DOI: 10.1002/adsc.200700168

Chemoenzymatic Synthesis and Inhibitory Activities of Hyacinthacines A_1 and A_2 Stereoisomers

Jordi Calveras,[a] Josefina Casas,[b] Teodor Parella,[c] Jesús Joglar,[a] and Pere Clapés[a,*]

[a] Biotransformation Unit, Institute for Chemical and Environmental Research (IIQAB)-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain
Fax: (+34)-93-204-5904; e-mail: pcsqbp@iiqab.csic.es
[b] Research Unit on Bioactive Molecules (RUBAM), Institute for Chemical and Environmental Research (IIQAB)-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain
[c] Servei de Ressonància Magnètica Nuclear, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain

Received: April 2, 2007

Dedicated to Prof. Vicente Gotor on the occasion of his 60th anniversary.

Supporting information for this article is available on the WWW under http://asc.wiley-vch.de/home/.

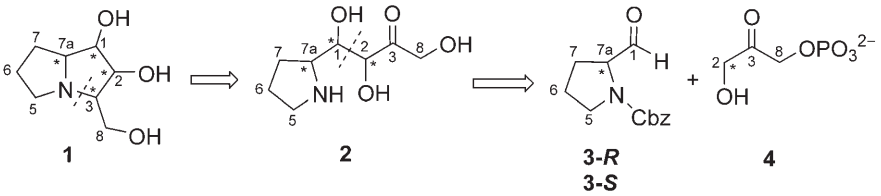
Abstract: A novel straightforward chemoenzymatic procedure for the synthesis of hyacinthacine stereoisomers based on the aldol addition of dihydroxyacetone phosphate (DHAP) to *N*-Cbz-prolinal under catalysis by L-rhamnulose 1-phosphate aldolase from *E. coli* is presented. The synthesis is complemented by a simple and effective purification protocol consisting of ion-exchange chromatography on CM-sepharose. As examples, (−)-hyacinthacine A_2 [the enantiomer of (+)-hyacinthacine A_2], 7-deoxy-2-epialexine (the enantiomer of 3-epihyacinthacine A_2), *ent*-7-deoxyalexine (the enantiomer of 7-deoxyalexine) and 2-epihyacinthacine A_2 were synthesized by these procedures and characterized for the first time. These new isomers were assayed as inhibitors of glycosidases. As a result, (−)-hyacinthacine A_2 demonstrated to be a good inhibitor of α-D-glucosidase from rice whereas the natural enantiomer, hyacinthacine A_2, was not. Moreover, a new family of inhibitors of α-L-rhamnosidase was uncovered.

Keywords: aldol reaction; aldolases; amino aldehydes; hyacinthacines; polyhydroxylated pyrrolizidine alkaloids

Polyhydroxylated pyrrolizidine alkaloids are naturally occurring compounds with a substantial interest as inhibitors of glycosidases and glycosyltransferases.[1,2] Like other polyhydroxylated alkaloids, they may lead to potential therapeutic drugs against diabetes II, cancer and viral infections including HIV.[3,4] Among the polyhydroxylated pyrrolizidine alkaloids, those possessing a hydroxymethyl substituent at C-3 are relatively rare in nature, alexine and australine being the first reported ones.[5] These compounds differ in the C-7a configuration and this feature causes a differential activity against disaccharidase type α-glucosidases.[6] The compounds with the (*S*) configuration at C-7a (alexine analogues) are scarce in nature, while a number with the (*R*) configuration have been reported.[4] Others, such as hyacinthacines A_1 and A_2, have been recently isolated from the bulbs of *Muscari armeniacum* (Hyacinthaceae) and demonstrated to be good inhibitors against rat intestinal lactase, rat epididymis α-L-fucosidase, and amyloglucosidase from *Arpergillus niger*.[2] The chemical syntheses of these compounds usually involve cumbersome protection-deprotection reactions, elaborated starting materials derived from the sugar chiral pool, and therefore, moderate stereoselection and global yields are achieved.[7–9] Importantly, the generation of configurational diversity on the stereogenic centers appears to be of paramount importance to optimize their activity and selectivity.[10] Consequently, novel methodologies for the preparation of these compounds with a broad configurational diversity are of increasing interest.[11]

