other stabilizers), the yield of the isolated high-quality spherical resin was rather low, too low to allow economic adaptation for use with complex functional co-monomers. However, it was realized that a major contribution to the seeding of aggregation was irregular agitation at the liquid–gas interface, especially when this interface was located too close to one of the oscillating baffles. Accordingly, therefore the two baffle set arrangement was designed, built, and commissioned and the volume of the total liquid phase adjusted to be sufficiently remote from the upper baffle to allow for efficient suspension within the bulk of the liquid and yet to avoid induction of particle aggregation at the surface. Since evaporative losses were also significant with an aqueous phase volume of only 25 mL, the level of liquid was maintained during the reaction by gradual addition of more aqueous phase (5 mL). These simple design and operational changes led to a significant improvement in the yields of the isolated high quality beads, despite the polymerization being performed with only approximately 2 g of comonomers (for example, G4, G7, and G8, Figure 3e–g). The most robust stabilizer used was poly(vinyl alcohol) (PVA, 88% hydrolyzed, M_{wt} = ca. 125000 Aldrich) at 0.75 wt%, with 3.3 wt% of NaCl in the aqueous phase, and with a baffle oscillation frequency of 6 Hz. Xanthan gum was also a useful stabilizer (G5) but a higher oscillation frequency, 8 Hz, was required because of the larger viscosity of this aqueous solution. By using the optimized procedure 2% cross-linked gel-type resin G9 was prepared using functional co-monomer **1**, with a good yield of isolated quality beads of about 1.5 g (ca. 75%; Figure 3h). Resins G1–G9 are all lightly cross-linked (DVB $\leq$ 5 vol%) and have been prepared in the absence of any porogen. Each would therefore be expected to have a gel-type morphology and a correspondingly very low dry-state surface area. This was confirmed for resin G8 whose dry-state surface area is less than 5 $m^2 g^{-1}$. The volume swelling of G4, G7, and G8 with cross-link ratios of 5, 2, and 1%, respectively, in toluene are 3.05, 4.52, and 7.59 $mL g^{-1}$, which is again consistent with the gel-type morphology of these species. Interestingly the corresponding swelling figure for the 2% cross-linked functional resin G9 is 3.37 $mL g^{-1}$, which suggests that the presence of the residues from the functional co-monomer **1** reduces somewhat the ability of toluene to solvate this resin.

In summary, we have developed a robust small-scale experimental procedure for synthesizing both macroporous and gel-type high-quality spherical particulate styrene–divinylbenzene resins in good yield. The methodology is tolerant to the use of functional co-monomers, and now offers the prospect of exploiting complex, structurally well-characterized monomers in suspension polymerization to produce resins for use in high-throughput discovery programs.

Received: June 18, 2002 [Z19552]

