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Original article

Synthesis and biological evaluation of a diazepanone-based library of liposidomycins analogs as MraY inhibitors

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ABSTRACT

New inhibitors of the bacterial tranferase MraY are described. A scaffold strategy based on the diazepanone central core of liposidomycins, natural inhibitors of MraY has been developed. It involves the introduction of key structural fragments required for biological activity on enantiopure diazepanones by reductive amination, esterification and glycosylation. Biological evaluation of these compounds on MraY enzyme revealed interesting inhibitory activity for compounds displaying three fragments on the scaffold: a palmitoyl chain, an aminoribose part and an alkyluracil moiety. The inhibitors were also evaluated on MurG enzyme. The best compounds resulted in inhibition with IC_{50} values in the 100 μM range for one or the other enzyme.

1. Introduction

Multidrug resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA) set a severe public health problem [1]. Therefore, designing and synthesizing new antibacterial agents is a priority and a challenge for the scientific community. Among the most validated targets for developing new antibacterial compounds are enzymes involved in the biosynthetic pathway of peptidoglycan. This polymer constitutes an essential part of the bacterial cell wall and protects the cell from osmotic pressure [2]. Indeed, enzymes involved in its biosynthesis have been demonstrated to be ubiquitous and essential to bacterial growth [3] and any anomaly in this biosynthetic process leads to cell lysis. Furthermore, peptidoglycan has no counterparts in eukaryotic cells. Of particular interest to address the problem of bacterial resistance is to focus on targets that have not been explored as much as before to delay the occurrence of resistance. Owing to its trans-membrane localization [4], the MraY transferase, which is an essential enzyme [5] that catalyzes the first membrane-associated step of peptidoglycan biosynthesis, has only recently been purified to homogeneity and characterized [6], with tests allowing high throughput screening of inhibitors being achieved [7]. MraY catalyzes the transfer of uridine di-phosphate-*N*-acetyl-muramoyl-pentapeptide from the cytoplasm to the membrane (Fig. 1) through its fixation on the undecaprenyl phosphate lipid carrier, leading to the formation of lipid I (undecaprenylpyrophosphate-*N*-acetyl-muramoyl-pentapeptide) and to the release of uridine monophosphate (UMP).

At the moment, neither is a crystal structure of MraY available, nor are there any MraY-directed antibiotics in clinical use as a consequence of the trans-membrane localization of this enzyme. Nevertheless, several families of natural antibiotics including liposidomycins [8], caprazamycins [9] or tunicamycins [10] have been identified (Fig. 2) which display high *in vitro* inhibition of the enzymatic activity, but modest antibacterial activity probably due to their high hydrophilicity limiting their passive diffusion through membranes [11]. Based on the structure of these inhibitors and in the context of a program directed to the synthesis and biological

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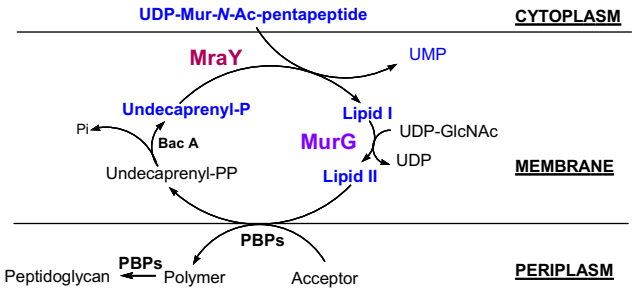
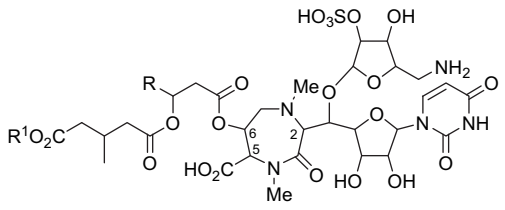


Fig. 1. Role of MraY in bacterial peptidoglycan biosynthesis.

evaluation of new antibacterials [12], our goal is to develop efficient access to libraries of compounds that inhibit the MraY enzymatic activity. The finality is to contribute not only to the discovery of new antibacterials, but also to the elucidation of the structure of the enzyme's active site through structure–activity relationships study of families of inhibitors [12,13]. A key point of this study is the development of scaffolds allowing structural changes that could permit further optimization to generate "lead" compounds [14].

Toward that goal, we recently described [15] straightforward access to enantiopure 1,4-diazepanone scaffolds (Fig. 3) that display differentiated functions such as amine, amide, primary and secondary alcohols and for which different configurations at asymmetric carbons are available.

Such heterocycles are the central core of liposidomycins, MraY natural inhibitors isolated from *Streptomyces griseosporeus.* With caprazamycins, they display a complex nucleosidic structure based on that heterocycle and also involve a uridine moiety, an aminoribosyl part, fatty acid chains and a methyl glucoside unit for caprazamycins. Knapp et al. [16] have determined the absolute configuration of these natural compounds as being 2*S*,5*S*,6*S*. Our goal is to develop a library of simplified analogs of liposidomycins based on the diazepanone scaffold. The choice of key fragments to be introduced on this polyfunctionalized platform took advantage of the structure–activity relationships study carried out by Aventis and involving an aminoribosyl uridine pharmacophore [17] (Fig. 4). Conclusions of this study were notably that the aminoribosyl moiety and the uracil part are important for activity [18]. Indeed, either uracil N-alkylation or double bond reduction led to loss of activity. Furthermore, it was shown that removal of both hydroxyl groups of uridine has no drastic impact on biological activity [19].



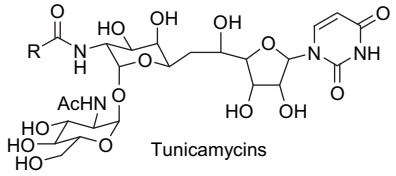


Fig. 2. Natural inhibitors of MraY.

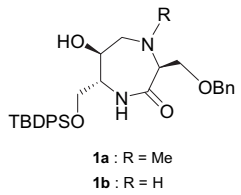


Fig. 3. Exemples of synthesized diazepanones.

Taking these results into account, we hypothesized that only the uracil template and not the uridine one, should be crucial to ensure a possible recognition of the resulting inhibitors by the enzyme and chose to replace the ribose part of uridine by a simple C_5-acyclic alkyl chain. Therefore, alkyluracil, aminoribose and fatty acid chain, all three mimicking or being key fragments of the naturally occurring inhibitors, were retained in the targeted compounds. Either one, two, three or four of these fragments were introduced on scaffold **1a** or **1b** (Fig. 5) in which the intracyclic nitrogen of the amine function was substituted or not by a methyl group. Sequential introduction of these fragments and biological evaluation of the corresponding inhibitors on the MraY transferase allowed the identification of their role in the biological activity.

2. Results and discussion

2.1. Chemistry

From the *N*-methyl diazepanone **1a** (Scheme 1), easily obtained from L-ascorbic acid and L-serine [15], it was possible to introduce either one or two key fragments. First, *O*-acylation of the free secondary alcohol function by palmitic acid in the presence of dicyclohexylcarbodiimide afforded the ester **2** in 93% yield. Successive deprotections of the primary alcohol functions involved hydrogenolysis of benzyl ether in the presence of palladium black in acetic acid giving **3** followed by silyl ether cleavage by ammonium fluoride affording **4** in 76% overall yield from **2**. It has to be noted that carrying out this reaction in the presence of tetrabutylammonium fluoride led to partial migration of the palmitoyl chain to the primary alcohol function. Thanks to ammonium fluoride, the intermediate alcoholate was directly protonated by the ammonium counter ion avoiding the chain migration. We next turned to the introduction of the aminoribosyl moiety through O-glycosylation [20, 13b, 13e] of the primary alcohol function of **3** by the fluoroazidoribose derivative **5** which was carried out in the presence of boron trifluoride etherate and molecular sieves in excess leading to **6** in 87% yield. The fluororibose **5** was readily obtained in four steps from D-ribose [13b, 13e]. The presence of the isopentylidene moiety on the β-face of this sugar derivative allows steric control of the β-selectivity during the ribosylation reaction. Indeed, only the β-anomer is formed during this reaction. Next, azide reduction by hydrogenation in the presence of palladium on charcoal (10%) afforded the corresponding primary amine **7** in 83% yield. Synthesis of the targeted inhibitor **9** first involved acidolysis of the

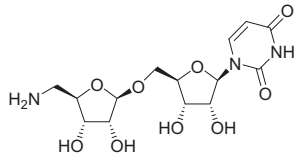


Fig. 4. Structure of Aventis pharmacophore.

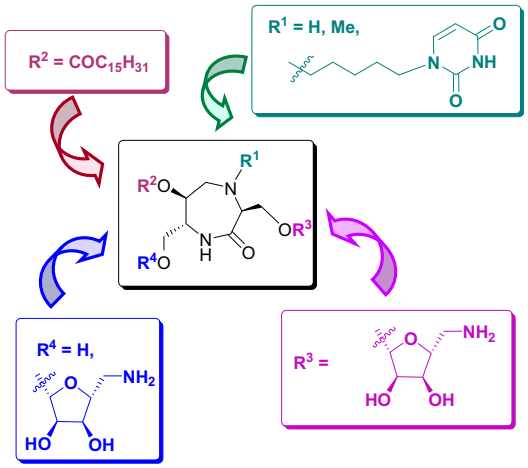


Fig. 5. Proposed scaffold strategy.

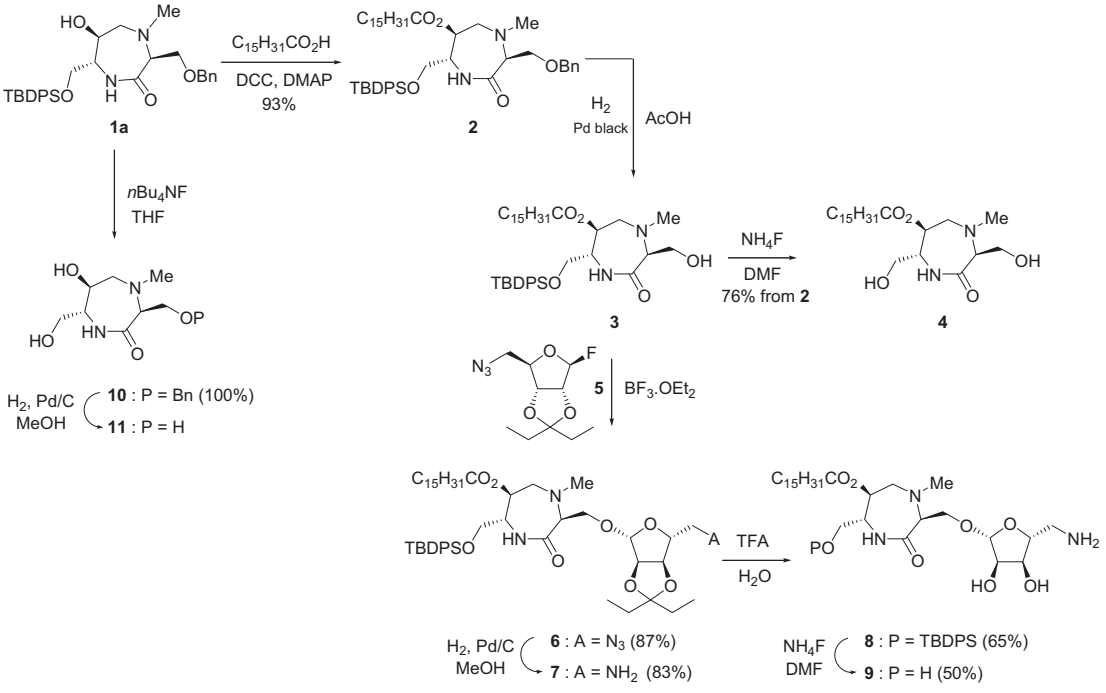
isopentylidene protecting group of the diol leading to **8** in 65% yield, followed by silyl ether deprotection as above giving **9** in 50% yield. In a complementary manner, in order to evaluate the biological activity of the diazepanone scaffold itself, and to compare its activity with that of the decorated scaffold with the different fragments, **1a** was submitted to sequential deprotections of its primary alcohol functions. Thus, silyl deprotection by tetrabutylammonium fluoride afforded **10** in quantitative yield and subsequent benzyl ether hydrogenolysis gave **11**.

Alternatively, toward the possible introduction of three key fragments, we embarked on chemical manipulation of the diazepanone **1b** (Scheme 2). Introduction of the alkyluracilyl moiety involved reductive amination of the diazepanone **1b** with uracil pentanal **12** [12a] in the presence of sodium triacetoxyborohydride [21] yielding **13** in 87% yield. Acylation of the secondary alcohol function with palmitic acid, as above, gave the ester **14** in 87% yield. Then, the removal of benzyl ether protection on the primary alcohol function was studied. Catalytic hydrogenation in various conditions (palladium on charcoal, palladium black, palladium dihydroxide, …) either failed or resulted in partial reduction of the uracil double bond, the obtained aminoalcohol probably poisoning the catalyst [22]. The best conditions for this reaction revealed to be boron trichloride in dichloromethane at low temperature [23]. In these conditions, the pure alcohol **15** could be isolated in 92% yield. Introduction of the aminoribose was then carried out by glycosylation with the azidofluororibose derivative **5**, as above, and afforded the protected inhibitor **16** displaying three structural fragments (97% yield). To overcome the already observed uracil double bond reduction in hydrogenolysis conditions, the azido group reduction was achieved in Staudinger conditions in the presence of 1,2-bis(diphenylphosphino)ethane [24] yielding **17** (92%). Acidolysis of the isopentylidene ketal, followed by silyl deprotection respectively gave **18** (57%) and **19** (87%).

