Total synthesis of the isoquinolinium metabolite ETM-204 of Trabectedin

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Abstract
Ecteinascidin-743 (Trabectedin, Trabectedin®, Yondelis®) is a synthetically obtained pharmaceutical drug originally isolated from a marine tunicate. Trabectedin is used for the chemotherapy of soft-tissue sarcoma and ovarian cancer. The isoquinolinium metabolite ETM-204 has been found in biotransformation and degradation studies of Trabectedin. We report the first total synthesis of ETM-204 and its full spectroscopic characterization confirming the postulated structure. Central elements of the 12-step synthesis starting from 2-methyl-6-nitrophenol are a Cu-mediated conversion of an iodoarene to a phenol, a Skattebøl-formylation, and a modified Pomeranz–Fritsch cyclization to assemble the isoquinoline ring. The pH-dependence of its visual absorbance could be clarified.

Graphic abstract

Keywords Cyclization · Heterocycles · Indicator dye · Medicinal chemistry · Ullman coupling

Introduction
Ecteinascidin-743 (ET-743) is a marine alkaloid originally isolated from the Caribbean tunicate Ecteinascidia turbinata. ET-743 binds covalently to guanine residues in the minor groove of the DNA double helix, which triggers a series of downstream events resulting in potent antitumor activity. ET-743 is marketed as Trabectedin® (Yondelis®) for the treatment of soft-tissue carcinoma and ovarian cancer. This remarkable biological activity with its unique mechanism of action together with its complex structure has made it an attractive target for total synthesis. Impressive total syntheses have been reported by Corey [1], Fukuyama [2, 3], Danishefsky [4, 5], Zhu [6, 7], Chen [8], Williams [9], Ma [10], and several synthetic studies towards its synthesis have been undertaken [11]. Commercial production involves the semi-synthesis starting from cyanosafracin B [12, 13].

In the course of the clinical development of Trabectedin, studies have been undertaken, which investigated the metabolism and degradation of this complex natural product [14–17]. Several metabolites have been identified and characterized via HPLC–MS. In Scheme 1 we outline the degradation pathway, which leads to the formation of ETM-204 and other metabolites proposed in the literature [14–17]. To confirm the structure of the key metabolite, which also represents a degradation product of the drug substance and product, we isolated ETM-204 and partially characterized it on the one hand and sought out to synthesize ETM-204 and confirm its structure by full spectroscopic characterization and make it accessible for biological testing and as reference standard on the other hand.
Results and discussion

Isolation and characterization of degradation product ETM-204

To verify and characterize ETM-204, one of the main degradation products, sufficient amounts of this substance needed to be isolated from a stressed Trabectedin solution. Thermal stress applied to a methanolic solution was found to be most efficient to generate high amounts of the target substance. Ideally, Trabectedin solution was incubated for 4 d at 60 °C yielding the degradation product in approx. 35–40% (based on relative area by HPLC). Higher temperatures and/ or longer incubation time did not improve the yield. Using semi-preparative HPLC 2 mg of material was purified which was subjected to NMR analysis for structure elucidation and confirmation.

Additionally HPLC–MS experiments using stressed Trabectedin solution were conducted to further characterize the degradation product. Signals from ESI positive mode at m/z 204 as well as m/z 189 and m/z 161 indicate the pseudomolecular ion (M + H), the loss of 15 Da (m/z 204 → 189) representing a methyl group and 43 Da (m/z 204 → 161) representing a CH₃CHN fragment all shown in Fig. 1. These results are in agreement with published data from Reid [14].

To confirm the structure and obtain sufficient quantities for analytical and biological studies we pursued a total synthesis of ETM-204 (Scheme 2).

As starting material for our synthesis, we used commercially available 2-methyl-6-nitrophenol and converted it into nitroanisole 1 via Williamson ether synthesis using 1.5 eq iodomethane and 2.0 eq potassium carbonate in DMF at 23 °C. This reaction delivered 1 in excellent 99% yield after aqueous workup and sufficient purity, which allowed to use the crude product of 1 directly in a reduction of the nitro group with 5.0 eq iron powder in an acetic acid/water mixture to produce α-anisidine 2. Although the
phase separation during aqueous workup took some time because of formation of an emulsion, the reaction provided 98% of the desired compound 2 as a red-brown oil, which was already pure according to NMR and used without further treatment. A colorless product sample was obtained via distillation with a Kugelrohr apparatus.

Next, we planned to convert the amino group of compound 2 into phenol-OH by first forming the corresponding aryl diazonium salt 3a and subsequent replacement by a hydroxy group via heating up the acidic aqueous solution to obtain 3-methylguajacol (4) directly. While the diazotation worked reliably, we failed to convert the diazonium species 3a to compound 4, as it either did not react at all, or decomposed after longer reaction times. Therefore, we decided to use iodo compound 3 as an intermediate, which was formed in 96% yield after addition of 2.1 eq sodium iodide solution to the diazonium intermediate 3a and stirring at 23 °C overnight. The conversion of iodoarene 3 into phenol 4 was planned using copper salts as catalyst and water as a nucleophile [18]. The best yield (70%) was achieved using a procedure of Xiao et al., with Cu(OH)2 in combination with glycolic acid as ligand [19].

