calculated according to the Cheng–Prusoff relationship,[36] assuming competitive interactions.

Biological Tests. The compound were assayed for their μ and δ opioid activities on the GPI and MVD respectively as previously described.[37] The agonist concentration that produces half-maximal inhibition of the electrically stimulated muscle twitch, IC_{50}, was determined from six to eight computed log dose–response curves with six different concentrations of the compound. Since the sensitivity varied from one tissue to another, [Met]enkephalin IC_{50} was determined on the same preparation, and the agonist IC_{50} was corrected by multiplication of the ratio: mean of all the [Met]enkephalin IC_{50}/[Met]enkephalin IC_{50} of the assay.

Analgesic Tests. Analgesic potencies were evaluated on the hot-plate test at 55 ± 0.2 °C by using albino mice (Depré, France) weighing 24–28 g.[28] The hot plate was surrounded by a cylindrical plexiglass chimney (14-cm diameter; 20-cm high). The jump latency time was measured by means of a stopwatch, 10 min after intracerebroventricular (icv) drug administration by hand (10-μL volume) with a modified 30-gauge needle and a Hamilton syringe.[38] Mice that did not jump within 180 s were removed (cut-off time). The experimenter was blind with respect to pretest manipulations. The percentage of analgesia was calculated according to the formula $(T_t - T_c)/(180 - T_c)$ (T_t and T_c are the jump latency times of treated and control animals respectively). The cut-off time (180 s) represents 100% analgesia. The ED_{50} and their 95% confidence limits were determined according to the method of Litchfield and Wilcoxon.[39] Each value represented 10–20 mice tested. Correlation between the analgesic effect of opioid peptides and their ability to inhibit [^{3}H]DAGO binding on crude brain membrane preparation was assessed by a multiple linear regression analysis.

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Registry No. 1, 111035-56-4; 2, 82015-09-6; 3, 114442-55-6; 4, 115141-25-8; 5, 115141-26-9; 6, 115141-27-0; 7, 115141-27-0; Cbz-Tyr-D-Ser(*t*-Bu)-OH, 114414-63-0; Cbz-Tyr-D-Thr(*t*-Bu)-OH, 115141-28-1; Cbz-Tyr-Thr(*t*-Bu)-OH, 115141-29-2; H-Gly-OMe·HCl, 5680-79-5; Cbz-Tyr-D-Ser(*t*-Bu)-Gly-OMe, 115141-30-5; Cbz-Tyr-D-Ser(*t*-Bu)-Gly-OH, 114414-62-9; BOC-Phe-Leu-OH, 33014-68-5; H-Thr-OCH_2Ph·$^1/_2$HOOCCOOH, 86088-59-7; BOC-Phe-Leu-Thr-OCH_2Ph, 115141-31-6; H-Phe-Leu-Thr-OCH_2Ph, 114414-66-3; Cbz-Tyr-D-Ser(*t*-Bu)-Gly-Phe-Leu-Thr-OCH_2Ph, 115141-32-7; BOC-D-Ser(CH_2Ph)-OH, 47173-80-8; H-Gly-Phe-Leu-Thr-OCH_2Ph·F_3CCOOH, 112550-44-4; BOC-D-Ser-(CH_2Ph)-Gly-Phe-Leu-Thr-OCH_2Ph, 115160-76-4; H-D-Ser-(CH_2Ph)-Gly-Phe-Leu-Thr-OCH_2Ph·F_3CCOOH, 115160-78-6; BOC-Tyr-OH, 3978-80-1; BOC-Tyr-D-Ser(CH_2Ph)-Gly-Phe-Leu-Thr-OCH_2Ph, 115141-33-8; BOC-Tyr-D-Ser(CH_2Ph)-Gly-Phe-Leu-Thr-OH, 115141-34-9; Cbz-Tyr-D-Thr(*t*-Bu)-Gly-Phe-Leu-Thr-OCH_2Ph, 114414-61-8; Cbz-Tyr-Thr(*t*-Bu)-Gly-Phe-Leu-Thr-OCH_2Ph, 115141-35-0; H-D-Ser-OMe·HCl, 5874-57-7; Cbz-Tyr-D-Ser-OMe, 102683-26-1; Cbz-Tyr-D-Ser-OH, 115141-36-1; Cbz-Tyr-D-Ser-Gly-OMe, 115141-37-2; Cbz-Tyr-D-Ser-Gly-OH, 115141-38-3; Cbz-Phe-OH, 1161-13-3; H-Leu-OMe·HCl, 7517-19-3; Cbz-Phe-Leu-OMe, 3850-45-1; Cbz-Phe-Leu-OH, 4313-73-9; H-Thr(*t*-Bu)-OMe·HCl, 71989-43-0; Cbz-Phe-Leu-Thr(*t*-Bu)-OMe, 115141-39-4; Cbz-Phe-Leu-Thr(*t*-Bu)-OH, 115141-40-7; H-Phe-Leu-Thr(*t*-Bu)-OH, 114414-64-1; Cbz-Tyr-D-Ser-Gly-Phe-Leu-Thr(*t*-Bu)-OH, 115141-41-8; Cbz-Tyr-D-Thr-OH, 112550-43-3; Cbz-Tyr-D-Thr-Gly-OMe, 115141-42-9; Cbz-Tyr-D-Thr-Gly-OH, 74086-15-0; Cbz-Tyr-D-Ser(*t*-Bu)-Gly-Phe-Leu-Thr(*t*-Bu)-OH, 114442-56-7; Cbz-Tyr-OH, 1164-16-5; H-D-Ser(*t*-Bu)-OMe·HCl, 78537-14-1; H-D-Thr(*t*-Bu)-OMe·HCl, 115141-43-0; Cbz-Tyr-D-Ser(*t*-Bu)-OMe, 115141-44-1; Cbz-Tyr-D-Thr(*t*-Bu)-OMe, 115141-45-2; Cbz-Tyr-Thr(*t*-Bu)-OMe, 111540-39-7; Cbz-Tyr-D-Thr-Gly-Phe-Leu-Thr(*t*-Bu)-OH, 115141-46-3.

Synthesis and Biological Evaluation of Isomeric Dinucleoside Monophosphates and Monomethylphosphonates of 9-β-D-Arabinofuranosyladenine and Related Analogues[1]

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Université des Sciences et Techniques du Languedoc, Laboratoire de Chimie Bio-Organique, U.A. 488 du CNRS, Place E.-Bataillon, 34060 Montpellier Cédex, France, and Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium. *Received January 25, 1988*

The 3′→5′, 3′→3′ and 5′→5′ dinucleoside monophosphates and methylphosphonates of 9-β-D-arabinofuranosyladenine, as well as its 5′-(hydrogen phosphonate) and 5′-(methyl methylphosphonate) derivatives have been the subject of a systematic synthesis and examination of their biological, i.e. antiviral and cytostatic, properties. First the properly protected monomeric building blocks were prepared and then condensed to give fully protected intermediates. These latter were then deblocked to afford the unprotected compounds, which were fully characterized. Only the 3′→5′ phosphodiester isomers **13** and **16** and, to a lesser extent, the 5′-(hydrogen phosphonate) derivative **21** showed marked biological activity.

Of the various nucleoside analogues that have been described as antiviral and antitumor agents,[2] 9-β-D-arabinofuranosyladenine (araA, vidarabine, VIRA-A) is one of the best known drugs with rather selective antiherpetic activity.[3] Although araA has been licensed for clinical use, i.e. for the topical treatment of herpetic keratitis and the systemic intravenous treatment of herpetic encephalitis,

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(1) Preliminary results of this work have been communicated by the authors at the 20th anniversary nucleotide group meeting celebrating 40 years of nucleic acids research at Birmingham University, Chemistry Department, 14–16 December 1987, Birmingham, England.

