

Supporting Information

Development of A By-product-Free Strategy for the Synthesis Oxazoline from *N*-Acetylglucosamine

Sen Zhou,^a Bo Ma,^a Feng Tang,^b Wenqiang Liu,^a Xin Li,^a Baoquan Chen,^a Shiyong Shang,^c
Wei Huang,^b Yaohao Li*^a and Zhongping Tan*^a

^aState Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

^bCAS Key Laboratory of Receptor Research, CAS Center for Excellence in Molecular Cell Science, Center for Biotherapeutics Discovery Research, Shanghai Institute of Material Medical, Chinese Academy of Sciences, Shanghai 201203, China

^cCenter of Pharmaceutical Technology, School of Pharmaceutical Sciences, Tsinghua University, Beijing 100084, China

*Email: yaohaoli@imm.ac.cn, zhongping.tan@imm.ac.cn

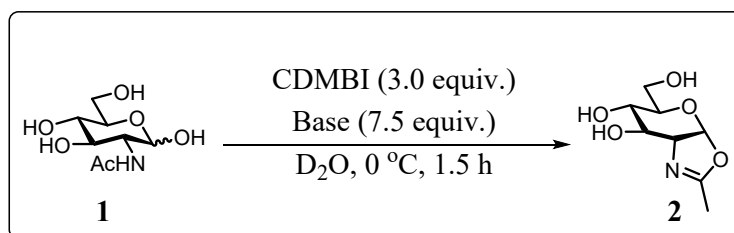
Table of Contents

1.General information.....	S2
2.Representative procedure I for Table 1	S2
3.Representative procedure II for Table 2	S2
4.Representative procedure III for Table 3	S4
5.NMR spectra of the reaction mixtures in Table 4	S5
6.Comparison of the effects of reaction time and equivalents of CDMBI	S6
7.Comparison of the effect of the equivalents of CMBI-BF₄	S7
8.DMC-mediated synthesis of oxazoline 2	S8
9.Representative procedure IV for the synthesis of CDMBI	S10
10.Representative procedure V for the synthesis of CMBI-BF₄	S11
11.Detailed spectroscopic analysis of GlcNAc 1 , oxazoline 2 and by product 3	S12
12. ¹ H NMR and ¹³ C NMR spectra.....	S15
13.References.....	S20

1. General Information

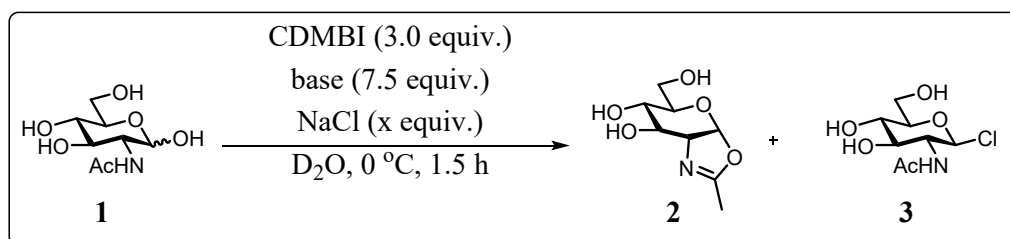
All reagents used in this study were purchased from commercial sources and used without further purification unless otherwise stated. Gel filtration chromatography separations were performed using Sephadex G-25. Reaction monitoring was carried out using NMR or TLC. Thin Layer Chromatography (TLC) was conducted on silica gel HSGF254 (HPTLC: $8\pm 2\ \mu\text{m}$ $\geq 80\%$). High-resolution mass spectrometry analysis was performed using a Waters Synapt G2-Si Q-TOF system. ^1H and ^{13}C NMR spectra were recorded on JNM-ECZ500R, Quantum-I 400, and Bruker AVANCE III-400 in deuterated solvents. Chemical shifts (δ) are reported in parts per million (ppm), and coupling constants (J) are in hertz (Hz). The following abbreviations were used to describe multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad.

2. Representative procedure I for Table 1



To a 2.5 ml centrifuge tube equipped with a magnetic stirring bar, GlcNAc (11 mg, 0.05 mmol), 2-chloro-1,3-dimethyl-1H-benzimidazol-3-ium chloride (CDMBI, 32.5 mg, 0.15 mmol, 3.0 equiv.), and PhSO₃Na (9.1 mg, 0.05 mmol, 1.0 equiv.) were added. Then, 0.5 ml of D₂O was added to the tube, and the resulting solution was vortexed for 0.5 min. Finally, the base (0.375 mmol, 7.5 equiv.) was added, and the resulting mixture was stirred for 1.5 h at 0 °C. After the addition of 0.3 ml of D₂O to the mixture, the supernatant was directly subjected to NMR analysis following centrifugation. The yield was determined by comparing the integration of the signals of the H-atoms of sodium benzenesulfonate and the anomeric H-atom of the GlcNAc-oxazoline 2.

3. Representative procedure II for Table 2



To a 2.5 ml centrifuge tube equipped with a magnetic stirring bar, GlcNAc (11 mg, 0.05 mmol), CDMBI (32.5 mg, 0.15 mmol, 3.0 equiv.), PhSO₃Na (9.1 mg, 0.05 mmol, 1.0 equiv.), and varying amounts of NaCl were added. The resulting mixture was vortexed for 0.5 min after adding 0.5 ml of D₂O, and base (0.375 mmol, 7.5 equiv.) was then added. The mixture was stirred for 1.5 h at 0 °C. After the addition of 0.3 ml of D₂O, the mixture was centrifuged and the supernatant was subjected to NMR analysis. The yields were determined by comparing the integration of the signals of the H-atoms of sodium benzenesulfonate and the anomeric H-atoms of the GlcNAc-oxa **2** and the by-product **3**.

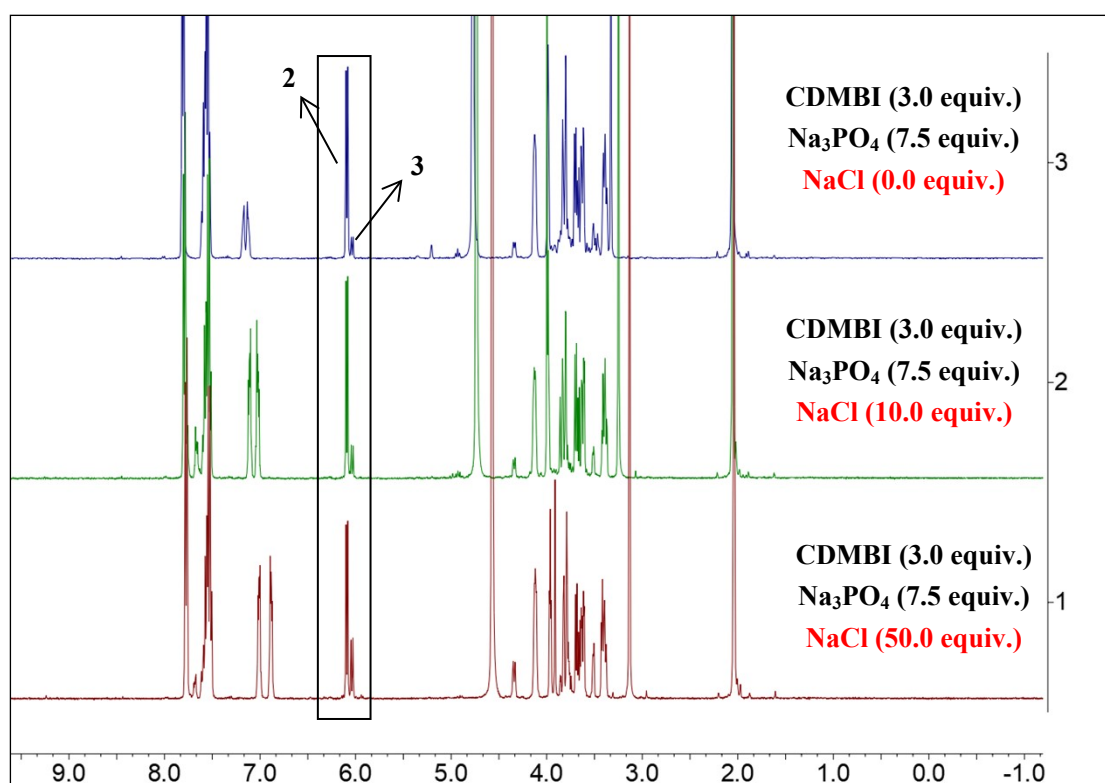


Figure S1. Comparison of ¹H NMR spectra of the reaction under different amounts of NaCl when Na₃PO₄ was used as base.

