Synthesis of (2*S*,3*R*)-3-Amino-2-hydroxy-5-methylhexanoic Acid Derivatives. Application to the Synthesis of Amastatin, an Inhibitor of Aminopeptidases[1]

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Received December 19, 1979

Methods are reported for the synthesis of optically pure derivatives of (2*S*,3*R*)-3-amino-2-hydroxy-5-methylhexanoic acid. [(Benzyloxy)carbonyl]-D-leucine methyl ester was reduced in 95% yield with diisobutylaluminum hydride to the aldehyde which was converted via the cyanohydrin to (2*RS*,3*R*)-3-amino-2-hydroxy-5-methylhexanoic acid (AHMHA). The mixture of diastereomers was converted to the corresponding Boc-AHMHA methyl ester derivatives and separated by chromatography over silica gel. The optical purity of the diastereomers at C-3 was established by converting each to diastereomeric Boc-AHMHA-Leu-OMe and separating these dipeptides by chromatography. Pure (2*S*,3*R*)-Boc-AHMHA was coupled with valyl-valyl-aspartic acid dibenzyl ester by using dicyclohexylcarbodiimide/1-hydroxybenzotriazole in 63% yield. The Boc group was removed by using trifluoroacetic acid, and the benzyl groups were removed by hydrogenolysis. The product, (2*S*,3*R*)-AHMHA-L-Val-L-Val-L-Asp, amastatin, was found to be identical with the natural product.

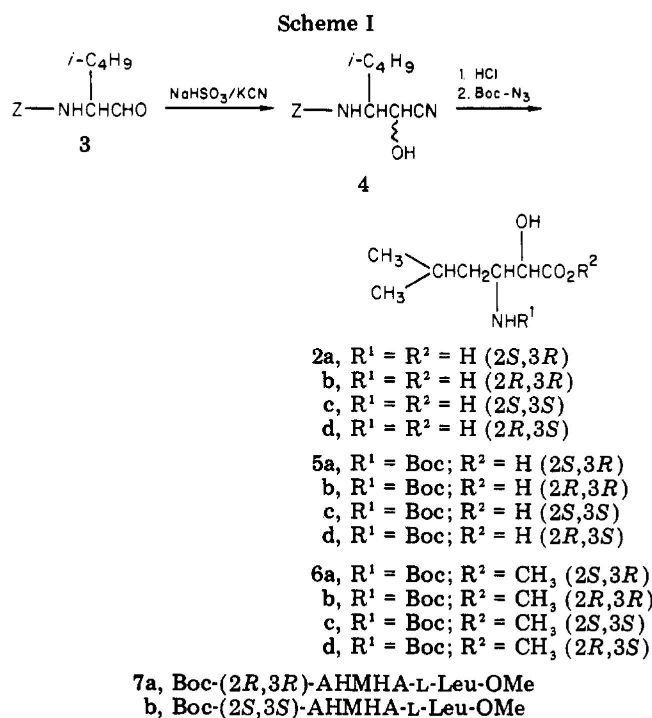
Amastatin, (2*S*,3*R*)-(3-amino-2-hydroxy-5-methylhexanoyl)-L-valyl-L-valyl-L-aspartic acid (**1**), is a low molecular weight inhibitor of leucine aminopeptidase and aminopeptidase A.[2] Amastatin contains the novel amino acid (2*S*,3*R*)-3-amino-2-hydroxy-5-methylhexanoic acid (**2a**), abbreviated AHMHA, which is essential for efficient inhibition of these enzymes. Amino acid **2a** has been synthesized as a mixture of the 2*S*,3*R* and 2*R*,3*R* diastereomers,[3] as have several closely related 2-hydroxy-3-amino acids. However, an efficient method for separating the diastereomers of **2** has not been developed. We report here the synthesis and resolution of derivatives of amino acid **2a** and utilization of optically pure **2a** to synthesize amastatin.

