Pancratistatin Analogues

Stereospecific Biocatalytic Synthesis of Pancratistatin Analogues**

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The *Amaryllidaceae* alkaloids constitute an important class of natural compounds.[1] Particularly, pancratistatin (**1**) and its closely related anhydro and deoxy congeners narciclasine (**3**), lycoricidine (**4**), and *trans*-dihydrolycoricidine (**2**) have been isolated from roots of the Hawaiian plant *Hymenocallis littoralis*.[2] These compounds have attracted considerable attention due to their interesting biological properties, such as potent cytotoxic, antiviral, and insect antifeedant activity.[1–3] Current knowledge suggests a mechanism that involves a disruption of protein biosynthesis through inhibition of ribosomal tRNA binding.[4] Thus, the unique, highly oxygenated phenanthridone skeleton constitutes a promising lead for new anticancer drugs.

The structures **1**–**4** have been the subject of several elegant total syntheses[5,6] that centered around the major difficulty of a controlled installation of the five to six

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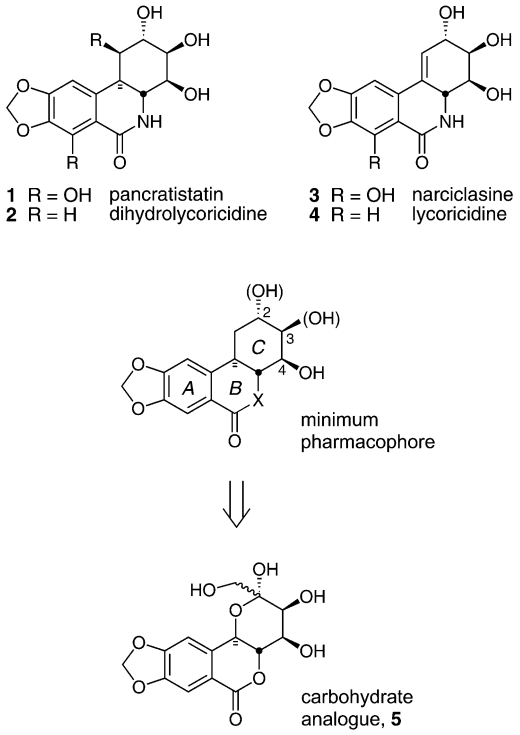
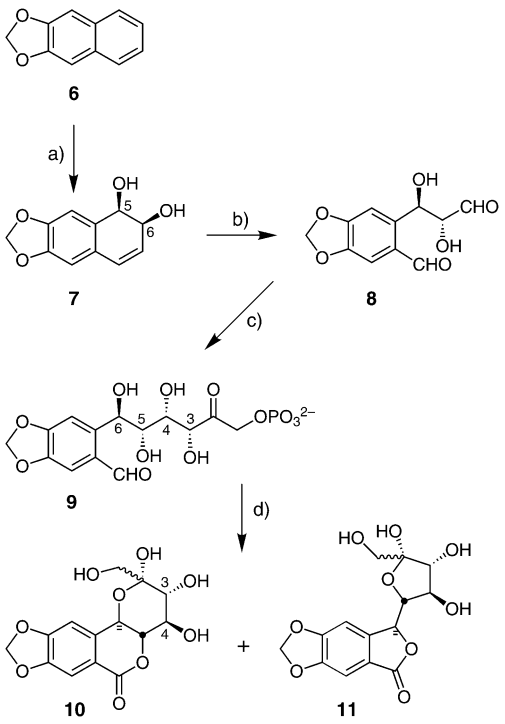


contiguous stereogenic centers, including the B/C ring junction. Systematic investigations to establish the minimum structure of the pharmacophore have been performed only very recently,[6–9] however, due to the limited natural supply and the complexity of the structural core. From these structure–activity studies it may be concluded that the crucial features for biological activity reside in 1) the piperonyl-type ring A, 2) the *trans* stereochemistry of the B/C ring junction that adjusts 3) the correct spatial orientation of peripheral hydroxy functions in the aminocyclitol-type ring C. Among the latter, it appears that at least the correctly positioned 4-OH and either or both of the 2-/3-hydroxyl groups are obligatory for the minimum pharmacophore.[9] While oxygenated seco-derivatives were found to be inactive,[6,8] however, a requirement for the lactam nature of ring B has not been demonstrated yet.

