The Stereocontrolled Synthesis of Phthalic Acid 4,5-*cis*-Dihydrodiol. An Unambiguous Structural Assignment of the Bacterial Metabolite of Phthalic Acid

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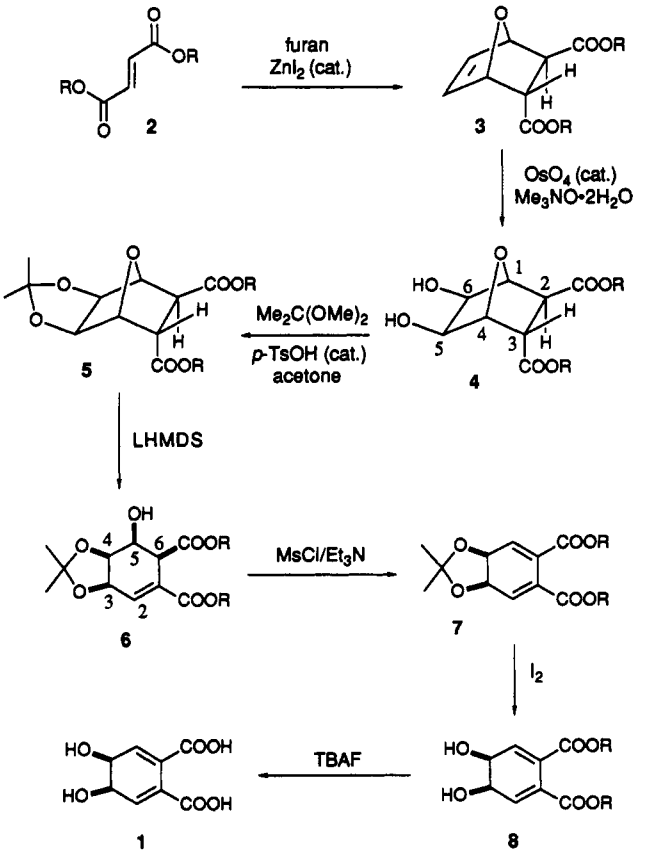
The stereocontrolled synthesis of the extremely labile phthalic acid 4,5-*cis*-dihydrodiol (1) has been achieved in a highly efficient manner in seven steps from bis[2-(trimethylsilyl)ethyl] fumarate (2). The Diels–Alder adduct 3 of the fumarate with furan has been converted into first *exo-cis*-diol 4 and then its acetonide 5. Treatment of acetonide 5 with lithium hexamethyldisilazide results in the regioselective formation of olefinic alcohol 6. Following the introduction of the second double bond into the cyclohexene ring system, the highly sensitive deprotection of the acetonide group of dihydrodiol diester acetonide 7 can be effected under iodine catalysis. The hydrolysis of the 2-(trimethylsilyl)ethyl ester group has been achieved by treatment with tetra(*n*-butyl)-ammonium fluoride in THF. This synthesis constitutes an unambiguous assignment of the structure and stereochemistry of the microbial metabolite of phthalic acid.

In recent years, there has been growing interest in the study of bacterial degradation of aromatic compounds in soil, particularly from an ecological standpoint.[1] Phthalic acid is one such aromatic compound of major importance since it is released in large quantities into the environment by both natural and industrial processes.[2] It is postulated that phthalate is first dihydroxylated by phthalate dioxygenase to give phthalic acid 4,5-*cis*-dihydrodiol, which is further degraded to β-carboxy-*cis*,*cis*-muconic acid.[3] However, the structure of this dihydrodiol, including its stereochemistry, remains to be unequivocally established. As an extension of our synthesis of shikimic acid with the use of the Diels–Alder reaction of 4-acetoxy-1-(phenyldimethylsilyl)-1,3-butadiene with an acrylate,[4] it was envisioned that adaptation of a similar Diels–Alder approach using furan would lead to the stereocontrolled synthesis of phthalic acid 4,5-*cis*-dihydrodiol (1), thus unambiguously assigning the structure of the above bacterial metabolite of phthalic acid.

