Nucleotides

Part LXXI[1])

A New Type of Labelling of Nucleosides and Nucleotides

by **Harald Sigmund** and **Wolfgang Pfleiderer***)

Fachbereich Chemie, Universität Konstanz, Postfach 5560, D-78457 Konstanz

A new labelling technique attaching fluorescein *via* a carbamoyl linker directly to the amino groups of the nucleobases was developed. The amino groups were first converted to the phenoxycarbonyl derivatives (→ **10**, **15**, **19**, **58**), which reacted under mild conditions with 5-aminofluorescein to give the corresponding *N*-[(fluorescein-5-ylamino)carbonyl] derivatives (→ **11**–**14**, **16**, **17**, **20, 59, 60**). The introduction of the 5-aminofluorescein residue into properly protected adenylyl-adenosine dimers (→ **39**, **40**) and trimer (→ **50**) worked well, and final deprotection of these uniformly blocked precursors led on treatment with DBU (1,8-diazabicyclo[5.4.0]undec-7-ene), in one step to dimer **41** and trimer **51**. Synthesis of an appropriately protected monomeric phosphoramidite building block (→ **75**) was more difficult, since introduction of the 2-(4-nitrophenyl)ethyl residue into the fluorescein moiety in **59** led mainly to trisubstitution to give **61** including the urea function. Formation of the adenylyl dimer **66** and trimer **67** proceeded in the usual manner by phosphoramidite chemistry; however, deprotection of **67** with DBU was incomplete since the *O*-alkyl group at the urea moiety was found to be very stable. Finally, the appropriate phosphoramidite building block **75** could be synthesized by the sequence **59** → **72** → **73** → **74** → **75**. The phosphoramidite **75** was used for the synthesis of dimer **77** and trimer **79** by solution chemistry, as well as for that of various oligonucleotides by the machine-aided approach on solid support carrying the fluorophore at different positions of the chain (→ **84**–**87**). The attachment of the fluorescein fluorophor *via* a short carbamoyl linker onto the 6-amino group of 2′-deoxyadenosine enables such molecules to function very well in fluorescence-polarization experiments.

1. Introduction. – Labelling of nucleosides, nucleotides, and oligonucleotides is a crucial procedure for identification of nucleic acid components in general. DNA sequencing and DNA probing play an important role in nucleic acid chemistry and molecular biology and can be achieved only when the molecules under investigation can be detected. The starting techniques [2] used radioactive nuclides, which reveal several disadvantages and have, therefore, been substitued by a broad variety of fluorophores [3]. The DNA-probing technique for gene analysis that was introduced by *Southern* in 1975 [2] applies the principle of hybridization of complementary oligonucleotide sequences. We have to differentiate between hybridizations in solution and those on solid-support material, depending on the purpose of the investigation. The use of nonradioactive markers for oligoncleotides was originally based upon biotin and its interaction with the proteins avidin and streptavidin [4]; however, this indirect method affords painful preparations of the probe, whereas the developed digoxigenin–UTP conjugate [5] allows more easily the introduction of a marker into oligonucleotides by chemical and enzymatic means. An ideal label for DNA probing should fulfill

[1]) Part LXX: [1]

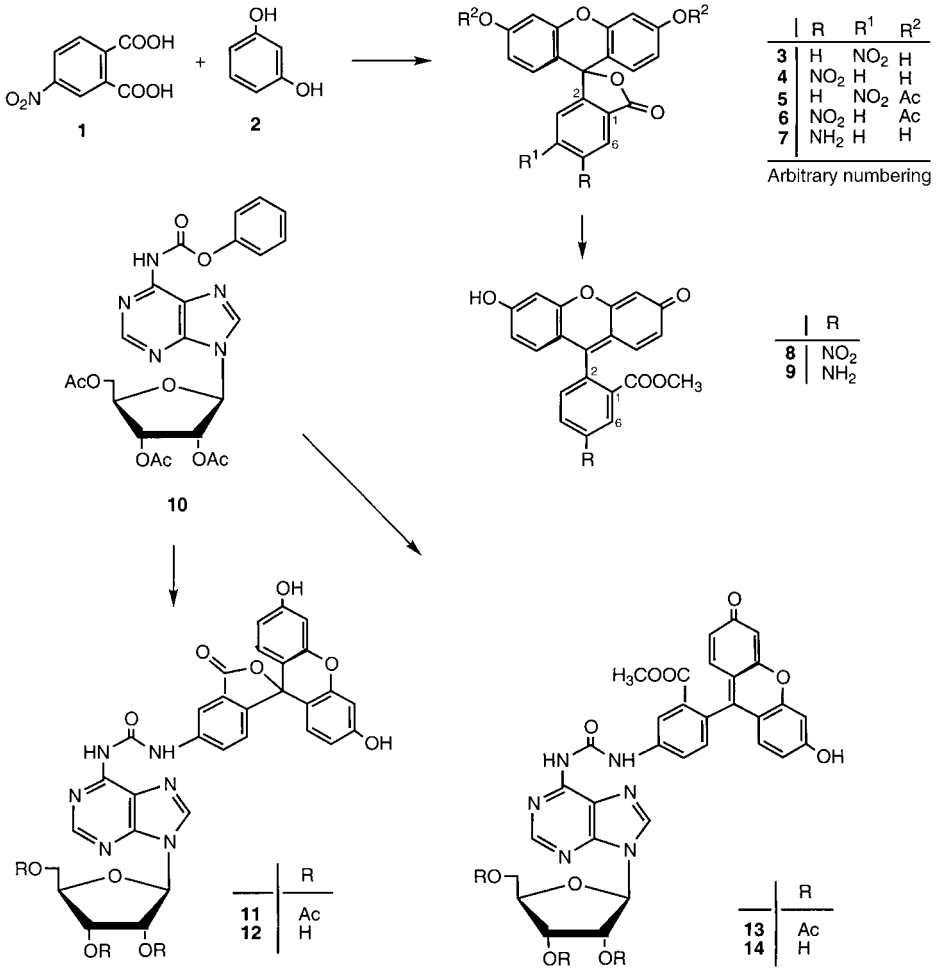
at least four criteria, such as easy introduction into DNA sequences, easy detection in low concentrations, unequivocal signal appearance showing hybridization, and reasonable stability towards increased hybridization temperatures. The crucial step is the former approach, whereby in principle a spacer between the nucleotide unit and the label is applied by attachment to the 3′- or 5′-end [7] of the oligonucleotide, its phosphate bridge [8], the nucleobase [9], and the sugar moiety [10]. We will describe a new type of labelling of nucleosides by omitting the spacer and attaching the fluorophore directly to the amino group of adenosine, cytidine, or guanosine *via* an urea function. These investigations are based upon our former findings [11] that N^6-(phenoxycarbonyl)adenosine and N^4-(phenoxycarbonyl)cytidine react under mild conditions with aromatic amines to the corresponding urea derivatives.

2. Synthesis. – The fluorophore of choice for the labelling experiments was 5-aminofluorescein (**7**), which was synthesized from 4-nitrophthalic acid (**1**) and resorcinol (= benzene-1,3-diol; **2**) in a fusion reaction [12] leading to a mixture of 4- and 5-nitrofluorescein (**3** and **4**, resp.) (*Scheme 1*). Acetylation to the diacetyl derivatives **5** and **6** allowed separation of the isomers by fractional crystallization.

Reduction of **6** is tricky [13] and worked best only with the system Na_2S/NaSH to give 5-aminofluorescein (**7**) in 77% yield. Esterification of **6** with MeOH/H_2SO_4 gave the open-form methylester **8** of 5-nitrofluorescein, which could, however, not been reduced to the corresponding 5-amino derivative **9**. Compound **9** was, therefore, prepared from **7** by an esterification analogous to that of **6**. The informative ^{1}H-NMR spectra of these compounds show clearly that 5-nitro-3′,6′-diacetylfluorescein (**6**) and 5-aminofluorescein (**7**) exist in their lactone forms, whereas **9** is methyl 5-amino-2-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)benzoate. The same structural conclusion can be drawn from the UV spectra. The UV absorption [14] and emission spectra [15] of fluorescein itself have been widely investigated in relationship to the pH. *Lindquist* [14c] determined the p*K* values describing the equilibria monocation to neutral form, neutral form to monoanion, and monoanion to dianion to be 2.2, 4.4, and 6.6, respectively. The neutral form exists thereby as an equilibrium mixture of the cyclic lactone and the open carboxy form [14d,e]. Little information is available about 5-aminofluorescein (**7**), since its use in fluorescence immunoassays is mainly mentioned in patents. We determined the p*K* values of the methyl ester **9** of 5-aminofluorescein to be 1.6, 3.1, and 6.6, showing that this molecule forms a dication at pH 0. At pH 2, the monocation exists, and, at pH 5, the neutral form is present. Finally, at pH 8, the monoanion exhibits the typical strong increase in extinction coefficient at 492 nm to 82500 $l \cdot mol^{-1} \cdot cm^{-1}$ (*Fig. 1*) and correlates expectedly with the dianion of fluorescein. An analogous pK_a determination of 5-aminofluorescein (**7**) is problematic since in the pH range 3–6, overlapping p*K* values do not allow a clear separation.

The adenosine conjugates were prepared from 2′,3′,5′-tri-*O*-acetyl-N^6-(phenoxycarbonyl)adenosine (**10**) with 5-aminofluorescein (**7**) and its methyl ester **9** to give **11** and **13**, respectively, in very good yields (*Scheme 1*). To facilitate workup, the educt **10** should be applied in an excess of 1.3–1.5 equiv. to get complete reaction of the dyestuff which has chromatographic properties similar to those of the conjugate. Deacetylation of **11** works well with methanolic ammonia to give **12**, whereas the cleavage of the acetyl groups of **13** required K_2CO_3/MeOH to give the methyl ester **14**. The NMR

Scheme 1



spectra of the adenosine–fluorescein conjugates show nicely separated signals of the nucleoside and of the dyestuff (*Fig. 2*) and allow easy assignment of the protons. The presence of 2 OH protons (10.1 ppm) of the xanthene moiety of **11** illustrates that this compound exists in the cyclic lactone form. Also, the signals of the NH functions of the urea moiety (12.2 and 10.5 ppm) are quite characteristic. Typical for this type of compounds are also the only hardly separated H–C(2) and H–C(8) protons of the purine ring.

The UV/VIS spectra depend on the molecular species present at the related pH. In the normal pH range, **13** shows 4 pK_a values at 0.96 (dication $\rightleftharpoons$ monocation), 2.48 (monocation $\rightleftharpoons$ neutral form), 6.57 (neutral form $\rightleftharpoons$ monoanion), and 11.9 (monoanion $\rightleftharpoons$ dianion). We assume that the dication is protonated at the N(1) atom of the purine ring and the carbonyl function of the xanthene moiety. The monocation is,

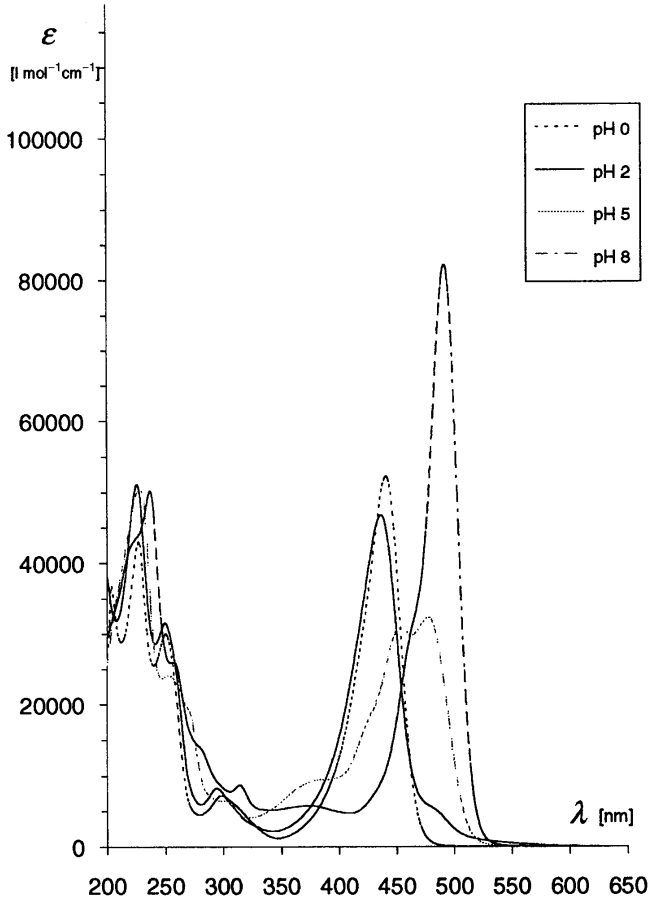


Fig. 1. *UV-Absorbtion spectra of the dication* (pH 0), *the monocation* (pH 2), *the neutral form* (pH 5), *and the monoanion* (pH 8) *of* **9**

according to the partially overlapping pK_a in strong acid, a mixture of two species with preference for the xanthene protonated form. The long-wavelength-absorption band of the cations is found at 440 nm and is shifted characteristically to a double maximum at 466 and 494 nm with lower extinction for the neutral species (see *Fig. 3,a*). Monoanion formation involving ionization at the xanthene OH group is associated with an enormous increase in extinction coefficient to 86000 $l \cdot mol^{-1} \cdot cm^{-1}$ at 494 nm and appearance of strong fluorescence. The dianion formation is related to deprotonation of the urea function, since N^6-(*N*-phenylcarbamoyl)adenosine [11] has, as a more-simple analog, a pK_a of 12.2.

A more-complex situation is present in compound **11**, since here the neutral species exists preferentially in the cyclic lactone form as established by the low extinction at 440 nm. At pH 5.5, the lactone ring has opened up to form a monoanion at the carboxy group characterized by the double maximum at 450 and 470 nm. Further deprotonation

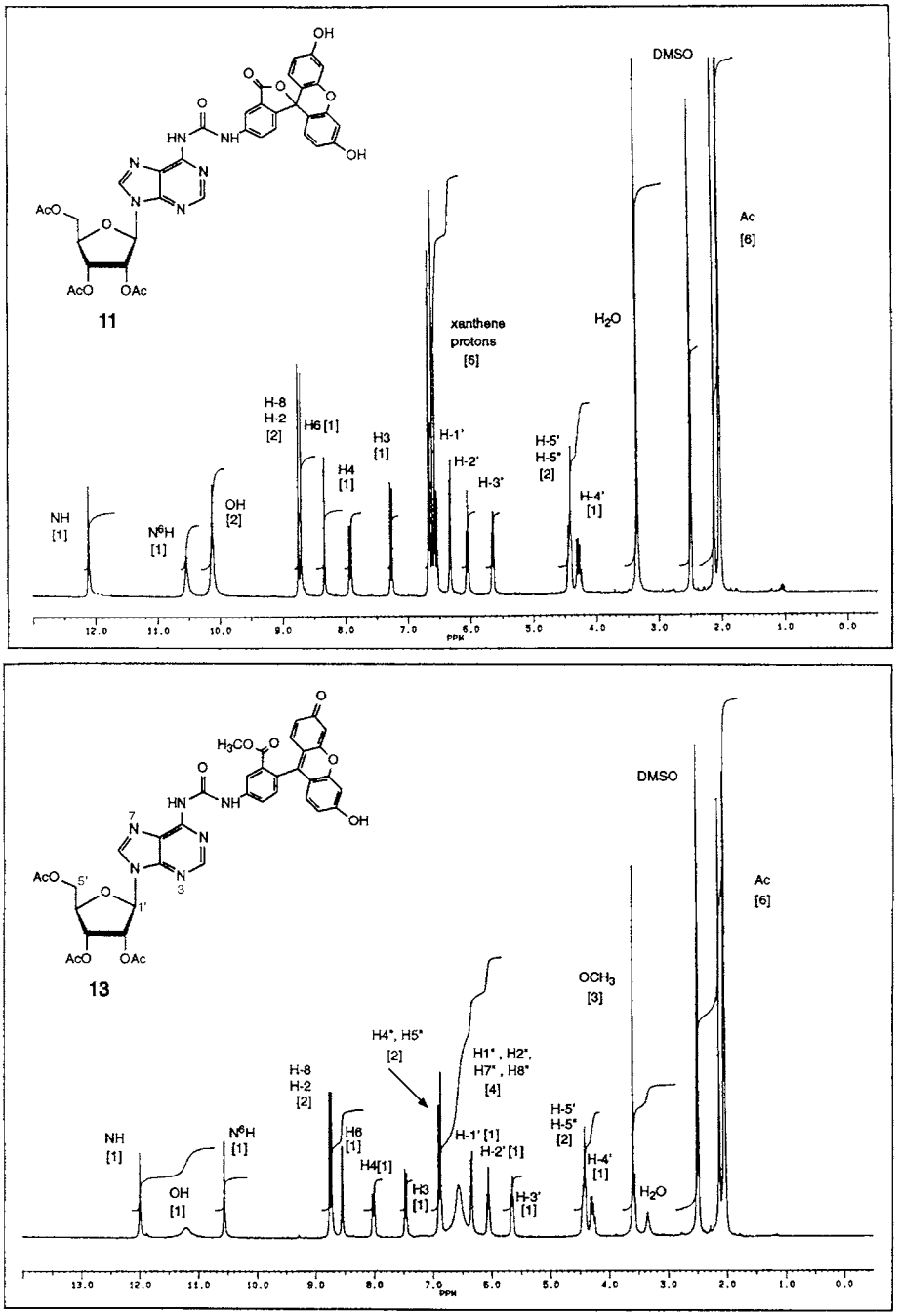


Fig. 2. *NMR Spectra of* **11** *and* **13** *in (D$_6$)DMSO.* H-2, H-8, N^{6}H: protons of the nucleobase; H-1′, H-2′, H-3′, H-4′, H-5′, H-5″: protons of the sugar moiety; H3, H4, H6: protons of the isobenzofuran or benzoic acid moiety (arbitrary numberings); H1″, H2″, H4″, H5″, H7″, H8″: protons of the xanthene moiety; integrations in brackets.

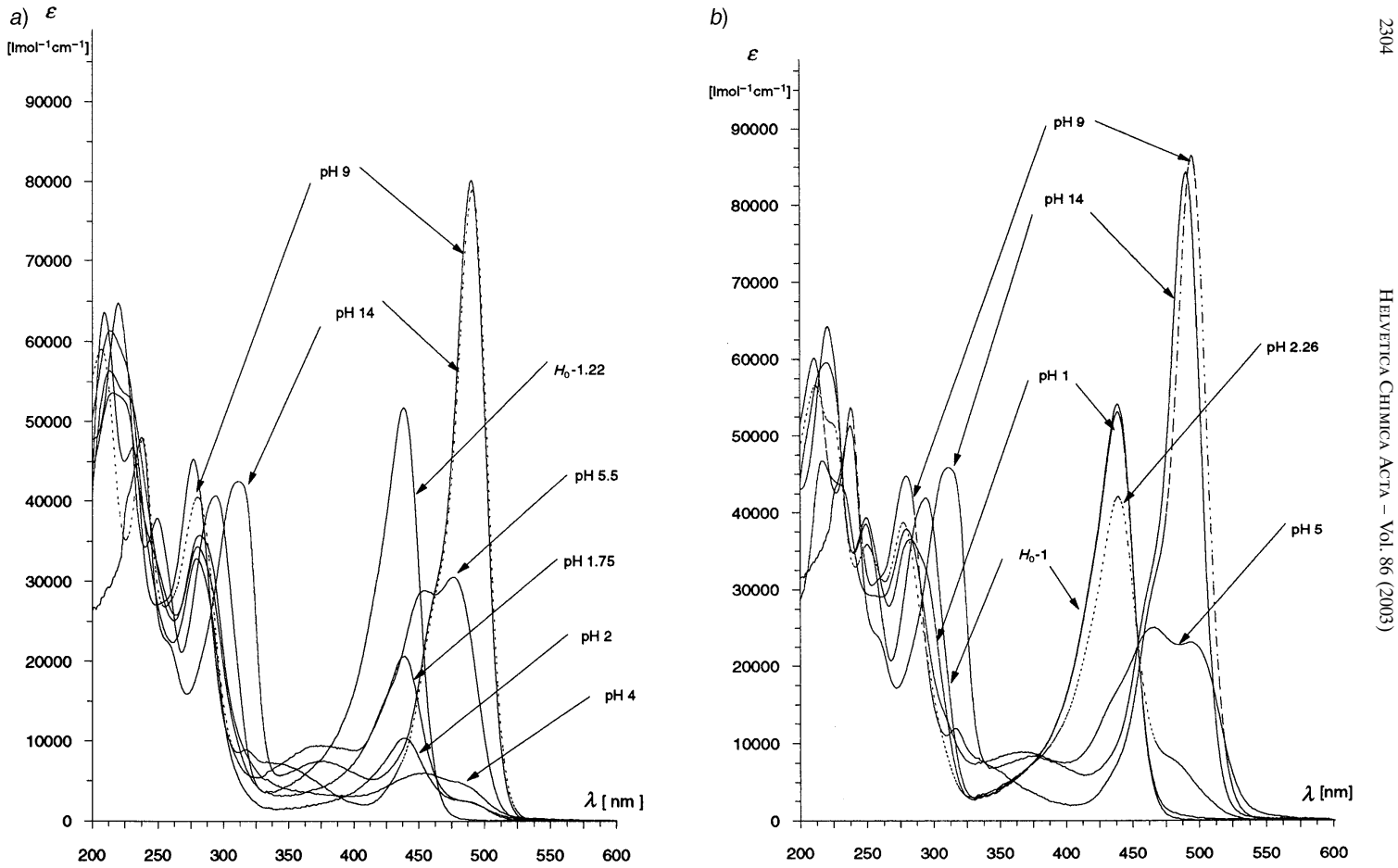


Fig. 3. *UV Spectra of* a) *the monocation* (pH 0), *the neutral form* (pH 2), *the monoanion* (pH 5.5), *and the dianion* (pH 9) *of* **11** *and* b) *of the monocation* (pH 1), *the neutral form* (pH 5), *and the monoanion* (pH 9) *of* **13**

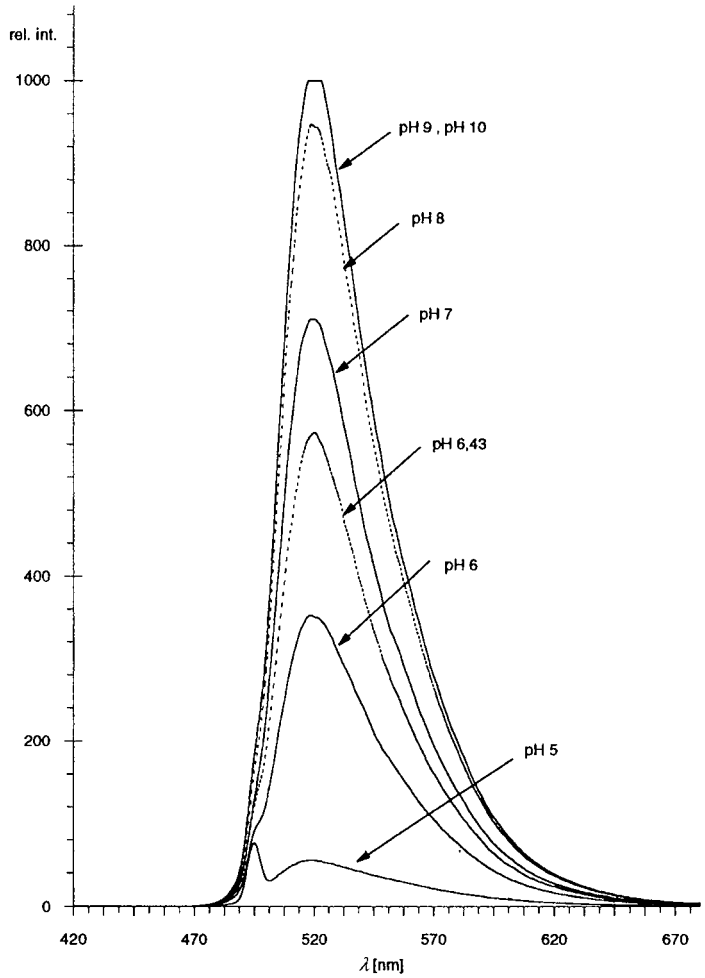


Fig. 4. *pH-Dependent fluorescence spectra of* **11**

at the xanthene moiety gives the strongly fluorescent dianion with a high extinction coefficient (*Fig. 3,b*). The emission properties of **11** reflect also the structural features, since the neutral species shows very weak fluorescence that increases systematically with higher pH (*Fig. 4*).

Cytidine carbamates are less investigated and known only in the form of N^4-fmoc [16] and N^4-npeoc [17] protecting groups (fmoc = (9*H*-fluoren-9-ylmethoxy)carbonyl, npeoc = [2-(4-nitrophenyl)ethoxy]carbonyl). Also, very few N^4-urea derivatives of cytosine and cytidine have been prepared by reaction with isocyanates [18]. Treatment of 5′-*O*-(dimethoxytrityl)-N^4-{[2-(4-nitrophenyl)ethoxy]carbonyl}cytidine with ammonia in H_2O/MeOH proceeded only by β-elimination of the protecting group [19], whereas the N^4-(phenoxycarbonyl)cytidine proved to be a versatile starting material for N^4-urea formation [11]. The 2′,3′,5′-tri-*O*-acetyl-N^4-(phenoxycarbonyl)cytidine (**15**)

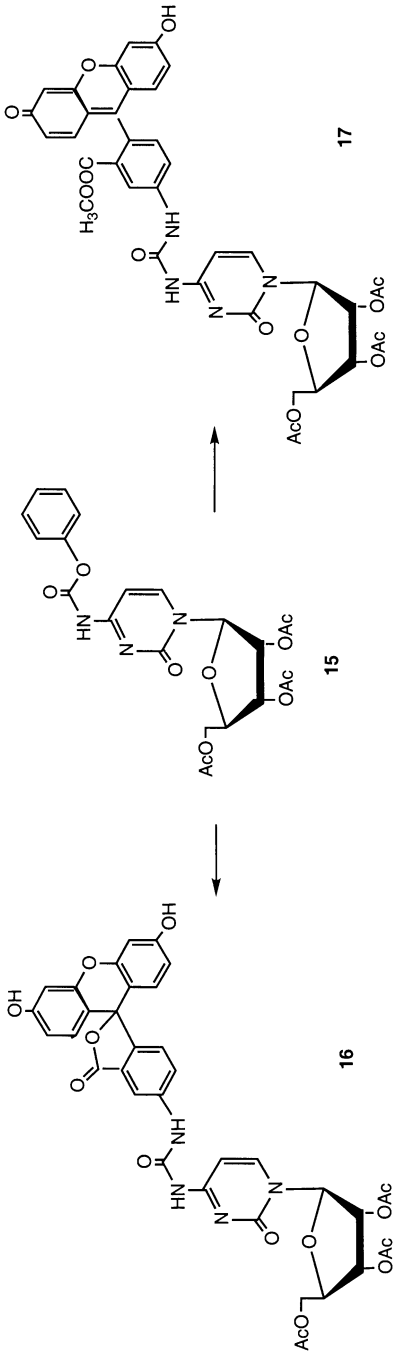
reacted with 5-aminofluorescein (**7**) methyl ester **9** of 5-aminofluorescein in pyridine at 70° in good yields to the corresponding conjugates **16** and **17**, respectively (*Scheme 2*). The ^{1}H-NMR- and UV data are in good agreement with the findings in the adenosine series. Compound **16** exists as neutral species mainly in the cyclic lactone form, but its UV spectrum is best characterized as a dianion at pH 9. The various molecular forms of **17** are better separated spectrophotometrically, as seen from the pK_a values 0.40, 2.79, 6.13, and 11.33. The less-basic position in the dication is the N(3) ring atom followed by the xanthene moiety. The neutral form at pH 4.5 has a relatively low extinction which raises very high on conversion to the monoanion at pH 9. The further deprotonation of the urea function can be recognized from the spectral change in the UV region at *ca.* 310 nm, whereas the characteristic long-wavelength band at 496 nm does not change.

N^2-Urea derivatives in the guanine/guanosine series have also not been systematically investigated, and the few examples have been derived from the reaction of guanine [18b] or guanosine [18e][20][21] with various isocyanates. Ammonolysis of carbamates have been only occassionally described [20]. We started our investigations in this respect from 3′,5′-di-*O*-acetyl-2′-deoxy-O^6-[2-(4-nitrophenyl)ethyl]guanosine, which is a reasonably soluble guanosine derivative prone to substitution reaction. Phenyl carbonochloridate reacted with **18** in pyridine at room temperature in good yield to the corresponding N^2-(phenoxycarbonyl) derivate **19**, which could be further converted to the deoxyguanosine conjugate **20** (*Scheme 3*). Its structure was established by ^{1}H-NMR and UV spectra, similarly as for the adenosine and cytidine analogs, as well as by elemental analysis.

