changed, but the plane of symmetry is lost if the hydroxyl group of **3** is esterified (as in **4**) with (*S*)-lactic acid.

In our full paper[2] we summarized the classification of steric centers by a chart which separated centers of stereoisomerism into chiral and achiral and then subdivided the achiral centers into those having and not having chiral configurations. Centers of prostereoisomerism were treated in an analogous manner. This classification brought out pherochiral properties only if the element was also graphochiral (and propherochiral properties only if it was also prographochiral). The present terminology[7] is therefore better balanced and it allows one to focus on the relevant property, as we have illustrated in discussing examples **1–4**.

(7) Of the examples listed in Chart 1,[2] Cghij, $Cg^{+}g^{-}hi$, **8a–c,f,g** are graphochiral; tetragonal Xghij, octahedral Xgghgig, **8d, e, h** are agraphochiral. (Of this last group **8d, e, h** can be further classed as prographochiral whereas the two others are not prographochiral.) Cgghi and $Cggh^{+}h^{-}$ are prographochiral; tetragonal Xgggh and octahedral Xgggggh are not prographochiral. In the alternative classification Cghij, **8a–e** are pherochiral; $Cg^{+}g^{-}hi$, tetragonal Xghij, **8f–h** and octahedral Xgghgig are apherochiral; Cgghi is propherochiral; $Cggh^{+}h^{-}$, tetragonal Xgggh, and octahedral Xgggggh are not propherochiral.

## Potential Inhibitors of L-Asparagine Biosynthesis. I. β-Elimination Reactions with β-Hydroxyaspartic Acid Derivatives[1a,b]

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In the course of a study aimed at preparing irreversible inhibitors of the enzyme L-asparagine synthetase we observed a β-elimination reaction with derivatives of β-hydroxyaspartic acids, the results of which form the text of this paper.

In view of the usefulness of the diazoacetate group in the design of irreversible enzyme inhibitors, we attempted to synthesize the *O*-diazoacetyl derivative of both *threo*- (**1a**) and *erythro*-β-hydroxyaspartic acid (**1b**). Initially we began with **1a** since it was readily obtainable,[2] whereas **1b** was more difficult to obtain. Because of contradictory reports[2,3] concerning the stereospecific synthesis of **1a** and **1b**, we used two methods to ascertain their stereointegrity, namely a chemical vanadate test[4] and analysis *via* an automatic amino acid analyzer;[5] both confirmed the stereopurity of **1a** and **1b**.

(1) (a) This work was supported by Grant M-28 from the Health Research and Services Foundation, Pittsburgh, Pa., and Grant CA-11714 from the National Cancer Institute, NIH, Bethesda, Md. (b) Presented in part at the 163rd National Meeting of the American Chemical Society, Boston, Mass., April 1972, Medi 26. (c) Taken in part from the M.S. Dissertation of B. S. P., University of Pittsburgh, July 1971.

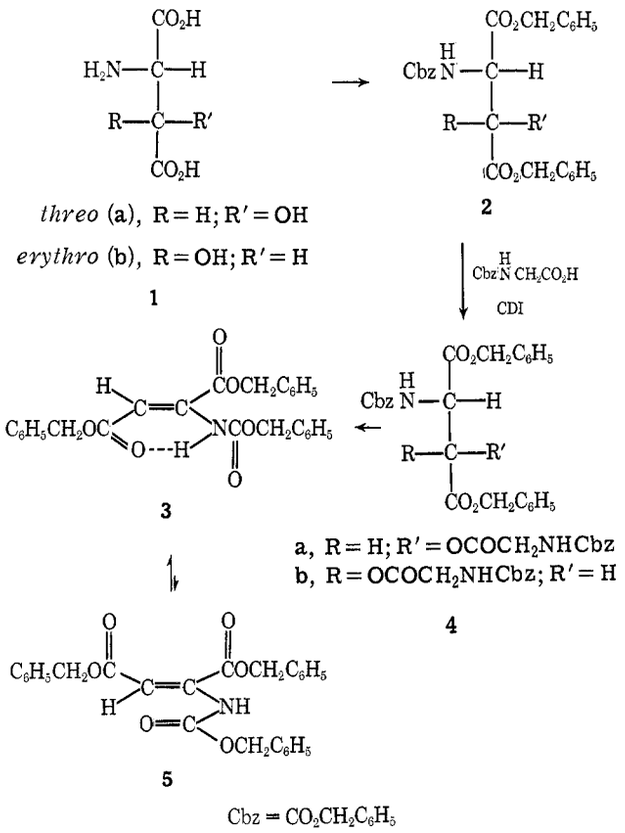
(2) C. W. Jones, III, D. E. Leyden, and C. H. Stammer, *Can. J. Chem.*, **47**, 4363 (1969).

