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Synthesis of [7-^{3}H]valienamine, [7-^{3}H]valienone, [7-^{3}H]valiolamine and [7-^{3}H]valiolone from validamycin A

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SUMMARY

To investigate the biosynthetic pathway to the cyclitol moieties of acarbose and validamycin A, [7-^{3}H]valienamine, [7-^{3}H]valienone, [7-^{3}H]valiolamine and [7-^{3}H]valiolone were synthesized as plausible precursors. Valienamine together with validamine was isolated from the degradation of validamycin A by *Flavobacterium saccharophilum* and served as starting material for the synthesis. Validamine was removed partially at the stage of tritylation and completely after the oxidation of the primary hydroxy group at C-7 to the aldehyde. The resulting valienamine aldehyde was reduced with tritiated sodium borohydride to produce [7-^{3}H]valienamine. The latter was converted to [7-^{3}H]valiolamine by a synthetic route described in the literature. The ^{3}H-labeled amines were oxidized to [7-^{3}H]valienone and [7-^{3}H]valiolone, respectively, using 3,5-di-*tert*-butyl-1,2-bezoquinone (DBQ) followed by hydrolysis with oxalic acid.

Key Words: acarbose, validamycin A, valienamine, valienone, valiolamine, valiolone, sodium borotritiide

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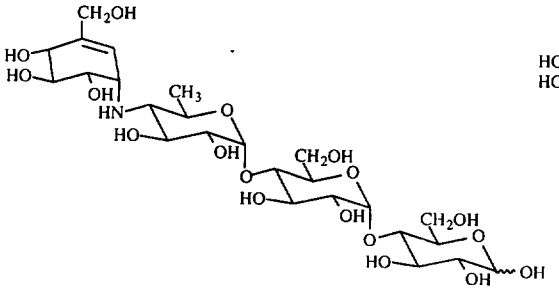
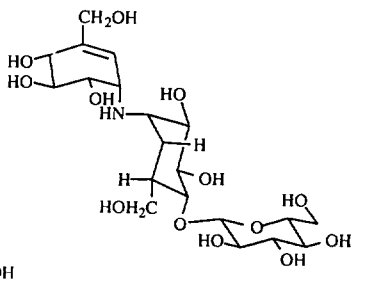
INTRODUCTION

Acarbose (**1**) isolated from *Actinoplanes* sp. is an α-glucosidase inhibitor and clinically useful drug for the treatment of type II insulin-independent diabetes (1), whereas validamycin A (**2**) is an important antibiotic from *Streptomyces hygroscopicus* var. *limoneus* used in the Orient for controlling Blight Sheath, a disease of rice plants caused by the phytopathogen, *Pellicularia sasakii* (2). Both compounds contain unsaturated aminocyclitols which are identical with valienamine, also found in other secondary metabolites such as salbostatin (3), adiposin (4), the amylostatins (5) and the trestatins (6). These aminocyclitol moieties are aliphatic analogs of *m*-C_7N units which are found in the ansamycin (7) and mitomycin (8) antibiotics. The *m*-C_7N unit in those antibiotics is synthesized via a branch of the shikimate pathway (9). However, based on experiments with stable isotope-labeled precursors the *m*-C_7N units of acarbose (10) and validamycin A (11) are derived from the pentose phosphate pathway. Specifically,



Acarbose (**1**)



Validamycin A (**2**)

Figure 1. Structures of acarbose and validamycin A.

it was proposed that either sedoheptulose 7-phosphate or *ido*-heptulose-7-phosphate is a precursor of these cyclitols. Valiolone (**16**), expected as the first intermediate of the cyclization of the heptulose, could be dehydrated to valienone (**13**) which is identical with the unsaturated cyclitols of acarbose and validamycin A. Alternatively, **16** could be aminated to form valiolamine (**15**) which could then be dehydrated after forming a Schiff's base. The unsaturated cyclitol is connected to a deoxyhexose in acarbose and to a saturated cyclitol in validamycin A by a nitrogen bridge. However, it is not known whether the unsaturated cyclitol or the partner moiety, i.e., the deoxyhexose for

acarbose and the saturated cyclitol for validamycin A, is aminated first to connect to each other. We therefore report here the synthesis of the radiolabeled cyclitols **4**, **13**, **15** and **16** as a prerequisite for testing *in vivo* or *in vitro* their involvement in the biosynthesis of acarbose in *Actinoplanes* sp. and of validamycin A in *S. hygroscopicus* var. *limoneus*.

