Enantiospecific Syntheses of Aristeromycin and Neplanocin A

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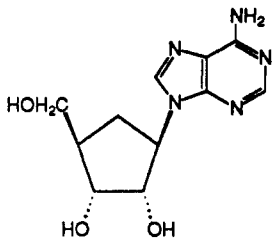
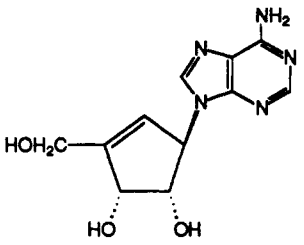
The carbocyclic nucleosides (-)-aristeromycin and (-)-neplanocin A were made enantiospecifically in nine steps and ten steps, respectively, from D-ribonic acid γ-lactone. Quenching of an organocuprate conjugate addition reaction with either acetic acid or methanesulfinyl chloride determines whether the divergent synthetic route branches toward (-)-aristeromycin or (-)-neplanocin A. An alternate synthesis of cyclopentenone 1, a common intermediate to these compounds, from D-gulonic acid γ-lactone is also described.

Introduction

The adenosine analogues (-)-aristeromycin (Ari) and (-)-neplanocin A (NpcA), isolated from *Streptomyces citricolor*[2] and *Ampullariella regularis*,[3] respectively, have



Ari



NpcA

elicited numerous biological studies due to their potent antiviral and antitumor activities.[4] The antiviral activities of a variety of adenosine analogues, including NpcA and 3-deazaAri, have been correlated with their inhibitory effects toward the cellular enzyme *S*-adenosylhomocysteine (AdoHcy) hydrolase, presumably due to subsequent decreases in viral 5'-cap mRNA methylation.[5] Recent studies in our laboratory suggested that the 4'-hydroxymethyl substituent of Ari and NpcA is responsible for part of the cytotoxic effects of these compounds and is not essential for inhibition of AdoHcy hydrolase and antiviral activity.[6]

In order to develop more selective chemotherapeutic agents based on Ari and NpcA, we wished to synthesize a series of Ari and NpcA analogues modified in the 4'-position. We envisioned that many of these analogues could be made from Ari or NpcA themselves. However, we desired an efficient synthetic route that was not only divergent and enantiospecific but also easily adjustable for obtaining some target 4'-modified Ari and NpcA analogues difficult to make from the parent compounds.

Although the literature abounds with syntheses of Ari[7] and NpcA,[8] none fulfilled all of our requirements. In a preliminary communication, we have reported a route to Ari from D-ribonic acid γ-lactone that met our demands.[9] Herein we describe in detail this synthesis of Ari as well as a related synthesis of NpcA. These nucleoside analogues have been prepared via a common cyclopentenone intermediate that can be derived from either D-ribonic acid γ-lactone or D-gulonic acid γ-lactone.

Results and Discussion

In designing a synthetic route to Ari and NpcA (Scheme I), it occurred to us that both compounds might be made from cyclopentenone 1, a useful chiral building block synthesized in four steps from D-ribonic acid γ-lactone as previously reported from this laboratory.[10] Enone 1 could be treated with lithium bis(*tert*-butoxymethyl)cuprate,[11] a hydroxymethyl equivalent. Quenching the reaction with a proton source would give cyclopentanone 2, a putative Ari precursor. Alternatively, quenching with methanesulfinyl chloride to yield β-keto sulfoxide 3 followed by pyrolytic syn elimination would result in cyclopentenone 4, a NpcA precursor. This route was attractive because it fulfilled the requirements mentioned above, especially the demand for easy access to 4'-modified Ari and NpcA analogues not readily obtainable from the parent compounds. Through the reactions described in Scheme I, a wide variety of such 4'-modified analogues might be synthesized simply by changing the alkyl portion of the organocuprate reagent.

Cyclopentenone 1 was added to a solution of lithium bis(*tert*-butoxymethyl)cuprate, formed by the procedure of Eckrich and Corey.[11] Quenching the reaction with acetic

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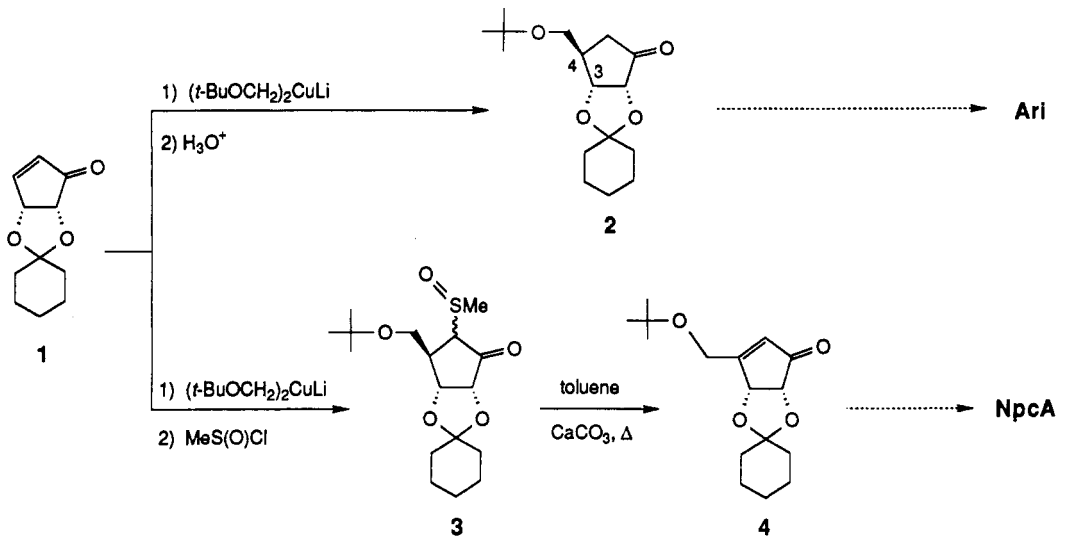
Scheme I

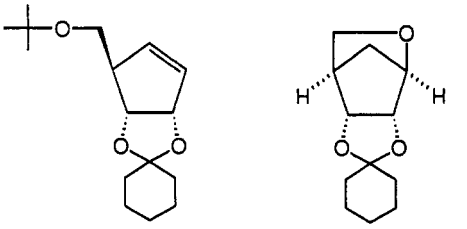


acid gave cyclopentanone **2** as one diastereomer in 81% purified yield. Decoupling experiments using proton NMR spectroscopy revealed that $J(H_3H_4) = 1$ Hz, corresponding to a dihedral angle of either 65° or 105° according to the Karplus equation. Molecular models illustrate that the former angle is not attainable with H_3 and H_4 in either a cis or a trans relationship. The latter angle is easily attainable with these protons trans to each other, but not when they are cis (i.e., the alkyl substituent of the organocuprate added to the less hindered face resulting in **2** with the depicted stereochemistry). Unambiguous proof of the stereochemical character of **2** is provided by the successful conversion of this ketone to Ari (vide infra).

