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Synthesis and Biological Activity of New Functionalized Epothilones for Prodrug Design and Tumor Targeting

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Abstract: Epothilones are potent antiproliferative agents, which have served as successful lead structures for anticancer drug discovery. However, their therapeutic efficacy would benefit greatly from an increase in their selectivity for tumor cells, which may be achieved through conjugation with a tumor-targeting moiety. Three novel epothilone analogs bearing variously functionalized benzimidazole side chains were synthesized using a strategy based on palladium-mediated coupling and macrolactonization. The synthesis of these compounds is described and their *in vitro* biological activity is discussed with respect to their interactions with the tubulin/microtubule system and the inhibition of human cancer cell proliferation. The additional functional groups may be used to synthesize conjugates of epothilone derivatives with a variety of tumor-targeting moieties.

Keywords: Antiproliferative agent · Drug conjugate · Epothilone · Total synthesis · Tumor targeting

1. Introduction

Epothilones (Fig. 1) are microtubule-stabilizing agents[1] which display very potent antiproliferative activity *in vitro*[2] and high *in vivo* antitumor activity in a variety of human tumor models.[3] They have been at the focus of extensive research for the past decade, which has led to the synthesis of a large number of diverse epothilone analogs;[4] several compounds of this family have reached clinical development and one has been approved recently for breast cancer therapy.[5,6]

Notwithstanding their promising properties, epothilones lack any inherent selectivity for malignant cells with respect to normal ones. As for many other cancer chemotheraputics, their clinical efficacy relies heavily on the increased proliferation rate of many types of tumors in comparison to the majority of healthy tissues. The latter are inevitably affected at some level of cytotoxicity during therapy, which often leads to significant side effects; in the case of epothilones, these include neurological and gastrointestinal toxicities.[3] The resulting need to balance efficacy against toxicity in oncological treatments is a well-known problem, which often limits the dosage that can be administered, and may ultimately lead to failure of therapy.

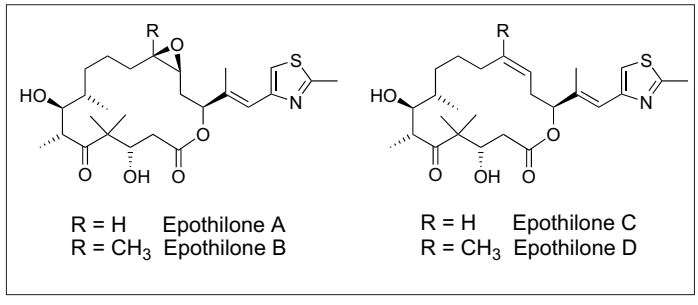

Fig. 1.

To reduce their side effects, it would be very desirable to confer to epothilones an intrinsic ability to discriminate between healthy and malignant cells. This may be achieved for instance by structural modifications affecting cellular uptake, in order to produce increased transport of the drug into malignant cells. Conjugation of an epothilone derivative as a cytotoxic 'warhead' to a tumor-targeting moiety may result in such preferential accumulation of the conjugate into cancer cells. This approach has been already applied in the past, using a variety of macromolecular and small-molecule targeting moieties to prepare conjugates of antiproliferative drugs for tumor targeting.[7]

The natural epothilones framework, however, offers little opportunity for easy conjugation chemistry. Two obvious functionalities that may lend themselves to derivatization are the two hydroxyl groups at the C(3) and C(7) positions, which might be converted, for instance, to esters or carbonates. Functionalization of these groups, however, appeared likely to be difficult, given that they are secondary alcohols in a relatively crowded steric environment. Moreover, derivatization at these positions entails the risk of side reactions, such as macrocycle opening through retro-aldol chemistry or elimination reactions. The aromatic side chain would appear comparatively less problematic for chemical modification, but it lacks a suitable functional group for derivatization; however, a variety of side-chain-modified epothilones are known,[8] including analogs with additional nucleophilic groups as part of the side chain, and it has been shown that even significant alterations of this part of the structure are

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often well tolerated in terms of activity.[9] Therefore, a promising approach for the development of tumor-targeted epothilones may consist in the utilization of side chain-modified analogs that incorporate an additional functionality that is amenable to derivatization and conjugation. Analogs incorporating a benzimidazole-based side chain have previously been shown to exhibit high cytotoxicity in combination with the epothilone D core macrocycle,[10] and the benzimidazole moiety offers a site for further modification at the N(1) position. Therefore, we envisioned compounds **1**–**3** (Fig. 2) as targets for the subsequent preparation of epothilone conjugates; in addition to providing the possibility of different conjugation chemistries, the different functionalities that decorate the N(1) appendage would offer further insight into the SAR of benzimidazole-based epothilone analogues. Should these derivatives (*i.e.* **1**–**3**) maintain high cytotoxicity, as we hoped would be the case, they would open the way to the easy synthesis of a large array of epothilone conjugates with a variety of targeting moieties.

