Table IV. Side Chain Bromination of Alkyl-Substituted Molecules with 1 under Reflux in $CCl_4$

| substrate | product | substrate (mmol):1 (g) | reac time, h | yield of pure product, % |
|---|---|---|---|---|
| methylbenzene | (bromomethyl)benzene | 3:3 | 4 | 78 |
| 1-methylnaphthalene | 1-(bromomethyl)naphthalene | 1:1 | 4 | 63 |
| 2-methylnaphthalene | 2-(bromomethyl)naphthalene | 1:1 | 24 | 79 |
| ethylbenzene | 1-phenyl-1-bromoethane | 10:10 | 4 | 81 |
| 1,2-dimethylbenzene | 1,2-bis(bromomethyl)benzene | 1:3 | 24 | 85 |
| 1,3-dimethylbenzene | 1,3-bis(bromomethyl)benzene | 1:3 | 24 | 75 |
| 1,4-dimethylbenzene | 1,4-bis(bromomethyl)benzene | 1:3 | 24 | 82 |
| 2,6-dimethylpyridine | 2,6-bis(bromomethyl)pyridine | 1:3 | 24 | 66 |
| hexamethylbenzene | 1-(bromomethyl)pentamethylbenzene | 1:1 | 4 | 75 |

in which the dibromide (**14a**) was formed as the only product.

We further searched for the optimal reaction conditions for converting alkyl-substituted benzene derivatives to mono- or dibromo products. The reaction conditions and the yields of the isolated pure products are presented in Table IV. The structure of the products were determined on the basis of their spectroscopic data and comparison with those of independently synthesized compounds. Many of the products mentioned in Table IV are usually obtained via reactions with two or even more steps, while the yields presented proved to be in almost all cases higher than those reported up to now in the literature.

The preparation of bromo-substituted products with the bromine complex of cross-linked poly(styrene-*co*-4-vinylpyridine) (1) is a manipulatively simple method; after completion of the reaction the polymer beads are filtered off, the solvent is evaporated in vacuo, and the crude products are pure enough for further reactions or can be easily purified by crystallization or distillation. The polymer resins can be easily recovered after the reaction and reused several times.

## Experimental Section

IR spectra were recorded using a Perkin-Elmer 727 B spectrometer and $^1H$ NMR spectra with a Jeol JNM-PS-100 spectrometer, with $Me_4Si$ as internal reference, while mass spectra and high-resolution measurements were taken with a CEC-21-11 spectrometer. GLC analysis was carried out on a Varian Aerograph 1800 instrument and TLC on Merck silica gel F 254. Cross-linked poly(styrene-*co*-4-vinylpyridine) and bromine complexes were prepared according to previously published procedures[11,13] and are commercially available.[15]

**Addition and Isolation Procedures.** Alkyl-substituted aromatic or heteroaromatic molecules (1–10 mmol) were dissolved in 20–200 mL of $CCl_4$ and 1–10 g of polymer reagent, 4–40 mg of dibenzoyl peroxide was added, and the reaction mixture was heated under reflux for from 1–24 h. In the case of light-initiated reactions, the reaction mixtures were stirred for 3 h and irradiated with 125-W HPQ lamps. Insoluble polymer was filtered off and the solvent evaporated under reduced pressure, and the reaction mixtures were analyzed by GLC or $^1H$ NMR. Crude reaction mixtures were purified by distillation or crystallization. The yields of pure products and the reaction conditions are listed in Table IV, while in Tables I–III the effect of the structure of the organic molecule on the course of bromination is presented.

**Benzyl Bromide**: 79%; bp 199 °C (lit.[16] bp 194–198 °C; yield 59%).

**1-(Bromomethyl)naphthalene**: crystallization from ethanol (63%); mp 58–60 °C (lit.[16] mp 55–56 °C; yield 57%); NMR $\delta$ 4.9 (br s, 2 H, $CH_2$) 7.5 (m, 7 H).

**2-(Bromomethyl)naphthalene**: crystallization from ethanol (79%); mp 60–62 °C (lit.[16] mp 54–55 °C; yield 62%); NMR $\delta$ 4.38 (br s, 2 H, $CH_2$), 7.4 (m, 7 H).

