Organic & Biomolecular Chemistry

Dynamic Article Links

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 825

www.rsc.org/obc

PAPER

A formal [3+3]-annulation-based approach to pancratistatins: total synthesis of (±)-7-deoxy-pancratistatin and its 2-*epi* and 2,4-di*epi* analogues†

Olaia Nieto-García,[a] Hugo Lago-Santomé,[a] Fernando Cagide-Fagín,[b] Juan Carlos Ortiz-Lara[c] and Ricardo Alonso*[a]

Received 12th August 2011, Accepted 4th October 2011
DOI: 10.1039/c1ob06374j

A full account is given for the total synthesis and the cytotoxic activity against the human lung tumoral cell line NCI-H460 of (±)-7-deoxy-pancratistatin and its 2-*epi*- and 2,4-di*epi*- unnatural analogues.

Introduction

Among the constituents isolated from plants of the *Amaryllidaceae* family, those with a partially hydrogenated and heavily oxygenated phenanthridin-6-one skeleton (most commonly referred to as the isocarbostyrils,[1] *e.g.* compounds **1**–**4**, Fig. 1), have been proposed as the most important metabolites responsible for the therapeutic benefits of some species of these plants in the folk medical treatment of cancer.[2,3]

Pancratistatin (**1**), with strong antitumoral activity, was identified as a most promising lead. In fact, pancratistatin has been in preclinical development for over 20 years. Its advancement to clinical trials has been prevented to date by two major obstacles: the lack of enough supply from natural resources and its poor aqueous solubility. Nowadays, both problems demand, more than ever, a practical and scalable synthetic route.[4,5]

Towards that end, we recently reported a novel procedure to build complex polyoxygenated nitrocyclohexanes. In the same communication, we succinctly described the conversion of one of such nitrocyclohexanes into a non-natural protected analogue of pancratistatin, namely the (±)-7-deoxy-2-*epi*-pancratistatin tetraacetate (**6**).[6]

Now, we show that the synthetic protocol developed for **6** is susceptible of modification at different stages to allow the preparation of other pancratistatins. Most importantly, it is possible to obtain the relative stereochemistry required by the natural products, as demonstrated with the synthesis of racemic (±)-7-deoxypancratistatin (*rac*-**2**). Non-natural epimers at C-4, *e.g.*, the 2,4-di*epi* analogue **7**, can also be prepared. As for *rac*-**2** and **7**, full details are given here for the synthesis of deprotected **6**: (±)-7-deoxy-2-*epi*-pancratistatin (**5**, Fig. 1).

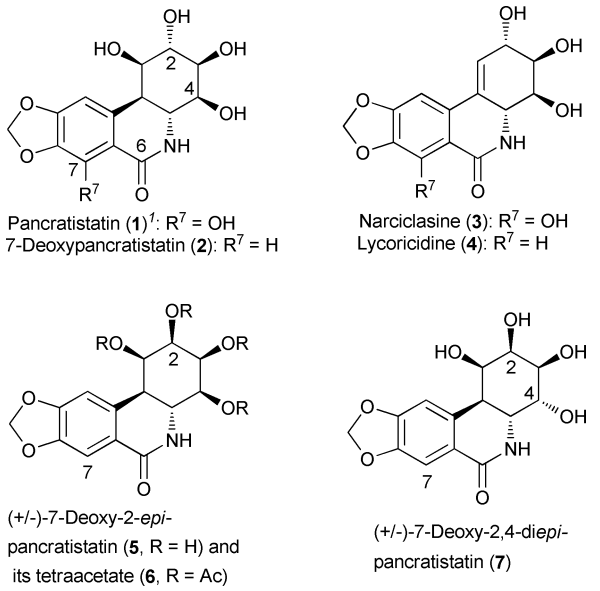


Fig. 1 Structures of some *Amaryllidaceae* constituents (1–4) and some synthetic analogues (5–7). [1]For a complete numbering of the carbon skeleton, see Scheme 1.

The cytotoxicity of the new compounds against the human tumoral cell line NCI-H460 was determined and the results discussed in relation with the pharmacophore of pancratistatin.

The synthetic plan

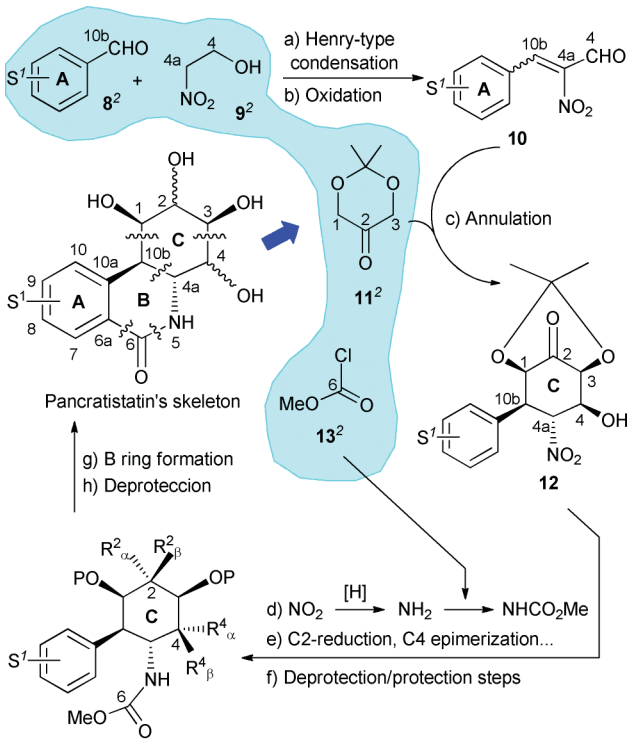
According to our plan (Scheme 1), the pancratistatin skeleton would be assembled from a total of four commercially available fragments: an aromatic aldehyde (**8**), 2-nitroethanol (**9**), 2,

[a]*Departamento de Química Orgánica, Universidad de Santiago, 15782, Santiago de Compostela, Spain. E-mail: r.alonso@usc.es; Fax: +34 981 595012; Tel: +34 981 563100 Ext 14250*

[b]*Currently at the University of Porto: CIQ/Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, 4169-007, Porto, Portugal*

[c]*Currently at Sintenovo S.A. de C.V.: Calle 4, no. 26, Naucalpan de Juárez, Estado de México, México, Código Postal 53370; Fax: 0052 55761358; Tel: 0052 55760396*

† Electronic supplementary information (ESI) available: Experimental procedures for **20a–b**, **22a–b**, 1,2-*cis*-**23**, **26–28**, **33** and **17**, including nOe data for 1,2-*cis*-**23** and X-ray data for 1,2-*cis*-**22a**; NMR spectra for compounds **17**, **15a**, **15b**, **6**, **5**, **20a**, **20b**, 1,2-*trans*-**22a**, 1,2-*cis*-**22a**, 1,2-*cis*-**23**, **24a**, **24b**, **14a**, **25**, **2**, **27**, **28**, **30**, **31**, **16a**, **16b**, **32**, **7** and **33**; and some additional data for the studies on cytotoxicity. CCDC reference number 839217. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c1ob06374j



Scheme 1 The synthetic plan to the pancratistatin's skeleton. [1]S = aromatic substituents. [2]Carbons in fragments **8**, **9**, **11** and **13** are numbered according to their final numbering in the benzophenanthridin-6-one system of pancratistatin.

2-dimethyl-1,3-dioxan-5-one (**11**) and an alkoxycarbonylating agent such as methyl chloroformate (**13**).

Briefly stated, a Henry-type condensation of **8** and **9** followed by oxidation of the terminal hydroxyl group, would render β-aryl-α-nitro-α,β-enals **10**, which have ring **A** and carbons 4, 4a and 10b in place, as well as the nitrogenated function required at position 4a in the form of a nitro group.

Ring **C** would then be formed by annulation of enals **10** with dioxanone **11** to give protected nitrocyclitols **12**. These intermediates would then be further elaborated to one of the diastereomeric compounds of type **14**, **15** or **16** in order to prepare the natural compounds or their 2-*epi* or 2,4-di*epi* analogues, respectively. Such elaboration would involve a number of operations including: d) the modification of the nitrogen functionality, from a nitro group to a carbamate, e) the stereoselective generation and/or modification of stereocenters at positions 2 and 4, as needed, and f) any required deprotection-protection steps along the sequence.

Finally, prior to the last deprotection step, the Banwell's modification[7] of the Bischler-Napieralsky type cyclization would be applied to **14**, **15** or **16** to build up the lactam ring **B**. Choice of this cyclization type for the late stages of the synthesis relied on its demonstrated success in a number of previous syntheses.[8]

Attractive as this plan could be, especially because of its convergence and the use of simple and readily available starting materials, a number of potential pitfalls were readily apparent.

Significantly, at the outset of our study, only one compound of type **10** had been named in a patent (with neither details nor references for its preparation, properties or use), thus increasing the doubts on the stability of such strongly polarized, electron-deficient olefins, not to say on their use in synthesis.

Even if the proposed annulation process, **10** + **11**, could be feasible, the possibility to achieve synthetically useful yields with the desired stereochemical outcomes was most uncertain; in fact, apart from **12**, seven other possible diastereoisomers could be formed.

Compound **12** could be prone to undergo aromatization of its heavily oxygenated **C** ring through elimination (facilitated by the presence of the keto, nitro and aryl groups), and keto-enol tautomerization. This synthetically fatal process could also take place in other compounds derived from **12** along the preparative sequence, especially if they maintain either the keto or the nitro group or both.

Proving the methodology: synthesis of (±)-7-deoxy-2-*epi*-pancratistatin (5)

To prove the validity of the plan proposed for pancratistatins (Scheme 1), we begun by studying the preparation and stability of β-aryl-α-nitro-α,β-enals **10**.

