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ARTICLE

Development and use of a general route to brassinolide, its biosynthetic precursors, metabolites and analogues

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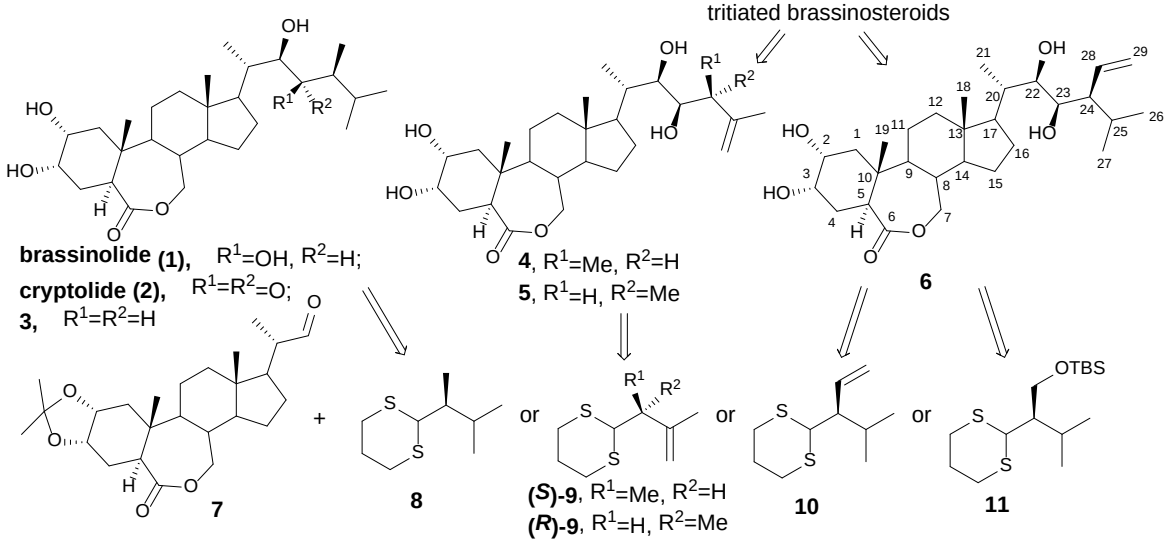
A new method for the construction of steroid side chains through the addition of lithium salts of dithianes to a C-22 aldehyde was developed. An efficient one-pot procedure for the preparation of a suitable C-22 aldehyde from commercial epibrassinolide in three steps in 86% isolated yield was described. Enantioselective hydroxymethylation of isovaleraldehyde and Kulinkovich cyclopropanation of silylated Roche esters were used as key steps for the dithiane syntheses. The method was applied for the preparation of brassinolide, its biosynthetic precursors and metabolites. In addition, a number of brassinosteroids with a double bond in the side chain were prepared as precursors for tritiated derivatives for biosynthetic studies.

Introduction

Brassinosteroids (BS) are a group of plant hormones,[1] which have been studied since the isolation of brassinolide (Bl)[2] in the late 1970's. To date, about 70 compounds related to Bl have been discovered in plant sources.[3] Chemical synthesis has been the only way to obtain BS in sufficient quantities. Bl is the most active among natural BS, and it has been the compound of choice for various in-depth investigations of this group of phytohormones.[4] Despite the progress made, synthesis of Bl and its congeners with a campestane skeleton is still not a trivial task due to the necessary de novo construction of the side chain.[5] The main drawbacks of the proposed approaches are: (1) a low stereoselectivity of the reactions used and (2) the lack of a general methodology for the preparation of a set of fully functionalized BS (including isotopically labeled compounds), their biosynthetic precursors and metabolites.

Recently, we have developed a convenient general approach to the synthesis of side chains of BS belonging to the campestane series.[6] In this article, we present its use for the synthesis of brassinolide (**1**), cryptolide (**2**), and 23-deoxybrassinolide (**3**) (Scheme 1). In addition, an identical tritiated brassinosteroids



Scheme 1 Retrosynthetic analysis of brassinosteroids **1-6**.

approach was applied for the preparation of the unsaturated analogs **4-6** as synthetic precursors for tritiated brassinolide, epibrassinolide, and 28-homobrassinolide.

