# Synthesis of 2′(3′)-O-Aminoacyl Triribonucleoside Diphosphates Using the Triester Method[1]

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Specific syntheses of 2′(3′)-O-aminoacyl triribonucleoside diphosphates, C-C-A-Phe (**16e**), C-C-A-Ala (**16f**), and C-C-A-Gly (**16g**), which are the terminal sequences of corresponding aa-tRNAs and potential substrates for ribosomal peptidyltransferase, are described. The compounds **16e–g** were synthesized by employing phosphotriester methods with a benzoyl group for protection of heterocyclic amino groups, a 2-chlorophenyl group for internucleotide phosphate protection, a monomethoxytrityl group for blocking of the 5′-hydroxy function, a 4-methoxytetrahydropyranyl group for protection of 2′-hydroxy functions, and an N-benzyloxycarbonyl group for blocking of the α-amino acid. Protected dinucleotide block C-Cp (**11b**) was synthesized via the triester method and was condensed by using mesitylenesulfonyl tetrazolide with nucleoside components **9b** and **10b**, which have aminoacyl residues incorporated in the molecule, to yield protected aminoacyl trinucleotides **13a** and **14** in 60–70% yields. The fully protected aminoacyl trinucleotides **13a** and **13b** were also obtained from the protected C-C-A derivative **12** (with a free 3′-OH group on the 3′-terminus) by the aminoacylation reaction with corresponding N-benzyloxycarbonyl amino acids and mesitylenesulfonyl tetrazolide in 50–70% yields. The protected derivatives **13a,b** and **14** were deblocked to form C-C-A(Z-Phe) (**16b**), C-C-A(Z-Ala) (**16c**), and C-C-A(Z-Gly) (**16d**) in 15–40% yields by reactions with $N_2H_4$, $F^-$, and $H^+$ (for **16b,c**) or $NH_4OH$ and $H^+$ (for **16d**). The final products **16e–g** were prepared by hydrogenolysis ($Pd/BaSO_4$) of **16b–d** in practically quantitative yields. The syntheses of all components (**3a,c**, **4**, **9a,b**, and **10b**) for the triester approach to aminoacyl trinucleotides are also described.

It is well established that the 3′-terminus of all transfer ribonucleic acids (tRNAs) contains the common C-C-A sequence and that its 3′-terminal adenosine *cis*-diol grouping is a site of attachment of the amino acid. 2′-(3′)-O-Aminoacyl oligonucleotides derived from the 3′-terminus of AA-tRNA are able to participate in various subreactions of protein biosynthesis, including the interactions with peptidyltransferase, and elongation factor $T_u$. Thus, these compounds serve as extremely important tools for the elucidation of the involvement of the 3′-terminus of AA-tRNA in a series of enzymatic reactions which lead to the specific incorporation of amino acids into proteins.[2]

In spite of considerable progress in the synthesis of oligoribonucleotides with defined sequences in recent years, the general method for specific syntheses of 2′(3′)-O-aminoacyl oligoribonucleotides with chain lengths longer than two units has not yet been reported. Mercer and Symons[3] have described the aminoacylation of partially protected C-C-A derivatives with *N-tert*-butyloxycarbonyl amino acids. The starting trinucleoside diphosphate had the amino groups of aglycons, as well as both 2′- and 3′-hydroxyl groups on the 3′ end and the phosphodiester functions left unprotected. Thus, this reaction resulted in formation of several side products and very low yields of desired compounds. Moreover, the enzymatic degradation[4] of the charged tRNA cannot provide all necessary compounds since this method is limited, in principle, to the sequences naturally occuring in particular tRNAs. A further limitation derives from the fact that the chain length of the resulting 2′(3′)-O-aminoacyl oligonucleotides would depend upon the specificity of the nucleolytic enzymes. Thus, there is a real need for the efficient and specific synthesis of 2′(3′)-O-aminoacyl oligoribonucleotides.

In our laboratory we have developed a general synthesis of 2′(3′)-O-aminoacyl dinucleoside phosphates.[5] This approach was based on the condensation of a specifically protected nucleoside 3′-phosphate with a 2′(3′)-O-[[(benzyloxycarbonyl)amino]acyl] nucleoside as effected by DCC. However, attempts to extend this relatively simple methodology to the synthesis of higher 2′(3′)-O-aminoacyl oligonucleotides were unfortunately not successful.

In this report, we describe two methods for the synthesis of 2′(3′)-O-aminoacyl trinucleoside diphosphates based on the triester method.[6] We also report on the synthesis of all components for the construction of the target compounds via the triester procedure.[7]

## Results and Discussion

**(1) Protecting Groups.** A successful scheme for the synthesis of 2′(3′)-O-aminoacyl oligoribonucleotides requires the judicious choice of protecting groups, due to the 2′(3′)-O-aminoacyl group which is extremely sensitive to hydrolysis even at the neutral pH. For achievement of this goal, the following functional groups have to be protected during the synthesis using the triester approach: (i) the hydroxyl groups of ribose moieties; (ii) the amino groups

(1) (a) Dedicated to the late Professor František Šorm. (b) This paper is No. 35 in the series "Aminoacyl Derivatives of Nucleosides, Nucleotides, and Polynucleotides". For a preceding report of this series see: Quiggle, K.; Kumar, G.; Ott, T. W.; Ryu, E. K.; Chládek, S. *Biochemistry* **1981**, *20*, 3480. (c) This investigation was generously supported by U.S. Public Health Service Research Grant No. GM-19111 from the National Institutes of Health, by Biomedical Research Grant No. SO-7-RR-05529, and by an institutional grant to the Michigan Cancer Foundation from the United Foundation of Greater Detroit. (d) For abbreviations used, see: "Handbook of Biochemistry"; Sober, H. A., Ed.; CRC Press: Cleveland, OH, 1970; Sections A and B. Other abbreviations: TEAB, triethylammonium bicarbonate; MeOThp, 4-methoxytetrahydropyran-4-yl; lev, levulinyl (4-oxopentan-1-yl); $C^{Bz}$, $N^4$-benzoylcytosine; $A^{Bz}$, $N^6$-benzoyladenine; Z-Phe, N-(benzyloxycarbonyl)-L-phenylalanine, and similar abbreviations for other amino acid derivatives; C-C-A-Phe, cytidylyl(3′–5′)cytidylyl(3′–5′)-2′(3′)-O-(L-phenylalanyl)adenosine, and similar abbreviations for other aminoacyl oligonucleotides; MST, mesitylenesulfonyl tetrazolide; DCC, dicyclohexylcarbodiimide.

(2) (a) Chládek, S. In "Biological Implications of Protein–Nucleic Acid Interactions"; Augustyniak, J., Ed.; Elsevier: Amsterdam, 1980; p 149. (b) Ringer, D.; Chládek, S. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2590.

(3) Mercer, T. F. B.; Symons, R. A. *Eur. J. Biochem.* **1972**, *28*, 38.

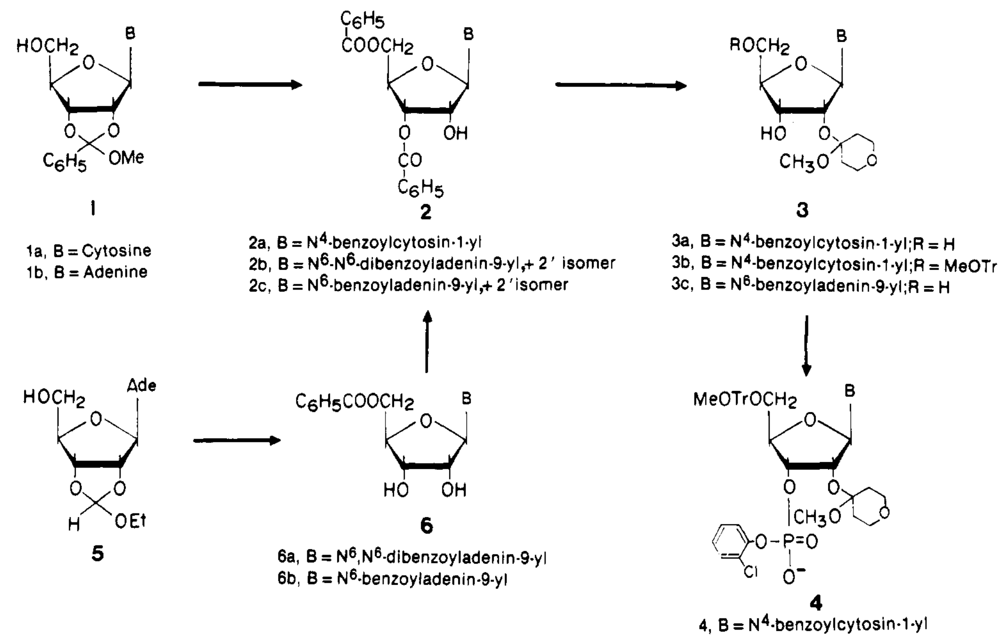
(4) Takanami, M. *Proc. Natl. Acad. Sci. U.S.A.* **1964**, *52*, 1271.

(5) (a) Chládek, S.; Žemlička, J. *J. Org. Chem.* **1974**, *39*, 2187. (b) Ryu, E. K.; Quiggle, K.; Chládek, S. *J. Carbohydr., Nucleosides, Nucleotides* **1977**, *4*, 387.

(6) Reese, C. B. *Tetrahedron* **1978**, *34*, 3143.

(7) The basic outline of this research has been presented at a meeting, and part of this work has been published in preliminary form. (a) Kumar, G.; Celewicz, L.; Chládek, S. 181st National Meeting of the American Chemical Society, Atlanta, GA, Apr 1981; American Chemical Society: Washington, DC, 1981; Abstract No. CARB 36. (b) Kumar, G.; Chládek, S. *Tetrahedron Lett.* **1981**, 827.

Scheme I




of aglycons; (iii) the phosphodiester bonds; (iv) the amino group of the amino acid.

In our blocking scheme, the 5′-hydroxyl group was protected by a monomethoxytrityl group, and the other positions (2′-OH) were blocked by a 4-methoxytetrahydropyran-4-yl group.[8] These groups are easily removable by using 0.05 N HCl without isomerization of phosphodiester linkages or the loss of the aminoacyl group.[5b,6] The exocyclic amino groups of nucleoside bases are usually protected against phosphorylation by using acyl groups such as the benzoyl group, and their removal is usually achieved with diluted ammonia.[6] Since these deblocking conditions are clearly not compatible with the preservation of the 2′(3′)-O-aminoacyl group, other deblocking conditions were sought. Hydrazine acetate is known to remove N-benzoyl groups from cytidine or adenosine without a significant cleavage of the O-benzoyl groups or the triester linkage.[9] However, it was found that the aminoacyl residue in, e.g., 2′(3′)-O-[N-(benzyloxycarbonyl)-L-phenylalanyl]adenosine was also hydrolyzed by hydrazine acetate under the conditions for removal of N-benzoyl groups (0.05 N $N_2H_4$ for 16 h), whereas the aminoacyl group in 3′-O-[N-(benzyloxycarbonyl)-L-phenylalanyl]-2′-deoxyadenosine proved to be stable. The same was found true if the neighboring (3′ or 2′) hydroxyl group of the 2′(3′)-O-[N-(benzyloxycarbonyl)-L-phenylalanyl]adenosine was blocked by a stable hydrazine protecting group such as, e.g., tetrahydropyranyl or 4-methoxytetrahydropyran-4-yl. Thus, it is evident that failure to block the 2′-OH results in the loss of the neighboring aminoacyl group in the reaction with hydrazine due to participation of the adjacent hydroxyl group.

