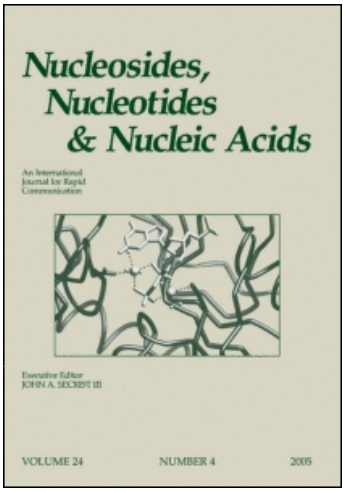

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A Rapid Synthetic Route to Conformationally Restricted [5,5]-Bicyclic Nucleoside-Amino Acid Conjugates

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A RAPID SYNTHETIC ROUTE TO CONFORMATIONALLY RESTRICTED [5,5]-BICYCLIC NUCLEOSIDE-AMINO ACID CONJUGATES

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□ *A concise synthetic route to a novel class of conformationally rigid 3′,4′-*cis*-fused bicyclic nucleoside derivatives has been developed. The synthetic strategy and approach involves initial synthesis of a key [5,5]-bicyclic 6-aminofurofuran-2-one scaffold, employing an L-serine derived aminobutenolide as a strategically functionalized chiral template. Subsequent utilization of the carbonyl functionality of the above bicyclic lactone toward nucleobase incorporation, and linking of the resident amine functionality with appropriately protected amino acids completed the syntheses of the target bicyclic nucleoside-amino acid conjugates. Following the above route, and utilizing a combination of easily available nucleobases (4) and amino acids (4) as the two diversity elements, combinatorial synthesis of a 16-member demonstration library of the title amino acid-linked nucleosides has been accomplished.*

Keywords Bicyclic nucleoside; conformational rigidity; nucleoside-amino acid conjugate; chiral aminobutenolide; combinatorial synthesis

INTRODUCTION

Construction of multifunctional scaffolds, grafting various biologically relevant structural units on a common framework, is an attractive strategy for the development of new chemical entities of potential biomedical application.[1] As amino acids and nucleosides are among the most common and fundamental building blocks of a vast array of natural macromolecules, creation of designer molecules via a judicious combination of the above building blocks provides an opportunity to access new and diverse classes of "nature-like" and yet nonnatural organic compounds.[2]

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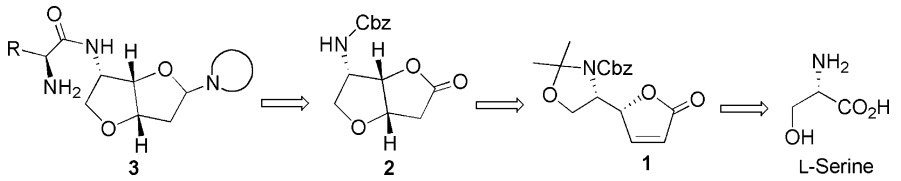


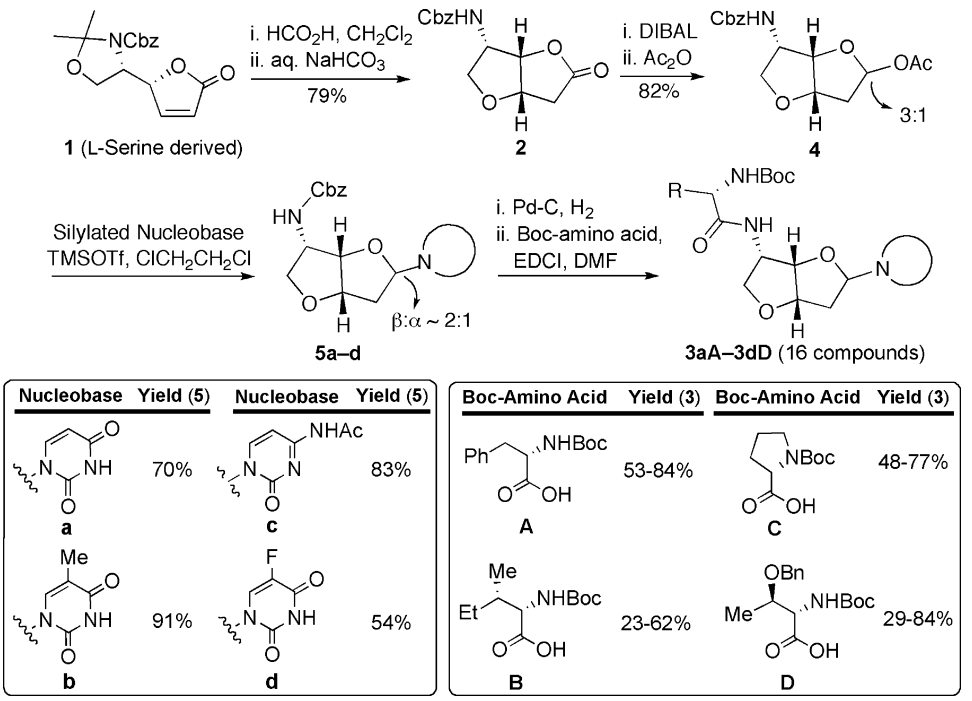
FIGURE 1 Strategy and approach towards amino acid-linked [5,5]-ring-fused bicyclic nucleosides.

In recent years, conformationally restricted nucleosides and oligonucleotides thereof have attracted considerable attention as biological tools in probing the furanose core conformational preferences, as exhibited by nucleosides/nucleotides in their interactions with the target enzymes.[3] For example, oligonucleotides constructed from comparatively rigid fused bicyclic carbohydrate core containing nucleosides have been found to display improved recognition of complementary RNA and DNA sequences.[3] In addition to their utility as biological probes, conformationally restrained non-natural nucleosides and their derivatives are also of potential interest as novel antiviral, anticancer, and antisense agents.[4] Consequently, design, synthesis, and biological evaluation of various conformationally restricted nucleosides, with ring-fused bicyclic sugar backbones, continue to be an active area of research.[5] In view of the above observations, and as part of an ongoing program investigating the complex peptidyl nucleoside antifungal antibiotics,[6,7] we are currently exploring development of synthetic routes to a new class of structurally unique amino acid-nucleoside hybrids. Initial results of the above studies leading to the development of a combinatorial synthetic route to a novel class of conformationally rigid [5,5]-bicyclic peptidyl nucleosides are reported herein.

Employing a de novo nucleoside synthesis protocol, our strategy and approach involved utilization of an L-serine derived 5-alkylamino substituted chiral butenolide **1** towards initial stereoselective formation of a strategic bicyclic furofuranone scaffold **2** (Figure 1). Subsequent incorporation of various nucleobases (first diversity element) via modification of the lactone carbonyl, and peptidic attachment of the resident amine functionality with appropriate amino acids (second diversity element) resulted in a 16-member (4-x-4) demonstration library of the desired [5,5]-bicyclic ring-fused nucleoside-amino acid conjugates **3**.

