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ORIGINAL PAPER

2′-*O*-Lysylaminohexyladenosine modified oligonucleotides

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Abstract Development of therapeutically active oligonucleotides for sequence-specific gene knockdown relies on chemical modifications that confer high stability and target affinity and ideally enable cellular uptake. 2′-*O*-Lysylaminohexyluridine-containing antisense and siRNA oligonucleotides have been shown to be well suited for gene knockdown. They are highly resistant to enzymatic degradation while having good affinity for the targeted RNA strand and efficiently down-regulate their target in cell culture tumor models. The 2′-*O*-lysylaminohexyl modification was expanded to adenosine nucleosides. The corresponding phosphoramidite building block was prepared in a straightforward procedure comprising six steps starting from adenosine. After 2′-*O*-alkylation with *N*-(6-bromohexyl)phthalimide and removal of the *N*-protecting group, the protected lysine was specifically attached to the alkylamino group. Incorporation of 2′-*O*-lysylaminohexyladenosine nucleotides in a test sequence confirmed that the cationic chains lead only to minor duplex destabilization and do not disturb the duplex structure. Results further emphasize the advantageous properties of 2′-*O*-lysylaminohexyl modified oligonucleotides for therapeutic applications.

Keywords Oligonucleotides · Zwitterions · Circular dichroism · Thermal denaturation · Alkylation

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Introduction

Although therapeutic gene silencing by nucleic acid analogues has been regarded as one of the great expectations in drug research for several decades, only one antisense oligonucleotide, fomivirsen, and one ribozyme oligonucleotide, pegaptanib, have been granted approval. Application of both drugs by injection into the eye reveals the still very limited therapeutic options. Apart from that, there is a rather long history of unsuccessful clinical trials of antisense oligonucleotides. Their use has been hampered by several factors, for example poor bioavailability, insufficient stability, substantial toxicity, insufficient targeting, and off-target effects, which are, at least in part, caused by the chemical modifications used.

Design of antisense drugs started with work on chemical modifications of the lead nucleic acid structure, which itself lacks sufficient stability against enzymatic degradation [1, 2]. A major step forward seemed to be achieved by replacement of one of the oxygen atoms of the phosphate bridge by a sulfur atom, which resulted in good plasma stability while only mildly interfering with base pairing. These phosphorothioate oligonucleotides are now referred to as first-generation antisense drugs. So far, most antisense agents used in late-stage clinical trials belong to this group. The clinical history of this class of drug candidates has revealed their drawbacks. The best example is oblimersen (Genasense, G3139). The largest study ever conducted for advanced melanoma using a combination of oblimersen and dacarbazine compared with dacarbazine monotreatment failed to reach the primary end point, overall survival [3]. In addition to its rational effect, several reports in recent years have linked oblimersen to unspecific or off-target effects [4–6]. A proteomic analysis of the effect of oblimersen on human melanoma cells has revealed several proteins that were down-regulated, with

many of them being involved in apoptosis resistance and cancer progression [7]. Additionally, a number of glycolytic enzymes were found to be down-regulated by oblimersen treatment, indicating a partial reversal of the Warburg effect. Using control oligonucleotides it was shown that the effect is indeed caused by the phosphorothioate backbone modification. As a matter of fact, it can be stated that the chemical modification used in more than half of all clinical studies over many years is directly associated with unwanted effects. In future, the phosphorothioate modification should only be used in selected cases, e.g. if induction of apoptosis is an immediate therapeutic objective.

As a consequence of this situation there is a heavy demand for appropriate chemical modifications to enable the design of effective nucleic acid drugs. The 2′-*O*-alkyl modification of oligonucleotides has been shown to be effective [8, 9]. The compounds impair duplex formation only weakly, and significantly increased plasma stability does not impede activation of the effector enzyme RNAseH. They have superior in vitro characteristics and have been shown to be devoid of the phosphorothioate-related off-target effects. 2′-*O*-Alkyl-modified compounds are therefore classified as second-generation antisense drugs. In the meantime—aiming at a third generation—more rigorously modified compounds have also been prepared on the basis of replacement of the furanose ring by other moieties, with peptide nucleic acids [10], morpholino oligonucleotides [11, 12], and locked nucleic acids (LNA) [13] as the most promising modifications. The advent of siRNA a decade ago [14] drew much of the attention away from the slowly developing antisense field. However, in the meantime it has become clear that—despite their different molecular effectors—the close structural relationship of antisense and siRNA results in the same hurdles in drug design [15–17].

