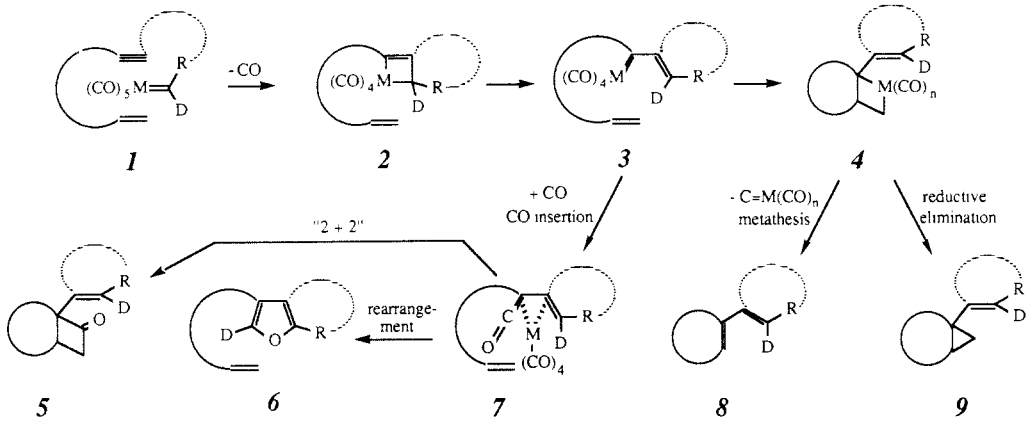
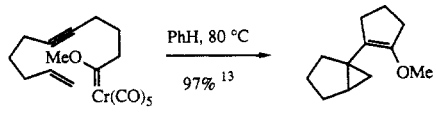
Scheme I



when it is possible to generate a new, electron-deficient carbene which bears one[5] or more stabilizing donor alkyl groups (i.e., Me versus H). Consistent with this is that substrate **11e**, containing a crotonate ester moiety, gave cyclopropanes **12e** as the only observed product; metathesis would have required generation of an unstable, carbomethoxy-bearing Fischer carbene. Enynes **11f** and **11g** are one- and two-carbon homologs of **11a** and should form bicyclic products more slowly. The former gave the bicyclo[4.1.0]heptanes **12f** as the dominant adducts, although the competition product, furan **15f**, was also observed. The latter produced furan **15g** as the only identifiable product; cyclization to the bicyclo[5.1.0]octane skeleton is now slowed to the point where other processes compete exclusively.

Covalent attachment of the carbene center to the enyne moiety (see dashed bond in **1**) provides an opportunity for formation of three rings during the reaction course. Carbene **16**[10b] was synthesized[11] to test this possibility. Warming **16**[12] provided the labile tricyclic enol ether **17**[10a] in excellent yield.[13] Thus, the viability of this potentially powerful tricyclization operation has been clearly demonstrated. Studies to delineate stereochemical,[14] mechanistic, and additional synthetic features of this process are in progress.



16 17

Acknowledgment. This investigation was supported by grants CA-24056 and GM-38854 awarded by the DHHS and by an award from the Alfred P. Sloan Foundation.

Supplementary Material Available: Spectral data for compounds **(*E*)-12a, (*Z*)-12a, 11b, (*E*)-12b, (*Z*)-12b, (*E*)-13b, (*Z*)-13b, 11c, (*E*)-12c, (*E*)-13c, (*E*)-14, (*Z*)-14, 15c, 11d, (*E*)-13d, 15d, 11e, (*E*)-12e, (*Z*)-12e, 11f, (*Z*)-12f, 15f, 11g, 15g, 16, 17**, i, and ii (16 pages). Ordering information is given on any current masthead page.

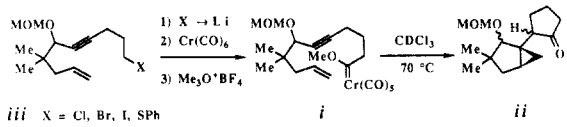
(10) This compound was characterized by ^{1}H NMR, IR, and (a) combustion and/or (b) HRMS analysis.

