times with the 1:2 mixture of 0.5 M K_2CO_3 and 0.5 M $KHCO_3$ solutions and finally with water. After the crude material was dried, it was boiled in 50 mL of ethanol, then cooled down, filtered off, and washed on the filter several times with ether to give 4.6 g (66%) of VII, mp 217–219 °C (lit.[11] mp 217–218 °C).

Z-Lys(For)-ONP (VIII). To the solution of 1.54 g (5 mmol) of Z-Lys(For)-OH[12] in 7 mL of absolute CH_2Cl_2 were added 0.69 g (5 mmol) of *p*-nitrophenol and 1.03 g (5 mmol) of dicyclohexylcarbodiimide with stirring and ice cooling. Stirring was continued for 2 h at 0 °C, and then the reaction mixture was left overnight at room temperature. The precipitated dicyclohexylurea was filtered off, and the solution was evaporated in vacuo. The residue was crystallized from acetone–petroleum ether to yield 1.5 g (70%) of VIII: mp 115–116 °C; $[\alpha]^{25}_D$ −30.12° (*c* 1, methanol). Anal. ($C_{21}H_{23}N_3O_7$) C, H, N, O.

Z-Pro-Lys(For)-Pro-Val-NH$_2$ (IX). The title compound was prepared from H-Lys(For)-Pro-Val-NH_2·HCl[12] and Z-Pro-OH by the same procedure as described for compound I. Purification was carried out on a silica gel column in solvent system A to give IX in 64% yield: R_f 0.33 (A), 0.76 (B); $[\alpha]^{25}_D$ −107.06° (*c* 1, methanol). Anal. ($C_{30}H_{44}N_6O_7$) C, H, N.

H-Pro-Lys(For)-Pro-Val-NH$_2$·HCl (X). Compound IX (1.2 g; 2 mmol) was suspended in 15 mL of methanol containing 10% formic acid and hydrogenolyzed in the presence of Pd/C catalyst for about 1 h. After filtration, the solution was evaporated in vacuo, the residue was dissolved in 10 mL of methanol, 2 mL of 1 N hydrochloric acid was added, and the solution was evaporated again. The residue was dissolved in water and lyophilized to give 1 g (94%) of X: R_f 0.15 (C), 0.45 (B); $[\alpha]^{25}_D$ −102.25° (*c* 1, methanol). Anal. ($C_{22}H_{39}N_6O_5Cl$) C, H, N, Cl.

Q(NO)-Lys(For)-Pro-Val-NH$_2$ (XI). To the solution of 1.1 g (2.7 mmol) of H-Lys(For)-Pro-Val-NH_2·HCl in 10 mL of absolute DMF were added 0.75 mL (5.4 mmol) of TEA and 675 mg (2.7 mmol) of *N*-(2-chloroethyl)-*N*-nitrosocarbamic acid succinimido ester with ice cooling and stirring. After 1 h, the reaction mixture was concentrated in vacuo. The residue was dissolved in cold ethyl acetate and washed three times with water. Sodium chloride was added to the combined water solution, which was extracted three times with ethyl acetate. The combined ethyl acetate solution was dried over Na_2SO_4, filtered, and evaporated in vacuo. The residue was chromatographed on a silica gel column in solvent system F to give 800 mg (59%) of XI: R_f 0.52 (F), 0.60 (A); $\epsilon_{400nm}{}^{max}$ 92. Anal. Calcd for $C_{20}H_{34}N_7O_6Cl$ (504.00): N, 19.45; Cl, 7.03. Found: N, 18.89; Cl, 6.73.

Q(NO)-Pro-Lys(For)-Pro-Val-NH$_2$ (XII). The compound was prepared from X by the same procedure as that used for XI. Purification was carried out on a silica gel column in solvent system G to give compound XII in 60% yield: R_f 0.78 (G), 0.28 (F); $\epsilon_{395nm}{}^{max}$ 86.6. Anal. Calcd for $C_{25}H_{41}N_8O_7Cl$ (601.12): N, 18.64; Cl, 5.89. Found: N, 18.10; Cl, 5.63.

