Bioorganic & Medicinal Chemistry 18 (2010) 4560–4569

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Bacterial transferase MraY inhibitors: Synthesis and biological evaluation

Delphine Lecerclé [a], Anthony Clouet [a], Bayan Al-Dabbagh [b,†], Muriel Crouvoisier [b], Ahmed Bouhss [b], Christine Gravier-Pelletier [a,*], Yves Le Merrer [a,*]

[a] Université Paris Descartes, UMR 8601 CNRS, Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, 45 rue des Saints-Pères, 75006 Paris, France
[b] Université Paris-Sud, IBBMC, UMR 8619 CNRS, Enveloppes Bactériennes et Antibiotiques, Bât. 430, 91405 Orsay, France

ARTICLE INFO

Article history:
Received 18 December 2009
Revised 6 April 2010
Accepted 7 April 2010
Available online 13 April 2010

Keywords:
Bacterial transferase MraY
Inhibition
Bis-epoxide
Triazole
Glycosylation
Cycloaddition

ABSTRACT

New inhibitors of the bacterial transferase MraY are described. Their structure is based on an aminoribosyl-*O*-uridine like scaffold, readily obtained in two key steps. The amino group can be coupled with proline or guanylated. Alternatively, these amino, prolinyl or guanidinyl groups can be introduced through a triazole linker. Biological evaluation of these compounds on MraY from *Bacillus subtilis* revealed interesting inhibitory activity for both amino compounds.

© 2010 Published by Elsevier Ltd.

1. Introduction

In an ongoing programme, undertaken to develop new potential antibiotics,[1] we are targeting the bacterial transferase MraY which is an essential enzyme[2] in peptidoglycan biosynthesis.[3] The latter is a unique feature in bacteria cell wall, without equivalent in eukaryotic cells. Enzymes involved in this biopolymer biosynthesis have been demonstrated to be ubiquitous and essential to bacterial growth[3a] and therefore represent challenging targets for the search of new antibiotics. The transferase MraY, which catalyses the first membrane step of peptidoglycan biosynthesis (Fig. 1), has been little exploited due to its transmembrane localisation,[4] so that it is currently the target of no antibiotics in clinical use. It catalyses the transfer of uridine mono-phosphate-*N*-acetyl-muramoyl-pentapeptide from its precursor UDP-Mur-*N*-Ac-pentapeptide onto undecaprenyl phosphate, resulting in the formation of lipid I.

We recently developed the synthesis of a new pharmacophore,[5] whose structure is bioinspired from both the south part of liposidomycins[6] (Fig. 2), which are naturally occurring inhibitors of MraY, and of a pharmacophore developed by Aventis.[7]

On the one hand, for this new pharmacophore, crucial residues such as aminoribosyl and uracil moieties have been retained. On the other hand, an inversion of the absolute configuration at one chiral center (C_2) and insertion of an extra methylene group between the sugar and the pyrimidic base, expected to enhance the stability of the resulting inhibitors,[8] have been introduced. Such an inversion of the absolute configuration at C_2 should not be prejudiciable to biological activity in reference to a structure–activity relationships study developed by Aventis[9] and related to their apparented pharmacophore. Our strategy (Fig. 3) presents the advantage that only two main key steps allow the obtention of

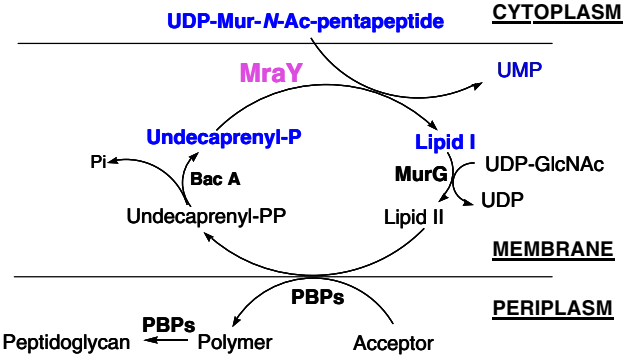


Figure 1. Role of MraY in bacterial peptidoglycan biosynthesis.

* Corresponding authors. Tel.: +33 142862181; fax: +33 142868387 (C.G.-P.); tel.: +33 142862176; fax: +33 142868387 (Y.L.M.).
E-mail addresses: christine.gravier-pelletier@parisdescartes.fr (C. Gravier-Pelletier), yves.le-merrer@parisdescartes.fr (Y. Le Merrer).
† Present address: Department of Internal Medicine, Faculty of Medicine and Health Sciences, United Arab Emirates University, PO Box 17666, Al Ain, United Arab Emirates.

0968-0896/$ - see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.bmc.2010.04.023

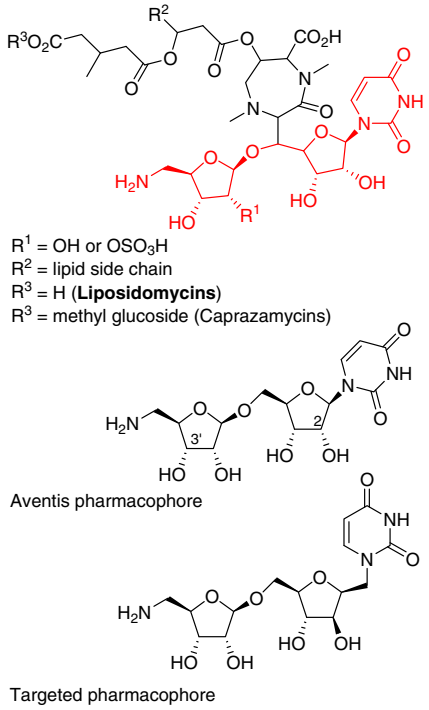


Figure 2. Natural inhibitors of MraY and pharmacophores.

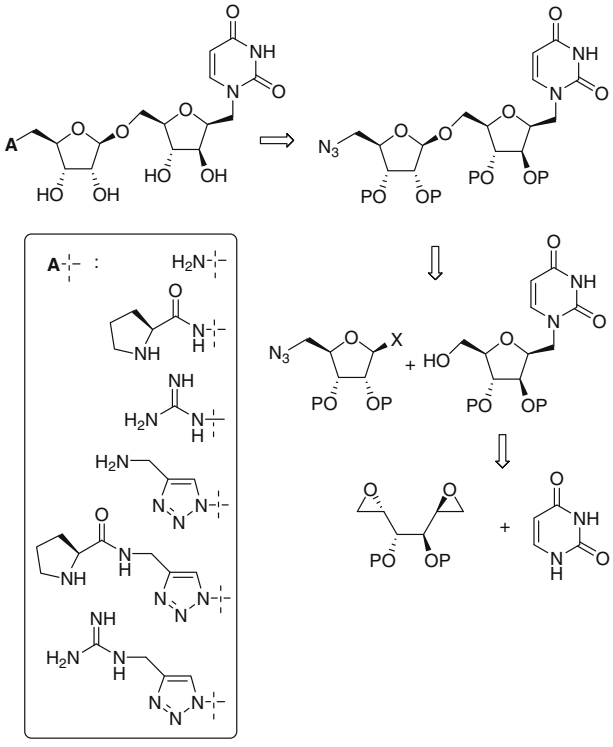


Figure 3. Retrosynthetic analysis.

the azido-pharmacophore,[5] namely a tandem N-alkylation—O-cyclisation of the C_2-symmetrical L-ido-bis-epoxide **1** with uracil, and O-glycosylation with a masked aminoribosyl derivative.[5] Its deprotection results in the amino scaffold whose biological evaluation on MraY from *Bacillus subtilis*[10] causes an inhibition of the enzymatic activity with an IC_{50} of 580 μM.

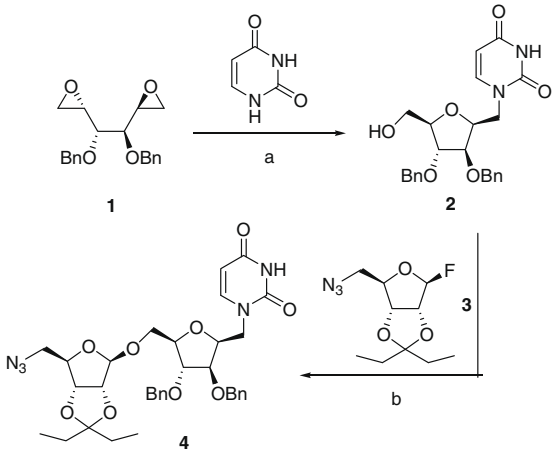
The present study is directed towards the introduction of structural diversity on the amino end of the scaffold expected to improve the resulting inhibitors' biological activity. Accordingly, compounds including other basic residues such as proline or guanidine have been targeted. Furthermore, to increase the number of compounds in this series, it was decided to take advantage of the Huisgen 1,3-dipolar cycloaddition[11,12] that involves the azido group, a precursor of the scaffold amino group, and various alkynes. The chosen alkynes were substituted by the same amine, proline and guanidine substituents, which resulted in the insertion of a triazole linker in the previous compounds. Indeed, numerous examples of interesting biological properties[13] displayed by such triazole-containing compounds have already been demonstrated. Such a unit is not just a passive linker but a rather active pharmacophore that may significantly contribute to protein binding.

2. Results and discussion

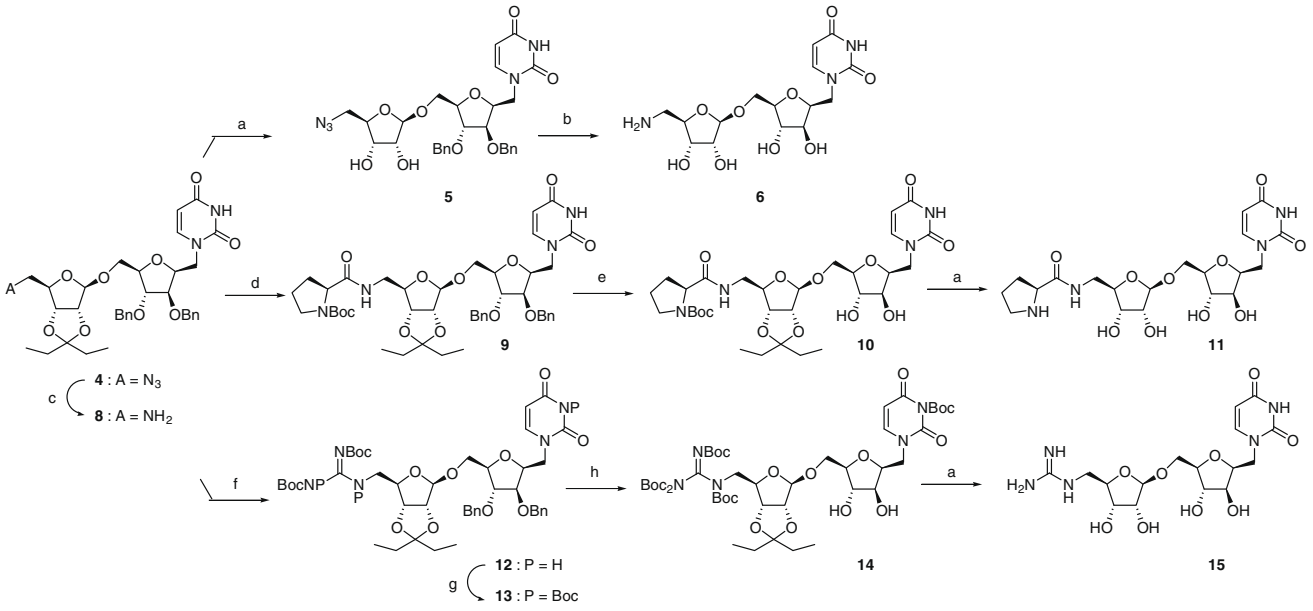
According to the proposed retrosynthetic plan, the azidopharmacophore **4** was efficiently obtained in only two steps (Scheme 1) from the C_2-symmetrical 1,2:5,6-dianhydro-3,4-di-*O*-benzyl-L-iditol readily available from D-mannitol.[14]

Tandem nucleophilic opening—O-cyclisation[15,16] of this bis-epoxide by bis-(trimethylsilyl) uracil, in situ prepared from uracil and *N*,*O*-bis-(trimethylsilyl) trifluoroacetamide (BSTFA), was performed in the presence of magnesium(II) perchlorate in refluxing acetonitrile. The resulting uridine-like derivative **2** was obtained in 46% yield. The latter was then glycosylated with 5-azido-1,5-di-deoxy-2,3-di-*O*-ethylpropylidene-1-fluoro-D-ribofuranose[17,18] **3** in the presence of boron trifluoride etherate leading to the major formation of the protected β-glycosylated azido scaffold **4**. Indeed, the presence of the hindered isopentylidene 1,2-diol protecting group on the sugar α face promotes major glycosylation on its β face (β/α = 13/1). The configuration in anomeric position was unambiguously assigned by ^1H NMR analysis: singlet or doublet for $H_{1''}$ in the β- or α-anomer, respectively.[18] The pure β-anomer was easily separated from its α-anomer by chromatographic purification and was isolated in 87% yield.

We next turned to the preparation of the first series of inhibitors (Scheme 2). Acidic hydrolysis of the di-*O*-ethylpropylidene protective group of **4** was carried out in the presence of aqueous trifluoroacetic acid giving the corresponding diol **5** in 80% yield. Finally, hydrogenolysis of the benzyl ethers and concomitant azido reduction were achieved in the presence of palladium black in acetic acid. It has to be pointed out that the partial reduction of the uracil



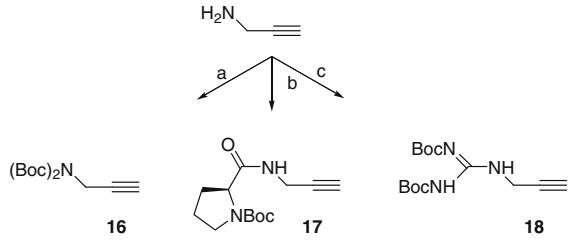
Scheme 1. Reagents and conditions: (a) BSTFA, CH_3CN then $Mg(ClO_4)_2$, Δ, 46%; (b) $BF_3{\cdot}OEt_2$, CH_2Cl_2, M.S., −78 °C, 87%.



