Table I. Reactivation of Inhibited Urease at 38 °C

inhibitor	$10^4 k_{react}$, s^{-1}
PPD[a]	0.36 ± 0.03
MBPT[a]	0.34 ± 0.04
2c[a]	0.36 ± 0.03
2b (1:1 complex)[b]	7 ± 1
1 (1:1 complex)[c]	8.2 ± 0.5

[a] In oxygen-free NEM buffer (pH 7.0; 1 mM in EDTA). [b] In oxygen-free NEM buffer, pH 7.01. [c] In oxygen-free NEM buffer (pH 7.11; 1 mM in EDTA, 5 mM in 2-mercaptoethanol, 0.1 M in KCl).[3]

Relevant first-order rate constants for reactivation are included in Table I, about which the following points are made: (i) the carboxamide from MBPT is released, while the inactivated urease contains intact **2b**; (ii) PPD and **2c** are initially hydrolyzed to diamidophosphate; (iii) the consonance of the first three rate constants in Table I identifies a diamidophosphate–nickel complex as the species responsible for inhibition; (iv) a different, significantly more labile diamidophosphate–nickel complex is formed when the inhibitor is not enzymatically derived from a substrate; (v) the consonance of the last two rate constants strongly suggests that **2b** is itself a substrate for the enzyme. The isomeric diamidophosphate–nickel complexes presumably differ in strength by virtue of N- vs. O-coordination to the metal ion and constitute the first such enzymatic data.

These results may be compared with the *N*-hydroxyurea inhibition of the enzyme where isomeric *primary substrate–nickel complexes* are responsible for inhibition and substrate activity, respectively.[8]

We earlier reported that approximately 50% of the phosphoramidate is released intact after reactivation of the isolated phosphoramidate–urease complex at 38 °C for 50 min but cautioned that catalysis of phosphoramidate decomposition by the tertiary amine buffer[9] or protein side chains may not be sufficient to account for the kinetics observed. Further work under conditions where the nonenzymatic decomposition of **1** is not catalyzed by buffer salts or other additives clearly demonstrates that **1** is itself a very poor substrate for urease. At 38 °C, $k_{cat}/K_m = 8 \pm 2$ M^{-1} s^{-1} ($[\mathbf{1}]_0 = 8.4$ mM), and this value may be compared with those for other substrates which range from 2.0×10^6 M^{-1} s^{-1} for urea to 0.34 M^{-1} s^{-1} for *N*-methylurea.[10] That this is a genuine substrate activity has been confirmed by its abolition after preequilibration of the enzyme with 0.9 mM acetohydroxamic acid.

Compounds **1**,[11] **2b**,[12] and **2c**[13] were prepared by published procedures. The value of k_{obsd} for the spontaneous hydrolysis of **1** (1.6 mM) in 0.3 M NEM (pH 7.0) at 38 °C was 6.5×10^{-5} s^{-1} (cf. 6.6×10^{-5} s^{-1} in water[14] at pH 7.0 and 36.8 °C). The concentration of **1** (elution time, 5.5 min) and/or of ammonia (elution time, 15 min) was measured in a single assay with an LKB Alpha-Plus amino acid analyzer using 0.2 M borate (pH 9.7, 0.05 M in NaCl) at 40.9 mL/h. Protein was removed from enzymatic reactions with a Centricon 30 microconcentrator (Amicon). Other enzymatic experiments used procedures similar to those previously reported.[1,3]

We have investigated the reaction of these inhibitors with urease from *jack beans* for the following reasons: (1) This nickel(II) metalloenzyme is readily available in high purity,[2] and the concentration of its active sites is easily measured. (2) Its reversible inhibition by 2-mercaptoethanol, fluoride, and hydroxamic acids involves direct coordination to active-site nickel ion.[2] (3) Nickel nutrition and/or inhibition studies strongly indicate that the active site of urease is essentially the same regardless of its source (mycoplasma, bacteria, fungi, algae, higher plants, invertebrates, and soil).[2c] The findings of this research can therefore be expected to be of general validity.