(3) H. Okai, N. Imamura, and N. Izumiya, *Bull. Chem. Soc. Jap.*, **40**, 2154 (1967).

(4) Y. Liwschitz, Y. Robinson, and A. Haber, *J. Chem. Soc.*, 3589 (1962).

(5) Recorded on a Beckman Model 116 A.A.A. The authors thank Mr. J. P. Vergnes, Department of Biochemistry, University of Pittsburgh, for these determinations.

The amino function of **1a** was readily protected by carbobenzoxylation followed by esterification of the carboxyl groups to give **2a**.[6] Esterification of **2a** with carbobenzoxyglycine in the presence of the condensing agent *N*,*N*′-carbonyldiimidazole (CDI)[7] afforded an oil which, according to tlc, was composed of three components. Separation by preparative tlc afforded starting material, the supposed **4a**, and compound **3**, a product of β elimination. Compound **4a** could not be




Cbz = $CO_2CH_2C_6H_5$

crystallized and attempts to prepare an analytical sample failed because of a tendency for it to decompose to **3**. The tentative assignment of the structure for **4a** was based on nmr data [δ 3.88 (d, $CH_2$ of glycyl, singlet after shaking with $D_2O$), 5.65 (m, β-H)] and the fact that stirring **4a** in THF with imidazole (this base is a side product of reactions with CDI) readily affords some of compound **3**.

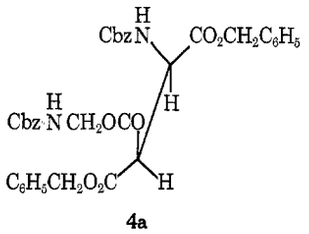
By a scheme similar to that used in the threo series, **2b** was obtained in a 72% overall yield from **1b**. In an attempt to synthesize the coupled product **4b**, similar results were obtained using CDI and carbobenzoxyglycine, namely, small amounts of starting material and unsaturated **3** were obtained and the major product presumably was **4b**. The latter was noncrystalline and attempts to prepare an analytical sample caused some decomposition to **3**. The tentative assignment of the structure for **4b** was based on nmr data [δ 3.92 (d, $CH_2$ of glycyl) and 5.66 (m, β-H)]. Furthermore, stirring the supposed **4b** in THF with imidazole very slowly (in contrast to the facile **4a**) formed some of **3**. As a control experiment both **2a** and **2b** were separately stirred with imidazole but only

(6) Y. Liwschitz and A. Singerman, *J. Chem. Soc.*, 1696 (1967); H. Okai and N. Izumiya, *Bull. Chem. Soc. Jap.*, **42**, 3550 (1969).

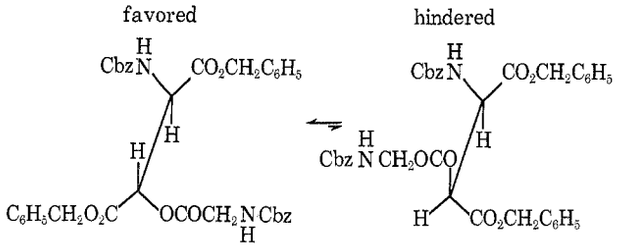
(7) H. A. Staab, *Angew. Chem., Int. Ed. Engl.*, **1**, 351 (1962).

starting material was recovered, thus implicating **4a** and **4b** as the source of **3**.

After several months in the refrigerator, compound **3** (from **4a** and **4b**) showed evidence on tlc for approx-



**4a**




**4b**

imately 5% of a lower $R_f$ component. Preparative tlc afforded a small sample of this new compound, whose structure was assigned as the cis isomer **5**. Evidently there is an equilibrium which lies far on the trans isomer (**3**) side. If **3** is dissolved in 15:1 isooctane–methylene chloride and allowed to remain for 1 week in sunlight[8] there is approximately a 25–35% conversion to **5**, and this ratio does not change on heating the mixture to 150°.

