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Synthesis of the Nucleoside Moiety of Liposidomycins: Elucidation of the Pharmacophore of this Family of MraY Inhibitors

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Abstract—Tunicamycins (TCMs) and liposidomycins (LPMs) are naturally occurring inhibitors of the bacterial translocase (MraY). Based on structure–activity relationship (SAR) studies, a molecular model has been proposed for their inhibitory mechanism. This study points out the importance of the nucleoside moiety of liposidomycins in the inhibition of MraY. A simplified molecule (**I**) based on the liposidomycin core structure has been synthesised and tested on MraY. The compound displayed a moderate inhibitory activity ($IC_{50} = 50\,\mu M$). The validation of the molecular model was then performed by synthesising higher homologues of **I**, containing an additional stereocentre in the 5′ position (**XIV** and **XV**). In agreement with the prediction, only the (*S*) isomer **XV** showed significant activity against MraY ($IC_{50} = 5\,\mu M$).

In the course of our investigations targeted at the discovery of new antibacterial entities able to overcome the problem of nosocomial bacterial resistance,[1] we have found translocase[2] (MraY) of interest as a potential target. This enzyme belongs to the bacterial peptidoglycan biosynthetic pathway, which is specific to bacteria.[3] In addition, it has been recently proved as essential for the survival of bacteria,[4] and displays the advantage of having known naturally occurring inhibitors[5] such as tunicamycins (TCMs), liposidomycins (LPMs) and mureidomycins (MRDs) (Fig. 1). TCMs are good inhibitors of translocase but are not selective. LPMs and MRDs are potent and selective inhibitors of MraY.[6–8] The low level or complete lack of activity against bacteria might be related to their high hydrophilicity which would be incompatible with a passive diffusion through the lipophilic cytoplasmic membrane.

Inspection of their chemical structures suggests that TCMs, LPMs and MRDs might be either substrates or transition state analogues of the reaction catalysed by MraY. This reaction consists of the replacement of a UMP unit from UDP-MurNAc pentapeptide (UMA5) by a phospho-lipid unit (dodecaprenyl phosphate: C55-P) to provide lipid **I** (Scheme 1).

The presence of uridine moieties in the inhibitor chemical structures suggests that they recognise the UMA5/UMP binding site. Moreover, the presence of lipid groups in TCMs and LPMs suggests their positioning in the C55-P binding site.

Because of the high flexibility of their peptide side chain, molecular modelling has predicted several low-energy conformations for MRDs. Therefore, TCM and LPM structures have been used for conformational studies. The structure of TCMs has been fully characterised. For LPMs, however, the configuration of four chiral centres remain four uncertain and chemical synthesis has never been completed.[9–16] Nevertheless, NMR studies carried out by Isono's group suggest that the configurations are *S*, *S*, *R*, *S*, for the 5′, 2″, 5″ and 6″ positions, respectively[9,17] (Fig. 2). We used these results for our model.

By using the *cvff* force field[18] and a conjugate gradient method with Insight/Discover, the lowest energy conformations of both LPMs and TCMs could be superimposed on the nucleoside substrate (UMA5) of the enzymatic reaction (Fig. 3).

