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ARTICLE

Synthesis of the first POSS cage-anthracycline conjugates via amide bond

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Silsesquioxane derivatives widely used as polymer modifiers and catalytic supports have many interesting features making them potential nano-carriers in biomedicine. Two alternative synthetic routes were described, leading to conjugates of functional POSS structures: octa(3-chloroammoniumpropyl)silsesquioxane and octa(carboxydecylthioethyl)silsesquioxane with anticancer drugs – anthracycylines.

Doxorubicin (DOX) and daunorubicin (DAU) are important anthracycline antibiotics produced by Streptomyces peucetius. These antitumor drugs, of a broad spectrum of action, are widely used in therapy[1], although they can cause also serious cardiovascular side effects leading to heart failure.[2] Thus, currently a focus is being made on development of smart delivery methods that would limit exposure of normal cells to DOX and DAU. Synthesis of nanoparticles bearing DOX and DAU is one of the proposed solutions. To this end, two pathways, encapsulation and covalent conjugation were studied in literature. Doxorubicin, for example, was H-bonded to the surface of graphene oxide[3], encapsulated in poly(lactide-co-glycolide[4], poly(ethylene glycol-co-lactide)[5] and solid lipid nanoparticles[6]. Various approaches have been also used in order to covalently attach doxorubicin to carrier particles (synthesis of prodrugs).

Such conjugates allow for slow release of drug that can be better controlled, compared to encapsulated systems. The carriers applied e.g. for chemical bonding of doxorubicin included polymeric and dendrimer nanoparticles[7], fatty acids[8] as well as other nanoparticles[9]. Surprisingly, polyhedral oligomeric silsesquioxanes (POSS) have been missing from the above list of anthracycline functionalized nanocarriers, although octameric POSS are considered as a next generation material in biological fields[10]. Additionally POSS T_8 allows for combined delivery of various drugs or/and imaging and targeting agents. A number of other POSS-based bio-conjugates, including dendrimer systems[11], bearing bio-active carbohydrates, peptides, drugs and bio-markers[12] have been reported. Herein we present the synthesis of the first two, according to our knowledge, T_8-POSS-doxorubicin and T_8-POSS-daunorubicin conjugates. T_8-POSS cages can be easily functionalised[13] and their small size (about 1.5 nm for the substrates used by us) makes them unique carriers, compared to the ones studied till now in binding anthracyclines. As oligomers of a well-defined structure, POSS cages allow to eliminate problems that arise from a multi-step synthesis of dendrimers as well as polydispersity of linear and graft polymer systems[14]. They are also well known to facilitate cell membrane penetration[13], the important feature in drug delivery processes, while on decay they form only a harmless silicic acid - $Si(OH)_4$.[15]

In order to produce such a new class of anthracycline conjugates two anthracyclines – daunorubicin hydrochloride and doxorubicin were used as substrates. Two POSS nanocarriers were applied, a commercially available octa(3-chloroammoniumpropyl)silsesquioxane $T_8[(CH_2)_3NH_2{\cdot}HCl]_8$ (1) and octa(carboxydecylthioethyl)silsesquioxane $T_8[(CH_2)_2S(CH_2)_{10}COOH]_8$ (2) (Fig 1). The latter one was made by thiol-ene[16] addition of 11-mercaptoundecanoic acid to octa(vinyl) silsesquioxane. Octa(3-chloroammoniumpropyl)silsesquioxane was reacted with succinic anhydride modified daunorubicin (SAMDAU), prepared according to a general method[17,18] described earlier for the modification of doxorubicin, to give a mixture of $(SAMDAU)_x$-$T_8[(CH_2)_3NH_2]_{8-x}$ nanoconjugates (x = 2-4) (1a-c). On the other hand, modified POSS (2) was coupled with doxorubicin hydrochloride in the presence of trimethylamine, N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride (EDC), yielding a mixture of $(DOX)_xT_8[(CH_2)_2S(CH_2)_{10}COOH]_{8-x}$ (x = 1-2) (2a-b). The progress of coupling could be followed by ^1H and ^{15}N NMR as formation of amide bonds was accompanied by shift of CH-NH_2, and -NH protons, respectively, from 3.35 ppm to 3.95 ppm, and from 7.89 ppm to 7.59 ppm for daunorubicin conjugate. For doxorubicin conjugate the respective resonances shift from 3.35 ppm to 3.94 ppm and from 8.02 ppm to 7.44 ppm. More drastic changes were observed for nitrogen shift, e.g. for the doxorubicin composite it shifts from 42.6 ppm in the substrate to 123.5 ppm in the products (2 a-b). The two types of conjugates - $(SAMDAU)_n$-$T_8[(CH_2)_3NH_2HCl]_{8-n}$ and $(DOX)_n$-$T_8[(CH_2)_2S(CH_2)_{10}COOH]_{8-n}$ were