Herein, we report a new straightforward chemoenzymatic methodology for the synthesis of polyhydroxylated pyrrolizidine alkaloids, in particular some stereoisomers of hyacinthacines A_1 and A_2. The synthetic strategy devised is based on the ability of dihydroxyacetone phosphate (DHAP) aldolases to accept *N*-Cbz-amino aldehydes as substrates.[12,13] Hence, the key step of our synthetic scheme was the aldol addition of DHAP to either (*R*)- or (*S*)-*N*-Cbz-prolinal, as shown in the retrosynthetic analysis (Scheme 1).

The stereochemistry at C-7a is fixed by the starting *N*-Cbz-prolinal, while those at positions C-1 and C-2



Scheme 1. Retrosynthetic analysis for hyacinthacine stereoisomers.

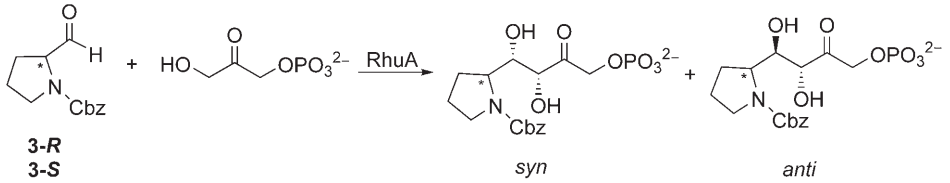
are controlled by the DHAP aldolase. The stereochemistry at C-3 depends on the reductive amination with Pd/C. This reaction is highly stereoselective, the hydrogen being delivered from the face opposite to the hydroxy group at the C-1 position.[13,14] Formally, this strategy might lead to obtain 8 out of 16 possible stereoisomers of hyacinthacines A_1 and A_2 starting from DHAP (**4**) and either (*R*)- or (*S*)-*N*-Cbz-prolinal (**3-*R*** or **3-*S***). The scope of this synthetic approach was investigated using D-fructose 1,6-bisphosphate aldolase from rabbit muscle (RAMA) as well as L-rhamnulose 1-phosphate aldolase (RhuA), and L-fuculose 1-phosphate aldolase (FucA) from *E. coli* as catalysts for the aldol addition of DHAP to **3-*S*** and **3-*R***. Both enantiomers of *N*-Cbz-prolinal (**3**) were easily accessible from (*S*)- or (*R*)-prolinol in 89% isolated yield.[15]

Emulsions and DMF/buffer (1:4 v/v) were the reaction systems of choice at both 25 °C and 4 °C. The literature precedent from enzymatic DHAP aldol addition studies demonstrated that the conversions may be influenced by the reaction media,[16] and improved at 4 °C.[17] The enzymatic reactions were incubated until constant conversion values were monitored.

RhuA tolerated (*S*)- and (*R*)-*N*-Cbz-prolinal in remarkably good conversions while no reaction was detected with both RAMA and FucA catalysts in any of the conditions assayed. As expected, conversions at 4 °C were much higher than those at 25 °C (Table 1) although they took a longer incubation period to reach constant conversion values. Moreover, the DMF/H_2O 1:4 mixture was the best reaction system although the aldehyde was not completely dissolved and milky solutions were observed at the beginning and during the reaction. This could be, among other reasons, because of changes in the active site solvation and ionization state caused by solvent molecules of DMF[18] which might favor *N*-Cbz-prolinal–aldolase interactions.