Proof for these structures is based on the following evidence. An nmr spectrum of **3** showed signals at $\delta$ 5.15, 5.25 (s, 6 H, benzylic), 5.51 (s, 1 H, vinyl), 7.34 (s, 15 H, aromatic), and 9.71 (broad, 1 H, NH), while in the ir ($CCl_4$) there was a broad NH band at 3.01 (H bonded) and carbonyl absorption at 5.73 and 5.92 $\mu$, the latter band due to the conjugated, H-bonded ester.[9] Furthermore, catalytic hydrogenation (Pt) of **3** gave aspartic acid (identical ir with ir of authentic sample). The cis isomer **5** showed resonance in the nmr at $\delta$ 5.00, 5.15 (m, 6 H, benzylic), 6.67 (s, 1 H, vinyl), 6.96 (broad, 1 H, NH, disappears with $D_2O$), and 7.33 (m, 15 H, aromatic). The ir ($CCl_4$) spectrum of **5** showed peaks at 2.92 $\mu$ (NH) and carbonyl absorption at 5.73 and 5.78 $\mu$.

The data for **3** and **5** deserve brief comment. In **3**, where a six-membered H-bonded ring can exist, the NH is broadened in the ir and further downfield in the nmr, and the H-bonded carbonyl appears at higher wavelength in the ir,[9] when compared to **5**. In **5**, where the carbamate carbonyl can assume a closer proximity to the vinyl hydrogen than the ester carbonyl can in **3**, there is greater deshielding[9,10] of this proton and it appears further downfield in the nmr.

The observation of $\beta$ elimination with certain amino acid derivatives is well documented.[11] The formation of **3** from **4a** could be explained by a one-step $\beta$-elimination mechanism (E2), since in the threo derivative the favored conformation has the leaving groups trans-coplanar. However, obtaining **3** from **4b** is not as readily explained. **4b** in its favored arrangement does not have the leaving groups coplanar, and rotation to bring about coplanarity followed by $\beta$ elimination would afford **5**. There are several possibilities[12] that could explain the formation of **3** from **4b** (cis elimination, E1 or E1cB), but in the absence of further experimental evidence it would be unwise to speculate.

(8) R. E. Lutz, D. F. Hindley, and R. H. Jordan *J. Amer. Chem. Soc.*, **73**, 4647 (1951).

(9) R. Huisgen, K. Herbig, A. Siegl, and H. Huber, *Chem. Ber.*, **99**, 2526 (1966).

(10) J. E. Dolfini, *J. Org. Chem.*, **30**, 1298 (1965).

(11) I. Photaki, *J. Amer. Chem. Soc.*, **85**, 1123 (1963); Y. Liwschitz, A. Singerman, and S. Sokoloff, *J. Chem. Soc.*, 1843 (1968); R. G. Hiskey, R. A. Upham, G. M. Beverly, and W. C. Jones, Jr., *J. Org. Chem.*, **35**, 513 (1970); R. K. Olsen, *ibid.*, **35**, 1912 (1970).

(12) J. March, "Advanced Organic Chemistry: Reactions, Mechanisms, and Structure," McGraw-Hill, New York, N. Y., 1968, pp 728–736.

(13) G. B. Payne and P. H. Williams, *J. Org. Chem.*, **24**, 54 (1959).

### Experimental Section

Melting points were taken on a Fisher-Johns apparatus and are not corrected. Ultraviolet spectra were determined in 95% ethanol on a Beckman DB-G recording spectrophotometer. Infrared absorption spectra were recorded on either a Perkin-Elmer Infracord or a Beckman IR-8 spectrophotometer. Nmr spectra were recorded on a Varian A-60 or A-60D recording spectrometer in $CDCl_3$ with tetramethylsilane as internal standard. Thin layer chromatography and preparative tlc (1.0 mm) were carried out with silica gel GF (Analtech, Inc.) and spots were located with either uv light or by spraying with 3% ceric sulfate in 3 *N* $H_2SO_4$ and then heating. Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich. Dioxane and tetrahydrofuran (THF) were purified by distillation from $LiAlH_4$. The petroleum ether used had a boiling point range of 30–60°. All concentrations were done under reduced pressure. Prior to concentration all organic layers were dried with anhydrous $Na_2SO_4$. Mass spectra were determined on an LKB Model 9000 spectrometer at 70 eV.