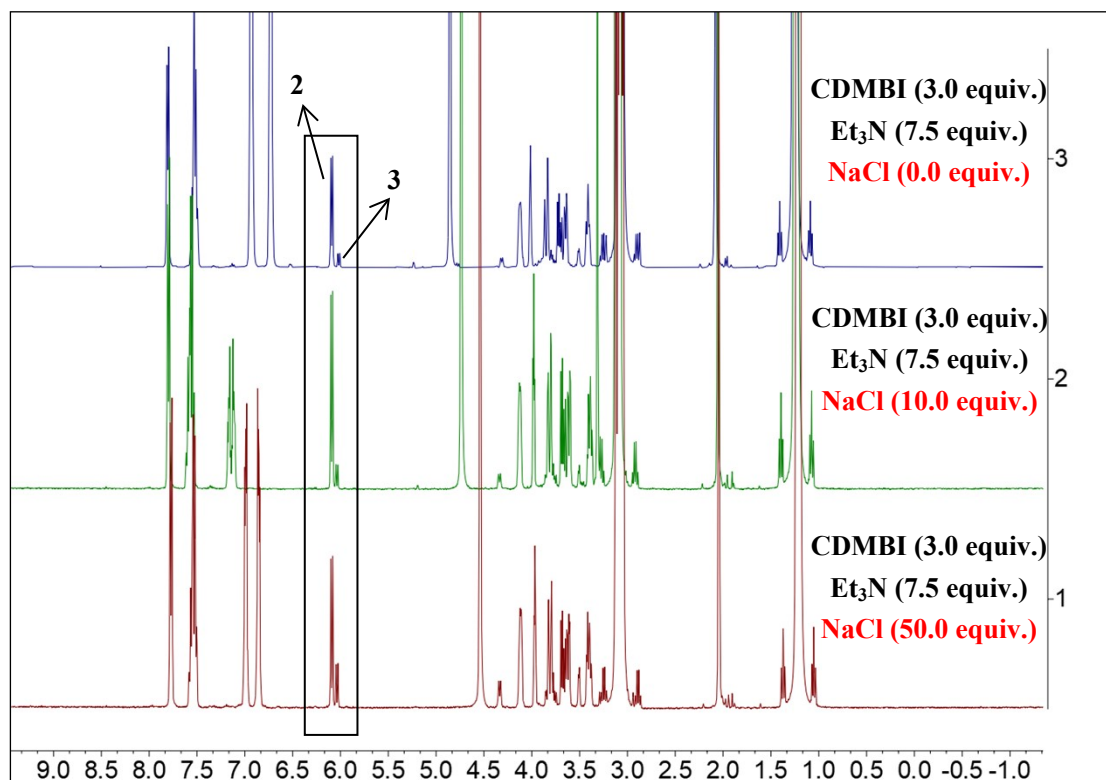


Figure S2. Comparison of ^1H NMR spectra of the reaction under different amounts of NaCl when Et_3N was used as base.

4. Representative procedure III for Table 3

The procedures for Table 3 (entries 1, 2, 5, and 6) are consistent with Representative Procedure I, except that the mixtures in entries 2 and 6 were reacted for 1.5 h in an ice water bath without stirring.

The procedures for Table 3 (entries 3, 4, 7, and 8) are as follows: GlcNAc (11 mg, 0.05 mmol), PhSO_3Na (9.1 mg, 0.05 mmol, 1.0 equiv.), and Na_3PO_4 or Et_3N (0.375 mmol, 7.5 equiv.) were added to a 2.5 ml centrifuge tube equipped with a magnetic stirring bar. Next, 0.5 ml of D_2O was added, and the resulting solution was vortexed for 0.5 min. Finally, CDMBI (32.5 mg, 0.15 mmol, 3.0 equiv.) was added. The resulting mixture was stirred for 1.5 h at 0°C for entries 3 and 7, while no stirring was applied for entries 4 and 8. After the addition of 0.3 ml of D_2O to the mixture, the supernatant was directly subjected to NMR analysis following centrifugation. The yields were determined by comparing the integration of the signals of the H-atoms of sodium benzenesulfonate and the anomeric H-atoms of the oxazoline **2** and by-product **3**.

5. NMR spectra of the reaction mixtures in Table 4

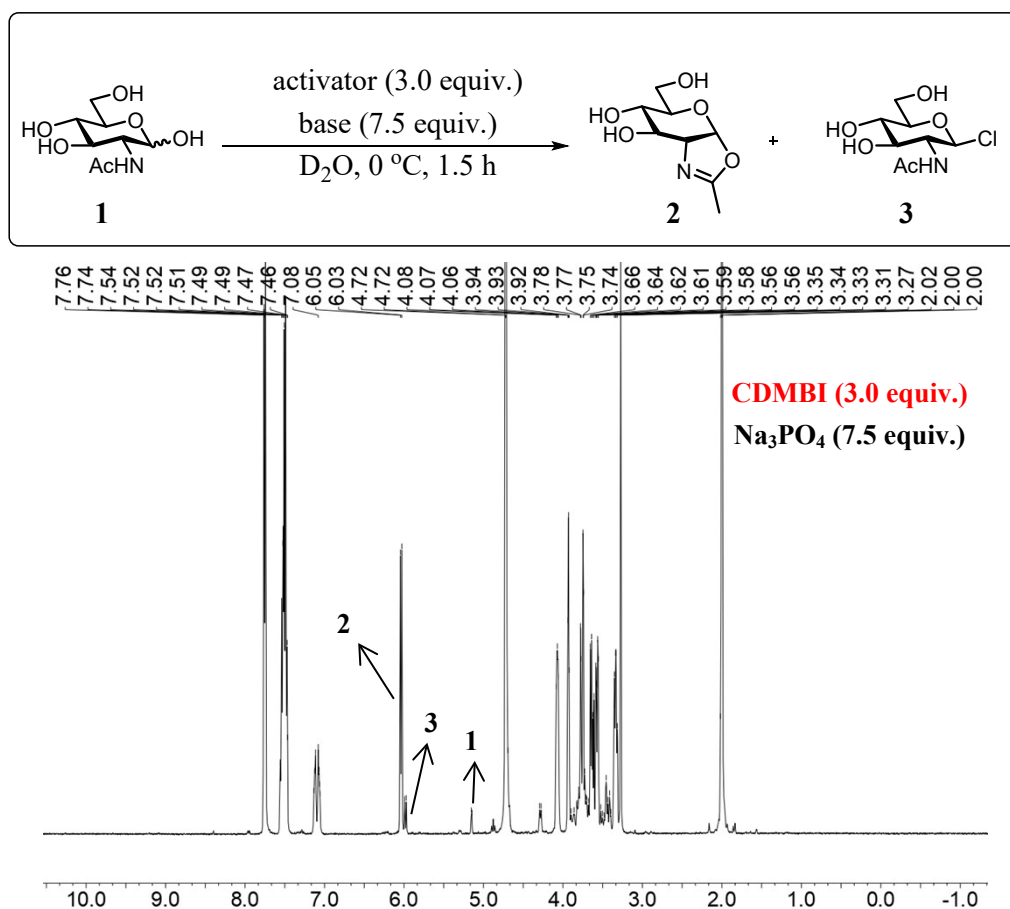


Figure S3. ¹H NMR spectrum of the reaction utilizing CDMBI as the activator.

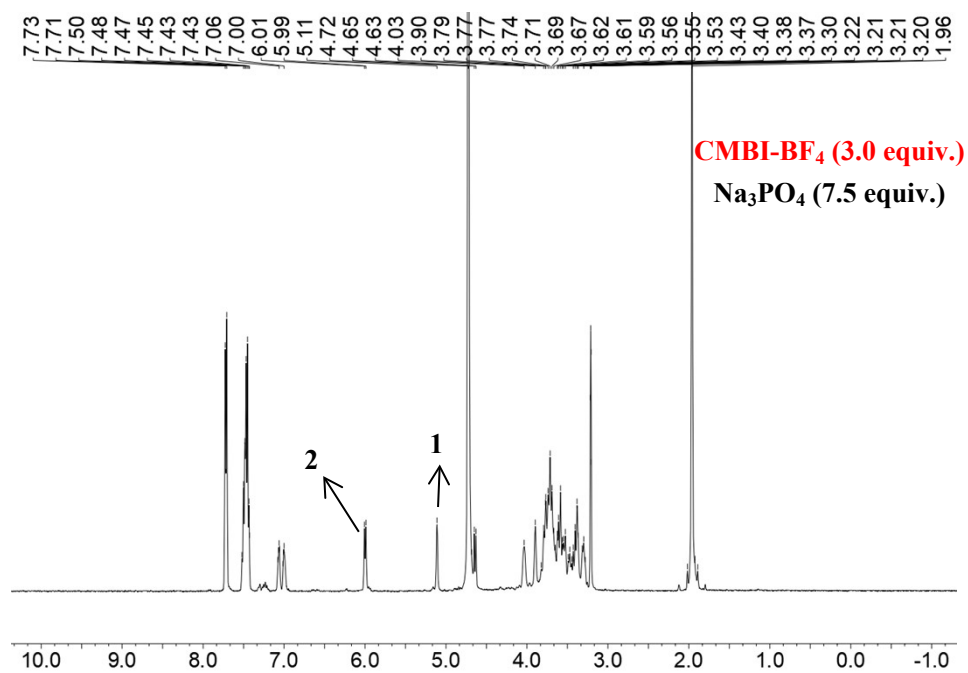
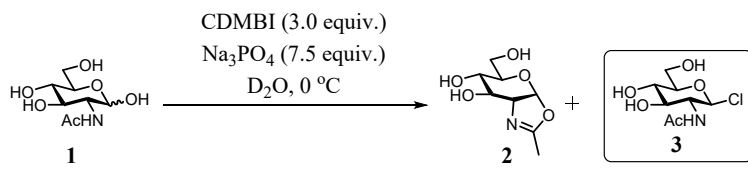


Figure S4. ¹H NMR spectrum of the reaction utilizing CMBI-BF₄ as the activator.

6. Comparison of the effects of reaction time and equivalents of CDMBI

To evaluate the potential impact of reaction time on the yield of by-product **3**, we compared its percentage at different intervals. Table S1 provides a summary of our findings. As the time increased from 5 to 15 minutes, 30 minutes, and 1 hour, we observed a slight increase in overall conversion and corresponding yields for both the desired product oxazoline **2** and by-product **3**. After 1 hour, the conversion was almost complete, and extending the reaction time did not result in a notable change in overall conversion. However, the percentage of by-product **3** did increase slightly, from 9% to 10%, although this increase may be within the margin of experimental error.

Table S1. Comparison of the effect of reaction time^a



Entry	Reaction time	Conv. (%) ^b	Yield (%) ^b	
			2	3
1	5 min	90	72	4
2	15 min	94	76	6
3	30 min	94	79	7
4	60 min	>95	81	9
5	90 min	>95	83	9
6	180 min	>95	83	10

^aReaction Conditions: **1** (0.05 mmol), CDMBI (0.15 mmol, 3.0 equiv.), and Na₃PO₄ (0.375 mmol, 7.5 equiv.) in 0.5 ml of D₂O at 0 °C. ^bDetermined by ¹H NMR with sodium benzenesulfonate as an internal standard.