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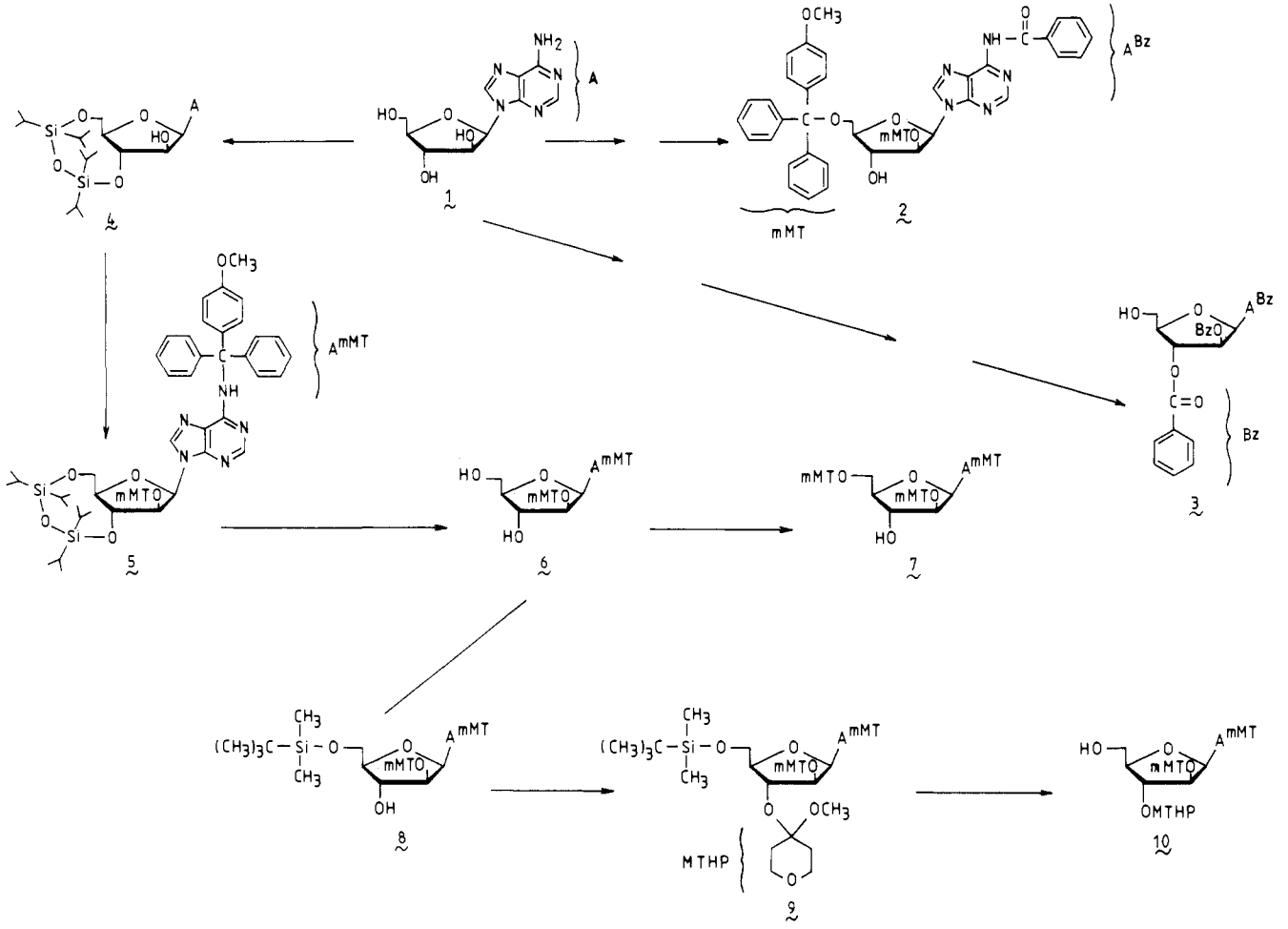
Scheme I



it suffers from several shortcomings,[4] including (i) a low aqueous solubility, which necessitates the administration of large volumes of fluid for systemic use; (ii) a low lipophilicity that limits the use of araA as a topical agent for the treatment of cutaneous herpesvirus infections; and (iii) a rapid deamination, and thus inactivation, by the ubiquitous adenosine deaminase. To overcome these problems, various derivatives of araA were synthesized and evaluated.[5] For example the first problem can be circumvented by using araA 5′-monophosphate (araAMP), the second by a number of *O*-acyl derivatives of araA, and the third by using carbocyclic araA. For neither compound, however, has it been established that they have clinical advantages over araA.

In the present work we have pursued a prodrug approach based on the development of new dimeric derivatives (**13**, **19**, **25**, **27**, **32**, and **34**) of araA. Several factors were taken into account in the design of such prodrugs: nature (phosphodiester or methylphosphonate) and position (3′→5′, 5′→5′, and 3′→3′) of the internucleotidic linkage. A 3′→5′-dinucleoside phosphate (**16**) containing both 9-β-D-xylofuranosyladenine and araA and two new derivatives of araAMP (**21** and **19**) were also synthesized.

Chemistry

Preparation of Properly Protected Monomeric Building Blocks. A successful scheme for the synthesis of the desired compounds requires preparation of properly protected derivatives of araA having a free 3′- or 5′-hydroxyl function. Initially we prepared, according to published procedures, the building blocks **2**[6] and **3**,[7] in which the exocyclic NH_2 was blocked with a base-labile benzoyl group and the other positions with benzoyl or acid-labile monomethoxytrityl (mMT) groups. Then, with the main aim to simplify the final deprotecting steps, we prepared new building blocks **7** and **10**, for which all protecting groups were acid-labile [mMT and methoxytetrahydropyranyl (MTHP) groups]. The synthesis of all building blocks started from commercially available araA (**1**) (Scheme I). Treatment of **1** with Markiewicz's reagent[8] resulted in its 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl) derivative (**4**).[9,10] Reaction of **4** with mMTCl gave

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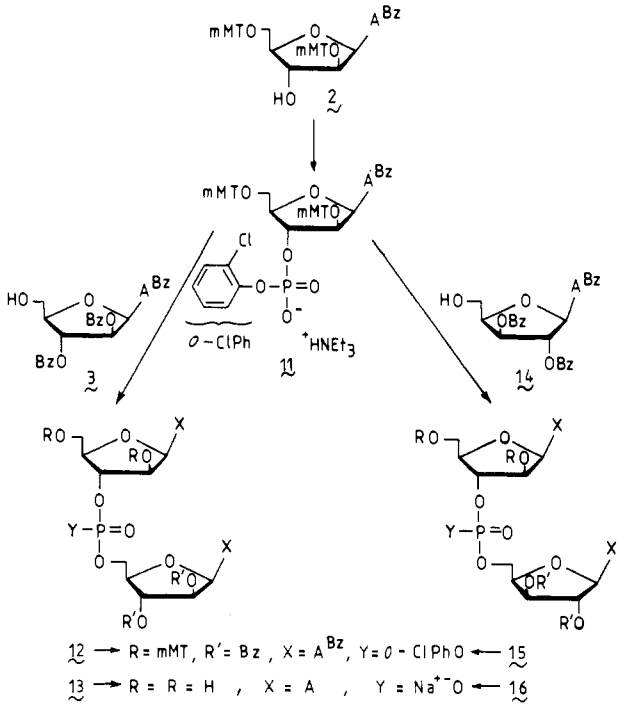
Scheme II



fully protected intermediate **5**. Purification at this stage was not attempted, and **5** was directly desilylated by using a combined reagent of tetraethylammonium bromide and potassium fluoride[11] to give **6**. The 5′-hydroxyl function of **6** was monomethoxytritylated to afford the building block **7** or temporarily protected with a *tert*-butyldimethylsilyl group to afford **8**. Attempted mMT introduction on the 3′-hydroxyl function of **8** was unsatisfactory, giving also the permonomethoxytrityl derivative, apparently owing to concomitant TBDMS loss (data not shown). This consideration led us to use the MTHP group in the 3′-OH protection of **8**, and the methoxytetrahydropyranylation of **8**, followed by the desilylation of intermediate **9**, afforded the desired building block **10**.

Synthesis of araA Dinucleoside Phosphates. Some araA-containing oligonucleotides have been synthesized previously.[12-21] Nevertheless a thorough literature survey revealed that, except for ref 19 and 20, no attention has been given to the synthesis and biological evaluation of araA dinucleoside phosphates.

The strategy followed for the chemical synthesis of the dissymmetrical araA-(3′→5′)-araA (and -xyloA) was based on the modified solution-phase phosphotriester method[22] (Scheme II). Phosphorylation of **2** with 2-chlorophenyl phosphoryl ditriazolide in pyridine[23] occurred readily to afford **11**. Solutions of the triethylammonium salt of **11** and the free 5′-OH building blocks **3** (and **14**[24]) treated with 2.5 molar equiv of 1-mesitylene-2-sulfonyl-3-nitro-1,2,4-triazole (MSNT)[25] in anhydrous pyridine at room temperature gave the fully protected dimers **12** (and **15**). The products, obtained as mixtures of diastereoisomers due to the chirality of the phosphorus triesters, were isolated in satisfactory yields by silica gel short column chromatography. For their deprotection, cleavage of the 2-chlorophenyl groups from phosphorus was accomplished with N^1,N^1,N^3,N^3-tetramethylguanidinium *syn*-4-nitrobenzaldoximate[25] in aqueous dioxane. Removal of *N*- as well as *O*-benzoyl with aqueous ammonia was followed by treatment with 80% acetic acid to remove the monomethoxytrityl groups. The mixtures obtained after deprotection were applied to a DEAE-Sephadex A-25 column and eluted with a linear gradient of triethylammonium bicarbonate to give, after precipitation from methanol–acetone, the pure deprotected **13** (and **16**).

The symmetrical araA-(5′→5′)- and -(3′→3′)-araA (**19** and **25**) were prepared from building blocks **10** and **7**, respectively (Scheme III). In the case of **19**, initially the modified solution-phase phosphotriester method was used as described above for the synthesis of **13** (and **16**). Then the hydrogen phosphonate method, recently developed for DNA[26-34] and RNA[35] synthesis, was investigated. The 5′- and 3′-(hydrogen phosphonates) **20** and **23** were prepared by using the tris(imidazoyl)phosphite procedure.[27] Reaction of **20** and **23** with **10** and **7** in the presence of chloro diphenyl phosphate and *N*-methylimidazole[26] led to the protected diaraA-(5′→5′)- and (3′→3′)-hydrogen phosphonates **22** and **24**, which were oxidized with aqueous iodine. On subsequent treatment with trifluoroacetic acid in methylene chloride, dinucleoside phosphodiesters **19** and **25** were isolated in satisfactory yields after workup and purification. Similar treatment of **20** with trifluoroacetic acid gave araA 5′-(hydrogen phosphonate) (**21**). It should be noted that all attempts to prepare unblocked diaraA hydrogen phosphonates from **22** and **24** in acidic conditions failed, the only products observed being araA (**1**) and its