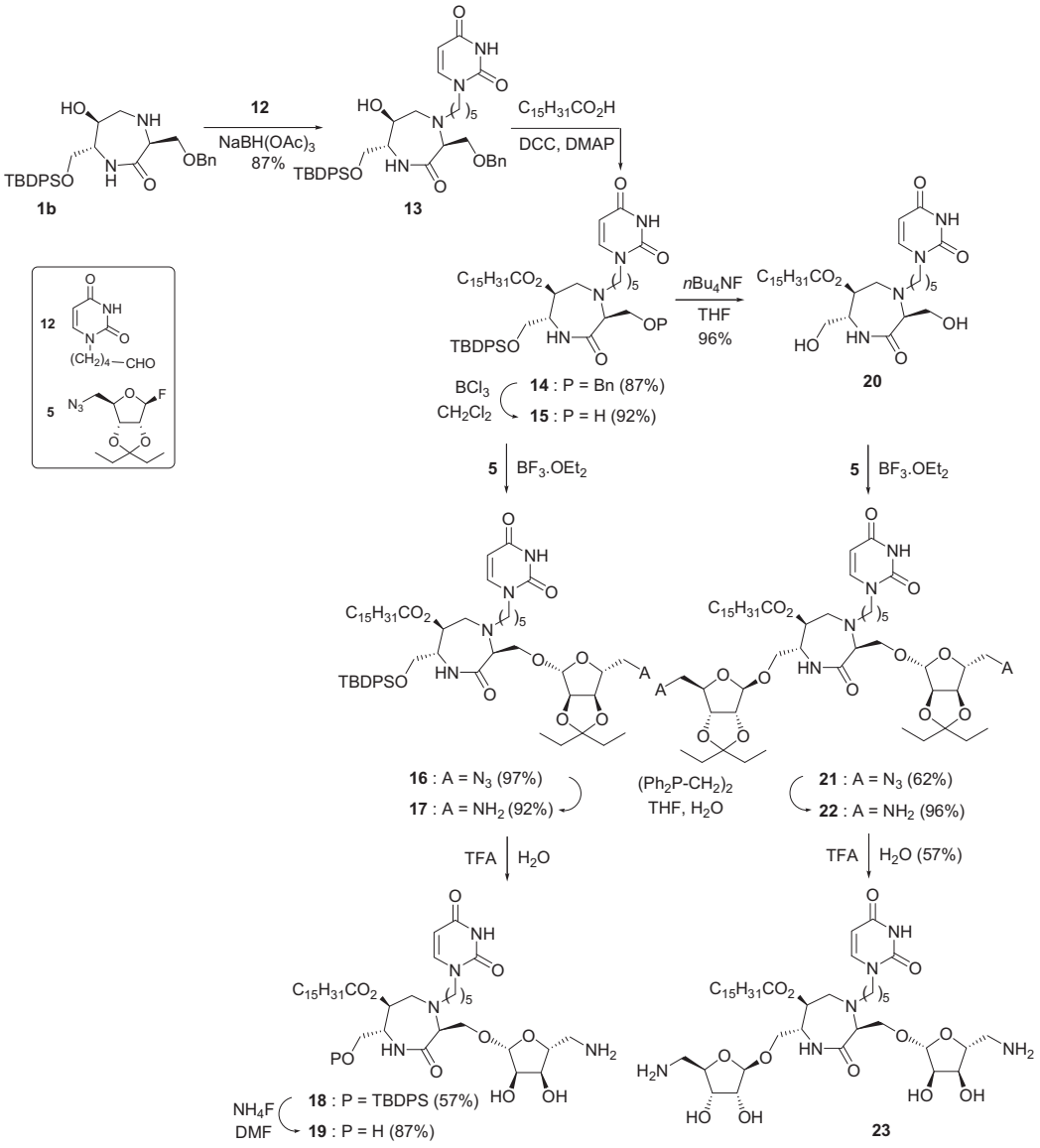
Alternatively, taking into account both the structure of the diol **20** and the presence of several sugars in some natural inhibitors of MraY such as tunicamycins (Fig. 2), we have been aiming at introducing two ribose derivatives on the scaffold. Thus, we turned to silyl ether deprotection of **15** that could be efficiently achieved in the presence of ammonium fluoride, as above, yielding **20** in 96% yield. The resulting diol **20** was then involved in a double glycosylation reaction with the fluoroazidoribose **5** affording **21** in 62% yield. Azide reduction in Staudinger conditions gave the bis-amino derivative **22** (96%) and was followed by acidic hydrolysis of both isopentylidene ketals giving the targeted inhibitor **23** in 57% yield.

2.2. Biological studies

The *in vitro* biological evaluation of the resulting compounds on purified MraY was carried out as described in the experimental section (Table 1). The residual activity of the enzyme was measured in the presence of 1 mM of the tested compounds. For the most active inhibitors, the IC_{50} values were determined. Furthermore,



Scheme 1. Synthesis of inhibitors with two key fragments from diazepanone **1a**.



Scheme 2. Synthesis of inhibitors with three or four key fragments from diazepanone **1b**.

the inhibitors were also tested on the MurG enzyme which catalyzes the second membrane step of peptidoglycan biosynthesis (Fig. 1), leading to lipid II formation.

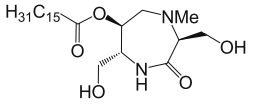
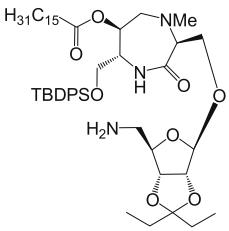
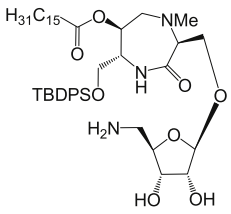
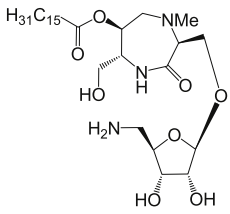
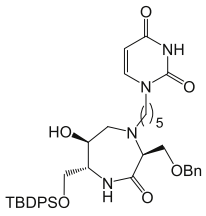
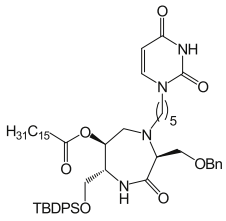
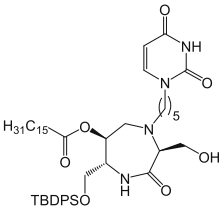
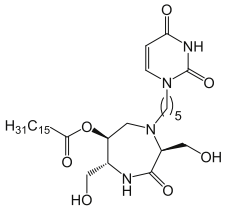
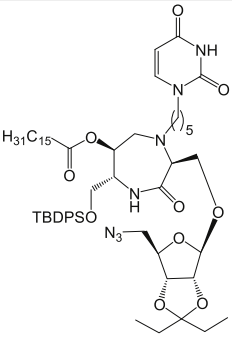
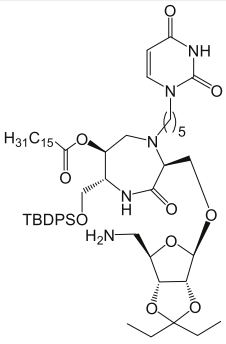
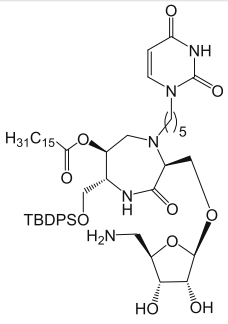
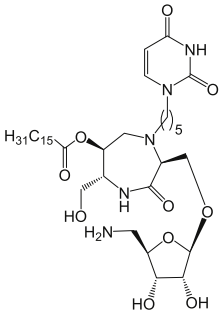
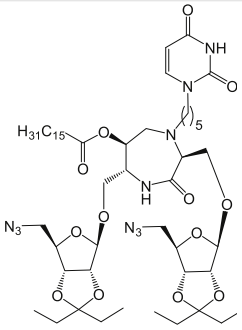
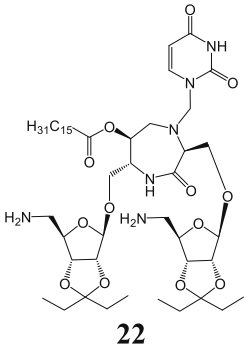
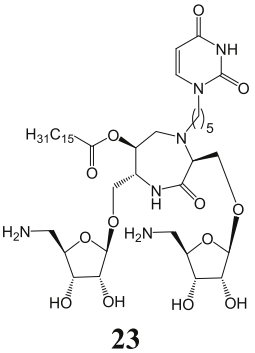
Concerning MraY inhibition, comparison of the results for both *N*-Me and *N*-alkyluracil-inhibitors shows that the latter are better inhibitors than the former as exemplified by the respective activities of **20** and **4**, **17** and **7**, **18** and **8**, **19** and **9**. In the *N*-alkyluracil series, the introduction of an aminoribose moiety on the diazepanone scaffold improves the inhibition. Indeed, compounds **16** and **18** exhibited better inhibitory activities than **15**, and **19** was a bit better than **20**. Finally, the best MraY inhibitor **18** resulted in an IC_{50} value of 120 μM and presented three key fragments on the scaffold: a palmitoyl chain, alkyluracil and aminoribose moieties, the remaining primary alcohol function being protected as a bulky hydrophobic silyl ether. Surprisingly, alcohol deprotection of compound **18** slightly decreased the inhibitory activity as compared to that of the parent compound, showing that a hydrophobic residue is better in this position. Furthermore, introduction of a supplementary aminoribose on the scaffold seems to lead to weakest inhibitors (compare the respective activities of **21** and **23** to that of **16** and **19**). Concerning MurG inhibition, several compounds caused enzymatic activity inhibition in the 100 μM range. It appears that reduction of the azido group into the corresponding amine(s) is crucial for inhibition as demonstrated by the respective activities of **17** and **16**, **22** and **21**.

3. Conclusion

The synthesis of a small library of MraY inhibitors has been developed in high yields according to a scaffold strategy based on diazepanone platforms. Indeed, such an heterocycle is the central core of liposidomycins, known natural inhibitors of MraY. Key structural fragments were introduced on these scaffolds to get simplified analogs of the natural compounds. Well-differentiated protected functions within these structures allowed sequential introduction of either one, two, three or four key fragments

Table 1

Inhibitory activity[a] of the synthesized compounds on the **MraY** and **MurG** enzymes

				
4	**7**	**8**	**9**	**13**
1740	1000	66% (2 mM)	75%	1600
N.D.[b]	N.D.	150	100%	100% (1 mM)
				
14	**15**	**20**	**16**	**17**
1400	380	270	190	620
100%	66%	100%	100%	110
				
18	**19**	**21**	**22**	**23**
120	220	420	310	2800
185	530	87%	100	130

[a]: Percentage of the residual activity measured at 1 mM concentration for MraY and at 2 mM concentration for MurG unless otherwise indicated or IC_{50} (μM). [b]: Not determined.

including a fatty acid chain, an alkyluracil residue mimicking the uridine present in liposidomycins and either one or two aminoribose moieties. Biological evaluation of the resulting inhibitors on both MraY and MurG activities resulted in IC_{50} values in the 100 μM range for the best compounds and showed that among the introduced fragments, three of them are important for activity since their absence led to lower activities, while the presence of a second aminoribose leads to weaker inhibitors. Interestingly the biological results suggest that the presence of a hydrophobic residue on the primary alcohol function of the scaffold is preferable to that of a free alcohol for MraY inhibition. Furthermore, a free primary amine on the ribose seems to be crucial to inhibit the MurG activity. Therefore, this study led to reaching some insight into the requirements for inhibition of the trans-membrane protein MraY.

4. Experimental

4.1. Chemical synthesis

^{1}H NMR (250 MHz) and ^{13}C NMR (63 MHz) spectra were recorded on a Bruker AM250 in $CDCl_3$ (unless otherwise indicated). ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded

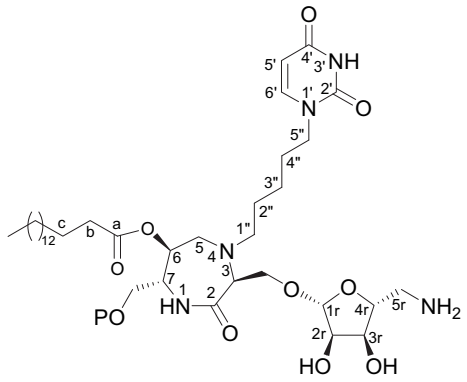


Fig. 6. Numbering of the compound **18**.

on a Bruker Avance or Avance II. Chemical shifts (δ) are reported in ppm and coupling constants are given in Hz. To facilitate the understanding of NMR spectroscopic data, the numbering of atoms for the following representative compound **18** is as indicated (Fig. 6).