The integrity of both the 2′-hydroxyl blocking group and the 3′-O-aminoacyl group must also be preserved during the removal of the groups protecting the phosphodiester linkage, which might otherwise lead to the cleavage and/or isomerization of the 3′,5′ phosphodiester linkage. Attempts to use the 2,2,2-trichloroethyl group for the protection of phosphate moiety[10] were, in general, unsuccessful due to low recovery, isolation problems, and partial reduction reactions of N-benzoylcytosine.[11] An alternative possibility of phosphorus protection by using an anilidate group[12] was also excluded because the deblocking conditions (amyl nitrite in slightly acidic medium) resulted in partial cleavage of the 2′-O-(4-methoxytetrahydropyran-4-yl) group.[13] The problem was ultimately resolved by the use of the 2-chlorophenyl group to protect the phosphodiester linkages. Although this group is normally removed during the oligonucleotide synthesis by using alkaline conditions,[6] it may also be displaced by $F^-$ under certain conditions[14] ($Bu_4N^+F^-$ in aqueous solution) without any adverse effect on the aminoacyl bond. It should be noted that the use of $F^-$ under anhydrous conditions, as suggested by Ogilvie et al.,[15] always resulted (in our hands) in partial cleavage of internucleotide bonds along with the complete loss of the amino acid (vide infra).

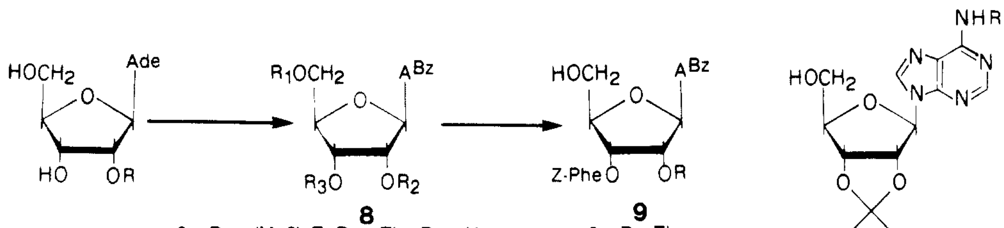
The α-amino group of the aminoacyl residue was protected with an N-benzyloxycarbonyl group which is uniquely suited for this purpose, since it is completely stable throughout the entire course of the synthesis and is removable by hydrogenolysis on palladium catalyst in the final step.[5]

**(2) Synthesis of Building Blocks.** The necessary reaction sequence dictates the use of the following building blocks for the synthesis of the 2′(3′)-O-aminoacyl derivatives of C-C-A: the 5′-terminal unit **4**, the extension unit **3a**, and the 3′-terminal units **3b**, **9a**,**b**, and **10b** (Schemes I and II). Components **4** and **3a** are similar to those used by other authors for the purpose of triester synthesis of oligoribonucleotides.[6,10] Nevertheless, we have developed new synthetic procedures to make these components much more readily available.

The key compound for the synthesis of cytidine components **3a** and **4** is $N^4,O^{3'},O^{2'}$-tribenzoylcytidine (**2a**).[16]

(8) Reese, C. B.; Saffhill, R.; Sulston, J. E. *Tetrahedron* **1970**, *26*, 1023.
(9) Letsinger, R. L.; Miller, P. S.; Grams, G. W. *Tetrahedron Lett.* **1961**, 2621.
(10) Neilson, T.; Werstriuk, E. S. *Can. J. Chem.* **1971**, *49*, 3004.
(11) Other authors have already reported relatively poor recoveries of unprotected oligoribonucleotides after the unblocking process involving Zn in DMF, as well as reductions of the $N^4$-benzoylcytidine moiety: (a) England, T. E.; Neilson, T. *Can. J. Chem.* **1976**, *54*, 1714. (b) Cook, A. T. *J. Org. Chem.* **1968**, *33*, 3589.
(12) Ohtsuka, E.; Murao, K.; Ubasawa, M.; Ikehara, M. *J. Am. Chem. Soc.* **1969**, *91*, 1537.
(13) Lee, H., unpublished experiments from our laboratory.
(14) Itakura, K.; Katagiri, N.; Bahl, C. P.; Whitman, R. H.; Narang, S. A. *J. Am. Chem. Soc.* **1975**, *97*, 7327.
(15) Ogilvie, K. K.; Beaucage, S. L.; Entuistle, D. N. *Tetrahedron Lett.* **1976**, 1255.

Scheme II




3c, R = MeOThp
7, R = Thp

8a, $R_1 = (MeO)_2Tr; R_2 = Thp; R_3 = H$
8b, $R_1 = Me_2(t\text{-}Bu)Si; R_2 = MeOThp; R_3 = H$
8c, $R_1 = MeCO(CH_2)_2CO; R_2 = MeOThp; R_3 = H$
8d, $R_1 = R_3 = MeCO(CH_2)_2CO; R_2 = MeOThp$
8e, $R_1 = (MeO)_2Tr; R_2 = Thp; R_3 = Z\text{-}Phe$
8f, $R_1 = Me_2(t\text{-}Bu)Si; R_2 = MeOThp; R_3 = Z\text{-}Phe$
8g, $R_1 = MeCO(CH_2)_2CO; R_2 = MeOThp; R_3 = Z\text{-}Phe$

9a, R = Thp
9b, R = MeOThp

10a, R = H
10b, $R = C_6H_5CO$

$A^{Bz}$ = $N^6$-benzoyladenin-9-yl

We have developed a short and efficient synthesis of **2a** via the benzoylation and acid-catalyzed opening of the cyclic ortho ester **1a**. The 3′ isomer **2a**, purified by crystallization, was shown by NMR spectroscopy to be completely free of the 2′ isomer. The reaction of **2a** with 4-methoxy-5,6-dihydro-2*H*-pyran catalyzed by anhydrous HCl followed by selective O-debenzoylation readily leads to derivative **3a**. The latter was readily converted to its 5′-*O*-monomethoxytrityl derivative **3b**, which on phosphorylation with (2-chlorophenyl)phosphobis[triazolide],[17] gave an excellent yield of the diester **4**. It is noteworthy that the diester **4** was free of side products, and, thereby, the possibility of the formation of compounds with a 3′–3′ bond is excluded.

Three different 3′-terminal units were employed in our present work. The component **10b** incorporates an alkali-stable ortho ester grouping which was readily prepared by benzoylation and O-debenzoylation of the glycine ortho ester derivative **10a**.[18] Due to the presence of the ortho ester linkage in **10b** and consequently in oligonucleotides incorporating **10b**, it was possible to hydrolyze both *N*-benzoyl and 2-chlorophenyl groups with ammonia. This approach is obviously precluded where ortho esters of the requisite amino acid are not available.[19] Therefore, a synthesis of the components of type **9** had to be developed for the optically active amino acids.[20] Compound **9a** was synthesized via 5′-dimethoxytritylation of starting material **7**, followed by the DCC-mediated aminoacylation with *N*-(benzyloxycarbonyl)-L-phenylalanine. The fully substituted intermediate **8e** was specifically deprotected at the 5′-position by brief treatment with 80% acetic acid without the cleavage of the 2′-*O*-tetrahydropyranyl group. We also sought a route which could employ the achiral 2′-*O*-(4-methoxytetrahydropyran-4-yl) group. Unfortunately, due to the increased acid lability of the 4-methoxytetrahydropyran-4-yl group relative to the tetrahydropyranyl group, the specific removal of the 5′-dimethoxytrityl group in the presence of the former was not possible. Therefore, the levulinic group was used for a block of the 5′-position, since this group is easily removed by brief treatment with hydrazine acetate.[21]

The reaction of **3c** with levulinic acid[21] resulted in the formation of both monolevulinyl (**8c**) and dilevulinyl (**8d**) derivatives as indicated by NMR data. The fact that **8c** is the 5′-*O*-levulinyl derivative follows from the following observations: (i) its reaction with monomethoxytrityl chloride was unsuccessful; (ii) the presence of a free *cis*-diol grouping (as indicated by positive reaction with periodate) in the compound obtained by acid hydrolysis from **8c**. The 5′-*O*-levulinyl derivative **8c** was aminoacylated with *N*-(benzyloxycarbonyl)-L-phenylalanine and mesitylenesulfonyl tetrazolide[22] (MST) to form the fully protected derivative **8g** in 84% yield. As was observed during the course of the other aminoacylation studies in the presence of MST (vide infra), these reactions were completed in a much shorter time and provided much better yields of products when compared to the use of DCC. Thus, in our opinion, MST appears to be an excellent aminoacylating reagent.

The selective removal of the 5′-*O*-levulinyl group[21] with $N_2H_4$ (2 min at room temperature) resulted in almost quantitative formation of **9b**, since both the aminoacyl residue and the *N*-benzoyl group are fully stable toward hydrazine under these conditions. On the other hand, the attempts to use 5′-*O*-*tert*-butyldimethylsilyl derivative **8f** for the preparation of **9b** were unsuccessful, since the aminoacyl group was also hydrolyzed under the conditions necessary for the removal of the silyl group from **8f** by using $F^-$ (cf. ref 23).

Component **3c** was used directly for the stepwise synthesis of the protected derivative of C-C-A (**12**) which was later aminoacylated (vide infra). In view of the relatively long synthetic routes described for **3c** or similar compounds,[24] a shorter route, analogous to that used for the synthesis of cytidine components (Scheme I), was developed. The adenosine cyclic orthobenzoate **1b**[25] was benzoylated, and the ortho ester intermediate thus obtained was hydrolyzed by brief treatment with acetic acid to form

(16) Fromageot, H. P. M.; Griffin, B. E.; Reese, C. B.; Sulston, J. E. *Tetrahedron* **1967**, *23*, 2315.

(17) (a) Katagiri, N.; Itakura, K.; Narang, S. A. *J. Am. Chem. Soc.* **1975**, *97*, 7332. (b) Chattopadhyaya, J. B.; Reese, C. B. *Tetrahedron Lett.* **1979**, 5059.

(18) Žemlička, J.; Chládek, S. *Collect. Czech. Chem. Commun.* **1966**, *31*, 3775.

(19) Only the synthesis of ortho esters of racemic amino acids has been described in the literature. Graham, W. H. *Tetrahedron Lett.* **1969**, 2233.

(20) Although the work on the 3′-terminal units **9** was done with isomerically pure compounds by starting from the single (2′) isomer of **3c** or **7**, the purity of the 3′-terminal unit **9** is really unimportant. After the final deblocking is accomplished, the aminoacyl residue will undoubtedly equilibrate between the 2′- and 3′-position of the adenosine *cis*-diol grouping: Griffin, B. E.; Jarman, M.; Reese, C. B.; Sulston, T. E.; Trenthan, P. R. *Biochemistry* **1966**, *5*, 3638.

(21) Van Boom, J. H.; Burgers, P. M. J. *Recl. Trav. Chim. Pays-Bas* **1978**, *97*, 73.

(22) Stawinski, J.; Hozumi, T.; Narang, S. A.; Bahl, C. P.; Wu, R. *Nucleic Acids Res.* **1977**, *4*, 353.

(23) Ogilvie, K. K.; Sadana, K. L.; Thompson, E. A.; Quillian, M. A.; Westmore, J. B. *Tetrahedron Lett.* **1974**, 2861.

(24) Neilson, T.; Werstiuk, E. S. *Can. J. Chem.* **1971**, *49*, 493.

