stirred overnight. The reaction was quenched with a saturated ammonium chloride/ammonium hydroxide solution (9/1) and extracted with two 50-mL portions of ether. The extracts were combined and dried over $MgSO_4$, and GC analysis was performed after concentration in vacuo. Isolation of the product was performed on a Chromatotron on a 4-mm plate of Kieselgel 60 PF_{254}, using hexane/ether (9/1) as the eluent. Complete spectral and elemental analyses confirmed the structure of the products. Yields are listed in Table I.

Simultaneous Addition of 5 and 7 to $(CH_2{=}CH)_2Cu(CN)Li_2$. To a well-stirred grey suspension of 5 mmol of $(CH_2{=}CH)_2Cu(CN)Li_2$ prepared by the aforementioned general procedure, contained in 30 mL of dry ether under argon at −70 °C, were added 5 mmol of **5** and **7** simultaneously via syringe. This was allowed to warm to room temperature and stir overnight. Workup in the usual manner afforded the residue which was submitted to gas chromatographic analysis. From the chromatogram, 1,4-conjugate addition to isophorone yielding **10** and ring cleavage of **5** to afford **9** had occurred (peaks were verified by coinjection with authentic specimens). Starting materials **5** and **7** were also present.

Addition of 5 to $(CH_2{=}CH)_2Cu(CN)Li_2$ with Added $(CH_3)_3SiCl$ or $BF_3{\cdot}Et_2O$. To a well-stirred grey suspension of 5 mmol of $(CH_2{=}CH)_2Cu(CN)Li_2$ prepared by the general procedure, contained in 30 mL of dry ether under argon at −70 °C, was added 1 equiv of $(CH)_3SiCl$ or $BF_3{\cdot}Et_2O$. This was permitted to stir for 15 min and 5 mmol of **5** was added neat. This was allowed to warm to room temperature and stir for 3 h, whereupon the reaction was quenched and worked up in the usual manner to afford the residue for gas chromatographic analysis. The gas chromatogram evidenced only starting materials.

2,2,6-Trimethyl-2,3-dihydro-4*H*-pyran-4-one (5):[24] GC 130 °C, 2.00 min; IR (cm^{-1}) 2960, 1660, 1600, 1390, 1150; ^1H NMR ($CDCl_3$) δ 5.30 (s, 1 H), 2.46 (s, 2 H), 2.00 (s, 3 H), 1.46 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 192.455, 172.190, 103.123, 80.852, 46.952, 26.162, 21.487; MS, *m/e* (relative abundance) 140 (2.01), 139 (23.41), 125 (2.13), 96 (7.27), 83 (100.00), 78 (2.58), 68 (4.34).

5-Carbethoxy-2,2,6-trimethyl-2,3-dihydro-4*H*-pyran-4-one (6):[24] GC 180 °C, 2.75 min; IR (cm^{-1}) 2980, 1720, 1670, 1590, 1390, 1080; ^1H NMR ($CDCl_3$) δ 4.28 (q, 2 H), 2.50 (s, 2 H), 2.15 (s, 3 H), 1.40 (s, 6 H), 1.30 (t, 3 H); ^{13}C NMR ($CDCl_3$) δ 188.074, 175.167, 165.665, 110.846, 81.537, 60.775, 47.083, 26.109, 20.777, 14.207.

4-Hydroxy-6-methyl-3,5-heptadien-2-one (9):[24] GC 130 °C, 2.38 min; IR (cm^{-1}) 3450, 1650, 1600, 1250, 1155; ^1H NMR ($CDCl_3$) δ 12.30 (exch.), 5.80 (m, 1 H), 5.50 (s, 1 H), 3.70 (s, exch.), 2.30 (s, 3 H), 2.10 (s, 3 H), 1.90 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 193.290, 183.413, 153.208, 121.631, 101.229, 28.003, 27.678, 25.534, 20.856; MS, *m/e* (relative abundance) 140 (16.85), 125 (100.00), 83 (37.60), 69 (41.87), 54 (41.20).

