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Macromolecular imidazole–tenside conjugates with carbamate linkage

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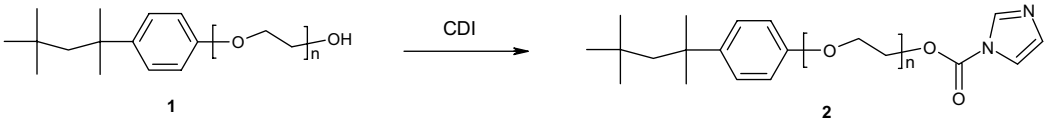
Abstract—Polyethylene glycol *tert*-octylphenyl ether and polyoxyethylenesorbitan trioleate are polydisperse macromolecular detergent molecules, containing a single hydroxyl function, which was transformed by 1,1-carbonyldiimidazole into imidazole–detergent conjugates with a carbamate linkage.

The two examined detergent molecules are of supramolecular size and widely used for integral membrane protein isolation.[1] Polyethylene glycol-*tert*-octylphenyl ether (Triton™ X-114) is linear containing an unpolar *tert*-octylphenyl part and a short PEG chain of various lengths (about 7.5 ethoxy groups in average) and carries a single hydroxyl function at the polar end. It forms miscellar structures and when heated above 22 °C, phase transfer occurs, which is observable as cloud point.

The second detergent molecule is polyoxyethylenesorbitan trioleate (Tween™ 85), equally polydisperse but branched containing in the core a sorbitan unit. The measured average molecular mass by Maldi-TOF MS is 1400 g/mol what is lower than expected and depends largely on the supplier. This structure carries equally a single hydroxyl function.

We functionalised both compounds to enlarge their application for bioseparation purposes in ongoing research. With carbonyldiimidazole (CDI) the hydroxyl functionality is reacted and an imidazole–detergent–carbamate is recovered.

To transform the hydroxyl function, the detergent was dissolved in dioxane at 37 °C and 1,1-carbonyldiimidazole[2] was added and a carbamate linkage between detergent and imidazole ring was formed (Scheme 1). An excess of CDI (30–60 equiv[3]) is employed to achieve a complete reaction in 2–8 h. To work with an excess of CDI appears not necessarily economic but an elevated reaction temperature may results in another product in which the sensible carbamate linkage is transformed. Different studies[4,5] show that heating at higher temperatures, carbamates connected by an imidazole ring are transformed completely to alkyl or aryl-imidazoles. The reaction is terminated by quenching of the CDI present in excess what generates a considerable amount of hydrolysed byproducts. The crude mixtures of **2** and **3** were therefore dialysed at 4 °C in 1 mM KH_2PO_4 solution. This purification procedure proves very efficient because the chosen dialysis membrane may contain larger holes than what one would choose in relation to the



1 2

Scheme 1.

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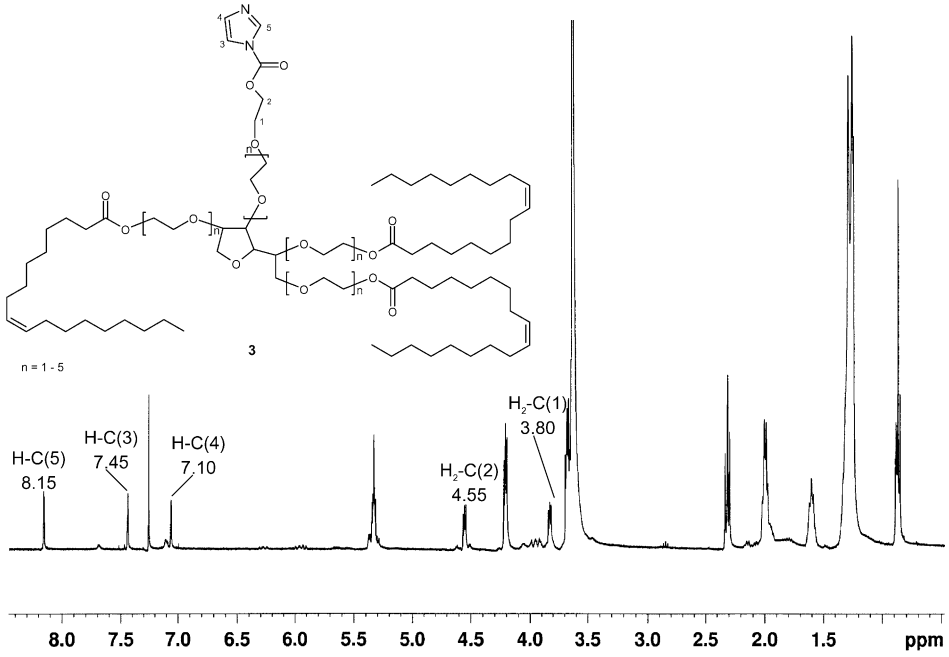
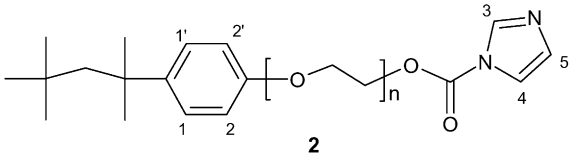


Figure 1. ^{1}H NMR (400 MHz, $CDCl_3$) of polyoxyethylenesorbitan trioleate imidazol carbamate **3**. The singlets H-3, H-4 and H-5 belong to the imidazol ring and the multiplets H-1 and H-2 belong to the ethoxy protons adjacent to the carbamate linkage. The other signals correspond to the substrate polyoxyethylenesorbitan trioleate.