The next step involved the ortho-formylation of phenol 4. First attempts using SnCl4/paraformaldehyde [20] or hexamethylenetetramine [21, 22] failed for this substrate. Next, we tried a procedure of Hofsløkken and Skattebøl, who described a convenient method for the ortho-formylation of phenols using MgCl2/Et3N and paraformaldehyde in dry MeCN [23]. First test reactions were encouraging, but the yield was very low. We finally achieved the best results with fresh batches of MgCl2 and paraformaldehyde, which have been dried for 90 min in oil-pump vacuum before they were used, and isolated 5 in moderate 48% yield.
Our initial strategy towards the N-methylated isoquinoline scaffold was based on the synthesis of compound 6a via reductive amination, followed by a Pictet-Spengler reaction with glyoxal (Scheme 3). As previous publications indicated that suitable reaction conditions could lead directly to the oxidized form of the heterocycle [24], we tried several conditions (variation of the temperature, changing the amount of glyoxal solution, using formic acid, acetic acid or 1,4-dioxane/HCl). Unfortunately, despite these efforts we could not detect any cyclized product. As an alternative cyclization method, we attempted a Pomeranz-Fritsch reaction [25] by condensing aldehyde 5 with aminoacetaldehyde dimethyl acetal to Schiff base 6b (Scheme 3). Unfortunately, it was not possible to cyclize 6b directly under acidic conditions as described by Woodward and Doering for a related, but less complex substrate [26]. Therefore, we followed a modified cyclization strategy of Birch and coworkers [27] by first reducing imine 6b with sodium borohydride to amine 6, which was directly N-tosylated without further purification to obtain 7 in excellent 99% yield over 2 steps. As many by-products were detected in the cyclization attempts of 7 we speculated that we could improve the selectivity of the reaction, by protecting the phenol-OH as a benzyl ether (Scheme 2). Indeed cyclization of 8 in 1,4-dioxane/6 M aqueous HCl at 110 °C led to cyclized product 9 in 48% yield. The expected elimination of the tosyl group did not proceed under acidic conditions, probably because of the electron donating effect of the benzyloxy substituent in position 8, which renders the proton in position 1 less acidic [27]. However, this could be easily overcome by treating 9 with potassium tert-butoxide in tert-butanol to form isoquinoline 10 in 87% yield (Scheme 2).

After deprotection of 10 via catalytic hydrogenation, using an H-cube® continuous-flow hydrogenation reactor with a 10% Pd on charcoal cartridge, 11 was obtained in moderate 55% yield after flash column chromatography, where also unreacted starting material 10 was collected and subjected to a second hydrogenation cycle via H-cube®.

With purified isoquinoline 11 in hand, a few test reactions were performed to find the best conditions for the methylation of the nitrogen atom. We had concerns that the phenolic OH could be methylated as well, which would complicate the purification of the final product. This issue could be avoided by exclusion of any addition of a base. Even with a large excess of iodomethane exclusive N-methylation was observed. Pure target compound ETM-204 was obtained in quantitative yield as an iodide salt by evaporation of the volatiles and subsequent drying in oil-pump vacuum (Scheme 2).

The final product was orange-colored as a solid and solutions were yellow (dissolved in methanol or chloroform), which was in contrast to the deep red color of the material isolated from degradation studies described above. Furthermore, all aromatic protons in our 1H spectrum were...
significantly shifted downfield compared to the $^1$H spectrum of Reid et al. [14], while the HPLC retention times of synthetic and isolated products were identical. To understand this different appearance, we performed experiments to study the acid/base behavior of ETM-204. Figure 2A shows the $^1$H NMR spectrum of the synthesized compound (with iodide as counter ion) and its solution in methanol. Next, we dissolved a small amount of our compound in 1 M NaOH (orange solution), and extracted it with chloroform, where the organic phase immediately turned red. After phase separation, drying of the organic phase over $\text{Na}_2\text{SO}_4$ and filtration, the volatiles were removed under reduced pressure and a dark red solid was obtained, which was dissolved in methanol-$d_4$ and a $^1$H NMR spectrum was recorded (Fig. 2B). The proton shifts of this betaine compound were in full alignment with the values previously described in literature [14].

After addition of a droplet of 4 M HCl in 1,4-dioxane to the red solution, the color immediately turned yellow again (Fig. 2C). After evaporation of the volatiles, a third $^1$H spectrum was recorded, which showed the same shifts as the initial spectrum and reflects the phenolic form of ETM-204.

**Conclusion**

In summary, we have successfully synthesized the central Trabectedin metabolite ETM-204 in a 12-step synthesis, assigned its structure via NMR spectroscopy and confirmed it with authentic material resulting from degradation studies. The synthesis started from commercially available 2-methyl-6-nitrophenol, which was converted into 2-methoxy-3-methylphenol (4) in four steps. *ortho*-Formylation with paraformaldehyde and $\text{MgCl}_2/\text{Et}_3\text{N}$ provided 2-hydroxy-3-methoxy-4-methylbenzaldehyde (5), which was further subjected to a reductive amination, followed by an N-tosylation. Key step of the synthesis was the cyclization to 1,2-dihydroisoquinoline derivative 9, which only succeeded after protection of the phenol-OH with a benzyl group. After aromatization and deprotection, isoquinoline 11 was finally N-methylated with an excess of iodomethane to yield ETM-204 as iodide. We could show that ETM-204 shows a pH-dependent behavior and can either exist as a cationic species in acidic pH or as a betaine under basic pH, which has to be considered when investigating its properties.
Experimental

