2′,3′,5′-Tri-O-benzoyl[4-13C]uridine. An Efficient, Regiospecific Synthesis of the Pyrimidine Ring[1]

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A synthesis of 2′,3′,5′-tri-O-benzoyl[4-13C]uridine is described in which sodium [1-13C]acetate is converted to the protected nucleoside in ten steps with an overall yield of 81%. The route is general and permits the regiospecific introduction of carbon or nitrogen into one or more positions in the pyrimidine ring.

Uracil and uridine are commonly used intermediates in chemical and biological syntheses. Although short, moderate-yield routes to these compounds are available,[3-8] there are no general high-yield, regiospecific approaches suitable for introducing isotopic labels into the pyrimidine ring. For example, the traditional one-step synthesis of uracil by the acid-catalyzed condensation of malic acid and urea gives an acceptable yield of ~60%,[5] but only permits C(2) to be conveniently labeled regiospecifically from commercially available, isotopically enriched material.[9] Longer, regiospecific routes such as the procedure developed by Shaw and coworkers[10] for building the pyrimidine ring onto the nitrogen atom of the amino sugar[11] or the stepwise construction of uracil from acetic acid suffer from several steps with unacceptably low yields. After surveying the literature, we decided that the pyrimidine ring could best be constructed via a condensation of potassium cyanate and β-alanine,[12] especially if the yields for the reduction of cyanoacetic acid (**3**) to β-alanine (**4**)[3,13,14] and conversion of dihydrouracil (**5**) to uracil (**8**)[3,7,15] could be improved, since each of the ring atoms in the base can be introduced unambigously. This has been accomplished, and we now report a ten-step synthesis of uridine tribenzoate from acetic acid which permits the atoms in the pyrimidine ring to be labeled selectively and proceeds in an overall yield of 81%.

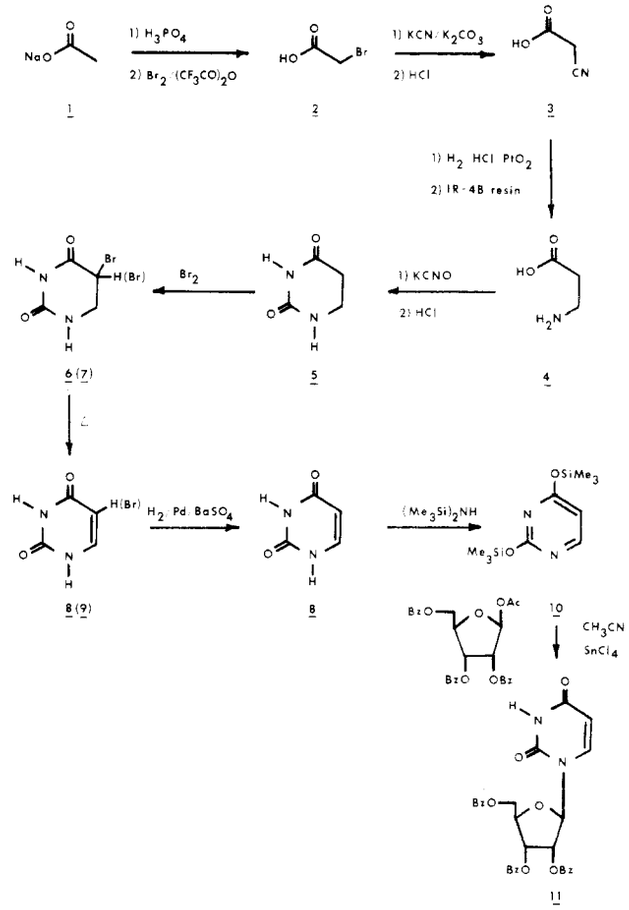
Results and Discussion

Synthesis. In selecting a synthetic route to 2′,3′,5′-tri-O-benzoyl[4-13C]uridine, we decided to use the sequence of reactions shown in Scheme I because of its versatility for labeling the carbons and nitrogens in the pyrimidine ring regiospecifically. C(4) and C(5) in the uracil moiety are derived from C(1) and C(2) in acetic acid, C(6) and N(1) from potassium cyanide, and C(2) and N(3) from potassium cyanate. The three building blocks are simple compounds and are available commercially with 13C and 15N in any position.

