Table II. Experimental Details

	5a	13c
cryst size, mm	0.05 × 0.20 × 0.5	0.12 × 0.20 × 0.30
max θ, deg	57	57
no. of rflctns	2506	4316
no. of obsd rflctns	1641	3199
abs cor	none	none
least squares	full matrix	block diagonal (two blocks)
refinement	anisotropic	anisotropic
heavier atoms	isotropic (fixed)	isotropic (fixed)
hydrogen atom	0.081	0.048
final R	0.085	0.047
final R_w	0.3	0.2
final difference ma--largest peak, e Å^{-3}		

(UV, IR, NMR, TLC) with an authentic sample.[8]

Anal. Calcd for $C_{11}H_{11}N_2O_2Cl$: C, 55.36; H, 4.65; N, 11.74; Cl, 14.85. Found: C, 55.45; H, 4.60; N, 11.64; Cl, 14.87.

Tryptophan (7a). Compound **13a** (20 g, 0.062 mol) was hydrogenated and worked up as described above to yield 7.98 g (63%) of recrystallized **7a**, mp 291 °C dec (lit.[5] mp 293 °C), which was identical (UV, IR, NMR, TLC) with an authentic sample (Eastman).

Anal. Calcd for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.72. Found: C, 64.64; H, 5.86; N, 13.67.

6-Methyltryptophan (7b). Compound **13b** (70 g, 0.21 mol) was hydrogenated and worked up as described above to yield 27.6 g (61%) of recrystallized **7b**, mp 297 °C dec (lit.[6] mp 298–300 °C), which was identical (UV, IR, NMR, TLC) with an authentic sample (Fluka).

Anal. Calcd for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47; N, 12.84. Found: C, 66.08; H, 6.62; N, 12.88.

Crystallography. Crystals of **5a** were prepared from ethanol, and the crystals of **13c** were obtained from *N,N*-dimethylformamide/benzene. Crystal data for **5a** and **13c** are listed in Table I. The intensity data were measured on a Hilger-Watts four-circle diffractometer (Ni filtered Cu Kα radiation, θ–2θ scans, pulse-height discrimination). Both structures were solved by a multiple-solution procedure.[11] Details of the analyses are summarized in Table II.

Registry No. **1a**, 88-72-2; **1b**, 89-58-7; **1c**, 89-59-8; **2a**, 32991-03-0; **2c**, 32989-56-3; **4a**, 71463-16-6; **4b**, 71463-17-7; **4c**, 71463-18-8; **5a**, 71463-19-9; **5b**, 71463-20-2; **6a**, 64258-95-3; **6b**, 71463-21-3; **7a**, 54-12-6; **7b**, 2280-85-5; **7c**, 17808-21-8; **9**, 71463-22-4; **10**, 71463-23-5; **12a**, 53868-36-3; **12b**, 70082-60-9; **12c**, 71463-24-6; **13a**, 71463-25-7; **13b**, 71463-26-8; **13c**, 71463-27-9; **14c**, 71463-28-0; *N,N*-dimethylformamide dimethyl acetal, 4637-24-5; diethyl formamidomalonate, 6326-44-9; methyl nitroacetate, 2483-57-0.

Supplementary Material Available: Tables of the final atomic parameters, bond lengths, and bond angles for compounds **5a** and **13c** (8 tables, 9 pages). Ordering information is given on any current masthead page.

(11) G. Germain, P. Main, and M. M. Woolfson, *Acta Crystallogr., Sect. A*, **27**, 368 (1971).

An Improved Synthesis of Agaritine[1]

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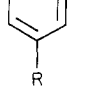
Eppley Institute, University of Nebraska Medical Center, Omaha, Nebraska, 68105

Received April 20, 1979

L-Glutamic acid 5-[2-[4-(hydroxymethyl)phenyl]hydrazide] (agaritine, **4a**), a compound present in *Agaricus bisporus*, the commercial edible mushroom, was synthesized for the bioassay of its possible tumorigenic properties. The mixed anhydride derived from 1-benzyl *N*-(benzyloxycarbonyl)-L-glutamate and ethyl chloroformate reacted with 4-carboxyphenylhydrazine (**1a**) to form the benzyl ester of *N*-(benzyloxycarbonyl)-L-glutamic acid 5-[2-(4-carboxyphenyl)hydrazide] (**3**). Reduction of **3** with BH_3/THF gave the corresponding 4-(hydroxymethyl)phenyl derivative (**5a**) which on hydrogenolysis in THF over Pd/C gave **4a**. The overall yield from **1a** was 25%, some 25-fold higher than previously obtained.

