Organic Syntheses

Preparation of Mono-Cbz Protected Guanidines

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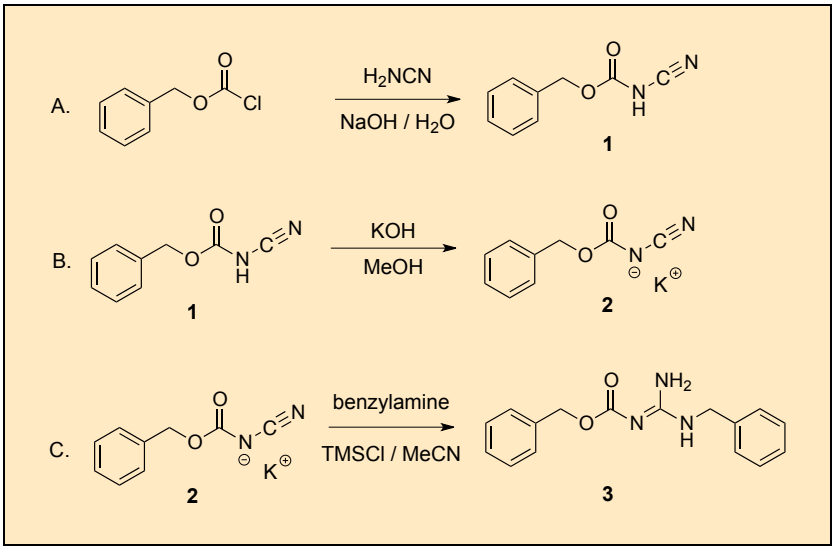
Checked by Joshua N. Payette and Mohammad Movassaghi



Procedure

A. *Carbonylbenzyloxycyanamide (**1**).* A one-necked 500-mL round-bottomed flask open to the atmosphere, equipped with a magnetic stirring bar (Note 1) is charged with cyanamide (50 weight % solution in H_2O) (12.6 g, 11.7 mL, 0.15 mol) (Notes 2 and 3) and distilled water (100 mL). Sodium hydroxide pellets (6.16 g, 0.154 mol, 2.05 equiv) are then added in portions (~3 x 2 g) over a 15 min period. The mixture is then stirred for 30 min at room temperature and then cooled to 0 °C (Note 4). The flask is fitted with a 100 mL addition funnel and the addition funnel charged with

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benzyl chloroformate (12.8 g, 10.7 mL, 0.075 mol, 1.00 equiv) (Note 4). The benzyl chloroformate solution is then added dropwise (~ 1 drop/second) over a span of 15 min. After addition of the benzyl chloroformate the reaction is stirred for an additional 3 h at room temperature. The mixture is transferred to a 250 mL separatory funnel and washed with diethyl ether (1 x 50 mL). The aqueous layer is then transferred to a 1-L Erlenmeyer flask equipped with a magnetic stirring bar (1.7 mm x 3.8 mm) and acidified to pH = 2 (Note 6) with conc. HCl (approx. 7 mL). Dichloromethane (100 mL) is then added to dissolve the solids and the mixture transferred to a 250 mL separatory funnel. After separation of the layers, the aqueous fraction is extracted with dichloromethane (2 x 50 mL) and the combined organics dried over anhydrous Na_2SO_4. The organics are filtered through a sintered glass funnel and the resultant sodium sulfate is washed with dichloromethane (2 x 25 mL). The solvent is removed on a rotary evaporator, and then the flask transferred to a high vacuum line (3.0 mmHg) for 3 h. The title compound is obtained as a viscous light-yellow oil. This material is used in the next step without further purification (Note 7).

