131. Nucleotides

Part XLV[1])

Synthesis of New (2′–5′)Adenylate Trimers, Containing 5′-Amino-5′-deoxyadenosine Residues at the 5′-End of the Oligoadenylate Chain, and of Its Analogues, Carrying a 9-[(2-Hydroxyethoxy)methyl]adenine Residue at the 2′-Terminus

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The 5′-amino-5′-deoxy-2′,3′-*O*-isopropylideneadenosine (**4**) was obtained in pure form from 2′,3′-*O*-isopropylideneadenosine (**1**), without isolation of intermediates **2** and **3**. The 2-(4-nitrophenyl)ethoxycarbonyl group was used for protection of the NH_2 functions of **4** (→ **7**). The selective introduction of the palmitoyl (= hexadecanoyl) group into the 5′-*N*-position of **4** was achieved by its treatment with palmitoyl chloride in MeCN in the presence of Et_3N (→ **5**). The 3′-*O*-silyl derivatives **11** and **14** were isolated by column chromatography after treatment of the 2′,3′-*O*-deprotected compounds **8** and **9**, respectively, with (*tert*-butyl)dimethylsilyl chloride and 1*H*-imidazole in pyridine. The corresponding phosphoramidites **16** and **17** were synthesized from nucleosides **11** and **14**, respectively, and (cyanoethoxy)bis(diisopropylamino)phosphane in CH_2Cl_2. The trimeric (2′–5′)-linked adenylates **25** and **26** having the 5′-amino-5′-deoxyadenosine and 5′-deoxy-5′-(palmitoylamino)adenosine residue, respectively, at the 5′-end were prepared by the phosphoramidite method. Similarly, the corresponding 5′-amino derivatives **27** and **28** carrying the 9-[(2-hydroxyethoxy)methyl]adenine residue at the 2′-terminus, were obtained. The newly synthesized compounds were characterized by physical means. The synthesized trimers **25**–**28** were 3-, 15-, 25-, and 34-fold, respectively, more stable towards phosphodiesterase from *Crotalus durissus* than the trimer (2′–5′)ApApA.

1. Introduction. – Two common problems are associated with the therapeutic application of (2′–5′)oligoadenylates and oligonucleotides, in general [2]. One of these is low permeability through the eucaryotic cell membranes. The second one is sensitivity to nucleases, leading to rapid degradation of oligonucleotides. Many attempts have been made to overcome these problems by chemical modification. It was shown that introduction of a lipophilic cholesterol group into oligonucleotides [3] and (2′–5′)oligoadenylate trimers [1] [4] [5] results in improvement of biological properties. Widely known is also the high stability of some sugar-modified analogues [6–8] of (2′–5′)oligoadenylates towards phosphodiesterases action. On the other hand, chemical modification of oligonucleotides [9], in general, and (2′–5′)oligoadenylates [10], in particular, through the substitution of the 5′- or 3′(2′)-end OH group by an amino group led to enhancement of stability of these compounds towards degradation by nucleases.

These data prompted us to synthesize a new type of (2′–5′)adenylate trimers containing 5′-amino-5′deoxyadenosine and 5′-deoxy-5′-(palmitoylamino)adenosine

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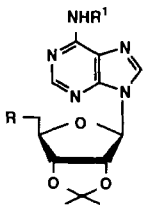
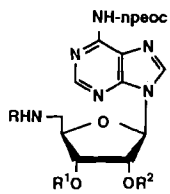
(= 5′-deoxy-5′-(hexadecanoylamino)adenosine) residues at the 5′-terminus, and their congeners with 9-[(2-hydroxyethoxy)methyl]adenine instead of the 2′-terminal adenosine residue.

2. Syntheses. – As a starting material, 2′,3′-*O*-isopropylideneadenosine (**1**) was taken and converted into the 5′-amino-5′-deoxy derivative **4** by a known method [11], slightly modified by us. Thus, treatment of **1** with TsCl in pyridine gave tosylate **2** which was reacted with NaN_3/DMF at 65–68° to give 5′-azido-5′-deoxy-2′,3′-*O*-isopropylidene-adenosine (**3**). Subsequent treatment of crude **3** with Ph_3P/pyridine/NH_4OH [12] afforded 5′-amino-5′-deoxy-2′,3′-*O*-isopropylideneadenosine (**4**) which was isolated by column chromatography (CC) in 50% overall yield. Reaction of **4** with palmitoyl chloride in MeCN in the presence of Et_3N followed by CC led to the 5′-palmitoylamino derivative **5** in 95% yield. The fully protected nucleosides **6** and **7** were obtained in 74 and 77% yield, respectively, by treatment of **4** and **5** with 3-methyl-1-[2-(4-nitrophenyl)ethoxycarbonyl]-1*H*-imidazol-3-ium chloride [13] in CH_2Cl_2.



	R	R^1
1	OH	H
2	TsO	H
3	N_3	H
4	NH_2	H
5	NHpalm	H
6	NHpalm	npeoc
7	NHnpeoc	npeoc



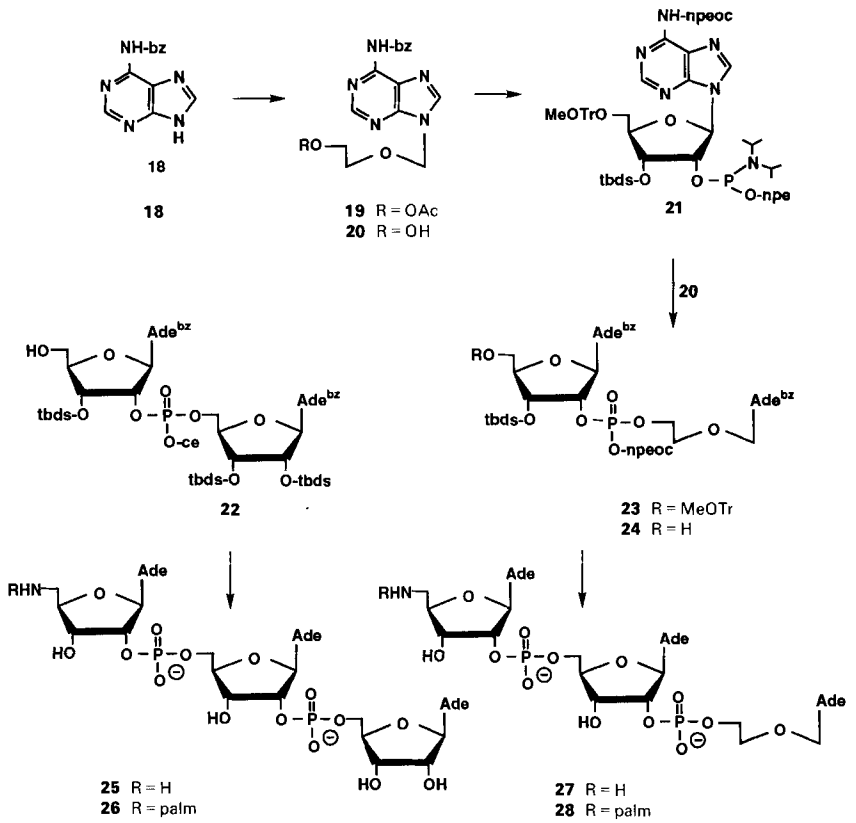
	R	R^1	R^2
8	npeoc	H	H
9	palm	H	H
10	npeoc	tbds	tbds
11	npeoc	tbds	H
12	npeoc	H	tbds
13	palm	tbds	tbds
14	palm	tbds	H
15	palm	H	tbds
16	npeoc	tbds	P< $^{Nipr_2}_{O\text{-}ce}$
17	palm	tbds	P< $^{Nipr_2}_{O\text{-}ce}$