RESULTS AND DISCUSSION

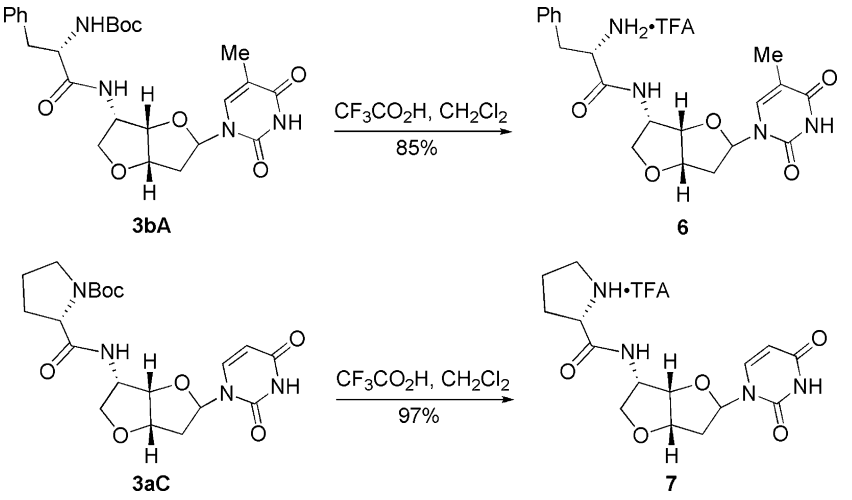
Following a recently developed protocol from our laboratory, initial synthesis of the L-serine derived chiral aminobutenolide **1** (8 steps from L-serine; 42% overall yield)[7c,8] was followed by acid assisted cleavage of the *N,O*-acetonide linkage and subsequent base mediated conjugate addition of the free hydroxy group to the enone moiety, providing the [5,5]-ring-fused bicyclic lactone **2** (Scheme 1) in good yield.[8] As evident, the lactone



SCHEME 1 Combinatorial synthesis of amino acid-linked [5,5]-bicyclic nucleosides.

carbonyl and the protected amine functionality as present in **2** provides convenient handles towards the desired synthesis of the target, conformationally rigid bicyclic peptidyl nucleosides. Towards nucleoside formation, partial reduction of the lactone **2** to lactol, followed by its treatment with acetic anhydride provided the corresponding acetate derivative **4** as an approximately 3:1 mixture (by ^{1}H NMR) of anomers. Subsequent nucleobase introduction was performed by reacting the acetate **4** with bis-silylated uracil in the presence of TMSOTf (Vorbrüggen protocol),[9] resulting in the regioselective (*N*-1 selective) formation of an inseparable anomeric mixture (~2:1 by ^{1}H NMR) of the corresponding nucleoside derivative **5a** in good yield. In high resolution NMR studies, NOE correlations observed between the anomeric proton of the minor isomer of **5a** (δ 6.28) and the ring-junction protons (H-3a and H-6a) indicated the minor isomer to be the α-anomer, thereby confirming the major isomer in the above nucleoside forming reaction to be the expected β-nucleoside. The poor stereoselectivity in the above *N*-glycosidation reaction can be attributed to the absence of any stereodirecting substituent at the adjacent C2′-position (nucleoside numbering) of the glycosyl donor **4**.

To achieve diversification, the nucleoside forming reaction was then extended to synthesize the corresponding thymidine, 5-fluorouridine, and cytidine analogs. Accordingly, reaction of **4** with the above bis-silylated



SCHEME 2 Removal of *N*-Boc-protection.

nucleobases resulted in the expected anomeric mixtures of the nucleoside derivatives **5b–d**, respectively, in moderate to high yields. The final steps towards completion of the synthesis and library construction involved hydrogenolytic unmasking of the side chain amine functionality of the above nucleosides, followed by standard peptidic coupling of the resulting free amine with a variety of suitably protected amino acids. Employing the above sequence of reactions, the combination of the four nucleosides **5a–d** with a representative set of four different *N*-Boc-amino acids (**A–D**) resulted in the construction of the 16-member library of amino acid-linked nucleosides **3aA-3aD**, **3bA-3bD**, **3cA-3cD**, and **3dA–3dD** (Scheme 1).

To demonstrate the feasibility of removal of the Boc-protecting group, two representative peptidyl nucleosides **3bA** and **3aC** (Scheme 2) were subjected to deprotection. Thus, treatment of the above compounds with trifluoroacetic acid under standard conditions cleanly afforded the corresponding trifluoroacetate salts of the free nucleoside derivatives **6** and **7** respectively in high yields (Scheme 2). The library members thus synthesized are presently being evaluated against various biological targets.

CONCLUSION

In conclusion, starting from an L-serine derived chiral aminobutenolide, a rapid, de novo synthetic route to a conformationally restricted [5,5]-bicyclic nucleoside scaffold has been developed. Subsequent anchoring of various amino acids on the side chain amine functionality of the nucleoside resulted in a structurally novel bicyclic nucleoside-peptide hybrid. Utilizing the above nucleoside intermediate, and employing readily available nucleobases (4) and amino acids (4) as the two diversity elements, the above

method was successfully extended to construct a 16-member demonstration library of bicyclic ring-fused novel peptidyl nucleosides. Starting from D-serine, the method can also be easily extended to obtain *ent*-**2** and subsequently the corresponding (3a*R*, 6*R*, 6a*R*)-diastereoisomers of **3**.

EXPERIMENTAL

All of the solvents and reagents used were obtained commercially and used as such unless noted otherwise. Moisture or air sensitive reactions were conducted under argon atmosphere in oven dried (120°C) glass apparatus. Diethyl ether and THF were distilled from sodium benzophenone ketyl, while dichloromethane was distilled over calcium hydride, prior to use. Solvents were removed under reduced pressure using standard rotary evaporators. Flash column chromatography was carried out using Silica gel 60 (230–400 mesh), while thin layer chromatography (tlc) was carried out on Silica Gel HLF, precoated glass plates. All yields reported refer to isolated material judged to be homogeneous by tlc and NMR spectroscopy. Proton and carbon nuclear magnetic resonance spectra were recorded using a Bruker DRX 400 MHz or Bruker DRX 500 MHz spectrometer (Bruker Biospin Corpn., Billerica, MA, USA). Unless noted otherwise, NMR spectra were recorded with the chemical shifts (δ) reported in ppm relative to Me_4Si (for ^1H) and $CDCl_3$ (for ^{13}C) as internal standards respectively. Mass spectra were obtained from a ZAB HS mass spectrometer (VG Analytical Ltd., Manchester, UK) equipped with a 11/250 data system. Fast-atom bombardment mass spectrometry (FAB-MS) experiments were performed with a Xenon gun operated at 8 Kev energy and 0.8 mA emission at the MS laboratory at the University of Kansas. Fast-atom bombardment high resolution mass spectra (FAB-HRMS) were recorded at 1:10,000 resolution using linear voltage scans under data system control and collected in a multi-channel analyzer mode (MCA).

Starting from L-serine, the aminobutenolide **1**, and the subsequent furofuranone **2** were prepared following earlier reported methods from our laboratory.[7c,8] The spectral and analytical data of both of the above compounds were in good agreement with the reported values.

