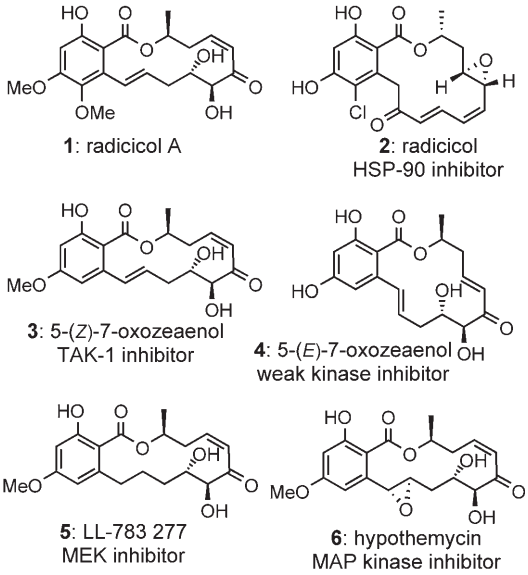
Total Synthesis

DOI: 10.1002/ange.200702406

Modular Synthesis of Radicicol A and Related Resorcylic Acid Lactones, Potent Kinase Inhibitors**

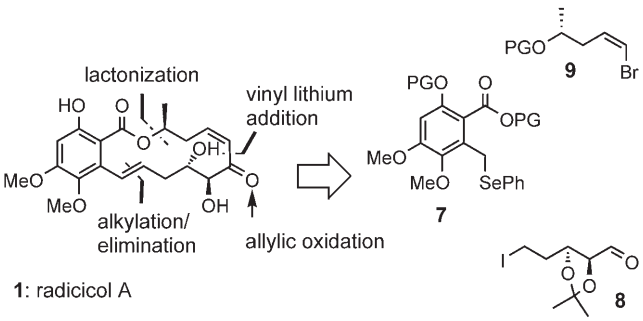
*Pierre-Yves Dakas, Sofia Barluenga, Frank Totzke, Ute Zirrgiebel, and Nicolas Winssinger**

Radicicol A (F87-25 09.04, **1**, Scheme 1) belongs to the family of resorcylic acid lactones[1] (RAL) and was first reported by researchers from Sandoz who identified this fungal metabolite from a screen for IL1β inhibition.[2] While it was observed that **1** accelerated the degradation of specific mRNA sequences including those of IL1β, its precise molecular target was not identified.[2,3] Subsequently, two other related resorcylic acid lactones containing a *cis*-enone (**3** and **5**, Scheme 1) were reported to be potent irreversible yet selective kinase inhibitors.[4,5] More recently, Santi and co-workers showed that hypothemycin, which also bears the *cis*-enone moiety, covalently inactivates ERK2 by reacting with a cysteine residue positioned in the active site (Cys166).[6]



Scheme 1. Structure of radicicol A and related resorcylides.

Our interest in RALs stems from the observation that a significant fraction of this family of natural products has been shown to inhibit kinases and ATPases (ATP = adenosine 5′-triphosphate). Despite the lack of obvious similarities between the RALs and ATP, these compounds have been shown to bind to the ATP-binding pocket of ATPases. We initiated a program to extend the diversity of RALs and to capitalize on this privileged structure.[1,7–9] A cornerstone of these efforts is the development of chemistry that is compatible with high-throughput synthesis. The disconnections that were envisioned for radicicol A and related *cis*-enone RALs are summarized in Scheme 2. A significant challenge is to control the *cis* geometry of the enone as it can isomerizes to the thermodynamically more favorable *trans* isomer as in compound **4**, which is known to be significantly less active.[5] It was thus anticipated that the enone should be revealed only at a late stage through a selective allylic oxidation and that the *cis*-alkene would originate from a vinyl lithium addition onto an aldehyde. As shown in Scheme 2, the molecule could thus be disconnected into three fragments (**7**, **8**, and **9**) of even complexity, in which the aldehyde or iodide of fragment **8** would need to be masked depending on the order of assembly. While the order of coupling of these three fragments is conceptually possible in all permutations, we reasoned that starting the sequence with the coupling of **8** + **9** would allow the use of a fluorinated protecting group for the alcohol **9**, thus enabling the use of fluorous isolation technology[10,11]



Scheme 2. Retrosynthetic disconnection of radicicol A. PG = protecting group.

