

# Synthetic Study toward Total Synthesis of $(\pm)$ -Germine: Synthesis of $(\pm)$ -4-Methylenegermine

Gilbert Stork,<sup>\*,†</sup> Ayako Yamashita,<sup>†©</sup> Robert M. Hanson, Ly Phan, Eifion Phillips, Daniel Dubé, Pieter H. Bos,<sup>‡</sup> Andrew J. Clark, Maxwell Gough, Mark L. Greenlee, Yimin Jiang, Keith Jones,<sup>®</sup> Masato Kitamura,<sup>®</sup> John Leonard, Tongzhu Liu, Philip J. Parsons, and Aranapakam M. Venkatesan

<sup>†</sup>Department of Chemistry and <sup>‡</sup>Chemical Probe Synthesis Facility, Department of Biological Sciences, Columbia University, New York, New York 10027, United States

**(5)** Supporting Information

**ABSTRACT:** The total synthesis of 4-methylenegermine is described.

G ermine  $(1, Figure 1)^1$  is the parent structure of a number of alkaloids that are esters or polyesters of its hydroxyl groups. Even though germine has been known for quite some time, no synthetic effort has been reported. There was great interest in the possible use of this and related substances in controlling blood pressure.<sup>2</sup> Our initial synthetic goal was to construct the germine-like structure 2 in which all relative stereocenters are introduced with the correct stereochemistry.<sup>3</sup> 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 at a later stage of the synthesis but retain the established relative stereochemical configuration at C4.



Figure 1. Structures of germine (1) and 4-methylenegermine (2).

The starting material chosen for the germine synthesis was the readily available tricyclic ketone 3.<sup>4</sup> We planned to use the A/B rings of ketone 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:<sup>5</sup> the reduction of the tosylhydrazone from 3 with catecholborane. This took place to give 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



Scheme 1. Conversion of 3 to Tricyclic Compound 9



in DCM, at -10 °C, gave a 4:1 mixture of the epoxides  $5\alpha$  and  $5\beta$ . The major component was isolated and analyzed by X-ray analysis to reveal the  $\alpha$ -epoxide  $5\alpha$ .<sup>7</sup> For conversion of  $5\alpha$  to the desired  $\beta$ -allylic alcohol 6, the mixture ( $5\alpha + 5\beta$ ) could be eliminated and isomerized/equilibrated<sup>8</sup> with aluminum isopropoxide, [Al(OiPr)<sub>3</sub>], in boiling *m*-xylene to provide a 1:4 mixture of the allylic alcohols. The major component was confirmed as the more stable  $3\beta$ -hydroxy-4-methylene compound 6 by X-ray analysis.

The small amount of unwanted  $5\alpha$  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

Received: August 7, 2017

recently, examples of this reaction have appeared in the literature.<sup>11</sup> 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 equiv of CAN in aq acetonitrile at -20 °C. This gave, in >90% yield, the crude dihydroxydienone 8 which was immediately transformed into its acetonide 9 in 71% overall yield from 6. As noted, 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 of the cyclohexenone system to introduce the  $\alpha$  hydroxyl group at C7; (3) reduction of the cyclohexenone ring C to form a required trans B/C system, the precursor of the five-membered ring needed for further elaboration (Scheme 2).





Reduction of the less substituted double bond could be done<sup>12</sup> 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 7 $\alpha$ -hydroxyl was initially attempted by peracid treatment of the enol acetate of 10, but that was unsatisfactory. We then followed a Marshall protocol:<sup>13</sup> 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<sub>3</sub> 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  $\alpha$  side because of interference on the  $\beta$  side by the methyl group, which is axial to the B ring. The next task was to reduce the enone in 13 to the saturated trans-fused ketone. Chemical reduction, such as Li/ NH<sub>3</sub>, 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 required, and we hoped that the desired stereochemistry would be favored by putting a bulky TBS group on the axial  $7\alpha$ -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<sub>3</sub>N), 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.<sup>14</sup> Treatment with cat. OsO<sub>4</sub> for 2 h at rt and reduction of the ketol 17 (NaBH<sub>4</sub>,

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).

## Scheme 3. Construction of C Ring and Preparation of Diene 22 and Dienophile 23



The idea had been to cyclize 19 to the cyclopentene aldehyde 20. There are two possible ways for the dialdehyde to cyclize. The undesired cyclization would involve attack by base on a methylene in a 1,3 relationship to the angular methyl group, and 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<sub>3</sub>N 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, was 20 obtained, only in 40-50% yield (overall from 19). This was a good place to confirm the geometry achieved so far in our synthesis of **20**, and an X-ray structure of the derivative **21**<sup>15</sup> was obtained.