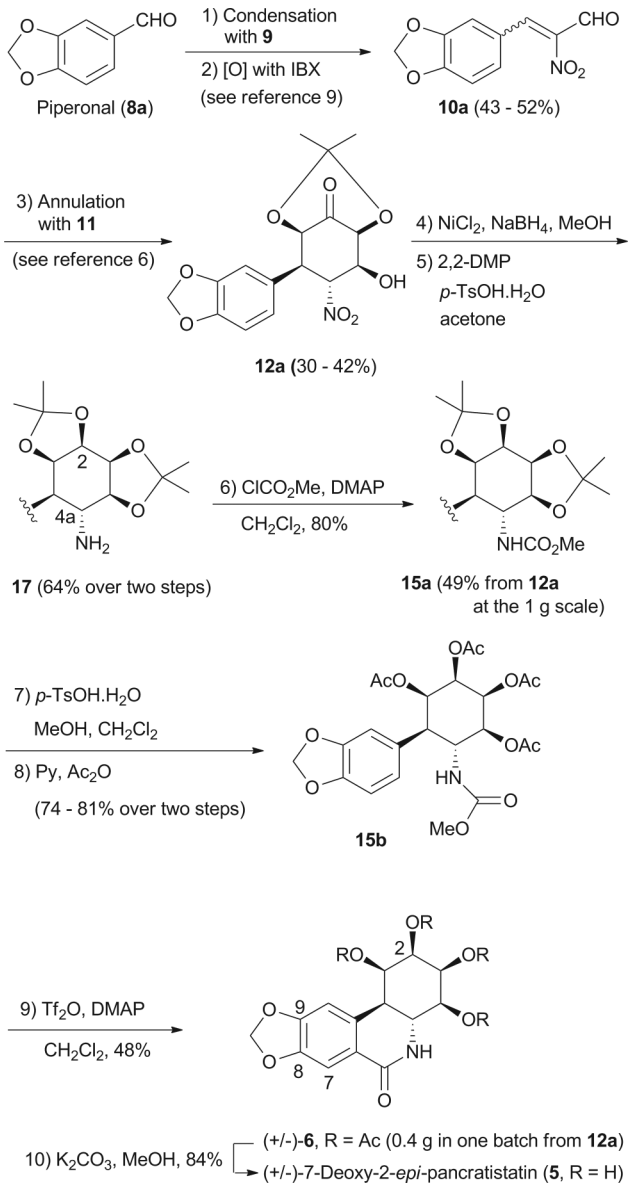
This largely overlooked type of compounds showed to be stable enough to be prepared in gram quantities by condensation of 2-nitroethanol with aromatic aldehydes, followed by oxidation with 2-iodoxybenzoic acid (IBX).[9]

Most relevant to our interest, β-aryl-α-nitro-α,β-enals **10** demonstrated to be capable to annulate with the pyrrolidine-derived enamine of dioxanone **11** to give the wanted protected nitrocyclitols **12** in a most stereoselective manner and with yields from 24% to 42% at the gram scale.[6]

Having access to intermediates **12**, we next studied their conversion into a pancratistatin analogue to verify the feasibility of the entire synthetic plan. In selecting the target at this early stage of our study, we considered significance (see below, under the heading "cytotoxic studies") but also convenience, reliability and practicality of the synthetic labour.

In the event, (±)-7-deoxy-2-*epi*-pancratistatin (**5**, Scheme 2) was chosen as the target, mainly because its preparation should be facilitated by three main reasons: (a) cheap and commercially available piperonal would be the starting material for the synthesis of the required intermediates: the nitroenal **10a** and the protected nitrocyclitol **12a**, (b) the reduction of the keto group in **12a** could be performed simultaneously with that of the nitro group immediately right after the preparation of this intermediate in order to minimize the risk of elimination or aromatization, and (c) hydride addition to the carbonyl group in **12a** would most likely be highly diastereoselective because shielding of its *Re* face by the isopropilidene bridge.

As concisely sketched in our previous communication,[6] the preparation of **5** from nitrocyclitol **12a** in the form of its protected tetraacetate **6**, was indeed possible. Complete details are given here, including the final deprotection of **6** to the desired (±)-7-deoxy-2-*epi*-pancratistatin analogue **5** (see the text below, Scheme 2 and the experimental section).



Scheme 2 Proving the synthetic methodology for 2-*epi* analogues: synthesis of (±)-7-deoxy-2-*epi*-pancratistatin (**5**).

As planned, we first addressed the simultaneous reduction of the keto and the nitro groups. Treatment of **12a**[10] with nickel boride,[11] and then with 2,2-dimethoxypropane led to the isolation of the diacetonide **17** as a single diastereoisomer, as a result of the exclusive addition of hydride to the *Si* face of the carbonyl group.

Application of the Bischler-Napieralsky protocol under Banwell's conditions for the formation of ring B required prior conversion of **17** into carbamate **15a** and change of the hydroxyl protection pattern from acetals to acetates, as in **15b**. Cyclization of **15b** took place with complete regiocontrol to give **6**, where the methylenedioxy group occupies positions 8 and 9, as it happens in the natural isocarbostyrils.

With the one-batch preparation of about 0.4 grams of **6** in ≈ 5% overall yield from **10a** and commercially available **11**, the proposed methodology demonstrated to be feasible but also practical, a major and important goal in the area.

Finally, tetraacetate **6** could be easily deprotected by acetate hydrolysis with K_2CO_3 in MeOH to give (±)-7-deoxy-2-*epi*-pancratistatin (**5**).

Application to the natural series

Once the general synthetic protocol was successfully tested for the 2-*epi* analogue **5**, we next moved on to evaluate its potential to prepare one member of the natural series. We selected (±)-7-deoxypancratistatin (*rac*-**2**) as the target, because, as for **5**, **12a** and piperonal would be the protected nitrocyclitol intermediate and the starting aldehyde, respectively.

On the other hand, establishing the natural relative configuration of *rac*-**2**, would require now to perform the hydride reduction of the C2 keto group of **12a** from its *Re* face.

Model studies

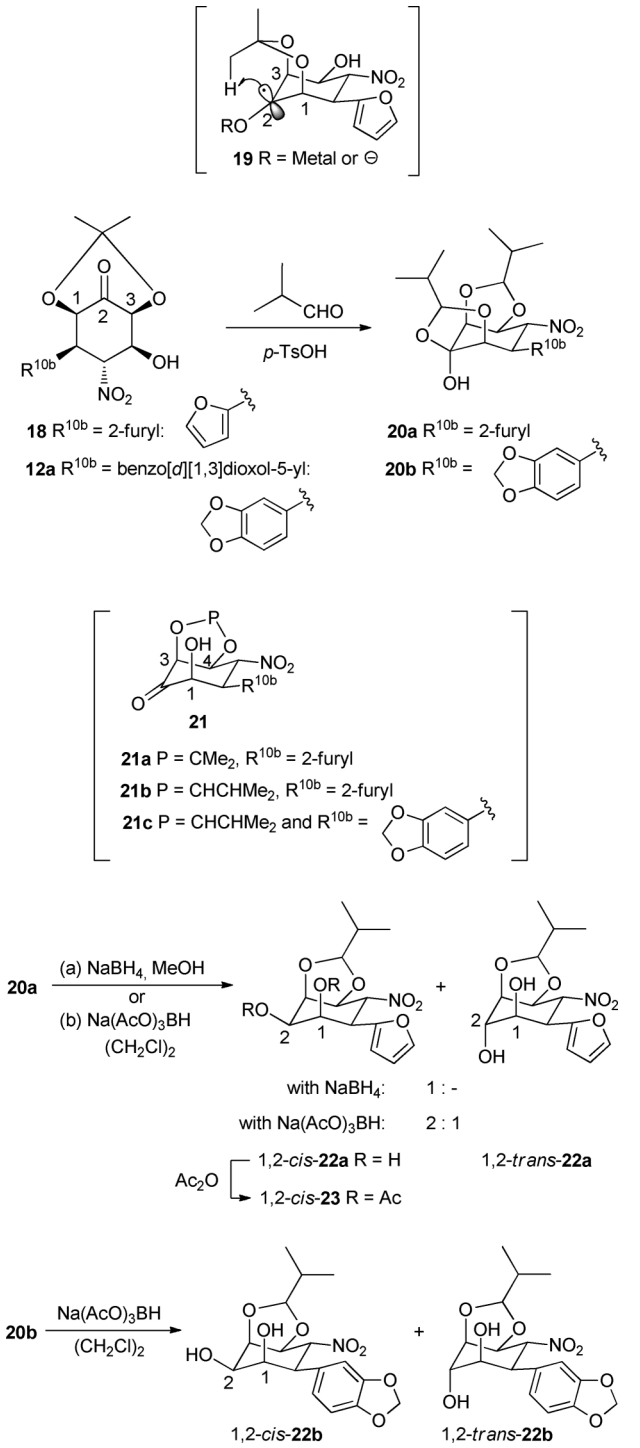
Re face-reduction at C2 was initially studied on protected nitrocyclitol **18** (Scheme 3), significantly easier to prepare than **12a** due to the higher stability of its nitroenal precursor.[12] Also, preparation of **18**[13] starts from furfural, a cheap starting material obtained from renewable sources.

Our first plan to perform the selective reduction at C2 of **18** from the *Re* face expected to take advantage of one structural factor revealed by its X-ray: its 1,3-dioxane ring is a boat where the carbonyl group and one of the methyl groups of the isopropylidene bridge are very close (C2 is just 2.9 and 2.3 Å away from the C and one of the H atoms of the CH_3 group, respectively).[6] Should such conformation be preponderant or even just easily attainable in solution, formation of a ketyl radical of type **19** could be followed by an intramolecular 1,5-hydrogen transfer to C2, thus resulting in the desired *Re*-face delivery. In the event, treatment of **18** in conditions reported to generate ketyl radicals[14] gave either no reaction or complex mixtures.

We then worked on the intermolecular hydride addition. For it to take place from the *Re* face of the C2 keto group in **18**, the previous removal of the isopropylidene bridge between positions 1 and 3 appeared to be mandatory. We reasoned that hydroxyketones of type **21** could be appropriate substrates: while expected to be manageable in terms of polarity, because the diol system at positions 3 and 4 would be in a protected form, the axial free hydroxyl group at position 1 could assist the reducing agent to deliver the hydride from the *Re* face, now accessible because of the absence of the acetal bridge.

