Natural Products

A Route to the Thapsigargins from (*S*)-Carvone Providing a Substrate-Controlled Total Synthesis of Trilobolide, Nortrilobolide, and Thapsivillosin F**

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Investigations into the biologically active components of the Mediterranean plant species *Thapsia* led to the isolation of thapsigargin (**1**) and fifteen closely related guaianolides,

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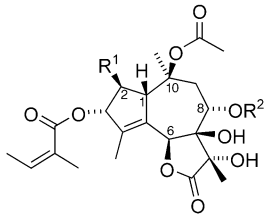
[**] This work was supported by a Wellcome Trust Research Fellowship (to S.F.O.), a Marie Curie Fellowship (to K.H.), the Deutsche Forschungsgemeinschaft (to O.S.), The Royal Society (to M.D.S.), and Novartis Research Fellowships (to S.V.L.). We thank Prof. S. B. Christensen for providing natural product samples for reference purposes and Dr. John Davis for X-ray crystallographic analyses. Early synthesis efforts by Dr. Helen Gold, Dr. Alison Woolford, and Emily Balskus are also gratefully acknowledged.

  DOI: 10.1002/ange.200353140

collectively termed "thapsigargins".[1] The members of this family of sesquiterpene lactones have a highly oxygenated tricyclic framework containing seven or eight stereogenic centers and are functionalized with a range of different acyl groups derived from acetic, butyric, angelic, and octanoic acids. The trilobolide subfamily (**2**–**4**) lack the acyloxy substituent at C2 and it is the first total synthesis of these



1: thapsigargin: R^1 = octanoyl, R^2 = butanoate
2: trilobolide: R^1 = H, R^2 = (*S*)-2-methylbutanoate
3: nortrilobolide: R^1 = H, R^2 = butanoate
4: thapsivillosin F: R^1 = H, R^2 = 3,3-dimethylacrylate

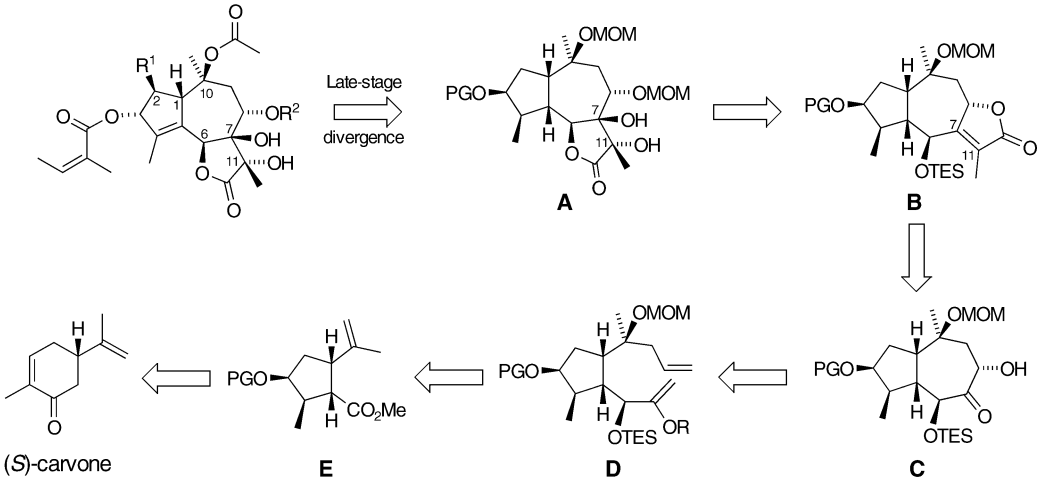
three thapsigargins that we report herein.

