Memorial Institute Medium 1640 supplemented with 10% heat-inactivated fetal bovine serum and 50 μg/mL of kanamycin was used as the cell cultured medium. L1210 or L5178Y cells (5 × $10^4$ cells/mL) were cultured in a $CO_2$ gas incubator at 37 °C for 48 h in 1 mL of medium containing various concentrations of test compound dissolved in 0.6% $Me_2SO$. In the periods of exposured tested, the volumes of $Me_2SO$ used for dissolving the water-insoluble portion of test compound had no toxicity on L1210 or L5178Y cells. The antitumor activity evaluated as $IC_{50}$ (the concentration in μg/mL required for 50% inhibition of cell growth). The $IC_{50}$ value was obtained by plotting the logarithm of concentration of test compound vs. the growth rate (percentage of control) of the treated cells.

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## An Unequivocal Synthesis of (Methylazoxy)methanol Acetate and ([$^{14}$C]Methylazoxy)methanol Acetate

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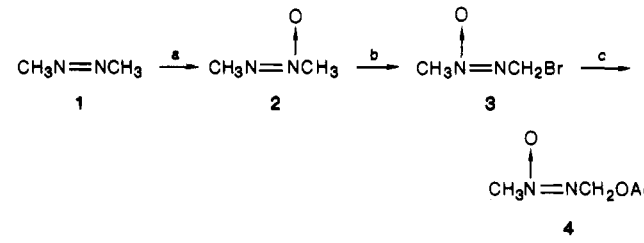
(Methylazoxy)methanol (**12**) is the aglycon of cycasin, the toxic constituent of the nuts of *Cycas circinalis*, which produces hepatoma in rats.[1] Biological studies with this material required a sample of (methylazoxy)methanol acetate (**4**) labeled specifically with carbon-14 in the methyl group.

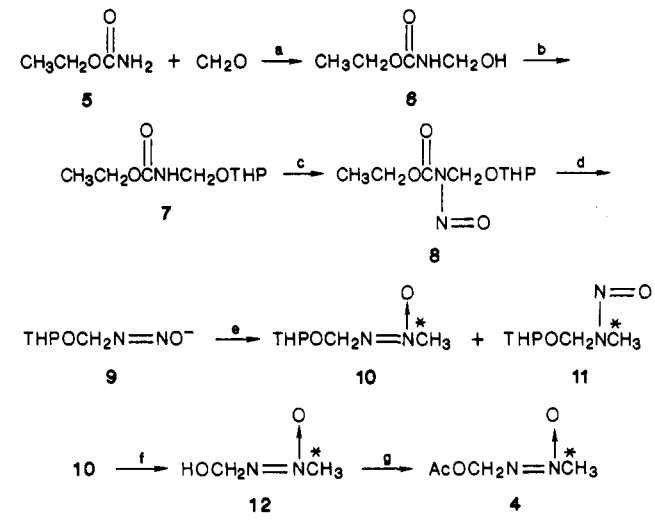
The preparation of **4** as shown in Scheme I has been reported.[2] These authors pointed out that this synthesis did not unequivocally establish the position of the oxygen atom in the azoxy moiety[3] because it was the first example of bromination of an azoxy compound with *N*-bromosuccinimide, and it was not clear what the preference, if any, for bromination of azoxymethane (**2**) would be. Horisberger and Matsumoto[4a] and Cazer et al.[4b] subsequently used this method to prepare **4** in which both carbons attached to nitrogen are labeled; however, this method is not suitable for preparing **4** specifically labeled in the methyl group because of the symmetry of **1**. Accordingly, we developed a scheme (Scheme II) based on Moss' directed synthesis of azoxyalkanes[5] that not only

**Scheme I**[a]




[a] (a) $C_6H_5COOOH$; (b) NBS, $(C_6H_5COO)_2$; (c) AcOAg.

