Natural Product Synthesis

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Syntheses of Xanthofulvin and Vinaxanthone, Natural Products Enabling Spinal Cord Regeneration**

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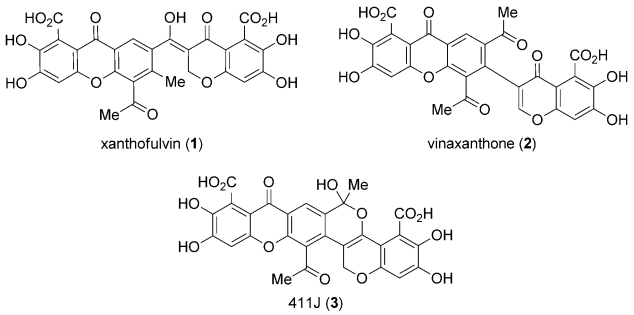
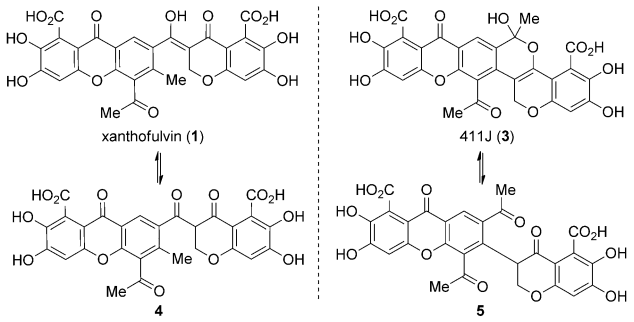
The failure of neurons in the central nervous system (CNS) to undergo regeneration following injury accounts for the permanent and debilitating effects that accompany spinal cord injury, for which there is no cure. Gene therapy, biologics, and stem-cell-based approaches have received considerable attention in promoting CNS regeneration, while the use of low-molecular-weight compounds has not been as extensively investigated.[1] However, in the context of spinal cord injury small molecules hold considerable potential for the accelerated development of new therapeutics. The delivery of drugs directly into the spinal cavity through spinal injection can expedite small-molecule-based drug development. Moreover, a variety of hydrogels and other polymers for continuous drug delivery, developed specifically for spinal cord therapy, when coupled with a validated small molecule will provide a unique and promising platform for therapeutic development.[2]

The natural product xanthofulvin (**1**, also named SM-216289) represents one of the most promising leads in the development of treatments for spinal cord injury. Xanthofulvin (**1**) and the related compound vinaxanthone (**2**) were isolated from fungal extracts of *Penicillium* sp. SPF-3059 (Scheme 1).[3] Both compounds strongly block the effects of the inhibitor of axonal regeneration semaphorin3A (Sema3A) with no observable cytotoxicity at concentrations above 1000 times the effective dose.[3b,4] Animal studies of xanthofulvin have demonstrated remarkable effects after complete spinal cord transection.[5] The dramatically improved functional recovery observed resulted from significant axonal regeneration and myelination, reduction of the number of apoptotic cells, and enhanced angiogenesis. While the pronounced effects of xanthofulvin have been attributed to the inhibition of Sema3A, removal of Sema3A function does not enhance regeneration after spinal cord injury, thus suggesting that the natural product functions through a more complex mode of action than initially described.



Scheme 1. Structures of xanthofulvin (**1**), vinaxanthone (**2**), and 411J (**3**).

In addition to questions surrounding the modes of action, the atomic connectivity of xanthofulvin was previously unclear, since there were two conflicting structures proposed. The isolation and structural characterization data of the natural products xanthofulvin (**1**) and 411J (**3**) reveal the structures show nearly identical spectral properties (Scheme 2).[6] In addition, the keto form **4** of xanthofulvin



Scheme 2. Equilibria of xanthofulvin (**1**) and **4** and 411J (**3**) and **5**.

and the keto form **5** of 411J also possess overlapping spectral properties.[7] This observation led us to the conclusion that the assignments for xanthofulvin and 411J were based on the same natural product. In both studies the natural product was co-isolated with vinaxanthone (**2**) as well. We sought to resolve these conflicting structural assignments through the synthesis of xanthofulvin (**1**), the structure that appeared the most plausible.

