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

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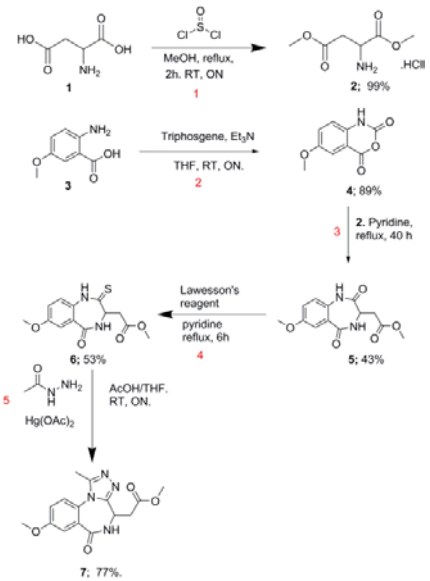
ABSTRACT: TC AC 28, 6-(1*H*-Indol-4-yl)-8-methoxy-1-methyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine-4-acetic acid methyl ester, has been synthesized on a near gram scale in seven steps with notable improvements in the reported poor yielding last 2 steps enabling this key chemical probe compound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

The 1,4-benzodiazepine scaffold is a well-established "privileged scaffold" in medicinal chemistry[1-16] 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 K_d 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 this molecule with the aim of improving the final two problematic and low yielding steps.[23]

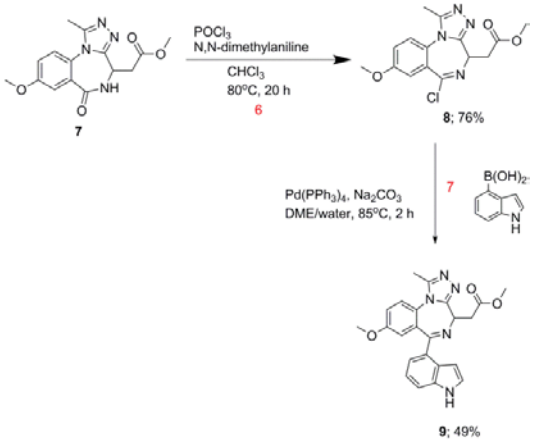
Our scale-up efforts (step 1, Scheme 1) started with a synthesis of the methyl ester hydrochloride salt **2**, which was formed in virtually quantitative yield, followed a cyclization step (step 2) to afford the isatoic anhydride **4**.[24]

Scheme 1. Synthesis of triazolo-benzadiazepinone, **7**.



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. 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 $POCl_3$ used and we were able to avoid the inefficient chromatographic step by carrying out a trituration in Et_2O (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.

Entry	$POCl_3$ (equiv.)	*N*,*N*-DMA (equiv.)	Work up	Purification	Isolated Yield (**8**) (%)
1	21	5.5	Quench (Et_3N)	Acetone/DCM (30% to 80%)	20[a]

				column	
2	10	3	Quench (water) extraction with $CHCl_3$	Trituration with diethyl ether.	50
3	1.5	2	Quench (water) extraction with $CHCl_3$	Trituration with diethyl ether.	76

[a]Material 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**.[27,29] Maintaining the original $Pd(PPh_3)_4$ catalyst, we obtained, by using a DME/water mixture with Na_2CO_3 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**.

Entry	Catalyst	Solvent	Base	Conditions	Isolated Yield (**9**) (%)
1	$Pd(PPh_3)_4$	DMF	Et_3N	100 °C, 24 h	27
2	$Pd(PPh_3)_4$	DME/ Water	Na_2CO_3	85 °C, 2 h	49

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.

Step	S.M. (g)[a]	Prod. (g)	yield (%)	S.M. (g)[b]	Prod.(g)	yield (%)
1	50.07	74.00	>99	-	-	-
2	50.02	57.03	89	-	-	>99
3	45.00	27.30	43[c]	3.70	1.77	36
4	15.01	8.30	53	1.86	1.12	57
5	8.00	6.57	77[d]	2.20	2.15	91
6	0.99	0.80	76[e]	2.04 (0.17 x 12)	0.619	29
7	1.33	0.81	49	-	-	27-31

[a]Scale up; S.M. =starting material, Prod.= product. [b]Original papers. [c]Trituration in ether as opposed to chromatography. [d]Reaction mixture quenched with $NaHCO_3$ extracted with ethyl acetate as opposed to no work-up. [e]$POCl_3$ (1.5 eq), DMA (2 eq.) quenched with water, extraction with $CHCl_3$ and trituration with diethyl ether as opposed to $POCl_3$ (21 eq). DMA (5.5 eq.), quenched with Et_3N 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 $CDCl_3$ and DMSO-d_6. 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. [28]