**Scheme II**[a]




[a] (a) $Ba(OH)_2$; (b) dihydropyran, $C_7H_7SO_3H$; (c) $N_2O_4$, $NaHCO_3$; (d) *t*-$BuO^-K^+$; (e) $C^*H_3I$, glyme; (f) MeOH, HCl; (g) $Ac_2O$, pyridine.

unequivocally establishes the relation of the nitrogen bearing oxygen and the methyl group through synthesis, but also allowed us to prepare **4** specifically labeled with carbon-14 in the methyl group.

(Hydroxymethyl)urethane (**6**) was prepared by base-catalyzed condensation of urethane and formaldehyde.[6] Treatment of **6** with dihydropyran using *p*-toluenesulfonic acid as catalyst gave the tetrahydropyranyl ether **7**. Compound **7** was purified by HPLC using conditions that gave relatively short retention times. This compound was thermally unstable and very sensitive to acids. Attempts to purify **7** by TLC or distillation resulted in decomposition.

Nitrosation of **7** was accomplished in good yield with dinitrogen tetraoxide in the presence of sodium bicarbonate.[7] The resulting nitrosourethane **8** was unstable at ambient temperatures but could be stored for several weeks at −70 °C.

Treatment of **8** with potassium *tert*-butoxide gave the diazotate **9**, which was alkylated with methyl iodide without isolation. When the alkylation was carried out in HMPA, the preferred solvent for azoxyalkane formation,[5] a very low yield (4%) was realized because of the difficulty of separating the sensitive product **10** from the solvent HMPA. When ether was used as solvent for the alkylation reaction, only trace amounts of **10** could be identified. The product from the ether reaction was assigned the nitros-

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(2) Matsumoto, H.; Nagahama, T.; Larsen, H. O. *Biochem. J.* **1965**, *95*, 13c.

(3) Although the relation of the nitrogen bearing oxygen and the methyl group has not been established by synthesis, this relationship has been clearly established by $^1$H NMR: Korsch, B. N.; Riggs, N. V. *Tetrahedron Lett.* **1964**, 523.

(4) (a) Horisberger, M.; Matsumoto, H. *J. Labelled Compd.* **1968**, *4*, 164. (b) Cazer, F. D.; Barnela, S. B.; Kumar, K. S.; Kamat, P. L.; Milo, G. E.; Witiak, D. T. *J. Labelled Compd. Radiopharm.* **1985**, *21*, 81.

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(6) Einhorn, A. *Justus Leibigs Ann. Chem.* **1908**, *361*, 113.

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amine structure 11[8] on the basis that nitrosamines are expected[5] side products from the alkylation of diazotates, and the $^1$H NMR spectra of the product exhibited a three-proton singlet at $\delta$ 3.06 for the *N*-methyl group and a two-proton AB quartet at $\delta$ 5.42 and 5.87 ($J$ = 10 Hz) for the $OCH_2N$ function, as well as the expected signals for the THP residue (see Experimental Section). The chemical shift of these signals compare favorably with the corresponding methyl resonance at $\delta$ 2.97 and methylene resonance at $\delta$ 5.45 reported[9] for methyl(methoxymethyl)nitrosamine.

When the reaction was carried out in glyme, a 2:1 mixture of **10** and **11** was obtained from which the desired **10** was isolated in 13% yield. Hydrolysis of **10** with dilute methanolic hydrochloric acid gave (methylazoxy)methanol (**12**), which was converted to its acetate **4** without isolation. The acetate had spectral and chromatographic properties which were identical with an authentic sample. ([$^{14}$C]-Methylazoxy)methanol acetate was prepared by the above procedure using carbon-14-labeled methyl iodide in the alkylation reaction. An overall radiochemical yield of 6% was realized, and **4** had a specific activity of 39.5 mCi/mmol.

## Experimental Section

Melting points were taken on a Kofler hot stage apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 267 grating spectrophotometer. $^1$H NMR spectra were taken on a Varian HA-100 or EM-360 spectrometer, and mass spectra were obtained on an AEI MS-902 instrument. E. Merck silica gel HF analytical plates were used for analytical TLC.

Radioactive samples were counted on a Packard Tricarb 3375 liquid scintillation spectrometer using an Omifluor–toluene 6 g/L cocktail. Developed TLC plates were scanned on a Varian Berthold radioscanner fitted with a Model LB 242 X dual rate meter.