Sodium [1-13C]acetate was selected as the starting material because the free acid is more expensive than its sodium salt. Although generation of acetic acid by treatment of sodium acetate with phosphoric acid[16] gave material containing a small amount of water, the "wet" acetic acid could be used in the bromination step to give [1-13C]bromoacetic acid (**2**) in an overall yield of 95%. In contrast to the recent report by Climie and Evans,[17] we found that the bromination proceeded smoothly in trifluoroacetic anhydride with a catalytic amount of phosphorus tribromide. The conversion of **2** to cyanoacetic acid (**3**) was quantitative.

Several procedures have been used to reduce cyanoacetic acid (**3**) to β-alanine (**4**), including reductions with Raney nickel[7,13,14,18] and palladium,[3] but the yields were unacceptable for our purposes. Previous workers[3,18] used large ratios of catalyst to cyanoacetic acid in the reduction; however, in our hands **3** was not completely consumed in the presence of palladium after 7 days at 50 psi, even when additional "catalyst" was added periodically. Alternatively we found that the nitrile was cleanly reduced to the desired amino acid in 16 h at 50 psi with Adam's catalyst. By carrying out the reduction in the presence of hydrochloric acid, it was possible to suppress the side reactions which are common during reduction of cyanides to primary amines[19] and to obtain a quantitative yield of β-alanine. Conversion of β-alanine to dihydrouracil proceeded smoothly, according to the procedure developed by Lengfield and Stieglitz.[12]

Scheme I. Synthesis of 2′,3′,5′-Tri-O-benzoyl[4-13C]uridine



The bromination–dehydrobromination sequence reported by Gabriel[15] appeared to be a logical procedure for introducing the C(4)–C(5) double bond. However, we found that it was impossible to carry out the bromination of dihydrouracil (**5**) under conditions where **5** was completely consumed without formation of at least 10% of the dibromo derivative **7**.[20] This problem was solved by deliberately overbrominating **5** and carrying out the thermal elimination of hydrogen bromide on **6** and **7**. The resulting mixture of **8** and **9** was then treated with hydrogen in the presence of palladium on barium sulfate.[21] Under these conditions, 5-bromouracil (**9**) was smoothly converted to uracil (**8**), which was resistant to hydrogenation. In this way, dehydrogenation of dihydrouracil was carried out

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Table I

Compd[f]	Assignment	Chemical shifts, ppm[c] δ ^{1}H	Chemical shifts, ppm[c] δ ^{13}C	Coupling constants, Hz[d] J_{HH}	$^2J_{^{13}CCH}$	$^3J_{^{13}CCCH}$	$^1J_{^{13}CC}$
[1-^{13}C]Bromoacetic acid[a] (2)	$-CH_2-$	3.90 (d)	27.83 (d)		4.6		62.4
	$-^{13}CO_2H$		168.06				
[1-^{13}C]Cyanoacetic acid[a] (3)	$-CH_2$	3.80 (d)	24.50 (d)		8.0		57.6
	$-^{13}CO_2H$		165.26				
[1-^{13}C]-3-Aminopropionic acid[a] (4)	$-^{13}CO_2H$		175.85				
	C(2)	2.60 (d of t)	32.94 (d)	6.5	5.1	6.3	50.5
	C(3)	3.21 (d of t)	35.93				
[4-^{13}C]-5,6-Dihydrouracil[a] (5)	N(1)	7.40 (br s)		2.6 ($J_{1,6}$)			
	C(2)		173.22				
	N(3)	9.83 (br s)					
	^{13}C(4)		170.87				
	C(5)	2.43 (d of t)	30.38 (d)	6.7	6.3	6.6	48.0
	C(6)	3.21 (d of t)	35.32	($J_{5,6}$)			
[4-^{13}C]Uracil[a] (8)	N(1), N(3)	10.90 (br s)					
	C(2)		151.43				
	^{13}C(4)		164.25				
	C(5)	5.46 (d of d)	100.15 (d)	7.6	1.9	10.6	65.1
	C(6)	7.33 (d of d)	142.09				
2′,3′,5′-Tri-*O*-benzoyl[4-^{13}C]-uridine[b,e] (11)	C(2)		150.07				
	N(3)	9.13					
	^{13}C(4)		162.49				
	C(5)	5.57 (d of d)	103.39 (d)	8.1	1.0	10.8	65.9
	C(6)	7.73 (d of d)	139.60				

