Table I. Analytical Data for Compounds I-VI

compd	yield, %	mp (solvent)	elemental anal.	^{1}H NMR	^{13}C NMR
I	85[a]	158-9 (ethyl acetate) (lit.[18] 158-9)		DMSO-d_6: 3.28 (s, 3 H), 8.12 (s, 4 H), 10.1 (s, 1 H)	DMSO-d_6: 43.13, 127.76, 130.22, 139.27, 145.27, 192.60
II	88[a]	132-3 (ethyl alcohol) (lit.[11] 124-5)		$CDCl_3$: 7.43 (m, 3 H), 7.95 (m, 2 H), 8.03 (AB, J_{AB} = 8.3, $\Delta\nu$ = 32.6, 4 H), 10.05 (s, 1 H)	$CDCl_3$: 127.44, 127.86, 129.14, 129.91, 133.42, 138.66, 140.02, 146.07, 190.50
III	50[b]	137-8 (ethyl alcohol)	calcd for $C_{14}H_{12}O_3S$: C, 64.60; H, 4.65; S, 12.32 (found: C, 64.56; H, 4.62; S, 12.20)	$CDCl_3$: 2.61 (s, 3 H), 7.55 (m, 3 H), 7.96 (m, 2 H), 8.05 (AB, J_{AB} = 6.1, $\Delta\nu$ = 6.9, 4 H)	$CDCl_3$: 26.48, 127.35, 127.49, 128.66, 129.07, 133.28, 139.86, 140.24, 144.85, 196.32
IV	84[b]	139-40 (ethyl alcohol) (lit.[14] 139-40)		$CDCl_3$: 7.56 (m, 2 H), 7.65 (m, 1 H), 7.98 (d, J = 8.7, 2 H), 8.24 (AB, J_{AB} = 8.8, $\Delta\nu$ = 71.11, 4 H)	$CDCl_3$: 124.41, 127.87, 128.85, 129.58, 134.02, 139.80, 147.14, 150.16
V	95[b]	126-7 (ethyl alcohol) (lit.[14] 125-6)		$CDCl_3$: 7.53 (m, 2 H), 7.59 (m, 1 H), 7.78 (d, J = 9, 2 H), 7.93 (d, J = 9, 2 H), 8.03 (d, J = 9, 2 H)	$CDCl_3$: 116.66, 117.15, 127.80, 128.14, 133.06, 133.95, 139.88, 145.58
VI	61[b]	151-2 (ethyl alcohol) (lit.[19] 144)		$CDCl_3$: 7.52 (m, 4 H), 7.61 (m, 2 H), 7.75 (m, 2 H), 7.95 (AB, J_{AB} = 8.14, $\Delta\nu$ = 55.35, 4 H), 7.97 (m, 2 H)	$CDCl_3$: 127.22, 127.43, 128.17, 129.11, 130.09, 132.89, 133.29, 135.85, 140.35, 141.21, 144.22, 194.63

[a] Reaction temperature = 100 °C. [b] Reaction temperature = 130 °C.

the reaction of 4-fluorobenzaldehyde with sodium benzenesulfinate at 130 °C gave 4-(phenylsulfonyl)benzaldehyde in 88% yield.

The reaction of sodium benzenesulfinate with different activated aromatic halides (Y = CN, NO_2, CH_3CO, and PhCO) was then examined, and the corresponding 4-phenylsulfonyl derivatives were isolated in 50% yield (for X = F, Y = CH_3CO) to 95% yield (for X = F, Y = CN).

We believe that the route described in this paper represents a significant improvement in the preparation of sulfone derivatives of benzaldehyde.

Experimental Section

Sodium methanesulfinate (Fairfield), sodium benzenesulfinate (Kodak), 4-fluorobenzaldehyde (Aldrich), 4-chlorobenzaldehyde (Kodak), 4-fluorobenzophenone (Aldrich), 4-fluoronitrobenzene (Kodak), 4-chloronitrobenzene (Kodak), 4-fluorobenzonitrile (Aldrich), 4-chlorobenzonitrile (Kodak), 4-fluoroacetophenone (Aldrich), 4-fluoropropiophenone (Aldrich), and 4-chlorophenyl phenyl sulfone (Aldrich) were used as received. DMSO (Kodak) was dried over 4-Å molecular sieves (Aldrich) for 24 h.