Interest in the thapsigargins arose after they were recognized to be potent histamine liberators[2] and selective and irreversible inhibitors of the ubiquitous sarco-endoplasmic reticulum Ca^{2+} ATP dependent pumps (SERCAs) up to subnanomolar concentrations.[3] When applied to intact cells, thapsigargin (**1**) is able to penetrate the cell where it binds to and locks the SERCA into a conformation that has a poor affinity for Ca^{2+} and ATP.[4] Trilobolide (**2**) ($K_D = 1.0$ nM) binds only 2.5 times less tightly to SERCA than **1** ($K_D =$ 0.4 nM).[5] Thapsigargins demonstrate a remarkable specificity for the SERCA isozymes, and have hence become powerful tools to manipulate and study intracellular Ca^{2+}-dependent signalling pathways.[6] Thapsigargin (**1**) has also been shown to restore apoptotic function in cancer cell lines.[7] This has recently led to the development of a potential treatment for prostate cancer using a prodrug conjugate of **1** that is cleaved and activated by prostate-specific antigen.[8]

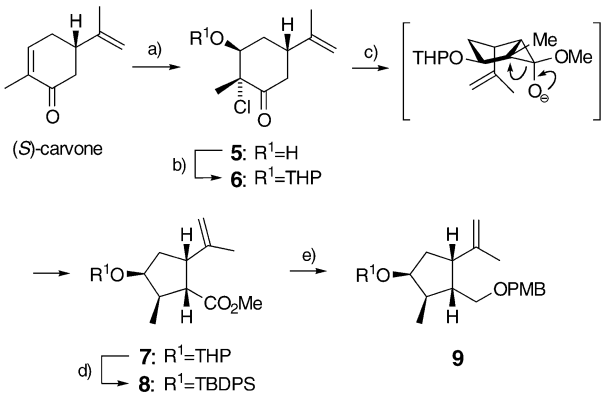
Despite the prevalence of the thapsigargins in the fields of molecular biology and oncology (a scan of the past 10 years' literature reveals almost 5000 hits for the keyword "thapsigargin"), the synthetic community has been slow to reflect its significance. To date, no total or partial synthesis of any member of this family has been reported. Using degradation,[1] Christensen and co-workers have carried out a number of modifications on the periphery of the natural substance to investigate some of the important structure–activity relationships. However, no modification of the core has been possible using this strategy. A flexible synthetic route to the thapsigargins would allow the preparation of simplified analogues and permit the investigation of detailed structure–activity relationships, which would not be otherwise possible.

Our general approach to the thapsigargins is outlined in Scheme 1. To allow access to all 16 natural compounds in the series and a number of potential analogues, our plan leads to the common intermediate **A** (PG = *tert*-butyldiphenylsilyl (TBDPS)) as the point of late-stage divergent functionalization of the cyclopentane ring prior to sequential introduction of the various ester functionalities required. We envisaged the installation of the two quarternary centers at C7 and C11 by a stereoselective *cis*-dihydroxylation of butenolide **B**. Assembly of this lactone should be possible from the α-hydroxy ketone **C**, which we planned to generate by an enol ether metathesis/oxidation sequence from diene **D**. Cyclopentane **E** (PG = tetrahydropyranyl (THP)), has already served as an intermediate in a synthesis of two other guaianolides: cladantholide and estafiatin.[9]

The synthesis begins with the stereoselective epoxidation of commercially available (*S*)-(+)-carvone with basic hydrogen peroxide (Scheme 2).[10] Opening of the resulting oxirane with lithium chloride in the presence of trifluoroacetic acid (TFA)[11] led exclusively to chlorohydrin **5**, which was protected to yield a diastereomeric mixture of the THP ethers **6**. Treatment of **6** with sodium methoxide effected a regio- and stereoselective Favorskii rearrangement to give methyl ester **7** as the sole product. At this point, the protecting group was exchanged to the more robust TBDPS group (**7**→**8**). It should be noted that the Favorskii rearrange-



Scheme 1. The synthesis plan. PG = protecting group, MOM = methoxymethyl, TES = triethylsilyl.

