87829-57-0; **6b**, 87829-58-1; **8a**, 87829-59-2; **8b**, 87829-60-5; *cis*-**9a**, 87829-61-6; *trans*-**9a**, 87829-62-7; *cis*-**9b**, 87829-63-8; *trans*-**9b**, 87829-64-9; **10**, 87829-65-0; **11**, 87829-66-1; **12**, 87829-67-2; **13**, 87829-68-3; **13**·HCl, 87829-69-4; **14**, 87829-70-7; **14**·HCl, 87841-51-8; (L)-**15**, 87829-71-8; **16**, 87829-72-9; (*S*)-**17**, 24325-17-5; (L)-**18**, 84246-49-1; (*S*)-**19**, 87829-73-0; (L)-**21**, 87829-74-1; **22**, 87829-75-2; *p*-nitrobenzaldehyde, 555-16-8; benzoyl chloride, 98-88-4; benzyl chloroformate, 501-53-1; *p*-coumaric acid, 7400-08-0.

Chemistry of Naturally Occurring Polyamines. 8.[1] Total Synthesis of (+)-Hypusine

Colin M. Tice and Bruce Ganem*

Department of Chemistry, Baker Laboratory, Cornell University, Ithaca, New York 14853

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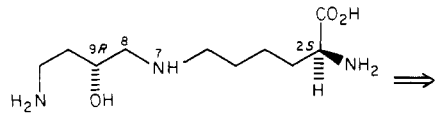
As part of a program to synthesize biologically interesting polyamines and their conjugates, we report a new approach to the synthesis of hypusine (1), an unusual amino acid constituent of the eucaryotic translation initiation factor elF-4D. Hypusine is formally a conjugate between (2*R*)-2-hydroxyputrescine and (2*S*)-lysine, and retrosynthetic analysis suggested that protected lysine **3** might be joined with isoxazolidine (**2**) to form the N7–C8 bond in 1. Chiral isoxazolidines described in this article were prepared by the cycloaddition of chiral nitrones such as **5** with alkenes. Allyl alcohol formed diastereomeric adducts **6** and **7**, which were easily separated by preparative HPLC. Levorotatory **6** could be oxidized to aldehyde **11** and then reductively condensed with lysine derivative **3** to furnish **13**. Exhaustive hydrogenation of the protecting groups in **13** led to (+)-hypusine (1).

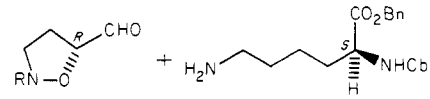
The amino acid hypusine (1) ((2*S*,9*R*)-2,11-diamino-9-hydroxy-7-azaundecanoic acid) is a rare and unusual naturally occurring polyamine, first isolated in 1971 by Nakajima et al. from extracts of bovine brain.[2] Ten years later, Folk et al. discovered radiolabeled hypusine in the protein fraction of human peripheral lymphocytes grown in the presence of ^{3}H-labeled putrescine or spermidine.[3] Structure 1 is formally a conjugate between 2-hydroxyputrescine and lysine, and recent evidence suggests that protein-bound hypusine in these lymphocytes indeed arises by the posttranslational modification of lysine residues. Moreover, deoxyhypusine lacking the C9-hydroxyl has been implicated as a key transitory intermediate.[4]

Hypusine in these lymphocytes occurs predominantly in a single low molecular weight protein. Earlier this year, it was suggested that this peptide serves as an important translation initiation factor, designated elF-4D, in all growing eucaryotic cells.[5] The unusual structure of 1, its atypical biosynthesis, and potential biochemical importance all stimulated our interest in the synthesis of this substance. One synthesis of 1 has been published.[6] Another, based on methods we developed for the selective functionalization of hydroxyputrescine, is described in an accompanying article.[1] Here we report yet a third approach using nitrone dipolar cycloadditions, which has culminated in the efficient, chiral synthesis of dextrorotatory hypusine.

