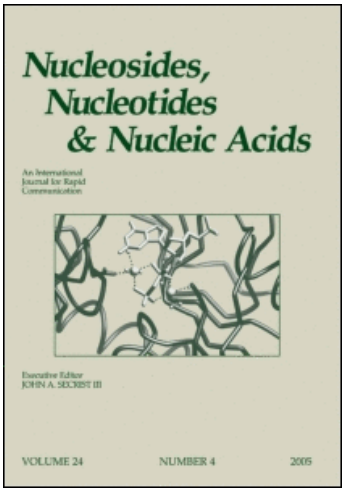

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ZWITTERIONIC OLIGONUCLEOTIDES: A STUDY ON BINDING PROPERTIES OF 2′-*O*-AMINOHEXYL MODIFICATIONS

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▫ *2′-O-Aminohexyl side chains provide excellent conditions for zwitterionic interstrand and intrastrand interactions of oligonucleotides. 2′-O-Aminoalkylated phosphoramidites of adenosine and uridine were synthesized and incorporated in increasing number into homo adenosine and homo uridine/thymidine dodecamers, respectively. CD spectra of these dodecamers with complementary sense DNA exhibited a B-DNA type structure. While duplex stability values of all tested oligonucleotides were lower than those of the native oligonucleotides, they were significantly higher than those of 2′-O-heptyl modified oligonucleotides. The destabilization amounted to 0.9, 1.5, and 2.7°C per modification for 2′-O-aminohexyl adenosine, 2′-O-aminohexyl uridine, and 2′-O-heptyl adenosine substitutions. These findings are pointing to a duplex stabilizing effect of the interaction of side chain amino groups with backbone phosphoric acid.*

INTRODUCTION

For about 20 years, gene therapy has been an important issue in the field of therapy of diseases. Somatic gene therapy would bear the attribute of a perfect therapy, if implemented in its theoretical perfection–a kind of molecular surgery.

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Not surprisingly, it was considered to be the ultimate goal during the first decade of therapeutic biotech research. Antisense drug therapy, the other gene therapeutic approach, is basically a hybrid between somatic gene therapy and drug therapy. On the one hand, silencing of genes by direct attack is the principle mode of action. On the other hand, the compounds are applied in therapeutic doses in a manner corresponding to that of regular drugs. While somatic gene therapy still may be seen as a concept of the future, the antisense drug approach has reached the market. Nevertheless, it was clear to experts in drug development from early phases that the "rush for clinical trials" with a class of absolutely new drug compounds of largely unknown pharmacokinetic and pharmacodynamic properties might easily lead to failure and a backlash for this remarkable approach in drug therapy.[1] Therefore, it is not surprising that the lack of success of the first drug fomivirsen and the failing of most other projects in preclinical or clinical phases still shed doubt on the ultimate success of this important therapeutic principle. The advent of siRNA (short interfering RNA)-induced gene silencing has given new impetus to the whole field and once more has opened a great chance for a class of drug compounds, which, as *nucleic acid drugs* may act either on genes as antisense compounds or on proteins as aptamers.

In terms of drug development, specific gene sequences are almost perfect drug targets and their antisense sequences are excellent lead structures. However, in any drug research project, optimizing the lead structure to achieve an optimum of pharmacological parameters is an indispensable prerequisite for success. As a rule, there is a significant difference between the structure of the lead compound and that of the preclinical development candidate (PDC). An abundant variety of chemical modifications of oligonucleotides has been reported.[2,3] It seems worthwhile to reconsider the material available and to choose promising options, which might be particularly suited as tools in nucleic acid drug lead optimization.

The present article summarizes some results of biophysical studies with a type of particularly promising nucleic acid drug modifications, the so-called zwitterionic oligonucleotides. A cationic substituent covalently linked to a nucleic acid, like an amino group protonated under physiological conditions, may neutralize an anionic phosphate group, thus decreasing the electrostatic repulsion between the two single strands[4] and/or strengthening interstrand binding in duplexes. In addition, such compounds may be expected to exhibit different protein-binding properties. The term "zwitterionic" oligonucleotide has been coined at almost the same time independently by two research groups: Hashimoto et al.[5,6] tethered aminohexyl groups to carbon 5 of uridine and prepared the corresponding modified oligonucleotides resulting in improved binding characteristics compared to oligonucleotides bearing alkyl chains of the same length. Our own group made also use of aminohexyl groups[4] but attached them to the 2′-*O*-position of the sugar moiety, which had been found previously to be an excellent position for ligand attachment by us and others.[7–9] The decision for a hexyl alkyl chain was, in our case, the result of a Monte Carlo simulation study[10] on nucleic acid duplexes. According

to the computed data, short alkyl chain up to four carbon atoms are too short to contribute to ionic interstrand stabilization of a duplex, while chain lengths from five to eight provide suitable conditions. A six-carbon tether gave optimal steric stabilization.

Apart from double strand stabilization it was also the realistic hope for improved cellular uptake that rendered the approach of zwitterionic oligonucleotides with an aminoalkyl side chain particularly promising. It had been established earlier that conjugation or complexation of antisense compounds to poly-L-lysine[11–14] increases the efficacy of cellular uptake to a great extent. In fact, during the last decade, our own group, together with the group of Kreuter and Zimmer, focused on the design of aminoalkyl modified nanoparticles and liposomes as oligonucleotide carriers.[15–18] Several promising "drug targeting" strategies including other polycationic peptides like protamine[19] have been reported.

In the meantime, several publications have appeared on "zwitterionic oligonucleotides" in general and on oligonucleotides bearing aminoalkyl groups in particular. 2′-*O*-Aminopropyl[20,21] modified oligonucleotides were found to have very favorable effects on both nuclease stability and hybridization properties. While exhibiting melting temperatures that are 1.0°C per modification higher than phosphorothioates,[22] their 3′-exonuclease resistance was 6–8 times better than that of 2′-desoxyphosphodiesters.[20] Structure-based studies showed that the cationic modification prevents the binding of a metal ion that is required for the enzyme to efficiently catalyze the phosphoryl transfer reaction.[23] Other reported zwitterionic 2′-modifications include the 2′-*O*-dimethylaminopropyl and 2′-*O*-guanidiniumethyl[22] groups. Spermidine and other polyamines have been conjugated to several positions of oligonucleotides.[5,24,25] However, only little data is published to date referring to hybridization properties of these compounds. Hashimoto et al. found that a duplex of his completely zwitterionic oligonucleotide with the natural DNA complement is insensitive to the ionic strength of the solution in contrast to wild-type DNA.[5] Two oligonucleotides containing four 2′-*O*-aminohexyl modified thymidine moieties each showed an increase in melting point by 3.2 and 4.4°C, respectively.[21]

Apart from the "ionic" function, aminoalkyl groups have been mainly reported as linkers to attach dyes, reactive groups or other macromolecules to oligonucleotides. 2′-*O*-Aminohexyl modified nucleotides were prepared and incorporated into oligonucleotides as a linker for attachment of cholesterol,[9] fluorescein,[21] biotin,[26] anthraquinone,[27] and pyrene.[28]