All aspects of this work continue under active investigation.

Acknowledgment. We are grateful to the Australian Research Grants Scheme and to the University of Queensland for financial support, to Dr. Tom Malefyt for preliminary nonenzymatic studies on PPD, and to Drs. Isao Yamatsu and Eric Craswell for generous gifts of MBPT and PPD, respectively. One of us (R.K.A.) acknowledges a Commonwealth Postgraduate Award.

(7) With PPD at 1.86×10^{-5} M and urease at 2.0×10^{-5} N, inhibition was virtually complete after 1 min at pH 7.0 and 38.0 °C. A second-order rate constant of $(4 \pm 0.5) \times 10^4$ M^{-1} s^{-1} was measured for the reaction between urease and MBPT at pH 7.0 and 38.0 °C, while the release of ammonia from **2c** (2.47×10^{-5} M) in the presence of urease (2.68×10^{-5} N) was complete within the time of mixing at pH 7.0 and 25.0 °C.

(8) Blakeley, R. L.; Hinds, J. A.; Kunze, H. E.; Webb, E. C.; Zerner, B. *Biochemistry* **1969**, *8*, 1991–2000.
(9) Jencks, W. P.; Gilchrist, M. *J. Am. Chem. Soc.* **1965**, *87*, 3199–3209.
(10) Dixon, N. E.; Riddles, P. W.; Gazzola, C.; Blakeley, R. L.; Zerner, B. *Can. J. Biochem.* **1980**, *58*, 1335–1344.
(11) Chambers, R. W.; Khorana, H. G. *J. Am. Chem. Soc.* **1958**, *80*, 3749–3752 (Method B).
(12) Klement, R.; Biberacher, G. *Z. Anorg. Allg. Chem.* **1956**, *285*, 74–85.
(13) Klement, R. In *Organic Syntheses*; Rochow, E. G., Ed.; McGraw-Hill: New York, 1960; Vol. VI, pp 108–111.
(14) Chanley, J. D.; Feageson, E. *J. Am. Chem. Soc.* **1963**, *85*, 1181–1190.

Generation and Trapping of Alkynolates from Alkynyl Tosylates: Formation of Siloxyalkynes and Ketenes

Peter J. Stang* and Kenneth A. Roberts

Chemistry Department, The University of Utah
Salt Lake City, Utah 84112

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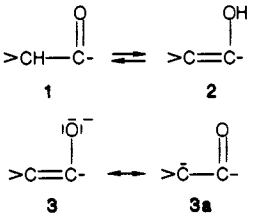
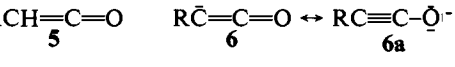
Alcohols and alkoxides are well-known organic compounds and reagents. Likewise, carbonyls, **1**, and their enols, **2**, are well-known and important species.[1] Yet despite the importance and wide spread use of enolates, **3**, simple enols, **2**, have only recently been prepared and fully characterized.[2]



With the exception of the recent observation[3] of the parent hydroxyacetylene, HC≡COH, by tandem mass spectrometry in the gas phase, hydroxyalkynes **4** are not known and outside of some theoretical calculations[4] little is known about the alkynol ketene **4–5** tautomerism of alkynolates, **6**, and their O-trapping in particular.



Alkynolates **6** have been generated previously by Rathke et al. via ketene **7**, by Hoppe and Schöllkopf[6] via isoxazoles **10**, and by Kowalski and co-workers[7] by rearrangement of α-keto dianions **14**, as shown in Scheme I.

However, in all previous cases trapping with a variety of electrophiles, including silicon in the case of **8** and **11**, gave only carbon-silylated (alkylated) ketenes **9** and **12** or ketene-derived products **16**.

