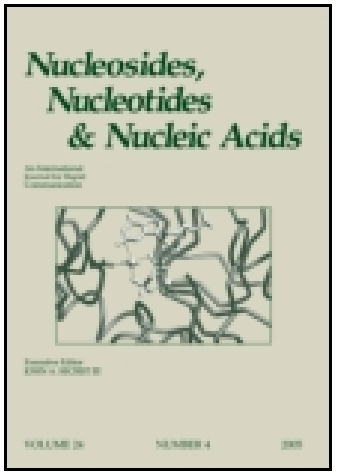

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Synthesis of Polyaminooligonucleotides and Their Combinatorial Libraries

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SYNTHESIS OF POLYAMINOOLIGONUCLEOTIDES AND THEIR COMBINATORIAL LIBRARIES

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ABSTRACT: A synthesis of phosphoramidites of 2'-deoxyadenosine and 2'-deoxyguanosine carrying a protected spermine moiety at *N*-6 and *N*-2 positions respectively is described. An approach to analyse properties of polyaminooligonucleotides using their synthetic combinatorial libraries is described and discussed. A synthesis of a polyaminooligonucleotide combinatorial library was carried out and the analysis of the library clearly showed that the presence of spermine moieties in oligodeoxyribonucleotides increases stability of their complexes.

Studies on oligonucleotides carrying polyamine residues of different structure at various positions of oligonucleotide chains were recently reported as modifications that ease forming stable duplex and/or triplex structure with natural DNA[1-3]. Thus, both 5'- and 3'-end modifications with natural polyamines were prepared and their analysis confirmed that formation of a triplex with double stranded DNA was easier than with unmodified oligodeoxynucleotides[4,5]. Polyamine moieties were attached either *via* an appropriate linker to a 5'- or 2'-hydroxyl function of a sugar moiety or directly to a heterocyclic base residue[4-14].

RESULTS AND DISCUSSION

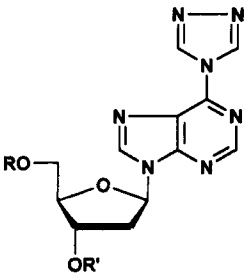
We undertook systematic studies of polyaminooligonucleotides as a promising class of oligonucleotide analogues which might be useful in antisense and antigene therapy. We decided to start with polyaminooligonucleotides carrying polyamine moieties at nucleobase residues. An attachment of a substituent either at exo-*N*-positions of nucleobases or at *C*-5 of uracil moiety should not disturb Watson-Crick base pairing. In our approach we rely on using phosphoramidites of deoxynucleosides carrying

protected polyamine moieties rather than on applying polyaminonucleobase precursors *e.g.* "tethered" oligonucleotides. This approach gives a possibility to use different polyamine modifications in a same oligonucleotide chain. It should also allow for a better control of a purity of a final product. The latter condition is of a special importance for us as we intend to use synthetic polyaminooligonucleotide combinatorial libraries of (SOCL) approach to find oligonucleotides with desired properties[13-15].

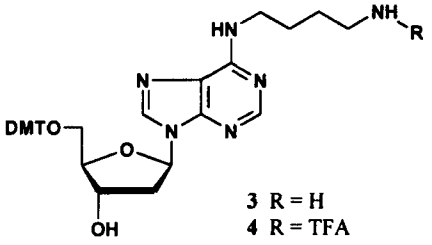
Thus, we undertook a synthesis of 3'-phosphoramidites of 2'-deoxynucleosides carrying a protected polyamine moiety at pyrimidine or purine residues. Recently, we described a new method of synthesis of a phosphoramidite of 2'-deoxycytydine carrying a protected spermine moiety at *N*-4 position[15]. We reported also a synthesis of a polyaminooligonucleotide combinatorial library (SOCL) which contained such modified deoxycytidine units[15]. The analysis of the above library clearly showed that the presence of spermine moieties in oligodeoxyribonucleotides increases stability of their duplexes and that the combinatorial approach can be very useful in studies of this class of compounds[15]. In this communication syntheses of 3'-phosphoramidites of deoxyadenosine and deoxyguanosine carrying spermine residues at *N*-6 and *N*-2 positions respectively are presented.