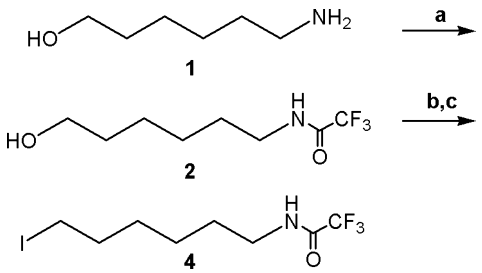
The activation of RNAse H, a nuclease specifically cleaving duplexes consisting of a DNA and an RNA strand, is deemed to be an important property for highly efficient antisense oligonucleotides. Owing to their RNA-like character, 2′-*O*-modified antisense oligonucleotides are generally unable to result in substrates for RNAse H after hybridizing to the targeted RNA.[29,30] However, by creating so-called gapmers, which incorporate a stretch of unmodified nucleotides flanked by several 2′-*O*-modified nucleotides, this drawback can be overcome.[31]

In this article, we give a detailed description of the preparation of zwitterionic oligonucleotides containing 2′-*O*-aminohexyl adenosine and uridine modifications and a systematic study of the biophysical properties of these antisense oligonucleotides. In contrast to the previously published characteristics of some 2′-*O*-aminoalkyl modified oligonucleotides, we focused on one specific duplex sequence, poly(A)-poly(U), and compared our results to those obtained with 2′-*O*-alkyl modifications of similar lengths.

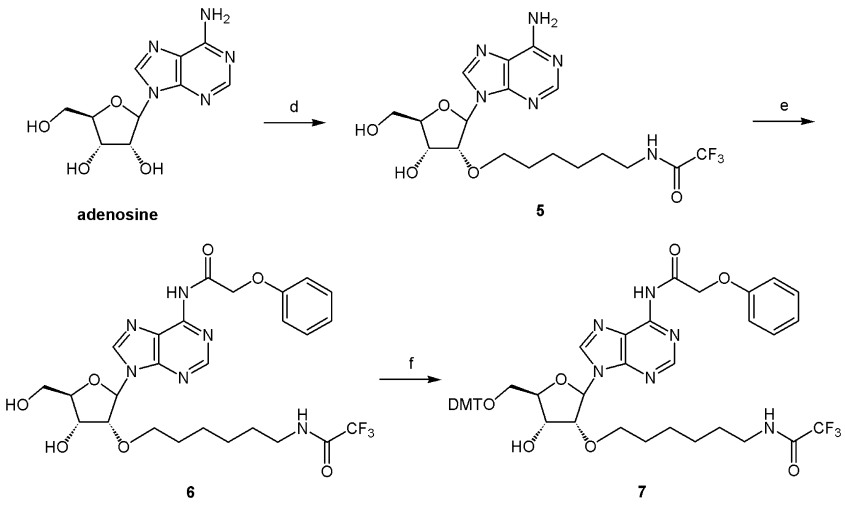
RESULTS AND DISCUSSION

The direct 2′-*O*-alkylation method was chosen to prepare the adenosine nucleoside[32] (Scheme 1). First, the amino group of 6-hydroxyhexylamine (**1**) was protected as trifluoracetamide using ethyl trifluoroacetate to give **2** (Scheme 2). Substitution of the hydroxy group by iodide was achieved via the tosylate **3** and subsequent reaction with potassium iodide giving 6-trifluoroacetamidohexane-1-iodide (**4**). This alkylating agent was reacted with deprotonated adenosine to afford 2′-alkylated product **5** in a 30% yield. Additionally, 8% of 3′-alkylated product was found after work-up as well as 2.5% of dialkylated adenosine. In the next step the exocyclic amino group of adenosine was protected with a phenoxyacetyl group to give **6** and the 5′-position was dimethoxytritylated resulting in **7**.

While the synthesis of 2′-*O*-aminohexyl adenosine derivatives proceeded straightforward in high analogy to that of 2′-alkylated ribonucleotides, amino-alkylation of uridine was less easily achieved. "Soft" alkylating agents react in a S_{N2}-manner with nucleosides and the pKa of the imido nitrogen in position 3 is lower than that of the hydroxyl group in 2′. Consequently, protection of the nucleobase at position 3 is necessary for the synthesis of 2′-*O*-alkyl derivatives of uridine when sodium hydride is used. Thus, we protected the imido group of 5′-DMT-uridine by reaction of methoxyethoxymethylchloride and triethylamine.[33] Subsequent alkylation with 6-phthalimidohexan-1-bromide gave rise to a mixture of 2′- and 3′-alkylated uridine derivatives with preferred alkylation of 2′ (2.3:1) and a total yield of 30%. The two isomers could be separated by MPLC (medium pressure liquid chromatography).

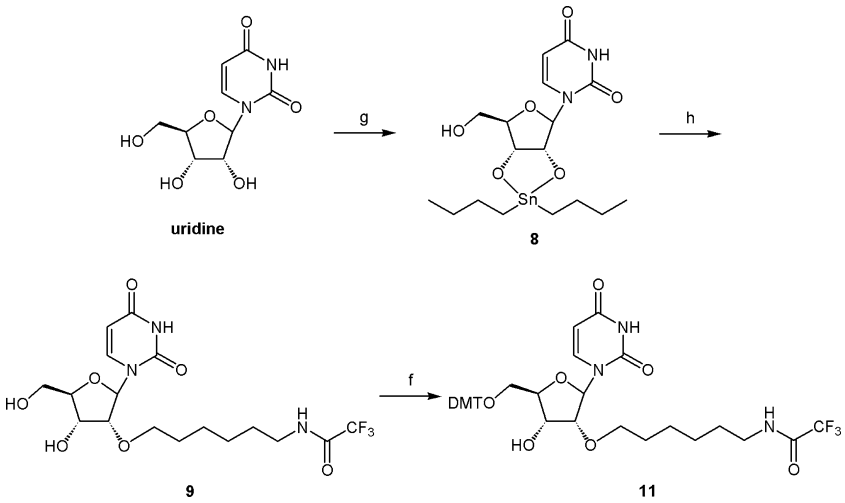


SCHEME 1 Synthesis of aminohexyl building block. a: CF_3COOEt, CH_2Cl_2 (96%); b: p-TosCl, CH_2Cl_2, pyr (85%); c: KI, acetone (85%).



SCHEME 2 Synthesis of adenosine building block **7**. d: NaH, **4** (30%); e: TMSCl, $(PhOCH_2CO)_2O$ (36%); f: DMTCl, pyr (73%).

With uridine an additional protecting step is required prior to 2′-*O*-alkylation. In spite of the good selectivity in favor of 2′-alkylated product, we decided to check the previously published procedure via a stannylene derivative (**8**)[13,33] to overcome poor overall yields. Alkylation of the stannylene derivative **8** (Scheme 3), achieved by reaction with dibutyltin oxide in nearly quantitative yield,[34] gave **9/10** in better total yield of 44%, but with loss of selectivity for the 2′-position (55:45). Separation of the two isomers by flash chromatography or MPLC did not succeed. Dimethoxytritylation products **11** and **12**, however, could be separated more



SCHEME 3 Synthesis of uridine building block **11**. g: dibutyltin (IV) oxide (98%); h: **4**; f: DMTCl, pyr (15% for two steps).

easily by column chromatography. While the selectivity of a 2′-alkylation upon activation with sodium hydride was higher, the overall yield of the stannylene process was higher, adding up to 15% compared to 8% of the sodium hydride method. Furthermore, trifluoracetic acid could not be used to protect the amino group, when sodium hydride was used for deprotonation. Trifluoracetic acid is superior to the phthalimido group, because it is cleaved readily after oligonucleotide synthesis under the same conditions as the base protecting groups (concentrated aqueous ammonia 55°C/16 h). Cleavage of the phthalimido groups requires an additional step with 10% aqueous methylamine,[35] because the deprotection with concentrated ammonia proceeds ineffectively.