Hydroxyketones of type **21** showed to be elusive and we were unable to prepare the *a priori* simpler **21a** as well as **21b**. We alternatively looked for ways to generate **21** *in situ* under the basic hydride reductive conditions, so that it would be immediately reduced as formed and its isolation would not be required. Precursors of type **20** appeared most suitable for such a purpose. Treatment of **20a** (prepared from **18** and isobutyraldehyde under acid catalysis), with $NaBH_4$ in MeOH gave the corresponding diol **22a**, presumably through hydride-promoted opening of the hemiketal function in **20a** to the putative hydroxyketone intermediate **21b** and subsequent reduction.

Diol **22a** was obtained as a single stereoisomer. Unfortunately, as supported by nOe data of its diacetate **23** and definitely proved by X-ray diffraction of **22a** itself,[15] it corresponded to the 1, 2-*cis*-isomer. Similar results were achieved using Red-Al in



Scheme 3 *Re* face-reduction at C2: model studies on **18** and preliminary studies with **12a**.

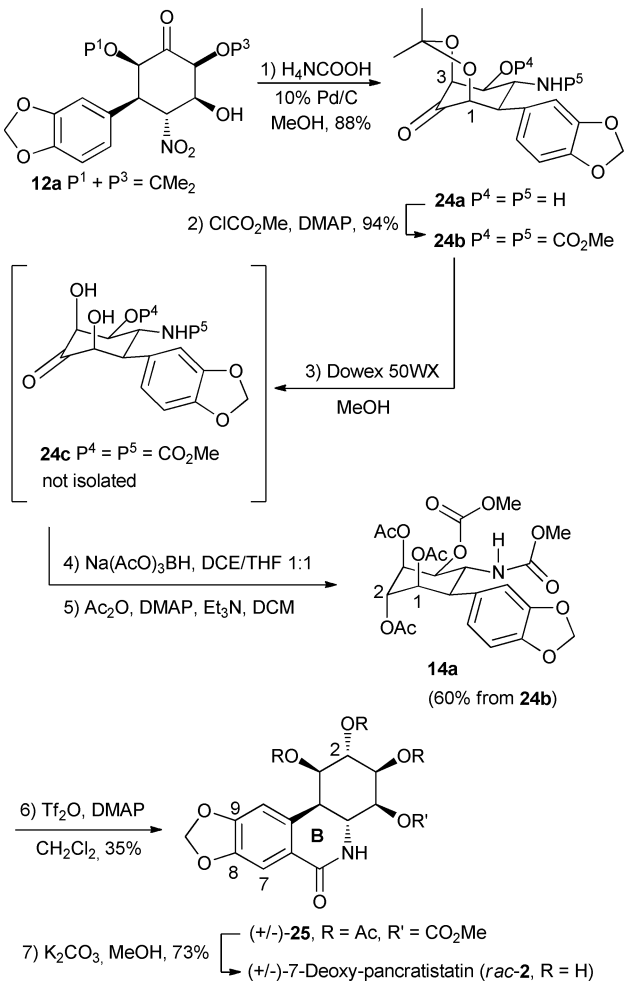
CH_2Cl_2. Use of $NaBH(AcO)_3$, known to promote hydroxyl-directed ketone reduction,[16] in 1,2-dichloroethane at 45 °C allowed to obtain the desired *trans*-diol **22a** as a 1 : 2 mixture with its *cis*-isomer.

Synthesis of (±)-7-deoxypancratistatin (*rac*-2)

Having proved the possibility to reduce the masked C2 keto group of a latent α-hydroxyketone of type **20** from its *Re* face through the corresponding putative ketone of type **21** with the help of $NaBH(AcO)_3$, we restarted our work on the preparation of *rac*-**2** from protected nitrocyclitol **12a**.

Under the conditions employed to transform **18** into **20a**, nitrocyclitol **12a** was converted into hemiketal **20b** (Scheme 3). On treatment with $NaBH(AcO)_3$, **20b** behaved as **20a** (suffering hemiketal deprotection and selective reduction of the putative α-hydroxyketone intermediate **21c** so formed), thus allowing access to *trans*-**22b** (isolated as a 1 : 1.7 mixture with 1,2-*cis*-**22b**) with the natural relative 1,2-*trans*-diol configuration required by *rac*-**2**.

Nevertheless, before further pursuing this route to complete the preparation of *rac*-**2** through 1,2-*trans*-**22b**, we decided to evaluate if the selectivity of the $NaBH(AcO)_3$ reduction step could be further increased when performed on compounds having an additional free OH α to the carbonyl group at C2, *i.e.* when carried out on α,α′-dihydroxyketones. To explore this option, we selected the 1,3-dihydroxyketone **24c** as the substrate and its corresponding acetonide **24b** as its direct precursor (Scheme 4).



Scheme 4 Validation of the strategy for the preparation of the natural series: total synthesis of (±)-7-deoxypancratistatin (*rac*-**2**).

While the incorporation of one methoxycarbonyl unit as P^5 in **24b** was required by our strategy (to later perform the formation of ring **B**), the attachment of a second methoxycarbonyl group (P^4) simply offered the advantage of carrying out the amino-functionalization and the C4–OH-protection in the same step.

Preparation of **24b** from **12a** was best accomplished by reduction of the nitro group through catalytic hydrogen transfer using ammonium formate to the intermediate amino alcohol **24a**, followed by the formation of both the carbamate at C4a and the carbonate at C4 on treatment with methyl chloroformate.

Liberation of the two hydroxyl groups at positions 1 and 3 of **24b** was performed by acid hydrolysis of the acetonide bridge under a variety of conditions: *p*-TsOH·H_2O, TFA, HCl and Dowex® 50WX. The putative dihydroxyketone **24c** thus formed was not isolated but directly reduced with $NaBH(AcO)_3$ to the corresponding triol, which was isolated and characterized as its triacetate **14a**. Compound **14a** was exclusively obtained as the desired 1,2-*trans* stereoisomer, the result of the exclusive addition of hydride to the *Re*-face of the keto group. The use of Dowex 50WX was particularly convenient in terms of operability; it also afforded the best overall yields for the three-step conversion (hydrolysis, reduction and acetylation) of **24b** into **14a**.

The last two operations, the formation of ring B on treatment with Tf_2O and DMAP, and the final hydrolysis of the acetates and the carbonate, proceeded uneventfully, thus concluding the total synthesis of (±)-7-deoxypancratistatin (*rac*-**2**) and validating the strategy for the preparation of *Amaryllidaceae* isocarbostyrils.

Synthesis of (±)-7-deoxy-2,4-di-*epi*-pancratistatin

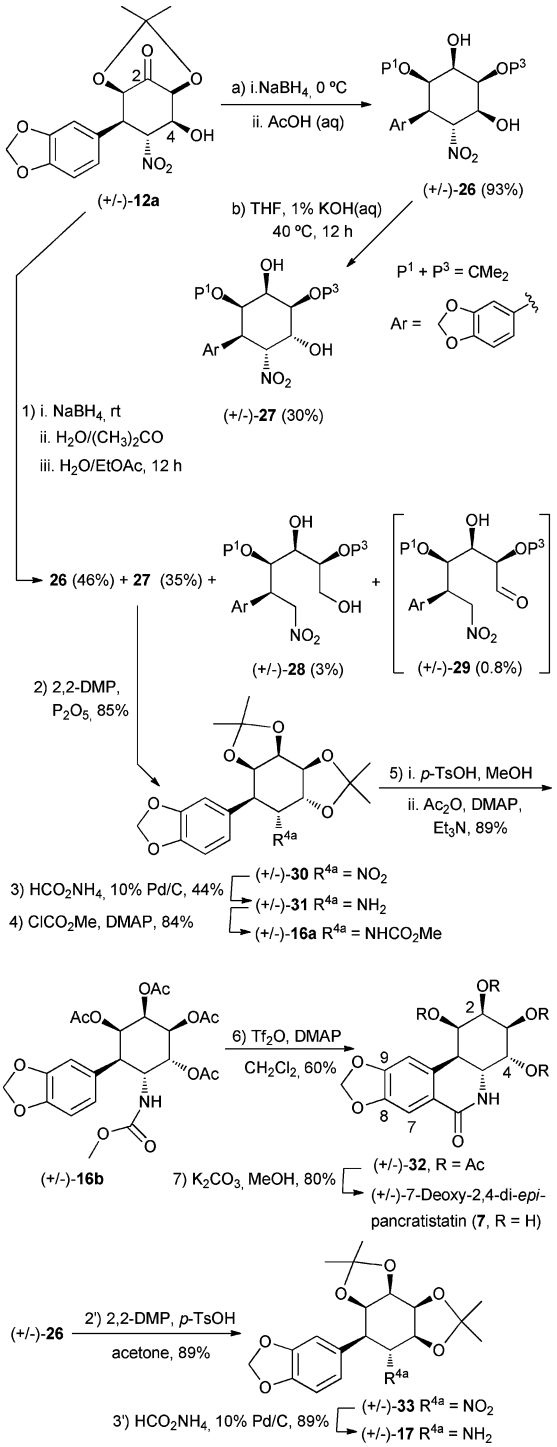
As discussed below, under the heading on cytotoxicity, it was of interest to determine the antitumoral activity of compound **7**, a new non-natural analogue of pancratistatin deoxygenated at C7 and epimer at positions 2 and 4.

Preparation of **7** from **12a** (Scheme 5) required inversion of configuration at C4. To evaluate the possibility of a base-promoted inversion, we first reduced the C2-keto group of **12a** from the unhindered *Si* face with $NaBH_4$. Treatment of the diol so formed **26** with aqueous KOH in TFH promoted its partial transformation into its C4 epimer **27**, thus proving that the desired base-promoted retro-Henry process to the putative aldehyde intermediate **29** followed by a Henry re-cyclization with overall C4 epimerization, was indeed achievable.

Compound **27** could also be directly obtained from **12a** with $NaBH_4$ at rt and prolonged stirring in a slightly basic biphasic media. Under these conditions, the open diol **28** was also formed apart from the cyclised ones **26** and **27**. On one occasion, we isolated a chromatographic fraction where the diol **28** was mixed with an aldehyde that, because it rendered **27** on treatment with DBU, it most likely corresponded to **29**.