Our early work on modification of oligonucleotides at the 2′-*O*-position included design and studies of zwitterionic compounds [2, 18–22]. With this group of compounds, particularly promising results were obtained by introduction of a lysine building block at the 2′-position of uridine. Solid-phase oligomerization was shown to result in highly stable antisense and siRNA oligonucleotides with good base-pairing properties and excellent target down-regulating properties in vitro [18, 19]. Building on the promising results obtained with the 2′-*O*-lysylaminohexyluridine nucleoside, we report the expansion of this chemistry to the purine nucleoside adenosine.

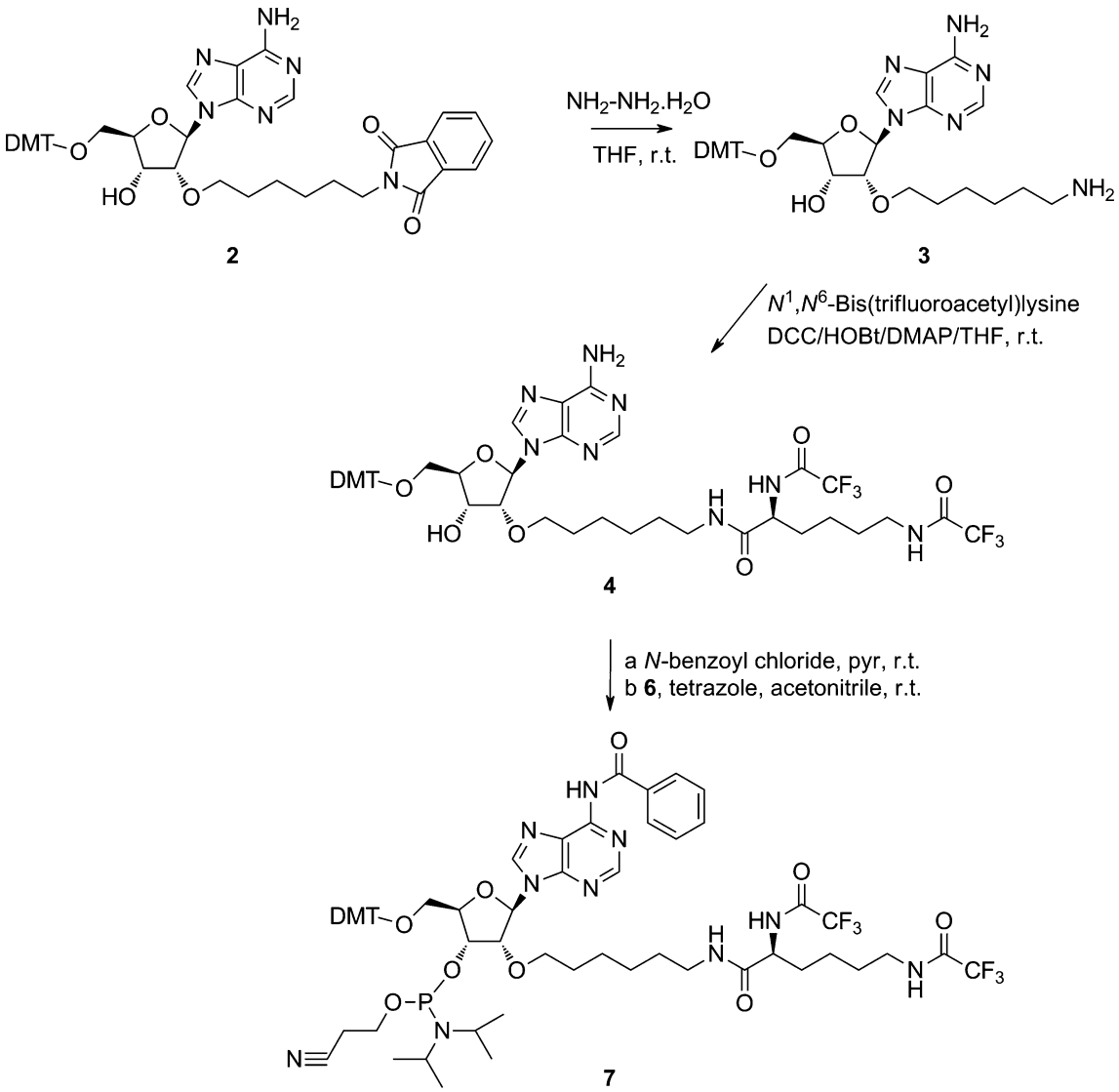
Results and discussion

The preparation of *N*-benzoyl-2′-*O*-[N^2,N^6-bis(trifluoroacetyl)lysyl]aminohexyl-5′-*O*-(4,4′-dimethoxytrityl)adenosine 3′-*O*-[*O*-(2-cyanoethyl)-*N*,*N*′-diisopropylphosphoramidite] (**7**) (Scheme 1) proceeded analogously to the respective uridine compound [18] with adjustments being made with regard to the exocyclic amino function of adenosine. After tritylation of adenosine, 2′-alkylation was achieved with NaI and 6-phthalimidohexyl chloride [9]. The phthalimido group was selected because of the easy and orthogonal deprotection by hydrazinolysis. Coupling of the lysyl moiety, protected as bis(trifluoroacetyl)amide, was performed in high yield with the classical peptide coupling reagents dicyclohexyl carbodiimide (DCC) and 1*H*-hydroxybenzotriazole (HOBt) and proceeded selectively at the alkyl amine without, at this stage, the need for protection of the aromatic amine of the nucleobase. Therefore, N^6-protection for automated oligonucleotide synthesis can conveniently be carried out after 2′-*O*-sidechain attachment. Finally, after *N*-benzoylation and 3′-phosphitylation according to standard procedures, the phosphoroamidite building block **7** suitable for solid phase oligonucleotide synthesis was prepared in a total yield of 13%.

The modified adenosine phosphoroamidite **7** was subsequently successfully incorporated into 2′-desoxyoligonucleotides using the standard coupling reagent 4,5-dicyanoimidazole (DCI) with a coupling time of 15 min. To assess the effect on oligonucleotide structure and target affinity, an increasing number of lysylaminohexyl-modified adenosine nucleosides was incorporated into a model dodecamer sequence (Table 1).

