Pentopyranosyl Oligonucleotide Systems

Communication No. 13[1])

The α-L-Arabinopyranosyl-(4′ → 2′)-oligonucleotide System: Synthesis and Pairing Properties[1])

by **Oliver Jungmann**[2a]), **Markus Beier**[2b]), **Anatol Luther**[2c]), **Hoan K. Huynh**[2d]), **Marc-Olivier Ebert**[2e]), **Bernhard Jaun**[2e]), **Ramanarayanan Krishnamurthy***[3]), and **Albert Eschenmoser***[3])

The *Skaggs* Institute for Chemical Biology at The *Scripps* Research Institute (TSRI) 10550 North Torrey Pines Road, La Jolla, CA-92037, USA

and

Laboratory of Organic Chemistry, Swiss Federal Institute of Technology, Hönggerberg, HCI-H309, CH-8093 Zürich

Dedicated to *Jack Dunitz*, teacher, colleague, and friend, on the occasion of his 80th birthday

Among the members of a family of diastereoisomeric pentopyranosyl-(4′ → 2′)-oligonucleotide systems derived from D-ribose, D-xylose, L-lyxose, and L-arabinose, the α-arabinopyranosyl system shows by far the strongest *Watson–Crick* base pairing. The system is, in fact, one of the strongest oligonucleotide-type base-pairing systems known. It undergoes efficient cross-pairing with all the other members of the pentopyranosyl family, but not with RNA and DNA. The paper describes the synthesis and pairing of the properties of α-L-arabinopyranosyl-(4′ → 2′)-oligonucleotides.

1. Introduction. – Among the six pentopyranosyl oligonucleotide systems studied in our laboratories [1][3–6], the α-L-arabinopyranosyl-(4′ → 2′)-oligonucleotide system turned out to be the most surprising of all: here, we encountered the largest gap between expectation and observation with respect to a system's base-pairing properties. The qualitative conformational pairing criteria that had been conspicuously successful in predicting the base-pairing capabilities of homo-DNA [7] and p-RNA [4a] had led us to expect that an arabino analog of p-RNA would be a weaker pairing system than pyranosyl-RNA [4a]. Fact is that α-L-arabinopyranosyl-(4′ → 2′)-oligonucleotides constitute the strongest base-pairing system among all potentially natural nucleic acid alternatives studied so far, surpassing the pairing strengths of both natural RNA and pyranosyl-RNA by a large margin [4]. Recently, *Jaun* and co-workers [8] determined the structure of an octamer duplex of the α-L-arabinopyranosyl-oligonucleotide series by NMR spectroscopy and provided therewith the basis for a rationalization of remarkable properties of the arabino system. Here, we report experimental aspects of

---

[1]) Communication No. 12: [1]. The paper is also communication No. 38 in the series 'Chemistry of α-Aminonitriles'. For communication No. 37, see [2].

[0]) [a]) TSRI October 1998 – June 2000; [b]) TSRI September 1996 – December 1998; [c]) TSRI October 1998 – October 2000; [d]) TSRI October 1998 – January 2001; [e]) ETH-Hönggerberg.

[3]) E-mail: eschenmoser@org.chem.ethz.ch; rkrishna@scripps.edu.

the synthesis and the pairing behavior of α-D- and α-L-arabinopyranosyl-(4′→2′)-oligonucleotides, complementing the two preliminary publications on the topic [3a][3b] and present a discussion of the system in comparison with the other members of the pentopyranosyl-(4′→2′)-oligonucleotide family.

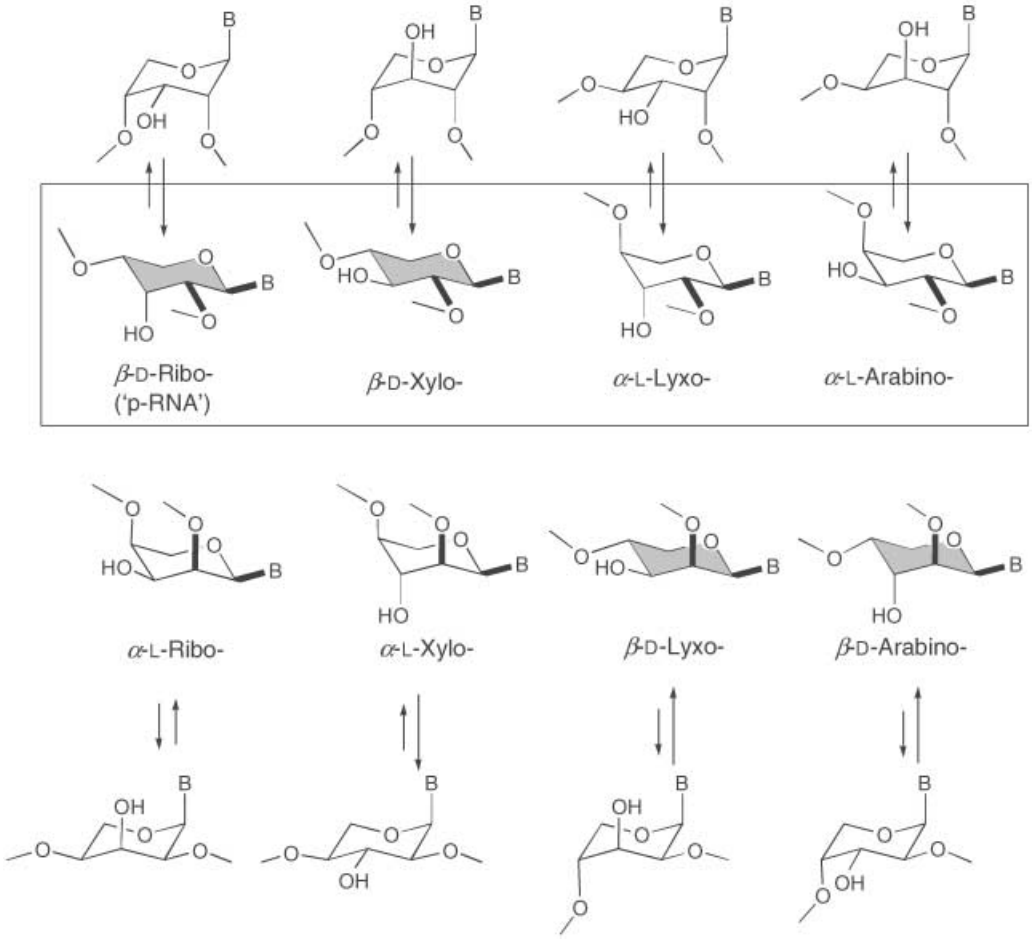
Scheme 1 illustrates the selection problem that arose when choosing the nucleoside configuration for each of the pentopyranosyl nucleoside members for a comparative study of four of the family's eight members. For the ribopyranosyl member [4], the first studied experimentally, the choice had been straightforward, since, of the two possible nucleoside D-diastereoisomers[4]), only the β-D-epimer could be expected to exist in a well-defined single-type of conformation. In this isomer, the nucleobase would unambiguously occupy an equatorial position at the pyranosyl chair. Furthermore, the β-D-nucleoside epimer promised to be the more accessible one, since, in this epimer, the nucleobase and the substituents at C(2′) are *trans* to each other. In choosing the other members of the family, the equatorial position of the nucleobase in the more-stable pyranose-chair conformation became the predominant selection criteria by reasons that were both chemical and etiological in nature. According to this criterion, the xylopyranosyl member had obviously to be the β-D-diastereoisomer [1]. Less certain was the choice in the cases of the remaining lyxo- and arabinopyranosyl members, since, for both, it could be expected that, in each of the two epimers, the nucleobase would occupy an equatorial position. In the case of lyxopyranose, the α-L-lyxopyranosyl series was chosen because its nucleosides were expected to be readily accessible (nucleobase *trans* to substituent at C(2′)), and, furthermore, the sense of chirality at the nucleobase-bearing anomeric center is the one corresponding to that in the β-D-ribo- and β-D-xylopyranosyl series. In the arabinose case, we originally chose to synthesize β-D-arabinopyranosyl-nucleosides to be able to study a quartet of pentopyranosyl-(4′→2′)-oligonucleotides in which the configurational differences between its members would follow a consistent and transparent pattern, allowing a meaningful comparison and interpretation of pairing properties. This pattern would have involved having the phosphodiester group 2′,4′-diequatorial in the β-D-ribo and β-D-xylo systems, 2′-equatorial/4′-axial in the α-L-lyxo system, 2′-axial/4′-equatorial in the β-D-*arabino* system, and the nucleobases equatorial in all four of them, with the anomeric center in all cases possessing the same sense of local chirality (1′*R*).

Serious experimental difficulties in attempts to prepare the thymine nucleoside of the β-D-arabinopyranosyl series with its *cis*-arrangement of nucleobase and the substituent at C(2′) made us reconsider the choice of target. Since the epimeric α-D-arabinopyranosyl nucleoside persistently dominated the product mixtures from such nucleosidation experiments, we eventually made this epimeric nucleoside (and its adenine analog) our new and much more conveniently accessible target. This switch in the configuration at the anomeric center demanded switching at the same time from the D- to the L-arabinopyranosyl series (*Scheme 2*). For practical reasons, we developed the methodology for the synthesis of α-arabinopyranosyl-(4′→2′)-oligonucleotides in the (chirally wrong) D-series and made the switch to the (chirally correct) α-L-arabinopyranosyl series in a later phase of our work. After this change, the pattern

---

[4]) α/β-Nomenclature: α-diastereoisomer in the pentopyranose series: nucleobase at C(1) is *cis* to the OH group at C(4), whereas these two substituents are *trans* in the β-diastereoisomer [9].

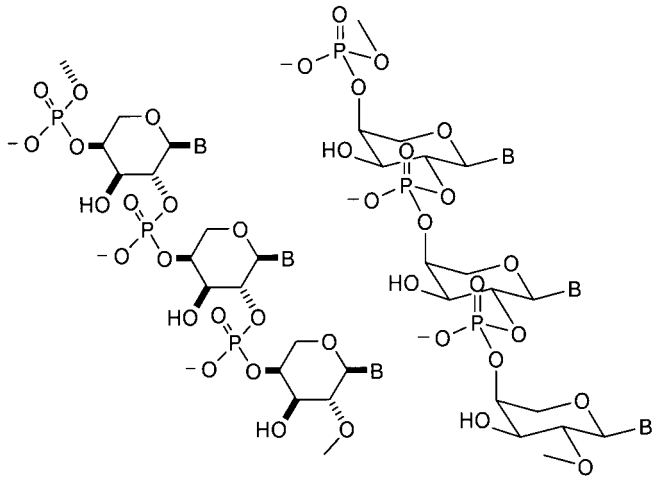
Scheme 1. *Configuration and (Idealized) Conformation of the Eight Diastereoisomeric Repeating Units of the Family of Pentopyranosyl-(4′ → 2′)-Oligonucleotides.* Pyranose chairs of the β-D-series are shaded. The set of the four members that were studied experimentally is framed. **B** = nucleobase.



of configurational differences within the family of the four pentopyranosyl-oligonucleotide systems becomes one in which both the nucleobase and the substituent at C(2′) are equatorial in all four systems and in which the configurational differences refer only to the substituents at C(3′) and C(4′). In all four systems, the local sense of chirality at the anomeric center is the same; this is essential for intersystem cross-pairing studies. The formulae of the four oligonucleotide systems studied experimentally are framed in *Scheme 1*.

2. Synthesis. – 2.1. *Preparation of α-D-Arabinopyranosyl Nucleosides Containing Adenine (A) and Thymine (T), and of α-L-Arabinopyranosyl Nucleoside Building Blocks Containing Adenine (A), Thymine (T), Cytosine (C), and Guanine (G)*. The

Scheme 2. *Constitution, Configuration, and Idealized Pairing Conformation of* α-L-*Arabinopyranosyl-(4′ → 2′)-oligonucleotides.* **B** = nucleobase.



synthesis of α-arabinopyranosyl-(4′ → 2′)-oligonucleotides to be described in *Sect. 2.2* was executed in two parts: the initial approach made use of a Fmoc-protecting-group strategy, resulting in the first preparations of adenine and thymine containing the α-arabinopyranosyl oligonucleotides in the D-series. The approach was then extended to the preparation of adenine, thymine, cytosine, and guanine oligonucleotides in the L-series. Eventually, a follow-up synthesis of α-arabinopyranosyl oligonucleotides containing all four canonical nucleobases in the L-arabinose series by a DMT-protecting-group strategy paralleling the syntheses of oligonucleotides in the ribo-, xylo-, and lyxopyranosyl series [1][4][5] was developed. This latter development was induced by the demands of material for the recently published NMR-structure analysis of the p**a**$[(CGAATTCG)]_2$ duplex [8].

Although a part of the work on α-arabinopyranosyl oligonucleotides described here had been initially carried out in the D-arabinose series (see above), all reactions reported in this paper in *Schemes 2–5* are uniformly formulated in terms of the L-series. Special mention will be made whenever data are presented that were collected in the D-series only. There are reports in the literature where arabinopyranosyl nucleosides have been prepared in the α-L-, α-D-, and β-D-series, but in a different context [10].

2.1.1. *Synthesis of* α-D- *and* α-L-*Arabinopyranosyl Nucleoside Building Blocks Containing the Fmoc Protecting Group.* Nucleosidation of the readily accessible 1,2,3,4-tetra-*O*-benzoyl-β-L-arabinopyranose (**1**) [11] with the appropriately protected nucleobases N^6-benzoyladenine (A^{Bz}), thymine (T), N^4-benzoylcytosine (C^{Bz}), and N^2-isobutyryl guanine ($G^{i\text{-}bu}$) under the *Vorbrüggen–Hilbert–Johnson* conditions [12] furnished the nucleoside derivatives **2a**–**2d** in modest (44%) to high (84%) yields (*Scheme 3*). Reaction with N^2-isobutyrylguanine proved to be low-yielding, with interference from the N^7-isomer **2e** necessitating time-consuming purification by

column chromatography (CC)[5]). The constitutional assignment of N^9- *vs.* N^7-guanine regioisomers was accomplished by NMR-spectral-data comparisons between **2d** and **2e** as documented in *Table 1*, and UV-spectral data comparisons of the completely deprotected guanine derivatives (*Table 1* [13]), obtained by hydrazinolysis ($NH_2NH_2 \cdot H_2O$, EtOH, 12 h, r.t.) of **2d** and **2e**. Selective deprotection of the BzO groups without touching acyl protection of nucleobases was brought about by basic treatment (with either NaOH or Na/MeOH) to afford the triols **3a**–**3d** with yields in the 80% range[6]). At this stage, configurational assignments of compounds **3a**–**3d** were accomplished by ^{1}H-NMR analysis: typically large coupling constants ($J = 9-12$ Hz) between the vicinal H–C(1′) and H–C(2′) as documented in *Table 2* confirmed that these nucleosides **3a**–**3d** have the α-configuration, and that the preferred conformation in solution ((D$_6$)DMSO) is the one depicted in their respective formulas of *Scheme 3*. For the thymine derivative **3b** in the D-arabinose series, an X-ray analysis provided the final confirmation (*Fig. 1*)[7]).

Table 1. *Constitutional Assignments of* N^7- *and* N^9-*(α-L-Arabinopyranosyl)guanine-Regioisomers Based on Comparison of Selected NMR Data of* **2d** *and* **2e**, *and UV Data of the Corresponding Free Triols*

Compound[a])	UV[b]) λ_{max} (ε) [nm]	^{1}H-NMR[c]) ((D$_6$)DMSO) δ [ppm]	^{13}C-NMR[c]) ((D$_6$)DMSO) δ [ppm]
N^7-Isomer **2e**		6.75 (br. *s*, H–C(1′)) 8.60 (*s*, H–C(8))	84.63 (C(1′))
7-(α-L-Arabinopyranosyl)guanine	285 (5300)		
N^9-Isomer **2d**		6.02 (*d*, $J = 9.5$, H–C(1′)) 8.49 (*s*, H–C(8))	81.31 (C(1′))
9-(α-L-Arabinopyranosyl)guanine	252 (11400)		

[a]) Free triols were obtained by hydrazinolysis ($NH_2NH_2 \cdot H_2O$, EtOH, 12 h, r.t.) of **2e** and **2d**, respectively. [b]) Measured in H_2O, pH 7, $c \approx 1$ mg/100 ml. Assignment based on the consistent behavior that N^9-guanine nucleosides exhibit a maxima around 250 nm, while, in the corresponding N^7-guanine isomers, it is shifted bathochromically to *ca.* 285 nm [13a]; also *cf.* Table 1 on page 1428 of [13c]. [c]) Assignment based on the fact that the H–C(1′), H–C(8), and C(1′) chemical shifts are consistently downfield for the N^7-guanine nucleosides as compared to the N^9-derivatives [13].

Exploratory experiments in the D-series with free nucleosides (mostly **3b**) and corresponding 2′-acylated derivatives of **3a**–**3d** with reagents such as BzCl and DMT-chloride led to the observation that the relative reactivities of the three OH groups follows the order HO–C(2′) ≈ HO–C(3′) ≥ HO–C(4′)[8]). It was decided, therefore, that the least-reactive 4′-OH function should carry the phosphoramidite group, while

[5]) Further improvements using 2-amino-6-chloropurine as a surrogate for guanine in the nucleosidation reaction were promising, but difficulties with purification led to their abandonment.

[6]) In the case of cytosine nucleoside **2c**, it was necessary to monitor the reaction carefully by TLC and stop the reaction before the loss of the N^4-benzoyl protecting group.

[7]) Carried out by *Raj K. Chadha*, TSRI. Crystallographic data (excluding structural factors) for the structure reported in this paper has been deposited with the *Cambridge Crystallographic Data Centre* as deposition No. CCDC 201895. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB12 1EZ, UK (fax: + 44 (1233) 336 0333; e-mail: deposit@ccdc.cam.ac.uk).

[8]) For example, tritylation of the 2′-acetate derivative of arabinopyranosyl thymine **3b** led to the tritylation at C(3′) in 38% yield with no tritylation occurring at the 4′-OH.

Table 2. *^{1}H-NMR* J*(H–C(1′),H–C(2′)) Values* [Hz] *for Selected Compounds* (in (D_6)DMSO) *Depicted in* Scheme 3

Compound	**a** (A)	**b** (T)	**c** (C)	**d** (G)
2	9.1	–	–	9.5
3	9.2	12.4	9.0	9.3
4	9.6	9.7	9.3	9.5
5	9.7	9.8	8.5 : 8.8 (*exo*/*endo*)	9.7
6	9.3	9.3	9.0	9.3
7	9.3	8.0	–	9.3

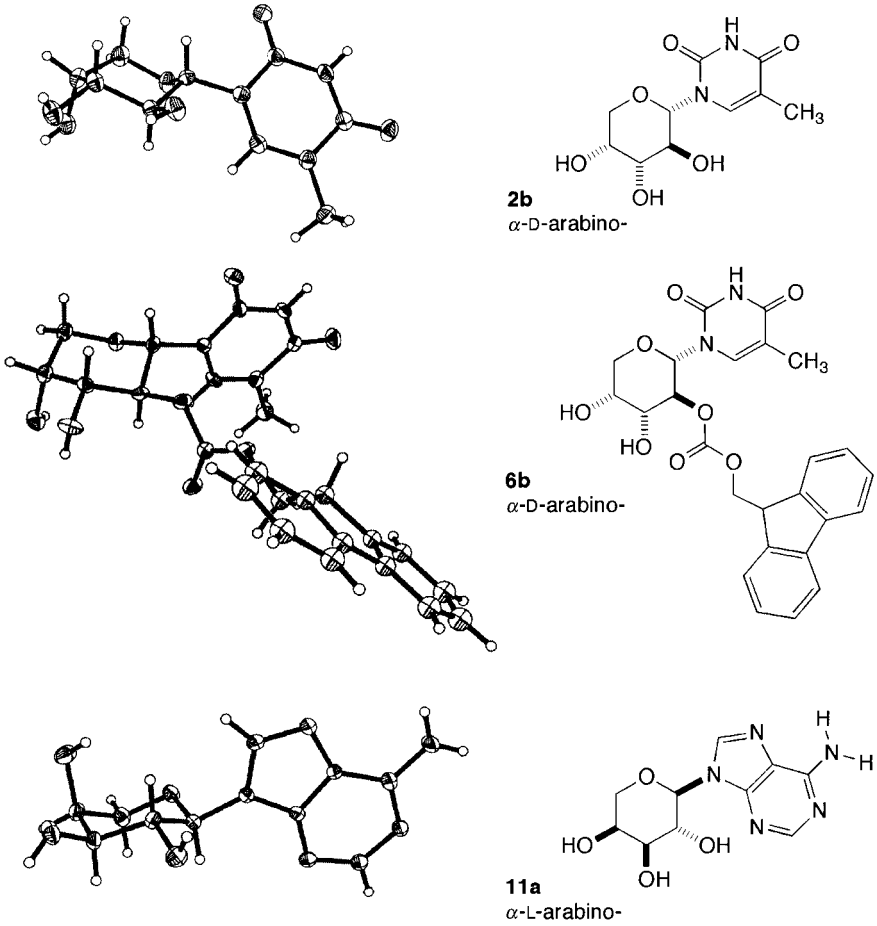
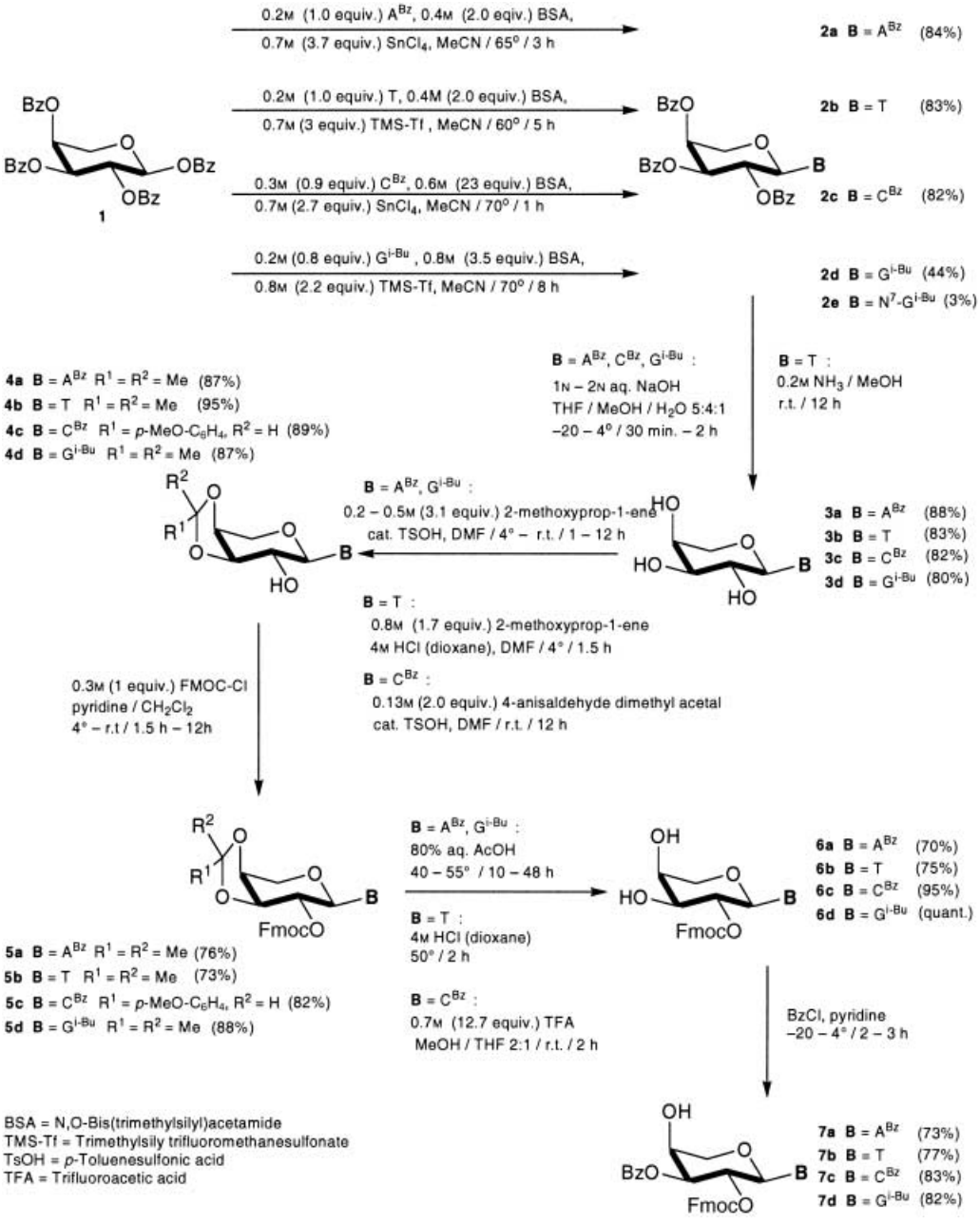


Fig. 1. *X-Ray structures of* **2b**, **6b**, *and* **11a**. Nucleosidic torsion angle (max ± are as follows): O–C(1′)–N(1)–C(2): **2b**: 86.2°; **6b**: 125.4°. O–C(1′)–N(9)–C(4): **11a**: – 102.1° (see *Footnotes 7*, *10*, and *14*).

Scheme 3. *Preparation of the A, T, C, and G Nucleoside Building Blocks for the Synthesis of α-L-Arabinopyranosyl-(4′ → 2′)-oligonucleotides* via *the Fmoc-Protecting-Group Strategy*



the more-reactive 2′-OH would act as the nucleophile on the crucial chain elongation on the CPG-solid-support synthesis; such an approach is the opposite of that undertaken in the synthesis of oligonucleotides in the ribo- [4], xylo- [1], and the lyxopyranosyl series [5]. A regioselective introduction of a protecting group at the 2′-OH position is to be achieved *via* a transient regioselective blockade of the *cis*-vicinal 3′-OH and 4′-OH groups by, *e.g.*, cyclic isopropylidine acetal group, easily introduced or removed under mildly acidic conditions. The choice of an acid-labile isopropylidine acetal protecting group for the 3′,4′-positions precluded the use of DMT- as a 2′-*O*-protecting group and thus dictated the choice of the Fmoc group as an acid resistant, base labile protecting group for the 2′-OH position.

Reaction of triols **3a**, **3b**, and **3d** with 2-methoxyprop-1-ene in DMF with HCl or TsOH as catalyst afforded the corresponding 3′,4′-isopropylidene derivatives **4a**, **4b**, and **4d**, respectively, in over 80% yields, as expected with no signs of a competing formation of a (*trans*!) 2′,3′-isopropylidene derivative. For triol **3c**, the use of 4-anisaldehyde (4-methoxybenzaldehyde) dimethyl acetal in place of 2-methoxyprop-1-ene under similar reaction conditions (DMF, TsOH) afforded a mixture of diastereoisomeric 3′,4′-benzylidene acetals **4c**, in 89% yield, as the sole product[9]). Introduction of Fmoc group at C(2′) was achieved by treatment of **4a**–**4d** with Fmoc-Cl in pyridine at 4° to give **5a**–**5d** in good yields (73–88%). Subsequent acid-catalyzed cleavage of the 3′,4′-acetal protecting groups by treatment with 80% aq. AcOH for **5a** and **5d**, with 4M HCl in dioxane for **5b**, and with 0.7M TFA in MeOH/THF 2:1 for **5d**, generated the 2′-Fmoc derivatives **6a**–**6d** in yields ranging from 70–99%[10]). As pointed to before, the lack of reactivity of the 4′-OH group towards electrophiles came in handy for the selective introduction of the Bz group at 3′-O-position by simply treating **6a**–**6c** with *ca.* 1 equiv. of BzCl at −20–4° to afford the 3′-*O*-Bz derivatives **7a**–**7d** in 70–83% yields[11]). No additional benzoylation at the nucleobases under these conditions were observed. The appropriately protected 3′-*O*-Bz-2′-Fmoc derivatives **7a**–**7d** were the starting materials for the preparation of building blocks suitable for automated solid-support synthesis.

The phosphitylations of **7a**–**7d** were conducted under standard conditions [14] with (2-cyanoethoxy)bis(diisopropylamino)phosphine and with 1*H*-tetrazole as a catalyst (*Scheme 4*). A full equivalent of 1*H*-tetrazole was used to prevent the loss of the Fmoc

---

9) 2-Methoxyprop-1-ene, in fact, could be used in the case of **3c** with no difficulty for the formation of 3′,4′-isopropylidene acetal derivative; but, after formation of 2′-Fmoc derivative, problems (side-product formation) were encountered in the subsequent deprotection of 3′,4′-isopropylidene acetal group to generate **6c**.

10) In the case of the thymine derivative **6b** in the D-arabinose series, X-ray analysis (carried out by *Raj K. Chadha*, TSRI) confirmed the constitution and configuration (*Fig. 1*). Crystallographic data (excluding structural factors) for the structure reported in this paper has been deposited with the *Cambridge Crystallographic Data Centre* as deposition No. CCDC 201894. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB12 1EZ, UK (fax: + 44 (1233) 336 0333; e-mail: deposit@ccdc.cam.ac.uk).

11) Among the benzoylating reagents tried BzCl, BzCN, and Bz_2O, BzCl was found to be the reagent of choice. While Bz_2O (in the presence of DMAP or 1-methyl-1*H*-imidazole) was unreactive and resulted in the cleavage of the base-sensitive Fmoc group, use of BzCN in the case of **6b** led to mixtures of 3′-,4′-, and 3′,4′-benzoylated products under the reaction conditions employed.

group by the $(i\text{-}Pr)_2NH$ generated during the reaction[12]). The purification and isolation of the phosphoroamidites were carried out by very quick (10–15 min.) CC over silica gel at room temperature (silica gel pretreated with toluene containing 1% *Hünig*'s base, followed by careful washing with toluene to remove all of the base) to afford **8a**–**8d** in yields of up to 95%. Such precautions were necessitated by the observations that these phosphoramidites readily decompose under normal chromatographic isolation procedures.

The sensitivity of the Fmoc group to base interfered, once again, with the loading of the solid support CPG with nucleobases **7a**–**7d**; compromises were nevertheless found, which led to satisfactory production of suitably loaded solid supports **10a**–**10d**. As the first step, the 4′-OH group was derivatized as the 4′-*O*-succinate by reacting **7a**–**7d** with 2.0 equiv. of succinic anhydride in the presence of 0.1–0.5 equiv. of DMAP in CH_2Cl_2 as solvent over a 5–7 day period at room temperature [15]. Minimizing the amount of DMAP (and monitoring the reaction by TLC) was found to eliminate the unwanted loss of the Fmoc group. The crude 4′-*O*-succinates **9a**–**9b** (confirmed by ^{1}H-NMR spectroscopy and mass spectrometry) were directly used in the next step of attachment to the specially derivatized LCAMA-CPG-solid support, which has been shown to be stable to the conditions of base induced eliminative deprotection of the Fmoc group [16]. Commercially available glyceryl-modified CPG was reacted with *N*,*N*′-dimethylhexane-1,6-diamine in the presence of 1,1′-carbonyldi[1*H*-imidazole] to generate the LCAMA-CPG (which has the advantages of a long spacer arm and the large pore size of 500 Å, characteristics considered to be advantageous for larger loading values and synthesizing long oligonucleotides). Reaction of the LCAMA-CPG with 4′-*O*-succinylated nucleosides **9a**–**9d** in the presence of TOTU [17] as a condensing agent, followed by capping with excess Ac_2O in the presence of 1-methyl-1*H*-imidazole, led to suitably derivatized LCAMA-CPGs **10a**–**10d**. The loading capacities were determined to range from 12–19 μmol/g by treatment of 5–10 mg of the CPG with 5 ml of 0.1M soln. of DBU in MeCN, and by measuring the absorbance of the solution at 305 nm with a molar absorptivity value (ε) for methylidene-fluorene of 9100 (see *Exper. Part*, *Sect. 2* for details).

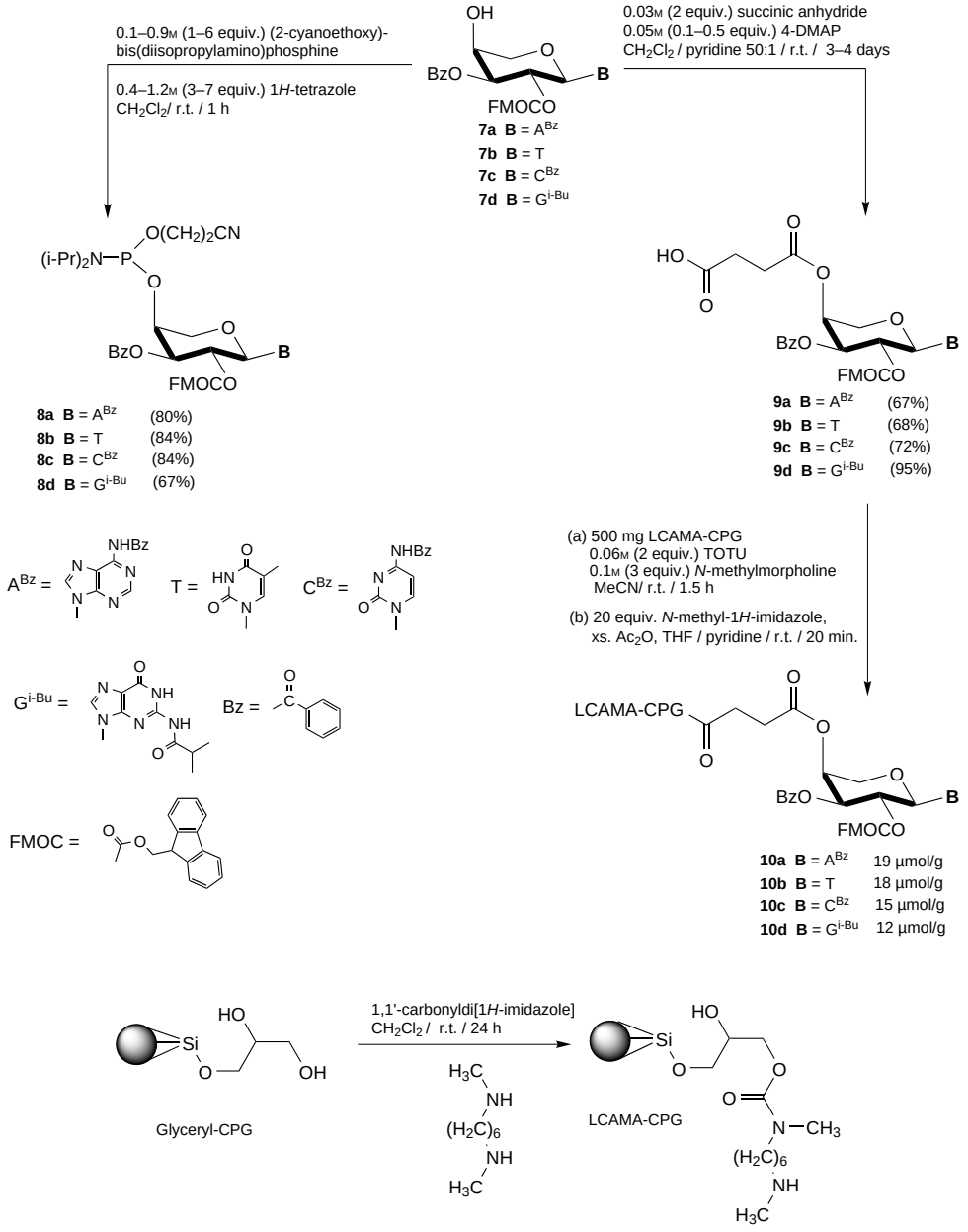
With derivatives **8a**–**8d** and **10a**–**10d**, the requisite building blocks, suitable for proceeding to the synthesis of α-D- and α-L-arabinopyranosyl-(4′ → 2′)-oligonucleotides by automated solid-phase synthesis and the Fmoc strategy, were in hand.

2.1.2. *Synthesis of* α-L-*Arabinopyranosyl Nucleoside Building Blocks Containing the DMT Protecting Group*. While the above Fmoc-protecting-group approach was useful for furnishing enough phosphoramidites for the synthesis of α-D-arabinopyranosyl-(4′ → 2′)-oligonucleotides in a small-scale (0.5–1 μmol) range (see *Sect. 2.2*), the low loading capacity of the CPG solid support and the problems presented by the Fmoc group due to its inherent instability under basic conditions made this approach

---

[12]) These conditions were chosen over the usual 'basic' reaction conditions, *i.e.*, phosphitylation with (chloro)(diisopropylamino)phosphine with either *Hünig*'s base or with collidine and *N*-methyl-1*H*-imidazole, because the sensitivity of the Fmoc group to the base used or produced in the phosphitylation reaction. These 'basic' reaction conditions were found to yield only *ca.* 60% of the phosphitylated products, compounded by complications due to loss of Fmoc group and the incomplete removal of the unreacted phosphitylating reagent by CC.