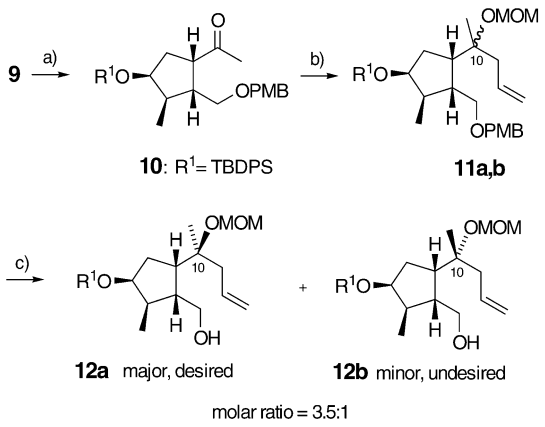


Scheme 2. Synthesis of left-hand fragment **9**. Reagents and conditions: a) 1. H_2O_2, NaOH, MeOH, 10°C, 88%, 2. LiCl, TFA, THF, RT, 95%; b) DHP, PPTS cat., CH_2Cl_2, RT, 87%; c) NaOMe, MeOH, 0°C, 95%, d.r. > 95:5; d) 1. PPTS cat., MeOH, 40°C, 84%; 2. TBDPSCl, imidazole, DMF, RT, 98%; e) 1. LAH, THF, 0°C; 2. NaH, PMBCl, DMF, RT. TFA = trifluoroacetic acid, DHP = dihydropyran, PPTS = pyridinium *p*-toluenesulfonate, THP = tetrahydropyranyl, TBDPS = *tert*-butyldiphenylsilyl, PMB = *p*-methoxybenzyl.

ment with the TBDPS-protected alcohol was also investigated, but the yields and selectivities were inferior to the THP case. Reduction of ester **8**, followed by protection of the primary alcohol gave 4-methoxybenzyl ether **9**.

As testament to the efficiency of this chemistry, no column chromatography was necessary in this opening eight-step sequence, allowing the preparation of more than 50 g of **9** per batch.

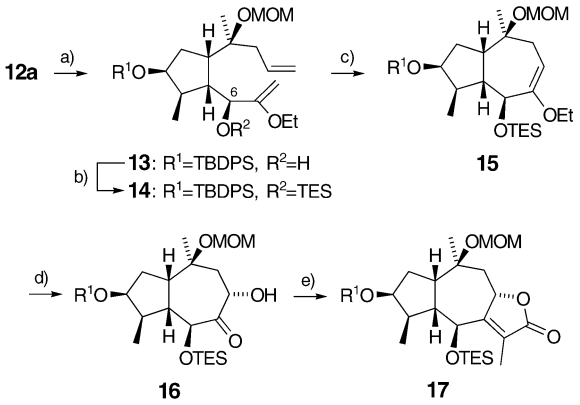
Osmylation and in situ diol cleavage provided the ketone **10** (Scheme 3). Addition of allyl magnesium bromide resulted in the formation of an inseparable diastereomeric mixture of homoallylic alcohols (d.r. = 3.5:1), with preference for the Felkin product.[12] After MOM protection of the tertiary alcohol (→**11a,b**) and removal of the PMB group, this



Scheme 3. Synthesis of the C10 epimeric alcohols **12a** and **12b**. Reagents and conditions: a) 1. OsO_4 cat., NMO, acetone, H_2O, RT; 2. $NaIO_4$, RT, 74% over four steps; b) 1. AllylMgBr, THF, −78°C, 99%, d.r. = 3.5:1; 2. MOMCl, DIPEA, DMAP cat., CH_2Cl_2, RT, 88%; c) DDQ, aq. pH 7 phosphate buffer, CH_2Cl_2, RT, 93%. DIPEA = *N*,*N*-diisopropylethylamine, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DMAP = 4-(*N*,*N*-dimethylamino)pyridine.

mixture could be separated by column chromatography to give the C10 epimeric alcohols **12a** and **12b**.

