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A versatile scaffold for a library of liposidomycins analogues: a crucial and potent glycosylation step

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Abstract—A key step for the synthesis of a diazepanone scaffold dedicated to a library of MraY inhibitors is described. It involves the *O*-glycosylation of a conveniently protected L-serine by a D-ribofuranose derivative. High yield and selectivity were obtained.

1. Introduction

Due to the emergence of bacterial resistance displayed by the known antibiotics,[1] there is an urgent need for identifying new potential targets and for developing antibacterial agents directed towards them. As an essential enzyme involved in the biosynthesis of bacterial cell wall peptidoglycan, the bacterial translocase MraY represents such a target. This enzyme, partially anchored or totally immersed in the membrane,[2] catalyzes the first membrane step of peptidoglycan biosynthesis. Three families of naturally occurring inhibitors of the translocase have been identified: Tunicamycins,[3] liposidomycins[4] and mureidomycins,[5] which show structural analogies with the natural substrate of this enzyme, UDP-Mur-*N*-Ac-pentapeptide (Fig. 1). However, although these compounds exhibit powerful activity on the translocase MraY in vitro,[6] none of them display high activity in vivo, probably due to their high hydrophily, which limits their passive diffusion through biological membranes. In order to develop new antibiotics dedicated to the translocase MraY, our goal is the design and synthesis of a relevant scaffold allowing great flexibility for the introduction of key structural fragments required for biological activity and mimicking either the whole or part of the natural inhibitors.

Fine tuning of physico-chemical properties is expected to provide inhibitors displaying improved biological activity. We have previously described access to the ribosyl diazepanone core of liposidomycins.[7]

We are now aiming at using this diazepanone core as a versatile scaffold taking advantage of both the various configurations available at stereogenic centres, according to the starting material (L-ascorbic or D-isoascorbic acid), and the high and orthogonal functionalization of this moiety. According to the Aventis group conclusions concerning the importance of the aminoribose part of liposidomycins for biological activity,[8] the structure of the retained scaffold is outlined in Figure 2. Retrosynthetic analysis shows two key fragments: an aminoepoxide readily available from L-ascorbic acid[9] and a serinyl aminoriboside. Access to this second fragment proved troublesome and we present herein our preliminary results concerning its synthesis in good yield and high selectivity.

2. Results and discussion

The synthesis of a glycoconjugate between the anomeric position of α-D-ribofuranose as a sugar donor and L-serine as a sugar acceptor requires the protection of both counterparts except the nucleophilic function of the acceptor and the anomeric position of the donor, which is usually activated. According to the nature of the protecting group at the α-position of the anomeric one, a possible anchimeric assistance can direct the reaction towards a single anomer. In this context, the convenient protection of L-serine was easily achieved from commercially available *N*-Boc-*O*-benzyl-L-serine

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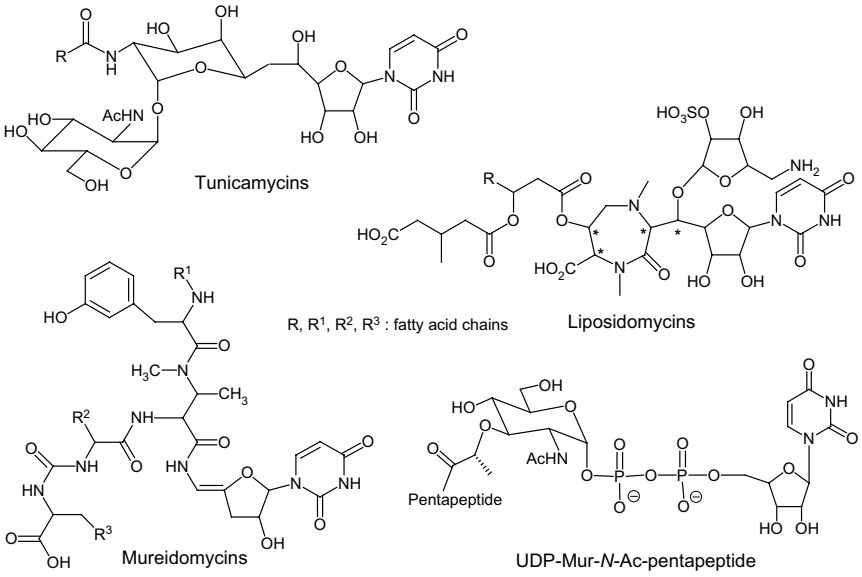


Figure 1. Structure of naturally occurring inhibitors of the translocase MraY and of the natural substrate.