B. *Potassium benzyloxycarbonylcyanamide (**2**).* A 250-mL round-bottomed flask open to the atmosphere is equipped with a magnetic stirring bar (1.0 mm x 2.5 mm) and charged with MeOH (100 mL) (Note 8). Potassium hydroxide flakes (4.20 g, 0.075 mol, 1.00 equiv) are then added in portions (~4 x 1 g) and the mixture stirred until all the KOH is dissolved. The flask is then cooled by placement in an ice bath, the internal temperature measured to be ~0 ºC, and the flask fitted with a 125 mL addition funnel. The crude material from Step A is dissolved in MeOH (25 mL) and added dropwise via the addition funnel over 30 min, at which point the solution turns a milky white. The flask is stoppered and allowed to stand in a –20 °C freezer overnight. The crude solid is collected on a Büchner funnel and washed with cold MeOH (2 x 25 mL) to give the first crop of the title compound (Note 9) as a fine white powder (9.46 g, 59%). The filtrate is further concentrated to half the original volume and stored in the freezer to yield a second crop (1.31 g, 8%) (Note 10). The combined solids are dried under air overnight (Note 11).

C. *N-Benzyl, N□-Cbz-guanidine (**3**).* A flame-dried 250-mL single-necked round-bottomed flask equipped with a magnetic stirring bar (1.0 mm x 2.5 mm), and a rubber septum is placed under a nitrogen atmosphere. The flask is then charged with potassium carbobenzyloxycyanamide (**2**) (6.42 g, 30.0 mmol). Acetonitrile (100 mL) is

then added and the mixture stirred vigorously for 15 min. Trimethylsilyl chloride (3.58 g, 4.20 mL, 33.0 mmol, 1.10 equiv) is then added dropwise via syringe over a period of 10 min. The mixture is stirred at room temperature for 30 min and the solution becomes milky. Benzylamine (3.54 g, 3.60 mL, 33.0 mmol, 1.10 equiv) is then added in a single portion, and the solution immediately becomes more opaque. The mixture is then stirred for 1 h. The reaction flask is then transferred to a rotary evaporator and concentrated to dryness under reduced pressure (25 mmHg). The resulting solid is slurried in dichloromethane (350 mL) and transferred to a 500 mL separatory funnel. The organic phase is washed with 1M Na_2CO_3 (100 mL), sat. NaCl (100 mL) and dried over anhydrous Na_2SO_4. The organics are decanted, and the sodium sulfate is washed with dichloromethane (3 x 50 mL). The combined organics are then concentrated under reduced pressure (25 mmHg) in a 1 L round-bottomed flask to give an off-white solid. The solid is slurried in ethyl acetate (100 mL) and transferred to a 500 mL Erlenmeyer flask. The evaporation flask is rinsed with an additional portion of ethyl acetate (40 mL). A stir bar (1.7 mm x 3.8 mm) is then added to the Erlenmeyer flask and the mixture heated to reflux in an oil bath. Methanol is added in portions (20 mL; 120 mL total) until all the solids dissolve. The solution is then passed through filter paper and the filtrate stored in the freezer (–20 °C) for 4 h. The resulting microcrystals were collected on a sintered glass funnel, washed with diethyl ether (40 mL) and then dried under vacuum to give 7.34 g (86%) of a white solid. The mother liquor is then concentrated until solid began to appear (~ 1/3 volume), and the mixture is then allowed to stand in the freezer (–20 °C) for 4 h. The resulting microcrystals were collected on a sintered glass funnel, washed with diethyl ether (40 mL) and then dried under vacuum to give 0.74 g (9%) of a white solid, which was combined with the original crop of crystals to provide 8.08 g (95%) of the product (Notes 12 and 13).

Notes

1. Mixtures were stirred between 600 and 1000 rpm any more or less would cause the stirring to cease when solid precipitates formed. The stir bar used was egg shaped and 3.3 cm in length.
2. All chemicals were purchased from Sigma Aldrich Chemical Co. and were used without further purification. Benzyl chloroformate was

purchased as a technical grade (≥98%) reagent and cyanamide was purchased as a 50% weight solution in water.