Scheme 4. *Preparation of the Phosphoroamidite and CPG Derivatives for the Automated Synthesis of* α-L-*Arabinopyranosyl-(4′ → 2′)-oligonucleotides* via *the Fmoc-Protecting-Group Strategy*



unsuitable for large-scale synthesis. Yet, the need for such a 'large-scale' production (*ca.* 10–15 mg) of p**a**(CGAATTCG) came up in the project to determine the structure of α-arabinopyranosyl-(4′ → 2′)-oligonucleotide duplex by NMR spectroscopy [8]. Therefore, we resumed our efforts to develop an approach based on the use of DMT as a protecting group with the sole purpose of having access to large quantities of α-L-arabinopyranosyl-(4′ → 2′)-oligonucleotides.

The first requirement of this approach was to find a way to produce the 2′-*O*-trityl derivatives with high selectivity and in high yields. Toward this goal, exploratory studies on the tritylation of triols **3a** and **3b** were conducted. Treatment of triol **3a** with Me_3SiNEt_2 (DMF/ − 40 to − 55°) followed by tritylation (DMTCl/AgOTf/collidine/MeCN/room temperature/3 d) furnished the 4′-*O*-DMT derivative, in low yields (31%). Attempts to achieve a regioselective benzoylation with BzCN *via* a cyclic tributyl stannane *cis*-diol derivative [18], furnished the 3′-*O*-benzoylated derivatives of **3a** and **3b**, subsequent tritylation (DMTCl/AgOTf/collidine) provided the 3′-*O*-benzoyl-4′-*O*-trityl derivative in 53% yield in the case of **3a**, while a mixture of the 3′-*O*-benzoyl-4′-*O*-trityl and the 3′-*O*-benzoyl-2′-*O*-trityl derivatives was obtained starting from **3b**. Once again, the formation of product mixtures and low yields in the tritylation step prompted us to abandon these approaches.

While searching for a selective protection group for the *cis*-vicinal 3′,4′-diol function, which could be removable again in the presence of a *O*-DMT group, we came across a recent paper by *R. A. Jones et al.* [19] describing the use of the reagent *N*,*N*-dimethylformamide dimethyl acetal for the reversible protection of vicinal diols. Cyclic (dimethylamino)methyleneacetals of *cis*-diols were found to be cleaved with simple protic solvents such as MeOH. The use of this reagent[13]) solved our problem for a selective and efficient tritylation of the 2′-OH group.

Another improvement we wanted to realize while shifting from the Fmoc- to the DMT-based strategy concerned the problems encountered in the nucleobase-protection step of the oligonucleotide synthesis (see *Sect. 2.2.1*). Those problems were to be overcome by replacing the NH_2 protecting groups for adenine (benzoyl), guanine (isobutyryl), and cytosine (benzoyl) by phenoxyacetyl ('PAC', for adenine as well as guanine [20]) and isobutyryl (for cytosine [20]), the former for the sake of being more easily removable, the latter for being more stable (see *Footnote 6*).

Scheme 5 summarizes the preparation of the four nucleoside building blocks **15a**–**15d** that were required for the DMT-based approach to α-arabinopyranosyl-(4′ → 2′)-oligonucleotides, work that was done now exclusively in the L-arabinose series. Central intermediates were the corresponding trihydroxy nucleosides **12a**, **3b**, **12c**, and **12d**, of which the thymine member was available from the earlier work (see *Scheme 3*), whereas the cytosine member was made from scratch by nucleosidation of L-arabinopyranosyl-tetrabenzoate **1** with *N*-isobutyrylcytosine to **11c**, followed by hydrolytic cleavage of all protecting groups and selective reprotection of the cytosine nucleus's primary amino group with isobutyric anhydride in DMF. The trihydroxypurine nucleosides **12a** and **12d**, on the other hand, were made by exchange of the *N*-protecting groups in the nucleosides **2a** and **2d** used earlier. This was accomplished by

---

[13]) Initiated by *A. L.* The publication [19] appeared while our work was in progress.

complete deprotection by treatment with 2M NH_3/MeOH to give **11a**[14]) and **11b**, followed by introduction of the new PAC protection group into the purine nuclei by a three-operation procedure consisting of *O*-silylation [21], *N*-acylation [20], and *O*-desilylation, producing the nucleosides **12a** and **12d** in high yields.

With the four trihydroxy nucleosides **12a**, **3b**, **12c**, and **12d** in hand, we were ready for the critical introduction of the trityl group the 2′-*O*-position *via* transient protection of the vicinal 3′,4′-diol groups utilizing the chemistry described by *R. A. Jones et al.* [19]. We were relieved to find that a one-pot procedure of 3′,4′-amide-acetal formation with nucleosides **12a**, **3b**, **12c**, and **12d** (1.5 equiv. *N*,*N*-dimethylformamide dimethyl acetal, pyridine, collidine, DMF, room temperature, 1–3 h) followed by tritylation (1.2–1.5 equiv. DMT-Cl, collidine, CH_2Cl_2, room temperature, 6–12 h) and subsequent careful methanolysis[15]) of the 3′,4′-amide-acetal group worked reliably and effectively furnished the 3′,4′-dihydroxy-2′-*O*-tritylated nucleosides **13a**–**13d** in yields of 71–61% after chromatographic purification[16]).

In our search for a solution to the last protection problem, that involving the 3′-OH function, we eventually had to accept a compromise. In deciding to stick to benzoate as the protection group, the strategy we had successfully used in the ribopyranosyl series [4], we first had to learn that, in this DMT-protected *arabino* series, it is not the equatorial 3′-OH group that can regioselectively be benzoylated but rather the axial 4′-OH group. The behavior is the opposite of what we had observed previously while exploring the 2′-Fmoc-protected 3′,4′-dihydroxy nucleosides **6a**–**6d** (see *Scheme 3*); it is clearly due to steric hindrance to 3′-benzoylation exerted by the trityl group. In welcome analogy to our experience in the synthesis of ribopyranosyl-(4′→2′)-oligonucleotide [4], where the observation of a (2′→3′)-migration of the *O*-Bz group had been crucial, we showed that the (under mild conditions[17])) regioselectively accessible 4′-monobenzoates **14a**–**14d** undergo a clean equilibration in CH_2Cl_2 solution and the presence of Et_3N at room temperature producing a *ca.* 1:1 mixture of 4′- and 3′-monobenzoates from which the pure 3′-monobenzoates **15a**–**15d** could be isolated by chromatography in yields of 35–38% (with respect to **14a**–**14d**). This we accepted as a compromise for the preparation of the desired nucleosides **15a**–**15d**, taking into account that the recovered 4′-benzoates could be (and were) recycled.

Phosphitylation of **15a**–**15d** with the commercially available chloro(2-cyanoethoxy)-(diisopropylamino)phosphane in the presence of $(i\text{-}Pr)_2NH$ gave phosphoroamidites **16a**–**16d** in 79–85% yields (*Scheme 6*). For the preparation of the CPG solid support,

---

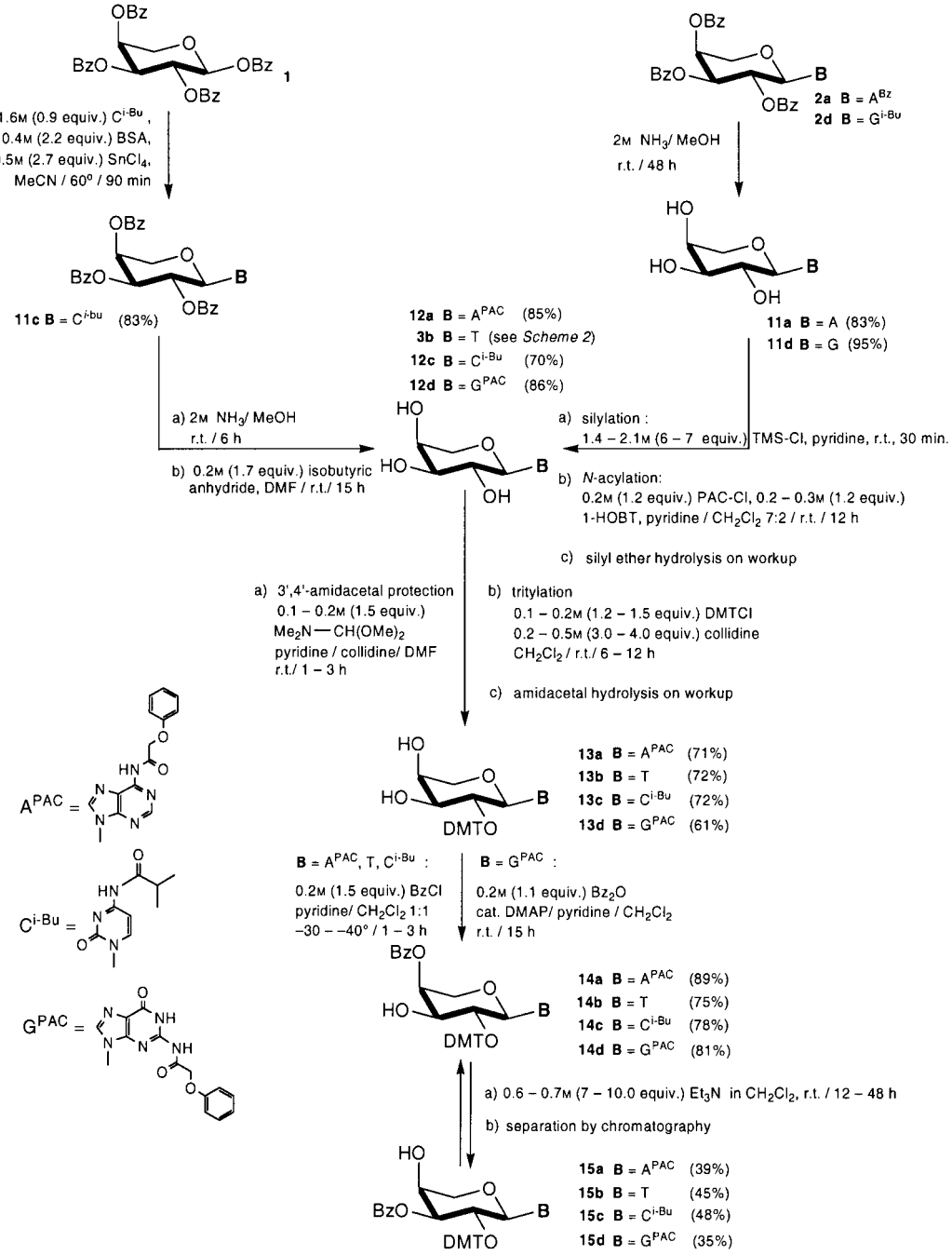
[14]) The structure was also confirmed by X-ray analysis (*Fig. 1*; carried out by *Raj K. Chadha*, TSRI). Crystallographic data (excluding structural factors) for the structure reported in this paper has been deposited with the *Cambridge Crystallographic Data Centre* as deposition No. CCDC 201893. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB12 1EZ, UK (fax: + 44 (1233) 336 0333; e-mail: deposit@ccdc.cam.ac.uk).

[15]) Continuous TLC monitoring of the hydrolytic workup is important due to the high lability of PAC protecting group. After addition of MeOH, a spot with lower R_f (corresponding to presumed hemi-acetal) appears within minutes, which, then, over a period of 30 min converts to a more-polar spot corresponding to the desired triols.

[16]) Chromatographic isolation in the case of **13a** and **13d** was performed with i-PrOH instead of MeOH to minimize the loss of PAC protecting groups.

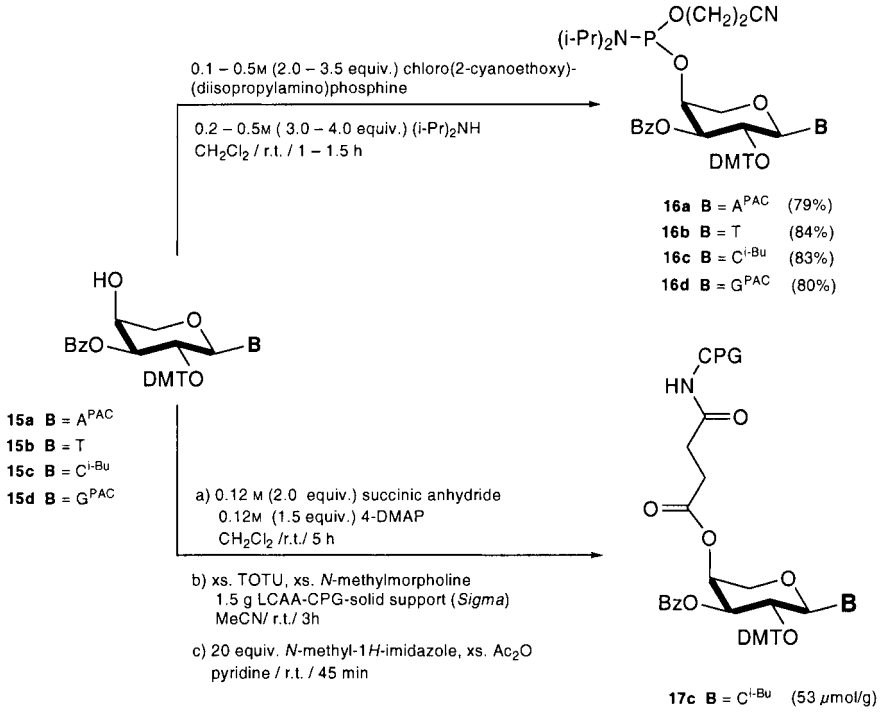
[17]) Benzoylation with BzCl/pyridine in CH_2Cl_2 at $-30°$ to $-40°$; the guanine derivative **13d** required special conditions (Bz_2O/pyridine, DMPA, r.t.).

Scheme 5. *Preparation of the A, T, C, and G Nucleoside Building Blocks for the Synthesis of α-L-Arabinopyranosyl-(4′ → 2′)-oligonucleotides* via *the DMT-Protecting-Group Strategy*



the standard one-pot protocol used previously for the pyranosyl *ribo*, *xylo*, and *lyxo* series [1][4][5], (formation of the 4′-*O*-succinic acid derivative by treatment with succinic anhydride in the presence of DMAP, followed by CPG derivatization with TOTU [17] as an activator and subsequent capping with Ac_2O), was found to work with **15c** to give **17c** without any problem. The loading capacity [13c] was determined to be 53 μmol/g, what we considered to be high. The cytosine-containing CPG solid support was the only one needed, since p**a**(4′-CGAATTCG-2′) was the only arabinopyranosyl-oligonucleotide strand we synthesized with the DMT-based strategy.

Scheme 6. *Preparation of the Phosphoroamidite and CPG Derivatives for the Automated Synthesis of α-*L*-Arabinopyranosyl-(4′ → 2′)-oligonucleotides* via *the DMT-Protecting-Group Strategy*



2.2. *Synthesis of α-Arabinopyranosyl-(4′ → 2′)-oligonucleotides. Table 3* lists all α-arabinopyranosyl sequences synthesized in the course of our study. By reasons mentioned in the *Introduction*, sequences made in the first phase of the project contained the D-arabinopyranose unit, while those prepared at a later stage belong to the L-arabinopyranosyl series. With the exception of the self-complementary sequence p**a**(CGAATTCG), which was prepared in 'large' amounts (*ca.* 10 mg) for the NMR structure analysis [8], the amount made in the case of all other sequences was in the range of 1 μmol. The methodology followed was in essence the one previously used in the ribo- [4], xylo- [1], and lyxopyranosyl [5] series on an automated DNA synthesizer (*Perseptive*'s *Expedite*), taking into account, as detailed in *Sect. 2.2.1* and *2.2.2* below, the specific aspects and demands of the two different protection strategies. In these two

strategies, the solid-phase synthesis of α-arabinopyranosyl oligonucleotide strands proceeds in opposite directions, namely, the 3′(phosphityl) → 2′(hydroxy) direction in the Fmoc-protection strategy and the 2′(phosphityl) → 3′(hydroxy) direction in the DMT-protection strategy. If, in a future preparation of α-arabinopyranosyl-(4′ → 2′)-oligonucleotides, one had to choose between the two strategies with respect to the criterion of expediency and yield, the DMT-protection strategy would clearly be preferred.

Table 3. *HPLC and MS Data of α-Arabinopyranosyl-(4′ → 2′)-oligonucleotides*

Entry	Base sequences[a]) (all 4′ → 2′)	Deprotection[b]) *Method*	O.D. 260 nm (yield)	Anal. HPLC ion exchange[c]) gradient, t_R [min]	MALDI-TOF-MS[d]) $[M+H]^+$	
					obs.	calc.
1	D-p**a**(A_8)	*A*	4.4 (5%)	10–50% in 30 min, t_R 14.8	2573	2572
2	L-p**a**(A_8)	*A*	4.8 (9%)	10–50% in 30 min, t_R 14.8	2571	2572
3	L-p**a**(A_{12})	*A*	4.9 (5%)	10–50% in 30 min, t_R 20.2	3890	3889
4	D-p**a**(T_4)	*B*	–	20–80% in 30 min, t_R 14.1	1223 (Na^+)	1224 (Na^+)
5	D-p**a**(T_8)	*A*	4.2 (16%)	0–100% in 30 min, t_R 21.0	2501	2500
6	L-p**a**(t_8)	*A*	8.8 (18%)	0–100% in 30 min, t_R 21.0	2501	2500
7	L-p**a**(T_{12})	*A*	4.8 (7%)	0–100% in 30 min, t_R 22.9	3780	3780
8	D-p**a**(T_4A)	*B*	–	10–50% in 30 min, t_R 11.5**	1547	1546
9	D-p**a**(T_4A_2)	*B*	–	10–50% in 30 min, t_R 15.2**	1876	1876
10	D-p**a**(T_4A_3)	*B*	–	10–50% in 30 min, t_R 25.9**	2206	2205
11	D-p**a**(TA_8)	*B*	–	–	2891	2892
12	D-p**a**((ATATA)	*B*	8.5 (31%)	10–50% in 30 min, t_R 17.5	1564	1566
13	D-p**a**(TATAT)	*A*	–	20–80% in 30 min, t_R 10.3	1557	1557
14	D-p**a**(ATATATA)	*A*	–	20–80% in 30 min, t_R 12.7	2214	2214
15	D-p**a**(A_4T_4)	*C*	7.6 (13%)	10–50% in 30 min, t_R 28.2	2534	2534
16	D-p**a**(T_4A_4)	*B*	7.6 (13%)	10–50% in 30 min, t_R 28.4**	2535	2534
17	D-p**a**$(AT)_4$	*A*	–	20–80% in 30 min, t_R 14.4	2536	2536
18	D-p**a**$(TA)_4$	*A*	–	20–80% in 30 min, t_R 15.1	2536	2536
19	L-p**a**(TATTTTAA)	*A*	7.4 (16%)	0–100% in 30 min, t_R 19.0**	2527	2527
20	L-p**a**(TTAAAATA)	*A*	13.1 (25%)	0–100% in 30 min, t_R 16.2	2544	2545
21	L-p**a**(G_8)	*A*	2.7 (9%)	0–100% in 30 min, t_R 26.6	2010	2009
22	L-p**a**(C_8)	*A*	4.3 (16%)	10–50% in 30 min, t_R 9.9	1771	1770
23	L-p**a**(ATTCAGCG)	*D*	4.3 (7%)	0–100% in 30 min, t_R 19.1	2538	2538
24	L-p**a**(CGCTGAAT)	*D*	2.2 (6%)	0–100% in 30 min, t_R 18.9	2536	2538
25	L-p**a**(CGAATTCG)*	*E*	446 (11%)	0–100% in 30 min, t_R 21.5	2537	2538

[a]) All sequences were synthesized by the Fmoc-protection strategy with the exception of the sequence 25, labeled *, which was synthesized by the DMT-protection strategy. [b]) *Method A:* 0.15M $MeONH_2$ in 25% aq. NH_3/EtOH 3 : 1 at r.t. for 10 h (24 h for mixed AT and homo-G sequences); *Method B:* 25% aq. NH_3 at 4°, 9–24 h; *Method C:* 25% aq. NH_3 at 4°, 21 h followed by 25% aq. NH_2NH_2, r.t., 10 min; *Method D:* ethylenediamine/EtOH 1 : 1, r.t., 2 h [22]; *Method E:* pyridine/Et_3N 4 : 1, r.t., 5 h; 40% aq. $MeNH_2$, r.t., 1 h. [c]) All oligonucleotides were purified by ion-exchange chromatography on a *Mono Q HR 5/5* column (58 × 6.0 mm, *Pharmacia*); elution with 10 mM Na_2HPO_4 in H_2O and a linear gradient of 1M NaCl with a flow of 1 ml/min. pH 10.5–11.5; followed by desalting on *Sep-Pak* cartridges. peak purity (260 nm) 95–99%. ** Elution with 25 mM *Tris* and 1.5M urea in H_2O, and a linear gradient of 1M NH_4Cl, pH 8.0. [d]) Matrix-assisted laser-desorption ionization time-of-flight mass spectroscopy; matrix: 3-hydropicolinic acid or α-cyanohydroxycinnamic acid or 2,4,6-trihydroxyacetophenone and ammonium citrate buffer.

2.2.1. *Oligonucleotide Synthesis with Fmoc-Protected Building Blocks.* Since MeCN solutions of Fmoc-phosphoramidites **8a**–**8d** (stored with 4-Å molecular sieves) were

found to decompose slowly (over periods of days), for their use in the solid-support synthesis, all solutions were freshly prepared and used within 24 h[18]). The syntheses were performed with *ca.* 30 mg of solid-support derivatives **10a** – **10d** with the following modifications to the protocols used earlier: *a*) coupling of phosphoroamidites over a period of 15 min with either 0.5M pyridine hydrochloride or 0.5M 1*H*-tetrazole in MeCN and *b*) cleavage of Fmoc with 0.1M DBU in MeCN over a 1.5-min period. Coupling efficiencies were monitored by measurement of UV absorbance of fulvene resulting from the cleavage of Fmoc group after each coupling (in analogy to *Trityl* assay). They ranged from 95 to 98% for T-, C-, and G-containing residues, while around 90% for A-containing residues[19]). Coupling yields were found, sporadically, to drop to *ca.* 80% in the synthesis of longer oligonucleotide strands.

The Fmoc groups of the last residue were removed before detaching the oligonucleotide strand from the solid support. Detachment and concomitant removal of all remaining groups posed a major problem. Under the base treatments normally used (such as aq. NH_3), some of the α-arabinopyranosyl-(4′ → 2′)-oligonucleotides were found to be extremely sensitive towards strand scission. Therefore, various conditions had to be explored and fine-tuned for the specific sequence under consideration, these conditions were – depending on the specific oligonucleotide sequence – either treatment of the dried CPG material with 0.15M $MeONH_2 \cdot HCl$ in 25% aq. NH_3/EtOH 3:1 at room temperature for 10–24 h (*Method A*, *Table 3*), or with 25% aq. NH_3 at 4° for 9–24 h (*Method B*, *Table 3*), or with 25% aq. NH_3 at 4° for 21 h, followed by 25% aq. $NH_2NH_2 \cdot H_2O$ at room temperature for 10 min (*Method C*, see *Table 3*), or with ethylenediamine/EtOH 1:1 at room temperature for 2 h (*Method D*, *Table 3*). All these treatments led to the deprotection of the Bz groups on the sugar, the 2-cyanoethyl group on the phosphate, as well as the acylamino groups of adenine, cytosine, and guanine with the concomitant detachment of the oligonucleotides from the CPG solid support. The homo-adenine sequences (*Entries 1–3*, *Table 3*) did not fare well under the standard aqueous NH_3 deprotection conditions, yielding lots of strand-scission products; milder treatment with $MeONH_2 \cdot HCl$ in combination with aq. NH_3 (*Method A*) resulted in the least strand scission. For the homo-guanine-containing sequence L-p**a**(G_8) (*Entry 21*), it was observed that the N^2-isobutyryl groups were not fully deprotected within 10 h; therefore, the deprotection time was prolonged, and a balance had to found between complete deprotection and the amount of strand-scission products. Thus, the deprotection was stopped after 24 h, and the peak corresponding to L-p**a**(G_8) was isolated[20]). In the case of L-p**a**(ATTCAGCG) and its complementary sequence where decomposition was most serious, treatment with ethylenediamine/EtOH 1:1 at room temperature for 2 h [22] led to a marked improvement in minimizing strand scission. In all cases, deprotections were monitored by ion-exchange HPLC for optimal deprotection time. While almost all of the oligonucleotide sequences

---

[18]) Decomposition of Fmoc-phosphoramidites as seen by TLC. The problem was most severe in the case of guanine derivative **7d**.

[19]) In a typical 0.5-μmol-scale synthesis, each Fmoc-derived fulvene eluate was diluted to 5 ml with MeCN, and the absorbance at 305 nm was measured against a 0.1M soln. of DBU in MeCN as a reference. The value obtained for each fulvene eluate was compared as a percentage to that of the eluate of the previous step.

[20]) Also isolated were L-p**a**(G_6) and L-p**a**(G_7) from this deprotection procedure as strand-scission products.

could be purified by ion-exchange HPLC (target purity 95%), on *Mono Q HR 5/5* column (*Pharmacia*) with 10 mM NaH_2PO_4 buffer at pH 10.5–11.5 with a linear gradient of 1M NaCl (see *Exper. Part*, *Sect. 6*), some sequences containing thymine and adenine (*Entries 8–10*, *16*, and *19*) remained still unresolved; these sequences were purified with 25 mM *Tris*·HCl buffer at pH 8.0 containing 1.5M urea with a linear gradient of 1M NH_4Cl. Product peaks (monitored at 260 nm) were collected in vials containing 20 µl of a 1M aq. AcOH (to prevent further strand scission) and desalted (see *Exper. Part*, *Sect. 7*), and the desalted oligonucleotides dissolved in 1 ml of H_2O. The concentrations of such purified oligonucleotide stock solutions were determined by UV (at *ca.* 80°) with the following molar extinction coefficients at 260 nm at pH 7: $\varepsilon(p\mathbf{a}(A)) = 15000$, $\varepsilon(p\mathbf{a}(T)) = 10000$, $\varepsilon(p\mathbf{a}(C)) = 8400$, $\varepsilon(p\mathbf{a}(G)) = 11900$. The purified oligonucleotides were checked for the correct mass by matrix-assisted laser-desorption ionization time-of-flight (MALDI-TOF) mass spectrometry [23]. For the oligonucleotides synthesized by this procedure and for their HPLC and MS data, see *Table 3*.

2.2.2. *Synthesis of α-L-Arabinopyranosyl-(4′-CGAATTCG-2′) with DMT-Protected Building Blocks.* This synthesis followed the previously established protocols [1][4][5] on the automated DNA synthesizers (*Perseptive*'s *Expedite*) on a 5-µmol scale, with the following modifications to the standard program: *a*) increase of the coupling time to 27 min with a 0.07M solution of phosphoramidite in MeCN (this modification allowed the parsimonious use of only *ca.* 5-fold excess of phosphoramidite), and *b*) use of 0.35M solution of 5-(ethylthio)-1*H*-tetrazole in MeCN as activator. Average coupling efficiencies were *ca.* 93%, as judged by trityl assay ($\varepsilon(500\text{ nm}) = 66000$). A total of nine such synthesis operations, carried out in the '*Trityl-Off*' mode, were performed to reach an overall 45-µmol scale.

The detachment from the solid support and the deprotection of the oligonucleotide was effected in two stages: first, treatment of the dried CPG solid support with pyridine/Et_3N 4:1 for 5 h at room temperature [24] (removal of the 2-cyanoethyl protecting group converting the phosphotriester group into the more-stable phosphodiester group) and, second, exposure to 40% aqueous $MeNH_2$, effecting the simultaneous deprotection of the sugar and nucleobase protecting groups along with detachment of the oligonucleotide from the solid support. The crude oligonucleotide was purified (target purity > 95%) by ion-exchange HPLC on *Mono Q HR 5/5* column (*Pharmacia*) with 10 mM NaH_2PO_4 buffer at pH 11.3 with a linear gradient of 1M NaCl (see *Exper. Part, Sect. 6*)[21]). The product peak (monitored at 260 nm) was collected in a vial containing 0.1M phosphate buffer at pH 7.0 to prevent any strand scission. The oligonucleotide was desalted (see *Exper. Part*, *Sect. 7*), and its integrity was checked by MALDI-TOF mass spectroscopy (*Entry 25*, *Table 3*). The counter ion was exchanged to Na^+ by treatment with Na^+ form of *DOWEX* (*50 W × 8*) to make it suitable for NMR measurements. The concentration of the oligonucleotide measured with $\varepsilon(260\text{ nm}) = 90600$, gave a total of 4.92 µmol of the oligomer (15.9 mg isolated; 11% overall yield).

---

[21]) This high pH was found to be more satisfactory in preventing the self-association of this self-complementary oligonucleotide than the usual pH of 10.5.

3. Base-Pairing Properties. – The pairing properties of α-D- and α-L-arabinopyranosyl-(4′ → 2′)-oligonucleotides were characterized by methods used in earlier studies in the ribo- [4], xylo- [1], and lyxopyranosyl oligonucleotide series [5]. These methods are: temperature-dependent UV spectroscopy (for T_m measurements [25]), concentration-dependent T_m measurement by UV spectroscopy (for determination of thermodynamic data [26]), molar-ratio-dependent UV spectroscopy (for checking stoichiometry of complex formation [27]), and temperature-dependent CD spectroscopy. All measurements were made in 'phosphate buffer' (10 mM aq. NaH_2PO_4 buffer containing 0.1 mM Na_2(EDTA), 150 mM (or 1M) NaCl at pH 7.0) with a total oligonucleotide concentration of *ca.* 10 μM, unless otherwise stated. *Tables 4* and *5* list the T_m and thermodynamic data collected for the intra- and intersystem pairing duplexes formed by the α-L-arabinopyranosyl-(4′ → 2′)-oligon ucleotides, while *Figs. 3–6* document pairing behavior, complementing information published in preliminary communications [3]. The data of *Table 4* indicate whether a given observation refers to the D- or L-series[22]); all data in *Table 5* refer to the L-arabinopyranosyl series.

The T_m values listed in *Tables 4* and *5* document the dominant characteristic of α-arabinopyranosyl-(4′ → 2′)-oligonucleotides, namely, that they form more-stable duplexes among all members of the pentopyranosyl-oligonucleotide family studied thus far. Duplexes of p**a**-octamers have T_m values on the average of *ca.* 20° higher than corresponding p**l**-octamers, their nearest corresponding competitors (*Table 5*). This property tends to extend to the phenomenon of self-association of non-self-complementary strands, both in homo-oligomer (*Table 4*, *Entries 2–4*) and hetero-oligomeric strands (*Entries 8, 11*, and *12*). While self-association is weak for (A_8) and (A_{12}) oligomers ($T_m < 5°$ and 7.8°, resp.), it is remarkably pronounced in (T_8) and (T_{12}) series ($T_m = 26.9°$ and 52.2°, resp., see *Fig. 2*). This behavior is also reflected in the behavior of the antiparallel-complementary, but not self-complementary, sequences (4′-TATTTTAA-2′) and (4′-TTAAAATA-2′); mixed together, they form a duplex that is stronger than the self-association of the individual strands (*Fig. 3,d*, and *Table 4*, *Entries 8–10*). The sequences (G_6) and (C_6), remarkably, do not exhibit discernable self-pairing; yet, mixing of these two sequences produces a very strong duplex ($T_m = 72.3°$ in 0.15M NaCl, *Entry 7*; *Fig. 3*). The tendency for self-pairing in a sequence with mixed A and T nucleobases is diminished by the introduction of G and C nucleobases. This is demonstrated by the low T_m values of self-association exhibited by the non-self-complementary sequence (4′-ATTCAGCG-2′) and its complementary sequence (4′-CGCTGAAT-2′) (*Entries 11* and *12*). Once again, the duplex formed after mixing these two sequences has high stability ($T_m = 80.5°$ in 0.15M NaCl, *Entry 13*; *Fig. 4*). Testifying to the superiority of the α-arabino-oligonucleotide sequences in terms of the strength of base-pairing, the self-complementary sequence (4′-CGAATTCG-2′) melts at a $T_m = 78.1$ (1 μM, 0.15M NaCl, *Entry 13*; *Fig. 4,c*), while the T_m value of the corresponding p-RNA sequence is 51°[23])!

The CD spectra of the α-L-arabino-oligonucleotide sequences are strikingly similar to those observed in the α-L-lyxo series, but markedly different from the β-D-ribo and β-

---

[22]) In one case, p**a**(A_8) + p**a**(T_8), UV-T_m measurements in both the D-α- and L-α-series were made, observed T_m values were: 71.1° in the D-α-case (0.15M NaCl, 0.01M *Tris* buffer) and 68.3° in the L-α-case (0.15M NaCl, 0.01M phosphate buffer).

[23]) Calculated from the thermodynamic data reported in [4b].

Table 4. T_m *Values and Thermodynamic Data of α-Arabinopyranosyl-(4′ → 2′)-duplexes*

Entry	Oligonucleotide System (L-series)	T_m [°][a] Duplexes, $c \approx 10$ μM		ΔG (25°)	ΔH [kcal/mol]	$T\Delta S$ (25°)	Ref.
		1M NaCl	0.15M NaCl	0.15M NaCl[a][b]			
1	p**a**(A_8)	< 5					
2	p**a**(A_{12})	7.8[c]					
3	p**a**(T_8)	26.9[c]					
4	p**a**(T_{12})	52.2[c]					
5	D-p**a**(A_8) + D-p**a**(T_8)	78.7[d]	71.1[d]	− 15.7[d]	− 60.6[d]	44.9[d]	[3a]
6	p**a**(A_{12}) + p**a**(T_{12})	> 95	74.3[e]				[3b]
7	p**a**(G_6) + p**a**(C_6)	–	72.3	− 12.1	− 30.7	− 18.6	
8	p**a**(TTAAAATA)	48.1[d]		− 8.2	− 31.1	− 22.9	
9	p**a**(TATTTTAA)	48.9[d]		− 9.4	− 53.2	− 43.8	
10	p**a**(TATTTTAA) + p**a**(TTAAAATA)	74.6	65.6	− 15.3	− 66.1	− 50.8	[3b]
11	p**a**(ATTCAGCG)	< 5					
12	p**a**(CGCTGAAT)	18.6[d]					
13	p**a**(ATTCAGCG) + p**a**(CGCTGAAT) Self-complementary sequences	> 88	80.5	− 17.6	− 63.0	− 45.4	[3b]
14	D-p**a**(A_4T_4)	–	61.2[d]	− 13.5[d]	− 59.9[d]	− 46.4[d]	[3a]
15	D-p**a**(T_4A_4)	–	69.4[d]	− 14.5[d]	− 57.6[d]	− 43.0[d]	[3a]
16	D-p**a**(T_4A_3)		47.5[d]				
17	D-p**a**(ATATA)	–	29.2[d]				
18	D-p**a**(TATAT)	–	21.0[d]				
19	D-p**a**(ATATATA)	–	57.4[d]				
20	D-p**a**(ATATATAT)	–	60.0[d]				[3a]
21	D-p**a**(TATATATA)	–	60.8[d]				[3a]
22	p**a**(CGAATTCG)	–	78.1 (1 μM)				

[a]) Measurements were made in 0.01M NaH_2PO_4, 0.1 mM Na_2(EDTA) buffer, pH 7.0, unless otherwise indicated. Estimated error of T_m determination ± 0.5°. – : not measured. [b]) Thermodynamic data from plots of T_m^{-1} *vs.* ln *c*; experimental error estimated in ΔH values ± 5%. [c]) Self-pairing. [d]) T_m measured in 0.01M *Tris* · HCl buffer, pH 7.0. [e]) T_m measured with no NaCl.

D-xylo series (*Fig. 5*; also Fig. 2 in [3b]). This parallels the conformational relationships that are presumed to prevail within the pentopyranosyl family (see *Scheme 1*). The parallelism reappears in intersystem cross-pairing studies (*Table 5* and *Fig. 6*). The T_m values of the duplexes formed between the p**a** and p**l** sequences are consistently higher than any duplex formed from any sequence combinations of p**a**- with either p**r**- or p**x**-oligomers (*Table 5*). This intersystem cross-pairing behavior pervades the entire family [3] (*Table 5*). Not surprisingly, there is no intersystem cross-pairing between α-L-arabinopyranosyl-(4′ → 2′)-oligonucleotides and the corresponding complementary RNA and DNA sequences; this is similar to the behavior exhibited by the other three pentopyranosyl-(4′ → 2′)-oligonucleotide members [1][3–5].

All observations made both in the inter- and intrasystem pairing studies are consistent with the *Watson–Crick* base-pairing mode in antiparallel strand orientation. This is confirmed for the α-L-arabinopyranosyl duplex formed by the self-complementary strand p**a**(4′-CGAATTCG-2′) by NMR structure analysis [8].

