a Spex Fluorolog II spectrofluorimeter. Details of the correction of emission spectra and the determination of luminescence quantum yields were as reported previously.<sup>[14]</sup> Luminescence lifetimes on the nanosecond timescale were determined with an IBH single photon counting apparatus ( $\lambda_{exc}$  = 337 nm) or a single-shot Nd:YAG laser apparatus ( $\lambda_{exc}$  = 532 nm). Transient absorption spectra and lifetimes with picosecond and nanosecond resolution were obtained with two pump and probe systems based on Nd:YAG laser; excitation with the second (532 nm) or third harmonic (355 nm) was used. Details of this time-resolved spectroscopy equipment were reported earlier.<sup>[14]</sup> Experimental uncertainties were estimated to be ±8% for lifetime determination, ±20% for quantum yields, and ±3 nm for emission and absorption peaks.

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- [1] G. R. Newkome, C. N. Moorefield, F. Vögtle, *Dendritic Molecules: Concepts, Synthesis, Perspectives*, VCH, Weinheim, **1996**.
- H.-B. Mekelburger, W. Jaworek, F. Vögtle, Angew. Chem. 1992, 104, 1609; Angew. Chem. Int. Ed. Engl. 1992, 31, 1571; J. Issberner, R. Moors, F. Vögtle, Angew. Chem. 1994, 106, 2507; Angew. Chem. Int. Ed. Engl. 1994, 33, 2413.
- [3] F. Zeng, S. C. Zimmerman, Chem. Rev. 1997, 97, 1681; D. K. Smith, F. Diederich, Chem. Eur. J. 1998, 4, 1353; A. Archut, F. Vögtle, Chem. Soc. Rev. 1998, 27, 233; H. Frey, Angew. Chem. 1998, 110, 2313; Angew. Chem. Int. Ed. 1998, 37, 2193; M. Fischer, F. Vögtle, Angew. Chem. 1999, 111, 934; Angew. Chem. Int. Ed. 1999, 38, 884; M. A. Hearshaw, J. R. Moss, Chem. Commun. 1999, 1.
- [4] S. Campagna, G. Denti, S. Serroni, A. Juris, M. Venturi, V. Ricevuto, V. Balzani, *Chem. Eur. J.* **1995**, *1*, 211; A. Bar-Haim, J. Klafter, R. Kopelman, *J. Am. Chem. Soc.* **1997**, *119*, 6197; S. Serroni, A. Juris, M. Venturi, S. Campagna, I. Resino Resino, G. Denti, A. Credi, V. Balzani, *J. Mater. Chem.* **1997**, *7*, 1227; D.-L. Jiang, T. Aida, *Nature* **1997**, *388*, 454; R. Kopelman, M. Shortreed, Z.-Y. Shi, W. Tan, Z. Xu, J. S. Moore, A. Bar-Haim, J. Klafter, *J. Phys. Chem. B* **1997**, *101*, 6318; V. Balzani, S. Campagna, G. Denti, A. Juris, S. Serroni, M. Venturi, *Acc. Chem. Res.* **1998**, *31*, 26.
- [5] Recent examples: C. J. Hawker, K. L. Wooley, J. M. J. Fréchet, J. Am. Chem. Soc. 1993, 115, 7638; P. J. Dandliker, F. Diederich, M. Gross, C. B. Knobler, A. Louati, E. M. Sanford, Angew. Chem. 1994, 106, 1821; Angew. Chem. Int. Ed. Engl. 1994, 33, 1739; P. J. Dandliker, F. Diederich, J.-P. Gisselbrecht, A. Louati, M. Gross, Angew. Chem. 1995, 107, 2906; Angew. Chem. Int. Ed. Engl. 1995, 34, 2725; H.-F. Chow, I. Y.-K. Chan, D. T. W. Chan, R. W. M. Kwok, Chem. Eur. J. 1996. 2, 1085; E. C. Constable, P. Haverson, M. Oberholzer, Chem. Commun. 1996, 1821; J. P. Collman, L. Fu, A. Zingg, F. Diederich, Chem. Commun. 1997, 193; J. Issberg, F. Vögtle, L. De Cola, V. Balzani, Chem. Eur. J. 1997, 3, 706; G. R. Newkome, E. He, J. Mater. Chem. 1997, 7, 1237; X. Camps, H. Schönberger, A. Hirsch, Chem. Eur. J. 1997, 3, 561; I. Jestin, E. Levillain, J. Roncali, Chem. Commun. 1998, 2655; A. P. H. J. Schenning, R. E. Martin, M. Ito, F. Diederich, C. Boudon, J.-P. Gisselbrecht, M. Gross, Chem. Commun. 1998, 1013; P. R. L. Malenfant, L. Groenendaal, J. M. J. Fréchet, J. Am. Chem. Soc. 1998, 120, 10990; F. Cardullo, F. Diederich, E. Echegoyen, T. Habicher, N. Jayaraman, R. M. Leblanc, J. F. Stoddart, S. Wang, Langmuir 1998, 14, 1955; P. Bhyrappa, G. Vaijayanthimala, K. S. Suslick, J. Am. Chem. Soc. 1999, 121, 262; M. Plevoets, F. Vögtle, L. De Cola, V. Balzani, New J. Chem. 1999, 63; M. Enomoto, T. Aida, J. Am. Chem. Soc. 1999, 121, 874.
- [6] J.-F. Nierengarten, D. Felder, J.-F. Nicoud, *Tetrahedron Lett.* 1999, 40, 273.
- [7] a) J.-F. Nierengarten, T. Habicher, R. Kessinger, F. Cardullo, F. Diederich, V. Gramlich, J.-P. Gisselbrecht, C. Boudon, M. Gross, *Helv. Chim. Acta* 1997, *80*, 2238; b) J.-F. Nierengarten, C. Schall, J.-F. Nicoud, *Angew. Chem.* 1998, *110*, 2037; *Angew. Chem. Int. Ed.* 1998, *37*, 1934; c) R. Kessinger, M. Gomez-Lopez, C. Boudon, J.-P.