6-(1,2,4-triazol-4-yl)purine 2'-deoxyribonucleoside[16,17] (**1**) was prepared from deoxyadenosine as described and then converted into its 5'-*O*-dimethoxytrityl derivative (**2**). Then, **2** was reacted with putrescine in pyridine at 70 °C to give 6-*N*-(5-azapentane-1-yl) derivative **3** as a main product. **3** was transformed into trifluoroacetyl derivative **4** when reacted with trifluoroacetic anhydride in pyridine. Its structure was confirmed by ^{1}H and ^{19}F NMR spectra. Then, **2** was reacted with spermine as above and resulted **5** was trifluoroacetylated as before to give **6** in 70% overall yield after chromatographic purification. NMR spectra of **6** confirm that a polyamine chain is attached to a purine residue through a terminal amino group. Then, the standard phosphitylation procedure with bis(*N*,*N*-diisopropylamino)(2-cyanoetoxy)phosphine and tetrazole gave a phosphoramidite **7**.

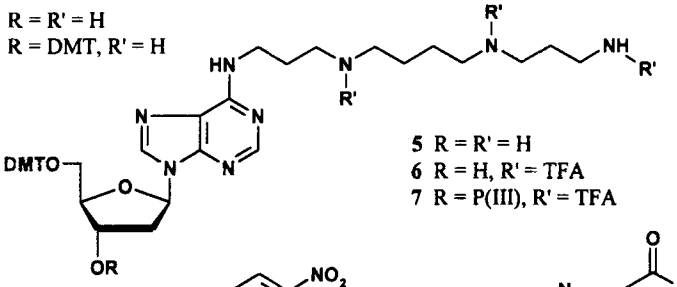
Introduction of *N*-2-polyamine deoxyguanosine units into oligonucleotides was reported previously[8,12]. However, all reports are based on a use of *O*-6-(*p*-nitrophenylethyl)-2-fluorodeoxyinosine units as tethers which were converted into *N*-2-polyamine deoxyguanosine units during a final deprotection of oligonucleotide synthesis. Thus, *O*-6-(*p*-nitrophenylethyl) deoxyguanosine (**8**) was obtained from trimethylsilyl protected deoxyguanosine (hexamethyldisalazane/DMF) and *p*-nitrophenylethanol[12]. **8** was converted into *O*-6-(*p*-nitrophenylethyl)-2-fluorodeoxyinosine (**9**) as described[8,12,18-21] using hydrogen fluoride in pyridine and t-butylnitrite at low temperatures (-50 to -25 °C). However, we could not achieve high yields. Additionally, a by-product, *O*-6-(*p*-



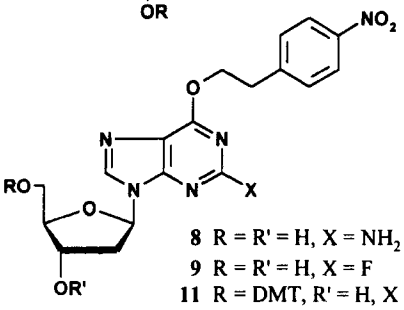
1 R = R' = H
2 R = DMT, R' = H



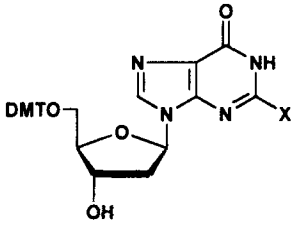
3 R = H
4 R = TFA



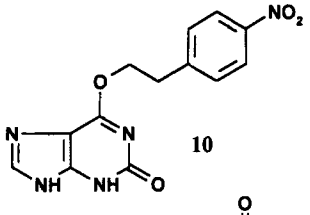
5 R = R' = H
6 R = H, R' = TFA
7 R = P(III), R' = TFA



