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Synthesis of proline derivatives of bile acids and their evaluation as organocatalysts in the asymmetric direct aldol reaction

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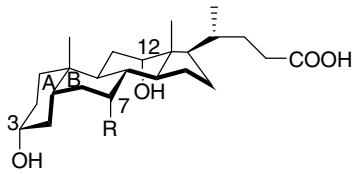
Abstract—A new family of bile acid derived organocatalysts was obtained by linking L- or D-proline to amino derivatives of cholic and deoxycholic acids, which were used to promote the asymmetric direct aldol reaction between acetone and 4-nitrobenzaldehyde. Both the activity and enantioselectivity of the organocatalytic systems were dependent not only on the position of the proline moiety on the cholestanic backbone and its absolute configuration, but also on the presence of free hydroxyl group on the steroidal skeleton. The cholic acid derivative bearing a D-prolinamide moiety at the 12-position and free hydroxyl groups at the 3- and 7-positions emerged as the best organocatalytic system giving complete conversion of the substrate, even when using only 2% of catalyst loading and ee up to 80%.

1. Introduction

The enantioselective formation of C–C bonds promoted by chiral organic molecules[1] (asymmetric organocatalysts) represents an attractive approach for the synthesis of enantiomerically enriched products. Organocatalysts are usually endowed with inertness and robustness, so that demanding reaction conditions, such as an inert atmosphere or absolute solvents, are not required.[2] These features make the use of organocatalysts advantageous instead of organic ligands in transition metal complexes to promote asymmetric transformations. For these reasons, since List and Barbas have reported the use of L-proline as an organocatalyst for the direct asymmetric aldol reaction,[3] the last seven years have witnessed an explosive growth with regards to asymmetric organocatalytic methods.[4] Much effort has been devoted towards the synthesis of new organocatalysts and, in this frame, a great deal of interest has been addressed to the development of proline containing systems to be used in the asymmetric formation of C–C bonds via an enamine pathway, such as aldol[5] and Mannich reactions[6] or conjugate additions.[7] The interest in the development of this kind of system lies in the consideration that some molecular architectures, where proline is linked, form a chiral cleft that can mimic the active site of the class I aldolase enzymes,[8] which catalyze enantioselective C–C bond forming reactions by an enamine pathway. Taking into account these considerations, and following our long-standing interest in the use of bile acid derivatives in enantioselective processes,[9] we became interested in developing proline containing bile acids, where, because of their concave structure, caused by the *cis* junction of the A and B cyclohexane rings (Fig. 1), the cholestanic backbone and the appended proline should form a chiral cleft that can help the enantioselection. In addition the presence of free hydroxyl groups can constitute a further advantage by controlling, via hydrogen bonds, the position of the substrate in the cavity of the organocatalyst.



1a R= OH cholic acid
1b R=H deoxycholic acid

Figure 1. Cholic and deoxycholic acid structure.

Since in the transition state of the reaction, proceeding via enamine pathway, a hydrogen bond is present between an acidic proton of the catalyst and an acceptor group on the

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substrate,[10] we judged that the proline moiety had to be linked to the cholestanic skeleton by means of an amide bond. This required that one of the hydroxyl groups of bile acids was selectively transformed into an amino group. In addition, since it is known that the enantioselectivity of the bile acid derivatives depends not only on the nature of the appended moieties, but also on their position on the cholestanic backbone,[9d] L- and D-proline containing derivatives at the 3-, 7- and 12-positions were synthesized, in order to find the best position and the best match between the stereochemistry of the bile acid and the absolute configuration of proline for asymmetric organocatalysis. The synthesis of analogous systems having free hydroxyl groups was also designed in order to evaluate the effect of the presence of these groups on asymmetric organocatalysis. The activity and enantioselectivity of this new class of organocatalysts (Fig. 2) were assayed in the asymmetric direct aldol reaction.

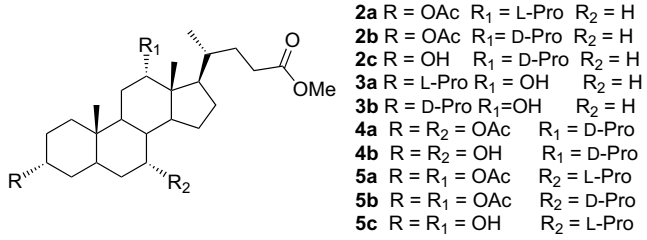


Figure 2. Structure of bile acid derived organocatalysts.