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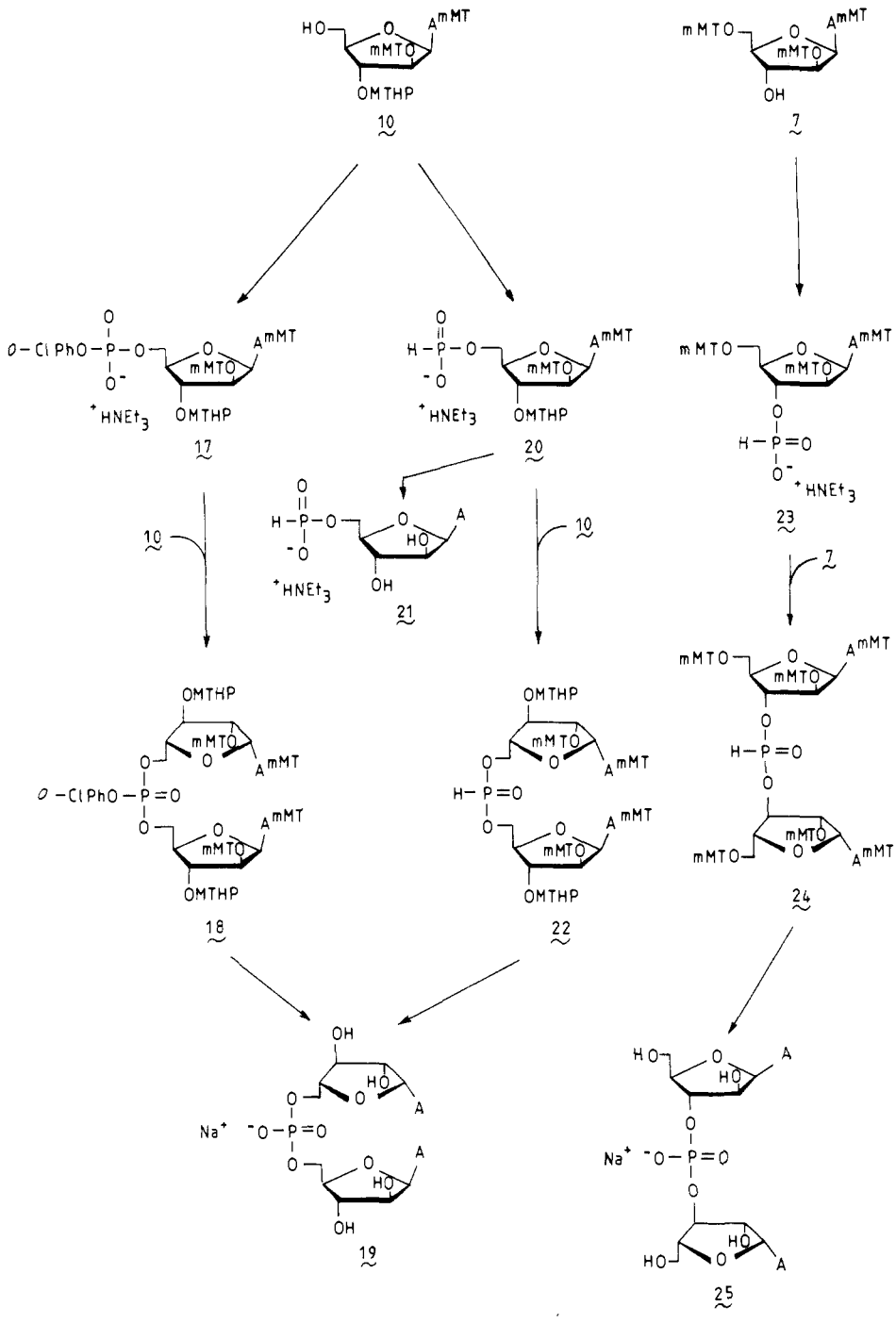
Scheme III



5′- (**21**) or 3′-(hydrogen phosphonate) derivative (data not shown).

Synthesis of araA Dinucleoside Methylphosphonates. During this decade there has been considerable interest in the synthesis, biophysical studies and biological evaluation of nonionic oligonucleoside methylphosphonates.[36-71] The preparation of these nucleic acid analogues can be accomplished either by a phosphite[46,47,53,54,57] or by a phosphotriester ap-

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Table I. Relevant Data for the Properties of Unprotected Derivatives of araA

compd	TLC[a] R_f values A	B	^{1}H NMR[b]	^{31}P NMR[c]	mass spectrum (FAB > 0)
13	0.69	0.63	8.20, 8.18, 8.13, and 8.12 (4 s, 4 × 1 H, 2 H-2 and 2 H-8), 7.19 and 7.17 (2 br s, 2 × 2 H, 2 NH_2), 6.79 (d, 1 H, OH), 6.29 and 6.27 (2 d, 2 × 1 H, 2 H-1′, $J_{1',2'}$ = 6.2 and 4.8 Hz), 5.75 (m, 2 H, 2 OH), 5.45 (t, 1 H, OH-5′, J = 5.4 Hz), 4.6 (dd, 1 H, H-3′), 4.4 and 4.2 (2 m, 2 × 1 H, 2 H-2′), 4.1, 4.0, 3.9 and 3.6 (4 m, 1 and 1 and 3 and 2 H, H-3′ and 2 H-4′,5′,5″)	0.21	597 $(M + H)^+$
16	0.71	0.64	8.34, 8.20, 8.15, and 8.12 (4 s, 4 × 1 H, 2 H-2 and 2 H-8), 7.24 and 7.18 (2 br s, 2 × 2 H, 2 NH_2), 7.0 (br s, 1 H, OH), 6.7–6.4 (br s, 1 H, OH), 6.30 and 5.94 (2 d, 2 × 1 H, 2 H-1′, $J_{1',2'}$ = 6.1 and 1.1 Hz) 5.8–4.6 (br s, 2 H, 2 OH), 4.6 (dd, 1 H, H-3′), 4.4 (m, 1 H, H-2′), 4.3, 4.1, 3.9, and 3.6 (4 m, 4 × 2 H, H-2′, H-3′, and 2 H-4′,5′,5″)	1.26	641 $(M - H + 2\ Na)^+$, 619 $(M + Na)^+$, 597 $(M + H)^+$
19	0.61	0.49	8.28 and 8.13 (2 s, 2 × 2 H, 2 H-2 and 2 H-8), 7.22 (br s, 4 H, 2 NH_2), 6.26 (d, 2 H, 2 H-1′, $J_{1',2'}$ = 4.9 Hz), 6.1–5.6 (br s, 4 H, 2 OH-2′ and 3′), 4.21, 4.12, and 3.9 (3 m, 2 and 2 and 6 H, 2 H-2′,3′,4′,5′, and 5″)	2.16	597 $(M + H)^+$
21	0.66	0.64	8.23 and 8.14 (2 s, 2 × 1 H, H-2 and H-8), 7.8–7.0 (br s, 1 H, OH-2′ or 3′), 7.3 (br s, 2 H, NH_2), 6.58 (d, 1 H, HP, J_{PH} = 593.6 Hz), 6.26 (d, 1 H, H-1′, $J_{1',2'}$ = 4.2 Hz), 6.2–5.4 (br s, 1 H, OH-2′ or 3′), 4.15 and 3.9 (2 m, 2 and 3 H, H-2′,3′,4′,5′, and 5″)	2.39	376 $(M - H + 2\ Na)^+$, 354 $(M + Na)^+$, 332 $(M + H)^+$
25	0.77	0.65	8.19 and 8.12 (2 s, 2 × 2 H, 2 H-2, 2 H-8), 7.21 (br s, 4 H, 2 NH_2), 6.7–6.6 (br s, 2 H, 2 OH-2′ or 5′), 6.33 (d, 2 H, 2 H-1′, $J_{1',2'}$ = 6.0 Hz), 5.5–5.3 (br s, 2 H, 2 OH-2′ or 5′), 4.6, 4.5, 3.95, and 3.75 (4 m, 2 and 2 and 2 and 4 H, 2 H-2′,3′,4′,5′, and 5″)	1.84	597 $(M + H)^+$
27	0.60	0.47	8.22, 8.18, 8.14, 8.13 (4 s, 4 H, 2 H-2 and 2 H-8), 7.22 (br s, 4 H, 2 NH_2), 6.32 (m, 2 H, 2 H-1′), 5.75 and 5.70 (2 br s, 2 × 2 H, 2 OH-2′ and 3′), 4.4–4.1 and 3.95 (2 m, 8 and 2 H, 2 H-2′,3′,4′,5′, and 5″), 1.46 (d, 3 H, CH_3P, J_{PCH_3} = 17.3 Hz)	34.19	595 $(M + H)^+$
29	0.47	0.15	8.20, 8.18, and 8.14 (3 s, 2 H, H-2 and H-8), 7.25 (br s, 2 H, NH_2), 6.32 (d, 1 H, H-1′, $J_{1',2'}$ = 3.6 Hz), 5.80 and 5.74 (2 d, 2 × 1 H, OH-2′ and 3′), 4.4–4.1 and 4.0 (2 m, 4 and 1 H, H-2′,3′,4′,5′, and 5″), 3.59 and 3.58 (2 d, 3 H, CH_3OP, J_{POCH_3} = 11.07 Hz), 1.45 (d, 3 H, CH_3P, J_{PCH_3} = 17.4 Hz)	33.03, 32.71	719 $(2M + H)^+$, 360 $(M + H)^+$
32	0.68	0.48 and 0.51	8.22, 8.21, 8.20, 8.18, 8.14, 8.13, and 8.12 (7 s, 4 H, 2 H-2 and 2 H-8), 7.26 and 7.23 (2 s, 2 × 2 H, 2 NH_2) 6.3 (m, 2 H, 2 H-1′), 6.2–5.4 (br s, 4 H, OH-5′, OH-3′, and 2 OH-2′), 4.8 and 4.5–3.5 (m and m, 10 H, 2 H-2′,3′,4′,5′, and 5″), 1.56 and 1.54 (2 d, 3 H, CH_3P, J_{PCH_3} = 17.6 and 17.5 Hz)	32.88, 32.09	595 $(M + H)^+$
34	0.74	0.50	8.21 and 8.13 (2 s, 2 × 2 H, 2 H-2 and 2 H-8), 7.24 (br s, 4 H, 2 NH_2), 6.33 (d, 2 H, 2 H-1′, $J_{1',2'}$ = 4.9 Hz), 6.0 and 5.2 (2 br s, 2 × 2 H, 2 OH-2′, and 5′), 4.95 (m, 2 H, 2 H-3′), 4.50 (m, 2 H, 2 H-2′), 4.07 (m, 2 H, 2 H-4′), 3.7 (m, 4 H, 2 H-5′,5″), 1.70 (d, 3 H, CH_3P, J_{PCH_3} = 17.6 Hz)	32.42	595 $(M + H)^+$

