87697-48-1; 6-3,3,5α-d_3, 87697-37-8; 6-ol-3,3,5α-d_3, 87697-66-3; 6-3,3,5α-d_3 Me_3Si ether, 87697-67-4; 6-3β-d, 87697-38-9; 6-ol-3β-d, 87697-68-5; 6-3β-d Me_3Si ether, 87697-69-6; 6-3α-d, 87697-39-0; 6-ol-3α-d, 87697-70-9; 6-3α-d Me_3Si ether, 87697-71-0; 6-5α-d, 87697-40-3; 6-ol-5α-d, 87697-72-1; 6-5α-d Me_3Si ether, 87697-73-2; 7, 54657-02-2; 7-ol, 19043-45-9; 7 (phenylacetate), 87697-49-2; 7-5α,7,7-d_3, 87697-41-4; 7-ol-5α,7,7-d_3, 87697-74-3; 7-5α,7,7-d_3 Me_3Si ether, 87697-75-4; 7-7β-d, 87697-42-5; 7-ol-7β-d, 87697-76-5; 7-7β-d Me_3Si ether, 87697-77-6; 7-7α-d, 87697-43-6; 7-ol-7α-d, 87697-78-7; 7-7α-d Me_3Si ether, 87697-79-8; 7-5α-d, 87697-44-7; 7-ol-5α-d, 74051-94-8; 7-5α-d Me_3Si ether, 87697-80-1; 8, 87697-45-8; 8-2α-d, 87697-46-9; 8-4α-d, 87697-47-0; 5α-Δ²-cholestene, 570-73-0; 5α-Δ³-cholestene, 28338-69-4; Δ⁴-cholestene, 16732-86-8; Δ⁵-cholestene, 570-74-1; cholesteryl chloride, 910-31-6; 5α-cholestanyl-6-tosylhydrazone, 18069-88-0; 5α-Δ⁶-cholestene, 28338-70-7; 5α-Δ³-cholestene-3-d, 59582-32-0; Δ⁴-cholesten-3β-ol-3α-d, 1251-63-4; 5α-Δ⁶-cholestene-7-d, 87697-50-5; Δ⁵-cholesten-7β-ol-7α-d, 87697-51-6; cholestan-4β-ol-3,3,5α-d_3, 87697-53-8; cholestan-4-one-3,3,5α-d_3, 87697-52-7; Δ⁴-cholestene-3,3-d_2, 87697-54-9; cholestan-6β-ol-5α,7,7-d_3, 87697-55-0; cholestan-6-one-5α,7,7-d_3, 4321-23-7; Δ⁵-cholestene-7,7-d_3, 87697-56-1; 5α-cholestan-3-one, 566-88-1; 5α-cholestan-3-one-2,2,4,4-d_4, 13976-58-4; 2α,3α-oxido-5α-cholestane, 1753-61-3; 3α,4α-oxido-5α-cholestane, 1249-56-5; 5α-cholestan-β-ol-2α-d, 87697-81-2; 5α-cholestanyl-2α-d 3β-tosylate, 87711-05-5; 5α-cholestan-3β-ol-4α-d, 87697-82-3; 5α-cholestanyl-4α-d 3β-tosylate, 87697-83-4; cholest-4-en-3-one, 601-57-0; Δ⁵-cholesten-7-one, 22033-90-5; 5α-cholestan-4-one, 566-51-8; 5α-cholestan-6-one, 570-46-7.

Synthesis of Ribooligonucleotides Using the 4-Methoxybenzyl Group as a New Protecting Group for the 2'-Hydroxyl Group[1]

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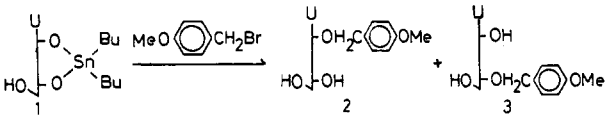
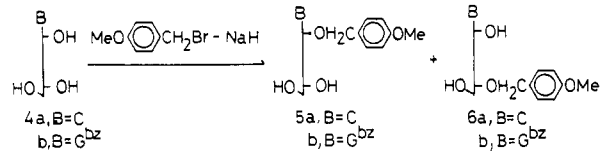
The 4-methoxybenzyl group was introduced to protect the 2'-hydroxyl group of uridine, cytidine, and N^2-benzoylguanosine by treatment of 2',3'-O-(dibutylstannylene)uridine or NaH-treated nucleosides with 4-methoxybenzyl bromide. The 2'-O-(4-methoxybenzyl)nucleosides can be used as useful starting materials for the synthesis of 3',5'-linked ribooligonucleotides. The 4-methoxybenzyl group was removed rapidly from the ribooligonucleotides by treatment with triphenylmethyl fluoroborate, and the completely deblocked ribooligonucleotides were characterized by enzymatic hydrolysis.

Tetrahydropyranyl[2] and methoxytetrahydropyranyl[3] groups have commonly been used to protect the 2'-hydroxyl group of ribonucleotsides in the synthesis of ribooligonucleotides. We have also reported[4] that the tetrahydropyranyl group can be used for synthesis of the 3'- and 5'-reiterated terminal sequences of Rous sarcoma virus 35S RNA. These protecting groups were introduced onto the 2'-hydroxyl group of ribonucleotides through 3',5'-protected ribonucleosides as intermediates.[2-5] Consequently, direct protection of the 2'-hydroxyl group of ribonucleosides has been a crucial problem in the chemical synthesis of ribooligonucleotides. However, only few examples of the direct protection of the 2'-hydroxyl group of ribonucleosides can be found in the literature.[6]

Scheme I



Scheme II



Recently, we found[7] that the 4-methoxybenzyl group can be introduced directly to protect the 2'-hydroxyl group of adenosine with 4-methoxybenzyl bromide and can again be removed rapidly by treatment with triphenylmethyl fluoroborate.[8] The 2'-protected adenosine has been shown to be a useful starting material for the synthesis of ribooligonucleotides.

In this paper, we report the synthesis of 2'-O-(4-methoxybenzyl)uridine, cytidine, and N^2-benzoylguanosine by using 4-methoxybenzyl bromide[9] and their employment in the synthesis of ribooligonucleotides.

Synthesis of 2'-O-(4-Methoxybenzyl)uridine (2). Moffatt and his co-workers have recently reported[6b] that

(1) This manuscript represents part 21 in a series on oligonucleotide synthesis. For the previous paper this series, see: Takaku, H.; Kamaike, K.; Suetake, M. *Chem. Lett.* **1983**, 111.