4. Discussion. – Among the four experimentally studied members of the family of pentopyranosyl-(4′ → 2′)-oligonucleotide systems, the α-arabinopyranosyl member is,

Table 5. T_m *Values of Duplexes Formed by Inter- and Intrasystem Base Pairing of* α-L-*Arabinopyranosyl-(4′ → 2′)-oligonucleotides*

Entry	Oligonucleotide systems	T_m [°] Intersystem duplexes 1M NaCl ($c \approx 5+5$ μM)[a]	For comparison: T_m of Intrasystem duplexes 1M NaCl ($c \approx 5+5$ μM)[a][b]	
	$(A_8) + (T_8)$			
1	p**a** + p**r**	44.7	p**a** + p**a**	78.7
2	p**r** + p**a**	59.4	p**l** + p**l**	51.0
3	p**a** + p**x**	42.5	p**x** + p**x**	47.3*
4	p**x** + p**a**	54.7	p**r** + p**r**	45.5
5	p**a** + p**l**	66.9	**r** + **r**	23.0
6	p**l** + p**a**	67.8		
	$(A_{12}) + (T_{12})$			
7	p**a** + p**r**	67.8	p**a** + p**a**	> 95
8	p**r** + p**a**	83.0	p**l** + p**l**	74.3
9	p**a** + p**x**	69.5	p**x** + p**x**	73.2
10	p**x** + p**a**	83.0	p**r** + p**r**	67.6
11	p**a** + p**l**	89.3	**r** + **r**	53.5
12	p**l** + p**a**	> 90		
	(TATTTTAA) + (TTAAAATA)			
13	p**a** + p**r**	45.3	p**a** + p**a**	74.6
14	p**r** + p**a**	53.6	p**l** + p**l**	46.4*
15	p**a** + p**x**	53.1	p**x** + p**x**	44.4
16	p**x** + p**a**	45.8	p**r** + p**r**	45.9
17	p**a** + p**l**	61.2	**r** + **r**	17.2
18	p**l** + p**a**	62.3		
	(ATTCAGCG) + (CGCTGAAT)			
19	p**a** + p**r**	70.2	p**a** + p**a**	> 88
20	p**r** + p**a**	66.8	p**l** + p**l**	61.9
21	p**a** + p**l**	73.3	p**x** + p**x**	
22	p**l** + p**a**	73.6	p**r** + p**r**	67.6
			r + **r**	52.0

[a]) Measurements were performed in 0.01M NaH_2PO_4, 0.1 mM Na_2(EDTA) buffer, pH 7.0, unless otherwise indicated. [b]) For T_m data and thermodynamic data of p**a**/p**a**, p**l**/p**l**, p**x**/p**x**, p**r**/p**r**, and **r**/**r** duplexes determined in 0.15M NaCl, see Table 2 in ref. [3b]. Estimated error of T_m determination ± 0.5°. All T_m values taken are from [3b]. p**r** = β-D-Ribopyranosyl, p**x** = β-D-xylopyranosyl, p**l** = α-L-lyxopyranosyl, p**a** = α-L-arabinopyranosyl, **r** = RNA. Values with (*) were measured in 0.01M *Tris*·HCl buffer, pH 7.0.

in a way, the most informative one when it comes to comparatively analyzing the family's base-pairing properties. Some of the structural factors deemed to be responsible for these remarkable properties express themselves most clearly in the structure of the α-arabinopyranosyl system. To these factors belong the conformational unambiguity and rigidity of the pyranosyl chairs, the fact that, in each member, the (relatively) small set of least-strained (idealized) conformations accessible to the repeating unit of a single strand contains at least one repetitive [3][7] pairing conformation, and, finally, that there is in each of these pairing conformations a large inclination between the (averaged) strand axis and the (averaged) nucleobase pairing axis. The first and second factors are expected to contribute to the conformational preorganization of pentopyranosyl-(4′ → 2′)-oligonucleotide single strands towards their duplex conformation. The third factor is responsible for the two most-characteristic features by which pentopyranosyl-(4′ → 2′)-oligonucleotide duplexes structurally distinguish themselves from the

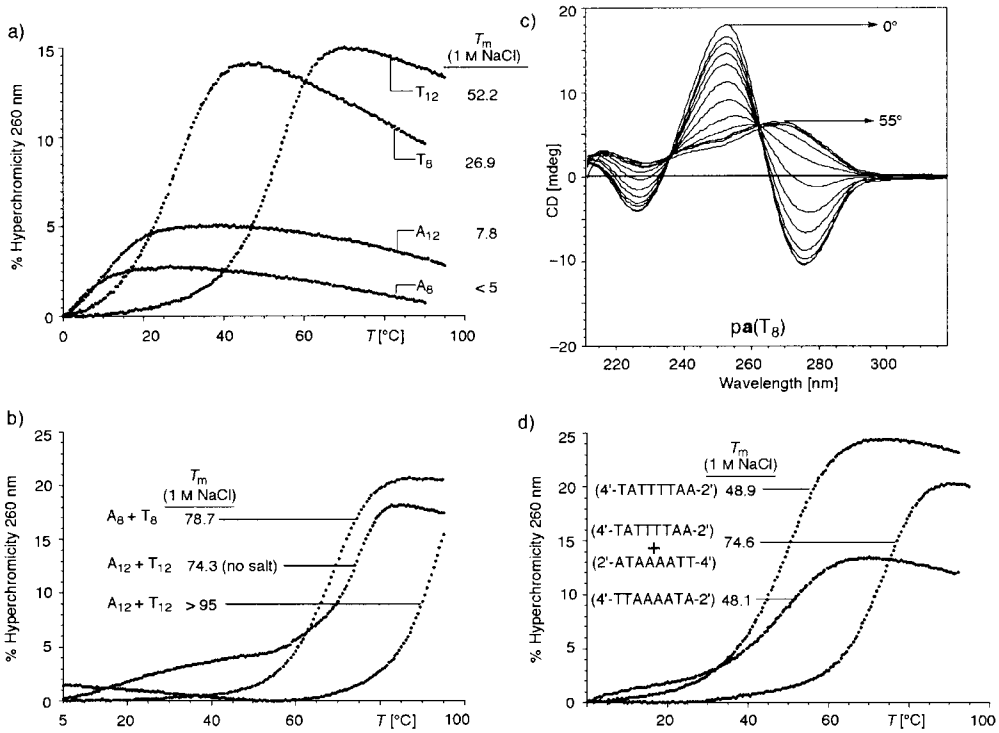


Fig. 2. *UV and CD data documenting the pairing behavior of A,T-containing (4′ → 2′)-α-L-arabinopyranosyl sequences. a*) Self-pairing of the homobasic strands p**a**(A_8), p**a** (A_{12}), p**a**(T_8), and p**a**(T_{12}) as demonstrated by UV-spectroscopic T_m curves (heating). *b*) UV-Spectroscopic T_m curves (heating) of selected duplex formation. *c*) Temperature-dependent CD curves of p**a**(T_8) demonstrating T/T self-pairing (temperature range: 0° → 55°). *d*) UV-Spectroscopic T_m curves of the duplex formed by p**a**(4′-TTAAAATA-2′) with its antiparallel complement p**a**(4′-TATTTTAA-2′) and self-pairing T_m curves of the individual strands. All measurements were made in 10 mM aq. NaH_2PO_4 buffer containing 0.1 mM Na_2(EDTA), 1M NaCl at pH 7.0, unless otherwise indicated. Total oligonucleotide concentrations in all measurements were *ca.* 10 μM. All T_m curves were fully reversible (no hysteresis). T_m Values are calculated from the maxima of the first derivative curve using kaleidagraph software program.

natural nucleic acids, namely, their quasi-linear (as against helical) duplex conformation and their exclusive adoption of an interstrand (as against intrastrand) base-stacking mode. A large inclination between backbone and nucleobase axes allows the nucleobases to reach optimal stacking distances without the need for strand helicalization[24]), implying that base stacking within the duplex has to be interstrand[25]).

---

[24]) Strand constriction by either helicalization or inclination are the two major pathways available to a 1,3-phosphodiester-based (6-bond) oligonucleotide strand to lower the distance between vicinal nucleobases and reach optimal stacking distances (see, *e.g.*, [28]).

[25]) To the extent that nucleobase stacking can be a source of stabilization due to attractive forces between the stacking partners π-systems, interstrand stacking in a duplex is expected to act as a glue between the strands, whereas intrastrand stacking is not, at least to the extent that it can persist in a single strand after de-duplexation.

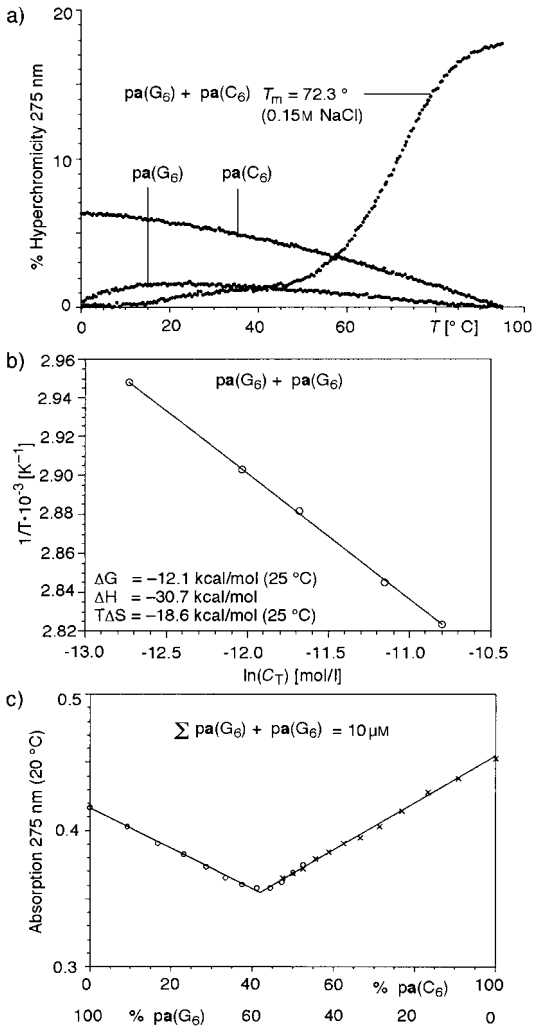


Fig. 3. *UV-*T_m*, Thermodynamic, and 'mixing-curve' data documenting the pairing behavior of G,C-containing (4′ → 2′)-α-*L*-arabinopyranosyl sequences. a*) UV-Spectroscopic T_m melting curves for pairing between p**a**(G_6) and p**a**(C_6), and corresponding curves of individual strands. For conditions of measurements, see caption of *Fig. 2*. T_m Curves fully reversible (no hysteresis). *b*) Thermodynamic data of the duplex p**a**(G_6)/p**a**(C_6) deduced from concentration dependence of UV-spectroscopically determined T_m values. T_m Values measured in 10 mM aq. NaH_2PO_4 containing 0.1 mM Na_2(EDTA), 150 mM NaCl at pH 7.0; for method, see [26]. *c*) Molar-ratiodependence of UV absorption (275 nm, 20°) ('mixing curve') considered to indicate a 1:1 duplex stoichiometry for the pairing between p**a**(G_6) and p**a**(C_6). Data measured in 10 mM aq. NaH_2PO_4 containing 0.1 mM Na_2(EDTA), 150 mM NaCl at pH 7.0 with a total oligonucleotide concentration of 10 μM; for method, see [27].

As delineated in the *Introduction*, there are two conformational subgroups to be distinguished among the four (4′ → 2′)-pentopyranosyl systems investigated (*Scheme 1*); they differ in the conformation of their 4′,2′-phosphodiester links; in one

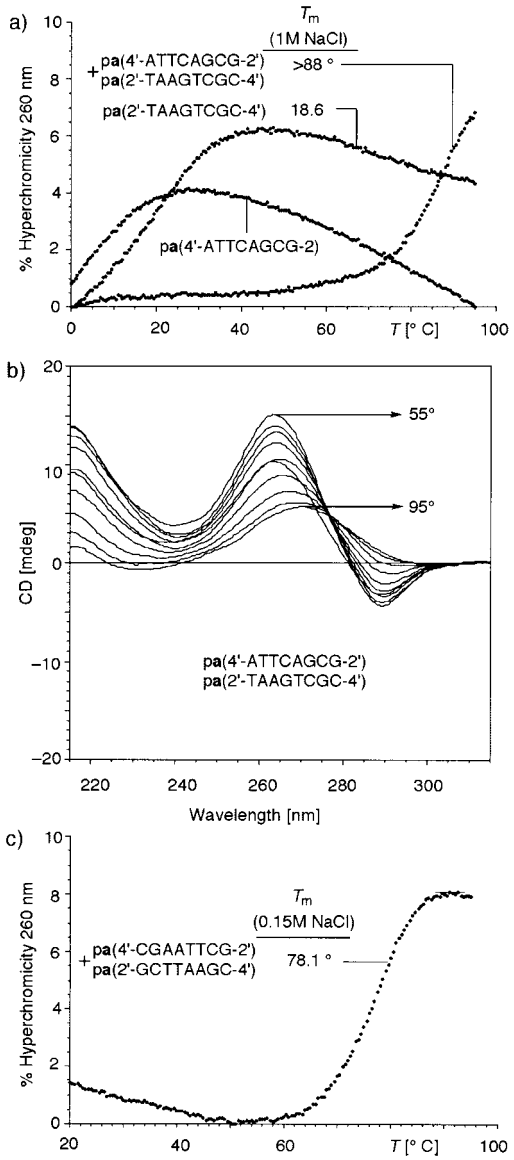


Fig. 4. *UV and CD data documenting the pairing behavior of indicated A,T,C,G-containing octamer sequences. a*) UV-Spectroscopic T_m curves and *b*) Temperature-dependent CD curves (temperature range: 55° → 95°). For conditions of measurements, see caption of *Fig. 2*. T_m Curves fully reversible (no hysteresis). *c*) UV-Spectroscopic T_m curves of duplex formed by the self-complementary p**a**(4′-ATTCAGCG-2′) sequence (*c* ≈ 1 μM, 0.15M NaCl, 10 mM aq. NaH_2PO_4 containing 0.1 mM Na_2(EDTA), 150 mM NaCl at pH 7.0) [8].

group (β-D-ribo and β-D-xylo), this link is 4′,2′-diequatorial, and 4′-axial/2′-equatorial in the other (α-L-lyxo and α-L-arabino). For each of these two groups, a representative NMR structure analysis has been carried out by *Jaun* and co-workers, with the duplex

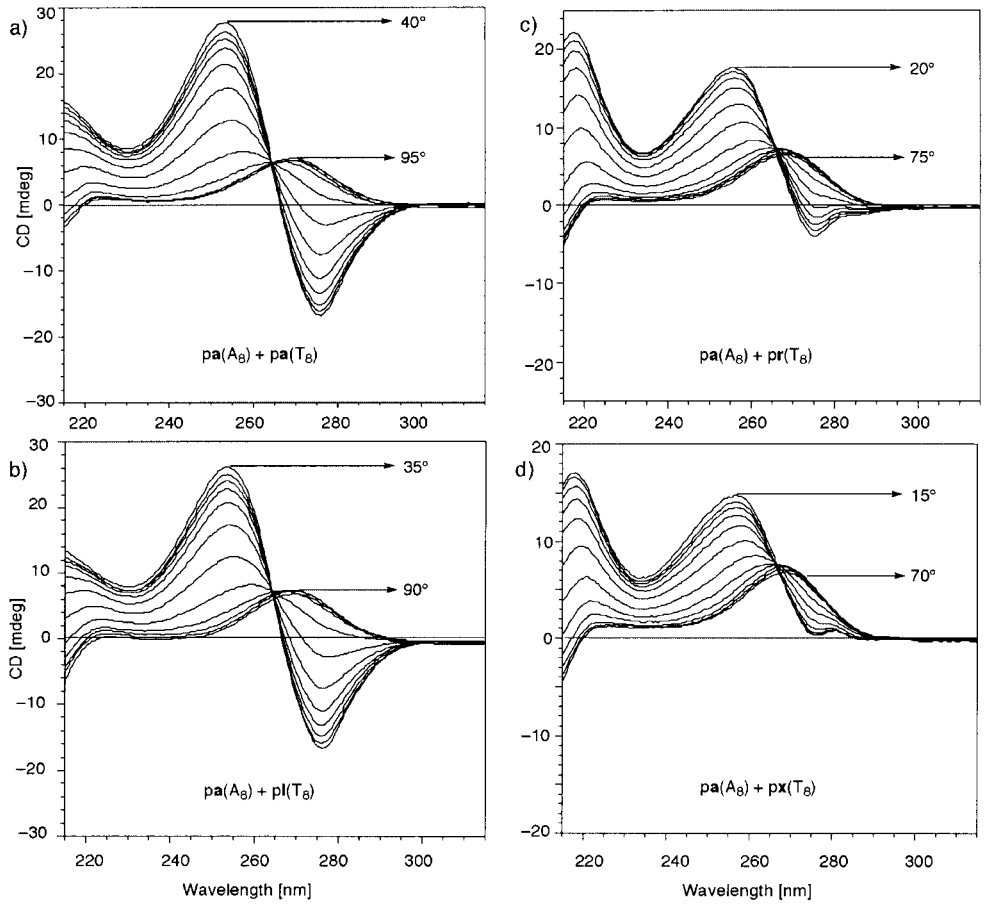


Fig. 5. *Temperature-dependent CD curves documenting intra- and intersystem pairing between:* a) ***pa****(A8) and* ***pa****(T8),* b) ***pa****(A8) and* ***pl****(T8),* c) ***pa****(A8) and* ***pr****(T8), and* d) ***pa****(A8) and* ***px****(T8).* Measurements in 10 mM aq. NaH_2PO_4 containing 0.1 mM Na_2(EDTA), 1M NaCl at pH 7.0. Total oligonucleotide concentrations were *ca.* 10 μM.

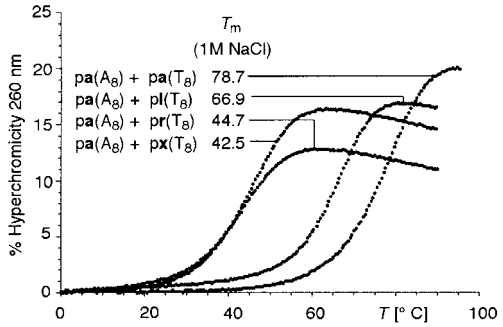


Fig. 6. *UV-Spectroscopic* T_m *curves documenting intra- and intersystem pairing between* ***pa****(A8) and* ***pa****(T8),* ***pa****(A8) and* ***pl****(T8),* ***pa****(A8) and* ***pr****(T8), and* ***pa****(A8) and* ***px****(T8).* For conditions of measurement, see caption of *Fig. 5.*

of the self-complementary octamer sequence 4′-CGAATTCG-2′ in the β-D-ribopyranosyl [4b] and the α-L-arabinopyranosyl series [8]. The latter study was described and compared with the former in a recent paper [8], so that we can restrict ourselves here to recapitulate those aspects of the α-L-arabinopyranosyl structure that are considered to have a direct bearing on the system's pairing properties.

The axial attachment of the phosphodiester bridge at C(4′) in the α-arabino structure compared to its equatorial attachment in the β-ribo series removes a specific source of steric encumberment in duplexes of the latter, namely, the intrastrand steric contact between the equatorial H-atom of the CH_2(5′) group of the pyranose chairs and the π-face of the neighboring nucleobase upstream[26]). The steric confrontation between these two groups is a direct consequence of the di-equatorial positioning of the phosphodiester bridge at C(4′) and C(2′). It disappears, as *Fig. 7* indicates, with the change of the phosphodiester attachment at C(4′) from equatorial to axial. The change clearly reflects itself in chemical shifts of the critical CH_2 H-atom in the ^{1}H-NMR spectra of the α-arabinopyranosyl *vs.* the β-ribopyranosyl octamer duplex: in the spectrum of the latter the critical H-atoms appear shielded by the ring current of a neighboring purine base, whereas the corresponding H-atoms in the α-arabinopyranosyl duplex are not (see *Fig. 7*).

Base pairing in α-L-arabinopyranosyl (4′-OP axial) duplexes is found to be always much stronger than in corresponding β-D-xylopyranosyl (4′-OP equatorial) duplexes (*Table 5* and [1]), and, furthermore, α-L-lyxopyranosyl duplexes tend to be more stable than β-D-ribopyranosyl and β-D-xylopyranosyl duplexes (*Table 5* and [5]). This pattern of relative strength of base pairing is consistent with the assumption that the intrastrand steric contact between pyranose rings and neighboring nucleobases in β-ribo- and β-xylopyranosyl duplexes does indeed act as steric repulsion that lowers duplex stability, and that it is the absence of this destabilization in the α-arabino- and α-lyxopyranosyl series that, at least in part, makes their duplexes more stable. The question, however, why it is that α-arabinopyranosyl duplexes are so much more stable than their α-lyxopyranosyl analogs, remains.

Fig. 8 points to a conformational divergence between α-arabino- and α-lyxopyranosyl duplexes that can be expected to contribute to this difference in pairing strengths. The figure indicates, within the simplifying constraints of idealized conformations, that the diversity of accessible conformers around the axial 4′-attachment of the phosphodiester bridge in a α-lyxopyranosyl single strand is larger than in a α-arabinopyranosyl single strand. The equatorial position of the 3′-OH group in the latter precludes a population of the family of conformers in which the 4′-O–P bond of the phosphodiester group is *anti*-periplanar to the arabinopyranose ring's endocyclic C(4′)–C(5′) bond, in contrast to the α-lyxopyranosyl series, where the population of this family of conformations is unfettered (*Fig. 8,b vs. a*, see also *e*). In both systems, on the other hand, the family of conformers that includes the actual pairing conformations[27]) is sterically unencumbered in both single and duplex strands of the arabino- and lyxopyranosyl series (*Fig. 8,d* and *c*). This means that, at least with respect to this C(4′)-domain of the conformational space of pentopyranosyl-(4′ → 2′)-

---

[26]) 'Upstream' = in the (2′ → 4′)-direction as against the (downstream) (4′ → 2′)-direction.

[27]) 4′O–P Bond *anti*-periplanar to endocyclic C(4′)–C(3′) bond.

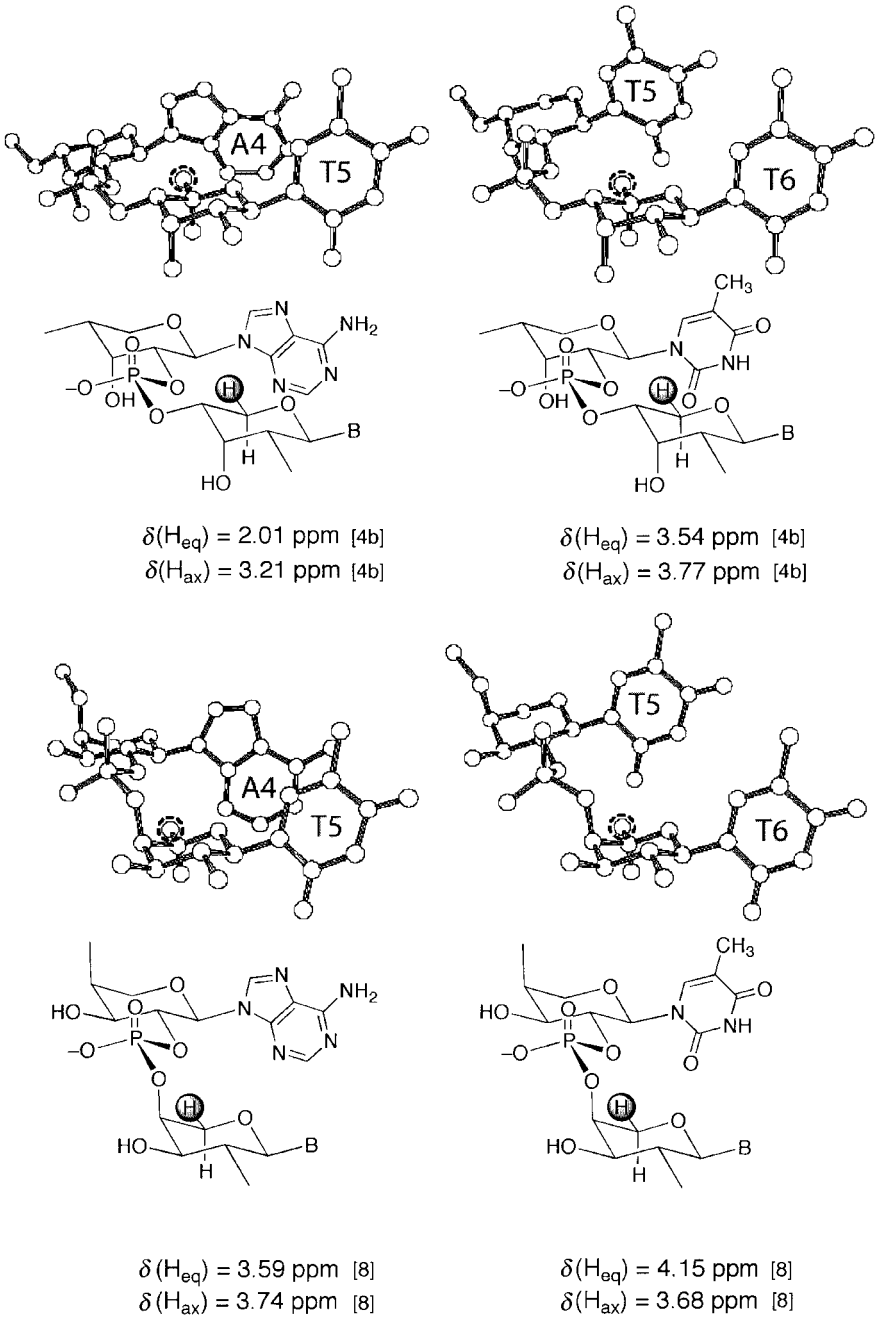


Fig. 7. *^{1}H-NMR Chemical shifts of equatorial and axial H-atoms at C(5′) in the (4′-CGAATTC-2′)$_2$ duplex in the β-*D*-ribopyranosyl-(4′ → 2′)- and the α-*L*-arabinopyranosyl-(4′ → 2′)-series.* The data are taken from [4b] and [8] and refer to the nucleoside-units T5 and T6, respectively.

oligonucleotide strands, the single strand of α-arabinopyranosyl nucleotides is more pre-organized toward the pairing conformation than the single strand of α-lyxopyranosyl nucleotides. This constraint in the population of single-strand conformers in the arabinopyranosyl series would be expected to contribute to higher duplex stability.

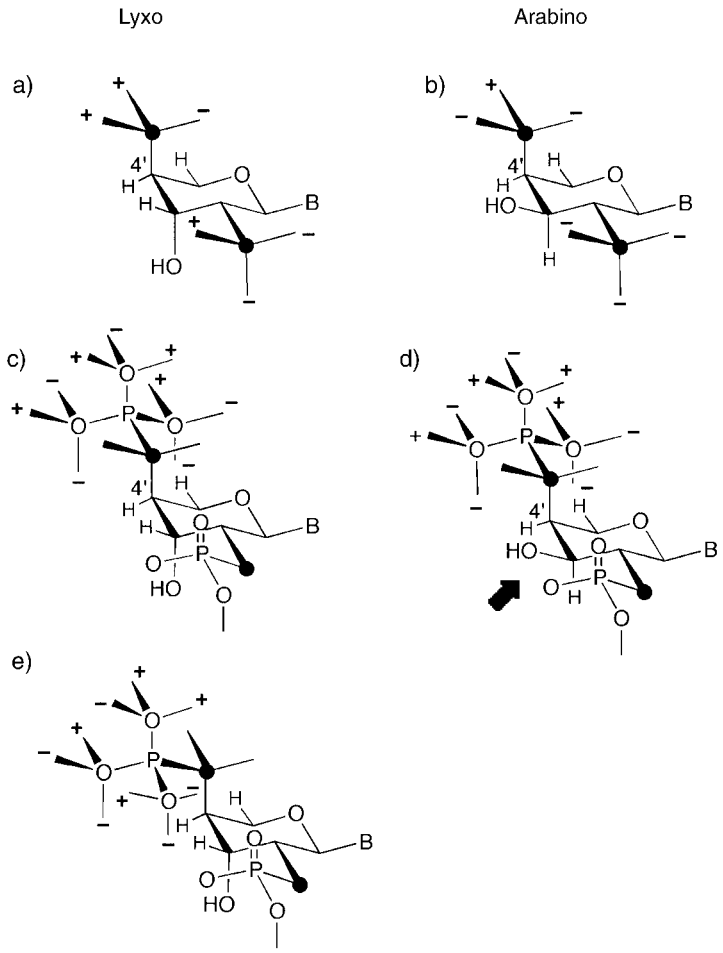


Fig. 8. *Qualitative conformational reasoning referring to the phosphodiester conformations in the C(4′)- and C(2′)-domains for both the* α-D-*lyxopyranosyl and* α-L-*arabinopyranosyl series.* The sign + denotes allowed and − disallowed conformational populations according to steric and stereoelectronic criteria. Bold arrow points to region of strong steric repulsion in (idealized) pairing conformation. For the rules followed in this conformational analysis, see [4a][7].

Appealing as the above argument may appear, it should not detract from recognizing the complexity of the problem of rationalizing relative base-pairing strengths, even in the case of structurally rather closely related oligonucleotide systems, as long as experimental structural information about the single strands involved is absent. One is quickly reminded of this complexity by inspecting *b* in *Fig. 8*, by which it

becomes clear that there is no way to reliably predict by qualitative conformational reasoning the type of conformation the phosphodiester group will assume in the C(2′)-domain in either a single- or a double-stranded α-arabinopyranosyl-(4′ → 2′)-oligonucleotide[28]). In fact, it is in this domain that the NMR structure analysis [8] in the arabino series uncovered a large deviation of the phosphodiester torsion angles from the values belonging to the corresponding (formally highly strained) idealized pairing conformation [8]. It was rather surprising to find, in this analysis, that the type of phosphodiester conformation adopted in the C(2′)-domain of the arabinopyranosyl duplex is the same as in the corresponding ribopyranosyl duplex. This can only mean that a pentopyranosyl-(4′ → 2′)-oligonucleotide strand has, in this domain, no significantly more-stable alternative conformation available; if it had, and that alternative would be a pairing conformation, the NMR structure analysis would have revealed it; if, however, that alternative were not a pairing conformation, the actually observed base-pairing strengths in α-arabinopyranosyl-(4′ → 2′)-oligonucleotide duplexes could not possibly be as high as observed. We are led to conclude, therefore, that, not only with respect to the domain of the C(4′)-phosphodiester attachment, but also with respect to the C(2′)-domain, α-arabinopyranosyl-(4′ → 2′)-oligonucleotide single strands are pre-organized toward duplexation in spite of the pairing conformation in the latter domain being highly strained. Such reasoning would lead to the expectation that arabinopyranosyl duplexes – irrespective of being more stable relative to their respective single strands – would have to be thermodynamically less stable against, *e.g.*, strand scission compared to duplexes of the β-ribopyranosyl series. Indeed, a much higher propensity of α-arabinopyranosyl strands toward strand scission under the basic conditions of protection-group removal after solid-support synthesis has been one of the specific observations made in the α-arabinopyranosyl-(4′ → 2′)-oligonucleotide series[29]).

The CD spectra reproduced in *Fig. 5* refer to intersystem cross-pairing within the pentopyranosyl-(4′ → 2′)-oligonucleotide family. These spectra convincingly support the assignments of pairing conformations for those two members of the family for which no NMR structure analysis is available (*Scheme 1*). In full harmony with the relationships expressed by these spectra, it is found that the combination of α-arabinopyranosyl strands and complementary α-lyxopyranosyl strands in intersystem cross-pairing leads to duplexes that are consistently more stable than the corresponding combination between α-arabinopyranosyl strands and β-ribo- or β-xylopyranosyl strands (*Table 5*, *Fig. 6*, and [3b]). In such intersystem cross-pairings between homobasic purine and homobasic pyrimidine strands duplex stabilities can strongly depend on which backbone bears the pyrimidine, and which bears the purine sequence. This remarkable phenomenon has been discussed at some length in a previous communication [3b].

Exceptionally strong base pairing such as that seen in α-arabinopyranosyl oligonucleotides can be accompanied by the occurrence of strong self-pairing of

---

[28]) Quite in contrast to the α-lyxopyranosyl series where the (C(2′)–O) torsion angles of both single and double strands are (qualitatively) clearly predictable (*Fig. 8,a*).

[29]) Not surprisingly, comparable lability toward strand scission has been observed in the β-xylopyranosyl series [1].

nucleobases. Such self-pairing may be quite selective for a given purine or pyrimidine base and be specific for a given type of backbone (see Table 1 in [3b]). For example, whereas homo-adenine sequences undergo strong (*Hoogsteen*) self-pairing in the homo-DNA series [28], no such pairing is observed in pyranosyl-RNA [4a], and almost none in the α-arabinopyranosyl series (*Table 4*). The situation is more or less inverse with regard to the self-pairing of thymine. The homo-pyrimidine sequence p**a**(T_{12}) shows the remarkable T_m value of 52° (10 μM, 1M NaCl), yet (fortunately) still surpassed by the value (> 95°) for the corresponding canonical A – T pairing in p**a**(A_{12})/p**a**(T_{12}) (*Table 4*). Understandably, no such self-pairing seems to occur with cytosine[30]). The possible self-pairing mode for thymine has been discussed in a previous communication (see Footnote 12 in [3b]).

An ominous case of noncanonical self-pairing of non-self-complementary A,T-containing strands was encountered in the behavior of the two antiparallel-complementary octamer sequence p**a**(TTAAAATA) and p**a**(TATTTTAA). Both undergo strong self-association to duplexes, which have T_m values of *ca.* 48° under conditions where the canonical cross-pairing duplex melts around 75°[31]). This case may be taken as a reminder of at least two questions that are of specific interest in the etiological context, namely, the one concerning the relationship between base-pairing strength and performance as an informational base-pairing system, and the other referring to the limitations of an informational system that would have to operate with two instead of four nucleobases. We have discussed the first question previously in the context of our work on template-directed sequence copying in the ribopyranosyl-(4′ → 2′)-oligonucleotide series [4c][4e]. With regard to the two-instead-of-four nucleobase question (see *e.g.*, [29]), the case of self-association of the two-base arabinopyranosyl strands mentioned above is just echoing a general experience we made in the course of our extensive studies on pentopyranosyl-(4′ → 2′)-oligonucleotides, namely: the higher the base-pairing strengths in an oligonucleotide system, the more difficult it becomes to construct (with only two nucleobases) a sequence that does not show self-pairing, even when the sequence is very short (*e.g.*, octamers). The task of constructing non-self-associating sequences of corresponding lengths with four instead of only two nucleobases is obviously much easier. This difference is assumed to be an important aspect in any conceptual and/or experimental evaluation of potentially primordial informational oligomers.

The work was supported by the *Skaggs Foundation* (*TSRI*). *O. J.*, *M. B.*, and *A. L.* thank the *Deutsche Forschungsgemeinschaft* for fellowship support. *O. J.*, *M. B.*, *A. L.*, and *H. K. H* were *Skaggs* postdoctoral fellows. We thank Dr. *A. Shivaniuk* for help in the ORTEP drawings in *Fig. 1*.

---

30) Neither p**a**(C_6) nor p**a**(G_6) show discernable self-pairing under identical conditions ($c \approx 10$ μM in 10 mM Na_2HPO_4 buffer, 0.1 mM Na_2(EDTA), pH 7.0 with 1M NaCl). The self-pairing mode conceivable for cytosine would require parallel strand orientation (forbidden in systems with large backbone inclination [28]).

31) The duplex strand formed by the A-rich sequence is presumably held together by four A/T base pairs, as well as four A/T and A/A interstrand base stacks.

