Synthetic Study Toward Total Synthesis of (±) Germine: Synthesis of (±) 4-Methylene germine

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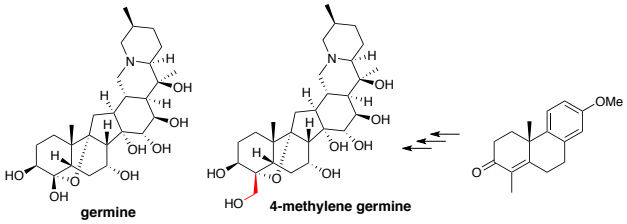
Supporting Information



ABSTRACT: The total synthesis of 4-methylene germine is described.

Germine (**1**, Fig 1)[1] is the parent structure of a number of alkaloids that are esters or polyesters of its hydroxyl groups. Even though it has been known for quite some time, no synthetic effort to germine has been reported. There was considerable interest in the possible use of these and related substances in controlling blood pressure[2]. Our initial synthetic goal was to construct the germine like structure **2**, in which all relative stereocenters are introduced with correct stereochemistry[3]. Note that one extra methylene group on C4 of the A ring was added to the hemiketal structure to make the intermediates more stable and to avoid the known sensitivity of such compounds to bases. We hoped to remove this superfluous methylene group in the later stage of the synthesis while retaining the established relative stereochemical configuration at C4.

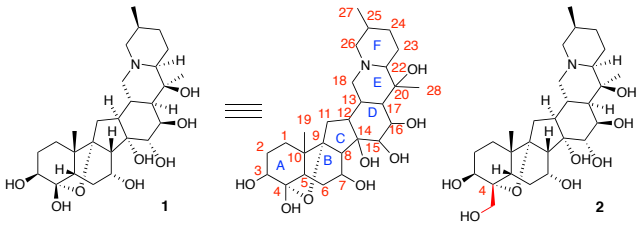
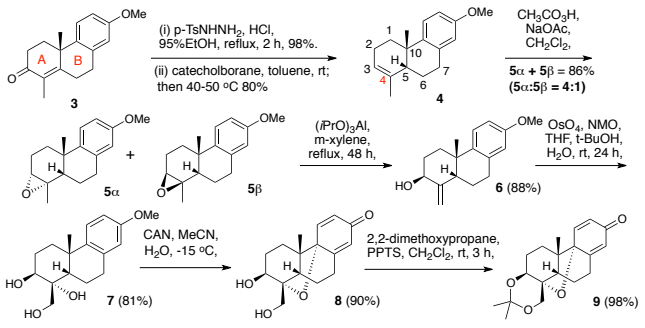


Fig 1: Structure of germine (**1**) and 4-methylene germine (**2**)

The starting material chosen for the germine synthesis was the readily available tricyclic ketone **3**[4]. We planned to use the A/B rings of ketone in **3** as the precursors of the A/B rings in germine (Scheme 1). Therefore, the starting enone had to be reduced, to produce the *cis* A/B fusion. We hoped to do this by applying Kabalka's method[5]: the reduction of the tosylhydrazone from **3** with catecholborane. This took place as anticipated, giving the tricyclic olefin **4**[6] in 73% yield. Not only was the *cis* stereochemistry of the A/B system produced, but the double bond was also placed correctly for the oxygenation of ring A. Oxidation of **4** with peracetic acid in DCM, at -10 °C, gave a 4:1 mixture of the epoxides **5α** and **5β**. The major component was isolated and analyzed by X-ray, to reveal the α-epoxide **5α**[7]. For conversion of **5α** to the desired β-allylic alcohol **6**, the mixture (**5α + 5β**) could be eliminated and isomerized/equilibrated[8] with aluminum isopropoxide, [Al(O*i*Pr)$_3$], in boiling *m*-xylene, providing a 1:4 mixture of the allylic alcohols. The major component was confirmed as the more stable 3β-hydroxy-4-methylene compound **6** by X-ray analysis.