*threo*-**β-Hydroxy-DL-aspartic Acid** (**1a**).—Maleic acid was converted[2] to **1a** in a 42% yield, and characterized by conversion to its dimethyl ester: HCl, mp 135–136° (lit.[2] mp 134–135°).

*erythro*-**β-Hydroxy-DL-aspartic Acid** (**1b**).—Fumaric acid was converted,[2] with much difficulty, to **1b** in a 13–15% yield, and characterized by conversion to its dimethyl ester: HCl, mp 149–150° (lit.[2] mp 152–153°). The difficulty encountered was in the conversion of fumaric acid to *trans*-epoxysuccinic acid.[13] In our hands, the epoxidation never proceeded as smoothly as reported,[13] while the conversion[2] of the epoxide to **1b** consistently (71% yield) gave good results.

**Dibenzyl *N*-Carbobenzoxy-*threo*-β-hydroxy-DL-aspartate** (**2a**).—This compound was prepared as reported[6] from **1a** in a yield of 88%: mp 90–91° (lit.[6] mp 88°); nmr $\delta$ 3.22 (broad, OH, exchangeable with $D_2O$), 4.6–4.8 (m, 2 H, H-$\alpha$, H-$\beta$), 5.12 (6 H, benzylic $-CH_2$), 5.75 (d, $J$ = 9 Hz, –NH), 7.33 (s, 15 H, phenyls); mass spectrum $m/e$ 463 ($M^+$).

**Dibenzyl *N*-Carbobenzoxy-*erythro*-β-hydroxy-DL-aspartate** (**2b**).—*N*-Carbobenzoxy-*erythro*-β-hydroxy-DL-aspartic acid was prepared[6] in a 78% yield from **1b**. This compound (0.50 g, 1.7 mmol) was heated under reflux in $CCl_4$ (10 ml) containing benzyl alcohol (0.68 ml, 6.8 mmol) and *p*-toluenesulfonic acid (0.05 g) and the $H_2O$ formed was removed *via* a Dean–Stark trap. After 72 hr the reaction solution was allowed to cool to room temperature. The resulting mixture, containing some crystallized product, was concentrated to dryness. The remaining oil was dissolved in $CHCl_3$, the solution was extracted twice with $NaHCO_3$ solution, dilute HCl solution, and $H_2O$, and the organic layer was dried. The solvent was evaporated and the product was crystallized from EtOAc–petroleum ether, affording 0.72 g (92%) of **2b**. The analytical sample had mp 74.5–75.5°; nmr $\delta$ 3.56 (broad, OH, exchangeable with $D_2O$), 4.57 (m, $H_\beta$, doublet after $D_2O$ exchange, $J$ = 2.5 Hz), 4.87 (m, $H_\alpha$), 5.08 (6 H, benzylic $-CH_2$), 5.83 (d, $J$ = 8 Hz, –NH), 7.30 (15 H, phenyls); mass spectrum $m/e$ 463 ($M^+$).

*Anal.* Calcd for $C_{26}H_{25}NO_7$: C, 67.37; H, 5.43; N, 3.02. Found: C, 67.49; H, 5.49; N, 2.90.

**Dibenzyl *O*-(*N*-Carbobenzoxyglycyl)-*N*-carbobenzoxy-*threo*-β-hydroxy-DL-asparate** (**4a**) **and Dibenzyl 2-Carbobenzoxyamino-**