(11) Metal-halogen exchange between *t*-BuLi (2 equiv) and 1-iododec-9-en-4-yne (made in five steps from pent-4-yn-1-ol and 1-bromopent-4-ene) at −78 °C in the presence of $Cr(CO)_6$, warming to 0 °C, and methylation (Vedejs, E. *Organic Syntheses*; Wiley: New York, 1987; p 140) provided **16** in 57% yield.

(12) Sealed tube in benzene under argon at 80 °C for 5 h.

(13) Filtration of the reaction mixture through florisil provided tricycle **17** in 97% yield as the sole species observable by ^{1}H NMR analysis. MPLC on florisil gave analytically pure **17** in 63% yield. Purification on silica gel was accompanied both by partial hydrolysis to the ketone and by rearrangement to less substituted enol ethers.

(14) The first carbene-induced cyclization examined in our laboratory was the intramolecular annulation of the chiral enyne i. An initial 3 mg sample of i was characterized by ^{1}H and ^{13}C NMR spectroscopy and then warmed



iii X = Cl, Br, I, SPh *i* *ii*

in $CDCl_3$ to ~70 °C for ~15 min. Formation of *two* diastereometric ketones of constitution ii was observed by ^{1}H NMR analysis. These were isolated by HPLC, and the ^{1}H NMR spectrum of each clearly indicated their epimeric nature at the stereogenic carbon atom adjacent to the ketone carbonyl. This means, of course, that a single diastereomeric relationship was established among the methoxymethoxylated and cyclopropyl carbons by the cyclopropanation event. The relative configuration of these centers has not been established because, despite *numerous* attempts, we have never been able to successfully repeat the metallation of iii, immediate precursors to carbene i. Nonetheless, this result portends some interesting and potentially useful stereochemical features for this class of cyclizations.

Lithiothioacetals as Carbenoids. Highly Selective One-Flask Conversion of Cyclohex-2-en-1-ones to Lithium Bicyclo[1.1.0]butan-2-olate Intermediates

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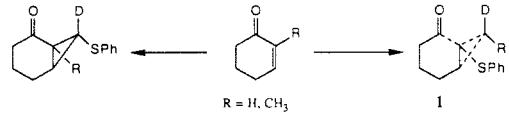
The formation of several carbon–carbon bonds in one flask is a desirable process in organic chemistry.[1] Herein we disclose unusual and synthetically promising reactions in which enones undergo novel types of cyclopropanation (eq 1). During the conversion of 2-cyclohexenones to **1**, a profound annulative rearrangement occurs, while three new C–C bonds (marked by dashed lines in **1**) and one new C–D bond are being formed.



The development of these remarkable reactions was made possible by the recent observation that lithio derivatives of phenyl thioacetals behave as selective carbenoids if another anionic site is positioned in the same molecule.[2] Subsequent elaboration of this principle has engendered synthetically useful and mechanistically interesting chemistry.[3] We now show that the manifold properties of the phenylthio group allow efficient production of homoenolates in the form of highly strained bicyclo[1.1.0]butan-2-olate derivatives which can lead to selective rearrangements.

In 1983, Cohen and Yu reported that the conjugate adduct of 2-cyclohexenone and tris(phenylthio)methyllithium,[4] when treated

(1) Posner, G. H. *Chem. Rev.* **1986**, *86*, 831.

(2) Cohen, T.; Ritter, R. H.; Oullette, D. *J. Am. Chem. Soc.* **1982**, *104*, 7142.

(3) (a) Cohen, T.; Ouellette, D.; Senaratne, K. P. A.; Yu, L.-C. *Tetrahedron Lett.* **1981**, *22*, 3377. (b) Cohen, T.; Yu, L.-C. *J. Am. Chem. Soc.* **1983**, *105*, 2811. (c) Cohen, T.; Yu, L.-C. *J. Org. Chem.* **1984**, *49*, 605. (d) Ritter, R. H.; Cohen, T. *J. Am. Chem. Soc.* **1986**, *108*, 3718.

