Studies on Transfer Ribonucleic Acids and Related Compounds. 29.[1] Synthesis of a Decaribonucleotide of *Escherichia coli* $tRNA_f^{Met}$ (Bases 11–20) Using a New Phosphorylating Reagent[2]

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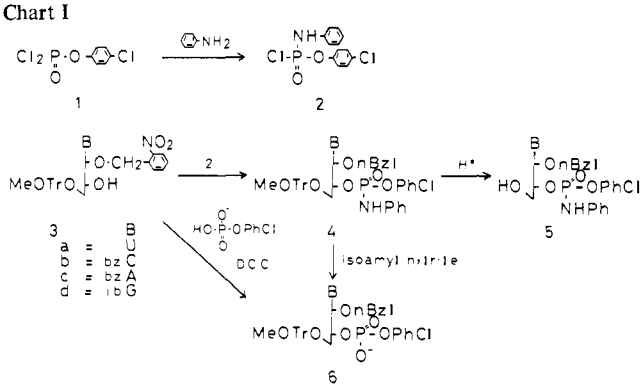
Abstract: A decanucleotide corresponding to the bases 11–20 of *Escherichia coli* $tRNA_f^{Met}$,[3] A-G-C-A-G-C-C-U-G-Gp[4], was synthesized by triester block condensations. Completely protected mononucleotides (**4**) were prepared as the key intermediates by phosphorylation of the 2′-*O*-(*o*-nitrobenzyl) nucleosides (**3**) with a new phosphorylating reagent, *p*-chlorophenyl *N*-phenylchlorophosphoramidate (**2**). The 5′-monomethoxytrityl group was removed and **5** were used as the 3′-terminal units in the synthesis of protected C-Up (**9**) and A-G-Cp (**13**). The diesterified mononucleotides (**6**) served as internal units. Successive coupling of these oligonucleotides to the dianilidated dinucleotide (**8**) yielded the protected decanucleotide (**16**). Mesitylenesulfonyl triazolide was used as the condensing reagent in both the synthesis of blocks and their condensation. The yields in condensations of monomers were 63–85% and those in block condensations were 27–60%. After deblocking of the terminal phosphate, amino, internucleotidic phosphate, and 5′-hydroxyl groups, the 2′-O-protected decanucleotide was irradiated with UV light to remove the *o*-nitrobenzyl ether. The deblocked decanucleotide was isolated by ion-exchange chromatography on DEAE-cellulose and characterized by complete hydrolysis with a base-nonspecific RNase to give a correct ratio of mononucleotides. The sequence was confirmed by mobility shift analysis. The decanucleotide is the largest naturally occurring fragment synthesized chemically.

Chemical synthesis of tRNA fragments is of importance for providing materials for a variety of biochemical studies or tRNA functions.[5] The discovery of RNA ligase[6] raises the possibility of constructing a total molecule from synthetic fragments.[7] We have been synthesizing $tRNA_f^{Met}$ fragments of *Escherichia coli* by various methods.[8] These fragments have been joined by using RNA ligase to yield the 3′-heptadecanucleotide (bases 61–77)[7] and 5′-eicosanucleotide (bases 1–20) of the tRNA. It is desirable to synthesize larger fragments chemically, even though RNA ligase can catalyze joining of ribotrinucleotides.[10] The triester approach[11] has been shown to be suitable for the preparation of large quantities of oligonucleotide blocks. We have previously reported a synthesis of 2′-*O*-(*o*-nitrobenzyl) nucleotides bearing 3′-phosphorodianilidates in the synthesis of triesterified ribonucleotide blocks.[12] The dianilidate could be converted to the phosphomonoester by isoamyl nitrite treatment[13] and the monoesterified oligonucleotide was used for triisopropylbenzenesulfonyl chloride[14] (TPS) catalyzed condensation in the synthesis of a heptanucleotide from a eukaryotic tRNA loop.[15] In the present paper, we wish to report a new phosphorylating reagent, *p*-chlorophenyl *N*-phenylchlorophosphoramidate (**2**), and its use for the preparation of fully protected mononucleotides (**4**), which serve as key intermediates in the synthesis of 3′-phosphodiesterified oligonucleotide blocks. Activation of these blocks by mesitylenesulfonyl triazolide[16] (MST) afforded a decanucleotide corresponding to bases 11–20 of $tRNA_f^{Met}$ from *E. coli* (Figure 1).