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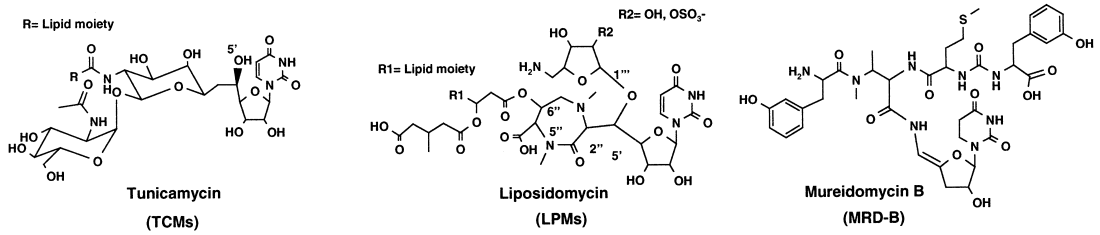
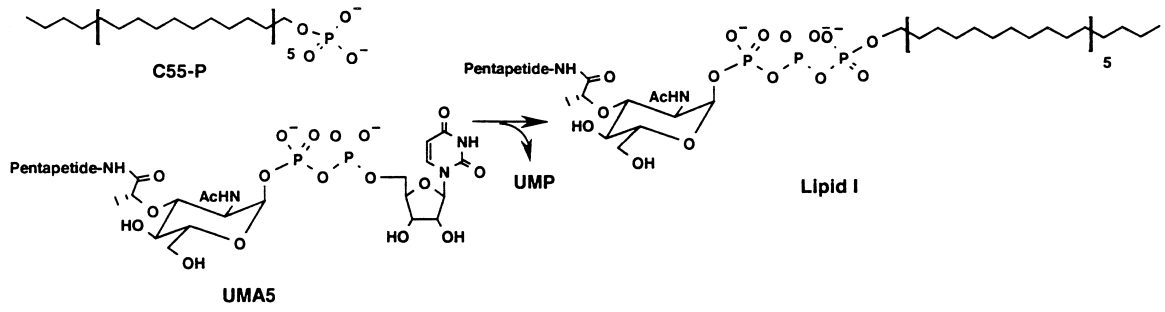


Figure 1.



Scheme 1.

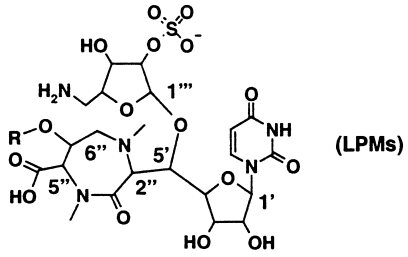
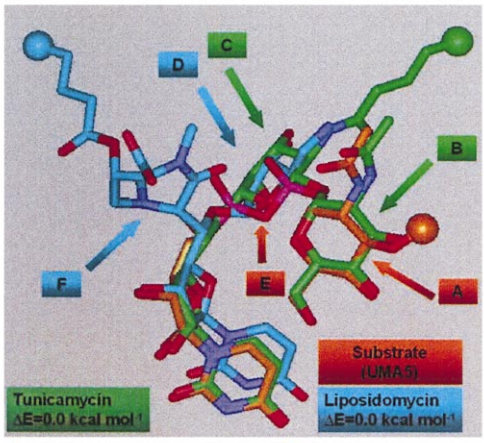


Figure 2.

Information given by this model can be summarised as follows: (a) Uridine moieties are superimposed for TCMs, LPMs and UMA5. (b) The muramoyl residue of UMA5 and the GlcNAc moiety of TCMs are overlaid (rings *A* and *B*, respectively). (c) The ribosamine unit of LPMs fits perfectly the *N*-acetyl galactosamine like central unit of TCMs (rings *D* and *C*), even at the level of stereochemistry, mimicking the diphospho unit (*E*) of UMA5. (d) TCM and LPM lipid chains point in different directions. (e) The 5′, 2″ bond that bears the diazepanone ring (*F*) of LPMs points in the same direction as the 5′ hydroxyl of TCMs.

Common features among these three families might reflect the minimal structure responsible for the activity displayed by both inhibitors. In order to confirm this hypothesis, we undertook the synthesis of **I**, a structure that might correspond to the minimal part of LPMs responsible for activity (Scheme 2).

