Synthesis of leinamicin antibiotic analogs

Ph.D. Theses

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1. History and aims

The conscious healing among the ancient day's people (Chinese, Indian and Egyptian) was started with applying elementary instruments, methods and agents. Alchemy and jatrochemistry promoted the evolution of healing in the middle ages.

Pharmacy research and the pharmaceutical industry became stronger and intensified at the turning point of the 19th and the 20th century. After discovering the first antibiotic penicillin, by FLEMING in 1929, the antibiotics spread quickly all around the world in the 20th century. It was facilitated (and gave a higher value and intensive orientation of research on them) by the fact that sometimes it is difficult or impossible to replace them with another pharmaceuticals.

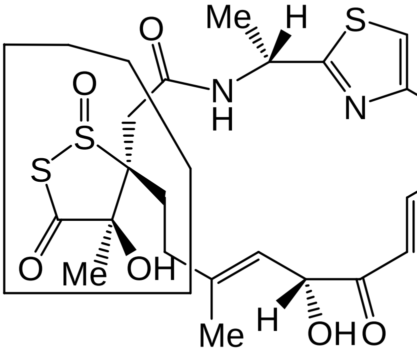
Nowadays the preparation and modification of a semi-synthetic or a full synthetic analog of an antibiotic become separated studies which are standing in a close relation with microbiology and biochemistry. Research of antibiotics gave a large measure to approve the biotechnology processes and modernize the fermentation industry. For today more than 10.000 antibiotics have been isolated and about 100 of them attained clinical adaptation. The number of the chemically or biologically modified derivatives is close to 100.000. The antibiotics dominate 25-30% of the world's pharmacy trade.

However, the adaptation of them resulted in the development of resistance. This suggests a leisurely usage of these pharmaceuticals. Even more and more new derivatives are prepared but the fight against the resistance becomes a general problem.

Choosing the antibiotic leinamycin (see in **Scheme 1**) to the subject of our research is expalined by different factors as follows:

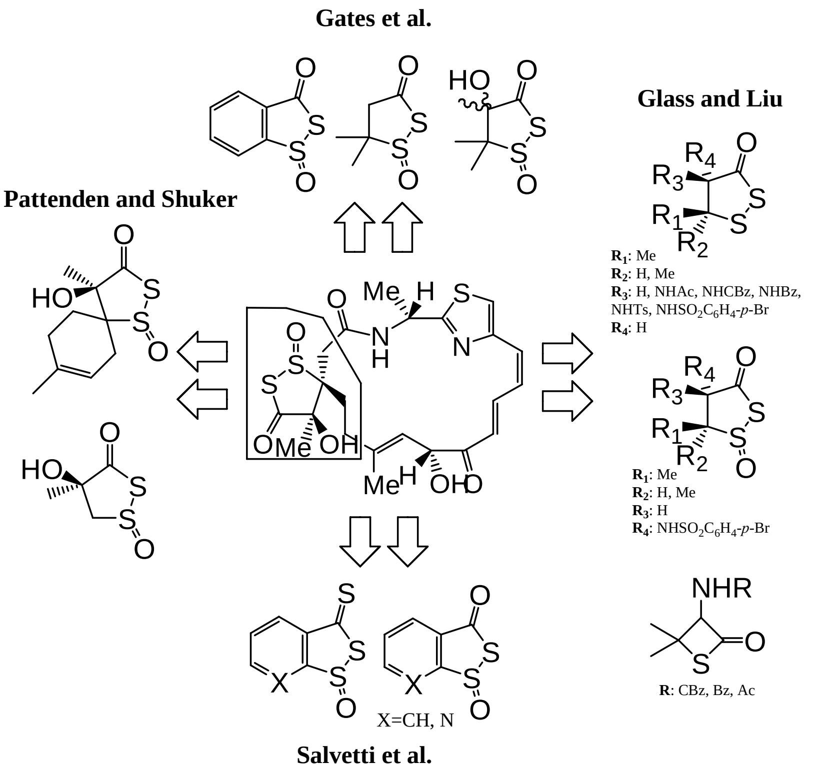
- The heteroring that connects to the macrolide moiety is unusual among the antibiotics (of course another sulfur-containing antibacterial compounds already exist). The synthesis of this ring will be valuable per se. Our first aim was to elaborate a new, short synthetic method to prepare the heteroring.

- Only a small structural portion of the macrocyclic antibiotic is responsible for the activity and this facilitates the synthesis of the analogs. During our work we aspired to make simple analogs and parallel with this keep the biologically important moieties.



Scheme 1

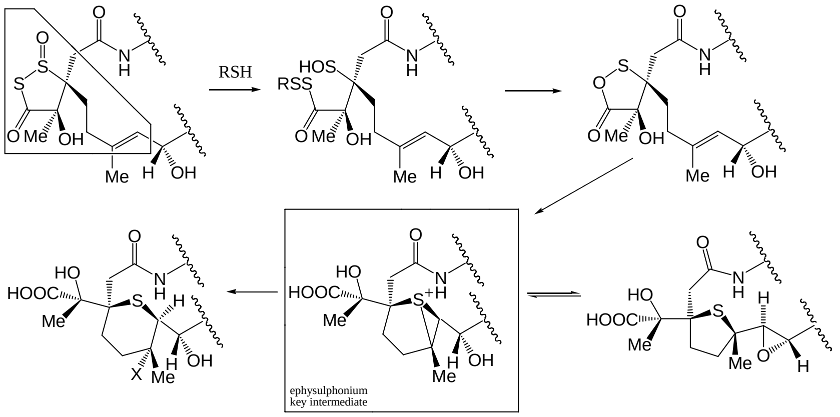
- Through the late discovery of leinamycin (in 1989) the different bacterial strains will be probably non- or barely resistant towards it. We think this will be verified among the derivatives, too. Another important thing that this – DNA-cleaver – antibiotic has only a few derivatives and analogs (see in **Scheme 2**) and its cytotoxicity was investigated only in a single case in the past. During our work we intended to achieve the formation of the biocative molecular elements on simple carriers with a new synthetic method. Together with this we wanted to analyse the cytotoxic activity–chemical structure relationship of the analogs.