Retrosynthetic analysis of 1 suggested that the N7–C8 bond might be formed by reductive amination of an aldehyde having general structure **2** with a suitably protected form of lysine **3**. Isoxazolidines such as **2** could arise by



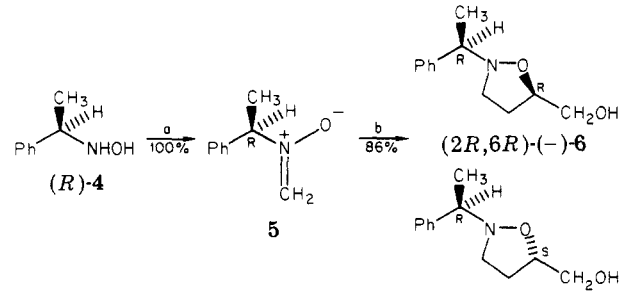
1



2 3

nitrone cycloadditions with alkenes.[7] We envisioned that a chiral R group on the nitrone might induce asymmetry at C9 (hypusine numbering).[8]

Both enantiomers of *N*-(α-methylbenzyl)hydroxylamine (**4**) were readily prepared.[9] Formaldehyde treatment of



(*R*)-4 5 (2*R*,6*R*)-(−)-6

(2*S*,6*R*)-(+)-7

(*R*)-**4** cleanly produced the optically active nitrone **5**,[10] which with allyl alcohol in refluxing benzene gave a 1:1 mixture of cycloadducts.

Diastereomeric alcohols **6** and **7** were the anticipated regioisomers on the basis of literature precedent.[7] Their

(1) For Part 7, see Tice, C. M.; Ganem, B. *J. Org. Chem.*, preceding article in this issue.
(2) Shiba, T.; Mizote, H.; Kaneko, T.; Nakajima, T.; Kakimoto, Y.; Sano, I. *Biochim. Biophys. Acta.* **1971**, *244*, 523.
(3) Park, M. H.; Cooper, H. L.; Folk, J. E. *J. Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 2869.
(4) Park, M. H.; Cooper, H. L.; Folk, J. E. *J. Biol. Chem.* **1982**, *257*, 7217.
(5) Cooper, H. L.; Park, M. H.; Folk, J. E.; Safer, B.; Braverman, R. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 1854.
(6) Shiba, T.; Akiyama, H.; Umeda, I.; Okada, S.; Wakamiya, T. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 899.
(7) Black, D. St. C.; Crozier, R. F.; Davis, V. C. *Synthesis* **1975**, 205.
(8) (a) Belzecki, C.; Panfil, I. *J. Org. Chem.* **1979**, *44*, 1212. (b) Vasella, A.; Voeffray, R. *J. Chem. Soc., Chem. Commun.* **1981**, 97.
(9) Polonski, T.; Chimiak, A. *Tetrahedron Lett.* **1974**, 2453.
(10) Fornefeld, E. J.; Pike, A. J. *J. Org. Chem.* **1979**, *44*, 835.

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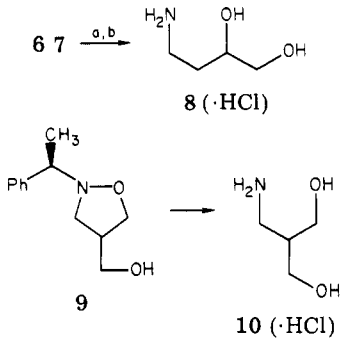
structures were assured by hydrogenolysis of the crude cycloaddition mixture to a single amino diol **8**, exhibiting

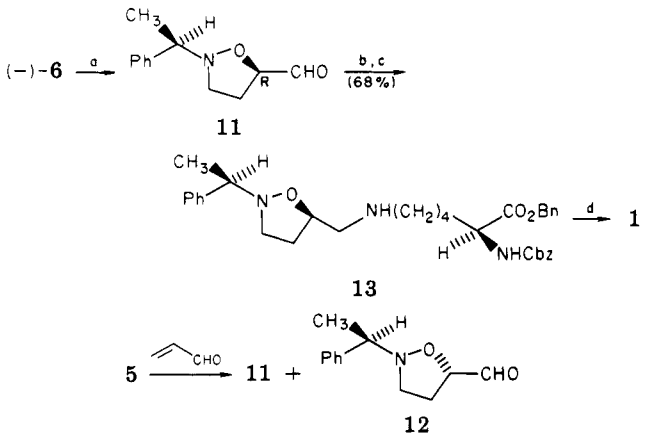


a, HCl, MeOH; b, H_2, 10% Pd-C, H_2O, EtOH

four resonances in its ^{13}C NMR spectrum. Regioisomeric adduct **9** would have produced symmetrical amino diol **10**, expected to display only three resonances in its ^{13}C NMR spectrum.

