5-Hydroxypentane-2,3-dione and 3-amino-1-hydroxypropan-2-one, putative precursors of vitamin B_6

Eckardt Wolf, Isaac A. Kennedy, Klaus Himmeldirk, and Ian D. Spenser

Abstract: New syntheses are described of 5-hydroxypentane-2,3-dione (**7**) (i.e., laurencione (**7** ⇄ **8**)) and of 3-amino-1-hydroxypropan-2-one (3-amino-1-hydroxyacetone) (**5**) hydrochloride, putative precursors of the C_5 unit, C-2′,2,3,4,4′, and of the C_3N unit, N-1,C-6,5,5′, respectively, of pyridoxine (**6**).

Key words: 3-amino-1-hydroxyacetone, 3-amino-1-hydroxypropanone, 5-hydroxypentane-2,3-dione, laurencione, vitamin B_6.

Résumé : On décrit de nouvelles synthèses de la 5-hydroxypentane-2,3-dione (**7**) (c'est-à-dire de la laurencione (**7** ⇄ **8**)) et du chlorhydrate de la 3-amino-1-hydroxypropan-2-one (3-amino-1-hydroxyacétone) (**5**), des précurseurs potentiels respectivement de l'unité en C_5, C-2′,2,3,4,4′, et de l'unité C_3N, N-1,C-6,5,5′, de la pyridoxine (**6**).

Mots clés : 3-amino-1-hydroxypropane-2-one, 3-amino-1-hydroxyacétone, 5-hydroxypentane-2,3-dione, laurencione, vitamine B_6.

[Traduit par la rédaction]

Introduction

Two precursors, one a modified pentose, 1-deoxy-D-xylulose (**1**), the other a non-protein amino acid, 4-hydroxy-L-threonine (**2**), account for the origin of the entire C_8N skeleton of vitamin B_6 (pyridoxol, pyridoxine) (**6**) (1). A hypothetical seven-step reaction sequence was advanced to account for the chemical mechanism of the formation of the pyridoxine skeleton from these two substrates (2). The first step in this sequence was postulated to be the union of the two precursors to form a Schiff base (**3**), which was then primed to generate the bond destined to become C-4—C-5 of pyridoxol, by intramolecular aldol condensation in a multistep reaction sequence. The fragment destined to become C-2′,2,3,4,4′ of pyridoxol, derived from 1-deoxy-D-xylulose (**1**), was modified by dehydration, and the fragment destined to yield N-1,C-6,5,5′, derived from 4-hydroxy-L-threonine (**2**), underwent dehydrogenation and decarboxylation.

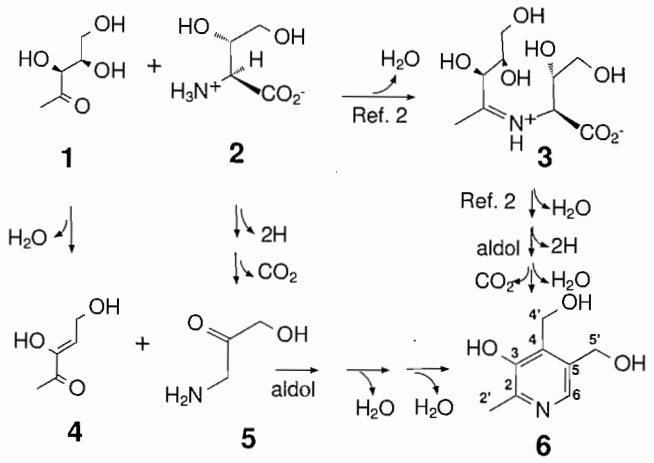
It is equally plausible, however, that the corresponding modifications of the two substrates take place ahead of Schiff base formation, so that the immediate precursors that undergo condensation to form the pyridoxine skeleton are the enol (**4**) of 5-hydroxypentane-2,3-dione (**7**), generated by dehydration of 1-deoxy-D-xylulose (**1**), rather than 1-deoxy-D-xylulose itself, and 3-amino-1-hydroxypropan-2-one (**5**), generated by dehydrogenation and decarboxylation of 4-hydroxy-L-threonine (**2**), rather than the intact amino acid (Scheme 1).

Received February 21, 1997.

E. Wolf, I.A. Kennedy, K. Himmeldirk, and I.D. Spenser.[1]
Department of Chemistry, McMaster University, Hamilton, ON L8S 4M1, Canada.

[1] Author to whom correspondence may be addressed: Telephone: (905) 525-9140, ext 23245. Fax: (905) 522-2509. E-mail: spenser@mcmaster.ca

Scheme 1. Alternative routes for the derivation of pyridoxol (**6**) from 1-deoxy-D-xylulose (**1**) plus 4-hydroxy-L-threonine (**2**).



In an effort to throw further light on the mechanism of formation of pyridoxol, we have prepared synthetic samples of **5** and **7**, the two compounds that incorporate these structural modifications.

5-Hydroxypentane-2,3-dione (**7**), in an equilibrium mixture with the isomeric furanose (**8**), i.e., laurencione (**7** ⇄ **8**) (3), was prepared in five steps from the commercially available pent-3-yn-1-ol (**18**) in an overall yield of 81%. Furthermore, a bond-labeled sample, [2,3-$^{13}C_2$]-5-hydroxypentane-2,3-dione (**7***) (or rather [2,3-$^{13}C_2$]laurencione (**7*** ⇄ **8***)) was prepared from [$^{13}C_2$]acetylene (**15***) in seven steps in an overall yield of 31% (cf. Scheme **4**). Earlier syntheses of laurencione (**7** ⇄ **8**) had given the product in overall yields of 21% (in six steps, starting from γ-butyrolactone), 19% (in eight steps from 2-acetylbutyrolactone) and 17% (in five steps from 1,1-dichloroacetone), respectively (4).

3-Amino-1-hydroxypropan-2-one (3-amino-1-hydroxyacetone) (**5**) hydrochloride, the compound derivable from 4-hydroxy-L-threonine (**2**) by dehydrogenation and decarboxylation, was synthesized in four steps from DL-3-aminopropane-1,2-diol (**23**) in an overall yield of 42% (cf. Scheme 5).

A discussion of the rationale for the selection of the two compounds as putative pyridoxine precursors, and of their synthesis follows.

Results and discussion

Rationale for the choice of 5-hydroxypentane-2,3-dione (7) and 3-amino-1-hydroxypropan-2-one (5) as putative pyridoxine precursors

In *Escherichia coli* only two genes, *pdxA* and *pdxJ*, and therefore only two enzyme-catalyzed steps, are implicated in the generation of pyridoxol (**6**) from 1-deoxy-D-xylulose (**1**) plus 4-hydroxy-L-threonine (**2**) (5).