Scheme 2

<u>The mechanism of the DNA-cleavage of leinamycin:</u>

In the presence of a thiol, the dithiolan ring opens and via an oxathiolan ring structure an episulphonium key intermediate is formed (**Scheme 3**) and this structure is responsible for the DNA-cleaving effect.



Scheme 3

The episulphonium intermediate is able to cleave the carbon-nitrogen bond and endows the DNA with a radical nature. This radical quickly eliminates from the living organism by rapid oxidation.

2. Applied methods

Chemical: The micro-, semimicro and macro methods of modern organic preparative chemistry were applied during our synthetic work. The reactions were monitored and the products pre-identified by thin layer chromatography and UV-detection. The isolation and purification of the crude products were carried out by crystallization or by column chromatography. Elemental analyses, melting point determination, ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry were applied to identify and characterize the prepared compounds.

Biological (cytotoxicity-test):

Materials: *HeLa* cell line human (*HeLa* cells are derived from a cervical carcinoma and showed epithelial cell morphology).

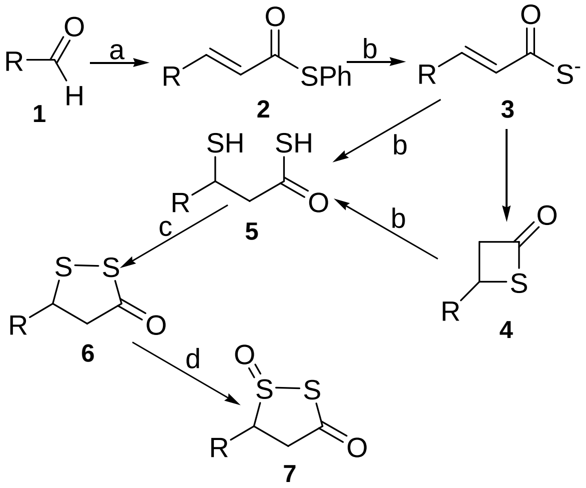
3. New results

In our work we incorporated the bioactive part of the antitumor antibiotic leinamycin into different simple carrier molecules and studied their cytotoxicity.

First we elaborated a new synthetic method to prepare the potentially bioactive 1,3-dioxo-1,2-dithiolan-3-on-*S*-oxide moiety of leinamycin. We used this method to form the dithiolan and dithiolan-*S*-oxide ring on different aromatic, aliphatic, carbohydrate and nucleoside carriers. We studied the cytotoxic activity of both of the dithiolanons and the *S*-oxides, because we assumed that the non-oxidized ring is potentially active, too. We wanted to attest that the oxidized form is more reactive. Our most important results concerning the synthesized new-type of analogs and their cytotoxicity are as follows.

3.1. The synthesis of the 1,2-dithiolan-3-on-*S*-oxide ring

With the method shown in **Scheme 4** the α,β-unsaturated *trans* (*E*) tiophenyl esters were prepared from various aldehydes (**1**) using phenylthiocarbonylmethylenetriphenylphosphorane in a Wittig reaction. The advantage of this reaction is the near 100% stereoselectivity for the *trans* derivative (**2**) with a good choice of the reagent. On the other hand, the Wittig derivative contains the –CH=CH– and the C=O parts of the heteroring. In the presence of a thiol (SH^{-}) we synthetized the β-mercapto thiolic acid (**5**) from the esters in a conjugate addition and a nucleophilic addition step.



(a) $Ph_3PCHCOSPh$ (1,2 – 1,4 equivalent), room temperature, 16-48 h; (b) Et_3N (2,1 equivalent), $H_2S_{(g)}$, 1,4-dioxane, room temperature, 2 h; (c) $K_3[Fe(CN)_6]$ (1,5 equivalent), 1,4-dioxane – H_2O, room temperature, 12-16 h; (d) dimethyldioxirane (1 equivalent), acetone, room temperature, 2 h.

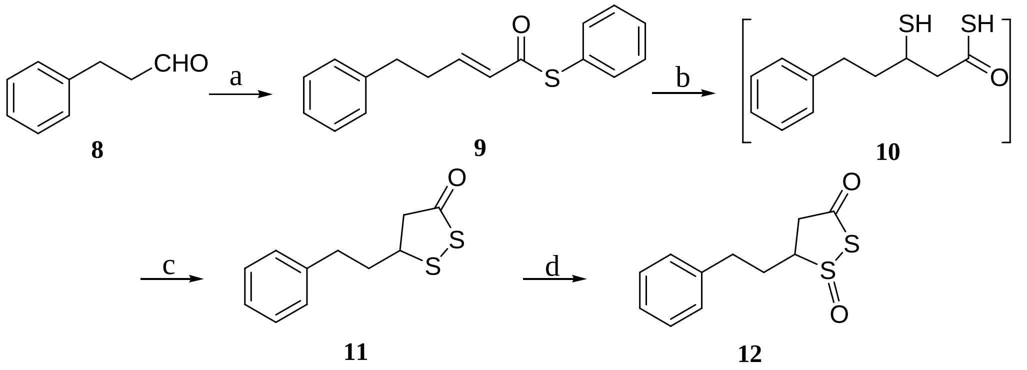
Scheme 4

The 1,2-dithiolane-3-one ring (**6**) was prepared from the thiolic acid with Fe(III)-ions. The *S*-oxide (**7**) was synthesized with dimethyldioxirane under mild conditions.

