Synthesis and Biological Evaluation of Aza-Epothilones

Dieter Schinzer,*[a] Karl-Heinz Altmann,[b] Friedrich Stuhlmann,[a] Armin Bauer,[a] and Markus Wartmann[b]

KEYWORDS:

antitumor agents · epothilones · lactams · natural products

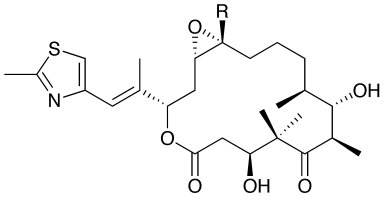
Epothilones are a new class of macrolides that were first isolated from the myxobacterium *Sorangium cellulosum* by Höfle, Reichenbach and co-workers[1] at the Gesellschaft für Biotechnologische Forschung (GBF) in Braunschweig (Germany) in 1993. In 1995, they were independently rediscovered by Bollag and co-workers[2] in a general screening program for compounds with a taxol-like biological activity. Upon more detailed biological profiling, these investigators found that epothilones are powerful cytotoxic agents that—like taxol—function through stabilization of celluar microtubules.[2, 3] However, unlike taxol, epothilones are capable of inhibiting the growth of multidrug-resistant human cancer cell lines, a finding that has generated tremendous excitement in the scientific community.[4–7]

Due to their relatively simple structure, combined with a unique biological in vitro profile and their enhanced solubility in water (as compared to taxol), epothilones are highly attractive lead structures for synthetic chemists, biologists, and clinical researchers in the search for useful new anticancer drugs.[8] Epothilone B itself exhibits potent antitumor activity in vivo, even in human tumor models that respond poorly or not at all to taxol.[8]

Besides the total syntheses of the naturally occurring epothilones, numerous studies have been directed at the design and synthesis of nonnatural epothilone analogues and the elucidation of structure – activity relationships (SARs) for this class of microtubule inhibitors.[9] Although no analogue with more potent in vitro activity than epothilone B has been identified to date, deoxyepothilone B (which is also a minor fermentation product; Figure 1) possesses an attractive pharmacological profile in vivo.[10]

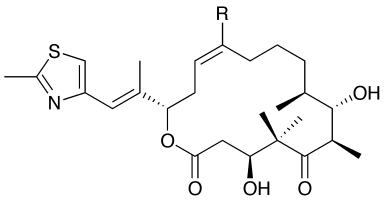
In this paper, we report on the synthesis and biological evaluation of a new type of epothilone A analogues **3**, which are based on a macrolactam rather than the natural macrolactone scaffold ("aza-epothilones"). Similar findings were reported in a patent application while this study was in progress.[11] Replacement of the ester bond in natural epothilones by an amide linkage as in the aza-epothilones **3** may lead to metabolically more stable compounds with an improved pharmacological profile in vivo.

The synthesis of the target structures **3** according to our previously developed building block strategy for the synthesis of epothilones[5] required an efficient access to the aza-fragments **10** (Scheme 1).[12] To this end, commercially available allylglycine **4** was first converted into the desired Weinreb amide[13] **6**, which was subsequently transformed into the optically active methyl ketone **7** by addition of two equivalents of methyllithium. Reaction of **7** with the ylide **8** provided the stereochemically homogeneous trisubstituted olefin **9** in high yield. In contrast, the corresponding phosphonate gave no reaction with **7**.[14]