(25) This compound was described previously by a different method, but no characterization was given: Reese, C. B.; Stewart, J. C. M.; van Boom, J. M.; de Leuw, H. P. M.; Nagel, J.; deRoy, J. F. M. *J. Chem. Soc., Perkin Trans. 1* **1975**, 934.

**2c** in ca. 50% yield along with **2b** (ca. 8% yield). The UV spectra of **2b** and **2c** differ considerably and are in good agreement with corresponding spectra of other similarly substituted derivatives of adenosine.[26] The structures of derivatives **2b** and **2c** were also established on the basis of elemental analyses and NMR spectra. It was of interest to find that **2b** easily undergoes the loss of one benzoyl group from the aglycon during brief treatment with trifluoroacetic acid. The loss of a single *N*-benzoyl group from compounds similar to **2b** under strongly alkaline conditions has been previously reported in the literature.[26,27] The target compound **2c** was also prepared via benzoylation of **5** or 2′,3′-*O*-isopropylideneadenosine. Removal of *cis*-diol protecting groups led to either compounds **6a** or **6b**, depending on whether trifluoroacetic step was included. Again, both **6a** and **6b** showed very distinct UV spectra which were in each case similar to those of corresponding compounds **2b** and **2c**. Compound **6a** could be converted to **2c** via a three-step procedure involving the ortho ester exchange reaction with methyl orthobenzoate, acid-catalyzed opening of the cyclic ortho ester, and trifluoroacetic acid promoted removal of one $N^6$-benzoyl group.

Repeated attempts to crystallize **2c** in order to obtain requisite pure 3′-isomer have, to date, been unsuccessful. Nevertheless, the route from compound **1b** to **2c** is of continued interest because it provides ready access to **3c**, via the methoxypyranylation and *O*-debenzoylation.[20]

**(3) Oligonucleotide Synthesis and Aminoacylation of the Protected C-C-A Derivative.** The general strategy of synthesis of 2′(3′)-*O*-aminoacyl oligonucleotides requires building of oligonucleotide chains from the 5′ to the 3′ position and adding the aminoacylated building block **9a**, **9b**, or **10b** as the last one. Thus, the 5′-terminal unit **4** and the extension unit **3a** were coupled first by using[22] MST to afford the protected C-C derivative **11a**. Incidentally, MST was found to be a superior condensing reagent relative to mesitylenesulfonyl triazolide.[7b] MST reduces reaction times to approximately 3–5 h, and the yields of coupling reaction are generally very satisfactory (55–70%). The lower yield of protected C-C derivative **11a** (55%), in comparison to those of trinucleotides **12–14**, was probably due to the difficult separation of **11a** from the starting material **3a**. Compound **11a** was isolated as a mixture of two diastereoisomers due to the chirality of the phosphorus triester; these diastereoisomers could be separated by TLC. The presence of two diastereoisomers in roughly equivalent amounts was also indicated in the splitting of the signal in the $^{31}$P NMR. The routine deblocking (ammonium hydroxide and 0.05 N HCl) of either the mixture or of separated diastereoisomers led to C-C (**15**) which is quantitatively degraded by pancreatic ribonuclease or snake venom diesterase, thus, proving the virtual absence of the unnatural 2′–5′ or 3′–3′ phosphodiester linkages in **11a** or **15**. Protected dinucleoside phosphate **11a** was smoothly phosphorylated with (2-chlorophenyl)phosphobis[triazolide][17] to give **11b** in 90% yield. The trinucleotide derivatives **12**, **13a**, and **14** were obtained via coupling of dinucleotide **11b** with appropriate 3′-terminal components **3c**, **9b**, and **10b** as effected by MST in 60–70% yields. $^{31}$P NMR spectra of compounds **12–14** show, again, the splitting of signals due to chirality of the phosphorus atoms.

In order to circumvent the relatively laborious synthesis of the 3′-terminal components **9** incorporating the aminoacyl residue, yet another route leading to protected aminoacyl derivatives of type **13** was studied. It was found that protected trinucleotide **12** (cf. ref 28) could be readily aminoacylated in the presence of MST as an activating agent with *N*-(benzyloxycarbonyl)-L-alanine or *N*-(benzyloxycarbonyl)-L-phenylalanine in very good yields. It is noteworthy that the present approach, unlike oligonucleotide aminoacylations used previously by other authors,[3,29] leads to a *single* product. It is apparent that the latter route, involving the aminoacylation of protected oligonucleotides, should be especially advantageous for the synthesis of various aminoacyl esters derived from a single oligonucleotide. Since the aminoacyl residue is introduced into the completed oligonucleotide chain, this method should be well suited to the synthesis of larger 2′(3′)-*O*-aminoacyl oligoribonucleotides or the 2′(3′)-*O*-aminoacyl oligoribonucleotides with the radioactive aminoacyl group.

**(4) Deblocking Procedures.** Although triester methodology greatly improves yields of condensation reactions, the deblocking procedures are naturally more complicated than those of diester methods. This is even a greater problem in the case of triester synthesis of 2′(3′)-*O*-aminoacyl oligonucleotides due to the necessity of removing all protecting groups independently and with preservation of the integrity of the aminoacyl ester linkage. This task was further complicated by the lack of suitable analytical methods necessary to monitor the deblocking steps. In our present scheme, four deblocking steps were used in the following order: (i) removal of benzoyl groups from aglycons with hydrazine; (ii) cleavage of 2-chlorophenyl groups from phosphorus with $F^-$; (iii) removal of 5′-*O*-monomethoxytrityl and 2′-*O*-(4-methoxytetrahydropyran-4-yl) groups with 0.01 N HCl; (iv) hydrogenolysis of the *N*-benzyloxycarbonyl group of the aminoacyl moieties to generate the final compounds **16e–g** (see Scheme III).

In the particular case of deblocking of derivative **14**, which incorporates the alkali-stable amino acid ortho ester moiety, steps i and ii could be connected, and both the *N*-benzoyl groups and the 2-chlorophenyl groups were removed with ammonium hydroxide.[6] The reaction of protected compounds **13a,b** with a large excess of hydrazine acetate (pH 5, 16 h) leads to the removal of the benzoyl groups from adenine and cytosine moieties. Although this reaction is quite specific at the nucleoside level (vide supra). Analysis of the reaction mixtures, derived from **13a** and **13b**, have shown the formation of a compound without an amino acid and other side products which are presumably still partially benzoylated. The desired aminoacylated intermediate, with a UV spectra consistent with C-C-A, was obtained in pure form after silica gel column chromatography in ca. 50% yield. The removal of 2-chlorophenyl groups from phosphorus (step ii) was smoothly accomplished by reaction with $Bu_4N^+F^-$ in an aqueous medium (0.05 M salt, 6 h). Since the unblocking of the triester function proceeds very cleanly, no purification step was included. In the next step (iii, including the opening of the ortho ester grouping of **14**) the acid-labile groups were removed with dilute HCl (pH 2.0),

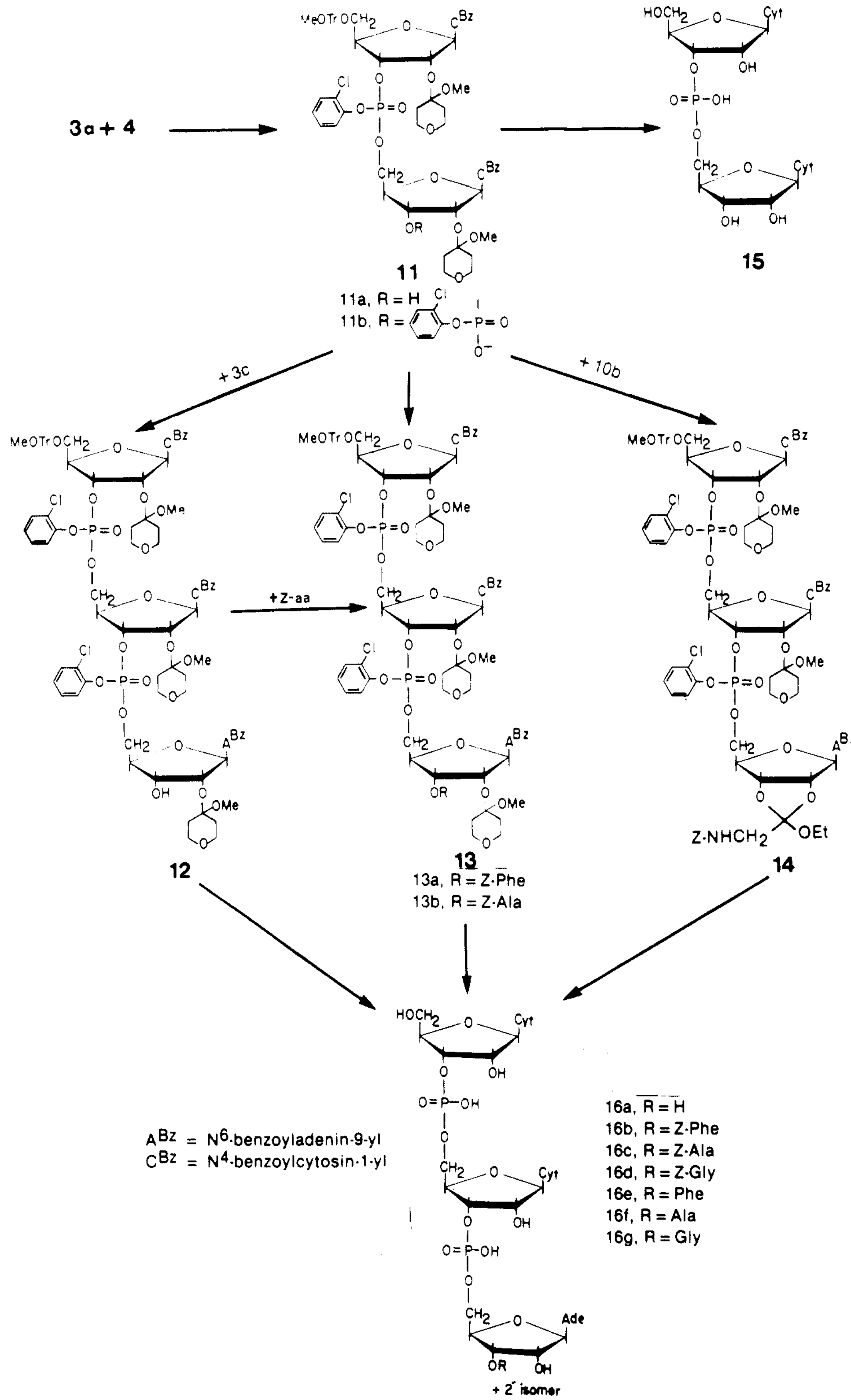
(26) (a) Chládek, S.; Žemlička, J.; Šorm, F. *Collect. Czech. Chem. Commun.* **1966**, *31*, 1785. The structure of **2b** should probably be assigned to $N^6,N^6,O^{2'}(O^{3'}),O^{5'}$-tetrabenzoyladenosine (and similarly for the related derivatives **6a**) on the basis of available evidence. (b) Lyon, P. A.; Reese, C. B. *J. Chem. Soc., Perkin Trans. 1* **1974**, 2645. On the other hand, unexplained differences exist between UV spectra of **2b**, **6b**, and analogous compounds of Lyon and Reese.

(27) Ralph, R. K.; Khorana, H. G. *J. Am. Chem. Soc.* **1961**, *83*, 2926.