[1] *A Practical Guide to Combinatorial Chemistry* (Eds.: A. W. Czarnik, S. H. DeWitt), American Chemical Society, Washington, DC, **1977**.
[2] *Solid-supported Combinatorial and Parallel Synthesis of Small-Molecular-Weight Compound Libraries* (Eds.: D. Obrecht, J. M. Villalgordo), Pergamon, Oxford, **1998**.
[3] P. Sereci, *Solid-Phase, Synthesis and Combinatorial Technologies*, Wiley, Chichester, **2000**.
[4] A. Akelah, D. C. Sherrington, *Chem. Rev.* **1981**, *81*, 557–587.
[5] S. V. Ley, I. R. Baxendale, R. N. Bream, P. S. Jackson, A. G. Leach, D. A. Longbottom, M. Nesi, J. S. Scott, R. I. Storer, S. J. Taylor, *J. Chem. Soc. Perkin 1* **2000**, 3815–4195.
[6] E. A. Grulke in *Encyclopedia of Polymer Science and Engineering, Vol. 16*, 2nd ed (Eds.: M. F. Mark, N. M. Bikales, C. G. Overberger, G. Menges, J. I. Kroschwitz), Wiley, New York, **1989**, p. 443.
[7] D. C. Sherrington in *Polymer-supported Reactions in Organic Synthesis* (Eds.: P. Hodge, D. C. Sherrington), Wiley, Chichester, **1980**, p. 469.
[8] C. R. Brunold, J. C. B. Hunns, M. R. Mackley, J. W. Thompson, *Chem. Eng. Sci.* **1989**, *44*, 1227–1244.
[9] A. W. Dickens, M. R. Mackley, H. R. Williams. *Chem. Eng. Sci.* **1989**, *44*, 1471–1479.
[10] M. R. Mackley, X. Ni, *Chem. Eng. Sci.* **1991**, *46*, 3139–3151.
[11] X. Ni, *J. Chem. Technol. Biotechnol.* **1994**, *59*, 213–221.
[12] X. Ni, H. Jian, A. W. Fitch, *Chem. Eng. Sci.* **2002**, *57*, 267–280.
[13] X. Ni, Y. Zhang, I. Mustafa, *Chem. Eng. Sci.* **1998**, *53*, 2903–2919.
[14] X. Ni, Y. Zhang, I. Mustafa, *Chem. Eng. Sci.* **1999**, *54*, 841–850.
[15] X. Ni, D. C. Bennett, K. C. Symes, B. D. Grey, *J. Appl. Polym. Sci.* **1999**, *76*, 1669–1676.
[16] X. Ni, J. C. Johnstone, K. C. Symes, B. D. Grey, D. C. Bennett, *AIChE J.* **2001**, *47*, 1746–1757.
[17] D. C. Sherrington, *Chem. Commun.* **1998**, 2275–2286.
[18] Monomer **1** was prepared in 91% yield by reaction of 3-bromo-4-(tetrahydropyran-2-yloxy)phenol with 4-chloromethylstyrene using NaH in DMF (4 h, RT). The synthesis of the phenol is reported by R. Kranich, K. Eis, O. Geis, S. Mühle, J. W. Bats, H.-G. Schmalz, *Chem. Eur. J.* **2000**, *6*, 2874–2894.

An Azulene Dimer as a Near-Infrared Quencher**

Wellington Pham, Ralph Weissleder, and Ching-Hsuan Tung*

Highly sensitive, efficient, and quenchable fluorochromes have become essential for acquiring genomic and proteomic data from biological samples.[1] Fluorescent compounds also have many other useful applications in medicine, biotechnology, and biological science.[2,3] Specifically, fluorochromes attached to various ligands have been used for detecting nucleic acid hybridization (molecular beacons),[4,5] in drug discovery,[6] for sensing molecular interactions,[7,8] and for deciphering biological pathways.[9] Typically, molecular DNA beacons[4,5] and enzyme-sensing probes[10] primarily rely on the fluorescence resonance energy transfer (FRET) between

[*] Prof. Dr. C.-H. Tung, Dr. W. Pham, Prof. Dr. R. Weissleder
Center for Molecular Imaging Research
Massachusetts General Hospital
149 13th Street, Room 5406, Charlestown, MA 02129 (USA)
Fax: (+1) 617-726-5708
E-mail: tung@helix.mgh.harvard.edu

[**] We would like to thank Dr. Fred Reynolds for helpful discussions. This research was supported by grants from the NIH (P50 CA86355, R33 CA88365, and T32 CA79443).

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author. It contains detailed information on the synthesis and characterization of the compounds discussed.

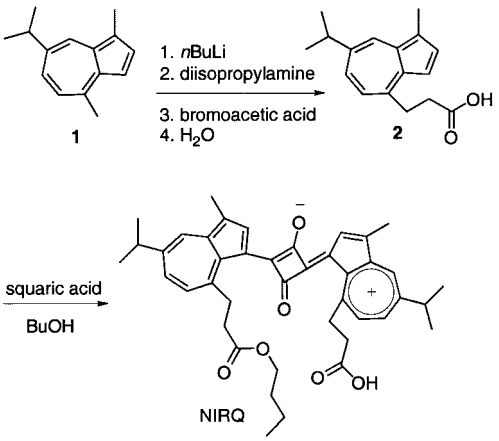
  0044-8249/02/4119-3659 $ 20.00+.50/0

a fluorochrome and a quencher. The vast majority of sensors fluoresce in the visible light range (400–650 nm) and commonly consist of organic fluorochrome or semiconductor materials.[11] The most commonly used quencher to pair with those fluorochromes is 4-(4′-dimethylaminobenzeneazo)benzoic acid (DABCYL), which has a broad absorption spectrum between 360 and 560 nm.

