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N-Glycosylation with Sulfoxide Donors for the Synthesis of Peptidonucleosides

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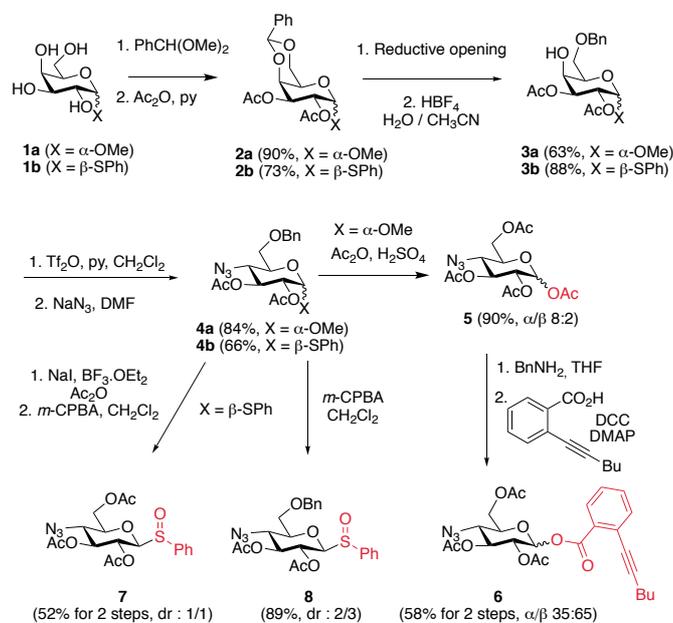
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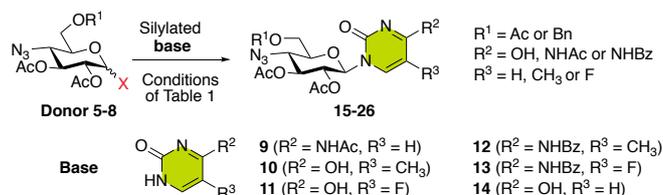
will also be replaced by a free hydroxymethyl group. To access these compounds, different bases will first be glycosylated with a donor carrying an azide group at the C4 position. Following this glycosylation step, the azide will be reduced to the corresponding amine allowing a dipeptide coupling. A potential impact of the dipeptide motif on the bioactivity will also be studied with the synthesis of compounds comprising L-serine instead of D-serine or a modified peptide.

Results and discussion

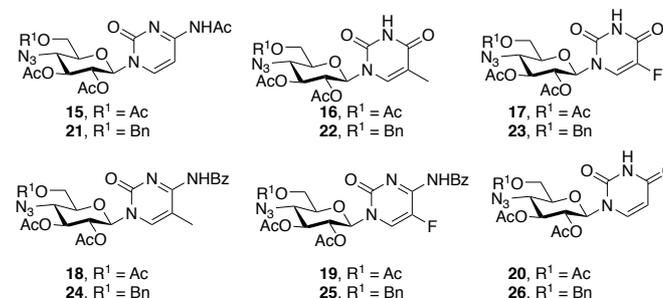
First of all, we prepared anomeric acetate donor **5**, which is the most conventionally activatable donor used to synthesize nucleoside derivatives under Vorbrüggen glycosylation conditions (Scheme 1).¹¹ Methyl α -D-galactopyranoside **1a** was first protected as a 4,6-benzylidene acetal to yield the corresponding product, which was esterified with acetic anhydride in pyridine. Compound **2a** was then engaged in a reductive opening reaction of the 4,6-*O*-benzylidene acetal to lead to galactopyranoside **3a** having a free hydroxyl at position 4. Best results for this step were obtained using triethylsilane (2.5 eq.) in combination with triflic acid (2 eq.) as described by Sakagami and Hamana.¹² Since we also observed the formation of the corresponding 4-*O*-triethylsilyl ether adduct, the reaction mixture was further treated with tetrafluoroboric acid to furnish the desired alcohol **3a** in 63% yield. The latter was then reacted with TiF_2O in the presence of pyridine to form the triflate in the 4-position, that was displaced by NaN_3 with inversion of configuration leading to 4-azido-4-deoxy glucopyranoside adduct **4a**. A final acetylation step led to donor **5** as a mixture of anomers ($\alpha/\beta = 8:2$).