(28) The compound **12** has furnished C-C-A (**16a**) in 60% yield upon successive deblocking with ammonium hydroxide and HCl. C-C-A (**16a**) was almost quantitatively degraded by pancreatic ribonuclease and snake venom phosphodiesterase to the expected products in proper ratios.

(29) Smrt, J.; Jonák, J. *Collect. Czech. Chem. Commun.* **1979**, *44*, 3321.

Scheme III




and the compounds **16b–d** were isolated by preparative TLC on cellulose in acetic acid containing systems. The recovery of compounds **16b–d** from the plates was lower than expected, apparently due to the poor solubility of these compounds.

The compounds **16b–d** were characterized by chromatography and by UV spectra; the yields of **16b,c** ranged from 15% to 20% for the three consecutive deblocking steps, whereas the higher yield of glycine derivative **16d** (40%) apparently reflects the absence of the hydrazine deblocking step. In the final step, the *N*-benzyloxycarbonyl group in **16b–d** was removed in practically quantitative yields by catalytic hydrogenolysis in acetic acid to generate target compounds **16e–g**. Although the compounds **16e–g** appeared to be uniform on TLC, another purification step (preparative paper electrophoresis in acidic medium) was included to assure the complete absence of any impurities which could possibly influence the biochemical investigations. The final compounds C-C-A-Phe (**16e**), C-C-A-Ala (**16f**), and C-C-A-Gly (**16g**) were characterized by the usual criteria, including TLC, electrophoresis, UV spectra, and alkaline hydrolysis to C-C-A and the parent amino acid. The almost quantitative degradation of **16e–g** with pancreatic ribonuclease and snake venom phosphodiesterase to the expected products in the correct ratio proves a virtual absence of unnatural phosphodiester linkages in the final products.

## Experimental Section

**General Methods.** The general methods were the same as those described in previous papers in this series.[5] Elemental analyses were performed by MHW Laboratories.

**Chromatography and Electrophoresis.** Thin-layer chromatography was performed on silica gel coated aluminum foils, on silica gel 60F-254 and HPTLC precoated plates, on silica gel 60F-254 (E. M. Laboratories, Inc.) and cellulose coated plastic foils, on 13254 cellulose with a fluorescent indicator (Eastman Kodak Co). Preparative TLC was performed on silica gel GF (precoated thin-layer chromatography plates, 2000 μm) and Avicel F (precoated thin-layer plates, 1000 μm), both products of Analtech. The following systems were used. For silica gel: $S_1$, $CH_2Cl_2$–5% $CH_3OH$; $S_2$, $CH_2Cl_2$–10% $CH_3OH$; $S_3$, $CH_2Cl_2$–2.5% $CH_3OH$; $S_4$, $CH_2Cl_2$–15% $CH_3OH$; $S_5$, 1-butanol saturated with water. For cellulose: $S_6$, 1-butanol–$H_2O$–$CH_3COOH$ (5:3:2); $S_7$, 1-butanol saturated with 10% $CH_3COOH$. For HPTLC silica gel, $S_8$, $CH_2Cl_2$–15% $CH_3OH$. Descending paper chromatography was performed on Whatman No. 1 paper in system $S_9$ [2-propanol–concentrated ammonium hydroxide–water (7:1:2)]. Paper electrophoresis was conducted on a Savant flat plate (at 4 °C) by using either 1 M acetic acid ($E_1$) or 0.02 M $Na_2HPO_4$ (pH 7.0, $E_2$) on Whatman No. 1 paper at 50 V/cm for 2 h. Electrophoretic mobilities are given in Table IV. Preparative paper electrophoresis was conducted in system $E_1$ on Whatman No. 3MM paper at 50 V/cm for 3–4 h. The bands of 2′(3′)-O-aminoacyl oligonucleotides were eluted by centrifugation with 80% acetic acid (at 4 °C), and the solutions were freeze–dried.

**Column Chromatography.** Column chromatography was performed on silica gel GF-254 (Type 60, Merck) by using a linear gradient of methylene chloride with methanol. A pumping pressure of ca. 20–50 psi was applied to give a flow rate of 2 mL/min, and 10-min fractions were collected.

**Spectra.** UV spectra were obtained by using a Cary Model 11 recording spectrophotometer. Yields of oligonucleotides were determined spectrophotometrically at pH 2.0 (0.01 N HCl) by using the following extinction coefficients: C-C, $\epsilon_{260}$ 13.6; C-C-A, $\epsilon_{260}$ 27.9 (pH 2.0) (neglecting ca. 6% or 2% hypochromicity for C-C-A or C-C, respectively[1c]). NMR spectra were recorded on a JEOL FX-100 and a Nicolet Model NT-300.

**Enzyme Degradation.** Pancreatic ribonuclease (ribonuclease A, Sigma) and snake venom diesterase (Worthington) degradations were performed as described previously[5] by using an incubation time of 3.5 h. The analysis of degradation was performed by electrophoresis in system $E_2$ on Whatman No. 3MM paper.

**Starting Materials.** Nucleosides, amino acids, and reagents were commercial preparations (Sigma and Aldrich).

**2′,3′-O-(α-Methoxybenzylidene)adenosine (1b) and 2′,3′-O-(α-Methoxybenzylidene)cytidine (1a).** Adenosine (3.24 g, 12 mmol) was mixed with DMF (60 mL), methyl orthobenzoate (8.72 g, 48 mmol), and methanesulfonic acid (2.7 mL). The reaction mixture was kept at room temperature for 6 h after TLC in system $S_2$ showed complete conversion to species with higher $R_f$ values. The reaction mixture was cooled with ice and neutralized with concentrated ammonia. After the mixture was kept at 0 °C overnight, the precipitate was filtered off and washed by DMF. DMF was evaporated in vacuo and the residue dissolved in methylene chloride. The solution was washed once with 5% sodium bicarbonate and once with water containing a drop of triethylamine and dried with magnesium sulfate. The solvent was evaporated, the residue was applied to a silica gel column (6 × 30 cm), and chromatography was performed by elution with a linear gradient of $CH_2Cl_2$ (with 0.1% triethylamine) and $CH_2Cl_2$–10% $CH_3OH$ (0.1% triethylamine; 2 L × 2 L). The bis ortho ester of adenosine[16] contained in the first fraction was obtained (0.62g) by evaporation of the eluent: UV (95% ethanol) $\lambda_{max}$ 260 nm ($\epsilon$ 14.86), $\lambda_{min}$ 226; NMR (acetone-$d_6$ + $D_2O$) $\delta$ 8.4 and 8.36 (2 s, 1 H, $H_8$), 8.15 and 8.13 (2 s, 1 H, $H_2$), 7.70–7.38 (m, 10 H, phenyls), 6.56 and 6.34 (2 d, 1 H, $H_{1'}$, $J_{1',2'}$ = 2.5 and 2.0 Hz), 3.26–3.03 (m, 9 H, $OCH_3$). Anal. Calcd for $C_{27}H_{29}O_7N_5$: C, 60.55; H, 5.46; N, 13.08. Found: C, 60.28; H, 5.35; N, 13.20.

The major product **1b** was obtained in 66% yield (3.06 g) as a white foam, which gives a double spot on TLC in system $S_2$ due to the existence of two diastereoisomers. Anal. Calcd for $C_{18}H_{19}O_5N_5 \cdot 0.5\,H_2O$: C, 54.81; H, 5.11; N, 17.75. Found: C, 55.3; H, 4.94; N, 17.83.

The cytidine derivative **1a** was prepared analogously in 71% yield and was obtained as a colorless hygroscopic syrup (pure by TLC but containing, according to NMR, traces of triethylamine) which did not give a good elemental analysis.

***N*,*O*$^{3'}$,*O*$^{5'}$-Tribenzoylcytidine (2a).** 2′,3′-O-(α-Methoxybenzylidene)cytidine (**1a**; (20.45 g, 56.6 mmol) was dissolved in anhydrous pyridine (500 mL), the solution was cooled in ice, and benzoyl chloride (19.72 mL, 170 mmol) was added dropwise under continuous external cooling. The reaction mixture was stirred in the dark at 0 °C for 1 h and then quenched by the addition of 2 M TEAB (740 mL) and methanol (800 mL). After ca. 30 min at room temperature, the solution was concentrated in vacuo. The resulting oil was taken up in methylene chloride, and the solution was washed with 10% sodium bicarbonate solution and water and dried with magnesium sulfate. The solution was evaporated in vacuo, and the residue was coevaporated repeatedly with toluene, dissolved in 80% acetic acid (880 mL), immediately concentrated in vacuo to an oil, and coevaporated with ethanol. The residue was triturated with ethanol (ca. 500 mL), and the solid that was precipitated (uniform on TLC in system $S_1$) was filtered off and dried in vacuo to yield a mixture of 2′- and 3′-benzoyl derivatives (yield 19.5 g, 63%). The pure title compound was obtained by crystallization from boiling ethanol [yield 13.3 g (42%); fibrous needles; mp 214–215.5 °C (lit.[16] mp 198–202 °C)] which was shown by NMR to be free of its $N,O^{2'},O^{5'}$ isomer. Anal. Calcd for $C_{30}H_{25}N_3O_8$: C, 64.85; H, 4.54; N, 7.56. Found: C, 64.92; H, 4.59; N, 7.64.

***N*$^4$-Benzoyl-2′-O-(4-methoxytetrahydropyran-4-yl)cytidine (3a).** $N,O^{3'},O^{5'}$-Tribenzoylcytidine (**2a**; 4.3 g, 7.74 mmol) was stirred in dry dioxane (980 mL) and a solution of HCl (6 M) in DMF (2.6 mL) until all solid material went into solution; then 4-methoxy 5,6-dihydro-2*H*-pyran (15.7 g, 137 mmol) was added. After 2 h at room temperature, when TLC in system $S_1$ indicated that no starting material remained, the reaction was neutralized with triethylamine after being cooled to 0 °C. The precipitated triethylamine hydrochloride was removed by filtration, the filtrate was concentrated in vacuo, the residue was dissolved in a mixture of ethanol (60 mL) and pyridine (40 mL), and a mixture of 2 M sodium hydroxide (20 mL) and ethanol (40 mL) was added. After exactly 5 min at room temperature, an excess of pyridinium Dowex 50 ion-exchange resin was added. The resin was removed by filtration and washed with a mixture of pyridine–ethanol, and the filtrate was concentrated in vacuo. The residue was dissolved in methylene chloride and extracted by water (3×), and the methylene chloride solution was dried with magnesium sulfate and evaporated in vacuo. Solid material was obtained after trituration with methylene chloride–petroleum ether; yield 2.81 g (79%) of chromatographically uniform **3a**.

The analytical sample was obtained by chromatography on a silica gel column under standard conditions to give the crystalline material, mp 181–183 °C. Anal. Calcd for $C_{22}H_{27}N_3O_8$: C, 57.26; H, 5.90; N, 9.11. Found: C, 57.10; H, 5.89; N, 8.95.

***N*$^4$-Benzoyl-2′-O-(4-methoxytetrahydropyran-4-yl)-5′-O-(methoxytrityl)cytidine (3b).** The title compound was prepared analogously as described for the corresponding 2′-O-tetrahydropyranyl derivative[24] as a solid powder in 75.5% yield, obtained after standard silica gel chromatography. Anal. Calcd for $C_{42}H_{43}N_3O_9 \cdot 0.5H_2O$: C, 67.91; H, 5.97; N, 5.66. Found: C, 68.06; H, 6.54; N, 5.12.

