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Photodimerisation of glycothymidines in solution and in micelles†‡

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Glycothymidines were designed and synthesized as a new class of functional glycomimetics in which a photochemical [2 + 2] cycloaddition of the thymine moiety induces structural changes of carbohydrate presentation. To test if photodimerisation of these glycothymidines is feasible within an array of molecules, the photochemical reaction was investigated using NMR and NMR diffusion experiments in solution as well as in the supramolecular context of detergent micelles that mimic cellular membranes.

Accessibility, density, and conformation of cell surface carbohydrates play essential roles in molecular recognition processes such as in cell/bacterial adhesion.[1] For their study, functional glycomimetics are of great interest as tools for examining structural details that influence interaction with carbohydrates. In this context, it has been our goal to design glycomimetics that feature a photosensitive functional group to control carbohydrate presentation on surfaces or in other model systems *via* irradiation.[2]

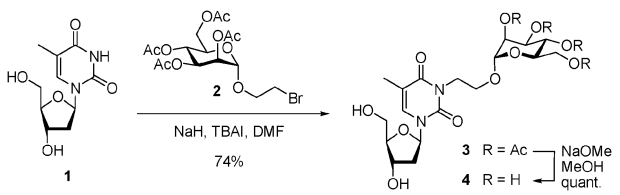
2′-Deoxythymidine was selected as a bio-compatible scaffold molecule for the design of photoswitchable glycothymidines. Like thymidine, glycothymidines should undergo a photochemical [2 + 2] cycloaddition reaction, thus leading to a conformational change within an assembly of glycothymidine molecules.[3]

The preparation of glycothymidines was easily achieved by a Williamson type synthesis with bromoalkyl glycosides such as **2** (Scheme 1).[4] It led to chemoselective *N*-alkylation of unprotected 2′-deoxythymidine. Deacetylation according to Zemplén[5] gave glycothymidine **4** in high yield.

The photodimerisation of glycothymidine **4** in solution was investigated, using acetone as triplet sensitizer. Upon irradiation with light of 295 nm wavelength **4** undergoes a [2 + 2] cycloaddition (Scheme 2), resulting in six different stereo- and regioisomers.[6] Conversion of the monomer into its dimeric cycloaddition product was clearly detected by the change of the respective UV/Vis spectra of the isomeric product mixture **5**. Whereas glycothymidine monomer **4** absorbs at 267 nm, the cyclobutane dimer derived from **4** shows maximal absorption at 240 nm. In the ^{1}H NMR spectra of **5**, seven triplets are resolved for the anomeric protons of the ribose rings (H-1′) in the range between 5.5 and 6.5 ppm, reflecting the formation of at least four of the six possible diastereomeric products (*cf.* ESI‡ and Scheme 2). The NMR spectrum of the monomer **4** shows a single triplet for H-1′ at 6.32 ppm. The dimeric products could be easily separated from the monomeric starting material by chromatography. The data demonstrate that this new class of functional glycomimetics readily undergo the desired photodimerisation reaction.

As this glycothymidine was designed to mimic structural changes within the glycocalyx, which may be utilized to affect density, availability, and presentation mode of surface-exposed carbohydrates, it was important to investigate whether photodimerisation occurs in a supramolecular context. Hence, the glycothymidine was furnished with a hydrophobic moiety, yielding an amphiphilic glycothymidine that associates with membranes or membrane mimics. To study the photochemical reactivity in a supramolecular context, micelle-forming detergents where utilized as a simplified ("minimal") model membrane, because these are amenable to NMR spectroscopy.

Synthesis of an amphiphilic glycothymidine started with nucleoside **1**, which was converted into its 5′-azidodeoxy derivative **6** employing an Appel reaction with sodium azide (Scheme 3).[7] Then, *N*-alkylation with the bromoethyl mannoside **2** led to the desired azido-functionalized glycothymidine **7**, which was *O*-acetylated and then converted into the fully protected isothiocyanate **9** in an aza-Wittig-type reaction. Usually, isothiocyanates can be converted with amines into thiourea derivatives in high yields. Here, **9** reacted with octylamine to furnish **10**. After de-*O*-acetylation, the unprotected, amphiphilic thiourea derivative **11** was isolated. Photodimerisation of **11** proceeded analogously to the



Scheme 1 Chemoselective *N*-alkylation of unprotected 2′-deoxythymidine **1** with bromoalkyl mannoside **2**.

