by 6-*epi*-dysidiolide **16** is considerably lower than the values recorded for the inhibition of cdc25A (13 μM) and cdc25B (18 μM). Furthermore, the most active compound in this enzyme-assay, ketone **18**, exhibited an IC_{50} value in the high nanomolar range (800 nM) and was 6.4 times more active than **16**. These results indicate that dysidiolide analogues and their derivatives can differentiate selectively between different types of phosphatases and conceivably among the three cdc25 family members. The data also indicate that substantial variation of the precise structural details of the natural product itself is tolerated and leads to inhibitors with significantly enhanced potency. Thus, replacement of the hydroxyethyl bridge between the annelated core ring system and the hydroxybutenolide present in compound **16** by an unsaturated three-carbon unit (see **21**) or introduction of a keto group (see **18** and **19**) leads to more potent cdc25C inhibitors.

The synthetic dysidiolide analogues also displayed considerable and differing biological activity in a cytotoxicity assay[11] of the colon cancer cell line SW480 (Table 1). Four of the eight compounds investigated showed IC_{50} values in the very low micromolar range and pronounced antitumor activity. In this cellular assay, alcohol **17** with a shortened carbon chain was the most active compound, whereas inhibitors **18** and **19** which had shown the lowest IC_{50} values and compounds **22** and **23** in which the alcohol is positioned differently between the hydroxybutenolide and the core structure of dysidiolide were considerably less active. This trend also became apparent when ketones **18** and **19** as well as *epi*-dysidiolide **16** were subjected to cytotoxicity assays of the colon cell line HCT116, the prostate cancer cell line PC3, and the breast cancer cell line MDA-MB231. Ketones **18** and **19** again were substantially less active than *epi*-dysidiolide **16**, which inhibits cell proliferation in all three cases with IC_{50} values in the very low micromolar range (Table 1).

Thus, the data indicate that the small library of natural product analogues already contains potent compounds with significantly different biological activities both in vitro and in vivo. The observation that the order of IC_{50} values determined in an enzyme assay does not necessarily parallel the outcome of cellular assays is not uncommon.

In conclusion, we have demonstrated that the synthesis of natural product derived libraries in long multistep sequences executed on a polymeric support and employing a variety of widely differing synthetic transformations is feasible and that it can deliver potent biologically active compounds with high frequency.

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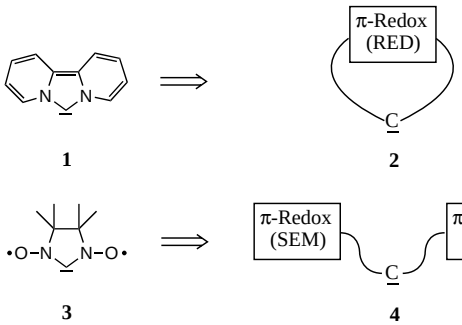
Dinitroxide Carbenes, A New Class of Carbenes with Autoumpolung Character: Preparation in Solution and Stabilization in Transition Metal Complexes**

Robert Weiss* and Norbert Kraut

With the synthesis of **1** we recently reported the prototype of a carbene with autoumpolung character.[1] As the general structure **2** shows, in such a system a singlet carbene center is conjugated with the termini of a two-step π-redox system in the reduced state (RED).[2] The electrons in this reservoir can be used in the sense of a redox umpolung to respond to electronic requirements of the coordination partner at the carbene center.[3] To extend this concept we report here the first synthesis of the dinitroxide carbene **3** in solution and its stabilization in transition metal complexes.



1 **2**

3 **4**

[*] Prof. Dr. R. Weiss, Dipl.-Chem. N. Kraut
Institut für Organische Chemie
Universität Erlangen-Nürnberg
Henkestrasse 42, 91054 Erlangen (Germany)
Fax: (+49) 9131-85-25876
E-mail: robert.weiss@chemie.uni-erlangen.de

