Proof of Principle in the Selective Treatment of Cancer by Antibody-Directed Enzyme Prodrug Therapy: The Development of a Highly Potent Prodrug**

Lutz F. Tietze,* Tim Feuerstein, Anja Fecher, Frank Haunert, Olaf Panknin, Ulrich Borchers, Ingrid Schuberth, and Frauke Alves

The antibody-directed enzyme prodrug therapy (ADEPT),[1] first described by Bagshawe,[2] is a new strategy for a selective treatment of cancer, in which a nontoxic prodrug is enzymatically converted into a cytotoxic compound selectively at the surface of malignant cells by employing an enzyme–immuno conjugate. As a proposed requirement for the prodrug the corresponding cytotoxic compound should posses an $IC_{50} < 10$ nM[3] (IC_{50}: drug concentration required for 50% inhibition of target cells) and the quotient of the IC_{50} of the prodrug and the prodrug in the presence of the related enzyme ($Q\,IC_{50}$) should be above 1000.[4]

Because of its high cytotoxicity with an IC_{50} of about 0.03 nM (cell line L1210) the antibiotic CC-1065 (**1**), which was isolated from *streptomyces zelensis*, is a good target.[5] It contains a spirocyclopropyl moiety which can alkylate N3 of adenine in AT-rich parts of the minor groove of DNA. Synthetically, the spirocyclopropyl moiety may be formed from a chloromethyl group, and such seco-compounds are also found in nature as duocarmycin SA.[6] It is assumed that the seco-compounds are first transformed into the spirocyclopropyl derivatives which then react with an adenine group in the DNA. We have shown that such seco-compounds may reversibly be detoxified by glycosylation of the phenolic hydroxyl group which prohibits the formation of the spirocyclopropyl group.[7]

However, the seco-CBI galactoside **2**, which by enzymatic cleavage leads to CBI **4** (Figure 1), a potent analogue of CC-1065 developed by Boger et al.[8], shows a rather high cytotoxicity with a low $Q\,IC_{50}$ of 32; this may be explained by a direct alkylation of an adenine group by the chloromethyl group in the protected seco-compound **2**.[4]

Here we describe the synthesis as well as the in vitro and in vivo investigations using an enzyme–immuno conjugate of the seco-CBI-Q-galactoside **3** (Figure 1), which shows excellent results. It is important to note that compound **3**, which in the presence of β-D-galactosidase leads to the highly cytotoxic CBI **4**, contains a secondary chloride moiety instead of a primary as in **2** suggesting that the direct alkylation should be diminished by steric hindrance.[9]

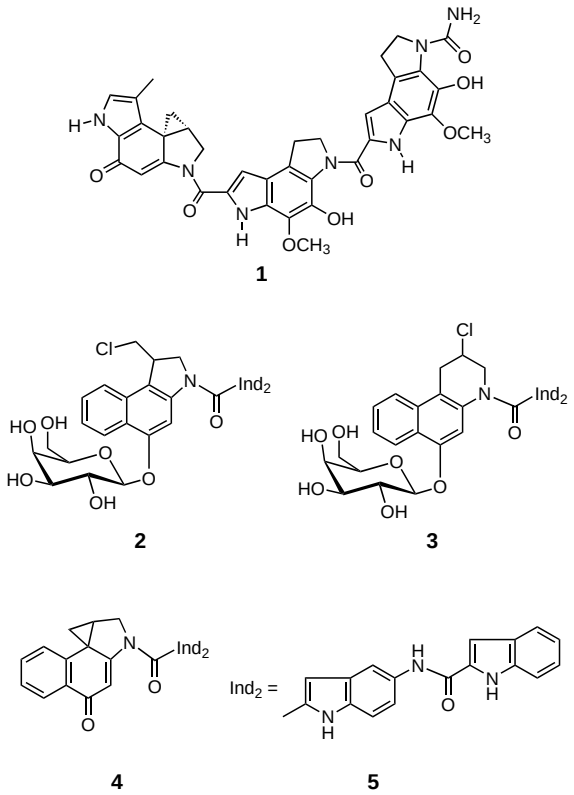

Figure 1. CC-1065 (**1**), seco-CBI-galactoside **2**, seco-CBI-Q-galactoside **3**, and CBI **4**.