Results and Discussion

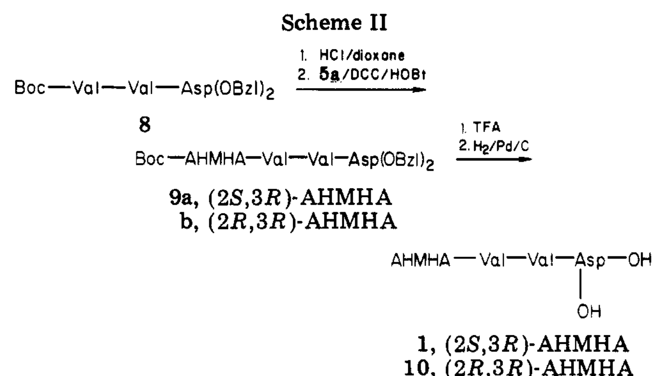
The preparation of 3-amino-2-hydroxy-5-methylhexanoic acid derivatives **2**, **5**, and **6** is outlined in Scheme I. The aldehyde **3**, prepared by reduction of Z-D-leucine methyl ester with diisobutylaluminum hydride in toluene (95% yield) as described for the Boc derivative,[4] was converted to the cyanohydrin **4** by following the method of Nishizawa et al.[3] Hydrolysis of the cyanohydrin gave the hydroxy amino acids **2a,b** as a mixture of diastereomers in 85–90% yield from Z-Leu-OMe. The mixture **2a,b** was converted by reaction with (*tert*-butyloxy)carbonyl azide (Boc-N_3)[5] to the Boc amino acid derivatives **5a,b** (73% yield) which were transformed to the methyl esters in quantitative yield by reaction with diazomethane.

Diastereomers **6a** and **6b** can be separated readily by column chromatography over silica gel.[6] Saponification of ester **6a** gives acid **5a** which, upon treatment with trifluoroacetic acid, is converted to the free acid **2a**. Treatment of optically pure acid **5a** or **5b** with diazomethane gave **6a** or **6b**, respectively, establishing that these

Scheme I



Scheme II



saponification conditions did not racemize **5a** or **5b** at C-2. This sequence of reactions also was carried out by using [(*tert*-butyloxy)carbonyl]-L-leucine methyl ester to prepare the corresponding series of 3*S* diastereomers **2c,d**, **5c,d**, and **6c,d**.

The optical purity of the amino acids at C-3 was established by converting enantiomers **5b** (2*R*,3*R*) and **5c** (2*S*,3*S*) to the corresponding protected diastereomeric

(1) The abbreviations used follow IUPAC-IUB tentative rules as described in *J. Biol. Chem.*, **247**, 977 (1972). Additional abbreviations used are as follows: DCC, dicyclohexylcarbodiimide; DCU, dicyclohexylurea; HOBt, 1-hydroxybenzotriazole; Boc, *tert*-butyloxycarbonyl; AHMHA, 3-amino-2-hydroxy-5-methylhexanoic acid; Z, (benzyloxy)carbonyl.

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Table I. Properties of 3-Amino-2-hydroxy-5-methylhexanoic Acid Derivatives[a]

	^1H NMR, δ						$[\alpha]^{24}_D$, deg (c = 1, MeOH)	mp, °C
	Boc	C-2	C-3	C-4	C-5	C-6,7[b]		
5a	1.45	4.32 (m)	4.03 (m)	1.24 (m)	1.65 (m)	0.91 (d, J = 6), 0.93 (d, J = 6)	+13.81	43-45
5b	1.43	3.87-4.26 (m)[c]		1.17-1.83 (m)[d]		0.96 (d, J = 6)	+50.09	37-39
6a	1.51	4.44 (m)	4.20 (m)	1.59 (m)	1.85 (m)	0.95 (d, J = 8), 0.92 (d, J = 6)	+10.80	84-85
6b	1.45	4.07-4.28 (m)[c]		1.60-1.75 (m)[d]		0.96 (d, J = 6)	+74.60	93-95

[a] The NMR data and melting points for the corresponding 3S derivatives are identical with the 3R derivatives. The optical rotations for the 3S derivatives **5c**, **5d**, **6c**, and **6d** were identical (±5%) in magnitude but opposite in sign to those of the 3R derivatives. [b] J values in hertz. [c] C-2 and C-3 protons in this region are not assigned. [d] C-4 and C-5 protons in this region are not assigned.

dipeptides **7a** (*R,R,S*) and **7b** (*S,S,S*) by reaction with L-leucine methyl ester. Dipeptides **7a** and **7b** were separable by TLC over silica gel (R_f(**7a**) 0.43; R_f(**7b**) 0.51; with 5% methanol in chloroform). Each sample was homogeneous and contained less than 2% of the diastereomer. Thus the sequence of reactions outlined in Scheme I gave less than 2% racemization at C-3 in amino acid derivatives **2**, **5**, and **6**. Physical constants for all compounds are presented in Table I.