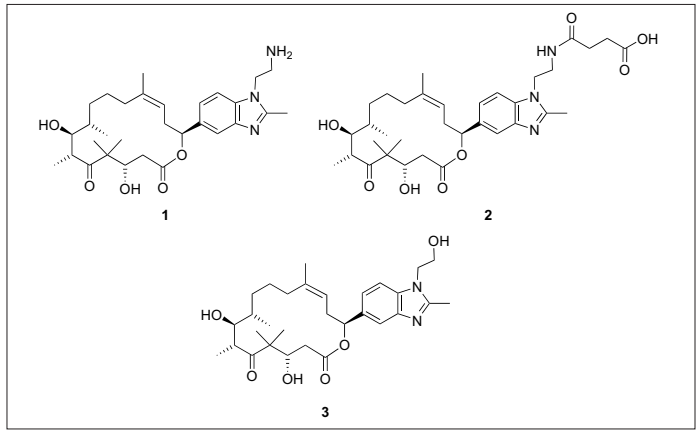
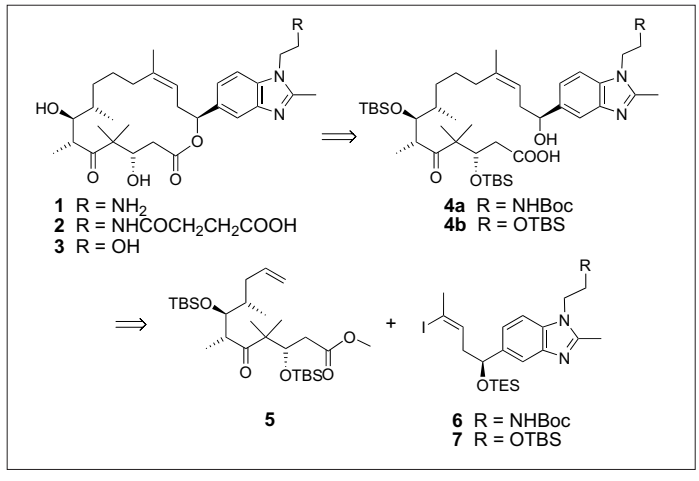


Fig. 2.

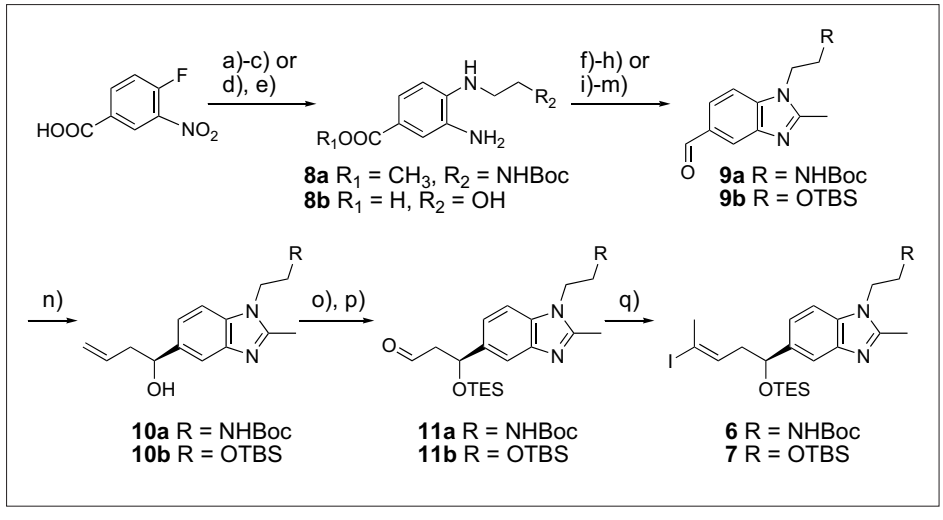


Scheme 1.