Scheme 2. Reagents and conditions: (a) TFA, H_2O, 80%, 70% and 60%, respectively, for **5**, **11** and **15**; (b) H_2, Pd, AcOH, 20%; (c) $(Ph_2PCH_2)_2$, CH_2Cl_2, H_2O, 90%; (d) *N*-Boc-L-proline, HATU, Et_3N, DMF, 68%; (e) H_2, $Pd(OH)_2$, EtOAc, EtOH, 96%; (f) BocNHC(SMe)NBoc, $HgCl_2$, CH_2Cl_2, Et_3N, 90%; (g) Boc_2O, DMAP, Et_3N, THF, 92%; (h) H_2, Pd/C, MeOH, 99%.

double bond was observed during this step affording **7** (**6**/**7** = 85/15). Nevertheless, the pure amino pharmacophore **6** could be isolated after careful flash chromatographic purifications. In a complementary manner, azido group reduction under Staudinger conditions, in the presence of bis-1,2-diphenyl phosphinoethane,[19] led to the corresponding amine **8** and was followed by coupling with *N*-Boc-proline in the presence of HATU as coupling agent[20] giving the protected proline derivative **9** (61% overall yield). Then, benzyl ethers hydrogenolysis in the presence of Pearlman's catalyst and acidic hydrolysis with aqueous trifluoroacetic acid of both proline *N*-Boc and isopentylidene diol protecting groups gave the proline targeted inhibitor **11** in good yield. Finally, guanylation of the amino scaffold **8** was carried out with *N,N′*-di-Boc-*S*-methyl isothiourea in the presence of mercuric(II) chloride giving **12** (90% yield). However, as precedently observed for the preparation of **6**, subsequent *O*-benzyl ethers deprotection by hydrogenolysis in the presence of Pearlman's catalyst, led to a slight reduction of the uracil double bond and total deprotection of benzyl ethers could not be achieved, whatever the conditions. To avoid this side reaction, the uracil and guanidine moieties were first protected as the *N*-Boc derivative **13** prior to hydrogenolysis, which could then be efficiently performed in the presence of 10% Pd/C in MeOH, without any reduction of the uracil double bond, giving **14**. Then, final acidic hydrolysis of both isopentylidene and *N*-Boc protecting groups was carried out to afford the expected guanidine inhibitor **15**.

We next tackled the synthesis of the triazole-containing inhibitors from the azido scaffold **4**. It first required the preparation of the corresponding alkynes (Scheme 3). They resulted from propargylamine by its bis-*N*-Boc protection with *tert*-butyldicarbonate for **16**, coupling with *N*-Boc-L-Pro-OH in the presence of HATU for **17** and reaction with *N,N′*-bis-(*tert*-butyloxycarbonyl)-1*H*-pyrazole-1-carboxamidine for **18**, respectively.

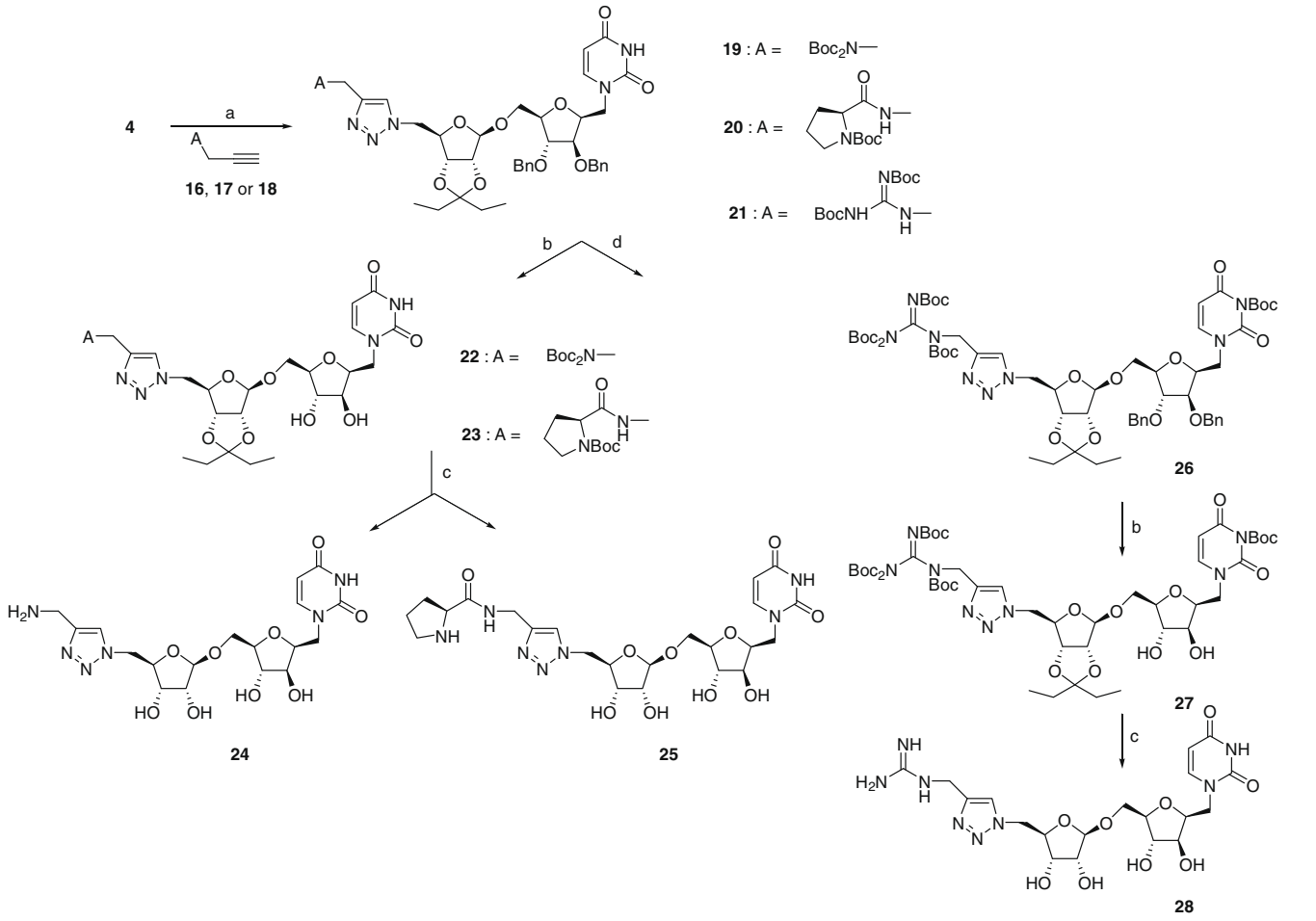


Scheme 3. Reagents and conditions: (a) Boc_2O, DMAP, Et_3N, THF; (b) *N*-Boc-L-proline, HATU, Et_3N, DMF, 99%; (c) *N,N′*-di-Boc-1*H*-pyrazole-1-carboxamidine, $(i\text{Pr})_2\text{NEt}$, DMF, 59%.

The 1,3-dipolar cycloaddition involving azido scaffold **4** and alkynes **16**, **17** or **18** was then carried out in the presence of copper(II) sulfate pentahydrate and sodium ascorbate in *t*-BuOH/H_2O to yield the expected 1,4-di-substituted triazoles **19**, **20** or **21** in moderate yield (Scheme 4). Then, two different pathways towards the final compounds were designed depending on the eventual uracil protection as its *tert*-butyl carbamate. On the one hand, hydrogenolysis of benzyl ethers of **19** and **20** could be efficiently carried out in the presence of 10% Pd on charcoal affording **22** and **23**, respectively. Final acidic hydrolysis with aqueous trifluoroacetic acid of both *N*-Boc and the ketal protecting groups afforded the targeted triazole substituted by an amine or a proline moiety, **24** or **25**. On the other hand, direct hydrogenolysis of benzyl ethers from **21** could not be achieved, since all the tested conditions resulted in the recovery of starting material, probably due to catalyst poisoning. Consequently, first *N*-Boc protection of both uracil and guanidine was performed which gave **26**, prior to benzyl ethers hydrogenolysis which led to **27** and subsequent acidic hydrolysis which afforded the targeted triazole substituted by a guanidine moiety **28**.

The in vitro biological evaluation of the resulting compounds **6**, **7**, **11**, **15**, **24**, **25**, **28** 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 2 mM concentration of the tested compounds. For the more active compounds, the IC_{50} values were determined.

The results indicate that the amino scaffold shows an interesting IC_{50} equal to 580 μM. The inhibition observed for compound **7** confirms that the uracil double bond is important for biological activity as already observed by Aventis.[21] However, the amine substitution with either a proline or a guanidine moiety led to the loss of activity. Interestingly, the triazole insertion improves activity for the inhibitor **25** or **28** as compared to the apparented compounds **11** or **15** that do not contain this linker. Nevertheless, the activity of the triazole-containing inhibitors remains comparable to that



Scheme 4. Reagents and conditions: (a) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, *t*-BuOH, H_2O, 29%, 29% and 57%, respectively, for **19**, **20** and **21**; (b) H_2, Pd/C, MeOH, 79%, 79% and 90% for, respectively, **22**, **23** and **26**; (c) TFA, H_2O, 47%, 64% and 50% for, respectively, **24**, **25** and **28**; (d) Boc_2O, DMAP, Et_3N, THF, 79%.

Table 1
Inhibitory activities of synthetized compounds on the MraY enzyme

Compound	6	7	11	15	24	25	28
IC_{50} (μM) or RA[a] (%)	580	946	−61%	1790	595	820	600

[a] Residual activity (RA) of MraY in the presence of compound **11** at a final concentration of 2 mM.

of the amino scaffold. Furthermore, it has to be pointed out that the observed activity seems to be selective towards MraY, since testing of these compounds at 1 mM on the MurG enzyme, catalyzing lipid II formation (Fig. 1) revealed no inhibition.

3. Conclusions

In this study, we have developed a straightforward synthetic route towards new inhibitors of the bacterial transferase MraY based on an aminoribosyl-*O*-uridine like scaffold. The latter is readily obtained through a tandem nucleophilic opening O-cyclisation of L-ido-bis-epoxide by uracil and subsequent O-glycosylation of the resulting primary alcohol with an azidoribosyl derivative. On the one hand, azide reduction led to the amino scaffold, which can be either coupled with proline or guanylated. On the other hand, the azido scaffold can be submitted to cycloaddition with several amino, prolinyl or guanidinyl substituted alkynes. Biological evaluation of these compounds on MraY from *B. subtilis* revealed that the amino scaffold presents an interesting inhibitory activity. However, the prolino or guanidino derivatives showed no or weak inhibitory activity. Nevertheless, the introduction of a triazole linker on the previous compounds improved the inhibitory activity, but that activity remained comparable to that of the amino scaffold, so that, future work in this series will be based on that scaffold.

4. Experimental

4.1. Chemical synthesis

^1H NMR (250 MHz) and ^{13}C NMR (63 MHz) spectra were recorded on a Bruker AM250 in $CDCl_3$ (unless indicated). ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker Avance or Avance II. Chemical shifts (δ) are reported in ppm and coupling constants are given in Hz. Mass spectra, electrospray (ESI) and high resolution (HRMS) were recorded by the service de Spectrométrie de Masse, ICSN Gif sur Yvette. Optical rotations were measured with Perkin–Elmer 241C polarimeter with a sodium (589 nm) or a mercury (365 nm) lamp at 20 °C. All reactions were carried out in anhydrous solvents under a nitrogen atmosphere (except for hydrogenolysis), and were monitored by thin-layer chromatography with Merck 60F-254 precoated silica (0.2 nm) on glass. Flash chromatography was performed with Merck Kieselgel 60 (40–63 μm) or octadecyl-functionalized silica gel (column C18). Spectroscopic ^1H and ^{13}C NMR, MS and/or analytical data were obtained using chromatographically homogeneous samples.

4.1.1. 2,5-Anhydro-3,4-di-*O*-benzyl-1-deoxy-1-(uracil-1′-yl)-D-glucitol 2

To a solution of uracil (560 mg, 5 mmol) in acetonitrile (5.0 mL), was added bis-(trimethylsilyl)trifluoroacetamide (BSTFA) (6.6 mL, 25 mmol). The mixture was refluxed for 30 min. The silylated uracil obtained after concentration in vacuo was dissolved in acetonitrile (5.0 mL), then a solution of bis-epoxide **1** (326 mg, 1 mmol) in acetonitrile (5.0 mL) was added. The mixture was heated to 85 °C, and magnesium(II) perchlorate (334 mg, 1.5 mmol) was added. The reaction was stirred overnight, then concentrated in vacuo. The residue was dissolved in EtOAc, washed with brine, dried ($MgSO_4$), and the solvent was evaporated in vacuo. Flash chromatography of the crude (EtOAc/cyclohexane/MeOH 6:4:0.3) afforded **2** (202 mg, 46%) as a foamy solid. R_f 0.37 (EtOAc/cyclohexane/MeOH 5:4:1); $[\alpha]_D^{20}$ −8 (*c* 1.0, MeOH); IR (neat): *ν* 2924, 1678, 1455, 1250 cm^{-1}; ^1H NMR ($(CD_3)_2CO$, 250 MHz): δ 3.68–3.75 (m, 2H, H_6), 3.90 (dd, $J_{H1\text{-}H1}$ = 14 Hz, $J_{H1\text{-}H2}$ = 9 Hz, 1H, H_1), 4.00–4.05 (m, 1H, H_5), 4.22 (dd, $J_{H1\text{-}H1}$ = 14 Hz, $J_{H1\text{-}H2}$ = 4 Hz, 1H, H_1), 4.25–4.29 (m, 2H, H_3, H_4), 4.36 (dd, $J_{H2\text{-}H1}$ = 9 Hz, $J_{H2\text{-}H1}$ = 4 Hz, 1H, H_2), 4.63 (d, *J* = 12 Hz, 1H, H_{CH2Ph}), 4.72 (s, 1H, H_{CH2Ph}), 4.73 (s, 1H, H_{CH2Ph}), 4.76 (d, *J* = 12 Hz, 1H, H_{CH2Ph}), 5.54 (d, $J_{H5'\text{-}H6'}$ = 8 Hz, 1H, $H_{5'}$), 7.33–7.48 (m, 10H, H_{Ar}), 7.57 (d, $J_{H6'\text{-}H5'}$ = 8 Hz, 1H, $H_{6'}$); ^{13}C NMR ($(CD_3)_2CO$, 62.5 MHz): δ 48.8 (C_1), 62.8 (C_6), 71.7 (C_{CH2Ph}), 78.8 (C_2), 83.2 (C_3, C_4), 85.4 (C_5), 101.1 ($C_{5'}$), 127.9, 128.0, 128.1, 128.4, 128.7, 128.8, 138.4, 138.8 (C_{Ar}), 146.5 ($C_{6'}$), 151.7 ($C_{2'}$), 163.9 ($C_{4'}$); HRMS calcd for $[M+Na]^+$ 461.1689, found 461.1676.