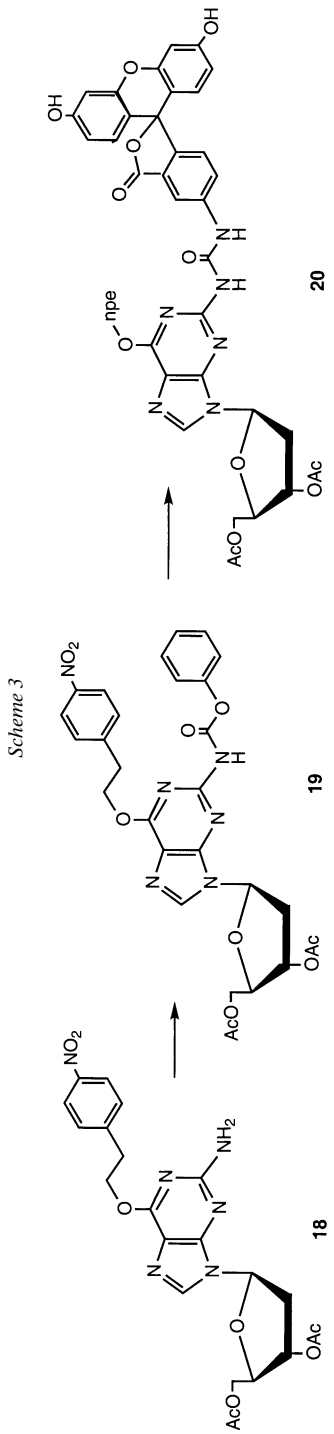
To allow the use of labelled adenosine derivatives in oligonucleotide synthesis, we developed a new strategy starting at the 3′-end with 2′-deoxy-N^6,3′-*O*-bis-{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine (**21**) [19] and condensation with 2′-deoxy-5′-*O*-(monomethoxytrityl)- (**22**) and 2′-deoxy-5′-*O*-(dimethoxytrityl)-N^6-[(9*H*-fluoren-9-ylmethoxy)carbonyl]adenosine 3′-[2-(4-nitrophenyl)ethyl diisopropylphosphoramidite] (**23**), which were derived from N^6-fmoc-protected 2′-deoxyadenosine **24** [22] first by monomethoxytritylation (→ **25**) and dimethoxytritylation (→ **26**), respectively, and followed by phosphitylation with 2-(4-nitrophenyl)ethyl diisopropylphosphoramidochloridite to give the dimers **27** and **28** in 70–80% yield (*Scheme 4*). Their detritylations led to the protected adenylyladenosine **29**, and their treatment with Et_3N in MeCN cleaved selectively the fmoc protecting group to form **30** and **31**, respectively. The introduction of the phenoxycarbonyl function onto the 6-amino group of **30** and **31** with 1-(phenoxycarbonyl)-1*H*-tetrazole was problematic since partial detritylation took place, and isolation of the products **32** and **33**, respectively, was difficult and gave only 65% yield when protic solvents like MeOH were omitted on chromatography.

To overcome these difficulties, N^6-fmoc-protected 2′-deoxyadenosine **24** was protected at the 5′-OH group by the npeoc group (→ **34**) and then phosphitylated to give **35**. The condensation of **21** with **35** gave the dimer **36**, which lost the fmoc group on treatment with Et_3N yielding **37**. The 6-amino group of **37** was then acylated by 5-(4-nitrophenyl)-1-(phenoxycarbonyl)-1*H*-tetrazole to form the fully protected dimer **38**. The introduction of the fluorophore was achieved by reaction of **33** or **38** with 5-aminofluorescein (**7**) to give the conjugates **39** and **40**, respectively. Compound **40** was then deprotected in one step by treatment with DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) in pyridine and subsequently purified by FP (fast-performance) liquid chroma-

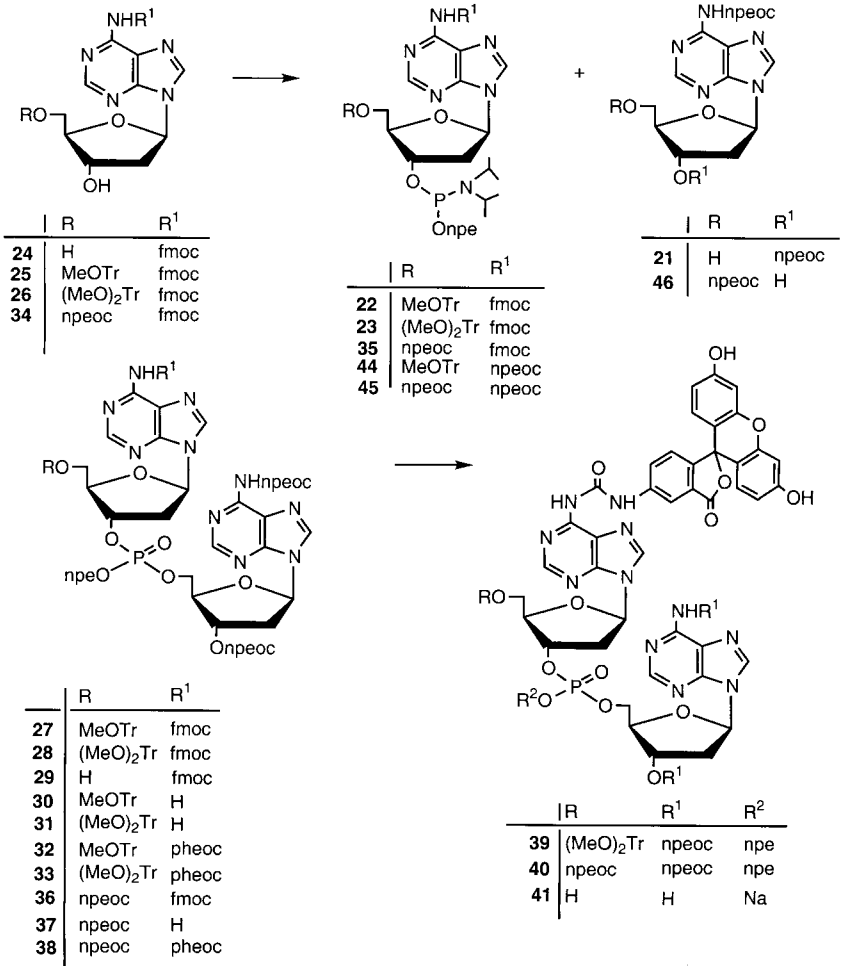
Scheme 2



Scheme 3



Scheme 4



	R	R^1
24	H	fmoc
25	MeOTr	fmoc
26	$(MeO)_2Tr$	fmoc
34	npeoc	fmoc

	R	R^1
22	MeOTr	fmoc
23	$(MeO)_2Tr$	fmoc
35	npeoc	fmoc
44	MeOTr	npeoc
45	npeoc	npeoc

	R	R^1
21	H	npeoc
46	npeoc	H

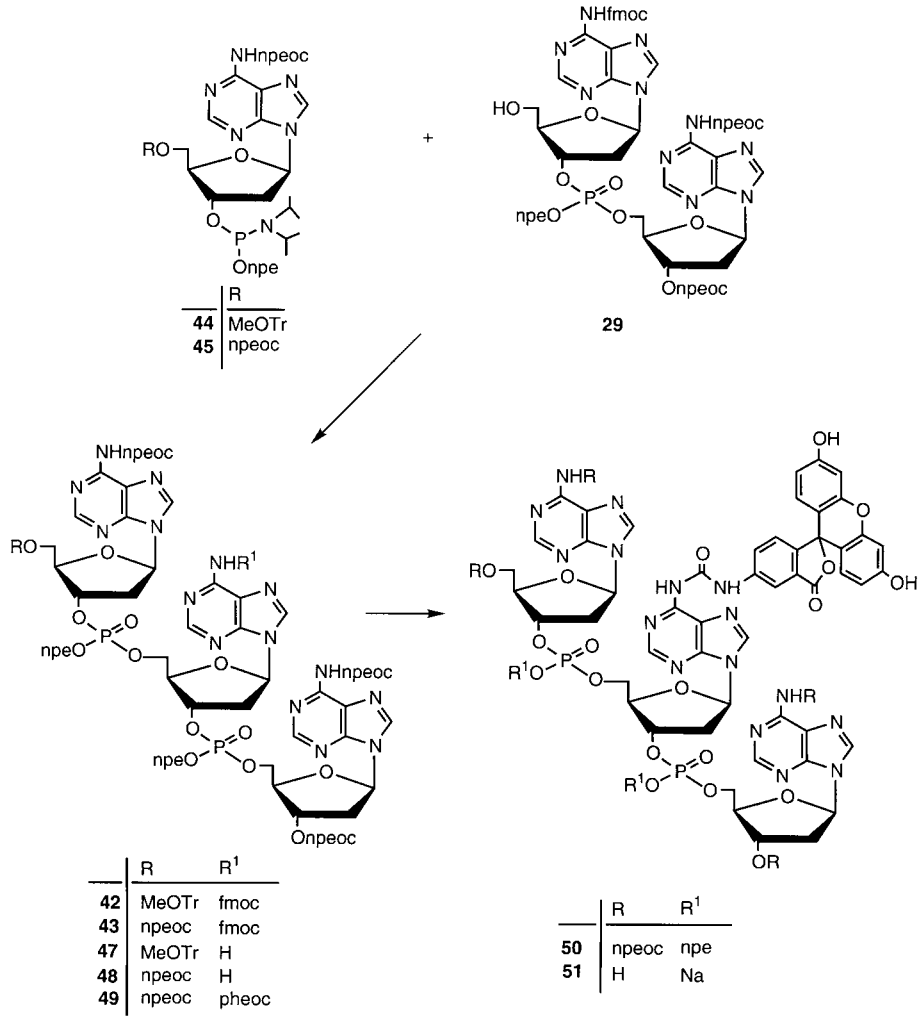
	R	R^1
27	MeOTr	fmoc
28	$(MeO)_2Tr$	fmoc
29	H	fmoc
30	MeOTr	H
31	$(MeO)_2Tr$	H
32	MeOTr	pheoc
33	$(MeO)_2Tr$	pheoc
36	npeoc	fmoc
37	npeoc	H
38	npeoc	pheoc

	R	R^1	R^2
39	$(MeO)_2Tr$	npeoc	npe
40	npeoc	npeoc	npe
41	H	H	Na

tography (DEAE-*Sephadex*; $(Et_3NH)HCO_3$ buffer (TBK)). The resulting triethylammonium salt was finally transformed into its more-stable sodium salt **41**.

The syntheses of the corresponding trimers **42** and **43** worked in an analogous manner by treatment of the dimer **29** with either 2′-deoxy-5′-*O*-(monomethoxytrityl)-N^6-[2-(4-nitrophenyl)ethoxy]carbonyl}- (**44**) [22] or 2′-deoxy-N^6,5′-*O*-bis-{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine 3′-[2-(4-nitrophenyl)ethyl diisopropylphosphoramidite] (**45**) (*Scheme 5*), which was prepared from its precursor **46** [23] (*cf. Scheme 4*). Deprotection of the fmoc group of **42** and **43** by Et_3N led in 94% yield to **47** and **48**, respectively. The anticipated acylation with 1-(phenoxycarbonyl)-5-(4-nitrophenyl)-1*H*-tetrazole was, again, unsuccessful with **47** but gave an excellent yield with **48** to form **49**.

Scheme 5



	R
44	MeOTr
45	npeoc

	R	R1
42	MeOTr	fmoc
43	npeoc	fmoc
47	MeOTr	H
48	npeoc	H
49	npeoc	pheoc

	R	R1
50	npeoc	npe
51	H	Na

Treatment of **49** with 5-aminofluorescein (**7**) gave the fluorescence-labelled trimer **50**, however, in only 22% isolated yield, showing that the labelling experiments proceed with decreasing yields going from the monomer **11** *via* the dimer **40** to the trimer conjugate **50**. Complete deprotection of **50** was achieved by DBU treatment, which cleaved in one step all npe and npeoc groups; DEAE-*Sephadex* purification and conversion of the resulting triethylammonium salt gave the sodium salt **51** in 60% yield (from **50**), which exhibited a very clean HPLC analysis (*Fig. 5*).

Characterization of the various dimers and trimers was done by ^{1}H-NMR and UV spectra as well as by elemental analysis. The ^{1}H-NMR spectra present quite complex patterns of which several characteristic signals can be assigned unambiguously.

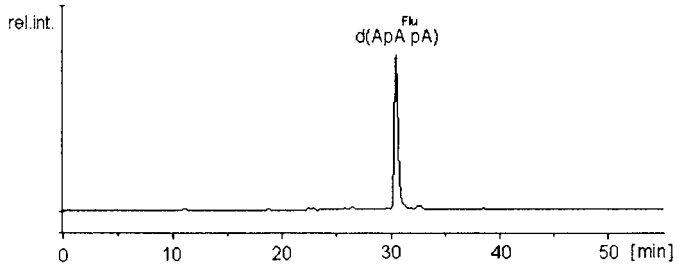


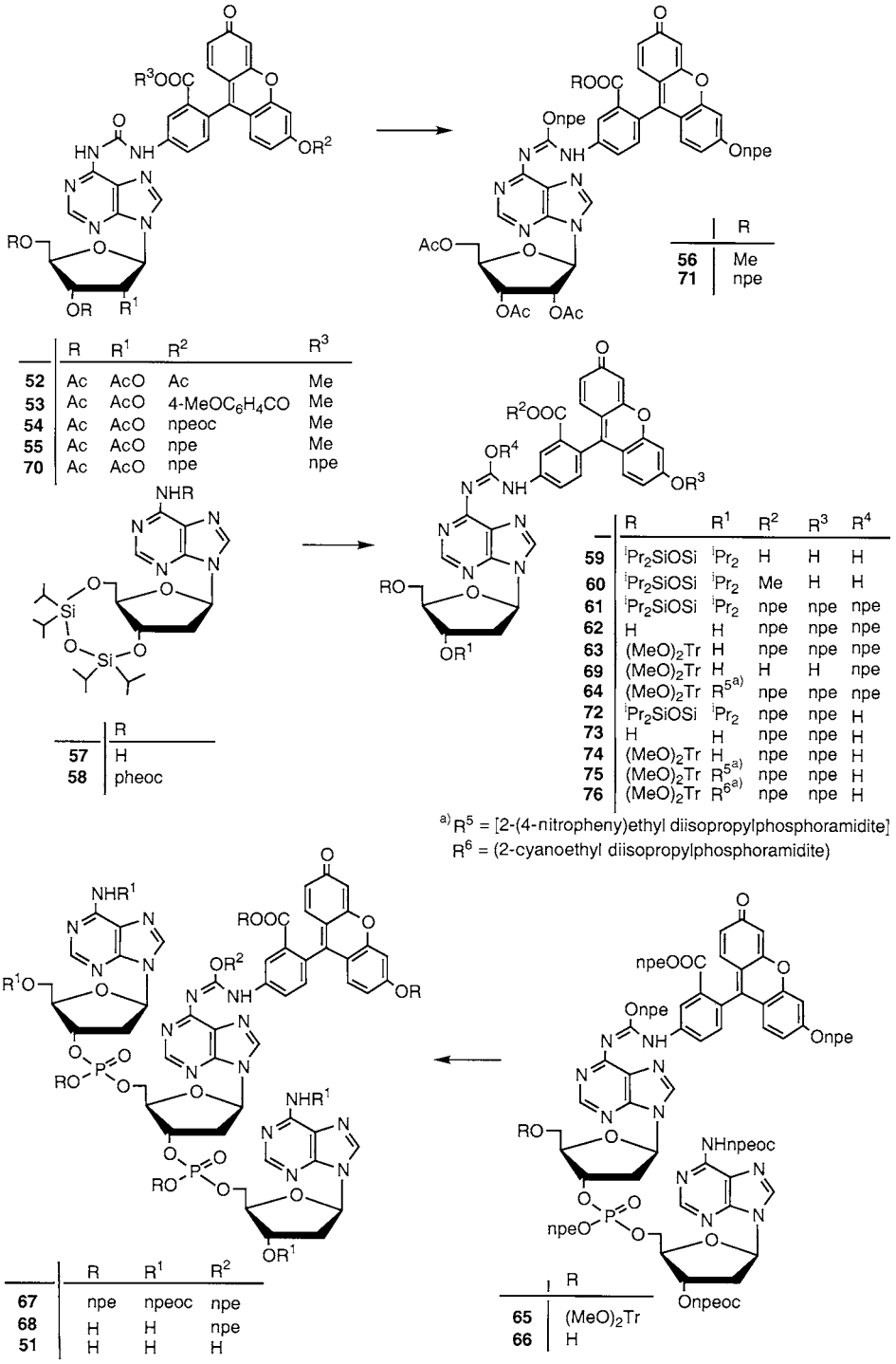
Fig. 5. *HPLC of* **51**

Since the above-described results have not been satisfactory in every respect, we decided to synthesize fluorescein-labelled 5′-*O*-(dimethoxytrityl protected 3′-phosphoramidites for use in a conventional DNA synthesizer. For this purpose, the xanthene moiety also had to be protected to avoid side reactions. The 2′,3′,5′-tri-*O*-acetyl-N^6-carbamoyladenosine–fluorescein (methyl ester) conjugate **13** was first treated as a model substance with various anhydrides to protect the OH group. The resulting compounds **52**–**54** (*Scheme 6*), which are formally vinylogous anhydrides, turned out to be too reactive for further use. Compound **52** could be seen only on TLC, but isolation in pure form was not possible. The next attempt was an esterification reaction of **13** with 2-(4-nitrophenyl)ethanol under *Mitsunobu* [24] conditions, which led to a mixture of two compounds of which the npe-protected 3-hydroxyxanthenyl derivative **55** was formed only in 8%, whereas the main reaction product **56** was additionally substituted at the urea O-atom. The structural assignment of the second npe group at this O-atom is based on the ^{1}H-NMR chemical shift of a *t* for a CH_2CH_2O moiety appearing at lower field than expected for a CH_2CH_2N group.

In the 2′-deoxyadenosine series, the 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl) derivative **57** was chosen as the starting material. Phenoxycarbonylation to **58** worked well, and subsequent treatment with 5-aminofluorescein (**7**) and methyl ester **9** of 5-aminofluorescein led to the conjugates **59** and **60**, respectively, in very good yields. The *Mitsunobu* reaction of **59** with 2-(4-nitrophenyl)ethanol afforded a threefold substitution to give **61** in 90% yield. Desilylation to **62** was achieved by Bu_4NF in AcOH/THF. The transformations into the 5′-*O*-(dimethoxytrityl) derivative **63** and the corresponding 3′-[2-(nitrophenyl)ethyl diisopropylphosphoramidite] **64** were performed routinely. The fully protected building block **64** was reacted with compound **21** to the dimer **65**, which was detritylated to **66** and then condensed with **45** to give the fully protected trimer **67**. A new surprise was encountered on DBU treatment, expected to remove all blocking groups. HPLC Analysis showed, on comparison with **51**, only 20% conversion to **51**, whereas the main peak was due to a more-lipophilic substance that still contained a protecting group and presumably has the isourea structure **68**.

To find out which function is relatively stable towards DBU, compound **63** was treated under the same reaction conditions as **67**: the *O*-[2-(4-nitrophenyl)ethyl]isourea **69** (*Scheme 6*) was isolated in 81% yield. The cleavage of the npe group from the isourea moiety under more-drastic conditions with 2000 equiv. of DBU was followed by HPLC. Even after 48 h, still some starting material **69** was present, indicating that

Scheme 6



	R	R^1	R^2	R^3
52	Ac	AcO	Ac	Me
53	Ac	AcO	4-$MeOC_6H_4CO$	Me
54	Ac	AcO	npeoc	Me
55	Ac	AcO	npe	Me
70	Ac	AcO	npe	npe

	R	R^1	R^2	R^3	R^4
59	$^iPr_2SiOSi$	$^iPr_2$	H	H	H
60	$^iPr_2SiOSi$	$^iPr_2$	Me	H	H
61	$^iPr_2SiOSi$	$^iPr_2$	npe	npe	npe
62	H	H	npe	npe	npe
63	$(MeO)_2Tr$	H	npe	npe	npe
69	$(MeO)_2Tr$	H	H	H	npe
64	$(MeO)_2Tr$	$R^{5a)}$	npe	npe	npe
72	$^iPr_2SiOSi$	$^iPr_2$	npe	npe	H
73	H	H	npe	npe	H
74	$(MeO)_2Tr$	H	npe	npe	H
75	$(MeO)_2Tr$	$R^{5a)}$	npe	npe	H
76	$(MeO)_2Tr$	$R^{6a)}$	npe	npe	H

[a] R^5 = [2-(4-nitropheny)ethyl diisopropylphosphoramidite]
R^6 = (2-cyanoethyl diisopropylphosphoramidite)

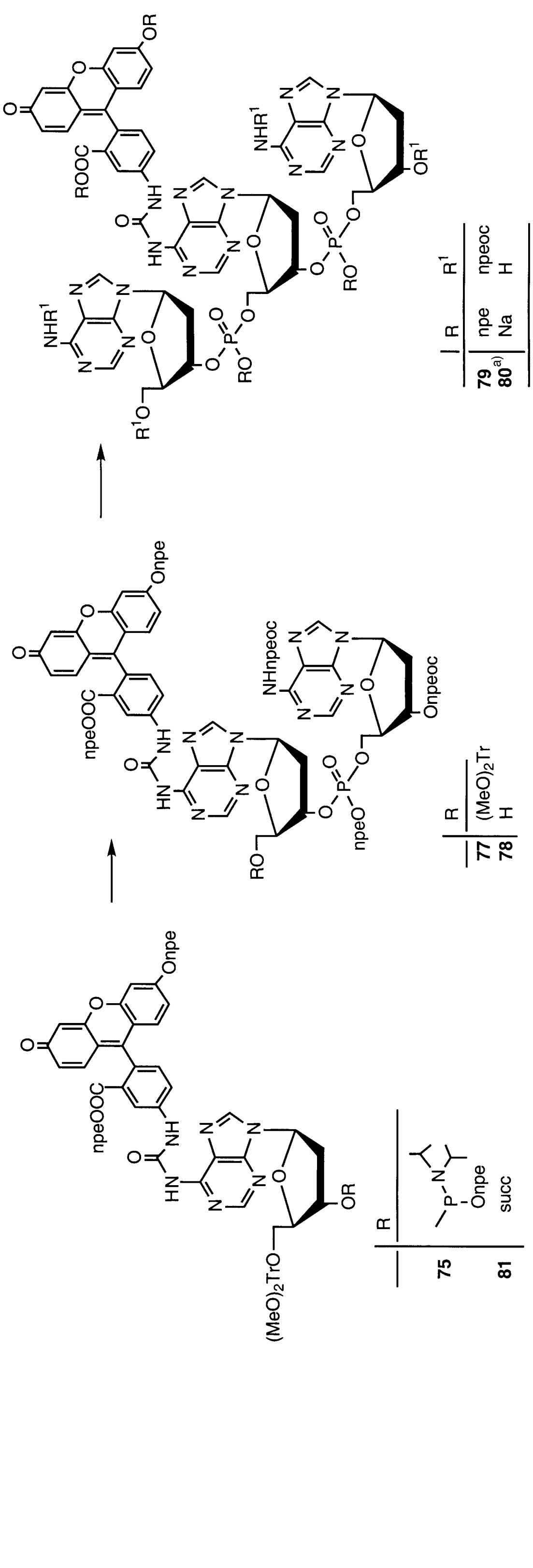
building block **64** is not suitable for oligonucleotide synthesis in a machine-aided approach.

To overcome this problem, we concentrated again on the *Mitsunobu* reaction of **11** and **59** by changing the reaction conditions to get preferentially disubstitution at the fluorescein moiety and less trisubstitution. It was found that the educt should be mixed in dioxane with 2-(4-nitrophenyl)ethanol and diethyl azodicarboxylate first, and then triphenylphosphine should be added in small portions controlling the progress of the reaction by TLC after each addition and waiting for 5 min. Compound **11** gave thus a mixture of 48% of the desired product **70** and 38% of **56** (*Scheme 6*). The analogous reaction of **11** with **59** applying 2.1 to 2.3 equiv. of triphenylphosphine led to the desired disubstituted product **72** in 64%, whereas the trisubstituted **61** was formed in only 16% yield. Desilylation of **72** gave **73** in 92% yield and subsequent dimethoxytritylation led in 76% yield to **74**. The anticipated labelled building block **75** for oligonucleotide synthesis was prepared by phosphitylation in the usual manner, and condensation with **21** led to the dimer **77**, which was again detritylated to **78** in 83% yield and used for the next condensation with the phosphoramidite **45** to give the fully protected dApdA^{flu}dA trimer **79** (*Scheme 7*). The DBU cleavage removed all protecting groups in one step, and, after DEAE-*Sephadex* purification and conversion into the sodium salt, **80** was isolated in 60% yield and established by physical means to be identical to compound **51** prepared by a different route.

The trimer **51** (= **80**) was also synthesized on an *ABI*-DNA synthesizer *392* starting with a modified CPG-solid support loaded with 2′-deoxy-5′-*O*-(dimethoxytrityl)-*N*^{6}-{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine 3′-succinate in the usual manner. For the next cycle, the 2-cyanoethyl phosphoramidite **76** was found to be more reactive than **75** and giving higher yields. Proceeding with the 2′-deoxy-5′-*O*-(dimethoxytrityl)-*N*^{6}-{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine 3′-(2-cyanoethyl diisopropylphosphoramidite) [22] gave the trimer, which was treated first with $CHCl_2COOH$ to remove the dimethoxytrityl group, then with DBU to cleave the ce, npe, and npeoc groups before detaching from the solid-support by ammonia. This sample was identical to both the preparatively synthesized materials **51** and **80** as shown by HPLC. The analogous trimer d(ApApA^{flu}) carrying the fluorophore at the 3′-end required first the synthesis of the 3′-succinate **81**, which was coupled to the CPG solid support; the following two conventional cycles yielded a high-quality product. The analogous combination with dA^{flu} led, to the trimer (dA^{flu})$_3$ that showed, however, some impurities in the HPLC.

3. Oligonucleotide Synthesis. – To establish the usefulness of the fluorescein-labelled dA as a new type of building block, several oligodeoxynucleotides carrying the fluorophore in different positions were synthesized from the npe- and npeoc-protected dA, dC, and dG phosphoramidites [22] (*Table 1*). First, we assembled the unlabeled 15-mer sequence **82** and its complementary strain **83** as a reference. Then, the dA^{flu} was attached to the 5′-end (→ **84**), the 3′-end (→ **86**), the 3′,5′-end (→ **87**), and the middle (→ **85**) of the chain. The complementary strains for hybridization studies carried on both ends a 10-mer (see **88**), a 20-mer (see **89**), or a 30-mer-T (see **90**) extension. The melting points (*Table 1*) were determined at a salt concentration of 0.12M (Na^+) and at pH 7.4. The unlabelled duplex **82** · **83** showed a T_m of 59.6°, and the attachment of one dA^{flu} unit at the 3′- or 5′-end forming the hybrids **83** · **84** and **83** · **86** caused a small

Scheme 7


npeOOC
Onpe
(MeO)2TrO
OR
R
75
81
Onpe
succ
RO
npeO
NHnpeoc
Onpeoc
R
77
78
(MeO)2Tr
H
ROOC
OR
NHR1
R1O
RO
OR1
R
R1
79
80a)
npe
Na
npeoc
H
a) 80 = 51

decrease of 1–2°, whereas labelling on both ends did not change the T_m of the standard. Labelling in the middle of the chain gave, expectedly, a strong depression to 45.7° (**83**·**85**), which is also recognized in the duplexes **84**·**88**, **84**·**89**, and **84**·**90**, where the label does not allow perfect H-bonding.

Table 1. *Oligodeoxynucleotide Sequences and* T_m *of Hybridazations*[a])

	Sequence	Hybrid	T_m [°]
82	5′-d(TCC CAG TCA CGA CGT)-3′		
83	5′-d(ACG TCG TGA CTG GGA)-3′	**82**·**83**	59.6
84	5′-d(A^{flu}TCC CAG TCA CGA CGT)-3′	**83**·**84**	58.5
85	5′-d(TCC CAG TCA^{flu} CGA CGT)-3′	**83**·**85**	45.7
86	5′-d(TCC CAG TCA CGA CGT-A^{flu})-3′	**83**·**86**	57.6
87	5′-d(A^{flu}-TCC CAG TCA CGA CGT-A^{flu})-3′	**83**·**87**	59.8
88	5′-d(T_{10}ACG TCG TGA CTG GGA T_{10})-3′	**84**·**88**	54.6
89	5′-d(T_{20}ACG TCG TGA CTG GGA T_{20})-3′	**84**·**89**	54.3
90	5′-d(T_{30}ACG TCG TGA CTG GGA T30)-3′	**84**·**90**	54.0

[a]) Salt concentration: 0.12M (Na^+).

4. Fluorescence Polarization. – Fluorescence-polarization spectroscopy (FPS) is used as a tool in biochemistry [25] and diagnostic [26] to detect interactions between big molecules such as proteins or molecule aggregates present in membranes based upon the mobility of the molecules. Fluorescing molecules excited by polarized light show in viscous solvents a strong polarization of the emitted light. Small molecules that rotate faster show, in general, almost complete fluorescence depolarization, whereas, with big molecules, the grade of polarization is increased due to their restricted rotation and mobility. Although nucleic acids belong to the group of large biomolecules, relatively little is known about the detection of interactions of nucleic acids with other nucleic acid components or other biomolecules by fluorescence polarization [27]. In the first experiments, the relationship between the fluorescence polarization and the molecular mass as well as the time scale of hybridization was studied (*Table 2*). The addition of T_{10} did not have any influence on the observed results. The fluorescence polarization was also dependent on the salt concentration and the pH of the solution (*Table 3*). Higher salt concentrations stabilized the hybridization and increased the viscosity of the solution, and a lower pH was consistent with stronger duplex stability.

Furthermore, the grade of fluorescence polarization was dependent on the size of the target molecule, as demonstrated by the duplexes **83**·**84**, **84**·**88**, **84**·**89**, and **84**·**90**.