1-Hydroxy-3,5,5-trimethyl-1-vinyl-2-cyclohexen-1-ol (12): GC 160 °C, 2.25 min; IR (cm^{-1}) 3420, 1660; ^1H NMR ($CDCl_3$) δ 6.20–5.80, 5.40–4.90 (m, 4 H), 1.75 (s, 2 H), 1.70 (s, 3 H), 1.60 (s, 2 H), 1.25 (s, 3 H), 0.95 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 145.985, 136.233, 124.225, 111.324, 71.892, 48.433, 44.310, 30.259, 28.284, 24.004; MS, *m/e* (relative abundance) 166 (14.54), 151 (72.58), 133 (75.40), 110 (80.75), 95 (100.00). Anal. Calcd for $C_{11}H_{18}O$: C, 79.46; H, 10.91. Found: C, 79.31; H, 10.85.

5-Carbethoxy-4-hydroxy-2,2,6-trimethyl-4-vinyl-2,3-dihydro-4*H*-pyran (13): GC 180 °C, 4.25 min; IR (cm^{-1}) 3500, 1710, 1600; ^1H NMR ($CDCl_3$) δ 6.10–5.80 (m, 1 H), 5.40–5.00 (m, 2 H), 4.25 (q, 2 H), 4.05 (s, 1 H), 2.20 (s, 3 H), 1.20–1.45 (m, 11 H); ^{13}C NMR ($CDCl_3$) δ 170.281, 168.891, 163.686, 145.582, 112.195, 76.100, 69.354, 60.066, 46.267, 29.341, 25.071, 21.060, 14.319. Anal. Calcd for $C_{13}H_{20}O_4$: C, 64.98; H, 8.39. Found: C, 64.88; H, 8.42.

3,5,5-Trimethyl-3-vinylcyclohexanone (10):[28] GC 160 °C, 3.00 min; IR (cm^{-1}) 1710, 1635; ^1H NMR ($CDCl_3$) δ 5.95–5.60, 5.10–4.85 (m, 3 H), 2.58, 2.14 (AB dd, J = 15 Hz, 2 H), 2.15 (s, 2 H), 1.67 (s, 2 H), 1.14 (s, 3 H), 1.07 (s, 3 H), 1.00 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 211.409, 147.015, 111.700, 54.238, 50.702, 49.708, 41.405, 36.174, 32.882, 31.419, 29.171; MS, *m/e* (relative abundance) 166 (72.59), 151 (44.08), 124 (16.37), 110 (48.28), 95 (60.67), 83 (90.08), 67 (100.00).

3-Carbethoxy-2,6,6-trimethyl-2-vinyltetrahydropyran-4-one (11): GC 180 °C, 0.79 min; IR (cm^{-1}) 1740, 1640, 1610; ^1H NMR ($CDCl_3$) δ 13.20 (exch.), 6.40–6.10 (m, 1 H), 5.40–5.00 (m, 2 H), 4.35 (q, 2 H), 2.35 (s, 2 H), 1.63 (s, 3 H), 1.40 (s, 6 H), 1.35 (t, 3 H); ^{13}C NMR ($CDCl_3$) δ 171.443, 170.943, 144.134, 112.385, 101.393, 74.109, 70.812, 60.595, 41.234, 29.523, 29.295, 28.912, 14.078; MS, *m/e* (relative abundance) 240 (2.54), 225 (40.56), 179 (84.48), 167 (62.80), 83 (100.00). Anal. Calcd for $C_{13}H_{20}O_4$: C, 64.98; H, 8.39. Found: C, 65.07; H, 8.40.

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Synthesis of New Amino Acids Mimicking Sulfated and Phosphorylated Tyrosine Residues

