The Free Internet Journal
for Organic Chemistry

Paper

Archive for
Organic Chemistry

Arkivoc **2017**, part iv, 12-19

Aldehyde-mediated N-nitrosation of an amino acid

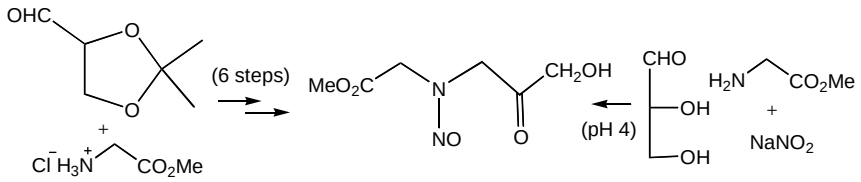
Jing Wang, Biying Wu, Mingmin Ding, Yue Yang, Zhiying Liu, Fang Zhang, Erqing Tang, and Jianli Duan*

School of Pharmaceutical Sciences, Wuhan University, Wuhan 430072, P.R. China
E-mail: jlduan@whu.edu.cn

Received 09-05-2016 **Accepted** 03-24-2017 **Published on line** 04-18-2017

Abstract

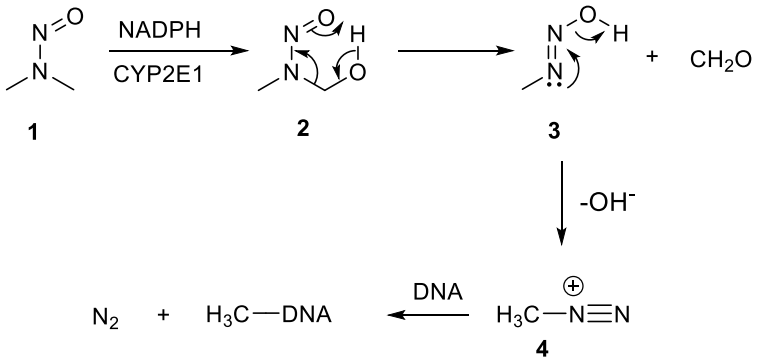
The N-nitroso Amadori compound is prepared by a multi-step synthetic strategy, using glyceraldehyde and glycine methyl ester hydrochloride as starting materials. Meanwhile, that the N-nitroso Amadori compound can be formed under the simulated gastric conditions in the presence of sodium nitrite is confirmed.



Keywords: N-Nitrosation, Amadori rearrangement, amino acids, sugars, carcinogenicity

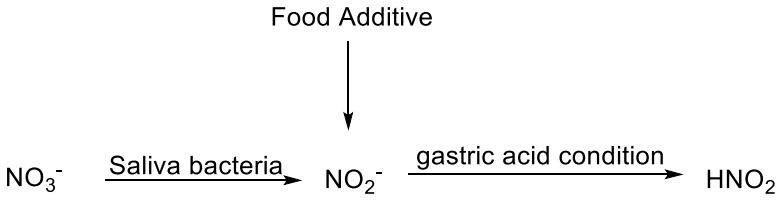
Introduction

The N-nitroso compounds, including nitrosamines, nitrosoamides and related compounds, have received considerable attention in recent years because many of them are potent carcinogens. *N*-nitrosodimethylamine (NDMA), produced as by-product of several industrial processes， was found to be carcinogenic to rats in toxicology studies.[1,2] The carcinogenicity of N-nitroso compounds is due to their ability to produce DNA alkylating agents directly[3] or by bioactivation.[4] Nitrosamines are relatively stable so as to require metabolic activation by enzymes to produce the alkylating agents. For example, NDMA **1** is transformed to α-hydroxy-nitrosamine **2** by the specific enzyme cytochrome P-450.[5] The α-hydroxynitrosamine **2** is highly unstable and quickly undergoes intramolecular rearrangement to form the diazohydroxides **3**. Then loss of the hydroxyl group leads to the formation of a methyldiazonium ion **4**. Alkyl diazonium ions are well known as powerful alkylating agents and are believed to be responsible species in DNA alkylation (Scheme 1).



Scheme 1. Metabolic activation of NDMA.