1.1. Synthesis of organocatalysts

The amino derivatives of the deoxycholic acid, from which organocatalysts **2a**–**c** and **3a**–**b** can be prepared, were synthesized as previously described.[11] The synthetic route to 12- and 7-amino derivatives of cholic acid is reported in Scheme 1 and it is similar for the two compounds, requiring the oxidation of 7 or 12 hydroxyl groups to ketones that can be transformed into oximes, easily reduced to amino groups.[11] In fact, due to the axial position of the 7 and 12 hydroxyl groups, the Mitsunobu transformation did not work; in addition, due to the asymmetric structure of the cholestanic backbone, the catalytic hydrogenation of an oxime at these positions proceeds with complete stereoselectivity.[11a]

To obtain ketone **8**, the carboxylic function of cholic acid was transformed in methylester, after which the 3- and 7-hydroxyl groups were acetylated by reacting **6** with acetic anhydride and pyridine:[11g] under these experimental conditions the 12-OH group does not react and **7** was obtained in 72% yield after chromatographic purification. The oxidation of the 12-OH group, performed with potassium dicromate,[11a] gave **8** in quantitative yield. Ketone **11** was obtained in four steps from cholic acid, which was reacted with methylacetate[11] to give **9** in 75% yield after chromatographic purification. The selective oxidation of the 7-hydroxy group with NBS in acetone solution[11a] to give **10** in quantitative yield, which was then reacted with acetic anhydride and triethylamine in the presence of DMAP giving **11** in 88% yield. Both 12- and 7-keto derivatives **8** and **11** were transformed in the corresponding oximes, under standard reaction conditions,[11] which were eventually reduced, with complete stereoselectivity, to the amino derivatives by means of catalytic hydrogenation followed by reaction with Zn in acetic acid.[11]

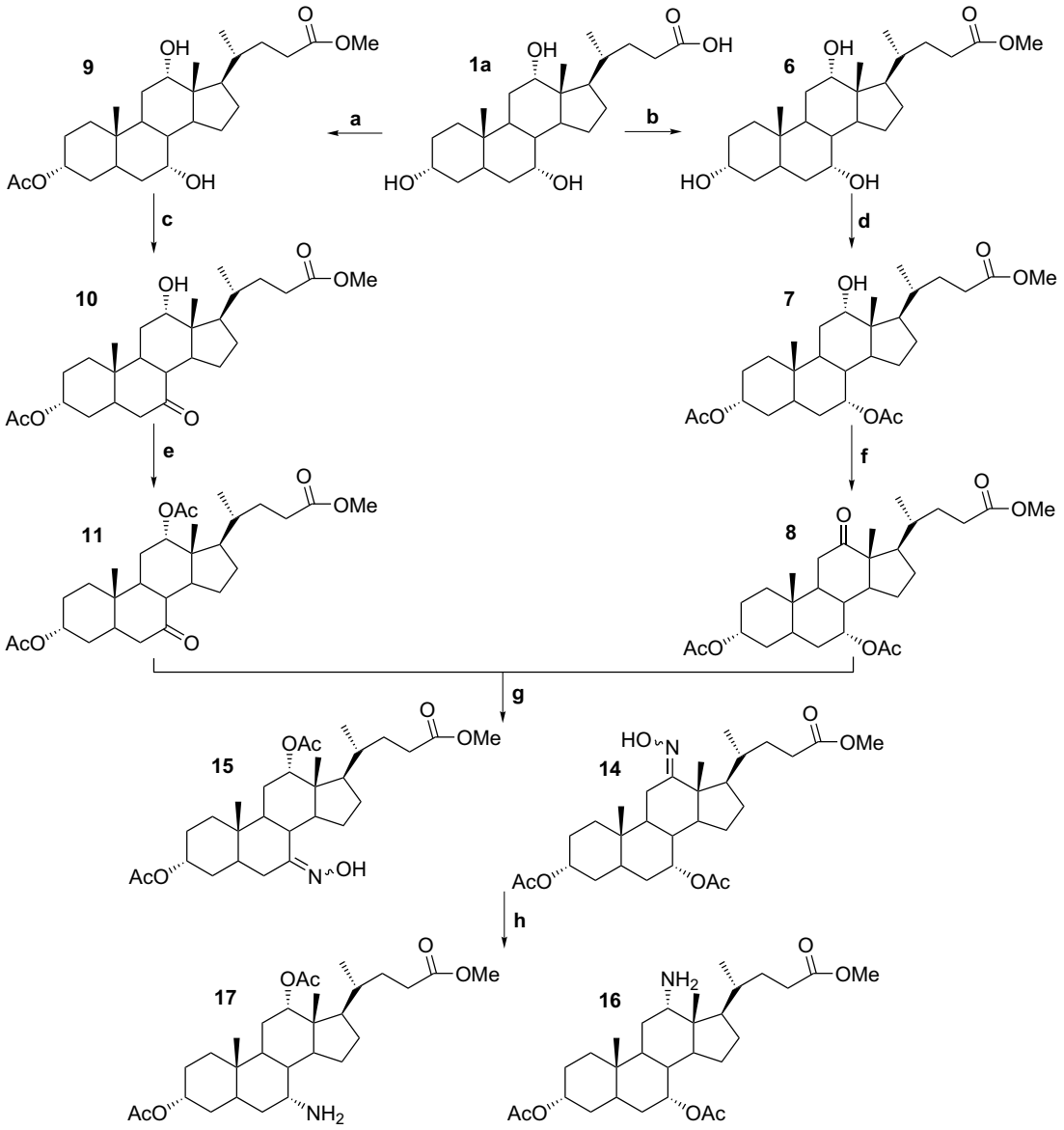
The proline containing bile acids **2a**–**b**, **3a**–**b**, **4a**, **5a**–**b** were obtained by starting from the corresponding amino derivatives in two steps (Scheme 2). By reacting **12**, **13**, **16**, **17** with L- or D-Boc-proline in the presence of isobutylchloroformate[12] at room temperature the corresponding prolinamides of bile acids were obtained in 50% yield, after chromatographic purification. The final products were obtained in quantitative yield by removing the Boc protecting group by means of trifluoroacetic acid in dichloromethane solution.[13]

