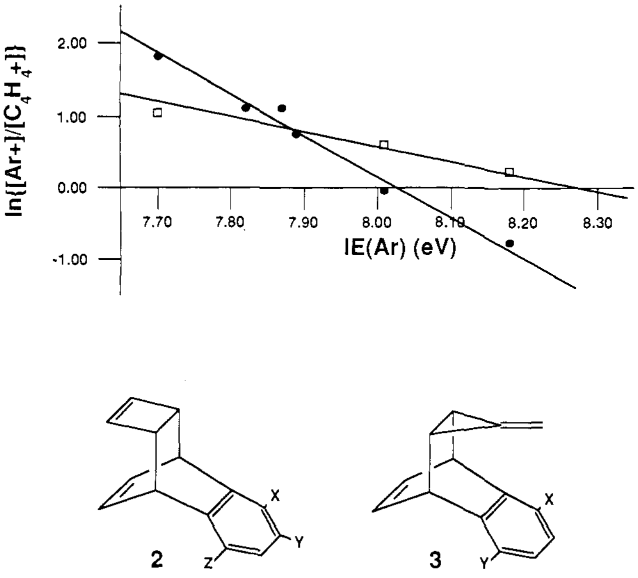

Figure 3. Plot of ln {[Ar+]/[C_4H_4+]} vs. IE(Ar) for compounds in the series **2** and **3**. Filled circles correspond to series **2**; substituents studied were X=Y=Z=H; X=Me, Y=Z=H; X=Y=Me, Z=H; X=Z=Me, Y=H; X=Y=Z=Me; X=Z=H, Y=OMe. Open squares correspond to series **3**; substituents studied were X=Y=H; X=Me, Y=H; X=Y=Me.

clobutadiene to an aromatic adjunct, with the prototype being Nenitezscu's hydrocarbon (**1**, Figure 1).[5] The 70-eV electron-ionization mass spectrum of **1** shows *m/z* 52 as the most intense peak. That this peak is derived from the cyclobutene moiety in **1** and not from fragmentation of the benzene adjunct is shown by deuteriation[6] at C3; **1**-*3-d* shows no more than 0.5% *m/z* 52—the most intense peak is now *m/z* 53. The CAD spectrum of the C_4H_3D ion from **1**-*3-d* shows a peak at *m/z* 26.5, corresponding to the C_4H_3D dication, when nitrogen is used as the collision gas but not when helium is used (Figure 2). Relatively easy formation of the nominally aromatic dication might be expected for cyclobutadiene. All other CAD spectra reported used helium to avoid interference from the dication. The *m/z* 26:27 ratio in the CAD spectrum of the C_4H_4 ion from **1** is high (>7), consistent with Bowers' criterion for nearly exclusive formation of cyclic ions.[2] We note, however, that this criterion cannot be expected to be useful in differentiating methylenecyclopropene from cyclobutadiene structures since a facile fragmentation of the latter to ions with *m/z* 26 would also be expected.[7] Double deuteriation[6] of **1** revealed that there cannot be rapid, reversible interconversion between cyclobutadiene and methylenecyclopropene structures; **1**-*2,4-d*$_2$ and its isomer, **1**-*3,4-d*$_2$, gave $C_2H_2D_2$ ions with notably different CAD spectra. The ion from **1**-*3,4-d*$_2$ had peaks at *m/z* 26, 27, and 28 in a ratio of 1:2.3:0.9 whereas that from **1**-*2,4-d*$_2$ had the same peaks in a ratio of 1:4:1 (Figure 2). Rapid reversible interconversion between cyclobutadiene and methylenecyclopropene structures would have resulted in ions exhibiting the same CAD spectrum.