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(3) Reese, C. B.; Saffhill, R.; Sulston, J. E. *Tetrahedron* **1970**, *26*, 1023; *J. Am. Chem. Soc.* **1967**, *89*, 3366.

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(5) Markiewicz, W. T. *J. Chem. Res., Synop.* **1979**, 24; *J. Chem. Res. Miniprint* **1979**, 0.81. Markiewicz, W. T.; Wiewioroski, M. *Nucleic Acids Res., Spec. Publ.* **1978**, *No. 4*, 185. Honda, S.; Terada, K.; Sato, Y.; Sekine, M.; Hata, T. *Chem. Lett.* **1982**, 15.

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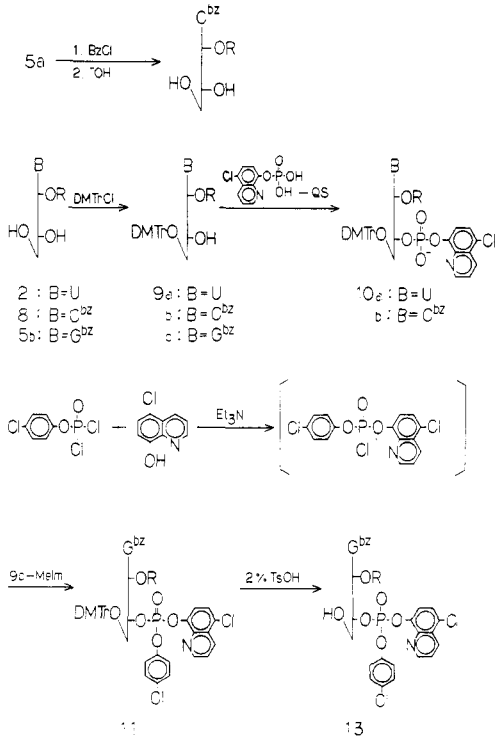
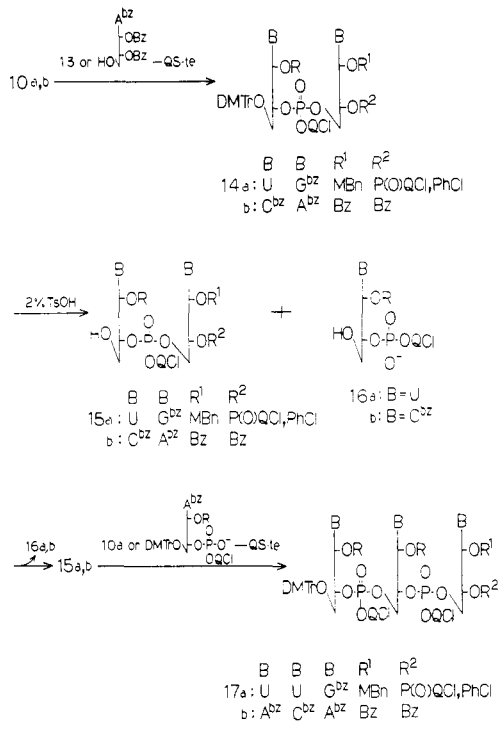
0022-3263/84/1949-0051$01.50/0

Scheme III



Scheme IV



when 2′,3′-*O*-(dibutylstannylene)uridine was treated with benzyl bromide in DMF, 2′(3′)-*O*-benzyluridine was obtained. We examined the preparation of 2′-*O*-(4-methoxybenzyl)uridine (**2**) by modification of the procedure of Moffatt. 2′,3′-*O*-(Dibutylstannylene)uridine (**1**) was treated with 4-methoxybenzyl bromide in dry DMF at 100 °C (Scheme I). After 2 h, the reaction mixture was worked up and purified to give 2′(3′)-*O*-(4-methoxybenzyl)uridines (**2** and **3** in a 1:1 ratio as estimated from the NMR signals of the H-1′ protons). The 2′-isomer **2** (δ 5.88) was at lower field than the 3′-isomer **3** (δ 5.77). The results were consistent with the rules proposed by Reese and his coworkers.[10] The 2′-isomer **2** was separated by crystallization from methanol in 21% yield by using the procedure of Ikehara.[6d]

Synthesis of 2′-*O*-(4-Methoxybenzyl) Derivatives of Cytidine (5a) and N^2-Benzoylguanosine (5b). Moffatt and his co-workers reported[6b] that the selective alkylation of adenosine and cytidine via 2′,3′-*O*-(dibutylstannylene) nucleosides was not successful. We therefore examined the alkylation of sodium hydride treated nucleosides with 2-nitrobenzyl bromide which has been developed by Ikehara.[6e] Cytidine (**4a**) was allowed to react with 4-methoxybenzyl bromide in the presence of sodium hydride in dry DMF at −5 °C (Scheme II). After 2 h, the reaction mixture was quenched with water and concentrated. The residue was dissolved in water and washed with CH_2Cl_2. The hydrochloride of the pure 2′-substituted **5a** was separated by crystallization from ethanol in 36% yield and reconverted into **5a** in 82% yield. The location of the 4-methoxybenzyl group was determined by NMR analysis of the 3′,5′-di-*O*-acetylated compound **7a**, the proton of low field was assigned to H-3′.

N^2-Benzoylguanosine (**5b**) was 4-methoxybenzylated similarly and the 2′-substituted compound **5b** was isolated by extraction with water after the mixture was neutralized with hydrochloric acid. The location of the substitution was determined by NMR decoupling experiments with 3′,5′-di-*O*-acetylated compound **7b**. The anomeric proton was decoupled by irradiation at 4.90 ppm (H-2′) which had been shifted slightly upon acetylation. Another signal corresponding to one proton showed a larger downfield shift and was assigned to H-3′.

Rapid Synthesis of Trinucleotide Blocks 17. The 2′-substituted compounds (**2**, **5a**, **5b**) were utilized as starting materials for forming 3′–5′ internucleotidic bonds. Reaction of **5a** with benzoyl chloride in dry pyridine gave the corresponding benzoylated product which on immediate de-*O*-benzoylation with 2-NaOH gave 2′-*O*-(4-methoxybenzyl)-N^4-benzoylcytidine (**8**, Scheme III). Treatment of the 2′-substituted compounds (**2**, **8**, **5b**) with dimethoxytrityl chloride in dry pyridine gave the corresponding 5′-*O*-(dimethoxytrityl)-2′-*O*-(4-methoxybenzyl) *N*-protected nucleosides **9**.

