Pure & Appl. Chem., Vol. 62, No. 7, pp. 1319–1324, 1990.
Printed in Great Britain.

Synthesis of brassinosteroids

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Abstract - Different approaches to the synthesis of brassinosteroids, new class of phytohormones, have been developed starting from accessible natural steroids. Several brassinosteroids of natural type (brassinolide, 28-homobrassinolide, 24-epibrassinolide, 28-norbrassinolide etc.), their analogues and intermediates have been synthesized for study as plant growth-promoting substances.

INTRODUCTION

The discovery of brassinolide (ref. 1), a new polyhydroxysteroid with high plant growth-promoting activity, in 1979 was the beginning of a new period in the investigation of hormonal system and chemical growth regulation in plants. Since then several related steroids have been isolated from plant sources (ref. 2). The characteristic feature of brassinosteroids (BS) is the ability to act in very low concentrations on the hormonal balance in plants, to activate the metabolic and growth processes, to increase the total plant productivity. These properties and extremely low content of BS in natural sources are the major reasons for the interest of many scientific groups in the synthesis of BS (ref. 3). In the extending of studies on natural and transformed steroids carried out by the school of Prof. A. Akhrem, the BS synthesis was the subject of study for a number of years in our laboratory. In the course of our investigation several synthetic approaches were developed and a lot of natural type BS and their analogues were synthesized from accessible natural steroids.

The employment of stigmasterol 1 as starting material in many synthetic schemes is due to its ready availability and the presence of the functional groups in its molecule permitting the needful transformations of cyclic part and the side chain. The conversion of 1 to 28-homobrassinosteroids with the retaining of side chain carbon sceleton was accomplished as follows (Fig. 1). Cycloketone 2, the key intermediate in BS synthesis from stigmasterol, was prepared from 1 *via* tosilation, iso-steroidal rearrangement of the tosylate and Jones oxidation of the corresponding 6-alcohol (ref. 4). Interaction of the cycloketone 2 with hydrogen bromide in acetic acid at room temperature proceeded regioselectively with cyclopropane ring opening to give the bromoketone 3 with almost quantitative yield. The dehydrobromination of 3 and further hydroxylation of the resultant dienoketone 4 gave (ref. 5) tetrahydroxyketone 5, the 22S,23S-isomer of natural hormone ethylbrassinone. Its transformation into 22S,23S-homobrassinolide 9 can be fulfiled by the successive steps of cis-diol groups protection, Baeyer-Villiger oxidation, diols deprotection and relactonization.

An alternative synthesis of 9 which permits to avoid the hydroxygroups protection and deprotection stages was then elaborated by us (ref. 6). This included the regioselective addition of bromine and hydrogen bromide to 22-bond and propan cycle of 2, respectively, Baeyer-Villiger oxidation of ketone 6, debromination with simultaneous dehydrobromination of 7 and hydroxylation of the dienolactone 8.

For the synthesis of BS, possessing the natural configuration of hydroxy functions in the side chain, we used an approach based on the transformation of 22R,23R-epoxides (ref. 7). The epoxidation of 3 with MCPBA gave 10 as the major reaction product. Dehydrobromination of 10 and further epoxide ring opening under the action of HBr followed by nucleophilic substitution with