ac = acetyl,
bz = benzoyl,
ce = 2-cyanoethyl,
MeOTr = monomethoxytrityl,
npe = 2-(4-nitrophenyl)ethyl,
npeoc = 2-(4-nitrophenyl)ethoxycarbonyl,
palm = palmitoyl,
tbds = (*tert*-butyl)dimethylsilyl

Removal of the isopropylidene groups from **6** and **7** with 80% HCOOH/H_2O gave compounds **8** and **9** in 76 and 82% yield, respectively. Silylation of **8** with (*tert*-butyl)-dimethylsilyl chloride in pyridine in the presence of 1*H*-imidazole gave a mixture of 2′,3′-bis-*O*-silyl, 3′-*O*-silyl, and 2′-*O*-silyl derivatives **10**–**12** which were isolated in an individual form by column chromatography in 20, 31, and 33% yield, respectively. Similarly, **9** was converted to the silyl derivatives **13**–**15** in 11, 36, and 43% yield, respectively. Reaction of 3′-*O*-silylated compounds **11** and **14** with (2-cyanoethoxy)-bis(diisopropylamino)phosphane [14] in CH_2Cl_2 in the presence of 1*H*-imidazole resulted in the formation of the phosphoramidites **16** and **17** in 91 and 83% yield, respectively. The latter were used as building blocks in the synthesis of (2′–5′)oligoadenylate analogues, *i.e.*, for the condensation with 5′-OH dimers **22** [14] and **24**.

The latter was obtained from N^6-benzoyladenine (**18**) [15] after trimethylsilylation and [2-(acetoxy)ethoxy]methyl bromide treatment [16] (→**19**; *Scheme*), deacetylation,

Scheme



and phosphoramidite condensation of the resulting N^6-benzoyl-9-[(2-hydroxyethoxy)-methyl]adenine (**20**) with N^6-benzoyl-3′-*O*-[(*tert*-butyl)dimethylsilyl]-5′-*O*-(mono-methoxytrityl)adenosine 2′-[2-(4-nitrophenyl)ethyl *N*,*N*-diisopropylphosphoramidite] (**21**) under 3-nitro-1*H*-1,2,4-triazole catalysis, subsequent I_2 oxidation (→fully protected dimer **23** in 85% yield), and detritylation (90% yield).

Condensation of amidite **16** with 5′-OH dimer **22** [14] in MeCN in the presence of 1*H*-tetrazole gave the corresponding fully protected trimer which was not isolated in a pure form (*Scheme*). Oxidation of the latter with I_2 in H_2O/CH_2Cl_2/pyridine and final removal of the various protecting groups using successive treatment with 0.5M DBU (1,8-diazabicyclo[5.4.0]undec-7-ene)/MeCN, 1M Bu_4NF/THF, and NH_3/MeOH, led to the fully deblocked trimer **25** in 17% overall yield which was purified by *DEAE*-cellulose CC. The synthesis of the (palmitoylamino)-containing trimer **26** was achieved in an analogous manner from phosphoramidite **17** and 5′-OH dimer **22** in 53% yield. Due to the poor solubility of the palmitoylamino derivative **26** in H_2O, a solution of $(Et_3NH)HCO_3$ in MeOH/H_2O was used for the *DEAE*-cellulose CC. In a similar way, the trimers **27** and **28** were obtained from 5′-OH dimer **24** and the phosphoramidites **16** and **17** in 11 and 58% overall yield, respectively.

All newly synthesized nucleosides and **23** and **24** were characterized by elemental analysis, TLC, UV, and ^{1}H-NMR, the amidites **16** and **17** also by ^{31}P-NMR, and the trimers **25–28** by UV, ^{1}H-NMR, and HPLC.

3. Biochemical Studies. – The stability of the newly synthesized trimers towards phosphodiesterase from *Crotalus durissus*, in comparison with the trimer (2′–5′)ApApA, was studied by means of preparative TLC. The synthesized trimers **25–28** were 3-, 15-, 25-, and 34-fold, respectively, more stable than the trimer (2′–5′)ApApA.

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Experimental Part

General. TLC: Precoated silica gel thin-layer sheets *F 1500 LS 254* from *Schleicher & Schüll.* Prep. column chromatography (CC): silica gel (*Merck 60*, 63–200 μm). Ion-exchange chromatography: *DEAE-Servacel 23 SS* (*Serva*). HPLC: *Merck-Hitachi, L-6200-Intelligent* pump, *D-2000* chromatointegrator, detection at 260 nm (*Uvikon 730 SLC*, Fa. *Kontron*); column *RP 18* (*LiChrospher* 125 × 4 mm, 5 m, *Merck 50943*); flow rate 1 ml/min; elution: *A* = 0.1M aq. (Et_3NH)OAc buffer (pH 7.0)/MeCN 1:1, *B* = 0.1M aq. (Et_3NH)OAc buffer; gradient for **20** and **22**: *A*/*B* 1:1 within 0–5 min, then *A*/*B* 6:4 within 5–35 min; gradient for **21** and **23**: *A*/*B* 1:1 within 0–5 min, then only *A* within 5–35 min. M.p.: *Gallenkamp* melting-point apparatus; no corrections. UV/VIS: *Perkin-Elmer Lambda 15*; λ_{max} in nm (log ε). ^{1}H-NMR: *Bruker WM-250*; δ in ppm rel. to $SiMe_4$. ^{31}P-NMR: *Jeol JM GX-400*; δ in ppm rel. to 85% H_3PO_4 soln.

