DOI: 10.1002/cmdc.200600284

Cis-Stilbene Derived Furopyranones Show Potent Antiproliferative Activity by Inducing G2/M Arrest

Cyril A. Fuhrer,[a] Eric Grüter,[a] Stephan Ruetz,[b] and Robert Häner*[a]

Stilbenoids, including resveratrol (**1**, Figure 1) and combretastatin A-4 (**2**), have been shown to be potent inhibitors of proliferation and to induce apoptosis in human cancer cell lines.[1]

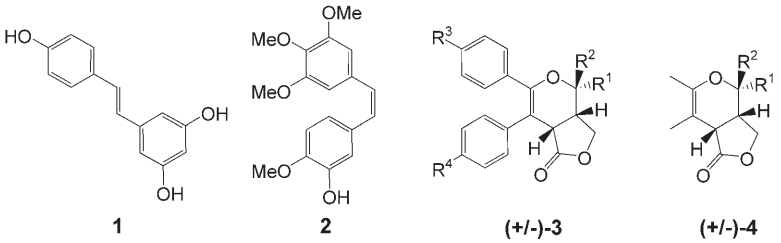


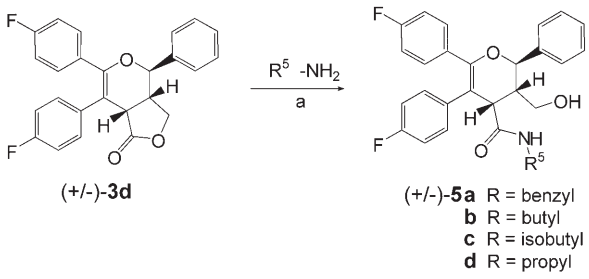
Figure 1. The naturally occurring stilbenoids resveratrol (**1**) and combretastatin A-4 (**2**) as well as the synthetic furopyranones **3** and **4**, of which compound **3** contains a *cis*-stilbenoid moiety.

Resveratrol (**1**) and its analogues are commonly found in various plants including grapes, berries, and peanuts. Many of these naturally occurring stilbenoids exhibit a variety of pharmaceutically interesting biological properties, such as a protective role in atherosclerosis and coronary heart disease, anti-inflammatory and antioxidant effects, as well as chemopreventive effects in carcinogenesis.[2,3] Although a detailed knowledge of the mode of action for the antiproliferative effects is still elusive, several reports indicate that inhibition of cell growth is a consequence of interference with cell-cycle progression, and induction of apoptosis.[2] Several structural aspects, such as the nature of the arylic substituents and geometrical isomerism were shown to have a significant influence on the inhibitory effects.[4–12] The *cis*-stilbenoid combretastatin A-4 (**2**) is a natural product isolated from the South African bush willow tree *Combretum caffrum* and strongly inhibits the polymerization of tubulin by binding to the colchicine-binding site.[7,12–16] The natural compound **2** also exerts potent cytotoxicity against a variety of human cancer cells including multidrug-resistant (MDR) cancer cell lines. As a result of its high biological activity combretastatin A-4 (**2**) serves as a starting point for the development of new antitubulin agents with a potential for cancer treatment.[1,8,13,17–26]

We recently reported the synthesis of 3a,7a-dihydro-3*H*,4*H*-furo[3,4-*c*]pyran-1-ones by a stereoselective, intramolecular *hetero* Diels–Alder reaction, including compounds of type **3**.[27] These compounds contain the *cis*-stilbene motif. In light of the clinical relevance of this class of compounds, we examined their effect on tumour cell growth. Herein, we report the inhibitory activities of this class of cyclic stilbenoid analogues and their influence on the cell cycle progression in A549 and KB31 cell lines.