[a] Me_2SO-d_6. [b] $CDCl_3$. [c] Shifts relative to Me_4Si, letters in brackets refer to coupling patterns observed. [d] Coupling values are estimated to be accurate within 0.2 Hz. [e] The rest of the ^{1}H and ^{13}C NMR spectra for the sugar moiety are as follows: ^{1}H NMR 4.70 (3, m, C(5′) and C(4′) protons), 5.73 (2, m, C(2′) and C(3′) protons, $J_{1'2'}$ = 5.3 Hz), 6.20 (1, d, C(1′), $J_{1'2'}$ = 5.3 Hz), 7.20–7.53 (9, m, *m*- and *p*-benzoate protons), 7.77–8.07 (6, m, *o*-benzoate protons), 9.07 (1, br s, N(3) proton); ^{13}C NMR 63.73 (C(5′)), 73.77 (C(2′)), 80.64 and 88.33 (C(4′) and C(1′)), 128.60 and 129.91 (ortho and meta carbons of benzoates at C(2′) and C(3′)), 128.86 and 129.71 (ortho and meta carbons of benzoate at C(5′)), 133.74 (*p*-benzoate carbons), 165.36 (carbonyl of benzoates at C(2′) and C(3′)), 166.06 (carbonyl of benzoate at C(5′)). [f] Registry no.: **2**, 57858-24-9; **3**, 65138-33-2; **4**, 65138-34-3; **5**, 65138-35-4; **8**, 61101-07-3; **11**, 65102-76-3.

in an excellent overall yield. Since we were interested in obtaining the nucleoside, we found it advantageous to convert dihydrouracil (**5**) to the trimethylsilyl derivative[22] of uracil (**10**) without purification at intermediate stages. **10** can be easily purified by distillation and used directly in the stannic chloride fusion[23,24] with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranoside.[25] We also found that it was more efficient to purify uracil via hydrolysis of the bistrimethylsilyl derivative (**10**) than by the usual crystallization or chromatography procedures. When conversion of **10** to **11** was carried out in acetonitrile, the reaction with the sugar was highly selective for the N(1) position of the pyrimidine.[23] Pure 2′,3′,5′-tri-*O*-benzoyl[4-^{13}C]uridine was obtained by medium-pressure chromatography on silica gel.

NMR Spectra. Chemical shifts (^{1}H and ^{13}C) and coupling constants for all of the labeled compounds that we prepared are listed in Table I. Assignments were based on coupling patterns, nuclear Overhauser enhancements, and comparisons with other compounds.[26,27,28]

Two- and three-bond ^{13}C–^{1}H coupling constants were obtained from ^{1}H spectra and confirmed by an analysis of the corresponding proton-coupled ^{13}C spectra.[29] Few values for the type of compounds listed in Table I are reported in the literature because of experimental problems involved in obtaining the data from ^{13}C in natural abundance. However, the coupling constants measured in this study are in agreement with trends reported by others.

Two-bond ^{13}C–^{1}H couplings between an sp^2-hybridized carbon, in particular a carbonyl carbon, and a proton attached to an sp^3-hybridized carbon usually lie between 4.0 and 8.0 Hz.[30] This range encompasses the values found for compounds **2–5**. Two-bond ^{13}C–^{1}H couplings where both carbons are sp^2 hybrids are noticably smaller (1.0–4.0 Hz),[27,32,33] as is seen for uracil (**8**) and 2′,3′,5′-tri-*O*-benzoyluridine (**10**).