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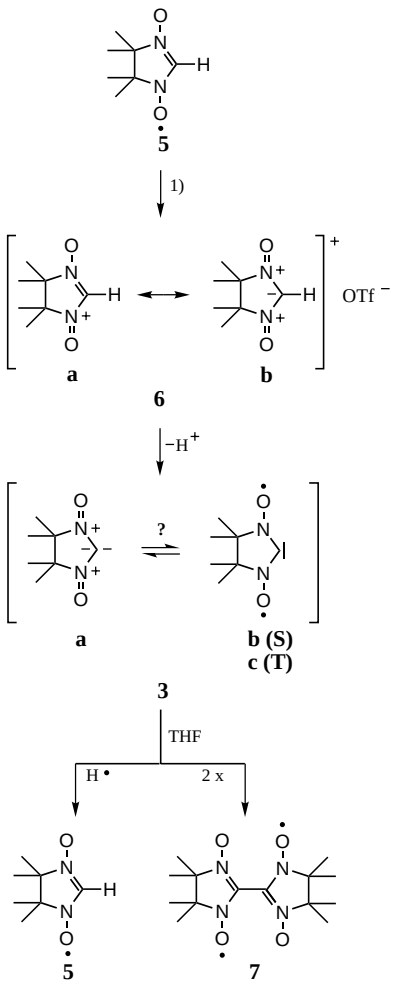
Compound **3** is the first example of a general class of compounds **4**, in which the two substituents at the carbene center can be looked upon as being the termini of two, mutually independent two-step π-redox systems. This electronic configuration should be able to interact with even higher flexibility than **2** with a coordination partner at the carbene center. In **3** the two redox systems exist formally in a semi-oxidized form (SEM form)[4] so that aside from the autoumpolung ability the multiplicity of this compound is also of importance. We have recently reported the synthesis and C2 functionalization of the radical anion of **3**.[5]

In the synthesis of **3** a suitable nitronylnitroxide is initially oxidized to the corresponding nitronylnitrosonium ion and then deprotonated at the central C2 unit. Whereas nitronylnitroxides, as a consequence of their magnetic properties, are of particular current interest as central building blocks in supramolecular materials,[6] little is known about the corresponding nitronylnitrosonium ions.[7a] Only one compound of this type has been unambiguously characterized by X-ray structure analysis.[7b] We have established that nitronylnitroxides may be readily transformed into the corresponding nitronylnitrosonium triflates with Br_2/Me_3SiOTf ($OTf^- =$ trifluoromethanesulfonate) in the ratio of 1:2.[8] With this method we obtained in good yield the highly oxidizing ($E_{1/2} =$ 0.80 V vs. saturated calomel electrode (SCE)) orange nitronylnitrosonium salt **6** from the stable, red-violet radical **5**.[9] According to semi-empirical calculations nitronylnitrosonium ions are best regarded as geminal dinitrosonium-stabilized carbanions ("dinitrosonium methides"). According to this description the resonance structure **b** from Scheme 1 dominates for **6** (charge on C2 (PM3): −0.86). From the calculations the C2 H atom is correspondingly highly positive (charge (PM3): +0.26); the reasons for this lie presumably in the strong −I effect of the neighboring nitrosonium functions and in ring-strain effects.[3] In agreement with this interpretation the resonance signal for this proton is at $\delta =$ 9.52 in the ^{1}H NMR spectrum.

As expected, **6** may be readily deprotonated: if KO*t*Bu is added to a solution of **6** in THF the red-violet biradical **7**, previously synthesized by Ullman et al. by an alternative route[10] is obtained in high yield (86%). Remarkably, with inverse addition (i.e. the addition of **6** to a solution of KO*t*Bu) 37% of **5** is obtained in addition to 34% of **7**. The experimental results can be interpreted as follows:

1) By deprotonation of **6** the dinitrosoniumdiide **3a** is first formed from which the dinitroxide carbene is generated by autoumpolung, either in the form of the singlet biradicaloid **3b** and/or the corresponding π-triplet carbene **3c**.[11] The determination of the relative energies of the electronic configuration of **3**—only possible for the gas phase—is a difficult theoretical problem which is currently occupying us.[12]
2) The subsequent reactions, that is, the dimerization of **3** to **7** and the formation of **5** from **3** by H abstraction from a solvent molecule,[13] strongly suggests the presence of an open-shell system in which the central C atom exhibits partial radical character. Because of spin delocalization in the π-system this takes place only with **3b**/**3c**, not, however, with **3a**.



Scheme 1. Preparation and deprotonation of **6**; 1) Br_2:TMSOTf 1:2. S = singlet, T = triplet.

3) The dependency of the observed product distribution on the mode of addition of the reactants in the deprotonation of **6** suggests that **7** is not only accessible by direct dimerization of **3b**/**3c**, but—energetically more favorably—also by recombination of the carbenes **3a**–**c** with the precursor **6** and subsequent deprotonation. This "proton-catalyzed" dimerization has also been observed with Arduengo carbenes.[14] With inverse addition (i.e. with base already present) **3** finds only a small amount of the precursor cation as reaction partner present so that H abstraction from the solvent can compete with the (uncatalyzed) dimerization.