Starting from the known racemic alcohol **6** (Figure 2), the silyl ether is synthesized by protection of the secondary hydroxyl group with *tert*-butyldiphenylsilylchloride (TBDPSCl). Hydrogenolytic cleavage of the benzylic ether in **7**, treatment of the obtained phenol using the Schmidt procedure[10] with the trichloroacetimidate of tetraacetylgalactose **8** in the presence of $BF_3 \cdot Et_2O$ followed by addition of bisindolylcarboxylic acid[11] and 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide (EDC) in an one pot procedure yields **9** in 38% overall yield. Deprotection of the secondary alcohol and substitution of the hydroxyl group by chloride using the Appel procedure[12] with triphenylphosphane in tetrachloromethane and acetonitrile for 5 h at 40 °C leads to **10**. Finally, solvolysis of the acetyl groups using sodium methoxide in methanol completes the synthesis to give **3** as a 1:1 mixture of two diastereomers in 17% yield over three steps.

The cytotoxicity of the prodrug **3** in the absence and presence of β-D-galactosidase was determined in a cellular cytotoxicity assay by exposure to the human bronchial carcinoma cell line A549 and the human pancreatic ductal adenocarcinoma cell line PancTu 1 for 24 h;[4, 7] As expected, the cytotoxicity of **3** in the presence of β-D-galactosidase with an $IC_{50} = 0.20$ nM (A549) and an $IC_{50} = 0.13$ nM (PancTu 1) is very high. In contrast, the prodrug **3** in the absence of β-D-galactosidase is less toxic by a factor of 1600 for the cell line A549 and by a factor of 3140 for the cell line PancTu 1 (Figure 3).

[*] Prof. Dr. Dr. h.c. L. F. Tietze, Dipl.-Chem. T. Feuerstein, Dr. A. Fecher, Dipl.-Chem. F. Haunert, Dipl.-Chem. O. Panknin, Dr. U. Borchers, Dr. I. Schuberth
Institut für Organische Chemie
der Georg-August-Universität Göttingen
Tammannstrasse 2, 37077 Göttingen (Germany)
Fax: (+49) 551-399476
E-mail: ltietze@gwdg.de
Dr. F. Alves
Zentrum Innere Medizin, Hämatologie und Onkologie
Georg-August-Universität Göttingen

[**] This work was supported by the Deutsche Forschungsgemeinschaft (SFB 416) and by the Fonds der Chemischen Industrie.

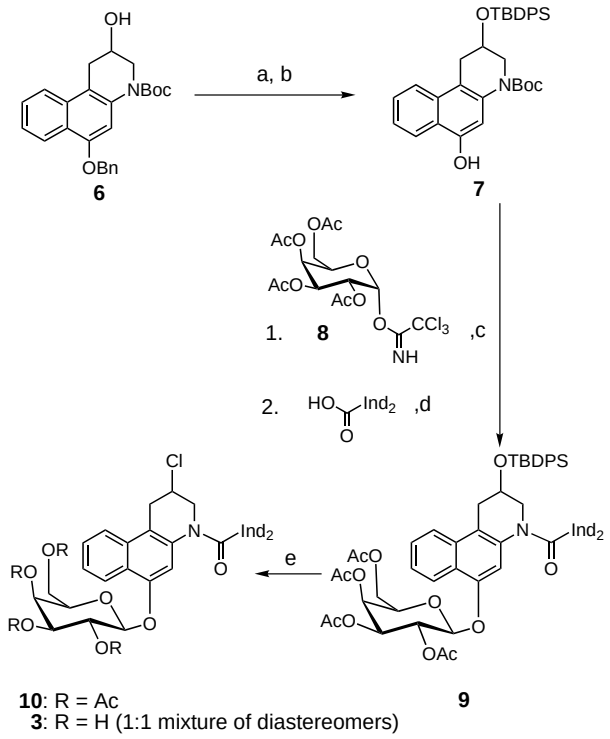

Figure 2. Synthesis of seco-CBI-Q-galactoside **3**: a) TBDPSCl, imidazol, DMF, RT, 3 days, 94 %; b) Pd/C, NH_4CO_2H, acetone, reflux, 1 h, 99 %; c) **8**, $BF_3 \cdot OEt_2$, CH_2Cl_2, $-10\,^{\circ}C \rightarrow$RT, 5 h; d) EDC, DMF, RT, 15 h, 41 % over two steps; e) 1. TBAF · SiO_2, THF, 6 h, 47 %, 2. Ph_3P, CCl_4:CH_3CN 1:1, 40 °C, 5 h, 38 %, 3. NaOMe, MeOH, RT, 30 min, 95 %. Boc = *tert*-butoxycarbonyl, TBAF = tetrabutylammonium fluoride.