3.2. The synthesis of the dithiolanon derivatives of D-arabinose and 3-phenylpropionaldehyde

We synthesized the dithiolan and dithiolan-*S*-oxide derivatives of 3-phenylpropionaldehyde (**8→11, 12**) with the method shown in **Scheme 5**. We observed that in contempt of the neutral carrier the derivatives had a well-defined cytotoxic effect.

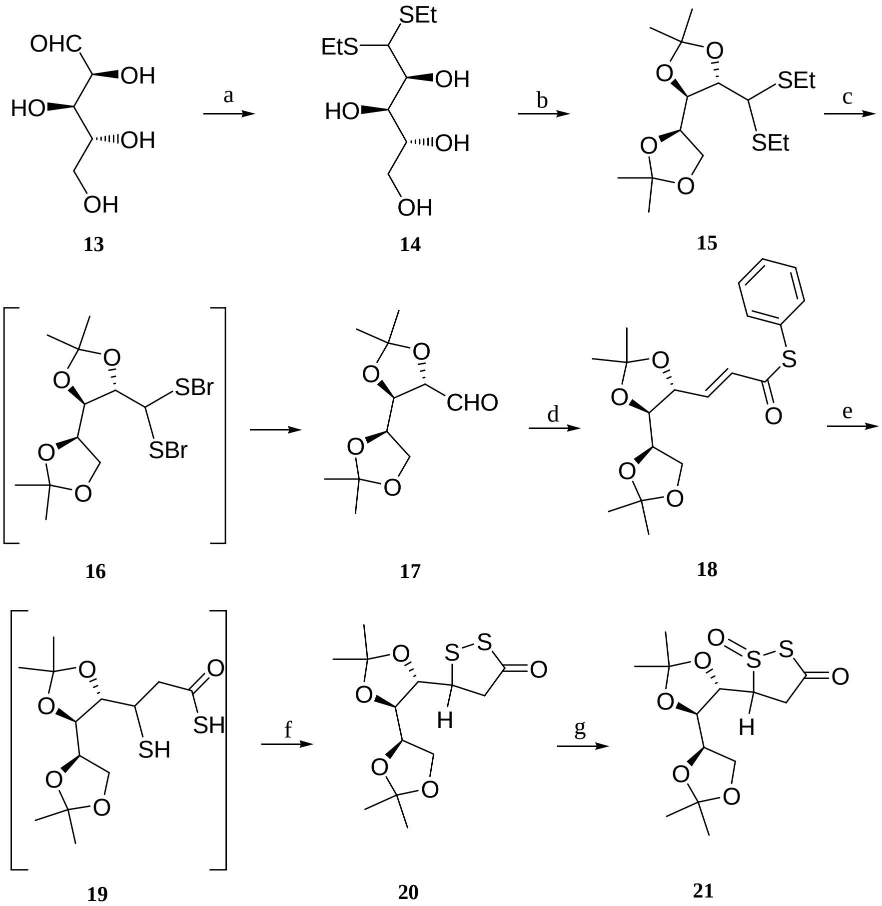
We established that in accord with our theory, the non-oxidized ring-containing derivative is biologically active, too. It was also demonstrated that the oxidized ring form is more reactive than the non-oxidized one. Finally we found that (just like in the case of the antibiotic) the presence of a thiol assured the highest activity.



(a) $Ph_3PCHCOSPh$ (1.3 equivalent), toluene, room temperature, 48 h, 80%; (b) Et_3N (2,1 equivalent), H_2S, 1,4-dioxane, room temperature, 2 h; (c) $K_3[Fe(CN)_6]$ (1,5 equivalent), 1,4-dioxane – H_2O, room temperature, 12 h, 60% (b+c); (d) dimethyldioxirane (1 equivalent), acetone, room temperature, 2 h, 97%.

Scheme 5

Scheme 6 shows the preparation of the D-arabinose dithiolanon and dithilanon-*S*-oxide derivatives (**13→20**, **21**). In the case of these derivatives we found that the heteroring is the potentially bioactive portion. As found in the case of 3-phenylpropionaldehyde the presence of the thiol increased the activity.