4.1.2. 2,5-Anhydro-3,4-di-*O*-benzyl-1-deoxy-1-(uracil-1′-yl)-6-(5″-azido-1″,5″-dideoxy-2″,3″-*O*-isopentylidene-β-D-ribos-1″-yl)-D-glucitol 4

To a solution of **2** (350 mg, 0.799 mmol) in CH_2Cl_2 (20 mL) were added the fluorinated ribose **3** (295 mg, 1.2 mmol) and molecular sieves 4 Å. The mixture was stirred for 1 h, then cooled to −78 °C and $BF_3{\cdot}OEt_2$ (152 μL, 1.2 mmol) was added. The mixture was stirred at rt overnight. The mixture was diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, and concentrated in vacuo. Flash chromatography of the crude (acetone/cyclohexane 4:6) afforded **4** as a white solid (462 mg, 87%). $[\alpha]_D^{20}$ −4 (*c* 1.0, MeOH); ^1H NMR ($CDCl_3$, 500 MHz): δ 0. 90, 0.93 (2t, *J* = 7 Hz, 6H, H_{CH3CH2}), 1.57, 1.71 (2qd, *J* = 7 Hz, 4 H, H_{CH2CH3}), 3.13 (dd, $J_{H5''\text{-}H5''}$ = 13 Hz, $J_{H5''\text{-}H4''}$ = 7 Hz, 1H, $H_{5''}$), 3.35 (dd, $J_{H5''\text{-}H5''}$ = 13 Hz, $J_{H5''\text{-}H4''}$ = 8 Hz, 1H, $H_{5''}$), 3.58 (dd, $J_{H6\text{-}H6}$ = 11 Hz, $J_{H6\text{-}H5}$ = 7 Hz, 1H, H_6), 3.72 (dd, $J_{H1\text{-}H1}$ = 14 Hz, $J_{H1\text{-}H2}$ = 8 Hz, 1H, H_1), 3.80 (dd, $J_{H6\text{-}H6}$ = 11 Hz, $J_{H6\text{-}H5}$ = 5 Hz, 1H, H_6), 3.89 (d, $J_{H4\text{-}H3}$ = 3 Hz, 1H, H_4), 4.03 (d, $J_{H3\text{-}H4}$ = 3 Hz, 1H, H_3), 4.05–4.07 (m, 1H, $H_{4''}$), 4.27–4.31 (m, 2H, H_2, H_5), 4.35 (dd, $J_{H1\text{-}H1}$ = 14 Hz, $J_{H1\text{-}H2}$ = 3 Hz, 1H, H_1), 4.41–4.48 (m, 2H, $H_{2''}$, H_{CH2Ph}), 4.50–4.62 (m, 3H, $H_{3''}$, H_{CH2Ph}); 5.16 (s, 1H, $H_{1''}$), 5.63 (d, *J* = 8 Hz, 1H, $H_{5'}$), 7.27–7.41 (m, 11H, H_{Ar}, $H_{6'}$), 9.24 (s, 1H, $H_{3'}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 7.6, 8.6 (C_{CH3CH2}), 29.1, 29.6 (C_{CH2CH3}), 48.5 (C_1), 53.6 ($C_{5''}$), 68.5 (C_6), 71.8, 71.9 (C_{CH2Ph}), 79.0 (C_2), 82.4 ($C_{2''}$), 82.8, 82.9 (C_3, C_4), 83.4 ($C_{4''}$), 85.6 ($C_{3''}$), 85.9 (C_5); 101.8 ($C_{5'}$), 109.3 ($C_{1''}$), 117.3 (C_{CEt2}), 127.9, 128.3, 128.4, 128.7, 128.8, 137.3, 137.5 (C_{Ar}), 145.8 ($C_{6'}$), 151.2 ($C_{2'}$), 164.0 ($C_{4'}$); HRMS calcd for $[M+Na]^+$ 686.2802, found 686.2814.

4.1.3. 2,5-Anhydro-3,4-di-*O*-benzyl-1-deoxy-1-(uracil-1′-yl)-6-(5″-azido-1″,5″-dideoxy-D-ribos-1″-yl)-D-glucitol 5

To a solution of **4β** (20 mg, 0.03 mmol) in CH_2Cl_2 (1.0 mL) were added TFA (30 μL) and water (two drops). The mixture was stirred at rt for 48 h, then concentrated, diluted with EtOAc, washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The crude was purified by flash chromatography (CH_2Cl_2/MeOH 95:5), yielding **5** as a colourless oil (11 mg, 62%). R_f 0.27 (CH_2Cl_2/MeOH 95:5); $[\alpha]_D^{20}$ −6 (*c* 1.0, MeOH); IR (neat): *ν* 3381, 2924, 2102, 1676, 1455*m*, 1250 cm^{-1}; ^1H NMR ($(CD_3)_2CO$, 250 MHz): δ 3.34 (m, 1H, $H_{5''}$), 3.41 (m, 1H, $H_{5''}$), 3.58 (m, 1H, H_6), 3.81 (m, 2H, H_1, H_6), 3.99–4.27 (m, 8H, H_1, H_2, H_3, H_4, H_5, $H_{2''}$, $H_{3''}$, $H_{4''}$), 4.61–4.76 (m, 4H, H_{CH2Ph}), 5.01 (s, 1H, $H_{1''}$), 5.51 (d, $J_{H5'\text{-}H6'}$ = 7.9 Hz, 1H, $H_{5'}$), 7.38 (m, 11H, H_{Ar}, $H_{6'}$); ^{13}C NMR ($(CD_3)_2CO$, 62.5 MHz): δ 48.6 (C_1), 54.5 ($C_{5''}$), 69.3 (C_6), 72.1, 72.2 (C_{CH2Ph}), 73.0 ($C_{2''}$), 75.6 ($C_{3''}$), 79.3 (C_2), 82.7, 83.2 (C_3, C_4), 83.8 ($C_{4''}$), 84.0 (C_5), 101.4 ($C_{5'}$), 108.8 ($C_{1''}$), 128.7, 128.9, 129.3, 129.4, 138.8, 139.1 (C_{Ar}), 147.0 ($C_{6'}$), 151.9 ($C_{2'}$), 164.5 ($C_{4'}$); HRMS calcd for $[M+Na]^+$ 618.2176, found 618.2170.

4.1.4. 2,5-Anhydro-1-deoxy-1-(uracil-1′-yl)-6-(5″-amino-1″,5″-dideoxy-D-ribos-1″-yl)-D-glucitol 6

Palladium black (150 mg) was added to a stirred solution of **5** (105 mg, 0.18 mmol) in acetic acid (10 mL). The mixture was stirred under H_2 for 6 h at rt. Acetic acid was removed in vacuo. The crude was purified first with a column HyperSep C_{18} with H_2O, then H_2O/MeOH (1:1). The cleanest fraction (first one) was purified on silica gel chromatography, using different eluent system; starting with CH_2Cl_2, then CH_2Cl_2/MeOH (8:2), then *i*PrOH, then *i*PrOH/NH_3 (100:25), and finally *i*PrOH/NH_3:MeOH (90:25:10) to afford pure **6** (13.9 mg, 20%). R_f 0.27 (*i*PrOH/NH_3 100:25); $[\alpha]_D^{20}$ −33 (*c* 1.0, H_2O); ^1H NMR (D_2O, 500 MHz): δ 3.13 (dd, $J_{H5''\text{-}H5''}$ = 14 Hz, $J_{H5''\text{-}H4''}$ = 6 Hz, 1H, $H_{5''}$), 3.43 (dd, $J_{H5''\text{-}H5''}$ = 14 Hz, $J_{H5''\text{-}H4''}$ = 3 Hz, 1H, $H_{5''}$), 3.73 (dd, $J_{H6\text{-}H6}$ = 11 Hz, $J_{H6\text{-}H5}$ = 8 Hz, 1H, H_6), 3.92–3.98 (m, 2H, H_5, H_1), 3.99 (dd, $J_{H6\text{-}H6}$ = 11 Hz, $J_{H6\text{-}H5}$ = 3 Hz, 1H, H_6), 4.06 (dd, $J_{H3\text{-}H2}$ = 4 Hz, $J_{H3\text{-}H4}$ = 3 Hz, 1H, H_3), 4.16 (d, $J_{H2''\text{-}H3''}$ = 5 Hz, 1H, $H_{2''}$), 4.17–4.22 (m, 2H, H_1, $H_{4''}$), 4.26 (dd, $J_{H4\text{-}H5}$ = 5 Hz, $J_{H4\text{-}H3}$ = 3 Hz, 1H, H_4), 4.28 (dd, *J* = 7 Hz, *J* = 5 Hz, 1H, $H_{3''}$), 4.35 (ddd, $J_{H2\text{-}H1}$ = 9 Hz, $J_{H2\text{-}H1}$ = $J_{H2\text{-}H3}$ = 4 Hz, 1H, H_2), 5.13 (s, 1H, $H_{1''}$), 5.86 (d, $J_{H5'\text{-}H6'}$ = 8 Hz, 1H, $H_{5'}$), 7.68 (d, $J_{H6'\text{-}H5'}$ = 8 Hz, 1H, $H_{6'}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 43.8 ($C_{5''}$), 48.9 (C_1), 69.3 (C_6), 73.1 ($C_{3''}$), 74.8 ($C_{2''}$), 77.2 (C_4), 78.7, 78.8 (C_2, C_3), 79.1 ($C_{4''}$), 84.1 (C_5), 102.1 ($C_{5'}$), 108.7 ($C_{1''}$), 148.4 ($C_{6'}$), 153.0 ($C_{2'}$), 167.5 ($C_{4'}$); HRMS calcd for $[M+Na]^+$, 412.1332, found 412.1327.

4.1.5. 2,5-Anhydro-3,4-di-*O*-benzyl-1-deoxy-1-(uracil-1′-yl)-6-(5″-amino-1″,5″-dideoxy-2″,3″-*O*-isopentylidene-β-D-ribos-1″-yl)-D-glucitol 8

To a solution of **4** (148 mg, 0.22 mmol) in THF (10 mL) was added 1,2-bis-(diphenylphosphino)ethane (53 mg, 0.13 mmol) and water (1 mL). The mixture was stirred overnight at rt, and then concentrated in vacuo. Flash chromatography of the crude (CH_2Cl_2/MeOH 95:5) afforded **8** as a white solid (128 mg, 90%). $[\alpha]_D^{20}$ −29 (*c* 1.0, MeOH); ^1H NMR ($CDCl_3$, 500 MHz):δ 0.88, 0.92 (2t, *J* = 7 Hz, 6H, H_{CH3CH2}), 1.56, 1.70 (2qd, *J* = 7 Hz, 4H, H_{CH2CH3}), 2.68–2.71 (m, 2H, $H_{5''}$), 3.57 (dd, $J_{H6\text{-}H6}$ = 11 Hz, $J_{H6\text{-}H5}$ = 6 Hz, 1H, H_6), 3.75 (dd, $J_{H1\text{-}H1}$ = 14 Hz, $J_{H1\text{-}H2}$ = 8 Hz, 1H, H_1), 3.78 (dd, $J_{H6\text{-}H6}$ = 11 Hz, $J_{H6\text{-}H5}$ = 5 Hz, 1H, H_6), 3.96 (d, $J_{H4\text{-}H3}$ = 3 Hz, 1H, H_4), 4.02 (d, $J_{H3\text{-}H4}$ = 3 Hz, 1H, H_3), 4.17 (t, $J_{H4''\text{-}H5''}$ = 7 Hz, 1H, $H_{4''}$), 4.26–4.30 (m, 2H, H_2, H_5), 4.33 (dd, $J_{H1\text{-}H1}$ = 14 Hz, $J_{H1\text{-}H2}$ = 3 Hz, 1H, H_1), 4.41–4.45 (m, 3H, $H_{2''}$,H_{CH2Ph}), 4.49–4.62 (m, $H_{3''}$, 3 H, H_{CH2Ph}), 5.10 (s, 1H, $H_{1''}$), 5.61 (d, *J* = 8 Hz, 1H, $H_{5'}$), 7.25–7.38 (m, 10H, H_{Ar}), 7.42 (d, *J* = 8 Hz, 1H, $H_{6'}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 7.5, 8.5 (C_{CH3CH2}), 29.0, 29.5 (C_{CH2CH3}), 45.3 ($C_{5''}$), 48.4 (C_1), 68.0 (C_6), 71.6, 71.9 (C_{CH2Ph}), 79.0 (C_2), 82.4, 82.7, 82.9, 83.3 (C_3, C_4, C_5, $C_{2''}$), 86.0 ($C_{3''}$), 89.3 ($C_{4''}$), 101.7 ($C_{5'}$), 109.0 ($C_{1''}$), 116.7 (C_{CEt2}), 127.8, 127.8, 128.1, 128.3, 128.7, 128.7, 137.4, 137.5 (C_{Ar}), 146.1 ($C_{6'}$), 151.3 ($C_{2'}$), 164.2 ($C_{4'}$); HRMS: Calcd for $[M+Na]^+$ 660.2897, found 660.2914.

