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Gram Scale Laboratory Synthesis of TC AC 28, a High Affinity BET Bromodomain Ligand.

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ABSTRACT: TC AC 28, 6-(1H-Indol-4-yl)-8-methoxy-1-methyl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine-4-acetic acid methyl ester, has been synthesized on a near gram scale in seven steps with notable improvements in the reported poor yielding last 2 steps enabling this key chemical probe compound to be available for researchers.

INTRODUCTION

The 1,4-benzodiazepine scaffold is a well-established “privileged scaffold” in medicinal chemistry1-16 and we have an active interest in synthesising libraries of such compounds.17-21 Our recently described triazolo-benzodiazepine derivative TC AC 28 is a potent, selective BET (Bromo and extraterminal) bromodomain inhibitor and a useful epigenetic tool compound, with a crystallographically defined binding mode to the target protein and displaying Kd values of 40 nM and 800 nM toward Brd2(2) and Brd2(1), respectively.22, 23 We sought to scale up the original seven-step-protocol towards the racemic product (as in the original manuscript) with the aim of improving the final two problematic and low yielding steps.23

RESULTS AND DISCUSSION

Our scale-up efforts (step 1, Scheme 1) started with a synthesis of the methyl ester hydrochloride salt 2, which was formed in virtually quantitative yield, followed a cyclization step (step 2) to afford the isoatoic anhydride 4.24


Reaction of the latter, formed the benzodiazepinedione 5, and we employed an ether trituration, as opposed to our earlier reported chromatographic purification work-up. This was followed by treatment with Lawesson’s reagent,25,26 then by mercury-mediated cyclization to afford the triazolo-analogue 7 (steps 3 – 5). At this stage, no significant differences in yields were noticed from our original report and we did not attempt less toxic routes to 7 given that the yield was acceptable and the chemistry scalable. However, the next two crucial steps were vital in our aims to obtain approximate gram quantities of product.

Scheme 2. Synthesis of TC AC 28 (9).
Step 6 (Scheme 2) was originally performed by combining 12 batches of ca. 170 mg amounts of precursor 7, yielding the key chloroimidate intermediate 8, which was obtained as a white solid in 619 mg amounts (29% yield). Careful re-examination of this step led us to significantly lower the amounts of POCl₃ used and we were able to avoid the inefficient chromatographic step by carrying out a trituration in Et₂O (Table 1, entry 3). Indeed, we were delighted to obtain a yield of 76% of 8 in nearly gram quantities (0.80 g) in a one-step protocol.

Table 1. Step 6 Optimization.

<table>
<thead>
<tr>
<th>Entry</th>
<th>POCI₃ (equiv.)</th>
<th>N.N-DMA (equiv.)</th>
<th>Work up</th>
<th>Purification</th>
<th>Isolated Yield (8) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21</td>
<td>5.5</td>
<td>Quench (Et₂N)</td>
<td>Acetone/DCM (30% to 80%) column</td>
<td>20°</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>3</td>
<td>Quench (water) extraction with CHCl₃</td>
<td>Trituration with diethyl ether.</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>2</td>
<td>Quench (water) extraction with CHCl₃</td>
<td>Trituration with diethyl ether.</td>
<td>76</td>
</tr>
</tbody>
</table>

aMaterial decomposes in silica

Buoyed by this result we next examined the final Pd-catalyzed Suzuki-Miyaura coupling reaction in order to install the indolyl group in 9. 27,28 Maintaining the original Pd(PPh₃)₄ catalyst, we obtained, by using a DME/water mixture with Na₂CO₃ as base, 9 in 49% yield (Table 3, Entry 2), which was scalable to 0.8 g of product (Table 2).

Table 2. Suzuki Coupling Optimization.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Solvent</th>
<th>Base</th>
<th>Conditions</th>
<th>Isolated Yield (9) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pd(PPh₃)₄</td>
<td>DMF</td>
<td>Et₂N</td>
<td>100 °C, 24 h</td>
<td>27</td>
</tr>
</tbody>
</table>

CONCLUSIONS

Overall, acceptable, near gram quantities of the final product 9 have been synthesized, benefitting ultimately from improved steps 6 and 7 of the original synthetic route (Table 3).

Table 3. Comparison of scale-up vs. original published route.