(3a*S*,6*S*,6a*S*)-2-Acetoxy-6-(benzyloxycarbonylamino)-hexahydrofuro[3,2-*b*]furan (4)

Step 1: To a cooled (−78°C) and stirred solution of the bicyclic lactone **2**[7c,8] (1.4 g, 5 mmol) in anhydrous CH_2Cl_2 (20 mL) was added DIBAL-H (1 M in toluene, 6.5 mL, 6.5 mmol) dropwise. The reaction was stirred at −78°C for 1.5 hours and then quenched by careful addition of MeOH (2 mL). The reaction mixture was allowed to warm to room temperature, followed by addition of EtOAc (100 mL) and saturated aqueous sodium

potassium tartrate solution (50 mL). The resulting mixture was stirred until clear separation into two layers. The organic layer was separated and the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined extracts were dried (Na_2SO_4) and solvent removed under reduced pressure. After drying under high vacuum for 2 hours, the crude lactol (1.27 g), without any further purification, was used directly for the next reaction.

Step 2: The crude lactol (1.27 g, 4.55 mmol) obtained from the above reaction was dissolved in anhydrous CH_2Cl_2 (15 mL) and cooled to 0°C (ice bath). To this stirring solution was added sequentially anhydrous pyridine (0.9 mL, 11.5 mmol), 4-DMAP (20 mg, catalytic), and freshly distilled Ac_2O (0.9 mL, 9.5 mmol). The resulting solution was allowed to attain room temperature and stirring continued for another 2 hours. The reaction was quenched by the addition of ice-cold water (20 mL) and stirred for 10 minutes. The two layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic extracts were washed sequentially with saturated aqueous $NaHCO_3$ (1 × 20 mL) and brine (1 × 20 mL), and dried over Na_2SO_4. After removal of solvent under vacuum, the residual oil was purified by flash chromatography (1% MeOH in CH_2Cl_2) to provide the acetate **4** as a white solid (1.3 g, 82% over 2-steps). ^{1}HNMR (500 MHz, $CDCl_3$, mixture of anomers): δ 7.32–7.36 (m, 5H), 6.43–6.34 (2m, 1H), 5.29–5.24 (m, 1H), 5.11–5.09 (m, 2H), 4.92–4.98 and 4.78–4.72 (2m, 1H), 4.70–4.65 (m, 1H), 4.40–4.28 (m, 1H), 4.10–4.04 (m, 1H), 3.72 & 3.38 (2t, J = 9 Hz, 1H), 2.42–2.25 (m, 2H), 2.04 (s, 3H). ^{13}CNMR (125.8 MHz, $CDCl_3$, mixture of anomers): δ 170.0 156.0, 136.2, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 99.4, 99.2, 85.0, 82.1, 81.4, 81.3, 70.2, 68.6, 67.2, 67.1, 67.0, 53.6, 53.5, 40.7, 40.6, 21.3, 21.1. HRMS (ES+) found 344.1099 (M+Na) (calc for $C_{16}H_{19}NO_6Na$: 344.1110).

Benzyl (3*S*,3a*S*,6a*S*)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl) hexahydrofuro[3,2-*b*]furan-3-ylcarbamate (5a)

To a solution of the acetate **4** (0.675 g, 2.1 mmol) in anhydrous $(CH_2Cl)_2$ (25 mL) was added bis(trimethylsilyl)uracil (1.41 g, 5.25 mmol; 2.5 equiv), followed by freshly distilled TMSOTf (2 mL, 10.5 mmol; 5 equiv). After stirring at room temperature for 2 hours, the reaction was quenched by the addition of saturated aqueous $NaHCO_3$ solution (10 mL). The organic layer was separated, the aqueous layer was saturated with solid NaCl and the solution extracted with EtOAc (3 × 50 mL). The combined organic extract was washed with brine (10 mL), dried over Na_2SO_4, concentrated under vacuum, and the residue was purified by flash chromatography (hexane/ethyl acetate: 1/2 to 1/4) to obtain the nucleoside **5a** as a white solid (0.55 g, 70% yield). ^{1}HNMR (400 MHz, CD_3OD, mixture of anomers): δ 7.87 and 7.63 (2d, J = 8 Hz, 1H), 7.34–7.28 (m, 5 H), 6.28 (dd, J = 8.4,

4.8 Hz, 0.35H), 6.14 (t, J = 6.8 Hz, 0.65H), 5.73–5.68 (m, 1H), 5.09 (br s, 3H), 4.72–4.24 (4m, 2H), 4.11–3.98 (2 m, 1H), 3.72 & 3.53 (2t, J = 8.8 Hz, 1H), 2.73–2.49 (2m, 1H), 2.37–2.13 (2m, 1H). ^{13}CNMR (125.8 MHz, CD_3OD, mixture of anomers): 166.3, 158.7, 152.6, 152.3, 142.9, 142.9, 143.0, 142.9, 138.4, 129.6, 129.2, 129.0, 103.4, 103.0, 89.9, 87.8, 84.8, 84.7, 84.2, 84.1, 71.0, 70.5, 67.8, 56.4, 55.9, 41.2, 39.1, HRMS (ES+) found 374.1354 (M+H) (calc for: $C_{18}H_{20}N_3O_6$: 374.1352).

General Procedure for the Synthesis of the Bicyclic Nucleosides 5b–d

Following the same procedure as in **5a** above, the acetate **4** was separately reacted with the corresponding bis-silylated nucleobases derived from thymine, N^4-acetylcytosine, and 5-fluorouracil respectively. After standard work-up of the reaction mixture as above, the crude products obtained were purified by flash chromatography (EtOAc: hexanes) to provide the nucleoside derivatives **5b–d**.

Benzyl (3S,3aS,6aS)-5-(5-Methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) hexahydrofuro-[3,2-b]furan-3-ylcarbamate (5b)

Purified by column chromatography (hexane/ethyl acetate: 1/4) to obtain the product as a white solid (0.448 g, 91% yield). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of anomers): δ 8.46–8.39 (2 br s, 1H), 7.45–7.30 (m, 5H), 7.02 (s, 1H), 6.46–6.12 (2m, 1H), 5.30 (br t, J = 7.4 Hz, 1 H), 5.10 (br s, 2H), 4.89–4.85 (m, 1.3H), 4.70–4.11 (m, 2.7H), 3.70–3.45 (2 t, J = 8.8 Hz, 1H), 2.71–2.59 (m, 1H), 2.30–2.23 (m, 1H), 1.93 (s, 3H). ^{13}C NMR (125.8 MHz, DMSO-d_6, mixture of anomers): δ 163.7, 155.8, 150.5, 150.3, 137.0, 136.8, 136.6, 136.3, 128.4, 128.3, 127.9, 127.8, 109.8, 98.0, 86.2, 84.8, 82.2, 82.1, 82.0, 81.5, 68.3, 67.9, 66.4, 65.7, 65.6, 65.5, 54.8, 54.0, 37.7, 12.4, 12.1. HRMS (ES+) found 388.1508 (M+H) (calc for $C_{19}H_{22}N_3O_6$: 388.1509).