***N*-(Hydroxymethyl)urethane (6).**[6] A slurry of 600 mg of barium hydroxide (3.5 mmol) in 6 mL of water was added to 20 g of 99% urethane (224 mmol), and 16.8 mL of 37% formaldehyde solution (224 mmol) was added in one portion with stirring. The mixture became cool and gradually formed a cloudy solution. The mixture was stirred at room temperture, and the disappearance of formaldehyde was followed by using Tollen's reagent. After 40 min, the reaction was stopped by adding solid $CO_2$ and filtering the precipitated barium carbonate. The solvent was removed under vacuum, and the oily product placed over $P_2O_5$ in a desiccator. Crystals formed in 2 days and were collected by filtration. **6** was recrystallized from peroxide-free ethyl ether to give 10.2 g (38%) of white crystals: mp 53–55 °C; IR ($CH_2Cl_2$) 3580, 3440, 2960, 1715, 1600, 1220, 1020 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 1.22 (t, 3, $J$ = 7 Hz, $OCH_2CH_3$), 3.85 (br s, 1), 4.11 (q, 2, $J$ = 7 Hz, $CH_3CH_2O$), 4.64 (d, 2, $J$ = 7 Hz, $NHCH_2OH$), 5.85 (br s, 1).

Anal. Calcd for $C_4H_9NO_3$: C, 40.33; H, 7.62; N, 11.76. Found: C, 40.49; H, 8.07; N, 11.84.

***N*-[(2-Tetrahydropyranyloxy)methyl]urethane (7).** *N*-(hydroxymethyl)urethane (1 g, 8.4 mmol) was dissolved in 15 mL of ether with 5 mg of *p*-toluenesulfonic acid and 1.7 mL of dihydropyran. The mixture was stirred at room temperature for 2 h, then diluted with ether, and extracted with half-saturated $NaHCO_3$. The ether solution was dried ($Na_2SO_4$) and evaporated to give a clear oil. Three products were shown by TLC (benzene–ethyl acetate, 1:1). The compound was unstable to silica gel, but purification (100 mg per injection) by HPLC using a 1 m × 8 mm Bio-Sil A (25–35 $\mu$m) column (benzene–ethyl acetate, 8:2) with a high flow rate (9.9 mL/min) and a short retention time (10 min) gave 577 mg (34%) of product, which was homogeneous by TLC (ether, $R_f$ 0.43): high-resolution mass spectrum gave mass 203.1154, calcd for $C_9H_{13}NO_4$, 203.1157; IR ($CH_2Cl_2$) 3440 (NH), 1720 (C=O), 1310 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 1.28 (t, 3, $J$ = 7 Hz, $OCH_2CH_3$), 1.63 (m, 6, THP, H-3, H-4, H-5), 3.8 (m, 1, THP, H-6), 4.12 (q, 2, $J$ = 7 Hz, $OCH_2CH_3$), 4.78 (m, 2, $NHCH_2O$), 5.82 (br s, NH).

***N*-[(2-Tetrahydropyranyloxy)methyl]-*N*-nitrosourethane (8).** Nitrosation was carried out by adaptation of a reported procedure.[7] *N*-[(2-Tetrahydropyranyl)methyl]urethane (203 mg, 1.0 mmol) was dissolved in 3 mL of ether, and 200 mg of solid sodium bicarbonate was added. The mixture was cooled to −30 °C, and 1.0 mL of a 17 M solution of nitrogen dioxide in ether was added dropwise through a septum. The temperature was maintained between −20 and −10 °C for 30 min. At this time the mixture was poured into 10 mL of saturated aqueous $NaHCO_3$ and was extracted with 15 mL of ether. The ether extract was dried ($Na_2SO_4$) and evaporated to give a yellow oil. Polar material was removed by quickly filtering the product through a column of 3 g of silica gel with ether as eluant. The yield was 173 mg (75%) of material that was homogeneous by TLC (ether): IR ($CH_2Cl_2$) 1750 (C=O) cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 1.38 (t, 3, $J$ = 7 Hz, $OCH_2CH_3$ overlapped with THP), 3.48 (m, 1, THP, H-6), 3.95 (q, 2, $J$ = 7 Hz, $OCH_2CH_3$), 5.02 (s, 2, $NCH_2O$). This compound was not stable at room temperature, but could be stored at −70 °C for several weeks.