In considering a possible mechanism of this process it is a reasonable assumption that each one of the two substrates is involved in one of these enzymic steps. If these steps occurred before the two substrates interacted, then the products of the two reactions would be intermediates in the formation of pyridoxol (**6**) from **1** plus **2**. Formally, the creation of the pyridoxol skeleton ($C_8H_{11}NO_3$) (**6**) from **1** ($C_5H_{10}O_4$) plus **2** ($C_4H_9NO_4$) requires a decarboxylation ($-CO_2$), the elimination of three molecules of water ($-3\ H_2O$), and a dehydrogenation ($-2H$). If these processes corresponded to the actual mechanistic steps, then two of them must be enzyme catalyzed.

In the formation of pyridoxol from the two acyclic substrates, **1** plus **2**, a new C—N bond and a new C—C bond must be formed. Formation of the new C—N bond, between the keto group, C-2, of **1** and the amino group of **2**, poses no problems. However, since the new C—C bond must be formed by interaction of C-4 of **1** with C-3 of **2**, both of which are secondary carbinols, activation at these sites is required, so that bond formation between them can take place.

One way of achieving activation of C-4 of **1** is by dehydration, to yield the enol (**4**), i.e., a tautomer of the diketone (**7**). This diketone, 5-hydroxypentane-2,3-dione, has recently been found in nature (3), existing as an equilibrium mixture with its furanose isomer (**8**). The mixture **7** ⇄ **8** is referred to as laurencione. The 5-phosphate ester of hydroxypentane-2,3-dione (**7**) had earlier been postulated (6) as the precursor of the C_5 chain, C-4′,4,5,6,7, of the thiazole unit of thiamin (**9**). 1-Deoxy-D-xylulose (**1**), the compound from which 5-hydroxypentane-2,3-dione (**7**) may be derived by dehydration, has been shown to be incorporated into the C_5 chain, C-4′,4,5,6,7, of the thiazole unit of thiamin (7–10) and concurrently also into C-2′,2,3,4,4′ of pyridoxol (10). Furthermore, 1-deoxy-D-xylulose (**1**) has been identified as an intermediate of the nonmevalonic acid route to isoprenoids (e.g., **10**) in *E. coli* (11) and 5-hydroxypentane-2,3-dione (**7**) was again invoked as an intermediate (12).

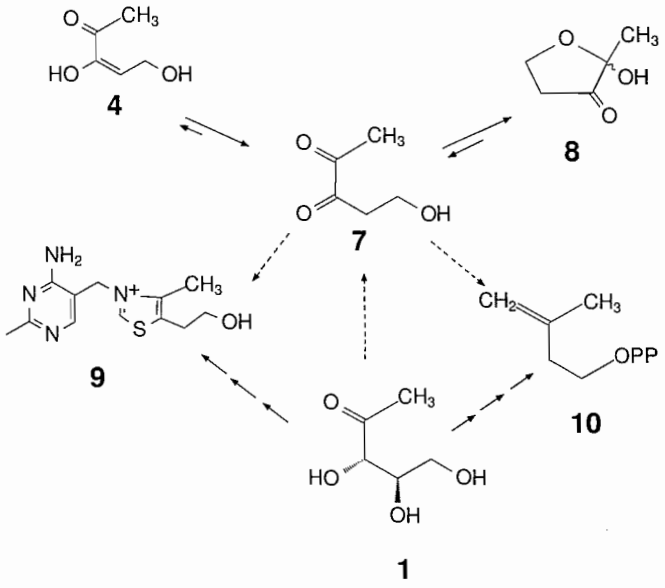
Thus, 5-hydroxypentane-2,3-dione (**7**) appeared to be a prime candidate in the choice of possible intermediates on the route from 1-deoxy-D-xylulose (**1**) into the C_5 unit, C-2′,2,3,4,4′, of pyridoxol (**6**).

In selecting the reaction that might serve to activate the carbinol carbon, C-3, of the other substrate, 4-hydroxy-L-threonine (**2**), for bond formation, we considered dehydrogenation, a process that is formally required in the conversion of the two substrates, **1** plus **2**, into pyridoxol (**6**) (Scheme 1). The product of dehydrogenation at C-3 of 4-hydroxy-L-threonine would be L-2-amino-4-hydroxy-3-oxobutanoic acid (**11**). This compound has not been described. The corresponding dehydrogenation product of threonine (**12**), L-2-amino-3-oxobutanoic acid (**13**), has been prepared (13, 14): It was found that this compound lacks stability, with a half life of ca. 10 min at physiological pH, undergoing spontaneous decarboxylation to yield 1-aminopropan-2-one (**14**) (Scheme 3). It is most likely that the corresponding dehydrogenation of 4-hydroxy-L-threonine (**2**) to the oxoamino acid (**11**) is accompanied by an analogous spontaneous decarboxylation, leading to 3-amino-1-hydroxypropan-2-one (**5**). Such a reaction sequence on the route to pyridoxol would then account not only for the required, presumably enzyme-catalyzed, dehydrogenation, but also for the required, but presumably spontaneous, decarboxylation.

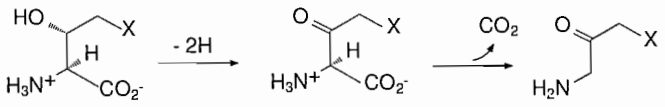
3-Amino-1-hydroxypropan-2-one (**5**) thus appeared to be a promising choice as a possible intermediate on the route from 4-hydroxy-L-threonine (**2**) into the C_3N unit, N-1,C-6,5,5′, of pyridoxol (**6**).

To date we have not been able to obtain evidence that either of the two compounds serves as precursor of pyridoxol in *Escherichia coli*. The reason for this lack of success may be

Scheme 2. 5-Hydroxypentane-2,3-dione (**7**) as a possible intermediate on the routes from 1-deoxy-D-xylulose (**1**) into thiamin (**9**) and into isoprenoids (e.g., **10**).

