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Efficient Preparation of Photolabile Agent MNI-glu by Regioselective Nitration of 4-Methoxyindoline Derivative

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Abstract: The present work investigates the nitration of 4-methoxy indoline derivative with different reagents and optimized conditions. We now report a simple methodology for nitration employing claycop in the presence of amine, which offers significant improvement with regard to the yield and regioselectivity, and a much more favorable isomeric ratio of products was obtained under mild conditions. Therefore, a useful method for preparation of a photolabile L-glutamate derivative was concurrently established.

Keywords: Aromatic nitration, caged L-glutamate, claypop, nitroindolines, photorelease agent

INTRODUCTION

Photorelease of biologically signaling molecules from photocleavable (caged) carriers is now a standard technique for physiologists, neuroscientists, and biochemists, but rapid and efficient release of these molecules faces lots of problems including lack of suitable carriers. In

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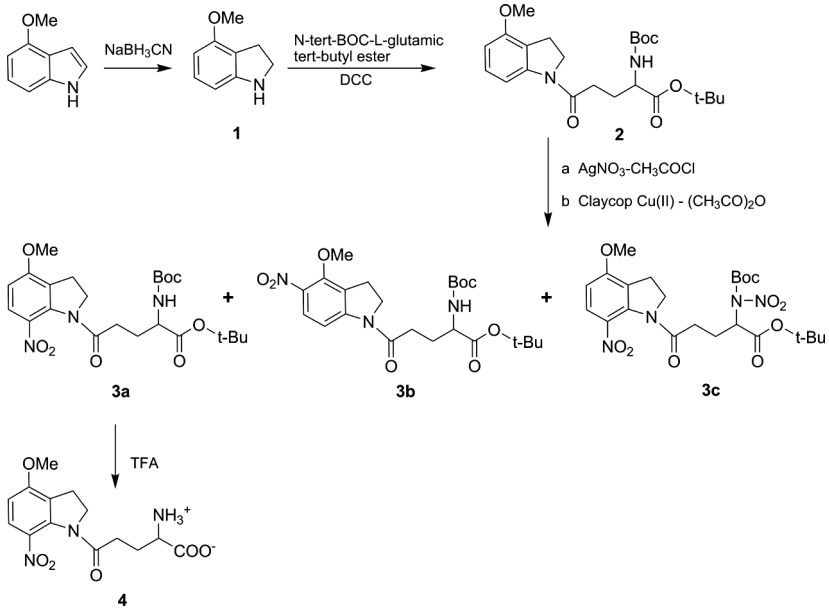
1999, Papageorgiou et al. developed a series of 7-nitroindolyl derivatives that rapidly and efficiently released carboxylates, such as L-glutamate, a key neurotransmitter, in synapses of the central nervous system.[1] To enhance the photorelease efficiency, many research groups focused on this kind of compound, especially 1-glutamine-4-methyloxy-7-nitroindoline (MNI-glu), a practical reagent that could improve the photolytic efficiency by two-folds.[2] At the same time, different methods have been developed for the nitration on the aromatic ring. Fedoryak[3] and coworkers used the traditional $AgNO_3$ method to perform the 7-nitration on the indole ring. The yield was blocked by side reactions including 5-nitro isomer (**3b**) and partial nitration of the Boc-protected amino function in the side chain. Hereafter, claycop-acetic anhydride, as a mild selective nitration reagent, was used to successfully decrease the amount of 5-nitro isomer,[4] but the *N*-nitroamide by-products still existed in a significant quantity. Herein, our strategy focused on giving more favorable regioselectivity on the 7-nitro isomer (**3a**) and avoiding the side reaction by optimizing experimental conditions and introducing amines as substrate of transition of the nitro group.