Optical rotations were measured on a Perkin-Elmer 341 polarimeter with sodium (589 nm) or mercury (365 nm) lamp at 20 °C. Mass spectra, electrospray, chemical ionization (CI) and high resolution (HRMS) were recorded by the Service de Spectrométrie de Masse, ICSN Gif sur Yvette or Ecole Normale Supérieure, Paris. All reactions were carried out under a nitrogen atmosphere, and were monitored by thin-layer chromatography with Merck 60F-254 precoated silica (0.2 mm) on glass. Flash chromatography was performed with Merck Kieselgel 60 (200–500 μm); the solvent systems were given v/v. Spectroscopic ^{1}H and ^{13}C NMR, MS and/or analytical data were obtained using chromatographically homogeneous samples.

4.1.1. (3S,6S,7R)-3-Benzyloxymethyl-7-tert-butyldiphenylsilyloxymethyl-4-N-methyl-6-palmitoyloxy-1,4-diazepan-2-one **(2)**

To a solution of the alcohol **1a** (670 mg, 1.26 mmol) in CH_2Cl_2 (15 mL) were successively added palmitic acid (645 mg, 2.51 mmol), dicyclohexylcarbodiimide (579 mg, 2.51 mmol) and dimethylaminopyridine (37 mg, 0.25 mmol) and the mixture was stirred for 20 h at r.t. After filtration through a celite pad and concentration in vacuo, flash chromatography of the residue (cyclohexane/EtOAc 8:2) afforded 902 mg (93%) of the palmitic ester **2** as a colorless oil. R_f 0.25 (cyclohexane/EtOAc 8:2); $[\alpha]_D$ +19 (*c* 1.0, CH_2Cl_2); ^{1}H NMR δ 7.70–7.24 (m, 15H, H_{ar}), 6.07 (d,1H, $J_{H1-H7} = 6$ Hz, H_1), 5.02 (dl, 1H, H_6), 4.52 (AB, 2H, $J_{AB} = 15$ Hz, CH_2Ph), 4.35 (m, 1H, H_7), 3.82 (d, 2H, $J_{CH2OBn-H3} = 3.3$ Hz, CH_2OBn), 3.63 (dd, 1H, $J_{CH2aOSi-H7} = 3.3$ Hz, $J_{CH2OSi} = 11.4$ Hz, $CH_{2a}OSi$), 3.61 (dd, 1H, $J_{CH2bOSi-H7} = 3.3$ Hz, $J_{CH2OSi} = 11.4$ Hz, $CH_{2b}OSi$), 3.31 (t, 1H, $J_{H3-CH2OBn} = 3.3$ Hz, H_3), 3.12 (dd, 1H, $^2J_{H5a-H5b} = 15.7$ Hz , $^3J_{H5a-H6} = 3.3$ Hz, H_{5a}), 2.85 (dd, 1H, $J_{H5a-H5b} = 15.7$ Hz, $J_{H5b-H6} = 1$ Hz, H_{5b}), 2.39 (s, 3H, NMe), 2.10 (t, 2H, $J_{CH2b-CH2c} = 7.7$ Hz, H_b), 1.52 (m, 2H, H_c), 1.24 (sl, 24H, $(CH_2)_{12}$), 1.03 (s, 9H, *t*Bu), 0.86 (t, 3H, $J_{CH3-CH2} = 6.7$ Hz, CH_3); ^{13}C NMR δ 173.5 (C_2), 173.1 (C_a), 138.2, 135.7, 130.2, 128.4, 128.1, 127.7 (C_{ar}), 74.6 (C_3), 73.6 (CH_2Ph), 71.3 (C_6), 70.2 (CH_2OBn), 60.9 (CH_2OSi), 58.0 (C_5), 52.3 (C_7), 44.6 (NMe), 32.1, 29.6, 29.9, 25.0, 22.9 (–$(CH_2)_{14}$), 27.0, 19.3 (*t*Bu), 14.3 (CH_3); MS (ESI$^+$) 771.5 $(M + H)^+$, 793.5 $(M + Na)^+$; HRMS (ESI$^+$) for $C_{47}H_{71}N_2O_5Si$ $(M + H)^+$ calcd 771.5132, found 771.5151.

4.1.2. (3S,6S,7R)-3-Hydroxymethyl-7-tert-butyldiphenylsilyloxymethyl-4-N-methyl-6-palmitoyloxy-1,4-diazepan-2-one **(3)**

A suspension of palladium black (100 mg) in acetic acid (5 mL) was saturated with dihydrogen. A solution of the benzyl ether **2** (110 mg, 0.14 mmol) in acetic acid (1 mL) was then added and the mixture was stirred under dihydrogen atmosphere for 20 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. Flash chromatography of the residue (cyclohexane/EtOAc 6:4) gave 74 mg (76%) of the alcohol **3** as a colorless oil. R_f 0.5 (cyclohexane/EtOAc 1:1); ^{1}H NMR δ 7.57–7.38 (m, 10H, H_{ar}), 6.03 (d,1H, $J_{H1-H7} = 5$ Hz, H_1), 5.04 (dl, 1H, H_6), 4.01 (m, 1H, H_7), 3.86 (d, 2H, $J_{CH2OH-H3} = 5$ Hz, CH_2OH), 3.71 (ABX, 2H, $J_{Ax} = 4$ Hz, $J_{AB} = 10.6$ Hz, CH_2OSi), 3.31 (t, 1H, $J_{H3-CH2OH} = 3.3$ Hz, H_3), 3.12 (dd, 1H, $J_{H5a-H5b} = 15.7$ Hz, $J_{H5a-H6} = 3.3$ Hz, H_{5a}), 2.85 (dd, 1H, $J_{H5a-H5b} = 15.7$ Hz, $J_{H5b-H6} = 1$ Hz, H_{5b}), 2.39 (s, 3H, NMe), 2.10 (m, 2H, H_b), 1.70–1.50 (m, 2H, H_c), 1.24 (sl, 24H, $(CH_2)_{12}$), 1.03 (s, 9H, *t*Bu), 0.86 (t, 3H, $J_{CH3-CH2} = 6.7$ Hz, CH_3); ^{13}C NMR δ 173.5 (C_2), 172.9 (C_a), 135.7, 132.5, 130.2, 128.0 (C_{ar}), 73.3 (C_3), 71.3 (C_6), 62.5 (CH_2OH , CH_2OSi), 58.0 (C_5), 53.7 (C_7), 44.5 (NMe), 35.0 (Cb), 32.1, 29.7, 25.0, 22.8 (-$(CH_2)_{13}$), 27.2, 19.5 (*t*Bu), 14.5 (CH_3); MS (ESI$^+$) 681.4 $(M + H)^+$, 703.3 $(M + Na)^+$; HRMS (ESI$^+$) for $C_{40}H_{65}N_2O_5Si$ $(M + H)^+$ calcd 681.4663, found 681.4639.

4.1.3. (3S,6S,7R)-3,7-Dihydroxymethyl-4-N-methyl-6-palmitoyloxy-1,4-diazepan-2-one **(4)**

To a solution of silyl ether **3** (50 mg, 72 μmol) in DMF (5 mL) was added ammonium fluoride (9 mg, 210 μmol) and the mixture was stirred for 18 h at r.t. After concentration in vacuo, trituration of the residue in diethyl ether led to 32 mg (100%) of the diol **4** as a hygroscopic solid. $[\alpha]_D$ +10 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (MeOD) δ 4.99 (dt, 1H, $J_{H6-H7} = 8.3$ Hz, $J_{H6-H5} = 2.7$ Hz, H_6), 4.17 (dt, 1H, $J_{H7-H6} = 8.3$ Hz, $J_{H7-H9} = 4.5$ Hz, H_7), 3.95 (dd, 1H, $J_{H3-H8a} = 4.5$ Hz, $J_{H8a-H8b} = 11.8$ Hz, H_{8a}), 3.85 (dd, 1H, $J_{H3-H8b} = 4.5$ Hz, $J_{H8a-H8b} = 11.8$ Hz, H_{8b}), 3.75 (dd, 1H, $J_{H7-H9a} = 4.3$ Hz, $J_{H9a-H9b} = 11.8$ Hz, H_{9a}), 3.67 (dd, 1H, $J_{H7-H9b} = 5.3$ Hz, $J_{H9a-H9b} = 11.8$ Hz, H_{9b}), 3.31 (t, 1H, $J_{H3-H8} = 4.4$ Hz, H_3), 3.17 (dd, 1H, $J_{H5a-H6} = 2.7$ Hz, $J_{H5b-H5a} = 15.5$ Hz, H_{5a}), 3.01 (dd, 1H, $J_{H5b-H6} = 2.7$ Hz, $J_{H5b-H5a} = 15.5$ Hz, H_{5b}), 2.47 (s, 3H, NMe), 2.37 (t, 2H, $J_{CH2b-CH2c} = 7.3$ Hz, H_b), 1.70–1.50 (m, 2H, H_c), 1.32 (s, 24H, $(CH_2)_{12}$), 0.93 (t, 3H, $J_{CH3-CH2} = 6.6$ Hz, CH_3); ^{13}C NMR (MeOD) δ 176.8 (C_2), 174.5 (C_a), 74.8 (C_3), 72.7 (C_6), 62.0 (C_8), 61.1 (C_9), 58.6 (C_5), 55.1 (C_7), 42.7 (NMe), 35.3 (C_b), 33.1, 30.8, 26.0, 23.7 (–$(CH_2)_{13}$), 14.4 (CH_3); MS (ESI$^+$) 465.3 $(M + Na)^+$; HRMS (ESI$^+$) for $C_{24}H_{46}N_2O_5Na$ $(M + Na)^+$ calcd 465.3304, found 465.3297.

4.1.4. (3S,6S,7R)-7-tert-Butyldiphenylsilyloxymethyl-4-N-methyl-6-palmitoyloxy-3-(5′-azido-5′-deoxy-2′,3′-O-isopentylidene-β-D-ribos-1′-yl-methyl)-1,4-diazepan-2-one **(6)**

To a solution of alcohol **3** (377 mg, 554 μmol) in CH_2Cl_2 (16 mL) at r.t. was added the fluororibose derivative **5** (204 mg, 831 μmol) and molecular sieves 4 Å (1.5 g). After 1 h stirring, the mixture was cooled to −78 °C and boron trifluoride etherate (104 μL, 830 μmol) was dropwise added. The mixture was slowly warmed to 20 °C in 5 h and was hydrolyzed by the addition of a saturated $NaHCO_3$ aqueous solution. After CH_2Cl_2 extractions, the combined organic layers were washed (H_2O), dried ($MgSO_4$), filtered and concentrated in vacuo. Flash chromatography of the residue (cyclohexane/EtOAc 7:3) afforded 435 mg (87%) of the ribosyl diazepanone **6** as an oil. R_f 0.37 (cyclohexane/EtOAc 7:3); $[\alpha]_D$ +13 (*c* 1.0, CH_2Cl_2); ^{1}H NMR δ 7.62–7.36 (m, 10H, H_{ar}), 6.01 (d, 1H, $J_{H1-H7} = 6.2$ Hz, H_1), 5.14 (s, 1H, $H_{1'}$), 4.97 (dd, 1H, $J_{H6-H7} = 9.5$ Hz, $J_{H6-H5} = 2$ Hz, H_6), 4.53 (d, 1H, $J_{H3'-H2'} = 6$ Hz, $H_{3'}$), 4.45 (d, 1H, $J_{H2'-H3'} = 6$ Hz, $H_{2'}$), 4.27 (t, 1H, $J_{H4'-H5'} = 7.7$ Hz, $H_{4'}$), 4.19 (m, 1H, H_7), 4.01 (dd, 1H, $J_{CH2OR} = 10.6$ Hz, $J_{CH2aOR-H3} = 3.6$ Hz, $CH_{2a}OR$), 3.74–3.66 (m, 3H, CH_2OSi, $CH_{2b}OR$), 3.41 (dd, 1H, $J_{H5'a-H5'b} = 12.8$ Hz, $J_{H5'a-H4'} = 7.7$ Hz, $H_{5'a}$), 3.29 (t, 1H, $J_{H3-CH2aOR} = 3.6$ Hz, H_3), 3.17–3.06 (m, 2H, H_{5b}, $H_{5'b}$), 2.83 (dd, 1H, $J_{H5a-H5b} = 15.7$ Hz, H_{5a}), 2.38 (s, 3H, NMe), 2.15 (m, 2H, H_b); 1.67, 1.46 (2q, 4H, $J_{CH2-CH3} = 6.7$ Hz, $C(\underline{CH_2}CH_3)_2$), 1.55–1.45 (m, 2H, H_c), 1.24 (sl, 24H, $(CH_2)_{12}$), 1.04 (s, 9H, *t*Bu), 0.90 (2t, 6H, $J_{CH3-CH2} = 6.7$ Hz, $C(CH_2\underline{CH_3})_2$), 0.86 (t, 3H, $J_{CH3-CH2} = 7.5$ Hz, CH_3);

^{13}C NMR δ 173.3, 173.0 (C_2, C_a), 136.0, 135.9, 132.9, 132.6, 130.5, 128.4, 128.3 (C_{ar}), 117.5 (C(CH$_2$CH$_3$)$_2$), 109.4 ($C_{1'}$), 86.2, 85.9 ($C_{4'}$, $C_{2'}$), 82.8 ($C_{3'}$), 74.2 (C_3), 71.7 (C_6), 67.4 (CH_2OR), 61.7 (CH_2OSi), 58.0 (C_5), 53.6 (C_7), 46.2 ($C_{5'}$), 44.8 (NMe), 32.3 (C_b), 30.5–23.1 (–(CH_2)$_{13}$, C(CH$_2$CH$_3$)$_2$), 27.3, 19.6 (*t*Bu), 14.3 (CH_3), 8.8, 7.8 (C(CH$_2$CH$_3$)$_2$); MS (ESI$^+$) 687.4 (100%), 928.6 (M + H)$^+$, 703.3 (M + Na)$^+$; HRMS (ESI$^+$) for $C_{50}H_{79}N_5O_8NaSi$ (M + Na)$^+$ calcd 928.5596, found 928.5523.