The impact of the equivalents of CDMBI was also evaluated and compared. As shown in Table S2, the overall conversion was only 58% with a yield of oxazoline **2** at 33% and by-product **3** at 1% when 1 equivalent of CDMBI was used in the reaction. When the amount of CDMBI was increased to 2 equivalents, the conversion rate was raised to 72%, with 54% of oxazoline **2** and 4% of by-product **3**. Further increase of CDMBI to 3 equivalents produced a conversion above 95%, resulting in approximately 83% of oxazoline **2** and 9% of by-product **3**. However, the use of four

equivalents of CDMBI did not significantly increase the yield of oxazoline **2**, and the proportion of by-product **3** reached 11%. These findings suggest that three equivalents of CDMBI are optimal for the conversion process.

Table S2. Comparison of the effect of the equivalents of CDMBI^a

Entry	CDMBI (equivalents)	Conv. (%) ^b	Yield (%) ^b	
			2	3
1	1	58	33	1
2	2	72	54	4
3	3	>95	83	9
4	4	>95	82	11

^aReaction Conditions: **1** (0.05 mmol), CDMBI with varying equivalents, and Na₃PO₄ (0.375 mmol, 7.5 equiv.) in 0.5 ml of D₂O at 0 °C with stirring for 1.5 hours. ^bDetermined by ¹H NMR with sodium benzenesulfonate as an internal standard.

7. Comparison of the effect of the equivalents of CMBI-BF₄

To investigate the potential for improving the relatively low conversion and yield of oxazoline **2** mediated by CDMBI-BF₄, we conducted an experiment to compare the effects of varying the equivalents of CDMBI-BF₄. As shown in Table S3, although

Table S3. Comparison of the effect of the equivalents of CMBI-BF₄^a

Entry	CMBI-BF ₄ (equivalents)	Conv. (%)	Yield (%)
			2
1	3	59	51
2	5	61	48
3	7	70	52

^aReaction Conditions: **1** (0.05 mmol), CMBI-BF₄ with varying equivalents, and Na₃PO₄ (0.375 mmol, 7.5 equiv.) in 0.5 ml of D₂O at 0 °C with stirring for 1.5 hours. ^bDetermined by ¹H NMR with sodium benzenesulfonate as an internal standard.

increasing the equivalents of CDMBI-BF₄ resulted in an increase in overall conversion, the yield of oxazoline **2** remained largely unchanged. These results suggest that factors other than equivalence may be responsible for the low yield of the reaction mediated by CDMBI-BF₄.

8. DMC-mediated synthesis of oxazoline **2**.^[1]

To determine whether the by-product **3** could also be formed in the 2-chloro-1,3-dimethylimidazolinium chloride (DMC)-mediated synthesis of oxazoline **2**, we conducted and analyzed the reaction using the previously reported protocol. Briefly, GlcNAc (27.7 mg, 0.125 mmol) and DMC (63.4 mg, 0.375 mmol, 3 equiv.) were added to a 2.5 ml centrifuge tube equipped with a magnetic stirring bar. Next, 0.5 ml of D₂O was added to the tube, and the resulting solution was swirled for 0.5 min. Finally, Et₃N (156 μ l, 0.375 mmol, 7.5 equiv.) was added, and the resulting mixture was stirred for 0.5 h at 0 °C. Subsequently, the reaction mixtures were filtered through a 0.45 μ m membrane and purified by gel filtration on a Sephadex G-25 column to afford oxazoline **2**. ¹H NMR (500 MHz, D₂O) δ 6.04 (d, *J* = 7.3 Hz, 1H), 4.11-4.03 (m, 1H), 3.92 (t, *J* = 3.5 Hz, 1H), 3.77 (dd, *J* = 12.3, 2.5 Hz, 1H), 3.62 (dd, *J* = 12.4, 6.6 Hz, 1H), 3.55-3.50 (m, 1H), 3.35-3.31 (m, 1H), 2.00 (s, 3H, Me). 3.36 (s, DMI (2*CH₂)), 2.73 (s, DMI (2*N-CH₃)). ¹³C NMR (126 MHz, D₂O) δ 168.00 (DMI, CO), 167.99, 100.56, 73.35, 72.03, 69.26, 66.21, 61.95, 45.10 (2*C, DMI, 2*CH₂), 30.90 (2*C, DMI, 2*N-CH₃), 13.10

The ¹H NMR spectrum showed the presence of a small amount of by-product **3** (Fig. S5), suggesting that the by-product can also form in the DMC-mediated synthesis of oxazoline **2**. This further indicates that the chloride atom in the by-product may come from the chloride counter-ion in the reagent.

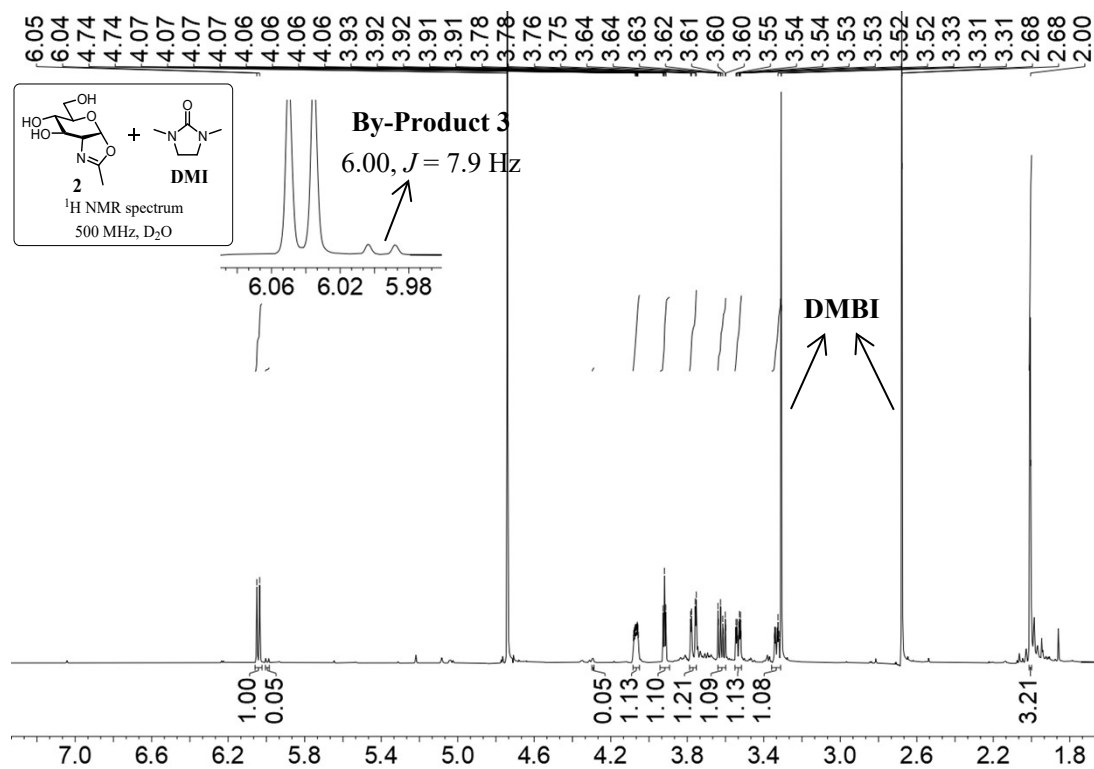


Figure S5. ¹H NMR spectrum of the mixture of oxazoline **2** and its corresponding by-product **3**.

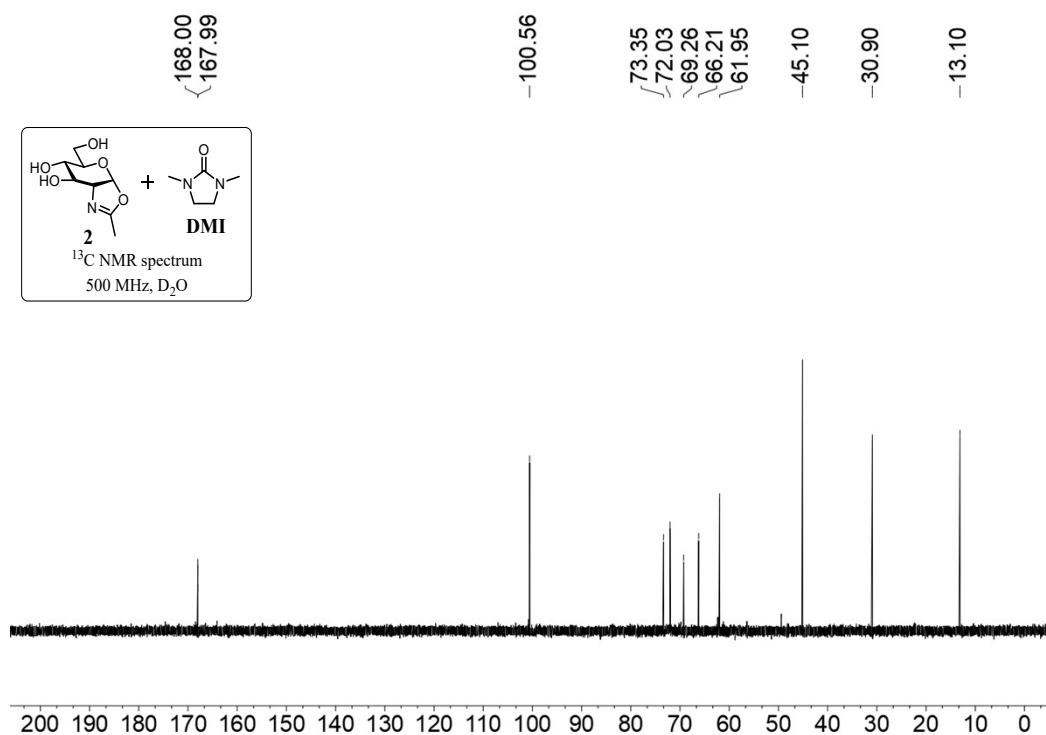
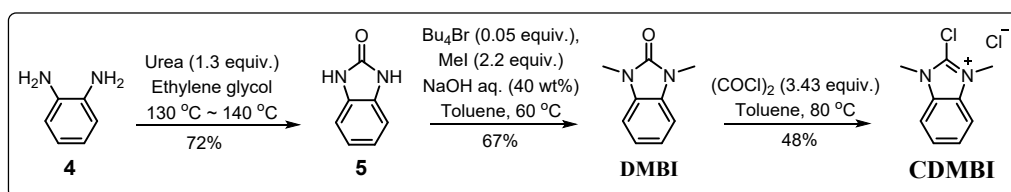


Figure S6. ¹³C NMR spectrum of the mixture of oxazoline **2** and its corresponding by-product **3**.