Scheme 1: Conversion of **3** to Tricyclic Compound **9**

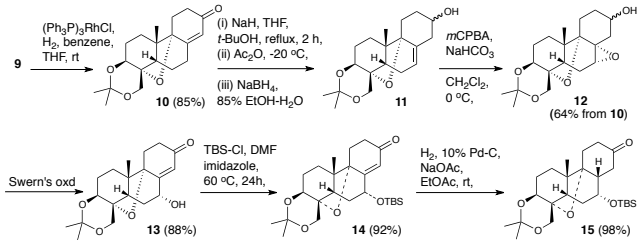


The small amount of unwanted **5α** isomer as well as an isomer of **6**, could be recycled with the next batch of the epoxides. Taking in account one recycling, the yield for the required conversion of **4** to **6** was 75-80%. Hydroxylation of **6** with osmium tetroxide (OsO_4)[9] in the presence of NMO gave a single triol in 81% yield. It was assumed to be **7**[10], and ready for attempted connection to ring C. At the time these experiments were done, there had not been a report of the trapping of an alcohol group by the cation, or

radical cation, formed from the oxidation of an anisole ring. More recently, examples of this reaction have appeared in the literature[11]. Several oxidations were carried out on **7**; some electrochemical, some with CAN, some on various derivatives of **7**, such as the diacetate, the acetonide, the benzylidene and the mono- and di-TBS derivatives. In the end, we selected direct oxidation of **7** with 2.1 equivalents of CAN in aq acetonitrile at -20 °C. This gave, in greater than 90% yield, the crude dihydroxydienone **8** which was immediately transformed into its acetonide **9**, in 71% overall yield from **6**. As noted above, the acetonide system of **9** involves a C4 hydroxymethyl group rather than the required hemiketal hydroxyl of **1**.

Now three problems had to be faced: (1) reduction of the dienone **9** to the enone **10**; (2) use the cyclohexenone system to introduce the α hydroxyl group at C7; (3) reduce the cyclohexenone ring C to form a required trans B/C system, the precursor of the five member ring needed for further elaboration (Scheme 2).

Scheme 2: Functionaliztion of Trycyclic system **15**

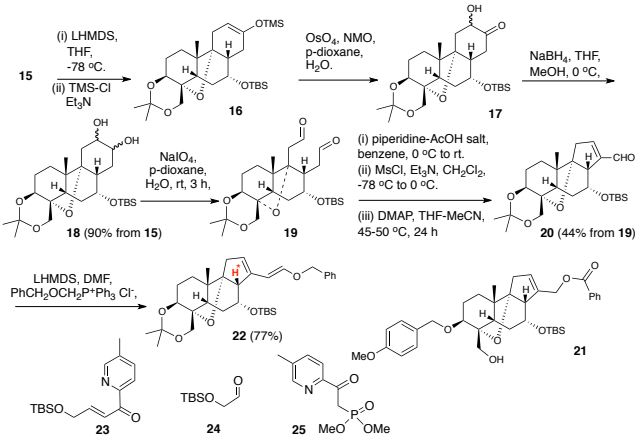


Reduction of the less substituted double bond could be done[12], by the use of the Wilkinson catalyst under hydrogen in equal amounts of benzene and THF. This gave **10** in 85% yield. Introduction of the 7α-hydroxyl was initially attempted by peracid treatment of the enol acetate of **10**, but that was unsatisfactory. We, therefore, followed a Marshall protocol[13]: initial reduction of the enol acetate of **10** in 85% ethanol with sodium borohydride, followed by peroxidation of the resulting mixture of homoallyl alcohols **11** with *m*CPBA in the presence of $NaHCO_3$ to **12**, and, finally, Swern oxidation to give the hydroxyenone **13** in 57% overall yield from **10**. Oxidation by the peracid was assumed (later confirmed) to take place from the α side, because of interference on the β side by the methyl group, which is axial to the B ring. The next task was to find a way to reduce the enone in **13** to the saturated *trans* fused ketone. Chemical reduction, such as Li/NH_3, would be unsuitable because of the elimination of the oxide bridge by any process that would add electrons to the enone system. Catalytic hydrogenation was obviously required, and we hoped that the desired stereochemistry would be favored by putting a bulky TBS group on the axial 7α-hydroxyl, as shown in **14**. In fact, reduction of **14** with 10% Pd on charcoal, in the presence of sodium acetate, gave a saturated ketone that, we assumed, could be represented by **15.** The correctness of the various assumptions implied in structure **15** was verified by an X-ray structure determination.