2. Results and Discussion

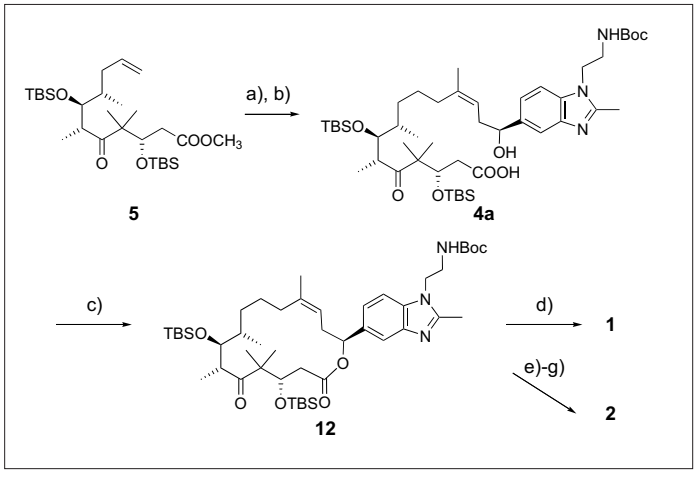
The synthesis of epothilone analogs **1**–**3** was based on a strategy previously applied to the preparation of other side-chain-modified epothilones,[11] and relied on the common intermediate **5**, to which the corresponding side-chain moieties **6** and **7** were connected *via* Suzuki-Miyaura palladium-mediated coupling (Scheme 1). Selective deprotection afforded *seco*-acids **4a**–**b**, then Yamaguchi macrolactonization[12] was applied to complete the epothilone structure; full deprotection afforded **1** and **3**, while **2** was synthesized through late-stage functionalization of the protected lactone precursor to **1** (*vide infra*).

Synthesis of the benzimidazole-containing vinyl iodides **6** and **7** started from 4-fluoro-3-nitrobenzoic acid (Scheme 2). Despite minor differences in the protection sequence, the synthesis was conducted in a very similar manner for both derivatives, with the introduction of the ethylendiamine or 2-aminoethanol handle at a very early stage through nucleophilic aromatic substitution. Subsequent reduction of the nitro group and cyclization with triethylorthoacetate easily afforded the desired functionalized benzimidazoles in very good yields (88% in four steps and 69% in three steps respectively). Interestingly, Swern oxidation afforded aldehyde **9b** smoothly from the corresponding alcohol, while it failed completely to convert its amino-analog to **9a**; the latter was obtained instead in excellent yield (96%) using manganese oxide as the oxidizing agent.

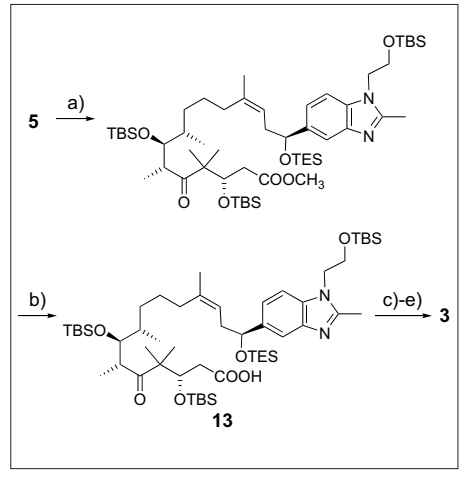


Scheme 2. a) H_2SO_4, MeOH, 65 °C, 6 h, 97%; b) $BocNHCH_2CH_2NH_2$, DCM, triethylamine, r.t., 25 h, 96%; c) H_2, Pd/C, MeOH, r.t., 17 h, 99%; d) ethanolamine, MeOH, r.t., 4 h, 89%; e) H_2, Pd/C, EtOH, r.t., 40 min, 83%; f) triethylorthoacetate, EtOH, reflux, 19.5 h, 96%; g) DIBAL-H, DCM, –78 °C → r.t., 17 h, 78%; h) MnO_2, DCM, 40 °C, 1 h, 96%; i) triethylorthoacetate, EtOH, reflux, 2 h, 94%; j) H_2SO_4, MeOH, 65 °C, 27 h, 98%; k) TBSCl, imidazole, DMF, r.t., 2.5 h, 90%; l) DIBAL-H, DCM, –78°C → r.t., 26 h, 82%; m) $(COCl)_2$, DMSO, DCM, –78 °C, 1 h, 67%; n) i. (–)-DIP-Cl, allylMgBr, Et_2O, 0 °C, 1 h, then –78 °C (solution A); ii. 9, Et_2O, –100 °C, dropwise addition of solution A, then –100 °C, 2 h, 89% (**10a**) and 95% (**10b**), *ee* 94% (**10a**) and 91% (**10b**) determined through Mosher ester analysis; o) TESCl, imidazole, DMAP, DMF, r.t., 4 h, 98% (**a**) and 92% (**b**); p) OsO_4, 2,6-lutidine, $NaIO_4$, DMF/water, r.t., 23 h, 74% (**11a**) and 1.5 h, 74% (**11b**); q) $[Ph_3PCH(CH_3)I]I$, NaHMDS, THF, –78 °C → –30 °C, 1 h, then –78 °C, **11**, 7 h, 42% (**6**) and 4 h, 37% (**7**).