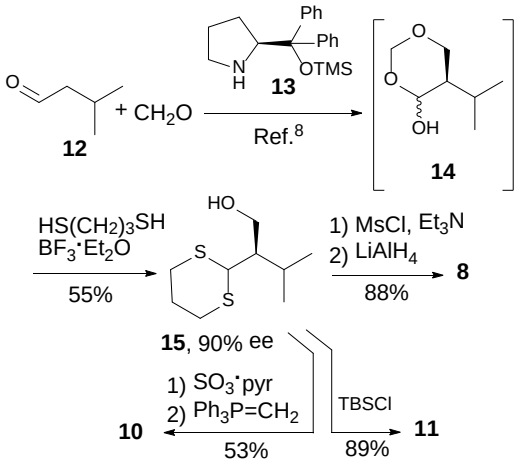
Results

In our retrosynthetic analysis (Scheme 1) we envisaged that the target molecules **1-6** could be synthesized from a steroidal aldehyde **7** through diastereoselective alkylation with lithium salts of sterically hindered dithianes **8-11**. A catalytic asymmetric aldol reaction seemed promising as the key step in the preparation of dithianes **8,10,11**. The unsaturated dithianes **(*S*)**- and **(*R*)-9** could be obtained from enantiomerically pure Roche esters.

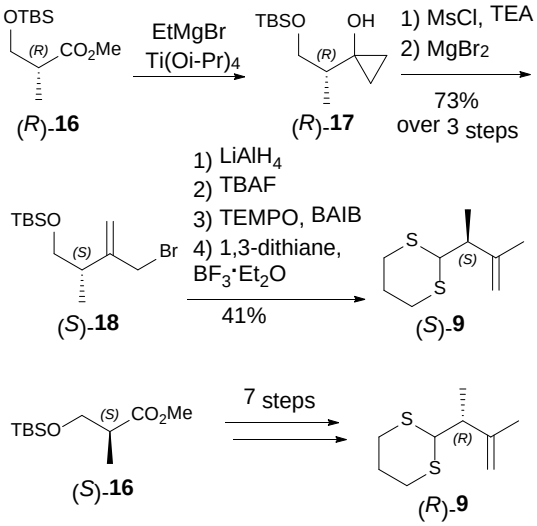
Enantioselective hydroxymethylation of isovaleraldehyde (**12**) with formaldehyde in the presence of diphenylprolinol ether **13**[7] led to the cyclic hemiacetal **14** that was directly subjected to thioacetal formation (Scheme 2). The hydroxymethyl thioacetal **15** was obtained in 90% ee and was used for the preparation of the dithianes **8,10,11**. A two step deoxygenation afforded compound **8**, an oxidation-methylenation sequence gave the unsaturated dithiane **10**, and silylation of the hydroxyl group resulted in ether **11**.

A pair of enantiomeric dithianes **(*S*)-9** and **(*R*)-9** was prepared starting from silylated Roche esters **(*R*)-16** and **(*S*)-16**, respectively, using the cyclopropyl-allyl rearrangement[8] of the cyclopropyl mesylates for the generation of the isopropylidene fragment (Scheme 3). The subjection of ester **(*R*)-16** to Kulinkovich cyclopropanation[9] conditions led to cyclopropanol **(*R*)-17**, which on mesylation and treatment with $MgBr_2$ furnished the allyl bromide **(*S*)-18**. At first, we tried to introduce the 1,3-dithiane group prior to the cyclopropanol opening to enable experiments with less volatile compounds. However, attempts to carry out the cyclopropyl-allyl rearrangement of compounds bearing the dithiane moiety were unsuccessful.

The allyl bromide **(*S*)-18**, after chromatographic purification, was transformed into the desired product **(*S*)-9** by four successive steps involving reductive dehalogenation,



Scheme 2 Synthesis of dithianes **8,10,11**.



Scheme 3 Synthesis of dithianes **(*S*)-9** and **(*R*)-9**.

desilylation, primary alcohol oxidation,[10] and conversion of the formed aldehyde into its 1,3-dithiane. The reaction sequence was carried out without isolation of the intermediates (because of their volatility) to give the dithiane **(*S*)-9** in 41% overall yield. The enantiomeric dithiane **(*R*)-9** was prepared starting from the ester **(*S*)-16** according to the same procedure.