Table 2. *Fluorescence Polarization: Dependence on Target Size and Hybridization Time in Buffer at pH 9*

Substance	*P*	Substance	Time	*P*
A^{flu} (**12**)	0.012	**83**·**84** + T_{10}	10 min	0.036
d($AA^{flu}A$) (**51**)	0.018	**83**·**84** + T_{10}	20 min	0.053
16-mer **84**	0.041	**83**·**84** + T_{10}	45 min	0.066
83·**84**	0.085	**83**·**84** + T_{10}	90 min	0.069
T_{10} + **84**	0.040	**83**·**84** + T_{10}	20 h	0.083

Table 3. *Fluorescence Polarization: Dependence of pH, Salt Concentration, and Oligonucleotide Sequence*

Sequence	pH	[NaCl]	*P*	Sequence	pH	[NaCl]	*P*	Sequence	pH	[NaCl]	*P*
84	7	–	0.076	**84**·**88**	7	–	0.122	**83**·**84**	7	0.06 M	0.106
84	7	0.1 M	0.083	**84**·**88**	7	0.1 M	0.184	**84**·**88**	7	0.06 M	0.154
84	7	0.2 M	0.094	**84**·**88**	7	0.2 M	0.194	**84**·**89**	7	0.06 M	0.167
84	7	0.4 M	0.108	**84**·**88**	7	0.4 M	0.205	**84**·**90**	7	0.06 M	0.170
84	7	1.0 M	0.130	**84**·**88**	7	1.0 M	0.223				
84	9	–	0.052	**84**·**88**	9	–	0.115	**83**·**84**	9	0.2 M	0.120
84	9	0.1 M	0.063	**84**·**88**	9	0.1 M	0.168	**84**·**88**	9	0.2 M	0.175
84	9	0.2 M	0.083	**84**·**88**	9	0.2 M	0.175	**84**·**89**	9	0.2 M	0.191
84	9	0.4 M	0.100	**84**·**88**	9	0.4 M	0.190	**84**·**90**	9	0.2 M	0.190
84	9	1.0 M	0.129	**84**·**88**	9	1.0 M	0.203				

Experimental Part

General. Products were dried under high vacuum. All solvents used were of anhydrous grade. TLC: precoated silica gel thin-layer sheets *60 F254* from *Merck*. Flash chromatography (FC): silica gel *Baker* (30–60 μm); 0.2–0.3 bar; FPLC = fast performance liquid chromatography; CC = column chromatography. HPLC: pump *L 6000*, autosampler *AS 4000*, UV detector *L 4000*, *Merck-Hitachi*; column *RP 18*, *Licrocart*, 125 × 4 mm, 5 μm, *Merck*; elution: *A* = 0.1M (Et_3NH)OAc buffer pH 7, *B* = 0.1M (Et_3NH)OAc buffer/MeCN 1:1; gradient: 5% *B* (0–2 min), 5–40% *B* (2–30 min), 40–100% *B* (30–50 min), 100% *B* (50–60 min); flow rate 1 ml/min. M. p.: *Gallenkamp* melting-point apparatus; no corrections. UV/VIS: *Perkin-Elmer Lambda 5*; λ_{max} in nm (log ε); the pK_a determinations were performed spectrophotometrically [28]. Fluorescence spectra: *Perkin-Elmer LS 50*. Fluorescence polarization: measured at *Merck* with *Merck vitalab eclair*. ^{1}H-NMR: *Bruker AC-250*; δ in ppm rel. to $SiMe_4$ or $CDCl_3$ ((D_6)DMSO) as internal standard; arbitrary numbering of the fluorescein (flu) moiety (*cf.* **3**–**7**); the same numbering is retained for the open form of fluorescein-derived substituents (*cf.* **8** and **9**). ^{31}P-NMR: *Jeol JMN-GX400*.

1. *3′,6′-Di*-O-*acetyl-5-nitrofluorescein* (= *3′,6′-Bis(acetyloxy)-5-nitrospiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthen]-3-one*; **6**). A mixture of 4-nitrophthalic acid (50 g, 0.24 mol) and resorcinol (53 g, 0.48 mol) was heated in an open *Erlenmeyer* flask to 180–190° for 3 h with stirring, whereby the melt solidified to a dark mass. The crude material was ground and then heated in 0.5N HCl (1 l) for 1 h under reflux. After cooling, the precipitate was collected, dried, then boiled in Ac_2O (110 ml) for 1 h, and filtered hot through a glass frit. The filtrate was kept several days in the icebox for crystallization. The separated solid was recrystallized first from Ac_2O (70 ml) and finally from toluene (70 ml): 17 g (28%) of **6**. M.p. 215–218°. TLC (toluene/AcOEt 7:1): R_f 0.64. ^{1}H-NMR ((D_6)DMSO): 8.70 (*d*, H–C(6)); 8.55 (*dd*, H–C(4)); 7.75 (*d*, H–C(3)); 7.30 (*d*, H–C(4′), H–C(5′)); 7.05–6.52 (*m*, H–C(1′), H–C(2′), H–C(7′), H–C(8′)); 2.28 (*s*, 2 Ac).

2. *3′,6′-Di*-O-*acetyl-4-nitrofluorescein* (= *3′,6′-Bis(acetyloxy)-4-nitrospiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthen]-3-one*; **5**). The mother liquor of **6** was concentrated to a smaller volume to give a second fraction of **6** (2 g). The filtrate was evaporated and the resulting syrup dissolved in hot toluene (200 ml). On cooling, **5** separated as a crystalline solid, which was again recrystallized from toluene: 15 g (25%) of **5**. M.p. 191°. TLC (toluene/AcOEt 7:1): R_f 0.51.

3. *5-Nitrofluorescein* (= *3′,6′-Dihydroxy-5-nitrospiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthen]-3-one*; **4**). Compound **6** (10 g, 20.4 mmol) was heated in a mixture of 10% NaOH (100 ml) and MeOH (500 ml) till a clear soln. was obtained (15 min). The warm soln. was diluted with H_2O (150 ml), acidified with AcOH (50 ml), heated again to boiling, and then slowly cooled and kept in the icebox overnight. The orange precipitate was collected and dried: 7.3 g (95%) of **4**. M.p. >250° (dec.). ^{1}H-NMR ((D_6)DMSO): 10.20 (br. *s*, 2 OH); 8.70 (*d*, H–C(6)); 8.62 (*dd*, H–C(4)); 7.65 (*d*, H–C(3)); 6.80–6.50 (*m*, H–C(1′), H–C(2′), H–C(4′), H–C(5′), H–C(7′), H–C(8′)).

4. *5-Aminofluorescein* (= *5-Amino-3′,6′-dihydroxyspiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthen]-3-one*; **7**). A mixture of 5-nitrofluorescein (**4**; 1.1 g, 3 mmol), $Na_2S \cdot 9\,H_2O$ (2.6 g, 11 mmol), and NaSH (1.2 g, 21 mmol) was heated in H_2O (45 ml) for 24 h under reflux. After cooling, the mixture was acidified with AcOH and the resulting dark precipitate collected. The solid was dissolved in 6% HCl soln. (50 ml), insoluble sulfur and charcoal were filtered off, and the filtrate was kept in the icebox overnight for crystallization. The precipitate

was again dissolved in 0.5N NaOH (40 ml) and then AcOH added to adjust the pH to 4. The precipitate was collected and dried: 0.8 g (77%) of **7**. UV (pH 9): 237 (4.63), 257 (4.29), 281 (4.12), 314 (3.78), 378 (3.70), 488 (4.87). ^{1}H-NMR ((D$_6$)DMSO): 10.05 (br. *s*, 2 OH); 7.00–6.80 (*m*, H–C(3), H–C(4), H–C(6)); 6.65–6.50 (*m*, 6 arom. H (xan)); 5.70 (*s*, NH$_2$).

5. *2-(6-Hydroxy-3-oxo-3*H*-xanthen-9-yl)-5-nitrobenzoic Acid Methyl Ester* (**8**). A mixture of **6** (1 g, 2.2 mmol), MeOH (30 ml), and conc. H_2SO_4 soln. (1 ml) was heated for 40 h under reflux. The yellow soln. was diluted with Et_2O to crystallize slowly by standing overnight in the icebox. The precipitate (0.86 g, 80%) consisted of the hydrogensulfate salt. Anal. calc. for $C_{21}H_{13}NO_7 \cdot H_2SO_4$ (489.4): C 51.54, H 3.09, N 2.86; found: C 52.35, H 3.40, N 2.53.

The hydrogensulfate salt (0.5 g) was suspended in MeOH (20 ml), then H_2O (20 ml) was added, the pH adjusted to 5 by addition of little Na_2HPO_4 soln., and the mixture stirred for 15 min. The dark red precipitate was collected and dried: 0.36 g (94%) of **8**. ^{1}H-NMR ((D$_6$)DMSO): 8.90 (*d*, H–C(6)); 8.72 (*dd*, H–C(4)); 8.70–8.00 (br. *s*, OH); 7.82 (*d*, H–C(3)); 7.10–6.80 (*m*, H–C(1′), H–C(2′), H–C(4′), H–C(5′), H–C(7′), H–C(8′)); 3.65 (*s*, MeO).

6. *5-Amino-2-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)benzoic Acid Ethyl Ester* (**9**). A mixture of **7** (3 g, 8.6 mmol), MeOH (60 ml), and conc. H_2SO_4 soln. (1 ml) was heated for 40 h under reflux. The soln. was poured on ice (300 g), and then, under stirring, a sat. $NaHCO_3$ soln. was slowly added to adjust the pH to 6.5. An orange precipitate separated, which was collected by centrifugation. The solid was washed twice with H_2O, centrifuged, and then heated in MeOH (100 ml) to boiling. After cooling, the solid was collected and dried under high vacuum at 50°: 2.2 g (70%) of **9**. M.p. 284° (dec.). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.28. UV (pH 9): 221 (4.63), 237 (4.70), 257 (4.41), 280 (4.15), 314 (3.94), 3.74 (3.76), 457 (4.40), 492 (4.91). pK_a Values: 1.61, 3.09, 5.99. ^{1}H-NMR ((D$_6$)DMSO): 11.0 (br. *s*, OH); 7.35 (*d*, H–C(6)); 7.05–6.52 (*m*, H–C(3), H–C(4), 6 arom. H (xan)); 5.90 (br. *s*, NH$_2$); 3.49 (*s*, MeO). Anal. calc. for $C_{21}H_{15}NO_5$ (361.4): C 69.80, H 4.18, N 3.88; found: C 69.17, H 4.33, N 3.52.

7. *2′,3′,5′-Tri-*O*-acetyl-*N^6*-[(fluorescein-5-ylamino)carbonyl]adenosine* (=*2′,3′,5′-Tri-*O*-acetyl-*N^6*-{[(3′,6′-dihydroxy-3-oxospiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthen]-5-yl)amino]carbonyl}adenosine*; **11**). A mixture of 2′,3′,5′-tri-*O*-acetyl-*N*^{6}-(phenoxycarbonyl)adenosine (**10**) [11] (1 g, 2 mmol) and **7** (0.347 g, 1 mmol) was heated in pyridine (10 ml) to 70° for 1 h with stirring. After evaporation and co-evaporation with toluene, the residue was dissolved in a little AcOEt and submitted to FC (silica gel, 11 × 3.5 cm; toluene (100 ml), toluene/AcOEt 4:1 (200 ml), toluene/AcOEt 1:1 (200 ml), toluene/AcOEt 1:1 + MeOH (10 ml) (200 ml). The product fraction was concentrated to a smaller volume, whereby **11** separated from toluene. The solid was collected and dried: 0.78 g (99%) of crude **11**, which was pure enough for further reactions. The substance was recrystallized from ^{i}PrOH: 0.61 g (79%) of **11**. Colorless powder. M.p. 188–190° (dec.). TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.16. UV (pH 9): 238 (4.69), 280 (4.62), 317 (3.96), 368 (sh., 3.70), 455 (4.35), 492 (4.91). pK_a Values: 1.07, 1.71, 4.92, 6.55, 12.2. ^{1}H-NMR ((D$_6$)DMSO): 12.1 (*s*, NH); 10.5 (*s*, H–N(6); 10.1 (*s*, 2OH); 8.74 (*s*, H–C(2)); 8.70 (*s*, H–C(8)); 8.33 (*dd*, H–C(6)(flu)); 7.93 (*dd*, H–C(4)(flu)); 7.25 (*d*, H–C(3)(flu)); 6.67–6.52 (*m*, 6 arom. H (xan)); 6.32 (*d*, H–C(1′)); 6.04 (*t*, H–C(2′)); 5.64 (*t*, H–C(3′)); 4.43 (*m*, 2 H–C(5′, H–C(5″)); 4.28 (*m*, H–C(4′)); 2.12, 2.04, 202 (3 *s*, 3 Ac). Anal. calc. for $C_{37}H_{30}N_6O_{13}$ (766.7): C 57.97, H 3.94, N 10.96; found: C 57.27, H 4.49, N 11.11.

8. *N^6-[(Fluorescein-5-ylamino)carbonyl]adenosine* (=*N^6-{[(3′,6′-Dihydroxy-3-oxospiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthen]-5-yl)amino]carbonyl}adenosine*; **12**). A soln. of **11** (0.6 g, 0.78 mmol), K_2CO_3 (0.2 g), and Bu_4NBr (50 mg) in MeOH (20 ml) was stirred at r.t. for 3.5 h and then diluted with H_2O (100 ml) and acidified with AcOH. The formed solid was collected by suction through a glass frit, washed with H_2O, and treated with EtOH to give a gelatinous precipitate, which was again collected and dried under high vacuum at 50°: 0.39 g (78%) of **12**. M.p. 207° (dec.). TLC (^{i}PrOH/conc. NH_3 soln./H_2O 7:1:2): R_f 0.46. UV (MeOH): 238 (4.67), 261 (sh., 4.44), 281 (4.61), 317 (3.95), 344 (sh., 4.36), 456 (sh., 4.36), 490 (4.89). ^{1}H-NMR ((D$_6$)DMSO): 12.2 (*s*, NH); 10.4 (br. *s*, H–N(6), 2 OH); 8.70 (*s*, H–C(2), H–C(8)); 8.30 (*dd*, H–C(6)(flu)); 7.90 (*dd*, H–C(4)(flu)); 7.21 (*d*, H–C(3)(flu)); 6.8–6.4 (*m*, 6 arom. H (xan)); 6.0 (*d*, H–C(1′)); 5.7–5.0 (*m*, H–C(2′), H–C(3′), OH); 3.91 (*m*, H–C(4′)); 3.60 (*m*, H–C(5′)). Anal. calc. for $C_{31}H_{24}N_6O_{10} \cdot 2\ H_2O$ (640.6): C 55.03, H 4.17, N 12.42; found: C 55.46, H 4.44, N 12.21.

9. *2′,3′,5′-Tri-*O*-acetyl-*N^6*-{[[4-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)-3-(methoxycarbonyl)phenyl]amino}carbonyl]adenosine* (**13**). A mixture of 2′,3′,5′-tri-*O*-acetyl-*N*^{6}-(phenoxycarbonyl)adenosine (**10**) [11] (2 g, 4 mmol) and **9** (0.347 g, 1 mmol) was heated in abs. dioxane to 70° for 1.5 h with stirring. After cooling and standing overnight, a precipitate separated and was collected and dried to give 1.5 g (96%) of almost pure material, which was recrystallized from dioxane (60 ml): 1.2 g (75%) of **13**. M.p. 190° (dec.). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.45. UV (pH 9): 238 (4.73), 280 (4.65), 317 (4.08), 356 (sh., 3.74), 460 (4.40), 492 (4.91). pK_a Values: 0.96, 2.48, 6.57,

11.9. ^{1}H-NMR ((D$_6$)DMSO): 12.1 (*s*, NH); 11.2 (br. *s*, OH); 10.5 (*s*, H–N(6); 10.1; 8.75 (*s*, H–C(2)); 8.71 (*s*, H–C(8)); 8.54 (*dd*, H–C(6)(flu)); 7.99 (*dd*, H–C(4)(flu)); 7.45 (*d*, H–C(3)(flu)); 6.89–6.52 (*m*, 6 arom. H (xan); 6.33 (*d*, H–C(1′)); 6.05 (*t*, H–C(2′)); 5.64 (*t*, H–C(3′)); 4.44 (*m*, 2 H–C(5′)); 4.28 (*m*, H–C(4′)); 3.59 (s, MeO); 2.12, 2.04, 202 (3 *s*, 3 Ac).

10. N^6-*{[[4-(6-Hydroxy-3-oxo-3*H*-xanthen-9-yl)-3-(methoxycarbonyl)phenyl]amino}carbonyl]adenosine* (**14**). A soln. of **13** (0.6 g, 0.77 mmol), K_2CO_3 (0.2 g), and Bu_4NBr (50 mg) in MeOH (30 ml) was stirred at r.t. for 2.5 h, then diluted with H_2O (100 ml), acidified with a few drops of AcOH, and concentrated to 20 ml. The resulting precipitate was collected, then stirred in MeOH for 2 h, filtered again, and dried under high vacuum: 0.38 g (73%) of **14**. M.p. 208–209°. TLC (^{i}PrOH/conc. NH_3 soln./H_2O 7:1:2): R_f 0.57. UV (pH 9): 238 (4.72), 258 (sh., 4.48), 280 (4.64), 316 (4.08), 348 (sh., 3.81), 460 (sh., 4.40), 494 (4.91). ^{1}H-NMR ((D$_6$)DMSO): 12.1 (*s*, NH); 10.4 (br. *s*, H–N(6), OH); 8.70 (*s*, H–C(2), H–C(8)); 8.5 (*dd*, H–C(6)(flu)); 8.00 (*dd*, H–C(4)(flu)); 7.51 (*d*, H–C(3)(flu)); 7.5–7.0 (*m*, 6 arom. H (xan); 6.05 (*d*, H–C(1′)); 5.7–5.0 (*m*, H–C(2′), H–C(3′, OH); 3.99 (*m*, H–C(4′)); 3.52 (*m*, 2 H–C(5′)). Anal. calc. for $C_{32}H_{26}N_6O_{10}\cdot H_2O$ (654.6): C 57.14, H 4.10, N 12.49; found: C 57.36, H 4.32, N 12.91.

11. *2′,3′,5′-Tri*-O-*acetyl*-N^4*-(phenoxycarbonyl)cytidine* (**15**). A mixture of 2′,3′,5′-tri-*O*-acetylcytidine (0.74 g, 2 mmol) [29] and 1-(phenoxycarbonyl)-1*H*-tetrazole (2.5 g, 13 mmol) in dioxane (10 ml) was stirred at 40° for 30 min. After evaporation, the residue was purified by FC (12 × 2.5 cm; toluene (50 ml), toluene/acetone 9:1 (200 ml), toluene/acetone 4:2 (600 ml); 50-ml fractions). *Fr. 13–16* gave 0.6 g (61%) of **15**. Colorless solid foam. TLC (toluene/acetone 1:1): R_f 0.5. UV (MeOH): 243 (4.28), 264 (3.89). ^{1}H-NMR ((D$_6$)DMSO): 11.4 (br. *s*, NH); 8.14 (*d*, H–C(6)); 7.47–7.17 (*m*, Ph); 7.06 (*d*, H–C(5)); 5.90 (*d*, H–C(1′)); 5.49 (*dd*, H–C(2′)); 5.36 (*t*, H–C(3′)); 4.38–4.17 (*m*, H–C(4′), 2 H–C(5′)); 2.07, 2.06 2.03 (3 *s*, 3 Ac). Anal. calc. for $C_{22}H_{23}N_3O_{10}$ (489.4): C 53.99, H 4.74, N 8.59; found: C 54.08. H 4.71, N 9.02.

12. *2′,3′,5′-Tri*-O-*acetyl*-N^4*-[(fluorescein-5-ylamino)carbonyl]cytidine* (=*2′,3′,5′-Tri*-O-*acetyl*-N^4*-[[(3′,6′-dihydroxy-3-oxospiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthen]-5-yl)amino]carbonyl]cytidine*; **16**). A mixture of **7** (0.52 g, 1.5 mmol) and **15** (1.2 g, 2.4 mmol) in pyridine (10 ml) was heated to 70° for 3 h. Stirring was continued overnight, the mixture evaporated and co-evaporated with toluene, and the residue dissolved in CH_2Cl_2/MeOH and submitted to FC (13 × 3.5 cm; CH_2Cl_2 (100 ml), CH_2Cl_2/MeOH 97:3 (200 ml), CH_2Cl_2/MeOH 95:5 (100 ml), CH_2Cl_2/MeOH 93:7 (200 ml), CH_2Cl_2/MeOH 9:1 (300 ml; 50-ml fractions). *Fr. 14–18* were evaporated and co-evaporated with toluene and then the residue dried to give 1.1 g (97%) of crude material. A sample (0.3 g) was heated in acetone (50 ml), the soln. filtered, and then Et_2O (30 ml) slowly added with stirring. The yellow precipitate was collected and dried under high vacuum at 50°: 0.26 g (85%) of pure **16**. TLC (CH_2Cl_2/MeOH 4:1): R_f 0.63. UV (pH 9): 238 (4.73), 260 (sh., 4.40), 288 (4.89), 316 (4.14), 348 (sh., 3.88), 428 (sh., 3.70), 456 (sh., 4.38), 491 (4.91). ^{1}H-NMR ((D$_6$)DMSO): 11.6 (br. *s*, NH); 10.5 (s, NH); 10.1 (s, OH); 8.22 (*d*, H–C(6)(flu)); 8.05 (*d*, H–C(6)); 7.67 (*m*, H–C(4)(flu)); 7.24 (*m*, H–C(3)(flu), H–C(5)); 6.67–6.48 (*m*, 6 arom. H (xan)); 5.91 (*d*, H–C(1′)); 5.50 (*dd*, H–C(2′)); 5.37 (*t*, H–C(3′)); 4.37–4.19 (*m*, H–C(4′), 2 H–C(5′)); 2.06, 2.04 (2 *s*, 3 Ac). Anal. calc. for $C_{36}H_{30}N_4O_{14}$ (742.7): C 58.22, H 4.07, N 7.54; found: C 57.59, H 4.25, N 7.46.

13. *2′,3′,5′-Tri*-O-*acetyl*-N^4*-{[[4-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)-3-(methoxycarbonyl)phenyl]amino}carbonyl]cytidine* (**17**). A mixture of **9** (0.2 g, 0.55 mmol) and **15** (0.5 g, 1 mmol) in pyridine (10 ml) was heated to 70° for 2 h. The soln. was evaporated and co-evaporated with toluene, the residue treated with warm dioxane by ultrasound, collected, and then dissolved in dioxane/MeOH 1:1 (50 ml). The soln. was filtered hot and MeOH carefully removed until a precipitate formed. After standing overnight, the solid was collected and dried: 0.26 g (63%) of **17**. Yellow powder. M.p. 188–190° (dec.). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.45. UV (H_o – 1.54): 226 (4.76), 249 (4.59), 310 (4.54), 376 (3.91), 439 (4.74). UV (pH 1): 223 (4.75), 248 (4.60), 297 (4.44), 360 (sh., 3.64), 439 (4.72). UV (pH 4): 216 (4.67), 232 (4.68), 256 (sh., 4.46), 275 (4.46), 292 (sh., 4.42), 372 (3.99), 458 (4.42), 474 (4.41). UV (pH 8): 216 (4.71), 238 (4.77), 259 (sh., 4.46), 288 (4.47), 318 (sh., 4.19), 346 (sh., 3.85), 434 (sh., 3.81), 464 (sh., 4.46), 496 (4.93). UV (pH 13): 219 (4.63), 239 (4.76), 258 (sh., 4.45), 288 (4.45), 309 (4.55), 313 (sh., 4.45), 365 (sh., 3.89), 434 (sh., 3.81), 464 (sh., 4.50), 494 (4.95). pK_a Values: 0.40, 2.79, 6.13, 11.33. ^{1}H-NMR ((D$_6$)DMSO): 11.6 (br. *s*, NH); 11.1 (*s*, OH); 10.5 (*s*, NH); 8.34 (*m*, H–C(6)(flu)); 8.08 (*d*, H–C(6)); 7.88 (*m*, H–C(4)(flu)); 7.43 (*m*, H–C(3)(flu)); 6.87–6.52 (*m*, H–C(5), 6 arom. H (xan); 5.92 (*d*, H–C(1′)); 5.50 (*dd*, H–C(2′)); 5.37 (*t*, H–C(3′)); 4.37–4.22 (*m*, H–C(4′, 2 H–C(5′)); 3.59 (*s*, MeO); 2.07, 2.05 (2 *s*, 3 Ac). Anal. calc. for $C_{37}H_{32}N_4O_{14}$ (756.7): C 58.73, H 4.26, N 7.40; found: C 58.59, H 4.24, N 6.96.

14. *3′,5′-Di*-O-*acetyl-2′-deoxy*-O^6*-[2-(4-nitrophenyl)ethyl]guanosine* (**18**). A mixture of 3′,5′-di-*O*-acetyl-2′-deoxyguanosine [30] (0.702 g, 2 mmol), PPh_3 (0.84 g, 3.2 mmol), and 2-(4-nitrophenyl)ethanol (0.501 g, 3 mmol) in dry dioxane (40 ml) was stirred for 5 min. Then, diethyl azodicarboxylate (0.558 g, 3.2 mmol) was added and stirred vigorously for 1 h at r.t. (→ clear soln.). The soln. was evaporated and the residue treated

with Et_2O to remove triphenylphosphine oxide. The resulting solid was recrystallized from MeOH: 0.78 g (78%) of **18**. Colorless crystals. M.p. 124°. ^{1}H-NMR ((D_6)DMSO): 8.17 (*d*, 2 H, *o* to NO_2); 8.06 (*s*, H–C(8)); 7.62 (*d*, 2 H, *m* to NO_2); 6.52 (*s*, NH_2); 6.22 (*dd*, H–C(1′)); 5.30 (*d*, H–C(3′)); 4.66 (*t*, CH_2); 4.30–4.16 (*m*, H–C(4′), 2 H–C(5′)); 3.24 (*t*, CH_2); 3.06–2.94 (*m*, H–C(2′)); 2.45 (*m*, 1 H–C(2′)); 2.07 (*s*, 1 Ac); 2.01 (*s*, 1 Ac). Anal. calc. for $C_{22}H_{24}N_6O_8$ (500.5): C 52.80, H 4.83, N 16.79; found: C 52.73, H 4.87, N 16.70.

15. *3′,5′-Di*-O-*acetyl-2′-deoxy*-O^6-*[2-(4-nitrophenyl)ethyl]*-N^2-*(phenoxycarbonyl)guanosine* (**19**). A soln. of **18** (0.751 g, 0.67 mmol) in abs. pyridine (10 ml) was cooled to 0°, and then phenyl carbonochloridate (260 µl, 1 mmol) was added dropwise within 4 min. The mixture was kept 30 min at 0° and another 30 min at r.t., then diluted with CH_2Cl_2 (70 ml), and washed with sat. $NaHCO_3$ soln. The org. layer was dried ($MgSO_4$), evaporated and co-evaporated with toluene and the residue dissolved in CH_2Cl_2 and submitted to FC (silica gel, 2.5 × 10 cm; toluene (20 ml), toluene/AcOEt 7:3 (200 ml), toluene/AcOEt 3:2 (200 ml), toluene/AcOEt 1:1 (200 ml), toluene/AcOEt 2:3 (200 ml), and toluene/AcOEt/MeOH 5:4:1 (200 ml); 50-ml fractions). *Fr. 6–19* gave 0.735 g (79%) of **19**. Colorless solid. TLC (CH_2Cl_2/AcOEt/MeOH 10:9:1): R_f 0.45. ^{1}H-NMR ((D_6)DMSO): 10.95 (br. *s*, NH); 8.40 (*s*, H–C(8)); 8.16 (*m*, 2 H *o* to NO_2); 7.60–7.20 (*m*, Ph, 2 H *m* to NO_2); 6.32 (*t*, H–C(1′)); 5.40 (*m*, H–C(3′)); 4.74 (*t*, CH_2CH_2O); 4.36 (*m*, H–C(4′), 2 H–C(5′)); 3.30 (*m*, 1 H–C(2′), CH_2CH_2O); 2.49 (*m*, 1 H–C(2′)); 2.06, 1.95 (2 *s*, 2 Ac). Anal. calc. for $C_{29}H_{28}N_6O_{10}\cdot 0.5$ toluene (666.6): C 58.26, H 4.78, N 12.12; found: C 58.55, H 4.84, N 12.61.

16. *3′,5′-Di*-O-*acetyl-2′-deoxy*-N^2-*{[(3′,6′-dihydroxy-3-oxospiro[isobenzofuran-1(3*H*),9′-[9H]xanthen]-5-yl)amino]carbonyl}*-O^6-*[2-(4-nitrophenyl)ethyl]guanosine* (**20**). A mixture of **7** (0.431 g, 1.3 mmol) and **19** (1.2 g, 1.9 mmol) was heated in pyridine (10 ml) to 70° for 2 h. After evaporation and co-evaporation with toluene, the residue was dissolved in CH_2Cl_2/MeOH and purified by FC (silica gel (30 g); CH_2Cl_2 (250 ml), CH_2Cl_2/MeOH 49:1 (200 ml), CH_2Cl_2/MeOH 95:5 (200 ml), CH_2Cl_2/MeOH 9:1 (50-ml fractions; 500 ml). *Fr. 10–15* gave a residue that was treated with MeOH (10 ml). Then the solid was collected and dried: 0.87 g (76%) of **20**. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.64. ^{1}H-NMR ((D_6)DMSO): 11.4 (br. *s*, NH); 10.3 (*s*, NH); 10.1 (*s*, 2 OH); 8.40 (*s*, H–C(8)); 8.32 (*d*, H–C(6)); 8.11 (*d*, 2 H *o* to NO_2); 7.75 (*dd*, H–C(4)); 7.65 (*d*, 2 arom. H *m* to NO_2); 7.19 (*d*, H–C(3)(flu)); 6.67–6.53 (*m*, 6 arom. H (xan); 6.42 (*t*, H–C(1′)); 5.35 (*m*, H–C(3′)); 4.85 (*t*, CH_2CH_2O); 4.36–4.10 (*m*, H–C(4′), 2 H–C(5′)); 3.30 (*m*, CH_2CH_2O); 3.13 (*m*, H–C(2′)); 2.60 (*dd*, 1 H–C(2′)); 1.95, 1.90 (2 *s*, 2 Ac). Anal. calc. for $C_{43}H_{35}N_7O_{14}\cdot 0.5\ H_2O$ (882.8): C 58.50, H 4.11, N 11.11; found: C 58.54, H 4.32, N 11.22.