RESULTS AND DISCUSSION

The overall strategy for the synthesis of [7-^{3}H]valienamine and [7-^{3}H]valienone is shown in Figure 2, and for [7-^{3}H]valiolamine and [7-^{3}H]valiolone in Figure 3. To obtain valienamine as a starting material, validamycin A was incubated with *Flavobacterium saccharophilum* which degrades validamycin A by the action of a D-glucoside 3-hydrogenase and a C-N lyase to valienamine (**4**), validamine (**3**) and other cyclitols (12).

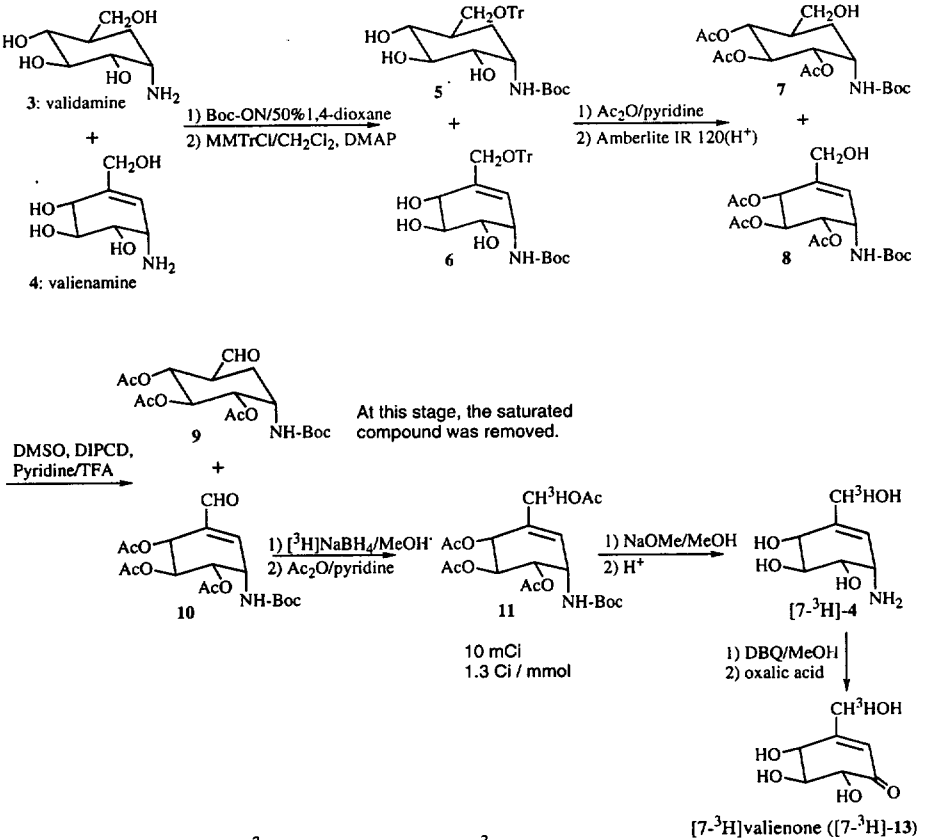


Figure 2. Synthesis of [7-^{3}H]valienamine and [7-^{3}H]valienone.

