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Template effect of vancomycin aglycon in the oxidative oligomerization of 1,6-dithio-D-mannitol: A MALDI-TOF MS and solvent effect study

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Abstract

Oxidative oligomerization of 1,6-dithio-D-mannitol (DTM) using air as oxidant is reported. It was found that both ring and chain oligomers were formed with a degree of polymerization up to 11–12 as determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). It was found that the relative amounts of rings to chains were significantly dependent on the solvent employed: in water or in dimethyl formamide (DMF) yielded ring oligomers with a relative amount of higher than 90% (on the average), and almost exclusive formation of chain oligomers was observed in methanol or in dioxane. The oxidative oligomerization of DTM was also carried out in the presence of vancomycin aglycon as a template. It was observed that in water the vancomycin aglycon induced the exclusive formation of chain oligomers from DTM. The non-covalent, strong interaction existing between the vancomycin aglycon and the chain dimer of DTM was further supported by electrospray ionization mass spectrometry (ESI-MS).

Keywords: 1,6-Dithio-D-mannitol; Oxidative polycondensation; Ring–chain equilibrium; MALDI-TOF MS; ESI-MS

1. Introduction

Oxidation of thiols has been found to play an important role in many essential biochemical processes (Antholine, Kalyanaraman, Templin, Byrnes, & Petering, 1991; Antholine & Petering, 1979; Firouzabadi, Iranpoor, & Zolfigol, 1998; Iranpoor & Zeynizadeh, 1999; Pispisa, Paradossi, Palleschi, & Desideri, 1988) and its significance in industrial procedures has also been highlighted (Noorduyn, Have, & Pieters, 1955; Urban, 1956). Oxidative couplings of thiols by molecular oxygen in the presence of catalysts such as iron complexes (Field & Barbee, 1969; Field & Khim, 1972; Walters et al., 2006) or by oxidizing agents, including I_2, to yield disulfides (McComas, Crowley, Hwang, & Boger, 2003; Nicolaou, Boddy, Bräse, & Winssinger, 1999) are well-known synthetic methods and their modification in order to increase the conversion of thiols to disulfides has been considerably improving (Walters et al., 2006).

Surprisingly, formation and preparation of linear and cyclic poly(disulfides) containing sugar units have not been reported yet. Such poly(disulfides) are expected to have good water-solubility and due to the non-covalent interaction existing between the oligomers of different sizes and drugs, e.g., vancomycin aglycon, enhanced solubilization of the title drug may be potentially achieved.

Vancomycin is a glycopeptide antibiotic used as a last resort for treatment of bacterial infections caused by Gram-positive bacteria resistant to other antibiotics (Nicolaou et al., 1999). The threat of vancomycin-resistant strains urges the search for new, semisynthetic glycopeptide antibiotics. Recently, we prepared several new squaric acid

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derivatives from the vancomycin aglycon (McComas et al., 2003), and some of them possessed fairly good antibacterial activity (Sztaricskai et al., 2006). One of the problems with such derivatives lacking sugar moieties is their poor water-solubility.

In this paper, we report the formation of linear and cyclic oligomers of 1,6-dithio-D-mannitol (DTM) by oxidative polycondensation using air as oxidant in the presence and absence of vancomycin aglycon.

2. Experimental

2.1. Chemicals

With the exception of 1,6-dideoxy-1,6-dibromo-D-mannitol (Szabo, Institoris, Dalmi, & Kaczmarek, 1987) all chemicals used were received from Aldrich (Seelze, Germany).

2.2. Preparation of the monomer

2.2.1. Preparation of 1,6-bis-triphenylmethyl-1,6-dithio-D-mannitol

NaH (50% in oil) (701 mg, 14.6 mmol) was washed with hexane (100 mL) and dried under argon stream followed by suspension in dioxane (100 mL). Triphenylmethanethiol (3.37 g, 12.2 mmol) was added to the suspension and stirred for 10 min followed by addition of 1,6-dideoxy-1,6-dibromo-D-mannitol (1.5 g, 4.9 mmol) and stirring was continued for additional 2 h. After completion of the reaction, methanol (2 mL) was added to the reaction mixture and stirred for 30 min to quench the unreacted NaH. The mixture was evaporated in vacuum and the residue was purified by silica gel chromatography (hexane/acetone 75/25 v/v) to remove the unreacted triphenylmethanethiol. (Yield: 3.4 g, 100%).

