Photo-Induced Macromolecular Functionalization of Cellulose via Nitroxide Spin Trapping

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ABSTRACT

A mild radical photo-induced method is presented to graft synthetic polymers onto cellulose. Solid cellulose is functionalized with a biocompatible Norrish type I radical photoinitiator (2-hydroxy-1-(4-(2-hydroxyethoxy)phenyl)-2-methylpropan-1-one, Irgacure® 2959). Following near-UV irradiation (λ_{max} ~ 311 nm), radicals are generated on the cellulose surface and trapped by a nitroxide-functionalized

polystyrene (M_n = 3800 g mol^{-1}, *PDI* = 1.09). The method was first evaluated for the chain-end functionalization of heterotelechelic functional poly(ethylene glycol)s (PEGs) (M_n ~ 2000 g mol^{-1}). Two PEGs bearing either a 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) nitroxide or Irgacure® 2959 at their chain termini were photochemically reacted with either molecular Irgacure® 2959 or TEMPO, respectively. Both reactions produced rapidly (30–120 min) and almost quantitatively the expected adduct with few by-products as evidenced by size-exclusion chromatography coupled to electrospray ionization-mass spectrometry (SEC/ESI-MS) analysis. The ligation of the two functionalized PEGs was observed to proceed in a similar fashion (45 min) as evidenced by the clear and nearly quantitative shift in SEC traces. Finally the successful grafting of solid cellulose substrates with TEMPO-polystyrene (M_n = 3800 g mol^{-1}, *PDI* = 1.09) was demonstrated by X-ray photoelectron spectroscopy (XPS) and water contact angle measurement.

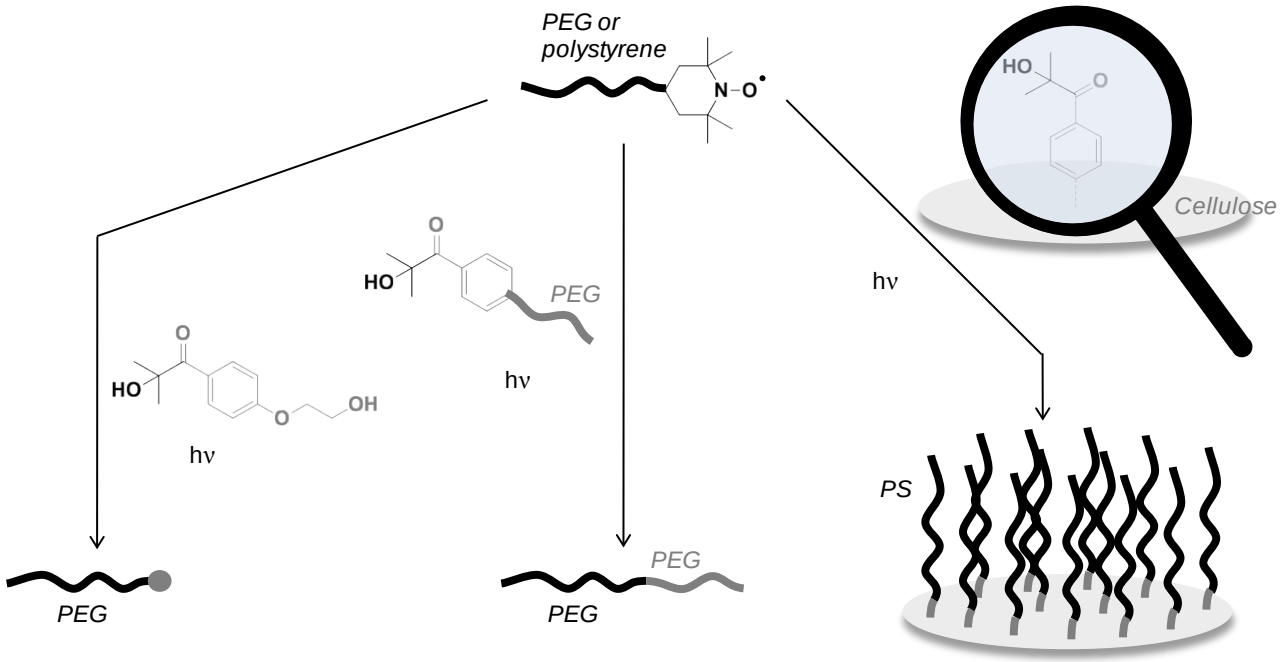
KEYWORDS Grafting onto, conjugation, photoinitiator, TEMPO, cellulose