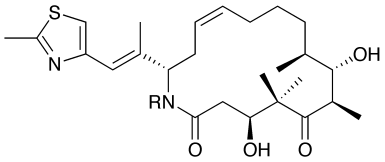
1

epothilone A: R = H; epothilone B: R = Me



2

deoxyepothilone A: R = H; deoxyepothilone B: R = Me



3a: R = H; **3b**: R = Me

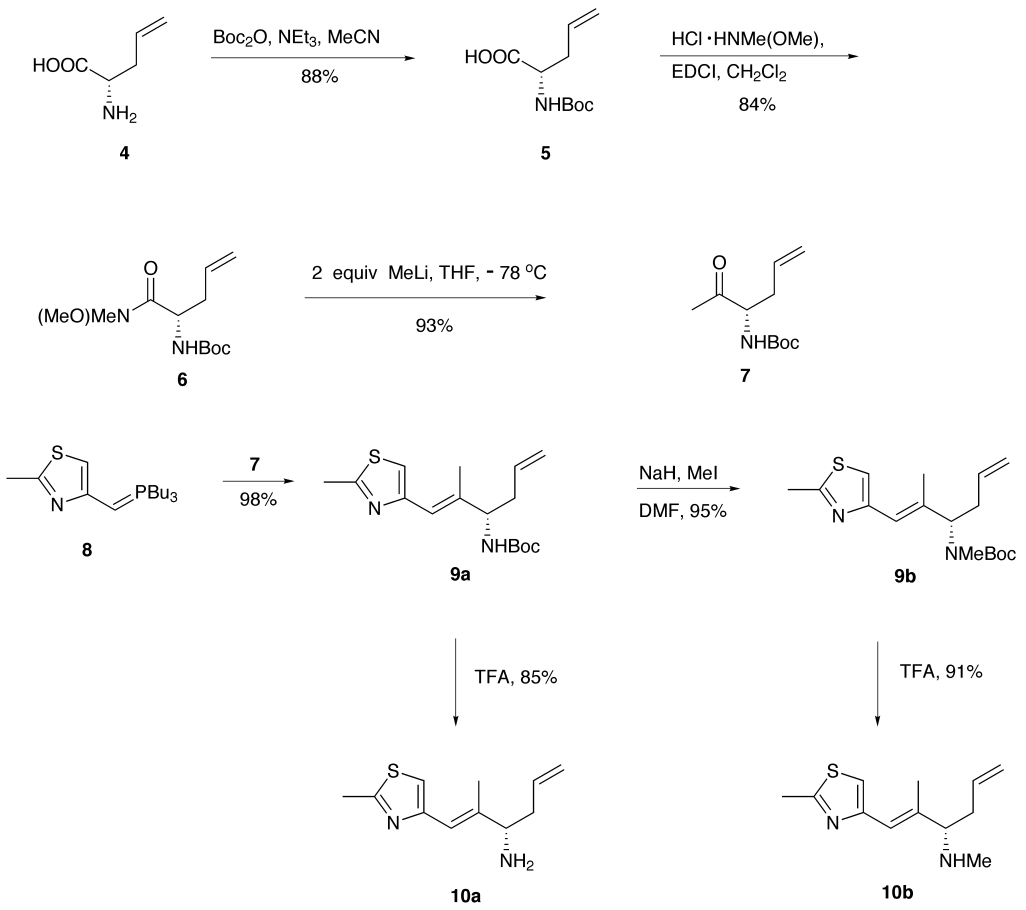
Figure 1. *Structures of selected naturally occurring epothilones and aza-epothilones A.*

Compound **9** can be directly deprotected with trifluoroacetic acid (TFA) to give the primary amine **10a**; alternatively, alkylation of **8** with methyl iodide prior to TFA treatment furnishes the secondary amine **10b**. The stereoselective synthesis of fragment C1 – C12 **11** has been previously reported by our laboratory.[5e]