Benzyl (3S,3aS,6aS)-5-(4-Ethanamido-2-oxopyrimidin-1(2H)-yl) hexahydrofuro[3,2-b]furan-3-ylcarbamate (5c)

Purified by column chromatography (ethyl acetate/MeOH: 98/2 to 96/4) to obtain the product as a white solid (0.423 g, 83% yield). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of anomers): δ 10.68 and 9.85 (2 br s, 1H), 8.25–8.05 (m, 0.5H), 7.78–7.63 (m, 1H), 7.55–7.32 (m, 5.5H), 6.44 and 5.80 (2 br s, 1H), 6.14 and 5.56 (2 br s, 1H), 5.17–5.09 (m, 2H), 4.88–4.76 (m, 1H), 4.67 & 4.54 (2brs, 1H), 4.39 (brs, 1H), 4.20–4.06 (m, 2H), 3.73–3.46 (2m, 1H), 2.86–2.74 (m, 1H), 2.23 and 2.17 (2s, 3H). ^{13}C NMR (125.8 MHz, $CDCl_3$, mixture of anomers): δ 171.3, 171.2, 162.8, 156.8, 156.0, 155.5, 154.9, 154.6, 144.9, 143.8, 143.4, 136.1, 128.5, 128.4, 128.2, 128.1, 128.0, 97.6, 96.9,

89.9, 88.3, 87.5, 84.0, 83.9, 83.3, 82.3, 71.1, 70.0, 68.9, 67.2, 67.1, 55.7, 54.6, 54.5, 42.3, 41.7, 39.9, 29.7, 24.8, 24.6. HRMS (ES+) found 415.1615 (M+H) (calc for $C_{20}H_{23}N_4O_6$: 415.1618).

Benzyl (3S,3aS,6aS)-5-(5-Fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) hexahydrofuro-[3,2-b]furan-3-ylcarbamate (5d)

Purified by column chromatography (hexane/1,4-dioxane: 2/1 to 1/1) to obtain the product as a white solid (0.39 g, 54% yield). ^{1}H NMR (500 MHz, Acetone-d_6, mixture of anomers): δ 10.4 (brs, 1H), 7.87 (d, J = 6.5 Hz, 1H), 7.38–7.28 (m, 5H), 6.45 (brd, J = 8 Hz, 1H), 6.21 (br t, J = 86.5 Hz, 1H), 5.06 (s, 2H), 4.96 (t, J = 4.5 Hz, 1H), 4.91–4.88 (m, 1H), 4.32–4.26 (m, 1H), 4.01 (apparent t, J = 8 Hz, 1H), 3.56 (apparent t, J = 9 Hz, 1H), 2.49 (dd, J = 14.5, 6 Hz, 1H), 2.42–2.35 (m, 1H). ^{13}C NMR (125.8 MHz, Acetone-d_6, mixture of anomers): δ 157.7, 156.9, 149.8, 142.5, 140.7, 138.2, 129.3, 128.8, 125.7, 125.5, 88.5, 84.0, 83.4, 70.4, 66.9, 55.9, 40.8. HRMS (ES+) found 392.1251 (M+H) (calc for $C_{18}H_{19}FN_3O_6$: 392.1258).

General Procedure for the Synthesis of the Bicyclic Peptidyl Nucleosides 3aA-3dD

Step 1 (Cbz-deprotection): Each of the side chain *N*-Cbz protected nucleoside derivatives **5a-d**, dissolved separately in 1:1 MeOH/dioxane (1 g/10 mL of sovent)), were treated with 10% palladium on activated carbon (wt. of nucleoside/wt. of Pd-C = 10/1), and the resulting mixture stirred at room temperature under H_2 atmosphere (balloon). After stirring for 2–3 hours (monitored by TLC), the reaction mixtures were filtered through Celite and the residue washed thoroughly with methanol (5x). Removal of solvent under vacuum and drying of the resulting oily residue under high vacuum provided the corresponding free amines. The crude amines thus obtained were used directly for the subsequent peptidic coupling reaction without any further purification.

Step 2 (Peptidic coupling): Each of the four aminoalkyl nucleoside derivatives as obtained above, were divided into four equal portions, dissolved in anhydrous DMF (10% solution by wt.), followed by sequential addition of the different *N*-Boc amino acids **A–D** (1.5 equiv) and EDCI (2 equiv) into the four different reaction flasks. The resulting mixtures were stirred at room temperature overnight and then quenched by the addition of water. The resulting mixtures were extracted thoroughly with ethyl acetate (4x), the extracts dried over anhydrous Na_2SO_4, and solvent removed under high vacuum. Purification of the crude residues by flash chromatography (EtOAc: hexanes) provided the pure peptidyl nucleoside derivatives **3aA-3dD** (16 compounds).

tert-Butyl (S)-1-((3S,3aS,6aS)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) hexahydrofuro- [3,2-b]furan-3-ylamino)-1-oxo-3-phenylpropan-2-ylcarbamate (3aA)

Obtained as a white solid (74 mg, 82%). ^{1}H NMR (500 MHz, CD_3OD, mixture of anomers): δ 7.80 & 7.64 (2 dd, J = 16, 8 Hz, & dd, J = 13, 8.3 Hz, 1H), 7.27–7.17 (m, 5H), 6.33–6.13 (2m, 1H), 5.77–5.71 (m, 1H), 4.85–4.27 (m, 4H), 4.11–3.85 (m, 1H), 3.72–3.42 (m, 1H), 3.11–3.02 (m, 1H), 2.85–2.79 (m, 1H), 2.74–2.60 (m, 1H), 2.53–2.21 (3m, 1H), 1.37–1.35 (m, 9H). ^{13}C NMR (125.8 Hz, CD_3OD, mixture of anomers): δ 174.6, 172.8, 166.2, 157.7, 155.1, 152.6, 152.2, 143.1, 138.7, 130.6, 130.5, 129.6, 127.9, 103.7, 103.1, 89.7, 89.5, 87.8, 87.7, 84.8, 84.7, 84.2, 84.1, 83.4, 83.3, 80.9, 71.6, 71.2, 70.7, 57.5, 54.8, 54.7, 54.6, 54.5, 54.2, 54.1, 41.0, 40.9, 39.6, 39.4, 39.3, 38.8, 38.7, 37.7, 37.6, 37.4, 32.1, 28.8. HRMS (ES+) found 509.2008 (M+Na) (calc for $C_{24}H_{30}N_4O_7Na$: 509.2012).

tert-Butyl (2S,3S)-1-((3S,3aS,6aS)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)hexahydro-furo[3,2-b]furan-3-ylamino)-3-methyl-1-oxopentan-2-ylcarbamate (3aB)

White solid (53 mg, 52%). ^{1}H NMR (500 MHz, CD_3OD, mixture of anomers): δ 7.88 & 7.66 (2 dd, J = 8.0, 5.0 Hz & 8.3 & 4.8 Hz, 1H), 6.34–6.19 (2m, 1H), 5.77 & 5.71 (t, J = 7.5 Hz & dd, J = 8.0, 2.0 Hz, 1H), 4.92–4.77 (m, 1H), 4.73–4.38 (2m, 2H), 4.16–3.90 (3m, 2H), 3.60–3.41 (2m, 1H), 2.76–2.51 (3m, 1H), 2.37–2.24 (2m, 1H), 1.77 (brs, 1H), 1.44–1.41 (m, 10H), 1.18–1.11 (m, 1H), 0.93–0.86 (m, 6H). ^{13}C NMR (125.8 Hz, CD_3OD, mixture of anomers): δ 175.0, 174.9, 173.0, 172.8, 166.2, 158.1, 155.1, 152.6, 152.2, 143.2, 143.1, 143.0, 103.7, 103.2, 89.6, 89.4, 87.9, 87.8, 87.7, 84.7, 84.2, 83.3, 80.8, 71.6, 71.3, 71.1, 60.9, 60.8, 55.0, 54.8, 54.6, 54.3, 54.1, 41.0, 38.8, 38.5, 38.4, 38.3, 37.7, 37.4, 37.1, 32.2, 28.8, 26.0, 25.9, 16.2, 11.8. HRMS (ES+) found 453.2353 (M+H) (calc for $C_{21}H_{33}N_4O_7$: 453.2349).