It was evident from the outset that this dihydrodiol metabolite is exceptionally labile under both acidic and basic conditions, undergoing facile dehydration to produce 4-hydroxyphthalic acid. Therefore, the choice of the carboxylic acid protecting group was of prime concern. For example, attempts at the hydrolysis of the methyl ester derivative of 4,5-dihydrodiol 1 under various conditions resulted uniformly in the clean formation of the aromatized compound. On the basis of our previous successful experience in the synthesis of shikimic acid, the 2-(trimethylsilyl)ethyl group was selected for protection of the carboxyl functionality.[4] This protecting group can be removed by treatment with fluoride ion under virtually neutral conditions. The completed synthesis of phthalic acid 4,5-*cis*-dihydrodiol (1) is outlined in Scheme I.

The starting fumarate ester 2 was obtained in 81 % yield by the treatment of fumaryl chloride with 2-(trimethylsilyl)ethanol. The Diels–Alder reaction of this ester with excess furan at room temperature, overnight, in the presence of a catalytic amount of zinc iodide[5,6] provided

Scheme I. Synthesis of Phthalic Acid *cis*-4,5-Dihydrodiol (1) (R = $CH_2CH_2SiMe_3$)



adduct 3 in 41% yield, with 45% recovery of the starting ester (75% yield based on recovered diester 2). Various attempts at improving the yield of this Diels–Alder reaction by changing the reaction conditions (higher reaction temperatures, longer reaction time, and larger amounts of the catalyst) were unsuccessful. As is apparent from the synthetic route summarized in Scheme I, it is conceivable that the cis isomer of 2 may be utilized for the synthesis of 1 following the same approach. Thus, the expected endo-adduct with furan may be subsequently converted into the intermediate 7. However, the use of the cis isomer, obtained by treatment of maleic anhydride with 2-(trimethylsilyl)ethanol[7] in 50% yield, in lieu of 2, was found

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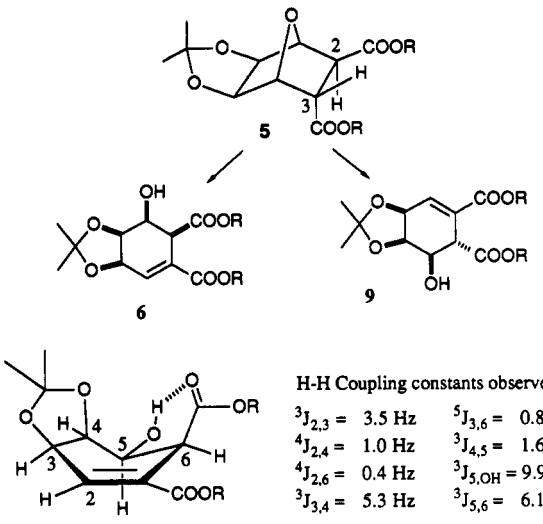
Scheme II. Two Possible Pathways of the Deprotonation–Ether Ring Opening Sequence from 5 (R = $CH_2CH_2SiMe_3$)



H-H Coupling constants observed:

$^3J_{2,3}$ =	3.5 Hz	$^5J_{3,6}$ =	0.8 Hz
$^4J_{2,4}$ =	1.0 Hz	$^3J_{4,5}$ =	1.6 Hz
$^4J_{2,6}$ =	0.4 Hz	$^3J_{5,OH}$ =	9.9 Hz
$^3J_{3,4}$ =	5.3 Hz	$^3J_{5,6}$ =	6.1 Hz

Figure 1. Stereostructure of olefinic alcohol 6 on the basis of the observed H–H coupling constants. NMR spectrum was obtained in $CDCl_3$ at 300 MHz.

to be more problematic due to the lower yield of the endo-Diels–Alder adduct (30%), and the formation of several unidentifiable side products. Cis-dihydroxylation of 3 with the catalytic osmium tetraoxide oxidation procedure[8] provided diol 4 in high yield, with virtually complete stereoselectivity. The stereochemistry of the cis diol group was ascertained as exo (see 4) on the basis of the lack of observable proton–proton vicinal couplings between protons at C-1 and C-6 and those at C-4 and C-5.