Continuing the synthesis, alcohol **12a** was oxidized with tetra-*n*-propylammonium perruthenate (TPAP)[13] and *N*-morpholine-*N*-oxide (NMO) (Scheme 4) and the resulting aldehyde was treated with the lithium anion of ethyl vinyl

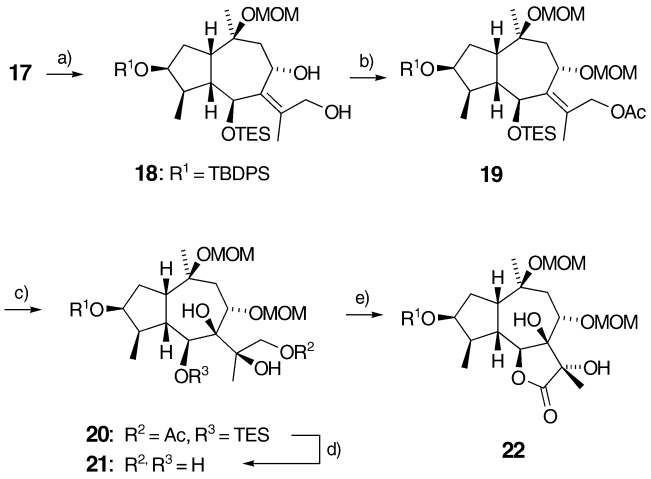


Scheme 4. Synthesis of the α,β-unsaturated lactone **17**. Reagents and conditions: a) 1. TPAP cat., NMO, 4 Å MS, CH_2Cl_2, RT, 90%, 2. CH_2=CHOEt, *t*BuLi, THF, −78°C, 96%, d.r. > 95:5; b) TESCl, imidazole, DMF, RT, 95%; c) 2.5 mol% Grubbs' dihydroimidazolidine Ru cat., CH_2Cl_2, reflux, 92%; d) $K_2OsO_2(OH)_4$ cat., $K_3Fe(CN)_6$, $NaHCO_3$, $MeSO_2NH_2$, K_2CO_3, *t*BuOH, H_2O, RT, 90%, d.r. = 16:1; e) 1. $HO_2CCH(Me)P(O)(OEt)_2$, EDCI, CH_2Cl_2, RT; 2. NaH, THF, reflux, 79% over two steps.

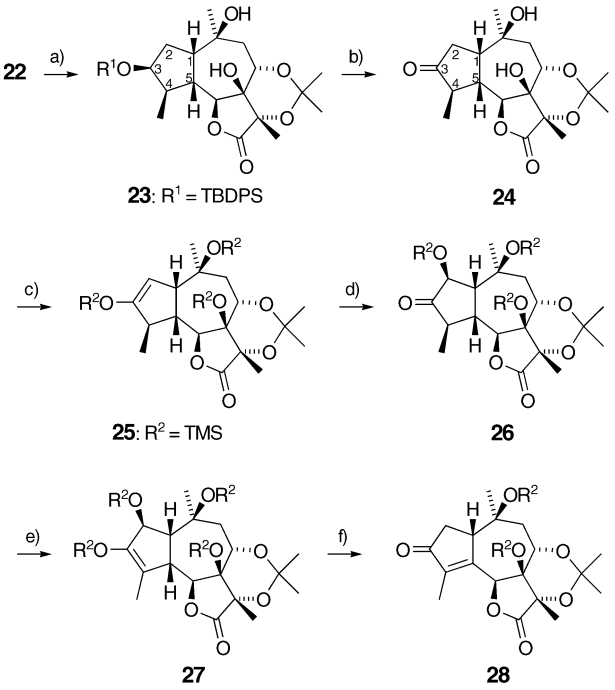
ether to give alcohol **13** as a single diastereoisomer. The stereochemical outcome of this addition is again consistent with the Felkin–Anh model. TES protection gave enol ether **14**, which was subjected to the key ring-closing metathesis reaction using only 2.5 mol% of Grubbs' dihydroimidazoline ruthenium catalyst[14] to form the required cyclic enol ether **15** in remarkable yield.[15]Osmylation in the absence of a chiral ligand led predominantly to the desired α-hydroxy ketone epimer **16** (d.r. = 16:1), resulting from attack on the concave face.[16] The C8 alcohol of **16** was then esterified with 2-(diethoxyphosphoryl)propionic acid, and a subsequent intramolecular Horner–Wadsworth–Emmons reaction resulted in the formation of butenolide **17**.