NMR spectra were obtained on a GE QE-300 instrument at 300 MHz for proton and 75 MHz for ^{13}C spectra in $CDCl_3$ solution, and shifts are referenced to TMS internal standard. All chemical shifts (δ) are in parts per million, and coupling constants are in hertz units. Melting points are not corrected. Elemental analyses were performed by Analytical Technologies Division, Eastman Kodak Company.

General Procedure. The substrate (0.1 mol) and sodium methane(benzene)sulfinate (0.11 mol) were dissolved in dry DMSO (75 mL), under nitrogen. The mixture was stirred at 100 or 130 °C (see Table I) for 16 h and then poured over ca. 200 g of ice. The solid thus formed was collected and crystallized. All the yields reported in this paper are of crystallized materials.

The following materials have been prepared: 4-(methylsulfonyl)benzaldehyde (I) from 4-fluorobenzaldehyde, 4-(phenylsulfonyl)benzaldehyde (II) from 4-fluorobenzaldehyde, 4-(phenylsulfonyl)acetophenone (III) from 4-fluoroacetophenone, 4-(phenylsulfonyl)nitrobenzene (IV) from 4-fluoronitrobenzene, 4-(phenylsulfonyl)benzonitrile (V) from 4-fluorobenzonitrile, and 4-(phenylsulfonyl)benzophenone (VI) from 4-fluorobenzophenone.

Table I summarizes the yields and analytical data for compounds I-VI. No attempt was made to improve reaction conditions and optimize yields.

Registry No. I, 5398-77-6; II, 66-39-7; III, 65085-83-8; IV, 1146-39-0; V, 28525-13-5; VI, 54687-39-7; 4-fluorobenzaldehyde, 459-57-4; 4-fluoroacetophenone, 403-42-9; 4-fluoronitrobenzene, 350-46-9; 4-fluorobenzonitrile, 1194-02-1; 4-fluorobenzophenone, 345-83-5; sodium methanesulfinate, 20277-69-4; sodium benzenesulfinate, 873-55-2; 4-chlorobenzaldehyde, 104-88-1; 4-chloronitrobenzene, 100-00-5; 4-chlorobenzonitrile, 623-03-0; 4-chlorophenyl phenyl sulfone, 80-00-2.

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A New Method for the Enzymatic Synthesis of Nucleosides Using Purine Nucleoside Phosphorylase

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Nucleoside analogues have been used extensively as antibiotic substances and as biological probes.[1-3] Recent interest in this class of compounds has been stimulated by the efficacy of certain nucleosides as antiparasitic[4] and antiviral[5-6] agents. Zidovudine (3′-azido-3′-deoxythymidine, AZT) and the various 2′,3′-dideoxynucleosides have received especial attention due to their virucidal activity in the treatment of AIDS patients.[7] The broad-spectrum antiviral activity of virazole (ribavirin, 1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide) has recently been shown to extend to the treatment of plant as well as animal viruses.[8]

Traditionally nucleosides have been prepared by various chemical methods.[3] Recently, however, a number of papers and patents have appeared reporting the enzymatic preparations of both natural and unnatural nucleosides.[9] These works employed two basic strategies (see Scheme I). The first strategy used a pyrimidine nucleoside as the glycosyl donor and a purine or purine analogue as the glycosyl acceptor. This was conducted as a one-pot reac-

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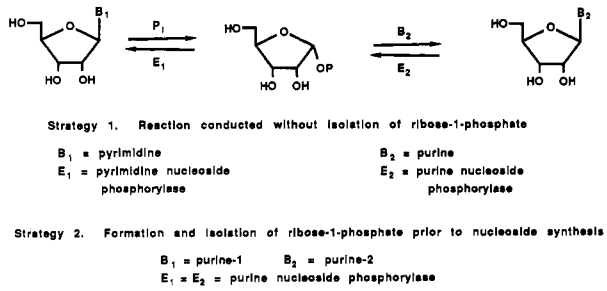
Scheme I. Strategies for the Enzymatic Syntheses of Nucleosides



Strategy 1. Reaction conducted without isolation of ribose-1-phosphate

B_1 = pyrimidine; E_1 = pyrimidine nucleoside phosphorylase; B_2 = purine; E_2 = purine nucleoside phosphorylase