Reactions were carried out under air, unless indicated otherwise. For inert reactions, standard Schlenk techniques under an inert atmosphere of N₂ or Ar and anhydrous solvents were used. The described nuclear resonance spectra were acquired with the following instruments: Bruker AVANCE III with autosampler: 300.36 MHz ¹H NMR, 75.53 MHz ¹³C NMR; chemical shifts δ [ppm] are referenced to residual protonated solvent signals as internal standard: CDCl₃: δ = 7.26 ppm (¹H), 77.16 ppm (¹³C); methanol-d₄: δ = 3.31 ppm (¹H), 49.00 ppm (¹³C). Signal multiplicities are abbreviated as bs (broad singlet), dd (doublet of doublet), dt (doublet of triplet), m (multiplet), s (singlet), t (triplet), and q (quadruplet). The deuterated solvent, the chemical shifts δ in ppm (parts per million), and the coupling constants J in Hertz (Hz) are given. Deuterated solvents for nuclear resonance spectroscopy were purchased from Euriso-top®.

Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60, F₂₅₄ plates and spots were visualized by UV-light (λ = 254 and/ or 366 nm), or by treatment with cerium ammonium molybdate solution (CAM: 5.0 g λ = 254 and/or 366 nm), or by treatment ized by UV-light (λ = 254 and/or 366 nm). As mobile phase acetonitrile (VWR HiPer-Solv, HPLC–MS grade) and water (Barnstead NANOpure®, ultrapure water system) with 0.05% trifluoroacetic acid THF was used. The following standard method was used: Column oven: 40 °C, flow rate 0.7 cm³/min; 0–0.5 min CH₃CN/0.05% TFA = 2:98 (v/v), 0.5–10.0 min linear increase to CH₃CN/0.05% TFA = 100:0 (v/v), 10.0–12.0 min hold CH₃CN/0.05% TFA = 100:0 (v/v), 12.5–13.0 min return to initial conditions. High-resolution mass spectrometry (HRMS): all HRMS measurements were done at Vienna BioCenter, using the following procedure: sample spectra were acquired by data-dependent high-resolution tandem mass spectrometry on a QExactive Focus (Thermo Fisher Scientific, Germany). The electrospray ionization potential was set to +3.5 or − 3.0 kV, the sheath gas flow was set to 20, and an auxiliary gas flow of 5 was used. Samples were diluted with an appropriate solvent (methanol or chloroform) and 1 mm³ was injected on a SeQuant® ZIC®-pHILIC HPLC column (Merck, 100 × 2.1 mm; 5 µm; 100 Å; peek coated; equipped with a guard column). The separation solvent (A: ACN, B: 25 mM ABC) was delivered through an Ultimate 3000 HPLC system (Thermo Fisher Scientific, Germany) with a flow rate of 100 mm³ min⁻¹ and appropriate gradients were used for proper sample elution. Acetonitrile (ACN) HiPerSolv CHROMANORM® for HPLC-Supergradient was obtained from VWR Chemicals, methanol Optima® LC/MS Grade from Fisher Chemicals, ammonium hydrogen carbonate for LC–MS LiChropur were purchased from Merck, chloroform Plus for HPLC from Sigma–Aldrich, and H₂O was obtained from a Milli-Q® Advantage A10 water purification system (Merck). Melting points were measured on a MEL-TEMP® apparatus with integrated microscopical support from Electrothermal in open capillary tubes. Reported values are uncorrected. Hydrogenation experiments were performed using the H-Cube® continuous-flow hydrogenation unit (HC-2.2S) from ThalesNano Inc. running with a Knauer Smartline pump 100 and equipped with a 10 cm³ ceramic pump head. As hydrogenation catalyst 10% Pd/C catalyst cartridges were used (ThalesNano Inc., THS01111, 10% Pd/C CatCart™). Chemicals were purchased from the companies ABCR, ACROS Organics, Alfa Aesar, Brenn, Fisher Scientific, Fluka, Merck, Roth, Sigma Aldrich or VWR and were used without further purification, unless oth- erwise stated. All solvents were purchased from the abovementioned companies and were used without further purification unless otherwise stated. For reactions where moisture was excluded, absolute solvents were used. For that purpose, the purchased solvents were dried using the following methods and stored in brown 1 dm³ Schlenk bottles under argon and over activated molecular sieves. For analytical applications solvents with analytical grade were purchased. Absolute DMF, MeCN, and MeOH were purchased in anhydrous quality and stored over activated molecular sieves (3 Å: MeCN, MeOH; 4 Å: DMF) under argon. Dichloromethane: CH₂Cl₂ (stabilized with EtOH) was first heated under reflux over P₂O₅ for 5 h, then heated under reflux over CaH₂ for 5 h and distilled under argon atmosphere into a brown 1 dm³ Schlenk bottle containing activated 4 Å molecular sieves. Absolute Et₃N was obtained by refluxing over CaH₂ and distilling into a 1 dm³ Schlenk bottle containing activated 4 Å molecular sieves. 1,4-Dioxane was distilled and stored over KOH.

