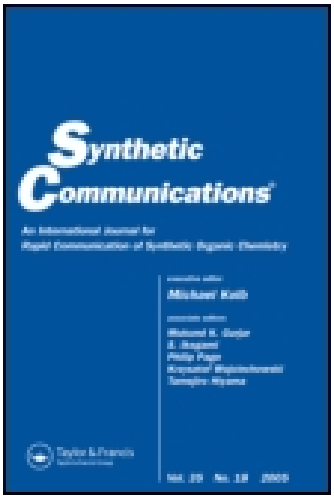

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Convenient Large-Scale Synthesis of Universal Solid Support

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Abstract: A simple, convenient large-scale synthesis of a universal solid support useful for the synthesis of oligonucleotides is described.

Keywords: Oligonucleotide, universal solid support, synthesis, antisense

Standard assembly of oligonucleotides of defined sequence and length involves use of a solid support that contains the first nucleoside covalently attached to a support via a cleavable linker such as a succinyl or oxalyl group.[1–3] This support-bound nucleoside becomes the 3′-terminal residue of the synthesized oligonucleotide after cleavage and purification steps. This methodology suffers from the fact that at least four solid supports for DNA synthesis and an additional four supports for RNA synthesis are needed. For therapeutic application of oligonucleotides containing sugar, backbones and base-modified versions are investigated. This necessitates making many different solid supports.[4] Recently, we have developed a versatile nonnucleoside molecule that functions as a universal linker and, when attached to a solid support, is capable of synthesizing various oligonucleotides and their analogs.[5] This eliminates the need to store different nucleoside-loaded solid supports.

We report here an easy and convenient method for the large-scale synthesis of this universal linker solid support. Diels–Alder reaction of N-phenylmaleimide with furan in acetonitrile at reflux for 5 h gave the adduct **1** in 78% yield. Cis-dihydroxylation of the olefin was achieved using

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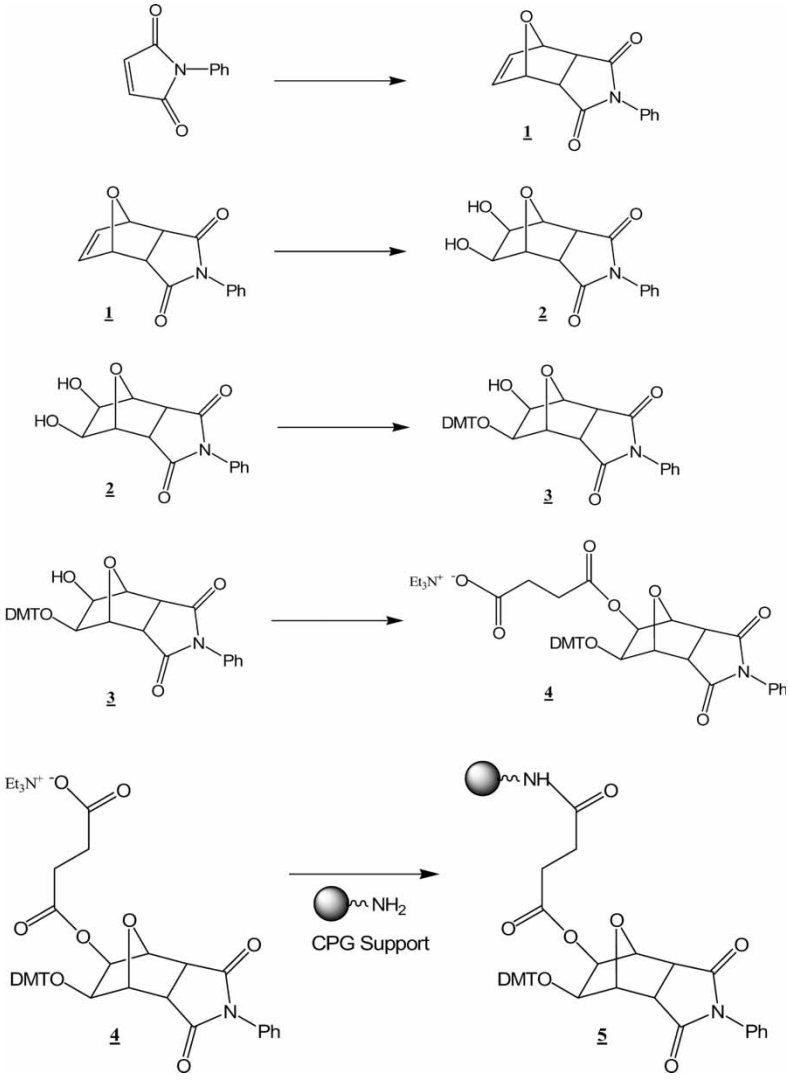
osmium tetroxide and hydrogen peroxide in acetone to give the diol **2** in 82% yield. Dimethoxytritylation was achieved using 4,4′-dimethoxytrityl chloride in anhydrous pyridine at room temperature for 2 h to afford product **3** in 83% yield. Treatment of compound **3** with succinic anhydride in the presence of triethylamine in anhydrous methylene chloride afforded the linker molecule **4** as the triethylammonium salt in 91% yield. Loading of the succinate linker molecule to controlled pore glass solid support was achieved using O-benzotriazol-1-yl-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HBTU) as activating agent to give **5**. The calculated molar equivalent of molecule **4** was taken to obtain a loading of 45 μmol/g.