Circular dichroism (CD) spectrometry is a sensitive method for determining secondary structures of oligonucleotides in solution. Duplexes of adenosine–thymidine homomers give a pronounced CD spectrum, in which subtle changes caused by chemical modifications can be detected. Gradual substitution of desoxyadenosine against 2′-*O*-lysylaminohexyladenosine resulted only in minor quantitative changes in the CD spectrum (Fig. 1) without alteration of the curve shape, indicating that the original duplex structure prevailed. Incorporation of up to four modifications (**12**) caused only minor changes of the CD spectra, whereas lower band intensities in the far-UV region and loss of the characteristic double peak of A/T homomer duplexes at 260 nm were observed for **14**, with the anionic phosphate backbone fully neutralized. This indicates a less defined duplex structure or incomplete hybridization to the counter strand, possibly caused by unspecific intrastrand interactions of **14**.

The duplex stabilities as determined by recording transition temperatures showed a gradual decrease with increasing number of cationic nucleotides (Fig. 2),whereas substitution of the first two 5′-terminal nucleosides results in a relatively pronounced effect on the transition temperature and thus on duplex stability. No further negative effect was observed when up to five nucleosides were modified. An even higher number of cationic modifications



Scheme 1

Table 1 Sequences and duplex transition temperatures (*Tm* ± SEM) of oligonucleotides

Compound No.	Sequence (5′–3′)	*Tm* (°C)
8	AAAAAAAAAAAA	34.7 ± 0.4
9	A*AAAAAAAAAA	31.4 ± 0.2
10	A*A*AAAAAAAAA	25.9 ± 0.9
11	A*A*A*AAAAAAAA	26.3 ± 0.6
12	A*A*A*A*AAAAAAA	25.4 ± 0.7
13	A*A*A*A*A*AAAAAA	27.5 ± 0.4
14	A*A*A*A*A*A*AAAAA	18.3 ± 0.7
15	A*A*A*A*A*A*A*AAAA	16.6 ± 0.3
16	TCTCCCAGCGTGCGCCAT	78.7 ± 0.5
17	U*CU*CCCA*GCGU*GCGCCA*T	66.6 ± 0.6

* 2′-*O*-Lysylaminohexyl modified nucleotide; others are 2′-desoxynucleotides

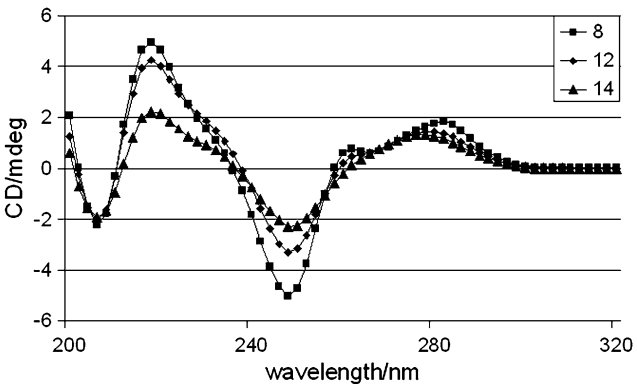


Fig. 1 Circular dichroism spectra of duplexes of dA_{12}–dT_{12} homomers with 2′-*O*-lysylaminohexyl modifications: *squares*, **8**; *rhombuses*, **12**; *triangles*, **14**