(S)-tert-Butyl 2-((3S,3aS,6aS)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) hexahydrofuro-[3,2-b]furan-3-ylcarbamoyl)pyrrolidine-1-carboxylate (3aC)

White solid (65 mg, 70%). ^{1}H NMR (500 MHz, CD_3OD, mixture of anomers): δ 7.91–7.64 (2m, 1H), 6.34–6.22 (2m, 1H), 5.78–5.70 (m, 1H), 4.82–4.71 (m, 1H), 4.60–4.54 (m, 1H), 4.47–4.38 (m, 1H), 4.22 (brs, 1H), 4.14–3.97 (2m, 1H), 3.79–3.57 (m, 1H), 3.54–3.38 (m, 2H), 2.76–2.50 (m, 1H), 2.42–2.09 (m, 2H), 2.02–1.82 (m, 3H), 1.43 and 1.39 (2s, 9H). ^{13}C NMR (125.8 Hz, CD_3OD, mixture of anomers): δ 176.1, 176.0, 172.8, 166.2, 156.5, 156.1, 155.1, 152.6, 152.2, 143.2, 103.7, 103.2, 89.7, 89.6, 88.1, 87.9, 87.7, 84.7, 84.6, 84.4, 84.2, 83.4, 83.3, 81.7, 72.1, 71.3, 71.0, 70.8, 70.4, 61.8, 61.7, 61.5, 55.0, 54.9, 54.8, 54.6, 48.1, 40.9, 40.8, 38.7, 38.5, 37.8, 37.4, 32.7,

32.6, 32.1, 28.8, 25.5, 24.8. HRMS (ES+) found 437.2048 (M+H) (calc for $C_{20}H_{29}N_4O_7$: 437.2036).

tert-Butyl (2S,3R)-3-(benzyloxy)-1-((3S,3aS,6aS)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)hexahydrofuro[3,2-b]furan-3-ylamino)-1-oxobutan-2-ylcarbamate (3aD)

White solid (72 mg, 75%). ^{1}H NMR (500 MHz, CD_3OD, mixture of anomers): δ 7.78 & 7.55 (t, J = 8.5 Hz & dd, J = 8.3, 3.3 Hz, 1H), 7.32–7.24 (m, 5H), 6.32–6.11 (m, 1H), 5.72–5.59 (m, 1H), 4.79–4.69 (2m, 1H), 4.60–4.35 (m, 3H), 4.19–3.96 (m, 3H), 3.75–3.44 (3m, 1H), 2.75–2.31 (2m, 2H), 2.24–2.22 (m, 1H), 1.46–1.42 (m, 9H), 1.22–1.16 (m, 3H). ^{13}C NMR (125.8 Hz, CD_3OD, mixture of anomers): δ 173.4, 172.8, 166.3, 158.2, 155.1, 154.9, 152.5, 152.2, 143.0, 139.9, 139.8, 129.5, 129.1, 129.0, 128.9, 128.8, 103.8, 103.7, 89.8, 89.5, 87.9, 87.8, 84.8, 84.2, 84.1, 83.4, 81.2, 76.7, 76.5, 76.4, 72.6, 72.5, 71.2, 71.1, 70.8, 60.6, 60.5, 60.4, 55.2, 54.9, 54.8, 54.5, 41.0, 40.8, 38.9, 38.6, 37.7, 37.5, 37.3, 32.0, 28.8, 16.8, 16.7. HRMS (ES+) found 553.2269 (M+Na) (calc for $C_{26}H_{34}N_4O_8$ Na: 553.2274).

tert-Butyl (S)-1-((3S,3aS,6aS)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)hexa-hydrofuro[3,2-b]furan-3-ylamino)-1-oxo-3-phenylpropan-2-ylcarbamate (3bA)

White solid (92 mg, 84%). ^{1}H NMR (500 MHz, CD_3OD, mixture of anomers): δ 7.61 & 7.47 (2d, J = 6.0 Hz, 1H), 7.28–7.14 (m, 5H), 6.35–6.12 (m, 1H), 4.83–4.69 (m, 2H), 4.60–4.26 (m, 2H), 4.15–3.88 (3m, 1H), 3.74–3.40 (3m, 1H), 3.07–2.91 (m, 1H), 2.88–2.80 (m, 1H), 2.71–2.43 (2m, 1H), 2.37–2.19 (m, 1H), 1.91 & 1.86 (2s, 3H), 1.34 (br s, 9H). ^{13}C NMR (125.8 MHz, CD_3OD, mixture of anomers): δ 174.7, 174.6, 166.5, 166.4, 157.7, 152.9, 152.8, 138.7, 130.6, 129.6, 127.9, 127.8, 112.5, 112.4, 89.1, 89.0, 87.5, 84.6, 84.4, 84.2, 80.9, 79.6, 71.4, 70.9, 70.8, 57.6, 57.5, 54.9, 54.7, 54.5, 40.8, 40.7, 39.4, 39.3, 38.6, 28.8, 12.9, 12.5. HRMS (ES+) found 501.2349 (M+H) (calc for $C_{25}H_{33}N_4O_7$: 501.2349).

(**3bB**). White solid (58 mg, 62%). ^{1}H NMR (500 MHz, CD_3OD, mixture of anomers &): δ 7.67 & 7.49 (d, J = 4.5 Hz & s, 1H), 6.37–6.19 (2m, 1H), 4.92–4.84 (m, 1H), 4.75–4.40 (m, 2H), 4.19–4.01 (2m, 1H), 3.95–3.89 (m, 1H), 3.78–3.53 (2m, 1H), 2.72–2.69 (m, 0.4H), 2.50 (dd, J = 14.5, 6.5 Hz, 0.6H), 2.37–2.24 (m, 1H), 1.91–1.89 (m, 3H), 1.77–1.74 (m, 1H), 1.52–1.44 (m, 1H), 1.42 & 1.40 (2s, 9H), 1.17–1.11 (m, 1H), 0.92–0.85 (m, 6H). ^{13}C NMR (125.8 Hz, CD_3OD, mixture of anomers): δ 175.1, 174.9, 166.5, 166.4, 158.1, 152.5, 152.4, 138.7, 138.6, 138.5, 138.4, 112.4, 112.1, 89.1, 88.9, 87.7, 87.5, 84.5, 84.4, 84.3, 80.8, 71.5, 71.4, 70.9, 60.9, 60.8, 54.8, 54.6, 54.3, 54.0, 40.8, 40.7, 38.7, 38.6, 38.4, 28.8, 26.0, 25.9, 16.2, 16.1, 12.9, 12.5, 11.8, 11.7. HRMS (ES+) found 467.2511 (M+H) (calc for $C_{22}H_{35}N_4O_7$: 467.2506).