Since the dinitroxide carbenes **3a**–**c** have a single n electron pair on the central C atom they should also be able to act as σ-electron donor systems towards ML_n units (where M = metal center, L_n = ligand set). Dependent upon the electronic nature of the respective ML_n fragment basically two electronically complementary classes of carbene complexes can be imagined using the autoumpolung concept (see Scheme 2). In **8** the carbene ligand acts as π-electron donor towards the ML_n unit (Schrock-type carbene complex), in **9**, however, as a π-electron acceptor (Fischer-type carbene complex). In the latter case, in addition to a singlet biradicaloid structure **9S**, a novel triplet carbene complex **9T** is also conceivable. The

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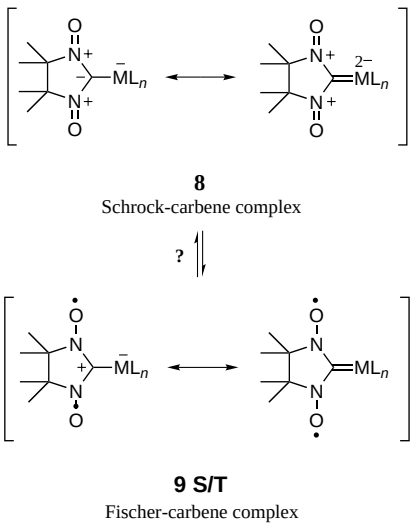
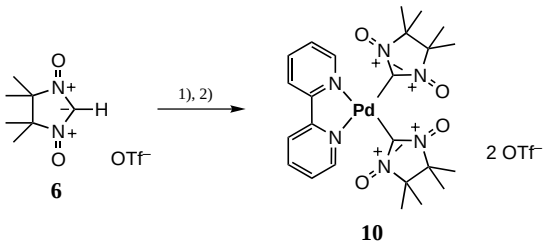


Scheme 2. Possibilities for autoumpolung in the transition metal complexes of **3**.

electronic properties of ML_n (oxidation state of M, type, number, and spatial arrangement of L) may well decide which of these electronic configurations is realized. Equilibria between the autoumpolung isomers **8** and **9S/9T** can also be envisaged. Semi-empirical calculations (PM3) with different ML_n units confirm this assumption at least qualitatively.

The synthesis of transition metal complexes of **3** by direct reaction of transition metal compounds with the dinitroxide carbene formed in situ failed due to the transient nature of the carbene. However, the synthesis succeeded by S_E reactions at the electron-rich C2 center of **6**. In this way we obtained the first dinitroxide carbene complex **10** in good yield (85%) by the reaction of **6** with palladium(II) acetate/2,2′-bipyridine in acetonitrile under mild conditions (Scheme 3, see also the Experimental Section and ref. [15]).



Scheme 3. Preparation of **10**: 1) $Pd(OAc)_2$, CH_3CN, reflux, 12 h; 2) 2,2′-bipyridine, THF, RT, 1 h.

Figure 1 shows the crystal structure of the cation of **10**.[16] Owing to their electronic character the carbene ligands, arranged almost vertically to the coordination plane of the palladium, should be regarded as nitronylnitrosonium systems. This assignment comes from spectroscopic measurements as well as the structural parameters of the five-membered ring in **10**, which resemble closely those of a previously described C2-phenylated nitronylnitrosonium compound. In particular, the N–O bond lengths in **10** at about 124 pm resemble those of this reference compound (ca. 122.5 pm).[7b] Moreover, **10** is

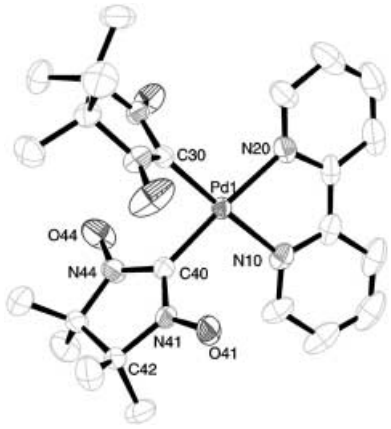


Figure 1. Molecular structure of the cation of **10**. Selected bond lengths [Å] and angles [°]: Pd1-N20 2.066(4), Pd1-C40 1.977(4), C40-N41 1.341(6), C40-N44 1.351(6), N44-O44 1.231(5), N41-O41 1.243(4); N20-Pd1-N10 79.8(16), C40-Pd1-C30 85.44(17), N44-C40-N41 104.6(4), C40-N41-C42 117.0(3), C40-N41-O41 122.8(3).

diamagnetic. Clearly in this complex the reversed-polarity singlet structure **3a** of a dinitroxide carbene is stabilized in accordance with the general structural type **8** (see also Scheme 2). The variable electronic nature of the dinitroxide carbenes predisposes this ligand type as a novel, electronically flexible coordination partner both for metallic and non-metallic centers.