9. Representative procedure IV for the synthesis of CDMBI. [2]



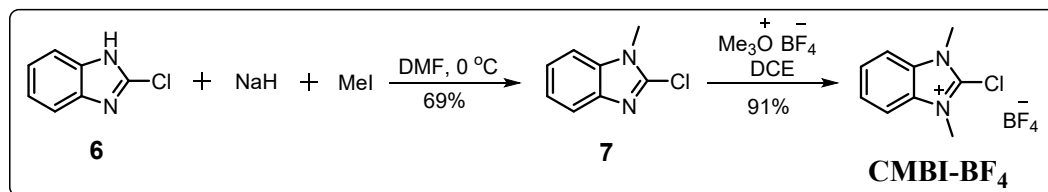
(1) A mixture of benzene-1,2-diamine (12.5 g, 115.5 mmol) and urea (8.33 g, 138.6 mmol, 1.2 equiv.) in 60 ml of ethylene glycol was heated at 135 °C for 25 h. At 10 and 18 h, an additional amount of urea (345.7 mg, 5.775 mmol, 0.05 equiv.) was added into the reaction. The reaction mixture was cooled to room temperature and H₂O was added with stirring to precipitate the product. The resulting precipitate was washed with H₂O and subsequently with toluene, then dried under vacuum to yield 11.2 g of 2-hydroxybenzimidazole **5** with a yield of 72%. ¹H NMR (400 MHz, DMSO-*d*6) δ 10.75-10.38 (m, 2H), 7.08-6.74 (m, 4H). ¹³C NMR (101 MHz, DMSO-*d*6) δ 155.38, 129.73, 120.48, 108.56.

(2) To a mixture of 2-hydroxybenzimidazole (4.024 g, 30 mmol), Bu₄NBr (484 mg, 1.5 mmol, 0.05 equiv.), 40 wt% aq. NaOH (7.6 ml), and toluene (20 ml) was added MeI (4.11 ml) dropwise at 60 °C, and the mixture was heated at the same temperature for 19 h. After cooling, the organic layer was washed with aq. HCl and aq. NaHCO₃, and dried over Na₂SO₄. The solvent was removed in vacuum, and the residue was recrystallized from a mixture of acetone and hexane (1/1, v/v) to yield 3.3 g of **DMBI** in 67% yield. ¹H NMR (400 MHz, DMSO-*d*6) δ 7.14-7.08 (m, 2H), 7.07-7.01 (m, 2H), 3.30 (s, 6H). ¹³C NMR (101 MHz, DMSO-*d*6) δ 153.78, 129.62, 120.81, 107.50, 26.84.

(3) To a solution of **DMBI** (3.2 g, 20 mmol) in toluene (13.3 ml), oxalyl chloride (4.4 ml, 50 mmol, 2.5 equiv.) was added at room temperature, and the resulting mixture was heated to 80 °C. After 49 h, an additional amount of oxalyl chloride (1.6 ml, 18.8 mmol, 0.94 equiv.) was added, and the mixture was stirred at 80 °C for 23 h. The resulting precipitate was washed with toluene and dried in vacuo to give 2.1 g of **CDMBI** (49%). ¹H NMR (400 MHz, D₂O) δ 7.84-7.72 (m, 2H), 7.71-7.57 (m, 2H), 4.03 (s, 6H). ¹³C NMR (101 MHz, D₂O) δ 140.45, 131.14, 127.06, 112.52, 32.36 (d, *J*

= 13.4 Hz).

10. Representative procedure VI for CMBI-BF₄.^[3]



In a Schlenk flask equipped with an overpressure valve, NaH (60%, 432 mg, 10.81 mmol, 1.1 equiv.) was suspended in DMF (10 ml) and cooled to 0 °C. Then 2-chloro-1H-benzo[*d*]imidazole (1.5 g, 9.83 mmol, 1.0 equiv.) was added in portions. The solution was stirred at 0 °C for 15 min. Subsequently, MeI (0.67 ml, 10.81 mmol, 1.1 equiv.) was added dropwise via a syringe. The cooling bath was removed, and the reaction mixture was stirred for an additional 15 min. The reaction mixture was then poured into 50 ml of H₂O, and the resulting precipitate was collected, washed with H₂O (3 x 15 ml), and dried in vacuo to yield product **7** (69%, 1.2 g). ¹H NMR (400 MHz, CDCl₃) δ 7.82-7.53 (m, 1H), 7.40-6.98 (m, 3H), 3.70 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 141.56, 140.84, 135.55, 123.05, 122.58, 119.22, 109.23, 30.39 (d, *J* = 12.8 Hz).

In a Schlenk flask, 2-chloro-1-methyl-1H-benzo[*d*]imidazole (1.2 g, 7.10 mmol, 1.0 equiv.) was dissolved in dry 1,2-dichloroethane (7.5 ml). Trimethyloxonium-tetrafluoroborate (1.21 g, 8.17 mmol, 1.15 equiv.) was then added. The reaction mixture was refluxed for 30 min and then stored overnight at -21 °C. The resulting precipitate was collected, washed with a cold mixture of 1,2-dichloroethane and diethyl ether (10:1, -10 °C, 3 x 3 ml), and dried under vacuum to yield **CMBI-BF₄** (1.73 g, 91% yield). ¹H NMR (500 MHz, D₂O) δ 7.89-7.81 (m, 2H), 7.74-7.67 (m, 2H), 4.07 (d, *J* = 0.9 Hz, 6H). ¹³C NMR (126 MHz, D₂O) δ 140.54, 131.35, 127.19, 112.59, 32.29.

11. Detailed spectroscopic analysis of GlcNAc **1**, oxazoline **2** and by

product 3

A detailed analysis of the mass spectrum presented in Figure 2c revealed that the peak height ratio between $[M(^{35}\text{Cl})\text{-H}^+]$ 238.0482 and $[M(^{37}\text{Cl})\text{-H}^+]$ 240.0456 is approximately 3:1. This ratio is in agreement with the isotopic ratio of chlorine ($^{35}\text{Cl}/^{37}\text{Cl} = 76.75\%/24.24\%$). The lighter isotope ratios of other atoms in the by-product are considerably higher ($^{12}\text{C}/^{13}\text{C} = 98.93\%/1.07\%$, $^{16}\text{O}/^{18}\text{O} = 99.76\%/0.20\%$, and $^1\text{H}/^2\text{H} = 99.985\%/0.015\%$), and therefore, their contribution to the peak height is insignificant. Thus, the observed peak height ratio further indicates that the by-product **3** is a compound containing one chlorine atom.⁴

To verify the identity of oxazoline **2** and its corresponding by-product **3**, their NMR signals were also tentatively assigned based on the ^1H , ^{13}C , and H-H COSY spectra of their mixture that was purified through Sephadex column chromatography.

Oxazoline 2: ^1H NMR (400 MHz, D_2O) δ 5.99 (d, $J = 7.3$ Hz, 1H, **H1**), 4.07-3.99 (m, 1H, **H2**), 3.88 (t, $J = 3.5$ Hz, 1H, **H3**), 3.72 (dd, $J = 12.1, 2.4$ Hz, **H6a**), 3.57 (dd, $J = 12.4, 6.5$ Hz, 1H, **H6b**), 3.51 (dd, $J = 9.0, 3.3$ Hz, 1H, **H4**), 3.29-3.25 (m, 1H, **H5**), 1.94 (s, 3H). ^{13}C NMR (101 MHz, D_2O) δ 168.00, 100.62, 71.54, 68.52, 65.68, 61.57, 56.21, 13.01.

By-product 3: ^1H NMR (400 MHz, D_2O) δ 5.94 (d, $J = 7.8$ Hz, 1H, **H1**), 4.25 (d, $J = 7.9$ Hz, 1H, **H2**), 4.06-4.00 (m, 1H, **H3**), 3.77-3.73 (m, 1H, **H6a**), 3.71-3.63 (m, 1H, **H6b**), 3.41 (dd, $J = 4.6, 2.1$ Hz, 1H, **H4**), 3.37-3.33 (m, 1H, **H5**), 3.32-3.30 (m, 1H), 1.95 (s, 3H). ^{13}C NMR (101 MHz, D_2O) δ 168.59, 100.32, 72.81, 66.05, 62.13, 49.24, 49.20, 12.92.