4.1.5. (3S,6S,7R)-7-tert-Butyldiphenylsilyloxymethyl-4-N-methyl-6-palmitoyloxy-3-(5′-amino-5′-deoxy-2′,3′-O-isopentylidene-β-D-ribos-1′-yl-methyl)-1,4-diazepan-2-one (7)

To a solution of the azido-ribosyl-diazepanone **6** (305 mg, 337 μmol) in methanol (7 mL) was added palladium black (200 mg) and the mixture was stirred under dihydrogen atmosphere for 1 h. The mixture was then filtered through a celite pad and concentrated in vacuo. Flash chromatography of the residue (CH_2Cl_2/MeOH/Et$_3$N 9:1:3‰) gave 245 mg (83%) of the expected amine **7** as a colorless oil. R_f 0.39 (CH_2Cl_2/MeOH/Et$_3$N 9:1:3‰); $[\alpha]_D$ +14 (*c* 1.0, CH_2Cl_2); ^{1}H NMR δ 7.60–7.34 (m, 10H, H_{ar}), 6.07 (d, 1H, J_{H1-H7} = 6.0 Hz, H_1), 5.07 (s, 1H, $H_{1'}$), 4.97 (dd, 1H, J_{H6-H7} = 9.5 Hz, J_{H6-H5} = 3.4 Hz, H_6), 4.51 (d, 1H, $J_{H3'-H2'}$ = 6 Hz, $H_{3'}$), 4.42 (d, 1H, $J_{H2'-H3'}$ = 6 Hz, $H_{2'}$), 4.24 (m, 1H, H_7), 4.17 (t, 1H, $J_{H4'-H5'}$ = 7.1 Hz, $H_{4'}$), 4.00 (dd, 1H, J_{CH2OR} = 10.5 Hz, $J_{CH2aOR-H3}$ = 3.6 Hz, $CH_{2a}OR$), 3.75–3.62 (m, 3H, CH_2OSi, $CH_{2b}OR$), 3.26 (t, 1H, $J_{H3-CH2OR}$ = 3.6 Hz, H_3), 3.07 (dd, 1H, $J_{H5a-H5b}$ = 15.6 Hz, J_{H5a-H6} = 3.4 Hz, H_{5a}), 2.83 (dl, 1H, $J_{H5a-H5b}$ = 15.6 Hz, H_{5b}), 2.66 (dl, 2H, $^3J_{H5'-H4'}$ = 7.1 Hz, $H_{5'}$), 2.37 (s, 3H, NMe), 2.13 (AB of ABX, 2H, J_{AB} = 15.8 Hz, $J_{Bx} = J_{Ax}$ = 7.7 Hz, H_b), 1.63, 1.47 (2q, 4H, $J_{CH2-CH3}$ = 7.4 Hz, C(CH$_2$CH$_3$)$_2$), 1.58–1.38 (m, 2H, H_c), 1.22 (sl, 24H, $(CH_2)_{12}$), 1.03 (s, 9H, *t*Bu), 0.87, 0.78 (2t, 6H, $J_{CH3-CH2}$ = 7.4 Hz, C(CH$_2$CH$_3$)$_2$), 0.84 (t, 3H, $J_{CH3-CH2}$ = 7.5 Hz, CH_3); ^{13}C NMR δ 173.0 (C_a), 170.7 (C_2), 135.7, 135.6, 132.6, 132.4, 130.2, 128.1 (C_{ar}), 116.7 (C(CH$_2$CH$_3$)$_2$), 109.2 ($C_{1'}$), 88.7 ($C_{4'}$), 86.0 ($C_{3'}$), 82.7 ($C_{2'}$), 73.9 (C_3), 71.4 (C_6), 67.4 (CH_2OR), 61.4 (CH_2OSi), 58.1 (C_5), 52.5 (C_7), 45.3 ($C_{5'}$), 44.2 (NMe), 34.4 (C_b), 32.03, 29.5, 25.0, 22.8 (–(CH_2)$_{13}$, C(CH$_2$CH$_3$)$_2$), 27.0, 19.3 (*t*Bu), 14.3 (CH_3), 8.5, 7.5 (C(CH$_2$CH$_3$)$_2$); MS (ESI$^+$) 681.4 (100%), 880.5 (M + H)$^+$, 902.5 (M + Na)$^+$; HRMS (ESI$^+$) for $C_{50}H_{81}N_3O_8NaSi$ (M + Na)$^+$ calcd 902.5691, found 902.5569.

4.1.6. (3S,6S,7R)-7-tert-Butyldiphenylsilyloxymethyl-4-N-methyl-6-palmitoyloxy-3-(5′-amino-5′-deoxy-β-D-ribos-1′-yl-methyl)-1,4-diazepan-2-one (8)

At 20 °C, the aminoribosyl-diazepanone **7** was stirred for 1.5 h in a 4/1 mixture of trifluoroacetic acid (4 mL) and water (1 mL). The mixture was then concentrated in vacuo and purification on Waters SEP-PAK® cartridge (CH_2Cl_2/MeOH 8:2) afforded 30 mg (65%) of **8** as a colorless oil. R_f 0.23 (CH_2Cl_2/MeOH 8:2); ^{1}H NMR (250 MHz, MeOD) δ 7.67–7.37 (m, 10H, H_{ar}), 5.06 (dl, 1H, J_{H6-H7} = 8.6 Hz, H_6), 4.92 (s, 1H, $H_{1'}$), 4.17 (m, 1H, H_7), 4.04–3.86 (m, 4H, $CH_{2a}OR$, $H_{4'}$, $H_{3'}$, $H_{2'}$), 3.83–3.74 (m, 3H, CH_2OSi, $CH_{2b}OR$), 3.38 (t, 1H, $J_{H3-CH2OR}$ = 4.1 Hz, H_3), 3.07 (m, 2H, H_{5a}, $H_{5'a}$), 2.92 (dd, 1H, $J_{H5b-H5a}$ = 15.4 Hz, J_{H5b-H6} = 2.0 Hz, H_{5b}), 2.82 (dd, 1H, $J_{H5'b-H5'a}$ = 13.1 Hz, $J_{H5'b-H4'}$ = 8.5 Hz, $H_{5'b}$), 2.40 (s, 3H, NMe), 2.20 (AB of ABX_2, 2H, J_{CH2b} = 15.7 Hz, $J_{CH2b-CH2c}$ = 7.5 Hz, H_b), 1.52 (m, 2H, H_c), 1.28 (s, 24H, $(CH_2)_{12}$), 1.07 (s, 9H, *t*Bu), 0.89 (t, 3H, $J_{CH3-CH2}$ = 7.5 Hz, CH_3); ^{13}C NMR (63 MHz, MeOD) δ 175.8 (C_2), 174.2 (C_a), 136.8, 133.9, 133.7, 131.2, 129.1, 129.0 (C_{ar}), 109.2 ($C_{1'}$), 81.7 ($C_{3'}$), 76.2 ($C_{4'}$), 74.1 ($C_{2'}$), 73.6 (C_6), 72.4 (C_3), 67.7 (CH_2OR), 63.1 (CH_2OSi), 58.6 (C_5), 54.9 (C_7), 44.9 ($C_{5'}$), 42.8 (NMe), 35.2 (C_b), 33.2–23.8 (-(CH_2)$_{13}$), 27.5, 20.1 (*t*Bu), 14.5 (CH_3); MS (ESI$^+$) 812.4 (100%, M + H)$^+$; HRMS (ESI$^+$) for $C_{45}H_{74}N_3O_8Si$ (M + H)$^+$: calcd 812.5245, found 812.5209.

4.1.7. (3S,6S,7R)-3-(5′-Amino-5′-deoxy-β-D-ribos-1′-yl-methyl)-7-hydroxymethyl-4-N-methyl-6-palmitoyloxy-1,4-diazepan-2-one (9)

To a solution of the silyl ether **8** (46 mg, 56 μmol) in DMF (4 mL) was added NH_4F (2.1 mg, 56 μmol) and the reaction mixture was stirred at r.t. for 15 h NH_4F was filtered off and the reaction mixture was concentrated under reduced pressure. The residue was than carefully rinsed with ether (3 × 2 mL) and dried in vacuo to afford the alcohol **9** (16 mg, 50%) as an oil. $[\alpha]_D$ +32 (*c* 1.0, MeOH); ^{1}H NMR (500 MHz, MeOD, 330 K) δ 4.94 (s, 1H, $H_{1'}$), 4.87 (dt, 1H, J_{H6-H7} = 9.2 Hz, J_{H6-H5} = 2 Hz, H_6), 4.18 (ddd, 1H, J_{H7-H6} = 9.2 Hz, $J_{H7-CHbOH}$ = 6.2 Hz, $J_{H7-CHaOH}$ = 3.6 Hz, H_7), 4.12–4.04 (m, 2H, $H_{3'}$, $H_{2'}$), 4.01 (m, 2H, $CH_{2a}Or$, $H_{4'}$), 3.88 (dd, 1H, J_{CH2Or} = 10.5 Hz, $J_{CHbOr-H3}$ = 3.7 Hz, $CH_{2b}OR$), 3.69 (dd, 1H, J_{CH2OH} = 11.5 Hz, $J_{CHaOH-H7}$ = 3.7 Hz, CH_aOH), 3.62 (dd, 1H, J_{CH2OH} = 11.5 Hz, $J_{CHbOH-H7}$ = 6.1 Hz, CH_bOH), 3.35 (t, 1H, $J_{H3-CH2OR}$ = 3.7 Hz, H_3), 3.19 (sl, 1H, $H_{5'a}$), 3.07 (dd, 1H, $J_{H5a-H5b}$ = 15.5 Hz, J_{H5a-H6} = 3.7 Hz, H_{5a}), 3.00 (m, 1H, H_{5b}), 2.89 (dd, 1H, $J_{H5a-H5b}$ = 15.5 Hz, J_{H5b-H6} = 2 Hz, H_{5b}), 2.41 (s, 3H, NMe), 2.34 (t, 2H, $J_{CH2b-CH2c}$ = 7.3 Hz, H_b), 1.63 (tt, 2H, H_c, J_{Hc-Hb} = J_{Hc-Hd} = 7.5 Hz), 1.28 (s, 24H, $(CH_2)_{12}$), 0.88 (t, 3H, $J_{CH3-CH2}$ = 7.5 Hz, CH_3); ^{13}C NMR (125 MHz, MeOD) δ 176.2 (C_2), 174.6 (C_a), 109.3 ($C_{1'}$), 80.9 ($C_{3'}$), 76.4 ($C_{4'}$), 74.6 ($C_{2'}$, C_3), 73.6 (C_6), 68.2 (CH_2OR), 61.4 (CH_2OH), 59.4 (C_5), 55.3 (C_7), 44.9 ($C_{5'}$), 44.1 (NMe), 35.6 (C_b), 33.4–24.1 (-(CH_2)$_{13}$), 14.8 (CH_3); MS (ESI$^+$) 574.2 (100%, M + H)$^+$; HRMS (ESI$^+$) for $C_{29}H_{55}N_3O_8Na$ (M + Na)$^+$ calcd 596.3887, found 596.3890.

4.1.8. (3S,6S,7R)-3-(Benzyloxymethyl)-7-(hydroxymethyl)-6-hydroxy-4-N-methyl-1,4-diazepan-2-one (10)

To a solution of the diazepanone **1a** (147 mg, 276 μmol) in CH_2Cl_2 (6.3 mL) at r.t. was added a solution of tetrabutylammonium fluoride in THF (1 M, 328 μL, 1.1 mmol). After 2 h stirring, the mixture was concentrated in vacuo and flash chromatography of the residue (CH_2Cl_2/MeOH/Et$_3$N 9:1:3‰) afforded 81 mg (100%) of the diol **10** as a white foam. R_f 0.3 (CH_2Cl_2/MeOH/Et$_3$N 9:1:3‰); $[\alpha]_D$ +13 (*c* 1.0, CH_2Cl_2); ^{1}H NMR δ 7.29 (m, 5H, H_{ar}), 6.75 (d, 1H, J_{H1-H7} = 3.8 Hz, H_1), 4.54 (s, 2H, CH_2Ph); 3.92 (m, 1H, H_7), 3.84–3.75 (m, 4H, H_6, CH_2OBn, $CH_{2a}OH$), 3.77 (dd, 1H, J_{CH2OH} = 11.3 Hz, $J_{CH2bOH-H7}$ = 3.4 Hz, $CH_{2b}OH$), 3.34 (t, 1H, $J_{H3-CH2OBn}$ = 3.7 Hz, H_3), 3.09 (dd, 1H, $J_{H5a-H5b}$ = 14.8 Hz, J_{H5a-H6} = 3.4 Hz, H_{5a}), 2.79 (dd, 1H, $J_{H5a-H5b}$ = 14.8 Hz, J_{H5b-H6} = 2.0 Hz, H_{5b}), 2.44 (s, 3H, NMe); ^{13}C NMR δ 174.4 (C_2), 137.9, 128.5, 127.8, 127.7 (C_{ar}), 74.1 (C_3), 73.5 (CH_2Ph), 69.4 (CH_2OBn), 68.2 (C_6), 60.2 (C_5, CH_2OH), 56.4 (C_7), 45.0 (NMe); MS (ESI$^+$) 295 (M + H)$^+$, 317 (M + Na)$^+$.