***N*$^6$,*O*$^{5'}$-Dibenzoyladenosine (6b).** 2′,3′-O-Isopropylideneadenosine (0.31 g, 1 mmol) was dissolved in pyridine (2 mL), the solution was cooled in ice, and benzoyl chloride (0.45 mL, 3.9 mmol) was added dropwise, with exclusion of atmospheric moisture, and the mixture was stirred for 40 h at room temperature. The reaction mixture was quenched by ice and evaporated to a syrup, and the residue was dissolved in methylene chloride. The solution was washed with saturated solution sodium bi-

Table I

| compd | UV data[a] | | ¹H NMR,[h] δ | | | | |
|---|---|---|---|---|---|---|---|
| | $\lambda_{max}$, nm ($\epsilon \times 10^{-3}$) | $\lambda_{min}$, nm ($\epsilon \times 10^{-3}$) | solvent | H-8 or H-2, H-6 or H-5 | aromatic protons | H-1′ | other signals |
| 1a | 268 | 246 | acetone-$d_6$ | 7.86, 7.84 (2 d, 1,[b] $J$ = 8) | 7.76–7.28 (m, 6)[c] | 6.04, 6.12 (2 d, 1, $J$ = 1)[b] | 2.5 (s, 3,[d] $OCH_3$) |
| 1b | 260 (14.45) | 226 | acetone-$d_6$ + $D_2O$ | 8.41, 8.36 (2 s, 1),[b] 8.24, 8.23 (2 s, 1) | 7.75–7.4 (m, 5) | 6.55, 6.22 (2 d, 1, $J$ = 3.1)[b] | 3.4 and 3.2 (2 s, 3, $OCH_3$) |
| 2a | 305 (8.07), 262 (21.8) | 294 (7.15) | $Me_2SO$-$d_6$ + $D_2O$ + $CD_3COOD$ | 8.22 (d, 1, $J$ = 7.6), 7.32 (d, 1, $J$ = 7.3) | 8.09–7.54 (m, 15) | 5.96 (d, 1, $J$ = 3) | |
| 2b | 249, (26.35), 273 (19.9), 231 (43.14) | 267 | acetone-$d_6$ + $D_2O$ | 8.69 (s, 1),[e] 8.58 (s, 1) | 8.19–7.40 (m, 20) | 6.60, 6.37 (2 d, 1,[f] $J$ = 5) | |
| 2c | 280 (19.48), 260 (13.8) | 254 | acetone-$d_6$ + $D_2O$ | 8.61 (s, 2) | 8.17–7.4 (m, 15) | 6.61, 6.39 (2 d, 1,[f] $J$ = 4) | |
| 3a | 307 (9.6), 262 (24.4) | 288, 229 | acetone-$d_6$ | 8.61 (d, 1, $J$ = 7.6) | 8.19–7.29 (m, 16)[c] | 6.28 (d, 1, $J$ = 5.9) | 3.1 (s, 3, $OCH_3$) |
| 3b | 309 (8.7), 263 (20.5), 233 (21.7) | 290, 248, 226 | acetone-$d_6$ | 8.34 (d, 1, $J$ = 7.7), 7.16 (d, 1, $J$ = 7.6) | 8.18–6.93 (m, 19) | 6.25 (d, 1, $J$ = 5) | 3.81 (s, 3, $OCH_3$ of methoxytrityl), 3.21 (s, 3, $OCH_3$ of MeOThp) |
| 3c | 281 (18.4), 260 (10.9) | 250 | acetone-$d_6$ | 8.68 (s, 1), 8.64 (s, 1) | 8.6–7.5 (m, 5) | 6.23 (d, 1, $J$ = 7.6) | 2.65 (s, 3, $OCH_3$), 1.87–1.46 (m, 4, C–$CH_2$ of MeOThp) |
| 3c + 2′ isomer | | | $CDCl_3$ | 8.82 (s, 1), 8.76 (s, 1) | 8.08–7.51 (m, 5) | 5.96, 5.84 (2 d, 1,[f] $J$ = 7.5) | 2.66 (s, 3, $OCH_3$), 1.84–1.54 (m, 4, C–$CH_2$ of MeOThp) |
| 4 | 309 (9.81), 262 (25.6), 235 (23.0) | 274, 247, 228 | acetone-$d_6$ | | 8.21–7.3 (m, 25)[g] | 6.93 (d, 1, $J$ = 8.8) | 3.81 (s, 3, $OCH_3$ of methoxytrityl), 3.12 (s, 3, $OCH_3$ of MeOThp) |
| 6a | 273 (17.0), 249 (22.30) | 268 | $Me_2SO$-$d_6$ + $D_2O$ | 8.81 (s, 1), 8.63 (s, 1) | 8.01–7.39 (m, 15) | 6.16 (d, 1, $J$ = 4.9) | |
| 6b | 280 (20.11), 260 (11.73), 229 (24.6) | 250 | $Me_2SO$-$d_6$ + $D_2O$ | 8.67 (s, 1), 8.64 (s, 1) | 8.0–7.53 (m, 10) | 6.10 (d, 1, $J$ = 4.9) | |
| 8c | 277 (18.40) | 245 (9.20) | $CDCl_3$ | 8.81 (s, 1), 8.27 (s, 1) | 7.51–8.06 (m, 5) | 6.18 (d, 1, $J$ = 5.62) | 2.9 (s, 3, $OCH_3$ of MeOThp), 2.19 (s, 3, $COCH_3$ of levulinyl) |
| 8g | 278 (18.05) | 245 (10.2) | $CDCl_3$ | 8.81 (s, 1), 8.22 (s, 1) | 7.97–7.25 (m, 15) | 5.89 (d, 1, $J$ = 6.35) | 5.1 (s, 4, $C_6H_5CH_2$), 2.6 (s, 3, $OCH_3$ of MeOThp), 2.19 (s, 3, $COCH_3$ of levulinyl) |
| 9a | 275 (18.29) | 245 (9.14) | $CDCl_3$ | 8.77 (s, 1) | 8.01–7.22 (m, 16) | 5.79 (d, 1, $J$ = 7.56) | 5.103 (s, 4, $C_6H_5CH_2$) |
| 9b | 275 (17.87) | 250 (11.17) | $CDCl_3$ + $D_2O$ | 8.81 (s, 1) | 7.99–7.26 (m, 16) | 5.97 (d, 1, $J$ = 7.0) | 5.09 (s, 4, $C_6H_5CH_2$), 2.47 (s, 3, $OCH_3$ of MeOThp) |
| 10b | 281 (19.1) | 248 | acetone-$d_6$ + $D_2O$ | 8.67 (2 s, 1),[b] 8.10 (2 s, 1)[b] | 7.67–7.25 (m, 10) | 6.48, 6.37 (2 d, 1, $J$ = 2.5)[b] | 5.15, 5.07 (2 s, 2, $C_6H_5CH_2$)[b] |

[a] 95% ethanol. [b] Doubling of signals due to diastereoisomers. [c] Also contains H-5. [d] Contains traces of triethylamine. [e] Signals are doubled in $Me_2SO$–$D_2O$ due to the presence of 2′- and 3′-benzoates. [f] Doubling of signals is due to the presence of 2′ and 3′ isomers. [g] Cytosine protons are buried in this region. [h] $J$ values are given in hertz.

carbonate and water and dried with magnesium sulfate. The solvent was removed by evaporation and the residue coevaporated with toluene. The residue was dissolved in trifluoroacetic acid (5 mL) and kept at room temperature for 30 min, and the solution was evaporated in vacuo and coevaporated several times with dioxane. The crude reaction product was purified on a column of silica gel (2.5 × 20 cm) with a linear gradient of $CH_2Cl_2$ and $CH_2Cl_2$–6% $CH_3OH$ (1 L × 1 L). The peak containing the main product was pooled, and the solvents were evaporated to yield compound **6b**, 0.15 g (32%). The final product was positive to *cis*-diol spray. The analytical sample was obtained by trituration of the foamy substance with $CH_2Cl_2$/petroleum ether. Anal. Calcd for $C_{24}H_{21}N_5O_6{\cdot}H_2O$: C, 58.41; H, 4.70; N, 14.19 Found: C, 58.23; H, 4.45; N, 14.23.

**$N^6$,$N^6$,$O^{5'}$-Tribenzoyladenosine (6a).** 2′,3′-*O*-(Ethoxymethylene)adenosine (5;[26a] 10.0 g, 31 mmol) was dissolved in pyridine (17 mL) and the solution cooled in ice. Benzoyl chloride (6.3 mL, 53.8 mmol) was added dropwise, and the reaction mixture was stirred overnight. Additional benzoyl chloride (8 mL, 72 mmol) was added, and the reaction mixture was allowed to stand for 5 h before being quenched by ice. The solution was evaporated to a small volume, and the residue was dissolved in methylene chloride and extracted with water. The $CH_2Cl_2$ solution was dried with magnesium sulfate and evaporated in vacuo, and the residue was repeatedly coevaporated with toluene. The residue was dissolved in 80% acetic acid (65 mL) and kept at room temperature for 48 h, followed by evaporation and coevaporation with toluene. The residue was dissolved in $CH_2Cl_2$ (100 mL) and the solution stirred with 5% sodium bicarbonate (50 mL) for 2 h. The organic layer was washed with water, dried with magnesium sulfate, and evaporated to dryness. The residue was dissolved in $CH_2Cl_2$ and applied to the silica gel column (6 × 30 cm), which was eluted with a linear gradient of $CH_2Cl_2$ and $CH_2Cl_2$–7% methanol (3 L × 3 L). The pooled fractions were evaporated to yield the title compound (6.2 g, 33%), which was chromatographically uniform in system $S_1$ and positive to *cis*-diol spray. Treatment of **6a** with an excess of trifluoroacetic acid for 10 min gave compound **6b** in quantitative yield. Anal. Calcd for $C_{31}H_{25}N_5O_7{\cdot}H_2O$: C, 62.30; H, 4.56; N, 11.72. Found: C, 62.15; H, 4.33; N, 11.38.

**$N^6$,$O^{3'}$($O^{2'}$),$O^{5'}$-Tribenzoyladenosine (2c). (A) Via Benzoylation of 2′,3′-*O*-(α-Methoxybenzylidene)adenosine (1b).** Compound **1b** (17.4 g, 45 mmol) was dissolved in pyridine (500 mL), and the solution was cooled in ice and treated with benzoyl chloride (15.69 mL, 135 mmol) for 1 h, after which the starting material was no longer detected by TLC in system $S_1$. The reaction was worked up as indicated for the synthesis of **2a**. The reaction products were isolated on a silica gel column (6 × 43 cm) which was eluted using a linear gradient of $CH_2Cl_2$ and $CH_2Cl_2$–5% $CH_3OH$ (4 L × 4 L). Two major fractions were collected and the solvents evaporated in vacuo. The yield from the first fraction, shown to be **2b**, was 2.78 g (8%), and the yield from the second fraction (**2c**) was 13.35 g (50.5%). Both compounds were chromatographically uniform in system $S_1$. Anal. Calcd for $C_{38}H_{29}N_5O_8{\cdot}3H_2O$ (**2b**): C, 61.86; H, 4.78; N, 9.49. Found: C, 62.2; H, 4.42; N, 8.87. Calcd for $C_{31}H_{25}O_7N_5{\cdot}0.5H_2O$ (**2c**): C, 63.25; H, 4.45; N, 11.90. Found: C, 63.56; H, 4.25; N, 11.18. **2c** was also obtained by the benzoylation of with benzoic acid anhydride, though in low yield. Neither compound **2b** nor **2c** migrates during electrophoresis in borate buffer.

