New Approach to the Synthesis of 2′(3′)-O-Aminoacyl Oligoribonucleotides[1]

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A new approach to the synthesis of 2′(3′)-O-aminoacyl oligoribonucleotides which are the 3′-terminal sequences of corresponding aa-tRNAs is described. It is exemplified by the synthesis of the trinucleotide sequence C-C-A-Phe (10). This compound was synthesized by employing the benzotriazolyl phosphotriester approach using the following protecting groups on the components: a (9-fluorenylmethyloxy)carbonyl for the cytosine and adenine amino groups; 2-chlorophenyl groups for the internucleotide bond protection; a dimethoxytrityl group for blocking of the 5′-hydroxy function; a 4-methoxytetrahydropyranyl group for protection of 2′-hydroxy functions; and a (2-(4-biphenylyl)isopropyloxy)carbonyl group for blocking of the α-amino acid. Protected C-C-A (7) was synthesized in a stepwise fashion and quantitatively aminoacylated with ((2-(4-biphenylyl)isopropyloxy)carbonyl)-L-phenylalanine in the presence of mesitylenesulfonyltetrazole. The resulting fully blocked derivative 9 was deprotected in *only two steps* via oximate and diluted formic acid treatment to obtain the desired C-C-A-Phe (10) in ca. 30% isolated yield by using a new isolation procedure. Thus this approach has potential to become a general method for the synthesis of various 2′(3′)-O-aminoacyl oligoribonucleotides which are important tools for mechanistic studies of protein synthesis in in vitro systems.

It is well established that 2′(3′)-O-aminoacyl oligoribonucleotides with a nucleotide sequence identical with or resembling that of the 3′-terminus of aa-tRNA are important tools for studying a role of the aa-tRNA 3′-end in protein biosynthesis.[2] The development of specific and general syntheses for 2′(3′)-O-aminoacyl oligoribonucleotides is thus of a considerable importance. An alternative to the chemical synthesis, an enzymic degradation of aa-tRNA, may provide fragments with only natural, thus unmodified sequences of a given aa-tRNA and in only very limited quantities.[3]

The development of chemical syntheses of 2′(3′)-O-aminoacyl oligoribonucleotides has been hampered by the presence in their molecules of two extremely sensitive functions, the aminoacyl ester bond and phosphodiester linkages, which have reverse sensitivity to the spectrum of usual deblocking reagents. This fact makes a selection of appropriate blocking groups a rather difficult task.[4]

Previous work from our laboratory[5] has shown that the phosphotriester method is suitable and advantageous for the synthesis of 2′(3′)-O-aminoacyl oligoribonucleotides. In that work an N-benzoyl group was used for the protection of the cytidine and adenosine aglycon moieties. Although removal of the benzoyl group with hydrazine could be accomplished selectively in the presence of, e.g., a 2′(3′)-O-(N-(benzyloxycarbonyl)aminoacyl) moiety in simple nucleoside models, this reaction becomes more complicated with longer oligoribonucleotide derivatives. The formation of side products and the presence of partly deprotected material was apparently responsible for lower yields of deblocking. Secondly, the protection of the α-amino function of the amino acid with the commonly used N-benzyloxycarbonyl group requires a catalytic hydrogenation in order to accomplish its removal. This heterogeneous reaction again appears to be more sluggish with larger oligomers.[1a] Lastly, removal of phosphate protecting groups with fluoride ions may, under certain conditions, lead to some degradation of the oligonucleotide chain.[5,6] Therefore, further progress in chemistry of 2′(3′)-O-aminoacyl oligoribonucleotides requires the development of new protecting groups for both amino groups of aglycons and the α-amino group of an amino acid. In this work we describe the development of such a blocking system which has general application for the synthesis of longer aminoacylated RNA fragments.

Results and Discussion

(1) Protecting Groups. The polyfunctional nature of nucleoside/nucleotide molecules requires the use of different blocking groups to protect reactive functions and, moreover, these groups have to be removable with preservation of the integrity of the highly unstable 2′(3′)-O-aminoacyl ester linkage. This fact, although rather trivial, presents a serious constraint for a judicious choice of blocking groups, since the aminoacyl ester bond is extremely sensitive to hydrolysis even at neutral pH. The following functional groups have to be protected during oligoribonucleotide synthesis using the triester approach: (i) hydroxy groups of ribose moieties; (ii) amino groups of aglycons; (iii) phosphodiester bonds; (iv) the α-amino group of the amino acid. It is also important to realize that the sequential removal of blocking groups must proceed in a selective manner. Thus, removal of blocking groups from phosphorus must not be accompanied by any cleavage of 2′-OH protecting groups at the ribose moieties, since this would certainly lead to a breakage and isomerization of internucleotide bonds and also to a loss of an aminoacyl residue.