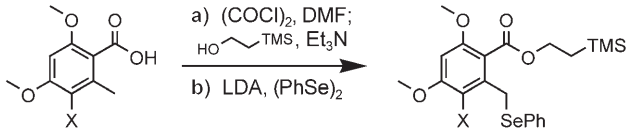
[*] P.-Y. Dakas, Dr. S. Barluenga, Prof. N. Winssinger
Institut de Science et Ingénierie Supramoléculaires
Université Louis Pasteur—CNRS UMR 7006
8 allée Gaspard Monge, 67000 Strasbourg (France)
Fax: (+33) 3-9024-5112
E-mail: winssinger@isis.u-strasbg.fr
Homepage: www-isis.u-strasbg.fr/winssinger/
F. Totzke, U. Zirrgiebel
ProQinase GmbH
Breisacher Strasse 117, Freiburg (Germany)
Homepage: www.proqinase.com

[**] This project was partly supported by grants from the Agence National de la Recherche (ANR) and Human Frontier Science Program (HFSP). An MENRT fellowship (P.Y.D.) is also gratefully acknowledged. The authors thank Kerstin Knepper for preliminary work on this project.

Supporting information for this article, including experimental procedures, physical characterization for the synthesis of **1**, **3**, and **31** as well as selectivity profile of compound **31** against a panel of 127 kinases, is available on the WWW under http://www.angewandte.org or from the author.

until the penultimate cyclization. Two features make this strategy particularly attractive. First and foremost, it has been shown that multiple components tagged with fluorinated tags of different lengths can be carried through a synthesis as a mixture and ultimately resolved using fluorous chromatography.[12,13] Second, this technology has now been automated, making it amenable to high-throughput synthesis.[14]

The aromatic fragment **7** was prepared with a silyl-based protecting group (TMSE, 2-(trimethylsilyl)ethyl) for the carboxylic acid. As shown in Scheme 3, this synthesis was



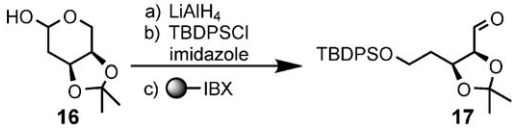
X = OMe (**10**), H (**11**), or Cl (**12**) X = OMe (**13**), H (**14**), or Cl (**15**)

Scheme 3. Synthesis of aromatic moieties **13**–**15**. a) $(COCl)_2$, (1.0 equiv), DMF (cat.), CH_2Cl_2, 0 °C, 1 h, then 2-(trimethylsilyl)ethanol (1.0 equiv), Et_3N (2.6 equiv), 4-DMAP (cat.), 23 °C, 1 h, 96–98 %; b) LDA (2.0 equiv), $(PhSe)_2$ (1.0 equiv), THF, −78 °C, 1 h, 89–91 %, 4-DMAP = 4-dimethylamino pyridine, LDA = lithium diisopropylamide, TMS = trimethylsilyl.

achieved in two steps from the different acids **10**–**12** by esterification with 2-(trimethylsilyl)ethanol and subsequent formation of the selenide via LDA deprotonation of the benzylic position and reaction with diphenyldiselenide. This procedure was found to be effective for the trimethoxy-substituted aryl ring corresponding to radicicol A (**10**) as well as for the dimethoxy aryl ring corresponding to 5-(*Z*)-7-oxozeaenol (**11**) and the chlorodimethoxy substitution present in radicicol (**12**). While several protecting groups for the *ortho*-phenol (MOM (methoxymethyl), EOM (ethoxymethyl), PMB (*para*-methoxybenzyl)) were found to be suitable in the case of the 2,4-dimethoxy substitution, these protecting groups were not stable for the 2,4,5-trimethoxy-subtituted aryl ring. The presence of the 5-methoxy substituent renders any acid-labile protecting group at the 2-position particularly susceptible to acids and, more generally, makes this aryl moiety prone to oxidation. Nevertheless, it was found that a methyl ether could be cleaved very selectively in the presence of the other methyl ethers using boron trichloride for all three aromatic systems.

Intermediate **8**, with the iodide masked in the form of a silyl-protected hydroxyl group, was conveniently obtained from acetal-protected deoxyribose[15] in three steps (Scheme 4). Protected deoxyribose (obtained in one step from 2-deoxy-D-ribose) was reduced using $LiAlH_4$, and the crude reaction product was selectively silylated on the sterically less encumbered alcohol using TBDPSCl (greater than 15:1 selectivity) in the presence of imidazole in DMF. The free alcohol was then oxidized using an immobilized version of IBX,[16] thus affording the aldehyde, which could be used directly in the subsequent reaction without workup or further purification.