Isolation of ETM-204 by degradation of Trabectedin

20 mg Trabectedin were placed into a 10 cm³ glass vial, dissolved in 8 cm³ MeOH, closed and put into an oven
for 4 d at 60 °C. Afterwards the reaction was stopped by placing the solution into a fridge. Semi-preparative chromatography of stressed solutions was conducted on an Agilent 1260 Infinity II instrument with quaternary pump G7111B, sampler G7129A, thermostatic column compartment G7116B and DAD G7115A using a Zorbax SB-Phenyl, 250×9.6 mm with 5 µm particles column. Respective fractions were collected automatically during several runs using a G1364 Agilent fraction collector. Mobile phase A was an aqueous solution with 16 mM ammonium for using a G1364 Agilent fraction collector. Mobile phase fractions were collected automatically during several runs 18% B and a flow rate of 4.5 cm3/min. After that the color of 10 min isocratic phase at a composition of 82% A and used (VWR, LC-grade). First step of the run consisted of 10 min isocratic phase at a composition of 82% A and 18% B and a flow rate of 4.5 cm3/min. After that the column was washed by changing to 90% mobile phase B for 11 min followed by an equilibration step of 5 min. Collected fractions were combined and concentrated using evaporator GeneVac EZ-2 (Bartelt). Remaining residues were resuspended in 1.5 cm3 dichloromethane (VWR, GC-grade). The supernatant was carefully removed from the precipitate and put into a fresh HPLC-vial. Again, material was evaporated to complete dryness to yield 2 mg and stored at 2–8 °C until NMR analysis. HPLC–MS analysis of solutions from degradation study was performed on an Agilent 1290 Infinity instrument with binary pump G4220A, sampler GG4226A, thermostatic column compartment G1316C and DAD G4212A coupled with a Sciex QTRAP 4500 operating in positive electron spray mode (ESI +). Components were separated on a Zorbax SB-Phenyl, 150×4.6 mm with 1.8 µm particles column. The same mobile phases were used as described above but in MS-grade quality. At a flow rate of 0.8 cm3/min initial conditions were 15% mobile phase B increasing to 27% after 45 min. A second steeper gradient was implemented until 75 min up to 65% B followed by an equilibration step for 12 min at initial conditions.

2-Methoxy-1-methyl-3-nitrobenzene (1) A dried and argon-flushed 100 cm3 Schlenk-flask with magnetic stirring bar was charged with 5.66 g 2-methyl-6-nitrophenol (37.0 mmol, 1.0 eq), which were then dissolved in 70 cm3 abs. DMF forming a yellow solution. Then 10.2 g K2CO3 (73.9 mmol, 2.0 eq) and 3.50 cm3 iodomethane (55.4 mmol, 1.5 eq) were added resulting in a red suspension, which was stirred for 60 h at 23 °C. The suspension, which had turned orange, was diluted with 300 cm3 EtOAc, poured into a 1000 cm3 separation funnel and washed with half-saturated NaCl solution (5×300 cm3) and brine (1×200 cm3). The resulting yellow organic layer was dried over Na2SO4, filtered and concentrated on a rotary evaporator furnishing 6.13 g 1 (36.7 mmol, 99%) as a red oil. Rf = 0.48 (cyclohexane/EtOAc = 10:1 (v/v); UV/KMnO4); 1H NMR (300 MHz, CDCl3): δ = 7.63 (d, J = 7.4 Hz, 1H, Ar–H), 7.41 (d, J = 7.2 Hz, 1H, Ar–H), 7.10 (t, J = 7.9 Hz, 1H, Ar–H), 3.90 (s, 3H, OCH3), 2.37 (s, 3H, CH3) ppm; 13C NMR (75.5 MHz, CDCl3): δ = 151.8 (CAr–OCH3), 144.5 (CAr–NO2), 135.7 (CAr–H), 134.6 (CAr–CH3), 123.9 (CAr–H), 123.0 (CAr–H), 62.0 (OCH3), 16.2 (CH3) ppm.

1-Iodo-2-methoxy-3-methylbenzene (3) In an inert 500 cm3 round-bottom flask equipped with a Schlenk adapter and a magnetic stirring bar 6.00 g 2-methoxy-1-methyl-3-nitrobenzene (1, 35.9 mmol, 1.0 eq) were dissolved in 180 cm3 glacial acetic acid and 18 cm3 dist. H2O. 10.0 g Fe powder (180 mmol, 5.0 eq) were added, the Schlenk adapter connected to a bubbler and the reaction mixture stirred for 17 h at 23 °C. The resulting grey-blue suspension was poured into a 2 dm3 Erlenmeyer flask and neutralized with 400 cm3 satd. K2CO3-solution under ice cooling. After further dilution with 500 cm3 dist. H2O the reaction mixture was transferred into a 2 dm3 separation funnel. The reaction mixture was extracted with 350 cm3 CH2Cl2 upon which a third intermediate phase was formed, which was collected separately. The aqueous phase was again extracted with 350 cm3 CH2Cl2 and the intermediate phase was extracted with 150 cm3 CH2Cl2. The combined organic phases were dried over Na2SO4, filtered and the solvent removed on a rotary evaporator (at water bath temperature ≤ 30 °C). The resulting 4.84 g red-brown oil of 2 (35.3 mmol, 98%) was used in the next step without further purification. Rf = 0.16 (cyclohexane/EtOAc = 10:1 (v/v); UV/KMnO4); 1H NMR (300 MHz, CDCl3): δ = 6.83 (t, J = 7.7 Hz, 1H, Ar–H), 6.59 (‘t’, J = 7.1 Hz, Ar–H), 3.76 (s, 3H, OCH3, overlapping with bs, 2H, NH2), 2.28 (s, 3H, CH3) ppm; 13C NMR (75.5 MHz, CDCl3): δ = 145.9 (CAr–OCH3), 139.9 (CAr–NH2), 131.2 (CAr–CH3), 124.5 (CAr–H), 120.9 (CAr–H), 113.9 (CAr–H), 59.8 (OCH3), 15.9 (CH3) ppm.