3. An excess (2 equiv) of sodium cyanamide are required as the second equivalent deprotonates the acidic acylcyanamide that is formed.
4. Temperature was monitored by a thermocouple placed directly in the reaction mixture.
5. Benzyl chloroformate is moisture sensitive and can produce toxic and corrosive fumes. When used it was open to the atmosphere; exercise caution as accidental contact with water can have severe consequences. Store and handle this chemical in a fume hood with adequate ventilation.
6. pH was determined by EMD Millipore colorpHast® pH Test Strips.
7. This material should be carried to the next step as soon as possible as it is subject to numerous decomposition pathways at room temperature but is reasonably stable in the freezer up to 5 days. The crude material displays the following spectroscopic properties: ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.22 (s, 2 H), 7.34–7.36 (m, 5 H).
8. The crude material from step 1 is very viscous and requires extensive hand stirring (5-20 min) in MeOH before it will completely dissolve. Once completely dissolved in the required amount of MeOH it is much easier to work with.
9. Potassium benzyloxycarbonylcyanamide has been previously shown to be an inhibitor of alcohol dehydrogenase; care should be exercised when handling this compound and it should be handled in a fume hood with adequate ventilation.
10. In a second experiment the checkers received 8.9 g (55%) in the first crop and 3.2 g (20%) in the second crop of crystals.
11. This compound exhibits the following physiochemical properties: R_f = 0.71 (ethyl acetate). mp 219–221 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.86 (s, 2 H), 7.24–7.35 (m, 5 H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 65.2, 122.1, 127.1, 127.3, 128.1, 138.7, 162.5; IR (powder) ν 3060, 3030 (both w), 2189 (s) 2143 (m), 1622 (s), 1398, 1342, 1306, 1178, 1147 (all m), 779, 732, 697 (all s) cm^{-1}. HRMS (ESI-) Calculated for $C_9H_7N_2O_2$ [M-] *m*/*z* 175.0513, obsd 175.0510. Anal calcd for $C_9H_7KN_2O_2$: C, 50.45; H, 3.29; N, 13.07. Found C, 50.56; H, 3.43; N, 13.22.

12. This compound exhibits the following physiochemical properties: R_f = 0.47 (ethyl acetate). mp 160–162 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.37 (d, J = 4.7 Hz, 2 H), 4.96 (s, 2 H), 7.23–7.36 (m, 10 H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 43.4, 65.2, 126.9, 127.1, 127.4, 127.6, 128.2, 128.4, 138.0, 161.5, 163.1. IR (powder) ν 3475, 3283 (both m), 1640, 1617, 1588, 1559 (all s), 1426 (m), 1275, 1131 (both s) cm^{-1}. HRMS (ESI+) Calculated for $C_{16}H_{18}N_3O_2$ m/z (M+H) 284.1394, obsd. 284.1385. Anal. calcd for $C_{16}H_{17}N_3O_2$: C, 67.83; H, 6.05; N, 14.83. Found C, 67.91; H, 6.16; N, 14.83.

13. The checkers obtained 4.17 g (77%) of the product when the reaction was performed on approximately half-scale.

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hazardous materials should be handled using the standard procedures for work with chemicals described in references such as "Prudent Practices in the Laboratory" (The National Academies Press, Washington, D.C., 2011; the full text can be accessed free of charge at http://www.nap.edu/catalog.php?record_id=12654). All chemical waste should be disposed of in accordance with local regulations. For general guidelines for the management of chemical waste, see Chapter 8 of Prudent Practices.

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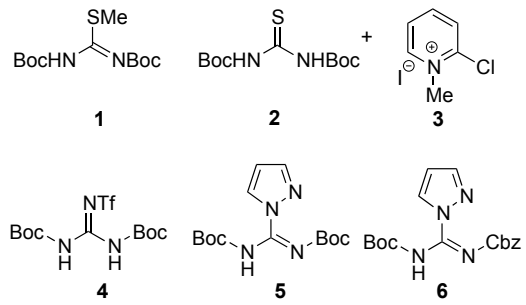
Discussion

The guanidinium ion has proven itself as a valuable motif for molecular recognition, through both electrostatic and hydrogen bond donor-acceptor patterns.[2] These properties have allowed this functional group to be deployed in a vast range of chemical fields from catalysis to medicinal chemistry.[3,4,5,6] It is thus not surprising that its utility has prompted the development of numerous reagents and synthetic methods to introduce the guanidine unit (Figure 1). To avoid problems associated with the high basicity and potential nucleophilicity of the guanidine, most methods typically install a diacylated (or di-protected) guanidine. The most commonly utilized reagent for guanylation is di-Boc-*S*-Me-pseudothiourea