Further information on the C_4H_4 ion derived from molecules such as **1** was obtained through study[6] of the series **2** (Figure 3). Fragmentation of the molecular ions of these compounds could be expected to give the C_4H_4 ion and the substituted naphthalene ion in a ratio that depended on the relative ionization energies of the C_4H_4 neutral and the naphthalene, according to Stevenson's rule.[8] In fact, a plot of ln {[Ar^+]/[$C_4H_4^+$]} vs. IE(Ar), where [Ar^+] is the relative intensity of the peak with *m/z* corresponding to the naphthalene ion and IE(Ar) is the ionization energy of the naphthalene,[9] was linear with a slope that depended on the energy of the ionizing electrons but with an intercept on the IE axis that did not. The intercept occurred at 8.02 ± 0.09 eV (Figure 3). The corresponding plot for compounds **3** gave an intercept at 8.28 ± 0.12 eV (Figure 3).[10] It seems reasonable to assume that compounds **3** would be more likely to afford methylenecyclopropene ions and compounds **2** to produce cyclobutadiene ions. To the extent that the intercepts of the Stevenson's rule plots differ by more than the experimental error, there is evidence that these ions retain their structural identity, at least temporarily. One can anticipate that the neutralization-reionization mass spectra[3] might provide more definitive evidence on the lifetimes of these isomeric, cyclic C_4H_4 ions. Experiments to obtain these spectra are in progress.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research.

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[5] Nenitzescu, C. D.; Avram, M.; Pogany, J. J.; Mateescu, G. D.; Farcasian, M. *Stud. Cercet. Chim. (Cluj)* **1963**, *11*, 7.

[6] Ray, J. C., Jr., Ph.D. Dissertation, Cornell University, Ithaca, NY, August 1986.

[7] The CAD spectra of the C_4H_4 ions from compounds in series **2** or **3** (Figure 3) are essentially identical.

[8] McLafferty, F. W. *Interpretation of Mass Spectra*, 3rd ed.; University Science Books: Mill Valley, CA, 1980.

[9] Heilbronner, E.; Hoshi, T.; von Rosenberg, J. L.; Hafner, K. *Nouv. J. Chim.* **1977**, *1*, 105. For aromatic hydrocarbons that lack serious nonbonded interactions in the planar geometry, vertical and adiabatic ionization energies are essentially identical: Boschi, R.; Clar, E.; Schmidt, W. *J. Chem. Phys.* **1974**, *60*, 4406.

[10] These intercepts could be identified with ionization energies of the C_4H_4 neutrals; however, some caution is required since the effective ionization energies of the naphthalene fragment and the C_4H_4 fragment will be perturbed by distortion away from their equilibrium geometries during the dissociation of the adduct molecular ion. If one assumed that comparable distortions occurred for the ions from **2** and **3** then the difference in ionization energies of cyclobutadiene and methylenecyclopropene would be equal to the difference between the intercepts for the Stevenson's rule plots. Experiments on the dissociation of butadiene dimer molecular ions suggest that such an assumption is likely to give relative ionization energies to within ±0.05 eV (Tureček, F.; Hanuš, V. *Mass Spec. Rev.* **1984**, *3*, 85). UV photoelectron spectroscopy on cyclobutadiene has revealed that the vertical ionization energy is 8.24 eV and that the adiabatic ionization energy is probably 8.10 eV (Kreile, J.; Münzel, N.; Schweig, A.; Specht, H. *Chem. Phys. Lett.* **1986**, *124*, 140).

Andrimid, a New Peptide Antibiotic Produced by an Intracellular Bacterial Symbiont Isolated from a Brown Planthopper

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Some homopterous insects such as planthoppers and leafhoppers transmit intracellular symbiotic microorganisms to offspring through their eggs.[1] However, their role and the nature of metabolites remain unknown because the microorganisms are difficult to isolate without extracellular contamination.[2] In the following we describe the isolation of andrimid (**1**) from the culture

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[1] Nasu, S. *Nippon Oyo Dobutsu Konchu Gakkaishi* **1965**, *9*, 225.

[2] Nasu, S.; Kusumi, T.; Suwa, Y.; Kita, H. *Appl. Entomol. Zool.* **1981**, *16*, 88.