Treatment of **27** with 2,2-dimethoxypropane and P_2O_5 in acetone followed by catalytic hydrogenation rendered amine **31**, which was converted into the suitable cyclization precursor **16b** by methoxycarbonylation to **16a** and subsequent change of the hydroxyl protecting groups from isopropylidene ketals to acetates. Cyclization of **16b** to **32** and final acetate removal took place as previously indicated for the transformation of **15b** into **5**, to finally render the desired 2,4-di-*epi* analogue **7**.

It should also be indicated here that submission of **26** to the same sequence of transformations employed to convert its C4-epimer **27** into **31**, completed an alternative way to obtain amine **17** from **12a**: **12a** → **26** → **33** → **17**, which is longer but more efficient (74%) than that previously described en route to (±)-7-deoxy-2-*epi*-pancratistatin (64%, Scheme 2).



Scheme 5 Total synthesis of (±)-7-deoxy-2,4-di-*epi*-pancratistatin (**7**).

Cytotoxic studies

To date, the study of the cytotoxicity of both natural and unnatural analogues of pancratistatin against tumoral cell lines allowed reaching a number of important conclusions on structure–activity relationships.[17]

Antitumoral activity appears to require at least: (a) the integrity of the tricyclic ABC benzophenanthridinone system, (b) a *trans*

B–C ring fusion, (c) one oxygenated (methoxy) substituent at position 9 of the aromatic ring A and (d) a 2,3-diol in ring C.

And potent activity was observed for compounds having the 2,3,4-triol subunit in ring C and two oxygenated substituents in ring A in the form of a methylenedioxy group between positions 8 and 9 (better if combined with a free phenol group at C7 as in pancratistatin itself).

At the outset of our work, no data was available on the influence that the relative configuration of the stereogenic carbons at positions 2, 3 and 4, *i.e.*, the relative orientation of the 2,3,4-triol-subunit in space, could have on antitumoral activity. In this respect, (±)-7-deoxy-2-*epi*-pancratistatin (**5**), where the relative configuration of only one of such centres, C2, is inverted while that of the others remains unchanged, offered a good opportunity to asses the impact of this structural factor on cytotoxicity (this issue added to other advantages on selecting **5** as our initial target, as previously indicated).

In evaluating the cytotoxicity of **5**, we required that of (±)-7-deoxypancratistatin (*rac*-**2**) as a reference. In fact, this reason, together with the preparative aspects mentioned above, contributed to the choice of *rac*-**2** as the synthetic goal to prove the capability of the new synthetic strategy to render the relative configuration of naturally occurring pancratistatins.

Besides, comparing the activity obtained for *rac*-**2** with that reported for natural **2**, could allow a crude estimation of the cytotoxicity of the enantiomer of 7-deoxypancratistatin (*ent*-**2**).[18]

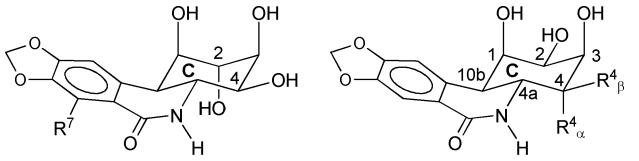
In our study, we employed the NCI-H460 human large cell lung carcinoma. Likewise, we used a natural sample of narciclasine[19] as a quantitative positive control for cytotoxicity; the IC_{50} (0.037 μg mL^{-1}) determined in our assays compared well with the reported literature value[20] (0.032 μg mL^{-1}).

As expected, racemic 7-deoxypancratistatin (*rac*-**2**) showed to be less active than the pure natural enantiomer **2**. However, the IC_{50} value obtained for *rac*-**2** (1.57 μg mL^{-1}) was surprisingly high (about 5 times higher than that reported for **2** (0.29 μg mL^{-1}).[21] This suggests that *ent*-**2** is neither cytotoxic nor even just a mere inactive spectator, in which case the IC_{50} for *rac*-**2** would be expected to be twice as much as that of **2**, *i.e.*, around 0.6 μg mL^{-1}; on the contrary, the presence of *ent*-**2** in the racemic material appears to have a detrimental effect on the cytotoxicity of its natural enantiomer **2**, lowering it by a factor of about 2.6.

As for compound **5**, the 2-*epi* analogue of racemic 7-deoxypancratistatin (*rac*-**2**), we observed a dramatic decrement in activity. In fact, while *rac*-**2** showed a NCI-H460 cell growth inhibition (% GI) of 85% (at 100 μM) with a IC_{50} value of 1.57 μg mL^{-1}, compound **5** displayed a %GI = 16% at the same concentration and a IC_{50} value higher than 30 μg mL^{-1}.

To adequately interpret this result in structural terms, the NMR data of *rac*-**2**, **5** and their corresponding tetraacetate precursors, **25** and **6**, were of help. In particular, the coupling constants observed for the signals of the protons attached to ring **C** were in agreement with $^3C_{10b}$ conformers, as shown for *rac*-**2** and **5** in Fig. 2. Thus, they appear to adopt the same conformation proposed for pancratistatin itself (**1**) and a number of natural and synthetic analogues having a rigid BC *trans*-fused system.

Comparison of the $^3C_{10b}$ conformers of *rac*-**2** and **5** in Fig. 2 clearly shows that the change in configuration at C2 in going from active *rac*-**2** to its essentially inactive C2-epimer **5**, removes the only hydroxyl group that *rac*-**2** had in its bottom face, thus firmly



1 (pancratistatin, PST) R^7 = OH
rac-**2** [(+/-)-7-deoxy-PST] R^7 = H
5 R^4_α = H, R^4_β = OH
7 R^4_α = OH, R^4_β = H

Fig. 2 Comparative arrangements of ring **C** substituents in pancratistatin (PST, **1**), *rac*-7-deoxypancratistatin (*rac*-**2**), (±)-7-deoxy-2-*epi*-pancratistatin (**5**) and (±)-7-deoxy-2,4-di-*epi*-pancratistatin (**7**).

associating activity with the presence of an axially oriented OH group at C2.

To further study this relationship, we next decided to explore if the activity could be restored by an additional epimerization at carbon 4, that is, in the 2,4-di*epi* analogue **7**, which recuperates an axially oriented OH group at the bottom of the molecule in a proximal 1,3-diaxial disposition with regards to the C2 hydroxyl group of *rac*-**2**. Surprisingly, compound **7** showed to be fully inactive, displaying no noticeable cytotoxicity against the NCI-H460 and the MCF-7 human tumoral cell lines at 100 mM.[22]

Thus, epimerization at C4 was not beneficial but further detrimental, at least when combined with C2 epimerization, for antitumoral activity. Taking together, the results obtained for compounds **5** and **7** reveal the fundamental role that the stereochemistry at positions 2 and 4 play on the interaction of these molecules with the putative biological target(s) responsible for their antitumoral activity.

Experimental

Total synthesis of (±)-7-deoxy-2-*epi*-pancratistatin (5)

(±)-Amine 17. A) From 12a in two steps and 64% overall (Scheme 2)[23]. $NaBH_4$ (0.40 g, 10.58 mmol) was added to a solution of $NiCl_2$ (0.37 g, 2.85 mmol) in MeOH (50 mL) and the suspension was sonicated for 0.5 h.[24] A mixture of $NaBH_4$ (0.40 g, 10.58 mmol) and **12a**[10] (1.19 g, 2.85 mmol) in MeOH (50 mL), prepared in a separated flask, was then added in one portion. Five additional portions of $NaBH_4$ (0.20 g each) were then added at 10 min intervals. The reaction mixture was diluted with water (100 mL), stirred for 3 h, treated with NaCl (1.45 g) and extracted with EtOAc (6 × 100 mL). The combined organic layers were dried and concentrated *in vacuo*, and the crude aminodiol dissolved in acetone (67 mL) and treated with *p*-toluenesulphonic acid (*p*-TsOH·H_2O, 0.466 g, 2.45 mmol) and 2,2-dimethoxypropane (13 mL, 106 mmol). The reaction mixture was stirred for 4.5 h at rt and then neutralized with Et_3N (0.368 mL, 2.65 mmol). Most of the volatiles were removed *in vacuo*, the residue diluted with 3 times its volume of water and extracted with CH_2Cl_2 (2 × 50 mL), and the extracts dried and concentrated. Chromatography (silica gel, 10% MeOH/EtOAc) afforded amine **17** (651 mg, 64%): mp = 75–76 °C (CH_2Cl_2/hexane); R_f 0.38 (10% MeOH/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.79 (s, 1H), 6.73 (d, J = 8.0 Hz, 1H), 6.67 (d, J = 8.0 Hz, 1H), 5.90 (s, 2H), 4.53 (m, 1H), 4.30 (m, 2H), 4.17 (t, J = 7.3 Hz, 1H), 3.60 (dd, J = 11.8, 7.3 Hz, 1H), 2.77 (br s, 2H), 2.44 (dd, J = 11.8, 3.2 Hz, 1H), 1.56 (s, 3H), 1.54 (s, 3H), 1.35 (s, 3H), 1.26 (s, 3H); ^{13}C NMR (101 MHz,

$CDCl_3$) δ 147.6, 146.8, 130.5, 123.4, 110.1, 109.9, 109.0, 108.2, 100.9, 81.3, 76.0, 73.7, 73.0, 49.6, 48.5, 26.3, 26.1, 25.4, 24.0; IR ($CHCl_3$) 3072 (NH_2) cm^{-1}; EMBR (ESI-TOF) *m/z* (%): 364.1748 (M+1, 100), 306.1339 (12), 245.0783(6); EMBR (CI) *m/z* (relative intensity) 364 ($(M+H)^+$; EMAR (ESI-TOF, M+1) *m/z*: calc. for ($C_{19}H_{26}NO_6$): 364.1755, found: 364.1748.