RESULTS AND DISCUSSION

Recently, a study found that 4-methoxy-7-nitroindoline glutamate has a remarkable photolytic efficiency.[2] Herein, we report a regioselective synthesis of the title compound from commercially available 4-methoxyindole. The synthetic route was outlined in Scheme 1. 4-methoxyindole was reduced in quantitative yield using sodium cyanoborohydride ($NaBH_3CN$) in acetic acid to 4-methoxyindoline, which was coupled to *N*-tert-BOC-L-glutamic acid a-*tert*-butyl ester under standard 1,3-dicyclohexylcarbodiimide (DCC) coupling conditions to give methoxyindoline **2**. Nitration of compound **2** was accomplished to yield nitro methoxyindoline **3**, which was deprotected by trifluoroacetic acit (TFA) treatment to obtain MNI-glu. However, the advantage could not readily be exploited because of the very poor yield for introduction of the nitro group. Although the detailed photolytic mechanism is not known, oxygen transfer takes place from the 7-nitro group to the adjacent 1-acyl group, so the 5-nitro isomer is not useful. Therefore, nitration of aromatic rings has received considerable attention for the regioselectivity. Some research groups tried to block the 5-position with a suitable substituent, such as 5-methyl group, but this caused a significant reduction in photoefficiency, which could be ascribed to steric inhibition of the resonance interaction between the methoxy group and the aromatic ring.[5] Thus, it is very important and urgent to explore a practical nitration agent and optimize the conditions.



Scheme 1. Synthetic route of MNI-glu.

We screened a wide range of nitration reagents and observed that with excess $AgNO_3$–acetic chloride, a complex mixture of products, which included the 7- and 5-nitro isomers in a ratio of nearly 1:1 together with a large part of additional products, was obtained under traditional conditions. In a previous report, the by-products were a mixture of *N*-nitroso and *N*-nitro species on the nitrogen of the protected amino acid residue.[6]

According to our experience, far more excess $AgNO_3$ may cause the problem. Therefore, we decreased the amount of $AgNO_3$ involved in this reaction from 2.5 to 1.0 equiv (Table 1), and performed the reaction at

Table 1. Nitration of **2** with $AgNO_3/CH_3COCl$ method

					Yield of products **3** (%)		
Reactant	Ratio	Temp.	Solvent	Time (h)	**3a** (7-isomer)	**3b** (5-isomer)	**3c** (*N*-nitro)
$2 + AgNO_3$	1:2–2.5	rt	CH_3CN	1	14	10	63
$2 + AgNO_3$	1:1.02	rt	CH_3CN	1	37	20	25
$2 + AgNO_3$	1:1.02	0°C	CH_3CN	1	64	10	6
$2 + AgNO_3$	1:1.02	0°C	CCl_4	48	—	—	—

room temperature. The *N*-nitro and nitroso by-products did not dominate the mixture, but the nitration on the 7- and 5- positions showed less selectivity. Because of the greater thermodynamic stability of 7-nitro isomer, the regioselectivity could be increased by lowering the reaction temperature. Meanwhile, it is appeared that formation of by-products would also be marred. Just as we expected, the best result was obtained when the reaction was performed at 0°C in acetonitrile. The *N*-nitro and nitroso by-products decreased significantly, and the desired 7-nitro isomer (**3a**) increased to some extent as indicated by the data presented in Table 1 (from 37% to 64%).

Furthermore, we found that the nitration process depended not only on the reaction temperature but also on the polarity of the solvent. The change of solvent from acetonitrile to tetrachloride decreased the reaction activity. Therefore, under this circumstance, a polar solvent favored the reaction more than a nonpolar one (Table 1); the polar solvent may be better at solvating the intermediate CH_3COONO_2, stabilizing the charged NO_2^+ electrophile and then reducing the activation energy.

