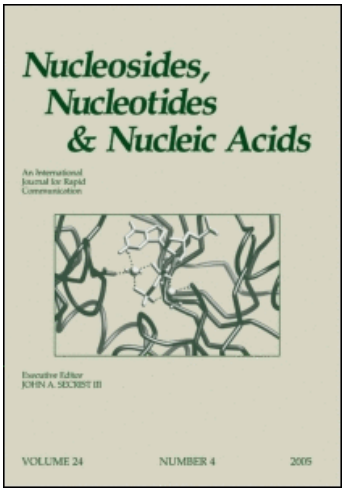

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Synthesis of Monomers Bearing at the 2′-Position Cyanomethoxycarbonyl Group for Phosphoramidite Oligonucleotide Synthesis

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ABSTRACT

A novel series of phosphoroamidites for the synthesis of 2′-modified oligonucleotides was designed and synthesized on the base of 2′-amino uridine and 2′-amino arabinoadenosine. The amino groups in these compounds were acidified by bis-cyanomethyl esters of different dicarbonic acids. Generated reactive linker groups containing cyanomethoxycarbonyl groups are stable under conditions of oligonucleotide synthesis but could be easily functionalised in post-synthetic stage by treatment with compounds bearing primary amino groups.

Key Words: Modified nucleosides; Cyanomethyl esters, 2′-amino uridine, 2′-amino arabinoadenosine, Cyanomethoxycarbonyl; Reactive linker groups.

INTRODUCTION

For the preparation of 2′-modified ODN a post-synthetic functionalization method was suggested[1] as it has one obvious advantage: the same parent compound could be applied to construct a vast number of differently modified products. This makes the approach especially favourable in scenarios where an optimisation process and thus, a

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pool of functionalized oligonucleotides are needed.[2] Toward this strategy we have incorporated one or two reactive methoxyoxalamido groups into the 2′-position of phosphoramidites on the base of uridine and cytidine.[3] These monomers for the phosphoramidite method of oligonucleotide synthesis allow the formation of conjugates of oligonucleotides with different reagents containing primary amino groups. Now we report the synthesis of a novel series of monomers bearing cyanomethoxycarbonyl group in the 2′-position of the nucleoside, connected by linkers of different length.

RESULTS AND DISCUSSION

The ester carbonyl carbon of the cyanomethoxycarbonyl residue as of the methoxyoxalyl residue is highly electrophilic due to the electron withdrawing effects of the adjacent methoxy and carbonyl groups. Indeed, rapid and quantitative reactions are observed with strong nucleophiles such as primary amines, ammonia or the hydroxyl anion.[4] On the other hand cyanomethyl esters of carbonic acids are stable towards the reagents used in solid-phase phosphoramidite synthesis as has been previously shown by Kohgo et al.[5] The introduction of monomers containing in the 2′-position the methoxyoxalamido group into an oligonucleotide requires the increase of the time of condensation and usage of 5-ethyltho tetrazole due to the steric difficulties. The cyanomethoxycarbonylamido group structure seems more flexible and should avoid the above mentioned problems.

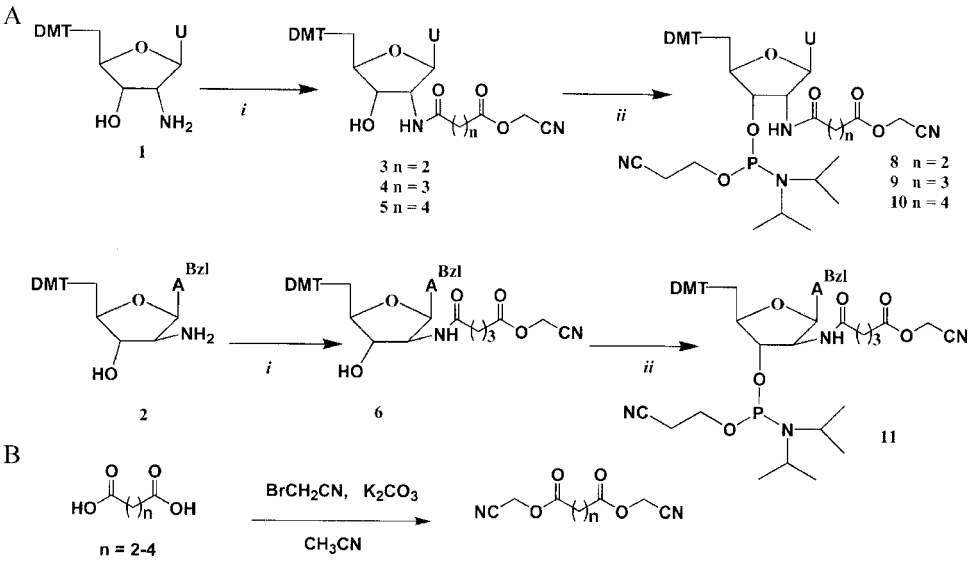

Figure 1. A: Synthesis of nucleosides containing in 2′-position reactive cyanomethyl esters of various carbonic acids and their phosphoramidites: i) $NCCH_2OC(O)-(CH_2)n-C(O)OCH_2CN$, where n = 2–4, DIPEA, CH_3CN; ii) 2-cyanoethyl N,N,N′,N′-tetraisopropylphosphoramidite, $NH(iPr)_2$ tetrazole. B: Synthesis of bis-cyanomethyl esters of succinic (n = 2), glutaric (n = 3), adipic (n = 4) acids.