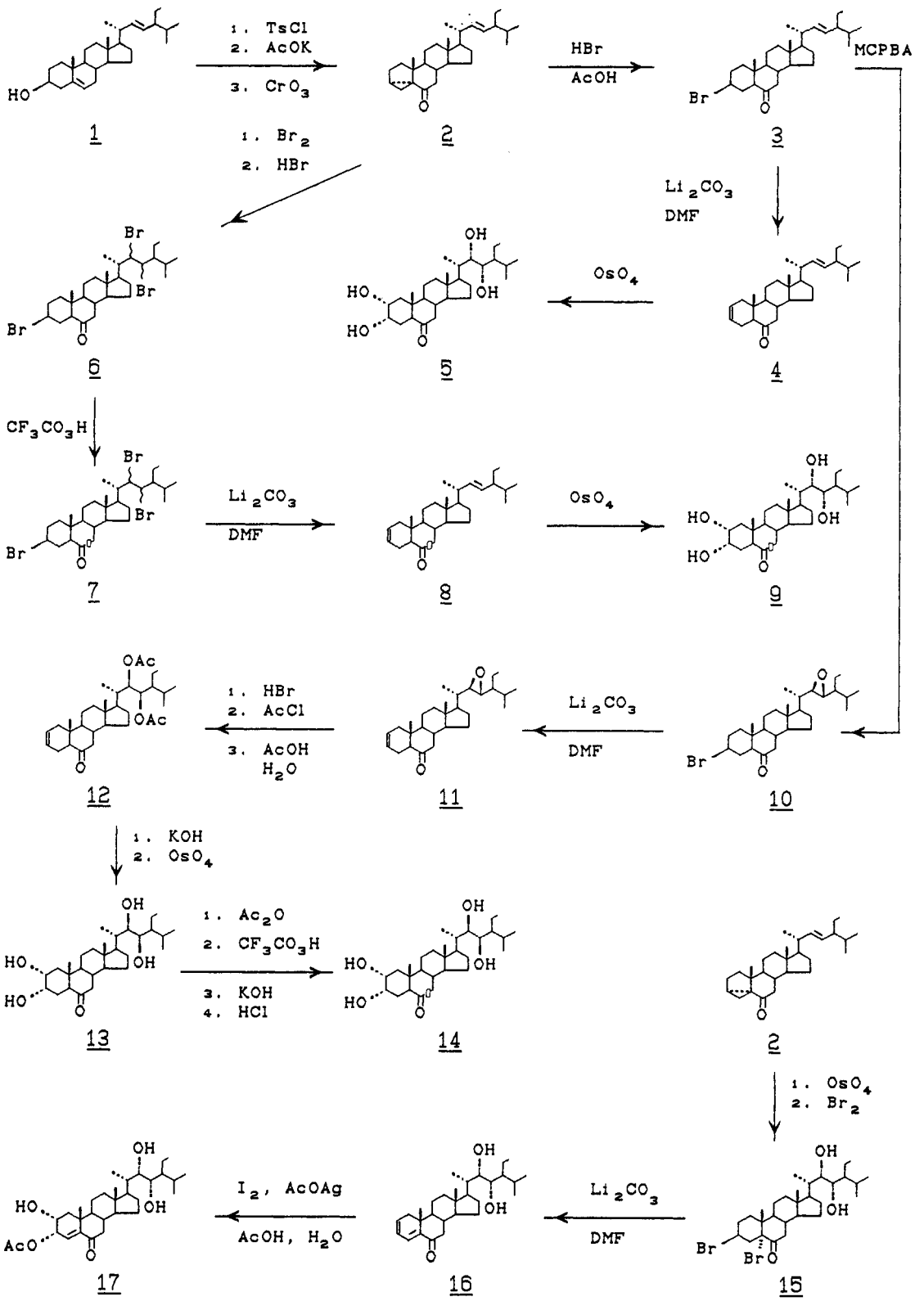

Fig. 1. Synthesis of 28-homobrassinosteroids from stigmasterol.

inversion at the carbon atom bearing the Br atom in bromoacetate intermediate gave the diacetate 12. The latter was easily transformed into ethylbrassinone 13 and homobrassinolide 14 by the usual way. This approach has been also used for the synthesis of 22S,23S-28-homobrassinosteroids (ref. 8).

The Δ^4-BS-analogues like 17 were synthesized by the employment of α-cis-hydroxylation method developed by us earlier (ref. 9). The dienoketone 16 obtained from 2 *via* 15 (in accordance with ref. 10) was treated with iodine and silver acetate in aqueous acetic acid to give the unsaturated ketone 17.

For the preparation of wide range of BS possessing both natural and unnatural side chains including brassinolide and its isomers we used an approach based on the reaction of sulfone derivatives 20 with steroidal 22-aldehydes (ref. 11) (Fig. 2). Sulfones 20 were obtained from isovaleric acid *via* successive stages of α-alkylderivatives, alcohols, tosilates 19 and corresponding sulfides. Addition of the aldehyde 21, obtained by the usual method from stigmasterol, with the carbanion derived from 20 was followed by acetylation to give β-acetoxy sulfone 22. Reduction of 22 with Na-Hg and further deprotection gave an olefinic product 23. Application of the method of 22,23-dihydroxyfunctionality construction *via* epoxides to this compound allowed us to obtain all the possible isomers of 22,23-cis-diols of ergostan, stigmastan and cholestan series. When the 22R,23R-epoxide 24 (R=(S)-Me) was subjected to dehydrobromination and epoxy ring transformation followed by treatment of the resultant 25 with osmium tetraoxide castasterone 26 was obtained. The Baeyer-Villiger oxidation of its tetraacetate, deprotection and relactonization yielded brassinolide 27. It should be pointed out that along with the products of the normal 20β-series the BS analogues with 20α-methyl group have been synthesized employing this approach.