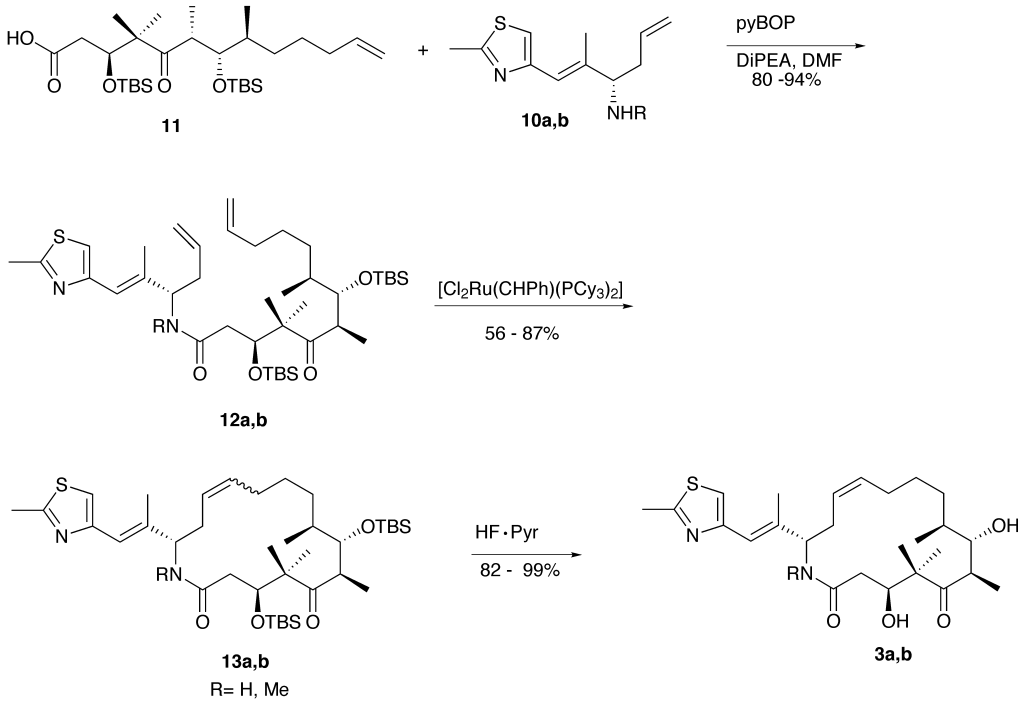
Compound **11** was coupled with **10a** and **10b** in the presence of pyBOP[15] in DMF to generate the fully functionalized amides **12a** and **12b**, respectively (Scheme 2). Olefin metathesis employing Grubbs' catalyst[16] provided the protected lactams **13** in very high yield, but as a 1:1 mixture of *Z* and *E* isomers which

[a] *Prof. Dr. D. Schinzer, Dr. F. Stuhlmann, Dr. A. Bauer*
Chemisches Institut der Otto-von-Guericke-Universität
Universitätsplatz 2, 39106 Magdeburg (Germany)
Fax: (+49) 391-67-12223
E-mail: Dieter.Schinzer@vst.uni-magdeburg.de

[b] *Dr. K.-H. Altmann, Dr. M. Wartmann*
Novartis Pharma AG, TA Oncology Research, 4002 Basel (Switzerland)



Scheme 1. *Synthesis of thiazole-containing aza-fragments* ***10****. Boc* = tert-*butoxycarbonyl; EDCI* = N′-*(3-dimethylaminopropyl)*-N-*ethylcarbodiimide.*



Scheme 2. *Coupling of aza-fragments* ***10*** *with epothilone fragment* ***11*** *to construct aza-epothilones* ***3****. Cy* = *cyclohexyl; DiPEA* = *diisopropylethylamine; pyBOP* = *benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate; TBS* = tert-*butyldimethylsilyl.*

could be separated by chromatography. Deprotection according to our standard protocol (HF · pyridine, pyridine) gave the desired aza-epothilones **3** in pure form and excellent yields.

Attempts to synthesize the corresponding epoxides under various conditions failed and generated only decomposed material.[14] Epothilone analogues **3a** and **3b** were evaluated for their ability to promote tubulin polymerization in vitro as well as for antiproliferative activity in a cell growth inhibition assay with human epidermoid carcinoma cell lines KB-31 and KB-8511. While KB-31 cells are highly sensitive to growth inhibition by taxol, the derived subline KB-8511 is ca. 250-fold more resistant to the action of this drug due to overexpression of the P glycoprotein (P-gp) efflux pump. As illustrated by the data in Table 1, **3a** and **3b** are significantly less potent inducers of tubulin polymerization in vitro than epothilone A or even deoxyepothilone A. Likewise, both compounds are also less potent inhibitors of cell proliferation, although **3a** (with a secondary amide bond) is only 10 – 15-fold less potent than the corresponding reference compound deoxyepothilone A. In contrast, the *N*-methyl derivative **3b** is at least 400-fold less active than deoxyepothilone A. It is thus clear that the replacement of the ester moiety in epothilones by a more polar amide group causes a drop in biological activity of at least one order of magnitude, even in the most favorable case. However, it should be kept in mind that

  1439-4227/00/01/01 $ 17.50+.50/0

within the epothilone B structural framework (which we have not addressed in this work), any analogue with a 10 – 20-fold reduced activity is still an inhibitor of human cancer cell proliferation in the low nanomolar range (cf. the IC_{50} value for epothilone B in Table 1). Such analogues could still be sufficiently potent to produce relevant pharmacological effects in vivo.