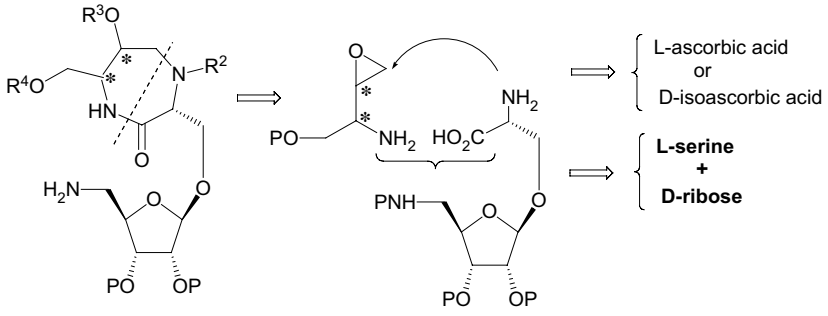
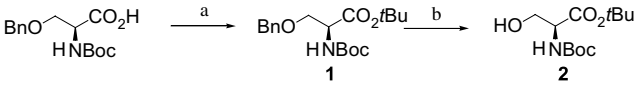


Figure 2. Structure and retrosynthetic analysis of the targeted scaffold.



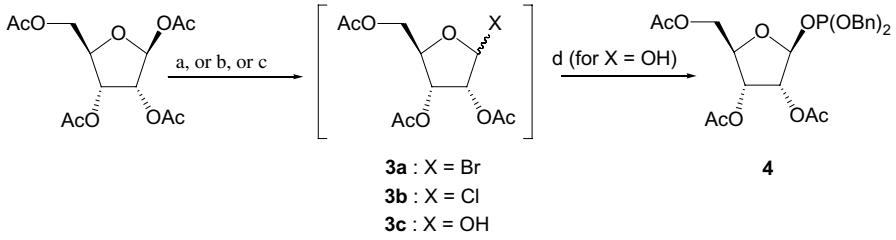
Scheme 1. Reagents and conditions: (a) Cl_3CCO_2tBu, $BF_3{\cdot}OEt_2$, C_6H_{12}/CH_2Cl_2 2/1, 96%; (b) H_2, $Pd(OH)_2/C$, EtOH/AcOH 5/1, 98%.

(Scheme 1) by successive protection of the acidic function as its *tert*-butyl ester **1** through treatment with *tert*-butyl trichloroacetimidate in the presence of boron trifluoride etherate and hydrogenolysis of the benzyl ether in the presence of Pearlman's catalyst in a 5/1 mixture of ethanol/acetic acid (94% overall yield of **2**).

In order to direct the glycosylation towards the exclusive formation of the β anomer, the commercial per-acetylated β-D-ribofuranose was first chosen as the sugar donor. Activation of its anomeric position (Scheme 2) involved either bromination (excess of bromotrimethylsilane in CH_2Cl_2, −40 to 20 °C)[10] or chlorination (hydrogen chloride in CH_2Cl_2, 0 °C)[11] with both leading to an α/β mixture of the corresponding halo derivative (**3a** or **3b**, respectively), in which the β-anomer was the major product (over 95% by ^1H NMR). However, these mixtures could not be purified by flash chromatography due to the instability of halo furanose.[12]

Various Lewis acids,[13] gathered in Table 1, were then tried to catalyze the glycosylation of **2**, while varying reaction time, temperature of the reaction and amount of catalyst.

However, none of these conditions afforded any of the expected product, mainly leading to a formal hydrolysis at the anomeric position or to an intractable mixture. In order to evaluate the behaviour of the glycosylation in the presence of a thioglycoside at the anomeric position, the introduction of a thiomethyl (TMS-SMe, TMSOTf, CH_2Cl_2, 36 °C)[14] or thiophenyl (PhSH, $SnCl_4$, C_6H_6, 60 °C)[15] residue in the anomeric position of per-acetylated β-D-ribofuranose was tentatively carried out, but was also unsuccessful. Both improving the stability of the activated sugar and increasing the nucleofugal character of the leaving group at anomeric position were then tried. For this purpose, the per-acetylated β-D-ribofuranose was selectively hydrolyzed at the anomeric position by successive bromination with a 30% hydrogen



Scheme 2. Reagents and conditions: (a) TMSBr, CH_2Cl_2, −40 °C to rt, 96%; (b) HCl_g, CH_2Cl_2, 0 °C; (c) (i) HBr·AcOH, CH_2Cl_2, (ii) $NaHCO_3$ neat then concentration in vacuo and THF/H_2O extraction; (d) $Et_2NP(OBn)_2$, 1,2,4-triazole, CH_2Cl_2, 20 °C.