Cellulose is the most abundant organic raw material on the planet. Due to its renewability and biodegradability it is currently attracting much interest for the production of biofuels or platform chemicals.[1-5] In addition, recent applications in the field of materials science have appeared, arising from the low density and the excellent thermal and mechanical properties of cellulose, particularly in the production of composites.[6] However, there are still some major drawbacks to using cellulose including its water-absorbing nature and its poor compatibility with other materials, *e.g.*, synthetic polymers. To combat these problems, grafting synthetic polymers onto cellulose is the most straightforward method to alter its surface properties and thus to control the wettability, adhesion, or hydrophobicity of the biopolymer.[7] Although *grafting from* methods such as surface-initiated nitroxide-mediated polymerization (NMP),[8] atom transfer radical polymerization (ATRP),[9-14] reversible addition-fragmentation transfer polymerization (RAFT),[15-19] or ring-opening polymerization (ROP)[20-24] are considered as the most efficient approaches – particularly in terms of grafting density – efficient *grafting to* methods have recently produced very good results. Cellulose has been successfully modified with pre-formed polymers by both hetero-Diels–Alder[25] and 1,3-dipolar nitrile imine-ene[26] cycloadditions. In the latter case, light was used as the grafting trigger. Importantly, employing light offers temporal and spatial control of the reaction.[27] In the present contribution we introduce a very facile *grafting to* protocol based on the generation of radicals at the surface of cellulose by mild UV irradiation (λ_{max} ~ 311 nm) of an immobilized photoinitiator, followed by radical trapping with a nitroxide-functionalized polymer (see Scheme 1). Previously, nitroxide radical coupling was employed to efficiently link polymer strands, however via a copper-catalyzed mechanism using ATRP-made

polymers to generate reactive radicals.[28-33] A rather similar philosophy based on spin capturing was reported employing nitrones which after a first radical reaction generate a nitroxide able to undergo a second radical coupling.[34-36]

Homogeneous photo-induced ligation in solution To evaluate the new photochemical *grafting to* method, preliminary studies were carried out through the end functionalization of a synthetic homopolymer, *i.e.*, poly(ethylene glycol) (PEG) (Scheme 1). Subsequently, the possibility of employing the light-induced radical technique to couple two synthetic macromolecular strands to produce a block copolymer was demonstrated (see Scheme 1). Finally, we applied the method to the grafting of a nitroxide-functionalized hydrophobic polymer (polystyrene) onto cellulose surface which was modified beforehand with a radical photoinitiator.

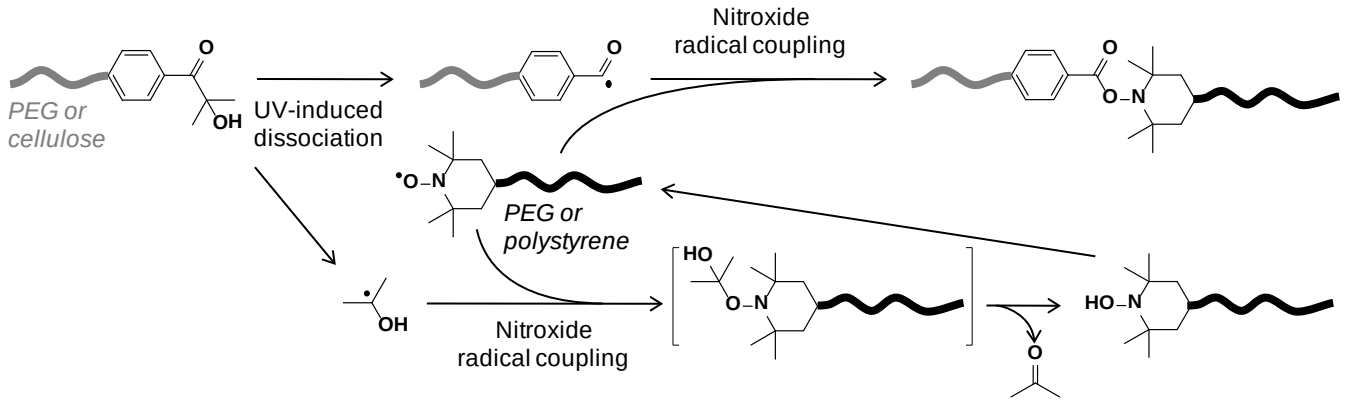
Scheme 1. General synthetic methodology for the UV-induced macromolecular conjugation by nitroxide spin trapping