Experimental Part

General. Solvents for extraction: ACS grade. Solvents for reaction: reagent grade. Reagents: unless otherwise noted, from *Acros*, *Fluka*, or *Aldrich*, highest quality available. Chloro(2-cyanoethoxy)(diisopropylamino)phosphine (97%) was purchased from *CheM – Impex Inc.*, Wood Dale, IL, USA. TLC: silica gel *60* F_{254} aluminum plates (*Whatman*, Type *Al Sil G/UV*, 250 μm layer); visualization by UV absorption and/or *A*) by dipping in a soln. of 4-anisaldehyde /H_2SO_4/AcOH/EtOH 5:5:1:18 or *B*) cerium(IV) sulfate (3 mM)/ammonium molybdate (250 mM) in aq. H_2SO_4 (10%), followed by heating. Flash column chromatography (CC) was performed on silica gel *60* (0.40–0.63 mm, 230–440 mesh; *EM Science*) at low pressure (max. 2 bar). In case of acid-sensitive compounds, the silica gel was pretreated with solvents containing *ca.* 0.5% Et_3N. Melting points (uncorrected) were measured with *MEL-TEMP II* (*Laboratory Devices Inc.*, USA). NMR: ^{1}H: δ values in ppm (TMS as internal standard); *J* [Hz], assignments of ^{1}H resonances were in some cases based on 2D experiments (^{1}H,^{1}H–COSY); ^{13}C: δ values in ppm (TMS as internal standard); *J* [Hz]; assignments and multiplicities were based on 2D experiments (^{1}H,^{13}C-COSY); ^{31}P: δ values in ppm (85% H_3PO_4 as external standard). FAB$^+$-MS (matrix-soln.): *m/z* (intensity in %); performed in the positive-ion mode, with 3-nitrobenzyl alcohol (3-NBA) as matrix, on a *VG ZAB-VSE* double-focusing high-resolution mass spectrometer equipped with a cesium ion gun. Matrix-assisted laser-desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was performed on a *Voyager-Elite* mass spectrometer (*Perseptive Biosystems*) with delayed extraction with THAP or DHB as the matrix with ammonium citrate added to the sample. Elemental analysis was performed with *Perkin-Elmer PE2400 CHN* analyzer. Oligonucleotides were synthesized on an *Expedite 8909 Nucleic Acid Synthesis* system (*Perseptive Biosystems*). HPLC: Ion exchange (IE) HPLC was performed on *Pharmacia Äkta Purifier (900)* controlled by *UNICORN* system. Columns: *Mono Q HR 5/5* (*Pharmacia*) or *SAX 1000-8* (*Macherey & Nagel*); Buffer *A:* 10 mM Na_2HPO_4 in H_2O, pH 11.5; Buffer *B:* 10 mM Na_2HPO_4 in H_2O, 1M NaCl, pH 11.5. UV Spectra: on a *Cary 1 C* spectrophotometer (*Varian*). Melting-point (T_m) measurements of oligonucleotides were determined with *Cary 1 Bio* spectrophotometer (*Varian*). CD Spectrum was measured on an *AVIV 61 DS* CD spectropolarimeter. All measurements were made with the 'phosphate buffer', 10 mM aq. NaH_2PO_4 buffer containing 0.1 mM Na_2EDTA, 150 mM (or 1M) NaCl at pH 7.0, with a total oligonucleotide concentration of *ca.* 10 μM, unless indicated otherwise, and the samples were thoroughly degassed, either by heating or by vacuum and ultrasonication. Concentrations of oligonucleotide solns. were calculated from the UV absorbance of the solns. at 260 nm (pH 7) at *ca.* 80° with the following molar extinction coefficients: ε(p**a**(A)) = 15000, ε(p**a**(T)) = 10000, ε(p**a**(C)) = 8400, ε(p**a**(G)) = 11900. *Abbreviations:* Ag-Tf = silver trifluoromethanesulfonate, BOP: (benzotriazol-1-yl)oxytris(dimethylamino)phosphonium hexafluorophosphate; BSA = bis(trimethylsilyl)acetamide, CPG = 'controlled-pore glass', DHB = 2,5-dihydroxybenzoic acid, DIBAL-H = diisobutylaluminium hydride, DMAP: 4-(dimethylamino)pyridine, DMT: 4,4′-dimethoxytrityl, Fmoc = [(9*H*-Fluoren-9-yl)methoxy]carbonyl, 1-HOBT = 1-Hydroxy-1*H*-benzotriazole, LCAA-CPG: long chain aminoalkyl-CPG (500 Å); LCAMA-CPG: long-chain aminomethylalkyl-CPG (500 Å); PAC = phenoxyacetyl, TMS-Tf = trimethylsilyl trifluoromethanesulfonate, THAP = 2,4,6-trihydroxyacetophenone, TOTU: *O*-{[(2-cyanoethoxycarbonyl)methylidene]amino}-1,1,3,3-tetramethyluronium tetrafluoroborate, TSOH = toluene-4-sulfonic acid, UF H_2O = ultra-filtered H_2O.

1. Experiments Referring to *Scheme 3.* – *1′,2′,3′,4′-Tetra*-O-*benzoyl-α/β*-L-*arabinopyranose* (**1**) [11]. To an ice-cooled mixture of 600 ml of dry pyridine and 500 ml of dry CH_2Cl_2, over a period of 15 min, were added 200 ml (1.7 mol) of BzCl. To this mixture, 50 g (0.33 mol) of (+)-L-arabinose was added in small portions over 5 h. The mixture was allowed to warm slowly to r.t., and stirred overnight. The syrupy mixture was poured into 1000 ml of ice H_2O, the org. layer was separated and washed twice with 400 ml of sat. aq. $NaHCO_3$ soln. The org. layer was dried (Na_2SO_4) and evaporated, and the pale yellow oil was co-evaporated twice with 100 ml of toluene. The residue was crystallized from 200 ml of hot EtOH. After storage at 4° overnight, the crystals were filtered, washed with cold EtOH, Et_2O and dried under high vacuum (0.03 Torr/r.t., 12 h) to yield 161.1 g (88%) of **1**. Colorless crystals. M.p. 174–176°. TLC (hexane/AcOEt 3:1): R_f 0.36. ^{1}H-NMR (600 MHz, $CDCl_3$): 4.20 (*dd*, *J* = 2.0, 13.4, H–C(5)); 4.42 (*dd*, *J* = 0.8, 13.4, H–C(5)); 5.92 (*m*, H–C(4)); 6.0 (*m*, H–C(3), H–C(2)); 6.88 (*d*, *J* = 1.6, H–C(1)); 7.32 (*m*, 4 arom. H); 7.49 (*m*, 2 arom. H); 7.54 (*m*, 4 arom. H); 7.66 (*m*, 2 arom. H); 7.90 (*m*, 4 arom. H); 8.15 (*m*, 4 arom. H). ^{13}C-NMR (150 MHz, $CDCl_3$): 63.42 (*t*, C(5′)); 68.15, 68.51 (2*d*, C(3′), C(2′)); 69.88 (*d*, C(4′)); 91.47 (*d*, C(1′)); 128.81, 128.83, 129.04, 129.16, 129.30, 129.50, 129.72, 130.15, 130.31, 130.31, 133.83, 133.99, 134.26 (arom. C); 165.11, 165.99, 166.15, 166.17 (4*s*, CO of Bz). ESI-MS (pos., MeOH): 605 (5, $[M+K]^+$), 589 (100, $[M+Na]^+$), 467 (4, $[M+Na-OBz]^+$), 445 (19, $[M-OBz]^+$), 323 (6, $[M-2OBz]^+$). Anal. calc. for $C_{33}H_{26}O_9$: C 69.96, H 4.63; found: C 70.17, H 4.39.

N^6-*Benzoyl-9-(2′,3′,4′-tri*-O-*benzoyl-α*-L-*arabinopyranosyl)adenine* (**2a**). To a suspension of 24.9 g (0.10 mol) of N^6-benzoyladenine and 63.7 g (0.10 mol) of **1** in 450 ml of dry MeCN, 50 ml (0.2 mol, 2 equiv.)

of BSA was added, and the mixture was heated to 65° in an oil bath. After 1.5 h, 42 ml (0.37 mol) of $SnCl_4$ was cautiously added (vigorous boiling) over 20 min. The clear soln. was maintained at 65° for further 90 min until TLC showed complete consumption of the starting material. The mixture was cooled to r.t. and poured into 500 ml of ice-cold sat. aq. $NaHCO_3$ soln. and 500 ml of AcOEt. The org. layer was separated, washed several times with sat. aq. $NaHCO_3$ soln., and washed with sat. aq. NaCl soln. The org. phase was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude product was purified by CC (silica gel; CH_2Cl_2/MeOH 100:1 to 100:4). The product fractions were combined and evaporated to yield 59.5 g (84%) of **2a**. Colorless foam. TLC (toluene/AcOEt 1:1): R_f 0.24. ^{1}H-NMR (600 MHz, (D_6)DMSO): 4.35 (*dd*, *J* = 1.9, 13.1, H–C(5′)); 4.49 (*d*, *J* = 13.1, H–C(5′)); 5.87 (*m*, H–C(4′)); 6.06 (*dd*, *J* = 3.4, 9.9, H–C(3′)); 6.46 (*d*, *J* = 9.1, H–C(1′)); 6.65 (*t*, *J* = 9.5, H–C(2′)); 7.37 (*m*, 3 arom. H); 7.54 (*m*, 5 arom. H); 7.62 (*m*, 1 arom. H); 7.69 (*m*, 5 arom. H); 7.77 (*m*, 1 arom. H); 7.93 (*dd*, 1 arom. H); 8.02 (*d*, 2 arom. H); 8.31 (*m*, 2 arom. H); 8.86 (*s*, H–C(8)); 8.89 (*s*, H–C(2)); 11.22 (*s*, H–N(6)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 67.38 (*t*, C(5′)); 69.61 (*d*, C(2′)); 69.77 (*d*, C(4′)); 72.72 (*d*, C(3′)); 82.38 (*d*, C(1′)); 126.33 (*s*, C(5)); 129.29, 129.32, 129.36, 129.41, 129.57, 129.63, 129.85, 129.89, 129.91, 130.11, 130.67, 133.31, 133.71, 134.63, 134.75, 134.80 (arom. C); 144.33 (*d*, C(8)); 151.43 (*s*, C(4)); 152.87 (*d*, C(2)); 153.07 (*s*, C(6)); 165.16, 165.46, 165.92 (*s*, CO of OBz); 168.18 (*s*, CO of NHBz). FAB-MS (pos., 3-NBA/CsI): 816.1035 (100, $[M + Cs]^+$). Anal. calc. for $C_{38}H_{29}N_5O_8$: C 66.76, H 4.28, N 10.24; found: C 66.40, H 4.43, N 10.46.

*1-(2′,3′,4′-Tri-*O*-benzoyl-α-*L*-arabinopyranosyl)thymine* **(2b)**. To a suspension of 13.4 g (0.11 mol) of thymine and 60.0 g (0.11 mol) of **1** in 450 ml of dry MeCN, 50 ml (0.2 mol) of BSA was added, and the mixture was heated to 60°, during which time all solid material dissolved. After 45 min, 57.7 ml (0.33 mol) of TMS-Tf was added, and the soln. was maintained at 60° for 4.5 h. The mixture was cooled to r.t., poured into a mixture of 600 ml of ice-cold sat. aq. $NaHCO_3$ soln. and 600 ml of AcOEt. The org. layer was separated, washed with 500 ml of sat. aq. $NaHCO_3$ soln., 500 ml of sat. aq. NaCl soln., and dried (Na_2SO_4). The solvent was removed *in vacuo*, and the residue was crystallized from 150 ml of AcOEt to yield 50.1 g (83%) of **2b**. Colorless needles. M.p. 222–224° TLC (toluene/AcOEt 1:1): R_f 0.59. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.85 (*s*, Me–C(5)); 4.28 (*dd*, *J* = 1.7, 13.2, H–C(5′)); 4.42 (*d*, *J* = 13.2, H–C(5′)); 5.75 (*m*, H–C(4′)); 5.86 (br. *s*, H–C(2′)); 6.02 (*dd*, *J* = 3.4, 9.8, H–C(3′)); 6.23 (br. *s*, H–C(1′)); 7.36 (*t*, 2 arom. H); 7.45 (*t*, 2 arom. H); 7.55 (*t*, 1 arom. H); 7.61 (*t*, 3 arom. H); 7.69 (*m*, H–C(6), 2 arom. H); 7.74 (*t*, 1 arom. H); 7.80 (*d*, 2 arom. H); 8.41 (*d*, 2 arom. H); 11.43 (*s*, H–N(3)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 13.02 (Me–C(5)); 67.45 (*t*, C(5′)); 69.75, 69.86 (C(4′), C(2′)); 72.28 (*d*, C(3′)); 80.92 (*d*, C(1′)); 111.40 (*s*, C(5)); 128.98, 129.38, 129.56, 129.74, 129.81, 129.89, 130.02, 130.49 (arom. C); 134.61, 134.73, 134.86 (arom. C, C(6)); 151.25 (C(2)); 164.24 (C(4)); 165.33, 165.58, 165.96 (*s*, CO of Bz). ESI-MS (pos., MeOH): 593 (100, $[M + Na]^+$), 571 (6, $[M + H]^+$), 471 (16, $[M + Na - OBz]^+$), 445 (12, $[M + H - Thy]^+$), 353 (10, $[M + H + Na - 2OBz]^+$), 323 (5, $[M + H - Thy - OBz]^+$). ESI-MS (neg., MeOH): 569 (100, $[M - H]^-$), 540 (26, $[M - H - CO]^-$). Anal. calc. for $C_{31}H_{20}N_2O_9$: C 65.26, H 4.59, N 4.91; found: C 65.23, H 4.81, 4.83.

N^4*-Benzoyl-1-(2′,3′,4′-tri-*O*-benzoyl-α-*L*-arabinopyranosyl)cytosine* (**2c**). A suspension of 16.5 g (74 mmol) of 4-*N*-benzoylcytosine, 45.52 g (81 mmol) of **1**, and 46 ml (185 mmol) of BSA was heated in 300 ml of dry MeCN at 80° in an oil bath. When all of the material dissolved (*ca.* 30 min), 26 ml (222 mmol) of $SnCl_4$ was cautiously added (vigorous boiling). Stirring at 80° was continued for 30 min, until TLC indicated complete reaction. The yellow soln. was allowed to cool to r.t., and poured into 600 ml of ice-cold sat. aq. $NaHCO_3$ soln. and 400 ml of AcOEt. The org. phase was separated, washed with sat. aq. $NaHCO_3$ soln., sat. aq. NaCl soln., and dried (Na_2SO_4). The solvent was removed under vacuum, and the residue was crystallized from 250 ml of MeOH to afford 40.2 g (82%) of **2c**. Colorless needles. TLC (toluene/AcOEt 1:1): R_f 0.3. ^{1}H-NMR (600 MHz, (D_6)DMSO): 4.34 (*d*, *J* = 13.2, H–C(5′)); 4.49 (*d*, *J* = 13.2, H–C(5′)); 5.81 (*m*, H–C(4′)); 5.86 (br. *s*, H–C(2′)); 6.08 (*m*, H–C(3′)); 6.48 (br. *s*, H–C(1′)); 7.36, 7.44, 7.49, 7.55 (4*m*, 7 arom. H, H–C(5)); 7.61, 7.69, 7.80, 7.97, 8.16 (4*m*, 13 arom. H); 8.40 (br. *s*, H–C(6)); 11.26 (br. *s*, H–N(4)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 67.81 (*t*, C(5′)); 69.92 (*d*, C(4′)); 70.66 (*d*, C(2′)); 72.34 (*d*, C(3′)); 82.07 (*d*, C(1′)); 98.53 (*d*, C(5)); 129.24, 129.35, 129.54, 129.65, 129.82, 129.88, 130.10, 130.56, 133.62, 134.70 (arom. C); 146.55 (*s*, C(4)); 155.20 (*s*, C(2)); 164.38 (*d*, C(6)); 165.40, 165.69, 165.98 (*s*, CO of OBz); 168.31 (*s*, CO of NHBz). ESI-MS (pos., MeOH): 682 (14, $[M + Na]^+$), 660 (3, $[M + H]^+$), 429 (100, $[M + H - H_2NBz]^+$). ESI-MS (neg., MeOH): 658 (100, $[M - H]^-$), 536 (8, $[M - H - OBz]^-$).

N^2*-Isobutyryl-9-(2′,3′,4′-tri-*O*-benzoyl-α-*L*-arabinopyranosyl)guanine* (**2d**) *and* N^2*-Isobutyryl-7-(2′,3′,4′-tri-*O*-benzoyl-α-*L*-arabinopyranosyl)guanine* (**2e**). To a suspension of 22.1 g (0.1 mol) of *N*^{2}-isobutyrylguanine and 67 g (0.12 mol) of **1** in 450 ml of dry MeCN, 77 ml (0.35 mol) of BSA was added, and the mixture was heated under reflux. After 2 h, 58 ml (0.3 mol) of TMS-Tf was added to the clear soln., and heating was continued for further 6 h. The soln. was allowed to cool to r.t., and poured into a mixture of 500 ml of ice-cold sat. aq. $NaHCO_3$ soln. and 500 ml of AcOEt. The org. layer was separated, washed with sat. aq. $NaHCO_3$ soln., sat. aq. NaCl soln.,

dried (Na_2SO_4), and evaporated *in vacuo*. The resulting foam was purified by CC (silica gel, 26 × 8 cm, toluene/AcOEt 10:1 to 10:4, 10:5 to 10:8 and 10:9 to 10:20). Fractions containing the N^9-isomer **2d** were combined, stored at 4° to afford crystals, which were filtered, washed with AcOEt, and dried *in vacuo* (0.03 Torr/r.t., 12 h) to yield 26.0 g (44%) of **2d** as colorless needles. The remaining fractions containing the N^7-isomer **2e** were combined, evaporated, and the residue was crystallized from 50 ml of AcOEt to afford 2.1 g (3%) of **2e** as colorless needles.

Data of **2d**. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.42. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.12, 1.13 (2*d*, J = 6.8, Me_2CH); 2.80 (*m*, Me_2CHCO); 4.39 (*dd*, J = 1.5, 13.1, H–C(5′)); 4.45 (*d*, J = 13.1, H–C(5′)); 5.86 (*m*, H–C(4′)); 6.00 (*m*, H–C(3′)); 6.02 (*d*, J = 9.5, H–C(1′)); 6.10 (*dd*, J = 9.5, 9.5, H–C(2′)); 7.37 (*m*, 4 arom. H); 7.55 (*m*, 2 arom. H); 7.63 (*m*, 2 arom. H); 7.68 (*m*, 4 arom. H); 7.76 (*m*, 1 arom. H); 8.18 (*d*, 2 arom. H); 8.49 (*s*, H–C(8)); 11.71, 12.04 (2 br. *s*, H–N(2), H–N(1)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 19.75 (*q*, Me_2CH); 19.64 (*q*, Me_2CH); 35.55 (*d*, Me_2CHCO); 68.01 (*t*, C(5′)); 69.78 (*d*, C(4′)); 70.74 (*d*, C(3′)); 72.29 (*d*, C(2′)); 81.31 (*d*, C(1′)); 120.52 (*s*, C(5)); 128.56, 129.25, 129.59, 129.82, 129.86, 129.94, 130.63 (arom. C); 134.70, 134.76, 134.84 (arom. C, C(8)); 149.32, 149.52 (C(2), C(4)); 155.47 (*s*, C(6)); 165.21, 165.35, 166.00 (*s*, CO of Bz); 181.02 (*s*, Me_2CHCO). ESI-MS (pos., MeOH): 688 (45, $[M + Na]^+$), 359 (100, $[M + Na - 2$ Obz – isobutyramide$]^+$), 323 (47, $[M + Na - 3$ OBz$]^+$). ESI-MS (neg., MeOH): 664 (100, $[M - H]^-$). Anal. calc. for $C_{35}H_{32}N_5O_9$: C 63.06, H 4.84, N 10.50; found: C 63.33, H 4.96, 10.41.

Data of **2e**. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.54. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.12 (2*d*, Me_2CH); 2.72 (*m*, Me_2CHCO); 4.34 (*d*, J = 12.8, H–C(5′)); 4.44 (br. *d*, H–C(5′)); 5.85 (*m*, H–C(4′)); 6.02, 6.33, 6.75 (3 br. *s*, H–C(3′), H–C(2′), H–C(1′)); 7.35 (*m*, 2 arom. H); 7.38 (*m*, 2 arom. H); 7.54 (*m*, 2 arom. H); 7.62 (*m*, 2 arom. H); 7.71 (*m*, 4 arom. H); 7.73 (*m*, 1 arom. H); 8.34 (*m*, 2 arom. H); 8.60 (*s*, H–C(8)); 11.61, 12.18 (2 br. *s*, H–N(2), H–N(1)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 19.63 (*q*, Me_2CH); 19.74 (*q*, Me_2CH); 35.56 (*d*, Me_2CHCO); 67.69 (*t*, C(5′)); 69.66, 70.94, 72.46 (*d*, C(4′), C(3′), C(2′)); 84.63 (*d*, C(1′)); 111.93, 128.80, 129.35, 129.52, 129.61, 129.81, 129.87, 129.95, 130.83, 134.59, 134.64, 134.72, 148.56, 152.67 (arom. C, C(5), C(8), C(2), C(4), C(6)); 165.13, 165.40, 166.16 (*s*, CO of Bz); 181.97 (*s*, Me_2CHCO). ESI-MS (pos., MeOH): 704 (17, $[M + K]^+$), 688 (100, $[M + Na]^+$). ESI-MS (neg., MeOH): 664 (100, $[M - H]^-$). Anal. calc. for $C_{35}H_{32}N_5O_9$: C 63.06, H 4.84, N 10.50; found: C 62.95, H 4.81, N 10.52.

9-(α-L-Arabinopyranosyl)-N^6*-benzoyl-adenine* (**3a**). To a soln. of 50 g (73.1 mmol) of **2a** in 600 ml of THF/MeOH/H_2O 5:4:1 (*v*/*v*/*v*), cooled to −20°, 150 ml of 2N aq. NaOH soln. was slowly added over a period of 25 min. Stirring at −20° was continued for further 75 min after which TLC showed complete reaction. The soln. was carefully neutralized with 35% aq. HCl soln. and evaporated, during which the product precipitated as a white solid. At this stage, the mixture was kept at 4° overnight, the precipitate was filtered, washed with acetone, and dried in high vacuum (0.03 Torr/r.t., 12 h) to yield 28.3 g (64.1 mmol, 88%) of **3a**. White solid. TLC ($CHCl_3$/MeOH 1:1): R_f 0.68. ^{1}H-NMR (600 MHz, (D_6)DMSO): 3.62 (*m*, H–C(3′)); 3.79 (*m*, H–C(4′), H–C(5′)); 3.85 (*d*, J = 12.2, H–C(5′)); 4.28 (*m*, H–C(2′)); 4.79 (*d*, J = 5.0, HO–C(4′)); 5.03 (*d*, J = 5.7, HO–C(3′)); 5.33 (*d*, J = 5.8, HO–C(2′)); 5.48 (*d*, J = 9.2, H–C(1′)); 7.55–8.06 (*t*, 5 arom. H); 8.62 (*s*, H–C(8)); 8.75 (*s*, H–C(2)); 11.17 (*s*, H–N(6)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 69.39 (*d*, C(2′)); 69.63 (*d*, C(4′)); 70.30 (*t*, C(5′)); 74.29 (*d*, C(2′)); 84.33 (*d*, C(1′)); 126.18 (C(5)); 129.33, 133.30, 134.19 (arom. C); 143.83 (*d*, C(8)); 151.07 (*s*, C(4)); 152.57 (*d*, C(2)); 153.54 (*s*, C(6)); 166.43 (*s*, CO of Bz). ESI-MS (pos., MeOH): 765 (48, $[2M + Na]^+$), 394 (46, $[M + Na]^+$), 240 (100, $[A^{Bz} + H]^+$). ESI-MS (neg., MeOH): 406 (14, $[M + Cl]^-$); 370 (100, $[M - H]^-$).

1-(α-L-Arabinopyranosyl)thymine (**3b**). To a soln. of MeONa prepared from 2.5 g (0.11 mol) of Na and 500 ml of dry MeOH was added 50.1 g (88 mmol) of **2b**. The mixture was stirred for 12 h at r.t., after which TLC showed complete consumption of the starting material. The white solid formed during the reaction was filtered and was shown to be the Na salt of the product isolated in almost quant. yield. To obtain the neutral form of the product, the suspension was neutralized with AcOH and 50 g of silica gel was added. The mixture was concentrated to dryness, and the residue was purified by CC (silica gel; $CHCl_3$/MeOH 1:1). Product fractions were combined, evaporated *in vacuo*, and the residue was crystallized from MeOH to yield 18.9 g (83%) of **3b**. Colorless needles. M.p. 249–250°. TLC ($CHCl_3$/MeOH 1:1): R_f 0.68. ^{1}H-NMR (600 MHz, (D_6)DMSO) 1.80 (*s*, Me–C(5)); 3.48 (*m*, H–C(3′)); 3.62 (*d*, J = 12.1, H–C(5′)); 3.67 (*m*, H–C(4′)); 3.70 (*m*, H–C(2′)); 3.75 (*dd*, J = 1.7, 12.1, H–C(5′)); 4.69, 4.93 (2*d*, J = 5.9, J = 5.6, HO–C(4′), HO–C(3′)); 5.22 (*d*, J = 2.1, HO–C(2′)); 5.22 (*d*, J = 12.4, H–C(1′)); 7.52 (*s*, H–C(6)); 11.40 (*s*, H–N(3)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 12.90 (*q*, Me–C(5)); 69.06, 69.36 (*d*, C(2′), C(4′)); 70.13 (*d*, C(5′)); 74.10 (*d*, C(3′)); 83.90 (*d*, C(1′)); 110.21 (*s*, C(5)); 137.44 (*d*, C(6)); 151.78 (*s*, C(2)); 164.56 (*s*, C(4)). FAB-MS (pos., 3-NBA/NaI): 281 (100, $[M + Na]^+$), 259.0937 (37, $[M + H]^+$). Anal. calc. for $C_{10}H_{14}N_2O_5$: C 46.51, H 5.46, N 10.85; found: C 46.54, H 5.27, N 10.94.

1-(α-L-Arabinopyranosyl)-N^4*-benzoyl-cytosine* (**3c**). To a soln. of 36.0 g (54.6 mmol) of **2c** in 500 ml of THF/MeOH/H_2O 5:4:1 (*v*/*v*/*v*), cooled in an ice bath, 100 ml of 1N NaOH soln. in MeOH/H_2O 1:1 (*v*/*v*) was

added during 5 min, and stirring at 4° was continued. After 45 min, TLC showed complete consumption of the starting material, and the mixture was neutralized (pH 7) by the addition of 6M aq. HCl. The pale yellow soln. was concentrated to a volume of 100 ml and kept at −20° overnight. The resulting precipitate was filtered, washed with EtOH, and dried under high vacuum (0.03 Torr/50°, 12 h) to yield 11.9 g of **3c**. Concentration of the mother liquor led to another crop of 3.54 g for an overall yield of 15.46 g (82 %) of **3c**. Colorless powder. TLC ($CHCl_3$/MeOH 6:1): R_f 0.2. ^{1}H-NMR (600 MHz, (D_6)DMSO): 3.53 (*m*, H−C(3′)); 3.66 (*d*, *J* = 12.1, H−C(5′)); 3.72 (*m*, H−C(4′)); 3.76 (*m*, H−C(2′)); 3.80 (*d*, *J* = 12.1, H−C(5′)); 4.77 (*d*, *J* = 5.3, HO−C(4′)); 4.98 (*d*, *J* = 5.7, HO−C(3′)); 5.24 (*d*, *J* = 5.4, HO−C(2′)); 5.47 (*d*, *J* = 9.0, H−C(1′)); 7.39 (*d*, *J* = 6.5, H−C(5)); 7.53 (*m*, 2 arom. H); 7.62 (*m*, 1 arom. H); 8.02 (*m*, 2 arom. H); 8.11 (*d*, *J* = 6.5, H−C(6)); 11.32 (*s*, H−N(4)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 69.37 (*t*, C(4′)); 69.85 (*d*, C(2′)); 70.47 (*d*, (C(5′)); 74.23 (*d*, C(3′)); 85.05 (*d*, C(1′)); 97.53 (*d*, C(5)); 129.30, 133.60, 133.92 (arom. C); 147.05 (*d*, C(6)); 155.89 (*s*, C(2)); 163.90 (*s*, C(4)); 168.17 (*s*, CO of Bz). ESI-MS (pos., MeOH): 281 (100, $[M+Na]^+$), 238 (27, $[Cyt^{Bz}+Na]^+$), 216 (10, $[Cyt^{Bz}+H]^+$).

*9-(α-L-Arabinopyranosyl)-*N^2*-isobutyryl-guanine* (**3d**). To a soln. of 25.0 g (37.5 mmol) of **2d** in 400 ml of a mixture of THF/MeOH/H_2O 5:4:1 (*v*/*v*/*v*), cooled in an ice bath, 80 ml of 1N NaOH (aq.) was added over 15 min. After complete addition, stirring was continued for further 15 min, followed by neutralization with 37% aq. HCl soln. The clear soln. was evaporated to a volume of 200 ml, adsorbed on 50 g of silica gel, and purified by CC (silica gel; CH_2Cl_2/MeOH 3:1 to 2:1). The product fractions were combined and evaporated to yield 10.6 g (80%) of **3d**. White solid. TLC ($CHCl_3$/MeOH 3:1): R_f 0.33. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.10 (*d*, *J* = 6.8, *Me*CHCO); 1.12 (*s*, *Me*$_2$CH); 2.77 (*m*, Me$_2$C*H*CO); 3.53 (*m*, H−C(3′)); 3.64 (*d*, *J* = 12.1, H−C(5′)); 3.77 (*m*, H−C(4′)); 3.86 (*dd*, *J* = 1.7, 12.1, H−C(5′)); 4.09 (*m*, H−C(2′)); 4.88 (*d*, *J* = 5.1, HO−C(4′)); 5.09 (*d*, *J* = 5.4, HO−C(3′)); 5.14 (*d*, *J* = 9.3, H−C(1′)); 5.39 (*d*, *J* = 5.4, HO−C(2′)); 8.12 (*s*, H−C(8)); 11.76, 12.14 (2 br. *s*, H−N(2), H−C(1)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 19.67 (*q*, *Me*$_2$CH); 19.71 (*q*, *Me*$_2$CH); 35.55 (*d*, Me$_2$*C*HCO); 69.24 (*d*, C(4′)); 69.74 (*d*, C(2′)); 70.36 (*t*, C(5′)); 74.35 (*d*, C(3′)); 84.06 (*d*, C(1′)); 120.53 (*s*, C(5)); 138.56 (*d*, C(8)); 149.01 (*s*, C(4)); 149.94 (*d*, C(2)); 155.70 (*s*, C(6)); 181.01 (*s*, Me$_2$CH*C*O). ESI-MS (pos., MeOH): 729 (100, $[2M+Na]^+$), 376 (24, $[M+Na]^+$). ESI-MS (neg., MeOH): 388 (6, $[M+Cl]^-$), 352 (100, $[M-H]^-$). Anal. calc. for $C_{14}H_{18}N_5O_8$: C 47.59, H 5.42, N 19.82; found: C 47.25, H 5.25, 19.66.

N^6*-Benzoyl-9-(3′,4′-*O*-isopropylidene-α-L-arabinopyranosyl)adenine* (**4a**). A soln. of 25.0 g (67 mmol) of **3a** and 400 mg (2.1 mmol) of TsOH · H_2O, in 200 ml of dry DMF, was cooled to 4° in an ice bath, and 20 ml (209 mmol) of 2-methoxyprop-1-ene was added over 30 min. Stirring at 4° was continued for another 30 min before the mixture was neutralized by the addition of 25% aq. NH_3 soln. The solvent was removed *in vacuo* and 50 ml of AcOEt was added to the residue. A fine precipitate formed was removed by filtration over silica gel, followed by washing the silica gel with $CHCl_3$/MeOH 10:1 (*v*/*v*). The filtrate was combined with the washings and evaporated *in vacuo*. The resulting foam was dissolved in 80 ml of hot AcOEt, from which crystals were formed overnight at r.t. The crystals were filtered and dried in high vacuum (0.03 Torr/r.t., 12 h) to yield 24.0 g (87%) of **4a**. Colorless crystals. TLC ($CHCl_3$/MeOH 9:1): R_f 0.59. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.35, 1.57 (2*s*, *Me*$_2$C); 4.09 (*dd*, *J* = 2.5, 13.4, H−C(5′)); 4.22 (*dd*, *J* = 6.3, 6.4, H−C(3′)); 4.25 (*d*, *J* = 13.4, H−C(5′)); 4.33 (*m*, H−C(4′), H−C(2′)); 5.52 (*d*, *J* = 9.6, H−C(1′)); 5.70 (*d*, *J* = 6.2, HO−C(2′)); 7.57–8.05 (*m*, 5 arom. H); 8.80 (*s*, H−C(8)); 8.93 (*s*, H−C(2)); 11.22 (H−N(6)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 27.07 (*q*, *Me*$_2$C); 28.87 (*q*, *Me*$_2$C); 66.13 (*t*, C(5′)); 70.76 (*d*, C(2′)); 74.11 (*d*, C(4′)); 79.87 (*d*, C(3′)); 83.19 (*d*, C(1′)); 109.72 (*s*, Me$_2$*C*); 126.34 (*s*, C(5)); 129.32, 133.30, 134.22 (arom C); 144.11 (*d*, C(8)); 151.12 (*s*, C(4)); 152.57 (*s*, C(2)); 153.50 (*s*, C(6)); 166.48 (*s*, CO of Bz). ESI-MS (pos., MeOH): 845 (55, $[2M+Na]^+$), 434 (21, $[M+Na]^+$), 240 (100, $[A^Bz+H]^+$). ESI-MS (neg., MeOH): 410 (100, $[M-H]^-$). Anal. calc. for $C_{20}H_{21}N_5O_5$: C 58.39, H 5.14, N 17.02; found: C 58.25, H 5.18, N 17.04.

*1-(3′,4′-*O*-Isopropylidene-α-L-arabinopyranosyl)thymine* (**4b**). To a suspension of 23.8 g (92 mmol) of **3b** in 200 ml of dry DMF, cooled in an ice bath, was added 27 ml of 4M HCl in dioxane followed by 15 ml (157 mmol) of 2-methoxyprop-1-ene over a 10 min. Stirring at 4° was continued for 1.5 h before the mixture was carefully neutralized with 25% aq. NH_3 soln. The mixture was filtered through silica gel, and the silica gel was washed with $CHCl_3$/MeOH 9:1 (*v*/*v*). The washings were combined with the filtrate, evaporated and 200 ml of Et_2O was added to the residue. The resulting white precipitate was filtered and dried in high vacuum (0.03 Torr/r.t., 12 h) to yield 26.2 g (95 %) of **4b**. White solid. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.46. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.30 (*s*, *Me*$_2$C); 1.51 (*s*, *Me*$_2$C); 1.81 (*s*, Me−C(5)); 3.69 (*m*, H−C(2′)); 3.91 (*dd*, *J* = 2.6, 13.5, H−C(5′)); 4.11 (*dd*, *J* = 6.0, 6.3, H−C(3′)); 4.14 (*d*, *J* = 13.5, H−C(5′)); 4.20 (*m*, H−C(4′)); 5.25 (*d*, *J* = 9.7, H−C(1′)); 5.53 (*d*, *J* = 5.8, HO−C(2′)); 7.50 (*s*, H−C(6)); 11.32 (br. *s*, H−N(3)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 12.75 (*q*, *Me*−(5)); 26.98 (*q*, *Me*$_2$C); 28.85 (*q*, *Me*$_2$C); 66.17 (*t*, C(5′)); 70.32 (*d*, C(2′)); 74.09 (*d*, C(4′)); 82.61 (*d*, C(1′)); (*d*, C(1′)); 109.50 (*d*, Me$_2$*C*H); 110.44 (*s*, C(5)); 137.30 (*d*, C(6)); 151.85 (*s*, C(2)); 164.25 (*s*, C(4)). ESI-MS (pos., MeOH): 321 (100, $[M+Na]^+$). ESI-MS (neg., MeOH): 297 (100, $[M-H]^-$). Anal. calc. for $C_{13}H_{18}N_2O_6$: C 52.34, H 6.08, N 9.39; found: C 51.93, H 6.18, N 9.17.

N^4-*Benzoyl-1-{3′,4′*-O-*[(4-methoxyphenyl)methylidene]-α*-L-*arabinopyranosyl}cytosine* (**4c**). A soln. of 11.41 g (33 mmol) of **3c**, 630 mg (3.3 mmol) of TsOH · H_2O, and 11.3 ml (66 mmol) of 4-anisaldehyde dimethyl acetal in 500 ml of dry DMF was stirred at r.t. overnight. The mixture was neutralized with conc. 25% aq. NH_3 soln. and evaporated (8 Torr 45°). The residue was co-evaporated twice with toluene, and 200 ml of MeOH was added, which caused spontaneous precipitation of a white solid. The solid was filtered, washed with MeOH, and dried under high vacuum (0.03 Torr/r.t., 12 h) to yield 13.6 g (89%) of **4c**. Mixture of diastereoisomers. TLC ($CHCl_3$/MeOH 9 :1): R_f 0.56, 0.64. ^{1}H-NMR (600 MHz, (D_6)DMSO): 3.79, 3.80 (2*s*, MeO); 3.83 (m, H−C(2′) of isomer a); 3.94 (*dd*, *J* = 2.1, 13.5, H−C(5′) of isomer a); 4.01 (*m*, H−C(2′) of isomer b); 4.08 (*dd*, *J* = 2.0, 12.5, H−C(5′) of isomer b); 4.32 (*m*, H−C(4′)); 4.36 (*d*, *J* = 12.5, H−C(5′) of isomer b); 4.54 (*dd*, *J* = 5.7, 6.9, H−C(3′)); 5.59 (2*d*, *J* = 9.3, H−C(1′)); 5.78, 5.81 (2*d*, *J* = 6.0, HO−C(2′)); 5.85, 6.19 (2*s*, ArC*H*); 6.99 (*m*, 2 arom. H); 7.39, 7.51 (2*m*, H−C(5), 4 arom. H); 7.63 (*m*, 1 arom. H); 8.01 (*m*, 2 arom. H); 8.15, 8.25 (2 br. *s*, H−C(6)); 11.29 (H−N(4)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 56.01 (MeO); 66.32 66.75 (C(5′)); 68.11, 71.92 (C(2′) isomer a and b); 74.32 (C(3′)); 76.37, 79.35 (C(4′)); 81.16 (C(3′)); 83.76, 83.91 (C(1′)); 97.60 (C(5)); 103.01, 104.29 (Ar*C*H); 114.49, 114.54, 127.57, 128.73, 129.30, 129.54, 130.00, 131.93, 133.64, 133.91 (arom. C); 147.10 (C(6)); 155.81 (C(2)); 160.70, 166.93 (arom. C); 164.02 (C(4)); 168.23 (CO of Bz). ESI-MS (pos., MeOH): 488 (100, $[M + Na]^+$), 238 (60, $[Cyt^{Bz} + Na]^+$), 216 (11, $[Cyt^{Bz} + H]^+$). ESI-MS (neg., MeOH): 464 (100, $[M - H]^-$). Anal. calc. for $C_{24}H_{23}N_3O_7$: C 61.93, H 4.98, N 9.03; found: C 61.70, H 5.05, N 8.93.