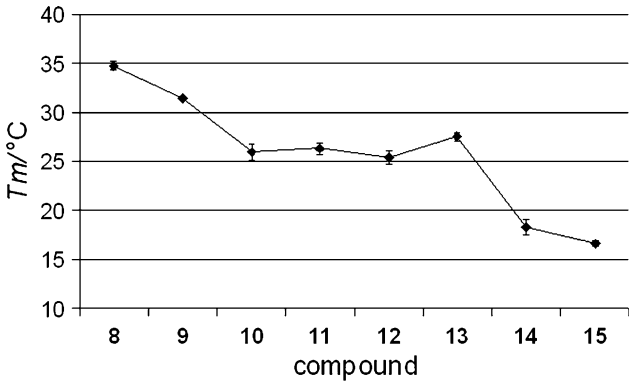


Fig. 2 Transition temperatures (*Tm*) of dA_{12}–dT_{12} duplexes with the indicated number of 2′-*O*-lysylaminohexyladenosine nucleosides

led to a significant decrease in transition temperatures. Similar to the results with 2′-*O*-lysylaminohexyluridine nucleosides [18], the decrease on the transition temperature amounted to 1.4 °C per modification when five neighboring cationic chains were present. Most of the destabilization can be attributed to the lower thermal stability of DNA/RNA hybrids. Substitution of dA by rA alone contributes to an overall decrease in melting temperature of 1.9 °C [18, 23]. This confirms the assumption that with isolated lysylaminohexyl chains the adverse effect on the counter-strand affinity, probably caused by the high steric bulk, overcomes positive effects of interstrand amine–phosphate interactions [19]. Several adjacent modifications seem to be necessary for effective interstrand charge interactions. The sharp decline in duplex stability of **14** and **15**, which was also reflected in CD data, can be attributed to charge interactions, which occur if the overall charge of the strand approaches neutrality.

Incorporation of 2′-*O*-lysylaminohexyladenosine and uridine nucleotides in a phosphodiester oligonucleotide isosequential to oblimersen (Table 1), a clinically relevant antisense oligonucleotide, confirmed that the reduction in melting temperature is higher for isolated modifications (2.4 °C per modification). The observed effect for five lysylalkyl chains is very similar to that of a full phosphorothioate backbone, the modification of oblimersen. This demonstrates that the reduction in counter-strand affinity is acceptable for clinical applications, especially because it is associated with higher stability against enzymatic degradation and better pharmacokinetics [18].

In conclusion, the 2′-*O*-lysylaminohexyl technology was expanded to adenosine. The respective building block for oligonucleotide synthesis was successfully prepared in good yield. Biophysical experiments further confirmed the usefulness of this type of modification in nucleic acid drug design.

Experimental

Reagents were purchased from Sigma-Aldrich in standard quality and used without purification. To prepare anhydrous solvents tetrahydrofuran (THF) was heated under reflux with metallic sodium until the indicator benzophenone turned blue, and then distilled, and dichloromethane and pyridine were distilled from calcium hydride. NMR spectra were recorded on a 200-MHz Bruker Spectrospin or a 500-MHz Bruker Advance. Shifts are reported relative to the solvent peak ($CHCl_3$ in $CDCl_3$: $\delta = 7.26$ and 77.00). All reactions were monitored by thin-layer chromatography (TLC) using silica gel 60-F254 precoated aluminum plates from Merck. Compounds were visualized by UV-deletion or using anisaldehyde–sulfuric acid. Column chromatography was performed with Merck silica gel 60 which was impregnated with diluted triethylamine before use. Elemental analyses (C, H, N) were conducted at the Microanalytical Laboratory of the University of Vienna using the 2400 CHN Elemental Analyzer (Perkin–Elmer); their results were in good agreement (±0.3%) with the calculated values.

5′-O-(4,4′-Dimethoxytrityl)-2′-O-(6-phthalimidohexyl)adenosine (**2**, $C_{45}H_{46}N_6O_8$)

5′-*O*-(4,4′-Dimethoxytrityl)adenosine [24] (**1**, 2.5 g, 4.39 mmol) was dissolved in 45 cm^3 DMF under an argon atmosphere and cooled in an ice bath. Sodium hydride (130 mg, 95% dispersion, 5.27 mmol) was added in portions. After intensive stirring for 30 min, 130 mg sodium iodide (0.88 mmol) and 3.4 g *N*-(6-bromohexyl)-phthalimide (10.97 mmol) dissolved in 5 cm^3 DMF were added. The solution was warmed to room temperature and stirred for a total of 67 h. The organic solvent was removed and the residue dissolved in ethyl acetate. The organic phase was washed successively with water, bicarbonate, and brine. After solvent removal in vacuo, the residue was purified on silica gel with dichloromethane–triethylamine 9:1 as mobile phase. The corresponding fractions were washed with bicarbonate and dried, affording 1.27 g **2** as a white foam (36%).