In order to study the efficiency of the reaction and to determine the optimal conditions, two ω-functional α-methoxy poly(ethylene glycol)s were synthesized. The former ω-hydroxyl extremity of monomethyl ether PEG was equipped with the TEMPO nitroxide or the photoinitiator Irgacure® 2959 (2-hydroxy-1-(4-(2-hydroxyethoxy)phenyl)-2-methylpropan-1-one, **PI**) (see Figures S1 and S2). To allow for a precise assignment of the reaction products by mass spectrometry, the homopolymers were first reacted independently with low-molecular-weight compounds, *e.g.*, TEMPO-functionalized PEG **1** with **PI** (see

Figure 1). The preliminary experiments were carried out in acetonitrile under nitrogen atmosphere and at ambient temperature, using a 36-Watt UV lamp with a maximum emission at 311 nm. The progress of the reaction was monitored by injecting periodically withdrawn samples in a size-exclusion chromatography system coupled to an electrospray ionization-mass spectrometer. We observed that the reaction between TEMPO-functionalized PEG **1** and **PI** occurred since the adduct **2** originating from the recombination of **1** and the benzoyl-based fragment of **PI** was unambiguously formed (Figure 1 and Table S3). However, the reaction did not proceed to completion due to a side reaction occurring between the TEMPO moieties and the tertiary radical fragment produced by photocleavage of the initiator **PI** (see Scheme 2). Indeed, the adduct formed during the side reaction is unstable and leads to non-reactive hydroxylamine species **1-H** *via* the stoichiometric elimination of acetone (refer to Figure 1). While the formation of **2** proceeded rather fast in the first 30 minutes (more than 50 % conversion according to SEC/ESI-MS), the yield did not appear to substantially increase after 60 or 130 min of irradiation (Figure 1). It must be noted that the reaction proceeded similarly under ambient atmosphere (in the presence of oxygen). In that case, however, more non-identified by-products were observed. Although the reaction was not yet optimized, it could already be employed for surface functionalization since washing procedures can readily remove non-grafted hydroxylamine species. Further improvement would be required if the reaction was to be employed for quantitative macromolecular conjugation in homogeneous conditions. Indeed, only the most powerful techniques such as liquid chromatography at critical conditions would be able to separate the products of block conjugation from hydroxylamine PEG by-products.

Scheme 2. Mechanisms involved in the UV-induced conjugation by nitroxide spin trapping



An avenue to reactivate the H-terminal TEMPO species is by the reoxidation of the hydroxylamine moiety to re-form the nitroxide, thus improving the functionalization efficiency.[37] After a 60-min irradiation at identical conditions as above, a 4.5-fold excess of lead dioxide (PbO_2) with respect to **1**

was added to the mixture. Re-formation of the TEMPO-functionalized PEG **1** was unambiguously observed (see Figure S4). After a 60-min re-irradiation, a substantially higher conversion of 80 % was obtained.