Generally three-bond ^{13}C–^{1}H coupling constants, where the carbon atom is in a carbonyl group, are comparable to or greater than the corresponding two-bond couplings.[30,31] However, values for three-bond couplings can vary considerably depending on hybridization, conformation, and the electronegativity of attached substituents.[32,34] Coupling constants reported in the literature range from 0.7 to 16 Hz, with the maximum values found for conformations where the C(2)–C(3) and C(1)–H dihedral angle is 180°. Larger couplings are found between a carbonyl carbon and a β-hydrogen on an sp^2-hydribized carbon where the dihedral angle is ~180° and range from 7 to 16 Hz.[27,32] In comparison, we found $^3J_{^{13}C-^1H}$'s of 10.6 and 10.8 for **8** and **10**, respectively. If the β carbon is sp^3 hybridized, the three-bond coupling is usually much smaller because of changes in electronegativity and conformation.[34] Thus, values of $^3J_{^{13}C-^1H}$ for **4** and **5** are significantly smaller when compared to those for **8** and **10**. Also, it is interesting to note that $^3J_{^{13}C-^1H}$ for **4** and **5** are almost identical. Since one would expect the heterocycle and the amino acid to adopt similar conformations, the fact that β-alanine is zwitterionic implies that the charged carboxylate and ammonium moieties have little effect on $^3J_{^{13}C-^1H}$.

The carbon–carbon one-bond couplings shown in Table I fall within the range reported for $^1J_{^{13}C-^{13}C}$ in compounds where one of the carbons is in a carbonyl group.[28] We did not see any $^2J_{^{13}C-^{13}C}$ couplings and conclude that their magnitudes were all less than ~1 Hz.

Conclusion

Since 2′,3′,5′-tri-*O*-benzoyluridine (**11**) is a precursor for the synthesis of a large number of biologically important pyrimidine nucleosides such as 4-thiouridine,[35,36] 2-thiouridine,[37] 2,4-dithiouridine,[36] cytidine,[35,38] and 2-thiocytidine,[38] this approach serves as a convenient, high-yield, regiospecific

entry into these compounds. We are presently using this approach to synthesize [1-^{15}N]- and [3-^{15}N]uracil.

Experimental Section

General. Boiling and melting points are uncorrected. ^{1}H NMR spectra were recorded on a Varian Associates Model EM-390 spectrometer. ^{13}C NMR spectra were recorded on a Varian Associates Model XL-100-15 spectrometer. The chemical shifts given are δ values in parts per million downfield relative to Me_4Si. Unless otherwise indicated, the purity of the reactions was determined by spectral comparison (NMR) with an unlabeled authentic sample, and isotopic purity was checked at each stage by careful integration of the expanded portion of the ^{1}H spectrum where $^2J_{^{13}C-^1H}$ couplings were observed. Isotopic enrichment (90 ± 1%) was also confirmed by mass spectral analysis. Sodium [1-^{13}C]acetate (90% isotopic enrichment) was obtained from Koch Isotopes. The ^{1}H NMR and ^{13}C NMR spectra are recorded in Table I.

The yields reported for these optimized procedures were duplicated at least five times with unlabeled material before committing labeled material to the sequence, and yields of the individual steps were reproducible to within 1%. In addition, two batches of labeled material were brought through the sequence with the same overall yield.

[1-^{13}C]Acetic Acid (12). A 50-mL recovery flask, fitted with a magnetic stirring bar, was charged with 25.95 g (0.183 mol) of phosphorus pentoxide and capped with a rubber septum. Air (20 cm^3) was removed via a syringe to create a partial vacuum, and 10.0 g (0.555 mol) of water was slowly added via a syringe to the cooled flask (ice bath) at a rate such that no appreciable buildup in pressure was observed. After addition was complete, the flask was heated (oil bath) at 70 °C until all the phosphorus pentoxide had dissolved (approximately 2 h). The contents of the flask were allowed to cool and 7.50 g (90.4 mmol) of fused anhydrous sodium [1-^{13}C]acetate was added to the flask. After the flask was fitted with a short-path distillation apparatus, the oil bath was heated to 210 °C and the [1-^{13}C]acetic acid (bp 103–105 °C) was collected in a tared receiver. The reaction flask was flamed *gently* with a bunsen burner to drive over the last of the acetic acid (this drives over a small amount of water which can be carried over into the bromination step), giving 6.2 g (theoretical 5.5 g) of distillate. Therefore, assuming quantitative collection of acetic acid (confirmed by NMR analysis of the remaining phosphoric acid residue), the distillate contained 0.7 g (39 mmol) of water. This "wet" acetic acid was used directly in the bromination reaction.