4.1.6. 2,5-Anhydro-3,4-di-*O*-benzyl-1-deoxy-1-(uracil-1′-yl)-6-(5″-(*N*-Boc-L-prolinyl-*N*′-amido)-1″,5″-dideoxy-2″,3″-*O*-isopentylidene-β-D-ribos-1″-yl)-D-glucitol 9

To a solution of Boc-L-Pro-OH (47 mg, 0.22 mmol) in DMF (4 mL) was added HATU (82 mg, 0.22 mmol) and TEA (40 μL, 0.28 mmol). To the mixture was added a solution of **8** (92 mg, 0.14 mmol) in DMF (2 mL). The mixture was stirred overnight at

rt, and then concentrated in vacuo. Flash chromatography (cyclohexane/acetone 65:35) afforded **9** as a white solid (81 mg, 68%). $[\alpha]_D^{20}$ −49 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 250 MHz): δ 0.87, 0.91 (2t, J = 7.4 Hz, 6H, H_{CH3CH2}), 1.46 (s, 9H, H_{Boc}), 1.54, 1.69 (2qd, J = 7.4 Hz, 4H, H_{CH2CH3}), 1.76–1.88 (m, 2H, H_γ), 1.92–2.02 (m, 1H, H_β), 2.06–2.24 (m, 1H, H_β), 3.38–3.52 (m, 5H, $H_{5''}$, H_δ, H_1), 3.60 (dd, $J_{H6\text{-}H6}$ = 11 Hz, $J_{H6\text{-}H5}$ = 6 Hz, 1H, H_6), 3.78–3.84 (m, 1H, H_6), 3.96 (d, $J_{H3\text{-}H4}$ = 3 Hz, 1H, H_3), 4.03 (d, $J_{H4\text{-}H3}$ = 3 Hz, 1H, H_4), 4.24–4.33 (m, 4H, H_1, H_2, $H_{5''}$), 4.35–4.62 (m, 8H, $H_{2''}$, $H_{3''}$, $H_{4''}$, H_5, H_{CH2Ph}), 5.21 (s, 1H, $H_{1''}$), 5.62 (d, J = 8 Hz, 1H, $H_{5'}$), 7.30–7.41 (m, 11H, H_{Ar}, $H_{6'}$), 9.4 (s, 1H, $H_{3'}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 7.8, 8.8 (C_{CH3CH2}), 28.8 (C_{Boc}), 28.8–29.7 (C_{CH2CH3}, C_γ, C_β), 42.5, 42.6 (C_δ, $C_{5''}$), 47.5 (C_1), 68.5 (C_6), 72.0, 72.3 (C_{CH2Ph}), 79.3 (C_2), 82.7, 82.7, 83.3, 83.6 (C_3, C_4, C_5, $C_{2''}$, $C_{3''}$, $C_{4''}$), 86.1 (C_{Boc}), 102.0 ($C_{5'}$), 109.4 ($C_{1''}$), 117.0 (C_{CEt2}), 128.1, 128.1, 128.4, 128.5, 128.9, 129.0, 137.7, 137.8 (C_{Ar}), 146.0 ($C_{6'}$), 151.5 ($C_{2'}$), 164.4 ($C_{4'}$); HRMS calcd for $[M+Na]^+$ 857.3949, found 857.3945.

4.1.7. 2,5-Anhydro-3,4-dihydroxy-1-deoxy-1-(uracil-1′-yl)-6-(5″-(*N*-Boc-L-prolinyl-*N*′-amido)-1″,5″-dideoxy-2″,3″-*O*-isopentylidene-β-D-ribos-1″-yl)-D-glucitol 10

A suspension of 20% $Pd(OH)_2$ (6 mg) and **9** (33 mg, 0.04 mmol) in a mixture of EtOAc (2 mL) and EtOH (0.75 mL) was saturated with dihydrogen and stirred for 3 h. The reaction mixture was then filtered through a Celite pad and the residue was washed with MeOH. The filtrate was concentrated in vacuo to afford **10** as a white solid (25 mg, 96%). $[\alpha]_D^{20}$ −77 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.84, 0.88 (2t, J = 7.4 Hz, 6H, H_{CH3CH2}), 1.44 (s, 9H, H_{Boc}), 1.54, 1.67 (2qd, J = 7.4 Hz, 4H, H_{CH2CH3}), 1.84–1.88 (m, 2H, H_γ), 1.96–2.08 (m, 1H, H_β), 2.12–2.21 (m, 1H, H_β), 3.36–3.46 (m, 4H, $H_{5''}$, H_δ), 3.55–3.60 (m, 1H,H_6), 3.75 (dd, $J_{H1\text{-}H1}$ = 13 Hz, $J_{H1\text{-}H2}$ = 6.5 Hz, 1H, H_1), 3.90–3.94 (m, 2H, H_5, H_6), 4.04–4.06 (m, 2H, H_4, H_2), 4.20–4.25 (m, 2H, H_1, $H_{2'''}$), 4.28–4.30 (m, 1H, $H_{4''}$), 4.65 (d, $J_{H2''\text{-}H3''}$ = 5.5 Hz, 1H, $H_{2''}$), 4.58 (d, $J_{H3''\text{-}H2''}$ = 5.5 Hz, 1H, $H_{3''}$), 5.12 (s, 1H, $H_{1''}$), 5.66 (d, $J_{H5'\text{-}H6'}$ = 8.5 Hz, 1H, $H_{5'}$), 7.39 (d, $J_{H6'\text{-}H5'}$ = 8.5 Hz, 1H, $H_{6'}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 7.3, 8.4 (C_{CH3CH2}), 22.7 (C_γ), 28.4 (C_{Boc}), 28.8 (C_{CH2CH3}), 29.0 (C_β), 29.3 (C_{CH2CH3}), 42.7 (C_δ), 47.3 ($C_{5''}$), 47.9 (C_1), 60.4 (C_α), 68.4 (C_6), 79.0 (C_3), 79.5 (C_4), 80.9 (C_{Boc}), 82.2 ($C_{3''}$), 84.6 (C_5), 85.6 ($C_{2''}$), 85.8 ($C_{4''}$), 102.0 ($C_{5'}$), 109.1 ($C_{1''}$), 116.9 (C_{CEt2}), 146.0 ($C_{6'}$), 151.5 ($C_{2'}$), 155.8 (C_{Boc}), 164.0 ($C_{4'}$); HRMS calcd for $[M+Na]^+$ 677.3010, found: 677.3000.

4.1.8. 2,5-Anhydro-3,4-dihydroxy-1-deoxy-1-(uracil-1′-yl)-6-(5″-(*N*-L-prolinyl-*N*′-amido)-1″,5″-dideoxy-2″,3″-dihydroxy-β-D-ribos-1″-yl)-D-glucitol 11

At 0 °C, to a solution of **10** (25 mg, 0.04 mmol) in water (0.55 mL) was added TFA (2.2 mL). The mixture was stirred for 1 h at rt, and then concentrated in vacuo. The residue was diluted with water and aqueous NH_4OH (28%, 6 mL) was added at 0 °C. The mixture was then concentrated in vacuo. Chromatography on reverse phase (C18) with H_2O, H_2O/MeOH (1:1) and then MeOH afforded **11** as a white solid (13 mg, 70%). $[\alpha]_D^{20}$ −1 (*c* 1.0, MeOH); ^{1}H NMR (D_2O, 500 MHz): δ 1.91–1.99 (m, 2H, H_γ), 2.07–2.16 (m, 1H, H_β), 2.21–2.29 (m, 1H, H_β), 3.37–3.49 (m, 4H, $H_{5''}$, H_δ), 3.54–3.61 (m, 1H, H_6), 3.77 (dd, $J_{H1\text{-}H1}$ = 13 Hz, $J_{H1\text{-}H2}$ = 6.5 Hz, 1H, H_1), 3.90–3.95 (m, 2H, H_5, H_6), 4.06–4.13 (m, 2H, H_4, H_2), 4.21–4.28 (m, 2H, H_1, $H_{2'''}$), 4.30–4.34 (m, 1H, $H_{4''}$), 4.65 (d, $J_{H2''\text{-}H3''}$ = 5.5 Hz, 1H, $H_{2''}$), 4.61 (d, $J_{H3''\text{-}H2''}$ = 5.5 Hz, 1H, $H_{3''}$), 5.02 (s, 1H, $H_{1''}$), 5.68 (d, $J_{H5'\text{-}H6'}$ = 8.5 Hz, 1H, $H_{5'}$), 7.41 (d, $J_{H6'\text{-}H5'}$ = 8.5 Hz, 1H, $H_{6'}$); ^{13}C NMR (D_2O, 125 MHz): δ 22.4 (C_γ), 29.1 (C_β), 42.7 (C_δ), 47.6 ($C_{5''}$), 47.9 (C_1), 59.5 (C_α), 68.5 (C_6), 79.0 (C_3), 79.8 (C_4), 82.3 ($C_{3''}$), 84.8 (C_5), 85.6 ($C_{2''}$), 85.8 ($C_{4''}$), 101.9 ($C_{5'}$), 109.1 ($C_{1''}$), 146.6 ($C_{6'}$), 153.2 ($C_{2'}$), 164.8 ($C_{4'}$); MS: *m*/*z* 487 (100%, $[M+1]^+$).

4.1.9. 2,5-Anhydro-3,4-di-*O*-benzyl-1-deoxy-1-(uracil-1′-yl)-6-(5″-(*N*,*N*′-bis-(*tert*-butyloxycarbonyl)guanidino)-1″,5″-dideoxy-2″,3″-*O*-isopentylidene-β-D-ribos-1″-yl)-D-glucitol 12

To a solution of **8** (91 mg, 0.14 mmol) in CH_2Cl_2 (10 mL) were successively added *N*,*N*′-bis-(*tert*-butyloxycarbonyl)-*S*-methylisothiourea (49 mg, 0.17 mmol), triethylamine (40 μL, 0.28 mmol) and $HgCl_2$ (58 mg, 0.21 mmol). The mixture was stirred for 36 h at rt, then filtered through a Celite pad, washed with CH_2Cl_2 and the filtrate was concentrated in vacuo. Flash column chromatography (cyclohexane/acetone 6:4) afforded **12** as a white solid (113 mg, 90%). $[\alpha]_D^{20}$ +5 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.87, 0.91 (2t, J = 7 Hz, 6H, H_{CH3CH2}), 1.46, 1.48 (2s, 18H, H_{Boc}), 1.54, 1.69 (2qd, J = 7 Hz, 4H, H_{CH2CH3}), 3.26 (ddd, $J_{H5''\text{-}H5''}$ = 14 Hz, $J_{H5''\text{-}H4''}$ = 9 Hz, $J_{H5''\text{-}NH}$ = 4 Hz,1H, $H_{5''}$), 3.61 (dd, $J_{H6\text{-}H6}$ = 11 Hz, $J_{H6\text{-}H5}$ = 6 Hz, 1H, H_6), 3.67–3.69 (m, 1H, H_1), 3.76–3.84 (m, 2H, H_6, $H_{5''}$), 3.93 (d, $J_{H4\text{-}H3}$ = 4 Hz, 1H, H_4), 4.01 (d, $J_{H3\text{-}H4}$ = 4 Hz, 1H, H_3), 4.05–4.07 (m, 1H, H_5), 4.27–4.35 (m, H_2, 2H, H_1, $H_{4''}$), 4.41 (d, J = 12 Hz, 1H, H_{CH2Ph}), 4.46–4.58 (m, 5H, H_{CH2Ph}, $H_{2''}$, $H_{3''}$), 5.14 (s, 1H, $H_{1''}$), 5.58 (dd, $J_{H5'\text{-}H6'}$ = 8 Hz, $J_{H5'\text{-}NH}$ = 2 Hz, 1H, $H_{5'}$), 7.27–7.36 (m, 11H, $H_{6'}$, H_{Ar}), 8.57–8.59 (m, 1H, NH), 9.16–9.18 (m, 1H, $H_{3'}$), 11.48 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 7.5, 8.5 (C_{CH3CH2}), 28.2, 28.4 (C_{Boc}), 29.0, 29.5 (C_{CH2CH3}), 42.3 ($C_{5''}$), 48.6 (C_1), 68.1 (C_6), 71.6, 72.0 (C_{CH2Ph}), 78.9 (C_2), 80.9, 81.7 (C_{Boc}), 82.5 ($C_{3''}$), 82.9, 83.0 (C_3, C_4), 83.3 (C_5), 85.3 ($C_{4''}$), 85.8 ($C_{2''}$), 101.7 ($C_{5'}$), 109.1 ($C_{1''}$), 116.9 (C_{CEt2}), 127.8, 127.9, 128.0, 128.2, 128.7, 128.8, 137.4, 137.4 (C_{Ar}), 146.0 ($C_{6'}$), 151.2 ($C_{2'}$), 153.1 (C_{gua}), 156.3 (C_{Boc}), 163.9 ($C_{4'}$); MS: *m*/*z* 880 (100%, $[M+1]^+$).