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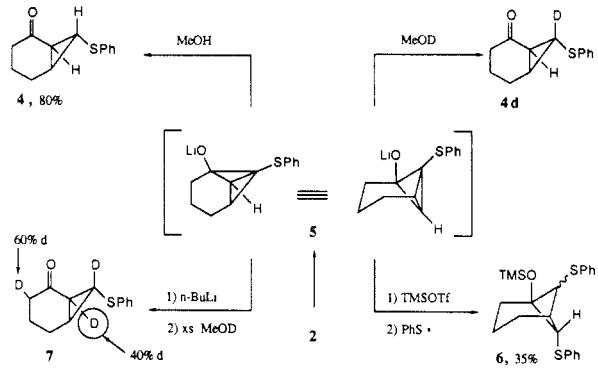
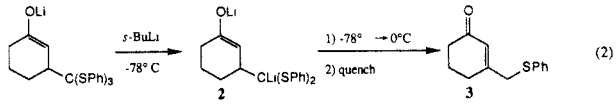
Scheme I



with *s*-BuLi at −78 °C followed by warming to −45 °C, gave enolate dianion **2** (eq 2).[3b] When the reaction mixture was



(2)

warmed to 0 °C and quenched, **3** was formed by an apparent 1,2-hydride transfer to the carbenoid carbon atom. Subsequently, it was found by us that −78 °C was sufficient for lithium–phenylthio exchange and that warming **2** to −45 °C followed by quenching gave an 80% yield of cyclopropyl ketone **4** (Scheme I).[5] An analogous reaction occurred, albeit at 0 °C, when the same sequence was performed starting with carvone.[3b]

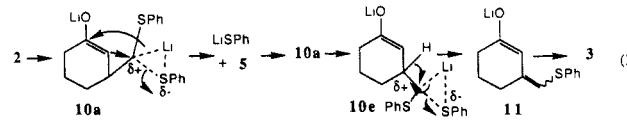
Before defining the scope of the cyclopropanation, we have studied in detail the conversion of **2** to **4**. The results in Scheme I implicate the intermediacy of bicyclo[1.1.0]butan-2-olate **5**. When **2** is warmed, the carbon atom bearing the phenylthio groups becomes electrophilic presumably by metal-ion-assisted thiophenoxide expulsion.[6] Cyclopropanation of the enolate group double bond completes the bicyclobutane nucleus.

Bicyclobutan-2-olates are rare and highly unstable species.[7] Indeed, heretofore they have never been trapped with added reagents. The formation of cyclopropane **4d** and bridged cyclobutane **6**[8] shows that **5** is a true ambident homoenolate;[9] the electrophile can be incorporated either at carbon yielding **4d** or at oxygen yielding **6**. Compound **6** is formed from the silylation product of **5** by attack of the phenylthiyl radical generated from thiophenoxide by oxygen during workup; such an attack is precedented.[10] The present report appears to be the first case in which a group I metal homoenolate reacts selectively at either carbon or oxygen with electrophiles, and thus a true analogy can be drawn between the ambidenticity of an enolate and homoenolate **5**. The bridgehead proton of **5** can be removed by 1 equiv of *n*-BuLi added after ring closure, a reaction which is well-known for other bicyclobutanes.[11] The resulting dianion is trapped to give **7**. We believe that the deuterium distribution in **7** can be explained by assuming an equilibrium between **5** and its open form **5a**. Although **5a** may be present only in minute amounts, the alkyllithium would remove its highly acidic protons α' to the carbonyl group much faster than the bridgehead proton of **5**.

Compound **5** and its bridgehead anion can be trapped with *p*-anisaldehyde to produce **8** and **9**, respectively, in one-flask

reactions. Conversion of 2-cyclohexenone and tris(phenylthio)methane to **8** or **9** can be dubbed a "substitutive cyclopropanation" because the cyclopropane ring is subsequently functionalized in the same pot as it is formed.

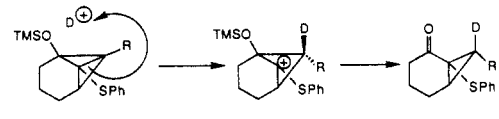
The conversion of **2** to **3** (eq 2) via **5** is particularly interesting. Decomposition of **2** at −45 °C must result in a species which can only undergo intramolecular ring closure to the kinetic product **5** if the carbenoid[6] appendage is in a quasiaxial conformation (drawn here as **10a**, eq 3). Upon decomposition of **5** at 0 °C, the



(3)

reverse process presumably occurs to form a species which is similar to, if not the same as, **10a**. When the latter equilibrates to the conformation **10e** in which the carbenoid group is quasiequatorial, a 1,2-hydride migration ensues to afford the thermodynamic product, dienolate **11**, which upon workup is converted to **3**. The transformation of **5** to **10** is an example of an extremely rare if not unique carbenoid retro-addition. This process, in a formal sense, is known for the transition-metal complex-catalyzed isomerization of bicyclobutanes[12] in which the d electrons of the metal, absent in the case of lithium, play the role that the sulfur p electrons do in the present case, stabilizing the carbenoid.