More recently fluorochromes fluorescing in the near-infrared (NIR) range have been developed to either 1) extend the number of color probes available for simultaneous sensing or encoding,[12,13] or 2) to image molecular interactions in vivo.[14–21] Most reported near-infrared fluorochromes contain bi- or tricarbocyanine rings and exhibit fluorescence between 650 and 900 nm.[20–22] One particular challenge, however, is the development of efficient, biocompatible, and conjugatable quenchers, analogous to DABCYL but with absorptions in the near-infrared range. We describe here the synthesis and properties of a generic type of compound that efficiently quenches NIR fluorescence. We furthermore show how derivatives of this model compound can be used for protease sensing in the near-infrared spectrum.

Based on prior observations that certain azulene precursors of antibacterial and anti-inflammatory agents[23] have strong absorption in the 500 to 600-nm range,[24] we hypothesized that it should be feasible to extend the conjugated π system to a linked bicyclic system resulting in higher wavelength absorption. We furthermore postulated that such compounds should be stable, biocompatible, and suitable for either solid-phase or aqueous chemistry, and also have a single attachment moiety for bioconjugation. Another consideration for constructing a bis-azulenyl compound was that the chemical reactivity of azulene has been studied extensively.[25–29] Absorbance of derivatives can be fine-tuned by replacing the substituents on the azulene rings.[24]

The synthesis of a nonfluorogenic near-infrared quencher (NIRQ) started from 1,4-dimethyl-7-isopropylazulene (**1**, Scheme 1). First a carboxylic acid handle was attached to the cycloheptatriene ring prior to condensation. The methyl group at the 4-position was deprotonated by the method developed by Schrott et al.[30] The nucleophilic moiety was generated by *n*BuLi at −40 °C in the presence of diisopropylamine. The subsequent in situ nucleophilic substitution was carried out by reaction with bromoacetic acid to make the carboxylic acid intermediate **2**. Two moles of **2** were then condensed with one mole of squaric acid by refluxing in the presence of BuOH. The dominant product of this reaction was the desired monosubstituted dimer NIRQ. Under the optimized conditions a disubstituted by-product was formed in less than 4% yield and was removed conveniently by flash column chromatography.



Scheme 1. Preparation of the azulene-based NIR quencher NIRQ.

As expected, upon dimerization we observed a significant shift in absorption from 550 nm for **2** to a broad peak around 700–800 nm for NIRQ (Figure 1). The absorption maximum changed little with different solvents; however, the extinction coefficient was solvent dependent (Table 1).

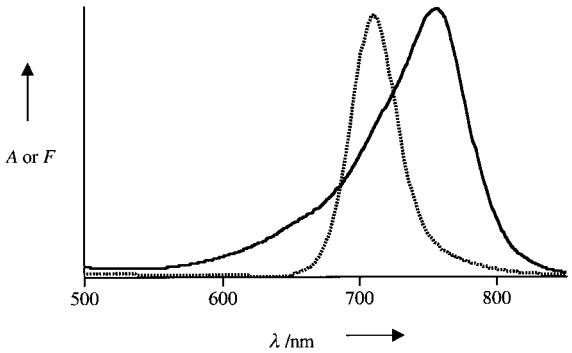


Figure 1. Absorption spectrum of quencher NIRQ (solid line) and emission spectrum of fluorochrome Alexa-680 (dashed line).