4.1.10. 2,5-Anhydro-3,4-di-*O*-benzyl-1-deoxy-1-(3′-(*N*-*tert*-butyloxycarbonyl)-uracil-1′-yl)-6-(5″-(*N*,*N*′,*N*″-*tetra*(*tert*-butyloxycarbonyl)guanidino)-1″,5″-dideoxy-2″,3″-*O*-isopentylidene-β-D-ribos-1″-yl)-D-glucitol 13

To a solution of **12** (105 mg, 0.12 mmol) in THF (10 mL) were added, di-*tert*-butyldicarbonate (78 mg, 0.36 mmol), DMAP (5 mg, 0.024 mmol) and triethylamine (50 μL, 0.36 mmol). The solution was stirred overnight at rt, then concentrated in vacuo. Flash column chromatography (cyclohexane/acetone 6:4) afforded **13** as a colourless oil (129 mg, 92%). $[\alpha]_D^{20}$ −23 (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.86, 0.91 (2t, J = 7 Hz, 6H, H_{CH3CH2}), 1.47, 1.50, 1.52 (3s, 36H, H_{Boc}), 1.53 (qd, J = 7 Hz, 2H, H_{CH2CH3}), 1.62 (s, 9H_{Boc}), 1.67 (qd, J = 7 Hz, 2H, H_{CH2CH3}), 3.56 (dd, $J_{H6\text{-}H6}$ = 11 Hz, $J_{H6\text{-}H5}$ = 7 Hz, 1H, H_6), 3.68 (dd, $J_{H1\text{-}H1}$ = 15 Hz, $J_{H1\text{-}H2}$ = 9 Hz, 1H, H_1), 3.79 (dd, $J_{H6\text{-}H6}$ = 11 Hz, $J_{H6\text{-}H5}$ = 5 Hz, 1H, H_6), 3.87 (dd, $J_{H5''\text{-}H5''}$ = 14 Hz, $J_{H5''\text{-}H4''}$ = 4 Hz, 1H, $H_{5''}$), 3.93 (d, $J_{H3\text{-}H4}$ = 4 Hz, 1H, H_4), 4.00 (d, $J_{H3\text{-}H4}$ = 4 Hz, 1H, H_3), 4.05–4.08 (m, 1H, H_5), 4.25 (dd, $J_{H5''\text{-}H5''}$ = 14 Hz, $J_{H5''\text{-}H4''}$ = 10 Hz, 1H, $H_{5''}$), 4.32–4.35 (m, 2H, H_1, H_2), 4.42 (d, J = 12 Hz, 1H, H_{CH2Ph}), 4.49–4.55 (m, 3H, H_{CH2Ph}, $H_{4''}$), 4.59 (d, J = 12 Hz, 1H, H_{CH2Ph}), 4.65 (d, $J_{H2''\text{-}H3''}$ = 6 Hz, 1H, $H_{2''}$), 4.93 (d, $J_{H3''\text{-}H2''}$ = 6 Hz, 1H, $H_{3''}$), 5.11 (s, 1H, $H_{1''}$), 5.67 (d, $J_{H5'\text{-}H6'}$ = 8 Hz, 1H, $H_{5'}$), 7.28 (d, $J_{H6'\text{-}H5'}$ = 8 Hz, 1H, $H_{6'}$), 7.28–7.34 (m, 6H, H_{Ar}), 7.36–7.39 (m, 4H, H_{Ar}); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 7.6, 8.6 (C_{CH3CH2}), 27.6, 28.1, 28.2 (C_{Boc}), 29.1, 29.4 (C_{CH2CH3}), 48.8 ($C_{5''}$), 49.3 (C_1), 68.0 (C_6), 71.7, 72.1 (C_{CH2Ph}), 78.7 (C_2), 82.3 ($C_{3''}$), 82.5 (C_3), 83.4 (C_4, C_5), 84.2 ($C_{4''}$), 84.3 ($C_{2''}$), 84.6, 85.9, 86.8 (C_{Boc}), 101.6 ($C_{5'}$), 109.3 ($C_{1''}$), 116.5 (C_{CEt2}), 127.9, 128.0, 128.2, 128.3, 128.7, 128.8, 137.6, 137.3 (C_{Ar}), 144.2 (C_{Boc}), 145.4 ($C_{6'}$), 147.6, 148.1 (C_{Boc}), 149.4 ($C_{2'}$), 151.6 (C_{gua}), 157.4 (C_{Boc}), 161.1 ($C_{4'}$); HRMS calcd for $[M+Na]^+$ 1202.5736, found 1202.5734.

4.1.11. 2,5-Anhydro-3,4-dihydroxy-1-deoxy-1-(3′-(*N*-*tert*-butyloxycarbonyl)-uracil-1′-yl)-6-(5″-(*N*-*N*′-*N*″-*tetra*(*tert*-butyloxycarbonyl)guanidino)-1″,5″-dideoxy-2″,3″-*O*-isopentylidene-β-D-ribos-1″-yl)-D-glucitol 14

A suspension of 10% Pd/C (40 mg) and **13** (40 mg, 0.04 mmol) in MeOH (2 mL) was saturated with dihydrogen and stirred for 3 h. The reaction mixture was then filtered through a Celite pad, the

residue was washed with MeOH and the filtrate was concentrated in vacuo affording **14** as a colourless oil (34 mg, 99%). $[\alpha]_D^{20}$ −43 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.82, 0.87 (2t, J = 7 Hz, 6H, H_{CH3CH2}), 1.45, 1.47, 1.50 (3s, 36H, H_{Boc}), 1.50 (qd, J = 7 Hz, 2H, H_{CH2CH3}), 1.58 (s, 9H, H_{Boc}), 1.65 (qd, J = 7 Hz, 2H, H_{CH2CH3}), 3.49 (dd, $J_{H6\text{-}H6}$ = 11 Hz, $J_{H6\text{-}H5}$ = 3 Hz, 1H, H_6), 3.73 (dd, $J_{H1\text{-}H1}$ = 13 Hz, $J_{H1\text{-}H2}$ = 6 Hz, 1H, H_1), 3.85–3.88 (m, 2H, $H_{5''}$, H_4), 3.94–3.99 (m, 2H, H_6, H_3), 4.19–4.25 (m, 4H, $H_{5''}$, H_1, H_2, H_5), 4.52 (dd, J = 11 Hz, J = 5 Hz, 1H, $H_{4''}$), 4.64 (d, $J'_{H2''\text{–}H3'}$ = 6 Hz, 1H, $H_{2''}$), 4.93 (d, $J_{H3''\text{-}H2''}$ = 6 Hz, 1H, $H_{3''}$), 5.10 (s, 1H, $H_{1'}$), 5.68 (d, $J_{H5'\text{-}H6'}$ = 8 Hz, 1H, $H_{5'}$), 7.32 (d, $J_{H6'\text{-}H5'}$ = 8 Hz, 1H, $H_{6'}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 7.4, 8.5 (C_{CH3CH2}), 27.6, 28.1 (C_{Boc}), 28.8, 29.4 (C_{CH2CH3}), 48.3 ($C_{5''}$), 48.7 (C_1), 67.4 (C_6), 77.5 (C_4), 79.1 (C_2), 79.3 (C_5), 82.6 ($C_{3''}$), 84.4 (C_3), 84.7 ($C_{4''}$), 84.8 ($C_{2''}$), 85.0 (C_{Boc}), 85.9, 86.8 (C_{Boc}), 101.5 ($C_{5'}$), 109.3 ($C_{1''}$), 116.7 (C_{CEt2}), 145.0 (C_{Boc}), 145.9 ($C_{6'}$), 148.1 (C_{Boc}), 149.5 ($C_{2'}$), 151.3 (C_{gua}), 157.4 (C_{Boc}), 161.1 ($C_{4'}$); HRMS calcd for $[M+Na]^+$ 1022.4797, found 1022.4802.

4.1.12. 2,5-Anhydro-3,4-dihydroxy-1-deoxy-1-(uracil-1′-yl)-6-(5″-guanidino)-1″,5″-dideoxy-2″,3″-dihydroxy-β-D-ribos-1″-yl)-D-glucitol 15

At 0 °C, to a solution of **14** (30 mg, 0.03 mmol) in water (0.3 mL) was added TFA (1.2 mL). Then, the mixture was stirred for 1 h at rt and concentrated in vacuo. The residue was diluted with water and aqueous NH_4OH (28%, 6 mL) was added at 0 °C prior to concentration in vacuo. Chromatography on reverse phase (C18) using H_2O, H_2O/MeOH (1:1), and then MeOH afforded **15** as a white solid (8 mg, 60%). $[\alpha]_D^{20}$ −8 (*c* 1.0, MeOH); ^{1}H NMR (D_2O, 500 MHz): δ 3.32 (dd, $J_{H5''\text{-}H5''}$ = 15 Hz, $J_{H5''\text{-}H4''}$ = 6 Hz, 1H, $H_{5''}$), 3.55 (dd, $J_{H5''\text{-}H5''}$ = 15 Hz, $J_{H5''\text{-}H4''}$ = 3 Hz, 1H, $H_{5''}$), 3.66 (dd, $J_{H6\text{-}H6}$ = 12 Hz, $J_{H6\text{-}H5}$ = 8 Hz, 1H, H_6), 3.85–3.90 (m, 4H, H_6, H_5, H_1), 3.96 (dd, $J_{H3\text{-}H2}$ = 4 Hz, $J_{H3\text{-}H4}$ = 3 Hz, 1H, H_3), 4.03–4.06 (m, 2H, $H_{4''}$, $H_{2''}$), 4.13 (dd, $J_{H1\text{-}H1}$ = 15 Hz, $J_{H1\text{-}H2}$ = 4 Hz, 1H, H_1), 4.16 (dd, $J_{H3''\text{-}H2''}$ = 7 Hz, $J_{H3''\text{-}H4''}$ = 5 Hz, 1H, $H_{3''}$), 4.19 (dd, $J_{H4\text{-}H5}$ = 4 Hz, $J_{H4\text{-}H3}$ = 3 Hz, 1H, H_4), 4.29 (ddd, $J_{H2\text{-}H1}$ = 9 Hz, $J_{H2\text{-}H1}$ = $J_{H2\text{-}H3}$ = 4 Hz, 1H, H_2), 5.03 (s, 1H, $H_{1''}$), 5.79 (d, $J_{H5'\text{-}H6'}$ = 8 Hz, 1H, $H_{5'}$), 7.62 (d, $J_{H6'\text{-}H5'}$ = 8 Hz, 1H, $H_{6'}$); ^{13}C NMR (D_2O, 125 MHz): δ 45.2 ($C_{5''}$), 49.9 (C_1), 70.5 (C_6), 73.0 ($C_{3''}$), 75.6 ($C_{4''}$), 78.0 (C_4), 79.5, 79.5 (C_2, C_3), 82.0 ($C_{2''}$), 84.8 (C_5), 102.9 ($C_{5'}$), 109.4 ($C_{1''}$), 149.1 ($C_{6'}$), 154.1 ($C_{2'}$), 158.8 (C_{gua}), 168.7 ($C_{4'}$); HRMS calcd for $[M+1]^+$ 432.1731, found 432.1737.

4.1.13. *N,N*-bis-(*tert*-Butyloxycarbonyl)allylamine 16

To a solution of propargylamine (250 μL, 3.9 mmol) in THF (30 mL) were successively added, di-*tert*-butyl dicarbonate (2 g, 9.4 mmol), DMAP (476 mg, 3.9 mmol) and triethylamine (1.3 mL, 9.4 mmol) and the solution was stirred overnight. After concentration in vacuo, flash column chromatography (cyclohexane/acetone 6:4) afforded **16** as a colourless oil (89 mg, 90%). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.49 (s, 18H, H_{Boc}); 2.15 (t, $J_{Ha\text{-}Hc}$ = 3 Hz, 1H, H_a), 4.31 (d, $J_{Hc\text{-}Ha}$ = 3 Hz, 2H, H_c); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 28.2 (C_{Boc}), 35.8 (C_c), 70.6 (C_a), 79.7 (C_{Boc}), 83.0 (C_b), 151.6 (C_{Boc}); MS: m/z 278 (100%, $[M+Na]^+$).

4.1.14. (*S*)-*tert*-Butyl 2(pro-2-ynylcarbamoyl)pyrrolidine-1-carboxylate 17

To a solution of *N*-Boc-L-proline (164 mg, 0.76 mmol) in DMF (10 mL) were added HATU (290 mg, 0.76 mmol) and TEA (141 μL, 1 mmol). To this mixture was added a solution of propargylamine (33 μL, 0.5 mmol) in DMF (5 mL). The mixture was stirred overnight at rt, and then concentrated in vacuo. Flash chromatography (cyclohexane/acetone 65:35) afforded **17** as a colourless solid (128 mg, 99%). ^{1}H NMR ($CDCl_3$, 500 MHz, rotamers mixture): δ 1.1 (s, 9H, H_{Boc}), 1.79–1.90 (m, 2H, H_δ), 1.98–2.21 (m, 2H, H_β), 2.23–2.35 (m, 1H, H_a), 3.19–3.39 (m, 2H, H_γ, 3.85–4.1 (m, 2H, H_c), 4.10–4.30 (m, 1H, H_α), 6.39, 7.20 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 23.8–24.7 (C_δ), 28.4 (C_{Boc}), 29.1 (C_c), 30.9–31.1 (C_β), 47.2 (C_δ), 59.9–61.1 (C_α), 71.4–71.8 (C_a), 79.6 (C_{Boc}), 80.7 (C_b), 154.8–156.1 (C_{Boc}), 172.0–172.5 ($C_{CO\text{-}NH}$); MS: m/z 275 (100%, $[M+Na]^+$).

4.1.15. *N,N′*-di-(*tert*-Butyloxycarbonyl)guanidine 18

To a solution of *N,N′*-bis-(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine (579 mg, 2.6 mmol) in DMF (2 mL) were added *N,N*-diisopropylethylamine (446 μL, 2.6 mmol) and propargylamine (50 μL, 2.4 mmol). The mixture was stirred overnight at rt, then extracted with EtOAc. Flash chromatography (cyclohexane/acetone 7:3) afforded **17** as a white solid (418 mg, 59%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.44, 1.48 (2s, 18H_{Boc}), 2.25 (t, $J_{Ha\text{-}Hc}$ = 2 Hz, 1H, H_a), 4.22 (dd, $J_{Hc\text{-}Hc}$ = 5 Hz, $J_{Hc\text{-}Ha}$ = 2 Hz, 2H, H_c), 8.43, 11.42 (s, 1H, NH), ^{13}C NMR ($CDCl_3$, 125 MHz): δ 28.2 (C_{Boc}), 28.4 (C_{Boc}), 30.9 (C_c), 72.4 (C_a), 79.7 (C_{Boc}), 83.6 (C_b), 153.2 (C_{gua}), 155.8, 163.4 (C_{Boc}).

