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Tetrahedron Letters 46 (2005) 7299–7302
Tetrahedron Letters

An efficient synthesis of LY544344·HCl: a prodrug of mGluR2 agonist LY354740

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Received 10 August 2005; revised 26 August 2005; accepted 26 August 2005
Available online 12 September 2005

Abstract—LY544344·HCl was efficiently prepared in two steps from LY354740. The key step highlighted the in situ masking of the carboxylic acid groups as trimethylsilyl esters to facilitate an effective acylation reaction.

LY354740 (**1**) is a selective and potent agonist for group II metabotropic glutamate (mGlu) receptors, specifically mGlu2 and mGlu3, and has demonstrated anxiolytic activity in several pre-clinical animal models and in patients suffering from Generalized Anxiety Disorder (GAD).[1] During the course of clinical development, however, it was determined that **1** had relatively low oral bioavailability (3–5%) in humans.[2] Thus, prodrugs of **1** were prepared and evaluated for their ability to deliver higher plasma concentrations of **1**. The functionality present in **1** made it an ideal substrate for the preparation of potential prodrugs. After a thorough structure–activity relationship study of various ester and amide derivatives, *N*-acyl derivative, LY544344·HCl (**2**) was found to serve as an effective prodrug of **1**.[3] Subsequent clinical studies revealed that administration of **2** resulted in a 13-fold increase of the estimated human bioavailability of **1** (Fig. 1).

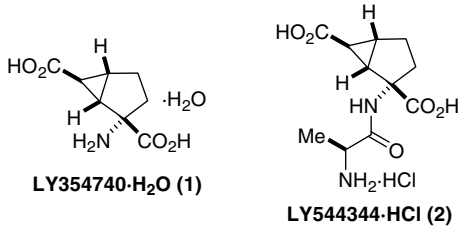

Figure 1. LY354740·H_2O and LY544344·HCl.

At the time of the discovery of **2**, LY354740 (**1**) had progressed well into clinical development. Since **2** appeared to be a superior clinical candidate, however, a very aggressive clinical development plan for **2** was assembled. A concise, efficient, and scalable synthesis of **2** was a key component of this aggressive development plan. After an extensive analysis of de novo synthetic routes to **2** that did not incorporate LY354740 (**1**), we determined that **2** could be prepared most efficiently from LY354740 (**1**). One complicating factor to this strategy was the limited availability of LY354740 (**1**). Furthermore, due to the length of the synthetic route to LY354740 (**1**),[4] the time constraints imposed by this aggressive development strategy would not allow for the preparation of more LY354740 (**1**). Thus, to conserve the inventory of LY354740 (**1**), a highly efficient synthesis of **2** became even more paramount.

The Discovery Chemistry synthesis of **2** is shown in Scheme 1 (route A).[3] It commenced with the esterification of LY354740 (**1**) to provide diester **3** in 98% yield. EDCI mediated coupling of **3** with *N*-(*tert*-butoxycarbonyl)-L-alanine (Boc-L-Ala) (**4**) provided **5** in 50% yield. Subsequent ester hydrolysis and *t*-butoxycarbonyl (Boc) group removal afforded the desired **2** in 80% yield. Given the limited availability of LY354740 (**1**), a more efficient synthesis was required to deliver the initial quantities of material for clinical development. In short order, route A was optimized as shown in Scheme 1 (route B). LY354740 (**1**) was esterified as previously described. Diester **6** was not isolated but taken directly into the next reaction. After a brief screening of coupling reagents and conditions, we found the coupling of Boc-L-Ala and **6** could be accomplished efficiently