2-Methoxy-3-methylaniline (2) In an inert 500 cm3 round-bottom flask equipped with a Schlenk adapter and a magnetic stirring bar 6.00 g 2-methoxy-1-methyl-3-nitrobenzene (1, 35.9 mmol, 1.0 eq) were dissolved in 180 cm3 glacial acetic acid and 18 cm3 dist. H2O. After completed addition the resulting slightly brownish solution was stirred for another 45 min at 0 °C. Then, a solution of 10.9 g NaI (72.6 mmol, 2.1 eq) in 35 cm3 dist. H2O were added dropwise with a rate of 40 cm3/h. After completed addition the solution turned dark-brown and foam formation was observed. After removal of the ice bath the septum of the flask was replaced with a bubbler and the reaction mixture was stirred for 19 h at 23 °C. The resulting two-phase mixture was evaporated to complete dryness to yield 2 mg and precipitate and put into a fresh HPLC-vial. Again, material was evaporated to complete dryness to yield 2 mg and stored at 2–8 °C until NMR analysis. HPLC–MS analysis of solutions from degradation study was performed on an Agilent 1290 Infinity instrument with binary pump G4220A, sampler GG4226A, thermostatic column compartment G1316C and DAD G4212A coupled with a Sciex QTRAP 4500 operating in positive electron spray mode (ESI +). Components were separated on a Zorbax SB-Phenyl, 150×4.6 mm with 1.8 µm particles column. The same mobile phases were used as described above but in MS-grade quality. At a flow rate of 0.8 cm3/min initial conditions were 15% mobile phase B increasing to 27% after 45 min. A second steeper gradient was implemented until 75 min up to 65% B followed by an equilibration step for 12 min at initial conditions.

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transferred into a 1000 cm$^3$ separation funnel and extracted with CH$_2$Cl$_2$ (2 x 200 cm$^3$). The combined organic phases were then washed with 1.8 M Na$_2$SO$_4$ solution (3 x 200 cm$^3$), dist. H$_2$O (1 x 200 cm$^3$), and brine (1 x 200 cm$^3$), dried over Na$_2$SO$_4$, filtrated and concentrated on a rotary evaporator resulting in 8.28 g 3 (33.4 mmol, 96%) and dark brown oil, which was used in the next step without further purification. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 7.61 (d, $J$ = 7.8 Hz, 1H, Ar–H), 7.14 (d, $J$ = 7.4 Hz, 1H, Ar–H), 6.75 (t, $J$ = 7.7 Hz, 1H, Ar–H), 3.78 (s, 3H, OCH$_3$), 2.35 (s, 3H, CH$_3$) ppm; $^{13}$C NMR (75.5 MHz, CDCl$_3$): $\delta$ = 158.2 (CAr–OCH$_3$), 137.3 (CAr–H), 132.5 (s, 1C, CAr–CH$_3$), 131.7 (s, 1C, CAr–H), 126.0 (s, 1C, CAr–H), 92.1 (s, 1C, CAr–I), 60.3 (s, 1C, CAr–OCH$_3$), 17.1 (s, 1C, CH$_3$) ppm.

2-Methoxy-3-methylphenol (4) A 250 cm$^3$ three-necked round-bottom flask with a gas adapter, reflux condenser, bubbler, and a magnetic stir bar was charged with a solution of 8.20 g 1-iodo-2-methoxy-3-methylbenzene (3, 33.1 mmol, 1.0 eq) and 12.1 cm$^3$ abs. MeCN, 3.18 g 2-methoxy-(156 mmol, 6.8 eq), which were then dried for 90 min in oil-pump vacuum. The solution was stirred for 19 h at 90 ºC in an oil bath. After full conversion 50 cm$^3$ H$_2$SO$_4$ (10% in H$_2$O) were added to the yellow suspension, and the mixture was transferred into a separation funnel and extracted with CH$_2$Cl$_2$ (4 x 90 cm$^3$). The combined organic phases were dried over Na$_2$SO$_4$, filtered, and the solvent was carefully removed under reduced pressure ($T \leq 30$ ºC). The crude product (4.18 g) was purified via flash column chromatography (420 g SiO$_2$; cyclohexane/EtOAc = 20:1 (v/v)) to yield 501 mg (2.76 mmol, 86%) of compound 6 as a pale brown oil. $R_f$ = 0.64 (cyclohexane/EtOAc = 10:1 (v/v); UV/CAM); $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 11.14 (s, 1H, OH), 9.82 (s, 1H, CHO), 7.19 (d, $J$ = 7.9 Hz, 1H, Ar–H), 6.81 (d, $J$ = 7.9 Hz, 1H, Ar–H), 3.89 (s, 3H, OCH$_3$), 2.33 (s, 3H, CH$_3$) ppm; $^{13}$C NMR (75.5 MHz, CDCl$_3$): $\delta$ = 196.2 (CHO), 154.9 (CAr–OH), 146.3 (CAr–OCH$_3$), 141.0 (CAr–CH$_3$), 128.2 (s, 1C, CAr–H), 121.9 (CAr–H), 120.4 (CAr–CHO), 60.2 (OCH$_3$), 16.9 (CH$_3$) ppm.