The cyanomethyl esters of the succinic, glutaric, adipic acids were introduced into the 2′-position of 2′-amino-2′-deoxyuridine, and the cyanomethyl ester of the glutaric acid into the 2′-position of 9-(2-amino-2-deoxy-β-D-arabinofuranosyl)-N^6-benzoyl adenine[6] (Fig. 1A). The 2′-amino group in compound 1 reacted with bis-cyanomethyl esters of varies dicarbonic acids prepared in situ in the presence of diisopropylehyl-amine (DIPEA) at room temperature. Bis-cyanomethyl esters of succinic, glutaric, adipic acids were prepared as follow (Fig. 1B): potassium carbonate and bromoacetonitrile were added to a solution of dicarbonic acid in anhydrous CH_3CN. The reaction mixture was stirred at room temperature for 12 h, the precipitate was removed and then washed with CH_3CN. Filtrates were used for reaction with 2′-amino-2′-deoxyuridine without additional purification. The reaction with 9-(2-amino-2-deoxy-β-D-arabinofuranosyl)-N^6-benzoyl adenine 2 was analogous. Nucleosides 3–7 were obtained in yields of 40, 30, 46, 47, 30% respectively. In this series we also synthesized the derivative of 2′-amino-2′-deoxyuridine bearing the monomethyl ester of malonic acid. Its reactivity is lower than the cyanomethyl esters, though it is reactive enough for successful functionalisation by primary amines.

GENERAL METHOD OF PREPARATION OF NUCLEOSIDES BEARING REACTIVE LINKER GROUPS

The nucleoside bearing an amino group at the 2′-position (1 mmol) was added to the bis-cyanomethyl ester of the corresponding dicarbonic acid prepared in situ (1.2 eq, 0.5 M CH_3CN solution) in the presence of DIPEA (2.4 eq) at room temperature. After 68 h, the reaction was complete (as monitored by TLC). The reaction mixture was evaporated to dryness in vacuo. The crude product was purified by silica-gel column chromatography using CH_2Cl_2 as eluent. The corresponding phosphoramidites were prepared using a standard procedure (Table 1).

5′-O-DMT-2′-deoxy-2′-(O-cyanomethyl)succinamidouridine 3: R_f 0.6 [CH_2Cl_2–CH_3OH (9:1)]; mp 98–102°C; UV (EtOH), λ_{max}/nm: 202 (73022), 234 (19584), 266 (8919). The yield of phosphoramidite 8 is 35%; R_f 0.7 [CH_2Cl_2–CH_3OH–Et_3N (9.4:0.5:0.1)]; ^{31}P NMR [$(CD_3)_2CO$]: δ 151.4, 152.4.

5′-O-DMT-2′-deoxy-2′-(O-cyanomethyl)glutaramidouridine 4: R_f 0.56 [CH_2Cl_2–CH_3OH (9:1)]; mp 90–95°C; UV (EtOH), λ_{max}/nm: 203 (76009), 234 (22519), 265 (10925). The yield of phosphoramidite 9 is 88%, R_f 0.63 [$CH_2Cl_2CH_3OH$–Et_3N (9.4:0.5:0.1)]; ^{31}P NMR [$(CD_3)_2CO$]: δ 151.2, 152.3.

5′-O-DMT-2′-deoxy-2′-(O-cyanomethyl)adipinamidouridine 5: R_f 0.7 [CH_2Cl_2–CH_3OH (9:1)]; mp 95–97°C; UV (EtOH), λ_{max}/nm: 201 (94372), 235 (24376), 266 (12450). The yield of phosphoramidite 10 is 47%, R_f 0.92 [CH_2Cl_2–CH_3OH–Et_3N (9.4:0.5:0.1)]; ^{31}P NMR [$(CD_3)_2CO$]: δ 151.3, 152.4.