Chart I



Synthesis of Protected Mononucleotides (5) by Phosphorylation with *p*-Chlorophenyl *N*-Phenylchlorophosphoramidate (2). A preliminary account of the synthesis of phosphorylating reagent (**2**) has been published.[17] Preparation of **2** and its reaction with 2′-*O*-(*o*-nitrobenzyl) nucleosides[12] (**3**) are shown in Chart I. The fully protected mononucleotides (**4**) were synthesized by treatment of **3** with **2** in pyridine and converted to **5** by removal of monomethoxytrityl groups. The 5′-deblocked mononucleotides (**5**) were key intermediates as 3′-terminal units in the synthesis of terminally diesterified blocks and were isolated by chromatography on silica gel. Diastereoisomers of each nucleotide were separated and identified by elemental analysis, UV, and NMR. The combined yields of **5** with high R_f (I) and low R_f (II) are shown in Table I together with elemental analysis and UV data. Table II gives NMR spectral properties of **5**-I and **5**-II. Compound **5a** could be isolated as crystals from chloroform without chromatography on silica gel and recrystallization from methanol-separated **5a**-I and **5a**-II.

The fully protected mononucleotides **4** can be converted to the diesters **6** which are intermediates in oligonucleotide synthesis as internal units. However, **6** were synthesized more easily from **3** by condensation with *p*-chlorophenyl phosphate using dicyclohexylcarbodiimide (DCC) as described in the synthesis of the guanosine derivative (**6d**).[15] Using these mononucleotides (**5, 6**), oligonucleotide blocks were synthesized and their condensation yielded a decanucleotide as shown below.

Synthesis of Oligonucleotide Blocks and Their Condensation to Yield the Decanucleotide (17). The protected decanucleotide (**16**) was synthesized by condensation of the tetra (**11**)- and tri (**14**)-nucleotides as shown in Chart II. The 3′ terminus of the decanucleotide (**16**) was blocked with phosphoramidate, since deblocking by isoamyl nitrite treatment of the anilidate gave the phosphomonoester without formation of the 2′,3′-cyclic phosphate which was found to be formed during alkaline removal of phenyl derivatives from the 3′-phosphate.[18] For the synthesis of the dianilidated dimer, **7** was condensed with **6d** by using MST[16] as the activating reagent and the 5′-mono-

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Table I. Yields, TLC, Elemental Analysis, and UV Spectral Data for 5′-Deblocked Mononucleotides (**5**)

	yield, %	TLC R_f value	elemental anal.	calcd (found) C	H	N	UV in 95% EtOH λ_{max} ($\epsilon \times 10^{-4}$), nm	λ_{min} ($\epsilon \times 10^{-4}$), nm
5a-I[a]	73	0.38[b]	$C_{28}H_{26}N_4O_{10}PCl$ (645.02)	52.13 (52.17)	4.07 (4.09)	8.69 (8.61)	260.5 (1.61)	241 (1.03)
5a-II		0.35[b]	$C_{28}H_{26}N_4O_{10}PCl\cdot 0.5H_2O$ (654.03)	51.42 (51.19)	4.17 (3.94)	8.57 (8.60)	260.5 (1.61)	241 (1.04)
5b-I	85	0.47[c]	$C_{35}H_{31}N_5O_{10}PCl$ (748.15)	56.19 (55.93)	4.19 (4.00)	9.36 (9.15)	261 (3.12) 302 (1.21)	240 (1.83) 291.5 (1.16)
5b-II		0.42[c]	$C_{35}H_{31}N_5O_{10}PCl$ (748.15)	56.19 (56.50)	4.19 (4.29)	9.36 (9.20)	261 (3.03) 302 (1.16)	240 (1.77) 291.5 (1.11)
5c-I	75	0.38[c]	$C_{36}H_3N_7O_9PCl$ (772.18)	55.99 (55.73)	4.05 (4.16)	12.70 (12.59)	279.5 (2.68)	247 (1.59)
5c-II		0.35[c]	$C_{36}H_{31}N_7O_9PCl$ (772.18)	55.99 (55.67)	4.05 (4.34)	12.70 (12.67)	279.5 (2.71)	247 (1.60)
5d-I	54	0.32[b]	$C_{33}H_{33}N_7O_{10}PCl$ (754.17)	52.55 (52.31)	4.42 (4.29)	13.00 (12.97)	260.5 (2.18)	240.5 (1.35)
5d-II		0.28[b]	$C_{33}H_{33}N_7O_{10}PCl\cdot 3/2H_2O$ (781.13)	50.74 (50.56)	4.64 (4.45)	12.55 (12.58)	260.5 (2.17)	240.5 (1.39)

