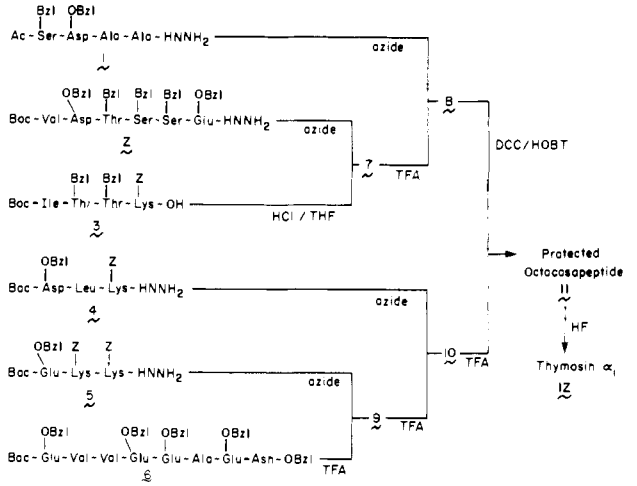
Scheme I. Synthesis of Thymosin α_1



Ile-Thr(Bzl)-Thr(Bzl)-Lys(Z)-OH (**8**): 89.4%; mp 297–298 °C; $[\alpha]^{25}_D$ +6.37° (*c* 1, Me_2SO). Anal. $C_{130}H_{159}N_{15}O_{30}$, C, H, N. Amino Acid Anal. $Asp_{1.97}$, $Ser_{2.96}$, $Glu_{1.08}$, $Ala_{1.85}$, $Val_{0.90}$, $Ile_{0.97}$, $Lys_{1.05}$, $Thr_{2.98}$.

Deblocking of Boc group with TFA from Boc-Glu(OBzl)-Val-Val-Glu(OBzl)-Glu(OBzl)-Ala-Glu(OBzl)-Asn-OBzl (**6**) provided the corresponding TFA octapeptide salt which was then condensed with Boc-Glu(OBzl)-Lys(Z)-Lys(Z)-$HNNH_2$ (**5**) via azide procedure to afford the protected undecapeptide Boc-Glu(OBzl)-Lys(Z)-Lys(Z)-Glu(OBzl)-Val-Val-Glu(OBzl)-Glu(OBzl)-Ala-Glu(OBzl)-Asn-OBzl (**9**) in good yield: 91.6%; mp 312–314 °C; $[\alpha]^{25}_D$ −13.68° (*c* 1, Me_2SO). Anal. $C_{117}H_{146}N_{14}O_{29}$, C, H, N. Amino Acid Anal. $Asp_{1.00}$, $Glu_{5.28}$, $Ala_{1.03}$, $Val_{1.78}$, $Lys_{1.90}$. Treatment of **9** with TFA and coupling of the resultant undecapeptide TFA salt with the tripeptide azide derived from Boc-Asp(OBzl)-Leu-Lys(Z)-$HNNH_2$ (**4**) yielded the protected C-terminal tetradecapeptide Boc-Asp(OBzl)-Leu-Lys(Z)-Glu(OBzl)-Lys(Z)-Lys(Z)-Glu(OBzl)-Val-Val-Glu(OBzl)-Glu(OBzl)-Ala-Glu(OBzl)-Asn-OBzl (**10**): 87.4%; mp 326–327 °C; $[\alpha]^{25}_D$ −15.71° (*c* 1, Me_2SO). Anal. $C_{148}H_{186}N_{18}O_{36}$, C, H, N. Amino Acid Anal. $Asp_{2.00}$, $Glu_{5.10}$, $Ala_{1.00}$, $Val_{1.93}$, $Leu_{1.03}$, $Lys_{2.89}$.