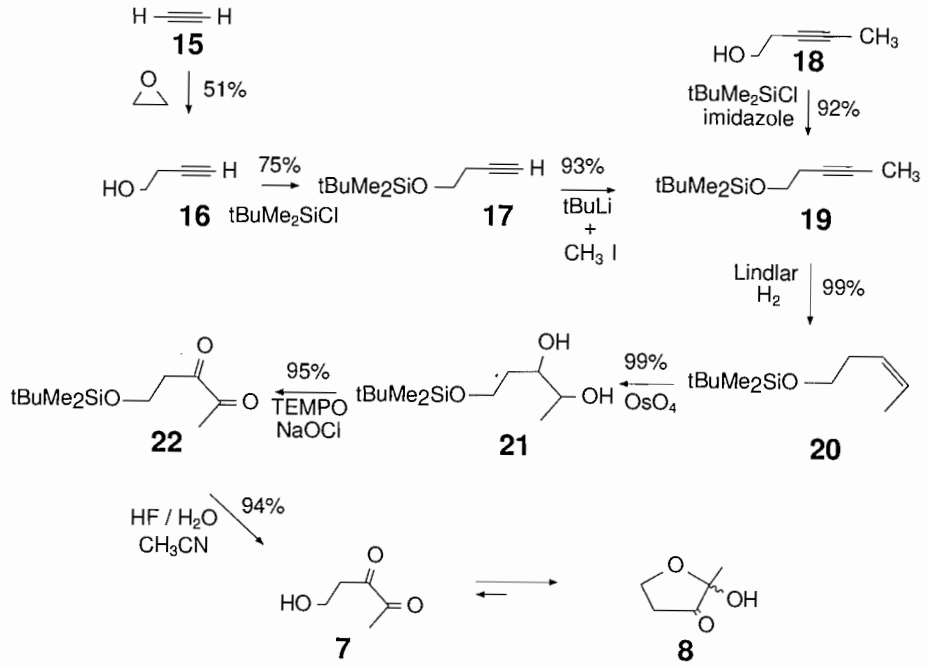


Scheme 3. The oxidative decarboxylation of threonine (**12**).



2 X = OH **11** X = OH **5** X = OH
12 X = H **13** X = H **14** X = H

Scheme 4. Synthesis of laurencione (**7** ⇄ **8**).



that the actual substrates for the enzymic reaction are the 5-phosphate ester of **7** and the 1-phosphate ester of **5**, rather than the non-phosphorylated species.

A discussion of the synthesis of the two putative intermediates of pyridoxol biosynthesis, **5** and **7**, follows.

Synthesis of 5-hydroxypentane-2,3-dione (7) (i.e., laurencione (7 ⇄ 8)) (Scheme 4)

Recent syntheses of laurencione (4) start from γ-butyrolactone, from 2-acetylbutyrolactone or from 1,1-dichloroacetone. These syntheses are not readily adaptable to the preparation of the [2,3-$^{13}C_2$]-labeled sample of 5-hydroxypentane-2,3-dione that we required. Our synthesis starts with [$^{13}C_2$]acetylene, one of the few bond-labeled compounds that are commercially available (Cambridge Isotope Laboratories).

[$^{13}C_2$]Acetylene (**15***) was converted into [3,4-$^{13}C_2$]but-3-yn-1-ol (**16***) by generating the mono lithium salt, which was reacted with ethylene oxide.[2] Reaction with *tert*-butyldimethylsilyl chloride in the presence of imidazole then protected the alcohol group. The resulting silyl ether (**17***) was treated with *tert*-butyllithium, followed by methyl iodide, to yield the silyl ether (**19***) of [3,4-$^{13}C_2$]pent-3-yn-1-ol. The corresponding unlabeled compound (**19**) is accessible by *tert*-butyldimethylsilyl chloride – imidazole treatment of pent-3-yn-1-ol (**18**), which is commercially available. Lindlar hydrogenation of the triple bond of **19*** gave [2,3-$^{13}C_2$]-(*Z*)-5-*tert*-butyldimethylsilyloxypent-2-ene (**20***), which was converted to the corresponding *cis*-diol (**21***) by means of osmium tetroxide. TEMPO oxidation (16) of the diol gave the corresponding dione (**22***), i.e., the silyl ether of the desired product. Removal of the silyl ether group by means of fluoride gave [2,3-$^{13}C_2$]-5-hydroxypentane-2,3-dione (**7***), together with the corresponding cyclic isomer, [2,3-$^{13}C_2$]-(2*RS*)-2-hydroxy-2-methyl-3-oxotetrahydrofuran (**8***), that is, [2,3-$^{13}C_2$]-laurencione. The overall yield in the seven-step synthesis of [2,3-$^{13}C_2$]laurencione (**7*** ⇄ **8***) from [$^{13}C_2$]acetylene (**15***) was 31%. The five-step synthetic route to unlabeled laurencione (**7** ⇄ **8**) from pent-3-yn-1-ol (**18**) gave the product in 81% yield and thus represents a significant improvement on the earlier syntheses (4). The composition of the equilibrium mixture **7** ⇄ **8** of laurencione, as determined by ^{13}C NMR spectroscopy, depends on the solvent. In $CDCl_3$ the ratio, **7** to **8**, was found (3) to be 17:83, whereas in C_6D_6 the **7** to **8** ratio was 40:60. Laurencione is unstable at room temperature, due to oligomerization. It can be stored in benzene (frozen) at ca. 0°C or in aqueous solution at 4°C. From aqueous solution it can be recovered by extraction into chloroform followed by evaporation of the solvent.

Synthesis of 3-amino-1-hydroxypropan-2-one (5) hydrochloride (Scheme 5)

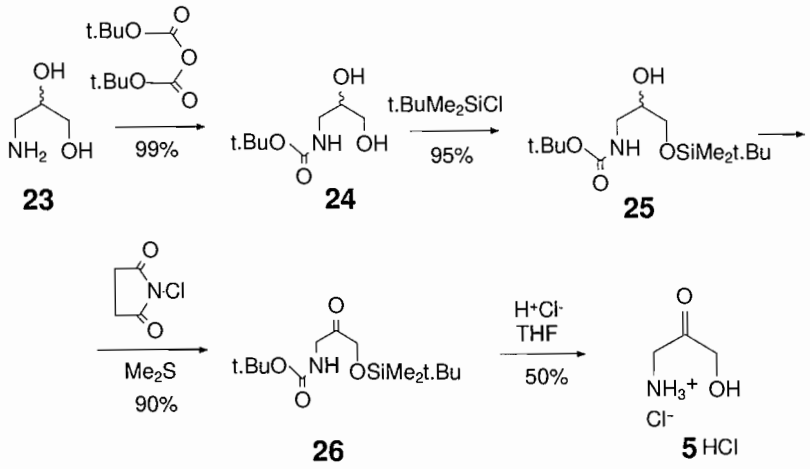
Surprisingly, neither Beilstein nor the Dictionary of Organic Compounds contains an entry for this simple three-carbon compound. Certainly, the compound is not described in the recent chemical literature. A very determined search did uncover a preparation of the compound by a three-step synthesis, in indifferent yield, from *N*-acetonylphthalimide, in connection with an early synthetic approach to ergothioneine (17).

3-Amino-1-hydroxypropan-2-one (**5**) hydrochloride was prepared starting from (±)-3-aminopropane-1,2-diol (**23**), a compound that is available commercially.