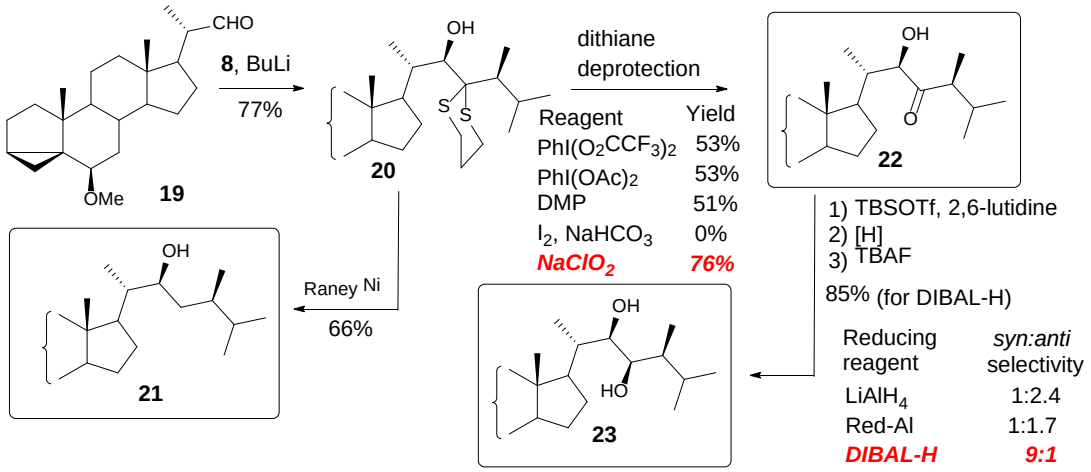
The initial attempts to construct side chains of cathasterone **21**, cryptolide **22**, and brassinolide **23** (Scheme 4) were performed on model aldehyde **19**[11], which is available in three steps from stigmasterol. The next step was the key to making the carbon skeleton of the side chain. The addition of lithiated 1,3-dithiane to C-22 aldehydes is known,[12] but compound **8** is sterically much more hindered. Fortunately, the reaction of **19** with the lithium salt of dithiane **8** proceeded smoothly to give hydroxy thioketal **20** in 77% yield as the main product along with a mixture of its isomers (6%). Reductive desulfurization of **20** with Raney nickel led to alcohol **21**.

The desulfurization of **20** to achieve ketone **22** proved to be more troublesome than initially expected. MeI and mercury salts were tried with no success. The oxidative desulfurization gave better results (see Scheme 4), and $NaClO_2$ finally proved to be the most effective.[13]

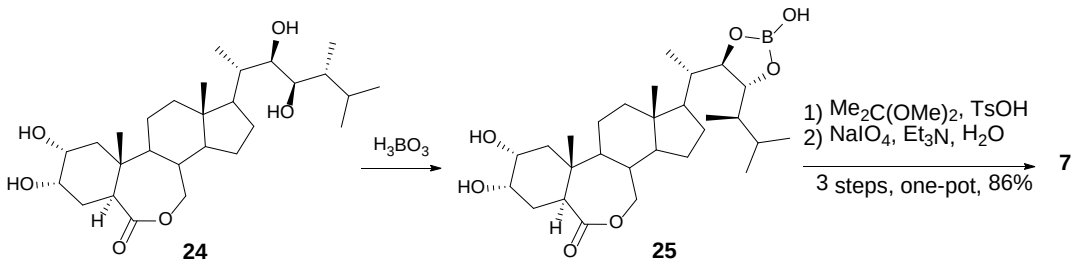
Reduction of hydroxy ketone **22** with $LiAlH_4$ or $NaBH_4$ led to *anti*-diol exclusively. The successful synthesis of *syn*-diol **23** required the prior protection of the hydroxyl group in **22** and a careful selection of the reducing agent. An acceptable result (85%) was achieved for this three-step procedure, using DIBAL-H for the reduction of the 23-keto group.

The developed methodology for BS side chains was then applied for the preparation of brassinolide (**1**) and its congeners **2-6**. Aldehyde **7** was estimated to be a particularly convenient intermediate for their synthesis as it already possesses the fully functionalized AB ring system that is characteristic for BS. However, the known method for its preparation is relatively laborious and inefficient (1.1% overall yield over 15 steps from stigmasterol).[5c, 14]

Therefore commercially available epibrassinolide (**24**)[15] was investigated to get a better access to aldehyde **7** (Scheme



Scheme 4 Model synthesis of the side chains of cathasterone, cryptolide, and brassinolide.