The bicyclic derivatives **3** and **4** were synthesized as previously reported.[27] Both types of compounds are accessible through an intramolecular *hetero* Diels–Alder reaction leading to single product diastereomers. In preliminary studies, compounds of type **3** showed antiproliferative activity in KB31 cells, whereas derivatives of type **4** lacking the *cis*-stilbene motif were inactive. To gain more insight in the relevance of the *cis*-stilbene moiety, further derivatives were synthesized to conduct a more detailed structure–activity relationship study. The synthesis of derivatives was carried out by considering the following questions: 1) to what extent does the stilbene motif influence the biological activity and 2) is the bicyclic structure required for the biological effect observed? To answer these questions, we undertook, on the one hand, the synthesis of derivatives of the parent structure **3** lacking one of the two phenyl substituents at the double bond of the dihydropyrane moiety. On the other hand, the bicyclic structure was further transformed into monocyclic compounds. To achieve the latter, the bicyclic compound **3d** was converted into monocyclic derivatives by ring opening aminolysis (Scheme 1). Thus, treatment with benzyl, butyl, isobutyl, or propyl amine in refluxing toluene in the presence of 2-hydroxypyridine[28] gave the four monocyclic derivatives **5a**–**d**. Treatment under the same conditions without a transacylation catalyst failed to give the ring-opened amides. The relative configuration of **5a** was verified by X-ray analysis.[29]

To establish the importance of the *cis*-stilbenoid motif, several C(7)-desphenyl derivatives were synthesised (Scheme 2). Their preparation involved a route similar to the one used for

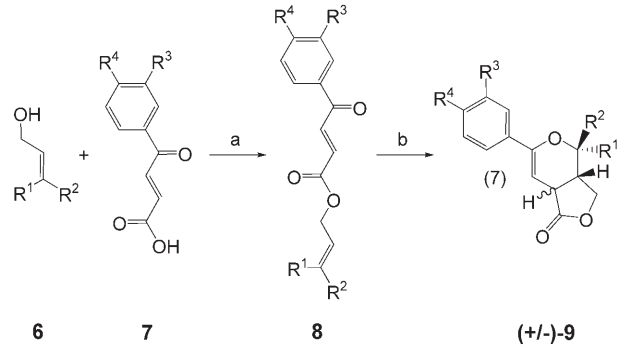


Scheme 1. Aminolysis of the γ-lactone ring of furo[3,4-*c*]pyranone **3d**. a) Toluene, 2-hydroxypyridine, R^5-NH_2, reflux, 20 h [yields: 47% (**5a**); 48% (**5b**); 52% (**5c**); 84% (**5d**)].

[a] *Dipl.-Chem. C. A. Fuhrer, Dr. E. Grüter, Prof. Dr. R. Häner*
Department of Chemistry, University of Bern, CH-3012 Bern (Switzerland)
Fax: (+41) 31-631-8057
E-mail: robert.haener@ioc.unibe.ch

[b] *Dr. S. Ruetz*
Novartis Institutes of Biomedical Research, CH-4002 Basel (Switzerland)

Supporting information for this article is available on the WWW under http://www.chemmedchem.org or from the author.



Scheme 2. Synthesis of furo[3,4-*c*]pyranones lacking the stilbene motif. a) Pivaloyl chloride, triethylamine, DMAP, 1,2-dichloroethane, 0 °C; b) *o*-xylene, reflux, 24–48 h (for yields see Table 1).

the synthesis of compounds **3** and **4**.[27] Starting from the allylic alcohols **6** and the γ-oxo-butenoic acids **7**, the corresponding esters **8** were prepared via the mixed anhydride using pivaloyl chloride. Esters **8** were subsequently transformed into the furopyranones **9** through an intramolecular *hetero* Diels–Alder reaction. The cyclisation was carried out in refluxing *o*-xylene. Yields of isolated products varied between 40 and 70% (see Table 1), which is acceptable in view of the relatively harsh reaction conditions.

The stereoselectivity of the *hetero* Diels–Alder reaction leading to products **9** varied greatly, depending on the substitution pattern of the allyl moiety of esters **8**. Whereas the previously reported reaction leading to compounds **3** and **4** provided a *cis*-configuration of the two rings only,[27] *cis*/*trans* ratios obtained in products **9a–f** varied between 97:3 and 34:66 (see Table 1). This indicates that the substituent at position C7 has a significant influence on the relative configuration of the formed products. The results obtained do not allow general conclusions to be drawn on the governing aspects of the stereochemical course. The relative configurations of compounds *cis*-**9c**, *cis*-**9f**, and *trans*-**9f** were confirmed by X-ray analysis.

