Identification of unexpected unlabeled *N,N*-dimethylamide formation in the synthesis of deuterated fragment of ribociclib by a HATU-mediated coupling reaction

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Abstract

Starting from *N*,*N*-dimethylamine and D_2O, deuterated fragment of ribociclib was synthesized for use as an mass spectroscopy (MS) internal standard. Furthermore, systematic studies on D_0 (unlabeled material) formation during the amidation reaction were performed, leading to the identification of a coupling reagent, HATU (*O*-(7-azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate), as main cause. Finally, an alternative route was designed using EDCI/HOBT as coupling reagents to produce the desired deuterated compound without D_0 residue.

Keywards: HATU, amidation, deuteration, *N*,*N*-dimethylamide, internal standard

Introduction

The *N*,*N*-dimethylamide motif is widely distributed in marketed drugs, such as bambuterol,

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jlcr.3690

camazepam, camostat, cariprazine, fimasartan, loperamide and ribociclib (**Figure 1**). The most common method for synthesis of the *N*,*N*-dimethylamide moiety could be dehydration condensation reaction of *N*,*N*-dimethylamine with carboxylic acid in the presence of condensation reagent. [1-4] In one of our projects, a key intermediate of ribociclib was required for use as a deuterated internal standard in further drug development.[5, 6] Then, a synthetic approach of HATU-mediated coupling reaction was designed (**Scheme 1**), and **3** was successfully synthesized in 88% yield.[7] However, trace amounts of **3'** (Mw = 292) was detected in the product **3** based on MS analysis, which didn't meet the criteria for use as a high-qualified deuterated internal standard because of the D_0 residue.[7] Therefore, a thorough investigation was performed in order to explore the cause of D_0 formation, and the result is reported herein.

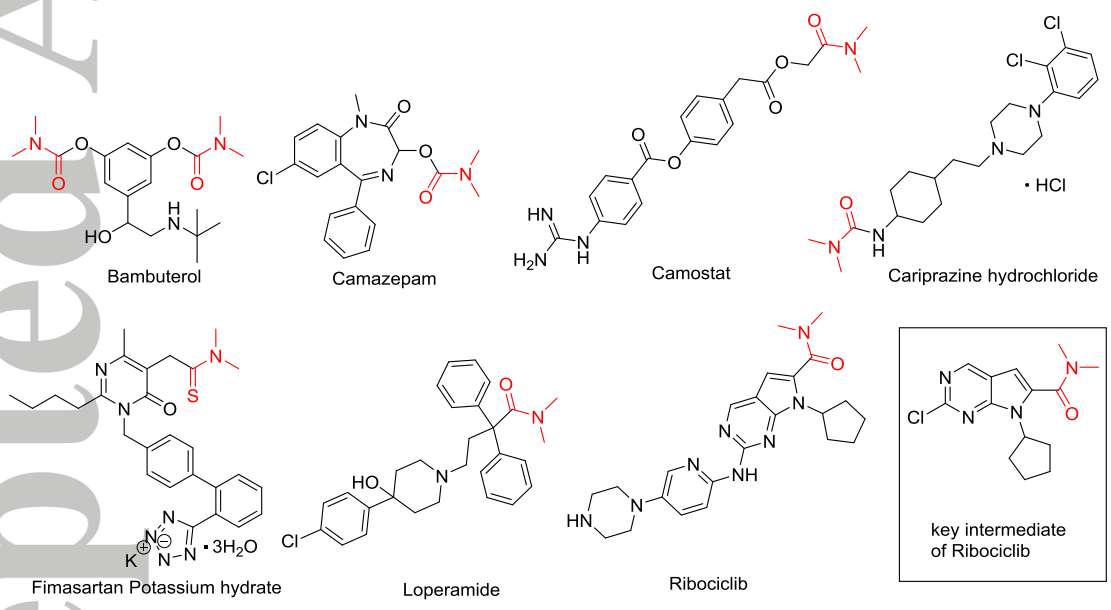
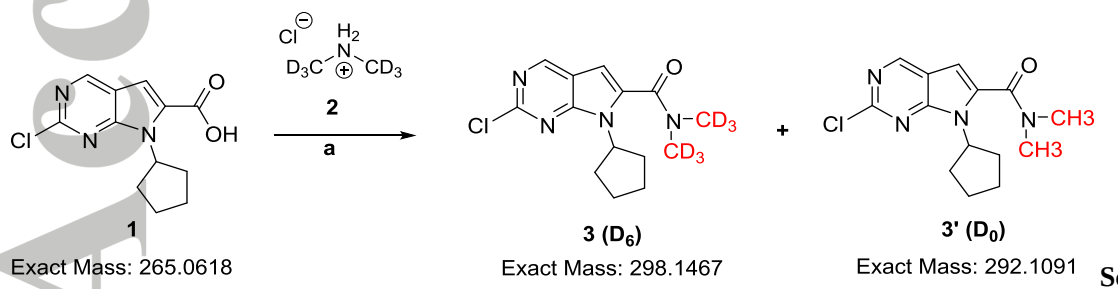