The phosphoramidites of modified adenosine and uridine derivatives (**13,14**) were prepared using the established method[36] in nearly quantitative yields. 2′-*O*-Ethyl adenosine phosphoramidite was prepared according to the previously published procedure.[32] All products were stored at −25°C in an argon atmosphere and were stable for months. Oligonucleotide syntheses were performed in an ABI 392 DNA synthesizer using the standard CE protocol. For coupling of the 2′-modified nucleotides, the coupling times were extended to 15 min. Still, average coupling yields were poorer than for unmodified base building blocks and amounted to only 92%. The steric hindrance of 3′ by the bulky 2′-*O*-substituent might explain these somewhat lower yields. Nevertheless, yields were high enough to provide the dodecamers after standard HPLC purification. We chose homomers of adenosine and its counterstrand, thymidine homomers, as the model substances because of the ease of analysis and purification. Circular dichroism spectra of poly(A)-poly(T) are well defined and an influence of the modifications on the secondary structure of the oligonucleotide duplex can easily be monitored.

One to six modified nucleotides were incorporated in oligonucleotides at the 5′-end (**15a,b–22a,b**, Table 1) and characteristics were compared to the unmodified DNA strands and to 2′-*O*-heptyl and 2′-*O*-ethyl modified adenosine dodecamers (**15c,d–22c,d**). For the adenosine homomers, further oligonucleotides bearing 2′-*O*-aminohexyl and 2′-*O*-ethyl modifications were prepared, one with unmodified

TABLE 1 Sequences of Synthesized Oligonucleotides

a, c, and d	b
15 5′-*A*AA AAA AAA AAA-3′	5′-*U*UU UUU UUU UUU-3′
16 5′-*AA*A AAA AAA AAA-3′	5′-*UU*U UUU UUU UUU-3′
17 5′-*AAA* AAA AAA AAA-3′	5′-*UUU* UUU UUU UUU-3′
18 5′-*AAA A*AA AAA AAA-3′	5′-*UUU U*UU UUU UUU-3′
19 5′-*AAA AA*A AAA AAA-3′	5′-*UUU UU*U UUU UUU-3′
20 5′-*AAA AAA* AAA AAA-3′	5′-*UUU UUU* UUU UUU-3′
a and c	
21 5′-AAA *AAA AAA* AAA-3′	
22 5′-*AA*A AA*A A*AA AA*A*-3′	

Underlined nucleotides are modified (**a**, **b**: 2′-*O*-aminohexyl, **c**: 2′-*O*-ethyl, **d**: 2-*O*-heptyl. (From Ref. [27].)

TABLE 2 HPLC Retention Times of Oligonucleotides Containing 2′-*O*-Aminohexyl Modification, Using a Linear Gradient 10–40% B in 0–30 min (A: 0.1 M TEAA, B: 0.1 M TEAA in 80% ACN)

2′-*O*-Aminohexyl adenosine series		2′-*O*-Aminohexyl uridine series		2′-*O*-Ethyl adenosine series	
15a	9.08	**15b**	17.35	**15c**	10.52
16a	10.70	**16b**	18.23	**16c**	13.40
17a	11.40	**17b**	18.08	**17c**	15.04
18a	12.11	**18b**	17.75	**18c**	16.50
19a	12.59	**19b**	17.36	**19c**	17.79
20a	14.47	**20b**	17.10	**20c**	18.69

triplets at both the 3′- and the 5′-end (**21a,d**) and one bearing modifications in an alternating pattern (**22a,d**). Retention times in HPLC increased with increasing number of 2′-*O*-aminohexyl adenosine nucleotides. However, in the uridine series, maximum retention time was reached with two modified nucleotides and then dropped with further increase of 2′-*O*-aminohexyl modifications (Table 2). MALDI mass spectrometry of HPLC-purified products was performed to confirm the structures. In contrast to reports by Griffey et al.,[20] but in accordance to the findings of Behr et al.,[24] no side products due to transamidation of trifluoroacetyl amides were observed in the capping step.

Circular dichroism spectra of the duplexes of these oligonucleotides with complementary DNA strands were recorded. The resulting spectra indicated a B-type DNA structure[37] for the duplexes of all compounds **15–20**. It is known that attachment of electron withdrawing moieties at the 2′ carbon shifts the conformational equilibrium toward the C3′-*endo* pucker, an RNA-like conformation. A shift toward an A-DNA shape might also be expected along with the incorporation of an increasing number of 2′-*O*-aminoalkyl modifications into oligodesoxynucleotides. A transition from B-type to A-type DNA occurs in similar hybrids with increasing

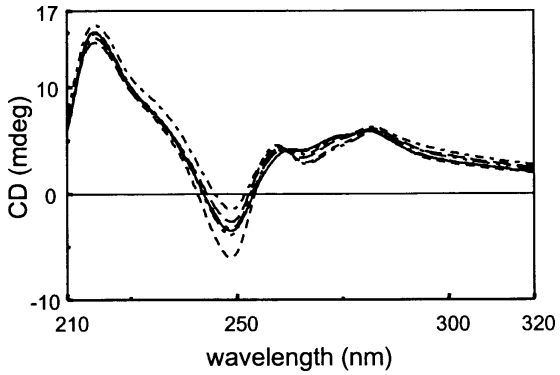


FIGURE 1 CD spectra of modified oligonucleotides **15b–20b** with complimentary DNA. **15b:** – – – **16b:** – – – **17b:** – – – – **18b:** – – – **19b:** – – **20b:** –. All determinations were carried out in a solution of 0.10 M Tris-HCl (pH 7.0) and 0.15 M NaCl.

number of RNA-like nucleosides.[38] However, no such occurrence could be observed for the tested compounds. Even the products consisting of 50% RNA-like nucleotides, the B-DNA structure was clearly favored (Figure 1). These findings are characteristic of the poly(A)-poly(T) duplex.[39] With these, the number of base pairs per helix turn is 10.5, compared to 10.1 for other B-DNA sequences.[40] Obviously, the conformational rigidity of a homo-A-homo-T duplex is exceptionally strong, because A-T-base pairs have a tendency to bind large amounts of water. The major and minor grooves of B-type DNA provide more room for inclusion of water than A-type DNA. B-type DNA is hence preferred. Similar shifts of DNA-types can be induced in sequences of mixed base composition.[41] Even increase of salt concentration does not induce conformational change. In poly(A)-poly(T)-duplexes, a triple helix is formed instead.