The 3′-phosphodiesters **10** and the phosphotriester **11** which are key starting materials for ribooligonucleotide synthesis were prepared as follows. The nucleoside derivatives **9** were phosphorylated with 5-chloro-8-quinolyl phosphate[11] in the presence of 8-quinolinesulfonyl chloride (QS)[12] in dry pyridine for 2 h to give the corresponding phosphodiester derivatives **10a,b** in almost quantitative yield, whereas **11** was prepared from **9c** and 4-chlorophenyl 5-chloro-8-quinolyl phosphorodichloridate by the method described previously.[13] TLC analysis shows that the O^6-phosphorylated guanosine derivative is formed in this reaction but is rapidly hydrolyzed with aqueous pyridine to give the corresponding **12** and 4-chlorophenyl 5-chloro-8-quinolyl phosphate.[14]

(10) Formageot, H. P. M.; Griffin, B. E.; Reese, C. B.; Sulston, J. E.; Trenthem, D. R. *Tetrahedron* **1966**, *22*, 705.

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(12) Takaku, H.; Kato, M.; Yoshida, M.; Hata, T. *Nucleic Acids Res., Symp. Ser.* **1978**, *No. 5*, s345. Takaku, H.; Yoshida, M.; Kato, M.; Hata, T. *Chem. Lett.* **1979**, 811. Takaku, H.; Kato, M.; Ishikaea, S. *J. Org. Chem.* **1981**, *46*, 4062.

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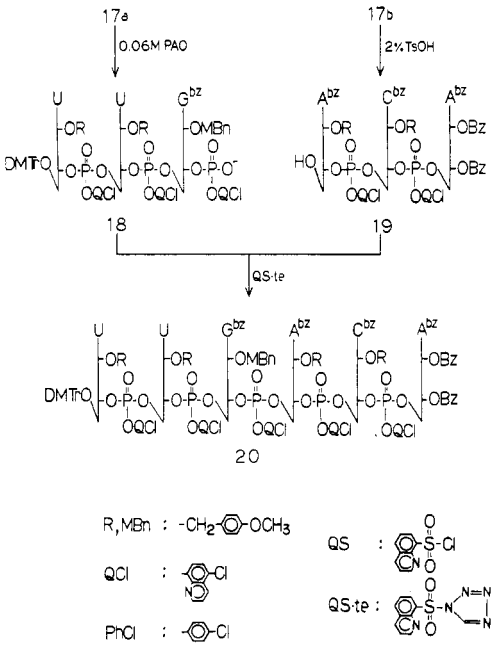
(14) Takaku, H.; Kamaike, K.; Kasuga, K. *Chem. Lett.* **1982**, 197.

Scheme V



Treatment of **12** thus obtained with 2% *p*-toluenesulfonic acid in a mixture of CH_2Cl_2 and MeOH (7:3 v/v) at 0 °C for 15 min gave the 5′-hydroxyl nucleotide **13** in 97% yield after silica gel column chromatography.[15] Reaction of the phosphodiester **10a** (1.5 molar equiv) with **13** (1.0 molar equiv) in the presence of 8-(quinolinylsulfonyl)-1*H*-tetrazole (QS-Te)[16] (3.75 molar equiv) in dry pyridine for 1 h, quenching with ice–water, extraction with CH_2Cl_2, washing of the CH_2Cl_2 solution with 0.1 M TEAB and then with water, and concentration by evaporation with toluene gave a residue which was treated with 2% *p*-toluenesulfonic acid in a mixture of CH_2Cl_2 and MeOH (7:3 v/v) at 0 °C for 15 min. The detritylated phosphodiester **16a** (Scheme IV) was removed from the reaction mixture by simple extraction with 5% $BaCl_2$ solution.[16] The 5′-hydroxyl dinucleotide **15a** was precipitated from a mixture of hexane and ether (95:5 v/v) and was used for the next coupling reaction without further purification. The triester derivative (**15a**) was treated with **10a** (1.5 molar equiv) and QS-te (3.75 molar equiv) in dry pyridine for 1 h to give, after the workup and chromatography of the reaction mixture, the trinucleotide derivative UUGp (**17a**) in 67% yield. By use of the same method, the trinucleotide derivative ACA (**17b**) was obtained in 61% yield by chromatography on silica gel. In this manner, trinucleotide blocks were produced within 1 day and isolated quite easily by silica gel chromatography on a short column.

Synthesis of the Hexanucleotide UUGACA (20). The dimethoxytrityl group of **17b** was selectively removed by treatment with 2% *p*-toluenesulfonic acid solution to give the 5′-hydroxyl phosphotriester intermediate **19** in 94% yield. On the other hand, the 4-chlorophenyl group could be selectively removed from **17a** by treatment with the 0.06 M N^1,N^1,N^3,N^3-tetramethylguanidium salt of pyridine-2-carboaldoxime in a mixture of dioxane and water (2:1 v/v) at room temperature for 16 h, giving the phosphodiester derivative **18** in an almost quantitative yield. The phosphodiester **18** (2.0 molar equiv) thus obtained was dissolved in dry pyridine, and the **19** (1.0 molar equiv) and QS-te (4.7 molar equiv) were added. The reaction was completed within 2 h, and the usual workup gave the desired hexamer **20** in 64% yield (Scheme V).

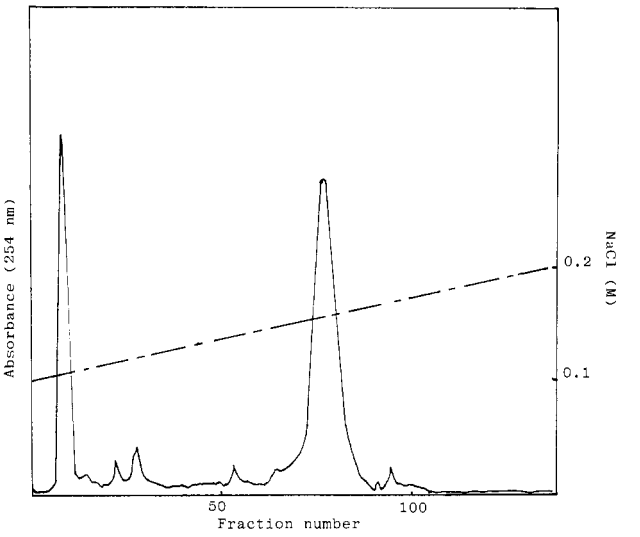
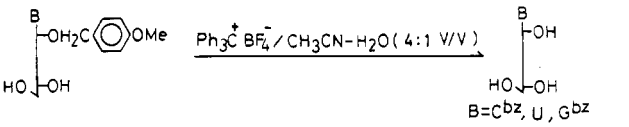
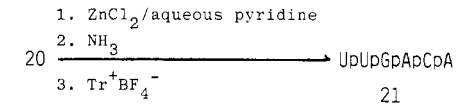


Figure 1. Chromatography of UpUpGpApCpA on a column (1 × 70 cm) of DEAE cellulose equilibrated with 7 M urea in 0.02 M Tris–HCl (pH 7.5). Elution was performed with a linear gradient of NaCl (0.1–0.3 M, total 2000 mL).

