Supporting Information

Functionalized C-nucleosides as remarkable RNA binders: targeting of prokaryotic ribosomal A-site RNA

Jean-Patrick Joly,[a] Marc Gaysinski,[a] Lorena Zara,[a] Maria Duca*,[a] and Rachid Benhida *,[a]

[a] Université Côte d'Azur, CNRS, Institute of Chemistry of Nice (ICN), Nice, France

Table of contents

Figure S1. Binding curves related to table 1 and obtained with fluorescence-based assay developed for the evaluation of dissociation constants (K_D, μM) for compounds **6a-c**, **11a-c** and **19a-c**.

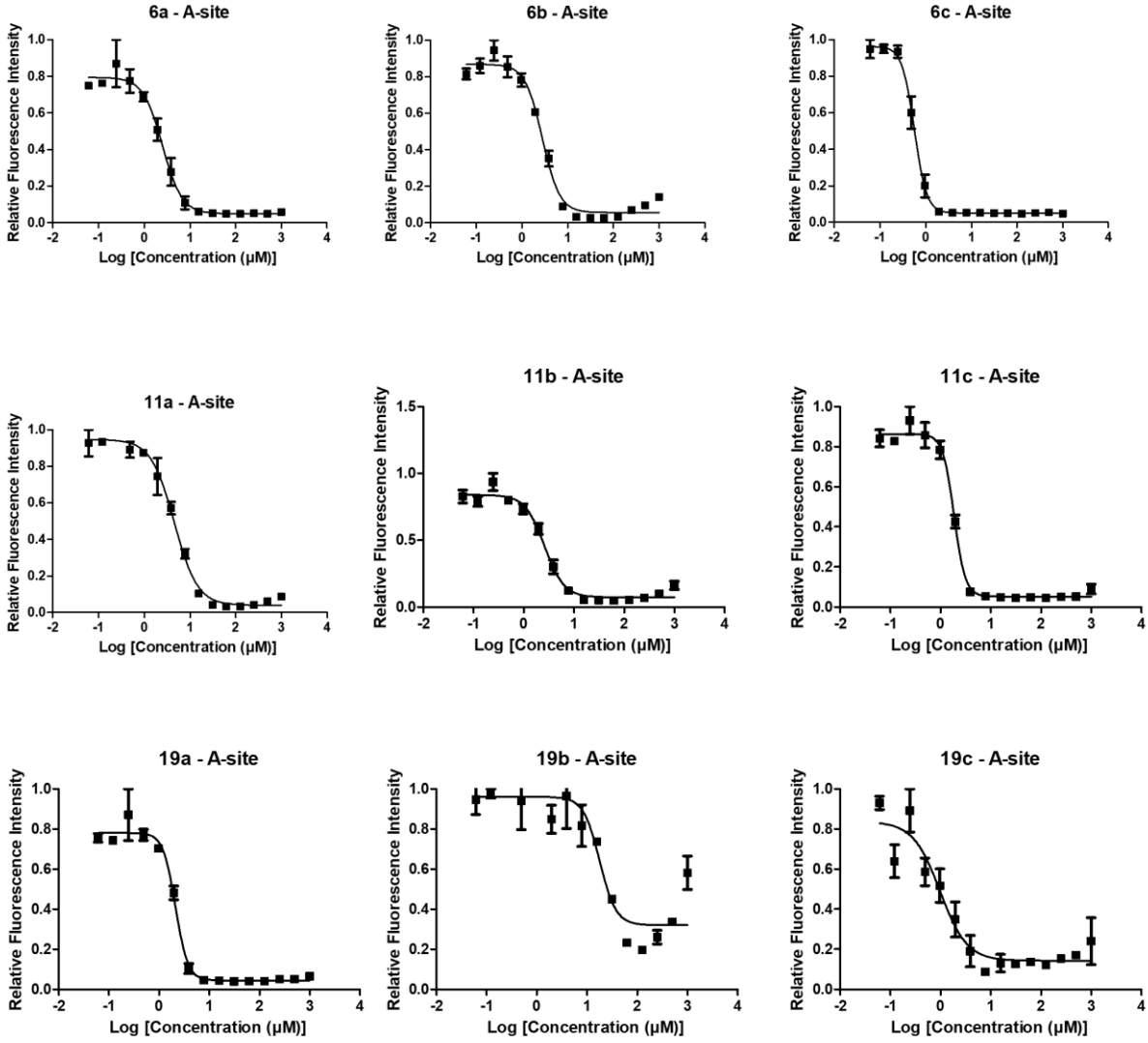


Figure S2. Circular dichroism curves for the interaction of compound **6c** (A) and **19c** (B) with A-site 27-mer RNA. RNA alone (black line) is compared to the addition of various equivalents (colored lines) of the compounds. Compounds alone do not have any circular dichroism signature.

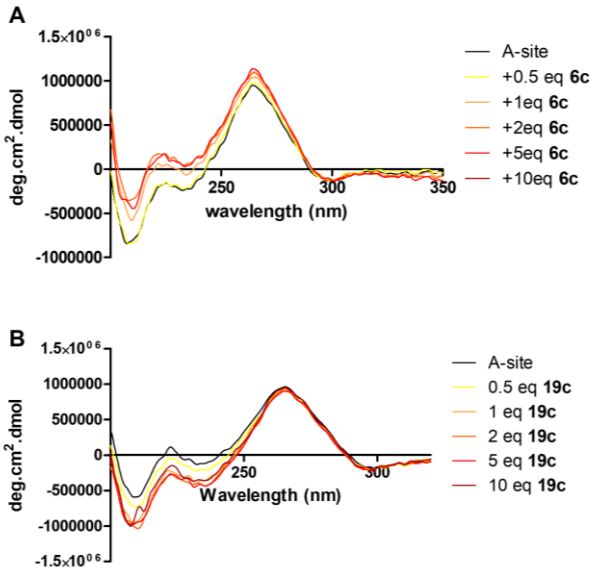


Figure S3. Chemical shifts variation in the ^{1}H NMR spectra of A-site 27-mer RNA upon interaction with various equivalents of compound **6c** (A) and **19c** (B).

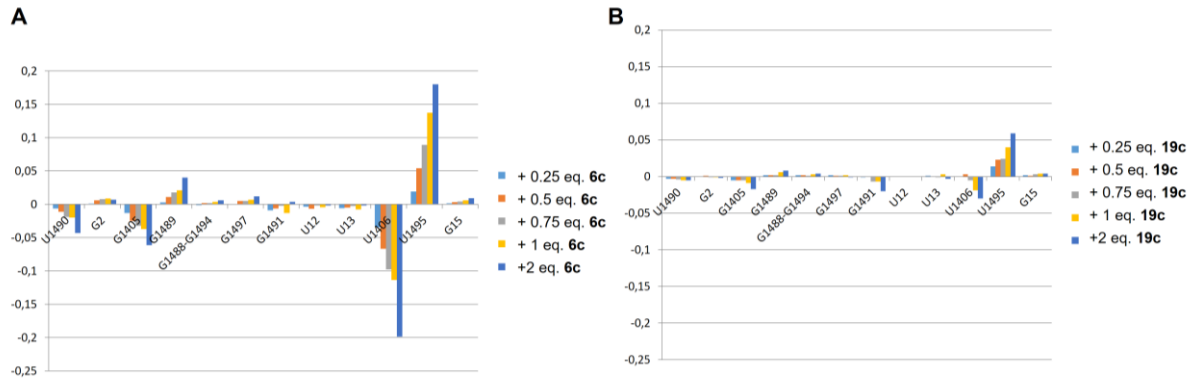


Figure S4. Staked plot of 1D NMR spectra of the imino region of 50 µM A-site RNA with increasing concentrations of **6c** (A) and **19c** (B). The spectra were collected at 286K in a H_2O/D_2O (90/10) buffer (20 mM phosphate and 50 mM NaCl, pH 7.4). Below, secondary structure of 27-mer A-site RNA fragment. Residues shown in grey are those exhibiting an imino resonance upon addition of **6c** (A) and **19c** (B).

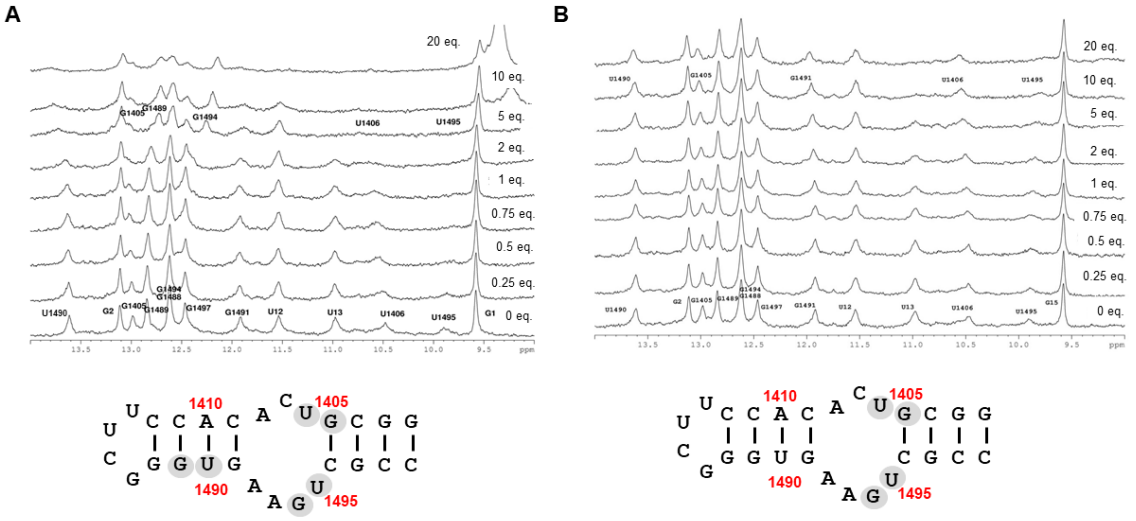
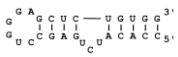
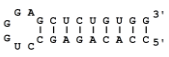
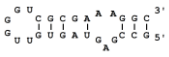
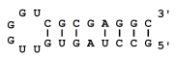


Table S1. Dissociation constants (K_D, μM) for the interaction of compounds **4**, **9**, **17** and **37** with 5 different RNAs[a]

Ligand		 **K_D (TAR)**	 **K_D (TARab)**	 **K_D (IRES IIId)**	 **K_D (IRES IIId ab)**	 **K_D (A-site)**
Neomycin		17.2 ± 1.2	17.2 ± 8.1	2.05 ± 0.1	7.55 ± 0.6	7.75 ± 0.6
Controls	**4**	61.5 ± 3.6	> 100	33.7 ± 4.2	> 100	12.3 ± 0.6
	9	nb	nb	nb	nb	nb
	17	nb	nb	51.5 ± 6.1	nb	39.4 ± 0.1
	37	nb	nb	nb	nb	nb

[a]Fluorescence measurements were performed in Buffer A (20 mM HEPES, pH 7.4, 20 mM NaCl, 140 mM KCl and 3 mM $MgCl_2$). K_D values are calculated at 5°C and represent the mean value calculated out of three independent experiments with standard deviation. nb = no binding

EXPERIMENTAL PROCEDURES

Materials. Solvents and most of the starting materials were purchased from Merck (Sigma Aldrich). All reactions involving air or moisture-sensitive reagents or intermediates were performed under an argon atmosphere. Flash column chromatographies were carried out on silica gel (Merck, SDS 60Å, 40-63 μm). Analytical thin-layer chromatography (TLC) was conducted on Merck precoated silica gel 60F254 plates and compounds were visualized with ninhydrin test and/or under ultraviolet light (254 nm). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE spectrometer (200 MHz or 500 MHz). Chemical shifts are reported in parts per million (ppm, δ) referenced to the residual ^{1}H resonance of the solvent ($CDCl_3$, δ 7.26; CD_3OD, δ 3.31; D_2O, δ 4.79; DMSO-d_6, δ 2.50). ^{13}C spectra were referenced to the residual ^{13}C resonance of the solvent ($CDCl_3$, δ 77.3; CD_3OD, δ 49.0; DMSO-d_6, δ 39.5). Splitting patterns have been designated as follow: s (singlet), d (doublet), dt (doublet of triplets), t (triplet), q (quartet), m (multiplet) and br (broad). Coupling constants (J values) are listed in hertz (Hz). High resolution mass spectra (HRMS) were obtained with a LTQ Orbitrap hybrid mass spectrometer with an electronspray ionization probe (Thermoscientific, San Jose, CA) by direct infusion from a pump syringe to confirm correct molar mass and high purity of compounds. Low resolution mass spectra (MS) were obtained with a Brucker Daltonics Esquire 3000+ electronspray spectrometer equipped with API ionization source. The Final products were analyzed by HPLC on a Waters Alliance 2695 pump coupled with a Waters 996 photodiode array detector and a Thermo Scientific, Betasil RP C18 (250 x 4.6 mm, 5μ). Solvent A (0.1% TFA in water and solvent B (0.1% TFA in acetonitrile) were used for HPLC studies. A gradient of A/B (100/0 to 40/60 for 30 min) was employed at a flow rate of 1 mL/min.

Synthetic procedures.

Activation of 5′-OH using tosyl chloride (general procedure A). To a solution of alcohols **20** and **25** (11.2 mmol) in pyridine (30 mL) at 0°C was added a solution of p-toluensulfonyl chloride (3.18 g, 16.7 mmol, 1.5 eq.) in pyridine (30 mL). After stirring 2 h at room temperature, the reaction mixture is cooled to 0°C and pyridine evaporated under reduced pressure. The crude product was washed three times with 15 mL of EtOAc and 20 mL of water. Organic phases were then washed twice with brine (20 mL) and dried over $MgSO_4$. After evaporation of the solvent under reduced pressure, compounds **21** and **26a-b** were purified by flash chromatography on a silica gel column.

Azide introduction (general procedure B). To a solution of tosylates **21**, **26** and **34** (8.0 mmol) in DMF (50 mL), were added sodium azide (2.08 g, 32.0 mmol, 4 eq.) and tetrabutylammonium iodide (177 mg, 0.48 mmol, 0.06 eq.). The reaction mixture is stirred at 80°C overnight. The solvent is then evaporated under reduced pressure and water (50 mL) was added. This crude product was washed with EtOAc (3 x 50 mL) and organic phases were dried over $MgSO_4$, then concentrated under reduced pressure. Crude products were then purified using silica gel chromatography affording pure compounds **22**, **27** and **34**.

Amide formation (general procedure C). To a solution of carboxylic acid (**1** or **7**, 1.5 mmol, 1.5 eq.) in DCM (10 mL) were added sequentially triethylamine (418 µL, 3.0 mmol, 2 eq.), chloromethylpyridinium iodide (766.0 mg, 3.0 mmol, 2 eq.) and compound **S**[1] (350.2 mg, 1.0 mmol). The reaction mixture was stirred under reflux 3 h then cooled to room temperature. The solvent was then concentrated under reduced pressure and the crude product was purified by silica gel chromatography affording pure compounds **2**, **8** and **13**.

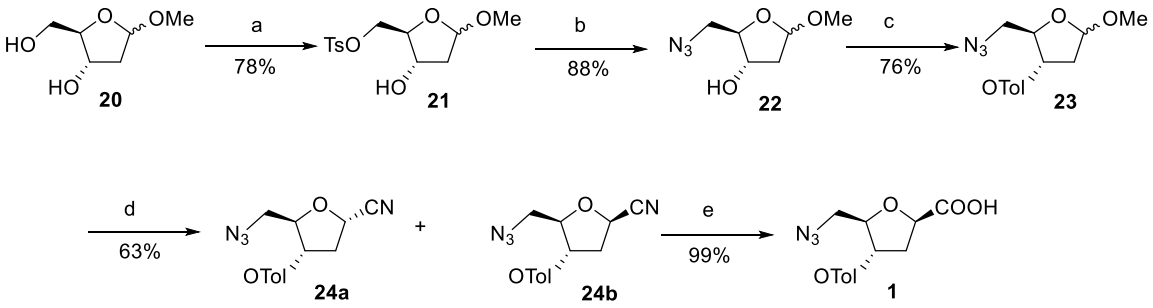
Reduction of the azido group (general procedure D). To a solution of compounds **3**, **8**, **16** and **33** (0.75 mmol) in a mixture DCM/MeOH 1:1 (20 mL) was added Pd/C (20% mol). The reaction mixture was stirred under an hydrogen atmosphere overnight then filtered on celite. Solvent was evaporated under reduced pressure leading to desired compounds **4**, **9**, **13** and **34** that were used in the following step without further purification.

Coupling between 5′-amine-*C*-nucleosides and protected amino acids (General procedure E). To a solution of *N*-protected amino acids (*Nα,Nε*-di-Boc-L-lysine, *Nα*-Boc-*Nω,Nε*-di-Cbz-L-arginine and *Nα,Nim*-di-Boc-L-histidine) (0.54 mmol, 1.2 eq.) in dry CH_2Cl_2 (5 mL) were added HOBt (73.0 mg, 0.54 mmol, 1.2 eq.), DIC (68.1 mg, 0.54 mmol, 1.2 eq.) and Et_3N (251 µL, 1.8 mmol, 4 eq.). Amines **4**, **9**, **17** and **34** (0.45 mmol) were added after 15 min stirring at room temperature. Stirring was then pursued at room temperature during 24h. The reaction mixture was then washed with water (3 times) and extracted twice with CH_2Cl_2. Organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The crude products were then purified by flash chromatography on a silica gel column using a mixture CH_2Cl_2/MeOH 95:5 as the eluent.

Deprotection of Boc and tBu protecting groups (general procedure F). To a solution of compounds **5a**, **5b'**, **5c**, **10a**, **10b'**, **10c**, **18a**, **18b'**, **18c** and **36** (0.024 mmol) in CH_2Cl_2 (1 mL) was added trifluoroacetic acid (0.24 mmol, 10 eq.). The reaction mixture was then stirred at room temperature overnight and the solvent concentrated under reduced pressure. Pure compounds were obtained after precipitation in CH_2Cl_2.

[1] Guianvarc'h D, Benhida R, Fourrey JL, Maurisse R, Sun JS. Incorporation of a novel nucleobase allows stable oligonucleotide-directed triple helix formation at the target sequence containing a purine.pyrimidine interruption. *Chem Commun (Camb).* **2001** 18, 1814-5.

Preparation of the modified 2´-desoxyribose scaffold 1.



Scheme S1. Synthesis of the modified 2'-deoxyribose scaffold **1**. Reagents: a) TsCl, Pyr, r.t., 2h; b) NaN_3, TBAI, DMF, 80°C, 2h; c) TolCl, Pyr, r.t., overnight; d) TMSCN, $BF_3{\cdot}Et_2O$, CH_2Cl_2, r. t., 45 min; e) HCl 37%, 1,4-dioxane, 90°C, 4h.

2′-deoxy-1′-*O*-methyl-5′-*O*-tosyl-(D)-ribofuranose (21). Compound **21** was obtained starting from compound **20** (1.66 g) using general procedure A. The crude product was purified by flash column chromatography using a mixture CHX/EtOAc 7:3 as the eluent affording pure compound **21** as a colorless solid. Yield 2.64 g (78%); R_f = 0.5 (CHX/EtOAc 1:1); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.00-2.15 (m, 2H), 2.45 (s, 3H), 3.21 and 3.33 (2s, 3H), 4.05-4.15 (m, 2H), 4.18-4.22 (m, 1H), 4.40-4.45 (m, 1H), 5.03 (d, *J* = 4.8 Hz, 1H), 7.35 (d, *J* = 7.9 Hz, 2H), 7.79 (dd, *J* = 8.3, 7.1 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 21.8, 41.1, 41.4, 55.1, 69.6, 70.4, 72.5, 72.8, 83.1, 84.5, 105.5, 105.8, 128.1, 130.0, 132.8, 145.2; MS (ESI) *m/z* = 324.4 $[M+Na]^+$.

