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An Efficient Method for the Synthesis of [4-^{15}N]Cytidine and [6-^{15}N]Adenosine Derivatives from Uridine and Inosine[1]

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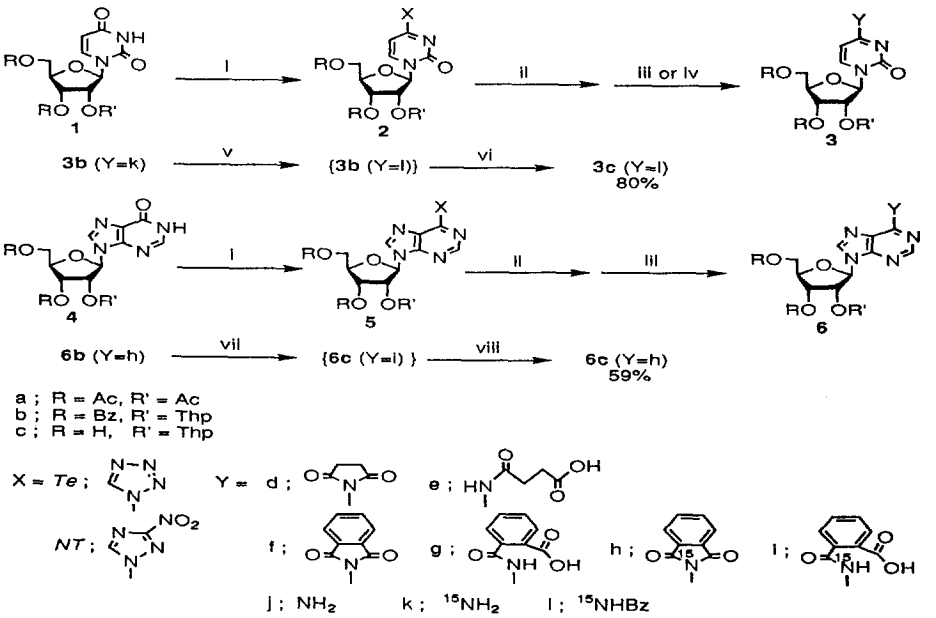
Abstract: Nucleophilic substitution reactions of 4-azolyl-1-β-D-ribofuranosylpyrimidin-2(1H)-one and 6-azolyl-9-β-D-ribofuranosyl-9H-purine derivatives, which were converted from uridine and inosine, with [^{15}N]phthalimide in the presence of triethylamine or DBU were found to give [4-^{15}N]-N^4-phthaloylcytidine and [6-^{15}N]-N^6-phthaloyladenosine derivatives, respectively, in high yields, while reactions with succinimide also afforded N^4-succinylcytidine and N^6-succinyladenosine derivatives, in high yields.

The importance of NMR spectroscopy in studies on the structure and, particularly, dynamic features of biopolymers, e.g., nucleic acids, has recently been acknowledged by the application of NMR pulse techniques in combination with the labeling of compounds with the stable isotopes of ^{13}C, ^{15}N, and ^{2}H.[2,3]

Two approaches to the chemical synthesis of ^{15}N-labeled nucleosides have been reported: the first one involves the synthesis of an appropriately ^{15}N-labeled heterocycle, followed by its glycosylation with an appropriately functionalized sugar derivative, giving the desired ^{15}N-labeled nucleoside;[4] the second one involves the chemical derivatization of an intact nucleoside to the corresponding ^{15}N-labeled nucleoside, via the reaction of an activated intermediate with [^{15}N]ammonia or [^{15}N]benzylamine.[5] The latter approach might be much more promising than the former for the synthesis of the subject. It would be synthetically advantageous if it were possible to introduce a ^{15}N-label into the exocyclic amino group of a base moiety of a nucleoside by the use of a solid nucleophile of [^{15}N]phthalimide, in place of [^{15}N]ammonia or [^{15}N]benzylamine.

We now communicate herein an efficient method for the synthesis of [4-^{15}N]cytidine (**3**) and [6-^{15}N]adenosine (**6**) derivatives from uridine and inosine derivatives, respectively, which is characterized by the nucleophilic substitution reaction of their azolyl derivatives with phthalimide or succinimide in the presence of triethylamine or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).