Scheme VI



Scheme VII



Removal of the 4-Methoxybenzyl Group from the Nucleoside Derivatives 2, 5b, and 8.[17] The nucleoside derivative **8** was treated with triphenylmethyl fluoroborate (5 molar equiv) in a mixture of CH_3CN and H_2O (4:1 v/v) at room temperature for 1.5 h (Scheme VI). N^4-Benzoylcytidine was obtained by paper chromatography in 99% yield. In a similar manner, uridine and N^2-benzoylguanosine were obtained in 95% and 98% yields, respectively. The *N*-benzoyl group and the glycosidic bond were stable during the above reaction. Cleavage of the glycosidic bond was less than 5% even after treatment with triphenylmethyl fluoroborate for 10 h.

Deblocking of the Fully Protected Hexanucleotide UUGACA (20). The hexamer **20** was deprotected by using first zinc chloride in aqueous pyridine at room temperature for 30 h to cleave the 5-chloro-8-quinolyl group, second with methnolic ammonia at 23 °C for 36 h to remove the benzoyl groups, and finally with triphenylmethyl fluoroborate in a mixture of CH_3CN and H_2O (4:1 v/v) at room temperature for 3 h to split off the 4-methoxybenzyl and dimethoxytrityl groups. The deblocked hexamer UUGACA (**21**, Scheme VII) was isolated by anion-exchange chromatography (Figure 1) and analyzed by HPLC on Finepak C_{18}[18] (Figure 2). **21** was digested with nuclease

(15) Takaku, H.; Yoshida, M.; Nomoto, T. *J. Org. Chem.* **1983**, *48*, 1399.
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(17) Barton, D. H. R.; Magnus, P. D.; Streckert, G.; Zurr, D. *J. Chem. Soc., Chem. Commun.* **1971**, 1109.
(18) Takaku, H.; Nomoto, T.; Kamaike, K. *Chem. Lett.* **1981**, 543.

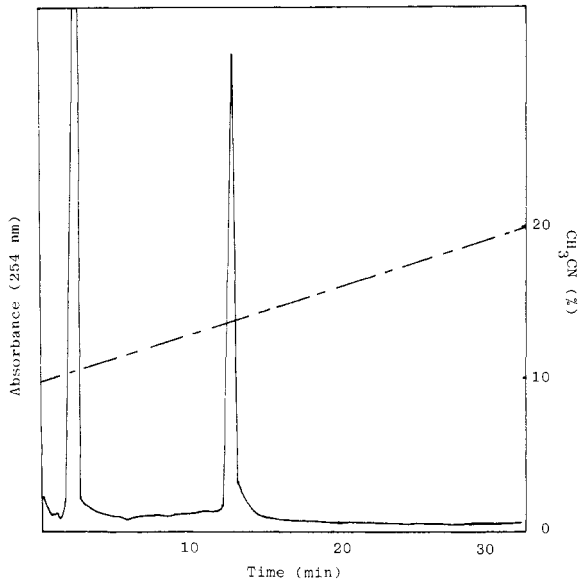

Figure 2. Reverse-phase HPLC of UpUpGpApCpA on Finepak SIL C_{18} (4.6 × 250 mm). Elution was performed with a linear gradient of acetonitrile (10–20%) in 0.1 M triethylammonium acetate (pH 7.0).

P1 to give the expected products in the correct ratio. This hydrolysis indicated that **21** had a 3′–5′ internucleotidic bond.

In conclusion, the 4-methoxybenzyl group described in this paper could be used for the 2′-hydroxyl of ribonucleosides in the synthesis of ribooligonucleotides. Especially, the 4-methoxybenzyl group can be introduced directly to protect the 2′-hydroxyl group of ribonucleotides. Since, the 2′-*O*-(4-methoxybenzyl)nucleosides are stable to acid and alkali, they can be used for the synthesis of ribooligonucleotides by the improved phosphotriester method. Finally, the 4-methoxybenzyl group was removed rapidly from the ribooligonucleotides by treatment with triphenylmethyl fluoroborate.

Experimental Section

General Methods. UV absorptions were measured with a Shimazu UV-200 recording spectrophotometer. ^{1}H NMR spectra were measured at 100 MHz with a JOEL JNMPS 100 spectrometer with Me_4Si as an internal standard.

High-pressure liquid chromatography (HPLC) was carried out by using a JASCO TWINCLE apparatus.

Paper chromatography was performed by the decending technique by using the following solvent systems: A, 1-PrOH–concentrated NH_4OH–H_2O (55:10:35 v/v); B, 2-PrOH–concentrated NH_4OH–H_2O (7:1:2 v/v). Paper electrophoresis was performed by using 0.05 M TEAB (pH 7.5) at 1100 V/40 cm.

Thin-layer chromatography (TLC) was performed on plates of silica gel (Merck $60F_{254}$) by using the following solvent systems: C, CH_2Cl_2–MeOH (9:1 v/v); D, CH_2Cl_2–MeOH (95:5 v/v); E, CH_2Cl_2–EtOH (10:1 v/v).

Tritylation of nucleosides is described in ref 15. Nuclease P1 was purchased from Yamasa Shouyu Co.

