JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
J. Am. Chem. Soc., 1998, 120(45), 11820-11821, DOI:10.1021/ja980730v

Manuscript JA980730V
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Supporting Synthesis Information:

Reagents can be obtained from a variety of commercial sources, e.g., Aldrich Chemical (Milwaukee, WI), TCI America (Portland, OR) and Monomer Sciences (New Market, AL). Dharmacon Research is in the process of making the final silyl-chloride and orthoester reagents readily available.

Synthesis of tris(2-acetoxyethoxy) orthoformate, ACE orthoester reagent: Acetic acid ethyl ester (85%) (5 eq., 323g) was treated with pyridinium p-toluene sulfonate (0.2eq, 30.8g) and trimethyl orthoformate (1eq., 67.8 ml). The reaction was heated to distill off the methanol product. The reaction was cooled and then neutralized with base. The product was purified on 300 grams of silica gel using solvent mixture of 20% hexanes and 80% ethylacetate, followed by high vacuum distillation (BP= 140 °C, 20 microns Hg). Final yield of product was 20%. Elemental Analysis: $C_{13}H_{22}O_9$, Calc.: C=48.44%, H=6.88%; Found: C=48.79%, H=6.91%

Synthesis of 2'-*O*-bis(2-acetoxyethoxy)methyl uridine (representative of general 2'-protection reaction): 5'-*O*-3'-*O*-tetraisopropyldisiloxyl uridine (TIPS-uridine) (1 eq., 4.86g) was reacted neat with tris(2-acetoxyethoxy) orthoformate (2.8 eq., 9g) and pyridinium p-toluene sulfonate (0.2eq., 0.5g) at 55 °C for 3 hours under high vacuum (<15 microns of Hg). The reaction was cooled to room temperature and neutralized with base. The crude reaction was passed over 100 grams of silica gel using a solvent mixture of 50% hexanes and 50% ethylacetate as a crude purification to remove the neutralized catalyst. The enriched mixture was treated with a freshly prepared solution of *N,N,N',N'*-tetramethylethylendiamine (TEMED) (9.05ml) and 48% hydrofluoric acid (1.08 ml) in acetonitrile (100ml) for 6 hours. The product, 2'-*O*-bis(2-acetyl-ethoxy)methyl uridine, was purified on 200 grams of silica gel using a solvent mixture of 95% ethyl acetate, 5% methanol, 0.1% TEMED. The yield for the combined two reactions was 65%. Adenosine (*N*-benzoyl), cytidine (*N*-acetyl) and guanosine (*N*-isobutyrl) 2'-ACE nucleosides were similarly synthesized and carried through the 5'- and 3'-derivatization reactions to produce final nucleoside phosphoramidites for use in RNA synthesis.

Elemental Analysis:

2'-*O*-ACE-uridine: $C_{18}H_{26}N_2O_{12}$

Calc., C=46.75%, H=5.67%, N=6.06%;

Found, C=46.67%, H=5.83%, N=5.95%

2'-*O*-ACE-*N*-benzoyl-adenosine: $C_{26}H_{31}N_5O_{11}$

Calc., C=52.97%, H=5.30%, N=11.88%

Found, C=52.72%, H=5.42%, N=11.49%

2'-*O*-ACE-*N*-acetyl-cytidine: $C_{20}H_{29}N_3O_{12}$

Calc., C=47.71%, H=5.81%, N=8.35%

Found, C=47.24%, H=5.98%, N=8.42%

2'-*O*-ACE-*N*-isobutyryl-guanosine: $C_{23}H_{33}N_5O_{12}$

Calc., C=48.33%, H=5.82%, N=12.25%

Found, C=48.42%, H=5.95%, N=12.51%

Synthesis of Bis(trimethylsiloxy)dichlorosilane:

Trimethylsilanol: Hexamethylsilazane (0.4 moles, 84 ml) was added to a stirred solution of glacial acetic acid (0.4 moles, 22.88 ml) in 400 ml water at 0°C. Two phases formed. The bottom aqueous phase was removed and washed twice with 100 mls of ether. The ether washes are combined with the silanol phase and stored at -20 C to remove excess water. The solution was decanted and dried over potassium carbonate for 75 min, diluted to 750ml with ether, filtered through 0.45um filter and used directly in the next synthesis. NMR showed formation of disiloxane routinely around 3-4%.

^{1}H NMR ($CDCl_3$) δ: 0.04 (s, hexamethyldisiloxane side product), 0.135 (s).

