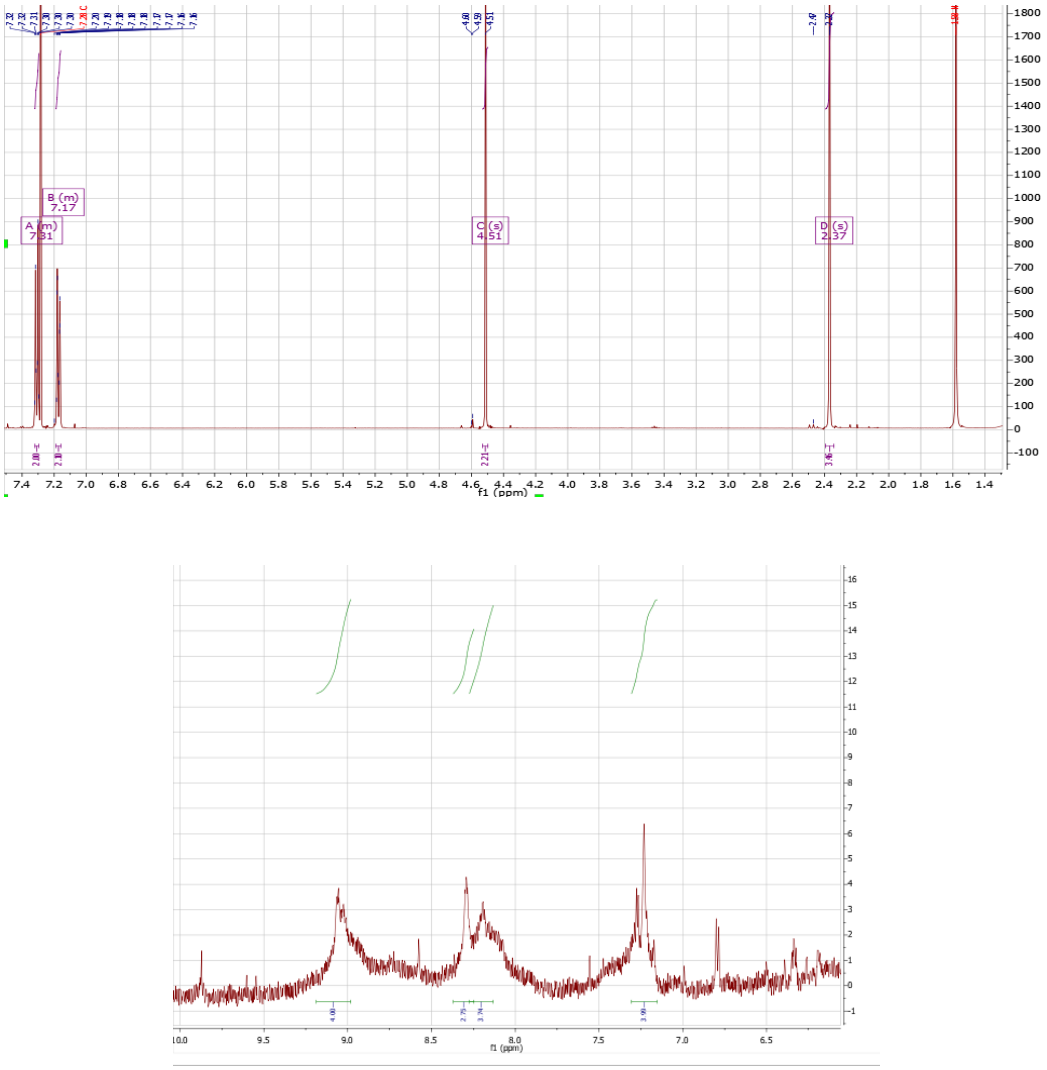
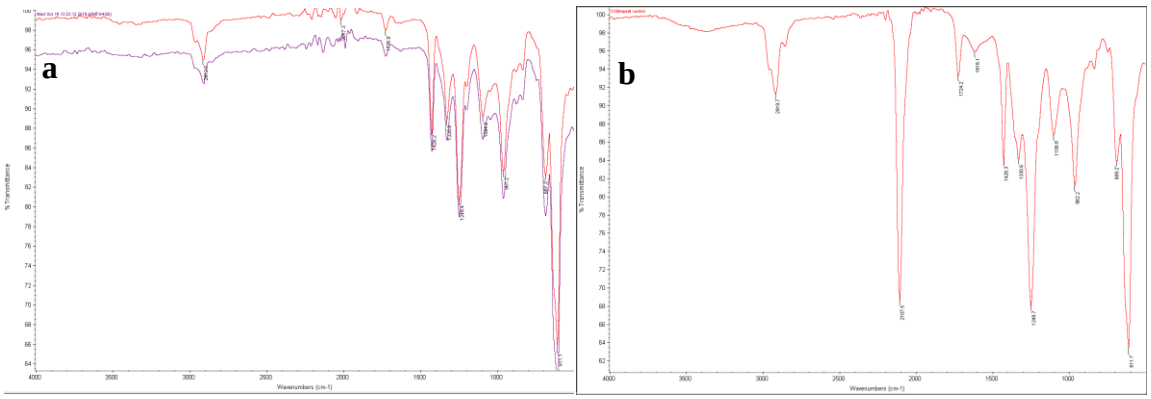