(S)-tert-Butyl 2-((3S,3aS,6aS)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)hexahydrofuro[3,2-b]furan-3-ylcarbamoyl)pyrrolidine-1-carboxylate (3bC)

Light yellow solid (69 mg, 77%). ^{1}H NMR (500 MHz, CD_3OD, mixture of anomers): δ 7.70–7.47 (2m, 1H), 6.35–6.24 (2m, 1H), 4.86–4.42 (m, 3H), 4.22–3.99 (m, 2H), 3.81–3.36 (m, 3H), 2.74–2.68 (m, 0.4H), 2.52–2.47 (m, 0.6H), 2.40–2.12 (m, 2H), 1.97–1.82 (m, 6H), 1.43–1.36 (m, 9H). ^{13}C NMR (125.8 MHz, CD_3OD, mixture of anomers): δ 176.1, 175.5, 166.5, 166.4, 156.7, 156.5, 152.8, 152.4, 138.8, 138.6, 138.5, 112.4, 112.1, 89.0, 88.8, 87.9, 87.7, 87.5, 84.8, 84.4, 84.2, 81.7, 81.6, 72.1, 71.7, 71.2, 71.0, 70.6, 61.8, 61.5, 61.4, 54.9, 54.8, 54.6, 54.3, 54.0, 48.1, 40.9, 40.8, 38.6, 38.4, 32.8, 32.7, 32.6, 31.6, 31.3, 30.9, 28.8, 28.7, 25.5, 24.8, 12.8, 12.5. HRMS (ES+) found 451.2184 (M+H) (calc for $C_{21}H_{31}N_4O_7$: 451.2193).

tert-Butyl (2S,3R)-3-(benzyloxy)-1-((3S,3aS,6aS)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimi-din-1(2H)-yl)hexahydrofuro[3,2-b]furan-3-ylamino)-1-oxobutan-2-ylcarbamate (3bD)

White solid (60 mg, 68%). ^{1}H NMR (500 MHz, CD_3OD, mixture of anomers): δ 7.59 & 7.54 (2s, 0.3H), 7.39 (d, $J = 9.0$ Hz, 0.7H), 7.32–7.22 (m, 5H), 6.33–6.09 (3m, 1H), 4.92–4.83 (m, 1H), 4.71–4.60 (2m, 1H), 4.57–4.52 (m, 1H), 4.48–4.39 (m, 2H), 4.21–3.95 (m, 3H), 3.75–3.50 (2m, 1H), 2.73–2.66 (m, 0.3H), 2.52–2.42 (m, 0.7H), 2.37–2.19 (2m, 1H), 1.84–1.82 (m, 3H), 1.45–1.41 (m, 9H), 1.21–1.19 (m, 3H). ^{13}C NMR (125.8 Hz, CD_3OD, mixture of anomers): δ 173.4, 173.3, 166.5, 166.4, 158.2, 152.7, 152.5, 152.3, 139.8, 139.7, 139.5, 138.5, 129.5, 129.4, 129.3, 129.1, 129.0, 128.9, 128.8, 112.5, 112.4, 112.1, 112.0, 89.2, 88.9, 87.7, 87.5, 84.6, 84.4, 84.3, 84.2, 81.2, 81.1, 76.8, 76.6, 76.4, 76.2, 72.6, 72.5, 72.4, 72.0, 71.7, 71.3, 70.9, 60.6, 60.4, 60.3, 59.9, 55.0, 54.6, 54.5, 54.0, 40.8, 40.5, 38.8, 38.5, 28.8, 16.8, 16.7, 16.6, 12.9, 12.7, 12.5. HRMS (ES+) found 545.2606 (M+H) (calc for $C_{27}H_{37}N_4O_8$: 545.2611).

tert-Butyl (S)-1-((3S,3aS,6aS)-5-(4-ethanamido-2-oxopyrimidin-1(2H)-yl)hexahydrofuro-[3,2-b]furan-3-ylamino)-1-oxo-3-phenylpropan-2-ylcarbamate (3cA)

White solid (48 mg, 53%). ^{1}H NMR (500 MHz, CD_3OD, mixture of anomers): δ 8.16–8.04 (m, 1H), 7.53–7.46 (m, 1H), 7.28–7.12, (m, 6H), 6.25–6.13 (m, 1H), 4.96–4.87 (m, 2H), 4.71–4.25 (m, 2H), 4.13–3.93 (m, 1H), 3.77–3.55 (m, 1H), 3.13–3.00 (m, 1H), 2.91–2.67 (m, 2H), 2.35–2.21 (m, 3H), 1.42–1.30 (m, 9H). ^{13}C NMR (125.8 Hz, CD_3OD, mixture of anomers): δ 174.8, 174.6, 173.2, 164.6, 164.5, 158.2, 157.9, 157.7, 146.6, 146.5, 138.6, 138.5, 130.7, 130.6, 130.5, 130.1, 129.6, 129.4, 128.0, 127.9, 126.5, 98.6, 98.5, 98.3, 91.4, 89.9, 85.5, 85.4, 84.3, 84.2, 84.0, 80.9, 71.5, 71.4, 70.7, 58.8, 57.5, 57.4, 56.9, 54.6, 54.5, 54.3, 45.0, 42.3, 42.2, 40.3, 40.2,

39.7, 39.4, 28.8, 24.7, 21.6. HRMS (ES+) found 528.2149 (M+H) (calc for: $C_{26}H_{34}N_5O_7$: 528.2458).

tert-Butyl (2S,3S)-1-((3S,3aS,6aS)-5-(4-ethanamido-2-oxopyrimidin-1(2H)-yl)hexahydro-furo[3,2-b]furan-3-ylamino)-3-methyl-1-oxopentan-2-ylcarbamate (3cB)

White solid (56 mg, 56%). ^{1}H NMR (500 MHz, CD_3OD, mixture of anomers): δ 8.30–8.08 (m, 1H), 7.53–7.42 (m, 1H), 7.27–7.09 (m, 1H), 6.25–6.09 (m, 1H), 4.99–4.97 (m, 1H), 4.72–4.47 (m, 2H), 4.14–3.94 (m, 1H), 3.83–3.57 (m, 2H), 2.85–2.69 (2m, 1H), 2.67–2.16 (m, 3H), 1.83–1.77 (m, 1H), 1.52–1.51 (m, 1H), 1.44–1.38 (m, 9H), 1.21–1.11 (m, 1H), 0.92–0.83 (m, 6H). ^{13}C NMR (125.8 Hz, CD_3OD, mixture of anomers): δ 175.2, 175.0, 173.2, 164.6, 158.1, 157.9, 146.6, 146.5, 146.4, 130.1, 129.4, 126.5, 98.5, 98.4, 91.4, 91.2, 90.0, 85.5, 85.3, 84.3, 84.2, 80.7, 71.4, 70.7, 60.8, 60.7, 59.1, 57.2, 54.8, 54.6, 54.4, 45.2, 44.4, 42.3, 42.1, 40.3, 38.7, 38.5, 38.3, 36.8, 28.8, 26.0, 25.9, 25.8, 24.7, 16.2, 16.1, 14.9, 11.7, 11.6. HRMS (ES+) found 494.2615 (M+H) (calc for $C_{23}H_{36}N_5O_7$: 494.2615).