The crucial ether ring opening was realized by the treatment of the diol acetonide 5 with 1.5 mol equiv of lithium hexamethyldisilazide,[5,6] resulting in the selective formation of olefinic alcohol 6 in 54% yield, together with the starting ether (20%) and a trace amount of aromatized compound(s). The alternative pathway involving deprotonation of 2-H followed by ether bridge opening should lead to the formation of the isomeric olefinic alcohol 9 (see Scheme II). The structure of the base-promoted ether ring-opened product was assigned on the basis of extensive proton–proton coupling constant analysis (see Figure 1). The most diagnostic coupling constants include the large (9.9 Hz) splitting between the OH and 5-H and the characteristic W-type coupling between 2-H and 4-H. These clearly define the stereo and conformational structure of the product as shown in Figure 1. The proposed conformation possessing the relatively strong intramolecular hydrogen bonding was further corroborated by the following spectroscopic observation: the low-field appearance of the hydroxylic proton (at 5.17 ppm) in its proton NMR spectrum and the lower frequency shift of the carbonyl C=O stretching absorption of the nonconjugated ester group to 1709 cm^{-1} in its IR spectrum. This complete regioselectivity may be a manifestation of the difference in accessibility of the two acidic, 2-endo and 3-exo, hydrogens for deprotonation by the base lithium hexamethyldisilazide.

The introduction of the second double bond into the cyclohexene ring system was achieved smoothly through the mesylate derivative[9] of 6, which spontaneously underwent elimination during the reaction and purification of the product by flash column chromatography on silica gel. Deprotection of the acetonide group of 7 proved to be highly problematic due to the highly sensitive nature of the *cis*-dihydrodiol system. Among numerous Lewis and Brönsted acid catalysts examined, iodine[10] was found to be ideal for the deprotection of the acetonide group in this labile molecule. Thus, refluxing of acetonide 7 in methanol for 3 h in the presence of 1.1 equiv of iodine provided dihydrodiol diester 8 in 70% yield, after purification by flash column chromatography, in addition to a trace amount (<5%) of the aromatized product(s).

Hydrolysis of the 2-(trimethylsilyl)ethyl ester of 8 was accomplished by its treatment with tetra(*n*-butyl)ammonium fluoride in THF at room temperature. The resulting diacid was found to be identical with the product obtained from phthalic acid by phthalate dioxygenase by proton NMR and reversed-phase HPLC analyses (see the Experimental Section). Further evidence for their structural unanimity was obtained by the observation that the synthetic diacid was a substrate for phthalate 4,5-dihydrodiol dehydrogenase.[3] Since the dihydrodiol diacid was found to gradually undergo dehydration to produce 4-hydroxyphthalic acid, upon standing even at 0 °C, an accurate yield of the resulting diacid could not be determined. However, it was estimated to be around 70% on the basis of a coupled enzyme assay and proton NMR analysis. This 7-step synthesis of the extremely labile phthalic acid *cis*-4,5-dihydrodiol described herein constitutes the first unambiguous assignment of the stereochemistry of the bacterial metabolite of phthalic acid.

