

Synthesis and Application of New Photocrosslinkers for Poly(ethylene glycol)

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Photocrosslinking of polyethylene glycol (PEG) using exogenous agents is a convenient way to produce branched PEG from commercial sources thus avoiding the tricky synthesis of new reactive and functional polymers. In this study, we synthesized two series of new photocrosslinkers, i.e. *bis*-fluorophenyl azide and *bis*-trifluoromethyl diazirine, which under soft UV-irradiation produce reactive species (i.e. nitrene and carbene respectively) that insert into the C–H bond of the polymer backbone, building new bridges between macromolecular chains. These photocrosslinkers are different in terms of behaviour under irradiation and affinity for the target substrate (i.e. PEG). Thus, practical conditions for photocrosslinking of a 10-kDa PEG were studied and followed by NMR and size-exclusion chromatography. In particular, we investigated irradiation in bulk or in solvent, at different irradiation times, with several concentrations of PEG and photolinkers. Finally, we were able to design a procedure to obtain soluble crosslinked PEGs of 300 kDa.

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Introduction

Polyethylene glycol (PEG) is a very popular polymer that has found numerous applications in e.g. drug-delivery systems^[1] and hydrogels for tissue engineering,^[2] thanks to its biocompatibility, chemical inertness and solubility in both organic and aqueous media. Branched and hyperbranched PEGs are desirable in particular cases for reducing the degree of crystallization of the polymer, increasing its loading capacity, and making copolymers, hydrogels, etc.^[3] Among the strategies developed to produce (hyper)branched PEGs, most are multistep procedures. For instance, crosslinking methods usually require first the synthesis of original new macromers such as multi-arm or star-shaped PEGs with reactive chain ends,^[4] PEG acrylate^[5] or α,ω -heterotelechelic PEG^[6,7] to mention but a few, and second, the conception of new and original reactants or crosslinker agents that will react with these macromers.^[8] For instance, in a very recent publication, Phelps and coworkers^[4] produced, with good control of crosslinking, a PEG hydrogel with ligands incorporated. For that purpose, they synthesized four-arm PEGs with maleimide reactive endings that were crosslinked using a dithiol peptide.

However, few strategies offer a simple and direct pathway. Wilms and coworkers^[3] present an elegant direct synthesis by random copolymerization of ethylene oxide and glycidol to produce, in a controlled manner, hyperbranched PEG-copolyglycerol. However, this methodology needs specific equipment for the manipulation of hazardous gases (i.e. ethylene oxide).

Therefore, the direct crosslinking of linear PEGs using an exogenous agent that avoids specialist handling could be an attractive way. Moreover, when a commercially available inexpensive PEG can be used as starting material instead of α,ω -heterotelechelic PEGs, tricky syntheses are avoided.

Photolinkers such as arylazides and diazirines, generating *in situ* nitrenes and carbenes respectively, are well-known reagents^[9] for photoaffinity labelling (PAL) of proteins^[10–12] and for the covalent surface modification of (bio)materials.^[13–15] Nevertheless, few examples of photoreactions performed in bulk have been described^[16] and only one publication reports the use of a *bis*-diazirine reagent as a photocrosslinker for PEG.^[17]

In this paper, we describe the synthesis of original *bis*-arylazides and *bis*-diazirines as potential crosslinking agents of polymers under mild conditions. Their reaction with PEG was studied in bulk and in solution, with the aim of forming defined, soluble, branched PEGs under light activation.

Results and Discussion

Synthesis of the Photocrosslinking Agents

Photoreactive compounds **1**^[18] and **2**^[19] are well-known photolinkers used in several applications ranging from biology to materials science. These two heterobifunctional molecules feature, on one side, an *N*-hydroxysuccinimidyl (NHS) ester that is prone to react with amino compounds, and on the other side, a photoreactive function, i.e. a perfluorophenyl azide (PFPA) for

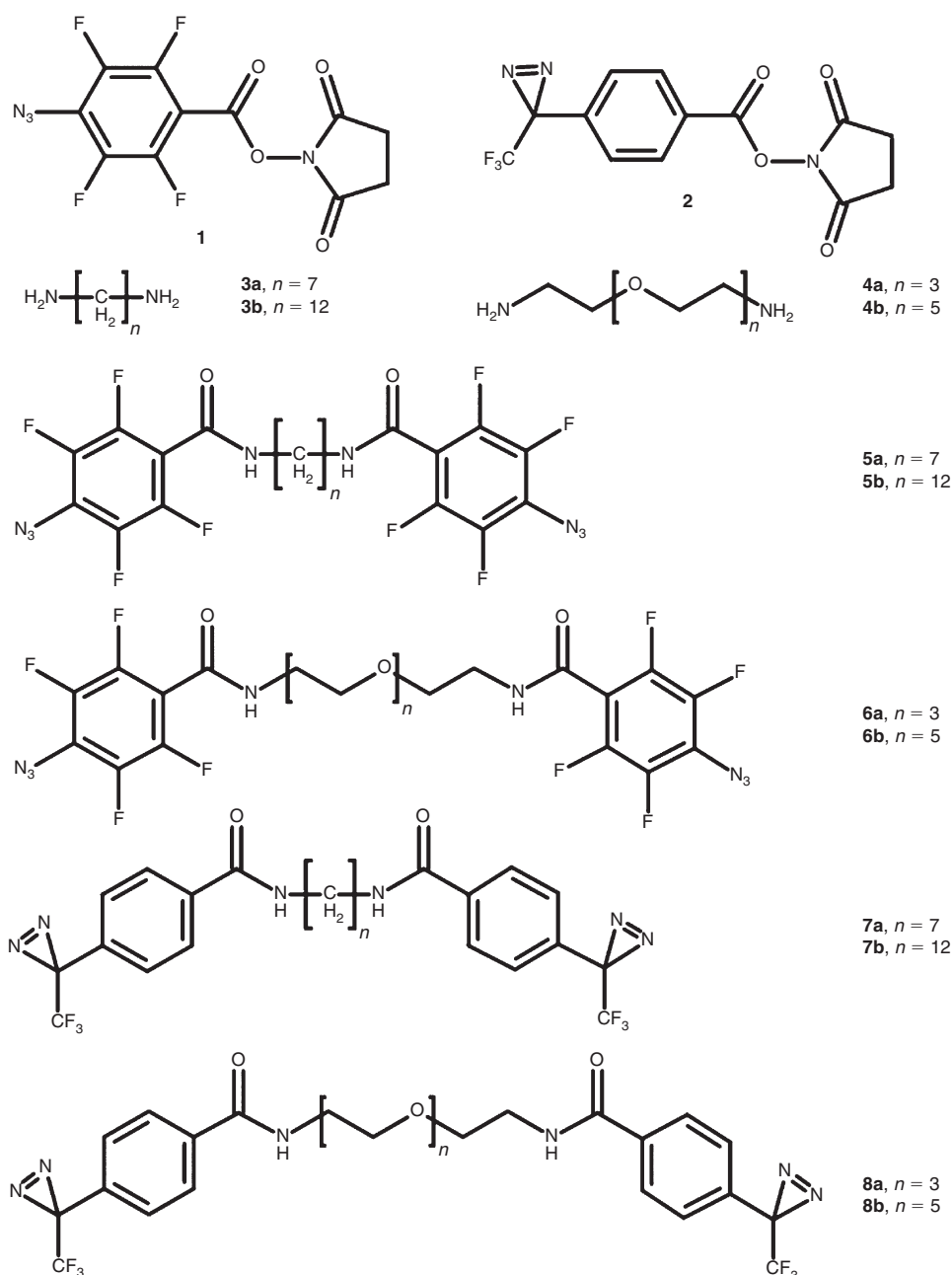


Fig. 1. Perfluorophenyl azide (PFPA) and trifluoromethyl phenyl diazirine (TPD) photocrosslinkers.