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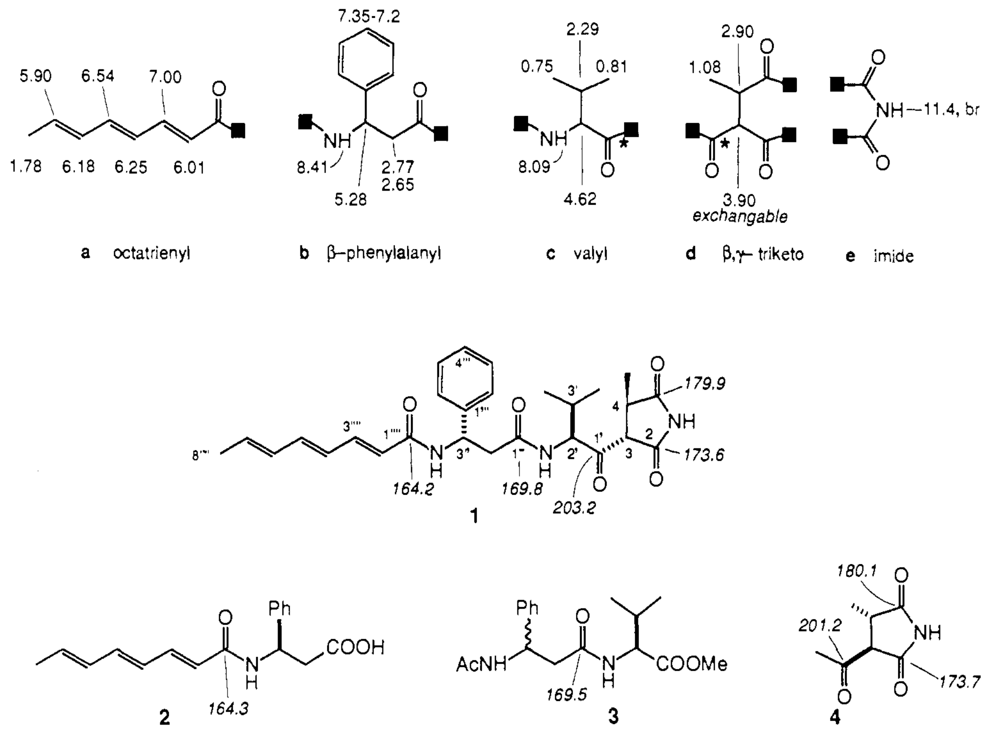

Figure 1. Partial structures, structure of andrimid **1**, and their NMR data.

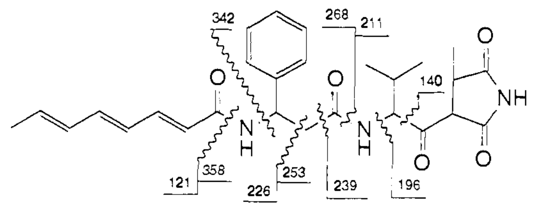

Figure 2. Mass spectroscopy fragmentation pattern of andrimid **1**.

broth of a symbiont of *Nilaparvata lugens* (brown planthopper). Andrimid is an antibiotic exhibiting strong specific activity against the white blight pathogen of rice plants, *Xanthomonas campestris* pv. *oryzae*; however, against Gram positive and negative bacteria including most plant pathogens it is inactive or only weakly active.

An *Enterobacter* sp.[3] microorganism present in the eggs of *N. lugens* (collected in Thailand) was isolated (on chocolate agar plate) and cultured in TSB[4] (5 days, 24 °C), and the purification of **1** was monitored by assaying against the pathogen. The CHCl$_3$ extract of the broth (24.5 L) was flash chromatographed through silica gel with water-saturated CH$_2$Cl$_2$/*i*-PrOH (97:3); purification by ODS HPLC (YMCpak, 60% MeOH in 0.05 M phosphate buffer, pH 5.0) and extraction with CHCl$_3$ yielded andrimid (**1**), 30 mg.