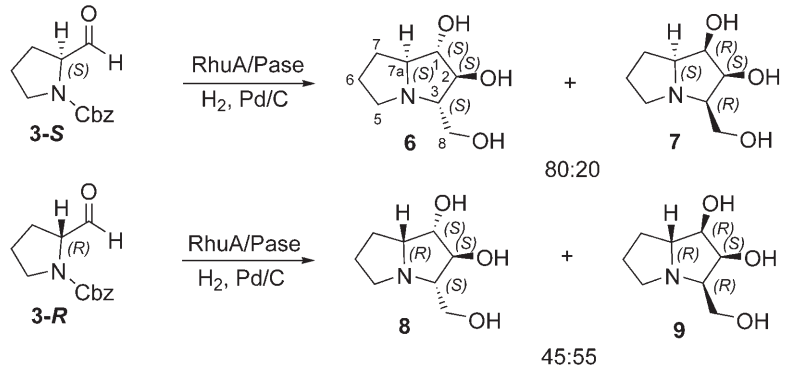
The corresponding adducts from the two enantiomers of **3** were purified and submitted to reductive amination following the methodology described.[13] The formation of four diastereoisomers of hyacinthacines A_1 and A_2 (Scheme 2, see experimental details in Supporting Information), namely (−)-hyacinthacine A_2 (**6**) [the enantiomer of (+)-hyacinthacine A_2[19]], 7-deoxy-2-epialexine (**7**) (the enantiomer of 3-epihyacinthacine A_2[9]), *ent*-7-deoxyalexine (**8**) (the enantio-

Table 1. Reaction medium and temperature effects on the conversion and steroselectivity of aldol additions of DHAP to (*S*)- or (*R*)-prolinal catalyzed by RhuA.



Aldehyde	*T* [°C]	Time [h][b]	Conversion [%][a]		*syn*/*anti*[d]
			Emulsions[c]	DMF/H_2O (1:4)	
3-*S*	25	20	40	58	66/34
3-*S*	4	44	70	81	80/20
3-*R*	25	20	37	69	66/34
3-*R*	4	44	68	80	45/55

[a] Molar percent conversion to the aldol adduct with respect to the starting DHAP concentration, determined by HPLC from the crude reaction mixture using purified standards.
[b] Incubation time required to reach constant substrate conversion.
[c] High water content emulsions. Mean conversion values obtained in three emulsion systems, $H_2O/C_{14}E_4$/tetradecane, $H_2O/C_{14}E_4$/hexadecane and $H_2O/C_{14}E_4$/squalane 90/4/6 wt% [$C_{14}E_4$: non-ionic polyoxyethylene ether surfactant, tetra(ethylene glycol) tetradecyl ether, $C_{14}H_{29}(OCH_2CH_2)_4OH$, with an average of 4 moles of ethylene oxide per surfactant molecule].
[d] For the determination on the diasteromeric ratio, see text.



Scheme 2. Chemoenzymatic synthesis of hyacinthacines A_1 and A_2 stereoisomers.

mer of 7-deoxyalexine[7]) and 2-epihyacinthacine A_2 (**9**) was confirmed by NMR analysis (see Supporting Information).

The absolute configurations at C-7a and C-2 are fixed by both the aldehyde and RhuA catalyst, respectively.[20] Consistent with previous observations the reductive amination gave the 1-/3-OH groups in a *syn* orientation. Inspection of the absolute configuration at C-1 (Table 1) reveals that the RhuA-DHAP complex may attack both enantiotopic faces of the acceptor aldehyde. Since the reactions were run to maximum conversion the stereochemical outcome of RhuA catalyst towards (*S*)- and (*R*)-prolinal may be close to thermodynamic control rather than kinetic control (Table 1). Therefore, the results achieved at 25 °C and 4 °C may reflect changes in the equilibrium position. Lowering the temperature favored the *syn* product when using *S*-prolinal. On the other hand, *R*-prolinal at 25 °C gave preferentially the *syn* product while a *ca.* 1:1 *syn*:*anti* ratio was observed at 4 °C.