If the organocuprate reaction is transferred to a solution of methanesulfinyl chloride[12] instead of being quenched with acetic acid, the β-keto sulfoxide **3** results as a mixture of four diastereomers due to variable chirality at the sulfur and α-carbon atoms. In refluxing toluene with calcium carbonate, compound **3** underwent pyrolytic syn elimination, yielding cyclopentenone **4** in an overall yield of 64% from **1**. The calcium carbonate apparently assists in the epimerization of the diastereomers of **3** unable to undergo syn elimination, converting them to the diastereomers capable of doing so.

Reduction of cyclopentanone **2** with diisobutylaluminum hydride (DIBAH; Scheme II) afforded alcohol **5** in 96% crude yield. The compound was 92% diastereomerically pure according to ^1H and ^{13}C NMR. After chromatographic separation of the two diastereomers, deuterium exchange of the hydroxyl proton and irradiation of $H_{5\alpha}$ and $H_{5\beta}$ revealed that $J(H_1H_2) = 5$ Hz for the major isomer and $J(H_1H_2) = 0$ Hz for the minor isomer. Again, using molecular models and the Karplus equation, we determined that the major isomer has H_1 and H_2 in a cis relationship (i.e., DIBAH approached selectively from the less hindered face). Further evidence was provided by nuclear Overhauser enhancement (NOE) experiments,[13] which showed that preirradiation of H-1 resulted in enhancement of the ether substituent methylene protons by 1.0% and 1.4%. Similar irradiation of H-1 in the minor diastereomer led to no such enhancement of these methylene protons. All other enhancements were also consistent with these stereochemical assignments. Reduction of cyclopentenone **4** under the same conditions yielded only diastereomer **8**, proton coupling constants again confirming that DIBAH approached from the less hindered face.

The alcohol **5** was then converted to its trifluoromethanesulfonate (triflate) **6** with trifluoromethanesulfonic anhydride. The triflate was subsequently displaced with adenide anion to give protected Ari **7** (46% overall yield from **5**). The ^{13}C NMR chemical shift values of the purine carbon atoms indicated that **7** is N^9-substituted; no N^7-substituted compound was detected.[14] Side products **9** and **10** were also isolated in 14% and 15% yields, respectively. Alkene **9** is the result of elimination of the



9 **10**

triflate, whereas bicyclic ether **10** may result from triflate displacement by the ether oxygen atom followed by elimination of isobutylene. Allylic alcohol **8** was likewise treated with methanesulfonyl chloride to give the methanesulfonate (mesylate) **11**. Displacement of the mesylate with adenide anion yielded protected NpcA **12** (45% overall yield from **8**). Again, ^{13}C NMR confirmed that this product was N^9-substituted. Deprotection of **7** and **12** with trifluoroacetic acid and water (2:1, 50 °C) resulted in Ari and NpcA, respectively. Spectroscopic and physical data (including optical rotation) of both final compounds matched literature values, and these compounds also coeluted with authentic samples of Ari and NpcA by HPLC.

During exploration of the feasibility of the synthetic pathways described above, the starting material, D-ribonic

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(13) The one-dimensional NOE spectra were obtained with approximately 50 mM solutions ($CDCl_3$, not degassed). The nonspinning samples (27 °C) were preirradiated for 5 s, followed by acquisition for 2.7 s (90° pulse). Blanks were run at the beginning and end of the list of frequencies. Each frequency used 16 real scans and 4 dummy scans, repeating this process 4 times. The spectra were transformed and subtracted and the percent enhancement calculated, correcting the intensities for percent saturation of the irradiated peak.

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acid γ-lactone, became unavailable from commercial sources. Undaunted, we developed an alternate method for the synthesis of the intermediate cyclopentenone 1 (Scheme III). Commercially available D-gulonic acid γ-lactone and cyclohexanone were heated in refluxing benzene with Dowex 50W (H^+) resin to yield diketal 13. The use of other acid sources (HCl, H_2SO_4, *p*-toluenesulfonic acid) resulted in poor yields and/or significant amounts of 3,5-cyclohexylidene γ-lactone. Compound 13 was then selectively deprotected in aqueous ethanol with Dowex 50W (H^+) as the acid source to afford 14. This 2,3-cyclohexylidene lactone was then dissolved in sodium hydroxide solution and treated with sodium periodate (2.4 equiv), resulting in L-erythruronolactone 15, an intermediate to 1 from D-ribonic acid γ-lactone. The L-erythruronolactone 15 was converted to cyclopentenone 1 as we have previously reported.[10]

To summarize, Ari can be synthesized in nine steps from D-ribonic acid γ-lactone or in ten steps from D-gulonic acid γ-lactone; NpcA is readily obtainable from Ari intermediate 1 using similar methodology. We are currently investigating the utility of organocuprate reagents in the formation of certain 4′-modified Ari and NpcA analogues with potential antiviral activity.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer Model 1420 spectrophotometer. NMR spectra were obtained on either a Varian XL-300 or a Bruker AM-500 spectrometer. All ^1H NMR chemical shifts are reported in δ relative to internal standard tetramethylsilane (TMS, δ 0.00); coupling constants are reported in hertz. ^{13}C NMR chemical shifts are reported in δ relative to $CDCl_3$ (center of triplet, δ 77.0) or relative to DMSO-d_6 (center of septet, δ 39.5). Mass spectra were recorded on a Ribermag R10-10 quadrupole spectrometer. All samples were ionized by electron impact at 70 eV. Optical rotations were determined by using the sodium-D line on a Perkin-Elmer Model 241 polarimeter. Elemental analyses were performed by Desert Analytics, Phoenix, AZ. Silica gel chromatography was accomplished with 70–230-mesh, 60-Å silica gel (Aldrich Chemical Co.). Ion-exchange chromatography was carried out with Dowex 50W (H^+), dry mesh 100–200, 4% cross-linked (Sigma Chemical Co.). All reactions were run under argon atmosphere except where water was used as solvent.