Bioassay. The phosphodiesterase from *Crotalus durissus* (oligonucleate-5′-nucleotidohydrolase EC 3.1.15.1) was obtained from *Boehringer Mannheim*, Germany. A soln. of the trimer (10–12 o.u.) in 100 l of 0.1M *Tris* · HCl buffer (pH 8.8), containing 0.002M $MgCl_2$, was incubated at 20° with $1 \cdot 10^{-2}$ units of the enzyme. The samples of the soln. were analyzed through the corresponding time, and the remaining concentration of the trimers was determined. The calculated half lifetime for the trimer (2′–5′)-ApApA and trimers **25–28** was found to be 8, 24, 120, 200, and 275 min, resp.

5′-Amino-5′-deoxy-2′,3′-O-isopropylideneadenosine (**4**). A soln. of 2′,3′-*O*-isopropylideneadenosine (**1** · 10 g, 0.032 mol) and TsCl (11.4 g, 0.06 mol) in dry pyridine (150 ml) was stirred at r.t. for 18 h, and a soln. of $NaHCO_3$ (4.7 g, 0.055 mol) in H_2O (500 ml) was added. The resulting mixture was extracted with $CHCl_3$ (4 × 200 ml) and the org. layer dried (Na_2SO_4), evaporated, and co-evaporated with toluene (2 × 100 ml). The residue (crude **2**) was dissolved in DMF (70 ml) and heated at 65–68° for 40 min in the presence of NaN_3 (6.5 g, 0.1 mol). The solvent was evaporated an the residue taken up in $CHCl_3$ (700 ml), washed with H_2O (3 × 100 ml), dried (Na_2SO_4), and evaporated. The mixture of conc. NH_4OH soln. (80 ml) and Ph_3P (18 g, 0.068 mol) was added to the crude azido derivative **3** in pyridine (70 ml), the mixture stirred at r.t. for 18 h, and the insoluble material filtered off. The filtrate soln. was evaporated and co-evaporated with toluene (2 × 100 ml), the resulting foam treated with hexane (300 ml), stirred for 1 h, and filtered, and the crude solid **4** purified by CC (30 × 2.5 cm, $CHCl_3$, then $CHCl_3$/MeOH 24:1) and finally crystallized from MeOH: 5 g (50%) of slightly yellow crystals. M.p. 205–207° ([11]: m.p. 203–207°).

5′-Deoxy-5′-(hexadecanoylamino)-2′,3′-O-isopropylideneadenosine (**5**). A mixture of **4** (0.92 g, 3.0 mmol) and hexadecanoyl chloride (1.29 g, 1.36 ml, 4.47 mmol) in MeCN (50 ml) in the presence of Et_3N (8 ml) was stirred at r.t. for 3 h and then evaporated. The residue was treated with $CHCl_3$ (100 ml), the org. phase washed with H_2O (2 × 50 ml), dried (Na_2SO_4), and evaporated, and the product purified by CC (40 × 1.5 cm, $CHCl_3$/MeOH 30:1) and finally crystallized from EtOH: 1.55 g (95%) of colorless crystals. M.p. 76–78°. UV (MeOH): 258 (4.13). ^{1}H-NMR ((D_6)DMSO): 8.31, 8.15 (2*s*, H–C(2), H–C(8)); 8.06 (br. *s*, N*H*palm); 6.10 (*s*, H–C(1′)); 5.40 (*d*, H–C(2′)); 4.87 (*m*, H–C(3′)); 4.14 (br. *s*, H–C(4′)); 3.33 (*m*, 2 H–C(5′)); 2.06 (br. *s*, 1 CH_2 of palm); 1.51, 1.29 (2*s*, Me_2C); 1.45 (br. *s*, 1 CH_2 of palm); 1.20 (*m*, 12 CH_2 of palm); 0.83 (*t*, Me of palm). Anal. calc. for $C_{29}H_{48}N_6O_4$ (544.7): C 63.94, H 8.88, N 15.42; found: C 63.66, H 8.85, N 15.22.

5′-Deoxy-5′-(hexadecanoylamino)-2′,3′-O-isopropylidene-N^6-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine (**6**). A mixture of **10** (1.35 g, 2.47 mmol) and 3-methyl-1-[2-(4-nitrophenyl)ethoxycarbonyl]-1*H*-imidazol-3-ium chloride (1.55 g, 4.97 mmol) in CH_2Cl_2 (30 ml) was stirred at r.t. for 18 h and evaporated. The product was purified by CC (25 × 2.5 cm, $CHCl_3$): 1.4 g (77%) of colorless foam. UV (MeOH): 266 (4.48), 274 (sh, 4.40). ^{1}H-NMR ((D_6)DMSO): 10.64 (*s*, N*H*npeoc); 8.64, 8.61 (2*s*, H–C(2), H–C(8)); 8.16 (*d*, 2 H *o* to NO_2); 8.10 (*t*, N*H*palm); 7.62 (*d*, 2 H *m* to NO_2); 6.22 (*d*, H–C(1′)); 5.46 (*dd*, H–C(2′)); 4.93 (*m*, H–C(3′); 4.39 (*t*, OCH_2CH_2); 4.16 (*m*,

H–C(4′)); 3.33 (*m*, 2 H–C(5′)); 3.11 (*t*, OCH_2CH_2); 2.04 (*t*, 1 CH_2 of palm); 1.53, 1.30 (2*s*, Me_2C); 1.44 (*m*, 1 CH_2 of palm); 1.19 (*m*, 12 CH_2 of palm); 0.83 (*t*, Me of palm). Anal. calc. for $C_{38}H_{55}N_7O_8$ (737.9): C 61.85, H 7.51, N 13.28; found: C 61.71, H 7.41, N 13.36.

*5′-Deoxy-2′,3′-O-isopropylidene-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-5′-{[2-(4-nitrophenyl)ethoxycarbonyl]amino}adenosine* (**7**). A mixture of **4** (0.7 g, 2.28 mmol) and 3-methyl-1-[2-(4-nitrophenyl)ethoxycarbonyl]-1*H*-imidazol-3-ium chloride (2.8 g, 8.98 mmol) in CH_2Cl_2 (40 ml) was stirred at r.t. for 18 h, filtered, and evaporated. The product was purified by CC (25 × 2.5 cm, AcOEt): 1.18 g (75%) of colorless foam. UV (MeOH): 267 (4.51), 274 (sh, 4.46). ^{1}H-NMR ((D_6)DMSO): 10.60 (*s*, NH–C(6)); 8.58, 8.56 (2*s*, H–C(2), H–C(8)); 8.13, 8.09 (2*d*, 4 H *o* to NO_2); 7.58, 7.49 (2*d*, 4 H *m* to NO_2); 7.37 (*t*, NH–C(5′)); 6.19 (*d*, H–C(1′)); 5.42 (*dd*, H–C(2′)); 4.93 (*dd*, H–C(3′)); 4.36 (*t*, OCH_2CH_2); 4.17 (*m*, OCH_2CH_2, H–C(4′)); 3.17 (*m*, 2 H–C(5′)); 3.08 (*t*, OCH_2CH_2); 1.50, 1.28 (2*s*, Me_2C). Anal. calc. for $C_{31}H_{32}N_8O_{11}$ (692.6): C 53.75, H 4.65, N 16.17; found: C 53.44, H 4.69, N 16.10.

