Synthesis of 1-Chloro-1-[^{15}N]nitrosocyclohexane, an Electrophilic Aminating Reagent.

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SUMMARY

We have prepared 1-chloro-1-[^{15}N]nitrosocyclohexane, an electrophillic aminating reagent useful for the stereoselective synthesis of α-[^{15}N]amino acids. Treatment of potassium [^{15}N]nitrate with Pb^{o} gave potassium [^{15}N]nitrite, which was converted into the intermediate hydroxylamine disulfonate by treatment with sodium bisulfite and sulfur dioxide. Addition of an excess of cylohexanone followed by heating gave a mixture of the cyclohexanone [^{15}N]oxime and unreacted cyclohexanone which was removed *in vacuo*. The resulting [^{15}N]oxime was then converted to 1-chloro-1-[^{15}N]nitrosocyclohexane in essentially quantitative yield by treatment with chlorine gas .

Keywords: 1-chloro-1-[^{15}N]nitrosocyclohexane, [^{15}N]cyclohexyloxime, potassium [^{15}N]nitrite, and potassium [^{15}N]nitrate

INTRODUCTION

Nitrogen-15 has been incorporated into organic compounds by the nucleophilic displacement of halogens with reduced nitrogen compounds such as ammonia[1,2], potassium phthalimide[3], benzylamine[4], formamide[5]. In addition, oxidized forms of labeled nitrogen such as nitrate[6] and nitrite[7] have been used to incorporate label directly. Recently, other electrophilic forms such as diazonium salts have proved useful in the synthesis of labeled compounds[8]. Reports by Oppolzer and coworkers[9-12] described an electrophilic nitrogen transfer using the α-chloro-α-nitroso group as a nitrogen source. Of particular interest to us was their use of 1-chloro-1-nitrosocyclohexane in the highly stereoselective synthesis of amino acids[9-11]. As diagrammed in Scheme 1, acyl derivatives of Oppolzer's camphor-10,2-sultam chiral auxiliary (**1**) are converted to the

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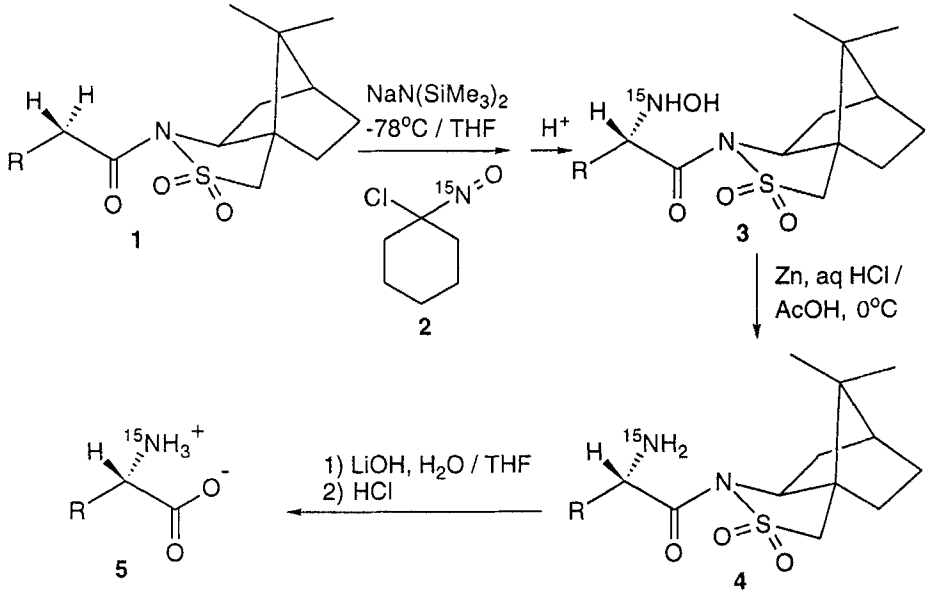
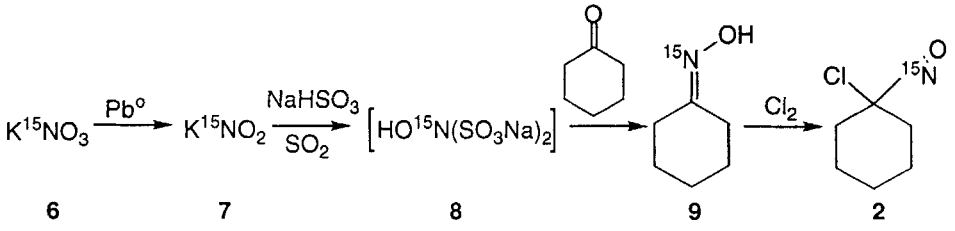
corresponding enolates using sodium *bis*(trimethylsilyl)amide. Titration of the enolates with a solution of the 1-chloro-1-nitrosocyclohexane yields sultam-linked N-hydroxy-α-amino acids. Reduction followed by cleavage of the chiral auxiliary, affords the corresponding α-amino acids with remarkable enantioselectivity (>99% enantiomeric excess). Based on Oppolzer's protocol the preparation of ^{15}N-labeled 1-chloro-1-nitrosocyclohexane provides a direct route to chiral [α-^{15}N]amino acids. Reported here is the preparation of 1-chloro-1-[^{15}N]nitrosocyclohexane from [^{15}N]nitric acid.