Sodium trimethylsilanate: Sodium hydride (0.88 moles, 21.1g) was suspended in 500 ml dry THF with vigorous stirring at 0 °C. To this was added freshly prepared trimethylsilanol (0.8 moles) in 500ml ether via cannula. Stirring was continued for one hour (RT) after addition. The solution was filtered through celite and used directly in the next synthesis. Disiloxane concentration 17% by ^{1}H NMR (NMR showed routine formation of disiloxane around 5-20%).

^{1}H NMR (of the crude reaction) ($CDCl_3$) δ: -0.06 (s, hexamethyldisiloxane side product), -0.19 (s).

Trichlorotrimethylsiloxysilane: Sodium trimethylsilanate (0.663 moles) from previous reaction was added by cannula to mechanically stirred solution of tetrachlorosilane(0.597 moles, 68.5 ml) in 500 ml ether at 0 °C. The salt was removed by centrifugation (rpm=8k, 10-15 min). and the solution decanted. The ether was removed *in vacuo* and the product distilled at atmospheric pressure (oil temperature 141 °C, vapor=122 °C). Distillation was continued to oil temperature of 185 °C to collect dichloro-bis(trimethylsiloxy)silane (87.91 molar% trichlorotrimethylsiloxy silane, 5.86 % dichloro-bis(trimethylsiloxy)silane, 6.22%disiloxane).

^{1}H NMR ($CDCl_3$) δ: 0.24 (s).

Bis(trimethylsiloxy)dichlorosilane: Trimethylsilanol (1.1 eq.) was synthesized fresh and cannulated to a mechanically stirred solution of trichlorotrimethylsilane (0.36 moles) from previous reaction and triethylamine (1.47 moles, 205 ml) in 1000 g ether at 0 °C. The solution was filtered under argon and the solvent removed *in vacuo*. The product was heated over calcium hydride at 4mm mercury and 60 °C. The product was then fractionally distilled with glass helices column under reduced pressure (BP=65 °C, 4mm Hg).

^{1}H NMR ($CDCl_3$) δ: 0.19 (s).

Synthesis of 5'-*O*-silyl-2'-*O*-ACE-uridine (representative of general 5'-protection reaction): To 2'-*O*-ACE-uridine (1 eq., 4.9g) and imidazole (4eq., 2.8g) in tetrahydrofuran was added bis(trimethylsiloxy)-cyclooctyloxy-silylchloride (OCT-Cl) (1.5eq., 5.86g in 20ml tetrahydrofuran) over 30 minutes with stirring. OCT-Cl is synthesized *in situ* from bis(trimethylsiloxy)-dichlorosilane and cyclooctanol. The 5'-silyl-2'-ACE uridine product was purified in 75-85% yield on 150 grams of silica gel using solvent mixture of 55% hexanes, 25% ethylacetate, 20% acetone.

Elemental Analysis:

5'-*O*-silyl-2'-*O*-ACE-uridine: $C_{32}H_{58}N_2O_{15}Si_3$

Calc., C=48.34%, H=7.35%, N=3.52%;

Found, C=47.99%, H=7.32%, N=3.54%

5'-*O*-silyl-2'-*O*-ACE-*N*-benzoyl-adenosine: $C_{44}H_{71}N_5O_{14}Si_3$

Calc., C=54.02%, H=7.31%, N=7.16%

Found, C=55.30%, H=7.59%, N=7.14%

5'-*O*-silyl-2'-*O*-ACE-*N*-acetyl-cytidine: $C_{38}H_{69}N_3O_{15}Si_3$

Calc., C=51.15%, H=7.79%, N=4.71%

Found, C=50.82%, H=7.90%, N=4.80%

5'-*O*-silyl-2'-*O*-ACE-*N*-isobutyryl-guanosine: $C_{37}H_{65}N_5O_{15}Si_3$

Calc., C=49.15%, H=7.25%, N=7.75%

Found, C=48.73%, H=7.47%, N=7.73%

Synthesis of 5'-*O*-silyl-2'-ACE-uridine-3'-*O*-(*N*,*N*-diisopropylmethoxy)phosphoramidite: To a solution of 5'-*O*-silyl-2'-*O*-ACE-uridine (1 eq., 6g) in 20ml of dichloromethane was added first bis(*N*,*N*-diisopropylamine)methoxy-phosphine (1.3eq., 2.7g) followed by tetrazole (0.8eq., 0.45g) with stirring. After 2 hours the reaction was quenched and the product isolated in 80-90% yield on 150 grams of silica gel using solvent mixture of 45% hexanes, 50% ethylacetate, 5% triethylamine.