COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Synthesis and biological evaluation of cytostatin analogues†

Laurent Bialy*[ab] and Herbert Waldmann[ab]

[a] *Max-Planck-Institut für molekulare Physiologie, Abteilung Chemische Biologie, Otto-Hahn-Str. 11, 44227 Dortmund, Germany. E-mail: herbert.waldmann@mpi-dortmund.mpg.de; Fax: 49 231 133 2499; Tel: 49 231 133 2400*

[b] *Universität Dortmund, Fachbereich 3, Organische Chemie, 44221 Dortmund, Germany*

Received (in Cambridge, UK) 12th May 2003, Accepted 3rd June 2003
First published as an Advance Article on the web 20th June 2003

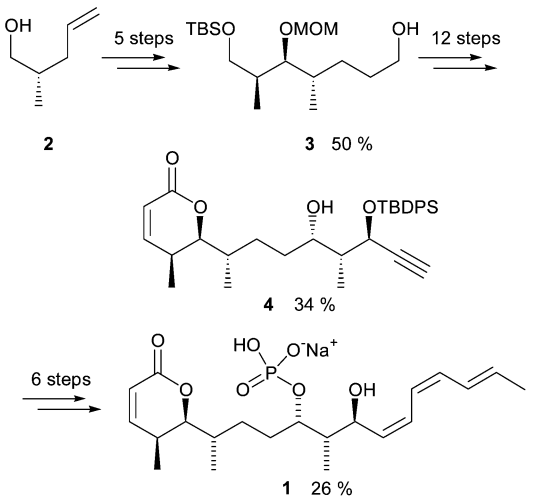
Analogues of the serine/threonine phosphatase 2A inhibitor cytostatin were synthesized and evaluated as inhibitors of various phosphatases in order to establish a basic structure–activity relationship (SAR) of the natural product.

Protein phosphorylation and dephosphorylation reactions are employed by living organisms for the regulation of innumerable cellular processes, and aberrant protein phosphorylation contributes to the development of many human diseases inducing cancer and diabetes.[1] Phosphorylation states are governed by protein kinases (PKs), which catalyze protein phosphorylation, and protein phosphatases (PPs), which are responsible for dephosphorylation. Naturally occurring PP inhibitors have widely been used to antagonize PP action in biology experiments.[2] In the light of this experience and the fact that potent and selective inhibitors of this enzyme class still are in short supply the development of small-molecule inhibitors of PPs is emerging as a very rapidly growing area of investigation.[3]

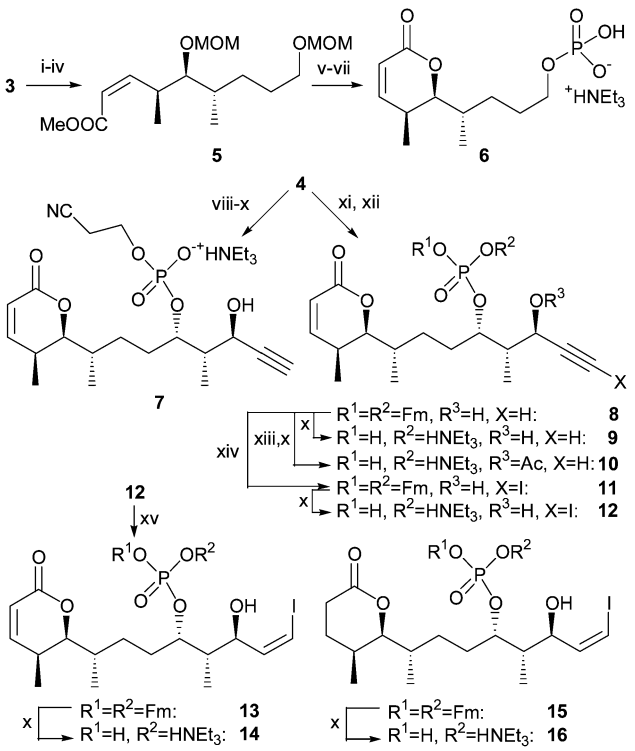
Cytostatin **1**, isolated from a *Streptomyces* strain,[4a] inhibits the serine/threonine phosphatase 2A at submicromolar concentration.[4b] It belongs to a family of natural products related to fostriecin,[5] which all inhibit PP2A with an unprecedented selectivity compared to other natural products like okadaic acid, calyculin or the microcystins.[6] Given the importance of PP inhibitors it is of major interest to medicinal chemistry and clinical biology to gain insight into the structural parameters that underlie the biological activity of these natural products.