The final coupling of N-terminal tetradecapeptide **8** and C-terminal tetradecapeptide **10** was achieved by the DCC–HOBT procedure.[6] Thus **10** was treated with TFA for removal of Boc group from the α-amino function and the tetradecapeptide TFA salt (69.35 g) obtained was allowed to react with the acetyl tetradecapeptide active ester derived from the reaction of 8 (59.27 g) with DCC and HOBT in a solvent mixture of DMF and Me_2SO. The fully protected acetyl octacosapeptide Ac-Ser(Bzl)-Asp(OBzl)-Ala-Ala-Val-Asp(OBzl)-Thr(Bzl)-Ser(Bzl)-Ser(Bzl)-Glu(OBzl)-Ile-Thr(Bzl)-Thr(Bzl)-Lys(Z)-Asp(OBzl)-Leu-Lys(Z)-Glu(OBzl)-Lys(Z)-Lys(Z)-Glu(OBzl)-Val-Val-Glu(OBzl)-Glu(OBzl)-Ala-Glu(OBzl)-Asn-OBzl (**11**) was obtained in satisfactory yield: 96.7 g (77.3%); mp 330 °C dec. Anal. $C_{273}H_{335}N_{33}O_{63}$, C, H, N. Amino Acid Anal. $Asp_{4.03}$, $Thr_{2.84}$, $Ser_{2.87}$, $Glu_{6.12}$, $Ala_{3.00}$, $Val_{2.89}$, $Ile_{0.98}$, $Leu_{0.97}$, $Lys_{4.02}$.

The fully protected thymosin α_1 (**11**) was then treated with anhydrous HF in the presence of anisole[7] to remove all the protecting groups. Typically, 10 g of **11** was mixed with 15 mL of anisole and stirred with 100 mL of HF at 0 °C for 30 min. The crude material obtained was then purified on a DEAE-Sephadex A-25 column (0.05 M Tris-HCl buffer, pH 8.0, linear gradient of NaCl 0–0.35 M) followed by gel filtration on a Sephadex G-10 column to give thymosin α_1 (**12**) as a white amorphous powder: 0.56 g (9.2%).[8] Amino Acid Anal. $Asp_{4.08}$, $Thr_{2.90}$, $Ser_{3.05}$, $Glu_{5.97}$, $Ala_{3.00}$, $Val_{3.04}$, $Ile_{0.98}$, $Leu_{1.00}$, $Lys_{4.02}$ (average recovery, 95%). The product migrated as a single spot[9] on acrylamide gel isoelectric focusing (pH 3.5–9.5) and on high voltage silica gel thin-layer electrophoresis (pH 1.9 and pH 5.6), indistinguishable from the natural thymosin α_1. The tryptic peptide maps[10] from synthetic and natural thymosin α_1 were also identical. Synthetic **12** shows activities equivalent to the natural compound in the MIF (macrophage migration inhibitory factor),[1] E-Rosette,[1] and other assays.

Acknowledgment. The authors thank Drs. F. Scheidl, T. Williams, and V. Toome and their associates for various physicochemical measurements and analyses; Dr. A. L. Goldstein for biological assays; Drs. J. Meienhofer, A. Ramel, and G. Saucy for advice and discussions.

Supplementary Material Available: Elemental analyses of compounds **1–11** (2 pages). Ordering information is given on any current masthead page.

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(3) Abbreviations used followed the recommendations of IUPAG–IUB Commision on Biochemical Nomenclature, *J. Biol. Chem.* **1972**, *247*, 977–983. Other abbreviations used follow: Ac, acetyl; Boc, *tert*-butyloxycarbonyl; Bzl, benzyl; DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide; HOBT, 1-hydroxybenzotriazole; TFA, trifluoroacetic acid; THF, tetrahydrofuran; Tris, tris(hydroxymethyl)aminomethane; Z, benzyloxycarbonyl.
(4) See for example, Finn, F. M.; Hofmann, K. "The Proteins". Third ed., Neurath, H., Hill, R. L., and Boeder, C. L., Ed.; Academic Press: New York, N. Y., 1976; Vol. II.
(5) Honzl, J.; Rudinger, J. *Collect. Czech. Chem. Commun.* **1961**, *26*, 2333–2344.
(6) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788–798.
(7) Sakakibara, S. "Chemistry and Biochemistry of Amino Acids, Peptides and Proteins", Weinstein, B., Ed.; Marcel Dekker: New York, 1971; Vol. 1, pp 51–85.
(8) The low yield in this step is probably due to side reactions occurring during HF treatment (Cf. the following. Tzougraki C., et al. *J. Am. Chem. Soc.* **1978**, *100*, 6248–6249. Atherton, E., et al. *J. Chem. Soc., Chem. Commun.* **1977**, 819–821. Yamashiro, D.; Li, C. H. *J. Am. Chem. Soc.* **1978**, *100*, 5174–5179. Feinberg, F. S.; Merrifield, R. B. *ibid.* **1975**, *97*, 3485–3496. Sano, S.; Kawanishi, S. *ibid.* **1975**, *97*, 3480–3484.) combined with the loss of material during the chromatographic purification procedures. Other deprotection processes such as hydrogenolysis (Pd catalyst at hydrogen pressures as high as 2500 psi), sodium–liquid ammonia reduction, and acidolysis with methanesulfonic acid, trifluoromethanesulfonic acid, and hydrobromic acid in acetic acid were investigated. None of these experiments gave better results, presumably owing to the extreme insolubility of the fully protected thymosin α_1 (**11**) in the solvents commonly used in peptide chemistry. For discussions on the general problems associated with deblocking protecting groups from fully protected larger polypeptides, see, for example, Finn, F. M.; Hofmann, K. ref 4, pp 105–253.
(9) The mixture of natural and synthetic thymosin α_1 migrated as a single spot in all the systems tested.
(10) To obtain the tryptic peptide map, thymosin α_1 was digested with TPCK–trypsin (100:1 ratio, pH 9.0 sodium borate buffer, 25 °C, 15 h), acidified to pH 2, and spotted on a silica gel TLC plate (Merck F-254). Electrophoresis (pH 1.9, 3000 V, 35 min) was carried out for the first dimension and thin-layer chromatography in *n*-BuOH–EtOAc–HOAc–H_2O (1:1:1:1) was carried out for the second dimension. The color was developed with ninhydrin or with chlorine–tolidine reagent.

