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Total synthesis of (+)-oxo-tomaymycin

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Supporting Information Placeholder

ABSTRACT: (+)-Oxo-tomaymycin, a naturally occurring substance of the pyrrolo-1,4-benzodiazepine (PBD) family, has been synthesized using a short and efficient route. The key construction of the 7-membered ring by amide bond formation has been realized *via* a chemoselective Ar-NO_2 reduction using $TiCl_3$ under acidic conditions, followed by a spontaneous cyclization. This synthesis has been easily scaled-up to 80 g, and should be amenable to the production of larger quantities.
KEYWORDS: natural product, oxo-tomaymycin, $TiCl_3$-mediated reduction, chemoselectivity.

Natural pyrrolo[1,4]benzodiazepine (PBD) antibiotics, including, among others, tomaymycin **1**[1] and anthramycin **3**[2] (Figure 1), are known to display some antitumor activity. They exert their biological effect by binding covalently to the minor groove of DNA *via* an exocyclic amino group of the guanine base, with selectivity for 5'-purine-guanine-purine sequence.[3] Contrary to **1** and **3**, oxo-tomaymycin **2**[4] is devoided of cytotoxic activity because it lacks the necessary labile aminal function that generates the reactive imine *in situ*.

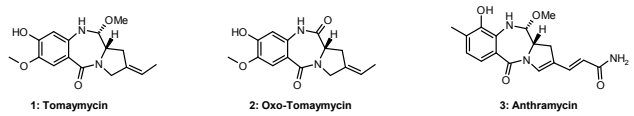
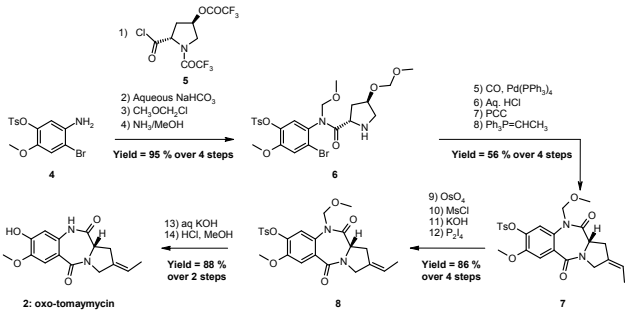


Figure 1. Pyrrolo[1,4]benzodiazepine most prominent members

The biological effect of PBDs can be increased by making dimers, leading to very potent cytotoxic compounds with pM activities[5] against a wide range of cancer cells. Based on these remarkable results, this family of compound is currently evaluated as promising payloads in the context of Antibody Drug Conjugates (ADC).[6]

For an internal research program dedicated to new payloads for ADCs, we needed rapid access to sizable quantities of oxo-tomaymycin **2**. This compound serves as a surrogate of **1** as it can be later on transformed into **1** using a known sequence (30 % overall yield) featuring an iminothioeter reduction to install the imine moiety which is in equilibrium with its MeOH-adduct.[7] To the best of our knowledge, there is only one report on the total synthesis of oxo-tomaymycin **2** that was realized three decades ago by Mori's group (Scheme 1).[8]

Scheme 1. Mori's synthesis of oxo-tomaymycin **2**

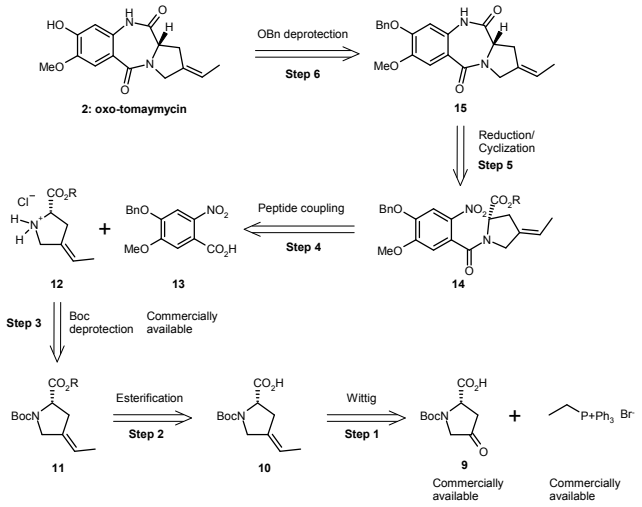


From a development point of view, their synthesis presents significant challenges, such as: length (14 steps, excluding the synthesis of **4** and **5**), use of genotoxic MOMCl (step 3) and toxic osmium derivative (OsO_4, step 9), to name a few. It is to be noted that the olefination reaction generates exclusively the undesired (*Z*)-isomer **7**. Conversion of **7** to the desired (*E*)-isomer **8** requires a 4-steps sequence of dihydroxylation/mesylation of secondary alcohol/epoxidation under basic conditions and finally epoxide deoxygenation to (*E*)-olefin. Cognizant of the vast body of chemical work performed around the pyrrolo-1,4-benzodiazepine scaffold,[5] we envisioned a more straightforward approach, that could be amenable to scale up without too much difficulty (Scheme 2).