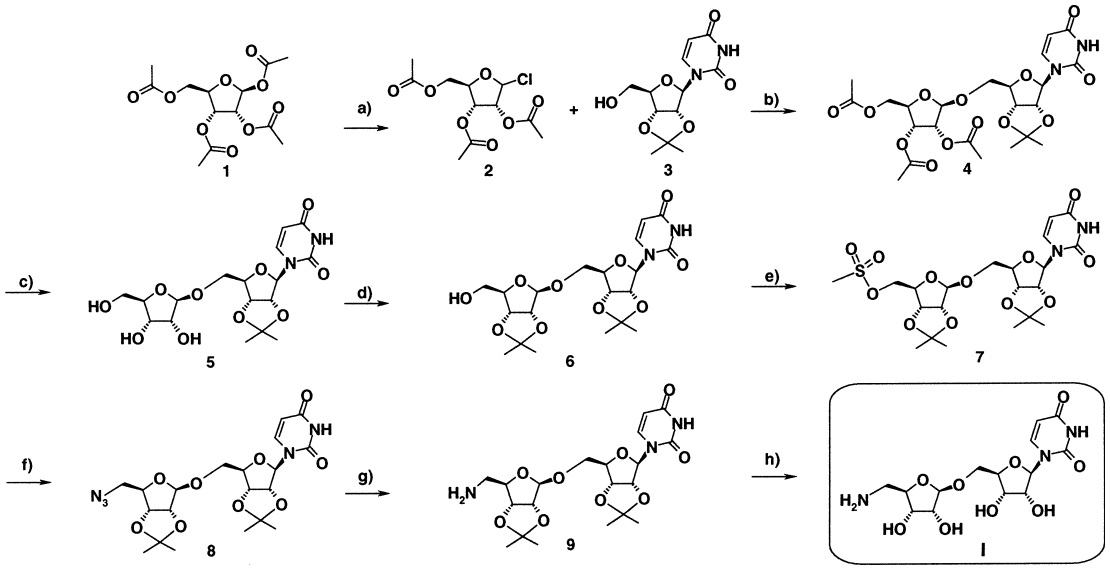
Chlorination of **1** (commercially available) with thionyl chloride gave **2**, which was stored and used as a 1 M methylene chloride solution. Condensation of **3** (commercially



● : Lipid side chain of TCMs ; ○ : Lipid side chain of LPMs ; ■ : Lactyl pentapeptide moiety of UMA5

Figure 3.

available) with **2** using mercury(II) cyanide led to **4**. Methanolysis of **4** gave **5**, which was reacted without purification with 2-methoxy propene in the presence of PTSA to provide the bis-acetonide **6**. Mesylation of the remaining primary alcohol of **6** was performed with methanesulfonyl chloride and TEA to provide **7**. Substitution of the mesylate **7** with sodium azide in DMF at 70 °C led to the azido compound **8**. Reduction of the azido group in the presence of PPh_3 and H_2O in THF gave **9**. Deprotection of both acetonide protecting groups by using 70% aqueous trifluoroacetic acid, gave rise to **I**.[19] The inhibitory activity (IC_{50}) of **I** was determined by using toluene-permeabilised cells of *Escherichia coli*[20,21] and the specific nucleoside substrate of the translocase UMA5. Tunicamycin (from Sigma) and mureidomycin-B were used as standards (Table 1).



Scheme 2. (a) $SOCl_2$, AcOH, CH_2Cl_2, 2 days, rt; (b) $Hg(CN)_2$, CH_2Cl_2, 2 days, rt, 80%; (c) NH_3, MeOH, rt, 2 days; (d) PTSA, acetone, 2-methoxypropene, 3 h, rt (72% for steps (c) and (d); (e) MsCl, Et_3N, 1.5 h, rt, 50%; (f) NaN_3, DMF, 70 °C, ~Q; (g) PPh_3, H_2O, THF, rt, 90%; (h) TFA:H_2O (7:3), 30 min, rt, ~Q.

Table 1.