Table 1. *Induction of tubulin polymerization and inhibition of human cancer cell proliferation by epothilones and aza-epothilones* ***3****.*

compound	Tubulin polymerization [%][a]	IC_{50} (KB-31) [nM][b]	IC_{50} (KB-8511) [nM][b]
epothilone A	63	2.10	1.90
epothilone B	85	0.19	0.19
deoxyepothilone A[c]	50	24.70	9.90
deoxyepothilone B[c]	93	2.70	1.44
3a	26	464	285
3b	< 10	$> 10\,000$	$> 10\,000$

[a] Induction of tubulin polymerization was determined by using a modified version of the microtubule protein centrifugation assay[17] at 2 μM compound concentrations for a 20 min incubation period. Percentage numbers indicate the relative degree of polymerization compared to 25 μM epothilone B, which under our experimental conditions caused $> 95\%$ of the total tubulin to polymerize. [b] IC_{50} values for growth inhibition of human epidermoid cancer cell lines KB-31 and KB-8511. Antiproliferative assays were performed as previously described.[18] Briefly, cells were seeded at 1.5×10^3 per well into 96-well microtiter plates and incubated overnight. Compounds were added in serial dilutions on day 1. Subsequently, the plates were incubated for two population doubling times (3 – 5 days) and then fixed with 3.3% (v/v) glutaraldehyde, washed with water, and stained with 0.05% methylene blue. After washing, the dye was eluted with 3% (v/v) HCl and the optical density measured at 665 nm. IC_{50} is defined as the drug concentration that leads to 50% of viable cells per well compared to control cultures (100%) at the end of the incubation period. The data represent the mean of three independent experiments. [c] See Figure 1 for structures.

This work was supported by the Fonds der Chemischen Industrie and the Novartis Pharma AG (Basel). The excellent technical assistance by J. Loretan (tubulin polymerization assay) and R. Reuter (cell growth inhibition assay) is gratefully acknowledged.

[1] a) G. Höfle, N. Bedorf, K. Gerth, H. Reichenbach (GBF), DE-B 4138042, **1993** (priority date: November 19, 1991); b) G. Höfle, N. Bedorf, H. Steinmetz, D. Schomburg, K. Gerth, H. Reichenbach, *Angew. Chem.* **1996**, *108*, 1671 – 1673; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1567 – 1569; c) K. Gerth, N. Bedorf, G. Höfle, H. Irschik, H. Reichenbach, *J. Antibiot.* **1996**, *49*, 560 – 563.

[2] D. M. Bollag, P. A. McQueney, J. Zhu, O. Jensens, L. Koupal, J. Liesch, M. E. Goetz, C. Lazarides, M. Woods, *Cancer Res.* **1995**, *55*, 2325 – 2333.

[3] R. J. Kowalski, P. Giannakakou, E. Hamel, *J. Biol. Chem.* **1997**, *272*, 2534 – 2541.

[4] Reviews: a) D. Schinzer, *Eur. Chem. Chron.* **1996**, *1*, 7 – 10; b) M. Kalesse, *Eur. Chem. Chron.* **1997**, *2*, 7 – 11; c) L. Wessjohann, *Angew. Chem.* **1997**, *109*, 739 – 742; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 739 – 742; d) K. C. Nicolaou, F. Roschangar, D. Vourloumis, *Angew. Chem.* **1998**, *110*, 2120 – 2153; *Angew. Chem. Int. Ed.* **1998**, *37*, 2014 – 2045.