On the basis of the chemistry revealed above, it can be seen that species such as **5**, which can be readily generated in a highly selective fashion, could become extremely versatile synthetic intermediates. However, all the cyclopropyl ketones discussed which are derived from **5** have incorporated the electrophile at the carbon atom bearing the phenylthio group. To increase the versatility even further, it would be desirable to direct the electrophilic attack to the other bridgehead carbon atom. With this in mind, **5** and its bridgehead methylated derivative were silylated with TMSCl and treated with excess DOAc at 0 °C. The gratifying result was conversion to **1** (eq 1, R = H, 37%; R = CH_3, 44% from the cyclohexenone). Apparently, reducing the ionic character of the oxygen–metal bond causes the silylated derivatives of **5** to revert to the general behavior of normal bicyclobutanes, i.e., the attacking electrophile is incorporated with inversion of stereochemistry at the bridgehead carbon atom which leads to the most highly stabilized cation (eq 4).[13]



(4)

In summary, enones were substitutively cyclopropanated with regio- and stereospecific creation of various types of new bonds. The latter were created at the expense of easily formed C–S and C–H bonds (tris(phenylthio)methane is simply prepared from thiophenol and trimethyl orthoformate[14]). Subsequent papers

(4) Manas, A. R.-B.; Smith, R. A. J. *J. Chem. Soc., Chem. Commun.* **1975**, 216. Cohen, T.; Nolan, S. M. *Tetrahedron Lett.* **1978**, 3533.

(5) Apparently the dry ice/hexanol bath used in ref 3b was at a lower temperature than −45 °C.

(6) Rachon, J.; Goedken, V.; Walborsky, H. M. *J. Am. Chem. Soc.* **1986**, *108*, 7435, and references cited therein.

(7) Nilsen, N. O.; Sydnes, L. K.; Skattebøl, L. *J. Chem. Soc., Chem. Commun.* **1978**, 128. Nilsen, N. O.; Skattebøl, L.; Sydnes, L. K. *Acta Chem. Scand. B* **1982**, *36*, 587. Jørgensen, E.; Sydnes, L. K. *J. Org. Chem.* **1986**, *51*, 1926.

(8) All new compounds gave satisfactory 300 MHz PMR, IR, and MS analyses.

(9) Review of homoenolates: Werstiuk, N. H. *Tetrahedron* **1983**, *39*, 205.

(10) Szeimies, G.; Schlosser, A.; Philipp, F.; Dietz, P.; Mickler, W. *Chem. Ber.* **1978**, *111*, 1922.

(11) Closs, G. L.; Closs, L. E. *J. Am. Chem. Soc.* **1963**, *85*, 2022.

(12) Gassman, P. G.; Williams, F. J. *J. Am. Chem. Soc.* **1970**, *92*, 7631. Gassman, P. G.; Atkins, T. J. *J. Am. Chem. Soc.* **1972**, *94*, 7748.

(13) Wiberg, K. B.; Szeimies, G. *J. Am. Chem. Soc.* **1970**, *92*, 571. Moore, W. R.; Taylor, K. G.; Muller, P.; Hall, S. S.; Gaibel, Z. L. F. *Tetrahedron Lett.* **1970**, *11*, 2365. Paquette, L. A.; Allen, G. R.; Broadhurst, M. J. *J. Am. Chem. Soc.* **1971**, *93*, 4503. Müller, E. *Tetrahedron Lett.* **1973**, *14*, 1201. Taylor, K. G.; Hobbs, W. E.; Clark, M. S.; Chaney, J. *J. Org. Chem.* **1972**, *37*, 2436. Hoz, S.; Livneh, M.; Cohen, D. *J. Am. Chem. Soc.* **1987**, *109*, 5149.

will describe the generalization of this scheme to other enones and the synthetic utilization of the strained products taking advantage of the great versatility of the phenylthio group.