We propose the formation of xanthofulvin can proceed through the union of the known natural product polivione with 5,6-dehydropolivione (**6**), a putative unsaturated derivative of the known natural product polivione.[8,9] Similarly, the

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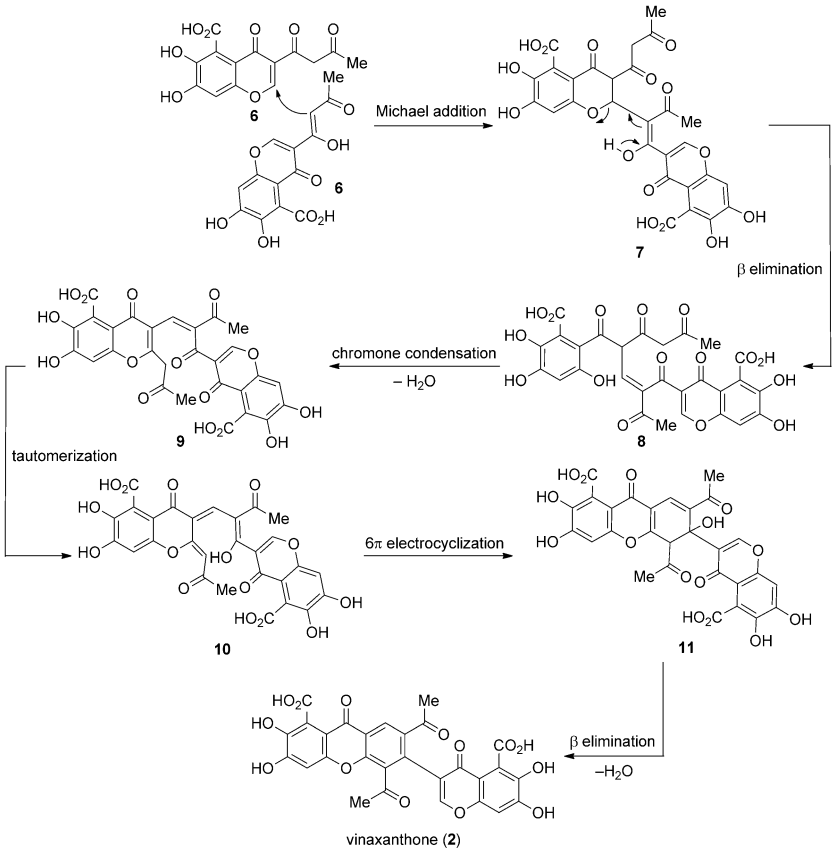
formation of vinaxanthone can be envisioned to occur through a dimerization of 5,6-dehydropolivione (Scheme 3). These proposals provided a template for the development of our synthetic routes. Previous proposals for the biosyntheses of xanthofulvin and vinaxanthone have been postulated to occur through a Knovenagel sequence[10] or, in the case of vinaxanthone, through a Diels–Alder/oxidative aromatization sequence. The Diels–Alder/oxidative aromatization sequence was applied in the sole existing synthesis of vinxanthone.[11] Previous to this report there was no laboratory preparation of xanthofulvin.



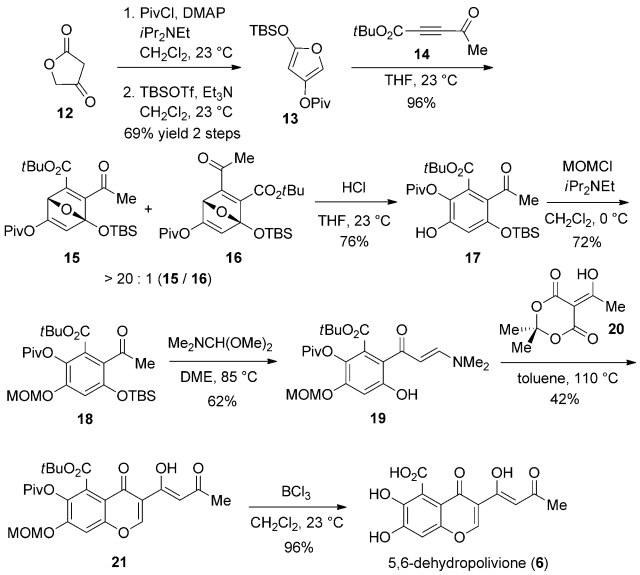
Scheme 3. Proposed dimerization of 5,6-dehydropolivione (**6**) generating vinaxanthone (**2**).