To obtain analogous systems with free hydroxyl groups, the acetyl moieties of compounds **2b**, **4a** and **5b** were removed (Scheme 3). The hydrolysis of the 3-acetyl group of **2b** was performed by reacting **2b** with HCl in methanol at room temperature.[11a] These reaction conditions performed well because the 3-acetyl group is equatorial and affords **2c** in quantitative yield. This experimental procedure cannot be used for removing the acetyl groups at the 7- and 12-positions, since they are axial and then less reactive. Therefore, to obtain **4b** and **5c**, the hydrolysis was carried out with sodium methoxide in methanol at room temperature.[14] Under these reaction conditions, the methylester group is stable and **22a** and **23a** can be obtained 46% and 93% yield, respectively, after chromatographic purification. Removing the Boc protecting group by means of trifluoroacetic acid in dichloromethane solution,[13] gave products **4b** and **5c** in quantitative yield.

1.2. Direct asymmetric aldol reaction

Organocatalysts **2**–**5** were assayed in the direct asymmetric aldol reaction between acetone and 4-nitrobenzaldehyde. The effects of the different reaction parameters, such as solvent, temperature, catalyst loading and the presence of additives, were investigated using **2b** as an organocatalytic system with the results obtained, listed in Table 1. All reactions were stopped when conversion of the substrate was complete or did not proceed further, as judged by TLC analysis. The first run, performed by mixing *p*-nitrobenzaldehyde (0.25 mmol) and **2b** (0.025 mmol) in acetone, used as reaction solvent,[3b] at room temperature gave complete conversion of the substrate in 48 h and the condensation product was obtained in 27% ee (entry 1). However, together with the aldol product, appreciable amounts of other condensation products together with the product coming from the dehydration of the aldol were obtained, and their formation was observed even when performing the reaction at 0 °C. These problems could be avoided by using a different reaction procedure, which involves the preactivation of the organocatalysts obtained by reacting **2b** and acetone for 1 h, then adding the aldehyde.[5c]