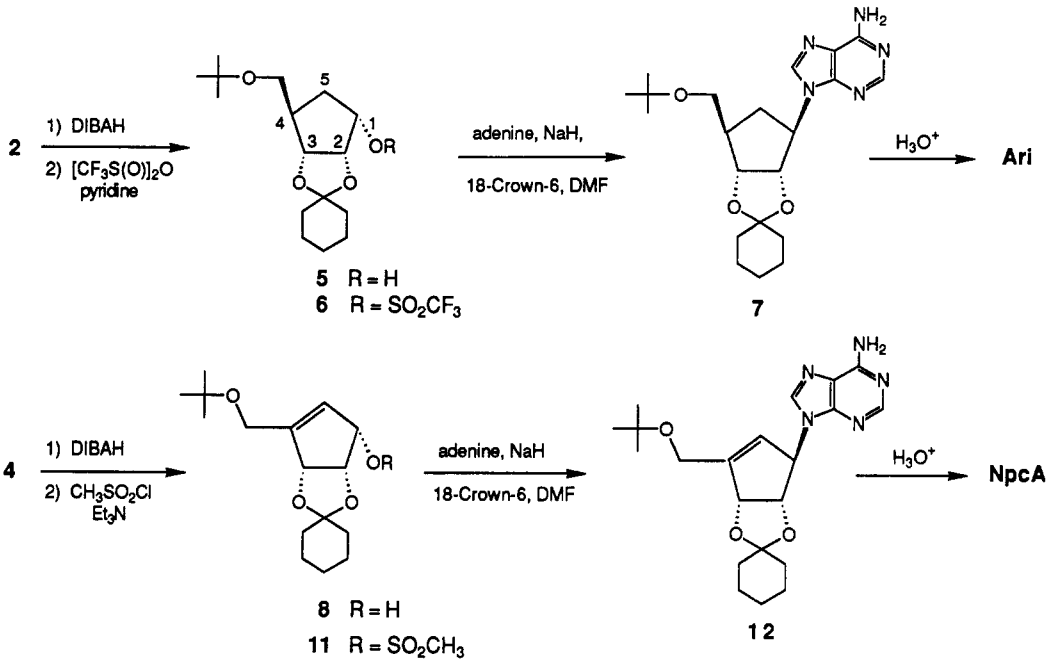
(2*R*,3*R*,4*R*)-4-(*tert*-Butoxymethyl)-2,3-(cyclohexylidenedioxy)-1-cyclopentanone (2). To a suspension of 1.94 g (17.3 mmol) potassium *tert*-butoxide and 75 mL anhydrous *tert*-butylmethyl ether cooled to −78 °C was added 12.3 mL (17.3 mmol) of *sec*-butyllithium (1.4 M in cyclohexane) dropwise over 5 min. The suspension immediately turned bright orange. After stirring 3.5 h at this temperature, 17.3 mL (34.6 mmol) of LiBr solution (2 M in THF) was added dropwise over 5 min. The dry ice/acetone bath was then exchanged with an ice/salt bath, and the white suspension was allowed to stir another 30 min. The suspension was recooled to −78 °C; then 1.78 g (8.65 mmol) of CuBr·SMe_2 (recrystallized from Me_2S and hexane) in 10 mL of diisopropyl sulfide was added dropwise over 5 min. The white suspension became a viscous, dark brown, homogeneous solution. After 1 h, 1.10 g (5.67 mmol) of enone 1 in 10 mL of THF was added dropwise over 5 min. The reaction mixture was allowed to warm to −30 °C over 15 min, stirred at this temperature for an additional 30 min, then quenched with 20 mL of AcOH/MeOH (1:1, degassed with sonicator), and poured into 200 mL of NH_4Cl/NH_4OH (pH 9). After removal of the aqueous layer, the organic phase was washed with a 1:1 mixture of saturated NH_4Cl and 3% NH_4OH solutions (3 × 50 mL) and then 50 mL of saturated NaCl solution. The organic phase was dried over $NaSO_4$, filtered, concentrated, and passed through silica gel (75 g, ether/hexane, 1:1) to obtain 1.30 g (81%) of 2: mp = 81–83 °C; IR (KBr) 2960, 2930, 2915, 2860, 1750, 1450, 1365, 1205, 1165, 1110, 1075, 1000 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.10 (s, 9 H), 1.30–1.70 (m, 10 H), 2.04 (d, J = 17, 1 H), 2.55 (dt, J_1 = 9, J_2 = 2, 1 H), 2.73 (dd, J_1 = 17, J_2 = 9, 1 H), 3.36 (dd, J_1 = 9, J_2 = 2, 1 H), 3.55 (dd, J_1 = 9, J_2 = 2, 1 H), 4.23 (d, J = 5, 1 H), 4.62 (d, J = 5, 1 H) [The doublet at δ 4.23 became a doublet of doublets upon irradiation of the δ 2.73 peak, apparently due to elimination of small virtual couplings.]; ^{13}C NMR ($CDCl_3$) δ 23.6, 23.9, 25.0, 27.1, 34.0, 36.4, 37.4, 37.6, 63.1, 73.3, 78.7, 81.6, 111.5, 213.1; MS, *m/z* 282 (M^+), 226, 197, 183, 140, 98, 69, 57; $[\alpha]_D$ = −154° (*c* = 0.184, $CHCl_3$). Anal. Calcd for $C_{16}H_{26}O_4$: C, 68.06; H, 9.28. Found: C, 67.95; H, 9.27.

