fractional recrystallization (4×) of the (-)-α-phenylethylamine salts from isopropyl alcohol. The less soluble salt (24%) had mp 154-157 °C and $[\alpha]^{25}_D$ +46° (c 1.0, CH_3CH_2OH). The mandelic acid (S)-**1f** was isolated as outline above for (S)-**1b** and recrystallized from toluene: mp 108-110 °C, $[\alpha]^{25}_D$ +137° (c 0.333, CH_3CH_2OH) [lit.[3] mp 109-112 °C, $[\alpha]^{25}_D$ -144° (c 0.3, CH_3CH_2OH) for the R enantiomer]; 95% ee; EA max (CH_3OH) 272 nm (ε 340), 265 (380), 258 (340) (sh); (CH_3OH-KOH) 273 (290), 265 (350), 258 (300) (sh); CD (CH_3OH, c 0.0251) $[\theta]_{277}$ ±0, $[\theta]_{274}$ -470, $[\theta]_{270}$ -130, $[\theta]_{267}$ -450, $[\theta]_{264}$ -200, $[\theta]_{261}$ -210, $[\theta]_{254}$ ±0; (c 0.00100) $[\theta]_{254}$ ±0, $[\theta]_{226}$ +1700, $[\theta]_{206}$ ±0; (CH_3OH-KOH, c 0.0500) $[\theta]_{280}$ ±0, $[\theta]_{274}$ -670, $[\theta]_{271}$ -280, $[\theta]_{266}$ -470, $[\theta]_{263}$ -300, $[\theta]_{261}$ -340, $[\theta]_{246}$ ±0, $[\theta]_{240}$ +670.

(R)-m-Chloromandelic acid[32] **((R)-1g)**: mp 103-105 °C, $[\alpha]^{23}_D$ -113° (c 4.40, CH_3CH_2OH) [lit.[3] mp 103-105 °C, $[\alpha]^{25}_D$ -116° (c 4, C_2H_5OH)]; 97% ee; EA max (CH_3OH) 275 nm (ε 260), 267 (310), 261 (270), 253 (390) (sh); (CH_3OH-KOH) 275 (240), 267 (310), 261 (250), 254 (220); CD (CH_3OH, c 0.0358) $[\theta]_{278}$ ±0, $[\theta]_{275}$ +620, $[\theta]_{272}$ +280, $[\theta]_{267}$ +650, $[\theta]_{264}$ +340, $[\theta]_{261}$ +370, $[\theta]_{256}$ +32 (sh), $[\theta]_{252}$ ±0, $[\theta]_{243}$ -1400; (CH_3OH-KOH, c 0.0358) $[\theta]_{281}$ ±0, $[\theta]_{276}$ +810, $[\theta]_{273}$ +400, $[\theta]_{268}$ +850, $[\theta]_{264}$ +420, $[\theta]_{262}$ +530, $[\theta]_{255}$ +260 (sh), $[\theta]_{247}$ ±0, $[\theta]_{243}$ -830.

(R)-m-Fluoromandelic Acid ((R)-1h). The racemic acid was prepared in 28% yield from m-fluorobenzaldehyde by way of the corresponding cyanohydrin.[33] Recrystallization from benzene gave a somewhat impure (±)-**1h**: mp 80-85 °C [lit.[9] mp 97 °C]. The acid was resolved by fractional crystallization of its (-)-ephedrine salt from 95% ethanol (2×). The less soluble salt (28%) had $[\alpha]^{25}_D$ -59° (c 4.33, CH_3OH). The mandelic acid (R)-**1h** was isolated as outlined above for the isolation of (S)-**1b** from its salt and recrystallized from benzene: mp 115-117 °C, $[\alpha]^{24}_D$ -119° (c 2.83, acetone) [lit.[9] mp 121 °C, $[\alpha]^{25}_{578}$ -129° (c 1, acetone)]; 92% ee; ^1H NMR (300 MHz, CD_3SOCD_3) δ 5.07 (s, 1, CHOH), 6.0 (v br s, 2), 7.07 (dt, 1, J = 2.2 and 8.4 Hz, ArH), 7.24 (m, 2, ArH), 7.36 (m, 1, ArH); EA max 269 nm (ε 980), 262 (1000), 257 (670), 251 (390), (CH_3OH-KOH) 269 (980), 263 (970), 257 (640), 252 (390); CD (CH_3OH, c 0.0325) $[\theta]_{273}$ ±0, $[\theta]_{270}$ +370, $[\theta]_{267}$ +90, $[\theta]_{263}$ +320, $[\theta]_{258}$ +200 (sh), $[\theta]_{252}$ ±0, $[\theta]_{243}$ -2700, (CH_3OH-KOH, c 0.0325) $[\theta]_{275}$ ±0, $[\theta]_{270}$ +840, $[\theta]_{267}$ +410, $[\theta]_{263}$ +880, $[\theta]_{259}$ +580, $[\theta]_{257}$ +610, $[\theta]_{252}$ +280 (sh), $[\theta]_{245}$ ±0, $[\theta]_{235}$ -3000.

(R)-o-Methylmandelic acid[32] **((R)-1i)**: mp 61-63 °C, $[\alpha]^{24}_D$ -171° (c 2.66, CH_3CH_2OH) [lit.[3] mp 61-63 °C, $[\alpha]^{25}_D$ -175° (c 2, CH_3CH_2OH)]; 98% ee; EA max (CH_3OH) 273 nm (ε 280), 265 (330), 261 (300); (CH_3OH-KOH) 273 (250), 264 (310); CD (CH_3OH, c 0.0537) $[\theta]_{280}$ ±0 $[\theta]_{275}$ -120, $[\theta]_{273}$ -20, $[\theta]_{269}$ -110, $[\theta]_{265}$ -20, $[\theta]_{263}$ -60, $[\theta]_{255}$ -20, $[\theta]_{245}$ -1100; (CH_3OH-KOH, c 0.0537) $[\theta]_{277}$ ±0, $[\theta]_{276}$ -30,[29] $[\theta]_{275}$ ±0, $[\theta]_{273}$ +150, $[\theta]_{269}$ +50, $[\theta]_{265}$ +120, $[\theta]_{263}$ +90, $[\theta]_{257}$ +100, $[\theta]_{247}$ ±0, $[\theta]_{238}$ -1000.

(R)-p-(Aminomethyl)mandelic acid[32] **((R)-3c)**: mp >260 °C, $[\alpha]^{23}_D$ -116° (c 1.5, 1 N HCl) [lit.[3] mp >200 °C, $[\alpha]^{25}_D$ -124° (c 1.5, 1 N HCl)]; 94% ee; EA max (H_2O) 270 nm (ε 160), 265 (250), 260 (280), 255 (230); (H_2O-HCl) 270 (160), 265 (240), 260 (240), 255 (180); (H_2O-KOH) 271 (140) (sh), 267 (200) (sh), 262 (250), 256 (230), 253 (190) (sh); CD (H_2O, c 0.0400) $[\theta]_{276}$ ±0, $[\theta]_{271}$ +310, $[\theta]_{268}$ +150, $[\theta]_{265}$ +380, $[\theta]_{261}$ +230, $[\theta]_{260}$ +260, $[\theta]_{253}$ +120 (sh), $[\theta]_{250}$ ±0, $[\theta]_{238}$ -2100; (H_2O-HCl, c 0.0400) $[\theta]_{274}$ ±0, $[\theta]_{271}$ +360, $[\theta]_{267}$ +170, $[\theta]_{264}$ +450, $[\theta]_{261}$ +250, $[\theta]_{258}$ +290, $[\theta]_{254}$ +150 (sh), $[\theta]_{250}$ ±0, $[\theta]_{249}$ -100; (H_2O-KOH, c 0.0400) $[\theta]_{280}$ ±0, $[\theta]_{273}$ -270, $[\theta]_{271}$ -140, $[\theta]_{265}$ -210, $[\theta]_{261}$ -120, $[\theta]_{259}$ -140, $[\theta]_{252}$ -75, $[\theta]_{243}$ -390.

p-Methylbenzylamine hydrochloride (5a): EA max (CH_3OH) 271 nm (ε 130), 266 (160), 262 (230), 255 (180), 251 (130) (sh), (CH_3OH-KOH) 273 (310), 267 (290), 264 (320), 259 (250), 252 (160) (sh).

Benzylamine hydrochloride (5b): EA max (CH_3OH) 267 nm (ε 120), 263 (170), 261 (170), 256 (210), 251 (150), 246 (110) (sh), 242 (72) (sh), (CH_3OH-KOH) 268 (100), 264 (140), 261 (150) (sh), 258 (190), 252 (150), 247 (110) (sh), 242 (70) (sh).

Toluene (5c): EA max (CH_3OH) 268 nm (ε 230), 265 (170), 262 (250), 260 (210), 255 (190), 249 (120) (sh), 243 (75) (sh).