In a similar manner, however without 2,2′-bipyridine, 2:1 carbene complexes of Hg^{II}, Ag^{I}, and Au^{I} could be obtained and characterized in good yield as stable, orange salts. These salts, similar to **10**, are highly oxidizing and, with respect to their electronic configuration, also appear to correspond to **10**. On-going investigations are concerned with the controlled reduction of these diamagnetic systems to the respective homoleptic nitronylnitroxide complexes, a previously unknown class of compounds.

Experimental Section

6: A solution of Br_2 (0.06 mL, 1.22 mmol) and Me_3SiOTf (0.48 mL, 2.68 mmol) in dichloromethane (5 mL) was added dropwise to a solution of **5** (383 mg, 2.44 mmol) in dichloromethane (10 mL) at −40 °C. After concentration of the solution diethyl ether (60 mL) was added. The orange precipitate was collected by filtration, washed with a little diethyl ether, and dried under high vacuum. Yield: 516 mg (69.1%); correct C,H,N,S analysis; ^{1}H NMR (400 MHz, CD_3NO_2, 32 °C): $\delta = 1.88$ (s, 12H; CH_3), 9.52 (s, 1H; CH); ^{13}C NMR (100 MHz, CD_3NO_2, 32 °C): $\delta = 24.6$ (s; CH_3), 93.2 (s; C), 122.2 (q, $^1J(C,F) = -318.9$ Hz; CF_3), 153.5 (s; CH); IR (KBr): $\tilde{\nu}$ = 3166m, 3060w, 1756w, 1627w, 1490s, 1410w, 1391m, 1381m, 1272s, 1229s, 1160s, 1034s, 813w, 757w, 640s, 575m, 540m, 519m cm^{-1}; EI-MS (70eV): *m*/*z* (%): 157 (40) [M^+ − OTf], 83 (55) [$C_6H_{11}^+$], 69 (100) [CF_3^+]; FAB-MS (NBA): *m*/*z* (%):463 (100) [$2M^+$ − OTf].

10: A solution of **6** (331 mg, 1.08 mmol) and $Pd(OAc)_2$ (121 mg, 0.54 mmol) in acetonitrile (20 mL) was heated under reflux for 12 h. The solution was then evaporated to a few mL and treated with diethyl ether. The orange solid was collected by filtration and washed with diethyl ether. The solid was then suspended in THF (10 mL), treated with 2,2′-bipyridine (84 mg, 0,54 mmol), and stirred at room temperature for 1 h. After the addition of diethyl ether (30 mL) the pale pink precipitate was collected by filtration, washed with a little diethyl ether, and dried under high vacuum. Yield: 401 mg (85.0%); correct C,H,N,S analysis; ^{1}H NMR (400 MHz, CD_3NO_2, −20 °C): $\delta = 1.74$ (s, 12H; CH_3), 1.86 (s, 12H; CH_3), 7.68 (m, 4H; CH), 8.45 (m, 4H; CH); ^{13}C NMR (100 MHz, CD_3NO_2, 20 °C): $\delta = 25.2$ (brs; CH_3), 92.2 (brs; C), 122.4 (q, $^1J(C,F) = -319.8$ Hz; CF_3), 125.7 (s; CH), 129.6 (s;

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CH), 144.0 (s; CH), 152.8 (s; CH), 157.5 (s; CH), 212.2 (s; CPd); IR (KBr): $\tilde{\nu}$ = 1637mb, 1450m, 1422s, 1384m, 1263s, 1225w, 1158m, 1033s, 860w, 772m, 637s, 549m, 519m cm^{-1}; FAB-MS (NBA): *m*/*z* (%): 727 (10) [M^+ – OTf+4H], 574 (90) [$M^+ - 2$OTf+H], 262 (100) [bipyPd$^+$].