2′-deoxy-1′-*O*-methyl-5′-azido-(D)-ribofuranose (22). Compound **22** was obtained following general procedure B starting from compound **21** (2.42 g). The crude product was purified by flash column chromatography using a mixture CHX/EtOAc 7:3 affording pure compound **22** as a colorless oil. Yield 1.22 g (88%); R_f = 0.80 (EtOAc 100%); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.00-2.15 (m, 2H), 2.78 (s, 1H), 3.34 (2s, 3H), 3.25-3.35 (m, 2H), 4.10-4.20 (m, 4H), 5.00-5.10 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 41.3, 41.6, 52.7, 53.8, 55.0, 55.4, 72.9, 73.4, 84.7, 85.6, 105.5, 105.6; MS (ESI) *m/z* = 196.0 $[M+Na]^+$.

2′-deoxy-1′-*O*-methyl-5′-azido-3′-(*O*-p-toluoyl)-(D)-ribofuranose (23). To a solution of compound **22** (1.21 g, 7.0 mmol) in pyridine (35 mL) toluoyl chloride (1.16 mL, 8.75 mmol, 1.25 eq.) was added at 0°C. The reaction mixture was stirred at room temperature overnight, then 30 mL of water were added. Pyridine was evaporated under reduced pressure and the crude product was washed with CH_2Cl_2 (3 x 40 mL). Organic phases were dried over $MgSO_4$ and the solvent was then evaporated under reduced pressure. The crude product was finally purified using flash column chromatography using a mixture CHX/EtOAc 8:2 as the eluent affording pure compound **23** as a colorless solid. Yield 1.55 g (76%); R_f = 0.75 (CHX/EtOAc 8:2); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.30-2.40 (m, 2H), 2.42-2.48 (m, 3H), 3.42 (2s, 3H), 3.60-3.65 (m, 2H), 4.30-4.35 (m, 1H), 5.20-5.25 (m, 1.5H), 5.35-5.40 (m, 0.5H), 7.23 (d, *J* = 7.9 Hz, 2H), 7.85 (dd, *J* = 8.0, 4.8 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 21.7, 39.1, 52.4, 54.2, 55.1,

55.5, 75.0, 75.8, 82.2, 83.4, 105.1, 106.0, 126.7, 126.9, 129.2, 129.8, 144.0, 144.2, 166.2, 166.6; MS (ESI) *m/z* = 291.1 $[M+K]^+$.

2′-deoxy-1′-cyano-5′-azido-3′-(*O*-*p*-toluoyl)-*α*-(D)-ribofuranose (24a) et 2′-désoxy-1′-cyano-5′-azido-3′-(*O*-*p*-toluoyl)-β-(D)-ribofuranose (24b). To a solution of compound **23** (1.45 g, 5.0 mmol) in CH_2Cl_2 (25 mL) were added trimethylsilyl cyanide (938 µL, 7.5 mmol, 1.5 eq.) and boron trifluoride diethyl etherate (528 µL, 5.0 mmol). The reaction mixture was stirred at room temperature 45 min, then washed with a saturated solution of $NaHCO_3$ (30 mL) and CH_2Cl_2 (3 x 25 mL). The organic phases were dried over $MgSO_4$ and evaporated under reduced pressure. Anomers **24a** α and **24b** β were separated by flash chromatography on a silica gel column using a mixture CHX/EtOAc 8:2 as the eluent and obtained as colorless oils. Yield 901 mg (63%). **24a**: R_f = 0.65 (CHX/ EtOAc 8:2); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.41 (s, 3H), 2.60-2.65 (m, 2H), 3.63 (qd, *J* = 13.2, 3.4 Hz, 2H), 4.47 (dd, *J* = 7.7, 1.7 Hz, 1H), 5.07 (dd, *J* = 5.9, 1.8 Hz, 1H), 5.42 (dt, *J* = 5.9, 1.8 Hz, 1H), 7.25 (d, *J* = 8.2 Hz, 2H), 7.98 (d, *J* = 8.2 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 21.9, 38.1, 52.0, 66.9, 75.4, 85.4, 118.5, 126.3, 129.5, 130.1, 144.7, 166.3; MS (ESI) *m/z* = 287.3 $[M+H]^+$. **24b**: R_f = 0.70 (CHX/ EtOAc 8:2); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.42 (s, 3H), 2.65-2.70 (m, 2H), 3.63 (d, *J* = 4.1 Hz, 2H), 4.30 (td, *J* = 4.1, 2.1 Hz, 1H), 4.87 (dd, *J* = 9.0, 6.8 Hz, 1H), 5.40 (dd, *J* = 3.5, 2.3 Hz, 1H), 7.25 (d, *J* = 8.2 Hz, 2H), 7.88 (d, *J* = 8.2 Hz, 2H).^{13}C NMR (50 MHz, $CDCl_3$) δ: 21.8, 37.9, 52.4, 66.1, 75.8, 84.9, 117.8, 126.2, 129.4, 129.8, 144.8, 166.0; MS (ESI) *m/z* = 287.1 $[M+H]^+$.

(2′-deoxy-5′-azido-3′-(*O*-*p*-toluoyl)-*β*-(D)-ribofuranos-1′-carboxylic acid (1). To a solution of compound **24b** (715 mg, 2.5 mmol) in dioxane (15 mL) was added HCl_{conc} (3 mL). The reaction mixture is stirred at reflux during 4 h, then cooled to room temperature. After the addition of cold water (15 mL), NaOH 2M was added until pH 5 and the resulting mixture was washed with EtOAc (3 x 10 mL). Organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. Compound **1** was obtained as a colorless oil. Yield 755 mg (99%); R_f = 0.70 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.42 (s, 3H), 2.45-2.55 (m, 2H), 3.60-3.65 (d, *J* = 4.3 Hz, 2H), 3.70-3.75 (m, 1H), 4.25-4.40 (m, 1H), 4.78 (t, *J* = 8.0 Hz, 1H), 5.35-5.40 (m, 1H), 7.25 (d, *J* = 8.2 Hz, 2H), 7.90 (d, *J* = 8.2 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 21.8, 29.8, 36.4, 71.3, 72.4, 84.7, 126.6, 129.4, 129.9, 144.6, 160.0, 166.4; MS (ESI) *m/z* = 345.2 $[M+K]^+$.

Synthesis of C-nucleosides belonging to Series A

***C*-[2′-deoxy-5′-azido-3′-(*O*-*p*-toluoyl)-*β*-(D)-ribofuranos-1′-yl]-*N*-[3-(2-*N*-acétylaminothiazol-4-yl)]phenylformamide (2).** Compound **2** was obtained following general procedure C starting from compound **1** (734 mg, 2.4 mmol) and compound **S**. Flash column chromatography using a mixture CHX/EtOAc 6:4 as the eluent afforded pure compound **2** as a yellow solid. Yield 761 mg (61%); R_f = 0.44 (CHX/EtOAc 7:3); ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.17 (s, 3H), 2.40 (s, 3H), 2.48-2.53 (m, 2H), 3.60-3.65 (m, 2H), 4.30-4.40 (m, 1H), 4.78 (t, *J* = 7.9 Hz, 1H), 5.30-5.40 (m, 1H), 7.30-7.40 (m, 3H), 7.43 (m, 2H), 7.52 (s, 1H), 7.61 (d, *J* = 7.4 Hz, 1H), 7.91 (d, *J* = 8.2 Hz, 2H), 8.35 (s, 1H), 9.98 (s,

1H), 12.31 (s, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 21.2, 22.5, 35.4, 51.9, 76.4, 78.3, 83.7, 108.1, 117.2, 119.1, 121.2, 126.6, 129.0, 129.3, 129.4, 134.8, 138.7, 144.0, 148.5, 158.0, 165.2, 168.6, 168.9; MS (ESI) *m/z* = 521.1 $[M+H]^+$.

***C*-[2′-deoxy-5′-azido-*β*-(D)-ribofuranos-1′-yl]-*N*-[3-(2-*N*-acetylaminothiazol-4-yl)]phenylformamide (3).** To a suspension of compounds **2** (754 mg, 1.45 mmol) in a mixture CH_2Cl_2/MeOH 1:1, potassium carbonate (440.9 mg, 3.19 mmol, 2.2 eq.) was added. The reaction mixture was stirred at room temperature overnight, then the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography using a mixture CH_2Cl_2/MeOH 95:5 as the eluent affording compound **3** as a colorless solid. Yield 513 mg (88%); R_f = 0.53 (CH_2Cl_2/MeOH 97:3); ^{1}H NMR (200 MHz, CD_3OD) δ:, 2.16 (s, 3H), 2.30-2.40 (m, 2H), 3.25-3.35 (m, 1H), 3.60 (dd, *J* = 12.9, 4.0 Hz, 1H), 4.00-4.10 (m, 1H), 4.20-4.25 (m, 1H), 4.60-4.70 (m, 1H), 7.30-7.40 (m, 2H), 7.50 (d, *J* = 8.1 Hz, 1H), 7.60-7.70 (m, 1H), 7.80 (s, 1H), 8.16 (t, *J* = 1.8 Hz, 1H); ^{13}C NMR (50 MHz, CD_3OD) δ: 22.5, 55.2, 54.9, 72.0, 78.0, 85.9, 108.1, 117.2, 119.2, 121.1, 129.3, 134.8, 138.8, 148.6, 158.0, 168.7, 170.1; MS (ESI) *m/z* = 403.0 $[M+H]^+$.

***C*-[2′-deoxy-5′-amino-*β*-(D)-ribofuranos-1′-yl]-*N*-[3-(2-*N*-acétylaminothiazol-4-yl)]phenylformamide (4).** Compound **4** was obtained starting from compound **3** (482 mg, 1.20 mmol) following general procedure D as a colorless solid. Yield 433 mg (96%); ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.16 (s, 3H), 2.65-2.75 (m, 2H), 3.25-3.45 (m, 3H), 3.79 (q, *J* = 4.2 Hz, 1H), 4.10-4.20 (m, 1H), 4.51 (t, *J* = 7.2 Hz, 1H), 5.12 (m, 2H), 7.36 (d, *J* = 7.8 Hz, 1H), 7.50-7.60 (m, 3H), 8.28 (s, 1H), 11.0 (s, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 22.5, 42.1, 70.7, 77.5, 87.3, 108.0, 117.2, 119.2, 121.0, 129.3, 134.8, 139.0, 148.6, 157.9, 168.7, 172.4; HRMS (ESI) *m/z* = 377.1278 $[M+H]^+$ ($C_{17}H_{21}N_4O_4S$ requires 377.1278).

***C*-[2′-deoxy-5′-(*Nα*,*Nε*-di-*tert*-butylcarbonyl-(L)-lysinamide)-*β*-(D)-ribofuranos-1′-yl]-*N*-[3-(2-*N*-acetylaminothiazol-4-yl)]phenylformamide (5a).** Compound **5a** was obtained starting from compound **4** (169.0 mg, 0.45 mmol) and *Nα*,*Nε*-di-Boc-L-lysine after applying general procedure E affording desired compound **5a** as a colorless solid. Yield 206 mg (65%); R_f = 0.65 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (500 MHz, CD_3OD) δ: 1.37 (s, 9H), 1.41 (s, 9H), 1.40-1.50 (m, 4H), 1.55-1.60 (m, 1H), 1.70-1.75 (m, 1H), 2.15-2.20 (m, 1H), 2.22 (s, 3H), 2.25-2.35 (m, 1H), 2.90-3.00 (m, 2H), 3.40 (d, *J* = 11.5 Hz, 1H), 3.55 (dd, *J* = 14.0, 7.0 Hz, 1H), 4.00-4.05 (m, 2H), 4.15-4.25 (m, 1H), 4.64 (dd, *J* = 8.8, 6.9 Hz, 1H), 7.35-7.40 (m, 2H), 7.69 (t, *J* = 6.3 Hz, 1H), 8.32 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ: 22.6, 24.1, 28.0, 28.7, 28.8, 30.6, 40.6, 41.0, 42.9, 56.4, 79.6, 79.9, 80.6, 88.6, 109.0, 119.5, 121.3, 123.3, 130.1, 136.6, 139.4, 150.9, 157.8, 158.5, 159.4, 170.9, 173.3, 176.1; HRMS (ESI) *m/z* = 705.3275 $[M+H]^+$ ($C_{33}H_{49}N_9O_6S$ requires 705.3276).

***C*-[2′-deoxy-5′-(*Nα*-*tert*-butylcarbonyl-*N*,*N*-(di-benzyloxycarbonylguanidino)-(L)-argininamide)-*β*-(D)-ribofuranos-1′-yl]-*N*-[3-(2-*N*-acetylaminothiazol-4-yl)]phenylformamide (5b).** Compound **5b** was obtained starting from compound **4** (169.0 mg, 0.45 mmol) and *Nα*-Boc-*Nω*,*Nε*-di-Cbz-L-arginine after applying general procedure E affording desired compound **5b** as a colorless solid. Yield

292 mg (72%); R_f = 0.60 (DCM/MeOH 9:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.31 (s, 9H), 1.45-1.50 (m, 3H), 2.00-2.10 (m, 2H), 2.16 (s, 3H), 3.15-3.20 (m, 1H), 3.40-3.45 (m, 2H), 3.75-3.85 (m, 3H), 3.85-3.95 (m, 1H), 4.05-4.10 (m, 1H), 4.50 (dd, *J* = 8.7, 6.8 Hz, 1H), 5.02 (s, 2H), 5.18 (s, 2H), 6.85 (d, *J* = 7.9 Hz, 1H), 7.25-7.35 (m, 10H), 7.48 (s, 1H), 7.55-65 (m, 2H), 8.17 (t, *J* = 5.9 Hz, 1H), 8.42 (t, *J* = 1.7 Hz, 1H), 9.12 (br s, 2H), 9.75 (s, 1H), 12.27 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 22.4, 23.3, 25.1, 28.1, 29.3, 41.1, 44.4, 54.3, 66.1, 68.1, 72.0, 77.9, 78.0, 87.1, 107.9, 117.5, 119.5, 121.1, 127.7, 127.8, 127.9, 128.2, 128.3, 128.5, 128.9, 134.7, 135.3, 137.1, 138.8, 148.7, 154.9, 155.3, 157.9, 159.6, 162.9, 168.6, 170.8, 172.9; MS (ESI) *m/z* = 923.7 $[M+Na]^+$.

***C*-[2′-deoxy-5′-(*Nα*,*Nδ*-di-*tert*-butylcarbonyl-(L)-histdinamide)-*β*-(D)-ribofuranos-1′-yl]-*N*-[3-(2-*N*-acetylaminothiazol-4-yl)]phenylformamide (5c).** Compound **5c** was obtained starting from compound **4** (169.0 mg, 0.45 mmol) and *Nα*,*Nim*-di-Boc-L-histidine after applying general procedure E affording desired compound **5c** as a colorless solid. Yield 238 mg (74%); R_f = 0.58 (DCM/MeOH 9:1); ^{1}H NMR (200 MHz, CD_3OD) δ: 1.32 (s, 9H), 1.48 (s, 9H), 2.05-2.20 (m, 5H), 2.90-3.00 (m, 2H), 3.50-3.55 (m, 1H), 3.95-4.00 (m, 1H), 4.10-4.15 (m, 1H), 4.35-4.40 (m, 1H), 4.55-4.60 (m, 1H), 7.20-7.30 (m, 3H), 7.50-7.60 (m, 2H), 7.95 (s, 1H), 8.15 (s, 1H); ^{13}C NMR (50 MHz, CD_3OD) δ: 22.7, 25.5, 28.6, 30.8, 31.7, 40.5, 43.3, 55.8, 73.8, 79.8, 80.8, 87.0, 108.9, 116.5, 119.4, 121.0, 123.2, 130.0, 136.5, 138.3, 139.7, 147.9, 150.8, 157.6, 170.9, 173.3, 174.7; MS (ESI) *m/z* = 736.8 $[M+Na]^+$.

***C*-[2′-deoxy-5′-(*Nα*-(*tert*-butylcarbonyl)-(L)-argininamide)-*β*-(D)-ribofuranos-1′-yl]-*N*-[3-(2-*N*-acetylaminothiazol-4-yl)]phenylformamide (5b′).** To a solution of compound **5b** (172 mg, 0.191 mmol) in a mixture CH_2Cl_2/MeOH 1:1 (10 mL) Pd/C 20% was added. The reaction mixture was stirred under an hydrogen atmosphere overnight, then filtered on celite. Solvent was evaporated under reduced pressure and the crude product purified by silica gel column chromatography using a mixture CH_2Cl_2/MeOH 9:1 as the eluent. Desired product **5b′** was obtained as a colorless solid and employed directly in the following reaction without further purification. Yield 107 mg (89%); R_f = (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.34 (s, 9H), 1.51 (m, 3H), 1.63 (m, 1H), 2.08 (m, 2H), 2.16 (s, 3H), 3.05 (m, 2H), 3.85 (m, 1H), 3.94 (m, 1H), 4.08 (m, 1H), 4.53 (t, *J* = 8.7 Hz, 1H), 6.96 (d, *J* = 7.7 Hz, 1H), 7.27 (broad s, 3H), 7.34 (t, J = 7.9 Hz, 1H), 7.46 (s, 1H), 7.62 (m, 2H), 8.00 (m, 1H), 8.35 (m, 1H), 8.40 (s, 1H), 9.82 (s, 1H), 12.29 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 22.8, 23.7, 25.4, 28.5, 29.4, 42.0, 54.4, 71.5, 72.3, 78.3, 87.4, 108.3, 117.9, 119.9, 121.5, 139.3, 135.1, 139.2, 149.1, 155.8, 157.3, 158.3, 166.3, 169.0, 171.3, 173.2; MS (ESI) *m/z* = 633.3 $[M+H]^+$.

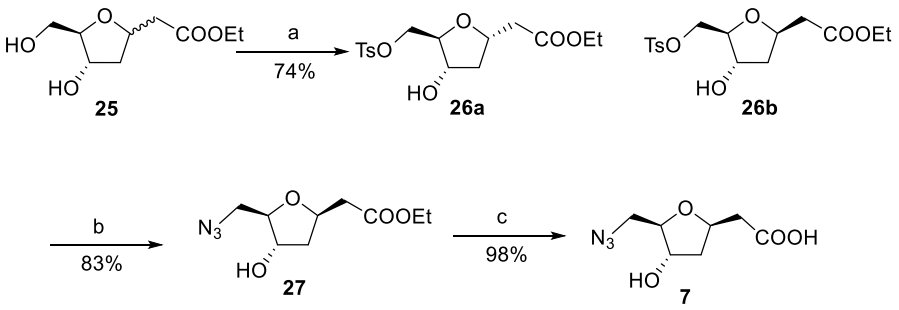
***C*-[2′-deoxy-5′-((L)-lysinamide)-*β*-(D)-ribofuranos-1′-yl]-*N*-[3-(2-*N*-acetylaminothiazol-4-yl)]phenylformamide (6a).** Deprotection of compound **5a** was obtained following general procedure F that led to desired compound **6a** as a colorless solid. Yield 12.0 mg (99%); Retention time 15.6 min; ^{1}H NMR (500 MHz, CD_3OD) δ: 1.50-1.55 (m, 2H), 1.65-1.70 (m, 2H), 1.85-1.95 (m, 2H), 2.23 (s, 3H), 2.25-2.30 (m, 1H), 2.32-2.37 (m, 1H), 2.85-2.90 (m, 2H), 3.35 (dd, *J* = 14.0, 3.5 Hz, 1H), 3.70 (dd, *J* = 14.0, 8.8 Hz, 1H), 3.90 (t, *J* = 6.6 Hz, 1H), 4.02 (dt, *J* = 8.7, 3.4 Hz, 1H), 4.18-4.22 (m, 1H), 4.67 (t, *J* = 7.5 Hz, 1H), 7.35-7.40 (m, 2H), 7.65-7.70 (m, 2H), 8.33 (t, *J* = 1.8 Hz, 1H); ^{13}C NMR (125 MHz,

CD_3OD) δ: 23.0, 23.2, 28.5, 32.7, 40.7, 43.9, 54.8, 74.2, 80.1, 88.8, 109.5, 117.5, 119.9, 121.8, 123.9, 130.5, 137.0, 139.8, 151.2, 159.9, 171.3, 171.7, 173.9; MS (ESI) *m/z* = 505.7 $[M+H]^+$.