Treatment of 2',3',5'-tri-*O*-acetyluridine (**1a**) with 1*H*-tetrazole or 3-nitro-1,2,4-triazole (2 mol. equiv.), diphenyl phosphate (1.2 mol. equiv.), and *p*-toluenesulfonyl chloride (2 mol. equiv.) in pyridine at room temperature for 1.5 days gave the corresponding 4-tetrazolyl [**2a** (*Te*)][6] and 4-(3-nitro-1,2,4-triazolyl) [**2a** (*NT*)][6] derivatives in 88% and 94% yields, respectively. Nucleophilic displacement of the 4-tetrazolyl group of **2a** (*Te*) with succinimide (1.5 mol. equiv.) took place in the presence of triethylamine (5 mol. equiv.) in methylene chloride at room temperature for 1.5 days to give 2',3',5'-tri-*O*-acetyl-N^4-succinylcytidine [**3a** (Y = d)]. The process of the reaction was followed by TLC and the partial conversion of **3a** (Y = d) to **3a** (Y = e) was indicated, so that acetic anhydride (2 mol. equiv.) and triethylamine (4 mol. equiv.) were added to the reaction mixture to induce the ring closure of **3a** (Y = e) back to **3a** (Y = d). After



Conditions ; (i) *p*-TsCl, $(PhO)_2P(O)OH$, 1*H*-tetrazole or 3-nitro-1,2,4-triazole, pyridine, r.t.
(ii) succinimide, phthalimide or [^{15}N]phthalimide, DBU or Et_3N, CH_2Cl_2, r.t.
(iii) acetic anhydride, Et_3N, CH_2Cl_2, r.t., 1 h
(iv) 1:1 H_2O - pyridine, r.t., 1 h
(v) BzCl, pyridine, r.t., 30 min
(vi) NaOH, H_2O - EtOH - pyridine, 0 °C, 15 min
(vii) NaOH, H_2O - pyridine, 0 °C, 1 h
(viii) $(CF_3CO)_2O$, pyridine, r.t., 30 min

Scheme 1

purification by silica gel column chromatography, **3a** (Y = d) was obtained in 88% yield (See Entry 1 in Table 1). In a similar manner, the reaction of **2a** (*NT*) with succinimide (1.5 mol. equiv.) in the presence of triethylamine (5 mol. equiv.) in methylene chloride at room temperature for 2 days, followed by treatment with acetic anhydride (2 mol. equiv.) and triethylamine (4 mol. equiv.), gave **3a** (Y = d) in 79% yield (Entry 2). Interestingly, after the reaction of **2a** (*Te*) or **2a** (*NT*) with phthalimide (1.5 mol. equiv.) in the presence of triethylamine (5 mol. equiv.), the treatment of the resulting solutions with 1:1 H_2O-pyridine for 1 h induced complete unmasking of the N^4-phthaloyl group of cytidine derivative [**3a** (Y = f)] to give 2',3',5'-tri-*O*-acetylcytidine **3a** (Y = j) in 94% and 96% yields (Entries 3 and 4), respectively. Similarly, 3',5'-di-*O*-benzoyl-2'-*O*-(tetrahydro-pyran-2-yl)uridine (**1b**),[7] after introducing the tetrazolyl group at its 4-position [**2b** (*Te*); 84% yield], was subjected to the displacement with succinimide or phthalimide in the presence of triethylamine, followed by the treatment with acetic anhydride - triethylamine or 1:1 H_2O - pyridine as described above, which gave 3',5'-di-*O*-benzoyl-N^4-succinyl-2'-*O*-(tetrahydropyran-2-yl)cytidine [**3b** (Y = d, 81% yield)] (Entry 5) and 3',5'-di-*O*-benzoyl-2'-*O*-(tetrahydropyran-2-yl)cytidine [**3b** (Y = j, 96% yield)] (Entry 7), respectively. Similar reactions using DBU[8] in the place of triethylamine took place more efficiently, although the reactions resulted in inevitable formation of a small amount of several by-products (Entries 6 and 8), which were impossible to purify.

Table 1 Synthesis of cytidine (**3**) and adenosine (**6**) derivatives.

Entry	Starting Materials	Imide (mol. equiv.)	Base (mol. equiv.)	Time (day)	Products	Yield (%)
1	**2a** (*Te*)	Suc (1.5)	Et3N (5.0)	1.5	**3a** (Y=d)	88[a]
2	**2a** (*NT*)	Suc (1.5)	Et3N (5.0)	2	**3a** (Y=d)	79[a]
3	**2a** (*Te*)	Phth (1.5)	Et3N (5.0)	1	**3a** (Y=j)	94[b]
4	**2a** (*NT*)	Phth (1.5)	Et3N (5.0)	1	**3a** (Y=j)	96[b]
5	**2b** (*Te*)	Suc (2.0)	Et3N (5.0)	3	**3b** (Y=d)	81[a]
6	**2b** (*Te*)	Suc (2.0)	DBU (1.2)	2 h	**3b** (Y=d)	79[a]
7	**2b** (*Te*)	Phth (1.5)	Et3N (5.0)	1	**3b** (Y=j)	96[b]
8	**2b** (*Te*)	Phth (1.5)	DBU (1.2)	30 min	**3b** (Y=j)	83[b]
9	**2b** (*Te*)	[^{15}N]Phth (1.5)	Et3N (5.0)	1	**3b** (Y=k)	96[a]
10	**5a** (*NT*)	Suc (2.0)	Et3N (5.0)	2	No Reaction	
11	**5a** (*NT*)	Suc (2.0)	DBU (3.0)	2	**6a** (Y=d)	60[a]
12	**5a** (*NT*)	Phth (2.0)	Et3N (5.0)	2	No Reaction	
13	**5a** (*NT*)	Phth (2.0)	DBU (3.0)	1	**6a** (Y=f)	74[a]
14	**5b** (*NT*)	Suc (2.0)	DBU (3.0)	1	**6b** (Y=d)	72[a]
15	**5b** (*NT*)	Phth (2.0)	DBU (3.0)	1	**6b** (Y=f)	88[a]
16	**5b** (*NT*)	[^{15}N]Phth (2.0)	DBU (3.0)	2	**6b** (Y=h)	99[a]