(1) House, H. O. *Modern Synthetic Reactions*, 2nd ed.; W. A. Benjamin, Inc.: Menlo Park, CA, 1972. March, J. *Advanced Organic Chemistry*, 2nd ed.; McGraw-Hill: New York, 1977.
(2) For a review on stable enols, see: Hart, H. *Chem. Rev.* **1979**, *79*, 515. For a more recent reference, see: Biali, S. E.; Rappoport, Z.; Hull, W. E. *J. Am. Chem. Soc.* **1985**, *107*, 5450 and references therein.
(3) Schwarz, H.; Weiske, T. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 282.
(4) Bouma, W. J.; Nobes, R. H.; Radom, L.; Woodward, C. E. *J. Org. Chem.* **1982**, *47*, 1869.
(5) Woodbury, R. P.; Long, N. R.; Rathke, M. W. *J. Org. Chem.* **1978**, *43*, 376.
(6) Hoppe, I.; Schöllkopf, U. *Justus Liebigs Ann. Chem.* **1979**, 219.
(7) Kowalski, C. J.; Fields, K. W. *J. Am. Chem. Soc.* **1982**, *104*, 321. Kowalski, C. J.; Haque, M. S.; Fields, K. W. *Ibid.* **1985**, *107*, 1429. Kowalski, C. J.; Haque, M. S. *J. Org. Chem.* **1985**, *50*, 5140. Kowalski, C. J.; Haque, M. S. *J. Am. Chem. Soc.* **1986**, *108*, 1325.

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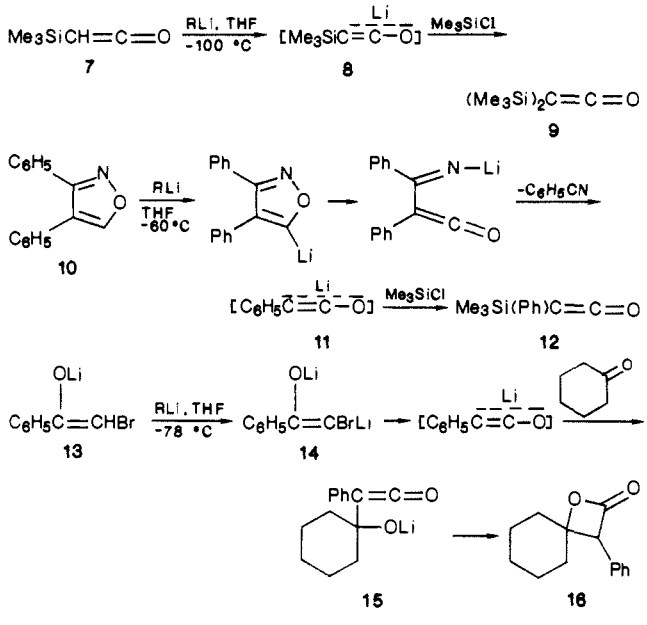
Table I. Physical and Spectral Data for Siloxyalkynes **19** and Ketenes **21** and **22**