**(B) Via Orthoesterification of $N^6$,$N^6$,$O^{5'}$-Tribenzoyladenosine (6a).** Compound **6a** (1.0 g, 1.66 mmol) was dissolved in DMF (15 mL), and methyl orthobenzoate (1.21 g, 6.64 mmol) and methanesulfonic acid (0.19 g, 2 mmol) were added. The reaction mixture was stirred for 4.5 h at room temperature and neutralized by addition of triethylamine (1.5 mL). After the solution was evaporated to dryness in vacuo, the residue was distributed between $CH_2Cl_2$ and water, and the organic layer was washed with water (3×), dried with magnesium sulfate, and evaporated. The residue was dissolved in 80% acetic acid (20 mL), freeze–dried after 15 min, and coevaporated with toluene. The crude intermediate **2b** was dissolved in trifluoroacetic acid (10 mL), allowed to stand for 10 min at room temperature, and treated as described for the synthesis of **6b**. Compound **2c** was isolated by silica gel chromatography (0.6 g, 62%). Repeated attempts at obtaining isomerically pure $N^6$,$O^{2'}$,$O^{5'}$-tribenzoyladenosine (**2c**) from the mixture of 2′ and 3′ isomers by crystallization were not successful.

**$N^6$-Benzoyl-2′-*O*-(4-methoxytetrahydropyran-4-yl)adenosine (3c).** The title compound was prepared by a modification of the described procedure[24] by starting from 3′,5′-di-*O*-acetyladenosine[16] (4 g, 11.4 mmol) which was suspended in dry dioxane (80 mL) and a 6 M solution of HCl in DMF (3.8 mL) and stirred until dissolved. 4-Methoxy-5,6-dihydro-2*H*-pyran (20.4 mL, 18.2 mmol) was added, and, after 4 h at room temperature, TLC in system $S_1$ indicated that the reaction was essentially complete. The reaction mixture was cooled with ice and neutralized by the addition of triethylamine (3.8 mL). Triethylamine hydrochloride was filtered off and the solution concentrated in vacuo. The residue was dissolved $CH_2Cl_2$, and chromatography was performed on a silica gel column (2.5 × 35 cm) eluted with a linear gradient of $CH_2Cl_2$–2% $CH_3OH$ and $CH_2Cl_2$–10% $CH_3OH$ (2.5 L × 2.5 L) under standard conditions. The fractions containing the product were pooled and evaporated, and the residue was dissolved in saturated methanolic ammonia (90 mL). After being allowed to stand for 16 h at room temperature, the solution was evaporated to dryness, yielding 2′-*O*-(4-methoxytetrahydropyran-4-yl)adenosine[8] (3.8 g, 87%), which was chromatographically uniform in system $S_2$. This product was directly used in the synthesis of **3c** by benzoylation and debenzoylation similar to the described procedure for *N*-benzoyl-2′-*O*-(tetrahydropyranyl)adenosine.[24] Compound **3c** was isolated by column chromatography on silica gel (2.5 × 50 cm) by using a linear gradient of $CH_2Cl_2$ and $CH_2Cl_2$–10% $CH_3OH$ (2 L × 2 L). The product, obtained in 70% yield, was chromatographically uniform in system $S_2$. Anal. Calcd for $C_{23}H_{27}N_5O_7{\cdot}0.5H_2O$: C, 53.89; H, 5.90; N, 13.66. Found: C, 54.09; H, 5.58; N, 13.54.

**$N^6$-Benzoyl-2′(3′)-*O*-(4-methoxytetrahydropyran-4-yl)adenosine (3c).** *N*,$O^{5'}$,$O^{3'}$($O^{2'}$)-Tribenzoyladenosine (**2c**) was converted to the title compound in 64% yield by the procedure described above for the analogous cytidine derivative **3a**. Compound **3c** appears as a double spot on TLC in system $S_2$ due to the presence of 2′ and 3′ isomers, the faster moving spot being identical with 2′ isomer from the previous preparation. Anal. Calcd for $C_{23}H_{27}O_7N_5{\cdot}H_2O$: C, 54.86; H, 5.80; N, 13.91. Found: C, 55.26; H, 5.64; N, 13.34.

**$N^6$-Benzoyl-2′,3′-*O*-[[[(benzyloxycarbonyl)amino]methyl]ethoxymethylene]adenosine (10b).** The compound **10a** (1.7 g, 3.5 mmol)[18] was dissolved in pyridine (5 mL), and benzoyl chloride (1.70 mL) was added dropwise with stirring and cooling to 0 °C. After 2 h at 0 °C, the reaction was quenched with ice and the mixture worked up as described for the synthesis of **3a**, including reaction with NaOH. The reaction product **10b** was isolated on a silica gel column (2.5 × 30 cm) eluted with a linear gradient of $CH_2Cl_2$ and $CH_2Cl_2$–7% $CH_3OH$ (2 L × 2 L). The fractions that contained pure **10b** (a double spot in $S_2$ due to diastereoisomers) were pooled and evaporated in vacuo. The product was obtained in solid form after trituration with methylene chloride–petroleum ether. The yield of **10b** was 1.48 g (71%). Anal. Calcd for $C_{29}H_{30}N_6O_8$: C, 58.97; H, 5.12; N, 14.23. Found: C, 58.99; H, 5.19; N, 14.04.

**$N^4$-Benzoyl-2′-*O*-(4-methoxytetrahydropyran-4-yl)-5′-*O*-(methoxytrityl)cytidine 3′-(4-Chlorophenyl phosphate) (4).** The triethylammonium salt of the title compound was prepared from **3a** essentially by the procedure of Chattopadhyaya and Reese[17b] in 95.5% yield. The compound **4** was chromatographically uniform in system $S_2$ ($R_f$ 0.1) and $S_5$ ($R_f$ 0.38): $^{31}$P NMR (acetone-$d_6$) −2.14 ppm (s). Anal. Calcd for $C_{54}H_{62}N_4PO_{12}Cl{\cdot}H_2O$: C, 62.15; H, 6.18; N, 5.37; P, 2.97. Found: C, 62.43; H, 6.06; N, 5.27; P, 3.19.

**$N^4$-Benzoyl-*P*-(4-chlorophenyl)-2′-*O*-(4-methoxytetrahydropyran-4-yl)-5′-*O*-(methoxytrityl)cytidylyl(3′–5′)-$N^4$-benzoyl-2′-*O*-(4-methoxytetrahydropyran-4-yl)cytidine 3′-(4-Chlorophenyl phosphate) (11b).** The triethylammonium salt of the title dinucleotide was prepared analogously to mononucleotide derivative **4** by starting from the protected derivative of CpC **11a** in 90% yield as a chromatographicly uniform product in systems $S_2$ ($R_f$ 0.05) and $S_5$ ($R_f$ 0.39): UV (95% ethanol) $\lambda_{max}$ 305 nm (14.63), 263 (41.56), 232 (29.47), $\lambda_{min}$ 290, 242, 228; $^{31}$P NMR (acetone-$d_6$) −2.25, −2.94, −3.38 ppm.

**$N^6$-Benzoyl-5′-*O*-levulinyl-2′-*O*-(4-methoxytetrahydropyran-4-yl)adenosine (8c) and $N^6$-Benzoyl-3′,5′-di-*O*-**

**levulinyl-2′-*O*-(4-methoxytetrahydropyran-4-yl)adenosine (8d).** Compound **3c** (0.2 g, 0.41 mmol) was dissolved in dioxane (2.0 mL) and DMF (5 mL), and levulinic acid (0.24 g, 2.05 mmol), 1,2-dimethylimidazole (0.038 g, 0.4 mmol), and 2,6-lutidine (3 mL) were added. The stirred solution was cooled to 0 °C, and a solution of DCC (0.7 g, 3.4 mmol) in dioxane (10 mL) was added over this period. The reaction mixture was further stirred at room temperature for 4–6 h until TLC in $S_2$ showed the formation of monolevulinyl (**8c**) and dilevulinyl (**8d**) derivatives along with some starting nucleoside. The precipitated dicyclohexylurea was then filtered off, and the filtrate was diluted with dichloromethane and extracted with water. The organic layer was washed with 2% sodium bicarbonate solution, dried over sodium sulfate, evaporated, and coevaporated with toluene in vacuo. The crude reaction product was chromatographed on a silica gel column (2.5 × 30 cm) by using a linear gradient of $CH_2Cl_2$ and $CH_2Cl_2$–5% $CH_3OH$ (2 L × 2 L) to afford **8d** (0.135 g, 49%) as the first fraction. Anal. Calcd for $C_{33}H_{39}N_5O_{11}$: C, 58.14; H, 5.72; N, 10.27. Found: C, 58.09; H. 5.57; N, 10.23. The second fraction contained **8c**, 0.07 g (29%). Anal. Calcd for $C_{28}H_{33}N_5O_9$: C, 57.63; H, 5.66; N, 12.00. Found: C, 57.83; H, 5.73; N, 10.81.

***N*[6]-Benzoyl-3′-*O*-[*N*-(benzyloxycarbonyl)-L-phenylalanyl]-5′-*O*-levulinyl-2′-*O*-(4-methoxytetrahydropyran-4-yl)adenosine (8g).** *N*-(Benzyloxycarbonyl)-L-phenylalanine (0.18 g, 0.6 mmol) and **8c** (0.3 g, 0.51 mmol) were coevaporated with dry pyridine several times and dissolved in anhydrous pyridine (10 mL), and the solution was treated with mesitylenesulfonyl tetrazolide (0.38 g, 1.5 mmol) in a drybox. The reaction mixture was kept in the drybox for 2 h, quenched with ice, and partitioned between water and methylene chloride. The organic layer was washed with water (3×), dried over sodium sulfate, evaporated, and coevaporated with toluene to dryness in vacuo. The crude reaction product was chromatographed on a silica gel column (2 × 35 cm) by using a linear gradient of $CH_2Cl_2$ and $CH_2Cl_2$–5% $CH_3OH$ (1.5 L × 1.5 L) to afford **8g**, 0.37 g (84%). Anal. Calcd for $C_{45}H_{48}N_6O_{12}\cdot0.5H_2O$: C, 61.80; H, 5.61; N, 9.62. Found: C, 61.31; H, 5.98; N, 9.61.

***N*[6]-Benzoyl-3′-*O*-[*N*-(benzyloxycarbonyl)-L-phenylalanyl]-2′*O*-(4-methoxytetrahydropyran-4-yl)adenosine (9b).** Compound **8g** (0.43 g, 0.5 mmol) was treated with ice-cold hydrazine buffer (5 mL; 0.5 M $N_2H_4$ in Py/AcOH, 3:2 v/v) for 2 min followed by the addition of acetylacetone (0.5 g, 5 mmol) at 0 °C. The reaction mixture was evaporated in vacuo and partitioned between methylene chloride and water. The organic layer was washed with water (3×), dried over sodium sulfate, and evaporated in vacuo. The solid product was chromatographed on a silica gel column (2.5 × 35 cm) by using a linear gradient of $CH_2Cl_2$ and $CH_2Cl_2$–4% $CH_3OH$ (2 L × 2 L) to afford pure **9b**, 0.32 g (83%). Anal. Calcd for $C_{40}H_{42}N_6O_{10}\cdot0.5H_2O$: C, 61.93; H, 5.54; N, 10.83. Found: C, 61.90; H, 6.03; N, 11.04.