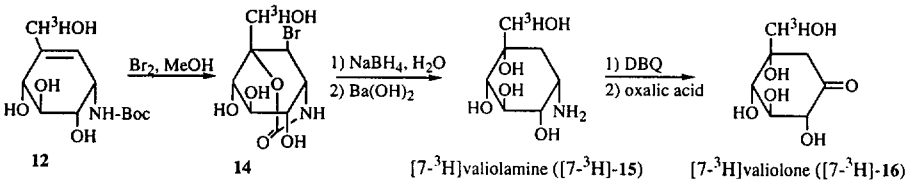


Figure 3. Synthesis of [7-^{3}H]valiolamine and [7-^{3}H]valiolone.

It was reported that valienamine and validamine can be separated on Dowex 1 (OH^-) (13) but this separation was found to be incomplete. Therefore, the mixture of valienamine and validamine from the Dowex 1 column was used directly for the synthesis of [7-^{3}H]valienamine without further purification, since the valienamine can be separated from validamine more effectively at a subsequent stage. After the aminocyclitols had been protected using 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (BOC-ON), the primary hydroxy groups at C-7 were tritylated with monomethoxytrityl chloride. This tritylation was not completed even after one week (14), but at this stage most of the validamine **3** had been tritylated, but only part of the valienamine **4**, based on the ^{1}H- and ^{13}CNMR data. Therefore, the tritylated compounds were isolated after one week to separate them from unreacted **4**, even though the tritylated valienamine **6** is lost. In a second tritylation of the unreacted material, a purer sample of **6** was obtained. After the tritylated mixture had been acetylated with acetic anhydride, detritylation using Amberlite IR-120 (H^+) in methanol quantitatively yielded a mixture of **7** and **8** despite the usually acid labile Boc-protection at the amino group. Moffatt oxidation of the primary hydroxy groups at C-7 to the aldehydes used N,N'-diisopropylcarbodiimide (DIPCD) with pyridium trifluoroacetate in dimethyl sulfoxide, which is a good reagent to oxidize primary hydroxy groups of carbohydrates without α,β-elimination (14, 15). Oxidation with MnO_2, which is a reagent to oxidize specifically allylic alcohols, failed due to competing elimination. Since aldehydo forms of pseudosugars are extensively hydrated (16), the compound could only be isolated as a mixture of the aldehyde and the corresponding hydrate. At this stage, aldehyde **10** was separated from aldehyde **9** completely by chromatography on silica (hexane : ethyl acetate = 1 : 2, R_f=0.87 for **10** and 0.38 for **9**). The R_f difference is due to the difference in structural properties, i.e., that the aldehyde **10** is conjugated

with a double bond, whereas the aldehyde **9** is not. After isolating the unsaturated aldehyde **10**, reduction with sodium borotritiide and acetylation of the resulting ^{3}H-alcohol produced ^{3}H-valienamine **11** with a high degree of stereoselective labeling (H_A : H_B = 8 : 2, 10 mCi, specific radioactivity = 1.3 Ci/mmol) compared to that of [6"-^{3}H] maltotriose (H_S : H_R = 6 : 4) in an earlier similar preparation (14). Deacetylation with NaOMe in MeOH and subsequent deprotection of the N-Boc-group with acid yielded quantitatively [7-^{3}H]valienamine **4**.

[7-^{3}H]Valiolamine was prepared from N-Boc-[7-^{3}H]valienamine **12**, diluted with unlabeled N-Boc-valienamine to a specific radioactivity of 2.5 mCi/mmole, followed by a synthetic sequence described by Horii and Fukase (17). The first step employed a stereospecific bromination to provide the corresponding cyclic carbamate **14**. Reductive amination using sodium borohydride and subsequent alkaline hydrolysis gave [7-^{3}H]valiolamine **15** in about 60 % radiochemical yield. To obtain [7-^{3}H]valienone **13** and [7-^{3}H]valiolone **16**, the [7-^{3}H]valienamine **4** and [7-^{3}H]valiolamine **15** were oxidized with 3,5-di-*tert*-butyl-1,2-benzoquinone (DBQ) (18) followed by hydrolysis with oxalic acid in 60 % overall yield. This represents the first reported preparations of unprotected valienone and valiolone.