5-Methoxyisatoic 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 H_2O (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 H_2O 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.[23]

Methyl-2-(7-methoxy-2,5-dioxo-2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepin-3-yl)acetate (5). 5-Methoxyisatoic anhydride **4** (45.00 g, 232.97 mmol) and DL-aspartic acid dimethyl ester hydrochloride (46.04 g, 232.99 mmol, 1 eq.) were suspended in pyridine (600 mL) and the reaction mixture was stirred at reflux for 18 hours. After cooling to ambient temperature, the reaction mixture was concentrated under reduced pressure. The residue was partitioned between ethyl acetate (500 mL) and 2 M HCl (500 mL). The organic layer was separated and the aqueous layer was further extracted with ethyl acetate (2 x 350 mL). Some solid material at the phase boundary was collected by filtration giving an initial crop of product. The combined organic phase of the filtrate was dried ($MgSO_4$) and concentrated under reduced pressure. Trituration with diethyl ether afforded the product as a white solid (27.30 g, 43%). LCMS purity (UV): 96%, tR 3.12 min. The NMR data were consistent with those reported.[23]

(+-)-Methyl-2-(7-methoxy-5-oxo-2-thioxo-2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepin-3-yl)acetate (6). To a suspension of

the previous compound **5** (15.01 g, 53.91 mmol) in pyridine (265 mL) was added Lawesson's reagent (19.62 g, 48.52 mmol, 0.9 eq.) and the reaction mixture was stirred at reflux for 6 hours. The reaction mixture was cooled to ambient temperature and concentrated under reduced pressure. The residue was suspended in CH_2Cl_2 (3 x 300 mL) and re-concentrated under reduced pressure. Trituration with CH_2Cl_2 afforded the product as a pale yellow solid (8.30 g, 53%). LCMS purity (UV): 92%, tR 3.51 min. The NMR data were consistent with those reported. [23]

(+-)-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 acethydrazide (6.04 g, 81.53 mmol, 3 eq.) in THF (120 mL) was added acetic acid (80 mL). The reaction mixture was cooled to 0ºC and mercury (II) acetate (12.91 g, 40.77 mmol, 1.5 eq.) was added to the reaction mixture portion-wise at such a rate that the temperature was maintained below 5ºC. Upon completion of the addition, the reaction mixture was stirred at 0ºC for 2 hours, and then allowed to warm to ambient temperature and stirred for 48 hours. The reaction mixture was concentrated under reduced pressure and the residue was partitioned between $NaHCO_3$ (sat. aq., 450 mL) and ethyl acetate (300 mL). The aqueous component was separated and extracted with ethyl acetate (2 x 300 mL). The combined organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The product was collected as a white solid (6.57 g, 77%) after flash column chromatography (95:5 CH_2Cl_2/MeOH). LCMS purity (UV): 95%, tR 3.15 min. The NMR data were consistent with those reported. [23]

(+-)-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 $CHCl_3$ (20 mL) was added N,N-dimethylaniline (0.79 g, 6.26 mmol) and $POCl_3$ (0.72 g, 4.70 mmol) under inert atm. and the reaction was heated at 80ºC for 18 hours. 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 $CHCl_3$ (50 mL) and the layers were separated. The aqueous layer was extracted with further $CHCl_3$ (50 mL). The combined organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was triturated with diethyl ether and to afford an off-white solid (0.80g, 76 %). The product was used without further purification. LCMS purity (UV): 97%, tR 3.94 min. The NMR data were consistent with those reported. [23]

(+-)-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 Na_2CO_3 (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 $Pd(PPh_3)_4$ (0.31 g, 0.27 mmol) the reaction was heated at 85ºC 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 ($MgSO_4$) 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 CH_2Cl_2/MeOH). ^{1}H-NMR (400 MHz) $CDCl_3$: 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). LCMS purity (UV): 99%, tR 4.12 min. Elemental Analysis: Calculated for $C_{23}H_{21}N_5O_3.^{3/4}H_2O$ (%): C, 64.4, H, 5.29, N, 16.33, found: C, 64.73, H, 5.12, N, 16.07. MS m/z (ES+) calculated for $C_{23}H_{21}N_5O_3$ $[+H]^+$: 416.3 found: 416.3; m/z (ES-) calculated for $C_{23}H_{21}N_5O_3$ $[-H]^+$: 414.3 found: 414.3.

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Notes

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

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ABBREVIATIONS

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

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