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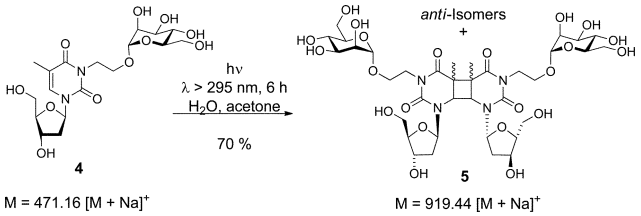
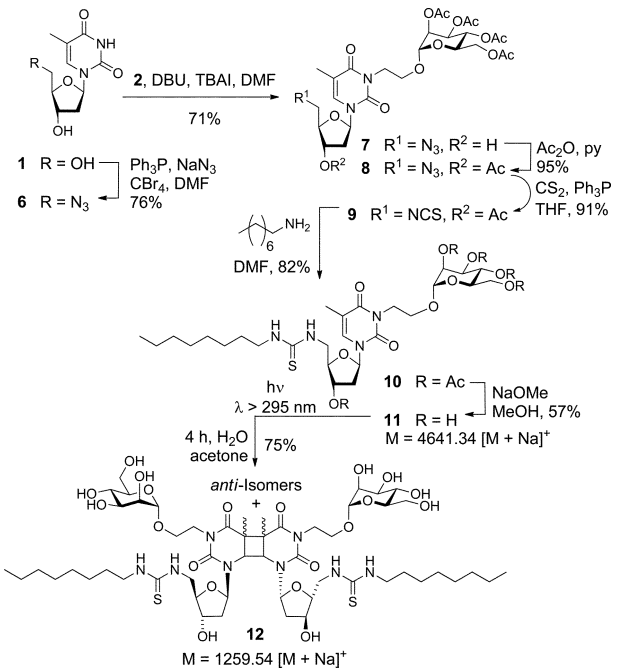


Scheme 2 Photodimerisation of glycothymidine **4**.



Scheme 3 Synthesis and photodimerisation of amphiphilic glycothymidine **11**.

photoaddition reaction of **4**, and led to the dimer **12** in comparable yield (Scheme 3).

The amphiphilic glycothymidine derivative was incorporated into well-defined SDS micelles,[8] directly irradiated in the NMR tube, and the samples characterized by NMR spectroscopy (Fig. 1).

In particular, a diffusion NMR experiment (DSTEBPLED)[9] was used to determine diffusion constants (*cf.* suppl. information‡). This experiment comprises a series of ^{1}H NMR spectra in which the signal intensity is attenuated by motion of

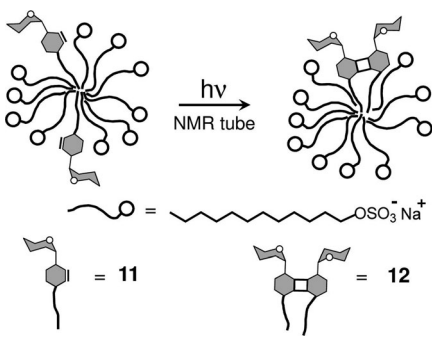


Fig. 1 Glycothymidine **11** could be incorporated into SDS micelles and photodimerised within the micellelar structure.

the molecules in a pulsed field gradient (PFG). The signal intensity attenuation in a PFG NMR experiment is described by the Stejskal–Tanner equation,[10] assuming a single component with a diffusion coefficient (D) is contributing to the signal according to eqn (1):

$$I = I_0 e^{-\gamma^2 G^2 \delta^2 (\Delta - \delta/3) D} \quad (1)$$

where γ is the gyromagnetic ratio, G the amplitude of the magnetic field gradient, δ denotes the effective duration of the gradient pulses and Δ is the effective diffusion time.