Scheme 1) Synthesis of ^{15}N-Labeled L-α-Amino Acids.

Results and Discussion

The efficient preparation of 1-chloro-1-nitrosocyclohexane (**2**) from cyclohexyl oxime (**9**) has been reported[13]. The standard preparation of oximes involves the condensation of hydroxylamine with ketones or aldehydes. Because of the expense of ^{15}N-labeled hydroxylamine hydrochloride we explored the utility of other labeling precursors. Eck and Marvel[14,15] described the conversion of sodium nitrite (**7**) to hydroxylamine disulfonate (**8**) by treatment with sodium bisulfite and sulfur dioxide (Scheme 2). Condensation of the hydroxylamine disulfonate with cyclohexanone yielded the oxime (**9**). This route was appealing because we could prepare the required potassium [^{15}N]nitrite from relatively inexpensive potassium [^{15}N]nitrate.



Scheme 2) Synthesis of 1-Chloro-1-[^{15}N]nitrosocyclohexane (**2**)

Eck and Marvel's protocol utilized an excess of nitrite and required purification of the oxime by distillation. Because of its cost, we used potassium [^{15}N]nitrite as the limiting reagent. Under these conditions, the product of the reaction was a mixture of the desired oxime and cyclohexanone. Based on the difference in their volatility, we were able to remove cyclohexanone from the cyclohexyl [^{15}N]oxime *in vacuo*. Our yields (47-77%) of the ^{15}N-labeled oxime were comparable to those reported by Eck and Marvel; the higher yields were obtained when using freshly crystallized potassium nitrite. Using minor modifications of the chlorination procedure described by Müller and coworkers, we converted the [^{15}N]oxime (**9**) into 1-chloro-1-[^{15}N]nitrosocyclohexane (**2**) in yields of 90-95%. We determined that vacuum distillation of 1-chloro-1-nitrosocyclohexane did not improve the yields of subsequent reactions. Because of the significant degradative loss of the chloronitroso compound during distillation, we did not distill this product. The presence of small amounts of diethyl ether in our product did not interfere with its subsequent reactions. The use of this reagent in the synthesis of ^{15}N-labeled L-α-amino acids is described in the following manuscript in this Journal[16].

Methods

Analytical Methods - Melting points were determined using a Thomas-Hoover apparatus and are uncorrected. Melting points are compared to that of unlabeled compounds.