Experimental Section

Bis[2-(trimethylsilyl)ethyl] Fumarate (2). To a cold (−42 °C), stirred solution of 10.0 mL (70 mmol) of 2-(trimethylsilyl)ethanol and 8.50 g (84 mmol) of triethylamine in 100 mL of dichloromethane was added dropwise 4.92 mL (46 mmol) of fumaryl chloride. After being stirred at −42 °C for 2 h, the reaction mixture was diluted with 200 mL of dichloromethane, the resulting mixture was washed successively with water (100 mL), brine (150 mL), and water (150 mL), and the organic layer was dried ($MgSO_4$). Rotary evaporation of the solvent left an oily crude product, which was purified by flash column chromatography on silica gel using ethyl acetate/hexanes (1:6) as the eluent to yield 9.0 g (81%) of diester 2 as a white solid: mp 32–33 °C (methanol–water); ^1H NMR (300 MHz, $CDCl_3$) δ 0.058 (s, 18 H), 1.01–1.08 (m, 4 H) and 4.26–4.33 (m, 4 H) [two AA'XX' spin systems], 6.82 ppm (s, 2 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ −1.55, 17.26, 63.61, 133.70, 165.20 ppm; IR (KBr) 2957 (m), 1733 (s), 1300 (s), 1257 (s) cm^{-1}. Anal. Calcd for $C_{14}H_{28}O_4Si_2$: C, 53.12; H, 8.92. Found: C, 52.73; H, 9.27.

Bis[2-(trimethylsilyl)ethyl] (2-*exo*,3-*endo*)-7-Oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate (3). A mixture of 3.70 g (11.6 mmol) of diester 2 and 1.58 g (23.2 mmol) of furan was stirred at room temperature for 2 days in the presence of 0.74 g (2.32 mmol) of anhyd zinc iodide. The reaction mixture was then diluted with 150 mL of ethyl acetate, and the resulting solution was washed with 50 mL of 0.1 M aqueous sodium thiosulfate, dried ($MgSO_4$), and concentrated by rotary evaporation. Purification by flash column chromatography using ethyl acetate/hexanes (1:9) as the eluent yielded, in addition to 1.67 g (45%) of 2, 1.50 g (41%) of 3 as a white solid, which was recrystallized from methanol at −42 °C: mp 48–49 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.046 (s, 9 H), 0.051 (s, 9 H), 1.00–1.03 (m, 2 H)/1.03–1.59 (m, 2 H) and 4.11–4.18 (m, 2 H)/4.21–4.27 (m, 2 H) [two sets of apparent AA'XX' spin systems], 2.83 (d, 1 H, J = 3.9 Hz), 3.60 (dd, 1 H, J = 5.0, 4.0 Hz), 5.22–5.24 (m, 2 H), 6.35 (dd, 1 H, J = 5.7, 1.5 Hz), 6.52 ppm (dd, 1 H, J = 5.7, 1.7 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ −1.52 (6 C), 17.42 (2 C), 47.64

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(2 C), 63.24, 63.58, 79.26, 82.54, 134.92, 136.69, 170.95, 172.11 ppm; IR (KBr) 2960 (m), 1726 (s), 1175 (s), 840 (s) cm^{-1}. Anal. Calcd for $C_{18}H_{32}O_5Si_2$: C, 56.21; H, 8.39. Found: C, 56.01; H, 8.49.