Table 1. Attempts of *N*-Boc-L-serine *tert*-butyl ester **2** glycosylation with **3a** or **3b** in CH_2Cl_2

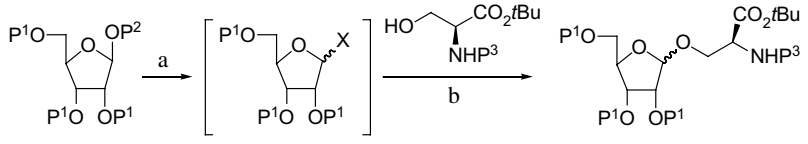
Entry	Lewis acid	Equivalents of **2**	Reaction conditions
1	$Hg(CN)_2$	0.67	24 h, rt
2	TMSOTf	1.5	24 h, rt
3	$HgClO_4$	0.5	14 h, −40 °C
4	$BF_3{\cdot}EtO_2$	1.2	4 h, 0 °C to rt
5	AgOTf	0.76	12 h, −10 °C

bromide solution in acetic acid and sodium hydrogenocarbonate treatment to afford the stable intermediate **3c**, in 86% overall yield. Activation at the anomeric position efficiently occurred via treatment with di-benzyl-*N*,*N*-diethyl-phosporamidite in the presence of 1,2,4-triazole[16] to give the expected phosphite **4**, which was isolated in 80% yield after chromatographic purification. Attempts to achieve glycosylation involved the *N*-Boc-L-serine *tert*-butyl ester **2** (1.23 equiv) in the presence of triflic acid (sub-stoichiometric amount) in CH_2Cl_2 at −15 °C and molecular sieves (4 Å),[17] but only led to degradation products. Finally, on the hypothesis that the *N*-Boc protective group of the serine derivative was sensitive to the Lewis acid used in the coupling reactions, further attempts were carried out from the commercially available *N*-benzyloxycarbonyl-serine *tert*-butyl ester. Thus, after careful tuning of the choice of both the protagonists of the glycosylation and the experimental conditions where we succeeded in obtaining the expected *O*-ribosyl-serine derivative in good yield and high selectivity (Scheme 3).

The best conditions (Table 2, entry 1[18]) involved 2,3,5-tri-*O*-benzoyl-ribofuranosyl bromide (1 equiv), prepared from the commercially available 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose under the same conditions as previously described (TMSBr excess, −40 to 20 °C, CH_2Cl_2), and *N*-Z-serine *tert*-butyl ester (1.3 equiv) in the presence of silver triflate (1.56 equiv) in CH_2Cl_2, at −15 °C, which successfully afforded the expected *O*-ribofuranosyl serine derivative **5a** in 75% yield as a single β-anomer. It has to be pointed out that starting from the corresponding analogous 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (entry 2), the same experimental conditions also led to the exclusive formation of the β-anomer due to the anchimeric assistance of the acetate at the C2 position but with a poorer isolated yield (32%). On the other hand, the 2,3,5-tri-*O*-benzyl-ribofuranosyl derivatives (entries 3 and 4) with the *N*-Boc-L-serine *tert*-butyl ester gave mixtures of α- and β-anomers in medium to good yield (^1H NMR for **5c**, H_1: $\delta = 4.83$ and 5.02 ppm, for α and β anomers, respectively). However, activation of the anomeric position as a fluoride (entry 4) instead of a bromide and coupling in the presence of stannous chloride/silver perchlorate as the Lewis acid[19] led to an inversion of the diastereoselectivity of glycosylation and to a slight excess of the targeted β-anomer.

3. Conclusion

An efficient glycosylation between the 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose, via its unstable 1-brominated derivative, and the *N*-benzyloxycarbonyl-L-serine *tert*-butyl ester was performed in high yield (75%) and selectivity. This reaction represents a key step towards the synthesis of the scaffold, which should allow the obtention of a library of liposidomycins analogues and work is currently in progress towards this goal. The study of this reaction has revealed the peculiar behaviour of furanosyl derivatives when compared to pyranosyl ones during the achievement of this reaction.



5a : P^1 = Bz, P^3 = Z
5b : P^1 = Ac, P^3 = Z
5c : P^1 = Bn, P^3 = Boc

Scheme 3. Reagents and conditions: see text and Table 2.

Table 2. Glycosylation of L-serine derivatives (see Scheme 3)

Entry	P^1	P^2	Activation conditions reaction a	X	P^3	Glycosylation conditions reaction b	Compound	Product ratio[a]	Yield (%)[b]
1	Bz	Ac	TMSBr, CH_2Cl_2, −40 °C to rt	Br	Z	AgOTf, CH_2Cl_2, −15 °C	**5a**	β: 100%	75
2	Ac	Ac	TMSBr, CH_2Cl_2, −40 °C to rt	Br	Z	AgOTf, CH_2Cl_2, −15 °C	**5b**	β: 100%	32
3	Bn	Ac	TMSBr, CH_2Cl_2, −40 °C to rt	Br	Boc	AgOTf, CH_2Cl_2, −15 °C	**5c**	β/α: 0.3	69
4	Bn	H	DAST, THF, −30 °C to rt	F	Boc	$SnCl_4$, $AgClO_4$, −15 °C to rt	**5c**	β/α: 1.2	45

[a] The product ratio was determined by ^{1}H NMR-spectral analysis of the crude reaction mixture, error ±5% of the stated value.
[b] Overall isolated yield.