The synthesis of 5,6-dehydropolivione (**6**) was accomplished in eight steps from tetronic acid (**12**; Scheme 4). A regioselective Diels–Alder reaction of furan **13**,[12] prepared from tetronic acid (**12**), with keto ester **14** (also prepared in as few as two steps[13]) provided the bicyclic adduct **15** in good yield and high regioselectivity (>20:1).[14] The high level of selectivity was possible owing to the polarization of the diene in concert with the ketone functioning as the dominant activating group. Treatment of Diels–Alder adduct **15** with hydrochloric acid induced aromatization, providing acetophenone **17** with migration of the pivaloyl group.[15] The first four steps of this sequence have been performed without chromatography and on scales above 50 g.

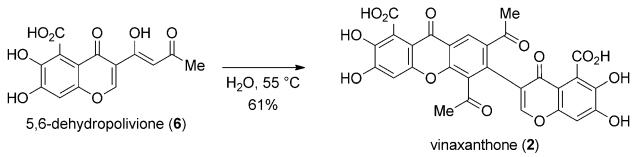
Protection of phenol **17** was required prior to chromone-triketone formation and was accomplished using methoxymethyl chloride and Hünig's base. Enaminone formation by using the dimethyl acetal of dimethylformamide in dimethoxyethane at elevated temperature occurred with simultaneous cleavage of the TBS group, thus generating enaminone **19**. Conversion of enaminone **19** to triketone **21** was achieved by treatment of **19** with 5-acetyl Meldrum's acid (**20**) in toluene heated to reflux, yielding protected 5,6-dehydropolivione (**21**) in 42% yield.[16] Simultaneous cleavage of the *tert*-butyl ester, pivaloyl, and methoxymethyl groups with boron trichloride in dichloromethane at 23°C provided 5,6-dehydropolivione (**6**) in 96% yield.

In agreement with our proposal, heating 5,6-dehydropolivione (**6**) in deionized water to 55°C provided vinaxanthone (**2**) in 61% yield (Scheme 5). Thus, vinaxanthone was synthesized in nine steps from tetronic acid, whereas the previous synthesis required 14 steps to provide the natural product.

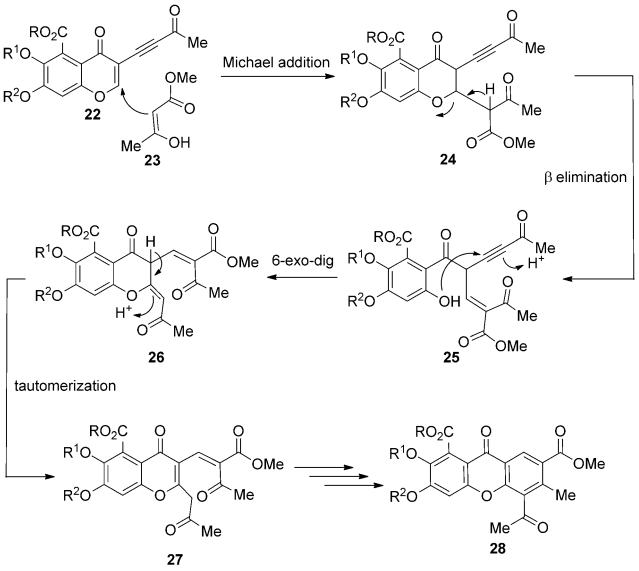
A related approach to access xanthofulvin failed owing to the facile dimerization of 5,6-dehydropolivione (**6**) forming vinaxanthone. Therefore, an ynone was envisioned as a surrogate for 5,6-dehydropolivione; this ynone would function only as a Michael-acceptor (Scheme 6).[17a] Related reactivity of 3-(1-alkynyl)chromones was previously described by Hu and co-workers.[17b] Michael addition into the chromone portion of ynone **22** and bond fragmentation provides phenol **25** that is poised to undergo conjugate addition and, after alkene



Scheme 4. Synthesis of 5,6-dehydropolivione (**6**) from tetronic acid (**12**). Piv = pivaloyl, DMAP = 4-(dimethylamino)pyridine, TBS = *tert*-butyldimethylsilyl, MOM = methoxymethyl, DME = 1,2-dimethoxyethane.



Scheme 5. Dimerization of 5,6-dehydropolivione (**6**) under neutral conditions to generate vinaxanthone (**2**).