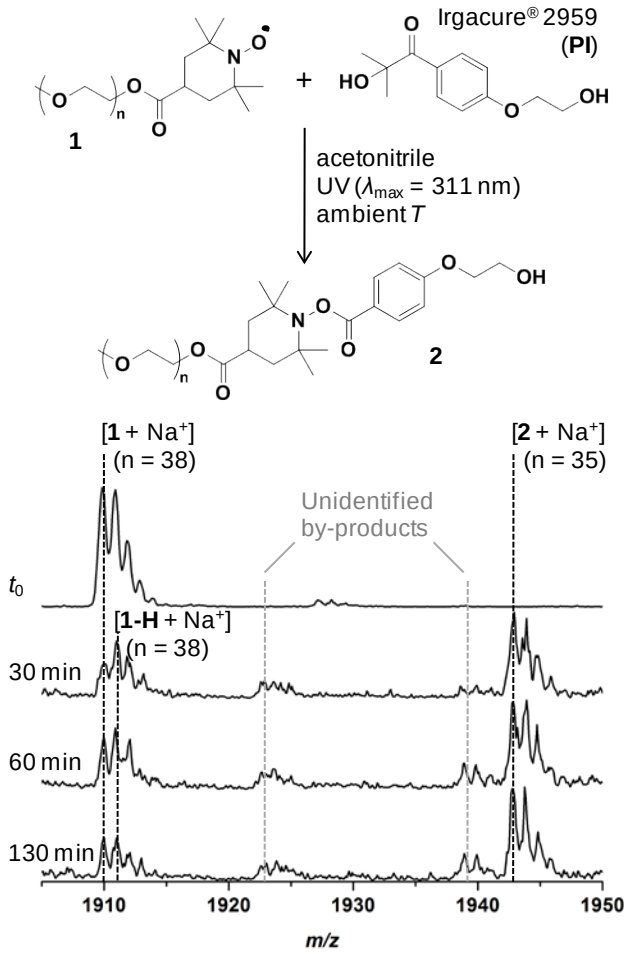


Figure 1. Macromolecular end-group functionalization by UV-induced nitroxide spin trapping. (Top) Reaction conditions for the coupling reaction between TEMPO-functionalized PEG **1** and photoinitiator **PI** (Irgacure® 2959) (5 eq.). (Bottom) SEC/ESI-MS spectra of TEMPO-functionalized PEG **1** before irradiation (top spectrum) and after variable irradiation times during the UV-triggered reaction with **PI**.

Employing PbO_2 *in situ* (2 eq. with respect to **1**) during the UV irradiation enabled us to reach quantitative functionalization of TEMPO-PEG **1** with **PI** as indicated by the clear shift of the PEG population peaks corresponding to the capping of the *p*-(2-hydroxyethoxy)benzoyl radicals by TEMPO located at the PEG chain end (see Figure 2b). It must be noted that the reaction proceeds at ambient temperature at a rather high rate (30 min) with a minimal proportion of by-products.

The analogous reaction between polymer-bound **PI** and free molecular TEMPO was also evaluated (without oxidizing agent) before conducting the macromolecular conjugation of TEMPO-PEG **1** with PI-PEG **3** (Figure 2a). Although substantially more by-products were present, the expected adduct could

again be observed with a satisfying yield (see Figure S6).