[a] Eluents (A) isopropanol–20% aqueous ammonia–water, 7:2:1 v/v/v; (B) 1 M aqueous ammonium acetate–ethanol, 2:8 v/v. R_f or araA: 0.72 (in system A) and 0.62 (in system B). R_f of araAMP: 0.19 (in system A) and 0.07 (in system B). [b] In Me_2SO-d_6. [c] In Me_2SO-d_6 + D_2O.

proach.[37,38,42,49,50,54,65] In the present work the former alternative was used to prepare symmetrical diaraA-(5′→5′)-(27) and -(3′→3′) (34) methylphosphonates and the latter to prepare dissymmetrical diaraA-(3′→5′)-methyl-

Table II. Inhibitory Effects of Unprotected Derivatives of araA on the Proliferation of Murine Leukemia (L1210), Murine Mammary Carcinoma (FM3A), Human B-Lymphoblast (Raji), and Human T-Lymphoblast (Molt/4F) Cells

compd[b]	ID_{50},[a] μg/mL L1210	FM3A	Raji	Molt/4F
13	22.2 ± 0.8	22.4 ± 2.0	30.0 ± 6.5	13.6 ± 3.7
16	10.0 ± 4.7	20.4 ± 4.4	32.3 ± 0.8	23.3 ± 6.6
19	>100	86.6 ± 9.5	>100	43.0 ± 9.4
21	30.2 ± 5.0	40.4 ± 2.3	64.0 ± 8.7	34.2 ± 17.0
25	>100	>100	>100	>100
27	>100	>100	>100	>100
29	>100	>100	>100	>100
32	>100	>100	>100	>100
34	>100	>100	>100	74.7 ± 18.6
araA (1)	21.0 ± 5.5	21.1 ± 2.3	25.9 ± 1.4	16.7 ± 7.9
araAMP	24.2 ± 2.9	23.9 ± 3.3	32.6 ± 4.5	18.5 ± 5.5

[a] 50% inhibitory dose ± standard deviation. [b] It was ascertained by TLC that synthesized compounds did not contain any visuable contamination of monomeric araA.

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Table III. Antiviral Activity of Unprotected Derivatives of araA in Primary Rabbit Kidney Cell Cultures against Herpes Simplex Virus, Vaccinia Virus, and Vesicular Stomatitis Virus[a]

		minimum inhibitory concentration,[c] μg/mL									
		herpes simplex virus type 1					herpes simplex virus type 2				
compd	minimum cytotoxic concentration,[b] μg/mL	(strain KOS)	(strain F)	(strain McIntyre)	TK⁻ (strain B2006)	TK⁻ (strain VMW1837)	(strain G)	(strain 196)	(strain Lyons)	vaccinia virus	vesicular stomatitis virus
13	>200	20	10	10	20	20	7	7	2	0.2	>100
16	100	20	10	7	20	20	7	20	2	2	>40
19	>400	70	70	70	300	150	70	100	20	7	>400
21	>400	300	150	200	300	150	70	150	70	20	>400
25	>400	>400	>400	>400	>400	>400	>400	>400	>400	300	>400
27	>400	>400	>400	>400	>400	>400	>400	>400	>400	100	>400
29	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
32	>200	150	150	150	>200	>200	150	>200	70	20	>200
34	>400	300	150	150	300	150	70	200	70	20	>400
araA (1)	>100	20	7	10	20	20	7	20	7	2	20
araAMP	>100	20	7	10	20	20	7	20	2	0.2	>400

[a] Methods are described in ref 76. [b] Required to cause a microscopically detectable alteration of normal cell morphology, when incubated with the cells for the same duration as required to measure antiviral activity. [c] Required to reduce virus-induced cytopathogenicity by 50%. Viral cytopathogenicity was recorded as soon as it reached completion in the control virus-infected cell cultures. The multiplicity of infection (MOI) was invariably 100 × $CCID_{50}$, that is 100 times the virus dose needed to infect 50% of the cells.

Table IV. Antivaccinia Activity of Unprotected Derivatives of araA in African Green Monkey Kidney (Vero), Aneuploid (Hela), and Diploid (E_6SM) Human Cell Cultures[a]

	minimum cytotoxic concentration,[b] μg/mL			minimum inhibitory concentration,[c] μg/mL (vaccinia virus)		
compd	Hela	Vero	E_6SM	Hela	Vero	E_6SM
13	≥200	>400	>400	20	20	2
16	100	>40	≥400	20	20	10
19	>400	>400	≥400	200	70	20
21	≥400	>400	>400	70	70	7
25	≥400	≥400	>400	>400	>400	>400
27	≥400	>400	>400	>400	>400	>400
29	≥400	≥200	>400	>400	>400	>400
32	>100	>100	>100	>40	>100	>40
34	≥400	≥400	>400	150	300	70
araA (1)	>200	≥400	>400	20	70	7
araAMP	≥400	≥200	≥400	20	20	7

[a-c] Footnotes as in Table III.

phosphonate (**32**).

On the one hand, methyldichlorophosphine was treated with 2 equiv of **10** or **7** at −10 °C in tetrahydrofuran. The resulting dinucleoside methylphosphonic acid diesters were not isolated but subsequently oxidized with *tert*-butyl hydroperoxide to afford the protected symmetrical (5′→5′) or (3′→3′) linked methylphosphonates **26** and **33**. When methyldichlorophosphine was treated successively with **10** and methanol in an one-pot reaction, the protected araA 5′-*O*-(methyl methylphosphonate) (**28**) was obtained.

On the other hand, reaction of **7** with in situ prepared methylphosphonic bis(triazolide) occurred readily to give after workup a mixture of araA 3′-methylphosphonate triethylammonium and pyridinium salts (**30**). The latter was coupled with 5′-unprotected building block **10** in the presence of the activating agent MSNT to afford the protected dissymmetrical (3′→5′) linked methylphosphonate (**31**) (Scheme IV).

All protected araA methylphosphonate derivatives **26**, **28**, **31**, and **33** were isolated in satisfactory yields after workup and purification by silica gel short column chromatography. They were deprotected by subsequent treatment with trifluoroacetic acid in methylene chloride to afford, after purification, the pure nonionic compounds **27**, **29**, **32**, and **34**. The diastereomer pairs of **29** and **32** were not separated.

Purity and structural assignments for the reported compounds are based on their physical properties (Table I).

Biological Evaluation

Antiproliferative Activity. Among the nine araA derivatives tested for their inhibitory effects on the proliferation of exponentially growing murine L1210 and FM3A and human Raji and Molt/4F cells, the araA-(3′→5′)-araA (**13**) and araA-(3′→5′)-xyloA (**16**) proved to be the most active (Table II). Their ID_{50} values for the different tumor cell lines were similar to those of the reference compounds araA (1) and araAMP. The monomeric araA 5′-(hydrogen phosphonate) (**21**) was also effective, but 2–3-fold less active than araA (1). All the other compounds were virtually inactive, except for the araA-(5′→5′)-araA (**19**) which showed a moderate inhibitory effect on FM3A and Molt/4F cell proliferation.

Antiviral Activity. The araA derivatives were evaluated in vitro for their antiviral activity in comparison with araA (1) and araAMP (Table III). The test system con-