Scheme 1. Preparation of different donors **5-8**. Conditions for the reductive opening: with **2a** ($X = \alpha$ -OMe) Et_3SiH (2.5 eq.), TfOH (2 eq.) in CH_3CN , with **2b** ($X = \beta$ -SPh) Et_3SiH (1 eq.), cat. $\text{Cu}(\text{OTf})_2$ in CH_3CN



Obtained Compounds



Scheme 2. Glycosylation of different pyrimidine bases **9-14** using donors **5-8**

Table 1. *N*-Glycosylation conditions of different pyrimidine bases

entry	Donor	Base ^a	Conditions ^b	Compound, Yield (%)
1	5	9	TMSOTf (1.5 eq.)	15, 36
2	5	9	SnCl_4 (3 eq.) ^c	15, 69
3	5	10	SnCl_4 (3 eq.)	16, 74
4	5	11 ^d	TMSOTf (1.5 eq.)	17, 71
5	5	12	SnCl_4 (3 eq.)	18, 45
6	5	13	SnCl_4 (3 eq.)	19, 51
7	5	14 ^d	TMSOTf (1.5 eq.)	20, 25
8	6	9 ^{e,f}	$\text{Ph}_3\text{PAuNTf}_2$ (10 mol%)	15, 49
9	6	10 ^{e,f}	$\text{Ph}_3\text{PAuNTf}_2$ (10 mol%)	16, 48
10	6	11 ^f	$\text{Ph}_3\text{PAuNTf}_2$ (10 mol%)	17, 40
11	6	12 ^{e,f}	$\text{Ph}_3\text{PAuNTf}_2$ (10 mol%)	18, 30
12	6	13 ^f	$\text{Ph}_3\text{PAuNTf}_2$ (10 mol%)	19, 9
13	6	14 ^{e,f}	$\text{Ph}_3\text{PAuNTf}_2$ (10 mol%)	20, 78
14	7	9	TMSOTf (1.5 eq.)	15, 95
15	7	10	TMSOTf (1.5 eq.)	16, 89
16	7	11	TMSOTf (1.5 eq.)	17, 71
17	7	12	TMSOTf (1.5 eq.)	18, 88
18	7	13	TMSOTf (1.5 eq.)	19, 75
19	7	14	TMSOTf (1.5 eq.)	20, 94
20	8	9	TMSOTf (1.5 eq.)	21, 54
21	8	10	TMSOTf (1.5 eq.)	22, 77
22	8	11 ^d	TMSOTf (1.5 eq.)	23, 76
23	8	12	TMSOTf (1.5 eq.)	24, 66
24	8	13	TMSOTf (1.5 eq.)	25, 50
25	8	14	TMSOTf (1.5 eq.)	26, 81

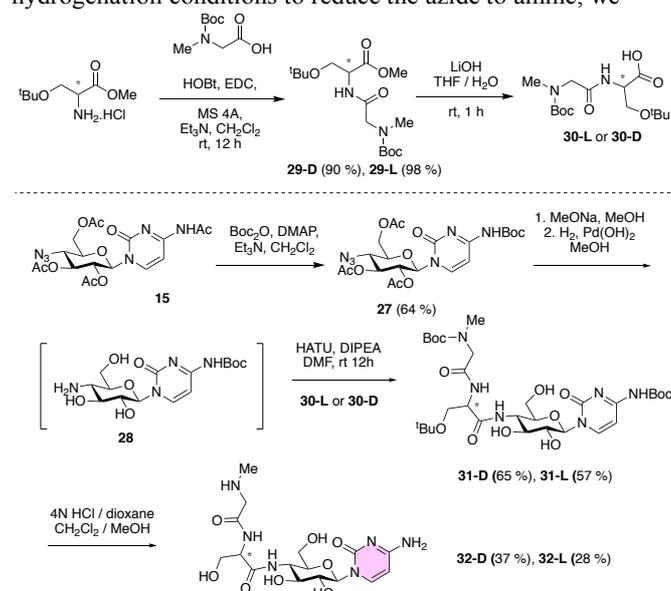
a) BSA (4 eq.) was used as silylating agent unless otherwise stated. b) the reaction mixture was heated for 12 h at 55 °C in MeCN unless otherwise stated c) the reaction was carried out in DCE. d) HMDS (1.8 eq.), saccharine (5 mol.%) e) with BSTFA (*N,O*-bis(trimethylsilyl)tri-fluoroacetamide). f) the reaction was carried out for 48 h at r.t.

With this donor in hand, we first performed the glycosylation of *N*⁴-Ac-cytosine **9**, the nucleic base naturally present in the gougertin. Reaction with *N,O*-bis(trimethylsilyl)acetamide (BSA) in MeCN gave the corresponding silylated cytosine, which was subsequently treated with **5** in the presence of