To enhance the ratio of **3a**/**3b**, a mild nitration reagent, "claycop," was introduced by Papageorgiou et al.[4] The major advantage is the increasing regioselectivity, which stems from microporous solids, such as montmorillonite K10 clay, with high diffusivity and shape selectivity. In addition, such procedures were often found to provide good yields under mild conditions, reduce the activation energy, and simplify the workup. We used the Laszlo's claycop (copper nitrate impregnated on montmorillonite K10) as the nitration agent and aimed to achieve higher regioselectivity and then increase the yield of 7-nitro isomer (**3a**). The experiment results showed that with the claycop method, the high-selective nitration reagent significantly increased the **3a**/**3b** isomer ratio, but the 5-nitro isomer **3b** still existed in a small quantity. Moreover, the *N*-nitro by-product **3c**, which was far more than *N*-nitroso of amide, formed in considerable amounts.

As discussed previously, lowering the temperature would be favorable for the reaction, so we carried out the reaction at 0°C and found that the ratio of **3a**/**3b** increased from 6:1 to 15:1 (Table 2), the *N*-nitro by-product decreased a little, but it was far from pure 7-nitro isomer. Additionally, the reaction time was also profoundly affected by solvent, and the result was very interesting. When compound **2** reacted with claycop in tetrachloride, the reaction was almost completed in 12 h and yielded a mixture of **3a** and **3b** in a 6:1 ratio, strongly favoring the 7-nitro isomer. In acetonitrile, the reaction was much more sluggish, and there was no benefit to the product ratio. These observations are the opposite of the former experiment; a nonpolar solvent would be favorable for the reaction, which could probably mean that a different mechanism dominated in the claycop method.

Table 2. Nitration of **2** with claycop method

Reactant	Ratio[a]	Temp.	Solvent	Time (h)	Yield of products **3** (%) **3a** (7-isomer)	**3b** (5-isomer)	**3c** (*N*-nitro)
2 + claycop	1:1	rt	CCl_4	12	45	8	30
2 + claycop	1:1	rt	CH_3CN	100	36	6	35
2 + claycop	1:1	0°C	CCl_4	48	59	4	21

[a]The molar ratio was calculated as the amount of nitrate anion contained in claycop.

Although the claycop nitration provided a satisfactory solution to the 7- and 5-isomer selectivity, we still encountered the problem of unwanted partial nitration/nitrosation of the Boc-protected amino function in the side chain during introduction of the 7-nitro group. In attempts to suppress these side reactions, other research groups tried to convert methoxyindoline **2** to its di-Boc derivative,[7] but only a slight improvement was observed. We decided to introduce the amine as a nitrotransition agent to the reaction.

We added aromatic and aliphatic amine respectively with **2** and claycop in one pot at room temperature, with the molar ratio of 1:2:1. As we know, *N*-nitrosoamide by-product would be little with claycop as nitration agent, so major by-products would be 5-nitro isomer **3b** and *N*-nitroamide **3c**. The results showed that the aliphatic amine decreased the *N*-nitration by-products more evidently than that of the aromatic amine, which indicated that t-butylamine favors the nitrotransition reaction (Table 3). It appears that the formation of the *N*-nitro and *N*-nitrosoamide by-products in small quantities is an inevitable

Table 3. Nitration of **2** with claycop method in the presence of amines at rt

Reactant	Ratio[a]	Solvent	Time (h)	Yield of products **3** (%) **3a** (7-isomer)	**3b** (5-isomer)	**3c** (*N*-nitro)
2 + claycop	1:1	CCl_4	12	45	8	30
2 + claycop + t-butylamine	1:2:1	CCl_4	12	48	2	11
2 + claycop + aniline	1:2:1	CCl_4	12	40	2	24

[a]The molar ratios were calculated as the amount of nitrate anion contained in claycop.

consequence when the substrate contains a secondary amide group. Further study on the mechanism of nitrotransition will be a future work.