(±)-Diketal carbamate 15a. A) From amine 17. To a solution of amine **17** (44 mg, 0.12 mmol) and DMAP (29 mg, 0.24 mmol) in CH_2Cl_2 (4 mL), $ClCO_2Me$ (44 µL, 0.57 mmol) was added at 0 °C under argon. After being stirred for 18 h, the reaction mixture was diluted with water (4 mL) and extracted with CH_2Cl_2 (3 × 10 mL). Chromatography (60% EtOAc/hexane) afforded carbamate **15a** like a pale yellow solid: mp = 201–202 °C (EtOAc/hexane); R_f 0.48 (50% EtOAc/hexane); $_1$H NMR ($CDCl_3$, 300 MHz) δ 6.76 (s, 1H), 6.69 (d, *J* = 7.4 Hz, 1H), 6.63 (d, *J* = 7.4 Hz, 1H), 5.89 (s, 2H), 4.88 (br s, 1H), 4.59 (m, 2H), 4.35 (m, 2H), 4.19 (m, 1H), 3.48 (s, 3H), 2.94 (m, 1H), 1.62 (s, 3H), 1.56 (s, 3H), 1.37 (s, 3H), 1.28 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.3, 147.1, 146.5, 130.5, 123.1, 110.2, 110.0, 109.0, 107.6, 100.7, 78.5, 76.2, 73.6, 73.2, 51.8, 50.7, 46.4, 26.2, 26.1, 25.4, 23.9; LRMS (ESI-TOF) *m/z* (%): 422.1808 (M+1, 82), 364.1394 (100), 289.1065 (13); HRMS (ESI-TOF, M+1) *m/z*: calc. for ($C_{21}H_{28}NO_8$): 422.1809, found: 422.1808. **B) From protected nitrocyclitol 12a at the gram scale**: $NaBH_4$ (0.63 g, 16.79 mmol) was added to a solution of $NiCl_2$ (0.72 g, 5.59 mmol) in methanol (98 mL) and the suspension was sonicated for 1 h.[24] In a separated flask, $NaBH_4$ (0.63 g, 16.79 mmol) was added to a solution of **12a**[10] (1.96 g, 5.59 mmol) in MeOH (98 mL). This reaction mixture was immediately added in one portion over the nickel derived suspension previously prepared. Five additional portions of $NaBH_4$ (0.63 g, 16.79 mmol) were added at 10 min intervals to the reaction mixture, which was then diluted with water (196 mL) and stirred for 3 h. Solid NaCl (1.96 g) was added and the reaction was extracted with EtOAc (12 × 196 mL). The combined organic layers were dried and concentrated and the crude aminodiol was dissolved in acetone (183 mL) and treated with *p*-TsOH·H_2O (1.29 g, 6.79 mmol) and 2,2-dimethoxypropane (34.83 mL, 284 mmol). The reaction mixture was stirred for 3 h at rt and neutralized with Et_3N (0.94 mL, 0.185 mmol). After solvent evaporation, the crude amine was dissolved in dichloromethane (94 mL), and DMAP (1.38 g, 11.32 mmol) and methyl chloroformate (2.09 mL, 22.61 mmol) were added at 0 °C under argon. After stirring for 18 h, water (94 mL) was added and the product was extracted with CH_2Cl_2 (6 × 94 mL). The organic extracts were collected, dried and concentrated. purification by chromatography (silica gel, 50% EtOAc/hexane), afforded **15a** together with some of its di-isopropylidene free amino precursor **17**, which was protected under the same carbamate-forming conditions to **15a** (1.17 g, 49% from **12a**).

(±)-Tetraacetate 15b. *p*-TsOH·H_2O (0.68 g, 3.57 mmol) was added to a solution of **15a** (1.17 g, 2.78 mmol) in MeOH/CH_2Cl_2 (149 mL, 1/1) at rt under argon. After stirring for 5 h, Et_3N (0.5 mL, 3.61 mmol) was added, the solvent evaporated and the residue dissolved in dry pyridine (46 mL) and treated with DMAP (0.33 g, 2.77 mmol) and Ac_2O (46 mL, 1.18 mmol). After being stirred for 2 h, the mixture was diluted with a saturated aqueous solution of $CuSO_4$ (92 mL)[25] and extracted with CH_2Cl_2 (6 × 46 mL). Chromatography (50% EtOAc/hexane) afforded **15b** (1.04 g, 2.06 mmol, 74%) as a white solid. R_f 0.35 (50% EtOAc/hexane); mp = 195–196 °C (CH_2Cl_2/hexane); R_f 0.4 (60% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.71 (s, 1H), 6.66 (s, 2H), 5.87 (s, 2H), 5.57 (s, 1H), 5.33 (s, 1H), 5.07 (dd, *J* = 7.3, 3.7 Hz, 1H), 5.02 (d, *J* = 2.9 Hz, 1H), 4.82 (d, *J* = 11.5 Hz, 1H), 4.63 (m, 1H), 3.47 (s, 3H), 3.04 (dd, *J* = 11.5, 2.1 Hz, 1H), 2.16 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.91 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.4, 170.2, 169.6, 169.5, 156.6, 147.6, 146.8, 129.6, 122.0, 108.9, 108.1, 101.0, 71.5, 71.4, 69.3, 68.3, 52.1, 48.0, 47.3, 20.8, 20.6, 20.5, 20.4; IR ($CHCl_3$) 1750, 1728 (CO) cm^{-1}; LRMS (ESI-TOF, M+1) *m/z* (%): 510.1599 (M+1, 100), 468.1501 (20), 450.1387 (56), 330.0965 (15); HRMS (ESI-TOF, M+1) *m/z*: calc. for ($C_{23}H_{28}NO_{12}$): 510.1606, found: 510,1599.

(±)-7-Deoxi-2-*epi*-pancratistatin tetraacetate (6). Triflic anhydride (Tf_2O, 99%+, 2.61 g, 9.25 mmol) was added to a solution of **15b** (0.94 g, 1.85 mmol) and DMAP (678 mg, 5.55 mmol) in CH_2Cl_2 (94 mL) at 0 °C under argon. After 16 h at rt, the solvent was evaporated *in vacuo* and the residue dissolved in 100 mL of a 1 : 1 mixture of THF and 0.1 M aqueous HCl. After stirring for 5 h at rt, the reaction mixture was adjusted to pH = 8 with a saturated aqueous solution of $NaHCO_3$, partially evaporated and extracted with CH_2Cl_2 (3 × 25 mL). Chromatography (70% EtOAc/hexane) afforded **6** (427 mg, 0.89 mmol, 48%) as a white solid; mp = 168 °C (EtOAc/hexane); R_f 0.29 (66% EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (s, 1H), 6.56 (d, *J* = 0.8 Hz, 1H), 6.25 (br s, 1H), 6.01 (m, 3H), 5.69 (dd, *J* = 3.5, 3.0 Hz, 1H), 5.14 (t, *J* = 3.5 Hz, 1H), 4.96 (dd, *J* = 10.8, 3.0 Hz, 1H), 4.26 (dd, *J* = 13.0, 10.8 Hz, 1H), 3.14 (ddd, *J* = 13.0, 2.5, 0.8 Hz, 1H), 2.10 (s, 3H), 2.09 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 170.1, 169.9, 169.4, 165.3, 151.6, 147.2, 131.1, 123.2, 108.2, 103.8, 101.8, 71.8, 68.2, 68.1, 65.6, 48.2, 40.1, 20.7, 20.6, 20.5, 20.4; IR ($CHCl_3$) 2923, 1748, 1668, 1258 cm^{-1}; LRMS (ESI-TOF) *m/z* (%): 478.1355 (M+1, 100); LRMS (CI): 478 $(M+H)^+$; HRMS (ESI-TOF, M+1) *m/z*: calc. for ($C_{22}H_{24}NO_{11}$): 478.1344, found: 478.1355.

(±)-7-Deoxy-2-*epi*-pancratistatin (5). (±)-7-Deoxy-2-*epi*-pancratistatin tetraacetate (**6**) (55 mg, 0.12 mmol) was added to a solution of K_2CO_3 in MeOH (15 mL). After stirring at rt for 5 min, filtration and washing with MeOH (3 × 5 mL), afforded **5** (30 mg, 0.10 mmol, 83%) as a white solid: mp = 295–296 °C (MeOH, decomposition); ^{1}H NMR (500 MHz, DMSO) δ 7.30 (s, 1H), 6.87 (s, 1H), 6.81 (s, 1H), 6.06 (s, 2H), 5.09 (d, *J* = 6.5 Hz, 1H), 5.02 (d, *J* = 6.4 Hz, 1H), 4.99 (d, *J* = 5.5 Hz, 1H), 4.86 (d, *J* = 6.6 Hz, 1H), 4.40 (br s, 1H), 3.89 (br s, 1H), 3.64 (dd, *J* = 12.9, 10.0 Hz, 1H), 3.55 (td, *J* = 6.4, 3.1 Hz, 1H), 3.48 (ddd, *J* = 10.0, 6.5, 2.5 Hz, 1H), 2.71 (d, *J* = 12.9 Hz, 1H); ^{13}C NMR (126 MHz, DMSO) δ 164.2, 150.5, 145.9, 134.8, 123.6, 106.8, 105.8, 101.6, 74.7, 72.0, 69.3, 68.8, 50.2, 41.7; LRMS (ESI-TOF) *m/z* (%): 310.0922 (M+1, 100), 245.0776 (86), 177.0549 (46), 149.0243 (91); HRMS (ESI-TOF, M+1) *m/z*: calc. for ($C_{14}H_{16}NO_7$): 310.0921, found: 310.0922.

Total synthesis of (±)-7-deoxypancratistatin (*rac*-2)

(±)-Amine 24a. A suspension of **12a** (100 mg, 0.28 mmol),[10] 10% Pd/C (150 mg) and ammonium formate (83 mg, 4.60 mmol) in dry methanol (2.8 mL) was stirred at rt. After completion of the reduction (as monitored by TLC), the catalyst was filtered off

and washed with methanol and EtOAc. The combined washings and the filtrate were evaporated *in vacuo*. Chromatography (1% Et_3N in EtOAc) afforded **24a** (80 mg, 88%) as a white solid: R_f 0.17 (EtOAc); ^1H NMR ($CDCl_3$, 400 MHz) δ: 6.98 (s, 1H), 6.80 (s, 2H), 6.05–5.90 (m, 2H), 4.52–4.46 (m, 1H), 4.31–4.10 (m, 1H), 3.73 (dd, J = 11.0, 9.1 Hz, 1H), 3.39 (dd, J = 9.1, 2.2 Hz, 1H), 2.36 (dd, J = 11.0, 1.2 Hz, 1H), 1.60 (s, 3H), 1.48 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 208.4, 148.1, 147.3, 130.8, 122.6, 108.8, 108.5, 101.1, 99.2, 80.2, 80.1, 78.7, 53.9, 53.0, 28.3, 25.4; LRMS (CI) m/z (%): 322.2 [$(M+H)^+$, 19], 246.1 (8); HRMS [CI, $(M+H)^+$] m/z: calc. for ($C_{16}H_{20}NO_6$): 322.1290, found: 322.1288.