Gisselbrecht, M. Gross, L. Echegoyen, F. Diederich, J. Am. Chem. Soc. 1998, 120, 8545.

- [8] The decrease in amplitude of redox processes in bulky systems is a complex process that is difficult to rationalize; for a straightforward discussion, see F. A. Armstrong, H. A. O. Hill, N. J. Walton, Acc. Chem. Res. 1988, 21, 407.
- [9] The accessible surface of the central core to the solvent molecules in the **CuG2** dendrimer was calculated with the MSEED program.<sup>[10]</sup> First we calculated the area of exposure to the solvent for **CuG0** and **G1CO<sub>2</sub>/Bu**, which amounted to 620 Å<sup>2</sup> and 840 Å<sup>2</sup>, respectively. Since for **CuG2** the surface available to the solvent is 6710 Å<sup>2</sup>, the exposure of the central core amounts, at most, to about 10%. For **CuG3** this can assumed to be less than 5%. If we consider that in the apolar CH<sub>2</sub>Cl<sub>2</sub> medium the counterion is likely to be in tight vicinity to the [Cu(phen)<sub>2</sub>]<sup>+</sup> central core, we can conclude that the interior of the dendrimer is virtually inaccessible to external contact.
- [10] G. Perrot, B. Cheng, K. D. Gibson, J. Vila, K. A. Palmer, A. Nayeem, B. Maigret, H. A. Sheraga, J. Comput. Chem. 1992, 13, 1.
- [11] C. C. Phifer, D. R. McMillin, Inorg. Chem. 1986, 25, 1329.
- [12] F. Vögtle, I. Lüer, V. Balzani, N. Armaroli, Angew. Chem. 1991, 30, 1367; Angew. Chem. Int. Ed. Engl. 1991, 30, 1333.
- [13] N. Armaroli, F. Diederich, C. O. Dietrich-Buchecker, L. Flamigni, G. Marconi, J.-F. Nierengarten, J.-P. Sauvage, *Chem. Eur. J.* 1998, 4, 406.
- [14] N. Armaroli, F. Diederich, L. Echegoyen, T. Habicher, L. Flamigni, G. Marconi, J.-F. Nierengarten, New J. Chem. 1999, 77.
- [15] A. D. Bacon, M. C. Zerner, Theor. Chim. Acta 1979, 53, 21.
- [16] Phosphorescence from  $C_{60}$  and its derivatives has rarely been reported and only in matrices below 5 K or at 77 K in presence of solvents that contain heavy atoms.
- [17] For the calculation of the thermodynamic driving force for electronic excited states from spectroscopic and/or electrochemical parameters, see V. Balzani, F. Scandola, *Supramolecular Photochemistry*, Ellis Horwood, Chichester, **1991**, p. 44.
- [18] For example, see a) G. L. Gaines III, M. P. O'Neil, W. A. Svec, M. P. Niemeczyk, M. R. Wasielewski, *J. Am. Chem. Soc.* **1991**, *113*, 719; b) F. Scandola, R. Argazzi, C. A. Bignozzi, M. T. Indelli, *J. Photochem. Photobiol. A* **1994**, *82*, 191.