Strategy 2. Formation and isolation of ribose-1-phosphate prior to nucleoside synthesis

B_1 = purine-1; B_2 = purine-2; $E_1 = E_2$ = purine nucleoside phosphorylase

tion without the isolation of α-ribose-1-phosphate (R-1-P) but required that both a pyrimidine nucleoside phosphorylase and purine nucleoside phosphorylase be present in the reaction media. The second strategy involved the enzymatic preparation of R-1-P from a nucleoside followed by the isolation of R-1-P. The isolated R-1-P was then used as the glycosyl donor in an enzymatic coupling reaction with added heterocycles. An overall purine to purine analogue exchange could be accomplished by this means. Of interest here is the report by Utagawa et al.[10] that the purine analogue 1,2,4-triazole-3-carboxamide (TCA, the aglycon component of virazole) could not be ribosylated to any measurable extent in a one-pot reaction using inosine as the ribosyl donor and purine nucleoside phosphorylase (PNPase) as the catalyst. These workers cited the low affinity of TCA (K_m = 167 mM) with respect to hypoxanthine (the phosphorolysis product of inosine, K_m = 5.6 mM) for PNPase. Hypoxanthine seems to act as a competitive inhibitor of virazole synthesis.

The disadvantages of the above enzymatic strategies are that they either require the presence of both pyrimidine and purine nucleoside phosphorylases or the isolation of R-1-P. New synthetic methods are needed which overcome these restrictions. We present in this paper a new approach to the enzymatic synthesis of nucleosides that overcomes the limitations of the current methods.

Our previous work in the development of irreversible acyl donors, such as isopropenyl acetate[11] and the vinyl esters,[12] led us to seek appropriate ribosyl donors wherein the leaving group would undergo an isomerization which would render the reaction essentially irreversible.

Table I. Nucleoside Syntheses Using N-7 Alkylated Guanosines and Inosines Catalyzed by Purine Nucleoside Phosphorylase

glycosyl donor[a]	glycosyl acceptor[b]	PNP,[c] units	time, days	yield,[d] %
1	adenine	25	4	100
2	adenine	25	4	100
3	adenine	25	2	90
1	3-deazaadenine[e]	250	2	65
2	3-deazaadenine	250	2	70
3	3-deazaadenine	250	2	73
1	TCA[f]	100	2	54
2	TCA	100	2	60
3	TCA	100	2	57

[a] 1000 μmol. [b] 250 μmol. [c] Purine nucleoside phosphorylase. [d] As determined by HPLC.[20] [e] 4-Amino-1*H*-imidazo[4,5-*c*]pyridine. [f] 1,2,4-triazole-3-carboxamide.

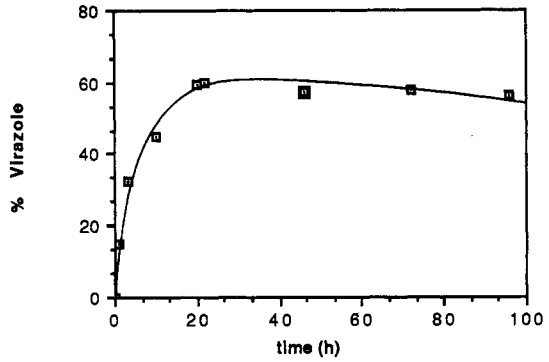


Figure 1. Enzyme-catalyzed synthesis of virazole from 7-methylguanosine. The initial reaction mixture contained 100 mM of 7-methylguanosine, 25 mM of 1,2,4-triazole-3-carboxamide, 25 mM phosphate, and 100 units of purine nucleoside phosphorylase/mmol of 7-methylguanosine at pH 7.4 and 25 °C. The formation of virazole was determined by HPLC.