(±)-Carbamate/carbonate 24b. Methyl chloroformate (200 μL, 1.99 mmol) and 4-DMAP (235 mg, 2.49 mmol) were added to a solution of **24a** (160 mg, 0.50 mmol) in dry CH_2Cl_2 (10 mL) under argon. After stirring for 2 h at rt, the reaction mixture was treated with a saturated aqueous solution of $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (3 × 8 mL). Chromatography (30% EtOAc/hexane) gave **24b** (204 mg, 94%) as white foam: R_f 0.37 (40% EtOAc/hexane); ^1H NMR ($CDCl_3$, 400 MHz) δ: 6.98–6.90 (m, 1H), 6.85–6.70 (m, 2H), 5.95 (m, 2H), 4.99–4.82 (m, 1H), 4.75–4.57 (m, 3H), 4.31–4.25 (m, 1H), 3.8 (s, 3H), 3.55 (br s, 3H), 3.13–2.96 (m, 1H), 1.64 (s, 3H), 1.47 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 206.6, 156.3, 155.0, 148.0, 147.4, 130.3, 122.7, 109.1, 108.5, 108.4, 101.2, 99.5, 79.9, 77.8, 55.4, 52.4, 51.3, 49.9, 28.5, 25.5; LRMS (CI) m/z (%): 438.2 [$(M+H)^+$, 8], 380.1 (40); HRMS [CI, $(M+H)^+$] m/z: calc. for ($C_{20}H_{24}NO_{10}$): 438.1400, found: 438.1398.

(±)-Triacetate 14a. A mixture of **24b** (190 mg, 0.43 mmol) and Dowex 50WX (930 mg) in MeOH (13 mL) was stirred for 2 days at 60 °C. After filtration, the solvent was evaporated *in vacuo* and the crude dissolved in DCE/THF (1 : 1, 12 mL) under argon. $NaBH(AcO)_3$ (460 mg, 2.17 mmol) was added and the mixture stirred at rt for 2 h and then quenched with 30% aqueous hydrogen peroxide (1 mL). After solvent evaporation, the crude was dissolved in dry CH_2Cl_2 (13 mL) and treated with Et_3N (1.6 mL), Ac_2O (0.8 mL) and 4-DMAP (11 mg, 0.09 mmol). After stirring for 4 h at rt, the mixture was treated with a saturated aqueous solution of $NaHCO_3$ (13 mL) and extracted (3 × 8 CH_2Cl_2). Chromatography (35% EtOAc/hexane) gave **14a** (210 mg, 92%) as a white foam: R_f 0.46 (60% EtOAc/hexane); ^1H NMR ($CDCl_3$, 500 MHz) δ: 6.81–6.66 (m, 3H), 5.93 (s, 2H), 5.50–5.41 (m, 1H), 5.16–5.04 (m, 2H), 5.03–4.97 (m, 1H), 4.72–4.56 (m, 1H), 4.48–4.34 (m, 1H), 3.78 (s, 3H), 3.55 (s, 3H), 3.41–3.24 (m, 1H), 2.19 (s, 3H), 2.16 (s, 3H), 2.00 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 169.5, 169.0, 168.4, 156.6, 155.5, 147.9, 147.2, 129.5, 122.4, 109.2, 108.4, 101.2, 75.1, 72.1, 68.6, 68.2, 55.4, 52.4, 48.4, 46.9, 21.0 (2C), 20.8; LRMS (CI) m/z (%): 526.1 [$(M+H)^+$, 66], 494.1 (22); HRMS [CI, $(M+H)^+$] m/z: calc. for ($C_{23}H_{28}NO_{13}$): 526.1561, found: 526.1557.

(±)-1,2,3-Triacetyl-7-deoxy-4-*O*-metoxycarbonylpancratistatin (25). Trifluoromethanesulfonic anhydride (159 μL, 0.97 mmol) was added to a solution of **14a** (100 mg, 0.19 mmol) and 4-DMAP (70 mg, 0.57 mmol) in dry CH_2Cl_2 (8 mL) at 0 °C under argon. After stirring for 4.5 h at rt, the solution was treated with a saturated aqueous solution of Na_2CO_3 (12 mL) and extracted (EtOAc, 3 × 12 mL). The combined organic extracts were dried, the solvent evaporated *in vacuo* and the crude dissolved in 1,4-dioxane (12 mL) and treated with 2 M HCl (1.3 mL). After stirring for 17 h, the mixture was neutralized with a saturated aqueous solution of $NaHCO_3$ (12 mL) and extracted (3 × 12 mL EtOAc). Chromatography (55% EtOAc/hexane) afforded **25** [33 mg, 35%, R_f 0.34 (60% EtOAc/hexane)] as a white solid: ^1H NMR ($CDCl_3$, 300 MHz) δ: 7.27 (s, 1H), 6.70 (s, 1H), 6.56 (s, 1H), 6.09–5.97 (m, 2H), 5.69–5.48 (m, 2H), 5.32–5.21 (m, 1H), 5.04 (dd, J = 10.8, 3.4 Hz, 1H), 4.32 (dd, J = 12.9, 10.8 Hz, 1H), 3.86 (s, 3H), 3.47 (dd, J = 12.9, 2.8 Hz, 1H), 2.17 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 169.7, 169.3, 168.4, 165.1, 154.6, 151.9, 147.4, 131.5, 123.5, 108.7, 103.8, 102.0, 75.6, 67.8, 66.8, 66.4, 55.6, 48.4, 39.5, 20.9, 20.8, 20.7; HRMS [ESI-TOF, $(M+H)^+$] m/z: calc. for ($C_{22}H_{24}NO_{12}$): 494.1267, found: 494.1266.

(±)-7-Deoxypancratistatin (*rac*-2). A solution of **25** (30 mg, 0.06 mmol) in MeOH saturated with K_2CO_3 (0.8 mL) was stirred for 20 min at rt. A drop of TFA was added and the volatiles removed *in vacuo*. Chromatography (10% MeOH/CH_2Cl_2) gave **2** [13 mg, 73%, R_f 0.39 (10% MeOH/CH_2Cl_2)] as a white solid: ^1H NMR (DMSO, 400 MHz) δ: 7.31 (s, 1H), 6.91 (s, 1H), 6.84 (s, 1H), 6.08 (s, 2H), 5.36 (br s, 1H), 5.06 (br s, 2H), 4.80 (br s, 1H), 4.38–4.28 (m, 1H), 4.02–3.94 (m, 1H), 3.91–3.80 (m, 1H), 3.77–3.60 (m, 2H), 2.99 (br d, J = 10.1 Hz, 1H); ^{13}C NMR (DMSO, 100 MHz) δ: 164.0, 150.5, 145.9, 135.3, 123.8, 106.8, 105.5, 101.5, 73.4, 70.3, 70.2, 68.7, 50.4, 40.1; LRMS (CI) m/z (%): 310.1 [$(M+H)^+$, 10], 115.0 (100); HRMS [CI, $(M+H)^+$] m/z: calc. for ($C_{14}H_{16}NO_7$): 310.0927, found: 310.0919.

Total synthesis of (±)-7-deoxy-2,4-di*epi*-pancratistatin (7)

(±)-Diol 26. $NaBH_4$ (14 mg, 0.37 mmol) was added to a solution of **12a** (100 mg, 0.29 mmol) in MeOH (5 mL) under Ar and the mixture stirred at rt until consumption of the starting material (TLC, 1 : 1 EtOAc/hexane, R_f 0.75), adjusted to pH 5.5 with 5% $AcOH_{(aq)}$ (10 mL), stirred for 30 min, partially evaporated and extracted with EtOAc (3 × 6 mL). The organic layers were dried and concentrated. Chromatography (50% EtOAc/hexane) afforded **26** (93 mg, 0.26 mmol, 93%) as a white solid: mp = 181–183 °C (EtOAc/hexane); ^1H NMR (400 MHz, CD_3CN) δ 6.96 (d, J = 1.4 Hz, 1H), 6.80 (dd, J = 8.0, 1.4 Hz, 1H), 6.77 (d, J = 8.0 Hz, 1H), 5.95 (d, J = 1.1 Hz, 1H), 5.94 (d, J = 1.1 Hz, 1H), 5.01 (dd, J = 12.0, 9.6 Hz, 1H), 4.28 (dd, J = 2.2, 3.3 Hz, 1H), 4.14–4.06 (m, 2H), 3.97 (s, 1H), 3.94 (s, 1H), 3.73 (d, J = 4.6 Hz, 1H), 3.22 (dd, J = 12.0, 1.0 Hz, 1H), 1.75 (s, 3H), 1.51 (s, 3H); ^{13}C NMR (100 MHz, CD_3CN) δ 148.6, 148.2, 131.5, 123.0, 109.4, 108.9, 102.4, 98.7, 93.7, 76.7, 76.4, 74.5, 66.4, 50.1, 32.7, 28.9; LRMS (ESI-TOF) m/z (%): 354.1180 (M+1, 100), 336.0716 (32), 278.0665 (27), 250.0844 (14); HRMS (ESI-TOF, M+1) m/z: calc. for ($C_{16}H_{20}NO_8$): 354.1183, found: 354.1180.