fumarate (3).—A solution of *N*-carbobenzoxyglycine[14] (0.23 g, 1.1 mmol) in anhydrous THF (1.5 ml) was added dropwise to a solution of *N,N'*-carbonyldiimidazole (CDI, Aldrich Chemical Co., 0.17 g, 1.1 mmol) in THF (2.5 ml) and stirred for 1 hr. To this was then added in one portion, a solution of **2a** (0.50 g, 1.1 mmol) in 2 ml of THF. After 3 days the solution was concentrated to dryness, redissolved in $CHCl_3$, washed twice with 5% HCl solution, saturated $NaHCO_3$ solution, and water and dried. Evaporation of the solvent afforded a syrup (0.61 g) whose tlc ($CHCl_3$) showed three spots. Preparative tlc ($CHCl_3$) of an aliquot (75 mg) of the syrup separated the three components; the one of lowest $R_f$ was shown by comparison ir to be recovered **2a** (10 mg), the middle $R_f$ compound was tentatively assigned (by physical data) as **4a** (10 mg, 11%), and the upper $R_f$ product was identified as the unsaturated **3** (20 mg, 32%). Compound **3** could not be induced to crystallize but an analytical sample was obtained by rechromatographing (1% $CH_3OH$–$CHCl_3$) on thin layer plates, and the extracted product was washed through a 1:1 charcoal–Celite column with $CHCl_3$. Evaporation of the solvent left a very pale yellow gum (**3**), uv $\lambda_{max}$ 212 mμ (ε 18,400) and 270 (13,900) with a shoulder at 259; mass spectrum *m/e* 445 ($M^+$), 430, 354 ($-CH_2C_6H_5$), 310 ($-CO_2CH_2C_6H_5$), 248, 140, 107 ($C_6H_5CH_2O^+$), 91 (base peak).

*Anal.* Calcd for $C_{26}H_{23}NO_6$: C, 70.11; H, 5.20; N, 3.14. Found: C, 70.10; H, 5.27; N, 3.07.

**Dibenzyl *O*-(*N*-Carbobenzoxyglycyl-*N*-carbobenzoxy-*erythro*-β-hydroxy-DL-aspartate (4b) and Dibenzyl 2-Carbobenzoxyaminofumarate (3).**—The above procedure was followed using CDI (2.30 g, 14.2 mmol) in 10 ml of THF, *N*-carbobenzoxyglycine[14] (2.96 g, 14.2 mmol) in 10 ml of THF, and **2b** (3.30 g, 7.1 mmol) in 10 ml of THF. The reaction mixture was worked up after 2.5 hr, as described above, to give a pale yellow oil (4.4 g). Tlc (1% MeOH–$CHCl_3$) of the oil indicated one major spot, tentatively assigned as **4b**, and traces of starting material (**2b**) and unsaturated **3**. From several purifications by preparative tlc (1.5% MeOH–$CHCl_3$, 0.21 g/three plates) we obtained a fairly pure (not analytical grade) sample of **4b** (0.18 g, 81% yield). Further attempts at purifying **4b** only led to some decomposition to the unsaturated **3**. By the above preparative tlc we obtained a sample of **3** (8 mg, 5%) which was identical in the ir, nmr, and uv with compound **3** as isolated from the reaction with **2a**.

**Dibenzyl 2-Carbobenzoxyaminofumarate (3) and Dibenzyl 2-Carbobenzoxyaminomaleate (5).**—A solution of **4a** (10 mg), contaminated with a small amount of **3**, in 1 ml of $CHCl_3$ was divided in half and a few crystals of imidazole were added to one portion. After both portions were stirred overnight a tlc examination indicated no change in the ratio of **4a** to **3** in the absence of imidazole, but about a 60–70% conversion to **3** in the presence of imidazole. Similarly, when **4b** contaminated with only a trace of **3** was stirred overnight with imidazole there was only a 20–30% increase in intensity of the spot on tlc corresponding to **3**, whereas in the absence of imidazole there was no change.

A pure sample of **3** (0.22 g), when allowed to remain in a refrigerator for about 3 months, was slowly converted to approximately 5% of the cis isomer **5**. Preparative tlc afforded 10 mg of **5**, which could not be induced to crystallize. An analytical sample of **5** was obtained by chromatography as reported above for **3**, uv $\lambda_{max}$ 211 mμ (ε 20,900) and 267 (14,000). Compound **3** (75 mg) was more readily converted to **5** by dissolving it in 1 ml of $CH_2Cl_2$, diluting with 15 ml of isooctane, and exposing it to daylight for 7 days. The solvent was removed by evaporation, and tlc of the resulting oil indicated a 25–35% enrichment of **5**: mass spectrum of **5** *m/e* 445 ($M^+$), 430, 354 ($-CH_2C_6H_5$), 310 ($-CO_2CH_2C_6H_5$), 248, 140, 107 ($C_6H_5CH_2O^+$), 91 (base peak).