Many of side chain formation methods in BS synthesis are based on the reactions of steroidal 22-aldehydes with various vinyl carbanions and futher transformations of allylic alcohol intermediates 3. However these stereo-selectivities were not high and gave relatively low yields of the desired 22α-alcohols along with substantial amounts of their 22β-isomers. We proposed that the reaction could be more stereoselective if the anionic centre of nucleophile was directly connected to some group other than H. The most suitable for this purpose it seemed us to be trimethylsilyl group because it can be easily removed on the next stages and furthermore it facilitates the formation of carbanion. That is why we synthesized (ref. 12) the vinylsilane 28 from the acetylene 29 and tested the stereoselectivity of its addition with aldehyde 21. The reaction of 21 with 28 proceeded smoothly at -78°C to give a 10:1 mixture of epimeric alcohols 30 and 31 in 97% total yield. Epoxidation of 30 followed by nucleophilic cleavage of Si-C-bond in 32 gave the epoxide 33 as a major product. On the other hand, an initial Si-C-bond cleavage in 30 followed by epoxidation of alkene 34 afforded the brassinosteroid intermediate 35. The further construction of the side chain of brasinolide from 35 is known (ref. 3).

The first step of the synthesis (ref. 13) of 24-epibrassinosteroids from ergosterol 36 was the reduction of diene system in B-cycle by the action of Li in diethylamine (Fig. 3). Steroid 38 was converted without isolation into 39 which was further transformed into dienoketone 40 by the usual way. The latter was hydroxylated to give the stereoisomeric tetraols 41 and 42, the synthetic precursors of 24-epibrassinolide 43 and its 22S,23S-isomer 44, respectively.

Among different approaches to the side chain construction one of the particular interest is the use of heterocyclic intermediates containing the desired functionality in a latent form. In this respect rather promising seems to be the application of isoxazole and 2-isoxazolines - the adducts of 1,3-dipolar cycloaddition reactions of nitrile oxides with unsaturated systems (ref. 14). That is why we studied the cycloaddition (ref. 15) of iso-butyronitrile oxide with the steroidal olefins 46 and acetylenes 47 prepared from pregnenolone acetate 45 (Fig. 4). Both reactions proceeded regiospecifically to give the cycloadducts 48 and 49 in high yields. The dehydration with simultaneous dehydrogenation of 48 under the action of thionyl chloride in DMF gave (ref. 16) the isoxazole 50. The same product was obtained from 49 in acidic conditions. The isoxazole 50 was catalitically hydrogenated on Raney nickel to give the enaminoketone 51 with natural configuration at C-20 (ref. 15b). The latter could be further hydrolised into diketone 52 or transformed into 53 by the usual way (ref. 14a). Unsaturated ketones like 53 can be used as intermediates for the synthesis of the side chains of BS (ref. 17). An alternative synthesis of 53 has been achieved