The proposed synthesis commenced (**step 1**) with the commercially available Boc L-Pro(4-oxo) **9**, on which a Wittig reaction should generate the *E* ethylidene function, with an *E/Z* ratio that would have to be evaluated. The synthesis of pure (*E*)-isomer of **10** (with *N*-PMB instead of *N*-Boc) by means of an Ireland-Claisen rearrangement conducted under cryogenic conditions (- 78° C) has been described (8 steps from (*S*)-ethyl lactate).[9] However, we felt that a direct approach based on simple one-step protocol starting from a commercially available substrate such as the Boc L-Pro(4-

oxo) **9** may be more advantageous from an economical and speed point of view (Scheme 2, **Step 1**).

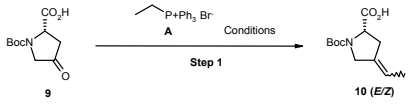
Scheme 2. Proposed retrosynthesis of oxo-tomaymycin **2**



Then (**step 2**), esterification of the carboxylic acid function of **10**, followed by deprotection of the *N*-Boc protecting group of **11** (**step 3**) would generate a chiral intermediate **12**, which could be coupled to the commercially available tetra-substituted phenyl **13**[10] to generate the chiral amide **14**. A one-pot chemoselective nitro reduction followed by intramolecular cyclization *via* amide bond formation (**step 5**) should generate the protected oxo-tomaymycin **15**. The use of nitro derivative **13** instead of its anthranilic acid counterpart was selected based on the following consideration: although coupling of anthranilic acid derivatives with proline of type **12** is described in the context of a pyrrolo[2,1-c][1,4]benzodiazepine synthesis,[11] yields are modest (~63 %) and coupling conditions are far from ideal (mutagenic solvent DMF was used, in conjunction with BOP dehydrating agent and potentially explosive HOBt). Lastly, OBn deprotection (**Step 6**) would complete the synthesis, leading to oxo-tomaymycin **2**.

Step 1 was evaluated, based on the literature precedent.[12] However, in our hands, the described conditions (**9** + ethyltriphenylphosphonium bromide + *t*BuOK in THF, reflux for 1h) failed to deliver the expected vinylidene product **10**. A small screening of conditions was thus performed, and the results are presented in Table 1.

Table 1. Screening of conditions for Wittig reaction[a]

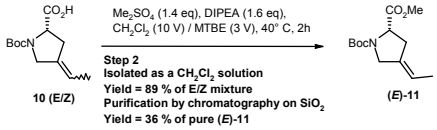


	solvent	Base (eq)	Conditions	yield[b] (Z/E ratio)
1	THF	*t*BuOK (2.5)	**9** + Base + **A**; 70° C, 1h	-
2	DMSO	NaH (2.5)	Base + **A**, 20° C, 1h, then **9**, 20° C, 18h	traces
3	THF	NaH (2.5)	Base + **A**, 50° C, 4h, then **9**, 50° C, 24h	51 % (1)
4[c]	THF	*t*BuOK (2.5)	Base + **A**, 50° C, 4h, then **9**, 50° C, 24h	65 % (1.4)
5[c,d]	MTBE	*t*BuOK (2.6)	Base + **A**, 50° C, 2h, then **9**, 35° C, 30 minutes	89 % (1.4)

a) All reactions were run under inert atmosphere. b) Isolated yields. c) tBuOK as a 1M solution in THF. d) 2.1 eq of **A** was used.

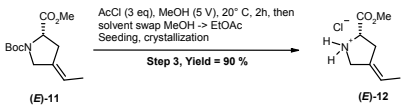
Switching from THF to DMSO and from *t*BuOK to NaH led to traces of **10** (Table 1, entry 2), with concomitant degradation. The real improvement in yield was observed when pre-forming the phosphorane derivative prior to addition of **9** (Table 1, entry 3). However, **10** was obtained as a 1/1 ratio of E and Z isomers. In the end, modifying the type of base (Table 1, entries 3 and 4) and changing the solvent and ratio of base/phosphonium salt and **9** (Table 1, entry 5) was found to be optimal, despite a Z/E ratio of 1.4, in disfavor of the desired **10*E***. Although other classical olefination methods (such as Julia-Kocienski or Peterson) may have been tested, it is known that they give predominantly the undesired (*Z*)-isomer on very close analogues.[13] Although not ideal, it was decided to pursue development with this ratio, as the two isomers could be easily separated later in the synthesis (*vide supra*). The mixture of **10 *(E/Z)*** was isolated as a MTBE solution, with high chemical purity (*see Supplementary Material*).