Keywords: Metabotropic glutamate receptors; Peptide coupling; Prodrugs; Trimethylsilyl esters.
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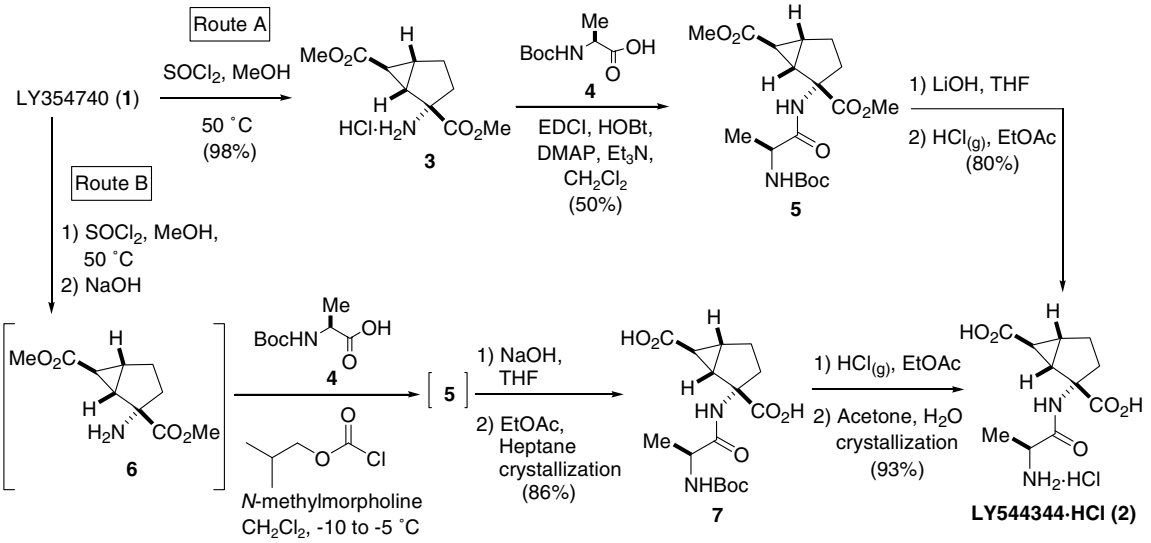
doi:10.1016/j.tetlet.2005.08.144



Scheme 1. First and second generation synthetic routes to LY544344·HCl.

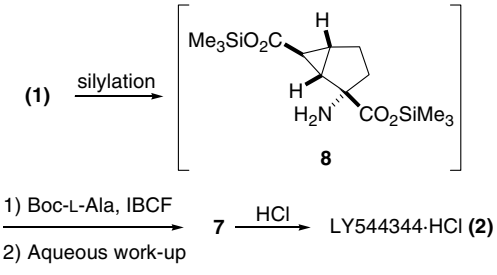
with isobutyl chloroformate (IBCF) affording **5**, which was not isolated, but also taken directly into the next step. Treatment of **5** with NaOH and subsequent crystallization from EtOAc and heptane afforded diacid **7** in 86% yield for the three steps. Boc group removal followed by a recrystallization from acetone and H_2O provided **2** in 93% yield.[5]

While route B in Scheme 1 was high yielding, it was four steps, and three of these steps involved protecting group manipulations. Thus, we embarked on the development of a more efficient preparation of **2**. Perhaps the most direct route to **2** would involve the coupling of the acid chloride of L-Ala and **1** under Schotten Baumann conditions.[6] In the event, the acid chloride of L-Ala was prepared.[7] Attempts to react it with **1** under typical Schotten Baumann conditions (THF, H_2O, and K_2CO_3 and NaOH), however, were completely unsuccessful. Additionally, the physical properties of **2** were not particularly amenable to this approach. It is insoluble in most organic solvents, but it is extremely water soluble (>650 mg/mL). Thus, even if the direct coupling under Schotten Baumann conditions had been successful, isolation of **2** may have been problematic.

We next examined coupling of the acid chloride of Boc-L-Ala with **1**.[8] While not as concise as the direct coupling of L-Ala with **1**, this approach would still avoid the carboxylic acid protection/deprotection sequence. In our hands, the preparation of the acid chloride of Boc-L-Ala proved problematic. There is precedent, however, for the reaction of the hydroxybenzotriazole (HOBt) ester of Cbz-L-Ala and glutamic acid under Schotten Baumann conditions.[9] In the event, the HOBt ester of Boc-L-Ala was prepared but, unfortunately, proved to be unreactive with **1**.

Another streamlined approach that was considered was the temporary or in situ protection of the carboxylic groups to enable the subsequent coupling with Boc-L-Ala. There are several examples in the literature where trimethylsilyl groups have been used for this purpose.[10] We envisioned an approach as described in Scheme 2. Since the carboxylic acid protection/deprotection sequence would occur in situ, this approach would essentially eliminate two steps from the synthetic routes shown in Scheme 1.