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Introduction

The presence of a sulfated tyrosine residue was first detected in fibrinopeptide B,[1] then on fibrinogens,[2] gastrin,[3] and cholecystokinin,[4] and more recently in several secretory proteins such as immunoglobin G[5] and fibronectin,[6] and in an insect neuropeptide designated leucosulfakinin.[7] Presumably, the *O*-sulfate ester group is introduced at a posttranslational stage of the biosynthesis but very little is known about the enzyme(s) responsible for this modification.[8] The ester bond of tyrosine *O*-sulfate is remarkably acid labile[1] and is therefore likely to be hydrolyzed in some of the common protein-chemical procedures.[9] The low chemical stability of ester sulfate groups has thus prevented the widespread use of natural compounds containing tyrosine *O*-sulfate residues for pharmacological investigations. Another posttranslational covalent modification of proteins that has been recognized as an important cell-regulation process and therefore extensively studied[10,11] is protein phosphorylation. The occurrence of phosphorylated tyrosine as a protein modification implies the existence of enzymes called tyrosine kinases capable of phosphorylating tyrosine.[12] The role of these tyrosine kinases is of great interest since tyrosine phosphorylation has been implicated in regulatory events such as cell transformation and hormone-induced cell growth.[13-15]

In order to elucidate the mechanisms of such phenomena that have not been clearly established yet, it appears to be essential to use analogues that cannot be hydrolyzed as substrates or inhibitors of sulfo-transferase or as substitutes of the Tyr(SO_3H) group in active peptides.

In order to enhance the chemical stability of compounds containing tyrosine *O*-sulfate and tyrosine *O*-phosphate residues, we have synthesized new amino acids related to sulfated and phosphorylated tyrosine residues, in which

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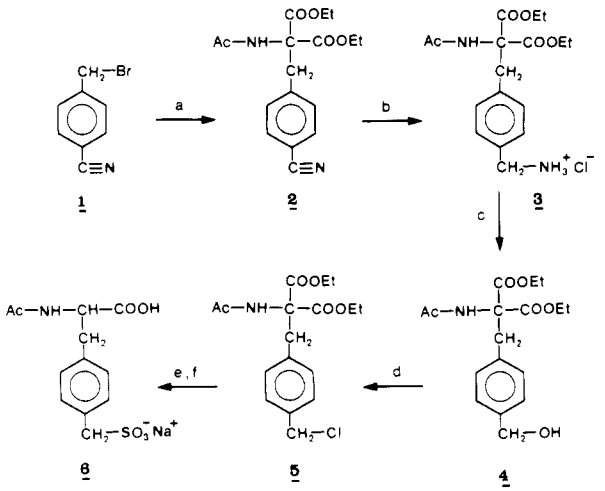
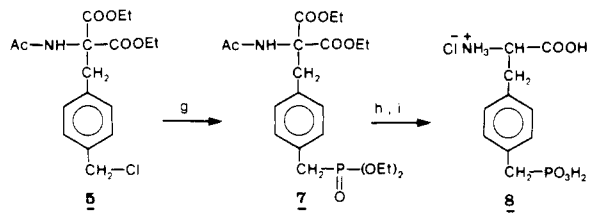
Scheme I



a. $Ac-NH-CH(COOEt)_2$, Na, EtOH.
b. H_2, Pd/C, HCl.
c. $NaNO_2$, H_2O, Δ.
d. $SOCl_2$, Δ.
e. Na_2SO_3, NaOH, Δ.
f. HCl, Δ.

Scheme II



g. $P(OEt)_3$, Δ.
h. NaOH, Δ.
i. HCl, Δ.

the OSO_3H and OPO_3H_2 groups are replaced by CH_2SO_3H and $CH_2PO_3H_2$ groups (compounds **6** and **8**). Besides their enhanced resistance in acid medium, these new compounds are able to mimic sulfated or phosphorylated tyrosine residues. Peptides containing these new derivatives therefore might display biological properties similar to those of the parent components, as was recently observed for cholecystokinin.[16]