4.1.9. (3S,6S,7R)-3,7-Dihydroxymethyl-6-hydroxy-4-N-methyl-1,4-diazepan-2-one (11)

To a solution of benzyl ether **10** (40 mg, 136 μmol) in THF (5 mL) was added palladium black (80 mg) and the mixture was stirred under dihydrogen atmosphere for 24 h. Monitoring of the reaction by thin-layer chromatography (CH_2Cl_2/MeOH 9:2) revealed that the conversion was not complete. The mixture was filtered through a celite pad, concentrated in vacuo and the same procedure was repeated. TLC control showed that the starting material was less than 10%. The mixture was filtered through a celite pad and concentrated in vacuo. Addition of cold CH_2Cl_2 allowed partial recovery of pure triol **11** as a solid. ^{1}H NMR (500 MHz, D_2O) δ 4.05 (dd, 1H, $J_{H8a-H8b}$ = 12.2 Hz, J_{H8a-H3} = 5.6 Hz, H_{8a}), 3.97–3.77 (m, 5H, H_{8b}, H_6, H_7, H_9), 3.42 (t, 1H, J_{H3-H8} = 5.6 Hz, H_3), 3.13 (dd, 1H, $J_{H5a-H5b}$ = 15.4 Hz, J_{H5a-H6} = 3.0 Hz, H_{5a}), 2.99 (dd, 1H, $J_{H5a-H5b}$ = 15.4 Hz, J_{H5b-H6} = 3.9 Hz, H_{5b}), 2.50 (s, 3H, NMe); ^{13}C NMR (D_2O) δ 177.4 (C_2), 73.0 (C_3), 67.4 (C_6), 60.7 (C_9), 59.9 (C_8), 58.9 (C_5), 56.6 (C_7), 42.5 (NMe); MS (ESI$^+$) 227.1 (M + Na)$^+$; HRMS (ESI$^+$) for $C_8H_{16}N_2O_4Na$ (M + Na)$^+$ calcd 227.1008, found 227.1005.

4.1.10. (3S,6S,7R)-3-(Benzyloxymethyl)-7-(tert-butyldiphenylsilyloxymethyl)-4-N-(5″-(uracil-1′-yl)pentyl)-6-hydroxy-1,4-diazepan-2-one ***(13)***

To a solution of the aldehyde **12** (353 mg, 1.02 eq., 1.80 mmol) in 1,2-dichloroethane (18.6 mL) was added sodium sulfate (5.11 g, 20 eq., 36.0 mmol). After stirring under argon atmosphere at r.t. for 10 min a solution of the diazepanone **1b** (917 mg, 1 eq., 1.77 mmol) in 1,2-dichloroethane (36.7 mL) was added and the reaction mixture was stirred for additional 19 h. Sodium triacetoxyborohydride (1.12 g, 3 eq., 5.26 mmol) was then added and the reaction mixture was stirred for additional 24 h. The resulting suspension was filtered through a celite pad and the reaction was quenched by addition of saturated aqueous solution of $NaHCO_3$ (20 mL). Layers were separated and the aqueous one was extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were dried ($MgSO_4$), filtered and concentrated in vacuo. Flash chromatography of the residue (AcOEt/MeOH/Et_3N 96:4:3‰) afforded **13** (1.07 g, 87%) as a white foam. R_f 0.26 (AcOEt/MeOH/Et_3N, 96:4:3‰); $[\alpha]_D$ +17 (*c* 1.0, CH_2Cl_2); ^1H NMR δ (500 MHz) 8.62 (*bs*, 1H, NH_{uracil}), 7.67–7.24 (m, 15H, H_{ar}), 7.06 (d, 1H, $J_{H6'-H5'}$ = 8.0 Hz, $H_{6'}$), 6.08 (d, 1H, J_{H1-H7} = 5.4 Hz, H_1), 5.65 (d, 1H, $J_{H5'-H6'}$ = 7.9 Hz, $H_{5'}$), 4.56, 4.52 (AB, 2H, J_{AB} = 12.1 Hz, CH_2Ph), 3.96–3.92 (m, 1H, H_7), 3.90–3.75 (m, 6H, CH_2OSi, CH_2OBn, H_6, OH), 3.67 (t, 2H, $J_{H5''-H4''}$ = 7.3 Hz, $H_{5''}$), 3.60 (t, 1H, $J_{H3-CH2OBn}$ = 3.8 Hz, H_3), 3.06 (dd, 1H, $J_{H5a-H5b}$ = 15.0 Hz, J_{H5b-H6} = 3.2 Hz, H_{5b}), 2.84 (dd, 1H, $J_{H5a-H5b}$ = 14.9 Hz, J_{H5a-H6} = 2.2 Hz, H_{5a}), 2.64–2.58 (m, 1H, $H_{1''}b$), 2.51–2.45 (m, 1H, $H_{1''}a$), 1.73–1.60 (m, 2H, $H_{4''}$), 1.57–1.42 (m, 2H, $H_{2''}$), 1.41–1.22 (m, 2H, $H_{3''}$), 1.09 (s, 9H, *t*Bu); ^{13}C NMR δ 173.8 (C_2), 163.6 ($C_{4'}$), 150.8 ($C_{2'}$), 144.3 ($C_{6'}$), 138.0, 135.7, 132.6, 130.1, 128.4, 128.0, 127.9, 127.6 (C_{ar}), 102.2 ($C_{5'}$), 73.3 (CH_2Ph), 72.2 (C_3), 70.4 (CH_2OBn), 69.4 (C_6), 61.8 (CH_2OSi), 57.0 (C_5), 55.5 (C_7), 54.7 ($C_{1''}$), 48.5 ($C_{5''}$), 29.6 ($C_{4''}$), 28.7, 19.3 (*t*Bu), 26.9 ($C_{2''}$), 23.8 ($C_{3''}$); MS (ESI^+) 699.3 $(M + H)^+$, 721.3 $(M + Na)^+$; HRMS (ESI^+) calcd for $C_{39}H_{51}N_4O_6Si$ $(M + H)^+$ 699.3578, found 699.3571.

4.1.11. (3S,6S,7R)-3-(Benzyloxymethyl)-7-(tert-butyldiphenylsilyloxymethyl)-4-N-(5″-(uracil-1′-yl)pentyl)-6-palmitoyloxy-1,4-diazepan-2-one ***(14)***

To a solution of the secondary alcohol **13** (1.06 g, 1.52 mmol) in CH_2Cl_2 (18 mL) was added palmitic acid (778 mg, 3.04 mmol), *N*,*N*′-dicyclohexylcarbodiimide (626 mg, 3.04 mmol) and 4-dimethylaminopyridine (37 mg, 0.30 mmol). After stirring at r.t. overnight and under argon atmosphere, the reaction mixture was filtered through a celite pad and concentrated under reduced pressure. Flash chromatography of the residue (EtOAc/cyclohexane 8:2) afforded **14** (1.24 g, 87%) as a white stable foam. R_f 0.18 (EtOAc/cyclohexane 7:3); $[\alpha]_D$ +47 (*c* 1.0, CH_2Cl_2); $[\alpha]_{365}$ + 161 (*c* 1.0, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ 8.58 (s, 1H, $H_{3'}$), 7.64–7.24 (m, 15H, H_{ar}), 7.02 (d, 1H, $J_{H6'-H5'}$ = 7.9 Hz, $H_{6'}$), 6.12 (d, 1H, J_{H1-H7} = 4 Hz, H_1), 5.63 (d, 1H, $J_{H5'-H6'}$ = 7.9 Hz, $H_{5'}$), 5.02–4.99 (m, 1H, H_6), 4.56, 4.54 (AB, 2H, J_{AB} = 12.0 Hz, CH_2Ph), 4.33–4.28 (m, 1H, H_7), 3.85 (d, 2H, J_{CH2OBn} = 3.8 Hz, CH_2OBn), 3.70–3.62 (m, 4H, CH_2OSi and $H_{5''}$), 3.60 (t, 1H, $J_{H3-CH2OBn}$ = 3.6 Hz, H_3), 3.07 (dd, 1H, $J_{H5b,H5a}$ = 15.6 Hz, $J_{H5b,H6}$ = 3.4 Hz, H_{5b}), 2.97 (dd, 1H, $J_{H5a,H5b}$ = 15.7 Hz, $J_{H5a,H6}$ = 2.3 Hz, H_{5a}), 2.63–2.58 (m, 1H, $H_{1b''}$), 2.52–2.47 (m, 1H, $H_{1a''}$), 2.16–2.06 (m, 2H, H_b), 1.66–1.61 (m, 2H, H_c), 1.52–1.50 (m, 2H, $H_{4''}$), 1.45–1.26 (m, 28H, $(CH_2)_{12}$, $H_{3''}$,$H_{2''}$), 1.08 (s, 9H, *t*Bu), 0.90 (t, 3H, $J_{CH3-CH2}$ = 6.9 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.7(C_3), 172.7(C_a), 163.1 ($C_{4'}$), 150.4 ($C_{2'}$), 144.2 ($C_{6'}$), 138.0, 135.6, 135.5, 132.5, 132.4, 130.1, 130.0, 128.3, 127.9, 127.8, 127.6, 127.4, (C_{ar}), 102.0 ($C_{5'}$), 77.2 (C_6), 73.3 (CH_2Ph), 71.8 (C_3), 70.5 (CH_2OBn), 61.0 (CH_2OSi), 53.8 (C_5), 53.6 ($C_{1''}$), 52.6 (C_7), 48.7 ($C_{5''}$), 34.3 (C_b), 31.9–29.2 (-$(CH_2)_{12}$), 28.8 ($C_{2''}$), 26.9, 19.2 (*t*Bu), 26.7 ($C_{4''}$), 24.8 (C_c), 23.7 ($C_{3''}$), 22.7 (CH_3-$\underline{C}H_2$), 14.1 (CH_3); MS (ESI^+) 937.5 $(M + H)^+$, 959.6 $(M + Na)^+$; HRMS (ESI^+) calcd. for $C_{55}H_{80}N_4O_7SiNa$ $(M + Na)^+$ 959.5694, found 959.5690.

4.1.12. (3S,6S,7R)-7-(tert-Butyldiphenylsilyloxymethyl)-3-(hydroxymethyl)-4-N-(5″-(uracil-1′-yl)pentyl)-6-palmitoyloxy-1,4-diazepan-2-one ***(15)***

To a solution of **14** (800 mg, 854 μmol) in anhydrous CH_2Cl_2 (17 mL) was added dropwise BCl_3 (1.0 M in CH_2Cl_2, 3.42 mL) at −78 °C under argon atmosphere. The resulting solution was allowed to warm to −65 °C in 3 h and was stirred for additional 2 h at the same temperature. The reaction mixture was quenched by the slow addition of a saturated aqueous $NaHCO_3$ solution (20 mL) at −78 °C, diluted with CH_2Cl_2 (17 mL), and warmed to room temperature. The layers were separated, and the aqueous layer was further extracted with CH_2Cl_2 (3 × 20 mL). The combined organic fractions were dried ($MgSO_4$) and concentrated in vacuo. Flash chromatography of the residue (CH_2Cl_2/MeOH 97:3 to 94:6) afforded **15** (666 mg, 92%) as a white stable foam. R_f 0.18 (CH_2Cl_2/MeOH 95:5); $[\alpha]_D$ +39 (*c* 1.0, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ 8.83 (s, 1H, $H_{3'}$), 7.64–7.38 (m, 10H, H_{ar}), 7.13 (d, 1H, $J_{H6'-H5'}$ = 7.9 Hz, $H_{6'}$), 6.15 (d, 1H, J_{H1-H7} = 5.4 Hz, H_1), 5.70 (dd, 1H, $J_{H5'-H6'}$ = 7.9 H, $J_{H5'-H3'}$ = 1.7 Hz, $H_{5'}$), 5.03 (td, 1H, J_{H6-H7} = 9.1 Hz, J_{H6-H5a} = J_{H6-H5b} ≈ 2.8 Hz, H_6), 4.09–4.05 (m, 1H, H_7), 3.92–3.90 (m, 2H, CH_2OH), 3.79 (dd, 1H, J_{CH2OSi} = 10.9 Hz, $J_{CHaOSi-H7}$ = 3.5 Hz, CH_aOSi), 3.72–3.67 (m, 3H, CH_bOSi, $H_{5''}$), 3.50 (t, 1H, $J_{H3-CH2OH}$ = 4.9 Hz, H_3), 3.09 (dd, 1H, $J_{H5a-H5b}$ = 15.7 Hz, J_{H5a-H6} = 3.2 Hz, H_{5a}), 3.01 (dd, 1H, $J_{H5b-H5a}$ = 15.7 Hz, J_{H5b-H6} = 2.6 Hz, H_{5b}), 2.68–2.63 (m, 2H, $H_{1b''}$, -OH), 2.54–2.49 (m, 1H, $H_{1a''}$), 2.19–2.09 (m, 2H, H_b), 1.74–1.64 (m, 2H, H_c), 1.54–1.43 (4H, $H_{2''}$, $H_{4''}$), 1.39–1.26 (m, 26H, $(CH_2)_{12}$, $H_{3''}$), 1.08 (s, 9H, *t*Bu), 0.90 (t, 3H, $J_{CH3-CH2}$ = 6.9 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3(C_2), 172.6(C_a), 163.7 ($C_{4'}$), 150.9 ($C_{2'}$), 144.2 ($C_{6'}$), 135.6, 135.5, 132.4, 132.2, 130.0, 127.9, 127.8 (C_{ar}), 102.2 ($C_{5'}$), 72.1 (C_3), 70.8 (C_6), 61.5 (CH_2OH), 61.4 (CH_2OSi), 53.4 (C_5), 53.2 (C_7), 52.6 ($C_{1''}$), 48.7 ($C_{5''}$), 34.2 (C_b), 31.9–28.6 ($(CH_2)_{12}$), 26.8, 19.1 (*t*Bu), 26.7 ($C_{2''}$), 24.7 ($C_{4''}$), 23.6 ($C_{3''}$), 22.6 (CH_3—$\underline{C}H_2$), 14.1 (CH_3); MS (ESI^+) 847.4 $(M + H)^+$, 1694.1 $(2M + 2H)^+$; HRMS (ESI^+) calcd. for $C_{48}H_{74}N_4O_7NaSi$ 869.5224 $(M + Na)^+$, found 869.5212.