1 and a trifluoromethyl phenyl diazirine (TPD) for **2**. In this contribution, the synthesis of novel *bis*-PFPA and *bis*-TPD photocrosslinkers is realized by reacting the NHS ester of **1** and **2** with a series of diamino spacers (**3a**, **3b**, **4a** and **4b**) to obtain eight compounds (Fig. 1) differing by the length and nature (lipophilic or hydrophilic) of the spacer.

PFPA leads to a reactive nitrene under irradiation at wavelengths of ~ 250 nm. PFPA is preferred to simple aryl azides^[20] because it undergoes 'singlet-like insertion' into C–H bonds mainly (i.e. in a concerted mechanism), instead of the stepwise reactions expected from triplet nitrene produced by irradiation of phenyl azide.^[21] PFPA derivatives are thus very attractive photolinkers, easily prepared, stable under storage and giving, under light excitation, very fast and efficient reactions. One major drawback is their excitation wavelength, which could damage some UV-sensitive substrates.

Under excitation at ~ 350 nm, TPD gives a very reactive singlet carbene that is able to insert into any X–H bonds in a concerted mechanism.^[9] Depending on the conditions (mostly the concentration of the photolinker), the photodecomposition of TPD can follow two pathways: (i) the direct elimination of dinitrogen leading to a singlet carbene, or (ii) the rearrangement into a diazo intermediate that either decomposes spontaneously into the desired carbene, or persists until it is subjected to supplementary irradiation at a wavelength of 300 nm.^[11,12] This phenomenon may sometimes preclude the use of TPD despite all its other advantages (i.e. high singlet reactivity, non-damaging irradiation wavelength to various substrates).

Under light excitation, the *bis*-PFPA and *bis*-TPD linkers designed herein are able to give two simultaneous insertions into C–H or X–H bonds of molecules present in their close vicinity. Consequently, when mixed with polymers, they can create

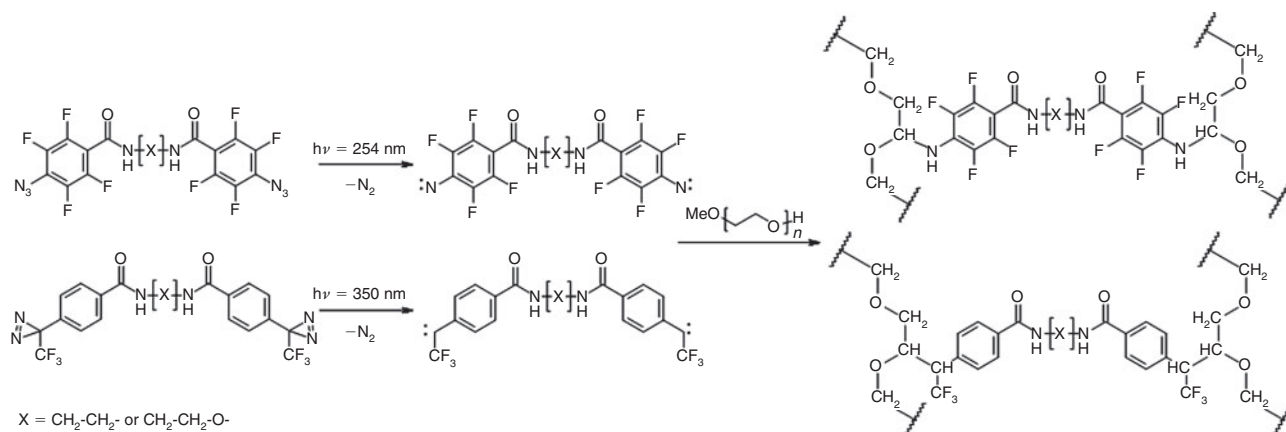


Fig. 2. Photocrosslinking of polyethylene glycol (PEG) induced by *bis*-PFPA (perfluorophenyl azide) and *bis*-TPD (trifluoromethyl phenyl diazirine).

junctions between two different macromolecular chains, as depicted in Fig. 2 in the case of PEG. In this respect, they may provide a convenient way to obtain crosslinked polymers.

The use of different kinds of spacers to separate the two photoreactive centres gives us the opportunity to control the behaviour of the photolinker in terms of solubility and affinity with the substrate. Indeed, because the species formed after irradiation (i.e. nitrenes and carbenes) are highly reactive, they would react in an unspecific way with the closest chemical bond; this means that, to be efficient, the photoreactive group has to be located just next to the targeted bond of the substrate. For that purpose, we used *bis*-amino aliphatic or *bis*-amino oligoether spacers in order to vary the hydrophilic–lipophilic balance of our compounds.

Photochemical Properties

The PFPA compounds **5** and **6** exhibit the characteristic phenyl azide UV absorption band at ~ 255 nm. Under irradiation in acetonitrile solution, a decrease of the absorption at 255 nm was observed (Fig. 3a) following apparent first-order kinetics, as shown in Fig. 3b for **5a**. From the slope of the curve, the rate constant could be calculated. We found a half-life of 18 s, and an irradiation time for total disappearance of the azide peak of 196 s. The decrease of the azide peak can also be observed by infrared spectroscopy thanks to the characteristic stretching band at $\sim 2100\text{ cm}^{-1}$ (Fig. 4a). The ratio of the absorbance corresponding to the N_3 stretching band (2125 cm^{-1}) to the absorbance of the CO amide band (1654 cm^{-1}) of compound **5a** gave a curve (Fig. 4b) comparable with the one observed in the UV spectrum. A total disappearance of the azide band was observed after 180 s, which is in agreement with the result calculated from UV measurements (Fig. 3).

The TPD compounds **7** and **8** show the characteristic peak of TPD absorption at ~ 355 nm (Fig. 5a). The photolysis of these compounds at 350 nm led to a decrease of the diazirine absorption peak with apparent first-order kinetics and a half-life of 1.1 min (Fig. 5b), in good agreement with published results.^[19,22] These observations prove that the introduction of *bis*-amino spacers (**3a**, **3b**, **4a** and **4b**) does not affect the behaviour of PFPA and TPD moieties under UV irradiation.

Photocrosslinking of PEG

Post-polymerization crosslinking with the help of an additive has the advantage, in comparison with other strategies,^[23] of offering several parameters that can be used to control the

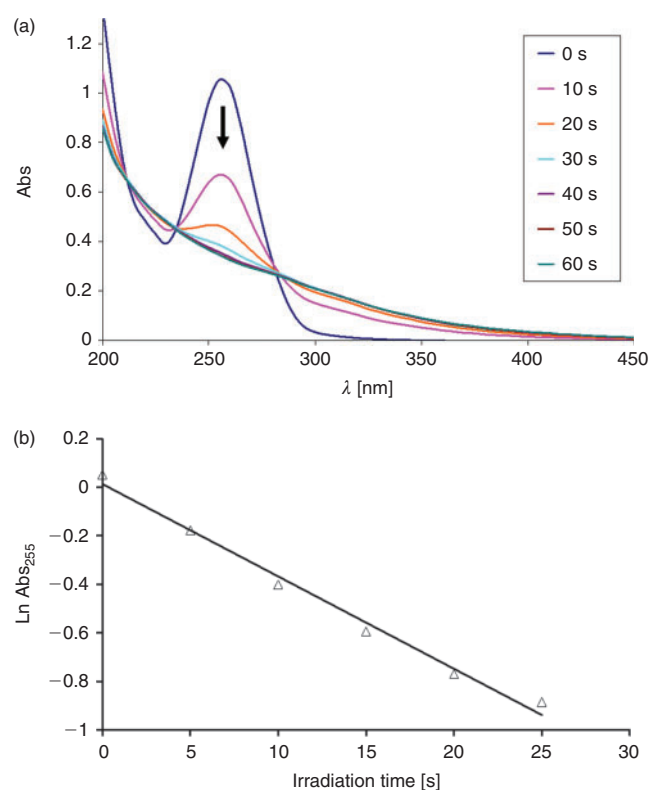


Fig. 3. (a) UV-vis spectra of **5a** (0.17 mM in CH_3CN) at different times of irradiation. (b) Logarithmic plot of the decrease of the absorption maximum at 255 nm of **5a** (0.17 mM in CH_3CN) consistent with first-order kinetics of the photolysis.