The physical data of **1**, C$_{27}$H$_{33}$N$_3$O$_5$ (479.2418, M$^+$ at 479.2415 by EI-MS), are as follows: mp 172–173.5 °C; UV λ_{max} 297 nm, ϵ 41 200 in MeOH, ϵ 51 100 in MeOH/aqueous NaOH, ϵ 38 000 in MeOH/AcOH; FTIR (film) 3280 (NH), 1730, 1705, 1647, 1610, 1535 cm^{-1}. ^{1}H NMR (Me$_2$SO-d_6) clarified partial structures **a**–**e** (Figure 1), of which **a**–**d** are self-explanatory; the low-field 11.4 ppm singlet together with the presence of all five oxygens as carbonyls (^{13}C NMR)[5] leads to the imide function **e**.

The mass spectroscopy (MS) fragmentation pattern (Figure 2) of **1** including B/E linked scans[6,7] revealed connectivities of moieties **a**–**b**–**c**. Reduction of **1** with NaBH$_4$ gave a *sec*-OH (C-1′ in **1**, 55% yield) originating from the asterisked carbonyl in structures **c**/**d**; prolonged reduction gave a hemiaminal[8] (C-2 in **1**), thus clarifying the link **c**–**d**–**e** and leading to the β-keto-succinimide moiety, pK_a 6.8[9] by UV titration in 60% aqueous MeOH. The planar structure of **1** was confirmed by syntheses of compounds **2**, **3** (diastereomeric) and **4** (partially racemic[10]) and comparison of ^{13}C NMR shifts (Figure 1).[5]

Amino acid analysis of **1** gave 0.5% L-(*S*)-valine formed as a side product by reversed Claisen condensation and 60% (3*S*)-3-amino-3-phenylpropionic acid (D-β-phenylalanine); the configurations were determined by comparisons of HPLC retention times of both synthetic enantiomers[11] on Chiralpak WH.[12] Although the acidic center at C-3 could lead to two diastereomers, only one is found (^{1}H NMR). As expected, this is the stabler trans isomer as shown by the 4% difference NOE observed at 3-H upon irradiation of 4-Me. The absolute configurations at C-3 and C-4 are probably *S* and *R* (as shown), respectively, because changes in the CD spectra accompanying basification were opposite to those of the synthetic fragment **4**[10] in the range 250–300 nm where this chromophore absorbs.

The activity of andrimid is highly specific. It was found to be active against all 418 strains and isolates of Southeast Asian and Japanese *X. campestris* pv. *oryzae*, the MIC value against the strain NIAES 1225 being 0.1 μg/mL; however, against other pathovars of the *X. campestris* species, the MIC is 20–60-fold. It is unclear why the brown planthopper lives on the rice plant and at the same time hosts a symbiont which yields an antibiotic against the plant pathogen. Preliminary studies have led to the isolation of several known antibiotics from a variety of other symbiont cultures.[13,14] The symbionts thus appear to be a

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(3) Identified as a close species to *E. agglomerans*. Detail will be published elsewhere.

(4) Difco Manual, 10th ed.; Difco Laboratories: Detroit, 1984; p 1027.

(5) ^{13}C NMR data of **1** (in Me$_2$SO-d_6): 203.2 (1′), 179.9 (5), 173.6 (2), 169.8 (1″), 164.2 (1⁗), 142.7 (1‴), 139.3 (3⁗), 138.8 (5⁗), 133.3 (7⁗), 131.4 (6⁗), 128.1 (3‴, 5‴, 4⁗), 126.7 (4‴), 126.3 (2‴, 6‴), 124.2 (2⁗), 63.0 (2′), 57.8 (3), 49.8 (3″), 41.1 (2″), 38.9 (4), 28.1 (3′), 19.3 (4′), 18.2 (8⁗), 17.2 (3′-Me), 14.5 (4-Me) ppm.

(6) Boyd, R. K.; Beynon, J. H. *Org. Mass Spectrom.* **1977**, *12*, 163.

(7) Bruins, A. P.; Jennings, K. R.; Evans, S. *Int. J. Mass Spectrom. Ion Phys.* **1978**, *26*, 395.