***N*[6]-Benzoyl-5′-*O*-(dimethoxytrityl)-2′-*O*-(4-tetrahydropyran-4-yl)adenosine (8a).** Compound 7 (0.85 g, 1.86 mmol)[24] and dimethoxytrityl chloride (0.76 g, 2.23 mmol) were reacted together in anhydrous pyridine (15 mL) for 16–20 h, and the reaction mixture was quenched with ice and partitioned between water and methylene chloride. The organic layer was washed with water, dried with sodium sulfate, evaporated, and coevaporated with toluene to dryness in vacuo. The crude product was chromatographed on a silica gel column (2.5 × 35 cm) by using a linear gradient of $CH_2Cl_2$ and $CH_2Cl_2$–4% $CH_3OH$ (2 L × 2 L) to afford **8a**, 0.9 g (63.9%). Anal. Calcd for $C_{43}H_{43}N_5O_8$: C, 68.16; H, 5.68; N, 9.24. Found: C, 67.90; H, 5.89; N, 9.12.

***N*[6]-Benzoyl-3′-*O*-[*N*-(benzyloxycarbonyl)-L-phenylalanyl]-2′-*O*-(4-tetrahydropyran-4-yl)adenosine (9a).** Compound **8a** (0.6 g, 0.79 mmol) and *N*-(benzoxycarbonyl)-L-phenylalanine (0.28 g, 0.95 mmol) were dissolved in pyridine (15 mL) after being coevaporated with anhydrous pyridine several times. The solution was stirred with 4-(dimethylamino)pyridine (0.05 g) and dicyclohexylcarbodiimide (0.35 g, 1.75 mmol) for 16 h. The reaction mixture was quenched with ice and filtered, and the filtrate was partitioned between water and methylene chloride. The organic layer was dried with sodium sulfate and evaporated to dryness in vacuo. The crude reaction product was coevaporated several times with toluene and dissolved in 80% acetic acid, and the solution was allowed to stand for 15 min and was freeze-dried. The residue was chromatographed on a silica gel column (2.5 ×

Table II. Protected Oligoribonucleotides and 3′-*O*-Aminoacyl Oligoribonucleotides

| compd | % yield[a] | formula | anal. calcd/found | | | | UV data[b] | | $^{31}$P NMR,[c] ppm |
|---|---|---|---|---|---|---|---|---|---|
| | | | % C | % H | % N | % Cl | $\lambda_{max}$, nm ($\epsilon \times 10^{-3}$) | $\lambda_{min}$, nm ($\epsilon \times 10^{-3}$) | |
| 11a | 55 | $C_{69}H_{72}N_6O_{19}ClP$ | 61.15/60.89 | 5.31/5.41 | 6.20/6.13 | 2.62/3.00 | 308 (16.2), 265 (46.7), 235 (41.97) | 298 (15.2), 245 (34.5) | −3.20, −3.34 |
| 12 | 61 | $C_{99}H_{101}N_{11}O_{28}Cl_2P_2$ | 58.69/58.38 | 5.02/5.16 | 7.61/7.46 | 3.50/3.81 | 263 (52.21) | 243 (36.55) | −2.87, −3.34, −3.42, −3.63 |
| 13a | 66, 51[d] | $C_{115}H_{121}N_{12}O_{31}Cl_2P_2$ | 60.05/60.18 | 5.26/5.17 | 7.31/7.06 | 3.08/3.32 | 265 (51.59) | 246 (38.94) | −2.98, −3.27, −3.45, −3.67[e] |
| 13b | 72[d] | $C_{110}H_{112}N_{12}O_{31}Cl_2P_2\cdot H_2O$ | 58.74/58.36 | 5.11/5.19 | 7.97/7.45 | 3.15/3.95 | 263 (50.57) | 243 (33.47) | −2.76, −3.34, −3.42, −3.56[f] |
| 14 | 61 | $C_{105}H_{104}N_{12}O_{29}Cl_2P_2$ | 59.18/58.81 | 4.92/5.16 | 7.89/7.77 | 2.91/3.19 | 262 (49.57) | 245 (39.09) | −3.34, −3.38 (double peak), −3.56, −3.63, −3.71, −3.78, −3.85[g] |

[a] TLC ($S_1$) homogenous, elongated or double spots due to diastereoisomers on phosphorus. [b] In 95% ethanol. [c] In acetone-$d_6$ with $H_3PO_4$ as an external standard; splitting of signals due to chirality of triester phosphorus. [d] Yields of aminoacylation of protected C-C-A derivative (12). [e] $^1$H NMR (acetone-$d_6$, $Me_4Si$ internal standard) 5.07 ppm (s, 2 p, $CH_2$ of benzyloxycarbonyl). [f] $^1$H NMR (acetone-$d_6$, $Me_4Si$ internal standard) 5.11 ppm (s, 2 p, $CH_2$ of benzyloxycarbonyl). [g] Two TLC-separated diastereoisomers (UV spectral also identical); $^1$H NMR spectra (acetone-$d_6$, $Me_4Si$ internal standard) 5.07 (s, 2 p, $CH_2$ of benzyloxycarbonyl).

Table III. 2′(3′)-O-Aminoacyl Trinucleoside Diphosphate Data

| compd | absorbance (0.01 N HCl solutions) $\lambda_{max}$, nm | 250/260 | 280/260 | 290/260 | % 2′-5′ isomer[a] | Cp/A[a] ratio | % 3′-3′ or 3′-2′ isomer[b] |
|---|---|---|---|---|---|---|---|
| C-C-A-Phe (16e) | 269 | 0.76 | 0.96 | 0.66 | 0 | 1.99 | 0 |
| C-C-A-Ala (16f) | 269 | 0.73 | 0.98 | 0.66 | 3.1 | 2.18 | c |
| C-C-A-Gly (16g) | 269 | 0.72 | 0.96 | 0.60 | 0 | 1.91 | 0 |

[a] Determined by pancreatic ribonuclease digestion. [b] Determined by snake venom phosphodiesterase digestion. [c] Not determined, since the starting C-C-A derivative (12) was free of 3′-3′ or 3′-2′ isomer (see Experimental Section).

35 cm) with a linear gradient of $CH_2Cl_2$ and $CH_2Cl_2$–7% $CH_3OH$ (2 L × 2 L) to afford pure **9a**, 0.3 g (51.6%). Anal. Calcd for $C_{39}H_{40}N_6O_9$: C, 63.58; H, 5.43; N, 11.41. Found: C, 63.21; H, 5.39; N, 10.96.

***N*⁶-Benzoyl-5′-*O*-(*tert*-butyldimethylsilyl)-2′-*O*-(4-methoxytetrahydropyran-4-yl)adenosine (8b).** Compound **3c** (3.9 g, 8 mmol), *tert*-butyldimethylsilyl chloride (1.4 g, 8.8 mmol), and imidazole (1.3 g, 19 mmol) were stirred in anhydrous DMF (20 mL) for 20 h, the solution was evaporated in vacuo, and the residue was partitioned between water and methylene chloride. The organic layer was washed with water, dried over sodium sulfate, and evaporated to dryness in vacuo. The crude reaction product was chromatographed on a silica gel column (2.5 × 50 cm) by using a linear gradient of $CH_2Cl_2$ and $CH_2Cl_2$–7% $CH_3OH$ (1:1 mixture) to afford pure **9b**, 2.78 g (62%). Anal. Calcd for $C_{29}H_{41}N_5O_7Si$: C, 58.09; H, 6.84; N, 11.68. Found: C, 57.85; H, 7.05; N, 11.48.

**General Method for Preparation of Fully Protected 3′-*O*-Aminoacyl Oligoribonucleotides 13 and 14 or Oligoribonucleotides 11a and 12.** The triethylammonium salt of the nucleotide component (4 or **11b**, 0.2 mmol) and the nucleoside component (**3a,c**, **9b**, or **10b**, 0.3 mmol) were dried by coevaporation with pyridine, and the residue was dissolved in pyridine (ca. 10 mL). MST (0.18 g, 0.7 mmol, ca. 3.5 equiv) was added to the reaction mixture (in a drybox). After ca. 4 h, the reaction was usually complete as evidenced by TLC in system $S_1$ (disappearance of the material with zero mobility and formation of a new spot, positive to perchloric acid spray). The reaction mixture was quenched by ice and concentrated to a small volume, and the residue was extracted by methylene chloride. The methylene chloride layer was washed with water (3×), the organic layer was dried with magnesium sulfate and evaporated in vacuo, and the residue was coevaporated with toluene (3×). The mixture was applied to a silica gel column (2.5 × 40 cm) which was eluted under standard conditions by using a linear gradient of $CH_2Cl_2$ and $CH_2Cl_2$–5% $CH_3OH$ (2 L × 2 L). Fractions containing the reaction products (**13**, **14**, **11a**, or **12**; positive to $HClO_4$ spray) were collected, evaporated, and dried in vacuo. For the yields and characterization of protected oligoribonucleotides, see Table II.

**Aminoacylation of Protected C-C-A Derivative 12.** Partially protected C-C-A derivative **12** (0.072 g, 0.035 mmol) and the *N*-(benzyloxycarbonyl)-L-amino acid (0.107 mmol, 3 equiv) were made anhydrous by coevaporation with pyridine and dissolved in pyridine (2 mL), and MST (0.036 g, 0.14 mmol, 4 equiv) was added to the solution in the drybox. The reaction mixture was kept in the drybox until TLC in system $S_1$ had shown the reaction to be complete (less than 2 h). Ice was added, and after ca. 30 min the reaction mixture was taken down to a small volume, the residue was partitioned between methylene chloride and water, and the organic layer was washed with water (3×) and dried with magnesium sulfate. The methylene chloride solution was evaporated in vacuo and the residue repeatedly coevaporated with toluene. The crude reaction product was purified as in the above preparation to yield compounds **13**.

**Cytidylyl(3′–5′)cytidylyl(3′–5′)-2′(3′)-*O*-[*N*-(benzyloxycarbonyl)glycyl]adenosine (16d).** Protected trinucleotide **14** (0.1 g, 0.048 mmol) was dissolved in dioxane (8 mL) and concentrated ammonium hydroxide (12 mL), and the resulting mixture was stirred at 50 °C for 24 h. The solution was concentrated in vacuo, the residue was dissolved in water and extracted three times with ether, and the water solution was freeze-dried. TLC in systems $S_4$ or $S_5$ have shown the essentially quantitative formation of slow-moving material. The residue was dissolved in a mixture 0.1 N HCl–dioxane (10 mL, 1:1 v/v), and the solution was kept at room temperature for 17 h before lyophilization. The residue was repeatedly cofreeze-dried with dioxane. TLC in system $S_6$ has indicated formation of a major nucleotide product with a mobility similar to that of adenosine which, upon chromatography in system $S_9$, gives C-C-A as the major product. The residue was dissolved in system $S_6$ and applied on two plates of cellulose which were developed in the same system. The major band of product **16d** was eluted by $S_6$, the solution evaporated in vacuo, and the residue coevaporated with water. The residue was dissolved in a water–acetic acid mixture with traces of methanol, the solution was filtered through a small cotton filter, and the yield of chromatographically and electrophoretically uniform **16d** was determined spectrophotometrically: 0.02 mmol (43%); UV (0.01 N HCl) $\lambda_{max}$ 268 nm; $A(250/260) = 0.74$, $A(280/260) = 0.91$, $A(290/260) = 0.56$.