4.1.13. (3S,6S,7R)-7-tert-Butyldiphenylsilyloxymethyl-4-N-(5″-(uracil-1′-yl)pentyl)-6-palmitoyloxy-3-(5-azido-5-deoxy-2,3-O-isopentylidene-β-D-ribos-1-yl-methyl)-1,4-diazepan-2-one ***(16)***

A mixture of the hydroxymethyl-diazepanone **15** (100 mg, 118 μmol), the azidofluororibose derivative **5** (43.5 mg, 177 μmol) and molecular sieves 4 Å (400 mg) in CH_2Cl_2 (3.7 mL) was stirred at r.t. for 1 h under argon atmosphere. The suspension was than cooled down to −78 °C and boron trifluoride etherate (100 μL, 0.81 mmol) was added dropwise. The reaction mixture was allowed to slowly warm up to r.t. and was stirred overnight. Saturated aqueous $NaHCO_3$ (20 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. Flash chromatography of the residue (CH_2Cl_2/MeOH 97:3 to 94:6) afforded **16** (123 mg, 97%) as a colorless solid. R_f 0.40 (CH_2Cl_2/MeOH 95:5); $[\alpha]_D$ +28 (*c* 1.0, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ 8.62 (s, 1H, $H_{3'}$), 7.65–7.38 (m, 10H, H_{ar}), 7.14 (d, 1H, $J_{H6'-H5'}$ = 7.9 Hz, $H_{6'}$), 6.10 (d, 1H, J_{H1-H7} = 5.9 Hz, H_1), 5.69 (d, 1H, $J_{H5'-H6'}$ = 7.9 Hz, $H_{5'}$), 4.98 (td, 1H, J_{H6-H7} = 9.8 Hz, J_{H6-H5a} = J_{H6-H5b} ≈ 2.7 Hz, H_6), 5.18 (s, 1H, H_{1r}), 4.59 (d, 1H, $J_{H2r-H3r}$ = 6.0 Hz, H_{2r}), 4.53 (d, 1H, $J_{H3r-H2r}$ = 6.1 Hz, H_{3r}), 4.34 (t, 1H, $J_{H4r-H5r}$ = 7.6 Hz, H_{4r}), 4.17–4.14 (m, 1H, H_7), 4.02 (dd, 1H, J_{CH2OSi} = 10.7 Hz, $J_{CHaOSi-H7}$ = 4.02 Hz, CH_aOSi), 3.77–3.65 (m, 5H, CH_bOSi, $H_{5''}$, CH_2OR), 3.54 (t, 1H, $J_{H3-CH2OR}$ = 4.0 Hz, H_3), 3.45 (dd, 1H, $J_{H5ar-H5br}$ = 12.6 Hz, $J_{H5ar-H4r}$ = 8.0 Hz, H_{5ar}), 3.20 (dd, 1H, $J_{H5br-H5ar}$ = 12.7 Hz, $J_{H5br-H4r}$ = 6.9 Hz, H_{5br}), 3.05 (dd, 1H, $J_{H5a-H5b}$ = 15.7 Hz, $J_{H5a,H6}$ = 3.3 Hz, H_{5a}), 2.94 (dd, 1H,

$J_{H5b-H5a}$ = 15.7 Hz, J_{H5b-H6} = 2.1 Hz, H_{5b}), 2.59–2.54 (m, 1H, $H_{1a''}$), 2.50–2.45 (m, 1H, $H_{1b''}$), 2.17–2.08 (m, 2H, H_b), 1.73–1.65 (m, 4H, $H_{4''}$, C(CH_2CH_3)$_2$), 1.56–1.51 (m, 4H, H_c, C(CH_2CH_3)$_2$), 1.46–1.36 (m, 2H, $H_{2''}$), 1.35–1.26 (m, 26H, $(CH_2)_{12}$, $H_{3''}$), 1.08 (s, 9H, *t*Bu), 0.92 (t, 3H, $J_{CH3-CH2}$ = 7.4 Hz, C(CH_2CH_3)$_2$), 0.90 (t, 3H, $J_{CH3-CH2}$ = 7.0 Hz, CH_3), 0.84 (t, 3H, $J_{CH3-CH2}$ = 7.5 Hz, C(CH_2CH_3)$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.9 (C_2) 172.6 (C_a), 163.2 ($C_{4'}$), 150.5 ($C_{2'}$), 144.1 ($C_{6'}$), 135.6, 135.5, 132.4, 132.2, 130.0, 127.9, 127.8 (C_{ar}), 117.2 (C(CH_2CH_3)$_2$), 109.0 (C_{1r}), 102.1 ($C_{5'}$), 85.7 (C_{4r}), 85.5 (C_{2r}), 82.3 (C_{3r}), 71.5 (C_3), 71.1 (C_6), 67.1 (CH_2OSi), 61.2 (CH_2OR), 53.7 (C_5), 53.3 (C_7, C_{5r}), 52.7 ($C_{1''}$), 48.7 ($C_{5''}$), 34.2 (C_b), 31.9–29.6 ((CH_2)$_8$), 29.5 (C(CH_2CH_3)$_2$), 29.4, 29.3, 29.2, 28.9 ((CH_2)$_4$), 28.8 (C(CH_2CH_3)$_2$), 26.9, 19.2 (*t*Bu), 26.8 ($C_{2''}$), 24.8 ($C_{4''}$), 23.8 ($C_{3''}$), 22.7 (CH_3-CH_2), 14.1 (CH_3), 8.3, 7.3 (C(CH_2CH_3)$_2$); MS (ESI$^+$) 1072.4 (M + H)$^+$, 1094.6 (M + Na)$^+$; HRMS (ESI$^+$) calcd. for $C_{58}H_{89}N_7O_{10}Si$ 1072.6518 (M + H)$^+$, found 1072.6514.

4.1.14. (3S,6S,7R)-7-tert-Butyldiphenylsilyloxymethyl-4-N-(5″-(uracil-1′-yl)pentyl)-6-palmitoyloxy-3-(5-amino-5-deoxy-2,3-O-isopentylidene-β-D-ribos-1-yl-methyl)-1,4-diazepan-2-one (17)

A solution of **16** (100 mg, 93.2 μmol) and 1,2-bis(diphenylphosphino)ethane (20 mg, 51.0 μmol) in THF (330 μL) and H_2O (33 μL) was stirred at r.t. overnight. Resulting suspension was concentrated in vacuo and the residue was suspended in ether. White precipitate was filtered off and the filtrate was concentrated in vacuo. Flash chromatography of the residue (CH_2Cl_2/MeOH/Et_3N 95:5:3‰) afforded **17** (90 mg, 92%) as a colorless solid. R_f 0.30 (CH_2Cl_2/MeOH 92:8); $[\alpha]_D$ +26 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64–7.38 (m, 10H, H_{ar}), 7.15 (d, 1H, $J_{H6'-H5'}$ = 7.9 Hz, $H_{6'}$), 6.11 (d, 1H, J_{H1-H7} = 5.7 Hz, H_1), 5.67 (d, 1H, $J_{H5'-H6'}$ = 7.9 Hz, $H_{5'}$), 5.12 (s, 1H, H_{1r}), 4.99 (td, 1H, J_{H6-H7} = 9.8 Hz, J_{H6-H5a} = J_{H6-H5b} ≈2.7 Hz, H_6), 4.56 (d, 1H, $J_{H2r-H3r}$ = 6.0 Hz, H_{2r}), 4.51 (d, 1H, $J_{H3r-H2r}$ = 6.1 Hz, H_{3r}), 4.24–4.21 (m, 2H, H_7, H_{4r}), 4.03 (dd, 1H, J_{CH2OSi} = 10.7 Hz, $J_{CHaOSi-H7}$ = 3.1 Hz, CH_aOSi), 3.78–3.65 (m, 5H, CH_bOSi, $H_{5''}$, CH_2OR), 3.54 (t, 1H, $J_{H3-CH2OR}$ = 3.3 Hz, H_3), 3.04 (dd, 1H, $J_{H5a-H5b}$ = 15.7 Hz, $J_{H5a,H6}$ = 3.3 Hz, H_{5a}), 2.94 (dd, 1H, $J_{H5b-H5a}$ = 15.7 Hz, J_{H5b-H6} = 1.5 Hz, H_{5b}), 2.77–2.70 (m, 2H, H_{5br}), 2.60–2.54 (m, 1H, $H_{1b''}$), 2.49–2.44 (m, 1H, $H_{1a''}$), 2.19–2.08 (m, 2H, H_b), 1.72–1.64 (m, 4H, $H_{4''}$, C(CH_2CH_3)$_2$)), 1.56–1.52 (m, 4H, H_c, C(CH_2CH_3)$_2$), 1.46–1.36 (m, 2H, $H_{2''}$), 1.33–1.27 (m, 26H, $(CH_2)_{12}$, $H_{3''}$), 1.08 (s, 9H, *t*Bu), 0.92 (t, 3H, $J_{CH3-CH2}$ = 7.4 Hz, C(CH_2CH_3)$_2$), 0.90 (t, 3H, $J_{CH3-CH2}$ = 6.0 Hz, CH_3), 0.84 (t, 3H, $J_{CH3-CH2}$ = 7.5 Hz, C(CH_2CH_3)$_2$); ^{13}C NMR (63 MHz, $CDCl_3$) δ 173.2 (C_2), 172.7 (C_a), 163.4 ($C_{4'}$), 150.7 ($C_{2'}$), 144.1 ($C_{6'}$), 135.6, 135.5, 132.4, 132.2, 130.0, 127.9, 127.8 (C_{ar}), 116.7 (C(CH_2CH_3)$_2$), 109.0 (C_{1r}), 102.1 ($C_{5'}$), 89.0 (C_{4r}), 85.8 (C_{2r}), 82.5 (C_{3r}), 71.5 (C_3), 71.1 (C_6), 67.4 (CH_2OSi), 61.2 (CH_2OR), 53.7 (C_5), 53.5 (C_7), 52.5 ($C_{1''}$), 48.5 ($C_{5''}$), 45.1 (C_{5r}), 34.2 (C_b), 31.9 ((CH_2)$_8$), 29.6 (C(CH_2CH_3)$_2$), 29.4, 29.3, 29.3, 29.1 ((CH_2)$_4$), 28.9 (C(CH_2CH_3)$_2$), 26.7, 19.2 (*t*Bu), 26.6 ($C_{2''}$), 24.8 ($C_{4''}$), 23.6 ($C_{3''}$), 22.6 (CH_3–CH_2), 14.1 (CH_3), 8.3, 7.4 (C(CH_2CH_3)$_2$); MS (ESI$^+$) 1046.7 (M + H)$^+$; HRMS (ESI$^+$) calcd. for $C_{58}H_{91}N_5O_{10}Si$ 1046.6613 (M + H)$^+$, found 1046.6658.