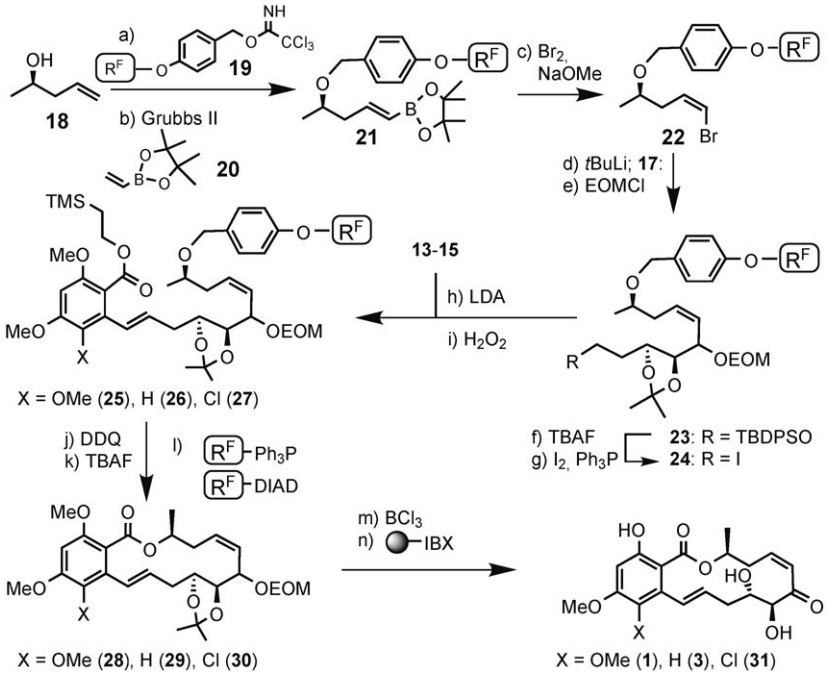
The synthesis of fragment **9** began with the protection of (*R*)-2-hydroxypentene (**18**, Scheme 5) with a fluorous version



Scheme 4. Synthesis of key intermediate **17**. a) $LiAlH_4$ (1.4 equiv), THF, from 0 °C to 23 °C, 2 h, 95 %; b) TBDPSCl (1.0 equiv), imid. (1.5 equiv), DMF, 23 °C, 2 h, 66 %; c) PS-IBX (3.0 equiv), CH_2Cl_2, 23 °C, 2 h, 100 %, DMF = dimethyl formamide, IBX = 2-iodoxybenzoic acid, TBDPS = *tert*-butyldiphenylsilyl.

of PMB trichloroacetimidate **19**.[12,17] Cross-metathesis with the vinyl borolane **20** afforded the *trans*-vinyl borolane[18] **21** in excellent yield and stereoselectivity (greater than 20:1 *E*/*Z*) using the second-generation Grubbs catalyst or even faster and with equally good stereoselectivity using the Grela modified catalyst.[19] Importantly, the *trans*-vinylborolane **21** could be stereospecifically converted to the *cis*-vinyl bromide **22** in excellent yield using the protocol of Brown et al.[20] Transmetalation of **22** with *t*BuLi and addition onto the crude aldehyde **17** afforded, after EOM protection, product **23** as a mixture of diastereoisomers (3:1). This lack of selectivity is inconsequential, as the stereogenic center in question will ultimately be oxidized to the ketone. Conversion of the silyl-protected hydroxyl group to the iodide (TBAF; Ph_3P, I_2) afforded compound **24**, which was alkylated in excellent yield with the three different aromatic fragments **13**–**15** previously deprotonated by LDA. The selenide was then oxidized and eliminated to afford compounds **25**–**27**. This reaction sequence did not require a single traditional workup. The crude reaction mixtures were simply loaded on fluorous-silica columns and eluted first with 75 % MeOH in water to remove all non-fluorous-tagged components and then washed with MeOH to recover the desired compounds. All the compounds (**21**–**27**) came at the solvent front with the MeOH wash and could be recovered in excellent yields. Reactions that were performed under basic conditions (**22** to **23** and alkylation with **24**) were quenched with benzoic acid resin prior to loading on the column. The PMB group was removed under the action of DDQ, and again, the byproducts of these reactions were easily sequestered using fluorous solid-phase extraction, while the TMSE ester was remove with TBAF. The hydroxyacids thus obtained were engaged in Mitsunobu macrocyclisations using fluorous-tagged triphenyl phosphine and diazo dicaboxylate, thus yielding the macrocycles **28**–**30** after a fluorous solid-phase extraction. The EOM and acetonide groups could be quantitatively deprotected using sulfonic acid resin in MeOH, while the *ortho*-phenol could be selectively cleaved in the presence of the other methyl ethers using BCl_3, or more conveniently, all operations could be achieved in one step with BCl_3. The allylic alcohols were then selectively oxidized with the polymer-bound version of IBX to afford radicicol A (**1**),[21] 5-(*Z*)-7-oxozeaenol (**3**),[22] and radicicol analogue **31** in nearly quantitative yield following a simple filtration.