EXPERIMENTAL

General. – Validamycin A (85 %) was a gift from Prof. Kenneth L. Rinehart, Jr., University of Illinois, IL. *Flavobacterium saccharophilum* (IFO 13984) was purchased from Osaka Institute of Fermentation. Valienamine was a gift from Takeda Chemical Industries Ltd., Osaka, Japan. All chemicals as well as Amberlite IR-120, Amberlite CG-50, Dowex 1 and Dowex 50 were obtained from Aldrich Chemical Co. Diaion WK-100 and Amberlite IRA-68 were from Supelco. Sodium borotritiide (5-10 Ci/mmol) was from Amersham. Tritiated samples were counted in Bio-Safe II biodegradable counting cocktail (Research Products International Corp) with a Beckman LS 1801 scintillation counter. ^{1}HNMR spectra, ^{13}CNMR spectra (acetone-d_6 or D_2O) and ^{3}HNMR spectra (acetone-d_6, D_2O, or H_2O) were recorded on an IBM-Bruker AF-300 NMR spectrometer. Reactions were monitored by TLC and compounds on chromatograms were visualized by UV light or alkaline permanganate spray.

Valienamine from the degradation of validamycin A by F. saccharophilum. —Validamycin A (5 g) was incubated with *F. saccharophilum* IFO 13984 in phosphate buffer (pH 7.1) containing ammonium sulfate and magnesium sulfate for 4 days as described (19). The culture was centrifuged and the supernatant was applied to a Dowex 50 column (2.5 x 20 cm, H^+). After washing the column with water, amines were eluted with 0.5 N NH_4OH. The fractions containing the amines were pooled and concentrated to dryness under reduced pressure. The residue was dissolved in water and applied to an Amberlite CG-50 column (2.5 x 20 cm, NH_4^+). The column was eluted with water to remove validamycin A and validoxylamine A and then with 0.5 N NH_4OH to obtain the primary amines. After concentrating the pooled amine fractions, the residue was dissolved in water and applied to a Dowex 1 column (1.5 x 45 cm, OH^-). The amines were eluted with water and evaporated to dryness under reduced pressure. In view of the difficulty of separating valienamine from validamine, the mixture was used for the following synthesis directly without further purification.

N-*(tert-Butyloxycarbonyl)-7-monomethoxytrityl-validamine* and *-valienamine* (**5** and **6**). —To a mixture of amines **3** and **4** (250 mg, 1.42 mmol) in water (5 mL) and 1,4-dioxane (5 mL) was added Boc-ON (360 mg, 1.46 mmol). The mixture was stirred at room temperature overnight and concentrated under reduced pressure. The residue was treated with water (5 mL) and washed with ethyl acetate (2 x 5 mL). The aqueous layer was applied to an Amberlite CG-50 column eluting with water. The fractions containing the product were concentrated to yield N-(tert-butyloxycarbonyl)-valienamine and -validamine (350 mg, 90 %); R_f 0.71 (ethyl acetate : EtOH = 1.5 : 1). To the solution of N-Boc-amines (300 mg, 1.09 mmol), triethylamine (150 µL, 1.1 mmol) and a catalytic amount of DMAP (10 mg) in dry CH_2Cl_2 (3 mL) was slowly added monomethoxytrityl chloride (123 mg, 0.4 mmol). The mixture was stirred at RT for 1 day and more trityl chloride (200 mg, 0.65 mmol) was added. After stirring for 1 week, the mixture was washed with water, NH_4Cl solution and brine. The organic layer was dried over Na_2SO_4 and concentrated to dryness under reduced pressure to give a mixture of **5** and **6** (160 mg). Unreacted starting material was recovered from the aqueous phase and tritylated again three more times to give 120 mg (total 280 mg, 48 %); R_f 0.31 (hexane : ethyl acetate = 1 : 2). **5**; ^{13}CNMR (acetone-d_6): δ 28.59, 30.46, 38.54, 51.74, 55.46, 64.75,

73.96, 74.27, 76.50, 78.91, 86.60, 113.72, 127.46, 128.43, 129.19, 131.15, 136.69, 145.95, 157.19, 159.48. **6**; δ 28.56, 49.49, 55.41, 64.96, 70.92, 71.43, 73.03, 79.01, 87.21, 113.82, 122.24, 127.60, 128.54, 129.09, 131.08, 136.49, 138.70, 145.76, 156.69, 159.59.