CONCLUSIONS

Our main emphasis was to achieve higher selectivity to meet market demand. We carried out nitration of compound **2** with different methods under various conditions. Claycop proved to be a better nitration agent in terms of regioselectivity. It is noteworthy that under typical claycop procedure, the **3a** and **3b** isomers were obtained in 6:1 ratio (i.e., 45% 7-nitro isomer compared to 8% 5-isomer), but the unwanted *N*-nitroamide by-product **3c** still existed in some quantity. Whereas when amine was added to the reaction mixture, especially aliphatic amine, the yield of **3c** decreased significantly (from 30% to 11%), while the ratio of **3a**/**3b** enhanced (to 24). In addition, our results illustrate that temperature and solvent could affect the reaction profoundly. Decreasing the reaction temperature would facilitate access to a higher regioselectivity. Because of different mechanisms of nitration methods, polar and nonpolar solvents would give opposite outcomes. With claycop as nitration agents, a nonpolar solvent would be favorable; it is just adverse to that of the $AgNO_3/CH_3COCl$ method. In conclusion, a simple methodology for nitration employing claycop in the presence of aliphatic amine provides a satisfactory solution to the 7- and 5-nitro isomer selectivity and offers an interesting chemoselectivity in some instances. It thus represents a valuable alternative to the existing approaches on both the laboratory and industrial scales.

EXPERIMENTAL

General

Melting points were determined with an electrothermal capillary melting-point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Jeol-AL-300 Fourier Transform (FT) spectrometer with tetramethylsilane (TMS) as the internal standard. The electrospray ionization/turnover frequency (ESI-TOF) spectra were taken on a Liner Scieneific LDI-1700 mass spectrometer. Silica gel (0.040–0.064 mm) was used for column chromatography. Analytical high-performance liquid chromatography (HPLC) was performed on a Shimazu LC-10AT with a reverse column of YMC-C8 (4.6×150 mm) at 1 ml/min flow rate. Some reagents were purchased from Aldrich. Organic solvents were dried

over anhydrous Na_2SO_4 and evaporated under reduced pressure. Claycop was prepared with a 2:3 (w/w) ratio of Cu $(NO_3)_2$ and montmorillonite K10 as described in Ref. 8.

Synthesis of 1-Glutamine-4-methyloxy-7-nitroindoline (4)

4-Methoxyindoline (**1**)

Sodium cyanoborohydride ($NaBH_3CN$) (1.56 g, 24.8 mmol) was added portionwise over 20 min to a solution of 4-methoxyindole (1.0 g, 6.79 mmol) in acetic acid (20 mL), and the mixture was stirred at room temperature for 0.5 h. Water was added, and the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate (25 mL) and washed with saturated $NaHCO_3$ and brine. The organic layer was dried with anhydrous $MgSO_4$ and concentrated in vacuo to give **1** as viscous oil (0.98 g, 98%), which was used immediately without purification.

1-[S-(4-t-Butoxycarbonyl)-4-(t-butoxycarbonylamino)]-butanoyl-4-methoxyindoline (**2**)

1,3-Dicyclohexylcarbodiimide (DCC) (0.29 g, 1.4 mmol), dimethylaminopyrimidine (DMAP) (0.37 g, 3.0 mmol), and N-BOC-l-glutamic acid a-t-butyl ester (0.36 g, 1.2 mmol) were added to a solution of 4-methoxylindoline **1** (0.15 g, 1.0 mmol) in acetonitrile (12 mL). The reaction mixture was stirred at room temperature for 16 h and then filtered. The filtrate was diluted with CH_2Cl_2 (25 mL) and washed with saturated $NaHCO_3$ solution, 0.5 N HCl, and brine. The organic layer was dried with anhydrous $MgSO_4$ and evaporated to dryness. The crude product was purified by column chromatography with EtOAc–petroleum ether (60–90°C) to yield product **2** as white solid (0.34 g, 78%), mp 129–130°C.