(1*S*,2*S*,3*R*,4*R*)-4-(*tert*-Butoxymethyl)-2,3-(cyclohexylidenedioxy)cyclopentan-1-ol (5). To a 5 °C solution of 400 mg (1.42 mmol) of ketone 2 in 40 mL of CH_2Cl_2 (stored over 4-Å molecular sieves) was added 2.13 mL (2.13 mmol) of diisobutylaluminum hydride (1 M in CH_2Cl_2) dropwise over 2 min. The reaction mixture was stirred for 3 h at this temperature and then cautiously quenched with 20 mL of MeOH. The colloidal suspension was concentrated, and the white solid was treated with ether. The suspension was filtered, washing thoroughly with Et_2O, and the filtrate was concentrated, leaving 385 mg (96%) of 5 as a clear, colorless oil. By ^1H and ^{13}C NMR, the product appeared to be 92% of one diastereomer. A small sample was passed through silica gel (Et_2O/hexane, 1:1) to obtain the diastereomerically pure 5: IR (neat) 3515, 2965, 2930, 2855, 1450, 1395, 1365, 1285, 1250, 1235, 1200, 1165, 1150, 1110, 1090, 1040, 1015, 955, 915, 890, 855 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.15 (s, 9 H), 1.35–1.75 (m, 10 H), 1.85 (m, 2 H), 2.20 (m, 1 H), 2.55 (d, J = 10, 1 H, exchanged with D_2O), 3.20 (dd, J_1 = 4, J_2 = 9, 1 H), 3.30 (dd, J_1 = 4, J_2 = 9, 1 H), 4.20 (m, 1 H), 4.45 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 23.6, 24.0, 25.2, 27.4, 33.8, 36.0, 36.1, 42.2, 63.1, 72.1, 72.5, 79.2, 83.1, 111.3; MS, *m/z* 284 (M^+), 255, 241, 229, 185, 167, 113, 99, 95, 57; $[\alpha]_D$ = −13° (*c* = 0.726, $CHCl_3$). Anal. Calcd for $C_{16}H_{28}O_4$: C, 67.57; H, 9.92. Found: C, 67.49; H, 10.12.

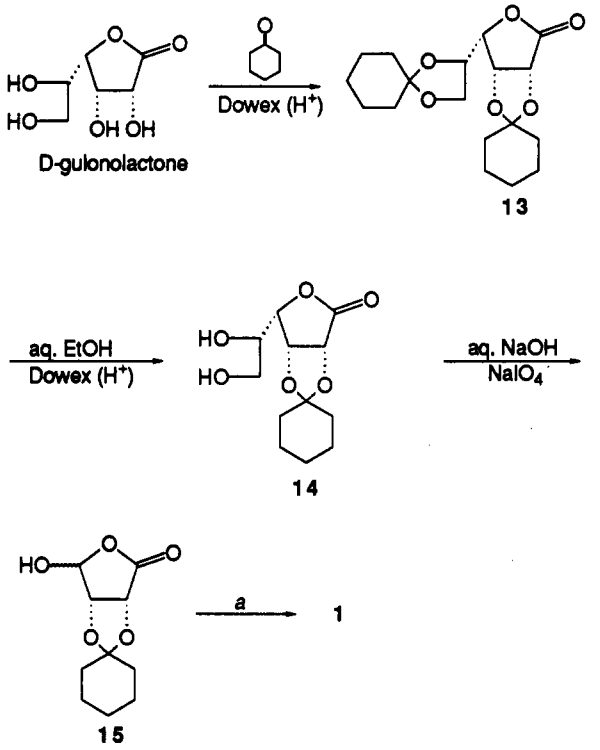
(1*S*,2*R*,3*R*,4*R*)-4-(*tert*-Butoxymethyl)-2,3-(cyclohexylidenedioxy)cyclopentan-1-ol Trifluoromethanesulfonate (6). To a 5 °C solution of 430 mg (1.51 mmol) of alcohol 5 in 6 mL of CH_2Cl_2 (stored over 3-Å molecular sieves) and 0.125 mL (123 mg, 1.55 mmol) of pyridine (distilled from BaO) was added 0.255 mL (428 mg, 1.52 mmol) of trifluoromethanesulfonic anhydride in 3 mL of CH_2Cl_2 dropwise over 8 min. The reaction mixture was stirred at 5 °C for 30 min and then quenched with 3 mL of cold H_2O. After stirring for several minutes, the aqueous layer was removed and the CH_2Cl_2 layer washed with cold H_2O (2 × 10 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated to give 598 mg (95%) triflate 6 as a clear, colorless oil: ^1H NMR ($CDCl_3$) δ 1.10 (s, 9 H), 1.25–1.75 (m, 10 H), 1.95 (m, 1 H), 2.25 (m, 2 H), 3.20 (dd, J_1 = 2, J_2 = 8, 1 H), 3.35 (dd, J_1 = 3, J_2 = 8, 1 H), 4.35 (d, J = 5, 1 H), 4.50 (dd, J_1 = 5, J_2 = 5, 1 H), 5.25 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 23.7, 23.9, 25.1, 27.2, 33.0, 34.0, 35.8, 41.4, 63.0, 73.0, 78.2, 83.2, 87.1, 112.3, 119.0 (q, CF_3).