(**1**), which generates a highly reactive carbodiimide intermediate upon reaction with a thiophilic metal salt such as Hg(II), Cu(I) or Ag(I).[7] The parent thiourea **2** can also be used in conjunction with an activating agent (typically Mukaiyama's reagent (**3**)[8] or other suitable peptide coupling reagents such as EDC[9]), although this reagent is very expensive. Goodman's reagent (**4**)[10] is very reactive and is capable of guanylating weakly nucleophilic amines. The pyrazole transfer reagents **5** and **6** have also been developed to obviate the use of toxic metal salts, but these reagents remain prohibitively expensive for use on a preparative scale.[11]

Figure 1. Common reagents for guanylation

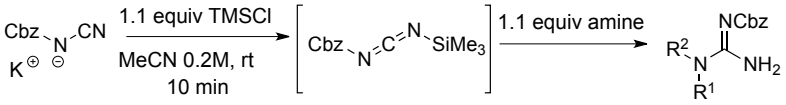


These reagents (**1-6**) are typically used to install the guanidine unit as an *N*,*N*-disubstituted guanidine. For more highly substituted substrates, however, it becomes useful to generate peripheral C-N bonds around the intact guanidine unit, in a selective manner.[12] We became interested in exploiting mono-*N*-acylguanidines in these methodologies, anticipating the ability to exploit a resultant open nitrogen valence for further derivitization. The synthesis of mono-*N*-acylguanidines, via acylation with acid chlorides or anhydrides is often complicated by over acylation due to the increased acidity of the initially formed mono-*N*-acylguanidine.[13] Alternatively they can be accessed by the controlled hydrolysis of diacyl-protected guanidines.[14] Several methods have also been developed for their synthesis from *N*-acyl-thioureas[15] or *N*-acyl-pseudothioureas.[15c] The use of the mono-protected versions of the reagent **5** have also been reported, but are unreactive or poorly reactive toward amines because of their decreased electrophilicity.[11,12i] The differentially protected pyrazole **6** can be employed followed by removal of the Boc or Cbz protecting group.[16] All in all, these methods are difficult to employ in a preparative setting as they utilize costly

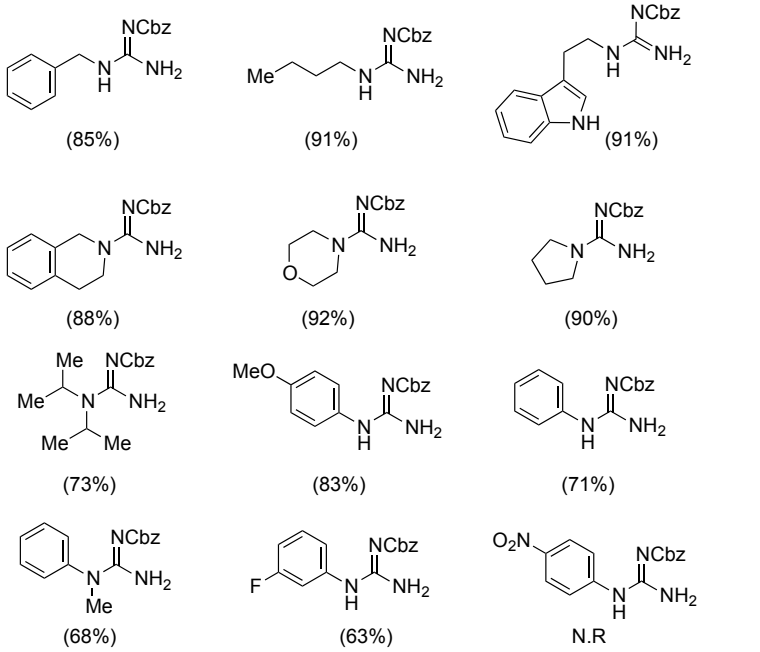
reagents, provide poor yields, and require multiple synthetic manipulations.