N-*(tert-Butyloxycarbonyl)-4,5,6-tri-O-acetyl-validamine and -valienamine* (**7** and **8**). —To a solution of **5** and **6** (160 mg, 0.29 mmol) in pyridine (1 mL) containing a catalytic amount of DMAP (5 mg) was added acetic anhydride (100 μL, 1.05 mmol). The mixture was stirred at room temperature overnight and treated with water (5 mL). The product was extracted with ethyl acetate (2 x 5 mL), and washed with $NaHCO_3$ and brine. The organic extracts were dried over Na_2SO_4, and concentrated to dryness under reduced pressure to give N-Boc-4,5,6-tri-O-acetyl-7-monomethoxytrityl-valienamine and -validamine (170 mg, 87 %); R_f 0.63 (hexane : ethyl acetate = 1 : 1). Validamine derivative; ^{1}HNMR (acetone-d_6): δ 1.44 (s, 9H), 1.7-2.1 (m, 12H), 3.05, (m, 2H), 3.78 (s, 3H), 4.37 (m, 1H), 4.93 (dd, J=4.5, 10.7 Hz, 1H), 5.13 (d, J=10.7 Hz, 1H), 5.23 (m, 1H), 6.9-7.5 (m, 14H). ^{13}CNMR (acetone-d_6): δ 20.60, 20.67, 20.77, 28.53, 30.44, 37.01, 47.81, 55.51, 62.91, 72.45, 72.69, 73.33, 78.91, 86.89, 113.79, 127.59, 128.52, 128.98, 131.20, 145.41, 145.46, 156.59, 170.16, 170.29, 170.40. Valienamine derivative; ^{1}HNMR (acetone-d_6): δ 1.41 (s, 9H), 1.75-2.0 (m, 9H), 3.57 (m, 2H), 3.79 (s, 3H), 4.64 (m, 1H), 5.10 (dd, J=4.9, 10.7 Hz, 1H), 5.34 (dd J=7.0, 10.7 Hz, 1H), 5.63 (d, J=7.0 Hz, 1H), 5.85 (dd, J=1.4, 5.4 Hz, 1H), 6.90-7.50 (m, 14H). ^{13}CNMR (acetone-d_6): δ 20.58, 20.64, 20.73, 28.53, 47.15, 55.46, 63.84, 69.67, 70.32, 71.67, 79.09, 87.50, 113.89, 124.51, 127.75, 128.62, 128.98, 131.11, 137.13, 145.27, 145.34, 156.34, 159.74, 169.82, 170.12, 170.24. To a solution of the above (170 mg, 0.25 mmol) in MeOH (2 mL) was added Amberlite IR-120 (H^+, 50 mg). The mixture was stirred at room temperature for 7 hr and the resin was filtered off. The solution was concentrated and the residue was chromatographed on a column of silica gel with hexane : ethyl acetate (1 : 1) as solvent to yield amines **7** and **8** (80 mg, 80 %); R_f 0.53 (hexane : EtOAc = 1 : 2). **7**; ^{1}HNMR (acetone-d_6): δ 1.39 (s, 9H), 1.80 (m, 1H), 1.90-2.05 (m, 10H), 2.20 (m, 1H), 3.45 (m, 2H), 4.35 (m, 1H), 4.83 (dd, J=4.4, 10.7 Hz, 1H), 4.90 (dd, J=9.5, 11.0 Hz, 1H), 5.29 (dd~t, J=10.1 Hz, 1H). ^{13}CNMR (acetone-d_6): δ 20.67-21.03 (3C), 28.52, 30.42, 38.63, 47.67, 62.24, 72.10, 73.12, 73.51, 78.84, 156.68, 170.21-170.89 (3C). **8**;