The amino acid L-glutamic acid 5-[2-[4-(hydroxymethyl)phenyl]hydrazide] (**4a**), called agaritine by its discoverer,[2] is a constituent of edible mushrooms classified as *Agaricus bisporus*. These are the ordinary mushrooms of commerce in the Western hemisphere. Because hydrazine and many of its derivatives have marked physiological activity, including the ability to induce cancers in laboratory animals,[3] a bioassay of agaritine for potential tumorigenic activity is presently underway in this Institute. More than 1 kg of agaritine may be required during the course of the bioassay and it seemed desirable to obtain these quantities by synthesis rather than by isolation from mushrooms. An earlier synthesis[4] was on a scale sufficient to provide proof of structure, but the yield of product based on 4-carboxyphenylhydrazine (**1a**) was only 1%.



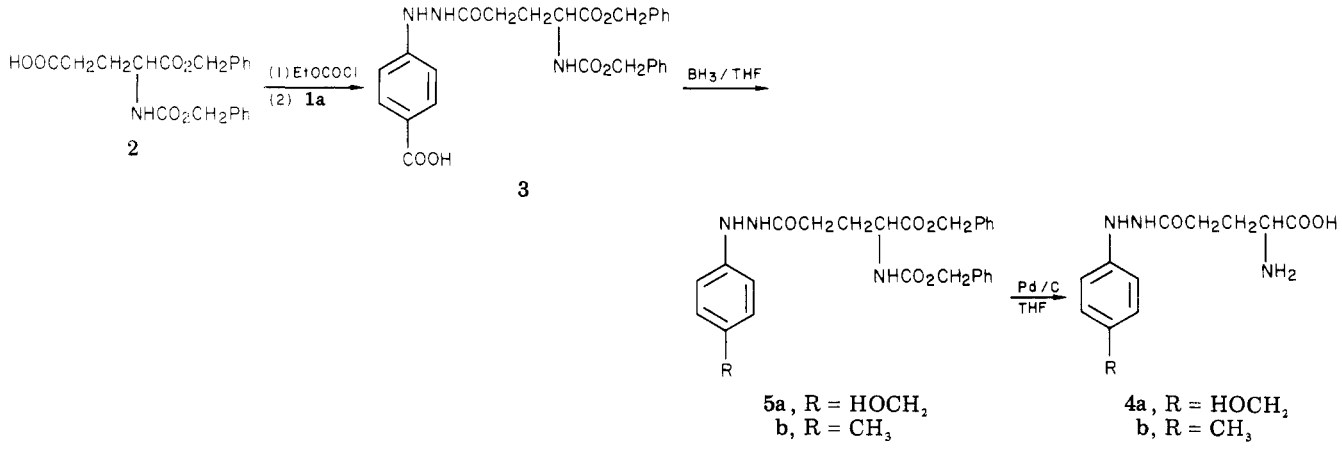
1a, R = HOOC-
b, R = $HOCH_2$-

Furthermore, ion-exchange column chromatography was used in the last step and required 5 L of packing to purify 1 g of crude agaritine. Scale-up of this synthesis to the

(1) This work was done under contract (N01 CP33278) with the Public Health Service (NIH-NCI).
(2) B. Levenberg, *J. Biol. Chem.*, **239**, 2267 (1964).
(3) B. Toth, *Cancer Res.*, **35**, 3693 (1975).
(4) R. B. Kelly, E. G. Daniels, and J. W. Hinman, *J. Org. Chem.*, **27**, 3229 (1962).

0022-3263/79/1944-3752$01.00/0

Scheme I



10-g level would have been a cumbersome undertaking in our laboratory. This paper describes a more efficient synthesis not requiring chromatographic purification of the final product.

The hydrazide bond in agaritine can be formed by classical methods used in peptide synthesis, but in attempting to couple glutamic acid in the 5-position to 4-(hydroxymethyl)phenylhydrazine (**1b**), the previous authors[4] encountered complications which we believe were largely responsible for the poor yields. First, **1b** is not stable[5] and was formed in situ by reduction of the corresponding ester with $LiAlH_4$. This reduction produces a considerable proportion of *p*-tolylhydrazine which, of course, also reacts with the activated glutamic acid. Secondly, **1b** has two sites for unwanted acylation: the hydroxyl group and the N-position of the hydrazine. Acylation at the N-position may actually be enhanced by the activating effect of the hydroxymethyl group.

