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Synthesis and cytotoxicity of (+/−)-7,9-dideoxy-pancratistatin analogues†

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Olaia Nieto-García‡ and Ricardo Alonso*

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Access to some 7,9-dideoxy analogues of pancratistatin was achieved by combining our previously reported nitroenal/dioxanone annulation (to form ring C) with a hetero-Diels–Alder/aromatization path to build the dihydroisoquinolinone subunit (rings A and B); testing of their antiproliferative activity afforded some clues about the role of aromatic substituents in pancratistatin's pharmacophore.

Introduction

The isocarbostyril constituents of the Amaryllidaceae plant family, with general structure **A** (Fig. 1),[1] keep receiving considerable attention due to their antitumoral properties.[2] Pancratistatin (**1**),[3] in particular, is at the focus of much research activity[4] because it selectively induces apoptosis in a wide variety of human cancer cell lines at low doses with minimal effect on normal cells,[5] it decreases the volume of human tumors *in vivo*[6] and does not inhibit CYP3A4.[7] A major and continuous effort is being dedicated to the total synthesis of **1** with the main goal of solving the supply problem derived from its minute occurrence in natural sources.[8,9] The synthetic work is also being directed to the preparation of unnatural derivatives to improve the pharmacological profile (in particular, to gain higher water solubility) and to define the pharmacophore.

To date, a significant number of pancratistatin derivatives have been prepared and tested.[10] Most of them have changes in rings B and C. In general, any departure from the B/C natural structure usually resulted in diminished activity. In fact, a number of structural elements were identified as critical motifs to be preserved for potent activity. They include the location and the stereochemistry of three hydroxyl groups in ring C (those at carbons C2, C3 and C4),[11] the *trans* B/C ring junction (positions C4a and C10b)[12] and the ring B-lactam functionality (positions C5 and C6).[13] In clear contrast, position C1 was demonstrated to be the only one that could be altered with advantage. This line of research, first initiated with the C1-benzoate of pancratistatin that showed ≈10^3 increments in antitumoral activities,[14] continues to be successfully pursued by several groups.[15] The participation of ring A in the pharmacophore has been much less studied. The phenolic C7-group is important for activity, as early established with the comparatively less active natural 7-deoxy analogues [*e.g.*, 7-deoxypancratistatin (**2**)[16] was shown to be less active than pancratistatin in lung NCI-H460 and colon KM20L2 human cancer cell lines].[17] Thus far, only three other derivatives of pancratistatin with variations in ring A (while keeping intact its B/C functionality) were synthesized: the indole mimic of 7-deoxypancratistatin **3**,[18] the bisTMS derivative **4**[19] and the

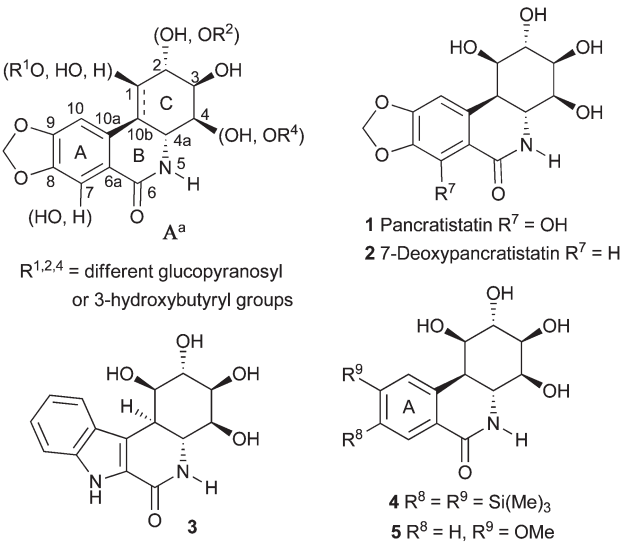
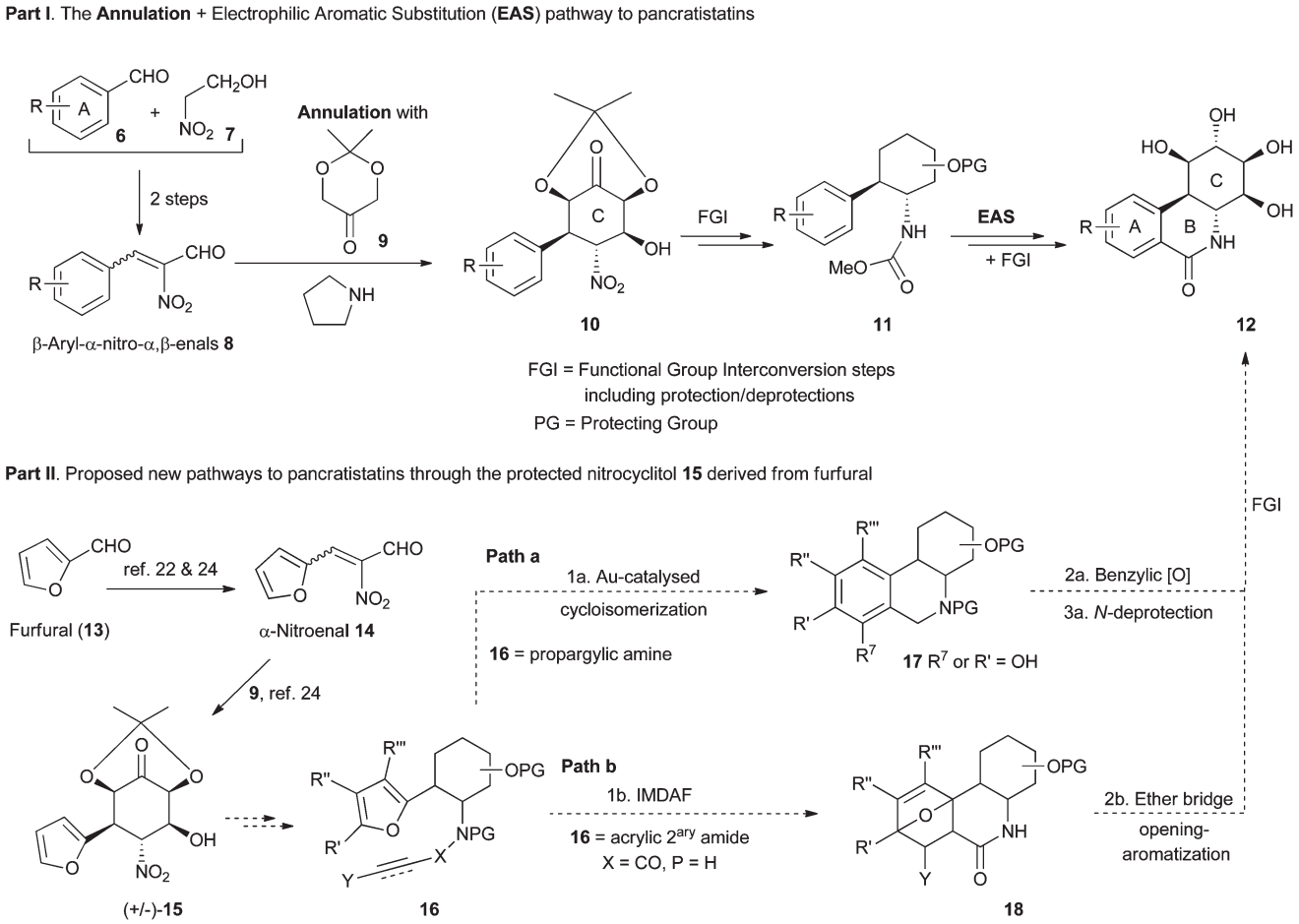