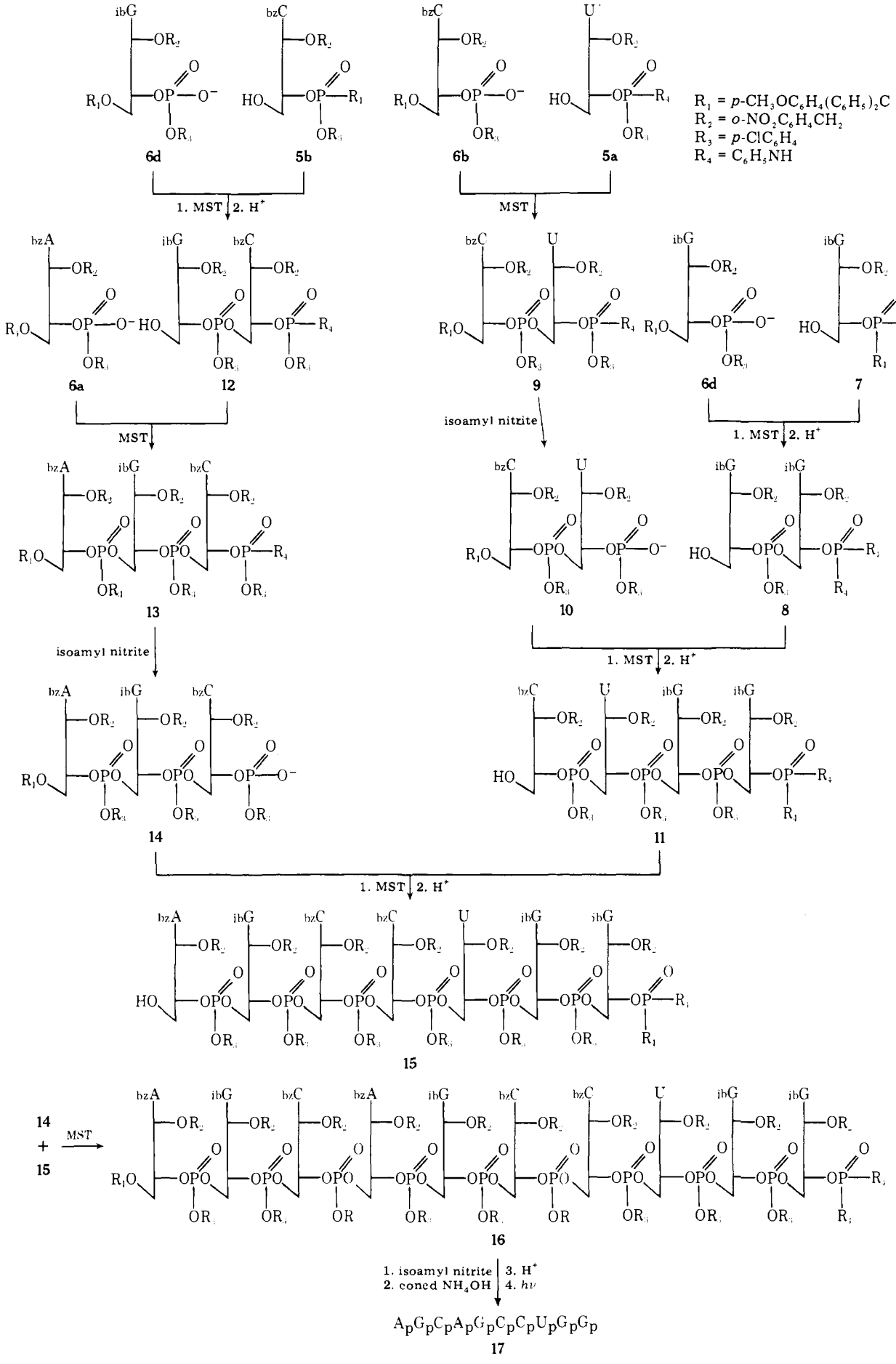
[a] I, higher R_f diastereoisomer; II, lower R_f diastereoisomer. [b] Solvent system, $CHCl_3$/MeOH (10:1). [c] Solvent system, $CHCl_3$/MeOH (15:1).

Table II. NMR Spectral Data for 5′-Deblocked Mononucleotides (**5**)