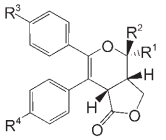
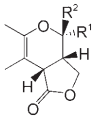
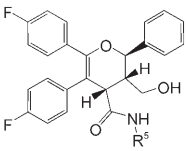
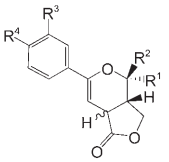
The biological activity of the obtained compounds were determined using the YO-PRO apoptosis/viability assay[30,31] in two different cell lines (A549: human nonsmall cell lung cancer, and KB31: human cervix carcinoma). The IC_{50} values indicating the antiproliferative activity of the synthesised compounds are shown in Table 2. The structure–activity profile is

Table 1. Preparation of (±)-3a,7a-dihydro-3*H*,4*H*-furo[3,4-*c*]pyran-1-ones **9a–f** by intramolecular *hetero* Diels–Alder reaction of α,β-unsaturated γ-ketoesters.

R^1	R^2	R^3	R^4	compd. **8** (% yield)	compd. **9** (% yield)[a]	*cis*/*trans* ratio
H	C_6H_5	H	NO_2	**8a** (63)	**9a** (55)	92:8
H	C_6H_5	NO_2	H	**8b** (67)	**9b** (46)	42:58
H	C_6H_5	H	H	**8c** (80)	**9c** (39)	37:63
CH_3	CH_3	H	NO_2	**8d** (58)	**9d** (66)	36:64
CH_3	CH_3	NO_2	H	**8e** (69)	**9e** (44)	97:3
CH_3	CH_3	H	H	**8f** (92)	**9f** (73)	34:66

[a] Combined isolated yield of *cis*- and *trans*-isomers.

Table 2. Antiproliferative activity of the synthesised compounds in A549 and KB31 cell lines.

		R^1	R^2	R^3	R^4	R^5	IC_{50} [μM][a] A549	KB31
	**3a**	CH_3	CH_3	H	H		18.72 ± 0.89	17.69 ± 3.34
	3b	CH_3	CH_3	F	F		14.77 ± 3.26	13.18 ± 3.28
	3c	H	C_6H_5	H	H		10.67 ± 1.36	8.54 ± 1.78
	3d	H	C_6H_5	F	F		8.78 ± 2.54	7.27 ± 1.58
	**4a**	CH_3	CH_3				> 20	> 20
	4b	H	C_6H_5				> 20	> 20
	**5a**					benzyl	n.t.[b]	> 20
	5b					butyl	n.t.[b]	15.85 ± 0.30
	5c					isobutyl	11.76 ± 1.68	10.41 ± 0.26
	5d					propyl	14.73 ± 0.64	12.32 ± 0.93
	**9a**[d]	H	C_6H_5	H	NO_2		> 20	> 20
	9b[d]	H	C_6H_5	NO_2	H		> 20	> 20
	9c[c]	H	C_6H_5	H	H		> 20	> 20
	9d[d]	CH_3	CH_3	H	NO_2		> 20	> 20
	9e[d]	CH_3	CH_3	NO_2	H		> 20	> 20
	9f[c]	CH_3	CH_3	H	H		> 20	> 20

[a] IC_{50} values were determined as described previously.[30] [b] Not tested. [c] *Cis*- and *trans*-isomers were tested separately and were both found to be inactive. [d] Only the *cis*-isomer was tested.

very similar for both cell lines. The bicyclic compounds **3a–d** containing the *cis*-stilbene motif were active in the assay with IC_{50} values in the range of 7–20 μM. Compounds **3c** and **3d** showed the highest antiproliferative activity. Absence of one or both of the phenyl substituents at the enol–ether double bond leads to loss of antiproliferative activity (compounds of type **4** and **9**). This clearly establishes the importance of the *cis*-stilbene motif. Furthermore, aminolysis of the lactone ring of the most active compound (**3d**) also reduces the activity partly (in the case of **5b**, **5c**, and **5d**) or completely (**5a**). This shows that the bicyclic nature of the structure also contributes to the activity.