We now faced the problem of converting the cyclohexanone ring of **15** into a cyclopentane system (the C ring), functionalized to allow the construction to proceed by transforming **15** into the cyclopentene aldehyde **20**. Conversion of **15** to **20** was started by the formation of the TMS enol ether (LHMDS, followed by TMS-Cl-Et_3N), which we expected would be mostly the single isomer **16** because of the preference for a double bond parallel to the ring junction in a trans decalin system[14]. Treatment with cat OsO_4 for 2 h at rt, and reduction of the ketol **17** ($NaBH_4$, MeOH-THF) gave a vicinal diol mixture **18** in 90% yield from **15**. Cleavage (sodium periodate/aq *p*-dioxane) of **18** gave the dialdehyde **19** (Scheme 3).

The idea had been to cyclize **19** to the cyclopentene aldehyde **20**. There are, of course, two possible ways for the dialdehyde to cyclize. Note that the undesired cyclization would involve attack by base on a methylene in 1,3 relationship to the angular methyl group, and that this would favor the alternative methylene thus leading to the correct aldol. Surprisingly, the cyclization with piperidine acetate salt stopped at the aldol state, rather than giving directly the unsaturated aldehyde. Indeed, dehydration was not simple, presumably reflecting the added strain a double bond would place in the *trans* hydrindane system: heating at reflux a DCM solution of the mesylate (from $MsCl/Et_3N$ on the aldol) left the mesylate mixture unchanged. Only by treatment of the mesylate with DMAP in an acetonitrile-THF mixture at 45-50 °C, for 24 h, **20** was obtained, only in 40-50 % yield (overall from **19**). It seemed to be a good place to confirm the geometry achieved so far in our synthesis of **20**, and an X-ray structure of the derivative **21**[15] was obtained.

Scheme 3: Construction of C ring and Preparation of Diene **22** and Dienophile **23**



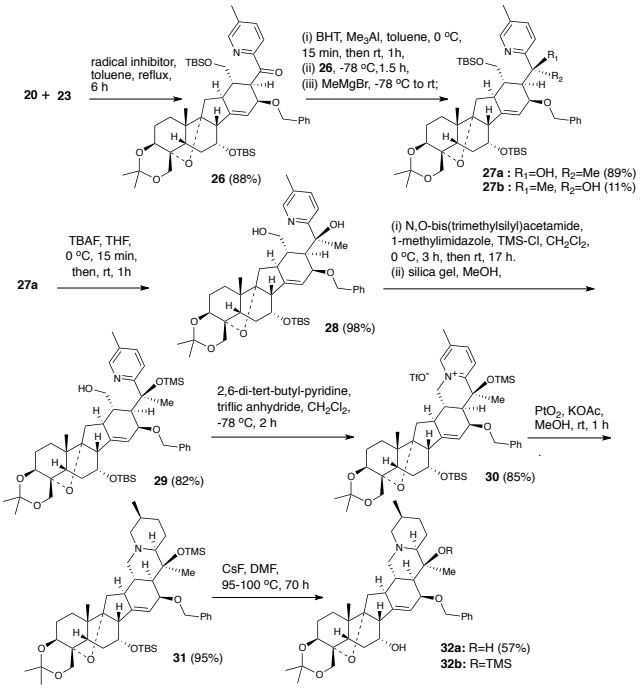
The further elaboration of **20** toward **2** involved transforming it into an alkoxydiene, which could take part in a Diels Alder cycloaddition with a suitable dienophile. This was done by Wittig reaction with benzyloxymethyl triphenylphosphorane, easily prepared by deprotonation of the alkylation product of triphenylphosphine with chloromethyl benzyl ether, to give a suitable diene **22** in 77 % yield. We would, of course, have liked to get a preponderance of the *E*-enol ether from the Wittig reaction, so that the following Diels Alder cycloaddition would lead to the correct hydroxyl stereochemistry, protected as its benzyl ether. The *E/Z* ratio of enol ethers expected from a Wittig reaction is, however, not clear (nor is it understood). Because such enol ethers are mostly made as intermediates to the aldehydes obtained by hydrolysis, characterization of the enol ether geometry is not usually of concern. In the few cases investigated, both isomers are formed[16]. Separation of the predominately *E*-enol ether (*E:Z* = 4:1, in our case **22**) was unnecessary, as the *Z*-isomer would be expected to undergo the planned Diels Alder addition with much more difficulty.