9-[5′-O-DMT-2′-deoxy-2′-(O-cyanomethyl)glutaramido-β-D-arabinofuranosyl]-N^6-benzoyladenine 6: R_f = 0.43 [CH_2Cl_2–CH_3OH–Et_3N (9.4:0.5:0.1)]; UV (EtOH), λ_{max}/nm: 206 (60080), 237 (27091), 277 (13571). The yield of phosphoramidite 11 is

Table 1. ^{1}H-NMR spectra of nucleosides bearing reactive linker groups at the 2′-position.*

Compound	U (A^{Bz})	DMTr-, Bz	1′-H (1H)	2′-H (1H)	3′-H (1H)	4′-H (1H)	5′-H (2H), $-(CH_2)_n-$	$-O-CH_2-CN$
3	8.07 [d, 6(U), $J_{5,6}$ 8.00], 5.38 [d, 5(U), $J_{5,6}$ 8.00]	7.46–6.88 (m, 9H), 6.93 (d, 4H, *J* 9.00), 3.79 (s, 6H, $-CH_3$)	5.87 (d, $J_{1',2'}$ 4.00)	4.92 (m)	4.73 (m)	4,17 (dd, $J_{3',4'}$ 3.00, $J_{4',5'}$ 3.50)	3.87 [m, NHC(O)CH_2CH_2–], 3.62–3.42 [m, 4H, 2H–5′, NHC(O)CH_2CH_2–]	4.84 (d, OCH_2CN, *J* 3.00)
4	8.07 [d, 6(U), $J_{5,6}$ 8.00], 5.40 [d, 5(U), $J_{5,6}$ 8.00]	7.46–6.88 (m, 9H), 6.93 (d, 4H, *J* 9.00), 3.78 (s, 6H, $-CH_3$)	5.87 (d, $J_{1',2'}$ 4.00)	4.95 (m)	4.74 (m)	4,20 (dd, $J_{3',4'}$ 3.00, $J_{4',5'}$ 3.50)	3.89 [m, NHC(O)$(CH_2)_3CH_2$–], 3.62–3.42 [m, 4H, 2H–5′; NHC(O)$CH_2(CH_2)_3$–], 2.56 [br, NHC(O)$CH_2CH_2CH_2$–]	4.84 (d, OCH_2CN, *J* 3.00)
5	8.07 [d, 6(U), $J_{5,6}$ 8.00], 5.40 [d, 5(U) $J_{5,6}$ 8.00]	7.46–6.88 (m, 9H), 6.93 (d, 4H, *J* 9.00), 3.78 (s, 6H, $-CH_3$)	5.87 (d, $J_{1',2'}$ 4.00)	4.93 (m)	4.75 (m)	4,20 (dd, $J_{3',4'}$ 3.00, $J_{4',5'}$ 3.50)	3.89 [m, NHC(O)$(CH_2)_3CH_2$–], 3.62–3.42 [m, 4H, 2H–5′; NHC(O)$CH_2(CH_2)_3$–], 2.75 [s, 4H, NHC(O)$CH_2CH_2(CH_2)_2$–; NHC(O)$(CH_2)_2CH_2CH_2$–]	4.84 (d, OCH_2CN, *J* 3.00)

6	8.51 [s, 8(A)], 8.46 [s, 2(A)]	7.77–7.15 (m, 14H, 9H–DMTr, 5H–Bz), 7.85–6.80 (m, 4H), 3.77 (s, 6H, $-CH_3$)	6.61 (d, $J_{1',2'}$ 6.00)	4.95 (d, $J_{1',2'}$ 6.00)	4.58 (m)	4,14 (m)	3.86–3.70 [m, NHC(O)$(CH_2)_2$CH_2–], 3.60–3.39 [m, 4H, 2H–5′; NHC(O)C$H_2(CH_2)_2$–], 2.85 [m, NHC(O)CH_2CH_2CH_2–];	5.35 (s, OCH_2CN)
7	7.74 [d, 6(U), $J_{5,6}$ 8.00], 5.37[d, 5(U), $J_{5,6}$ 8.00]	7.51–7.32 (m, 9H), 6.92–6.87 (d, 4H, *J* 9.00), 3.77 (s, 6H, $-CH_3$)	6.07 (d, $J_{1',2'}$ 9.00)	4.87 (m)	4.47 (dd, $J_{2',3'}$ 6.00, $J_{3',4'}$ 2.20)	4,18 (m)	3.50–3.30 [m, 4H, 2H–5′, NHC(O)CH_2–]	3.64 (s, OCH_3)

*^{1}H-NMR spectra were obtained on a ''Bruker AM-400'' using TMS as an internal standard, [$(CD_3)_2$CO]: δ, *j*(Hz).

30%; R_f 0.75 [CH_2Cl_2–CH_3OH–Et_3N (9.65:0.25:0.1)]; ^{31}P NMR [$(CD_3)_2CO$]: δ 150.2, 150.0.

5′-O-DMT-2′-deoxy-2′-(O-Me)malonamidouridine 7. R_f 0.33 [CH_2Cl_2–CH_3OH–Et_3N (9.4:0.5:0.1)]; UV (EtOH), λ_{max}/nm: 204 (63398), 235 (20670), 269 (8008). The yield of phosphoramidite 12 is 67%; R_f 0.43 [CH_2Cl_2–CH_3OH–Et_3N (9.4:0.5:0.1)]; ^{31}P NMR [$(CD_3)_2CO$]: δ 151.2, 150.2.

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