**1-Phenyl-1-bromoethane**: distillation under reduced pressure [$T$ = 107–109 °C, (15 mm Hg); 81%] [lit.[17] bp 92–94 °C (8 mmHg); yield from 1-phenylethanol, yield 74%]; NMR $\delta$ 2.18 (d, 3 H, $J$ = 6 Hz, $CH_3$), 5.64 (q, 1 H CH), $\delta$ 8.08 (m, 5 H, Ph).

**2,6-Bis(bromomethyl)pyridine**: crystallization from petroleum ether (66%); mp 84–87 °C (lit.[18] mp 83–86 °C; yield 20%).

**Pentamethylbenzyl bromide**: crystallization from petroleum ether (75%); mp 83–85 °C (lit.[19] mp 84–85 °C; yield 94% from methyl ether).

**1,2-Bis(bromomethyl)benzene**: crystallization from chloroform (85%); mp 92–93 °C (lit.[20] mp 98–99 °C; yield 56%); NMR $\delta$ 5.05 (s, 4 H, $CH_2$), $\delta$ 7.98 (m, 4 H, Ph).

**1,3-Bis(bromomethyl)benzene**: crystallization from chloroform (75%); mp 74–76 °C (lit.[20] mp 70–72 °C; yield 44%); NMR $\delta$ 4.85 (s, 4 $CH_2$) H, 7.92 (m, 4 H Ph).

**1,4-Bis(bromomethyl)benzene**: crystallization from chloroform (82%); mp 143–145 °C (lit.[20] mp 142–144 °C; yield 50%); NMR $\delta$ 4.85 (s, 4 H, $CH_2$), 8.08 (s, 4 H, Ph).

**Regeneration of Resins.** Beads were washed with methanol and water, then suspended in 2 N NaOH, stirred for 1 h at 50 °C, washed with water, methanol, and chloroform, and dried at room temperature to constant weight. No loss of polymer activity for the further preparation of complex 1 was found.

**Registry No.** **3** (Ar = Ph), 108-88-3; **3** (Ar = 1-naphthalene), 90-12-0; **3** (Ar = 2-naphthalene), 91-57-6; **3** (Ar = pentamethylbenzene), 87-85-4; **4** (Ar = Ph), 100-39-0; **4** (Ar = 1-naphthalene), 3163-27-7; **4** (Ar = 1-pentamethylbenzene), 53442-65-2; **4** (Ar = 2-naphthalene), 939-26-4; **5**, 98-82-8; **6**, 3575-19-7; **7**, 98-83-9; **8**, 36043-44-4; **9**, 3360-54-1; **12a**, 95-47-6; **12b**, 108-38-3; **12c**, 106-42-3; **13a**, 89-92-9; **14a**, 91-13-4; **14b**, 626-15-3; **14c**, 623-24-5; $Br_2$, 7726-95-6; ethyl benzene, 100-41-4; 1-phenyl-1-bromoethane, 585-71-7; 2,6-dimethylpyridine, 108-48-5; 2,6-bis(bromomethyl)-pyridine, 7703-74-4.

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# (Methoxyethoxy)methyl Group: New Amide and Hydroxyl Protecting Groups of Uridine in Oligonucleotide Synthesis[1]

Tsunehiko Ito, Souichi Ueda, and Hiroshi Takaku*

*Laboratory of Bioorganic Chemistry, Chiba Institute of Technology, Tsudanuma, Narashino-shi, Chiba 275, Japan*

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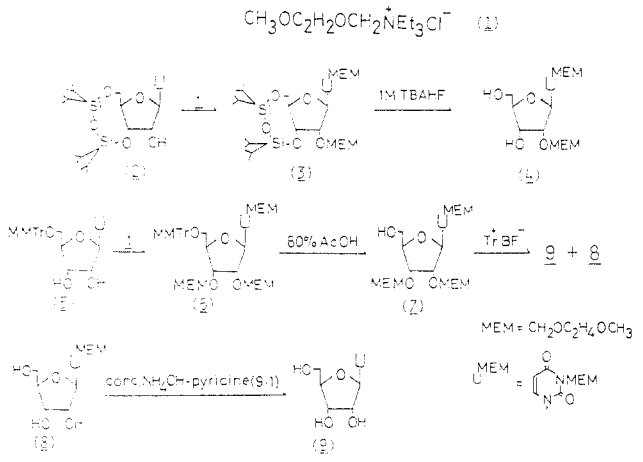
Synthesis of oligonucleotides containing uridine has revealed the occurrence of side reactions involving the amide function of the uracil residue during phosphorylating and coupling reactions. Many workers have re-