Hydrogenolysis of (R)-m-Fluoromandelic Acid ((R)-1h). A mixture of (R)-**1h**, $[\alpha]^{24}_D$ -119° (c 2.83, acetone) (0.30 g, 1.8 mmol), potassium hydroxide (1.12 g, 19.9 mmol), 90% aqueous ethanol (50 mL), and Raney nickel (2.0 g, wet) was stirred under an atmosphere of hydrogen (680 mmHg, 25 °C). An equivalent amount of hydrogen (47 mL, 1.7 mmol) was consumed in 1.5 h, and the catalyst was removed by filtration. The filtrate was acidified (pH <2) and evaporated to about 15 mL, and water (30 mL) was added. The solution was extracted with ether (2 × 25 mL). The dried ($MgSO_4$) ethereal solution was evaporated at reduced pressure. Recrystallization of the solid residue from benzene gave (R)-mandelic acid ((R)-**1a**) (90 mg, 34%): mp 122-124 °C, $[\alpha]^{25}_D$ -139° (c 1.14, H_2O) [lit.[17] $[\alpha]^{26}_D$ -130° (c 3.24, H_2O)]; ^1H NMR (300 MHz, CD_3SOCD_3) δ 5.02 (s, 1, CHOH), 7.30 (m, 3, ArH), 7.42 (m, 2, ArH). This ^1H NMR spectrum was different from that of (R)-**1h** but identical with that of (R)-**1a** under the same conditions of observation.

(32) Purchased from Niels Clauson-Kaas A/S Chemical Research Laboratory, Rugmarken 28, DK-3520 Farum, Denmark.

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Pentacovalent Oxaphosphorane Chemistry in Organic Synthesis. 2. Total Syntheses of (±)-*trans*- and (±)-*cis*-Neocnidilides

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Both (±)-*trans*- and (±)-*cis*-neocnidilides (1 and 2) have been synthesized via the route illustrated in Scheme III. The condensation of the 1,2λ⁵-oxaphospholene **5b** with valeraldehyde produced the highly substituted phosphonates **12s** and **12a**, which were transformed via an intramolecular Wadsworth-Horner-Emmons olefination reaction to the title compounds.

The carbon analogue[1] of the Ramirez carbonyl condensation reaction forms the basis of the syntheses of both (±)-*trans*- and (±)-*cis*-neocnidilides (1 and 2). These compounds are major constituents of the volatile oil of most representatives of *Apium graveleues* L (Umbelliferae) and have been found to inhibit both the growth and the toxin production of mycotoxin-producing fungi.[2] The structure of *trans*-neocnidilide, isolated from *Cnidium officinale*, was reported by Mitsuhashi and Muramatsu in

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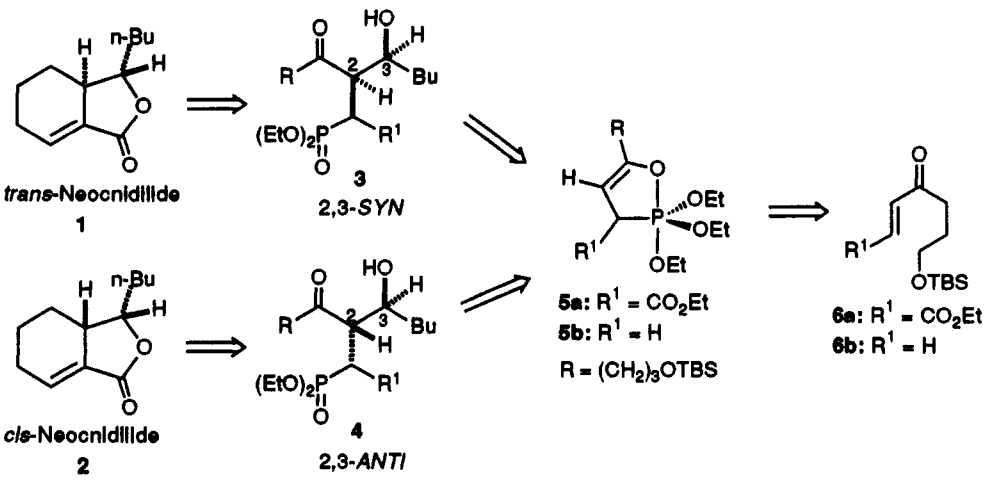
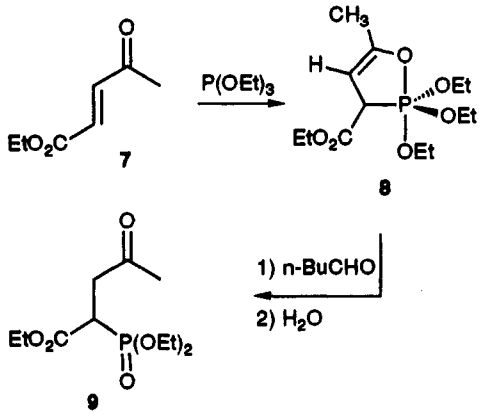
(2) Suzuki, H.; Tanaka, A.; Yamashita, K. *Agric. Biol. Chem.* **1987**, *51*, 3369.

0022-3263/91/1956-2326$02.50/0

Scheme I



Scheme II



1964.[3] More recently, Fischer and Gijbels[4] have isolated and reported the structure of *cis*-neocnidilide from *A. graveolens* L and shown it to be identical with the previously isolated isocnidilide. Both *cis*- and *trans*-neocnidilides have previously been synthesized.[2,5]

Our route to **1** and **2** utilizes the condensation of 1,2λ⁵-oxaphospholene **5** with valeraldehyde to prepare the highly functionalized phosphonates **3** and **4** (Scheme I). *trans*-Neocnidilide can then be produced from the syn diastereomer **3**, and *cis*-neocnidilide from the anti diastereomer **4**. Ideally, we would like the ester ($R^1 = CO_2Et$) to be present in the starting enone **6a**. However, the condensation of valeraldehyde with the model compound **8**, prepared from *trans*-ethyl-4-oxo-2-pentenoate[6] (**7**) and triethyl phosphite, has to date not produced any recognizable products due to the thermal instability of **8** (see Scheme II). The ester was successfully introduced at a later stage in the synthesis by alkylating α to the phosphonate with methyl cyanoformate.

The enone **6b** needed for the synthesis of the 1,2λ⁵-oxaphospholene **5b** was prepared in three steps from 2-hydroxytetrahydrofuran (**10**)[7] (see Scheme III). The 1,4-diol **11a** was formed via addition of vinylmagnesium bromide to **10**, followed by selective silylation to produce **11b**. Subsequent oxidation with PDC yielded the desired enone **6b**. Formation of the 1,2λ⁵-oxaphospholene **5b** proceeded by reaction of **6b** with neat triethyl phosphite. Condensation of **5b** with valeraldehyde under neat conditions followed by hydrolysis produced a mixture of the keto phosphonates **12s** and **12a** in a ratio of 1.78:1.0. Assignment of stereochemistry was by comparison with similar compounds made previously by us via this method (the syn isomer generally has a smaller proton–proton coupling constant, $J_{2,3}$, than the anti isomer),[1] as well as by NMR spectral analysis of the acetonides **14s** and **14a** (vide infra). The diastereomers were separated via HPLC (semipreparative column) at this point, but could in theory be carried on as a mixture through the subsequent reactions. While Scheme III only illustrates the conversion of **12s** to **1**, we have converted the anti diastereomer **12a** to **2** via the same reactions in approximately the same overall yield.

Attempted reduction of the carbonyl in a model compound containing a benzyl-protected C3 hydroxyl function via the modified Wolff–Kischner reaction[8] led to intractable material. We therefore envisioned removal of the oxygen functionality at C4 (neocnidilide numbering) via a Barton radical deoxygenation procedure. Stereoselective reduction of the ketone in **12s** with tetramethylammonium triacetoxyborohydride[9] produced the anti diol **13s**,[10] which was subsequently protected as the acetonide to give **15s**. One- and two-dimensional (COSY) proton NMR spectral analysis of the acetonide–alcohol **14s** supported our structural assignments. The proton–proton coupling constant (J) between H4 and H5 was 3.0 Hz, while that between H5 and H6 was 8.5 Hz, as would be expected for equatorial–axial and equatorial–equatorial orientations of the protons in a chair conformation of the acetonide.[11] See the Experimental Section for more details. Ultimate conversion to the respective neocnidilide isomers also supported our stereochemical assignments in our initial condensation products **12s** and **12a**.