size of **2** and **3**. In fact, a cut off size of 12,000–14,000 was applied, this is far beyond the size of a single conjugate but appropriate because triton X-114 and Tween 85 derivatives form micelles in aqueous media, whose diameter exceed the diameter of the membrane holes. The recorded ^{1}H NMR spectra show that pure carbamates **2** and **3** were generated (Fig. 1). The ^{1}H NMR measurement at 400 MHz of **3** shows at 8.15, 7.45 and 7.10 ppm the singlets of the imidazole ring. Beside these protons two new signals (multiplets) appear at 4.55 and 3.80 ppm, which belong to the ethoxy group adjacent to the carbamate **3**. This interpretation is enforced by an H–H-COSY NMR, which shows that there is only coupling between these two signals indicating an isolated group. All, but not labelled signals correspond to Tween 85 structure. The same signal pattern[6] is found for the ethoxy part of triton–imidazole conjugate **2**.

The conversion of the detergents to conjugates **2** and **3** was examined with ^{1}H NMR, and according to the spectra, all hydroxyl groups appear to be activated what can easily be seen in the spectra of triton–imidazole conjugate **2** (Fig. 2). The integrals of the imidazole ring protons are in perfect ratio of 1:2 with the doublets of *para*-benzene. A Maldi TOF MS spectra shows[7] that the triton derivative yields carbamate **2**.



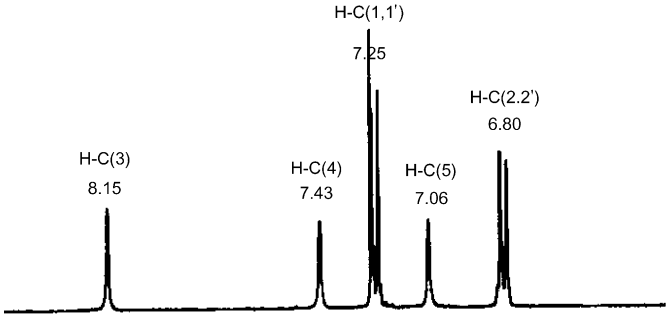
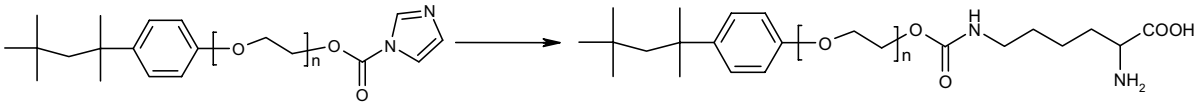


Figure 2. ^{1}H NMR (400 MHz, $CDCl_3$) shows that the conversion of the limiting reagent **1** is quantitative. The integrals of the singlets at 8.15, 7.43 and 7.06 ppm correspond to one proton and the integrals of the doublet at 7.25 and 6.80 ppm correspond to two protons.



Scheme 2.

Carbamates are reactive compounds and represent an activated alcohol. To demonstrate that **2** contains a reactive carbamate linkage the conjugation with L-lysine was examined (Scheme 2). For this purpose, a 1 M L-lysine solution was mixed with a 50% solution of **2** in a borate buffer and stirred for one day.[8] The purification was executed by dialysis as described before. A Maldi TOF MS reveals that the coupling yields bioconjugate **4**[9] fairly well.

Acknowledgements

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References and notes

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3. General procedure: 1 mmol detergent was dissolved in 20 ml dioxane at 37 °C and 1.62 g (100 mmol) of 1,1-carbonyldiimidazole was added and stirred for 3 h. KH_2PO_4 (10 ml, 0.1 M) was added to quench CDI in excess. The resulting mixture was dialysed (MWCO 12,000–14,000) at 4 °C in 1 mM KH_2PO_4 for 18 h changing the buffer three times and the remaining detergent–imidazol-carbamate, was lyophilised for 1 day yielding 80–90%.
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6. ^{1}H NMR (400 MHz, $CDCl_3$) of activated triton X-114 **2**: 8.15 (s, 1H), 7.43 (s, 1H), 7.25 (d, $J = 8.96$, 2H), 7.06 (s, 1H), 6.80 (d, $J = 8.77$, 2H), 4.55 (m, 2H), 4.09 (t, 2H), 3.72 (m, 4H), 3.66 (m, 22H), 1.68 (s, 2H), 1.33 (s, 6H), 0.70 (s, 9H).
7. MALDI TOF MS of activated triton X-114 **2** with applied biosystems voyager 2016. $M+H^+$: 476.7 (37), 520.7 (92), 564.7 (100), 608.8 (88), 652.8 (64), 696.9 (43), 740.9 (23), 785(14).
8. Two hundred and fifty microlitres (0.25 mmol) of a 1 M L-lysine solution was reacted with 15 μL (0.0115 mmol) 50% solution of activated triton **2** with 235 μL 0.1 M borate buffer (pH 8) was mixed and reacted at room temperature for one day yielding 40–50%.
9. MALDI TOF MS of lysine-triton X-114 **4** conjugate with applied biosystems voyager 2016. $M+H^+$: 466.6 (9), 510.7 (31), 554.7 (66), 598.8 (93), 730.9 (79), 775 (52), 819 (36), 863.1 (23), 907.1 (11).