***C*-[2′-deoxy-5′-((L)-argininamide)-*β*-(D)-ribofuranos-1′-yl]-*N*-[3-(2-*N*-acetylaminothiazol-4-yl)]phenylformamide (6b).** Deprotection of compound **5b′** was obtained following general procedure F that led to desired compound **6b** as a colorless solid. Yield 88.7 mg (98%); Retention time 15.7 min; ^1H NMR (500 MHz, CD_3OD) δ: 1.70-1.75 (m, 2H), 1.95-2.00 (m, 2H), 2.23 (s, 3H), 2.25-2.30 (m, 1H), 2.32-2.42 (m, 1H), 3.21 (t, *J* = 6.9 Hz, 2H), 3.37 (dd, *J* = 13.8, 3.3 Hz, 1H), 3.70 (dd, *J* = 14.0, 8.9 Hz, 1H), 3.98-4.05 (m, 2H), 4.21 (dt, *J* = 5.7, 3.7 Hz, 1H), 4.68 (t, *J* = 7.6 Hz, 1H), 7.35 (s, 1H), 7.37 (t, *J* = 7.9 Hz, 1H), 7.65-7.70 (m, 2H), 8.32 (t, J = 5.0 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ: 22.7, 25.2, 29.8, 40.2, 41.7, 43.6, 54.2, 73.8, 79.6, 88.3 109.1, 119.6, 121.5, 123.5, 136.5, 139.3, 150.7, 158.6, 159.4, 170.7, 170.9, 173.6; HRMS (ESI) *m/z* = 533.2271 $[M+H]^+$ ($C_{23}H_{33}N_5O_8S$ requires 533.2289).

***C*-[2′-deoxy-5′-((L)-histidinamide)-*β*-(D)-ribofuranos-1′-yl]-*N*-[3-(2-*N*-acetylaminothiazol-4-yl)]phenylformamide (6c).** Deprotection of compound **5c** was obtained following general procedure F that led to desired compound **6c** as a colorless solid. Yield 92.6 mg (95%); Retention time 15.9 min; ^1H NMR (500 MHz, CD_3OD) δ: 2.22 (s, 3H), 2.25-2.35 (m, 2H), 3.35-3.45 (m, 4H), 3.69 (dd, *J* = 14.0, 9.0 Hz, 1H), 3.90-3.95 (m, 1H), 4.12-4.18 (m, 1H), 4.29 (t, *J* = 6.8 Hz, 1H), 4.71 (t, *J* = 7.5 Hz, 1H), 7.33 (s, 1H), 7.35 (t, *J* = 8.0 Hz, 1H), 7.44 (s, 1H), 7.64 (dd, *J* = 22.4, 7.9 Hz, 2H), 8.28 (s, 1H), 8.77 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ: 22.6, 27.8, 40.1, 43.7, 53.5, 73.7, 79.6, 88.1, 109.1, 119.6, 119.7, 121.5, 123.5, 128.3, 130.1, 135.7, 136.6, 139.3, 150.4, 169.4, 170.9, 173.8; HRMS (ESI) *m/z* = 514.1869 $[M+H]^+$ ($C_{23}H_{28}N_7O_5S$ requires 514.1867).

Preparation of the modified 2´-desoxyribose scaffold 7.



Scheme S2. Synthesis of 2'-deoxyribose scaffold **7**. Reagents: a) TsCl, Pyr, r.t., 2h; b) NaN_3, DMF, 80°C, overnight; c) HCl 37%, dioxane, r.t., overnight.

2-[2′-deoxy-5′-*O*-tosyl-*α*-(D)-ribofuranos-1′-yl] ethyl acetate (26a) et 2-[2′-désoxy-5′-*O*-tosyl-*β*-(D)-ribofuranos-1′-yl] ethyl acetate (26b). Compounds **26a** and **26b** were obtained starting from compound **25** (2.29 g) and following general procedure A. Flash column chromatography using a mixture CHX/EtOAc 1:1 as the eluent afforded pure compounds **26a** α and **26b** β as colorless solids. Yield 2.97 g (74%); **26a**: R_f = 0.65 (CHX/EtOAc 6:4); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.22 (t, *J* = 7.2 Hz, 3H), 1.65-1.75 (m, 1H), 2.45 (s, 3H), 2.50-2.60 (m, 3H), 3.80-3.85 (m, 2H), 3.90-4.00 (m, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 4.30-4.35 (m, 1H), 4.50-4.60 (m, 1H), 7.31 (d, *J* = 8.2 Hz, 2H), 7.80 (d, *J* = 8.3 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 13.6, 21.7, 39.4, 40.5, 60.8, 69.1, 73.5, 74.4, 84.4, 128.3, 131.2, 132.5, 147.1, 171.7; MS (ESI) *m/z* = 358.9 $[M+H]^+$; **26b**: R_f = 0.55 (CHX/EtOAc 6:4); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.24 (t, *J* = 7.1 Hz, 3H), 1.65-1.75 (m, 1H), 1.90-2.00 (m, 1H), 2.44 (s, 3H), 2.47-2.50 (m, 2H), 3.80-3.90 (m, 2H), 4.00-4.05 (m, 1H), 4.12 (q, *J* = 7.2 Hz, 2H), 4.25-4.35 (m, 1H), 4.45-4.55 (m, 1H), 7.34 (d, *J* = 8.0 Hz, 2H), 7.78 (d, *J* = 8.3 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 14.3, 21.8, 40.3, 40.7, 60.8, 69.6, 73.6, 75.1, 84.0, 128.1, 130.1, 132.7, 145.2, 170.9; MS (ESI) *m/z* = 358.9 $[M+H]^+$.

2-[2′-désoxy-5′-azido-*β*-(D)-ribofuranos-1′-yl] ethyl acetate (27). Compound **27** was obtained starting from compound **26b** (2.86 g) following general procedure B. Flash column chromatography using a mixture CHX/EtOAc 6:4 as the eluent afforded compound **27** as a colorless solid. Yield 1.52 g (83%); R_f = 0.60 (CHX/EtOAc 6:4); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.25 (t, *J* = 7.1 Hz, 3H), 1.75-1.90 (m, 1H), 2.08 (ddd, *J* = 13.2, 5.7, 2.4 Hz, 1H), 2.40-2.45 (m, 1H), 2.60 (qd, *J* = 15.7, 6.5 Hz, 2H), 3.35 (ddd, *J* = 32.4, 12.9, 4.5 Hz, 2H), 3.90-3.95 (m, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 4.20-4.30 (m, 1H), 4.45-4.60 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 14.2, 40.4, 52.8, 60.8, 74.1, 75.1, 85.6, 171.1; MS (ESI) *m/z* = 252.8 $[M+Na]^+$.

2-[2′-désoxy-5′-azido-*β*-(D)-ribofuranos-1′-yl] acetic acid (7). Compound **27** (1.5 g, 6.55 mmol mmol) was stirred overnight in a mixture of dioxane (20 mL) and HCl 37% (5 mL) leading to desired carboxylic acid **7** as a colorless solid. Yield 1.29 g (98%); R_f = 0.60 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (200 MHz, CD_3OD) δ: 1.75-1.85 (m, 1H), 2.00-2.10 (m, 1H), 2.40-2.45 (m, 1H), 2.55-2.60 (m, 2H), 3.25-

3.35 (m, 3H), 3.80-3.90 (m, 1H), 4.10-4.30 (m, 1H), 4.40-4.55 (m, 1H); ^{13}C RMN (50 MHz, CD_3OD) δ: 41.2, 41.7, 53.9, 74.5, 76.6, 87.1, 174.8; MS (ESI) *m/z* = 202.3 $[M+H]^+$.

Synthesis of C-nucleosides belonging to Series B.

***C*-[2′-deoxy-5′-azido-*β*-(D)-ribofuranos-1′-yl]-*N*-[3-(acethylaminothiazole-4-yl)]phenylacetamide (8).** Compound **8** was obtained starting from compounds **7** (301 mg, 1.5 mmol) and **S** following general procedure C. Flash column chromatography using a mixture CHX/EtOAc 7:3 as the eluent afforded compound **8** as a white solid. Yield 362 mg (58%); R_f = 0.55 (CHX/EtOAc 7:3); ^{1}H NMR (500 MHz, CD_3OD) δ: 1.90-2.00 (m, 2H), 2.17 (s, 3H), 2.20-2.30 (m, 1H), 2.65-2.75 (m, 3H), 3.43 (dd, *J* = 13.0, 4.0 Hz, 2H), 4.00-4.10 (m, 1H), 4.25-4.35 (m, 1H), 4.55-4.65 (m, 1H), 7.14 (s, 1H), 7.40-7.50 (m, 2H), 8.07 (s, 1H), 8.21 (s, 1H), 9.95 (br s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ: 22.5, 41.6, 44.0, 74.3, 76.9, 87.1, 108.2, 119.0, 120.7, 122.9, 130.0, 136.6, 139.9, 150.7, 159.2, 170.7, 171.3; MS (ESI) *m/z* = 417.1 $[M+H]^+$.

***C*-[2′-désoxy-5′-amino-*β*-(D)-ribofuranos-1′-yl]-*N*-[3-(acethylaminothiazole-4-yl)]phenylacetamide (9).** Compound **9** was obtained starting from compound **8** (312 mg, 0.75 mmol) and following general procedure D as a colorless solid. Yield 275 mg (94%); ^{1}H NMR (200 MHz, CD_3OD) δ: 1.85-1.95 (m, 1H), 2.00-2.10 (m, 1H), 2.22 (s, 3H), 2.60-2.70 (m, 3H), 3.35 (s, 2H), 3.75-3.85 (m, 1H), 4.10-4.20 (m, 1H), 4.50-4.60 (m, 1H), 7.25-7.35 (m, 2H), 7.45-7.50 (m, 1H), 7.60-7.70 (m, 1H), 8.14 (s, 1H); ^{13}C NMR (50 MHz, CD_3OD) δ: 22.6, 41.5, 44.1, 45.1, 74.6, 76.8, 88.1, 108.9, 118.9, 120.7, 122.9, 130.0, 136.6, 140.0, 150.8, 159.4, 170.9, 171.8; HRMS (ESI) *m/z* = 391.1437 $[M+H]^+$ ($C_{18}H_{23}N_4O_4S$ requires 391.1434).

***C*-[2′-deoxy-5′-(*Nα*,*Nε*-di-*tert*-butylcarbonyl)-(L)-lysinamide)-*β*-(D)-ribofuranos-1′-yl]-*N*-[3-(acetylaminothiazole-4-yl)]phenylacetamide (10a).** Compound **10a** was obtained starting from compound **9** (175 mg, 0.45 mmol) and *Nα*,*Nε*-di-Boc-L-lysine after applying general procedure E affording desired compound **10a** as a colorless solid. Yield 242 mg (75%); R_f = 0.67 (DCM/MeOH 9:1); ^{1}H NMR (500 MHz, CD_3OD) δ: 1.30-1.40 (m, 4H), 1.42 (s, 18H), 1.50-1.60 (m, 1H), 1.65-1.75 (m, 1H), 1.90-1.95 (m, 1H), 2.00-2.10 (m, 1H), 2.23 (s, 3H), 2.60-2.65 (m, 2H), 2.90-3.00 (m, 2H), 3.35-3.40 (m, 2H), 3.80-3.90 (m, 1H), 3.95-4.00 (m, 1H), 4.10-4.20 (m, 1H), 4.50-4.60 (m, 1H), 7.30-7.40 (m, 2H), 7.49 (d, *J* = 7.5 Hz, 1H), 7.63 (d, J = 5.0 Hz, 1H), 8.15 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ: 23.1, 25.5, 29.7, 29.9, 31.0, 32.5, 40.7, 42.0, 45.7, 56.8, 75.4, 81.1, 81.6, 88.6, 109.4, 120.1, 122.4, 125.2, 131.3, 139.0, 139.7, 152.1, 157.8, 159.1, 159.8, 170.2, 174.8, 176.6; MS (ESI) *m/z* = 741.5 $[M+Na]^+$.

***C*-[2′-deoxy-5′-(*Nα*-*tert*-butylcarbonyl-*N*,*N*-(di-benzyloxycarbonylguanidino)-(L)-argininamide)-*β*-(D)-ribofuranos-1′-yl]-*N*-[3-(acethylaminothiazole-4-yl)]phenylacetamide (10b).** Compound **10b** was obtained starting from compound **9** (175 mg, 0.45 mmol) and *Nα*-Boc-*Nω*,*Nε*-di-Cbz-L-arginine after applying general procedure E affording desired compound **10b** as a colorless solid. Yield 288 mg (70%); R_f = 0.64 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (500 MHz, CD_3OD) δ: 1.40 (s, 9H), 1.50-1.65 (m, 4H),

1.80-1.90 (m, 1H), 2.00-2.05 (m, 1H), 2.21 (s, 3H), 2.55-2.65 (m, 2H), 3.25-2.30 (m, 2H), 3.80-3.85 (m, 3H), 3.95-4.00 (m, 1H), 4.05-4.10 (m, 1H), 4.45-4.55 (m, 1H), 5.08 (s, 2H), 5.20 (s, 2H), 7.25-7.40 (m, 12H), 7.47 (d, *J* = 7.3 Hz, 1H), 7.62 (d, *J* = 7.4 Hz, 1H), 7.98 (s, 1H), 8.16 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ: 23.0, 23.9, 26.7, 29.1, 32.0, 37.3, 41.8, 43.1, 46.0, 55.2, 56.4, 68.7, 70.3, 75.2, 77.1, 81.1, 87.3, 109.4, 119.3, 121.1, 123.4, 129.4, 129.8, 129.9, 137.0, 138.8, 165.3, 171.3, 172.0; MS (ESI) *m/z* = 937.6 $[M+Na]^+$.

***C*-[2′-deoxy-5′-(*Nα*,*Nδ*-di-*tert*-butylcarbonyl-(L)-histdinamide)-*β*-(D)-ribofuranos-1′-yl]-*N*-[3-(acetylaminothiazole-4-yl)]phenylacetamide (10c).** Compound **10c** was obtained starting from compound **9** (175 mg, 0.45 mmol) and *Nα*,*Nim*-di-Boc-L-histidine after applying general procedure E affording desired compound **10c** as a colorless solid. Yield 229 mg (70%); R_f = 0.57 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (500 MHz, CD_3OD) δ: 1.36 (s, 9H), 1.58 (s, 9H), 1.80-1.90 (m, 1H), 2.00-2.05 (m, 1H), 2.21 (s, 3H), 2.60-2.65 (m, 2H), 2.75-2.80 (m, 1H), 2.90-3.00 (m, 1H), 3.33-3.37 (m, 2H), 3.75-3.85 (m, 1H), 4.10-4.15 (m, 1H), 4.30-4.35 (m, 1H), 4.50-4.60 (m, 1H), 7.20 (s, 1H), 7.30-7.40 (m, 2H), 7.48 (d, *J* = 7.6 Hz, 1H), 7.60 (d, *J* = 7.5 Hz, 1H), 8.01 (s, 1H), 8.06 (s, 1H), 8.12 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ: 22.7, 28.0, 28.7, 40.5, 43.3, 55.8, 73.8, 79.8, 80.8, 87.0, 108.9, 116.5, 119.4, 121.0, 123.2, 130.0, 136.5, 138.3, 139.7, 147.9, 150.8, 157.6, 170.9, 173.3, 174.7; MS (ESI) *m/z* = 750.6 $[M+Na]^+$

***C*-[2′-deoxy-5′-(*Nα*-(*tert*-butylcarbonyl)-(L)-argininamide)-β-(D)-ribofuranos-1′-yl]-*N*-[3-(acethylaminothiazole-4-yl)]phenylacetamide (10b′).** To a solution of compound **10b** (0.16 mmol) in a mixture CH_2Cl_2/MeOH 1:1 (10 mL) Pd/C 20% was added. The reaction mixture is then stirred under an hydrogen atmosphere overnight, then filtered on celite. Solvent was evaporated under reduced pressure and the crude production purified by silica gel column chromatography using a mixture CH_2Cl_2/MeOH 9:1 as the eluent. Desired product **10b′** was obtained as a white solid and employed directly in the following reaction without further purification. Yield 185 mg (91%); R_f = 0.15 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (500 MHz, CD_3OD) δ: 1.40 (s, 9H), 1.54 (m, 4H), 1.85 (m, 1H), 2.05 (m, 1H), 2.22 (s, 3H), 2.62 (m, 2H), 3.29 (m, 2H), 3.78 (m, 3H), 3.92 (m, 1H), 4.14 (m, 1H), 4.54 (m, 1H), 7.31 (m, 12H), 7.47 (d, *J* = 7.2 Hz, 1H), 7.62 (d, *J* = 7.1 Hz, 1H), 8.18 (s, 1H); MS (ESI) *m/z* = 646.8 $[M+H]^+$.

***C*-[2′-deoxy-5′-((L)-lysinamide)-*β*-(D)-ribofuranos-1′-yl]-*N*-[3-(acethylaminothiazole-4-yl)]phenylacetamide (11a).** Deprotection of compound **10a** was obtained following general procedure F that led to desired compound **11a** as a colorless solid. Yield 12.0 mg (97%); Retention time 16.0 min; ^{1}H NMR (500 MHz, CD_3OD) δ: 1.35-1.40 (m, 2H), 1.55-1.60 (m, 2H), 1.75-1.85 (m, 2H), 1.90-1.95 (m, 1H), 2.09 (ddd, *J* = 13.2, 5.8, 2.3 Hz, 1H), 2.23 (s, 3H), 2.60-2.70 (m, 2H), 2.87 (t, *J* = 7.7 Hz, 2H), 3.35-3.45 (m, 2H), 3.80-3.85 (m, 2H), 4.10-4.15 (m, 1H), 4.55-4.65 (m, 1H), 7.30-7.35 (m, 2H), 7.50 (ddd, *J* = 8.1, 2.0, 0.9 Hz, 1H), 7.65-7.75 (m, 1H), 8.13 (t, *J* = 5.0 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ: 23.0, 23.1, 28.4, 32.4, 40.7, 41.6, 44.0, 44.6, 54.7, 75.2, 77.1, 86.7, 109.4, 119.3, 121.1, 123.5, 130.6, 137.1, 140.5, 151.2, 159.9, 170.7, 171.2, 172.3; MS (ESI) *m/z* = 519.5 $[M+H]^+$.