2.2.2. Preparation of 1,6-dithio-D-mannitol

To a solution of 1,6-bis-triphenylmethyl-1,6-dithio-D-mannitol (1.0 g, 1.43 mmol) in dichloromethane (15 mL), trifluoroacetic acid (0.5 mL) and then triethylsilane (0.25 mL, 0.183 g, 1.57 mmol) were added. After 1 h stirring additional 0.25 mL of triethylsilane was added to the mixture. The reaction mixture was stirred for 1 h at ambient temperature and evaporated in vacuum. The excess of the reactants was removed by coevaporation with toluene. The residue was washed with ether and dried at ambient temperature. (Yield: 0.27 g, 88%).

2.3. Oligomerization reaction

After dissolution of 1–5 mg 1,6-dithio-D-mannitol in solvents H_2O, or dimethyl formamide, or methanol (1 mL), triethylamine (10 μL) was added to the solution and stirred vigorously for a predetermined interval and the products were analyzed by MALDI-TOF MS.

2.4. Instrumentation

2.4.1. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS)

The MALDI-MS measurements were performed with a Bruker BIFLEX III™ mass spectrometer equipped with a time-of-flight (TOF) analyzer (Bruker Daltonik, Bremen, Germany). In all cases 19 kV acceleration voltage was used with pulsed ion extraction (PIE™). The positive ions were detected in the reflection mode (20 kV). A nitrogen laser (337 nm, 3 ns pulse width, 10^6–10^7 W/cm^2) operating at 4 Hz was applied to produce laser desorption. The *m/z* values were determined with an accuracy of ±0.2 Th. The samples were prepared with a 2,5-dihydroxybenzoic acid (DHB) matrix (20 mg/mL) in dimethyl formamide. The analyte solutions were added to the matrix solution from the reaction mixture, or the solution was evaporated in vacuo and dissolved in dimethyl formamide at a concentration of 5 mg/mL and then added to the matrix solution. The samples were cationized with sodium trifluoroacetate dissolved in methanol (5 mg/mL). The matrix, the analyte solution and the NaTFA solution were mixed in a 50:10:5 v/v ratio. A volume of 0.5 μL of these solutions was deposited onto the sample plate (stainless steel), and allowed to air-dry.

2.5. Electrospray quadrupole time-of-flight MS/MS (ESI-QqTOF)

MS and MS/MS measurements were performed with a MicroTOF-Q type QqTOFMS instrument equipped with an ESI source from Bruker (Bruker Daltoniks, Bremen, Germany). The sample solutions were introduced directly into the ESI source with a syringe pump (Cole-Parmer Ins. Co., Vernon Hills, IL, USA) at a flow rate of 2 μL/min. The temperature of the drying gas (N_2) was maintained at 100 °C. The needle voltage was 4 kV. For MS/MS experiments, nitrogen gas was used as the collision gas and the collision energy of 20 eV was applied. The pressure in the collision cell was determined to be 9×10^{-3} mbar. The precursor ions for the MS/MS experiments were selected with an isolation width of 2 Th. All the MS and MS/MS spectra were accumulated and recorded by a digitizer at a sampling rate of 2 GHz. Each spectrum was calibrated externally with the salt clusters produced from the electrosprayed solution of sodium trifluoroacetate. The accuracy of mass determination was within ±8 ppm. The mass spectra recorded were evaluated by the DataAnalysis 3.1 software from Bruker.

3. Results and discussion

3.1. MALDI-TOF MS investigations

The oxidative polycondensation of 1,6-dithio-D-mannitol in the presence of triethylamine yielded linear (chain) and cyclic (ring) oligomers. The MALDI-TOF mass

spectrum of the reaction mixture obtained by oxidative polycondensation of 1,6-dithio-D-mannitol in dimethyl formamide (DMF) is shown in Fig. 1 and the formation of chain and ring oligomers is depicted in Scheme 1.