On the assumption of such a hypothetical minimum structure, we reasoned that replacement of the cyclitol moiety by a carbohydrate ring structure might be a permissible structural variation due to the fact that most hexoaldoses and -ketoses preferentially adopt a cyclic pyranoid structure in aqueous solution. In addition to the anomeric hydroxy group, which would mimic the 2-OH group of pancratistatin, a ketose unit as in **5** would further offer the possibility that the primary CH_2OH moiety may be positioned in a way to replace hydrophilic contacts made by the extra 1-OH in **1** upon effector binding. Thus, the B-ring lactone analogue **5** is an interesting synthetic target (as well as related stereoisomers). The major advantage of such a synthetic strategy lies in the rather simple task of attaching the aromatic nucleus to a linear carbohydrate side chain. The carbohydrate fragment itself could be established enzymatically by an aldolase reaction, which would effect a chain extension by a dihydroxyacetone fragment with simultaneous adjustment of the desired absolute configuration.[10] In this respect, the well-characterized dihydroxyacetone phosphate dependent aldolases[11] have been shown to accept an unusually broad variety of aldehyde acceptors in place of the natural substrate but maintain complete stereocontrol over the asymmetric centers at the newly formed C–C bond.

Retrosynthetically, this strategy called for an aldehyde acceptor component of type **8** that in turn might be generated by ozonolytic ring cleavage of the unsaturated *cis*-diol precursor **7** (Scheme 1). Dihydroarene-*cis*-diols, derived

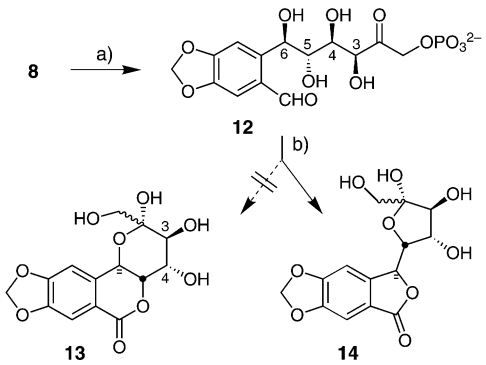


Scheme 1. Sequence of enzymatic dihydroxylation and aldolization to create four contiguous stereocenters. a) Naphthalene dioxygenase (*E. coli*), aeration, 24 h at 37 °C; 64% yield (85% based on conversion). b) O_3, −78 °C, MeOH; then Me_2S, room temperature. c) Rhamnulose 1-phosphate aldolase, dihydroxyacetone phosphate, pH 7.0, room temperature, two days. d) Acid phosphatase, pH 5.9, three days, then Br_2/$BaCO_3$; product ratio 1:1, flash chromatography; 10% overall yield (four steps from **7**).

from oxidative microbial arene degradation, have recently been developed as synthetically valuable building blocks.[12] Particularly, naphthalene dioxygenases[13] from *Pseudomonas* strains have been shown to be highly effective for dihydroxylation of polycyclic arenes and derivatives. The dioxole **6** required for this study, however, posed a considerable challenge owing to its linearly extended substrate structure, because known biocatalysts prefer angular substrates.[12,14] Screening experiments revealed that toluene dioxygenases were ineffective but that indeed a naphthalene dioxygenase from *Pseudomonas putida* G7 was able to oxidize **6**. Preparative whole-cell biooxidation, using recombinant *E. coli* cells that harbored the gene for dioxygenase overproduction,[15] was performed on multigram scale to produce the desired diol

7 in enantiopure form (>98% *ee*) which was isolated by extraction and flash chromatography.[16] The compound was found to be rather sensitive to traces of acid, which causes decomposition to the corresponding naphthol and diminished total yields (85% based on conversion). The absolute (5*R*,6*S*) configuration of **7** follows from its positive sign of optical rotation that is shared by all related *cis*-dihydrodiol metabolites from polycyclic aromatic hydrocarbons, and by the notion that such metabolites had previously been shown to have an identical configuration, independent from the type and source of enzyme.[12]

