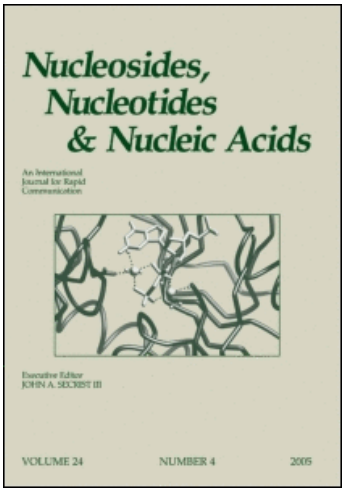


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

The RNA World: Functional Diversity in a Nucleoside by Carboxyamidation of Uridine

Torin M. Dewey[a]; Carina M. Zyzniewski[a]; Bruce E. Eaton[a]
[a] Medicinal Chemistry Department, NeXstar Pharmaceuticals, Inc., Boulder, CO

To cite this Article Dewey, Torin M. , Zyzniewski, Carina M. and Eaton, Bruce E.(1996) 'The RNA World: Functional Diversity in a Nucleoside by Carboxyamidation of Uridine', Nucleosides, Nucleotides and Nucleic Acids, 15: 10, 1611 — 1617
To link to this Article: DOI: 10.1080/07328319608002461
URL: http://dx.doi.org/10.1080/07328319608002461

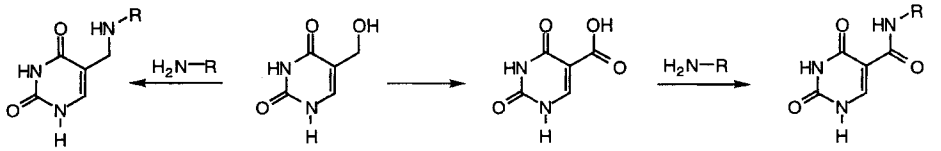
NUCLEOSIDES & NUCLEOTIDES, 15(10), 1611-1617 (1996)

THE RNA WORLD: FUNCTIONAL DIVERSITY IN A NUCLEOSIDE BY CARBOXYAMIDATION OF URIDINE

Torin M. Dewey, M. Carina Zyzniewski, and Bruce E. Eaton
Medicinal Chemistry Department
NeXstar Pharmaceuticals, Inc.
2860 Wilderness Pl.
Boulder, CO 80301

Abstract: A facile method for the 5-carboxyamidation of protected uridines is described allowing synthesis of an array of uridines with diverse functionality.

Recently it has been proposed that under prebiotic conditions 5-uracil derivatives are formed that increase the structural diversity of nucleic acids.[1] These modified uracils may have served as an intermediate structural class with both nucleic acid and protein functionality, serving as a bridge between the RNA world and the DNA-protein world. The central structure of this molecular evolution is 5-hydroxymethyl-uracil **1**, which is formed spontaneously from formaldehyde and uracil.[1] Solvolysis of the 5-hydroxyl group in the presence of amino groups (Scheme 1) and other nucleophiles could have been a route to numerous 5-position modified uracils (**2**) and their nucleic acid descendants.



2 **1** **3** **4**

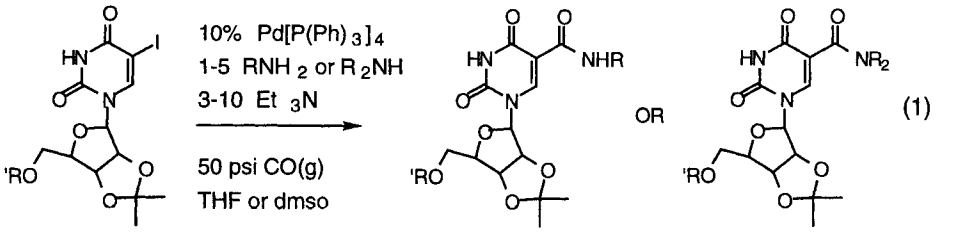
Scheme 1

An alternative and equally probable path to diversification of **1** under prebiotic conditions would be oxidation to **3** and subsequent condensation with amines to give 5-carboxamide derivatives (**4**). While most of the known RNA pyrimidine base modifications known today can be derived from the solvolysis of **1**, 5-formylcytidine may be a survivor of the structural class containing 5-carbonyl functionality.[2]