The mechanisms of the purine nucleoside phosphorylase enzymes are not known in detail;[13] therefore, a reasonable mechanism was postulated and from this mechanism potential irreversible ribosyl donors were designed. The postulated mechanism is shown in Scheme II. The proposed proton transfer from an enzyme carboxylic acid side chain to the electron-rich N^7 of the purine would create an overall positive charge largely delocalized in the imidazole ring on nitrogens 7 and 9. The partial positive charge on N^9 would serve to weaken the glycosyl bond. A slight conformational change by the enzyme could then cause the two subunits to be pulled apart and the glycosidic bond broken. Trapping the intermediate ribosyl cation by phosphate would then lead to formation of R-1-P. Alternatively the last two steps may occur simultaneously as an S_N2 reaction.[13]

The first step in the proposed mechanism is the protonation of the imidazole nitrogen to give a positively charged intermediate. This intermediate and its attendant positive charge could be mimicked by the alkylation on N^7 of the purine nucleosides. Methylation occurs readily at the N^7 positions of guanosine and inosine.[14] Shugar and co-workers[15] have recently demonstrated that both 7-methylguanosine (**2**) and 7-methylinosine (**3**) are substrates for purine nucleoside phosphorylase catalyzed phosphorolysis. A V_{max} = 3.3 μmol/min per mg of enzyme

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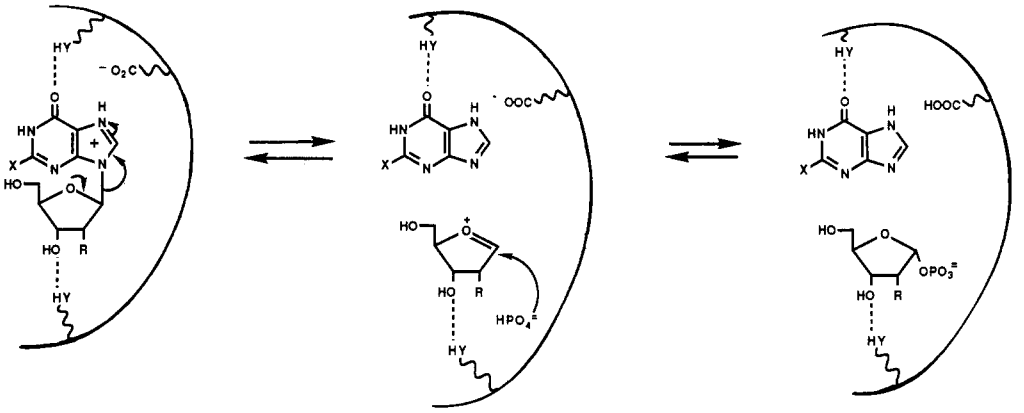
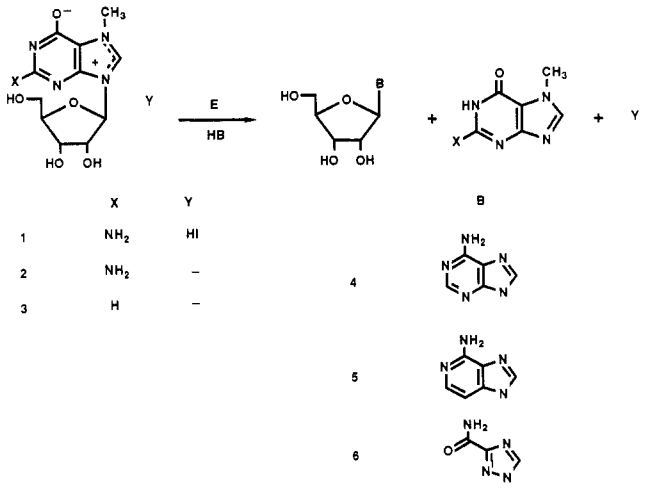
Scheme II. Hypothetical Mechanism for Purine Nucleoside Phosphorylase



Scheme III. Enzymatic Transribosylation Using 7-Methylpurines



and a K_m = 14.7 μM were reported for 7-methylguanosine. In addition, the corresponding heterocycle 7-methylguanine did not show product inhibition of the enzyme at concentrations up to the solubility limit of the heterocycle.[15]

The 7-alkylated ribosyl donors are both acid labile (cleavage of the glycosidic bond) and base labile (imidazole ring opening).[14] The enzymatic phosphorolyses of 7-methylguanosine and 7-methylinosine are also pH dependent.[15] Consequently it was found best to use a weakly buffered (0.1 M) phosphate solution corresponding to 1 mol of phosphate per mole of alkylated donor. The solution pH was monitored and adjusted to pH 7–7.4 by periodic additions of 0.5 N sodium hydroxide. Under these conditions adenine was completely converted to adenosine (4) (Scheme III). High conversions of 1,2,4-triazole-3-carboxamide[16] to virazole (5)[17] and 3-deazaadenine[18] to 3-deazaadenosine (6)[19] were also accomplished (see Table I). The time-dependent formation of virazole is shown in Figure 1. The yield of the reaction reached a maximum after approximately 24 h. The slight drop in yield after 24 h is probably due to the degradation of R-1-P in the reaction media and the enzyme-catalyzed equilibration of the reaction mixture.