Suc = Succinimide Phth = Phthalimide [^{15}N]Phth = [^{15}N]Phthalimide

a) Yield of *N*-succinyl or phthaloyl derivative after the nucleophilic displacement reaction and treatment with acetic anhydride - triethylamine.

b) Yield of cytidine derivative after the nucleophilic displacement reaction and deprotection of the N^4-phthaloyl group by treatment with 1 : 1 H_2O - pyridine.

[4-^{15}N]-3',5'-Di-*O*-benzoyl-2'-*O*-(tetrahydropyran-2-yl)cytidine [**3b** (Y = k)] was prepared in 96% yield (Entry 9) by the nucleophilic substitution of **2b** (*Te*) with [^{15}N]phthalimide in the presence of triethylamine and subsequent unmasking of the N^4-phthaloyl group. Compound **3b** (Y = k) was further subjected to N^4-benzoylation and *O*-debenzoylation as usual to give [4-^{15}N]-N^4-benzoyl-2'-*O*-(tetrahydropyran-2-yl)cytidine [**3c** (Y = l)], which is a useful intermediate for the RNA oligonucleotide synthesis, in 80% yield.

2',3',5'-Tri-*O*-acetylinosine (**4a**) was treated with 1*H*-tetrazole or 3-nitro-1,2,4-triazole (2 mol. equiv.), diphenyl phosphate (1.2 mol. equiv.), and *p*-toluenesulfonyl chloride (2 mol. equiv.) in pyridine at room temperature. Although the reaction with 1*H*-tetrazole was unsuccessful, that with 3-nitro-1,2,4-triazole for 7 days gave 6-(3-nitro-1,2,4-triazolyl) derivative [**5a** (*NT*)][9] in 84% yield. Nucleophilic displacement reaction of the 3-nitro-1,2,4-triazolyl group of **5a** (*NT*) with succinimide or phthalimide (2 mol. equiv.) took place in the presence of DBU (3 mol. equiv.) in methylene chloride at room temperature to give 2',3',5'-tri-*O*-acetyl-N^6-succinyl- [**6a** (Y = d) in 60% yield; Entry 11] or -phthaloyladenosine [**6a** (Y = f) in 74% yield; Entry 13], after treatment with acetic anhydride - triethylamine and subsequent purification by silica gel column chromatography. Similarly, 3',5'-di-*O*-benzoyl-2'-*O*-(tetrahydropyran-2-yl)inosine (**4b**)[10] was, after introducing the 3-nitro-1,2,4-triazolyl group at 6-position of the hypoxanthine moiety [**5b** (*NT*); 79% yield], subjected to the displacement reaction of the 3-nitro-1,2,4-triazolyl group with succinimide or phthalimide in the presence of DBU followed by treatment with acetic anhydride - triethylamine to give 3',5'-di-*O*-benzoyl-N^6-succinyl- [**6b** (Y = d) in 72% yield; Entry 14] or -phthaloyl-2'-*O*-(tetrahydropyran-2-yl)adenosine [**6b** (Y = f) in 88% yield; Entry 15]. [6-^{15}N]-3',5'-Di-*O*-benzoyl-N^6-phthaloyl-2'-*O*-(tetrahydropyran-2-yl)adenosine [**6a** (Y = h)] was prepared by the displacement reaction of **5b** (*NT*) with [^{15}N]phthalimide as described above in 99% yield (Entry 16). *O*-Debenzoylation of **6b** (Y = h) to **6c** (Y = i), followed by treatment with trifluoroacetic anhydride (5 mol. equiv.) in pyridine, gave

[6-^{15}N]-N^6-phthaloyl-2'-*O*-(tetrahydropyran-2-yl)adenosine [**6c** (Y=h), 59% overall yield], which is a synthetic intermediate for oligoribonucleotides.

It is thus concluded the present procedure provides us with very useful synthetic intermediates leading to an oligoribonucleotide functionalized by ^{15}N labels in the exocyclic amino groups of cytidine and adenosine. In addition, it is an alternative way for N^6-succinyl protection of 2'-deoxyadenosine, which has been reported as a countermeasure for minimizing the depurination in the course of an oligodeoxyribonucleotide synthesis.[11]

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