17. *2′-Deoxy*-N^6,*3′*-O-*bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**21**) [23].

18. *2′-Deoxy*-N^6-*[(9*H*-fluoren-9-ylmethoxy)carbonyl]-5′*-O-*(monomethoxytrityl)adenosine 3′-[2-(4-Nitrophenyl)ethyl Diisopropylphosphoramidite]* (**22**). A soln. of 2′-deoxy-*N*^{6}-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-5′-*O*-(monomethoxytrityl)adenosine (**24**; 0.53 g, 0.7 mmol) in CH_2Cl_2 (10 ml) and Et^iPr_2N (0.6 ml) was treated under N_2 with 2-(4-nitrophenyl)ethyl diisopropylphosphoramidochloridite (0.6 g, 1.8 mmol), and the mixture stirred at r.t. for 1.5 h. The orange soln. was diluted with $CHCl_3$ (300 ml), washed with phosphate buffer pH 7 (70 ml), dried ($MgSO_4$), and evaporated, and the resulting brown oil dissolved in a little CH_2Cl_2 and purified by CC (Alox (neutral; 60 g), hexane (50 ml), hexane/acetone 19:1 (200 ml), hexane/acetone 9:1 (200 ml), hexane/acetone 17:3 (200 ml), hexane/acetone 15:5 (200 ml), hexane/acetone 1:1 (200 ml; 50-ml fractions)). *Fr. 18–28* gave 0.4 g (55%) of **22**. TLC (Alox, hexane/acetone 4:1): R_f 0.45. UV (MeOH): 226 (4.45), 237 (4.39), 256 (4.59), 265 (4.67), 270 (4.62), 288 (4.12), 298 (4.01). ^{1}H-NMR ($CDCl_3$): 8.71 (*s*, H–C(2)); 8.47 (*s*, NH); 8.13–8.07 (*m*, H–C(8), 2 H *o* to NO_2); 7.78–7.63 (*m*, all fmoc, H–C(1), H–C(4), H–C(5), H–C(8)); 7.43–7.16 (*m*, H–C(2), H–C(3), H–C(6), H–C(7) (all fmoc), 10 arom. H, 2 H *m* to NO_2, 2 H *o* to MeO); 6.78 (*d*, 2 H *m* to MeO); 6.45 (*d*, H–C(1′)); 4.70 (*m*, H–C(3′)); 4.62 (*d*, CH_2–C(9)(fmoc)); 4.32 (*m*, H–C(9), H–C(4′)(fmoc)): 3.90–3.71 (*m*, CH_2CH_2O); 3.78 (*s*, MeO); 3.52 (*m*, Me_2CH); 3.37 (*m*, 2 H–C(5′)); 3.00, 2.90 (2 *t*, CH_2CH_2O); 2.80–2.57 (*m*, 2 H–C(2′)); 1.10 (*m*, 2 Me_2CH). Anal. calc. for $C_{59}H_{60}N_7O_9P$ (1042.2): C 68.00, H 5.80, N 9.41; found: C 66.97, H 6.02, N 9.02.

19. *2′-Deoxy-5′*-O-*(dimethoxytrityl)*-N^6-*[(9*H*-fluoren-9-ylmethoxy)carbonyl]adenosine 3′-[2-(4-Nitrophenyl)ethyl 4-Diisopropylphosphoramidite]* (**23**). As described for **22**, with 2′-Deoxy-5′-*O*-(dimethoxytrityl)-[*N*^{6}-[(9*H*-(fluoren-9-ylmethoxy)carbonyl]adenosine (**26**; 3 g, 3.9 mmol), CH_2Cl_2 (20 ml), Et^iPr_2N (3 ml) and 2-(4-nitrophenyl)ethyl diisopropylphosphoramidochloridite (3 g, 9 mmol). CC (Alox (150 g), hexane (100 ml), hexane/acetone 19:1 (400 ml), hexane/acetone 9:1 (400 ml), hexane/acetone 17:3 (400 ml), hexane/acetone 4:1 (400 ml), hexane/acetone 3:1 (400 ml), hexane/acetone 1:1 (200 ml); 50-ml fractions). *Fr. 26–40* gave 2.9 g (70%) of **23**. TLC (Alox, hexane/acetone 4:1): R_f 0.45. UV (MeOH): 226 (4.49), 238 (4.49), 254 (4.59), 265 (4.67), 270 (4.64), 288 (4.13), 298 (4.00). ^{1}H-NMR ($CDCl_3$): 8.71 (*s*, H–C(2)); 8.32 (*s*, NH); 8.13–8.07 (*m*, H–C(8), 2 H *o* to NO_2); 7.78–7.63 (*m*, H–C(1), H–C(4), H–C(5), H–C(8)(all fmoc)); 7.44–7.15 (*m*, H–C(2), H–C(3), H–C(6), H–C(7) (all fmoc), 2 H *m* to NO_2, 4 H *m* to MeO); 6.74 (*d*, 4 H *o* to MeO);

6.45 (*d*, H–C(1′)); 4.70 (*m*, H–C(3′)); 4.62 (*d*, CH_2–C(9)(fmoc)); 4.32 (*m*, H–C(9), H–C(4′)): 3.90–3.71 (*m*, CH_2CH_2O); 3.76 (2 *s*, 2 MeO); 3.55–3.46 (*m*, Me_2CH); 3.41–3.27 (*m*, 2 H–C(5′)); 3.00, 2.90 (2 *t*, CH_2CH_2O); 2.80–2.57 (*m*, 2 H, H–C(2′, 2″)); 1.10 (*m*, 2 Me_2 CH). Anal. calc. for $C_{60}H_{61}N_7O_{10}P$ (1072.2): C 67.22, H 5.83, N 9.15; found: C 66.83, H 5.91, N 9.03.

20. *2′-Deoxy-*N^6*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]adenosine* (**24**). According to [31]. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.25. UV (MeOH): 226 (4.00), 254 (4.46), 264 (4.57), 274 (4.43), 288 (3.72), 299 (3.74). ^{1}H-NMR ((D$_6$)DMSO): 10.9 (*s*, NH); 8.67 (*s*, H–C(2)); 8.65 (*s*, H–C(8)); 7.90–7.30 (*m*, 8 H (fmoc)); 6.45 (*d*, H–C(1′)); 5.36 (*d*, OH–C(3′)); 5.03 (*t*, OH–C(5′)); 4.42–4.27 (*m*, H–C(3′), H–C(9)(fmoc), CH_2–C(9)); 3.88 (*q*, H–C(4′)): 3.67–3.49 (*m*, 2 H–C(5′)); 2.76 (*m*, H–C(2′)); 2.32 (*m*, 1 H–C(2′)). Anal. calc. for $C_{25}H_{23}N_5O_5$ (473.5): C 63.42, H 4.90, N 14.79; found: C 63.04, H 5.06, N 14.65.

21. *2′-Deoxy-*N^6*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-5′-*O*-(monomethoxytrityl)adenosine* (**25**) [31]. A mixture of **24** (1.42 g, 3 mmol; co-evaporated with pyridine) and monomethoxytrityl chloride (1.2 g, 4 mmol) in pyridine (50 ml) was stirred at r.t. for 18 h. The soln. was concentrated to 10 ml, diluted with AcOEt (200 ml), washed with phosphate buffer pH 7, dried ($MgSO_4$), evaporated, and co-evaporated with toluene. The residue was dissolved in a little CH_2Cl_2 and submitted to FC (silica gel (50 g); toluene (100 ml), toluene/AcOEt 1:1 (300 ml), toluene/AcOEt 1:1 (295 ml) + MeOH (5 ml), toluene/AcOEt 1:1 (290 ml) + MeOH (10 ml); 50-ml fractions. *Fr. 13–27* gave 1.8 g (80%) of **25**. Colorless solid foam. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.50. UV (MeOH): 227 (4.34), 236 (4.30), 254 (4.46), 265 (4.55), 271 (4.47), 287 (3.73), 299 (3.70). ^{1}H-NMR ((D$_6$)DMSO): 10.9 (*s*, NH); 8.57 (*s*, H–C(2)); 8.56 (*s*, H–C(8)); 7.90–7.83 (*m*, H–C(1), H–C(4), H–C(5), H–C(8)(all fmoc)); 7.44–7.09 (*m*, H–C(2), H–C(3), H–C(6), H–C(7)(all fmoc), 12 arom. H); 6.79 (*d*, 2 H *o* to MeO); 6.48 (*d*, H–C(1′)); 5.43 (*d*, OH–C(3′)); 4.52 (*m*, H–C(3′); 4.42–4.30 (*m*, H–C(9)(fmoc), CH_2–C(9)(fmoc)); 4.04 (*m*, H–C(4′)): 3.69 (*s*, MeO); 3.19 (*m*, 2 H–C(5′)); 2.94 (*m*, H–C(2′)); 2.40 (*m*, 1 H–C(2′)). Anal. calc. for $C_{45}H_{39}N_5O_6 \cdot 0.5$ toluene (491.3): C 73.56, H 5.47, N 8.84; found: C 73.35, H 5.51, N 8.86.

22. *2′-Deoxy-*N^6*-5′-*O*-(dimethoxytrityl)-[(9*H*-fluoren-9-ylmethoxy)carbonyl]adenosine* (**26**). As described for **25**, with **24** (3.5 g, 7.4 mmol), dimethoxytrityl chloride (3 g, 9 mmol), and pyridine (80 ml). Purification by FC (silica gel (80 g); toluene (100 ml), toluene/AcOEt 1:1 (500 ml), toluene/AcOEt 1:1 (490 ml) + MeOH (10 ml), toluene/AcOEt 1:1 (480 ml) + MeOH (20 ml); 100-ml fractions). *Fr. 12–18* gave 4.7 g (82%) of **26**. Yellowish solid foam. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.48. UV (MeOH): 226 (4.41), 237 (4.42), 254 (4.47), 265 (4.55), 270 (4.48), 288 (3.73), 299 (3.71). ^{1}H-NMR ((D$_6$)DMSO): 10.9 (*s*, NH); 8.58 (*s*, H–C(2)); 8.57 (*s*, H–C(8)); 7.90–7.83 (*m*, H–C(1), H–C(4), H–C(5), H–C(8)(all fmoc)); 7.44–7.09 (*m*, H–C(2), H–C(3), H–C(6), H–C(7)(all fmoc), 9 arom. H); 6.79 (*m*, 4 H *o* to MeO); 6.47 (*d*, H–C(1′)); 5.41 (*d*, OH–C(3′)); 4.52 (*m*, H–C(3′); 4.41–4.27 (*m*, H–C(9)(fmoc), CH_2–C(9)(fmoc)); 4.02 (*m*, H–C(4′)): 3.69 (2 *s*, 2 MeO); 3.18 (*m*, 2 H–C(5′)); 2.94 (*m*, H–C(2′)); 2.40 (*m*, 1 H–C(2′)). Anal. calc. for $C_{46}H_{41}N_5O_7 \cdot 0.5$ toluene (821.4): C 72.33, H 5.52, N 8.52; found: C 72.71, H 5.61, N 8.33.

23. *2′-Deoxy-*N^6*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-5′-O-(monomethoxytrityl)adenylyl-{3′-{*O^P*-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy-*N^6*,3′-*O*-bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**27**). To a mixture of **22** (0.5 g, 0.5 mmol) and **21** (0.32 g, 0.5 mmol) in MeCN (10 ml) and CH_2Cl_2 (3 ml) under Ar was added under stirring 1*H*-tetrazole (0.21 g, 3 mmol). Stirring was continued for 1.5 h at r.t. Then I_2 (0.5 g) in pyridine/CH_2Cl_2/H_2O 5:1:1 (7 ml) was added and the mixture stirred for 15 min. After dilution with $CHCl_3$ (300 ml), the soln. was decolorized by washing with a $Na_2S_2O_3$/NaCl soln., dried ($MgSO_4$), and evaporated. The resulting oil was purified by FC (silica gel (20 g), toluene (50 ml), toluene/acetone 9:1 (100 ml), toluene/acetone 4:1 (100 ml), toluene/acetone 7:3 (100 ml), toluene/acetone 3:2 (100 ml), toluene/acetone 1:1 (200 ml), toluene/acetone 2:3 (100 ml), toluene/acetone 3:7 (100 ml; 50-ml fractions). *Fr. 12–17* were evaporated and co-evaporated with CH_2Cl_2: 0.55 g (70%) of **27**. Solid foam. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.24. UV (MeOH): 224 (4.56), 237 (4.52), 256 (4.80), 265 (4.90), 276 (4.79), 289 (4.41), 299 (4.22). ^{1}H-NMR ((D$_6$)DMSO): 10.9 (*s*, NH); 10.6 (*s*, NH); 8.58–8.45 (4 *s*, H–C(2), H–C(8)); 8.16–7.11 (*m*, 32 arom. H); 6.74 (*m*, 2 H *o* to MeO); 6.40 (*m*, H–C(1′)); 5.35 (*m*, H–C(3′)); 5.15 (*t*, OH–C(5′)); 5.00 (*m*, 1 H–C(3′)); 4.41–4.21 (*m*, 12 H, CH_2CH_2O, H–C(9)(fmoc), CH_2, H–C(4′), H–C(5′)); 4.06 (*m*, H–C(4′)); 3.49 (*m*, 2 H, H–C(5′)); 3.15–2.87 (*m*, 8 H, CH_2CH_2O, H–C(2′)); 2.58–2.50 (*m*, H–C(2′)). Anal. calc. for $C_{81}H_{72}N_{13}O_{21}P \cdot H_2O$ (1594.5): C 60.33, H 4.63, N 11.29; found: C 60.56, H 4.70, N 11.04.

24. *2′-Deoxy-5′-*O*-(dimethoxytrityl)-*N^6*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]adenylyl-{3′-{*O^P*-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy-*N^6*,3′-*O*-bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**28**). As described for **27**, with **23** (2.9 g, 2.7 mmol), **21** (1.4 g, 2.2 mmol), MeCN (10 ml), CH_2Cl_2 (3 ml), 1*H*-tetrazole (0.92 g), and I_2 soln. (15 ml). After FC (silica gel (60 g); toluene (100 ml), toluene/acetone 9:1 (200 ml), toluene/acetone 4:1 (200 ml), toluene/acetone 7:3 (200 ml), toluene/acetone 3:2 (400 ml), toluene/acetone 1:1 (200 ml), toluene/

acetone 2:3 (200 ml), MeOH (100 ml); 50-ml fractions). *Fr. 21–32* were evaporated and co-evaporated with CH_2Cl_2 and EtOH: 2.8 g (78%) of **28**. Solid foam. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.31. UV (MeOH): 238 (4.62), 256 (4.84), 266 (4.92), 272 (4.86), 289 (4.01), 299 (4.23). ^{1}H-NMR ((D$_6$)DMSO): 10.9 (*s*, NH); 10.6 (*s*, NH); 8.59–8.47 (4 *s*, 4 H, H–C(2), H–C(8)); 8.16–7.13 (*m*, 29 arom. H); 6.74 (*m*, 4 H *o* to MeO); 6.41 (*m*, H–C(1′)); 5.31 (*m*, 1 H–C(3′)); 5.15 (*m*, H–C(3′)); 4.40–4.15 (*m*, 13 H, CH_2CH_2O, H–C(9)(fmoc), CH_2, H–C(4′), H–C(5′)); 3.66 (*s*, 2 MeO); 3.18–2.89 (*m*, 10 H, CH_2CH_2O, H–C(2′), H–C(5′)); 2.62–2.48 (*m*, 2 H, H–C(2′)). ^{31}P-NMR ((D$_6$)DMSO): −1.38; −1.45. Anal. calc. for $C_{82}H_{74}N_{13}O_{22}P$ (1624.6): C 60.63, H 4.59, N 11.21; found: C 60.12, H 4.70, N 11.03.

25. *2′-Deoxy*-N^6-*[(9*H*-fluoren-9-ylmethoxy)carbonyl]adenylyl-{3′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy*-N^6,3′-O-*bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**29**). A soln. of **28** (1.0 g, 0.62 mmol) in CH_2Cl_2 (30 ml) containing 1.5% of CF_3COOH was stirred at r.t. for 1 h, then diluted with $CHCl_3$ (300 ml), and washed several times with phosphate buffer pH 7. The org. layer was dried ($MgSO_4$) and evaporated. The residue was dissolved in a little CH_2Cl_2 and purified by FC (silica gel (30 g), toluene (100 ml), toluene/AcOEt 1:1 (200 ml), toluene/AcOEt 1:1 (95 ml) + MeOH (5 ml), toluene/AcOEt 1:1 (90 ml) + MeOH (10 ml); toluene/AcOEt 1:1 (160 ml) + MeOH (40 ml); 100-ml fractions). *Fr. 7* and *8* were evaporated and co-evaporated with CH_2Cl_2: 0.78 g (96%) of **29**. Solid. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.2. UV (CH_2Cl_2): 266 (4.89), 272 (sh., 4.83), 289 (sh., 4.39), 299 (4.21). ^{1}H-NMR ((D$_6$)DMSO): 10.9 (*s*, NH); 10.6 (*s*, NH); 8.63–8.59 (*m*, 4 H–C(2), H–C(8)); 8.18–7.29 (*m*, 20 arom. H); 6.40 (*m*, 2 H, H–C(1′)); 5.35 (*m*, 1 H, H–C(3′)); 5.16 (*m*, 1 H, H–C(3′)); 5.00 (*m*, 1 H, H–C(3′)); 4.41–4.21 (*m*, 12 H, CH_2CH_2O, H–C(9)(fmoc), CH_2, H–C(4′), H–C(5′)); 4.06 (*m*, 1 H, H–C(4′)); 3.50 (*m*, 2 H, H–C(5′)); 3.15–2.87 (*m*, 10 H, CH_2CH_2O, H–C(2′), H–C(5′)); 2.58–2.50 (*m*, 2 H, H–C(2′)). ^{31}P-NMR ((D$_6$)DMSO): −1.45. Anal. calc. for $C_{61}H_{56}N_{13}O_{20}P$ (1322.2): C 55.42, H 4.27, N 13.77; found: C 55.94, H 4.65, N 13.45.

26. *2′-Deoxy-5′*-O-*(monomethoxytrityl)adenylyl-{3′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy*-N^6,3′-O-*bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**30**). A soln. of **27** (0.46 g, 0.29 mmol) in MeCN (8 ml) and Et_3N (2 ml) was stirred at r.t. for 2.5 h. After evaporation, the residue was dissolved in little CH_2Cl_2 and submitted to FC (silica gel (15 g); toluene (50 ml), toluene/AcOEt 1:1 (150 ml), toluene/AcOEt 1:1 (95 ml) + MeOH (5 ml), toluene/AcOEt 1:1 (95 ml) + MeOH (5 ml), toluene/AcOEt 1:1 (90 ml) + MeOH (10 ml), toluene/AcOEt 1:1 (85 ml) + MeOH (15 ml), toluene/AcOEt 1:1 (80 ml) + MeOH (20 ml); 40-ml fractions). *Fr. 8–12* were evaporated and co-evaporated with CH_2Cl_2: 0.36 g (91%) of **29**. Solid foam. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.53. UV (MeOH): 240 (4.57), 266 (4.79). ^{1}H-NMR ((D$_6$)DMSO): 10.6 (*s*, NH); 8.59 (2 *s*, 2 H, H–C(2)); 8.20–8.11 (*m*, 5 H, H–C(8), H *o* to NO_2);); 8.02 (*m*, 3 H, H–C(8), H *o* to NO_2); 7.56 (*m*, 4 H *m* to NO_2); 7.42–7.12 (*m*, 20 H, arom. H, NH_2); 6.75 (*d*, 2 H, H *o* to MeO); 6.40 (*t*, 2 H, H–C(1′)); 6.30 (*t*, 1 H, H–C(1′)); 5.30 (*m*, 1 H, H–C(3′)); 5.10 (*m*, 1 H, H–C(3′)); 4.36–4.13 (*m*, 10 H, CH_2CH_2O, H–C(4′), H–C(5′)); 3.67 (*s*, MeO); 3.17–2.87 (*m*, 10 H, CH_2CH_2O, H–C(2′), H–C(5′)); 2.50 (*m*, 2 H, H–C(2′)); 2.28 (*s*, Me (toluene)). Anal. calc. for $C_{66}H_{62}N_{13}O_{19}P \cdot 0.5$ toluene (1418.4): C 58.86, H 4.69, N 12.84; found: C 58.87, H 4.82, N 12.91.

27. *2′-Deoxy-5′*-O-*(dimethoxytrityl)adenylyl-{3′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy*-N^6,3′-O-*bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**31**). As described for **30**, with **28** (0.74 g, 0.46 mmol), in MeCN (20 ml), and Et_3N (5 ml). After FC (silica gel (20 g); CH_2Cl_2 (100 ml), CH_2Cl_2/MeOH 95:5 (100 ml), CH_2Cl_2/MeOH 9:1 (100 ml), CH_2Cl_2/MeOH 85:15 (100 ml); 50-ml fractions). *Fr. 5–7* gave 0.58 g (91%) of **31**. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.53. UV (MeOH): 240 (4.57), 266 (4.79). ^{1}H-NMR ((D$_6$)DMSO): 8.80 (*s*, NH); 8.66 (2 *s*, 2 H, H–C(2)); 8.19–7.83 (*m*, 8 H, H–C(8), H *o* to NO_2);) 7.38–7.13 (*m*, 15 H, arom. H, H *m* to NO_2, H *m* to MeO); 6.75 (*m*, 4 H, H *o* to MeO); 6.40 (*m*, 2 H, H–C(1′)); 5.91 (*s*, NH_2); 5.32–3.18 (*m*, 2 H, H–C(3′)); 4.51–4.17 (*m*, 10 H, CH_2CH_2O, H–C(4′), H–C(5′)); 3.72 (2 *s*, 2 MeO); 3.33 (*m*, 2 H, H–C(5′)); 3.11–2.87 (*m*, 8 H, CH_2CH_2O, H–C(2′)); 2.60 (*m*, 2 H, H–C(2′)). Anal. calc. for $C_{67}H_{64}N_{13}O_{20}P \cdot H_2O$ (1420.3): C 56.66, H 4.68, N 12.82; found: C 56.50, H 4.64, N 12.81.

28. *2′-Deoxy-5′*-O-*(monomethoxytrityl)*-N^6-*(phenoxycarbonyl)adenylyl-{3′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy*-N^6,3′-O-*bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**32**). To a soln. of **30** (0.1 g, 0.071 mmol) in dioxane (2 ml), 1-(phenoxycarbonyl)-1*H*-tetrazole (0.27 g, 1.4 mmol) was added and the mixture stirred for 14 h at 40°. After evaporation, the residue was dissolved in CH_2Cl_2 (5 ml) and submitted to FC (silica gel (10 g); toluene (30 ml), toluene/acetone 3:1 (100 ml), toluene/acetone 1:1 (100 ml), toluene/acetone 1:3 (100 ml); 30-ml fractions). *Fr. 6–8* gave 70 mg (65%) of **33**. TLC (silica gel, toluene/acetone 1:1): R_f 0.23. The substance decomposed on storage.

29. *2′-Deoxy-5′*-O-*(dimethoxytrityl)*-N^6-*(phenoxycarbonyl)adenylyl-{3′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy*-N^6,3′-O-*bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**33**). As described for **32**, with **31** (0.1 g,

0.07 mmol) and 1-(phenoxycarbonyl)-1*H*-tetrazole (0.27 g, 1.4 mmol). TLC (silica gel, toluene/acetone 1:1): R_f 0.25. The substance decomposed on storage.

30. *2′-Deoxy-*N^6*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-5′-*O*-{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**34**). Compound **24** (0.95 g, 2 mmol) was co-evaporated twice with dry pyridine (10 ml). The residue was dissolved in pyridine (20 ml), the soln. cooled to $-40°$, 2-(4-nitrophenyl)ethyl carbonochloridate (0.6 g, 2.6 mmol) in CH_2Cl_2 (10 ml) added, and the mixture stirred for 30 min at $-30°$, and for 30 min at $-10°$. Then another 0.15 g of the reagent was added and stirring continued for 3 h at r.t. The mixture was diluted with CH_2Cl_2 (300 ml) and washed several times with phosphate buffer pH 7. The org. phase was dried (Na_2SO_4), evaporated, and co-evaporated with toluene (3×10 ml) and the residue purified by FC (silica gel (40 g); toluene (50 ml), toluene/AcOEt 1:1 (300 ml), toluene/AcOEt 1:1 (290 ml) + MeOH (10 ml), toluene/AcOEt 1:1 (280 ml) + MeOH (20 ml), toluene/AcOEt 1:1 (100 ml) + MeOH (100 ml); 100-ml fractions). *Fr. 8–10* were evaporated, and the residue was treated with Et_2O by ultrasound to give, after drying under high vacuum, 1.05 g (78%) of **34**. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.27. UV (MeOH): 226 (4.11), 256 (4.52), 265 (4.59), 287 (4.03), 298 (3.93). ^{1}H-NMR ((D$_6$)DMSO): 10.9 (*s*, NH); 8.65 (*s*, H–C(2)); 8.60 (*s*, H–C(8)); 8.15 (*d*, 2 H, H *o* to NO_2); 7.90–7.81 (*m*, H–C(1), H–C(4), H–C(5), H–C(8)(all fmoc)); 7.51–7.32 (*m*, H–C(2), H–C(3), H–C(6), H–C(7)(all fmoc), 2 H *m* to NO_2); 6.46 (*m*, H–C(1′)); 5.55 (*d*, OH–C(3′)); 4.52–3.99 (*m*, H–C(9)(fmoc), CH_2, H–C(3′), H–C(4′), 2 H–C(5′), CH_2CH_2O); 3.01 (*m*, CH_2CH_2O); 2.94 (*m*, H–C(2′)); 2.40 (*m*, H–C(2′)). Anal. calc. for $C_{34}H_{30}N_6O_9$ (660.6): C 61.26, H 4.54, N 12.61; found: C 61.95, H 4.72, N 12.58.

31. *2′-Deoxy-*N^6*-[(9*H*-fluoren-9-yloxy)carbonyl]-5′-*O*-{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine 3′-[2-(4-Nitrophenyl)ethyl Diisopropylphosphoramidite]* (**35**). To a soln. of **34** (0.4 g, 0.6 mmol) in CH_2Cl_2 (10 ml) and Et^{i}Pr$_2$N (0.5 ml), 2-(4-nitrophenyl)ethyl diisopropylphosphoramidochloridite (0.4 g, 1.2 mmol) was added under Ar and then stirred for 70 min at r.t. The mixture was diluted with $CHCl_3$ (200 ml) and extracted with phosphate buffer pH 7 (70 ml). The aq. phase was washed again with $CHCl_3$ and the combined org. layer dried ($MgSO_4$) and evaporated *in vacuo* followed by high vacuum to remove Et^{i}Pr$_2$N. The residue was dissolved in a little CH_2Cl_2 and submitted to FC (Alox (70 g): hexane (50 ml), hexane/acetone 7:3 (200 ml), hexane/acetone 2:3 (200 ml), acetone (200 ml), acetone/MeOH 95:5 (400 ml); 50-ml fractions). *Fr. 17–21* were evaporated and co-evaporated with CH_2Cl_2: 0.35 g (60%) of **35**. TLC (Alox, hexane/acetone 4:1): R_f 0.35. ^{1}H-NMR ((D$_6$)DMSO): 10.9 (*s*, NH); 8.73 (*s*, H–C(2)); 8.29 (*s*, NH); 8.18–8.04, 8.15 (*m*, H–C(8), 4 H *o* to NO_2)); 7.77–7.65 (*m*, H–C(1), H–C(4), H–C(5), H–C(8)(all fmoc)); 7.42–7.24 (*m*, H–C(2), H–(3), H–C(6), H–C(7)(all fmoc), 4 H *m* to NO_2); 6.43 (*m*, H–C(1′)); 4.60 (*m*, CH_2–C(9)(fmoc), H–C(3′)); 4.38 (*m*, H–C(9)(fmoc), H–C(4′), 2 CH_2CH_2O); 3.89–3.76 (*m*, 2 H–C(5′)); 3.52 (*m*, 2 Me_2CH); 3.08–2.97 (*m*, 2 CH_2CH_2O); 2.85–2.61 (*m*, 2 H–C(2′)); 1.13 (*m*, 2 Me_2CH). Anal. calc. for $C_{48}H_{51}N_8O_{12}P$ (962.9): C 59.87, H 5.34, N 11.64; found: C 58.98, H 5.48, N 10.80.