Fig. 1 **A**: general structure for the isocarbostyril constituents of the Amaryllidaceae family; [a] the C3-absolute configuration is as shown except for natural 3-*epi*-pancratistatin. Compounds **1**, **2**, **4** and **5**: hitherto known ring-A substitution patterns for the B/C amide–cyclohexanetetrol pancratistatin system [in natural (**1**, **2**) and synthetic (**4**, **5**) derivatives]. Compound **3**: indole mimic of **2**.

Department of Organic Chemistry, University of Santiago de Compostela, Campus Vida, 15782 Santiago de Compostela, Spain. E-mail: r.alonso@usc.es; Fax: +34 981 595012; Tel: +34 981 563100 Ext 14250

†Electronic supplementary information (ESI) available: Experimental procedures for **19**, **16a**, **20** and **16b**, copies of NMR spectra for all new compounds and cytotoxicity data. See DOI: 10.1039/c2ob27127c

‡Currently at Freie Universität Berlin, Institut für Chemie and Biochemie, Takustrasse 3, 14195 Berlin, Germany. E-mail: olaia.nieto@fu-berlin.de



Scheme 1 Previously-reported (part **I**, above) and herein-explored (part **II**, below) pathways to pancratistatins.

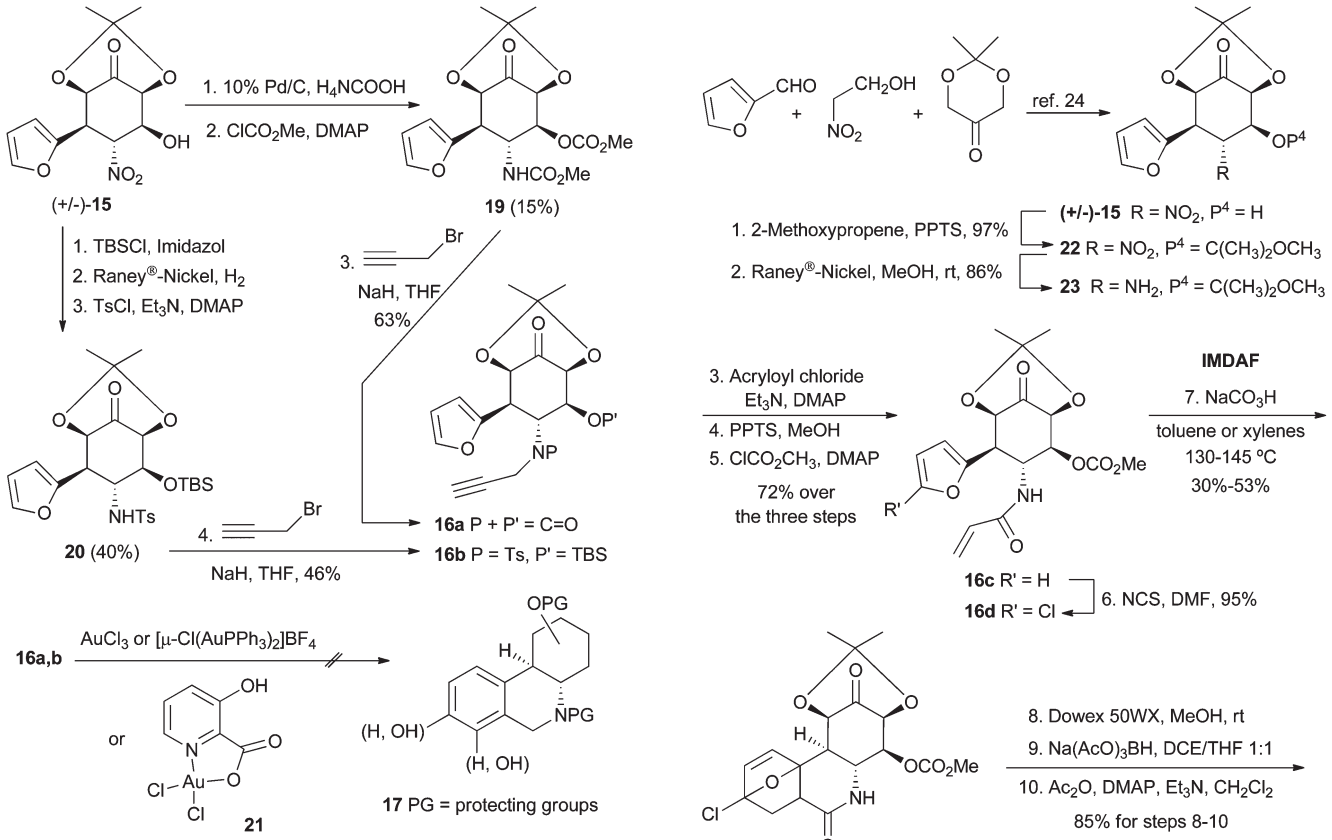
7,8-deoxy-9-methoxypancratistatin **5**[20] (Scheme 1). Replacement of the highly oxygenated phenyl ring A with a plain indole unit as in **3** resulted in the drop of activity against a number of human cancer lines to GI_{50} values higher than 10 μg mL^{-1}. Complete deoxygenation of ring A as in **4** (with the incorporation of two trimethylsilyl groups in positions 8 and 9) also caused loss of activity. Interestingly, the presence of a single oxygenated substituent at position C9 in ring A, as it happens in the methoxy analogue **5**, can be enough to keep the activity in the micromolar range (GI_{50} = 2.6–4.9 μg mL^{-1}).

Further understanding of the role that ring A plays in the pharmacophore requires the synthesis of new derivatives with different aromatic substituents. To that end, the development of preparative methods that could eventually allow the access to diverse substitution patterns through common intermediates is most desirable.