***C*-[2′-deoxy-5′-((L)-argininamide)-*β*-(D)-ribofuranos-1′-yl]-*N*-[3-(acethylaminothiazole-4-yl)]phenylacetamide (11b).** Deprotection of compound **10b′** was obtained following general procedure F that led to desired compound **11b** as a colorless solid. Yield 135 mg (93%); Retention time 16.0 min; ^{1}H NMR (500 MHz, CD_3OD) δ: 1.60-1.65 (m, 2H), 1.80-1.85 (m, 2H), 1.90-1.95 (m, 1H), 2.05-2.10 (m, 1H), 2.22 (s, 3H), 2.60-2.70 (m, 2H), 3.05-3.10 (m, 2H), 3.35-3.45 (m, 3H), 3.80-3.90 (m, 2H), 4.10-4.15 (m, 1H), 4.55-4.60 (m, 1H), 7.30-7.35 (m, 2H), 7.48 (d, *J* = 8.6 Hz, 1H), 7.65 (d, *J* = 7.8 Hz, 1H), 8.13 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ: 23.0, 25.6, 30.1, 41.7, 42.1, 44.0, 44.6, 54.5, 75.1, 77.1, 86.7, 109.4, 119.4, 121.2, 123.5, 130.6, 137.1, 140.4, 151.2, 159.1, 159.9, 170.4, 171.2, 172.3; HRMS (ESI) *m/z* = 547.2447 $[M+H]^+$ ($C_{24}H_{35}N_8O_5S$ requires 547.2446).

***C*-[2′-deoxy-5′-((L)-histdinamide)-*β*-(D)-ribofuranos-1′-yl]-*N*-[3-(acethylaminothiazole-4-yl)]phenylacetamide (11c).** Deprotection of compound **10c** was obtained following general procedure F that led to desired compound **11c** as a colorless solid. Yield 103 mg (98%); Retention time 15.9 min; ^{1}H NMR (500 MHz, CD_3OD) δ: 1.85-1.95 (m, 1H), 2.08 (ddd, *J* = 13.1, 5.7, 2.3 Hz, 1H), 2.22 (s, 3H), 2.67 (ddd, *J* = 22.5, 14.4, 6.3 Hz, 2H), 3.15-3.25 (m, 2H), 3.30-3.35 (m, 1H), 3.40-3.45 (m, 1H), 3.75-3.85 (m, 1H), 4.05-4.15 (m, 2H), 4.55-4.65 (m, 1H), 7.25-7.30 (m, 2H), 7.35 (s, 1H), 7.46 (dd, *J* = 4.4, 3.6 Hz, 1H), 7.61 (d, *J* = 7.7 Hz, 1H), 8.09 (s, 1H), 8.73 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ: 23.0, 28.3, 41.6, 44.0, 44.5, 54.1, 75.1, 77.3, 86.6, 109.4, 119.3, 119.9, 121.1, 123.5, 128.9, 130.5, 136.2, 140.4, 151.1, 159.9, 169.2, 171.3, 172.3; HRMS (ESI) *m/z* = 528.2026 $[M+H]^+$ ($C_{24}H_{30}N_7O_5S$ requires 528.2024).

Synthesis of C-nucleosides belonging to Series C.

***C*-[2′-désoxy-5′-azido-3′-(*O*-*p*-toluoyl)-*β*-(D)-ribofuranos-1′-yl]-2-*N*-[*O*-(triisopropylsilyl)-4-(phenylureido)-phenol]-carboxamide (13).** Compound **13** was obtained starting from compounds **1** and **12** following general procedure C. Flash column chromatography using a mixture CHX/EtOAc 8:2 as the eluent afforded desired compound **13** as a slight yellow solid: yield 700.3 mg (68%); R_f = 0.80 (CHX/EtOAc 7:3); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.13 (t, J = 6.0 Hz, 18H), 1.20-1.30 (m, 3H), 2.30-2.40 (m, 4H), 2.68 (dd, *J* = 13.8, 6.0 Hz, 1H), 3.71 (d, J = 4.0 Hz, 2H), 4.30-4.40 (m, 1H), 4.78 (dd, *J* = 10.7, 5.9 Hz, 1H), 5.33 (d, J = 6.0 Hz, 1H), 6.85 (d, *J* = 8.8 Hz, 1H), 7.20-7.25 (m, 4H), 7.40-7.60 (m, 4H), 7.95 (t, J = 8.0 Hz, 1H), 8.45 (d, J = 2.0 Hz, 1H), 8.89 (s, 1H), 9.07 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 13.0, 17.9, 21.7, 26.9, 36.6, 52.4, 79.0, 84.8, 113.5, 117.2, 117.5, 119.9, 126.5, 127.8, 128.9, 129.2, 129.7, 132.1, 138.8, 141.4, 144.4, 154.1, 166.2, 169.2; MS (ESI) *m/z* = 708.9 $[M+Na]^+$.

***C*-[2′-deoxy-5′-azido-3′-(*O*-*p*-toluoyl)-*β*-(D)-ribofuranos-1′-yl]-2-*N*-[4-(phenylureido)-phénol]-carboxamide (14).** To a solution of compound **13** (0.12 mmol) in THF (10 mL) was added a 1M solution of tetrabutylammonium fluoride in THF (0.14 mmol, 1.2 eq.). The reaction mixture was stirred 1 h at room temperature then the solvent was concentrated under reduced pressure and the crude product purified by flash column chromatography using a mixture CH_2Cl_2/MeOH 95:5 as the eluent leading to desired compound **14** as a slight yellow solid: yield 42.9 mg (87%); R_f = 0.78 (CH_2Cl_2/MeOH 97:3); ^{1}H

NMR (200 MHz, DMSO-d_6) δ: 2.39 (s, 3H), 2.40-2.50 (m, 2H), 3.70 (d, *J* = 5.0 Hz, 2H), 4.35-4.40 (m, 1H), 4.81 (dd, *J* = 9.3, 7.0 Hz, 1H), 5.30-5.35 (m, 1H), 6.87 (d, *J* = 8.7 Hz, 1H), 7.22 (dd, *J* = 8.0, 2.2 Hz, 1H), 7.36 (d, J = 8.0 Hz, 2H), 7.50-7.65 (m, 3H), 7.90-8.05 (m, 4H), 8.29 (d, J = 4.0, 1H), 9.15 (s, 1H), 10.1 (s, 1H), 10.6 (s, 1H), 11.0 (s, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ: 21.2, 35.8, 52.1, 76.1, 78.3, 83.6, 112.6, 114.7, 116.4, 125.8, 126.6, 128.3, 128.6, 129.0, 129.3, 129.5, 132.4, 143.3, 144.1, 151.1, 165.3, 168.7, 169.0; MS (ESI) *m/z* = 553.9 $[M+Na]^+$.

2-*C*-[2′-désoxy-5′-azido-3′-(*O*-*p*-toluoyl)-*β*-(D)-ribofuranos-1′-yl]-5-(phenylureido)-benzoxazole (15). To a solution of compound **14** (0.104 mmol) in dry THF (10 mL) at 0°C were added sequentially triphenylphosphine (0.52 mmol, 5 eq.) and diisopropylazodicarboxylate (0.52 mmol, 5 eq.). The reaction mixture was stirred at room temperature during 4 h, then neutralized using a mixture AcOH/MeOH 1:1 (1 mL). Solvent was then evaporated under reduced pressure and the resulting oil was purified by flash column chromatography using a mixture CHX/EtOAc 7:3 as the eluent leading to desired compound **15** as a colorless solid: yield 41.5 mg (78%); R_f = 0.62 (CHX/EtOAc 7:3); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.37 (s, 3H), 2.66 (ddd, *J* = 13.8, 6.2, 1.6 Hz, 1H), 2.88 (ddd, *J* = 14.0, 9.6, 6.2 Hz, 1H), 3.55-3.65 (m, 2H), 4.41 (ddd, *J* = 6.2, 4.1, 2.2 Hz, 1H), 5.40-5.45 (m, 2H), 6.97 (t, *J* = 7.3 Hz, 1H), 7.29 (t, *J* = 7.9 Hz, 2H), 7.30-7.40 (m, 3H), 7.46 (d, *J* = 13.8 Hz, 2H), 7.65 (d, *J* = 8.8 Hz, 1H), 7.95 (d, J = 13.8 Hz, 2H), 7.98 (d, J = 2.0 Hz, 1H), 8.69 (s, 1H), 8.83 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 21.2, 36.1, 52.0, 73.4, 76.3, 83.9, 109.2, 110.9, 117.2, 118.3, 121.8, 126.6, 128.8, 129.3, 129.5, 136.7, 139.7, 140.6, 144.0, 145.8, 152.7, 164.4, 165.3; MS (ESI) *m/z* = 535.2 $[M+Na]^+$.

2-*C*-[2′-deoxy-5′-azido-*β*-(D)-ribofuranos-1′-yl]-5-(phenylureido)-benzoxazole (16). To a suspension of compounds **15** (1.45 mmol) in a mixture CH_2Cl_2/MeOH 1:1, was added potassium carbonate (440.9 mg, 3.19 mmol, 2.2 eq.). The reaction mixture was stirred at room temperature overnight, then the solvent was evaporated under reduced pressure. The crude product was then purified by flash column chromatography using a mixture CH_2Cl_2/MeOH 9:1 as the eluent afforded pure compound **16** as a colorless solid: yield 45.5 mg (77%); R_f = 0.35 (CH_2Cl_2/MeOH 95:5); ^{1}H NMR (200 MHz, CD_3OD) δ: 2.36 (m, 1H), 2.61 (ddd, *J* = 13.4, 8.6, 5.9 Hz, 1H), 3.42 (d, *J* = 5.1 Hz, 2H), 4.09 (td, *J* = 5.1, 3.0 Hz, 1H), 4.40 (dt, *J* = 5.9, 3.0 Hz, 1H), 5.35 (dd, *J* = 8.6, 6.8 Hz, 1H), 7.00 (m, 1H), 7.37 (m, 6H), 7.90 (d, *J* = 1.8 Hz, 1H); ^{13}C NMR (50 MHz, CD_3OD) δ: 40.0, 53.8, 74.2, 74.8, 87.8, 111.6, 111.7, 119.5, 120.5, 123.9, 129.9, 137.8, 140.4, 142.0 148.2, 155.6, 167.3; MS (ESI) *m/z* = 417.3 $[M+Na]^+$, 811.4 $[2M+Na]^+$.

2-*C*-[2′-deoxy-5′-amino-*β*-(D)-ribofuranos-1′-yl]-5-(phenylureido)-benzoxazole (17). General procedure D was applied to compound **16** (0.75 mmol) leading to desired compounds **17** as a colorless solid: yield 242.9 mg (88%); ^{1}H RMN (200 MHz, CD_3OD) δ: 2.48 (dd, *J* = 7.6, 4.3 Hz, 2H), 3.60 (dd, *J* = 13.4, 5.1 Hz, 1H), 4.20-4.30 (m, 1H), 4.40-4.50 (m, 2H), 5.45 (t, *J* = 7.6 Hz, 1H), 6.95-7.05 (m, 3H), 7.40-7.50 (m, 7H), 7.98 (s, 1H); ^{13}C NMR (50 MHz, CD_3OD) δ: 40.3, 43.0, 74.2, 74.5, 84.9, 111.3, 111.8, 120.4, 123.7, 129.8, 132.9, 138.0, 140.4, 141.7, 148.0, 155.6, 168.2; HRMS (ESI) *m/z* = 369.1560 $[M+H]^+$ ($C_{19}H_{21}N_4O_4$ requires 369.1557).

2-*C*-[2′-deoxy-5′-(*Nα*,*Nε*-di-*tert*-butylcarbonyl-(L)-lysinamide)-*β*-(D)-ribofuranos-1′-yl]-5-(phenylureido)-benzoxazole (18a). Compound **18a** was obtained starting from compound **17** (65.6 mg, 0.45 mmol) and *Nα*,*Nε*-di-Boc-L-lysine after applying general procedure E. Flash column chromatography using a mixture CH_2Cl_2/MeOH 95:5 as the eluent afforded desired compound **18a** as a colorless solid (Yield 213.1 mg 68%). R_f = 0.70 (DCM/MeOH 9:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.32 (m, 4H), 1.35 (2s, 18H), 1.48 (m, 2H), 2.23 (m, 1H), 2.46 (m, 1H), 2.87 (m, 2H), 3.16 (m, 1H), 3.32 (m, 1H), 3.87 (m, 2H), 4.21 (m, 1H), 5.24 (dd, *J* = 9.8, 5.7 Hz, 1H), 6.75 (m, 2H), 6.97 (t, *J* = 7.4 Hz, 1H), 7.28 (t, *J* = 8.9 Hz, 2H), 7.37 (dd, *J* = 8.8, 2.1 Hz, 1H), 7.46 (d, *J* = 7.6 Hz, 2H), 7.63 (d, *J* = 8.8 Hz, 1H), 7.85 (t, *J* = 5.6 Hz, 1H), 7.94 (d, *J* = 2.1 Hz, 1H), 8.31 (s, 1H), 8.70 (s, 1H), 8.84 (s, 1H); ^{13}C RMN (125 MHz, DMSO-d_6) δ: 23.2, 28.6, 29.6, 32.1, 38.9, 41.7, 54.7, 72.8, 73.1, 78.3, 86.4, 109.5, 111.2, 117.3, 118.6, 112.2, 129.1, 137.2, 140.1, 141.1, 146.1, 153.1, 155.7, 155.9, 166.1, 172.8; MS (ESI) *m/z* = 718.4 $[M+Na]^+$.

2-*C*-[2′-deoxy-5′-(*Nα*-*tert*-butylcarbonyl-*N*,*N*-(di-benzyloxycarbonylguanidino)-(L)-argininamide)-*β*-(D)-ribofuranos-1′-yl]-5-(phenylureido)-benzoxazole (18b). Compound **18b** was obtained starting from compound **17** (65.6 mg, 0.45 mmol) and *Nα*-Boc-*Nω*,*Nε*-di-Cbz-L-arginine after applying general procedure E affording desired compound **18b** as a colorless solid (Yield 272.8 mg, 68%); R_f = 0.67 (DCM/MeOH 9:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.33 (s, 9H), 1.50-1.60 (m, 4H), 2.20-2.30 (m, 1H), 2.40-2.50 (m, 1H), 3.05-3.15 (m, 1H), 3.25-3.35 (m, 1H), 3.80-3.95 (m, 4H), 4.20-4.25 (m, 1H), 5.05 (s, 2H), 5.23 (s, 2H), 5.26 (dd, *J* = 6.2, 2.9 Hz, 1H), 6.81 (d, *J* = 8.6 Hz, 1H), 6.98 (t, *J* = 7.4 Hz, 1H), 7.25-7.45 (m, 14H), 7.62 (d, *J* = 8.8 Hz, 1H), 7.89 (t, *J* = 5.8 Hz, 1H), 7.96 (d, *J* = 2.0 Hz, 1H), 8.69 (s, 1H), 8.83 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 21.9, 22.0, 25.5, 28.5, 29.7, 38.9, 41.8, 44.8, 54.5, 66.5, 68.5, 72.8, 73.0, 78.4, 86.4, 109.5, 111.2, 117.3, 118.4, 118.6, 122.2, 128.1, 128.2, 128.6, 128.9, 129.1, 135.7, 137.1, 137.4, 140.1, 141.1, 146.1, 153.1, 155.3, 160.0, 163.3, 166.0, 172.5; MS (ESI) *m/z* = 915.4 $[M+Na]^+$.

2-*C*-[2′-deoxy-5′-(*Nα*,*Nδ*-di-*tert*-butylcarbonyl-(L)-histdinamide)-*β*-(D)-ribofuranos-1′-yl]-5-(phenylureido)-benzoxazole (18c). Compound **18c** was obtained starting from compound **17** and *Nα*,*Nim*-di-Boc-L-histidine after applying general procedure E affording desired compound **18c** as a colorless solid (Yield 238.0 mg, 75%); R_f = 0.60 (DCM/MeOH 9:1); ^{1}H NMR (500 MHz, CD_3OD) δ: 1.34 (s, 9H), 1.58 (s, 9H), 2.25-2.35 (m, 1H), 2.40-2.45 (m, 1H), 2.80-2.90 (m, 1H), 3.00 (dd, *J* = 10.7, 6.0 Hz, 1H), 3.40-3.45 (m, 2H), 4.00-4.10 (m, 1H), 4.29 (dt, *J* = 5.8, 2.9 Hz, 1H), 4.34 (dd, *J* = 15.4, 7.7 Hz, 1H), 5.32 (dd, *J* = 8.7, 6.8 Hz, 1H), 7.00-7.10 (m, 1H), 7.25-7.30 (m, 2H), 7.35-7.40 (m, 1H), 7.43 (d, J = 9.6 Hz, 1H), 7.54 (d, J = 4.8 Hz, 1H), 7.93 (s, 1H), 8.06 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ: 28.4, 29.0, 32.0, 40.9, 43.0, 56.3, 74.5, 74.9, 79.9, 81.1, 87.4, 87.9, 112.0, 112.2, 116.6, 119.8, 120.7, 120.9, 124.3, 130.2, 130.3, 138.3, 138.7, 140.9, 142.4, 148.5, 155.9, 158.0, 168.4, 173.7, 174.7; MS (ESI) *m/z* = 728.5 $[M+Na]^+$.

2-*C*-[2′-deoxy-5′-(*Nα*-(*tert*-butylcarbonyl)-(L)-argininamide)-*β*-(D)-ribofuranos-1′-yl]-5-(phenylureido)-benzoxazole (18b′). To a solution of compound **18b** (142.6 mg, 0.16 mmol) in a

mixture CH_2Cl_2/MeOH 1:1 (10 mL) Pd/C 20% was added. The reaction mixture is then stirred under an hydrogen atmosphere overnight, then filtered on celite. Solvent was evaporated under reduced pressure and the crude product purified by silica gel column chromatography using a mixture CH_2Cl_2/MeOH 9:1 as the eluent. Desired product **18b′** was obtained as a colorless solid. Yield 92.7 mg (93%).; R_f = (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (500 MHz, CD_3OD) δ: 1.37 (s, 9H), 1.60-1.70 (m, 4H), 2.35-2.40 (m, 1H), 2.45-2.50 (m, 1H), 3.10-3.15 (m, 2H), 3.45-3.50 (m, 2H), 4.00-4.10 (m, 2H), 4.30-4.35 (m, 1H), 5.35 (dd, *J* = 8.4, 7.0 Hz, 1H), 6.85 (d, *J* = 8.6 Hz, 1H), 7.02 (t, *J* = 7.4 Hz, 1H), 7.25-7.30 (m, 1H), 7.34 (dd, *J* = 8.7, 1.6 Hz, 1H), 7.40-7.45 (m, 2H), 7.55 (d, *J* = 8.8 Hz, 1H), 7.98 (d, *J* = 1.7 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ: 19.2, 22.1, 26.0, 28.5, 30.6, 40.2, 41.8, 42.6, 55.7, 74.4, 74.6, 79.4, 80.7, 87.5, 111.7, 111.9, 119.4, 120.8, 123.4, 129.8, 137.8, 140.5, 141.8, 148.0, 155.4, 168.3; MS (ESI) *m/z* = 625.8 $[M+H]^+$.