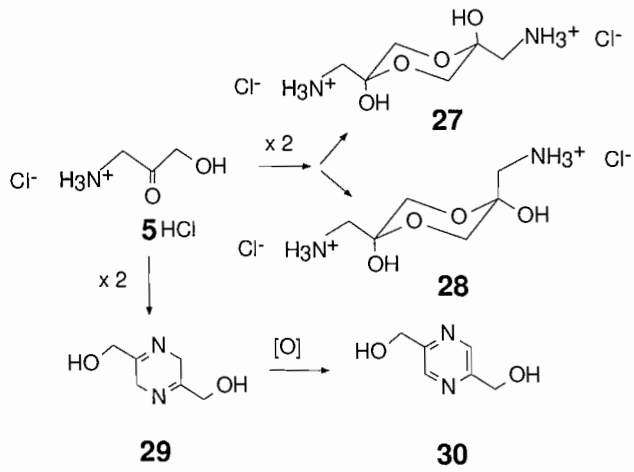
Conversion of (±)-3-aminopropane-1,2-diol (**23**) into the desired product requires preferential oxidation of the secondary alcohol group. To achieve this the primary alcohol and the amino group of the starting material had to be protected.

[2] The preparation of unlabeled but-3-yn-1-ol by this method is described by Verkruijsse and Brandsma (15*a*); see also ref. 15*b*.

Scheme 5. Synthesis of 3-amino-1-hydroxypropan-2-one (**5**).



Scheme 6. Dimers of 3-amino-1-hydroxypropan-2-one (**5**).



Protection of the amino group in the presence of the two alcohol functions was implemented by means of di-*tert*-butyl dicarbonate (18), to yield (±)-*N*-*tert*-butyloxycarbonyl-3-aminopropane-1,2-diol (**24**). The primary alcohol group of the latter was protected selectively by means of Corey silylation in the presence of 4-dimethylaminopyridine (19). The resulting doubly protected product (**25**) was then oxidized with *N*-chlorosuccinimide in the presence of dimethyl sulfide (20) to yield the protected aminoketone (**26**), which was deprotected by acid hydrolysis. 3-Amino-1-hydroxyacetone (**5**) hydrochloride was obtained as slightly yellow crystals that darkened on standing.

Slow recrystallization from ethanol-*tert*-butyl methyl ether gave the product as long, slightly yellow needles. X-ray crystallography of a 0.02 mm thick single crystal of the crystalline product shows it to be pure 3-amino-1-hydroxyacetone (**5**) hydrochloride.

However, the ^{1}H NMR spectrum in D_2O of every sample of the product **5** showed, in addition to the COCH_2O (δ 4.35 ppm) and the COCH_2N (δ 4.03 ppm) singlet signals of equal intensity, two additional singlets, each ca. one fifth as intense as the other two, at δ 3.54 and 3.06 ppm, indicating the presence of another component, with which **5** was in equilibrium in aqueous solution. The structure of the minor component was deduced from the ^{13}C NMR spectrum. In addition to the three major signals at δ 204.6 (*C*O), 65.7 (*C*H_2O), 44.9 (*C*H_2N), the spectrum showed a less intense signal at δ 92.7 (ketal). Furthermore, the two *C*H_2 signals show satellites of lower intensity: three satellites (δ 66.2, 65.6, 65.3 ppm) straddle the *C*H_2O signal, two (δ 45.3, 44.2 ppm) appear at the *C*H_2N signal.

We conclude that in aqueous solution the aminoketone exists in equilibrium with the two stereoisomers of a dimer, *trans*- and *cis*-2,5-dihydroxy-2,5-aminomethyl-1,4-dioxane dihydrochloride (**27** and **28**). Since these ring compounds are fluxional, the ^{1}H NMR spectrum does not show evidence of diastereotopic protons. When the D_2O solution of the sample is kept for 3 weeks at room temperature, the COCH_2N signals (δ 4.03 and 3.04 ppm) in the ^{1}H NMR spectrum have almost completely disappeared, indicating deuterium exchange. In addition, weak signals in the aromatic region of the spectrum are observable. These become prominent when the sample is exposed to air for several weeks at elevated temperature. This suggests formation of another dimer (**29**), which eventually oxidizes to the pyrazine (**30**). We did not attempt to isolate samples of the decomposition products.

Experimental

Synthesis of 5-hydroxypentane-2,3-dione (7), in equilibrium with (2*RS*)-2-hydroxy-2-methyl-3-oxotetrahydrofuran [(2*RS*)-2-hydroxy-2-methyl-dihydrofuran-3(2*H*)-one] (8) (i.e., laurencione (7 ⇄ 8)) (Scheme 4)

5-tert-Butyldimethylsilyloxypent-2-yne (19)

Pent-3-yn-1-ol (**18**) (3.21 g, 38.1 mmol) was dissolved in dry *N,N*-dimethylformamide (100 mL). Imidazole (5.71 g, 83.9 mmol) was added, followed by *tert*-butyldimethylsilyl chloride (6.36 g, 42.2 mmol) in small portions. The mixture was stirred overnight at room temperature, was then poured into petroleum ether (150 mL), and the solution was extracted with water (750 mL), aqueous HCl (ca. 5%, 2 × 100 mL), and water. The organic phase was dried ($MgSO_4$) and the solvent distilled off. The residue was distilled at reduced pressure

(bp 80–85°C at 10 Torr (1 Torr = 133.3 Pa)) to yield the silyl ether (**19**) (7.00 g, 92.5%). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.66 (t, $^3J_{H,H}$ = 7.0 Hz, 2H), 2.31 (tq, $^3J_{H,H}$ = 7.0 Hz, $^5J_{H,H}$ = 2.5 Hz, 2H), 1.74 (t, $^5J_{H,H}$ = 2.5 Hz, 3H), 0.87 (s, 9H), 0.04 (s, 6H).

^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 76.6, 76.1, 62.3, 25.8, 23.1, 18.3, 3.4, −5.3. MS (CI) *m/z*: 199 (100%, m + H$^+$).

*(Z)-5-*tert*-Butyldimethylsilyloxypent-2-ene (**20**)*

Alkyne **19** (3.97 g, 20 mmol) and quinoline (1 mL) were dissolved in ethyl acetate (100 mL). Lindlar catalyst (450 mg, Aldrich) was added and the mixture hydrogenated at ca. 1 atm (101.3 kPa) until uptake of hydrogen ceased. The catalyst was filtered off and washed with ethyl acetate, and the filtrate was extracted with HCl (ca. 5%, 2 × 50 mL) and brine. The organic layer was dried over K_2CO_3, and the solvent was evaporated, to yield the alkene (**20**) (4.00 g, 99%). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 5.33–5.56 (m, 2H), 3.59 (t, $^3J_{H,H}$ = 7.0 Hz, 2H), 2.26 (t, $^3J_{H,H}$ = 7.0 Hz, 2H), 1.60 (d, $^3J_{H,H}$ = 6.5 Hz, 3H), 0.88 (s, 9H), 0.04 (s, 6H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 126.6, 125.7, 62.8, 30.8, 25.9, 18.4, 12.9, −5.3. MS (CI) *m/z*: 201 (80%, m + H$^+$), 135 (100%).