2′-*O*-(4-Methoxybenzyl)uridine (2). 2′,3′-*O*-(Dibutylstannylene)uridine (1;[6b] 8.55 g, 18 mmol) was treated with 4-methoxybenzyl bromide (5.07 g, 25 mmol) in dry DMF at 100 °C. After 2 h, the reaction mixture was quenched with ice–water and evaporated in vacuo. The residue was dissolved in water (160 mL) and washed with ether (200 mL), and the aqueous solution was concentrated in vacuo. The residue was dissolved in CH_2Cl_2 and applied to a silica gel column (5 × 15 cm). The column was eluted with 500 mL of CH_2Cl_2, followed by a stepwise gradient of MeOH (0–2%) in CH_2Cl_2. The appropriate fractions were evaporated, and the residue was dissolved in MeOH (30 mL). The solution was left at room temperature for overnight and then cooled in a refrigerator for 1 day. The resulting precipitate was collected by filtration to give pure prisms: 1.40 g (21%): mp 146–148 °C; R_f 0.28 (solvent C), 0.23 (solvent E); UV (95% EtOH) λ_{max} 262 nm (ϵ 12 000), 225, λ_{min} 240 nm; ^{1}H NMR (Me_2SO-d_6) δ 3.60 (m, 2 H, 5′-H), 3.76 (s, 3 H, OCH_3), 3.80–4.38 (m, 5 H, 2′-H, 3′-H, 4′-H, 3′-OH, 5′-OH), 4.62 (d, 2 H, $ArCH_2$), 5.72 (d, 1 H, $J_{5,6}$ = 8 Hz, 5-H), 5.88 (d, 1 H, $J_{1',2'}$ = 6 Hz, 1′-H), 6.94 (d, 2 H, J = 8 Hz, Ar), 7.33 (d, 2 H, J = 8 Hz, Ar), 7.90 (d, 1 H, $J_{5,6}$ = 8 Hz, 6-H), (3.80–4.38, 2 H, disappeared with D_2O). Anal. Calcd for $C_{17}H_{20}N_2O_7 \cdot {}^1/_2H_2O$: C, 54.69; H, 5.67; N, 7.50. Found: C, 54.99; H, 5.56; N, 7.26.

2′-*O*-(4-Methoxybenzyl)cytidine Hydrochloride. Cytidine (4.86 g, 20 mmol) was dissolved in dry DMF (60 mL) and cooled to −5 °C. Sodium hydride (50% oil dispersion, 1.24 g, 26 mmol, washed with dry benzene) was added under stirring. After 1 h, 4-methoxybenzyl bromide (4.82 g, 24 mmol) in dry DMF (40 mL) was added dropwise to the reaction mixture during 1 h and allowed to stand at −5 °C for 1 h. The reaction was quenched with ice–water (20 mL), and the solution was concentrated to oil. The oil was dissolved in water (160 mL) and extracted with CH_2Cl_2 (2 × 40 mL). The aqueous solution was evaporated in vacuo until it became a syrup, and then EtOH (3 mL) and 1 N HCl (19 mL) were added. The solution was left in refrigerator overnight. The precipitate was collected by filtration, and recrystallization from EtOH–H_2O (95:5 v/v) gave 2.88 g (36%) of the pure 2′-isomer: mp 196–199 °C; UV (95% EtOH) λ_{max} 274 nm, 225, λ_{min} 246; ^{1}H NMR (Me_2SO-d_6) δ 3.62 (br s, 2 H, 5′-H), 3.70 (s, 3 H, OCH_3), 3.75–4.21 (m, 3 H, 2′-H, 3′-H, 4′-H), 4.55 (s, 2 H, $ArCH_2$), 5.88 (d, 1 H, $J_{1',2'}$ = 3 Hz, 1′-H), 6.18 (d, 1 H, $J_{5,6}$ = 8 Hz, 5-H), 6.89 (d, 2 H, J = 8 Hz, Ar), 7.32 (d, 2 H, J = 8 Hz, Ar), 8.87 (br s, 1 H, NH, disappeared by addition of D_2O), 9.92 (br s, 1 H, NH, disappeared by addition of D_2O). Anal. Calcd for $C_{17}H_{21}N_3O_6$·Hcl: C, 51.06; H, 5.56; N, 10.51. Found: C, 50.92; H, 5.61; N, 10.27.

2′-*O*-(4-Methoxybenzyl)cytidine (5a). The hydrochloride (2.88 g, 7.2 mmol) was dissolved in water (148 mL) and neutralized with 2 N NaOH. The precipitate was collected by filtration and recrystallization from water and gave 2.15 g (82%) of pure **5a**: mp 101–102 °C; R_f 0.21 (solvent C), R_f 0.05 (solvent E); UV (95% EtOH) λ_{max} 273 nm (ϵ 10 200), 226, λ_{min} 250; ^{1}H NMR (Me_2SO-d_6) δ 3.44 (m, 2 H, 5′-H), 4.31 (s, 3 H, OCH_3), 3.84 (m, 2 H, 2′-H, 4′-H), 4.04 (m, 1 H, 3′-H), 4.59 (s, 2 H, $ArCH_2$), 5.06–5.20 (m, 2 H, 3′-OH, 5′-OH), 5.71 (d, 1 H, $J_{5,6}$ = 8 Hz, 5-H), 5.92 (d, 1 H, $J_{1',2'}$ = 4 Hz, 1′-H), 6.84 (d, 2 H, J = 8 Hz, Ar), 7.28 (d, 4 H, J = 8 Hz, Ar, NH_2), 7.81 (d, 1 H, $J_{5,6}$ = 8 Hz, 6-H). Anal. Calcd for $C_{17}H_{21}N_3O_6 \cdot 3H_2O$: C, 48.91; H, 6.53; N, 10.07. Found: C, 48.65; H, 6.09; N, 10.11.

2′-*O*-(4-Methoxybenzyl)-*N*^{4},*O*^{3},*O*^{5}-triacetylcytidine (7a). Compound **5a** (189 mg, 0.5 mmol) was treated with acetic anhydride (1.34 mL) in dry pyridine (1.8 mL) at room temperature for 20 h. The completion of the reaction was checked by TLC (solvent C). The solution was evaporated, and the residue was coevaporated with aqueous EtOH. Recrystallization from EtOH gave **7a**: 151 mg (71%); mp 156–157 °C; UV (95% EtOH) λ_{max} 297 nm, 253; ^{1}H NMR ($CDCl_3$) δ 2.08 (s, 3 H, Ac), 2.12 (s, 3 H, Ac), 2.17 (s, 3 H, Ac), 3.79 (s, 3 H, OCH_3), 4.38 (m, 4 H, 5′-H, 4′-H, 2′-H), 4.58 (s, 2 H, $ArCH_2$), 5.20 (t, 1 H, 3′-H), 6.03 (d, 1 H, $J_{1',2'}$ = 4 Hz, 1′-H), 6.90 (d, 2 H, J = 8 Hz, Ar), 7.18 (d, 2 H, J = 8 Hz, Ar), 7.18 (d, 3 H, Ar, 5-H), 7.90 (d, 1 H, $J_{5,6}$ = 8 Hz, 6-H). Anal. Calcd for $C_{23}H_{26}N_3O_{10}$: C, 54.76; H, 5.19; N, 8.33. Found: C, 54.98; H, 5.52; N, 7.95.