Fig 23 1H NMR spectrum of 4-methy-benzylazide (a) and aromatic region of the 1H NMR spectrum of the product of the CuAAC reaction between polymer a and p-methylbenzylazide (b).



c

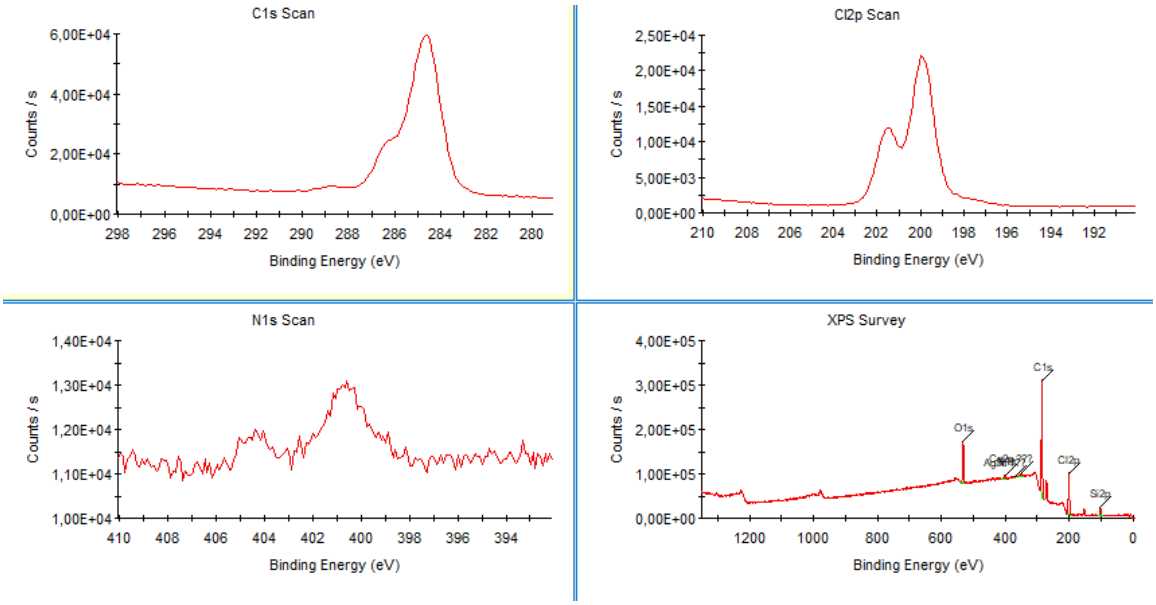
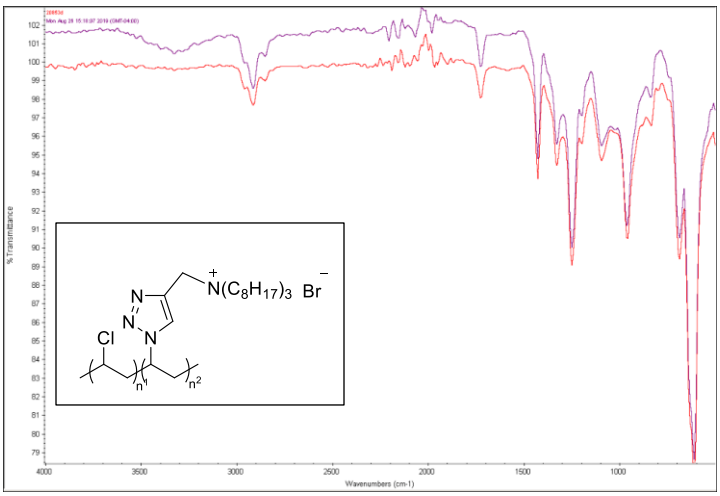