*5′-Deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-5′-{[2-(4-nitrophenyl)ethoxycarbonyl]amino}adenosine* (**8**). A soln. of **7** (0.7 g, 1.01 mmol) in 80% formic acid (50 ml) was stirred at r.t. for 5 h, evaporated, and co-evaporated with MeOH (2 × 30 ml). The product was crystallized from MeOH: 0.5 g (76%) of colorless crystals. M.p. 106–107°. UV (MeOH): 267 (4.50), 274 (sh, 4.42). ^{1}H-NMR ((D_6)DMSO): 10.60 (*s*, NH–C(6)); 8.63, 8.56 (2*s*, H–C(2), H–C(8)); 8.16, 8.12 (2*d*, 4 H *o* to NO_2); 7.56 (*m*, 4 H *m* to NO_2, NH–C(5′)); 5.93 (*d*, H–C(1′)); 5.54 (*d*, OH–C(2′)); 5.32 (*d*, OH–C(3′)); 4.70 (*m*, H–C(2′)); 4.38, 4.21 (2*t*, 2 OCH_2CH_2); 4.07 (*m*, H–C(3′)); 3.94 (*m*, H–C(4′)); 3.27 (*m*, 2 H–C(5′)); 3.10, 3.00 (2*t*, 2 OCH_2CH_2). Anal. calc. for $C_{28}H_{28}N_8O_{11}$ (652.5): C 51.53, H 4.32, N 17.17; found: C 51.39, H 4.31, N 17.15.

*5′-Deoxy-5′-(hexadecanoylamino)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**9**). A soln. of **6** (0.9 g, 1.21 mmol) in 80% formic acid (70 ml) was stirred at r.t. for 8 h, evaporated, and co-evaporated with MeOH (2 × 40 ml). The product was crystallized from EtOH: 0.7 g (82%) of colorless crystals. M.p. 142–143°. UV (MeOH): 267 (4.38), 275 (sh, 4.31). ^{1}H-NMR ((D_6)DMSO): 10.60 (*s*, NH–C(6)); 8.66, 8.62 (2*s*, H–C(2), H–C(8)); 8.16 (*d*, 2 H *o* to NO_2); 7.99 (*t*, NH–C(5′)); 7.61 (*d*, 2 H *m* to NO_2); 5.94 (*d*, H–C(1′)); 5.52 (*d*, OH–C(2′)); 5.29 (*d*, OH–C(3′)); 4.70 (*m*, H–C(2′)); 4.38 (*t*, OCH_2CH_2); 4.06 (*m*, H–C(3′)); 3.93 (*m*, H–C(4′)); 3.42 (*m*, 2 H–C(5′)); 3.11 (*t*, OCH_2CH_2); 2.06 (*t*, CH_2 of palm); 1.46 (*m*, CH_2 of palm); 1.19 (*m*, 12 CH_2 of palm); 0.83 (*t*, Me of palm). Anal. calc. for $C_{35}H_{51}N_7O_8$ (697.8): C 60.24, H 7.36, N 14.05; found: C 59.99, H 7.46, N 13.99.

*2′,3′-Bis-*O*-[(*tert*-butyl)dimethylsilyl]-5′-deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-5′-{[2-(4-nitrophenyl)ethoxycarbonyl]amino}adenosine* (**10**), *3′-*O*-[(*tert*-Butyl)dimethylsilyl]-5′-deoxy-*N^6*-[2-(4-nitrophenyl)-ethoxycarbonyl]-5′-{[2-(4-nitrophenyl)ethoxycarbonyl]amino}adenosine* (**11**), *and 2′-*O*-[(*tert*-Butyl)dimethylsilyl]-5′-deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-5′-{[2-(4-nitrophenyl)ethoxycarbonyl]amino}adenosine* (**12**). A soln. of **8** (0.41 g, 0.62 mmol) and (*tert*-butyl)dimethylsilyl chloride (0.162 g, 1.07 mmol) in pyridine (4.5 ml) in the presence of 1*H*-imidazole (0.15 g, 2.2 mmol) was stirred at r.t. for 18 h. The mixture was diluted with $CHCl_3$ (80 ml), the extract washed with H_2O, dried (Na_2SO_4), and evaporated, and the residue co-evaporated with toluene (2 × 30 ml) to remove pyridine. Purification by CC (30 × 2.5 cm, $CHCl_3$) gave, after drying under high vacuum, 0.11 g (20%) of **10**, 0.15 g (31%) of **11**, and 0.16 g (33%) of **12**.

10: UV (MeOH): 267 (4.57), 275 (sh, 4.50). ^{1}H-NMR ((D_6)DMSO): 10.61 (*s*, NH–C(6)); 8.73, 8.60 (2*s*, H–C(2), H–C(8)); 8.17, 8.13 (2*d*, 4 H *o* to NO_2); 7.54 (*m*, 4 H *m* to NO_2, NH–C(5′)); 5.97 (*d*, H–C(1′)); 4.97 (*m*, H–C(2′)); 4.39 (*t*, OCH_2CH_2); 4.25 (*m*, OCH_2CH_2, H–C(3′)); 3.92 (*m*, H–C(4′)); 3.38 (*m*, 2 H–C(5′), H_2O); 3.10, 3.01 (2*t*, 2 OCH_2CH_2); 0.87, 0.83, 0.62 (3*s*, 2 *t*-Bu); 0.04, 0.01, –0.17, –0.56 (4*s*, 2 $SiMe_2$). Anal. calc. for $C_{40}H_{56}N_8O_{11}Si_2$ (881.1): C 54.52, H 6.40, N 12.71; found: C 54.50, H 6.60, N 12.59.

11: UV (MeOH): 267 (4.53), 275 (sh, 4.46). ^{1}H-NMR ((D_6)DMSO): 10.60 (*s*, NH–C(6)); 8.68, 8.58 (2*s*, H–C(2), H–C(8)); 8.16, 8.13 (2*d*, 4 H *o* to NO_2); 7.61 (*d*, 2 H *m* to NO_2); 7.52 (*m*, 2 H *m* to NO_2, NH–C(5′)); 5.91 (*d*, H–C(1′)); 5.44 (*d*, OH–C(2′)); 4.90 (*t*, H–C(2′)); 4.38 (*t*, OCH_2CH_2); 4.21 (*m*, OCH_2CH_2, H–C(3′)); 3.90 (*m*, H–C(4′)); 3.33 (*m*, 2 H–C(5′), H_2O); 3.12, 3.00 (2*t*, 2 OCH_2CH_2); 0.84 (*s*, *t*-Bu); 0.05, 0.03 (2*s*, $SiMe_2$). Anal. calc. for $C_{34}H_{42}N_8O_{11}Si$ (766.8): C 53.25, H 5.52, N 14.61; found: C 53.22, H 5.76, N 14.26.