N^2-*Isobutyryl-9-(3′,4′*-O-*isopropylidene-α*-L-*arabinopyranosyl)guanine* (**4d**). To a soln. of 9.3 g (26.2 mmol) of **3d** and 262 mg (1.4 mmol) of TsOH · H_2O in 260 ml of dry DMF, cooled in an ice bath, 4.5 ml (47 mmol) of 2-methoxyprop-1-ene was added, during 2 h, through a syringe. After complete addition, the soln. was allowed to cool to r.t. and stirred overnight. The mixture was neutralized with 25% aq. NH_3 soln., 20 g of silica gel was added, and the mixture was evaporated to dryness. The residue was purified by CC (silica gel; CH_2Cl_2/MeOH 10 :1 (*v*/*v*)). The product fractions were collected, combined, and evaporated, and the colorless foam was dried in high vacuum (0.03 Torr/r.t., 12 h) to yield 9.06 g (87%) of **4d**. TLC ($CHCl_3$/MeOH 6:1): R_f 0.37. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.12 (*d*, *J* = 6.9, 2 *Me*$_2$CH); 1.33 (*s*, *Me*$_2$C); 1.55 (*s*, *Me*$_2$C); 3.92 (*dd*, *J* = 2.6, 13.3, H−C(5′)); 4.08 (*m*, H−C(2′)); 4.15 (*t*, *J* = 7.0, H−C(3′)); 4.23 (*d*, *J* = 13.3, H−C(5′)); 4.30 (*m*, H−C(4′)); 5.17 (*d*, *J* = 9.5, H−C(1′)); 5.68 (*d*, *J* = 6.0, HO−C(2′)); 8.21 (*s*, H−C(8)); 11.65, 12.11 (2*s*, H−N(1), H−N(2)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 19.69 (*Me*$_2$CH); 26.99 (*Me*$_2$C); 28.76 (*Me*$_2$C); 35.96 (Me$_2$*C*H-CO); 66.17 (C(5′)); 71.07 (C(2′)); 73.93 (C(4′)); 79.78 (C(3′)); 82.87 (C(1′)); 109.77 (Me$_2$*C*H); 120.78 (C(5)); 138.81 (C(8)); 148.96 (C(4)); 149.89 (C(2)); 155.68 (C(6)); 180.99 (Me$_2$CH*C*O). ESI-MS (pos., MeOH): 416 (100, $[M + Na]^+$), 394 (38, $[M + H]^+$), 222 (68, $[G^{ibu} + H]^+$). ESI-MS (neg., MeOH): 392 (100, $[M - H]^-$). Anal. calc. for $C_{17}H_{23}N_5O_6$: C 51.90, H 5.89, N 17.80; found: C 51.60, H 6.22, N 17.59.

N^6-*Benzoyl-9-(2′*-O-*{[(9*H*-fluoren-9-yl)methoxy]carbonyl}-3′,4′*-O-*isopropylidene-α*-L-*arabinopyranosyl)-adenine* (**5a**). To a soln. of 9.0 g (21.9 mmol) of **4a** in 80 ml of dry pyridine, cooled to 4° in an ice bath, a soln. of 6.2 g (24.1 mmol) of (9*H*-fluoren-9-yl)methyl chloroformate in 40 ml of dry CH_2Cl_2 was added, over a period of 1.5 h *via* a dropping funnel. The pale yellow soln. was stirred at r.t. overnight, and the reaction was quenched by the addition of 10 ml of MeOH. The solvent was evaporated *in vacuo*, followed by co-evaporation with toluene. The residue was dissolved in 250 ml of CH_2Cl_2, and washed with sat. aq. $NaHCO_3$ soln. and sat. aq. NaCl soln. The org. layer was dried (Na_2SO_4), evaporated, and the crude product was purified by CC (silica gel; toluene/AcOEt 5 :1 to 1 :1). The product fractions were combined, evaporated, and dried under high vacuum (0.03 Torr/r.t., 12 h) to afford 10.6 g (76%) of **5a**. Colorless foam. TLC (toluene/AcOEt/MeOH 5 :4 :1): R_f 0.55. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.35 (*s*, *Me*$_2$CH); 1.58 (*s*, *Me*$_2$CH); 4.04 (*t*, *J* = 6.5, CH of Fmoc); 4.18 (*dd*, *J* = 2.3, 13.5, H−C(5′)); 4.25 (*dd*, *J* = 6.5, 10.6, 1 H, C*H*$_2$ of Fmoc); 4.30 (*dd*, *J* = 6.5, 10.6, 1 H, C*H*$_2$ of Fmoc); 4.35 (*d*, *J* = 13.5, H−C(5′)); 4.40 (*m*, H−C(4′)); 4.55 (*dd*, *J* = 5.6, 7.4, H−C(3′)); 5.44 (*dd*, *J* = 7.4, 9.7, H−C(2′)); 5.96 (*d*, *J* = 9.7, H−C(1′)); 7.21 (*m*, 3 arom. H); 7.35 (*m*, 3 arom. H); 7.55 – 8.01 (*m*, 7 arom. H); 8.67 (*s*, H−C(8)); 8.74 (*s*, H−C(2)); 11.25 (*s*, H−N(6)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 27.05, 28.58 (*Me*$_2$CH); 46.71 (CH of Fmoc); 66.25 (C(5′)); 69.94 (CH_2 of Fmoc); 74.30 (C(4′)); 76.66 (C(3′)); 76.96 (C(2′)); 80.23 (C(1′)); 110.55 (Me$_2$*C*H); 120.93, 121.00, 125.38 (arom. C); 125.77 (C(5)); 127.89, 127.94, 128.44, 128.56, 129.30, 129.36, 133.34, 134.14, 141.40, 141.53 (arom. C); 143.46 (C(8)); 143.73, 143.76 (arom. C); 151.38 (C(4)); 152.83 (C(2)); 153.03 (C(6)); 154.28 (CO of Fmoc); 166.41 (CO of Bz). ESI-MS (pos., MeOH): 634 (87, $[M + H]^+$), 418 (19, $[M + H - CO_2 -$ (9-(hydroxymethyl)-9*H*-fluorene)$]^+$), 240 (48, $[A^{Bz} + H]^+$); 179 (100, [9-methylidene-9*H*-fluorene + H]$^+$). ESI-MS (neg., MeOH): 632 (9, $[M - H]^-$); 410 (100, $[M - H - Fmoc]^-$); 238 (87, $[A^{Bz} - H]^-$). Anal. calc. for $C_{35}H_{31}N_5O_7$: C 66.34, H 4.93, N 11.05; found: C 66.20, H 5.28, N 10.75.

1-(2′-O-*{[(9*H*-Fluoren-9-yl)methoxy]carbonyl}-3′,4′*-O-*isopropylidene-α*-L-*arabinopyranosyl)thymine* (**5b**). To an ice-cooled soln. of 24.0 g (80.5 mmol) of **4b** in 200 ml of dry pyridine was added, over a period of 2 h, a soln. of 20.8 g (80.5 mmol) of (9*H*-fluoren-9-yl)methyl chloroformate in 80 ml of dry CH_2Cl_2. The yellow

soln. was stirred at 4° for further 30 min, and the reaction was stopped by the addition of 10 ml of MeOH. The soln. was evaporated to a volume of 50 ml, and 400 ml of CH_2Cl_2 was added. The org. layer was washed twice with sat. aq. $NaHCO_3$ soln., sat. aq. NaCl soln., dried (Na_2SO_4), and evaporated *in vacuo*, followed by the co-evaporation with toluene. The resulting yellow oil was dissolved in 100 ml of toluene, from which the product crystallized. After storage at 4° overnight, the product was filtered, washed with Et_2O, and dried in vacuum (0.03 Torr/r.t., 24 h) to yield 30.6 g (73%) of **5b**. Colorless crystals. M.p. 197–198°. TLC (toluene/AcOEt 1:1): R_f 0.46. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.31 (*s*, Me_2C); 1.52 (*s*, Me_2C); 1.76 (*s*, Me–C(5)); 4.04 (*dd*, *J* = 2.3, 13.6, H–C(5′)); 4.23 (*m*, CH of Fmoc, H–C(5′)); 4.29 (*m*, H–C(4′)); 4.38 (*dd*, *J* = 6.7, 10.6, 1 H, CH_2 of Fmoc); 4.46 (*dd*, *J* = 5.7, 7.4, H–C(3′)); 4.50 (*dd*, *J* = 6.6, 10.6, 1 H, CH_2 of Fmoc); 4.91 (*dd*, *J* = 7.4, 9.8, H–C(2′)); 5.65 (*d*, *J* = 9.8, H–C(1′)); 7.31 (*m*, 2 arom. C); 7.41 (*m*, 2 arom. H); 7.45 (*s*, H–C(6)); 7.50–7.88 (*m*, 4 arom. H); 11.42 (*s*, H–N(3)). ^{13}C-NMR (150 MHz, (D_6)DMSO):12.77 (*q*, Me–C(5)); 26.93 (*q*, Me_2C); 28.56 (*q*, Me_2C); 46.88 (*d*, CH of Fmoc); 66.18 (*d*, C(5′)); 70.13 (*t*, CH_2 of Fmoc); 74.26 (*t*, C(4′)); 75.97 (*d*, C(2′)); 76.76 (*d*, C(3′)); 79.96 (*d*, C(1′)); 110.33, 110.99 (C(5), Me_2*C*H); 121.04, 121.09, 125.50, 125.57, 128.00, 129.09, 128.66 (arom. C); 136.50 (*d*, C(6)); 141.50, 141.58, 143.78, 143.89 (arom. C); 151.37 (*s*, C(2)); 154.57 (*s*, CO of Fmoc); 164.28 (*s*, C(4)). FAB-MS (pos., 3-NBA/CsI): 653.0920 ([*M* + Cs]$^+$). Anal. calc. for $C_{28}H_{28}N_2O_8$: C 64.61, H 5.42, N 5.38; found: C 64.71, H 5.29, N 5.12.

N^4-*Benzoyl-1-(2′*-O-*{[(9*H*-fluoren-9-yl)methoxy]carbonyl}-3′,4′*-O-*[(4-methoxyphenyl)methylidene]-α*-L-*arabinopyranosyl)cytosine* (**5c**). To a soln. of 12.0 g (25.8 mmol) of **4c** in 120 ml of dry pyridine, cooled in an ice bath, was added a soln. of 7.34 g (28.4 mmol) of (9*H*-fluoren-9-yl)methyl chloroformate in 50 ml of dry CH_2Cl_2, during 1.5 h. Stirring at 4° was continued for another 30 min before the reaction was quenched by the addition of 20 ml of MeOH. The mixture was evaporated under reduced pressure, and the residue was taken up in CH_2Cl_2. The CH_2Cl_2 soln. was washed with sat. aq. $NaHCO_3$ soln. and sat. aq. NaCl soln., and dried (Na_2SO_4). The org. phase was evaporated *in vacuo*, and the residue was co-evaporated with toluene to remove the pyridine. The resulting foam was purified by CC (silica gel; toluene/AcOEt 5:1 to 1:2 (*v*/*v*)). The product fractions were collected, combined, and evaporated to colorless foam, which was dried under high vacuum (0.03 Torr/r.t., 12 h) to yield 15.8 g (82%) of **5c**. TLC (toluene/AcOEt 1:2): R_f 0.34, 0.44. ^{1}H-NMR (600 MHz, (D_6)DMSO): 3.77, 3.78 (2*s*, MeO); 4.08 (*d*, *J* = 11.7, H–C(5′)); 4.18 (*t*, *J* = 6.8, CH of Fmoc); 4.22 (*m*, H–C(5′), CH of Fmoc); 4.34 (*m*, 1 H, CH_2 of Fmoc, H–C(4′), H–C(5′)); 4.44 (*m*, 1 H, CH_2 of Fmoc, H–C(4′), H–C(5′)); 4.67 (*dd*, *J* = 6.5, 6.6, H–C(3′)); 4.92 (*dd*, *J* = 5.8, 6.7, H–C(3′)); 5.04 (br. *t*, *J* = 8.5, H–C(2′)); 5.20 (br. *t*, *J* = 8.8, H–C(2′)); 5.89 (*s*, ArC*H*); 5.99 (2*d*, *J* = 8.5, 8.8, H–C(1′)); 6.26 (*s*, ArC*H*); 6.98 (*m*, 2 arom. H); 7.36 (*m*, 2 arom. H); 7.39 (*m*, H–C(5), arom. H); 7.51 (*m*, 5 arom. H); 7.61 (*m*, 1 arom. H); 7.85 (*m*, 2 arom. H); 7.96 (*m*, 2 arom. H); 8.15, 8.33 (2*m*, H–C(6)); 11.36, 11.39 (2*s*, H–N(4)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 46.87 (CH of Fmoc); 56.02 (MeO); 66.41, 66.72 (*t*, C(5′)); 70.33 (*t*, CH_2 of Fmoc); 74.30 (*d*, C(4′) of one isomer); 74.50 (*d*, C(2′) of one isomer); 76.12 (*d*, C(3′) of one isomer); 76.47 (*d*, C(4′) of one isomer); 77.98 (*d*, C(3′) of one isomer); 78.17 (*d*, C(2′) of one isomer); 81.04 (*d*, C(1′)); 97.84, 98.00 (C(5)); 103.49, 104.84 (*d*, ArCH); 114.59, 114.58, 120.99, 121.05, 125.55, 125.57, 125.79, 127.79, 127.98, 128.13, 128.56, 128.83, 129.29, 129.31, 129.57, 131.35, 133.69, 133.81, 141.45, 141.48, 141.54, 143.82, 143.85, 143.87 (arom. C); 146.69, 146.93 (C(6)); 154.58, 154.64 (CO of Fmoc); 155.29, 155.37 (C(2)); 160.81, 161.07 (arom. C); 164.36 (C(4)); 168.27 (CO of Bz). FAB-MS (pos., NBA/NaI): 710 (100 [*M* + Na]$^+$), 688 (45, [*M* + H]$^+$), 216 (17, [Cyt^{Bz} + H]$^+$), 179 (97, [9-methylidene-9*H*-fluorene + H]$^+$).

9-(2′-O-*{[(9*H*-fluoren-9-yl)methoxy]carbonyl}-3′,4′*-O-*isopropylidene-α*-L-*arabinopyranosyl)*-N^2-*isobutyrylguanine* (**5d**). To a soln. of 5.4 g (13.7 mmol) of **4d** in 50 ml of dry pyridine and cooled to 4° in an ice bath was slowly added, a soln. of 3.6 g (13.9 mmol) of (9*H*-fluoren-9-yl)methyl chloroformate dissolved in 20 ml of dry CH_2Cl_2, through a dropping funnel during 2 h. Stirring at 4° was continued for further 30 min, when TLC showed complete consumption of the starting material. The reaction was stopped by the addition of 5 ml of MeOH, and the solvents were removed under vacuum. The residue was dissolved in 300 ml of $CHCl_3$, washed with sat. aq. $NaHCO_3$ soln. and sat. aq. NaCl soln., and dried (Na_2SO_4). The solvent was evaporated to yield pale yellow foam, which was purified by CC (silica gel; 17 × 5.5 cm, toluene/AcOEt/MeOH 2:1:0 to 4:5:1). The product fractions were collected, combined, and evaporated. During the evaporation, a white solid precipitated, and the mixture was kept at 4° overnight, and the product was filtered and dried in vacuum (0.03 Torr/r.t., 12 h) to yield 7.99 g (88%) of **5d**. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.37. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.13 (*d*, *J* = 6.9, Me_2CH); 1.17 (*d*, *J* = 6.9, Me_2CH); 1.34 (*s*, Me_2C); 1.56 (*s*, Me_2C); 2.81 (*m*, Me_2C*H*CO); 4.00 (*t*, *J* = 6.2, CH of Fmoc); 4.03 (*dd*, *J* = 2.4, 14.0, H–C(5′)); 4.27 (*dd*, *J* = 6.2, 10.7, 1 H, CH_2 of Fmoc); 4.36 (*d*, *J* = 14.0, H–C(5′)); 4.38 (*m*, H–C(4′)); 4.47 (*m*, 1 H, CH_2 of Fmoc, H–C(3′)); 5.17 (*dd*, *J* = 7.5, 9.7, H–C(2′)); 9.70 (*d*, *J* = 9.7, H–C(1′)); 7.13 (*m*, 1 arom. H); 7.24 (*m*, 2 arom. H); 7.30–7.84 (*m*, 5 arom. H); 8.17 (*s*, H–C(8)); 11.67, 12.07 (2*s*, H–N(1), H–N(2)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 19.69 (Me_2CH); 19.74 (Me_2CH); 27.01 (Me_2C); 28.53 (Me_2C); 35.61 (Me_2*C*HCO); 46.75 (CH of Fmoc); 66.38 (C(5′)); 69.88 (CH_2 of Fmoc); 74.15

(C(4′)); 76.34 (C(3′)); 77.29 (C(2′)); 79.83 (C(1′)); 110.61 (Me_2CH); 120.39 (C(5)); 120.87, 120.93, 121.03, 125.02, 125.32, 127.52, 127.93, 128.31, 128.58 (arom. C); 138.30 (C(8)); 141.47, 141.56, 143.60, 143.90 (arom. C); 149.18 (C(4)); 149.37 (C(2)); 154.27 (CO of Fmoc); 155.47 (C(6)); 181.09 (Me_2CHCO). ESI-MS (pos., MeOH): 654 (22, $[M+K]^+$), 638 (100, $[M+Na]^+$), 616 (100, $[M+H]^+$), 179 (95, [(9-methylidene-9*H*-fluorene + H]$^+$). ESI-MS (neg., MeOH): 614 (3, $[M-H]^-$), 392 (100, $[M-G^{ibu}-H]^-$). Anal. calc. for $C_{32}H_{33}N_5O_6$ (615.64) C 62.53, H 5.46, N 11.38; found: C 62.53, H 5.28, N 11.29.

N^6-*Benzoyl-(2′*-O-*{[9*H*-fluoren-9-yl)methoxy]carbonyl}-α*-L-*arabinopyranosyl)adenine* (**6a**). A soln. of 10 g (15.8 mmol) of **5a** in 150 ml of 80% aq. AcOH soln. was stirred at 40°. After 48 h, the solvent was removed *in vacuo*, followed by co-evaporation with toluene. The residue was purified by CC (silica gel; CH_2Cl_2/MeOH 100:2 to 100:5). The product fractions were combined, evaporated, and dried under high vacuum (0.03 Torr/r.t., 12 h) to yield 6.77 g (70%) of **6a**. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.39. ^{1}H-NMR (600 MHz, (D_6)DMSO): 3.85 (*m*, H–C(4′)); 3.91 (br. *s*, H–C(5)); 3.97 (*m*, H–C(3′)); 4.00 (*t*, *J* = 6.4, CH of Fmoc); 4.20 (*m*, CH_2 of Fmoc); 5.15 (*d*, *J* = 6.0, HO–C(4′)); 5.44 (*m*, HO–C(3′), H–C(2′)); 5.87 (*d*, *J* = 9.3, H–C(1′)); 7.19 (*m*, 2 arom. H); 7.28–8.02 (*d*, 11 arom. H); 8.62 (*s*, H–C(8)); 8.71 (*s*, H–C(2)); 11.24 (*s*, H–N(6)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 46.72 (CH of Fmoc); 69.44 (C(4′)); 69.66 (CH_2 of Fmoc); 70.46 (C(5′)); 71.48 (C(3′)); 76.55 (C(2′)); 81.24 (C(1′)); 120.98, 120.89 (arom. C); 125.45, 125.50, 125.65 (arom C., C(5)); 127.90, 128.41, 128.54, 129.31, 129.34, 141.37, 141.53 (arom. C); 143.33 (C(8)); 143.73, 143.90 (arom. C); 151.28 (C(4)); 152.80 (C(2)); 153.04 (C(6)); 154.67 (CO of Fmoc); 166.38 (CO of Bz). ESI-MS (pos., MeOH): 632 (6, $[M+K]^+$), 616 (11, $[M+Na]^+$), 594 (73, $[M+H]^+$), 240 (100, $[A^{Bz}+H]^+$), 179 (89, [9-methylidene-9*H*-fluorene + H]$^+$). ESI-MS (neg., MeOH): 628 (33, $[M+Cl]^-$), 592 (28, $[M-H]^-$); 370 (100, $[M-Fmoc]^-$), 238 (28, $[A^{Bz}-H]^-$). Anal. calc. for $C_{32}H_{27}N_5O_7$ (593.59): C 64.75, H 4.58, N 11.80; found: C 64.76, H 4.69, N 11.77.

1-(2′-O-*{[(9*H*-Fluoren-9-yl)methoxy]carbonyl}-α*-L-*arabinopyranosyl)thymine* (**6b**). To a suspension of 28.0 g (53.8 mmol) of **5b** in 450 ml of dry MeOH, 3 ml of 4M HCl in dioxane was added. The mixture was heated to 50° in an oil bath for 2 h. The obtained soln. was allowed to cool to r.t. and was evaporated *in vacuo* to a pale yellow syrup, which was dissolved in 1000 ml of CH_2Cl_2. The org. layer was washed with sat. aq. $NaHCO_3$ soln., followed by drying (Na_2SO_4). Evaporation of the solvent to a volume of 100 ml resulted in spontaneous precipitation of the product, which was collected by filtration. After drying in vacuum (0.03 Torr/r.t., 12 h) 19.5 g (75%) of **6b** was obtained. White solid. TLC ($CHCl_3$/MeOH 1:1): R_f 0.45. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.77 (*s*, Me–C(5)); 3.78 (*m*, H–C(4′), 2 H–C(5′)); 3.86 (*m*, H–C(3′)); 4.22 (*dd*, *J* = 6.8, 7.0, CH of Fmoc); 4.35 (*dd*, *J* = 6.8, 10.5, 1 H, CH_2 of Fmoc); 4.44 (*dd*, *J* = 7.0, 10.5, 1 H, CH_2 of Fmoc); 5.00 (*dd*, *J* = 9.3, 9.4, H–C(2′)); 5.06 (*d*, *J* = 6.5, HO–C(4′)); 5.35 (*d*, *J* = 6.0, HO–C(3′)); 5.60 (*d*, *J* = 9.3, H–C(1′)); 7.32 (*m*, 2 arom. H); 7.42 (*t*, 2 arom. H); 7.52 (*s*, H–C(6)); 7.56 (*m*, 2 arom. H); 7.88 (*m*, 2 arom. H); 11.42 (*s*, H–N(3)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 12.95 (*q*, Me–C(5)); 46.92 (*d*, CH of Fmoc); 69.37 (*t*, C(4′)); 69.91 (*t*, C(5′)); 70.15 (*t*, CH_2 of Fmoc); 71.47 (*d*, C(3′)); 75.47 (*d*, C(2′)); 81.03 (*d*, C(1′)); 110.58 (*s*, C(5)); 120.99, 121.05, 125.66, 125.80, 128.02, 128.10, 128.64, 128.78 (arom. C); 137.00 (*d*, C(6)); 141.48, 141.58, 143.82, 143.82, 144.06 (arom. C); 151.41 (*s*, C(2)); 154.94 (*s*, CO of Fmoc); 164.37 (*s*, C(4)). FAB-MS (pos., 3-NBA/CsI): 613.0606 ($[M+Cs]^+$). Anal. calc. for $C_{25}H_{24}N_2O_8$: C 62.50, H 5.03, N 5.83; found: C 62.20, H 5.41, N 5.67.

N^4-*Benzoyl-(2′*-O-*{[(9*H*-fluoren-9-yl)methoxy]carbonyl}-α*-L-*arabinopyranosyl)cytosine* **(6c).** To a soln. of 5.7 g (8.2 mmol) of **5c** in 100 ml of MeOH and 50 ml of THF, cooled in an ice bath, 8 ml (104 mmol) of TFA was added, the ice bath was removed, and stirring was continued at r.t. After 2 h, when TLC indicated complete consumption of the starting material, the mixture was neutralized carefully with a 2M soln. of NH_3 in MeOH under ice cooling. The mixture was evaporated, and the residue was taken up in 400 ml of CH_2Cl_2 and adsorbed on 25 g of silica gel. The resulting white solid was purified by CC (silica gel; CH_2Cl_2/MeOH 50:1 to 50:4 (*v*/*v*). The product fractions were collected, combined, and evaporated to dryness (high vacuum, 0.03 Torr/50°, 12 h) to afford 4.5 g (95%) **6c**. White solid. TLC ($CHCl_3$/MeOH 9:1): R_f 0.42. ^{1}H-NMR (600 MHz, (D_6)DMSO): 3.81 (*m*, H–C(4′)); 3.83 (*d*, *J* = 12.0, H–C(5′)); 3.88 (*d*, *J* = 12.0, H–C(5′)); 3.93 (*m*, H–C(3′)); 4.20 (*t*, *J* = 7.0, CH of Fmoc); 4.29 (*dd*, *J* = 7.0, 10.3); 4.38 (*dd*, *J* = 7.0, 10.3, CH_2 of Fmoc); 5.04 (*t*, *J* = 9.0, H–C(2′)); 5.16 (*d*, *J* = 6.0, HO–C(3′)); 5.44 (*d*, *J* = 6.1, HO–C(4′)); 5.87 (br. *d*, *J* = 9.0, H–C(1′)); 7.32 (*m*, 2 arom. H); 7.38 (*m*, 3 arom. H); 7.51 (*m*, H–C(5′), 2 arom. H); 7.58–7.96 (*m*, 6 arom. H); 8.17 (br. *d*, *J* = 6.8, H–C(6)); 11.34 (*s*, H–N(4)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 46.89 (*d*, CH of Fmoc); 69.35 (*d*, C(4′)); 70.04 (*t*, CH_2 of Fmoc); 70.48 (*t*, C(5′)); 71.46 (C(3′)); 76.60 (C(2′)); 82.14 (C(1′)); 97.83 (C(5)); 120.97, 121.03, 125.72, 126.03, 128.01, 128.17, 128.56, 128.60, 129.30, 133.67, 133.83, 141.45, 141.53, 143.90, 150.60 (arom. C); 146.83 (C(6)); 154.98 (CO of Fmoc); 155.37 (C(2)); 164.25 (C(4)); 168.24 (CO of Bz). ESI-MS (pos., MeOH): 592 (100 $[M+Na]^+$), 570 (11, $[M+H]^+$), 216 (5, $[Cyt^{Bz}+H]^+$). ESI-MS (neg., MeOH): 604 (26, $[M+Cl]^-$), 568 (66, $[M-H]^-$).

9-(2′-O-*{[(9*H*-Fluoren-9-yl)methoxy]carbonyl}-α*-L-*arabinopyranosyl)*-N^2-*isobutyrylguanine* (**6d**). A soln. of 7.3 g (11.9 mmol) of **5d** in 800 ml of 80% aq. AcOH was stirred at 55° in an oil bath. After 10 h, TLC

indicated complete reaction, and the solvent was removed *in vacuo*. To remove last traces of AcOH, the residue was co-evaporated several times with toluene and MeOH, resulting in a colorless foam, which was dried in high vacuum (0.03 Torr/r.t., 12 h) to yield 6.81 g (quant.) of **6d**. TLC ($CHCl_3$/MeOH 9:1): R_f 0.53. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.12 (*d*, $J = 6.8$, Me_2CH); 1.16 (*d*, $J = 6.8$, Me_2CH); 2.78 (*m*, Me_2CHCO); 3.73 (*d*, $J = 12.1$, H–C(5′)); 3.84 (*m*, H–C(3′)); 3.90 (*d*, $J = 12.1$, H–C(5′)); 3.97 (*t*, $J = 6.1$, CH of Fmoc); 4.22 (*dd*, $J = 6.6$, 10.7, 1 H, CH_2 of Fmoc); 4.43 (*dd*, $J = 5.9$, 10.7, 1 H, CH_2 of Fmoc); 5.17 (*d*, $J = 5.9$, HO–C(3′)); 5.30 (*t*, $J = 9.3$, H–C(2′)); 5.39 (*d*, $J = 9.3$, H–C(1′)); 5.44 (*d*, $J = 5.7$, HO–C(2′)); 7.12 (*t*, 1 arom. H); 7.25 (*m*, 3 arom. H); 7.38–7.83 (*m*, 4 arom H); 8.12 (*s*, H–C(8)); 11.75, 12.06 (2*s*, H–N(1), H–N(2)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 19.66 (Me_2CH); 19.76 (Me_2CH); 35.63 (Me_2CHCO); 46.78 (CH of Fmoc); 69.30 (C(4′)); 69.57 (CH_2 of Fmoc); 70.68 (C(5′)); 71.52 (C(3′)); 76.51 (C(2′)); 81.07 (C(1′)); 120.18 (C(5)); 120.89, 121.03, 125.10, 125.39, 127.49, 127.90, 128.26, 128.56 (arom. C); 138.35 (C(8)); 141.43, 141.56, 143.69, 143.93 (arom. C), 149.22, 149.39 (C(2), C(4)); 154.60 (CO of Fmoc); 155.49 (C(6)); 181.07 (Me_2CHCO). ESI-MS (pos., MeOH): 614 (4, $[M+K]^+$), 598 (24, $[M+Na]^+$), 576 (49, $[M+H]^+$), 240 (33, $[G^{ibu}+H]^+$), 179 (100, [(9-methylidene-9*H*-fluorene + H]$^+$). ESI-MS (neg., MeOH): 574 (28, $[M-H]^-$); 352 $[M-G^{ibu}-H]^-$). Anal. calc. for $C_{29}H_{29}N_6O_6 \cdot H_2O$ (593.58): C 58.68, H 5.26, N 11.80; found: C 58.82, H 5.66, N 11.96.

N^6-*Benzoyl-9-(3′-*O*-benzoyl-2′-*O*-{[(9*H*-fluoren-9-yl)methoxy]carbonyl}-α-*L*-arabinopyranosyl)adenine* (**7a**). To a soln. of 6.5 g (11 mmol) of **6a** in 100 ml of dry pyridine, cooled in an ice/salt bath, 1.42 ml (12.1 mmol) of BzCl was slowly added through a syringe during 2 h. Stirring at 0° was continued for another 30 min, when TLC showed complete reaction. The mixture was poured into 100 ml of ice-cold sat. aq. $NaHCO_3$ soln. and 200 ml of CH_2Cl_2. The org. layer was washed with sat. aq. $NaHCO_3$ soln. and sat. aq. NaCl soln., and dried (Na_2SO_4). The org. layer was evaporated *in vacuo*, followed by co-evaporation with toluene, and the residue was purified by CC (silica gel; CH_2Cl_2/MeOH 100:2 to 100:5). Product fractions were combined, evaporated, and the colorless foam was dried in high vacuum (0.03 Torr/r.t., 12 h) to yield 5.57 g (73%) of **7a**. TLC ($CHCl_3$/MeOH 9:1): R_f 0.56. ^{1}H-NMR (600 MHz, (D_6)DMSO): 3.92 (*t*, $J = 6.5$, CH of Fmoc); 4.00 (*dd*, $J = 1.0$, 12.1, H–C(5′)); 4.15 (*d*, $J = 12.1$, H–C(5′)); 4.20 (*m*, CH_2 of Fmoc); 4.26 (*m*, H–C(4′)); 5.55 (*dd*, $J = 3.3$, 9.6, H–C(3′)); 5.68 (*d*, $J = 6.4$, HO–C(4′)); 6.04 (*t*, $J = 9.6$, H–C(2′)); 6.24 (*d*, $J = 9.3$, H–C(1′)); 7.00–8.04 (*m*, 18 arom. H); 8.75 (*s*, H–C(8)); 8.78 (*s*, H–C(2)); 11.27 (*s*, H–N(6)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 46.58 (*d*, CH of Fmoc); 66.80 (*t*, C(4′)); 70.06 (*d*, C(5′)); 70.19 (*t*, CH_2 of Fmoc); 73.33 (*d*, C(2′)); 75.27 (*d*, C(3′)); 81.02 (*d*, C(1′)); 120.84, 120.91 (arom. C); 125.24, 125.30, 125.81 (arom. C, C(5)); 127.73, 127.78, 128.42, 129.33, 129.36, 129.53, 129.98, 130.23, 133.37, 134.09, 134.49, 141.31, 141.39 (arom. C); 143.43 (*d*, C(8)); 143.49, 143.63 (arom. C); 151.45 (*s*, C(4)); 152.98 (*s*, C(2)); 153.09 (*s*, C(6)); 154.41 (*s*, CO of Fmoc); 165.81, 166.42 (*s*, CO of Bz). ESI-MS (pos., MeOH): 720 (9, $[M+Na]^+$), 698 (100, $[M+H]^+$), 418 (10, $[M+H-CO_2-$ (9-(hydroxymethyl)-9*H*-fluorene)]$^+$). ESI-MS (neg., MeOH): 696 (14, $[M+Cl]^-$), 474 (41, $[M-H-$Fmoc$]^-$), 352 (100, $[M-$Fmoc$-$Bz$]^-$). Anal. cal. for $C_{39}H_{31}N_5O_6$ (697.70): C 67.14, H 4.48, N 10.04; found: C 66.80, H 4.75, N 9.85.

*1-(3′-*O*-Benzoyl-2′-*O*-{[(9*H*-fluorenyl)methoxy]carbonyl}-α-*L*-arabinopyranosyl)thymine* (**7b**). To a soln. of 17.0 g (35.4 mmol) of **6b** in 250 ml of dry pyridine, cooled in an ice bath, 5 ml (42.5 mmol) of BzCl was slowly added, over 30 min, through a dropping funnel. Stirring at 4° was continued for another 1.5 h, and the mixture was poured into a mixture of 300 ml of ice-cold sat. aq. $NaHCO_3$ soln. and 300 ml of CH_2Cl_2. The org. layer was separated, washed with sat. aq. $NaHCO_3$ soln. and sat. aq. NaCl soln., and dried (Na_2SO_4). The solvent was evaporated *in vacuo*, and the residue was co-evaporated twice with toluene. A colorless foam was obtained, which was purified by CC (silica gel; toluene/AcOEt 1:0 to 1:1). The product fractions were collected, evaporated, and dried under high vacuum (0.03 Torr/r.t.) to yield 15.9 g (77%) of **7b**. Colorless foam. TLC (toluene/AcOEt/MeOH 5:4:1): R_f 0.7. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.80 (*s*, Me–C(5)); 3.89 (*dd*, $J = 1.23$, 12.1, H–C(5′)); 4.02 (*d*, $J = 12.1$, H–C(5′)); 4.09 (*t*, $J = 6.7$, CH of Fmoc); 4.14 (*d*, $J = 6.8$, H–C(4′)); 4.32 (*dd*, $J = 6.7$, 10.6, 1 H, CH_2 of Fmoc); 4.39 (*dd*, $J = 6.9$, 10.6, 1 H, CH_2 of Fmoc); 5.44 (*m*, H–C(2′), H–C(3′)); 5.57 (*d*, $J = 7.1$, HO–C(4′)); 5.94 (*d*, $J = 8.0$, H–C(1′)); 7.10–7.93 (*m*, 14 H, arom. H, H–(C(6)); 11.48 (*s*, H–N(3)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 12.96 (*q*, Me–C(5)); 46.75 (*d*, CH of Fmoc); 66.75 (*t*, C(4′)); 69.89 (*d*, C(5′)); 70.21 (*t*, CH_2 of Fmoc); 72.60 (*d*, C(3′)); 75.14 (*d*, C(2′)); 80.64 (C(1′)); 111.1 (*d*, C(5′)); 120.93, 120.97, 125.37, 125.51, 127.85, 127.88, 128.52, 128.55, 129.41, 129.54, 129.98, 130.17, 134.47 (arom. C); 136.56 (*d*, C(6)); 141.40, 141.45, 143.58, 143.76 (arom. C); 151.33 (*s*, C(2)); 154.69 (*s*, CO of Fmoc); 164.33 (*s*, C(4)); 165.72 (*s*, CO of Bz). ESI-MS (pos., MeOH): 623 (9, $[M+K]^+$), 607 (100, $[M+Na]^+$), 179 (54, [9-methylidene-9*H*-fluorene + H]$^+$). ESI-MS (neg., MeOH): 619 (73, $[M+Cl]^-$), 361 (100, $[M-$Fmoc$]^-$). Anal. calc. for $C_{32}H_{28}N_2O_9$ (584.58): C 65.75, H 4.83, N 4.79; found: C 65.40, H 5.12, N 4.57.