Su-Sun Wang,* Irina Douvan Kulesha, Donald P. Winter
Chemical Research Department, Hoffmann-La Roche Inc. Nutley, New Jersey 07110
Received August 13, 1978

Synthesis and Chemistry of *cis*- and *trans*-2,3-Divinylthiirane

Sir:

In recent years, there has been a considerable interest in the thermal rearrangements of divinylcyclopropanes and their heteroanalogues. Next to *cis*-1,2-divinylcyclopropane,[1] the Cope rearrangement of *cis*-2,3-divinyloxirane[2] and *cis*-2,3-divinylaziridine[3] (eq 1) has been investigated in detail. Simi-

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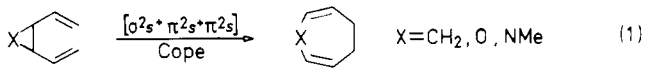
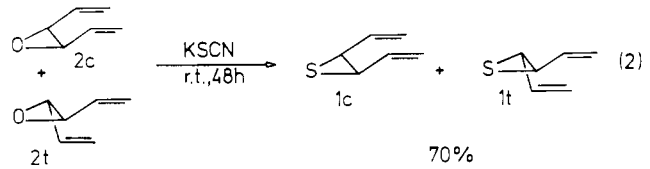


larly the reactions of the corresponding trans compounds have been studied.[4]

Nothing is known, however, about the chemistry of compounds of this type containing third-row elements, e.g., divinylthiiranes.[5] Although there have been reports on the intermediacy of *cis*-2,3-divinylthiirane 1,1-dioxides in the synthesis of 4,5-dihydrothiepin 1,1-dioxides,[6] the isolation or characterization of the intermediates were unsuccessful.

We report here the synthesis and thermal rearrangement of *cis*- and *trans*-2,3-divinylthiirane (**1c** and **1t**). Thiiranes are generally prepared by conversion of the corresponding oxiranes with thiourea or inorganic thiocyanates. The neat mixture of the *cis*- and *trans*-2,3-divinyloxiranes (**2c** and **2t**)[2a] was stirred at room temperature under nitrogen with a saturated aqueous solution of KSCN for a total of 48 h (eq 2). Thiourea or the use