[1-^{13}C]Bromoacetic Acid (2). (A) From "Wet" [1-^{13}C]Acetic Acid. Trifluoroacetic anhydride (44.90 g, 0.214 mol) was carefully added to the cooled (ice bath) [1-^{13}C]acetic acid from the previous experiment. (*Caution.* The reaction is very exothermic due to hydrolysis of some of the trifluoroacetic anhydride by the water in the acetic acid and due also to the formation of the mixed anhydride. Since this anhydride is low boiling, care must be taken to prevent any loss. It is recommended that the trifluoroacetic anhydride be slowly added to the magnetically stirred acetic acid by way of a dropping funnel, protected with a dry ice condenser and drying tube.) After addition of trifluoroacetic anhydride was complete, 0.40 g (1.5 mmol) of phosphorus tribromide was added to the reaction mixture and the dropping funnel was charged with 14.43 g (90.2 mmol) of bromine. The reaction flask was then heated with an oil bath (maintained at 60 °C). When trifluoroacetic anhydride started to reflux, bromine was slowly added to the reaction flask at a rate such that a pale bromine color is just maintained in the flask. After addition was complete and the bromine color had been completely discharged (6–7 h),[39] 3.39 g (0.188 mol) of water was carefully added to the cooled reaction mixture (*caution:* vigorous reaction). This amount of water represents the theoretical amount[40] required to hydrolyze all of the anhydrides and phosphorus tribromide left at the end of the reaction. Trifluoroacetic acid (along with hydrogen bromide) was removed by distillation and the remaining colorless liquid solidified on standing to afford [1-^{13}C]bromoacetic acid (mp 46–48 °C). The distillate, after evaporation at room temperature under a slow stream of nitrogen, afforded a small amount of [1-^{13}C]bromoacetic acid which had azeotroped with solvent.[41] The total yield of [1-^{13}C]bromoacetic acid was 11.81 g (85.7 mmol, 95%).

(B) From Anhydrous Acetic Acid.[42] Trifluoroacetic anhydride (25 g, 0.119 mol) was carefully added to 2.40 g (40 mmol) of acetic acid and the mixture was allowed to cool to room temperature. To the magnetically stirred solution 6.40 g (40 mmol) of bromine was slowly added, and stirring was continued at room temperature for 22 h, by which time all the bromine color had been dissipated. The mixture was cooled, hydrolyzed with 2.25 g (0.125 mol) of water, and worked up as before, giving 5.56 g (40 mmol, 100%) of bromoacetic acid.

[1-^{13}C]Cyanoacetic Acid (3). A stirred solution of 2.78 g (20 mmol) of [1-^{13}C]bromoacetic acid in water (6 mL) was carefully neutralized with 1.49 g (11 mmol) of anhydrous potassium carbonate, followed by slow addition of 1.40 g (21.5 mmol) of potassium cyanide in water (5 mL). The mixture was allowed to stir at ambient temperature (22 °C) for 15 min and heated at 60 °C (oil bath) for 25 min. The solution was cooled to 10 °C and acidified by the addition of 3.63 mL of an 18.5% aqueous solution (21.8 mmol) of hydrochloric acid.

The product was extracted with diethyl ether in a continuous extractor for 72 h, the ether extract was dried over anhydrous sodium sulfate, and solvent was removed to afford 1.71 g (20 mmol, 100%) of [1-^{13}C]cyanoacetic acid. NMR analysis indicated that no [1-^{13}C]-bromoacetic acid remained.