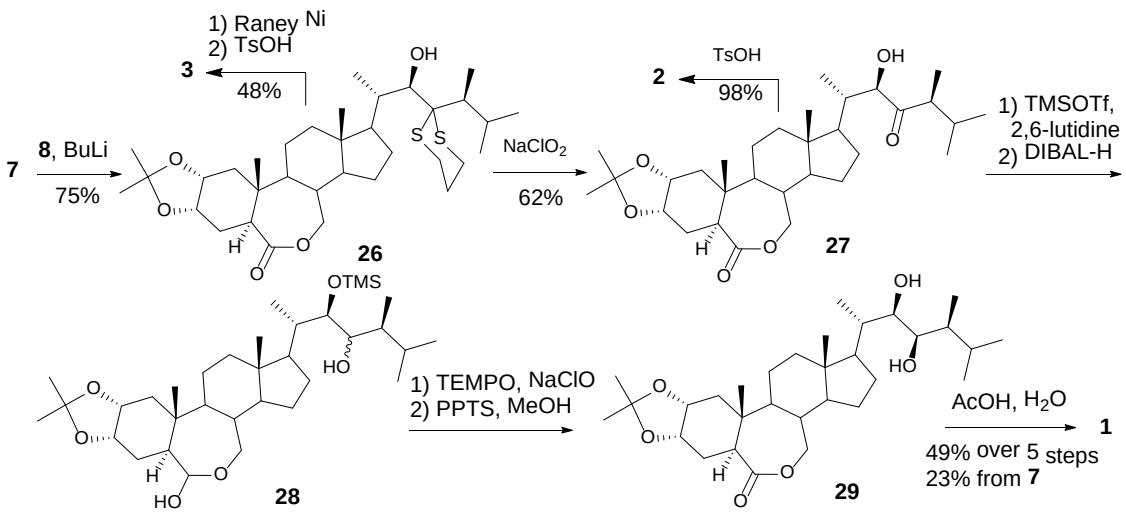
Scheme 5 Synthesis of the aldehyde **7**.

5). To differentiate the two diol moieties in **24**, regioselective formation of the thermodynamically more stable 22,23-boronate was explored.[16] A previous study made use of methylboronic acid esters, but these were not satisfactory for the present purpose because of their high stability and the necessity of an additional oxidation step for their removal.

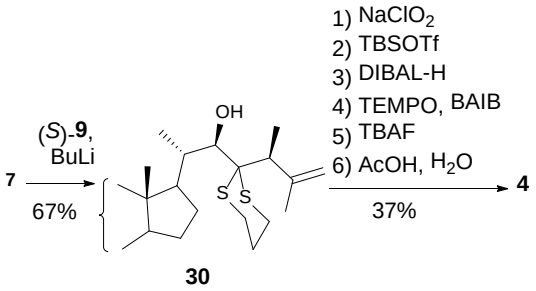
A good variant for the temporary protection of the 22,23-diol was found using the ester of boric acid.[17] Compound **25** was obtained by mixing epibrassinolide (**24**) with an equivalent amount of boric acid in THF. The crude reaction mixture was subsequently treated with 2,2-dimethoxypropane and the obtained 2,3-acetonide was treated with triethylamine, water and sodium periodate. Under these reaction conditions, the 22,23-boric ester was in equilibrium with the parent diol, which subsequently was cleaved to give the aldehyde **7**. Through this one-pot synthesis, the desired aldehyde **7** was obtained in 3 steps and 86% overall yield starting from commercially available epibrassinolide **24**.

The reaction of aldehyde **7** with the lithium salt of dithiane **8** proceeded without affecting the lactone and led to coupling product **26** in good yield (Scheme 6).