^{1}HNMR (acetone-d_6): δ 1.40 (s, 9H), 1.95-2.05 (m, 9H), 4.00 (m, 2H), 4.60 (m, 1H), 5.03 (dd, J=4.9, 10.4 Hz, 1H), 5.39 (dd, J=6.8, 10.4 Hz, 1H), 5.58 (d, J=6.8 Hz, 1H), 5.86 (dd, J=1.5, 5.3 Hz, 1H). ^{13}CNMR(acetone-d_6): δ 20.67-21.03 (3C), 28.52, 47.03, 61.84, 69.84, 70.32, 71.65, 79.06, 122.34, 139.99, 156.35, 170.21-170.89 (3C).

N-*(tert-Butyloxycarbonyl)-4,5,6,-tri*-O-*acetyl-valienamine-3-aldehyde* (**10**). —To a solution of **7** and **8** (10 mg, 0.025 mmol) in dry dimethyl sulfoxide (100 μL) and dry benzene (200 μL) was added N,N'-diisopropylcarbodiimide (DIPCD, 23 μL, 0.15 mmol), dry pyridine (4 μL, 0.049 mmol), and trifluoroacetic acid (2 μL, 0.026 mmol). The reaction mixture was stirred at room temperature overnight, then cooled to 0 °C, and a solution of oxalic acid (13.5 mg, 0.15 mmol) in methanol (500 μL) was added to destroy excess DIPCD. After 30 min the solution was diluted with cold water (5 mL) and extracted with ethyl acetate (3 x 5 mL). The combined organic extracts were washed with water, dried over Na_2SO_4, and concentrated to dryness. The residue was chromatographed on silica gel with hexane : ethyl acetate (1 : 1) as solvent to give **10** (8 mg, 80 %); R_f 0.84-0.93 (hexane : ethyl acetate = 1 : 2). ^{1}HNMR (acetone-d_6): δ 1.44 (s, 9H), 1.97-2.0 (3s, 9H), 4.91 (dd, J=4.2, 8.9 Hz, 1H), 5.17 (dd, J=4.5, 7.3 Hz, 1H), 5.32 (m, 1H), 5.70 (d, J=4.2 Hz), 6.73 (d, J=8.9 Hz, NH), 7.04 (d, J=3.4 Hz, 1H), 9.58 (s, 1H). **9**; R_f 0.33-0.43.

[7-^{3}H]-N-*(tert-Butyloxycarbonyl)-4,5,6,7-tetra*-O-*acetyl-valienamine* ([7-^{3}H]-**11**). —To a solution of **10** (3 mg, 7.5 μmol) in methanol (200 μL) at 0 °C was added sodium borotritiide (100 mCi, specific radioactivity 5-10 Ci/mmol) dissolved in water (150 μL). The mixture was stirred for 4 hr and neutralized with 0.05 N HCl (350 μL) and the product was extracted with ethyl acetate (3 x 1 mL). After drying over Na_2SO_4 and concentrating under reduced pressure, the residue was treated with dry pyridine (100 μL) and acetic anhydride (5 μL). The mixture was stirred at room temperature overnight and evaporated in a vacuum. The residue was dissolved in water (1 mL) and extracted with ethyl acetate (3 x 1 mL). The organic extract was washed with brine, dried over Na_2SO_4, and evaporated to dryness under reduced pressure. The residue was chromatographed on silica gel (2.5 mL) with hexane : ethyl acetate (2 : 1) to give [7-^{3}H]-**11** (10 mCi, specific radioactivity 1.3 Ci/mmol); R_f 0.45 (hexane : ethyl acetate = 1 : 1).