Nitration of **2** with Different Agents

$AgNO_3/CH_3COCl$ Method. Compound **2** (2.17 g, 5.0 mmol) and silver nitrate (0.86 g, 5.1 mmol) in 20 mL acetonitrile (or CCl_4) were stirred at room temperature (or 0°C) to give a clear solution, and then a solution of acetyl chloride (0.4 g, 5.1 mmol) was added. A greyish precipitate formed upon addition of the first few drops. The reaction was complete when thin-layer chromatography (TLC) showed that compound **2** totally disappeared, which depended on the temperature and solvent (see Table 1). The mixture was filtered, and the filtrate was washed with

saturated $NaHCO_3$ solution and brine. The organic layer was dried with $MgSO_4$ and evaporated to dryness. The crude product was purified by column chromatography with EtOAc–petroleum ether (60–90°C) to yield product **3a** as yellow solid (for the corresponding yields, see Table 1), mp 142–144°C (lit.[4] 145–147°C).

Claycop Nitration Method. Compound **2** (2.17 g, 5.0 mmol) was added to a suspension of claycop [1.2 g, 2.55 mmol $Cu(NO_3)_2$] in 20 mL CCl_4 (or CH_3CN) and acetic anhydride (10 mL) at room temperature (or 0°C). The mixture was stirred until compound **2** totally disappeared on TLC. It took 12–100 h typically, as shown in Table 2. The solid was filtered and washed with EtOAc. The filtrate was washed with saturated $NaHCO_3$ and brine. The organic layer was dried with $MgSO_4$ and evaporated to dryness. The crude product was purified by column chromatography with EtOAc–petroleum ether (60–90°C) to yield product **3a** as yellow crystals (for the corresponding yields, see Table 2), mp 143–145°C (lit.[4] 145–147°C).

^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.74$ (d, $J = 9.0$ Hz, 1 H, 6-H), 6.63 (d, $J = 9.0$ Hz, 1 H, 5-H), 5.15 (br s, 1 H, NH), 4.17–4.23 (m, 3 H, CH and 2-H), 3.90 (s, 3 H, OCH_3), 3.26 (t, 2 H, 3-H), 2.54–2.60 (t, 2 H, $COCH_2$), 2.26–2.34 and 1.96–2.04 (m, 2 H, CH_2), 1.46 (s, 9 H, CH_3), 1.42 (s, 9 H, CH_3) ppm; ESI-TOF$^+$: 480.35 $(M + H)^+$.

Hydrolysis of **3a** to Prepare 1-[S-(4-Amino-4-carboxybutanoyl)]-4-methoxy-7-nitroindoline (**4**)

TFA (1.5 mL in 1 mL CH_2Cl_2) was added dropwise to a solution of **3a** (0.26 g, 0.5 mmol) in CH_2Cl_2 (2 mL). The mixture was stirred at room temperature for 1.5 h and concentrated in vacuo. The residue was dissolved in water (20 mL) and adjusted to pH 7.0 with 1 M aq. NaOH. The solution was washed with ether, lyophilized, and stored at −20°C as a pale yellow powder (129 mg, 80%).

^{1}H NMR (300 MHz, D_2O): $\delta = 7.57$ (d, $J = 9.0$ Hz, 1 H, H6), 6.67 (d, $J = 9.0$ Hz, 1 H, H5), 4.11 (t, $J = 7.8$ Hz, 2 H, H2), 3.76 (s, 3 H, OMe), 3.51 (t, 1 H, CH), 2.88 (t, $J = 7.8$ Hz, 2 H, H3), 2.56 (t, 2 H, $COCH_2$), 1.95 ± 2.08 (m, 2 H, CH_2CH).

Nitration of 2 with Claycop Method in the Presence of Amines

Compound **2** (2.17 g, 5.0 mmol) was added to a suspension of claycop [2.4 g, 5.1 mmol $Cu(NO_3)_2$] in CCl_4 (25 mL) and stirred at room

temperature. Two additional experiments were carried out at the same time with identical amounts of reagents, but t-butylamine (0.5 ml, 5.0 mmol) and aniline (0.47 ml, 5.0 mmol) were, respectively, added to such solutions. After 12 h, the mixtures were detected by HPLC with methanol–water as mobile phase, gradient elution, rate 1 ml/min, detection wavelength: 314 nm. The results are shown in Table 3.

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