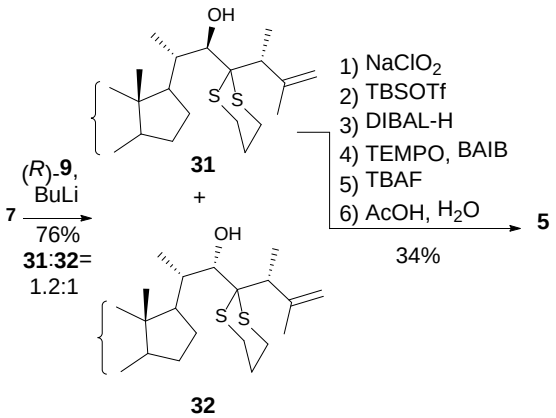
Its reductive desulfurization followed by deprotection of the acetonide afforded 23-deoxy-brassinolide (**3**). Oxidative hydrolysis of the 1,3-dithiane derivative **26** in the presence of



Scheme 6 Synthesis of brassinolide (**1**), cryptolide (**2**), and 23-deoxybrassinolide (**3**).



Scheme 7 Synthesis of Δ^{25}-brassinolide (**4**).



Scheme 8 Synthesis of Δ^{25}-epibrassinolide (**5**).

$NaClO_2$ yielded the carbonyl compound **27**, which after the removal of the acetonide provided cryptolide (**2**). Silylation of **27** and successive treatment of the resulting product with DIBAL-H at -78 °C led to compound **28**. Its lactol group was converted back to the lactone by TEMPO/NaClO oxidation to give brassinolide (**1**) after removal of the protecting group.

Radioactive labeled BS are valuable tools in studies of the mechanistic aspects of these phytohormones in the regulation of plant processes and in pharmacokinetic studies.[18] Therefore, we have prepared a series of unsaturated analogs of BS as direct precursors of the corresponding tritiated derivatives.[18a, 19] Compound **4** was prepared starting from **(*S*)-9** (Scheme 7) according to the above procedure. The only difference was the use of bis[(acetoxy)iodo]benzene (BAIB) as a stoichiometric oxidant in the conversion of the intermediate lactol into the lactone.

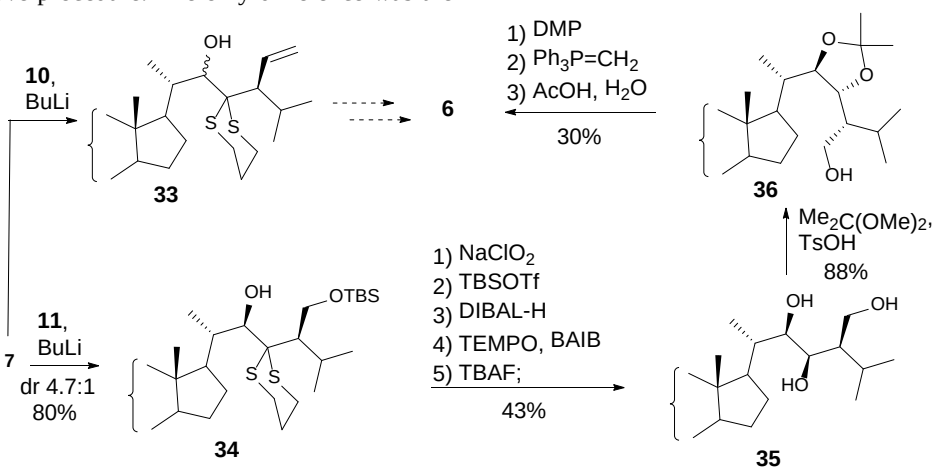
Contrary to the results obtained for the preparation of the campestane derivatives **1** and **4**, the addition of the lithium salt of dithiane **(*R*)-9** to the aldehyde **7**, which is necessary for the synthesis of Δ^{25}-epibrassinolide, proceeded less stereoselectively and gave a mixture of adducts **31** and **32** in the ratio 1.2:1 (Scheme 8). These were separated by chromatography and compound **31** was successfully converted to the target product **5**. The asymmetric center at C-24 had no influence on the stereoselectivity of the reduction of the intermediate 23-ketone. To confirm the configuration of the stereocenters at C-22 and C-23, the Δ^{25}-unsaturated compounds **4** and **5** were hydrogenated over a Pd/C catalyst to give brassinolide (**1**) and epibrassinolide (**24**), respectively.