9-[(1′*R*,2′*S*,3′*R*,4′*R*)-4-(*tert*-Butoxymethyl)-2,3-(cyclohexylidenedioxy)cyclopentan-1-yl]adenine (7). To a suspension of 6.42 g (47.5 mmol) of adenine in 200 mL of anhydrous DMF was added 1.89 g (47.2 mmol) of sodium hydride (60% dispersion in mineral oil) and 11.9 g (45.0 mmol) of 18-crown-6. The mixture was heated to 70 °C for 4 h. The white suspension was cooled to 0 °C; then 6.6 g (15.9 mmol) of triflate 6 was dissolved in 20 mL of DMF and added to the adenide solution. The reaction mixture was stirred at 0 °C for 32 h and then room temperature for 12 h. The mixture was then filtered through a Büchner funnel (washing with 750 mL of CH_2Cl_2). The filtrate was poured into a separatory funnel (total of 1.5 L of CH_2Cl_2), washed with 2 × 250 mL of saturated KCl solution, and dried over Na_2SO_4. After filtration, the solution was concentrated and the residue passed twice through flash silica gel (150 g, CH_2Cl_2/EtOH, 9:1) to give 3.1 g of material with 4% 18-crown-6 by weight (yield of 7: 46% from 5). Preparative silica gel plates provided an analytically pure sample of 7 as a glassy solid: IR (KBr) 3320, 3160, 2960, 2930, 2855, 1640, 1595, 1575, 1470, 1365, 1100, 650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (s, 9 H), 1.35–1.90 (m, 10 H), 2.25–2.65 (m, 3 H), 3.50 (dd, J_1 = 6, J_2 = 9, 1 H), 3.60 (dd, J_1 = 4, J_2 = 9, 1 H), 4.65 (dd, J_1 = 4, J_2 = 6, 1 H), 4.85 (m, 1 H), 5.05 (dd, J_1 = 5, J_2 = 6, 1 H), 6.40 (br s, 2 H), 7.95 (s, 1 H), 8.35 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 23.4, 23.9, 25.0, 27.4, 34.3, 34.6, 37.4, 44.0, 61.8, 62.3, 72.8, 81.2, 83.5, 113.9, 120.1, 139.4, 150.0, 152.6, 155.7; MS, *m/z* 402 (M + 1), 358, 344, 246, 216, 136, 57, 41; $[\alpha]_D$

Scheme II



Scheme III[a]



[a] See ref 10.

= −32° (c = 0.630, $CHCl_3$). Anal. Calcd for $C_{21}H_{31}N_5O_3$: C, 62.82; H, 7.78; N, 17.44. Found: C, 62.71; H, 7.88; N, 17.06.

(−)-Aristeromycin (Ari). Compound 7 (101 mg, 0.25 mmol) was dissolved in 15 mL of CF_3COOH/H_2O (2:1) and heated to 50 °C for 3 h. The solution was then concentrated and the residue passed through a Dowex 50W (H^+) column to obtain 53 mg (79%) of Ari as a yellowish brown foam. Passage through an HPLC column (Hamilton PRP-1 semipreparative column, H_2O/MeCN, 19:1) resulted in analytically pure material: mp = 211–213 °C dec (lit.[2b] mp 213–215 °C); IR (KBr) 3325, 3205, 2945, 2845, 1660, 1610, 1575, 1420, 1345, 1295, 1260, 1105, 1050, 910, 835, 735, 715, 670, 655 cm^{-1}; ^1H NMR (DMSO-d_6 + D_2O) δ 1.70 (m, 1 H), 2.03 (m, 1 H), 2.20 (m, 1 H), 3.46 (m, 2 H), 3.83 (dd, J_1 = 5.1, J_2 = 2.9, 1 H), 4.33 (dd, J_1 = 9.1, J_2 = 5.3, 1 H), 4.67 (dd, J_1 = 9.0, J_2 = 9.1, 1 H), 8.11 (s, 1 H), 8.19 (s, 1 H); ^{13}C NMR (DMSO-d_6 + D_2O) δ 29.3, 45.4, 59.3, 63.1, 71.7, 74.6, 118.9, 140.1, 149.8, 152.1, 156.0; MS, m/z 266 (M + 1), 265 (M^+), 248, 234, 218, 206, 190, 178, 162, 136, 135, 108, 81; $[\alpha]_D$ = −56.0° (c = 0.366, DMF) (lit.[2b,7f] −52.5° and −53.0°). Anal. Calcd for $C_{11}H_{15}N_5O_3$: C, 49.81; H, 5.70; N, 26.40. Found: C, 49.73; H, 5.94; N, 26.43.

(4*R*,5*R*)-3-(*tert*-Butoxymethyl)-4,5-(cyclohexylidenedioxy)-2-cyclopenten-1-one (4). Cyclopentenone 1 (3.4 g, 17.5 mmol) in 25 mL of THF was added to 26.6 mmol of bis(*tert*-butoxymethyl)cuprate, prepared as described for the synthesis of 2 above. Instead of being quenched with acetic acid, the reaction mixture was cooled to −78 °C and transferred over 10 min via cannula to a −78 °C solution of methanesulfinyl chloride[15] (7.33 mL, 10.0 g, 102 mmol) in 800 mL of *tert*-butyl methyl ether. The dry ice/acetone bath was removed and replaced with an ice bath. The reaction mixture reached 0 °C and was stirred a total of 30 min, whereupon it was quenched and extracted as 2 above. The aqueous layers were combined and extracted with 6 × 150 mL of CH_2Cl_2. The ether and CH_2Cl_2 layers were combined, dried over Na_2SO_4, filtered, and concentrated. The residue was passed through silica gel (150 g, ether/hexane, 1:1 followed by CH_2Cl_2/EtOH 19:1) to give β-keto sulfoxide 3 as a mixture of four diastereomers. This material was dissolved in 600 mL of distilled toluene with 1.97 g (19.7 mmol) of $CaCO_3$ and refluxed for 15 h. The reaction was filtered, concentrated, and passed through silica gel (150 g, ether/hexane, 1:1), yielding 3.33 g (68%) of 4 as a clear, colorless oil: IR (neat) 2980, 2930, 2855, 1720, 1625, 1450, 1365, 1280, 1255, 1245, 1190, 1160, 1145, 1105, 1055, 980, 940, 930, 910, 850, 600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (s, 9 H), 1.30–1.70 (m, 10 H), 4.25 (dd, J_1 = 18, J_2 = 1, 1 H), 4.45 (dd, J_1 = 18, J_2 = 1, 1 H), 4.50 (d, J = 6, 1 H), 5.10 (d, J = 6, 1 H), 6.15 (dd, J_1 = J_2 = 1); ^{13}C NMR ($CDCl_3$) δ 23.6, 23.8, 24.8, 27.3, 35.8, 37.2, 60.2, 74.2, 77.4, 77.6, 116.1, 128.1, 176.1, 202.0; MS, m/z 280 (M^+), 251, 237, 181, 126, 109, 57, 55, 41; $[\alpha]_D$ = −30° (c = 0.836, $CHCl_3$). Anal. Calcd for $C_{16}H_{24}O_4$: C, 68.55; H, 8.63. Found: C, 68.40; H, 8.63.