4.1.15. (3S,6S,7R)-7-tert-Butyldiphenylsilyloxymethyl-4-N-(5″-(uracil-1′-yl)pentyl)-6-palmitoyloxy-3-(5-amino-5-deoxy-β-D-ribos-1-yl-methyl)-1,4-diazepan-2-one (18)

A solution of **17** (57 mg, 53.0 μmol) in TFA (4 mL) and H_2O (1 mL) was stirred at r.t. for 1.5 h. The reaction mixture was then concentrated in vacuo. Saturated aqueous $NaHCO_3$ (5 mL) was added to the residue and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated in vacuo. Purification on Waters SEP-PAK® cartridge (CH_2Cl_2/MeOH 8:2) afforded **18** (30 mg, 57%) as a colorless solid. R_f 0.37 (CH_2Cl_2/MeOH 8:2); $[\alpha]_D$ +35 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (500 MHz, CD_3OD) δ 7.69–7.63 (m, 4H, H_{ar}), 7.58 (d, 1H, $J_{H6'-H5'}$ = 7.8 Hz, $H_{6'}$), 7.49–7.41 (m, 6H, H_{ar}), 5.65 (d, 1H, $J_{H5'-H6'}$ = 7.8 Hz, $H_{5'}$), 5.03 (td, 1H, J_{H6-H7} = 9.3 Hz, J_{H6-H5a} = J_{H6-H5b} ≈2.6 Hz, H_6), 4.95 (s, 1H, H_{1r}), 4.24–4.23 (m, 1H, H_7), 4.02–3.99 (m, 2H, CH_aOR, H_{4r}), 3.96–3.91 (m, 2H, H_{2r}, H_{3r}), 3.87–3.78 (m, 3H, CH_2OSi, CH_bOR), 3.75 (t, 2H, $J_{H5''-H4''}$ = 7.2 Hz, $H_{5''}$), 3.50 (t, 1H, $J_{H3-CH2OR}$ = 3.9 Hz, H_3), 3.04 (dd, 1H, $J_{H5a-H5b}$ = 13.1 Hz, J_{H5a-H6} = 3.1 Hz, H_{5a}), 2.97 (d, 2H, *J* = 11.6 Hz, H_{5r}), 2.81 (dd, 1H, $J_{H5b-H5a}$ = 8.4 Hz, J_{H5b-H6} = 13.1 Hz, H_{5b}), 2.68–2.62 (m, 1H, $H_{1b''}$), 2.45–2.40 (m, 1H, $H_{1a''}$), 2.23–2.11 (m, 2H, H_b), 1.73–1.62 (m, 2H, $H_{4''}$), 1.54–1.51 (m, 2H, H_c), 1.47–1.39 (m, 2H, $H_{2''}$), 1.35–1.27 (m, 26H, $(CH_2)_{12}$, $H_{3''}$), 1.09 (s, 9H, *t*Bu), 0.91 (t, 3H, $J_{CH3-CH2}$ = 6.9 Hz, CH_3); ^{13}C NMR (125 MHz, CD_3OD) δ 176.2 (C_2), 174.3 (C_a), 166.7 ($C_{4'}$), 152.8 ($C_{2'}$), 147.3 ($C_{6'}$), 136.8, 136.7, 133.9, 133.7, 131.3, 129.2, 129.1 (C_{ar}), 109.1 (C_{1r}), 102.3 ($C_{5'}$), 82.8 (C_{4r}), 76.5 (C_{2r}), 74.1 (C_{3r}), 72.9 (C_3), 72.8 (C_6), 68.4 (CH_2OR), 62.8 (CH_2OSi), 54.7 (C_{5r}), 54.6 (C_7), 54.1 ($C_{1''}$), 49.6 ($C_{5''}$), 45.4 (C_5), 35.2 (C_b), 33.1–30.3 ((CH_2)$_{11}$), 29.9 ($C_{4''}$), 28.3 ($C_{2''}$), 27.5, 20.1 (*t*Bu), 25.9 (C_c), 24.8 (C_d), 23.8 ($C_{3''}$), 14.5 (CH_3); MS (ESI$^+$) 978.5 (M + H)$^+$; HRMS (ESI$^+$) calcd. for $C_{53}H_{84}N_5O_{10}Si$ 978.5987 (M + H)$^+$, found 978.5986.

4.1.16. (3S,6S,7R)-3-(5-Amino-5-deoxy-β-D-ribos-1-yl-methyl)-7-hydroxymethyl-4-N-(5″-(uracil-1′-yl)pentyl)-6-palmitoyloxy-1,4-diazepan-2-one (19)

To a solution of **18** (18 mg, 18.4 μmol) in DMF (1.3 mL) was added NH_4F (0.7 mg, 0.0184 μmol) and the reaction mixture was stirred at r.t. overnight. Reaction mixture was then concentrated in vacuo and the residue was carefully rinsed with ether (3 mL). Product was dissolved in methanol, filtered through the cotton and concentrated in vacuo to afford **19** (12 mg, 87%) as a colorless solid. R_f 0.15 (CH_2Cl_2/MeOH/NH_4OH 7:3:0.3); $[\alpha]_D$ +25 (*c* 1.0, MeOH); ^{1}H NMR (CD_3OD) δ 7.60 (d, 1H, $J_{H6'-H5'}$ = 7.8 Hz, $H_{6'}$), 5.68 (d, 1H, $J_{H5'-H6'}$ = 7.8 Hz, $H_{5'}$), 4.97 (s, 1H, H_{1r}), 4.83 (td, 1H, J_{H6-H7} = 9.8 Hz, J_{H6-H5a} = J_{H6-H5b} ≈2.5 Hz, H_6), 4.35–4.31 (m, 1H, H_7), 4.16–4.13 (m, 1H, H_{3r}), 4.12–4.08 (m, 1H, H_{4r}), 4.05–4.02 (m, 2H, CH_aOR, H_{2r}), 3.91 (dd, 1H, J_{CH2OR} = 10.6 Hz, $J_{CHbOR-H3}$ = 3.2 Hz, CH_bOr), 3.79 (t, 2H, $J_{H5''-H4''}$ = 7.2 Hz, $H_{5''}$), 3.68 (dd, 1H, J_{CH2OH} = 11.5 Hz, $J_{CHaOH-H7}$ = 2.8 Hz, CH_aOH), 3.61 (dd, 1H, J_{CH2OH} = 11.5 Hz, $J_{CHbOH-H7}$ = 6.2 Hz, CH_bOH), 3.48 (t, 1H, $J_{H3-CH2OR}$ = 3.1 Hz, H_3), 3.23 (dd, 1H, $J_{H5a-H5b}$ = 11.4 Hz, J_{H5a-H6} = 2.4 Hz, H_{5a}), 3.00 (dd, 1H, $J_{H5b-H5a}$ = 11.4 Hz, J_{H5b-H6} = 2.1 Hz, H_{5b}), 2.96 (d, 2H, *J* = 2.2 Hz, H_{5r}), 2.69–2.63 (m, 1H, $H_{1b''}$), 2.43–2.31 (m, 3H, H_b, $H_{1a''}$), 1.78–1.69 (m, 2H, $H_{4''}$), 1.67–1.61 (m, 2H, H_c), 1.56–1.45 (m, 2H, $H_{2''}$), 1.42–1.31 (m, 26H, $(CH_2)_{12}$, $H_{3''}$), 0.92 (t, 3H, $J_{CH3-CH2}$ = 7.0 Hz, CH_3); ^{13}C NMR (125 MHz, CD_3OD) δ 176.4 (C_2), 174.6 (C_a), 166.7 ($C_{4'}$), 152.8 ($C_{2'}$), 147.3 ($C_{6'}$), 109.0 (C_{1r}), 102.2 ($C_{5'}$), 80.3 (C_{4r}), 76.3 (C_{2r}), 74.3 (C_{3r}), 73.6 (C_3, C_6), 68.4 (CH_2OR), 61.0 (CH_2OH), 55.3 (C_{5r}), 54.6 (C_7), 54.1 ($C_{1''}$), 49.7 ($C_{5''}$), 44.5 (C_5), 35.2 (C_b), 33.0–30.3 ((CH_2)$_{10}$), 29.9 ($C_{4''}$), 28.3 ($C_{2''}$), 26.0 (C_c), 24.8 (C_d), 23.6 ($C_{3''}$), 14.4 (CH_3); MS (ESI$^+$) 740.5 (M + H)$^+$; HRMS (ESI$^+$) calcd. for $C_{37}H_{66}N_5O_{10}$ 740.4810 (M + H)$^+$, found 740.4814.

4.1.17. (3S,6S,7R)-3,7-Dihydroxymethyl-6-palmitoyloxy-4-N-(5″-(uracil-1′-yl)pentyl)-1,4-diazepan-2-one (20)

To a solution of **15** (176 mg, 208 μmol) in DMF (15 mL) was added NH_4F (24 mg, 0.645 μmol) and the reaction mixture was stirred at r.t. overnight. NH_4F was filtered off and the reaction mixture was then concentrated in vacuo. The residue was carefully rinsed with ether (3 × 2 mL) and dried in vacuo to afford **20** (122 mg, 96%) as a colorless solid. R_f 0.38 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D$ +68 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (500 MHz) δ 10.05 (s, 1H, $H_{3'}$), 7.33 (d, 1H, J_{H1-H7} = 4.5 Hz, H_1), 7.22 (d, 1H, $J_{H6'-H5'}$ = 7.9 Hz, $H_{6'}$), 5.73 (d, 1H, $J_{H5'-H6'}$ = 7.8 Hz, $H_{5'}$), 4.87 (td, 1H, J_{H6-H7} = 5.3 Hz, J_{H6-H5a} = J_{H6-H5b} ≈2.8 Hz, H_6), 4.26–4.21 (m, 1H, H_7), 4.01–3.70 (m, 6H, CH_2OH, $H_{5''}$), 3.44 (t, 1H, $J_{H3-CH2OH}$ = 4.0 Hz, H_3), 3.05 (dd, 1H, $J_{H5a-H5b}$ = 15.4 Hz, J_{H5a-H6} = 2.8 Hz, H_{5a}), 2.93 (dd, 1H,

$J_{H5b-H5a}$ = 15.4 Hz, J_{H5b-H6} = 2.6 Hz, H_{5b}), 2.67–2.62 (m, 2H, $H_{1b''}$, -OH), 2.47–2.43 (m, 1H, $H_{1a''}$), 2.35–2.24 (m, 3H, H_b, -OH), 1.82–1.73 (m, 1H, $H_{4''a}$), 1.71–1.651 (m, 1H, $H_{4''b}$), 1.63–1.58 (m, 2H, H_c), 1.47–1.42 (m, 4H, $H_{2''}$, $H_{3''}$), 1.30–1.27 (m, 24H, $(CH_2)_{12}$,), 0.89 (t, 3H, $J_{CH3-CH2}$ = 6.9 Hz, CH_3); ^{13}C NMR δ 175.5(C_2), 173.2 (C_a), 164.4 ($C_{4'}$), 151.3 ($C_{2'}$), 144.8 ($C_{6'}$), 102.1 ($C_{5'}$), 73.4 (C_3), 71.5 (C_6), 61.8 (CH_2OH), 60.6 (CH_2OH), 54.0 (C_5), 53.7 (C_7), 52.5 ($C_{1''}$), 48.8 ($C_{5''}$), 34.3 (C_b), 31.9–29.2 (($CH_2)_{11}$), 28.4 (C_c), 26.6 ($C_{2''}$), 24.8 ($C_{4''}$), 23.2 ($C_{3''}$), 22.6 (CH_3-$\underline{C}H_2$), 14.1 (CH_3); MS (ESI$^+$) 631.4 $(M + Na)^+$; HRMS (ESI$^+$) calcd. for $C_{32}H_{56}N_4O_7Na$ 631.4047 $(M + Na)^+$, found 631.4065.

4.1.18. (3S,6S,7R)-3,7-Di-(5-azido-5-deoxy-2,3-O-isopentylidene-β-D-ribos-1-yl-methyl)-4-N-(5″-(uracil-1′-yl)pentyl)-6-palmitoyloxy-1,4-diazepan-2-one (21)