*Anal.* Calcd for $C_{26}H_{23}NO_6$: C, 70.11; H, 5.20. Found: C, 69.92; H, 5.48.

**DL-Aspartic Acid from Dibenzyl 2-Carbobenzoxyaminofumarate (3).**—To a solution of **3** (0.10 g, 0.22 mmol) in 50% ethanol–dioxane (4 ml) was added 50 mg of $PtO_2$. The mixture was hydrogenated at 1 atm pressure for 70 min, during which the calculated amount of $H_2$ was consumed. Removal of the catalyst by filtration and concentration of the filtrate gave a residue which was crystallized from $H_2O$–ethanol, yielding 12 mg (41%) of DL-aspartic acid (identical in the ir with an authentic sample).

(14) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 2, Wiley, New York, N. Y., 1961, p 891.

**Registry No.**—**2a**, 16712-81-5; **2b**, 34910-00-4; **3**, 34910-01-5; **5**, 34910-02-6; L-asparagine, 70-47-3.

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## Reaction of 3β-Acetoxy-8α,9α-oxido-5α-lanostane with Grignard Reagents[1]

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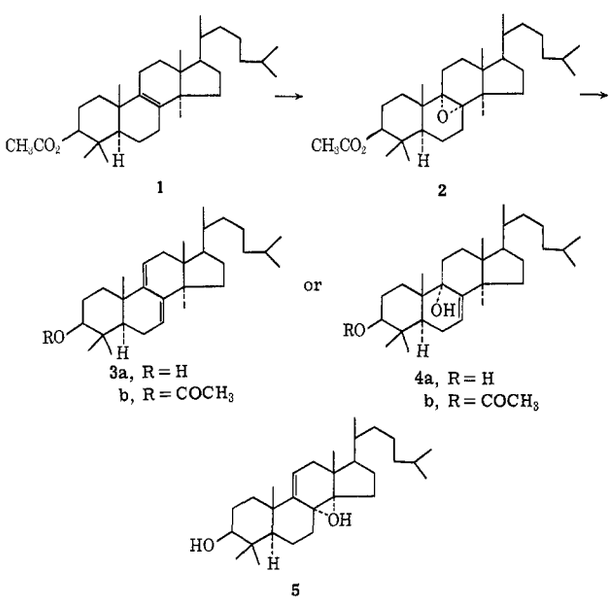
*Received April 13, 1970*

Synthetic degradation of lanosterol has been used to prepare otherwise difficultly accessible 14α-methyl steroids.[2] With the prospect of readily obtaining B/C ring juncture modifications of lanosterol for similar purposes, we decided to explore the oxirane ring opening reactions of an 8α,9α-oxidolanostane with methyl and allyl Grignard reagents. For this purpose dihydrolanosterol acetate (**1**) was oxidized in excellent yield to 3β-acetoxy-8α,9α-oxido-5α-lanostane (**2**).[3]




Methyllithium in ether did not attack the oxirane ring over a period of 19 days. With methylmagnesium iodide in refluxing toluene the product was dihydroagnosterol (**3a**). Allylmagnesium bromide[4] in ether at 25° reacted completely in a few hours with epoxy

(1) Steroids and Related Natural Products. 79. For Part 78 see G. R. Pettit and Y. Kamano, *Experientia*, **28**, in press. This investigation was supported by Public Health Service Research Grants RO1 CA08705-01, R01 CA08705-02, and R01 CA11451-01.

(2) *Cf.* G. R. Pettit and P. Hofer, *Helv. Chim. Acta*, **41**, 2142 (1963); *J. Chem. Soc.*, 4439 1963.

(3) J. Fried, J. W. Brown, and M. Applebaum, *Tetrahedron Lett.*, 849 (1965); I. G. Guest and B. A. Marples, *J. Chem. Soc. C*, 1468 (1971).

(4) H. Felkin and G. Roussi, *Tetrahedron Lett.*, 4153 (1965). The reactivity of allyl Grignard reagents toward epoxide ring-opening is much greater than that of methyl or isopropyl Grignard reagents. The stereochemical course leads to trans diaxial products.