2′-*O*-(4-Methoxybenzyl)-*N*^{2}-benzoylguanosine (5b). *N*^{2}-Benzoylguanosine (**4b**, 2.33 g, 6.0 mmol) was dissolved in dry DMF (50 mL) and cooled to −5 °C. Sodium hydride (50% oil dispersion, 1.24 g, 26.0 mmol, washed with dry benzene) was added under stirring. After 1 h, 4-methoxybenzyl bromide (1.44 g, 7.2 mmol) in dry DMF (10 mL) was added dropwise to the reaction mixture for 1 h and allowed to stand at −5 °C for 4 h. The reaction was quenched with EtOH and concentrated to oil. The oil was dissolved in water (160 mL) and washed with CH_2Cl_2. The aqueous solution was neutralized with 3 N HCl and evaporated in vacuo. The residue was dissolved in CH_2Cl_2 (100 mL) and washed with water (2 × 30 mL). The organic layer was dried with Na_2SO_4, concentrated to dryness, and purified on a silica gel column (5 × 15 cm). The column was eluted with 300 mL of CH_2Cl_2 followed by 300 mL of CH_2Cl_2–MeOH (98:2 v/v). The appropriate fractions were evaporated and recrystallized from water and gave pure **5b**: 1.72 g (57%); mp 126–127 °C; R_f 0.53 (solvent C), 0.23 (solvent E); UV (95% EtOH) λ_{max} 290 nm, 260

(ε 19 300), λ_{min} 272, 251; ^{1}H NMR (Me_2SO-d_6) δ 3.50 (br s, 2 H, 5′-H), 3.70 (s, 3 H, OCH_3), 4.05 (br s, 1 H, 4′-H), 4.50 (m, 2 H, 3′-H, 2′-H), 4.61 (s, 2 H, $ArCH_2$), 5.11 (t, 1 H, 5′-OH), 5.31 (m, 1 H, 3′-OH), 6.09 (d, 1 H, $J_{1',2'}$ = 6 Hz, 1′-H), 6.75–8.05 (m, 9 H, Ar), 8.24 (s, 1 H, 8-H), 5.11 and 5.31 disappeared on addition of D_2O. Anal. Calcd for $C_{25}H_{25}N_5O_7 \cdot H_2O$: C, 57.13; H, 5.19; N, 13.33. Found: C, 57.11; H, 5.10; N, 12.98.

2′-*O*-(4-Methoxybenzyl)-3′,5′-di-*O*-acetyl-*N*^{2}-benzoylguanosine (7b). The nucleoside derivative **5b** (221 mg, 0.44 mmol) was treated with acetic anhydride (1.2 mL) in dry pyridine (1.6 mL) at room temperature for 10 h. The product was isolated as described for the synthesis of **7a**: mp 94–96 °C; UV (95% EtOH) λ_{max} 288 nm, 259; ^{1}H NMR ($CDCl_3$) δ 2.04 (s, 3 H, Ac), 2.28 (s, 3 H, ac), 3.61 (s, 3 H, OCH_3), 4.22 (br s, 2 H, 5′-H), 4.50 (br s, 3 H, 4′-H, $ArCH_2$), 4.89 (m, 1 H, 2′-H), 5.48 (d, 1 H, 3′-H), 6.00 (d, 1 H, $J_{1',2'}$ = 6 Hz, 1′-H), 6.60–8.08 (m, 10 H, Ar, 8-H). Anal. Calcd for $C_{29}H_{28}N_5O_9$: C, 58.98; H, 4.78; N, 11.86. Found: C, 58.85; H, 4.83; N, 11.73.

2′-*O*-(4-Methoxybenzyl)-*N*^{4}-benzoylcytidine (8). The nucleoside derivative **5a** (911 mg, 2.50 mmol) was treated with benzoyl chloride (1.75 mL, 10 mmol) in dry pyridine (5 mL) at room temperature. After 3 h, the reaction mixture was quenched with ice–water (2 mL) and extracted with CH_2Cl_2 (2 × 25 mL). The organic layer was washed with 5% $NaHCO_3$ solution (2 × 30 mL) and water (30 mL), and the organic solution was evaporated in vacuo. The residue was dissolved in EtOH (13 mL)–pyridine (5 mL) and the solution was treated with 2 N NaOH (14 mL) at 0 °C for 15 min. The reaction mixture was neutralized with Amberlite IR 120 (pyridinium form). The supernatant was passed through column (30 mL) of Amberlight IR 120 (pyridinium form), and the resin was washed with pyridine–water (3:1 v/v, 50 mL). The eluate was evaporated and recrystallized from EtOH and gave 1.013 g (87%) of pure **8**: mp 160–162 °C; UV (95% EtOH) λ_{max} 305 nm, 262, 228, λ_{min} 289, 241; ^{1}H NMR (Me_2SO-d_6) δ 3.72 (br s, 2 H, 5′-H), 3.78 (s, 3 H, OCH_3), 3.90–4.20 (m, 3 H, 4′-H, 3′-H, 2′-H), 4.75 (s, 2 H, $ArCH_2$), 5.10–5.40 (m, 2 H, 5′-OH, 3′-OH), 6.04 (d, 1 H, $J_{1',2'}$ = 2 Hz, 1′-H), 6.92 (d, 2 H, J = 8 Hz, Ar), 7.32 (d, 1 H, $J_{5,6}$ = 8 Hz, 5-H), 7.35 (d, 2 H, J = 8 Hz Ar), 7.60–8.00 (m, 5 H, Ar), 8.04 (d, 1 H, $J_{5,6}$ = 8 Hz, 6-H), 5.10–5.40 disappeared by addition of D_2O. Anal. Calcd for $C_{24}H_{25}N_3O_7$: C, 61.55; H, 5.40; N, 8.99. Found: C, 61.50; H, 5.41; N, 8.79.