*(3R4S,3S4R)-1-*O*-*tert*-Butyldimethylsilylpentane-1,3,4-triol (**21**)*

Alkene **20** (2.77 g, 13.8 mmol) was dissolved in *tert*-butanol (28 mL) and tetrahydrofuran (12 mL). Water (4 mL) and 4-methylmorpholine-*N*-oxide (NMO) (2.10 g) was added. Air was displaced by passing a stream of nitrogen through the mixture. An aqueous solution of osmium tetroxide (4%, 1 mL) was added and the mixture stirred 5 h at room temperature. An aqueous solution of sodium bisulfite (150 mg in 5 mL water) was added, followed by Florisil R (5.0 g). The mixture was stirred 10 min and then filtered through silica gel, which was then washed with ethyl acetate. Evaporation of the filtrate in vacuo yielded the colourless oily product (**21**) (3.20 g, 99%). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.78–3.91 (m, 4H), 2.11 (s (br), 2H), 1.60–1.72 (m, 2H), 1.16 (d, $^3J_{H,H}$ = 6.0 Hz, 3H), 0.89 (s, 9H), 0.07 (s, 6H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 75.7, 70.0, 62.2, 32.5, 25.8, 18.0, 17.7, −5.7. MS (CI) *m/z*: 235 (100%, m + H$^+$).

*5-*tert*-Butyldimethylsilyloxypentane-2,3-dione (**22**)*

Compound **21** (938 mg, 4 mmol) was dissolved in methylene chloride (60 mL). 2,2,6,6-Tetramethyl-1-piperidinyloxy (TEMPO, 30 mg), water (4 mL), and KBr (240 mg) were added and the mixture was cooled in an ice bath. Ice-cold aqueous solutions of saturated $NaHCO_3$ (48 mL) and of NaOCl (Cl_2 content ca. 4%, 48 mL) were added and the mixture, cooled in an ice bath, was stirred 10 min. The layers were separated, the aqueous layer was extracted with methylene chloride (50 mL), and the combined organic phases were dried ($MgSO_4$). The mixture was filtered through silica gel, which was then washed with methylene chloride until the washings emerged colourless. Concentration in vacuo gave the pure product **22** as a yellow liquid (892 mg, 95%). UV–VIS: λ_{max} = 422 nm, $\varepsilon_0 = 1.63 \times 10^6$. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.91 (t, $^3J_{H,H}$ = 6.2 Hz, 2H), 2.91 (t, $^3J_{H,H}$ = 6.1 Hz, 2H), 2.29 (s, 3H), 0.83 (s, 9H), 0.02 (s, 6H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 197.8, 196.7, 59.8, 38.5, 25.1, 22.9, 17.5, −6.2. MS (CI) *m/z*: 231 (100%, m + H$^+$).

*5-Hydroxypentane-2,3-dione (**7**), in equilibrium with (2*RS*)-2-hydroxy-2-methyl-3-oxotetrahydrofuran (**8**)*

Freshly distilled (Kugelrohr, bp 120°C at 0.7 Torr) diketone **22** (230 mg, 1 mmol) was dissolved in acetonitrile (10 mL). The solution was cooled in an ice bath and aqueous hydrofluoric acid (49% w/v, 0.6 mL) was added with stirring. The ice bath was removed after 15 min and stirring continued for 45 min. The mixture was poured into chloroform (30 mL), sodium bicarbonate (1.5 g) followed by saturated aqueous bicarbonate solution (1 mL) was added, and the mixture was stirred until evolution of CO_2 had ceased (ca. 10 min). Solid $MgSO_4$ (ca. 5 g) was added to remove water and the mixture was filtered after standing overnight. Evaporation of the solvent in vacuo gave the product as a yellow oily liquid (108 mg, 94%). Fast distillation in vacuo (Kugelrohr, 130°C, 0.5 Torr) gave pure product (92 mg, 80%) consisting of 5-hydroxypentane-2,3-dione (**7**) (17%), in equilibrium with (2*RS*)-2-hydroxy-2-methyl-3-oxotetrahydrofuran (**8**) (83%), as determined on the basis of an NMR spectrum in $CDCl_3$. In C_6D_6 the ratio of **7** to **8** was 40:60.

***7** (cf. ref. 3)*: ^{1}H NMR (200 MHz, $CDCl_3$) δ: ~4.2 (OH, overlapping OCH_2 of **8**), 3.84 (t, $^3J_{H,H}$ = 5.5 Hz, 2H), 2.91 (t, $^3J_{H,H}$ = 5.5 Hz, 2H), 2.24 (s, 3H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 198.4, 197.1, 56.9, 38.2, 23.3.

***8** (cf. ref. 3)*: ^{1}H NMR (200 MHz, $CDCl_3$) δ: 4.00–4.27 (m, ca. 3H; OCH_2 of **8**, overlapped by OH of **7** and **8**), 2.37–2.55 (m, 2H), 1.29 (s, 3H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 210.2, 96.3, 61.6, 33.4, 20.9.

***7** + **8** (cf. ref. 3)*: MS (CI, NH_3) *m/z*: 116 (100%, m − OH + NH_3^+).

Synthesis of [2,3-$^{13}C_2$]-5-hydroxypentane-2,3-dione (7*), in equilibrium with [2,3-$^{13}C_2$]-(2*RS*)-2-hydroxy-2-methyl-3-oxotetrahydrofuran ([2,3-$^{13}C_2$]-(2*RS*)-2-hydroxy-2-methyldihydrofuran-3(2*H*)-one) (8*) (*i.e.*, [$^{13}C_2$]laurencione (7* ⇄ 8*))

*[2,3-$^{13}C_2$]-5-*tert*-Butyldimethylsilyloxypent-2-yne (**19***) from [1,2-$^{13}C_2$]acetylene (**15***)*

*[3,4-$^{13}C_2$]But-3-yn-1-ol (**16***) (cf. ref. 15)*

[$^{13}C_2$]Acetylene (**15***) (0.5 L, ca. 22 mmol) was condensed from the breakseal flask in which it was supplied (Cambridge Isotope Laboratories) into an evacuated 250 mL reaction flask, immersed in a liquid nitrogen bath. After complete transfer (ca. 1 h) the breakseal flask was disconnected and the reaction vessel was flushed with dry nitrogen while still maintained at ca. −180°C. Dry NH_3 (ca. 50 mL) was condensed into the system and $LiNH_2$ (0.55 g, 22.8 mmol) was then added. The liquid nitrogen bath was replaced by an ethanol – solid CO_2 bath that had been precooled to ca. −50°C, and the mixture was refluxed (Dry Ice condenser) for 2 h. Ethylene oxide (ca. 1.5 mL, 30 mmol) was then condensed into the system and the mixture was refluxed for 40 h. NH_3 was evaporated and water (30 mL) and saturated NH_4Cl solution (10 mL) were then added. The aqueous phase was continuously extracted with diethyl ether (2 days); the ether was then evaporated at atmospheric pressure to yield the crude product (823 mg) as a