trimethylsilyl triflate (TMSOTf, 1.5 eq.) for 12 h at 55 °C.¹³ After workup and purification, the nucleoside **15** was isolated in 36% yield (Scheme 2, Table 1, entry 1) and as a single β -anomer due to the anchimeric assistance of the 2-OAc group. This was confirmed by the ¹H NMR spectrum showing a large coupling constant between protons H-1 and H-2 (³J_{1,2} = 9.5 Hz) indicating an axial orientation of both protons. The yield could be increased to 69% by using the Vorbrüggen method with SnCl₄ (3 eq.) in MeCN at 55°C for 12 h (Table 1, entry 2).¹⁴ The glycosylation of other pyrimidine bases was then tested and for each base, the reaction conditions had to be readjusted accordingly. The results are summarized in Table 1. Similar yields were obtained with thymine **10** (entry 3) and 5-F-uracil **11** (entry 4), which led respectively to the β -glycosylated adducts **16** and **17** in 74 and 71% yield respectively. Note that 5-F-uracil **11** was silylated in the presence of 1,1,1,3,3,3-hexamethylidisilazane (HMDS) in combination with saccharin (5 mol%) as catalyst.¹⁵ With 5-Me-N⁴-Bz-cytosine **12** (entry 5) and 5-F-N⁴-Bz-cytosine **13** (entry 6), which are rarely glycosylated with pyranosyl donors, moderate yields of 45 and 51% were achieved respectively for **18** and **19**. A much lower yield of 25% was also obtained with silylated uracil **14**, which led to the corresponding β -nucleoside **20** (entry 7). This disappointing result led us to consider the use of other donors for these glycosylations. We started our investigations with the preparation of glycosyl *ortho*-hexynyl benzoate **6**, readily accessible from **5** after selective deacetylation at the anomeric position and subsequent esterification of the corresponding hemiacetal (Scheme 1). Recently, these donors have been shown to be superior donors for the *N*-glycosylation of nucleobases using gold catalysis under very mild conditions.¹⁶ Donor **6** was then subjected to glycosylation with silylated pyrimidine bases under the catalysis of Ph₃PAuNTf₂ (10 mol%) at room temperature in MeCN. In all cases the corresponding nucleosides were isolated with an excellent β -selectivity but with variable yields depending on the acceptor. For example, a good 78% yield was obtained with uracil **14** (entry 13). Moderate results (30 to 49%) were achieved with other bases (entries 8-11) and, with 5-F-N⁴-Bz cytosine **13**, the reaction led to a low conversion with the desired β -glycosylated adduct **19** been isolated in only 9% yield (entry 12). We then turned our attention to the use of sulfoxide donors¹⁷ that we recently exploited with success in problematic glycosylations¹⁸ and which have been very rarely used for the *N*-glycosylation with furanosyl or pyranosyl derivatives.¹⁹ Donor **7** was prepared from phenyl β -D-thiogalactoside **1b**, following the same strategy as for **5**, *i.e.* 4,6-*O*-benzylidene acetal formation, *O*-2/*O*-3 acetylation (to **2b**), reductive opening of the acetal (Et₃SiH with cat. Cu(OTf)₂ to **3b**)²⁰ and introduction of the azide at C-4 through triflate displacement to afford **4b** (Scheme 1). The obtained **4b** was further treated by NaI/BF₃•OEt₂ in acetic anhydride leading to the acetolysis of the 6-*OBn* position.²¹ Thioether oxidation was carried out under common conditions (*m*-CPBA) smoothly giving sulfoxides **7** as a mixture of two diastereomers (dr of 1:1). Donor **7** was then subjected to glycosylation after prior silylation of the pyrimidine bases with BSA in MeCN. In all cases, TMSOTf

(1.5 eq.) was used as promoter and after 12 h at 55 °C, we were pleased to obtain the corresponding β -nucleosides in good to excellent yields ranging from 71 to 95% (entries 14-19). We also prepared 6-*OBn* sulfoxide derivatives **8** (dr of 2:3) by direct oxidation of **4b**. *N*-glycosylation with this donor **8** efficiently led to 6-*OBn* β -nucleosides in comparable yields (50-81%, entries 20-25).

In order to carry out the peptide coupling and access analogues, we first examined azide reduction directly on compound **15**. However, while using Staudinger conditions²² or different hydrogenation conditions to reduce the azide to amine, we