The synthesis of the unsaturated stigmastane derivative **6** was initially attempted through the coupling of the lithium salt of dithiane **10** with aldehyde **7** (Scheme 9). However, the reaction produced a mixture of C-22 isomers **33** that proved inseparable in our hands. An alternative approach involved the use of dithiane **11**. Fortunately, the addition of its lithium salt to aldehyde **7** proceeded smoothly to give the desired adduct **34** in 66% isolated yield with dr=4.7:1. Standard manipulations allowed access to triol **35** which was regioselectively protected as 22,23-acetonide **36**. Primary alcohol oxidation followed by Wittig methylenation and acetonide deprotection led to Δ^{28}-homobrassinolide **6**.

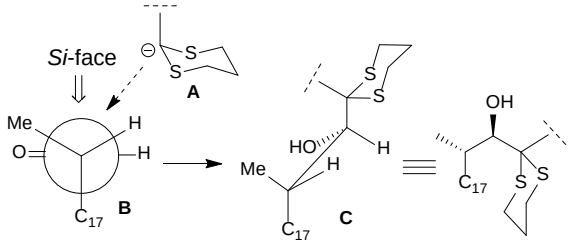
Discussion

The initial premise of this study was based on the hypothesis that bulky nucleophiles **A** (derived from *n*-BuLi and dithianes **8-11**) in the reaction with 22-aldehydes **B** should give Felkin-Anh adducts **C** in a highly diastereoselective manner (Scheme 10).

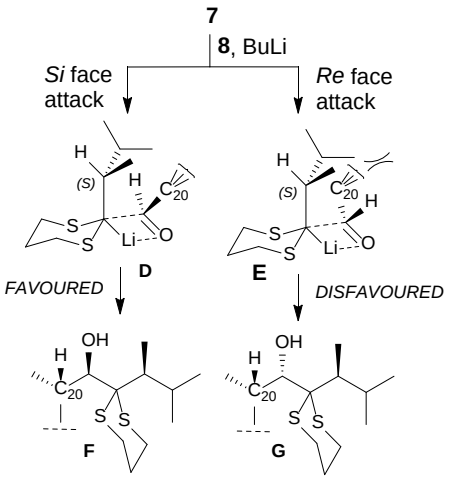
This was indeed the case with the lithium salts of dithianes **8** and **(*S*)-9**, but for **(*R*)-9** and **10** the diastereoselectivity was poor, thus showing the influence of an asymmetric center in the



Scheme 9 Synthesis of Δ^{28}-homobrassinolide (**6**).



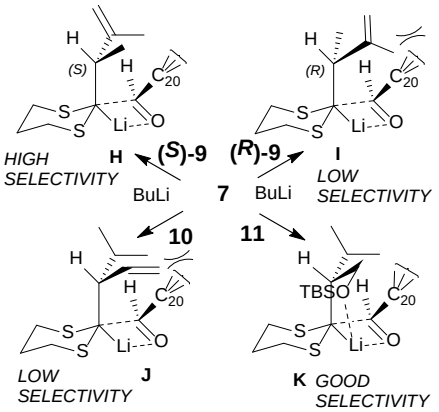
Scheme 10 Addition of carbanions **A** to aldehydes **B** under Felkin-Anh control.



Scheme 11 Facial selectivity in the addition of lithiated dithiane **8** to aldehyde **7**.

nucleophile on the stereochemical course of the addition. This influence may be caused by conformational peculiarities of the 2-lithio-2-alkyl-1,3-dithianes. These compounds are held in a fixed conformation with an alkyl group in the axial position (Scheme 11).[20] The most stable conformation is that in which the hydrogen atom is oriented toward the 1,3-dithiane cycle. Additions of organolithium reagents to carbonyl compounds are thought to occur through four-center transition structures.[21] Because of their compactness, the reaction is potentially sensitive to steric factors. In the addition of the lithium salt of dithiane **8** to aldehyde **7**, there are two possible transition structures **D** and **E**, corresponding to *Si* face and *Re* face attack, respectively. Transition structure **E** is disfavored by steric interactions between the isopropyl group of the reagent and C-20 of the steroidal molecule. The major product **F** thus corresponds to that (**C**) predicted by the Felkin-Anh model.