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(1) (a) This paper is 43 in the series Aminoacyl Derivatives of Nucleosides, Nucleotides, and Polynucleotides. For a preceding report in this series, see: Scalfi-Happ, C.; Happ, E. Ghag, S., Chládek, S. *Biochemistry* **1987**, *26* 4682. (b) This investigation was supported, in part by the U.S. Public Health Service Research Grant GM 19111 from the National Institutes of Health and by an Institutional Grant to the Michigan Cancer Foundation from the United Foundation of Greater Detroit. This work was presented at the International Round Table: Nucleosides, Nucleotides and their Biological applications, Konstanz (Germany), cf. Scalfi-Happ, C.; Happ, E.; Chládek, S. *Nucleosides Nucleotides*, **1987**, *6*, 345. (c) For abbreviations used, see: *Handbook of Biochemistry*; Sober, H. A., Ed.; CRC Press: Cleveland, OH, Sections A and B. Other abbreviations: A^{Fmoc}, N^6-((9-fluorenylmethoxy)-carbonyl)adenin-9-yl; BPOC, (2-(4-biphenylyl)isopropyloxy)carbonyl; BT, benzotriazolyl; C^{Fmoc}, N^4-((9-fluorenylmethyloxy)carbonyl)cytosin-1-yl; C-C-A-Phe, cytidylyl(3′-5′)cytidylyl(3′-5′)-2′(3′)-O-(L-phenylalanyl)-adenosine; 2-ClPh, 2-chlorophenyl; DMT, 4,4′-dimethoxytrityl; Fmoc, (9-fluorenylmethyloxy)carbonyl; MST, (mesitylenesulfonyl)tetrazole; Mthp, 4-methoxytetrahydropyran-4-yl; $TIPDSiCl_2$, 1,1,3,3-dichlorotetraisopropyldisiloxane. A_{260} unit is a quantity of material contained in 1 mL of solution which has an absorbance of 1.00 at 260 nm when measured in a 1-cm path length cell.

(2) Chládek, S.; Sprinzl, M. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 371.

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

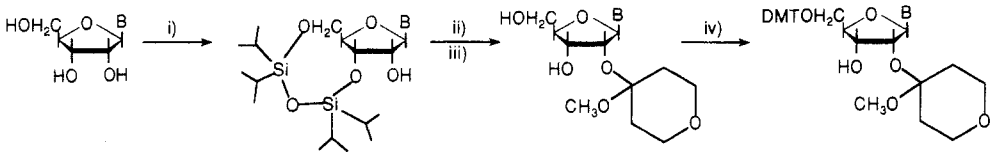
(4) Chládek, S. In *Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum Publishing Co.; New York, in press.

(5) Kumar, G.; Celewicz, L.; Chládek, S. *J. Org. Chem.* **1982**, *47*, 634.

(6) Reese, C. B.; Titmas, R. C.; Yau, L. *Tetrahedron Lett.* **1978**, 2727.

0022-3263/87/1952-5387$01.50/0

Scheme I



1 2 3 4

i) $TIPDSiCl_2$
ii) methoxydihydropyran + H^+
iii) F^-
iv) DMTCl

a) B = Ade^{Fmoc}
b) B = Cyt^{Fmoc}

Table I. ^1H NMR Spectral Data of Nucleoside Derivatives (δ)[a]

compd	solvent	H_8 or H_2; H_6 or H_5	aromatic protons	$H_{1'}$	fluorene protons	OCH_3 (Mthp)	other signals
2a	$CDCl_3$	8.89 (s; 1)	7.68, 7.36	6.04 (d; 1)	5.09 (m; 1)		1.11 (m, 28)
		8.21 (s; 1)	(m; 8)	J = 1	4.62 (m; 2)		isopropyls
2b	$CDCl_3$ + D_2O	8.23–7.21	(m; 10)	5.81 (s; 1)	4.42 (m; 1)		1.03 (m; 28)
					4.25 (m; 2)		isopropyls
3a	$CDCl_3$	8.81 (s; 1)	7.78, 7.41	5.99 (d; 1)	4.73 (d; 1)	2.68	
		8.07 (s; 1)	(2 m; 8)	J = 8	4.44 (m; 2)		
3b	$CDCl_3$	8.32 (d; 1)	7.84, 7.37	6.05 (d; 1)	4.65 (m; 1)	3.08	
		7.00 (d; 1)	(2 m; 8)	J = 7	4.53 (m, 2)		
4a	$CDCl_3$	8.78 (s; 1)	7.8–6.8	5.93 (d; 1)	4.68 (m; 1)	2.67 (s; 3)	3.84 (s; 6) CH_3O of DMT
		8.01 (s; 1)	(m; 21)	J = 7	4.43 (m; 2)		
4b	$CDCl_3$	8.33–7.24	(m; 23)	6.26 (d; 1)	4.65–4.18	3.2 (s; 3)	3.80 (s; 6) CH_3O of DMT
			(d; 1)	J = 4	(m; 3)		

[a] δ given in ppm; J values in hertz.