The lack of selectivity of RhuA towards (*R*)- and (*S*)-prolinal was probably due to the structural differences between these aldehydes and the natural acceptor L-lactaldehyde. Based on the studies of Kroemer et al.,[21] the L-lactaldehyde may be fixed at the active site by two-hydrogen bonds, the methyl group of the aldehyde being oriented into a hydrophobic environment. Hence, it is effectively fixed at the active enzyme pocket favoring the nucleophilic attack on a single enantiotopic face. The conformational restriction imposed by the five-membered ring of *N*-Cbz-prolinal as well as the lack of any directing hydroxy group may make difficult an effective orientation of the carbonyl group at the enzyme active pocket. Moreover, docking simulations of *N*-protected 3-aminopropionaldehyde into RhuA suggested that the protecting group cannot get into the catalytic site and therefore cannot drive any preferred orientation.[22] Hence, the loss of interaction points between the RhuA and the *N*-Cbz-prolinal may lead to less restricted rotation around C-7a/C-1.

On the other hand, the low stereoselectivity of RhuA towards the acceptor aldehyde furnished four hyacinthacine stereoisomers which could be assayed if a good separation method was available. In this regard, ion exchange chromatography on a CM-separose in the NH_4^+ form,[2] eluted isocratically with 0.01 M NH_4OH, gave an excellent separation of diastereoisomers **8** and **9.** The same conditions furnished pure **6** but **7** was not recovered. Alternatively, the separation of the **6**/**7** mixture was accomplished by eluting with 6 mM to 8 mM CH_3COONH_4 (pH 6).

Compound **6** had good inhibitory activity against α-D-glucosidase from rice (IC_{50} 30 μM, Table 2). Interestingly, it has been reported[2] that hyacinthacine A_2, the enantiomer of **6**, does not inhibit α-D-glucosidase from rice. Moreover, **6** and **8** were active against α-L-rhamnosidase from *Penicillium decumbens* (Table 2) but to a lower degree. Furthermore, these compounds are quite selective since they did not show inhibitory activity against α-L-fucosidase from bovine kidney, α-mannosidase from jack bean, β-D-galactosidase from bovine liver or *Aspergillus oryzae*, and β-D-glucosidase from sweet almonds among the glycosidases assayed. Only compounds **8** and **9** caused 50 % inhibition of α-mannosidase at 0.6 mM. In this context, it

Table 2. Inhibition of glycosidases by compounds **6**–**9**.

Compound	α-D-Glucosidase from Rice		α-L-Rhamnosidase from *Penicillium decumbens*	
	IC_{50} [μM]	K_i [μM]	IC_{50} [μM]	K_i [μM]
6	30	4.7 ± 0.5[a]	115	57.0 ± 0.9[d]
7	700	nd[b]	700	nd
8	800	nd	90	33.0 ± 0.7[d]
9	ni[c]	nd	300	nd

[a] Competitive inhibition.
[b] nd, not determined.
[c] ni, no inhibition.
[d] Mixed type inhibition.

has been reported that australine analogues, that have the same configuration at C-7a, also inhibited human α-mannosidases.[23]

Next, K_i and type of inhibition were determined for the active compounds (Table 2, and the Lineweaver–Burke plots in Figure 1, see the Dixon plots in the Supporting information). Compound **6**, $K_i = 4.7$ μM, is a rather potent competitive inhibitor of α-D-glucosidase from rice, indicating that it would mimic the substrate and interact with the enzyme active site. Hydroxy substituents of the pyrrolizidine ring of **6** are in pseudo-equatorial position as are the ones in α-D-glucose. In this case, it is likely that the inhibition activity was due to substrate mimicking rather than to a transition state analogue. This is not the case for hyacinthacine A_2 and other compounds tested in this study. α-D-Glucosidase inhibitors are effective in lowering the postprandial glycemic rise after carbohydrate ingestion as well as the insulin release and requirement. They are also active against tumor metastasis. Hence, they are leads for potential antiobesity, antidiabetes type II and antimetastasic therapeutic agents.[24]

The compounds **6** and **8**, $K_i = 57$ and 33 μM, respectively, behaved as mixed type α-L-rhamnosidase inhibitors, suggesting that they may bind to the free enzyme but also to the enzyme-substrate complex. Remarkably, only the compounds with the (*S*)-configurations at C-1, C-2 and C-3 were active, being, to the best of our knowledge, the first hyacinthacine analogues active against α-L-rhamnosidase reported until now. α-L-Rhamnosidase is involved in the hydrolysis of both rhamnogalacturonans which have a key role as immunomodulators enhancing the cytotoxicity of NK cells and O-antigen tetrasaccharide, the first stage of bacterial invasion of a host cell. Thus, inhibitors of this glycosidase are potential leads for therapeutic agents against cancer and bacillary dysentery.[25]