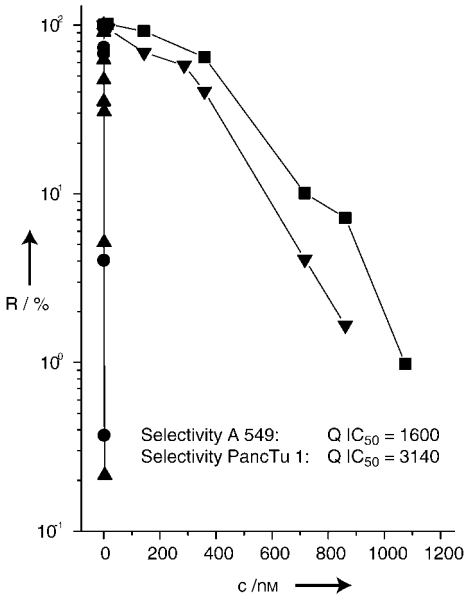

Figure 3. In vitro cytotoxicity of **3** against human bronchial carcinoma cells of line A549 and human pancreatic ductal adenocarcinoma cells of line PancTu 1. (▼): Cytotoxicity of **3** against A549. (●): Cytotoxicity of **3** against A549 in the presence of β-D-galactosidase (*Escherichia coli*, 0.4 U mL^{-1}). (■): Cytotoxicity of **3** against PancTu 1. (▲): Cytotoxicity of **3** against PancTu 1 in the presence of β-D-galactosidase (*Escherichia coli*, 0.4 U mL^{-1}). Cells were exposed to various concentrations of the test substance for 24 h at 37 °C; after 12 days of incubation clone formation was compared to untreated control assay and the relative clone-forming rate was determined; R = relative colony-forming rate, c = concentration.

The acute toxicity of seco-CBI-Q-galactoside **3** was evaluated by administration to young male and female SCID mice using DMSO:0.9 % saline solution (1:200) as vehicle.[13] Each animal received either a single dose (group 1), or three (group 2), or five doses (group 3), respectively, on successive days with each dose containing 20 μg kg^{-1} of **3**. Control animals (group 4) received five intraperitoneal (i.p.) doses of vehicle solution, equivalent to the volume of the test solutions administered.

All the animals tolerated the treatment well without any complications, such as, body weight loss or apathetic behavior. In all organs examined (e.g. lung, liver, spleen, kidneys) no macroscopical or microscopical changes could be detected by histological examination. Thus, the study shows that the prodrug **3** is nontoxic to normal organs and blood parameters of SCID mice using therapeutic doses; this indicates that **3** is not converted into **4** by enzymes expressed by the SCID mice. This is a necessary prerequisite for application within ADEPT.

To evaluate the potency of prodrug **3** for an in vivo application, orthotopic tumors of the human bronchial carcinoma cell line A549 and the pancreatic ductal adenocarcinoma cell line PancTu 1 in SCID mice were generated.[14] The treatment started on day eight after orthotopic implantation by intravenous (i.v.) administration of the immunoconjugate of galactosidase and a human epithelial monoclonal antibody. After a clearance time of 84 h[15] the galactoside **3** (25 μg kg^{-1}, using DMSO:0.9 % saline solution (1:200) as vehicle) was applied i.p. to the mice and given every second day until sacrifice on day 30. Primary tumor volume in treated mice was significantly decreased whereas tumor invasion in the control animals caused complications such as acute dyspnoea and weight loss (Figure 4).

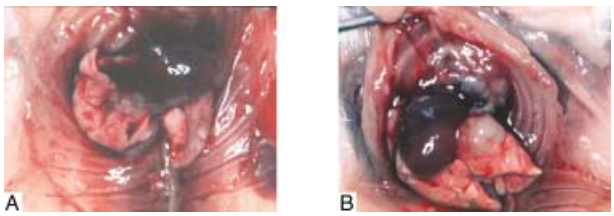

Figure 4. Macroscopic appearance of tumors: A) Lung tumor in a treated mouse. B) Lung tumor in a control mouse.

Histological examination of lung tissue shows that in treated animals the tumor is only located in small areas (Figure 5). In contrast, the lung tumor in controls invades the whole lung.