Scheme 3. Esterification of carboxylic acid **10** followed by separation of Z/E isomers



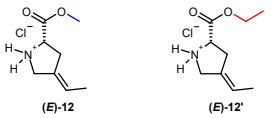
The next step was the esterification of the carboxylic acid function of **10** (used as a mixture of Z/E isomers) (Scheme 3). The retained reaction conditions were a combination of dimethylsulfate and DIPEA in a mixture of MTBE and dichloromethane under reflux. After acid washes with citric acid and concentration to dryness, the resulting crude Z/E mixture obtain with a yield of 89 % was purified by chromatography. This was initially performed using the SFC technique.[14] The low productivity of this method was deemed not suitable for quick delivery of material. Thus, a switch from SFC to HPLC was envisioned, and, after further development, a global yield of 91 % was obtained (based on a maximum recovery yield of 40 % of **(*E*)-11**). This switch increases productivity from 0.36 KKD (Kg of product per Kg of stationary phase per Day) to 0.55 KKD (*see Supplementary Informations*). Finally, pure **(*E*)-11** (optical purity > 99.5 %, chemical purity > 99 %) was isolated with a 36 % overall yield from compound **9**.

Scheme 4. *N*-Boc deprotection and isolation of **(*E*)-12**



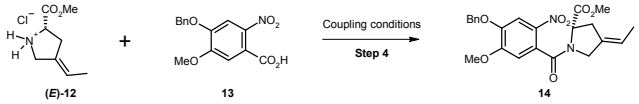
Armed with large quantities of **(*E*)-11**, the following NBoc deprotection step was then studied, using *in situ* generated dry HCl by employing acetyl chloride in MeOH (Scheme 4).The initial conditions led to the formation of a large amount (as high as 8.1 % surface area) of an ethyl ester impurity (**(*E*)-12'**) (Scheme 5).

Scheme 5. Ethyl ester impurity **(*E*)-12'*E***



It was anticipated that this non-robust process could potentially lead to a crystallization problem of the hydrochloride of **(*E*)-12** (lower yield and/or lower purity). The root cause of the formation of this ethyl ester impurity was traced back to the solvent swap step (MeOH ➔ EtOAc). It was hypothesized that the residual amount of HCl present in MeOH could lead to acidic degradation of EtOAc into EtOH and acetic acid. The EtOH would then transesterify the methyl ester and eventually lead to **(*E*)-12'**. In order to circumvent this problem, a preliminary distillation step was performed, before the solvent swap, by adding extra amount of MeOH, once the reaction was complete, and distilling half of it twice in order to remove excess HCl. Using this procedure, and by adding a seeding step, the amount of **(*E*)-12'** dropped from 8.1 % down to an acceptable 2 %. Of note is that, conducting this reaction in *i*PrOAc, a solvent more stable towards transesterification than EtOAc did not lead to clean crystallization of **(*E*)-12**. Next, we turned our attention to the amide bond formation between **(*E*)-12** and **13** (Table 2).

Table 2. Screening of coupling conditions[a]

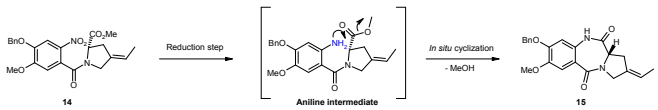


	solvent	coupling agent[b]	base	yield[c]
1	CH_2Cl_2	DCC (2 eq)	TEA (1.1 eq)	93 %
2	CH_2Cl_2	T_3P[d] (3 eq)	DIPEA (3 eq)	52 %
3	EtOAc/ DMF	CMPI (1.2 eq)	DIPEA (3 eq)	76 %
4	2-MeTHF	CMPI (1.2 eq)	DIPEA (3 eq)	90 %
5[e]	2-MeTHF	CMPI (1.3 eq)	DIPEA (3 eq)	95 %

a) All reactions were run under inert conditions at 20° C. b) *DCC* = DiCyclohexyl Carbodiimide; *T_3P* = 1-Propanephosphonic anhydride solution, 2,4,6-Tripropyl-1,3,5,2,4,6-trioxatriphosphorinane -2,4,6-trioxide; *CMPI* = 2-chloromethylpyridnium iodide. c) Isolated yields after purification. d) Used as a 50 % w/w solution in dichloromethane. e) Reaction performed at ~400 mmoles scale.

Whatever the coupling agent, the yields were fair (entries 2 and 3) to very good (entries 1, 4 and 5), with no signs of epimerization. The reactions were generally complete within 2 hours at 20° C (except in the case of T_3P, entry 2, for which only half-conversion was reached after 24 hours), with very few side products. Based on our own experience, CMPI (2-chloro-1-methylpyridinium iodide, also known as Mukaiyama's reagent)[15] was considered as the coupling agent of choice for further scale up. The purity of crude **14** was sufficient (97.4 % + 2 % of the ethyl ester impurity coming from **(*E*)-12'** as determined by HPLC) to carry out the next step without further purification/isolation, *i.e.* to keep **14** as a solution in 2-MeTHF. The one-pot nitro-reduction/cyclization step could then be studied (Table 3).