**([$^{14}$C]Methylazoxy)methanol Tetrahydropyranyl Ether (10).** A solution of 224 mg of potassium *tert*-butoxide (2 mmol) in 2 mL of dry (3A molecular sieve) dimethoxyethane was cooled to −30 °C, and 232 mg of **8** (1 mmol) in 3.5 mL dimethoxyethane was added dropwise over 5 min. The mixture was stirred at −20 to −30 °C for 30 min. At this time 50 mCi of [$^{14}$C]methyl iodide (1 mmol) was added to the mixture by vacuum transfer. The reaction mixture was allowed to warm to room temperature and was stirred for 2 h. A further 71 mg (0.5 mmol) of unlabeled methyl iodide was added, and the reaction was allowed to proceed for another 2 h. The reaction mixture was filtered through Celite with ether, and the filtrate was evaporated to dryness. The residue was dissolved in $CCl_4$ and filtered and the solvent removed to give 172 mg of yellow oil. The product was chromatographed on two preparative 20 × 20 cm silica gel GF plates eluted with ether to afford 29.2 mg (6.5 mCi) of **10** in 17% chemical yield (13.2% radiochemical yield). The product **10** ($R_f$ 0.80) was shown to be free of **11** ($R_f$ 0.71) by radio-TLC (ether). The following spectral data were obtained on an unlabeled sample prepared in the same manner and are identical with an authentic sample prepared from (methylazoxy)methanol: $^1$H NMR ($CDCl_3$) $\delta$ 1.65 (m, 6, THP, H-3, H-4, H-5), 3.4–4.0 (m, 2, THP, H-6), 4.05 (t, 3, $J$ = 1.5 Hz, $CH_3NO$), 4.9 (m, 1, THP, H-2) 5.03 (q, 2, $J$ = 1.5 Hz, $NCH_2O$). IR ($CH_2Cl_2$) showed the absence of a carbonyl function. This material was carried immediately on to the next step. Also obtained was 5.8 mg (1.3 mCi) of [$^{14}$C]methyl[(tetrahydropyranyloxy)methyl]nitrosamine (**11**): $^1$H NMR ($CDCl_3$) $\delta$ 1.67 (br s, 6, THP H-3, H-4, H-5), 3.06 (s, 3 $CH_3NN{=}O$), 3.4–4.0 (m, 2 THP, H-6), 4.6–5.0 (m, 1, THP, H-2), 5.42 and 5.87 (AB q, 2, $J$ = 10 Hz, $THPOCH_2NN{=}O$).

**([$^{14}$C]Methylazoxy)methanol Acetate (4).** The 29.2 mg (6.5 mCi) of **10** from the preceding reaction was dissolved in 1 mL of anhydrous methanolic HCl (prepared by adding 1 mL of acetyl chloride to 100 mL of anhydrous methanol), and the mixture was stirred at room temperature for 30 min. A TLC (ether) at this time showed only a single radioactive component with $R_f$ 0.29 corresponding to **12**. Two drops of pyridine were added, and the mixture was taken almost to dryness under vacuum. Chloroform (1 mL) was added, and then 0.5 mL of a 2:1 solution of pyridine–acetic anhydride was added. The mixture was stirred for 2 h at ambient temperature and taken to dryness under vacuum, and the residues was chromatographed on a 20 × 20 cm silica gel HF plate with ether as eluant. The band corresponding to (methylazoxy)methanol acetate ($R_f$ 0.64) was eluted from the silica

(8) It should be pointed out that **11** is an interesting compound in its own right since it is a derivative of methyl(hydroxymethyl)nitrosamine, the proposed ultimate carcinogen of dimethylnitrosamine.[10] Appropriate modification of the synthetic procedure described here, i.e., replacing the solvent dimethoxyethane with ether in the alkylation reaction, would allow the preparation of **11** specifically labeled with carbon-14 in the methyl group.