## 2-Phenylquinoline – Carbohydrate Hybrids: Molecular Design, Chemical Synthesis, and Evaluation of a New Family of Light-Activatable DNA-Cleaving Agents\*\*

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The development of photochemical DNA-cleaving agents, which selectively cleave DNA by irradiation with light with a specific wavelength under mild conditions and without any additives such as metals and reducing agents, is very interest-

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## COMMUNICATIONS

ing from both chemical and biological standpoints and offers considerable potential in medicine.<sup>[1]</sup> Here, we discuss the molecular design, chemical synthesis, DNA-photocleaving properties, and cytotoxicity of two novel and artificial light-activatable DNA-cleaving agents, namely, the 2-phenylquino-line – carbohydrate hybrids **1** and **2**.



In our approach to create novel DNA-cleaving molecules, we designed artificial intercalator-carbohydrate hybrid systems,<sup>[2–4]</sup> because many clinically useful antitumor antibiotics such as anthracyclines<sup>[5]</sup> and aureolic acids,<sup>[6]</sup> which interact with DNA, were commonly found to contain both aromatic and carbohydrate domains. Since Denny et al.<sup>[7]</sup> demonstrated the efficacy of 2+1 unfused tricyclic aromatic systems such as phenylquinolines as "minimal intercalators", 2-phenylquinoline<sup>[8, 9]</sup> was selected as the DNA intercalator. The conjugated C=N bond in the 2-phenylquinoline unit was also expected to generate the photoexcited  ${}^{\scriptscriptstyle 3}\!(n\!\rightarrow\!\pi^*)$  state upon photoirradiation, which may have a radical character and could be capable of cleaving DNA. On the other hand, certain 2,6-dideoxy amino sugars seemed appropriate as the carbohydrate source, since they are DNA groove binders in some DNA-binding antitumor antibiotics<sup>[10]</sup> and our previously reported artificial DNA-interactive intercalator-carbohydate molecules.<sup>[4]</sup> Therefore, we designed novel, artificial intercalator-carbohydrate hybrids that consist of 2-phenylquinoline and a 2,6dideoxy amino sugar, which are connected by an ethylene glycol linker.

The designed 2-phenylquinoline – carbohydrate hybrids **1** and **2** were synthesized by a short reaction sequence (Scheme 1). Thus, the glycosidation of the phenylthio sugar  $\mathbf{3}^{[11]}$  (1.0 equiv) with ethylene glycol (**4**, 5.0 equiv) using *N*-



Scheme 1. Synthesis of **1** and **2**. a) NBS (1.5 equiv with respect to **3**), 4-Å molecular sieves, MeCN, 0°C, 1 h, 79%, **5**:6 = 1.4:1; b) H<sub>2</sub>, cat. Pd/C, 35% HCHO (aq). MeOH, 25 °C, 18 h, 81%; c) **9**, DCC (1.0 equiv), cat. 4-DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 0°C, 4.5 h, 93% for **10**, 90% for **11**; d) HF/Py, Py, 25 °C, 15 h, 99% for **1**, 94% for **2**. TBS = *tert*-butyldimethylsilyl.

bromosuccinimide (NBS)<sup>[12]</sup> in MeCN gave a mixture of the  $\alpha$ glycoside **5** and the  $\beta$ -glycoside **6** in 79% yield in a ratio of 1.4:1. The azide groups in **5** and **6** were next converted into *N*,*N*-dimethylamino groups by treatment with 35% HCHO (aq) and a catalytic amount of Pd/C in MeOH under a hydrogen atmosphere to afford **7** and **8** in 81% yield. After their separation by column chromatography, **7** (1.0 equiv) and **8** (1.0 equiv) were esterified with 2-phenylquinoline-4-carboxylic acid (**9**, 1.5 equiv) by using *N*,*N*-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (4-DMAP) in CH<sub>2</sub>Cl<sub>2</sub> to give the hybrids **10** and **11** in 93 and 90% yields, respectively. Finally, removal of the silyl groups in **10** and **11** with HF/pyridine (Py) furnished the desired 2-phenylquinoline – carbohydrate hybrids **1** and **2**, respectively, in high yields.

