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# *N*-Glycosylation with sulfoxide donors for the synthesis of peptidonucleosides†‡

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The synthesis of glycopyranosyl nucleosides modified in the sugar moiety has been less frequently explored, notably because of the lack of a reliable method to glycosylate pyrimidine bases. Herein we report a solution in the context of the synthesis of peptidonucleosides. They were obtained after glycosylation of different pyrimidine nucleobases with glucopyranosyl donors carrying an azide group at the C4 position. A methodological study involving different anomeric leaving groups (acetate, phenylsulfoxide and *ortho*-hexynylbenzoate) showed that a sulfoxide donor in combination with trimethylsilyl triflate as the promoter led to the best yields.



## Introduction

Fungicides represent a class of pesticides used to prevent the development of fungi in plants and/or to fight fungal infection caused by pathogens.[1] Their use has become essential over time to avoid contamination of whole crops and to improve agricultural yields in the context of increasing urbanization that continues to decrease the area of cultivable land.[2] The continuous use of agrochemicals has led to the development of pest resistance to active substances, which makes some pesticides ineffective.[3] Thus, finding new structural motifs with new antifungal modes of action to fight against pathogens is essential to circumvent resistance. Natural products remain an important source of inspiration for the discovery of new active molecules. Among them gougerotin, isolated for the first time in 1962 from the strains of *Streptomyces gougerotii*,[4] caught the attention of researchers.[5] This peptidylnucleoside consists of a glucan-type pyranose saccharide motif that has a carboxamide group at the 6-position. It is also substituted at the 4-position by a dipeptide made of D-serine and sarcosine and *N*-linked at the anomeric position by a cytosine base. Since its discovery, gougerotin has been the subject of three total syntheses, two were described in the 1970s[6] and a more recent one was described in 2005 using solid- and solution-phase methodologies.[7] Gougerotin has a very broad spectrum of biological activities: antiviral,[8] antifungal,[5] antiparasitic and antibacterial[9] and acts by inhibiting protein synthesis in procaryotic and eucaryotic systems. It is active on several varieties of plants whether in preventive or curative tests but its phytotoxicity limits its direct use on plants.[10] In order to optimize its crop specificity, we were interested in the preparation of a few gougerotin analogues. The main modifications will concern the replacement of natural nucleic bases by other pyrimidine bases while preserving the glucopyranosyl skeleton of the parent molecule (Fig. 1). The carboxamide function at C5 will also be replaced by a free hydroxymethyl group. To access these com-

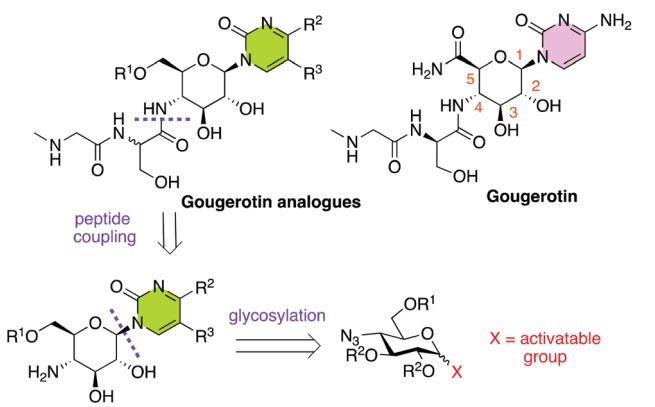


**Fig. 1** Retrosynthetic scheme of gougerotin analogues.

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†This article is dedicated to the memory of our esteemed colleague Dr Mazen Es-Sayed.

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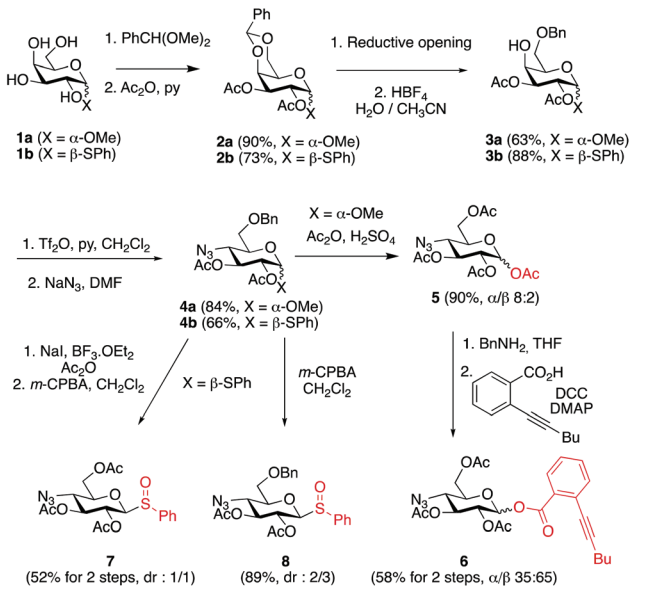
pounds, different bases will first be glycosylated with a donor carrying an azide group at the C4 position. Following this glycosylation step, the azide will be reduced to the corresponding amine allowing a dipeptide coupling. A potential impact of the dipeptide motif on the bioactivity will also be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