The dienophile **23** was synthesized by a Wadsworth-Emmons condensation between siloxyaldehyde **24**[17] and the phosphonate **25**, accessible by the coupling of 2-cyano-5-methylpyridine[18] with dimethyl methylphosphonate. The Diels Alder adduct **26** was then obtained, in refluxing toluene with **23**, in the presence of the inhibitor, 3-*tert*-butyl-4-hydroxy-5-methylphenylsulfide, in about 88% yield, apparently as a single substance (Scheme 4). We were confident that the correct stereoisomer had resulted because: (1) the dienophile would approach the more accessible face of the diene, i.e. *cis* to the starred hydrogen (H*) in **22**; (2) there should be exclusive *endo* addition because *exo* addition would lead to

very severe interference between the TBSO substituent on the dienophile and the diene. As a result of such *endo* addition, the benzyloxy group in the diene and the acyl pyridine of the dienophile would end up *cis* to each other in the adduct.

The adduct **26** is obviously capable of easy elimination of the benzyloxy group, so that risk must be reduced by making the next step, the addition of the methyl Grignard reagent at low temperature to the keto group of **26**. This could, of course, give either of two stereoisomers, or a mixture of the two. After many experiments, it was concluded that the major product from MeMgBr with **26** had the *undesired* stereochemistry at the newly formed tertiary alcohol center[19]. While this was disappointing, it had been shown by H. Yamamoto[20] that the stereochemistry of Grignard reactions with *cyclic* ketones could be reversed by previous complexation with the trimethylaluminum salt of 2,6-di-*tert*-butyl-4-methylphenol (BHT). It was gratifying that this process (toluene, -78 °C) gave, in 89% yield, a tertiary methyl carbinol different from the one previously obtained, and clearly the desired tertiary alcohol **27a**, in addition to 11% of **27b**, the *undesired* isomer obtained previously.

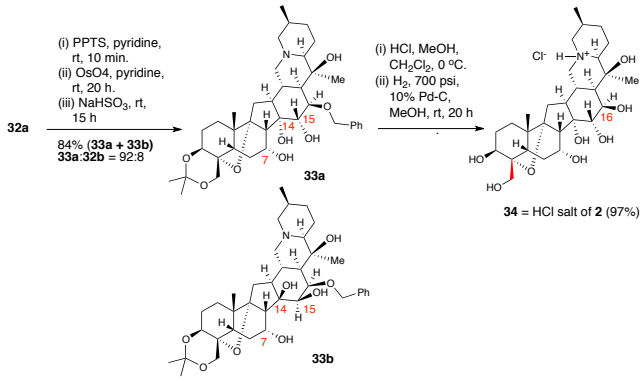
Scheme 4: Diels Alder Cycloaddition, Grinard Reaction and Construction of E-F ring (Completion of A-B-C-D-E-F)