Attempts to effect the intended *syn*-dihydroxylation of lactone **17** proved unsuccessful, most likely due to a combination of unfavorable steric and electronic factors. After some experimentation, we found that carrying out the osmium-mediated dihydroxylation of a reduced derivative was possible (Scheme 5). Thus, formation of diol **18** by lithium borohydride reduction of lactone **17** was followed by orthogonal protection of the primary and secondary alcohol functions to give olefin **19**. At this stage, the dihydroxylation proceeded smoothly to give diol **20** as a single diastereoisomer.[17] Concomitant removal of TES and acetate protecting groups from **20** afforded the tetraol **21**.

The carbon framework was completed by a highly selective TPAP oxidation that first oxidized the primary alcohol of **21**, facilitated intermediate lactol formation, and



Scheme 5. Completion of the tricyclic framework. Reagents and conditions: a) $LiBH_4$, THF, reflux, 92%; b) 1. Ac_2O, DMAP, 2,6-lutidine, CH_2Cl_2, RT, 95%, 2. MOMCl, DIPEA, DMAP, CH_2Cl_2, RT, 92%; c) 20 mol% $K_2OsO_2(OH)_4$, quinuclidine, K_2CO_3, $MeSO_2NH_2$, $K_3Fe(CN)_6$, *t*BuOH, H_2O, RT, d.r. > 95:5; d) K_2CO_3, MeOH, RT, 85% over two steps; e) 10 mol% TPAP, 40 equivalents NMO, 4 Å MS, MeCN, RT, 73%.



Scheme 6. Synthesis of ketone **28**. Reagents and conditions: a) Amberlyst-15, acetone, RT, 85%; b) 1. TBAF, THF, RT, 98%, 2. TPAP cat., NMO, 4 Å MS, CH_2Cl_2, RT, 93%; c) TMSCl, Et_3N, DMF, 120°C, 88%; d) dimethyldioxirane, acetone, CH_2Cl_2, 0°C, 99%, d.r. > 95:5; e) TMSCl, Et_3N, DMF, 150°C, 90%; f) 10 mol% PhSeBr, CH_2Cl_2, 0°C to RT, 94%.

effected a second oxidation to lactone **22** in a single transformation. The 26-step sequence to the core **22** has been carried out on multigram scale (to make > 10 g of **22**) with a minimum of chromatography. It is intended that this intermediate will also serve as a precursor for a number of designed analogues.

Removal of the MOM groups in **22** with simultaneous acetonide formation gave **23** (Scheme 6). Of particular note here is the observation that the acetonide protecting group imparted remarkable chemical stability and crystallinity to all tetracyclic products in the sequence thereafter. Consequently, unmasking of the C3 hydroxy group and TPAP oxidation furnished crystalline ketone **24**, which provided proof for the correct configuration of all stereocenters.