It was not difficult to obtain substantial quantities of alcohols **6** and **7** since these diastereomers were easily separated by preparative HPLC. Both **6** and **7** were separately submitted to the remaining steps of the synthesis. Conversion to the natural product demonstrated that the less polar diastereomer possessed the 2*R*,6*R* configuration as in (−)-**6**.

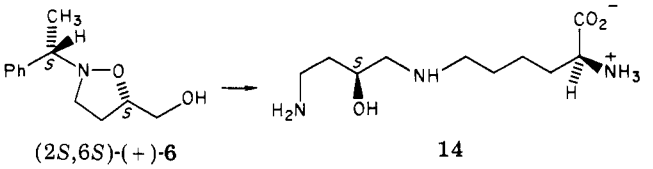
Oxidation of (−)-**6** with PDC[11] and Collins reagent[12] failed. However, Swern oxidation[13] smoothly converted alcohol (−)-**6** to the aldehyde (2*R*,6*R*)-**11** without epimerization. The same aldehyde **11** could be obtained as a



a, $(COCl)_2$, Me_2SO, Et_3N, CH_2Cl_2, −70 °C; b, **3**, 3-Å sieves, C_6H_6, room temperature 1 h; c, $NaBH_4$, MeOH, 5 °C; d, H_2, 10% Pd-C, HCl, H_2O, EtOH

mixture with **12** by the reaction of nitrone **5** with acrolein. In this case, only a modest degree of asymmetric induction was observed.[14] More importantly, it proved much more difficult to separate **11** and **12** (or subsequent synthetic mixtures) chromatographically on a preparative scale.

Reductive amination of **11** with lysine derivative **3**[15] under the conditions described by Borch[16] did not give **13** cleanly. Superior results are obtained in a stepwise process when **11** and **3** were first condensed to form an imine which was subsequently treated with sodium borohydride to form amine **13** in 68% overall yield from alcohol (−)-**6**. Finally, exhaustive hydrogenation of **13** furnished material identical in all respects with natural hypusine (**1**). The unnatural 9-epihypusine (**14**) was synthesized in a similar fashion from (+)-**6**.



(2*S*,6*S*)-(+)-**6** **14**

With synthetic material in hand, it now becomes possible to investigate the biological and pharmacological properties of both **1** and **14**.

Experimental Section

General Section. Dichloromethane, benzene, triethylamine, pyridine, oxalyl chloride, and dimethyl sulfoxide, (Me_2SO) were distilled from CaH_2 prior to use. All reactions were conducted under a nitrogen or argon atmosphere. IR spectra were determined on a Perkin Elmer 681 infrared spectrophotometer. ^1H NMR spectra were recorded on a Varian CFT-20 spectrometer at 80 MHz or on a Bruker WM300 spectrometer at 300 MHz. Chemical shifts are expressed relative to internal tetramethylsilane ($CDCl_3$), to HOD at 4.60 ppm (D_2O), or to Me_2SO at 2.49 ppm (Me_2SO-d_6). ^{13}C NMR spectra were recorded on a JEOL FX90Q spectrometer at 22.49 MHz relative to $CDCl_3$ at 77.0 ppm or to internal dioxane at 67.4 ppm (D_2O). Mass spectra were obtained on a computerized AEI MS902 instrument using isobutane as reagent gas. Thin-layer chromatography was carried out on Merck precoated silica gel 60F-254 plates. Flash chromatography refers to the technique described by Still et al.[17] Analytical high-pressure liquid chromatography (HPLC) was carried out on a Waters 6000A system. Preparative HPLC was performed on a Waters Prep LC/500. Optical rotations were determined on a Perkin Elmer Model 141 polarimeter.