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 8.27$ (s, 1H, H-8), 8.04 (s, 1H, H-2), 7.84–7.80 (m, 2H, Phth H-4,7), 7.69–7.65 (m, 2H, Phth H-5,6), 7.43–7.23 (m, 9H, Ar-H, amide-H), 6.81 (d, 4H, Ar-H), 6.12–6.10 (m, 1H, H-1′), 4.49 (m, 2H, H-2′, H-3′), 4.24–4.22 (ddd, 1H, H-4′), 3.78 (s, 6H, $2OCH_3$), 3.71–3.43 (m, 6H, H-5′, alkyl-H-1, alkyl-H-6), 1.67–1.26 (m, 8H, alkyl-H) ppm; ^{13}C-NMR (50 MHz, $CDCl_3$): $\delta = 168.42$ (s, Phth: C-1, C-3), 158.46 (s, Ar-4), 155.49 (s, C-6), 149.46 (s, C-4), 144.52 (Ar-C-1), 135.67, 135.59 (Ar-C-1), 133.81 (d, Ar-C), 132.01 (s, Ar-C), 130.05 (Ar-C), 128.12 (Ar-C), 127.82 (Ar-C), 126.85 (Ar-C), 123.12 (Ar-C), 120.00 (Ar-C), 113.10 (Ar-C), 86.84

(C-1′), 86.47 (Ar-C), 83.83 (C-4′), 81.57 (C-2′), 71.07 (OCH_2), 69.82 (C-3′), 63.03 (C-5′), 55.15 (OCH_3), 37.65 (NCH_2), 29.34, 28.29, 26.37, 25.32 (CH_2) ppm; ESI MS: $m/z = 800.0$ (M^+).

2′-O-Aminohexyl-N-benzoyl-5′-O-(4,4′-dimethoxytrityl)adenosine (**3**, $C_{44}H_{48}N_6O_7$)

Alkylated adenosine derivative **2** (450 mg, 0.563 mmol) was dissolved in 10 cm^3 tetrahydrofuran. After addition of 250 mm^3 hydrazine hydrate, the reaction mixture was heated to reflux under an argon atmosphere for 4 h. After cooling, $NaHCO_3$–K_2CO_3 buffer (1 M, pH 9, 5 cm^3) was added. After extraction with dichloromethane, drying of the organic phase, and evaporation, 340 mg (0.508 mmol, 90%) of a white foam of **3** resulted.

^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 8.31$ (s, 1H, H-8), 8.01 (s, 1H, H-2), 7.42–7.21 (m, 9H, Ar-H), 6.74 (d, 4H, Ar-H), 6.12 (d, 1H, H-1′), 4.51-4.42 (m, 2H, H-2′, H-3′), 4.23 (d, 1H, H-4′), 3.51 (s, 6H, OCH_3), 3.67–3.31 (m, 6H, H-5′, alkyl-H-2, alkyl-H-6), 1.51–1.19 (m, 8H, alkyl-CH_2) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 158.2$ (Ar-C-4), 155.5 (C-6), 152.7 (C-2), 149.3 (C-4), 144.3 (Ar-C-1), 135.5 (Ar-C-1), 131.4-126.8 (Ar-C-2,3,4,5,6), 113.0 (Ar-C-3,5), 86.5 (C-1′), 86.4 (CPh_3), 83.7 (C-4′), 81.3 (C-2′), 70.8 (OCH_2), 69.6 (C-3′), 62.9 (C-5′), 55.0 (OCH_3), 41.5 (NCH_2), 29.2–25.3 (4 CH_2) ppm; ESI MS: $m/z = 670.0$ (M^+).

2′-O-[N^2,N^6-bis(trifluoroacetyl)lysyl]aminohexyl-5′-O-(4,4′-dimethoxytrityl)adenosine (**4**, $C_{47}H_{54}F_6N_8O_9$)

2′-*O*-Aminohexyladenosine derivative **3** (250 mg, 0.318 mmol) was dissolved in 20 cm^3 dry THF. 1*H*-1-Hydroxybenzotriazole (50 mg), 45 mg diisopropylcarbodiimide, 106 mg N^2,N^6-bis(trifluoroacetyl)lysine (0.313 mmol), and 4 mg 4-dimethylaminopyridine were added sequentially. The reaction mixture was stirred at r.t. for 20 h. After solvent evaporation, the residue was chromatographed on a silica column with dichloromethane–methanol 97:3 as mobile phase. The fractions containing the product ($R_f = 0.32$) were pooled and evaporated to give 239 mg **4** as a white foam (0.242 mmol, 76%).