Based on the total synthesis of cytostatin (Scheme 1),[7] we now describe the synthesis of structural analogues of the molecule and their evaluation as phosphatase inhibitors.

Dephosphorylated cytostatin is not active as a phosphatase inhibitor.[4b] To establish whether the lactone and phosphate moieties alone would suffice to confer activity, a corresponding truncated analogue was synthesized starting from alcohol **3**. The alcohol was protected as the methoxymethyl (MOM) ether and after cleavage of the *tert*-butyldiphenylsilyl (TBDPS) group, oxidation to the corresponding aldehyde was carried out. Subsequent Still–Gennari olefination[8] yielded (*Z*)-configured unsaturated ester **5** (Scheme 2). **5** was converted to the lactone with hot aqueous HCl. Treatment with phosphoramidite $(FmO)_2PN(iPr)_2$ (Fm = 9-fluorenylmethyl)[9] and subsequent oxidation with *meta*-chloroperbenzoic acid (*m*-CPBA) gave the desired phosphotriester which was deprotected with an excess



Scheme 1 Synthesis of (4*S*,5*S*,6*S*,9*S*,10*S*,11*S*)-cytostatin (**1**).



Scheme 2 Synthesis of cytostatin analogues: (i) 10 eq. MOMCl, 13 eq. $(iPr)_2NEt$, CH_2Cl_2, 0 °C, 1 h, room temp., 13 h; (ii) 1.2 eq. TBAF, THF, room temp., 15 h, 90% over 2 steps; (iii) 1.5 eq. Dess–Martin periodinane, 11.5 eq. $NaHCO_3$, CH_2Cl_2, room temp., 90 min; (iv) 2 eq. $(CF_3CH_2O)_2P(O)CH_2C(O)Me$, 4 eq. 18-crown-6, 1.5 eq. KHMDS, THF, −78 °C, 35 min, aldehyde, −78 °C, 3 h, 80% over 2 steps; (v) 1 M HCl, H_2O, THF, 15 h, 60 °C, 88%; (vi) 4 eq. $(iPr_2)NP(OFm)_2$, 3 eq. tetrazole, CH_2Cl_2, 270 min, 10 eq. *m*-CPBA, −78 °C, 0 °C, 90 min; 88%; (vii) NEt_3/CH_3CN 1/4.8 (v/v), room temp., 18 h, 75%; (viii) 6.1 eq. $(\beta\text{-CE})_2PN(iPr)_2$, 4.45 eq. tetrazole, CH_3CN, 0 °C to room temp., 105 min; 6.1 eq. I_2, THF/pyridine/H_2O 7/2/1, room temp., 5 min, quantitative; (ix) 70% HF.pyridine/THF, 17/83, room temp., 24 h, 84%; (x) NEt_3/CH_3CN 1/4.6 (v/v), room temp., 15 h, 85% (**7**), 98% (**9**), 49% (**10**), quantitative (**12**, **14**), 92% (**16**);: (xi) 3 eq. $(iPr)_2NP(OFm)_2$, 2.7 eq. tetrazole, CH_3CN/CH_2Cl_2 5/4 (v/v), room temp., 330 min, 3 eq. I_2, THF/pyridine/H_2O 7/2/1 (v/v), 5 min, 95%; (xii) HF.pyridine/THF 1/4.75 (v/v), room temp., 24 h, then 1/2.4, room temp., 8 h, 82%; (xiii) Ac_2O, pyridine, cat. DMAP, 1 h, 60%; (xiv) 1.5 eq. *N*-iodosuccinimide (NIS), 0.15 eq. $AgNO_3$, DMF, 90 min, quantitative; (xv) 1.73 eq. $K^+(^-OOCN{=}NCOO^-)^+K$, 3.47 eq. HOAc, 2-propanol/dioxane 11/1 (v/v), 870 min, 63%.

† Electronic supplementary information (ESI) available: experimental details. See http://www.rsc.org/suppdata/cc/b3/b305308n/