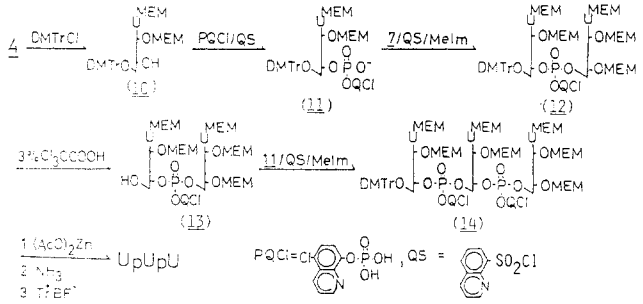
(1) This manuscript represents part 34 in a series on oligonucleotide synthesis. For the previous paper this series, see: Imai, K.; Ito, T.; Kondo, S.; Takaku, H. *Nucleosides Nucleotides* **1985**, *4*, 669.

0022-3263/86/1951-0931$01.50/0 

**Scheme I**



**Scheme II**



ported[2] protecting groups for the uracil residue in order to overcome these side reactions. More recently, we found that the (methoxyethoxy)methyl group is an easily introduced protecting group for the uracil residue which can be removed under mild conditions.[3]

In this paper, we report the use of the (methoxyethoxy)methyl (MEM) group[4] as a new amido and hydroxyl protecting group for uridine.

The MEM group can be introduced selectively onto the $N^3$-position of the uracil moiety of 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine (**2**)[5] by treatment with (methoxyethoxy)methyl chloride (MEM-Cl) in the presence of triethylamine. Unfortunately, $N^3$,$O^2$-bis[(methoxyethoxy)methyl]uridine (**4**), envisioned as an important starting material for oligoribonucleotides containing uridine units, could not be obtained in this way. However, the triethylammonium salt of **1**[4] effects introduction of the MEM group onto both the amido and the hydroxyl group of uridine. MEM-Cl (8 molar equiv) was added dropwise to a solution of triethylamine (8 molar equiv) in dry THF over a period of 10 min and allowed to stand at 20 °C. After 16 h, the silylated nucleoside **2** (1 molar equiv) was added to the mixture and heated under reflux. After 16 h, compound **3** was converted into desilylated compound **4** in 92% yield by treatment with 1 M tributylammonium fluoride (TBAHF) for 4 h. The 3′-terminal nucleoside **8** was similarly prepared from the reaction of **1** and 5′-*O*-(monomethoxytrityl)uridine (**5**). The tritylated nucleoside **5** (1 molar equiv) was treated with 1 prepared from MEM-Cl (14 molar equiv) and triethylamine (14 molar equiv) by heating in dry THF at reflux to afford the corresponding fully protected nucleoside **6**, which was further converted to the detritylated product **7** in 79% yield by treatment with 80% AcOH. When **2** was exposed to MEM-Cl in the presence of diisopropylethylamine at room temperature, the yield of **4** was not satisfactory.

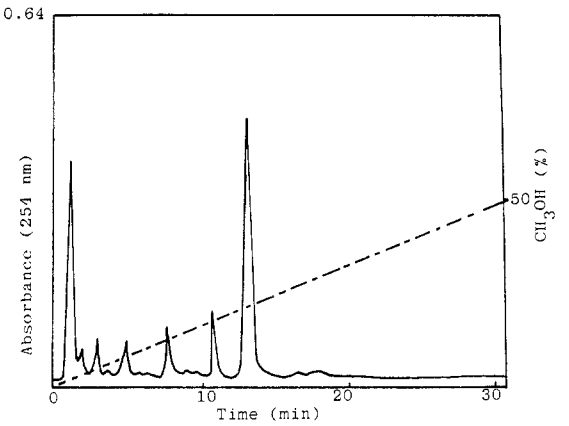



**Figure 1.** Reverse phase HPLC of UpUpU on Finepack $C_{18}$ (4.6 × 250 mm). Elution was performed with a linear gradient of MeOH (0–50%) in 0.05 M ammonium phosphate (pH 7.0) over 32 min at flow rate of 1 mL/min.