compd	1′-H	2′-H	3′-H	4′-H	5′-H	CH_2Ar	5′-OH	5-H	6-H	2-H	8-H	Ar-H	NH	Ar-NH
5a-I	6.02 d 1 $J_{1'2'}$ = 7 Hz	4.33 m 1	5.17 m 1	4.33 m 1	3.66 m 2	4.87 s 2	5.32 bs 1	5.64 d 1 J_{56} = 8 Hz	7.80 d 1 J_{56} = 8 Hz			6.67–8.05 m 13	11.27 bs 1	8.66 d 1 J_{HP} = 10 Hz
5a-II	6.07 d 1 $J_{1'2'}$ = 7 Hz	4.40 m 1	5.20 m 1	4.29 m 1	3.60 m 2	4.96 s 2	5.32 bs 1	5.67 d 1 J_{56} = 8 Hz	7.84 d 1 J_{56} = 8 Hz			6.65–8.10 m 13	11.32 bs 1	8.62 d 1 J_{HP} = 10 Hz
5b-I	6.12 d 1 $J_{1'2'}$ = 4 Hz	4.40 m 1	5.19 m 1	4.38 m 1	3.78 m 2	4.87, 5.03 ABq 2 J_{gem} = 14 Hz	5.41 bs 1	7.32 d 1 J_{56} = 7 Hz	8.45 d 1 J_{56} = 7 Hz			6.73–8.16 m 18	11.28 bs 1	8.63 d 1 J_{HP} = 10 Hz
5b-II	6.16 d 1 $J_{1'2'}$ = 5 Hz	4.48 m 1	5.19 m 1	4.32 m 1	3.75 m 2	4.95, 5.11 Abq 2 J_{gem} = 14 Hz	5.36 bs 1	7.36 d 1 J_{56} = 7 Hz	8.52 d 1 J_{56} = 7 Hz			6.67–8.10 m 18	11.23 bs 1	8.61 d 1 J_{HP} = 10 Hz
5c-I	6.28 d 1 $J_{1'2'}$ = 7 Hz	5.04 m 1	5.40 m 1	4.44 m 1	3.73 m 2	4.90 s 2	5.40 m 1			8.70 s 2		6.69–8.16 m 18	11.16 bs 1	8.70 d 2 J_{HP} = 10 Hz
5c-II	6.30 d 1 $J_{1'2'}$ = 7 Hz	5.11 m 1	5.40 m 1	4.38 m 1	3.66 m 2	4.96 s 2	5.40 m 1			8.71 s 2		6.68–8.11 m 18	11.13 bs 1	8.66 d 2 J_{HP} = 10 Hz
5d-I	6.01 d 1 $J_{1'2'}$ = 8.4 Hz	4.82 m 1	5.35 m 1	4.42 m 1	3.69 m 2	4.89 s 2	5.35 m 1				8.20 s 1	6.68–8.00 m 13	11.53, 12.03 bs 1 (each)	8.71 d 1 J_{HP} = 10 Hz
5d-II	6.05 d 1 $J_{1'2'}$ = 8 Hz	4.88 m 1	5.38 m 1	4.37 m 1	3.64 m 2	4.97 s 2	5.38 m 1				8.27 s 1	6.63–8.09 m 13	11.58, 12.04 bs 1 (each)	8.70 d 1 J_{HP} = 10 Hz

methoxytrityl group was removed with 80% acetic acid to yield the dimer **8**. The dinucleotide **10** was synthesized by condensation of **5a** with **6b** and subsequent removal of the terminal anilidate by isoamyl nitrite treatment in pyridine–acetic acid overnight. **10** was then allowed to react with **8** to give the tetranucleotide (**11**). The conditions for these reactions and yields are summarized in Table III. The trinucleotide (**14**) was synthesized by a similar method and **14** was condensed with

Chart II


R1 = p-CH3OC6H4(C6H5)2C
R2 = o-NO2C6H4CH2
R3 = p-ClC6H4
R4 = C6H5NH
6d
5b
6b
5a
1. MST 2. H+
MST
6a
12
9
6d
7
isoamyl nitrite
1. MST 2. H+
13
10
8
isoamyl nitrite
1. MST 2. H+
14
11
1. MST 2. H+
15
14 + 15
MST
16
1. isoamyl nitrite 2. coned NH4OH
3. H+ 4. hν
ApGpCpApGpCpCpUpGpGp
17

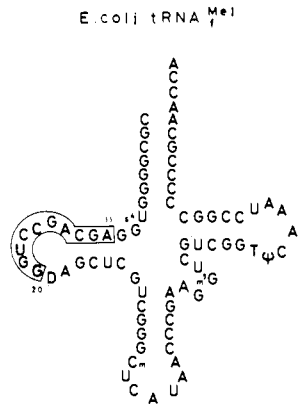


Figure 1.

the tetranucleotide (**11**) to yield the heptanucleotide (**15**) which was then subjected to a repeat condensation for the synthesis of the decanucleotide (**16**). Deblocking of **16** by successive treatment with isoamyl nitrite, concentrated ammonia, 80% acetic acid, and UV irradiation yielded the unprotected product (**17**). **17** was isolated by chromatography on DEAE-cellulose in the presence of 7 M urea in a yield of 39%. The base composition was analyzed by anion-exchange chromatography using a nucleic acid analyzer after digestion with base-nonspecific RNase M.[19] The sequence was confirmed by mobility shift analysis[20] after ^{32}P labeling of the 5′ end and nuclease P1[21] digestion.