32. *2′-Deoxy-*N^6*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-5′-*O*-{[2-(4-nitrophenyl)ethoxy]carbonyl}adenylyl-{3′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy-*N^6*,3′-*O*-bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**36**). A mixture of **21** (0.528 g, 0.83 mmol) and 1*H*-tetrazole (0.2 g) was stirred in MeCN (5 ml) for 5 min and then evaporated. A soln. of **35** (1.6 g, 1.7 mmol) in CH_2Cl_2 (10 ml) was added and then stirred at r.t. for 2 h. Subsequent oxidation with I_2 (0.2 g) in pyridine/CH_2Cl_2/H_2O 5:1:1 (3 ml) by stirring for 15 min gave a brown mixture that was diluted with $CHCl_3$ (300 ml) and then decolorized by washing with a $Na_2S_2O_3$/NaCl. soln. The org. layer was dried ($MgSO_4$) and evaporated and the obtained oil purified by FC (silica gel (50 g); toluene (50 ml), toluene/AcOEt 1:1 (200 ml), toluene/AcOEt 1:1 (190 ml) + MeOH (10 ml), toluene/AcOEt 1:1 (185 ml) + MeOH (15 ml), toluene/AcOEt 1:1 (180 ml) + MeOH (20 ml), toluene/AcOEt 1:1 (190 ml) + MeOH (10 ml), toluene/AcOEt 1:1 (180 ml) + MeOH (10 ml) and toluene/AcOEt 1:1 (190 ml) + MeOH (20 ml); 50-ml fractions). Fr. *16–20* were evaporated and the residue was treated with Et_2O and then the solid dried *in vacuo:* 0.78 g of **36** (63%). TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.24. UV (MeOH): 227 (4.37), 265 (4.94), 286 (4.51), 298 (4.27). ^{1}H-NMR ((D$_6$)DMSO): 10.9 (*s*, NH); 10.9 (*s*, NH); 8.58–8.45 (4 *s*, 4 H, H–C(2), H–C(8)); 8.16–7.29 (*m*, 24 arom. H); 6.40 (*m*, 2 H, H–C(1′)); 5.30 (*m*, 1 H, H–C(3′)); 5.12 (*m*, 1 H, H–C(3′)); 4.40–4.14 (*m*, 17 H, CH_2CH_2O, H–C(9)(fmoc), CH_2, H–C(4′), H–C(5′)); 3.15–2.87 (*m*, 10 H, CH_2CH_2O, H–C(2′)); 2.58–2.50 (*m*, 2 H, H–C(2′)). Anal. calc. for $C_{70}H_{63}N_{14}O_{24}P$ (1515.3): C 55.49, H 4.19, N 12.84; found: C 55.20, H 4.33, N 12.99.

33. *2′-Deoxy-5′-*O*-{[2-(4-nitrophenyl)ethoxy]carbonyl}adenylyl-{3′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy-*N^6*,3′-*O*-bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**37**). A soln. of **36** (0.8 g, 0.54 mmol) in MeCN (20 ml), CH_2Cl_2 (20 ml), and Et_3N (10 ml) was stirred at r.t. for 2 h. After evaporation, the residue was dissolved in CH_2Cl_2 and submitted to FC (silica gel; CH_2Cl_2 (100 ml), CH_2Cl_2/MeOH 98:2 (200 ml), CH_2Cl_2/MeOH 96:4 (200 ml), CH_2Cl_2/MeOH 94:6 (200 ml), CH_2Cl_2/MeOH 1:1 (100 ml); 100-ml fractions). *Fr. 6* and *7* were

evaporated, and the residue was treated with Et_2O and dried: 0.57 g (87%) of **37**. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.24. UV (MeOH): 265 (4.80). ^{1}H-NMR ((D$_6$)DMSO): 10.9 (*s*, NH); 10.6 (*s*, NH); 8.59 (*s*, 2 H, H–C(2)); 8.23–8.02 (*m*, 10 H, H–C(8), H *o* to NO_2); 7.31 (br. *s*, 2 H, NH_2); 6.43 (*t*, 1 H, H–C(1′)); 6.31 (*t*, 1 H, H–C(1′)); 5.34 (*m*, 1 H, H–C(3′)); 5.07 (*m*, 1 H, H–C(3′)); 4.51–4.17 (*m*, 14 H, CH_2CH_2O, H–C(4′), H–C(5′)); 3.11–2.88 (*m*, 10 H, CH_2CH_2O, H–C(2′)); 2.60 (*m*, 2 H, H–C(2′)). Anal. calc. for $C_{55}H_{53}N_{14}O_{22}P$ (1293.1): C 51.09, H 4.13, N 15.17; found: C 50.87, H 4.28, N 15.31.

34. *2′-Deoxy-5′*-O-*{[2-(4-nitrophenyl)ethoxy]carbonyl}*-N^6-*(phenoxycarbonyl)adenylyl-{3′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy*-N^6,3′-O-*bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**38**). To a soln. of **37** (0.13 g, 0.1 mmol) in dry dioxane (5 ml) was added 5-(4-nitrophenyl)-1-(phenoxycarbonyl)-1*H*-tetrazole (0.16 g, 0.16 mmol) and then stirred at 40° for 20 h. After evaporation, the residue was dissolved in little CH_2Cl_2 and purified by FC (silica gel (15 g); CH_2Cl_2 (25 ml), CH_2Cl_2/MeOH 98:2 (100 ml), CH_2Cl_2/MeOH 9:3 (200 ml), CH_2Cl_2/MeOH 9:3 (200 ml); 25-ml fractions). *Fr. 7–11* were evaporated, and the residue was treated with Et_2O and then the solid collected and dried: 0.14 g (93%) of **38**. TLC (silica gel, CH_2Cl_2/MeOH 9:1): R_f 0.68. UV (MeOH): 267 (4.80), 296 (sh., 4.22). ^{1}H-NMR ((D$_6$)DMSO): 11.0 (*s*, NH); 10.6 (*s*, NH); 8.61 (*m*, 4 H, H–C(2), H–C(8)); 8.17–8.02 (*m*, 8 H *o* to NO_2); 7.60–7.40 (*m*, 10 H *m* to NO_2, H_o (Ph)); 7.25 (*m*, 3 arom. H); 6.40 (*m*, 2 H, H–C(1′)); 5.33 (*m*, 1 H, H–C(3′)); 5.11 (*m*, 1 H, H–C(3′)); 4.40–4.21 (*m*, 14 H, CH_2CH_2O, H–C(4′), H–C(5′)); 3.09–2.89 (*m*, 10 H, CH_2CH_2O, H–C(2′)); 2.63–2.50 (*m*, 2 H, H–C(2′)). Anal. calc. for $C_{62}H_{57}N_{14}O_{24}P$ (1413.2): C 52.70, H 4.07, N 13.88; found: C 51.94, H 4.21, N 14.46.

35. *2′-Deoxy*-N^6-*{[(3′,6′-dihydroxy-3-oxospiro[isobenzofuran-1(3*H*),9′-[9*H*]xanthen]-5-yl)amino]carbonyl}-5′*-O-*(dimethoxytrityl)adenylyl-{3′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy*-N^6,3′-O-*bis{[2-(4-nitrophenyl)-ethoxy]carbonyl}adenosine* (**39**). A mixture of **33** (0.1 g, 0.066 mmol) and **7** (0.068 g, 0.2 mmol) in pyridine (2 ml) was heated to 65° for 1.5 h with stirring. The soln. was evaporated and co-evaporated with toluene and the residue dissolved in a little CH_2Cl_2/EtOH and submitted to FC (silica gel (20 g); toluene (25 g), toluene/AcOEt 1:1 (100 ml), toluene/AcOEt 1:1 (95 ml) + MeOH (5 ml), toluene/AcOEt 1:1 (90 ml) + MeOH (10 ml), toluene/AcOEt 1:1 (85 ml) + MeOH (15 ml), toluene/AcOEt 1:1 (80 ml) + MeOH (20 ml), toluene/AcOEt 1:1 (75 ml) + MeOH (25 ml), toluene/AcOEt 1:1 (70 ml) + MeOH (30 ml), toluene/AcOEt 1:1 (60 ml) + MeOH (40 ml), toluene/AcOEt 1:1 (120 ml) + MeOH (80 ml); 25-ml fractions). *Fr. 26–37* were evaporated, and the residue was treated with a little MeOH: 0.08 g (68%) of **39**. TLC (silica gel, CH_2Cl_2/MeOH 9:1): R_f 0.60–0.65. ^{1}H-NMR ((D$_6$)DMSO): 12.2 (*s*, NH); 10.25–10.20 (*s*, 4 H, NH, OH); 8.60–6.50 (*m*, 38 H, arom. H, H–C(2), H–C(8) (4 H), npe/npeoc (12 H), $(MeO)_2$Tr (13 H), flu (9 H)); 6.75 (*m*, 4 H *o* to MeO); 6.40 (*m*, 2 H, H–C(1′)); 5.32–5.03 (*m*, 2 H, H–C(3′)); 4.40–4.11 (*m*, 10 H, CH_2CH_2O, H–C(4′), H–C(5′)); 3.75 (2 *s*, 2 MeO); 3.33–2.88 (*m*, 10 H, CH_2CH_2O, H–C(5′), H–C(2′)); 2.60 (*m*, 2 H, H–C(2′)). Anal. calc. for $C_{88}H_{75}N_{14}O_{26}P \cdot 2\ H_2O$ (1811.7): C 58.34, H 4.39, N 10.82; found: C 58.23, H 4.40, N 10.65.

36. *2′-Deoxy*-N^6-*{[(3′,6′-dihydroxy-3-oxospiro[isobenzofuran-1(3*H*),9-[9*H*]xanthen]-5-yl)amino]carbonyl}-5′*-O-*{[2-(4-nitrophenyl)ethoxy]carbonyl}adenylyl-{3′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy*-N^6,3′-O-*bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**40**). A mixture of dry **7** (0.2 g, 0.59 mmol) and **38** (0.1 g, 0.07 mmol) in abs. pyridine (2 ml) was heated to 70° for 45 min. After evaporation and co-evaporation with toluene, the residue was treated with MeOH (3 ml). The solid was washed with MeOH to remove excess of the dye and then dissolved in CH_2Cl_2 for purification by FC (silica gel (25 g); CH_2Cl_2/MeOH 98:2 (100 ml), CH_2Cl_2/MeOH 97:3 (100 ml), CH_2Cl_2/MeOH 95:5 (100 ml), CH_2Cl_2/MeOH 94:6 (100 ml), CH_2Cl_2/MeOH 9:1 (100 ml), CH_2Cl_2/MeOH 4:1 (100 ml), and CH_2Cl_2/MeOH 1:1 (100 ml); 50-ml fractions). *Fr. 10–13* were evaporated, and the residue was treated with MeOH, filtered, and dried: 0.06 g (51%) of **40**. TLC (silica gel, CH_2Cl_2/MeOH 9:1): R_f 0.48 UV (MeOH/CH_2Cl_2 1:1): 224 (4.89), 227 (sh., 4.87), 271 (sh., 4.94), 275 (4.95), 347 (3.53), 424 (sh., 3.59), 455 (3.91), 483 (sh., 3.96). ^{1}H-NMR ((D$_6$)DMSO): 12.1 (*s*, NH); 10.6 (*s*, 3 H, NH, OH); 10.4 (*s*, NH); 8.65–8.55 (*m*, 4 H, H–C(2), H–C(8)); 8.30 (*s*, H–C(6)(flu)); 8.12–8.02 (*m*, 8 H *o* to NO_2); 7.91 (*d*, H–C(4)(flu)); 7.60–7.43 (*m*, 8 H *m* to NO_2); 7.22 (*d*, H–C(3)(flu)); 6.64–6.37 (*m*, 8 H, xan, H–C(1′)); 5.33 (*m*, 1 H, H–C(3′)); 5.13 (*m*, 1 H, H–C(3′)); 4.34–4.19 (*m*, 14 H, CH_2CH_2O, H–C(4′), H–C(5′)); 3.11–2.93 (*m*, 10 H, CH_2CH_2O, H–C(5′), H–C(2′)); 2.53 (*m*, 2 H, H–C(2′)). Anal. calc. for $C_{76}H_{65}N_{15}O_{28}P \cdot 2\ H_2O$ (1703.4): C 53.58, H 4.08, N 12.33; found: C 53.35, H 4.00, N 12.25.

37. *2′-Deoxy*-N^6-*{[(3′,6′-dihydroxy-3-oxospiro[isobenzofuran-1(3*H*),9-[9*H*]xanthen]-5-yl)amino]carbonyl}-adenylyl-(3′-5′)-2′-deoxyadenosine Sodium Salt* (**41**). A soln. of **40** (50 mg, 0.03 mmol) in 0.5M DBU in pyridine (10 ml) was stirred at r.t. for 4 h. After dilution with H_2O (50 ml) and neutralization with AcOH (1 ml), the mixture was extracted with $CHCl_3$ (100 ml). Phase separation was achieved by addition of conc. NH_3 soln. (10 ml). The aq. layer was again extracted with $CHCl_3$ and finally evaporated. The residue was purified by FPLC (DEAE-*Sephadex A25*, column 50 × 2 cm; gradient of 100% H_2O to 100% TBK buffer (1M, pH 8) within 32 h). The fluorescent fractions were eluted with 80–90% buffer concentration, evaporated, and co-evaporated with

H_2O. The resulting triethylammonium salt was dissolved in MeOH (5 ml) and treated with NaI (0.1 g) in acetone to give **41**, which was collected and dried: 20 mg (67%) of **41**. Yellow powder. UV (pH 9): 264 (4.33), 280 (sh., 4.33), 492 (4.62).

38. *2′-Deoxy-5′-*O*-(monomethoxytrityl)-*N^6*-{[2-(4-nitrophenyl)ethoxy]carbonyl}adenylyl-{3′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy-*N^6*-[(9*H*)-fluoren-9-ylmethoxy)carbonyl]adenylyl-{3′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy-*N^6*,3′-O-bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**42**). A soln. of **29** (0.53 g, 0.4 mmol) and **44** (0.842 g, 0.8 mmol) in dry MeCN (10 ml) and CH_2Cl_2 (10 ml) was treated under N_2 with 1*H*-tetrazole (0.17 g) by stirring at r.t. for 2 h. Then a soln. of I_2 (0.5 g) in pyridine/CH_2Cl_2/H_2O 5:1:1 (7 ml) was added and stirred for 15 min for oxidation. The mixture was diluted with $CHCl_3$, extracted with a $Na_2S_2O_3$/NaCl soln. until decolorazition was achieved, the org. layer dried ($MgSO_4$), evaporated, and co-evaporated with toluene, and the resulting syrup dissolved in little CH_2Cl_2 and purified by FC (silica gel (25 g); toluene (50 ml), toluene/AcOEt 1:1 (100 ml), toluene/AcOEt 1:1 (95 ml) + MeOH (5 ml), toluene/AcOEt 1:1 (90 ml) + MeOH (10 ml), toluene/AcOEt 1:1 (85 ml) + MeOH (15 ml), toluene/AcOEt 1:1 (80 ml) + MeOH (20 ml), toluene/AcOEt 1:1 (160 ml) + MeOH (40 ml); 50-ml fractions. *Fr. 8–12* were evaporated and co-evaporated with CH_2Cl_2: 0.87 g (96%) of **42**. Colorless solid foam. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.2. UV (CH_2Cl_2): 258 (sh., 4.97), 265 (5.04), 274 (sh., 4.97), 299 (sh., 4.39). ^{1}H-NMR ((D$_6$)DMSO): 10.9 (*s*, NH); 10.6 (*s*, NH); 8.58–8.41 (*m*, 6 H, H–C(2), H–C(8)); 8.15–7.97 (*m*, 14 H, H *o* to NO_2, H–C(1), H–C(4), H–C(5), H–C(8)(all fmoc)); 7.61–7.09 (*m*, 26 H, arom. H, H *m* to NO_2, H–C(2), H–C(3), H–C(6), H–C(7)(all fmoc)); 7.91 (*d*, H–C(4)(flu)); 7.72 (*m*, 2 H *o* to MeO)); 6.40 (*m*, 3 H, H–C(1′)); 5.33 (*m*, 1 H, H–C(3′)); 5.13 (*m*, 2 H, H–C(3′)); 4.39–4.14 (*m*, 20 H, CH_2CH_2O, H–C(4′), H–C(5′), H–C(9)(fmoc), CH_2); 3.64 (*s*, MeO); 3.17–2.88 (*m*, 15 H, CH_2CH_2O, H–C(5′), H–C(2′)); 2.62–2.48 (*m*, 3 H, H–C(2′)). Anal. calc. for $C_{108}H_{98}N_{20}O_{32}P_2 \cdot H_2O$ (2250.1): C 57.19, H 4.44, N 12.35; found: C 56.97, H 4.61, N 12.28.

39. *2′-Deoxy-*N^6*,5′-*O*-bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenylyl-{3′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy-*N^6*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]adenylyl-{3′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy-*N^6*,3′-*O*-bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**43**). As described for **42**, with **29** (0.6 g, 0.45 mmol), **45** (0.85 g, 0.91 mmol), 1*H*-tetrazole (0.19 g, 2.8 mmol), MeCN (10 ml), and CH_2Cl_2 (10 ml), followed by I_2 oxidation. After FC (silica gel (30 g); toluene/AcOEt 1:1 (400 ml), toluene/AcOEt 1:1 (195 ml) + MeOH (5 ml), toluene/AcOEt 1:1 (190 ml) + MeOH (10 ml), toluene/AcOEt 1:1 (180 ml) + MeOH (20 ml), toluene/AcOEt 1:1 (340 ml) + MeOH (60 ml), toluene/AcOEt 1:1 (160 ml) + MeOH (40 ml); 100-ml fractions). *Fr. 10–15* were evaporated and co-evaporated with AcOEt: 0.92 g (93%) of **43**. Solid foam. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.11. UV (CH_2Cl_2): 266 (5.06), 271 (sh., 4.97), 291 (4.61), 298 (sh., 4.43). ^{1}H-NMR ((D$_6$)DMSO): 10.9 (*s*, NH); 10.6 (*s*, 2 H, NH); 8.56 (*m*, 6 H, H–C(2), H–C(8)); 8.15–7.12 (*m*, 32 H, arom. H, H *o* to NO_2, H–C(1) to H–C(8)(fmoc)); 6.40 (*m*, 3 H, H–C(1′)); 5.33 (*m*, 1 H, H–C(3′)); 5.15 (*m*, 1 H, H–C(3′)); 5.08 (*m*, 1 H, H–C(3′)); 4.40–4.15 (*m*, 24 H, CH_2CH_2O, H–C(4′), H–C(5′), H–C(9)(fmoc), CH_2); 3.18–2.94 (*m*, 15 H, CH_2CH_2O, H–C(5′), H–C(2′)); 2.63–2.50 (*m*, 3 H, H–C(2′)). Anal. calc. for $C_{97}H_{89}N_{21}O_{35}P_2$ (2170.9): C 53.67, H 4.13, N 13.55; found: C 52.96, H 4.13, N 13.08.

40. *2′-Deoxy-5′-*O*-(monomethoxytrityl)-*N^6*-{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine 3′-[2-(4-Nitrophenyl)ethyl Diisopropylphosphoramidite]* (**44**) [22].

41. *2′-Deoxy-*N^6*,5′-*O*-bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine 3′-[2-(4-Nitrophenyl)ethyl Diisopropylphosphoramidite]* (**45**). A soln. of **46** (1.2 g, 1.9 mmol) [23] in CH_2Cl_2 (10 ml) and Et^{i}Pr$_2$N (1.2 ml) was treated under N_2 with 2-(4-nitrophenyl)ethyl diisopropylphosphoramidochloridite (1.2 g, 4 mmol) with stirring at r.t. for 1.5 h. After dilution with $CHCl_3$ (300 ml), the mixture was extracted with sat. $NaHCO_3$ soln. (70 ml), the aq. phase extracted with $CHCl_3$, the combined org. phase dried ($MgSO_4$) and evaporated finally under high vacuum to remove Et^{i}Pr$_2$N. Purification was achieved by FC (silica gel (30 g), prepared with hexane + 1% Et_3N; hexane + 1% Et_3N (100 ml), hexane + 1% Et_3N/AcOEt 2:1 (100 ml), hexane + 1% Et_3N/AcOEt 1:1 (100 ml), hexane + 1% Et_3N/AcOEt 1:2 (100 ml), AcOEt + 1% Et_3N (200 ml), AcOEt/acetone 1:1 (200 ml); 50-ml fractions). *Fr. 10–14* were evaporated and co-evaporated with CH_2Cl_2: 1.42 g (81%) of **45**. Solid foam. TLC (silica gel, hexane/AcOEt 1:2 + 1% Et_3N): R_f 0.25. UV (MeOH): 267 (4.63), 272 (sh., 4.61). ^{1}H-NMR ($CDCl_3$): 8.73, 8.72 (2 *s*, H–C(2)); 8.37 (*s*, NH); 8.13–8.03 (*m*, H–C(8), 6 H *o* to NO_2)); 7.48–7.36 (*m*, 6 H *m* to NO_2); 6.44 (2 *t*, H–C(1′)); 4.58–4.15 (*m*, 3 CH_2CH_2O, H–C(3′), H–C(4′)); 3.96–2.99 (*m*, 2 CMe_2CH, 3 CH_2CH_2O, 2 H–C(5′)); 2.80–2.60 (*m*, 2 H–C(2′)); 1.10 (*m*, 2 Me_2CH). Anal. calc. for $C_{42}H_{48}N_9O_{14}P$ (933.9): C 54.02, H 5.18, N 13.50; found: C 53.88, H 5.50, N 12.99.

42. *2′-Deoxy-*N^6*,5′-*O*-bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**46**) [23].

43. *2′-Deoxy-5′-*O*-(monomethoxytrityl)-*N^6*-{[2-(4-nitrophenyl)ethoxy]carbonyl}adenylyl-{3′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxyadenylyl-{3′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy-*N^6*,3′-*O*-bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**47**). A soln. of **42** (0.75 g, 0.33 mmol) in MeCN/CH_2Cl_2 1:1 (40 ml) was

treated with Et_3N (10 ml) and stirred at r.t. for 2.5 h. After evaporation and co-evaporation with EtOH, the residue was dissolved in a little CH_2Cl_2 for purification by FC (silica gel (20 g); CH_2Cl_2 (40 ml), CH_2Cl_2/MeOH 95:5 (200 ml), CH_2Cl_2/MeOH 9:1 (200 ml); 40-ml fractions). *Fr. 6–9* were evaporated, and the residue was treated with a little EtOH by ultrasound and then the precipitate collected: 0.62 g (93%) of **47**. TLC (silica gel, CH_2Cl_2/MeOH 9:1): R_f 0.53. UV ($CHCl_3$): 238 (sh., 4.61), 266 (4.94), 275 (sh., 4.87). ^{1}H-NMR ((D$_6$)DMSO): 10.6 (s, 2 H, NH); 8.58–8.41 (*m*, 4 H, 3 H–C(2), 1 H–C(8)); 8.24 (*s*, 1 H, H–C(8)); 8.16–7.97 (*m*, 11 H, H *o* to NO_2, H–C(8)); 7.61–7.09 (*m*, 24 H, H *m* to NO_2, arom. H, NH_2); 6.71 (*m*, 2 H *o* to MeO); 6.43–6.32 (*m*, 3 H, H–C(1′)); 5.32 (*m*, 1 H, H–C(3′)); 5.11 (*m*, 2 H, H–C(3′)); 4.37–4.21 (*m*, 17 H, CH_2CH_2O, H–C(4′), H–C(5′)); 3.64 (*s*, MeO); 3.16–2.88 (*m*, 15 H, CH_2CH_2O, H–C(5′)), H–C(2′)); 2.62–2.50 (*m*, 3 H, H–C(2′)). Anal. calc. for $C_{93}H_{88}N_{20}O_{30}P_2 \cdot 2\ H_2O$ (2063.8): C 54.12, H 4.49, N 13.64; found: C 53.48, H 4.51, N 13.40.

44. *2′-Deoxy-*N^6*,5′-*O*-bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenylyl-{3′-{*O^P*-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxyadenylyl-{3′-{*O^P*-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy-*N^6*,3′-*O*-bis{[2-(4-nitrophenyl)ethoxy]carbonyl}-adenosine* (**48**). A soln. of **43** (0.6 g, 0.28 mmol) in MeCN/CH_2Cl_2 1:1 and Et_3N (10 ml) was stirred at r.t. for 4 h. The mixture was evaporated and the residue dissolved in CH_2Cl_2 for FC (silica gel (30 g); CH_2Cl_2 (50 ml), CH_2Cl_2/MeOH 98:2 (200 ml), CH_2Cl_2/MeOH 96:4 (400 ml), CH_2Cl_2/MeOH 95:5 (200 ml), CH_2Cl_2/MeOH 9:1 (200 ml); 50-ml fractions) *Fr. 10–19* were evaporated. The residue was treated with CH_2Cl_2 (25 ml) and the soln. filtered and then treated with EtOH until the soln. became turbid. The CH_2Cl_2 was removed *in vacuo* at r.t. whereby **48** separated at the glaswall. The EtOH was decanted and the residue treated with Et_2O: 0.51 g (94%) of **48**. Colorless solid. TLC (silica gel, CH_2Cl_2/MeOH 9:1): R_f 0.55. UV ($CHCl_3$): 266 (4.95), 270 (sh., 4.93). ^{1}H-NMR ((D$_6$)DMSO): 10.6 (*s*, 2 H, NH); 8.55 (*m*, 4 H, 3 H–C(2), 1 H–C(8)); 8.27 (*s*, 1 H, H–C(8)); 8.16–8.00 (*m*, 13 H, H *o* to NO_2, H–C(8)); 7.60–7.40 (*m*, 12 H, H *m* to NO_2); 7.32 (br. *s*, 2 H, NH_2); 6.43–6.36 (*m*, 3 H, H–C(1′)); 5.33 (*m*, 1 H, H–C(3′)); 5.11 (*m*, 2 H, H–C(3′)); 4.40–4.21 (*m*, 21 H, CH_2CH_2O, H–C(4′), H–C(5′)); 3.09–2.96 (*m*, 15 H, CH_2CH_2O, H–C(5′), H–C(2′)); 2.63–2.50 (*m*, 3 H, H–C(2′)). ^{31}P-NMR ((D$_6$)DMSO): −1.41, −1.48, −1.56. Anal. calc. for $C_{82}H_{79}N_{21}O_{33}P_2$ (1948.6): C 50.54, H 4.09, N 15.10; found: C 50.55, H 4.27, N 15.09.

45. *2′-Deoxy-*N^6*,5′-*O*-bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenylyl-{3′-{*O^P*-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy-*N^6*-(phenoxycarbonyl)adenylyl-{3′-{*O^P*-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy-*N^6*,3′-*O*-bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**49**). To a soln. of **48** (0.3 g, 0.15 mmol) in dry dioxane (5 ml), 5-(4-nitrophenyl)-1-(phenoxycarbonyl)-1*H*-tetrazole (0.2 g, 0.6 mmol) was added and then stirred at 40° for 48 h. The mixture was evaporated and the residue dissolved in little CH_2Cl_2 and purified by FC (silica gel (7 g); CH_2Cl_2 (40 ml), CH_2Cl_2/MeOH 97:3 (100 ml), CH_2Cl_2/MeOH 95:5 (200 ml); 20-ml fractions). *Fr. 7–10* were treated with some AcOEt and then concentrated to a small volume whereby a precipitate separated on the glasswall. The yellowish residual AcOEt was decanted and then the solid treated with Et_2O: 0.29 g (93%) of colorless **49**. TLC (silica gel, CH_2Cl_2/MeOH 9:1): R_f 0.63, 0.68. UV ($CHCl_3$): 267 (4.99), 272 (sh., 4.95). ^{1}H-NMR ((D$_6$)DMSO): 11.1 (*s*, 2 H, NH); 10.6 (*m*, 2 H, NH); 8.63–8.51 (*m*, 6 H, H–C(2), H–C(8)); 8.15–7.99 (*m*, 12 H *o* to NO_2); 7.60–7.39 (*m*, 14 H, H *m* to NO_2, H_o of (Ph)); 7.28 (*m*, 3 H, H_m, H_p (Ph)); 6.40 (*m*, 3 H, H–C(1′)); 5.33 (*m*, 1 H, H–C(3′)); 5.11 (*m*, 2 H, H–C(3′)); 4.40–4.21 (*m*, 21 H, CH_2CH_2O, H–C(4′), H–C(5′)); 3.09–2.95 (*m*, 15 H, CH_2CH_2O, H–C(5′), H–C(2′)); 2.56 (*m*, 3 H, H–C(2′)). ^{31}P-NMR ((D$_6$)DMSO): −1.34; −1.42. Anal. calc. for $C_{89}H_{83}N_{21}O_{35}P_2$ (2068.7): C 51.67, H 4.04, N 14.22; found: C 51.09, H 4.09, N 13.97.

46. *2′-Deoxy-*N^6*,5′-*O*-bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenylyl-{3′-{*O^P*-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy-*N^6*-{[3′,6′-dihydroxy-3-oxospiro[isobenzofuran-1(3*H*),9-[9*H*]xanthen]-5-yl)amino]carbonyl}adenylyl-{3′-{*O^P*-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy-*N^6*,3′-*O*-bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**50**). A mixture of **49** (0.15 g, 0.073 mmol) and **7** (0.051 g, 0.146 mmol) in pyridine (2 ml) was heated to 60–70° for 2 h with stirring. The soln. was evaporated and co-evaporated with toluene. The residue was dissolved in a little CH_2Cl_2 and submitted to FC (silica gel (20 g); CH_2Cl_2/MeOH 99:1 (50 ml), CH_2Cl_2/MeOH 98:2 (100 ml), CH_2Cl_2/MeOH 95:5 (100 ml), CH_2Cl_2/MeOH 9:1 (100 ml), CH_2Cl_2/MeOH 1:1 (100 ml); 30-ml fractions). *Fr. 11–13* were evaporated, and the residue was treated with Et_2O: 0.075 g (44%) of **50**. Yellow powder. TLC (silica gel, CH_2Cl_2/MeOH 9:1): R_f 0.39. ^{1}H-NMR ((D$_6$)DMSO): 12.1 (*s*, 1 H, NH); 10.4 (*m*, 5 H, NH, OH); 8.63–8.51 (*m*, 6 H, H–C(2), H–C(8)); 8.30 (*s*, H–C(6)(flu)); 8.15–8.02 (*m*, 12 H *o* to NO_2); 7.88 (*s*, H–C(4)(flu)); 7.60–7.43 (*m*, 12 H *m* to NO_2); 7.22 (*s*, H–C(3)(flu)); 6.64–6.42 (*m*, 9 H, xan, H–C(1′)); 5.33 (*m*, 1 H, H–C(3′)); 5.13 (*m*, 2 H, H–C(3′)); 4.34–4.22 (*m*, 21 H, CH_2CH_2O, H–C(4′), H–C(5′)); 3.05–2.93 (*m*, 15 H, CH_2CH_2O, H–C(5′), H–C(2′)); 2.53 (*m*, 3 H, H–C(2′)). ^{31}P-NMR ((D$_6$)DMSO): −1.36; −1.40. Anal. calc. for $C_{103}H_{90}N_{22}O_{39}P_2 \cdot H_2O$ (2339.9): C 52.87, H 3.96, N 13.17; found: C 52.66, H 4.04, N 13.02.