The photoinduced DNA-cleaving activities of the hybrids **1** and **2** along with the components of these hybrids, 12-15,<sup>[11]</sup> were assayed with supercoiled  $\Phi X174$  DNA. As apparent



from Figure 1, the 2-phenylquinoline – carbohydrate hybrids 1 (500  $\mu$ M) and 2 (500  $\mu$ M) caused significant cleavage of DNA, leading to small fragments upon photoirradiation with long-wavelength UV light (365 nm), while 12–15 did not show



Figure 1. Photocleavage of supercoiled  $\Phi$ X174DNA.  $\Phi$ X174DNA (50µm per base pair) was incubated with various compounds in 20% acetonitrile in Tris-HCl buffer (Tris = tris(hydroxymethyl)aminomethane, pH 7.5, 50mM) at 25°C for 1 h under UV irradiation (365 nm, 15 W) from a lamp placed 10 cm from the mixture, and analyzed by gel electrophoresis (0.9% agarose gel, ethidium bromide stain). Lane 1: DNA alone; lane 2: DNA with irradiation; lanes 3– 8: compounds **1**, **2**, **12**, **13**, **14**, and **15** (500µM), respectively. Form I = covalently closed supercoiled DNA, Form II = open circular DNA, and Form III = linear DNA.

DNA-cleaving activity under the same conditions. These results clearly indicate the importance of the hybrid structure for DNA cleaving. These results also strongly suggest that the 2,6-dideoxy amino sugar works as the DNA groove binder and significantly enhances the intercalating ability of the 2-phenylquinoline. In the absence of light no DNA cleavage by **1** and **2** was observed. Furthermore, the DNA-cleaving ability of the  $\beta$ -anomer hybrid **2** was found to be stronger than that of the  $\alpha$ -anomer hybrid **1** (Figure 2). This result demon-



Figure 2. Photocleavage of supercoiled  $\Phi$ X174DNA.  $\Phi$ X174DNA (50 µm per base pair) was incubated with the hybrid in 20% acetonitrile in Tris – HCl buffer (pH 7.5, 50 mM) at 25 °C for 1 h under UV irradiation (365 nm, 15 W) from a lamp placed 10 cm from the mixture, and analyzed by gel electrophoresis (0.9% agarose gel, ethidium bromide stain). a) Lane 1: DNA alone; lane 2: DNA with irradiation; lane 3: DNA + 1 without irradiation; lanes 4–9: 1 (1000), 1 (500), 1 (300), 1 (100), 1 (30), and 1 (10 µm), respectively. b) Lane 1: DNA alone; lane 2: DNA with irradiation; lane 3: DNA + 2 without irradiation; lanes 4–9: 2 (1000), 2 (500), 2 (300), 2 (100), 2 (30), and 2 (10 µm), respectively.

strates that the DNA-cleaving activity is definitely dependent on the configuration of the sugar moiety in the hybrid. Since the DNA-cleaving activity of **1** and **2** significantly decreased in the presence of a radical scavenger, dimethyl sulfoxide, the DNA cleavage must arise from the photoexcited 2-phenylquinoline radical. The DNA-cleaving site specificity of the hybrids **1** and **2** was also analyzed according to the Sanger protocol.<sup>[13]</sup> The results in Figure 3 clearly show the identical high guanine selectivity.

The cytotoxicity of the DNA-cleaving hybrids **1** and **2** was next examined using HeLa S3 cells exposed to each agent for 72 h with or without 1 h of photoirradiation.<sup>[14]</sup> The IC<sub>50</sub> values of **1** and **2** without photoirradiation were 12 and 18 $\mu$ M, respectively, and those with photoirradiation were 0.44 and 0.25  $\mu$ M, respectively. These results indicate that the cytotoxic



Figure 3. Autoradiogram of slab gel electrophoresis with 12% polyacrylamide/8M urea for sequence analysis. The 5'-end-labeled M13mp18 DNA was cleaved by the hybrids at pH 7.5 and  $25 \,^{\circ}$ C for 1 h under UV irradiation (365 nm, 15 W) from a lamp placed 10 cm from the mixture (bases 49–105 are shown). Lanes A, G, C, and T: Sanger A, G, C, and T reactions, respectively; lanes 1 and 2: 1 and 2 (1000 µM), respectively. activities of **1** and **2** with photoirradiation were much higher than those without, and correlated with their capacity to cleave DNA. Furthermore, we found that when the HeLaS3 cells were exposed to  $1 \mu M$  of hybrid **1** or **2** without photoirradiation, practically all of the cells survived, while similar treatment combined with photoirradiation wiped out the cells. These results clearly show that the DNA-cleaving activity induced by photoirradiation significantly affects the cytotoxicity of the hybrids, and the life of the cancer cells can be controlled by treatment with an appropriate amount of the 2-phenylquinoline – carbohydrate hybrid with or without the photoirradiation.