Conclusion

The 2′-*O*-(*o*-nitrobenzyl) nucleosides allow use of the acid-labile monomethoxytrityl group for 5′ protection. By using two types of key intermediates **5** and **6**, oligonucleotide blocks (e.g., **9** or **13**) suitable for condensation reaction have been synthesized in high yield. These fully protected blocks are versatile, allowing elongation in either the 3′ or 5′ directions. Removal of the anilido group from **9** by isoamyl nitrite treatment[13] yielded the diesterified block (**10**). **13** was synthesized by elongation of **12** in the 5′ direction. The 3′-*p*-chlorophenyl phosphoranilidates such as that in **12** were stable during chromatography on silica gel and could be isolated in high yields. Decrease in yields which occurred during isolation of 3′-cyanoethyl *p*-chlorophenyl oligonucleotides[15] were not observed in the present experiments. Although this type of anilidate was stable during acid treatment for removal of the trityl derivative, it was less susceptible to isoamyl nitrite when compared with the phosphomono- or dianilidate. Removal of the anilido group from **14** required a larger excess of the reagent than for **5**. This may become a drawback with this approach when a block becomes larger. Otherwise, block condensation using the present approach appears to be a method suitable for the synthesis of longer fragments. The 5′-monomethoxytrityl protection and 3′-phosphate should prevent attack by hydroxyl groups during removal of *p*-chlorophenyl groups on internucleotidic linkages with concentrated ammonia.[22] Fluoride ion has been used to remove the *o*-chlorophenyl groups from the internucleotide linkages without attacking the 5′-levulinyl group in the synthesis of a repeated U-A tetradecanucleotide and cleavage of internucleotide bonds during the deblocking was reported to be 2%.[23] In the present experiment the overall yield in deblocking was ca. 40%. Improvements in these conditions to prevent internucleotide cleavage during removal of aryl protecting groups[24] is necessary if larger oligonucleotides are to be synthesized by the triester approach. The present decanucleotides is the largest naturally occurring segment obtained chemically. Synthesis of other $tRNA_f^{Met}$ fragments by block condensation and their joining with RNA ligase are in progress.

Experimental Section

General Methods. Paper chromatography was performed by the descending technique by using solvent systems: A, isopropyl alcohol–concentrated ammonia–water (7:1:2, v/v); B, *n*-propyl alcohol–concentrated ammonia–water (55:10:35, v/v). Paper electrophoresis was performed by using 0.05 M triethylammonium bicarbonate (pH 7.5) at 900 V/40 cm. R_f values and relative mobilities of compounds are collected in Table IV. Thin-layer chromatography (TLC) was performed on plates on silica gel (Merck HF 254) by using a mixture of chloroform–methanol. For columns, silica gel G (Merck, type 60) was used. Preparation of **6**[15] and **7**[12] was as described. Removal of the 5′-*O*-monomethoxytrityl, 2′-*O*-(*o*-nitrobenzyl), phosphorodianilidate, and other general methods were as described previously.[15] Enzymatic hydrolyses were also described in ref 25.

***p*-Chlorophenyl *N*-Phenylchlorophosphoramidate (2).** *p*-Chlorophenyl phosphorodichloridate (**1**) was synthesized by treatment of *p*-chlorophenol and phosphoryl trichloride as described for other phosphorylating reagents.[26] The dichloridate (**1**) (6.135 g, 25 mmol) in benzene (140 mL) was cooled in an ice bath and aniline (4.7 g, 50 mL) in benzene (10 mL) was added dropwise with stirring during 30 min. The stirring was continued for 36 h at room temperature in the dark. The product was collected by filtration, washed with water, and dried over P_2O_5.[17] The yield was 5.75 g (19 mmol, 76%), mp 145–147 °C. UV: λ_{max}^{EtOH} 225, 272 (sh), $\lambda_{max}H^+$ 225, $\lambda_{max}^{OH^-}$ 223, 247 nm. Paper electrophoresis after aqueous pyridine treatment showed *p*-chlorophenylphosphoramidic acid ($Rm_{U\text{-}Up}$, 0.53).