A mixture of the dihydroxymethyl-diazepanone **20** (102 mg, 167 μmol), the fluororibose derivative **5** (123 mg, 0.502 mmol) and molecular sieves 4 Å (900 mg) in CH_2Cl_2 (8 mL) was stirred at r.t. for 30 min under argon atmosphere. The suspension was then cooled down to −78 °C and boron trifluoride etherate (80 μL, 0.65 mmol) was added dropwise. The reaction mixture was allowed to slowly warm up to r.t. and was stirred overnight. Saturated aqueous $NaHCO_3$ (20 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. Flash chromatography of the residue (CH_2Cl_2/MeOH 99:1 to 92:8) afforded **21** (110 mg, 62%) as a colorless solid. R_f 0.32 (CH_2Cl_2/MeOH 95:5); $[\alpha]_D$ +14 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.45 (s, 1H, $H_{3'}$), 7.14 (d, 1H, $J_{H6'-H5'}$ = 7.9 Hz, $H_{6'}$), 6.48 (d, 1H, J_{H1-H7} = 5.1 Hz, H_1), 5.68 (d, 1H, $J_{H5'-H6'}$ = 7.8 Hz, $H_{5'}$), 5.15 (s, 1H, H_{1r1}), 5.08 (s, 1H, H_{1r2}), 4.85 (td, 1H, J_{H6-H7} = 10.0 Hz, J_{H6-H5a} = J_{H6-H5b} ≈2.6 Hz, H_6), 4.67 (d, 1H, J_{H2-H3} = 6.0 Hz, H_{2r1}), 4.63–4.58 (m, 3H, H_{2r2}, H_{3r1}, H_{3r2}), 4.38 (t, 1H, J_{H4-H5} = 6.4 Hz, H_{4r1}), 4.32 (t, 1H, J_{H4-H5} = 7.5 Hz, H_{4r2}), 4.28–4.25 (m, 1H, H_7), 4.03 (dd, 1H, *J* = 10.5 Hz, *J* = 3.6 Hz, CH_aOR_1), 3.86 (dd, 1H, *J* = 10.4 Hz, *J* = 2.8 Hz, CH_aOR_2), 3.78–3.64 (m, 3H, CH_bOR_1, $H_{5''}$), 3.53 (t, 1H, $J_{H3-CH2OR}$ = 3.5 Hz, H_3), 3.48–3.33 (m, 4H, H_{5ar1}, H_{5ar2}, H_{5br1}, CH_bOR_2), 3.18 (dd, 1H, $J_{H5b-H5a}$ = 12.6 Hz, J_{H5b-H4} = 7.8 Hz, H_{5br2}), 3.01 (dd, 1H, $J_{H5a-H5b}$ = 15.5 Hz, J_{H5a-H6} = 3.2 Hz, H_{5a}), 2.91 (dd, 1H, $J_{H5b-H5a}$ = 15.6 Hz, J_{H5b-H6} = 2.0 Hz, H_{5b}), 2.60–2.54 (m, 1H, $H_{1b''}$), 2.48–2.42 (m, 1H, $H_{1a''}$), 2.36–2.25 (m, 2H, H_b), 1.72–1.55 (m, 10H, H_c, $C(\underline{C}H_2CH_3)_2$), 1.44–1.38 (m, 4H, $H_{2''}$, $H_{4''}$), 1.29–1.26 (m, 26H, $(CH_2)_{12}$, $H_{3''}$), 0.93–0.86 (m, 15H, $C(CH_2\underline{C}H_3)_2$, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.4 (C_2), 172.8 (C_a), 163.7 ($C_{4'}$), 150.7 ($C_{2'}$), 144.2 ($C_{6'}$), 117.3, 117.2 ($\underline{C}(CH_2CH_3)_2$), 109.7, 108.8 ($C_{1r}$), 102.1 ($C_{5'}$), 85.9, 85.5 ($C_{4r}$), 85.4 ($C_{2r}$), 82.4, 82.0 ($C_{3r}$), 71.2 ($C_6$), 71.1 ($C_3$), 67.0, 66.4 ($CH_2OR$), 53.8 ($C_5$), 53.7, 53.5 ($C_{5r}$), 52.9 ($C_7$), 51.5 ($C_{1''}$), 48.7 ($C_{5''}$), 34.3 ($C_b$), 31.8–29.4 (($CH_2)_9$), 29.4, 29.3 ($C(\underline{C}H_2CH_3)_2$), 29.3, 29.2 28.9 (($CH_2)_3$), 28.9, 28.8 ($C(\underline{C}H_2CH_3)_2$), 26.8 ($C_{2''}$), 24.9 ($C_c$), 23.7 ($C_{4''}$), 22.6 ($C_{3''}$), 14.0 ($CH_3$), 8.3 ($C(\underline{C}H_2CH_3)_2$), 7.3 ($C(CH_2\underline{C}H_3)_2$); MS (ESI$^+$) 1081.7 $(M + Na)^+$; HRMS (ESI$^+$) calcd. for $C_{52}H_{86}N_{10}O_{13}Na$ 1081.6274 $(M + Na)^+$, found 1081.6267.

4.1.19. (3S,6S,7R)-3,7-Di-(5-amino-5-deoxy-2,3-O-isopentylidene-β-D-ribos-1-yl-methyl)-4-N-(5″-(uracil-1′-yl)pentyl)-6-palmitoyloxy-1,4-diazepan-2-one (22)

A solution of **21** (62.0 mg, 58.5 μmol) and 1,2-bis(diphenylphosphino)ethane (25.6 mg, 64.4 μmol) in THF (0.7 mL) and H_2O (70 μL) was stirred at r.t. overnight. The resulting suspension was concentrated in vacuo and the residue was suspended in ether (3 mL). White precipitate was filtered off and the filtrate was concentrated in vacuo. Flash chromatography of the residue (CH_2Cl_2/Et_3N 1:3‰ to CH_2Cl_2/MeOH/Et_3N 80:20:3‰) afforded **22** (56.9 mg, 96%) as a colorless solid. R_f 0.21 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D$ +11 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.15 (d, 1H, $J_{H6'-H5'}$ = 7.9 Hz, $H_{6'}$), 5.67 (d, 1H, $J_{H5'-H6'}$ = 7.8 Hz, $H_{5'}$), 5.11 (s, 1H, H_{1r1}), 5.02 (s, 1H, H_{1r2}), 4.88 (td, 1H, J_{H6-H7} = 9.9 Hz, J_{H6-H5a} = J_{H6-H5b} ≈2.6 Hz, H_6), 4.64–4.57 (m, 4H, H_{3r1}, H_{3r2}, H_{2r1}, H_{2r2}), 4.31–4.29 (m, 1H, H_7), 4.28–4.21 (m, 2H, H_{4r1}, H_{4r2}), 4.03 (dd, 1H, *J* = 10.5 Hz, *J* = 3.3 Hz, CH_aOR_1), 3.80 (dd, 1H, *J* = 10.5 Hz, *J* = 2.7 Hz, CH_aOR_2), 3.77–3.74 (m, 3H, CH_bOR_1, $H_{5''}$), 3.52 (dd, 1H, *J* = 10.3 Hz, *J* = 4.2 Hz, CH_bOR_2), 3.48 (t, 1H, $J_{H3-CH2Or}$ = 3.2 Hz, H_3), 3.23 (bs, 4H, NH_2), 2.98 (dd, 1H, $J_{H5a-H5b}$ = 15.6 Hz, J_{H5a-H6} = 3.3 Hz, H_{5a}), 2.91 (d, 1H, $J_{H5b-H5a}$ = 15.6 Hz, J_{H5b-H6} = 1.9 Hz, H_{5b}), 2.88–2.75 (m, 4H, H_{5r1}, H_{5r2}), 2.59–2.54 (m, 1H, $H_{1b''}$), 2.47–2.42 (m, 1H, $H_{1a''}$), 2.35–2.24 (m, 2H, H_b), 1.73–1.54 (m, 10H, H_c, $C(\underline{C}H_2CH_3)_2$), 1.49–1.37 (m, 4H, $H_{2''}$, $H_{4''}$), 1.30–1.26 (m, 26H, $(CH_2)_{12}$, $H_{3''}$), 0.93–0.86 (m, 15H, $C(CH_2\underline{C}H_3)_2$, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.6 (C_2), 172.8 (C_a), 163.7 ($C_{4'}$), 150.9 ($C_{2'}$), 144.1 ($C_{6'}$), 116.7, 116.6 ($\underline{C}(CH_2CH_3)_2$), 109.0 ($C_{1r}$), 102.1 ($C_{5'}$), 89.0, 88.3 ($C_{4r}$), 85.9, 85.5 ($C_{2r}$), 82.6, 82.3 ($C_{3r}$), 71.7 ($C_3$), 71.3 ($C_6$), 67.4, 65.7 ($CH_2OR$), 53.8 ($C_5$), 53.3 ($C_{1''}$), 51.2 ($C_7$), 48.6 ($C_{5''}$), 45.1, 44.8 ($C_{5r}$), 34.4 ($C_b$), 31.8–29.4 (($CH_2)_9$), 29.3, 29.3 ($C(\underline{C}H_2CH_3)_2$), 29.2, 29.0 (($CH_2)_2$), 28.9, 28.8 ($C(\underline{C}H_2CH_3)_2$), 26.7 ($C_{2''}$), 24.9 ($C_c$), 23.7 ($C_{4''}$), 22.6 ($C_{3''}$), 14.0 ($CH_3$), 8.3, 7.4, 7.28 ($C(CH_2\underline{C}H_3)_2$); MS (ESI$^+$) 1007.7 $(M + H)^+$; HRMS (ESI$^+$) calcd. for $C_{52}H_{91}N_6O_{13}$ 1007.6644 $(M + H)^+$, found 1007.6629.

4.1.20. (3S,6S,7R)-3,7-Di-(5-amino-5-deoxy-β-D-ribos-1-yl-methyl)-4-N-(5″-(uracil-1′-yl)pentyl)-6-palmitoyloxy-1,4-diazepan-2-one (23)

A solution of **22** (46.3 mg, 46 μmol) in TFA (4 mL) and H_2O (1 mL) was stirred at r.t. for 2 h. The reaction mixture was then concentrated in vacuo. The crude product was purified by column chromatography on octadecyl-functionalized silica gel (Aldrich) by using gradient elution (MeOH/H_2O/AcOH 4:4:2 to 7:1:2) to afford **23** (30 mg, 57%) as a diacetate. R_f 0.20 (CH_2Cl_2/MeOH/NH_4OH 6:4:0.5); $[\alpha]_D$ +26 (*c* 1.0, MeOH); ^{1}H NMR (500 MHz, MeOD) δ 7.61 (d, 1H, $J_{H6'-H5'}$ = 7.8 Hz, $H_{6'}$), 5.69 (d, 1H, $J_{H5'-H6'}$ = 7.8 Hz, $H_{5'}$), 4.99 (s, 1H, H_{1r1}), 4.94 (s, 1H, H_{1r2}), 4.83 (bs, 1H, H_6), 4.43–4.40 (m, 1H, H_7), 4.13–4.07 (m, 4H, H_{4r1}, H_{4r2}, H_{3r1}, H_{3r2}), 4.05–4.00 (m, 3H, CH_aOR_1, H_{2r1}, H_{2r2}), 3.93–3.86 (m, 2H, CH_bOR_1, CH_aOR_2), 3.82–3.75 (m, 2H, $H_{5''}$), 3.55 (dd, 1H, *J* = 10.6 Hz, *J* = 6.7 Hz, CH_bOR_2), 3.48 (t, 1H, $J_{H3-CH2OR}$ = 3.0 Hz, H_3), 3.25 (dd, 1H, $J_{H5a-H5b}$ = 12.9 Hz, J_{H5a-H6} = 4.3 Hz, H_{5a}), 3.08–2.92 (m, 5H, H_{5r1}, H_{5r2}, H_{5b}), 2.69–2.63 (m, 1H, $H_{1b''}$), 2.42–2.30 (m, 3H, H_b, $H_{1a''}$), 1.96 (s, 6H, AcO^-),1.77–1.69 (m, 2H, $H_{4''}$), 1.67–1.61 (m, 2H, H_c), 1.56–1.45 (m, 2H, $H_{2''}$), 1.34–1.31 (m, 26H, $(CH_2)_{12}$, $H_{3''}$), 0.92 (t, 3H, $J_{CH3-CH2}$ = 6.9 Hz, CH_3); ^{13}C NMR (125 MHz, MeOD) δ 178.8 (C_3), 176.4 (CH_3COO^-),174.7 (C_a), 166.8 ($C_{4'}$), 153.0 ($C_{2'}$), 147.5 ($C_{6'}$), 110.2, 109.0 (C_{1r}), 81.1, 80.8 (C_{4r}), 76.4, 76.0 (C_{2r}), 74.6, 74.4 (C_{3r}), 73.9 (C_3), 73.8 (C_6), 68.6, 68.3 (CH_2OR), 55.4 (C_5), 54.2 ($C_{1''}$), 52.8 (C_7), 49.7 ($C_{5''}$), 44.9, 44.8 (C_{5r}), 35.3 (C_b), 33.2–28.5 (($CH_2)_{12}$), 26.1 ($C_{2''}$), 24.9 (C_c), 23.9 (AcO^-), 23.2 ($C_{4''}$), 23.2 ($C_{3''}$), 14.6 (CH_3); HRMS (ESI$^+$) calcd. for $C_{42}H_{75}N_6O_{13}$ 871.5392 $(M + H)^+$, found 871.5406.

4.2. Enzymatic assays

The activities of the compounds against MraY transferase were tested as previously described [6,25]. The assay was performed in a reaction mixture of 10 μL containing, in final concentrations, 100 mM Tris-HCl, pH 7.5, 40 mM $MgCl_2$, 1.1 mM C_{55}-P, 250 mM NaCl, 0.25 mM UDP-MurNAc-[^{14}C]pentapeptide (337 Bq), and 8.4 mM *N*-lauroyl sarcosine. The reaction was initiated by the addition of MraY enzyme, and the mixture was incubated for 30 min at 37 °C under shaking with a thermomixer (Eppendorf). The reaction was stopped by heating at 100 °C for 1 min. The compounds were also tested against MurG as previously described [26,27]. Reaction mixtures contained, in a final volume of 12.5 μl, 200 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 16 μM UDP-[^{14}C]GlcNAc (1.7 kBq), 16 μM lipid I analog, 30% (v/v) dimethyl sulfoxide and

MurG. After 30 min at 37 °C, it was stopped by boiling for 3 min. Then, the mixture was lyophilized and taken up in 10 μl of 2-propanol/ammonium hydroxide/water (6:3:1; v/v/v). In both cases, the radiolabeled substrate (UDP-MurNAc-pentapeptide in the case of MraY, UDP-GlcNAc in the case of MurG) and reaction product (lipid I, product of MraY, and lipid II, product of MurG) were separated by TLC on silica gel plates LK6D (Whatman) using 2-propanol/ammonium hydroxide/water (6:3:1; v/v/v) as the mobile phase. The radioactive spots were located and quantified with a radioactivity scanner (model Multi-Tracemaster LB285; EG&G Wallac/Berthold). Residual activities were calculated with respect to a control assay without inhibitors. IC_{50} values were determined with 7 inhibitor concentrations. Data represent the mean of independent triplicate determinations, and the standard deviations were less than 10%.

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Appendix. Supplementary material

Supplementary material related to this article can be found online at doi:10.1016/j.ejmech.2011.02.006.

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