12: UV (MeOH): 267 (4.54), 275 (sh, 4.48). ^{1}H-NMR ((D_6)DMSO): 10.63 (*s*, NH–C(6)); 8.69, 8.60 (2*s*, H–C(2), H–C(8)); 8.18, 8.15 (2*d*, 4 H *o* to NO_2); 7.62 (*m*, 4 H *m* to NO_2, NH–C(5′)); 6.00 (*d*, H–C(1′)); 5.27 (*d*, OH–C(3′)); 4.78 (*t*, H–C(2′)); 4.41 (*t*, OCH_2CH_2); 4.24 (*m*, OCH_2CH_2, H–C(3′)); 4.04 (*m*, H–C(4′)); 3.36 (*m*, 2 H–C(5′), H_2O); 3.13, 3.04 (2*t*, 2 OCH_2CH_2); 0.68 (*s*, *t*-Bu); –0.12, –0.26 (2*s*, $SiMe_2$). Anal. calc. for $C_{34}H_{42}N_8O_{11}Si$ (766.8): C 53.25, H 5.52, N 14.61; found: C 53.57, H 5.69, N 14.56.

*2′,3′-Bis-*O*-[(*tert*-butyl)dimethylsilyl]-5′-deoxy-5′-(hexadecanoylamino)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**13**), *3′-*O*-[(*tert*-Butyl)dimethylsilyl]-5′-deoxy-5′-(hexadecanoylamino)-*N^6*-[2-(4-nitrophenyl)-ethoxycarbonyl]adenosine* (**14**), *and 2′-*O*-[(*tert*-Butyl)dimethylsilyl]-5′-deoxy-5′-(hexadecanoylamino)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]adenosine* (**15**). As described for **10–12**, with **9** (0.6 g, 0.85 mmol), (*tert*-butyl)dimethylsilyl chloride (0.15 g, 1.02 mmol), pyridine (6 ml), and 1*H*-imidazole (0.14 g, 2.05 mmol). After

dilution with $CHCl_3$ (100 ml) and workup as described, drying under high vacuum at 40° gave 0.09 g (11%) of **13**, 0.25 g (36%) of **14**, and 0.3 g (43%) of **15**.

13: UV (MeOH): 267 (4.39), 275 (sh, 4.27). ^{1}H-NMR ((D$_6$)DMSO): 10.63 (*s*, NH–C(6)); 8.75, 8.63 (2*s*, H–C(2), H–C(8)); 8.16 (*d*, 2 H *o* to NO_2); 8.11 (br. *s*, NH–C(5′)); 7.61 (*d*, 2 H *m* to NO_2); 5.98 (*d*, H–C(1′)); 5.00 (*dd*, H–C(2′)); 4.38 (*t*, OCH_2CH_2); 4.26 (*d*, H–C(3′)); 3.94 (*t*, H–C(4′)); 3.43 (*m*, 2 H H–C(5′)); 3.10 (*t*, OCH_2CH_2); 2.10 (*t*, 1 CH_2 of palm); 1.48 (*m*, 1 CH_2 of palm); 1.21 (*m*, 12 CH_2 of palm); 0.83 (*t*, Me of palm); 0.90, 0.64 (2*s*, 2 *t*-Bu); 0.09 (*s*, $SiMe_2$, SiMe); –0.14 (*s*, SiMe). Anal. calc. for $C_{47}H_{79}N_7O_8Si_2$ (926.4): C 60.93, H 8.59, N 10.58; found: C 60.92, H 8.49, N 10.29.

14: UV (MeOH): 267 (4.40), 275 (sh, 4.34). ^{1}H-NMR ((D$_6$)DMSO): 10.62 (*s*, NH–C(6)); 8.71, 8.63 (2*s*, H–C(2), H–C(8)); 8.17 (*d*, 2 H *o* to NO_2); 8.06 (*t*, NH–C(5′)); 7.62 (*d*, 2 H *m* to NO_2); 5.93 (*d*, H–C(1′)); 5.47 (*d*, OH–C(2′)); 4.94 (*m*, H–C(2′)); 4.39 (*t*, OCH_2CH_2); 4.24 (*d*, H–C(3′)); 3.91 (*m*, H–C(4′)); 3.40 (*m*, 2 H–C(5′)); 3.11 (*t*, OCH_2CH_2); 2.07 (*t*, 1 CH_2 of palm); 1.46 (*t*, 1 CH_2 of palm); 1.21 (*m*, 12 CH_2 of palm); 0.90 (*s*, *t*-Bu); 0.84 (*t*, Me of palm); 0.10 (*s*, $SiMe_2$). Anal. calc. for $C_{41}H_{65}N_7O_8Si$ (812.1): C 60.64, H 8.06, N 12.07; found: C 60.55, H 8.01, N 11.89.

15: UV (MeOH): 264 (4.43), 275 (sh, 4.36). ^{1}H-NMR ((D$_6$)DMSO): 10.63 (*s*, NH–C(6)); 8.68, 8.62 (2*s*, H–C(2), H–C(8)); 8.15 (*d*, 2 H *o* to NO_2); 8.07 (*t*, NH–C(5′)); 7.61 (*d*, 2 H *m* to NO_2); 5.98 (*d*, H–C(1′)); 5.22 (*d*, OH–C(3′)); 4.75 (*t*, H–C(2′)); 4.38 (*t*, OCH_2CH_2); 4.01 (*m*, H–C(3′), H–C(4′)); 3.43 (*m*, 2 H–C(5′)); 3.10 (*t*, OCH_2CH_2); 2.10, 1.48 (2*t*, 2 CH_2 of palm); 1.19 (*m*, 12 CH_2 of palm); 0.82 (*t*, Me of palm); 0.67 (*s*, *t*-Bu); –0.11, –0.26 (2*s*, $SiMe_2$). Anal. calc. for $C_{41}H_{65}N_7O_8Si$ (812.1): C 60.64, H 8.06, N 12.07; found: C 60.77, H 8.00, N 11.84.