Fig. 1 shows that a series of chain and ring oligomers was formed that appear with decreasing intensities in the MALDI-TOF mass spectrum. The masses of the chain and ring oligomers appeared in the MALDI-TOF mass spectra can be described by Eqs. (1) and (2), respectively.

$$M_C(n) = 212n + 2 + 23 \quad (1)$$

$$M_R(n) = 212n + 23 \quad (2)$$

where $M_C(n)$ and $M_R(n)$ are the masses of the chain and the ring oligomers with number of repeating units n, respectively. 212, 2 and 23, respectively, are the masses of the repeating unit, the endgroups (2H) and the sodium ion attached to the oligomers.

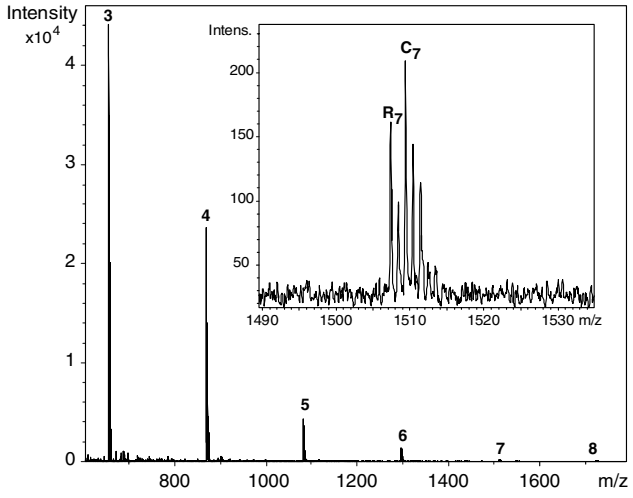


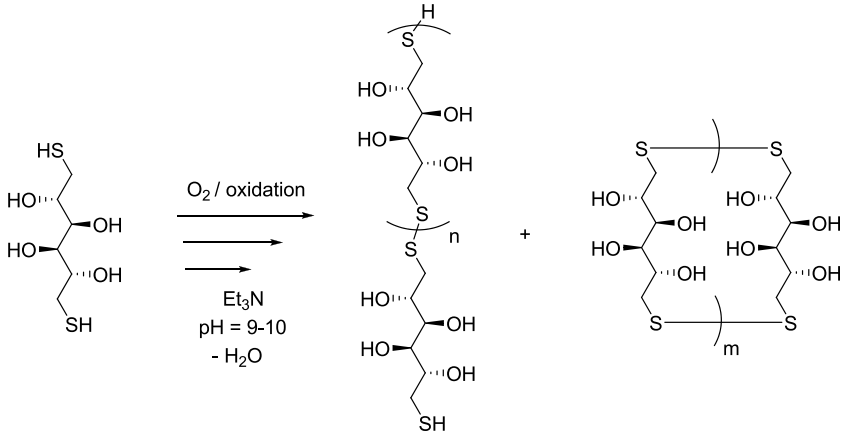
Fig. 1. MALDI-TOF mass spectrum of the reaction mixture obtained by the oxidative polycondensation of 1,6-dithio-D-mannitol. The numbers at the top of the peaks represent the number of repeating units. Inset shows the zoomed MALDI-TOF mass spectrum presented in Fig. 1 in the range of m/z 1490–1540. R_7 and C_7 denote the ring and chain oligomers, respectively, with $n = 7$. Experimental conditions: initial concentration: 5 mg/mL, solvent: *N*,*N*-dimethylformamide (DMF), reaction time: 7 days.

As an example, in Fig. 1 inset the presence of both chain and ring oligomers with $n = 7$ can be clearly observed. On the other hand, since all of the chain and ring oligomers are well resolved, MALDI-TOF MS offers a unique possibility for the determination of the ratio of the ring to chain oligomers by using the corresponding MALDI-TOF MS intensities (in the case of equal ionization probabilities). However, it should be taken into account that mainly due to isotopic contribution of the M+2 peak of sulfur atoms present in the oligomers, the intensity of the monoisotopic peaks of chain oligomers should be corrected. Therefore, the corrected intensity for the chain oligomers can be expressed by Eq. (3)

$$I_{C,n} = I^*_{C,n} - vI_{R,n} \quad (3)$$

where $I_{C,n}{}^*$ and $I_{R,n}$ are the measured intensity for the chain and ring oligomer, respectively, with n repeating units. v is the relative intensity of the M+2 peak of ring R_n to its monoisotopic peak.