mobile liquid. The product from several runs (2.93 g) was pooled and distilled to yield [3,4-$^{13}C_2$]but-3-yn-1-ol (**16***) (2.75 g, 51%, purity >95%, main contaminant diethyl ether); bp 125–130°C (lit. (15) bp of the unenriched compound 128.9°C). IR; ν (cm^{-1}): 3279, 2947, 2889, 1048 (ν_{max}). ^1H NMR (200 MHz, $CDCl_3$) δ: 3.71 (dt(q), $^3J_{H,H} = {}^3J_{C,H} = 6.1$, 2H), 2.36–2.44 (m, 2H), 2.22 (br s), 1H), 2.01 (ddt, $^4J_{H,H} = 2.6$ Hz, $^1J_{C,H} = 244.2$ Hz, $^2J_{C,H} = 54.2$ Hz, 1H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 81.3 (enriched)(d, $^1J_{C,C} = 171$ Hz), 69.7 (enriched)(d, $^1J_{C,C} = 171$ Hz), 60.8, 22.7 (dd, $^1J_{C,C} = 65.4$ Hz, $^2J_{C,C} = 14$ Hz). MS (EI) *m/z*: 73 (5%, m + H^+), 71 (15%), 55 (30%), 45 (100%).

*[1,2-$^{13}C_2$]-4-*tert*-Butyldimethylsilyloxybut-1-yne (**17***)*
[3,4-$^{13}C_2$]But-3-yn-1-ol (**16***) (1.25 g, 17.3 mmol) was dissolved in dry dimethylformamide (40 mL). Imidazole (2.50 g, 36.7 mmol) was added, followed by *tert*-butyldimethylsilyl chloride (3.00 g, 19.9 mmol). The mixture was stirred overnight and was then poured into petroleum ether (40–60°C) (50 mL). The mixture was extracted with water (400 mL), dilute HCl (5% v/v, 2 × 25 mL), and water (50 mL). The petroleum ether solution was then dried (anhydrous $MgSO_4$), the solvent distilled off, and the residue distilled in vacuo. [1,2-$^{13}C_2$]-4-*tert*-Butyldimethylsilyloxybut-1-yne (**17***) (2.65 g, purity 92% by NMR, yield 75%) was obtained from the fraction, bp 65–70°C at 10 Torr. IR (film) ν (cm^{-1}): 2956, 2932, 2859, 1111, 839 (ν_{max}). ^1H NMR (200 MHz, $CDCl_3$) δ: 3.72 (dt, $^3J_{H,H} = 7.1$ Hz, $^3J_{C,H} = 4.3$, 2H), 2.30–2.45 (m, 2H), 1.93 (ddt, $^4J_{H,H} = 2.6$ Hz, $^1J_{C,H} = 244.4$ Hz, $^2J_{C,H} = 53.4$ Hz, 1H), 0.88 (s, 9H), 0.05 (s, 6H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 81.7 (enriched)(d, $^1J_{C,C} = 172$ Hz), 69.0 (enriched) (d, $^1J_{C,C} = 172$ Hz), 61.8, 25.9, 22.8 (dd, $^1J_{C,C} = 64$ Hz, $^2J_{C,C} = 10$ Hz), 18.3, −5.3. MS (CI) *m/z*: 187 (100%, m + H^+), 132 (60%).

*[2,3-$^{13}C_2$]-5-*tert*-Butyldimethylsilyloxypent-2-yne (**19***)*
[1,2-$^{13}C_2$]-4-*tert*-Butyldimethylsilyloxybut-1-yne (**17***) (1.78 g, 8.8 mmol) was dissolved in dry tetrahydrofuran (100 mL). The mixture was cooled to −20°C, a solution of *tert*-butyllithium (5.5 mL, 1.7 M in pentane) was added over a period of 5 min, and the mixture was allowed to stand for 1 h. Methyl iodide (1.0 mL, 16 mmol) was added, the cooling bath was removed after 30 min and stirring was continued for another 30 min. The mixture was poured into petroleum ether (40–60°C, 150 mL), followed by extraction with half-concentrated NaCl solution (2 × 150 mL). The organic phase was dried (anhydrous $MgSO_4$) and the solvent distilled off. The residual liquid was distilled in vacuo (Kugelrohr, 85°C, 10 Torr), yielding [2,3-$^{13}C_2$]-5-*tert*-butyldimethylsilyloxypent-2-yne (**19***) (1.80 g, 93%). IR (film); ν (cm^{-1}): 2956, 2930, 2858, 1103, 837 (ν_{max}). ^1H NMR (200 MHz, $CDCl_3$) δ: 3.76 (dt, $^3J_{H,H} = 7.4$ Hz, $^3J_{C,H} = {}^4J_{C,H} = 1.8$ Hz, 2H), 2.24–2.39 (m, 2H), 1.74 (q, $^4J_{H,H} = 2.5$ Hz, $^2J_{C,H} = 2.5$ Hz, 3H), 0.88 (s, 9H), 0.05 (s, 6H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 78.1 (enriched)(d, $^1J_{C,C} = 176$ Hz), 74.6 (enriched)(d, $^1J_{C,C} = 176$ Hz), 62.3, 25.9 (dd, $^1J_{C,C} = 45$ Hz, $^2J_{C,C} = 34$ Hz), 23.0 (dd, $^1J_{C,C} = 45$ Hz, $^2J_{C,C} = 34$ Hz), 18.3, 3.4, −5.3. MS (CI, NH_3) *m/z*: 218 (20%, m + NH_4^+), 201 (100%, m+H^+), 132 (20%).

The experimental procedure for the conversion of [2,3-$^{13}C_2$]-5-*tert*-butyldimethylsilyloxypent-2-yne (**19***) into [2,3-$^{13}C_2$]-(*Z*)-5-*tert*-butyldimethylsilyloxypent-2-ene (**20***) and thence, via **21*** and **22***, into bond-labeled laurencione (**7*** ⇄ **8***), was similar to that described above for the preparation of the corresponding unenriched compounds. Spectral data for the enriched compounds are given below.

*[2,3-$^{13}C_2$]-(Z)-5-*tert*-Butyldimethylsilyloxypent-2-ene (**20***)*: IR (film), ν (cm^{-1}): 3004, 2956, 2931, 2859, 1255, 1089, 837 (ν_{max}). ^1H NMR (200 MHz, $CDCl_3$) δ: 6.06–4.83 (m, 2H), 3.59 (dt, $^3J_{H,H} = 7.1$ Hz, $^3J_{C,H} = 3.4$ Hz, 2H), 2.20–2.39 (m, 2H), 1.60 (ddd, $^3J_{H,H} = 5.0$ Hz, $^2J_{C,H} = 6.8$ Hz, $^4J_{H,H} = 0.9$ Hz, 3H), 0.86 (s, 9H), 0.04 (s, 6H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 126.9 (enriched)(d, $^1J_{C,C} = 70$ Hz), 125.2 (enriched)(d, $^1J_{C,C} = 70$ Hz), 62.8 (d, $^2J_{C,C} = 3$ Hz), 30.8 (dd, $^1J_{C,C} = 33$ Hz, $^2J_{C,C} = 10$ Hz), 26.0 18.4, 12.9 (dd, $^1J_{C,C} = 32$ Hz, $^2J_{C,C} = 10$ Hz), −5.2. MS (CI), *m/z*: 203 (100%, m + H^+), 162 (20%), 130 (60%).