Radicicol A and the analogues bearing different substituents on the aromatic ring as well as their corresponding allylic alcohols were tested in vitro against a panel of 24 kinases to assess their activity and specificity. In agreement with the



Scheme 5. Synthesis of radicicol A (**1**), 5-(*Z*)-7-oxozeaenol (**3**), and radicicol A analogue **31**. a) **19** (1.0 equiv), CSA (cat.), CH_2Cl_2, 23 °C, 12 h, 92%; b) **20** (2.0 equiv), Grubbs II (2.5 mol%); toluene, 80 °C, 12 h,92%; c) Br_2 (1.0 equiv, 1 M in CH_2Cl_2), Et_2O, −20 °C, 10 min, then NaOMe (2.2 equiv, 1 M in MeOH), −20 °C, 30 min, 89%; d) *t*BuLi (2.0 equiv), THF/Et_2O, −100 °C, 15 min, then **17** (1.0 equiv), −100 °C, 15 min, 88%; e) EOMCl (8.0 equiv), *i*Pr_2EtN (8.0 equiv), TBAI (cat.), DMF, 23 °C, 12 h, 96%; f) TBAF (2.5 equiv), THF, 23 °C, 12 h, 92%; g) PPh_3 (1.5 equiv), I_2 (1.5 equiv), imidazole (2.5 equiv), THF, 0 °C, 1 h, 91%; h) **13**–**15** (1.0 equiv), LDA (2.0 equiv), THF/HMPA 10:1, −78 °C, 20 min, 88–91%, i) H_2O_2 (2.0 equiv), THF, 23 °C, 2 h, 79–82%, j) DDQ (1.2 equiv), CH_2Cl_2/H_2O 2:1, 23 °C, 2 h, 70–80%; k) TBAF (3.0 equiv), THF, 23 °C, 2 h, 87%; l) R^FPh_3P (2.0 equiv), R^FDEAD (2.0 equiv), toluene (10 mM), 23 °C, 2 h, 81%; m) BCl_3 (3.0 equiv), CH_2Cl_2, 0 °C, 15 min, 86%; PS-IBX (3.0 equiv), CH_2Cl_2, 23 °C, 1 h, greater than 90%. CSA = camphor-10-sulfonic acid, DEAD = ethoxycarbonylazocarboxymethyl, DDQ = dichlorodicyanoquinone, Grubbs II = ruthenium[1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(phenylmethylene)(tricyclohexylphosphine), HMPA = hexamethylphosphoramide, LDA = lithium diisopropylamide, $R^F = C_3H_6C_6F_{13}$, TBAF = tetrabutylammonium fluoride, TBAI = tetrabutylammonium iodide, TBDPS = *tert*-butyldiphenylsilyl.