N^4-*Benzoyl-(3′-*O*-benzoyl-2′-*O*-{[(9*H*-fluoren-9-yl)methoxy]carbonyl}-α-*L*-arabinopyranosyl)cytosine* (**7c**). BzCl (1.3 ml, 10.18 mmol) was slowly added to an ice-cold mixture of 5.8 g (11.2 mmol) of **6c** in 100 ml of dry pyridine during 1.5 h. Stirring at 4° was continued for another 30 min, when TLC showed complete reaction.

The mixture was poured into 100 ml of ice-cold sat. aq. $NaHCO_3$ soln. and 200 ml of CH_2Cl_2. The org. layer was separated, washed with sat. aq. $NaHCO_3$ soln. and sat. aq. NaCl soln., dried (Na_2SO_4), and evaporated. Last traces of pyridine were removed by co-evaporation with toluene, and a pale yellow foam was obtained, which was purified by CC (silica gel; toluene/AcOEt 5:1 to 1:1). The product fractions were collected, combined, and evaporated. During the evaporation, a white solid precipitated, which was filtered, washed with Et_2O, and the mixture was kept at 4° overnight. Drying of the product under vacuum (0.03 Torr/r.t., 12 h) yielded 5.69 g (83%) of **7c**. TLC (toluene/AcOEt 1:2): R_f 0.43. ^{1}H-NMR (600 MHz, (D_6)DMSO): 3.96 (*d*, *J* = 11.31, H–C(5′)); 4.08 (*m*, H–C(4′), H–C(5′)); 4.19 (br. *d*, *J* = 6.2, CH of Fmoc); 4.28 (*dd*, *J* = 7.0, 10.4, 1 H, CH_2 of Fmoc); 4.33 (*dd*, *J* = 7.0, 10.4, 1 H, CH_2 of Fmoc); 5.49 (*m*, H–C(3′), H–C(2′)); 5.65 (*d*, *J* = 6.6, HO–C(4′)); 6.18 (br. *d*, H–C(1′)); 7.11 (*m*, 2 arom. H); 7.27 (*t*, 1 arom. H); 7.31 (*m*, 2 arom. H); 7.44 (*m*, H–C(5), 1 arom. H); 7.50 (*m*, 4 arom. H); 7.64 (*m*, 2 arom. H); 7.79 (*m*, 2 arom. H); 7.94 (*m*, 2 arom. H); 7.99 (*d*, 2 arom. H); 8.27 (*d*, *J* = 7.3, H–C(6)); 11.36 (*s*, H–N(4)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 46.71 (CH of Fmoc); 66.76 (C(4′)); 70.24, 70.32 (C(2′), C(3)); 73.63, 75.06 (C(5′), CH_2 of Fmoc); 81.77 (C(1′)); 98.17 (C(5)); 120.91, 120.95, 125.42, 125.65, 127.86, 127.95, 128.44, 128.52, 129.32, 129.56, 129.98, 130.20, 133.70, 133.84, 134.49, 141.37, 141.41, 143.63, 143.73 (arom. C); 146.50 (C(6)); 154.69 (CO of Fmoc); 155.69 (CO of Fmoc); 155.26 (C(2)); 164.38 (C(6)); 165.75 (CO of BzO); 168.27 (CO of NHBz). ESI-MS (pos., MeOH): 696 (100, $[M+Na]^+$), 674 (17, $[M+H]^+$). ESI-MS (neg., MeOH): 672 (100, $[M-H]^-$), 450 (25, $[M-H\text{-Fmoc}]^-$), 432 (21, $[M-2\,OBz]^-$). Anal.: cal. for $C_{38}H_{31}N_3O_9$ (673.56); C 67.76, H 4.64, N 6.24; found: C 67.73, H 4.73, N 6.25.

*9-(3′-*O*-Benzoyl-2′-*O*-{[(9*H*-fluoren-9-yl)methoxy]carbonyl}-α-*L*-arabinopyranosyl)-*N*^{2}-isobutyrylguanine* (**7d**). A soln. of 5.35 g (12.6 mmol) of **6d** in 100 ml of dry pyridine and 50 ml of dry CH_2Cl_2 was cooled to −20° (i-PrOH / dry ice), and 1.1 ml (9.4 mmol) of BzCl was slowly added during 2 h. After complete addition, stirring was continued for another h, when TLC showed consumption of the starting material. The soln. was poured into 200 ml of ice-cold sat. aq. $NaHCO_3$ soln. and 200 ml of CH_2Cl_2. The org. phase was extracted with sat. aq. $NaHCO_3$ soln. and sat. aq. NaCl soln., dried (Na_2SO_4), and evaporated. The crude product was purified by CC (silica gel; CH_2Cl_2/MeOH 95:5 (*v*/*v*)). The product fractions were collected, combined, and evaporated to yield the product as colorless foam, which was dried in high vacuum (0.03 Torr/r.t.) overnight to yield 5.2 g (82%) of **7d**. TLC ($CHCl_3$/MeOH 6:1): R_f 0.55. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.12, 1.14 (2*d*, *J* = 6.9, *Me*$_2$CH); 2.82 (*m*, *Me*$_2$CHCO); 3.92 (br. *t*, *J* = 6.2, CH of Fmoc); 4.00 (br. *s*, H–C(5′)); 4.22 (*m*, 1 H, CH_2 of Fmoc), H–C(4′)); 4.38 (*dd*, *J* = 6.2, 10.7, 1 H, CH_2 of Fmoc); 5.43 (*dd*, *J* = 3.4, 9.8, H–C(3′)); 5.69 (br. *d*, *J* = 4.8, HO–C(4′)); 5.74 (*d*, *J* = 9.3, H–C(1′); 5.80 (br. *t*, *J* = 9.5, H–C(2′)); 7.04–7.94 (*m*, 13 arom. H); 8.25 (*s*, H–C(8)); 11.76, 12.13 (2*s*, HN–C(1), HN–C(2)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 19.53 (*Me*$_2$CH); 19.71 (*Me*$_2$CH); 35.56 (*Me*$_2$CHCO); 46.66 (CH of Fmoc); 66.71 (C(4′)); 69.99 (CH_2 of Fmoc); 70.37 (C(5′)); 73.43 (C(2′)); 75.14 (C(3′)); 80.78 (C(1′)); 120.51, 120.84, 120.93 (C(5), arom. C); 125.10, 125.16, 127.56, 127.73, 128.34, 128.45, 129.39, 129.59, 129.92, 130.10, 130.20, 133.66, 134.56 (arom. C); 138.25 (C(8)); 141.35, 141.43, 143.50, 143.63 (arom. C); 149.32, 149.51 (C(2), C(4)); 154.39 (CO of Fmoc); 155.54 (C(6)); 165.76 (CO of Bz); 181.14 (Me$_2$CH*C*O). ESI-MS (pos., MeOH): 702 (100, $[M+Na]^+$), 680 (58, $[M+H]^+$); 222 (19, $[G^{ibu}+H]^+$), 179 (24, [9-methylidene-9*H*-fluorene + H]$^+$). ESI-MS (neg., MeOH): 678 (9, $[M-H]^-$), 456 (100, $[M-G^{ibu}-H]^-$). Anal. calc. for $C_{36}H_{33}N_5O_9 \cdot H_2O$ (697.70): C 61.97, H 5.06, N 10.04; found: C 61.82, H 5.45, N 9.90.

2. Experiments Referring to *Scheme 4*. – *N*^{6}*-Benzoyl-9-(3′-*O*-benzoyl-4′-*O*-[(2-cyanoethoxy)(diisopropylamino)phosphino]-2′-*O*-{[(9*H*-fluoren-9-yl)methoxy]carbonyl}-α-*L*-arabinopyranosyl)adenine* (**8a**). A soln. of 1.0 g (1.43 mmol) of **7a** in 15 ml of dry CH_2Cl_2 was stirred over molecular sieves (4 Å) for 1 h at r.t. To this soln., 100 mg (1.43 mmol) of 1*H*-tetrazole and 682 ml (2.15 mmol, 1.5 equiv.) of (2-cyanoethoxy)bis(diisopropylamino)phosphine were added, and stirring at r.t. was continued for another h. The mixture was washed with sat. aq. $NaHCO_3$ soln. and sat. aq. NaCl soln. The org. layer was dried (Na_2SO_4) and evaporated *in vacuo* to afford colorless foam. This residue was purified rapidly (10–15 min.) by CC (silica gel; 7 × 2 cm, conditioned with toluene containing 1% of EtN(i-Pr)$_2$, toluene/AcOEt 1:1). The product fractions were combined, and the solvent was removed *in vacuo* and dried under high vacuum (0.03 Torr/r.t.) overnight to yield 1.02 g (80%) **8a** as a mixture of diastereoisomers. TLC (toluene/AcOEt 1:2): R_f 0.52, 0.59. ^{1}H-NMR (600 MHz, $CDCl_3$): 1.15, 1.22 (4*d*, *J* = 6.8, *Me*$_2$CH); 2.37 (*m*, CH_2CH_2CN); 2.63 (*t*, *J* = 6.2, CH_2CH_2CN); 3.58 (*m*, CH_2CH_2CN); 3.71 (*m*, Me$_2$C*H*, CH_2CH_2CN); 3.78 (*m*, CH_2CH_2CN); 3.87 (*m*, CH_2CH_2CN); 3.88 (*t*, *J* = 7.5, CH of Fmoc); 3.93 (*t*, *J* = 7.5, CH of Fmoc); 4.05 (2*d*, *J* = 12.7, H–C(5′)); 4.11 (*dd*, *J* = 3.7, 7.5, 1 H, CH_2 of Fmoc); 4.15 (*m*, 1 H, CH_2 of Fmoc); 4.25, 4.39 (2*dd*, *J* = 1.8, 12.7, H–C(5)); 4.63, 4.73 (2*m*, H–C(4′)); 5.39, 5.46 (2*dd*, *J* = 3.0, 9.6, H–C(3′)); 6.03 (*m*, H–C(2′), H–C(1′)); 7.01, 7.10, 7.22, 7.25, 7.30, 7.36, 7.41, 7.52, 7.63 (9*m*, 13 arom. H); 8.31, 8.36 (2*s*, H–C(8)); 8.83, 8.85 (2*s*, H–C(2)); 9.03, 9.05 (2 br. *s*, H–N(6)). ^{13}C-NMR (150 MHz, $CDCl_3$): 20.34, 20.77 (CH_2CH_2CN); 24.94, 25.07, 25.12 (*Me*$_2$CH); 43.72, 43.76, 43.80, 43.84 (Me$_2$*C*H); 46.50 (CH of Fmoc); 57.91, 58.81 (2*d*, *J*(C,P) = 19.5, CH_2CH_2CN); 69.24 (C(4′)); 69.54, 69.60 (C(5′)); 69.66 (C(4′)); 70.95, 71.00 (CH_2 of

Fmoc); 72.45, 72.66 (C(2′)); 73.91, 74.40 (C(3′)); 81.34, 81.50 (C(1′)); 117.95, 120.24, 120.28, 123.04, 123.11, 125.11 (CN, C(5), arom. C); 127.40, 127.43, 128.14, 128.18, 128.28, 128.76, 129.22, 129.44, 130.12, 130.35, 133.19, 133.92, 134.02, 134.13 (arom. C); 141.27, 141.35, 141.41, 141.57 (C(8), arom. C); 142.96, 143.01, 143.17 (arom. C); 150.02 (C(4)); 152.53 (C(2)); 153.55 (C(6)); 154.43, 154.50 (*s*, CO of Fmoc); 164.96, 165.84, 165.93 (*s*, CO of Bz). ^{31}P-NMR (242 MHz, $CDCl_3$): 151.40; 152.74. ESI-MS (pos., MeOH): 920 (100, $[M+Na]^+$), 898 (95, $[M+H]^+$). ESI-MS (neg., MeOH): 874 ($[M-H]^-$).

*1-(3′-*O*-Benzoyl-4′-*O*-[(2-cyanoet hoxy)(diisopropylamino)phospino]-2′-*O*-[[(9*H*-fluoren-9-yl)methoxy]-carbonyl]-α-*L*-arabinopyranosyl)thymine* (**8b**). To a soln. of 1.0 g (1.71 mmol) of **7b** in 15 ml of dry CH_2Cl_2, stirred over molecular sieves (4 Å) for 1 h at r.t., 119 mg (1.71 mmol) of 1*H*-tetrazole and 0.82 ml (2.57 mmol, 1.5 equiv.) of (2-cyanoethoxy)bis(diisopropylamino)phosphine were added. The mixture was stirred for 60 min at r.t., until TLC showed complete consumption of the starting material. The mixture was diluted with 20 ml of CH_2Cl_2 and washed twice with sat. aq. $NaHCO_3$ soln., followed by sat. aq. NaCl soln. The org. phase was dried (Na_2SO_4) and evaporated *in vacuo* to afford colorless foam, which was purified rapidly (10–15 min.) by CC (silica gel; 7 × 2 cm, conditioned with toluene containing 1% of $EtN(i\text{-}Pr)_2$, toluene/AcOEt 3:1). The product fractions were combined, evaporated, and dried under high vacuum (0.03 Torr/r.t., 12 h) to afford 1.12 g (84%) of **8b** as a mixture of diastereoisomers. TLC (toluene/AcOEt 1:1): R_f 0.63, 0.72. ^{1}H-NMR (600 MHz, $CDCl_3$): 1.20 (4*d*, *J* = 6.8, Me_2CH); 1.95, 1.96 (2*s*, Me–C(5)); 2.35 (*m*, CH_2CH_2CN); 2.61 (*t*, *J* = 6.2, CH_2CH_2CN); 3.55 (*m*, CH_2CH_2CN); 3.67 (*m*, Me_2CH, CH_2CH_2CN); 3.76 (*m*, CH_2CH_2CN); 3.82 (m, CH_2CH_2CN); 3.92 (2*d*, *J* = 11.6, 12.1, H–C(5′)); 4.09 (2*t*, *J* = 7.8, *J* = 7.9, CH of Fmoc); 4.16 (*d*, *J* = 11.6, H–C(5′)); 4.29 (*m*, H–C(5′), CH_2 of Fmoc); 4.55, 4.65 (2*m*, H–C(4′)); 5.31, 5.37 (2*dd*, *J* = 3.1, 10.0, H–C(3′)); 5.47, 5.54 (2*t*, *J* = 9.6, H–C(2′)); 5.93, 5.95 (2*d*, *J* = 9.3, H–C(1′)); 7.10–8.00 (8*m*, 13 arom. H, H–C(6)); 8.45 (br. *s*, H–N(3)). ^{13}C-NMR (150 MHz, $CDCl_3$): 12.91, 13.00 (*q*, Me–C(5) of both isomers); 20.32, 20.75 (*t*, CH_2CH_2CN of both isomers); 24.92, 25.13, 25.19 (*q*, Me_2CH of both isomers); 43.67, 43.70, 43.75, 43.78 (*d*, Me_2CH of both isomers); 46.65 (*d*, CH of Fmoc of both isomers); 58.00, 58.93 (*t*, *J*(C,P) = 18.7, 20.0, CH_2CH_2CN of both isomers); 68.55 (*t*, C(4′) of isomer a); 68.80, 69.17 (*d*, C(5′) of both isomers); 69.53 (*t*, C(4′) of isomer b); 71.09, 71.12 (*t*, CH_2 of Fmoc of both isomers); 71.80, 72.07 (*d*, C(2′) of both isomers); 73.70, 74.29 (*d*, C(3′) of both isomers); 81.17 (*d*, C(1′) of both isomers); 112.27, 112.46 (*s*, CN of both isomers); 117.76 (*d*, C(5)); 120.33, 120.37, 125.34, 125.44, 127.48, 127.52, 128.22, 128.29, 128.73, 128.97, 129.28, 129.48, 130.07, 130.30, 133.95, 134.07 (arom. C); 135.39 (*t*, C(6)); 141.49, 141.53, 143.18, 143.27 (arom. C); 150.64, 150.72 (*s*, C(2)); 154.79, 154.81 (*s*, CO of Fmoc); 163.51 (*s*, C(4)); 165.79, 165.88 (*s*, CO of Bz). ^{31}P-NMR (242 MHz, $CDCl_3$): 151.64, 153.03. ESI-MS (pos., MeOH): 807 (100, $[M+Na]^+$). Anal. calc. for $C_{41}H_{45}N_4O_{10}P$: C 62.71, H 5.78, N 7.13; found: C 62.79, H 5.81, N 7.13.

N^4*-Benzoyl-1-(3′-*O*-benzoyl-4′-*O*-[(2-cyanoethoxy)(diisopropylamino)phosphino]-2′-*O*-[[(9*H*-fluoren-9-yl)methoxy]ca rbonyl]-α-*L*-arabinopyranosyl)cytosine* (**8c**). A suspension of 1.0 g (1.5 mmol) of **7c** in 15 ml of dry CH_2Cl_2 was stirred over molecular sieves (4 Å) for 1 h at r.t., and 105 mg (1.5 mmol) of 1*H*-tetrazole and 715 µl (2.25 mmol, 1.5 eq.) of (2-cyanoethoxy)bis(diisopropylamino)phosphine were added, and stirring at r.t. was continued for another h, until TLC showed complete consumption of the starting material. The mixture was washed with sat. aq. $NaHCO_3$ soln. and sat. aq. NaCl soln., dried (Na_2SO_4), and was evaporated to a colorless foam. The crude product was purified by CC (silica gel; 7 × 2 cm, toluene/AcOEt 2:1 (*v*/*v*)). To avoid acid-catalyzed decomposition of the phosphoramidite the silica gel was first washed with toluene containing 1% of $EtN(i\text{-}Pr)_2$, and CC was performed within 10 to 15 min. The product fractions were collected, combined, and evaporated to yield colorless foam, which was dried under high vacuum (0.03 Torr/r.t., 12 h) to afford 990 mg (84%) of **8c** as a mixture of diastereoisomers. TLC (toluene/AcOEt 1:1): R_f 0.41, 0.49. ^{1}H-NMR (600 MHz, $CDCl_3$): 1.16, 1.19, 1.24 (4*d*, Me_2CH); 2.37, 2.63 (*t*, *J* = 6.2, CH_2CH_2CN); 3.56 (*m*, CH_2CH_2CN); 3.69 (*m*, Me_2CH, CH_2CH_2CN); 3.77, 3.84 (*m*, CH_2CH_2CN); 3.97 (2*d*, *J* = 12.5, H–C(5′)); 4.09 (2*t*, *J* = 7.7, *J* = 7.9, CH of Fmoc); 4.19, 4.29 (*m*, CH_2 of Fmoc, H–C(5′)); 4.58, 4.68 (2*m*, H–C(4′)); 5.35, 5.41 (2*dd*, *J* = 3.2, 10.0, H–C(3′)); 5.49, 5.57 (2*t*, *J* = 9.8, H–C(2′)); 5.23 (br. *t*, *J* = 9.8, H–C(1′)); 7.08, 7.18, 7.27, 7.31, 7.37, 7.40, 7.43, 7.50, 7.62, 7.66, 7.87, 7.95, 8.00 (18*m*, 2 br. *s*, 13 arom. H, H–C(5), H–C(6)); 8.70 (br. *s*, H–N(4)). ^{13}C-NMR (150 MHz, $CDCl_3$): 20.38, 20.76 (CH_2CH_2CN); 23.00, 24.94, 25.08 (Me_2CH); 43.69, 43.77 (Me_2CH); 46.68 (CH of Fmoc); 58.00, 58.90 (2*d*, *J*(C,P) = 18.2, 20.0, CH_2CH_2CN); 68.55 (*d*, *J*(C,P) = 16.3, C(4′)); 69.18, 69.45 (C(5′)); 69.65 (*d*, *J*(C,P) = 23.7, C(4′)); 71.13, 71.17 (CH_2 of Fmoc); 72.73, 73.08 (C(2′)); 73.76, 74.30 (C(3′)); 82.21 (C(1′)); 120.18, 120.23, 120.26, 125.44, 125.47, 125.54, 125.57, 127.53, 128.11, 128.13, 128.74, 128.97, 129.32, 129.40, 129.49, 130.06, 130.30, 133.67, 133.93, 134.05, 141.37, 141.19, 143.17, 143.48, 143.51 (arom. C); 154.84, 154.88 (CO of Fmoc); 165.80, 165.89 (CO of BzO). ^{31}P-NMR (240 MHz, $CDCl_3$): 151.73, 152.79. ESI-MS (pos., MeOH): 896 (80, $[M+Na]^+$), 874 (100, $[M+H]^+$). Anal. calc. for $C_{41}H_{45}N_4O_{10}P$ (785.30): C 64.60, H 5.54, N 8.01; found: C 64.25, H 5.79, N 8.21.

9-(3′-O-Benzoyl-4′-O-[(2-cyanoethoxy)(diisopropylamino)phosphino]-2′-O-[[(9H-fluoren-9-yl)methoxy]-carbonyl]-α-L-arabinopyranosyl)-N²-isobutyrylguanine (**8d**). To a soln. of 679 mg (1 mmol) of **7d** in 20 ml of dry CH_2Cl_2 stirred over molecular sieves (4 Å) for 1 h at r.t, were added 77 mg (1.1 mmol) of 1*H*-tetrazole and 476 µl (1.5 mmol) of (2-cyanoethoxy)bis(diisopropylamino)phosphane, and stirring was continued for another h. The mixture was diluted with CH_2Cl_2, washed with sat. aq. $NaHCO_3$ soln. and sat. aq. NaCl soln., and evaporated. The colorless foam obtained was purified by CC (silica gel; 7×2 cm) with toluene/AcOEt 1:1 (*v*/*v*). The product fractions were collected, combined, and evaporated to yield 588 mg (67%) of **8d**. Colorless foam. TLC (toluene/AcOEt 1:2): R_f 0.47. ^{1}H-NMR (600 MHz, $CDCl_3$): 1.13, 1.17, 1.23 (4*d*, $J = 6.8$, 2 Me_2CH); 2.36, 2.60 (*t*, $J = 6.1$, CH_2CH_2CN); 2.72 (*m*, Me_2CHCO); 3.59 (m, CH_2CH_2CN); 3.67 (*m*, Me_2CH, CH_2CH_2CN); 3.73, 3.80 (m, CH_2CH_2CN); 3.86 (*d*, $J = 12.6$, H–C(5′)); 3.92 (*t*, $J = 7.3$, CH of Fmoc); 3.99 (t, $J = 7.3$, CH of Fmoc); 4.11 (*dd*, $J = 7.5$, 10.6, CH_2 of Fmoc); 4.11 (*dd*, $J = 7.5$, 10.6, CH_2 of Fmoc); 4.25, 4.29 (2*dd*, $J = 2.1$, 12.6, H–C(5)); 4.57, 4.70 (2 br. *m*, H–C(4′)); 5.38, 5.41 (2*dd*, $J = 3.1$, 9.9, H–C(3′)); 5.64, 5.66 (2*d*, $J = 9.6$, H–C(1′)); 5.87, 6.20 (2*t*, $J = 9.6$, H–C(2′)); 7.06, 7.10, 7.22, 7.26, 7.30, 7.36, 7.49, 7.52, 7.64, 7.97 (10*m*, 13 arom. H); 7.92, 7.93 (2*s*, H–C(8)); 9.34 (br. *s*, H–N). ^{13}C-NMR (150 MHz, $CDCl_3$): 19.31, 20.52 (Me_2CH); 20.78, 20.84 (CH_2CH_2CN); 24.90, 25.04, 25.09 (Me_2CH); 36.46, 36.69 (Me_2CHCO); 43.75, 43.82 (M_2CH); 46.60 (CH of Fmoc); 57.80 (*d*, J(C,P) = 19.5, CH_2CH_2CN); 68.84 (C(4′)); 69.23 (C(5′)); 69.65 (d, J(C,P) = 14.0, C(4′)); 70.87 (CH_2 of Fmoc); 72.46 (C(2′)); 73.81 (C(3′)); 81.11 (C(1′)); 118.20, 120.28, 120.31, 120.33 (CN, C(5), arom. C); 127.39, 127.47, 128.18, 128.23, 128.77, 128.97, 129.16, 129.39, 130.06, 130.29, 134.07 (arom. C); 137.02 (C(8)); 141.40, 142.98, 142.98, 143.14 (arom. C); 148.57, 149.12 (C(4), C(2)); 154.29 (CO of Fmoc); 155.97 (C(6)); 166.02 (CO of Bz); 179.36 (Me_2CHCO). ^{31}P-NMR (242 MHz, $CDCl_3$): 152.88. ESI-MS (pos., MeOH): 918 ($[M+K]^+$), 902 ($[M+Na]^+$), 880 ($[M+H]^+$). Anal. calc. for $C_{45}H_{50}N_6O_{10}P$ (879.90): C 61.43, H 5.73, N 11.11; found: C 61.22, H 5.91, N 11.08.

General Method for the Preparation of Nucleoside-Derivatized LCAMA-Controlled-Pore-Glass Solid Supports (LCAMA-CPG) (**10a**–**10d**) [15][16]. A mixture of 0.2–0.3 g (0.29–0.5 mmol) of **7a**–**7d**, 57–100 mg (0.57–1.0 mmol) of succinic anhydride, 3.5–30 mg (0.03–0.25 mmol) of 4-DMAP, and 0.14–0.24 ml of pyridine in 10 ml of CH_2Cl_2 was stirred at r.t. for 3–4 days (monitored by TLC for consumption of starting material). The mixture was extracted with sat. aq. $NaHCO_3$ soln., followed by 5% aq. citric acid soln. The org. phase was dried (Na_2SO_4) and concentrated *in vacuo* to afford 67–95% of **9a**–**9d**, containing traces of starting material as impurity.

A suspension of 2 g of glyceryl-CPG (500 Å) and 3 g of *N*,*N*-carbonyldi[1*H*-imidazole] in 50 ml CH_2Cl_2 were shaken for 24 h at r.t. The solvent was filtered, the CPG was washed with CH_2Cl_2 and dried. The activated CPG was suspended in CH_2Cl_2, and 3 ml of 1,6-bis-methylamino hexane was added and the mixture was shaken for 24 h at r.t. The solvent was filtered, the support was washed carefully with DMF, MeOH, acetone, Et_2O, and dried. To 300 mg of the derivatized LCAMA-CPG (500 Å), 30 mg (*ca.* 0.05 mmol) of nucleosides **9a**–**9d**, 30 mg TOTU, and 0.01 ml *N*-methylmorpholine in 5 ml of MeCN were added, and the mixture was shaken for 1.5 h. The solvent was filtered, the support was washed with DMF, MeOH, acetone, Et_2O, and dried. To this dried CPG was added 2 ml of 1-methyl-1*H*-imidazole in pyridine/THF (capping soln. *A*; *c.f. Sect. 5.3*) and Ac_2O in THF (capping soln. *B*; *c.f. Sect. 5.3*), and the mixture was shaken for 20 min. The solvent was filtered, the support was washed with DMF, MeOH, acetone, Et_2O, and dried to afford **10a**–**10d**.

Loading-Capacity Determination. Solid support (5–10 mg) was suspended in 5 ml of 0.1M DBU in MeCN, and the absorbance was measured at 305 nm ($\varepsilon = 9100$). The loading capacity was calculated from the following formula,

Loading capacity [µmol/g] = $A_{305\,nm} \times 5/9100 \times$ mg(support) and determined to be 19 µmol/g for **10a**, 18 µmol/g for **10b**, 15 µmol/g for **10c**, and 12 µmol/g for **10d**.

3. Experiments Referring to *Scheme 5*. – *9-(α-L-Arabinopyranosyl)adenine* (**11a**). A suspension of 37.0 g (54.1 mmol) **2a** in 500 ml of 2.0M NH_3 in MeOH was stirred at r.t. for 2 days and concentrated to 60 ml. The resulting white precipitate was filtered, washed with AcOEt, Et_2O, and dried in high vacuum (0.03 Torr/r.t.) overnight to yield 12.0 g (83%) of **11a**. TLC (CH_2Cl_2/MeOH 3:1) : R_f 0.40. ^{1}H-NMR (600 MHz, (D_6)DMSO): 3.58 (*m*, H–C(3′)); 3.73 (*d*, $J = 12.1$, H–C(5′)); 3.77 (*m*, H–C(4′)); 3.80 (*dd*, $J = 1.7$, 12.1, H–C(5′)); 4.19 (*m*, H–C(2′)); 4.80 (*d*, $J = 5.0$, HO–C(4′)); 5.05 (*d*, $J = 5.7$, HO–C(3′)); 5.26 (*d*, HO–C(2′)); 5.31 (*d*, $J = 9.3$, H–C(1′)); 8.15 (*s*, H–C(2)); 8.26 (*s*, H–C(8)). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 69.40 (C(4′)); 69.64 (C(2′)); 70.10 (C(5′)); 74.37 (C(3′)); 83.98 (C(1′)); 119.30 (C(5)); 139.99 (C(8)); 150.70 (C(4)); 153.47 (C(2)); 156.79 (C(6)). ESI-MS (pos.): 290 (10, $[M+Na]^+$), 268 (100, $[M+H]^+$). ESI-MS (neg.): 302 (100, $[M+Cl]^-$).

9-(α-L-Arabinopyranosyl)guanine (**11d**). A suspension of 12.8 g (19.2 mmol) of **2d** in 200 ml of sat. NH_3 in MeOH was stirred at r.t. for 2 days. The resulting white precipitate was filtered, washed with AcOEt, Et_2O, and dried in high vacuum (0.03 Torr/r.t.) to afford 5.17 g (95%) of **11d**. TLC (CH_2Cl_2/MeOH 3:1): R_f 0.5. ^{1}H-NMR

(600 MHz, (D_6)DMSO): 3.49 (*dd*, *J* = 3.2, 9.2, H–C(3′)); 3.61 (*d*, *J* = 12.2, H–C(5′)); 3.73 (br. *s*, H–C(4′)); 3.79 (*dd*, *J* = 1.1, 12.2, H–C(5′)); 4.02 (*dd*, *J* = 9.3, H–C(2′)); 4.35 (br. *s*, OH); 4.77 (br. *s*, OH); 5.04 (*d*, *J* = 9.3, H–C(1′)); 5.28 (br. *s*, OH); 6.51 (br. *s*, NH_2–C(2)); 7.78 (*s*, H–C(8)). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 69.33 (C(4′)); 69.61 (C(2′)); 70.10 (C(5′)); 74.42 (C(3′)); 83.48 (C(1′)); 116.95 (C(5)); 136.04 (C(8)); 152.55 (C(4)); 154.94 (C(2)); 158.19 (C(6))). ESI-MS (pos.): 322 (6, $[M+K]^+$), 306 (22, $[M+Na]^+$), 284 (70, $[M+H]^+$), 152 (100). ESI-MS (neg.): 318 (6, $[M+Cl]^-$), 282 (100, $[M-H]^-$), 150 (26).

*9-(α-*L*-Arabinopyranosyl)-*N^2*-(phenoxyacetyl)adenine* (**12a**). To a soln. of 4.5 g (16.8 mmol) of **11a** (co-evaporated with 2 × 50 ml pyridine) in 70 ml dry pyridine, under Ar, was added 12.8 ml (0.1 mol) of TMSCl, and the mixture was stirred at r.t. for 30 min. In a separate flask under Ar, 2.73 g (20.2 mmol) of 1-HOBT (co-evaporated with 2 × 40 ml pyridine) was dissolved in 1.63 ml (20.2 mmol) pyridine and 20 ml dry CH_2Cl_2, and 2.79 ml (20.2 mmol) of phenoxyacetyl chloride was added. This soln. was cooled to 0°, and the pyridine soln. containing TMS-**11a** was added. The mixture was stirred overnight at ambient temp., then cooled to 0°, and 20 ml H_2O was added, and the mixture was stirred for 20 min. The solvent was evaporated, the residue co-evaporated with 2 × 20 ml of H_2O under reduced pressure, and 20 ml of H_2O was added to the residue. The resulting crystals were filtered, washed with 2 × 40 ml of i-PrOH, 3 × 100 ml of Et_2O, and dried under high vacuum (0.03 Torr/r.t.) overnight to afford 5.8 g (85.1%) of **12c**. TLC (CH_2Cl_2/MeOH 3:1) : R_f 0.75. ^{1}H-NMR (600 MHz, (D_6)DMSO): 3.61 (*dd*, *J* = 3.2, 9.2, H–C(3′)); 3.79–3.82 (*m*, H–C(4′), H–C(5′)); 3.84 (*dd*, *J* = 1.8, 12.5, H–C(5′)); 4.27 (*dd*, *J* = 9.2, 9.2, H–C(2′)); 5.05 (*s*, ArOCH_2); 5.47 (*d*, *J* = 9.1, H–C(1′)); 6.97 (*m*, 3 arom. H); 7.31 (*m*, 2 arom. H); 8.73 (*s*, H–C(8)); 8.82 (*s*, H–C(2)); 10.99 (br. *s*, NHCO). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 68.00 (CH_2O); 69.36 (C(4′)); 69.65 (C(2′)); 70.30 (C(5′)); 74.24 (C(3′)); 84.41 (C(1′)); 115.39 (arom. C); 121.94 (arom. C); 123.58 (C(5)); 130.36 (arom. C); 143.85 (C(8)); 149.61 (C(4)); 152.59 (arom. H); 153.07 (C(2)); 158.64 (C(6)); 168.34 (CO of PhO). ESI-MS (pos): 424 (4, $[M+Na]^+$), 402 (93, $[M+H]^+$), 270 (100, $[G^{PAC}]^+$). ESI-MS (neg.): 436 (100, $[M+Cl]^-$), 400 (59, $[M-H]^-$), 174 (20).

*N^4-Isobutyryl-1-(2′,3′,4′-tri-*O*-benzoyl-α-*L*-arabinopyranosyl)cytosine* (**11c**). A suspension of 20 g (35.3 mmol) of **1** and 5.8 g (31.8 mmol) of 4-*N*-Isobutyrylcytosine in 200 ml dry MeCN was warmed to 60° (oil bath). Addition of 19.6 ml (79.4 mmol) BSA resulted in a clear soln. After 30 min, 11.1 ml (95.3 mmol) of $SnCl_4$ was added dropwise (→ exothermic reaction), and stirring was continued for another 60 min. The mixture was cooled to r.t. and poured into a mixture of cold sat. aq. $NaHCO_3$ soln./AcOEt 1:1, (*v*/*v*) with stirring. The aq. phase was extracted with 3 × 150 ml AcOEt, and washed successively with sat. aq. Na_2CO_3 (2×), H_2O and sat. aq. NaCl soln. The org. phase was dried ($MgSO_4$) and evaporated. The resulting oil was purified by CC (silica gel; hexane/AcOEt 1:2). The product fractions were combined, evaporated (rotavap), and dried under high vacuum (*ca.* 0.5 Torr/r.t.) to furnish 18.3 g (83%) of **11c**. Colorless amorphous solid. TLC (hexane/AcOEt 1:2): R_f 0.35. ^{1}H-NMR (600 MHz, $CDCl_3$): 1.17 (2*d*, *J* = 6.6, *Me*$_2$CH); 2.84 (*m*, H–C(9)); 4.22 (*d*, *J* = 13.2, H–C(5′)); 4.43 (*dd*, *J* = 13.8, 1.20, H–C(5′)); 5.83–5.86 (*m*, H–C(3′), H–C(4′)); 5.98 (*t*, *J* = 9.6, H–C(2′)); 6.45 (*d*, *J* = 9.6, H–C(1′)); 7.27–7.48 (*m*, 6 arom. H); 7.55–7.59 (*m*, H–C(5), 2 arom. H); 7.63–7.94 (*m*, 6 arom.); 7.97 (*d*, *J* = 7.8, H–C(6)); 8.11–8.13 (*m*, 4 arom. H). ^{13}C-NMR (150.9 MHz, $CDCl_3$): 19.29 (*Me*$_2$CH); 36.92 (Me$_2$*C*HCO); 67.96 (C(5′); 69.43, 69.63, 72.08 (C(4′), C(3′), C(2′)); 82.39 (C(1′)); 98.16 (C(5)); 128.58; 128.68; 128.79; 128.90; 129.04; 129.18; 129.69; 130.14; 130.17; 130.38; 130.50; 133.65; 133.68; 134.13; 145.07 (C(6)); 154.95 (C(2)); 163.78 (C(4)); 165.70, 165.94, 166.07, 171.63 (4 CO of Bz), 178.33 (C(8)). MALDI-FTMS (DHB): 648.1976 ($[M+Na]^+$, calc. 648.1958).