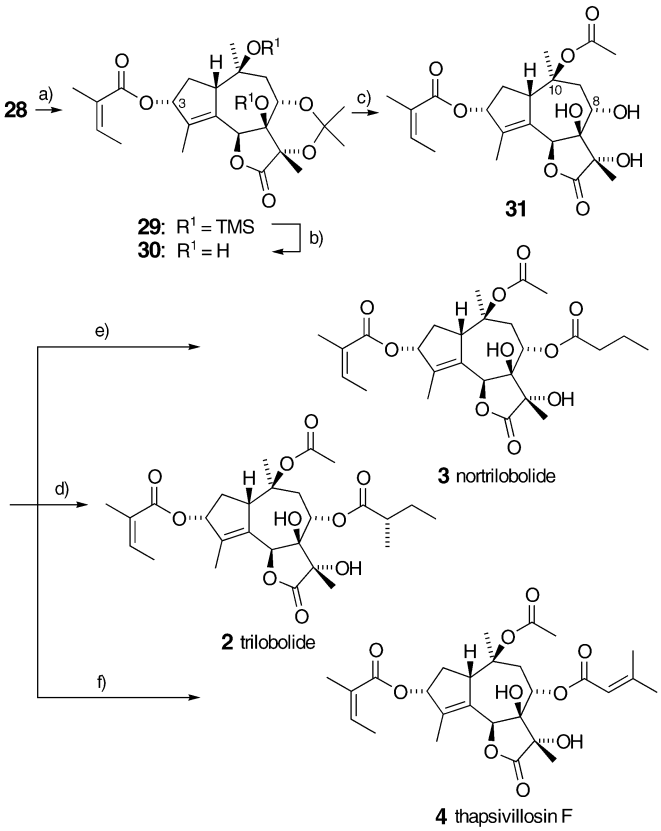
Generation of the C4,C5 α,β-unsaturation from **24** either by a direct (IBX) or indirect (via enol ether) approach was unsuccessful due to an inherent preference for enolization to the less substituted (C2) side. However, a previous model study carried out within our laboratory[18] had shown that enolization in the desired sense could be achieved after installation of the C2 hydroxy group.

Accordingly, ketone **24** was transformed into silyl enol ether **25** which was oxidized with dimethyldioxirane to afford α-silyloxy ketone **26**. X-ray crystal structure analysis of **26** confirmed that oxygen incorporation occurred exclusively from the convex face. Submitting ketone **26** to TMSCl/Et_3N at 150°C afforded the tetrasubsitituted silyl enol ether **27** in excellent yield.

A range of standard conditions was then tested for the oxidation of enol ether **27** to its corresponding α,β-unsaturated ketone. This screening process led to the discovery of an unprecedented catalytic selenium reaction that provides a direct route to the trilobolide series of the thapsigargins. Treatment of **27** with a catalytic quantity of phenylselenyl bromide gave enone **28** as the sole product, in which the C2 silyloxy group has been lost. The exact mechanism of this formal elimination reaction is still subject to investigation and will be reported later.

Ketone **28** was then stereoselectively reduced with sodium borohydride (d.r. = 4:1) to give alcohol **29** (Scheme 7). To distinguish the five hydroxyl groups in the molecule, the endgame strategy had been designed to minimize protecting group manipulation and take advantage of the inherent order of reactivity of the alcohols in a concise fashion. Accordingly, the C3 hydroxy group was first esterified with angelic acid under Yamaguchi conditions[19] to provide **29** before unmasking the tertiary alcohols with TBAF to yield **30**. Selective acylation of the C10 hydroxy group was then achieved with isopropenyl acetate mediated by polymer-supported tosic acid. Removal of the acetonide led to triol **31**. Finally, esterification with (*S*)-2-methylbutyric anhydride occurred exclusively at the secondary alcohol of C8 to provide trilobolide (**2**). Reaction of **31** with butyric anhydride and senecioic anhydride also furnished nortrilobolide (**3**) and thapsivillosin F (**4**), respectively. All spectral and analytical characteristics of synthetic **2**–**4** are in full accordance with the data obtained from the samples from natural sources.

In summary, the total synthesis of three thapsigargins has been achieved. Stereocontrol during this synthesis relies entirely upon efficient substrate control with all seven stereocentres of the natural product core set from the initial stereochemistry in (+)-carvone. The synthesis is robust (average yield of 90.3% per step), amenable to scale up,



Scheme 7. Completion of the synthesis of trilobolide, nortrilobolide, and thapsivillosin F. Reagents and conditions: a) 1. $NaBH_4$, MeOH, 0°C, 86%, d.r. = 4:1, 2. angelic acid, Et_3N, 2,4,6-trichlorobenzoyl chloride, toluene, 80°C, 76%; b) TBAF, THF, RT, quant; c) 1. isopropenyl acetate, PS-TsOH, CH_2Cl_2, RT, 68%, 2. HCl (aq), MeOH, 40°C; d) (*S*)-2-methylbutyric anhydride, DMAP, CH_2Cl_2, RT, 78% over two steps; e) butyric anhydride, DMAP, CH_2Cl_2, RT, 72% over two steps; f) senecioic anhydride, DMAP, CH_2Cl_2, RT, 73% over two steps. TBAF = tetrabutylammonium fluoride.

and its divergent nature will allow the syntheses of other thapsigargins as well as a number of analogues. After biological assessment of these compounds, further insight into the remarkable biological properties of this substance class should be possible.