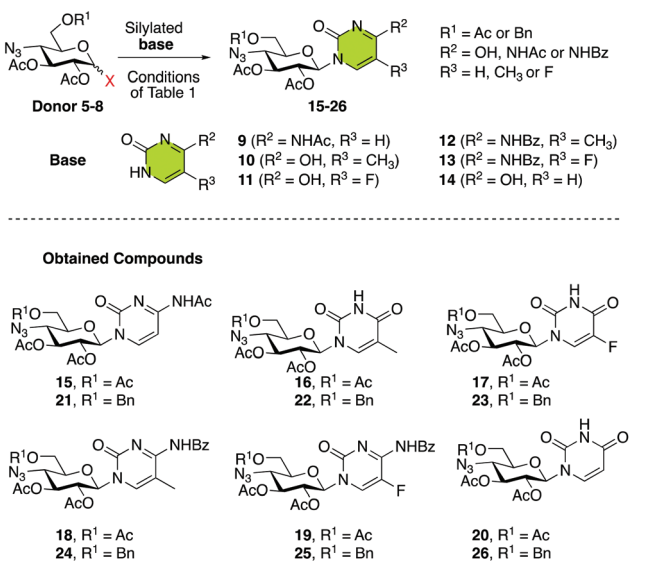
## Results and discussion

First, we prepared the anomeric acetate donor **5**, which is the most conventionally activatable donor used to synthesize nucleoside derivatives under the 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 with 4,6-*O*-benzylidene acetal to yield galactopyranoside **3a** having a free hydroxyl at position 4. The 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 $Tf_2O$ in the presence of pyridine to form the triflate at the 4-position, which was displaced by $NaN_3$ with the inversion of the configuration leading to the 4-azido-4-deoxy glucopyranoside adduct **4a**. A final acetolysis step led to donor **5** as a mixture of anomers (α/β = 8 : 2).




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

With this donor in hand, we first performed the glycosylation of $N^4$-Ac-cytosine **9**, a nucleic base naturally present in gougerotin. The 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 from 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 the Vorbrüggen method with $SnCl_4$ (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 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-hexamethyldisilazane (HMDS) in combination with saccharin (5 mol%) as a catalyst.[15] With 5-Me-$N^4$-Bz-cytosine **12** (entry 5) and 5-F-$N^4$-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




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

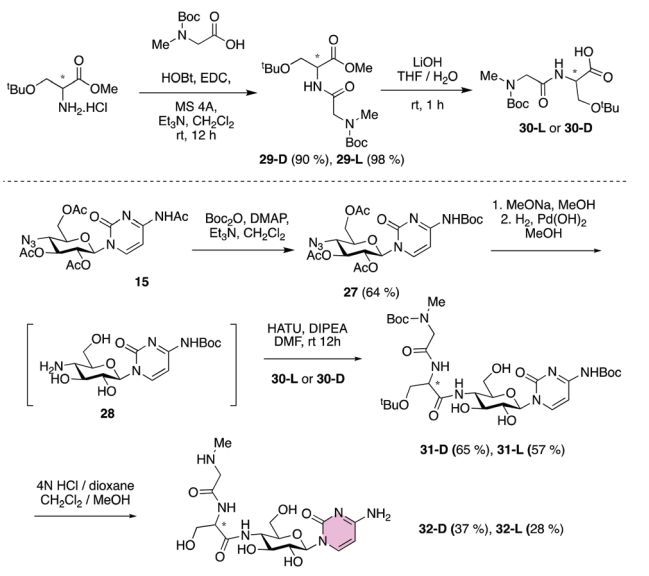
**Table 1** *N*-glycosylation conditions of different pyrimidine bases

| 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] | $Ph_3PAuNTf_2$ (10 mol%) | **15**, 49 |
| 9 | **6** | **10**[e,f] | $Ph_3PAuNTf_2$ (10 mol%) | **16**, 48 |
| 10 | **6** | **11**[f] | $Ph_3PAuNTf_2$ (10 mol%) | **17**, 40 |
| 11 | **6** | **12**[e,f] | $Ph_3PAuNTf_2$ (10 mol%) | **18**, 30 |
| 12 | **6** | **13**[f] | $Ph_3PAuNTf_2$ (10 mol%) | **19**, 9 |
| 13 | **6** | **14**[e,f] | $Ph_3PAuNTf_2$ (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 the 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)trifluoroacetamide). [f] The reaction was carried out for 48 h at r.t.