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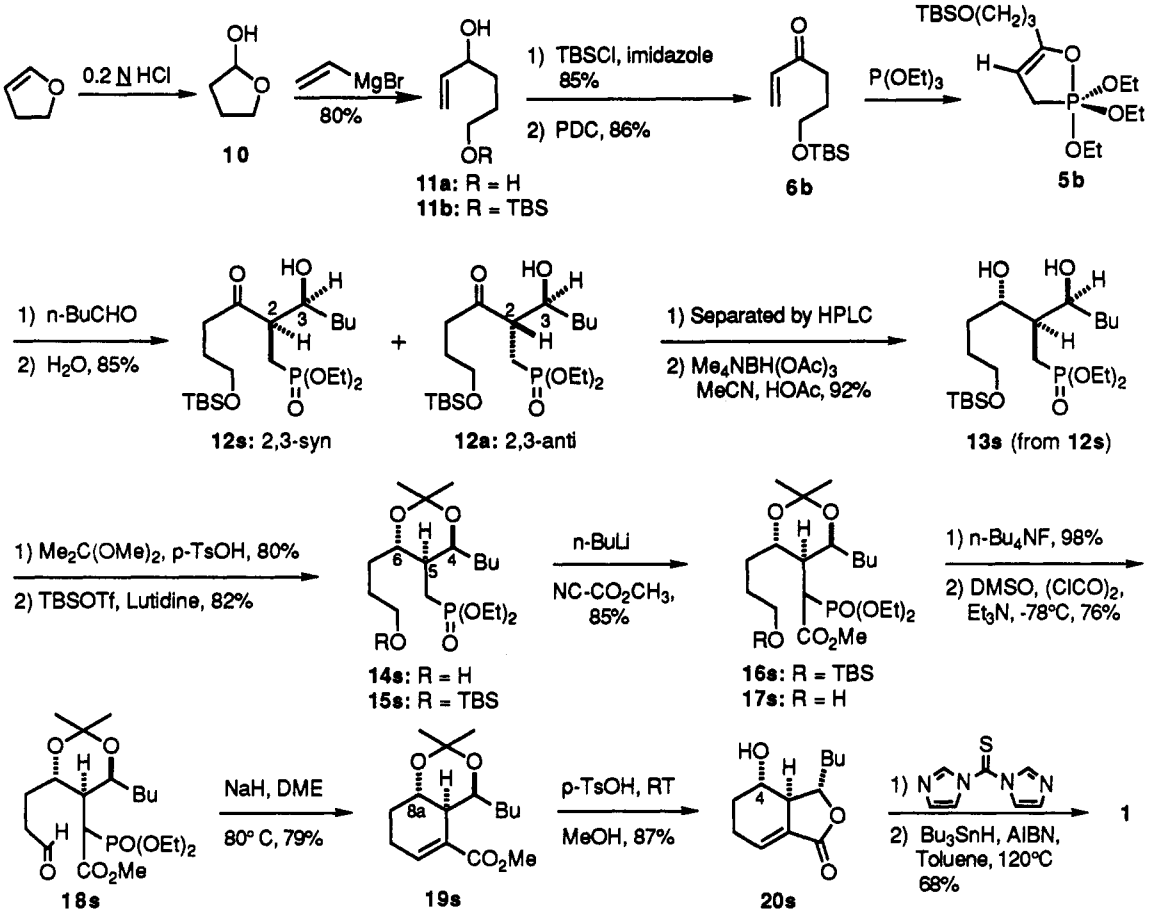
(9) Evans, D. A.; Chapman, K. T. *Tetrahedron Lett.* **1986**, *27*, 5939.
(10) The reduction of **13s** produced a small amount (<10%) of the other alcohol diastereomer, while the reduction of **13a** was completely stereoselective.
(11) (a) Sohár, P. *Nuclear Magnetic Resonance*, Vol. II; CRC Press: Boca Raton, Fl, 1983. (b) Booth, H. *Prog. NMR Spectr.* **1969**, *5*, 149. (c) For a recent example of NMR spectral assignments in acetonides, see: Ziegler, F. E.; Cain, W. T.; Kneisley, A.; Stirchak, E. P.; Wester, R. T. *J. Am. Chem. Soc.* **1988**, *110*, 5442.

Scheme III



Assembly of the functionalized cyclohexene was performed at this point. Treatment of the phosphonate **15s** with *n*-butyllithium at −78 °C followed by Mander's reagent,[12] methyl cyanoformate, produced the phosphono ester **16s**. Removal of the silyl protecting group and subsequent Swern oxidation[13] of the primary hydroxyl group to the aldehyde set the stage for ring formation via an intramolecular Wadsworth–Horner–Emmons olefination reaction.[14] Treatment of **18s** with sodium hydride in DMF at 80 °C produced the desired cyclohexene **19s** in good yield.

We were able to effect deprotection of the diol and lactonization to 4-hydroxy-*trans*-neocnidilide (**20s**) in one step by treatment of the acetonide **19s** in methanol with a catalytic amount of *p*-toluenesulfonic acid. Deoxygenation at C4 via Barton's modified procedure[15] proceeded smoothly to produce *trans*-neocnidilide (**1**). The same sequence of reactions produced *cis*-neocnidilide (**2**) from **12a**. Spectroscopic data for the synthetic materials **1** and **2** were identical in all respects with those reported for the isolated natural compounds.[2]

This straightforward route to substituted cyclohexene derivatives illustrates the synthetic utility of pentacovalent phosphorus reagents. We are presently investigating the effect of substitution on the diastereomeric ratio of the condensation products, as well as the possibility of using configurationally defined phosphorus precursors to produce enantiomerically pure synthetic targets.

Experimental Section

General. Triethyl phosphite was treated with sodium prior to distillation. THF and Et_2O were distilled from sodium–benzophenone. CH_2Cl_2 and CH_3CN were distilled from calcium hydride. $CHCl_3$ was distilled from P_2O_5 under nitrogen. All reactions were carried out under a dry nitrogen or argon atmosphere in flame-dried one- or two-necked round-bottom flasks.

Proton, carbon, and phosphorus NMR spectra were obtained in $CDCl_3$. Carbon signals were obtained from normal ^{13}C spectra and supported by an INEPT pulse sequence. ^{31}P chemical shifts are reported in ppm downfield from H_3PO_4. All two-dimensional spectra were obtained in absolute value mode and were multiplied by a sine-bell apodization function prior to Fourier transformation. For COSY[16] data, the matrix consisted of 256 t_1 increments containing 1K complex points each and was zero-filled to 512 real points in both dimensions. The sweepwidths were 1748 Hz in both dimensions, with a relaxation delay of 3 s. A total of 32 scans per t_1 value were recorded. 2D *J*-resolved spectra[17] were acquired, using sweepwidths of 2000 Hz and 62.5 Hz (64 t_1 values) in the chemical shift (f_2) and *J*-coupling (f_1) dimensions, respectively. The matrix was zero-filled to 1K by 128 real data points. A total of 54 transients per t_1 value were recorded. Melting points are

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(17) Aue, W. P.; Karhan, J.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 4226.

uncorrected. Column chromatography was performed on Kiesel Gel 60 PF_{254}, using a step gradient of CH_3OH in CH_2Cl_2. R_f values indicated refer to thin-layer chromatography on Analtech 2.5 × 10 cm, 250 M analytical plates coated with silica gel GF. High pressure liquid chromatography was done on a Rainin Dynamax-60A semipreparative silica gel column.

5-Hexene-1,4-diol (11a). To a solution of vinylmagnesium bromide in THF (130 mL, 0.72 M, 0.94 mmol) at rt was added a solution of butyrolactol[7] (**10**) (5.5 g, 62.5 mmol) in 63 mL of THF (exothermic). After being stirred for 14 h, the reaction was quenched slowly with 150 mL of 0.2 M HCl. The organic layer was separated, and the aqueous layer was extracted with Et_2O (6 × 50 mL). The resulting crude oil was purified via column chromatography on 100 g of silica gel with 20% (200 mL), 30% (200 mL), and 50% (800 mL) EtOAc/petroleum ether. Concentration in vacuo gave 5.4 g of **11a** as a clear oil (47 mmol, 75% yield): R_f(70% EtOAc/petroleum ether) = 0.38; ^{1}H NMR 5.95–5.80 (1 H, m), 5.31–5.05 (2 H, m), 4.15 (1 H, m), 3.65 (2 H, m), 3.05 (2 H, bs), 1.65 (4 H, m); ^{13}C NMR 141.0, 114.6, 72.8, 62.7, 34.1, 28.7; IR: 3325, 2937, 2875, 1643, 1425, 1056, 993 cm^{-1}; exact mass calcd for $C_6H_{12}O_2$ $(M - H_2O)^+$ 98.0729, found 98.0723.

6-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-1-hexen-3-ol (11b). To **11a** (8.1 g, 69.8 mmol) in DMF (70 mL) under argon and at −5 °C were added imidazole (5.7 g, 84.0 mmol) and TBDMSCl (10.5 g, 69.8 mmol). After being stirred for 15 min at −5 °C, the reaction mixture was diluted with 200 mL of Et_2O and 200 mL of distilled H_2O. Aqueous workup yielded 15.6 g of an oil. Purification via column chromatography on silica gel (500 mL of petroleum ether, then 700 mL of 3% EtOAc/petroleum ether) yielded **11b** (13.7 g, 85% yield) as an oil, along with diprotected starting material (0.8 g): R_f(20% EtOAc/petroleum ether) = 0.58; ^{1}H NMR 5.92–5.78 (1 H, m), 5.25–5.01 (2 H, m), 4.08 (1 H, m), 3.65 (2 H, t, J = 5.8 Hz), 2.88 (1 H, bs), 1.58 (4 H, m), 0.86 (9 H, s), 0.02 (6 H, s); ^{13}C NMR 141.3, 114.3, 72.6, 63.3, 34.3, 28.7, 25.9, 18.3, −5.4; IR 3377, 2945, 1641, 1473, 1106, 989, 835 cm^{-1}; exact mass calcd for $C_{12}H_{26}O_2Si$ $(M + 1)^+$ 230.1695, found 231.1773.

6-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-1-hexen-3-one (6b). To **11b** (10 g, 43.5 mmol) in CH_2Cl_2 (1000 mL) under argon and at 0 °C were added PDC (32.7 g, 87.0 mmol) and Celite 545 (327 g). The reaction mixture was allowed to stir for 10 min at 0 °C and 26 h at rt. Dilution with 300 mL of Et_2O, filtration through a short column of Celite, and purification via column chromatography on 100 g of silica gel (3% EtOAc/petroleum ether) yielded **6b** as a clear oil (8.5 g, 86% yield): R_f(20% EtOAc/petroleum ether) = 0.83; ^{1}H NMR 6.32 (1 H, dd, J = 17.7, 10.1 Hz), 6.18 (1 H, dd, J = 17.7, 1.7 Hz), 5.78 (1 H, dd, J = 10.1, 1.7 Hz), 3.60 (2 H, t, J = 6.1 Hz), 2.63 (2 H, t, J = 7.3 Hz), 1.79 (2 H, quin, J = 7.3 Hz), 0.85 (9 H, s), 0.01 (6 H, s); ^{13}C NMR 194.4, 136.7, 127.6, 62.1, 35.8, 27.0, 25.9, 18;2, −5.4; IR 3377, 2959, 2887, 1703, 1683, 1402, 1094 cm^{-1}; exact mass calcd for $C_{12}H_{24}O_2Si$ $(M - C_4H_9)^+$ 171.0841, found 171.0852.