In our approach, the 2′-hydroxy functions of ribose moieties were blocked with the 4-methoxytetrahydropyranyl group[7] and the 5′-hydroxy function was masked with the dimethoxytrityl group. These groups are easily removable at acidic pH without isomerization of the phosphodiester linkages or loss of an aminoacyl moiety.[4,8] We have investigated different N-acyl groups for protection of exocyclic amino groups of nucleoside bases to replace commonly used N-benzoyl groups, whose selective removal appeared to be problematic (vide supra). We hoped that a removal of some other group via, e.g., a mild β-elimination procedure in an anhydrous medium, would not interfere with a sensitive aminoacyl function. Indeed, the (9-fluorenylmethoxy)carbonyl group[9] fulfilled such a requirement since we have found that it is removable via the agency of N^1,N^1,N^3,N^3-tetramethylguanidine in an anhydrous medium without significant loss of a protected aminoacyl ester group. Incidentally, N^1,N^1,N^3,N^3-tetramethylguanidine has been employed previously as a base during the 2-nitrobenzaldoxime-promoted cleavage of 2-chlorophenyl phosphotriesters.[6,10] Thus, we employed once again the 2-chlorophenyl group[5] for internucleotide protection. Contrary to our previous work, in which we used fluoride ions for removal of such a group,[5] we now resorted to the oximate treatment (vide supra and ref 10) as a selective way to remove both the (9-fluorenylmethyloxy)carbonyl and 2-chlorophenyl groups and thus avoid use of fluoride ions completely. In fact, fluoride ions are difficult to remove from a reaction mixture and their use may result, under certain conditions, in a cleavage of internucleotide bonds.[5,6]

The α-amino group of the aminoacyl residue must be protected during the aminoacylation reaction and use of urethane-type groups seems to be the most advantageous since the racemization of the L-aminoacyl residue could be avoided.[4] Our search for a suitable group led to the application of the (2-(4-biphenylyl)isopropyloxy)carbonyl group.[11] We have found that this group, previously employed in peptide chemistry, can be easily cleaved with a brief acidic treatment, thus replacing the rather inconvenient hydrogenation procedure necessary for the removal of the N-benzyloxycarbonyl group (vide supra).

(2) Synthesis of Building Blocks. Scheme I shows the synthesis of cytidine- and adenosine-derived building blocks for 2′(3′)-*O*-aminoacyl oligonucleotide synthesis. In order that this method becomes general, analogous derivatives of uridine and guanosine must be also prepared. The appropriate derivative of uridine is now available[1b] and the synthesis and use of the corresponding guanosine synthon will be reported subsequently.

The *N*-(9-fluorenylmethyloxy)carbonyl nucleosides[9b] **1** were first converted into 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl) derivatives **2** via the reaction with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in the presence of pyridine.[12] In the next step the 4-methoxytetrahydropyranyl group was introduced into the 2′-position via addition of **2** to 4-methoxy-5,6-dihydro-2*H*-pyran in presence of mesitylenesulfonic acid. The protecting 3′,5′-tetraisopropyldisiloxane group was removed quantitatively with deactivated fluoride ions[5] (in the presence of water and pyridine) without significantly affecting the N-(9-fluorenylmethyloxy)carbonyl group which is otherwise known to be easily removable by nucleophiles.[9b]

Thus, compounds **3** could be used as extension units for the oligonucleotide synthesis while the synthons **4** obtained