47. *2′-Deoxyadenylyl-(3′-5′)-2′-deoxy-*N^6*-{[(3′,6′-dihydroxy-3-oxospiro[isobenzofuran-1(3*H*),9-[9*H*]xanthen]-5-yl)amino]carbonyl}adenylyl-(3′-5′)-2′-deoxyadenosine* (**51** (= **80**)). Compound **50** (90 mg, 0.039 mmol)

was dissolved in 0.5M DBU in pyridine (10 ml) and stirred at r.t. for 3 h. A Na_2HPO_4 soln. was added and then the mixture extracted with CH_2Cl_2 (3×30 ml). The aq. phase was treated with conc. NH_3 soln. (5 ml), the strongly fluorescent soln. extracted again with CH_2Cl_2, the aq. layer evaporated, and the residue purified by FPLC (DEAE-*Sephadex 25*; gradient $H_2O/(Et_3NH)HCO_3$ buffer (pH 7)). The product fraction was evaporated and several times co-evaporated with H_2O. The resulting residue was dissolved in a little MeOH and then treated with NaI (0.2 g) in acetone (50 ml) under N_2 for 20 h. The formed orange precipitate was collected and dried under high vacuum: 21 mg (60%) of **51**.

Analogous treatment with **79** (80 mg, 0.03 mmol) gave 26 mg (65%) of **51**. UV (buffer pH 9): 261 (4.55), 280 (sh., 4.41), 492 (4.75). ^{1}H-NMR ((D_6)DMSO/H_2O 1:1): 8.7–8.05 (*m*, 7 H, H–C(2), H–C(8), H–C(6)(flu)); 7.70 (*d*, 1 H, H–C(4)(flu)); 7.10 (*d*, H–C(3)(flu)); 6.7–6.2 (*m*, 9 H, xan, H–C(1′)); 5.0–3.5 (*m*, 12 H, H–C(3′), H–C(4′), H–C(5′)); 2.7–2.3 (*m*, 6 H, H–C(2′)).

48. *2′,3′,5′-Tri*-O-*acetyl*-N^6-*{{{4-[3-(acetyloxy)-6-hydroxy-9*H*-xanthen-9-yl]-3-(methoxycarbonyl)phenyl}amino}carbonyl}adenosine* (**52**). Not isolated since the vinylogous AcO group turned out to be too reactive.

49. *2′,3′,5′-Tri*-O-*acetyl*-N^6-*{{{4-{3-hydroxy-6-{[(4-methoxyphenyl)carbonyl]oxy}-9*H*-xanthen-9-yl}-3-(methoxycarbonyl)phenyl}amino}carbonyl}adenosine* (**53**). A soln. of **13** (0.1 g, 13 μmol) in dry pyridine (5 ml) was treated with anisoyl chloride (20 μl) with stirring at r.t. for 5 min. After dilution with CH_2Cl_2 (30 ml), the mixture was extracted with 0.1M KH_2PO_4, the org. phase dried ($MgSO_4$), evaporated, and co-evaporated with toluene, the residue treated with a little EtOH, and the solid collected and dried: 0.1 g (85%) of **53**. A sample was purified by prep. TLC (CH_2Cl_2/MeOH 95:5). TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.39. UV (CH_2Cl_2): 260 (sh., 4.69), 276 (4.78), 338 (4.15), 352 (sh., 4.07), 402 (sh., 4.04), 431 (4.24), 453 (4.24), 486 (sh., 3.97). ^{1}H-NMR ((D_6)DMSO)[2]: 12.0 (*s*, NH); 10.6 (*s*, H–N(6)); 8.76 (*s*, H–C(2)); 8.71 (*s*, H–C(8)); 8.60 (*d*, H–C(6)(flu)); 8.10 (*d*, 2 H *o* to MeO); 8.04 (*dd*, H–C(4)(flu)); 7.64 (*d*, H–C(4)(xan)); 7.52 (*d*, H–C(3)(flu)); 7.26–6.87 (*m*, 2 H *m* to MeO, H–C(1), H–C(2), H–C(8)(all xan)); 6.45 (*dd*, H–C(7)(xan)); 6.34 (*d*, H–C(1′)); 6.25 (*d*, H–C(5)(xan)); 6.05 (*t*, H–C(2′)); 5.65 (*t*, H–C(3′)); 4.46–4.24 (*m*, H–C(4′), 2 H–C(5′)); 3.86 (*m*, 1 MeO); 3.61 (*s*, 1 MeO); 2.12, 2.04, 2.02 (3 *s*, 3 Ac). Anal. calc. for $C_{46}H_{38}N_6O_{15} \cdot 1.5\ H_2O$ (941.9): C 58.66, H 4.38, N 8.92; found: C 58.68, H 4.22, N 8.71.

50. *2′,3′,5′-Tri*-O-*acetyl*-N^6-*{{{4-{3-hydroxy-6-{[2-(4-nitrophenyl)ethoxy]carbonyl}oxy}-9*H*-xanthen-9-yl}-3-(methoxycarbonyl)phenyl}amino}carbonyl}adenosine* (**54**). A soln. of **13** (0.16 g, 0.2 mmol) in CH_2Cl_2 (5 ml) was treated with 3-methyl-1-{[2-(4-nitrophenyl)ethoxy]carbonyl}-1*H*-imidazolium chloride (0.2 g) by stirring at r.t. for 3 h. The undissolved reagent was filtered off and the filtrate evaporated. The residue was treated with EtOH (5 ml) and the orange solid collected and dried: 0.2 g (99%) of chromatographically pure **54**. TLC (silica gel, CH_2Cl_2/MeOH 95:5): R_f 0.23. UV (CH_2Cl_2): 276 (4.74), 337 (4.08), 352 (sh., 3.99), 400 (sh., 3.98), 429 (4.16), 452 (4.16), 482 (sh., 3.90). ^{1}H-NMR ((D_6)DMSO)[2]: 12.0 (*s*, NH); 10.5 (*s*, H–N(6)); 8.75 (*s*, H–C(2)); 8.71 (*s*, H–C(8)); 8.58 (*d*, H–C(6)(flu)); 8.18 (*d*, 2 H *o* to NO_2); 8.04 (*dd*, H–C(4)(flu)); 7.60–7.48 (*m*, H–C(3)(flu), H–C(4)(xan), 2 H *m* to NO_2); 7.16–6.90 (*m*, H–C(1), H–C(2), H–C(8)(all xan)); 6.45 (*dd*, H–C(7)(xan)); 6.33 (*d*, H–C(1′)); 6.25 (*d*, H–C(5)(xan)); 6.05 (*t*, H–C(2′)); 5.64 (*t*, H–C(3)); 4.55 (*t*, CH_2CH_2O); 4.46–4.23 (*m*, H–C(4′), 2 H–C(5′)); 3.61 (*s*, MeO); 3.16 (*t*, CH_2CH_2O); 2.12, 2.04, 2.02 (3 *s*, 3 Ac). Anal. calc. for $C_{47}H_{39}N_7O_{17}$ (973.9): C 57.97, H 4.04, N 10.07; found: C 57.54, H 4.20, N 9.99.

51. *2′,3′,5′-Tri*-O-*acetyl*-N^6-*{{{4-{3-hydroxy-6-[2-(4-nitrophenyl)ethoxy]-9*H*-xanthen-9-yl}-3-(methoxycarbonyl)phenyl}amino}carbonyl}adenosine* (**55**). A mixture of **13** (0.1 g, 0.13 mmol), PPh_3 (67 mg, 0.26 mmol) and 2-(4-nitrophenyl)ethanol (0.106 g, 0.65 mmol) in dioxane (10 ml) was heated to 60° with stirring, and then diethyl azodicarboxylate (40 μl) was added. After 1 h, more PPh_3 (60 mg) and diethyl azodicarboxylate (100 ml) were added, and stirring was continued for 2 h. The soln. was diluted with CH_2Cl_2 (30 ml) and washed with phosphate buffer (pH 7). The org. phase was dried ($MgSO_4$) and evaporated and the orange oil separated by prep. TLC (CH_2Cl_2/MeOH 95:5): products **55** and **56**. The slower moving product was eluted and, after evaporation, treated with MeOH to give a solid: 0.07 g (51%) of **55**. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.27. UV (CH_2Cl_2): 231 (4.65), 256 (sh., 4.55), 277 (4.71), 307 (sh., 4.22), 335 (4.06), 404 (sh., 4.01), 436 (4.25), 458 (4.31), 488 (sh., 4.10). ^{1}H-NMR ((D_6)DMSO)[2]: 12.0 (*s*, NH); 10.5 (*s*, H–N(6); 8.75 (*s*, H–C(2)); 8.71 (*s*, H–C(8)); 8.54 (*d*, H–C(6)(flu)); 8.17 (*d*, 2 H *o* to NO_2); 8.00 (*dd*, H–C(4)(flu)); 7.62 (*m*, 2 H *m* to NO_2); 7.46 (*m*, H–C(3)(flu)); 7.26 (*m*, H–C(4)(xan)); 6.94–6.83 (*m*, H–C(1), H–C(2), H–C(8)(all xan)); 6.35 (*m*, H–C(7)(xan), H–C(1′)); 6.25 (*s*, H–C(5)(xan)); 6.02 (*t*, H–C(2′)); 5.64 (*t*, H–C(3′)); 4.45–4.26 (*m*, CH_2CH_2O, H–C(4′), 2 H–C(5′)); 3.58 (*s*, MeO); 3.24 (*m*, CH_2CH_2O); 2.12,

---

[2]) For convenience and deviating from the systematic name, the OH or =O group is considered to be at C(6) of the xanthene moiety.

2.04, 2.02 (3 *s*, 3 Ac). Anal. calc. for $C_{46}H_{39}N_7O_{15} \cdot H_2O$ (947.9): C 58.30, H 4.36, N 10.34; found: C 58.15, H 4.30, N 9.97.

52. *2′,3′,5′-Tri*-O-*acetyl*-N^6-*{{{4-{3-hydroxy-6-[2-(4-nitrophenyl)ethoxy]-9*H-*xanthen-9-yl}-3-(methoxycarbonyl)phenyl}amino}[2-(4-nitrophenyl)ethoxy]methylene}adenosine* (**56**). The faster-moving product from *Exper. 51* was eluted and, after evaporation, treated with a little MeOH to give a precipitate that was dried: 10 mg (8%) of **56**. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.42. UV (CH_2Cl_2): 230 (4.79), 260 (sh., 4.70), 284 (4.82), 338 (4.17), 356 (sh., 4.11), 404 (sh., 4.11), 434 (4.25), 437 (4.31), 458 (4.43), 488 (sh., 4.10). ^{1}H-NMR ((D$_6$)DMSO)^2): 11.6 (*s*, NH); 8.78 (*s*, H–C(2)); 8.75 (*s*, H–C(8)); 8.45 (*d*, H–C(6)(flu)); 8.19–8.08 (*m*, 4 H *o* to NO_2); 7.94 (*dd*, H–C(4)(flu)); 7.61 (*m*, 4 H *m* to NO_2); 7.40 (*m*, H–C(3)(flu)); 7.25 (*m*, H–C(4)(xan)); 6.92–6.83 (*m*, H–C(1), H–C(2), H–C(8)(xan)); 6.35 (*m*, H–C(7)(xan), H–C(1′)); 6.22 (*d*, H–C(5)(xan)); 6.03 (*t*, H–C(2′)); 5.65 (*t*, H–C(3′)); 4.68 (*m*, 1 CH_2CH_2O); 4.43–4.23 (*m*, 1 CH_2CH_2O, H–C(4′), 2 H–C(5′)); 3.56 (*s*, MeO); 3.23 (*m*, 2 CH_2CH_2O); 2.12, 2.06, 2.03 (3 *s*, 3 Ac). Anal. calc. for $C_{54}H_{46}N_8O_{17}$ (1079.1): C 60.11, H 4.30, N 10.39; found: C 59.69, H 4.38, N 10.50.

53. *2′-Deoxy-3′,5′*-O-*(tetraisopropyldisiloxane-1,3-diyl)adenosine* (**57**). Twice, 2′-deoxyadenosine (12.5 g, 50 mmol) was co-evaporated with dry pyridine, then dissolved in pyridine (200 ml), and treated with 1,1-dichloro-1,1,3,3-tetraisopropyldisiloxane (17.3 ml, 55 mmol). After stirring at r.t. for 12 h, the mixture was diluted with CH_2Cl_2 (300 ml) and extracted with $NaHCO_3$ soln. (100 ml). The org. phase was dried ($MgSO_4$), evaporated, and co-evaporated with toluene, the residue dissolved in CH_2Cl_2, and the soln. evaporated: 24.5 g (98%) of crude **57**, which was used without further purification for the following experiments.

54. *2′-Deoxy*-N^6-*(phenoxycarbonyl)-3′,5′*-O-*(tetraisopropyldisiloxane-1,3-diyl)adenosine* (**58**). A mixture of **57** (5 g, 10 mmol) and 1-(phenoxycarbonyl)-1*H*-tetrazole (3.8 g, 20 mmol) was stirred in dry dioxane (30 ml) at 40° for 12 h. After evaporation, the residue was treated with toluene (50 ml), the insoluble material filtered off, and the filtrate concentrated to *ca.* 20 ml and submitted to FC (silica gel; toluene/AcOEt 5:1 (250 ml), toluene/AcOEt 2:1 (600 ml); 100-ml fractions). *Fr. 4–9* were evaporated, and the residue was dried under high vacuum: 4.5 g (77%) of **58**. An anal. sample was prepared by recrystallization of 0.5 g from Et_2O (20 ml): 0.3 g of colorless crystals. M.p. 106–107°. TLC (silica gel, toluene/AcOEt/MeOH 5:4:0.5): R_f 0.70. UV (MeOH): 253 (4.15), 267 (4.29), 274 (sh., 4.18). ^{1}H-NMR ((D$_6$)DMSO): 11.1 (*s*, NH); 8.60 (*s*, H–C(2)); 8.57 (*s*, H–C(8)); 7.47–7.22 (*m*, 5 arom. H); 6.38 (*dd*, H–C(1′)); 5.17 (*q*, H–C(3′)); 3.92–3.80 (*m*, H–C(4′), 2 H–C(5′)); 2.92 (*m*, 1 H–C(2′)); 2.60 (*m*, 1 H–C(2′)); 1.03 (*m*, 2 ^{i}Pr$_2$Si). Anal. calc. for $C_{29}H_{43}N_5O_6Si_2$ (613.9): C 56.74, H 7.06, N 11.41; found: C 56.67, H 7.10, N 11.56.

55. N^6-*{{[3-Carboxy-4-(6-hydroxy-3-oxo-3*H-*xanthen-9-yl)phenyl]amino}carbonyl}-2′-deoxy-3′,5′*-O-*(tetraisopropyldisiloxane-1,3-diyl)adenosine* (**59**). Separately, **7** (1.4 g, 4 mmol) and **58** (2 g, 4 mmol), were co-evaporated with dry pyridine. Then **7** and **58** were dissolved together in dry pyridine (10 ml) and heated to 60° for 1 h. After evaporation and co-evaporation with toluene, the mixture was dissolved in CH_2Cl_2/MeOH and mixed with silica gel (10 g). This solid was put onto a FC column (silica gel (60 g); CH_2Cl_2 (100 ml), CH_2Cl_2/MeOH 98:2 (200 ml), CH_2Cl_2/MeOH 97:3 (200 ml), CH_2Cl_2/MeOH 96:4 (200 ml), CH_2Cl_2/MeOH 95:5 (200 ml), CH_2Cl_2/MeOH 9:1 (200 ml), CH_2Cl_2/MeOH 4:1 (100 ml); 100-ml fractions). *Fr. 9–11* were evaporated and co-evaporated with dioxane, and the residue was dried under high vacuum: 2.7 g (80%) of **59**. Colorless solid. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.44. UV (CH_2Cl_2): 228 (4.76), 277 (4.61), 281 (sh., 4.58), 310 (sh., 3.37), 458 (2.90). ^{1}H-NMR ((D$_6$)DMSO)^2): 12.1 (*s*, NH); 10.2 (*s*, NH, OH); 8.65 (*s*, H–C(2)); 8.56 (*s*, H–C(8)); 8.36 (*d*, H–C(6)(flu)); 7.93 (*dd*, H–C(4)(flu)); 7.25 (*d*, H–C(3)(flu)); 6.67–6.52 (*m*, 6 arom. H (xan)); 6.37 (*dd*, H–C(1′)); 5.17 (*q*, H–C(3′)); 3.92–3.79 (*m*, H–C(4′), 2 H–C(5′)); 2.90 (*m*, 1 H–C(2′)); 2.67 (*m*, 1 H–C(2′)); 1.05 (*m*, 2 ^{i}Pr$_2$Si). Anal. calc. for $C_{43}H_{50}N_6O_{10}Si_2 \cdot H_2O$ (885.1): C 58.35, H 5.92, N 9.50; found: C 58.35, H 6.06, N 9.43.

56. *2′-Deoxy*-N^6-*{{[4-(6-hydroxy-3-oxo-3*H-*xanthen-9-yl)-3-(methoxycarbonyl)phenyl]amino}carbonyl}-3′,5′*-O-*(tetraisopropyldisiloxane-1,3-diyl)adenosine* (**60**). A mixture of **9** (0.361 g, 1 mmol) and **58** (1 g, 1.6 mmol) in pyridine (10 ml) was heated to 60° with stirring for 45 min. After evaporation and co-evaporation with toluene, the residue was dissolved in a little CH_2Cl_2 and submitted to FC (silica gel (20 g); CH_2Cl_2 (150 ml), CH_2Cl_2/MeOH 98:2 (100 ml), CH_2Cl_2/MeOH 96:4 (100 ml), CH_2Cl_2/MeOH 94:6 (100 ml), CH_2Cl_2/MeOH 9:1 (200 ml); 50-ml fractions). *Fr. 7–11* were evaporated, and the residue was treated with MeOH: 0.75 g (85%) of **60**. Orange powder. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.31. UV (CH_2Cl_2): 230 (4.61), 253 (4.45), 277 (4.65), 307 (sh., 4.08), 341 (3.99), 404 (4.01), 433 (4.24), 454 (4.30), 484 (sh., 4.09). ^{1}H-NMR ((D$_6$)DMSO)^2): 12.1 (*s*, NH); 11.2 (br. *s*, OH); 10.4 (*s*, NH); 8.65 (*s*, H–C(2)); 8.57 (*s*, H–C(8), H–C(6)(flu)); 8.03 (*dd*, H–C(4)(flu)); 7.45 (*d*, H–C(3)(flu)); 6.89–6.55 (*m*, 6 arom. H (xan)); 6.38 (*dd*, H–C(1′)); 5.17 (*q*, H–C(3′)); 3.94–3.80 (*m*, H–C(4′), 2 H–C(5′)); 2.90 (*m*, 1 H–C(2′)); 2.62 (*m*, 1 H–C(2′)); 1.06 (*m*, 2 ^{i}Pr$_2$Si). Anal. calc. for $C_{44}H_{52}N_6O_{10}Si_2$ (881.1): C 59.98, H 5.95, N 9.54; found: C 59.95, H 5.94, N 9.43.

57. *2′-Deoxy-*N^6*-{[2-(4-nitrophenyl)ethoxy]{[3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-[6-[2-(4-nitrophenyl)-ethoxy]-3-oxo-3*H*-xanthen-9-yl}phenyl}amino}methylene}-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)adenosine* (**61**). A mixture of **59** (2.7 g, 3.1 mmol), PPh_3 (4.8 g, 18 mmol), and 2-(4-nitrophenyl)ethanol (4.1 g, 24 mmol) in dry dioxane (50 ml) was heated to 60° with stirring till a clear dark red soln. was obtained. Diethyl azodicarboxylate (3 ml, 18 mmol) was added and heating at 60° continued for 1.5 h (→ orange). The mixture was diluted with CH_2Cl_2 (300 ml) and washed with phosphate buffer (pH 7; 100 ml). The org. phase was dried ($MgSO_4$) and evaporated, and the resulting oil dissolved in toluene (20 ml) and submitted to FC (silica gel (100 g), toluene (200 ml), toluene/AcOEt 4:1 (500 ml), toluene/AcOEt 1:1 (500 ml), toluene/AcOEt 4:1 (480 ml) + MeOH (20 ml) and toluene/AcOEt 4:1 (450 ml) + MeOH (50 ml); 100-ml fractions). *Fr. 13–20* were evaporated and co-evaporated with EtOH, and the residue was treated with MeOH by ubltrasound. The precipitate was stirred in MeOH for 1 h, collected, and dried under high vacuum: 3.7 g (90%) of **61**. Orange solid. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.69. UV (CH_2Cl_2): 230 (4.71), 262 (sh., 4.71), 284 (4.80), 338 (4.10), 406 (sh., 4.06), 436 (4.30), 458 (4.35), 486 (sh., 4.14). ^{1}H-NMR ((D$_6$)DMSO)^2): 11.7 (*s*, NH); 8.66 (*s*, H–C(2)); 8.63 (*s*, H–C(8)); 8.50 (*d*, H–C(6)(flu)); 8.19–8.01 (*m*, 6 H *o* to NO_2); 7.88 (*dd*, H–C(4)(flu)); 7.64–7.55 (*m*, 4 H *m* to NO_2); 7.35 (*m*, 2 H *m* to NO_2, H–C(3)(flu)); 7.18 (*d*, H–C(4)(xan)); 6.87–6.77 (*m*, H–C(1), H–C(2), H–C(8)(all xan)); 6.42–6.33 (*m*, H–C(1′), H–C(7)(xan)); 6.16 (*d*, H–C(5)(xan)); 5.17 (*q*, H–C(3′)); 4.73–4.22 (*m*, 3 CH_2CH_2O); 3.92–3.83 (*m*, H–C(4′), 2 H–C(5′)); 3.22 (*m*, 2 CH_2CH_2O); 2.82 (*m*, 1 CH_2CH_2O, 1 H–C(2′)); 2.65 (*m*, H–C(2′)); 1.05 (*m*, 2 ^{i}Pr$_2$Si). Anal. calc. for $C_{67}H_{71}N_9O_{16}Si_2$ (1314.5): C 61.22, H 5.44, N 9.59; found: C 61.07, H 5.42, N 9.00.

58. *2′-Deoxy-*N^6*-{[2-(4-nitrophenyl)ethoxy]{[3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-[6-[2-(4-nitrophenyl)-ethoxy]-3-oxo-3*H*-xanthen-9-yl]phenyl}amino}methylene}adenosine* (**62**). To a soln. of **61** (1 g, 0.76 mmol) in THF (10 ml), AcOH (400 µl) and $Bu_4NF \cdot 3\ H_2O$ (0.718 g, 2.28 mmol) were added, and the mixture was stirred at r.t. for 12 h. After dilution with CH_2Cl_2 (300 ml) and washing with $NaHCO_3$ soln. (100 ml), the org. phase was dried ($MgSO_4$), and the resulting oil dissolved in CH_2Cl_2 (10 ml) and submitted to FC (silica gel (20 g); CH_2Cl_2 (100 ml), CH_2Cl_2/MeOH 98:2 (100 ml), CH_2Cl_2/MeOH 95:5 (200 ml), CH_2Cl_2/MeOH 9:1 (200 ml); 50-ml fractions). *Fr. 7–10* were evaporated and co-evaporated with EtOH, and the residue was treated in EtOH with ultrasound: 0.68 g (84%) of **62**. Orange solid. TLC (silica gel, CH_2Cl_2/MeOH 9:1): R_f 0.35. UV (H_o −4): 222 (4.80), 253 (4.70), 298 (4.65), 444 (4.71). UV (pH 0): 222 (4.80), 259 (4.68), 290 (4.70), 431 (sh., 4.45), 455 (4.59), 504 (sh., 3.56). UV (pH 3–13): 283 (4.69), 356 (4.03), 440 (sh., 4.21), 465 (4.33), 495 (4.23). Basic pK_a: −1.54, 0.68. ^{1}H-NMR ((D$_6$)DMSO)^2): 11.9 (*s*, NH); 8.76 (*s*, H–C(2)); 8.74 (*s*, H–C(8)); 8.50 (*d*, H–C(6)(flu)); 8.19–8.01 (*m*, 6 H *o* to NO_2); 7.88 (*dd*, H–C(4)(flu)); 7.70 (*m*, 4 H *m* to NO_2); 7.38–7.31 (*m*, 2 H *m* to NO_2, H–C(3)(flu)); 7.17 (*d*, H–C(4)(xan)); 6.84 (*m*, H–C(1), H–C(2), H–C(8)(all xan)); 6.48 (*t*, H–C(1′)); 6.35 (*dd*, H–C(7)(xan)); 6.16 (*d*, H–C(5)(xan)); 5.38 (*d*, OH–C(3′)); 5.04 (*t*, OH–C(5′)); 4.75–4.22 (*m*, H–C(3′), 3 CH_2CH_2O); 3.90 (*m*, H–C(4′)); 3.56 (*m*, 2 H–C(5′)); 3.23 (*m*, 2 CH_2CH_2O); 2.76 (*m*, 1 CH_2CH_2O, 1 H–C(2′)); 2.47 (*m*, 1 H–C(2′)). Anal. calc. for $C_{55}H_{45}N_9O_{15}$ (1072.2): C 61.62, H 4.23, N 11.76; found: C 61.41, H 4.42, N 11.73.

59. *2′-Deoxy-5′-*O*-(dimethoxytrityl)-*N^6*-{[2-(4-nitrophenyl)ethoxy]{[3-{[2-(4-nitrophenyl)ethoxy]carbon-yl}-4-[6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl}phenyl}amino}methylene}adenosine* (**63**). Compound **62** (10.7 g, 10 mmol) was twice co-evaporated with pyridine (30 ml), then dissolved in dry pyridine (100 ml), and treated with dimethoxytrityl chloride (4.1 g, 12 mmol) under stirring at r.t. for 3 h. The soln. was concentrated to 50 ml, diluted with CH_2Cl_2 (300 ml), and extracted with $NaHCO_3$ soln. The org. phase was separated, dried ($MgSO_4$), evaporated, and co-evaporated with toluene. The residue was dissolved in CH_2Cl_2 (50 ml) and submitted to FC (silica gel (150 g); toluene/EtOH 4:1 (200 ml), toluene/EtOH 1:1 (500 ml), toluene/EtOH 1:1 (195 ml) + MeOH (5 ml), toluene/EtOH 1:1 (190 ml) + MeOH (10 ml), toluene/EtOH 1:1 (185 ml) + MeOH (15 ml), toluene/EtOH 1:1 (360 ml) + MeOH (40 ml), toluene/EtOH 1:1 (450 ml) + MeOH (50 ml), EtOH/MeOH 4:1 (300 ml); 100-ml fractions). *Fr. 14–20* were evaporated and co-evaporated with EtOH, and the residue was suspended in EtOH and treated with ultrasound. The mixture was stirred overnight and the precipitate collected and dried: 12.0 g (87%) of **63**. M.p. starting at 139° (dec.). TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.42. UV (CH_2Cl_2): 231 (4.82), 282 (4.79), 340 (4.08), 406 (sh., 4.03), 436 (4.27), 490 (sh., 4.12). ^{1}H-NMR ((D$_6$)DMSO)^2): 12.1 (*s*, NH); 8.70 (*s*, H–C(2)); 8.66 (*s*, H–C(8)); 8.52 (*d*, H–C(6)(flu)); 8.18–8.01 (*m*, 6 H *o* to NO_2); 7.88 (*dd*, H–C(4)(flu)); 7.60 (*m*, 4 H *m* to NO_2); 7.38–7.13 (*m*, 2 H *m* to NO_2, H–C(3)(flu), H–C(4)(xan), 9 arom. H); 6.86–6.75 (*m*, H–C(1), H–C(2), H–C(8)(all xan), 4 H *m* to MeO); 6.51 (*t*, H–C(1′)); 6.34 (*dd*, H–C(7)(xan)); 6.15 (*d*, H–C(5)(xan)); 5.4 (*d*, OH–C(3′)); 4.75–4.22 (*m*, H–C(3′), 3 CH_2CH_2O); 4.04 (*m*, H–C(4′)); 3.66 (2 *s*, 2 MeO); 3.23 (*m*, 2 CH_2CH_2O, 2 H–C(5′)); 2.76 (*m*, 1 CH_2CH_2O, 1 H–C(2′)); 2.45 (*m*, 1 H–C(2′)). Anal. calc. for $C_{76}H_{63}N_9O_{17} \cdot H_2O$ (1392.4): C 65.56, H 4.71, N 9.05; found: C 65.80, H 4.84, N 8.88.