(1*S*,4*R*,5*S*)-3-(*tert*-Butoxymethyl)-4,5-(cyclohexylidenedioxy)-2-cyclopenten-1-ol (8). To a −78 °C solution of 3.20 g (11.4 mmol) of enone 4 in 160 mL of CH_2Cl_2 (stored over 3-Å molecular sieves) was added 17.1 mL (17.1 mmol) of diisobutylaluminum hydride (1.0 M in CH_2Cl_2) via syringe. After 4 h, the reaction was cautiously quenched with 30 mL of methanol and warmed to room temperature. Water (100 mL) was added,

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and the slurry was stirred for 15 min and filtered through a Büchner funnel, rinsing the solid material several times with CH_2Cl_2 and H_2O. The filtrate was transferred to a separatory funnel and shaken, and the organic phase was removed. The aqueous phase was re-extracted with 2 × 300 mL of CH_2Cl_2. The combined CH_2Cl_2 layers were dried over $NaSO_4$, filtered, and concentrated, and the residue was passed through silica gel (200 g, ether/hexane, 1:1) to result in 2.65 g (82%) of **8** as a clear, colorless oil: IR (neat) 3530, 2970, 2930, 2860, 1500, 1390, 1365, 1280, 1250, 1230, 1195, 1165, 1110, 1090, 1055, 1015, 965, 930, 905, 885, 600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (s, 9 H), 1.30–1.70 (m, 10 H), 2.80 (d, J = 10, 1 H, exchanged with D_2O), 4.00 (d, J = 18, 1 H), 4.10 (d, J = 18, 1 H), 4.55 (m, 1 H), 4.75 (dd, $J_1 = J_2$ = 5.5), 4.95 (d, J = 5.5, 1 H), 5.75 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 23.8, 24.1, 25.0, 27.4, 36.4, 37.4, 58.2, 73.4, 73.5, 77.5, 82.7, 113.0, 130.4, 144.3; MS, m/z 282 (M^+), 253, 239, 165, 128, 111, 99, 81, 57; $[\alpha]_D$ = −18° (c = 1.29, $CHCl_3$). Anal. Calcd for $C_{16}H_{26}O_4$: C, 68.06; H, 9.28. Found: C, 67.96; H, 9.42.

(1*S*,4*R*,5*R*)-3-(*tert*-Butoxymethyl)-4,5-(cyclohexylidenedioxy)-2-cyclopenten-1-ol Methanesulfonate (11). To a 5 °C solution of 387 mg (1.37 mmol) of **8** and 0.28 mL (203 mg, 2.01 mmol) of triethylamine in 7 mL of CH_2Cl_2 (stored over 3-Å molecular sieves) was added dropwise 0.11 mL (163 mg, 1.42 mmol) of methanesulfonyl chloride. A white precipitate developed during the course of the reaction. After 45 min, the reaction mixture was poured into a separatory funnel and washed with 15 mL of cold H_2O. The aqueous layer was extracted with 25 mL of CH_2Cl_2, and the combined organic layers were washed with 15 mL of cold H_2O, dried over Na_2SO_4, filtered, and concentrated to give 504 mg (100%) of **11** as a tan oil: IR (neat) 2970, 2930, 2860, 1660, 1460, 1450, 1365, 1345, 1280, 1250, 1230, 1170, 1125, 1095, 1055, 995, 950, 910, 885, 860, 820, 730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (s, 9 H), 1.30–1.70 (m, 10 H), 3.15 (s, 3 H), 4.05 (d, J = 14, 1 H), 4.15 (d, J = 14, 1 H), 4.90 (dd, $J_1 = J_2$ = 5.5, 1 H), 4.95 (d, J = 5.5, 1 H), 5.40 (m, 1 H), 5.75 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 23.9, 24.0 24.9, 27.4, 36.5, 37.2, 39.0, 58.4, 73.7, 76.8, 81.1, 82.6, 114.1, 124.3, 148.9; MS, m/z 360 (M^+), 331, 317, 189, 165, 111, 79, 66, 57.

9-[(1′*R*,4′*R*,5′*S*)-3-(*tert*-Butoxymethyl)-4,5-(cyclohexylidenedioxy)-2-cyclopenten-1-yl]adenine (12). To a suspension of 560 mg (4.15 mmol) of adenine in 10 mL of anhydrous DMF was added 124 mg (3.10 mmol) of sodium hydride (60% dispersion in mineral oil) and 183 mg (0.70 mmol) of 18-crown-6. The mixture was heated to 60 °C for 6 h. The white suspension was cooled to room temperature; then 504 mg (1.37 mmol) of mesylate **11** was dissolved in 3 mL of DMF and added to the adenide solution. The reaction mixture was stirred at room temperature for 48 h, at which time TLC showed **11** still present. The mixture was then heated to 65 °C for 6 h, during which time **11** disappeared by TLC. The mixture was then filtered through a Büchner funnel (washing with CH_2Cl_2). The filtrate was poured into a separatory funnel and washed with 75 mL of H_2O. The aqueous phase was extracted with 2 × 75 mL of CH_2Cl_2, and the organic layers were combined, washed with 75 mL of brine, and dried over Na_2SO_4. After filtration, the solution was concentrated and the residue passed through a silica gel column (40 g, CH_2Cl_2/EtOH, 19:1 then 9:1), resulting in 250 mg of **12** (45%) as a glassy solid: mp = 80–83 °C; IR (KBr) 3310, 3165, 2960, 2925, 2845, 1640, 1595, 1570, 1470, 1415, 1365, 1330, 1295, 1245, 1195, 1165, 1105, 945, 910, 890, 800, 650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (s, 9 H), 1.35–1.75 (m, 10 H), 4.20 (m, 2 H), 4.70 (d, J = 6.0, 1 H), 5.35 (d, J = 6.0, 1 H), 5.60 (s, 1 H), 5.70 (br s, 2 H, exchanges with D_2O), 5.80 (s, 1 H), 7.70 (s, 1 H), 8.40 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 23.6, 24.0, 24.9, 27.4, 35.7, 37.2, 58.8, 64.3, 73.7, 83.5, 84.0, 113.2, 119.9, 121.6, 138.4, 149.6, 151.2, 153.0, 155.7; MS, m/z 399 (M^+), 356, 342, 326, 301, 272, 259, 244, 228, 208, 167, 136, 111, 81, 57; $[\alpha]_D$ = −77° (c = 0.206, $CHCl_3$). Anal. Calcd for $C_{21}H_{29}N_5O_3$: C, 63.14; H, 7.32; N, 17.53. Found: C, 63.44; H, 7.32; N, 17.49.