While it is entertaining to speculate on the chemical nature of the prebiotic RNA world, a more contemporary use of 5-carboxamid-uridines stems from their potential as antiviral drugs. Secondary 5-carboxamid-uridines are of particular interest, especially if the methodology used to prepare them can tolerate a variety of functional groups. Previous to our work, only primary carboxamid-uridine had been prepared, both by standard synthetic methods,[3] and by carboxyesterification in methanol followed by amidation with ammonia.[4] We desired to prepare a variety of secondary and tertiary 5-carboxamid-uridines and determine if they were in fact stable compounds, even if they contained reactive groups. Previous experience with the preparation of 5-carbonyluridines[5] and 5-carbonyl-2'-deoxyuridines[6] suggested that palladium catalyzed carboxyamidation might be possible using 5-iodouridine. In addition, precedent existed for the formation of ester[7] or carboxamide[8] products from alkenyl and aryl halides using Pd(II) catalysts. However, carboxyamidation reactions with 5-halouridines were unknown and precedent existed for direct substitution of iodide with amines to give the corresponding 5-aminouridines,[7] making it impossible to predict whether carboxyamidation or direct substitution would occur. Herein we describe the successful syntheses of a diverse array of C-5 position uridine analogs via palladium(0) catalyzed carboxyamidation.

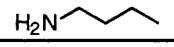
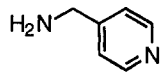
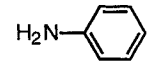
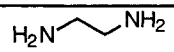
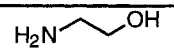
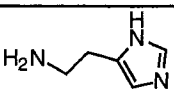
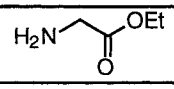
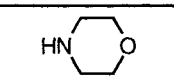
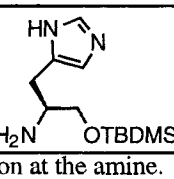
Treatment of uridines **5** - **7** (equation 1, Table 1) with a catalytic amount (10 mol%) of *tetrakis*-[triphenylphosphine]palladium(0) in either THF or DMSO, under 50 psi of CO at 70 °C, using 1 - 5 equivalents of amine and 3 - 10 equivalents of triethylamine gave the uridine analogs of Table 1 (yields not optimized) where the amide product is the result of reaction of the amine functionality. By this method, amines containing an assortment of functional groups can be introduced: amino acids, alcohols, protected amines, aryl rings, esters, ethers and heterocycles. No direct coupling products of the amine with the 5-position of the uridine were detected in the reaction mixtures. In several cases (**11** - **16**) the 5'-hydroxyl was protected to ease purification of the products, but the reaction proceeds for several amines even when the 5'-hyroxyl is not protected (**8** - **10**).

The 5-carboxamid-uridines are stable under varying conditions, including acid or base treatment. For example, the hydrolytic stability of the 5-carboxamide functionality was tested with **9**, in the presence of concentrated NH_4OH at room temperature over 48 h, during which time there was no detectable decomposition by ^1H NMR analysis. These compounds represent a new class of 5-modified uridine analogs, which may resemble



5, R'= H; **6**, R'=TBDMS; **7**, R'=DMT

TABLE 1. Summary of uridine carboxyamidation products.

Product	Starting Uridine	Amine	Yield (%)
8	**5**		65
9	**5**		89
10	**5**		42
11	**6**		78(30)#
12	**6**		61
13	**6**		68
14	**6**		80
15	**6**		68
16	**7**		69

All product yields refer to carboxyamidation at the amine. # Overall isolated yield for trifluoroacetyl-protected carboxamide from **6**.

nucleosides that bridged the prebiotic RNA and protein worlds. We are currently investigating the biological activity of these uridines as well their incorporation into oligonucleotides by use of the SELEX protocol.

Experimental:

General. *tetrakis*-[Triphenylphosphine]palladium(0) was used as received from Strem Chemicals or prepared by a literature method.[8] All compounds were prepared from

reagent grade starting materials purchased from Aldrich unless otherwise noted. 5-Iodo-2',3'-*O*-isopropylideneuridine (**5**) was prepared according to literature methods.[9-10] Product purification was accomplished on Merck grade 60 flash silica gel. NMR spectra were obtained on a 300 MHz Bruker ARX spectrometer and are reported as δ values, referenced to solvent resonances. ^{1}H NMR spectra are reported as δ values (multiplicity, *J* value, and integrated number of protons). Mass spectra were performed at the UC-Berkeley Mass Spec. Facility in Berkeley, CA. Elemental Analyses were performed by Desert Analytics in Tucson, AZ.