Acknowledgment. We thank the National Science Foundation for support of this work and Dr. Alvin Marcus for recording the mass spectra.

Supplementary Material Available: Experimental procedures and spectral data for **1** (R = H and R = CH_3), **4**, **4d**, and **6-9** (3 pages). Ordering information is given on any current masthead page.

(14) Nolan, S. M. Ph.D Thesis, University of Pittsburgh, 1982.

Stereochemical Course of the Phospho Group Transfer Catalyzed by cAMP-Dependent Protein Kinase

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Received January 4, 1988

Protein phosphorylation by cyclic 3',5'-adenosine monophosphate (cAMP)-dependent protein kinase (E.C. 2.7.1.37) is the major pathway by which cAMP regulates cellular metabolism. The activation of the enzyme by cAMP is known to occur in a cAMP-promoted dissociation of the holoenzyme into two catalytic subunits and a dimer of regulatory subunits.[1] The catalytic subunit catalyzes the transfer of a phospho group from adenosine triphosphate (ATP) to serine or threonine residues of appropriate peptide and protein substrates.

The mechanism of action of cAMP-dependent protein kinase has been extensively studied and reviewed.[2] Two mechanistic pathways have been considered: (i) a double displacement mechanism involving a phospho-enzyme intermediate and (ii) a single displacement mechanism in which the phospho group is transferred directly between bound substrates. The available experimental data do not, however, distinguish unambiguously between these mechanisms. Thus the presence of a low ATPase activity in cAMP-dependent protein kinase[3] and the relatively long distance between the γ-phosphorus of an enzyme-bound ATP analogue and the hydroxyl oxygen of a bound acceptor peptide substrate, estimated by NMR,[4] are both consistent with a mechanism involving a phospho-enzyme intermediate. Yet no direct evidence for such a phospho-enzyme has ever been obtained. Moreover, the steady-state kinetic behavior of the enzyme,[2,5] the apparent lack of an ATP/ADP exchange reaction in the absence of an acceptor substrate,[6] and the failure of the enzyme to catalyze positional isotope exchange of labeled ATP[7] are all consistent with a direct transfer pathway.

To clarify the reaction mechanism of the cAMP-dependent protein kinase, we have determined the stereochemical course of the phospho group transfer reaction by the use of chiral [γ-(*S*)-^{16}O,^{17}O,^{18}O]ATP.[8] For a double displacement mechanism, overall retention of the configuration at phosphorus is expected, while the single displacement pathway is predicted to result in inversion of the configuration at phosphorus.[9] We chose the most efficient acceptor peptide substrate, Leu-Arg-Arg-Ala-Ser-Leu-Gly,[10] and used the purified catalytic subunit of the protein kinase in the presence of ATP chiral at the γ-phospho group by virtue of the three stable isotopes of oxygen.[11,12] The configuration of the resulting [^{16}O,^{17}O,^{18}O]phosphopeptide was then to be established by the transfer of the chiral phospho group to (*S*)-butane-1,3-diol catalyzed by *E. coli* alkaline phophatase, followed by the determination of the absolute configuration at phosphorus.[8] We found, however, that the phosphoheptapeptide is an unsuitable substrate[13] for alkaline phosphatase in the transfer reaction. Indeed, it is known that although cationic amino alcohols such as ethanolamine or tris(hydroxymethyl)aminomethane are good phosphate acceptors, their *O*-phosphate esters are relatively poor substrates for alkaline phosphatase.[14] We suspected, therefore, that the two guanidinium groups of the heptapeptide could be the reason why the phosphoheptapeptide is such a poor phospho group donor in this reaction.