It has been reported that N-nitroso compounds can be formed endogenously.[6] There are two major sources of nitrosating agents (Scheme 2): (1) Sodium nitrite from food. It has been a common practice in food industry to add sodium nitrite to processed meats to inhibit the growth of the bacteria responsible for botulism poisoning. (2) The bioreduction of nitrate both digested and biosynthesized leads to nitrite.

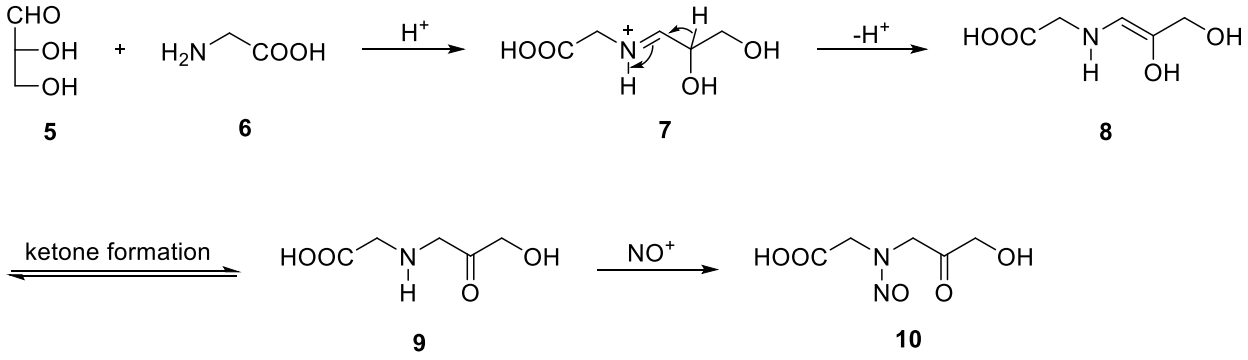


Scheme 2. Formation of the active nitrosating agent.

N-Nitrosation involves the biomolecular reaction of a substrate having at least one nitrogen atom with a pair of electrons with a nitrosating reagent. Human food contains amino acids and sugars. Amino acids contain a primary amine group, while the sugar has a carbonyl group. It was hypothesized that amino acids can react with sugars to form imines under gastric conditions. When the imines of amino acids and sugars encounter nitrous acid endogenously generated, N-nitroso compound can be formed. The goals of this subject are to

synthesize specific N-nitroso Amadori compound and to determine whether the compound can be formed under the endogenous conditions in the simulated gastric conditions.

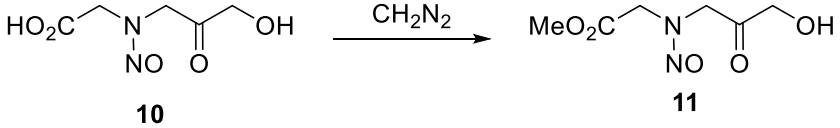
Glyceraldehyde **5** was chosen as representative of dietary aldehydes because it has fewest hydroxyl groups, which causes fewer problems in synthesis. Basically, our synthetic target is the N-nitroso Amadori compound *N*-(3-hydroxy-2-oxopropyl)-*N*-nitrosoglycine (**10**). The possible route to the formation of compound **10** is shown in Scheme 3**.** With this standard, we will be able to determine whether this compound can be formed under simulated gastric conditions. The results will be important for the evaluation of the toxicological significance of endogenous nitrosation.



Scheme 3. Possible route to the formation of *N*-nitroso Amadori compound.