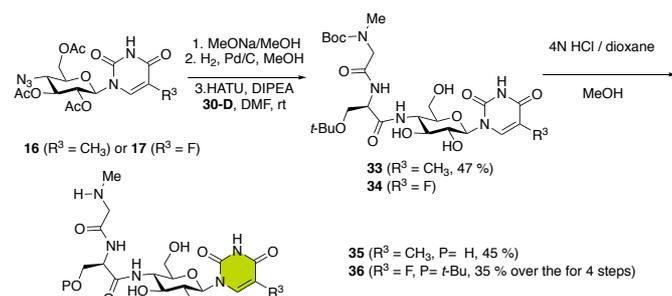


Scheme 3. Preparation of analogues **32-D** and **32-L**

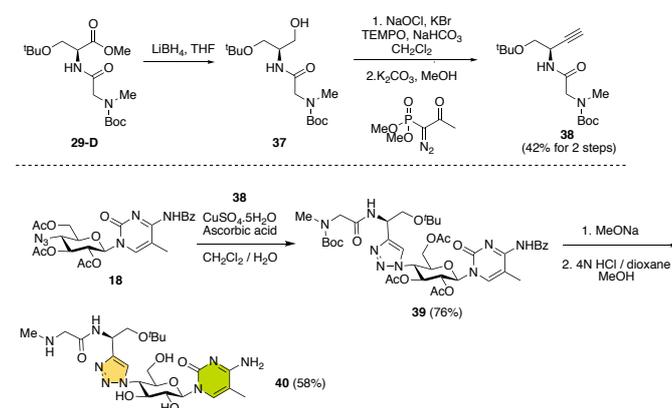
obtained complex mixtures that probably result from deacetylation and acetate migration. To solve this problem, we decided to protect first the N⁴-cytosine with a *tert*-butylcarbamate group and remove all the acetyl groups before hydrogenation (Scheme 3). Therefore, compound **15** was treated with Boc₂O in the presence of NEt₃ and 4-dimethylaminopyridine (DMAP) to provide the corresponding derivative **27** by concomitant deprotection of N⁴-Ac-cytosine. Zemlén deacetylation was carried out followed by hydrogenation with palladium hydroxide in methanol. This sequence led to the corresponding amine **28** that was used without purification in the following peptide coupling. The synthesis of the dipeptide fragment **30-D**, naturally present in the gougertoin, started with the coupling of the methyl ester of D-*O*-*tert*-butyl-serine with Boc-sarcosine using hydroxybenzotriazole (HOBt) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) in the presence of triethylamine in CH₂Cl₂. The obtained dipeptide **29-D** was then saponified with lithium hydroxide to carboxylic acid **30-D**. The same sequence was also performed with the methyl ester of L-*O*-*tert*-butyl-serine providing the corresponding dipeptide fragment **30-L**. The peptide coupling of amine **28** was then carried out with both dipeptides **30-D** and **30-L** using the coupling agent HATU²³ in the presence of Hünig's base in DMF to furnish **31-D** and **31-L** in 65 and 57% yield

respectively. Both compounds were then treated with 4N HCl in MeOH/CH₂Cl₂ to remove the Boc and *t*-butyl groups allowing the preparation of analogues **32-D** and **32-L**. Due to the high polarity of these products and associated difficulties to their purification, these compounds were obtained in 37 and 28% yields respectively.

To access other compounds, the two uracil derivatives **16** and **17**, were subjected to a similar reaction sequence, namely Zemplén deacetylation, hydrogenation followed by dipeptide coupling with carboxylic acid **30-D**, affording the corresponding peptidonucleosides **33** and **34** (Scheme 4). Further deprotection with HCl 4N allowed us to obtain analogues **35** and **36**. For **34**,



Scheme 4. Preparation of analogues **35-36**



Scheme 5. Preparation of analogue **40**

a partial deprotection was obtained leading to **36** as the major adduct still having the *t*-butyl group on the serine. Attempts to further deprotect compound **36** led to a very low yield of desired product due to degradation.

Taking advantage of the presence of the azide in position C-4 of pyranosyl compound **18**, we also prepared compound **40** containing a triazole unit (Scheme 5). To this end we synthesized alkyne **38** from **29-D** after its reduction to alcohol **37**, oxidation to the aldehyde²⁴ and treatment with dimethyl (1-diazo-2-oxopropyl)phosphonate²⁵ in the presence of potassium carbonate in MeOH. The CuAAC reaction²⁶ between alkyne **38** and azide **18** was carried out in a water/dichloromethane mixture with CuSO₄·5H₂O and ascorbic acid to smoothly give the triazole derivative **39** in 76% yield. Final removal of the acyl groups with MeONa in methanol followed by treatment with 4N HCl at room temperature gave, as for **36**, a partial deprotection leading to **40** having the *t*-butyl group on the serine as the major adduct in 58% yield.

The antifungal activities of some of the synthesized analogues including intermediates or not fully deprotected compounds were evaluated in preventive tests against a panel of different pathogens relevant to agriculture practices such as *Podosphaera fuliginea* (SPHRFU), *Uromyces appendiculatus* (UROMAP), *Puccinia triticina* (PUCCRT), *Alternaria brassicae* (ALTEBA), *Botryotiniacineal* (BOTRCI), *Zymoseptoria tritici* (SEPPTR) (Table 2). Although some of the analogs were found to have good bioactivities, none of them showed superior activity to gougerotin itself. To be noted that only the activity against *Podosphaera fuliginea* was retained at 500 ppm for certain analogues. These results show the importance of the carboxamide function and of the presence of the dipeptide chain in retaining all the activities.

Table 2. Antifungal activities of a selection of synthesized compounds

Compound	[C] (ppm)	SPHRFU	UROMAP	PUCCRT	ALTEBA	BOTRCI	SEPPTR
Gougerotin ^a	250	83 %	100 %	100 %	-	-	86 %
22	500	70 %	0 %	0 %	0 %	0 %	20 %
23	500	0 %	0 %	0 %	0 %	0 %	0 %
24	500	83 %	0 %	0 %	0 %	0 %	0 %
26	500	83 %	0 %	0 %	0 %	0 %	0 %
32-L	500	90 %	11 %	67 %	25 %	45 %	79 %
32-D	500	75 %	86 %	0 %	0 %	0 %	43 %
33	500	0 %	0 %	0 %	0 %	0 %	0 %
34	500	44 %	0 %	0 %	0 %	0 %	0 %
36	500	80 %	0 %	0 %	0 %	0 %	20 %