2,2,2-Triethoxy-2,3-dihydro-5-[3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]propyl]-1,2λ^5-oxaphospholene (5b). A flame-dried, 25-mL flask under argon was charged with enone **6b** (0.5 g, 2.19 mmol) and $P(OEt)_3$ (0.5 mL, 2.85 mmol). The reaction mixture was allowed to stir for 52 h at 40 °C. The crude product was purified by bulb-to-bulb distillation (80 °C, 0.05 mmHg) to give **5b** as a clear oil (553 mg, 69%): ^{1}H NMR 4.58–4.39 (1 H, dm, J_{H-P} = 46.8 Hz), 3.85 (6 H, m), 3.59 (2 H, t, J = 6.4 Hz), 2.55–2.46 (2 H, dm, J_{H-P} = 18.7 Hz), 2.12 (2 H, t, J = 7.7 Hz), 1.71 (2 H, m), 1.25 (2 H, m), 1.12 (9 H, t, J = 7.0 Hz), 0.87 (9 H, s), 0.02 (6 H, s); ^{13}C NMR 154.8 (d, J_{P-C} = 15.4 Hz), 89.7 (d, J_{P-C} = 5.9 Hz), 62.6, 61.9 (d, J_{P-C} = 10.3 Hz), 29.7, 27.6, 28.8 (d, J_{P-C} = 168.2 Hz), 25.8, 18.2, 16.5 (d, J_{P-C} = 7.3 Hz), −5.4; IR 2968, 1676, 1458, 1317, 1186, 1086, 1053, 968 cm^{-1}.

Diethyl (2*R,3*S**)-[2-[4-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-1-oxobutyl]-3-hydroxyheptyl]phosphonate (12s) and Diethyl (2*R**,3*R**)-[2-[4-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-1-oxobutyl]-3-hydroxyheptyl]phosphonate (12a).** To the oxaphospholene **5b** (400 mg, 1.02 mmol) under argon was added freshly distilled valeraldehyde (0.4 mL, 3.76 mmol). The reaction was followed by ^{1}H NMR. After 38 h at rt, the reaction was stirred with distilled water (20 equiv) for 14 h. This solution was extracted with CH_2Cl_2 (5 × 10 mL), dried ($MgSO_4$), and concentrated in vacuo. The crude products were purified via column chromatography on 30 g of silica gel with CH_2Cl_2 (100 mL), 0.5% CH_3OH/CH_2Cl_2 (200 mL), and 1% CH_3OH/CH_2Cl_2 (500 mL) to give an oil as a mixture of the diastereomers **12s** and **12a** (394 mg, 0.87 mmol, 85% yield). HPLC separation (2% CH_3OH/CH_2Cl_2) yielded a syn:anti ratio (**12s**:**12a**) of 1.78:1.0 by weight. For **12s** (syn isomer): R_f (5% MeOH/CH_2Cl_2) = 0.48; ^{1}H NMR 4.01 (4 H, m), 3.63 (1 H, m, H3, $J_{2,3}$ = 3.4 Hz from decoupling studies), 3.54 (2 H, t, J = 6.1 Hz), 2.87 (1 H, m, H2), 2.61 (2 H, m), 2.23–1.82 (2 H, m), 1.68 (2 H, m), 1.40–1.21 (6 H, m), 1.15 (6 H, td, J = 7.0, 3.0 Hz), 0.82 (12 H, bs), −0.03 (6 H, s); ^{13}C NMR 213.1, 73.2 (d, J_{P-C} = 12.6 Hz), 62.1, 61.9 (d, J_{P-C} = 6.7 Hz), 61.8 (d, J_{P-C} = 6.5 Hz), 50.9 (d, J_{P-C} = 3.1 Hz), 41.1, 34.5, 28.1, 26.3, 25.9, 24.6 (d, J_{P-C} = 142.5 Hz), 22.5, 18.3, 16.3 (d, J_{P-C} = 5.9 Hz), 13.9, −5.4; ^{31}P NMR 31.9 ppm; IR 3331, 2962, 1713, 1235, 1051, 1030, 962; exact mass calcd for $C_{21}H_{45}O_6PSi$ $(M - C_4H_9)^+$ 395.2017, found 395.2023. For **12a** (anti isomer): R_f(5% MeOH/CH_2Cl_2) = 0.52; ^{1}H NMR 4.07 (4 H, m), 3.68 (1 H, m, H3, $J_{2,3}$ = 4.7 Hz from decoupling studies), 3.60 (2 H, t, J = 6.2 Hz), 3.05 (1 H, m, H2), 2.66 (2 H, td, J = 7.3, 2.6 Hz), 2.25–1.61 (4 H, m), 1.55–1.12 (6 H, m), 1.25 (6 H, td, J = 7.0, 1.0 Hz), 0.85 (12 H, bs), 0.01 (6 H, s); ^{13}C NMR 212.1, 71.5 (d, J_{P-C} = 12.9 Hz), 62.0, 61.8 (d, J_{P-C} = 6.6 Hz), 61.6 (d, J_{P-C} = 6.5 Hz), 50.9 (d, J_{P-C} = 2.8 Hz), 40.1, 34.2, 28.1, 26.4, 25.9, 22.4 (d, J_{P-C} = 142.6 Hz), 22.5, 18.3, 16.3 (d, J_{P-C} = 6.0 Hz), 13.9, −5.4; ^{31}P NMR 31.2 ppm; IR 3331, 2962, 1713, 1235, 1051, 1030, 962 cm^{-1}; exact mass calcd for $C_{21}H_{45}O_6PSi$ $(M - C_4H_9)^+$ 395.2017; found 395.2023.

Diethyl (2*R,3*S**)-[2-[4-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-1(*S**)-hydroxybutyl]-3-hydroxyheptyl]phosphonate (13s).** $Me_4NBH(OAc)_3$ (6.7 g, 25.5 mmol) was dissolved in a mixture of HOAc and CH_3CN (18 mL:18 mL) and cooled to 5 °C under argon. To this mixture was added a solution of **12s** (2.2 g, 5.1 mmol) in CH_3CN (5.0 mL). After 30 min at −5 °C, the reaction was allowed to stir for 16 h at rt and then quenched with 70 mL of 0.5 N sodium potassium tartrate. This mixture was stirred for 1 h and extracted with CH_2Cl_2 (5 × 50 mL). The combined organic extracts were washed with 50 mL of saturated $NaHCO_3$ and dried (anhyd Na_2SO_4). The crude product was purified via column chromatography (100 g of silica gel, 1.5% MeOH/CH_2Cl_2) to give **13s** (2.14 g, 92% yield): R_f(5% MeOH/CH_2Cl_2) = 0.42; ^{1}H NMR 4.06 (4 H, m), 3.95–3.52 (4 H, m), 2.18–1.15 (13 H, m), 1.30 (6 H, t, J = 7.1 Hz), 0.89 (12 H, bs), 0.06 (6 H, s); ^{13}C NMR 74.1 (d, J_{P-C} = 3.9 Hz), 70.4 (d, J_{P-C} = 12.3 Hz), 63.6, 61.6, (d, J_{P-C} = 2.7 Hz), 6.15 (d, J_{P-C} = 2.1 Hz), 42.9 (d, J_{P-C} = 2.6 Hz), 34.9, 32.8, 29.7, 28.2, 26.9 (d, J_{P-C} = 142.4 Hz), 25.8, 22.7, 18.2, 16.3 (d, J_{P-C} = 6.1 Hz), 14.0, −5.5; ^{31}P NMR 35.9 ppm; IR 3367, 2931, 1251, 1100, 1054, 1033, 962 cm^{-1}; exact mass calcd for $C_{21}H_{47}O_6PSi$ $(M - C_4H_9)^+$ 397.2029, found 397.2173.