Bis[2-(trimethylsilyl)ethyl] (2-*exo*,3-*endo*,5-*exo*,6-*exo*)-5,6-Dihydroxy-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylate (4). To a stirred solution of 2.22 g (5.7 mmol) of **3** and 639 mg (5.7 mmol) of trimethylamine *N*-oxide dihydrate in 60 mL of dry acetone was added 580 mg (0.057 mmol) of 2.5 wt % osmium tetraoxide in 2-methyl-2-propanol at room temperature under nitrogen. After being stirred overnight at room temperature, the reaction was quenched by successive additions of 300 mg of solid sodium hydrosulfite, 2.0 g of 230–400-mesh silica gel, and 12 mL of water. The resulting mixture was filtered, and the acetone was removed by rotary evaporation. The aqueous solution thus obtained was extracted with dichloromethane (2 × 60 mL), and the combined organic layers were dried over $MgSO_4$. Rotary evaporation of the solvent left a solid residue, which was purified by flash column chromatography on silica gel with ethyl acetate/hexanes (1:2) as the eluent to yield 1.90 g (80%) of diol **4** as a white crystalline solid: mp 94–95 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.043 (s, 9 H), 0.059 (s, 9 H), 0.97–1.06 (m, 4 H), 2.95 (d, 1 H, J = 5.5 Hz), 3.41 (dd, 1 H, J = 5.5, 5.5 Hz), 3.75–3.87 (br m, 2 H), 3.88–3.94 (br s, 1 H), 3.95–3.99 (br s, 1 H), 4.15–4.27 (m, 4 H), 4.58 (dd, 1 H, J = 5.5, 1.0 Hz), 4.64 ppm (d, 1 H, J = 1.0 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ −1.51 (6 C), 17.36, 17.39, 46.65, 47.58, 63.97 (2 C), 71.34, 73.80, 82.93, 85.87, 170.38, 171.54 ppm; IR (KBr) 3377 (m), 3300 (m), 2954 (m), 1730 (s), 1177 (s), 835 (s) cm^{-1}. Anal. Calcd for $C_{18}H_{34}O_7Si_2$: C, 51.64; H, 8.19. Found: C, 51.56; H, 8.29.

Bis[2-(trimethylsilyl)ethyl] (2-*exo*,3-*endo*,5-*exo*,6-*exo*)-5,6-Dihydroxy-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylate Acetonide (5). A solution of 300 mg (0.72 mmol of diol **4** and 0.263 mL (2.16 mmol) of 2,2-dimethoxypropane in 10 mL of dry acetone was heated at 60 °C for 4 h in the presence of 2.0 mg (0.007 mmol) of *p*-toluenesulfonic acid monohydrate. After the solution was cooled to room temperature, the solvent was removed by rotary evaporation and the resulting oily residue was dissolved in 30 mL of dichloromethane. The solution was washed with 20 mL of saturated aqueous $NaHCO_3$ and was dried over $MgSO_4$. Rotary evaporation of the solvent left a crude oil, which was purified by flash column chromatography on silica gel using ethyl acetate/hexanes (1:4) as the eluent to afford 329 mg (quantitative) of **5** as a colorless liquid: bp 135 °C (1.5 Torr); ^1H NMR (300 MHz, $CDCl_3$) δ 0.036 (s, 9 H), 0.056 (s, 9 H), 0.96–1.05 (m, 4 H), 1.28 (s, 3 H), 1.46 (s, 3 H), 2.88 (d, 1 H, J = 5.3 Hz), 3.44 (dd, 1 H, J = 5.6, 5.3 Hz), 4.17–4.24 (m, 4 H), 4.26 (1 H), and 4.35 (1 H) [ABq, J_{AB} = 5.5 Hz], 4.62 (d, 1 H, J = 5.6 Hz), 4.71 ppm (s, 1 H); ^{13}C NMR (75.5 MHz, CDC_3) δ −1.51 (6 C), 17.43 (2 C), 25.19, 25.85, 45.75, 46.99, 63.93, 63.96, 79.44, 79.85, 81.64, 83.03, 119.96, 170.45, 171.41 ppm; IR (neat) 2956 (m), 1726 (s), 1265 (s), 738 (s) cm^{-1}. Anal. Calcd for $C_{21}H_{38}O_7Si_2$: C, 54.99; H, 8.35. Found: C, 55.04; H, 8.37.