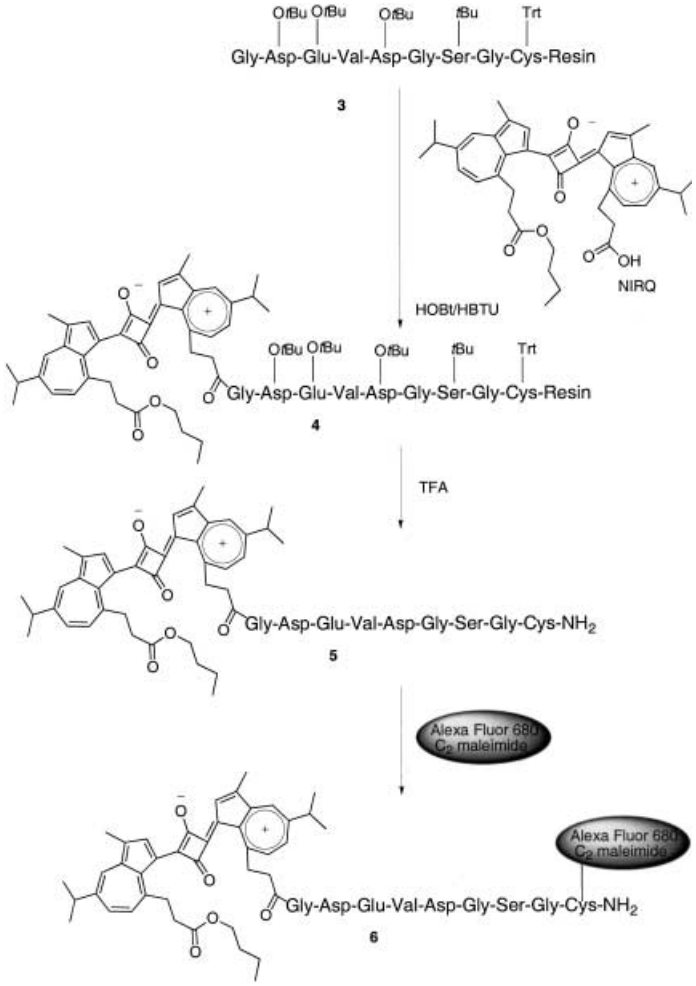
Table 1. Absorption maximum and extinction coefficient of NIRQ.

Solvent	λ_{max} [nm]	ε [$M^{-1}cm^{-1}$]
acetonitrile	760	69000
MeOH	757	82000
DMF	768	50000
DMSO	773	41000

In order to test the utility of the NIR quencher we designed an activatable NIR-fluorescent caspase-3 substrate. NIRQ activated with HBTU/HOBt was directly anchored to the N-terminus of a capase-3 cleavable peptide substrate G*DEVD*GSGC (cleavage site in italics, Scheme 2). The labeled peptide was then cleaved from the solid support and purified by reversed-phase HPLC. NIRQ retained its optical property despite the harsh acid treatment during peptide cleavage and deprotection. Thereafter, a near-infrared fluorochrome, Alexa-680 C_2 maleimide ($\lambda_{ex} = 679$ nm and $\lambda_{em} = 702$ nm), was reacted with the cystein residue of the opposite end of the peptide chain. Alexa-680 was chosen as the fluorescence donor because its emission spectrum overlaps with the absorption spectrum of NIRQ (Figure 1). Two other probes containing Alexa-680, either without the quencher (**7**) or with DABCYL (**8**), were also prepared as controls (Figure 2a).

The HPLC-purified NIRQ probes were tested against caspase-3 with and without an inhibitor of the enzyme in pH 7.2 buffer (20 mM PIPES, 100 mM NaCl, 10 mM DTT, 1 mM EDTA, 0.1% CHAPS, 10% sucrose).[31] Within 180 min the

  0044-8249/02/4119-3660 $ 20.00+.50/0



Scheme 2. Synthesis of NIR-fluorescent caspase-3 substrates. HBTU = *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOBt = 1-hydroxy-1*H*-benzotriazole, TFA = trifluoroacetic acid.