The new amino acids were synthesized according to Scheme I (compound **6**) and Scheme II (compound **8**). Derivative **2** was prepared by base-catalyzed alkylation of diethyl acetamidomalonate with α-bromo-*p*-tolunitrile (**1**) (87% yield) and converted to compound **3** by catalytic hydrogenation in the presence of Pd/C catalyst in acid medium (85% yield) as previously described for the synthesis of diethyl [3-(aminomethyl)benzyl]acetamidomalonate.[17] A similar synthesis route was used by Nutt et al.[18] for the preparation of conformationally constrained amino acids in somatostatin analogues. By use of sodium nitrite in aqueous medium, the hydroxymethyl derivative **4** was prepared from **3** in 89% yield. *N*-Acetyl-DL-*p*-(hydroxymethyl)phenylalanine has previously been synthesized by Smith and Sloane[19] but by a different method. Treatment of hydroxymethyl derivative **4** with thionyl chloride afforded diethyl [4-(chloromethyl)benzyl]acetamidomalonate (**5**) in a yield of 72%. Conversion to the desired methanesulfonic acid derivative **6** was performed with sodium sulfite in 10% sodium hydroxide solution, as previously described by Johnson and Ambler for the synthesis of phenylmethanesulfonic acid.[20] During the reaction, sodium hydroxide also allowed saponification of the two ester functions of compound **5**. After acid treatment with 1 M HCl and heating, compound **6** was extracted with ethanol and chromatographed on silica gel, with a 46% yield. *N*-Acetamido-*p*-(sulfomethyl)-DL-phenylalanine ethyl ester was isolated as a side product. Another usual method for the synthesis of sulfonic acids is oxidation of mercaptans,[21] which can easily be prepared as previously described.[22]

The synthesis of compound **8** (Scheme II) used a classic Arbuzov reaction with triethyl phosphite and compound **5** as the alkyl chloride moiety.[23] The intermediate diethyl [4-[(diethoxyphosphinyl)methyl]benzyl]acetamidomalonate (**7**) was converted to the corresponding phosphonic acid **8** by acid-catalyzed hydrolytic desalkylation.[24] The vigorous conditions required were effective for this purpose. Numerous alkyl phosphonates, however, contain functional groups that are too delicate to survive the harsh reaction conditions involved. If an acidic treatment cannot be carried out, Hata et al.[25] suggested replacing the trialkyl phosphite usually employed in the Arbuzov reaction by tris(trimethylsilyl) phosphite, which has a similar nucleophilic reactivity. The resulting bis(trimethylsilyl) phosphonate esters are easily hydrolyzed under mild conditions in the presence of other labile groups. Several authors have also reported convenient methods for the conversion of dialkyl phosphonates to bis(trimethylsilyl) phosphonates with chloro-, bromo-, or iodotrimethylsilane.[26-28]

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The two analogues of tyrosine described in this paper (compounds **6** and **8**) are obtained as racemic forms. A detailed procedure for the resolution of the *N*-acetyl DL isomers of *p*-(hydroxymethyl)phenylalanine by use of hog kidney acylase I has already been described by Smith and Sloane.[19] In our synthesis, the hydroxymethyl derivative **4** is easily converted to *N*-acetyl-DL-*p*-(hydroxymethyl)-phenylalanine by saponification and decarboxylation. Thus, the method of Smith and Sloane can be rigorously applied.

Moreover, the new tyrosine analogues can be incorporated into a peptidic sequence by coupling methods usually employed in peptidic synthesis. Ac-DL-Phe(*p*-CH_2SO_3H) (compound **6**) was introduced into the sequence of CCK_8 without any side-chain protection.[16] However, in order to work with more easily removable protecting groups, we verified that the amine and the carboxylic acid functions of the amino acid **8** could selectively be blocked by a *tert*-butyloxycarbonyl group (compound **9**) and a methyl ester function (compound **10**), without any side-chain protection. The synthesis of the partially protected analogues **9** and **10** is described. Analogues of CCK_8 containing the Phe(*p*-CH_2SO_3H) group in place of the Tyr-(SO_3H) moiety were shown to be chemically stable for several days and equipotent to the parent compound.[16]

In this paper we propose an easy access to novel amino acids mimicking sulfated and phosphorylated tyrosine residues, with enhanced chemical stability. Considering the growing interest related to posttranslational modifications of proteins such as sulfation and phosphorylation of tyrosine residues, the preparation of analogues of sulfated and phosphorylated tyrosine, structurally very similar and more stable, provides new challenges in numerous biochemical and pharmacological fields.