We recently reported a synthetic protocol to the pancratistatin skeleton (Scheme 1, part **I**)[21] in which ring C is assembled through a formal [3 + 3] annulation of β-aryl-α-nitro-α,β-enals (**8**)[22] with 2,2-dimethyl-1,3-dioxan-5-one (**8 + 9 → 10**), and ring B is formed by intramolecular electrophilic aromatic substitution (EAS) of appropriately-protected methyl carbamates of type **11**. In this approach, the substituents present in the starting aldehydes **6** and the activation-requirements and regioselectivity of the EAS step dictate and restrict the attainable substitution pattern of ring A in the final products **12**.

Looking for more flexible pathways, we identified compounds of type **16** as attractive intermediates because their unsaturated furylamine or furylamide functionality could in principle be transformed into differently-substituted dihydroisoquinolin-1(2*H*)-ones (the A/B ring system of **12**) by a number of procedures. In particular, we set out to explore the two paths, a and b, indicated in part **II** of Scheme 1. In path a, a furan–alkyne gold-catalysed cycloisomerization[23] of **16** would directly afford phenolic tetrahydroisoquinolines **17** (R^7 or R′ = OH), which should then be oxidized at their benzylic position and N-deprotected to render the corresponding dihydroisoquinolinone units. In path b, **16** would have to undergo an intramolecular Diels–Alder cycloaddition of its furan ring with the *N*-tethered unsaturated chain and the resulting oxabicyclic systems **18** would then need to be aromatized.

Herein, we report an initial study of both paths, the first successful implementation of path b and its application to the preparation of (+/−)-7,9-dideoxy derivatives of pancratistatin, which were tested against the NCI-H460 and the MCF-7 tumoral cell lines.



Scheme 2 Synthesis of furylpropargylamines **16a** and **16b** and attempts of gold-catalysed cycloisomerization to phenols **17**.

Exploratory studies of the gold-catalysed cycloisomerization pathway

All intermediates of type **16** used in this work were synthesized from the furyl-derived nitrocyclitol (+/−)-**15**, in turn prepared as previously reported[24] in three steps from furfural and dioxanone **9** through the β-furyl-α-nitroenal **14** (Scheme 1, part **II**). In particular, as indicated in Scheme 2, the application of two different NO_2-reduction and protection protocols to **15** led to intermediates **19** and **20**, which were respectively converted into the desired furylpropargylamines **16a** and **16b** by incorporation of a propargyl group at their nitrogen atoms.