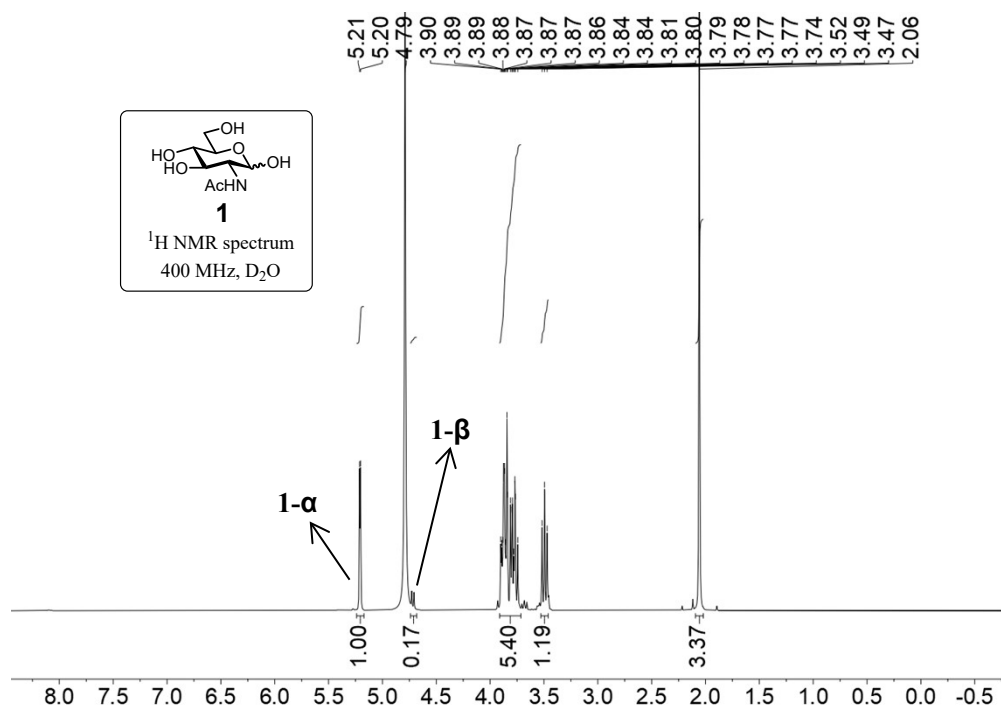


Figure S7. ¹H NMR spectrum of GlcNAc **1** where exist two isomers (**1-α** and **1-β**).

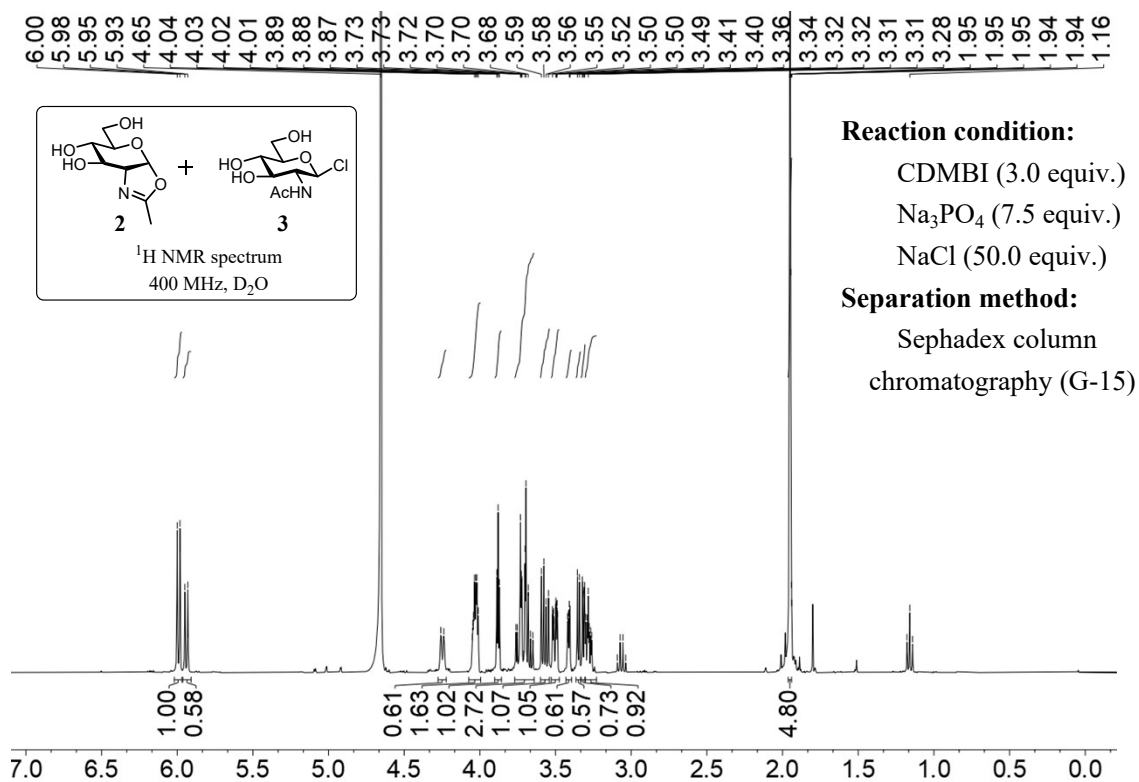


Figure S8. ¹H NMR spectrum of the mixture of oxazoline **2** and its corresponding by-product **3**.

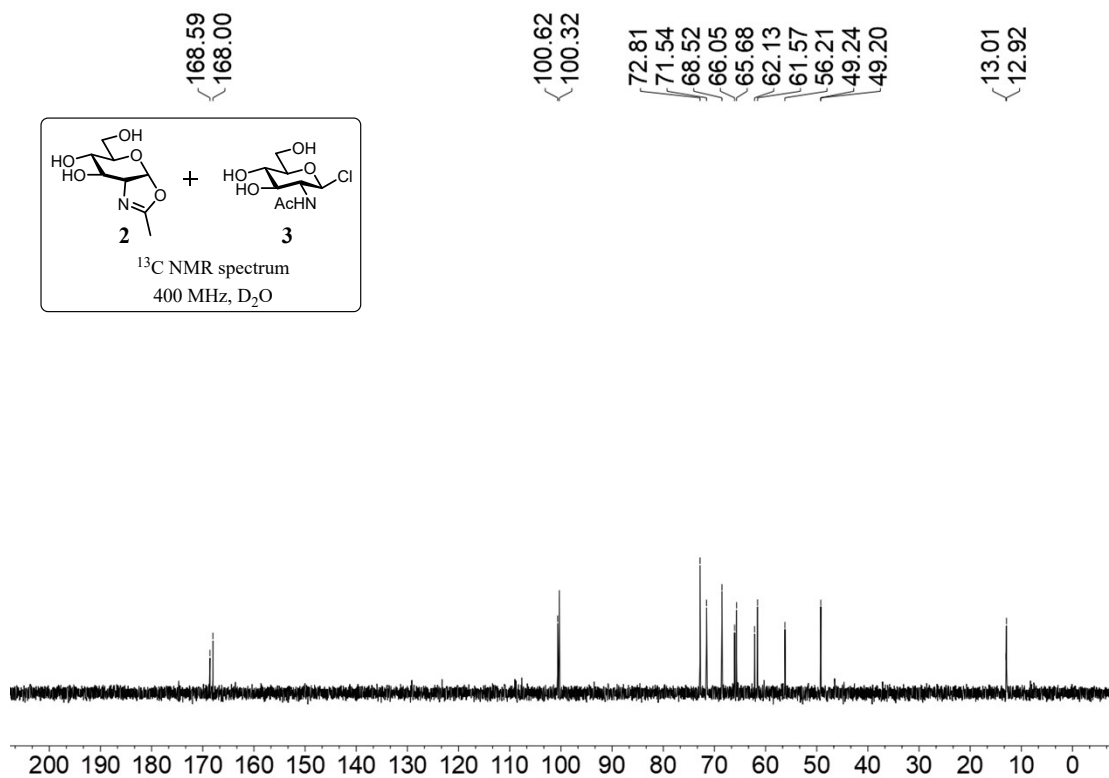


Figure S9. ^{13}C NMR spectrum of the mixture of oxazoline **2** and its corresponding by-product **3**.

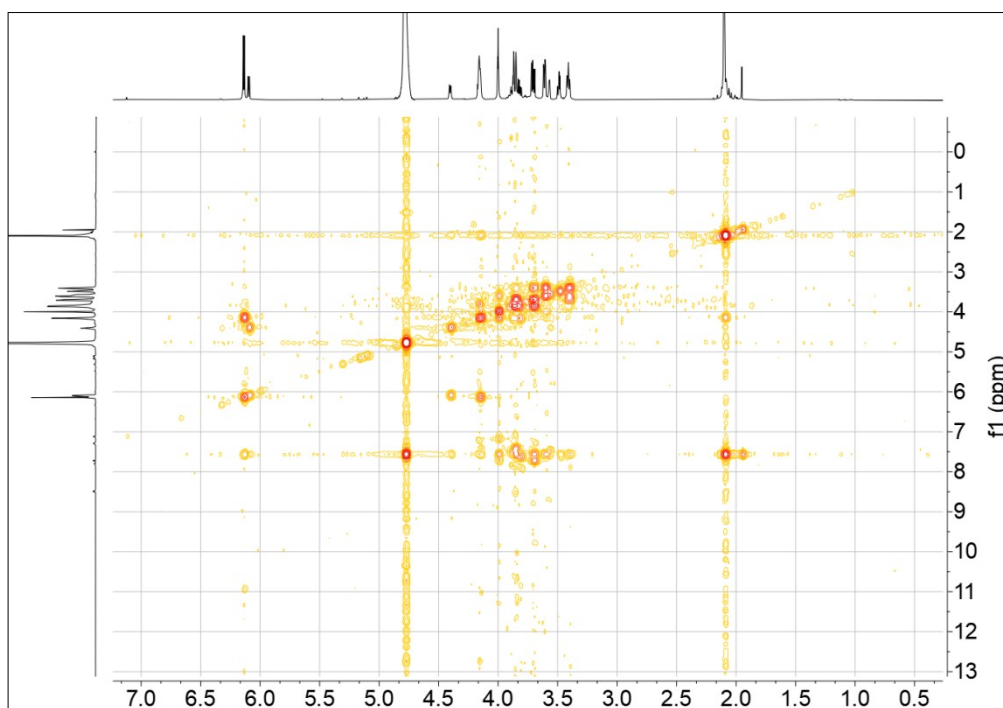


Figure S10. H-H COSY spectrum of the mixture of oxazoline **2** and its corresponding by-product **3**.