60. *2′-Deoxy-5′*-O-*(dimethoxytrityl)*-N^6-*{[2-(4-nitrophenyl)ethoxy]{[3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H-*xanthen-9-yl}phenyl}amino}methylene}adenosine 3′-[2-(4-Nitrophenyl) Diisopropylphosphoramidite]* (**64**). To a soln. of **63** (0.5 g, 0.36 mmol) in CH_2Cl_2 (7 ml) under N_2 Et^iPr_2N (250 µl, 1.4 mmol) and 2-(4-nitrophenyl)ethyl diisopropylphosphoramidochloridite (0.242 g, 0.72 mmol) were added and stirred at r.t. for 1 h. After dilution with CH_2Cl_2 (100 ml) and washing with $NaHCO_3$ soln., the org. phase was dried ($MgSO_4$), and evaporated and the Et^iPr_2N removed under high vacuum. The residue was purified by FC (Al_2O_3 (neutral, 75 g); CH_2Cl_2/AcOEt 1:1 (200 ml), AcOEt (200 ml), AcOEt (100 ml) + MeOH (3 ml) and AcOEt/MeOH 95:5 (300 ml); 50-ml fractions). *Fr. 9–16* were evaporated and co-evaporated with CH_2Cl_2: 0.48 g (79%) of **64**. Solid foam. TLC (silica gel, toluene/AcOEt/MeOH 10:9:1): R_f 0.57. ^{1}H-NMR ($CDCl_3$)^2): 13.71 (*s*, NH); 8.64 (*s*, H–C(2)); 8.43 (*d*, H–C(6)(flu)); 8.33 (*s*, H–C(8)); 8.20–8.01 (*m*, 8 H *o* to NO_2, H–C(4)(flu), H–C(3)(flu)); 7.60–7.16 (*m*, 21 H, 8 H *m* to NO_2, arom. H); 6.92–6.50 (*m*, H–C(1), H–C(2), H–C(4), H–C(7), H–C(8)(all xan), 4 H *m* to MeO, H–C(1′)); 6.39 (*d*, H–C(5)(xan)); 4.99 (*m*, 1 CH_2CH_2O); 4.73 (*m*, H–C(3′)); 4.31 (*m*, 2 CH_2CH_2O); 3.73 (2 *s*, 2 MeO); 3.77–2.61 (*m*, 3 CH_2CH_2O, H–C(4′), 2 H–C(5′), 2 H–C(2′), 2 Me_2CH); 1.1–1.0 (*m*, 2 *Me*$_2$CH). ^{31}P-NMR ($CDCl_3$): 148.3; 148.2. Anal. calc. for $C_{90}H_{84}N_{11}O_{20}$ (1670.7): C 64.70, H 5.07, N 9.22; found: C 62.97, H 5.07, N 8.73.

61. *2′-Deoxy-5′*-O-*(dimethoxytrityl)*-N^6-*{[2-(4-nitrophenyl)ethoxy]{[3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H-*xanthen-9-yl}phenyl}amino}methylene}adenylyl-{3′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy*-N^6,3′-O-*bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**65**). To a mixture of **64** (0.63 g, 0.37 mmol) and **46** (0.12 g, 0.19 mmol) in MeCN (5 ml) and CH_2Cl_2 (5 ml) under N_2, 1*H*-tetrazole (0.1 g) was added and stirred for 30 min. Then a soln. of I_2 (0.5 g) in pyridine/CH_2Cl_2/H_2O 5:1:1 (7 ml) was added and stirred for 15 min for oxidation. After dilution with CH_2Cl_2 (200 ml) and decolorization with $Na_2S_2O_3$ soln., the org. phase was dried ($MgSO_4$), evaporated, and co-evaporated with toluene to remove pyridine. The crude material was dissolved in CH_2Cl_2 and submitted to FC (silica gel (25 g); toluene (50 ml), toluene/AcOEt 1:1 (100 ml), toluene/AcOEt 1:1 (97 ml) + MeOH (3 ml), toluene/AcOEt 1:1 (190 ml) + MeOH (10 ml), toluene/AcOEt 1:1 (180 ml) + MeOH (20 ml); 40-ml fractions). *Fr. 12–15* were evaporated and co-evaporated with EtOH. The residue was suspended in MeOH and exposed to ultrasound and the solid collected and dried under high vacuum: 0.25 g of **65**. *Fr. 11* was rechromatographed to give another 0.06 g. Total yield 74%. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.20. UV ((D$_6$)DMSO): 234 (4.81), 274 (4.93), 342 (sh., 4.01), 412 (sh., 4.00), 434 (sh., 4.30), 458 (4.26), 490 (sh., 4.04). ^{1}H-NMR ($CDCl_3$)^2): 12.1 (*s*, NH); 10.6 (br. *s*, NH); 8.61–8.52 (*m*, 5 H, H–C(2), H–C(8), H–C(6)(flu)); 8.18–7.99 (*m*, 12 H *o* to NO_2); 7.88 (*m*, H–C(4)(flu)); 7.64–7.10 (*m*, 23 H, H *m* to NO_2, arom. H, H–C(3)(flu), H–C(4)(xan)); 6.86–6.71 (*m*, 7 H, H–C(1), H–C(2), H–C(8)(all xan), H *m* to MeO, H–C(1′)); 6.44–6.32 (*d*, 3 H, H–C(1′), H–C(7)(xan)); 6.15 (*d*, H–C(5)(xan)); 5.32 (*m*, H–C(3′)); 5.09 (*m*, H–C(3′)); 4.73 (*m*, 2 H, CH_2CH_2O); 4.44–4.19 (*m*, 14 H, CH_2CH_2O, H–C(4′), H–C(5′)); 3.64 (2 *s*, 2 MeO); 3.23–2.57 (*m*, 18 H, CH_2CH_2O, H–C(5′), H–C(2′)). Anal. calc. for $C_{112}H_{96}N_{17}O_{32}P_2$ (2223.1): C 60.51, H 4.35, N 10.71; found: C 60.34, H 4.35, N 10.99.

62. *2′-Deoxy*-N^6-*{[2-(4-nitrophenyl)ethoxy]{[3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)-ethoxy]-3-oxo-3*H-*xanthen-9-yl}phenyl}amino}methylene}adenylyl-{3′-{O^P-[2-(4-nitrophenyl)ethyl}-5′}-2′-deoxy*-N^6,3′-O-*bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**66**). A soln. of **65** (1.1 g, 0.495 mmol) in 3% Cl_3CCOOH/CH_2Cl_2 (50 ml) was stirred at r.t. for 30 min. After washing with $NaHCO_3$ soln., the org. phase was dried ($MgSO_4$) and evaporated. The residue was dissolved in CH_2Cl_2 and purified by FC (silica gel (45 g); toluene (50 ml), toluene/AcOEt 1:1 (380 ml) + MeOH (20 ml), toluene/AcOEt 1:1 (270 ml) + MeOH (30 ml), toluene/AcOEt 1:1 (255 ml) + MeOH (45 ml), toluene/AcOEt/MeOH 1:1:1 (200 ml); 100-ml fractions). *Fr. 12* and *13* were evaporated and co-evaporated with EtOH, and the residue was treated with MeOH and exposed to ultrasound: 0.73 g of **66**. *Fr. 11* and *14* were rechromatographed and gave a second crop (0.15 g) for a total yield of 0.88 g (92%) of **66**. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.37. UV (CH_2Cl_2): 270 (sh., 4.90), 274 (4.98) 292 (sh., 4.86), 337 (4.10), 408 (sh., 4.02), 436 (sh., 4.24), 459 (4.30), 488 (sh., 4.09). ^{1}H-NMR ((D$_6$)DMSO)^2): 12.0 (*s*, NH); 10.6 (br. *s*, NH); 8.72–8.50 (*m*, 5 H, H–C(2), H–C(8), H–C(6)(flu)); 8.18–8.01 (*m*, 12 H *o* to NO_2); 7.88 (*m*, H–C(4)(flu)); 7.64–7.32 (*m*, 13 H, H *m* to NO_2, H–C(3)(flu); 7.18 (*m*, H–C(4)(xan)); 6.84 (*m*, H–C(1), H–C(2), H–C(8)(all xan)); 6.42–6.33 (*d*, 3 H, H–C(1′), H–C(7)(xan)); 6.15 (*d*, H–C(5)(xan)); 5.34 (*m*, 1 H, H–C(3′)); 5.17 (*t*, 1 H, OH–C(5′)); 5.00 (*m*, 1 H, H–C(3′)); 4.75 (*m*, 2 H, CH_2CH_2O); 4.44–4.04 (*m*, 14 H, CH_2CH_2O, H–C(4′), H–C(5′)); 3.51 (*m*, 2 H, H–C(5′)); 3.23–2.57 (*m*, 16 H, CH_2CH_2O, H–C(5′), H–C(2′)). Anal. calc. for $C_{91}H_{78}N_{17}O_{30}P_2$ (1920.7): C 56.91, H 4.09, N 12.40; found: C 56.35, H 4.15, N 12.99.

63. *2′-Deoxy*-N^6,*5′*-O-*bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenylyl-{3′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy*-N^6-*{[2-(4-nitrophenyl)ethoxy]{[3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H-*xanthen-9-yl}phenyl}amino}methylene}adenylyl-{3′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy*-N^6,3′-

O-*bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**67**). To a mixture of **45** (0.5 g, 0.54 mmol) and **66** (0.4 g, 0.21 mmol) in MeCN (7 ml) and CH_2Cl_2 (7 ml) under N_2, 1*H*-tetrazole (0.2 g) was added and stirred at r.t. for 90 min. After oxidation with I_2 (0.5 g) in pyridine/CH_2Cl_2/H_2O 5:1:1 (7 ml) and stirring for 15 min, the mixture was diluted with CH_2Cl_2 (200 ml) and then decolorized with $Na_2S_2O_3$ soln. The org. phase was dried ($MgSO_4$), evaporated, and co-evaporated with toluene until the pyridine was completely removed. The residue was dissolved in CH_2Cl_2 (10 ml) and purified by FC (silica gel (30 g); toluene (50 ml), toluene/AcOEt 1:1 (100 ml), toluene/AcOEt 1:1 (190 ml) + MeOH (10 ml), toluene/AcOEt 1:1 (360 ml) + MeOH (40 ml), toluene/AcOEt 1:1 (340 ml) + MeOH (60 ml), toluene/AcOEt/MeOH 1:1:1 (100 ml); 50-ml fractions). *Fr. 16–23* were evaporated and co-evaporated with EtOH, and the resulting solid was treated in MeOH by ultrasound: 0.49 g (84%) of **67**. TLC (silica gel, CH_2Cl_2/MeOH 95:5): R_f 0.32. UV (MeOH/CH_2Cl_2 1:1): 269 (5.16), 346 (4.09), 405 (sh., 3.91), 434 (sh., 4.25), 460 (4.40), 488 (4.29). ^{1}H-NMR ((D$_6$)DMSO)^2): 12.3 (*s*, NH); 10.6 (br. *s*, NH); 8.68–8.48 (*m*, 7 H, H–C(2), H–C(8), H–C(6)(flu)): 8.18–7.96 (*m*, 18 H *o* to NO_2); 7.86 (*m*, H–C(4)(flu)); 7.64–7.10 (*m*, 19 H, H *m* to NO_2, H–C(3)(flu)); 7.16 (*m*, H–C(4)(xan)); 6.81 (*m*, H–C(1), H–C(2), H–C(8)(all xan)); 6.45–6.33 (*d*, 4 H, H–C(1′), H–C(7)(xan)); 6.15 (*d*, H–C(5)(xan)); 5.34 (*m*, 1 H, H–C(3′)); 5.14 (*m*, 2 H, H–C(3′)); 4.72 (*m*, 2 H, CH_2CH_2O); 4.42–4.21 (*m*, 25 H, CH_2CH_2O, H–C(4′), H–C(5′)); 3.32–2.57 (*m*, 24 H, CH_2CH_2O, H–C(2′)). Anal. calc. for $C_{127}H_{111}N_{25}O_{45}P_2$ (2769.4): C 55.08, H 4.04, N 12.64; found: C 54.95, H 4.23, N 12.68.

64. *2′-Deoxyadenylyl-(3′-5′)*-N^6-*{{[3-carboxy-4-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)phenyl]amino}[2-(4-nitrophenyl)ethoxy]methylene}-2′-deoxyadenylyl-(3′-5′)-2′-deoxyadenosine* (**68**). This compound was only chromatographically detected but not isolated.

65. N^6-*{{[3-Carboxy-4-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)phenyl]amino}[2-(4-nitrophenyl)ethoxy]methylene}-2′-deoxy-5′*-O-*(dimethoxytrityl)adenosine* (**69**). A soln. of **63** (0.5 g, 0.36 mmol) in 0.5M DBU in pyridine (50 ml) was stirred at r.t. for 4 h. AcOH (1 ml) was added and then the mixture extracted with CH_2Cl_2/KH_2PO_4 soln. The org. phase was dried and evaporated. The residue was dissolved in CH_2Cl_2 and purified by FC (silica gel (45 g); CH_2Cl_2 (50 ml), CH_2Cl_2/MeOH 95:5 (150 ml), CH_2Cl_2/MeOH 1:1 (150 ml), CH_2Cl_2/MeOH 4:1 (100 ml); 50-ml fractions). *Fr. 4–9* were evaporated, and the residue was treated in MeOH with ultrasound: 0.31 g (80%) of **69**. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.69. UV (CH_2Cl_2): 284 (4.65), 362 (3.63), 425 (sh., 3.84), 453 (4.10), 480 (4.11). ^{1}H-NMR ((D$_6$)DMSO)^2): 12.2 (*s*, OH); 10.1 (*m*, 2 H, HN); 8.70 (*s*, H–C(2)); 8.64 (*s*, H–C(8)); 8.28 (*d*, H–C(6)(flu)); 8.09 (*d*, 2 H *o* to NO_2); 7.85 (*d*, H–C(4)(flu)); 7.55 (*d*, 2 H *m* to NO_2); 7.31 (*d*, H–C(3)(flu)); 7.22–7.13 (*m*, 9 arom. H); 6.81–6.74 (*m*, 4 H *o* to MeO); 6.66–6.47 (*t*, H–C(1′), 6 arom. H (xan)); 5.43 (*d*, OH–C(3′)); 4.74 (*t*, CH_2CH_2O); 4.49 (*m*, H–C(3′)); 4.02 (*m*, H–C(4′)); 3.67 (2 *s*, 2 MeO); 3.40–3.19 (*m*, CH_2CH_2O, 2 H–C(5′)); 2.87 (*m*, H–C(2′)); 2.40 (*m*, H–C(2′)). Anal. calc. for $C_{60}H_{49}N_7O_{13} \cdot H_2O$ (1094.1): C 65.60, H 4.70, N 8.96; found: C 65.60, H 5.15, N 9.18.

66. *2′,3′,5′-Tri*-O-*acetyl*-N^6-*{{[4-{3-hydroxy-6-[2-(4-nitrophenyl)ethoxy]-9*H*-xanthen-9-yl}-3-{[2-(4-nitrophenyl)ethoxy]carbonyl}phenyl}amino}carbonyl}adenosine* (**70**). To a mixture of **11** (0.1 g, 1.3 mmol) and 2-(4-nitrophenyl)ethanol (0.022 g, 1.3 mmol) in dioxane (3 ml) was added diethyl diazocarboxylate (123 µl, 0.78 mmol). The mixture was heated to 60° with stirring until a clear soln. was obtained. Then PPh_3 (0.16 g, 0.6 mmol) was added in small portions within 3 h. After dilution with CH_2Cl_2 (30 ml) and washing with phosphate buffer (pH 7; 10 ml), the org. phase was separated and dried ($MgSO_4$) and the resulting dark orange oil dissolved in toluene/AcOEt 1:1 (10 ml) and submitted to FC (silica gel (25 g); toluene (50 ml), toluene/AcOEt 1:1 (50 ml), toluene/AcOEt 1:1 (97 ml) + MeOH (3 ml), toluene/AcOEt 1:1 (190 ml) + MeOH (10 ml), toluene/AcOEt 1:1 (150 ml) + MeOH (15 ml); 30-ml fractions). *Fr. 9–12* yielded 0.07 g (48%) of **70**. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.33. UV (CH_2Cl_2): 256 (sh., 4.63), 277 (4.81), 338 (4.07), 354 (sh., 4.02), 404 (sh., 4.04), 437 (4.31), 458 (4.38), 487 (4.15). ^{1}H-NMR ((D$_6$)DMSO)^2): 11.7 (*s*, NH); 10.3 (*s*, H–N(6); 8.73 (*s*, H–C(2)); 8.69 (*s*, H–C(8)); 8.55 (*d*, H–C(6)(flu)); 8.15 (*d*, 2 H *o* to NO_2); 8.02 (*d*, 2 H *o* to NO_2); 7.95 (*dd*, H–C(4)(flu)); 7.62 (*m*, 2 H *m* to NO_2); 7.40 (*d*, H–C(3)(flu)); 7.32 (*m*, 2 H *m* to NO_2); 7.14 (*m*, H–C(4)(xan)); 6.90–6.78 (*m*, H–C(1), H–C(2), H–C(8)(all xan)); 6.35 (*m*, H–C(1′), H–C(7)(xan)); 6.14 (*d*, H–C(5)(xan)); 6.06 (*t*, H–C(2′)); 5.66 (*t*, H–C(3′)); 4.45–4.24 (*m*, 2 CH_2CH_2O, H–C(4′), 2 H–C(5′)); 3.32 (*m*, 1 CH_2CH_2O); 2.82 (*t*, 1 CH_2CH_2O); 2.13, 2.05, 202 (3 *s*, 3 Ac). Anal. calc. for $C_{53}H_{44}N_8O_{17}$ (1064.95): C 59.77, H 4.16, N 10.52; found: C 59.49, H 4.30, N 10.52.

67. *2′,3′,5′-Tri*-O-*acetyl*-N^6-*{{[4-{3-hydroxy-6-[2-(4-nitrophenyl)ethoxy]-9*H*-xanthen-9-yl}-3-{[2-(4-nitrophenyl)ethoxy]carbonyl}phenyl}amino}[2-(4-nitrophenyl)ethoxy]methylene}adenosine* (**71**). *Fr. 6–8* from *Exper. 64* were evaporated and dried: 60 mg (38%) of **71**. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.44. UV (CH_2Cl_2): 260 (sh., 4.67), 283 (4.77), 338 (4.08), 354 (sh., 4.03), 406 (sh., 4.03), 437 (sh., 4.28), 459 (4.33), 481 (4.11). ^{1}H-NMR ((D$_6$)DMSO)^2): 11.6 (*s*, NH); 8.75 (*s*, H–C(2)); 8.47 (*s*, H–C(8)); 8.20–8.02 (3*d*, 6 H *o* to NO_2); 7.86 (*dd*, 1 H, H–C(4)(flu)); 7.64–7.31 (*m*, 6 H *m* to NO_2, H–C(3)(flu)); 7.17 (*m*, H–C(4)(xan)); 6.84

(*m*, H–C(1), H–C(2), H–C(8)(all xan)); 6.35 (*m*, H–C(1′), H–C(7)(xan)); 6.14 (*d*, H–C(5)(xan)); 6.06 (*t*, H–C(2′)); 5.66 (*t*, H–C(3′)); 4.71–4.24 (*m*, 3 CH_2CH_2O, H–C(4′), 2 H–C(5′)); 3.24 (*m*, 2 CH_2CH_2O); 2.82 (*t*, 1 CH_2CH_2O); 2.13, 2.05, 2.02 (3 *s*, 3 Ac). Anal. calc. for $C_{61}H_{51}N_9O_{19}$ (1214.1): C 60.34, H 4.23, N 10.38; found: C 60.29, H 4.45, N 10.02.

68. *2′-Deoxy*-N^6-*{{{3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H-*xanthen-9-yl}phenyl}amino}carbonyl}-3′,5′*-O-*(tetraisopropyldisiloxane-1,3-diyl)adenosine* (**72**). A mixture of **59** (3 g, 3.5 mmol) and 2-(4-nitrophenyl)ethanol (3 g, 18 mml) in dry dioxane (20 ml) was heated to 60° with stirring till a clear soln. was obtained. Then diethyl diazodicarboxylate (3 ml, 18 mmol) and PPh_3 (1.7 g, 7.3 mmol) were added and stirred for 10 min, whereby all educt had disappeared, and very little tris-substituted product could be detected by TLC. After dilution with CH_2Cl_2 (300 ml) and washing with phosphate buffer (pH 7; 100 ml), the org. phase was dried ($MgSO_4$) and evaporated and the obtained orange oil dissolved in toluene (20 ml) and purified by FC (silica gel (70 g); toluene (200 ml), toluene/AcOEt 7:3 (200 ml), toluene/AcOEt 1:1 (200 ml), toluene/AcOEt 1:1 (295 ml) + MeOH (5 ml), toluene/AcOEt 1:1 (380 ml) + MeOH (20 ml) and toluene/AcOEt 1:1 (360 ml) + MeOH (40 ml); 100-ml fractions). *Fr. 13–17* were evaporated and co-evaporated with EtOH, and the residue was treated with MeOH by ultrasound to give, after drying, 2.6 g (64%) of **72**. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.78. UV (CH_2Cl_2/MeOH 1:1): 231 (4.76), 278 (4.84), 310 (sh., 4.18), 355 (4.06), 406 (sh., 3.99), 436 (sh., 4.30), 460 (4.44), 489 (4.33). ^{1}H-NMR ((D$_6$)DMSO)^2): 12.0 (*s*, NH); 10.3 (*s*, NH); 8.65 (*s*, H–C(2)); 8.57 (*s*, H–C(8)); 8.56 (*d*, H–C(6)(flu)); 8.17–8.02 (*m*, 4 H *o* to NO_2); 7.96 (*dd*, H–C(4)(flu)); 7.61 (*m*, 2 H *m* to NO_2); 7.39 (*d*, H–C(3)(flu)); 7.33 (*m*, 2 H *m* to NO_2); 7.15 (*d*, H–C(4)(xan)); 6.89–6.78 (*m*, H–C(1), H–C(2), H–C(8)(all xan)); 6.37 (*m*, H–C(1′), H–C(7)(xan)); 6.15 (*d*, H–C(5)(xan)); 5.16 (*q*, H–C(3′)); 4.42 (*t*, 1 CH_2CH_2O); 4.25 (*t*, 1 CH_2CH_2O); 3.92–3.83 (*m*, H–C(4′), 2 H–C(5′)); 3.22 (*t*, 1 CH_2CH_2O); 2.97–2.82 (*m*, 1 H–C(2′), 1 CH_2CH_2O); 2.65 (*m*, H–C(2′)); 1.05 (*m*, 2 ^{i}Pr$_2$Si). Anal. calc. for $C_{59}H_{64}N_8O_{14}Si_2$ (1165.4): C 60.81, H 5.54, N 9.62; found: C 60.47, H 5.63, N 9.68.

69. *2′-Deoxy*-N^6-*{{{3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H-*xanthen-9-yl}phenyl}amino}carbonyl}adenosine* (**73**). To a soln. of **72** (3 g, 2.6 mmol) in THF (30 ml) were added AcOH (1.5 ml) and $Bu_4NF \cdot 3\ H_2O$ (2.1 g, 6.6 mmol), and the mixture was stirred at r.t. for 16 h. After dilution with CH_2Cl_2 (300 ml), and washing with $NaHCO_3$ soln. (100 ml), the org. phase was dried ($MgSO_4$) and evaporated. The obtained crude orange oil was treated in EtOH by ultrasound and the precipitate collected and dried: 2.2 g (89%) of **73**. The substance was pure enough for the dimethoxytritylation to **74**. A sample (0.5 g) was purified by FC (silica gel (50 g); CH_2Cl_2 (100 ml), CH_2Cl_2/MeOH 98:2 (200 ml), CH_2Cl_2/MeOH 95:5 (200 ml), CH_2Cl_2 (555 ml) + MeOH (45 ml) and CH_2Cl_2 (170 ml) + MeOH (30 ml); 50-ml fractions). *Fr. 15–22* were evaporated and co-evaporated with EtOH, and the residue was treated in EtOH by ultrasound: 0.38 g of **73**. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.25. UV (CH_2Cl_2/MeOH 1:1): 231 (4.70), 278 (4.80), 308 (sh., 4.19), 358 (4.02), 408 (sh., 3.97), 435 (sh., 4.28), 460 (4.43), 488 (4.32). ^{1}H-NMR ((D$_6$)DMSO)^2): 12.1 (*s*, NH); 10.5 (*s*, NH); 8.71 (*s*, H–C(2), H–C(8)); 8.57 (*d*, H–C(6)(flu)); 8.17 (*d*, 2 H *o* to NO_2); 8.03 (*d*, 2 H *o* to NO_2); 7.95 (*dd*, H–C(4)(flu)); 7.62 (*d*, 2 H *m* to NO_2); 7.41 (*d*, H–C(3)(flu)); 7.33 (*m*, 2 H *m* to NO_2); 7.17 (*d*, H–C(4)(xan)); 6.84 (*m*, H–C(1), H–C(2), H–C(8)(all xan)); 6.46 (*t*, H–C(1′)); 6.35 (*m*, H–C(7)(xan)); 6.15 (*d*, H–C(5)(xan)); 5.37 (*d*, OH–C(3′)); 5.03 (*t*, OH–C(5′)); 4.43 (*m*, H–C(3′), 1 CH_2CH_2O); 4.25 (*t*, 1 CH_2CH_2O); 3.90 (*m*, H–C(4′)): 3.58 (*m*, 2 H–C(5′)); 3.22 (*m*, 1 CH_2CH_2O); 2.97–2.82 (*m*, 1 H–C(2′), 1 CH_2CH_2O); 2.65 (*m*, 1 H–C(2′)). Anal. calc. for $C_{47}H_{38}N_8O_{13} \cdot H_2O$ (940.9): C 60.00, H 4.28, N 11.91; found: C 60.41, H 4.79, N 11.87.

70. *2′-Deoxy-5′*-O-*(dimethoxytrityl)*-N^6-*{{{3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)-ethoxy]-3-oxo-3*H-*xanthen-9-yl]phenyl}amino}carbonyl}adenosine* (**74**). A soln. of **73** (1.5 g, 2.6 mmol) was co-evaporated twice in dry pyridine (10 ml). Then the residue was dissolved in pyridine (20 ml), and dimethoxytrityl chloride (0.61 g, 1.8 mmol) was added and stirred at r.t. for 16 h. After dilution with CH_2Cl_2 (100 ml) and washing with $NaHCO_3$ soln., the org. phase was dried ($MgSO_4$), evaporated, and co-evaporated with toluene and the residue dissolved in a little CH_2Cl_2 and purified by FC (silica gel (50 g); toluene (50 ml), toluene/AcOEt 1:1 (200 ml), toluene/AcOEt 1:1 (195 ml) + MeOH (5 ml), toluene/AcOEt 1:1 (190 ml) + MeOH (10 ml), toluene/AcOEt 1:1 (555 ml) + MeOH (45 ml), toluene/AcOEt 1:1 (150 ml) + MeOH (50 ml); 100-ml fractions). *Fr. 10–12* were evaporated and co-evaporated with EtOH, and the residue was treated in EtOH by ultrasound: 1.5 g (76%) of **74**. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.57. UV (CH_2Cl_2/MeOH 1:1): 232 (4.86), 278 (4.85), 308 (sh., 4.21), 356 (4.04), 406 (sh., 3.97), 434 (sh., 4.30), 459 (4.45), 489 (4.34). ^{1}H-NMR ((D$_6$)DMSO)^2): 12.1 (*s*, NH); 10.5 (*s*, NH); 8.63 (*s*, H–C(2)); 8.62 (*s*, H–C(8)); 8.58 (*d*, H–C6)(flu)); 8.17 (*d*, 2 H *o* to NO_2); 8.03 (*d*, 2 H *o* to NO_2); 7.95 (*dd*, H–C(4)(flu)); 7.62 (*d*, 2 H *m* to NO_2); 7.42 (*d*, H–C(3)(flu)); 7.36–7.16 (*m*, 2 H *m* to NO_2, 9 arom. H, H–C(4)(xan)); 6.89–6.73 (*m*, 4 H *o* to MeO,

H–C(1), H–C(2), H–C(8)(all xan)); 6.48 (*t*, H–C(1′)); 6.34 (*dd*, H–C(7)(xan)); 6.15 (*d*, H–C(5)(xan)); 5.41 (*d*, OH–C(3′)); 4.52 (*m*, H–C(3′)); 4.42 (*t*, 1 CH_2CH_2O); 4.25 (*t*, 1 CH_2CH_2O); 4.02 (*m*, H–C(4′)): 3.69 (*s*, 2 MeO); 3.25–3.16 (*m*, 2 H–C(5′), 1 CH_2CH_2O); 3.00 (*m*, 1 H–C(2′)); 2.82 (*t*, 1 CH_2CH_2O); 2.40 (*m*, 1 H–C(2′)). Anal. calc. for $C_{68}H_{56}N_8O_{15}$ (1225.3): C 66.66, H 4.61, N 9.15; found: C 66.12, H 4.79, N 9.06.

71. *2′-Deoxy-5′*-O-*(dimethoxytrityl)*-N^6-*{{{3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl}phenyl}amino}carbonyl}adenosine 3′-[2-(4-Nitrophenyl) Diisopropylphosphoramidite]* (**75**). To a soln. of **74** (0.2 g, 0.16 mmol) and 1*H*-tetrazole (0.01 g, 0.14 mmol) in CH_2Cl_2 (3 ml), 2-(4-nitrophenyl)ethyl tetraisopropylphosphorodiamidite (0.125 g, 0.4 mmol) was added and stirred at r.t. for 1.5 h. After dilution with $CHCl_3$ (50 ml) and washing with $NaHCO_3$ soln. (2×), the org. phase was dried ($MgSO_4$), concentrated to *ca.* 5 ml, and added dropwise to dry Et_2O/hexane 4:1 (100 ml). The precipitate was collected and dried under high vacuum: 0.18 g (85%) of **75**. TLC (silica gel, toluene/AcOEt/MeOH 10:8:1): R_f 0.39/0.41. ^{1}H-NMR ((D$_6$)DMSO)^2): 12.2 (*s*, NH); 8.58 (*s*, H–C(2); 8.45 (*d*, H–C(6)(flu)); 8.25 (br. *s*, NH); 8.23–8.00 (*m*, H–C(8), 6 H *o* to NO_2, H–C(4)(flu)); 7.48–7.11 (*m*, 6 H *m* to NO_2, 9 arom. H, H–C(3)(flu), H–C(4)(xan)); 6.90–6.48 (*m*, 4 H *o* to MeO, H–C(1), H–C(2), H–C(5), H–C(7), H–C(8)(all xan), H–C(1′)); 4.70 (*m*, H–C(3′)); 4.27 (*m*, 2 CH_2CH_2O, H–C(4′)); 3.72 (*s*, 2 MeO); 3.90–2.50 (*m*, 3 CH_2CH_2O, CH_2CH_2O, 2 H–C(2′), 2 H–C(5′), 2 Me_2CH); 1.12 (*m*, 2 Me_2CH).