Unfortunately, all our attempts to promote the gold-catalysed cycloisomerization of either the cyclic-carbamate protected propargylamine **16a** or its less-rigid *N*-tosyl analogue **16b** under a variety of conditions, using $AuCl_3$, $[\mu\text{-}Cl(AuPPh_3)_2]BF_4$ or salt **21**, failed thus far to render the desired phenolic tetrahydroisoquinolines **17**; either non-reaction/deprotection or complex mixtures were observed.

The hetero-Diels–Alder/aromatization pathway

To explore path b, we selected acrylamide **16d** (Scheme 3). We chose to use a chlorine substituent next to the oxygen atom of its furan ring with a double purpose; first, to facilitate the IMDAF cycloaddition (*i.e.*, the intramolecular Diels–Alder reaction of furan, step 1b in path b, Scheme 1);[25] second, to enable the aromatization process (step 2b) to directly afford C8-phenolic-7,9-dideoxygenated isoquinolinone systems, which we judged useful for pharmacophore studies (*vide infra*).

Scheme 3 Incorporation of the **IMDAF**/**aromatization** protocol to dihydroisoquinolin-1(2*H*)-ones into a new synthetic scheme for 7,9-dideoxy pancratistatin analogues (+/−)-**12** from furfural.

For the synthesis of **16d** from (+/−)-**15**, we first addressed the reduction of the nitro group, which was best performed with RANEY®-Nickel on its methoxyisopropyl ether derivative **22** (Scheme 3). We then introduced an acryloyl group at the nitrogen atom of the resulting amine **23**, replaced the acetal function at C4 with a methylcarbamate, and finally chlorinated the furan ring.

Acrylamide **16d** showed no change on heating in toluene in a closed flask at 120 °C for 3 days, but decomposed at higher temperature (xylenes, 130–145 °C), or when smoothly warmed (60–90 °C) in the presence of $Yb(OTf)_3$. However, when it was heated in the presence of $NaHCO_3$ (closed flask, toluene or xylenes, external bath temperature = 130–145 °C), the desired cycloadduct **18a** was obtained as a single stereoisomer (30–53%, Scheme 3).[26]

The conversion of **16d** into **18a** is the first example of an IMDAF process of a *secondary* amide *N*-tethered to a 2-furyl ring to directly render the corresponding *N*-deprotected oxabicyclic-fused γ-lactam.[27]

Once the viability of the IMDAF process was demonstrated, we were ready to study the following steps in the way to **12**, *i.e.*, the conversion of cycloadduct **18a** into the corresponding pancratistatin analogues. In practice, we found it convenient to first elaborate ring C before proceeding to the opening-aromatization of the oxanorbornene unit. In the event, successive treatment of **18a** with acid (to cleave the acetonide), $Na(AcO)_3BH$ (to reduce the keto group from the β face) and Ac_2O (steps 8–10) led to triacetate **18b** (85% overall) with the natural relative configuration at every stereocenter of ring C.

Aromatization of **18b** was simply achieved with NaMeO, which also hydrolysed the ester and carbamate groups, rendering the desired 7,9-dideoxy-8-phenolic pancratistatin analogue **12a**. By inverting the order of the reducing and deprotection steps in the synthetic sequence (steps a–d at the bottom of Scheme 3), **18a** was converted into **12c**, the C2-epimer of **12a**. We also prepared the analogue **12b** by alkylation of **12a** with benzyl bromoacetate.

Antiproliferative activity

The antiproliferative data of compounds (+/−)-**12a–c** against the tumoral cell line NCI-H460 (human large-cell lung carcinoma) are collected in Table 1. The activity data for some reference compounds are also included.

As compared to (+/−)-7-deoxypancratistatin [(+/−)-**2**], which showed an NCI-H460 cell growth inhibition (%GI) of 85% (at 100 μM) and an IC_{50} value of 1.57 μg mL^{-1},[28] its 9-deoxy-8-hydroxy analogue **12a** as well as its 9-deoxy-8-[2-(benzyloxy)-2-oxoethoxy] analogue **12b** displayed %GI values of only 3.8% and 7% (respectively, at the same concentration) and IC_{50} values higher than 30 μg mL^{-1}. Thus, removing the oxygenated substituent at C9 is extremely detrimental to activity (>12 fold), regardless of whether the remaining C8 oxygenated function is a free phenol, as in **12a**, or an alkylated form of it, as in **12b**. This decrease in activity is by far more pronounced than those reported for the removal of the oxygenated function at C7 [a ≈ 6 fold drop in going from pancratistatin (**1**, IC_{50} = 0.048 μg mL^{-1}) to its 7-deoxy analogue **2** (IC_{50} = 0.29 μg mL^{-1})], or at C8 [a ≈ 10 fold cut in going from 7-deoxypancratistatin (**2**, IC_{50} = 0.29 μg mL^{-1}) to its 8-deoxy-9-methoxy analogue **5** (IC_{50} = 2.8 μg mL^{-1})].