6. Prepared by silylation of **5** with 2 eq. of *tert*-butyldimethylsilyl chloride in pyridine at room temperature. The product was purified on silica gel with 30% ethyl acetate/hexanes. ^{1}H NMR ($CDCl_3$) δ 8.86 (s, 1H), 7.91 (s, 1H), 5.84 (d, *J* = 2.7 Hz, 1H), 4.69 (m, 2H), 4.37 (m, 1H), 3.90 (dd, *J* = 11.7, *J* = 2.3 Hz, 1H), 3.77 (dd, *J* = 11.7, *J* = 3.0 Hz, 1H), 1.56 (s, 3H), 1.33 (s, 3H), 0.88 (s, 9H), 0.10 (s, 6H).

7. To a stirred solution of 0.82 g of **5** (2.0 mmol) in 1.0 mL of anhydrous DMF and 1.8 mL of anhydrous pyridine, under argon, was added 24 mg of 4-dimethylaminopyridine (0.2 mmol) and 0.75 g of DMT-Cl (2.2 mmol). The solution was stirred at room temperature overnight, diluted with 150 mL of ethyl acetate, washed with 3x75 mL of H_2O, 1x50 mL of brine and concentrated *in vacuo*. The residue was purified on silica gel with 40% EtOAc/hexanes to give 1.28 g (90% yield) of the product as a white solid. ^{1}H NMR (dmso-d_6) δ 11.70 (s, 1H), 8.22 (s, 1H), 7.4-7.2 (m, 9H), 6.8 (m, 4H), 5.80 (d, *J* = 1.6 Hz, 1H), 4.99 (dd, *J* = 6.4, *J* = 1.6 Hz, 1H), 4.61 (m, 1H), 4.10 (m, 1H), 3.72 (s, 3H), 3.71 (s, 3H), 3.29 (m, 1H), 3.06 (m, 1H), 1.44 (s, 3H), 1.23 (s, 3H).

Standard Synthesis Procedure. In a heavy-walled glass bomb under argon, a solution was prepared of **5**, **6**, or **7**, 3 equiv. of amine, 5 equiv. of triethylamine, and 0.1 equiv. of *tetrakis*-[triphenylphosphine]palladium in 3 mL of THF. The bomb was evacuated and filled with 50 psi of CO three times, then sealed and heated at 70°C for 48 hrs. The reaction was cooled, vented carefully, the volatiles removed *in vacuo* and the residue purified on a flash silica gel column.

8. The product was purified on silica gel with 5% MeOH/CH_2Cl_2 to give a yellow solid (0.251 g, 65% yield). Analytical sample from methanol as fluffy white needles. ^{1}H NMR (dmso-d_6) δ 11.92 (br s, 1H), 8.68 (t, *J* = 5.4 Hz, 1H), 8.61 (s, 1H), 5.85 (d, *J* = 1.8 Hz, 1H), 5.09 (t, *J* = 4.5 Hz, 1H), 4.91 (dd, *J* = 6.3, 1.8 Hz, 1H), 4.74 (dd, *J* = 6.0, 2.7 Hz, 1H), 4.19 (m, 1H), 3.56 (m, 2H), 3.24 (m, 2H), 1.47 (s, 3H), 1.4 (m, 2H), 1.3 (m, 2H), 1.27 (s, 3H), 0.9 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (dmso-d_6) δ 163.3, 161.3, 149.4, 146.9, 112.5, 105.0, 92.8, 87.4, 84.4, 80.7, 61.2, 38.0, 31.2, 26.9, 25.0, 19.5,

13.6; FAB$^+$ HRMS calcd (found) for $C_{17}H_{26}N_3O_7$: 384.1771(384.1772). Anal. Calcd. (found) for $C_{17}H_{25}N_3O_7$: C, 53.26 (53.46); H, 6.57 (6.53); N, 10.96 (10.98).