Experimental Section

Solvents were of analytical grade from Prolabo. Chromatography was carried out with Merck silica gel (230–400 mesh). For thin-layer chromatography, Merck plates precoated with F254 silica gel were used with the following solvent systems (by volume): A, $CHCl_3$–MeOH (90:10); B, hexane–EtOAc–MeOH (60:40:4); C, $CHCl_3$–MeOH–H_2O–AcOH–EtOAc (35:15:3:1.5:1); D, CH_2Cl_2–MeOH (80:20); E, CH_2Cl_2–MeOH–H_2O–AcOH (70:30:6:3); F, 2-propanol–NH_4OH (28%) (60:40); G, 2-propanol–NH_4OH (28%) (50:50). Plates were developed with UV, iodine vapor, or ninhydrin. The structure of the compounds was confirmed by ^1H NMR spectroscopy (Brucker WH 270 MHz). Mass spectra were recorded on a double-focussing VG 70-250 instrument. The fast atom bombardment (FAB) ionization were obtained with a FAB saddle field source (Ion Tech Ltd., Teddington, UK) operated with xenon at 8 kV and 1 mA. Glycerol or cesium iodide was used for calibration. Accelerating voltage was set at 6 kV and resolution was 1200.

Diethyl (4-Cyanobenzyl)acetamidomalonate (2). A solution of Na (0.26 g, 0.011 mol), diethyl acetamidomalonate (2.17 g, 0.01 mol), and *p*-cyanobenzyl bromide (**1**) (1.96 g, 0.01 mol) in 25 mL of ethanol was refluxed and stirred for 17 h at 110 °C. After cooling and addition of water (50 mL), the crystalline material was collected by filtration and washed twice with water, to yield 2.89 g (87%) of a white solid: R_f 0.73 (A), R_f 0.29 (B); ^1H NMR (DMSO-d_6) δ 1.10 (t, 6 H, 2 CH_3CH_2O), 1.90 (s, 3 H, CH_3CO), 3.45 (s, 2 H, $PhCH_2C$), 4.10 (q, 4 H, 2 CH_3CH_2O), 7.12 and 7.70 (2 d, 4 H, aromatic protons), 8.12 (s, 1 H, CONH). Anal. Calcd for $C_{17}H_{20}N_2O_5$: C, 61.45; H, 6.02; N, 8.43. Found: C, 61.22; H, 6.19; N, 8.20.

Diethyl [4-(Aminomethyl)benzyl]acetamidomalonate (3). Compound **2** (996 mg, 3 mmol) was hydrogenated at atmospheric pressure and room temperature for 22 h in ethanol (25 mL) and concentrated HCl (1.5 mL) with Pd/C 10% as catalyst (200 mg). After filtration, the solution was taken to dryness. Water (60 mL) was added to the residue and unreacted material was removed by filtration. The filtrate was again concentrated to dryness, giving 956 mg (85%) of a white solid: R_f 0.28 (C); ^1H NMR (DMSO-d_6) δ 1.10 (t, 6 H, 2 CH_3CH_2O), 1.90 (s, 3 H, CH_3CO), 3.42 (s, 2 H, $PhCH_2C$), 3.90 (s, 2 H, NH_3^+-CH_2Ph), 4.10 (q, 4 H, 2 CH_3CH_2O), 6.98 and 7.34 (2 d, 4 H, aromatic protons), 7.93 (s, 1 H, CONH), 8.25 (large s, 3 H, NH_3^+-CH_2Ph). Anal. Calcd for $C_{17}H_{24}N_2O_5$·HCl: C, 54.76; H, 6.44; N, 7.52. Found: C, 54.62; H, 6.38; N, 7.29.

Diethyl [4-(Hydroxymethyl)benzyl]acetamidomalonate (4). A solution of compound **3** (2.06 g, 5.5 mmol), $NaNO_2$ (535 mg), and water (100 mL) was heated for 2 h at 110 °C, cooled, and extracted with ethyl acetate. The extract was washed with 1 M HCl, water, 5% $NaHCO_3$, water and brine, dried over Na_2SO_4, filtrated, and taken to dryness, giving a white crystallized solid: 1.67 g (89%); R_f 0.15 (B); ^1H NMR (DMSO-d_6) δ 1.10 (t, 6 H, 2 CH_3CH_2O), 1.90 (s, 3 H, CH_3CO), 3.36 (s, 2 H, $PhCH_2C$), 4.10 (q, 4 H, 2 CH_3CH_2O), 4.40 (d, 2 H, $HOCH_2Ph$), 5.05 (t, 1 H, $HOCH_2Ph$), 6.88 and 7.16 (2 d, 4 H, aromatic protons), 7.92 (s, 1 H, CONH). Anal. Calcd for $C_{17}H_{23}NO_6$: C, 60.53; H, 6.82; N, 4.15. Found: C, 60.43; H, 6.65; N, 3.87.