[5] Total syntheses of epothilone A: a) A. Balog, D. Meng, T. Kamenecka, P. Bertinato, D.-S. Su, E. J. Sorensen, S. J. Danishefsky, *Angew. Chem.* **1996**, *108*, 2976 – 2978; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2801 – 2803; b) D. Meng, P. Bertinato, A. Balog, D.-S. Su, T. Kamenecka, E. J. Sorensen, S. J. Danishefsky, *J. Am. Chem. Soc.* **1997**, *119*, 10073 – 10092; c) Z. Yang, Y. He, D. Vourloumis, H. Vallberg, K. C. Nicolaou, *Angew. Chem.* **1997**, *109*, 170 – 172; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 166 – 168; d) K. C. Nicolaou, Y. He, D. Vourloumis, H. Vallberg, F. Roschangar, F. Sarabia, S. Ninkovic, Z. Yang, J. I. Trujillo, *J. Am. Chem. Soc.* **1997**, *119*, 7960 – 7973; e) D. Schinzer, A. Limberg, A. Bauer, O. M. Böhm, M. Cordes, *Angew. Chem.* **1997**, *109*, 543 – 544; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 523 – 524; f) K. C. Nicolaou, S. Ninkovich, F. Sarabia, Z. Yang, *Angew. Chem.* **1997**, *109*, 539 – 540; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 525 – 527; g) K. C. Nicolaou, S. Ninkovich, F. Sarabia, D. Vourloumis, Y. He, H. Vallberg, M. R. V. Finlay, Z. Yang, *J. Am. Chem. Soc.* **1997**, *119*, 7974 – 7991; h) R. E. Taylor, G. M. Galvin, K. A. Hilfiker, Y. Chen, *J. Org. Chem.* **1998**, *63*, 9580 – 9583; i) S. C. Sinha, C. F. Barbas III, R. A. Lerner, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 14603 – 14608; j) D. Schinzer, A. Bauer, O. M. Böhm, A. Limberg, M. Cordes, *Chem. Eur. J.* **1999**, 2483 – 2491; k) M. Shibasaki, D. Sawada, *Angew. Chem.* **2000**, *112*, 215 – 219; *Angew. Chem. Int. Ed.* **2000**, *39*, 209 – 213.

[6] Total syntheses of epothilone B: a) D.-S. Su, D. Meng, P. Bertinanto, A. Balog, E. J. Sorensen, S. J. Danishefsky, Y.-H. Zheng, T.-C. Chou, L. He, S. B. Horwitz, *Angew. Chem.* **1997**, *109*, 775 – 777; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 775 – 777; b) D. Meng, D.-S. Su, A. Balog, P. Bertinato, E. J. Sorensen, S. J. Danishefsky, Y.-H. Zheng, T.-C. Chou, L. He, S. B. Horwitz, *J. Am. Chem. Soc.* **1997**, *119*, 2733 – 2734; c) see ref. [5b]; d) K. C. Nicolaou, N. Winssinger, J. Pastor, S. Ninkovic, F. Sarabia, Y. He, D. Vourloumis, Z. Yang, T. Li, P. Giannakakou, E. Hamel, *Nature* **1997**, *387*, 268 – 272; e) see ref. [5g]; f) D. Schinzer, A. Bauer, J. Schieber, *Synlett* **1998**, 861 – 864; g) S. A. May, P. A. Grieco, *Chem. Commun.* **1998**, 1597 – 1598; h) A. Balog, C. Harris, K. Savin, X.-G. Zhang, T.-C. Chou, S. J. Danishefsky, *Angew. Chem.* **1998**, *110*, 2821 – 2824; *Angew. Chem. Int. Ed.* **1998**, *37*, 2675 – 2678; i) J. Mulzer, A. Mantoulidis, E. Öhler, *Tetrahedron Lett.* **1998**, *39*, 8633 – 8636; j) D. Schinzer, A. Bauer, J. Schieber, *Chem. Eur. J.* **1999**, 2492 – 2500; k) J. D. White, K. F. Sundermann, R. G. Carter, *J. Org. Chem.* **1999**, *64*, 684 – 685; l) J. D. White, K. F. Sundermann, R. G. Carter, *Org. Lett.* **1999**, *1*, 1431 – 1434.