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 have liked to obtain 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 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.<sup>16</sup> 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<sup>17</sup> and the phosphonate 25, accessible by the coupling of 2-cyano-5-methylpyridine<sup>18</sup> 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-methylphenyl sulfide, in about 88% yield, apparently as a single substance (Scheme 4).

Scheme 4. Diels–Alder Cycloaddition, Grignard Reaction, and Construction of E-F Ring (Completion of A-B-C-D-E-F)



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, and (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 acylpyridine of the dienophile would end up *cis* to each other in the adduct.

The adduct 26 is 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 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.<sup>19</sup> While this was disappointing, it had been shown by H. Yamamoto<sup>20</sup> 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.

We now undertook the reduction of the pyridine ring. First, 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  $\alpha$  side to give **31** because of the hindrance by the C20 axial tertiary TMS ether on the  $\beta$  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 C20 hydroxyl and the TBS group on the C7 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).

We were conscious that the osmylation could add hydroxyls either from the  $\alpha$  side, as desired, or from the  $\beta$  side (Scheme 5).



Some assistance to the  $\alpha$  side hydroxylation might come from the 7-hydroxyl in ring B, and, additionally, there was interference to coming from the  $\beta$  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<sub>4</sub>, 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).<sup>21</sup> 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),<sup>1c</sup> 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

### Scheme 5. Introduction of Diol and Deprotection

stereocenters at C14 and C15 are described in the S1). Thus, the minor component **33b** was determined to be the undesired glycol.

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**, Figure 1).

In conclusion, compound **34** is racemic germine hydrochloride having an extra methylene group at C4 but with all of 15 of germine's asymmetric centers<sup>3</sup> correctly established relative to the single asymmetric center of the starting material 3.<sup>22</sup>

#### ASSOCIATED CONTENT

#### **Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b02434.

X-ray structures for 5, 6, 15, 21, 31 and 35 (ZIP)

Experimental procedures and characterization data, <sup>1</sup>Hand <sup>13</sup>C NMR spectra, LCMS data; 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 benzene- $d_6$  (PDF)

#### AUTHOR INFORMATION

#### **Corresponding Author**

\*E-mail: gjs8@columbia.edu. ORCID <sup>©</sup>

Ayako Yamashita: 0000-0002-7601-9969 Keith Jones: 0000-0002-9440-4094 Masato Kitamura: 0000-0002-0543-8795

#### Notes

The authors declare no competing financial interest.

#### ACKNOWLEDGMENTS

This research was supported by the NIH (R01 GM05147, R01 HL25635) and NSF (CHE-86-12434). We acknowledge our colleagues Professor Gerard Parkin for the X-ray analyses, Professor James Leighton for helping with 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 Corp. is thanked for a generous supply of germine.

#### REFERENCES

(1) (a) Greenhill, J. V.; Graysham, P. T. Alkaloids; Academic Press, 1992; Vol. 41, pp 177–187. (b) Kupchan, S. M.; Narayanan, C. R. J. Am. Chem. Soc. **1959**, 81, 1913. (c) Tang, J.; Li, H.-L.; Shen, Y.-H.; Jin, H.-Z.; Yan, S.-K.; Liu, R.-H.; Zhang, W.-D. Helv. Chim. Acta **2007**, 90, 769 (CCDC-635769).

(2) (a) Baker, P. D. Southern Med. Surg. **1859**, 15, 579. (b) Renforado, J. M.; Flacke, W.; Swaine, C. R.; Mosimann, W. J. Pharmacol. Exp. Ther. **1960**, 130, 311. (c) Saksena, A. K.; McPhail, A. T. Tetrahedron Lett. **1982**, 23, 811.

(3) The equilibrium between hemiketal  $\rightleftharpoons$  hydroxy ketone reduced the number of asymmetric centers to 15 in (+)-germine. Our interest was in

the synthesis of the  $(\pm)$ -germine derivative rather than the (+)-isomer, reducing the relative asymmetric center from 15 to 14.

(4) Stork, G.; Meisels, A.; Davies, J. E. J. Am. Chem. Soc. 1963, 85, 3419.
(5) Kabalka, G. W.; Yang, D. T. C.; Baker, J. D. J. Org. Chem. 1976, 41, 574.

(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. Acta* **1974**, *57*, 351.