(−)-Neplanocin A. Compound **12** (200 mg, 0.50 mmol) was treated in a similar manner to **7** to give 95 mg of (−)-neplanocin A (72%): recrystallized from MeOH; mp = 211–213 °C (lit.[8e] mp 212–213 °C); IR (KBr) 3380, 3305, 3210, 2930, 2845, 1650, 1605, 1575, 1480, 1420, 1335, 1300, 1255, 1155, 1115, 1065, 1045, 710 cm^{-1}; ^1H NMR (DMSO-d_6 + D_2O) δ 4.12 (s, 2 H), 4.32 (dd, $J_1 = J_2$ = 5.5, 1 H), 4.45 (d, J = 5.5, 1 H), 5.35 (d, J = 2.7, 1 H), 5.75 (d, J = 1.7, 1 H), 8.16 (s, 1 H), 8.15 (s, 1 H); ^{13}C NMR (DMSO-d_6 + D_2O) δ 58.6, 64.5, 72.3, 76.7, 119.5, 123.8, 139.9, 149.8, 150.1, 152.5, 156.0; MS, m/z 264 (M + 1), 245, 228, 216, 186, 136, 135, 111, 108, 81; $[\alpha]_D$ = −153.3° (c = 0.30, H_2O) (lit.[3b,8e] −157° and −153.8°). Anal. Calcd for $C_{11}H_{13}N_5O_3$: C, 50.19; H, 4.98; N, 26.60. Found: C, 50.40; H, 5.13; N, 26.18.

2,3:5,6-Di-*O*-cyclohexylidene-D-gulonic Acid γ-Lactone (13). To a 2-L, three-necked round-bottomed flask equipped with a Dean-Stark trap and reflux condenser was added 50.0 g (0.28 mol) of D-gulonic acid δ-lactone (Aldrich Chemical Co.), 1 L of benzene, 165 mL of cyclohexanone, and Dowex 50W (H^+) (100 g, which was thoroughly washed with 3 N HCl, H_2O to pH 7, EtOH, and finally benzene). The well-stirred suspension was refluxed for 6 h, at which point the rate of H_2O entering the trap slowed to 1 mL/h. The reaction mixture was allowed to cool, whereupon it was filtered through a Büchner funnel and brought to pH 7 with triethylamine. The filtrate was concentrated to a gummy, yellow material, dissolved in 1.5 L of ether, and washed with 7 × 400 mL of H_2O. The ether layer was dried over Na_2SO_4, filtered, and concentrated. The yellow oil was then recrystallized from CH_2Cl_2 (100 mL) and hexane (500 mL) to give 48.8 g (51%) of **13** as a white solid: mp = 163–165 °C; IR (KBr) 2925, 2845, 1765, 1445, 1370, 1340, 1280, 1230, 1195, 1160, 1140, 1125, 1100, 1070, 1040, 1005, 975, 930, 905, 850, 830, 805, 775, 715 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30–1.80 (m, 20 H), 3.84 (m, 1 H), 4.22 (m, 1 H), 4.44 (m, 2 H), 4.74 (dd, J_1 = 4, J_2 = 6, 1 H), 4.85 (d, J = 6, 1 H); ^{13}C NMR ($CDCl_3$) δ 23.6, 23.7, 23.8, 23.9, 24.6, 25.0, 34.7, 35.3, 36.2, 36.4, 64.9, 75.1, 75.3, 75.7, 81.2, 111.0, 115.2, 173.3; MS, m/z 338 (M^+), 309, 295, 99, 81, 69, 55; $[\alpha]_D$ = −47° (c = 2.16, acetone). Anal. Calcd for $C_{18}H_{26}O_6$: C, 63.89; H, 7.74. Found: C, 64.28; H, 7.91.

2,3-*O*-Cyclohexylidene-D-gulonic Acid γ-Lactone (14). A suspension of 40.5 g (120 mmol) of **13** in 850 mL of EtOH was warmed to 50 °C until dissolution occurred. Water was slowly added until a precipitate appeared, which would not redissolve (total H_2O: 225 mL). Dowex 50W (H^+) (40 g, prewashed with 3 N HCl, H_2O to pH 7, and finally EtOH) was added and the solution stirred at 45 °C for 16 h. The reaction mixture was cooled to room temperature and filtered through a Büchner funnel. The filtrate was brought to pH 7 with triethylamine and then concentrated. The residue was taken up in 500 mL of CH_2Cl_2 and then filtered. The filtrate was washed with 50 mL of H_2O, and the aqueous phase was re-extracted with 3 × 250 mL of CH_2Cl_2. The combined CH_2Cl_2 layers were dried over Na_2SO_4, filtered, and concentrated to a syrup, which was recrystallized from CH_2Cl_2 (125 mL) and hexane (125 mL) to give 15.7 g (51%) of **14** as a white solid: mp = 114–116 °C; IR (KBr) 3460, 3410, 2960, 2930, 2860, 1760, 1460, 1450, 1415, 1375, 1345, 1295, 1275, 1265, 1230, 1210, 1160, 1115, 1095, 1050, 1015, 980, 930, 915, 890, 815, 725, 680, 640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30–1.80 (m, 10 H), 3.55 (br s, 1 H, exchanged with D_2O), 3.85 (m, 2 H), 4.10 (m, 1 H), 4.20 (m, 1 H, exchanged with D_2O), 4.70 (dd, J_1 = 9, J_2 = 6, 1 H), 4.85 (dd, $J_1 = J_2$ = 6, 1 H), 4.95 (d, J = 6, 1 H); ^{13}C NMR ($CDCl_3$) δ 23.7, 23.8, 24.6, 35.1, 36.3, 62.6, 71.1, 75.7, 76.2, 80.0, 115.0, 174.6; MS, m/z 258 (M^+), 229, 215, 99, 83, 69, 55; $[\alpha]_D$ = −62° (c = 2.16, acetone). Anal. Calcd for $C_{12}H_{18}O_6$: C, 55.81; H, 7.02. Found: C, 55.68; H, 7.08.