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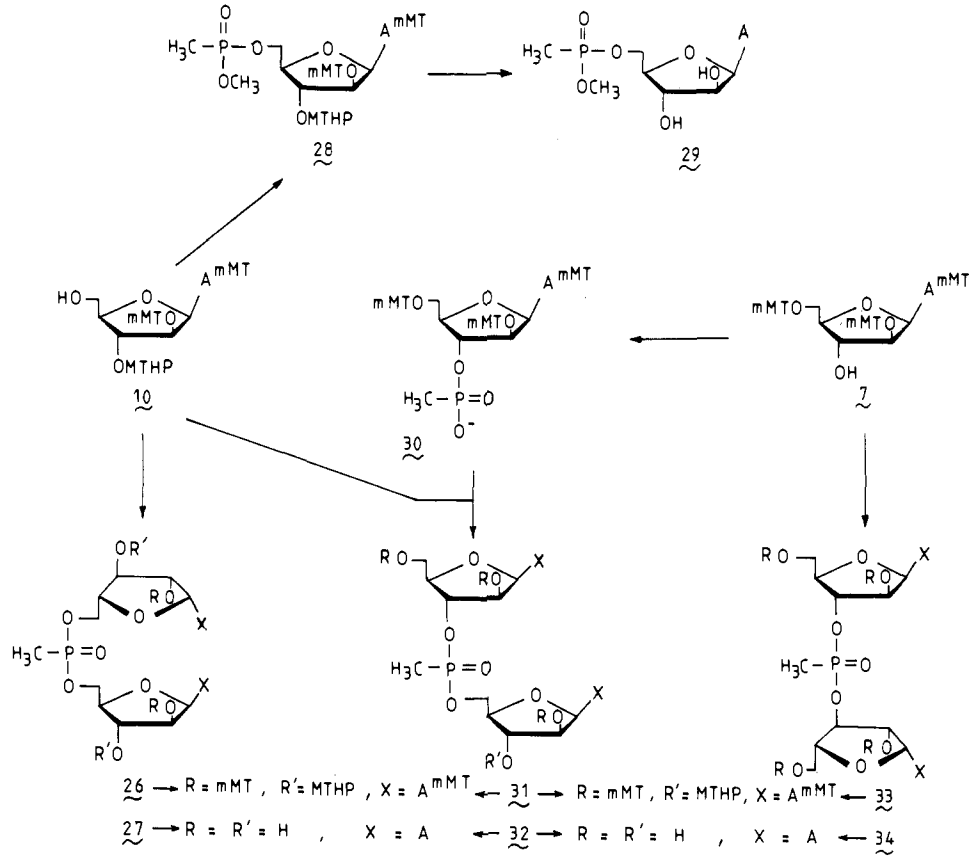
Scheme IV



sisted of primary rabbit kidney (PRK) cells infected with herpes simplex virus type 1 [HSV-1 (strains KOS, F or McIntyre)], herpes simplex virus type 2 [HSV-2 (strains G, 196 or Lyons)], vaccinia virus, vesicular stomatitis virus, or thymidine kinase (TK) deficient HSV-1 (strains B2006 and VMW 1837). None of the test compounds caused a microscopically detectable alteration of host-cell morphology at a concentration lower than 100 μg/mL. The relatively poor cytotoxicity of araA and its derivatives for PRK cells is due to the confluent state and thus the resting condition of the cells. Antimetabolites such as araA are known to possess little, if any, toxicity for cells with a low metabolic activity such as confluent PRK, HeLa, Vero or E_6SM cells (Tables III and IV). None of the compounds exhibited a significant activity against vesicular stomatitis virus. Akin to the reference compounds araA and araAMP, derivatives **13** and **16** exhibited significant antiviral activity against DNA viruses (HSV-1, HSV-2 and vaccinia). Compounds **19**, **21**, **32**, and **34** were also active against vaccinia virus, albeit at higher concentrations than **13** and **16**.

In additional experiments aimed at assessing the antivaccinia activity of the compounds, it was ascertained that **13** and **16**, and to a lesser extent **19** and **21**, showed an inhibitory effect on vaccinia growth in test systems other than PRK cells, namely African green monkey kidney (Vero), aneuploid (Hela), and diploid (E_6SM) human cell cultures (Table IV).

In terms of a structure–function relationship, it follows from these studies that (i) the methylphosphonate derivatives are devoid of activity, possibly because of their nuclease resistance[37,38] [this may also apply for the symmetrical (3′→3′) dinucleoside monophosphate (**25**)]; (ii) the dissymmetrical (3′→5′) dinucleoside monophosphates (**13** and **16**) are the more active compounds [as has been stipulated for the araC dinucleoside phosphates,[72,73] these compounds may enter the cell as such or be first cleaved to the nucleoside and nucleotide before entering the cell]; and (iii) the monoionized araA 5′-(hydrogen phosphonate) (**21**) has some cytostatic and antiviral effects, in particular against vaccinia virus.

Enzymatic Hydrolysis of araA Dinucleoside Methylphosphonate Derivatives. Phosphodiesterase I progressively converted araAMP and araA to araHX. This is obviously due to contaminating phosphatase and adenosine deaminase activity in the enzyme preparation. When compounds **13**, **27**, **32**, and **34** were incubated in the presence of phosphodiesterase I, araHX could be detected after 4 or 24 h in the incubation mixture of compounds **13** and **34**, respectively. These observations indicate that compounds **13** and **34** are first hydrolyzed to araA and araAMP (or araA 3′-methylphosphonate), whereafter araAMP is dephosphorylated by contaminating phosphatases followed by deamination of araA to araHX.

Phosphodiesterase II had no effect on araA, araHX, and compounds **27** and **32**. However, araAMP was extensively converted to araA presumably by contaminating phosphatases. Also **13**, and to a much lesser extent **34**, released araA as the principal reaction product, indicating an initial hydrolysis of **13** and **34** to araA and araA 3′-phosphate (or araA 3′-methylphosphonate) and subsequent dephosphorylation of araA 3′-phosphate to araA.

Freshly prepared human serum did not affect araHX, slowly deaminated araA, and converted araAMP after 24 h to araHX presumably via araA. Incubation of compound **13** with human serum released small amounts of araA after 4 h incubation and considerable amounts of araA (and

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araHX) after 48 h incubation. While compounds **27** and **32** were not affected after 48 h of incubation, compound **34** showed traces of hydrolysis products after 4–24 h.

In conclusion, while phosphodiesterase I (that releases 5′-nucleotides from 3′,5′-phosphodiesters of poly- or oligonucleotides), phosphodiesterase II (that releases 3′-nucleotides from 3′,5′-phosphodiesters of poly- or oligonucleotides), and human serum easily hydrolyzed the 3′,5′-phosphodiester dimer of araA (**13**), and left the 5′,5′- and 3′,5′-methylphosphonate diester dimers (compounds **27** and **32**) unaffected after 48 h of incubation, they released some hydrolysis products from compound **34** after 24–48 h of incubation. This means that the 5′,5′- and 3′,5′-methylphosphonate araA diesters are highly resistant to enzymatic attack by phosphodiesterases, while the 3′,3′-methylphosphonate araA diester is slowly degraded. These observations are clearly reflected by the similar biological (i.e. antiviral and cytostatic) activities of araA and its 3′,5′-phosphodiester dimer **13**, while in contrast, the methylphosphonate araA dimers (**27**, **32**, and **34**) had only poor, if any, biological activity. Thus, it became clear from this study that the main inhibitory effects of the dinucleotides are due to the intra- or extracellular release of araAMP or araA.

Experimental Section

Chemical Synthesis. General procedures and instrumentation used were described in ref 74. Proton nuclear magnetic resonance of unprotected compounds was determined at ambient temperature with a Bruker WM 360 WB spectrometer. ^{31}P NMR spectra were recorded with proton decoupling on a Bruker WP 200 SY instrument at 81.015 MHz; chemical shifts (parts per million) are reported relative to external H_3PO_4. Fast-atom bombardment mass spectra (FAB-MS) were recorded in the positive-ion mode on a JEOL DX300 mass spectrometer, with a JMA-DA 5000 mass data system; xenon was used for the atom gun at 3 kV with a total discharge current of 20 mA and the matrix was glycerol. The FAB-MS of **13** has been previously described.[75]

***N*^{6}-(4-Monomethoxytrityl)-9-[2-*O*-(4-monomethoxytrityl)-β-D-arabinofuranosyl]adenine (6).** Compound **4**[9,10] (36.2 g, 71.0 mmol) was first coevaporated three-times with anhydrous pyridine and then dissolved in pyridine (560 mL), and 4-monomethoxytrityl chloride (mMTCl, 43.85 g, 142 mmol) was added to the stirred solution at 65 °C. Heating of the reaction mixture was continued for 10 h, during this time additional mMTCl (7 × 21.9 g, 7 × 71.0 mmol) was added equivalent by equivalent. After 3 days (total reaction time) at 65 °C, the reaction mixture was cooled at room temperature and poured into saturated aqueous sodium bicarbonate, and the mixture was extracted into the organic phase with methylene chloride. The combined organic layers were washed once with water, dried over sodium sulfate, evaporated to dryness, and reevaporated three times in toluene solvent and then in acetonitrile. The residue containing **5** was dissolved in acetonitrile (1 L), and tetraethylammonium bromide (88.7 g, 422 mmol), potassium fluoride (24.5 g, 422 mmol), and water (21.3 mL) were added. The resulting mixture was stirred at 50 °C for 1 h and then poured into saturated aqueous sodium bicarbonate after being cooled at room temperature. Successive usual extractive workup gave a syrup, which was precipitated from hexane to afford crude **6**. Further purification was accomplished by silica gel column chromatography using as eluent a stepwise gradient of ethyl acetate (5–50%) in methylene chloride. Pooling and evaporation of the appropriate fractions as indicated by TLC gave pure **6** (41.6 g, 72%), which was precipitated from hexane: mp 150–152 °C; UV (MeOH) 274 nm (26.7), λ_{min} 250 nm (13.9); ^1H NMR ($CDCl_3$) δ 7.92 and 7.44 (s and s, 1 and 1 H, H-2 and H-8), 7.4–6.4 (m, 29 H, 2 mMT and NH), 5.57 (d, 1 H, H-1′, $J_{1',2'}$ = 6.0 Hz), 4.4–4.9 (m, 5 H, H-2′,3-′,4′ and OH-3′,5′), 3.5–3.7 (m partially obscured by CH_3O, 2 H, H-5′, 5″), 3.61 and 3.68 (s and s, 3 and 3 H, 2 CH_3O). Anal. ($C_{50}H_{45}O_6N_5$) C, H, N.