Recent reports describe that stilbenoids show potent inhibition of cell growth in different cancer cell lines.[1,4,18] The nature of the substituents and conformational aspects were shown to significantly influence the regulatory effects the compounds exert on the cell cycle. Thus, replacement of the hydroxy-groups of resveratrol by methoxy-groups greatly enhances the inhibition of A549 cell growth.[5] In addition, combretastatin and its analogues, which contain a *cis*-orientation, show a pronounced increase in activity, with an IC_{50} value up to 100 times lower than that of the *trans*-configured resveratrol.[6,10,12] To gain further information on the effect of the described compounds on the cell cycle progression, a cell cycle analysis was performed with the two most active compounds **3c** and **3d** (Figure 2 and Supporting Information). As shown in Figure 2 for **3d**, a 24 h treatment of KB31 cells leads to a significant and dose dependent cell cycle arrest. At the highest concentration tested (20 μM), the population of cells in the G2-phase increases from 20% to 40% (Table 3). Similar results were also obtained with A549 cells (see Supporting Information), suggesting a similar effect on cell cycle progression in both cell lines. These findings are in good agreement with published reports. Whereas resveratrol causes A549 cells to be blocked in the S phase,[14] *cis*-configured analogues seem to have a different mode of action leading to G2/M arrest.[5,6,11,22] Interestingly, the cell viability tests show that both compounds, **3c** and **3d**, induce apoptosis in KB31 cells whereas no programmed cell death was observed in A549 cells (see Supporting Information). It is at present unclear whether the underlying mechanism of action of these compounds is the same as the one reported for combretatstatin analogues.

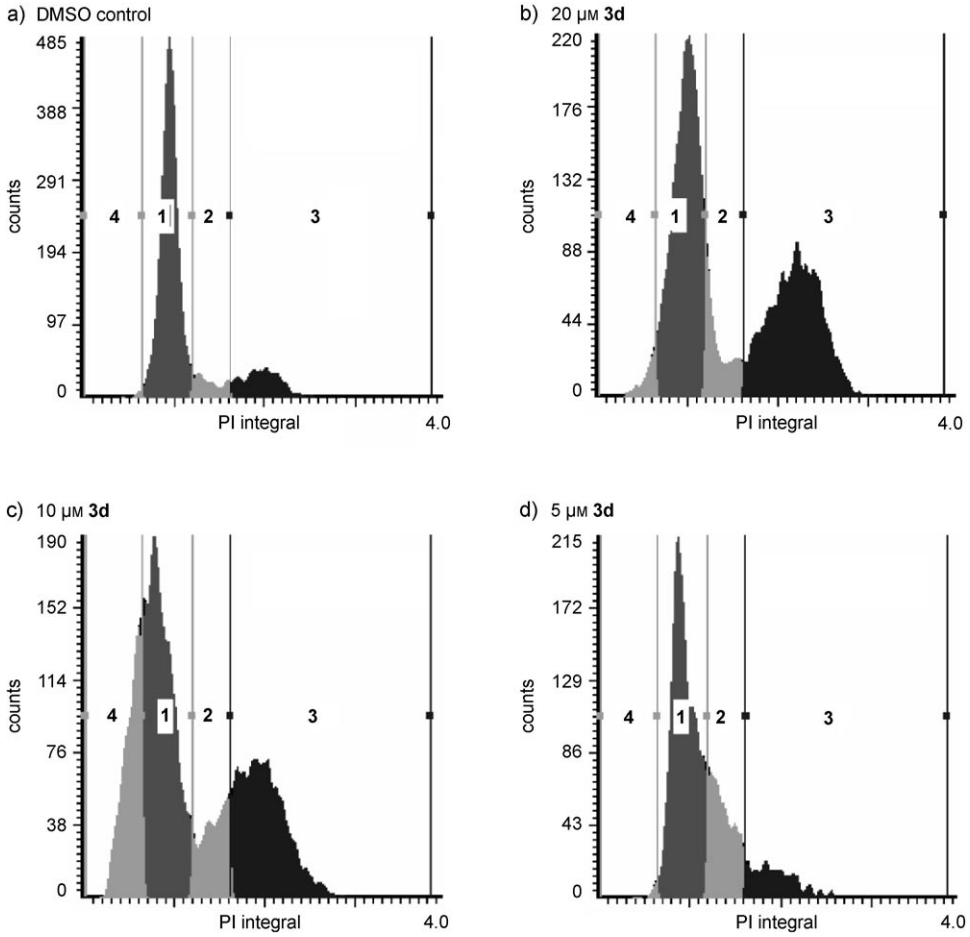


Figure 2. Cell cycle analysis (Laser Scanning Cytometry) after treatment of KB31 cells with compound **3d** for 24 h; concentrations as indicated. a) to d) correspond with Table 3.