*1-(α-*L*-Arabinopyranosyl)-*N^4*-isobutyrylcytosine* (**12c**). A soln. of 18 g (28.8 mmol) of **11c** in 300 ml 2.0M NH_3 in MeOH was stirred for 6 h at r.t. The clear soln. was concentrated *in vacuo*, and the residual gum was dissolved in H_2O (300 ml) and Et_2O (100 ml). The resulting aq. phase was lyophilized, and the resulting material was dissolved in 200 ml of dry DMF, and 8 ml (48 mmol) isobutyric anhydride was added dropwise under Ar. The mixture was stirred at r.t. for 15 h and concentrated *in vacuo*. The resulting residue was purified by CC (silica gel; CH_2Cl_2/MeOH 6:1) to give 6.4 g (70%) of **12c**. TLC (CH_2Cl_2/MeOH 6:1): R_f 0.22. ^{1}H-NMR (600 MHz, (D_6)DMSO): ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.05 (2*d*, *J* = 6.6, *Me*$_2$CH); 2.74 (*m*, Me$_2$*C*HCO); 3.50 (*dd*, *J* = 9.0; 2.4, H–C(2′)); 3.63 (*d*, *J* = 11.4, H–C(5′)); 3.70–3.73 (*m*, H–C(3′), H–C(4′)); 3.97 (*dd*, *J* = 12.0, 1.20, H–C(5′)); 4.74 (*d*, *J* = 4.2, HO–C(4′)); 4.97 (*s*, H–C(3′)); 5.21 (*d*, *J* = 4.8, HO–C(2′)); 5.44 (*d*, *J* = 9.0, H–C(1′)); 7.27 (*d*, *J* = 7.2, H–C(5)); 8.03 (*d*, *J* = 7.2, H–C(6)); 10.86 (*s*, H–N(7)). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 19.71, 19.89 (Me$_2$CH); 35.73 (Me$_2$CHCO); 69.35, 69.86, 70.41 (C(4′), C(2′), C(5′)); 74.19 (C(3′)); 84.95 (C(1′)); 96.86 (C(5)); 146.98 (C(6)); 155.17 (C(2)); 163.36 (C(4)); 178.70 (C(8), C=O). ESI-MS (pos.): 336 (100, $[M+Na]^+$), 314 (65, $[M+H]^+$).

*9-(α-*L*-Arabinopyranosyl)-*N^2*-(phenoxyacetyl)guanine* (**12d**). To a soln. of 8.4 g (29.6 mmol) of **11d** (co-evaporated with 2 × 100 ml pyridine) in 100 ml dry pyridine, under Ar was added 26.2 ml (0.21 mol) of TMSCl, and the mixture was stirred at r.t. for 30 min. In a separate flask under Ar, 5.2 g (38.4 mmol) of 1-HOBT (co-

evaporated with 2 × 40 ml pyridine) was dissolved in 3.1 ml (38.4 mmol) pyridine and 20 ml dry CH_2Cl_2, and 5.3 ml (38.4 mmol) of phenoxyacetyl chloride was added. This soln. was cooled to 0°, and the pyridine soln. containing TMS-**11d** was added. The mixture was stirred overnight at r.t., cooled to 0°, 20 ml H_2O was added, and the mixture was stirred for 20 min. The solvent was evaporated, the residue was co-evaporated with 2 × 20 ml of H_2O under reduced pressure, and 40 ml of H_2O was added to the residue. The resulting white crystals were filtered, washed with 2 × 40 ml of i-PrOH, 3 × 100 ml of Et_2O, and dried under high vacuum (0.03 Torr/r.t.) overnight to afford 10.6 g (85.9%) of **12d**. TLC (CH_2Cl_2/MeOH 3:1): R_f 0.8. ^{1}H-NMR (600 MHz, (D_6)DMSO): 3.53 (*dd*, *J* = 3.1, 9.3, H–C(3′)); 3.66 (*d*, *J* = 12.3, H–C(5′)); 3.76 (br. *s*, H–C(4′)); 3.84 (*dd*, *J* = 1.7, 12.3, H–C(5′)); 4.10 (*dd*, *J* = 9.3, H–C(2′)); 4.86 (*s*, PhOCH_2); 5.14 (*d*, *J* = 9.3, H–C(1′)); 6.97 (*m*, 3 arom. H); 7.31 (*m*, 2 arom. H); 8.14 (*s*, H–C(8)); 11.80 (*s*, NH(1)); 11.89 (*s*, NHCOCH_2). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 67.00 (CH_2O); 69.27 (C(4′)); 69.68 (C(2′)); 70.38 (C(5′)); 74.32 (C(3′)); 84.22 (C(1′)); 115.38 (arom. C); 120.81 (C(5)); 122.17 (arom. C); 130.41 (arom. C); 138.83 (C(8)); 148.15 (arom. C); 149.75 (C(4)); 155.76 (C(2)); 158.44 (C(6)); 171.88 (CO of PhO). ESI-MS (pos.): 440 (100, $[M+Na]^+$), 418 (54, $[M+H]^+$), 308 (51), 286 (80). ESI-MS (neg.): 452 (8, $[M+Cl]^-$), 416 (100, $[M-H]^-$).

*9-[2′-*O*-[(4″,4‴-Dimethoxytriphenyl)methyl]-α-*L*-arabinopyranosyl]-*N^2*-(phenoxyacetyl)adenine* (**13a**). To a soln. of 6.5 g (16.2 mmol) of **12a** in 140 ml dry pyridine/DMF 1:1, cooled to 0°, was added 3.2 ml (24.3 mmol) of *N*,*N*-dimethylformamide dimethyl acetal slowly, and the soln. was stirred for 1 h. The solvent was evaporated under high vacuum at r.t., and to the resulting residue was added a soln. of 6.6 g (19.4 mmol) of DMT-Cl in 50 ml dry CH_2Cl_2 with stirring, followed by 6.4 ml (48.6 mmol) of collidine. After consumption of the starting material (TLC, *ca.* 6 h), 10 ml MeOH was added, and the soln. was stirred for another 30 min. The mixture was diluted with 300 ml of CH_2Cl_2, and the soln. was washed sequentially with sat. aq. $NaHCO_3$ soln., 10% aq. citric acid soln., sat. aq. $NaHCO_3$ soln., and sat. aq. NaCl soln. The org. phase was evaporated to yield a yellow foam, which was purified by CC (silica gel; washed with AcOEt until yellow color is removed and then eluted with 20:1 CH_2Cl_2/i-PrOH) to afford 8.1 g (71%) of **13a**. TLC (CH_2Cl_2/MeOH 7:1): R_f 0.7. ^{1}H-NMR (600 MHz, (D_6)DMSO): 3.62–3.70 (*m*, H–C(5′)); 3.63, 3.67 (2*s*, MeO); 3.88 (*m*, H–C(4′)); 4.00 (*m*, H–C(3′)); 4.09 (*dd*, *J* = 5.9, H–C(2′)); 4.49 (*d*, *J* = 5.3, HO–C(3′)); 4.80 (*d*, *J* = 6.0, HO–C(4′)); 5.00 (*s*, PhOCH_2); 5.64 (*d*, *J* = 5.4, H–C(1′)); 6.61–7.31 (*m*, 18 arom. H); 8.28 (*s*, H–C(8)); 8.62 (*s*, H–C(2)); 10.86 (br. *s*, NHCO). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 55.71 (MeO); 55.75 (MeO); 65.81 (C(5′)); 67.51 (C(4′)); 67.98 (CH_2O); 72.34 (C(3′)); 73.84 (C(2′)); 82.35 (C(1′)); 113.65; 113.50; 115.40; 121.92; 123.34; 127.26; 128.23; 128.42; 130.34; 130.91; 131.18; 136.38; 136.55; 143.83 (C(8)); 146.89; 149.52, 152.31 (C(2)); 152.66; 158.66; 158.82; 158.91; 168.11 (CO of PhO). ESI-MS (pos.): 726 (16, $[M+Na]^+$), 704 (100, $[M+H]^+$), 424 (54). ESI-MS (neg.): 738 (6, $[M+Cl]^-$), 702 (40, $[M-H]^-$), 552 (98), 436 (100).

*1-[2′-*O*-[(4″,4‴-Dimethoxytriphenyl)methyl]-α-*L*-arabinopyranosyl]thymine* (**13b**). The reaction was performed as described for **13c** in 200 ml of dry DMF in the presence of molecular sieves (4 Å) with 10 g (38.8 mmol) of **3b**, 20 ml of collidine, 7.7 ml (58.1 mmol) of *N*,*N*-dimethylformamide dimethyl acetal, 100 ml of CH_2Cl_2, 20 ml of collidine, and 20 g (58.1 mmol) of DMT-Cl at r.t. Workup as described for **13c** and purification by CC (silica gel; hexane/AcOEt 1:4) gave 16.2 g (72%) of **13b**. TLC (hexane/AcOEt 1:4): R_f 0.45. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.57 (*s*, Me–C(5)); 3.52 (*t*, *J* = 9.0, H–C(2′); 3.58 (*d*, *J* = 6.6, H–C(5′)); 3.60 (*m*, H–C(4′)); 3.68 (*m*, H–C(5′), HO–C(4′)); 3.72, 3.73 (2*s*, 2 MeO); 3.86 (*m*, H–C(3′)); 4.60 (*d*, *J* = 4.8, HO–C(3′)); 5.72 (*d*, *J* = 9.0, H–C(1′)); 6.50 (*s*, H–C(6)); 6.77–6.83 (*m*, 4 arom. H); 7.13–7.20 (*m*, 7 arom. H); 7.31–7.33 (*m*, 2 arom. H); 11.38 (*s*, H–N(3)). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 12.97 (*Me*–C(5)); 55.71 (MeO); 69.24 (C(5′)); 69.32, 72.71, 74.11 (C(2′), C(3′), C(4′)); 82.69 (C(1′)); 87.92 (Ar_3C); 109 (C(5)); 113.35; 127.01; 128.04; 128.65; 129.76; 131.28; 131.57; 137.05 (C(6)); 137.05; 137.28; 147.61; 152.09 (C(2)); 158.66; 158.73; 164.18 (C(4)). ESI-MS (pos.): 583 (92, $[M+Na]^+$), 303 (100, $[DMT]^+$).

*1-[2′-*O*-[(4″,4‴-Dimethoxytriphenyl)methyl]-α-*l*-arabinopyranosyl]-*N^4*-isobutyrylcytosine* (**13c**). To the soln. of 10.5 g (33.5 mmol) of **12c** in 200 ml of dry DMF were added molecular sieves (4 Å), 20 ml of collidine, and 6.7 ml (50.3 mmol) of *N*,*N*-dimethylformamide dimethyl acetal at r.t. The soln. was stirred for 3 h and then concentrated to dryness. The residue was dissolved in 100 ml of CH_2Cl_2, and 20 ml of collidine was added, followed by 17 g (50.3 mmol) of DMT-Cl. The mixture was stirred overnight, quenched with MeOH, and filtered through *Celite*. The filtrate was washed successively with 10% aq. citric acid, sat. aq. $NaHCO_3$ soln. and sat. aq. NaCl soln., and dried (Na_2SO_4) and concentrated to dryness. The residue was purified by CC (silica gel; hexane/EtOAc 1:4) to give 15 g (72%) of **13c**. TLC (hexane/EtOAc 1:4): R_f 0.4. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.04 (2*d*, *J* = 6.6, *Me*$_2$CH), 2.70 (*m*, Me$_2$C*H*CO); 3.56 (*d*, *J* = 6.4, H–C(5′)); 3.61 (*t*, *J* = 9.0, H–C(2′); 3.64 (br. *s*, H–C(4′)); 3.70–3.72 (*m*, 2 MeO, H–C(5′), HO–C(3′)); 3.82 (*m*, H–C(3′)); 4.65 (*d*, *J* = 5.4, HO–C(4′)); 5.90 (*d*, *J* = 8.4, H–C(1′)): 6.71–6.78 (*m*, 4 arom. H); 6.83 (*d*, *J* = 9.0, 1 arom. H); 6.93 (*d*, *J* = 7.8, H–C(5)); 7.04–7.36 (*m*, H–C(6), 8 arom. H); 10.87 (*s*, H–N(7)). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 19.65, 19.88 (*Me*$_2$CH);

35.71 (Me_2CHCO); 55.74, 55.82 (MeO); 69.01, 69.25, 73.41, 73.89 (C(5′), C(4′), C(2′), (C(3′)); 83.90 (C(1′)); 87.96 (Ar_3C); 96.76 (C(5)); 113.49; 113.59; 126.99; 127.27; 128.13; 128.25; 128.49; 128.71; 129.76; 131.25; 131.51; 136.88; 137.12; 141.07; 146.08 (C(6)); 147.38; 149.20; 155.72 (C(2)); 158.64; 158.73; 163.18 (C(4)); 178.60 (C(8), C=O). ESI-MS (pos.): 638 (100, $[M+Na]^+$), 303 (30, $[DMT]^+$).

*9-{2′-*O*-[(4″,4‴-Dimethoxytriphenyl)methyl]-α-l-arabinopyranosyl}-*N^2*-(phenoxyacetyl)guanine* (**13d**). To a soln. of 5.8 g (13.9 mmol) of **12d** in 140 ml dry pyridine/DMF 1:1, cooled to 0°, was added slowly 2.8 ml (20.9 mmol) of *N,N*-dimethylformamide dimethyl acetal, and the soln. was stirred for 1 h. The solvent was evaporated under high vacuum at r.t., and to the resulting residue was added a soln. of 5.7 g (16.7 mmol) of DMT-Cl in 50 ml dry CH_2Cl_2 with stirring, followed by 5.5 ml (41.7 mmol) of collidine. After consumption of the starting material (TLC, *ca.* 6 h), 10 ml MeOH was added, and the soln. was stirred for another 30 min. The mixture was diluted with 300 ml of CH_2Cl_2, and the soln. was washed sequentially with sat. aq. $NaHCO_3$ soln., 10% aq. citric acid soln., sat. aq. $NaHCO_3$ soln., and sat. aq. NaCl soln. The org. phase was evaporated to yield a yellow foam, which was purified by CC (silica gel; washed with AcOEt containing 1% Et_3N, until yellow color is removed and then eluted with CH_2Cl_2/i-PrOH containing 1% Et_3N 20:1 to afford 18.3 g (61%) of **13d**. TLC (CH_2Cl_2/i-PrOH 10:1): R_f 0.5. ^{1}H-NMR (600 MHz, (D_6)DMSO): 3.57–3.69 (*m*, 2 H–C(5′)); 3.64, 3.68 (2*s*, MeO); 3.84 (br. *s*, H–C(4′)); 3.90 (*m*, H–C(2′)); 3.96 (br. *s*, H–C(3′)); 4.52 (br. *s*, HO–C(3′)); 4.80 (*d*, $J=4.9$, HO–C(4′)); 4.87 (*d*, $J=15.8$, 1 H, $PhOCH_2$); 4.93 (*d*, $J=15.9$, 1 H, $PhOCH_2$); 5.42 (*d*, $J=5.3$, H–C(1′)); 6.68–7.33 (*m*, 18 arom. H); 7.76 (*s*, H–C(8)); 11.90 (br. *s*, 2 NH). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 55.71 (MeO); 55.77 (MeO); 65.94 (C(5′)); 67.29 ($ArOCH_2$); 67.60 (C(4′)); 72.52 (C(3′)); 74.17 (C(2′)); 82.15 (C(1′)); 113.19; 113.49; 113.65; 115.36; 120.46; 122.15; 127.31; 128.23; 130.41; 131.01; 131.29; 136.35; 139.24 (C(8)); 146.90; 148.33; 149.60; 155.87; 158.52; 158.64; 158.87; 158.96; 172.16 (CO of PhO). ESI-MS (pos.): 821 (100, $[M+Et_3NH]^+$), 742 (16, $[M+Na]^+$), 720 (40, $[M+H]^+$), 435 (44). ESI-MS (neg.): 754 (10, $[M+Cl]^-$), 718 (100, $[M-H]^-$), 584 (20).

*9-{4′-*O*-Benzoyl-2′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-α-*L*-arabinopyranosyl}-*N^2*-(phenoxyacetyl)adenine* (**14a**). To a soln. of 1.8 g (2.6 mmol) **13a** in 10 ml of dry CH_2Cl_2 and 10 ml of dry pyridine, cooled to −40° (acetone/dry ice bath), was added 330 µl (2.8 mmol) of BzCl slowly through a syringe over 60 min. The soln. was allowed to warm to −10° and quenched with 10 ml of sat. aq. $NaHCO_3$ soln., and 100 ml of CH_2Cl_2 were added. The org. layer was washed with sat. aq. $NaHCO_3$ soln. and dried (Na_2SO_4). The solvent was evaporated, and the residue was purified by CC (silica gel; eluted with AcOEt/hexane 2:1) to yield 1.9 g (89%) of **14a**. White foam. TLC: (CH_2Cl_2/i-PrOH 10:1): R_f 0.85. ^{1}H-NMR (600 MHz, (D_6)DMSO): 3.68 (*s*, MeO); 3.70 (*s*, MeO); 3.96 (*d*, $J=12.1$, H–C(5′)); 4.02 (*d*, $J=12.8$, H–C(5′)); 4.30 (*m*, H–C(3′)); 4.44 (*m*, H–C(2′)); 4.58 (br. *s*, HO–C(3′)); 5.01 (*s*, $PhOCH_2$); 5.23 (*s*, H–C(4′)); 5.98 (*d*, $J=7.8$, H–C(1′); 6.53–8.06 (*m*, 23 arom. H); 8.32 (*s*, H–C(8)); 8.45 (*s*, H–C(2)); 10.84 (br. *s*, NHCO). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 55.71 (MeO); 55.72 (MeO); 65.62 (C(5′)); 68.02 (CH_2O); 71.92 (C(3′)); 72.72 (C(2′)); 72.87 (C(4′)); 84.61 (C(1′)); 113.12; 113.22; 115.39; 121.92; 123.93; 126.90; 127.96; 128.31; 136.61; 144.27 (C(8)); 147.43; 149.45; 152.00 (C(2)); 152.33; 158.66; 158.74; 165.97 (CO of Bz); 168.13 (CO of PhO). ESI-MS (pos.): 846 (5, $[M+K]^+$), 830 (15, $[M+Na]^+$), 808 (85, $[M+H]^+$), 581 (100). ESI-MS (neg.): 806 (35, $[M-H]^-$), 656 (100).

*1-{4′-*O*-Benzoyl-2′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-α-*L*-arabinopyranosyl}thymine* (**14b**).The reaction was performed as described for **14c** with 18 g (31.1 mmol) of **13b** in 100 ml of dry pyridine, 100 ml of CH_2Cl_2 and 5.2 ml (45 mmol) of BzCl at −10° for 3 h. Workup as described for **14c** with 200 ml of CH_2Cl_2 and 200 ml of sat. aq. $NaHCO_3$ soln., followed by CC (silica gel; hexane/AcOEt 1:3), gave 16 g (75%) of **14b** and 3.1 g (16%) of **13b**.

Data of **14b**. TLC (hexane/AcOEt 1:3): R_f 0.42. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.57 (*s*, Me–C(5)); 3.69 (*dd*, $J=9.0$, H–C(2′)); 3.73 (*s*, 2 MeO); 3.85 (*d*, $J=12.6$, H–C(5′)); 3.96 (*d*, $J=13.2$, H–C(5′)); 4.23 (*m*, H–C(3′)); 4.56 (*d*, $J=4.2$, HO–C(3′)); 5.05 (*s*, H–C(4′)); 5.93 (*d*, $J=9.0$, H–C(1′)); 6.24 (*s*, H–C(6)); 6.77–6.81 (*m*, 4 arom. H); 7.15–7.21 (*m*, 7 arom. H); 7.34–7.35 (*m*, 2 arom. H); 7.52–7.54 (*m*, 2 arom. H); 7.71–7.75 (*m*, 3 arom. H); 11.40 (*s*, H–N(3)). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 13.27 (Me–C(5)); 55.51 (MeO); 66.05 (C(5′)); 72.05, 72.38, 73.82 (C(2′), C(3′), C(4′)); 82.40 (C(1′)); 88.35 (Ar_3C); 110.20 (C(5)); 113.32; 127.04; 128.05; 129.45; 130.00; 130.45; 131.35; 131.65; 134.39; 135.99 (C(6)); 151.73 (C(2)); 158.69; 158.83; 164.05 (C(4)); 165.68 (C=O). ESI-MS (pos.): 687 (26, $[M+Na]^+$), 303 (100). ESI-MS (neg.): 663 (100, $[M-H]^-$).

*1-{4′-*O*-Benzoyl-2′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-α-*L*-arabinopyranosyl}-*N^4*-isobutyrylcytosine* **(14c)**. To the soln. of 7 g (11.4 mmol) of **13c** in 50 ml of dry pyridine and 50 ml of CH_2Cl_2 was added dropwise 1.96 ml (17 mmol) of BzCl at −10°. The soln. was stirred for 3 h, diluted with 100 ml of CH_2Cl_2, and washed with 100 ml of sat. aq. $NaHCO_3$ soln. The org. phase was dried (Na_2SO_4), filtered, evaporated, and purified by CC (silica gel; hexane/AcOEt 1:3) to give 6.4 g (78%) of **14c** and 0.9 g (15%) of **13c**.

Data of **14c**. TLC (hexane/AcOEt 1:3): R_f 0.45. ^{1}H-NMR (600 MHz, (D_6)DMSO): 1.04 (2*d*, $J=6.6$, Me_2CH); 2.70 (*m*, Me_2CHCO); 3.71 (*s*, 2 MeO); 3.79 (*m*, H–C(2′)); 3.90–3.99 (*m*, 2 H–C(5′)); 4.24 (br. *s*,

H–C(3′)); 4.48 (br. *s*, HO–C(3′)); 5.12 (br. *s*, H–C(4′)); 6.12 (*d*, *J* = 8.4, H–C(1′)); 6.73–6.75 (*m*, H–C(5), H–C(6), 18 arom. H); 10.89 (*s*, H–N(7)). ^{13}C-NMR (150.9 MHz, (D$_6$)DMSO): 19.62, 19.83 (*Me*$_2$CH); 35.75 (Me$_2$C*H*CO); 55.76 (MeO); 66.74, 72.19, 72.95, 73.66 (C(2′), C(4′), C(5′), (C(3′)); 83.65 (C(1′)); 88.40 (Ar$_3$C); 97.24 (C(5)); 113.57; 126.12; 126.99; 128.11; 128.25; 128.49; 128.76; 129.02; 129.44; 129.71; 130.16; 130.40; 130.78; 131.32; 131.63; 134.24; 145.32 (C(6)); 155.82 (C(2)); 158.71; 158.81; 163.31 (C(4)); 165.89 (CO of Bz); 178.67 (C(8)). ESI-MS (pos.): 742 (100, [*M* + Na]$^+$), 303 (25, [DMT]$^+$).

*9-{4′-*O*-Benzoyl-2′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-α-*L*-arabinopyranosyl}-*N^2*-(phenoxyacetyl)guanine* (**13d**). To 100 ml of dry CH_2Cl_2, 14.4 g (20.0 mmol) of **13d**, 244 mg (2 mmol) 4-DMAP, 9.7 ml (120 mmol) dry pyridine, and 4.98 g (22.0 mmol) BzOBz were added sequentially. The soln. was stirred for 15 h at r.t. and quenched with 5 ml i-PrOH for 30 min. The mixture was washed with sat. aq. $NaHCO_3$ soln. and sat. aq. NaCl soln., and the org. phase was dried (Na_2SO_4). The solvent was evaporated to yield a yellow foam, which was purified by CC (silica gel; washed with AcOEt containing 1% Et_3N, until the yellow color is removed and then eluted with CH_2Cl_2/i-PrOH containing 1% Et_3N 30:1) to afford 13.3 g (80.7%) of **13d**. TLC (CH_2Cl_2/i-PrOH 20:1): R_f 0.65. ^{1}H-NMR (600 MHz, (D$_6$)DMSO): 3.66, 3.70 (2*s*, MeO); 3.89 (*dd*, *J* = 8.0, H–C(2′)); 3.92–3.99 (*m*, 2 H–C(5′)); 4.29 (*m*, H–C(3′)); 4.91 (br. *s*, HO–C(3′); 4.92 (*d*, *J* = 15.7, 1 H, PhOC*H*$_2$); 4.99 (*d*, *J* = 15.8, 1 H, PhOC*H*$_2$); 5.21 (br. *s*, H–C(4′)); 5.63 (*d*, *J* = 7.7, H–C(1′)); 6.67–7.80 (*m*, 24 arom. H, H–C(8)); 11.85 (br. *s*, NH); 11.96 (br. *s*, NH). ^{13}C-NMR (150.9 MHz, (D$_6$)DMSO): 55.72 (MeO); 55.79 (MeO); 65.78 (C(5′)); 68.09 (PhOCH$_2$), 71.83 (C(3′)); 73.11 (C(4′)); 74.13 (C(2′)); 82.27 (C(1′)); 113.16; 113.34; 115.34; 120.09; 121.86; 127.09; 127.93; 128.61; 129.45; 130.18; 130.33; 130.42; 131.14; 131.49; 134.29; 137.12; 149.86; 150.39; 151.08; 156.55; 158.79; 158.90; 165.88 (CO of Bz); 173.32 (CO of PhO). ESI-MS (pos.): 926 (100, [*M* + Et$_3$NH]$^+$), 824 (18, [*M* + H]$^+$), 303 (100, [DMT]$^+$). ESI-MS (neg.): 822 (100, [*M* – H]$^-$).

*9-{3′-*O*-Benzoyl-2′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-α-*L*-arabinopyranosyl}-*N^2*-(phenoxyacetyl)adenine* (**15a**). To a soln. of 3.8 g (4.7 mmol) **14a** in 50 ml of dry CH_2Cl_2 was added 4.5 ml (32.6 mmol) of dry Et_3N, and the mixture was stirred for 48 h at r.t. The solvent was evaporated to yield a white foam, which was purified by CC (silica gel; AcOEt/hexane 3:1) to afford 1.5 g (39%) of **15a** and 1.6 g (39%) of starting material.

Data of **15a**: TLC (CH_2Cl_2/i-PrOH 10:1) : R_f 0.7. ^{1}H-NMR (600 MHz, (D$_6$)DMSO): 3.55 (*s*, MeO); 3.65 (*s*, MeO); 3.84 (*dd*, *J* = 12.0, 2.1, H–C(5′)); 4.04–4.08 (*m*, H–C(4′), H–C(5′)); 4.51 (*dd*, *J* = 8.2, H–C(2′)); 5.01 (*s*, PhOCH$_2$); 5.09 (*d*, *J* = 6.8, HO–C(4′); 5.51 (*dd*, *J* = 8.9, 3.7, H–C(3′)); 6.11 (*d*, *J* = 8.2, H–C(1′)); 6.48–8.59 (*m*, 23 arom. H); 8.03 (*s*, H–C(8)); 8.63 (*s*, H–C(2)); 10.87 (br. *s*, NHCO). ^{13}C-NMR (150.9 MHz, (D$_6$)DMSO): 55.51 (MeO); 55.69 (MeO); 66.25 (C(4′)); 67.99 (CH$_2$O); 69.09 C(5′); 70.81 (C(2′)); 77.26 (C(3′)); 83.86 (C(1′)); 113.40; 115.38; 121.92; 123.61; 127.03; 127.96; 128.69; 129.79; 130.23, 130.34; 130.51; 131.01; 133.46; 143.48 (C(8)); 146.72; 149.55; 152.36 (C(2)); 152.41; 158.56; 158.66; 158.80; 165.94 (CO of Bz); 168.12 (CO of Ph). ESI-MS (pos.): 846 (60, [*M* + K]$^+$), 830 (93, [*M* + Na]$^+$), 808 (100, [*M* + H]$^+$). ESI-MS (neg.): 842 (11, [*M* + Cl]$^-$); 806 (100, [*M* – H]$^-$).

*1-{3′-*O*-Benzoyl-2′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-α-*L*-arabinopyranosyl}thymine* (**15b**). To the soln. of 5 g (7.5 mmol) of **14b** in 100 ml of dry CH_2Cl_2 was added 10.5 ml (75 mmol) of Et_3N at r.t. The soln. was stirred for 12 h and then concentrated. The residue was purified by CC (silica gel; hexane/AcOEt 1:3) to give 2.25 g (45%) of **15b** and 2.15 g (43%) of **14b**.

Data of **15b**. TLC (hexane/AcOEt 1:3): R_f 0.40. ^{1}H-NMR (600 MHz, (D$_6$)DMSO): 1.57 (*s*, Me–C(5)); 3.57, 3.68 (2*s*, MeO); 3.73 (*d*, *J* = 10.8, H–C(5′)); 3.92–3.94 (*m*, H–C(5′), H–C(4′)); 4.06 (*t*, *J* = 9.0, H–C(2′)); 5.12 (*d*, *J* = 7.2, HO–C(4′)); 5.38 (*dd*, *J* = 9.0, 3.6, H–C(3′)); 5.96 (*d*, *J* = 9.0, H–C(1′)); 6.50 (*s*, H–C(6)); 6.57–6.58 (*m*, 1 arom. H); 6.77–6.78 (*m*, 2 arom. H); 6.97–7.19 (*m*, 9 arom. H); 7.39–7.41 (*m*, 3 arom. H); 7.54–7.59 (*m*, 3 arom. H); 11.33 (*s*, H–N(3)). ^{13}C-NMR (150.9 MHz, (D$_6$)DMSO): 13.04 (Me–C(5)); 55.51 (MeO); 55.71 (MeO); 66.29 (C(5′)); 69.12, 69.86 (C(2′), C(4′)); 77.55 (C(3′)); 82.79 (C(1′)); 88.12 (Ar$_3$C); 110.20 (C(5)); 113.40; 113.47; 127.11; 128.07; 128.38; 128.64; 129.82; 130.58; 131.40; 133.42; 136.46 (C(6)); 151.63 (C(2)); 158.58; 158.85; 164.17 (C(4)); 165.90 (C=O). ESI-MS (pos.): 687(30, [*M* + Na]$^+$), 303 (100)

*1-{3′-*O*-Benzoyl-2′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-α-l-arabinopyranosyl}-*N^4*-isobutyrylcytosine* **(15c)**. To the soln. of 4.0 g (5.5 mmol) of **14c** in 100 ml of dry CH_2Cl_2 was added 7.7 ml (55.5 mmol) of Et_3N at r.t. The soln. was stirred for 12 h and then concentrated. The residue was purified by CC (silica gel; hexane/AcOEt 1:3) to give 1.9 g (48%) of **15c** and 1.83 g (46%) of **14c**.

Data of **15c**. TLC (hexane/AcOEt 1:3): R_f 0.43. ^{1}H-NMR (600 MHz, (D$_6$)DMSO): 1.04 (2*d*, *J* = 6.6, *Me*$_2$CH); 2.69 (*m*, Me$_2$C*H*CO); 3.55, 3.67 (2*s*, 2 MeO); 3.77 (*dd*, *J* = 12.0, 1.8, H–C(5′)); 3.94–3.99 (*m*, H–C(4′), H–C(5′)); 4.10 (*t*, *J* = 9.0, H–C(2′)); 5.15 (*d*, *J* = 6.6, HO–C(4′)); 5.38 (*dd*, *J* = 9.0, 3.6, H–C(3′)); 6.15 (*d*, *J* = 9.0, H–C(1′)); 6.53 (*d*, *J* = 8.4, 2 arom. H); 6.67 (*d*, *J* = 7.8, 2 arom. H); 6.90 (*d*, *J* = 7.2, H–C(5)); 6.95–7.59 (*m*, H–C(6′), 14 arom. H); 10.88 (*s*, H–N(7)). ^{13}C-NMR (150.9 MHz, (D$_6$)DMSO): 19.64, 19.92 (*Me*$_2$CH); 35.74 (Me$_2$C*H*CO); 55.46, 55.66 (MeO); 66.22, 69.37, 70.57 (C(2′), C(4′), C(5′)); 77.50 (C(3′)); 83.91

(C(1′)); 88.16 (Ar_3C); 97.26 (C(5′)); 113.51; 113.66; 126.15; 127.05; 128.13; 128.39; 128.63; 129.04; 129.74; 129.82; 130.41; 130.55; 131.39; 133.41; 137.02; 145.68 (C(6)); 155.46 (C(2)); 158.52; 158.82; 163.16 (C(4)); 165.87 (CO of Bz); 178.69 (C(8)). ESI-MS (pos.): 742 (32, $[M+Na]^+$), 454 (37), 303 (100).

*9-{3′-*O*-Benzoyl-2′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-α-*L*-arabinopyranosyl}-N²-(phenoxyacetyl)guanine* (**15d**). To a soln. of 6.8 g (8.3 mmol) **14d** in 100 ml of dry CH_2Cl_2 was added 8.1 ml (57.7 mmol) of dry Et_3N, and the mixture was stirred for 48 h at r.t. The solvent was evaporated to yield a white foam, which was purified by CC (silica gel; eluted with 40:1 CH_2Cl_2/i-PrOH containing 1% Et_3N) to afford 2.8 g (41%) of **15d** (contains *ca.* 14.5% of **14d**, by ^{1}H-NMR). Also, 2.9 g (43%) of starting material **14d** was recovered.

Data of **15d**. TLC (CH_2Cl_2/i-PrOH 20:1): R_f 0.50. ^{1}H-NMR (600 MHz, (D_6)DMSO): 3.56, 3.65 (2*s*, MeO); 3.85 (*d*, *J* = 10.5, H–C(5′)); 3.97 (*d*, *J* = 11.5, H–C(5′)); 4.00 (*m*, H–C(4′)); 4.29 (*m*, H–C(2′)); 4.95 (*d*, *J* = 16.0, 1 H, PhOCH_2); 5.01 (*d*, *J* = 16.0, 1 H, PhOCH_2); 5.03 (br. *s*, HO–C(4′)); 5.40 (*dd*, *J* = 9.2, 3.4, H–C(3′)); 5.79 (*d*, *J* = 8.5, H–C(1′)); 6.53–7.60 (*m*, 24 arom. H, H–C(8)); 11.81 (br. *s*, 2 NH). ^{13}C-NMR (150.9 MHz, (D_6)DMSO): 55.54 (MeO); 55.71 (MeO); 66.45 (C(4′)); 67.50 ($PhOCH_2$); 69.72 (C(5′)); 71.11 (C(2′)); 77.53 (C(3′)); 83.62 (C(1′)); 113.34; 113.61; 115.23; 115.41; 120.71; 122.12; 127.12; 127.91; 128.12; 128.68; 129.85; 130.40; 130.51; 130.74; 131.25, 133.52; 136.89; 138.69; 146.66; 147.00; 149.42; 155.89; 158.60; 158.86; 166.05 (CO of Bz); 172.26 (CO of PhO). ESI-MS (pos.): 926 (100, $[M+Et_3NH]^+$), 846 (18, $[M+Na]^+$), 824 (20, $[M+H]^+$), 303 (100, $[DMT]^+$). ESI-MS (neg.): 822 (100, $[M-H]^-$).