architecture of the final polymers. In our case, the parameters that might be interesting to investigate are: polymer : crosslinker ratio, irradiation time, neat solid or solvent media, and affinity between the crosslinker and the substrate. As very reactive species are created during the irradiation process, it is crucial to avoid side reactions as much as possible. So, in a first approach, it seems obvious that the reaction should be carried out in neat mixtures, as pure as possible to avoid insertion of the nitrenes into undesired molecules (i.e. impurities, solvent molecules). However, the reaction yields are expected to be low because, to be efficient, the double insertion process requires two reactive species in close proximity to C–H bonds of the substrate at the

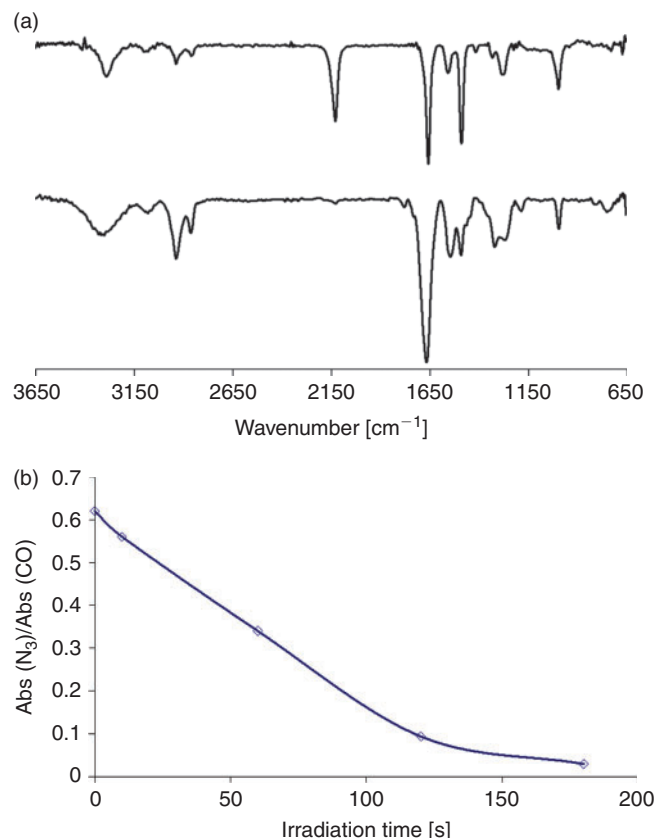


Fig. 4. (a) IR spectra of **5a** at 0 s (upper line) and 180 s (lower line) of irradiation (3 mM in CH₃CN). (b) Absorbance ratio of the azide band to the amide band of **5a** (3 mM in CH₃CN) at different irradiation times at 255 nm.

same moment; otherwise they miss their target, leading to several uncontrolled side reactions (i.e. rearrangement, self-coupling of reactive species, hydrogen abstraction...).

In order to evaluate the efficiency of our photocrosslinkers, they were tested on PEG either in bulk or in solution.

Photocrosslinking in Bulk

We first attempted the photocrosslinking in bulk of neat mixtures of PEG (10 kDa) and crosslinker **6a** that were obtained after solvent casting and careful removal of solvent traces under high vacuum. As can be seen on Fig. 6 from ¹⁹F NMR, the conversion of the PFPA groups was complete after 25 min of irradiation and the two new peaks that appeared at -143 and -161 ppm could be attributed to covalently linked products. The peak at -76 ppm originates from the CF₃COOH used as internal standard. After irradiation, the crude mixture (soluble in the analysis solvent) was analysed by size-exclusion chromatography (SEC), revealing a peak at ~10 kDa (Fig. 7) that corresponds to the initial PEG sample. For the sake of comparison, pure PEG samples were also irradiated under the same conditions (i.e. 250 nm, 25 min) and no other peaks than the one at 10 kDa appeared in the SEC elution curve, meaning that no chain scission occurred. One explanation of this unsuccessful photocrosslinking could be that, during solvent evaporation, PEG chains crystallize, leading to the expulsion of 'impurities' (i.e. the photocrosslinkers) from the spherulites to form segregated domains. So, under irradiation, the reactive species would tend to react among themselves in those domains instead of reacting with PEG chains.

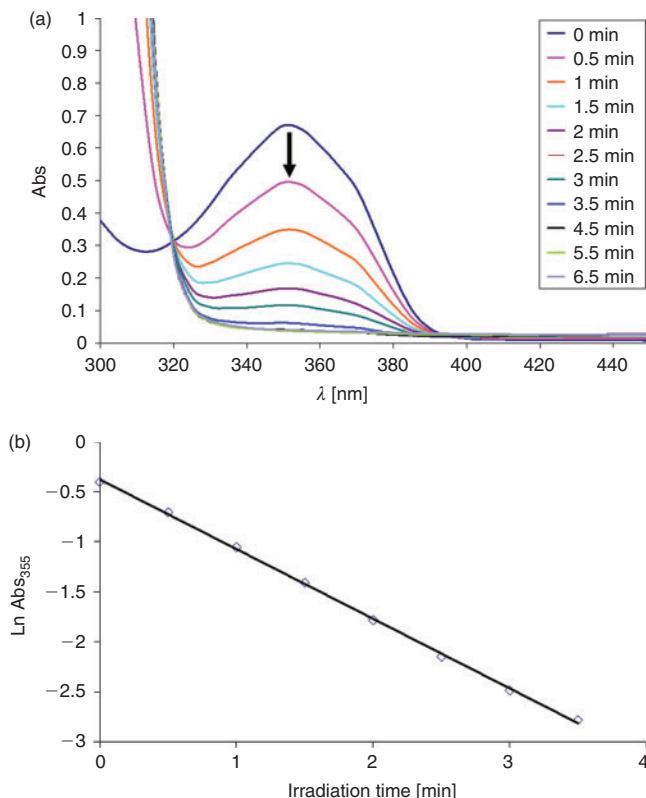


Fig. 5. (a) UV-vis spectra of **7a** (0.2 mM in CH₃CN) at different times of irradiation. (b) Logarithmic plot of the decrease of the absorption maximum at 355 nm of **7a** (0.2 mM in CH₃CN) consistent with first-order kinetics of the photolysis.