4.1.16. 2,5-Anhydro-3,4-di-*O* -benzyl-1-deoxy-1-(uracil-1′-yl)-6-[5″-(4‴-(*N,N-di-tert* -butyloxyaminomethyl)-1*H*,1‴,2‴,3‴-triazol-1‴-yl)-1″,5″-dideoxy-2″,3″-*O* -isopentylidene-β-D-ribos-1″-yl]-D-glucitol 19

A mixture of azide **4** (119 mg, 0.179 mmol), alkyne **16** (55 mg, 0.215 mmol), sodium ascorbate (11 mg, 0.054 mmol) and $CuSO_4{\cdot}5H_2O$ (9 mg, 0.036 mmol) in *t*-BuOH/H_2O (2:1, 4.5 mL) was vigorously stirred at rt for 3 days. After disappearance of azide, the mixture was diluted with H_2O and extracted with CH_2Cl_2. The combined organic layers were washed with H_2O, dried ($MgSO_4$) and concentrated in vacuo. Flash column chromatography (cyclohexane/acetone 1:1) afforded **19** as a white powder (48 mg, 29%). $[\alpha]_{Hg}^{20}$ −12 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.83, 0.86 (2t, J = 7 Hz, 6H, H_{CH3CH2}), 1.47 (s, 18H, H_{Boc}), 1.50, 1.64 (2qd, J = 7 Hz, 4H, H_{CH2CH3}), 3.54 (dd, $J_{H6\text{-}H6}$ = 11 Hz, $J_{H6\text{-}H5}$ = 6 Hz, 1H, H_6), 3.70 (dd, $J_{H1\text{-}H1}$ = 14 Hz, $J_{H1\text{-}H2}$ = 8 Hz, 1H, H_1), 3.82 (dd, $J_{H6\text{-}H6}$ = 11 Hz, $J_{H6\text{-}H5}$ = 4 Hz, 1H, H_6), 3.96 (d, $J_{H4\text{-}H3}$ = 3 Hz, 1H, H_4), 3.99 (d, $J_{H3\text{-}H4}$ = 3 Hz, 1H, H_3), 4.03–4.06 (m, 1H, $H_{4''}$), 4.24–4.32 (m, 3H, H_2, $H_{5''}$, H_1), 4.38–4.45 (m, 3H, H_5, $H_{5''}$, H_{CH2Ph}), 4.49–4.60 (m, 5H, $H_{2''}$, $H_{3''}$, H_{CH2Ph}), 4.88 (s, 2H, $H_{6'''}$), 5.11 (s, 1H, $H_{1''}$), 5.55 (d, J = 8 Hz, 1H, $H_{5'}$), 7.2 (d, J = 8 Hz, 1H, $H_{6'}$), 7.24–7.37 (m, 10H, H_{Ar}), 7.54 (s, 1H, $H_{5'''}$), 8.96 (s, 1H, $H_{3'}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 7.4, 8.5 (C_{CH3CH2}), 28.2 (C_{Boc}), 28.9, 29.5 (C_{CH2CH3}), 41.8 ($C_{6'''}$), 48.4 (C_1), 53.1 ($C_{5''}$), 68.2 (C_6), 71.6, 72.1 (C_{CH2Ph}), 79.1 (C_2), 82.1 (C_{Boc}), 82.7 ($C_{2''}$), 82.9 (C_{Boc}), 83.0 (C_3, C_4), 83.5 (C_2), 85.5 ($C_{3''}$), 85.8 (C_5), 101.9 ($C_{5'}$), 109.2 ($C_{1''}$), 117.4 (C_{CEt2}), 122.6 ($C_{5'''}$), 127.8, 127.9, 128.3, 128.4, 128.8, 128.8, 137.4, 137.5 (C_{Ar}), 145.7, 145.8 ($C_{4'''}$, $C_{6'}$), 151.1 ($C_{2'}$), 152.5 (C_{Boc}), 163.7 ($C_{4'}$); HRMS calcd for $[M+Na]^+$ 941.4273, found 941.4281.

4.1.17. 2,5-Anhydro-3,4-di-*O* -benzyl-1-deoxy-1-(uracil-1′-yl)-6-[5″-(4‴-(*N*-Boc-L-prolinyl-*N′*-amidomethyl)-1*H* ,1‴,2‴,3‴-triazol-1‴-yl)-1″,5″-dideoxy-2″,3″-*O* -isopentylidene-β-D-ribos-1″-yl]-D-glucitol 20

A mixture of **4** (120 mg, 0.18 mmol), **17** (55 mg, 0.216 mmol), sodium ascorbate (11 mg, 0.054 mmol), $CuSO_4{\cdot}5H_2O$ (9 mg, 0.036 mmol) and *N,N*-diisopropylethylamine (93 μL, 0.54 mmol) in *t*-BuOH/H_2O (2:1; 9 mL) was vigorously stirred at 50 °C for 2 h. After disappearance of azide, the mixture was diluted with H_2O and extracted with CH_2Cl_2. The combined organic layers were washed with H_2O, dried ($MgSO_4$) and concentrated in vacuo. Flash column chromatography (cyclohexane/acetone 1:1) afforded **20** as a white powder (44 mg, 29%). $[\alpha]_{Hg}^{20}$ −46 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz, rotamers mixture): δ 0.85, 0.86 (2t, J = 7 Hz, 6H, H_{CH3CH2}), 1.34 (s, 9H, H_{Boc}), 1.50, 1.65 (2qd, J = 7 Hz, 4H, H_{CH2CH3}), 1.83–1.89 (m, 4H, H_β, H_γ), 3.32–3.42 (m, 1H, H_α), 3.43–3.51 (m, 2H, H_δ), 3.62 (dd, $J_{H6\text{-}H6}$ = 11 Hz, $J_{H6\text{-}H5}$ = 6 Hz, 1H, H_6), 3.73 (dd, $J_{H1\text{-}H1}$ = 14 Hz, $J_{H1\text{-}H2}$ = 8 Hz, 1H, H_1), 3.83 (dd, $J_{H6\text{-}H6}$ = 11 Hz,

$J_{H6\text{-}H5}$ = 4 Hz, 1H, H_6), 3.89–3.96 (m, 1H, H_4), 4.02 (d, $J_{H3\text{-}H4}$ = 3 Hz, 1H, H_3), 4.08–4.12 (m, 1H, $H_{4''}$), 4.20–4.35 (m, 3H, H_2, $H_{5''}$, H_1), 4.40–4.46 (m, 3H, H_5, $H_{5''}$,H_{CH2Ph}), 4.54–4.65 (m, 6H, $H_{2''}$, $H_{3''}$, H_{CH2Ph}, $H_{6'}$), 5.14 (s, 1H, $H_{1''}$), 5.46–5.50 (m, 1H, $H_{5'}$), 7.16–7.2 (m, 1H, $H_{6'}$), 7.26–7.37 (m, 10H, H_{Ar}), 7.62 (br s, 1H, $H_{5'''}$), 8.52, 8.64 (br s, 1H, $H_{3'}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 8.7, 9.8 (C_{CH3CH2}), 28.3 (C_β, C_γ), 29.8 (C_{Boc}), 30.2, 30.8 (C_{CH2CH3}), 44.9 ($C_{6'''}$), 48.5, 48.7 (C_α, C_δ), 49.6 (C_1), 54.1 ($C_{5''}$), 70.0 (C_6), 73.0, 73.4 (C_{CH2Ph}), 78.7 (C_2), 81.9 (C_{Boc}), 83.5, 84.1, 84.6 ($C_{2''}$, C_3, C_4, $C_{4''}$), 86.8, 87.0 ($C_{3''}$, C_5), 103.0 ($C_{5'}$), 111.0 ($C_{1''}$), 118.6 (C_{CEt2}), 129.1, 129.2, 129.6, 129.7, 130.0, 130.1 (C_{Ar}), 132.3 ($C_{5'''}$), 138.6, 138.7 (C_{Ar}), 146.9 ($C_{6'}$), 152.3 ($C_{2'}$); HRMS calcd for $[M+Na]^+$ 938.4276, found 938.4258.

4.1.18. 2,5-Anhydro-3,4-di-*O*benzyl-1-deoxy-1-(uracil-1′-yl)-6-[5″-(4‴-(*N*-*N*′-bis-(*tert*-butyloxycarbonyl)guanidinomethylene)-1*H*1‴,2‴,3‴-triazol-1‴-yl)-1″,5″-dideoxy-2″,3″-*O*isopentylidene-β-D-ribos-1″-yl]-D-glucitol 21

A mixture of **4** (108 mg, 0.163 mmol), **18** (58 mg, 0.196 mmol), sodium ascorbate (10 mg, 0.054 mmol), $CuSO_4{\cdot}5H_2O$ (8 mg, 0.033 mmol) and diisopropylethylamine (84 μL, 0.49 mmol) in *t*-BuOH/H_2O (2:1, 9 mL) was vigourously stirred at 50 °C for 3 h. After disappearance of azide, the mixture was diluted with H_2O and extracted with CH_2Cl_2. The combined organic layers were washed with H_2O, dried ($MgSO_4$) and concentrated in vacuo. Flash column chromatography (cyclohexane/acetone 1:1) afforded **21** as a white powder (89 mg, 57%). $[\alpha]_{Hg}^{20}$ −8 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.84, 0.85 (2t, *J* = 7 Hz, 6H, H_{CH3CH2}), 1.45, 1.49 (2s, 18H, H_{Boc}), 1.51, 1.64 (2qd, *J* = 7 Hz, 4H, H_{CH2CH3}), 3.54 (dd, $J_{H6\text{-}H6}$ = 11 Hz, $J_{H6\text{-}H5}$ = 6 Hz, 1H, H_6), 3.68 (dd, $J_{H1\text{-}H1}$ = 14 Hz, $J_{H1\text{-}H2}$ = 8 Hz, 1H, H_1), 3.82 (dd, $J_{H6\text{-}H6}$ = 11 Hz, $J_{H6\text{-}H5}$ = 4 Hz, 1H, H_6), 3.94 (d, $J_{H3\text{-}H4}$ = 3 Hz, 1H, H_3), 3.98 (d, $J_{H4\text{-}H3}$ = 3 Hz, 1H, H_4), 4.03–4.07 (m, 1H, $H_{4''}$), 4.23–4.33 (m, 3H, H_2, $H_{5''}$, H_1), 4.38–4.42 (m, 2H, $H_{5''}$, H_{CH2Ph}), 4.46 (dd, $J_{H5\text{-}H6a}$ = 7 Hz, $J_{H5\text{-}H6b}$ = 7 Hz, 1H, H_5), 4.49–4.59 (m, 5H, $H_{2''}$, $H_{3''}$, H_{CH2Ph}), 4.88 (br s, 2H, $H_{6'''}$), 5.12 (s, 1H, $H_{1''}$), 5.54 (d, *J* = 8 Hz, 1H, $H_{5'}$), 7.16 (d, *J* = 8 Hz, 1H, $H_{6'}$), 7.23–7.34 (m, 10H, H_{Ar}), 7.57 (s, 1H, $H_{5'''}$), 8.82 (s, 1H, $H_{3'}$), 9.39, 11.43 (2s, 2H, NH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 7.4, 8.5 (C_{CH3CH2}), 28.2, 28.4 (C_{Boc}), 28.9, 29.5 (C_{CH2CH3}), 36.6 ($C_{6'''}$), 48.3 (C_1), 53.1 ($C_{5''}$), 68.4 (C_6), 71.6, 72.0 (C_{CH2Ph}), 79.0 (C_2), 79.6 (C_{Boc}), 82.1 ($C_{2''}$), 82.7, 82.8 (C_3, C_4), 83.4, 83.5 ($C_{4''}$, C_{Boc}), 85.5 (C_5), 85.6 ($C_{3''}$), 101.8 ($C_{5'}$), 109.3 ($C_{1''}$), 117.3 (C_{CEt2}), 122.5 ($C_{5'''}$), 127.7, 127.9, 128.2, 128.3, 128.7, 128.8, 137.3, 137.5 (C_{Ar}), 144.3 ($C_{4'''}$), 145.7 ($C_{6'}$), 151.2 ($C_{2'}$), 153.0 (C_{gua}), 156.0 (C_{Boc}), 163.9 ($C_{4'}$); HRMS calcd for $[M+Na]^+$ 983.4491, found 983.4464.

4.1.19. 2,5-Anhydro-3,4-dihydroxy-1-deoxy-1-(uracil-1′-yl)-6-[5″-(4‴-(*N*,*N*-*di*-*ter* t-butyloxyaminomethyl)-1*H*,1‴,2‴,3‴-triazol-1‴-yl)-1″,5″-dideoxy-2″,3″-*O* -isopentylidene-β-D-ribos-1″-yl]-D-glucitol 22

A suspension of 10% Pd/C (45 mg) and **19** (47 mg, 0.05 mmol) in MeOH (2 mL) was saturated with dihydrogen and stirred for 3 h. The reaction mixture was then filtered through a Celite pad, the residue was washed with MeOH and the filtrate was concentrated in vacuo affording **22** as a colourless oil (30 mg, 79%). $[\alpha]_D^{20}$ −16 (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.83, 0.86 (2t, *J* = 7 Hz, 6H, H_{CH3CH2}), 1.46 (s, 18H, H_{Boc}), 1.53, 1.65 (2qd, *J* = 7 Hz, 4H, H_{CH2CH3}), 3.56 (dd, $J_{H6\text{-}H6}$ = 10 Hz, $J_{H6\text{-}H5}$ = 6 Hz, 1H, H_6), 3.71 (dd, $J_{H1\text{-}H1}$ = 15 Hz, $J_{H1\text{-}H2}$ = 8 Hz, 1H, H_1), 3.84 (dd, $J_{H6\text{-}H6}$ = 10 Hz, $J_{H6\text{-}H5}$ = 3 Hz, 1H, H_6), 3.91–3.96 (m, 1H, $H_{4''}$), 4.06 (br s, 1H, H_4), 4.09 (br s, 1H, H_3), 4.16–4.24 (m, 2H, H_2, H_1), 4.43–4.60 (m, 3H, H_5, $H_{5''}$), 4.72–4.76 (m, 2H, $H_{2''}$, $H_{3''}$), 4.86 (s, 2H, $H_{6'''}$), 5.16 (s, 1H, $H_{1''}$), 5.60 (d, *J* = 8 Hz, 1H, $H_{5'}$), 7.34 (d, *J* = 8 Hz, 1H, $H_{6'}$), 7.66 (s, 1H, $H_{5'''}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 7.5, 8.6 (C_{CH3CH2}), 28.2 (C_{Boc}), 29.0, 29.6 (C_{CH2CH3}), 41.8 ($C_{6'''}$), 48.2 (C_1), 53.4 ($C_{5''}$), 68.9 (C_6), 77.3 (C_3), 79.1 (C_2), 79.4 (C_4), 82.2 ($C_{2''}$), 83.3 (C_{Boc}), 84.6 ($C_{4''}$), 85.5 ($C_{3''}$), 85.8 (C_5), 102.2 ($C_{5'}$), 109.5 ($C_{1''}$), 117.5 (C_{CEt2}), 122.9 ($C_{5'''}$), 145.6, 146.2 ($C_{4'''}$, $C_{6'}$), 152.0 ($C_{2'}$), 152.6 (C_{Boc}), 164.7 ($C_{4'}$); HRMS calcd for $[M+Na]^+$ 761.3334, found 761.3348.