(±)-Bis[2-(trimethylsilyl)ethyl] 3β,4β,5β-Trihydroxy-1-cyclohexene-1,6-dicarboxylate 3,4-*O*-Acetonide (6). To a cold (−42 °C), stirred 1.0 M solution of lithium hexamethyldisilazide in 0.656 mL of THF (0.656 mmol) was added 201 mg (0.438 mmol) of acetonide **5** in 2 mL of THF. After 4 h at −42 °C, the reaction mixture was warmed to −23 °C and was kept at that temperature for 1.5 h. The reaction was then quenched by addition of 15 mL of saturated aqueous ammonium chloride, and the resulting mixture was extracted with dichloromethane (2 × 20 mL). The combined organic layers were washed in series with water (20 mL), brine (20 mL), and water (20 mL) and dried over $MgSO_4$. Rotary evaporation of the solvent left crude product, which was purified by flash column chromatography on silica gel using ethyl acetate/hexanes (1:3) as the eluent to yield 107 mg (54%) of olefinic alcohol **6** as a white solid: mp 86–87 °C (petroleum ether); ^1H NMR (300 MHz, $CDCl_3$) δ 0.0072 (s, 9 H), 0.0423 (s, 9 H), 0.89–1.06 (m, 4 H), 1.30 (s, 3 H), 1.35 (s, 3 H), 3.84 (ddd, 1 H, J = 6.1, 0.8, 0.4 Hz), 3.93–4.04 (m, 2 H), 4.23–4.35 (m, 3 H), 4.53 (ddd, 1 H, J = 5.3, 1.6, 1.0 Hz), 4.68 (ddd, 1 H, J = 5.3, 3.5, 0.8 Hz), 5.17 (d, 1 H, J = 9.9 Hz), 6.76 ppm (ddd, 1 H, J = 3.5, 1.0, 0.4 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ −1.87 (3 C), −1.68 (3 C), 16.97, 17.21, 26.01, 26.69, 39.99, 63.15, 63.61, 68.85, 72.80, 76.19, 110.55, 128.43, 134.62, 165.49, 172.15 ppm; IR (KBr) 3430 (s), 2957 (s), 1716 (s), 1709 (s), 1232 (br, s) cm^{-1}. Anal. Calcd for $C_{21}H_{38}O_8Si_2$: C, 54.99; H, 8.35. Found: C, 55.11; H, 8.36.

Bis[2-(trimethylsilyl)ethyl] *cis*-5,6-Dihydroxy-1,3-cyclohexadiene-2,3-dicarboxylate *O*-Acetonide (7). To a cold (0 °C), stirred solution of 279 mg (0.609 mmol) of olefinic alcohol **6** and 0.255 mL of triethylamine (1.83 mmol) in 3 mL of dry dichloromethane was added dropwise 0.141 mL (1.83 mmol) of methanesulfonyl chloride. After 1 h at 0 °C, the solution was diluted with 20 mL of dichloromethane and the resulting mixture was washed in series with ice–water (10 mL), 1 M aqueous HCl (10 mL), saturated aqueous $NaHCO_3$ (10 mL), and water (10 mL), and the organic layer was dried over $MgSO_4$. Rotary evaporation of the solvent left an oily crude product, which was purified by flash column chromatography using ethyl acetate/hexanes (1:4) as the eluent to yield 238 mg (88%) of diene **7** as a colorless liquid: ^1H NMR (300 MHz, $CDCl_3$) δ 0.040 (s, 18 H), 0.99–1.06 (m, 4 H) and 4.22–4.29 (m, 4 H) [two AA'XX' spin systems], 1.38 (s, 3 H), 1.41 (s, 3 H), 4.74 (dd, 2 H, J = 2.3, 1.4 Hz), 6.58 ppm (dd, 2 H, J = 2.3, 1.4 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ −1.50 (6 C), 17.34 (2 C), 24.94, 26.67, 63.63 (2 C), 70.07 (2 C), 106.75, 128.96 (2 C), 131.40 (2 C), 166.21 (2 C) ppm; IR (film) 2956 (m), 1724 (s), 1252 (s) cm^{-1}; HRMS calcd for $C_{21}H_{36}O_6Si_2$-H [$(M + H)^+$] m/z 441.2128, found m/z 441.2098.