9. Purified on silica gel with 5% MeOH/CH_2Cl_2 to give 0.201 g (89% yield) as a pale yellow solid. Analytical sample from methanol as white needles. ^{1}H NMR (dmso-d_6) δ 11.98 (s, 1H), 9.19 (t, J = 6.3 Hz, 1H), 8.66 (s, 1H), 8.48 (d, J = 4.5 Hz, 2H), 7.25 (d, J = 5.7 Hz, 2H), 5.86 (d, J = 2.2 Hz, 1H), 5.10 (t, J = 4.8 Hz, 1H), 4.93 (dd, J = 6.2, 2.2 Hz, 1H), 4.73 (dd, J = 6.3, 3.0 Hz, 1H), 4.49 (d, J = 6.3 Hz, 2H), 4.20 (m, 1H), 3.56 (t, J = 4.5 Hz, 2H), 1.47 (s, 3H), 1.27 (s, 3H). ^{13}C NMR (dmso-d_6) δ 163.2, 161.9, 149.5, 149.4, 148.4, 147.4 , 122.1, 112.6 , 104.8, 92.9, 87.4, 84.4, 80.7, 61.2, 41.2, 26.9, 25.0; FAB$^+$ HRMS calcd (found) for $C_{19}H_{23}N_4O_7$: 419.1567(419.1569).

10. Purified on silica gel with 4-6.5% MeOH·NH_3/CH_2Cl_2 to give an off-white solid, 0.107 g, (42% yield). Recrystrallized from methanol to give fine white needles. ^{1}H NMR (dmso-d_6) δ 12.16 (br s, 1H), 10.88 (s, 1H), 8.79 (s, 1H), 7.63 (d, J = 7.8 Hz, 2H), 7.34 (m, 2H), 7.09 (t, J = 7.4 Hz, 1H), 5.88 (d, J = 2.1 Hz, 1H), 5.16 (t, J = 4.7 Hz, 1H), 4.95 (dd, J = 6.3, 2.1 Hz, 1H), 4.76 (dd, J = 6.3, 2.7 Hz, 1H), 4.25 (m, 1H), 3.59 (m, 2H), 1.48 (s, 3H), 1.29 (s, 3H); ^{13}C NMR (dmso-d_6) δ 163.6, 159.9, 149.3, 147.8, 138.1, 129.0, 124.0, 119.5, 112.5, 104.6, 93.2, 87.6, 84.5, 80.7, 61.2, 26.9, 25.0. FAB$^+$ HRMS calcd (found) for $C_{19}H_{22}N_3O_7$: 404.1458(404.1468).

11. Purified on silica gel with 25% MeOH·NH_3/EtOAc to give 0.381 g (78% yield) of white solid. This material was protected by treatment with trifluoroacetic anhydride in anhydrous pyridine at 0°C, and purified on silica gel with 40% EtOAc/hexanes to give 0.173 g (30% yield) as a white solid. ^{1}H NMR (dmso-d_6) δ 11.95 (s, 1H), 9.48 (t, J = 5.0 Hz, 1H), 8.81 (t, 5.8 Hz, 1H), 8.49 (s, 1H), 5.75 (d, J = 1.6 Hz, 1H), 4.89 (dd, J = 6.1, 1.7 Hz, 1H), 4.67 (dd, J = 6.1, 2.2 Hz, 1H), 4.36 (m, 1H), 3.77 (m, 2H), 3.4 (m, 2H), 3.3 (m, 2H), 1.48 (s, 3H), 1.29 (s, 3H), 0.78 (s, 9H), 0.00 (s, 3H), -0.04 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 163.9, 162.7, 157.4 (q, J = 37 Hz), 149.1, 147.5, 115.9 (q, J = 288 Hz), 113.5, 104.4, 96.2, 88.4, 86.3, 81.7, 63.9, 42.1, 38.6, 27.1, 25.6, 25.0, 18.2, -5.8, -5.8. FAB$^+$ HRMS calcd (found) for $C_{23}H_{36}F_3N_4O_8Si$: 581.2254(581.2249).