*[2,3-$^{13}C_2$]-(2R3S,2S3R)-5-*O*-tert-Butyldimethylsilylpentane-2,3,5-triol (**21***)*: IR (film), ν (cm^{-1}): 3385, 2956 (ν_{max}), 2931, 2859, 1256, 1090, 836. ^1H NMR (200 MHz, $CDCl_3$) δ: 4.16–3.33 (m, 2H), 3.77–3.96 (m, 2H), 2.54 (br s), OH, 2H), 1.58–1.78 (m, 2H), 1.15 (dt, $^3J_{H,H} = 6.4$ Hz, $^3J_{C,H} = {}^2J_{C,H} = 2.1$ Hz, 3H), 0.88 (s, 9H), 0.07 (s, 6H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 75.7 (enriched)(d_{AB}, $^1J_{C,C} = 43$ Hz), 70.0 (enriched)(d_{AB}, $^1J_{C,C} = 42$ Hz), 62.5, 31.8 (d, $^1J_{C,C} = 37$ Hz), 25.8, 18.0, 17.7 (d, $^1J_{C,C} = 39$ Hz), −5.7. MS (CI), *m/z*: 237 (100%, m + H^+), 140 (30%).

*[2,3-$^{13}C_2$]-5-*tert*-Butyldimethylsilyloxypentane-2,3-dione (**22***)*: IR (film), ν (cm^{-1}): 2957, 2932, 2887, 2859, 1677 (ν_{max}). ^1H NMR (200 MHz, $CDCl_3$) δ: 3.40 (dt, $^3J_{H,H} = 6.1$ Hz, $^3J_{C,H} = 4.0$ Hz, 2H), 2.91 (q, $^3J_{H,H} = 6.1$ Hz, $^2J_{C,H} = 6.1$ Hz, 2H), 2.30 (d, $^2J_{C,H} = 5.5$ Hz, 3H), 0.83 (s, 9H), 0.01 (s, 6H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 198.7 (enriched)(d_{AB}, $^1J_{C,C} = 46$ Hz), 197.2 (enriched)(d_{AB}, $^1J_{C,C} = 46$ Hz), 58.4, 39.4 (dd, $^1J_{C,C} = 39$ Hz, $^2J_{C,C} = 15$ Hz), 25.8, 22.4 (d, $^1J_{C,C} = 35$ Hz), 18.2, −5.7. MS (CI, NH_3), *m/z*: 250 (100%, m + NH_4^+), 233 (100% m + H^+).

[2,3-$^{13}C_2$]-5-Hydroxypentane-2,3-dione (7*), in equilibrium with [2,3-$^{13}C_2$]-(2*RS*)-2-hydroxy-2-methyldihydrofuran-3(2*H*)-one ([2,3-$^{13}C_2$]-(2*RS*)-2-hydroxy-2-methyl-3-oxotetrahydrofuran) (8*)

7*: ^1H NMR (200 MHz, C_6D_6) δ: 3.69 (q, $^3J_{H,H} = 7.8$ Hz, 2H), 2.55–2.64 (m, 2H), 1.85 (d, $^2J_{C,H} = 4.2$ Hz, 3H). ^{13}C NMR (50.3 MHz, C_6D_6) δ: 196.2 (enriched)(d_{AB}, $^1J_{C,C} = 46$ Hz), 195.2 (enriched)(d_{AB}, $^1J_{C,C} = 46$ Hz), 55.2, 35.2 (dd, $^1J_{C,C} = 31$ Hz, $^2J_{C,C} = 22$ Hz), 21.9 (dd, $^1J_{C,C} = 33$ Hz, $^2J_{C,C} = 24$ Hz).

8*: ^1H NMR (200 MHz, C_6D_6) δ: 3.43–3.60 (m, 2H), 1.70–1.90 (m, 2H), 1.27 (dd, $^2J_{C,H} = 5.0$ Hz, $^3J_{C,H} = 2.8$ Hz, 3H). ^{13}C NMR (50.3 MHz, C_6D_6) δ: 206.0 (enriched)(d, $^1J_{C,C} = 51$ Hz), 98.3 (enriched)(d, $^1J_{C,C} = 51$ Hz), 60.7, 32.1 (dd, $^1J_{C,C} = 39$ Hz, $^2J_{C,C} = 13$ Hz), 15.8 (d, $^1J_{C,C} = 47$ Hz).

7* + **8***: IR (film), ν (cm^{-1}): 3939, 2990, 2900, 1725 (ν_{max}), 1678, 1075. MS (CI, NH_3), *m/z*: 118 (100%, m − OH + NH_3^+).

Synthesis of 3-amino-1-hydroxypropan-2-one (3-amino-1-hydroxyacetone) (5) hydrochloride from (±)-3-aminopropane-1,2-diol (23) (Scheme 5)

*(±)-*N*-tert-Butyloxycarbonyl-3-aminopropane-1,2-diol (24)*
Di-*tert*-butyl dicarbonate (18) (25 g, 114.6 mmol), dissolved

in CH_2Cl_2 (25 mL), was added slowly to a stirred solution of (±)-3-aminopropane-1,2-diol (**23**) (8.7 g, 95.5 mmol) in CH_2Cl_2–MeOH (20 mL, 1:5) and triethylamine (1.3 mL, 9.6 mmol). The reaction, which was followed by TLC, was complete in 2 h. Evaporation of the solvents gave the product **24** (18 g, 99%) as a colourless solid that was used without further purification; mp 55–58°C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 5.12 (br s, 1H), 3.18–3.76 (m, 5H), 1.39 (s, 9H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 157.4, 80.0, 71.3, 63.6, 42.8, 27.9.