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 $Ph_3PAuNTf_2$ (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-$N^4$-Bz cytosine **13**, the reaction led to a low conversion with the desired β-glycosylated adduct **19** isolated in only 9% yield (entry 12). We then turned our attention to the use of sulfoxide donors[17] which we recently exploited with success in problematic glycosylations[18] and which have been very rarely used for *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 ($Et_3SiH$ 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 with NaI/$BF_3{\cdot}OEt_2$ in acetic anhydride, leading to the acetolysis of the 6-*O*Bn position.[21] Thioether oxidation was carried out under common conditions (*m*-CPBA), smoothly affording 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 the 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-*O*Bn sulfoxide derivatives **8** (dr of 2 : 3) by direct oxidation of **4b**. *N*-glycosylation with this donor **8** efficiently led to 6-*O*Bn β-nucleosides in comparable yields (50–81%, entries 20–25).

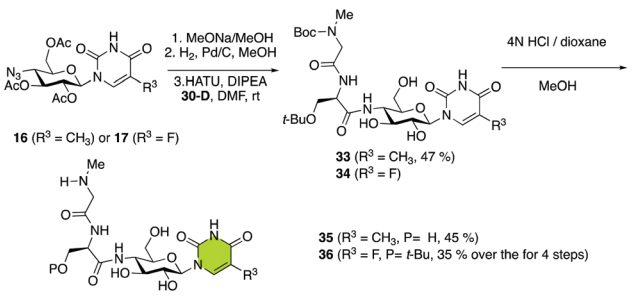
In order to carry out the peptide coupling and access analogues, we first examined azide reduction directly on compound **15**. However, while using the Staudinger conditions[22] or different hydrogenation conditions to reduce the azide to an amine, we obtained complex mixtures that probably result from deacetylation and acetate migration. To solve this problem, we decided to protect first the $N^4$-cytosine with a *tert*-butylcarbamate group and remove all the acetyl groups before hydrogenation (Scheme 3). Therefore, compound **15** was treated with $Boc_2O$ in the presence of $NEt_3$ and 4-dimethylaminopyridine (DMAP) to provide the corresponding derivative **27** by concomitant deprotection of $N^4$-Ac-cytosine. The Zemplén deacetylation was carried out followed by hydrogenation with palladium hydroxide in methanol. This reaction 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 gougerotin, 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 $CH_2Cl_2$. 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 dipep-



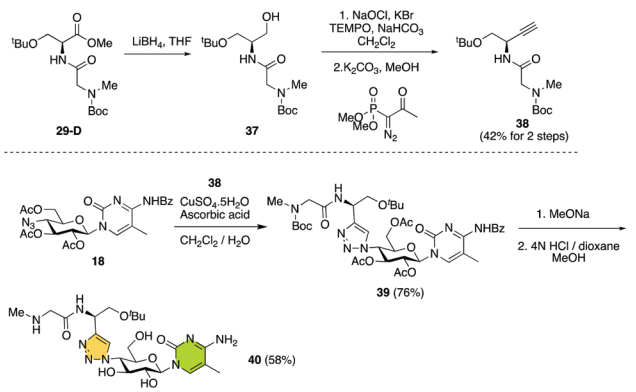

**Scheme 3** Preparation of analogues **32-D** and **32-L**.

tides **30-D** and **30-L** using the coupling agent HATU[23] 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 4 N HCl in $MeOH/CH_2Cl_2$ 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 the difficulties associated with 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 the Zemplén deacetylation and hydrogenation followed by dipeptide coupling with carboxylic acid **30-D**, affording the corresponding peptidonucleosides **33** and **34** (Scheme 4). Further deprotection with 4 N HCl allowed us to obtain analogues **35** and **36**. For **34**, 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 the desired product due to degradation.