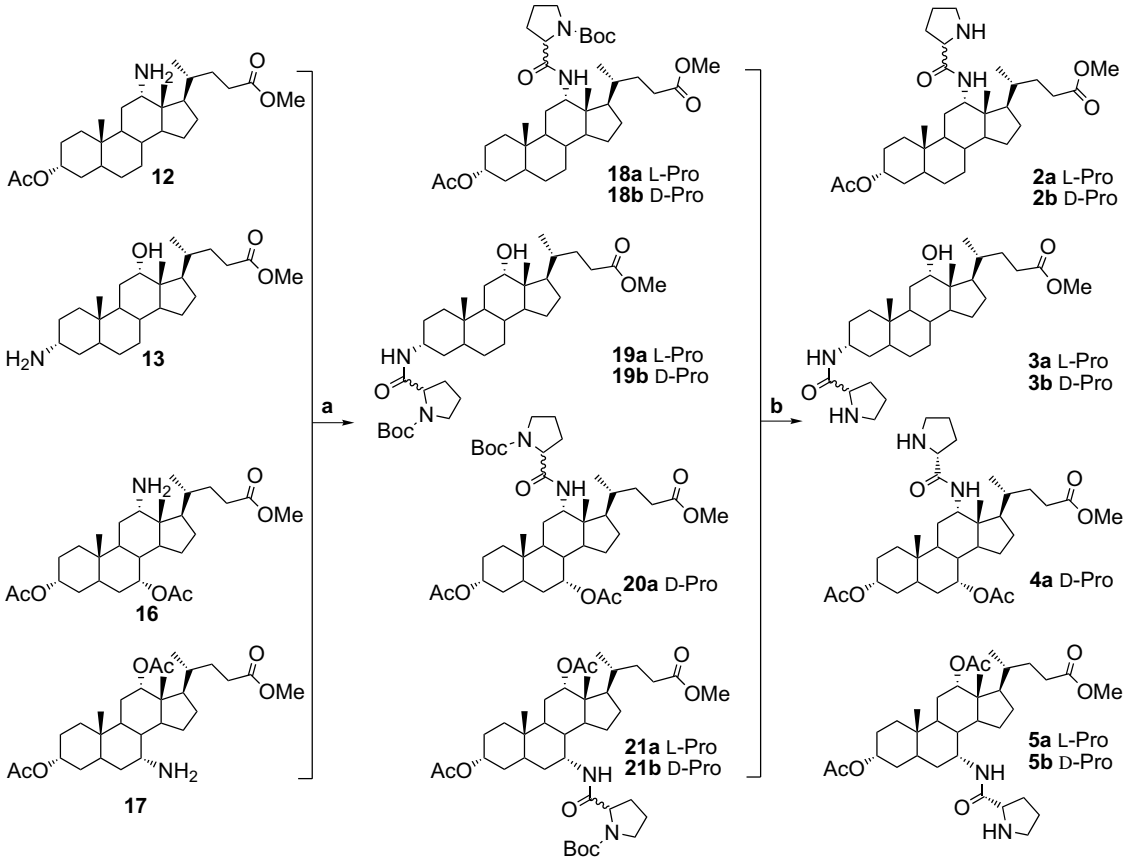
Under these experimental conditions at 0 °C, complete conversion of the aldehyde was reached in 48 h, with



Scheme 1. Synthesis of amine derivatives of cholic acid. Reagents and conditions: (a) AcOMe, TsOH, H_2O, reflux, 24 h; (b) MeI, DBU, CH_2Cl_2, rt, 12 h; (c) NBS, acetone/H_2O, rt, 30 h; (d) Ac_2O, Py, toluene, rt, 24 h; (e) Ac_2O, DMAP, Et_3N, THF, rt, 24 h; (f) $K_2Cr_2O_7$, H_2O, AcOH, rt, 20 h; (g) NH_2OH HCl, NaOAc, MeOH, reflux, 3 h; (h) $PtO_2{\cdot}xH_2O$, AcOH, H_2 (2 bar), rt, 6 d, then Zn powder, rt, 12 h.