DOI: 10.1039/b305308n

of triethylamine in dry acetonitrile to yield the phosphate **6** as monotriethylammonium salt. The advanced intermediate **4** was used to generate more complex analogues. The mono-protected phosphate **7** could readily be synthesized by making use of the less base-labile β-cyanoethyl protecting group. After phosphitylation with the phosphoramidite $(NCCH_2CH_2O)_2PN(i\text{Pr})_2$, oxidation with I_2/pyridine/water, cleavage of the TBDPS group with HF–pyridine and treatment with an excess of triethylamine in dry acetonitrile, the phosphodiester **7** was obtained in high yield. Also the 9-fluorenylmethyl protected phosphotriester **8** was cleanly deprotected to alkyne **9** under basic conditions. In order to assess the importance of the C-11–OH group for biological activity acetylated compound **10** was synthesized in 2 steps in satisfactory yield from alcohol **8**. The iodoalkyne **11** and iodoalkene **13**, both intermediates in the synthesis of the natural product, were deprotected under standard conditions to the phosphates **12** and **14**. The saturated lactone **15** was obtained as a by-product during the diimide reduction of iodoalkyne **11** to iodoalkene **13**. The corresponding deprotected phosphate **16** was obtained by treatment with triethylamine and was considered useful to evaluate the relevance of the double-bond for biological activity.

The cytostatin analogues were evaluated by means of *in vitro* inhibition assays of the serine–threonine phosphatases of type 2A (PP2A) and 1 (PP1). These are of particular interest because cytostatin has been described as a highly selective PP2A, but not PP1, inhibitor.[4d] In addition, the inhibition of protein tyrosine phosphatases PTP1B (which is considered as a potential target for diabetes therapy[10]) and CD45 (a positive regulator of T-cell activation and therefore a potential target for treatment of autoimmune diseases and suppression of graft rejection[11]), and the dual-specifity phosphatase VHR (which dephosphorylates ERK, a member of the ras signal transduction pathway[12]) was examined. The enzymatic activity was determined by hydrolysis of *para*-nitrophenyl phosphate in standard buffers for $PP2A_1$,[13a] PP1,[13a] VHR,[13b] PTP1B,[13b] and of a commercial, phosphorylated peptide for CD45.‡

The shortened fragments of (4*S*,5*S*,6*S*,9*S*,10*S*,11*S*)-cytostatin (**1**) (Table 1, entry 1) show a lower activity against PP2A, albeit still in the nanomolar range. It is interesting to note that the alkyne **9** is less active than the more hydrophobic iodoalkyne **12**. This analogue in turn is less active than the (*Z*)-iodoalkene **14** which is nearly as potent as **1** (entries 4, 6 and 7). The acetylated derivative **10** is totally inactive (entry 5), while the saturated lactone **16** and the phosphodiester **7** show very weak activities with IC_{50} values in the high micromolecular range (entries 3, 8). From these results, a basic structure–activity relationship can be derived (Fig. 1). The unsaturated lactone is necessary for the biological activity of the natural product. It is prudent to speculate that covalent modification of the enzyme may take place, for example by nucleophilic attack of a cysteine residue in a Michael-type reaction.[14] Furthermore, the phosphate group must be fully deprotected, suggesting that a very tight interaction of the phosphate with the enzyme is required for inhibition. The C-11-hydroxy group seems to be essential for activity, as suggested by the lack of inhibition by the truncated compound **6** (entry 2) and the acetylated compound

Table 1 IC_{50} values (in μM) for inhibition of different phosphatases with cytostatin analogues

Entry	Compound	PP2A	PP1	VHR	PTP1B	CD45
1	**1**	0.033 ± 0.003	> 25[a]	> 25[a]	> 25[a]	> 25[a]
2	**6**	> 100[a]	> 100[a]	> 100[a]	> 100[a]	> 100[a]
3	**7**	42 ± 7	> 100[a]	> 100[a]	> 100[a]	—[b]
4	**9**	0.37 ± 0.05	> 100[a]	> 100[a]	> 100[a]	> 100[a]
5	**10**	> 100[a]	> 100[a]	> 100[a]	> 100[a]	—[b]
6	**12**	0.079 ± 0.009	> 100[a]	> 100[a]	> 100[a]	> 100[a]
7	**14**	0.039 ± 0.004	> 100[a]	> 100[a]	> 100[a]	> 100[a]
8	**16**	*ca.* 100	> 100[a]	> 100[a]	> 100[a]	—[b]

[a] Highest concentration investigated. [b] Not investigated.

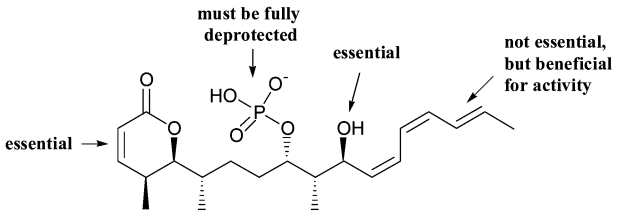


Fig. 1 SAR of (4*S*,5*S*,6*S*,9*S*,10*S*,11*S*)-cytostatin.