(±)-Diol 27. A solution of **26** (1.79 g, 5.06 mmol) in THF/1% $KOH_{(aq)}$ (9 : 1, 25 mL) was stirred for 12 h at 40 °C. The mixture was diluted with a saturated aqueous solution of NH_4Cl (12 mL), adjusted to pH = 7 with 5% $AcOH_{(aq)}$ and extracted with CH_2Cl_2 (3 × 20 mL). Chromatography (silica gel, 50% EtOAc/Hexane) afforded **27** (537 mg, 30%): mp = 175–176 °C (EtOAc/hexane); ^1H NMR (400 MHz, CD_3CN) δ 6.91 (d, J = 1.8 Hz, 1H), 6.84 (dd, J = 8.1, 1.8 Hz, 1H), 6.79 (d, J = 8.1 Hz, 1H), 5.95 (d, J = 1.1 Hz, 1H), 5.94 (d, J = 1.1 Hz, 1H), 5.35 (dd, J = 12.1, 4.0 Hz, 1H), 4.57 (td, J = 4.8, 4.3, 4.3 Hz, 1H), 4.32 (td, J = 3.9, 3.8,

2.0 Hz, 1H), 4.21 (ddd, J = 4.3, 2.0, 1.5 Hz, 1H), 4.13 (dd, J = 3.8, 1.5 Hz, 1H), 3.96 (d, J = 4.8 Hz, 1H), 3.81 (d, J = 3.9 Hz, 1H), 3.61 (dd, J = 12.1, 1.5 Hz, 1H), 1.72 (d, J = 0.7 Hz, 3H), 1.44 (d, J = 0.7 Hz, 3H); ^{13}C NMR (100 MHz, CD_3CN) δ 148.6, 147.6, 133.8, 122.2, 109.3, 108.8, 102.3, 99.0, 87.1, 77.1, 76.1, 72.5, 63.6, 46.8, 33.0, 28.6; LRMS (ESI-TOF) m/z (%): 354.1181 (M+1, 7), 336.0701 (28), 245.0789 (100), 177.0565 (11); HRMS (ESI-TOF, M+1) m/z: calc. for ($C_{16}H_{20}NO_8$): 354.1183, found: 354.1181.

(±)-Nitrodiacetal 30. 2,2-Dimethoxypropane (12.5 mL, 102 mmol) and P_2O_5 (400 mg) were added to a solution of **27** (450 mg, 1.27 mmol) in acetone (30 mL) at 0 °C and under argon. After stirring for 40 min at 0 °C, the mixture was diluted with a saturated aqueous solution of $NaHCO_3$ (40 mL) and adjusted to pH = 7 with Et_3N. Then, the solvent was partially evaporated *in vacuo* and the mixture extracted with CH_2Cl_2 (2 × 50 mL). Chromatography (30% EtOAc/hexane) afforded **30** [348 mg, 0.89 mmol, 85% (besides 80 mg of recovered starting material)]: mp = 224–225 °C (EtOAc/hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.94 (s, 1H), 6.73 (s, 2H), 5.95 (s, 2H), 5.26 (dd, J = 9.0, 7.8 Hz, 1H), 4.71 (dd, J = 7.5, 3.4 Hz, 1H), 4.61 (dd, J = 7.5, 2.1 Hz, 1H), 4.37 (dd, J = 10.3, 7.8 Hz, 1H), 4.27 (dd, J = 10.3, 3.4 Hz, 1H), 3.81 (dd, J = 9.0, 2.1 Hz, 1H), 1.57 (s, 3H), 1.48 (s, 3H), 1.39 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.1, 147.5, 131.1, 122.2, 113.1, 110.2, 108.8, 108.3, 101.2, 87.7, 78.5, 73.1, 70.6, 68.8, 45.7, 26.8, 26.4, 25.7, 23.4.

(±)-Amine 31. MeOH (5 mL) was added to a mixture of **30** (327 mg, 0.83 mmol), 10% Pd/C (266 mg, 0.25 mmol) and HCO_2NH_4 (629 mg, 9.97 mmol). After stirring for 18 h, the reaction mixture was filtered through a pad of celite, the filtered solids washed with MeOH (2 × 10 mL) and the filtrate concentrated. Chromatography (10% MeOH/EtOAc) afforded **31** (132 mg, 0.36 mmol, 44%): R_f 0.6 (10% MeOH/EtOAc); ^{1}H NMR (500 MHz, CD_3OD) δ 7.01 (d, J = 1.8 Hz, 1H), 6.89 (dd, J = 8.0, 1.8 Hz, 1H), 6.82 (d, J = 8.0 Hz, 1H), 5.97 (s, 2H), 4.64 (dd, J = 7.4, 3.7 Hz, 1H), 4.49 (dd, J = 7.4, 2.2 Hz, 1H), 4.38 (dd, J = 10.7, 7.7 Hz, 1H), 3.99 (dd, J = 8.8, 7.7 Hz, 1H), 3.97 (dd, J = 10.7, 3.7 Hz, 1H), 3.01 (dd, J = 8.8, 2.2 Hz, 1H), 1.57 (s, 3H), 1.52 (s, 3H), 1.48 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 149.5, 148.9, 133.6, 123.9, 113.6, 111.0, 110.4, 109.1, 102.5, 80.8, 74.8, 71.9, 71.3, 51.7, 48.5, 27.1, 27.1, 26.0, 23.8; LRMS (ESI-TOF) m/z (%): 364.1755 (M+1, 100), 324.1387 (32), 306.1274 (42), 284.1040 (19); HRMS (ESI-TOF, M+1) m/z: calc. for ($C_{19}H_{26}NO_6$): 364.1755, found: 364.1755.

(±)-Carbamate 16a. $ClCO_2Me$ (53 µL, 0.68 mmol) was added to a solution of **31** (124 mg, 0.34 mmol) and DMAP (29 mg, 0.82 mmol) in CH_2Cl_2 (5 mL) at 0 °C under argon. After stirring for 13 h, the reaction mixture was diluted with water (5 mL) and extracted with CH_2Cl_2 (3 × 10 mL). Chromatography (60% EtOAc/hexane) afforded **16a** as a pale yellow solid: mp = 212–213 °C (EtOAc/hexane); R_f 0.33 (60% EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 (d, J = 1.5 Hz, 1H), 6.77 (dd, J = 8.0, 1.5 Hz, 1H), 6.72 (d, J = 8.0 Hz, 1H), 5.95 (d, J = 1.1 Hz, 1H), 5.94 (d, J = 1.1 Hz, 1H), 4.83 (br s, 1H), 4.58 (dd, J = 7.5, 3.6 Hz, 1H), 4.45 (dd, J = 7.5, 2.1 Hz, 1H), 4.39 (t, J = 8.4 Hz, 1H), 4.29 (dd, J = 10.3, 8.4 Hz, 1H), 3.86 (dd, J = 10.3, 2.1 Hz, 1H), 3.56 (s, 3H), 2.84 (br s, 1H), 1.57 (s, 3H), 1.48 (s, 3H), 1.45 (s, 3H), 1.29 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.0, 147.6, 146.7, 133.6, 122.3, 111.8, 109.7, 109.4, 107.8, 100.9, 78.7, 74.2, 70.9, 70.7, 52.1, 51.5, 48.2, 26.9, 26.8, 25.8, 23.6; LRMS (ESI-TOF) m/z (%): 422.1814 (M+1, 8), 364.1013 (95), 361.0916 (M+2, 100); HRMS (ESI-TOF, M+1) m/z: calc. for ($C_{21}H_{28}NO_8$): 422.1809, found: 422.1814.

(±)-Carbamate/tetraacetate 16b. p-TsOH·H_2O (97 mg, 0.51 mmol) was added to a solution of **16a** (90 mg, 0.21 mmol) in MeOH/CH_2Cl_2 (1/1, 10.5 mL) at rt under argon. After being stirred for 30 min, Et_3N (0.5 mL) was added. The reaction mixture was evaporated and the residue dissolved in dry CH_2Cl_2 (4 mL) and treated with DMAP (21 mg, 0.17 mmol), Et_3N (333 µL, 2.39 mmol) and Ac_2O (112 µL, 1.18 mmol). After stirring for 2 h, the mixture was diluted with a saturated aqueous solution of NH_4Cl (4 mL), adjusted to pH = 7 with 5% $AcOH_{(aq)}$ and extracted with CH_2Cl_2 (3 × 10 mL). Chromatography (60% EtOAc/hexane) afforded **16b** (95 mg, 0.19 mmol, 89%) as a white solid: mp = 123–124 °C (CH_2Cl_2/hexane); R_f 0.4 (60% EtOAc/hexane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.83–6.62 (m, 3H), 5.94 (s, 2H), 5.40–5.38 (m, 2H), 5.34 (dd, J = 5.6, 2.8 Hz, 1H), 5.21 (dd, J = 6.2, 2.8 Hz, 1H), 4.79 (dd, J = 12.6, 8.6 Hz, 1H), 4.40 (d, J = 8.6 Hz, 1H), 3.57 (s, 3H), 3.21 (d, J = 12.6 Hz, 1H), 2.18 (s, 3H), 2.18 (s, 3H), 2.00 (s, 3H), 1.96 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.8, 169.5, 169.2, 169.1, 156.1, 147.9, 147.1, 129.6, 122.0, 108.7, 108.3, 101.1, 71.4, 70.9, 67.9, 67.4, 52.4, 46.1, 44.6, 21.0, 20.9, 20.7, 20.6; LRMS (ESI-TOF) m/z (%):532.1425 (M+Na, 100), 510.1626 (22), 468.1539 (11), 450.1424 (11); HRMS (ESI-TOF, M+Na) m/z: calc. for ($C_{23}H_{27}NNaO_{12}$): 532.1425, found: 532.1430.

(±)-7-Deoxi-2,4-di-*epi*-pancratistatin tetraacetate (32). Tf_2O (99%+, 111 µL, 0.67 mmol) was added to a solution of **16b** (69 mg, 0.14 mmol) and DMAP (50 mg, 0.41 mmol) in CH_2Cl_2 (3 mL) at 0 °C under argon. After stirring for 16 h at rt, the reaction mixture was evaporated *in vacuo* and the residue dissolved in a mixture of THF and 0.1 M $HCl_{(aq)}$ (1 : 1, 3 mL). The mixture was stirred for 5 h at rt, adjusted to pH = 8 with $NaHCO_{3(aq)}$, partially evaporated and extracted (CH_2Cl_2, 2 × 5 mL). Chromatography (70% EtOAc/hexane) afforded **32** [29 mg, 0.06 mmol, 60% (17 mg of starting material were also recovered)] as a white solid: mp = 290–291 °C (decomposition, EtOAc/hexane); R_f 0.5 (70% EtOAc/hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (s, 1H), 6.60 (s, 1H), 6.06 (dd, J = 3.0, 2.2 Hz, 1H), 6.03 (d, J = 1.1 Hz, 1H), 6.01 (d, J = 1.1 Hz, 1H), 5.94 (s, 1H), 5.36 (m, 2H), 5.33 (dd, J = 6.1, 3.0 Hz, 1H), 4.35 (dd, J = 13.3, 1.6 Hz, 1H), 3.52 (dd, J = 13.3, 1.6 Hz, 1H), 2.18 (s, 3H), 2.12 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 170.2, 169.7, 169.0, 165.0, 151.8, 147.2, 131.4, 122.9, 108.6, 103.8, 101.8, 68.7, 68.1, 67.7, 66.0, 49.4, 37.4, 20.9, 20.7, 20.6, 20.6. LRMS (ESI-TOF) m/z (%): 478.1329 (M+1, 100), 418, 1143 (7); HRMS (ESI-TOF, M+1) m/z: calc. for ($C_{22}H_{24}NO_{11}$): 478.1344, found: 478.1329.