Diethyl (4*S,5*R**,6*S**)-[[4-Butyl-6-[3-[[1,1-dimethylethyl)dimethylsilyl]oxy]propyl]-2,2-dimethyl-1,3-dioxan-5-yl]methyl]phosphonate (15s).** To a solution of **13s** (200 mg, 0.44 mmol) in CH_2Cl_2 (4.4 mL) were added 0.44 mL of 2,2-dimethoxypropane and a catalytic amount of *p*-TsOH (10 mg) under argon. The reaction mixture was allowed to stir overnight at rt and quenched with 6 mL of distilled H_2O. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The crude product was purified via column chromatography (10 g of silica gel, 1.5% MeOH/CH_2Cl_2) to give the acetonide that has lost the silyl protecting group **14s** (94 mg, 57% yield) as well as triol (45 mg, 0.13 mmol) as oils. The triol was converted to the acetonide (39 mg, 77% yield) via this same method. **14s**: R_f(5% MeOH/CH_2Cl_2) = 0.45; ^{1}H NMR 4.11 (4 H, m), 3.89 (1 H, m, H4, $J_{4,5}$ = 3.0 Hz from decoupling studies), 3.78 (1 H, m, H6, $J_{5,6}$ = 8.5 Hz from decoupling studies), 3.65 (2 H, m), 2.55 (1 H, bs), 2.05–1.15 (25 H, m), 0.91 (3 H, t, J = 7.1 Hz); ^{13}C NMR 99.3, 74.2 (d, J_{P-C} = 5.1 Hz), 74.0 (d, J_{P-C} = 7.3 Hz), 62.3, 61.8 (d, J_{P-C} = 6.3 Hz), 61.4 (d, J_{P-C} = 6.9 Hz), 35.4, 32.5, 29.8, 29.5, 28.8, 27.7, 22.6, 19.5, 18.1 (d, J_{P-C} = 143.8 Hz), 16.4 (d, J_{P-C} = 4.0 Hz), 16.3 (d, J_{P-C} = 4.3 Hz), 14.0; ^{31}P NMR 34.8 ppm; IR 3431, 2931, 1381, 1231, 1056, 1025, 962, 838 cm^{-1}; exact mass calcd for $C_{18}H_{37}O_6P$ $(M - CH_3)^+$ 365.2060, found 365.2084. Proton–proton decoupling studies were performed by irradiation at 1.85 and 1.38 ppm. Assignments of protons H4 and H6 were substantiated by COSY spectra. The $J_{4,5}$ and $J_{5,6}$ values indicate that the conformation of the acetonide six-membered ring is a chair, with H4 equatorial and H6 axial. It has also been observed that equatorial protons have a larger chemical shift value

than axial protons in variously substituted cyclohexanes and six-membered heterocycles.[11]

Triol: R_f(5% MeOH/CH_2Cl_2) = 0.27; ^1H NMR 4.31 (1 H, bs), 4.11–3.89 (7 H, m), 3.83–3.25 (3 H, m), 2.31–1.19 (13 H, m), 1.33 (6 H, td, J = 7.05, 1.27), 0.92 (3 H, t, J = 6.9 Hz); ^{13}C NMR 73.9 (d, J_{P-C} = 4.1 Hz), 7.07 (d, J_{P-C} = 12.4 Hz), 62.6, 61.9 (d, J_{P-C} = 6.7 Hz), 61.7 (d, J_{P-C} = 6.9 Hz), 41.7 (d, J_{P-C} = 2.8 Hz), 34.5, 32.1, 29.8, 28.5, 22.6, 21.5 (d, J_{P-C} = 139.3 Hz), 16.4 (d, J_{P-C} = 5.9 Hz), 14.0; ^{31}P NMR 34.3 ppm; IR 3362, 2937, 1231, 1056, 1031, 963 cm^{-1}.

The TBS group was reintroduced by reaction of the acetonide–alcohol **14s** (240 mg, 0.63 mmol) from above with TBSOTF (0.29 mL, 1.26 mmol) and lutidine (0.15 mL, 1.26 mmol) in CH_2Cl_2 (6 mL) at −78 °C. After being stirred for 10 h at −78 °C, the reaction mixture was warmed to rt, quenched with pH 7 buffer solution (2.0 mL), and extracted with CH_2Cl_2 (3 × 10 mL). The crude product was purified by column chromatography (10 g of silica gel, 1% MeOH/CH_2Cl_2) to give pure product **15s** (255 mg, 82% yield) as an oil: R_f(5% MeOH/CH_2Cl_2) = 0.77; ^1H NMR 4.01 (4 H, m), 3.77 (2 H, m), 3.56 (2 H, t, J = 6.6 Hz), 1.87–1.05 (19 H, m), 1.28 (3 H, s), 1.25 (3 H, s), 0.83 (12 H, s), −0.02 (6 H, s); ^{13}C NMR 99.0, 74.0 (d, J_{P-C} = 6.2 Hz), 73.8 (d, J_{P-C} = 6.2 Hz), 63.0, 61.2 (d, J_{P-C} = 5.4 Hz), 35.8, 32.5, 29.8, 29.0, 28.8, 27.7, 25.9, 22.5, 19.4, 18.3, 18.2 (d, J_{P-C} = 143.6 Hz), 16.3 (d, J_{P-C} = 6.1 Hz), 13.9, −5.5; ^{31}P NMR 34.7 ppm; IR 2955, 2860, 1464, 1381, 1254, 1093, 1059, 1029, 961, 834 cm^{-1}; exact mass calcd for $C_{24}H_{51}O_6PSi$ $(M - CH_3)^+$ 479.2955, found 479.2927.

Diethyl (4*S,5*R**,6*S**)-[[4-Butyl-6-[3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]propyl]-2,2-dimethyl-1,3-dioxan-5-yl]carbomethoxymethyl]phosphonate (16s).** To **15s** (311 mg, 0.63 mmol) in THF (12 mL) at −78 °C under argon was added 1.6 M of *n*-BuLi (1.6 mL, 2.50 mmol). After 75 min at −78 °C, a solution of $NCCO_2Me$ (0.2 mL, 2.50 mmol) in THF (3 mL) was added. The reaction mixture was stirred for 2 h at −78 °C, warmed to −10 °C, and quenched with 10 mL of distilled H_2O. Extraction with CH_2Cl_2 and purification via column chromatography (10 g of silica gel, 1% MeOH/CH_2Cl_2) yielded the oil **16s** (241 mg, 85% yield based on reacted **15s**) as a mixture of diastereomers at the ester group and unreacted **15s** (60 mg). **16s**: R_f(5% MeOH/CH_2Cl_2) = 0.79; ^1H NMR (major) 4.08 (4 H, m), 3.82 (2 H, m), 3.67 (3 H, s), 3.55 (2 H, m), 3.19 (1 H, bd, J_{P-H} = 24.8 Hz), 2.13–1.11 (17 H, m), 1.34 (3 H, s), 1.25 (3 H, s), 0.83 (12 H, s), −0.01 (6 H, s); ^{13}C NMR 169.4, 99.5, 74.1 (d, J_{P-C} = 6.4 Hz), 73.9 (d, J_{P-C} = 6.3 Hz), 62.6, 61.3 (d, J_{P-C} = 5.4 Hz), 52.2, 41.1, 40.8 (d, J_{P-C} = 138.8 Hz), 35.9, 29.3, 29.1, 28.9, 27.7, 25.9, 22.5, 19.3, 18.3, 16.3 (d, J_{P-C} = 4.1 Hz), 14.1, −5.3; ^{31}P NMR (major) 34.8 ppm, (minor) 34.6 ppm; IR 2956, 2857, 1738, 1469, 1380, 1254, 1100, 1053, 1027, 963, 837 cm^{-1}; exact mass calcd for $C_{26}H_{53}O_8PSi$ $(M - CH_3)^+$ 537.3010, found 537.3051.

Diethyl (4*S,5*R**,6*S**)-[[4-Butyl-6-(3-hydroxypropyl)-2,2-dimethyl-1,3-dioxan-5-yl]carbomethoxymethyl]-phosphonate (17s).** To a solution of **16s** (105 mg, 0.19 mmol) in THF (1.9 mL) at rt was added *n*-Bu_4NF (90 mg, 0.29 mmol). After being stirred for 2 h, the reaction mixture was quenched with 5 mL of NH_4Cl and extracted with CH_2Cl_2 (5 × 15 mL). The crude product was purified via chromatography on 5 g of silica gel with 50 mL of 0.5% MeOH/CH_2Cl_2 and 200 mL of 1.0% MeOH/CH_2Cl_2 to give a clear oil (81 mg, 98% yield): R_f(5% MeOH/CH_2Cl_2) = 0.44; ^1H NMR (major) 4.59 (1 H, m), 4.15 (4 H, m), 3.94 (1 H, m), 3.75 (2 H, s), 3.61 (2 H, m), 3.16 (1 H, dd, J = 23.9, 2.5 Hz), 2.07 (1 H, m), 2.05–1.15 (22 H, m), 0.92 (3H, t, J = 7.0 Hz); ^{13}C NMR: 169.0, 100.9, 69.1 (d, J_{P-C} = 16.8 Hz), 67.1, 63.2 (d, J_{P-C} = 6.9 Hz), 62.6 (d, J_{P-C} = 6.9 Hz), 60.8, 52.5, 45.7 (d, J_{P-C} = 4.2 Hz), 42.5 (d, J_{P-C} = 138.9 Hz), 32.3, 30.0, 29.0, 28.3, 25.6, 23.5, 22.6, 16.4 (d, J_{P-C} = 2.4 Hz), 16.3 (d, J_{P-C} = 3.1 Hz), 13.9; ^{31}P (major) 33.2 ppm, (minor) 33.0 ppm; IR: 3431, 2943, 1737, 1381, 1250, 1150, 1056, 1031, 962 cm^{-1}; exact mass calcd for $C_{20}H_{39}O_8P$ $(M - CH_3)^+$ 423.2146, found 423.2133.