The next step was the crucial introduction of the stereogenic center at the future position C(15) of the epothilone. Our initial approach made use of Oppolzer's bornane sultam auxiliary[13] in an aldol reaction with aldehyde **9a**; stereoselectivity, however, proved to be rather disappointing with a diastereomeric ratio of only 2:1. We therefore turned our attention towards Brown allylation[14] of **9** to install the de-



Scheme 3. a) i. 9-BBN, THF, r.t., 3 h (solution A); ii. $AsPh_3$, $CsCO_3$, $[PdCl_2(dppf)]\cdot DCM$, DMF, water, **6**, solution A, –5 °C → r.t., 12 h, 83%; b) i. $LiOH\cdot H_2O$, dioxane/water, 60 °C, 11.5 h; ii. DCM, water, HCl, pH 2, r.t., 6 h, 85%; c) i. 2,4,6-trichlorobenzoylchloride, triethylamine, THF, –10 °C → 0 °C, 1 h; ii. dilution with toluene (solution A); iii. DMAP, toluene, slow addition of solution A over 3 h, r.t., then r.t., 2 h, 57%; d) TFA, DCM, 0 °C → r.t., 2 h, 44% after HPLC purification; e) $ZnBr_2$, DCM, 0 °C to 4 °C, 72 h, 57%; f) succinic anhydride, diisopropylethylamine, DMF, r.t., 2 h, 92%; g) TFA, DCM, 0 °C → r.t., 5 h, 23% after HPLC purification.



Scheme 4. a) i. 9-BBN, THF, r.t., 3 h (solution A); ii. $AsPh_3$, $CsCO_3$, $[PdCl_2(dppf)]\cdot DCM$, DMF, water, **7**, solution A, –10 °C → r.t., 2.5 h, 77%; b) $LiOH\cdot H_2O$, isopropanol/water, r.t., 72 h, 26%; c) DCM/isopropanol/1M HCl 2:1:1, r.t, 2.5 h, not purified; d) i. 2,4,6-trichlorobenzoylchloride, triethylamine, THF, –10 °C → 0 °C, 1 h; ii. dilution with toluene (solution A); iii. DMAP, toluene, slow addition of solution A over 1.5 h, r.t., 54% over two steps; e) HF·Py, THF, r.t., 26 h, 84%.

sired chirality and we obtained a much more satisfying enantiomeric excess for both intermediates **10a** and **10b**. Oxidation of the latter with osmium tetroxide/periodate followed by Wittig reaction of the resulting aldehydes with iodoethyltriphenylphosphonium iodide[15a] successfully completed the synthesis of **6** and **7**. The Wittig step gave the desired *Z* product exclusively, albeit in modest yields. This is consistent with a number of literature reports on this type of reaction, and there is some indication that it may be inherently limited to about 50% yield.[15b] Despite its moderate yield, the reaction is valuable for the present synthesis because it affords straightforward access to the *Z*-configured methyl vinyliodide motif, which is otherwise is rather cumbersome to access.

With **6** in hand, the backbone of **1** could be assembled *via* Suzuki-Miyaura coupling with **5** (Scheme 3); subsequent basic hydrolysis of the methyl ester proceeded smoothly and also allowed selective cleavage of the silyl ether at position 15 by simply prolonging the acidic workup step. The resulting *seco*-acid was cyclized in a Yamaguchi reaction[12] and acidic deprotection of all remaining groups in one step afforded **1** in a satisfactory 25% yield over two steps after HPLC purification.