In a previous paper,[3] we reported that the *N*-MEM protecting group was stable under standard operations required for oligonucleotide synthesis and can be removed by the action of triphenylmethyl fluoroborate (TrBF). We have now found that concentrated ammonia–pyridine (9:1, v/v) is much more effective for the removal of the *N*-MEM protecting group than TrBF. Thus, removal of the MEM group from $N^3$-[(methoxyethoxy)methyl]uridine (**8**)[3] was carried out by treatment with concentrated ammonia–pyridine (9:1 v/v) at 50 °C for 5 h to give uridine in 94% yield (Scheme I). Treatment of **1** with TrBF in $CH_3CN$–$H_2O$ (4:1 v/v) at room temperature for 2 h gives **9** and **8** in 85% and 10% yields, respectively. The nucleoside derivative **7** when subject to basic and acidic conditions (0.2 N NaOH in dioxane–MeOh, 15:5, v/v, 2 h, or hydrochloric acid, pH 2.0, 24 h) at 20 °C was recovered quantitatively.

The utility of these nucleotides (**4** and **7**) can be demonstrated by the synthesis of UpUpU (**14**). Nucleoside **4** was treated with DMTrCl for 2 h to give the tritylated product **10** in 91% yield. The compound **10** was phosphorylated with 5-chloro-8-quinolyl phosphate (PQCl)[6] in the presence of 8-quinolinesulfonyl chloride (QS-Cl)[7] in dry pyridine for 2 h to give the triethylammonium salt of **11** in 88% yield (Scheme II). The 3′-phosphate component (**11**) and 5′-hydroxyl component (**7**) were coupled by using QS-Cl and *N*-methylimidazole (MeIm) in dry pyridine at room temperature.[8] The condensation reaction was completed in 1.5 h and the usual workup gave the desired dimer (**12**) in 83% yield. Further, the fully protected trimer (**14**) was prepared in 87% yield according to our original method using 3% TCA[9] in $CH_3NO_2$–MeOH

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(95:5, v/v) for selective removal of the DMTr group from **12**. During these condensations, we did not observe any side reactions.

Removal of all the protecting groups from **14** was performed as follows: The trimer **14** was deprotected by using first zinc acetate in aqueous pyridine at room temperature for 24 h to cleave the 5-chloro-8-quinolyl group, second with concentrated ammonia at 50 °C for 4 h to remove the MEM groups, and finally with TrBF in $CH_3CN$–$H_2O$ (4:1, v/v) at room temperature for 2 h to split off the MEM and the DMTr groups. The deblocked trimer UpUpU was isolated in 69% (98 OD) yield after separation by HPLC on Finepack C-18 column (Figure 1). The trimer was completely degraded by nuclease P1 to U and pU in the ratio of 1.00:2.01.

In conclusion the MEM group is a suitable protecting group for both the amide and the hydroxyl groups of uridine, because the MEM group can be introduced easily to both the amide and the hydroxyl groups of uridine and the MEM group is removed rapidly under mild conditions. Since the MEM protected uridine derivatives are stable to acid and alkali, they can be used for the synthesis of oligouridylate by the phosphotriester approach, and the yield of oligouridylate is dramatically high.

## Experimental Section

Ultraviolet spectra were recorded on a Shimazu UV-200 spectrometer. $^1H$ NMR spectra were recorded on a Hitachi R-24B spectrometer. Thin layer chromatography (TLC) was performed on precoated TLC plates of silica gel 60 F-254 (Merck Art. No. 5715) and the $R_f$ values of the protected nucleoside and nucleotide derivatives were measured after development with solvent A ($CH_2Cl_2$–MeOH, 9:1, v/v). Column chromatography was performed with silica gel C-200 purchased from Wako Co. Ltd. Paper chromatography was performed by the descending technique with Whatman 3 MM paper using solvent B (1-PrOH–concentrated $NH_4OH$–$H_2O$, 55:10:35, v/v). HPLC was performed on a Finepack C-18 reverse phase column using elution with a linear gradient of 0–50% MeOH in 0.05 M ammonium phosphate (pH 7.0) over 32 min at a flow rate of 1.0 mL/min. Pyridine was distilled twice from *p*-toluenesulfonyl chloride and from $CaH_2$ and then stored over molecular sieve (4 Å). THF was distilled from $LiAlH_4$ and stored over sodium wire and molecular sieve (4 Å). 8-Quinolinesulfonyl chloride (QS-Cl) was purchased from Aldrich Chemical Co. Nuclease P1 was purchased from Yamasa Co.