compd	yield	bp (mmHg)	IR,[a] cm^{-1}	mass spectrum,[b] *m/e* (%)	^1H NMR,[c] δ	^{13}C NMR,[d] δ
19a	55%	73–74 °C (10)	2980–2860 (vs), 2270 (vs), 1470 (s), 1390 (m), 1360 (m), 1320 (s), 1260 (s), 1150 (vs), 860–780 (vs), 720 (s), 670 (m)	212 (14.6, M^+), 197 (39.1), 141 (7.4), 115 (8.2), 99 (17.8), 84 (10.9), 73 (100), 57 (8.7)	0.2 (s, 6 H, $SiMe_2$), 1.0 (s, 9 H, Si-*t*-Bu), 1.2 (s, 9 H, *t*-Bu)	−5.7 ($SiMe_2$), 18.5, 25.4 (Si-*t*-Bu), 26.5, 32.3 (*t*-Bu), 40.3 (β-C), 85.5 (α-C)
19b	52%	70–71 °C (2)	2980–2860 (vs), 2270 (vs), 1460 (m), 1400–1350 (w), 1250 (vs), 1150 (w), 1100–1060 (w), 995 (w), 870–780 (s), 740 (w), 680 (w)	212 (14.2, M^+), 197 (19.1), 183 (17.5), 155 (15.9), 127 (17.6), 75 (67.2), 73 (100)	0.2 (s, 6 H, $SiMe_2$), 0.9 (s, 9 H, Si-*t*-Bu), 0.9, 1.2, 1.3, 2.2 (m, *sec*-Bu)	−5.6 ($SiMe_2$), 25.5 (Si-*t*-Bu), 36.0 (β-C), 86.7 (α-C)
21a	87%	53–54 °C (0.5)	2980–2820 (vs), 2070 (vs), 1455 (m), 1420 (w), 1360 (m), 1300 (m), 1220–1180 (m), 1010 (m), 945 (m), 850 (m), 805 (w), 700 (s)	256 (4.6, M^+), 229 (100), 227 (74.8), 225 (55.3), 173 (5.6)	0.9–1.1 (overlapping *m*, 15 H, $GeEt_3$), 1.2 (s, 9 H, *t*-Bu)	6.3, 8.7 ($GeEt_3$), 33.0, 33.3 (*t*-Bu), 23.5 (sp^2 C), 181.2 (sp C)
21b	75%	55–56 °C (0.5)	2980–2820 (vs), 2070 (vs), 1455 (m), 1420 (m), 1375 (m), 1150–1100 (m), 1010 (s), 850 (m), 700 (s)	256 (4.5, M^+), 229 (100), 227 (75.2), 225 (27.3)	0.8–2.0 (overlapping m)	5.4, 8.7 ($GeEt_3$), 11.8, 23.0, 31.7, 32.3 (*sec*-Bu), 16.3 (sp^2 C), 180.3 (sp C)
22a	93%	86–87 °C (0.1)	2980–2840 (vs), 2060 (vs), 1460 (s), 1415 (w), 1375–1360 (m), 1180, 1150 (m), 1070 (m), 870 (m), 800 (w), 700–650 (s)	386 (2.6, M^+), 331 (100), 329 (75.2), 327 (41.1), 275 (5.3), 200 (7.5), 155 (8.8)	0.8–1.8 (overlapping m), 1.2 (s, *t*-Bu)	12.0, 13.7, 27.3, 28.8 ($SnBu_3$), 32.2, 34.2 (*t*-Bu), 20.5 (sp^2 C), 176.4 (sp C)

[a] Neat oil. [b] Electron impact, 70 eV. [c] Solvent $CDCl_3$, 90 MHz. [d] Solvent $CDCl_3$, 300 MHz, decoupled.

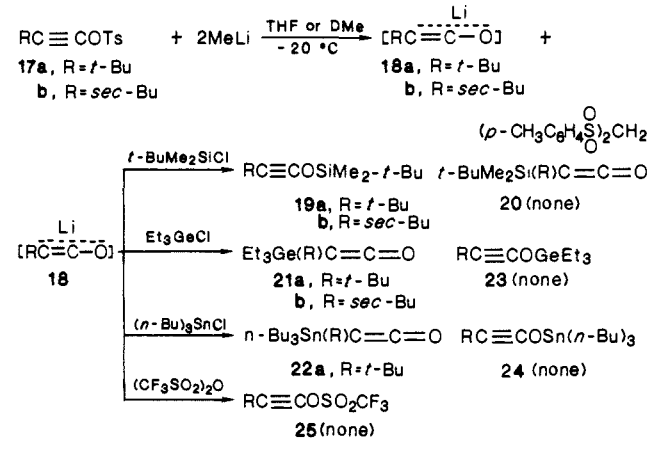
Scheme I



Hence, in this paper we wish to report the first[8] O-trapping via silylation of alkynolates **6** and the formation of new siloxyalkynes as well as ketene-derived products.