In the event, **1** was silylated using several different silylating reagents and conditions (Table 1), and subsequently converted to **7** via an isobutyl chloroformate mediated peptide coupling reaction. Gratifyingly, **7** could be prepared directly from **1** using various sets of conditions. The conditions in entry 4 (TMS–Cl and *N*-methylmorpholine (NMM)) provided **7** in yields that were competitive with the previous synthetic routes described in Scheme 1. There were, however, potential problems with the silylation reaction. It produced a heterogeneous mixture that could prove difficult to transfer on a larger scale. Additionally, there was no good method for monitoring the silylation. The conditions described in entry 5 (1,1,1,3,3,3-hexamethyldisilazane, (HMDS)) proved even more appealing. A slurry of **1** was heated to reflux in HMDS. Fortunately, **8**[11] was soluble in HMDS, and, thus, a clear solution resulted upon completion of the silylation reaction. This solution was then added to the isobutyl mixed anhydride of Boc-L-



Scheme 2. Trimethylsilyl ester strategy.

Table 1. Conversion of **1** → **7** via Scheme 2

Entry	Silylation conditions	%Yield **7**
1	BSU (2.5–5.0 equiv), DMAC, 60 °C	45
2	BSA (5.0 equiv), CH_2Cl_2, rt	76
3	TMS–Cl (3.0 equiv), Et_3N (5.0 equiv), CH_2Cl_2, rt	45
4	TMS-Cl (5.0 equiv), CH_2Cl_2, reflux, 3.5 h; cool to rt, add NMM (6.0 equiv), reflux 1 h; cool to rt	80
5	HMDS (5.0 equiv), reflux	82

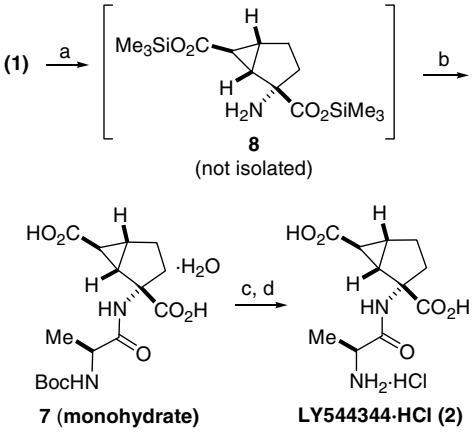
TMS–Cl = chlorotrimethylsilane, BSU = 1,3-bis(trimethylsilyl)urea, BSA = *N*,*O*-bis(trimethylsilyl)acetamide, HMDS = 1,1,1,3,3,3-hexamethyldisilazane.

Ala to afford **7** after work-up. This initial result was very encouraging and warranted further optimization.

In short order, we confirmed that **1** could be esterified by treatment with 5 vol[12] of HMDS at reflux. Upon completion of the silylation, CH_2Cl_2 (5 vol) was added, and this resulting solution was added to the corresponding isobutyl mixed anhydride at −10 °C. Addition of CH_2Cl_2 to the HMDS solution mixture prior to addition to the mixed anhydride aided in transfer and increased the yield of the peptide coupling. Other solvents such as THF and toluene were examined, but CH_2Cl_2 proved optimal at this stage. The mixture was then allowed to warm to rt. Upon completion of the reaction, 5.0 M NaOH was added resulting in hydrolysis of the trimethylsilyl esters. A simple layer separation was effective in removing the silyl related impurities with the organic layer leaving biscarboxylate of **7** in the aqueous layer. Following acidification of the aqueous layer, the monohydrate of **7** was isolated in 82–85% yield (Scheme 3).[13]

Completion of the synthesis was accomplished by treating the monohydrate of **7** with aqueous HCl in acetone to afford technical grade **2**.[8] Subsequent recrystallization afforded LY544344·HCl in 90–94% yield and >99.8% purity.[15]

In conclusion, we have demonstrated a very efficient synthesis of LY544344·HCl from LY354740 that highlights the use of in situ formation and subsequent hydrolysis of trimethylsilyl esters to enable an efficient peptide coupling sequence. While the overall yields of the syntheses of LY544344·HCl via methyl esters and trimethylsilyl esters are approximately equivalent, the trimethylsilyl ester route is more streamlined and efficient. Results from the application of this methodology on pilot plant scale will be reported in due course.