^{3}HNMR (acetone-d_6): δ 4.54 (0.15H) , 4.82 (0.85H). ^{1}HNMR (unlabeled **11**, acetone-d_6): δ 1.42 (s, 9H), 1.95-2.05 (4s, 12H), 4.41 (d, J=12.9 Hz, 1H), 4. 65 (m, 1H), 4.67 (d, J=12.9 Hz, 1H), 5.07 (dd, J=4.9, 10.1 Hz, 1H), 5.39 (dd, J=6.7, 10.1 Hz, 1H), 5.55 (d, J=6.7 Hz, 1H), 5.94 (m, 1H). ^{13}CNMR (unlabeled **11**, acetone-d_6): δ 20.60-20.65 (4C), 28.51, 47.05, 63.35, 69.50, 70.15, 71.05, 79.18, 126.49, 134.94, 156.29, 170.11-170.44 (4C).

[7-^{3}H]Valienamine ([7-^{3}H]-**4**). —To a solution of [7-^{3}H]-**11** (1.5 mCi; 10.5 mg, specific radioactivity 60 mCi/mmol) in methanol (0.5 mL) was added NaOMe (50 μL, 25 % in methanol). After stirring at room temperature for 2 hr, the solution was neutralized with 1N HCl and concentrated to dryness under reduced pressure. The residue was taken up in MeOH and ethyl acetate (1 : 1) and filtered to remove salt. The filtrate was concentrated to give tritiated compound **12**, which was dissolved in 70 % TFA (0.6 mL) at 0 °C. After stirring for 2 hr, the solution was diluted with water (2 mL), neutralized with 1N NaOH and applied to a Dowex 50 column (5 mL, H^+). The column was washed with water (20 mL) and eluted with 1 % NH_4OH. The fractions containing [7-^{3}H]-**4** were concentrated to give 1.14 mCi of product (76 % radiochemical yield). ^{3}HNMR (D_2O): δ 4.09 (0.2H), 4.14 (0.8H). ^{1}HNMR (unlabeled **4**, D_2O): δ 3.44 (m, 1H), 3.55-3.65 (m, 2H), 4.02 (d, J=5.14 Hz, 1H), 4.04 (d, J=12.8 Hz, 1H), 4.15 (d, J=12.8 Hz, 1H), 5.74 (m, 1H).

[7-^{3}H]Valienone ([7-^{3}H]-**13**). —To a solution of [7-^{3}H]-**4** (60 μCi; 4.2 mg, specific radioactivity 2.5 mCi/mmol) in methanol (0.5 mL) was added DBQ (5.8 mg, 26.4 μmmol) methanol (0.3 mL) at room temperature. After stirring for 2 hr water (1 mL) was added and the pH lowered to 4 using an aqueous solution of oxalic acid. After the mixture had been stirred for another 2 hr, the pH was adjusted to 6 using Dowex 1 (OH^-). After diluting with water (5 mL), the mixture was washed with ethyl acetate (3 x 5 mL). The aqueous phase was passed through an Amberlite CG-50 weak cation exchange column (5 mL, NH_4^+) to give pure [7-^{3}H]valienone, 35 μCi (60 % radiochemical yield); R_f 0.64 (n-BuOH : EtOH : H_2O = 9 : 7 : 4). ^{1}HNMR (D_2O): δ 3.68 (dd, J=8.6, 11.0 Hz, 1H), 4.16 (d, J=11.0 Hz, 1H), 4.37 (d, J=12.5 Hz, 1H), 4.39 (d, J=12.5 Hz, 1H), 4.55 (d, J=8.6 Hz, 1H), 6.12 (m, 1H). ^{13}CNMR (unlabeled **13**, D_2O): δ 58.82, 70.09, 74.29, 75.21, 118.76, 164.33, 197.77.