In summary, we have developed a new and simple stereoselective chemoenzymatic synthesis and purification methodology for the preparation of new hyacinthacines A_1 and A_2 stereoisomers. This work demonstrates that analogues of these natural compounds can be prepared in a few synthetic steps along with an effective and simple purification procedure. As a result, the enantiomer of the natural hyacinthacine A_2 was prepared and demonstrated to be a good inhibitor of α-D-glucosidase from rice and a new family of inhibitors of α-L-rhamnosidase has been revealed.

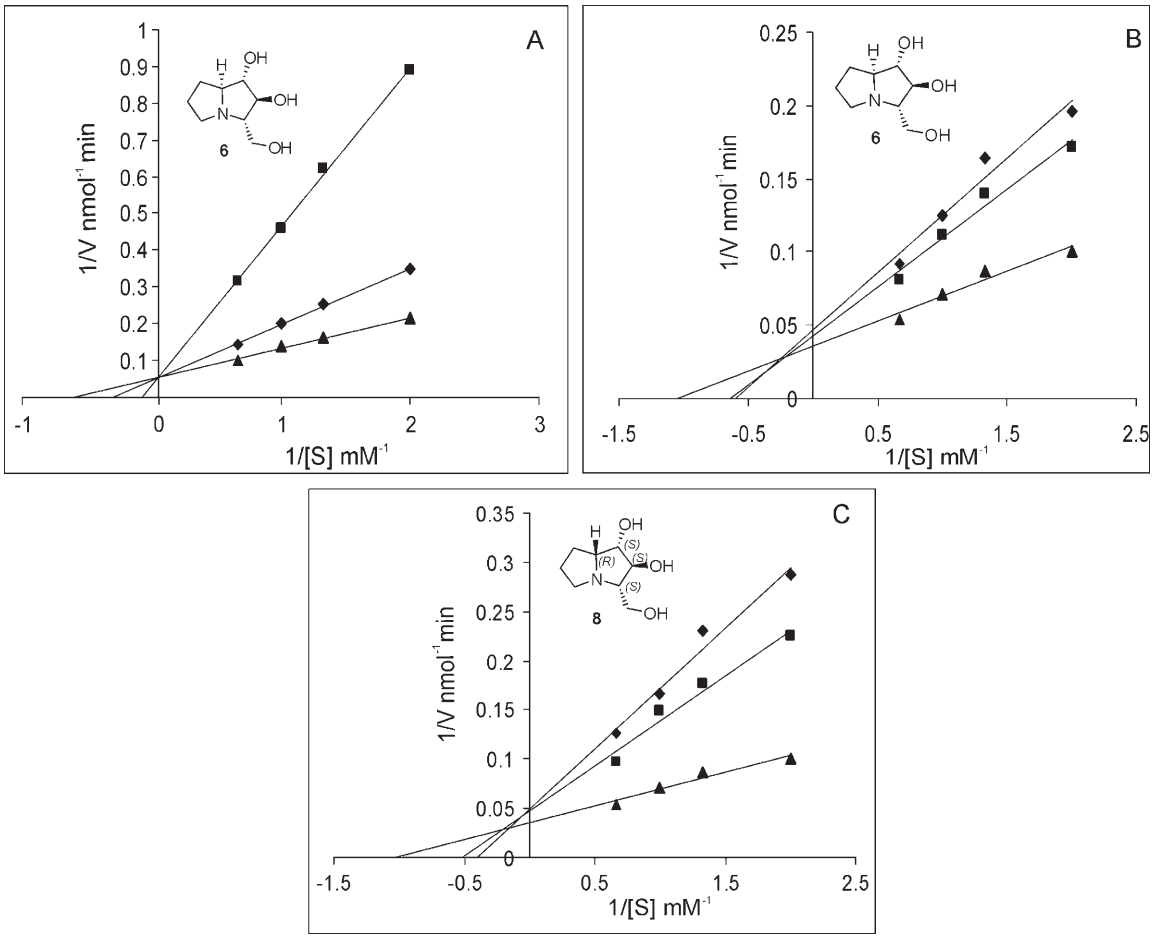


Figure 1. Lineweaver-Burk double reciprocal plots for the determination of inhibition constant (K_i) of **6** against α-D-glucosidase from rice (**A**) and that of **6** and **8** against α-L-rhamnosidase from *Penicillium decumbens* (**B**) and (**C**), respectively.

This methodology may become more advantageous with the use of dihydroxyacetone (DHA) and borate as DHAP mimic in RhuA-catalyzed reactions.[26] Modification of FruA and FucA by protein engineering to increase their tolerance towards (*R*)- and (*S*)-prolinal and their derivatives is currently in progress. This may lead to new derivatives with optimized inhibitory activity against glycosidases.

Experimental Section

Synthetic and analytical procedures are described in the Supporting Information. A typical synthesis of hyacinthacine stereoisomers is described below.

Enzymatic Aldol Condensations

(*S*)-Prolinal (16.1 mmol, 3.75 g) was dissolved in DMF (22.5 mL) and cooled to 0 °C. Then, the DHAP solution (9.5 mmol, 90 mL of a 105.2 mM solution, at pH 6.9, freshly prepared) was added at 4 °C under vigorous agitation. Then, RhuA (225U) was added and mixed again. The reaction was shaken (100 rpm) at 4 °C and when it reached a constant conversion, MeOH (115 mL) was added. Then, excess of aldehyde was removed by extractions with ethyl acetate (2 × 50 mL). The solution of the product obtained (100 mL) was treated with acid phosphatase (50 mg, 7 U mg^{-1}) at pH 5.5. The unphosphated product was desalted by RP-HPLC and after lyophilization a pale brown solid was obtained; yield: 1.03 g (58 % overall).