For example, in Fig. 1 inset ring oligomers with $n = 7$ appear at m/z 1507 with an intensity of 150, while chain oligomers are present at m/z 1509 with an intensity of 260. However, the intensity of 260 is the sum of intensities originating from the monoisotopic peak of C_7 and the M+2 peak of R_7. Using the elemental composition of R_7 ($C_{42}H_{84}O_{28}S_{14}Na$) one can calculate that the value of v is 0.86 in this case, i.e., the expected intensity due to the presence of the M+2 peak of R_7 is ca. 130, so the real intensity ratio of ring to chain oligomers is ca. 150/130 instead of 150/260. In the case of equal ionization probabilities the intensity ratio of ring to chain will reflect to their relative amount in the sample. Table 1 summarizes the ratio of ring to chain oligomers consisting of three repeating units ($n = 3$), i.e., the corrected relative intensities $I_{R,n3}/I_{C,3}$ obtained from the products of polycondensation reactions performed in different solvents and at different reaction times.



Scheme 1. Schematic representation of the formation of chain and ring oligomers by the oxidative polycondensation of 1,6-dithio-D-mannitol.

Table 1
The dependence of the ring to chain ratio for the oligomers composed of $n = 3$ repeating units (based on the corresponding MALDI-TOF MS intensities) as a function of the solvents and the reaction time

Solvent	Time (day)	Ring/chain
DMF	1	0.5
DMF	7	8.2
DMF	42	10.1
MeOH	1	0.1
MeOH	7	0.2
H_2O	1	3.8
H_2O	7	5.3
Pyridine	7	7.3
Dioxane	7	0.3

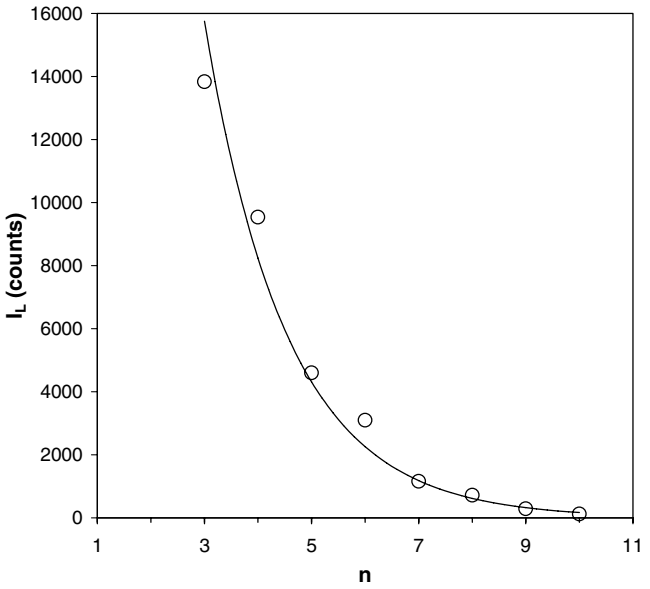


Fig. 2. MALDI-TOF MS intensities as a function of n for the reaction mixture obtained by the oxidative polycondensation of 1,6-dithio-D-mannitol in methanol. The solid line represents the fitted intensities by Eq. (4). Experimental conditions: initial concentration: 5 mg/mL, reaction time: 7 days.

As it turns out from the data of Table 1, the ratio of ring to chain is increased with elongation of the reaction time. On the other hand, the solvent also greatly affects the resulting ring to chain ratio. In solvents such as DMF or water ring oligomers dominates over the linear ones, while in methanol or in dioxane linear oligomers were formed overwhelmingly.

For the description of the linear series formed in methanol "the most probable distribution" (Flory, 1953, chap. 8) was applied using Eq. (4).

$$I_{Cx,\mathrm{calc}} = A_C p^n \tag{4}$$

where $I_{Cx,\mathrm{calc}}$ is the intensity of the oligomer peaks appeared in the MALDI-TOF mass spectrum, p is the extent of the polycondensation, x is the number of the repeating units and A_C is the normalization factor.