5′-*O*-(Dimethoxytrityl)-2′-*O*-(4-methoxybenzyl) *N*-Protected Nucleoside 3′-(5-Chloro-8-quinolyl) Phosphates (10). The tritylated compounds **9a,b** (1.0 mmol) and 5-chloro-8-quinolyl phosphate (312 mg, 1.2 mmol) were coevaporated with dry pyridine and dissolved in dry pyridine (10 mL). To the solution was added QS (546 mg, 2.4 mmol). After the mixture was stirred at room temperature for 2 h, TLC (solvent C, R_f 0.00–0.10) showed that the reaction was completed. The reaction mixture was quenched with ice–water (2 mL) and then with water (30 mL). The solution was dried with Na_2SO_4, evaporated in vacuo, and precipitated with hexane. The triethylammonium salts of **10** were obtained in almost quantitative yields as stable colorless solids, uncontaminated with undesired side products. ^{31}P NMR spectral data of **10a** and **10b** are given below. **10a**: δ 5.45 (pyridine-d_5-C_6D_6, 85% H_3PO_4); **10b**: δ 6.42 (pyridine-d_5–C_6D_6, 85% H_3PO_4).

Synthesis of the Fully Protected Mononucleotide 11. To a dry THF (4.0 mL) solution of 4-chlorophenyl phosphodichloride (0.3 mL, 2.0 mmol) was added a dry THF (5 mL) solution of 5-chloro-8-hydroxyquinoline (395 mg, 2.2 mmol) and triethylamine (0.31 mL, 2.2 mmol) at −10 °C. The reaction temperature was gradually raised to room temperature, and the mixture was stirred for 45 min.[18] The reaction was monitored by TLC. After the completion of the reaction, triethylammonium hydrochloride was removed by filtration. To the filtrate was added **9c** (810 mg, 1.0 mmol) and 1-methylimidazole (0.24 mL, 3.0 mmol). The mixture was kept for 1 h. The reaction mixture was quenched with ice–water (1 mL) and extracted with CH_2Cl_2 (2 × 25 mL). The CH_2Cl_2 extract was washed with water (2 × 25 mL), dried with Na_2SO_4, and evaporated in vacuo. The residue was dissolved again in CH_2Cl_2 and chromatographed on a silica gel column. The appropriate fractions [eluted with CH_2Cl_2–MeOH (98:2 v/v)] were evaporated to give the fully protected mononucleotide **11** which was isolated as a solid (952 mg, 82%) by precipitation from hexane (100 mL): mp 111–113 °C; R_f 0.50 (solvent C), 0.25 (solvent D); UV (MeOH) λ_{max} 295 nm (sh), 282, 264, 232, λ_{min} 271, 258. Anal. Calcd for $C_{61}H_{52}N_6O_{12}PCl_2$: C, 63.05; H, 4.51; N, 7.23. Found: C, 63.41; N, 4.62; N, 7.48.

Rapid Synthesis of Trinucleotide Blocks 17. The fully protected mononucleotide **11** (2.322 g, 2.0 mmol) was treated with 2% *p*-toluenesulfonic acid in a mixture of CH_2Cl_2 and MeOH (7:3 v/v 50 mL) at 0 °C for 15 min.[15] The reaction mixture was neutralized with phosphate buffer (pH 7.0) and transferred into CH_2Cl_2. The organic layer was washed with water, dried with Na_2SO_4, and evaporated in vacuo. The residue was precipitated from hexane–ether (95:5 v/v, 150 mL) and used as the 5′-hydroxyl component in the subsequent condensation without purification. The yield of **12** was 1.575 g (95%). The 5′-hydroxyl compound **12** (0.829 g, 1.0 mmol) thus obtained was combined with the phosphodiester **10a** (1.669 g, 1.5 mmol), rendered anhydrous by coevaporation of dry pyridine (three times), and then treated with QS-te (0.979 g, 3.75 mmol) in dry pyridine (5 mL) at room temperature. After 1 h, no 5′-hydroxyl component **12** could be detected on TLC of the reaction mixture. The reaction mixture was quenched with ice–water (2 mL) and extracted with CH_2Cl_2 (2 × 25 mL). The CH_2Cl_2 extract was washed with 0.1 M TEAB solution (pH 7.5, 2 × 30 mL) and water (30 mL) and then concentrated. The residue was coevaporated with toluene and treated with 2% *p*-toluenesulfonic acid in a mixture of CH_2Cl_2 and MeOH (7:3 v/v, 21 mL) at 0 °C for 15 min. The reaction mixture was neutralized with phosphate buffer (pH 7.0). The detritylated phosphodiester derivative **16a** was removed from the reaction mixture by simple extraction with 5% $BaCl_2$ solution (4 × 10 mL) in CH_2Cl_2. The organic layer was dried with Na_2SO_4 and evaporated in vacuo. The residue was dissolved in a small amount of CH_2Cl_2 and poured into hexane–ether (95:5 v/v, 150 mL). The resulting precipitate was collected by filtration and was used for the next coupling reaction without further purification. The 5′-hydroxyl dinucleotide **15a** was treated with the phosphodiester **10a** (1.669 g, 1.5 mmol) in the presence of QS-te (0.980 g, 3.75 mmol) in dry pyridine (5 mL) for 1 h. The reaction was quenched with ice–water (2 mL) and extracted with CH_2Cl_2 (2 × 40 mL). The organic layer was washed with 0.1 M TEAB solution (pH 7.5, 2 × 50 mL) and water (50 mL), dried with Na_2SO_4, filtered, and evaporated in vacuo. The residue was dissolved in a small amount of CH_2Cl_2 and purified by short column chromatography (4 × 14 cm) on silica gel. The appropriate fractions were evaporated to give the corresponding trinucleotide **17a** which was isolated as a solid (1.556 g, 67%) by precipitation from hexane (200 mL); R_f 0.41 (solvent C), 0.23 (solvent D).

In a same way, the fully protected trinucleotide **17a** was isolated as a solid: 1.398 g (61%); R_f 0.35 (solvent C), 0.14 (solvent D).

Synthesis of Hexanucleotide 20. Treatment of **17b** (0.801 g, 0.35 mmol) with 2% *p*-toluenesulfonic acid solution as described for **13** afforded the corresponding 5′-hydroxyl component **19** (0.655 g, 94%). On the other hand, the triester derivative **17a** (1.533 g, 0.66 mmol) was treated with a 0.06 M solution of the N^1,N^1,N^3,N^3-tetramethylguanidium salt of pyridine-2-carboaldoxime in a mixture of dioxane and water (2:1 v/v, 20 mL) at room temperature for 16 h. The reaction mixture was treated with Dowex 50W-X2 (pyridinium form), and the resin was removed by filtration and washed with 50% aqueous pyridine (15 mL). The filtrate was washed with ether (3 × 10 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The CH_2Cl_2 extract was dried with Na_2SO_4 and evaporated in vacuo. The phosphodiester **18** thus obtained was combined with **19** (0.655 g, 0.33 mmol) and rendered anhydrous by coevaporation with pyridine (3.3 mL) for 2 h. The reaction mixture was then worked up as described for the preparation of **17** and purified by silica gel column (4 × 20 cm) chromatography. The appropriate fractions [eluted with a stepwise gradient of MeOH (0–5%) in CH_2Cl_2] were evaporated to ca. 3–4 mL and poured into hexane (70 mL). A white precipitate was collected to give **20**: 1.007 g (73%); R_f 0.35 (solvent C), R_f 0.17 (solvent D).