EXPERIMENTAL

Furan, *N*-phenylmaleimide, pyridine, diisopropylethylamine, triethylamine, succinic anhydride, hydrogen peroxide, *tert*-butyl alcohol, 4-dimethylamino-pyridine, *N*-methylimidazole, anhydrous acetonitrile, methylene chloride, and toluene were purchased from Aldrich Chemical Company, Milwaukee, Wisconsin, and used as received. Osmium tetroxide was purchased from Sterm Company, Newburyport, MA. 4,4′-Dimethoxytriphenylmethyl chloride was purchased from ChemGenes, Wilmington, MA. HBTU reagent was purchased from SENN chemicals, San Diego, CA. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 200-MHz or Bruker AM 300-MHz spectrometer. Chemical shifts are reported in parts per million (ppm) using tetramethylsilane as an internal standard with DMSO-d_6 or $CDCl_3$ as solvent.

N-Phenyl-tetrahydro-4,7-epoxyisobenzopyrrole-1,3-dione (1)

A three-necked, round-bottomed flask (5 L), equipped with a magnetic stirring bar, a heating mantle, a reflux condenser, and a dropping funnel, was charged with a solution of *N*-phenylmaleimide (0.5 kg, 2.89 mol) in acetonitrile (1.6 L). Following the addition of furan (0.5 L), the stirred solution was heated at reflux for 5 h. Then the reaction mixture was cooled to room temperature, when a colorless solid precipitated out. The material was filtered and washed with acetonitrile (0.5 L). The filtrate solution was concentrated to afford more of product, which was also filtered and washed with acetonitrile (0.3 L). The combined solid portions were dried under high vacuum at room temperature overnight to afford 0.54 kg (78%) of product. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.06 (s, 2H), 5.22 (s, 2H), 6.58 (s, 2H), 7.18–7.58 (m, 5H). ^{13}C NMR (DMSO-d_6, 75.5 MHz) δ: 47.41, 80.75, 126.76, 128.38, 128.92, 132.09, 136.58, 175.66. MS (ESI, m/z): 241. Anal. calcd. (%) for $C_{14}H_{11}NO_3$: C, 69.70; H, 4.60; N, 5.81. Found: C, 69.74; H, 4.69; N, 5.88.

N-Phenyl-5,6-dihydroxy-hexahydro-4,7-epoxy-isobenzopyrrole-1,3-dione (2)

The content of a 1-g sealed vial of osmium tetroxide was dissolved in purified *ter*t-butyl alcohol (0.2 L). The pale green solution was treated with 3–5 drops of 30% hydrogen peroxide and allowed to remain at room temperature for 1 day. If the solution became dark, the dropwise addition of 30% hydrogen peroxide was repeated until the pale green color persisted. This solution is stable for at least 1 year at room temperature. Each mL contains 2×10^{-5} mol of osmium tetroxide.

A 5-L three-necked flask, fitted with a mechanical stirrer, reflux condenser with ice water cooling, and a heating mantle, was charged with a solution of olefin **1** (0.23 kg, 0.93 mol) in acetone (2.5 L). A 30% hydrogen peroxide solution (0.5 L) was added, followed by an osmium tetroxide solution prepared earlier (0.18 L). *Warning*: For large scales, the reaction could be exothermic! Slow addition (1–2 h) of osmium tetroxide solution is recommended. Gentle refluxing of reaction mixture with stirring was maintained for 7–8 h. During this period, reaction color changed from brown to pale brown to colorless, and the solid started crashing out. Vigorous stirring was maintained throughout the period. TLC indicated the disappearance of the starting material. The reaction mixture was cooled to room temperature, and the precipitated solid was filtered. The solid was washed with ether (2 L) and dried in vacuum oven at room temperature overnight. The filtrate solution was concentrated, and ether (1 L) was added when an additional solid precipitated out, which was filtered and washed with ether (0.3 L). The combined solid product was dried in an oven at 45°C for 2 days to afford a total of 211 g (82%) of colorless product **2**. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.14 (s, 2H), 3.88 (d, 2H), 4.39 (s, 2H), 5.1 (d, 2H), 7.18–7.58 (m, 5H). ^{13}C NMR (DMSO-d_6, 75.5 MHz) δ: 45.47, 71.82, 84.08, 126.72, 128.40, 128.90, 132.16, 176.26. MS (ESI, m/z): 275. Anal. calcd. (%) for $C_{14}H_{13}NO_5$: C, 61.09; H, 4.76; N, 5.09. Found: C, 61.21; H, 4.84; N, 5.17.