(8) Wijnberg, J. B. P. A.; Speckamp, W. N.; Schoemaker, H. E. *Tetrahedron Lett.* **1974**, 4073.

(9) 3-Acetylsuccinimide: pK_a = 7.5. Champion, J. *Ann. Chim.* (*Rome*) **1954**, *12*, 649.

(10) During the course of preparation of **4** from L-(*S*)-lactic acid, partial racemization of the methyl group occurred; however, the methyl and acetyl groups always maintain a trans relationship.

(11) Cohen, S.; Weinstein, S. *J. Am. Chem. Soc.* **1964**, *86*, 725.

(12) Technical Brochure, No. 2; Daicel Chemical Industries, Ltd.: Tokyo 100, Japan.

promising new source for bioactive compounds. It would be interesting if the ability of the intracellular symbionts to produce some antimicrobial substances is one of the defense mechanisms of the insects which lack immunological defensive systems.

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Supplementary Material Available: Positive and negative EI-MS and proposed fragmentation mechanisms of some predominant ions (1 page). Ordering information is given on any current masthead page.

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(13) Fredenhagen, A.; Kenny, P.; Kita, H.; Komura, H.; Naya, Y.; Nakanishi, K.; Nishiyama, K.; Sugiura, M.; Tamura, S. In IUPAC Proceedings, Pesticide Chemistry, Ottawa, 1986, in press.

(14) Jigami, Y.; Harada, N.; Uemura, H.; Tanaka, H.; Ishikawa, K.; Nakasato, S.; Kita, H.; Sugiura, M. *Agr. Biol. Chem.* **1986**, *50*, 1637.

The First Stable Germene: A Compound with a Germanium-Carbon Double Bond

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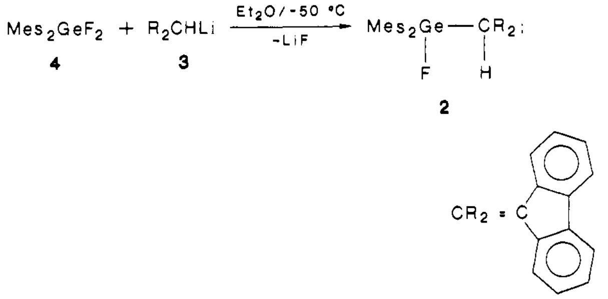
There is now a great deal of interest in group 13, 14, and 15 elements in low coordination states.[1] Since 1981, several new compounds of the type M=M′ (M and M′: group 13, 14, and 15 elements) with pπ–pπ bonds have been reported. In organogermanium chemistry, the first stable derivatives with a sp^2-hybridized germanium have only been prepared very recently: they are the digermenes $R_2Ge{=}GeR_2$[2] (R = bis(trimethylsilyl)methyl,[2a] 2,6-dimethylphenyl,[2b] 2,6-diethylphenyl[2c]), the germaphosphene $R_2Ge{=}PR'$[3a] (R = mesityl, R′ = 2,4,6-tri-*tert*-butylphenyl), and the germaimines $R_2Ge{=}NR'$[3b] (R = $(Me_3Si)_2N$, R′ = N=C(R″)(R‴)). Germenes $R_2Ge{=}CR_2'$ have long been speculated as reactive intermediates and could only be characterized by trapping reactions.[1c,d,4,5]

In the present paper we describe the synthesis and some reactions of the first stable compound with a germanium–carbon double bond, the germene **1**. The highly air-sensitive **1** is stabilized owing to large steric hindrance around the double bond and high mesomeric effects; it has been isolated as adducts (**1b**, **1c**, **1d**) with bases and characterized by its physicochemical data and its expected trend in chemical reactivity.