**$N^3$,$O^{2'}$-Bis[(methoxyethoxy)methyl]uridine (4).** MEM-Cl (1.62 g, 12 mmol) was added dropwise to a solution of triethylamine (0.88 mL, 12 mmol) in dry THF (6 mL) over a period of 10 min and allowed to stand at 20 °C. After 16 h, 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)uridine (**2**) (739 mg, 1.5 mmol) was added to the mixture and heated under reflux for 16 h. The mixture was quenched with ice–water (2 mL) and extracted with $CH_2Cl_2$ (40 mL). The organic layer was dried with $Na_2SO_4$ and evaporated in vacuo. The residue was dissolved in THF (1.5 mL) and the solution was treated with 1 M tributylammonium fluoride (TBAHF) (3 mL) at room temperature for 3 h. The solution was diluted with pyridine–water–methanol (3:1:1, v/v, 30 mL) and treated with Dowex 50W-X2 (pyridinium form). The resin was removed by filtration and the filtrate was evaporated in vacuo. The residue was dissolved in $CH_2Cl_2$ and applied to silica gel column (3 × 15 cm). The column was eluted with 200 mL of $CH_2Cl_2$ followed by a stepwise gradient (1 L) of MeOH (0–3%) in $CH_2Cl_2$. The appropriate fractions were evaporated to give **4** in 92% (570 mg) yield: $R_f$ 0.36 (solvent A); UV $\lambda_{max}$ (MeOH) 263 nm, $\lambda_{min}$ 231 nm; $^1H$ NMR ($Me_2SO$-$d_6$) $\delta$ 8.04 (d, 1 H, $J_{5,6}$ = 7 Hz, 6-H), 5.95 (d, 1 H, $J_{1',2'}$ = 5 Hz, 1′-H), 5.80 (d, 1 H, $J_{5,6}$ = 7 Hz, 5-H), 5.27 (s, 2 H, $CH_2$), 4.25–3.80 (m, 3 H, 2′-H, 3′-H, and 4′-H), 3.70–3.30 (m, 10 H, $C_2H_4$ × 2, 5′-H), 3.23 (s, 6 H, $OCH_3$ × 2). Anal. Calcd for $C_{17}H_{28}O_{10}N_2 \cdot {}^1/_2CH_3OH$: C, 48.16; H, 6.93; N, 6.42. Found: C, 48.29; H, 6.89; N, 6.40.

**$N^3$,$O^{2'}$,$O^{3'}$-Tris[(methoxyethoxy)methyl]uridine (7).** To a dry THF (20 mL) solution of **1** (42 mmol) prepared from the above described method was added **5** (1.55 g, 3.0 mmol), and it was heated under reflux for 12 h. The mixture was quenched with ice–water (6 mL) and extracted with $CH_2Cl_2$ (60 mL). The $CH_2Cl_2$ extract was dried with $Na_2SO_4$ and evaporated in vacuo. The residue was treated with 80% AcOH (25 mL) at room temperature. After 6 h, the solution was evaporated to dryness under reduced pressure. The residue was dissolved in $CH_2Cl_2$ and chromatographed on a silica gel column (3 × 15 cm) with $CH_2Cl_2$–MeOH (97:3, v/v) to give **7** (1.22 g, 79%), $R_f$ 0.41 (solvent A); UV $\lambda_{max}$ (MeOH) 264 nm; $^1H$ NMR ($Me_2SO$-$d_6$) $\delta$ 8.01 (d, 1 H, $J_{5,6}$ = 8 Hz, 6-H), 5.93 (d, 1 H, $J_{1',2'}$ = 4 Hz, 1′-H), 5.22 (m, 3 H, $CH_2$ and 5′-OH), 4.71 (s, 4 H, $CH_2$ × 2), 4.40–4.00 (m, 3 H, 2′-H, 3′-H, and 4′-H), 3.70–3.30 (m, 14 H, $C_2H_4$ × 2 and 5′-H), 3.23 (s, 9 H, $OCH_3$ × 3). Anal. Calcd for $C_{21}H_{36}O_{12}N_2 \cdot {}^1/_2H_2O$: C, 48.74; H, 7.21; N, 5.41. Found: C, 48.67; H, 7.31; N, 5.31.