Table 3. Screening of conditions for one-pot two step sequence



	reducing agent (eq)	conditions	Aniline (area % in crude)	yield[a]
1	$FeSO_4$ (11)	NH_4OH (15 eq), EtOH/water, reflux, 16h	20 %	25 %
2	Pt/C + Me_2S	H_2 (4 bars), THF, 40° C, 18h	20 %	45 %
3	$Na_2S_2O_4$ (3)	THF/Water, 50° C, 24h	-	< 1 %
4	$Na_2S_2O_4$ (3)	THF/Water, NaOH (0.1 N), TBAB (0.2 eq) 60° C, 24h	-	20 %
5	$TiCl_3$ (8)	AcOH (7 V)/THF (10 V), 20° C, 24h	< 1 %	87 %
6	$TiCl_3$ (8)	AcOH (3 V)/2-MeTHF (3 V), 20° C, 24h	< 1 %	54 %
7	$TiCl_3$ (7)	AcOH (3 V)/2-MeTHF (26 V), 20° C, 24h	< 1 %	68 %
9[b]	$TiCl_3$ (5.8)	AcOH (2 V)/2-MeTHF (21 V), 35° C, 4.5 h	< 1 %	87 %

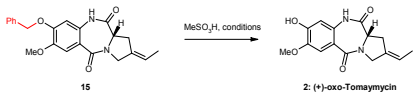
a) Isolated yield after purification. b) Reaction performed at 200 mmoles scale.

Although a large number of reagents are able to perform the reduction of $ArNO_2$ into $ArNH_2$, only a limited number of them are known to be chemoselective enough to expect to leave the other reducible functionalities of the molecule (*i.e.* benzylaryl ether and exocyclic double bond) untouched. Among them, both $FeSO_4$ in the presence of NH_4OH[16] and $Na_2S_2O_4$[17,20b] gave low yield of cyclized product (entries 1 and 3) with the aniline intermediate (*i.e.* non-cyclized compound) as a major impurity. Me_2S-poisoned Pt/C catalyst[18] in the presence of hydrogen led to an increase in cyclized product (entry 2). Finally, $TiCl_3$ (as 20 % w/w solution in 2N HCl)[19] proved to be the reagent of choice to affect both $ArNO_2$ reduction and cyclization in a one-pot manner. The reduction was fast, and the acidic conditions facilitated the cyclization step.[20b] Increasing the concentration from 17 to 6 volumes (entries 6 *vs* 5), as well as diminishing the equivalents of $TiCl_3$ (from 8 to 7, entries 7 *vs* 6) both have a negative impact on conversion. With the optimized procedure in hand (entry 9), the 2-MeTHF solution of **14,** prepared in Step 3, was reduced and cyclized in less than 5 hours at 35° C (compared to 3.5

days in the case of a close analogue using $Na_2S_2O_4$ followed by acidic cyclization)[20b] to deliver compound **15**, after work-up, concentration and crystallization from 2-MeTHF. The yield was 86.6 % with a chemical purity of 99.6 % (HPLC). The final debenzylation step, leading to (+)-oxo-tomaymycin **2** could then be tackled.

As for the reduction of the NO_2 function in **14**, chemoselective conditions during debenzylation were necessary in order to leave the exocyclic double bond untouched, hence the resort to acidic conditions, namely $MeSO_3H$, already applied to a close analogue of **15**.[6b] Optimization of this reaction in terms of the amount of $MeSO_3H$, additive, the volume of reaction and the isolation procedure was thus evaluated, and the results are reported in Table 4.

Table 4. Optimization of debenzylation under acidic conditions[a]



	$MeSO_3H$ (eq)	solvent (volume) / quench	additive (eq)	yield[b]
1	33	CH_2Cl_2 (100) / aq. $NaHCO_3$	-	66 %
2	33	CH_2Cl_2 (50) / Water	-	11 %
3	33	CH_2Cl_2 (100) / aq. AcONa	-	64 %
4	20	CH_2Cl_2 (20) / aq. AcONa	-	66 %
5[c]	20	THF (10) / -	-	< 1 %
6[c]	20	2-MeTHF (10) / -	-	~1.1 %
7[c,d]	20	CH_2Cl_2 (20) / -	-	< 1 %
8	10	CH_2Cl_2 (20) / -	-	9 %
9	17	CH_2Cl_2 (20) / aq. AcONa	-	80 %
10	17	CH_2Cl_2 (10) / aq. AcONa	-	77 %
11[e]	17	CH_2Cl_2 (10) / Water	Anisole (17)	91 %

a) All the reactions are performed at 20° C for 2 hours. b) Isolated yield. c) Almost no conversion observed. d) Trifluoroacetic acid used instead of $MeSO_3H$. e) Reaction performed at ~160 mmoles scale

Both the solvent and the acidic reagent had a strong influence on the reaction. CH_2Cl_2 was the best solvent (entries 1 *vs* 5 and 6) when used with $MeSO_3H$ (trifluoroacetic acid didn't promote debenzylation, entries 7 *vs* 4). The dilution has also been optimized, from a highly diluted reaction (100 volumes of dichloromethane, entries 1 & 3) to a more acceptable 10 volumes (entries 10 & 11). We also tried to replace dichloromethane for greener solvents (entries 5 & 6 *vs* 10) but no reaction occurred in either THF or 2-MeTHF, as **15** is poorly soluble in those solvents compared to dichloromethane. The number of equivalents of acid had a strong influence on the kinetics of the reaction (entries 8 *vs* 9). Finally, an additive such as anisole (a known trap for benzyl carbocation) led to an increase in the isolated yield (entries 11 *vs* 10), avoiding quenching with large volume of aqueous sodium acetate solution and increasing the purity of the isolated compound. Under optimized conditions (entry 11), and with recrystallization from THF/MTBE, pure (+)-oxo-tomaymycin **2** (see Figure 2) was isolated with 83 % yield as THF hemi-solvate.