***N*^{6}-(4-Monomethoxytrityl)-9-[2,5-bis-*O*-(4-monomethoxytrityl)-β-D-arabinofuranosyl]adenine (7).** Compound **6** (10.6 g, 13.1 mmol) was dissolved in anhydrous pyridine (150 mL). mMTCl (4.85 g, 15.72 mmol) was added, and the reaction mixture was stirred at room temperature for 10 h. Saturated aqueous sodium bicarbonate was added, and the resulting mixture was extracted with methylene chloride. The combined organic layers were washed with water and then dried over sodium sulfate and evaporated to leave crude **7**, which was precipitated from hexane. Pure **7** (11.1 g, 78%) was isolated after silica gel column chromatography of the precipitate, using as eluent a stepwise gradient of ethyl acetate (0–50%) in hexane. The compound was lyophilized from benzene: UV (MeOH) λ_{max} 274 nm (18.8), λ_{min} 251 nm (11.0); ^1H NMR ($CDCl_3$) δ 8.08 and 7.96 (s and s, 1 and 1 H, H-2 and H-8), 7.5–6.5 (m, 43 H, 3 mMT and NH), 5.90 (d, 1 H, H-1′, $J_{1',2'}$ = 5.1 Hz), 4.35 (m, 1 H, H-2′), 3.80 (m, 2 H, H-3′ and 4′), 3.77 and 3.64 (s and s, 6 and 3 H, 2 and 1 CH_3O), 3.48 and 3.12 (m and m, 1 and 1 H, H-5′ and 5″), 1.89 (br s, 1 H, OH-3′). Anal. ($C_{70}H_{61}N_5O_7$) C, H, N.

***N*^{6}-(4-Monomethoxytrityl)-9-[2-*O*-(4-monomethoxytrityl)-5-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl]adenine (8).** A solution of **6** (30.0 g, 36.95 mmol) and *tert*-butyldimethylsilyl chloride (6.12 g, 40.6 mmol) in anhydrous pyridine (150 mL) was stirred at room temperature for 5 h. Saturated aqueous sodium bicarbonate was added, and the resulting mixture was extracted with methylene chloride. The combined organic layers were washed once with water, dried over sodium sulfate, evaporated to dryness, and reevaporated three times in toluene solvent. The residue was chromatographed on a silica gel column with methylene chloride–ethyl acetate (95:5) to afford 30.8 g (90%) of pure **8**. This compound was lyophilized from benzene: UV (MeOH) λ_{max} 275 nm (25.3), λ_{min} 252 nm (13.0); ^1H NMR ($CDCl_3$) δ 7.98 and 7.87 (s and s, 1 and 1 H, H-2 and H-8), 7.6–6.4 (m, 29 H, 2 mMT and NH), 5.75 (d, 1 H, H-1′, $J_{1',2'}$ = 5.0 Hz), 4.6–3.3 (m partially obscured by CH_3O, 5 H, H-2′,3′,4′,5′ and 5″), 3.70 and 3.56 (s and s, 3 and 3 H, 2 CH_3O), 2.55 (d, 1 H, OH-3′, J = 2 Hz), 0.87 (s, 9 H, $SiC(CH_3)_3$), 0.02 (s, 6 H, $Si(CH_3)_2$. Anal. ($C_{56}H_{59}O_6N_5Si$) C, H, N, Si.

***N*^{6}-(4-Monomethoxytrityl)-9-[2-*O*-(4-monomethoxytrityl)-3-*O*-(methoxytetrahydropyranyl)-β-D-arabinofuranosyl]adenine (10).** Compound **8** (25.0 g, 27.0 mmol) was dissolved in anhydrous dioxane (270 mL), and then 5,6-dihydro-4-methoxy-2*H*-pyran (MDHP, 15.4 g, 135 mmol) and *p*-toluenesulfonic acid monohydrate (2.57 g, 13.5 mmol) were added to the stirred solution at room temperature. Additional MDHP (2 × 4.9 g, 2 × 43.2 mmol) was added after 1 and 2.5 h. When TLC indicated the reaction was complete (8.5 h total reaction time), the mixture was neutralized with methanolic ammonia (previously saturated at −10 °C, ca. 4 mL) and poured into saturated aqueous sodium bicarbonate. The product was extracted into the organic phase with methylene chloride. The combined organic layers were washed with water, dried over sodium sulfate, evaporated to dryness, and reevaporated with tetrahydrofuran (THF). The residue containing **9** was dissolved in dry THF (150 mL), and a 1 M solution of tetra-*n*-butylammonium fluoride in THF (150 mL) was added. The solution was stirred for 1.5 h at ambient temperature and then poured into saturated aqueous sodium bicarbonate and extracted with methylene chloride. The organic layers were combined, washed with water, dried over sodium sulfate, and evaporated to dryness. The resulting crude material was first precipitated from hexane and then chromatographed on a silica gel column with a stepwise gradient of acetone (0–25%) in cyclohexane to afford 22.3 g (89%) of pure **10**. This compound was lyophilized from benzene: UV (MeOH) λ_{max} 275 nm (25.6), λ_{min} 251 nm (13.9); ^1H NMR ($CDCl_3$) δ 7.94 and 7.82 (s and s, 1 and 1 H, H-2 and H-8), 7.4–6.5 (m, 29 H, 2 mMT and NH), 5.69 (d, 1 H, H-1′, $J_{1',2'}$ = 4.9 Hz), 4.82 (m, 1 H, H-3′), 4.62 (m, 1 H, H-2′), 4.05 (m, 2 H, H-4′ and 5′), 3.90 (m, 1 H, H-5″), 3.8–3.7 and 3.6–3.4 (m and m partially obscured by CH_3, 4 H, H-α of MTHP), 3.76 and 3.61 (s and s, 3 and 3 H, CH_3O of mMT), 3.07 (s, 3 H, CH_3O of MTHP), 3.0 (br s, 1 H, OH-5′),

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1.9–1.7 (m, 4 H, H-β of MTHP). Anal. ($C_{56}H_{55}N_5O_8$)C, H, N.

Arabinofuranosyladenylyl(3′→5′)arabinofuranosyladenine (13). A solution of 1,2,4-triazole (3.23 g, 47.53 mmol), 2-chlorophenyl phosphorodichloridate (4.48 g, 18.28 mmol) and triethylamine (3.88 g, 38.39 mmol) in anhydrous acetonitrile (36 mL) was stirred for 0.5 h at room temperature. This mixture was added to a solution of N^6-benzoyl-9-[2,5-di-*O*-(4-monomethoxytrityl)-β-D-arabinofuranosyl]adenine[6] (**2**) (6.7 g, 7.31 mmol) in pyridine (40 mL). The reaction mixture was stirred for 0.5 h, and a solution of triethylamine (4.6 g) and water (3 mL) in pyridine (15 mL) was then added. After 15 min the mixture was diluted with saturated aqueous sodium bicarbonate (700 mL) and repeatedly extracted with chloroform. The combined extracts were washed with saturated aqueous sodium bicarbonate and water, dried over sodium sulfate, and evaporated. The resulting gum was precipitated from petroleum ether to afford 11 (7.9 g, 90% after exhaustive drying in vacuo), which was chromatographically pure. This material was used directly without further purification.

Compound 11 (3.3 g, 2.73 mmol) and N^6-benzoyl-9-(2,3-di-*O*-benzoyl-β-D-arabinofuranosyl)adenine[7] (**3**) (1.43 g, 2.47 mmol) were first coevaporated three times with anhydrous pyridine and then dissolved in pyridine (14 mL), and 1-mesitylene-2-sulfonyl-3-nitro-1,2,4-triazole[25] (MSNT, 1.99 g, 6.83 mmol) was added to the stirred solution at room temperature. After 40 min, saturated aqueous sodium bicarbonate (3 mL) was added and the stirring continued for 15 min. The reaction mixture was poured into saturated aqueous sodium bicarbonate and repeatedly extracted with chloroform. The combined chloroform extracts were dried over sodium sulfate, filtered, and evaporated to dryness. The residue was reevaporated three times in toluene solvent and then chromatographed on a silica gel column with chloroform–methanol (99:1) to afford 3.23 g (78%) of fully blocked dimer **12** as a mixture of diastereoisomers.

A solution of *syn*-4-nitrobenzaldoxime[25] (9.56 g, 57.5 mmol) and triethylamine (5.81 g, 57.5 mmol) in dioxane–water (1:1 v/v, 180 mL) was added to dimer **12**, and the reaction mixture was stirred at 40 °C in a stopped flask for 16 h. The product was then concentrated under reduced pressure, and the resulting gum was redissolved in aqueous ammonia (*d* 0.92, 400 mL) in a pressure bottle. After 20 h at 40 °C, the solvent was removed in vacuo and coevaporated with water. The residue was dissolved in 80% aqueous acetic acid (200 mL) and stirred at room temperature for 10 h. The reaction mixture was diluted with water and repeatedly extracted first with chloroform and then with ether. The aqueous layer was evaporated to dryness, and the residue was coevaporated three times with water. The product was chromatographed on a DEAE-Sephadex A-25 column with triethylammonium bicarbonate buffer (TEAB, pH 7.5, linear gradient from 0.002 to 0.3 M) as eluent. The appropriate fractions were combined, evaporated, and reevaporated in water and then lyophilized to give **13**. The latter was contaminated by nonnucleotidic triethylammonium salt as verified by ^{1}H NMR spectroscopy. Precipitation from methanol–acetone (3:1 v/v, 100 mL) afforded pure **13** (triethylammonium form), which was two times passed through a column of Dowex 50 W × 2 (sodium form) ion-exchange resin to yield the sodium salt of **13** (0.51 g, 33% from **3** after lyophilization from water). The properties of **13** are presented in Table I.