Diethyl (4*S,5*R**,6*S**)-[[4-Butyl-6-(3-propanoyl)-2,2-dimethyl-1,3-dioxan-5-yl]carbomethoxymethyl]phosphonate (18s).** To a cold (−78 °C) solution of CH_2Cl_2 (2.0 mL) and oxalyl chloride (192 mL, 2.23 mmol) was added a solution of DMSO (0.31 mL, 4.45 mmol) in CH_2Cl_2 (4 mL). The reaction mixture was stirred for 30 min at −78 °C. A solution of **17s** (487 mg, 1.11 mmol) in CH_2Cl_2 (11 mL) was added quickly, and the mixture was stirred for 1 h at −78 °C. Et_3N (1.54 mL, 11.1 mmol) was added, and the mixture was stirred for 65 min at −78 °C. After being warmed to rt, the reaction mixture was quenched with 6 mL of distilled H_2O and extracted with CH_2Cl_2 (3 × 30 mL). The crude product was purified via column chromatography (10 g of silica gel, 1% MeOH/CH_2Cl_2) to give pure product as an oil (371 mg, 76% yield): R_f(5% MeOH/CH_2Cl_2) = 0.72; ^1H NMR 9.76 (1 H, t, J = 1.5 Hz), 4.41 (1 H, m), 4.11 (4 H, m), 3.89 (1 H, m), 3.73 (3 H, s), 3.24 (1 H, dd, J = 24.1, 2.6 Hz), 2.51 (2 H, m), 2.38–1:02 (21 H, m), 0.91 (3 H, t, J = 7.1 Hz); ^{13}C NMR 201.8, 170.1, 100.8, 69.1 (d, J_{P-C} = 15.7 Hz), 68.4, 63.2 (d, J_{P-C} = 6.9 Hz), 62.6 (d, J_{P-C} = 7.1 Hz), 52.5, 45.1 (d, J_{P-C} = 4.2 Hz), 42.5 (d, J_{P-C} = 138.8 Hz), 41.0, 36.1, 28.3, 25.5, 23.9, 23.3, 22.7, 16.4 (d, J_{P-C} = 2.6 Hz), 16.3 (d, J_{P-C} = 3.2 Hz), 13.9; ^{31}P NMR (major) 33.7 ppm, (minor) 33.5 ppm; IR 2960, 2727, 1761, 1734, 1460, 1379, 1249, 1096, 1051, 1029, 966 cm^{-1}; exact mass calcd for $C_{20}H_{37}O_8P$ $(M - CH_3)^+$ 421.1989, found 421.1973.

Methyl (4*S,4a*R**,8a*S**)-4-Butyl-4a,7,8,8a-tetrahydro-2,2-dimethyl-4*H*-1,3-benzodioxin-5-carboxylate (19s).** To a hot (80 °C) solution of **18s** (117 mg, 0.27 mmol) in DME (27 mL) under argon was added NaH (80% mineral oil dispersion, 33 mg, 1.08 mmol). After being stirred at 80 °C for 40 min, the reaction mixture was slowly poured into ice–water and extracted with Et_2O. Purification via column chromatography (5% EtOAc/petroleum ether) gave the desired product **19s** (60 mg, 79% yield) as a mixture of isomers at C8a: R_f(20% EtOAc/petroleum ether) = 0.75; ^1H NMR (major) 6.89 (1 H, m), 4.57 (1 H, m), 3.98 (1 H, m), 3.72 (3 H, s), 2.96 (1 H, m), 2.34 (2 H, m), 1.93–0.91 (8 H, m), 1.47 (3 H, s), 1.46 (3 H, s), 0.88 (3 H, d, J = 7.1 Hz), (minor) 6.78 (1 H, m), 4.27 (1 H, m), 3.97 (1 H, m), 3.71 (3 H, s), 2.73 (1 H, m), 2.42 (2 H, m), 1.93–0.91 (8 H, m), 1.47 (3 H, s), 1.46 (3 H, s), 0.88 (3 H, t, J = 7.1 Hz); ^{13}C NMR (major) 167.3, 140.6, 129.5, 99.0, 72.9, 66.4, 51.3, 42.6, 30.1, 29.9, 28.3, 27.5, 26.9, 24.7, 22.5, 14.0, (minor) 169.4, 139.8, 130.0, 97.9, 71.9, 67.0, 37.4, 33.3, 30.1, 29.7, 28.8, 27.7, 27.0, 21.4, 21.3, 13.8; IR 2956, 1721, 1640, 1433, 1379, 1258, 1217, 1190, 1092, 1042 cm^{-1}; exact mass calcd for $C_{16}H_{26}O_4$ $(M - CH_3)^+$ 267.1595, found 267.1592.

(3*S,3a*R**,4*S**)-3-Butyl-3a,4,5,6-tetrahydro-4-hydroxy-1-(3*H*)-isobenzofuranone (4-Hydroxy-*trans*-neocnidilide) (20s).** To a solution of **19s** (100 mg, 0.35 mmol) in MeOH (3.5 mL) at rt was added a catalytic amount of *p*-TsOH (10 mg). After being stirred for 1 h, the reaction was quenched with 10 mL of distilled H_2O and extracted with Et_2O. The crude product was purified via column chromatography (silica gel, 20% Et_2O/petroleum ether) to yield an oil (65 mg, 87% yield) as a mixture of isomers at C_4: R_f(50% EtOAc/petroleum ether) = 0.54; ^1H NMR (major) 6.72 (1 H, q, J = 3.3 Hz), 4.25 (1 H, td, J = 8.9, 2.7 Hz), 3.68 (1 H, m), 2.81 (1 H, bs), 2.70–1.15 (11 H, m), 0.91 (3 H, t, J = 7.1 Hz), (minor) 6.85 (1 H, q, J = 3.3 Hz), 4.51 (1 H, m), 3.68 (1 H, m), 2.81–1.15 (12 H, m), 0.92 (3 H, t, J = 6.9 Hz); ^{13}C NMR (major) 169.8, 135.0, 128.9, 85.5, 70.8, 49.7, 35.5, 31.0, 27.7, 26.0, 22.4, 13.8, (minor) 170.1, 135.4, 127.3, 80.3, 62.3, 47.6, 34.5, 30.9, 27.4, 25.9, 20.8, 13.8; IR 3425, 3031, 2962, 1744, 1681, 1463, 1337, 1250, 1106, 1090, 1044 cm^{-1}; exact mass for $C_{12}H_{18}O_3$ $(M)^+$ 210.1254, found 210.1246.

***trans*-Neocnidilide (1).** To a solution of **20s** (31 mg, 0.148 mmol) in CH_2Cl_2 (0.8 mL) at rt and under argon atmosphere was added 1,1′-thiocarbonyldiimidazole (53 mg, 0.295 mmol). After being stirred at rt for 22 h, the reaction was concentrated in vacuo and diluted with 5 mL of freshly distilled toluene. To this solution were added AIBN (20 mg, 0.122 mmol) and Bu_3SnH (59 μL, 0.218 mmol). After being refluxed for 4 h at 120 °C, the reaction mixture was poured into a mixture of Et_2O (5.0 mL) and 0.5 N HCl (5.0 mL). The aqueous layer was neutralized with 20% aq KOH solution and thoroughly extracted with Et_2O (5 × 10 mL). Crude product was purified by column chromatography (10% Et_2O/petroleum ether) to give pure *trans*-neocnidilide as an oil (18.8 mg, 68% yield): R_f(20% EtOAc/petroleum) = 0.74; ^1H NMR 6.75 (1 H, q, J = 3.2 Hz), 3.96 (1 H, m), 2.56–1.05 (13 H, m), 0.90 (3 H, t, J = 7.1 Hz); ^{13}C NMR 170.2, 135.1, 131.3, 85.8, 43.1, 34.3, 27.5, 25.4, 25.0, 22.5, 20.7, 13.8; IR 2934, 2867, 1764, 1680, 1461, 1245, 1184, 1025, 974 cm^{-1}; exact mass calcd for $C_{12}H_{18}O_2$ $(M)^+$ 194.1305, found 194.1322.

Diethyl (2*R,3*R**)-[2-[4-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-1(*R**)-hydroxybutyl]-3-hydroxyheptyl]-phosphonate (13a).** Diol **13a** (570 mg, 94% yield), was prepared from **12a** (600 mg, 1.33 mmol) by following the same procedure

as for the preparation of **13s**. **13a**: R_f(5% MeOH/CH_2Cl_2) = 0.42; ^{1}H NMR 4.05 (4 H, m), 3.94 (1 H, m), 3.79 (1 H, m), 3.63 (2 H, t, J = 5.7 Hz), 2.30–1.29 (13 H, m), 1.29 (6 H, t, J = 7.1 Hz), 0.89 (12 H, bs), 0.03 (6 H, s); ^{13}C NMR 73.8 (d, J_{P-C} = 3.6 Hz), 70.4 (d, J_{P-C} = 12.9 Hz), 63.3, 61.7 (d, J_{P-C} = 6.3 Hz), 61.6 (d, J_{P-C} = 6.7 Hz), 41.3 (d, J_{P-C} = 2.9 Hz), 34.6, 31.9, 29.5, 28.6, 25.9, 22.6, 21.4 (d, J_{P-C} = 140.9 Hz), 18.3, 16.4 (d, J_{P-C} = 5.9 Hz), 14.0, –5.4; ^{31}P NMR 34.8 ppm; IR 3363, 2956, 2931, 1256, 1100, 1057, 1031, 963 cm^{-1}; exact mass calcd for $C_{21}H_{47}O_6PSi$ (M – C_4H_9)$^+$ 397.2029, found 397.2173.