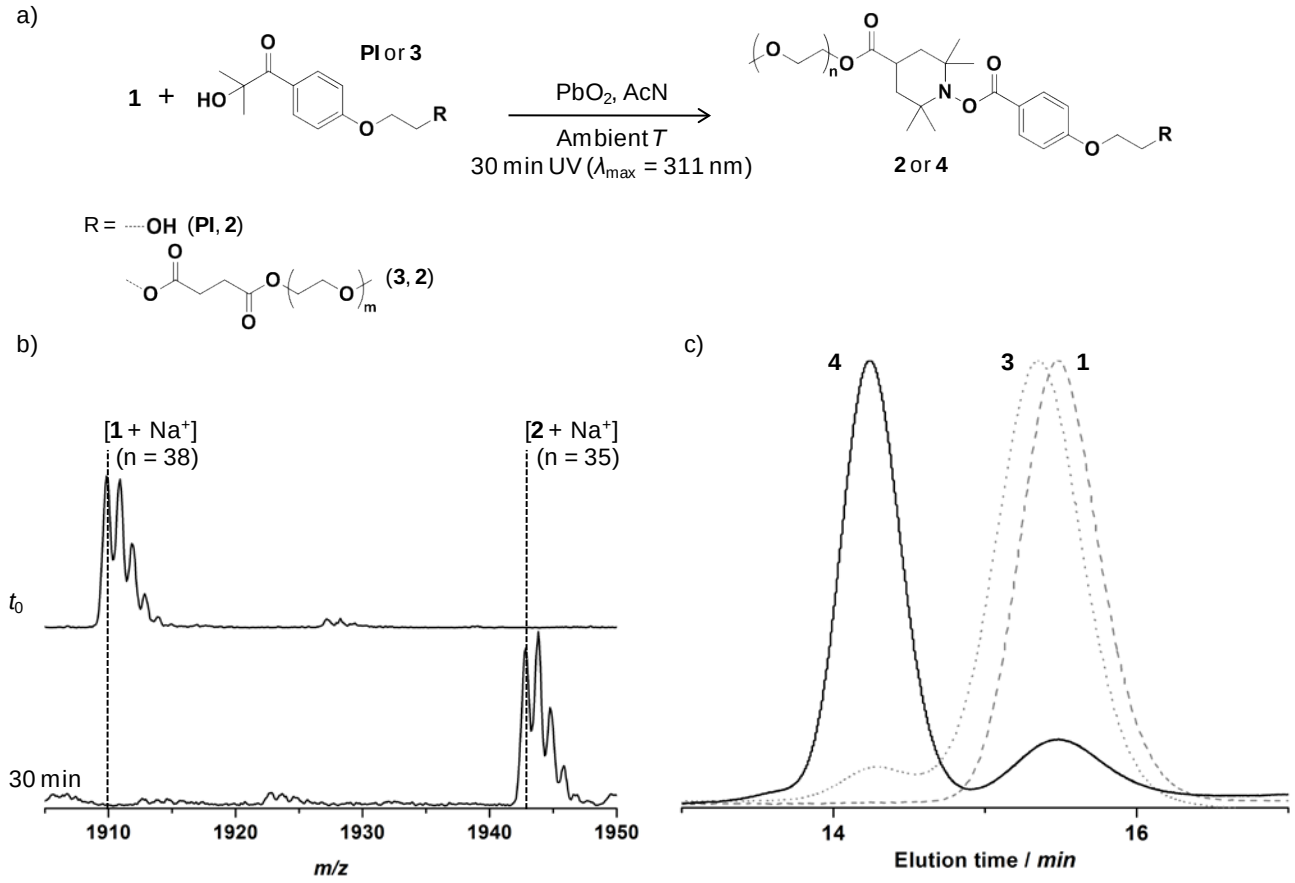


Figure 2. Modular block copolymer synthesis by UV-induced nitroxide spin trapping. (a) Coupling reaction between TEMPO-functionalized PEG **1** and Irgacure® 2959-based (macro)molecules **PI** or **3** in the presence of PbO_2. (b) SEC/ESI-MS spectra of TEMPO-functionalized PEG **1** before (top spectrum) and after (bottom spectrum) UV-triggered reaction with **PI** (5 eq.) in the presence of PbO_2 (2 eq. with respect to **1**). (c) Overlay of SEC traces of end-functionalized homopolymers **1** (grey dashed line) and **3** (grey dotted line) and corresponding coupling product **4** (black straight line) formed after 30 min of irradiation in the presence of PbO_2 (3 eq.).

Subsequently, the reaction between the two functionalized PEGs **1** and **3** was conducted employing the established optimum conditions, *i.e.*, in the presence of PbO_2. Stoichiometric equivalents of the polymers were mixed in a dispersion of lead oxide (3 eq.) in acetonitrile. Size-exclusion chromatography was performed on the filtered raw mixture and compared to the starting materials chromatograms (Figure 2c). It must be noted that **3** contained a small fraction of high molecular weight polymer, probably originating from limited dimerization under natural light exposure of either **PI** or **3**.

After only 30 min of irradiation, a clear shift towards higher molecular weights was observed and about 75 % of the starting materials have been converted. The shape of the residual starting material suggests that **3** has been almost entirely consumed while remaining **1** is present, which could be explained by the hydroxylamine species formation. Indeed while **1** can be involved in this side reaction, radicals are continuously formed at the chain end of **3** and could recombine or undergo other side reactions before reacting with initial or most likely re-oxidized PEG-TEMPO **1**. In the polymer-small molecule experiments, an excess of low-molecular-weight photoinitiator was employed. Thus re-oxidized PEG-TEMPO **1** has access to photo-generated radicals for an extended period of time. However, during macromolecular conjugation, those radicals having a relatively low concentration might have recombined before all PEG-TEMPO molecules were reacted. This was confirmed by time-dependant SEC/ESI-MS monitoring. The examination of the ESI-mass spectrum of the SEC low-molecular-weight region indicated that PEG-PI **3** was entirely consumed while remaining PEG-TEMPO **1** was present after 45 min (see Figure S7b). Analysis of the high-molecular-weight population by ESI-MS indicated that the product formed corresponded to the adduct **4** (Figure S7c and d).