The Boc 2*S*,3*R* amino acid **5a** was used to synthesize amastatin (**1**) (Scheme II). Stepwise addition of Boc-L-Val to aspartic acid dibenzyl ester gave the protected tripeptide **8** in 50% overall yield. The (*tert*-butyloxy)carbonyl group was removed with 4 N hydrochloric acid in dioxane, the salt neutralized with triethylamine, and the tripeptide coupled with **5a** by using dicyclohexylcarbodiimide/*N*-hydroxybenzotriazole[7] in methylene chloride to give protected tetrapeptide **9a** in 63% yield after chromatography. Removal of the (*tert*-butyloxy)carbonyl group from **9a** followed by hydrogenation over palladium on carbon gave amastatin (**1**) in 95% yield. When the synthesis of tetrapeptide **9** was carried out by using the diastereomeric mixture of amino acids **5a,b**, both **9a** and the 2*R*,3*R* diastereomer **9b** were obtained in 60 and 25% yields, respectively. These could be separated by column chromatography.[6] Tetrapeptide **9b** was converted by sequential deprotection to epiamastatin (**10**, 95%). Synthetic amastatin inhibits leucine aminopeptidase ($IC_{50} = 4 \times 10^{-7}$ M) at a concentration comparable to that reported for the isolated substance ($IC_{50} = 10.5 \times 10^{-7}$ M).[2] Epiamastatin is a much weaker inhibitor of this enzyme. The kinetics of inhibition of leucine aminopeptidase by these compounds will be reported separately.

Experimental Section

Melting points were determined on a Fisher-Johns melting point apparatus and are corrected. The ^1H NMR spectra were recorded on a Bruker HX-90E pulse Fourier transform NMR spectrometer inferfaced with a Nicolet 1080 computer and disk unit. Optical rotations were measured at the sodium D line by a Perkin-Elmer 241 polarimeter. Microanalyses were performed by Galbraith Laboratories. Thin-layer chromatography (TLC) was performed on silica gel G plates.

[(*tert*-Butyloxy)carbonyl]-L-valyl-L-valyl-L-aspartic Acid Dibenzyl Ester (8). Aspartic acid dibenzyl ester *p*-toluenesulfonate (2.42 g, 5 mmol) was neutralized with triethylamine and coupled with [(*tert*-butyloxy)carbonyl]-L-valine (1.07 g, 5 mmol) by using DCC (1.24 g, 6 mmol) and HOBt (1.15 g, 7.5 mmol) in methylene chloride overnight at 5 °C. The reaction mixture was filtered, and the methylene chloride layer was washed with distilled water, saturated sodium bicarbonate, and 1 N citric acid. The solution was dried ($MgSO_4$) and the solvent evaporated in vacuo to give the protected dipeptide. The protected dipeptide was purified by column chromatography over silica gel, eluting with 10% ethyl acetate in benzene (80% yield). After removal of the (*tert*-butyloxy)carbonyl group from Boc-Val-Asp($OBzl)_2$ with 4 N hydrochloric acid in dioxane (30 min, 25 °C), the salt (1.12 g, 2.5 mmol) was neutralized with triethylamine and coupled with [(*tert*-butyloxy)carbonyl]-L-valine (0.543 g, 2.5 mmol) as described above to give protected tripeptide Boc-Val-Val-Asp($OBzl)_2$: 61% yield; mp 93–94 °C; R_f (5% CH_3OH in $CHCl_3$) 0.63; NMR ($CDCl_3$) δ 0.83–0.97 (4 s, 12 H, CH_3), 1.43 (s, 9 H, $(CH_3)_3C$), 1.67–2.40 (m, 2 H), 2.65–3.25 (AB, 2 H), 3.73–4.02 (m, 1 H), 4.19–4.42 (m, 1 H), 4.76–5.04 (m, 2 H), 5.07 (s, 2 H), 5.12 (s, 2 H), 6.50 (d, J = 10 Hz, 1 H), 6.82 (d, J = 10 Hz, 1 H), 7.26–7.33 (m, 10 H).