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




**Scheme 5** Preparation of analogue **40**.

Taking advantage of the presence of the azide at position C-4 of the 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 aldehyde[24] and treatment with dimethyl (1-diazo-2-oxopropyl)phosphonate[25] in the presence of potassium carbonate in MeOH. The CuAAC reaction[26] between alkyne **38** and azide **18** was carried out in a water/dichloromethane mixture with $CuSO_4{\cdot}5H_2O$ and ascorbic acid to smoothly give the triazole derivative **39** in 76% yield. The final removal of the acyl groups with MeONa in methanol followed by treatment with 4 N HCl at room temperature led to, 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), *Botryotiniacinereal* (BOTRCI), and *Zymoseptoria tritici* (SEPPTR) (Table 2). Although some of the analogs were found to have good bioactivities, none of them showed superior activity to gougerotin. It is 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.

## 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 bases by other pyrimidine bases and the replacement of the carboxa-

**Table 2** Antifungal activities of selected 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.

mide function at C5 by a 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. 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 on other gougerotin analogues is still ongoing.

# Experimental

### General remarks

All non-aqueous reactions were run under an inert atmosphere (argon) using standard techniques for manipulating air-sensitive compounds and the glassware was stored in an 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 in a microwave oven for 45 seconds. The reactions were monitored with analytical Merck TLC silica gel 60 F254 plates, visualized under UV (254 nm) and stained with $KMNO_4$ or vanillin. Column chromatography was performed using 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 the addition of anhydrous and granular $K_2CO_3$. NMR spectra were recorded with AM 300, AVANCE 300 and AVANCE 500 Brüker spectrometers. Chemical shifts are given in parts per million and referenced to the solvent peak of $CDCl_3$, defined at 77.2 ppm ($^{13}$C NMR) and 7.26 ppm ($^{1}$H NMR), or to the solvent peak of $CD_3OD$, defined at 49.9 ppm ($^{13}$C NMR) and 3.34 ppm ($^{1}$H NMR), or to the solvent peak of $D_2O$, defined at 4.79 ppm ($^{1}$H NMR), or to the solvent peak of DMSO-$d_6$, defined at 39.5 ppm ($^{13}$C NMR) and 2.50 ppm ($^{1}$H NMR). Data are reported as follows: 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 PerkinElmer Spectrum BX instrument with an FT-IR system. Optical rotation was measured on an Anton Paar MCP300 polarimeter using a cell of 1-dm-length path. Mass spectra were recorded with the 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 ($2/3V_{tot}$) under an argon atmosphere was added BSA (4 eq.). The resulting mixture was heated at 60 °C for 1 h and then cooled to room temperature. The donor (1 eq.) was stirred with 4 Å molecular sieves in dry MeCN ($1/3V_{tot}$) 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 $NaHCO_3$. The reaction mixture was filtered and the aqueous phase was extracted with EtOAc (5×). The organic layers were combined, washed with NaCl, dried over $Na_2SO_4$, 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), and 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. $[\alpha]_D^{25}$ +56.6 ($c$ = 1.0, $CHCl_3$); $^{1}$H NMR (300 MHz, $CD_3OD$) $\delta$ 8.14 (d, 1H, $J_{HAr,HAr}$ = 7.5 Hz, $H_{Ar}$), 7.49 (d, 1H, $J_{HAr,HAr}$ = 7.5 Hz, $H_{Ar}$), 6.12 (d, 1H, $J_{1,2}$ = 9.0 Hz, *H*1), 5.54 (t, 1H, $J_{3,2}$ = $J_{3,4}$ = 9.5 Hz, *H*3), 5.32 (t, 1H, $J_{2,3}$ = $J_{2,1}$ = 9.5 Hz, *H*2), 4.47 (dd, 1H, $J_{6,6'}$ = 13.0 Hz and $J_{6,5}$ = 1.0 Hz, *H*6), 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, *H*4, *H*5), 2.21 (s, 3H, NHCOC$H_3$), 2.14 (s, 3H, OCOC$H_3$), 2.12 (s, 3H, OCOC$H_3$), 1.94 (s, 3H, OCOC$H_3$); $^{13}$C NMR (75 MHz, $CD_3OD$) $\delta$ 173.9 (*C*═O), 173.1 (*C*═O), 172.0 (*C*═O), 172.0 (*C*═O), 165.5 ($Cq_{Ar}$), 158.6 ($Cq_{Ar}$), 147.5 ($CH_{Ar}$), 99.8 ($CH_{Ar}$), 83.6 (*C*1), 77.1 (*C*4), 75.4 (*C*3), 73.2 (*C*2), 64.8 (*C*6), 61.9 (*C*5), 25.4 (COC$H_3$), 21.4 (COC$H_3$), 21.1 (COC$H_3$); IR $\nu$ (film, cm$^{-1}$) 3239 (═C–H), 2112 ($N_3$), 