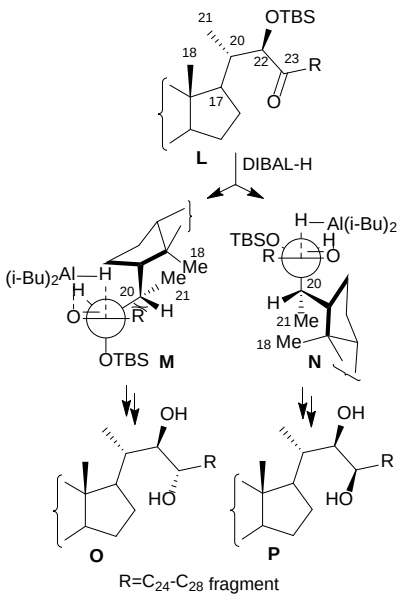
The same diastereoselectivity was observed in the addition of lithiated dithiane **(*S*)-9** to aldehyde **7** as a result of the preferential formation of the *Si* face transition structure **H** (Scheme 12). The corresponding *Si* face transition structure **I** for the similar reaction of the enantiomeric lithiated dithiane **(*R*)-9** is disfavored due to a repulsive interaction between the isopropenyl group of the reagent and C-20 of aldehyde **7**. The poor diastereoselectivity of the addition of lithiated dithiane **10** to **7** can be attributed to steric hindrance imposed by the isopropyl and ethylene groups in **J**. The coordination of lithium with OTBS in the transition structure **K** was thought to be the



Scheme 12 Transition structures for the *Si* face addition of lithiated dithianes **9-11** to aldehyde **7**.

reason for the good diastereoselectivity for the reaction of lithiated dithiane **11** with aldehyde **7**.

Much to our delight, the reduction of the silyl ethers of the 22-hydroxy-23-ketones with DIBAL-H proceeded with high stereoselectivity to provide the diols **P** with the required *syn* stereochemistry (Scheme 13). The stereochemical course of the reaction was not influenced by the configuration at C-24 and the bulkiness of the silyl group affected the selectivity only to a small extent (*syn:anti* = 9:1 for TBS and 3:1 for TMS). According to the Houk model,[22] the addition of DIBAL-H to silyl ethers of 22-hydroxy-23-ketones **L** should proceed through the transition structure **M** with the carbonyl group being perpendicular to the C-OTBS bond. The reaction starts with the formation of a Lewis acid-base complex between the reducing agent and the carbonyl compound. The complex arranges itself so to minimize repulsive interactions between ^{i}Bu and C_{20}-substituents. The hydride transfer should occur in the anti-position with respect to the OTBS group to give the *anti*-diol **O**. However, the major diastereomer **P** had the



Scheme 13 Transition structures for the reduction of silyl ethers of 22-hydroxy-23-ketones **L** with DIBAL-H.

opposite configuration at C-23 to that predicted. The observed stereoselectivity of the reaction can be explained by a strong steric repulsion between the branched side chain and the substituents at C-20 thus destabilizing conformation **M**. These steric repulsions are minimized in conformation **N** where the carbonyl group is perpendicular to the plane formed by C-17, C-20 and C-22. Hydride attack from the less hindered side of the molecule leads then to the required *syn*-diol **P**.

Conclusions

In conclusion, we have developed an efficient procedure for the stereoselective construction of steroidal side chains. Its scope and limitations were examined in the synthesis of steroidal plant hormones. The total number of steps was reduced considerably through application of epibrassinolide as starting material. The use of boric acid for selective protection of the 22,23-diol group in epibrassinolide enabled the preparation of the key intermediate C-22 aldehyde in 86% overall yield *via* a one-pot three-step procedure.

The key reaction in the syntheses of the steroidal side chains was the addition of lithiated dithianes to the C-22 aldehyde. The stereochemical course of the reaction proved to be dependent on the asymmetric center in the nucleophile. A good diastereoselectivity was observed for the addition of the lithium salts of (*S*)-dithianes to give adducts with a campestane carbon skeleton. An important advantage of the proposed method is its applicability for the preparation of a broad spectrum of steroidal plant hormones, including biosynthetic precursors and metabolites. The validity of the procedure was demonstrated by the synthesis of brassinolide, cryptolide and 23-deoxybrassinolide. In addition, a number of brassinosteroids with a double bond in the side chain were prepared as precursors for tritiated derivatives for biosynthetic and pharmacokinetic studies.

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Notes

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† Electronic Supplementary Information (ESI) available: copies of ^{1}H and ^{13}C NMR spectra. See DOI:

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