The neutralized zwitterionic form of N-7 methylated guanosine (2)[14] provided a slight advantage over its hydroiodide salt (1) both in ease of preparation and in yield of transribosylation products. Transribosylation using 7-methylinosine (3)[14] was also found to be effective. Two factors favored the use of the methylated guanosines over the methylated inosine. First, the commercial cost of guanosine is approximately half that of inosine. Second, 7-methylguanine is extremely insoluble. During the transribosylation reaction the phosphorolysis of 7-methylguanosine produces 7-methylguanine, which precipitates out of solution. Upon completion of the reaction the solid 7-methylguanine can be nearly quantitatively removed by filtration or centrifugation.

The procedures described in the Experimental Section were found to be amenable to modification. For example, replacement of up to 50% of the aqueous solution volume with acetonitrile was tolerated by the enzyme. In the presence of high concentrations of acetonitrile, the reaction yields were unaffected, or in some cases slightly enhanced (i.e. 3-deazaadenosine). The reaction rates, however, were 2–4-fold slower than in the aqueous solution. Also the initial phosphate ratios were varied from 0.25 to 1.0, with respect to the amount of ribosyl donor used, without noticeably affecting the overall yields.

In summary, the use of 7-methylguanosine and 7-methylinosine as effective ribosyl donors has been established. The methods reported herein should be generally applicable to the enzymatic ribosylation of purine analogs. The enzymic phosphorolysis of 7-methylguanosine and 7-methylinosine should also provide a good in situ preparation of α-D-ribosyl-1-phosphate.[21]

Experimental Section

General. Purine nuceoside phosphorylase (PNPase) was obtained from Toyoba Chemical Co. Enzyme activities were determined using a standard inosine phosphorylase–xanthine oxidase coupled spectrophotometric assay[22] or by spectrofluorimetric assay using 7-methylguanosine as the substrate.[15] One unit of PNPase phosphorolyzes 1 μmol of inosine per minute under standard assay conditions.[22] The purine nucleosides were alkylated according to the procedure of Jones and Robins.[12] TCA[14] and 3-deazaadenine[16] were synthesized as previously reported. pH adjustments were made by the addition of 0.5 N sodium hydroxide using a Chemtrix pH controller. Reactions were monitored by

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HPLC on a Resolvex Sil 4.6 mm × 24 cm column using a mixture of 0.5 N ammonium formate (pH 4.2), methanol, and dichloromethane 2:18:80 as the mobile phase.[20] A UV detector set at 254 nm was used to monitor the column effluent.

Synthesis of 4-Amino-1-β-D-ribofuranosyl-1*H*-imidazo[4,5-*c*]pyridine (3-Deazaadenosine, 5). A solution of 333 mg (1.0 mmol) of 7-methylguanosine (2) and 42.5 mg (0.25 mmol) of 4-amino-1*H*-imidazo[4,5-*c*]pyridine hydrochloride in 10 mL of 1.0 M phosphate buffer pH 7.4 was treated with 10 mg (250 units) of purine nucleoside phosphorylase. Almost immediately a copious precipitate of 7-methylguanine began to separate from the solution. The solution was maintained at pH 7.0–7.4 for 2 days after which no further increase in product formation was observed. The solution was filtered, and the solid was washed with 2 mL of water. The combined filtrates were lyophylized, and the residue was dissolved in water–methanol (3 mL, 2:1) and loaded onto a 0.9 × 15 cm Dowex 1 (OH^-) column which had been washed with water and methanol and equilibrated in 7:3 water–methanol. The column was washed with 1:9 water–methanol prior to eluting the product with 0.07 N formic acid–methanol, 1:9. The product fractions were pooled and evaporated to give 35 mg (53 %) of 5: mp 225–230 °C [lit.[18b] 225–231 °C]; UV pH 1 λ_{max} 260 nm (ϵ 1.1 × 104), pH 13 λ_{max} 266 nm (ϵ 1.05 × 10^4) [lit.[19a] pH λ_{max} 261 nm (ϵ (1.0–1.14) × 10^4), pH 13 λ_{max} 265 nm (ϵ 1.07 × 10^4)].