Q(NO)-Pro-NH$_2$ (XIII). The title compound was prepared from H-Pro-NH_2 by the same procedure as that used for III. Column chromatography on silica gel was performed in solvent system E: yield, 70%; R_f 0.8 (E); $\epsilon_{395nm}{}^{max}$ 87; mp 94–95 °C (dec). Anal. ($C_8H_{13}N_4O_3Cl$) C, H, N, Cl.

Q(NO)-Pro-Val-NH$_2$ (XIV). H-Pro-Val-NH_2·HCl (1.25 g; 5 mmol) was suspended in 15 mL of absolute DMF; 1.4 mL (10 mmol) of TEA and 1.25 g (5 mmol) of *N*-(2-chloroethyl)-*N*-nitrosocarbamic acid succinimido ester were added to it with stirring and ice cooling. After 1 h, the reaction mixture was diluted with ice-cold water and the precipitate was filtered off and washed well on the filter with water. The product was dried over P_2O_5 to give 0.91 g (53%) of XIV: R_f 0.8 (G); mp 130 °C (dec); $\epsilon_{395nm}{}^{max}$ 87. Anal. ($C_{13}H_{22}N_5O_4Cl$) N, Cl.

Measurements on L1210 Leukemia in Mice. Six to ten week old C57B1 × DBA_2 F_1 hybrid mice of either sex, bred in our institute, were inoculated intraperitoneally (ip) with 10^6 cells of L1210 leukemia. The L1210 was maintained by weekly transplantation in DBA_2 mice. Compounds in the amount equivalent to the dose related to kilograms of body weight were dissolved in 0.2 mL of dimethyl sulfoxide and diluted to 10 mL with 0.9% NaCl. Treatment consisted of a single ip injection 1 day after transplantation in a volume of 0.1 mL/10 g of body weight. The percentage increase in life span (% ILS) of treated animals was compared with the untreated tumor bearer and was calculated as follows: % ILS = [(T − C)/C] × 100 (T and C = median survival in days for treated and control groups, respectively). The experiments were repeated three times.

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Registry No. I, 106039-75-2; II, 106039-76-3; III, 106039-77-4; IV, 106039-78-5; V, 106039-79-6; VI, 106039-80-9; VII, 106039-81-0; VIII, 14373-54-7; IX, 106039-82-1; X, 106039-83-2; XI, 106039-84-3; XII, 106039-85-4; XIII, 81965-44-8; XIV, 97209-67-1; BOC-Pro-OH, 15761-39-4; H-Lys(BOC)-Pro-Val-NH_2·AcOH, 92603-22-0; H-Pro-Lys-Pro-Val-NH_2·2CF_3COOH, 106039-87-6; Z-Pro-OH, 1148-11-4; H-Lys(BOC)-Pro-Val-NH_2, 53267-79-1; H-Pro-Val-NH_2·HCl, 51165-72-1; Z-Lys(For)-OH, 20807-05-0; H-Lys(For)-Pro-Val-NH_2·HCl, 106039-88-7; H-Pro-NH_2, 7531-52-4; Q(NO)-Lys(QNO)-Pro-Val-NH_2, 87230-62-4; Q(NO)-Lys-Pro-Val-NH_2·HCl, 87230-64-6; *N*-(2-chloroethyl)-*N*-nitrosocarbamic acid succinimido ester, 80354-49-0.

Antitumor Agents. 86.[1,2] Synthesis and Cytotoxicity of α-Methylene-γ-lactone-Bearing Purines

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α-Methylene-γ-lactones covalently linked to adenine (**3**), hypoxanthine (**4**), and guanine (**5**) were synthesized by using the convenient Reformatsky-type reaction between ethyl α-(bromomethyl)acrylate and the proper purine ketones. In vitro cytotoxicity data demonstrated that these compounds were active against L-1210 tissue culture cells with **3** being most potent (ED_{50} = 0.3 μg/mL).