a) Gougerotin 80 % from fermentation broth

ARTICLE

Conclusions

In this article, we have successfully prepared a series of peptidonucleosides, analogues of gougerotin. The main modifications relate to the replacement of the natural nucleic base by other pyrimidine bases and the replacement of the carboxamide function at C5 by an hydroxymethyl group. For the glycosylation stage, a methodological study involving different anomeric leaving groups (acetate, phenylsulfoxide and *ortho*-hexynylbenzoate) was carried out. Using six different pyrimidines, the sulfoxide donor in combination with TMSOTf as a promoter most generally led to the best yields. Unfortunately, none of the prepared compounds showed superior antifungal activity to gougerotin itself. In order to fully explore and optimize the structural features of this family leading to new compounds towards the potential development of a new class of therapeutically useful antifungal agents, an investigation of other gougerotin analogues is still ongoing.

Experimental

General remarks

All non-aqueous reactions were run under an inert atmosphere (argon), by using standard techniques for manipulating air-sensitive compounds and the glassware was stored in the oven prior to use. All reagents and solvents were commercially available and were used without further purification. Molecular sieves 4 Å were used as a powder and were activated overnight at 250 °C and under reduced pressure, in a Kugelrohr apparatus or with a micro-wave for 45 seconds. Reactions were monitored with analytical Merck TLC silica gel 60 F254 plates and visualized under UV (254 nm) and stained with KMnO₄ or vanillin. Column chromatography was done with Merck Geduran silical gel Si 60 (40-63 μm) and Redisep Rf columns (silica gel Si 60, 40-63 μm) on an Interchim puriFlash[®] apparatus and on a Teledyne Isco combiflash Rf. Preparative thin-layer chromatography was performed on silica gel 60 F254 0.5 mm 20×20 cm plates and visualised under UV (254 nm). Deuterated chloroform used for NMR analyses was generally neutralized by addition of anhydrous and granular K₂CO₃. NMR spectra were recorded with AM 300, AVANCE 300 and AVANCE 500 Bruker spectrometers. Chemical shifts are given in parts per million, referenced to the solvent peak of CDCl₃, defined at 77.2 ppm (¹³C NMR) and 7.26 ppm (¹H NMR) or to the solvent peak of CD₃OD, defined at 49.9 ppm (¹³C NMR) and 3.34 ppm (¹H NMR) or to the solvent peak of D₂O, defined at 4.79 ppm (¹H NMR) or to the solvent peak of DMSO-d₆,

defined at 39.5 ppm (¹³C NMR) and 2.50 ppm (¹H NMR). Data are reported as follow: chemical shifts, multiplicity (s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, bs = broad singlet), coupling constant (in Hz) and integration. IR spectra were recorded on a Perkin-Elmer Spectrum BX instrument with an FT-IR system. Optical rotation were measured on an Anton Paar MCP300 polarimeter using a cell of 1-dm-length path. Mass spectra were recorded with Waters Micromass LCT Premier mass spectrometer.

General Procedure for the glycosylation of sulfoxide donors.

To a stirred solution of the nitrogen base (1.6 eq.) in dry MeCN (2/3 V_{tot}) under argon atmosphere was added BSA (4 eq.). The resulting mixture was heated at 60 °C for 1h and then cooled to room temperature. The donor (1 eq.) is stirred with 4 Å molecular sieves in dry MeCN (1/3 V_{tot}) under argon for 1 h. The solution of the nitrogen base was added to the donor and then TMSOTf (1.5 eq.). The resulting mixture was heated at 55 °C overnight and then quenched with aqueous NaHCO₃. The reaction mixture was filtered and the aqueous phase was extracted with EtOAc (5x). The organic layers were combined, washed with NaCl, dried over Na₂SO₄, filtered and concentrated under vacuum. The residue was purified by flash chromatography on silica gel to afford the clean product.

2,3,6-Tri-*O*-acetyl-4-azido-1-*N*-(*N*-acetyl-cytosine)-β-*D*-glucopyranoside **15**.