Diethyl [4-(Chloromethyl)benzyl]acetamidomalonate (5). A solution of compound **4** (210 mg, 0.6 mmol) and thionyl chloride (1.4 mL, 30 equiv) in dichloromethane (15 mL) was refluxed for 23 h and concentrated to dryness. The residue was washed twice with diethyl ether, to give a white solid: 161 mg (72%); R_f 0.62 (D); ^1H NMR (DMSO-d_6) 1.10 (t, 6 H, 2 CH_3CH_2O), 1.90 (s, 3 H, CH_3CO), 3.40 (s, 2 H, $PhCH_2C$), 4.10 (q, 4 H, 2 CH_3CH_2O), 4.65 (s, 2 H, $ClCH_2Ph$), 6.95 and 7.3 (2 d, 4 H, aromatic protons), 8.03 (s, 1 H, CONH). Anal. Calcd for $C_{17}H_{22}NO_5Cl$: C, 57.38; H, 6.19; N, 3.94. Found: C, 57.45; H, 5.91; N, 3.78.

***N*-Acetamido-*p*-(sulfomethyl)-DL-phenylalanine (6).** A solution of compound **5** (1.1 g, 3.1 mmol), Na_2SO_3 (3.3 g), 10% NaOH (8 mL), and water (20 mL) was heated at 120 °C for 3 h, cooled, and acidified with 1 M HCl (pH 1). The reaction mixture was still heated for 1 h at 120 °C, cooled, and extracted with ethanol. After filtration, ethanol was removed and the product was purified by chromatography on silica gel with CH_2Cl_2–CH_3OH–H_2O–AcOH (70:30:6:3) as eluent, to yield 424 mg (46%) of a white solid: R_f 0.16 (E); ^1H NMR (D_2O) (TMS as external reference) δ 1.53 (s, 3 H, CH_3CO), 2.55 and 2.81 (dd, 2 H, $PhCH_2CH<$), 3.78 (s, 2 H, $PhCH_2SO_3Na$), 4.11 (m, 1 H, CH<), 6.88 and 6.97 (2 d, 4 H, aromatic protons); mass spectrum (FAB), m/e $(MH)^+$ calcd 324, found 324.

Diethyl [4-[(Diethoxyphosphinyl)methyl]benzyl]acetamidomalonate (7). Compound **5** (50 mg, 0.14 mmol) was dissolved in triethyl phosphite (4 mL) and refluxed for 17 h. After removal of triethyl phosphite, the oily residue was purified by flash chromatography on silica gel with CH_2Cl_2–CH_3OH (90:10) as eluent, to yield 42.3 mg (66%) of a white solid: R_f 0.17 (A); ^1H NMR (DMSO-d_6) δ 1.13 (t, 12 H, 4 CH_3CH_2O) 1.92 (s, 3 H, CH_3CO), 3.08 and 3.13 (s, 2 H, $OCH_2PO_3Et_2$), 3.35 (s, 2 H, $PhCH_2C$), 3.87 (m, 4 H, $PO(OCH_2CH_3)_2$), 4.09 (q, 4 H, 2 $COOCH_2CH_3$), 6.86 and 7.12 (d, 4 H, aromatic protons), 7.99 (s, 1 H, CONH). NMR 2D $^{31}P/^1H$ (400 MHz): protons near phosphorus atom have chemical shifts of 3.08 and 3.13 ppm ($CH_2PO(OCH_2CH_3)_2$) and 3.87 ppm ($CH_2PO(OCH_2CH_3)_2$). Anal. Calcd for $C_{21}H_{32}NO_8P$: C, 55.14; H, 7.00; N, 3.06. Found: C, 54.89; H, 7.22; N, 2.87.