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gel with ethanol–ether (1:1) and afforded 3.0 mCi of **4**, which was both chemically and radiochemically pure by TLC (ether) and GC (2.5% Carbowax 20M on 100–120 Varaport-30, 6 ft × $^1/_8$ in. column, 140 °C, $N_2$ flow 30 mL/min, $t_R$ 3.6 min). The material had specific activity of 39.5 mCi/mmol.

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## Activated Anhydrides of Tartaric and Malic Acids

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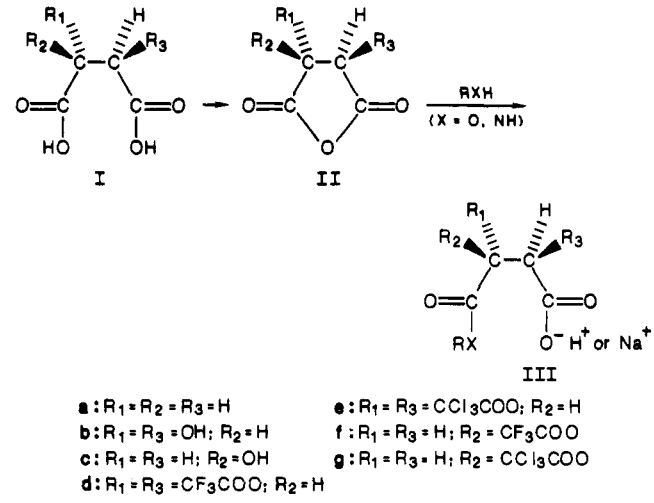
The monoesterification of dicarboxylic acids can often be achieved by preliminary intramolecular anhydride formation. Thus, monoesters (IIIa, X = O) of succinic acid (Ia) can be made[2] by reacting 1 equiv of alcohol with succinic anhydride (IIa). Application of this approach to tartaric (Ib) and malic (Ic) acids has been limited. Their anhydrides (IIb and IIc) are known, but they are either difficult to make or impossible to isolate.[3] Stable derivatives of these anhydrides are known wherein the hydroxyls have been esterified.[4] However, these derivatives are useless for forming esters with free hydroxyls in the acid moiety since deprotection of the hydroxyls would also cleave the esters. We report below the syntheses and characterizations of anhydrides IId-g and their use for the syntheses of IIIb and IIIc.

Of the four compounds IId–g, only IIf was a known material before the start of our work. There are two reports of its use as a reactive intermediate in the synthesis of malate half acid/half amides[5b] and half acid/half esters.[5a] Though no detailed study of its reactivity was reported, it was clear that the trifluoroacetate (TFA) group activated the anhydride toward nucleophile attack. Partial




a: $R_1 = R_2 = R_3 = H$
b: $R_1 = R_3 = OH$; $R_2 = H$
c: $R_1 = R_3 = H$; $R_2 = OH$
d: $R_1 = R_3 = CF_3COO$; $R_2 = H$
e: $R_1 = R_3 = CCl_3COO$; $R_2 = H$
f: $R_1 = R_3 = H$; $R_2 = CF_3COO$
g: $R_1 = R_3 = H$; $R_2 = CCl_3COO$

TFA removal also consumed added nucleophilic reagent, so excess alcohol or amine was used. Nevertheless, these reactions proceeded with good regiochemical integrity and acceptable yields of ester or amide. The goals of our work were to extend this approach to tartrate derivatives and to improve it by the use of trichloroacetate (TCA) instead of TFA (IIe and IIg). We reasoned that TCA should also activate the ring-opening process but not be as labile to nucleophilic attack as TFA. This would permit the use of only 1 equiv of nucleophile. TCA removal could also be achieved under conditions that would not cleave a simple ester. We therefore set out to develop efficient syntheses for these trihaloacetylated anhydrides.