The General Procedure was followed using **7** (300 mg, 0.68 mmol), **9** (184 mg, 1.09 mmol), BSA (0.67 mL, 2.72 mmol), TMSOTf (0.18 mL, 1.02 mmol), 4 Å molecular sieves (200 mg) in dry MeCN (13.6 mL). The residue was purified by flash chromatography on silica gel (EtOAc) to afford product **15** (304 mg, 0.65 mmol, 95%) as a colorless oil. [α]_D²⁵ + 56.6 (*c* = 1.0, CHCl₃); ¹H NMR (300 MHz, CD₃OD) δ 8.14 (d, 1H, *J*_{H_{Ar},H_{Ar}} = 7.5 Hz, H_{Ar}), 7.49 (d, 1H, *J*_{H_{Ar},H_{Ar}} = 7.5 Hz, H_{Ar}), 6.12 (d, 1H, *J*_{1,2} = 9.0 Hz, H₁), 5.54 (t, 1H, *J*_{3,2} = *J*_{3,4} = 9.5 Hz, H₃), 5.32 (t, 1H, *J*_{2,3} = *J*_{2,1} = 9.5 Hz, H₂), 4.47 (dd, 1H, *J*_{6,6'} = 13.0 Hz and *J*_{6,5} = 1.0 Hz, H₆), 4.31 (dd, 1H, *J*_{6',6} = 13.0 Hz and *J*_{6',5} = 4.5 Hz, H_{6'}), 4.00-3.93 (m, 2H, H₄, H₅), 2.21 (s, 3H, NHCOCH₃), 2.14 (s, 3H, OCOCH₃), 2.12 (s, 3H, OCOCH₃), 1.94 (s, 3H, OCOCH₃); ¹³C NMR (75 MHz, CD₃OD) δ 173.9 (C=O), 173.1 (C=O), 172.0 (C=O), 172.0 (C=O), 165.5 (C_{qAr}), 158.6 (C_{qAr}), 147.5 (CH_{Ar}), 99.8 (CH_{Ar}), 83.6 (C₁), 77.1 (C₄), 75.4 (C₃), 73.2 (C₂), 64.8 (C₆), 61.9 (C₅), 25.4 (COCH₃), 21.4 (COCH₃), 21.1 (COCH₃); IR ν (film, cm⁻¹) 3239 (=C-H), 2112 (N₃), 1747 (C=O), 1663 (NH-C=O); ESIHRMS *m/z* = 467.1486 [M+H]⁺. C₁₈H₂₃N₆O₉ requires 467.1527.

2,3,6-Tri-*O*-acetyl-4-azido-1-*N*-(*N*-*tert*-butyloxycarbonyl--cytosine)- β -D-glucopyranoside 27. To a stirred solution of **15** (40 mg, 0.09 mmol) in CH₂Cl₂ (1 mL) was added di-*tert*-butyldicarbonate (40 μ L, 0.17 mmol, 2 eq.), triethylamine (12 μ L, 0.09 mmol, 1 eq.) and 4-dimethylaminopyridine (10.5 mg, 0.09 mmol, 1 eq.). The resulting mixture was stirred at room temperature for 4 h and then concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (CH₂Cl₂/MeOH 99:1 to 98:2) to afford product **27** (29 mg, 0.06 mmol, 64%) as a yellow powder. [α]_D²⁵ + 41.7 (*c* = 1.2, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 7.62 (d, 1H, *J*_{H_{Ar},H_{Ar}} = 7.5 Hz, *H*_{Ar}), 7.30 (d, 1H, *J*_{H_{Ar},H_{Ar}} = 7.5 Hz, *H*_{Ar}), 6.05 (d, 1H, *J*_{1,2} = 9.5 Hz, *H*₁), 5.40 (t, 1H, *J*_{2,1} = *J*_{2,3} = 9.5 Hz, *H*₂), 5.07 (t, 1H, *J*_{3,2} = *J*_{3,4} = 9.5 Hz, *H*₃), 4.39 (d, 1H, *J*_{6,6'} = 12.5 Hz, *H*₆), 4.27 (dd, 1H, *J*_{6,6'} = 12.5 Hz, *J*_{6,5} = 4.0 Hz, *H*_{6'}), 3.76-3.67 (m, 2H, *H*₄, *H*₅), 2.11 (s, 6H, OCOCH₃), 1.97 (s, 3H, OCOCH₃), 1.51 (s, 9H, OC(CH₃)₃); ¹³C NMR (75 MHz, CDCl₃) δ 170.5 (C=O), 170.2 (C=O), 169.5 (C=O), 143.8 (CH_{Ar}), 96.3 (CH_{Ar}), 83.7 (C_q), 81.3 (C₁), 75.5 (C₄), 73.5 (C₃), 70.7 (C₂), 62.7 (C₆), 60.2 (C₅), 28.2 (C(CH₃)₃), 20.9 (COCH₃), 20.8 (COCH₃), 20.6 (COCH₃); IR ν (film, cm⁻¹) 2987 (C-H), 2109 (N₃), 1751 (C=O), 1731 (C=O), 1667 (NH-C=O), 1626 (NH-C=O); ESIHRMS *m/z* = 525.1945 [M+H]⁺. C₂₁H₂₉N₆O₁₀ requires 525.1953.