Anal. Calcd for $C_{33}H_{45}N_3O_8$: C, 64.79; H, 7.40; N, 6.87. Found: C, 64.65; H, 7.58; N, 6.93.

(2*RS*,3*R*)-3-Amino-2-hydroxy-5-methylhexanoic Acid (2a,b). To a solution of Cbz-D-Leu-OMe (1.37 g, 5 mmol) in toluene (40 mL) was added 6.7 mL of DIBAL (10 mmol), and the reaction mixture was stirred for 6 min at −78 °C under N_2. The reaction was worked up as described for an analogue[4] to yield aldehyde **3**. The aldehyde was dried for 30 min in vacuo and then added to a cold solution of $NaHSO_3$ (4 mmol) as described by Nishizawa et al.[3] The reaction mixture was stirred at 5 °C overnight, ethyl acetate (100 mL) and KCN (0.32 g, 5 mmol in 25 mL of water) were added, and the reaction mixture was stirred at room temperature for 4 h. The organic phase was washed with water, dried ($MgSO_4$), and evaporated to give cyanohydrins **4a,b** as an oil.

Cyanohydrin **4** was dissolved in 40 mL of dioxane–concentrated HCl (1:1) and the solution heated to reflux for 12 h. The hydrolysate was washed with Et_2O and dried in vacuo. The residue was dissolved in water (5 mL) and acetone (50 mL) was added. The solution was adjusted to pH 5.5 with 2 N NaOH and was allowed to stand at 5 °C overnight. The crystals of product **2a,b** (80–90%) were collected by filtration and washed with acetone.

(2*RS*,3*R*)-3-[[(*tert*-Butyloxy)carbonyl]amino]-2-hydroxy-5-methylhexanoic Acid (5a,b). A mixture of amino acid hydrochlorides **2a,b** (0.593 g, 3 mmol) was neutralized with triethylamine and reacted with Boc azide (0.644 g) in H_2O–dioxane (1:1, 4 mL) at 25 °C overnight. The reaction was worked up in the usual fashion[4] to give the Boc amino acids **5a,b** (0.588 g, 73%).

(2*S*,3*R*)-3-[[(*tert*-Butyloxy)carbonyl]amino]-2-hydroxy-5-methylhexanoic Acid Methyl Ester (6a) and (2*R*,3*R*)-3-[[(*tert*-Butyloxy)carbonyl]amino]-2-hydroxy-5-methylhexanoic Acid Methyl Ester (6b). To a solution of Boc amino acids **5a,b** (0.52 g, 2 mmol) at 0 °C was added ethereal diazomethane until the solution was slightly yellow. The solution was allowed to stand at 25 °C for 1 h. The solvent was removed by evaporation (100% yield) and the mixture of esters **6a,b** chromatographed over silica gel, eluting with a gradient of 10–20% ethyl acetate in benzene [R_f(**6a**) 0.22, R_f(**6b**) 0.28]. Fractions containing the Boc amino acid esters **6a** and **6b** were combined and concentrated, and the products were crystallized from ethyl acetate–hexane: **6a**, 60%; **6b**, 25%. Physical constants for **6a** and **6b** are given in Table I.

Anal. Calcd for $C_{13}H_{25}NO_5$: C, 56.71; H, 9.15; N, 5.09. Found: C, 57.06; H, 9.40; N, 5.08.

Saponification of **6a** and **6b** in aqueous dioxane (1:1) made basic with sodium hydroxide (pH 10) gave the optically pure Boc amino acids **5a** and **5b** in quantitative yield. Physical properties of these compounds are given in Table I.

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(2*RS*,3*R*)-[3-[[(*tert*-Butyloxy)carbonyl]amino]-2-hydroxy-5-methylhexanoyl]-L-valyl-L-valyl-aspartic Acid Dibenzyl Ester (9a,b). Boc-Val-Val-Asp(OBzl)$_2$ (8; 0.612 g, 1 mmol) was deprotected by using 4 N hydrochloric acid in dioxane (30 min, 25 °C). The salt (0.548 g, 1 mmol) was neutralized with triethylamine and coupled with Boc acids **5a,b** (0.261 g, 1 mmol) in 5 mL of methylene chloride with DCC (0.248 g, 1.2 mmol) and HOBt (0.23 g, 1.5 mmol) as coupling reagents. The product was isolated as described above for the tripeptide. Separation of diastereomers was achieved by chromatography over silica gel, eluting with 1% methanol in chloroform.