11. The triene moiety is not essential for PP2A inhibition. The presence of a (*Z*)-configured double bond with a hydrophobic group attached (here an iodine) seems to be sufficient for high PP2A-inhibitory activity.

Notably protein phosphatase 1 (PP1) was not inhibited by any of the cytostatin analogues in a *p*-NPP based assay identical to the PP2A assay. This demonstrates that variation of the structure of the natural product can be carried out without compromising the high selectivity described for the natural products of the fostriecin class. Furthermore VHR, PTP1B and CD45 were also not inhibited by any of the analogues. Clinical phase I trials of fostriecin have been stopped due to impurities in the natural product samples.[15] Most probably the triene moiety in fostriecin and cytostatin is responsible for the instability of the natural products upon storage. Thus, the finding that it may be replaced by simpler structural elements without loss of biological activity is of particular importance for the design of more stable but still active analogues.[16]

This research was supported by the Fonds der Chemischen Industrie. L. B. is grateful to the Fonds der Chemischen Industrie for a scholarship.

Notes and references

‡ The assay was performed using the commercially available BIOMOL GREEN™ CD45 Tyrosine Phosphatase Assay Kit.

1 T. Hunter, *Cell*, 2000, **100**, 113.
2 (*a*) K. Hinterding, D. Alonzo-Díaz and H. Waldmann, *Angew. Chem. Int. Ed.*, 1998, **37**, 668; (*b*) J. E. Sheppeck II, C.-M. Gauss and A. R. Chamberlin, *Bioorg. Med. Chem.*, 1997, **5**, 1739.
3 See for instance: J. S. Lazo, D. C. Aslan, E. C. Southwick, K. A. Cooley, A. P. Ducruet, B. Joo, A. Vogt and P. Wipf, *J. Med. Chem.*, 2001, **44**, 4042.
4 (*a*) M. Amemiya, M. Ueno, M. Osono, T. Masuda, N. Kinoshita, C. Nishida, M. Hamada, M. Ishizuka and T. Takeuchi, *J. Antibiot.*, 1994, **47**, 536; (*b*) M. Kawada, M. Amemiya, M. Ishizuka and T. Takeuchi, *Biochim. Biophys. Acta*, 1999, **1452**, 209.
5 review: D. S. Lewy, C.-M. Gauss, D. R. Soenen and D. L. Boger, *Curr. Med. Chem.*, 2002, **9**, 2005and references cited therein.
6 J. E. Sheppeck, II, C.-M. Gauss and A. R. Chamberlin, *Bioorg. Med. Chem.*, 1997, **5**, 1739.
7 L. Bialy and H. Waldmann, *Angew. Chem. Int. Ed.*, 2002, **41**, 1748.
8 W. C. Still and C. Gennari, *Tetrahedron Lett.*, 1983, **41**, 4405.
9 Y. Watanabe, T. Nakamura and H. Mitsumoto, *Tetrahedron Lett.*, 1997, **38**, 7407.
10 A. Cheng, N. Dubé, F. Gu and M. L. Tremblay, *Eur. J. Biochem.*, 2002, **269**, 1050.
11 A. I. Lazarovitz, S. Poppema, Z. Zhang, M. Khandaker, C. E. Le Feuvre, S. K. Singhal, B. M. Garcia, N. Ogasa, A. M. Jevnikar and M. H. White, *Nature*, 1996, **380**, 717.
12 T. Ishibashi, D. P. Bottaro, A. Chan, T. Miki and S. A. Aaronson, *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 12170.
13 (*a*) A. Takai and G. Mieskies, *Biochem. J.*, 1991, **275**, 233; X. Huang, M. R. Swingle and R. E. Honkanen, in *Methods in Enzymology, Vol. 315*, (Ed.: K. Palczewski), Academic Press, San Diego, p. 589; (*b*) T. Hamaguchi, A. Masuda, T. Morino and H. Osada, *Chem. Biol.*, 1997, **4**, 279.
14 The possible role of a cysteine residue in the PP2A inhibition of fostriecin has been discussed: D. R. H. Evans and J. A. Simon, *FEBS Letters*, 2001, **498**, 110.
15 R. S. de Jong, N. H. Mulder, D. R. A. Uges, D. Th. Sleijfer, F. J. P. Höppener, H. J. M. Groen, P. H. B. Willemse, W. T. A. van der Graaf and E. G. E. de Vries, *Brit. J. Canc.*, 1999, **79**, 882.
16 In this respect we note that cytostatin derived inhibitors **9**, **12** and **14** are significantly more stable upon storage that the natural product.