Fig 24 IR spectrum of a sample disc of unreacted, unmodified PVC as provided by the manufacturer. Red and blue spectra belong to the scans of the two faces of a single disc (a); IR spectrum of a sample disc of azidated PVC (PVC-N_3). Red and blue spectra belong to the scans of the two faces of a single disc (b). XPS elemental mapping analysis of PVC-N_3 (c).

a



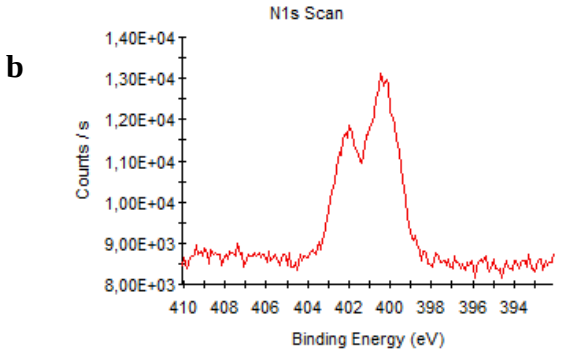


Fig 25 IR spectrum of a sample disc of trioctyl-propargyl bromide- modified PVC (PVC-oct). Red and blue spectra belong to the scans of the two faces of a single disc (a). XPS spectrum for N1s of PVC-oct (b).

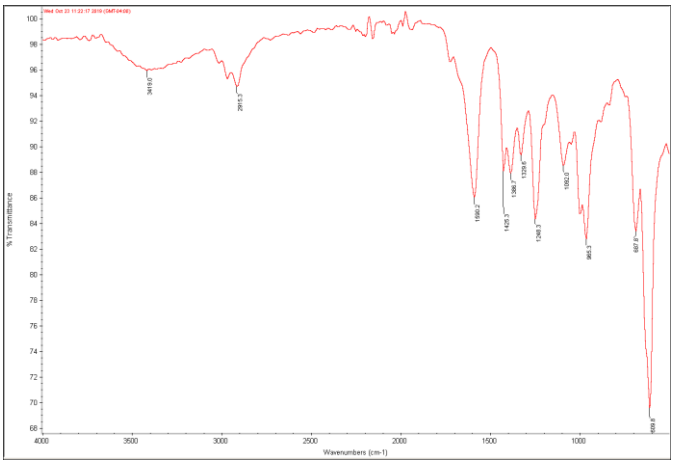


Fig 26 IR spectrum of a sample disc of trimethyl-propargyl bromide- modified PVC (PVC-met).

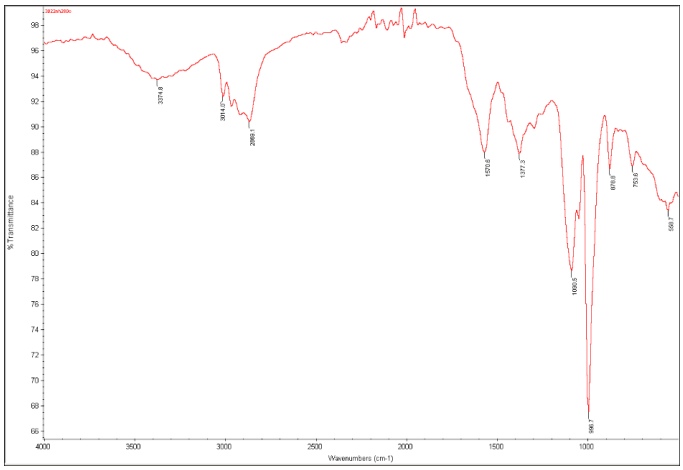


Fig 27 IR spectrum of a sample disc PEG-modified PVC (PVC-PEG).

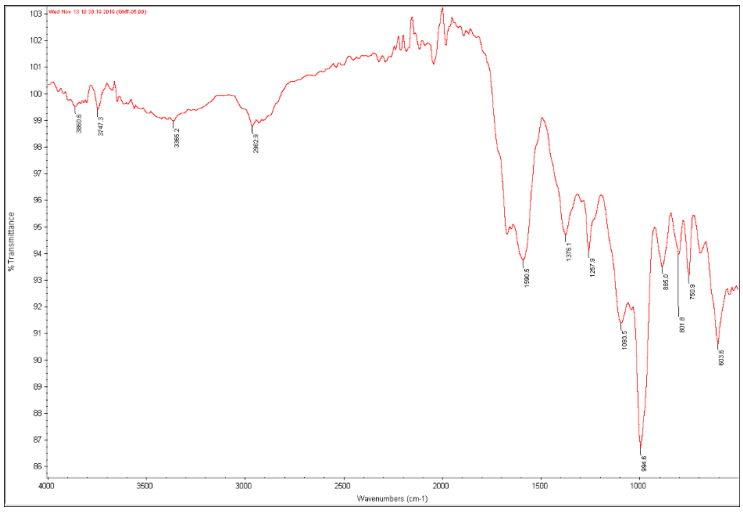


Fig 28 IR spectrum of a sample disc of PEI-modified PVC (PVC-PEI).