Thus, of the three oxygenated substituents that natural pancratistatin has in its aromatic ring A, the one at C9 appears to be the most important for activity. Its location, at position *para* with respect to the amide carbonyl function, suggests that it could act, independently of other functions, by tuning the acceptor capabilities of the mentioned carbonyl group through a donor resonance effect.

Removal of the oxygenated function in C9 was also adverse in the (comparatively inactive) 2-*epi* series: the activity dropped in going from (+/−)-7-deoxy-2-*epi*-pancratistatin [(+/−)-2-*epi*-**2**, %GI = 16%] to its 9-deoxy-8-hydroxy analogue **12c** (%GI = 3.5%).

Similar data were obtained against the MCF-7 (breast) tumoral cell line: **12a** and **12b** showed %GI values of 4.6% and 16%, respectively, while **12c** was found to be completely inactive (for details, see ESI†).

Conclusions

The capability of compounds of type **16** to serve as intermediates for the preparation of pancratistatin analogues was initially evaluated by exploring two different paths to build the A/B ring system. In spite of initial failure, the furan–alkyne gold-catalysed cycloisomerization route (path a) deserves to be further studied because of its high potential. The Diels–Alder/aromatization pathway (path b) was reduced to practice and successfully incorporated into a new synthetic scheme for pancratistatin analogues from furfural. Because the IMDAF process takes place with *secondary* acrylamides of type **16** (NPG = NH), the synthetic protocol does not require protection–deprotection steps at nitrogen. Testing of the cytotoxic activities of the 7,9-dideoxy analogues allowed further

Table 1 Antiproliferative data for **12a–c** and some reference compounds against the tumoral cell line NCI-H460 (large-cell lung human carcinoma)

	(+/−)-**2**[a]	(+/−)-**12a**	(+/−)-**12b**	(+)-**1**[b]	(+)-**2**[b]	(+/−)-**5**[c]	(+/−)-2-*epi*-**2**[a]	(+/−)-**12c**
%GI (at 100 μM)	85%	3.8%	7%	—	—	—	16%	3.5%
IC_{50} (μg mL^{-1})	1.57	>30	>30	0.048	0.29	2.8	>30	>30

[a] From ref. 28. [b] From ref. 17. [c] From ref. 19*b*.

discussion of the role of aromatic substituents in the pharmacophore of pancratistatin.

Experimental section

(1*S,5*R**,6*R**,7*R**,8*S**)-6-(Furan-2-yl)-8-((2-methoxypropan-2-yl)-oxy)-3,3-dimethyl-7-nitro-2,4-dioxabicyclo[3.3.1]nonan-9-one (+/−)-22**

2-Methoxypropene (966 μL, 10.09 mmol) and PPTS (84 mg, 0.34 mmol) were added to a solution of (+/−)-**15** (1 g, 3.36 mmol) in dry CH_2Cl_2 (16.8 mL). After stirring for 1 h at rt, the reaction mixture was neutralized with Et_3N (0.5 mL) and the solvent evaporated *in vacuo*. Chromatography (15% EtOAc–hexane) afforded **22** (1.22 g, 97%) as a white solid: R_f = 0.49 (20% EtOAc–hexane); mp: 137–139 °C (EtOAc–hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.37 (dd, J = 1.8 Hz, 0.7 Hz, 1H), 6.41 (d, J = 3.3 Hz, 1H), 6.35 (dd, J = 3.3, 1.8 Hz, 1H), 5.45 (dd, J = 11.7, 9.6 Hz, 1H), 4.69 (dd, J = 1.9, ≈1.9 Hz, 1H), 4.45 (dd, J = 2.2, ≈2.2 Hz, 1H), 4.30 (dd, J = 9.6, 1.9 Hz, 1H), 3.51 (dd, J = 11.7, 1.9 Hz, 1H), 3.24 (s, 3H), 1.57 (s, 3H), 1.47 (s, 3H), 1.37 (s, 3H), 1.30 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 206.7, 147.6, 143.0, 110.7, 108.9, 102.8, 99.3, 86.9, 79.1, 76.7, 76.5, 49.7, 44.4, 28.3, 25.3, 24.9, 24.1; LRMS (IE) m/z (%): 369.0 [$(M)^+$, 8]; HRMS [IE, $(M)^+$] m/z: calcd for ($C_{17}H_{23}NO_8$): 369.1424, found: 369.1414.

(1*S,5*R**,6*R**,7*R**,8*S**)-7-Amino-6-(furan-2-yl)-8-((2-methoxypropan-2-yl)oxy)-3,3-dimethyl-2,4-dioxabicyclo[3.3.1]-nonan-9-ona (+/−)-23**

A suspension of **22** (3.52 g, 9.52 mmol) and RANEY®-Nickel (≈15 mL) in MeOH–THF (1 : 1, 40 mL) was stirred at rt under a H_2 atmosphere. After completion of the reduction (as monitored by TLC), the catalyst was filtered off and washed with MeOH (200 mL) and EtOAc (200 mL). Evaporation of the combined washings and the filtrate afforded **23** (2.76 g, 86%) as a pale brown solid; R_f = 0.42 (80% EtOAc–hexane): mp = 137–139 °C (EtOAc–hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.35 (d, J = 1.7 Hz, 1H), 6.35 (dd, J = 3.2, 1.7 Hz, 1H), 6.32 (d, J = 3.2 Hz, 1H), 4.60 (dd, J = 2.3, 1.8 Hz, 1H), 4.25 (dd, J = 2.3, 1.9 Hz, 1H), 3.89 (dd, J = 11.0, 9.2 Hz, 1H), 3.51 (dd, J = 9.2, 1.9 Hz, 1H), 3.27 (s, 3H), 2.66 (dd, J = 11.0, 1.8 Hz, 1H), 1.51 (s, 3H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 209.4, 152.0, 142.0, 110.6, 107.6, 102.2, 98.8, 80.2, 79.7, 77.9, 50.0, 49.6, 48.0, 28.4, 25.4, 25.2, 25.2; LRMS (EI) m/z (%): 340.1 [$(M + H)^+$, 12].