For **9a**: R_f 0.51; NMR ($CDCl_3$) δ 0.83–1.01 (6 s, 18 H, CH_3), 1.44 (s, 9 H, $(CH_3)_3C$), 1.64–1.71 (m, 3 H), 2.03–2.31 (m, 2 H), 2.82–3.26 (AB, 2 H), 3.86–4.01 (m, 1 H), 4.18–4.29 (m, 1 H), 4.30–4.43 (m, 2 H), 4.87–4.96 (m, 1 H), 5.12–5.29 (m, 4 H), 5.35 (d, J = 10 Hz, 1 H), 5.54 (d, J = 10 Hz, 1 H), 6.83 (d, J = 10 Hz, 1 H), 7.06 (d, J = 10 Hz, 1 H), 7.38–7.61 (m, 10 H).

Anal. Calcd for $C_{40}H_{58}N_4O_{10}$: C, 63.64; H, 7.74; N, 7.42. Found: C, 63.57; H, 7.90; N, 7.46.

For **9b**: R_f 0.56; NMR ($CDCl_3$) δ 0.77–1.11 (m, 18 H, 6 CH_3), 1.44 (s, 9 H, $(CH_3)_3C$), 1.58–1.81 (m, 3 H), 2.01–2.21 (m, 1 H), 2.28–2.45 (m, 1 H), 2.84–3.26 (AB, 2 H), 3.69–3.91 (m, 1 H), 4.04–4.33 (m, 2 H), 4.33–4.44 (m, 1 H), 4.96–5.07 (m, 1 H), 5.11–5.30 (m, 4 H), 5.75 (d, J = 4 Hz, 1 H), 6.86 (d, J = 8 Hz, 1 H), 6.76 (d, J = 10 Hz, 1 H), 6.99 (d, J = 10 Hz, 1 H), 7.35–7.58 (m, 10 H).

(2*S*,3*R*)-(3-Amino-2-hydroxy-5-methylhexanoyl)-L-valyl-L-valyl-L-aspartic Acid (1, Amastatin). Tetrapeptide **9a** (0.189 g, 0.25 mmol) was stirred in trifluoroacetic acid at room temperature for 30 min. After removal of the solvent, the residue was dissolved in methanol (15 mL) and hydrogenated (40 psi of hydrogen, 0.05 g of 10% palladium on carbon) for 24 h. The solution was filtered and amastatin (1) isolated by crystallization in 95% overall yield: mp 265–267 °C dec; $[\alpha]^{25}_D$ −40.0° (c 0.44, acetic acid) (lit.[2] $[\alpha]^{22}_D$ −44.3°); R_f 0.46 (n-butyl alcohol–acetic acid–water, 4:1:1) (lit.[2] R_f 0.46); IC_{50} (leucine aminopeptidase) = 4×10^{-7} M (lit.[2] $IC_{50} = 1 \times 10^{-6}$ M).

(2*R*,3*R*)-(3-Amino-2-hydroxy-5-methylheptanoyl)-L-valyl-L-valyl-L-aspartic Acid (10). By use of the procedure described for the synthesis of 1, tetrapeptide **9a** was converted to epiamastatin (**10**) in 95% yield: mp 267–269 °C dec; $[\alpha]^{25}_D$ −26.23° (c 0.44, acetic acid); R_f 0.50 (n-butyl alcohol–acetic acid–water, 4:1:1).

(2*R*,3*R*)-[3-[[(*tert*-Butyloxy)carbonyl]amino]-2-hydroxy-5-methylhexanoyl]-L-leucine Methyl Ester (7a). Boc acid **5b** (0.20 g, 0.82 mmol) was coupled with L-leucine methyl ester hydrochloride (0.137 g, 0.82 mmol) by using DCC (0.202 g, 0.98 mmol) and HOBt (0.187 g, 1.23 mmol) in methylene chloride. The product was isolated in crude form without chromatography as described for peptide **9** and characterized by NMR and TLC data; R_f 0.43 (5% methanol in chloroform). The presence of Boc-(2*S*,3*S*)-AHMHA-L-Leu-OMe compound **7b** was not detected in the crude isolate.