To the measured intensities Eq. (4) was fitted. Fig. 2 shows the plots of MALDI-TOF MS intensity versus number of repeating units together with the fitted curves calculated by Eq. (4).

Fig. 2 shows that the peak MALDI-TOF MS intensities obey "the most probable distribution" as it is typical for linear polycondensation reactions.

It was found that the intensity ratio of the ring to chain oligomers is significantly dependent on the number of the repeating units. In Fig. 3 the MALDI-TOF MS intensity ratios of the ring (I_R) and the chain (I_C) are shown, i.e., I_R/I_C are plotted as a function of n for the oligomer mixtures formed in DMF and in water at the same initial concentrations.

Fig. 3 shows that there is a significant decrease in the value of I_R/I_C as the number of the repeating units increases. The observed decrease of I_R/I_C values with increasing n is in line with the Jacobson–Stockmayer theory developed for the description of polycondensation reactions leading to the formation of ring and chain oligomers (Jacobson & Stockmayer, 1950). According to this theory (Jacobson & Stockmayer, 1950), under equilibrium conditions the mole fraction (N_R) of rings composed of n repeating units is proportional to n as given by Eq. (5)

$$N_R \propto p^n n^{-\alpha} \tag{5}$$

where p is the extent of reaction and α is the scaling factor.

The theoretical value of n for long, flexible polymers is 2.5. By combining Eqs. (4) and (5), the I_R/I_C values can be expressed by Eq. (6) in the case of equal ionization probabilities for all oligomers.

$$I_R/I_C = A_{R/L} n^{-\alpha} \tag{6}$$

where $A_{R/L}$ is the proportionality constant.

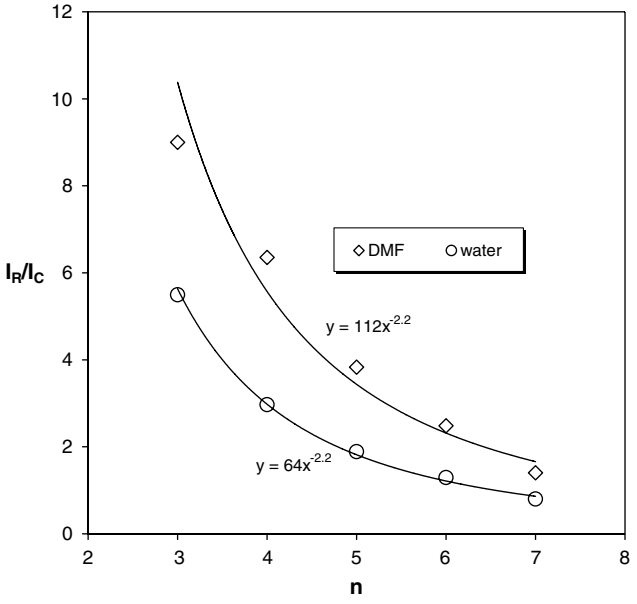


Fig. 3. I_R/I_C versus n plots for the reaction mixture obtained by the oxidative polycondensation of 1,6-dithio-D-mannitol in water and DMF at initial concentration of 5 mg/mL. The solid line represents the fitted I_R/I_C values by Eq. (6). Reaction time: 7 days.

Eq. (6) was fitted to the experimental I_R/I_C values as it is indicated by the solid line in Fig. 3. As it can be seen in Fig. 3 for the value of α 2.2 were obtained, which is very close to the theoretical value (2.5) predicted by the Jacobson–Stockmayer theory. On the other hand, it can also be concluded from the results presented in Fig. 3 that oligomerization conducted in DMF yields more cyclics than oligomerization performed in water (higher I_R/I_C ratio) under the same experimental conditions.