Melting curves of these duplexes, except for that of **15a**, all exhibit a slight decrease of the melting temperature (*Tm*) with increasing number of modified nucleotides (Table 3) compared to the native A_{12}–T_{12} duplex. For both the adenosine and the uridine series, the extent of decrease was less than that for 2′-*O*-alkyl and ethylene glycol modified oligonucleotides with comparable alkyl length.[42] For **20a**, the decrease per modification was only 0.9°C, and for **20b**, 1.5°C, whereas homo-adenosine dodecamers with six 2′-*O*-butyl modifications showed a destabilization of 11.9°C (2.0°C per modification) and with six 2′-*O*-heptyl chains even 16.1°C (2.7°C per modification). Interestingly, the location of modified nucleosides is crucial with regard to their hybridization affinity. When comparing the melting temperatures of **20a,c**, **21a,c**, and **22a,c**, all adenosine dodecamers with six 2′-*O*-aminohexyl modifications, differences of five degrees are observed. The highest melting temperature was found for **21a,c**, the products with six consecutive internal modifications. In comparison, when the modifications were placed at the end of the adenosine dodecamer (**20a,c**), the melting temperatures were 2 and 3°C lower for the aminohexyl and the ethyl series, respectively. Modified nucleosides alternating with unmodified ones (**22a,c**) were the least favored in that respect. The distribution of steric hindrance across the oligonucleotide strand obviously eases thermal denaturation. It is evident that the amino

TABLE 3 Melting Temperatures of Duplexes of 2′-*O*-Modified Oligonucleotides

T12-A12	34.7						
15a	35.7	**15b**	32.5	**15c**	36.2	**15d**	31.8
16a	33.5	**16b**	33.0	**16c**	34.4	**16d**	29.5
17a	32.6	**17b**	30.2	**17c**	32.3	**17d**	26.4
18a	31.3	**18b**	28.6	**18c**	32.0		
19a	30.7	**19b**	27.7	**19c**	29.1	**19d**	23.0
20a	29.5	**20b**	25.6	**20c**	28.4	**20d**	18.6
21a	31.7			**21c**	31.9		
22a	26.6			**22c**	25.9		

(**a**: Aminohexyl modified adenosine, **b**: aminohexyl modified uridine, **c**: ethyl modified adenosine **d**: heptyl modified adenosine[27]) with complimentary DNA.

group can lead to duplex stabilization by reducing the net charge of the oligonucleotide and compensate for the unfavorable effect of the steric bulk of the 2′-*O*-alkyl substiment. The increase in melting point when compared to a modified oligonucleotide with 2′-*O*-heptyl groups (same number of side chain atoms) amounts to 10.9°C (1.8°C per modification). This increase of melting temperature is attributed to the neutralizing effect of the protonated amino group.

Phosphorothioates, widely used in antisense experiments, are known to exhibit a decrease in melting temperature of at least 0.5°C per modified nucleotide in comparison to phosphodiester oligonucleotides.[2] A substitution of 2′-desoxyuridine for thymidine accounts for an average reduction of 0.5°C, shown for a considerable number of different sequences.[2] Hence, duplex destabilization of the 2′-*O*-aminohexyl modification amounts to only about 0.5°C per modification for both adenosine and uridine/thymidine dodecamers when compared to phosphorothioates, the current standard for antisense treatment. Griffey et al. found an increase of 0.4°C for fully 2′-*O*-aminopropyl modified 15- and 17-mers when compared to the corresponding phosphorothioate, but even higher melting temperatures for 2′-*O*-propyl and 2′-*O*-methyl modified oligonucleotides of the same sequence.[20] They used a lower salt concentration during their experiments, possibly accounting for the differences in comparison to our results (Figure 2).

The nuclease resistance of the uridine series **15b–20b** was tested using 5′-exonuclease. The test substances were incubated with 200 μU 5′-exonuclease from calf spleen for 30 min at 37°C. The extent of degradation was determined by HPLC and can be seen in Figure 3. The unmodified dodecamer was nearly fully degraded (90%) after 30 min, and **15b** was partially (15%) degraded. Three modified nucleotides at the 5′-end (**17b**) were sufficient to provide nearly full 5′-exonuclease stability, with only 2% of oligonucleotide losing the 5′-terminal nucleotide. These results are in accordance with the findings of Griffey et al. for aminopropyl modified oligonucleotides.[20] In the molecule with a single terminal 2′-modification, the aminohexyl groups seem to account for the higher nuclease resistance. After incubation for 30 min, only 15% of the oligonucleotide showed a loss of the terminal nucleotide, while a $t_{1/2}$ of 20 min was determined for the aminopropyl modification.

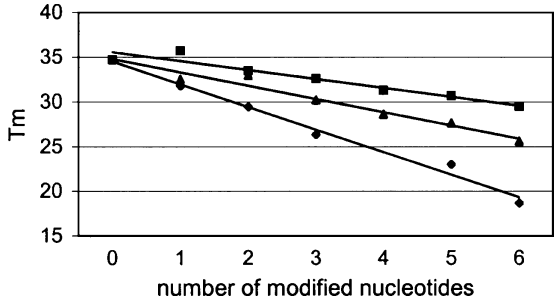


FIGURE 2 Melting temperatures of 2′-*O*-aminohexyl modified adenosine (squares) and uridine series (triangles) compared to 2′-*O*-heptylmodified adenosine series (rhombuses).

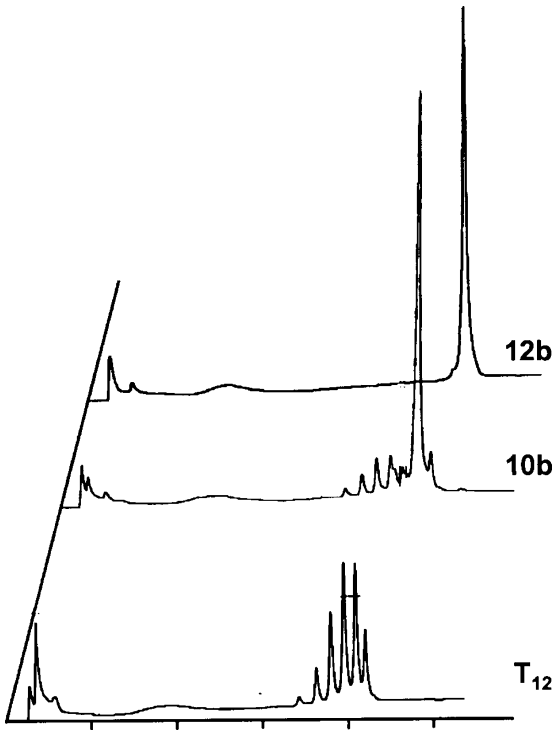


FIGURE 3 HPLC after incubation of oligonucleotides **15b**, **17b**, and control T12 with 5′-exonuclease (calf spleen) for 30 min at 37°C.

In both experiments, the incorporation of three terminal modified nucleotides was enough to achieve full resistance against nuclease degradation. However, these results do not give a straightforward comparison on the effect of the length of the alkyl linker on nuclease resistance because Griffey et al. used snake venom phosphodiesterase and 3′-terminal modifications.

EXPERIMENTAL

Reagents were purchased from Merck or Aldrich in standard quality and used without purification. Melting points were measured in a Büchi melting point apparatus and are uncorrected. Anhydrous solvents were obtained as follows: tetrahydrofurane was refluxed on sodium and then distilled, and pyridine, dichloromethane and triethylamine were distilled from calcium hydride. Purified water was obtained from a Milli-Q-apparatus. NMR spectra were recorded on a Bruker AC-200 MHz. Shifts are reported relative to the solvent peak ($CHCl_3$ in CDCl3: δ 7.26 and 77.00, DMSO in d6-DMSO: δ 2.50 and 39.50), and coupling constants are in Hz. Thin-layer chromatography (TLC) was performed using silica gel 60-F_{254} precoated aluminium plates by Merck. Column chromatography was

performed with Merck silica gel 60. Elemental analyses were done at the Institut für Organische Chemie der Johann Wolfgang Goethe Universität Frankfurt/Main with a Heraeus CHN Rapid.