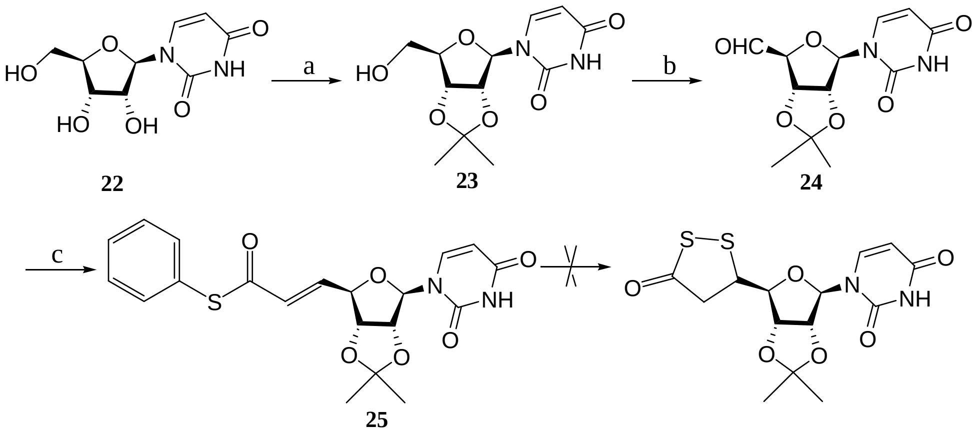


(a) cc. HCl, EtSH, 0 °C, 15 min; (b) 2,2-dimethoxypropane (1 equivalent), *p*TsOH (cat. amm.), acetone, room temperature, 6 h, 97%; (c) $CdCO_3$, NBS (2,2 equivalent), acetone:H_2O 9:1, room temperature, 45 min, 98%; (d) $Ph_3PCHCOSPh$ (1,3 equivalent), toluene, room temperature, 48 h, 81%; (e) Et_3N (2,1 equivalent), H_2S, 1,4-dioxane, room temperature, 2 h; (f) $K_3[Fe(CN)_6]$ (1,5 equivalent), 1,4-dioxane – H_2O, room temperature, 14 h, 73% (e+f); (g) dimethyldioxirane (1 equivalent), acetone, room temperature, 2 h, 95%.

Scheme 6

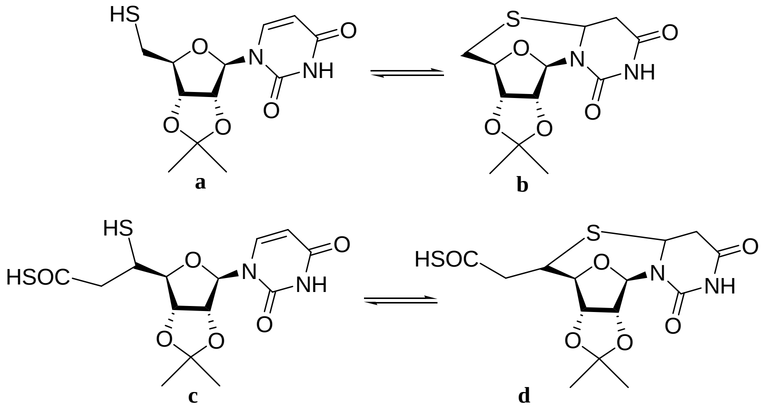
3.3. Synthesis of nucleoside dithiolanon derivatives

During the synthesis of the uridine derivatives (**Scheme 7**, **22→25**) we found that in the presence of the isopropylidene protecting group the reaction gave only a polycyclic structure in the conjugate addition step (**Scheme 7a**). We established that the strained structure gave rise to the formation of this bridged form. The double bond of the nucleobase is close enough to the 5’ β-mercapto thiolic moiety to form the bridged derivative (equilibra **a→b** and **c→d** in **Scheme 7a**).



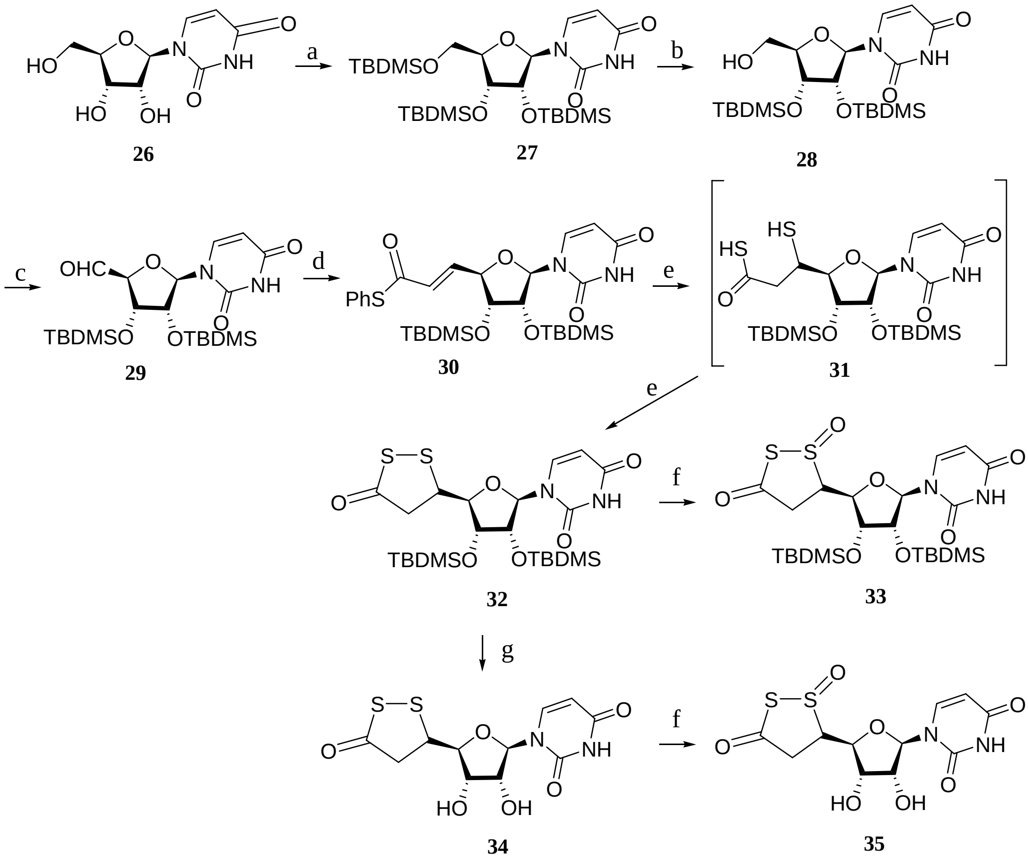
(a) HC(OEt)$_3$ (2 equivalent), *p*TsOH (cat. amm.), acetone, room temperature, 1,5 h, 97%; (b) DCC (1 quivalent), dichloro-aceticacid (1 equivalent), DMSO:EtOAc = 1:5, room temperature, 1,5 h; oxalic acid, room temperature, 30 min; pyridine, room temperature, 14 h; (c) Ph$_3$PCHCOSPh (1,2 equivalent), ethylacetate, room temperature, 15 h, 71% (b+c).