4. Experiments Referring to *Scheme 6*. – *9-{3′-*O*-Benzoyl-4′-*O*-[(2-cyanoethoxy)(diisopropylamino)phosphino]-2′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-α-*L*-arabinopyranosyl}-N²-(phenoxyacetyl)adenine* (**16a**). To a soln. of 1.1 g (1.4 mmol) **15a** in 30 ml of dry CH_2Cl_2 was added 0.95 ml (5.4 mmol) of EtN(i-Pr_2) slowly, followed by 0.61 ml (4.9 mmol) of chloro(2-cyanotheoxy)(diisopropylamino)phosphine by syringe. The mixture was stirred for 1.5 h at r.t., diluted with 50 ml of CH_2Cl_2, and washed with sat. aq. $NaHCO_3$ soln. The org. phase was dried (Na_2SO_4), concentrated, and subjected to CC (silica gel; AcOEt/hexane 2:1) to afford 1.1 g (79%) of **16a** as a mixture of diastereoisomers (a/b 0.7:1). TLC (AcOEt: hexanes 3:1): R_f 0.75. ^{1}H-NMR (600 MHz, $CDCl_3$): 1.11, 1.12 (2*d*, *J* = 6.9, *Me*$_2$CH); 1.14, 1.15 (2*d*, *J* = 7.2, Me$_2$CH); 2.13 (*dt*, *J* = 16.7, 6.5, 1 H, CH_2CH$_2$CN of isomer b); 2.26 (*dt*, *J* = 6.5, 16.7, 1 H, CH_2CH$_2$CN of isomer b); 2.51 (t, *J* = 6.1, 2 H, CH_2CH$_2$CN of isomer a); 3.49 (*m*, CH$_2$CH_2CN of isomer b); 3.58 (*m*, CH$_2$CH_2CN of both isomers, Me$_2$C*H* of both isomers); 3.60 (*s*, MeO); 3.61 (*s*, MeO); 3.69 (*s*, MeO); 3.70 (*s*, MeO); 3.71 (*m*, CH$_2$CH_2CN of isomer a); 3.94 (*d*, *J* = 12.5, H–C(5′) of isomer b); 3.96 (*d*, *J* = 12.4, H–C(5′) of isomer a); 4.06 (*m*, H–C(5′) of isomer b); 4.19 (*dd*, *J* = 12.5, 2.3, H–C(5′) of isomer a); 4.34 (br. *d*, *J*(H,P) = 8.6, H–C(4′) of isomer a); 4.47 (br. *d*, *J*(H,P) = 11.0, H–C(4′) of isomer b); 4.59 (*m*, H–C(2′) of isomer a); 4.67 (*m*, H–C(2′) of isomer b); 4.87 (br. *s*, ArOCH_2 of both isomers); 5.53 (*m*, H–C(3′) of both isomers); 5.98 (*d*, *J* = 8.1, H–C(1′) of isomer b); 6.00 (*d*, *J* = 8.1, H–C(1′) of isomer a); 6.48–7.66 (*m*, 46 arom. H); 8.83 (*s*, H–C(2) of isomer b); 8.84 (*s*, H–C(2) of isomer a); 9.34 (br. *s*, NHCO of both isomers). ^{13}C-NMR (150.9 MHz, $CDCl_3$): 20.16, 20.21 (*d*, *J*(C,P) = 7.0, *C*H$_2$CH$_2$CN of isomer b); 20.67, 20.71 (*d*, *J*(C,P) = 7.2, *C*H$_2$CH$_2$CN of isomer a); 24.84, 24.88, 24.92 (*Me*$_2$CH); 43.58, 43.67 (Me$_2$*C*H of isomer a); 43.62, 43.70 (Me$_2$*CH* of isomer b); 55.28, 55.44 (MeO); 55.33, 55.51 (MeO); 57.71, 57.84 (CH$_2$*C*H$_2$CN of isomer a, *J*(C,P) = 20.2); 58.41, 58.53 (CH$_2$*C*H$_2$CN of isomer b, *J*(C,P) = 18.4); 68.24 (C(5′) of isomer b); 68.53 (CH_2O of both isomers); 68.66 (C(5′) of isomer a); 69.03 (C(4′), *J*(C,P) = 15.6 of isomer b); 69.44 (C(4′), *J*(C,P) = 9.8 of isomer a); 70.42 (C(2′) of isomer b); 70.56 (C(2′) of isomer a); 75.90 (C(3′) of both isomers); 83.64 (C(1′) of both isomers); 113.21; 115.38; 117.84; 117.90;122.41; 122.82; 127.05; 127.11; 127.80; 128.03; 128.12; 128.21; 129.77; 129.82; 129.94; 130.19; 130.23; 131.02; 132.09; 133.13; 133.17; 135.31; 136.50; 141.95 (C(8) of both isomers); 145.41; 148.40; 148.43; 152.35; 152.38; 152.84 (C(2) of both isomers); 157.43; 157.46; 158.59; 158.67; 158.86; 158.95; 166.03, 166.06 (CO of Bz of both isomers); 166.94 (CO of PhO of both isomers). ^{31}P-NMR (241 MHz, $CDCl_3$): 150.10, 152.46. ESI-MS (pos.): 1030 (100, $[M+Na]^+$), 1008 (75, $[M+H]^+$). ESI-MS (neg.): 1042 (9, $[M+Cl]^-$), 1006 (100, $[M-H]^-$).

*1-{3′-*O*-benzoyl-4′-*O*-[(2-cyanoethoxy)(diisopropylamino)phosphino]-2′-*O*-[(4″,4‴-dimethoxytriphenyl)methyl]-α-*L*-arabinopyranosyl}thymine* (**16b**). To a soln. of 1.67 (2.5 mmol) of **15b** in 15 ml of dry CH_2Cl_2 was added successively 1.3 ml (7.5 mmol) of EtN(i-Pr_2) and 1.7 ml (7.5 mmol) of chloro(2-cyanotheoxy)(diisopropylamino)phosphine at r.t. The soln. was stirred for 1 h, diluted with 30 ml AcOEt and washed with sat. aq. $NaHCO_3$. The org. phase was dried (Na_2SO_4), filtered, and evaporated. Purification by CC (silica gel; hexane/AcOEt 1:1) gave 1.82 g (84%) of **16b** as a mixture of diastereoisomers. TLC (hexane/EtOAc 1:1): R_f 0.41. ^{1}H-NMR (600 MHz, $CDCl_3$): 1.09–1.14 (4 br. *s*, 4 *Me*$_2$CH); 1.68 (*s*, Me–C(5)); 2.05 (*dt*, *J* = 16.8, 6.5, 1 H, CH_2CH$_2$CN of isomer a); 2.21 (*dt*, *J* = 16.8, 6.6, 1 H, CH_2CH$_2$CN of isomer a); 2.49 (*t*, *J* = 16.8, 6.6, 2 H, CH_2CH$_2$CN of isomer b); 3.43 (*m*, 1 H, CH$_2$CH_2CN of isomer a); 3.54–3.68 (*m*, 2 H, CH$_2$CH_2CN of both isomers, Me$_2$C*H* of both isomers, MeO, CH$_2$CH_2CN of isomer b); 3.83 (*d*, *J* = 12.6, 1 H, H–C(5′) of isomer a); 3.84 (*d*, *J* = 12.6, 1 H, H–C(5′) of isomer b); 3.99 (*d*, *J* = 12.6, 1 H, H–C(5′) of isomer a); 4.08 (*dd*, *J* = 12.6, 1.8,

1 H, H–C(5′) of isomer b); 4.21 (*t*, *J* = 9.0, H–C(2′) of isomer a); 4.23–4.28 (*m*, H–C(2′), H–C(4′) of isomer b); 4.39 (br. *d*, *J* = 11.4, H–C(4′) of isomer a); 5.40 (*dd*, *J* = 9.6, 3.0, H–C(3′) of isomer a); 5.43 (*dd*, *J* = 9.6, 3.0, H–C(3′) of isomer b); 6.00 (*d*, *J* = 9.0, H–C(1′) of isomer a); 6.01 (*d*, *J* = 9.0, H–C(1′) of isomer b); 6.29 (*s*, H–C(6) of isomer a); 6.31 (*s*, H–C(6) of isomer b); 6.58–7.66 (*m*, 38 H). ^{13}C-NMR (150.9 MHz, $CDCl_3$): 12.80 (*Me*–C(5) of isomer a); 12.87 (*Me*–C(5) of isomer b); 20.05, 20.09 (*d*, *J*(C,P) = 6.9, *C*$H_2$$CH_2$CN of isomer a); 20.61, 20.65 (*d*, *J*(C,P) = 6.9, *C*$H_2$$CH_2$CN of isomer b); 24.85, 24.95, 25.34, 25.39 (*Me*$_2$CH); 43.49, 43.57, 43.66 (Me$_2$*C*H of both isomers); 55.21, 55.34, 55.40 (MeO of both isomers); 57.64, 57.78 (*d*, *J*(C,P) = 21.1, CH_2*C*H_2CN of isomer a); 58.33, 58.45 (*d*, *J*(C,P) = 19.4, CH_2*C*H_2CN of isomer b); 67.67 (C(5′) of isomer a); 67.97 (C(5′) of isomer b); 69.22 (C(2′) of isomer a); 69.39 (C(2′) of isomer b); 69.39, 69.45 (*d*, *J*(C,P) = 10.41, C(4′) of isomer a); 69.64, 69. 70 (*d*, *J*(C,P) = 9.8, C(4′) of isomer b); 76.18 (C(3′) of isomer a); 76.30 (C(3′) of isomer b); 83.14 (C(1′) of isomer a); 83.26 (C(1′) of isomer b); 88.63 (Ar$_3$C); 110.93 (C(5) of isomer a); 111.08 (C(5) of isomer b); 113.19; 117.71; 117.78; 127.01; 127.90; 127.98; 128.17; 128.56; 129.83; 130.06; 130.50; 133.09; 135.79 (C(6) of isomer a); 135.91 (C(6) of isomer b); 150.79 (C(2) of both isomers); 158.56; 158.77, 163.63 (C(4) of both isomers); 166.04 (CO of Bz of both isomers). ^{31}P-NMR (242.9 MHz, $CDCl_3$): 149.95, 152.57 ESI-MS (neg.): 900 (16, $[M-Cl]^-$, 864 (100, $[M-H]^-$).

*1-{3′-*O*-Benzoyl-4′-*O*-[(2-cyanoethoxy)(diisopropylamino)phosphino]-2′-*O*-[(4″, 4‴-dimethoxytriphenyl)-methyl]-α-*L*-arabinopyranosyl}-*N*^{4}-isobutyrylcytosine* (**16c**). To a soln. of 1.9 g (2.6 mmol) of **15c** in 20 ml of dry CH_2Cl_2 were added successively 1.4 ml (7.9 mmol) of EtN(i-Pr$_2$) and 1.8 ml (7.9 mmol) of chloro(2-cyanotheoxy)(diisopropylamino)phosphine at r.t. The soln. was stirred for 1 h, diluted with 50 ml of AcOEt, and washed with sat. aq. $NaHCO_3$ soln. The org. phase was dried (Na_2SO_4), filtered, evaporated, and the residue was purified by CC (silica gel; hexane/AcOEt 1:2) gave 2.0 g (83%) of **16c** as a mixture of diastereoisomers.

Data of Major Isomer of **16c**: TLC (hexane/AcOEt 1:2): R_f 0.41. ^{1}H-NMR (600 MHz, $CDCl_3$): 1.07–1.25 (*m*, 6 *Me*$_2$CH); 2.06, 2.21 (*dt*, *J* = 16.8, 6.6, C*H*$_2$CH$_2$CN); 2.74 (*m*, 1 H, Me$_2$C*H*); 3.43–3.38 (*m*, CH_2C*H*$_2$CN, 2 Me$_2$C*H*); 3.58 (*s*, MeO); 3.66 (*s*, MeO); 3.84 (*d*, *J* = 12.6, 1 H, H–C(5′)); 3.96 (*d*, *J* = 12.0, 1 H, H–C(5′)); 4.24 (*t*, *J* = 9.0, H–C(2′)); 4.36 (*m*, H–C(4′)); 5.39 (*dd*, *J* = 9.0, 3.0, H–C(3′)); 6.26 (*d*, *J* = 9.0, H–C(1′)); 6.57 (*d*, *J* = 7.8, 2 arom. H); 6.66 (*d*, *J* = 7.8, 2 arom. H); 6.74 (*d*, *J* = 7.2, H–C(5)); 6.93–7.27 (*m*, H–C(6), 9 arom. H), 7.33 (*t*, *J* = 7.8, 2 H); 7.49 (*t*, *J* = 7.2, 1 H); 7.64 (*d*, *J* = 7.2, 2 arom. H). ^{13}C-NMR (150.9 MHz, $CDCl_3$): 19.30, 19.52 (*Me*$_2$CH); 20.07, 20.11 (*d*, *J*(C,P) = 6.5, *C*$H_2$$CH_2$CN); 24.85 (Me$_2$*C*H); 37.04 (Me$_2$*C*HCO); 43.51, 43.59 (*d*, *J*(C,P) = 12.2, Me$_2$*C*HP); 55.27, 55.41 (MeO of both isomers); 58.37, 58.50 (*d*, *J*(C,P) = 19.2, CH_2*C*H_2CN); 68.37 (C(5′)); 69.27, 69.28 (*d*, *J*(C,P) = 16.7, C(4′)); 69.96 (C(2′)); 76.36 (C(3′)); 84.17 (C(1′)); 88.68 (Ar$_3$C); 97.53 (C(5)); 113.26; 113.35; 118.49; 127.03; 127.99; 128.13; 129.76; 130.02; 130.03; 133.08; 144.76 (C(6)); 155.76 (C(2)); 158.57; 158.79; 162.31(C(4)); 166.02 (CO of Bz); 177.47 (C(8)). ^{31}P-NMR (242.9 MHz, $CDCl_3$): 150.05. ESI-MS (pos.): 943 (100, $[M+Na]^+$), 840 (44), 441 (44), 303 (25).

Diagnostic Data of Minor Isomer of **16c**. TLC (hexane/AcOEt 1:2): R_f 0.46. ^{1}H-NMR (600 MHz, $CDCl_3$): 2.48 (*m*, C*H*$_2$CH$_2$CN); 5.42 (*dd*, *J* = 8.9, 3.4, H–C(3′)); 6.26 (d, *J* = 8.7, H–C(1′)). ^{31}P-NMR (242.9 MHz, $CDCl_3$) 152.7.

*9-{3′-*O*-Benzoyl-4′-*O*-[(2-cyanoethoxy)(diisopropylamino)phosphino]-2′-*O*-[(4″,4‴-dimethoxytriphenyl)-methyl]-α-l-arabinopyranosyl}-*N^2*-(phenoxyacetyl)guanine* (**16d**). To a soln. of 2 g (2.4 mmol) **15d** in 50 ml of dry CH_2Cl_2 was added 1.7 ml (9.7 mmol) of EtN(i-Pr$_2$) slowly, followed by 1.08 ml (4.86 mmol) of chloro(2-cyanotheoxy)(diisopropylamino)phosphine by syringe. The mixture was stirred for 1.5 h at r.t., diluted with 50 ml of CH_2Cl_2, and washed with sat. aq. $NaHCO_3$ soln. The org. phase was dried (Na_2SO_4), concentrated, and subjected to CC (silica gel; eluted with 40:1 CH_2Cl_2/i-PrOH containing 1% Et_3N) to afford 2 g (80.4%) of **16d** as a mixture of diastereoisomers (a/b 1:0.9). TLC (CH_2Cl_2/i-PrOH 20:1): R_f 0.75. ^{1}H-NMR (600 MHz, $CDCl_3$): 1.07, 1.08, 1.10, 1.12 (br. *s*, Me$_2$CH), 2.10 (*dt*, *J* = 16.7, 6.5, 1 H, C*H*$_2$CH$_2$CN of isomer a); 2.22 (*dt*, *J* = 16.7, 6.5, 1 H, C*H*$_2$CH$_2$CN of isomer a); 2.50 (*t*, *J* = 6.1, C*H*$_2$CH$_2$CN of isomer b); 3.48 (*m*, CH_2C*H*$_2$CN, of isomer a); 3.55 (*m*, CH_2C*H*$_2$CN of both isomers, Me$_2$C*H* of both isomers); 3.57 (*s*, MeO of isomer b); 3.58 (*s*, MeO); 3.67 (*s*, MeO); 3.68 (*s*, MeO); 3.70 (*m*, CH_2C*H*$_2$CN of isomer b); 3.88 (*d*, *J* = 12.2, H–C(5′) of isomer a); 3.89 (*d*, *J* = 12.2, H–C(5′) of isomer b); 4.01 (*dd*, *J* = 12.4, 3.1, H–C(5′) of isomer a); 4.14 (*dd*, *J* = 2.7, 12.4, H–C(5′) of isomer b); 4.30 (br. *d*, *J*(H,P) = 8.5, H–C(4′) of isomer b); 4.37 (*m*, H–C(2′) of isomer b); 4.45 (br. *d*, *J*(H,P) = 11.0, H–C(4′), of isomer a); 4.55 (*m*, H–C(2′) of isomer a); 4.83 (br. *s*, PhOCH_2 of both isomers); 5.49 (*m*, H–C(3′) of both isomers); 5.67 (*d*, *J* = 7.7, H–C(1′) of isomer a); 5.72 (*d*, *J* = 8.1, H–C(1′) of isomer a); 6.52-7.62 (*m*, 48 arom. H). ^{13}C-NMR (150.9 MHz, $CDCl_3$): 20.10, 20.15 (*d*, *J*(C,P) = 7.0, *C*$H_2$$CH_2$CN of isomer a); 20.64, 20.69 (*d*, *J*(C,P) = 7.1, *C*$H_2$$CH_2$CN of isomer b); 24.78, 24.83, 24.88, 24.92 (Me$_2$CH); 43.53, 43.62 (Me$_2$*C*H of isomer b); 43.58, 43.66 (Me$_2$*C*H of isomer a); 55.29, 55.49 (MeO); 55.32, 55.55 (MeO); 57.64, 57.77 (CH_2*C*H_2CN, *J*(C,P) = 20.0 of isomer b); 58.39, 58.51 (CH_2*C*H_2CN, *J*(C,P) = 18.9 of isomer a); 67.75 (CH_2O of both isomers); 67.84 (C(5′) of isomer a); 68.39 (C(5′) of isomer b); 68.81 (C(4′) of isomer a); 69.36 (C(4′) of

isomer b); 70.40 (C(2′) of isomer a); 70.78 (C(2′) of isomer b); 75.81 (C(3′) of both isomers); 83.18 (C(1′) of isomer b); 83.74 (C(1′) of isomer a); 113.23; 115.36; 115.41; 117.83; 117.87; 121.15; 121.40; 123.24; 126.98; 127.03; 127.67; 127.74; 128.03; 128.23; 129.52; 129.68; 129.75; 129.90; 130.34; 130.40; 130.42; 131.29; 133.18; 133.24; 136.44; 137.71; 137.99; 145.85; 147.46; 148.78; 148.84; 156.75; 157.14; 157.22; 158.63; 158.68; 158.94; 158.99; 166.12 (CO of Bz of both isomers); 170.48, 170.55 (CO of PhO of both isomers). ^{31}P-NMR (241 MHz, $CDCl_3$): 150.30; 152.50. ESI-MS (pos.): 1126 (100, $[M + Et_3NH]^+$), 1047 (45, $[M + Na]^+$), 1025 (50, $[M + H]^+$). ESI-MS (neg.): 1023 (100, $[M - H]^-$), 970 (50), 822 (90).

Preparation of Nucleoside-Derivatized Controlled-Pore Glass (CPG) **17c**. To a soln. of 300 mg (0.416 mmol) of **15c** in 5 ml of dry CH_2Cl_2 were added successively 76 mg (0.62 mmol) of DMAP and 83 mg (0.83 mmol) of succinic anhydride at r.t. The mixture was stirred for 5 h, diluted with 40 ml of CH_2Cl_2, and washed with 20 ml of 10% aq. citric acid soln. The org. layer was dried (Na_2SO_4), filtered, and evaporated. The residue was purified by CC (silica gel; CH_2Cl_2/MeOH 9 : 1). The product fractions were collected, evaporated, and dried for 1 h *in vacuo* (0.2 Torr). This material was dissolved 10 ml dry MeCN, followed by successive addition of 104 µl (949 mmol) *N*-methylmorpholine, 155 mg (475 mmol) TOTU, and 1.5 g LCAA-CPG. The suspension was gently shaken for 3 h at r.t. Filtration and washing with DMF, MeOH, acetone, and Et_2O afforded **17c** after drying *in vacuo* (0.2 Torr). A suspension of the nucleoside-derivatized solid support **17c** in 25 ml dry pyridine and 3.0 ml 1-methyl-1*H*-imidazole was treated with 3.0 ml Ac_2O for 45 min. Filtration and washing with DMF, MeOH, acetone, and Et_2O afforded capped **17c** after drying *in vacuo* (0.2 Torr) for 3 h. The loading capacity was determined to be 52.5 µmol/g (by the method described in [13c]).

5. Automated Solid-Phase Synthesis on a *Perseptive Expedite* Gene Synthesizer. – Oligonucleotide syntheses were carried out on a 0.5-, 1-, and 10-µM scales. The DNA/RNA synthesizer column was filled with the CPG solid support loaded with the appropriate nucleobase. The substrates and reagents required were prepared as follows.

5.1. *With the Fmoc-Protected Building Blocks.* To perform the oligonucleotide synthesis in a 0.5-µmol scale, 30–40 mg of the corresponding solid support were filled in a commercially available column (height 1.5 mm, diameter 0.3 mm). Before placing the appropriate column on the DNA synthesizer in the reaction chamber, the desired synthesis parameters (sequence, synthesis cycle) were specified, and the reagents/solvents required for the oligonucleotide synthesis were prepared and installed as follows.

Pre-Automation Procedures. 5.1.1. *Fmoc-Phosphoramidites.* A 0.075 M soln. of phosphoroamidates in dry MeCN (dried over 3-Å molecular sieves (8–12 mesh), freshly activated by heating at *ca.* 300° under high vacuum overnight) was prepared. The number of couplings for the particular phosphoramidite was increased by two additional couplings to give a factor, which was multiplied by 16.0 mg for the thymine (**8b**) and 18.0 mg for the adenine (**8a**), cytosine (**8c**), and guanine (**8d**) phosphoramidites. The amount of the corresponding phosphoramidite was dissolved in 289 µl dry MeCN (17 pulses at 17 µl each); this resulted in a 22-fold excess of phosphoramidite over the CPG-solid support. The solns. were kept over molecular sieves (4 Å, mesh 8–12) for 2 h prior to use, and used within 24 h[18]).

5.1.2. *Washing.* MeCN (wash) was purchased from *PerSeptive Biosysytem*™ and stored over molecular sieves (4 Å, mesh 8–12) at least 2 h before use (*GEN089865*).

5.1.3. *Oxidizing Soln.* Oxidizer soln. from *PerSeptive Biosysytem*™ containing I_2 in pyridine/H_2O/THF (*GEN089850*).

5.1.4. *Deblocking Soln.* 0.1M DBU in MeCN was prepared by dissolving 1.5 ml of DBU (*Aldrich*) in 100 ml of MeCN. The soln. was kept over molecular sieves (4 Å, mesh 8–12) at least 2 h before use.

5.1.5. *Activator Soln.* 0.5M pyridine hydrochloride in MeCN was prepared by dissolving 5.78 g of pyridine hydrochloride (*Fluka*) in 100 ml of anh. MeCN. The activator soln. was stored 12 h over molecular sieves (4 Å, mesh 8–12) before use. Subsequently, it was replaced by a 0.5M soln. of 1*H*-tetrazole in MeCN as activator from *PerSeptive Biosysytem*™ (*GEN089880*).

5.1.6. *Capping Soln.* A: capping reagent *A* from *PerSeptive Biosysytem*™ containing Ac_2O in THF (*GEN089810*).

5.1.7. *Capping Soln.* B: from *PerSeptive Biosysytem*™ containing 1-methyl-1*H*-imidazole in a mixture of THF/pyridine (*GEN089820*).

The synthesis of oligonucleotides with the *Perseptive Expedite Gene Synthesizer* required the following modifications to the protocol provided by *Perseptive* for the DNA/RNA synthesis: *a*) the duration of the coupling time of phosphoramidite was *ca.* 15 min with either 0.5M pyridine hydrocloride or 0.5M 1*H*-tetrazole in MeCN, and *b*) the 'deblocking' was accomplished by 0.1M DBU in MeCN over a 1.5-min period. All oligonucleotides were synthesized in the 'Fmoc-off' mode, where the final Fmoc group was removed. The progress of the synthesis was followed by UV monitoring of the fulvene ion resulting from the cleavage of the

Fmoc group after each coupling. In a typical 0.5-μmol scale synthesis, each Fmoc-derived fulvene eluate (collected in a glass tube) was diluted to 5 ml with MeCN, and the absorbance at 305 nm ($\varepsilon = 9100$) was measured with a 0.1M soln. of DBU in MeCN as reference. The value obtained for each fulvene eluate was compared to that of the fulvene eluate from the previous step, and the coupling efficiency was determined.

5.2. *Post-Automation Procedures.* 5.2.1. *Removal of 2-Cyanoethyl Protecting Group*, *Removal of Sugar and Nucleobase Protecting Groups, and Detachment from CPG Solid Support.* After the automated synthesis was completed, the CPG solid support containing the oligonucleotide ('Trityl-on') was dried *in vacuo* for 30 min, transferred to a pear-shaped 10-ml flask, and one of the following procedure was used depending on the sequence of the oligonucleotides (*Table 3* lists the specific deprotection method for the specific sequence):

Method A. To the flask containing the dry CPG solid support was added 2.4 ml of a soln. of 0.15M $MeONH_2 \cdot HCl$ in 25%. aq. NH_3/EtOH 3:1, and and the mixture was shaken at r.t. for 2–10 h. After deprotection, the suspension was diluted with H_2O, filtered (*Nalgene*, PTFE (0.2 μm)), and evaporated to dryness to afford the crude oligonucleotides.

Method B. To the flask containing the dry CPG solid support was added 2 ml of 25% aq. NH_3 and shaken at 4° for 7–24 h. After deprotection, the suspension was diluted with H_2O, filtered (*Nalgene*, PTFE (0.2 μm)), and evaporated to dryness to afford the crude oligonucleotides.

Method C. To the flask containing the dry CPG solid support was added 2 ml of 25% aq. NH_3 and shaken at 4° for 21 h, and evaporated to dryness. The residue was treated with 25% aq. $NH_2NH_2 \cdot H_2O$ at r.t. for 10 min. After deprotection, the suspension was diluted with H_2O, filtered (*Nalgene*, PTFE (0.2 μm)), and evaporated to dryness to afford the crude oligonucleotides.

Method D. To the flask containing the dry CPG solid support was added 2 ml of a mixture of ethylenediamine/EtOH (1:1) and shaken at r.t. for 2 h. After deprotection, the suspension was diluted with H_2O, filtered (*Nalgene*, PTFE (0.2 μm)), and evaporated to dryness to afford the crude oligonucleotides.

All of the above deprotections were monitored by anion-exchange HPLC for optimum deprotection time, and the crude oligonucleotides were taken to the next step, HPLC purification.

5.3. *With the Phosphoramidite Building Blocks: Pre-Automation Procedures.* 5.3.1 *Phosphoroamidites.* The phosphoramidite soln. (*ca.* 0.07M) was dried over 3-Å molecular sieves (8–12 mesh, freshly activated by heating at *ca.* 300° under high vacuum overnight) overnight at r.t. prior to use. An excess of *ca.* 5 equiv. of phosphoroamidites was used.

5.3.2. *Activator Soln.:* 5-(ethylthio)-1*H*-tetrazole in dry MeCN (0.35M), which was dried over freshly activated 4-Å molecular sieves for 24 h at r.t.

5.3.3. *Capping Soln.* A: 1-methyl-1*H*-imidazole in pyridine/THF, supplied by *Perseptive Biosystems*™ was used (*GEN089810*).

5.3.4. *Capping Soln.* B: Ac_2O in THF, supplied by *Perseptive Biosystems*™ was used (*GEN089820*).

5.3.5. *Oxidizing Soln.:* I_2 in pyridine/THF, supplied by *Perseptive Biosystems*™ was used (*GEN089850*).

5.3.6. *Detritylation Reagent.*: 6% (*v*/*v*) Cl_2CHCO_2H soln. in $ClCH_2CH_2Cl$.

The synthesis of oligonucleotides with the *Perseptive Expedite Gene Synthesizer* required the following modifications to the protocol provided by *Perseptive* for the DNA/RNA synthesis: *a*) the duration of the coupling time of phosphoramidite was about 27 min. *b*) use of 0.35M soln. of 5-(ethylthio)-1*H*-tetrazole in MeCN as activator, and *c*) the detritylation was accomplished by 6% (*v*/*v*) Cl_2CHCO_2H in $ClCH_2CH_2Cl$ over a 3-min period. All oligonucleotides were synthesized in the 'Trityl-off' mode.

5.4. *Post-Automation Procedures.* 5.4.1. *Removal of 2-Cyanoethyl Protecting Group*, *Sugar and Nucleobase Protecting Groups, and Detachment from CPG Solid Support* (*Table 3* lists the specific deprotection method for the specific sequence). *Method E.* After the automated synthesis was completed, the CPG solid support containing the oligonucleotide was dried *in vacuo* for 30 min, transferred to a pear-shaped 10-ml flask, and treated with 2.4 ml of pyridine/Et_3N 5:1 for 5.5 h at r.t. Evaporation of pyridine and Et_3N *in vacuo*, followed by co-evaporation with DMF – avoiding temp. over 35° – resulted in dry CPG solid support. To the flask containing the dry CPG solid support was added 2.4 ml of a mixture of 40% aq. $MeNH_2$ conc. aq. NH_3 1:1 and shaken at r.t. for 1 h. The mixture was filtered, washed with H_2O, the washings and filtrates were combined and concentrated *in vacuo* to afford the crude oligonucleotides. The residue was dissolved in *ca.* 2 ml of H_2O, filtered (*Nalgene* syringe filter, 0.2 μM), and taken to the next step, HPLC purification.

All of the above deprotections were monitored by anion-exchange HPLC for optimum deprotection time.

6. HPLC Purification of Oligonucleotides. – Oligonucleotides were purified by ion-exchange HPLC. Anion-exchange HPLC was performed either on *Pharmacia Äkta Purifier (900)* controlled by *UNICORN* system. Columns: *Mono Q HR 5/5* (*Pharmacia*); buffer *A:* 10 mM Na_2HPO_4 in H_2O, pH 10.5–11.5; buffer *B:*

10 mM Na_2HPO_4 in H_2O, 0.1M NaCl, pH 10.5–11.5. The product fractions were collected in a vial containing either 0.1M aq. AcOH soln. or 0.1M aq. phosphate buffer soln.

For certain A,T-containing sequences (see *Table 3*), the following buffer afforded better separation: buffer *A:* 25 mM *Tris* · HCl buffer and 1.5M urea in H_2O, pH 8.0; buffer *B:* 25 mM *Tris* · HCl buffer and 1.5M urea in H_2O, 1M NH_4Cl, pH 8.0. The product fractions were collected in a vial containing 20 µl of a 1.0M aq. AcOH soln.

7. Desalting of Oligonucleotides [13c]. – The product fractions from HPLC purification were combined and diluted with 0.5–1M aq. $Et_3NH \cdot HCO_3$ buffer to twice the volume, and was the soln. applied to a previously conditioned reverse-phase *Sep-Pak* cartridge (*C18 Waters*). Successive elution with 0.1–0.5M aq. $Et_3NH \cdot HCO_3$ buffer soln., MeCN/H_2O 1:1 and lyophilization of the MeCN/H_2O fractions containing the product (monitored by UV at 260 nm) afforded the salt-free oligonucleotides. The residue was dissolved in 1 ml of H_2O and stored at $-20°$ as stock soln.

REFERENCES

[1] T. Wagner, H. K. Huynh, R. Krishnamurthy, A. Eschenmoser, *Helv. Chim. Acta* **2002**, *85*, 399–416.

[2] K.-U. Schöning, P. Scholz, X. Wu, S. Guntha, G. Delgado, R. Krishnamurthy, A. Eschenmoser. *Helv. Chim. Acta* **2002**, *85*, 4111–4153.

[3] a) M. Beier, F. Reck, T. Wagner, R. Krishnamurthy, A. Eschenmoser, *Science* **1999**, *283*, 699–703; b) O. Jungmann, H. Wippo, M. Stanek, H. K. Huynh, R. Krishnamurthy, A. Eschenmoser, *Org. Lett.* **1999**, *10*, 1527–1530.

[4] a) S. Pitsch, S. Wendeborn, B. Jaun, A. Eschenmoser, *Helv. Chim. Acta* **1993**, *76*, 2161–2183; b) I. Schlönvogt, S. Pitsch, C. Lesueur, A. Eschenmoser, R. M. Wolf, B. Jaun, *Helv. Chim. Acta* **1996**, *79*, 2316–2345; c) S. Pitsch, R. Krishnamurthy, M. Bolli, S. Wendeborn, A. Holzner, M. Minton, C. Lesueur, I. Schlönvogt, B. Jaun, A. Eschenmoser, *Helv. Chim. Acta* **1995**, *78*, 1621–1635; d) M. Bolli, R. Micura, A. Eschenmoser, *Chem. Biol.* **1997**, *4*, 309–320; e) M. Bolli, R. Micura, S. Pitsch, A. Eschenmoser, *Helv. Chim. Acta* **1997**, *80*, 1901–1951.

[5] F. Reck, H. Wippo, R. Kudick, R. Krishnamurthy, A. Eschenmoser, *Helv. Chim. Acta* **2001**, *84*, 1778–1804.

[6] H. Wippo, F. Reck, R. Kudick, M. Ramaseshan, G. Ceulemans, M. Bolli, R. Krishnamurthy, A. Eschenmoser, *Bioorg. Med. Chem*. **2001**, *9*, 2411–2428.

[7] A. Eschenmoser, M. Dobler, *Helv. Chim. Acta* **1992**, *75*, 218-259.

[8] M.-O. Ebert, A. Luther, H. K. Huynh, R. Krishnamurthy, A. Eschenmoser, B. Jaun, *Helv. Chim. Acta* **2002**, *85*, 4055–4073.

[9] P. Collins, R. Ferrier in 'Monosaccharides-Their Chemistry and Their Roles in Natural Products', Wiley, West Sussex, England, 1996, pp. 11–15; Nomenclature of Carbohydrates (IUPAC recommendations 1996), *Anon. UK. Chemistry International* **1997**, *19*, 52–54.

[10] A. P. Martinez, W. W. Lee, L. Goodman, *J. Org. Chem*. **1969**, *34*, 92–97; A. P. Martinez, W. W. Lee, *J. Org. Chem*. **1969**, *34*, 416–421; A. P. Martinez, W. W. Lee, L. Goodman, *J. Med. Chem*. **1969**, *12*, 1125; M. Fuertes, J. T. Witkowski, R. K. Robins, *J. Org. Chem*. **1975**, *40*, 2372–2377.

[11] M. L. Wolfrom, C. C. Christman, *J. Am. Chem. Soc*. **1936**, *58*, 39–43; H. G. Fletcher Jr., R. K. Ness, C. S. Hudson, *J. Am. Chem. Soc.* **1951**, *73*, 3698–3699.

[12] G. E. Hilbert, T. B. Johnson, *J. Am. Chem. Soc*. **1930**, *52*, 4489–4494; H. Vorbrüggen, B. Bennua, *Chem. Ber.* **1981**, *114*, 1279–1286.

[13] a) P. Garner, S. Ramakanth, *J. Org. Chem.* **1988**, *53*, 1294–1298; b) J. Kjellberg, N. G. Johansson, *Tetrahedron* **1986**, *42*, 6541–6544; c) M. Böhringer, H.-J. Roth, J. Hunziker, M. Göbel, R. Krishnan, A. Giger, B. Schweizer, J. Schreiber, C. Leumann, A. Eschenmoser, *Helv. Chim. Acta* **1992**, *75*, 1416–1477.

[14] A. Kraszewski, K. E. Norris, *Nucleic Acids Sym. Ser.* **1987**, *18*, 177–180.

[15] M. J. Gait, M. Singh, R. C. Sheppard, M. D. Edge, A. R. Greene, G. R. Heathcliffe, T. C. Atkinson, C. R. Newton, A. F. Markham, *Nucleic Acids Res.* **1980**, *8*, 1081–1096.

[16] K. P. Stengele, W. Pfleiderer, *Nucleic Acids Sym. Ser.* **1989**, *21*, 101–102; K. P. Stengele, W. Pfleiderer, *Tetrahedron Lett.* **1990**, *31*, 2549–2552.

[17] R. Pon, S. Yu, Y. S. Sanghvi, *Bioconjugate Chem.* **1999**, *10*, 1051–1057; O. Jungmann, Ph.D. thesis, Universität Konstanz, UFO Atelier für Gestaltung und Verlag, 1997, Band 322.

[18] T. Ogawa, M. Matsui, *Tetrahedron* **1981**, *37*, 2363–2369; S. David, S. Hanessian, *Tetrahedron* **1985**, *41*, 643–663.

[19] Q. Song, W. Wang, A. Fischer, X. Zhang, B. L. Gaffney, R. A. Jones, *Tetrahedron Lett*. **1999**, *40*, 4153–4156.

[20] J. C. Schulhof, D. Molko, R. Teoule, *Nucleic Acids Res.* **1987**, *15*, 397–416; N. N. Polushin, A. M. Morocho, B. Chen, J. S. Cohen, *Nucleic Acids Res.* **1994**, *22*, 639–645; N. D. Sinha, P. Davis, N. Usman, J. Perez, R. Hodge, J. Kremsky, R. Casale, *Biochimie* **1993**, *75*, 13–23.
[21] G. S. Ti, B. L. Gaffney, R. A. Jones, *J. Am. Chem. Soc.* **1982**, *104*, 1316–1319.
[22] P. S. Miller, M. P. Reddy, A. Murakami, K. R. Blake, S. B. Lin, C. H. Agris, *Biochemistry* **1986**, *25*, 5092–5097.
[23] U. Pieles, W. Zürcher, M. Schär, H. E. Moser, *Nucleic Acids Res.* **1993**, *21*, 3191–3196.
[24] A. K. Sood, S. A. Narang, *Nucleic Acids Res.* **1977**, *4*, 2757–2765.
[25] I. Tinoco Jr., K. Sauer, J. C. Wang, in 'Physical Chemistry: Principles and Applications in Biological Sciences', Eds. D. Young, D. Cavanaugh, Prentice Hall, Upper Saddle River, NJ, 1995, pp. 559–589.
[26] L. A. Marky, K. J. Breslauer, *Biopolymers* **1987**, *26*, 1601–1620.
[27] C. R. Cantor, P. R. Schimmel, 'Biophysical Chemistry, Part III: The Behavior of Biological Macromolecules', Freeman, San Francisco, CA, 1980, pp. 1135–1139.
[28] J. Hünziker, H. J. Roth, M. Böhringer, A. Giger, U. Diederichsen, M. Göbel, R. Krishnan, A. Eschenmoser, *Helv. Chim. Acta* **1993**, *76*, 259–352.
[29] J. S. Reader, G. F. Joyce, *Nature* **2002**, *420*, 841–844.

Received January 31, 2003