Arabinofuranosyladenylyl(3′→5′)xylofuranosyladenine (16). This compound was prepared from 11 (3.3 g, 2.73 mmol) and N^6-benzoyl-9-(2,3-di-*O*-benzoyl-β-D-xylofuranosyl)adenine[24] (**14**) (1.43 g, 2.47 mmol) as described above for the synthesis of **13**. The properties of **16** (sodium salt, 30% yield from **14**) are presented in Table I.

Arabinofuranosyladenylyl(5′→5′)arabinofuranosyladenine (19). Method A. The following modifications were made in the procedure described above for the synthesis of **13**. Phosphorylation of **10** (0.128 g, 0.138 mmol) with 2-chlorophenyl phosphoryl ditriazolide in pyridine gave its 3′-(2-chlorophenyl) phosphate triethylammonium salt **17** (0.145 g, 89%). Subsequent reaction between **17** (0.125 g, 0.106 mmol) and the free 5′-OH nucleoside **10** (0.088 g, 0.095 mmol) in the presence of MSNT gave, after standard workup and silica gel column chromatography with a stepwise gradient of acetone (0–40%) in cyclohexane as eluent, the fully protected dimer **18** (0.163 g, 85%). Selective removal of the o-chlorophenyl group from phosphorus of **18** was accomplished with tetramethylguanidinium *syn*-4-nitrobenzaldoximate in aqueous dioxane. After the 2-chlorophenyl cleavage was complete, the solvent was removed in vacuo and the resulting gum was taken up in chloroform and water. The aqueous layer was extracted twice with chloroform, and the combined organic layers were dried over sodium sulfate, filtered, and evaporated to dryness. For the removal of 4-methoxytrityl and methoxytetrahydropyranyl groups the residue was dissolved in a solution of 2% trifluoroacetic acid in methylene chloride, and the reaction mixture was stirred at room temperature for 14 h. Water was added, and the reaction mixture was neutralized to pH ca. 7.0 by addition of 10% aqueous ammonia. After chloroform extraction, the aqueous layer was evaporated to dryness and the residue chromatographed on a DEAE-Sephadex A-25 column with TEAB buffer as eluent. Further purification of **19** was accomplished by preparative TLC (*i*-PrOH–NH_4OH (20%)–H_2O, 8:1:1; silica gel). Pure **19** was dissolved in water, filtered (HV-4 Millipore), transformed into its sodium salt, and lyophilized (0.036 g, 72% from **18**).

Method B. To a solution of imidazole (0.793 g, 11.67 mmol) in anhydrous acetonitrile (22 mL) were added, with stirring and cooling in an ice bath, PCl_3 (0.305 mL, 3.50 mmol) and triethylamine (1.71 mL, 12.28 mmol). The mixture was stirred for 15 min, and then a solution of dried 5′-OH-free nucleoside **10** (0.752 g, 0.81 mmol) in acetonitrile (22 mL) was added dropwise. After the addition of **10** was complete, the reaction mixture was stirred at room temperature for 4 h. Water (6 mL) was added, and the solution was stirred for another 30 min. The solvent was removed under vacuum, and the residue was reevaporated with a mixture of pyridine–triethylamine (4:1, v/v) three times and then taken up in chloroform (ca. 40 mL) and water (ca. 40 mL). The aqueous layer was extracted twice with chloroform. The combined organic layers were dried over sodium sulfate, filtered, and evaporated to dryness. After coevaporation with toluene, the residue was dissolved in a small amount of chloroform and precipitated from hexane to afford **20** (0.79 g, 90%). This compound was sufficiently pure (TLC and ^{1}H NMR analysis) to be used without further purification for the preparation of **21** and **22**.

Compounds **20** (0.34 g, 0.312 mmol) and **10** (0.317 g, 0.343 mmol) were coevaporated three times with anhydrous pyridine and then dissolved in the same solvent (6 mL). To this solution was added with stirring chloro diphenyl phosphate (0.214 mL, 1.03 mmol) and *N*-methylimidazole (0.177 g, 2.15 mmol). The resulting mixture was stirred at ambient temperature for 2 h, poured into water, and extracted with chloroform. The organic layers were dried over sodium sulfate, evaporated to dryness, and coevaporated with toluene. The residue was chromatographed on a silica gel column with a stepwise gradient of ethyl acetate (0–70%) in methylene chloride as eluent to afford pure (TLC and ^{1}H NMR analysis) **22** (0.36 g, 60%).

To a stirred solution of **22** (0.160 g, 0.084 mmol) in pyridine (1.5 mL) was added a mixture of iodine (0.047 g, 0.19 mmol) in pyridine–water (98:2, 0.3 mL). After 0.5 h, 0.5 M aqueous sodium bisulfite (0.6 mL) was added to destroy excess iodine, and the solution was diluted with water and then extracted with chloroform. The combined organic layers were washed with saturated aqueous sodium bicarbonate, dried over sodium sulfate, filtered, and evaporated to dryness. The continuation of the synthesis and the purification of **19** (0.039 g, 75% from **22** after lyophilization as its sodium salt) was carried out as described above in method A. The properties of **19** are presented in Table I.

9-β-D-Arabinofuranosyladenine 5′-(Hydrogen phosphonate) (Triethylammonium salt) (21). Compound **20** (0.44 g, 0.40 mmol) was dissolved in a solution of 1% trifluoroacetic acid in chloroform (12 mL), and the reaction mixture was stirred first at 0 °C for 4 h and then at room temperature for 8 h. The reaction mixture was neutralized to pH ca. 7.0 by addition of $^1/_{15}$ M phosphate buffer (pH 7.5), evaporated to dryness, and then coevaporated successively with pyridine, toluene, and ethanol. The residue was dissolved in water, and the aqueous solution was extracted with chloroform. The aqueous layer was evaporated to dryness, and the residue was chromatographed on a DEAE-Sephadex A-25 column with TEAB buffer as eluent. Further purification of **21** was accomplished by preparative TLC (*i*-PrOH–NH_4OH (20%)–H_2O, 7:2:1; silica gel). Pure **21** was dissolved in water, filtered (HV-4 Millipore), and lyophilized (0.085

g, 49% from **20**). The properties of **21** are presented in Table I.

Arabinofuranosyladenylyl(3′→3′)arabinofuranosyladenine (25). This compound was prepared as described above for the synthesis of **19** (method B). Phosphitylation of **7** (1.0 g, 0.922 mmol) with PCl_3 and triethylamine gave its 3′-(hydrogen phosphonate) triethylammonium salt **23** (1.08 g, 91%). Subsequent reaction between **23** (0.51 g, 0.408 mmol) and the free 3′-OH nucleoside **7** (0.40 g, 0.367 mmol) in the presence of chloro diphenyl phosphate and *N*-methylimidazole gave, after standard workup and silica gel column chromatography with a stepwise gradient of acetone (0–20%) in cyclohexane as eluent, the fully protected dimer **24** (0.50 g, 62%). From **24** (0.46 g, 0.208 mmol), oxidation with iodine followed by unblocking with trifluoroacetic acid gave crude **25**, which was chromatographed on a DEAE-Sephadex A-25 column with TEAB buffer as eluent. Further purification of **25** was accomplished by silanized silica gel column chromatography (RP-2, Merck No. 7719; eluent, water). Pure **25** was dissolved in water, filtered (HV-4 Millipore), transformed into its sodium salt, and lyophilized (0.092 g, 72% from **24**). The properties of **25** are presented in Table I.

Arabinofuranosyladenine 5′-(Arabinofuranosyladenylyl 5′-Methylphosphonate) (27). Methyldichlorophosphine (24.2 μL, 0.27 mmol) was added, with stirring and cooling at −10 °C under argon, to a solution of nucleoside **10** (0.50 g, 0.54 mmol) and collidine (178.4 μL, 1.35 mmol) in dry tetrahydrofuran (6 mL). The resulting mixture was allowed to warm up slowly to room temperature, and the stirring was continued for an additional 4 h. The resulting dinucleoside methylphosphonic acid diester was not isolated but oxidized to the corresponding methylphosphonate by addition of *tert*-butyl hydroperoxide (70%, 55.5 μL, 0.405 mmol). After the oxidation was complete (20 mn), the mixture was diluted with water and extracted with chloroform. The combined extracts were dried over sodium sulfate, filtered, and evaporated. The residue was chromatographed on a silica gel column with a stepwise gradient of ethyl acetate (50–90%) in cyclohexane as eluent. The fractions containing pure compound were pooled and evaporated to give **26** (0.37 g, 72%), which was dissolved in a solution of 1% trifluoroacetic acid in methylene chloride (35 mL). The reaction mixture was stirred first at 0 °C for 2 h and then at room temperature for 6 h. The reaction mixture was neutralized to pH ca. 7.0 by addition of $^1/_{15}$ M phosphate buffer (pH 7.5), diluted with methylene chloride, and extracted with water. The aqueous layer was evaporated to dryness, and the residue was chromatographed on a silanized silica gel column (RP-2, Merck No. 7719) with a stepwise gradient of methanol (0–20%) in water as eluent. The fractions containing the pure compound were pooled, evaporated, dissolved in water, filtered (HV-4 Millipore), and lyophilized to give **27** (0.071 g, 61%). The properties of **27** are presented in Table I.