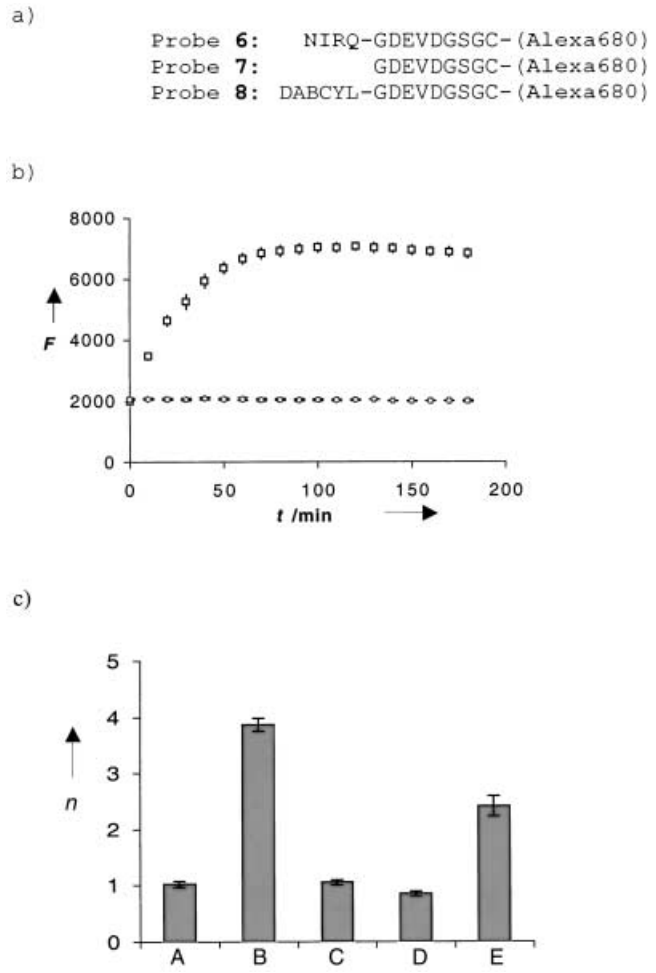


Figure 2. Activation of various NIR probes with caspase-3. a) List of tested probes. b) Fluorescence observed on activation of probe **6** with (square) and without (circle) specific caspase-3 inhibitor. c) Changes (*n*-fold) in the intensity of the NIR fluorescence signal at 180 min for a series of experiments: A) **6**, B) **6** + caspase-3, C) **6** + caspase-3 + inhibitor, D) **7** + caspase-3, E) **8** + caspase-3.

fluorescence signal of **6** had increased about fourfold while no activation was observed when the caspase-3 specific inhibitor was present (Figure 2b). Since DABCYL is not an ideal quencher in this range of wavelengths, the fluorescence signal of probe **8** was only increased by 2.4-fold (Figure 2c, Experiment E). As expected, the fluorescence signal of both negative controls, probe **7** with enzyme (Experiment D) and probe **6** without enzyme (Experiment A), were unchanged. The result indicates that these probes are stable in DTT-containing buffer. An initial study relying on a Stern–Volmer plot suggests that fluorescence quenching is caused by resonance energy transfer.[2]

Overall, our results show that the bis-azulenyl compound developed is an extremely stable and efficient generic NIR-quenching molecule. Its maximum absorption is in the range around 700 to 800 nm, the best window for in vivo imaging.[32] Transformation of the carboxylic acid group into a hydroxysuccinimide ester allows ready conjugation to peptides, oligonucleotides, and potentially other target ligands. The material can be synthesized in 53% yield, and synthesis can be scaled up easily. Additional modifications to side chains and unsaturated spacers are conceivable to further fine-tune absorption maxima and/or shift them further into the near-infrared region. While the current compound was designed to be lipophilic (primarily for solid-phase coupling), additional modification of side chains (e.g. by sulfonation, pegylation, hydroxylation) could render the material hydrophilic. With the extension of fluorochromes into the near-infrared spectrum for broader coverage and in vivo applications this quencher should find widespread use in imaging molecular targets in vivo and in multiwavelength sensing.