Diethyl (4*R,5*R**,6*R**)-[[4-Butyl-6-[3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]propyl]-2,2-dimethyl-1,3-dioxan-5-yl]methyl]phosphonate (15a).** Acetonide–alcohol **14a** (87 mg, 85% yield) was prepared from **13a** (120 mg, 0.26 mmol) by following the same procedure as for the preparation of **14s**. **14a**: R_f(5% MeOH/CH_2Cl_2) = 0.45; ^{1}H NMR 4.09 (4 H, m), 3.85 (1 H, m, H6, $J_{5,6}$ = 3.5 Hz from decoupling studies), 3.65 (2 H, t, J = 5.9 Hz), 3.58 (1 H, m, H4, $J_{4,5}$ = 5.4 Hz from decoupling studies), 2.68 (1 H, bs), 2.05–1.21 (25 H, m), 0.91 (3 H, t, J = 6.9 Hz); ^{13}C NMR 100.2, 74.6 (d, J_{P-C} = 6.4 Hz), 68.8 (d, J_{P-C} = 12.8 Hz), 62.3, 61.6 (d, J_{P-C} = 7.7 Hz), 61.5 (d, J_{P-C} = 7.4 Hz), 39.6, 34.8, 29.5, 28.2, 27.7, 25.5, 24.7, 23.9 (d, J_{P-C} = 142.7 Hz), 22.6, 16.4 (d, J_{P-C} = 6.0 Hz), 14.0; ^{31}P NMR 32.7 ppm; IR 3431, 2931, 1381, 1231, 1056, 1025, 962, 838 cm^{-1}; exact mass calcd for $C_{18}H_{37}O_6P$ (M – CH_3)$^+$ 365.2060, found 365.2084. Proton–proton decoupling studies were performed by irradiation at 1.50 ppm. Assignments of protons H4 and H6 were substantiated by COSY and 2D J-resolved spectra. The $J_{4,5}$ and $J_{5,6}$ values indicate that the conformation of the acetonide six-membered ring is a chair, with H4 axial and H6 equatorial. It is also known that equatorial protons have a larger chemical shift value than axial protons in variously substituted cyclohexanes and six-membered heterocycles.[11]

To a solution of the acetonide–alcohol from above (1.65 g, 4.34 mmol) in DMF (4.5 mL) at 0 °C was added imidazole (0.36 g, 5.21 mmol). After 5 min at 0 °C, TBDMSCl (0.72 g, 4.78 mmol) was added. The reaction mixture was warmed to rt after 1 h and quenched with 5 mL of distilled H_2O. Extraction with CH_2Cl_2 (5 × 30 mL) and purification via column chromatography (100 g of silica gel, 1% MeOH/CH_2Cl_2) gave **15a** (1.72 g, 80% yield) as an oil: R_f(5% MeOH/CH_2Cl_2) = 0.77; ^{1}H NMR 4.05 (4 H, m), 3.85 (1 H, m), 3.56 (3 H, m), 1.86–1.16 (25 H, m), 0.83 (12 H, s), –0.01 (6 H, s); ^{13}C NMR 100.0, 74.4 (d, J_{P-C} = 4.5 Hz), 68.4 (d, J_{P-C} = 14.7 Hz), 62.9, 61.4 (d, J_{P-C} = 6.4 Hz), 61.3 (d, J_{P-C} = 6.6 Hz), 39.5, 35.0, 29.2, 28.2, 27.4, 25.9, 25.5, 24.7, 23.7 (d, J_{P-C} = 140.7 Hz), 18.3, 16.4 (d, J_{P-C} = 5.9 Hz), 14.1, –5.4; ^{31}P NMR (major) 23.7 ppm; IR 2955, 2860, 1464, 1381, 1254, 1093, 1059, 1029, 961, 834 cm^{-1}; exact mass calcd for $C_{24}H_{51}O_6PSi$ (M – CH_3)$^+$ 479.2955, found 479.2927.

Diethyl (4*R,5*R**,6*R**)-[[4-Butyl-6-[3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]propyl]-2,2-dimethyl-1,3-dioxan-5-yl]carbomethoxymethyl]phosphonate (16a).** Phosphono ester **16a** (254 mg, 82% yield based on reacted **15a**) was prepared from **15a** (338 mg, 0.68 mmol) as a mixture of diastereomers at the ester group, using the same procedure as for the preparation of **16s**. Unreacted starting material **15a** (61 mg) was isolated. **16a**: R_f(5% MeOH/CH_2Cl_2) = 0.79; ^{1}H NMR (major) 4.36 (1 H, m), 4.08 (4 H, m), 3.89 (1 H, m), 3.69 (3 H, s), 3.55 (2 H, m), 3.40 (1 H, dd, J = 22.4, 9.6 Hz), 2.29 (1 H, m), 2.11–1.11 (22 H, m), 0.83 (12 H, s), 0.01 (6 H, s), (minor) 4.08 (5 H, m), 3.89 (1 H, m), 3.68 (3 H, s), 3.55 (2 H, m), 3.16 (1 H, dd, J = 23.9, 2.4 Hz), 2.11–1.11 (23 H, m), 0.87 (12 H, s), –0.01 (6 H, s); ^{13}C NMR (major) 169.2, 100.6, 69.2 (d, J_{P-C} = 5.6 Hz), 69.1 (d, J_{P-C} = 9.8 Hz), 63.2 (d, J_{P-C} = 4.6 Hz), 62.6 (d, J_{P-C} = 7.8 Hz), 62.5, 52.4, 44.9 (d, J_{P-C} = 4.4 Hz), 42.4 (d, J_{P-C} = 137.8 Hz), 36.1, 29.6, 28.3, 27.3, 25.6, 24.2, 22.7, 19.5, 18.3, 16.4 (d, J_{P-C} = 5.6 Hz), 16.3 (d, J_{P-C} = 5.8 Hz), 14.1, –5.4; ^{31}P NMR (major) 23.9 ppm, (minor) 23.3 ppm; IR 2956, 2857, 1738, 1469, 1380, 1254, 1100, 1053, 1027, 963, 837 cm^{-1}; exact mass calcd for $C_{26}H_{53}O_8PSi$ (M – CH_3)$^+$ 537.3010, found 537.3051.

Diethyl (4*R,5*R**,6*R**)-[[4-Butyl-6-(3-hydroxypropyl)-2,2-dimethyl-1,2-dioxan-5-yl]carbomethoxymethyl]-phosphonate (17a).** Deprotected phosphono ester (oil, 180 mg, 90% yield) was prepared from **16a** (250 mg, 0.45 mmol), following the same procedure as for the preparation of the syn isomer using nBu$_4$NF. **17a**: R_f(5% MeOH/CH_2Cl_2) = 0.44; ^{1}H NMR (major) 4.39 (1 H, m), 4.12 (4 H, m), 3.96 (1 H, m), 3.74 (3 H, s), 3.67 (2 H, t, J = 5.9 Hz), 3.20 (1 H, dd, J = 23.9, 2.5 Hz), 2.13 (1 H, m), 1.98–1.12 (22 H, m), 0.92 (3 H, t, J = 7.0 Hz), (minor) 4.16 (5 H, m), 3.98 (1 H, m), 3.73 (3 H, s), 3.61 (2 H, t, J = 6.5 Hz), 3.42 (1 H, dd, J = 22.7, 8.9 Hz), 2.35 (1 H, m), 2.18–1.05 (22 H, m), 0.91 (3 H, t, J = 7.0 Hz); ^{13}C NMR (major) 169.2, 100.6, 69.2 (d, J_{P-C} = 5.5 Hz), 69.1 (d, J_{P-C} = 9.9 Hz), 63.2 (d, J_{P-C} = 4.4 Hz), 62.6 (d, J_{P-C} = 7.6 Hz), 62.5, 52.4, 44.9 (d, J_{P-C} = 4.3 Hz), 42.4 (d, J_{P-C} = 137.9 Hz), 36.1, 29.6, 28.3, 27.3, 25.6, 24.2, 22.7, 16.4 (d, J_{P-C} = 5.5 Hz), 16.3 (d, J_{P-C} = 5.9 Hz), 14.1, (minor) 170.6, 100.5, 70.6 (d, J_{P-C} = 6.4 Hz), 69.3 (d, J_{P-C} = 12.7 Hz), 62.9 (d, J_{P-C} = 7.0 Hz), 62.7 (d, J_{P-C} = 7.7 Hz), 62.5, 52.4, 45.1 (d, J_{P-C} = 3.9 Hz), 44.9 (d, J_{P-C} = 132.9 Hz), 35.8, 30.3, 28.1, 27.1, 26.3, 24.7, 22.7, 16.3 (d, J_{P-C} = 6.2 Hz), 14.1; ^{31}P NMR (major) 23.6 ppm, (minor) 23.0 ppm; IR 3421, 2935, 1736, 1379, 1231, 1163, 1053, 1028, 966 cm^{-1}; exact mass calcd for $C_{20}H_{39}O_8P$ (M – CH_3)$^+$ 423.2146, found 423.2133.