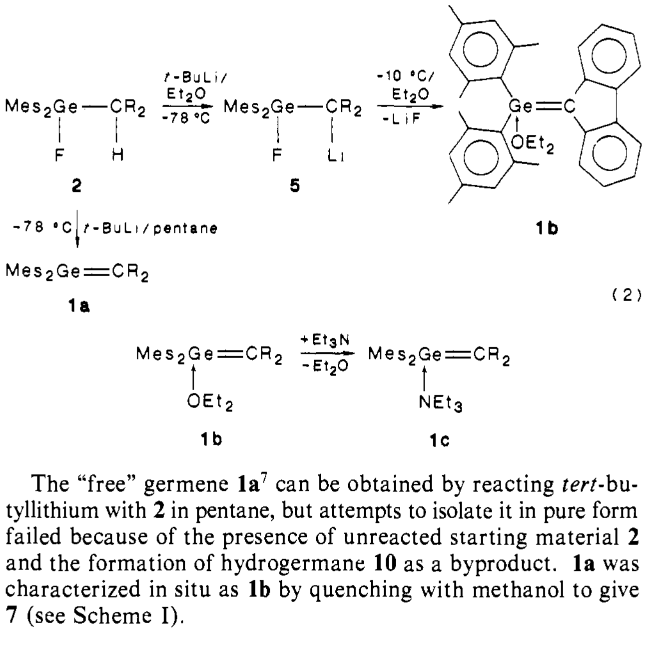
The first step in the synthesis of **1** involves the preliminary preparation of organofluorogermane **2**[6] by addition of the fluorenyllithium **3** (prepared by reacting *n*-butyllithium (1.6 M in hexane) with fluorene) to dimesityldifluorogermane **4**[3]) (eq 1).



$$\underset{\mathbf{4}}{Mes_2GeF_2} + \underset{\mathbf{3}}{R_2CHLi} \xrightarrow[-LiF]{Et_2O/-50\ ^\circ C} \underset{\mathbf{2}}{Mes_2Ge(F)-CR_2(H)};$$

(1)

1 was formed by dehydrofluorination of **2** with *tert*-butyllithium: 1 equiv of *t*-BuLi was added to **2** in ethereal solution at −78 °C under nitrogen. A yellow-orange color due to **5** developed immediately. Elimination of LiF occurred at −10 °C to give **1b**,[7] which crystallizes on cooling (eq 2).



(2)

The "free" germene **1a**[7] can be obtained by reacting *tert*-butyllithium with **2** in pentane, but attempts to isolate it in pure form failed because of the presence of unreacted starting material **2** and the formation of hydrogermane **10** as a byproduct. **1a** was characterized in situ as **1b** by quenching with methanol to give **7** (see Scheme I).

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(1) For reviews on M=M compounds, see the following. (a) Si=Si: West, R. *Science (Washington, DC)* **1984**, *225*, 1109-1114. (b) Si=M (M, group 14 and 15 elements): Raabe, G.; Michl, J. *Chem. Rev.* **1985**, *85*, 419-509. Brook, A. G.; Baines, K. M. *Adv. Organomet. Chem.* **1986**, *25*, 1-44. (c) Si=C (or N) and Ge=C (or N): Wiberg, N. *J. Organomet. Chem.* **1984**, *273*, 141-177. (d) Ge=M (M, group 14 and 15 elements): Satgé, J. *Adv. Organomet. Chem.* **1982**, *21*, 241-287. (e) M=M′ (group 14 and 15 elements): Cowley, A. H. *Acc. Chem. Res.* **1984**, *17*, 386-392. Cowley, A. H. *Polyhedron* **1984**, *3*, 389-432. (f) P=M (group 15 elements): Cowley, A. H.; Kilduff, J. E.; Lasch, J. G.; Mehrotra, S. K.; Norman, N. C.; Pakulski, M.; Whittlesey, B. R.; Atwood, J. L.; Hunter, W. E. *Inorg. Chem.* **1984**, *23*, 2582-93.

(2) (a) Hitchcock, P. B.; Lappert, M. F.; Miles, S. J.; Thorne, A. J. *J. Chem. Soc., Chem. Commun.* **1984**, 480-482. (b) Masamune, S.; Hanzawa, Y.; Williams, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 6136-6137. (c) Snow, J. T.; Murakami, S.; Masamune, S.; Williams, D. J. *Tetrahedron Lett.* **1984**, *25*, 4191-4194.