Received: March 1, 2002
Revised: May 17, 2002 [Z18797]

[1] S. L. Beaucage, *Curr. Med. Chem.* **2001**, *8*, 1213.
[2] J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, *2nd ed.*, Kluwer, New York, **1999**.
[3] P. Mitchell, *Nat. Biotechnol.* **2001**, *19*, 1013.
[4] D. L. Sokol, X. Zhang, P. Lu, A. M. Gewirtz, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 11538.
[5] S. Tyagi, F. R. Kramer, *Nat. Biotechnol.* **1998**, *14*, 303.
[6] J. L. Harris, P. B. Alper, J. Li, M. Rechsteiner, B. J. Backes, *Chem. Biol.* **2001**, *8*, 1131.
[7] G. Zlokarnik, P. A. Negulescu, T. E. Knapp, L. Mere, N. Burres, L. Feng, M. Whitney, K. Roemer, R. Y. Tsien, *Science* **1998**, *279*, 84.

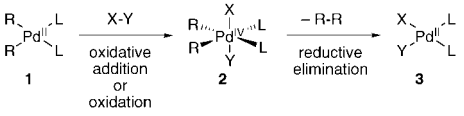
  0044-8249/02/4119-3661 $ 20.00+.50/0

[8] M. Zaccolo, F. De Giorgi, C. Y. Cho, L. Feng, T. Knapp, P. A. Negulescu, S. S. Taylor, R. Y. Tsien, T. Pozzan, *Nat. Cell Biol.* **2000**, *2*, 25.
[9] M. Zaccolo, T. Pozzan, *IUBMB Life* **2000**, *49*, 375.
[10] G. T. Wang, E. Matayoshi, H. J. Huffaker, G. A. Krafft, *Tetrahedron Lett.* **1990**, *31*, 6493.
[11] W. C. Chan, D. J. Maxwell, X. Gao, R. E. Bailey, M. Han, S. Nie, *Curr. Opin. Biotechnol.* **2002**, *13*, 40.
[12] P. E. Klein, R. R. Klein, S. W. Cartinhour, P. E. Ulanch, J. Dong, J. A. Obert, D. T. Morishige, S. D. Schlueter, K. L. Childs, M. Ale, J. E. Mullet, *Genome Res.* **2000**, *10*, 789.
[13] A. K. Tong, Z. Li, G. S. Jones, J. J. Russo, J. Ju, *Nat. Biotechnol.* **2001**, *19*, 756.
[14] R. Weissleder, C.-H. Tung, U. Mahmood, A. Bogdanov, Jr., *Nat. Biotechnol.* **1999**, *17*, 375.
[15] C.-H. Tung, S. Bredow, U. Mahmood, R. Weissleder, *Bioconjugate Chem.* **1999**, *10*, 892.
[16] C. Bremer, S. Bredow, U. Mahmood, R. Weissleder, C.-H. Tung, *Radiology* **2001**, *221*, 523.
[17] C.-H. Tung, U. Mahmood, S. Bredow, R. Weissleder, *Cancer Res.* **2000**, *60*, 4953.
[18] K. Marten, C. Bremer, K. Khazaie, M. Sameni, B. Sloane, C.-H. Tung, R. Weissleder, *Gastroenterology* **2002**, *122*, 406.
[19] C. Bremer, C.-H. Tung, R. Weissleder, *Nat. Med.* **2001**, *7*, 743.
[20] K. Licha, C. Hessenius, A. Becker, P. Henklein, M. Bauer, S. Wisniewski, B. Wiedenmann, W. Semmler, *Bioconjugate Chem.* **2001**, *12*, 44.
[21] S. Achilefu, R. B. Dorshow, J. E. Bugaj, R. Rajagopalan, *Invest. Radiol.* **2000**, *35*, 479.
[22] S. R. Mujumdar, R. B. Mujumdar, C. M. Grant, A. S. Waggoner, *Bioconjugate Chem.* **1996**, *7*, 356.
[23] A. E. Asato, A. Peng, M. Z. Hossain, T. Mirzadegan, J. S. Bertram, *J. Med. Chem.* **1993**, *36*, 3137.
[24] W. Pham, R. Weissleder, C.-H. Tung, *Tetrahedron Lett.* **2002**, *43*, 19.
[25] S. L. Chen, R. Klein, K. Hafner, *Eur. J. Org. Chem.* **1998**, 423.
[26] S. Ito, M. Fujita, N. Morita, T. Asao, *Bull. Chem. Soc. Jpn.* **1995**, *68*, 3611.
[27] S. Ito, H. Kobayashi, S. Kikuchi, N. Morita, T. Asao, *Bull. Chem. Soc. Jpn.* **1996**, *69*, 3225.
[28] S. Ito, N. Morita, T. Asao, *Bull. Chem. Soc. Jpn.* **1999**, *72*, 2543.
[29] T. Tomiyama, M. Yokota, S. Wakabayashi, K. Kosakai, T. Yanagisawa, *J. Med. Chem.* **1993**, *36*, 791.
[30] W. Schrott, P. Neumann, S. Brosius, H. Barzynski, K. D. Schomann, H. Kuppelmaier, US patent 5084592 **1989** [*Chem. Abstr.* **1989**, *111*, 196757].
[31] Abbreviations: CHAPS = 3-[(3-cholamidopropyl)dimethylammonio] propanesulfonic acid, DTT = dithiothreitol, EDTA = ethylenediaminetetraacetic acid, PIPES = piperazine-1,4-bis-(2-ethanesulfonic acid).
[32] R. Weissleder, *Nat. Biotechnol.* **2001**, *19*, 316.