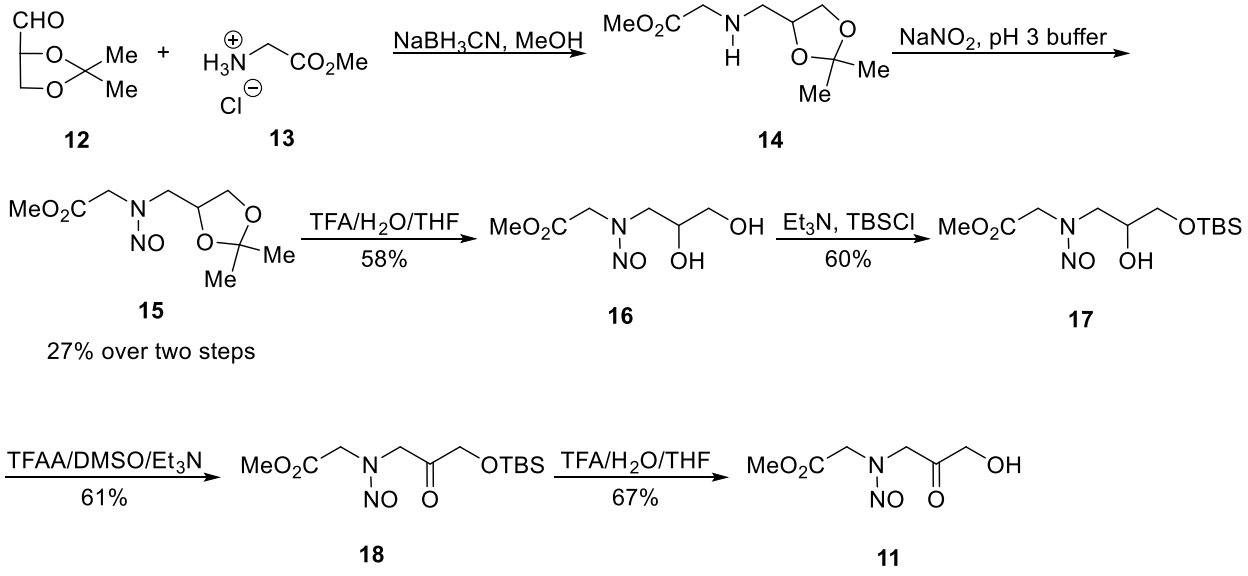
Results and Discussion

The synthesis of N-nitroso Amadori compound **10** proved to be very difficult. According to Schieberle's method,[7] a mixture of glyceraldehyde **5** and glycine **6** was refluxed in a mixture of anhydrous methanol and DMF. The Amadori compound may be formed in very low yield in this process. Unfortunately, none of the expected Amadori compounds could be isolated since the high polarity of the expected product could make it very hard to be separated from the complex product mixture. Other conditions[8,9] for Amadori rearrangement were also applied and none of them gave the desired product in a reasonable yield. An alternative synthetic target is methyl *N*-(3-hydroxy-2-oxopropyl)-*N*-nitrosoglycinate (**11**), the methyl ester of compound **10** （Scheme 4）. The nitrosation product mixture produced under simulated gastric conditions should be readily methylated by CH_2N_2 *in situ* without affecting other functional groups. So if compound **11** can be found in the mixture of methylation products, it will be enough to conclude that compound **10** can be formed in that process. Additionally, esterifying the corresponding carboxylic function group considerably reduces the polarity.



Scheme 4. Methylation of compound **10**.

Our synthesis of the N-nitroso Amadori compound **11** is summarized in Scheme 5. The first step is the reductive amination reaction to form methyl ((2, 2-dimethyl-1, 3-dioxolan-4-yl) methyl) glycinate (**14**). Later, it was found that it was not necessary to separate the product **14**; the crude product mixture directly underwent the nitrosation reaction with nitrous acid to afford methyl *N*-((2, 2-dimethyl-1, 3-dioxolan-4-yl) methyl)-*N*-nitrosoglycinate (**15**), which was easily deprotected to give methyl *N*-(2, 3-dihydroxypropyl)-*N*-nitrosoglycinate (**16**) under acidic conditions. Using Et_3N as the base, the primary hydroxyl group of diol **16** was selectively protected by the TBDMS group to afford methyl *N*-(3-((tert-butyldimethylsilyl)oxy)-2-hydroxypropyl)-*N*-nitrosoglycinate （**17**）. CrO_3[10] and $KMnO_4$[11] have been used to oxidize β-hydroxynitrosamine. But the reaction was slow to give *N*-(3-((tert-butyldimethylsilyl)oxy)-2-oxopropyl)-*N*-nitrosoglycinate （**18**） with less than 10% yield after 24 hours along with some decomposition products. Then we found that Swern-type oxidation can transform the β-hydroxynitrosamine **17** to β-ketonitrosamine **18** at a much faster reaction rate and also in good yield. At last, the TBDMS group was successfully removed under acidic conditions using TFA/THF/H_2O. It is noteworthy to mention that using HF or TBAF, common reagents for desilylation, caused the total decomposition of the starting material. A possible reason is that the fluoride ion can react with the nitroso group as a nucleophile to cause the decomposition.