*3′-O-[(*tert*-Butyl)dimethylsilyl]-5′-deoxy-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-5′-{[2-(4-nitrophenyl)-ethoxycarbonyl]amino}adenosine 2′-O-[2-Cyanoethyl* N,N*-Diisopropylphosphoramidite]* (**16**). To a soln. of **11** (0.26 g, 0.33 mmol) in anh. CH_2Cl_2 (3 ml), 1*H*-tetrazole (0.011 g, 0.16 mmol) and (2-cyanoethoxy)bis-diisopropylamino)phosphane (0.51 g, 1.65 mmol) was added under Ar. After stirring at r.t for 18 h, the mixture was diluted with $CHCl_3$ (100 ml), the org. phase washed with sat. $NaHCO_3$ soln. (30 ml), dried (Na_2SO_4), and evaporated. Purification by CC (silica gel, 30 × 1.5 cm, AcOEt/hexane 1:2) gave, after drying at 40° *in vacuo*, **16** (0.3 g, 91%). Colorless foam. UV (MeOH): 267 (4.54), 274 (sh, 4.48). ^{31}P-NMR ($CDCl_3$): 149.606, 150.951 (2 diastereoisomers). Anal. calc. for $C_{43}H_{59}N_{10}O_{12}PSi$ (967.1): C 53.40, H 6.14, N 14.48; found: C 53.18, H 6.15, N 14.28.

*3′-O-[(*tert*-Butyl)dimethylsilyl]-5′-deoxy-5′-(hexadecanoylamino)-*N^6*-[2-(4-nitrophenyl)ethoxycarbonyl]-adenosine 2′-O-[2-Cyanoethyl* N,N*-Diisopropylphosphoramidite]* (**17**). As described for **16**, with **14** (0.29 g, 0.357 mmol), 1*H*-tetrazole (0.012 g, 0.17 mmol), CH_2Cl_2 (3 ml), and (2-cyanoethoxy)bis(diisopropylamino)phosphane (0.53 g, 1.785 mmol). Workup (washing with sat. $NaHCO_3$ soln. (20 ml)) and purification gave **17** (0.3 g, 83%). Colorless foam. UV (MeOH; mixture of 2 diastereoisomers): 267 (4.41), 275 (sh, 4.35). Anal. calc. for $C_{50}H_{82}N_9O_9PSi$ (1012.3): C 59.32, H 8.16, N 12.45; found: C 59.02, H 8.11, N 12.17.

More polar diastereoisomer: ^{1}H-NMR ($CDCl_3$): 8.62, 8.05 (2*s*, H–C(2), H–C(8)); 8.13 (*d*, 2 H *o* to NO_2); 8.04 (br. *s*, NH–C(6)); 7.73 (br. *s*, NH–C(5′)); 7.38 (*d*, 2 H *m* to NO_2); 5.90 (*d*, H–C(1′)); 4.82 (*m*, H–C(2′)); 4.48 (*t*, OCH_2CH_2Ar); 4.30 (*m*, H–C(3′)); 4.18 (*m*, H–C(**4′**)); 4.05 (*m*, $CNCH_2CH_2$); 3.66 (*m*, 1 H–C(5′)); 3.39 (*m*, 1 H–C(5′), 2 Me_2CH); 3.10 (*t*, OCH_2CH_2Ar); 2.68, 2.51 (2*t*, 2 H, $CNCH_2CH_2$); 2.21 (*t*, 1 CH_2 of palm); 1.55 (br. *s*, 1 Me_2CH, 1 CH_2 of palm); 1.16 (*m*, 1 CH_2 of palm); 0.97, 0.67 (2*d*, 1 Me_2CH); 0.88 (*s*, *t*-Bu); 0.81 (*t*, Me of palm); 0.11, 0.09 (2*s*, $SiMe_2$). ^{31}P-NMR ($CDCl_3$): 150.193.

Less polar diastereoisomer: ^{1}H-NMR ($CDCl_3$): 8.62, 7.96 (2*s*, H–C(2), H–C(8)); 8.07 (*d*, 2 H *o* to NO_2); 8.03 (br. *s*, NH–C(6)); 7.62 (*m*, NH–C(5′)); 7.32 (*d*, 2 H *m* to NO_2); 5.87 (*d*, H–C(1′)); 4.69 (*m*, H–C(2′)); 4.42 (*t*, OCH_2CH_2Ar); 4.25 (*m*, H–C(3′)); 4.12 (*m*, H–C(4′)); 4.04 (*m*, $CNCH_2CH_2$); 3.35 (*m*, 2 H–C(5′), 2 Me_2CH); 3.04 (*t*, OCH_2CH_2Ar); 2.63 (*t*, $CNCH_2CH_2$); 2.16 (*m*, 1 CH_2 of palm); 1.51 (br. *s*, Me_2CH, 1 CH_2 of palm); 1.12 (*m*, 12 CH_2 of palm); 0.91 (*dd*, Me_2CH); 0.81 (*s*, *t*-Bu); 0.74 (*t*, Me of palm); 0.01, 0.00 (2*s*, $SiMe_2$).

*9-[(2-Acetoxyethoxy)methyl)]-*N^6*-benzoyladenine* (**19**). A suspension of N^6-benzoyladenine (**18**) [15] (3 g, 12.55 mmol) in 1,2-dichloroethane (75 ml) was refluxed with *N,O*-bis(trimethylsilyl)acetamide (7.5 ml, 30 mmol) for 45 min. After removal of the solvent, the residue was co-evaporated with abs. toluene (2 × 20 ml) and finally taken up in anh. toluene (75 ml). To this soln. were added dropwise with stirring within 1 h 4 drops of Et_3N and (2-acetoxyethoxy)methyl bromide [16] (3 g, 15.15 mmol) in anh. toluene (20 ml), under Ar at r.t. The mixture was refluxed for 2 h, the solvent evaporated, the residue extracted with $CHCl_3$ (100 ml), the extract washed with sat. $NaHCO_3$/NaCl soln. (2 × 25 ml), dried (Na_2SO_4), and again evaporated. Crystallization of the residue from AcOEt gave **19** (3.6 g, 80%). TLC ($CHCl_3$/MeOH 9:1): R_f 0.68. UV (MeOH): 229 (4.12), 279 (4.30). ^{1}H-NMR ($CDCl_3$): 8.94 (*s*, NH); 8.81, 8.16 (2*s*, H–C(2), H–C(8)); 7.49–8.02 (2*m*, benzoyl); 5.70 (*s*, 1 CH_2); 4.18 (*m*, 1 CH_2); 3.78 (*t*, 1 CH_2); 2.02 (*s*, Ac). Anal. calc. for $C_{17}H_{17}N_5O_4$ (355.3): C 57.46, H 4.82, N 19.71; found: C 57.43, H 4.85, N 19.53.