<table>
<thead>
<tr>
<th>Step</th>
<th>S.M. (g)</th>
<th>Prod. (g)</th>
<th>Yield (%)</th>
<th>S.M. (g)</th>
<th>Prod. (g)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50.07</td>
<td>74.00</td>
<td>&gt;99</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>50.02</td>
<td>57.03</td>
<td>89</td>
<td>-</td>
<td>-</td>
<td>&gt;99</td>
</tr>
<tr>
<td>3</td>
<td>45.00</td>
<td>27.30</td>
<td>43°</td>
<td>3.70</td>
<td>1.77</td>
<td>36</td>
</tr>
<tr>
<td>4</td>
<td>15.01</td>
<td>8.30</td>
<td>53</td>
<td>1.86</td>
<td>1.12</td>
<td>57</td>
</tr>
<tr>
<td>5</td>
<td>8.00</td>
<td>6.57</td>
<td>77°</td>
<td>2.20</td>
<td>2.15</td>
<td>91</td>
</tr>
<tr>
<td>6</td>
<td>0.99</td>
<td>0.80</td>
<td>76°</td>
<td>2.04</td>
<td>0.619</td>
<td>29</td>
</tr>
<tr>
<td>7</td>
<td>1.53</td>
<td>0.81</td>
<td>49</td>
<td>-</td>
<td>-</td>
<td>27-31</td>
</tr>
</tbody>
</table>

a Scale up; S.M. = starting material, Prod. = product. bOriginal papers. cTrituration in ether as opposed to chromatography. dReaction mixture quenched with NaHCO₃, extracted with ethyl acetate as opposed to no work-up. ePOCl₃ (1.5 eq), DMA (2 eq.) quenched with water, extraction with CHCl₃ and trituration with diethyl ether as opposed to POCI₃ (21 eq.). DMA (5.5 eq.), quenched with Et₂N and purified by chromatography.

EXPERIMENTAL SECTION

All commercially purchased materials and solvents were used without further purification unless specified otherwise. NMR spectra were recorded on a Bruker Avance III HD 400 MHz spectrometer and prepared in deuterated solvents such as CDCl₃ and DMSO-d₆. LC-MS spectra were acquired using an Agilent 6120 (600 Bar) HPLC with Agilent 1290 MCT column compartment oven and Agilent 6120 Quad Mass Spectrometer and percentage purities were run on a Zorbax SB C18 2.1x 50 mm 1.8 μm column (0.1% Aq Formic Acid 0.1% Formic Acid in MeCN 5-95 %, 0.1% TFA/MeCN, over 5 min, held at 100 % for 2 min, flow rate – 0.5mL/min) with the UV detector at 250 nm, bandwidth 100 nm. Purifications were performed by flash chromatography on silica gel columns using a Reveleris PREP purification system.

(DL)-Aspartic acid dimethyl ester hydrochloride (2). To a suspension of DL-Aspartic acid (50.00 g, 375.65 mmol) in methanol (300 mL) at 0°C was dropwise added thionyl chloride (68.50 mL, 939.14 mmol, 2.5 eq.) at such a rate that the temperature was maintained below 10°C. Upon completion of the addition, the reaction mixture was stirred at reflux for 2 hours, and then allowed to cool to ambient temperature and stirred overnight. The reaction mixture was concentrated under reduced pressure and the
resulting viscous oil was triturated from diethyl ether, filtered and dried at 40°C under vacuum, affording the product as a white solid (74.00 g, > 99%). The spectral data were consistent with those reported. The aqueous component was partitioned between NaHCO₃ (450 mL) and ethyl acetate (300 mL). The reaction mixture was concentrated under reduced pressure. The residue was triturated with H₂O and the resulting solid was collected by filtration and dried at 50°C under vacuum, affording the product as a brown solid (17.00 g, 89%). LCMS purity (UV): 99%, tR 3.24 min. The NMR data were consistent with those reported.