(1*R,5*S**,6*S**,7*R**,8*R**)-7-Acrylamido-8-(furan-2-yl)-3,3-dimethyl-9-oxo-2,4-dioxabicyclo[3.3.1]nonan-6-yl methyl carbonate (+/−)-16c**

Acryloyl chloride (400 μL, 4.99 mmol), Et_3N (696 μL, 4.99 mmol) and DMAP (110 mg, 0.91 mmol) were added to a solution of **23** (1.54 g, 4.54 mmol) in dry CH_2Cl_2 (22 mL) under argon. After stirring for 2 h, 2,6-di-*tert*-butyl-4-methylphenol (200 mg, 0.91 mmol) was added, the solvent was evaporated and the residue dissolved in dry MeOH (30 mL). PPTS (228 mg, 0.91 mmol) was added and the mixture stirred at rt for 1 h and then neutralized with Et_3N. After solvent evaporation, the residue was dissolved in dry CH_2Cl_2 (30 mL) and treated with methyl chloroformate (420 μL, 5.44 mmol) and DMAP (664 mg, 5.44 mmol). After stirring for 1.5 h, 2,6-di-*tert*-butyl-4-methylphenol (200 mg, 0.91 mmol) was added and the mixture treated with a saturated aqueous solution of $NaHCO_3$ (30 mL) and extracted with CH_2Cl_2 (3 × 20 mL). Chromatography (40% EtOAc–hexane) afforded **16c** (1.24 g, 72%) as a white solid: R_f = 0.67 (60% EtOAc–hexane); ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.33 (s, 1H), 6.44 (d, J = 3.2 Hz, 1H), 6.37–6.29 (m, 1H), 6.17 (dd, J = 17.0, 1.3 Hz, 1H), 5.95 (dd, J = 17.0, 10.2 Hz, 1H), 5.82 (d, J = 8.8 Hz, 1H), 5.58 (dd, J = 10.2, 1.3 Hz, 1H), 5.12 (ddd, J = 11.8, 10.3, 8.8 Hz, 1H), 4.94 (dd, J = 10.3, 2.1 Hz, 1H), 4.68 (s, 1H), 4.44 (s, 1H), 3.76 (s, 3H), 3.32 (br d, J = 11.8 Hz, 1H), 1.59 (s, 3H), 1.48 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 206.4, 165.7, 154.9, 150.0, 142.1, 130.4, 127.0, 110.8, 108.5, 99.4, 79.1, 77.7, 77.5, 55.3, 48.6, 44.5, 28.2, 25.4; LRMS (CI) m/z (%): 380.1 [$(M + H)^+$, 100]; HRMS [CI, $(M + H)^+$] m/z: calcd for ($C_{18}H_{22}NO_8$): 380.1345, found: 380.1340.

(1*R,5*S**,6*S**,7*R**,8*R**)-7-Acrylamido-8-(5-chlorofuran-2-yl)-3,3-dimethyl-9-oxo-2,4-dioxabicyclo[3.3.1]nonan-6-yl methyl carbonate (+/−)-16d**

N-Chlorosuccinimide (262 mg, 1.96 mmol) was added to a solution of **16c** (620 mg, 1.63 mmol) in dry DMF (8 mL) under argon. After stirring for 12 h at rt, the reaction mixture was neutralized with Et_3N, treated with 2,6-di-*tert*-butyl-4-methylphenol (72 mg, 0.33 mmol) and the solvent was evaporated *in vacuo*. Chromatography (40% EtOAc–hexane) afforded **16d** (640 mg, 95%) as a white solid: R_f = 0.51 (50% EtOAc–hexane); mp: 139–145 °C ($CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 6.46 (d, J = 3.3 Hz, 1H), 6.20 (dd, J = 17.0, 1.3 Hz, 1H), 6.09 (d, J = 3.3 Hz, 1H), 5.97 (dd, J = 17.0, 10.3 Hz, 1H), 5.79–5.65 (m, 1H), 5.62 (dd, J = 10.3, 1.3 Hz, 1H), 5.10 (ddd, J = 11.7, 10.3, 9.8 Hz, 1H), 4.90 (dd, J = 10.2, 2.0 Hz, 1H), 4.67 (dd, J = 2.0, 2.0 Hz, 1H), 4.43 (dd, J = 2.0, 1.3 Hz, 1H), 3.76 (s, 3H), 3.24 (dd, J = 11.7, 1.3 Hz, 1H), 1.59 (s, 3H), 1.48 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 206.2, 166.0, 154.8, 149.6, 135.3, 130.4, 127.0, 110.9, 107.4, 99.3, 79.4, 77.3, 77.2, 55.2, 47.5, 44.6, 28.1, 25.3; LRMS (CI) m/z (%): 414.1 [$(M + H)^+$, 22], 356.0 (37), 280.0 (46), 267.0 (95); HRMS [CI, $(M + H)^+$] m/z: calcd for ($C_{18}H_{21}ClNO_8$): 414.0956, found: 414.0956.