In conclusion, with the galactoside **3** we have developed a new highly potent prodrug, which meets all the necessary requirements for a successful use in ADEPT: excellent selectivity (Q IC_{50} = 1600 and 3140 for the used cell lines), low systemic toxicity and high cytotoxicity of the corresponding drug (IC_{50} = 0.20 and 0.13 nM, respectively). In addition, first preclinical investigations on mice show promising results. A further advantage of this compound is its reasonable water

  1433-7851/02/4105-0760 $ 17.50+.50/0

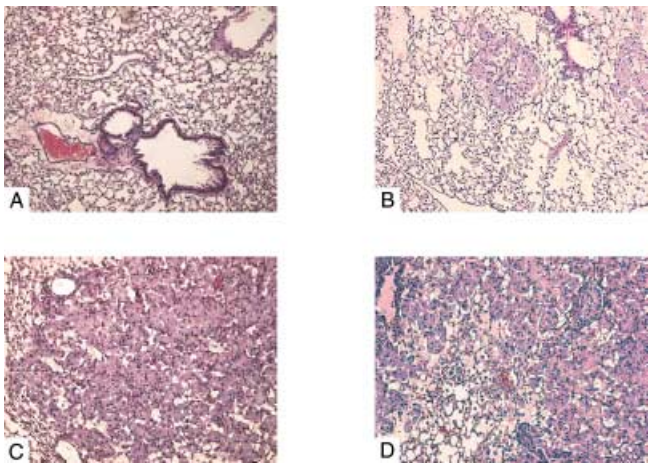

Figure 5. Histological characteristics of the lung tumors: The tumor tissue shows prominently roundly formed nuclei and a solid structure; operatively expansive. A) and B) Haematoxylin and Eosin staining of lung tissue of a treated mouse; the tumor tissue is only located in small areas. C) and D) Haematoxylin and Eosin staining of lung tissue of a control mouse; tumor invading the bronchius.

solubility arising from its hydrophilic sugar moiety and the fact that it seems not to penetrate the cell membrane. Thus, cellular human galactosidase may be used for an activation. Finally, the synthesis allows the preparation of analogues containing different sugar moieties.

Received: September 27, 2001 [Z17982]

[1] Reviews: a) L. N. Jungheim, T. A. Shepherd, *Chem. Rev.* **1994**, *94*, 1553 – 1566; b) C. J. Springer, I. Niculescu-Duvaz, *Adv. Drug Delivery Rev.* **1997**, *26*, 151 – 172; c) K. N. Syrigos, A. A. Epenetos, *Anticancer Res.* **1999**, *19*, 605 – 614; G. M. Dubowchik, M. A. Walker, *Pharmacol. Ther.* **1999**, *83*, 67 – 123.

[2] K. D. Bagshawe, *Br. J. Cancer* **1987**, *56*, 531 – 532.

[3] Our early investigations using acetal glycosides of aldophosphamide as prodrugs were not successful probably because of the relatively low cytotoxicity of aldophosphamide: a) L. F. Tietze in (Eds.: E. Borowski, D. Shugar), *Molecular aspects of chemotherapy*, Pergamon Press, Oxford, **1990**, p. 55; b) L. F. Tietze, R. Fischer, *Angew. Chem.* **1981**, *93*, 1002; *Angew. Chem. Int. Ed. Engl.* **1981**, *20*, 969; c) L. F. Tietze, R. Fischer, H. J. Guder, A. Goerlach, M. Neumann, T. Krach, *Carbohydr. Res.* **1987**, *164*, 177 – 194; d) L. F. Tietze, M. Beller, R. Fischer, M. Lögers, E. Jähde, K. H. Glüsenkamp, M. F. Rajewsky, *Angew. Chem.* **1990**, *102*, 812 – 813; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 782 – 783.

[4] L. F. Tietze, T. Herzig, A. Fecher, F. Haunert, I. Schuberth, *ChemBioChem* **2001**, *2*, 758 – 765.

[5] Reviews: a) D. L. Boger, D. S. Johnson, *Angew. Chem.* **1996**, *108*, 1542 – 1580; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1438 – 1474; b) D. L. Boger, R. M. Garbaccio, *Acc. Chem. Res.* **1999**, *32*, 1043 – 1052.

[6] M. Ichimura, T. Ogawa, K. Takahashi, E. Kobayashi, I. Kawamoto, T. Yasuzawa, I. Takahashi, H. Nakano, *J. Antibiot.* **1990**, *43*, 1037 – 1038.