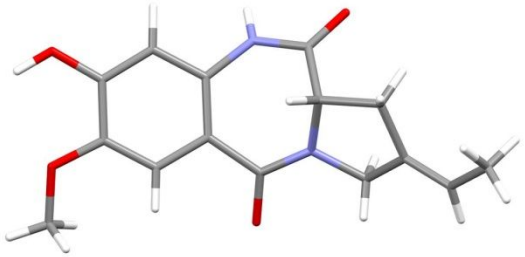


Figure 2. 3D Molecular configuration of synthetic (+)-oxo-tomaymycin **2** as determined from X-Ray single crystal diffraction data

In conclusion, the present article has described a 6-step synthesis of (+)-oxo-tomaymycin **2** with an overall yield of 22 % that is amenable to decagram-scale synthesis. This includes the implementation of a process for the efficient separation of Z and E isomers of compound **11** by chiral chromatography. The most notable feature of this work is the chemoselective reduction of nitro compound **14** mediated by $TiCl_3$, followed by *in situ* cyclization under acidic conditions. Efforts are currently underway in order to increase *E-to-Z* ratio of Step 1 and to replace the chromatographic purification by selective crystallization of diastereoisomeric salts of the **10(E/Z)** mixture. Results will be reported in due course.

EXPERIMENTAL SECTION

General procedures. All reactions were performed under a nitrogen atmosphere. All reagents and chemicals were bought from chemical suppliers and used without further purification (unless otherwise stated). Thin layer chromatography (TLC) was performed using commercially available pre-coated plates (Merck Silica Gel 60 F254). Visualization was via UV light (at 254 nm) or by staining with phosphomolybdic acid or iodine vapors. Purification via chromatography was performed on PuriFlash PF450 system using cartridges pre-loaded with silica gel, 60 Å particle size, 30 or 50 µm from Interchim and compounds were loaded as saturated solutions in the appropriate solvent. Optical rotation ($[\alpha]_D$) were measured on a Perkin-Elmer polarimeter 382 at 589 nm and 20° C. Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker Advance 200 or 400 MHz instrument at 303° K and calibrated using residual undeuterated solvent as an internal reference. Chemical shifts, δ, are quoted in parts per million (ppm) downfield of tetramethylsilane. Coupling constants (J) are reported to the nearest 0.1 Hz. The splitting patterns for the spectra assignment are abbreviated to: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br) and some as a combination of these. Infrared spectra (IR) were recorded on a Thermo Nexus T-IR spectrometer using a universal ATR accessory for sampling with absorption of most relevance quoted as ν in cm^{-1} and the samples were run as solids (in KBr pellets) or in solution (CH_2Cl_2). Low resolution mass

spectrometry was conducted on a Waters UPLC-SQD spectrometer, in positive (ES+) or negative (ES-) electrospray mode. High-resolution mass spectrometry (HRMS) was conducted on a Waters Xevo Q TOF spectrometer, in positive (ES+) electrospray mode.

Synthesis of (2S, 4E)-4-Ethylidene-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester 2-carboxylic acid (10 (E/Z))

To a suspension of Ph_3PEtBr (1.26 kg, 3.4 mol) and MTBE (4.4 L, 12 vols) heated at 50°C *via* a heating mantle was slowly added a 1.6 M KOtBu in THF solution (2.63 L, 4.2 mol). More THF (185 mL, 0.5 vols) was used to rinse the equipment. The suspension is aged for further 2 hours at 50°C before being cooled down to 35°C for the slow introduction of the THF (2.4 L, 6.5 vols) solution of N-tBoc-4-Oxo-L-Proline (370 g, 1.61 mol, 99.8 % purity by HPLC, 99.9 % e.e. by chiral GC). THF (185 mL, 0.5 vols) is added to rinse the dropping funnel and the medium is stirred for further 1 hour at 35°C. After cooling to 20°C, water (3.7 L, 10 vols) is added to quench the reaction. The rich aqueous layer is washed 7 times by toluene (7 x 2.6 L, 7 x 7 vols) followed by adding MTBE (3.7 L, 10 vols) and adjusting the pH to 3/3.5 using aqueous 1N HCl solution. The aqueous layer is extracted by MTBE (1.85 L, 5 vols) and the combined organic layers are finally washed by 1 M citric acid solution (3 x 1.85 L, 3 x 5 vols). The resulting compound **10 (E/Z)** is isolated as a MTBE solution (347 g, 89 %).

^{1}H NMR (200 MHz, DMSO-d6, mixture E/Z and rotamers) δ 12.6 (s broad, 1H), 5.37 (m, 1H), 4.24 (m, 1H), 3.89 (m, 2H), 2.88 (m, 1H), 2.50 (m, 1H), 1.55 (s broad, 3H), 1.41 (m, 9H).