***p*-(Phosphonomethyl)-DL-phenylalanine (8).** A solution of compound **7** (27.5 mg, 0.06 mmol), 1 N NaOH (0.24 mL, 4 equiv) in water (1 mL), and methanol (1 mL) was stirred for 3 h at room temperature. After addition of 4 mL of water and 2.5 mL of concentrated HCl, the reaction mixture was refluxed for 4 h (110 °C), cooled, and, after addition of 10 mL of water, adjusted to pH 4 with 10% NaOH. After lyophilization and purification by chromatography on silica gel with 2-propanol–NH_4OH (28%) (60:40) as eluent, a white product (9.7 mg, 55% yield) was obtained: R_f 0.10 (F); ^1H NMR (D_2O) (TMS as external reference) δ 2.72 and 2.80 (s, 2 H, $CH_2PO_3^-$), 2.84 and 3.08 (dd, 2 H, $CH_2CH<$), 3.74 (m, 1 H, CH<), 7.00 and 7.10 (d, 4 H, aromatic protons); mass spectrum (FAB), m/e $(MH)^+$ calcd 296, found 296.

***N*-(*tert*-Butyloxycarbonyl)-*p*-(phosphonomethyl)-DL-phenylalanine (9).** To a solution of compound **8** (32 mg, 0.108

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mmol) in the solvent mixture H_2O–dioxane (1 mL:2 mL) were added, at 0 °C, 1 N NaOH (0.9 mL, 8 equiv and $(Boc)_2O$ (42 mg, 1.8 equiv). The reaction mixture was stirred for 1 h at 0 °C followed by stirring overnight at room temperature. After acidification with 1 M HCl (pH 4) and evaporation of the solvents, the residue was dissolved in water. Compound **9** was extracted from EtOAc to yield, after evaporation of EtOAc, 16 mg (41%) of a pale yellow solid: R_f 0.25 (F); ^{1}H NMR (DMSO-d_6) δ 1.25 (s, 9 H, Boc), 3.80 and 3.87 (s, 2 H, CH_2PO_3), 3.74 and 3.94 (dd, 2 H, $CH_2CH<$), 3.98 (m, 1 H, CH<), 7.03 (d, 1 H, NH), 7.10 (s, 4 H, aromatic protons); mass spectrum (FAB), m/e $(MH)^+$ calcd 360, found 360.

***p*-(Phosphonomethyl)-DL-phenylalanine Methyl Ester (10).** Compound **8** (50 mg, 0.169 mmol) was lyophilized in 1 N NaOH (5 mL) to eliminate NH_4OH in order to prevent amidification of the COOH function during the reaction. After acidification with 1 M HCl (pH 2–3) and evaporation to dryness, compound **8** was dissolved in dry MeOH (5 mL). Then, thionyl chloride (20 μL, 1.6 equiv) was carefully added at 0 °C. The reaction mixture was stirred for 1 h at 0 °C, 1 h at room temperature, and 6 h at 110 °C. After filtration of mineral salts and evaporation of MeOH, the product was washed with ether, to yield 27 mg (52%) of a white solid: R_f 0.41 (G); ^{1}H NMR (DMSO-d_6) δ 2.86 and 2.94 (s, 2 H, CH_2PO_3), 3.02 and 3.15 (dd, 2 H, $CH_2CH<$), 3.60 (s, 3 H, OCH_3), 4.12 (m, 1 H, CH<), 7.10 and 7.17 (d, 4 H, aromatic protons), 8.80 (large s, 3 H, NH_3^+); mass spectrum (FAB), m/e $(MH)^+$ calcd 310, found 310.