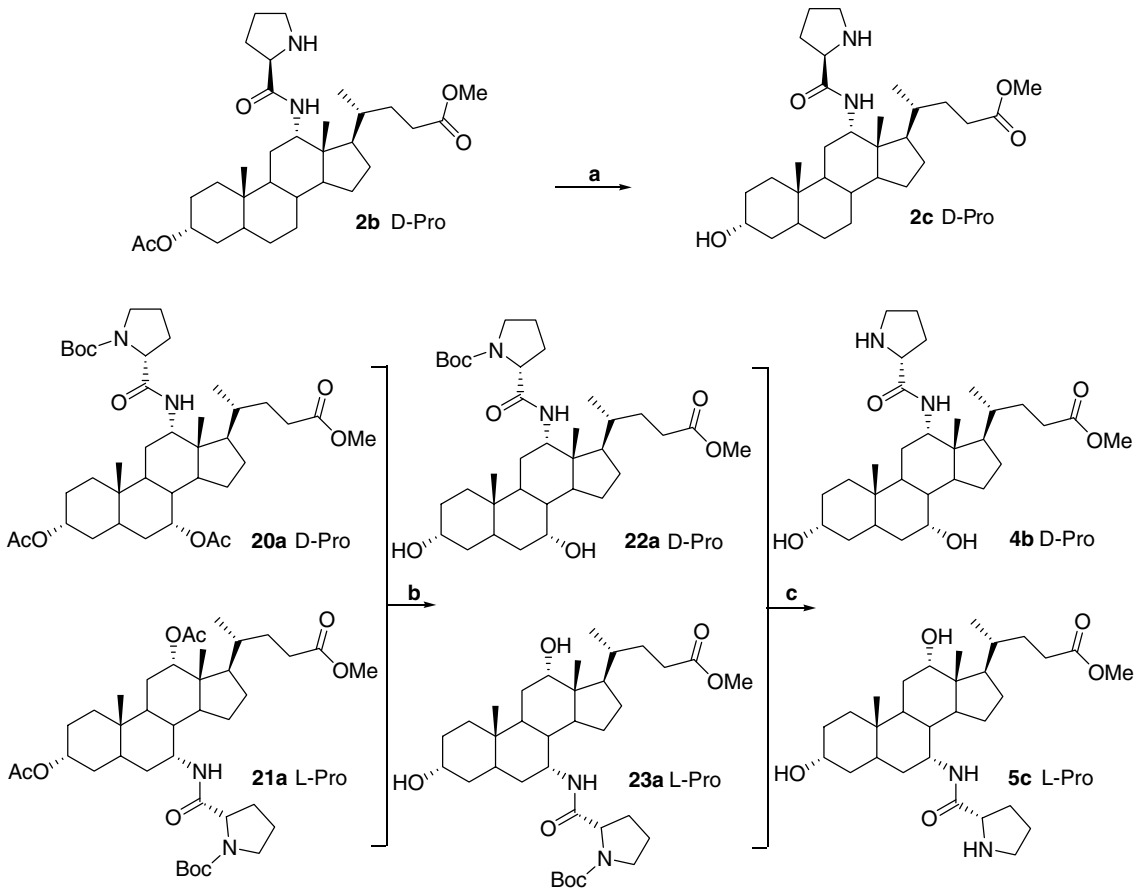
neither condensation products nor dehydration product being observed. In addition the aldol was obtained in 46% ee (entry 2). Further lowering of the reaction temperature, as well as increasing the catalyst loading, caused the enantioselectivity to decrease (entries 3 and 4). The use of a lower amount of catalyst did not improve the enantioselectivity, even if the activity of the catalyst remained unchanged, with complete conversion being reached in 48 h (entry 5). Changing the solvent gave, in general, inferior results both in terms of substrate conversion and enantioselectivity (entries 6–13). In fact, when using $CHCl_3$ or toluene, complete conversion of the substrate was observed, although 96 h were required in toluene solution and in both cases the ees were lower (entries 6 and 9). Even worse results were obtained when using THF or DMSO (entries 8 and 10), whereas a DMF–water mixture gave a slightly higher ee, unfortunately associated to lower substrate conversion (entry 11). Increasing the temperature in the presence of this solvent mixture gave complete conversion of the substrate, but lower enantioselectivity (entry 12). The use of trifluoroacetic acid as an additive did not afford positive results, either in DMF-H_2O solution (entry 14) where no reaction took place, or in acetone (entry 15), where a very low conversion of the substrate and 36% ee of the product were obtained.

On the basis of these results, for comparative purposes, the other organocatalysts were assayed under the experimental conditions which afforded the best results in terms of conversion and enantioselectivity, that is, the use of acetone as solvent at 0 °C with the pre-activation of the catalyst. The results are listed in Table 2.



Scheme 2. Synthesis of **2a**–**b**, **3a**–**b**, **4a** and **5a**–**b**. Reagents and conditions: (a) (L)- or (D)-Boc-Pro, isobutylchloroformate, NMM, CH_2Cl_2, 0 °C, 26 h; (b) CH_2Cl_2, TFA, rt, 15 min.

The activity of the different organocatalysts was comparable, with complete conversion of the substrate being reached in 48 h in all cases. Compound **2a**, which possesses an L-proline moiety linked at the 12-position of the deoxycholic acid, gave a remarkably lower enantioselectivity (entry 2), suggesting that a mismatched couple can be obtained when L-proline is linked at the 12-position. However the sense of asymmetric induction remained unchanged, with the (*S*)-enantiomer being obtained with both the diastereoisomers (entries 1 and 2): this result suggests that the sense of asymmetric induction depends mainly on the stereochemistry of the cholestanic backbone. A lower enantioselectivity is obtained when the proline moiety is linked at the 3-position of deoxycholic acid, as in **3a** and **3b** (entries 3 and 4), suggesting that, as observed with other kind of bile acid derivative,[9g] derivatization at the 3-position affords less enantioselective chiral auxiliaries. Again, the matched couple is constituted by the diastereoisomer bearing D-proline, although, in this case, the difference in the extent of asymmetric induction is smaller. The sense of asymmetric induction changes in passing from one to another diastereoisomer, suggesting that it depends on the aminoacid moiety. Very unusual results were obtained when using, **5a** and **5b** as organocatalysts, where the L- or D-proline moiety is linked at the 7-position of the cholic acid. Both diastereoisomers gave products with the same ee (entries 5 and 6) but opposite absolute configuration, suggesting a scarce influence of the cholestanic moiety on the asymmetric induction, which seems dependent only on the aminoacid moiety. The use of **2c**, the analogue of **2b** possessing a free OH group at the 3-position of the deoxycholic moiety, did not give better results than **2b** (entry 8), suggesting that a free OH group at this position does not help the enantioselectivity of the reaction, by controlling the position of the substrate at the inner of the cavity of the catalyst. This is likely due to the high distance between the moieties linked at positions 3 and 12 of the cholestanic backbone, which prevents interaction of the 3-OH group with a substrate that lies near the 12-position, where the active moiety of the catalyst is located. Even the use of **4a**, the analogue of **2b**, bearing a D-proline moiety linked to the 12-position of 3,7-diacetyl cholic acid, did not work better than **2b**, affording the aldol product in 42% ee. On the contrary, the use of **4b**, the analogue of **4a** bearing two free OH groups at positions 3 and 7, gave the best enantioselectivity (entry 10). The higher ee can be explained by taking into account that groups at positions 7 and 12 are closer than groups at positions 3 and 12,[15] therefore the 7-OH group can interact with a substrate reacting with the group linked at 12-position, by controlling its position inside the chiral cavity and then affording higher asymmetric induction. This result suggested that the behaviour of **4b** could be different from that one exhibited by the other organocatalysts and prompted