The unique ability of diborane to selectively reduce carboxylic acids in the presence of many other functional groups, including hydrazides,[6a] esters, and amides,[6b] suggested that the reduction could be accomplished after formation of the hydrazide bond between **1a** and a suitably activated and protected glutamic acid. It was expected that the deactivating effect of the carboxyl group in **1a** would decrease the tendency for acylation to occur in the N-position, while reduction of the carboxyl group in the N′-acylated product would be less likely to proceed beyond the alcohol stage[7,8] than in the free hydrazine. The sensitivity of agaritine to both acids and bases dictated the choice of a benzyl ester for protection of the 1-position in **3** since this group like the benzyloxycarbonyl group is readily removed by hydrogenolysis. Although there was some apparent reduction of the protecting groups by diborane, the selectivity of the carboxyl reduction remained high enough to afford reasonable yields of the desired product.

The mixed anhydride formed from **2** and ethyl chloroformate reacted readily with **1a** to give the *N*′-hydrazide **3** (Scheme I). Reduction of **3** with BH_3/THF gave about a 50% yield of "protected" agaritine **5a** after removal by chromatography of the corresponding tolyl derivative **5b**,[9] benzyl alcohol, and unidentified byproducts. Hydrogenolysis of **5a** removed the protecting groups. When the hydrogenolysis was conducted in aqueous or anhydrous alcohol, the usual solvents for similarly protected peptides, about 15% of the product was L-glutamic acid 5-[2-(4-methylphenyl)hydrazide] (**4b**),[9] which could not be efficiently separated from agaritine by either recrystallization or column chromatography. When THF was used as the solvent, agaritine precipitated as it formed, decreasing the amount of **4b** in the product to less than 2%. This was removed, along with other impurities, by fractional precipitation.

The reaction product was analyzed by high-pressure LC. Chromatographically homogeneous material was isolated from this system for use as a reference standard. On the basis of this standard, the purity of the unchromatographed product has been consistently greater than 98%.

^{13}C NMR assignments for agaritine and related compounds are shown in Table I. Peak assignments were based on several standard techniques, including single-frequency off-resonance decoupling (SFORD), relative peak heights, and model compounds. ^{13}C substituent effects for phenylhydrazine, benzyl alcohol, benzoic acid, and toluene were calculated.[10] The peak assignments of the para-substituted aromatic rings in Table I were verified by calculations which assumed additivity of the ^{13}C substituent effects of the model monosubstituted benzenes mentioned.[10]

Experimental Section

THF was dried over sodium and distilled, taking precautions to protect personnel against possibly explosive peroxide decomposition. Silica gel was J. T. Baker No. 3405 to which 5% water was added. Palladium on active carbon was obtained from Engelhard Industries. High-pressure LC utilized a 250 mm × 9.4 mm Partisil-10 ODS C_{18} reversed-phase column. At a flow rate of 5.0 mL/min with 0.1 M phosphate buffer (pH 6.7) the retention times for **4a** and **4b** were 9.0 and 19.5 min. TLC utilized Brinkmann Instruments precoated Sil G-25 UV_{254} plates. Development was with CH_2Cl_2 (15)/THF (5)/AcOH (0.2), yielding R_f values for **3**, **5a**, and **5b** of 0.64, 0.57, and 0.71, respectively.

(5) **1b** has never been isolated. The *N*′-acetyl derivative has been described.[6a]

(6) (a) B. Toth, D. Nagel, K. Patil, J. Erickson, and K. Antonson, *Cancer Res.*, **38**, 177 (1978); (b) H. O. House, "Modern Synthetic Reactions", W. A. Benjamin, Menlo Park, Calif., 1972.

(7) Thus, BH_3/THF reduced 4-carboxyphenylhydrazine nearly quantitatively to *p*-tolylhydrazine: D. L. Nagel, unpublished work. Under the same conditions *N*′-acetyl-4-carboxyphenylhydrazine gave good yields of the hydroxymethyl derivative.[6a] Although diborane normally reduces arylcarbonyl functions to the corresponding benzylic alcohol, it has been shown[8] that the presence on the ring of electron-donating groups facilitates reductive cleavage of the alcohol.