2-Methoxy-3-methyl-6-[(methylamino)methyl]phenol (6a) An inert 25 cm$^3$ Schlenk tube with magnetic stirring bar was charged with a solution of 535 mg 2-hydroxy-3-methoxy-4-methylbenzaldehyde (5, 3.22 mmol, 1.0 eq) in 4.0 cm$^3$ abs. MeOH and 441 mm$^3$ methylamine solution (33% in abs. EtOH, 3.54 mmol, 1.1 eq) were added. The yellow solution was stirred for 60 min at 23 ºC, and after full conversion (reaction control via TLC; cyclohexane/ EtOAc = 10:1 (v/v); $R_f$ = 0.10; UV/CAM) the reaction mixture was cooled to 0 ºC (ice bath) and treated with 122 mg NaBH$_4$ (3.22 mmol, 1.0 eq). The ice bath was removed and the pale yellow solution was stirred at 23 ºC for additional 45 min. Subsequently, the volatiles were removed under reduced pressure. The colorless residue was partitioned between 15 cm$^3$ EtOH and 15 cm$^3$ H$_2$O in a 50 cm$^3$ separation funnel and the aqueous phase was extracted with EtOAc (4 x 100 cm$^3$). The combined organic phases were dried with half-saturated NaCl solution (200 cm$^3$) and brine (2 x 200 cm$^3$), dried over Na$_2$SO$_4$, filtered, and the solvent was carefully removed under reduced pressure ($T \leq 30$ ºC). The crude product (6.83 g) was purified via flash column chromatography (410 g SiO$_2$; cyclohexane/ EtOAc = 10:1 (v/v); UV/KMnO$_4$); $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 6.93 (t, $J$ = 7.8 Hz, 1H, Ar–H), 6.82 (d, $J$ = 6.8 Hz, 1H, Ar–H), 6.71 (d, $J$ = 7.4 Hz, 1H, Ar–H), 5.76 (bs, 1H, OH), 3.81 (s, 3H, OCH$_3$), 2.32 (s, 3H, CH$_3$) ppm; $^{13}$C NMR (75.5 MHz, CDCl$_3$): $\delta$ = 149.0 (CAr–OH), 145.6 (CAr–OCH$_3$), 131.0 (CAr–CH$_3$), 124.7 (CAr–H), 122.6 (CAr–H), 113.3 (CAr–H), 60.7 (OCH$_3$), 15.9 (CH$_3$) ppm.