Scheme 3. Synthesis of organocatalysts **2c**, **4b**, **5c**. Reagents and conditions: (a) HCl concd, MeOH, rt, 24 h; (b) MeONa/MeOH 10%, rt, 24 h; (c) TFA, CH_2Cl_2, rt, 15 min.

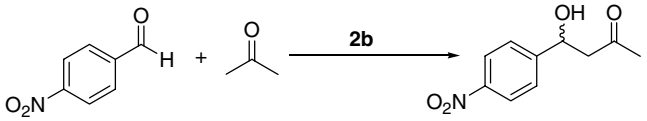
us to investigate the effect of various reaction parameters on activity and enantioselectivity of this system. The results are reported in Table 3.

Changing of solvent from acetone to THF or DMF did not improve the previous result. In fact when using THF as solvent, the activity dramatically dropped and the extent of asymmetric induction was lower (entry 2). On the contrary, the use of DMF did not change the enantioselectivity to a significant extent, but still worsened the activity of the catalyst (entry 3), although to a lesser extent. The use of THF/H_2O or DMF/H_2O mixtures afforded better results in terms of catalyst activity, to give complete substrate conversion in 24 h instead of 48 (entries 4 and 5). In addition, the presence of water avoided the formation of by-products coming from the acetone auto-condensation to give a cleaner reaction. Using the DMF/H_2O mixture as a reaction solvent allowed us to lower the catalyst loading to 5% without the loss of activity and with a slight improvement of asymmetric induction (entry 6). Further lowering of the catalyst loading to 2% did not give rise to loss of asymmetric induction and complete conversion of the substrate was still obtained, even if longer reaction time was required (entry 7). Performing the reaction in water greatly improved the catalyst activity, to afford complete substrate conversion in 15 h (entry 8): unfortunately the extent of asymmetric induction was lower. These results suggest the importance of the role of water in enhancing the catalyst activity even if the contemporary presence of an organic polar solvent is mandatory for the enantioselectivity.[16] Improvement of the asymmetric induction was obtained by lowering the temperature to −20 °C, without the loss of catalytic activity (entry 9). The best result in terms of asymmetric induction was reached by lowering the reaction temperature to further −40 °C (entry 10), but at this temperature the reaction was slower, affording complete substrate conversion in 48 h.

2. Conclusion

The synthesis of bile acid proline derivatives has allowed us to obtain a new class of organocatalysts, able to promote the direct asymmetric aldol reaction. Both activity and enantioselectivity depended not only on the absolute configuration of the proline moiety but also on the position, where it is linked on the cholestanic backbone. As already observed with other type of chiral auxiliaries derived from bile acids, the derivative bearing the catalyst moiety at the 12-position of the steroid skeleton was the most active and enantioselective. In addition, the presence of free OH groups at the 3- and 7-positions of cholic acid afforded the most efficient organocatalyst, able to promote the

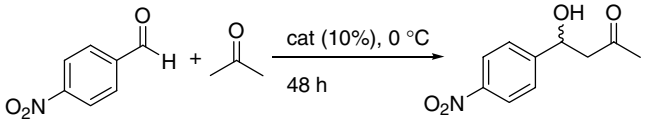
Table 1. Reaction of *p*-nitrobenzaldehyde with acetone in the presence of organocatalyst **2b**