12. ^1H NMR and ^{13}C NMR spectra

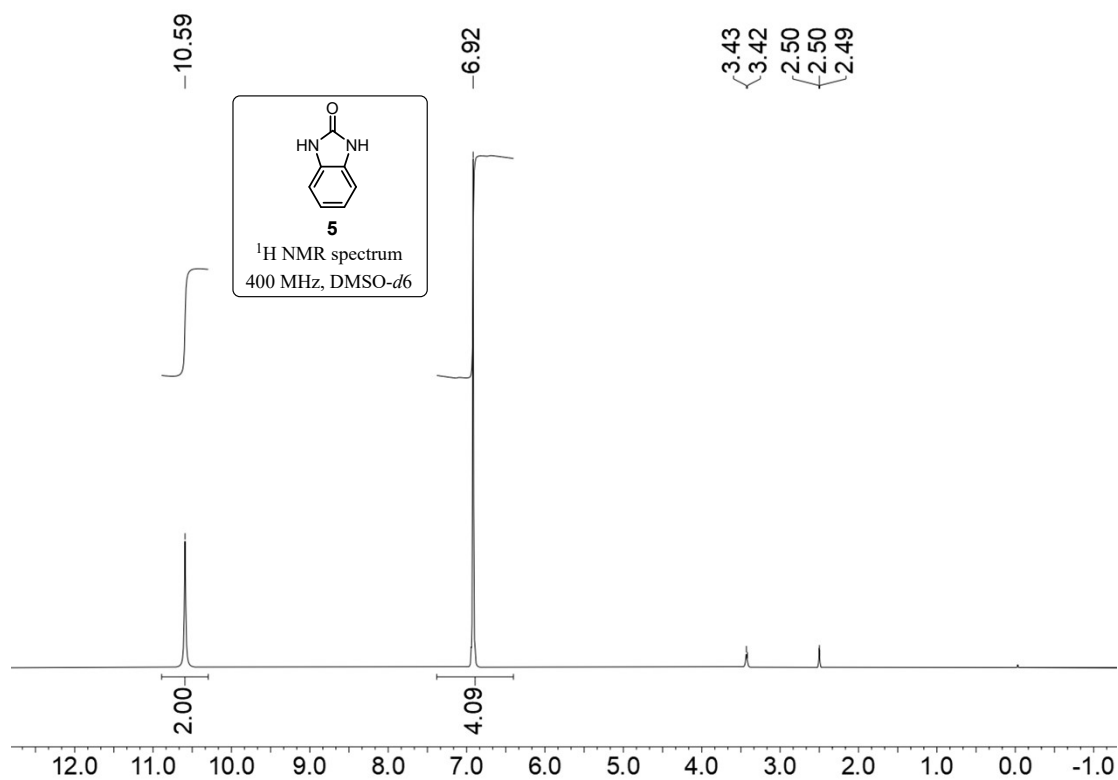


Figure S11. ^1H NMR spectrum of **5**.

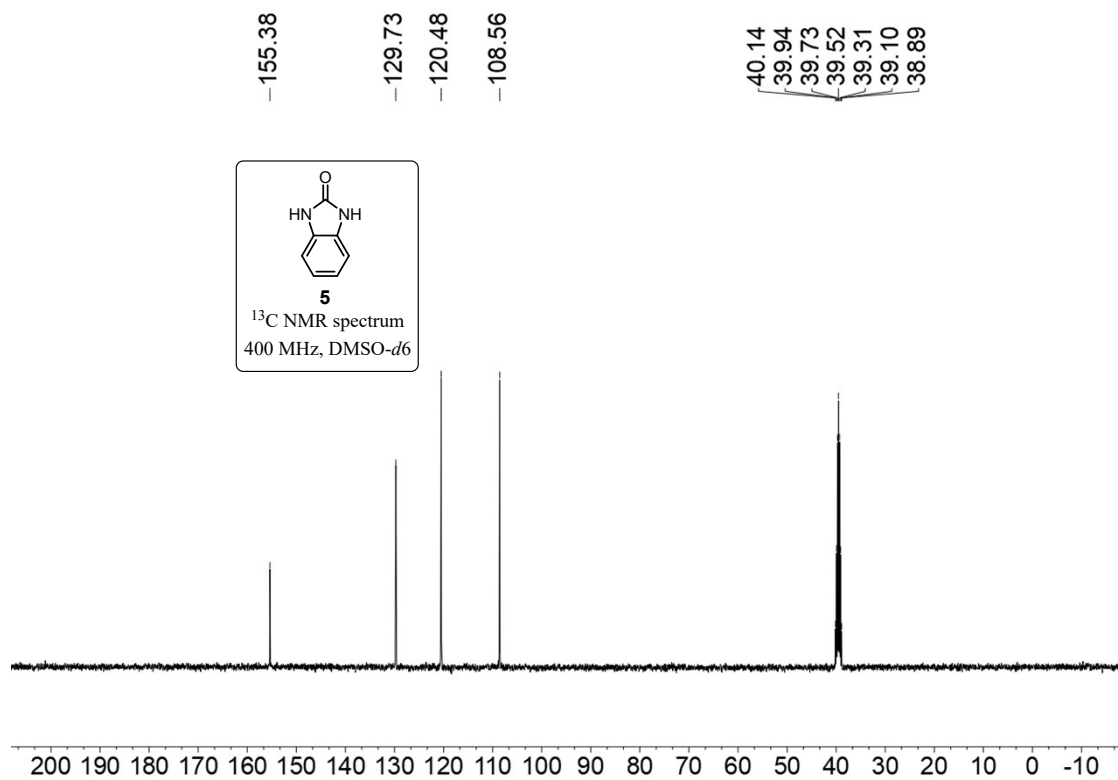


Figure S12. ^{13}C NMR spectrum of **5**.

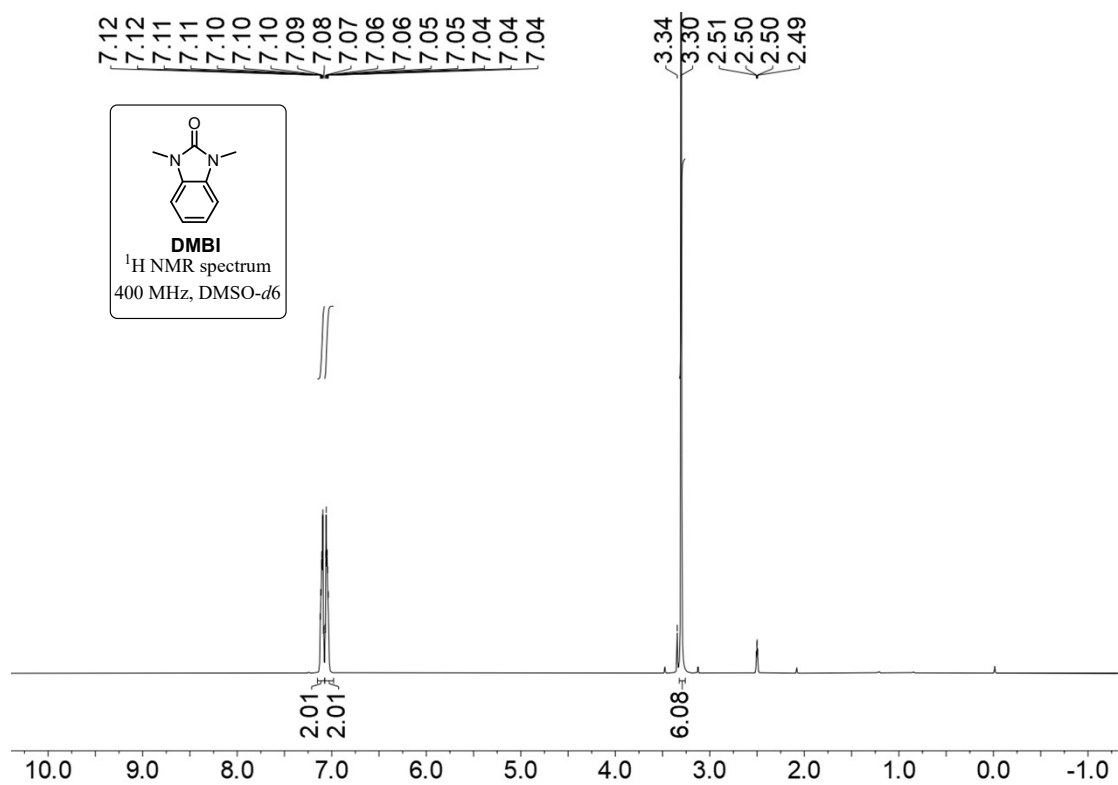


Figure S13. ¹H NMR spectrum of **DMBI**.