Preparation of Nucleoside 3′-*p*-Chlorophenyl Phosphoranilidates (5), 5c, 3c (871 mg, 1.1 mmol) was coevaporated with pyridine and treated with **2** (514 mg, 1.7 mmol) in pyridine (10 mL) for 8 h at 20 °C. Since **3c** and **4c** gave almost the same R_f values in TLC, an aliquot was treated with 80% acetic acid to check the extent of reaction. The diastereoisomers of **5c** were separated (I, R_f 0.38; II, 0.35) in TLC (15:1). **2** (0.85 mmol) was added to complete the reaction. After 16 h, no starting material was detected and the reaction mixture was treated with sodium acetate (0.56 g in 50 mL of water) which was added with cooling over 30 min. The phosphorylated product **4c** was extracted with chloroform (20 mL) three times, washed with water (10 mL) three times, and evaporated, and a trace of pyridine was removed by coevaporation of toluene. The residue was treated with 80% acetic acid (60 mL) at 30 °C overnight. Acetic acid was evaporated after completion of the demonomethoxytritylation was confirmed by TLC. The residue was dissolved in chloroform and the crystallized *p*-chlorophenyl phosphoranilidate was removed. The solution was applied to a column of silica gel (50 g) and elution was performed with chloroform–methanol (40:1). Fractions containing **5c** were combined, concentrated, and precipitated with *n*-pentane (100 mL) from their solution in chloroform (5 mL). The yield was 639 mg, 0.83 mmol, 75%.

Synthesis of Oligonucleotide Blocks, for Example, 9. The triethylammonium salts of **6b** (2.3 g, 65 400 A_{260}, 2.05 mmol) and **5a** (1.02 g, 1.58 mmol) were dried by coevaporation of pyridine and treated with MST (1.54 g, 6.15 mmol) at 30 °C for 36 h. The extent of reaction was checked by the presence of a trityl positive product (**9**) R_f 0.54) and disappearance of **5a** (R_f 0.13) in TLC (15:1). The reaction was stopped by addition of water (20 mL) with cooling and the mixture was kept at room temperature for 1 h. The product **9** was extracted with chloroform (50 mL), washed with 0.1 M triethylammonium bicarbonate (20 mL), then with water (10 mL) twice, and the chloroform was evaporated with addition of toluene. The residue was dissolved in chloroform and applied to a column (3.3 × 18.5 cm) of silica gel. The product was eluted with chloroform–methanol (50:1), detected by the trityl color reaction, combined and precipitated with *n*-pentane (200 mL) from its solution in chloroform (10 mL). The yield was 2.13 g, 1.35 mmol, 85%. An aliquot was treated with isoamyl nitrite in 1:1 pyridine–acetic acid overnight, then with concentrated ammonia at 55 °C for 3 h and with 80% acetic acid at 30 °C for 2 h. The partially protected dinucleotide was applied to paper chromatography in solvent A. The spot (R_f 0.73) was eluted and transferred to paper electrophoresis, and the nucleotide was irradiated with UV light for 2 h in water. Paper chromatography of the product showed two spots corresponding to C-U > p (R_f 0.14) and C-Up (R_f 0.04) in a ratio of 4:1 in solvent A. C-Up was identified by removal of the 3′-phosphate with alkaline phosphatase and by subsequent isolation and RNase A hydrolysis. Cp (0.205 A_{278}^{pH2}) and U (0.158 A_{260}^{pH2}) were separated by paper chromatography and found to give a molar ratio of 1:1.

Table III. Reaction Conditions and Yields in the Synthesis of Oligonucleotides

5′-protected component		3′-protected component		MST, mmol	reaction time, day	product		yield, %	TLC, R_f
compd	mmol	comp	mmol			compd	mmol		
6d	1.69	**7**	1.35	5.07	2	**8**	1.14	84	0.28, 0.26[a]
6b	2.05	**5a**	1.58	6.15	2	**9**	1.35	85	0.42[b]
10	0.70	**8**	0.50	2.04 + 1.02	2 + 2	**11**	0.36	60	0.26, 0.24[a]
6d	2.73	**5b**	2.10	8.21	2	**12**	1.58	75	0.31, 0.30, 0.23[b]
6a	1.58	**12**	1.20	4.66 + 2.33	2 + 2	**13**	0.75	63	0.38, 0.34[b]
14	0.41	**11**	0.246	1.64 + 1.64 + 0.82	2.5 + 3 + 3	**15**	0.101	38	0.30[a]
14	0.090	**15**	0.030	0.18 + 0.18	2 + 4	**16**	0.0082	27	0.31[a]

[a] Chloroform–methanol (10:1). [b] Chloroform–methanol (15:1).