Scheme 5. Synthesis of N-nitroso Amadori compound **11**.

With the standard compound **11** in hand, we have tried to determine whether it can be produced under the simulated gastric conditions. At last, we demonstrated that N-nitroso Amadori compound **11** can be produced under the simulated gastric conditions. Our preliminary experiments used glycine methyl ester and glyceraldehyde. The mixture of glycine methyl ester and glyceraldehyde in pH 4 buffer was stirred for 40 hours at 37 ºC. Then sodium nitrite was added to the solution. The reaction continued for another hour. Then the extract was analyzed by HPLC and one peak had the same retention time as the standard **11**. The compound was isolated by column chromatography and the ^1H NMR and ^{13}C NMR are identical with the standard **11** previously synthesized.

The result supports our hypothesis that imines of glyceraldehyde and glycine can undergo Amadori rearrangement *in vivo* and then following nitrosation can give N-nitroso Amadori compound as one of the products.

Conclusions

N-Nitroso Amadori methyl ester **11** was successfully synthesized by a multi-step synthetic strategy or under the simulated gastric conditions. The results indicated that the regular consumption of amino acids and sugar aldehydes in the diet in the presence of sodium nitrite may produce N-nitroso Amadori compounds which could be potentially toxic substances. Further investigation will be reported on the chemical and biological properties of these compounds in due course.

Experimental Section

General. All air- and moisture-sensitive reactions were carried out in flame-dried glassware under a nitrogen atmosphere. Reactive liquid compounds were measured and transferred by gas-tight syringes and added to the reaction flask through rubber septa. Moisture-sensitive and hygroscopic solid compounds were transferred under a nitrogen atmosphere in a glove bag. The reaction mixture was concentrated by using a rotary evaporator attached to a water aspirator. Residual solvents were usually removed under reduced pressure using a vacuum pump. Analytical thin-layer chromatography (TLC) was performed on glass-backed silica gel plates. Compounds were visualized under a UV lamp or by developing in iodine, vanillin, phosphomolybdic acid solution or with the potassium permanganate solution followed by heating on a hotplate to ~350 °C. Flash chromatography was performed on 230–400mesh silica gel with technical grade solvents which were distilled prior to use. 1H NMR spectra were recorded on a Bruker (Billerica, MA) AMX-250, Bruker AMX-300, Bruker AMX-500 at 250, 300, or 500 MHz, respectively, as $CDCl_3$ solutions with tetramethylsilane (δ = 0 ppm) as the internal standard. ^{13}C spectra were obtained on the same instruments at 62.5, 75, or 125MHz, respectively, with $CDCl_3$ (δ= 77 ppm) as the internal reference. Chemical shifts are reported in parts per million (ppm). Multiplicities are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), etc. Infrared spectra were recorded on a Thermo Nicolet NEXUX 670 FT-IR spectrometer as neat liquids with NaCl cells. Optical rotations were measured on a Jasco DIP-370 digital polarimeter.