(3) (a) Escudié, J.; Couret, C.; Satgé, J.; Andrianarison, M.; Andriamizaka, J. D. *J. Am. Chem. Soc.* **1985**, *107*, 3378-3379. (b) Glidewell, C.; Hursthouse, M. B.; Lloyd, D.; Lumbard, K. W.; Short, R. L. *J. Chem. Res. Synop.* **1986**, 400-401. Glidewell, C.; Lloyd, D.; Lumbard, K. W.; McKechnie, J. S. *Tetrahedron Lett.* **1987**, *28*, 343-344.

(4) Barton, T. J.; Kline, E. A.; Garvey, P. M. *J. Am. Chem. Soc.* **1973**, *95*, 3078. Rivière, P.; Castel, A.; Satgé, J. *J. Am. Chem. Soc.* **1980**, *102*, 5413-5415.

(5) Wiberg, N.; Kim, Ch. K. *Chem. Ber.* **1986**, *119*, 2966-2979, 2980-2994.

(6) **2**: white crystals, mp 64-65 °C; ^1H NMR (C_6D_6) δ 2.03 (s, 6 H, *p*-Me), 2.15 (d, $^5J_{HF}$ = 1.0 Hz, 12 H, *o*-Me), 4.86 (d, $^3J_{HF}$ = 4.4 Hz, 1 H, CH), 6.63 (s, 4 H, aromatic Mes), 6.80-7.86 (m, 8 H, CR_2); ^{19}F NMR −99 (reference CF_3COOH). Anal. Calcd for $C_{31}H_{31}GeF$: C, 75.19; H, 6.31. Found: C, 75.32; H, 6.45.

(7) **1a** ^1H NMR (C_6D_6) δ 2.06 (s, 6 H, *p*-Me), 2.20 (s, 12 H, *o*-Me), 6.63 (s, 4 H, aromatic Mes), 6.80-7.96 (m, 8 H, CR_2). **1b**: orange crystals, mp 85-86 °C; ^1H NMR (C_6D_6) δ 0.70-1.26 (m, 6 H, OCH_2CH_3), 1.80 (s, 6 H, *p*-Me), 2.30 (s, 12 H, *o*-Me), 2.76-3.43 (m, 4 H, OCH_2CH_3), 6.63 (s, 4 H, aromatic Mes), 6.76-7.90 (m. 8 H, CR_2); mass spectrum (EI), *m/e* (relative intensity) 476 (M, 20), 312 (Mes_2Ge, 100). Anal. Calcd for $C_{35}H_{40}GeO$: C, 76.53; H, 7.34. Found: C, 76.14; H, 7.01. **1c**: orange crystals, mp 74-75 °C; ^1H NMR (C_6D_6) δ 0.60-1.06 (m, 6 H, NCH_2CH_3), 1.96 (s, 6 H, *p*-Me), 2.33 (s, 12 H, *o*-Me), 2.26-2.80 (m, 4 H, NCH_2CH_3), 6.63 (s, 4 H, aromatic Mes), 6.76-7.90 (m, 8 H, CR_2); mass spectrum (EI), *m/e* (relative intensity) 476 (M, 20), 312 (Mes_2Ge, 100). Anal. Calcd for $C_{37}H_{45}GeN$: C, 77.10; H, 7.87. Found: C, 76.88; H, 7.62. **1d**: orange crystals, mp 78-80 °C; ^1H NMR (C_6D_6) δ 1.42-1.69 (m, 4 H, CCH_2), 2.30 (s, 6 H, *p*-Me), 2.63 (s, 12 H, o-Me), 3.51-3.78 (m, 4 H, OCH_2), 6.90 (s, 4 H, aromatic Mes), 7.10-8.15 (m, 8 H, CR_2); mass spectrum (EI), *m/e* (relative intensity) 476 (M, 20), 312 (Mes_2Ge, 100). Anal. Calcd for $C_{35}H_{38}GeO$: C, 76.81; H, 7.00. Found: C, 77.17; H, 6.88.

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