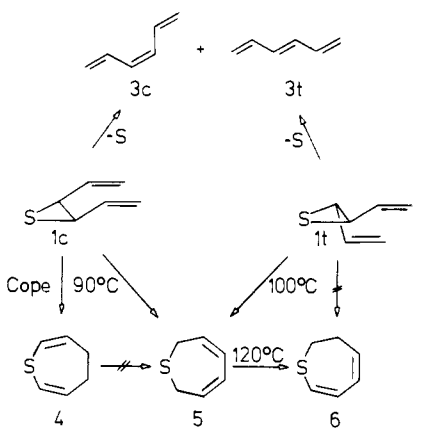


of a homogeneous reaction mixture proved to be unsuccessful. The aqueous layer was replaced every 12 h by a new batch and the reaction was followed by VPC until all of the starting material was consumed. Workup, followed by trap-trap distillation on a high vacuum line led to a mixture of 38% **1c** and 62% **1t** in 70% yield. The pure isomers were obtained via preparative VPC (10% OV 17 on Chromosorb P, 80 °C, moderate decomposition). The structures of **1c** and **1t** were assigned on the basis of their spectral data;[7] and spectra of **2c** and **2t** were useful for comparison.[2a,d] Similarly to those of the oxiranes, the absorptions of the allylic protons in **1c** appear at lower field than in **1t**. **1c:** ^{1}H NMR (90 MHz, $CDCl_3$, δ_{Me_4Si}) 3.64 (m, 2 H, allylic H), 5.20–5.93 (m, 6 H, ABX, vinyl H). **1t:** δ 3.32 (m, 2 H, allylic H), 5.10–5.54 (m, 6 H, vinyl H).

Pure **1c** and **1t** are stable at room temperature if stored under nitrogen and can be kept in a refrigerator for several weeks without appreciable loss of sulfur. Without purification or in solution, however, sulfur loss occurs readily, accompanied by polymerization of the so produced *cis*- and trans-1,3,5-hexatrienes (**3c** and **3t**). No appreciable reactions of **1c** and **1t** can be detected below 80 °C. Above that temperature two types of reactions are observed (Scheme I): (a) decomposition with loss of sulfur; (b) rearrangements without loss of sulfur.

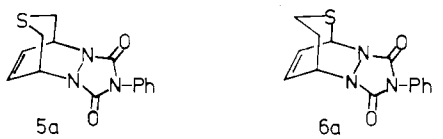
Decomposition reactions with loss of sulfur total between 20 and 25% in the gas-phase thermolysis (static system, break-seal technique) of **1c** and **1t** between 90 and 120 °C.

Scheme I



Although cheletropic sulfur elimination may play a role in these reactions, no stereospecific olefin formation is observed. Both **1c** and **1t** are yielding the same proportions of 20% **3c** and 80% **3t**. They do, however, not necessarily represent the true proportions. As shown by control experiments, **3c** and **3t** are undergoing secondary reactions (dimerization and polymerization) under the employed conditions.

About 75% of the thermolysis of **1c** and **1t** proceeds without loss of sulfur. **1c** rearranges smoothly at 90 °C with the formation of 52% 4,5-dihydrothiepin (**4**) and 48% 2,7-dihydrothiepin (**5**). **1t**, in contrast, produces a mixture of **5** and 2,3-dihydrothiepin (**6**) if thermolized between 100 and 125 °C, the proportions of **5** and **6** being variable and temperature/time dependent. A careful study showed, however, that **6** is *not* a primary reaction product, but is formed by thermal rearrangement of **5** at temperatures above 100–110 °C. Careful low conversion studies at 100 °C revealed that indeed **5** is the *only* reaction product. In a control experiment we could show that pure **5** is cleanly converted into the thermodynamically more stable **6** at 120 °C ($t_{1/2} \sim 3$ h). **4**, under the same conditions, is absolutely stable and does not rearrange into either **5** or **6**. The structures of **4**, **5**, and **6** have been assigned on the basis of their spectroscopic data;[7] the spectra of 4,5-dihydrooxepin[2b] and 4,5- and 2,7-dihydrothiepin 1,1-dioxide[6] were useful for comparison. **4:** ^{1}H NMR (90 MHz, $CDCl_3$, δ_{Me_4Si}) 2.49 (dd, 4 H, allylic H), 5.95 (m, 4 H, olefinic H). **5:** δ 3.19 (d, 4 H, J = 5.6 Hz, allylic H), 6.05 (m, 4 H, olefinic H). **6:** δ 2.95 (m, 4 H), 5.90 (m, 2 H), 6.10 (m, 2 H). Further proof for the structures was obtained by their reaction with *N*-phenyltriazolinedione at 0 °C in $CDCl_3$. Whereas **4** does not undergo any reaction, **5** and **6** are readily transformed into the corresponding derivatives **5a** and **6a**, whose structures were unambiguously assigned by NMR and high resolution mass spectra.[8]