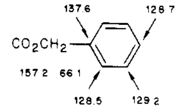
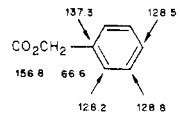
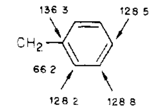
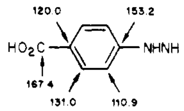
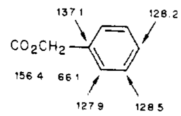
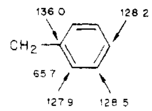
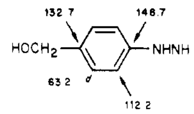
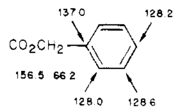
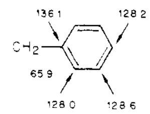
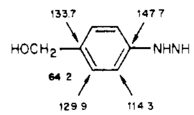
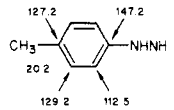
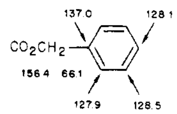
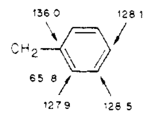
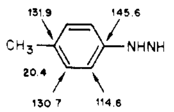
(8) K. M. Biswas, L. E. Houghton, and A. H. Jackson, *Tetrahedron, Suppl. No. 7*, **22**, 261 (1966).

(9) To confirm these structures, we synthesized **4b** by substituting *p*-tolylhydrazine for **1a** in the procedure described for **3**: yield 60%; mp 135–136 °C. Anal. Calcd for $C_{27}H_{29}N_3O_5$: C, 68.19; H, 6.15; N, 8.84. Found: C, 68.11; H, 6.18; N, 8.87. Hydrogenolysis of **4b** in THF over 10% Pd/C gave **3b**: mp 186–187 °C (lit.[4] mp 183–184 °C).

(10) G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists", Wiley-Interscience, New York, 1972.

Table I. ^{13}C Chemical Shifts of Substituted Glutamic Acids[a]

$$\overset{1}{R}\overset{2}{C}O\overset{3}{C}H_2\overset{4}{C}H_2\overset{5}{C}H(NHR')\overset{}{C}O_2R''$$

cmpd	C_1	C_2	C_3	C_4	C_5	R	R'	R''
6[b]	174.6[c]	30.9	27.0	54.0	175.0[c]	OH		H
2[b]	174.2[c]	30.4	26.6	53.9	172.4[c]	OH		
3[b]	171.3[c]	29.0	26.4	53.6	172.2[c]			
5a[b]	171.3[c]	29.8	26.7	53.8	172.3[c]			
4a[e]	175.0	30.4	27.0	55.1	174.6[e]		H	H
5b[b]	171.3[e]	29.7	26.7	53.8	172.2[e]			
4b[e]	175.4[c]	30.4	27.1	55.1	174.6[c]		H	H

[a] In parts per million downfield relative to Me_4Si. [b] Me_2SO-d_6 as solvent. [c] Assignments for C_1 and C_5 may be reversed. [d] Resonance obscured by carbons of groups R' and R'' at 128.0 to 128.6 ppm. [e] H_2O/D_2O (10/1) as solvent.

^{13}C NMR and ^1H NMR spectra were obtained with a Varian CFT-20 spectrometer and optical rotations with a Polyscience SR-6 polarimeter. Melting points were corrected. Elemental analyses were by Micro-Tech Laboratories.

1-Benzyl Ester of *N*-(Benzyloxycarbonyl)-L-glutamic Acid 5-[2-(4-Carboxyphenyl)hydrazide] (3). A solution of 72.5 g (0.19 mol) of 1-benzyl *N*-(benzyloxycarbonyl)-L-glutamate (**2**)[11] in 250 mL of THF was cooled to -10 °C and, with stirring, 27.2 mL (0.19 mol) of triethylamine in 18 mL of THF was added dropwise in 15 min. After 30 min, a solution of 18.8 mL (0.19 mol) of ethyl chloroformate in 40 mL of THF was added dropwise, maintaining the temperature below -5 °C. The mixture was stirred for 1 h at -10 °C after completion of the addition. To the thick white suspension thus formed was added, in 10-mL portions, with stirring, a suspension made from a mixture of 30 g (0.20 mol) of **1a**, THF (200 mL), water (75 mL), and triethylamine (20 mL). All solids dissolved during the addition. Cooling was discontinued and the solution was stirred overnight. A two-phase mixture resulted. The lower phase (ca. 25 mL) was extracted with 150 mL of ethyl acetate, and the extract was combined with the upper phase and evaporated to dryness at reduced pressure. The residue was stirred with 600 mL of 0.5 M HCl, filtered, and washed with water. The well-drained product was recrystallized from 95% ethanol to give 63 g (60% based on **1a**) of an off-white powder, mp 211–213 °C, suitable for use in the next step. Recrystallization three times from ethanol gave a white granular material, mp 214.5–215 °C.