[7] Partial syntheses: a) D. Schinzer, A. Limberg, O. M. Böhm, *Chem. Eur. J.* **1996**, *2*, 1477 – 1482; b) K. C. Nicolaou, Y. He, D. Vourloumis, H. Vallberg, Z. Yang, *Angew. Chem.* **1996**, *108*, 2554 – 2556; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2399 – 2401; c) D. Meng, E. J. Sorensen P. Bertinato, S. J. Danishefsky, *J. Org. Chem.* **1996**, *61*, 7998 – 7999; d) D. Meng, E. J. Sorensen, P. Bertinato, S. J. Danishefsky, *J. Org. Chem.* **1996**, *61*, 8000 – 8001; e) J. Mulzer, A. Mantoulidis, *Tetrahedron Lett.* **1996**, *37*, 9179 – 9182; f) E. Claus, A. Pahl, P. G. Jones, H. M. Meyer, M. Kalesse, *Tetrahedron Lett.* **1997**, *38*, 1359 – 1362; g) T. Gabriel, L. Wessjohann, *Tetrahedron Lett.* **1997**, *38*, 1363 – 1366; h) R. E. Taylor, J. D. Haley, *Tetrahedron Lett.* **1997**, *38*, 2061 – 2064; i) J. D. Brabander, S. Rosset, G. Bernardinelli, *Synlett* **1997**, 824 – 826; j) J. Mulzer, A. Mantoulidis, E. Öhler, *Tetrahedron Lett.* **1997**, *38*, 7725 – 7728; k) T. K. Chakraborty, S. Dutta, *Tetrahedron Lett.* **1998**, *39*, 101 – 104; l) P. Bijoy, M. A. Avery, *Tetrahedron Lett.* **1998**, *39*, 209 – 212; m) Z.-Y. Liu, C.-Z. Yu, J.-D. Yang, *Synlett* **1997**, 1383 – 1384; n) Z.-Y. Liu, C.-Z. Yu, R.-F. Wang, G. Li, *Tetrahedron Lett.* **1998**, *39*, 5261 – 5264; o) K. Gerlach, M. Quitschalle, M. Kalesse, *Synlett* **1998**, 1108 – 1110; p) U. T. Bornscheuer, J. Altenbuchner, H. H. Meyer, *Biotechnol. Bioeng.* **1998**, *5*, 554 – 559; q) S. C. Sinha, J. Sun, G. Miller, C. F. Barbas III, R. A. Lerner, *Org. Lett.* **1999**, *1*, 1623 – 1626.

[8] K.-H. Altmann, M. Wartmann, T. O'Reilly, *Rev. Cancer Online*, in press.

[9] a) For an overview, see ref. [4d]; recent publications in this field: b) K. C. Nicolaou, M. R. V. Finlay, S. Ninkovich, N. P. King, Y. He, T. Li, F. Sarabia, D. Vourloumis, *Chem. Biol.* **1998**, *5*, 365 – 372; c) M. Sefkow, M. Kiffe, D. Schummer, G. Höfle, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3025 – 3030; d) M. Sefkow, M. Kiffe, G. Höfle, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3031 – 3036.

[10] a) T.-C. Chou, X.-G. Zhang, A. Balog, D.-S. Su, D. Meng, K. Savin, J. R. Bertino, S. J. Danishefsky, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 9642 – 9647; b) C. R. Harris, S. J. Danishefsky, *J. Org. Chem.* **1999**, *64*, 8434 – 8456.

[11] Lactam-based epothilone analogues were reported in a patent application by the Bristol-Myers Squibb company: PCT application WO-A 99/02514, January 21, **1999**.

[12] All new compounds gave spectral and analytical data that were consistent with the assigned structures.

[13] A. Basha, M. Lipton, S. M. Weinreb, *Tetrahedron Lett.* **1977**, *18*, 4171 – 4174.

[14] F. Stuhlmann, unpublished results.

[15] J. Coste, D. Le-Nguyen, B. Castro, *Tetrahedron Lett.* **1990**, *31*, 205 – 208.

[16] W. J. Zuercher, M. Hashimoto, R. H. Grubbs, J. W. Ziller, *J. Am. Chem. Soc.* **1996**, *118*, 100 – 110.
[17] C. M. Lin, Y. Q. Yian, A. G. Chadhary, J. M. Rimoldi, D. G. I. Kingston, E. Hamel, *Cancer Chem. Pharm*. **1996**, *38*, 136 – 140.
[18] T. Meyer, U. Regenass, D. Fabbro, E. Alteri, J. Rösel, M. Müller, G. Caravatti, A. Matter, *Int. J. Cancer* **1989**, *43*, 851 – 856.