3.2. ESI-QqTOF MS/MS experiments

The structure of the ring oligomers was studied by ESI-QqTOF tandem mass spectrometry. In these experiments to the oligomerization mixture which was obtained in DMF (in this case mostly ring oligomers were formed) LiCl in methanol was added to facilitate the formation of lithiated adduct ions ($[M+Li]^+$). The $[M+Li]^+$ ions of the ring oligomers ($n = 3$–7) were selected in the Q part, then collisionally activated in the Q part of the instrument to get product ions, i.e., MS/MS spectra. It was found that the ring oligomers with $n = 3$–4 dissociate by elimination R_1 and R_2 cyclics, while oligomers consisting of $n > 4$ repeat units fragmented by the loss of R_2 cyclic. These results may indicate the preferential stability of the neutral R_2 ring oligomer over the ring oligomer with $n > 2$.

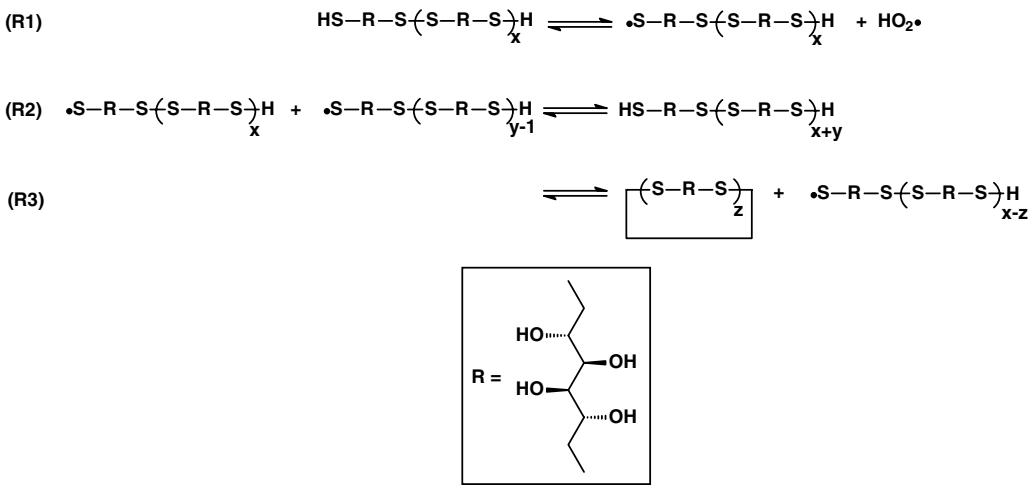
3.3. Mechanistic considerations and oligomerization in the presence of the vancomycin aglycon

As we have seen earlier the fraction of the ring to chain oligomers is greatly dependent on the solvent and the reaction time. In DMF or in water mostly macrocyclic oligomers were obtained after 7 days reaction time, while in methanol practically chain oligomers were obtained. For the formation of macrocyclic and chain oligomers a mechanism depicted in Scheme 2 is proposed.

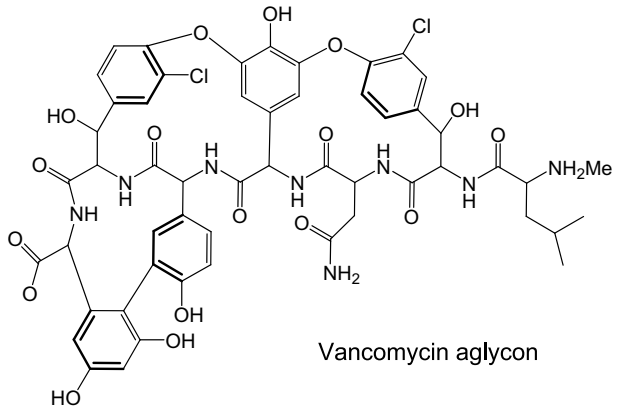
According to Scheme 2, in **R1** the thiol endgroup can react with O_2 to afford the thiolate and an HO_2 radical. In **R2** the chain oligomers can recombinate with thiolate radicals formed by process **R1** to produce growing chain oligomers. However, the thiolate radical ends can self-bite onto the backbone giving rise to the formation cyclics and chain oligomers as outlined in process **R3** in Scheme 2. The role of backbiting reaction in the production of macrocyclics was demonstrated by conducting the oligomerization in the presence of the vancomycin aglycon (Scheme 3) in water. It can be expected that the OH side groups of the oligomer can interact with the NH groups of the vancomycin aglycon producing vanconmycin + oligomer molecular complexes.