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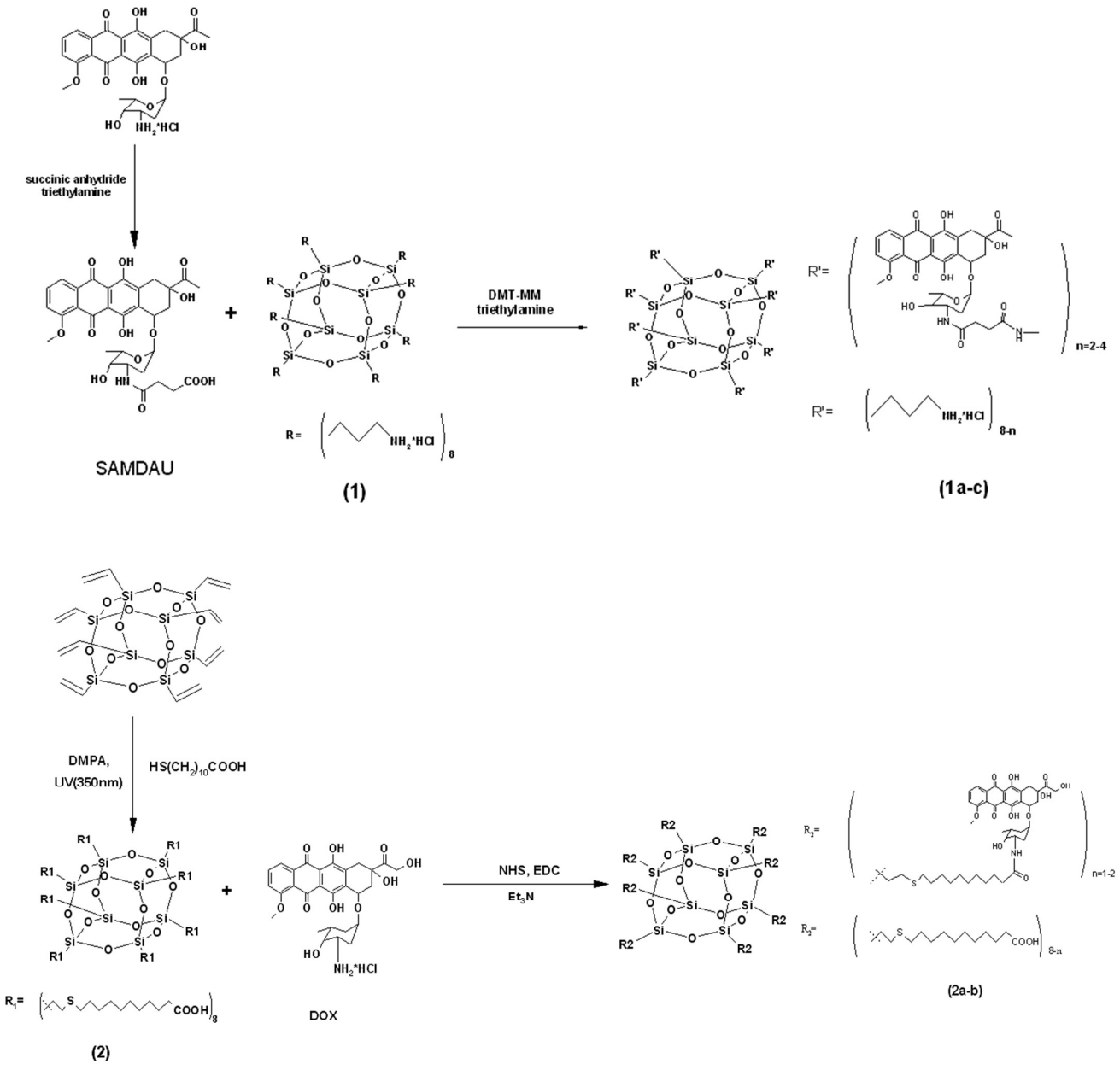
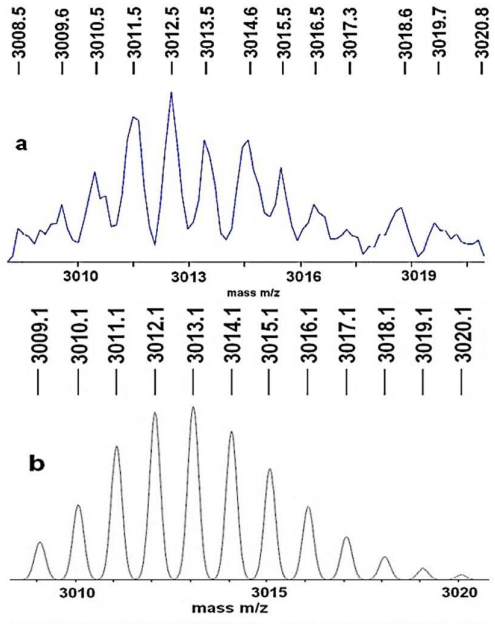
†Electronic Supplementary Information (ESI) available: synthetic and analytical data.