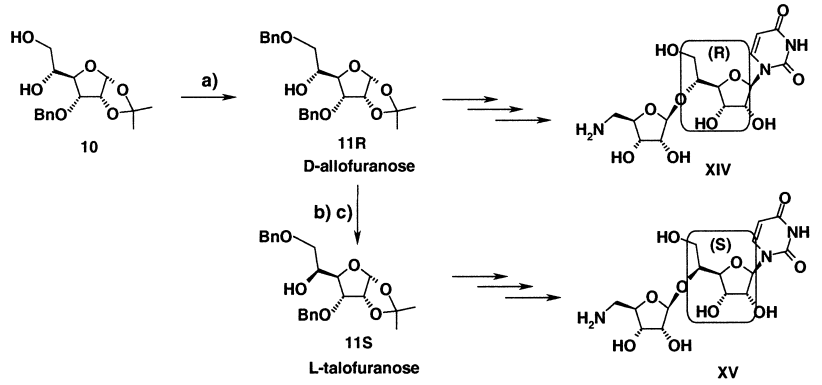
	Tunicamycins	Mureidomycin B	**I**
IC_{50} (μM)	0.48	0.065	50

As **I** showed a reasonable level of activity (50 μM) against translocase, we hypothesised that the introduction of a chiral centre between the two sugar units could further improve the activity, by decreasing the conformational flexibility. **XIV** and **XV** as (*R*) and (*S*) (Scheme 3) hydroxymethyl homologues, respectively, were chosen to provide an unambiguous answer for the configuration of the chiral centre. **XIV** and **XV** were prepared from intermediates **11***R* and **11***S*, corresponding respectively to the D-allofuranose and L-talofuranose series. These intermediates are accessible from a unique compound **10**.[22]

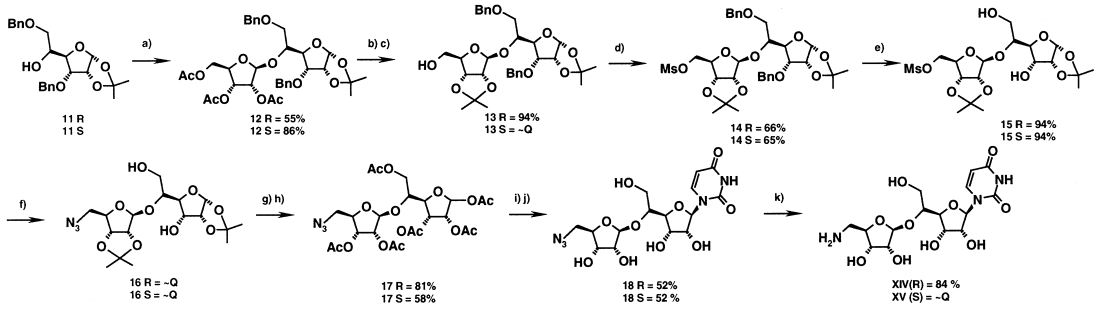
10 was subjected to monobenzylation by using the stannylene acetal method modified by Ohno et al.[23] Addition of dibutyltin oxide and azeotropic removal of water in toluene was followed by sequential addition of cesium fluoride in DMF and benzyl bromide, at room temperature. **11***S* was prepared from **11***R*, by inversion of configuration using Mitsunobu conditions and *p*-nitrobenzoic acid. Subsequent methanolysis of the resulting crude gave **11***S*.

The same chemical strategy as that used to give **I** was then applied to both stereoisomers (**11***R* and **11***S*) to provide respectively, compounds **XIV** and **XV** (Scheme 4).

Condensation of **11** with **2** under Koenigs–Knoor conditions led to **12**. Methanolysis of **12**, followed by acetonide protection gave **13**. Mesylation of the remaining primary alcohol was performed with methanesulfonyl chloride and TEA to give **14**. Subsequent hydrogenolysis of benzyl groups afforded the diol **15**. Mesylate substitution with sodium azide in DMF led to the azido compound **16**. Deprotection of both acetonide-protecting groups was accomplished by using 60% aqueous acetic acid. Acetylation of the crude reaction product gave **17**. The



Scheme 3. (a) Bu_2SnO, PhMe/azeotropic removal, then BnBr, CsF, DMF; (b) *p*NO_2-$PhCO_2H$, DEAD, PPh_3, PhMe; (c) MeONa, MeOH.