4.3 Bacterial assays

4.3.1 General procedure

A bacterial culture (*E.coli*) was grown overnight in Mueller Hinton Broth (MHB) with no antibiotic. A bacterial stock was prepared by diluting 10 µL culture in 2 mL MHB and shaking at 37°C for 2 h; then centrifuging at 4000 g at 4°C for 3 min, discarding the medium, adding 2 mL phosphate buffer saline (PBS) for three times, centrifuging each time changing the PBS. The culture was finally dispersed in 2 mL PBS and diluted to OD 0.06. In sterile tubes, the PVC disc of choice and 1.1 mL bacterial stock were introduced so that the disc was totally immersed, and then shaken at 37°C for 3 h. Then, 25 µL of the supernatant from each disc liquid was plated on an agar spot plate, and incubated at 37°C for 24 h. A negative control was implemented for each experiment, where a sterile tube

was only shaken with the bacterial stock and no disc, and then plated as described. 2 repeats were performed for each disc. Bacterial colony counts were calculated as follows: in the Fiji software, the control plates (only containing the bacteria stock not contacted with any disc) were uploaded, and the total area of the disc covered by bacteria colonies was calculated. For the test plates, when needed, the number of colonies were counted with the Fiji multi-point counter option. The areas of 10 different colonies were recorded, and their average multiplied for the total number of colonies. The results are reported in Table 3. The bacterial viability (%) was calculated as follows:

$$\%= \frac{number\ of\ colonies \times average\ area\ of\ a\ colony}{area\ of\ control\ plates} \times 100$$

4.3.2 Bacterial Assay Results

Table 2 Pictures of the agar plates after incubation at 37°C for 24 h.

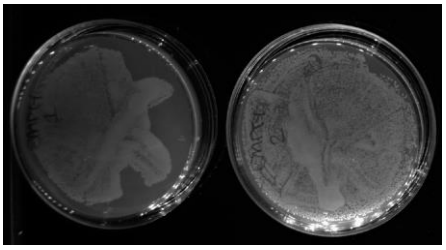
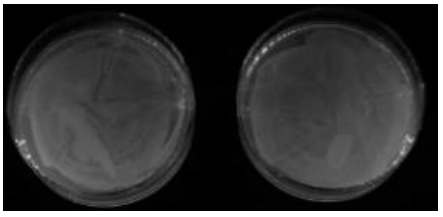
Bacteria stock, only	
PVC, unmodified	

Table 2 continued

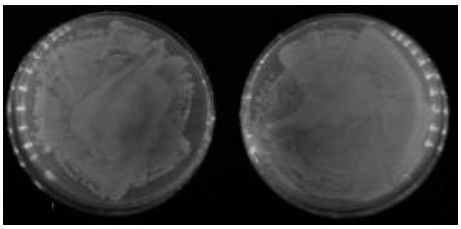
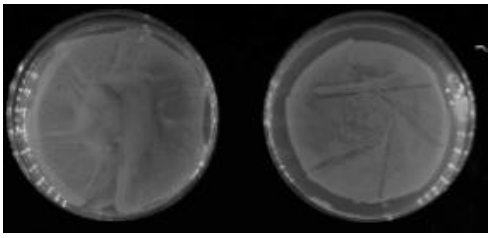
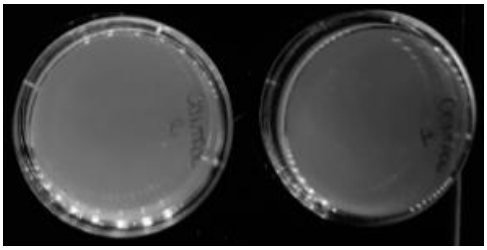
PVC-N_3	
PVC-N_3 , stirred at 80°C in H_2O, $CuSO_4$ only, **washed** by sonication in sat EDTA(aq)	
PVC , stirred at 80°C in H_2O, Bu_4NBF_4 only, **washed** in 10%DMF(aq) at 80°C for 24h	
PVC-N_3 , stirred at 80°C in H_2O, Bu_4NBF_4 only, **washed** in 10%DMF(aq) at 80°C for 24h	
PVC-N_3 , stirred at 80°C in H_2O, Bu_4NBF_4 only, **not washed** in 10%DMF(aq) at 80°C for 24h	