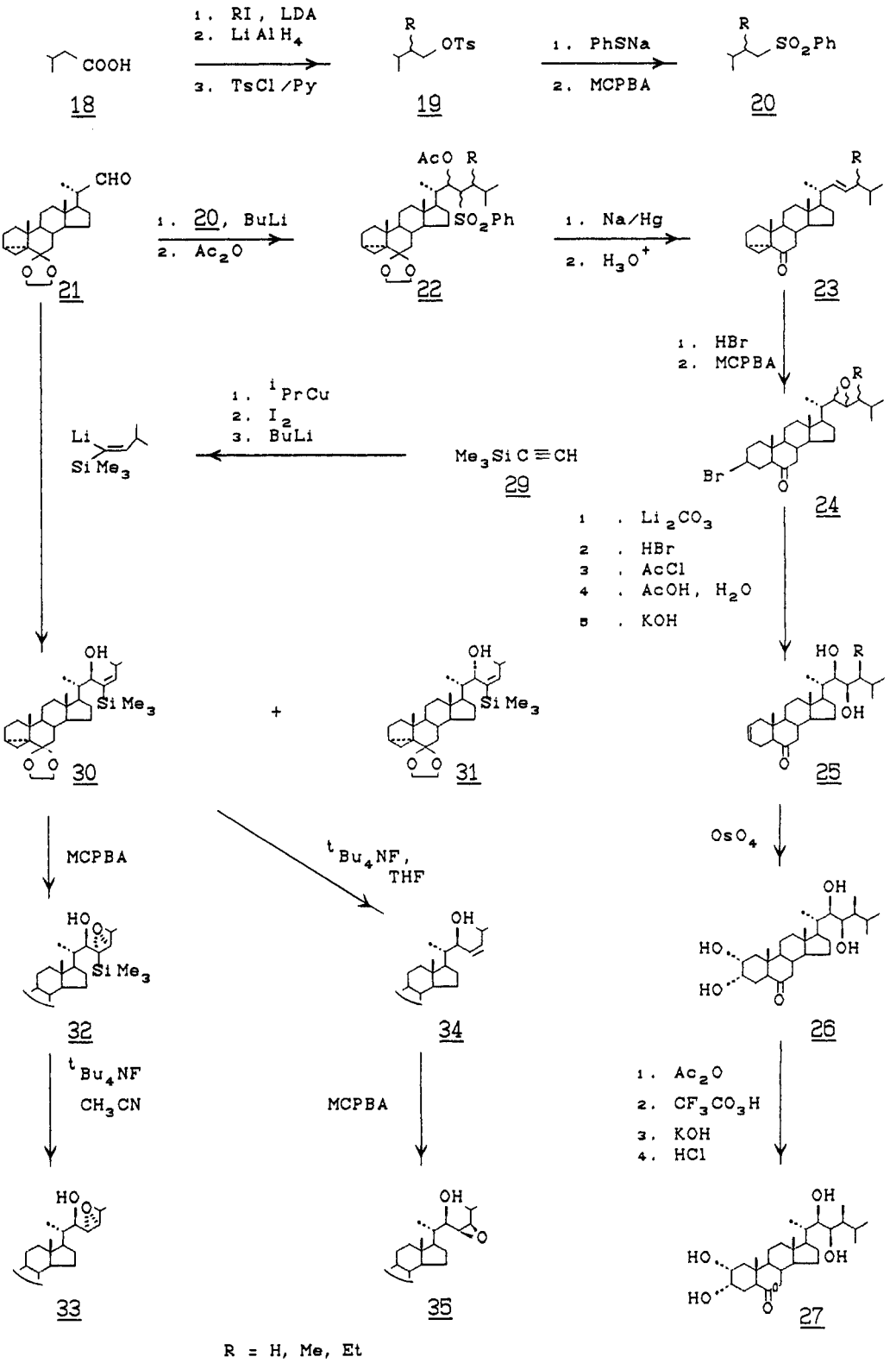

Fig. 2. Synthesis of brassinolide, its homologues, isomers and intermediates *via* 22-aldehydes