^{13}C NMR (50 MHz, DMSO-d6, mixture E/Z and rotamers) δ 173.8/173.5 (Z/E), 153.6/153.4 (E/Z), 135.1/134.2 (E/Z), 116.7/116.4 (Z/E), 79.0/78.8 (E/Z), 58.2/57.8 (E/Z), 48.7/47.6 (E/Z), 35.8/35.2 (Z/E), 28.0/27.9 (×2), 27.9, 14.3.

IR (CH_2Cl_2, cm^{-1}): ν max = 2976, 1748, 1701, 1654, 1477, 1391 & 1366

LCMS (ESI+) [M+Na]+= 264.1, [2M+Na]+= 505.2

HRMS (ESI): m/z calculated for $C_{12}H_{19}NO_4$: $[M+Na]^+$: 264.1212, found: 264.1209.

Synthesis of (2S, 4E)-4-Ethylidene-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester 2-methyl ester (11 E)

The previous MTBE solution of **10 (E/Z)** (347 g, 1.44 mol) was concentrated to 3 residual volumes. Dichloromethane (3.9 L 10.5 vols), diisopropylethylamine (0.45 L, 2.3 mol) and dimethyl sulfate (**Caution! CMR reagent, should be handle with care** 0.215 L, 2.02 mol) were added at 35°C. The mixture was heated to reflux for 1 hour via a heating mantle and quenched with an aqueous 10 %w/w citric acid solution (1.85 L, 5 vols). The resulting organic layer was further washed by an aqueous 10 %w/w citric acid solution (2 x 1.85 L, 2 x 5 vols), water (1.85 L, 5 vols) and 1N aqueous HCl (if necessary, to reach pH = 6.5-7.5). The solvent was removed by concentration to dryness to give crude **11 (E/Z)** as a liquid (yield is 89 %). **11 (E/Z)** was then purified by chiral column chromatography: 420 g of **11 (E/Z)** (as a dichloromethane solution, 111 g/L) were purified on a 5 cm i.d. column filled with 295 g of Chiralpak IC (20µm). For each run, 2.4 g of **11 (E/Z)** (in solution at 111 g per liter of dichloromethane) were injected on the column. The elution was performed at 35°C with a binary mixture made with n-heptane (90 vol.%) and isopropanol (10 vol.%). The elution flow rate was 7.1 L/h. The compound of interest **(*E*)-11** was recovered by fractioning and collecting the column outflow, based on a UV signal (210 nm). To remove the solvents, the purified stream of **(*E*)-11** was then concentrated to dryness under reduced pressure, to afford pure **(*E*)-11** as pale yellow oil (132 g, 36 %).

$[\alpha]_{20D}$= -5° (c= 3mg/mL, MeOH)

Enantiomeric excess = 99.2 % (as determined on Chiralpak IA 5µm 250 x 4.6mm, using Heptane 50 / iPrOH (v/v) as the elution mixture in isocratic mode, with a flow rate of 1 mL / min, column oven at 20° C and UV detection at 210 nm. Run time = 20 minutes.

^{1}H NMR (200 MHz, DMSO-d6, mixture of rotamers) δ 5.43 (m, 1H), 4.38 (m, 1H), 3.92 (s, 2H), 2.85 (m, 1H), 2.50 (m, 1H), 1.57 (d, J= 7 Hz, 3H), 1.32 (m, 9H).

^{13}C NMR (50 MHz, DMSO-d6, mixture of rotamers) δ 173.0/172.6, 153.5/152.9, 134.6/133.7, 116.7, 79.2/79.0, 58.4/58.0, 51.8, 50.5, 31.8/31.1, 28.0, 27.8 (×2), 14.05.

IR (CH_2Cl_2, cm^{-1}): ν max = 2975, 1749, 1698, 1436 & 1365.

LCMS (ESI+) [M+Na]+= 278.3, [2M+Na]+= 533.6.

HRMS (ESI): m/z calculated for $C_{13}H_{21}NO_4$: $[M+Na]^+$: 278.1368, found: 278.1364.

Synthesis of (2S, 4E)- 4-Ethylidene-pyrrolidine-2-methyl ester hydrochloride (*E*)-12

To a solution of **(*E*)-11** (100 g, 0.39 mol) in anhydrous methanol (0.5 L, 5 vols) at 5°C was added acetyl chloride (83 mL, 1.17 mol). The mixture was warmed-up to 20°C and stirred for 3 hours. Nitrogen bubbling was applied in the mixture and two addition/distillation of methanol was performed (2 x 0.5 L, 2 x 5 vols). A solvent swap for ethyl acetate by three addition/distillation of ethyl acetate (3 x 1 L, 3 x 10 vols) was performed, and the solution was then cool down to 0° C in 1h at which point seeding was added (0.5 % by weight of previously prepared **(*E*)-12** was added). The suspension was then stirred for 1 h at 0° C, before being filtered. The solid was washed with cold (0° C) EtOAc (2 x 0.25 L, 2 x 2.5 vols) and dried at 40 ± 2° C under vacuum (< 10 mbars) to give **(*E*)-12** as a white solid (68 g, 90 %).