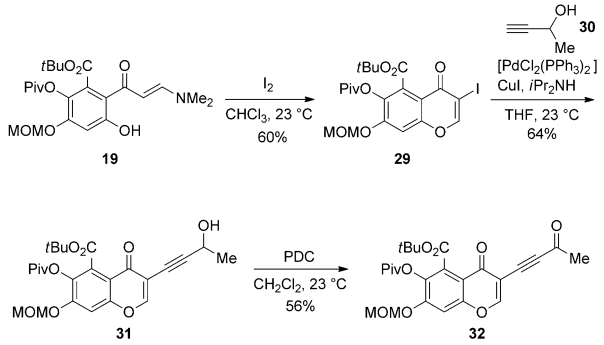


Scheme 6. Coupling of ynone **22** with methyl acetoacetate (**23**).

isomerization, generate adduct **27**. The tetracarbonyl-containing portion of adduct **27** can tautomerize and react through a 6π electrocyclization and subsequent dehydration as described in the biosynthetic proposal (Scheme 3), providing the xanthone core of xanthofulvin (**28**).

Reaction of the previously prepared enaminone **19** with iodine in chloroform at 23 °C generated iodochromone **29** (Scheme 7).[18] Sonogashira coupling of iodochromone **29** with 3-butyn-2-ol (**30**)[19] and subsequent oxidation[20] yielded ynone **32** as a solid (m.p. 178–179 °C).

As proposed in Scheme 6 addition of the sodium anion of methylacetoacetate into ynone **32** proceeded to generate the desired pentasubstituted arene **33** in 83% yield (Scheme 8).

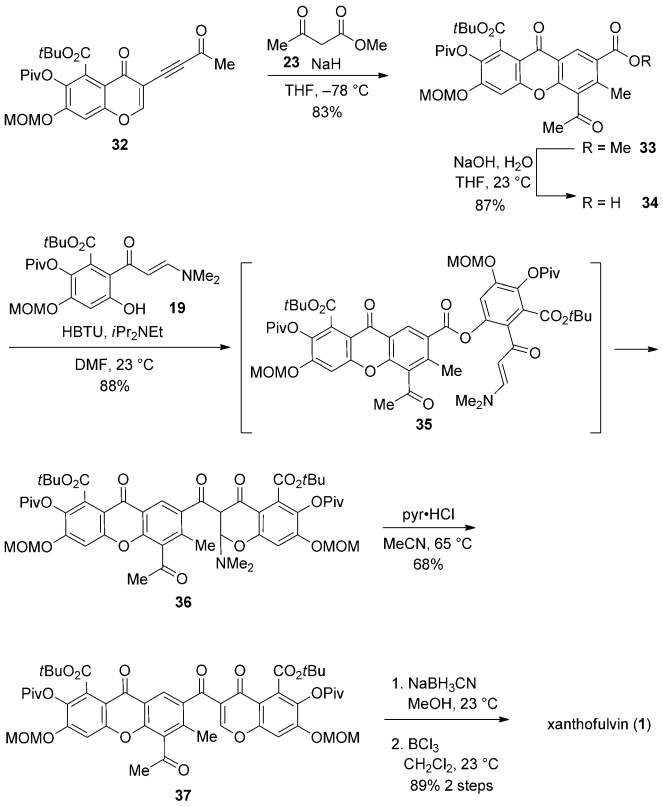


Scheme 7. Synthesis of ynone **32**. PDC = pyridinium dichromate.

The major by-product of the reaction (14%) was the derivative of **33** lacking the acetyl group. Saponification of the methyl ester with sodium hydroxide in a 3:1 tetrahydrofuran/water mixture yielded the corresponding carboxylic acid **34**. After optimization, coupling of carboxylic acid **34** and enaminone **19** using HBTU and Hünig's base in dimethylformamide at 23 °C generated diketone **36** in 88% yield.[21] Existing methods for coupling *ortho*-hydroxy aryl enaminones with carboxylic acid derivatives to initiate O-to-C carboxyl transfers employ anhydrides and other activated carboxylic derivatives.[22] The strategy of using coupling reagents proved more direct and tolerant of sensitive functionality. Elimination of dimethylamine using pyridinium chloride in acetonitrile generated endione **37** that underwent conjugate reduction with sodium cyanoborohydride yielding xanthofulvin in protected form.