Scheme 7



Scheme 7a

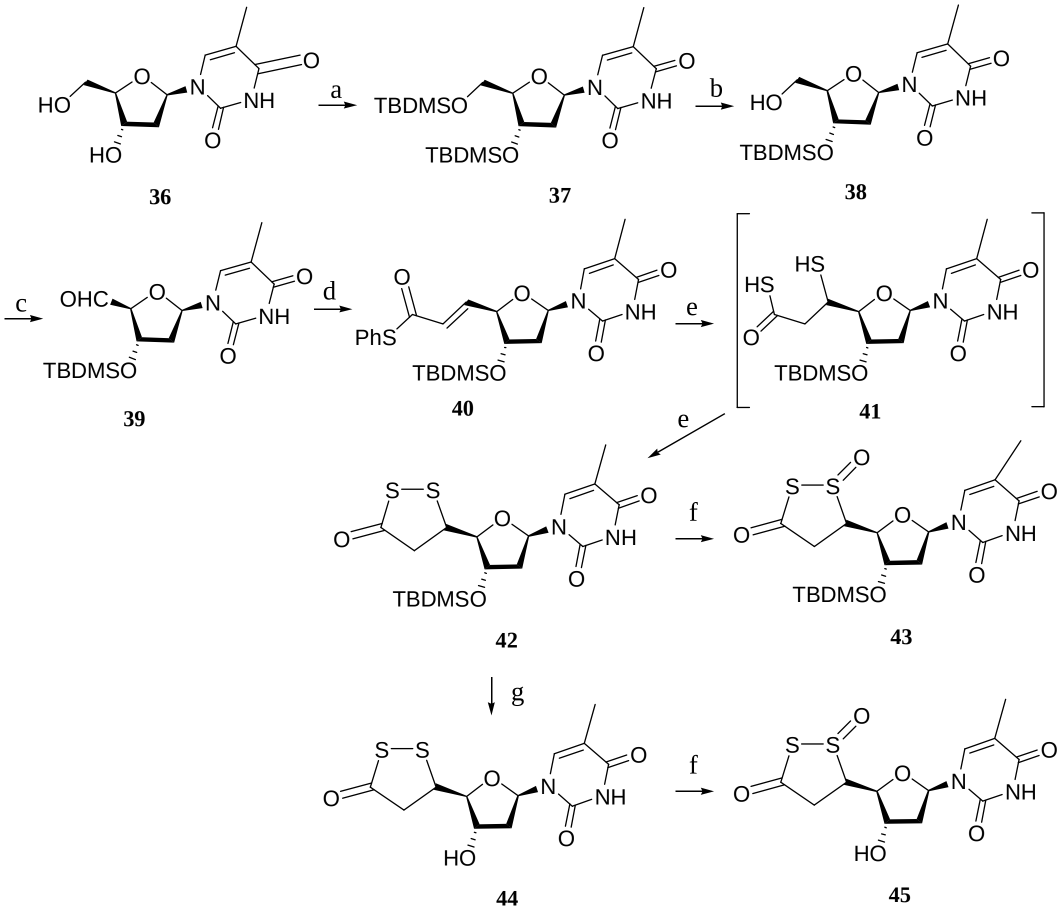
In an earlier publication PADRÓN et al. discussed that compounds carrying silyl ether groups (for example *tert.*-butyldimethylsilyl) possessed higher activity in some cases. Based on this, we changed the isopropylidene group to *tert.*-butyldimethylsilyl and compared the cytotoxic effects of the silylated and the non-silylated derivatives. The uridine 5'-dithiolanon and dithiolanon-*S*-oxide derivatives (**Scheme 8**: **32**, **33**, **34** and **35**) show better or equal cytotoxic effect than daunomycin as a reference material. The results supported our theory that in the case of nucleosides the base of the molecule forms a hydrogen-bonded adduct with the appropriate function of the DNA and carries the molecule to the site of action so the efficacy of the DNA-cleaving becomes higher.