N-tert-*Butyloxycarbonyl-3-amino-1*-tert-*butyldimethyl-silyloxy-2-propanol (25)*

N-tert-Butyloxycarbonyl-3-amino-1,2-propanediol (**24**) (18 g, 94.2 mmol) was dissolved in CH_2Cl_2 (150 mL) and *tert*-butyldimethylsilyl chloride (11 g, 103.7 mmol) and triethylamine (15.7 mL, 113 mmol) were added with stirring, followed by 4-dimethylaminopyridine (19) (0.46 g, 3.7 mmol), and stirring was continued overnight. Water (20 mL) was then added to quench the reaction. The layers were separated, the aqueous layer was washed with CH_2Cl_2 (3 × 10 mL), and the combined organic fraction was washed with water (3 × 10 mL) and brine and dried with anhydrous $MgSO_4$. Concentration of the solution gave a light yellow oil that was distilled at reduced pressure to give the product (**25**) (27.3 g, 95%) as a colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 5.26 (br s, 1H), 2.80–3.71 (m, 5H), 1.41 (s, 9H), 0.86 (s, 9H), 0.04 (s, 6H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 156.7, 79.4, 71.2, 64.8, 43.3, 28.3, 25.8, 18.2, −5.5. MS (EI), *m/z*: 306 (m + H^+), 250, 232, 206, 148.

N-tert-*Butyloxycarbonyl-3-amino-1*-tert-*butyldimethyl silyloxy-2-propanone (26)*

A suspension of *N*-chlorosuccinimide (16 g, 120.5 mmol) in CH_2Cl_2 (100 mL) was cooled to 0°C, dimethyl sulfide (12.2 mL, 166 mmol) (20) was added, and the mixture was cooled further to −20°C (CCl_4 – Dry Ice). *N-tert*-Butyloxycarbonyl-3-amino-1-*tert*-butyldimethylsilyloxy-2-propanol (**25**) (24.5 g, 80.3 mmol) in CH_2Cl_2 (20 mL) was added and the mixture was stirred at −20°C for 2 h. Triethylamine (16.8 mL, 120.5 mmol) was then added, the cooling mixture was removed, and stirring continued for 1 h. The reaction mixture was then diluted with ether (100 mL), washed with 1 M HCl, water, and brine, and dried with anhydrous $MgSO_4$. The solution was concentrated and the oily residue was chromatographed (5% diethyl ether – hexanes) on silica gel (200–400 mesh). The product **26** (21.9 g, 90%) was obtained as a colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 4.19 (s, 4H), 1.39 (s, 9H), 0.86 (s, 9H), 0.04 (s, 6H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 206.7, 115.6, 79.6, 68.2, 48.0, 28.2, 25.7, 18.1, −5.7. MS (EI), *m/z*: 246 (m − *t*.Bu^+), 230, 190, 129. MS (CI, NH_3), *m/z*: 321 (100%, m + NH_4^+).

3-Amino-1-hydroxyacetone (3-amino-1-hydroxypropan-2-one) (5) hydrochloride

N-tert-Butyloxycarbonyl-3-amino-1-*tert*-butyldimethylsilyloxy-2-propanone (**26**) (11.2 g, 37 mmol) was dissolved in a mixture containing aqueous 20% HCl and THF (50 mL, 2:5) and stirred for 24 h. Removal of the solvent gave a brownish crystalline solid that was recrystallized from EtOH-*tert*-butyl methyl ether to afford the product **5** (2.3 g) as light yellow needles; m.p. 134–136°C (lit. (8) mp 136–137°C). ^{1}H NMR (200 MHz, D_2O) δ: 4.35 (s, 2H, CH_2O), 4.03 (s, 2H, $CH_2NH_3^+$), 3.54 (s, CH_2O of dimer, intensity ca. 20% of 4.35 ppm signal), 3.06 (s, CH_2N of dimer, intensity ca. 20% of 4.05 ppm signal). ^{13}C NMR (50.3 MHz, D_2O), major signals δ: 204.6, 65.7, 44.9; minor signals δ: 92.7, 66.2, 65.6, 65.3, 45.3, 44.5. MS (CI, NH_3), *m/z*: 107 (5%, m (of free base) + NH_4^+), 90 (100%, m + H^+).

Acknowledgments

Research grants from the Natural Sciences and Engineering Research Council of Canada and from the National Institute of General Medical Sciences, U.S. Public Health Service, are gratefully acknowledged. We thank Dr. James F. Britten for confirming the structure of the synthetic sample of **5** HCl by X-ray crystallography.

References

1. R.E. Hill, K. Himmeldirk, I.A. Kennedy, R.M. Pauloski, B.G. Sayer, E. Wolf, and I.D. Spenser. J. Biol. Chem. **271**, 30426 (1996).
2. I.D. Spenser and R.E. Hill. Nat. Prod. Rep. **12**, 555 (1995).
3. M.W. Bernart, W.H. Gerwick, E.E. Corcoran, A.Y. Lee, and J. Clardy. Phytochemistry, **31**, 1273 (1992).
4. N. De Kimpe, A. Georgieva, M. Boeykens, and L. Lazar. J. Org. Chem. **60**, 5262 (1995); N. De Kimpe, A. Georgieva, M. Boeykens, I. Kozekov, and W. Aelterman. Synthesis, 1131 (1996).
5. T.-K. Man, G. Zhao, and M.E. Winkler, J. Bacteriol. **178**, 2445 (1996), and refs. cited therein.
6. R.H. White. Biochemistry, **17**, 3833 (1978).
7. S. David, B. Estramareix, J.-C. Fischer, and M. Therisod. J. Am. Chem. Soc. **103**, 7341 (1981).
8. M. Therisod, J.-C. Fischer, and B. Estramareix. Biochem. Biophys. Res. Commun. **98**, 374 (1981).
9. S. David, B. Estramareix, J.-C. Fischer, and M. Therisod. J. Chem. Soc. Perkin Trans. I, 2131 (1982).
10. K. Himmeldirk, I.A. Kennedy, R.E. Hill, B.G. Sayer, and I.D. Spenser. J. Chem. Soc. Chem. Commun. 1187 (1996).
11. S.T.J. Broers. Dissertation Nr. 10978, ETH Zürich, Switzerland. 1994.
12. M.K. Schwarz. Dissertation Nr. 10951, ETH Zürich, Switzerland. 1994.
13. W.G. Laver, A. Neuberger, and J.J. Scott. J. Chem. Soc. 1483 (1959).
14. J.P. Marcus and E.E. Dekker. Biochem. Biophys. Res. Commun. **190**, 1006 (1993).
15. (*a*) H.D. Verkruijsse and L. Brandsma. Synth. Commun. **21**, 235 (1991); (*b*) L. Brandsma. Preparative acetylenic chemistry. Elsevier, Amsterdam. 1971. pp. 60–61.
16. A.E.J. de Nooy, A.C. Besemer, and H. van Bekkum. Synthesis, 1153 (1996).
17. A.O. Jackson and C.S. Marvel. J. Biol. Chem. **103**, 191 (1933).
18. O. Keller, W.E. Keller, G. van Look, and G. Wersin. Org. Synth. **63**, 160 (1985).
19. S.K. Chaudhary and O. Hernandez. Tetrahedron Lett. 99 (1979).
20. K.K. Ogilvie, N. Nguyen-Ba, and R.G. Hamilton. Can. J. Chem. **62**, 1622 (1984).