(7) Reese, C. B.; Saffhill, R.; Sulston, J. E. *Tetrahedron* **1970**, *26*, 1023.
(8) Reese, C. B. *Tetrahedron* **1978**, *34*, 3143.
(9) (a) Caprino, L. A.; Han, G. Y. *J. Am. Chem. Soc.* **1970**, *92*, 5748. (b) The only applications of the Fmoc group in the nucleoside chemistry to date consist in preparation and stability studies of N-Fmoc derivatives of the common ribo- and deoxynucleosides [Heikkilä, J.; Chattopadhyaya, J. *Acta Chem. Scand., Ser. B* **1983**, *B 37*, 263] and the use of the later for the deoxyribooligonucleotide synthesis: (c) Webb, T. R.; Matteucci, M. D. *Nucleic Acids Res.* **1986**, *14*, 7661.
(10) Wreesmann, C. T. J.; Fidder, A. van der Marel, G. A.; van Boom, J. H. *Nucleic Acids Res.* **1983**, *11*, 8389.
(11) Sieber, P.; Iselin, B. *Helv. Chim. Acta* **1968**, *51*, 614.
(12) Markiewicz, W. T.; Wiewiorowski, M. In *Nucleic Acids Chemistry*; Townsend, L. B., Tipson, R. S., Eds.; Wiley: New York, 1986; pp 229.

from **3** by a reaction with dimethoxytrityl chloride serve as the 5′-terminal units.

(3) Oligonucleotide Synthesis and Aminoacylation of the Protected C-C-A Derivative. As in the previous communications of this series, our general strategy requires building of an oligonucleotide in the 5′-3′ direction.[1a,5] We have used the hydroxybenzotriazolyl method[1a,10] for the phosphorylation of the 5′-terminal component **4b** and the condensation of the intermediary formed activated phosphodiester with the synthon **3b** to form the dinucleotide **6** or a repetition of this reaction to produce the trinucleotide derivative **7** (Scheme II). Employing the bifunctional phosphorylating reagent **5** enables achievement of a two-step reaction without isolation of an intermediate diester in a good yield.[10] The intermediate **7** has only the 3′-OH group left unprotected and this was easily aminoacylated with *N*-[(2-(4-biphenylyl)isopropyloxy)-carbonyl]-L-phenylalanine activated by (mesitylenesulfonyl)tetrazole[5] to form the fully protected aminoacylated trinucleotide **9** in a quantitative yield.

(4) Deblocking Procedures. The major advantage of the new blocking system, as described herein, resides in the fact that it leads to a greatly simplified deblocking procedure: only *two simple steps*, both proceeding quantitatively, are required for removal of all the protecting groups. In the first step, the dry oximate treatment[6,10] removes both 2-chlorophenyl groups from internucleotide linkages and N-(9-fluorenylmethyloxy)carbonyl groups from exocyclic amino groups of aglycons. The workup of the reaction mixture is extremely simple and does not require the use of chromatography, since ether precipitation is sufficient to remove lipophilic products. It is noteworthy that only a small amount of the BPOC-phenylalanyl moiety was hydrolyzed under the reaction conditions employed and no other side products were observed while analyzing the reaction mixture by using HPLC. The small amount of deacylated trinucleotide is easily removed in a final purification step, which follows the hydrolysis with formic acid of dimethoxytrityl, 4-methoxytetrahydropyranyl, and BPOC groups. The final purification of the compound **10** was achieved by a simple and well-reproducible procedure using chromatography on a Baker's 10 SPE C18 column.

C-C-A-Phe (**10**) was quantitatively split with pancreatic ribonuclease and snake venom phosphodiesterase to expected products in correct ratios, which proves a virtual absence of unnatural linkages. Further characterization criteria included TLC, alkaline hydrolysis to C-C-A and phenylalanine, UV spectra, and paper electrophoresis.

We feel that the method outlined here has the potential for being extended to the preparation of longer aminoacylated oligonucleotide sequences, containing all common nucleosides. Work along these lines is in active progress in our laboratory.

Experimental Section

General Methods. The general methods were the same as those described in previous papers of this series.[1a,5] Elemental analyses were performed by MHW Laboratories.

Chromatography and Electrophoresis. Thin layer chromatography (TLC) was performed on silica gel (60F-254) coated aluminum foils containing fluorescent indicator (E.M. Laboratories) and on cellulose plates (Avicel F Uniplate; Analtech). Preparative TLC was performed on silica gel GF (precoated thin layer chromatography plates, 2000 μm; Analtech). The following chromatographic systems were used on silica gel, S_1, CH_2Cl_2–5% CH_3OH; S_2, CH_2Cl_2–10% CH_3OH, and on cellulose, S_3, 1-butanol–water–acetic acid (5:3:2), and S_4, 2-propanol–water–NH_4OH (7:2:1). Paper electrophoresis was conducted on a Savant flat plate (at 4 °C) by using 1 M acetic acid on Whatman no. 1 paper at 50 V/cm for 2 h. Column chromatography was performed on silica gel (E.M. Reagent, 70–230 mesh) using a step gradient of methanol in CH_2Cl_2. The 2′(3′)-O-aminoacyl oligonucleotides were also purified on a Baker's 10 SPE Octadecyl column (J.T. Baker Chemical Company) by using a step gradient of CH_3CN in ammonium acetate buffer (5 mM; pH 4.5). High performance liquid chromatography (HPLC) was conducted on an Altex-Spectraphysics instrument with a Kratos Spectroflow 773 absorbance detector (at 254 nm).