The peak areas of the series of ^{1}H spectra of a diffusion experiment were plotted against $\gamma^2 G^2 \delta^2 (\Delta - \delta/3)$, and fitted to a monoexponential decay to obtain the diffusion coefficient (D). In the case of more than one differently diffusing species contributing to the analyzed peak, a systematic deviation of the monoexponential decay described by eqn (1) is observed.

For determination of the diffusion coefficient, the methyl signals of the alkyl chains of the investigated compounds at 0.82 ppm (SDS) and 0.89 ppm (**11**) were used. At first, the diffusion of the substances **4**, **11**, and deuterated SDS were determined separately. These measurements expectedly show that the micelle forming SDS diffuses an order of magnitude slower than the glycothymidines **4** and **11** (Table 1). Also, the molecular diffusion of the alkylated glycothymidine **11** (14×10^{-11} m^2 s^{-1}) is about 2.5-times slower than that of the non-alkylated species **4** (38×10^{-11} m^2 s^{-1}). This large decrease in diffusion can not be explained based on the increase in the molecular size due to addition of the alkyl chain alone. Rather, this slower diffusion originates from a partial aggregation of the amphiphilic glycothymidine **11**. Another indication for the presence of aggregates are the strongly broadened signals in the spectra.

To investigate the incorporation of **11** into SDS micelles, the diffusion PFG NMR spectra of **11** were recorded at two different concentrations in SDS solutions (Table 1). The analysis shows a large decrease in the diffusion constants (5.7×10^{-11} m^2 s^{-1}; 5.3×10^{-11} m^2 s^{-1}). A comparison with the diffusion of the pure glycothymidine **11** (14×10^{-11} m^2 s^{-1}) and pure SDS (6.3×10^{-11} m^2 s^{-1}) shows that the glycothymidine **11** is incorporated quantitatively into the SDS micelles.

Subsequently, the micellar glycothymidine **11** was irradiated, and re-examined by NMR. The successful dimerization inside the micelles is evident by the appearance of multiple anomeric ribose protons (H′-1) of the dimeric thymidine in the expected area. The rate of dimerization inside the micelle is comparable to that in the absence of SDS (Fig. 2). The determined diffusion constants after dimerization are essentially unaltered (Table 1).

To further corroborate the incorporation of the glycothymidine into the SDS micelles as well as to demonstrate that the carbohydrate moiety remains accessible, relaxation broadening titrations with 12 mM Mn^{2+}-EDTA solution were performed. Addition of Mn^{2+}-EDTA, followed by ^{1}H–^{1}H gCOSY NMR experiments led to partial line broadening of cross peaks of the ethyl mannoside (M, L) and ribose (R) portions of glycothymidine **11**, while cross peaks of the octyl alkyl chain (A) remain unaffected (Fig. 3). Expectedly, similar

Table 1 Overview of diffusion coefficients determined by PFG NMR spectroscopy in D_2O

	D (m^2 s^{-1}) (fitting errors) before irradiation	D (m^2 s^{-1}) (fitting errors) after 230 min of irradiation at 295 nm
Pure SDS	6.3×10^{-11} ($\pm 1.9 \times 10^{-13}$)	—
Pure **4**	38×10^{-11} ($\pm 4.2 \times 10^{-12}$)	—
Pure **11**	14×10^{-11} ($\pm 6.7 \times 10^{-13}$)	9.6×10^{-11} ($\pm 2.1 \times 10^{-12}$)
11 in SDS solution[a]		
SDS	5.9×10^{-11} ($\pm 7.4 \times 10^{-14}$)	6.2×10^{-11} ($\pm 2.1 \times 10^{-13}$)
11	5.7×10^{-11} ($\pm 5.6 \times 10^{-14}$)	5.5×10^{-11} ($\pm 1.8 \times 10^{-13}$)
11 in SDS solution[b]		
SDS	5.7×10^{-11} ($\pm 2.7 \times 10^{-13}$)	5.7×10^{-11} ($\pm 2.2 \times 10^{-13}$)
11	5.3×10^{-11} ($\pm 5.9 \times 10^{-14}$)	5.3×10^{-11} ($\pm 4.2 \times 10^{-14}$)

[a] Concentration was chosen to give two molecules of **11** per SDS micelle at an average. [b] Concentration was chosen to give eight molecules of **11** per SDS micelle at an average.