Heterogeneous photo-induced ligation on cellulose After the successful evaluation of the reaction for homogeneous macromolecular conjugation, we implemented the new radical-trapping method for the functionalization of cellulosic substrates. For this purpose, we chose to immobilize carboxyl-modified Irgacure® 2959 **5** onto cellulose (refer to Figure 3). Classical DCC coupling was thus performed on NaOH-treated cellulose **Cel-OH** suspended in dichloromethane. After extensive rinsing with fresh dichloromethane (DCM), acetone, and toluene, the modified cellulose **Cel-PI** was analyzed by X-ray photoelectron spectroscopy (XPS) (Figure 3). Cellulose – being a fibrous material and thus having a non-homogeneous planar structure – can be challenging to analyze by XPS especially when functionalized with polystyrene. It is nevertheless possible to compare spectra obtained before and after modification to assess the success of the *grafting to* reaction. In the particular case of the present polystyrene grafting, we focused on the C 1s region of the spectra for a simple reason: all molecules to be grafted are mainly composed of carbon atoms and the C 1s peak of cellulose will evolve with the proportion of the different carbon-based bonds present on its surface following each modification step. The main contributions that can be detected on the non-modified cellulose **Cel-OH** are those involving oxygen-based bonds (289.6, 288.2, and 286.7 eV for O=C-O, O-C-O, and C-O-C/C-OH respectively).[38] Although the theoretical structure of cellulose does not comprise carbonyl moieties, the latter can be detected and certainly originate from oxidation that can readily occur on natural fibers such as cellulose.[38] The esterification of **Cel-OH** with **5** can be readily detected since the C-C/C-H contribution increases by a factor of close to 6 taking the main cellulose peak (286.7 eV) as a reference. Thus, the

cellulose substrate has been efficiently decorated with photoinitiating sites at its surface.

The macromolecular coating of cellulose was finally carried out by irradiation of **Cel-PI** placed in a solution of TEMPO-functionalized polystyrene **6** in toluene. In contrast to the macromolecular conjugation described above, no oxidizing agent was employed since PbO_2 is toxic and could give rise to purification problems due to adsorption onto the cellulose. Another less toxic oxidizing agent (MnO_2) was also evaluated during the polymer-small molecule conjugation studies but less satisfying results were obtained, particularly regarding the product stability (see Figure S5). As previously stated, a lower conjugation efficacy does not pose a significant issue: an excess of soluble species can be employed for heterogeneous surface modification since the non-reacted molecules or the possible by-products can readily be washed out. In the present case, the piece of cellulose was irradiated 10 minutes on each side under ambient conditions. The bottom graph of Figure 3 corresponds to the C 1s region of the XPS spectrum acquired on the sample **Cel-PS** after thorough rinsing with fresh DCM and acetone. The observed spectrum is substantially different from the spectra of **Cel-OH** and **Cel-PI**. Indeed, the main peak is now clearly associated with hydrocarbons (285.0 eV), revealing an efficient coverage of the surface with a carbon-rich material, *i.e.*, polystyrene. Furthermore, a new low-intensity signal appeared close to 291.8 eV and can unambiguously be assigned to a π-π^*-transition arising from the presence of an aromatic system.[39] Although **PI** also possesses an aromatic cycle and thus the latter is also present in **Cel-PI**, its concentration is too low to be detected. Control experiments were conducted where virgin cellulose is irradiated in presence of **6** or **Cel-PI** is suspended in a solution of **6** without irradiation. In both cases, the XPS spectrum of the cellulose samples, **Cel-OH** and **Cel-PI**, respectively, remained unchanged. A further proof of the macroscopic modification of the cellulosic substrate with polystyrene was obtained by water contact angle measurement. While it was impossible to measure any contact angle for **Cel-OH** or **Cel-PI** – the latter showed a slightly slower rate of water droplet absorption though – **Cel-PS** exhibited a contact angle of 86°.