Photocrosslinking in Solution

PFPA molecules, initially developed for PAL applications by biologists, are currently employed in aqueous media. So we tested the photocrosslinking reaction in an aqueous solution of PEG and the crosslinker **6a**. After 90 min of irradiation, the formation of a precipitate was observed in the solution. This indicated the formation of highly crosslinked PEG particles as this precipitate could not be resolubilized in aqueous or in organic solvents. Therefore, it was not possible to characterize this precipitate in more detail by solution techniques. Indeed, Blencowe et al.^[17] also obtained insoluble crosslinked PEGs by using *bis*-diazirines as photocrosslinkers. The swelling properties of their crosslinked PEGs were measured in a wide range of solvents including water, dimethylformamide and tetrahydrofuran. They demonstrated that the extent of swelling decreased as a function of the amount of photocrosslinkers used as a result of the increased crosslinking density. Unfortunately, they were neither able to quantify the crosslinking density nor the mean distance between crosslinking points. In this study, we employed higher PEG photocrosslinker ratios and longer PEG chain length than Blencowe et al.; consequently, we decided to focus on the soluble part of the crosslinked PEGs that could be analysed by SEC and so, in the following discussion, only soluble fractions are considered. As can be seen from the ¹⁹F NMR spectra (Fig. 8), several compounds were obtained after 90 min of irradiation. In SEC (Fig. 9), a shoulder can be seen next to the peak of the starting PEG and peaks of higher molar masses (100 to 1000 kDa) appeared, indicating a successful crosslinking of PEG chains. Unfortunately, we did not observe a sharp peak

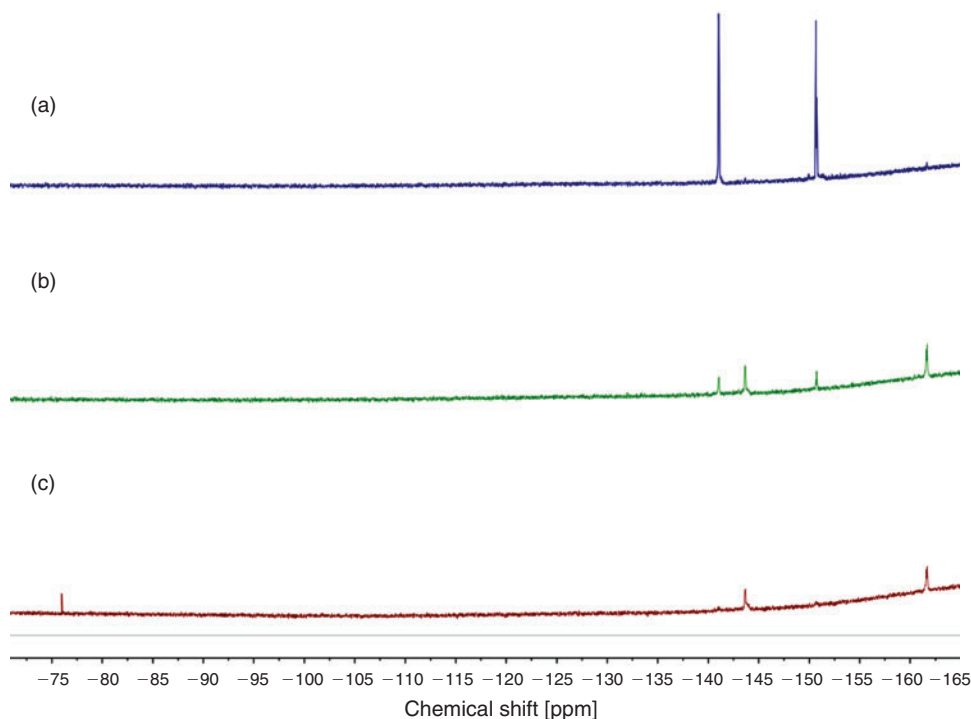


Fig. 6. ^{19}F NMR spectra (287 MHz, CDCl_3 , 25°C) of the bulk mixture of 10-kDa PEG (polyethylene glycol) with **6a** (16 : 1, **6a** : PEG) at different irradiation times (254 nm): (a) 0 min; (b) 15 min; (c) 25 min.

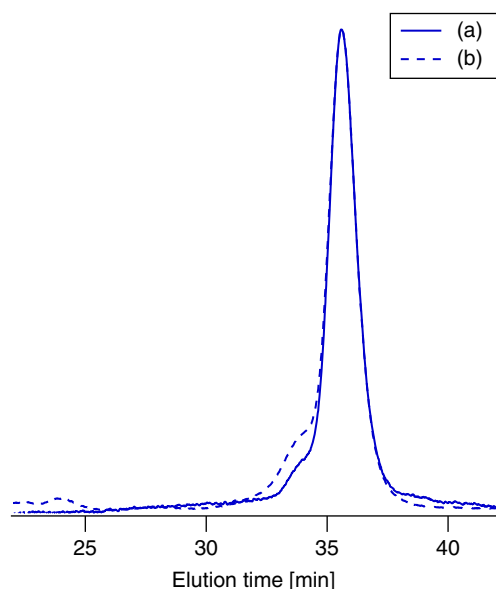


Fig. 7. Size-exclusion chromatography (SEC) chromatograms of (a) 10-kDa PEG (polyethylene glycol) and (b) the bulk mixture of 10-kDa PEG with **6a** (16 : 1, **6a** : PEG) irradiated (254 nm) for 25 min.

with a clear distribution of molar masses, meaning that crosslinking was happening in an uncontrolled way. Consequently, in order to obtain a controlled and reproducible protocol, several parameters needed to be investigated.

*Effect of the Photografting Conditions of **6a** on the Molar Mass of the Soluble Crosslinked PEG*

We hypothesized that controllable architecture of photocrosslinked PEG may be obtained with optimized experimental

parameters. Several factors, i.e. irradiation time, ratio of PEG to photocrosslinker, concentration of PEG, were looked at in term of molar mass obtained in the soluble fraction.

From Fig. 10, it is obvious that for a given PEG concentration and a given photocrosslinker ratio, the irradiation time has no effect, as after 5 min of irradiation, no further changes are observed in SEC chromatograms. From Fig. 11, it can be seen that, for a given irradiation time and a given PEG concentration, higher amounts of photocrosslinker lead to a decrease in the molar masses. This can be explained by a loss of insertion yield due the higher probability of reaction of reactive species between themselves. Consequently, it seemed logical that, at a low PEG : photocrosslinker ratio, the insertion yield could be improved with more concentrated PEG solutions. Surprisingly, the increase of PEG concentration (at given ratio of crosslinker and irradiation time) led to a drop in the molar mass (Fig. 12).

The trends discussed here were similarly observed with the other PFPA photocrosslinkers **5a**, **5b** and **6b**. This exploration of the different parameters that govern the photocrosslinking finally enabled us to elaborate a reproducible protocol to obtain soluble crosslinked PEG of ~ 300 kDa with PFPA photocrosslinkers. The conditions were as follows: 10 min of irradiation at 254 nm, PEG solution of 2 mg mL^{-1} , photolinker : PEG molar ratio of 3 : 2, solution in $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ (3 : 1 v/v).

The TPD crosslinkers **7–8** were tested under the same conditions (i.e. in bulk mixtures and in aqueous solvent) but in all cases, no photocrosslinked PEG product was detected but only low molar masses species certainly due to the coupling of photocrosslinkers between themselves. The TPD series is thus not useful for making crosslinked PEGs.