We now undertook the reduction of the pyridine ring. Before that it was necessary to protect the tertiary alcohol of the diol **28** to prevent the participation of the tertiary hydroxyl in reactions such as cyclization to a tetrahydrofuran ring. A TMS ether was selected for that role (excess TMS-Cl, followed by treatment with silica gel that removed the primary TMS), to give the alcohol **29**. Cyclization, followed by treatment with triflic anhydride and 2,6-di-*tert*-butylpyridine in DCM, 1 h at -78 °C, led to the pyridinium salt **30,** which was immediately hydrogenated, using platinum oxide in MeOH in the presence of potassium acetate. We expected the hydrogenation to take place from the α side to give **31** because of the hindrance by the C20 axial tertiary TMS ether on the β side. Survival of the olefinic bond in the ring D was, presumably, the result of hindrance by the axial benzyl ether group on one side, and the C7 axial TBS ether group on the other. All of these anticipations were satisfied by the X-ray structure determination of **31**.

Before OsO_4 oxidation, which was to follow, removal of both silyl groups, the TMS group (E ring) and the TBS group (B ring), seemed desirable. But, after many attempts, removal of the tertiary TMS group was unsuccessful, presumably because of the high steric hindrance in this particular case. We had partial success with cesium fluoride (CsF); a large excess of CsF and **31**, in anhyd DMF solution, were heated at 95-100 °C for 70 h. At this point, 57% of **32a** in which both the TMS on the C-20 hydroxyl and the TBS group on the C-7 hydroxyl had been removed, was yielded along with **32b** in which only the TBS group was removed. Compound **32b** was converted to **32a**, by applying the same reaction conditions (CsF, 95-100 °C, 70 h).

Scheme 5: Introduction of Diol and Deprotection



We were conscious that the osmylation could add hydroxyls either from the α side, as desired, or from the β side (Scheme 5). Some assistance to the α side hydroxylation might come from the 7-hydroxyl in ring B, and, additionally, there was interference to coming from the β side because of the benzyloxy group emerging from that side. Anyway, we were ready to try the osmylation: **32a** was first protonated with a slight excess of PPTS in dry pyridine, followed by stirring for 20 h at rt under argon, with about a 20% excess of OsO_4, to give a high yield of two glycols (correct MW) in a 92:8 (**33a**:**33b**) ratio. The major isomer **33a** was obtained as solid, but, unfortunately, could not be crystallized. Our expectation that **33a** was the desired glycol was confirmed by NOE interproton distance calculations, combined with DFT calculations (QM/NMR)[21]. The distance between the hydrogens at C15 and C7 in the DFT-optimized geometry of diastereomer **33a** (desired glycol) was measured as 2.58 Å, as it is in the X-ray of germine (**1**),[1(c)] while the distance between the same hydrogens in the undesirable glycol **33b** was measured as 3.68 Å. The interproton distance between C15 and C7 calculated from the NOE effect in our synthetic glycol (**33a**) was 2.58 Å, matching to that for the desired glycol **33a** (the details of the study on the stereochemistry of the two newly created stereocenters at C14 and C15 are described in Supporting Information). Thus, the minor component **33b** was determined to be the undesired glycol[22].

Now only one step remained, removal of the benzyl protecting group from the C16 hydroxyl. Because of the well-known poisoning effect of free amino groups on palladium hydrogenation, the amino group in **34a** was first covered by making its hydrochloride, by treatment with hydrogen chloride in MeOH (the acetonide was also eliminated by that operation). This was followed by hydrogen, pressurized to 700 psi, for 20 h at rt with 10% Pd on charcoal. The yield of debenzylation was essentially quantitative, giving the structure **34** (the hydrogen chloride salt of **2**, Fig 1).