Enediol **7** had to be ozonized under carefully controlled conditions because of its tendency for naphthol formation and facile overoxidation of the electron-rich arene by excess ozone. Without further purification, the aqueous solution of dialdehyde **8** (mixture of several hydrate constitutional isomers according to ^{1}H NMR analysis) was immediately treated with a solution of dihydroxyacetone phosphate[17] in the presence of an aldolase. Both the stereocomplementary fructose 1,6-bisphosphate (FruA)[11b] and rhamnulose 1-phosphate (RhuA))[11a] aldolases were found to accept **8** as a substrate to produce monophosphorylated products (TLC control). Conversion was rather sluggish and remained incomplete, which is probably due to the poor solubility of **8** in water that limits the effective substrate concentration. Regiospecific aldolase-catalyzed addition to the aliphatic aldehyde moiety was expected because of the excellent substrate quality of hydroxyaldehydes and the known unreactivity of aromatic carbaldehydes.[10] Spectroscopic analysis of the aldol adducts **9** and **12** gave complex spectra that were inconclusive for product identification, likely because of intramolecular cyclization to equilibrating five- or six-membered-ring hemiacetals of varying diastereomeric composition. Therefore, after enzymatic dephosphorylation, analysis was simplified by mild oxidation to furnish stable lactones. Interestingly, from the RhuA-catalyzed reaction both the desired pyranoid isomer **10** along with an equal fraction of the furanoid isomer **11** were formed (10% overall yield from **7**),[16] while from the FruA-catalyzed reaction only the undesired furanoid product **14** (10% overall yield) but no δ-lactone **13** resulted (Scheme 2). As a plausible explanation, in the case of the FruA-induced configuration the 3-/4-OH groups of **13** become enforced in a diaxial orientation, which destabilizes pyranose formation in favor of the competing furanoid structures. Furthermore, oxidation of furanoid hemiacetals may be at a certain kinetic advantage and thus favor the formation of γ-lactones.



Scheme 2. γ-Lactone formation from FruA-catalyzed aldolization. a) Fructose 1,6-bisphosphate aldolase, dihydroxyacetone phosphate, pH 7.0, room temprature, two days. b) Acid phosphatase, pH 5.9, three days, then Br_2/$BaCO_3$; 10% yield (four steps from **7**).

The δ-lactone **10** resembles (+)-pancratistatin in overall absolute configuration but deviates at 3-OH which is inverted. Correct overall configuration **5** (3/4-OH *cis*-vicinal) would call for catalysis by a tagatose 1,6-diphosphate aldolase (TagA). However, known TagA enzymes show low stereoselectivity for non-natural substrate analogues,[10,18] which currently renders such an option not viable but requires screening for new stereoselective aldolases.[19] Chemical inversion at this position would probably be rather difficult because of its low steric accessibility. Thus, **10** seems the so far best approximation that is accessible by this approach.

In summary, we have developed a new strategy for the stereospecific synthesis of novel pancratistatin analogues. Our study demonstrates that structures of high molecular complexity, incorporating a manifold of contiguous chiral centers, can be readily prepared in a few synthetic steps by a combination of enzymatic dihydroxylation and aldolization, without recourse to protective group manipulations. Screening of the new compounds for their biological activity, particularly with respect to anti-neoplastic effectivity, is currently in progress and will be reported in due course.

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[1] a) S. F. Martin in *The Alkaloids, Vol. 40* (Ed.: A. R. Brossi), Academic Press, New York, **1987**, pp. 251–376; b) O. Hoshino in *The Alkaloids, Vol. 51* (Ed.: G. A. Cordell), Academic Press, New York, **1998**, pp. 324–424.

[2] a) G. R. Pettit, V. Gaddamidi, D. L. Herald, S. B. Singh, G. M. Cragg, J. M. Schmidt, F. E. Boettner, M. Williams, Y. Sagawa, *J. Nat. Prod.* **1986**, *49*, 995–1002; b) G. R. Pettit, G. R. Pettit III, R. A. Backhaus, M. R. Boyd, A. W. Meerow, *J. Nat. Prod.* **1993**, *56*, 1682–1687.