(a) TBDMSCl (3 equivalent), pyridine, room temperature, 1 h; 65 °C, 22 h, 97%; (b) TFA:H_2O 10:1, DCM, room temperature, 45 min; 0 °C, 30 min, 89%; (c) Dess-Martin-perjodinane (1,5 equivalent), DCM, 0 °C/room temperature, 1,5 h; (d) $Ph_3PCHCOSPh$ (1,4 equivalent), 1,4-dioxane, room temperature, 22 h, 79% (c+d); (e) Et_3N (2,1 equivalent), H_2S, 1,4-dioxane, room temperature, 2 h; $K_3[Fe(CN)_6]$ (1,5 equivalent), 1,4-dioxane – water, room temperature, 16 h, 63%; (f) dimethyldioxirane (1 equivalent), acetone, room temperature, 2 h, 96%, 95%; (g) TFA:H_2O:THF 1:1:4, 0 °C, 6 h, 94%;

Scheme 8

The method for the preparation of the thymidine 5' derivatives (dithiolanons and their *S*-oxide pairs: **42**, **43**, **44** and **45**) is shown in **Scheme 9**. The biological tests showed good, but lower activity than for the corresponding uridine derivatives. Upon comparsion of the cytotoxicity of compounds **32**, **33**, **34**, **35** and **42**, **43**, **44**, **45** we established that the presence of the silyl group influenced the activity and more silyl groups enhanced the efficiency.



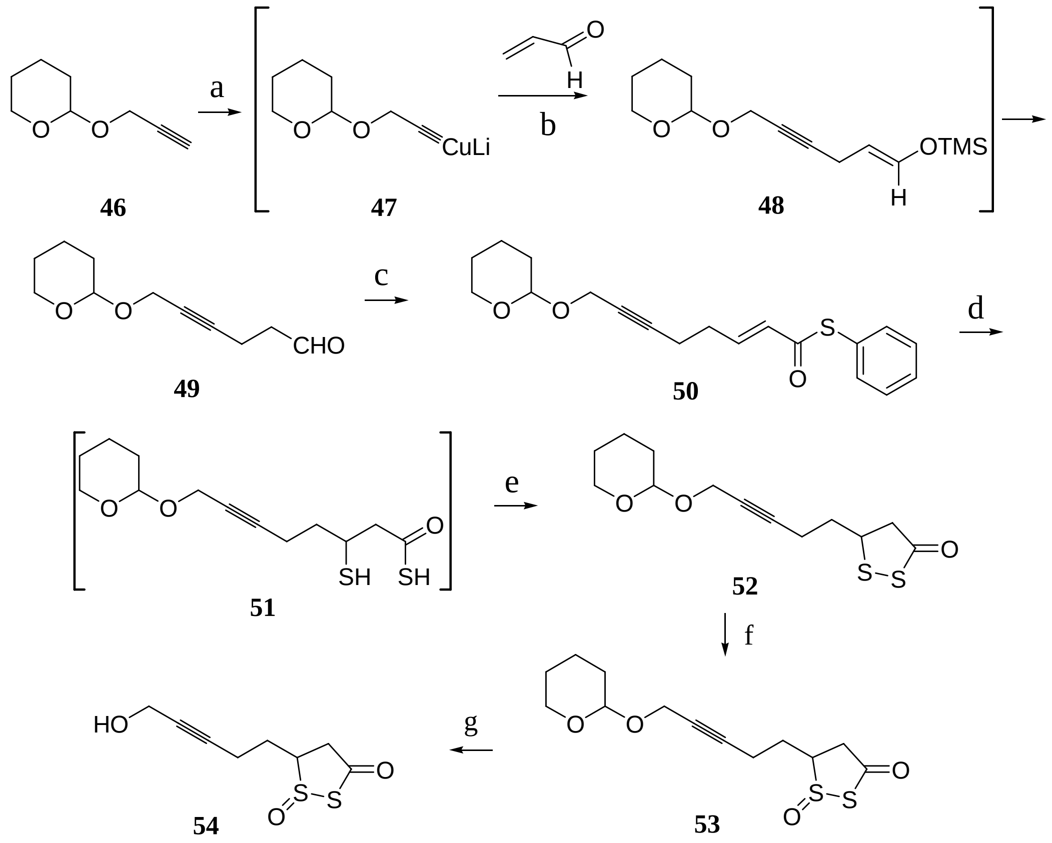
(a) TBDMSCl (2 equivalent), pyridine, room temperature, 1 h; 65 °C, 22 h, 94%; (b) TFA:H_2O 10:1, DCM, room temperature, 45 min; 0 °C, 30 min, 85%; (c) Dess-Martin-perjodinane (1,5 equivalent), DCM, 0 °C/room temperature, 1,5 h; (d) $Ph_3PCHCOSPh$ (1,4 equivalent), 1,4-dioxane, room temperature, 22 h, 77% (c+d); (e) Et_3N (2,1 equivalent), H_2S, 1,4-dioxane, room temperature, 2 h; $K_3[Fe(CN)_6]$ (1,5 equivalent), 1,4-dioxane – water, room temperature, 15 h, 68%; (f) dimethyldioxirane (1 equivalent), acetone, room temperature, 2 h, 94%, 95%; (g) TFA:H_2O:THF 1:1:4, 0 °C, 6 h, 95%.

Scheme 9

To summarize the results we assumed that the mechanism of action of the prepared derivatives is the same to that of leinamycin. The only difference is that the double bond of the pyrimidinedion ensured the function of the original γ-positional double bond and that gave rise to the formation of the episulphonium key intermediate.