[1-^{13}C]-β-Alanine Hydrochloride, [1-^{13}C]-3-Aminopropionic Acid Hydrochloride (13). To a solution of 1.70 g (20 mmol) of [1-^{13}C]cyanoacetic acid in water (25 mL) was added 6 mL of concentrated hydrochloric acid. The mixture was hydrogenated at 50 psi over platinum oxide (0.25 g) and the theoretical uptake of hydrogen was realized after 16 h.

The catalyst was filtered off and washed with water (4 × 15 mL). The filtrate and washings were combined and water was removed at reduced pressure giving 3.0 g of a pale yellow solid. (The theoretical yield was 2.47 g; thus, not all the water had been removed.)

[1-^{13}C]-β-Alanine, [1-^{13}C]-3-Aminopropionic Acid (4). The hygroscopic [1-^{13}C]-β-alanine hydrochloride from the reduction was dissolved in deionized water (40 mL) and applied to 25 mL of IR-4B resin [previously washed successively with 1% aqueous hydrochloric acid (250 mL), 1% aqueous sodium hydroxide, and finally deionized water (500 mL)]. The free amino acid was eluted from the column with deionized water (250 mL). Removal of water under reduced pressure gave 1.78 g (100%) of a pale yellow solid.

[4-^{13}C]-5,6-Dihydrouracil, [4-^{13}C]-5,6-Dihydro-2,4-dihydroxypyrimidine (5). To a solution of 1.78 g (20 mmol) of [1-^{13}C]-β-alanine in water (20 mL) was added 1.64 g (20 mmol) of potassium cyanate dissolved in 20 mL of water. The solution was *slowly* evaporated to dryness by heating in an oil bath (100 °C) under a stream of nitrogen. A syrupy residue, consisting of the potassium salt of [1-^{13}C]-β-ureidopropionic acid, solidified on standing. This potassium salt was acidified with 40 mL of 18% hydrochloric acid (6 N), the solution was evaporated to dryness, and the residue was heated at 170 °C for 30 min. The solid residue was thoroughly washed with 5-mL portions of water until all of the potassium chloride had been removed (negative silver nitrate test), and the residue was dried over phosphorus pentoxide to afford 1.85 g of [4-^{13}C]-5,6-dihydrouracil. The water washings were continuously extracted with ether for 72 h to afford a further 0.42 g (100%) of crude [4-^{13}C]-5,6-dihydrouracil.

(Bis-O-trimethylsilyl)[4-^{13}C]uracil, 2,4-Bis(trimethylsiloxy)[4-^{13}C]pyrimidine (10). A magnetically stirred suspension of 1.14 g (10 mmol) of [4-^{13}C]-5,6-dihydrouracil in 15 mL of glacial acetic acid was heated in an oil bath (maintained at 105 °C) until all the solid had dissolved. The flask was fitted with a dropping funnel and protected with an efficient dry-ice condenser. The dropping funnel was charged with 2.40 g (15 mmol) of bromine dissolved in 10 mL of glacial acetic acid and the solution was added slowly to the solution of dihydrouracil at 105 °C at such a rate to just maintain the bromine color. After all of the bromine color had been dissipated (3–4 h), the solvent was removed on a rotary evaporator under reduced pressure to afford a white solid. ^{1}H NMR analysis confirmed the absence of any starting dihydrouracil and showed the solid to contain 5-bromodihydrouracil and 5,5-dibromodihydrouracil in approximately equal proportions. The solid mixture was heated at 210 °C (oil bath, preheated) for 25 min under a stream of nitrogen to assist in the removal of the hydrogen bromide generated during the elimination, giving a mixture of [4-^{13}C]uracil and [4-^{13}C]-5-bromouracil. The mixture was dissolved by gentle heating in 100 mL of 50% aqueous ethanol and the resulting solution was hydrogenated over 1.0 g of 5% palladium on barium sulfate for 5 h (theoretical uptake was complete after 2 h). The solution was heated to boiling and the catalyst was removed by filtration and washed with hot water (4 × 15 mL). The combined filtrate and washings were evaporated to dryness under reduced pressure to afford crude [4-^{13}C]uracil (1.10 g, 99%) as a pale yellow solid. The [4-^{13}C]uracil was silylated by heating a stirred suspension in hexamethyldisilazane (8 mL) at reflux under a dry nitrogen atmosphere for 16 h. The solution was concentrated at reduced pressure [90 °C (50 mm)] before the vacuum was lowered to 7 mm to remove the remaining solvent. The residue was distilled to afford 2.40 g (94% from dihydrouracil) of 2,4-bis(trimethylsiloxy)[4-^{13}C]pyrimidine [bp 125 °C (5 mm) (lit.[22] 135 °C (7 mm))] as a colorless liquid (extremely moisture sensitive, collected and stored under dry nitrogen).