Oxanorbornene (+/−)-18a

A suspension of **16d** (80 mg, 0.19 mmol) and $NaHCO_3$ (19 mg, 0.23 mmol) in xylenes (2 mL) was stirred in a closed glass tube for 5 h at 140 °C (external bath temperature). Rotary evaporation of the solvent and chromatography (40% EtOAc–hexane) afforded **18a** (42 mg, 53%) as a white solid: R_f = 0.57 (50% EtOAc–hexane); mp = 143–145 °C (EtOAc–hexane, decomposition); ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.62 (d, J = 6.9 Hz, 1H), 6.78 (d, J = 5.6 Hz, 1H), 6.38 (d, J = 5.6 Hz, 1H), 4.65 (dd, J = 2.3, 2.3 Hz, 1H), 4.54 (dd, J = 9.9, 2.3 Hz, 1H), 4.49 (br s, 1H), 4.34 (ddd, J = 11.5, 9.9, 6.9 Hz, 1H), 3.80 (s, 3H), 2.65 (dd, J = 11.8, 4.0 Hz, 1H), 2.49 (dd, J = 8.3, 4.0 Hz, 1H), 2.11 (dd, J =

11.8, 8.3 Hz, 1H), 1.91 (dd, J = 11.5, 2.3 Hz, 1H), 1.48 (s, 3H), 1.44 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 204.7, 171.7, 154.9, 140.9, 136.8, 99.7, 98.5, 88.4, 79.6, 77.0, 75.9, 55.9, 49.0, 48.2, 47.5, 38.2, 28.7, 25.6; LRMS (CI) m/z (%): 414.14 [$(M + H)^+$, 10], 267.0 (15), 56.0 (100); HRMS [CI, $(M + H)^+$] m/z: calcd for ($C_{18}H_{21}ClNO_8$): 414.0956, found: 414.0950.

Triacetate (+/−)-18b

A mixture of **18a** (118.4 mg, 0.29 mmol) and Dowex 50WX (438 mg) in MeOH (2.9 mL) was stirred for 24 h at 60 °C. After filtration, the solvent was evaporated *in vacuo* and the crude dissolved in DCE–THF (1 : 1, 2.9 mL) under argon. $Na(AcO)_3BH$ (303.7 mg, 1.45 mmol) was added and the mixture stirred at rt for 2 h and then quenched with 30% aqueous hydrogen peroxide (0.8 mL). After solvent evaporation, the crude was dissolved in dry CH_2Cl_2 (2.9 mL) and treated with Et_3N (1.2 mL), Ac_2O (0.6 mL) and DMAP (6.8 mg, 0.06 mmol). After stirring for 12 h at rt, the mixture was treated with a saturated aqueous solution of $NaHCO_3$ (2.9 mL) and extracted with CH_2Cl_2 (3 × 2 mL). Chromatography (60% EtOAc–hexane) gave **18b** (121.8 mg, 85%) as a white foam: R_f = (20% EtOAc–hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.41 (s, 1H), 6.50 (d, J = 5.7 Hz, 1H), 6.32 (d, J = 5.7 Hz, 1H), 5.53 (dd, J = 3.7, 2.9 Hz, 1H), 5.30 (dd, J = 2.9, 2.9 Hz, 1H), 5.14 (br s, 1H), 4.89 (dd, J = 10.6, 3.7 Hz, 1H), 4.20 (dd, J = 11.9, 10.6 Hz, 1H), 3.84 (s, 3H), 2.80 (dd, J = 12.0, 3.8 Hz, 1H), 2.65 (dd, J = 11.9, 2.3 Hz, 1H), 2.54 (dd, J = 8.3, 3.8 Hz, 1H), 2.22 (dd, J = 12.0, 8.3 Hz, 1H), 2.18 (s, 3H), 2.14 (s, 3H), 2.11 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.1, 168.9, 168.8, 168.1, 154.4, 141.8, 134.6, 97.7, 88.2, 75.3, 69.2, 66.9, 66.5, 55.7, 47.7, 46.7, 41.4, 38.8, 21.0, 20.7, 20.6; LRMS (CI) m/z (%): 502.1 [$(M + H)^+$, 22], 460.1 (16), 123.1 (18); HRMS [ICI, $(M + H)^+$] m/z: calcd for ($C_{21}H_{25}ClNO_{11}$): 502.1116, found: 502.1110.