Entry[a]	Solvent	*T* (°C)	Time (h)	ee[b] (%)	Conversion[c] (%)
1	Neat	rt	48	27	>99
2	Neat	0	48	46	>99
3	Neat	−20	48	34	>99
4[d]	Neat	0	48	37	>99
5[e]	Neat	0	48	44	>99
6[f]	$CHCl_3$	0	48	32	>99
7[f]	CH_3CN	0	48	38	50
8[f]	THF	0	48	17	73
9[f]	Toluene	0	96	29	>99
10[f]	DMSO	10	48	23	26
11[f]	H_2O/DMF 2:1	0	48	49	72
12[f]	H_2O/DMF 2:1	rt	48	36	>99
13[f]	DMF	0	48	37	36
14[f,g]	H_2O/DMF 1:1	0	48	n.d.	0
15[e,g]	H_2O/acetone 4:1	rt	120	14	26

[a] Reagents and conditions: *p*-nitrobenzaldehyde (0.5 mmol), acetone (0.5 mL), catalyst (10 mol %); reactions were monitored by TLC.
[b] Determined by HPLC on chiral stationary phase (Chiralpack AS); the absolute configuration of the prevailing enantiomer was (*S*).
[c] Determined by ^1H NMR.
[d] With 20% of catalyst.
[e] With 5% of catalyst.
[f] Solvent: 1 mL. Solvent/acetone ratio 4:1.
[g] With 10% of TFA as additive.

Table 2. Reaction of *p*-nitrobenzaldehyde with acetone in the presence of organocatalyst **2a–c**, **3a–b**, **4a–b**, **5a–c** at 0 °C

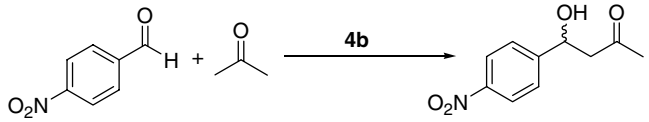


Entry[a]	Catalyst	ee[b] (%)	Absolute configuration[c]
1	**2b**	46	*S*
2	**2a**	12	*S*
3	**3a**	32	*R*
4	**3b**	38	*S*
5	**5a**	21	*R*
6	**5b**	21	*S*
7	**5c**	20	*R*
8	**2c**	41	*S*
9	**4a**	42	*S*
10	**4b**	64	*S*

[a] Reaction conditions: *p*-nitrobenzaldehyde (0.5 mmol), acetone (solvent), organocatalyst (10 mol %), 0 °C. All the reactions were stopped after 48 h at complete conversion.
[b] Determined by HPLC on chiral stationary phase (Chiralpack AS).
[c] Determined by comparison with the literature data.[4e]

asymmetric aldol reaction between acetone and 4-nitrobenzaldehyde with ee up to 80%. These results point out the importance of the presence of OH groups on the cholestanic backbone, able to control the position of the substrate, that allows the reaction to take place into the chiral cleft formed by the steroidal skeleton and the appended proline moiety, giving rise to higher levels of asymmetric induction. Finally, the possibility to obtain complete substrate conversion with a very low catalyst loading (2%) makes this system very appealing also for other reaction proceeding via enamine pathway: work is in progress on this topic.

Table 3. Reaction of *p*-nitrobenzaldehyde with acetone in the presence of organocatalyst **4b**



Entry[a]	Catalyst (mol %)	*T* (°C)	Time (h)	Solvent	Conversion[b] (%)	ee[c] (%)
1	10	0	48	Acetone	>99	64
2	10	0	48	THF	8	45
3	10	0	48	DMF	53	65
4	10	0	48	THF/H_2O[d]	>99	67
5	10	0	24	DMF/H_2O[d]	>99	64
6	5	0	24	DMF/H_2O[d]	>99	67
7	2	0	48	DMF/H_2O[d]	>99	65
8	5	0	15	H_2O	>99	27
9	5	−20	24	DMF/H_2O[d]	>99	75
10	5	−40	48	DMF/H_2O[d]	>99	80

[a] Reagents and conditions: *p*-nitrobenzaldehyde (0.5 mmol), acetone (0.5 mL); reactions were monitored by TLC.
[b] Determined by ^{1}H NMR.
[c] Determined by HPLC on chiral stationary phase (Chiralpack AS); the absolute configuration of the prevailing enantiomer was (*S*).
[d] Solvent/H_2O 2:1: the same results were obtained using solvent/H_2O 1:2.