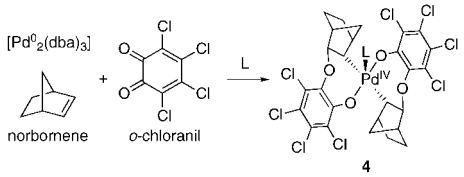
Single-Step Assembly of a C_2-Symmetrical Palladium(IV) Spirocyclic Complex

Yoshihiko Yamamoto,* Takuya Ohno, and Kenji Itoh

Palladium is an extensively studied transition element in organometallic chemistry as a versatile promoter of various stoichiometric and catalytic organic transformations.[1] Although palladium generally prefers low oxidation states 0, +1, and +2, the involvement of organopalladium(IV) intermediates have been claimed in important catalytic processes.[2,3] In contrast to platinum, which has an extensive organometallic chemistry in the +4 oxidation state,[4] organopalladium(IV) complexes had hardly been studied until the first unequivocal trialkylpalladium(IV) complex, [$PdIMe_3$(bpy)], was isolated and characterized by X-ray analysis.[5] Since this report, various polyalkylpalladium(IV) complexes **2** were synthesized from dialkylpalladium(II) precursors **1** by oxidative addition of alkylhalides, or oxidation by H_2O, halogens, dichalcogenides, etc., and most of them are thermally labile with the reductive elimination of an alkane readily occuring at or below room temperature to produce the corresponding palladium(II) fragments **3** [Eq. (1)].[6] In striking contrast, we found that the reaction of a readily available palladium(0) complex, [Pd_2(dba)$_3$ (dba = *trans*,*trans*-dibenzilideneacetone), with tetrachloro-1,2-benzoquinone (*o*-chloranil) and bicyclo[2.2.1]hept-2-ene (norbornene) directly produced a novel spirocyclic palladium(IV) dialkyl complex **4** in high yield [Eq. (2)].



(1)



(2)

Quinones have received considerable attention in coordination chemistry as redox-active, strongly electron-accepting ligands. Various transition metals have been shown to coordinate to *p*-quinones at their localized ring olefinic bonds.[7] On the other hand, *o*-quinones usually form complexes containing semiquinone or hydroquinone chelate ligands with the concomitant oxidation of transition-metal centers.[8] This was the case for palladium. The reaction of the Pd^0–phosphane complex [$Pd(PPh_3)_4$] with *o*-chloranil has already been reported to furnish a palladium(II) catecholate complex [$(Ph_3P)_2Pd(O_2C_6Cl_4)$].[9] Remarkably, the treatment

[*] Dr. Y. Yamamoto, T. Ohno, Prof. Dr. K. Itoh
Department of Molecular Design and Engineering
Graduate School of Engineering
Nagoya University
Chikusa, Nagoya 464-8603 (Japan)
Fax: (+81) 52-789-3205
E-mail: yamamoto@apchem.nagoya-u.ac.jp

  0044-8249/02/4119-3662 $ 20.00+.50/0