Alkylating agents have played a major role in the development of antitumor agents.[3] One member of this class of chemotherapeutic agents is α-methylene-γ-lactone-containing sesquiterpenes, such as the cytotoxic antitumor helenalin.[4-6] It has been demonstrated that the O=C—C=CH_2 system which is present in helenalin may act as an alkylating center through a Michael-like reaction with

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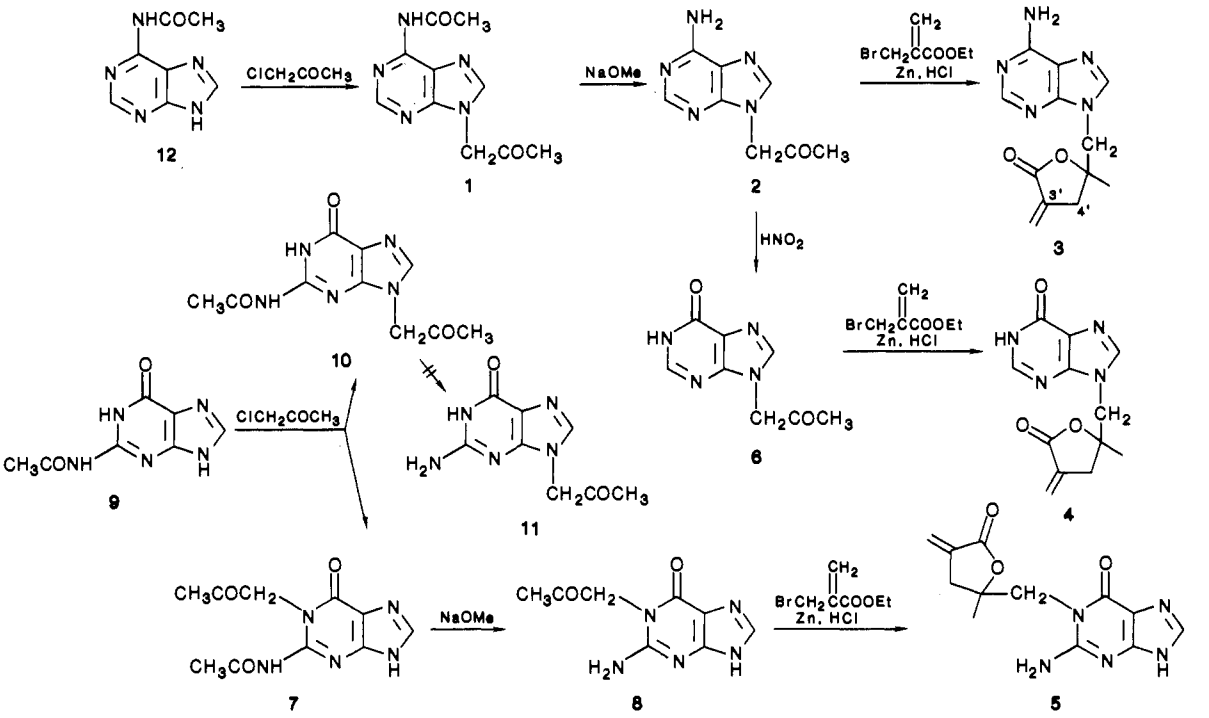
Scheme I



biological nucleophiles.[7-9] We have previously reported the synthesis of compounds derived from the combination of an above active alkylating center and a carrier moiety, such as a steroidal hormone[10] and a pyrimidine.[11] The significant antitumor activity demonstrated by uracil and thymine α-methylene-γ-lactones and related derivatives[11] encouraged us to further synthesize and evaluate α-methylene-γ-lactone-bearing purines.[12]

Chemistry

The use of a Reformatsky reaction with ethyl α-(bromomethyl)acrylate to form α-methylene-γ-lactone is similar to our previous work on preparing derivatives of the reactive lactone.[10,11] The remaining task was to prepare purine derivatives (**2**, **6**, **8**, and **11**) containing an acetone moiety. N^6-Acetyladenine (**12**) was alkylated with chloroacetone to give **1** (Scheme I), which was deprotected to 9-(2-oxopropyl)adenine (**2**). The amino group in **2** was hydrolyzed with nitrous acid to yield **6**. When N^2-acetylguanine (**9**) was treated with chloroacetone, two alkylated products were obtained, which were separated by crystallization. The chemical shifts for the 8-protons of the isomers were 8.05 and 7.85 ppm. Structure **10** with 9-substitution was assigned to the isomer that exhibited a downfield shift. N^2-Acetyl-1-(2-oxopropyl)guanine (**7**) was deprotected with sodium methoxide at room temperature to **8**. However, hydrolysis of the N^2-acetyl in **10** needed more drastic conditions. Hydrolysis in acidic or basic solution was always accompanied by extensive degradation of **11**. Treatment of **2**, **6** and **8** with ethyl α-(bromomethyl)acrylate in the presence of activated zinc yielded the target compounds **3**–**5**, respectively.