Scheme 3. Reagents and conditions: (a) HMDS, reflux; (b) (i) Boc-L-Ala, isobutyl chloroformate, *N*-methylmorpholine, CH_2Cl_2, −10 °C, (ii) 82–85% for two steps; (c) $HCl_{(aq)}$, acetone; (d) acetone, H_2O recrystallization, 90–94% for two steps.

Acknowledgements

The authors would like to thank Drs. Eric Moher and Jared Fennell for helpful discussions. We would also like to thank Steve Bandy, Paul Dodson, and Brian Scherer for analytical assay support.

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11. A sample of the HMDS solution was concentrated and analyzed. ^{1}H NMR data was consistent with compound **8**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.09–1.91 (s, 5H), 1.76 (br s, 2H), 1.69 (t, $J = 2.8$ Hz, 1H), 1.16 (ddd, $J = 14$, 10.5, 8 Hz, 1H), 0.31 (s, 9H), 0.27 (s, 9H).
12. 1 g (**1**)/5 mL HMDS.
13. Experimental procedure for the preparation of **7**: A slurry of HMDS (25 mL) and LY354740·H_2O (**2**) (5.00 g, 24.6 mmol) was heated at reflux (~125 °C) for 7 h. The resulting solution was cooled to 40 °C, CH_2Cl_2 (25 mL) was then added and the resulting solution was cooled to approximately −10 °C. In a separate flask, a solution of Boc-L-Ala (4.98 g, 26.3 mmol) in CH_2Cl_2 (40 mL) was cooled to approximately −10 °C. *N*-Methylmorpholine (2.87 mL, 26.1 mmol) was added, and the solution was stirred for 30 min. Isobutyl chloroformate (3.35 mL, 25.8 mmol) was added rapidly to the −10 °C solution while maintaining the temperature below 10 °C.[14] The mixture was then cooled to −10 °C and stirred for 30 min. The silyl ester solution prepared previously was added in one portion (~14 °C exotherm). The resulting mixture was allowed to warm to rt and then added to H_2O (50 mL). The pH of the aqueous layer was adjusted to approximately pH 8.5 with 5.0 N NaOH (~5 mL) and the mixture was stirred for 30 min. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (40 mL). H_2O (~15 mL) was added to the aqueous layer. The pH of the solution was adjusted to ~pH 4.5 by slow addition of 3.0 N HCl over a period of approximately 30 min. The solution was stirred until a precipitate formed (~2 h). The resulting slurry was stirred for approximately 4 h. The pH was then adjusted to pH 2 by the addition of 3.0 N HCl over a period of 1 h. The slurry was filtered, washed with H_2O, and then dried under vacuum at 40 °C overnight affording 7.57 g (82%) of the monohydrate of **7** as a white solid: $[\alpha]_D$ −13.8 (*c* 10, DMSO); ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.20 (s, 2H), 8.40 (s, 0.85H), 8.36 (s, 0.15H), 6.69 (d, $J = 8.2$ Hz, 0.85H), 6.33 (br d, 0.15H), 3.99 (quintet, $J = 7.2$ Hz, 0.85H), 3.84 (br m, 0.15H), 2.18–2.13 (m, 2H), 1.91–1.84 (m, 1H), 1.82–1.75 (m, 2H), 1.46 (br s, 0.85H), 1.43 (br s, 0.15H), 1.35 (s, 9H), 1.23–1.15 (m, 1H), 1.13 (d, $J = 6.9$ Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 176.4, 176.0 (2C), 157.5, 80.5, 67.3 (minor rotamer), 67.2 (major rotamer), 50.9, 35.6, 32.8, 29.3, 28.7, 27.4, 22.1, 18.5. HRMS calcd for $C_{16}H_{24}N_2O_7Na$ (M+Na) 379.1482, found 379.1468 *m*/*z*. Anal. Calcd for $C_{16}H_{26}N_2O_8$: C, 51.33; H, 7.00; N, 7.48. Found: C, 51.37; H, 6.84; N, 7.48.
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15. See Ref. 8 for experimental details and characterization data.