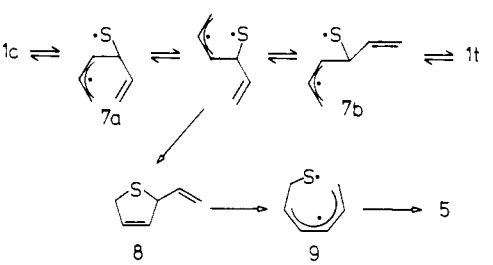


From the obtained results it is clear that two types of rearrangements are occurring simultaneously. The formation of **4**, which is only produced in the thermolysis of **1c**, can readily be interpreted in terms of a thermally allowed $[_\sigma 2_s + _\pi 2_s + _\pi 2_s]$ (Cope) rearrangement. The temperature required (90 °C) is clearly higher than for other *cis*-divinyl derivatives,[1-3] demonstrating the ability of sulfur to accommodate considerably more angle strain.

5, however, which is formed from both **1c** and **1t** under similar conditions, must have arisen via a different route. At the employed temperatures the relative lability of C–S bonds becomes apparent, allowing other processes to compete with the Cope rearrangement. Since **4** is stable under the reaction conditions (130 °C, 8 h, no change), it can be definitely ruled out as a precursor for **5**.[9]

One possible explanation for the observed results is outlined in Scheme II. Opening of the carbon–sulfur bond in **1c** and **1t** would lead to the isomeric diradicals **7a** and **7b**.[10] Rotation and ring closure[11] would lead to 2-vinyl-2,5-dihydrothiophene (**8**).

Scheme II



Although **8** is not known and its thermal behavior is uncertain, one can assume that it may undergo ring opening under the reaction conditions to produce the highly stabilized pentadienyl radical **9**[12] which could then close under the formation of **5**.[13]

We are currently engaged in a series of experiments to gain further insight into the detailed mechanism of this system.

Acknowledgment. We thank the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie for support of this research.

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(5) This is probably due to an observed predominance of sulfur extrusion leading to the corresponding olefins. Loss of sulfur occurs readily in a large number of thiiranes with electron-withdrawing and conjugated substituents. (a) Braslavsky, S.; Heicklen, J. *Chem. Rev.* **1977**, *77*, 473. (b) Sander, M. *ibid.* **1965**, *65*, 297. The synthesis of **1c** was claimed in a footnote,[3a] but no details were ever published.
(6) (a) Mock, W. L.; *J. Chem. Soc., Chem. Commun.* **1970**, 1254. (b) Paquette, L. A.; Maiorana, S. *ibid.* **1971**, 313. We thank Professor Paquette for copies of the NMR spectra.
(7) All new compounds gave satisfactory analytical data and all spectroscopic properties were in good agreement with the proposed structures.
(8) NMR data were readily interpreted by comparison with the derivative obtained from cyclohepta-1,3-diene, accounting for the sulfur bridge.
(9) One referee suggested that **1t** could be a precursor for **5** in the thermolysis of **1c**. While our control experiments gave no evidence for such an interconversion, the possibility cannot be ruled out completely.
(10) **7a** and **7b** are stabilized by allylic delocalization; similarly, S radicals are known to be quite stable.[5a]
(11) This process is well established in the vinylcyclopropane–cyclopentene rearrangement.
(12) Pentadienyl radicals are stabilized by between 4 and 9 kcal/mol (depending on the source) over allylic radicals and could well account for a lowering of the activation enthalpy for the reaction **8** → **9**: Pettus, J. A. Jr.; Moore, R. E. *J. Am. Chem. Soc.* **1971**, *93*, 3037.
(13) Alternatively, conrotatory opening for the C–C bond in **1c** and **1t**, a process well documented in the thermolysis of oxiranes,[2d] would lead to isomeric thiocarbonyl ylides.[14] Their disrotatory ring closure would lead to 2-vinyl-2,3-dihydrothiopene, which could, via a similar sequence of diradicals, provide another route to **5**.
(14) For formation and reactions of thiocarbonyl ylides, see Buter, J.; Wassenar, S.; Kellogg, R. M. *J. Org. Chem.* **1972**, *37*, 4045.