(±)-7-Deoxi-2,4-di-*epi*-pancratistatin (7). (±)-7-Deoxi-2,4-di-*epi*-pancratistatin tetraacetate (**32**) (28 mg, 0.06 mmol) was added to a saturated solution of K_2CO_3 in MeOH (1 mL). After stirring for 5 min at rt, filtration and washing with MeOH (3 × 5 mL) afforded **7** as a white solid (15 mg, 0.05 mmol, 80%): mp = 290–291 °C (decomposition, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (s, 1H), 7.25 (s, 1H), 6.84 (s, 1H)), 6.02 (d, J = 0.9 Hz, 1H), 6.01 (d, J = 0.9 Hz, 1H), 4.99 (s, 1H), 4.96 (d, J = 4.3 Hz, 1H), 4.85 (s, 1H), 4.74 (d, J = 6.0 Hz, 1H), 4.39 (s, 1H), 3.91 (s, 1H), 3.80

(dd, J = 13.4, 2.4 Hz, 1H), 3.77 (s, 1H), 3.68 (s, 1H), 3.00 (dd, J = 13.4, 1.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.2, 150.2, 145.6, 135.1, 123.5, 106.6, 105.6, 101.3, 74.1, 69.7, 69.1, 66.7, 48.3, 37.8; LRMS (ESI-TOF) m/z (%): 310.0921 (M+1, 100), 261.0520 (43), 213.0493 (6), 177.0562 (8), 149.0253 (16); HRMS (ESI-TOF, M+1) m/z: calc. for ($C_{14}H_{16}NO_7$): 310.0921, found: 310.0921.

Acknowledgements

Financial support by the Ministry of Science and Innovation (Project CTQ2008-03253) and by the Xunta de Galicia (Projects 08CSA046209PR and 2007/XA084) is gratefully acknowledged. ONG acknowledges fellowships from the Xunta de Galicia (Lucas Labrada grant) and from the "Diputación de A Coruña". We also thank Dr A. Jiménez of CBM-CSIC (Madrid, Spain) for a sample of natural narciclasine.

Notes and references

1 The name isocarbostyril stands for 1-oxo-1,2-dihydroisoquinoline or its proton tautomer, 1-hydroxyisoquinoline.
2 For reviews on the isolation, biosynthesis, synthetic studies, biological activity and medical potential, mainly as antitumoral agents, of the isocarbostyril constituents of the *Amaryllidaceae*, see: (*a*) L. Ingrassia, F. Lefranc, V. Mathieu, F. Darro and R. Kiss, *Translational Oncology*, 2008, **1**, 1–13; (*b*) A. Kornienko and A. Evidente, *Chem. Rev.*, 2008, **108**, 1982–2014See also the "Introduction" section of: (*c*) J. Collins, U. Rinner, M. Moser, T. Hudlicky, I. Ghiviriga, A. E. Romero, A. Kornienko, D. Ma, C. Griffin and S. Pandey, *J. Org. Chem.*, 2010, **75**, 3069–3084 and references cited therein.
3 For an account covering the progress in the synthesis of *Amaryllidaceae* constituents and some truncated derivatives from 1996 to the fall of 2004, with a detailed treatment of the total synthesis of pancratistatin, narciclasine, 7-deoxy-pancratistatin and lycoricidine, see: U. Rinner and T. Hudlicky, *Synlett*, 2005, 365–387.
4 For studies on the anticancer activity and the pharmaceutical potential of pancratistatin, see: (*a*) C. Griffin, C. Hamm, J. McNulty and S. Pandey, *Cancer Cell Int.*, 2010, **10**, 6; (*b*) C. Griffin, A. Karnik, J. McNulty and S. Pandey, *Mol. Cancer Ther.*, 2011, **10**, 57–68 and references therein. See also the "Introduction and Biomedical Significance" and the "Conclusion" sections of reference 5a, and the "Introduction" section of reference 5b.
5 For a review on the total synthesis of pancratistatin including data on its isolation from natural sources and biomedical significance, see: (*a*) M. Manpadi and A. Kornienko, *Org. Prep. Proced. Int.*, 2008, **40**, 107–161. For the last total synthesis reported to date and not included in ref. 4a, see: (*b*) D. Johan Hygum and M. Robert, *Eur. J. Org. Chem.*, 2009, 4666–4673.
6 J. C. Ortiz, L. Ozores, F. Cagide-Fagin and R. Alonso, *Chem. Commun.*, 2006, 4239–4241.
7 (*a*) M. G. Banwell, B. D. Bissett, S. Busato, C. J. Cowden, G. C. R. Hockless, J. W. Holman, R. W. Read and A. W. Wu, *J. Chem. Soc., Chem. Commun.*, 1995, 2551–2553; (*b*) M. G. Banwell, C. J. Cowden and R. W. Gable, *J. Chem. Soc., Perkin Trans. 1*, 1994, 3515–3518.
8 Several examples can be found in the account of Hudlicky and Rinner: reference 4b. For a recent application, see: S. L. Poe and J. P. Morken, *Angew. Chem., Int. Ed.*, 2011, **50**, 4189–4192.
9 P. Martínez-Bescos, F. Cagide-Fagín, L. F. Roa, J. C. Ortiz-Lara, K. Kierus, L. Ozores-Viturro, M. Fernández-González and R. Alonso, *J. Org. Chem.*, 2008, **73**, 3745–3753.
10 A detailed preparation procedure and full characterization data for **12a** can be found in reference 6 (in its reference 16).
11 See: (*a*) J. O. Osby and B. Ganem, *Tetrahedron Lett.*, 1985, **26**, 6413–6416; (*b*) H. Krawczyk, L. Albrecht, J. Wojciechowski, W. M. Wolf, U. Krajewska and M. Rózalski, *Tetrahedron*, 2008, **64**, 6307–6314 and references cited therein.
12 In our hands, β-furyl-α-nitro-α,β-enals (*e.g.*, the precursor of **18**) showed to be both more stable and convenient to prepare than the corresponding β-aryl-α-nitro-α,β-enals (*e.g.*, **10a**, the precursor of **12a**): see reference 9.
13 A multigram-scale detailed preparation of **18** from furfural, including a timetable, is described in the experimental section and the supporting information of: F. Cagide-Fagin and R. Alonso, *Eur. J. Org. Chem.*, 2010, **35**, 6741–6747.
14 (*a*) J. Cossy and D. Belotti, *Tetrahedron*, 2006, **62**, 6459–6470; (*b*) M. Inui, A. Nakazaki and S. Kobayashi, *Org. Lett.*, 2007, **9**, 469–472.
15 For an X-ray structure and a cif file, see the ESI†.
16 For a review on acyloxyborohydrides, see: (*a*) G. W. Gribble, *Chem. Soc. Rev.*, 1998, **27**, 395–404. For examples, see: (*b*) A. K. Saksena and P. Mangiaracina, *Tetrahedron Lett.*, 1983, **24**, 273–276; (*c*) M. D. Turnbull, G. Hatter and D. E. Ledgerwood, *Tetrahedron Lett.*, 1984, **25**, 5449–5452; (*d*) Y.-T. Liu, J. K. Wong, M. Tao, R. Osterman, M. Sannigrahi, V. M. Girijavallabhan and A. Saksena, *Tetrahedron Lett.*, 2004, **45**, 6097–6100.
17 For a discussion of the pharmacophore of the isocarbostyril constituents of *Amaryllidaceae* see, for example, ref. 2b (section 7, pp 1998–2002), 2c (Introduction, pp 109–111) and 5a, and references cited therein.
18 *ent*-7-Deoxypancratistatin was reported to exhibit cytotoxic potencies 10-fold lower than its corresponding natural enantiomer when evaluated in a minipanel of human cancer cell lines T. Hudlicky, U. Rinner, D. Gonzalez, H. Akgun, S. Schilling, P. Siengalewicz, T. A. Martinot and G. R. Pettit, *J. Org. Chem.*, 2002, **67**, 8726–8743.
19 Natural narciclasine was kindly supplied by Dr A. Jiménez, of CBM-CSIC (Madrid, Spain).
20 (*a*) G. R. Pettit, N. Melody, D. L. Herald, J. M. Schmidt, R. K. Pettit and J. C. Chapuis, *Heterocycles*, 2002, **56**, 139–155; (*b*) G. R. Pettit, S. A. Eastham, N. Melody, B. Orr, D. L. Herald, J. McGregor, J. C. Knight, D. L. Doubek, G. R. Pettit, L. C. Garner and J. A. Bell, *J. Nat. Prod.*, 2006, **69**, 7–13.
21 U. Rinner, H. L. Hillebrenner, D. R. Adams, T. Hudlicky and G. R. Pettit, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 2911–2915.
22 Some additional data for the studies on cytotoxicity, including a table of IC50s and %GIs for the new compounds, can be found on page S67 of the ESI†.
23 For the alternative preparation of **17** from **12a** in 3 steps (**12a** → **26** → **33** → **17**) and 74% overall yield (Scheme 5), see the ESI†.
24 A black solid is formed according to the protocol described in reference 11a.
25 The reaction was alternatively worked out with the addition of a saturated aqueous solution of NH_4Cl and then adjusted to pH 7 with 5% $AcOH_{aq}$.