Table IV. Paper Chromatography and Electrophoresis[a]

compound	solvent A	solvent B	rel mobility pH 7.5
A	0.68	0.67	−0.27
G	0.40	0.51	0
C	0.57	0.58	−0.19
U	0.56	0.58	0.04
A > p			0.41
G > p			0.61
C > p	0.36		0.53
U > p	0.35		0.73
Ap	0.26	0.44	0.91
Gp	0.13	0.33	1.00
Cp	0.22	0.43	0.99
Up	0.22	0.43	1.07
C(nBzl)-U(nBzl)p	0.85		0.51
C-U > p	0.22		0.76
C-Up	0.07		1.04
G(nBzl)-G(nBzl)p	0.15	0.44	0.94
G-Gp	0.07	0.17	1.01
G(nBzl)-C(nBzl)p	0.78		
G-C > p	0.12		0.71
G-Cp	0.07	0.26	1.00
A(nBzl)-G(nBzl)p	0.69		0.52
A-G-C > p	0.10		0.76
A-G-Cp	0.05	0.20	1.10
C(nBzl)-U(nBzl)-G(nBzl)-G(nBzl)p		0.55	
C-U-G-Gp		0.14	1.03
A(nBzl)-G(nBzl)-C(nBzl)-C(nBzl)-U(nBzl)-G(nBzl)-G(nBzl)p		0.50	0.75
A-G-C-C-U-G-Gp		0.04	0.92

[a] p, *p*-chlorophenyl phosphate.

Synthesis and Deblocking of the Decanucleotide (16). The triethylammonium salts of **14** (214 mg, 6300 $A_{262.5}$, 0.090 mmol) and **15** (137 mg, 0.03 mmol) were dried by coevaporation of pyridine. The mixture was treated with MST (45 mg, 0.18 mmol) at 26–33 °C for 2 days and checked by TLC 10:1). The product (**16**) and the starting material (**15**) traveled together (R_f 0.32). The mixture was kept another 2 days after MST (45 mg) had been added. Water (1 mL) was added with cooling and the mixture was extracted with chloroform (20 mL). The chloroform solution was washed with 0.1 M triethylamminoum bicarbonate (pH 7.5, 30 mL) and the aqueous layer was back-extracted with chloroform (10 mL) three times. The combined chloroform layer was washed with water (10 mL) three times, concentrated, coevaporated with toluene to remove pyridine, dissolved in chloroform (3 mL), and applied to a column of silica gel (15 g). The elution was performed with 25:1 chloroform–methanol. The trityl positive fractions were combined, concentrated, dissolved in chloroform (3 mL), and precipitated with *n*-pentane (45 mL). The yield was 117.7 mg (0.0082 mmol), 27%. An aliquot (68.5 mg, 0.0048 mmol) was treated with isoamyl nitrite (1.2 mL) in 1:1 pyridine–acetic acid (5 mL) at 20–30 °C for 1 day. Water (5 mL) was added when all trityl positive material remained at the origin in TLC. The nucleotide was extracted with chloroform (20 mL) washed with water. Chloroform was evaporated and the residue was treated with concentrated ammonia (50 mL) at 50 °C for 4 h. Volatile materials were removed and the residue was coevaporated with toluene. The monomethoxytrityl group was then removed by treatment with 80% acetic acid (40 mL) at room temperature overnight and the reaction was checked by TLC. Acetic acid was removed and the 2′-protected oligonucleotide was dissolved in 0.1 M triethylammonium bicarbonate (pH 7.5, 40 mL). The aqueous solution was washed with ether (20 mL) three times to remove the monomethoxytritanol and benzamide. One-third of the aqueous solution (407 A_{259}) was concentrated, dissolved in water (100 mL), irradiated with UV through a Pyrex filter for 2 h, concentrated, dissolved in 7 M urea–20 mM Tris-HCl (pH 8.0)–0.1 M NaCl (20 mL), and applied to a column (1.0 × 120 cm) of DEAE-cellulose. The elution was performed with a linear gradient of NaCl (0.2–0.5 M, total 1.2 L) in 7 M urea and 20 mM Tris-HCl. Three-milliliter fractions were collected every 30 min and fractions 183–203 contained the decanucleotide (**17**). The product was desalted by adsorbing to DEAE-cellulose, washing with 0.05 M triethylammonium bicarbonate, and eluting with the same buffer (1 M). The yield was 61 A_{256}, 0.62 μmol, assuming 10% hypochromicity. The base composition was analyzed by anion-exchange chromatography of the RNase M digested decanucleotide by using a nucleic acid analyzer. The sequence was confirmed by two-dimensional homochromatography of nuclease P_1 partially digested material after labeling by phosphorylation of the 5′ end with [γ-^{32}P]ATP and polynucleotide kinase.