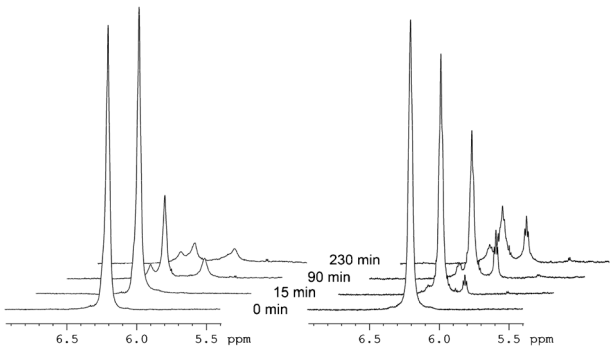


Fig. 2 Irradiation of glycothymidine **11** (pure) in D_2O-solution (left spectra) and with SDS micelles (right spectra), followed over time by 1D-^{1}H NMR spectroscopy at 25 °C.

results were obtained with the dimer in micellar solution (see suppl. information‡). This implies that both the monomeric and dimeric form of the investigated amphiphilic glycothymidine are embedded into the SDS micelle *via* the lipophilic alkyl chain, while the hydrophilic mannose is surface-exposed and accessible to the paramagnetic relaxation agent, and thus available for lectin binding.

In conclusion, glycothymidines are easily obtained from 2′-deoxythymidine and appropriate glycosides. They undergo a photosensitized [2 + 2] dimerisation in solution, and are thus in principle suited to test the influence of accessibility change and conformational alterations on carbohydrate recognition within a glycosylated surface. This is important in the context of cell adhesion and microbial adhesion to cell surfaces, *e.g.* lectin binding to the glycocalyx. To investigate if glycothymidines can be used in such a more complex supramolecular

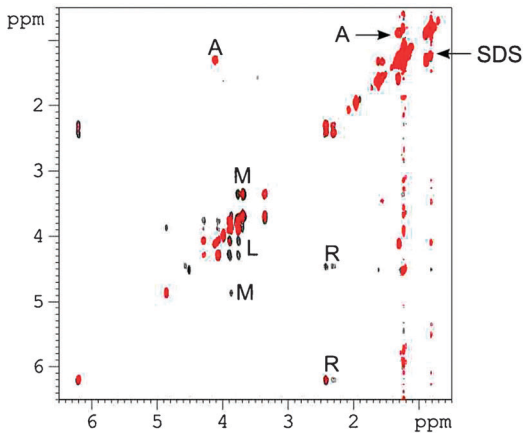


Fig. 3 Overlay of ^{1}H–^{1}H gCOSY experiments of glycothymidine **11** in SDS detergent micelles, in the absence (black contours), and in the presence of 2.6 mM Mn^{2+}-EDTA (red contours). Correlations are labeled as follows: R: deoxyribose, M: mannose, A: octyl chain, L: ethyl linker, SDS: cross peaks of the residual protons in the deuterated SDS.

context, they were incorporated into SDS micelles, which serve as a model system for the *in vivo* situation of the glycosylated cell surface. Using 1D- and diffusion NMR experiments it is demonstrated that glycothymidines undergo [2 + 2] cycloaddition when incorporated into micelles, thus leading to an altered pattern of surface-exposed carbohydrates. As a glyco-micelle might be regarded as a minimal glycocalyx model, this investigation represents the first step towards light switching of glycosylated surfaces, and elucidation of the role of conformational control in glycobiology.

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