Methyl *N*-((2, 2-dimethyl-1, 3-dioxolan-4-yl) methyl)-*N*-nitrosoglycinate (**15**). To a solution of 2 g (15.5mmol) glycine methyl ester hydrochloride and 2 g (15.4 mmol) glyceraldehyde acetonide in 20 mL of methanol, $NaBH_3CN$ (2 g, 33.3 mmol) was added. The reaction mixture was stirred at room temperature for 24 hours. Then methanol was evaporated under reduced pressure. The resulting solid was redissolved in ethyl acetate and the undissolved solid was filtered off. Then the solvent of the filtrate was removed again. The resulting oil was dissolved in 20 mL of pH 4 buffer (0.05 M) and 3.2 g (46 mmol) $NaNO_2$ was added in three portions. During addition of the sodium nitrite, H_2SO_4 was added to keep the pH of the solution at 3. Then the reaction mixture was stirred under room temperature for another 1 hour. The reaction mixture was extracted with ethyl ether (2 × 20 mL). The organic layer was dried with $MgSO_4$. Column chromatography (EtOAc/hexane, 1:3) gave 970 mg, 27%, of the desired product **15**. In $CDCl_3$, **15** exists as 4:1 mixture of Z and E isomers (due to N-N=O). The major product is the Z-isomer.^{1}H NMR (250 MHz， $CDCl_3$) δ(ppm) 4.54 (dd, *J* 3.8 Hz, 14.3Hz, 1H, CH_2-dioxolane-H_a), 4.39 (dd, *J* 6.4 Hz, 14.3 Hz, 1H, CH_2-dioxolane-H_b), 4.40 (d, *J* 16.5 Hz, 1H, $COCH_{2a}$), 4.25 (d, *J* 16.5 Hz, 1H, $COCH_{2b}$), 4.14-4.06 (m, 2H, $N(NO)CH_2$), 3.74-3.71 (m, 1H, CH), 3.65 (s, 3H, OCH_3), 1.36 (s, 3H, CH_3), 1.28

(s, 3H, CH_3); ^{13}C NMR (62.5MHz, $CDCl_3$) δ (ppm) 166.2, 110.2, 74.5, 66.7, 54.8, 52.4, 46.6, 26.7, 25.1; IR (neat) 2255, 1753(strong), 1456, 1065 cm^{-1}. HRMS (FAB): *m/z* calc'd for $[C_9H_{16}N_2O_5+H]^+$ 233.1137, found 233.1146.

Methyl *N*-(2, 3-dihydroxypropyl)-*N*-nitrosoglycinate (**16**). The protected nitrosamine **15** (140 mg，0.6 mmol) was dissolved in a mixture of 2 mL THF, 2 mL water and 2 mL TFA. The reaction mixture was stirred at room temperature for 8 hours. Then it was poured into 10 mL of water. The aqueous solution was extracted with ethyl ether (4×20 mL). The organic layer was separated, combined and dried over $MgSO_4$. Ether was removed by purging with nitrogen gas. Flash chromatography (EtOAc/hexane, 1:1) gave 68 mg (58.7%) desired *N*-nitrosodiol **16**. In $CDCl_3$, compound **16** exists as 5:1 mixture of Z and E isomers. Major product, Z-isomer, ^1H NMR (500 MHz, $CDCl_3$) δ(ppm) 4.42 (dd, *J* 8.8 Hz, 14.3 Hz, 1H, OCH_{2a}), 4.41 (d, *J* 16.3 Hz, 1H, $COCH_{2a}$), 4.28 (dd, *J* 3.2 Hz, 14.3 Hz, 1H, OCH_{2b}), 4.23 (d, *J* 16.3 Hz, 1H, $COCH_{2b}$), 4.12-4.06 (m, 1H, $N(NO)CH_{2a}$), 3.95-3.92 (m, 1H, $N(NO)CH_{2b}$), 3.76 (s, 3H, CH_3), 3.69-3.94 (m, 1H, CH), 3.09 (s, 1H, OH); ^{13}C NMR (125 MHz, CDCl3) δ(ppm) 167.6, 70.0, 63.8, 56.0, 52.9, 47.6; IR (neat) 3450(broad, strong)，1736, 1228 cm^{-1}. HRMS: *m/z* calc'd for $[C_6H_{12}N_2O_5+H]^+$ 193.0824, found 193.0816.