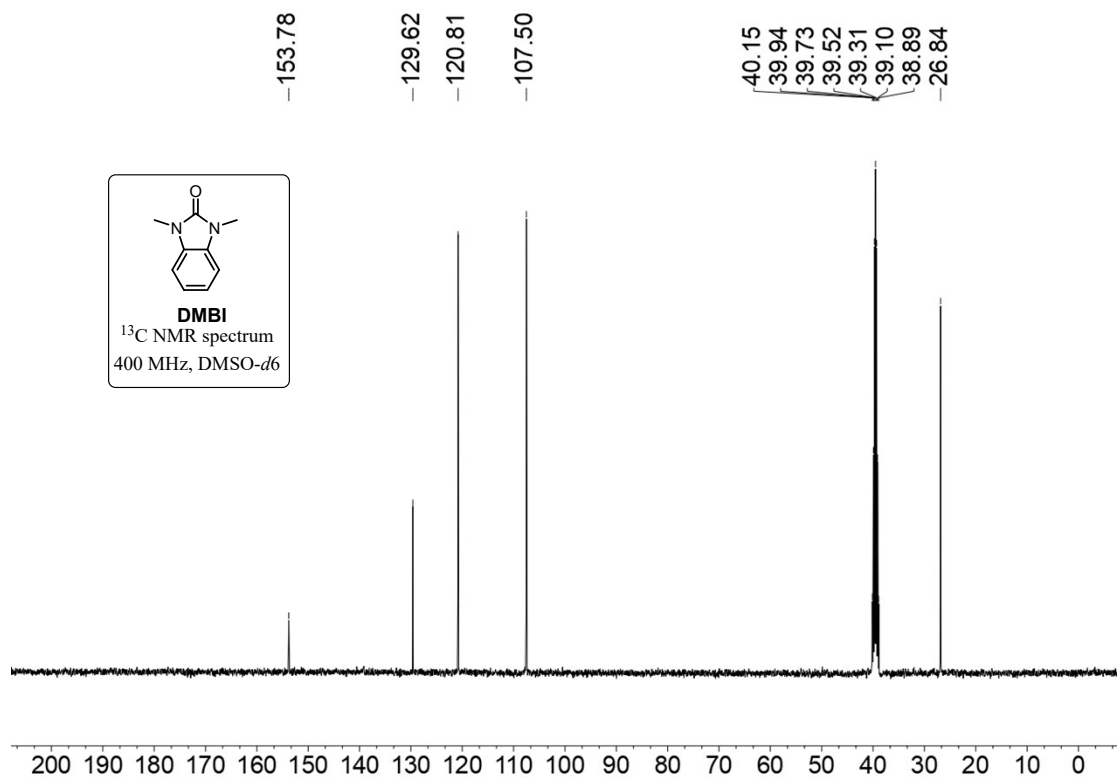


Figure S14. ¹³C NMR spectrum of **DMBI**.

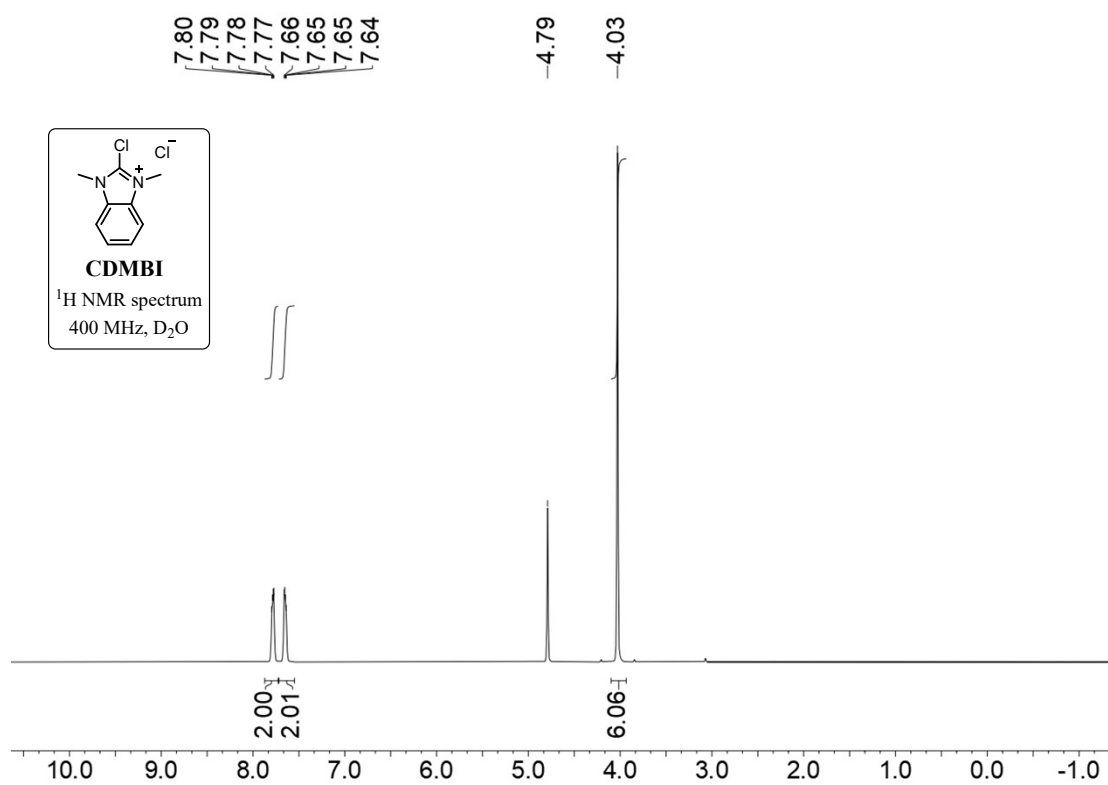


Figure S15. ¹H NMR spectrum of CDMBI.

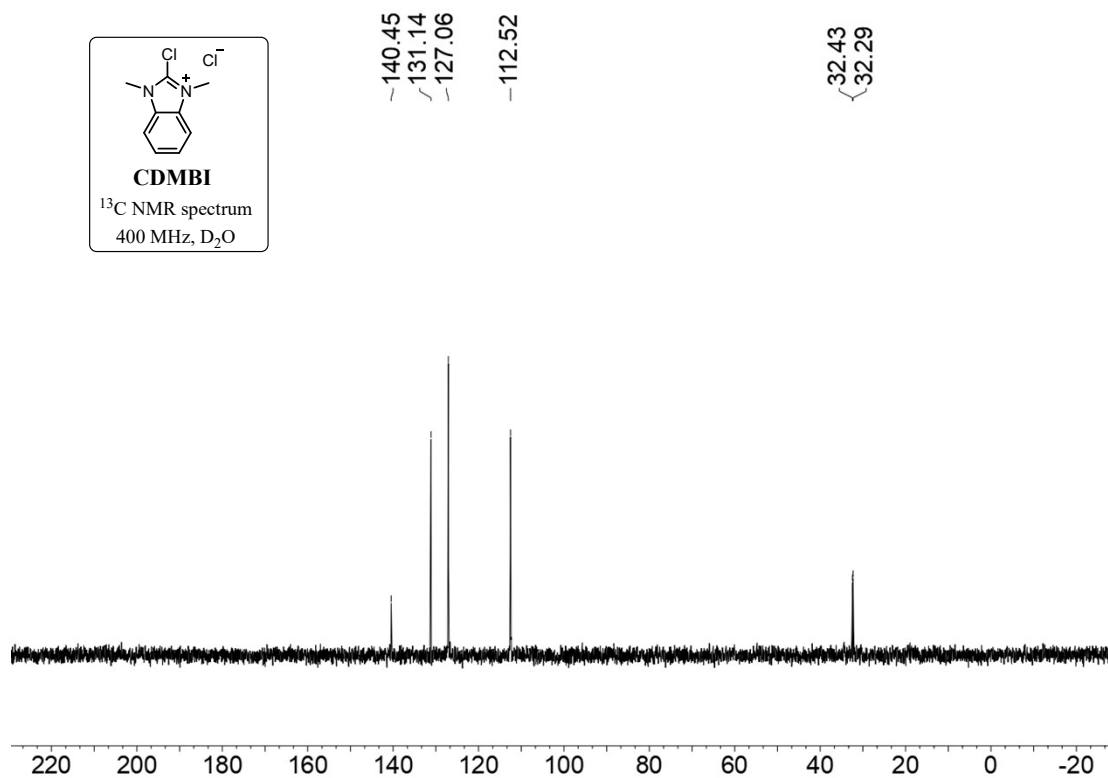


Figure S16. ¹³C NMR spectrum of CDMBI.

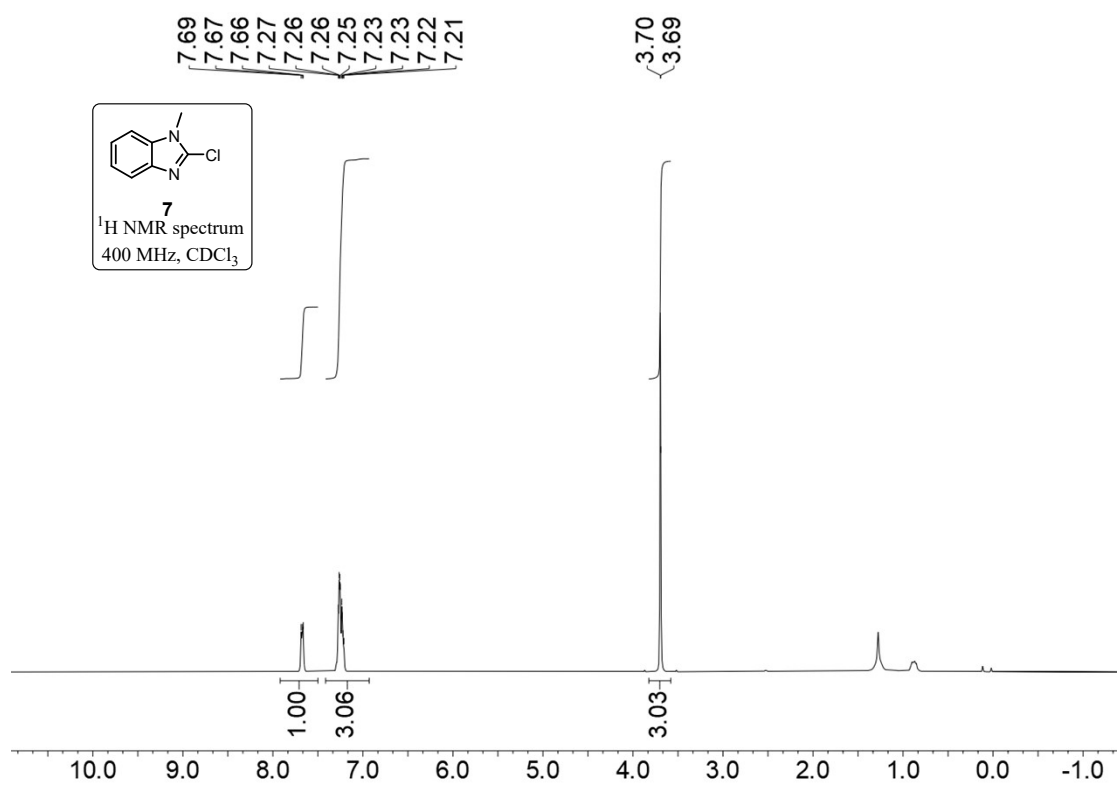


Figure S17. ¹H NMR spectrum of **7**.