N-(6-Hydroxyhexyl)trifluoroacetamide (2). To a solution of 6-hydroxyhexyl-1-amine (**1**, 100 g, 0.853 mol) in dichloromethane (200 mL) ethyl trifluoroacetate was added slowly. The mixture was stirred for 5 h and concentrated to 100 mL in vacuo. Storage at room temperature resulted in precipitation of a white solid. Collection by filtration, washing with CH_2Cl_2, and drying in vacuo afforded 174.6 g (96%) pure product 2. m.p.: 49–50°C, ^{1}H NMR ($CDCl_3$): 7.16 (bs, 1H, H-N), 3.63 (t, J = 7.1, 2H, CH20H), 3.34 (q, J = 7.1, 2H, CH_2NH), 1.60–1.40 (m, 8H, H-C(2), H-C(3), H-C(4), H-C(5)). ^{13}C NMR ($CDCl_3$): 157.34 (q, J = 36.5, CO), 115.87 (q, J = 285.8, CF_3), 62.50 (t, C(6)), 39.77 (t, C(1)), 32.29, 28.74, 26.26, and 25.15 (4t, C(2), C(3), C(4), C(5)). ESI-MS: 214.0 (calc. 213.20).

N-Trifluoroacetyl-(6-aminohexyl)-1-tosylate (3). Dried **2** (50 g, 0.235 mol) was dissolved in dichloromethane and *p*-toluenesulfonyl chloride (49.57 g, 0.260 mol) was added to the stirred solution. Pyridine (34 g, 0.430 mol) was added dropwise at 0°C and the resulting mixture was kept at 0°C for 1 h, then was allowed to warm to room temperature. Stirring was continued for 21 h. The reaction was quenched by pouring onto 0.5 M HCl/ice/H_2O. The product was extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, and the solvent was removed in vacuo. Recrystallization was afforded by stirring a suspension in 500 mL cyclohexane at 4°C for 18 h. Filtration of crystals, washing with cyclohexane and drying in vacuo gave product **3** (73.61 g, 85%). Fp: 33–36°C, ^{1}H NMR ($CDCl_3$): 7.75 (d, J = 8.1, 2H, Ar-H), 7.33 (d, J = 8.1, 2H, Ar-H), 6.64 (bs, 1H, NH), 3.99 (t, J = 6.3, 2H, H-6), 3.29 (m, 2H, CH_2NH), 2.43 (s, 3H, CH_3), 1.68–1.19 (m, 8H, H-2, H-3, H-4, H-5). ^{13}C NMR ($CDCl_3$): 157.34 (q, J = 36.5, CO), 144.84 (ArC), 132.96 (ArC), 129.85 (d, ArC), 127.78 (d, ArC), 115.87 (q, J = 285.75, CF_3), 70.32 (t, C-6), 39.63 (t, CH_2NH), 28.61, 28.56, 25.83, and 24.83 (4t, C-2, C-3, C-4, C-5), 21.60 (q, CH_3).

6-Trifluoroacetamidohexane-1-iodide (4). Tosylate **3** (73.6 g, 0.20 mol) was dissolved in 500 mL acetone and potassium iodide (35.97 g, 0.24 mol) was added. The reaction mixture was heated to reflux for 5 h, cooled to room temperature, and filtered. The filtrate was dried in vacuo and the residue was dissolved in dichloromethane. The solution was washed with 0.1 M sodium thiosulfate solution until it was colorless, dried over Na_2SO_4 and the solvent was removed in vacuo. Crystallization from dichloromethane/diethyl ether afforded **4** (55.0 g, 85%) as colorless needles. Fp: 57–58°C, ^{1}H NMR ($CDCl_3$): 6.38 (bs, 1H, NH), 3.40–3.31 (m, 2H, CH_2NH), 3.18 (t, J = 6.93, 2H, CH_2I), 1.90–1.20 (m, 8H, H-2, H-3, H-4, H-5). ^{13}C NMR ($CDCl_3$): 157.58 (q, J = 36.5, CO), 115.85 (q, J = 286.0, CF_3) 39.80 (t, C-1), 33.10, 29.92, 28.78, 25.56 (4t, C-2, C-3, C-4, C-5). ESI-MS (negative mode): 322.1 (calc. 323.10).

Alkylation of Adenosine by Sodium Hydride Method

Adenosine (50 g, 0.187 mol) was dried over phosphorous pentoxide for 20 h, partially dissolved in 400 mL DMF, and cooled to 0°C. Sodium hydride (9.0 g, 0.225 mol, 60% suspension in oil) was washed with *n*-hexane and added to the suspension of adenosine. The reaction mixture was stirred for 30 min. Iodide **4** (60.5 g, 0.187 mol) was, added and the mixture was heated to 40°C for 5 h. The reaction was quenched by adding 10 mL methanol and solvents were removed in vacuo. Repeated flash chromatography (5–10% methanol in dichloromethane) gave **5** (25.9 g, 30%) as a yellow foam. ^{1}H NMR (DMSO-d_6): 8.84 (bs, 1H, NH), 8.12 (s, 1H, H-2), 8.00 (s, 1H, H-8), 6.96 (m, 2H, NH_2), 6.27 (m, 1H, OH-5), 5.90 (d, J = 6, 1H, H-1′), 4.48 (m, 1H, H-2′), 4.36 (m, 1H, H-3′),4.12 (s, 1H, H-4′), 3.80–3.60 (m, 2H, H-5′a, H-5′b), 3.48 (m, 1H, OH-3′), 3.27 (m, 2H, CH_2NH), 3.06 (m, 2H, OCH_2), 1.30–1.10 (m, 8H, 4 CH_2). ^{13}C NMR (DMSO-d_6): 156.17 (CO), 156.04 (C-6), 151.85 (d, C-2), 148.06 (C-4), 139.84 (d, C-8), 120.11 (C-5), 115.69 (q, J = 286.0, CF_3), 87.89 (C-1′), 87.41 (C-4′), 80.65 (C-2′), 69.85 (t, OCH_2), 69.50 (C-3′), 62.12 (t, C-5′), 39.51 (t, CH_2NH), 28.67, 28.02, 25.79, 24.68 (4t, CH_2). $C_{18}H_{25}N_6O_5F_3$: calc. C 46.75, H 5.45, N 18.17, found C 46.75, H 5.56, N 18.05.

Alkylation of uridine by stannylene method (34). Dibutyltin (IV) oxide is toxic! Protective gloves should be worn during handling and dispersion of dust has to be prevented. Residual reagent has to be collected in a moistured container. Uridine (25 g, 0.102 mol) and dibutyltin (IV) oxide (25.38 g, 0.102 mol) were suspended in 2000 mL dried methanol. After refluxing for 4 h, a clear solution was formed. After cooling to room temperature, the solvent was removed in vacuo giving stannylene derivative **8** (47.4 g, 98%) as colorless crystals, which were used for the next reaction without further purification. m.p.: 232°C, ^{1}H NMR (MeOH-d_4): 7.93 (bs, 1H, H-6), 5.84 (bs, 1H, H-5), 5.71 (d, J = 7.6, 1H, H-1′), 4.40–3.70 (m, 5H, H-2′, H-3′, H-4′, H-5′), 1.90–1.10 (m, 13H, CH_2, OH-5′), 0.94 (t, J = 7.2, 6H, CH_3). $C_{17}H_{28}N_2O_6Sn$: calc. C 42.98, H 5.95, N 5.90; found C 43.14, H 6.08, N 5.79.