Spectra. UV spectra were obtained by using a Perkin-Elmer Lambda 5 UV/vis spectrophotometer. Yields of oligonucleotides were determined spectrophotometrically at pH 2.0 (0.01 N HCl) by using $\epsilon_{260} = 27.9 \times 10^{-3}$ for C-C-A. ^1H NMR spectra were recorded on a QE-300 instrument (General Electric) at 300 MHz with tetramethylsilane as an internal standard.

Enzymic Digestions. Digestions with ribonuclease A and snake venom phosphodiesterase (Russel venom) were performed with ca. 0.5–2.0 A_{260} units of oligonucleotides as described previously.[1a] The digestion mixtures were analyzed by HPLC on a μ-Bondapak C18 analytical column (Waters Associates) using a linear gradient of methanol (1–5%) in 50 mM ammonium acetate, pH 4.5. The peaks of digestion products were identified by a comparison with authentic samples and the ratio was obtained by using appropriate extinction coefficients.[13]

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

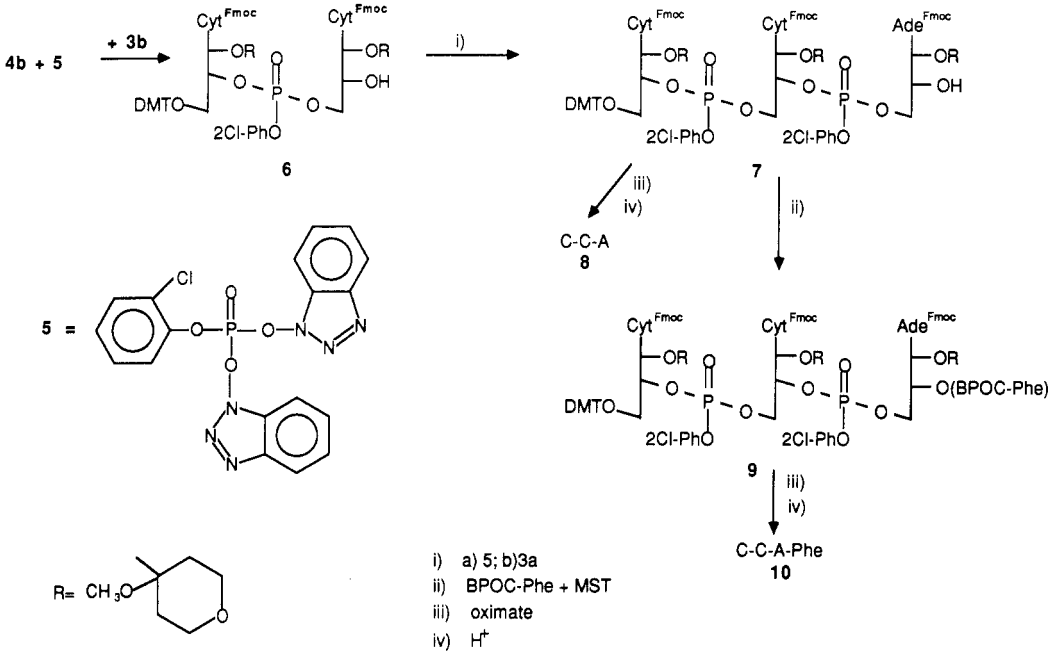
***N*-(9-Fluorenylmethyloxy)carbonyl Nucleosides (1a and 1b).** These two compounds were briefly described by Heikkilä and Chattopadhyaya[9b] without giving experimental details. Trimethylsilyl chloride (10 mL) was added dropwise under exclusion of atmospheric moisture during 30 min into a precooled solution of predried adenosine or cytidine (15 mmol) in anhydrous pyridine (125 mL). After ca. 1 h at room temperature, TLC in system S_2 has indicated the quantitative conversion into a fast moving material and (9-fluorenylmethoxy)carbonyl chloride (4.39 g) was added and the mixture was stirred for addition 3 h at room temperature. The ice-cooled reaction mixture was quenched with 5% ammonium bicarbonate and extracted with CH_2Cl_2. A trace of tetrabutylammonium fluoride solution (0.05 M; in tetrahydrofuran–pyridine–water, 8:1:1) was added to the combined methylene chloride extracts. After ca. 10 min, the reaction mixture was concentrated in vacuo and treated with CH_2Cl_2–CH_3OH (9:1) at 4 °C overnight and a solid precipitated material was recovered by filtration and dried in vacuo. Compound **1b** was recrystallized from ethanol. The yield of chromatographically uniform **1a** was 58% and **1b** was 90%. The mother liquor of **1a** contained some product with a high R_f on TLC, presumably the bis[(fluorenylmethyloxy)carbonyl] derivative. This product was purified on a column of silica gel (2.8 × 12 cm) by using a step gradient of CH_3OH in CH_2Cl_2. The main product (ca. 2 g) was dissolved in CH_2Cl_2 (25 mL) and under cooling treated with triethylamine for 8 h. After evaporation in vacuo and precipitation as above, another crop of product **1a** was obtained, raising the total yield to 75%.

