Gram scale laboratory synthesis of TC AC 28, a high affinity BET bromodomain ligand

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

Rayssa Khan,† Graham Marsh,§ Robert Felix,§ Paul D. Kemmitt,‡ Matthias G. J. Baud,¶ Alessio Ciulli,|| and John Spencer†,*

†Department of Chemistry, School of Life Sciences, University of Sussex, Falmer, Brighton BN1 9QJ, U.K.
‡Oncology, AstraZeneca, 310 Cambridge Science Park, Milton Road, Cambridge CB4 0WG, U.K.
§Tocris Bioscience, the Watkins Building, Atlantic Road, Avonmouth, Bristol BS11 9QD, U.K.
¶Division of Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, James Black Centre, Dow Street, Dundee DD1 5EH, U.K.

Supporting Information

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 two 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 chemistry,1–16 and we have an active interest in synthesizing libraries of such compounds.17–21 Our recently described triazolo-benzodiazepine derivative TC AC 28 is a potent, selective bromo and extraterminal bromodomain inhibitor and a useful epigenetic tool compound, with a defined binding mode to the target protein and displaying Kd values of 40 and 800 nM toward Brd2(2) and Brd2(1), respectively.22,23 We sought to scale up the original seven-step-protocol toward 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 by a cyclization step (step 2) to afford the isoisoic 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 workup. This was followed by treatment with Lawesson’s reagent25,26 and then 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. Step 6 (Scheme 2) was originally performed by combining 12 batches of ca. 170 mg of precursor 7, producing the key chlorimidate intermediate 8, which was obtained as a white solid in 29% yield (619 mg). Careful reexamination 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 near-gram quantities (0.80 g) in a one-step protocol.

Buoyed by this result, we next examined the final Pdcatalyzed Suzuki–Miyaura coupling reaction to install the indolyl group in 9.27,28 Maintaining the original Pd(PPh3)4 catalyst, we obtained, by using a 1,2-dimethoxyethane (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).

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).

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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 dimethyl sulfoxide (DMSO)-d₆. Liquid chromatography mass spectra (LCMS) 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 2.1 × 50 mm² 1.8 μm column (0.1% aq formic acid, 0.1% formic acid in MeCN 5→95%, 0.1% trifluoroacetyl (TFA)/MeCN, over 5 min, held at 100% for 2 min; flow rate, 0.5 mL/min) with UV detector at 250 nm and 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, thionyl chloride (68.50 mL, 939.14 mmol, 2.5 equiv) was dropwise added 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 h 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 (>99%). The spectral data were consistent with those reported.

5-Methoxyisatoic Anhydride (4). To a stirred solution of 2-amino-5-methoxy-benzoic acid (15.00 g, 99.23 mmol) and triethylamine (13.80 mL, 99.23 mmol, 1 equiv) in tetrahydrofuran (THF) (500 mL) at 0 °C, triphosgene (29.45 g, 99.23 mmol, 1 equiv) was portionwise added at such a rate that the temperature was maintained below 5 °C. Upon completion of the addition, the reaction mixture was stirred for 18 h at ambient temperature. The reaction was recooled 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 a further 30 min at ambient temperature, 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%, t<sub>R</sub> 3.24 min. The NMR data were consistent with those reported. 23

Scheme 2. Synthesis of TC AC 28 (9)

Table 1. Step 6 Optimization

<table>
<thead>
<tr>
<th>entry</th>
<th>POCl&lt;sub&gt;3&lt;/sub&gt; (equiv)</th>
<th>dimethylaniline (N,N-DMA) (equiv)</th>
<th>workup</th>
<th>purification</th>
<th>isolated yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21</td>
<td>5.5</td>
<td>quench (Et&lt;sub&gt;3&lt;/sub&gt;N)</td>
<td>acetone/DCM (30–80%) column</td>
<td>20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>3</td>
<td>quench (water) extraction with CHCl&lt;sub&gt;3&lt;/sub&gt;</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 CHCl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>trituration with diethyl ether</td>
<td>76</td>
</tr>
</tbody>
</table>

<sup>a</sup> “Material decomposes on silica.”

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 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pd(PPh&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;4&lt;/sub&gt;</td>
<td>dimethylformamide</td>
<td>Et&lt;sub&gt;3&lt;/sub&gt;N</td>
<td>100 °C, 24 h</td>
<td>27</td>
</tr>
<tr>
<td>2</td>
<td>Pd(PPh&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;4&lt;/sub&gt;</td>
<td>DME/water</td>
<td>Na&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>85 °C, 2 h</td>
<td>49</td>
</tr>
</tbody>
</table>