proposal that the enone moiety acts as a Michael acceptor, none of the products lacking the *cis*-enone showed significant activity (data not shown). Radicicol A, on the other hand, exhibited potent inhibitory activity (low nanomolar range) against VEGF-R2, VEGF-R3, FLT3, and PDGFR-β (Table 1). From a therapeutic perspective, radicicol A's 2-hydroxy-5-methoxy aryl substitution may present a liability, as such electron-rich aryl moieties may be oxidized to the corresponding quinones. We have also noted that compounds related to **3** could be oxidized to the quinone, albeit with stronger oxidizing agents, such as *m*CPBA (*m*-chloroperbenzoic acid). A number of RALs, such as pochonins C and D, are substituted with a chlorine atom at the 5-position rather than a methoxy group. Inspired by these analogues, we tested compound **31** in the same panel of kinases. While it was overall slightly less active than **1** and **3**, it remained a potent inhibitor of therapeutically important kinases (Table 1). The activity of this compound was further evaluated in a panel of 127 kinases, which showed that the only kinases inhibited at nanomolar concentrations were VEGF-R2 and 3, PDGFR-α and β, as well as MEK1 (see the Supporting Information), all of which have a cysteine residue in the active site. Kinases inhibited at low micromolar concentrations included FLT3, KIT, and VEGF-R1, which also bear a cysteine residue in the active site. The discrimination by **31** among the subset of kinases bearing an appropriately positioned cysteine residue indicates that unique interactions with its target are necessary for the Michael addition to proceed and that its selectivity could be honed or tuned to other kinases. The potential efficacy of this compound in vivo was then assessed by measuring the level of VEGF-R2 autophosphorylation in the presence of its ligand ($VEGF_{165}$). Immortalized human umbilical vein endothelial cells (HUVECs) known to express high levels of VEGF-R2 were incubated with inhibitor **31** for 90 min and then stimulated with $VEGF_{165}$ for 7 min. The level of autophosphorylation was measured by ELISA using anti-VEGF-R2 as capture antibody and anti-phosphotyrosine as detection antibody. Results are expressed as a percentage of maximal autophosphorylation in the absence of inhibitor (Figure 1). Compound **31** was found to have a cellular IC_{50} of 440 nM, which is consistent with its inhibition at the enzymatic level (90 nM). Consistently, PDGFR-β autophosphorylation was mildly inhibited by **31** (IC_{50} =

Table 1: IC_{50} profile (nM) against a panel of 24 therapeutically relevant kinases.[a]

	AKT1	ARK5	Aurora-A	Aurora-B	B-RAF VE	CDK2/CycA	CDK4/CycD1	CK2-α1	FAK	EPHB4	ERBB2	EGF-R	IGF1-R	SRC	VEGF-R2	VEGF-R3	FLT3	INS-R	MET	PDGFR-β	PLK1	SAK	TIE2	COT
radicicol A (**1**)									9 100				9 600	26	66	110			210					
oxozeaenol (**3**)				6 300									6 600	52	110	170			340					
Cl-radicicol A (**31**)														90	210	1 800			370					

[a] Empty fields: $IC_{50} > 100000$ nM.

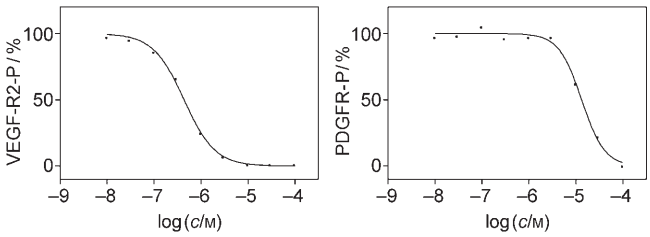


Figure 1. Cellular inhibition of VEGF-R2 (left) and PDGFR-β (right) autophosphorylation upon stimulation with their cognate ligand after preincubation with compound **31** (the *y* axis represents the percentage of phosphorylation compared to maximal phosphorylation).

13 μM), whereas autophosphorylation of TIE2 was unaffected. The demonstration that radicicol A inhibits key kinases involved in MAPK cascades can rationalize the original observation that it accelerates the degradation of Il1β's RNA, as there is mounting evidence that MAP kinases are involved in the regulation of protein translation by controlling the stability of specific mRNAs.[23,24]

In conclusion, we have developed a concise synthesis of radicicol A and related resorcylic acid lactones using a combination of fluorous tag-isolation and polymer-bound reagents, thus making the chemistry amenable to high-throughput synthesis. In addition to the therapeutic potential of this family of natural products, the irreversible nature of these inhibitors also offers a new lead structure for the design of selective inhibitors towards engineered kinases[25,26] as well as for chemical proteomics.[27]

Received: June 3, 2007
Published online: August 3, 2007

Keywords: inhibitors · resorcylic acid lactones · solid-phase extraction · total synthesis