1747 (C═O), 1663 (NH–C═O); ESIHRMS $m/z$ = 467.1486 $[M + H]^+$. $C_{18}H_{23}N_6O_9$ 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 $CH_2Cl_2$ (1 mL) were added di-*tert*-butyldicarbonate (40 μL, 0.17 mmol, 2 eq.), triethylamine (12 μL, 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 ($CH_2Cl_2$/MeOH 99 : 1 to 98 : 2) to afford product **27** (29 mg, 0.06 mmol, 64%) as a yellow powder. $[\alpha]_D^{25}$ +41.7 ($c$ = 1.2, $CHCl_3$); $^{1}$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.62 (d, 1H, $J_{HAr,HAr}$ = 7.5 Hz, $H_{Ar}$), 7.30 (d, 1H, $J_{HAr,HAr}$ = 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, OCOC$H_3$), 1.97 (s, 3H, OCOC$H_3$), 1.51 (s, 9H, OC(C$H_3$)$_3$); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 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 (*C*1), 75.5 (*C*4), 73.5 (*C*3), 70.7 (*C*2), 62.7 (*C*6), 60.2 (*C*5), 28.2 (C(C$H_3$)$_3$), 20.9 (COC$H_3$), 20.8 (COC$H_3$), 20.6 (COC$H_3$); IR $\nu$ (film, cm$^{-1}$) 2987 (C–H), 2109 ($N_3$), 1751 (C═O), 1731 (C═O), 1667 (NH–C═O), 1626 (NH–C═O); ESIHRMS $m/z$ = 525.1945 $[M + H]^+$. $C_{21}H_{29}N_6O_{10}$ 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 $CH_2Cl_2$ (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 $Et_3N$ (0.49 mL, 3.54 mmol, 3 eq.) was added. After being stirred overnight at room temperature, the mixture was diluted with aqueous saturated $NaHCO_3$ (8 mL). The aqueous phase was then extracted with EtOAc (3 × 10 mL). The organic layers were combined, dried over $Na_2SO_4$, 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. $[\alpha]_D^{25}$ −26.4 ($c$ = 1.0, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 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, $OCH_3$), 3.53 (dd, 1H, $J_{1,1'}$ = 9.0 Hz, $J_{1',1}$ = 3.0 Hz, *H*1), 2.93 (s, 3H, $NCH_3$), 1.46 (s, 9H, $CO_2C(CH_3)_3$), 1.10 (s, 9H, $OC(CH_3)_3$); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 62.1 (*C*1), 53.4 (*C*3), 52.8 (*C*2), 52.6 ($OCH_3$), 35.7 ($NCH_3$), 28.5 ($CO_2C(CH_3)_3$), 27.5 ($OC(CH_3)_3$); IR $\nu$ (film, cm$^{-1}$) 3314 (N–H), 2975 ($CH_3$), 2935 ($CH_2$), 1751 (C=O), 1684 (NH–C=O); ESIHRMS $m/z$ = 347.2182 $[M + H]^+$. $C_{16}H_{31}N_2O_6$ 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 a THF/$H_2O$ 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. 1 N HCl was then added until the pH value was 2. The aqueous layer was extracted with EtOAc (3 × 10 mL). The organic layers were combined, dried over $Na_2SO_4$ and concentrated under vacuum to afford the clean product **30-D** (351 mg, 1.06 mmol, quantitative). The product was used without further purification. $[\alpha]_D^{25}$ −32.8 ($c$ = 1.0, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 6.90 (d, 1H, $J_{NH,H2}$ = 8.0 Hz, *N*H), 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, $NCH_3$), 1.44 (s, 9H, $CO_2C(CH_3)_3$), 1.12 (s, 9H, $OC(CH_3)_3$); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 173.7 (*C*=O), 169.9 (*C*=O), 81.2 ($Cq_{CO_2C(CH_3)_3}$), 74.1 ($Cq_{OC(CH_3)_3}$), 61.7 (*C*1), 53.3 (*C*3), 52.7 (*C*2), 35.8 ($NCH_3$), 28.5 ($CO_2C(CH_3)_3$), 27.5 ($OC(CH_3)_3$); IR $\nu$ (film, cm$^{-1}$) 3320 (O–H), 2975 ($CH_3$), 2935 ($CH_2$), 1739 (C=O), 1670 (NH–C=O); ESIHRMS $m/z$ = 355.1842 $[M + Na]^+$. $C_{15}H_{28}N_2O_6Na$ 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 reduced pressure to afford the 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) were 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. The 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; $[\alpha]_D^{25}$ +14.6 ($c$ = 1.1, MeOH). $^1$H NMR (500 MHz, $CD_3OD$)[27] $\delta$ 8.11 (d, 1H, $J_{HAr,HAr}$ = 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, $NCH_3$), 1.56 (s, 9H, $CO_2C(CH_3)_3$), 1.50–1.41 (m, 9H, $OC(CH_3)_3$), 1.20 (s, 9H, $CO_2C(CH_3)_3$); $^{13}$C NMR (75 MHz, $CD_3OD$)[28] $\delta$ 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 (*C*1), 83.4 ($C(CH_3)_3$), 80.2 (*C*H), 75.8 (*C*H), 74.4 ($CH_2$), 73.9 ($C(CH_3)_3$), 73.8 ($C(CH_3)_3$), 71.6 ($CH_2$), 62.8 ($CH_2$), 56.1 (*C*7), 53.4 (*C*H), 53.1 ($CH_2$), 36.8 ($NCH_3$), 28.9 ($C(CH_3)_3$), 28.5 ($C(CH_3)_3$), 27.8 ($C(CH_3)_3$); IR $\nu$ (film, cm$^{-1}$) 3264 (N–H), 2976 (CH), 2926 (CH), 1758 (C=O), 1656 (NH–C=O); ESIHRMS $m/z$ = 687.3566 $[M + H]^+$. $C_{30}H_{51}N_6O_{12}$ requires 687.3565.