Scheme 4. (a) **2**, $Hg(CN)_2$, CH_2Cl_2, MS 4 Å, rt; (b) MeONa, MeOH, rt; (c) 2-methoxypropene, PTSA, acetone, rt; (d) MsCl, TEA, CH_2Cl_2, rt; (e) Pd/C, H_2, MeOH, rt; (f) NaN_3, DMF, 70 °C; (g) 60% AcOH aq. 70 °C; (h) Ac_2O, Pyr., rt; (i) *O*,*O*′-bis(trimethylsilyl)uracil,TMSOTf, CH_3CN, rt; (j) MeONa, MeOH, rt; (k) PPh_3, H_2O, THF, rt.

Table 2.

Compound no.	Tunicamycins	Mureidomycin B	**I**	**XIV**	**XV**
IC_{50} (μM)	0.48	0.065	50	425	5

introduction of the uracil moiety was performed using the standard Vorbruggen procedure.[24] Subsequent hydrolysis of the crude compound led to the nucleoside **18**. The azido group was reduced in the presence of PPh_3 and H_2O in THF to give the desired isomers **XIV**[25] and **XV**.[26]

The inhibitory activity (IC_{50}) of **XIV** and **XV** on translocase was determined and final results are summarised in Table 2.

Only the (*S*) isomer **XV** displays an efficient inhibitory activity on the enzyme. This result is in agreement with the molecular model we propose, and also with the configuration proposed by Isono's group for liposidomycin. Moreover, modification of **I** to give **XV** resulted in a 10-fold improvement of the inhibitory activity.

In conclusion, the model based on stable conformations of two highly potent enzyme inhibitors (TCMs and LPMs) with that of the natural substrate (UMA5) has led to the synthesis of smaller molecules which still display useful enzyme inhibitory activity. The model also supports Isono's work on the configuration of the 5′ chiral centre. The next step will be to elucidate the role of each functional group in such molecules.

Acknowledgement

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25. Analytical data for **XIV** (acetate salt): ^{1}H NMR (400 MHz, D_2O): 1.91 (s, 3H, CH_3 acetate salt), 3.09 (dd, 1H, $J=9$, 13 Hz, H5″a), 3.36 (dd, 1H, $J=2.5$, 13 Hz, H5″b), 3.74 (dd, 1H, $J=5$, 12.5 Hz, H6′a), 3.89 (dd, 1H, $J=3.5$, 12.5 Hz, H6′b), 4.06 (dt, 1H, $J=3.5$, 5 Hz, H5′), 4.10 to 4.20 (m, 4H,

H4″, H3″, H2″, H4′), 4.39 (dd, 1H, $J=5$, 6 Hz, H3′), 4.41 (dd, 1H, $J=6$, 4.5 Hz, H2′), 5.29 (s, 1H, H1″), 5.81 (d, 1H, $J=4.5$ Hz, H1′), 5.90 (d, 1H, $J=8$ Hz, H5), 7.66 (d, 1H, $J=8$ Hz, H6); MS (ESI): 406^+ ($M+H^+$).

26. Analytical data for **XV** (acetate salt): ^{1}H NMR (300 MHz, D_2O): 1.92 (s, 3H, CH_3 acetate salt), 3.19 (dd, 1H, $J=8$, 13.5 Hz, H5″a), 3.41 (dd, 1H, $J=3$, 13.5 Hz, H5″b), 3.80 (dd, 1H, $J=6.5$, 12 Hz, H6′a), 3.87 (dd, 1H, $J=4$, 12 Hz, H6′ b), 4.01 to 4.30 (m, 6H, H4″, H3″, H2″, H4′, H3′, H5′), 4.35 (dd, 1H, $J=3$, 5 Hz, H2′), 5.20 (s, 1H, H1″), 5.79 (d, 1H, $J=3$ Hz, H1′), 5.89 (d, 1H, $J=8$, H5), 7.82 (d, 1H, $J=8$ Hz, H6); MS (FAB): 406^+ ($M+H^+$).