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Preparation of 6-(Silyloxy)-6-alkylfulvenes. A Novel in Situ Trapping of an Enolate with *tert*-Butyldimethylsilyl Chloride

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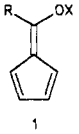
Fulvenes have long attracted the interest of theoreticians as well as inorganic and organic chemists.[1] They have served our research efforts as starting materials in the preparation of molecules of mechanistic interest and natural occurrence.[2] Our development of a new synthetic strategy required a flexible preparation of 6-oxyfulvenes (e.g., **1**, X = alkyl, acyl, or SiR_3) that would allow the



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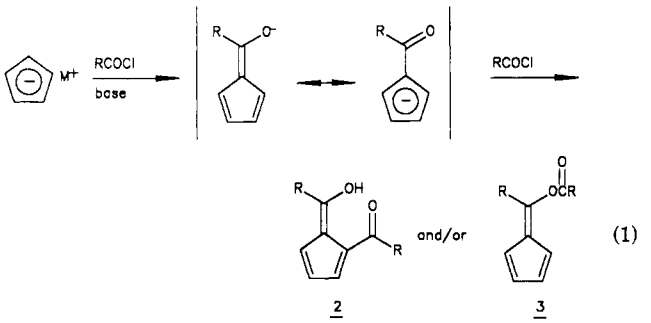
incorporation of a variety of alkyl groups and protection of the oxygen moiety. Herein we report that (1) cyclopentadiene anion (Cp^-) adds efficiently to acid chlorides, leading to the preparation of 6-oxyfulvenes; (2) the product distribution, viz., the formation of **2** vs **3** formed by addition of the presumed enolate intermediate to a second equivalent of the acid chloride, was found to be highly dependent upon the counterion used; (3) *tert*-butyldimethylsilyl (TBDMS) chloride is stable in the presence of CpK at −78 °C and serves admirably in a novel in situ trapping of the intermediate enolate, thereby providing high yields of 6-(silyloxy)-6-alkylfulvenes (**1**, X = TBDMS).

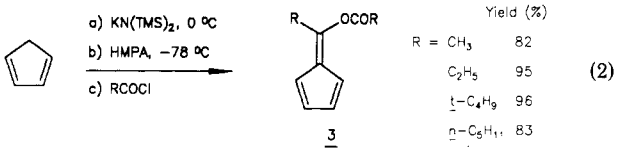


(1)

2 **3**

Although the addition of CpNa to esters has been used for the preparation of 6-oxyfulvenes,[3] we were unable to generalize the reaction and were unable to intercept the acylated intermediates with trapping/protecting agents. The addition of benzoyl chloride to a solution of CpLi, on the other hand, has been known for some time to provide 1-benzoyl-6-hydroxy-6-phenylfulvene (**2**, R = Ph).[4] Presumably, the initially formed benzoylcyclopentadiene adduct is readily deprotonated; the incipient enolate is then C-acylated by a second equivalent of the acid chloride. We reasoned that the nature of the products of the reaction of Cp^- with acid chlorides might be altered by conditions that favor O-acylation. Indeed, when conditions were employed that are known to favor O-acylation (viz., potassium counterion, THF and HMPA as cosolvent), 6-(acyloxy)-6-alkylfulvenes (e.g., **3**) were obtained in high yield, *exclusive* of other fulvene products.

The reaction of CpK in THF containing 1–2 equiv of hexamethylphosphoramide (HMPA) with acid chlorides (−78 °C to room temperature) was found to provide high yields of the 6-(acyloxy)-6-alkylfulvenes (eq 2). Either



(2)

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potassium hydride or potassium hexamethyldisilazane was found to be suitable for the generation of CpK. Reactions run without HMPA present were found to contain significant amounts of the C-acylated fulvene **2** and generally resulted in lower overall yields of fulvene products. The use of CpNa under the identical reaction conditions, with and without HMPA, gave a mixture of the fulvenes, but favored formation of **3**. Reactions in which the acid chloride was added rapidly or at temperatures significantly above −78 °C also provided mixtures of the **2** and **3**, though again the O-acylated product **3** predominated.

We desired that *only a single equivalent of the acid chloride be consumed* and that the oxygen be suitably protected, thus enabling further synthetic manipulation of the products. A variety of in situ trapping agents were used in attempts at capturing the presumed intermediate enolate. Various electrophilic agents, including methyl iodide, dimethyl sulfate, and benzyl bromide, were added

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