Mp = 124.23°C (DSC).

$[\alpha]_{20D}$ = -10° (c= 3 mg/mL, MeOH).

^{1}H NMR (200 MHz, DMSO-d6, mixture of rotamers) δ 10.19 (s broad, 2H), 5.53 (m, 1H), 4.54 (t, J= 8.5 Hz, 1H), 3.75 (m, 5H), 2.89 (dt, J= 17, 9.5 Hz, 1H), 2.60 (dt, J= 16, 8.5 Hz, 1H), 1.60 (d, J= 7 Hz, 3H).

^{13}C NMR (50 MHz, DMSO-d6, mixture of rotamers) δ 168.5, 131.4, 118.8, 58.0, 52.9, 48.3, 29.7, 14.5.

IR (CH_2Cl_2, cm^{-1}): ν max = 2774, 1747, 1440, 1256 & 1082.
LCMS (ESI+) $[M+H]^+$= 156.1.

HRMS (ESI): m/z calculated for $C_8H_{13}NO_2$: $[M+H]^+$: 156.1025, found: 156.0986.

Synthesis of (2S, 4E)-4-Ethylidne-2-methyl ester-1-(2-nitro, 4-benzyloxy, 5-methyl ester) (14)
4-Benzyloxy-5-methoxy-2-nitro-benzoic acid **13** (190 g, 0.627 mol), ***(E)*-12** (126 g, 0.66 mol) and CMPI (209 g, 0.82 mol) were suspended in 2-MeTHF (3.42 L, 18 vols) at 20°C. Diisopropylamine (0.33 L,1.88 mol) was added and the medium was aged for 4 hours under stirring. Water (2.85 L, 15 vols) was next added to quench the reaction mixture. The resulting organic layer was successively washed by aqueous 0.1N NaOH (2.85 L, 15 vols), aqueous 0.5N HCl (1.90 L, 10 vols) and water (2 x 1.90 L, 2 x 10 vols). Compound **14** was obtained as a 2-MeTHF solution (12 % w/w, 263 g, 95 %).

[α]20D = -37.7° (c= 3 mg/mL, MeOH).

^{1}H NMR (200 MHz, DMSO-d6, mixture of rotamers) δ 7.85 (s, 1H), 7.41 (m, 5H), 6.94 (m, 1H), 5.45 (m, 1H), 5.26 (s, 3H), 4.75/4.35 (m, 1H), 4.38 (m, 1H), 3.94 (m, 5H), 3.71 (s, 2H), 3.54 (s, 1H), 2.92 (m, 1H), 2.71 (m, 1H), 1.57 (m, 3H).

^{13}C NMR (50 MHz, DMSO-d6, mixture of rotamers) δ 171.7, 165.3, 154.2, 147.7, 137.0, 135.9, 133.8, 128.5 (×2), 128.2, 128.0 (×2), 126.8, 117.4, 109.5, 108.7, 70.45, 57.7, 56.5, 52.0, 30.9, 14.1.

IR (CH_2Cl_2, cm^{-1}): ν max = 2950, 1740, 1645, 1331 & 1059.

LCMS (ESI+) $[M+H]^+$= 441.1.

HRMS (ESI): m/z calculated for $C_{23}H_{25}N_2O_7$: $[M+H]^+$: 441.1662, found: 441.1670.

Synthesis of (6aS, 8E)-3-benzyloxy-8-ethylidene-2-methoxy-5-6a,7,9-tetrahydropyrrolo[2,1-c][1,4] benzo diazepine-6,11-dione (15)
To the previous 2-MeTHF solution of **14** (86 g, 0.195 mol) was added extra 2-MeTHF (1.72 L, 20 vols), followed by acetic acid (172 mL, 2 vols) at 20°C. The reaction mixture was then warmed to 30-35° C, and a 20% w/v 2N HCl aqueous solution of $TiCl_3$ (1.08 kg, 1.12 mol) was added in ~10 minutes (mild exotherm observed ~2° C), under stirring, to the reaction mixture and followed by a rinse with 2-MeTHF (86 mL, 1 vol). The medium was stirred for 3 hours at 35°C by a heating mantle. After cooling to 20°C, the reaction mixture was quenched by a 2M aqueous solution of NaOAc (2.58 L, 30 vols) and decanted. The aqueous phase was extracted with 2-MeTHF (1.29 L, 15 vols) and the combined organic layers were washed with water (0.86 L, 10 vols), aqueous 1N NaOH (2 x 0.86 L, 2 x 10 vols), saturated aqueous $NaHCO_3$ (2 x 1.12 L, 2 x 15 vols) and finally by water (0.86 L, 10 vols). **15** was crystallized from the 2-MeTHF solution by concentrating the solution to 4 residual volumes (around 0.35 L), cooling down the suspension at 0-5° C, and maintaining at 0-5° C for 10 hours. The suspension was then filtered, and **15** was obtained as a white, crystallin material (61 g, 87 %).

Mp = 213.06°C (DSC).

$[\alpha]_{20D}$ = +312.7° (c= 3mg/mL, MeOH).