[3] a) B. Gabrielsen, T. P. Monath, J. W. Huggins, D. F. Kefauver, G. R. Pettit, G. Groszek, M. Hollingshead, J. J. Kirsi, W. M. Shannon, E. M. Schubert, *J. Nat. Prod.* **1992**, *55*, 1569–1581; b) M. Ouarzane-Amara, J.-F. Franetich, D. Mazier, G. R. Pettit, L. Meijer, C. Doerig, I. Desportes-Livage, *Antimicrob. Agents Chemother.* **2001**, *45*, 3409–3415.

[4] a) L. Carrasco, M. Fresno, D. Vazquez, *FEBS Lett.* **1975**, *52*, 236–239; b) A. Mondon, K. Krohn, *Chem. Ber.* **1975**, *108*, 445–463; c) A. Jimenez, L. Sanchez, D. Vazquez, *FEBS Lett.* **1975**, *60*, 66–70; d) A. Baez, D. Vazquez, *Biochim. Biophys. Acta* **1978**, *518*, 95–103.

[5] R. Polt in *Organic Synthesis: Theory and Applications, Vol. 3* (Ed.: T. Hudlicky), JAI, Greenwich, CT, **1997**, pp. 109–148.

[6] T. Hudlicky, U. Rinner, D. Gonzalez, H. Akgun, S. Schilling, P. Siengalewicz, T. A. Martinot, G. R. Pettit, *J. Org. Chem.* **2002**, *67*, 8726–8743, zit. Lit.

[7] a) A. Evidente, O. Arrigoni, R. Liso, G. Calabrese, G. Randazzo, *Phytochemistry* **1986**, *25*, 2739–2743; b) G. R. Pettit, N. Melody, D. L. Herald, J. M. Schmidt, R. K. Pettit, J.-C. Chapuis, *Heterocycles* **2002**, *56*, 139–155.