8 R = R' = H, X = NH_2
9 R = R' = H, X = F
11 R = DMT, R' = H, X = F



12 X = $NH(CH_2)_4NH_2$
13 X = $NH(CH_2)_4NHTFA$
15 X = F



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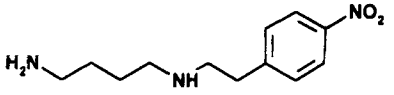
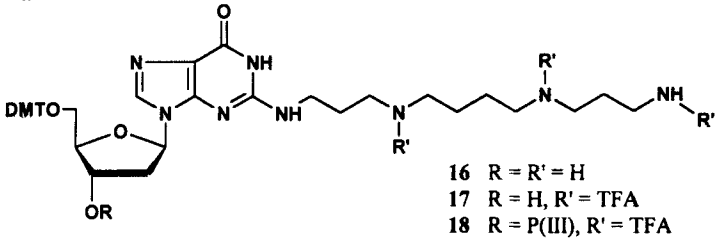


14



16 R = R' = H
17 R = H, R' = TFA
18 R = P(III), R' = TFA

DMT = 4,4'-dimethoxytrityl; TFA = trifluoroacetyl; P(III) = $-P(OCH_2CH_2CN)N(i\text{-}Pr)_2$

nitrophenylethylxanthine (**10**), was isolated, presumably as a product of depurination of a hydrolysed intermediate diazonium salt produced from **8**. Then, **9** was transformed into dimethoxytrityl derivative **11** and reacted overnight with putrescine in pyridine at 70 °C to give **12** (characterised as its TFA derivative **13**). However, when **8** was treated with putrescine a formation of 4-(3,8-diazaoctyl)nitrobenzene (**14**) resulting presumably from addition of 4-nitrostyrene to putrescine was observed. We are aware of a formation of impurities of a similar structure that could contaminate polyaminodeoxyguanosine phosphoramidites. Therefore, we decided to perform a polyamine substitution reaction on *O*-6-unprotected 2-fluorodeoxyinosine derivative **15** obtained after DBU treatment of **11**. Thus, treatment of **15** in pyridine with spermine (2 days, 70°C) gave desired **16**. Its trifluoroacetylation gave **17** which was then phosphitylated to produce the desired phosphoramidite **18**.

EXPERIMENTAL

All the solvents used in the reactions were purified and dried as described in earlier published papers. Spermine (Fluka) and trifluoroacetic anhydride (Merck) were used directly. ^{1}H NMR spectra were recorded on a Varian Unity 300 NMR spectrometer operating at 299.9 MHz using tetramethylsilane (TMS) as an internal standard. ^{31}P and ^{19}F NMR spectra were recorded on the same apparatus operating at 121.4 or 282,2 MHz respectively using either 85% ortophosphoric acid or trifluoroacetic acid as external standards. Mass spectra were determined on AMD Interactra (Germany) Spectrometer Model 402.

6-(1,2,4-triazol-4-yl)purine 2'-deoxyribonucleoside (1)

^{1}H NMR (DMSO-d_6): δ (ppm) 9.65 (s, 2H, H-3, H-5 from triazole), 9.02 (s, 1H, H-8), 8.94 (s, 1H, H-2), 6.54 (t, 1H, J 6.3 Hz, H-1'), 5.43 (d, 1H, J 4.5 Hz, OH-3'), 5.03 (t, 1H, J 5.4 Hz, OH-5'), 4.51-4.45 (sextet, 1H, J 1.8 Hz, H-3'), 3.95-3.91 (q, 1H, J 3.3 Hz, H-4'), 3.69-3.52 (m, 2H, H-5', H-5"), 2.85-2.76 (m, 1H, H-2'), 2.46-2.38 (m., 1H, H-2").

^{13}C NMR (DMSO-d_6/D_2O): δ (ppm) 153.28 (C-4), 151.99 (C-2), 146.00 (C-8), 142.69 (C-6), 141.03 (2C of triazole), 122.67 (C-5), 88.09 (C-4'), 84.27 (C-1'), 70.42 (C-3'), 61.31 (C-5'), 39.49 (C-2').

5'-*O*-Dimethoxytrityl-6-(1,2,4-triazol-4-yl)purine 2'-deoxyribonucleoside (2)

^{1}H NMR (DMSO-d_6): δ (ppm) 9.65 (s, 2H, H-3, H-5 of triazole), 8.91 (s, 1H, H-8), 8.84 (s, 1H, H-2), 6.69-7.31 (m, 13H, DMT arom.), 6.56 (t, 1H, J 6.6 Hz, H-1'), 5.46 (d, 1H, J 4.5 Hz, OH-3'), 4.49-4.57 (quintet, 1H, J 5.1 Hz, H-3'), 4.05-4.1- (quintet, 1H, J 3.6 Hz, H-4'), 3.68 (d, 6H, 11.4 Hz, 2×OCH_3 of DMT), 3.14-3.27- (m, 2H, H-5', H-5"), 2.95-3.03- (m, 1H, H-2'), 2.4-2.46- (m., 1H, H-2").