5-Methoxysiazoic anhydride (4). To a stirred solution of 2-amino-5-methoxy-benzoic acid 3 (15.00 g, 99.23 mmol) and triethylamine (13.80 mL, 99.23 mmol, 1 eq.) in THF (500 mL) at 0°C was portion-wise added triphosgene (29.45 g, 99.23 mmol, 1 eq.) at such a rate that the temperature was maintained below 5°C. Upon completion of the addition, the reaction mixture was stirred for 18 hours at ambient temperature. The reaction was re-cooled to 0°C and H₂O (15 mL) was added in a dropwise fashion at such a rate that the temperature was maintained below 10°C. After stirring for further 30 min at ambient temperature, the reaction mixture was concentrated under reduced pressure. The residue was triturated with H₂O and the residue was partitioned between EtOAc/water. The layers were separated and the residue was collected by filtration and dried at 50°C under vacuum, affording the product as a brown solid (17.00 g, 89%). LCMS purity (UV): 99%, tR 3.24 min. The NMR data were consistent with those reported.

Methyl-2-(7-methoxy-2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepin-3-yl)acetate (2). To a stirred suspension of compound 1 (45.00 g, 232.97 mmol) and DL-aspartic acid dimethyl ester hydrochloride (46.04 g, 232.99 mmol, 1 eq.) were suspended in diethyl ether (600 mL) and the reaction mixture was stirred at reflux for 6 hours. To the resulting viscous oil was triturated from diethyl ether, filtered and dried at 50°C under vacuum, affording the product as a white solid (74.00 g, > 99%). The spectral data were consistent with those reported.

(+/-)-Methyl-2-(6-chloro-8-methoxy-1-methyl-4H-benzo[f][1,2,4]triazolo[4,3-a][1,4]diazepin-4-yl)acetate (9). To a stirred suspension of compound 8 (1.33 g, 3.97 mmol) in DME (14 mL) was added a solution of Na₂CO₃ (0.76 g, 7.17 mmol) in water (6 mL), followed by the addition of indole-4-boronic acid (0.77 g, 4.76 mmol) and Pd(PPh₃)₄ (0.31 g, 0.27 mmol) the reaction was heated at 85°C for 2.5 hours. After cooling to ambient temperature it was filtered over celite and the filtrate was partitioned between EtOAc/water. The layers were separated and the organic layer was further washed with water and brine. The organic layer was dried (MgSO₄) and concentrated under reduced pressure. The product was collected as a white solid (0.81 g, 49%) after flash column chromatography (rf = 0.35; 95:5 CH₂Cl₂/MeOH). ¹H-NMR (400 MHz, CDCl₃): δ = 8.40 (s, 1H), 7.52 (d, J = 8.0, 1H), 7.42 (d, J = 9.0, 1H), 7.24 (d, J = 3.0, 1H), 7.20 (dd, J = 3.0, J = 9.0, 1H), 7.15 (t, J = 7.5, 1H), 7.08 (d, J = 7.5, 1H), 6.92 (d, J = 3.0, 1H), 6.58 (s, 1H), 4.78 (dd, J = 5.5, J = 9.0, 1H), 3.81 (s, 3H), 3.72 – 3.78 (m, 4H), 3.63 (dd, J = 5.5, J = 16.5, 1H), 2.64 (s, 3H). ¹³C NMR (101 MHz, CDCl₃): δ = 172.5, 168.1, 157.9, 156.4, 150.5, 136.5, 131.9, 130.8, 126.9, 126.4, 125.5, 124.3, 121.4, 117.7, 116.5, 113.6, 103.1, 55.8, 53.4, 51.9, 36.9, 12.2. LCMS purity (UV): 99%. tR 3.94 min. Elemental Analysis: Calculated for C₂₂H₁₈N₂O₃: C, 64.40, H, 5.29, N, 16.33. Found: C, 64.40, H, 5.29, N, 16.33; C, 64.73, H, 5.12, N, 16.07. MS m/z (ES+) calculated for C₂₂H₁₈N₂O₃ (+H)+: 416.3 found: 416.3; m/z (ES-) calculated for C₂₂H₁₈N₂O₃ (−H)+: 414.3 found: 414.3.

Author Contributions
All authors have given approval to the final version of the manuscript.

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Notes
The title product, TC AC 28, is sold under license from the University of Dundee.

ACKNOWLEDGMENTS
Supporting Information
The Supporting Information is available free of charge on the DOI:

Scanned NMR spectra and HPLC purity for all compounds.

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ABBREVIATIONS
TLC, thin layer chromatography. N,N-DMA: dimethylaniline