Stannylene derivative **8** (9.93 g, 20.9 mmol) was dissolved in 100 mL DMF and iodide **4** (14.45 g, 44.7 mmol) was added under an atmosphere of argon. The mixture was refluxed for 18 h. DMF was removed in vacuo and the residue was distributed between ethyl acetate and water and extracted five more times with ethyl acetate. Organic phases were dried over Na_2SO_4 and the solvent was removed in vacuo. Purification with flash chromatography (5% methanol in dichloromethane) afforded a mixture of 2′-(**9**) and 3′-alkylated (**10**) product (4.06 g, 44%, **9:10** = 55:45).

9: ^{1}H NMR (DMSO-d_6): 11.29 (bs, 1H, NH), 9.34 (bs, 1H, HNCF_3), 7.92 (d, J = 8.1, 1H, H-6), 5.82 (d, J = 4.5, 1H, H-1′), 5.62 (m, 1H, H-5), 5.10 (m, 1H, OH-5′), 5.00 (d, J = 5.9, 1H, OH-3′), 4.04 (m, 1H, H-3′), 3.95–3.20 (m, 6H, H-2′, H-4′, H-5′, OCH_2), 3.15 (t, J = 6.4, 2H, CH_2NH), 1.60–1.20 (m, 8H, CH_2). ^{13}C NMR (DMSO-d_6): 163.09 (C-4), 156.07 (q, J = 35.7, $COCF_3$), 150.47 (C-2), 140.33 (C-6), 115.93 (q,

J = 286.6, CF_3), 101.72 (C-5), 86.16 (C-1′), 85.04 (C-4′), 81.08 (C-2′), 69.63 (CH_2N), 68.32 (C-3′), 60.45 (t, C-5′), 39.03 (t, CH_2O), 29.15, 28.93, 25.94, 25.07 (4t, CH_2).

10: ^{1}H NMR (DMSO-d_6): 11.29 (bs, 1H, NH), 9.34 (bs, 1H, $HNCF_3$), 7.86 (d, J = 8.1, 1H, H-6), 5.73 (d, J = 4.5, 1H, H-1′), 5.62 (m, 1H, H-5), 5.30 (d, J = 5.7, 1H, OH-2′), 5.10 (m, 1H, OH-5′), 4.12 (m, 1H, H-3′), 3.95–3.20 (m, 6H, H-3′, H-4′, H-5′, OCH_2), 3.15 (t, J = 6.4, 2H, CH_2NH), 1.60–1.20 (m, 8H, CH_2). ^{13}C NMR (DMSO-d_6): 163.09 (C-4),156.07 (q, J = 35.7, $COCF_3$), 150.67 (C-2), 140.50 (C-6), 115.93 (q, J = 286.6, CF_3), 101.64 (C-5), 88.00 (C-1′), 82.76 (C-4′), 77.44 (C-3′), 72.59 (C-2′), 69.57 (CH_2N), 60.75 (t, C-5′), 39.03 (t, CH_2O), 29.15, 28.11, 25.89, 24.90 (4t, CH_2).

N^6-(2-Phenoxyacetyl)-2′-*O*-(6-trifluoroacetamido)hexyladenosine (6). 2′-*O*-Alkylated derivative **5** (25.0 g, 54 mmol) was dissolved in 100 mL dry pyridine. Chlorotrimethylsilane (24.7 g, 227 mmol) was added dropwise and the mixture was stirred at room temperature for 2 h. Phenoxyacetic anhydride (21.7 g, 76 mmol) was added and stirring was continued for 5 h. The reaction was quenched by pouring the mixture onto iced water/methanol (100 mL). After 30 min sodium bicarbonate (40 g) was added and the solution was extracted with dichloromethane. Organic layers were combined, dried over Na_2SO_4, and the solvent was removed in vacuo. Flash chromatography (3% i-PrOH in CH_2Cl_2) afforded N^6-protected adenosine derivative **6** (11.6 g, 36%) as a yellow foam. ^{1}H NMR ($CDCl_3$): 9.72 (bs, NH), 8.78 (s, 1H, H-2), 8.21 (s, 1H, H-8), 7.40–7.00 (m, 5H, Ar-H), 6.84 (m, 1H, OH-5′), 5.98 (d, J = 6.0, 1H, H-1′), 4.91 (s, 2H, CH_2O), 4.77 (m, 1H, H-2′), 4.55 (m, 1H, H-3′), 4.40 (s, 1H, H-4′), 4.00–3.80 (m, 2H, H-5′a, H-5′b), 3.56 (m, 1H, OH-3′), 3.37 (m, 2H, CH_2NH), 3.29 (m, 2H, OCH_2), 1.50–1.20 (m, 8H, CH_2). ^{13}C NMR ($CDCl_3$): 167.03 (N–C = O), 157.45 (CO), 156.94 (ArC), 151.99 (C-2), 150.62 (d, C-6), 149.07 (C-4), 143.62 (d, C-8), 129.80 (d, ArC), 124.19 (C-5),122.45 (d, ArC), 116.12 (q, J = 285, CF_3), 114.86 (d, ArC), 89.51 (d, C-1′), 87.98 (d, C-4′), 81.07 (d, C-2′), 71.13 (t, OCH_3), 70.62 (d, C-3′), 68.07 (t, CH_2O), 63.05 (t, C-5′), 39.59 (t, CH_2NH), 29.16, 28.66, 26.07, and 25.24 (4t, CH_2). $C_{26}H_{31}N_60_7F_3$: calc. (0.2 H_20) C 52.13 H 5.26 N 13.31, found C 52.22 H 5.34 N 13.26.

5′-*O*-Dimethoxytrityl-N^6-(2-phenoxyacetyl)-2′-*O*-(6-trifluoroacetamido)hexyladenosine (7). **6** (11.0 g, 18 mmol) was dissolved in 200 mL of a 50% mixture of pyridine and dichloromethane. 4-Dimethylaminopyridine (0.11 g, 0.9 mmol) and 4,4′-dimethoxytriphenylmethyl chloride (7.5 g, 22 mmol) were added to the stirred solution under argon at room temperature. After 2 h the reaction was quenched by addition of CH_2Cl_2 and washed with 0.1 M $NaHCO_3$, dried over Na_2SO_4, and the solvent was removed in vacuo. Purification by flash chromatography (3.5% i-PrOH in CH_2CL_2) gave fully protected adenosine derivative **7** (12.2 g, 73%) as a yellow foam. ^{1}H NMR ($CDCl_3$): 9.45 (bs, 1H, NH), 8.73 (s, 1H, H-2), 8.25 (s, 1H, H-8), 7.40–6.80 (m, 18H, Ar-H), 6.16 (d, J = 7.8, 1H, H-1′), 4.85 (m, 1H, H-2′), 4.84 (s, CH_2O), 4.62 (m, 1H, H-3′), 4.43 (m, 1H, H-4′), 4.40–3.80 (m, 2H, H-5′a, H-5′b), 3.95 (s, 6H, OCH_3), 3.60 (m, 1H, OH-3′), 3.40 (m,