5'-*O*-Dimethoxytrityl-6-*N*-(5-azapentane-1-yl)-2'-deoxyadenosine (3)

^{1}H NMR (DMSO-d_6): δ (ppm) 8.22 (s, 1H, H-8), 8.14 (s, 1H, H-2), 7.84 (s, 1H, NH), 6.77-7.34 (m, 13H, DMT arom.), 6.36 (t, 1H, J 6.6 Hz, H-1'), 5.38 (m, 1H, OH-3'), 4.47 (m, 1H, H-3'), 3.95-3.99 (q, 1H, J 4.2 Hz, H-4'), 3.71 (d, 6H, J 1.8 Hz, 2×OCH_3 of DMT), 3.33-3.44 (m, 6H, H-1,-4 aliph. CH_2, NH_2), 3.15-3.17 (d, 2H, J 4.8 Hz, H-5', H-5"), 2.85-2.92 (m, 1H, H-2'), 2.49-2.56 (m, 1H, H-2"), 1.54-1.64 (quintet, 2H, J 7.2 Hz, H-2 aliph. CH_2), 1.32-1.42 (quintet, 2H, J 7.2 Hz, H-3 aliph. CH_2).

5'-*O*-Dimethoxytrityl-6-*N*-[(*N*-trifluoroacetyl)-5-azapentane-1-yl]-2'-deoxyadenosine (4)

^{1}H NMR (DMSO-d_6): δ (ppm) 9.41 (t, 1H, J 5.7 Hz, NH-5), 8.24 (s, 1H, H-8), 8.15 (s, 1H, H-2), 7.85 (s, 1H, NH), 6.77-7.27 (m, 13H, DMT arom.), 6.37 (t, 1H, J 6.3 Hz, H-1'), 5.38 (m, 1H, OH-3'), 4.86 (s, 1H, H-3'), 3.96-4.01 (q, 1H, J 4.2 Hz, H-4'), 3.72 (d, 6H, J 1.5 Hz, 2×OCH_3 of

DMT), 3.16-3.48 (m, 6H, H-1,-4 aliph. CH_2, H-5', H-5"), 2.84-2.92 (m, 1H, H-2'), 2.32-2.36 (m, 1H, H-2"), 1.56 (m, 4H, H-2,-3 aliph. CH_2). ^{19}F NMR (DMSO-d_6): δ (ppm) 1.784 (s, CF_3).

5'-*O*-Dimethoxytrityl-6-*N*-[tris(*N,N',N''*-trifluoroacetyl)-4,9,13-triazatridecane-1-yl]-2'-deoxyadenosine (6)

^{1}H NMR (DMSO-d_6): δ (ppm) 9.45-9.53 (m, 1H, NH-13 aliph.), 8.25 (t, 1H, J 2.4 Hz, H-8), 8.16 (s, 1H, H-2), 7.89 (d, 1H, J 21.3 Hz, C(6)-NH), 6.77-7.27 (m, 13H, DMT arom.), 6.37 (t, 1H, J 6,0 Hz, H-1'), 5.37 (d, 1H, J 4.5 Hz, OH-3'), 4.48 (m, 1H, H-3'), 3.98 (q, 1H, J 4.2 Hz, H-4'), 3.72 (t, 6H, J 1.5 Hz, 2×OCH_3 of DMT), 3.16-3.47 (m, 14H, H-1,-3,-5,-8,-10,-12 aliph. CH_2, H-5', H-5"), 2.83-2.92 (m, 1H, H-2'), 1.74-1.93 (m, 4H, H-6,-7 aliph. CH_2), 1.53 (s, 4H, H-2,-11 aliph. CH_2).