Conclusions

Two series of photocrosslinkers have been synthesized and tested for their capacity to produce soluble crosslinked PEG

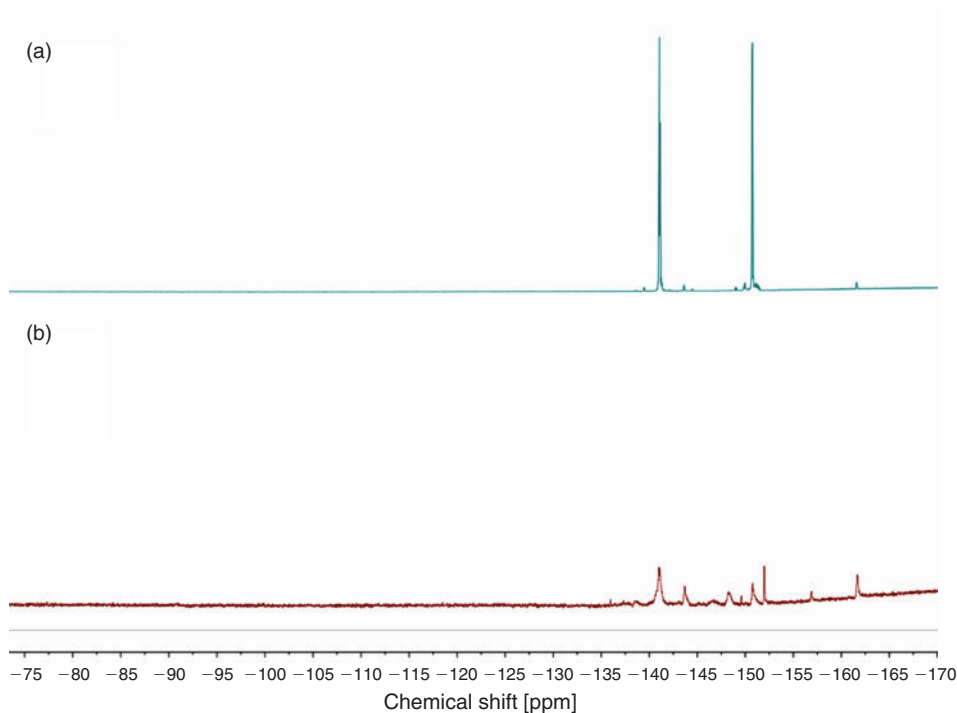


Fig. 8. ^{19}F NMR spectra (287 MHz, CDCl_3 , 25°C) of crosslinked 10-kDa PEG (polyethylene glycol) with **6a** (16 : 1, **6a** : PEG) at different irradiation times (254 nm, H_2O : CH_3CN solution (3 : 1 v/v)): (a) 0 min, and (b) 90 min.

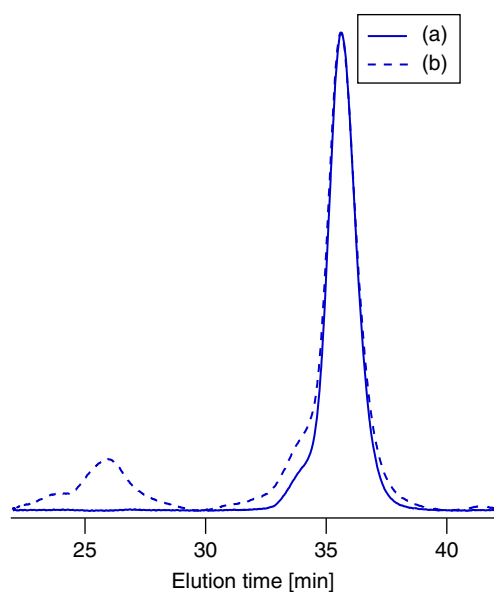


Fig. 9. Size-exclusion chromatography (SEC) chromatograms of (a) 10-kDa PEG (polyethylene glycol), and (b) the crosslinked 10-kDa PEG with **6a** (16 : 1, **6a** : PEG) obtained after 90 min of irradiation at 254 nm in H_2O : CH_3CN (3 : 1 v/v) solution.

starting from a 10-kDa PEG. Considering the previous literature, the diazirine derivatives should be more efficient than the aryl azides in insertion reactions into C–X bonds under light activation.^[9,14] However, at high concentration, diazirines can lead to persistent diazo compounds,^[11,12] thus reducing the yields of insertion. Moreover, the behaviour of the reactive intermediates (nitrenes, carbenes) changes when reactions are performed in diluted or concentrated solution, in the bulk or at the surface of a solid material.^[14,16,24] In the absence of reliable

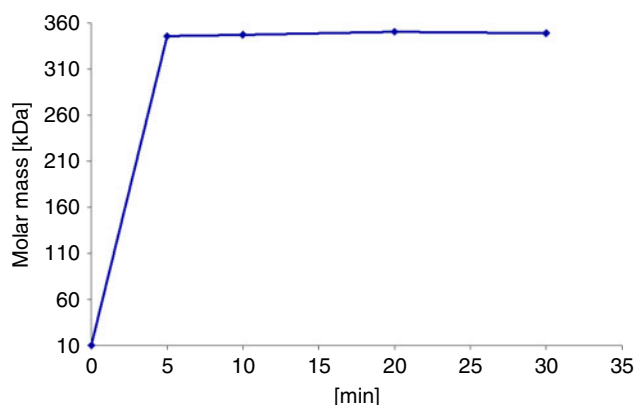


Fig. 10. Molecular mass of soluble crosslinked PEG (polyethylene glycol) for different times of irradiation at 254 nm in H_2O : CH_3CN (3 : 1 v/v) solution with a **6a** : PEG molar ratio of 4 : 1 at a PEG concentration of 2 mg mL^{-1} .

predictions, the reactivity of compounds **5–8** was examined by trial and error. In our hands, the *bis*-diazirines **7–8** could not be used to crosslink PEG; we surmise that self-condensation of the photolinkers and side reactions are the major processes whatever the nature and length of the spacer connecting the two light-sensitive endings. The *bis*-arylazides **5–6** showed globally the same behaviour and enabled the photocrosslinking of PEG in aqueous acetonitrile solution, but not in bulk owing again to self-coupling of the photolinkers, as seen by other groups.^[25] Best results were found by using **6a** (i.e. two 4-azido-2,3,5,6-tetrafluorobenzoyl motifs connected with a tetraethyleneglycol spacer via amide bonds), allowing the production of soluble crosslinked PEG of $\sim 300\text{ kDa}$, together with highly branched insoluble PEG that we were not able to characterize further.

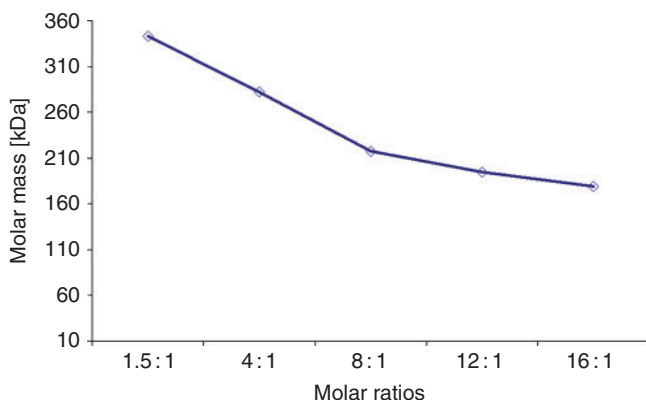


Fig. 11. Molecular mass of soluble crosslinked PEG (polyethylene glycol) for different **6a**:PEG molar ratios after 10 min of irradiation at 254 nm in H₂O:CH₃CN (3:1 v/v) solution at a PEG concentration of 2 mg mL⁻¹.