^{1}H NMR (200 MHz, DMSO-d6) δ 10.31 (s, 1H), 7.45 (m, 5H), 7.26 (s; 1H), 6.82 (s, 1H), 5.4_ (m, 1H), 5.09 (m, 2H), 4.24 (m, 2H), 3.97 (d, J= 17 Hz, 1H), 3.79 (s, 3H), 3.26 (d, J= 16.5 Hz, 1H), 2.61 (m, 1H), 1.66 (d, J= 6.5 Hz, 3H).

^{13}C NMR (50 MHz, DMSO-d6) δ 170.0, 164.3, 150.7, 145.5, 136.1, 133.9, 128.4 (×2), 128.1, 128.0 (×2), 118.3, 116.7, 111.9, 105.6, 70.0, 56.4, 55.6, 51.1, 27.1, 14.2.
IR (CH_2Cl_2, cm^{-1}): ν max = 2912, 1683, 1610, 1606, 1426 & 1227.

LCMS (ESI+) $[M+H]^+$= 379.1.

HRMS (ESI): m/z calculated for $C_{22}H_{22}N_2O_4$: $[M+H]^+$: 379.1658, found: 379.1655.

Synthesis of (6aS, 8E)-8-ethylidene-3-hydroxy-2-methoxy-5,6a,7,9-tetrahydropyrrolo[2,1-c][1,4]benzo diazepine-6,11-dione (2, (+)-oxo-tomaymycin)
To a solution of **15** (60 g, 0.21 mol) in dichloromethane (0.60 L, 10 vols) was added anisole (294 mL, 3.57 mol), followed by the dropwise addition of methane sulfonic Acid (175 mL, 3.57 mol), under stirring at 20°C. The reaction mixture was held for 1 hour and cooles down to 0-5°C. Water (0.60 L, 10 vols) was slowly added (**Caution ! highly exothermic reaction during the first ¼ of the addition**) and stirring was pursued for 1 hour at 20-25° C, before being cool down to 10 ± 2° C, maintained at that temperature for 1 hour, and then filtered. The solid was washed with MTBE (0.30 L, 5 vols) and dried over nitrogen. The crude product (59.9 g) thus obtained was suspended in THF (0.60 L, 10 vols) and warmed to 90° C. The solution was then concentrated to half its volume (0.30 L, 5 vols), and this operation was repeated 2 more times using 0.30 L (5 vols) o THF. At the end of the last concentration, the solution was cooled to 45° C, and MTBE (0.60 L, 10 vols) was added. The solution was further cooled to 0-5° C in 2 hours and stirred for one more hour before being filtered. The solid thus obtained was then washed with cold (0-5° C) MTBE (2 x 0.18 L, 2 x 3 vols) and dried at 45° C under vacuum (5 mbars) for 18 hours, to afford **2** ((+)-oxo-tomaymycin, (hemi solvate of THF) as a white powder (41,2 g, 91 %).

Mp = 264.1°C (DSC).

$[\alpha]_{20D}$ = +388.2° (c= 3mg/mL, MeOH).

Enantiomeric excess > 99.5 % (as determined on Chiralpak AY-H 5µm 250 x 4.6mm, using Heptane 50 / EtOH 50 / TEA 0.1 (v/v/v) as the elution mixture, with a flow rate of 1 mL / min and UV detection at 265 nm. (*S*)-enantiomer: Tr = 12.4 min; (*R*)-enantiomer: Tr = 5.4 min.

^{1}H NMR (400 MHz, DMSO-d6) δ 10.24 (s, 1H), 9.91 (s, 1H), 7.20 (s, 1H), 6.56 (s, 1H), 5.46 (d, J= 6.5 Hz, 1H), 4.21 (m,

2H), 3.94 (d, J= 16 Hz, 1H), 3.77 (s, 3H), 3.24 (d, J= 16 Hz, 1H), 2.56 (m, 1H), 1.65 (d, J= 6.5 Hz, 3H).

^{13}C NMR (50 MHz, DMSO-d6) δ 170.0, 164.55, 150.2, 144.6, 134.1, 130.9, 117.1, 116.6, 112.2, 107.7, 56.4, 55.65, 51.1, 27.1, 14.2.

IR (CH_2Cl_2, cm^{-1}): ν max = 3251, 2979, 1699, 1638, 1618, 1483, 1276 & 1207.

LCMS (ESI+) $[M+H]^+$= 289.1.

HRMS (ESI): m/z calculated for $C_{15}H_{16}N_2O_4$: $[M+H]^+$: 289.1188, found: 289.1205.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Copies of ^{1}H and ^{13}C NMR spectra for compounds **10 E/Z**, **11 E/Z**, **11 E**, **13**, **14**, **15** and **2** as well as preparative SFC and HPLC data for separation of crude **11(E/Z)** mixture (PDF).

X-ray single-crystal crystallography of (+)-oxo-tomaymycin **2**; CCDC Deposition Number 1972508 contains the supplementary crystallographic data (cif file excluding structure factors) for (+)-oxo-tomaymycin **2**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures.

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The authors declare no competitive financial interests.

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