Table I. Cytotoxicity of **3**–**5** (N = 8)[a]

compd	ED_{50}, μg/mL		
	KB	P-388	L-1210
3	5.0	>20	0.3
4	>10	>20	2.0
5	>10	>20	1.3
etoposide		1.71	1.58

[a] For significant activity, an ED_{50} value ≤4 μg/mL is required.

Biological Results and Discussion

The α-methylene-γ-lactone-bearing purines were selective for their tumor cytotoxicity (Table I). Compounds **3**–**5** demonstrated ED_{50} values greater than 20 μg/mL in the murine P-388 lymphocytic leukemia screens, indicating they were essentially inactive. In the KB human carcinoma of the nasopharynx screen, compound **3** afforded an ED_{50} value of 5 μg/mL, which demonstrated marginal activity, whereas **4** and **5** had greater than 10 μg/mL ED_{50} values. The compounds demonstrated the most potent activity against the growth of murine L-1210 lymphoid leukemia. Compound **3** afforded the best activity with an ED_{50} value of 0.3 μg/mL. Compound **5** gave an ED_{50} value of 1.3 μg/mL followed by **4**, which afforded a value of 2.0 μg/mL. Significant activity in these screens requires an ED_{50} value of less than 4 μg/mL.

The α-methylene-γ-lactone-bearing purines as a chemical class demonstrated selectivity for the growth of L-1210 lymphoid leukemia. The adenine (**3**) and guanine (**5**) derivatives may be more active in the biological screen becuase they are taken up more rapidly by the cells by a carrier-mediated process since they are normal endogenous

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metabolic precursors to nucleic acid synthesis.

Experimental Section

A. Synthetic Methods. Melting points were determined on a Fisher-Jones apparatus and are uncorrected. NMR spectra in Me_2SO-d_6 were run on a JEOL JNM-FX60 spectrometer, while UV maxima were measured on a Cary 2200 spectrophotometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. All starting materials were dried in vacuo at 110 °C in the presence of phosphorus pentoxide for 18 h. Tetrahydrofuran for Reformatsky reactions was distilled from lithium aluminum hydride just before use.

N^6-Acetyl-9-(2-oxopropyl)adenine (1). N^6-Acetyladenine (**12**) (5.32 g, 30 mmol) and anhydrous potassium carbonate (4.14 g, 30 mmol; well-powdered) were taken in dry DMF (150 mL), and a solution of chloroacetone (5.1 mL, 60 mL) in dry DMF (25 mL) was added with stirring over a period of 1 h. The stirring was continued after the completion of addition for 8 h, and the reaction mixture was filtered through a bed of Celite and washed with DMF (30 mL). The filtrate was evaporated, and the residue was coevaporated with xylene (2 × 25 mL) and ethanol (2 × 25 mL). The resulting solid was crystallized from methanol to yield **1** (4.2 g; 60%): mp 200–201 °C; ^{1}H NMR δ 2.29 (s, 6 H, 2 CH_3CO), 5.33 (s, 2 H, CH_2), 8.33 (s, 1 H, H-8), 8.63 (s, 1 H, H-2), 10.72 (s, 1 H, NH). Anal. ($C_{10}H_{11}N_5O_2$) C, H, N.

9-(2-Oxopropyl)adenine (2). Compound **1** (1.87 g, 8 mmol) was added to a solution of sodium (230 mg, 10 mmol) in absolute methanol (50 mL) with stirring. The resulting solution was stirred when a solid was slowly generated. After 1 h the suspension was cooled in ice and filtered. The solid was washed with cold methanol and dried. The filtrate was neutralized with 2 N acetic acid, concentrated to 5 mL, diluted with water (20 mL), and extracted with chloroform (6 × 30 mL). The extracts were combined, dried, and concentrated to 15 mL. Addition of hexane (100 mL) and subsequent cooling afforded more solid. The total yield of **2** was 1.45 g (94%): mp 247–248 °C; ^{1}H NMR δ 2.22 (s, 3 H, CH_3), 5.15 (s, 2 H, CH_2), 7.98 (s, 1 H, H-8), 8.09 (s, 1 H, H-2). Anal. ($C_8H_9N_5O$) C, H, N.