Treatment of alkynyl tosylates[9] **17** with 2 equiv of CH_3Li in THF or DME at −20 °C gave clear, pale yellow, solutions of alkynolates **18**. Reaction with *t*-$BuMe_2SiCl$ and workup gave siloxyalkynes **19**, with *no* indication of C-silylation nor any formation[10] of ketenes **20**. In contrast, quenching with either Et_3GeCl or *n*-Bu_3SnCl gave only the corresponding ketene products **21** and **22**, respectively, with no indication[11] of any alkynyl products **23** or **24**. Attempted quenching with $(CF_3SO_2)_2O$ in hopes of preparing presently unknown alkynyl triflates **25** resulted in only black tarry products as summarized in Scheme II.

Scheme II



Structural assignments for siloxyalkynes **19** and ketenes **21** and **22** are based upon spectral data as summarized in Table I.

Siloxyalkynes **19** show the expected[11] very strong absorption in the IR at 2270 cm^{-1} due to the unsymmetrically substituted C≡C.[12] The mass spectra show the proper molecular ion and appropriate fragmentation patterns.

Likewise, the ^1H as well as ^{13}C NMR spectrum are consistent with the proposed structures. Particularly noteworthy and characteristic are the acetylenic carbon signals in the ^{13}C NMR. The α-C's for **19a** and **19b** are in the normal alkyne region at 85.4 and 86.7 ppm, respectively, whereas the β-C's are at 40.3 and 31.0 ppm, respectively. This considerable upfield shift of the β-C's is caused by the electron-rich nature of the siloxyalkynes due to resonance donation, **19**, in analogy with both alkynyl tosylates **17** and alkoxyalkynes **26**, where the β-C for EtC≡COEt is at 36 ppm.[13]

(8) For similar trapping of alkynolates derived from α-keto dianions, see: Kowalski, C. J.; Haque, M. S. *J. Am. Chem. Soc.*, following paper in this issue. We thank Dr. Kowalski for exchange of information and preprints prior to publication.

(9) Stang, P. J.; Surber, B. W. *J. Am. Chem. Soc.* **1985**, *107*, 1452. Stang, P. J.; Surber, B. W.; Chen, Z. C.; Roberts, K. A.; Anderson, A. G. *Ibid.*, in press.

(10) Ketenes have very characteristic strong absorption in the IR around 2050 cm^{-1}. We did not observe any IR absorptions in this region in the crude reaction mixture where 3–5% of ketene would have been easily detected.

(11) All O-substituted alkynes, such as RC≡COTs,[9] RC≡COSi≡,[12] etc., have characteristic very *strong* IR absorptions between 2260 and 2290 cm^{-1}. No such absorption was observed in the crude reaction mixtures.

(12) Maas, G.; Brückmann, R. *J. Org. Chem.* **1985**, *50*, 2801.

$$RC\overset{\beta}{\equiv}C\overset{\alpha}{-}OSi \leftrightarrow RC^{-}=C=O^{+}Si$$
19

$$RC\overset{\beta}{\equiv}C\overset{\alpha}{-}OTs \leftrightarrow RC^{-}=C=O^{+}Ts$$
17

$$RC\overset{\beta}{\equiv}C\overset{\alpha}{-}OR' \leftrightarrow RC^{-}=C=O^{+}R'$$
26

Likewise, ketenes **21** and **22** show characteristic, very strong IR absorptions at 2050–2070 cm^{-1} due to the ketene vibrations. Their mass spectra show respectable molecular ions and appropriate fragmentation patterns. Proton and ^{13}C NMR are in concert with the proposed structures and the C=C=O signals at 16–23 and 175–181 ppm are particularly characteristic for ketenes.