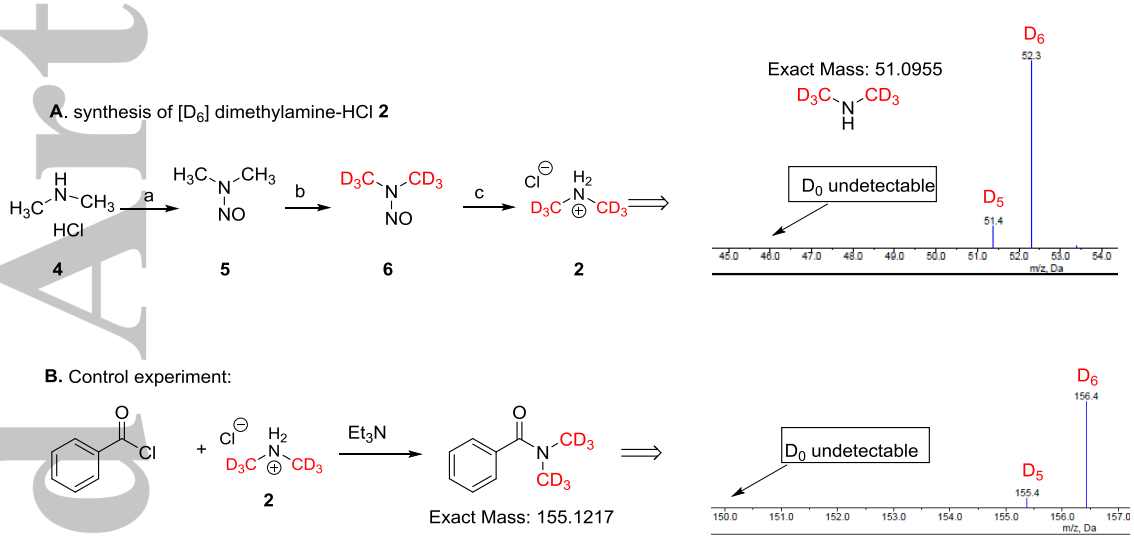
Figure 1. Marketed drugs containing *N*,*N*-dimethylamide motif



Scheme 1. Synthesis of **3** by HATU-mediated coupling reaction. Reagents and conditions: (a) [D_6] dimethylamine hydrochloride **2**, HATU, DIPEA, DMF, r.t., 1 h, 88% yield.

Results and discussions

Firstly, we suspected that our own-prepared [D_6]dimethylamine hydrochloride **2** might contain trace amounts of [D_0]dimethylamine due to the uncompleted deuteration since we started with unlabeled [D_0]dimethylamine hydrochloride to synthesize the [D_6]dimethylamine hydrochloride via a 3-step synthetic sequence: *N*-nitroso formation, base-catalyzed H-D exchange and DCl-promoted hydrolysis as depicted in **Scheme 2**.



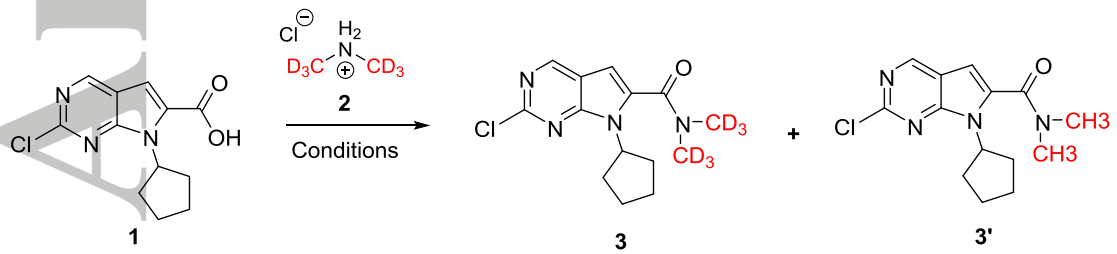
Scheme 2. Synthesis of [D_6] dimethylamine hydrochloride **(2)** and control experiment. Reagents and conditions: (a) $NaNO_2$, 37% HCl, r.t., 17 h, 22% yield; (b) CH_3ONa, D_2O, 80 ^oC, 5 h, 2 cycles, 50% yield; (c) 36% DCl, 90 ^oC, 16 h, 48% yield.