Table 2 continued

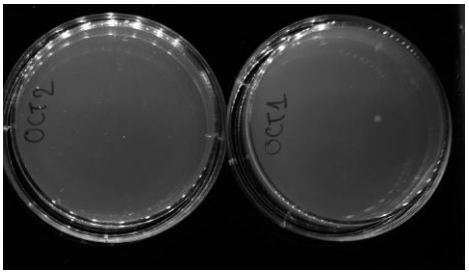
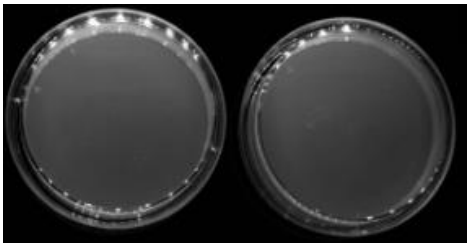
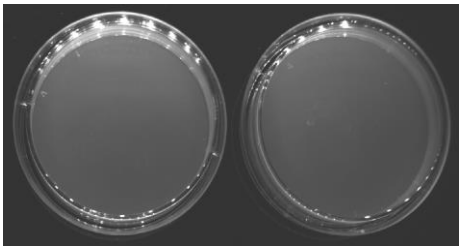
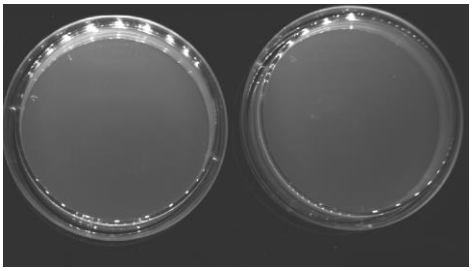
Oct-PVC made **with** Bu_4NBF_4, **not washed** in 10%DMF(aq) at 80°C for 24h and **washed** by sonication in sat EDTA(aq)	
Oct-PVC made **with** Bu_4NBF_4, **washed** in 10%DMF(aq) at 80°C for 24h and **washed** by sonication in sat EDTA(aq)	
Oct-PVC made **without** Bu_4NBF_4 and **washed** by sonication in sat EDTA(aq)	
Met-PVC made **without** Bu_4NBF_4 and **washed** by sonication in sat EDTA(aq)	

Table 2 continued

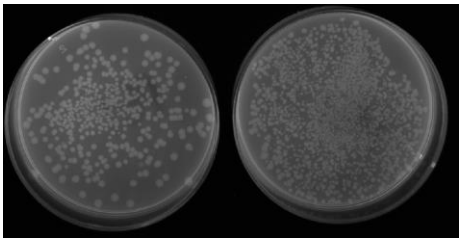
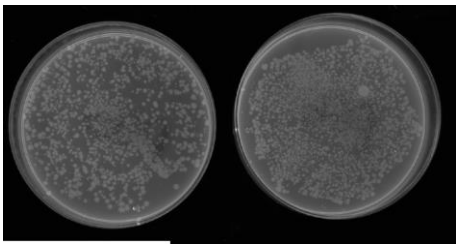
PEG-PVC	
PEI-PVC	

Table 3 semi-quantification of the bacterial viability for different samples:

Sample	Number of colonies (unitless)	Average area of 10 colonies (unitless)	Viability (%)
Bacteria stock, only	-	1.767	100%
PVC, unmodified	-	-	100%
PVC-N_3	-	-	100%
Oct-PVC made **with** Bu_4NBF_4, **not washed** in 10%DMF(aq) at 80°C for 24 h and **washed** by sonication in sat EDTA(aq)	-	-	0%

Table 3 continued

Oct-PVC made **with** Bu_4NBF_4, **washed** in 10%DMF(aq) at 80°C for 24 h and **washed** by sonication in sat EDTA(aq)	-	-	0%
Oct-PVC made **without** Bu_4NBF_4 and **washed** by sonication in sat EDTA(aq)	-	-	0%
Met-PVC made **without** Bu_4NBF_4 and **washed** by sonication in sat EDTA(aq)	-	-	0%
PEG-PVC	342	0.00183	62,5%
PEI-PVC	873	0,00091	44,9%

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