Table 3. Comparison of Scale-Up vs Original Published Route

<table>
<thead>
<tr>
<th>step</th>
<th>S.M. (g)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>prod. (g)</th>
<th>yield (%)</th>
<th>S.M. (g)&lt;sup&gt;b&lt;/sup&gt;</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></td>
</tr>
<tr>
<td>3</td>
<td>45.00</td>
<td>27.30</td>
<td>43&lt;sup&gt;c&lt;/sup&gt;</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&lt;sup&gt;d&lt;/sup&gt;</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&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.04 (0.17 × 12)</td>
<td>0.619</td>
<td>29</td>
</tr>
<tr>
<td>7</td>
<td>1.33</td>
<td>0.81</td>
<td>49</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> “Scale-up (this work); S.M. = starting material, prod. = product.
<sup>b</sup> Original papers.
<sup>c</sup> Trituration in ether as opposed to chromatography.
<sup>d</sup> Reaction mixture quenched with NaHCO<sub>3</sub> extracted with ethyl acetate as opposed to no workup. 21 POCl<sub>3</sub> (1.5 equiv), DMA (2 equiv) quenched with water, extraction with CHCl<sub>3</sub> and trituration with diethyl ether as opposed to POCl<sub>3</sub> (21 equiv). DMA (5.5 equiv), quenched with Et<sub>3</sub>N and purified by chromatography.

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mmol) in pyridine (265 mL), Lawesson’s reagent (19.62 g, 48.82 mmol, 0.9 equiv) was suspended in DCM, and the reaction mixture was stirred at reflux for 6 h. The reaction mixture was cooled to ambient temperature and concentrated under reduced pressure. The residue was dissolved in CHCl₃ (3 × 300 mL) and re-concentrated under reduced pressure. Trituration with CHCl₃ afforded the product as a pale yellow solid (8.30 g, 53%). LCMS purity (UV): 92%, R₉ 3.51 min. The NMR data were consistent with those reported.

(+/−)-Methyl-2-(8-methoxy-1-methyl-6-oxo-5,6-dihydro-4H-benzo[f][1,2,4]triazolo[4,3-a][1,4]diazepin-4-yl)acetate (7). To a stirred suspension of compound 6 (8.00 g, 27.18 mmol) and acetylazide (6.04 g, 81.53 mmol, 3 equiv) in THF (120 mL), the reaction mixture was cooled to 0 °C, and mercury (II) acetate (12.91 g, 40.77 mmol, 1.5 equiv) was added to the reaction mixture portionwise at such a rate that the temperature was maintained below 5 °C. Upon completion of the addition, the reaction mixture was stirred at 0 °C for 2 h and then allowed to warm to ambient temperature and stirred for 48 h. The reaction mixture was concentrated under reduced pressure, and the residue was partitioned between NaHCO₃ (sat. aq., 450 mL) and ethyl acetate (300 mL). The aqueous component was separated and washed with water and brine. The organic layer was dried (MgSO₄) and concentrated under reduced pressure. The product was collected as a white solid (0.80 g, 76%). The product was used without further purification. LCMS purity (UV): 95%, R₉ 3.15 min. The NMR 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 (8). To a solution of compound 7 (0.99 g, 3.13 mmol) in CHCl₃ (20 mL), N,N-dimethylaniline (0.79 g, 6.26 mmol) and POCl₃ (0.72 g, 4.70 mmol) were added under inert atmosphere, and the reaction was heated at 80 °C for 18 h. After cooling to room temperature, the reaction was slowly poured into lukewarm water (80 mL) with stirring. After stirring for 15 min, it was diluted with CHCl₃ (50 mL) and the layers were separated. The aqueous layer was extracted with ethyl acetate (2 × 300 mL). The combined organic layer was dried (MgSO₄) and concentrated under reduced pressure. The residue was triturated with diethyl ether to afford an off-white solid (0.80 g, 76%). The product was used without further purification. LCMS purity (UV): 97%, R₉ 3.94 min. The NMR 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), a solution of NaN₂CO₃ (0.76 g, 7.17 mmol) in water (6 mL) was added, 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), and the reaction was heated at 85 °C for 2.5 h. 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 (r₁ = 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 (t, 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, 123.4, 121.2, 117.7, 116.5, 113.6, 103.1, 55.8, 53.4, 51.9, 36.9, 12.2. LCMS purity (UV): 99%, R₉ 4.12 min. Elemental analysis: calculated for C₂₃H₂₁N₂O₃: 23H₂O (%): C, 46.40, H, 5.29, N, 16.33, found: C, 46.73, H, 5.12, N, 16.07. MS m/z (ES⁺) calculated for C₂₃H₂₁N₂O₃·[H⁺]: 416.3 found: 416.3; m/z (ES⁻) calculated for C₂₃H₂₂N₂O₃·[−H]⁻: 414.3 found: 414.3.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.omega.7b00780

Scanned NMR spectra and HPLC purity for all compounds (PDF)

AUTHOR INFORMATION

Corresponding Author
j.spencer@sussex.ac.uk

ORCID

Matthias G. J. Baud: 0000-0003-3714-4350
Alessio Ciulli: 0000-0002-8654-1670
John Spencer: 0000-0001-5231-8836

Present Address
Chemistry Department, Faculty of Natural and Environmental Sciences, University of Southampton, Southampton SO17 1BJ, U.K. (M.G.J.B.).

Author Contributions

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

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Notes

The authors declare the following competing financial interest(s): the title product, TC AC 28, is sold under license from the University of Dundee and is available at Tocris on: https://www.tocris.com/dispprod.php?itemId=519094#.WShYEU3rvIU.

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ABBREVIATIONS

TLC, thin-layer chromatography; N,N-DMA, dimethylaniline

REFERENCES