**Cytidylyl(3′–5′)cytidine (15) and Cytidylyl(3′–5′)cytidylyl(3′–5′)adenosine (16a).** The protected derivative **11a** or **12** (ca. 0.025 mmol) was dissolved in dioxane (2 mL) and concentrated aqueous ammonia (8 mL), and the sealed reaction mixture was stirred at 50 °C for 24 h. The solution was evaporated, residue was partitioned between water and ether, and the aqueous layer was freeze-dried. The residue was dissolved in a mixture of dioxane–0.1 N HCl (10 mL, 1:1), and after 12.5 h at room temperature the solution was neutralized with 0.5 N ammonia and freeze-dried. The residue was dissolved in methanol–water and applied on one plate of cellulose which was developed in system $S_9$. The band of product **15** or **16a** was eluted with water. The product C-C (**15**), obtained in 74% yield, was chromatographically ($S_9$) and electrophoretically ($E_2$; mobility = 0.41, relative to Cp) uniform and had the following UV spectral properties: $\lambda_{max}$ 279 nm, $\lambda_{min}$ 241; $A(250/260) = 0.45$, $A(280/260) = 2.00$, $A(290/260) = 1.45$. C-C (**15**) is cleaved (96.9%) by pancreatic ribonuclease to form Cp and C (Cp/C ratio of 0.98) and is also cleaved (100%) by snake venom phosphodiesterase to form pC and C. C-C-A (**16a**) was obtained in 60.5% yield and was chromatographically ($S_6$ and $S_9$) and electrophoretically $E_2$; mobility = 0.68, relative to Cp) uniform: UV spectra $\lambda_{max}$ 270 nm, $\lambda_{min}$ = 236; $A(250/260) = 0.71$, $A(280/260) = 1.01$, $A(290/260) = 0.61$. C-C-A (**16a**) was cleaved (97.8%) with pancreatic ribonuclease to Cp and A (Cp/A ratio of 2.01) and also with snake venom phosphodiesterase (100%) to form C, pC, and pA.

**Cytidylyl(3′–5′)cytidylyl(3′–5′)-2′(3′)-*O*-[*N*-(benzyloxycarbonyl)-L-phenylalanyl]adenosine (16b) and Cytidylyl(3′-5′)cytidylyl(3′-5′)-2′(3′)-*O*-[*N*-(benzyloxycarbonyl)-L-alanyl]adenosine (16c). (a) Removal of *N*-Benzoyl Groups with Hydrazine.** The fully protected aminoacyl derivative **13** (ca. 0.025 mmol) was dissolved in hydrazine buffer (15 mL; 0.5 M $N_2H_4$ in pyridine–acetic acid, 3:2 v/v), and the reaction mixture was kept at room temperature for 16 h. The solution was cooled to 0 °C, acetylacetone was added (0.5 mL, approximately 2 equiv relative to hydrazine), the solution was concentrated in vacuo, and the residue was partitioned between water and methylene chloride. The organic layer was washed with water, dried with magnesium sulfate, evaporated in vacuo, and coevaporated with toluene. The residue was dissolved in methylene chloride, applied on a silica gel column (1.5 × 30 cm), and eluted with a linear gradient of $CH_2Cl_2$ and $CH_2Cl_2$–25% MeOH (1 L × 1 L). The product was recovered by pooling and evaporation of the appropriate fractions. It appeared to be uniform on TLC in systems $S_2$, $S_5$, and $S_9$ and has a UV spectrum (95% ethanol–0.01 N HCl) similar to that of C-C-A. A small amount of slower migrating material, which does not contain an amino acid (as evidenced by comparison on TLC in system $S_8$ with the intermediate obtained by $N_2H_4$ deblocking from trinucleotide **12**), can be recovered from the later fractions from the silica gel column. The yields of the

Table IV. Electrophoretic and Chromatographic Mobilities of Products and Standard Specimens (Electrophoresis in $E_1$ and TLC System $S_6$)

| compd | electrophoretic mobility[a] | $R_f$ |
|---|---|---|
| Cp | 1.00 | 0.41 |
| A | 2.73 | 0.69 |
| C-C-A (16a) | 1.85 | 0.20 |
| C-C-A(Z-Gly) (16d) | 1.53 | 0.71 |
| C-C-A-Gly (16g) | 2.45 | 0.25 |
| C-C-A(Z-Ala) (16c) | 1.32 | 0.68 |
| C-C-A-Ala (16f) | 2.20 | 0.23 |
| C-C-A(Z-Phe) (16b) | 1.12 | 0.75 |
| C-C-A-Phe (16e) | 2.05 | 0.45 |

[a] Relative mobility toward cathode; mobility of Cp = 1.00.

desired intermediate products range between 35% and 50%.

**(b) Removal of 2-Chlorophenyl Groups from Phosphorus with $F^-$.** The protected derivative from the previous experiment (approximately 0.01 mmol) was dissolved in 0.05 M tetrabutylammonium fluoride in a mixture of tetrahydrofuran-pyridine-water (1.2 mL, 3 equiv, 8:1:1 v/v/v) and allowed to stand for 6 h at room temperature. After this time, TLC in systems $S_5$ and $S_4$ indicated essentially quantitative conversion of the starting triester to diester. The solution was evaporated in vacuo and the residue partitioned between methylene chloride and water. The organic layer was washed with water (3×) and back-extracted with ethyl acetate (2×), and the combined organic layers were dried with sodium sulfate and evaporated in vacuo. The residue was directly used in the next step without further purification.

**(c) Removal of the Methoxytetrahydropyranyl and Methoxytrityl Groups in Acidic Medium.** The residue from the previous step was dissolved in a mixture of 0.1 N HCl and dioxane (5 mL, 1:1 v/v), and the reaction mixture was allowed to stand at room temperature for 16 h. The workup of the reaction was as described above for the synthesis of C-C-A(Z-Gly) (**16d**). The yields of chromatographically and electrophoretically uniform compounds **16b** and **16f** were in the 15-20% range in three consecutive deblocking steps. For C-C-A(Z-Phe) (**16b**): UV (0.01 N HCl) $\lambda_{max}$ 269 nm; $A(250/260) = 0.76$, $A(280/260) = 0.96$, $A(290/260) = 0.66$. For C-C-A(Z-Ala) (**16c**): UV (0.01 N HCl) $\lambda_{max}$ 269 nm; $A(250/260) = 0.76$, $A(280/260) = 1.04$, $A(290/260) = 0.69$.

**2′(3′)-*O*-Aminoacyl Derivatives of Cytidylyl(3′-5′)cytidylyl(3′-5′)adenosine (16e-g).** The *N*-benzyloxycarbonyl derivatives **16b-d** (10-20 μmol) were hydrogenated as described previously,[5] with the exception that the reaction time was approximately 2-3 h (until TLC in system $S_6$ showed quantitative conversion to the slower moving aminoacyl derivative). The products were further purified (for the purpose of biochemical investigations) by preparative electrophoresis in system $E_1$. For the characterization of the final products, see Tables III and IV. The compounds **16e-g** were quantitatively hydrolyzed during paper chromatography in system $S_9$ to C-C-A and the corresponding amino acid.

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**Registry No. 1a** (isomer 1), 80185-90-6; **1a** (isomer 2), 80185-91-7; **1b** (isomer 1), 80226-97-7; **1b** (isomer 2), 80226-98-8; **1b** 5′-(α,α-dimethoxybenzyl) ether, 80185-92-8; **2a** (2′-isomer), 6554-16-1; **2a** (3′-isomer), 6554-17-2; **2b** (2′-isomer), 80185-93-9; **2b** (3′-isomer), 80185-94-0; **2c** (2′-isomer), 80185-95-1; **2c** (3′-isomer), 80185-96-2; **3a**, 77451-35-5; **3b**, 80185-97-3; **3c** (2′-isomer), 80185-98-4; **3c** (3′-isomer), 72677-41-9; **4** triethylammonium salt, 80186-00-1; **5**, 3250-02-0; **6a**, 55697-22-8; **6b**, 33485-36-8; **7**, 80186-01-2; **8a**, 80186-02-3; **8b**, 80186-03-4; **8c**, 80186-04-5; **8d**, 80186-05-6; **8g**, 80186-06-7; **9a**, 80186-07-8; **9b**, 78272-39-6; **10a**, 3309-58-8; **10b** (isomer 1), 80226-99-9; **10b** (isomer 2), 80227-00-5; **11a** (isomer 1), 80186-08-9; **11a** (isomer 2), 80227-01-6; **11b** triethylammonium salt, 80206-09-3; **12**, 80206-10-6; **13a**, 78272-40-9; **13a** $N^4,N^4,N^6$-tridebenzoyl, 80186-09-0; **13a** $N^4,N^4,N^6$-tridebenzoyl, bis(de-2-chlorophenyl), 80186-10-3; **13b**, 80186-11-4; **13b** $N^4,N^4,N^6$-tridebenzoyl, 80206-11-7; **13b** $N^4,N^4,N^6$-tridebenzoyl, bis(de-2-chlorophenyl), 80186-12-5; **14**, 80186-13-6; **15**, 2536-99-4; **16a**, 2866-39-9; **16b** 2′-isomer, 80186-14-7; **16b** 3′-isomer, 78280-90-7; **16c** 2′-isomer, 80186-15-8; **16c** 3′-isomer, 80186-16-9; **16d** 2′-isomer, 80186-17-0; **16d** 3′-isomer, 78280-88-3; **16e** 2′-isomer, 80186-18-1; **16e** 3′-isomer, 78280-91-8; **16f** 2′-isomer, 80186-19-2; **16f** 3′-isomer, 80186-20-5; **16g** 2′-isomer, 80186-21-6; **16g** 3′-isomer, 78280-89-4; adenosine, 58-61-7; methyl orthobenzoate, 707-07-3; 4-methoxy-5,6-dihydro-2*H*-pyran, 17327-22-9; 2′,3′,-*O*-isopropylideneadenosine, 362-75-4; 3′,5′-di-*O*-acetyladenosine, 6554-24-1; 2′-*O*-(4-methoxytetrahydropyran-4-yl)adenosine, 28219-91-2; levulinic acid, 123-76-2; *N*-(benzyloxycarbonyl)-L-phenylalanine, 1161-13-3; *N*-(benzoyloxycarbonyl)-L-alanine, 1142-20-7; cytidine, 65-46-3.

# Alkoxyl Migration in Displacement of a 5-Trifluoromethanesulfonyloxy Group from Ribofuranosides[1a]

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Methyl 2,3-*O*-isopropylidene-5-*O*-triflyl-β-D-ribofuranoside (**2**) reacts at room temperature with primary aralkanols such as benzyl alcohol and the steroid alcohols **3a,b** in dichloromethane and in the presence of sodium sulfate to give the corresponding aralkyl 2,3-*O*-isopropylidene-5-*O*-methyl-β-D-ribofuranosides **5a,b** and **11** in 40-45% yields. Migration of the methoxyl group from C-1 to C-5, via a tricyclic oxonium ion (**7a**), is suggested as the basis of formation of the new β-glycoside. Anchimeric assistance by a benzyloxy group in the displacement of the sulfonate is observed in the reaction of benzyl 2,3-*O*-isopropylidene-5-*O*-triflyl-β-D-ribofuranoside (**14**) with methanol, which affords methyl 5-*O*-benzyl-2,3-*O*-isopropylidene-β-D-ribofuranoside (**15**) in 40% yield on treatment with $Na_2SO_4$ in $CH_2Cl_2$. The elements of anomeric control in these facile transformations remain to be elaborated.

Trifluoromethanesulfonate (triflate) esters are exceedingly useful substrates in nucleophilic substitution reactions because of their high level of reactivity and ready accessibility.[2] These considerations have attracted interest

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