9-Bromo-6,7,8-trihydroxy-1-[^{3}H]hydroxymethyl-2-oxa-4-azabicyclo-[3.3.1]nonan-3-one ([7-^{3}H]-**14**). —To a stirred solution of N-Boc-[7-^{3}H]valienamine [7-^{3}H]-**12** (1 mCi; 100 mg, specific radioactivity 2.5 mCi/mmol) in dry methanol (3 mL), bromine (21 µL, 0.41 mmol) in dry methanol (3 mL) was added dropwise at -5 °C. This mixture was stirred at -5 to 0 °C for 90 min and then the volatile compounds were evaporated under reduced pressure to yield a reddish-brownish solid. Recrystallization from a mixture of methanol (1.5 mL), chloroform (20 mL) and hexane (10 mL) gave after centrifugation (10 min, 7000 x g) quantitatively the pure bromide [7-^{3}H]-**14** as a slightly brownish solid; ^{13}CNMR (DMSO-d_6): δ 41.48, 56.21, 63.85, 73.04, 74.24, 75.36, 87.10, carbonyl carbon not detected.

[7-^{3}H]Valiolamine ([7-^{3}H]-**15**). —Sodium borohydride (60 mg, 1.5 mmol) was added to a vigorously stirred solution of [7-^{3}H]-**14** (107 mg, 0.37 mmol) in water (5 mL) at room temperature. After stirring for 2 hr, the pH was adjusted to 6 using glacial acetic acid. Then the solvents were evaporated to furnish a reddish oil which was dissolved in water (4 mL). Barium hydroxide (316 mg, 1.8 mmol) was added and the mixture was stirred at 90 °C for 4 hr. After cooling to room temperature carbon dioxide was bubbled through the reaction mixture for 5 min to form insoluble barium carbonate. Water (10 mL) was then added and the suspension was centrifuged (10 min, 7000 x g). The resulting supernatant was separated and the residue was resuspended in water (10 mL). After another centrifugation the combined supernatant was concentrated to dryness under reduced pressure. The residue was dissolved in water and applied to an Amberlite CG-50 column (8 mL, NH_4^+). Elution with water gave the reduced cyclic carbamate (300 µCi). Subsequently [7-^{3}H]valiolamine was eluted with 0.2 N ammonium hydroxide (300 µCi). Both fractions were further purified by an IRA-68 anion exchange column (9 mL, OH^-) eluting with water. The unreacted cyclic carbamate was again subjected to hydrolysis to give more [7-^{3}H]-**15** (270 µCi); total 570 µCi, 57 % based on [7-^{3}H]-**12**. ^{13}CNMR (D_2O): δ 32.14, 51.13, 65.89, 71.62, 73.04, 74.00, 76.41.

[7-^{3}H]Valiolone ([7-^{3}H]-**16**).—To a stirred solution of [7-^{3}H]valiolamine (90 µCi; 6 mg, specific radioactivity 2.5 mCi/mmol) in methanol (1 mL) was added DBQ (10 mg, 45 µmol) dissolved in methanol (0.3 mL) at room temperature. After stirring for 90 min

water (1 mL) was added and the pH lowered to 5 using an aqueous solution of oxalic acid. After the mixture had been stirred for 1 hr, the pH was adjusted to 8 using ammonium hydroxide. After dilution with water (10 mL) the mixture was washed with chloroform (4 x 5 mL). The aqueous phase was concentrated to approximately 5 mL *in vacuo* and applied to an Amberlite IRA-68 anion exchange column (8 mL, OH^-). Elution with water gave a fraction containing 62 μCi of ^{3}H. This solution was concentrated to 5 mL and further purified by a Diaion WK-100 weak cation exchange column (8 mL, NH_4^+-form) to give pure [7-^{3}H]-**16**, 54 μCi (60 % radiochemical yield); R_f 0.45 (n-BuOH : EtOH : H_2O = 9 : 7 : 4). ^{1}HNMR (D_2O): δ 2.39 (d, J=14.6 Hz, 1H), 2.79 (d, J=14.6 Hz, 1H), 3.39 (d, J=11.5 Hz, 1H), 3.62 (d, J=11.3 Hz, 1H), 3.63 (t, J=9.5 Hz, 1H), 3.84 (d, J=9.4 Hz, 1H), 4.19 (d, J=9.7 Hz, 1H). ^{13}CNMR (unlabeled **16**, D_2O): δ 45.45, 65.37, 72.91, 75.79, 76.01, 78.62, 209.43.

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