Anal. Calcd for $C_{27}H_{27}N_3O_7$: C, 64.15; H, 5.38; N, 8.31. Found: C, 64.33; H, 5.44; N, 8.43.

Benzyl Ester of *N*-(Benzyloxycarbonyl)-L-glutamic Acid 5-[2-(4-Hydroxymethyl)phenyl]hydrazide] (5a). To a stirred, N_2-blanketed suspension of 63 g (0.12 mol) of **3** in 750 mL of THF, cooled to -10 °C, was added 400 mL of 1 M BH_3/THF over a 3-h period. Stirring was continued for 3.5 h more at 0–5 °C and the solution recooled to -10 °C. A cold mixture of 400 mL of water and 400 mL of THF was added, slowly at first until the very vigorous evolution of H_2 subsided. After addition of 1500 mL of ethyl acetate, the cooling bath was removed and stirring was continued overnight during which time a nearly clear two-phase solution resulted. After transfer of the solution to a separatory funnel with 900 mL of additional ethyl acetate, the lower phase (ca. 250 mL) was discarded and the organic layer washed cautiously with 600 mL of 1.0 N NaOH and twice with 600 mL of water. After the solution was dried (Na_2SO_4), it was evaporated at reduced pressure to yield 51 g of an orange-yellow solid. This was dissolved in a minimum of warm CH_2Cl_2 and chromatographed on 500 g of silica gel, using, in sequence, 5 L of 5% acetone in CH_2Cl_2, 1 L of 10% acetone, and 3 L of 30% acetone for development. The desired product (TLC check) appeared in the last portion of the 10% fraction and in the 30% fraction. Evaporation of these fractions at reduced pressure gave 39 g of a light yellow solid which on recrystallization from acetone–ether (1:4) yielded 29.5 g (48%) of a nearly colorless material of a hard, waxy consistency. Sintering began at 90 °C, mp 123–125 °C.

Anal. Calcd for $C_{27}H_{29}N_3O_6$: C, 65.97; H, 5.95; N, 8.55. Found: C, 66.10; H, 6.02; N, 8.49.

Agaritine (4a). A continuous flow of H_2 was introduced above the surface of a rapidly stirred solution of 24.6 g (0.05 mol) of **5a**

(11) G. H. L. Nefkens and R. J. F. Nivard, *Recl. Trav. Chim. Pays-Bas*, **83**, 199 (1964). *N*-(Benzyloxycarbonyl)-L-glutamic acid (**6**) used in this synthesis was prepared by the method of M. Bergmann and L. Zervas, *Ber. Dtsch. Chem. Ges. B*, **65**, 1192 (1932). The overall yield of **2** from L-glutamic acid was 50%.

in 270 mL of THF containing 4.1 g of 10% Pd/C in suspension. After 24 h the suspension of product and catalyst was filtered on sintered glass under N_2 pressure and washed with 150 mL of THF and twice with 150 mL of acetonitrile. The grayish mixture was thoroughly dried on the filter in the N_2 stream and then kept under vacuum overnight. The precipitate was treated on the filter with two 100-mL portions of water. The resulting filtrate was stirred briefly with 600 mL of acetonitrile, forming two phases. The upper phase was separated and mixed with 1 L of acetonitrile. After 30 min the agaritine was collected on sintered glass, washed with 200 mL of acetonitrile and 200 mL of ether, and vacuum dried at room temperature for 24 h. An additional 10% of agaritine was obtained by again treating the catalyst on the filter with 300 mL of water, evaporating the filtrate under reduced pressure at room temperature to 50 mL, and precipitating agaritine by addition of 450 mL of acetonitrile. The second crop was filtered, washed, dried, and combined with the main portion. Agaritine was obtained as fine white needles containing 1 mol of water of crystallization:[12] yield 12.6 g (88%); mp 203–206 °C dec; $[\alpha]_D^{23}$ + 8° (c 9.89 in water) [corrected for water of crystallization: $[\alpha]_D^{23}$ + 9° (lit.[4] $[\alpha_D^{23}]$ +7°)].

Chromatographic properties and ^1H NMR spectra were identical with those of agaritine isolated from mushrooms[13] and agaritine obtained through the courtesy of the Upjohn Co.