(S)-tert-Butyl 2-((3S,3aS,6aS)-5-(4-ethanamido-2-oxopyrimidin-1(2H)-yl) hexahydrofuro-[3,2-b]furan-3-ylcarbamoyl)pyrrolidine-1-carboxylate (3cC)

White solid (59 mg, 48%). ^{1}H NMR (500 MHz, CD_3OD, mixture of anomers): δ 8.32–8.05 (m, 1H), 7.53–7.42 (m, 1H), 7.23–7.10 (m, 2H), 6.28–6.07 (m, 1H), 5.00–4.98 (m, 1H), 4.74–4.46 (m, 2H), 4.23–4.01 (m, 2H), 3.79–3.58 (m, 1H), 3.52–3.40 (m, 2H), 2.86–2.66 (m, 1H), 2.32–2.18 (m, 5H), 1.98–1.81 (m, 3H), 1.46–1.33 (m, 9H). ^{13}C NMR (125.8 Hz, CD_3OD, mixture of anomers): δ 176.3, 176.1, 173.2, 164.6, 158.2, 158.0, 156.6, 156.2, 146.7, 146.6, 139.1, 130.1, 129.4, 126.5, 98.5, 98.4, 91.5, 91.4, 91.0, 90.2, 90.1, 89.8, 85.6, 85.3, 84.5, 84.4, 81.7, 81.5, 72.4, 71.9, 71.3, 71.1, 70.8, 61.7, 61.6, 61.5, 61.2, 57.6, 54.7, 54.6, 54.5, 48.1, 42.5, 42.1, 42.0, 40.2, 40.1, 39.9, 32.8, 32.7, 31.6, 31.4, 28.8, 25.5, 24.8, 24.7, 21.6. HRMS (ES+) found 478.2277 (M+H) (calc for $C_{22}H_{32}N_5O_7$: 478.2302).

tert-Butyl (2S,3R)-3-(benzyloxy)-1-((3S,3aS,6aS)-5-(4-ethanamido-2-oxopyrimidin-1(2H)-yl)hexahydrofuro[3,2-b]furan-3-ylamino)-1-oxobutan-2-ylcarbamate (3cD)

White solid (78 mg, 84%). ^{1}H NMR (500 MHz, CD_3OD, mixture of anomers): δ 8.14, 8.08 & 7.94 (3d, J = 7.5 Hz, 1H), 7.48–7.10 (m, 6H), 6.25–6.08 (m, 1H), 5.07–4.92 (m, 1H), 4.75–4.41 (m, 3H), 4.32–3.89 (m, 2H), 3.71–3.50 (m, 2H), 3.28–3.22 (m, 1H), 2.83–2.65 (2m, 1H), 2.31–2.23 (m, 1H), 2.20–2.16 (2s, 3H), 1.46–1.41 (m, 9H), 1.32–1.19 (m, 3H). ^{13}C NMR (125.8 Hz, CD_3OD, mixture of anomers): δ 173.5, 173.0, 164.4, 158.5, 158.0, 146.9, 146.2, 140.1, 139.6, 129.9, 129.8, 129.6, 129.5, 129.3, 129.2, 129.0, 128.8, 128.7, 126.3, 98.8, 98.5, 98.3, 91.3, 91.1, 89.7, 85.7, 85.2, 84.1,

84.0, 81.0, 80.8, 76.7, 76.5, 76.1, 72.7, 72.4, 72.3, 72.1, 70.6, 60.3, 60.0, 59.7, 56.6, 54.8, 54.7, 54.4, 45.4, 44.9, 44.4, 41.9, 40.1, 39.9, 37.1, 36.6, 29.1, 25.0, 24.5, 17.2, 16.7, 16.5, 15.3. HRMS (ES+) found 572.2717 (M+H) (calc for $C_{28}H_{38}N_5O_8$: 572.2721).

tert-Butyl (S)-1-((3S,3aS,6aS)-5-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)hexa-hydrofuro[3,2-b]furan-3-ylamino)-1-oxo-3-phenylpropan-2-ylcarbamate (3dA)

White solid (59 mg, 66%). ^{1}H NMR (500 MHz, DMSO-d_6, mixture of anomers): δ 11.9 & 10.8 (2s, 1H), 8.15–7.82 (2m, 1H), 7.26–7.16 (m, 5H), 6.96–6.89 (m, 1H), 6.17–6.10 (m, 1H), 5.29–5.10 (m, 1H), 4.84–4.72 (m, 1H), 4.66–4.62 (m, 1H), 4.28–4.24 (m, 2H), 3.94–3.57 (4m, 2H) 3.43–3.23 (2m, 1H), 2.98–2.87 (m, 1H), 2.74–2.69 (m, 1H), 2.16–2.14 (m, 1H), 1.28–1.19 (m, 9H). ^{13}C NMR (125.8 Hz, DMSO-d_6, mixture of anomers): δ171.9, 171.8, 166.1, 166.0, 165.9, 165.8, 155.2, 155.1, 151.3, 151.2, 149.0, 138.1, 138.0, 129.4, 129.2, 128.0, 127.9, 126.1, 86.8, 85.9, 85.8, 83.5, 83.4, 82.8, 82.2, 82.1, 82.0, 81.8, 78.3, 69.1, 69.0, 68.9, 68.8, 55.6, 55.5, 52.9, 52.8, 37.8, 37.7, 37.6, 37.4, 36.6, 36.5, 28.1, 27.8. HRMS (ES+) found 505.2074 (M+H) (calc for $C_{24}H_{30}FN_4O_7$: 505.2099).

tert-Butyl (2S,3S)-1-((3S,3aS,6aS)-5-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-hexahydrofuro[3,2-b]furan-3-ylamino)-3-methyl-1-oxopentan-2-ylcarbamate (3dB)

White solid (38 mg, 23%). ^{1}H NMR (500 MHz, CD_3OD, mixture of anomers): δ. 7.89 (br s, 1H), 6.23–6.19 (m, 1H), 4.92–4.89 (m, 1H), 4.83 (brs, 1H), 4.47–4.44 (m, 1H), 4.06–3.92 (2m, 2H), 3.59–3.52 (m, 1H), 2.53 (dd, J = 14, 5.8 Hz, 1H), 2.34–2.29 (m, 1H), 1.77 (brs, 1H), 1.54–1.46 (m, 1H), 1.44–1.39 (m, 9H), 1.17–1.13 (m, 1H), 0.93–0.86 (m, 6H). ^{13}C NMR (125.8 Hz, CD_3OD, mixture of anomers): δ 175.1, 174.9, 159.7, 159.6, 159.4, 158.1, 151.2, 150.9, 143.0, 141.2, 141.1, 127.0, 127.8, 126.7, 126.5, 126.4, 89.4, 89.2, 88.1, 84.9, 84.7, 84.3, 84.2, 80.8, 79.6, 71.1, 60.7, 55.0, 54.8, 54.6, 40.9, 40.7, 38.6, 38.5, 38.4, 28.8, 26.1, 26.0, 16.2, 16.1 11.7, 11.6. HRMS (ES+) found 471.2251 (M+H) (calc for $C_{21}H_{32}FN_4O_7$: 471.2255).