obtained, respectively with 40 and 70% yield, and their formation was confirmed by the relevant MALDI-TOF MS spectra (Fig. 2, Table 1)†.



Scheme 1. Synthetic routes for preparation of POSS – anthracycline conjugates.



Scheme 2. MALDI-TOF: (**a**) isotopic pattern of molecular ion [Dox-$T^8[(CH_2)_2S(CH_2)_{10}COOH]_7$ +Ag]$^+$ (2a); (**b**) simulated isotopic pattern

An interesting feature appears to be the multi-substitution of anthracyclines to POSS cages. Two to four molecules of SAMDAU in the ratio of 1 : 2.6 : 1 coupled with one POSS (based on the peak hight in the MALDI TOF spectra). In the case of DOX, single and double substitutions to POSS were observed in 3 : 1 ratio. Similar multi-coupling of ferrocene carboxylic acid to octa(3-chloroammoniumpropyl)silsesquioxane was found earlier by Chujo et al[19], however, they used over 4.5 molar excess of the acid per 1 mol of ammonium POSS. In our case the equimolar amounts of both reactants were used.

Table 1. MALDI TOF analysis of POSS anthracycline nanoconjugates

Reactants	MALDI TOF
POSS + SAMDAU	[POSS-$(SAMDAU)_2$ + K^+] calcd 2139.66, found 2141.20, [POSS-$(SAMDAU)_3$ + K^+] calcd 2749.24], found 2751.79, [POSS-$(SAMDAU)_4$ + K^+] calcd 3358.82 found 3362.23
POSS + DOX	[POSS-DOX + Ag^+] calcd 3013.1 found 3012.5, [POSS-$(DOX)_2$ + Ag^+] calcd 3538.59 found 3539.4

For POSS-$(SAMDAU)_n$ conjugates, the ions corresponding to the products of reaction between OH groups of daunorubicin and 4-(4,6-Dimethoxy[1,3,5]triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM)[20] were also observed that subsequently increased the values in Table 1 by 139 units (mass of the triazynyl fragment), e.g. in the simplest case, apart from 2141.20 [POSS-$(SAMDAU)_2$ + K^+] the ion at 2280.59 [POSS-$(SAMDAU)_2$ + K^+ + 139,11] was observed.

In conclusion, we have presented two alternative routes leading to POSS nano-composites bearing anticancer drug moieties. Such the systems, incorporating two anthracyclines – doxorubicin and daunorubicin, anchored via cleavable linkers, can assure a more efficient way of delivery of these drugs. Current research is directed on in vitro studies of the novel composites.