Anal. Calcd for $C_{12}H_{17}N_3O_4 \cdot H_2O$: C, 50.52; H, 6.71; N, 14.73. Found: C, 50.54; H, 6.73; N, 14.98.

Registry No. **1a**, 619-67-0; **2**, 3705-42-8; **3**, 71426-47-6; **4a**, 2757-90-6; **4b**, 13523-77-8; **5a**, 71426-48-7; **5b**, 71426-49-8; **6**, 1155-62-0.

(12) The water of crystallization could not be removed by the usual drying methods without decomposition. An anhydrous product was obtained from the hydrated material by precipitating it from saturated aqueous solution with 4 volumes of 1-butanol–ethanol (1:3) and drying under vacuum at 40 °C: mp 206–209 °C dec (lit.[4] mp 205–208 °C). Anal. Calcd for $C_{12}H_{17}N_3O_4$: C, 53.92; H, 6.41; N, 15.72. Found: C, 53.70; H, 6.39; N, 15.61.

(13) Agaritine was isolated and purified by a modification of the method described in ref 4: P. Issenberg, unpublished work.

A Convergent Total Synthesis of (±)-Prostaglandin $F_{2\alpha}$ via Conjugate Addition and Regiospecific Enolate Trapping

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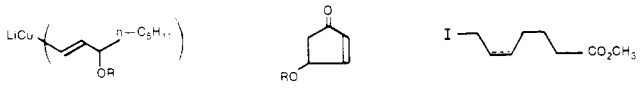
Contribution No. 528, Institute of Organic Chemistry, Syntex Research, Palo Alto, California 94304

Received April 19, 1979

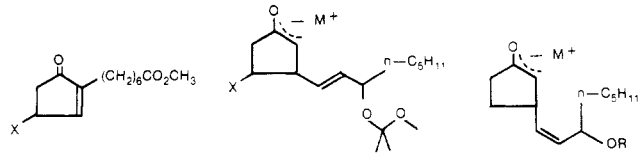
A convergent total synthesis of (±)-$PGF_{2\alpha}$ via the conjugate addition of the dioctenylcuprate reagent **7a**, derived from 1-iodo-3-hydroxyoct-1-*cis*-ene, to 4-[(*tert*-butyldimethylsilyl)oxy]cyclopent-2-en-1-one (**2a**) followed by regiospecific enolate trapping with ketene bis(methylthio)acetal monoxide (**18**) and stereospecific sulfenate–sulfoxide transformation is reported. The thioacetal intermediate **22**, after stereospecific reduction and hydrolysis, is converted to the known ketol **24** and then to (±)-$PGF_{2\alpha}$.

One of the simplest converging syntheses of prostaglandins is the regiospecific alkylation of the enolate initially generated by the conjugate addition of a selected vinyl cuprate (e.g., **1**, β chain) to a protected 4-hydroxycyclopent-2-enone (**2**) with an appropriate allyl or saturated halide (e.g., **3**, α chain). This attractive route has been investigated in our laboratories[1a,b] and by others[2a,b] for several years without success.[2c] The obstacles to overcome are alkylating the initially formed nonequilibrated enolate in any kind of resonable yield and retaining the 4-oxygen substituent under conditions where alkylation does occur.



1 **2a** R = $SiMe_2Bu$ **3**

Our attempts to realize this enolate trapping route were directed first to obtaining the requisite lithium dialkenylcuprate (**1**, R = CMe_2OMe) for conjugate addition to a 2-[(carbomethoxy)hexyl]-4-protected hydroxycyclopent-2-enone (**4a**). This was achieved by us[3] and others.[4]



4a X = $OCMe_2OMe$ **5a** X = H **6a** R = CMe_2OMe
b X = H **b** X = $OSiMe_2Bu$ **b** R = $SiMe_2Bu$

We found next that a lithium *cis*-dialkenylcuprate gave higher yields of conjugate addition which led to 13-*cis*-PGs with a high degree of stereoselectivity at C-15[5] as an added important benefit. Knowing that the cuprate-generated enolate (e.g., **6**) was present in the reaction mixtures in large amounts (≥70%) prior to protic quench, we attempted alkylations with allyl bromide and iodide under a variety of conditions but obtained no alkylation. The same results (no alkylation) were obtained when the cyclopentenone **2a** was used.[1b] We knew that the enolate, **5a**, could be trapped efficiently as the (trimethylsilyl)enol ether, and the regenerated enolate could then be alkylated.[1a] Later, reports[6] of α-alkylations of cuprate-

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