Proton and proton-decoupled ^{13}C FT-NMR spectra were obtained using Bruker (AC-250, and AM-200 WB) NMR spectrometers. ^{15}N FT-NMR spectra were obtained on the Bruker AM-200 operating at 28.283 MHz. ^{15}N NMR spectra were referenced with respect to a D_2O solution of sodium nitrate (0 ppm). Acquisition parameters were as follows: ^{1}H NMR --- 3 KHz sweep width, 32 K data points, 5.11 s acquisition time, 0.196

Hz/pt data point resolution, and 25°C; proton-decoupled ^{13}C NMR --- 10.869 KHz sweep width, 32 K data points, 1.51 s acquisition time, 5 s relaxation delay, 0.663 Hz/pt data point resolution, and 25°C. The ^{13}C NMR spectrum of the product 1-chloro-1-nitrosocyclohexane (2) was obtained on a neat sample; intermediates were dissolved in D_2O or $CDCl_3$.

Potassium [^{15}N]Nitrite (6) - An aqueous solution containing [^{15}N]Nitric acid (7.81) g, 122 mmol; 99% ^{15}N) was neutralized (pH = 7) with 6.83 g of KOH at 0°C. The solution was reduced (rotary evaporator) and the resulting white solid was dried *in vacuo* at room temperature (mechanical pump, liquid nitrogen trap, 12 h) to give 12.15 g of K[^{15}N]NO_3 (118.3 mmol, 97.5% yield). The solid was finely powdered and mixed with 123.5 g (596 mmol, 5 equiv) of lead (325 mesh). The resulting mixture was added to a 250-mL single-necked flask and placed under a nitrogen atmosphere. The temperature of the flask was brought to 360°C and maintained there with a themocouple/temperature controller for 1 h. The reaction was then cooled to 60°C and 100 mL of water was added. The suspension was stirred for 1 h, while cooling to ambient temperature, and filtered through a pad of Celite™. The filtrate was reduced and 25 mL of water was added to dissolve the solids. Methanol was added to this solution until it became cloudy. The solution was filtered through Celite™ and evaporated. The residue was then taken up in 5 mL of water and 5 mL of methanol was added. Ethanol was added to initiate precipitation. The precipitate was collected by filtration to give 8.88 g K$^{15}NO_2$ (87% yield). ^{15}N NMR δ +234 ppm; MS *m/z* (negative ion FAB $^{15}NO_2$) 47.

Cyclohexanone [^{15}N]oxime (9) -- A solution of 6.20 g (72.0 mmol) of K$^{15}NO_2$ and 3.88 g (36.6 mmol) of anhydrous Na_2CO_3 in 60 mL of water was added to a 250-mL, single-necked flask and cooled to 0°C using an ice/acetone bath. A #20-gauge needle was used to bubble SO_2 slowly and continuously into the solution. During the addition of SO_2, great care was taken to maintain the temperature of the reaction between 0°C and 4°C. The addition was continued until the reaction solution was quite acidic (pH 1 - 2). A slight brown color was observed just as the solution became acidic. Cyclohexanone (7.4 mL; 7.0 g; 71 mmol, Aldrich Chemical Co.) was added, followed by 8 mL of ethanol. The reaction mixture was heated to 50°C and maintained at this temperature for 1 h. The progress of the reaction was monitored by TLC (diethyl ether:hexane 30:70). After cooling to room temperature, the mixture was extracted with four 25 mL portions of ether. The

aqueous phase was made alkaline (pH = 12) with 6 M NaOH and extracted with two additional 25 mL portions of ether. The six ether extracts were combined, dried ($MgSO_4$), filtered, and evaporated (rotary evaporator) to give 7.10 g of crude oily product. This oil contained excess cyclohexanone which was removed from the much less volatile oxime *in vacuo* at room temperature (mechanical pump, liquid nitrogen trap, 16 h). The yield was 4.95 g (60%); mp. 85-87°C (lit.[14]: 86-88°C). The yield of this reaction varied from 47-77% with better yields (65-77%) obtained when freshly crystallized potassium nitrite was used. IR 3600-3000, 2900, 1670, 1500, 1470, 1000 cm^{-1} ; ^{13}C NMR ($CDCl_3$) 160.35 (d, J_{C-N} = 3.43 Hz), 31.88 (d, J_{C-N} = 0.74 Hz), 26.72, 25.64, 25.42, 24.33. ^{1}H NMR ($CDCl_3$) 1.59 (bs, 6H), 2.20 (bs, 2H), 2.49 (bs, 2H); ^{15}N NMR ($CDCl_3$) -51 ppm; HRMS *m/z* calcd. for $C_6H_{11}{}^{15}NO$ (M+) 114.0811, obsd 114.0811; Analysis Calcd for $C_6H_{11}{}^{15}NO$ (114.0811): C, 63.12; H, 9.71; N, 13.14. Found: C, 63.04; H, 9.74; N, 13.42.