N^6-*Benzoyl-9-[(2-hydroxyethoxy)methyl]adenine* (**20**). A suspension of **19** (2 g, 5.62 mmol) in EtOH (60 ml) was treated with 1N NaOH (8 ml; → clear soln.). After 15 min stirring, the mixture was neutralized with pyridinium *Dowex* (50 × 4). The *Dowex* was filtered off and washed with EtOH (5 × 10 ml) and the filtrate evaporated. The residue was triturated with $CHCl_3$ to give **22** (1.58 g, 89%). Colorless solid. TLC ($CHCl_3$/MeOH 9:1): R_f 0.41. UV (MeOH): 230 (4.12), 279 (4.30). ^{1}H-NMR ($(D)_6$DMSO): 11.20 (*s*, NH); 8.77, 8.61 (2*s*, H–C(2),H–C(3)); 7.54–8.05 (*m*, *d*, benzoyl); 5.69 (*s*, 1 CH_2); 4.69 (*t*, OH–C(5′)); 3.54, 3.35 (2*m*, 2 CH_2). Anal. calc. for $C_{15}H_{15}N_5O_3 \cdot 0.5\ H_2O$ (322.3): C 55.89, H 5.00, N 21,72; found: C 55.64, H 4.80, N 21.66.

N^6-*Benzoyl-3′*-O-*[(*tert*-butyl)dimethylsilyl]-5′*-O-*(monomethoxytrityl)adenylyl-{2-{O^P-[2-(4-nitrophenyl)-ethyl]}→2″}-N^6-benzoyl-9-[(2″-hydroxyethoxy)methyl]adenine* (**23**). A soln. of N^6-benzoyl-3′-*O*[(*tert*-butyl)-dimethylsilyl]-5′-*O*-(monomethoxytrityl)adenosine 2′-[2-(4-nitrophenyl)ethyl *N*,*N*-diisopropylphosphoramidite] (**21**) [14] (8.22 g, 7.78 mmol), **20** (1.63 g, 5.21 mmol), and 3-nitro-1*H*-1,2,4-triazole (2.6 g, 22.8 mmol) in anh. MeCN (78 ml) was stirred at r.t. under Ar for 2 h. Then I_2 in CH_2Cl_2/pyridine/H_2O 1:3:1 was added till persistence of the brown color, and stirring was continued at r.t. for 30 min. The soln. was diluted with $CHCl_3$ (100 ml), washed with sat. $Na_2S_2O_3$/NaCl soln. (2 × 50 ml), dried (Na_2SO_4), and evaporated. Final co-evaporation was done with toluene (2 × 20 ml) to remove pyridine. Purification by CC (silica gel, 40 × 3.5 cm, $CHCl_3$ (1 l), then $CHCl_3$/MeOH 97:3 (2 l)) gave **23** (5.6 g, 85%). Colorless foam. TLC ($CHCl_3$/MeOH 9:1): R_f 0.72. UV (MeOH): 234 (4.62), 278 (4.66). ^{1}H-NMR ($CDCl_3$: 9.03, 8.98 (2 br. *s*, 2 NH); 8.73, 8.64 (2*d*, 2 H–C(2)); 8.23–7.16 (2*m*, 24 H, 2 H–C(8), bz. MeO*Tr*); 6.78 (*d*, 2 H *o* to *MeO*); 6.26 (*m*, H–C(1′)); 5.52, 5.55 (*m*, 2*s*, 3 H, H–C(2′), CH_2 (diastereoisomers)); 4.65 (*m*, H–C(3′) (diastereoisomers)); 4.18 (*m*, H–C(4′)); 4.12 (*m*, 2 H, OCH_2CH_2 (npe)); 3.80 (*m*, 1 CH_2); 3.74 (*s*, MeO); 3.61, 3.53, 3.29 (3*m*, 2 H–C(5′), 1 CH_2); 2.93, 2.84 (2*t*, 2 H, OCH_2CH_2 (npe)-(diastereoisomers)); 0.82, 0.80 (2*s*, *t*-Bu); 0.05, 0.04, 0.01 (3*s*, SiMe2). Anal. calc. for $C_{66}H_{68}N_{11}O_{13}PSi$ (1282.4): C 61.81, H 5.34, N 12.01; found: C 61.45, H 5.23, N 11.87.

N^6-*Benzoyl-3′*-O-*[(*tert*-butyl)dimethylsilyl]adenylyl-{2′-{O^P-[2-(4-nitrophenyl)ethyl]→2″}-N^6-benzoyl-9-[(2″-hydroxyethoxy)methyl]adenine* (**24**). A soln. of **23** (6.41 g, 5 mmol) was stirred with 2% TsOH in CH_2Cl_2/MeOH (120 ml) for 45 min. The mixture was diluted with $CHCl_3$ (100 ml), the org. phase washed with H_2O (2 × 50 ml), dried (Na_2SO_4), and evaporated and the crude product purified by FC (35 × 3.5 cm, $CHCl_3$ (1 l), then $CHCl_3$/MeOH 97:3 (2 l)). Drying *in vacuo* gave **24** (4.54 g, 90%). TLC ($CHCl_3$/MeOH 95:5): R_f 0.41 UV (MeOH): 234 (4.56), 278 (4.66). ^{1}H-NMR ($CDCl_3$): 9.09, 9.00 (*d*, *m*, 2 NH); 8.75 (2*s*, 2 H–C(2)); 8.18–7.13 (2*m*, 14 H, aryl, H–C(8)); 6.05 (*d*, H–C(1′)); 5.56, 5.52 (*m*, 2*s*, 3 H–C(2′), 1 CH_2 (diastereoisomers)); 4.52 (*m*, H–C(3′)); 4.10 (*m*, OH–C(5′)); 4.03–3.66 (*m*, 9 H, H–C(4′), 2 CH_2, 2 H–C(5′), OCH_2CH_2 (npe)): 2.88 (*m*, 2 H, OCH_2CH_2 (npe)); 0.90 (*s*, *t*-Bu); 0.09, 0.08, 0.07 (3*s*, $SiMe_2$). Anal. calc. for $C_{46}H_{52}N_{11}O_{12}PSi$ (1010.0): C 54.70, H 5.19, N 15.25; found: C 54,23 G 5.18, N 14.91.

5′-Amino-5′-deoxyadenylyl(2′–5′)adenylyl(2′–5′)adenosine (**25**). A soln. of **16** (0.135 g, 0.13 mmol) and **22** (0.12 g, 0.1 mmol) in abs. MeCN (2 ml) in the presence of 1*H*-tetrazole (0.035 g, 0.5 mmol) was stirred at r.t. for 4 h under Ar. The mixture was oxidized with I_2 (0.1 g) in CH_2Cl_2/H_2O/pyridine 1:3:1 (1 ml), stirred for 15 min, and diluted with $CHCl_3$ (100 ml), the org. phase washed with sat. $Na_2S_2O_3$ soln. (2 × 20 ml), dried (Na_2SO_4), and evaporated, and the residue dissolved in 0.5M DBU/MeCN (5 ml) and stirred for 24 h. Then, the soln. was neutralized with 1M AcOH/MeCN (2.5 ml) and evaporated. The residue was dissolved in 1M Bu_4NF/THF (5 ml) and stirred at r.t. for 60 h. After evaporation, the residue was dissolved in sat. NH_3/MEOH (30 ml), the soln. kept for 24 h and then evaporated, and the residue taken up in $CHCl_3$/H_2O 1:3 (100 ml). The aq. phase was applied onto a *DEAE-Servacel-23-SS* column (30 × 1.5 cm, linear gradient of 0.005–0.2M $(Et_3NH)HCO_3$ buffer (pH 7.5)). The product fractions were evaporated and coevaporated with NH_4OH soln. (3 × 30 ml). The residual NH_4^+ salt was lyophilized (H_2O); 16.9 mg (17%) of **25**. HPLC (see *General*): 10.88 min. UV (H_2O): 257 (4.55). ^{1}H-NMR (D_2O, *t*-BuOH as internal standard): 7.95, 7.90, 7.82, 7.80, 7.77, 7.67 (6*s*, 6 H, H–C(2), H–C(8)); 5.97, 5.88, 5.70 (3*d*, 3 H, H–C(1′)).