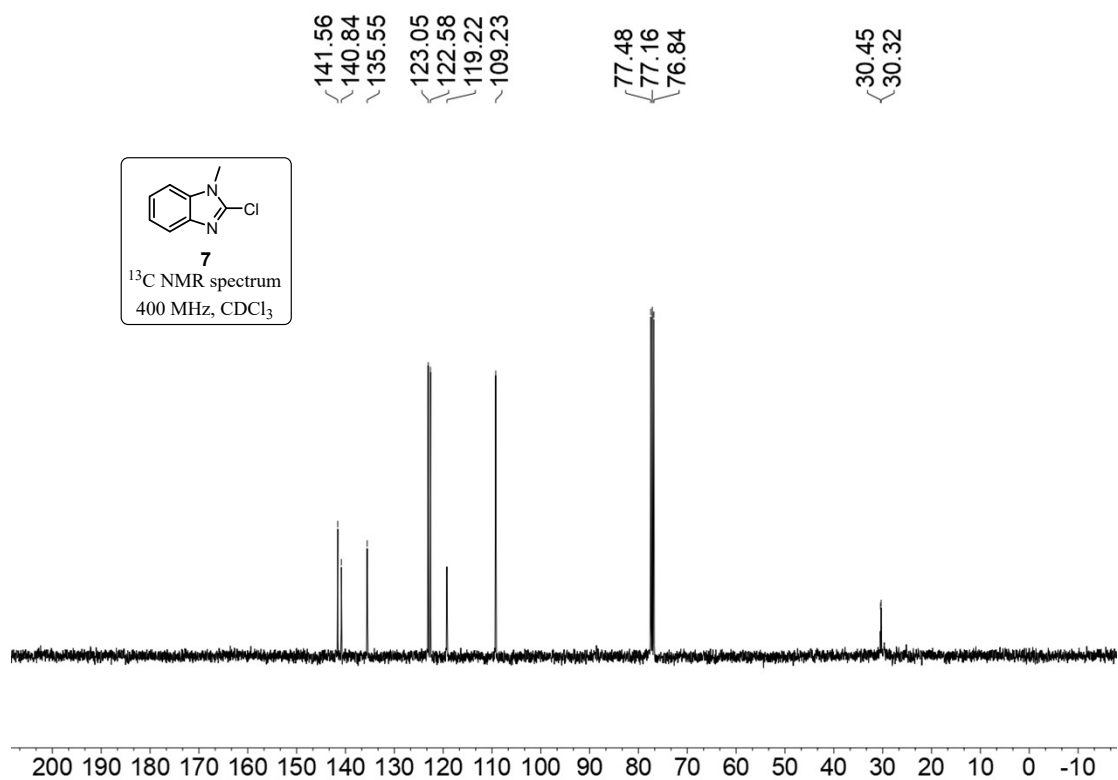


Figure S18. ¹³C NMR spectrum of **7**.

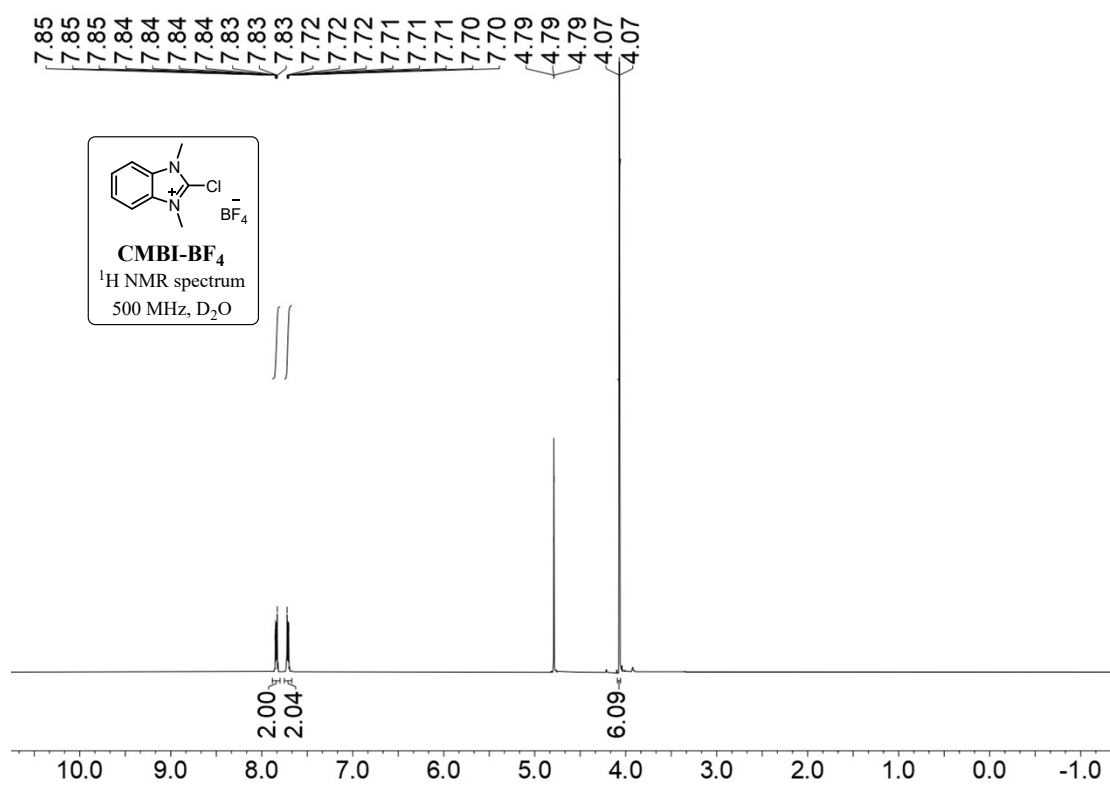


Figure S19. ¹H NMR spectrum of **CMBI-BF₄**.

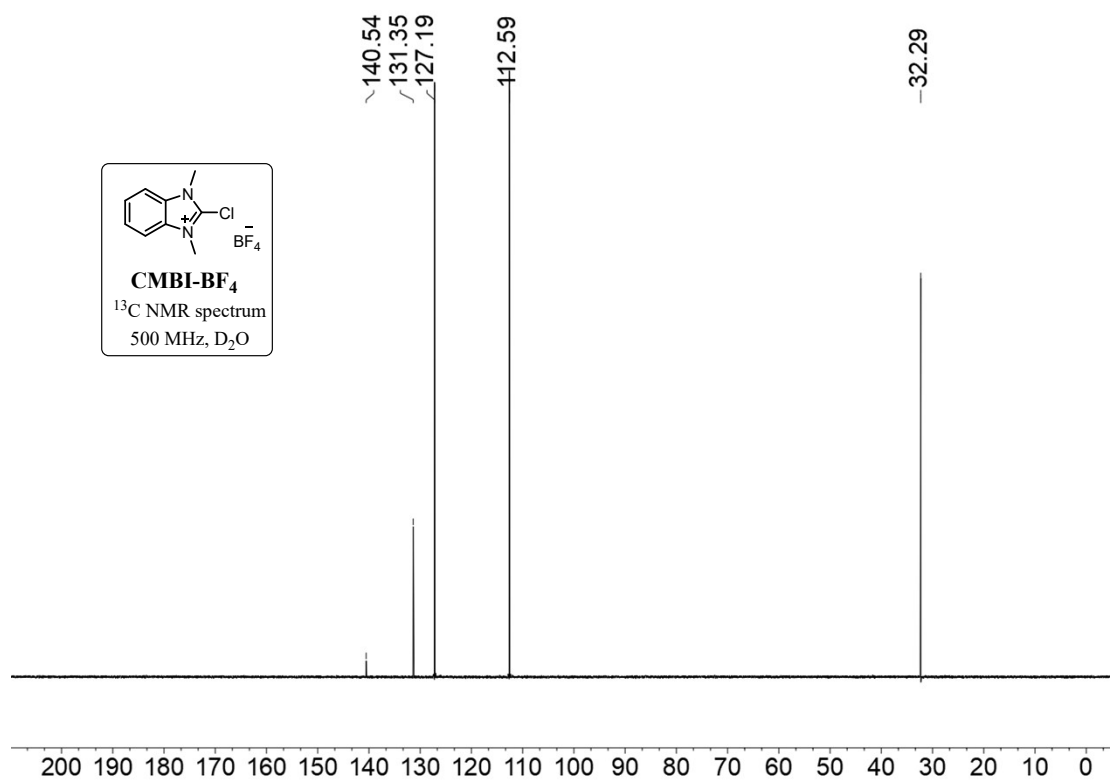


Figure S20. ¹³C NMR spectrum of **CMBI-BF₄**.

13. References

- [1] M. Noguchi, T. Tanaka, H. Gyakushi, A. Kobayashi and S.-i. Shoda, *J. Org. Chem.* 2009, **74**, 2210-2212.
- [2] M. Noguchi, T. Fujieda, W. C. Huang, M. Ishihara, A. Kobayashi and S.-i. Shoda, *Helv. Chim. Acta.* 2012, **95**, 1928-1936.
- [3] M. Werr, E. Kaifer, H. Wadepohl and H.-J. Himmel, *Chem. Eur. J.* 2019, **25**, 12981-12990.
- [4] R. A. Gross, Jr. *J. Chem. Educ.* 2004, **81**, 1161-1168.