Received: February 28, 2000 [Z 7]

Synthetic Carbohydrate Dendrimers, Part 8[+]

Toward the Synthesis of Large Oligosaccharide-Based Dendrimers

W. Bruce Turnbull, Anthony R. Pease, and J. Fraser Stoddart*[a]

KEYWORDS:

carbohydrates · chemoselective ligation · dendrimers · oligosaccharides · reductive amination

It is now generally acknowledged that cell surface protein – carbohydrate interactions play a crucial role in a wide range of biological processes[1] necessary for both normal physiological function and the onset of disease. Although protein – carbohydrate interactions typically display high dissociation constants with K_d values in the mM to μM range,[2] Nature can still attain physiologically useful avidities through the cooperative binding[3] of multiple copies of the ligands and receptors—the so-called multivalent effect.[4]

Synthetic chemists have successfully exploited this trick from Nature's toolbox in the synthesis of neoglycoconjugates ranging from small cluster glycosides[5] through neoglycoproteins[6] to polymers bearing many pendant saccharide residues.[7] These synthetic tools have not only provided inhibitors with greatly enhanced affinities over monovalent ligands, but they have also led to considerable advances in our understanding[4, 8] of the nature of multivalent interactions. Glycodendrimers[9] are a special class of neoglycoconjugates that combines the well-defined structural homogeneity of small clusters with the nanoscale dimensions of glycopolymers. Most of the glycodendrimer research reported to date has involved the construction of dendrimers bearing saccharide residues only on their peripheries through the use of either i) a convergent strategy[10] or by ii) the divergent modification of preexisting dendrimers.[11] However, monosaccharides are multifunctional building blocks and, as such, they are often found in Nature in branched oligomers and polymers.[1b] Indeed, the use of carbohydrates as building blocks for dendrimer construction confers a number of desirable properties on the dendrimer. In addition to water solubility and biocompatibility, it should be possible to exploit the known conformational preferences[12] of oligosaccharide monomers in order to mold the dendrimer's size and shape in a predetermined manner.

We recently described[13] the synthesis of oligosaccharide dendrimers based on β-D-glucopyranosyl repeating units. This synthesis, however, fell foul of the dogma of chemical carbohydrate synthesis—protecting group chemistry. In addition to the extensive chemical manipulations required to effect both regio- and stereoselectivity, the bulky appendages associated with the saccharide unit introduce considerable steric crowding which restricts the growth of highly branched molecules. Here, we report a very different strategy for the synthesis of oligosaccharide-based glycodendrons using reductive amination[14] as a chemoselective coupling reaction which allows the synthesis to be undertaken using a minimal number of i) protecting groups and ii) protecting-group manipulations.

Since one of our aims is to address the synthesis of large glycodendrimers, it seemed appropriate to base our synthetic strategy on an *oligosaccharide*—rather than on a monosaccharide[13]—*monomer*. We chose a linear trisaccharide, in the first instance, as it leads to the construction of glycodendrons and glycodendrimers with open and extended branches, while allowing the key reactions to be performed at the primary centers on hexopyranose units. In particular, we wanted to locate amino functions at the C-6 positions of the two non-reducing saccharide units: These two B groups find a complementary A function in the masked formyl group associated with the reducing sugar. Convergent dendron synthesis results[15] typically in a higher homogeneity of structure as a consequence of only a few reactions at a time being performed on each molecule. To achieve such a synthesis, we require an orthogonal protecting-group strategy—albeit with a minimal number of protecting groups present overall. UV-active *N*-benzoyl groups were chosen i) to allow easy detection following HPLC, as well as ii) to provide the necessary orthogonal protection of the primary amino functions with respect to the reducing terminal isopropylidene acetals, which can be hydrolyzed selectively in the presence of acid catalysts. We chose a maltosylgalactose derivative as the first AB_2 *trisaccharide monomer* to investigate because of i) the ease of its synthesis and ii) its relatively simple ^{1}H NMR spectrum that results from having distinctive and well resolved "signature" resonances associated with the α- and β-anomeric protons. The result of a molecular modeling study[16] of a first-generation dendrimer, comprising three first-generation 9-mer wedges attached to a trivalent benzeneoid core, is shown in Figure 1. While the "diameter" of this first-generation dendrimer is of the order of 8 nm, the second one has a "diameter" in the region of 11 – 12 nm. The modeling studies also indicate that such dendrimers have extended open structures which, although flexible about their galactityl linkers, still retain the conformational preferences of the maltosyl branching units.

[a] *Prof. J. F. Stoddart, Dr. W. B. Turnbull, A. R. Pease*
Department of Chemistry and Biochemistry
University of California, Los Angeles
405 Hilgard Avenue, Los Angeles, CA 90095-1569 (USA)
Fax: (+1) 310-206-1853
E-mail: stoddart@chem.ucla.edu

[+] Part 7: see ref. [13].

  1439-4227/00/01/01 $ 17.50+.50/0