Indeed, performing the oligomerization of 1,6-dithio-D-mannitol in water in the presence of the vancomycin aglycon furnished linear chain oligomers almost exclusively in contrast to the case when oligomerization was conducted in the absence of the vancomycin aglycon. (Note that in the absence of vancomycin the oligomerization yields cyclic oligomers overwhelmingly). The presence of the vancomycin aglycon–oligomer molecular complexes in the oligomerization mixture was further supported by electrospray ionization mass spectrometry (ESI-MS). The partial ESI-MS spectrum from the solution consisting of the vancomycin aglycon and the oligomerization mixture is shown in Fig. 4.

As it turns out from Fig. 4 protonated and sodiated peaks of the vancomycin aglycon (vma) + C_2 molecular complex, i.e. $[vma+C_2+H]^+$ and $[vma+C_2+Na]^+$ appear at *m/z* 1569.3454 and 1591.3279, respectively. The measured *m/z* values agree well with those calculated on the basis of the elemental composition of $[vma+C_2+H]^+$ ($C_{65}H_{79}Cl_2N_8O_{25}S_4$) and $[vma+C_2+Na]^+$ ($C_{65}H_{78}Cl_2N_8O_{25}S_4Na$), i.e., 1569.3411 and 1591.3230, respectively. These results clearly indicate the relatively strong interaction between the vancomycin aglycon and the oligomers



Scheme 2. Proposed mechanism for the oxidative polycondensation of 1,6-dithio-D-mannitol.



Scheme 3. Representation of the structure of the vancomycin aglycon.

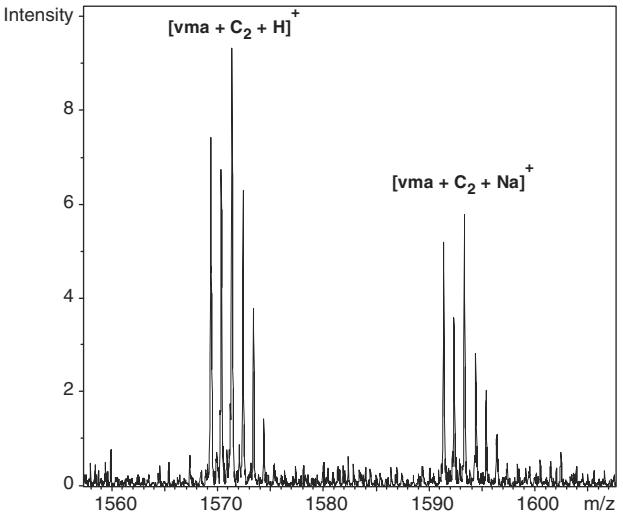


Fig. 4. Partial ESI-MS spectrum of the solution containing the vancomycin aglycon (0.01 mg/mL) and chain oligomers (0.01 mg/mL) in methanol. Vma represents the vancomycin aglycon and C_2 stands for the linear dimer of 1,6-dithio-D-mannitol.

of 1,6-dithio-D-mannitol. It can be expected that further polymerization proceeded starting from those adducts. In this way, the peptide type aglycon sterically hinders the back-biting reactions, i.e., resulting in the formation of linear oligomers.

4. Conclusions

The oligomerization of 1,6-dithio-D-mannitol was achieved by oxidative polycondensation using air and the reaction products were studied by MALDI-TOF MS. It was shown that the resulting ring to chain oligomer ratio could be effectively varied by performing the oligomerization in different solvents: in DMF or water ring oligomers were obtained exclusively, while the products of the oligomerization reaction conducted in methanol were chain oligomers dominantly. It was also shown that the chain-length dependence of the ring to chain ratio obeyed the Jacobson–Stockmayer theory. Based on the experimental results a mechanism was proposed for the oxidative polycondensation reaction of 1,6-dithio-D-mannitol. It was further supported that in the presence of the vancomycin aglycon formation of cyclics was considerably suppressed due to the relatively strong interaction between the aglycon and the linear oligomers. From these observations it can be concluded that in oligomerization reactions leading to the formation of cyclics, the resulting ring to chain fraction can be controlled by applying a suitable template. In addition, encapsulation of title drug by cyclic oligomers of different sizes may potentially enhance the drug solubilization.

Acknowledgments

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