**Tritylation of 4.** Compound **4** (1.02 g, 2.5 mmol), precoevaporated with dry pyridine, was dissolved in dry pyridine (12 mL) and treated with DMTrCl (1.35 g, 4.0 mmol) for 1 h. The usual workup gave **10** (1.66 g, 95%): $R_f$ 0.41 (solvent) A; $^1H$ NMR ($CDCl_3$) $\delta$ 7.90 (d, 1 H, $J_{5,6}$ = 8 Hz, 6-H), 7.39–6.75 (m, 14 H, Ar), 6.00 (d, 1 H, $J_{1',2'}$ = 4 Hz, 1′-H), 5.43 (s, 2 H, $CH_2$), 5.35 (d, 1 H, $J_{5,6}$ = 8 Hz, 5-H), 4.95 (d, 2 H, $CH_2$), 3.80 (s, 6 H, $OCH_3$ × 2), 3.38 (s, 6 H, $OCH_3$ × 2).

**Synthesis of the Fully Protected Dimer 12 and Trimer 14.** The tritylated uridine (**10**) (1.66 g, 2.3 mmol) was treated with PQCl (0.90 mg, 3.5 mmol) in the presence of QS-Cl (1.57 g, 7.0 mmol) in dry pyridine (23 mL) at room temperature for 2 h. After the usual workup, the triethylammonium salt of **11** (2.16 g, 88%) was purified by precipitation from its $CH_2Cl_2$ solutions to hexane–ether (95:5, v/v) and used as the 3′-phosphodiester component in the subsequent condensation without purification. The 3′-phosphodiester **11** (960 mg, 0.90 mmol) was treated with **7** (340 mg, 0.67 mmol) in the presence of QS-Cl (560 mg, 2.5 mmol) and MeIm (0.16 mL, 2.5 mmol) in dry pyridine (5 mL) for 1.5 h. The usual workup followed by chromatography gave **12** (1.09 g, 83%): $R_f$ 0.47 (solvent A); UV $\lambda_{max}$ (MeOH) 300 (sh), 264, 234 nm, $\lambda_{min}$ 249 nm.

The dimer **12** (1.09 g, 0.75 mmol) was treated with 3% TCA in $CH_3NO_2$–MeOH (95:5, v/v, 15 mL) at room temperature for 5 min. The mixture was quenched with pyridine and extracted with $CH_2Cl_2$ (25 mL). The usual workup followed by chromatography gave **13** (742 mg, 87%), $R_f$ 0.41 (solvent A). A solution of **11** (930 mg, 0.87 mol) and **13** (660 mg, 0.58 mmol) in dry pyridine (1.8 mL) was then condensed in the presence of QS-Cl (499 mg, 2.2 mmol) and MeIm (0.18 mL, 2.2 mmol) for 1.5 h. The usual workup followed by chromatography gave **14** (1.09 g, 89%): $R_f$ 0.45 (solvent A); UV $\lambda_{max}$ (MeOH) 300 (sh), 263, 234 nm, $\lambda_{min}$ 245 nm.

**Removal of All Protecting Groups from 14.** To a solution of **14** (105 mg, 50 μmol) in pyridine–water (9:1, v/v, 4 mL) was added zinc acetate (702 mg, 3.2 mmol). The mixture was stirred at room temperature for 24 h and then was treated with Dowex 50W-X2 (pyridinium form, 20 mL). The resin was removed by filtration and the filtrate was concentrated. To the residue was added concentrated ammonia (5 mL), and the mixture was kept 50 °C for 4 h. The solution was concentrated and the residue was treated with $Tr^+BF_4^-$ (330 mg, 1.0 mmol) in $CH_3CN$–$H_2O$ (4:1, v/v, 1 mL) at room temperature for 2 h. The solution was evaporated in vacuo and the residue was dissolved in 0.1 M TEAB solution (2 mL) and washed with ether. The solution was concentrated to an oil. The deblocked trimer UpUpU was isolated in 69% (98 OD) yield after separation by HPLC on Finepack C-18 column (Figure 1). UpUpU was characterized base composition analysis by paper chromatography after complete digestion with nuclease P1.[11] The ratio of U/pU was 1.00:2.01: UV $\lambda_{max}$ ($H_2O$, pH 7.0) 262 nm, $\lambda_{min}$ 231 nm.

**Registry No.** **1**, 60043-43-8; **2**, 69304-38-7; **3**, 99328-69-5; **4**, 99328-70-8; **5**, 51600-12-5; **6**, 99328-77-5; **7**, 99343-92-7; **10**, 99328-73-1; **11**, 99328-74-2; **11** (triethylammonium salt), 100190-49-6; **12**, 99363-39-0; **13**, 99328-75-3; **14**, 99328-76-4; **14** (deprotected phosphate), 100205-39-8; UpUpU, 3152-53-2.

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