In conclusion, Compd **34** is racemic germine hydrochloride having an extra methylene group at C4, but with all of 15 of germine's asymmetric centers[3] correctly established relative to the single asymmetric center of the starting material **3**[23].

Supporting Information (SI):
Available free of charge on the ACS Publications website.

(1) Experimental procedures and Characterization data, ^{1}H- and ^{13}C-NMR spectra, LCMS data, X-ray data for **5**, **6**, **15**, **21**, **31** and **35**[19], and (2) Study on the stereochemistry of the C14-OH and the C15-OH in **33a** using QM calculation and NOE effects. NMR-NOE spectra of **33a** in MeOH-d_4 and benene-d_6 (PDF).

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ACKNOWLEDGMENT

This research was supported by NIH grants; (R01 GM05147, R01 HL25635) and NSF (CHE-86-12434). The authors acknowledge our colleagues, Professor Gerard Parkin for the X-ray analyses, Professor James Leighton for helping the high pressure hydrogenation, Professor W. Clark Still for helping distinguishing **33a,b**, and Dr. John Decatur for NOE experiments for **33a**. Dr. Anil K. Saksena of Schering-Plough Corporation is thanked for a generous supply of germine.

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(3) The equilibrium between hemiketal ⇌ hydroxy ketone reduced the number of asymmetric centers to 15 in (+) germine. Our interest was in the synthesis of the (±) germine derivative rather than the (+)-isomer, reducing the relative asymmetric center from 15 to 14.

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(6) Compound **4** was previously observed in the mixture of products from the degradation of abietc acid: Wirthlin, T.; Wehrli, H.; Jeger, O. *Helv. Chim. Act*. **1974**, *57*, 351.

(7) Epoxidation from the α-face of the olefin in **4** is favored due to a steric hindrance from the angular methyl group at the C10, blocking the β-face (more than a steric hindrance from the C6 methylene group on the α-face of the olefin).

(8) Treatment with either lithium diethylamide in THF or aluminum *t*-butoxide in refluxing toluene, undesired α-expoxide **5α** can be opened to give the undesired α-allylic alcohol (an epimer of **6**). Further treatment of the undesired α-allylic alcohol with Al(*i*PrO)$_3$ (*m*-xylene, 135 °C), equilibrates to the desired more stable β-allylic alcohol **6** (a 1:4 = α:β). Further heating did not alter the ratio. Thus, a mixture of **5α** and **5β** was treated directly with Al(*i*PrO)$_3$ in boiling *m*-xylene. (a) Dauben, W. G.; Fonken, G. J.; Novce, D. S. *J. Am. Chem. Soc.* **1956**, *78*, 2579. (b) Eliel, E. L.; Ro, R. S. *J. Am. Chem. Soc.* **1957**, *79*, 5992.

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(10) We predicted that OsO_4 oxidation might be favored to proceed from the α-side of the olefin, because the β-face of the olefin is severely hindered by an angular (axial) methyl group and the *cis* A/B ring junction. This suggestion was supported by the success of the subsequent aromatic ring oxidation, a reaction possible only when the newly formed tertiary hydroxy group is both α and axial on the A ring.

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(15) We also made **21** by a different route: sodium borohydride, then benzoyl chloride on the aldehyde **20**, in which the ring A glycol had been deprotected and transformed into a mono *p*-methoxybenzyl ether. See **SI** for the conversion of **20** to **21**.

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(19) The stereochemistry of **27b** was confirmed by X-ray analysis of the derivative **35**. See **SI** for the conversion of **27b** to **35**.

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(22) The OsO_4 reaction using **32b** gave a single product, presumably the desired glycol. But the yield was lower than that from **32a**; also removal of TMS at the hindered C20 alcohol after osmylation was inefficient.

(23) At this point we realized that we did not have enough material (a few milligrams) to go through the several steps needed to remove the extra methylene group on C-4. One would have to restart the whole synthesis. But I (GS) am now 95 years old……..