9-β-D-Arabinofuranosyladenine 5′-*O*-(Methyl methylphosphonate) (29). Methyldichlorophosphine (48.5 μL, 0.54 mmol) in dry tetrahydrofuran (5 mL) was added under argon, with stirring and cooling at −78 °C, to a solution of nucleoside **10** (0.50 g, 0.54 mmol) and collidine (71.4 μL, 0.54 mmol) in tetrahydrofuran (5 mL). The mixture was stirred at −78 °C for 0.5 h, and then a mixture of methanol (43.7 μL, 1.08 mmol) and collidine (71.4 μL, 0.54 mmol) in tetrahydrofuran (3 mL) was added. The solution was allowed to warm up slowly to room temperature, and then *tert*-butyl hydroperoxide (70%, 110.9 μL, 0.81 mmol) was added. After 20 min the mixture was diluted with water and extracted with chloroform. The combined extracts were dried over sodium sulfate and evaporated. TLC of the residue (cyclohexane–acetone–ethylacetate, 1:1:8) showed a major spot (R_f 0.13), which corresponded to **28**, and two minor spots (R_f 0.42 and 0.25), which corresponded to **10** and **26**. This residue was chromatographed on a silica gel column with a stepwise gradient (50–90%) of ethyl acetate in cyclohexane as eluent. The combined fractions (0.23 g), containing **28** contaminated by some **26**, were dissolved in a solution of 1% trifluoroacetic acid in methylene chloride (30 mL). The reaction mixture was stirred first at 0 °C for 1 h and then at room temperature for 3 h. The reaction mixture was neutralized to pH ca. 7.0 by addition of $^1/_{15}$ M phosphate buffer (pH 7.5), diluted with methylene chloride, and extracted with water. The aqueous layer was evaporated to dryness, and the residue was chromatographed on a silica gel column with a stepwise gradient of methanol (0–20%) in chloroform as eluent. The combined fractions containing compound **29** were rechromatographed on a silanized silica gel column (RP-2, Merck No. 7719) with a linear gradient of methanol (0–50%) in water. The fractions containing the pure compound were pooled, evaporated, dissolved in water, filtered (HV-4 Millipore), and lyophilized to give **29** (0.042 g, 22% from **10**). The properties of **29** are presented in Table I.

Arabinofuranosyladenine 5′-(Arabinofuranosyladenylyl 3′-Methylphosphonate) (32). Methyl phosphonodichloridate (1.47 g, 11.07 mmol) was added to a solution of triazole (1.53 g, 22.14 mmol) and collidine (2.93 mL, 22.14 mmol) in dry pyridine (8 mL), and the resulting mixture was stirred at 0 °C for 1 h under argon. A solution of nucleoside **7** (0.80 g, 0.738 mmol) in dry pyridine (4 mL) was added, and the mixture was stirred at room temperature for 14 h. Solvent was evaporated under vacuum, and the residue was coevaporated successively with a mixture of pyridine–triethylamine (4:1, v/v) and toluene. The residue was dissolved in chloroform, and the solution was extracted with water. The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. The residue was dissolved in a small amount of chloroform and precipitated from pentane to afford **30** (0.91 g, quantitative). This compound was sufficiently pure (TLC and ^{1}H NMR analysis) to be used without further purification. Compound **30** (0.80 g, 0.65 mmol) and nucleoside **10** (0.54 g, 0.58 mmol) were first coevaporated three times with anhydrous pyridine and then dissolved in pyridine (8 mL), and MSNT (0.48 g, 1.63 mmol) was added to the stirred solution at room temperature. After 14 h saturated aqueous sodium bicarbonate (0.7 mL) was added, and the stirring was continued for 15 min. The reaction mixture was poured into saturated aqueous sodium bicarbonate and repeatedly extracted with chloroform. The combined chloroform extracts were dried over sodium sulfate, filtered, and evaporated to dryness. The residue was reevaporated three times in toluene solvent and then chromatographed on a silica gel column with a stepwise gradient of ethyl acetate (0–40%) in cyclohexane containing 10% of acetone as eluent to afford 0.70 g (58%) of fully blocked dimer **31** as a mixture of diastereoisomers. Unblocking of **31** and purification of **32** (0.10 g, 50% from **31**) were accomplished as described above for the synthesis of **27**. The properties of **32** are presented in Table I.

Arabinofuranosyladenine 3′-(Arabinofuranosyladenylyl 3′-Methylphosphonate) (34). This compound was prepared as described above for the synthesis of **27**. Nucleoside **7** (0.50 g, 0.46 mmol) in solution in tetrahydrofuran (6 mL) with collidine (152.0 μL, 1.15 mmol) was reacted with methyldichlorophosphine (20.6 μL, 0.23 mmol). Subsequent oxidation by addition of *tert*-butyl hydroperoxide (70%, 47.3 μL, 0.345 mmol) gave, after standard workup and silica gel column chromatography, the fully protected dimer **33** (0.31 g, 60%). Unblocking of **33** with trifluoroacetic acid afforded **34**, which was purified by RP-2 silanized silica gel column chromatography. The properties of **34** (41% yield from **33**) are presented in Table I.

Biological Methods. Assays on Cell Culture. The origin of the viruses and the techniques used for measuring inhibition of virus-induced cytopathogenicity have been described previously.[76,77]

Cytostatic assays were performed according to previously established procedures.[78,79]

Enzyme Hydrolysis Studies. The procedures to determine hydrolysis products of compounds **13**, **27**, **32**, and **34** were performed as recently described.[80] Briefly, compounds **27**, **32**, and

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34 were subjected to treatment with spleen phosphodiesterase I (10 units/mg of protein) (Merck, Darmstadt, West-Germany), snake venom phosphodiesterase II (0.019 unit/mg of protein) (Sigma, St. Louis, MO), and human serum. Incubation with the test compounds (1 mg/mL) were done with phosphodiesterase I (at 0.002 unit/mL) and phosphodiesterase II (at 0.2 units/mL) for 0, 1, 4, 24, and 48 h at 37 °C. Hydrolysis products were separated by thin-layer chromatography (TLC) and characterized by running appropriate standards in parallel. They were quantitated by estimation of the intensity of the spots on the TLC plates under UV. For comparison, compound 13, araAMP, araA, and ara-hypoxanthine (araHX) were incubated with the enzymes under identical experimental conditions, and the reaction products were analyzed.

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Registry No. 2, 82144-93-2; 3, 57018-83-4; 4, 87792-02-7; 5, 115561-31-4; 6, 115561-32-5; 7, 115561-33-6; 8, 115561-34-7; 9, 115561-35-8; 10, 115561-36-9; 11, 115590-40-4; 12 (diastereomer 1), 115590-41-5; 12 (diastereomer 2), 115650-42-5; 13, 88066-31-3; 14, 31079-98-8; 16, 115649-60-0; 17, 115561-38-1; 18, 115561-39-2; 19, 115561-40-5; 20, 115590-43-7; 21, 115561-42-7; 22, 115590-13-1; 23, 115561-44-9; 24, 115561-45-0; 25, 115561-46-1; 26, 115590-44-8; 27, 115561-47-2; 28, 115561-48-3; 29, 115561-49-4; 30, 115561-50-7; 31 (diastereomer 1), 115561-51-8; 31 (diastereomer 2), 115649-61-1; 32 (diastereomer 1), 115561-52-9; 32 (diastereomer 2), 115650-43-6; 33, 115561-53-0; 34, 115561-54-1.

Synthesis and Immunological Evaluation of N-Terminal, Noncrossreactive Tachykinin Antigens

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The N-terminal hexa- or pentapeptide sequences of the three mammalian tachykinins substance P, neurokinin A, and neurokinin B have been synthesized by the conventional solid-phase procedure with 6-aminocaproyl-*S*-(acetamidomethyl)cysteine as a C-terminal spacer and attachment function. A fourth sequence, with an additional N-terminal 6-aminocaproyl residue on the substance P–hapten sequence, was cyclized N- to C-terminally. For this purpose, a four-level protection scheme has been applied: BOC-TFA for N-terminal protection and cleavage; TFA-stable but HF-labile anchoring function and side-chain protection; *S*-acetamidomethyl for semipermanent thiol protection. The side chain amino function of Lys was protected with NO_2Z, stable against HF but readily cleaved with hydrogenation. The hapten sequences were coupled to maleimidated BSA, after the Acm group was removed by mercury/hydrogen sulfide treatment. Mice immunized with the three linear hapten sequences produced sera that were specific in enzyme-linked immunosorbant assay for the presented hapten and the respective tachykinin but displayed no crossreactivity at all toward the other haptens nor to one of the other tachykinins. It is concluded that this approach produced antisera, specific and selective for its respective mammalian tachykinins.

During the last few years, mammalian tachykinins have gained widespread attention. Substance P (SP) has been known for a very long time[1] and its physiological role is now considered to be that of a neurotransmitter[2] or a neuromodulator[3] peptide. Apart from SP, several other peptides from lower vertebrates belong to this family, all characterized by the same common C-terminal sequence—Phe-Xxx-Gly-Leu-Met-NH_2 (Xxx is either Val, Phe, or Tyr). For many years SP was the only tachykinin known to exist in mammals, and radioimmunoassays (RIA) were conducted assuming no other peptides reacted with the antisera. Recently, however, two new members of this family were discovered in mammals: neurokinin A (NKA)[4] and neurokinin B (NKB).[5] These new compounds have still mostly unknown, probably neurophysiological functions. Therefore, highly specific assays for each mammalian tachykinin are necessary in order to carry out conclusive studies on these agents.

Therefore, due to the particular chemistry of the tachykinins, most of the hitherto available immunoassays for SP were crossreactive with the other tachykinins. The chemically inert C-terminal heptapeptide of SP (see structural formulas below) did not permit the preparation of haptens with anything else exposed other than the C-terminus. All classical conjugation methods, like those using glutaraldehyde or dicyclohexylcarbodiimide conjugation, have always coupled the N-terminal part of SP and the other tachykinins to the carrier (e.g. BSA or thyroglobulin).

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