Removal of the 4-Methoxybenzyl Group from 2, 5b, and 8. Compound **2** (11 mg, 0.03 mmol) was treated with triphenylmethyl fluoroborate (33 mg, 0.15 mmol) in CH_3CN–H_2O (4:1 v/v, 1.2 mL) at room temperature. After 1.5 h, pyridine (1 mL) was added to the reaction mixture, and the mixture was evaporated in vacuo. The residue was dissolved in a small amount of pyridine and chromatographed on Toyo Roshi No. 51A paper developed with solvent B. Uridine was obtained in 95% yield

(estimated spectrophotometrically). In a similar manner, N^4-benzoylcytidine and N^2-benzoylguanosine were obtained in 99% and 98% yields, respectively.

Deblocking of the Fully Protected Hexanucleotide UUGACA (20). The hexamer **20** (41.8 mg, 10 μmol) was treated with $ZnCl_2$ (244 mg, 1.8 mmol) in aqueous pyridine (90%, 15 mL) at room temperature for 30 h. Then water (5 mL) and Dowex 50W-X2 (pyridinium form) were added with stirring. The resin was removed by filtration and washed with aqueous pyridine (50%). The filtrate and washings were collected and then evaporated to dryness. The residue was dissolved in methanolic ammonia (10 mL), and the mixture was kept at 23 °C for 36 h. The solution was concentrated, and the residue was treated with triphenylmethyl fluoroborate (44 mg, 200 μmol) in CH_3CN–H_2O (4:1 v/v, 2 mL) at room temperature for 3 h. Pyridine was added to the reaction mixture, and the solution was evaporated in vacuo. The deblocked product **21** was isolated by ion-exchange chromatography on a column (1.0 × 70 cm) of DEAE cellulose. The main part of the peak in Figure 1 (303 A_{254}) was purified and isolated by HPLC (Figure 2) in 70% yield. U-U-G-A-C-A (**21**) was characterized by base composition analysis by anion-exchange HPLC after complete digestion with nuclease P1. The ratio of pU/pG/pC/pA was 1.00:0.91:1.14:2.19: R_f 0.26 (solvent A); paper electrophoresis 0.83 (to Cp); UV (H_2O, pH 7.0) λ_{max} 258 nm, λ_{min} 234.

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Registry No. 1, 42822-78-6; **2**, 80015-57-2; **3**, 85193-75-5; **4b**, 3676-72-0; **5a**, 80015-55-0; **5a**·HCl, 87985-90-8; **5b**, 80015-56-1; **7a**, 87985-91-9; **7b**, 87985-92-0; **8**, 80015-59-4; **9a**, 85193-78-8; **9b**, 80015-61-8; **9c**, 87985-93-1; **10a**, 85193-79-9; **10b**, 80015-63-0; **11**, 87985-94-2; **12**, 87985-95-3; **13**, 88015-19-4; **14a**, 87999-43-7; **14b**, 80015-66-3; **15a**, 87985-97-5; **15b**, 80015-68-5; **16a**, 87985-96-4; **17a**, 87999-40-4; **17b**, 88015-18-3; **18**, 87999-41-5; **19**, 80015-70-9; **20**, 88083-16-3; **21**, 87999-42-6; 4-methoxybenzyl bromide, 2746-25-0; cytidine, 65-46-3.

Convergent and Efficient Synthesis of Spiro[benzofuran-3(2*H*),4′-piperidines]

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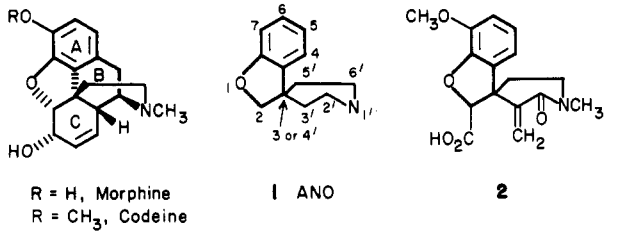
A synthesis is presented of spiro[7-methoxybenzofuran-3(2*H*),4′-piperidines], tricyclic analogues containing the A, N, and O rings of codeine. Two successive 1,4-additions into a pyridine residue, an intermolecular aryllithium addition followed by an intramolecular ester enolate ring closure, establish the spiro[benzofuran–dihydropyridine] system. Reduction and finally α-methylene lactam rearrangment provide the necessary functionalization for further elaboration and C-ring closure.

A broad class of biologically potent compounds are the 4,4-disubstituted piperidines. Although certain members are well documented for analgesic activity,[1] it is only recently that the wide-ranging pharmacological properties and commercial value of these compounds have been demonstrated.[2] An important group of analgesics within the disubstituted piperidines are the spiro[benzofuran-3-(2*H*),4′-piperidines] **1** containing three rings (ANO) of the pentacyclic morphine skeleton. Recently this fragment has been proven a viable intermediate for further elaboration into the codeine system.[3]

Early syntheses of the spiro[benzofuran-3(2*H*),4′-piperidines] have employed a geminal alkylation by a bis(2-haloethyl)amine to produce a structure containing a fully saturated, unsubstituted nitrogen ring. Functionalization of both the C-2 side chain and of the nitrogen ring has been achieved with a sequence in which the C-4′ position is quaternized through an α-chloro ortho ester rearrangement.[3] Recently a route involving an intramolecular addition to a pyridinium species has appeared.[4] The last method provides functionalization of the piperidine ring in the form of unsaturation but does not incorporate the C-7 oxygen substituent necessary for the codeine functional group pattern.

Enolate additions to 3-carbonylpyridinium salts have been employed in a variety of natural product preparations.[5] We now present a route to the synthesis of **2**, which



R = H, Morphine
R = CH_3, Codeine

1 ANO

2

has been elaborated to an octahydro-1*H*-benzofuro[3,2-*e*]isoquinoline,[3] by employing this methodology along with the well-known α-methylene lactam rearrangement.[6]

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