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

(8) Treatment with either lithium diethylamide in THF or aluminum *tert*-butoxide in refluxing toluene, undesired  $\alpha$ -expoxide  $5\alpha$  can be opened to give the undesired  $\alpha$ -allylic alcohol (an epimer of 6). Further treatment of the undesired  $\alpha$ -allylic alcohol with Al(*i*PrO)<sub>3</sub> (*m*-xylene, 135 °C) equilibrates to the desired more stable  $\beta$ -allylic alcohol 6 (1:4 =  $\alpha/\beta$ ). Further heating did not alter the ratio. Thus, a mixture of  $5\alpha$  and  $5\beta$  was treated directly with Al(*i*PrO)<sub>3</sub> in boiling *m*-xylene. (a) Dauben, W. G.; Fonken, G. J.; Noyce, D. S. J. Am. Chem. Soc. 1956, 78, 2579. (b) Eliel, E. L.; Ro, R. S. J. Am. Chem. Soc. 1957, 79, 5992.

(9) Van Rheenen, V.; Kelly, R. C.; Cha, D. Y. Tetrahedron Lett. 1976, 17, 1973.

(10) We predicted that  $OsO_4$  oxidation might be favored to proceed from the  $\alpha$ -side of the olefin because the  $\beta$ -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  $\alpha$  and axial on the A ring. (11) (a) Hata, K.; Hamamoto, H.; Shiozaki, Y.; Cämmerer, S. B.; Kita, Y. *Tetrahedron* **2007**, *63*, 4052. (b) Danishefsky, S. J.; Dai, M.

Heterocycles 2009, 77, 157. (12) This process was well known in the case of steroid dienones:

Djerassi, C.; Gutzwiller, J. J. Am. Chem. Soc. **1966**, 88, 4537. (13) Marshall, J. A.; Greene, A. E. J. Org. Chem. **1971**, 36, 2035.

(13) Marshan, J. A.; Greene, A. E. J. Org. Chem. **19**/1, 30, 2035. (14) House, H. O.; Trost, B. M. J. Org. Chem. **1965**, 30, 1341. Mazur,

Y.; Sondheimer, F. J. Am. Chem. Soc. **1958**, 80, 5220. (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 the SI for the conversion of **20** to **21**.

(16) (a) Mandai, T.; Osaka, K.; Wada, T.; Kawada, M.; Otera, J. *Tetrahedron Lett.* **1983**, *24*, 1171 (E/Z = 2.5/1). (b) Mandai, T.; Osaka, K.; Kawagishi, M.; Kawada, M.; Otera, J. J. Org. Chem. **1984**, *49*, 3595. This paper is perhaps the most relevant: it makes conjugated alkoxydienes via triphenylphosphoranes and obtains the dienes in an E/Z ratio of 2.5:1. (c) Ikeda, S.; Shibuya, M.; Kanoh, N.; Iwabuchi, Y. Chem. Lett. **2008**, *37*, 962 (E/Z = 5/2). (d) Patel, P. R.; Boger, D. L. Org. Lett. **2010**, *12*, 3540 (E/Z = 1/1.5). (e) Earnshaw, C.; Wallis, C. J.; Warren, S. J. Chem. Soc., Perkin Trans. 1 **1979**, 3099.

(17) (a) McDougal, P. G.; Rico, J. G.; Oh, Y.-I.; Condon, B. J. Org. Chem. **1986**, *51*, 3388. (b) Aszodi, J.; Bonnet, A.; Teutsch, G. Tetrahedron **1990**, *46*, 1579. We made this compound, originally, by ozonolysis of the bis TBS derivative of *cis* 1,4-butenediol.

(18) Crabb, T. A.; Heywood, G. C. Org. Magn. Reson. 1982, 20, 242.
(19) The stereochemistry of 27b was confirmed by X-ray analysis of the derivative 35. See the SI for the conversion of 27b to 35.

(20) Maruoka, K.; Itoh, T.; Sakurai, M.; Nonoshita, K.; Yamamoto, H. J. Am. Chem. Soc. **1988**, 110, 3588.

(21) Chini, M. G.; Jones, C. R.; Zampella, A.; D'Auria, M. V.; Renga, B.; Fiorucci, S.; Butts, C. P.; Bifulco, G. J. Org. Chem. **2012**, 77, 1489.

(22) A plan for conversion of **33a** to **1** was (with various deprotections/protections) C4-CH<sub>2</sub>OH to C4-CO<sub>2</sub>H, followed by Barton's conditions to change C4-CO<sub>2</sub>H to C4-OH. Barton, D. H. R.; Gero, S. D.; Holliday, P.; Quiclet-Sire, B.; Zard, S. Z. *Tetrahedron* **1998**, *54*, 6751. At this point, we realized that we did not have enough material (a few milligrams) to go through the several steps for this conversion. One would have to restart the whole synthesis. But I (G.S.) am now 95 years old...