Methyl *N*-(3-((tert-butyldimethylsilyl)oxy)-2-hydroxypropyl)-*N*-nitrosoglycinate (**17**). The *N*-nitroso diol **16** (50 mg, 0.026 mmol), TBDMSCl (80 mg, 0.53 mmol) and catalytic amount of DMAP were dissolved in 5 mL CH_2Cl_2 under argon. Then the solution was cooled to 0 ºC and 50 µL Et_3N was added to the solution. Then the solution was naturally warmed to RT and stirred for 4 hours. TLC was used to monitor the completion of the reaction. Upon completion, the reaction mixture was poured into 10 mL water and extracted by CH_2Cl_2 (3 × 10 mL). The extracts were combined, washed with 10 mL water, and dried with $MgSO_4$. The solvents were removed under reduced pressure at room temperature. Flash chromatography (ethyl acetate/hexane, 1:1) gave 47 mg (60%) of the desired product. In $CDCl_3$, compound **17** exists as 5:1 mixture of Z and E isomers. Major product, Z-isomer, ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 4.45 (dd , *J* 3.4, 14.8 Hz, 1H, $O(Si)CH_{2a}$), 4.38 (d, *J* 16.3 Hz, 1H, $COCH_{2a}$), 4.34 (d, *J* 16.3 Hz, 1H, $COCH_{2b}$), 4.24 (dd , *J* 7.6, 14.8Hz, 1H, $O(Si)CH_{2b}$), 4.08-4.01 (m, 1H, CH), 3.74 (s, 3H, OCH_3), 3.74-3.70 (m, 2H, $N(NO)CH_2$), 2.96 (d, *J* 4.6 Hz, 1H, OH), 0.91 (s, 9H, $C(CH_3)_3$), 0.10 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 167.0, 70.5, 64.4, 55.9, 52.6, 47.2, 25.8, 18.2, -5.4; IR (neat) 3428 (broad, strong), 1747（strong）, 1467, 1124 cm^{-1}. HRMS: m/z calc'd for $[C_{12}H_{26}N_2O_5+Si+H]^+$ 307.1689, found 307.1675.

Methyl *N*-(3-((tert-butyldimethylsilyl)oxy)-2-oxopropyl)-*N*-nitrosoglycinate (**18**). A solution of trifluoroacetic anhydride (525mg, 2.5 mmol) in 0.5 mL CH_2Cl_2 was added to a solution of dimethylsulfoxide (180 µL, 2.5 mmol) in 2.5 mL CH_2Cl_2 at -78 ºC. The mixture was stirred for 10 min, and a solution of alcohol **17**（140 mg, 0.5 mmol）in 1.5 mL CH_2Cl_2 was added. After stirring for 2h at –45 ºC, trimethylamine（560 µL, 4 mmol） was added, and the solution was allowed to warm to room temperature. Then saturated aqueous $NaHCO_3$ solution (5 mL) was added and the mixture was separated. The aqueous layer was extracted with ethyl ether, and the combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. Flash chromatography (ethyl acetate/hexane, 1:3) gave 85 mg (61%) desired product. In $CDCl_3$, compound **18** exists as 5:3 mixture of Z and E isomers. Major product, Z-isomer,^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 5.43 (s, 2H, $O(Si)CH_2$), 4.35 (s, 2H, $N(NO)CH_2$), 4.33(s, 2H, CH_2COO), 3.71 (s, 3H, OCH_3), 0.96 (s, 9H, $C(CH_3)_3$), 0.14 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 205.2, 166.3, 68.4, 58.1, 52.4, 45.4, 25.7, 18.1, -5.7; E isomer：^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 5.08 (s, 2H, $O(Si)CH_2$), 4.66 (s, 2H, $N(NO)CH_2$), 4.25 (s, 2H, CH_2COO), 3.81 (s, 3H, OCH_3), 0.93 (s, 9H, $C(CH_3)_3$), 0.12 (s, 6H, $Si(CH_3)_2$）; ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 201.7, 168.2, 68.5, 53.2, 52.6, 50.5, 25.7, 18.1, -5.7; IR (neat) 1747 (strong), 1461, 1106 cm^{-1}. HRMS (FAB): *m/z* calc'd for $[C_{12}H_{26}N_2O_5Si+H]^+$ 307.1689, found 307.1675.

Methyl *N*-(3-hydroxy-2-oxopropyl)-*N*-nitrosoglycinate (**11**). Mono-TBDMS protected *N*-nitroso Amadori compound **18** (24 mg, 0.079 mmol) was dissolved in a mixture of 2 mL of THF, 2 mL of water and 2 mL of TFA.