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.24$ (s, 1H, H-8), 8.06 (s, 1H, H-2), 7.42–7.22 (m, 12H, Ar-H, amide-H), 6.80 (d, 4H, Ar-H), 6.14 (d, 1H, H-1′), 4.57–4.46 (m, 2H, H-2′, H-3′), 4.25 (d, 1H, H-4′), 3.76 (s, 6H, OCH_3), 3.67–3.17 (m, 9H, H-5′, alkyl-H-2, alkyl-H-6, lysyl-H-2, lysyl-H-6), 1.89–1.26 (m, 14H, alkyl-CH_2, lysyl-CH_2) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 170.1$ (lysylamide), 158.5 (Ar-C-4), 155.5 (C-6), 149.5 (C-4), 144.5 (Ar-C-1), 135.6 (Ar-C-1), 130.0 (Ar-C-2,6), 128.1, 127.8, 126.9 (Ar-C-2,3,4,5,6), 113.1 (Ar-C-3,5), 86.6 (C-1′), 86.5 (CPh_3), 84.2 (C-4′), 81.7 (C-2′), 70.9 (OCH_2), 69.9 (C-3′), 63.1 (C-5′), 55.1 (OCH_3), 39.3, 39.1 (NCH_2), 31.7–22.1 (CH_2) ppm; ESI MS: $m/z = 990.1$ (M^+).

N-Benzoyl-2′-O-[N^2,N^6-bis(trifluoroacetyl)lysyl]aminohexyl-5′-O-(4,4′-dimethoxytrityl)adenosine (**5**, $C_{54}H_{58}F_6N_8O_{10}$)

2′-*O*-Lysylaminohexyladenosine derivative **4** (312 mg, 0.317 mmol) was dissolved under argon in 6 cm^3 of a mixture of pyridine and dichloromethane (5:1). Chlorotrimethylsilane (13 mg, 1.2 mmol) was added dropwise while stirring constantly. After 30 min, 95 mg benzoyl chloride (0.671 mmol) was added and the resulting mixture was stirred at r.t. under argon for 16 h. After addition of 5 cm^3 5% $NaHCO_3$ solution and evaporation of the organic solvent, the residue was dissolved in dichloromethane and extracted with $NaHCO_3$ solution. After drying and evaporation of the organic phase, the residue was dissolved in 3 cm^3 THF, and 0.5 cm^3 of a 1 M solution of tetrabutylammonium fluoride in THF was added. After stirring at r.t. for 20 h, dichloromethane was added. After repeated extraction with $NaHCO_3$, the organic phase was dried under reduced pressure. The residue was chromatographed using a mixture of dichloromethane and methanol (98:2) as mobile phase. The fractions containing the product were pooled and evaporated to dryness to give 280 mg **5** as a white foam (0.284 mmol, 89%).

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.49$ (s, 1H, H-8), 8.18 (s, 1H, H-2), 7.45–7.12 (m, 18H, Ar-H, amide-H), 6.68 (d, 4H, Ar-H), 6.12 (d, 1H, H-1′), 4.56–4.44 (m, 2H, H-2′, H-3′), 4.21 (d, 1H, H-4′), 3.64 (s, 6H, OCH_3), 3.50–2.92 (m, 9H, H-5′, alkyl-H-2, alkyl-H-6, lysyl-H-2, lysyl-H-6), 1.67–0.93 (m, 14H, alkyl-CH_2, lysyl-CH_2) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 172.6$ (benzoylamide), 169.7 (lysylamide), 158.5 (Ar-C-4), 155.3 (C-6), 149.6 (C-4), 144.4 (Ar-C-1), 135.5 (Ar-C-1), 133.7 (Ar-C-1), 133.2 (Ar-C-2-6), 130.0 (Ar-C-2,6), 128.3, 127.7, 126.9 (Ar-C-2,3,4,5,6), 113.1 (Ar-C-3,5), 86.6 (C-1′), 86.5 (CPh_3), 84.2 (C-4′), 81.7 (C-2′), 70.9 (OCH_2), 69.9 (C-3′), 63.1 (C-5′), 55.1 (OCH_3), 39.5, 39.1 (NCH_2), 31.9–21.9 (CH_2) ppm; ESI MS: $m/z = 1094.1$ (M^+).