Bis[2-(trimethylsilyl)ethyl] *cis*-5,6-Dihydroxy-1,3-cyclohexadiene-2,3-dicarboxylate (8). Diene acetonide **7** (200 mg, 0.454 mmol) and iodine (129 mg, 0.499 mmol) were dissolved in 13 mL of dry methanol. The solution was cooled to −78 °C and degassed by several evacuation–argon purge sequences using a vacuum pump, followed by flushing with a stream of deoxygenated argon delivered through a syringe needle. The degassed solution was heated at reflux for 3 h. After the solution was cooled to room temperature, solid sodium thiosulfate was added until the solution turned from deep red to white. The methanol was removed by rotary evaporation, the resulting residue was dissolved in 30 mL of dichloromethane, and the solution was dried over $MgSO_4$. Rotary evaporation of the solvent left the crude solid residue, which was purified by flash column chromatography on silica gel using ethyl acetate/dichloromethane (1:2) as the eluent to yield 127 mg (70%) of diol **8** as a white crystalline solid: mp 104.0–104.5 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.048 (s, 18 H), 0.98–1.05 (m, 4 H) and 4.21–4.28 (m, 4 H) [two AA'XX' spin systems], 2.92–3.04 (br s, 2 H), 4.34 (br s, 2 H), 6.73 ppm (dd, 2 H, J = 2.0, 2.0 Hz); ^{13}C NMR (75.5 MHz, $CDCL_3$) δ −1.52 (6 C), 17.37 (2 C), 63.74 (2 C), 67.16 (2 C), 129.55 (2 C), 136.52 (2 C), 165.95 (2 C) ppm; IR (KBr) 3340–3314 (br, s), 2955 (s), 1726 (s), 1252 (s), 1080 (s), 864 (s), 837 (s) cm^{-1}. Anal. Calcd for $C_{18}H_{32}O_6Si_2$: C, 53.97; H, 8.05. Found: C, 53.76; H, 7.94.

***cis*-5,6-Dihydroxy-1,3-cyclohexadiene-2,3-dicarboxylic Acid (Phthalic Acid *cis*-4,5-Dihydrodiol) (1).** To a solution of 29 mg (0.073 mmol) of diester **8** in 2 mL of dry THF was added 154 mg (0.42 mmol) of tetra(*n*-butyl)ammonium fluoride at room temperature. The mixture was kept stirring at room temperature for 30 min. The solvent was then removed by rotary evaporation to dryness, and the resulting viscous oily residue was dissolved in 2 mL of distilled water and applied to a fast-flow DEAE-Sepharose anion exchange column (2.5 cm × 8 cm). The column was flushed with a 0–1 M NH_4OAc gradient (100 mL). The product eluted at 0.5 M NH_4OAc. The product was detected by absorbance at 280 nm. The yield for the ester hydrolysis was estimated to be around 70% on the basis of the product concentration determined by a coupled enzyme assay.[3] NH_4OAc was removed by rotary evaporation. Product was exchanged into D_2O by five successive rotary evaporations for its ^1H NMR analysis. The HPLC trace generated from the synthetic sample was superimposable with that of the phthalic acid *cis*-4,5-dihydrodiol from phthalic acid by phthalate dioxygenase.[3] HPLC conditions are as follows: retention time 3.8 min on a C_{18} Microsorb column (5 mm i.d. × 25 cm, particle size 35–40 μm) with the use of methanol/water (20:80) containing 0.1% of trifluoroacetic acid as the eluent, and a flow rate of 0.50 mL/min. The freeze-dried sample was submitted for ^1H NMR analysis in D_2O using 4,4-dimethyl-4-silapentane sodium sulfonate as internal reference (δ 0.015 ppm for the trimethylsilyl protons): ^1H NMR (300 MHz, D_2O) δ 4.08 (apparent dd with J = 2.6, 1.7 Hz, 2 H) and 6.05 ppm (apparent dd with J = 2.6, 1.7 Hz, 2 H) [AA'XX' spin system].