[8] F. Chretien, S. I. Ahmed, A. Masion, Y. Chapleur, *Tetrahedron* **1993**, *49*, 7463 – 7478.
[9] J. McNulty, J. Mao, R. Gibe, R. Mo, S. Wolf, G. R. Pettit, D. L. Herald, M. R. Boyd, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 169 – 172.
[10] a) W.-D. Fessner, C. Walter, *Top. Curr. Chem.* **1996**, *184*, 97 – 194; b) W.-D. Fessner in *Stereoselective Biocatalysis* (Ed.: R. N. Patel), Marcel Dekker, New York, **2000**, S. 239 – 265.
[11] a) W.-D. Fessner, G. Sinerius, A. Schneider, M. Dreyer, G. E. Schulz, J. Badía, J. Aguilar, *Angew. Chem.* **1991**, *103*, 596 – 599; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 555 – 558; b) M. T. Zannetti, C. Walter, M. Knorst, W. D. Fessner, *Chem. Eur. J.* **1999**, *5*, 1882 – 1890; c) A. C. Joerger, C. Gosse, W.-D. Fessner, G. E. Schulz, *Biochemistry* **2000**, *39*, 6033 – 6041; d) M. Kroemer, G. E. Schulz, *Acta Crystallogr. Sect. D* **2002**, *58*, 824 – 832.
[12] a) H. A. J. Carless, *Tetrahedron: Asymmetry* **1992**, *3*, 795 – 826; b) S. M. Brown, T. Hudlicky, in *Organic Synthesis: Theory and Applications, Vol. 2* (Ed.: T. Hudlicky), JAI, Greenwich, CT, **1993**, S. 113 – 176; c) D. R. Boyd, G. N. Sheldrake, *Nat. Prod. Rep.* **1998**, *15*, 309 – 324; d) T. Hudlicky, D. Gonzalez, D. T. Gibson, *Aldrichimica Acta* **1999**, *32*, 35 – 62.
[13] A. Karlsson, J. V. Parales, R. E. Parales, D. T. Gibson, H. Eklund, S. Ramaswamy, *Science* **2003**, *299*, 1039 – 1042.
[14] D. R. Boyd, N. D. Sharma, J. S. Harrison, M. A. Kennedy, C. C. R. Allen, D. T. Gibson, *J. Chem. Soc. Perkin Trans. 1* **2001**, 1264 – 1269.
[15] a) W.-C. Suen, D. T. Gibson, *Gene* **1994**, *143*, 67 – 71; b) C. M. Serdar, D. C. Murdock, B. D. Ensley, U.S. Patent 5 173 425, **1992** [*Chem. Abstr.* **1993**, *118*, 100551].
[16] Selected data for new compounds: **7**, m.p. 132–133 °C; $[\alpha]_D^{20} = +180.6°$ ($c = 1$, $CHCl_3$); ^{1}H NMR (300 MHz, CD_3OD, TMS): $\delta = 5.66$, 5.31 (2 s, 1-,4-H), 5.08 (dd, 7-H), 4.66 (s, 2H, CH_2), 4.59 (dd,8-H), 3.19 (d, 5-H), 2.97 ppm (dt, 6-H), $J_{5,6} = 4.9$, $J_{6,7} = 0.8$, $J_{6,8} = 4.0$, $J_{7,8} = 9.7$ Hz; ^{13}C NMR (75 MHz, CD_3OD, TMS): $\delta =$ 147.2, 147.1 (C-2,-3), 130.4 (C-4a), 128.1, 126.9 (C-1,-4), 126.5 (C-1a), 108.0, 107.0 (C-7,-8), 101.0 (CH_2), 70.6, 67.8 ppm (C-5,-6); ESI-MS: m/z: 206 (17) $[M]^+$, 188 (100) $[M-H_2O]^+$. **10**, ^{1}H NMR (500 MHz, D_2O, TSP): $\delta = 7.18$, 7.13 (2 s, $2H_{ar}$), 6.05 (s, 2H, OCH_2O), 5.24 (d, 6-H), 3.89 (t, 4-H), 3.74 (d, 1-H_a), 3.71 (d, 3-H), 3.68 (t, 5-H), 3.54 ppm (d, 1-H_b), $J_{1a,1b} = 11.8$, $J_{3,4} = 9.6$, $J_{4,5} = 9.4$, $J_{5,6} = 10.0$ Hz; ^{13}C NMR (125.6 MHz, D_2O, TSP): $\delta = 188.4$ (C=O), 151.2, 150.5 ($2OC_{ar}$), 132.4, 118.6 ($2C_{ar}$), 115. 5, 111.4 ($2HC_{ar}$), 105.1 (OCH_2O), 101.0 (C-2), 76.9 (C-5), 76.5 (C-4), 75.6 (C-6), 73.4 (C-3), 66.5 ppm (C-1); ESI-MS: m/z: 349 (100) $[M+Na]^+$. **11**, ^{1}H NMR (500 MHz, D_2O, TSP): $\delta = 7.16$, 7.12 (2 s, $2H_{ar}$), 6.17 (s, 2H, OCH_2O), 5.60 (d, 6-H), 4.25 (t, 4-H), 4.13 (d, 3-H), 4.00 (dd, 5-H), 3.65 (d, 1-H_a), 3.60 ppm (d, 1-H_b), $J_{1a,1b} =$ 12.1, $J_{3,4} = 7.9$, $J_{4,5} = 6.8$, $J_{5,6} = 5.8$ Hz; ^{13}C NMR (125.6 MHz, D_2O, TSP): $\delta = 175.6$ (C=O), 157.0, 152.4 ($2OC_{ar}$), 147.1, 121.2 ($2C_{ar}$), 106.5, 106.2 ($2HC_{ar}$), 106.1 (OCH_2O), 104.9 (C-2), 83. 7 (C-6), 83.4 (C-5), 78.3 (C-3), 77.7 (C-4), 65.5 ppm (C-1); ESI-MS: m/z: 349 (100) $[M+Na]^+$.
[17] W.-D. Fessner, G. Sinerius, *Angew. Chem.* **1994**, *106*, 217 – 220; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 209 – 212.
[18] W.-D. Fessner, O. Eyrisch, *Angew. Chem.* **1992**, *104*, 76 – 78; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 56 – 58.
[19] E. Gonzalez-Garcia, V. Helaine, G. Klein, M. Schuermann, G. A. Sprenger, W.-D. Fessner, J.-L. Reymond, *Chem. Eur. J.* **2003**, *9*, 893 – 899.