2-Cyanoethoxybis(diisopropylamino)phosphan (**6**)

Phosphorus trichloride (10 g, 73 mmol) was dissolved in 125 cm^3 dry acetonitrile and stirred under an argon atmosphere. Diisopropylamine (45 g, 445 mmol) was added dropwise over a period of 1 h. After addition of 125 cm^3 hexane, 5.2 g hydroxypropionitrile (73 mmol) was added dropwise over a period of 30 min. After stirring for 1 h, the solution was filtered and the liquid phases separated. The acetonitrile phase was washed three times with fresh *n*-hexane, and the combined *n*-hexane phase was dried to give 9.2 g of a yellow oil (**7**, 42%). ^{1}H, ^{13}C, and ^{31}P NMR spectra were found to be identical with reports in the literature [25].

N-Benzoyl-2′-O-[N^2,N^6-bis(trifluoroacetyl)lysyl]amino-hexyl-5′-O-(4,4′-dimethoxytrityl)adenosine 3′-O-[O-(2-cyanoethyl)-N,N-diisopropylphos-phoramidite](**7**, $C_{63}H_{75}F_6N_{10}O_{11}P$)

N -Benzoyl-2′-*O*-[N^2,N^6-bis(trifluoroacetyl)lysyl]aminohexyl-5′-*O*-(4,4′-dimethoxytrityl)adenosine (**5**, 120 mg, 0.122 mmol) was dissolved under moisture-free conditions under an argon atmosphere in 5 cm^3 dry dichloromethane. After the addition of 9 mg 1*H*-tetrazole and 36.5 mg 2-cyanoethoxy-bis(diisopropylamino)phosphane (**6**), the mixture was stirred at r.t. for 16 h. After washing with $NaHCO_3$ solution, the organic phase was evaporated to dryness and the residue was purified on a silica column with dichloromethane–methanol (98:2) as mobile phase. Drying of the respective fractions gave 85 mg of a white foam (0.072 mmol, 59%).

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.59$ (s, 1H, H-8), 8.30 (s, 1H, H-2), 7.43–7.19 (m, 18H, Ar-H, amide-H), 6.78 (d, 4H, Ar-H), 6.18 (d, 1H, H-1′), 4.58–4.47 (m, 2H, H-2′, H-3′), 4.23 (d, 1H, H-4′), 3.73 (s, 6H, OCH_3), 3.66–3.16 (m, 13H, H-5′, alkyl-H-2, alkyl-H-6, lysyl-H-2, lysyl-H-6, isopropyl-CH, cyanoethyl-CH_2), 2.74 (t, 2H, NC-CH_2), 1.70–1.24 (m, 26H, alkyl-CH_2, lysyl-CH_2, isopropyl-CH_3) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta =$ 172.4 (benzoylamide), 169.9 (lysylamide), 158.4 (Ar-C-4), 155.6 (C-6), 149.9 (C-4), 144.3 (Ar-C-1), 135.5 (Ar-C-1), 133.7 (Ar-C-1), 133.0 (Ar-C-2-6), 129.9 (Ar-C-2,6), 128.6, 127.5, 126.8 (Ar-C-2,3,4,5,6), 113.1 (Ar-C-3,5), 86.9 (C-1′), 86.4 (CPh_3), 84.3 (C-4′), 81.3 (C-2′), 70.9 (OCH_2), 69.6 (C-3′), 63.0 (C-5′), 55.0 (OCH_3), 46.0 (cyanoethyl-OCH_2), 45.2 (isopropyl-CH), 39.5, 39.2, 39.1 (cyanoethyl-CH_2, alkyl-NCH_2), 31.9–21.9 (CH_2), 22.7 (isopropyl-CH_3) ppm; ESI MS: $m/z = 1293.7$ (M^+).

Oligonucleotide synthesis

DNA-based oligonucleotides were prepared on a Polygen 10-column DNA synthesizer on 0.2 mM scale using standard synthetic procedures. 2′-*O*-Lysylaminohexyl-modified nucleotides were coupled using extended coupling times of 15 min. Oligonucleotides were prepared in DMT-off mode and cleaved from the solid support by use of concentrated ammonia (1 h at r.t.). Deprotection was achieved by heating the resulting solution to 55 °C for 18 h. Ammonia was removed in vacuo and the residue was redissolved in 0.5 cm^3 water. Crude oligonucleotide products were desalted using Sephadex G-25 (GE Life Sciences, UK). Oligonucleotide structures were confirmed by MALDI-ToF-MS on a Kratos Seq using 3-hydroxypicolinic acid as matrix [21]: **9**: calc. 3938.10, found 3938.7; **10**: calc. 4181.4, found 4182.1; **11**: calc. 4424.8, found 4425.9; **12**: calc. 4668.1, found 4668.0; **13**: calc. 4911.5, found 4912.8; **14**: calc. 5154.8, found 5154.5; **15**: calc. 5398.1, found 5398.5; **17**: calc. 6535.6, found 6535.3.