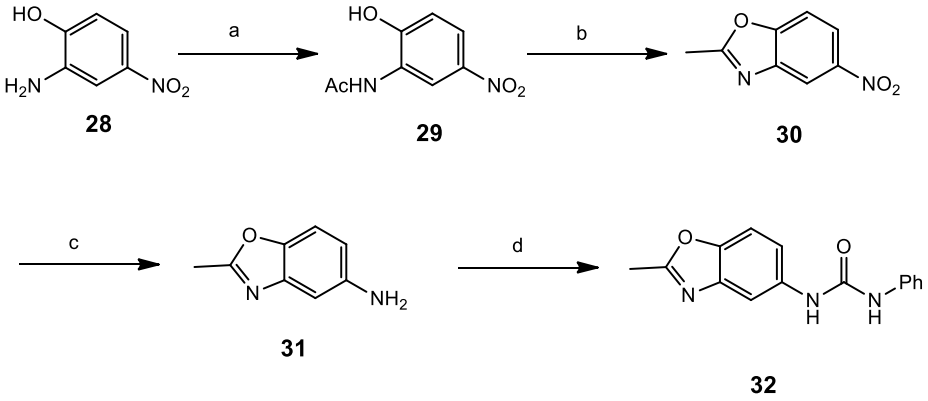
2-*C*-[2′-deoxy-5′-((L)-lysinamide)-*β*-(D)-ribofuranos-1′-yl]-5-(phenylureido)-benzoxazole (19a). Deprotection of compound **18a** was obtained following general procedure F that led to desired compound **19a** as a colorless solid. Yield 10.8 mg (91%); Retention time 12.5 min; ^{1}H NMR (500 MHz, CD_3OD) δ: 1.30-1.35 (m, 2H), 1.50-1.55 (m, 2H), 1.60-1.70 (m, 2H), 2.10-2.20 (m, 2H), 2.70-2.75 (m, 2H), 3.20-3.30 (m, 2H), 3.70-3.75 (m, H), 3.80-3.90 (m, 1H), 4.00-4.10 (m, 1H), 5.34 (dd, *J* = 8.4, 7.0 Hz, 1H), 6.79 (d, *J* = 8.7 Hz, 1H), 6.91 (t, *J* = 7.3 Hz, 1H), 7.04 (dd, *J* = 8.7, 2.5 Hz, 1H), 7.23 (t, *J* = 7.9 Hz, 2H), 7.41 (d, *J* = 7.7 Hz, 2H), 8.06 (d, *J* = 2.5 z, 1H), 8.74 (s, 1H), 8.81 (s, *J* = 7.7 Hz, 1H), 9.07 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ: 23.1, 23.5, 29.6, 31.7, 39.2, 42.2, 56.8, 73.5, 73.8, 86.2, 110.2, 111.5, 116.1, 119.2, 112.1, 130.7, 137.3, 140.1, 142.7, 148.1, 153.9, 156.5, 167.2, 172.8; MS (ESI) *m/z* = 497.1 $[M+H]^+$.

2-*C*-[2′-deoxy-5′-((L)-argininamide)-*β*-(D)-ribofuranos-1′-yl]-5-(phenylureido)-benzoxazole (19b). Deprotection of compound **18b′** was obtained following general procedure F that led to desired compound **19b** as a colorless solid. Yield (94%); Retention time 11.9 min; ^{1}H NMR (500 MHz, CD_3OD) δ: 1.40-1.50 (m, 2H), 1.60-1.70 (m, 2H), 2.30-2.35 (m, 1H), 2.50-2.55 (m, 1H), 3.10-3.10 (m, 2H), 3.40-3.50 (m, 2H), 4.10-4.20 (m, 2H), 4.35-4.40 (m, 1H), 5.36 (dd, *J* = 8.3, 7.2 Hz, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 7.01 (t, *J* = 7.2 Hz, 1H), 7.20-7.30 (m, 1H), 7.35 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.40-7.50 (m, 2H), 7.57 (d, *J* = 8.6 Hz, 1H), 8.01 (d, *J* = 1.9 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ: 19.3, 21.9, 26.3, 29.9, 41.1, 41.9, 42.5, 56.8, 74.4, 74.8, 81.5, 89.2, 111.8, 112.0, 112.5, 121.3, 123.4, 130.2, 138.6, 140.5, 140.9, 148.5, 169.2; MS (ESI) *m/z* = 525.6 $[M+H]^+$.

2-*C*-[2′-deoxy-5′-((L)-histidinamide)-*β*-(D)-ribofuranos-1′-yl]-5-(phenylureido)-benzoxazole (19c). Deprotection of compound **18c** was obtained following general procedure F that led to desired compound **19c** as a colorless solid. Yield (91%); Retention time 11.2 min; ^{1}H NMR (500 MHz, CD_3OD) δ: 2.30-2.40 (m, 1H), 2.40-2.45 (m, 1H), 2.80-2.90 (m, 1H), 2.95-3.05 (m, 1H), 3.40-3.50 (m, 2H), 4.00-4.05 (m, 1H), 4.28 (dt, *J* = 5.5, 2.6 Hz, 1H), 4.36 (dd, *J* = 15.4, 7.7 Hz, 1H), 5.32 (t, *J* = 8.6 Hz, 1H), 6.95-7.05 (m, 1H), 7.20-7.30 (m, 3H), 7.35-7.40 (m, 1H), 7.43 (d, J = 7.2 Hz, 2H), 7.54 (d, J = 7.2 Hz, 1H), 7.93 (s, 1H), 8.11 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ: 31.5, 41.9, 42.8, 55.4, 74.1, 74.9, 80.9,

87.5, 111.8, 113.2, 115.8, 120.1, 120.9, 122.4, 125.8, 130.2, 131.5, 136.2, 139.3, 141.7, 144.6, 149.1, 168.1, 173.3, 176.2; HRMS (ESI) *m/z* = 524.2255 $[M+H+H_2O]^+$ ($C_{25}H_{30}N_7O_6$ requires 524.2257).

Synthesis of HB nucleobase (33).



Scheme S3. Synthesis of HB nucleobase **32** as a negative control. Reagents: a) Ac_2O, pTsOH cat., toluene, 100°C, overnight; b) P_2O_5, pTsOH, toluene, 100°C, overnight; c) $NaBH_4$, Pd/C, CH_2Cl_2/MeOH, t.a., 3h; d) PhNCO, Et_3N, DMF, t.a., 2h.

2-acetamide-4-nitrophenol (29). To a suspension of commercially available 2-aminonitrophenol **28** (4.87 mmol, 1 eq.) in toluene (40 mL), acetic anhydride (5.84 mmol, 1.2 eq.) and p-toluensulfonic acid (5.84 mmol, 1.2 eq.) were added and the reaction mixture was stirred overnight at 100 °C. After returning the reaction mixture at room temperature, solvent was removed under reduced pressure and the crude product was purified using flash column chromatography on a silica gel column using a mixture CH_2Cl_2/MeOH 97:3 and leading to pure compound **29** as a yellow solid: 82% yield. Rf = 0.40 (DCM/MeOH 98:2); ^{1}H RMN (200 MHz, DMSO-d_6) δ: 2.14 (s, 3H), 7.01 (d, *J* = 9.0 Hz, 1H), 7.88 (dd, *J* = 9.0, 2.9 Hz, 1H), 8.94 (d, *J* = 2.8 Hz, 1H), 9.48 (s, 1H), 11.61 (s, 1H); ^{13}C RMN (50 MHz, DMSO-d_6) δ: 23.8, 115.0, 116.4, 120.3, 126.8, 139.0, 153.7, 169.3; MS (ESI) *m/z* = 219.2 $[M+Na]^+$.

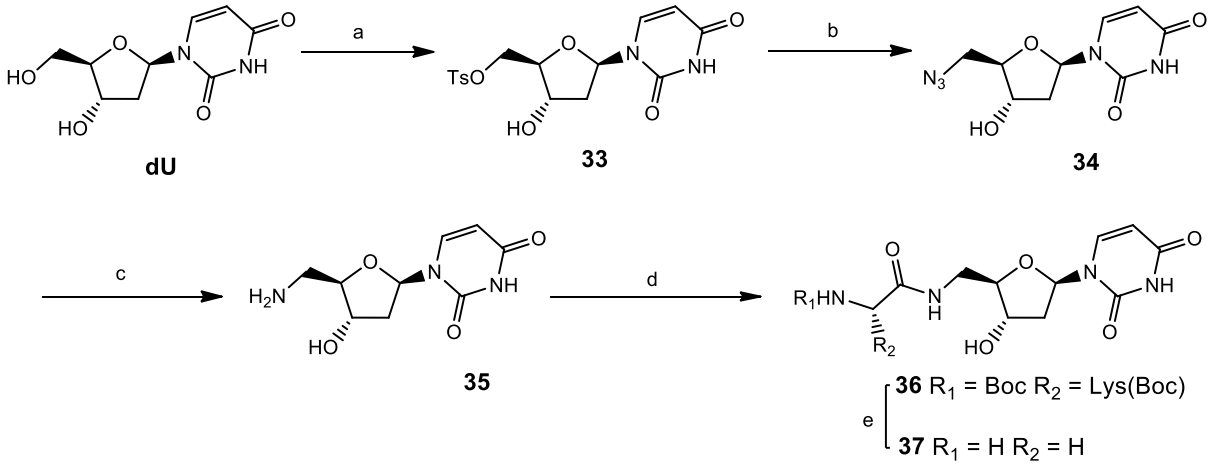
2-methyl-5-nitrobenzoxazole (30). To a suspension of compound **29** (1.58 mmol) in 30 mL of toluene, phosphorous pentoxide (4.74 mmol, 3eq.) and p-toluensulfonic acid (0.32 mmol, 0.2 eq.) were added and the reaction mixture was stirred at 100 °C overnight. After removal of the solvent under reduced pressure, the crude product was suspended in wáter (50 mL) and washed with ethyl acetate (3 x 50 mL). Organic phases were dried using Mg_2SO_4, the solvent was removed under reduced pressur and the crude product was purified using flash chromatography on a silica gel column using a mixture CHX/EtOAc 8 :2) leading to desired compound **30** in 55% yield. Rf = 0.55 (CHX/AcOEt 8:2); ^{1}H RMN (200 MHz, DMSO-d_6) δ: 2.69 (s, 3H), 7.94 (d, *J* = 8.9 Hz, 1H), 8.29 (dd, *J* = 8.9, 2.4 Hz, 1H), 8.55 (d, *J* = 2.3 Hz, 1H); ^{13}C RMN (50 MHz, DMSO-d_6) δ: 15.3, 111.6, 115.3, 119.2, 120.9, 142.4, 156.1, 165.5; MS (ESI) *m/z* = 201.8 $[M+Na]^+$.

2-methyl-5-aminobenzoxazole (31). To a solution of compound **30** (7.2 mmol) in a mixture CH_2Cl_2/MeOH (1:1, 30 mL), sodium boronhydride (36.0 mmol, 5 eq.) and 10% Pd/C were added. The

reaction mixture was stirred at room temperature 3h then filtered on a pad of celite. Solvent was removed under reduced pressure and the crude product was purified by flash chromatography on a silica gel column using a mixture CH_2Cl_2/MeOH 98:2 leading to desired compound **31** in 79% yield. Rf = 0.60 (DCM/MeOH 98:2); ^{1}H RMN (200 MHz, $CDCl_3$) δ: 2.55 (s, 1H), 3.54 (s, 1H), 6.61 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.91 (d, *J* = 2.3 Hz, 1H), 7.20 (d, *J* = 8.6 Hz, 1H); ^{13}C RMN (50 MHz, $CDCl_3$) δ: 14.9, 102.3, 106.2, 112.3, 140.2, 142.4, 144.8, 165.5; MS (ESI) *m/z* = 148.1 $[M+H]^+$.

2-methyl-5-phenylureidobenzoxazole (32). Compound **31** (2.89 mmol) was dissolved in DMF (120 mL), and triethylamine (4.33 mmol, 1.5 eq.), phenylisocyanate (3.46 mmol, 1.2 eq.) were added sequentially. The reaction mixture was stirred at room temperature 2h, solvent was then removed under reduced pressure and the crude product was purified by flash chromatography on a silica gel column using a mixture CHX/EtOAc 1:1 leading to desired compound **32** in 84% yield. ^{1}H RMN (500 MHz, CD_3OD) δ: 2.61 (s, 3H), 7.02 (m, 1H), 7.29 (m, 3H), 7.44 (m, 3H), 7.82 (d, *J* = 1.9 Hz, 1H); ^{13}C RMN (126 MHz, CD_3OD) δ: 14.7, 105.4, 111.1, 113.5, 122.7, 129.0, 129.5, 135.1, 139.1, 141.8, 146.6, 165.2; MS (ESI) *m/z* = 268.4 $[M+H]^+$, 290.6 $[M+Na]^+$.

Synthesis of a uridine conjugated in 5' position with lysine (37).



Scheme S4. Synthesis of 5'-lysinamide-2'-deoxyuridine **37**. Reagents: a) TsCl, pyr, t.a.; b) NaN_3, TBAI, DMF, 80°C; c) H_2, Pd/C, CH_2Cl_2/MeOH, t.a.; d) *Nα,Nε*-di-Boc-L-lysine, HOBt, DIC, Et_3N, CH_2Cl_2, t.a.

2'-deoxy-5'-(*O*-tosyl)-uridine (33). To a solution of 2'-deoxyuridine (**dU**, 11.2 mmol) in pyridine (30 mL) at 0°C was added a solution of tosyl chloride in pyridine (16.7 mmol, 1.5 eq.). After 2h stirring at room temperature, the solvent was removed under reduced pressure and the crude product dissolved in EtOAc (15 mL) and washed twice with water (20 mL). Organic phases were then dried over $MgSO_4$ and the solvent removed under reduced pressure. Purification of the crude product by flash chromatography on a silica gel column using a mixture CH_2Cl_2/MeOH 98:2 led to desired compound **33** in 69% yield. Rf = 0.85 (DCM/MeOH 9:1) ; ^{1}H RMN (200 MHz, DMSO-d_6) δ: 2.07 (m, 2H), 2.40 (s, 3H), 3.84 (m, 1H), 4.18 (m, 3H), 5.44 (d, *J* = 4.4 Hz, 1H), 5.53 (t, *J* = 11.5 Hz, 1H), 6.09 (t, *J* = 6.8 Hz, 1H), 7.45 (m,

3H), 7.78 (d, *J* = 8.3 Hz, 2H), 11.33 (s, 1H); ^{13}C RMN (50 MHz, DMSO-d_6) δ: 20.7, 69.9, 83.2, 84.2, 101.9, 125.4, 127.6, 130.1, 132.0, 137.8, 140.5, 145.1, 150.3, 163.0; MS (ESI) *m/z* = 405.5 $[M+Na]^+$.

2'-deoxy-5'-azido-uridine (34). Compound **34** was obtained from compound **33** following general procedure B and using a mixture of DCM/MeOH 98:2 as the eluent for column chromatography. Compound **35** was obtained as a yellow solid in 76% yield and it was employed in the following reaction without further purification. R_f = 0.80 (DCM/MeOH 9:1) ; ^{1}H RMN (200 MHz, DMSO-d_6) δ: 2.19 (m, 2H), 3.55 (d, *J* = 5.2 Hz, 2H), 3.85 (m, 1H), 4.18 (m, 1H), 5.44 (d, *J* = 4.3 Hz, 1H), 5.66 (d, *J* = 8.1 Hz, 1H), 6.18 (t, *J* = 6.9 Hz, 1H), 7.68 (d, *J* = 8.1 Hz, 1H), 11.36 (s, 1H); MS (ESI) *m/z* = 276.5 $[M+Na]^+$, 529.5 $[2M+Na]^+$.

2'-désoxy-5'-amino-uridine (35). Compound **35** was obtained starting from compound **34** following general procedure D in 95% yield and employed in the following reaction without further purification. ^{1}H RMN (200 MHz, DMSO-d_6) δ: 2.07 (dd, *J* = 6.7, 5.0 Hz, 1H), 2.70 (d, *J* = 5.2 Hz, 1H), 3.65 (dd, *J* = 8.4, 5.1 Hz, 1H), 4.13 (m, 1H), 5.60 (d, *J* = 8.0 Hz, 1H), 6.10 (t, *J* = 6.8 Hz, 1H), 7.78 (d, *J* = 8.1 Hz, 1H). SM (ESI) *m/z* = 228.9 $[M+H]^+$.

2'-désoxy-5'-[*Nα*,*Nε*-di-*tert*-butylcarbonyl)-L-lysinamide]-uridine (36). Compound **36** was obtained starting from compound **35** following general procedure E using a mixture DCM/MeOH 95:5 as the eluent for column chromatography. Desired compound **36** was obtained as a yellow solid in 71% yield. R_f = 0.65 (DCM/MeOH 9:1); ^{1}H RMN (200 MHz, DMSO-d_6) δ: 1.16 (m, 2H), 1.34 (s, 18H), 1.60 (m, 4H), 2.02 (m, 2H), 2.80 (m, 2H), 3.26 (m, 2H), 3.81 (m, 2H), 4.10 (m, 1H), 5.60 (d, J = 8.1 Hz, 1H), 6.08 (t, J = 7.0 Hz, 1H), 7.64 (d, J = 8.0 Hz); ^{13}C RMN (50 MHz, DMSO-d_6) δ: 21.4, 26.9, 28.4, 30.6, 40.1, 42.6, 60.2, 72.2, 79.4, 87.4, 95.6, 102.8, 141.2, 150.8, 155.7, 165.6, 177.3; MS (ESI) *m/z* = 578.6 $[M+Na]^+$.

2'-désoxy-5'-((L)-lysinamide)-uridine (37). Compound **37** was obtained starting from compound **36** following general procedure F as a slightly yellow solid in 97% yield. ^{1}H RMN (200 MHz, CD_3OD) δ: 1.16 (m, 2H), 1.64 (m, 4H), 2.03 (m, 2H), 2.83 (m, 2H), 3.21 (m, 2H), 3.92 (m, 2H), 4.15 (m, 1H), 5.58 (d, J = 8.0 Hz, 1H), 6.09 (t, J = 7.4 Hz, 1H), 7.64 (d, J = 8.0 Hz); ^{13}C RMN (50 MHz, CD_3OD) δ: 22.4, 27.8, 29.6, 39.6, 42.6, 60.2, 72.4, 87.4, 95.6, 102.8, 142.3, 151.2, 165.9, 176.8; MS (ESI) *m/z* = 356.9 $[M+H]^+$.

Biochemical procedures

Unless otherwise stated, all reagents and solvents were of analytical grade and from Merck (Sigma Aldrich). HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] and all inorganic salts for buffers were purchased from Merck (Sigma Aldrich) (molecular biology grade). RNA oligonucleotides whose sequences are indicated in Table 1 and DNA oligonucleotides were purchased from IBA GmbH and used without further purification. A mixture of pre- and mature yeast tRNAs (containing over 30 different species) was purchased from Sigma (type X-SA). Stocks of tRNAmix can be quantified in its native form (without base hydrolysis) using an extinction coefficient of 9640 cm-1 M-1 per base[2].

Buffers

All buffers were filtered through 0.22-µm Millipore filters (GP ExpressPLUS membrane). A small aliquot (50–100 ml) was first filtered and then discarded to avoid any contaminants that might be leached from the filter. The solutions to be used in the fluorescence experiments were prepared by diluting the concentrated stocks in Milli-Q water and filtered again as described above. All standard fluorescence measurements were performed in buffer A (20 mM HEPES (pH 7.4 at 25 °C), 20 mM NaCl, 140 mM KCl and 3 mM MgCl2). For competitive experiments in the presence of a dsDNA, a 15-mer sequence (5'-CGTTTT TATTTTTGC-3') and its complement, annealed beforehand, were added to buffer A to obtain a 100-fold nucleotide excess regarding TAR RNA (900 nM duplex; 5 nM RNA). For competitive experiments in the presence of a tRNA, a mixture of pre- and mature yeast tRNAs (containing over 30 different species from baker's yeast (S. cerevisiae, Sigma, type X-SA)) was added to buffer A to obtain a 100-fold nucleotide excess regarding TAR RNA. Stock solutions of tRNA were prepared in water and quantified using an extinction coefficient of 9640 cm-1 M-1 per base2. CD experiments were performed in buffer B (20 mM potassium phosphate buffer (pH 7.4 at 25 °C), 10 mM NaCl and 1 mM MgCl2).