Experimental section

General remarks

All reagents were purchased from commercial sources and used as supplied, while sovents were purified as described in literature[21]. ^1H, ^{13}C and ^{29}Si nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III DRX-600 and 500 MHz spectrometer, using deuterated THF, DMSO as solvents. Matrix-assisted laser desorption/ionization-time of flight mass spectra (MALDI-TOF-MS) were run on Voyager-Elite instrument. Syntheses of succinic anhydride-modified daunorubicin (SAMDAU) and octa[10-(carboxydecylthio)ethyl]silsesquioxane (2) were made according to general methods[22,16] described in the literature.

Synthesis of $(SAMDAU)_n$-$T_8[(CH_2)_3NH_2HCl]_{8-n}$ nanoconjugates (1a-c).

Octa(3-chloroammoniumpropyl)silsesquioxane (0.048 g, $4\cdot10^{-5}$ mol), Et_3N (0.017 g, $1.22\cdot10^{-4}$ mol) were dissolved in methanol (25 ml) and stirred in the dark for 1hr at room temperature. Then, SAMDAU (0.025 g, $4\cdot10^{-5}$ mol) and DMT-MM (0.069 g, $2.5\cdot10^{-4}$ mol) were added to the reaction mixture and it was stirred for 1 hr at room temperature. Progress of the reaction was followed by TLC using CH_2Cl_2/MeOH (9:1, v/v) as a mobile phase. The reaction mixture was filtered, concentrated under reduced pressure and dialised in Biotech tubing (MWCO 0.5-1 kD, Spectrum Laboratories) in MeOH for 24 hrs. Then it was dried on vacuum, yielding 0.044 g of a mixture of nanocomposites (1a-c).

Synthesis of DOX_n-$T_8[(CH_2)_2S(CH_2)_{10}COOH]_{8-n}$ nanoconjugates (2a-b).

All the synthetic steps were carried out in the dark. $T_8[(CH_2)_2S(CH_2)_{10}COOH]_8$ (0.4 g, $1.68\cdot10^{-4}$ mol), NHS (0.021 g, $1.80\cdot10^{-4}$ mol) and EDC (0.035 g, $1.8\cdot10^{-4}$ mol) were placed in a flask, dissolved in 12 ml of DMF and stirred under nitrogen for 2 hrs. Then a solution of Dox·HCl (0.1 g,$1.72\cdot10^{-4}$ mol) and Et_3N (0.055 g, $5.38\cdot10^{-4}$ mol) in DMF (8 ml) was added and the reaction mixture was stirred for 40 hrs at room temperature. Progress of the reaction was followed by TLC using CH_2Cl_2/MeOH (9:1, v/v) as a mobile phase. The product was precipitated in CH_2Cl_2/H_2O (1:3 v/v) (75 ml), filtered and washed three times with CH_2Cl_2/H_2O (1:3 v/v) (20 ml). Volatiles were removed on vacuum, yielding 0.368 g of a mixture of 2a and 2b.

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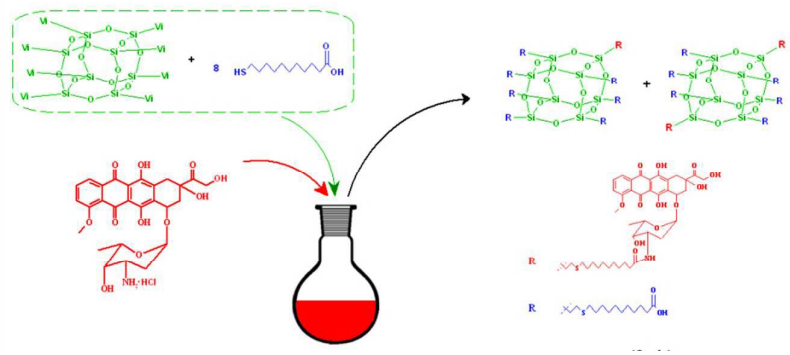
Graphic



TEXT

Synthetic routes leading to nano-cojugates of polyhedral silsesquioxane T_8 with doxorubicin and daunorubicin have been developed.