Diethyl (4*R,5*R**,6*R**)-[[4-Butyl-6-(3-propanoyl)-2,2-dimethyl-1,3-dioxan-5-yl]carbomethoxymethyl]phosphonate (18a).** Aldehyde **18a** (88 mg, 74% yield) was prepared from **17a** (120 mg, 0.27 mmol), following the same procedures for the preparation of **18s**. **18a**: R_f(5% MeOH/CH_2Cl_2) = 0.72; ^{1}H NMR 9.75 (1 H, t, J = 1.5 Hz), 4.35 (1 H, m), 4.12 (4 H, m), 3.89 (1 H, m), 3.73 (3 H, s), 3.24 (1 H, dd, J = 24.1, 2.6 Hz), 2.49 (2 H, m), 2.18–1.09 (21 H, m), 0.91 (3 H, t, J = 6.9 Hz), (minor) 4.12 (5 H, m), 3.90 (1 H, m), 3.77 (3 H, s), 3.41 (1 H, dd, J = 22.6, 8.9 Hz), 2.18–1.09 (21 H, m), 0.91 (3 H, t, J = 6.9 Hz); ^{13}C NMR 201.9, 169.1, 100.7, 69.1, 68.5 (d, J_{P-C} = 15.7 Hz), 63.2 (d, J_{P-C} = 6.6 Hz), 62.6 (d, J_{P-C} = 7.2 Hz), 52.5, 45.1 (d, J_{P-C} = 4.5 Hz), 42.5 (d, J_{P-C} = 138.3 Hz), 41.0, 36.1, 28.3, 25.5, 23.9, 23.3, 22.7, 16.4 (d, J_{P-C} = 6.0 Hz), 16.3 (d, J_{P-C} = 6.4 Hz), 14.2; ^{31}P NMR (major) 23.6 ppm, (minor) 23.5 ppm; IR 2960, 2727, 1761, 1734, 1460, 1379, 1249, 1096, 1051, 1029, 966 cm^{-1}; exact mass calcd for $C_{20}H_{37}O_8P$ (M – CH_3)$^+$ 421.1989, found 421.1973.

Methyl (4,*R,4a*R**,8a*R**)-4-Butyl-4a,7,8,8a-tetrahydro-2,2-dimethyl-4*H*-1,3-benzodioxin-5-carboxylate (19a).** Intramolecular Wadsworth–Emmons olefination product **19a** (oil, 55 mg, 85% yield) was prepared from **18a** (100 mg, 0.229 mmol), following the same procedure as for the preparation of **19s**. **19a**: R_f(20% EtOAc/petroleum ether) = 0.75; ^{1}H NMR 7.02 (1 H, dd, J = 6.5, 2.4 Hz), 4.28 (1 H, m), 3.72 (3 H, s), 3.42 (1 H, td, J = 8.5, 2.8 Hz), 2.79 (1 H, m), 2.39 (2 H, m), 2.15–1.11 (8 H, m), 1.37 (3 H, s), 1.29 (3 H, s), 0.89 (3 H, t, J = 7.0 Hz); ^{13}C NMR 168.6, 140.8, 131.3, 99.2, 72.1, 64.3, 51.3, 41.8, 34.7, 27.8, 26.9, 25.6, 24.3, 22.5, 21.1, 14.0; IR 2956, 1723, 1641, 1433, 1379, 1258, 1217, 1190, 1092, 1042 cm^{-1}; exact mass calcd for $C_{16}H_{26}O_4$ (M – CH_3)$^+$ 267.1595, found 267.1592.

(3*R,3a*R**,4*R**)-3-Butyl-3a,4,5,6-tetrahydro-4-hydroxy-1-(3*H*)-isobenzofuranone (4-Hydroxy-*cis*-neocnidilide) (20a).** To a solution of **19a** (155 mg, 0.55 mmol) in MeOH (3.5 mL) at rt was added p-TsOH (105 mg, 0.55 mmol). After being stirred for 2 days at rt, the reaction was quenched with 10 mL of distilled H_2O and extracted with EtO. The crude product was purified via column chromatography (silica gel, 20% Et_2O/petroleum ether) yielded **20a** (105 mg, 91% yield) as a white solid: mp 80–82 °C (recrystallized from hexane); R_f(50% EtOAc/petroleum ether) = 0.54; ^{1}H NMR 6.93 (1 H, q, J = 3.5 Hz), 4.78 (1 H, td, J = 9.5, 4.4 Hz), 4.48 (1 H, bs), 3.15 (1 H, m), 2.42 (2 H, m), 2.05–1.15 (8 H, m), 0.91 (3 H, t, J = 7.1 Hz); ^{13}C NMR 169.8, 136.2, 125.9, 82.7, 63.8, 43.3, 32.8, 29.4, 28.6, 22.5, 21.1, 13.9; IR 3425, 3031, 2962, 1744, 1681, 1463, 1337, 1250, 1106, 1090, 1044 cm^{-1}; exact mass calcd for $C_{12}H_{18}O_3$ (M)$^+$ 210.1254, found 210.1246.

***cis*-Neocnidilide (2).** To a solution of **20a** (30 mg, 0.143 mmol) in CH_2Cl_2 (0.8 mL) at rt and under an argon atmosphere was added 1,1′-thiocarbonyldiimidazole (51 mg, 0.29 mmol). After being stirred at rt for 22 h, the reaction was diluted with CH_2Cl_2 (1.0 mL) and applied directly to a silica gel chromatography column to yield pure thiocarbonylimidazolide (36 mg, 79% yield). A solution of the thiocarbonylimidazolide (35 mg, 0.109 mmol), Bu_3SnH (59 μL, 0.218 mmol), and AIBN (10 mg, 0.061 mmol) in 5 mL of toluene was refluxed for 2.5 h. The reaction was quenched and worked up as described for the preparation of **1**. Purification by column chromatography (10% Et_2O/petroleum ether) gave pure *cis*-neocnidilide (16 mg, 75% yield) as an oil: R_f(20% EtOAc/petroleum ether) = 0.74; ^{1}H NMR 6.84 (1 H, q, J = 3.4 Hz), 4.66 (1 H, td, J = 8.8, 2.8 Hz), 3.01 (1 H, m), 2.45–1.21 (12 H, m), 0.90 (3 H, t, J = 6.9 Hz); ^{13}C NMR 170.1, 136.1, 129.7, 81.8, 39.8,

31.5, 27.5, 25.1, 22.6, 22.5, 21.2, 13.9; IR 2933, 2867, 1764, 1684, 1452, 1245, 1184, 1025 cm^{-1}; exact mass calcd for $C_{12}H_{18}O_2$ $(M)^+$ 194.1305, found 194.1322.

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Supplementary Material Available: ^1H and ^{13}C NMR spectra for all compounds (55 pages). Ordering information is given on any current masthead page.

A Reagent for Reduction of Disulfide Bonds in Proteins That Reduces Disulfide Bonds Faster Than Does Dithiothreitol

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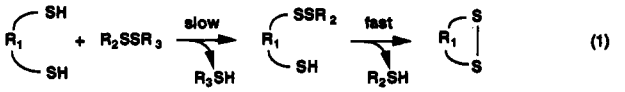
We have synthesized a new reagent—*N,N'*-dimethyl-*N,N'*-bis(mercaptoacetyl)hydrazine (DMH)—for the reduction of disulfide bonds in proteins. DMH reduces disulfide bonds 7 times faster than does dithiothreitol (DTT) in water at pH 7. DMH reduces mixed disulfides of cysteine proteases (papain and ficin) especially rapdily (30 times faster than DTT). DMH (ϵ° = -0.300 V) reduces noncyclic disulfides completely, although it is less strongly reducing than DTT (ϵ° = -0.356 V).

Introduction

This report describes the synthesis of *N,N'*-dimethyl-*N,N'*-bis(mercaptoacetyl)hydrazine (DMH) and the use of this reagent for reduction of disulfide bonds in water at pH ~7. Disulfide-reducing reagents are used in biochemistry for a number of purposes, especially in reduction of cystine groups in proteins and in maintaining essential thiol groups in reduced state.[1-3] The requirements for an optimal reducing reagent for cystine groups in proteins are as follows: (i) a reduction potential higher than the cystine group(s) to be reduced; (ii) a pK_a for thiol groups close to the pH of the solution in which the protein is to be manipulated; (iii) convenient physical properties (a crystalline solid with low odor and adequate solubility in water).[4,5]

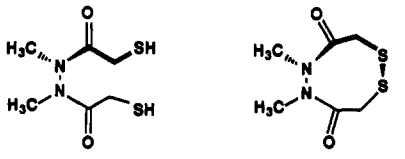
Thiol-disulfide interchange involves the nucleophilic attack of thiolate anion along the S-S bond axis of the disulfide.[5-9] The pK_a of simple alkanethiols in water is ~9-10; for these thiols, only a small fraction (1-0.1%) is present as thiolate at pH 7. The apparent rate of thiol-disulfide interchange is maximum when the pK_a of the thiol is close to the pH of the solution.[5,6] α,ω-Dithiols are more strongly reducing than monothiols, because the intramolecular reaction (the second step in eq 1) is faster



(1)

than the corresponding intermolecular reaction for monothiols. Among α,ω-dithiols, the dithiols that form cyclic six-membered disulfide are the most reducing, reflecting a balance between the thermodynamic stability of the CSSC dihedral angle and the entropy of formation of the ring from the acyclic reduced form.[6,10,11]

Dithiothreitol (DTT)[4] and β-mercaptoethanol (ME) are the favorite disulfide-reducing reagents in biochemistry.[1] DTT reduces noncyclic disulfides to thiols completely, and intermediate mixed disulfides are absent in any significant concentration (eq 1).[4] DTT has, however, some shortcomings: the value of its first thiol pK_a is 9.2,[6] and it is therefore relatively slow as a reducing reagent at pH ~7. It is expensive.[12] Its ability to chelate metals and generate H_2O_2 on exposure to air can cause problems.[13] Because mercaptoethanol is inexpensive, it can be used in large amounts (0.1-0.7 M) in biochemical manipulations (for example, in conjunction with SDS gel electrophoresis).[1] The pK_a of mercaptoethanol is 9.6.[6] The disadvantages of mercaptoethanol are that it is a weak reducing reagent and is foul-smelling. Because it is weakly reducing, it often generates complex reaction mixtures containing mixed disulfides.[6]



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