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A HIGHLY EFFECTIVE ROUTE TO N,N'–DISUBSTITUTED UREAS UNDER MILD CONDITIONS. AN APPLICATION TO THE SYNTHESIS OF tRNA ANTICODON LOOP FRAGMENTS CONTAINING UREIDONUCLEOSIDES.

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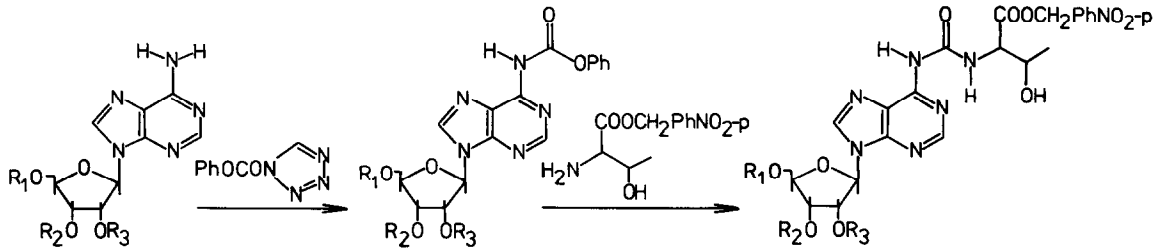
None of the methods described for synthesis of N,N'-disubstituted urea system which is present in hypermodyfied nucleosides (known as ureidonucleosides e. g. N^6-(N-threonylcarbonyl)adenosine) can be applied to such fragile and rich in reactive sites molecule as protected oligoribonucleotide. Two methods used up to now on nucleoside level [1,2] proceed in drastic conditions and give moderate yields.

We wish to report an efficient method for the synthesis of phenyl carbamates (IIa, b) and their quantitative aminolysis by appropriate amino acid ester under mild conditions as a new route to naturally occurring N,N'-disubstituted ureas (IIIa, b).

We have found that phenyl chloroformate in anhydrous pyridine, a reagent previously proposed [2,3] for synthesis of IIa, can not be generally used because of its high reactivity towards N–H bonds present in suitable protected uridine and N-acylated cytidine, adenosine and guanosine building units for oligoribonucleotide synthesis. As a result several side reactions were observed including degradation of guanosine derivatives.

To avoid these difficulties we have developed phenoxycarbonyltetrazole [4,5] as a crystalline reagent capable to transform free exo–NH_2 group of adenosine residue to a corresponding phenyl carbamate but unreactive towards the mentioned above N–H bonds (even if used in large excess).



I II III

a : $R_1 = R_2 = R_3 = Ac$ b : R_1 = 5'-O-monomethoxytrityl-2'-O-acetyluridine-3'(2,2,2-trichloroethyl)-phosphoryl, R_2, R_3 = $>C(CH_3)_2$

Suitable protected nucleoside Ia and dinucleosidemonophosphate Ib [6] as model substrates carrying free exo–NH_2 group react with 3 equiv of phenoxycarbonyltetrazole in anhydrous dioxane for 18 hr at 37° C to produce phenyl carbamates (IIa, b) as a sole products (TLC analysis). Isolation from excess of reagent [7] by silica gel short column chromatography in chloroform containing methanol followed by precipitation from hexane afforded IIa (90% yield) and IIb (88% yield). Phenyl carbamates (IIa, b) after aminolysis with 3 equiv of crystalline L-threonine p-nitrobenzyl ester [8] in anhydrous dioxane for 18 hr at 37° C gave N,N'-disubstituted ureas IIIa and IIIb, when isolated as above, in 92 and 91% yields respectively.

Structures of all synthesized compounds were proved by PMR, UV and IR spectroscopy [9] and elemental analyses.

The noteworthy features of this method are; (1) quantitative transformations in both steps (indicated by TLC) under mild conditions, (2) no side products formation and (3) stability of all protecting groups used in our approach to oligoribonucleotide synthesis [10]. Thus apart from other applications it opens the way to a chemical synthesis of anticodon loop of tRNA's containing hypermodyfied nucleosides with N,N'-disubstituted urea system.

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4. Prepared from equimolar amounts of phenyl chloroformate, tetrazole and triethylamine in anhydrous dioxane when stirred for 3 min. at 10° C. Triethylamine hydrochloride was filtered, solution evaporated without heating and residue crystallized from chloroform–hexane. Yield 92% (m. p. 126-8° C ; IR ($CDCl_3$)cm^{-1} 3160 (N–H), 1830 and 1805 (C=O) ; PMR ($CDCl_3$) δ 9.35(1H, s, 5H-tetrazole), 7.43(5H, m, phenyl).

5. Other applications of aryloxy(alkoxy)carbonyltetrazoles are under studies.

6. 5'-O-Monomethoxytrityl-2'-O-acetyluridine-3'(2,2,2-trichloroethyl)-phosphate [10] (1 equiv) and 2',3'-isopropylideneadenosine (1.05 equiv) coupled in the presence of 2,4,6-triisopropylbenzenesulfonyltetrazole [11] (3 equiv) in anhydrous pyridine for 3 hr at 22° C gave, after isolation by silica gel short column chromatography and precipitation from hexane, Ib in 71% yield. No N-phosphorylation was observed (to be published).

7. IIa can be alternatively isolated (92% yield) by fractional crystalization of excess of phenoxycarbonyltetrazole in hexane containing chloroform (10-20%).

8. This ester was choosen (m. p. 85° C, prepared as for glycine acc. to H. Schwartz and K. Arakawa, J. Amer. Chem. Soc., 81, 5691 (1959)) to ensure synchronization with all protecting groups present in oligoribonucleotide to be synthesized. L-Threonine hydroxyl group was not protected to avoid a steric hindrance during aminolysis.

9. Diagnostic spectral properties :
IIa, PMR ($CDCl_3$) δ 9.31(1H, s, N^6–H, rapid exchange in D_2O), 8.88(1H, s, H–8), 8.26(1H, s, H–2), 7.55(5H, m, phenyl), 6.30(1H, d, J=5.5 Hz, H–1') ; IR ($CDCl_3$)cm^{-1} 3410(N–H), 1755(C=O carbamate & acetyl).
IIb, PMR ($CDCl_3$) δ 9.56(1H, s, N^6–H, rapid exchange in D_2O), 8.85(1H, s, H–8), 8.26(1H, s, H–2), 7.65(1H, d, J=7.0 Hz, H–6 uridine), 2.10(3H, s, Ac), 1.61, 1.36(6H, 2s, isopropylidene).
IIIa, PMR ($CDCl_3$) δ 10.40(1H, d, J=9.0 Hz, αN–H, slow exchange in D_2O), 9.45(1H, s, N^6–H, rapid exchange in D_2O), 8.60(1H, s, H–8), 8.51(1H, s, H–2), 6.28(1H, d, J=5.5 Hz, H–1'), 5.35(2H, s, CH_2PhNO_2-p), 1.36(3H, d, J=6.0 Hz, Me-Thr) ; UV (pH 12.3) λ_{max} nm (ϵ) 270 (27700), 276 (25300), 300 (7100).
IIIb, PMR ($CDCl_3$) δ 10.36(1H, d, J=9.0 Hz, αN–H, slow exchange in D_2O), 9.23(1H, s, N^6–H, rapid exchange in D_2O), 8.60(1H, s, H–8), 8.33(1H, s, H–2), 2.14(3H, s, Ac), 1.61, 1.38(6H, 2s, isopropylidene), 1.33(3H, d, J=6.0 Hz, Me-Thr) ; UV (pH 12.3) λ_{max} nm (ϵ) 269 (40400), 276 (35600), 300 (8000).

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