While it is known that anilines undergo addition to acylcyanamides, these reactions typically require harsh reaction conditions which can significantly decompose the cyanamide.[17] These addition reactions are limited to anilines because acylcyanamides are quite acidic with pK_a's ~ 2-4.[18] If this reaction could be extended to a variety of amines it would provide an important cheap and practical method for both the installation of a guanidine unit, but also to a selectively protected unit. We considered that silylation might be capable of temporarily activating the

Table 1. TMSCl-mediated Guanylation of Amines with Potassium Benzyloxycarbonylcyanamide



Substrate Scope



cyanamide and masking the acidic proton. Indeed, treatment of potassium carbobenzyloxycyanamide with TMSCl is capable of generating a silylcarbodiimide intermediate which is highly reactive and capable of guanylating a variety of amines except very electron deficient aniline derivatives (Table 1).

References

1. Department of Chemistry, University of Utah, Salt Lake City, UT, 84112. r.looper@utah.edu; REL is grateful to the National Institutes of Health, General Medical Sciences (R01-GM090082) for financial support of this research.
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Appendix
Chemical Abstracts Nomenclature (Registry Number)

Cyanamide; (420-04-2)
Benzyl chloroformate: Benzyloxycarbonyl chloride; (501-53-1)

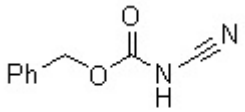
Carbonylbenzyloxycyanamide: Carbamic acid, *N*-cyano-, phenylmethyl ester; (86554-53-2)
Potassium benzyloxycarbonylcyanamide: Carbamic acid, *N*-cyano-, phenylmethyl ester, potassium salt; (50909-46-1)
N-Benzyl, *N'*-Cbz-guanidine: Carbamic acid, *N*-[imino[(phenylmethyl)amino]methyl]-, phenylmethyl ester; (22102-72-3)

Ki Hyeok Kwon was born in 1976 in Seoul, Korea. He studied chemical engineering at the University of Seoul, where he received his B.S. degree in 2002 and M.S. degree in 2005 under the supervision of Prof. Do. W. Lee. After received his degree, he moved to Marquette University where he earned his Ph. D. degree in 2011 for the catalytic coupling reaction involving C-H bond activation under the guidance Dr. Chae S. Yi. He is now pursuing post-doctoral research in the group of Prof. Ryan Looper at the University of Utah. His research focuses on the synthesis and methodology development of complex molecules with biological activity.

Travis Haussener was born in Elmira, NY in 1987. He attended The Pennsylvania State University where he earned a B.S. degree in chemistry in 2009. He is currently working on his Ph. D. at The University of Utah under the direction of Ryan Looper. Travis is interested in the total synthesis of Pactamycin. In his spare time he enjoys climbing, backcountry skiing, and running ultramarathons.

Ryan Looper was born in Banbury, England in 1976. He returned to the U.S. to attend Western Washington University where he earned a B.S. degree in Chemistry in 1998 and an M.S. degree in 1999 under the guidance of J. R. Vyvyan. He obtained his Ph.D. degree in 2004 at Colorado State University in the laboratories of R. M. Williams. After an NIH post-doctoral fellowship at Harvard University with S. L. Schreiber, he began his independent career at the University of Utah in 2007. He is primarily interested in the synthesis of complex natural products with biomedical significance.

Joshua Payette was born in Memphis, Tennessee and graduated from Wheaton College, Illinois in 2005 with a B.S. in Chemistry. In 2005 he joined the research group of Professor Hisashi Yamamoto at The University of Chicago. In 2010 he earned a PhD for his studies in chiral oxazaborolidinium catalyzed cycloaddition reactions. As a postdoctoral research associate in Professor Mohammad Movassaghi 's group at MIT, he is currently pursuing the total synthesis of alkaloid natural products.