2-Hydroxy-3-methoxy-4-methylbenzaldehyde (5) An inert 250 cm$^3$ three-necked round-bottom flask equipped with reflux condenser, bubbler, Schlenk line adapter, and magnetic stirring bar was charged with 3.29 g anhydrous MgCl$_2$ (34.5 mmol, 1.5 eq) and 4.70 g paraformaldehyde (156 mmol, 6.8 eq), which were then dried for 90 min in oil-pump vacuum. Then 60 cm$^3$ abs. MeCN, 3.18 g 2-methoxy-3-methylphenol (4, 23.0 mmol, 1.0 eq), and 12.1 cm$^3$ abs. triethylamine (87.4 mmol, 3.8 eq) were added and the reaction mixture was stirred for 19 h at 90 ºC in an oil bath. After 2-Hydroxy-3-methoxy-4-methylbenzaldehyde (5) was evaporated, the reaction mixture was partitioned between 15 cm$^3$ EtOH and 15 cm$^3$ H$_2$O in a 50 cm$^3$ separation funnel and the aqueous phase was extracted with EtOAc (4 x 100 cm$^3$). The combined organic phases were dried over Na$_2$SO$_4$, filtered, and the solvent was removed under reduced pressure. The colorless residue was partitioned between 15 cm$^3$ EtOH and 15 cm$^3$ H$_2$O in a 50 cm$^3$ separation funnel and the aqueous phase was extracted with EtOAc (4 x 100 cm$^3$). The combined organic phases were dried over Na$_2$SO$_4$, filtered, and the solvent was removed under reduced pressure. The crude product (566 mg) was dried in oil-pump vacuum and purified via flash column chromatography (57 g SiO$_2$; CH$_2$Cl$_2$/MeOH = 15:1 + 1% NH$_4$OH (v/v)) to yield 501 mg (2.76 mmol, 86%) of 6a as a pale brown oil. $R_f$ = 0.20 (CH$_2$Cl$_2$/MeOH = 15:1 + 1% NH$_4$OH (v/v); UV/KMnO$_4$); $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 6.64 (d, $J$ = 7.6 Hz, 1H, Ar–H), 6.57 (d, $J$ = 7.6 Hz, 1H, Ar–H), 3.94 (s, 2H, CH$_2$), 3.86 (s, 3H, OCH$_3$), 2.47 (s, 3H, NCH$_3$), 2.25 (s, 3H, CAr–CH$_3$) ppm; $^{13}$C NMR (75.5 MHz, CDCl$_3$): $\delta$ = 151.3 (CAr–OCH$_3$), 146.3 (CAr–OH), 131.0 (CAr–CH$_3$), 123.0 (CAr–H), 121.3 (CAr–H), 120.2 (CAr–H), 59.9 (OCH$_3$), 54.4 (CH$_2$), 35.1 (NCH$_3$), 15.9 (CAr–CH$_3$) ppm.
2-hydroxy-3-methoxy-4-methylbenzaldehyde (5, 10.7 mmol, 1.0 eq) in 13 cm³ abs. MeOH and 1.86 cm³ aminoacetaldehyde diethyl acetal (12.9 mmol, 1.2 eq) were added. The yellow solution was stirred for 30 min at 23 °C, and after full conversion (reaction control via TLC; cyclohexane/EtOAc = 10:1 (v/v); R₂ = 0.17; UV/KMnO₄) the reaction mixture was cooled to 0 °C (ice bath) and treated quant.) was directly used in the next step without further oil-pump vacuum, the red-brown oil (3.03 g, 10.7 mmol, 1.0 eq) in 13 cm³ abs. MeOH and 1.86 cm³ benzyl bromide (13.0 mmol, 1.5 eq) were added to the solution, the flask was equipped with a bubbler, and the reaction mixture was stirred for 3 h in oil-pump vacuum. Then 1.94 g potassium carbonate (13.0 mmol, 1.5 eq) and 1.54 g sodium bicarbonate (7.9 mmol, 1.05 eq) and 0.81 g sodium bicarbonate (8.66 mmol, 1.0 eq) in 43 cm³ abs. DMF. Then 1.80 g potassium carbonate (13.0 mmol, 1.5 eq) and 1.54 cm³ benzyl bromide (13.0 mmol, 1.5 eq) were added to the colorless solution and the suspension was stirred at 40 °C (oil bath) overnight. The reaction mixture was transferred into a 250 cm³ separation funnel, diluted with 130 cm³ EtOAc, and washed with H₂O (5 x 90 cm³) and brine (90 cm³). The organic phase was dried over Na₂SO₄, filtered, and the solvent was removed under reduced pressure. The crude product (4.75 g) was purified by flash column chromatography (475 g SiO₂; cyclohexane/EtOAc = 7:1 (v/v)) to yield 4.25 g (8.05 mmol, 93%) of 8 as a colorless oil. R₁ = 0.31 (cyclohexane/EtOAc = 7:1 (v/v); UV/KMnO₄); ¹H NMR (300 MHz, CDCl₃): δ = 151.4 (CAr–OCH₃), 149.9 (CAr–OBn), 143.1 (C–SO₂), 137.7 (Bn–Cq), 137.6 (CAr–CH₃ of Ts), 131.8 (CAr–CH₃ of Bn), 129.8 (CAr–H of Bn), 128.4 (2C, CAr–H of Bn), 128.1 (Cq–Ar), 128.5 (2C, CAr–H of Bn), 128.7 (7.82 mmol, 1.0 eq) in 80 cm³ 1,4-dioxane. After addition of 6.5 cm³ 6 M HCl
(39 mmol, 5.0 eq) the mixture was heated to 110 °C (oil bath) and heated under reflux for 6.5 h. The brown solution was cooled to 23 °C, the solvent removed under reduced pressure, and the crude residue (3.95 g) dried in oil-pump vacuum. Purification via flash column chromatography (475 g SiO2, cyclohexane/ EtOAc = 10:1 (v/v)) provided 1.65 g (3.79 mmol, 48%) as a colorless solid. Rf = 0.27 (cyclohexane/EtOAc = 10:1 (v/v); UV/KMnO4); m.p.: 104–109 °C; 1H NMR (300 MHz, CDCl3); δ = 7.64 (d, J = 8.2 Hz, 2H, Ts–H), 7.50–7.30 (m, 5H, Bn–H), 7.25 (d, J = 8.1 Hz, 2H, Ts–H), 6.69 (d, J = 7.7 Hz, 1H, NCH=CH), 6.54 (s, 1H, Ar–H), 5.72 (d, J = 7.8 Hz, 1H, NCH=CH), 4.94 (s, 2H, Bn–CH2), 4.44 (s, 2H, NCH2), 3.80 (s, 3H, OCH3), 2.39 (s, 3H, CH3 of Ts), 2.20 (s, 3H, CH3) ppm; 13C NMR (75.5 MHz, CDCl3): δ = 150.9 (Cq–OCH3), 147.6 (Cq–OBn), 144.0 (Cq of Ts), 137.3 (Cq of Bn), 134.9 (CqSO2), 131.6 (Cq–CH2), 129.8 (2C, Cq–H of Ts), 128.7 (2C, Cq–H of Bn), 128.6 (2C, Cq–H of Bn), 127.6 (Cq–H of Ts), 126.7 (Cq–Ar), 125.6 (NCH=CH), 122.3 (Cq–Ar), 119.7 (Cq–Ar), 109.6 (NCH=CH), 75.2 (Bn–CH2), 60.4 (OCH3), 42.2 (N–CH3), 21.6 (CH3 of Ts), 15.8 (CH3) ppm; HR-ESI-MS: calcd. [C12H14NO2]⁺ 204.1019, found 204.1019.