However, when **2** was subjected to MS analysis, no D_0 molecular weight (Mw = 46) was detected (see Scheme 2A). Also, the control experiment by acylation of **2** with benzoyl chloride showed no D_0 formation (Mw = 149) based on MS (see Scheme 2B). Repeated preparation of deuterated *N*,*N*-dimethylamine **2** was conducted for a few times, and the results that D_0 was detected in the synthesis of **3** were identical after coupling with **1** using HATU as a coupling reagent and *N*,*N*-dimethyl formamide (DMF) as a solvent (**Table 1, entry 1**). Therefore, we speculated that the unexpected [D_0] dimethylamine formation could result from an intermediate produced during the process of HATU-mediated coupling reaction, which rules out the possibility of starting material source **2**.

Next, the possibilities of [D_0]dimethylamine coming from solvents and bases were

investigated. We wondered if there was [D_0]dimethylamine residue in the solvent *N*,*N*-dimethyl formamide (DMF). Thus, dicloromethane (DCM) was used as solvent instead of DMF. However, trace amount of **3'** (D_0) was still detected in the reaction mixture (**entry 2**). The result ruled out the possibility of dimethylamine-contaminated DMF. Similarly, we switched *N*,*N*-diisopropylethylamine (DIPEA) to *N*-methylmorpholine (NMM) as base to find out whether dimethylamine residue existed in DIPEA (**entry 3**). Using NMM as a base, the reaction became sluggish, and more impurities were observed in the reaction mixture. However, LC-MS analysis still indicated the D_0 formation in the synthesis of **3**. .

Table 1. Reaction conditions screening[a]



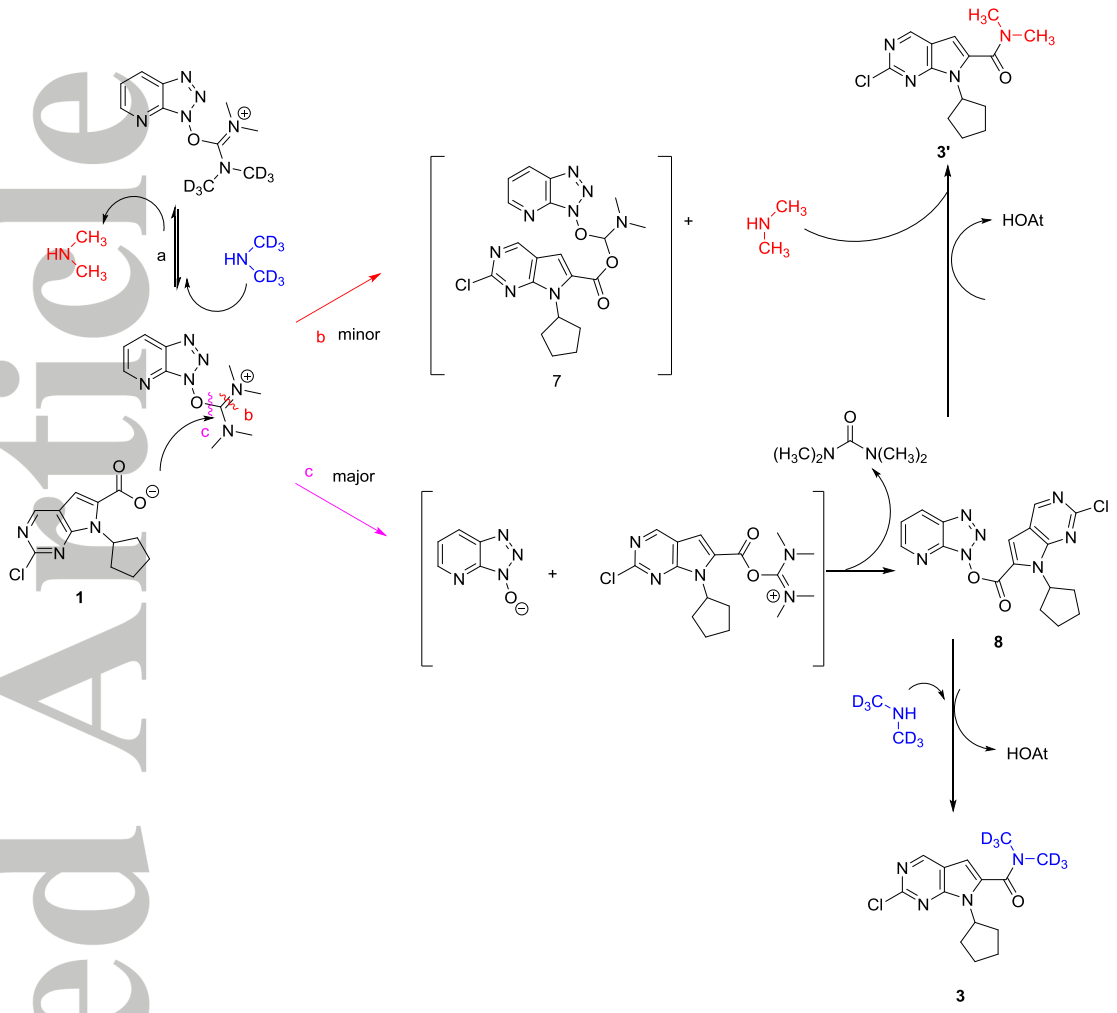
Entry	Reagent	Base	Solvent	D_0%[d]
1	HATU	DIPEA	DMF	1.1%
2	HATU	DIPEA	DCM	0.26%
3	HATU	NMM	DCM	0.75%
4	EDCI/HOBT	NMM	DCM	ND
5	HATU[b]	DIPEA	DCM	1.2%
6	HATU[c]	DIPEA	DCM	0.45%