(2*R*,3*S*,4*RS*)-2,3-(Cyclohexylidenedioxy)-4-oxo-4-butanoic Acid γ-Lactone (15). To a solution of 7.99 g (0.20 mol) of NaOH in 500 mL of H_2O was added 43.0 g (0.17 mol) of **14**, warming slightly to dissolution. This solution was cooled to 5 °C; then a solution of 85.6 g (0.40 mol) of $NaIO_4$ in 500 mL of H_2O was added dropwise over 1.5 h. During the addition, pH 7 was maintained with 2 N NaOH solution, and the temperature rose as high as 13 °C. The reaction mixture was stirred an additional 30 min; then 7.16 g of $BaCl_2$ was added, whereupon large amounts of white precipitate appeared. After being stirred another 15 min, the suspension was filtered through Celite, and the filtrate brought to pH 3 with 3 N HCl. The filtrate was then extracted with 4 × 1 L of cold EtOAc, reacidifying after each extraction. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated to 31.5 g (88%) of **15** as a white solid. Physical and spectral data matched previously reported values.[10]

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Supplementary Material Available: Physical and spectroscopic characterizations of **9** and **10** and complete, tabulated data from the NOE experiments performed on **5** and its C-1 diastereomer (1 page). Ordering information is given on any current masthead.

A Study on the Alkylsilyl Groups in Oligoribonucleotide Synthesis

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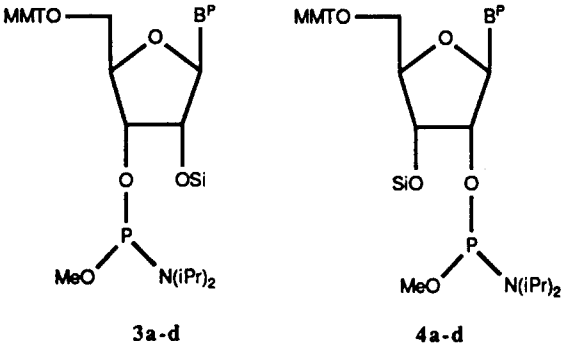
A detailed study has been carried out to show that the fidelity of the 3'-5' phosphate linkage is preserved during oligoribonucleotide synthesis when alkylsilyl groups are used to protect the 2'-hydroxyl groups. The isomeric purity of the 2'-silylated ribonucleoside 3'-phosphoramidites (**3a–d**), the key intermediates in oligoribonucleotide synthesis, have been established by comparing them with the 3'-silylated ribonucleoside 2'-phosphoramidites (**4a–d**) using ^1H and ^{31}P NMR spectroscopy. Using these 3'-amidites, a series of natural dinucleotides (A_pU, C_pU, G_pU, U_pU) were synthesized in solution. Isomeric dinucleotides with 2'-5' phosphate linkages (A_pU, C_pU, G_pU, U_pU) were prepared using the 3'-silylated nucleoside 2'-phosphoramidites. The intermediates during the syntheses and the final products were characterized by ^1H and ^{31}P NMR spectroscopy and HPLC. Comparison of the data from these two series of compounds provided unambiguous evidence for the fidelity of phosphate linkages in both the intermediates and the final products. To complete the comparison, a dinucleotide (U_pU) was prepared on a solid support.

Introduction

Some time ago, we introduced the alkylsilyl groups, principally *tert*-butyldimethylsilyl (TBDMS) and triisopropylsilyl (TIPS) groups, as 2'-hydroxyl protecting groups in oligoribonucleotide synthesis.[1] We extensively investigated the alkylsilyl groups in oligoribonucleotide synthesis both in solution and on the solid support using the phosphite triester coupling procedures.[2,3] We recently adapted the phosphoramidite coupling procedure, originally developed for the oligodeoxyribonucleotide synthesis,[4] to oligoribonucleotide synthesis on solid supports using the alkylsilyl groups as the 2'-protecting group.[5] Following this strategy, we were able to achieve the total chemical synthesis of an RNA molecule whose sequence corresponded to that of a 77-unit *E. coli* tRNA.[6]

To this point synthetic RNAs have been characterized in a number of ways. Enzymatic sequencing of synthetic RNA molecules indicated the correct ribonucleotide sequences.[5a,6] Synthetic RNAs were found to have the distinctive biological activity of natural RNAs. For example, the synthetic analogue of a tRNA retains some amino acid acceptance activity[6] even without the incorporation of modified bases common to the natural tRNA. Recently, several ribozymes have been synthesized.[7] These catalytic RNAs do not have rare bases in their sequences. Synthetic ribozymes have an activity comparable to those made by biochemical methods.[8] These examples have clearly demonstrated the success of the silyl protecting groups in oligoribonucleotide synthesis. Others have also described the successful use of the silyl protecting groups in oligoribonucleotide synthesis using the H-phosphonate coupling procedure.[9]

Scheme I. Ribonucleoside 3'-Phosphoramidites and 2'-Phosphoramidites



3a-d **4a-d**

a: B^P = N^6-benzoyladenine, Si = TBDMS
b: B^P = N^4-benzoylcytosine, Si = TBDMS
c: B^P = N^2-phenoxyacetylguanine, Si = TIPS
d: B^P = uracil, Si = TBDMS

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