5′-Deoxy-5′-(hexadecanoylamino)adenylyl(2′–5′)adenylyl(2′–5′)adenosine (**26**). As described for **25**, with **17** (0.065 g, 0.064 mmol), **22** (0.036 g, 0.03 mmol), MeCN (1 ml), 1*H*-tetrazole (0.011 g, 0.157 mmol; 3 h), I_2 (0.1 g), CH_2Cl_2/H_2O/pyridine 1:3:1 (0.5 ml), $CHCl_3$ (30 ml), sat. $Na_2S_2O_3$ soln. (2 × 10 ml), 0.5M DBU/MeCN (3 ml), 1M AcOH/MeCN (1.5 ml), 1M Bu_4NF/THF (1.5 ml; 48 h), and sat. NH_3/MeOH (20 ml). The residue in MeOH/H_2O 1:1 (30 ml) was applied onto a *DEAE-Servacel-23-SS* column (20 × 1.5 cm, linear gradient of 0.005–0.2M $(Et_3NH)HCO_3$ buffer (pH 7.5) in MeOH/H_2O 1:1). The product fractions were evaporated and co-evaporated with NH_4OH (3 × 20 ml) and then with MeOH (3 × 20 ml). The residual NH_4^+ salt was lyophilized (MeOH/H_2O 1.1): 19 mg (53%) of **26**. HPLC (see *General*): 32.83 min. UV (MeOH/H_2O 1:1): 257 (4.54). ^{1}H-NMR ((D_6)DMSO): 8.40, 8.35, 8.30 (3*s*, 3 H, H–C(2), H–C(8)); 8.09, 8.07 (br. *s*, 3 H, H–C(2), H–C(8)); 5.98 (br. *s*, 2 H, H–C(1′)); 5.82 (*s*, 1 H, H–C(1′)); 4.95 (*m*, 2 H, H–C(2′)); 4.52 (*m*, 1 H, H–C(2′)); 2.03, 1.37 (2*m*, 4 H, CH_2 of palm); 1.15 (*m*, 12 CH_2 of palm); 0.80 (*m*, Me of palm).

5′-Amino-5′-deoxyadenylyl(2′–5′)adenylyl(2′–2″)-N⁹-[(2″-hydroxyethoxy)methyl]adenine (**27**). As described for **25**, with **16** (0.135 g, 0.13 mmol), **24** (0.1 g, 0.098 mmol), 1*H*-tetrazole (0.035 g, 0.5 mmol), MeCN (3 ml), I_2 (0.1 g), CH_2Cl_2/H_2O/pyridine 1:3:1 (1 ml), $CHCl_3$ (100 ml), sat. $Na_2S_2O_3$ soln. (2 × 20 ml), 0.5M DBU/MeCN (4 ml), 1M AcOH/MeCN (2 ml), 1M Bu_4NF/THF (3 ml), sat. NH_3/MeOH (40 ml), and $CHCl_3/H_2O$ 1:1 (100 ml). *DEAE-Servacel-23-SS* CC, evaporation, co-evaporation, and lyophilization as described for **25** gave 10.2 mg (11%) of **27**. HPLC (see *General*): 13.26 min. UV (H_2O): 257 (4.54). ^{1}H-NMR (D_2O, *t*-BuOH as internal standard): 8.15, 8.12 (2 H), 8.08, 8.05, 7.87 (5*s*, 6 H, H–C(2), H–C(8)); 6.13 (*d*, 1 H, H–C(1′)); 5.92 (*d*, 1 H, H–C(1′)); 5.46 (*d*, 2 H, OCH_2–N(9)).

5′-Deoxy-5′-(hexadecanoylamino)adenylyl(2′–5′)adenylyl(2′–2″)-N⁹-[(2″-hydroxyethoxy)methyl]adenine (**28**). As described for **25**, with **17** (0.17 g, 0.167 mmol), **24** (0.1 g, 0.098 mmol), and 1*H*-tetrazole (0.035 g, 0.5 mmol), MeCN (3 ml), I_2 (0.1 g), CH_2Cl_2/H_2O/pyridine 1:3:1 (1.5 ml), $CHCl_3$ (100 ml), sat. $Na_2S_2O_3$ soln. (2 × 30 ml), 0.5M DBU/MeCN (6 ml), 1M AcOH/MeCN (3 ml), 1M Bu_4NF/THF (3 ml), and sat. NH_3/MeOH (40 ml). The residue in MeOH/H_2O 1:1 (50 ml) was applied onto a *DEAE-Servacel-23-SS* column (30 × 1.5 cm, linear gradient of 0.005–0.2M $(Et_3NH)HCO_3$ buffer (pH 7.5) in MeOH/H_2O 1:1). The product fractions were evaporated and co-evaporated with NH_4OH (3 × 20 ml) and then with MeOH (3 × 30 ml). The residual NH_4^+ salt was lyophilized (MeOH/H_2O 1:1): 65 mg (58%) of **28**. HPLC (see *General*): 34.27 min. UV (MeOH/H_2O): 257 (4.55). ^{1}H-NMR ((D_6)DMSO): 8.41, 8.29, 8.23, 8.12, 8.08, 8.04 (6*s*, 6 H, H–C(2), H–C(8)); 5.99 (*d*, 1 H, H–C(1′)); 5.97 (*d*, 1 H, H–C(1′)); 5.44 (*s*, 2 H, OCH_2–N(9)); 4.97 (*m*, 2 H, H–C(2′)); 4.29 (br. *s*, 2 H, $POCH_2CH_2$); 3.94 (br. *s*, 2 H, $POCH_2CH_2$); 2.03 (*t*, 1 CH_2 of palm); 1.37 (br. *s*, 1 CH_2 to palm); 1.13 (*m*, 12 CH_2 to palm); 0.78 (*t*, Me of palm).

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