1-Chloro-1-[^{15}N]nitrosocyclohexane[12] (2) -- AN IMPORTANT SAFETY NOTE: Müller, *et al.*[13] observed that 1-chloro-1-nitrosocyclohexane is a powerful lachrymator, irritating to the nasal membranes and causing a dull headache. Animals were also tested and eye irritation was observed. We therefore advise handling this material carefully. Our preparations were always carried out in an efficient hood, as were the subsequent reactions of this substance.

A 250 mL, three-necked flask was fitted with a magnetic stir bar, a sparging tube (center neck), an efficient reflux condenser, and a gas inlet adapter. A solution (pale brown) of 6.14 g (53.8 mmol) of [^{15}N]cyclohexyloxime (**9**) in 50 mL of reagent grade anhydrous diethyl ether was added to the flask. The sparging tube was adjusted so that the glass frit was fully immersed . A slow stream of argon was continually flushed into the gas inlet adapter and out of the condenser. Chlorine gas (Aldrich Chemical Co.) was bubbled through the sparging tube into the stirring oxime solution which was at room temperature. After a few min a muddy green color developed followed immediately by the appearance of a whitish solid. The ether began to reflux gently. After 5 min, the voluminous precipitate stopped the stir bar. As more chlorine was added, the center of the solid mass began to redissolve (a blue solution) and stirring recommenced. Refluxing had ceased. After about five minutes, the solid had all dissolved and the resulting solution was deep blue. Chlorine addition was continued until the solution had an obvious green cast. An ice-cold solution of 2 M NaOH (20 mL) was cautiously added with

continuous magnetic stirring to the reaction vessel. After a few mL of the sodium hydroxide solution had been added, vigorous refluxing of the ether occurred. The ether layer lost its green cast and developed a clear blue color. The base layer was yellow-brown. After the refluxing ceased, the aqueous layer was removed with a pipet. This procedure was repeated three more times by adding 20 mL of ice-cold 2M NaOH, stirring for several min, and withdrawing the aqueous layer with a pipet. No further refluxing of the ether was observed. The now intensely brilliant blue ether solution was decanted into a 125-mL Erlenmeyer flask and dried with sodium sulfate. After rapid filtration with vacuum through a medium fritted glass funnel, the dried solution was evaporated (rotary evaporator). The evaporation was monitored by weighing, and was stopped when the net weight was less than the theoretical weight and the blue liquid residue had begun to volatilize throughout the evaporator system and was beginning to be visible as a blue solid in the rotary evaporator trap (liquid nitrogen). The final residue was a blue liquid (7.64 g). Subtraction of the mass of the small amount of residual ether (c.a. 5%, ^{1}H NMR integration), the overall yield was 48 mmol (~90%). A ^{13}C NMR spectrum of the neat product showed no resonances due to oxime or cyclohexanone. The ^{13}C NMR shifts, relative to the ether methyl group (14.5 ppm) were: 116.58 ($^{1}J_{C\text{-}N}$ = 17 Hz), 32.64 ($^{2}J_{C\text{-}N}$ = 2.5 Hz), 24.04, 21.20. The ^{15}N NMR spectrum contained one resonance at +520 ppm. This blue product was dissolved in 44 mL of dry THF to make a 1.0 M solution which was used in subsequent nitrogen-transfer reactions.

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