Hence, there is no doubt about the identity of these stable, readily isolable siloxyalkynes and ketene derivatives. Therefore, alkynolates, like enolates, can react via O-alkylation (silylation) or C-alkylation,[5-7] although at the moment we neither understand nor can we control the exact site of reaction. In analogy with enolate chemistry we expect that the counterion, solvent, complexing agents, etc. play a crucial role in the control of site selection and these factors as well as the rich chemistry we expect from alkynolates are under investigation.

In summary, we have developed a simple, general means of alkynolate generation from readily available alkynyl tosylates. Reaction with *t*-$BuMe_2SiCl$ results in little-known[12] stable, novel siloxyalkynes, whereas trapping with R_3GeCl or R_3SnCl gives C-alkylation and stable ketenes as products. The full scope of alkynolate chemistry will be the subject of future reports.

Acknowledgment. Financial support by the National Cancer Institute of the NIH (CA16903) is gratefully acknowledged.

(13) Levy, G. T.; Lighter, R. L.; Nelson, G. L. *Carbon-13 Nuclear Magnetic Resonance Spectroscopy*, 2nd ed.; Wiley: New York, 1980; pp 90–95.

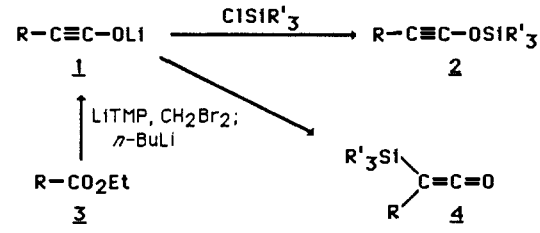
Ynol Silyl Ethers via O-Silylation of Ester-Derived Ynolate Anions

Conrad J. Kowalski,* G. Sankar Lal,[1a] and M. Serajul Haque[1b]

Synthetic Chemistry Department
Smith Kline and French Laboratories
Philadelphia, Pennsylvania 19101

Received July 14, 1986

Herein we report that lithium ynolate anions **1**, derived from esters **3** using our previously described homologation method,[2] react with chlorotrialkylsilanes on oxygen to afford silyl ynol ethers **2**. This represents the first silylation of ynolate anions on oxygen,[3] in contrast to literature reports[4] in which reactions with chlorotrimethylsilane resulted in formation of only the corresponding ketenes (**4**). In our hands, chlorotrimethylsilane treatment of ynolate anion **1** (R = CH_2CH_2Ph) in THF/hexane at −78 °C,



followed by warming to room temperature, similarly afforded ketene **4** (R = CH_2CH_2Ph, R′ = $SiMe_3$; IR 2080 cm^{-1}); however, when a solution of the same ynolate was treated with chlorotrimethylsilane at −78 °C, but then diluted with pentane at −78 °C, and quenched into aqueous bicarbonate without warming, silyl ynol ether **2** (R = CH_2CH_2Ph, R′ = $SiMe_3$; IR 2280 cm^{-1}) was obtained along with ketene **4** (in a ratio of about 2:1). It appears that silylation by $ClSiMe_3$ had occurred kinetically on oxygen to afford ynol ether **2**, but upon warming the reaction mixture in the first case, isomerization to the more stable ketene **4** had occurred. Previous workers apparently observed only the thermodynamic (ketene) products from ynolate anion silylation.

Unfortunately, our attempts to purify the very sensitive trimethylsilyl ynol ether **2** (R = CH_2CH_2Ph) were unsuccessful. It seemed to us that more bulky silylating reagents might also react kinetically with ynolate anions on oxygen but without subsequent rearrangement to ketene products. The literature had recently shown that ynol silyl ethers **2** were indeed thermally stable when a triisopropylsilyl or *tert*-butyldimethylsilyl group was attached[5] (in contrast to the triethylsilyl derivatives[5a]). It was gratifying to find, therefore, that quenching solutions of ynolate anion **1** (R = CH_2CH_2Ph) with either chlorotriisopropylsilane or chloro-*tert*-butyldimethylsilane afforded exclusively the ynol silyl ether products **2**, and, moreover, if excess chlorosilane was removed in vacuo from the crude product after workup, the ynol ethers could be purified by rapid flash chromatography on silica gel to afford the triisopropylsilyl and *tert*-butyldimethylsilyl ynol ethers (R = CH_2CH_2Ph) in 66% and 53% yield, respectively.