Received: October 22, 2003 [Z53140]

Keywords: asymmetric synthesis · natural products · terpenoids · total synthesis

[1] S. B. Christensen, A. Andersen, U. W. Smitt, *Fortschr. Chem. Org. Naturst.* **1997**, *71*, 129, and references therein.
[2] S. A. Patkar, U. Rasmussen, B. Diamant, *Agents Actions* **1979**, *9*, 53.
[3] a) J. Lytton, M. Westlin, M. Hanley, *J. Biol. Chem.* **1991**, *266*, 17067–17071; b) C. Caspersen, M. Treiman, *FEBS Lett.* **1995**, *377*, 31–36.
[4] Y. Sagara, J. B. Wade, G. Inesi, *J. Biol. Chem.* **1992**, *267*, 1286–1292.
[5] M. Wictome , Y. M. Khan, J. M. East, A. G. Lee, *Biochem. J.* **1995**, *310*, 859–868.
[6] M, Treiman, C. Caspersen, S. B. Christensen, *Trends Pharmacol. Sci.* **1998**, 19, 131–135, and references therein.
[7] Y. Furuya, P. Lundmo, A. D. Short, D. L. Gill, J. T. Isaacs, *Cancer Res.* **1994**, *54*, 6167–6175.
[8] S. R. Denmeade, C. M. Jakobsen, S. Janssen, S. R. Khan, E. S. Garrett, H. Lilja, S. B. Christensen, J. T. Isaacs, *J. Natl. Cancer Inst.* **2003**, *95*, 990–1000.
[9] E. Lee, J. W. Lim, C. H. Yoon, Y. Sung, Y. K. Kim, *J. Am. Chem. Soc.* **1997**, *119*, 8391–8392.
[10] K. C. Nicolaou, J. Y. Xu, S. Kim, J. Pfefferkorn, T. Ohshima, D. Vourloumis, S. Hosokawa, *J. Am. Chem. Soc.* **1998**, *120*, 8661–8673.
[11] J. S. Bajwa, R. C. Anderson, *Tetrahedron Lett.* **1991**, *32*, 3021–3024.
[12] The configuration of C10 and all subsequently formed stereocenters was unambiguously proven by X-ray analysis of **24** and **26** (vide infra).
[13] S. V. Ley, J. Norman, W. P. Griffith, S. P. Marsden, *Synthesis* **1994**, *7*, 639–666.
[14] M. Scholl, S. Ding, C. W. Lee, R. H. Grubbs, *Org. Lett.* **1999**, *1*, 953–956.
[15] Slow addition of catalyst was required to obtain high yield at low catalyst loading. For examples of enol ether metathesis reactions with Grubbs' Ru catalysts see: J. D. Rainier, J. M. Cox, S. P. Allwein, *Tetrahedron Lett.* **2001**, *42*, 179–181; A. Okada, T. Ohshima, M. Shibasaki, *Tetrahedron Lett.* **2001**, *42*, 8023–8027; V. K. Aggarwal, A. M. Daly, *Chem. Commun.* **2002**, *21*, 2490–2491.
[16] Force field calculations suggest that the OTES group blocks the convex face. Using AD mix α, **16** could be obtained as a single diastereomer (matched case).
[17] An inconsequential side product resulting from protecting group migration under the reaction conditions was also observed. As its deprotection also gave tetraol **21**, the separation from **20** was not necessary.
[18] E. P. Balskus, University of Cambridge, unpublished results.
[19] B. Hartmann, A. M. Kanazawa, J. P. Depres, A. E. Greene, *Tetrahedron Lett.* **1991**, *32*, 5077–5080.