8-Hydroxy-7-methoxy-2,6-dimethyisoquinolinium iodide (ETM-204, C12H11INO2) A 25 cm³ round-bottom flask was charged with a solution of 300 mg 7-methoxy-6-methylisoquinolin-8-ol (11, 1.59 mmol, 1.0 eq) in 7.9 cm³ MeOH. Iodomethane (987 mm³, 15.9 mmol, 10 eq) was added to the stirred orange solution (partially undissolved) and the tightly closed flask was stirred at 23 °C for 27 h. The resulting orange solution was concentrated via a Schlenk line by condensing the volatiles in an intermediate cooling trap and the remaining yellow solid was dried in oil-pump vacuum to give 523 mg (1.58 mmol, quant.) of the final compound ETM-204, which did not require further purification. Rf = 0.30 (strong tailing; CH2Cl2/MeOH = 20:1 (v/v); UV/CAM); m.p.: 201–203 °C (decomposition); 1H NMR (300 MHz, methanol-d4); δ = 9.73 (s, 3H, H–1), 8.32 (dd, J = 6.8, 1.2 Hz, 1H, H–3), 8.16 (d, J = 6.8 Hz, 1H, H–4), 7.56 (s, 1H, H–5), 4.46 (s, 3H, N–CH3), 3.88 (s, 3H, OCH3), 2.60 (s, 3H, CH3) ppm; 13C NMR (75.5 MHz, methanol-d4); δ = 149.7 (C–3), 148.0 (C–7), 146.9 (C–6), 146.6 (C–1), 135.1 (2C, C–3 and C–4a), 125.5 (C–4), 121.6 (C–8a), 121.2 (C–4), 118.9 (C–5), 61.0 (OCH3), 17.2 (CH3) ppm; HR-ESI-MS: calcd. m/z for [C12H11INO2]⁺ 204.0983, found 204.0982.

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Total synthesis of the isoquinolinium metabolite ETM-204 of Trabectedin

References

1. Corey EJ, Gin DY, Kania RS (1996) J Am Chem Soc 118:9202
2. Endo A, Yanagisawa A, Ab M, Tohma S, Kan T, Fukuyama T (2002) J Am Chem Soc 124:6552
3. Kawagishi F, Toma T, Inui T, Yokoshima S, Fukuyama T (2013) J Am Chem Soc 135:13684
4. Zheng S, Chan C, Furuuchi T, Wright BJD, Zhou B, Guo J, Danishefsky SJ (2006) Angew Chem Int Ed 45:1754
5. Zheng S, Chan C, Furuuchi T, Wright BJD, Zhou B, Guo J, Danishefsky SJ (2006) Angew Chem 118:1786
6. Chen J, Chen X, Bois-Choussy M, Zhu J (2005) J Am Chem Soc 128:87
7. Chen J, Chen X, Willot M, Zhu J (2006) Angew Chem Int Ed 45:8028
8. Jia J, Chen R, Jia Y, Gu H, Zhou Q, Chen X (2019) J Org Chem 84:13696
9. Fishlock D, Williams RM (2008) J Org Chem 73:9594
10. He W, Zhang Z, Ma D (2019) Angew Chem Int Ed 58:3972
11. Avendano C, de la Cuesta E (2010) Chem Eur J 16:9722
12. Cuevas C, Perez M, Martin M, Chicharro J, Fernandez-Rivas C, Flores M, Francesch A, Gallego P, Zarzuelo M, De La Calle F, Garcia J, Polanco C, Rodriguez I, Manzanares I (2000) Org Lett 2:2545
13. Menchaca R, Martinez V, Rodriguez A, Rodriguez N, Flores M, Gallego P, Manzanares I, Cuevas C (2003) J Org Chem 68:8859
14. Reid JM, Kuffel MJ, Ruben SL, Morales JJ, Rinehart KL, Squillace DP, Ames MM (2002) Clin Cancer Res 8:2952
15. Brandon EFA, Sparidans RW, Guijt KJ, Löwenthal S, Meijerman I, Beijnen JH, Schellens JHM (2006) Investigat New Drugs 24:3
16. Beumer JH, Rademaker-Lakhai JM, Rosing H, Hillebrand MJX, Bosch TM, Lopez-Lazaro L, Schellens JHM, Beijnen JH (2007) Cancer Chemother Pharmacol 59:825
17. Vermeir M, Hemeryck A, Cuypens F, Francesch A, Bockx M, Van Hoult J, Steemans K, Mannens G, Aviles P, De Coster R (2009) Biochem Pharmacol 77:1642
18. Cohen T, Dietz AG Jr, Miser JR (1977) J Org Chem 42:2053
19. Xiao Y, Xu Y, Cheon H-S, Chae J (2013) J Org Chem 78:5804
20. Werlé C, Yin C-JM, Heinemann FW, Hauser C, Meyer K (2017) Tetrahedron Lett 58:2715
21. Larrow JF, Jacobsen EN (2004) Org Synth Coll 10:96
22. Duff JC, Bills EJ (1934) J Chem Soc 1305. https://pubs.rsc.org/en/content/articlelanding/1932/JR/jr9320001987
23. Hofsløkken NU, Skattebøl L (1999) Acta Chem Scand 53:258
24. Wang Y-X, Wang L, Xu Y-N, Li Y-H, Jiang J-D, Si S-Y, Li Y-B, Ren G, Shan Y-Q, Hong B, Song D-Q (2011) Eur J Med Chem 46:1066
25. Pomeranz C (1893) Monatsh Chem 14:116
26. Woodward RB, Doering WE (1945) J Am Chem Soc 67:860
27. Birch AJ, Jackson AH, Shannon PVR (1974) J Chem Soc. Perkin Trans 1:2185

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