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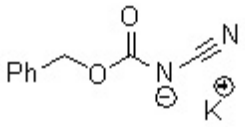
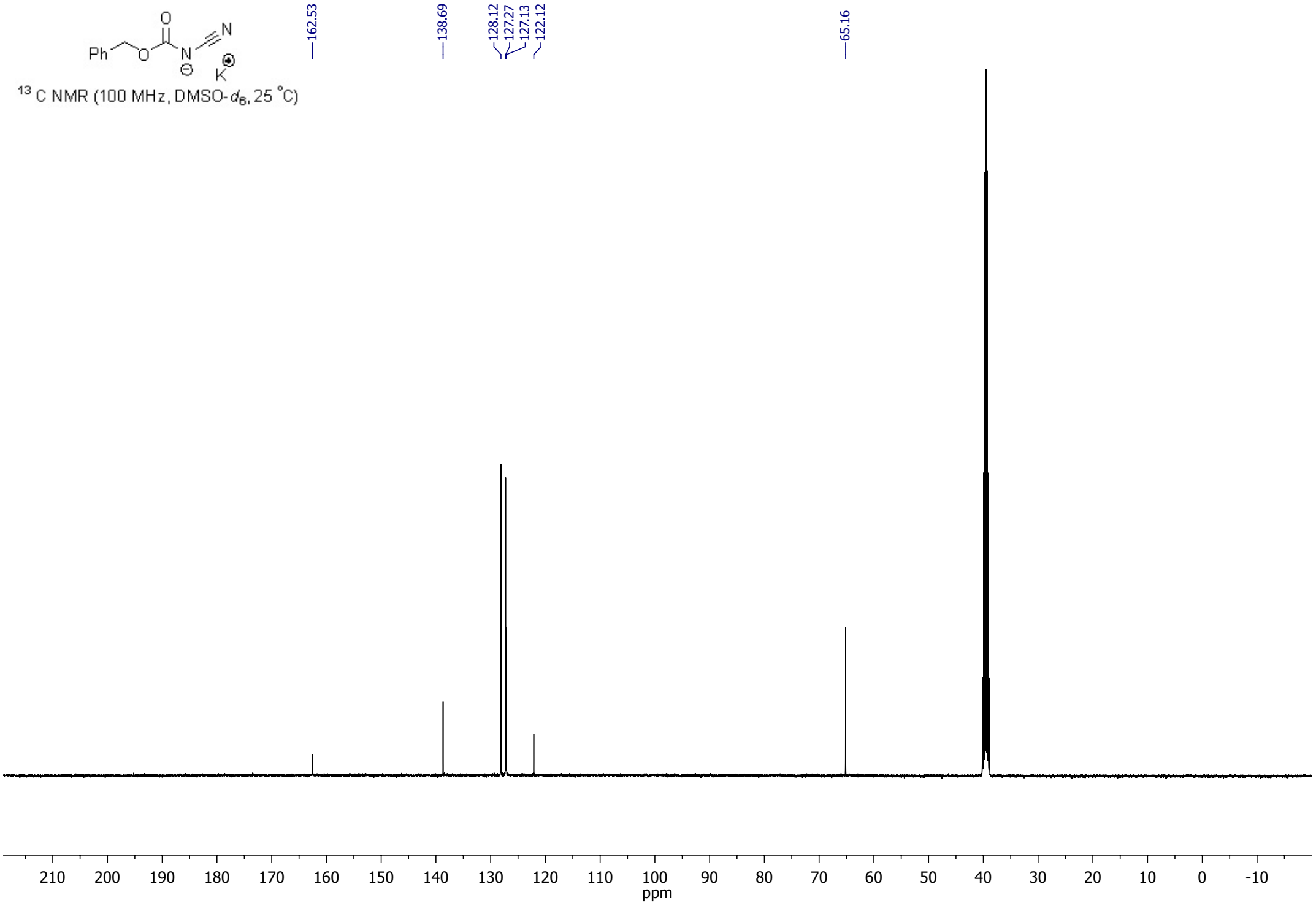