Triisopropylsilyl ynol ethers are expected to be less sensitive than the *tert*-butyldimethylsilyl analogues.[6] Since we sought a stable class of silyl ynols as reagents for subsequent studies, the triisopropylsilyl compounds were selected as targets for testing the generality of this new synthetic approach. Several ynolate anions, prepared from esters, were therefore treated with chlorotriisopropylsilane to afford ynol ethers as indicated in Table I.

The method[7] proved successful for preparing ynols **2** having R groups attached at primary, secondary, tertiary, alkenyl, and aryl carbon centers, as well as for functionalized R groups derived from lactone starting materials. While the yields were moderate (50–74%), the directness of this "one-pot" conversion of esters into triisopropylsilyl ynol ethers (i.e., **3** → **1** → **2**), coupled with its generality, should serve to make it an important method for

(1) Smith Kline & French Postdoctoral Research Scientist (a) 1985–1986, (b) 1983–1985.

(2) Kowalski, C. J.; Haque, M. S.; Fields, K. W. *J. Am. Chem. Soc.* **1985**, *107*, 1429–1430.

(3) Near the conclusion of this work, we learned from Professor Peter Stang that he and his co-workers had independently observed silylation of ynolate anions on oxygen. His results are reported in the preceding paper in this issue. We thank Professor Stang for sharing this information prior to publication.

(4) (a) Hoppe, I.; Schollkopf, U. *Justus Liebigs Ann. Chem.* **1979**, 219–226. (b) Woodburgy, R. P.; Long, N. L.; Rathke, M. W. *J. Org. Chem.* **1978**, *43*, 376.

(5) (a) Maas, G.; Bruckmann, R. *J. Org. Chem.* **1985**, *50*, 2801–2802. (b) Dr. Michael Pirrung has informed us that his previously reported preparation of some ynol silyl ethers was in error. For that report see: Pirrung, M. C.; Hwu, J. R. *Tetrahedron Lett.* **1983**, *24*, 565–568.

(6) Cunico, R. F.; Bedell, L. *J. Org. Chem.* **1980**, *45*, 4797–4798.

(7) In a typical procedure, performed under N_2, 4.4 mmol of 2.5 M *n*-butyllithium solution in hexane was added dropwise to a stirred solution of 4.8 mmol of 2,2,6,6-tetramethylpiperidine in 6 mL of THF with ice bath cooling. This mixture was then cooled with a −78 °C (dry ice/acetone) bath and added dropwise via syringe to a solution of 4.4 mmol of dibromomethane in 6 mL of THF, cooled with a −78 °C bath. After 5 min, a solution of 2.0 mmol of starting ester **3** in 5 mL of THF was added dropwise, and 10 min later a solution of 10 mmol of 2.5 M *n*-butyllithium in hexane was added dropwise. The −78 °C bath was replaced with a 30 °C water bath for 30 min and then returned, after which 10 mmol of chlorotriisopropylsilane was added. The temperature was raised to 0 °C (room temperature for **5b** and **13**) and stirring was continued for 1.5–24 h (see Table I). The mixture was diluted with 200 mL of petroleum ether and then washed with three 50-mL portions of aqueous sodium bicarbonate solution and two 50-mL portions of water. After drying over $MgSO_4$, removal of the solvent in vacuo, and removal of remaining chlorosilane under high vacuum, the residue was passed through a silica gel (15 g/g) flash column and eluted with 0.5% ether in petroleum ether (<5 min residence time). Evaporation of solvent in vacuo then afforded pure product. Some modifications of the reagent amounts were required for certain esters as previously discussed for simple homologation.[2]

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