7,9-Dideoxy-8-hydroxypancratistatin (+/−)-12a

A solution of **18b** (84 mg, 0.17 mmol) in MeOH (1.6 mL) was treated with NaMeO in MeOH (5.4 M, 186 μL) and stirred for 6 h at 50 °C. The pH of the mixture was adjusted to 3 with a 1 M aqueous solution of HCl and the solvent evaporated *in vacuo*. Chromatography (20% MeOH–CH_2Cl_2–1% TFA) afforded **12a** (14 mg, 93%) as a white solid: R_f = 0.28 (20% MeOH–CH_2Cl_2–1% TFA); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.42 (d, J = 2.7 Hz, 1H), 7.27 (d, J = 8.4 Hz, 1H), 7.00 (dd, J = 8.4, 2.7 Hz, 1H), 4.52 (br s, 1H), 4.18 (dd, J = 3.2, 3.2 Hz, 1H), 4.02 (br s, 1H), 3.93–3.88 (m, 2H), 3.21–314 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 168.4, 157.4, 140.9, 131.3, 127.4, 121.1, 115.3, 75.2, 72.2, 72.0, 70.4, 51.9, 40.8; LRMS (CI) m/z (%): 282.0 [$(M + H)^+$, 100], 203.0 (100), 167.0 (37); HRMS [CI, $(M + H)^+$] m/z: calcd for ($C_{13}H_{16}NO_6$): 282.0978, found: 282.0972.

7,9-Dideoxy-8-[2-(benzyloxy)-2-oxoethoxy]pancratistatin (+/−)-12b

K_2CO_3 (30 mg, 0.17 mmol), benzyl bromoacetate (21 μL, 0.13 mmol) and $Bu_4N^+I^-$ (12 mg, 0.03 mmol) were added to a solution of **12a** (23 mg, 0.08 mmol) in dry DMF (1 mL). After stirring for 9 h at rt, the pH was adjusted to 3 with 1 M aqueous HCl and the solvent evaporated *in vacuo*. Chromatography (20% MeOH–CH_2Cl_2–1% TFA) gave **12b** (9.1 mg, 25%) as a white solid: R_f = 0.62 (20% EtOAc–hexane); ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.51 (d, J = 2.8 Hz, 1H), 7.38 (d, J = 8.6 Hz, 1H), 7.35–7.22 (m, 5H), 7.16 (dd, J = 8.6, 2.8 Hz, 1H), 4.76 (s, 2H), 4.59 (s, 2H), 4.53 (br s, 1H), 4.18 (dd, J = 3.2, 3.2 Hz, 1H), 4.02 (br s, 1H), 3.96–3.87 (m, 2H), 3.26–3.17 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 170.0, 166.5, 157.0, 141.5, 132.3, 130.5, 128.1 (2C), 127.1, 126.8 (2C), 126.3, 119.7, 112.5, 74.0, 70.9, 70.8, 69.1, 64.9, 64.0, 50.6, 39.7.

7,9-Dideoxy-2-*epi*-8-hydroxypancratistatin (+/−)-12c

A mixture of **18a** (45 mg, 0.11 mmol) and $NaBH_4$ (8 mg, 0.22 mmol) in dry MeOH (0.7 mL) was stirred for 10 min at rt. The reaction was neutralized with 5% aqueous AcOH, evaporated *in vacuo* and extracted with CH_2Cl_2 (3 × 0.5 mL). The residue was dissolved in CH_2Cl_2 (0.5 mL) and stirred with *p*-TsOH·H_2O (25 mg, 0.13 mmol) for 1 h at rt. After neutralization with Et_3N and evaporation of the volatiles *in vacuo*, dry CH_2Cl_2 (0.5 mL), Et_3N (121 μL, 0.87 mmol), Ac_2O (41 μL, 0.43 mmol) and DMAP (3 mg, 0.022 mmol) were added and the mixture stirred for 1 h at rt, diluted with a saturated aqueous solution of NH_4Cl (0.5 mL) and extracted with CH_2Cl_2 (3 × 0.5 mL). Chromatography (60% EtOAc–hexane) gave protected **12c** [27 mg, 50% for the three steps, R_f = 0.24 (70% EtOAc–hexane)], which was dissolved in MeOH (1 mL), treated with NaMeO (5.4 M in MeOH, 60 μL) and stirred for 6 h at 50 °C. pH adjustment to 3 with aqueous HCl (1 M), solvent evaporation *in vacuo* and chromatography (20% MeOH–CH_2Cl_2–1% TFA) afforded **12c** (13 mg, 93% for the last step) as a white solid: R_f = 0.25 (20% MeOH–CH_2Cl_2–1% TFA); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.39 (d, J = 2.6 Hz, 1H), 7.26 (d, J = 8.4 Hz, 1H), 6.98 (dd, J = 8.4, 2.6 Hz, 1H), 4.61 (s, 1H), 4.09 (s, 1H), 3.83 (dd, J = 13.3, 10.2 Hz, 1H), 3.68 (br s, 1H), 3.59 (dd, J = 10.2, 2.6 Hz, 1H), 2.78 (br d, J = 13.3 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 168.1, 157.6, 131.4, 130.3, 127.5, 121.0, 115.2, 76.3, 73.9, 70.8, 51.5, 42.5; LRMS (ESI-TOF) m/z (%): 304.1 [$(M + Na)^+$, 36], 282.1 [$(M + H)^+$, 58], 245.1 (100), 149.0 (49); HRMS [ESI-TOF, $(M + H)^+$] m/z: calcd for ($C_{13}H_{16}NO_6$): 282.0978, found: 282.0972.

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