Table 3. Cell cycle distribution (KB31 cells) after treatment with compound **3d**.[a]

	Treatment	subG1 (Region 4)[b]	G1 (Region 1)[b]	S (Region 2)[b]	G2/M (Region 3)[b]
a)	DMSO control	1.60%	71.50%	7.30%	20.00%
b)	20 μM **3d**	3.30%	48.60%	9.10%	40.60%
c)	10 μM **3d**	21.20%	40.30%	12.30%	29.30%
d)	5 μM **3d**	1.60%	61.20%	22.60%	16.20%

[a] Determined by Laser Scanning Cytometry after treatment with various concentrations of **3d** for 24 h. [b] Regions 1–4 correlate with Figure 2.

In conclusion, we have described a new type of bicyclic scaffold containing the *cis*-stilbene motif. The derivatives show biological activity by arresting cell cycle progression at the G2/M transition. The presence of the *cis*-stilbene motif was shown to be critical for biological activity. Further modification of the described compounds with substituents that have been shown to critically influence the biological activity of *cis*-stilbenoids[22] is currently underway.

Acknowledgements

*Financial support by the Swiss National Foundation (grant 200021-101861) is gratefully acknowledged. We thank Luzia Moesch for excellent technical assistance. We thank Prof. H. Stoeckli-Evans, University of Neuchâtel, for determination of molecular structures of compounds **5a**, **9c**, cis-**9f**, and trans-**9f** by X-ray diffraction.*

Keywords: antiproliferative · apoptosis · cancer cell lines · *cis*-stilbenoids · G2/M arrest