Methyl Boc-sarcosinyl-*O*-*tert*-butyl-D-serinate 29-D. To a stirred solution of *O*-*tert*-butyl-D-serine methyl ester (250 mg, 1.18 mmol, 1 eq.) and Boc-sarcosine (290 mg, 1.54 mmol, 1.3 eq.) in dry CH₂Cl₂ (9.8 mL) were added 4 Å molecular sieves (400 mg), hydroxybenzotriazole (239 mg, 1.77 mmol, 1.5 eq.) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (452 mg, 2.36 mmol, 2 eq.). The resulting mixture was cooled to 0 °C and Et₃N (0.49 mL, 3.54 mmol, 3 eq.) was added. After being stirred overnight at room temperature, the mixture was diluted with aqueous saturated NaHCO₃ (8 mL). The aqueous phase was then extracted with EtOAc (3 x 10 mL). The organic layers were combined, dried over Na₂SO₄, filtered and concentrated under vacuum. The residue was purified by flash chromatography on silica gel (Heptane/EtOAc 80:20 to 60:40) to afford product **29-D** (367 g, 90%) as a colorless oil. [α]_D²⁵ - 26.4 (*c* = 1.0, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 4.69 (m, 1H, *H*₂), 4.17-3.81 (m, 2H, *H*₃), 3.80 (dd, 1H, *J*_{1,1'} = 9.0 Hz, *J*_{1',1} = 3.0 Hz, *H*₁), 3.72 (s, 3H, OCH₃), 3.53 (dd, 1H, *J*_{1,1'} = 9.0 Hz, *J*_{1',1} = 3.0 Hz, *H*₁), 2.93 (s, 3H, NCH₃), 1.46 (s, 9H, CO₂C(CH₃)₃), 1.10 (s, 9H, OC(CH₃)₃); ¹³C NMR (75 MHz, CDCl₃) δ 62.1 (C₁), 53.4 (C₃), 52.8 (C₂), 52.6 (OCH₃), 35.7 (NCH₃), 28.5 (CO₂C(CH₃)₃), 27.5 (OC(CH₃)₃); IR ν (film, cm⁻¹) 3314 (N-H), 2975 (CH₃), 2935 (CH₂), 1751 (C=O), 1684 (NH-C=O); ESIHRMS *m/z* = 347.2182 [M+H]⁺. C₁₆H₃₁N₂O₆ requires 347.2191.

Boc-sarcosinyl-*O*-*tert*-butyl-D-serine 30-D. To a stirred solution of **29-D** (367 mg, 1.06 mmol, 1 eq.) in THF/H₂O mixture (8.8 mL/1.8 mL 5:1), was added lithium hydroxide (33 mg, 1.38 mmol, 1.3 eq.). The resulting mixture was stirred for 1 h at room temperature and then concentrated under vacuum until THF was evaporated. HCl 1 N was then added until the

pH 2. The aqueous layer was extracted with EtOAc (3 x 10 mL). The organic layers were combined, dried over Na₂SO₄ and concentrated under vacuum to afford the clean product **30-D** (351 mg, 1.06 mmol, quantitative). The product is used without further purification. [α]_D²⁵ - 32.8 (*c* = 1.0, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 6.90 (d, 1H, *J*_{NH,H2} = 8.0 Hz, NH), 4.68 (m, 1H, *H*₂), 4.07-3.64 (m, 2H, *H*₃, *H*_{3'}), 3.85 (dd, 1H, *J*_{1,1'} = 9.0 Hz, *J*_{1,2} = 3.0 Hz, *H*₁), 3.55 (dd, 1H, *J*_{1,1'} = 9.0 Hz, *J*_{1,2} = 4.0 Hz, *H*_{1'}), 2.92 (s, 3H, NCH₃), 1.44 (s, 9H, CO₂C(CH₃)₃), 1.12 (s, 9H, OC(CH₃)₃); ¹³C NMR (75 MHz, CDCl₃) δ 173.7 (C=O), 169.9 (C=O), 81.2 (C_qCO₂C(CH₃)₃), 74.1 (C_qOC(CH₃)₃), 61.7 (C₁), 53.3 (C₃), 52.7 (C₂), 35.8 (NCH₃), 28.5 (CO₂C(CH₃)₃), 27.5 (OC(CH₃)₃); IR ν (film, cm⁻¹) 3320 (O-H), 2975 (CH₃), 2935 (CH₂), 1739 (C=O), 1670 (NH-C=O); ESIHRMS *m/z* = 355.1842 [M+Na]⁺. C₁₅H₂₈N₂O₆Na requires 355.1845.