^{1}H NMR (400 MHz, DMSO-d_6, 25 °C)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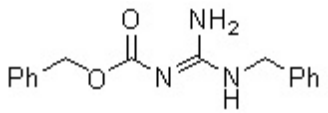

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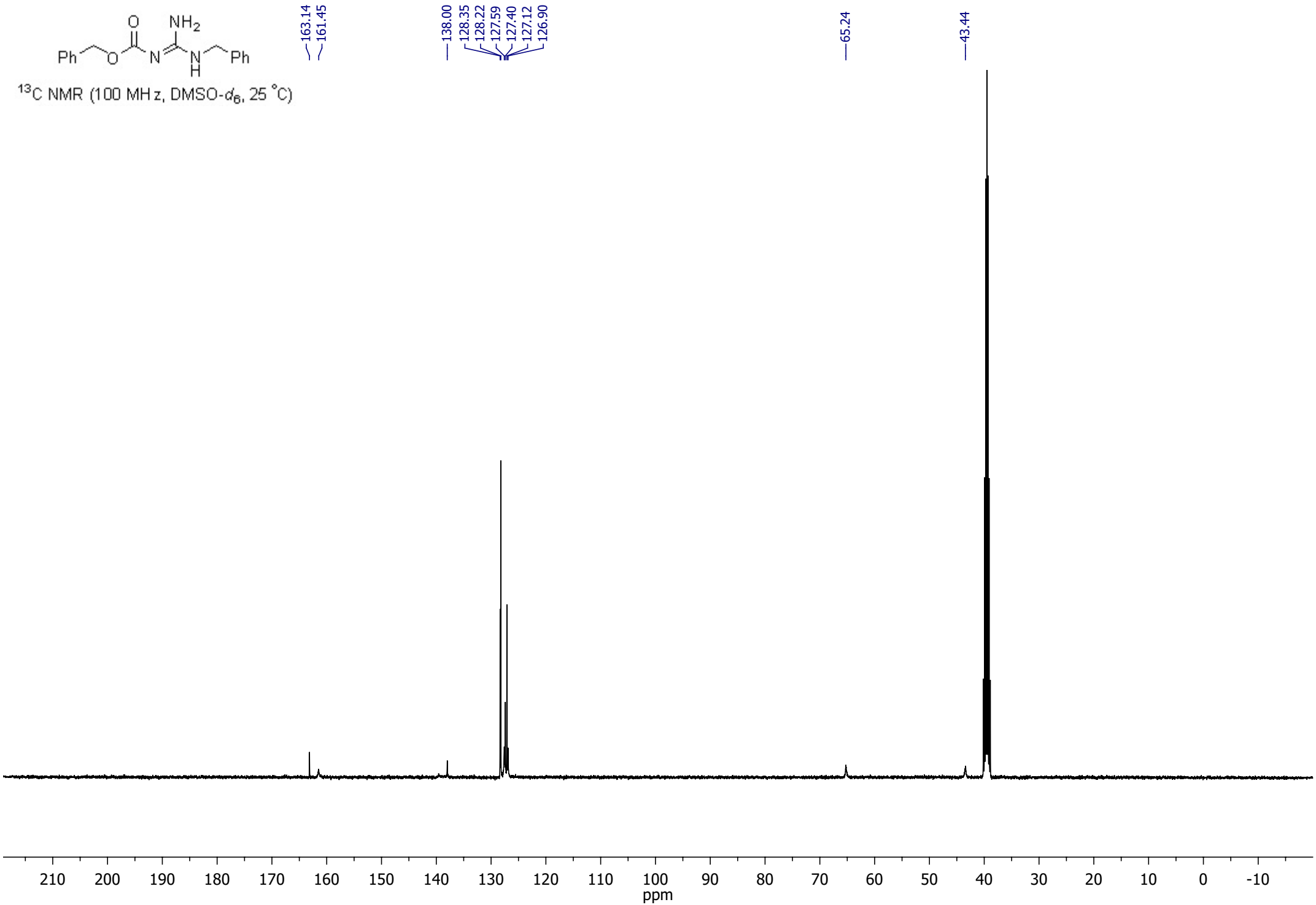
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