9-[(2-Methyl-4-methylene-5-oxotetrahydrofuran-2-yl)-methyl]adenine (3). 9-(2-Oxopropyl)adenine (**2**) (573 mg, 3 mmol), activated zinc dust (255 mg), and hydroquinone (6 mg) were stirred in THF (60 mL), and ethyl α-(bromomethyl)acrylate (0.9 mL) in THF (20 mL) was added slowly with vigorous stirring for a period of 1 h. The reaction mixture was heated to a vigorous reflux for 50 min when the gray suspension became clear. The reaction mixture was cooled before being poured slowly over ice-cold 5% hydrochloric acid (300 mL). The acidic solution was stirred in ice, neutralized with solid sodium bicarbonate, and extracted with chloroform (5 × 150 mL). The organic layers were combined, dried, concentrated to 25 mL, and slowly mixed with hexane (150 mL). The precipitated white solid was filtered and washed with hexane to give **3**: 520 mg (67%); mp 234–235 °C (methanol); ^{1}H NMR δ 1.41 (s, 3 H, CH_3), 2.96 (m, 2 H, 4′-CH_2), 4.42 (s, 2 H, NCH_2), 5.50 and 5.81 (t, 1 H each, J = 2.6 Hz, =CH_2), 7.93 (s, 1 H, H-8), 8.07 (s, 1 H, H-2). Anal. ($C_{12}H_{13}N_5O_2$·0.4H_2O) C, H, N.

9-(2-Oxopropyl)hypoxanthine (6). To **2** (573 mg, 3 mmol) in water (72 mL) were added sodium nitrite (1.8 g) and acetic acid (2.4 mL), and the mixture was stirred for 12 h at room temperature. The solution was neutralized and evaporated. The residue was coevaporated with ethanol (2 × 30 mL), stirred with methanol (50 mL), filtered, and washed with the same solvent (2 × 15 mL). The filtrate was concentrated to 5 mL and purified on a column of silica gel with 8:2 chloroform–methanol to yield **6**: 346 mg (60%); mp 282–284 °C; ^{1}H NMR δ 2.21 (s, 3 H, CH_3), 5.16 (s, 2 H, CH_2), 7.91 (s, 1 H, H-8), 7.98 (s, 1 H, H-2). Anal. ($C_8H_8N_4O_2$) C, H, N.

9-[(2-Methyl-4-methylene-5-oxotetrahydrofuran-2-yl)-methyl]hypoxanthine (4). 9-(2-Oxopropyl)hypoxanthine (**6**) (273 mg, 1.5 mg), activated zinc dust (130 mg), and hydroquinone (3 mg) were taken in dry THF (50 mL) and ethyl α-bromomethyl)acrylate (0.45 mL) in dry THF (15 mL) was added with stirring over 45 min. The suspension was heated to reflux for 2 h, cooled, and poured into 75 mL of ice-cold 1 N acetic acid with stirring. The resulting solution was neutralized with saturated sodium bicarbonate solution and evaporated (bath temperature 30 °C). The residue was extracted with methanol (50 mL) and concentrated to ca. 5 mL for column chromatography (silica gel in 9:1 chloroform–methanol). The fractions containing the product were combined, evaporated, and tritiated with chloroform (25 mL) to give 145 mg (37%) of **4**: mp 268–270 °C; ^{1}H NMR δ 1.42 (s, 3 H, CH_3), 2.97 (m, 2 H, 4′-CH_2), 4.43 (s, 2 H, NCH_2), 5.51 and 5.82 (t, 1 H each, J = 2.3 Hz, =CH_2), 7.94 and 8.08 (s, 7 H each, H-8 and H-2). Anal. ($C_{12}H_{12}N_4O_3$) C, H, N.