The mixture was stirred at room temperature for 1 hour. The solvents were removed either by vacuum or flowing nitrogen into the solution at room temperature. Flash chromatography (EtOAc/hexane, 1:1) gave 10 mg (66.6%) of the desired N-nitroso Amadori compound methyl ester **11**. In $CDCl_3$, compound **11** exists as 10:7 mixture of E and Z isomers. Major product, E-isomer, ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm): 5.13 (s, 2H, OCH_2), 4.45 (s, 2H, $N(NO)CH_2$), 4.35 (s, 2H, CH_2COO), 3.82 (s, 3H, OCH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ (ppm): 201.2, 166.3, 67.0, 53.4, 52.9, 50.5; Minor products, Z isomer, ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 5.28 (s, 2H, OCH_2), 4.46 (s, 2H, $N(NO)CH_2$), 4.38 (s, 2H, CH_2COO)，3.72 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 204.0, 168.2, 66.9, 57.4, 52.7, 45.4；IR (neat) 3541 (broad, strong), 2250, 1753 (strong), 1461, 1222 cm^{-1}; The compound was too unstable for a determination for elemental composition.

Nitrosation of methyl glycine and glyceraldehyde under simulated gastric conditions

Methyl glycine hydrochloride (1 g, 7.8 mmol) and 1.6g (17.7 mmol) D, L-glyceraldehyde were dissolved in 10 mL pH 4 buffer (0.05 M). The resulting solution was heated to 37 ºC and stirred for 40 hours at 37 ºC. The solution turned to a light yellow color. Then the reaction mixture was cooled to room temperature and sodium nitrite (2 g, 29 mmol) was added. The solution was stirred for another hour. The reaction was monitored by TLC. When TLC showed the completion of the reaction, the solution was extracted with ethyl acetate (3 × 10 mL). The existence of the compound **11** was determined by HPLC. The HPLC separation was performed on a reverse phase HPLC C-18 column (10 mm×25 cm). The program was as follows: mobile phase: water/CH_3CN; flow rate: 1 mL/ min; isocratic: 20% CH_3CN/80% water; standard **11** RT: 11.8 min. The combined organic layer was washed with 10 mL water, dried over $MgSO_4$ and concentrated under reduced pressure. Flash chromatography (ethyl acetate/hexane, 1:1) separated the product (5 mg) which has the same R_f value as the synthesized standard. The separated product's ^{1}H NMR and ^{13}C NMR are identical with those of the previously synthesized standard **11**.

References

1. Magee, P. N.; Farber, E. *J. Biochem.* **1962**, *83*, 114. https://doi.org/10.1042/bj0830114
2. Preussmann, R. *Chem. Ber.* **1962**, *95*, 1571. https://doi.org/10.1002/cber.19620950640
3. Mirvish, S. S. *J. Natl. Cancer I.* **1983**, *71*, 630.
4. Mochizuki, M.; Anjo, T.; Okada, M. *Tetrahedron Lett.* **1980**, *21*, 3696.
5. Yang, C. S.; Yoo, J. S. H.; Ishizaki, H.; Hong, J. *Drug Metab. Rev.* **1990**, *22,* 147. https://doi.org/10.3109/03602539009041082
6. Spiegelhalder, B.; Eisenbrand, G.; Preussmann, R. *Food Cosmet. Toxicol.* **1976**, *14*, 545. https://doi.org/10.1016/S0015-6264(76)80005-3
7. Hofmann, T.; Schieberle, P. *J. Agric. Food Chem.* **2000**, *48*, 4301. https://doi.org/10.1021/jf000076e
8. Hofmann, T.; Bors, W.; Stettmaier, K. *J. Agric. Food Chem.* **1999**, *47,* 379. https://doi.org/10.1021/jf980626x
9. Roper, H.; *Carbohyd. Res.* **1987**, *164,* 207. https://doi.org/10.1016/0008-6215(87)80131-3

10. Okada, M.; Suzuki,E.; Iiyoshi, M. *Chem. Pharm. Bull.* **1978**, *26*, 3891. https://doi.org/10.1248/cpb.26.3891
11. Saavedra, J. E.; Temu, C.T.; Farnsworth, D.W. *Synthetic Commun.* **1989**, *19*, 215. https://doi.org/10.1080/00397918908050972