Succinic acid derivative **2** was synthesized from **12** through selective removal of the BOC-group with zinc bromide. While partial loss of TBS groups also occured, sufficiently diluted reaction conditions provided roughly 60% of the desired TBS-protected, free amine product. Acylation of the amino group with succinic anhydride was achieved smoothly in 92% yield; deprotection under the same conditions used for **1** afforded **2** in a moderate but still acceptable yield of 23% after HPLC purification.

The synthesis of **3** was planned along the same sequence of reactions that led to **1** (Scheme 4); after a successful palladium-mediated coupling of **5** and **7**, however, the deprotection step proved to be unselective, with silyl ether cleavage occurring at both position 15 and the ethanolamino moiety during the basic hydrolysis of the methyl ester. Despite our attempts to optimize the reaction conditions, the result was always a mixture of derivatives with a modest yield of the desired product; under milder saponification conditions, one of the side products even presented a free alcohol at the ethanolamino moiety, while both the TES ether and the methyl ester were still intact. However, **13** could be isolated from these mixtures through flash chromatography, and sufficient material was eventually obtained to complete the synthesis. Selective cleavage of the TES ether in **13** was achieved under acidic conditions; the resulting *seco*-acid was cyclized successfully under Yamaguchi conditions, then deprotection with hydrofluoric acid/pyridine afforded the desired analog **3**.

Compounds **1**–**3** were evaluated *in vitro* for their effect on tubulin polymerization and on human cancer cell growth against several cell lines (Table 1). While **1** and **2** induce tubulin polymerization with similar potency and in the same range as Epo A, their antiproliferative activity is significantly different. Compound **1** has IC_{50} only about three times higher than that of Epo A, while for **2** this value is about 20 times higher than for Epo A. This discrepancy between the effects displayed on tubulin

Table 1. Tubulin-polymerizing and antiproliferative activity of epothilones **1**, **2** and **3**

Compound	EC_{50} tubulin polymerization [μM][a]	IC_{50} [nM][b]		
		MCF-7	A549	HCT116
1	4.3 ± 0.8[d]	10.5 ± 3.0[d]	13.0 ± 4.8[d]	n. d.[c]
2	4.1 ± 0.5[d]	65 ± 12[d]	108 ± 14[d]	n. d.[c]
3	n. d.[c]	0.52 ± 0.018	0.35 ± 0.019	0.38 ± 0.029
Epothilone A	3.9 ± 0.6[d]	2.9 ± 0.3[d]	5.0 ± 1.4	2.8 ± 0.4[d]
Epothilone B	3.0 ± 0.3[d]	0.33 ± 0.01[d]	0.34 ± 0.03[d]	0.16 ± 0.01[d]

[a]Concentration required to induce 50% of the maximum tubulin polymerization achievable with the respective compound (10 μM of porcine brain tubulin). [b]IC_{50}-values for human cancer cell growth inhibition. MCF-7: breast; A549: lung; HCT116: colon. [c]n. d. = not determined. [d]See ref. [16].

and on cells is not unprecedented,[8b,16] and may reflect differences in the cellular uptake of these compounds, but its precise reasons remain unclear. Strikingly, derivative **3** is significantly more active than **1**, with an IC_{50} value 20 to 40 times lower, coming closer to the values of the more potent Epothilone B. Although the chemical structures of **1** and **3** would seem very similar, it appears that a hydroxyl group in this particular position is much more advantageous than an amine.

The reduction in antiproliferative activity of compounds **1** and **2** in comparison with natural epothilones is somewhat more pronounced than we had anticipated, based on the nanomolar cytotoxicity displayed by the dimethylbenzimidazole analog of epothilone D.[10] The increased bulkiness of these derivatives might play a role, although literature precedents would indicate a good tolerance to large substituents in this region of the molecule.[9] Besides, comparison of **1** with **3** indicates that steric hindrance is very unlikely to be the only factor. In addition to their size, the acid-base properties of the newly introduced functional groups should also be considered, as they may have a significant influence on the transport of these analogs into and inside cells. This may be particularly important in the case of **1** and **2**, which possess ionizable groups in addition to the benzimidazole nitrogen, whose precise pKa is unknown in these compounds.

Overall, however, compounds **1**–**3** all display potent antiproliferative activity, and, therefore, are interesting candidates for the development of drug conjugates for tumor targeting.