[7] a) L. F. Tietze, R. Hannemann, W. Buhr, M. Lögers, P. Menningen, M. Lieb, D. Starck, T. Grote, A. Döring, I. Schuberth, *Angew. Chem.* **1996**, *108*, 2840 – 2842; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2674 – 2677; b) L. F. Tietze, M. Lieb, T. Herzig, F. Haunert, I. Schuberth, *Bioorg. Med. Chem.* **2001**, *9*, 1929 – 1939.

[8] D. L. Boger, S. W. Boyce, R. M. Garbaccio, M. Searcey, Q. Jin, *Synthesis* **1999**, 1505 – 1509.

[9] We have also prepared a methyl-CBI-galactoside with a secondary chloride moiety, which also shows a higher selectivity than **2** but with a significantly reduced cytotoxicity of the corresponding drug, see ref. [4].

[10] a) R. R. Schmidt, *Angew. Chem.* **1986**, *98*, 213 – 236; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 212 – 235; b) W. Dullenkopf, J. C. Castro-Palomino, L. Manzoni, R. R. Schmidt, *Carbohydr. Res.* **1996**, *296*, 135 – 147.

[11] a) M. A. Warpehoski, *Tetrahedron Lett.* **1986**, *27*, 4103 – 4106; b) S. Rajeswari, A. A. Adesomoju, M. P. Cava, *J. Heterocycl. Chem.* **1989**, *20*, 557 – 564; c) D. L. Boger, W. Yun, N. Han, *Bioorg. Med. Chem.* **1995**, *3*, 1429.

[12] R. Appel, *Angew. Chem.* **1975**, *87*, 863 – 874; *Angew. Chem. Int. Ed. Engl.* **1975**, *15*, 801 – 812.

[13] Severe combined immunodeficient (SCID) mice, strain C.B-17/Ztm-scid, 10 – 12 weeks old, were used.

[14] F. Alves, U. Borchers, B. Pladge, H. Augustin, K. Nebendahl, G. Klöppel, L. F. Tietze, *Cancer Lett.* **2001**, *165*, 161 – 170.

[15] M. P. Napier, S. K. Sharma, C. J. Springer, K. D. Bagshawe, A. J. Green, J. Martin, S. M. Stribbling, N. Cushen, D. O'Malley, R. H. J. Begent, *Clin. Cancer Res.* **2000**, *6*, 765 – 772.

Magnetization of Chiral Monolayers of Polypeptide: A Possible Source of Magnetism in Some Biological Membranes**

Itai Carmeli, Viera Skakalova, Ron Naaman,* and Zeev Vager

Some biological membranes are affected by weak magnetic fields.[1] In particular, magnet-induced orientation was observed in the outer segments of retinal rods,[2] chloroplasts,[3] bacterial chromatophores,[4] purple membranes,[5] and recently in black lipid membranes.[6] The membrane surfaces tend to align perpendicular to the magnetic field. Alignment due to anisotropic magnetic susceptibility,[7] which explains alignment effects in liquid crystals, requires much higher magnetic fields than were used in the above experiments. Hence, no existing theory could explain these significant observations. The lack of physico-microscopic understanding of the effect of magnetic fields on biological membranes prevents the comprehension of its significance.

By studying well-characterized monolayers of polyalanine we were able not only to obtain results similar to those observed in biological membranes, but also to gain an insight into the details of a mechanism that may account for the previously observed magnetic behavior of membranes. We propose an explanation of these phenomena in terms of a physical model, the components of which are supported by the experimental results presented here.

Self-assembled monolayers of L- or D-polyalanine polypeptides in the form of α-helices were prepared on glass slides coated with a 100-nm thick annealed gold film. The orientation of the molecules was monitored by IR spectroscopy in the presence and absence of a magnetic field. In addition, we

[*] Prof. R. Naaman, I. Carmeli, Dr. V. Skakalova, Prof. Z. Vager
Department of Chemical Physics
Weizmann Institute of Science
Rehovot 76100 (Israel)
Fax: (+972) 8-9344123
E-mail: ron.naaman@weizmann.ac.il

[**] We are grateful to Prof. M. Fridkin and his group for helping us in the synthesis of the polyalanine. Partial support from the US – Israel Binational Science Foundation is acknowledged.

  1433-7851/02/4105-0761 $ 17.50+.50/0