N-Phenyl-5-(4,4′-dimethoxytriphenylmethyloxy)-6-hydroxy-hexahydro-4,7-epoxyisobenzopyrrole-1,3-dione (3)

The dihydroxy compound **2** (0.28 g, 1 mol) was taken in a 5-L round-bottomed flask and co-evaporated with anhydrous pyridine (1.2 L). This step was repeated one more time to render the material anhydrous. Pyridine (3 L) was added and stirred using a magnetic stirrer at room temperature. 4,4′-Dimethoxytrityl chloride (0.5 kg, 1.5 mol, 1.5 equiv) was slowly added as solid over a period of 2 h. The solution was stirred overnight. TLC indicated the disappearance of the starting material. All volatiles were removed; toluene (2 L) was added and concentrated under vacuum using a rotary evaporator. This step was repeated one more time. The remaining crude material was purified by flash silica-gel chromatography using hexane–ethyl acetate. Triethylamine (1%) was used throughout purification to afford 0.47 kg (83%) of product **3**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.42 (d, 1H), 3.78 (d, 1H), 3.45 (s, 1H), 3.62 (d, 1H), 3.81 (s, 6H), 3.85 (s, 2H), 4.72 (s, 1H), 6.82 (d, 4H), 7.1–7.55 (m, 14H). ^{13}C NMR (DMSO-d_6, 75.5 MHz) δ: 20.99, 45.27, 45.68, 55.06, 73.92, 75.45, 81.96, 84.40, 87.40, 113.48, 113.60, 125.28, 126.73, 127.72, 127.98, 128.16, 128.34, 128.85, 129.79, 129.89, 132.11, 135.84, 136.34, 145.44, 158.47, 175.30, 176.03. MS (ESI, m/z): 577.5. Anal. calcd. (%) for $C_{35}H_{31}NO_7$: C, 72.78; H, 5.41; N, 2.42. Found: C, 72.93; H, 5.54; N, 2.47.

Triethylammonium *N*-Phenyl-5-(4,4′-dimethoxytriphenyl)-6-[2-carboxypropanoyloxy]-hexahydro-4,7-epoxyisobenzopyrrole-1,3-dione (4)

A 500-mL round-bottomed flask equipped with a magnetic stirrer was charged with a solution of *N*-phenyl-5-(4,4′-dimethoxytriphenylmethyloxy)-6-hydroxy-hexahydro-4,7-epoxyisobenzopyrrole-1,3-dione 3 (0.05 kg, 0.09 mol) in methylene chloride (0.48 L). Triethylamine (0.07 L, 0.51 mol, 6 equiv with respect to the starting DMT compound) was added at room temperature. To this clear solution, succinic anhydride (0.03 kg, 0.36 mol, 4 equiv with respect to the starting DMT compound) was added as a solid all at once. Stirring was continued overnight. TLC indicated the disappearance of the starting material. The reaction mixture was diluted with ethyl acetate (0.3 L), washed with water (2 × 0.2 L) and brine (0.12 L), and dried with magnesium sulfate. If colored, the material was passed through a short pad of silica gel using methylene chloride–methanol to afford 0.061 kg (91%) of product 4 as a pale yellow solid. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.96 (t, 9H), 2.58 (q, 4H), 2.59–2.78 (m, 8H), 2.91 (s, 1H), 3.17 (s, 1H), 3.75 (s, 6H), 4.09 (d, 1H), 4.54 (s, 1H), 5.18 (d, 1H), 6.92 (d, 4H), 7.08–7.55 (m, 14H). ^{13}C NMR (DMSO-d_6, 75.5 MHz) δ: 10.16, 29.87, 30.19, 45.03, 45.15, 45.75, 55.04, 74.39, 75.29, 81.75, 81.90, 87.17, 113.56, 113.64, 126.72, 126.82, 127.51, 128.03, 128.14, 128.40, 128.87, 129.67, 129.76, 132.04, 135.46, 135.93, 145.16, 158.50, 172.55, 174.18, 175.02, 175.52. MS (ESI, m/z): 778.4. Anal. calcd. (%) for $C_{45}H_{50}N_2O_{10}$: C, 69.39; H, 6.47; N, 3.60. Found: C, 69.61; H, 6.77; N, 3.87.

Loading of Linker Molecule (4) to Controlled Pore Glass Support (5)

Controlled pore glass (CPG) (100 g), succinate molecule **4** (4.05 g, 5.2 mmol), HBTU (1.97 g, 5.2 mmol), diisopropylethylamine (1.86 mL, 10.4 mmol), and anhydrous acetonitrile (500 mL) were successively added to a 1-L round-bottom flask. The resulting mixture was then tightly capped and shaken overnight (more than 16 h) at room temperature. The support was then filtered and washed with acetonitrile.

Capping of Unreacted Hydroxyl Sites on CPG Support

The loaded CPG, 4-dimethylaminopyridine (DMAP) (2.44 g, 20.0 mmol), CAP A (250 mL), and CAP B (250 mL) solutions were successively added to a 1-L round-bottom flask. CAP A is a mixture of *N*-methylimidazole, pyridine and acetonitrile (20 : 30 : 50, v : v : v); CAP B is a mixture of acetic anhydride and acetic acetonitrile (20 : 80, v : v). The resulting mixture was

then tightly capped and shaken overnight at room temperature. The support was filtered, washed with acetonitrile, and dried thoroughly under high vacuum. Loading of the linker molecule was determined by UV and found to be 45 μmol/g.

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