6-*O*-*p*-nitrophenylethyl-2-fluoro-2'-deoxyinosine (9)

^{1}H NMR (DMSO-d_6): δ (ppm) 8.57 (s, 1H, H-8), 8.17-8.2 (d, 2H, J 8.7 Hz, 2×C-H arom. of *p*-NPE), 7.63-7.66 (d, 2H, J 8.7 Hz, 2×C-H arom. of *p*-NPE), 6.31 (t, 1H, J 6.9 Hz, H-1'), 5.35 (d, 1H J 4.2 Hz, OH-3'), 4.94 (t, 1H, J 5.7 Hz, OH-5'), 4.83 (t, 2H, J 6.3 Hz, CH_2 aliph. of *p*-NPE), 4.37-4.43 (m, 1H, H-3'), 3.84-3.88 (q, 1H, J 3.3 Hz, H-4'), 3.47-3.64 (m, 2H, H-5', H-5"), 3.31 (t, 2H, J 6.6 Hz, CH_2 aliph. of *p*-NPE), 2.62-2.71 (m, 1H, H-2'), 2.28-2.35 (m, 1H, H-2").

^{19}F NMR (DMSO-d_6): δ (ppm) 25.231 (s, C(2)-F).

***O*-6-(*p*-nitrophenylethyl)xanthine (10)**

decomp. t. 225 °C; EI MS m/z (% rel. intens.): M^+ 301.0 (4.2), 152.0 (100); calc. for $C_{13}H_{11}N_5O_4$ 301.08.

5'-*O*-Dimethoxytrityl-2-fluoro-2'-deoxyinosine (11)

^{1}H NMR (DMSO-d_6): δ (ppm) 8.58 (d, 1H, J 3.9 Hz, NH), 7.98 (s, 1H, H-8), 6.78-7.36 (m, 13H, DMT arom.), 6.24 (t, 1H, J 6.3 Hz, H-1'), 5.36 (s, 1H, OH-3'), 4.38 (d, 1H, J 3.9 Hz, H-3'), 3.91-3.96 (q, 1H, J 5.7 Hz, H-4'), 3.72 (d, 6H, J 0.6 Hz, 2×OCH_3 DMT), 3.11-3.22 (m, 2H, H-5', H-5"), 2.65-2.74 (m, 1H, H-2'), 2.25-2.33 (m, 1H, H-2").

^{19}F NMR (DMSO-d_6): δ (ppm) 24.754 (m, C(2)-F).

4-(3,8-diazaoctyl)nitrobenzene (14)

^{1}H NMR (DMSO-d_6): δ (ppm) 8.14 (d, 2H, J 9.0 Hz, 2×C-H arom. of *p*-NPE), 7.5 (d, 2H, J 8.7 Hz, 2×C-H arom. of *p*-NPE), 2.72-2.88 (m, 4H ,CH_2 aliph. of *p*-NPE), 1.28-1.44 (m., 8H, CH_2 of putrescine residue).

5'-*O*-Dimethoxytrityl-2-*N*-[tris(*N,N',N''*-trifluoroacetyl)-4,9,13-triazatridecane-1-yl]-2'-deoxyguanosine (17)

^{1}H NMR (DMSO-d_6): δ (ppm) 10.72-10.75 (dd, 1H, J 11.4 Hz, NH-1), 9.47-9.54 (d, 1H, J 23 Hz, NH-13 aliph.), 7.8 (s, 1H, H-8), 6.78-7.35 (m, 13H, DMT arom.), 6.58 (t, 1H, C(2)-NH), 6.19 (t, 1H, J 6.3 Hz, H-1'), 5.34 (d, 1H, J 4.8 Hz, OH-3'), 4.35-4.44 (sextet, 1H, J 5.4 Hz, H-3'), 3.91-3.95 (quintet, 1H, J 3.3 Hz, H-4'), 3.72 (d, 6H, J 1.2 Hz, 2×OCH_3 DMT), 3.16-3.35 (m, 14H, H-1,-3,-5,-8,-10,-12 aliph. CH_2, H-5', H-5"), 2.69-2.74 (m, 1H, H-2'), 2.24-2.28 (m, 1H, H-2"), 1.73-1.84 (m, 4H, H-6,-7 aliph. CH_2), 1.51 (s, 4H, H-2,-11 aliph. CH_2).

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