[1] N. Winssinger, S. Barluenga, *Chem. Commun.* **2007**, 22.
[2] T. Kastelic, J. Schnyder, A. Leutwiler, R. Traber, B. Streit, H. Niggli, A. Mackenzie, D. Cheneval, *Cytokine* **1996**, *8*, 751.
[3] D. Cheneval, P. Ramage, T. Kastelic, T. Szelestenyi, H. Niggli, R. Hemmig, M. Bachmann, A. Mackenzie, *J. Biol. Chem.* **1998**, *273*, 17846.
[4] J. Ninomiya-Tsuji, T. Kajino, K. Ono, T. Ohtomo, M. Matsumoto, M. Shiina, M. Mihara, M. Tsuchiya, K. Matsumoto, *J. Biol. Chem.* **2003**, *278*, 18485; for the original isolation of 5-(*Z*)-7-oxozeaneol (also known as LL-Z1640-2), see: G. A. Ellestad, F. M. Lovell, N. A. Perkinson, R. T. Hargreaves, W. J. McGahren, *J. Org. Chem.* **1978**, *43*, 2339.
[5] A. Zhao, S. H. Lee, M. Mojena, R. G. Jenkins, D. R. Patrick, H. E. Huber, M. A. Goetz, O. D. Hensens, D. L. Zink, D. Vilella, A. W. Dombrowski, R. B. Lingham, L. Huang, *J. Antibiot.* **1999**, *52*, 1086.
[6] A. Schirmer, J. Kennedy, S. Murli, R. Reid, D. V. Santi, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 4234.
[7] S. Barluenga, P. Y. Dakas, Y. Ferandin, L. Meijer, N. Winssinger, *Angew. Chem.* **2006**, *118*, 4055; *Angew. Chem. Int. Ed.* **2006**, *45*, 3951; .
[8] E. Moulin, S. Barluenga, F. Totzke, N. Winssinger, *Chem. Eur. J.* **2006**, *12*, 8819.
[9] E. Moulin, V. Zoete, S. Barluenga, M. Karplus, N. Winssinger, *J. Am. Chem. Soc.* **2005**, *127*, 6999.
[10] D. P. Curran, *Handbook of Fluorous Chemistry*, Wiley-VCH, Weinheim, **2004**, p. 101.
[11] W. Zhang, D. P. Curran, *Tetrahedron* **2006**, *62*, 11837.
[12] S. Dandapani, M. Jeske, D. P. Curran, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 12008.
[13] Z. Luo, Q. Zhang, Y. Oderaotoshi, D. P. Curran, *Science* **2001**, *291*, 1766.
[14] W. Zhang, Y. Lu, *J. Comb. Chem.* **2006**, *8*, 890.
[15] J. Barbat, J. Gelas, D. Horton, *Carbohydr. Res.* **1983**, *116*, 312.
[16] G. Sorg, A. Mengei, G. Jung, J. Rademann, *Angew. Chem.* **2001**, *113*, 4532; *Angew. Chem. Int. Ed.* **2001**, *40*, 4395.
[17] Q. Zhang, H. Lu, C. Richard, D. P. Curran, *J. Am. Chem. Soc.* **2004**, *126*, 36.
[18] C. Morrill, R. H. Grubbs, *J. Org. Chem.* **2003**, *68*, 6031.
[19] A. Michrowska, R. Bujok, S. Harutyunyan, V. Sashuk, G. Dolgonos, K. Grela, *J. Am. Chem. Soc.* **2004**, *126*, 9318.
[20] H. C. Brown, C. Subrahmanyam, T. Hamaoka, N. Ravindran, D. H. Bowman, S. Misumi, M. K. Unni, V. Somayaji, N. G. Bhat, *J. Org. Chem.* **1989**, *54*, 6068.
[21] The synthetic product was found to be identical to a natural sample kindly provide by Dr. Traber (Novartis).
[22] For previous syntheses, see: K. Tatsuta, S. Takano, T. Sato, S. Nakano, *Chem. Lett.* **2001**, 172; P. Selles, R. Lett, *Tetrahedron Lett.* **2002**, *43*, 4627.
[23] M. Gaestel, *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 120.
[24] D. V. Henley, C. J. Bellone, D. A. Williams, M. F. Ruh, *Arch. Biochem. Biophys.* **2004**, *424*, 112.
[25] J. A. Blair, D. Rauh, C. Kung, C. H. Yun, Q. W. Fan, H. Rode, C. Zhang, M. J. Eck, W. A. Weiss, K. M. Shokat, *Nat. Chem. Biol.* **2007**, *3*, 229.
[26] M. S. Cohen, C. Zhang, K. M. Shokat, J. Taunton, *Science* **2005**, *308*, 1318.
[27] C. Drahl, B. F. Cravatt, E. J. Sorensen, *Angew. Chem.* **2005**, *117*, 5936; *Angew. Chem. Int. Ed.* **2005**, *44*, 5788.