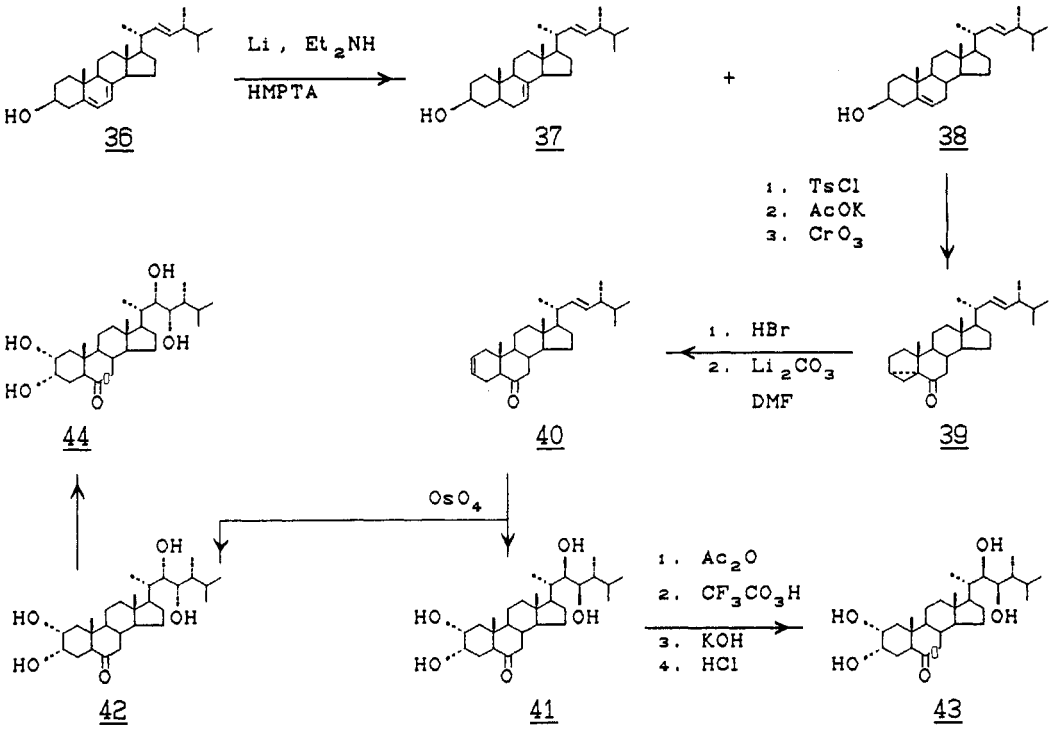

Fig. 3. Synthesis of 24-epibrassinosteroids from ergosterol

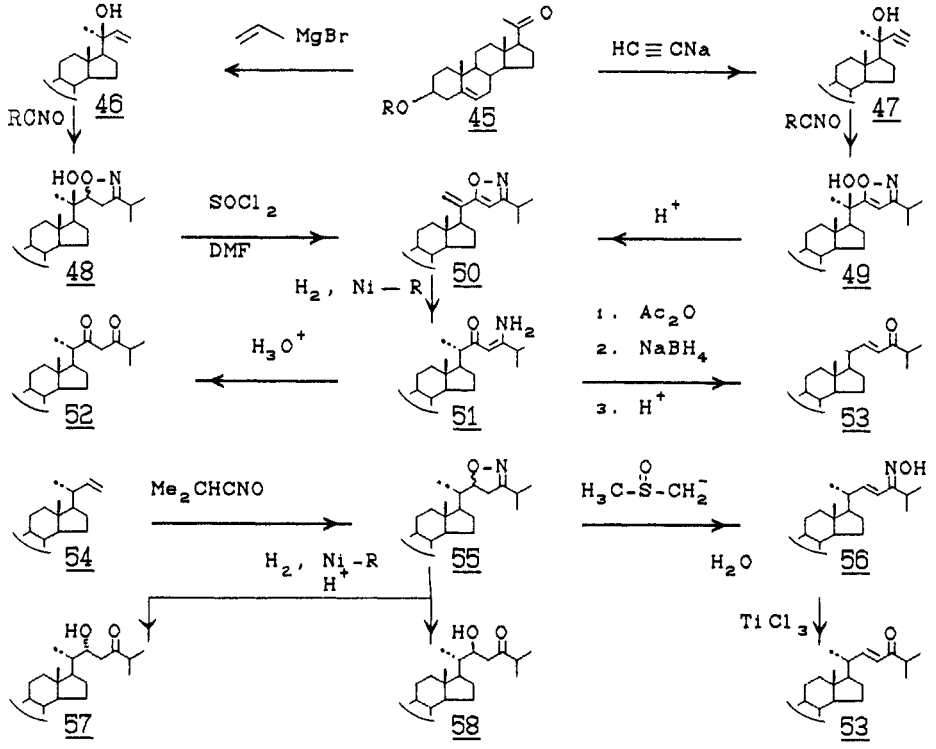

Fig. 4. Synthesis of brassinosteroidal intermediates *via* cycloadducts of nitrile oxides with steroidal olefins and acetylenes

(ref. 18) by the isoxazoline ring opening in 55 under the action of strong base (anion of dimethylsulfoxide) followed by hydrolysis of the resultant oxime 56. In this respect the β-ketols 57 and 58 obtained from the adduct 55 by the method developed by us earlier (ref. 19) are also perspective.

Acknowledgements

I thank Prof. A. Akhrem and Prof. F. Lakhvich, with whom much of this work was done in collaboration, for many stimulating discussions, and for their constant interest for this theme. I also thank the group of co-workers without whom none of this work would have been possible: their names are recorded in the references below.

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