4.1.20. 2,5-Anhydro-3,4-dihydroxy-1-deoxy-1-(uracil-1′-yl)-6-[5″-(4‴-(*N*-Boc-L-prolinyl-*N*′-*amidomethyl*)-1*H* ,1‴,2‴,3‴-triazol-1‴-yl)-1″,5″-dideoxy-2″,3″-*O* -isopentylidene-β-D-ribos-1″-yl]-D-glucitol 23

A suspension of 10% Pd/C (25 mg) and **20** (24 mg, 0.026 mmol) in MeOH (2 mL) was saturated with dihydrogen and stirred for 12 h. The reaction mixture was then filtered through a Celite pad, the residue was washed with MeOH and the filtrate was concentrated in vacuo affording **23** as a colourless oil (15 mg, 79%). ^{1}H NMR (MeOD, 500 MHz): δ 0.88, 0.90 (2t, *J* = 7 Hz, 6H, H_{CH3CH2}), 1.34 (s, 9H, H_{Boc}), 1.61, 1.69 (2qd, *J* = 7 Hz, 4H, H_{CH2CH3}), 1.83–1.95 (m, 3H, H_β, H_γ), 2.17–2.24 (m, 1H, H_γ, 3.40–3.45 (m, 1H, H_δ), 3.51–3.55 (m, 1H, H_δ), 3.65–3.69 (m, 1H, H_6), 3.80 (dd, $J_{H1\text{-}H1}$ = 15 Hz, $J_{H1\text{-}H2}$ = 8 Hz, 1H, H_1), 3.89–3.93 (m, 2H, $H_{4''}$, H_6), 3.98 (br s, 1H, H_4), 4.04 (br s, 1H, H_3), 4.14–4.16 (m, 1H, H_α), 4.19–4.26 (m, 4H, H_1, H_2, H_5), 4.39–4.67 (m, 6H, $H_{2''}$, $H_{3''}$, $H_{5''}$, $H_{6''}$), 5.21 (s, 1H, $H_{1''}$), 5.56 (d, *J* = 8 Hz, 1H, $H_{5'}$), 7.54 (d, *J* = 8 Hz, 1H, $H_{6'}$), 8.0 (s, 1H, $H_{5'''}$); ^{13}C NMR (MeOD, 125 MHz): δ 7.8, 8.8 (C_{CH3CH2}), 24.7 (C_β), 28.7 (C_{Boc}), 29.9, 30.3 (C_{CH2CH3}), 32.5 (C_γ), 35.5 ($C_{6'''}$), 47.9 (C_δ), 48.2 (C_1), 54.8 ($C_{5''}$), 61.9 (C_α), 70.0 (C_6), 78.5 (C_3), 80.2, 80.3 (C_2, C_4, C_5), 83.5 ($C_{4''}$), 85.9, 85.9 ($C_{2''}$, $C_{3''}$), 86.8 (C_{Boc}), 102.1 ($C_{5'}$), 111.0 ($C_{1''}$), 118.1 (C_{CEt2}), 125.3 ($C_{5'''}$), 146.0, 147.7 ($C_{4'''}$, $C_{6'}$), 156.0 ($C_{2'}$), 169.1 ($C_{4'}$).

4.1.21. 2,5-Anhydro-3,4-dihydroxy-1-deoxy-1-(uracil-1′-yl)-6-[5″-(4‴-aminomethyl-1*H*,1‴,2‴,3‴-triazol-1‴-yl)-1″,5″-dideoxy-2″,3″-dihydroxy-β-D-ribos-1″-yl]-D-glucitol 24

At 0 °C, to a solution of **22** (30 mg, 0.04 mmol) in water (0.3 mL) was added TFA (1.2 mL). The mixture was stirred for 1 h at rt, and then concentrated in vacuo. The residue was diluted with water and aqueous NH_4OH (28%, 6 mL) was added at 0 °C. The mixture was concentrated in vacuo. Chromatography on reverse phase (C18) using H_2O, H_2O/MeOH (1:1), and then MeOH afforded **24** as a white solid (19 mg, 47%). $[\alpha]_D^{20}$ −2 (c 1.0, MeOH); ^{1}H NMR (D_2O, 500 MHz): δ 3.65 (dd, $J_{H6\text{-}H6}$ = 12 Hz, $J_{H6\text{-}H5}$ = 9 Hz, 1H, H_6), 3.83–3.90 (m, 3H, H_1, H_6, H_5), 3.98 (dd, $J_{H4''\text{-}H5''}$ = 4 Hz, $J_{H4''\text{-}H5''}$ = 2 Hz, 1H, $H_{4''}$), 4.08 (d, $J_{H3\text{-}H4}$ = 4 Hz, 1H, H_3), 4.16 (dd, $J_{H1\text{-}H1}$ = 14 Hz, $J_{H1\text{-}H2}$ = 4 Hz, 1H, H_1), 4.22 (dd, $J_{H2\text{-}H1}$ = 4 Hz, $J_{H2\text{-}H1}$ = 2 Hz, 1H, H_2), 4.28–4.34 (m, 3H, $H_{3''}$, H_4, $H_{2''}$), 4.36 (s, 2H, $H_{6'''}$), 4.61 (dd, $J_{H5''\text{-}H5''}$ = 14 Hz, $J_{H5''\text{-}H4''}$ = 7 Hz, 1H, $H_{5''}$), 4.80 (dd, $J_{H5''\text{-}H5''}$ = 14 Hz, $J_{H5''\text{-}H4''}$ = 3 Hz, 1H, $H_{5''}$), 5.04 (s, 1H, $H_{1''}$), 5.77 (d, *J* = 8 Hz, 1H, $H_{5'}$), 7.63 (d, *J* = 8 Hz, 1H, $H_{6'}$), 8.14 (s, 1H, $H_{5'''}$); ^{13}C NMR (D_2O, 125 MHz): δ 35.5 ($C_{6'''}$), 49.9 (C_1), 54.7 ($C_{5''}$), 70.5 (C_6), 73.4 ($C_{3''}$), 75.5 (C_3), 78.1 (C_2), 79.5, 79.6 ($C_{4''}$, $C_{2''}$), 84.8 (C_4), 86.6 (C_5), 102.7 ($C_{5'}$), 109.5 ($C_{1''}$), 127.2 ($C_{5'''}$), 141.3 ($C_{4'''}$), 149.2 ($C_{6'}$), 153.6 ($C_{2'}$), 168.1 ($C_{4'}$); HRMS calcd for $[M+Na]^+$ 493.1659, found 493.1664.

4.1.22. 2,5-Anhydro-3,4-dihydroxy-1-deoxy-1-(uracil-1′-yl)-6-[5″-(4‴-(*N*-L-prolinyl-*N*′-amidomethyl)-1*H*,1‴,2‴,3‴-triazol-1‴-yl)-1″,5″-dideoxy-2″,3″-dihydroxy-β-D-ribos-1″-yl]-D-glucitol 25

At 0 °C, to a solution of **23** (15 mg, 0.002 mmol) in water (0.1 mL) was added TFA (0.4 mL). The mixture was stirred at rt for 1 h, and then concentrated in vacuo. The residue was diluted with water and aqueous NH_4OH (28%, 6 mL) was added at 0 °C. The mixture was then concentrated in vacuo and chromatography on reverse phase (C18) using H_2O, H_2O/MeOH (1:1), and then MeOH afforded **25** as a white solid (7 mg, 64%). $[\alpha]_D^{20}$ −6 (*c* 1.0, MeOH); ^{1}H NMR (D_2O, 500 MHz): δ 1.99–2.04 (m, 3H, H_β, H_γ), 2.38–2.40 (m, 1H, H_γ, 3.36–3.40 (m, 2H, H_δ), 3.62 (dd, $J_{H6\text{-}H6}$ = 11 Hz, $J_{H6\text{-}H5}$ = 8 Hz, 1H, H_6), 3.78–3.86 (m, 3H, H_1, H_6, H_5), 3.92–3.95 (m, 1H, $H_{4''}$), 4.01 (d, $J_{H3\text{-}H4}$ = 4 Hz, 1H, H_3), 4.12–4.31 (m, 5H, H_4, H_2, H_1, $H_{2''}$, $H_{3''}$), 4.34–4.37 (m, 1H, H_α), 4.49 (s, 2H, $H_{6'''}$), 4.53 (dd, $J_{H5''\text{-}H5''}$ = 14 Hz, $J_{H5''\text{-}H4''}$ = 7 Hz, 1H, $H_{5''}$), 4.71–4.73

(m, 1H, $H_{5''}$), 5.0 (s, 1H, $H_{1''}$), 5.72 (d, J = 8 Hz, 1H, $H_{5'}$), 7.58 (d, J = 8 Hz, 1H, $H_{6'}$), 7.91 (s, 1H, $H_{5'''}$); ^{13}C NMR (D_2O, 125 MHz): δ 25.2 (C_β), 31.0 (C_γ), 36.0 ($C_{6'''}$), 47.9 (C_δ), 49.9 (C_1), 54.4 ($C_{5''}$), 61.3 (C_α), 70.6 (C_6), 73.3 (C_2), 75.5 (C_3), 78.1 (C_4), 79.6, 79.6 ($C_{2''}$, $C_{4''}$), 81.8 ($C_{3''}$), 84.9 (C_5), 102.7 ($C_{5'}$), 109.5 ($C_{1''}$), 125.9 ($C_{5'''}$), 145.5 ($C_{4'''}$), 149.2 ($C_{6'}$), 153.6 ($C_{2'}$), 168.1 ($C_{4'}$); HRMS calcd for $[M+1]^+$ 568.2367, found 568.2378.

4.1.23. 2,5-Anhydro-3,4-di-*O*-benzyl-1-deoxy-1-(3′-(*N*-*tert*-butyloxycarbonyl)-uracil-1′-yl)-6-[5″-(4‴-(*N*-*N*′-*N*″-*tetra*(*tert*-butyloxycarbonyl)guanidinomethylene)-1*H*,1‴,2‴,3‴-triazol-1‴-yl)-1″,5″-dideoxy-2″,3″-*O*-isopentylidene-β-D-ribos-1″-yl]-D-glucitol 26

To a solution of **21** (41 mg, 0.042 mmol) in THF (5 mL) were added, di-*tert*-butyl dicarbonate (47 mg, 0.21 mmol), DMAP (5 mg, 0.042 mmol), then triethylamine (30 μL, 0.21 mmol) and the solution was stirred overnight at rt. After concentration in vacuo, flash column chromatography (cyclohexane/acetone 7:3) afforded **26** as a colourless oil (51 mg, 79%). $[\alpha]_D^{20}$ +2 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.83, 0.84 (2t, J = 7 Hz, 6H, H_{CH3CH2}), 1.46, 1.48, 1.49 (3s, 36H, H_{Boc}), 1.51 (qd, J = 7 Hz, 2H, H_{CH2CH3}), 1.59 (s, 9H, H_{Boc}), 1.63 (qd, J = 7 Hz, 2H, H_{CH2CH3}), 3.54 (dd, $J_{H6\text{-}H6}$ = 11 Hz, $J_{H6\text{-}H5}$ = 6 Hz, 1H, H_6), 3.65 (dd, $J_{H1\text{-}H1}$ = 14 Hz, $J_{H1\text{-}H2}$ = 8 Hz, 1H, H_1), 3.87 (dd, $J_{H6\text{-}H6}$ = 11 Hz, $J_{H6\text{-}H5}$ = 4 Hz, 1H, H_6), 3.95 (d, $J_{H3\text{-}H4}$ = 3 Hz, 1H, H_3), 3.97 (d, $J_{H4\text{-}H3}$ = 3 Hz, 1H, H_4), 4.04–4.07 (m, 1H, $H_{4''}$), 4.26–4.34 (m, 3H, H_2, $H_{5''}$, H_1), 4.37–4.42 (m, 3H, H_5, $H_{5''}$,H_{CH2Ph}), 4.51–4.59 (m, 5H, $H_{2''}$, $H_{3''}$, 3H_{CH2Ph}), 5.06 (d, $J_{H6'''\text{-}H6'''}$ = 15 Hz 1H, $H_{6'''}$), 5.12 (s, 1H, $H_{1''}$), 5.13 (d, $J_{H6'''\text{-}H6'''}$ = 15 Hz 1H, $H_{6'''}$), 5.57 (d, J = 8 Hz, 1H, $H_{5'}$), 7.17 (d, J = 8 Hz, 1H, $H_{6'}$), 7.16–7.38 (m, 10H, H_{Ar}), 7.80 (s, 1H, $H_{5'''}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 8.7, 9.8 (C_{CH3CH2}), 28.3, 28.4, 29.3, 29.4 (C_{Boc}), 30.2, 30.8 (C_{CH2CH3}), 43.9 ($C_{6'''}$), 50.2 (C_1), 54.3 ($C_{5''}$), 69.6 (C_6), 72.9, 73.3 (C_{CH2Ph}), 80.2 (C_2), 83.4, 83.5 ($C_{2''}$, C_{Boc}), 84.0, 84.1 (C_3, C_4), 84.8, 85.1 (C_{Boc}), 85.6 ($C_{4''}$), 86.8, 87.0 (C_5, $C_{3''}$), 88.1 (C_{Boc}), 102.8 ($C_{5'}$), 110.5 ($C_{1''}$), 118.5 (C_{CEt2}), 125.1 ($C_{5'''}$), 129.1, 129.2, 129.5, 129.7, 130.0, 130.1, 138.6, 138.8 (C_{Ar}), 145.4, 145.7 ($C_{4'''}$, C_{Boc}), 146.3 ($C_{6'}$), 148.9, 149.3 (C_{Boc}), 150.6 ($C_{2'}$), 152.3 (C_{gua}), 158.8 (C_{Boc}), 162.2 ($C_{4'}$); HRMS calcd for $[M+Na]^+$ 1283.6063, found 1283.6051.