12. Purified on silica gel with 6% MeOH/CH_2Cl_2 to give 0.173 g (61% yield) of colorless white solid. ^{1}H NMR (dmso-d_6) δ 11.93 (s, 1H), 8.80 (t, J = 5.6 Hz, 1H), 8.48 (s, 1H), 5.75 (d, J = 1.8 Hz, 1H), 4.89 (dd, J = 6.1, 1.8 Hz, 1H), 4.78 (t, J = 5.1 Hz, 1H), 4.67 (m, 1H), 4.34 (m, 1H), 3.76 (d, J = 3.8 Hz, 2H), 3.44 (m, 2H), 3.31 (m, 2H), 1.47 (s, 3H), 1.28 (s, 3H), 0.78 (2, 9H), -0.02 (s, 3H), -0.04 (s, 3H). ^{13}C NMR (dmso-d_6) δ 163.2 , 161.5, 149.4, 146.8, 112.3, 104.6, 94.5, 87.7, 84.9, 80.9, 63.4, 59.7, 41.2, 26.8, 25.6, 24.9, 17.9, -5.7, -5.8. Analytical sample from EtOAc/Hexanes.

FAB$^+$ HRMS calcd (found) for $C_{21}H_{36}N_3O_8Si$: 486.2272(486.2271). Anal. Calcd. (Found) for $C_{21}H_{35}N_3O_8Si$: C, 51.94 (52.03); H, 7.26 (7.36); N, 8.65 (8.61).

13. Purified on silica gel with 12% MeOH/CH_2Cl_2 to give 181.0 mg (68% yield) of a slightly yellow solid. ^{1}H NMR (dmso-d_6) δ 8.76 (t, J = 5.7 Hz, 1H), 8.47 (s, 1H), 7.52 (s, 1H), 6.78 (s, 1H), 5.73 (d, J = 1.5 Hz, 1H), 4.88 (dd, J = 6.1, 1.6 Hz, 1H), 4.66 (dd, J = 6.1, 2.1 Hz, 1H), 4.35 (m, 1H), 3.77 (m, 2H), 3.48 (m, 2H), 2.67 (t, J = 6.9 Hz, 2H), 1.46 (s, 3H), 1.28 (s, 3H), 0.77 (s, 9H), -0.01 (s, 3H), -0.04 (s, 3H). ^{13}C NMR (dmso-d_6) δ 163.2, 161.3, 149.4, 146.7, 134.7, 112.2, 111.8, 104.6, 94.6, 87.8, 84.9, 81.0, 63.4, 48.5, 27.0, 26.8, 25.6, 24.9, 17.9, -5.7; FAB$^+$ HRMS calcd (found) for $C_{24}H_{37}N_5O_7Si$: 535.2462(535.2456).

14. Purified on silica gel with 4% MeOH/CH_2Cl_2 to give 0.262 g (80% yield) of colorless white solid. ^{1}H NMR ($CDCl_3$) δ 8.96 (t, J = 5.5 Hz, 1H), 8.72 (s, 1H), 8.65 (s, 1H), 5.74 (d, J = 2.2 Hz, 1H), 4.85 (dd, J = 6.0, 2.2 Hz, 1H), 4.72 (dd, J = 6.0, 1.6 Hz, 1H), 4.51 (m, 1H), 4.22 (q, J = 7.1, 2H), 4.14 (d, J = 5.6 Hz, 2H), 3.96 (m, 1H), 3.78 (m, 1H), 1.58 (s, 3H), 1.36 (s, 3H), 1.28 (t, J = 7.1 Hz, 3H), 0.82 (s, 9H), 0.04 (s, 3H), -0.01 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 169.6, 163.1, 161.8, 149.4, 147.3, 112.3, 104.1, 94.6, 87.8, 84.9, 81.0, 63.4, 60.4, 40.8, 26.9, 25.6, 24.9, 17.9, 14.0, -5.8. Analytical sample from EtOAc/Hexanes. FAB$^+$ HRMS calcd (found) for $C_{23}H_{38}N_3O_9Si$: 528.2377(528.2382). Anal. Calcd. (Found) for $C_{23}H_{37}N_3O_9Si$: C, 52.36 (52.19); H, 7.07 (6.93); N, 7.96 (7.85).