Binding studies and K_d determination. Ligand solutions were prepared as serial dilutions in buffer A at a concentration four times higher than the desired final concentration to allow for the subsequent dilution before the addition of the RNA solution. An automated pipetting system epMotion 5075 (Eppendorf®) was used in order to perform these binding studies on 384-well plates (Greiner bio-one). Refolding of the RNA was performed using a thermocycler (ThermoStat Plus Eppendorf®) as follow: the 5'-Alexa^{488}-TAR RNA (0.2 nmol) was diluted in 1 mL of buffer A, denatured by heating to 90°C for 2 min, then cooled to 4°C for 10 min followed by incubation at 25°C for 15 min. After refolding, the RNA was diluted to a working concentration of 20 nM through addition of the appropriate amount of buffer A. After addition of 30 µL of each ligand on the 384-well plates in 15 dilutions (from 1 mM to 61 nM as final concentrations) and in duplicates, 30µL of the RNA solution were added to each well containing ligand leading to a final concentration of 10 nM. The fluorescence was measured on a GeniosPro (Tecan) with an excitation filter of 485±10 nm and an emission filter of 535±15 nm. Each point was measured 5 times with a 500 µs integration time and averaged. Binding was allowed to

proceed overnight at 5°C to achieve equilibrium. To study the temperature dependence, the plates were incubated after overnight equilibrium at different temperature ranging from 5°C to 35°C. Neomycin was taken as a control as its binding to TAR has already been studied using several methods. Its K_d value (12.0 ± 3.6 μM) is in good agreement with previously reported values.

Data analysis. Binding data were analyzed using Graphpad Prism 5 software. Unless otherwise stated, binding profiles were well modeled using a simple model assuming the one to one stoichiometry. A higher initial fluorescence value is observed in the presence of tRNA, which is consistent with the modification of the polarity of the solvent and a small fluorescence of the tRNA mixture.

NMR experiments. High resolution NMR experiments were recorder on a BRUKER AVANCE Ultra shield DRX 500 spectrometer operating at 500.13 MHz for ^{1}H, equipped with a temperature control unit (BCU 6.0, BVT 3000), and an inverse probe head (5mm PHTXI 1H-13C/15N Z-GRD). Proton chemical shift was referenced internally by setting the carrier frequency on water at the center of the spectrum (4,71ppm at 5°C). Chemical shifts (δ) are expressed in parts per million (ppm). All NMR experiments were carried out using standard pulse sequences supplied by the spectrometer manufacturer (BRUKER). 1D spectra were processed using TOPSPIN 2.1 NMR Software (BRUKER).

^{1}H NMR imino proton spectra were recorded in H_2O/D_20 (90/10) at 278°K (5°C) by using a WATERGATE 3-9-19 water suppression. Each proton NMR spectrum was acquired using 10,964 KHz Spectral Width (SW), 64K complex data point, acquisition time (aq) of 2,98s, relaxation delay (D1) of 1s, number of scan (ns) 2000, number of dummy scan (ds) 4 and a 90° flip angle pulse width. Water suppression was achieved using WATERGATE pulse sequence. Gradient pulse were sine shape (SINE.100), 1.5 ms long (P16) with 200 μs gradient recovery delay (D16) and strengths set to 8,44 Gauss.cm-1 (20%). A 45,6 μs delay (D19) was used for binomial water suppression. Prior to Fourrier transformation, the fids were multiplied by an exponential line broadening function of 3Hz.

Thermodynamic calculations

For thermodynamic analysis, ΔG° values were plotted versus T for the three-parameter fit.[2]

Nonlinear regression in Prism 4 (GraphPad Software) was used to fit the following equation to the data:

$\Delta G°T = \Delta H°Tr + \Delta CP\ (T - Tr) – T\Delta S°Tr - T\Delta CP \ln (T/Tr)$

where Tr is a constant reference temperature (in our study Tr = 293.15 K), and the three fit parameters are ΔH°Tr, the change in enthalpy upon binding at Tr ; ΔS°Tr, the change in entropy upon binding at Tr; and ΔCP, the change in heat capacity. Starting values for the three parameters did not affect the final values. ΔCP was assumed to be independent of temperature; inclusion of a ΔCP/ΔT term in the analysis did not improve the quality of the fits and gave larger standard errors for the returned parameters.

ΔH°T and ΔS°T were calculated by using:

[2] J. J. Boniface, Z. Reich, D. S. Lyons and M. M. Davis, Proc. Natl. Acad. Sci. U S A 1999, 96, 11446-11451; S. H. Yoo and M. S. Lewis, Biochemistry 1995, 34, 632-638; M. T. Record, Jr., C. F. Anderson and T. M. Lohman, Q. Rev. Biophys. 1978, 11, 103-178

$\Delta H°T = \Delta H°Tr + \Delta CP\ (T - Tr)$

and

$\Delta S°T = \Delta S°Tr + \Delta CP \ln (T/Tr)$.

Salt dependence of Kd was analyzed by the following equation4:

$\log(Kd) = \log(Knel) - Z\psi \log [KCl]$

where Knel is the dissociation constant at the standard state in 1 M KCl, Z is the number of ions displaced from the nucleic acid (essentially the number of intermolecular ion pairs) and ψ is the fractional probability of a counterion being thermodynamically associated with each phosphate of the RNA number of cations. Knel and Zψwere treated as fitting parameters.

UV-melting curves

UV-melting curves were recorded on aVarian Cary 300 UV/vis spectrophotometer. Absorbances were monitored at 260 nm, and the heating rate was set to 0.2 °C/min.

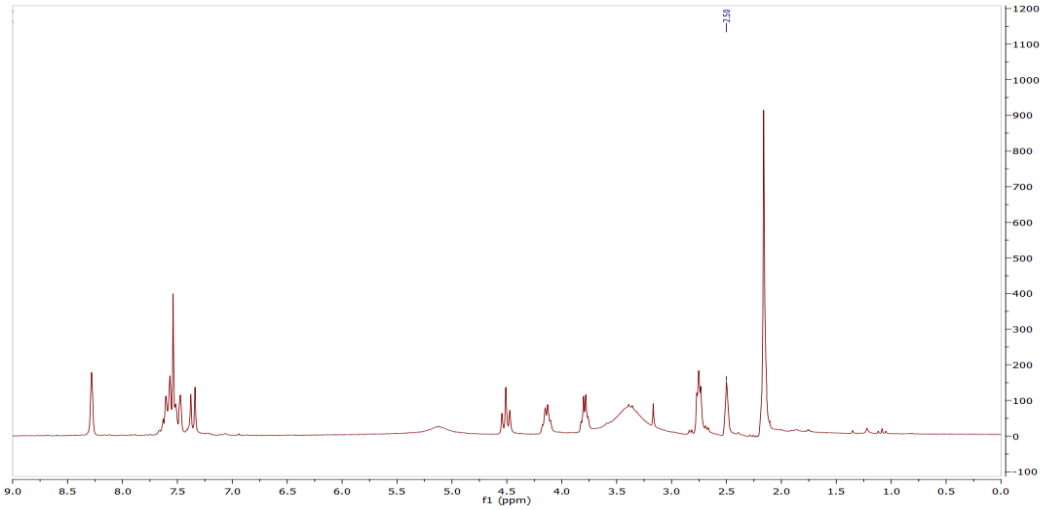
A cooling-heating-cooling cycle in the temperature range 10–90°C was applied.

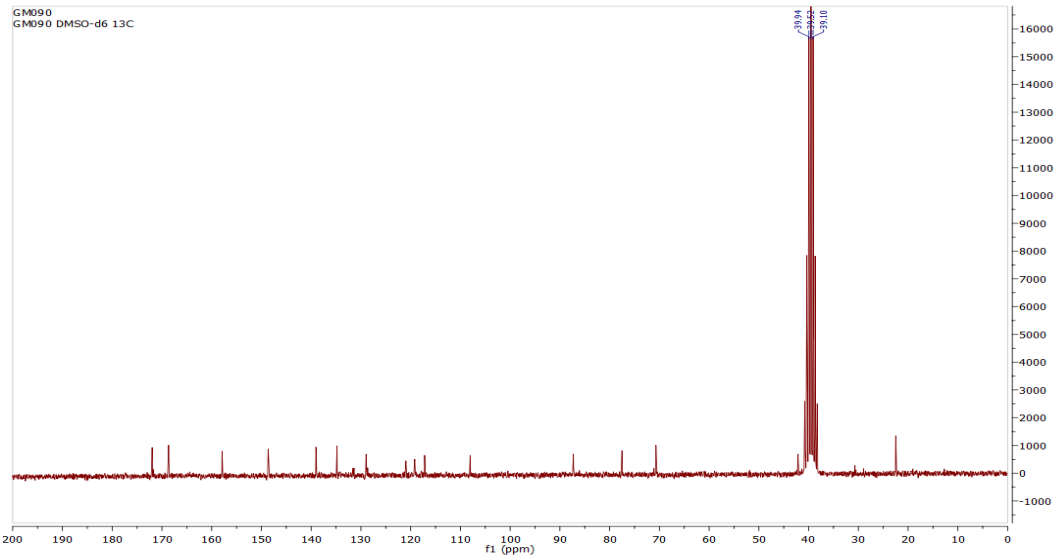
Tm values were obtained from the maximum of the first derivative curves and reported as the average of at least three ramps. To avoid evaporation of the solution, the sample solutions were covered with a layer of dimethylpolysiloxane. All measurements were carried out using a solution of A-site RNA at 1 μM in a 10 mM sodium cacodylate buffer at pH 7.4 containing 50 mM NaCl and 0.1 mM d'EDTA.

Circular dichroism spectra

CD measurements were performed with a Jasco J-810 spectropolarimeter equipped with a Jasco PTC 423S Peltier temperature controller. Samples were prepared in buffer B. Spectra were obtained at 3 μM RNA or compounds **6c** and **19c** (for individual spectra) or a molar ratio of 1:1, 1:10 RNA:**6c** and **19c** for the complexes in a 1-mm path-length cell. RNA samples were heat-denatured and allowed to refold as described above prior measurement. Spectra were recorded at 20°C from 360 to 200 nm at 1 nm intervals with an integration time of 4 sec and a 50 nm/min speed. CD scans were repeated five times, then averaged and corrected by the subtraction of buffer background or the spectra of the appropriate ligand alone.

Figure S5. ^{1}H, ^{13}C NMR and HRMS spectra for compound **4**.





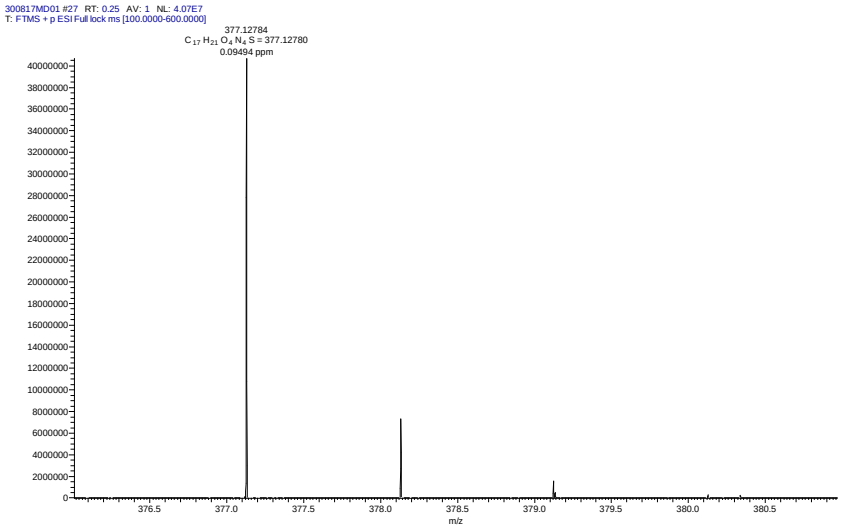


Figure S6. ^{1}H and ^{13}C NMR spectra for compound **6a**.

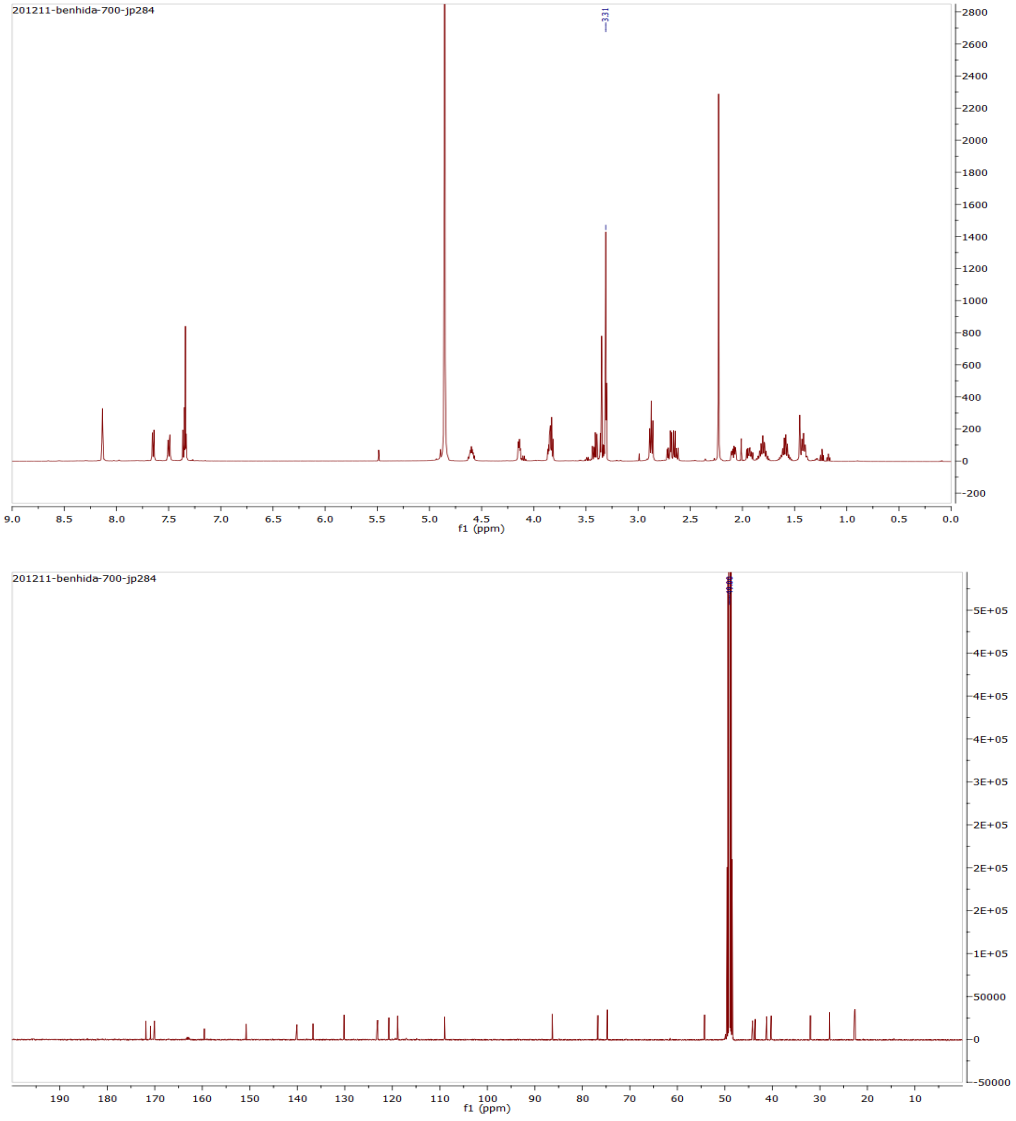
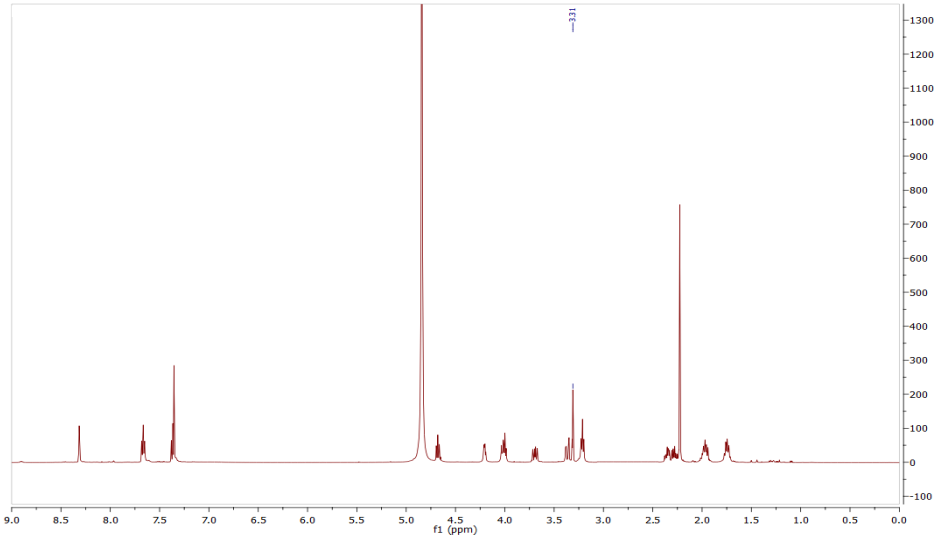
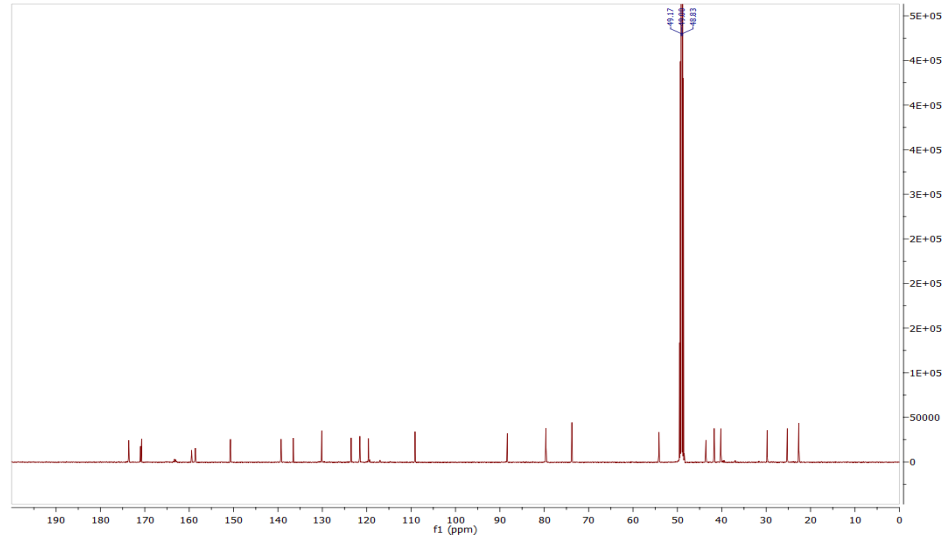


Figure S7. ^{1}H, ^{13}C NMR and HRMS spectra for compound **6b**.