(2*S*,3*S*)-[3-[[(*tert*-Butyloxy)carbonyl]amino]-2-hydroxy-5-methylhexanoyl]-L-leucine Methyl Ester (7b). By use of the procedure developed for the synthesis of dipeptide **7a**, Boc acid **5c** was converted to dipeptide **7b**: R_f 0.51 (5% methanol in chloroform). The presence of diastereomer **7a** was not detected in isolates of dipeptide **7b**.

Acknowledgment. This work was supported in part by a grant to D.H.R. from the National Institutes of Health (AM 20100-01) and a grant from Merck Sharp and Dohme, Inc., to Professor T. O. Goodfriend.

Registry No. 1, 67655-94-1; **2a**, 70853-11-1; **2b**, 70853-17-7; **2c**, 73397-20-3; **2d**, 73397-21-4; **4a**, 73397-23-6; **4b**, 73397-24-7; **5a**, 73397-25-8; **5b**, 73397-26-9; **5c**, 73397-27-0; **5d**, 73397-28-1; **6a**, 73397-29-2; **6b**, 73397-30-5; **6c**, 73397-31-6; **6d**, 73397-32-7; **7a**, 73397-33-8; **7b**, 73465-21-1; **8**, 70853-22-4; **9a**, 73397-34-9; **9b**, 73465-22-2; 10, 73465-23-3; Asp(OBzl)$_2$ p-toluenesulfonate, 2886-33-1; Boc-L-Val, 13734-41-3; Boc-Val-Asp(OBzl)$_2$, 70853-19-9; Cbz-D-Leu-OMe, 73397-22-5; L-Leu-OMe hydrochloride, 7517-19-3; Boc-L-Leu-OMe, 63096-02-6.

Lineatin: Regioselective Synthesis and Resolution Leading to the Chiral Pheromone of *Trypodendron lineatum*[1a]

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Received November 5, 1979

The aggregation pheromone of a major timber beetle pest, *Trypodendron lineatum* (Oliver), has been synthesized. Racemic lineatin (3,3,7-trimethyl-2,9-dioxatricyclo[3.3.1.0^{4,7}]nonane, **1**) has been prepared in 2.8% overall yield via an eight-step synthesis commencing from 5-hydroxy-3,5-dimethyl-3-hexenoic acid lactone (**2**), an easily obtainable condensation product of ketene and mesityl oxide. Dichlorocyclopropanation of **3** followed by low-temperature metalation–methylation gave the chlorobicyclo[4.1.0]heptane derivatives **5a** and **5b**, which upon treatment with potassium *tert*-butoxide in Me$_2$SO gave the expected methylenebicyclo[4.1.0]heptanes **11a** and **11b**. Buffered epoxidation gave the tricyclic oxaspiropentane **12** which was converted to oxabicyclo[4.2.0]octanones **13** and **14**. Borohydride reduction of **13** and **14** gave *endo*-oxabicyclo[4.2.0]octanols **18** and **19**, respectively, which were easily separated by rapid-flow chromatography. Treatment of alcohol **18** with p-toluenesulfonic acid (PTSA) gave lineatin whereas **19** gave 3,3,7-trimethyl-2,9-dioxatricyclo[4.2.1.0^{4,7}]nonane (**20**), originally considered as one of the two probable structures of lineatin. Production of the optical isomers of lineatin was achieved by resolution of the penultimate synthetic intermediate in the sequence. Diastereomeric carbamates derived from bicyclic alcohol **18** and (R)-(−)-1-(1-naphthyl)ethyl isocyanate were prepared and separated by preparative high-pressure LC on silica. LAH reduction of each diastereomer gave the optical isomers of **18** which were cyclized (PTSA) to the enantiomers of lineatin. Congruent arguments based on carbamate chromatographic properties and ^{1}H NMR data as well as optical rotatory power of the chiral centers of lineatin led to the assignment of the 1R,4S,5R,7R configuration to the dextrorotatory enantiomer of lineatin, (+)-**1**, which was attractive in the field.

The ambrosia beetle, *Trypodendron lineatum* (Oliver), is a major timber pest in the northern hemisphere and primarily attacks fallen and sawn timber. Although both host- and beetle-produced volatiles[1b,2] stimulate attack by

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