**Peptidonucleoside 32-D.** To a stirred solution of **31-D** (90 mg, 0.131 mmol, 1 eq.) in $CH_2Cl_2$/MeOH (2 : 1 v/v, 1.3 mL) was added a solution of 4 M 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 $H_2O$ 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 ($H_2O$/EtOH/EtOAc 4 : 4 : 2, pH 9) to afford **32-D** as a white powder (21 mg, 0.05 mmol, 37%). $[\alpha]_D^{25}$ −31.3 ($c$ = 1.1, $H_2O$); $^1$H NMR (300 MHz, $D_2O$) $\delta$ 7.73 (d, 1H, $J_{HAr,HAr}$ = 7.5 Hz, $H_{Ar}$), 6.07 (bs, 1H, $H_{Ar}$), 5.64 (d, 1H, $J_{1,2}$ = 9.5 Hz, *H*1), 4.45 (t, 1H, $J_{7,8}$ = $J_{7,8'}$ = 5.5 Hz, *H*7), 3.94 (s, 2H, *H*9), 3.91–3.80 (m, 1H, *H*4), 3.86 (d, 2H, $J_{8,7}$ = 5.5 Hz, *H*8), 3.80–3.71 (m, 3H, *H*2, *H*3, *H*5), 3.68 (dd, 1H, $J_{6,6'}$ = 12.5 Hz, $J_{6,5}$ = 1.5 Hz, *H*6), 3.57 (dd, 1H, $J_{6',6}$ = 12.5 Hz, $J_{6',5}$ = 5.5 Hz, *H*6′), 2.74 (s, 3H, $NCH_3$); $^{13}$C NMR (75 MHz, $D_2O$) $\delta$ 172.0 (*C*=O), 166.7 (*C*=O), 166.0 (*C*=O), 157.9 ($Cq_{Ar}$), 141.7 ($CH_{Ar}$), 97.0 ($CH_{Ar}$), 83.3 (*C*1), 77.5 (*C*5), 73.7 (*C*3), 71.7 (*C*2), 61.0 (*C*8), 60.6 (*C*6), 55.9 (*C*7), 51.3 (*C*4), 49.4 (*C*9), 32.8 ($NCH_3$); IR $\nu$ (film, cm$^{-1}$) 3310 (O–H), 3282 (N–H), 2976 ($CH_3$), 2933 ($CH_2$), 1744 (C=O), 1653 (NH–C=O); ESIHRMS $m/z$ = 431.3424 $[M + H]^+$. $C_{16}H_{28}N_6O_8$ requires 431.1890.

## Conflicts of interest

There are no conflicts to declare.

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