This solid was dissolved in H_2O/EtOH (4:1; 160 mL) and Pd/C (200 mg) added. The reaction mixture was stirred under hydrogen gas (50 psi) overnight at room temperature. After removal of the catalyst by filtration through neutralized and deactivated aluminum, the diasteromeric mixture of **6** and **7** was obtained as a brown solid; yield: 514 mg (77 %).

A similar procedure was followed starting with the (*R*)-Cbz-prolinal obtaining the mixture of diastereoisomers **8** and **9** as pale brown solid; yield: 656 mg (90 %).

Purification by Ion Exchange Chromatography

The diasteromeric mixture **8**+**9** (50 mg) was separated by ion exchange chromatography on a FPLC system. CM-Sepharose CL-6B (Amersham Pharmacia) in the NH_4^+ form as stationary phase was packed into a glass column (160 × 20 mm) to a final bed volume of 50 mL. The flow rate was 0.9 mL min^{-1}. The CM-Shepharose-NH_4^+ was washed initially with H_2O. Then, an aqueous solution of the crude material at pH 7 was loaded onto the column. Minor colored impurities were washed away with H_2O (150 mL, 3 bed volumes). The retained compounds **8** and **9** were released with 125 mL and 285 mL of aqueous 0.01 M NH_4OH, respectively. Pure fractions were pooled and lyophilized affording **8** (20 mg) and **9** (26 mg). The operation was repeated until the whole mixture was consumed.

A similar procedure was employed with the **7**+**9** mixture.

Supporting Information

Experimental preparations, analytical data, biological data and ^1H, ^{13}C NMR spectra for all compounds; COSY, HSQC and NOE spectra, are described in detail.

Acknowledgements

Financial support from the Spanish MEC (CTQ2005–25182-E, CTQ2006–01080 and CTQ2006–01345/BQU), La Marató de TV3 foundation (Ref: 050931) and Generalitat de Catalunya DURSI 2005-SGR-00698 is acknowledged. J. Calveras acknowledges the CSIC UAs pre-doctoral scholarship programs.