We therefore synthesized the truncated phosphotetrapeptide, Ala-Ser-[OP(O)(OH)$_2$]Leu-Gly,[15] and found it to be a much

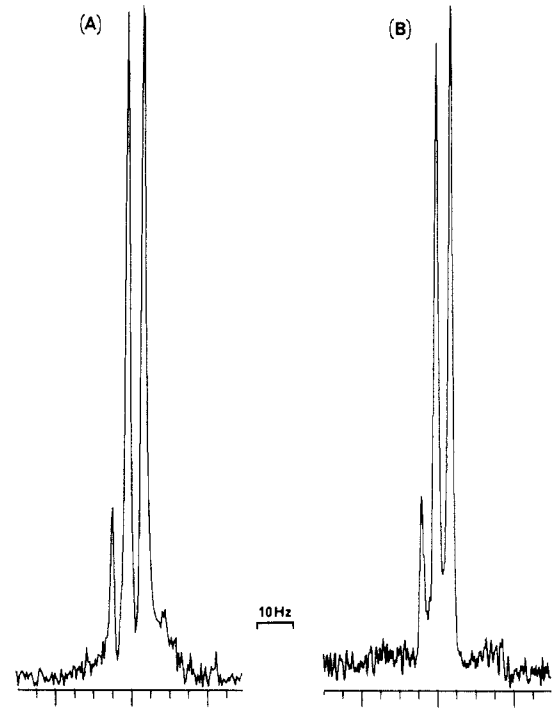

Figure 1. ^{31}P NMR spectra of (A) *O*-[^{16}O,^{17}O,^{18}O]phosphoheptapeptide (180 mg/2.5 mL) and (B) *O*-[^{16}O,^{17}O,^{18}O]phosphotetrapeptide (35 mg/2.5 mL) in D_2O. The spectra shown were recorded on a Nicolet 360 instrument at 146.1 MHz in a bilevel broad band decoupling mode. The spectrum of unreacted phosphotetrapeptide recovered after incubation with alkaline phosphatase was essentially unchanged.

[†] The Rockefeller University.
[§] Harvard University.

(1) Armstrong, R. N.; Kaiser, E. T. *Biochemistry* **1978** *17*, 2840-2845.
(2) Bramson, H. N.; Kaiser, E. T.; Mildvan, A. S. *C.R.C. Crit. Rev.* **1984**, *15*, 93-124.
(3) Armstrong, R. N.; Kondo, H.; Kaiser, E. T. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 722-725.
(4) Granot, J.; Mildvan, A. S.; Bramson, H. N.; Kaiser, E. T. *Biochemistry* **1980**, *19*, 3537-3543.
(5) Moll, G. W., Jr.; Kaiser, E. T. *J. Biol. Chem.* **1976**, *251*, 3993-4000.
(6) Bolen, D. W.; Stingelin, J.; Bramson, H. N.; Kaiser, E. T. *Biochemistry* **1980**, *19*, 1176-1182.
(7) Kaiser, E. T., unpublished results.
(8) Buchwald, S. L.; Hansen, D. E.; Hassett, A.; Knowles, J. R. *Methods Enzymol.* **1982**, *87*, 279-301. Friedman, J. M.; Freeman, S.; Knowles, J. R. *J. Am. Chem. Soc.* **1988**, *110*, 1268-1275.
(9) Knowles, J. R. *Ann. Rev. Biochem.* **1980**, *49*, 877-919.
(10) Kemp, B. E.; Graves, D. J.; Benjamini, E.; Krebs, E. G. *J. Biol. Chem.* **1977**, *252*, 4888-4893. The heptapeptide was prepared either by the solid-phase method on an oxime resin or in solution as Cbz-Leu-Arg-(NO$_2$)-Arg-(NO$_2$)-Ala-Ser(OBn)-Leu-Gly-OBn which was then hydrogenolyzed in acetic acid with Pd/C as catalyst.
(11) Blättler, W. A.; Knowles, J. R. *Biochemistry* **1979**, *18*, 3927-3933.
(12) The reaction mixture containing 3.2 mM peptide, 12.5 mM chiral ATP, 10 mM $MgCl_2$, and 250 nM enzyme was allowed to stand overnight at room temperature.
(13) The progress of hydrolysis was monitored by ^{31}P NMR. The reaction became sluggish after 10% of the substrate was consumed. The release of additional P_i was detected only immediately after addition of fresh enzyme. HPLC studies showed that at pH values above 10, elimination of P_i occurs.
(14) Wilson, I. B.; Dayan, J.; Cyr, K. *J. Biol. Chem.* **1964**, *239*, 4182-4185.
(15) Phosphotetrapeptide was synthesized in solution as *t*-Boc-Ala-Ser-[OP(O)(OPh)$_2$]-Leu-Gly-OBn, which was hydrogenolyzed in trifluoroacetic acid with PtO_2 as catalyst.

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