Synthesis of (2*R*,6*R*)-(−)-6 and (2*S*,6*R*)-(+)-7. To a solution of formalin (0.77 mL, 12.3 M, 9.47 mmol) in ethanol (30 mL) was added dropwise over 1 h a solution of (*R*)-(+)-*N*-(α-methylbenzyl)hydroxylamine (**4**) (1.23 g, 9.03 mmol) in 14:1 ethanol:water (30 mL). The mixture was stirred for 3 h, concentrated in vacuo to remove the bulk of the ethanol, and partitioned between CH_2Cl_2 (70 mL) brine (30 mL). The organic layer was dried over $MgSO_4$ and concentrated to leave 1.355 g (102%) of nitrone **5** as an oil: ^1H NMR ($CDCl_3$) δ 1.80 (3 H, d, J = 8.3 Hz), 5.07 (1 H, q, J = 8.3 Hz), 6.36 (1 H, d, J = 6.5 Hz), 6.44 (1 H, d, J = 6.5 Hz), 7.37 (3 H), 7.46 (2 H).

To a stirred solution of nitrone **5** (1.36 g) in benzene (50 mL) was added allyl alcohol (0.80 mL, 11.78 mmol). The mixture was heated at reflux for 3 h, cooled, and concentrated to leave 1.73 g of a yellow oil. Flash chromatography on 30 g of silica gel eluting with 90% ethyl acetate in hexanes furnished 1.61 g (86%) of alcohols **6** and **7** as a 1:1 mixture.

Preparative HPLC of 1.837 g of the **6**/**7** mixture using 9:1 ethyl acetate:hexanes as eluent, with peak shaving and two recycles, afforded 0.554 g of pure **6** and 1.153 g of mixed fractions. Adjustment of the HPLC conditions allowed separation of pure **7**.

Alcohol **6** displayed the following spectral characteristics: ^1H NMR ($CDCl_3$) δ 1.47 (3 H), 2.09 (1 H), 2.32 (1 H), 2.58 (1 H), 2.89 (1 H), 3.60 (1 H), 3.73 (1 H, q), 3.81 (1 H), 4.33 (1 H), 7.30 (5 H); ^{13}C NMR ($CDCl_3$) δ 21.7, 29.7, 52.9, 64.6, 65.9, 77.0, 127.1, 128.2, 142.8; IR (film) 3380 cm^{-1}; CIMS (isobutane) 208 (100, M + 1), 105 (19.65), 104 (13.82); HPLC retention time (μ-Porasil, ethyl acetate, 1.0 mL min^{-1}) 8.4 min; $[\alpha]^{23}_D$ −24° (*c* 1.3, $CHCl_3$).

Alcohol **7** displayed the following spectral characteristics: ^1H NMR ($CDCl_3$) δ 1.35 (3 H), 3.57 (1 H, dd), 3.69 (1 H, q), 7.35 (5 H); ^{13}C NMR ($CDCl_3$) δ 21.8, 30.1, 53.2, 64.9, 66.1, 66.7, 77.0, 127.0, 127.2, 128.3, 142.9; IR (film) 3390 cm^{-1}; CIMS (isobutane) 208

(11) Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979**, 399.
(12) Collins, J. C.; Hess, W. W.; Frank, F. J. *Tetrahedron Lett.* **1968**, 3363.
(13) Mancuso, A. J.; Huang, S.-L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480.
(14) In benzene at room temperature, a 1.5:1 mixture of diastereomers was formed; in CH_2Cl_2 or CH_3CN, the ratio was reversed.
(15) Bezas, B.; Zervas, L. *J. Am. Chem. Soc.* **1961**, *83*, 719.
(16) Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897.
(17) Still, W. C.; Kahn, M. *J. Org. Chem.* **1978**, *43*, 2923.

(100, M + 1), 105 (16.59), 104 (18.14); HPLC retention time (μ-Porasil, ethyl acetate, 1.0 mL min^{-1}) 10.0 min; $[\alpha]^{23}_D$ +84° (*c* 1.57, $CHCl_3$).

Synthesis of (2*S*,6*S*)-(+)-6 and (2*R*,6*S*)-(−)-7. These nitrone adducts were prepared by the above procedure starting with (*S*)-*N*-(α-methylbenzyl)hydroxylamine (**4**) and were identical with (2*R*,6*R*)-(−)-**6** and (2*S*,6*R*)-(+)-**7**, respectively except for their optical rotations. (2*S*,6*S*)-(+)-**6**: $[\alpha]^{23}_D$ +20° (*c* 1.25, $CHCl_3$). (2*R*,6*S*)-(+)-**7**: $[\alpha]^{23}_D$ +94° (*c* 0.80, $CHCl_3$).