References

[1] A. Kato, I. Adachi, M. Miyauchi, K. Ikeda, T. Komae, H. Kizu, Y. Kameda, A. A. Watson, R. J. Nash, M. R. Wormald, *Carbohydr. Res.* **1999**, *316*, 95–103.

[2] N. Asano, H. Kuroi, K. Ikeda, H. Kizu, Y. Kameda, A. Kato, I. Adachi, A. A. Watson, R. J. Nash, G. W. J. Fleet, *Tetrahedron: Asymmetry* **2000**, *11*, 1–8.

[3] T. D. Heightman, A. T. Vasella, *Angew. Chem.* **1999**, *111*, 794–815; *Angew. Chem. Int. Ed.* **1999**, *38*, 750–770; A. Vasella, G. J. Davies, M. Bohm, *Curr. Opin. Chem. Biol.* **2002**, *6*, 619–629; T. D. Butters, R. A. Dwek, F. M. Platt, *Curr. Top. Med. Chem.* **2003**, *3*, 561–574; N. G. Oikonomakos, C. Tiraidis, D. D. Leonidas, S. E. Zographos, M. Kristiansen, C. U. Jessen, L. Norskov-Lauritsen, L. Agius, *J. Med. Chem.* **2006**, *49*, 5687–5701.

[4] A. A. Watson, G. W. J. Fleet, N. Asano, R. J. Molyneux, R. J. Nash, *Phytochemistry* **2001**, *56*, 265–295.

[5] R. J. Molyneux, M. Benson, R. Y. Wong, J. E. Tropea, A. D. Elbein, *J. Nat. Prod.* **1988**, *51*, 1198–1206; J. E. Tropea, R. J. Molyneux, G. P. Kaushal, Y. T. Pan, M. Mitchell, A. D. Elbein, *Biochemistry* **1989**, *28*, 2027–2034; R. J. Nash, L. E. Fellows, J. V. Dring, G. W. J. Fleet, A. E. Derome, T. A. Hamor, A. M. Scofield, D. J. Watkin, *Tetrahedron Lett.* **1988**, *29*, 2487–2490.

[6] R. J. Nash, L. E. Fellows, J. V. Dring, G. W. J. Fleet, A. Girdhar, N. G. Ramsden, J. M. Peach, M. P. Hegarty, A. M. Scofield, *Phytochemistry* **1990**, *29*, 111–114.

[7] I. Izquierdo, M. T. Plaza, R. Robles, F. Franco, *Tetrahedron: Asymmetry* **2001**, *12*, 2481–2487.

[8] I. Izquierdo, M. T. Plaza, F. Franco, *Tetrahedron: Asymmetry* **2003**, *14*, 3933–3935; L. Rambaud, P. Compain, O. R. Martin, *Tetrahedron: Asymmetry* **2001**, *12*, 1807–1809; T. Yamashita, K. Yasuda, H. Kizu, Y. Kameda, A. A. Watson, R. J. Nash, G. W. J. Fleet, N. Asano, *J. Nat. Prod.* **2002**, *65*, 1875–1881.

[9] L. Chabaud, Y. Landais, P. Renaud, *Org. Lett.* **2005**, *7*, 2587–2590.

[10] G. Dickson Lucas, E. Leroy, J.-L. Reymond, *Org. Biomol. Chem.* **2004**, *2*, 1217–1226; A. Kato, N. Kato, E. Kano, I. Adachi, K. Ikeda, L. Yu, T. Okamoto, Y. Banba, H. Ouchi, H. Takahata, N. Asano, *J. Med. Chem.* **2005**, *48*, 2036–2044; H. Ouchi, Y. Mihara, H.

Watanabe, H. Takahata, *Tetrahedron Lett.* **2004**, *45*, 7053–7056; N. Asano, K. Ikeda, L. Yu, A. Kato, K. Takebayashi, I. Adachi, I. Kato, H. Ouchi, H. Takahata, G. W. J. Fleet, *Tetrahedron: Asymmetry* **2005**, *16*, 223–229; H. Takahata, Y. Banba, H. Ouchi, H. Nemoto, A. Kato, I. Adachi, *J. Org. Chem.* **2003**, *68*, 3603–3607.