Synthesis of Virazole (6). To a stirred solution of 333 mg (1.0 mmol) of 7-methylguanosine (2) and 28 mg (0.25 mmol) of 1,2,4-triazole-3-carboxamide in 10 mL of 0.25 M phosphate buffer, pH 7.8, was added 4 mg (100 units) of purine nucleoside phosphorylase. Within 5 min a copious precipitate of 7-methylguanine began to separate from the solution. The attendent drop in pH was compensated for by addition of 0.5 N NaOH. The solution was maintained at pH 7.4 for 2 days after which no further increase in product formation was observed. The insoluble materials were removed by centrifugation. The residual pellet was resuspended in 2 mL of water and centrifuged, and the supernatant liquid was combined with the original solution. The combined solutions were lyophylized, and the residue was dissolved in water (2 mL) and added to the top of a 0.9 × 15 cm Dowex 1 (OH^-) column. The column was washed with water (25 mL) and then eluted with 0.05 M ammonium phosphate buffer (pH 9). The product fractions were pooled, concentrated to a small volume, and desalted on a 1 × 15 cm Sehadex G-10 column. The product fractions were pooled and lyophylized to give 26 mg (44%) of virazole. The product was crystallized by dissolving the solid in a small amount of water and diluting the solution with absolute ethanol. The solid that separated was filtered, washed with absolute ethanol, and ether, and then dried over P_2O_5: mp 164–165 °C [lit.[17a] mp 166–168 °C]. Fast atom bombardment mass spectrometry indicated a molecular weight of 244, and the ^1H NMR spectrum agreed with the reported values.[17a]

With a proportional increase of each component, both 5 and 6 can be prepared in gram quantities.

Acknowledgment. Support of this work by W. R. Grace Co. is gratefully acknowledged.

Short Intramolecular Diels–Alder Approach to Spirovetivanes. Total Synthesis of *dl*-Hinesol[1]

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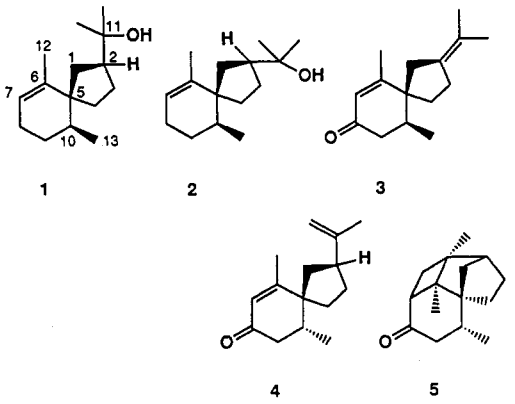
Received December 15, 1988

The spiro[4.5]decane skeleton[2] is present in several groups of sesquiterpenoids such as the spirovetivanes, acoranes, alaskanes, and laurenones.[3] Spirovetivanes are structurally recognized by a methyl group at C-10 and an isopropyl group at C-2. Depending on the relative stereochemistry at C-10, the spirovetivanes are divided into two groups. The more numerous group (at present), here represented by hinesol (1),[4,5] agaspirol (2),[6] and β-vetivone (3),[7] has the methyl group β (cis). The other group, here represented by solavetivone (4)[7] and solanascone (5),[7] has the methyl group α (trans).



Our synthetic plan (Scheme I) relies on the keto aldehyde 6 as a key precursor, which can be recognized as an ozonolysis product of the norbornene derivative 7, which in turn is the result of a hydrogenation of the disubstituted double bond in the norbornadiene derivative 8. Retrosynthetic disconnection a in 8 suggests an intramolecular cycloaddition transform leading to the 1-substituted cyclopentadiene 9 (R = Me) as a precursor. This strategy inherently permits control of the relative stereochemistry at C2 and C5, which we recently demonstrated in a model study in which 6 ($R_1 = R_2 = H$) was synthesized.[8] Herein we apply this intramolecular Diels–Alder-based[9] route to the specific case of *dl*-hinesol (1).[10,11]

The Diels–Alder substrate 9 (R = Me) was prepared in 48% overall yield from 3-methyl-2-cyclohexenone (Aldrich)

† Stockholm, Sweden.
‡ Notre Dame, IN.

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