Circular dichroism and transition temperature

Concentrations of purified and desalted oligonucleotides were determined by UV measurement at 260 nm. Molar extinction coefficents were calculated by addition of nucleotides (184,800 cm^{-1} M^{-1} for adenosine dodecamers, 105,600 cm^{-1} M^{-1} for thymidine dodecamers). CD spectra were recorded on a Jasco J-810 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 200 mm^3. Complementary strands were hybridized for 5 min at 80 °C, then slowly cooled to room temperature to ensure duplex formation. Measurements were conducted in a quartz cuvette with a path length of 1 mm, and the wavelength range was set to 320–200 nm with a scanning speed of 50 nm min^{-1}. For determination of the transition temperature the duplex solution was heated from 0 to 80 °C at 50 °C h^{-1}. Reported denaturation temperatures are means from triplicate experiments.

References

1. Urban E, Noe CR (2003) Farmaco 58:243
2. Winkler J, Urban E, Losert D, Wacheck V, Pehamberger H, Noe CR (2004) Nucleic Acids Res 32:710
3. Bedikian AY, Millward M, Pehamberger H, Conry R, Gore M, Trefzer U, Pavlick AC, De Conti R, Hersh EM, Hersey P, Kirkwood JM, Haluska FG (2006) J Clin Oncol 24:4738
4. Gjertsen BT, Bredholt T, Anensen N, Vintermyr OK (2007) Curr Pharm Biotechnol 8:373
5. Anderson EM, Miller P, Ilsley D, Marshall W, Khvorova A, Stein CA, Benimetskaya L (2006) Cancer Gene Ther 13:406
6. Lai JC, Benimetskaya L, Khvorova A, Wu S, Hua E, Miller P, Stein CA (2005) Mol Cancer Ther 4:305
7. Stessl M, Marchetti-Deschmann M, Winkler J, Lachmann B, Allmaier G, Noe CR (2009) J Proteomics 72:1019
8. Cotten M, Oberhauser B, Brunar H, Holzner A, Issakides G, Noe CR, Schaffner G, Wagner E, Birnstiel ML (1991) Nucleic Acids Res 19:2629
9. Wagner E, Oberhauser B, Holzner A, Brunar H, Issakides G, Schaffner G, Cotten M, Knollmueller M, Noe CR (1991) Nucleic Acids Res 19:5965
10. Porcheddu A, Giacomelli G (2005) Curr Med Chem 12:2561
11. Summerton J (1999) Biochim Biophys Acta 1489:141
12. Summerton JE (2007) Curr Top Med Chem 7:651
13. Jepsen JS, Wengel J (2004) Curr Opin Drug Discov Devel 7:188
14. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC (1998) Nature 391:806
15. Jackson AL, Bartz SR, Schelter J, Kobayashi SV, Burchard J, Mao M, Li B, Cavet G, Linsley PS (2003) Nat Biotechnol 21:635
16. Hoerter JAH, Walter NG (2007) RNA 13:1887
17. Eberle F, Giessler K, Deck C, Heeg K, Peter M, Richert C, Dalpke AH (2008) J Immunol 180:3229

18. Winkler J, Gilbert M, Kocourková A, Stessl M, Noe CR (2008) ChemMedChem 3:102
19. Winkler J, Noe CR (2007) Nucleosides Nucleotides Nucleic Acids 26:939
20. Noe CR, Winkler J, Urban E, Gilbert M, Haberhauer G, Brunar H (2005) Nucleosides Nucleotides Nucleic Acids 24:1167
21. Winkler J, Saadat K, Díaz Gavilán M, Urban E, Noe CR (2009) Eur J Med Chem 44:670
22. Winkler J, Urban E, Noe CR (2004) Monatsh Chem 135:109
23. Sugimoto N, Nakano S, Katoh M, Matsumura A, Nakamuta H, Ohmichi T, Yoneyama M, Sasaki M (1995) Biochemistry 34:11211
24. Song Q, Wang W, Fischer A, Zhang X, Gaffney BL, Jones RA (1999) Tetrahedron Lett 40:4153
25. Bannwarth W, Trzeciak A (1987) Helv Chim Acta 70:175