3.4. Preparation of a second generation leinamycin analog

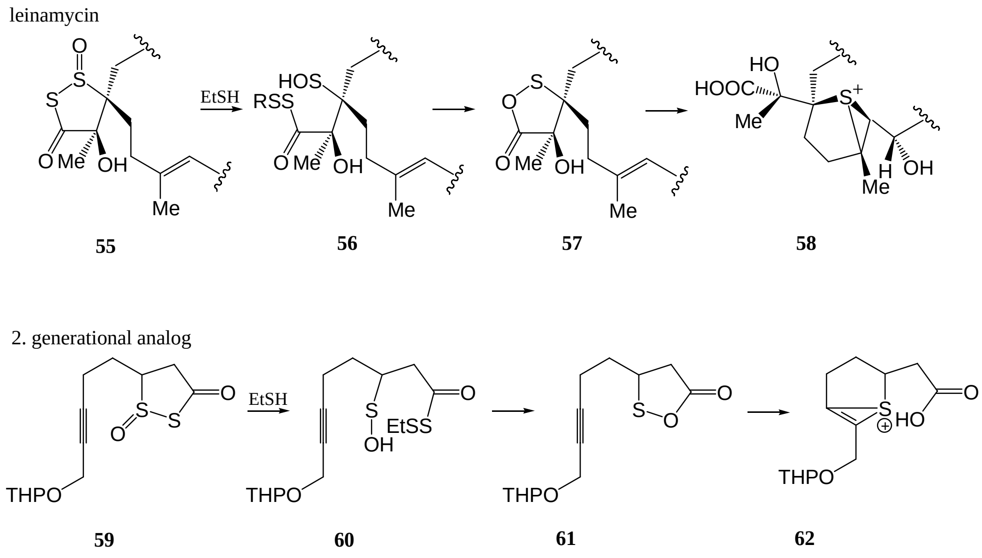
Based on a published 15-step synthesis in 2003 by LEE et al. we prepared a similar leinamycin analog containing a chemically more reactive triple bond instead of the double bond in six steps (**Scheme 10**).



(a) n-BuLi (1 equivalent), THF, -10 °C, 15 min; Cu(I)I•0.75Me_2S (1,1 equivalent), THF, -10 °C, 45 min; (b) Me_3SiI (1 equivalent), THF, -78 °C, 10 min; acrolein (1 equivalent), THF, -78 °C; -30 °C, 2,5 h, 42% (a+b); (c) $Ph_3PCHCOSPh$ (1,25 equivalent), 1,4-dioxane, room temperature, 16 h, 75%; (d) Et_3N (2,1 equivalent), H_2S, 1,4-dioxane, room temperature, 2 h; (e) $K_3[Fe(CN)_6]$ (1,5 equivalent), 1,4-dioxane – water, room temperature, 17 h, 62% (d+e); (f) dimethyldioxirane (1 equivalent), acetone, room temperature, 2 h, 98%; (g); Dowex 50W X4 ion-exchange resin, DCM, room temperature, 2 h, 94%.

Scheme 10

Good cytotoxicity results supported our theory that in the presence of a triple bond there is a possible chance for the formation of the episulphonium key intermediate on the way **59→62** (see in **Scheme 11.**)



Scheme 11

3.5. Results of the biological tests of the prepared compounds

Compound	IC_{50}	IC_{50} (+EtSH)	ClogP
Daunomycin	**2,96**	-	-1,621
129	257,7	65,54	3,607
130	68,43	24,75	1,875
138	35,93	16,87	2,338
139	18,15	12,60	0,634
154	10,04	7,84	5,397
155	3,68	2,67	3,694
156	62,23	20,72	-1,449
157	50,71	7,01	-3,152
164	n.a.	16,69	2,766
165	43,12	6,61	1,063
166	298,8	54,62	-0,615
167	118,7	29,69	-2,318
179	123,7	20,11	2,831
180	41,31	8,33	1,009

The results were given in IC_{50}-values (inhibition concentration) the presented inhibitation values in the Table are in micromolar concentrations (*μmol/L)*. For a comparable standard we used *daunomycin*, useful in tumor chemotherapy as reference material. The data show the average values of three parallel experiments.

Table 1

- It is also clear that ethanethiol significantly enhanced the cytotoxic effect in each case, so the assumption that for the activity of the dithiolanon-*S*-oxides the presence of a thiol is necessary is correct.

- At the same time, it is very interesting that the cyclic disulfide-type intermediates (**11**, **12**, **32**, **34**, **42**, **44** and **52**) are also active, although to a less extent, than the corresponding *S*-oxide analogs (**12**, **21**, **34**, **35**, **43**, **45** and **53**), which are chemically more reactive.

- All of the derivatives show cytotoxic effect in micromolar concentrations. In the case of compounds **11**, and **12** the "warhead" was coupled to molecules that are not as important as considered on the basis of the mechanism of action, but a slight activity was still observed also in these cases.

- The nucleoside derivatives (**32**, **33**, **34**, **35**, **42**, **43**, **44** and **45**) possessed strong cytotoxicity. The silyl ether-containing **33** was more active than the antibiotic daunomycin and the activity of the compounds **32**, **35** and **43** was comparable to that. In agreement with our working hypothesis, it is assumed that the comparatively good efficacy of these nucleoside-leinamycin analogs is attributed to the formation of base-pairs with DNA.