Peptidonucleoside 31-D. The solution **1** was prepared with Na (10 mg) in dry MeOH (2 mL, C = 0.22 M). To a stirred solution of the protected nucleoside **27** (147 mg, 0.28 mmol) in dry MeOH (5 mL) was added the solution **1** (0.26 mL, 20 mol%). The resulting mixture was stirred at room temperature for 1 h and then neutralized with Dowex® H⁺, filtered on celite and concentrated under reduce pressure to afford clean product without further purification. The obtained product (105 mg) was then hydrogenated at atmospheric pressure in the presence of Pd(OH)₂ (40%w/w, 42 mg) in MeOH (2.6 mL) for 12 h. The resulting mixture was then filtered on celite® and concentrated under reduced pressure to afford the clean corresponding amine (94 mg). To a stirred solution of the latter in DMF (4 mL) was added the dipeptide (109 mg, 0.33 mmol, 1.3 eq.) and DIPEA (0.17 mL, 1.01 mmol, 4 eq.). After 1 min, HATU (144 mg, 0.32 mmol, 1.5 eq.) was added and the resulting mixture was stirred at room temperature for 18 h. Solvent was removed and the crude product was purified by flash chromatography on silica gel (EtOAc/EtOH 99:1 to 92:8) to afford product **31-D** (113 mg, 0.16 mmol, 65%) as a yellow powder; [α]_D²⁵ + 14.6 (*c* = 1.1, MeOH). ¹H NMR (500 MHz, CD₃OD)²⁷ δ 8.11 (d, 1H, *J*_{H_{Ar},H_{Ar}} = 7.5 Hz, *H*_{Ar}), 7.33-7.26 (m, 1H, *H*_{Ar}), 5.77 (d, 1H, *J*_{1,2} = 9.0 Hz, *H*₁), 4.60-4.49 (m, 1H, *H*₇), 4.10-3.53 (m, 10H, *H*₂, *H*₃, *H*₄, *H*₅, *H*₆, *H*₈, *H*₉), 3.03-2.83 (m, 3H, NCH₃), 1.56 (s, 9H, CO₂C(CH₃)₃), 1.50-1.41 (m, 9H, OC(CH₃)₃), 1.20 (s, 9H, CO₂C(CH₃)₃); ¹³C NMR (75 MHz, CD₃OD)²⁸ δ 172.1 (C=O), 163.7 (C_q), 157.0 (C_q), 156.9 (C_q), 151.9 (C=O), 144.9 (CH, C_{Ar}), 96.1 (CH, C_{Ar}), 85.2 (C₁), 83.4 (C(CH₃)₃), 80.2 (CH), 75.8 (CH), 74.4 (CH₂), 73.9 (C(CH₃)₃), 73.8 (C(CH₃)₃), 71.6 (CH₂), 62.8 (CH₂), 56.1 (C₇), 53.4 (CH), 53.1 (CH₂), 36.8 (NCH₃), 28.9 (C(CH₃)₃), 28.5 (C(CH₃)₃), 27.8 (C(CH₃)₃); IR ν (film, cm⁻¹) 3264 (N-H), 2976 (CH), 2926 (CH), 1758 (C=O), 1656 (NH-C=O); ESIHRMS *m/z* = 687.3566 [M+H]⁺. C₃₀H₅₁N₆O₁₂ requires 687.3565.

Peptidonucleoside 32-D. To a stirred solution of **31-D** (90 mg, 0.131 mmol, 1 eq.) in CH₂Cl₂/MeOH (2:1 v/v, 1.3 mL) was added a solution of 4M HCl in dioxane (0.23 mL, 0.92 mmol, 7 eq.). The resulting mixture was stirred at room temperature for 2 days and then diluted with H₂O and then neutralized with

DOWEX® MONOSPHERE® 550A (OH) anion exchange resin. The mixture was filtered on celite and then concentrated under vacuum. The crude product was purified by preparative TLC (H₂O/EtOH/EtOAc 4:4:2, pH 9) to afford **32-D** as a white powder (21 mg, 0.05 mmol, 37%). [α]_D²⁵ – 31.3 (*c* = 1.1, H₂O); ¹H NMR (300 MHz, D₂O) δ 7.73 (d, 1H, *J*_{H_{Ar},H_{Ar}} = 7.5 Hz, H_{Ar}), 6.07 (bs, 1H, H_{Ar}), 5.64 (d, 1H, *J*_{1,2} = 9.5 Hz, H1), 4.45 (t, 1H, *J*_{7,8} = *J*_{7,8'} = 5.5 Hz, H7), 3.94 (s, 2H, H9), 3.91–3.80 (m, 1H, H4), 3.86 (d, 2H, *J*_{8,7} = 5.5 Hz, H8), 3.80–3.71 (m, 3H, H2, H3, H5), 3.68 (dd, 1H, *J*_{6,6'} = 12.5 Hz, *J*_{6,5} = 1.5 Hz, H6), 3.57 (dd, 1H, *J*_{6,6'} = 12.5 Hz, *J*_{6,5} = 5.5 Hz, H6'), 2.74 (s, 3H, NCH₃); ¹³C NMR (75 MHz, D₂O) δ 172.0 (C=O), 166.7 (C=O), 166.0 (C=O), 157.9 (C_{qAr}), 141.7 (CH_{Ar}), 97.0 (CH_{Ar}), 83.3 (C1), 77.5 (C5), 73.7 (C3), 71.7 (C2), 61.0 (C8), 60.6 (C6), 55.9 (C7), 51.3 (C4), 49.4 (C9), 32.8 (NCH₃); IR ν (film, cm⁻¹) 3310 (O-H), 3282 (N-H), 2976 (CH₃), 2933 (CH₂), 1744 (C=O), 1653 (NH-C=O); ESIHRMS *m/z* = 431.3424 [M+H]⁺. C₁₆H₂₈N₆O₈ requires 431.1890.

Conflicts of interest

There are no conflicts to declare

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