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Synthesis of DNA Containing Modified Bases by Postsynthetic Substitution. Synthesis of Oligomers Containing 4-Substituted Thymine: O^4-Alkylthymine, 5-Methylcytosine, N^4-(Dimethylamino)-5-methylcytosine, and 4-Thiothymine

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A strategy is described for synthesis of oligomers modified in the 4-position of thymine by postsynthetic substitution. 4-Triazolothymine phosphoramidite monomer has been prepared in one step from thymine amidite monomer and incorporated into a 12 mer AGCGAAXTCGCT using a DNA-synthesizer. The fully protected oligomer containing 4-triazolothymine, while still bound to CPG-support, was treated at 25 °C with either alcohol/DBU, dilute aqueous NaOH, concentrated aqueous ammonia, 1,1-dimethylhydrazine, or thiolacetic acid, to produce essentially pure oligodeoxynucleotides containing O^4-alkylthymine, thymine, 5-methylcytosine, N^4-(dimethylamino)-5-methylcytosine [i.e., 4-(2,2-dimethylhydrazino)-5-methylpyrimid-2-one (T^{DH})], or 4-thiothymine respectively. This first and efficient synthesis of T^{DH} oligomers indicates that this may be a general route to the synthesis of oligomers containing thymine with a reactive group at the 4-position. The melting temperature (Tm) of a DNA duplex containing T^{DH}:G or T^{DH}:A pairs was similar to that of a duplex with A:C mismatch.

Introduction

Because of the importance of modified DNA bases in carcinogenesis, mutation, and the action of some cancer chemotherapeutic agents, there has recently been great interest in synthesis of oligodeoxyribonucleotides containing modified bases.[1,2] In general these oligomers were made by a route in which the modified nucleoside was prepared, converted to the phosphoramidite or phosphotriester monomer, and then incorporated during the synthesis of the oligomer. Each new modified base required the synthesis of a modified monomer suitable for incorporation into DNA, and often a desired modified monomer is not stable under the conditions used in DNA synthesis.

An attractive alternative strategy for synthesis of oligomers containing modified bases is to incorporate a versatile base which combines the properties of stability to the normal procedures of DNA synthesis with sufficient chemical reactivity to allow one to convert it into a number of desirable products after synthesis of the oligomer. The strategy has the following potential advantages: (1) it offers the possibility of making DNA containing a labile or chemically reactive base; (2) a single synthesis of an oligomer containing the versatile base could provide a source of oligomers each containing a different modified base; and (3) special atoms, e.g., NMR sensitive ^{17}O, ^{15}N, ^{13}C, or radioactive ^{35}S, could be introduced by simple treatment with appropriate reagents at the last step.

Because of our particular interest in O^4-alkylthymine (T^{OR}), which plays a prominent role in nitrosamine carcinogenesis[3], we started with 4-substituted thymines. We now describe the synthesis, using phosphoramidite chemistry, of the dodecadeoxyribonucleotide AGCGAAXTCGCT containing 4-triazolothymine (T^{Tri}) and the easy conversion, in high yield, of this into the parent oligomer containing thymine (T), or into five oligomers each containing a different modified base: O^4-methylthymine (T^{OMe}), O^4-ethylthymine (T^{OEt}), 5-methylcytosine (T^{NH2}), N^4-(dimethylamino)-5-methylcytosine [i.e., 4-(2,2-dimethylhydrazino)-5-methylpyrimid-2-one] (T^{DH}), or 4-thiothymine (T^{S}). So far as we know this is the first chemical synthesis of an oligonucleotide containing a base with the chemically active hydrazino side chain, and its synthesis suggests that this strategy may allow the synthesis of oligomers containing other labile bases. Preliminary results of this work have been reported.[4] While this manuscript was in preparation, MacMillan and Verdine[5] suggested a similar strategy. They incorporated 4-O-(2,4,6-trimethylphenyl)-2'-deoxyuridine into oligomers, deprotected the oligomers, and then substituted them with various amines.

4-Triazolothymidine was introduced by Reese and Skone[6] as a precursor for the synthesis of 4-substituted thymidines. It is easily and quantitatively produced and has previously been used for synthesis of O^4-alkylthymine

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