[1] S. H. Inayat-Hussain, N. F. Thomas, *Expert Opin. Ther. Pat.* **2004**, *14*, 819–835.
[2] B. B. Aggarwal, A. Bhardwaj, R. S. Aggarwal, N. P. Seeram, S. Shishodia, Y. Takada, *Anticancer Res.* **2004**, *24*, 2783–2840.
[3] M. S. Jang, E. N. Cai, G. O. Udeani, K. V. Slowing, C. F. Thomas, C. W. W. Beecher, H. H. S. Fong, N. R. Farnsworth, A. D. Kinghorn, R. G. Mehta, R. C. Moon, J. M. Pezzuto, *Science* **1997**, *275*, 218–220.
[4] M. Cushman, D. Nagarathnam, D. Gopal, A. K. Chakraborti, C. M. Lin, E. Hamel, *J. Med. Chem.* **1991**, *34*, 2579–2588.
[5] E. J. Lee, H. Y. Min, H. J. Park, H. J. Chung, S. Kim, Y. N. Han, S. K. Lee, *Life Sci.* **2004**, *75*, 2829–2839.
[6] Y. Schneider, P. Chabert, J. Stutzmann, D. Coelho, A. Fougerousse, F. Gosse, J. F. Launay, R. Brouillard, F. Raul, *Int. J. Cancer* **2003**, *107*, 189–196.
[7] G. R. Pettit, B. E. Toki, D. L. Herald, M. R. Boyd, E. Hamel, R. K. Pettit, J. C. Chapuis, *J. Med. Chem.* **1999**, *42*, 1459–1465.
[8] A. Cirla, J. Mann, *Nat. Prod. Rep.* **2003**, *20*, 558–564.
[9] F. Minutolo, G. Sala, A. Bagnacani, S. Bertini, I. Carboni, G. Placanica, G. Prota, S. Rapposelli, N. Sacchi, M. Macchia, R. Ghidoni, *J. Med. Chem.* **2005**, *48*, 6783–6786.
[10] D. Simoni, M. Roberti, F. P. Invidiata, E. Aiello, S. Aiello, P. Marchetti, R. Baruchello, M. Eleopra, A. Di Cristina, S. Grimaudo, N. Gebbia, L. Crosta, F. Dieli, M. Tolorneo, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3245–3248.
[11] D. Simoni, R. Romagnoli, R. Baruchello, R. Rondanin, M. Rizzi, M. G. Pavani, D. Alloatti, G. Giannini, M. Marcellini, T. Riccioni, M. Castorina, M. B. Guglielmi, F. Bucci, P. Carminati, C. Pisano, *J. Med. Chem.* **2006**, *49*, 3143–3152.
[12] G. R. Pettit, M. R. Rhodes, D. L. Herald, E. Hamel, J. M. Schmidt, R. K. Pettitt, *J. Med. Chem.* **2005**, *48*, 4087–4099.
[13] J. P. Liou, Y. L. Chang, F. M. Kuo, C. W. Chang, H. Y. Tseng, C. C. Wang, Y. N. Yang, J. Y. Chang, S. J. Lee, H. P. Hsieh, *J. Med. Chem.* **2004**, *47*, 4247–4257.
[14] S. Kim, S. Y. Min, S. K. Lee, W. J. Cho, *Chem. Pharm. Bull.* **2003**, *51*, 516–521.
[15] J. A. Hadfield, K. Gaukroger, N. Hirst, A. P. Weston, N. J. Lawrence, A. T. Mcgown, *Eur. J. Med. Chem.* **2005**, *40*, 529–541.
[16] C. Borrel, S. Thoret, X. Cachet, D. Guenard, F. Tillequin, M. Koch, S. Michel, *Bioorg. Med. Chem.* **2005**, *13*, 3853–3864.
[17] J. A. Hadfield, S. Ducki, N. Hirst, A. T. Mcgown, *Prog. Cell Cycle Res.* **2003**, *5*, 309–325.
[18] G. C. Tron, T. Pirali, G. Sorba, F. Pagliai, S. Busacca, A. A. Genazzani, *J. Med. Chem.* **2006**, *49*, 3033–3044.
[19] P. K. Li, Z. L. Xiao, Z. G. Hu, B. Pandit, Y. J. Sun, D. L. Sackett, K. Werbovetz, A. Lewis, J. Johnsamuel, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5382–5385.
[20] K. A. Monk, R. Siles, M. B. Hadimani, B. E. Mugabe, J. F. Ackley, S. W. Studerus, K. Edvardsen, M. L. Trawick, C. M. Garner, M. R. Rhodes, G. R. Pettit, K. G. Pinney, *Bioorg. Med. Chem.* **2006**, *14*, 3231–3244.
[21] G. Dupeyre, G. G. Chabot, S. Thoret, X. Cachet, J. Seguin, D. Guenard, F. Tillequin, D. Scherman, M. Koch, S. Michel, *Bioorg. Med. Chem.* **2006**, *14*, 4410–4426.
[22] T. Pirali, S. Busacca, L. Beltrami, D. Imovilli, F. Pagliai, G. Miglio, A. Massarotti, L. Verotta, G. C. Tron, G. Sorba, A. A. Genazzani, *J. Med. Chem.* **2006**, *49*, 5372–5376.
[23] J. Y. Chang, M. F. Yang, C. Y. Chang, C. M. Chen, C. C. Kuo, J. P. Liou, *J. Med. Chem.* **2006**, *49*, 6412–6415.
[24] R. Romagnoli, P. G. Baraldi, V. Remusat, M. D. Carrion, C. L. Cara, D. Preti, F. Fruttarolo, M. G. Pavani, M. A. Tabrizi, M. Tolomeo, S. Grimaudo, J. Balzarini, M. A. Jordan, E. Hamel, *J. Med. Chem.* **2006**, *49*, 6425–6428.
[25] R. Romagnoli, P. G. Baraldi, M. G. Pavani, M. A. Tabrizi, D. Preti, F. Fruttarolo, L. Piccagli, M. K. Jung, E. Hamel, M. Borgatti, R. Gambari, *J. Med. Chem.* **2006**, *49*, 3906–3915.
[26] H. Nakamura, H. Kuroda, H. Saito, R. Suzuki, T. Yamori, K. Maruyama, T. Haga, *ChemMedChem* **2006**, *1*, 729–740.
[27] C. Fuhrer, R. Messer, R. Häner, *Tetrahedron Lett.* **2004**, *45*, 4297–4300<
[28] D. S. Tan, M. A. Foley, B. R. Stockwell, M. D. Shair, S. L. Schreiber, *J. Am. Chem. Soc.* **1999**, *121*, 9073–9087.
[29] CCDC 613295 (**5a**), CCDC 613296 (*cis*-**9c**), CCDC 613297 (*cis*-**9f**), and CCDC 613298 (*trans*-**9f**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre.http://www.ccdc.cam.ac.uk/data_request/cif
[30] I. Beuvink, A. Boulay, S. Fumagalli, F. Zilbermann, S. Ruetz, T. O'Reilly, F. Natt, J. Hall, H. A. Lane, G. Thomas, *Cell* **2005**, *120*, 747–759.
[31] T. Idziorek, J. Estaquier, F. De Bels, J. C. Ameisen, *J. Immunol. Methods* **1995**, *185*, 249–258.

Received: December 6, 2006
Published online on February 26, 2007