Manfred P. Schneider,* Margit Schnaithmann
Institut für Chemie, Universität Hohenheim
D-7000 Stuttgart-70, West Germany
Received August 17, 1978

Photolysis of Aryldiazo Compounds in Rigid Matrices. Temperature and Matrix Effects on the Selectivities of Insertion of Arylcarbenes into Carbon–Hydrogen Bonds

Sir:

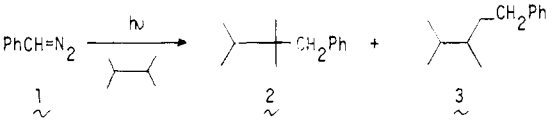
Recent reports from this[1] and other[2] laboratories have demonstrated that the low-temperature photolysis of aryldiazo compounds in rigid matrices can be a unique and widely applicable method for detecting triplet arylcarbene chemistry, especially in the systems in which competitive singlet and triplet reactions occur. The C–H "insertion" products derived from triplet carbene in rigid matrices of olefins and alcohols, which apparently arise via an abstraction–recombination mechanism, are in many cases entirely different from those observed[3] in liquid phase experiments. The difference may be explained mainly in terms of a difference in diffusibility between two reaction phases. In order to obtain more precise insight into the mechanism by which the matrix controls C–H insertion processes within it, we have investigated carbene processes in matrices of alkane which are, unlike other systems thus far reported,[1,2] not able to lead to *multiplicity-specific* products. We find that the matrix not only selects the multiplicity of arylcarbenes, but it also imposes a severe steric demand on carbene processes within it.

Table I. Temperature and Matrix Effects on C–H Insertion Selectivity of Phenylcarbene[a]

substrate	ratio	rel ratio[b] 0 °C	rel ratio[b] −196 °C
isobutane	tertiary:primary	118	4.1
2,3-dimethylbutane	tertiary:primary	69	6.5
isopropyl ether	tertiary:primary	194	4.3
isopropyl alcohol[c]	tertiary:primary	194	26
n-butane	secondary:primary	9.6	2.7
n-pentane	secondary:primary	9.0	4.9
	d	1.4	0.3
ethyl ether[e]	secondary:primary	148	17
ethyl alcohol[c]	secondary:primary	150	20

[a] All products were identified by GC comparisons with authentic samples and GC–mass spectral techniques. Other minor products detected in each experimental condition were toluene, bibenzil, benzaldehyde, benzaldazine, and stilbenes. [b] Corrected for number of hydrogens. Averages of triplicate runs are tabulated; reproducibility was <±3%. [c] The O–H insertion product was main one (>73%) in 0 °C photolysis and C–H insertion became dominant (>70%) in the solid run.[1a] [d] Ratio of 2- to 3-benzylpentanes. [e] Benzyl ethyl ether was formed in 30% yield of total products at 0 °C, but was not detected in −196 °C photolysis.

Direct irradiation of phenyldiazomethane (**1**) in degassed 2,3-dimethylbutane in a sealed Pyrex tube at 20 °C was carried out by a 300-W high-pressure mercury arc without filter until all of the diazo compound was destroyed. GC analysis of the resulting mixture showed that two C–H insertion products (**2** and **3**) were formed (~75%) in a ratio of 11.5:1.0. In contrast,



(1)

irradiation of **1** in frozen 2,3-dimethylbutane matrix at −196 °C resulted in a dramatic increase in the relative yield of the primary C–H insertion product (**3**), the ratio of **2**:**3** being 1.08:1.0. A similar but less dramatic increase in the relative yield of primary C–H insertion product was also observed in *n*-butane. The product distributions in other alkanes were also examined as a function of temperature and are given in Table I on a "per bond" basis. Included for comparison are the results for ether and alcohols. A more extensive temperature study has been performed on 2,3-dimethylbutane (mp −129 °C) and the results are shown in Figure 1. There is a sharp discontinuity (maximum) in the graph as the reaction phase changes from liquid to solid. This clearly indicates that the observed change in the insertion selectivity in the solid is ascribable not to a simple temperature effect at all but totally to a change in environment.

Which multiplicity is responsible for the emerging dominance of the primary C–H insertion product in the solid-phase experiments? It is important to note that, in alcoholic system, the C–H insertion products which were believed to be derived from triplet carbene showed a similar decrease in the insertion selectivity in the solid phase. Equally noteworthy are the results in the ether; the C–O displacement product, i.e., ethyl benzyl ether, formed in the photolysis of **1** in ether solution, apparently via attack of singlet carbene on oxygen atom,[4] was almost

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