Physical constants and spectral data of **1a** and **1b** agree with published values.[9b]

***N*-((9-Fluorenylmethyloxy)carbonyl)-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl) Nucleosides (2a and 2b).** Compound **1a** or **1b** (6.0 mmol) was made anhydrous by repeated coevaporation with pyridine and dissolved in anhydrous pyridine (50 mL), and the solution was cooled down in an ice bath. 1,1,3,3-Dichlorotetraisopropyldisiloxane (2 mL) was added dropwise under exclusion of atmospheric moisture and the reaction mixture stirred at room temperature until TLC in system S_2 showed complete conversion to a fast moving material. This required, in the case of **2b**, an addition of another portion of the silylating reagent (0.5 mL). The reaction mixture was quenched under ice-cooling with a solution of ammonium bicarbonate (5%; 50 mL) and extracted with CH_2Cl_2, and the combined organic extracts were dried with magnesium sulfate. The solution of the crude reaction product was concentrated in vacuo and the product was purified on a column (5 × 7 cm) of silica gel by using a step gradient of CH_3OH in CH_2Cl_2 (0.5% to 5%). The fractions containing chromatographically pure product were evaporated

(13) van Boom, J. H.; De Rooy, J. F. M. *J. Chromatogr.* **1977**, *131*, 169.

Scheme II



to obtain **2a** and **2b** in 80–85% yield. Anal. **2a**, calcd for $C_{37}H_{49}N_5O_7Si_2$: C, 60.71; H, 6.75; N, 9.57. Found: C, 60.54; H, 6.92; N, 9.71. **2b**, calcd for $C_{36}H_{49}N_3O_8Si_2 + H_2O$: C, 59.56; H, 7.08; N, 5.79. Found: C, 59.16; H, 7.00; N, 5.78.

***N*-((9-Fluorenylmethyloxy)carbonyl)-2′-*O*-(4-methoxytetrahydropyran-4-yl) Nucleosides (3a and 3b).** Intermediate **2a** or **2b** (8.5 mmol) was dissolved in anhydrous dioxane (50 mL) and mesitylenesulfonic acid (1.0 g) and 4-methoxydihydropyran (13 mL) were added in three portions. The reaction was shown to be completed by TLC in system S_1 in 2–4 h. The reaction mixture was quenched with ammonium bicarbonate (5%; 50 mL) which was added dropwise under cooling and extracted with CH_2Cl_2. The combined organic extracts were treated with 0.8 M tetrabutylammonium fluoride solution (tetrahydrofuran–pyridine–water, 8:1:1; 37 mL) until complete desilylation occurred (60 to 90 min). The reaction mixture was treated with ammonium bicarbonate solution (5%; 200 mL) and extracted with CH_2Cl_2, and the extracts were dried with magnesium sulfate and evaporated in vacuo. The crude reaction product was purified by chromatography on a silica gel column (5 × 9 cm) by applying a step gradient of CH_3OH in CH_2Cl_2 (0% to 5%). The fractions containing the desired product **3a** or **3b** were pooled and evaporated to recover the product as a chromatographically uniform foam in 40–50% yield. Anal. **3a**, calcd for $C_{31}H_{33}N_5O_8$: C, 61.68; H, 5.51; N, 11.60. Found: C, 61.49; H, 5.58; N, 11.37. **3b**, calcd for $C_{30}H_{33}N_3O_9 \cdot {}^1/_2H_2O$: C, 61.21; H, 5.82; N, 7.14. Found: C, 60.93; H, 6.54; N, 7.16.