- The cytotoxicity data of our compounds was also investigated in relation with their liphophilic character. Data show that the liphophilic silyl group-containing compounds were more reactive against human tumor cells than the desilylated ones. In the case of the pair of compounds **32**−**34**, **33**−**35**, **42**−**44** and **43**−**45**, carrying free hydroxyl and silyl-protecting group, the cytotoxicity of the lipophilic silylated compounds is higher than that of the respective pair with a negative CLogP value.

- Compound **53** that carries a triple bond-analog of the molecular fragment of leinamycin responsible for the DNA cleaving effect is also a comparatively active member of the series.

- We can note that the cytotoxicity of simple leinamycin analogs has not been extensively investigated. The results presented here demonstrate that some derivatives of leinamycin with very simplified structures, such as the dithiolanon-*S*-oxides possess comparative efficacy. Therefore further investigations of related compounds may be worthwile. For this purpose noncomplicated, convenient synthetic procedures like that was described in this work can be extended to additional aldehydes.

- It is believed that we have also succeeded in proving the cytotoxicity-enhancing properties of silyl ethers, first reported by PADRÓN et al. by using completely different structures, and this encourages further systematic studies on the topic.

4. Publications

Papers underlying the Theses

1. **Ákos Szilágyi**, István F. Pelyvás, Orsolya Majercsik, Pál Herczegh, *Incorporation of the bioactive moiety of leinamycin into thymidine*, Tetrahedron Letters, *45*, 4307-4309, **2004**. IF: **2.484**

2. **Ákos Szilágyi**, Ferenc Fenyvesi, Orsolya Majercsik, István F. Pelyvás, Ildikó Bácskay, Pálma Fehér, Judit Váradi, Miklós Vecsernyés, Pál Herczegh, *Synthesis and cytotoxicity of leinamycin antibiotic analogs*, Journal of Medicinal Chemistry, *49*, 5626-5630, **2006**. IF: **5.104**

Presentations at scientific meetings: oral lectures and posters

Oral lectures

1. Herczegh Pál, **Szilágyi Ákos**: *Leinamicin analógok szintézise*, A Magyar Kemoterápiai Társaság XVI. Konferenciája, Hajdúszoboszló, Magyarország, május 24-26., **2001.**

2. Herczegh Pál, **Szilágyi Ákos**: *Leinamicin analógok szintézise*, A Magyar Kemoterápiai Társaság XVII. Konferenciája, Szeged, Magyarország, június 7-8., **2002**.

3. Pál Herczegh, **Ákos Szilágyi**: *Synthesis of new leinamycin analogs*, 9th Blue Danube Symposium on Heterocyclic Chemistry, Tatranská Lomnica, Szlovák Köztársaság, június 16-20., **2002**.

4. **Ákos Szilágyi**, Pál Herczegh: *Synthesis of new leinamycin analogs*, 1st Austria-Hungary Carbonhydrate Chemistry Conference, Burgsteining, Ausztria, szeptember 10-12., **2003**.

5. **Szilágyi Ákos**, Herczegh Pál: *Új leinamicin analógok szintézise*, Szénhidrátkémiai Munkabizottsági ülés, Debrecen, Magyarország, november 5., **2004**.

6. **Ákos Szilágyi**: *Synthesis of new leinamycin analogs*, Sugars in the Synthesis of Natural Products Conference, Paszkówka, Lengyelország, június 8-12., **2005**.

7. Gábor Pintér, **Ákos Szilágyi**, Gyula Batta, Pál Horváth, István Löki, Tibor Kurtán, Sándor Antus, Sándor Kéki, Miklós Zsuga, Gábor Nagy, János Aradi, Pál Herczegh, *Synthesis of New Polyethylene Glycol Derivatives: Aggregates, Antibiotics*, Second German-Hungarian Workshop, Somogyaszaló, Magyarország, április 4-8., **2006**.

8. Pál Herczegh, **Ákos Szilágyi**, István Pelyvás, Gyula Batta, Gábor Pintér, Pál Horváth, Sándor Antus, Tibor Kurtán, Sándor Kéki, Miklós Zsuga, *Nucleosides in antibiotic analogs and in new nano-aggregates*, VIII. Jornadas de carbohidratos (RSEQ), Alcalá de Henares (Madrid), Spanyolország, szeptember 13-15., **2006**.

9. **Szilágyi Ákos**, Fenyvesi Ferenc, Majercsik Orsolya, Pelyvás F. István, Bácskay Ildikó, Fehér Pálma, Váradi Judit, Vecsernyés Miklós, Herczegh Pál, *A leinamicin antibiotikum analógjainak szintézise és biológiai vizsgálata*, MTA Antibiotikum Munkabizottsági Tudományos Ülés, Debrecen, Magyarország, Szeptember 21., **2006**.

Posters

1. **Ákos Szilágyi**, Ferenc Fenyvesi, Orsolya Majercsik, Ildikó Bácskay, Pálma Fehér, Miklós Vecsernyés, Pál Herczegh, *Synthesis and cytotoxicity of leinamycin antibiotic analogs*, 1st BBBB Conference of Pharmaceutical Sciences, Siófok, Magyarország, szeptember 26-28., **2005**.

2. **Ákos Szilágyi**, Pál Herczegh, *Synthesis and cytotoxicity of leinamycin antibiotic analogs*, 9th International Conference on the Chemistry of Antibiotics and other Bioactive Compounds (ICCA), Bordeaux-Arcachon, Franciaország, szeptember 25-29., **2005**.