[a]The reaction was performed with **1** (0.1 mmol), **2** (0.15 mmol), reagent (0.12 mmol), base (0.2 mmol) in solvent (1 mL) at r.t. for 1 h.[b] purchased from J&K, Lot: LOB0Q86 [c]purhcased from Alfa Aesar, Lot: 5019F05S; [d]The percentage was calculated based on the intensity relative to D_6.

Based on the screening study above, we concluded that the issue could be attributed to the use of HATU. To test this hypothesis, an alternative choice of coupling reagents, namely EDCI and HOBT, were chosen instead of HATU (**entry 4**). Indeed, no D_0 formation was observed by MS. Furthermore, to test whether this batch of HATU (purchased from Energy

Chemical) has accidental contamination with [D_0] dimethylamine, another two batches of HATU from different commercial suppliers (J&K and Alfa Aesar) were surveyed, and the result remained the same, showing trace amount of D_0 formation detected by MS (**entry 5, 6**). Consequently, it was confirmed that the D_0 formation (**3'**) was correlated with use of HATU, but did not result from contamination of *N*,*N*-dimethylamine in any solvents and reagents.

Having identified the effect of HATU in the synthesis of **3**, we proposed a mechanism on how D_0 was produced (**Scheme 2**).[9] Firstly, HATU is attacked by the carboxylate anion (deprotonated by DIPEA) to form the unstable O-acyl(tetramethyl)isouronium salt (path c). Then, the OAt anion rapidly attacks the isouronium salt, affording the OAt-activated ester **8**. Alternatively, HATU can be attacked by the carboxylate anion to form intermediate **7**, and *N*,*N*-dimethylamine is liberated in this way (path b). We may not rule out the possibility that a simple [D_o] and [D_6]dimethylamine exchange (path a) can also generate [D_0]dimethylamine. Finally, addition of [D_6]dimethylamine (major source) and [D_0]dimethylamine (trace amount generated during the amidation) to **8** results in the formation of **3** and **3'**.



Scheme 2. Proposed mechanism on D_0 formation (**3'**) in HATU-mediated synthesis of **3**

Conclusions

In summary, we conducted a systematic study to identify the unexpected D_0 formation in the synthesis of a deuterated compound for use as an MS internal standard. A possible mechanism is proposed for D_0 formation in the HATU-mediated synthesis of *N*,*N*-dimethylamide moiety. The result prompted us to believe that the use of HATU-like coupling reagents, such as HBTU, TBTU, AOP and so on[10], should be avoided in the synthesis of deuterated internal standard compounds containing *N*,*N*-dimethylamide moiety.