2H, CH_2NH), 3.31 (m, OCH_2), 1.60–1.20 (m, 8H, 4 CH_2). ^{13}C NMR ($CDCl_3$): 167.12 (N–C = O), 158.45 (ArC), 157.52 (CO), 157.03 (ArC), 152.06 (C-2),150.71 (d, C-6), 149.14 (C-4), 144.69 (ArC), 143.71 (d, C-8), 137.36, 130.89, 130.82, 130.62, 128.85, and 127.21 (ArC), 124.25 (C-5), 124.61 and 117.79 (ArC), 116.22 (q, J = 286, CF_3), 113.96 (ArC), 89.14 (d, C-1′), 85.63 (CPh_3), 84.03 (d, C-4′), 81.97 (d, C-2′), 71.22 (t, OCH_2), 70.68 (d, C-3′), 68.19 (t, CH_2O), 63.19 (t, C-5′), 55.99 (q, OCH_3), 39.64 (t, CH_2NH), 29.18, 28.69, 26.10, 25.27 (4t, CH_2). $C_{47}H_{49}N_6O_9F_3$: calc. C 62.18 H 5.55 N 9.26, found C 62.20 H 5.79 N 8.97.

5′-*O*-Dimethoxytrityl-2′-*O*-(6-trifluoroacetamido)hexyluridine (11). The mixture of 2′-and 3′-alkylated uridine **9/10** (4.0 g, 9.10 mmol) was reacted as described for the adenosine derivative in 50 mL pyridine and with DMTCl (4.62 g, 13.7 mmol). Workup and purification using MPLC (0–5% methanol in CH_2Cl_2) gave dimethoxytritylated products **11** (3.37 g, 35.1%) and **12** (1.85 g, 274%).

11: ^1H NMR (DMSO-d_6): 11.34 (bs, 1H, NH-3), 9.35 (bs, 1H, NH), 7.71 (d, J = 8.1, 1H, H-6), 7.73–7.22 (m, 9H, Ar-H), 6.88 (d, J = 8.8, 4H, Ar-H), 5.78 (d, J = 3.7, 1H, H-1′), 5.28 (d, J = 8.1, H-5), 5.07 (d, J = 6.7, 1H, OH-3′), 4.15 (m, 1H, H-3′), 3.94 (m, 1H, H-4′), 3.87 (m, 1H, H-2′), 3.73 (s, 6H, OCH_3), 3.53 (m, 2H, CH_2O), 3.23 (m, 2H, H-5′a, H-5′b), 3.14 (m, 2H, CH_2NH), 1.60–1.20 (m, 8H, 4 CH_2). ^{13}C NMR (DMSO-d_6): 162.89 (q, C-4), 158.10 (q, ArC), 156.05 (q, J = 35.6, CO), 150.21 (q, C-2), 144.56 (q, ArC), 140.12 (d, C-6), 135.31 and 135.05 (2q, ArC), 129.70, 127.81, 127.65, and 126.72 (4d, ArC), 115.98 (q, J = 279.0, CF_3), 113.19 (d, ArC), 101.41 (d, C-5), 87.10 (d, C-1′), 85.84 (q, ArC), 82.59 (d, C-4′), 80.81 (d, C-2′), 69.74 (t, OCH_2), 68.45 (d, C-3′), 62.60 (C-5′), 54.98 (OCH_3), 39.08 (CH_2NH), 28.90, 28.09, 25.88, and 24.91 (4t, CH_2). $C_{38}H_{42}F_3N_3O_9$: calc. (0.52 H_2O) C 60.76 H 5.78 N 5.59, found C 60.82 H 6.04 N 5.18.

12: ^1H NMR (DMSO-d_6): 11.33 (bs, 1H, 3-NH), 9.35 (bs, 1H, $NHCF_3$), 7.74 (d, J = 8.1, 1H, H-6), 7.74–7.20 (m, 9H, Ar-H), 6.88 (d, J = 8.7, 4H, Ar-H), 5.69 (d, J = 3.7, 1H, H-1′), 5.38 (d, J = 5.7, 1H, OH-2′), 5.30 (d, J = 8.0, H-5), 4.25 (m, 1H, H-2′), 3.91 (m, 1H, H-4′), 3.88 (m, 1H, H-3′), 3.73 (s, 6H, OCH_3), 3.53 (m, 2H, CH_2O), 3.23 (m, 2H, H-5′a, H-5′b), 3.14 (m, 2H, CH_2NH), 1.60–1.20 (m, 8H, 4 CH_2). ^{13}C NMR (DMSO-d_6): 162.92 (q, C-4), 158.11 (q, ArC), 156.05 (q, J = 35.6, CO), 150.37 (q, C-2), 144.51 (q, ArC), 140.38 (d, C-6), 135.36 and 135.11 (2q, ArC), 129.81, 127.80, 127.63, and 126.73 (4d, ArC), 115.98 (q, J = 279.0, CF_3), 113.18 (d, ArC), 101.33 (d, C-5), 87.29 (d, C-1′), 85.89 (q, ArC), 80.38 (d, C-4′), 76.81 (d, C-3′), 71.98 (d, C-2′), 69.57 (t, OCH_2), 62.41 (C-5′), 54.97 (OCH_3), 39.08 (CH_2NH), 29.05, 28.07, 25.92, and 25.00 (4t, CH_2). $C_{38}H_{42}F_3N_3O_9$: calc. C 61.53 H 5.71 N 5.66, found C 61.15 H 5.80 N 5.66.

5′-*O*-Dimethoxytrityl-*N*^{6}-(2-phenoxyacetyl)-2′-*O*-(6-trifluoroacetamido)hexyladenosine-3′-*O*-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (13). Protected adenosine derivative **11** (1.0 g, 1.1 mmol)

was dissolved in a mixture of diisopropylethyl amine (0.42 g, 3.5 mmol) and 5 mL dry CH_2Cl_2. 2-Cyanoethyl-*N*,*N*-diisopropylchloro phosphoramidite (0.4 g, 1.65 mmol) was added dropwise to the stirred solution under an argon atmosphere. After stirring at room temperature for 5 h, the reaction was quenched by adding 0.2 mL isopropanol and the solvents were removed in vacuo. Purification of the residue by MPLC (75–0% petrol ether in ethyl acetate) afforded diastereomeric phosphoramidite **13** (1.0 g, 82%) as a colorless foam. ^{1}H NMR ($CDCl_3$): 9.42 (bs, NH), 8.70 (s, 1H, H-2), 8.21 (s, 1H, H-8), 7.40–6.80 (m, 18H, Ar-H), 6.12 (d, J = 5.0, 1H, H-1′), 4.83 (s, CH_2O), 4.79 (m, 1H, H-2′), 4.58 (m, 1H, H-3′), 4.40 (m, 1H, H-4′), 3.80–3.30 (m, 16H, H-5′a, H-5′b, CH-CNE, $CH(CH_3)_2$, OCH_3, CH_2NH, OCH_2), 2.65 (m, CH-CNE), 2.65 (m, CH-CNE), 1.50–1.10 (m, 20H, CH_3, CH_2). ^{13}C NMR ($CDC1_3$): 166.63 (N–C = O), 158.54 (ArC), 156.92 (C-6), 151.85 (C-2), 148.21 (C-4), 144.38 (C-8), 135.56, 130.06, 129.83, 128.12, 127.83, and 122.46 (ArC), 117.63 (C-5), 114.90 and 113.11 (ArC), 86.66 (C-1′), 84.04 (C-4′), 80.50 (C-3′), 70.87 (O-CH_2), 70.70 (C-2′), 67.95 ($CHPh_3$), 63.01 (O-CH_2), 62.71 (C-5′), 55.18 (OCH_3), 43.16 and 43.06 ($CH(CH_3)_3$), 39.75 (CH_2-NH), 29.20, 28.60, 26.23, and 25.36 (CH_2), 24.59 and 24.53 (CH_3), 20.35 and 20.29 (CH_2-CN). ^{31}P NMR ($CDC1_3$): 151.27, 150.79. ESI-MS: 1099.8. $C_{56}H_{64}N_8O_{10}F_3P$: calc. C 59.11 H 5.91 N 9.85, found C 59.07 H 6.20 N 10.15.