Deprotonation of **6**: 1) A solution of **6** (193 mg, 0.63 mmol) in THF (5 mL) was added dropwise to a solution of KO*t*Bu (71 mg, 0.63 mmol) in THF (10 mL) at −78 °C. The solution was then evaporated to dryness and the residue was separated by chromatography on silica gel (CH_2Cl_2:ethyl acetate 4:1). Yields: 34 mg **7** (34%), 37 mg **5** (37%). 2) KO*t*Bu (58 mg, 0.52 mmol) was added slowly to a solution of **6** (160 mg, 0.52 mmol) in THF (10 mL) at −78°C. The solution was then evaporated to dryness and the residue separated by chromatography on silica gel (CH_2Cl_2:ethyl acetate 4:1). Yield: **7** (70 mg, 86.2%). Compounds **5** and **7** have been described in the literature and could be unambiguously identified by spectroscopic data (EI-MS, ESR, and UV measurements).[9, 10]

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[11] The conventional triplet compound with σ – π configuration can be ignored here because the single n electron pair on C2 is not only highly stabilized by ring-strain effects but also inductively and hyperconjugatively by the NO group.[12]

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[13] In a control experiment it was demonstrated that **5** is not formed simply by the reduction of **6** by the base: if the corresponding C2-deuterated salt is used instead of **6**, in addition to the dimer **7**, the radical **5** is obtained by inverse base addition. (MS analysis).

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[16] Crystal structure data for **10**: $C_{26}H_{32}F_6N_6O_{10}PdS_2$, M_r = 873.10, monclinic, space group *C*2/*c*, *a* = 40.219(8), *b* = 7.5618(15), *c* = 23.737(5) Å, β = 108.16(3)°, *V* = 6859(2) Å^3, *Z* = 8, ρ_{calcd} = 1.691 Mg m^{-3}, crystal dimensions: 0.20 × 0.20 × 0.10 mm, $Mo_{K\alpha}$ radiation (λ = 0.71073 Å), *T* = 173(2) K, *F*(000) = 3536; the data were collected with a Nonius Kappa CCD Area Detector in the range of 2.13° < θ < 25.02°(11 453 reflections were measured, of which 6004 were independent and 4783 with $I > 2\sigma(I)$). The absorption correction was carried out with the program Scalepack (Z. Otwinowski, W. Minor, *Methods Enzymol.* **1997**, *276*, 307). The structure was solved by direct methods (SHELXS 97); structure refinement against F^2 (SHELXL 97). The hydrogen atoms were fixed at idealized positions (riding-model). Refinement *R* values: *R*1 = 0.0477 for $I > 2\sigma(I)$, *wR*2 = 0.1420 for all data. Residual electron density 1.665/ – 0.890 e Å^{-3}. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-164924. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

Visualization of Molecular Recognition Events on Microstructured Lipid-Membrane Compartments by In Situ Scanning Force Microscopy**

Stephanie Künneke and Andreas Janshoff*

Cell membranes are the most important interfaces in biological systems. A variety of reactions, such as molecular biorecognition of soluble proteins by receptor lipids, take place at this unique surface. Artificial solid-supported lipid bilayers are well suited model systems that allow the study of certain reactions under defined quasi-native conditions. Additionally, they enable the assembly of biosensor surfaces with ordered receptor molecules in a densely packed matrix efficiently suppressing nonspecific adsorption of biomolecules.[1]

Arrays of microstructured individually addressable lipid-membrane compartments meet the requirements given by analytical applications, combinatorial libraries, or pharmacological screening, which create a demand for high-throughput analysis and highly comparable and reliable results.[2] A structured surface that enables a direct comparability of biomaterials and a parallel analysis of protein – receptor interactions on different membranes or receptors under identical ambient conditions is well suited if not required especially for scanning force microscopy of material contrasts and visualization of lipid – protein interactions. Because lipid membranes rarely show topographical features, a microstructured lipid bilayer with defined boundaries becomes necessary to compare contrasts qualitatively and to enable parallel control measurements. While the structuring of proteins and nucleic acids is relatively straightforward, the preparation and structuring of lipid bilayers requires handling in aqueous solution.

Methods based on contact printing for structuring lipid membranes are limited to a very small number of different lipid compositions, moreover the individual addressibility of the segments is cumbersome.[3]

We have developed a general microstructuring procedure for generating numerous selectively functionalized lipid-

[*] Prof. Dr. A. Janshoff, S. Künneke
Johannes Gutenberg Universität Mainz
Institut für Physikalische Chemie
Jakob Welder Weg 11, 55128 Mainz (Germany)
Fax: (+49) 6131-39-22970
E-mail: janshoff@mail.uni-mainz.de

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