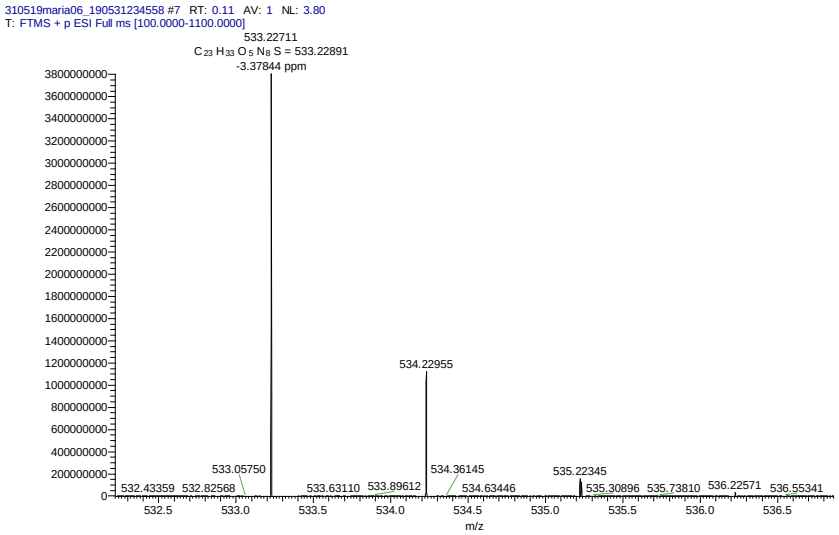
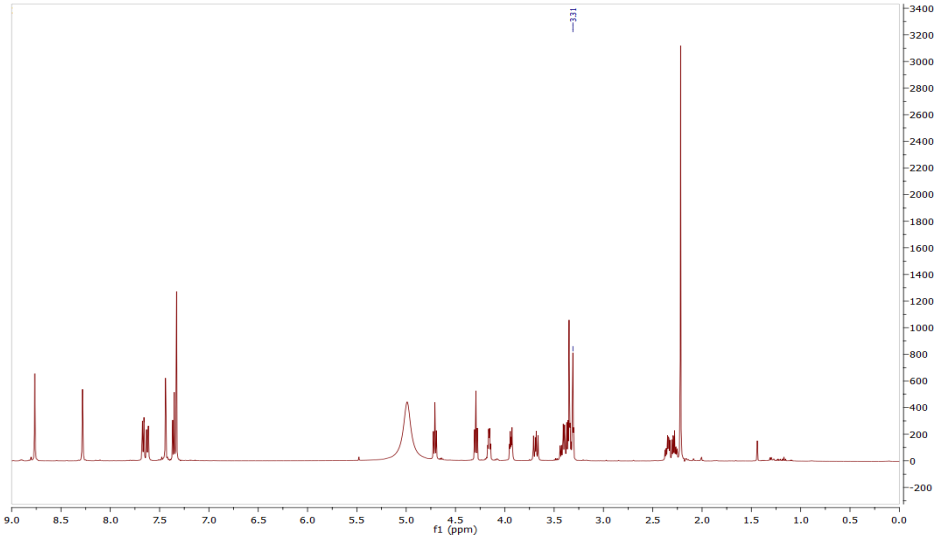
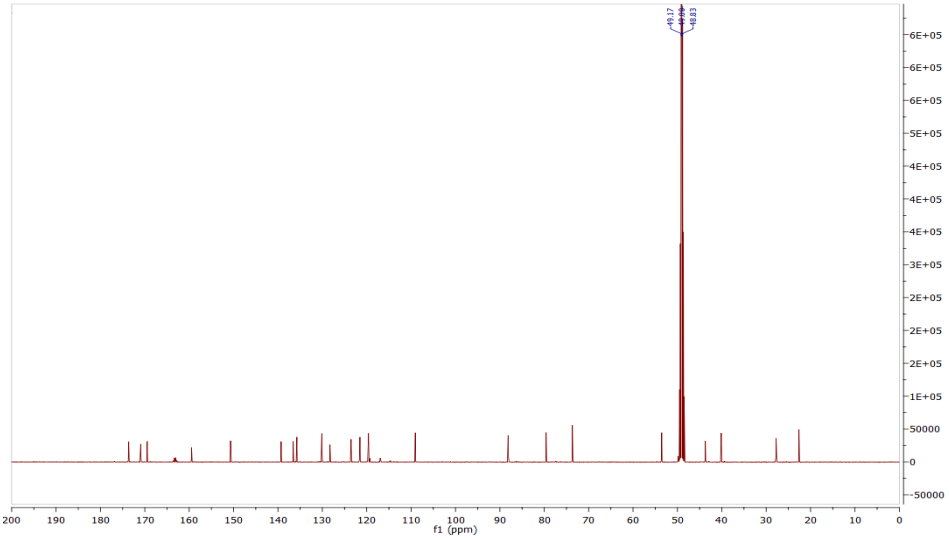


Figure S8. ^{1}H, ^{13}C NMR and HRMS spectra for compound **6c**.





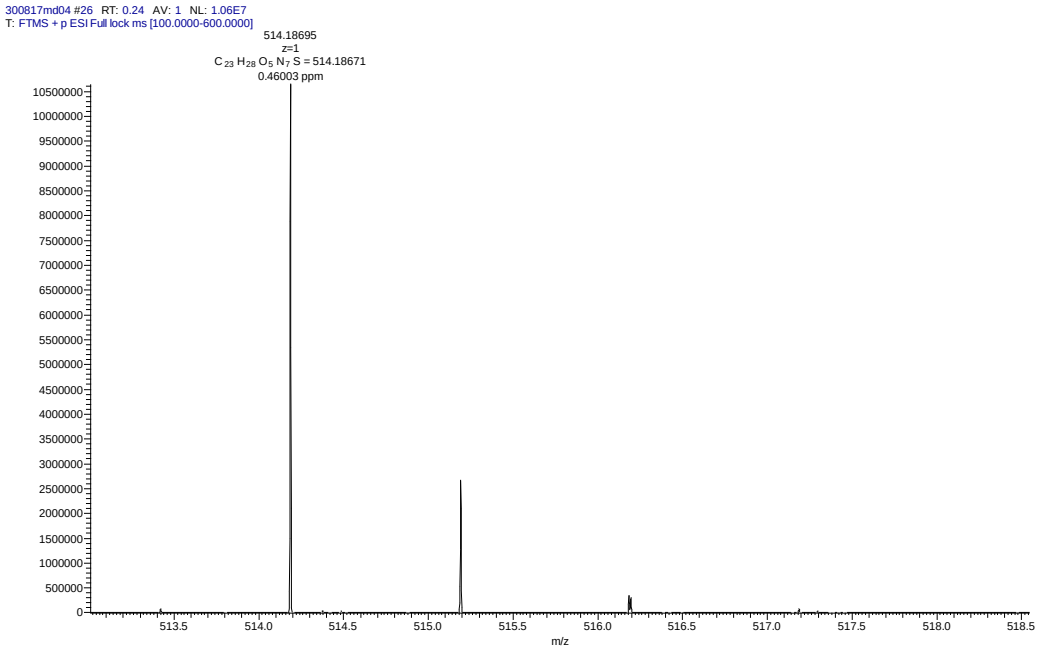
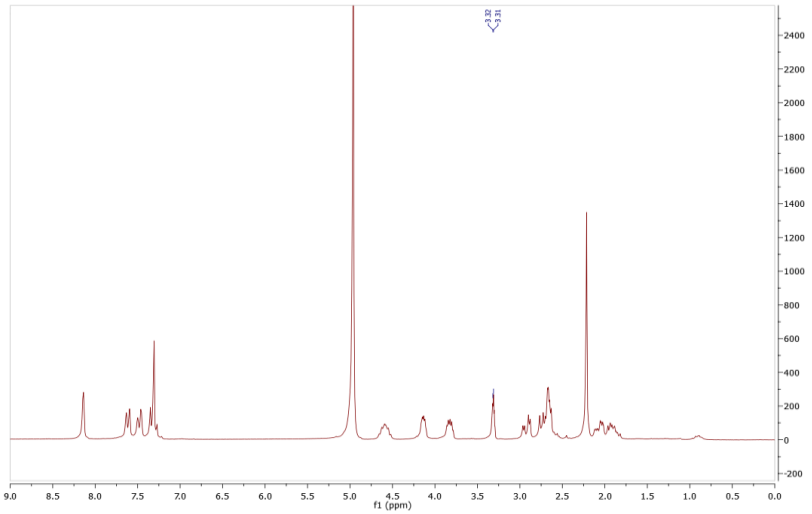
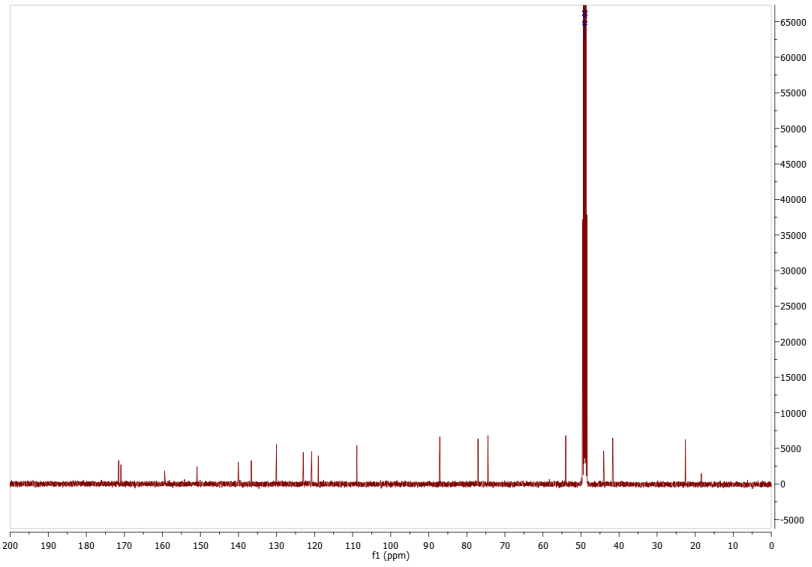


Figure S9. ^{1}H, ^{13}C NMR and HRMS spectra for compound **9.**





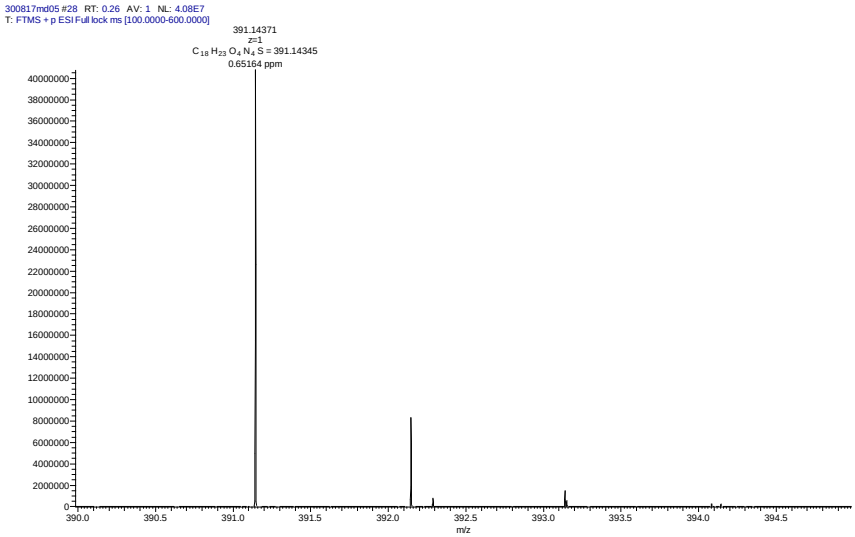


Figure S10. ^{1}H and ^{13}C NMR spectra for compound **11a**.

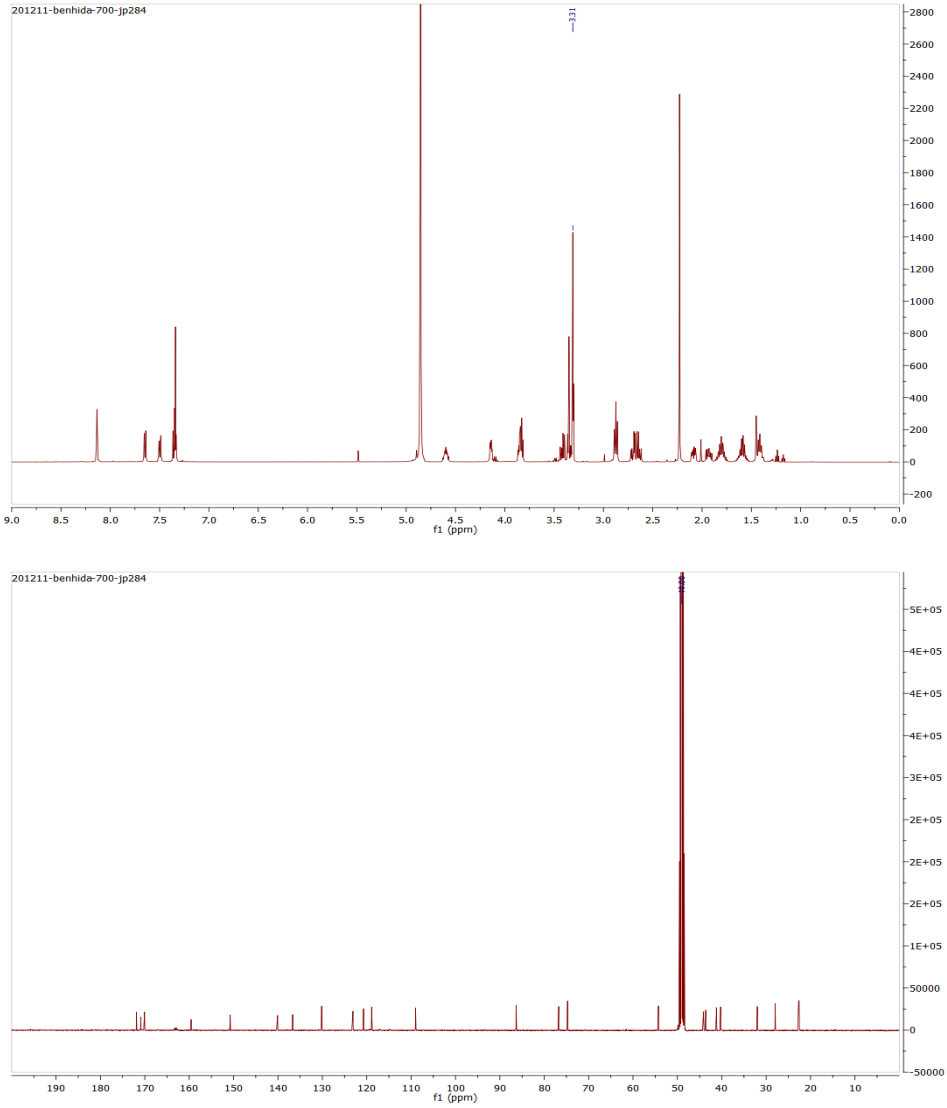
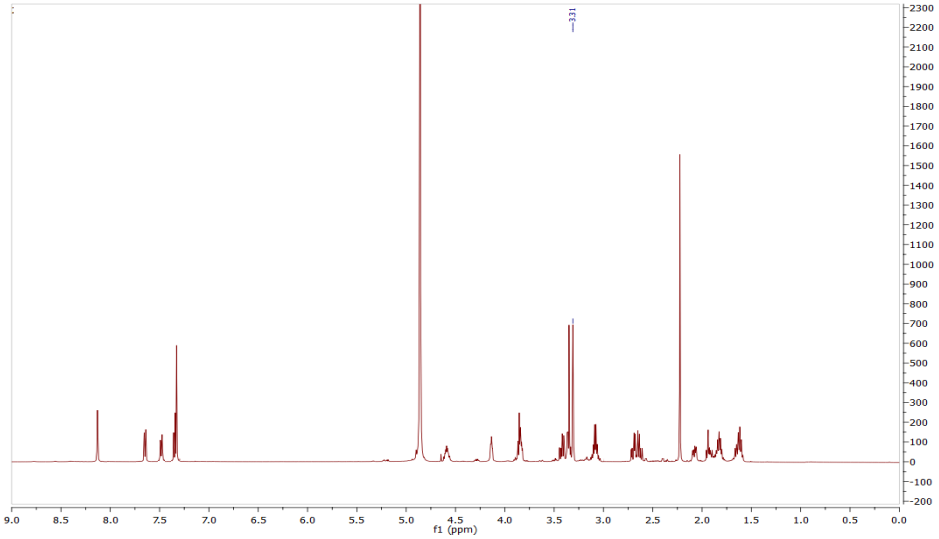
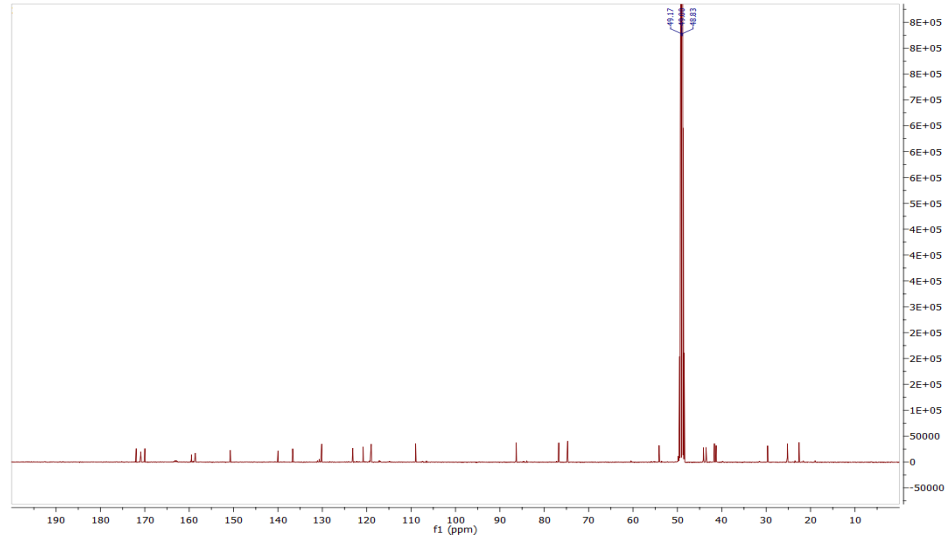


Figure S11. ^{1}H, ^{13}C NMR and HRMS spectra for compound **11b**.