3. Conclusions

We have synthesized three novel side-chain-modified Epo D analogs and we have established their tubulin-polymerizing and antiproliferative activity *in vitro*. Due to the additional functional group they bear, these analogs may now be used to prepare conjugates with appropriately functionalized tumor-targeting molecules. The different functional groups displayed by compounds **1**–**3** as an appendix to their benzimidazole side chain offer a significant degree of flexibility in terms of the conjugation chemistry that may be chosen. The synthesis of tumor-targeted conjugates based on **1**–**3** is currently in progress in our laboratory, and the results of these efforts will be disclosed in future publications.

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[1] D. M. Bollag, P. A. McQueney, J. Zhou, O. Hensens, L. Koupal, J. Liesch, M. Goez, E. Lazarides, C. M. Woods, *Cancer Res.* **1995**, *55*, 2325.
[2] a) R. J. Kowalski, P. Giannakakou, E. Hamel, *J. Biol. Chem.* **1997**, *272*, 2534; b) K.-H. Altmann, M. Wartmann, T. O'Reilly, *Biochim. Biophys. Acta Rev. Cancer* **2000**, *1470*, M79.
[3] S. Goodin, M. P. Kane, E. H. Rubin, *J. Clin. Onc.* **2004**, *22*, 2015.
[4] For a review on the epothilone SAR see for example: K.-H. Altmann, B. Pfeiffer, S. Arseniyadis, B. A. Pratt, K. C. Nicolaou, *ChemMedChem* **2007**, *2*, 396.
[5] E. Kaminskas, X. Jiang, R. Aziz, J. Bullock, R. Kasliwal, R. Harapanhalli, S. Pope, R. Sridhara, J. Leighton, B. Booth, R. Dagher, R. Justice, R. Pazdur, *Clin. Cancer Res.* **2008**, *14*, 4378.
[6] For a recent review on epothilone clinical trials: M. Harrison, C. Swanton, *Exp. Opin. Invest. Drugs* **2008**, *17*, 523.
[7] For a review on this topic see for example: a) S. Jaracz, J. Chen, L. V. Kuznetsova, I. Ojima, *Bioorg. Med. Chem.* **2005**, *13*, 5043 and references cited therein; for more recent examples see, for instance: b) P. S. Low, W. A. Henne, D. D. Doorneweerd, *Acc. Chem. Res.* **2008**, *41*, 120; c) G. Pasut, F. M. Veronese, *Prog. Polym. Sci.* **2007**, *32*, 933.
[8] a) See, for example: K. C. Nicolaou, R. Scarpelli, B. Bollbuck, B. Werschkun, M. M. A. Pereira, M. Wartmann, K.-H. Altmann, D. Zaharevitz, R. Gussio, P. Giannakakou, *Chem. Biol.* **2000**, *7*, 593; b) G. Bold, S. Wojcik, G. Caravatti, R. Lindauer, C. Stierlin, J. Gertsch, M. Wartmann, K.-H. Altmann, *ChemMedChem* **2006**, *1*, 37.
[9] K. C. Nicolaou, B. A. Pratt, S. Arseniyadis, M. Wartmann, A. O'Brate, *ChemMedChem* **2006**, *1*, 41.
[10] K.-H. Altmann, G. Bold, G. Caravatti, A. Flörsheimer, V. Guagnano, M. Wartmann, *Bioorg. Med. Chem.* **2000**, *10*, 2765.
[11] a) K.-H. Altmann, G. Bold, G. Caravatti, D. Denni, A. Flörsheimer, A. Schmidt, G. Rihs, M. Wartmann, *Helv. Chim. Acta* **2002**, *85*, 4086; b) F. Cachoux, T. Isarno, M. Wartmann, K.-H. Altmann, *ChemBioChem* **2006**, *7*, 54.
[12] J. Inanaga, K. Hirata, H. Saeki, T. Katsuki, M. Yamaguchi, *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989.
[13] W. Oppolzer, *Pure Appl. Chem.* **1990**, *62*, 1241.
[14] H. C. Brown, P. K. Jadhav, *J. Am. Chem. Soc.* **1983**, *105*, 2092.
[15] a) J. Chen, T. Wang, K. Zhao, *Tetrahedron Lett.* **1994**, *35*, 2827; b) H. Arimoto, M. D. Kaufman, K. Kobayashi, Y. Qiu, A. B. Smith III, *Synlett* **1998**, 765.
[16] S. Anthoine Dietrich, R. Lindauer, C. Stierlin, J. Gertsch, R. Matesanz. S. Notararigo, J. F. Dìaz, K.-H. Altmann, *Chem. Eur. J.* **2009**, *15*, 10144.