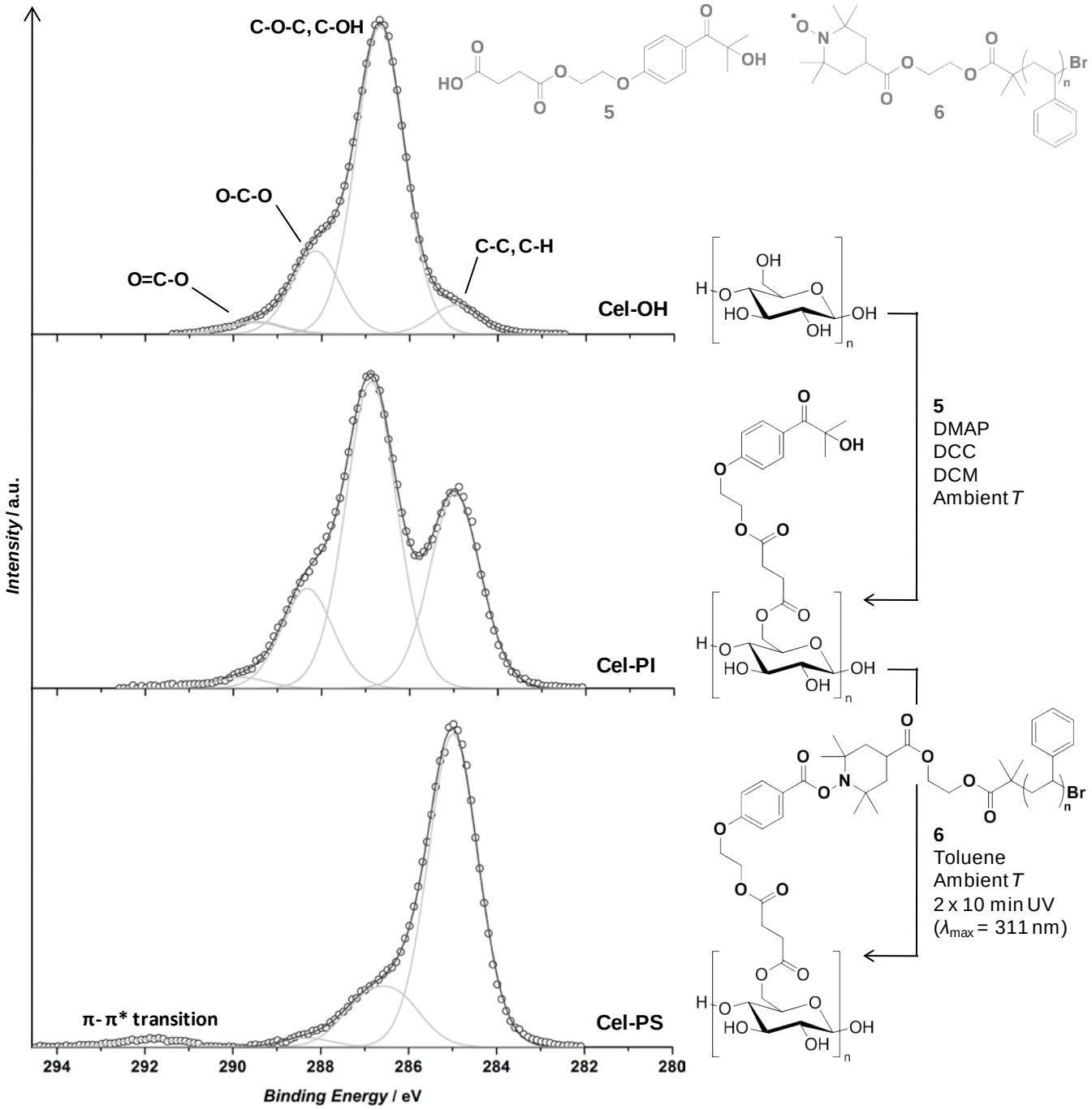


Figure 3. C 1s region of the XPS spectra of cellulose before modification (top, **Cel-OH**), after esterification with modified Irgacure 2959 photoinitiator **5** (middle, **Cel-PI**), and after UV-induced nitroxide radical trapping/grafting with TEMPO-functionalized PS **6** (bottom, **Cel-PS**). All spectra are normalized to maximum intensity.

In the present contribution, we have presented an original method for the modification of cellulose by UV-induced functionalization of photoinitiator-modified substrates with pre-formed nitroxide-functionalized macromolecules. Importantly, commercially available photoinitiator and nitroxide were

employed. The method was first evaluated with small molecules and modified poly(ethylene glycol)s in homogeneous conditions where all starting materials are soluble in the medium. It was found that – although the expected adducts are generated – lowered yields were obtained due to the formation of hydroxylamine species from the nitroxide-based molecules. The *in situ* utilization of an oxidizing agent such as PbO_2 proved to be very efficient for the re-formation of the nitroxide species leading to close-to-quantitative yields during polymer-polymer conjugation. In the subsequent key step a hydrophobic TEMPO-functionalized polystyrene was successfully grafted onto cellulose without the need for any additive as demonstrated by conclusive XPS data and contact angle measurements. The presented method can certainly be applied to the photopatterning of (macro)molecules onto a range of diverse substrates. Particularly protein patterning could potentially be achieved since the photoinitiator employed in the present study is biocompatible[40-42] and TEMPO-labeled proteins have been used earlier for protein structure investigations.[43-47]

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SUPPORTING INFORMATION AVAILABLE

Full experimental procedures, NMR spectra, and additional mass spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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