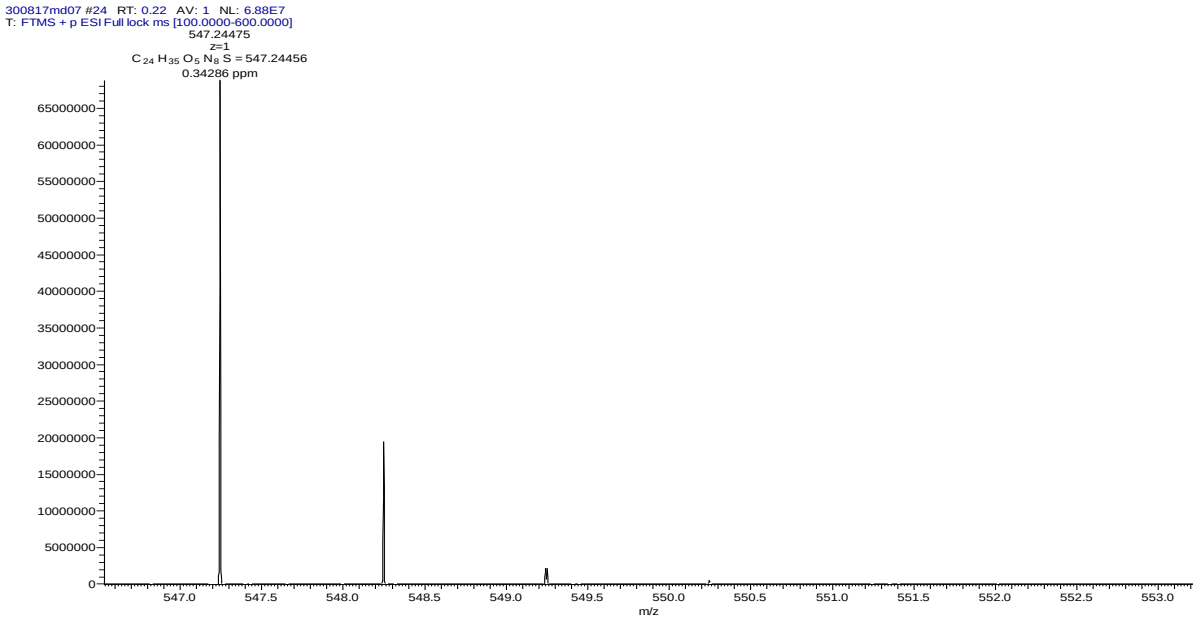
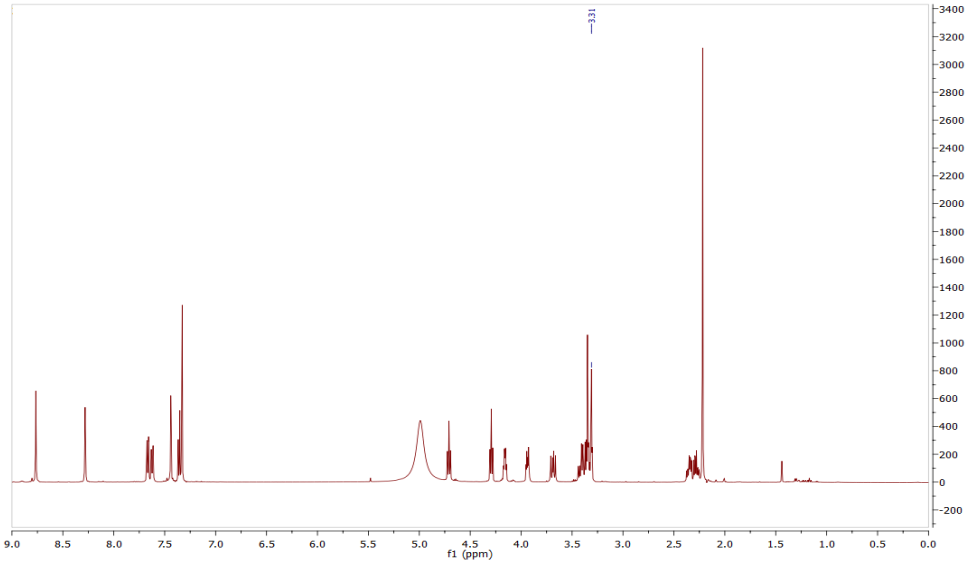
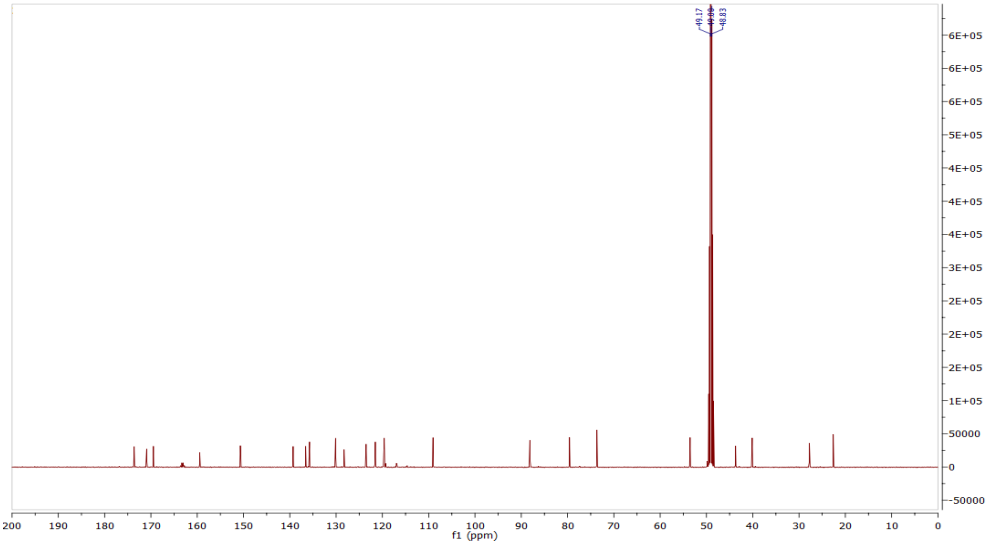


Figure S12. ^{1}H, ^{13}C NMR and HRMS spectra for compound **11c**.





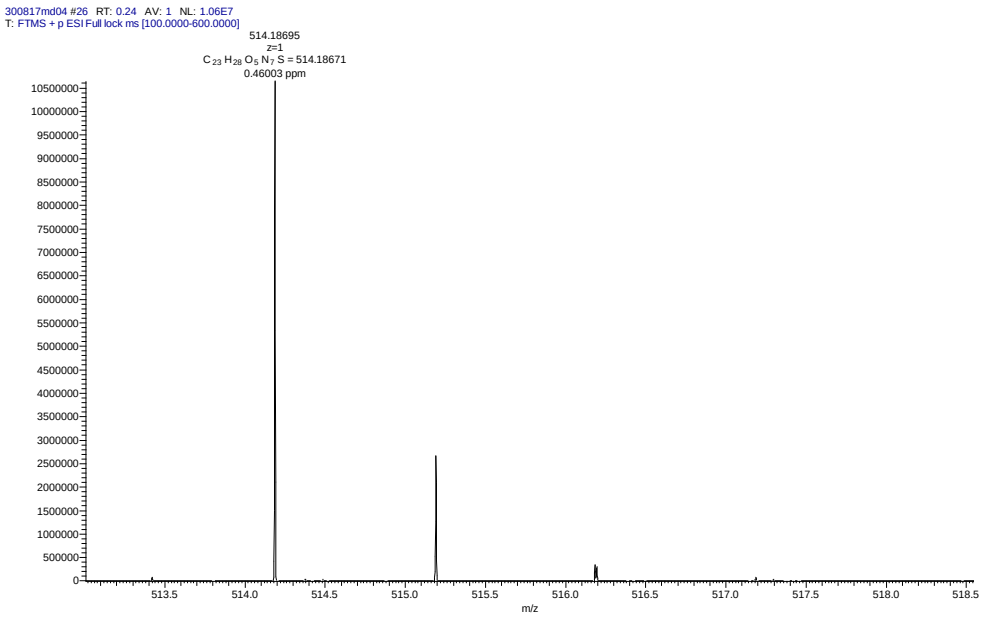
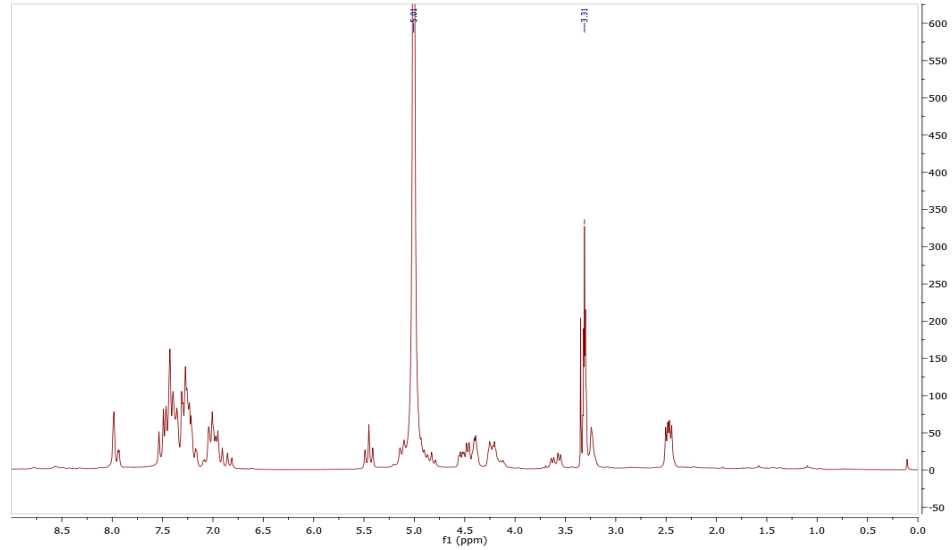
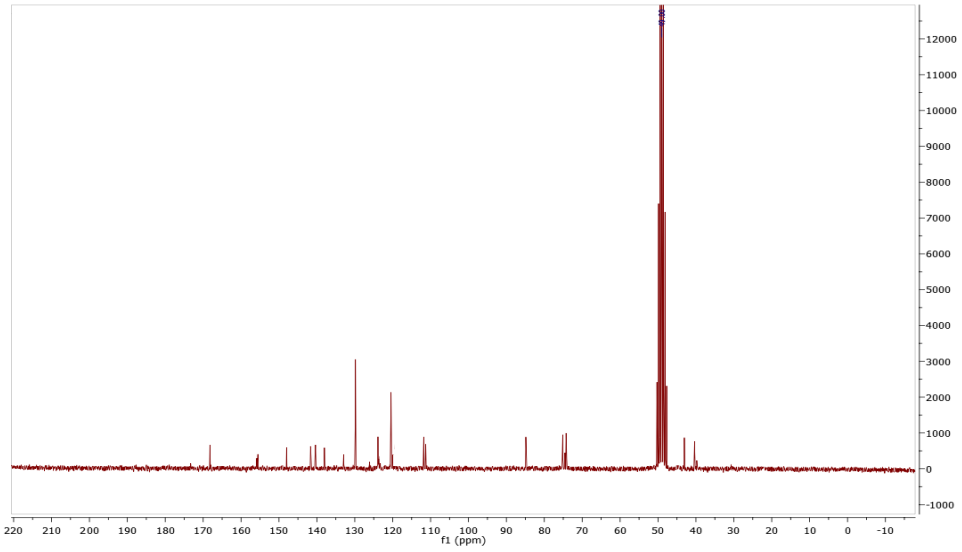


Figure S13. ^{1}H, ^{13}C NMR and HRMS spectra for compound **17**.





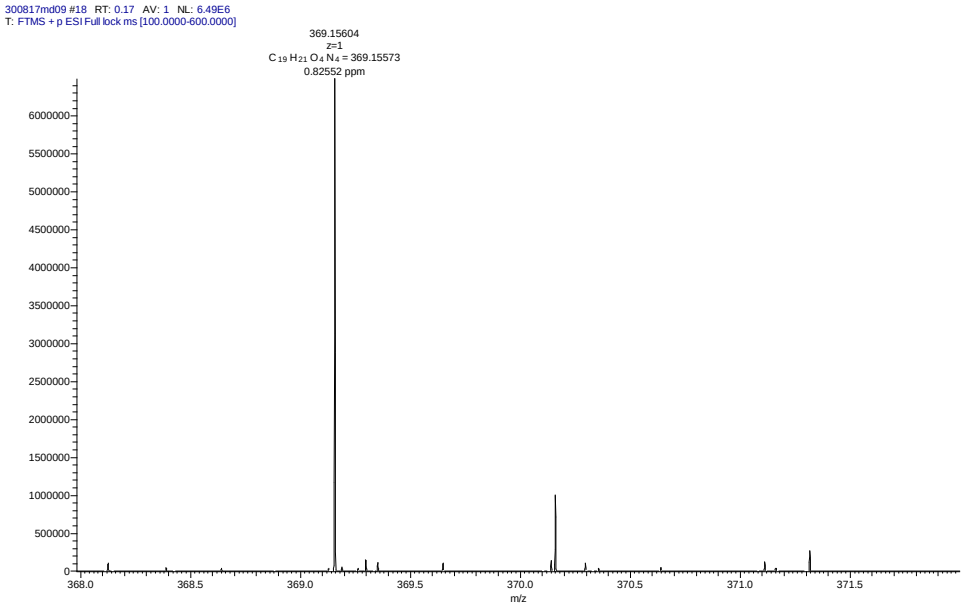


Figure S14. ^{1}H and ^{13}C NMR spectra for compound **19a**.

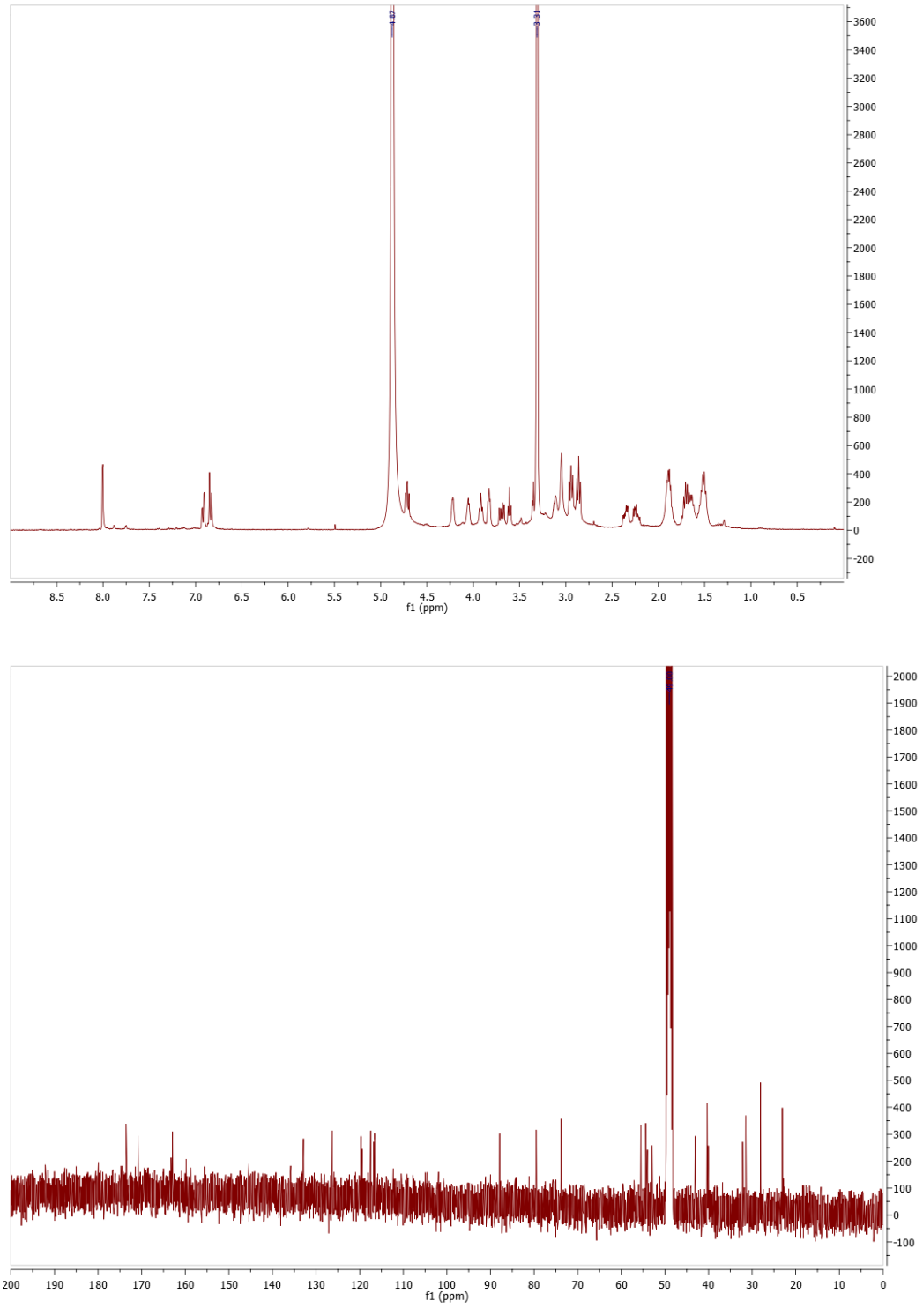
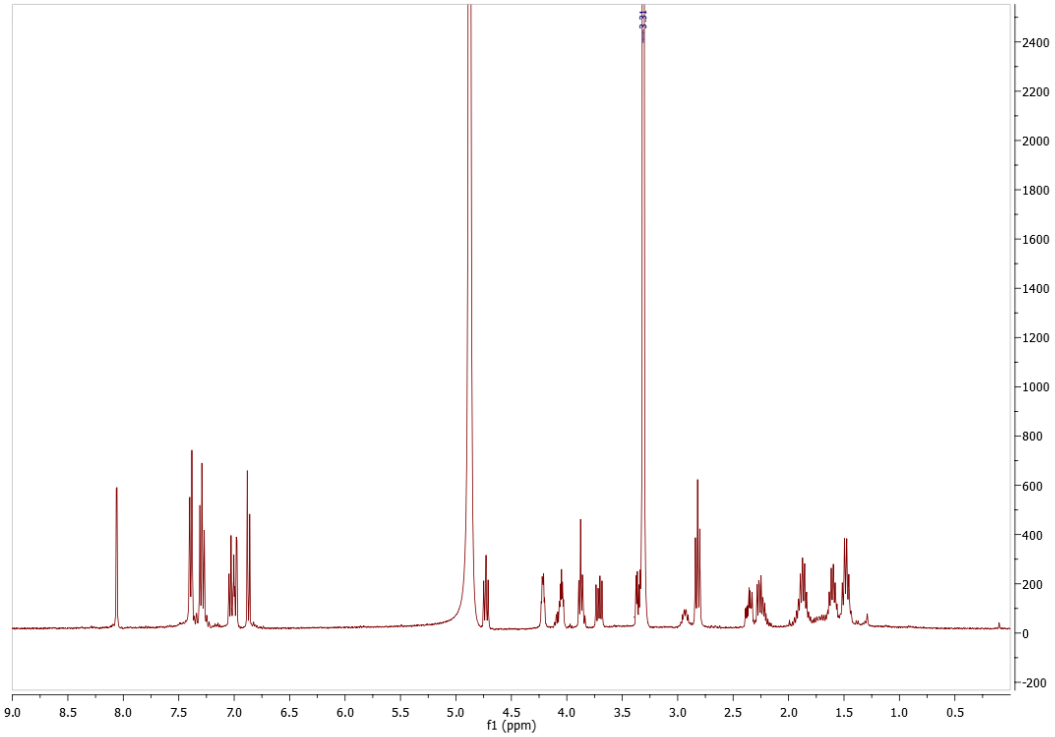


Figure S15. ^{1}H and ^{13}C NMR spectra for compound **19b**.



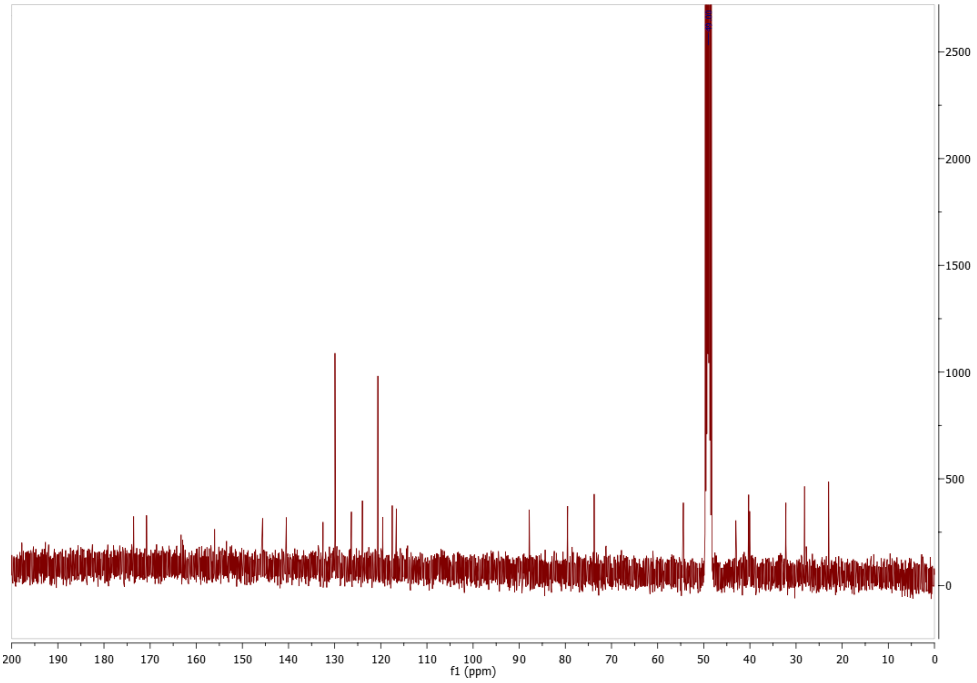


Figure S16. ^{1}H, ^{13}C NMR and HRMS spectra for compound **19c**.