[11] A. Romero, C. H. Wong, *J. Org. Chem.* **2000**, *65*, 8264–8268.

[12] C. H. Von der Osten, A. J. Sinskey, C. F. Barbas, III, R. L. Pederson, Y. F. Wang, C. H. Wong, *J. Am. Chem. Soc.* **1989**, *111*, 3924–3927; L. Espelt, J. Bujons, T. Parella, J. Calveras, J. Joglar, A. Delgado, P. Clapés, *Chem. Eur. J.* **2005**, *11*, 1392–1401.

[13] L. Espelt, T. Parella, J. Bujons, C. Solans, J. Joglar, A. Delgado, P. Clapés, *Chem. Eur. J.* **2003**, *9*, 4887–4899.

[14] T. Kajimoto, L. Chen, K. K. C. Liu, C. H. Wong, *J. Am. Chem. Soc.* **1991**, *113*, 6678–6680.

[15] M. Ocejo, J. L. Vicario, D. Badia, L. Carrillo, E. Reyes, *Synlett* **2005**, 2110–2112.

[16] L. Espelt, P. Clapés, J. Esquena, A. Manich, C. Solans, *Langmuir* **2003**, *19*, 1337–1346.

[17] T. Suau, G. Alvaro, M. D. Benaiges, J. Lopez-Santin, *Biotechnol. Bioeng.* **2006**, *93*, 48–55.

[18] C. Mattos, D. Ringe, *Curr. Opin. Struct. Biol.* **2001**, *11*, 761–764; C. Mattos, C. R. Bellamacina, E. Peisach, A. Pereira, D. Vitkup, G. A. Petsko, D. Ringe, *J. Mol. Biol.* **2006**, *357*, 1471–1482.

[19] S. Desvergnes, S. Py, Y. Vallee, *J. Org. Chem.* **2005**, *70*, 1459–1462.

[20] W. D. Fessner, G. Sinerius, A. Schneider, M. Dreyer, G. E. Schulz, J. Badia, J. Aguilar, *Angew. Chem.* **1991**, *103*, 596–599; *Angew. Chem. Int. Ed.* **1991**, *30*, 555–558; D. R. Hall, G. A. Leonard, C. D. Reed, C. I. Watt, A. Berry, W. N. Hunter, *J. Mol. Biol.* **1999**, *287*, 383–394; T. D. Machajewski, C. H. Wong, *Angew. Chem.* **2000**, *112*, 1406–1430; *Angew. Chem. Int. Ed.* **2000**, *39*, 1353–1374.

[21] M. Kroemer, I. Merkel, G. E. Schulz, *Biochemistry* **2003**, *42*, 10560–10568.

[22] J. Calveras, J. Bujons, T. Parella, R. Crehuet, L. Espelt, J. Joglar, P. Clapés, *Tetrahedron* **2006**, *62*, 2648–2656.

[23] B. Winchester, S. Al Daher, N. C. Carpenter, I. Cenci di Bello, S. S. Choi, A. J. Fairbanks, G. W. J. Fleet, *Biochem. J.* **1993**, *290*, 743–749.

[24] E. S. H. El Ashry, N. Rashed, A. H. S. Shobier, *Pharmazie* **2000**, *55*, 251–262; E. S. H. El Ashry, N. Rashed, A. H. S. Shobier, *Pharmazie* **2000**, *55*, 331–348; N. Asano, *Glycobiology* **2003**, *13*, 93R-104R.

[25] J. H. Kim, M. J. Curtis-Long, W. D. Seo, J. H. Lee, B. W. Lee, Y. J. Yoon, K. Y. Kang, K. H. Park, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4282–4285.

[26] M. Sugiyama, Z. Hong, L. J. Whalen, W. A. Greenberg, C.-H. Wong, *Adv. Synth. Catal.* **2006**, *348*, 2555–2559.