Indeed, many examples of glycosylation have been described in literature from pyranosyl compounds,[20] furanosyl derivatives are mainly involved into the *N*-glycosylation reactions with purine or pyrimidine moieties to afford nucleosidic compounds and only few examples are dedicated to *O*-glycoside derivatives of furanose. The reaction described in this paper is to our knowledge the first example of serine *O*-glycosylation with a D-ribofuranose derivative.

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18. Experimental procedure for the preparation of the pure β-anomer **5a**: Both the 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose and the *N*-Z-serine *tert*-butyl ester were submitted to azeotropic evaporation in the presence of CH_3CN before using. To a solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (650 mg, 1.29 mmol, 1 equiv) in CH_2Cl_2 (2.5 mL) at −40 °C, was dropwise added bromotrimethylsilane (650 μL, 4.8 mmol, 3.7 equiv) and the temperature was allowed to rise to 20 °C. After 1 h stirring at 20 °C, monitoring of the reaction by thin layer chromatography revealed that it was not complete and an excess of bromotrimethylsilane was added (1.3 mL, 9.6 mmol, 7.4 equiv) at −40 °C after which the temperature was again increased to 20 °C. After another 2 h stirring, in order for completion of the reaction, we were required to repeat the TMSBr addition (7.4 equiv, −40 then 20 °C, 2 h). Then, both excess of TMSBr and trimethylsilyl acetate were removed in vacuo at 20 °C. The crude 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide in CH_2Cl_2 (5 mL) was then added at −10 °C to a mixture of *N*-Z-serine *tert*-butyl ester (495 mg, 1.68 mmol, 1.3 equiv) and silver

triflate (517.7 mg, 2.01 mmol, 1.56 equiv) in CH_2Cl_2 (4 mL), which was first stirred at −20 °C for 1 h in the presence of molecular sieves 4 Å (Lancaster, powder, 6.5 g). The reaction mixture was then stirred at −10 °C for 14 h. Addition of triethylamine (2 mL) was followed by filtration through a Celite pad. A saturated aqueous solution of $NaHCO_3$ was added and subsequent CH_2Cl_2 extractions, drying over $MgSO_4$ and concentration in vacuo gave crude **5a** as a single β-anomer. Flash chromatography (cyclohexane/EtOAc 8/2) afforded the pure serinyl riboside derivative **5a** (719 mg) in 75% isolated yield. Selected data for **5a**: $[\alpha]_D$ +16 (*c* 1.0, CH_2Cl_2); ^1H NMR δ 8.04–7.29 (m, 20H, Ph), 6.51 (s, 1H, H_1), 5.86–5.77 (m, 2H, $H_{2,3}$), 5.62 (d, 1H, $J_{NH,2'} = 8.8$ Hz, NH), 5.14 (s, 2H, CH_2Ph), 4.76 (dd, 1H, $J_{4,5b} = 5.7$ Hz, $J_{4,5a} = 4.5$ Hz, H_4), 4.69 (dd, 1H, $J_{5a,5b} = 11.7$ Hz, $J_{5a,4} = 4.5$ Hz, H_{5a}), 4.53 (dd, 1H, $J_{5b,5a} = 11.7$ Hz, $J_{5b,4} = 5.7$ Hz, H_{5b}), 4.44 (m, 1H, $H_{2'}$), 3.74 (dd, 1H, $J_{3'a,3'b} = 8.9$ Hz, $J_{3'a,2'} = 1.5$ Hz, $H_{3'a}$), 3.49 (dd, 1H, $J_{3'b,3'a} = 8.9$ Hz, $J_{3'b,2'} = 2.6$ Hz, $H_{3'b}$), 1.09 (s, 9H, *t*-Bu); ^{13}C NMR δ 169.1 ($C_{1'}$), 165.8, 165.1, 164.8 (COPh), 156.1 (CONH), 136.3, 133.5, 133.4, 133.2, 129.8, 129.7, 128.9, 128.7, 128.4, 128.3, 128.1 (Ph), 99.6 (C_1), 80.2 (C_4), 75.0 (C_2), 73.5 (*t*-Bu), 72.1 (C_3), 67.0 (CH_2Ph), 64.9 (C_5), 61.6 ($C_{3'}$), 54.7 ($C_{2'}$), 27.1, 26.8 (*t*-Bu); HRMS (chemical ionization NH_3) for $C_{41}H_{45}N_2O_{12}$ $(M+18)^+$ calcd 757.2973; found 757.2964.

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