5′-*O*-Dimethoxytrityl-*N*-((9-fluorenylmethyloxy)carbonyl)-2′-*O*-(4-*O*-methoxytetrahydropyran-4-yl) Nucleosides (4a and 4b). Compound **3a** or **3b** (0.5 mmol) was dissolved in dry pyridine (3 mL) and treated with dimethoxytrityl chloride (0.7 mmol, 0.24 g) and a catalytic amount of 4-(dimethylamino)pyridine. After ca. 1 h TLC in system S_2 showed quantitative conversion to a fast moving product. The reaction mixture was quenched with 5% aqueous ammonium bicarbonate (25 mL) and extracted with CH_2Cl_2, the extracts were dried with magnesium sulfate and evaporated in vacuo, and the residue was coevaporated with toluene. The crude reaction product was purified on a column (0.5 × 5 cm) of silica gel by using a step gradient of CH_3OH in CH_2Cl_2 (1–3%). The fractions containing the product were pooled and evaporated in vacuo to recover 75–90% of product **4a** or **4b** which could be obtained as a white powder after trituration with CH_2Cl_2 and light petroleum ether. Anal. **4a**, calcd for $C_{52}H_{51}N_5O_{10}$: C, 68.93; H, 5.67; N, 7.73. Found: C, 68.77; H, 6.00; N, 7.48. **4b**, calcd for $C_{51}H_{51}N_3O_{11}$: C, 69.45; H, 5.83; N, 4.76. Found: C, 69.54; H, 6.04; N, 4.75.

General Method for Preparation of Protected Oligoribonucleotides. The procedure was essentially the same as used in the previous report.[1a] Compound **4** or **6** (0.11 mmol), predried by coevaporation with absolute pyridine, was phosphorylated with reagent **5** (0.6 mL of a 2 M solution in THF ref 10). The component with free 5′-OH (**3**, 0.1 mmol), predried by coevaporation with pyridine, and 1-methylimidazole (20 μL) in ca. 0.5 mL of THF were added after TLC in system S_2 showed a quantitative conversion to a base-line material. All operations were performed under complete exclusion of atmospheric moisture. After one to several hours at room temperature TLC in S_2 showed formation of a new fast moving product and almost complete disappearance of slow moving diester. The cooled reaction mixture was quenched with 5% ammonium bicarbonate (2–3 mL) and extracted with CH_2Cl_2, the extracts were dried with magnesium sulfate and evaporated in vacuo, and the residue was coevaporated with toluene. Products were separated by chromatography on a silica gel column (3 × 5 cm) by using a step gradient of CH_3OH (0–5%) in CH_2Cl_2. The fractions were checked by TLC and those containing product **6** or **7** were pooled and evaporated. The yields of chromatographically uniform vacuum-dried products were in the 60–75% range.

Aminoacylation of Protected C-C-A 7. The trinucleotide **7** (0.060 g; 0.025 mmol) was predried by coevaporation with absolute pyridine and treated in CH_2Cl_2 (1.0 mL) with the dicyclohexylammonium salt of BPOC-Phe (0.044 g; 0.075 mmol), (mesitylenesulfonyl)tetrazole (0.028 g; 0.11 mM), and 1-methylimidazole (30 μL). The reaction was shown to be quantitative by TLC in S_2 after 1–2 h. The mixture was diluted with CH_2Cl_2 and applied directly on the small column of silica gel, which was eluted with a step gradient of CH_3OH (0–5%) in CH_2Cl_2. The chromatographically uniform product **9** was isolated after pooling and evaporation of appropriate fractions. The yield was 0.064 g (92%).

Cytidylyl(3′-5′)cytidylyl(3′-5′)-2′(3′)-*O*-(L-phenylalanyl)-adenosine (10). The protected trinucleotide **9** (ca. 0.01 mmol) was treated with a freshly prepared solution of N^1,N^1,N^3,N^3-tetramethylguanidine (0.33 M) and *o*-nitrobenzaldoxime (0.38 M) in dry acetonitrile (1.0 mL) for 3 h at room temperature. After this time, TLC in S_2 showed a complete conversion to a base-line material. The reaction product was precipitated with dry ether (20 mL), isolated by centrifugation, and washed twice with dry ether. The pellet was dissolved in cold 80% formic acid (2.5 mL) and after 30 min at 0 °C ether (10–15 mL) was added under stirring. The product was isolated by centrifugation and after dissolving in 80% acetic acid (0.2 mL) reprecipitated with an excess of ether, isolated by centrifugation and washed repeatedly with dry ether. TLC in S_3 indicated the formation of product **10** together with a small amount of C-C-A (**8**). The pellet was dissolved in ammonium acetate buffer (5 mM; pH 4.5; ca. 1 mL)