2′,3′,5′-Tri-*O*-benzoyl[4-^{13}C]uridine, 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-2,4-dihydroxy[4-^{13}C]pyrimidine (11). To a solution at 10 °C containing 4.68 g (9.3 mmol) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranoside[25] and 2.0 g (8.6 mmol) of bis(*O*-trimethylsilyl)[4-^{13}C]uracil (**10**) in 100 mL of dry acetonitrile under a dry nitrogen atmosphere was added 1.6 g (6.1 mmol) of freshly distilled (from P_2O_5) stannic chloride in 50 mL of dry acetonitrile. The resulting solution was stirred at room temperature (22 °C) for 16 h and the solvent was removed at 22 °C under reduced pressure. The residue was dissolved in 300 mL of 1,2-dichloroethane and shaken with 250 mL of a saturated sodium bicarbonate solution. The resulting emulsion was allowed to settle and as much of the clear organic layer as possible was removed. A further 100 mL of 1,2-dichloroethane was added to the emulsion and the process was repeated. After the fifth extraction, the remaining bicarbonate layer was filtered through Whatman No. 1 filter paper to break up the emulsion and the resulting clear bicarbonate layer was extracted twice more with 50 mL of organic solvent. The combined organic extract was dried (sodium sulfate and magnesium sulfate) and the solvent was removed to afford 4.81 g of a light, creamy white crystalline solid. Medium-pressure chromatography on silica gel (ICN, 0.032–0.063 mm or 230–400 mesh) using methylene chloride–2% methanol as the eluant afforded 4.30 g (7.74 mmol, 90%) of 2′,3′,5′-tri-*O*-benzoyl[4-^{13}C]uridine as a white crystalline solid: mp 142–143 °C (lit.[35] 142–143 °C).

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Registry No.—**10**, 65102-77-4; **12**, 1563-79-7; **13**, 65138-36-5; trifluoroacetic acid anhydride, 407-25-0; 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranoside, 6974-32-9.

References and Notes

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(41) The amount of material which azeotroped over is effected by the amount of water present in the mixture. Hence, the amount of water added for the hydrolysis was carefully calculated to minimize the water left after hydrolysis.
(42) It is necessary to use anhydrous acetic acid to obtain the reported yield. We have not used this procedure with [1-^{13}C]acetic acid because of the high cost of the labeled material.

Steroid Total Synthesis. 11.[1,2] (+)-Estr-4-ene-3,17-dione from a Chiral Lactone

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Optically pure (+)-estr-4-ene-3,17-dione and (−)-estra-4,9-diene-3,17-dione have been synthesized from the prochiral 5,9-diketoheptanoic acid via the lactone **11**. The selective microbiological reduction of **10** produced optically pure **11**, which was converted to the masked Mannich base **16** and subsequently condensed with 2-methylcyclopentane-1,3-dione to give predominantly the trans diene **17**. This key intermediate was then transformed into (+)-estr-4-ene-3,17-dione via **24** and also to (−)-estra-4,9-diene-3,17-dione by the cyclization of the polyketone **20**.

An asymmetric synthesis of (+)-estr-4-ene-3,17-dione (**27**) is described starting from the chiral lactone **11** (Scheme I) obtained by the selective microbiological reduction of 5,9-diketoheptanoic acid. Condensation of the amine **16**,

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