Syntheses of the anhydrides were each achieved by heating the diacids with trifluoroacetic anhydride or trichloroacetic anhydride in dioxane at 75 °C. Completion of cyclic anhydride formation was monitored by noting the disappearance of diacid crystals; the cyclic anhydrides are very soluble in dioxane while the diacids are only sparingly soluble. We also ascertained that neat reaction of TFA anhydride and tartaric acid (pressure bottle, 75 °C) leads to formation of IId. Thus, while the dioxane solvent simplifies the procedure by allowing the use of an open system with only a reflux condenser, its use can be avoided. In either case, vacuum removal of trihaloacetic acid and unreacted trihaloacetic anhydride (and dioxane) leaves virtually clean anhydrides IId–g (crude yields >95%). Sublimation gives (>85% yields) analytically pure material. These sharp-melting, white, crystalline solids were characterized by their specific rotations, IR and NMR ($^1H$ and $^{13}C$) spectra, and elemental analyses. The absence of epimerization during anhydride formation was demonstrated by hydrolysis back to Ib and Ic and comparison of the rotations to those of the acids.

The ring openings of IId–g were compared to that of IIa and to each other. Each of the anhydrides was dissolved at room temperature in $CD_3OD$ and its reaction was monitored by NMR. Tartrate anhydrides were completely consumed in <90 s and malate anhydrides showed >80% ring opening in <90 s and complete reaction in <5 min. In contrast, IIa was 39% reacted in 3 h and 90% in 19 h.

The $CD_3OD$ reactions also shed light on the trihaloacetyl removal process. While more than half of the malate TFA was removed in 40 min and 88% in 3 h, there was only 6% TCA removal in 3 h. A similar trend is seen for the two tartrate trihaloacetyl groups. After 5 min there was 25% loss of one of the tartrate TCA groups vs. 66% loss of one TFA group and 21% loss of both TFA groups. Similarly, 87% double deacylation takes 3 h with TFA and 19 h with TCA.

(1) NIH Research Career Development Awardee (1983–1988).
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(3) (a) Malic anhydride was first reported in 1913 (Denham, W. S.; Woodhouse, H. *J. Chem. Soc.* **1913**, *103*, 1861–1870). Its synthesis requires formation of the disilver salt of malic acid and gives anhydride in 50% yield. Recent reports of its use are the following: Matsoukas, J. M.; Burnell, D. J.; Theodoropoulos, D. *Spectrosc. Lett.* **1983**, *16*, 933. Cordopatis, P.; Matsoukas, J.; Michel, A.; Janen, J.; Theodoropoulos, D. *Experientia* **1983**, *39*, 353–355. (b) There is one literature report that infers the existence of tartaric anhydride as a reactive intermediate; neither its isolation nor its direct characterization is reported: Higuchi, T.; Uno, H.; Shimada, I. *J. Pharm. Sci.* **1965**, *54*, 302. A second possible report of tartaric anhydride is suggested in *Chem. Abstr.* **1979** *91*, 221691; however, the original paper itself makes no mention of tartaric anhydride: Pankratova, V. N.; Goreva, T. F.; Krasnov, Yu. N. *Khim. Elementoorg. Soedin.* **1978**, *6*, 20. Hence we conclude that IIb has never been isolated or characterized.
(4) (a) Other than IIf,[5] the acetyl, propionyl, stearyl, and palmityl derivatives of IIc are known. The first reported and still most prominent derivative is the acetyl compound: Perkin, W. H. *Ber.* **1881**, *14*, 2540–2549. Anschutz, R. *Ber.* **1881**, *14*, 2789–2792. An improved preparation of this material appears in Jones, B. *J. Chem. Soc.* **1933**, 788–796. (b) At least nine dicarboxylated derivatives of IIb are in the literature, with the dibenzoyl and diacetyl the most prominent. An early report of both of them is Anschutz, R.; Pictet, A. *Ber.* **1880**, *13*, 1175–1178. An improved synthesis of the dibenzoyl compound is found in Butler, C. L.; Cretcher, L. H. *J. Am. Chem. Soc.* **1933**, *55*, 2605. A useful reference to the preparation of the diacetyl anhydride is Shriner, R.L.; Furrow, C. L. *Organic Syntheses*; Wiley: New York; 1963; Collect. Vol. IV, p 242.
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