5′-*O*-Dimethoxytrityl-2′-*O*-(6-trifluoroacetamido)hexyluridine-3′-*O*-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (14). Synthesis of uridine phosphoramidite **14** after the method described above starting with 1.0 g **11** gave diastereomeric **14** (1.25 g, 98%) as colorless foam. ^{1}H NMR ($CDC1_3$): 8.09 and 8.00 (d, J = 8.1, H-6), 7.44–7.22 (m, 9H, Ar-H), 6.95 (bs, 1H, NH-CF_3), 6.88 (m, 4H, Ar-H), 5.97 (d, J = 2.8, H-1′), 5.22 and 5.19 (d, J = 8.1, H-5), 4.65–3.40 (m, 15H, H-2′, H-3′, H-4′, H-5′a, H-5′b, OCH_3, OCH_2, $CH(CH_3)_2$), 3.39 (m, 2H, CH_2NH), 2.76, 2.61, and 2.44 (3m, 4H, OCH_2CH_2CN), 1.80–1.00 (m, 20H, CH_2, CH_3). ^{13}C NMR ($CDC1_3$): 163.09 (q, C-4), 158.79 (q, ArC), 157.25 (q, J = 36.4, CO), 150.27 (q, C-2), 144.32, 144.18 (2q, ArC), 140.12 and 140.06 (2d, C-6), 135.23, 135.14, 135.06, 134.95 (4q, ArC), 130.26, 128.28, 127.95, and 127.21 (4q, ArC), 117.58 and 117.46 (CN), 115.98 (q, J = 279.0, CF_3), 113.25 (d, ArC), 102.16 (d, C-5), 88.26 and 88.08 (d, C-1′), 87.18 and 87.02 (CPH_3), 82.29 (d, C-4′), 81.61 (C-2′), 70.86 and 70.57 (t, OCH_2), 69.91 and 69.82 (d, J = 14.9, C-3′) 61.43 and 60.87 (C-5′), 58.23 (t, OCH_2), 55.23 (q, OCH_3), 45.40 and 45.28 (t, $POCH_2$) 43.28 and 43.21 (d, J = 12.6, $CH(CH_3)_2$), 39.82 and 39.76 (t, NCH_2), 29.33, 28.66, 28.60, 26.10, 25.47 and 25.36 (t, CH_2), 24.69, 24.59, and 24.43 (q, $CH(CH_3)_2$), 20.24 (m, CH_2CN). ^{31}P NMR ($CDC1_3$): 150.85, 150.56. ESI-MS: 942.6.

Synthesis of Oligonucleotides

Oligonucleotides were prepared on an ABI 392B DNA Synthesizer in 1.0 μM scale using standard synthesis protocols. 2′-*O*-Aminohexyl modified nucleotides were coupled using extended coupling times of 5 min resulting in an average yield

of 92% as determined by trityl cation assay. Oligonucleotides were prepared in DMT-off mode and cleaved from solid support using concentrated ammonia (1 h/r.t.). Deprotection was afforded by heating the resulting solution to 55°C for 18 h. Ammonia was removed in vacuo and the residue was redissolved in 0.5 mL water.

HPLC Analysis and Purification

Crude oligonucleotides were filtered on Schleicher & Schuell Spartan 13/20, 0.45 μm Braunrand H filters, concentrated on a speed vac, and redissolved in 300 μL water. Concentrations were determined by measuring, UV-absorption at 260 nm. Analytical HPLC of a 20-μL sample (0.2 mg/mL) was performed on an ET 250/8/4 Nucleosil® 100-5 C18 column (Macherey Nagel) with a flow rate of 1 mL/min and a linear gradient of 10–40% B in 30 min (A: 0.1 M triethylammonium acetate in water, B: 0.1 M triethylammonium acetate in 80% acetonitrile). Preparative HPLC was done on a Aquapore Octyl Prep 20 Cartridge 250 × 10 mm (Applied Biosystems) with a flow rate of 4 mL/min and the same gradient as mentioned above. All oligonucleotides were desalted using Sep-Pak-Cartridges® (Waters, Milford, MA).

CD Spectroscopy

Concentrations of purified and desalted oligonucleotides were determined by UV measurement at 260 nm. Molar extinction coefficients were calculated by addition of nucleotides (184,800 $cm^{-1}M^{-1}$ for homo adenosine dodecamers, 105,600 $cm^{-1}M^{-1}$ for homo thymidine dodecamers). CD spectra were recorded on a Jasco J-710 spectropolarimeter.

Oligonucleotides were diluted to a concentration of 9 μM in a solution of 0.15 M NaCl and 0.01 M Tris-HCl (pH 7.0) of a total volume of 300 μL. Complementary strands were hybridized for 5 min at 80°C, then cooled to room temperature to ensure duplex formation. Measurements were done in a quartz cuvette of a path length of 1 mm. Wavelength range was set to 320–210 nm with a scanning speed of 50 nm/min. For determination of melting temperature, the duplex solution was heated from 0 to 80°C with a slope of 50°C per hour.

Nuclease Stability Test

To a solution of uridine dodecamer (2.71 nmol, 0.33 OD) in an Eppendorf cap, 25 μL PDE-incubation buffer was added. The solution was diluted with Milli-Q-Water to a total volume of 240 μL. After 10 min incubation at 37°C, 10 μL (200 μU) of phosphodiesterase solution (calf spleen, Boehringer Mannheim, 4 U/mL, diluted 1:200) was added. After mixing briefly, the resulting solution was incubated at 37°C for 30 min. PDE-stop buffer (50 μL) was added and the mixture was heated to 90°C for 10 min. Quantification of degradation was done using HPLC as described above.

CONCLUSION

A thorough study comparing 2′-*O*-alkyl– and 2′-*O*-aminohexyl–modified oligonucleotides is presented. The modifications tested were dodecamers of poly(A)-poly(T). Apart from studies on the secondary structure using circular dichroism, melting points were primarily used as an indicator of the hybridization affinity. 2′-*O*-Aminoalkyl–substituted oligonucleotides clearly offer more auspicious characteristics in view of the therapeutic application compared to 2′-*O*-alkyl–modified derivatives. The protonation of the negatively charged backbone by use of the 2′-*O*-aminohexyl modification is a useful technique toward increasing the stability of DNA-RNA duplexes. The destabilizing effect caused by the steric bulk of the 2′-*O*-alkyl chain is greatly diminished by the stabilizing effect of the cationic amino group. We have also shown that the incorporation of an increasing number of zwitterionic nucleoside building blocks efficiently augments stability against degrading 5′-exonucleases.

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