N^2-Acetyl-9-(2-oxopropyl)guanine (10) and N^2-Acetyl-1-(2-oxopropyl)guanine (7). N^2-Acetylguanine (**9**) (3.86 g, 20 mL) and anhydrous potassium carbonate (2.78 g, 20 mmol; well-powdered) were taken in dry DMF (400 mL), and the mixture was warmed to 60 °C with stirring. After 30 min the heating was terminated and a solution of chloroacetone (3.4 mL, 40 mmol) in DMF (40 mL) was added in drops (1 h). The stirring was continued for an additional 1 h, and the inorganic salts were filtered off and washed with DMF (2 × 40 mL). The filtrate was evaporated and the residue was coevaporated with xylene (2 × 25 mL) and ethanol (2 × 25 mL). The solid was stirred in boiling ethanol (250 mL) and filtered. The solid was the pure 9-alkylated isomer (**10**): 1.5 g (30%); UV λ_{max} (ϵ) 263 nm (16 950) at pH 1 and 268 nm (11 340) at pH 13; ^{1}H NMR δ 2.17 and 2.23 (s, 3 H each, 2 CH_3CO), 5.29 (s, 2 H, CH_2), 8.05 (s, 1 H, H-8). Anal. ($C_{10}H_{11}N_5O_3$) C, H, N.

The filtrate was cooled to obtain the 1-alkylated isomer **7**: 2 g (40%); UV λ_{max} (ϵ) 262 nm (16 300) at pH 1 and 263 nm (11 340) at pH 13; ^{1}H NMR δ 2.17 and 2.23 (s, 3 H each, 2 CH_3CO), 5.10 (s, 2 H, CH_2), 7.85 (s, 1 H, H-8). Anal. ($C_{10}H_{11}N_5O_3$) C, H, N.

1-(2-Oxopropyl)guanine (8). A solution of sodium (149.5 mg, 6.5 mmol) in methanol (130 mL) containing **7** (1.44 g, 5.88 mmol) was stirred at room temperature for 2.5 h, neutralized with 2 N acetic acid, and cooled with ice. The precipitated solid was filtered, washed with cold methanol, and dried to yield **8**: 900 mg (74%); mp 300 °C; ^{1}H NMR δ 2.18 (s, 3 H, CH_3), 4.94 (s, 2 H, CH_2), 6.44 (s, 2 H, NH_2), 7.56 (s, 1 H, H-8). Anal. ($C_8H_9N_5O_2$) C, H, N.

1-[(2-Methyl-5-methylene-5-oxotetrahydrofuran-2-yl)-methyl]guanine (5). To a stirred suspension containing **8** (208 mg, 1 mmol), activated zinc dust (85 mg), and hydroquinone (2 mg) in dry THF (50 mL) was added ethyl α-(bromomethyl)-acrylate (300 μL) in THF (15 mL) dropwise over 45 min. The suspension was heated to reflux for 24 h. The workup was similar to that of **4**. The crude product was purified on a column of silica gel using 7:3 chloroform–methanol for elution to afford 69 mg (25%) of a white solid (**5**): mp >280 °C; ^{1}H NMR δ 1.41 (s, 3 H, CH_3), 2.95 (m, 2 H, 4′-CH_2), 4.21 (s, 2 H, NCH_2), 5.54 and 5.84 (t, 1 H each, J = 2.5 Hz, =CH_2), 6.62 (s, 2 H, NH_2), 7.52 (s, 1 H, H-8). Anal. ($C_{12}H_{13}N_5O_3$) C, H, N.

Biological Screens. The cytotoxic screening of the α-methylene-γ-lactone-bearing purines was conducted according to the NIH protocol.[14] Drugs were tested from 0.1 to 20 μg/mL (N = 8) concentration and were incubated with 10^4 tumor cells grown in MEM + 10% fetal calf serum with penicillin and streptomycin on day 3; the number of viable cells as determined by the trypan blue exclusion method was determined for each concentration of drug and expressed as a percentage of those viable cells from the 0.05% Tween 80–water control. The ED_{50} values were estimated from a semilog plot of the drug concentration (μg/mL) vs. the percent of viable cells.

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Registry No. 1, 105970-01-2; **2**, 105970-02-3; **3**, 105970-03-4; 4, 100682-44-8; **5**, 105970-04-5; **6**, 105970-05-6; **7**, 105991-07-9; **8**, 105970-06-7; **9**, 19962-37-9; **10**, 105970-07-8; **12**, 6034-68-0; ClC-H_2COCH_3, 78-95-5; H_2C=C(CH_2Br)COOEt, 17435-72-2.

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