4.1.24. 2,5-Anhydro-3,4-dihydroxy-1-deoxy-1-(3′-(*N*-*tert*-butyloxycarbonyl)-uracil-1′-yl)-6-[5″-(4‴-(*N*-*N*′-*N*″-*tetra*(*tert*-butyloxycarbonyl)guanidinomethylene)-1*H*,1‴,2‴,3‴-triazol-1‴-yl)-1″,5″-dideoxy-2″,3″-*O*-isopentylidene-β-D-ribos-1″-yl]-D-glucitol 27

A suspension of 10% Pd/C (50 mg) and **26** (51 mg, 0.04 mmol) in MeOH (2 mL) was saturated with dihydrogen and stirred for 3 h. The reaction mixture was then filtered through a Celite pad, the residue was washed with MeOH and the filtrate was concentrated in vacuo affording **27** as a colourless oil (39 mg, 90%). $[\alpha]_D^{20}$ +6 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.85, 0.86 (2t, J = 7 Hz, 6H, H_{CH3CH2}), 1.43, 1.45, 1.49 (3s, 36H, H_{Boc}), 1.56 (qd, J = 7 Hz, 2H, H_{CH2CH3}), 1.58 (s, 9H_{Boc}), 1.66 (qd, J = 7 Hz, 2H, H_{CH2CH3}), 3.55 (dd, $J_{H6\text{-}H6}$ = 10 Hz, $J_{H6\text{-}H5}$ = 6 Hz, 1H, H_6), 3.72 (dd, $J_{H1\text{-}H1}$ = 14 Hz, $J_{H1\text{-}H2}$ = 6 Hz, 1H, H_1), 3.84 (dd, $J_{H6\text{-}H6}$ = 10 Hz, $J_{H6\text{-}H5}$ = 4 Hz, 1H, H_6), 3.93–3.97 (m, 1H, H_5), 4.05 (d, $J_{H4\text{-}H3}$ = 3 Hz, 1H, H_4), 4.09 (d, $J_{H3\text{-}H4}$ = 3 Hz, 1H, H_3), 4.21–4.24 (m, 1H, H_2), 4.27 (dd, $J_{H1\text{-}H1}$ = 14 Hz, $J_{H1\text{-}H2}$ = 5 Hz, 1H, H_1), 4.44 (dd, $J_{H5''\text{-}H5''}$ = 14 Hz, $J_{H4''\text{-}H5''}$ = 10 Hz, 1H, $H_{4''}$), 4.5 (dd, $J_{H5''\text{-}H5''}$ = 14 Hz, $J_{H5''\text{-}H4''}$ = 5 Hz, 1H, $H_{5''}$), 4.60 (dd, $J_{H4''\text{-}H5''}$ = 10 Hz, $J_{H4''\text{-}H5''}$ = 5 Hz, 1H, $H_{4''}$), 4.72–4.74 (m, 3H, $H_{2''}$, $H_{3''}$), 5.08 (s, 2H, $H_{6'''}$), 5.14 (s, 1H, $H_{1''}$), 5.66 (d, J = 8 Hz, 1H, $H_{5'}$), 7.31 (d, J = 8 Hz, 1H, $H_{6'}$), 7.87 (s, $H_{5'''}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 7.5, 8.6 (C_{CH3CH2}), 27.7, 28.0, 28.2 (C_{Boc}), 29.0, 29.7 (C_{CH2CH3}), 43.2 ($C_{6'''}$), 48.5 (C_1), 53.6 ($C_{5''}$), 68.9 (C_6), 77.5 (C_4), 79.1 (C_2), 79.9 (C_3), 82.2 ($C_{2''}$), 83.0, 84.3, 84.7 (C_{Boc}), 85.1 (C_5), 85.5 ($C_{3''}$), 85.9 ($C_{3''}$), 87.0 (C_{Boc}), 101.9 ($C_{5'}$), 109.3 ($C_{1''}$), 117.6 (C_C Et2), 124.0 ($C_{5'''}$), 144.1 ($C_{4'''}$), 145.0 (C_{Boc}), 145.1 ($C_{6'}$), 147.9, 148.0 (C_{Boc}), 149.6 ($C_{2'}$), 151.0 (C_{gua}), 158.2 (C_{Boc}), 161.0 ($C_{4'}$); HRMS calcd for $[M+1]^+$ 1081.5305, found 1083.5311.

4.1.25. 2,5-Anhydro-3,4-dihydroxy-1-deoxy-1-(uracil-1′-yl)-6-[5″-(4‴-guanidinomethylene)-1*H*,1‴,2‴,3‴-triazol-1‴-yl)-1″,5″-dideoxy-2″,3″-dihydroxy-β-D-ribos-1″-yl]-D-glucitol 28

At 0 °C, to a solution of **27** (39 mg, 0.036 mmol) in water (0.3 mL) was added TFA (1.2 mL). The mixture was stirred at rt for 1 h, and then concentrated in vacuo. The residue was diluted with water and aqueous NH_4OH (28%, 6 mL) was added at 0 °C. The mixture was then concentrated in vacuo and chromatography on reverse phase (C18) using H_2O, H_2O/MeOH (1:1) and then MeOH afforded **28** as a white solid (9 mg, 50%). $[\alpha]_D^{20}$ +3 (*c* 1.0, MeOH); ^{1}H NMR (D_2O, 500 MHz): δ 3.63 (dd, $J_{H6\text{-}H6}$ = 11 Hz, $J_{H6\text{-}H5}$ = 8 Hz, 1H, H_6), 3.80 (dd, $J_{H6\text{-}H6}$ = 11 Hz, $J_{H6\text{-}H5}$ = 3 Hz, 1H, H_6), 3.81–3.83 (m, 1H, H_5), 3.87 (dd, $J_{H1\text{-}H1}$ = 11 Hz, $J_{H1\text{-}H2}$ = 8 Hz, 1H, H_1), 3.95 (dd, $J_{H4''\text{-}H5''}$ = 4 Hz, $J_{H4''\text{-}H5''}$ = 2 Hz, 1H, $H_{4''}$), 4.02 (d, $J_{H3\text{-}H4}$ = 4 Hz, 1H, H_3), 4.11–4.23 (m, 3H, H_1, H_2, H_4), 4.25–4.30 (m, 2H, $H_{3''}$, $H_{2''}$), 4.50 (s, 2H, $H_{6'''}$), 4.56 (dd, $J_{H5''\text{-}H5''}$ = 14 Hz, $J_{H5''\text{-}H4''}$ = 7 Hz, 1H, $H_{5''}$), 4.76 (dd, $J_{H5''\text{-}H5''}$ = 14 Hz, $J_{H5''\text{-}H4''}$ = 3 Hz, 1H, $H_{5''}$), 5.01 (s, 1$H_{1''}$), 5.73 (d, J = 8 Hz, 1H, $H_{5'}$), 7.59 (d, J = 8 Hz, 1H, $H_{6'}$), 7.98 (s, 1H, $H_{5'''}$); ^{13}C NMR (D_2O, 125 MHz): δ 37.7 ($C_{6'''}$), 49.9 (C_1), 54.4 ($C_{5''}$), 70.6 (C_6), 73.3 (C_2), 75.5 (C_3), 78.1 (C_4), 79.5 ($C_{4''}$), 81.8 ($C_{2''}$, $C_{3''}$), 84.8 (C_5), 102.7 ($C_{5'}$), 109.5 ($C_{1''}$), 126.0 ($C_{5'''}$), 144.6 ($C_{4'''}$), 149.1 ($C_{6'}$), 153.6 ($C_{2'}$), 158.4 (C_{gua}), 168.1 ($C_{4'}$); HRMS calcd for $[M+1]^+$ 513.2057, found 513.2045.

4.2. Enzymatic assays

The activities of the compounds against MraY from *B. subtilis* were tested as previously described.[10,22] 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 from *Escherichia coli* as previously described.[23,24] 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 analogue, 35% (v/v) dimethyl sulfoxide and MurG. After 30 min at 37 °C, it was stopped by boiling for 3 min. Then, the mixture was lyophilised and taken up in 10 μL of 2-propanol/ammonium hydroxide/water (6:3:1; v/v/v). In both cases, the radiolabeled substrates (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 a mobile phase. The radioactive spots were located and quantified with a radioactivity scanner (model Multi-Tracemaster LB285; EG&G Wallac/Berthold). For MraY activity, residual activities and IC_{50} values were calculated with respect to a control assay without the inhibitors. Data represent the mean of independent triplicate determinations, and the standard deviation was less than 10%.

Acknowledgements

We gratefully acknowledge the European Community for the financial support of the Eur-INTAFAR integrated project within the 6th PCRDT framework (contract N° LSHM-CT-2004-512138) and for post-doctoral Grants to D.L., A.C. and B.A. We thank L. Le Corre for his generous gift of compound **17**.

References and notes

1. Monasson, O.; Ginisty, M.; Mravljak, J.; Bertho, G.; Gravier-Pelletier, C.; Le Merrer, Y. *Tetrahedron: Asymmetry* **2009**, *20*, 2320. and references cited.
2. Boyle, D. S.; Donachie, W. D. *J. Bacteriol.* **1998**, *180*, 6429.
3. (a) van Heijenoort, J. *Nat. Prod. Rep.* **2001**, *8*, 503; Bouhss, A.; Trunkfield, A. E.; Bugg, T. D. H.; Mengin-Lecreulx, D. *FEMS Microbiol. Rev.* **2008**, *32*, 208.
4. Bouhss, A.; Mengin-Lecreulx, D.; Le Beller, D.; van Heijenoort, J. *Mol. Microbiol.* **1999**, *34*, 576.
5. Clouet, A.; Gravier-Pelletier, C.; Al-Dabbagh, B.; Bouhss, A.; Le Merrer, Y. *Tetrahedron: Asymmetry* **2008**, *19*, 397.
6. For liposidomycins, see: (a) Isono, K.; Uramoto, M.; Kusakabe, H.; Kimura, K.; Izaki, K.; Nelson, C. C.; McCloskey, J. A. *J. Antibiot.* **1985**, *38*, 1617; (b) For caprazamycins, see: Takeuchi, T.; Igarashi, M.; Naganawa, H.; Hamada, M. PCT Int. Appl. 2001, pp 73, WO 2001012643.; (c) Igarashi, M.; Nakagawa, N.; Doi, N.; Hattori, S.; Naganawa, H.; Hamada, M. *J. Antibiot.* **2003**, *56*, 580.
7. Dini, C.; Colette, P.; Drochon, N.; Guillot, J.-C.; Lemoine, G.; Mauvais, P.; Aszodi, J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1839.
8. For correlation of molecular flexibility and inhibitory activities, see for example: (a) Mai, A.; Massa, S.; Ragno, R.; Cerbara, I.; Jesacher, F.; loidl, P.; Brosch, G. *J. Med. Chem.* **2003**, *46*, 512; (b) Narsinghani, T.; Chaturvedi, S. C. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 461.
9. Dini, C.; Drochon, N.; Guillot, J.-C.; Mauvais, P.; Walter, P.; Aszodi, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 533.
10. Bouhss, A.; Crouvoisier, M.; Blanot, D.; Mengin-Lecreulx, D. *J. Biol. Chem.* **2004**, *279*, 29974.
11. Huisgen, R. In Padwa, A., Ed.; 1,3-Dipolar Cycloadditional Chemistry; Wiley: New York, 1984; pp 1–176.
12. For review of click chemistry, see: (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004; (b) Kolb, H. C.; Sharpless, I. B. *Drug Discovery Today* **2003**, *8*, 1128.
13. For antibacterial activity of triazole compounds, see: Genin, M. J.; Allwine, D. A.; Anderson, D. J.; Barbachyn, M. R.; Emmert, D. E.; Garmon, S. A.; Graber, D. R.; Grega, K. C.; Hester, J. B.; Hutchinson, D. K.; Morris, J.; Reischer, R. J.; Ford, C. W.; Zurenko, G. E.; Hamel, J. C.; Schaadt, R. D.; Stapert, D.; Yagi, B. H. *J. Med. Chem.* **2000**, *43*, 953.
14. Poitout, L.; Le Merrer, Y.; Depezay, J.-C. *Tetrahedron Lett.* **1994**, *35*, 3293.
15. For analogous tandem reaction on bis-epoxides, see: (a) Poitout, L.; Le Merrer, Y.; Depezay, J.-C. *Tetrahedron Lett.* **1995**, *36*, 6887; (b) Le Merrer, Y.; Sanière, M.; McCort, I.; Dupuy, C.; Depezay, J.-C. *Tetrahedron Lett.* **2001**, *42*, 2661; (c) Saladino, R.; Ciambecchini, U.; Hanessian, S. *Eur. J. Org. Chem.* **2003**, 4401; (d) Busca, P.; McCort, I.; Prangé, T.; Le Merrer, Y. *Eur. J. Org. Chem.* **2006**, 2403.
16. There are some typographical errors in Ref. 15c. For example, in Scheme 1, bis-epoxide 3 and related compounds 4–9 are described with a L-manno and a D-gulo configuration, respectively, instead of a D-manno or a L-gulo configuration and in Scheme 2, compounds 13–18 should have an L-gluco configuration.
17. Hirano, S.; Ichikawa, S.; Matsuda, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 1854.
18. For similar glycosylation reaction involving the 5-azido-1,5-dideoxy-2,3-di-*O*-isopropylidene-1-fluoro-D-ribofuranose, see: Ginisty, M.; Gravier-Pelletier, C.; Le Merrer, Y. *Tetrahedron: Asymmetry* **2006**, *17*, 142.
19. Monasson, O.; Ginisty, M.; Bertho, G.; Gravier-Pelletier, C.; Le Merrer, Y. *Tetrahedron Lett.* **2007**, *48*, 8149.
20. (a) Albericio, F.; Bofill, J. M.; El-Faham, A.; Kates, S. A. *J. Org. Chem.* **1998**, *63*, 9678; (b) Nefzi, A.; Ostresh, J. M.; Houghten, R. A. *Tetrahedron Lett.* **1997**, *38*, 4943.
21. Dini, C.; Drochon, N.; Feteanu, S.; Guillot, J.-C.; Peixoto, C.; Aszodi, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 529.
22. Al-Dabbagh, B.; Henry, X.; El Ghachi, M.; Auger, G.; Blanot, D.; Parquet, C.; Mengin-Lecreulx, D.; Bouhss, A. *Biochemistry* **2008**, *47*, 8919.
23. Auger, G.; van Heijenoort, J.; Mengin-Lecreulx, D.; Blanot, D. *FEMS Microbiol. Lett.* **2003**, *219*, 115.
24. Crouvoisier, M.; Auger, G.; Blanot, D.; Mengin-Lecreulx, D. *Biochimie* **2007**, *89*, 1498.