Synthesis of 8. A solution of the hydrochloride salts of **6** and **7** (0.375 g, 1.54 mmol) in 3:1 ethanol:water (7 mL) was stirred with 10% palladium on carbon (0.340 g) under 2.7 atm of hydrogen for 4 days. The product was isolated (incomplete reaction) and resubmitted to similar conditions for a further 2 days. The mixture was filtered through Celite and concentrated to give 0.206 g (94%) of **8** as a colorless oil: ^{1}H NMR (D_2O) δ 1.5–1.75 (2 H), 2.97 (2 H, m), 3.37 (2 H, m), 3.63 (1 H), m); ^{13}C NMR (D_2O) δ 30.2, 37.6, 65.6, 70.1. Hydrochloride **8** was converted to the free amine using Dowex-1 (OH^- form): IR (film) 3300 cm^{-1}; CIMS (isobutane) 106 (M + 1, 100).

Synthesis of 11. A solution of oxalyl chloride (0.23 mL, 2.60 mmol) in CH_2Cl_2 (8 mL) was cooled to −70 °C and a solution of Me_2SO (0.38 mL, 5.37 mmol) in CH_2Cl_2 (2 mL) was added dropwise. After stirring for 10 min, a solution of alcohol (2*R*,6*R*)-(−)-**6** (0.287 g, 1.34 mmol) in CH_2Cl_2 (2.5 mL) was added dropwise. The mixture was stirred for 20 min at −70 °C and triethylamine (1 mL, 7.2 mmol) was added. After the solution stirred for 30 min at −70 °C, the cooling bath was removed and stirring continued for 20 min. The mixture was poured into water (50 mL) and extracted with ether (2 × 40 mL). The combined ether extracts were dried over $MgSO_4$ and concentrated to leave 0.325 g of the sensitive aldehyde **11** as a yellow oil. No attempt was made to purify **11**: the ^{1}H NMR spectrum of **11** was broad and unresolved; ^{13}C NMR ($CDCl_3$) δ 21.9, 30.8, 52.3, 66.9, 79.9, 127.1, 127.4, 128.4, 142.5; IR (film): 1735 cm^{-1}; CIMS (isobutane) 206 (M + 1, 100); R_f (ethyl acetate) 0.48.

Synthesis of 13. A mixture of crude aldehyde **11** (0.326 g, 1.34 mmol) and of amine **3** (0.567 g, 1.53 mmol) in benzene (12 mL) was stirred at room temperature with powdered 3-Å molecular sieves for 1 h. The mixture was filtered and concentrated to leave 0.922 g of crude imine as an oil: IR (film) 3420, 3340, 1730, 1675 cm^{-1}.

A solution of the crude imine in CH_3OH (10 mL) was cooled to 5 °C and solid sodium borohydride (0.56 g, 1.48 mmol) was added. The mixture was stirred for 25 min at 5 °C, poured into 3% aqueous HCl (60 mL), washed with two 40-mL portions of ether, made basic by addition of solid Na_2CO_3, and extracted with three 40-mL portions of CH_2Cl_2. The combined CH_2Cl_2 extracts were dried over $MgSO_4$ and the solvent was removed to leave 0.659 g of a yellow oil. Flash chromatography on 20 g of silica gel eluting with 47:2:1 CH_2Cl_2:MeOH:NH_4OH (lower layer used) afforded 0.516 g of **13** (68% from **6**) and 0.116 g of recovered amine **3**.

The reductive amination product **13** displayed the following spectral data: ^{1}H NMR (Me_2SO-d_6, 380 K) δ 1.33 (3 H, d, *J* = 6.5 Hz), 3.72 (1 H, q, *J* = 6.5 Hz), 4.10 (2 H, m), 5.05 (2 H, s), 5.13 (2 H, s), 7.33 (5 H, s); IR (film) 3330, 1745 (shoulder), 1725 cm^{-1}; CIMS (isobutane 421 (40.48), 371 (24.33, 188 (13.01, 122 (12.46), 108 (12.73), 105 (38.66), 91 (100); HPLC retention time (μ-Bondapak NH_2, acetonitrile, 1.5 mL min^{-1}) 17.0 min; $[\alpha^{23}_D$ 6.0° (*c* 3.41, $CHCl_3$).