Simultaneous removal of all six oxygen-bound groups on the phenols and carboxylic acids was accomplished with boron trichloride in dichloromethane at 23 °C providing material that matched all the reported spectral values for xanthofulvin (**1**) and 411J (**3**; Scheme 8). Synthesis has confirmed the structural assignment as that described for xanthofulvin (**1**), thus indicating the structural reassignment of 411J (**3**) to that of xanthofulvin (**1**) is required.

Through our in vivo outgrowth assay xanthofulvin and vinaxanthone were found to enhance neuronal outgrowth. This assay utilizes green fluorescent protein (GFP)-labeled



Scheme 8. Completion of the synthesis of xanthofulvin (**1**). HBTU = *O*-(1*H*-benzotriazoyl-1-yl)-*N*,*N*,*N*′,*N*,′-tetramethyluronium hexafluorophosphate.

cholinergic neurons in *C. elegans* to visualize enhanced branching after treatment with neurotrophic compounds.[23] Similar to other known neurotrophic compounds, xanthofulvin and vinaxanthone were found to increase the rate of branching in *C. elegans* with 32% and 31% of the treated animals displaying outgrowth at 2 μM respectively. This activity is comparable to dibutyryl cAMP,[24] which promotes branching in 36% of animals at 2 μM (Figure 1).

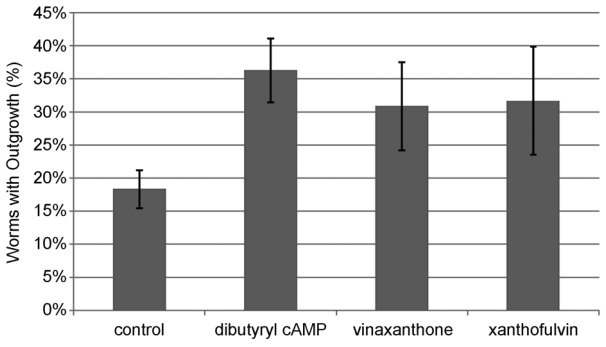


Figure 1. Outgrowth of GFP-labled cholinergic neurons in vivo in *C. elegans* after treatment with dibutyryl cAMP, xanthofulvin, and vinaxanthone. Control: 0.2% DMSO in M9 buffer.

The synthesis of xanthofulvin has provided laboratory access to this promising regenerative natural product for the first time. The second synthesis of vinaxanthone was achieved and provides the natural product in 2/3 the number of steps previously achieved. The chemical transformations employed in the syntheses included: a highly regioselective Diels–Alder reaction of an ynone ester, tandem reaction sequences for the formation of vinaxanthone from 5,6-dehydropolivione and the core of xanthofulvin, and an HBTU-mediated coupling of carboxylic acid derivative **33** and *ortho*-hydroxy enaminone **19** with subsequent O-to-C transfer, bringing two functionalized fragments together. The validation of the structural assignment of the natural product as that described for xanthofulvin has resolved ambiguity regarding the alternative assignment of 411J (**3**). Tests of synthetic xanthofulvin and vinaxanthone in vivo demonstrated the compounds possess growth-promoting activity. With synthetic access future efforts to optimize the biological performance of the natural products and to describe the modes of action will be pursued.