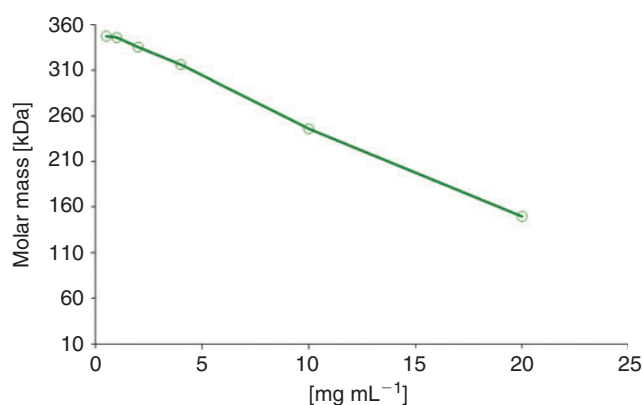


Fig. 12. Molecular mass of soluble crosslinked PEG (polyethylene glycol) for different PEG concentrations at a **6a**:PEG molar ratio of 1.5:1 after 10 min of irradiation at 254 nm in H₂O:CH₃CN (3:1 v/v) solution.

It is not obvious why in our case *bis*-arylazides were more efficient than *bis*-diazirines as the answer to this question would necessitate a dedicated study.^[14] One explanation could be that nitrenes are more persistent than carbenes.^[9,16] As we need a 'double insertion process', requiring two reactive species in close proximity to two C–H bonds at the same instant, nitrenes might be superior because they would have more time to undergo this double process, whereas in the same time, carbenes would lead to several uncontrolled side reactions (i.e. rearrangement, self-coupling of reactive species, hydrogen abstraction...). Another explanation could be the above-mentioned formation of persistent diazo species when diazirines are reacted at high concentration in solution.^[11]

Experimental

General

Solvents were of analytical grade and obtained from Aldrich–Fluka, Acros or ROCC. 1,7-Diaminoheptane **3a**, 1,12-diaminododecane **3b**, α,ω -dihydroxytetraethylene glycol, α,ω -dihydroxyhexaethylene glycol and α -methoxy- ω -hydroxy-PEG (10000 g mol⁻¹) were purchased from Aldrich. ¹H (300 MHz), ¹³C (75 MHz) and ¹⁹F (282 MHz) NMR spectra were recorded on a Bruker Avance II 300 spectrometer. ¹H (500 MHz) and ¹³C (125 MHz) NMR spectra were recorded on a Bruker AM-500 spectrometer. Spectra were obtained with

broad-band proton decoupling. Spectra were obtained in CDCl₃, D₂O or (CD₃)₂CO at room temperature. Chemical shifts δ are reported in ppm and are calibrated on the solvent signal at 7.26, 4.79 or 2.05 ppm for CDCl₃, D₂O or (CD₃)₂CO respectively. Coupling constants *J* are given in Hz. UV-visible spectra were recorded on a UV-vis-NIR Varian–Cary spectrophotometer (λ given in nm). Gel permeation chromatography (GPC) was carried at 45°C at a flow rate of 1 mL min⁻¹ out on a system composed of two PSS Gram columns (100 and 1000 Å) connected to a Waters 410 differential refractometer and a Waters UV detector, with dimethylformamide as the carrier solvent. Polystyrene standards were used for calibration. Low-resolution mass spectra (MS) were acquired using a Thermo Finnigan LCQ spectrometer in positive-APCI mode (atmospheric pressure chemical ionization), positive-CI mode (chemical ionization) or positive-ESI mode (electrospray ionization). Melting points were recorded with a calibrated Büchi melting point B-540 apparatus. Flash chromatography was performed on Merck 60 (40–63 μ m) silica gel. The reacting and rinsing solutions containing the polymer samples were shaken (200 rpm) with an Edmund Bühler stirrer (model KL-2).

Synthesis of Precursors

O-succinimidyl-4-azido-2,3,5,6-tetrafluorobenzoate **1** was synthesized according to a literature protocol.^[18] The synthesis of *O*-succinimidyl-4-(1-azido-2,2,2-trifluoroethyl) benzoate **2** was carried out in seven steps as described elsewhere (V. Pourcelle et al., unpubl. data).

α,ω -diaminotetraethylene glycol **4a** and α,ω -diamino-hexaethylene glycol **4b** were synthesized from α,ω -dihydroxy-tetraethylene glycol and α,ω -dihydroxyhexaethylene glycol respectively, according to procedures published elsewhere.^[26]

General Procedures for the Synthesis of Photocrosscoupling Agents **5a**, **5b**

Into a flame-dried round-bottomed flask and under dimmed light was introduced a solution of 2 equiv. of tetrafluoroarylazide **1** in dry acetonitrile (10 mL). Then, at room temperature under stirring and an argon atmosphere, a solution containing 1 equiv. of diamine spacer arms **3a** or **3b** in dry acetonitrile (10 mL) was added dropwise. After 2 to 4 h stirring, the mixture was filtered and the white precipitate obtained was dissolved in distilled water and extracted with chloroform. The organic layer was washed with water, dried over MgSO₄ and evaporated under vacuum. Compounds **5a** and **5b** were purified by crystallization in a minimum of a mixture of CHCl₃/hexane.

Synthesis of 4-Azido-N-[7-[(4-azido-2,3,5,6-tetrafluorobenzoyl)amino]heptyl]-2,3,5,6-tetrafluorobenzamide **5a**

The *title compound* (70 mg, 70%) was obtained as a white solid from **1** (118 mg, 0.36 mmol) and diamine spacer arm **3a** (23.1 mg, 0.18 mmol). mp 127.8°C. ν_{max} /cm⁻¹ 3294, 2923, 2854, 2125, 1654, 1554, 1485, 995, 724. λ_{max} /nm (ϵ /M⁻¹ cm⁻¹) 260 (67017). δ_{H} (300 MHz, CDCl₃) 1.40 (m, 6H, –CH₂–), 1.62 (m, 4H, NHCO–CH₂–CH₂–), 3.46 (dt, *J* 6.6, 4H, NHCO–CH₂–), 6.13 (s, 2H, NHCO). δ_{C} (125 MHz, CDCl₃) 158.1 (C=O), 144.4 (ddd, ¹*J*_{C–F} 250, ²*J*_{C–F} 14, ³*J*_{C–F} <5, CF), 140.8 (dd, ¹*J*_{C–F} 250, ²*J*_{C–F} 14, CF), 122.1 (m, ArC–C=O), 112 (t, ²*J*_{C–F} 19, CN₃), 40.2 (NHCO–CH₂–), 29.3 (NHCO–CH₂–CH₂–), 28.3 (–CH₂–), 26.5 (–CH₂–). δ_{F} (282 MHz, CDCl₃) –141.1 (dd, *J* 23, 12, 4F), –150.6 (dd, *J* 23, 12, 4F). *m/z* (ESI) 587 (100%, MNa⁺).

Synthesis of 3-Azido-N-[12-[(4-azido-2,3,5,6-tetrafluorobenzoyl)amino]dodecyl]-2,4,5,6-tetrafluorobenzamide 5b

The *title compound* (66 mg, 57 %) was obtained as a white solid from **1** (120 mg, 0.36 mmol) and diamine spacer arm **3b** (36.3 mg, 0.18 mmol). mp 140.2°C. $\nu_{\max}/\text{cm}^{-1}$ 3315, 2921, 2852, 2123, 1652, 1544, 1475, 991. λ_{\max}/nm ($\epsilon/\text{M}^{-1}\text{cm}^{-1}$) 260 (38725). δ_{H} (300 MHz, CDCl_3) 1.39–1.28 (m, 16H, $-\text{CH}_2-$), 1.61 (m, 4H, $\text{NHCO}-\text{CH}_2-\text{CH}_2-$), 3.45 (dt, J 6.3, 7.2, 4H, $\text{NHCO}-\text{CH}_2-$), 5.95 (s, 2H, NHCO). δ_{C} (125 MHz, $\text{C}_2\text{D}_6\text{O}$) 157.3 (C=O), 143.4 (ddd, $^1J_{\text{C-F}}$ 246, $^2J_{\text{C-F}}$ 14, $^3J_{\text{C-F}} < 5$, CF), 143.8 (dd, $^1J_{\text{C-F}}$ 248, $^2J_{\text{C-F}}$ 17, CF), 121.5 (t, $^2J_{\text{C-F}}$ 11, $\text{ArC}-\text{C}=\text{O}$), 113.6 (t, $^2J_{\text{C-F}}$ 20, CN_3), 40 ($\text{NHCO}-\text{CH}_2-$), 29.76 ($-\text{CH}_2-$), 29.74 ($\text{NHCO}-\text{CH}_2-\text{CH}_2-$), 29.4 ($-\text{CH}_2-$), 26.9 ($-\text{CH}_2-$). δ_{F} (282 MHz, CDCl_3) -141.0 (4F, dd, J 23, 12), -150.5 (4F, dd, J 20, 9). m/z (APCI) 635 (100 %, MH^+).