(S)-tert-Butyl 2-((3S,3aS,6aS)-5-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)hexa-hydrofuro[3,2-b]furan-3-ylcarbamoyl)pyrrolidine-1-carboxylate (3dC)

White solid (44 mg, 50%). ^{1}H NMR (500 MHz, DMSO-d_6, mixture of anomers): δ 11.9 & 10.8 (2s, 1H), 7.93–7.65 (m, 1H), 6.17–6.10 (m, 1H), 5.28–5.04 (m, 1H), 4.84–4.57 (m, 2H), 4.29–4.11 (m, 2H), 3.92–3.83 (m, 1H), 3.75–3.59 (m, 1H), 3.51–3.38 (m, 1H), 3.31–3.22 (m, 1H), 2.14–1.98 (m, 2H), 1.83–1.69 (m, 3H), 1.37–1.23 (m, 10H). ^{13}C NMR (125.8 Hz, DMSO-d_6, mixture of anomers): δ 172.8, 172.7, 166.1, 166.0, 165.8, 157.1,

156.9, 153.6, 153.3, 151.3, 151.2, 149.0, 141.1. 139.2, 131.5, 128.7, 125.8, 125.7, 86.7, 85.9, 85.8, 83.5, 83.4, 82.9, 82.7, 82.2, 78.6, 78.5, 78.4, 69.2, 68.9, 68.7, 68.6, 59.5, 59.3, 59.1, 59.0, 52.9, 52.8, 46.7, 46.5, 36.6, 36.4, 36.3, 36.1, 31.1, 30.0, 27.9, 27.7, 23.9, 23.8. HRMS (ES+) found 455.1940 (M+H) (calc for: $C_{20}H_{28}FN_4O_7$: 455.1942).

tert-Butyl (2S,3R)-3-(benzyloxy)-1-((3S,3aS,6aS)-5-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)hexahydrofuro[3,2-b]furan-3-ylamino)-1-oxobutan-2-ylcarbamate (3dD)

White solid (36 mg, 29%). ^{1}H NMR (500 MHz, CD_3OD, mixture of anomers): δ 7.77 (dd, J = 11.0 and 6.5 Hz, 1H), 7.32–7.16 (m, 5H), 6.17 & 6.10 (2t, J = 6.5 Hz, 1H), 4.91–4.89 (m, 1H), 4.83–4.81 (m, 1H), 4.59–4.55 (m, 1H), 4.48–4.38 (m, 2H), 4.18 (d, J = 15 Hz, 1H), 4.06–3.98 (m, 2H), 3.54–3.48 (m, 1H), 2.49 (dd, J = 14, 6.5 Hz, 1H), 2.34–2.22 (m, 1H), 1.45 & 1.42 (2s, 9H), 1.22–1.19 (m, 3H). ^{13}C NMR (125.8 Hz, CD_3OD, mixture of anomers): δ 173.4, 173.3, 159.6, 159.4, 158.1, 150.8, 150.7, 142.9, 141.1, 139.8, 130.1, 129.5, 129.4, 129.2, 129.1, 128.8, 126.7, 126.6, 89.4, 89.2, 84.7, 84.6, 84.1, 84.0, 81.1, 76.7, 76.6, 76.3, 72.5, 72.4, 71.1, 70.9, 60.5, 60.2, 55.1, 55.0, 54.8, 40.8, 40.6, 28.8, 16.7, 16.6. HRMS (ES+) found 549.2358 (M+H) (calc for $C_{26}H_{34}FN_4O_8$: 549.2361).

General Boc-Deprotection Procedure to Form the Free Peptidyl Nucleosides 6 and 7

The *N*-Boc peptidyl nucucleoside derivatives **3bA** and **3aC** were dissolved separately in anhydrous CH_2Cl_2 (10 mL of solvent / gm of compound), cooled to 0°C, followed by addition of an equal volume of TFA. The resulting solution was stirred at the same temperature for 30 minutes followed by 1 hour at room temperature. Excess solvent was removed under vacuum and the residue triturated with CH_2Cl_2 and diethyl ether. The resulting solids were dried under high vacuum to provide the fully deprotected peptidyl nucleosides **6** and **7** as their respective trifluoroacetic acid salts.

(S)-2-Amino-N-((3S,3aS,6aS)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)hexahydrofuro[3,2-b]furan-3-yl)-3-phenylpropanamide-TFA salt (6)

Obtained as a white solid (39 mg, 85%). ^{1}H NMR (500 MHz, CD_3OD, mixture of anomers): δ 7.59 & 7.45 (2s, 1H), 7.43–7.20 (m, 6H), 6.34–6.10 (2m, 1H), 4.87–4.82 (m, 1H), 4.76–4.56 (2m, 1H), 4.47–4.34 (2m, 1H), 4.15–3.94 (2m, 2H), 3.86 & 3.72 & 3.53 (3t, J = 5 Hz, 1H), 3.18–3.03 (m, 2H), 2.71–2.42 (2m, 1H), 2.38–2.20 (m, 1H), 1.93–1.86 (3s, 3H). ^{13}C NMR (125.8 MHz, CD_3OD, mixture of anomers): δ 170.0, 169.9, 166.5, 166.4,

162.3, 153.0, 152.8, 152.5, 138.6, 138.5, 135.8, 135.7, 135.6, 130.9, 130.8, 130.7, 130.6, 130.3, 129.6, 129.2, 129.0, 119.2, 116.4, 112.7, 112.5, 112.4, 89.1, 89.0, 87.5, 84.5, 84.4, 84.3, 70.9, 70.8, 70.7, 60.7, 55.7, 55.6, 54.8, 54.2, 54.1, 40.5, 40.3, 38.9, 38.8, 38.5, 38.3, 15.8, 12.9, 12.8, 12.5. HRMS (ES+) found 401.1808 (M+H) (calc for free amine $C_{20}H_{25}N_4O_5$: 401.1825).

(S)-N-((3S,3aS,6aS)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl)hexahydrofuro[3,2-b]-furan-3-yl)pyrrolidine-2-carboxamide-TFA salt (7)

Obtained as a white solid (25 mg, 97%). ^{1}H NMR (500 MHz, CD_3OD, mixture of anomers): δ. 7.90–7.65 (2m, 1H), 6.38–6.19 (2m, 1H), 5.77 & 5.71 (d, $J = 8.0$ Hz & dd, $J = 8.0$, 2.5 Hz, 1H), 4.78–4.72 (m, 1H), 4.66–4.52 (2m, 1H), 4.50–4.41 (m, 1H), 4.31–4.23 (m, 1H), 4.17–3.98 (2m, 1H), 3.76–3.61 (2m, 1H), 3.45–3.38 (m, 2H), 2.76–2.61 (2m, 1H), 2.54–2.36 (2m, 2H), 2.31–2.24 (m, 1H), 2.08–1.96 (m, 3H). ^{13}C NMR (125.8 Hz, CD_3OD, mixture of anomers): δ 172.7, 170.1, 166.2, 162.0, 155.3, 155.1, 152.7, 152.3, 143.1, 143.0, 118.8, 116.2, 103.7, 103.2, 89.6, 89.5, 87.8, 87.7, 84.7, 84.6, 84.3, 84.2, 83.2, 71.1, 71.0, 70.9, 70.7, 70.5, 61.3, 61.2, 61.1, 55.4, 55.2, 55.1, 55.0, 54.7, 47.6, 40.5, 38.6, 37.5, 37.4, 37.3, 32.0, 31.4, 25.2, 25.1. HRMS (ES+) found 337.1510 (M+H) (calc for free amine $C_{15}H_{21}N_4O_5$: 337.1512).

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