72. *2′-Deoxy-5′*-O-*(dimethoxytrityl)*-N^6-*{{{3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)-ethoxy]-3-oxo-3*H*-xanthen-9-yl}phenyl}amino}carbonyl}adenosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**76**). As described for **75**, with **74** (0.816 g, 0.66 mmol), 1*H*-tetrazole (0.05 g, 0.7 mmol), CH_2Cl_2 (5 ml), and 2-cyanoethyl tetraisopropylphosphorodiamidite (0.39 g, 1.9 mmol) for 24 h: 0.8 g (85%) of **76**. TLC (silica gel, toluene/AcOEt/MeOH 10:8:1): R_f 0.35, 0.37. ^{1}H-NMR ((D$_6$)DMSO)^2): 12.2 (*s*, NH); 8.58 (*s*, H–C(2)); 8.45 (*d*, H–C(6)(flu)); 8.25 (br. *s*, NH); 8.23–8.00 (*m*, H–C(8), 4 H *o* to NO_2, H–C(4)(flu)); 7.48–7.11 (*m*, 4 H *m* to NO_2, 9 arom. H, H–C(3)(flu), H–C(4)(xan)); 6.90–6.48 (*m*, 4 H *o* to MeO, H–C(1), H–C(2), H–C(5), H–C(7), H–C(8)(all xan), H–C(1′)); 4.70 (*m*, H–C(3′)); 4.28 (*m*, 2 CH_2CH_2O, H–C(4′)); 3.72 (*s*, 2 MeO); 3.70–3.20 (*m*, 2 CH_2CH_2O, $NCCH_2CH_2$, 2 H–C(5′)); 2.90–2.40 (*m*, $NCCH_2CH_2$, 2 Me_2CH, 2 H–C(2′)); 1.12 (*m*, 2 Me_2CH). Anal. calc. for $C_{77}H_{73}N_{10}O_{16}P$ (1425.5): C 64.88, H 5.16, N 9.83; found: C 64.63, H 5.10, N 9.93.

73. *2′-Deoxy-5′*-O-*(dimethoxytrityl)*-N^6-*{{{3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)-ethoxy]-3-oxo-3*H*-xanthen-9-yl}phenyl}amino}carbonyl}adenylyl-{3′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy*-N^6,*3′*-O-*bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**77**). A mixture of **46** (50 mg, 0.4 mmol) and 1*H*-tetrazole (0.1 g, 1.4 mmol) was stirred in very dry MeCN (2 ml) for 10 min and then degassed. Then crude **75** (0.74 g) in CH_2Cl_2 was added and stirred at r.t. for 2 h. After oxidation with I_2 (0.3 g) in pyridine/CH_2Cl_2/H_2O 5:1:1 (6 ml) and stirring for 15 min, the mixture was diluted with CH_2Cl_2 (200 ml) and then decolorized with $Na_2S_2O_3$ soln. The org. phase was dried ($MgSO_4$), evaporated and co-evaporated with toluene until the pyridine was completely removed. The residue was dissolved in CH_2Cl_2/MeOH 3:1 (10 ml) and purified by FC (silica gel (50 g); toluene (50 ml), toluene/AcOEt 1:1 (200 ml), toluene/AcOEt 1:1 (190 ml) + MeOH (10 ml), toluene/AcOEt 1:1 (370 ml) + MeOH (30 ml), toluene/AcOEt 1:1 (360 ml) + MeOH (40 ml) and toluene/AcOEt 1:1 (300 ml); 100-ml fractions). *Fr. 12–15* were evaporated and co-evaporated with EtOH, and the residue was treated in MeOH by ultrasound. The resulting precipitate was dried under high vacuum: 0.45 g (59%) of **77**. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.33. UV (CH_2Cl_2/MeOH 1:1): 224 (5.00), 232 (sh., 4.95), 275 (5.04), 308 (sh., 4.36), 358 (4.09), 404 (sh., 3.97), 436 (sh., 4.30), 459 (4.46), 489 (4.35). ^{1}H-NMR ((D$_6$)DMSO)^2): 12.1 (*s*, NH); 10.6 (br. *s*, NH); 10.4 (br. *s*, NH); 8.54 (*s*, 5 H, H–C(2), H–C(8), H–C(6)(flu)); 8.19–8.02 (*m*, 10 H *o* to NO_2); 7.95 (*m*, H–C(4)(flu)); 7.64–7.13 (*m*, 21 H, H *m* to NO_2, arom. H, H–C(3)(flu), H–C(4)(xan)); 6.68–6.71 (*m*, 11 H, H *o* to MeO, H–C(1), H–C(2), H–C(8)(all xan)); 6.44–6.32 (*m*, 3 H, H–C(1′), H–C(7)(xan)); 6.15 (*d*, H–C(5)(xan)); 5.32 (*m*, 1 H, H–C(3′)); 5.09 (*m*, 1 H, H–C(3′)); 4.44–4.19 (*m*, 14 H, CH_2CH_2O, H–C(4′), H–C(5′)); 3.65 (*s*, 2 MeO); 3.23–2.57 (*m*, 16 H, CH_2CH_2, H–C(5′), H–C(2′)). Anal. calc. for $C_{104}H_{89}N_{16}O_{30}P \cdot 2\ H_2O$ (2109.9): C 59.20, H 4.44, N 10.62; found: C 59.00, H 4.49, N 10.29.

74. *2′-Deoxy*-N^6-*{{{3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H*-xanthen-9-yl}phenyl}amino}carbonyl}adenylyl-{3′-{O^P-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy*-N^6,*3′*-O-*bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**78**). A soln. of **77** (0.5 g, 0.24 mmol) in 3% CCl_3COOH/CH_2Cl_2 (30 ml) was stirred at r.t. for 15 min. After dilution with CH_2Cl_2 (100 ml) and washing with $NaHCO_3$ soln., the org. phase was dried ($MgSO_4$) and evaporated. The residue was dissolved in CH_2Cl_2 (10 ml) and submitted to FC (silica gel (35 g); toluene (100 ml), toluene/AcOEt 1:1 (300 ml), toluene/AcOEt 1:1 (190 ml) + MeOH (10 ml), toluene/AcOEt 1:1 (185 ml) + MeOH (15 ml), toluene/AcOEt 1:1 (180 ml) + MeOH (20 ml), toluene/AcOEt 1:1 (175 ml) + MeOH (25 ml), toluene/AcOEt 1:1 (340 ml) + MeOH (60 ml); 100-ml fractions). *Fr. 12–16* were evaporated, and the residue was treated in MeOH by ultrasound: 0.355 g (83%) of **78**. TLC (silica gel, toluene/

AcOEt/MeOH 5:4:1): R_f 0.25. UV (CH_2Cl_2/MeOH 1:1): 224 (4.90), 232 (sh., 4.83), 276 (5.05), 308 (sh., 4.37), 350 (4.07), 408 (sh., 4.00), 436 (sh., 4.31), 460 (4.46), 487 (4.35). ^{1}H-NMR ((D_6)DMSO)[2]): 12.1 (*s*, NH); 10.6 (br. *s*, NH); 10.4 (br. *s*, NH); 8.68–8.58 (*s*, H–C(2), H–C(8), H–C(6)(flu)); 8.19–8.03 (*m*, 10 H *o* to NO_2); 7.95 (*dd*, H–C(4)(flu)); 7.64–7.32 (*m*, 11 H, H *m* to NO_2, H–C(3)(flu)); 7.18 (*m*, H–C(4)(xan)); 6.86 (*m*, H–C(1), H–C(2), H–C(8)(all xan)); 6.44–6.33 (*m*, 3 H, H–C(1′), H–C(7)(xan)); 6.15 (*d*, H–C(5)(xan)); 5.35 (*m*, 1 H, H–C(3′)); 5.17 (*t*, 1 H, OH); 5.00 (*m*, 1 H, H–C(3′)); 4.41–4.04 (*m*, 13 H, CH_2CH_2, H–C(4′), H–C(5′)); 3.51 (*m*, 2 H, H–C(5′)); 3.23–2.57 (*m*, 14 H, CH_2CH_2O, H–C(2′)). Anal. calc. for $C_{83}H_{71}N_{16}O_{28}P \cdot H_2O$ (1789.6): C 55.71, H 4.11, N 12.52; found: C 55.48, H 4.16, N 12.36.

75. *2′-Deoxy*-N[6],*5′*-O-*bis-{[2-(4-nitrophenyl)ethoxy]carbonyl}adenylyl-{3′-{O[P]-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy*-N[6]-*{{{3-{[2-(4-nitrophenyl)ethoxy]carbonyl}-4-{6-[2-(4-nitrophenyl)ethoxy]-3-oxo-3*H-*xanthen-9-yl}phenyl}amino}carbonyl}adenylyl-{3′-{O[P]-[2-(4-nitrophenyl)ethyl]}-5′}-2′-deoxy*-N[6],*3′*-O-*bis{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine* (**79**). A soln. of **78** (60 mg, 0.034 mmol) in very dry toluene/CH_2Cl_2 was twice co-evaporated, then once with dry MeCN/CH_2Cl_2, and finally dissolved in MeCN (3 ml). After addition of 1*H*-tetrazol (30 mg), the mixture was stirred for 10 min under N_2 and then diluted with CH_2Cl_2 (5 ml). Then **45** (0.12 g, 0.13 mmol) was added and stirred for 2.5 h. After oxidation with I_2 (0.1 g) in pyridine/CH_2Cl_2/H_2O 5:1:1 (5 ml) and stirring for 15 min, the mixture was diluted with CH_2Cl_2 (200 ml) and then decolorized with $Na_2S_2O_3$ soln. The org. phase was dried ($MgSO_4$), evaporated, and co-evaporated with toluene until the pyridine was completely removed. The residue was dissolved in CH_2Cl_2 (10 ml) and purified by FC (silica gel (45 g); toluene (50 ml), toluene/AcOEt 1:1 (100 ml), toluene/AcOEt 1:1 (190 ml) + MeOH (10 ml), toluene/AcOEt 1:1 (540 ml) + MeOH (60 ml), toluene/AcOEt 1:1 (350 ml) + MeOH (50 ml), toluene/AcOEt 1:1 (170 ml) + MeOH (30 ml), toluene/AcOEt/MeOH 1:1:1 (100 ml); 80-ml fractions). *Fr. 15–19* were evaporated and co-evaporated with EtOH, and the residue was treated in MeOH by ultrasound. The resulting precipitate was dried under high vacuum: 0.07 g (78%) of **79**. TLC (silica gel, toluene/AcOEt/MeOH 5:4:1): R_f 0.22. UV (CH_2Cl_2/MeOH 1:1): 234 (sh., 4.88), 270 (5.16), 308 (sh., 4.45), 352 (4.08), 413 (sh., 4.02), 434 (sh., 4.27), 459 (4.43), 488 (4.31). ^{1}H-NMR ((D_6)DMSO)[2]): 11.9 (*s*, NH); 10.4 (br. *s*, NH); 10.1 (br. *s*, NH); 8.68–8.48 (*m*, 7 H, H–C(2), H–C(8), H–C(6)(flu)); 8.18–7.96 (*m*, 16 H *o* to NO_2); 7.92 (*dd*, H–C(4)(flu)); 7.64–7.29 (*m*, 17 H, H *m* to NO_2, H–C(3)(flu)); 7.19 (*s*, H–C(4)(xan)); 6.81 (*m*, H–C(1), H–C(2), H–C(8)(all xan); 6.45–6.33 (*m*, 4 H, H–C(1′), H–C(7)(xan)); 6.15 (*d*, H–C(5)(xan)); 5.34 (*m*, 1 H, H–C(3′)); 5.17 (*m*, 2 H, H–C(3′)); 4.42–4.21 (*m*, 25 H, CH_2CH_2O, H–C(4′), H–C(5′)); 3.23–2.57 (*m*, 22 H, CH_2CH_2O, H–C(2′)). Anal. calc. for $C_{119}H_{104}N_{24}O_{43}P_2 \cdot 2\ H_2O$ (2656.3): C 53.81, H 4.09, N 12.65; found: C 53.29, H 4.16, N 12.69.

76. *2′-Deoxy-5′*-O-*(dimethoxytrityl)-N[6]-{{{3-{[2-(4-nitrophenyl)ethoxycarbonyl}-4-{6-[2-(4-nitrophenyl)-ethoxy]-3-oxo-3*H-*xanthen-9-yl}phenyl}amino}carbonyl}adenosine 3′-(Hydrogen Butanedioate)* (**81**). A mixture of **74** (0.3 g, 0.25 mmol), *N*,*N*-dimethylpyridin-4-amine (DMAP; 40 mg, 0.32 mmol) and succinic anhydride (32 mg) were stirred in CH_2Cl_2 (3 ml) at r.t. for 20 h. After dilution with CH_2Cl_2 and washing with 10% aq. citric acid soln. (2 ×) followed by $NaHCO_3$ soln., the org. layer was dried (Na_2SO_4) and evaporated: 0.3 g (92%) of **81**. Orange foam. TLC (silica gel, CH_2Cl_2/MeOH 9:1): R_f 0.60. UV (CH_2Cl_2/MeOH 1:1): 231 (sh., 4.84), 277 (4.81), 308 (sh., 4.16), 361 (4.00), 405 (sh., 3.94), 431 (sh., 4.25), 458 (4.43), 487 (4.33). ^{1}H-NMR ((D_6)DMSO)[2]): 12.2 (br. *s*, COOH); 12.0 (*s*, NH); 10.5 (*s*, NH); 8.62 (*s*, H–C(2), H–C(8), H–C(6)(flu)); 8.15, 8.03 (2*d*, 4 H *o* to NO_2); 7.95 (*dd*, H–C(4)(flu)); 7.62 (*d*, 2 H *m* to NO_2); 7.39 (*d*, H–C(3)(flu)); 7.36–7.16 (*m*, 2 H *m* to NO_2, 9 arom. H, H–C(4)(xan)); 6.89–6.73 (*m*, 4 H *o* to MeO, H–C(1), H–C(2), H–C(8)(all xan)); 6.51 (*t*, H–C(1′)); 6.34 (*m*, H–C(7)(xan)); 6.15 (*d*, H–C(5)(xan)); 5.41 (*d*, H–C(3′)); 4.42 (*t*, 1 CH_2CH_2O); 4.25 (*t*, 1 CH_2CH_2O, H–C(4′)): 3.69 (2 *s*, 2 MeO); 3.25–3.16 (*m*, 1 CH_2CH_2O, 2 H–C(5′)); 2.82 (*t*, 1 CH_2CH_2O); 2.40 (*m*, CH_2CH_2COOH, 2 H–C(2′)). Anal. calc. for $C_{72}H_{60}N_8O_{18} \cdot H_2O$ (1325.3): C 64.37, H 4.65, N 8.34; found: C 64.27, H 4.69, N 8.10.

REFERENCES

[1] Part LXX: R. Charubala, W. Pfleiderer, R. J. Suhadolnik, K. T. Iacono, N. F. Muto, J. W. Homan, C. Martinand-Mari, S. E. Horvath, E. E. Henderson, A. Steele, T. J. Rogers, *Helv. Chim. Acta* **2002**, *85*, 2284.

[2] E. M. Southern, *J. Biol. Chem.* **1975**, *98*, 503.

[3] L. Smith, J. Z. Sanders, R. J. Kaiser, P. Hughes, C. Dodd, C. R. Connell, C. Heiner, S. B. H. Kent, L. E. Hood, *Nature (London)* **1986**, *321*, 674; A. E. Karger, J. M. Harris, R. F. Gesteland, *Nucleic Acids Res.* **1991**, *19*, 4955; W. Ansorge, B. Sproat, J. Steegemann, C. Schwager, M. Zenke, *Nucleic Acids Res.* **1987**, *15*, 4593.

[4] I. C. Gillam, *Trends Biotechnol* **1987**, *5*, 332; R. H. Symons, 'Nucleic Acids Probes', CRC Press 1989; G. H. Kellar, M. M. Manak, 'DNA Probes', Stockton Press, 1989.
[5] User Manual, 'DIG DNA-Labelling and Detection Kits, Nonradioactive', Boehringer-Mannheim.
[6] J. A. Matthews, I. J. Kricka, *Anal. Biochem.* **1988**, *169*, 1.
[7] T. R. Broker, L. M. Amgerer, P. H. Yen, N. D. Hershey, N. Davidson, *Nucleic Acids Res.* **1978**, *5*, 363; J. P. Schreiber, N. Hsiung, C. R. Cantor, *Nucleic Acids Res.* **1979**, *6*, 181; J. G. J. Baumann, J. Wiegant, P. van Duijn, *J. Histochem. Cytochem.* **1981**, *29*, 227; R. W. Richardson, R. J. Gumport, *Nucleic Acids Res.* **1983**, *11*, 6167; L. M. Smith, S. Fung, M. W. Hunkapiller, T. J. Hunkapiller, L. E. Hood, *Nucleic Acids Res.* **1985**, *13*, 2399; B. A. Connolly, P. Rider, *Nucleic Acids Res.* **1985**, *13*, 4485; S. Agrawal, C. Christodoulou, M. Gait, *Nucleic Acids Res.* **1986**, *14*, 6227; P. Li, P. P. Medon, D. C. Skingle, J. A. Lanser, R. H. Symons, *Nucleic Acids Res.* **1987**, *15*, 5275; R. Zuckermann, D. Corey, P. Schultz, *Nucleic Acids Res.* **1987**, *15*, 5305; B. S. Sproat, B. Beijer, P. Rider, *Nucleic Acids Res.* **1987**, *15*, 6181; V. K. Kansal, T. Huynh-Dinh, J. Igolen, *Tetrahedron Lett.* **1988**, *29*, 5537; T. Tanaka, Y. Yamada, M. Ikehara, *Chem. Pharm. Bull.* **1988**, *36*, 1386; M. J. de Vos, A. Cravador, J. P. Lenders, S. Houard, A. Bollen, *Nucleosides Nucleotides* **1990**, *9*, 259; R. K. Gaur, *Nucleosides Nucleotides* **1991**, *10*, 895; A. Kumar, S. Advani, *Nucleosides Nucleotides* **1992**, *11*, 999; A. Kumar, S. Malhotra, *Nucleosides Nucleotides* **1992**, *11*, 1003; A. Murakami, J. Tada, K. Yamagata, J. Takano, *Nucleic Acids Res.* **1989**, *17*, 5587; N. D. Sinha, R. M. Cook, *Nucleic Acids Res.* **1988**, *16*, 2659; B. C. F. Chu, L. E. Orgel, *Nucleic Acids Res.* **1988**, *16*, 3671; W. Bannwarth, D. Schmidt, *Tetrahedron Lett.* **1989**, *30*, 1513; U. Pieles, U. Englisch, *Nucleic Acids Res.* **1989**, *17*, 285; N. T. Thuong, M. Chassignol, *Tetrahedron Lett.* **1988**, *29*, 5905; U. Englisch, D. H. Gauss, *Angew. Chem.* **1991**, *103*, 629; M. Bengtström, A. Jungell-Nortamo, A. C. Syvänen, *Nucleosides Nucleotides* **1990**, *9*, 123; F. Schubert, K. Ahlert, D. Cech, A. Rosenthal, *Nucleic Acids Res.* **1990**, *18*, 3427; U. Möller, D. Cech, F. Schubert, *Liebigs Ann. Chem.* **1990**, 1221.
[8] J. Y. Tang, S. Agrawal, *Nucleic Acids Res.* **1990**, *18*, 6461; R. T. Pon, *Tetrahedron Lett.* **1991**, *32*, 1715; H. Bazin, A. Roget, R. Teoule, *Nucleosides Nucleotides* **1991**, *10*, 363; U. Asseline, N. T. Thuong, *Nucleosides Nucleotides* **1991**, *10*, 359; P. S. Nelson, R. Sherman-Gold, R. Leon, *Nucleic Acids Res.* **1989**, *17*, 7179; K. Misiura, I. Durrant, M. R. Evans, M. Gait, *Nucleic Acids Res.* **1990**, *18*, 4345; S. Agrawal, P. C. Zamecnik, *Nucleic Acids Res.* **1990**, *18*, 5419; K. Misiura, I. Durrant, M. R. Evans, M. Gait, *Nucleosides Nucleotides* **1991**, *10*, 671; S. Agrawal, J.-Y. Tang, *Tetrahedron Lett.* **1990**, *31*, 1543; J. A. Fidanza, L. W. McLaughlin, *J. Org. Chem.* **1992**, *57*, 2340.
[9] H. Inoue, A. Imura, E. Ohtsuka, *Nucleic Acids Res.* **1985**, *13*, 7119; E. Jablonski, E. W. Moomaw, R. H. Tullis, J. L. Ruth, *Nucleic Acids Res.* **1986**, *14*, 6115; J. Haralambidis, M. Chai, G. W. Tregear, *Nucleic Acids Res.* **1987**, *15*, 4857; G. I. Trainor, F. W. Hobbs, A. J. Cocuzza, P. N. Confalone, *Nucleic Acids Res. Symp. Ser.* **1988**, *20*, 119; S. R. Sarfati, S. Pochet, C. Guerreiro, A. Namane, T. Huynh-Dinh, J. Igolen, *Tetrahedron* **1987**, *43*, 3491; K. J. Gibson, S. J. Benkovic, *Nucleic Acids Res.* **1987**, *15*, 6455; K. A. Cruickshank, D. J. Stockwell, *Tetrahedron Lett.* **1988**, *29*, 5221; D. J. Allen, P. L. Darke, S. J. Benkovic, *Biochemistry* **1989**, *28*, 4601; A. Kumar, P. Tchen, F. Roullet, J. Cohen, *Anal. Biochem.* **1988**, *169*, 376; A. Oser, W. K. Roth, G. Valet, *Nucleic Acids Res.* **1988**, *16*, 1181; M. S. Urdea, B. D. Warner, J. A. Running, M. Stemplen, J. Clyne, T. Horn, *Nucleic Acids Res.* **1988**, *16*, 4937; J. Telser, K. A. Cruickshank, L. E. Morrison, T. L. Netzel, *J. Am. Chem. Soc.* **1989**, *111*, 6966; A. Roget, H. Bazin, R. Teoule, *Nucleic Acids Res.* **1989**, *17*, 7643; S. R. Sarfati, A. Namane, *Tetrahedron Lett.* **1990**, *31*, 2581; D. Singh, V. Kumar, K. N. Ganesh, *Nucleic Acids Res.* **1990**, *18*, 3339; U. Pieles, B. S. Sproat, G. M. Lamm, *Nucleic Acids Res.* **1990**, *18*, 4354; P. Hurskainen, P. Dahlen, J. Ylikoski, M. Kwiatkowski, H. Silitari, T. Lövgren, *NucleicAcids Res.* **1991**, *19*, 1057; H. Eshaghpour, D. Söll, D. M. Crothers, *Nucleic Acids Res.* **1979**, *7*, 1485; G. Gebeyehu, P. Y. Rao, P. SooCan, D. A. Simms, L. Klevan, *Nucleic Acids Res.* **1987**, *15*, 4513.
[10] K. Yamana, Y. Ohashi, K. Nunota, M. Kitamura, H. Nakano, O. Sangen, T. Shimidzu, *Tetrahedron Lett.* **1991**, *32*, 6346; M. Manoharan, C. J. Guinosso, P. D. Cook, *Tetrahedron Lett.* **1991**, *32*, 7171; K. Yamana, T. Gokota, Y. Ohashi, H. Czaki, M. Kitamura, H. Nakano, O, Sangen, T. Shimidzu, *Nucleic Acids Res.* Symp. Ser. **1990**, *22*, 103; K. Yamana, T. Gokota, H. Ozaki, H. Nakano, O. Sangen, T. Shimidzu, *Nucleosides Nucleotides* **1992**, *11*, 383.
[11] H. Sigmund, W. Pfleiderer, *Helv. Chim. Acta* **1994**, *77*, 1267.
[12] A. H. Coons, M. H. Kaplan, *J. Exp. Med.* **1950**, *91*, 1; G. Steinbach, *Acta Histochem.* **1974**, *50*, 19.
[13] R. M. McKinney, F. C. Churchill II, *J. Chem. Soc. (C)* **1970**, 654; R. M. McKinney, J. T. Spillane, G. W. Pearce, *J. Org. Chem.* **1962**, *27*, 3986.
[14] a) V. Zanker, W. Peter, *Chem. Ber.* **1958**, *91*, 572; b) F. M. Abdel-Halim, R. M. Issa, M. S. El-Ezaby, A. A. Hasanein, *Z. Phys. Chem. N. F.* **1970**, *73*, 59; c) M. M. Martin, L. Lindquist, *J. Lumin.* **1975**, *10*, 381; d) Z. G.

Zhao, T. Shen, H-J. Xu, *Spectrochim. Acta, Part A* **1989**, *45*, 1113; e) S. C. Chen, H. Nakamura, *Chem. Pharm. Bull.* **1979**, *27*, 647.
[15] H. Diehl, R. Markuszewski, *Talanta* **1989**, *36*, 416; G. Guyot, R. Arnaud, J. Lemaire, *J. Chim. Phys.* **1975**, *72*, 647; I. Martin, A. Prado, M. S. Guijaro, J. I. Fernandez-Alonso, *J. Mol. Struct.* **1986**, *142*, 197; H. Leonardt, L. Gordon, R. Livingston, *J. Phys. Chem.* **1971**, *76*, 245.
[16] M. P. H. P. van Genderen, L. H. Koole, M. H. Buck, *Rec. Trav. Chim. Pays Bas* **1989**, *108*, 28; R. K. Gaur, V. Bobde, M. Atreyi, K. C. Gupta, *Ind. J. Chem., Sect. B* **1990**, *29*, 108.
[17] F. Himmelsbach, B. S. Schulz, T. Trichtinger, R. Charubala, W. Pfleiderer, *Tetrahedron* **1984**, *40*, 76.
[18] a) S. P. Dutta, G. B. Chheda, *J. Carbohydr. Nucleosides Nucleotides* **1980**, *7*, 217; b) A. S. Jones, J. H. Warren, *Tetrahedron* **1970**, *26*, 791; c) S. Kumar, N. J. Leonard, *J. Org. Chem.* **1988**, *53*, 3959; d) W. T. Markiewicz, N. S. Padyukova, Z. Samek, J. Smrt, *Coll. Czech. Chem. Chommun.* **1980**, *45*, 1860; e) K. L. Agarwal, H. G. Khorana, *J. Am. Chem. Soc.* **1972**, *94*, 3578.
[19] F. Himmelsbach, Ph. D. Thesis, Konstanz University, 1984.
[20] B. W. Watkins, H. Rapoport, *J. Org. Chem.* **1982**, *47*, 4471.
[21] P. Camus, M. F. Lhomme, J. Lhomme, *Tetrahedron Lett.* **1989**, *30*, 467.
[22] H. Lang, M. Gottlieb, M. Schwarz, S. Farkas, B. S. Schulz, F. Himmelsbach, R. Charubala, W. Pfleiderer, *Helv. Chim. Acta* **1999**, *82*, 2172.
[23] H. Schirmeister, F. Himmelsbach, W. Pfleiderer, *Helv. Chim. Acta* **1993**, *76*, 385.
[24] O. Mitsunobu, *Synthesis* **1981**, 1.
[25] G. Weber, *Adv. Protein Chem.* **1953**, *8*, 415.
[26] U. Landegren, R. Kaiser, C. T. Caskey, L. Hood, *Science (Washington, D.C.)* **1988**, *242*, 229; B. S. Reckmann, *Nachr. Chem. Tech. Lab.* **1989**, *37*, 692; L. E. Morrison, T. C. Halder, L. M. Stols, *Anal. Chem.* **1989**, *183*, 231.
[27] B. D. Wells, C. R. Cantor, *Nucleic Acids Res.* **1980**, *8*, 3229; J. A. Plumbridge, H. G. Bäumert, M. Ehrenberg, R. Rigler, *Nucleic Acids Res.* **1980**, *8*, 827; A. Murakami, M. Nakaura, Y. Nakatsuji, S. Nagahara, Q. Tran-Cong, K. Makino *Nucleic Acids Res.* **1991**, *19*, 4097; S. Nagaharra, A. Murakami, K. Makino, *Nucleosides Nucleotides* **1992**, *11*, 889; A. Murakami, S. Nagahara, M. Nakaura, H. Uematsu, M. Mukae, K. Makino, *Nucleic Acids Res. Symp. Ser.* **1990**, *22*, 27.
[28] A. Albert, E. P. Serjeant 'The Determination of Ionization Constants', Chapman & Hall, London, 1971.
[29] S. P. Dutta, C. I. Hong, G. P. Murphy, A. Mittelman, G. B. Chheda, *Biochemistry* **1975**, *14*, 3144.
[30] H. Schaller, G. Weimann, B. Lerch, H. G. Khorana, *J. Am. Chem. Soc.* **1963**, *85*, 3821.
[31] L. H. Koole, H. M. Moddy, N. L. H. L. Broeders, P. J. L. Quaedflieg, W. H. A. Kuijpers, M. H. P. van Genderen, A. J. J. M. Coenen, S. van der Wal, H. M. Buck, *J. Org. Chem.* **1989**, *54*, 1657; T. R. Webb, M. D. Matteucci, *Nucleic Acids Res.* **1986**, *14*, 7661.

Received November 26, 2002