15. Purified on silica gel with 4% MeOH/CH_2Cl_2 to give 0.202 g (68% yield) of colorless white solid. ^{1}H NMR (dmso-d_6) δ 11.65 (s, 1H), 7.90 (s, 1H), 5.80 (d, J = 2.2 Hz, 1H), 4.93 (dd, J = 6.2, 2.2 Hz, 1H), 4.68 (dd, J = 6.2, 3.4 Hz, 1H), 4.12 (m, 1H), 3.76 (m, 2H), 3.5 (br m, 6H), 3.29 (br m, 2H), 1.47 (s, 3H), 1.28 (s, 3H), 0.84 (s, 9H), 0.03 (s, 6H). ^{13}C NMR (dmso-d_6) δ 162.3, 160.3, 149.7, 142.2, 112.9, 111.1, 66.2, 65.9, 63.0, 47.0, 41.9, 26.9, 25.8, 25.1, 18.0, -5.5, -5.6. Analytical sample from EtOAc/Hexanes. FAB$^+$ HRMS calcd (found) for $C_{23}H_{38}N_3O_8Si$: 512.2428(512.2436).

16. Prepared from **7** (5'-DMT protected 5-iodouridine), and purified on silica gel with 0-5% MeOH/CH_2Cl_2 to give 0.294 g (69% yield) of a white solid. ^{1}H NMR ($CDCl_3$/CD_3OD) δ 9.20 (d, J = 8.4 Hz, 1H), 8.57 (s, 1H), 7.45 (s, 1H), 7.3-7.1 (m, 9H), 6.77 (m, 4H), 6.71 (s, 1H), 5.73 (d, J = 2.0 Hz, 1H), 4.94 (dd, J = 6.1, 2.0 Hz, 1H), 4.39 (m, 1H), 4.33 (m, 1H), 4.27 (m, 1H), 3.72 (s, 6H), 3.63 (d, J = 3.5 Hz, 2H), 3.41 (d, J = 3.8Hz, 2H), 2.94-2.74 (m, 2H), 1.49 (s, 3H), 1.25 (s, 3H), 0.89 (s, 9H), 0.03 (s, 3H), 0.02 (s, 3H). ^{13}C NMR (dmso-d_6) δ 163.2, 160.8, 158.0, 149.2, 148.0, 144.7, 135.3, 135.2, 134.7, 129.7, 129.5, 127.7, 127.5, 126.6, 113.1, 112.9, 105.2, 93.8, 86.4, 85.7, 83.9, 80.7, 63.9, 63.1, 54.9, 50.1, 28.5, 26.8, 25.7, 25.0, 17.9, -5.6, -5.6. FAB$^+$ HRMS calcd (found) for $C_{46}H_{58}N_5O_{10}Si$: 868.3953(868.3963).

REFERENCES

(1) Robertson, M. P.; Miller, S. L. *Science* **1995**, *268*, 702-705.

(2) Limbach, P. A.; Crain, P. F.; McCloskey, J. A. *Nucleic Acids Res.* **1994**, *22*, 2183-2196.

(3) Fukuda, T.; Hamana, T.; Kikuchi, K.; Marumoto, R. *Z. Naturforsch* **1986**, *41b*, 1571.

(4) Ono, A.; Haginoya, N.; Kiyokawa, M.; Minakawa, N.; Matsuda, A. *Bioorganic & Medicinal Chemistry Lett.* **1994**, *4*, 361.

(5) Dewey, T. M.; Mundt, A.; Crouch, G. J.; Zyzniewski, C.; Eaton, B. E. *J. Am. Chem. Soc.* **1995**, *117*, 8474-8475.

(6) Crouch, G. J.; Eaton, B. E. *Nucleosides Nucleotides* **1994**, *13*, 939.

(7) *Nucleic Acid Chemistry,* Eds. Townsend, L. B. and Tipson, R. S., Eds.; Wiley Interscience, New York, 1978; Vol 1, Part 1, p 59.

(8) Coulson, D. R. *Inorg. Syn.* **1972**, *13*, 121-124.

(9) Schoenberg, A.; Bartoletti, I.; Heck, R. F. *J. Org. Chem.* **1974**, *39*, 3318-3326.

(10) Schoenberg, A.; Heck, R. F. *J. Org. Chem.* **1974**, *39*, 3327-3331.

Received January 29, 1996
Accepted July 15, 1996