and purified on a C_{18} SPE Baker column (3 mL) by using a step gradient (0, 5, 10, 20, 50, and 100%) of CH_3CN in 5 mM ammonium acetate (pH 4.5). The fractions were concentrated in vacuo, freeze–dried, and analyzed by TLC in S_3. C-C-A (8) was removed by elution with 5% CH_3CN and the product was eluted with 20% CH_3CN. The total yield of the deblocking and purification was 28%. The product **10** was chromatographically and electrophoretically uniform and was characterized by alkaline hydrolysis (0.1 N KOH, 100 °C, 20 min) to Cp and A (ratio Cp/A = 2.22); complete digestion with RNAse A (ratio Cp/A = 2.03); complete digestion with snake venom phosphodiesterase (ratio Cp + C/pA = 1.05) and by UV spectra in 0.01 N HCl (λ_{max} = 269 nm; λ_{min} = 236 nm; $A_{250/260}$ = 0.72; $A_{280/260}$ = 1.01; $A_{290/260}$ = 0.69). The product **10** is also identical in several chromatographic and electrophoretic systems with a compound prepared previously by a different approach.[5]

Optically Active Pyrromethenone Amides. Exciton Coupling in Hydrogen-Bonded Dimers

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The amides of xanthobilirubic acid with (*R*)-(+)- and (*S*)-(−)-α-methylbenzylamine (**1** and **2**, respectively) and (*R*)-(+)- and (*S*)-(−)-1-(1-naphthyl)ethylamine (**3** and **4**, respectively) have been synthesized and found to exhibit moderate circular dichroism Cotton effets (CEs) (**1** and **3**: $\Delta\epsilon_{max}^{413}$ −1.3) in dimethyl sulfoxide solvent for the pyrromethenone long-wavelength transitions and stronger bisignate CEs (**1** and **3**: $\Delta\epsilon_{max}^{402} \simeq +3.1$, $\Delta\epsilon_{max}^{446} \simeq +1.3$) in CH_2Cl_2 solvent. The bisignate CE, whose intensity decreases with decreasing pyrromethenone concentration, is assigned to the pyrromethenone–pyrromethenone hydrogen-bonded dimer.

The pyrromethenone chromophore (Figure 1) is found in nature in the bile pigment (4*Z*,15*Z*)-bilirubin IXα, which is the yellow-orange, cytotoxic pigment of jaundice.[1] Pyrromethenones have therefore served as useful adjuncts in studies of jaundice phototherapy and bilirubin structure–biological function relationships.[2] The structures of typical 5*H*-pyrromethenones are known from crystallographic[3,4] studies which show the presence of (1) a lactam tautomer, (2) substantial double bond and single bond character in the C_4–C_5 and C_5–C_6 bonds, respectively, (3) a syn-*Z* configuration C=C at C_4, and (4) essentially planar, dimeric structures with extended pyrrole interplanar angles, $\phi \simeq 4°$.[5] Most of these structural features persist in solution where the H-bonded dimers are in equilibrium with their component monomers in nonpolar solvents (in $CHCl_3$, the association constant, $K_A \simeq 1700$ M^{-1} from vapor phase osmometry studies),[6a] and the monomers exhibit substantially greater twisting ($\phi \simeq 40°$) about C_5–C_6, as deduced from LIS–NMR studies.[6] Additional support for the presence of twisting (calculated $\phi \simeq 33°$) in the monomer and its near absence (calculated $\phi \simeq 5$–$8°$) in the dimer comes from molecular mechanics calculations.[7] These data reveal much about the pyrromethenone pigment and show that in the intermolecularly hydrogen-bonded dimer it is structurally similar to the planar pyrromethenone units found connected as a molecular dimer in the stable, intramolecularly hydrogen-bonded conformer of bilirubin (Figure 2).[9]

Pyrromethenones are typically bright-yellow compounds, possessing a conjugated π-electron system, and they exhibit an intense long-wavelength UV–vis absorption near 400 nm with $\epsilon \simeq 30\,000$. Although they may adopt a dissymmetric (twisted) conformation (Figure 2) and are therefore potentially chiral molecules, the conformational interconversion energy is calculated to be very small, ~1 kcal/mol.[7b] Consequently, their solutions in isotropic media are optically inactive. However, in optically active mesophases, weak circular dichroism (CD) has been detected for the kryptopyrromethenone dimer (Figure 2),[6a,b] but no induced CD (ICD) could be detected for xanthobilirubic acid (XBR, Figure 2) in sodium deoxycholate micelles in pH 7.7–8.0 buffer solution.[10a] Similarly, where heteroassociation, complexation with a chiral solvating agent might be expected, XBR showed no ICD for solutions with β-cyclodextrin;[10b] however, the noncovalent complex with human serum albumin[11] shows a moderately strong ICD.[2d]

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