Synthesis of Hypusine (1) Dihydrochloride. A solution of amine (2*S*,9*R*,13*R*)-**13** (0.067 g, 0.12 mmol) in 9:2:1 ethanol:H_2O:1.0 M HCl (3.0 mL) was stirred with 10% palladium-on-carbon (0.052 g) under 2.7 atm of hydrogen. After 72 h, the catalyst was removed by filtration and replaced with fresh catalyst (0.048 g) and the hydrogenation continued. After a further 72 h, the used palladium was again replaced with fresh catalyst (0.042 g) and hydrogenation was continued for another 110 h. The mixture was then filtered through Celite and concentrated to an oily solid (0.043 g). This crude sample of **1** was taken up in 1:1 H_2O:CH_3OH (10 mL) and the pH was adjusted to 5.2 with dilute HCl. The solvent was removed, the residue taken up in 9:1 CH_3OH:H_2O (4 mL), and ether (2.5 mL) was added. After storage in the refrigerator for several hours, white crystals appeared. These were collected and again recrystallized to afford 0.017 g (46%) of hypusine (**1**) as its dihydrochloride: mp 239–241 °C dec (lit.[6] mp 234–236 °C); ^{1}H NMR (D_2O) δ 1.27 (2 H, m), 1.65 (6 H, m), 2.95 (6 H, m), 3.56 (1 H, 6, *J* = 6.1), 3.86 (1 H, m); IR (HBr) 3400, 3040, 1610 cm^{-1}; $[\alpha]^{23}_D$ +8.3° (*c* 0.96, 6 M HCl) [lit.[6] $[\alpha^{23}_D$ +6.8°, +9.9° (*c* 0.12, 6 M HCl)].

Synthesis of 9-Epihypusine (14). The unnatural epimer of hypusine was prepared from (*S*)-(−)-*N*-(α-methylbenzyl)-hydroxylamine (**4**) via (2*S*,9*S*,13*S*)-(−)-**13** by procedures identical with those described for the synthesis of hypusine (**1**). Levorotatory **13** displayed the following spectral data: ^{1}H NMR (Me_2SO-d_6, 380 K) δ 1.33 (3 H, d, *J* = 6.5 Hz), 3.72 (1 H, q, *J* = 6.5 Hz), 4.10 (2 H, m), 5.05 (2 H, s), 5.13 (2 H, s), 7.33 (5 H, s); IR (film) 3330, 1745 (shoulder), 1725 cm^{-1}; CIMS (isobutane) 560 (71, M + 1), 421 (91), 371 (68), 105 (100), 91 (95); HPLC retentiontime (μBondapak-NH_2, CH_3CN, 1.5 mL min^{-1}) 19 min; $[\alpha]^{23}_D$ −1.9° (*c* 4.58, $CHCl_3$).

Amino acid **14** was indistinguishable from hypusine (**1**) either by ^{1}H NMR spectroscopy at 300 MHz or by TLC. Its IR spectrum and specific rotation were in good agreement with published values: found, $[\alpha]^{23}_D$ +15° (*c* 0.23, 6 M HCl); reported[6] $[\alpha]^{23}_D$ +16° (*c* 0.10, 6 M HCl).

Acknowledgment. We thank the National Institutes of Health (Grant AM-26754) for financial assistance. Support of the Cornell Nuclear Magnetic Resonance Facility by the National Science Foundation (Grants CHE 7904825 and PCM 8018643) is gratefully acknowledged.

Registry No. **1**, 34994-11-1; **1**·2HCl, 82310-93-8; **3**, 5591-94-6; (*R*)-**4**, 67377-55-3; (*S*)-**4**, 53933-47-4; (*R*)-**5**, 87681-42-3; (2*R*,6*R*)-**6**, 87681-43-4; (2*S*,6*S*)-**6**, 87681-44-5; (2*S*,6*R*)-**7**, 87681-45-6; (2*R*,6*S*)-**7**, 87681-46-7; **8**, 83430-32-4; **8**·HCl, 87681-47-8; **11**, 87681-48-9; (2*S*,9*R*,13*R*)-**13**, 87681-49-0; (2*S*,9*S*,13*S*)-**13**, 87727-38-6; **14**, 87681-50-3; formalin, 50-00-0; allyl alcohol, 107-18-6; (2*S*,9*R*,13*R*)-**13** imine, 87681-51-4.