General Procedures for the Synthesis of Photocrosscoupling Agents 6a, 6b, and 8b

In a flame-dried round-bottomed flask and under dimmed light was introduced a solution of 2 equiv. of tetrafluoroarylazide **1**, or trifluorophenyl diazirine **2**, in dry acetonitrile (10 mL). Then, at room temperature under stirring and an argon atmosphere, a solution containing 1 equiv. of diamine spacer arms **4a** or **4b** in dry acetonitrile (10 mL) was added dropwise. After 2 to 4 h stirring, distilled water (20 mL) was added and the mixture was extracted with CHCl_3 . The organic layer was washed with distilled water, dried over MgSO_4 and evaporated under vacuum to obtain compounds **6a**, **6b** and **8b** with no further purification.

Synthesis of 4-Azido-N-[2-[2-[2-[2-[(4-azido-2,3,5,6-tetrafluorobenzoyl)amino]ethoxy]ethoxy]ethoxy]ethyl]-2,3,5,6-tetrafluorobenzamide 6a

The *title compound* (84 mg, 74 %) was obtained as a white solid from **1** (120 mg, 0.36 mmol) and diamine spacer arm **4a** (34.7 mg, 0.18 mmol). mp 96.7°C. $\nu_{\max}/\text{cm}^{-1}$ 3292, 2923, 2854, 2125, 1652, 1558, 1488, 1101, 993. λ_{\max}/nm ($\epsilon/\text{M}^{-1}\text{cm}^{-1}$) 260 (62170). δ_{H} (300 MHz, CDCl_3) 3.64–3.62 (m, 16H, $-\text{CH}_2\text{CH}_2\text{O}-$), 6.74 (s, 2H, $-\text{NHCO}-$). δ_{C} (125 MHz, CDCl_3) 158.2 (C=O), 145.4 (ddd, $^1J_{\text{C-F}}$ 250, $^2J_{\text{C-F}}$ 24, $^3J_{\text{C-F}} < 5$, CF), 141.7 (dd, $^1J_{\text{C-F}}$ 237, $^2J_{\text{C-F}}$ 15, CF), 122.1 (t, $^2J_{\text{C-F}}$ 12, $\text{ArC}-\text{C}=\text{O}$), 111.9 (t, $^2J_{\text{C-F}}$ 18, CN_3), 70.8 ($-\text{CH}_2-\text{CH}_2-\text{O}-$), 70.6 ($-\text{CH}_2-\text{CH}_2-\text{O}-$), 69.7 ($-\text{CH}_2-\text{CH}_2-\text{O}-$), 40.4 ($\text{NHCO}-\text{CH}_2-$). δ_{F} (282 MHz, CDCl_3) -141.08 (dd, J 20, 11, 4F), -150.73 (dd, J 23, 11, 4F). m/z (APCI) 627 (100 %, MH^+).

Synthesis of 4-Azido-N-[2-[2-[2-[2-[2-[2-[(4-azido-2,3,5,6-tetrafluorobenzoyl)amino]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]-2,3,5,6-tetrafluorobenzamide 6b

The *title compound* (98 mg, 76 %) was obtained as a yellowish oil from **1** (120 mg, 0.36 mmol) and diamine spacer arm **4b** (50.6 mg, 0.18 mmol). $\nu_{\max}/(\text{cm}^{-1})$ 3299, 2931, 2871, 2125, 1652, 1550, 1488, 1107, 993. λ_{\max}/nm ($\epsilon/\text{M}^{-1}\text{cm}^{-1}$) 260 (1630). δ_{H} (300 MHz, CDCl_3) 3.62–3.53 (m, 24H, $-\text{CH}_2\text{CH}_2\text{O}-$), 7.1 (s, 2H, NHCO). δ_{C} (125 MHz, CDCl_3) 158.1 (C=O), 144.4 (ddd, $^1J_{\text{C-F}}$ 202, $^2J_{\text{C-F}}$ 12, $^3J_{\text{C-F}} < 5$, CF), 140.8 (dd, $^1J_{\text{C-F}}$ 249, $^2J_{\text{C-F}}$ 16, CF), 121.8 (t, $^2J_{\text{C-F}}$ 12, $\text{ArC}-\text{C}=\text{O}$), 112.4 (t, $^2J_{\text{C-F}}$ 19, CN_3), 70.8 ($-\text{CH}_2\text{CH}_2\text{O}-$), 70.8 ($-\text{CH}_2\text{CH}_2\text{O}-$), 70.7 ($-\text{CH}_2\text{CH}_2\text{O}-$), 70.6 ($\text{NHCO}-\text{CH}_2-\text{CH}_2-$), 69.8 ($-\text{CH}_2\text{CH}_2\text{O}-$), 40.4 ($\text{NHCO}-\text{CH}_2-$). δ_{F} (282 MHz, CDCl_3) -141.06 (dd, J 20, 12, 4F), -150.9 (dd, J 20, 9, 4F). m/z (APCI) 715 (50 %, MH^+).

Synthesis of 4-[3-(Trifluoromethyl)diazirin-3-yl]-N-[2-[2-[2-[2-[2-[2-[4-[3-(trifluoromethyl)diazirin-3-yl]benzoyl amino]ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]ethyl]benzamide 8b

The *title compound* (78 mg, 60 %) was obtained as yellowish oil from **2** (120 mg, 0.37 mmol) and diamine spacer arm **4b** (51.4 mg, 0.18 mmol). $\nu_{\max}/\text{cm}^{-1}$ 3326, 2914, 2871, 1645, 1548, 1186, 1153. λ_{\max}/nm ($\epsilon/\text{M}^{-1}\text{cm}^{-1}$) 350 (277). δ_{H} (300 MHz, CDCl_3) 3.62–3.58 (m, 24H, $-\text{CH}_2\text{CH}_2\text{O}-$) 7.22 (d, J 8, 4H, ArH), 7.31 (s, 2H, NHCO), 7.87 (d, J 8, 4H, ArH). δ_{C} (125 MHz, CDCl_3) 166.7 (C=O), 136.1 ($\text{ArC}-\text{CNN}$), 132.4 ($\text{ArC}-\text{C}=\text{O}$), 128.1 (ArCH), 126.8 (ArCH), 122.3 (q, $^1J_{\text{C-F}}$ 273, CF_3), 70.9 ($-\text{CH}_2\text{CH}_2\text{O}-$), 70.5 ($\text{NHCO}-\text{CH}_2-\text{CH}_2-$), 70.1 ($\text{NHCO}-\text{CH}_2-\text{CH}_2-$), 40.3 ($\text{NHCO}-\text{CH}_2-$), 28.7 (q, $^2J_{\text{C-F}}$ 40, CN_2). δ_{F} (282 MHz, CDCl_3) -65 (s, CF_3). m/z (APCI) 705 (30 %, MH^+).