EXPERIMENTAL

Materials and Instruments

Unlabeled intermediate (**1**) and authentic reference standards were supplied by Process Chemistry Institute of Chia Tai Tianqing Pharmaceutical Group Co. All other reagents and solvents were commercially available and used without further purification. NMR spectra were recorded on a Bruker Avance 500 spectrometer. Chemical shifts (δ) in ppm are quoted relative to D_2O or DMSO-d_6. High-resolution mass spectra were recorded on AB SCIEX Triple TOF 4600. TLC analysis and visualization were performed using UV light (254 nm). Column chromatography was performed using a TELEDYNE ISCO CombiFlash Rf+ Flash Chromatography system.

N,*N*-Dimethylnitrous amide **(5)**

Dimethylamine hydrochloride (40.0 g, 491 mmol) was dissolved in water (200 mL), then 37% hydrochloric acid was added. A solution of $NaNO_2$ (37.2 g, 540 mmol) in water (70 mL) was slowly added into the mixture at 0 ^oC over 10 min. The reaction mixture was stirred overnight (17 h) at room temperature. On completion, the mixture was extracted with DCM, and combined organic phases were dried over Na_2SO_4. After filtration, the mixture was concentrated under vacuum to afford crude yellow oil (29.8 g). The crude oil was purified by reduced pressure distillation (80 ^oC, 31 mbar) to afford the light yellow oil (7.6 g, 22% yield). ^1H NMR (300 MHz, $CDCl_3$): δ 3.76 (s, 3H), 3.04 (s, 3H); GCMS: calculated for $C_2H_6N_2O$ ($[M]^+$): 74, found 74 $[M]^+$.

N,*N*-Bis([D_6]methyl) nitrous amide **(6)**

A solution of **2** (5.0 g, 67.5 mmol) in D_2O (50 mL) was added CH_3ONa (10.94 g, 202 mmol), and the mixture was stirred under N_2 at 80 ^oC for 5 h. Then the mixture was concentrated under vacuo, and D_2O (50 mL) was added. The reaction mixture was stirred under N_2 at 80 ^oC for another cycle. This procedure was repeated until no D_0 was detected by GCMS. After completion, the mixture was extracted with DCM (100 mL x 3), and combined organic phases were dried over Na_2SO_4. After filtration, the mixture was concentrated under vacuum to afford yellow oil (2.7 g, 50%).[11] No α-CH_3 resonances were detectable by

^{1}H-NMR ($CDCl_3$). GCMS: calculated for $C_2D_6N_2O$ ($[M]^+$): 80, found: 80 $[M]^+$.

[D_6] Dimethylamine hydrochloride **(2)**

DCl (2.34 g , 62.5 mmol) was slowly added into **3** (2.0 g, 25.0 mmol) at 0 ^{o}C over 5 min. The mixture was then stirred overnight at 90 ^{o}C. The solvent was evaporated under vacuum, and white solid was obtained (2.1 g, 48% yield). The product was stored under vacuum to avoid air contact. No α-CH_3 resonances were detectable by ^{1}H-NMR (D_2O). [12] MS (ESI): *m/z* 52 $[M+H]^+$.

Procedure for the synthesis of **3 (entry 4)**

A solution of **1** (500 mg, 1.882 mmol) in DCM (15 mL) was added EDCI (433 mg, 2.258 mmol), HOBT (346 mg, 2.258 mmol) and NMM (381 mg, 3.76 mmol). The resulting mixture was stirred at room temperature for 10 min, and **2** (247 mg, 2.82 mmol) was added. After stirring for 1 h, it was diluted with DCM (100 mL) and washed with water (100 mL x 3). The organic fractions were combined and dried over Na_2SO_4. After filtration, the mixture was concentrated and purified by flash chromatography. The product was obtained as light yellow solid (260 mg, 48% yield). **^{1}H NMR** (500 MHz, DMSO-d_6): δ 8.97 (s, 1H), 6.80 (s, 1H), 4.82 (quint, J = 8.5 Hz, 1H), 2.26 - 2.20 (m, 2H), 2.07 - 1.99 (m, 2H), 1.97 - 1.91 (m, 2H), 1.67 - 1.62 (m, 2H); **^{13}C NMR** (125 MHz, DMSO-d_6): δ 162.67, 153.03, 152.80, 151.76, 136.46, 117.67, 100.23, 57.52, 30.98, 24.87; **HRMS** (ESI) calculated for $C_{14}H_{11}D_6ClN_4O$ ($[M+H]^+$) *m/z*: 299.1546, found 299.1598.

Acknowledgement

The authors would like to thank Jie Wang, Yuhan Li and Jianghao Ma for their generous help on Mass and NMR analysis, as well as Process Chemistry Institute of Chia Tai Tianqing Pharmaceutical Group Co. (CTTQ) for their supply of starting materials.

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