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References and Notes

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(2) Supported by a Grant-in-Aid for Scientific Research from the Ministry of Education.
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Synthesis of the Nontryptamine Moiety of the *Aspidosperma*-Type Indole Alkaloids via Cleavage of a Cyclic α-Diketone Monothioketal. An Efficient Synthesis of (±)-Quebrachamine and a Formal Synthesis of (±)-Tabersonine

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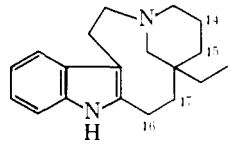
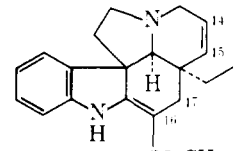
Abstract: The details of an efficient synthesis of (±)-quebrachamine and a formal synthesis of (±)-tabersonine via the cleavage of a cyclic α-diketone monothioketal are described.

Total syntheses of quebrachamine (**1**)[1-5] and tabersonine (**2**),[6] both being the parent bases of the *Aspidosperma* alkaloids and the latter being an in vivo progenitor of the iboga alkaloids,[7,8] have been completed by several groups in interesting manners; however, the overall yields reported are far from practical. Because of the highly efficient synthesis of tryptamine from tryptophan by decarboxylation which has been developed by this group,[9] our present concern lies in constructing the nontryptamine moiety leading to these alkaloids.[10]

As the pivotal synthetic intermediate for quebrachamine (**1**) and tabersonine (**2**), we chose the tetracyclic lactam **12** which possesses a hold for establishing the C-14,15 double bond in the latter as the lactam carbonyl group. Hence a nine-carbon tricarbonyl compound **9** would be required as a nontryptamine moiety in which three carbonyl groups must be differentiated chemically. Since Marshall has reported a cleavage reaction of a cyclic diketone monothioketal easily accessible from the corresponding ketone to give an ω-carboxy thioacetal,[11-14] it became apparent that the reaction would be suitable for the construction of the nine-carbon compound **8** possessing three different carbonyl groups being applied to a cyclic α-diketone monothioketal as **7**.



1



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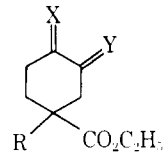
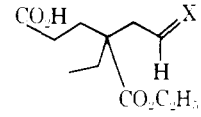
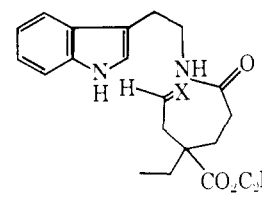


3, R = H, X = O, Y = H_2
4, R = H, X = $-O(CH_2)_2O-$, Y = H_2
5, R = C_2H_5, X = $-O(CH_2)_2O-$, Y = H_2, R = C_2H_5
6, R = C_2H_5, X = O, Y = H_2
7a, R = C_2H_5, X = O, Y = $-S(CH_2)_3S-$
7b, R = C_2H_5, X = O, Y = $(SCH_3)_2$



8a, X = $-S(CH_2)_3S-$
8b, X = $(SCH_3)_2$
9, X = O



10a, X = $-S(CH_2)_3S-$
10b, X = $(SCH_3)_2$
11, X = O

Our initial target, the α-diketone monothioketal **7**, was prepared from the known 4-ethoxycarbonylcyclohexanone ethylene ketal[15] **4** in a satisfactory yield. Thus, **4** was alkylated with ethyl bromide in tetrahydrofuran in the presence of lithium diisopropylamide[16] to produce 4-ethoxycarbonyl-4-ethylcyclohexanone ethylene ketal (**5**) in 92% yield which on hydrolysis with 1 N sulfuric acid in boiling ethanol afforded 4-ethoxycarbonyl-4-ethylcyclohexanone (**6**) in 95% yield. Direct alkylation of the ketoester **3** through a dianion intermediate by using 2 equiv of lithium diisopropylamide, which would save the protection-deprotection sequence, also provided the desired product **6** in a low yield; however, its optimization could not be achieved. Subsequent dithioketalization of **6** was performed to give the α-diketone monothioketal **7a** in 65% yield with trimethylene dithiotosylate through the pyrrolidine enamine intermediate.[17,18] A synthetically equivalent α-diketone monothioketal **7b** was also obtained through the hydroxymethylene ketone intermediate[17,18] with 2 equiv of methyl thiotosylate[19,20] in 57% yield.

Cleavage of the α-diketone monothioketal **7a** proceeded almost quantitatively to give the half-ester **8a** with three different carbonyl groups by treatment with sodium hydride in *tert*-butyl alcohol containing 3 equiv of water. Similarly **7b** afforded **8b** in excellent yield. Marshall reported a more convenient cleavage condition using sodium hydroxide in place of sodium hydride;[13] however, this could not be applied to the present synthesis as concomitant hydrolysis of the ester group occurred to give the dicarboxylic acid **8** (CO_2Et=CO_2H).

With the requisite half-ester **8** in hand, it was now possible to condense it with tryptamine at the specific position of the molecule. Treatment of **8a** with tryptamine in the presence of

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