General Procedures for the Synthesis of Photocrosscoupling Agents 7a, 7b, and 8a

In a flame-dried round-bottomed flask, under dimmed light and an argon atmosphere, 2 equiv. of trifluorophenyl diazirine **2**, 1 equiv. of diamine spacer arms **3a**, **3b** or **4a** and 6 equiv. of Et_3N were introduced in dry dichloromethane or dry DMF (4 mL). After 20 h stirring at room temperature CHCl_3 (50 mL) was added. The organic layer was washed with water, dried over MgSO_4 and evaporated under vacuum. Compounds **7a**, **7b** and **8a** were chromatographed on flash silica gel with hexane/ethyl acetate (1 : 1, 2 : 1, 18 : 1 respectively).

Synthesis of 4-[3-(Trifluoromethyl)diazirin-3-yl]-N-[7-[4-[3-(trifluoromethyl)diazirin-3-yl]benzoyl]aminoheptyl]benzamide 7a

The *title compound* (41 mg, 82 %) was obtained, after chromatography on silica gel with hexane/ethyl acetate (1 : 1) followed by crystallization in a mixture of CHCl_3 /hexane, as a white solid from **2** (59 mg, 0.18 mmol) and diamine spacer arm **3a** (11.7 mg, 0.09 mmol). mp 134°C. $\nu_{\max}/\text{cm}^{-1}$ 3311, 2923, 2852, 1637, 1548, 1191, 1155. λ_{\max}/nm ($\epsilon/\text{M}^{-1}\text{cm}^{-1}$) 350 (506). δ_{H} (300 MHz, CDCl_3) 1.38 (m, 6H, $-\text{CH}_2-$), 1.60 (m, 4H, $\text{NHCO}-\text{CH}_2-\text{CH}_2-$), 3.43 (dt, J 7, 13, 4H, $\text{NHCO}-\text{CH}_2-$), 6.39 (m, 2H, NHCO), 7.39 (d, J 8, 4H, ArH), 8.03 (d, J 8, 4H, ArH). δ_{C} (125 MHz, CDCl_3) 166.7 (C=O), 136.2 ($\text{ArC}-\text{CNN}$), 132.6 ($\text{ArC}-\text{C}=\text{O}$), 127.7 (ArCH), 126.9 (ArCH), 122.3 (q, $^1J_{\text{C-F}}$ 273, CF_3), 40.3 ($\text{NHCO}-\text{CH}_2-$), 29.7 ($\text{NHCO}-\text{CH}_2-\text{CH}_2-$), 28.8 ($-\text{CH}_2-$), 28.7 (q, $^2J_{\text{C-F}}$ 40, CN_2), 26.8 ($-\text{CH}_2-$). δ_{F} (282 MHz, CDCl_3) -65 (s, CF_3). m/z (APCI) 555 (100 %, MH^+).

Synthesis of 4-[3-(Trifluoromethyl)diazirin-3-yl]-N-[12-[4-[3-(trifluoromethyl)diazirin-3-yl]benzoyl]aminododecyl]benzamide 7b

The *title compound* (32 mg, 66 %) was obtained, after chromatography on silica gel with hexane/ethyl acetate (2 : 1) followed by crystallization in a mixture of CHCl_3 /hexane, as a white solid from **2** (51 mg, 0.16 mmol) and diamine spacer arm **3b** (15.6 mg, 0.08 mmol). mp 129°C. $\nu_{\max}/\text{cm}^{-1}$ 3311, 2921, 2852, 1633, 1539, 1190, 1147. λ_{\max}/nm ($\epsilon/\text{M}^{-1}\text{cm}^{-1}$) 350 (482.5). δ_{H} (300 MHz, CDCl_3) 1.33–1.27 (m, 16H, $-\text{CH}_2-$), 1.61 (m, 4H, $\text{NHCO}-\text{CH}_2-\text{CH}_2-$), 3.44 (dt, J 7, 13, 4H, $\text{NHCO}-\text{CH}_2-$), 6.13 (m, 2H, NHCO), 7.24 (d, J 8, 4H, ArH), 7.78 (d, J 9, 4H, ArH). δ_{C} (125 MHz, CDCl_3) 166.6 (C=O), 136.3 ($\text{ArC}-\text{CNN}$), 132.5 ($\text{ArC}-\text{C}=\text{O}$), 127.7 (ArCH), 127.0 (ArCH), 122.3

(q, $^1J_{C-F}$ 273, CF₃), 40.6 (NHCO–CH₂–), 29.9 (NHCO–CH₂–CH₂–), 29.7 (–CH₂–), 29.5 (–CH₂–), 28.7 (q, $^2J_{C-F}$ 40, CN₂), 27.3 (–CH₂–). δ_F (282 MHz, CDCl₃) –65.01 (s, CF₃). *m/z* (APCI) 625 (100 %, MH⁺).

Synthesis of 4-[3-(Trifluoromethyl)diazirin-3-yl]-N-[2-[2-[2-[2-[4-[3-(trifluoromethyl)diazirin-3-yl]benzoyl]amino]ethoxy]ethoxy]ethoxy]ethyl]benzamide 8a

The title compound (33 mg, 57 %) was obtained, after chromatography on silica gel with hexane/ethyl acetate (1 : 18), as a yellowish oil from **2** (61.5 mg, 0.18 mmol) and diamine spacer arm **4a** (18.1 mg, 0.09 mmol). ν_{max}/cm^{-1} 3342, 2923, 2854, 1647, 1550, 1186, 1155. λ_{max}/nm ($\epsilon/M^{-1}cm^{-1}$) 350 (490.5). δ_H (CDCl₃, 300 MHz) 3.63 (m, 16H, –CH₂CH₂O–), 6.82 (s, 2H, NHCO), 7.21 (d, *J* 8, 4H, ArH), 7.80 (d, *J* 8, 4H, ArH). δ_C (125 MHz, CDCl₃) 166.6 (C=O), 135.9 (ArC–CNN), 132.6 (ArC–C=O), 127.9 (ArCH), 127.0 (ArCH), 122.3 (q, $^1J_{C-F}$ 273, CF₃), 70.8 (–CH₂CH₂O–), 70.5 (–CH₂CH₂O–), 70.1 (NHCO–CH₂–CH₂–), 40.2 (NHCO–CH₂–), 28.7 (q, $^2J_{C-F}$ 40, CN₂). δ_F (282 MHz, CDCl₃) –65 (s, CF₃). *m/z* (APCI) 617 (100 %, MH⁺).

10-kDa PEG Photocrosslinking with Compound 6a

Photocrosslinking in Bulk

Under dimmed lighting, in a round-bottomed quartz flask, **6a** (1 mg, 1.5 μ mol) and 10-kDa PEG (11 mg, 1.1 μ mol) were introduced in dry dichloromethane (2 mL). After evaporation of the solvent under vacuum, the mixture was irradiated (3 \times 8 W, 254 nm, placed at a distance of 10 cm) in a home-made reactor (rotating 15-mL quartz flask) under an argon atmosphere for 25 min.

Photocrosslinking in Solution

Under dimmed lighting, in a round-bottomed quartz flask, **6a** (11 mg, 17.5 μ mol) in acetonitrile (2 mL) was mixed with 10-kDa PEG (11 mg, 1.1 μ mol) in Milli-Q water (6 mL). The mixture was irradiated (3 \times 8 W, 254 nm, placed at a distance of 10 cm) under stirring for 90 min. The water/acetonitrile mixture was evaporated at 50°C under reduced pressure to yield an orange solid.

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