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- [1] B. Armitage, Chem. Rev. 1998, 98, 1171-1200.
- [2] a) D. J. Mincher, G. Shaw, E. D. Clercq, J. Chem. Soc. Perkin Trans. 1
   1983, 613–618; b) D. J. Mincher, G. Shaw, J. Chem. Soc. Perkin Trans. 1
   1984, 1279–1282.
- [3] a) D. B. Berkowitz, S. J. Danishefsky, G. K. Schulte, J. Am. Chem. Soc. 1992, 114, 4518-4529; b) C. J. Roche, D. Berkowitz, G. A. Sulikowski, S. J. Danishefsky, D. M. Crothers, Biochemistry 1994, 33, 936-942; c) K. M. Depew, S. M. Zeman, S. H. Boyer, D. J. Denhart, N. Ikemoto, S. J. Danishefsky, D. M. Crothers, Angew. Chem. 1996, 108, 2972-2975; Angew. Chem. Int. Ed. Engl. 1996, 35, 2797-2801.
- [4] K. Toshima, H. Ouchi, Y. Okazaki, T. Kano, M. Moriguchi, A. Asai, S. Matsumura, Angew. Chem. 1997, 109, 2864–2866; Angew. Chem. Int. Ed. Engl. 1997, 36, 2748–2750.
- [5] a) F. Arcamone in Doxorubicin Anticancer Antibiotics. Medicinal Chemistry Series of Monographs, Vol. 17 (Ed.: G. Stevens), Academic Press, New York, 1981; b) Anthracycline and Anthracenedione Based Anticancer Agents (Ed.: J. W. Lown), Elsevier, Amsterdam, 1988; c) Anthracycline Antibiotics (Ed.: W. Priebe), ACS Symposium Ser. No. 574, American Chemical Society, Washington DC, 1995.
- [6] J. D. Skarbek, M. K. Speedie in Antitumor Compounds of Natural Origin: Chemistry and Biochemistry (Ed.: A. Aszalos), CRC Press, Boca Raton, FL, 1981, pp. 191–235.
- [7] a) G. J. Atwell, C. D. Bos, B. C. Baguley, W. A. Denny, J. Med. Chem.
  1988, 31, 1048-1052; b) G. J. Atwell, B. C. Baguley, W. A. Denny, J. Med. Chem. 1989, 32, 396-401; c) W. A. Denny, G. W. Rewcastle, B. C. Baguley, J. Med. Chem. 1990, 33, 814-819.
- [8] Y. Mikata, M. Yokoyama, S. Ogura, I. Okura, M. Kawasaki, M. Maeda, S. Yano, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1243–1248.
- [9] U. Henriksen, C. Larsen, G. Karup, C. Jeppesen, P. E. Nielsen, O. Buchardt, *Photochem. Photobiol.* 1991, 53, 299-305.
- P. Jÿtten, R. Greven in *Polysaccharide in Medicinal Applications* (Ed.: S. Dumitriu), Marcel Dekker, New York, **1996**, pp. 339–410.
- [11] The synthesis of the compound will be reported in detail elsewhere.[12] K. C. Nicolaou, S. P. Seitz, D. P. Papahatjis, J. Am. Chem. Soc. 1983,
- 105, 2430-2434.
  [13] F. Sanger, S. Nicklen, A. R. Coulsen, *Proc. Natl. Acad. Sci. U.S.A.* **1977**, 74, 5463-5467. Since the Sanger sequencing reactions result in base incorporation, cleavage at nucleotide N (sequencing) represents the site cleaved by the agent or the Maxam Gilbert reaction at N+1. Also, see: D. L. Boger, S. A. Munk, H. Zarrinmayeh, T. Ishizaki, J. Haught, M. Bina, *Tetrahedron* **1991**, *47*, 2661-2682.
- [14] D. A. Scudiero, R. H. Shoemaker, K. D. Paull, A. Monks, S. Tierney, T. H. Nofziger, M. J. Currens, D. Seniff, M. R. Boyd, *Cancer Res.* 1988, 48, 4827–4833.