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[1] R. M. Wilson, S. J. Danishefsky, *Acc. Chem. Res.* **2006**, *39*, 539–549.
[2] a) N. N. Madigan, S. McMahon, T. O'Brien, M. J. Yaszemski, A. J. Windebank, *Respir. Physiol. Neurobiol.* **2009**, *169*, 183–199; b) J. Baier Leach, K. A. Bivens, C. W. Patrick, Jr., C. E. Schmidt, *Biotechnol. Bioeng.* **2003**, *82*, 578–589; c) J. Struve, P. C. Maher, Y. Q. Li, S. Kinney, M. G. Fehlings, C. t. Kuntz, L. S. Sherman, *Glia* **2005**, *52*, 16–24; d) T. Gros, J. S. Sakamoto, A. Blesch, L. A. Havton, M. H. Tuszynski, *Biomaterials* **2010**, *31*, 6719–6729.
[3] a) M. Aoki, Y. Itezono, H. Shirai, N. Nakayama, A. Sakai, Y. Tanaka, A. Yamaguchi, N. Shimma, K. Yokose, H. Seto, *Tetrahedron Lett.* **1991**, *32*, 4737–4740; b) K. Kumagai, N. Hosotani, K. Kikuchi, T. Kimura, I. Saji, *J. Antibiot.* **2003**, *56*, 610–616.
[4] K. K. Kumagai, K. Kishino, A. Hosotani, N. Ito, A. Saji, I. Kimura, *Sumitomo Kagaku* **2005**, 1–8.
[5] S. Kaneko, A. Iwanami, M. Nakamura, A. Kishino, K. Kikuchi, S. Shibata, H. J. Okano, T. Ikegami, A. Moriya, O. Konishi, C. Nakayama, K. Kumagai, T. Kimura, Y. Sato, Y. Goshima, M. Taniguchi, M. Ito, Z. He, Y. Toyama, H. Okano, *Nat. Med.* **2006**, *12*, 1380–1389.
[6] S. K. Wrigley, M. A. Latif, T. M. Gibson, M. I. Chicarellirobinson, D. H. Williams, *Pure Appl. Chem.* **1994**, *66*, 2383–2386.
[7] See the Supporting Information for a comparison of the spectra of xanthofulvin and 411J.
[8] a) A. K. Demetriadou, E. D. Laue, F. J. Leeper, J. Staunton, *J. Chem. Soc. Perkin Trans. 1* **1988**, 763–768; b) A. K. Demetriadou, E. D. Laue, J. Staunton, *J. Chem. Soc. Perkin Trans. 1* **1988**, 769–772; c) A. K. Demetriadou, E. D. Laue, F. J. Leeper, J. Staunton, *J. Chem. Soc. Chem. Commun.* **1985**, 762–764; d) A. K. Demetriadou, E. D. Laue, J. Staunton, *J. Chem. Soc. Chem. Commun.* **1985**, 764–766.
[9] See the Supporting Information for full schemes for the proposed dimerization of 5,6-dehydropolivione and the reaction of polivione with dehydropolivione.
[10] S. L ösgen, O. *Schlörke*, K. Meindl, R. Herbst-Irmer, A. Zeeck, *Eur. J. Org. Chem.* **2007**, 2191–2196.
[11] K. Tatsuta, S. Kasai, Y. Amano, T. Yamaguchi, M. Seki, S. Hosokawa, *Chem. Lett.* **2007**, *36*, 10.
[12] T. M. Balthazor, E. L. Williams, *Synth. Commun.* **1992**, *22*, 1023–1026.
[13] L. A. M. Cornelius, R. G. A. Bone, R. H. Hastings, M. A. Deardorff, R. A. Scharlach, B. E. Hauptmann, C. J. Stankovic, H. W. Pinnick, *J. Org. Chem.* **1993**, *58*, 4774–4774.
[14] A. Gorgues, A. Simon, A. Lecoq, A. Hercouet, F. Corre, *Tetrahedron* **1986**, *42*, 351–370.
[15] T. R. Kelly, S. H. Bell, N. Ohashi, R. J. Armstrong-Chong, *J. Am. Chem. Soc.* **1988**, *110*, 6471–6480.
[16] a) Y. S. Oikawa, K. Sugano, O. Yonemitsu, *J. Org. Chem.* **1978**, *43*, 2087–2088; b) F. Xu, J. D. Armstrong, G. X. Zhou, B. Simmons, D. Hughes, Z. H. Ge, E. J. J. Grabowski, *J. Am. Chem. Soc.* **2004**, *126*, 13002–13009.
[17] a) F. Eiden, H. Fenner, *Chem. Ber.* **1968**, *101*, 2894–2895; b) L. Z. Zhao, F. C. Xie, G. Cheng, Y. H. Hu, *Angew. Chem.* **2009**, *121*, 6642–6645; *Angew. Chem. Int. Ed.* **2009**, *48*, 6520–6523.
[18] R. B. Gammill, *Synthesis* **1979**, 901–903.
[19] K. Sonogashira, Y. Tohda, N. Hagihara, *Tetrahedron Lett.* **1975**, *16*, 4467–4470.
[20] E. J. Corey, G. Schmidt, *Tetrahedron Lett.* **1979**, *20*, 399–402.
[21] V. Dourtoglou, J. C. Ziegler, B. Gross, *Tetrahedron Lett.* **1978**, *19*, 1269–1272.
[22] I. Yokoe, K. Maruyama, Y. Sugita, T. Harashida, Y. Shirataki, *Chem. Pharm. Bull.* **1994**, *42*, 1697–1699.
[23] K. Zlotkowski, D. Siegel, unpublished results..
[24] R. E. Rydel, L. A. Greene, *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 1257–1261.