3. Experimental

3.1. General procedures and materials

TLC analyses were performed on Silica Gel 60 sheets; flash chromatography separations were carried out on columns using Silica Gel 60 (230–400 mesh). Toluene was refluxed over sodium and distilled before the use. THF was refluxed over Na/K alloy and distilled before use. CH_2Cl_2, triethylamine, pyridine and *N*-methylmorpholine were refluxed over CaH_2 and distilled before use. MeOH was refluxed over magnesium methoxide and distilled before the use. Acetic anhydride was distilled before the use. NBS was recrystallized by boiling water before the use. Zn powder was washed with concentrated HCl, water, acetone and diethylether before the use. MeONa was prepared immediately before the use with dry MeOHa and metallic Na at 0 °C. Glacial acetic acid was used. Unless otherwise specified, the reagents were used without any purification. Methyl-3α-amino-12α-hydroxy-5β-cholan-24-oate **12**,[9b] methyl 3α-acetyloxy-12α-amino-5β-cholan-24-oate **13**,[9b] methyl 3α,7α-diacetyloxy-12α-keto-5β-cholan-24-oate **8**,[11g] methyl 3α,12α-diacetyloxy-7-keto-5β-cholan-24-oate **11**[11a] were obtained as previously described and matched the reported characteristics.

3.2. Instrumentation

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Varian Gemini-300 300 MHz NMR spectrometer, using TMS as external standard. The following abbreviations are used: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br = broad. HPLC analysis were performed on a JASCO PU-980 intelligent HPLC pump equipped with JASCO UV-975 detector. Optical rotations were measured with a JASCO DIP-360 digital polarimeter. Melting points were taken using a Kopfler Reichert-Jung apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer 1710 spectrophotometer.

3.3. General procedure for oxime synthesis

Trihydrated sodium acetate (5.5 equiv) and hydroxylamine hydrochloride (1.8 equiv) were added to a solution of ketone **8** or **11** (1.5 g, 1 equiv) in methanol (35 mL). The reaction mixture was stirred at reflux for 4.5 h. The solvent was removed under vacuum and the residue dissolved in CH_2Cl_2. The organic solution was washed with water then dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the crude product recrystallized by methanol to afford chemically pure oxime.

3.3.1. Methyl 3α,7α-diacetyloxy-12-oxime-5β-cholan-24-oate 14. Yield 1.60 g, 97%. Mp 94–96 °C. $[\alpha]_D^{22} = +130.0$ (*c* 1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.91 (s, 3H, CH_3), 0.92 (d, 3H, 21-CH_3), 1.00 (s, 3H, CH_3), 0.90–2.40 (m, 28H, steroidal CH and CH_2), 2.00 (s, 3H, 3-CH_3CO), 2.03 (s, 3H, 7-CH_3CO), 3.35 (br dd, 1H, NOH), 3.64 (s, 3H, CH_3OCO), 4.57 (m, 1H, 3-CH), 4.92 (br d, 1H, 7-CH). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.17, 19.17, 19.93, 21.63, 21.75, 22.31, 23.76, 26.78, 28.16, 30.73, 31.53, 34.67, 35.22, 35.63, 35.83, 36.03, 38.21, 40.92, 46.99, 49.75, 51.68, 53.68, 70.92, 73.99, 165.94 (C=NOH), 170.51 (acetate C=O), 170.85 (acetate C=O), 174.94 (24C=O). IR (KBr, cm^{-1}): 2949, 2868, 1734 (br C=O), 1714 (C=O), 1557, 1537, 1502, 1472, 1457, 1437, 1376, 1361, 1250, 1230, 1169, 1059, 1023, 963, 917.

3.3.2. Methyl 3α,12α-diacetyloxy-7-oxime-5β-cholan-24-oate 15. Yield 3.30 g, 80%. Mp 94–96 °C. $[\alpha]_D^{22} = +12.0$ (*c* 1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.75 (s, 3H, CH_3), 0.82 (d, 3H, 21-CH_3), 1.05 (s, 3H, CH_3), 0.90–2.40 (m, 28H, steroidal CH and CH_2), 2.00 (s, 3H, 3-CH_3CO), 2.12 (s, 3H, 12-CH_3CO), 3.10 (br dd, 1H, NOH), 3.66 (s, 3H, CH_3OCO), 4.68 (m, 1H, 3-CH), 5.10 (t, 1H, 12-CH). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.75, 17.86, 21.54, 21.60, 23.18, 24.79, 25.96, 26.48, 27.49, 27.61, 31.04, 31.21, 32.80, 34.10, 34.78, 35.16, 37.44,

42.10, 42.63, 44.59, 45.32, 47.03, 51.74, 73.33, 75.17, 160.59 (C=NOH), 170.70 (acetate C=O), 170.80 (acetate C=O), 174.81 (24C=O). IR (KBr, cm^{-1}): 2952, 2867, 1735 (br C=O), 1650, 1449, 1382, 1243, 1188, 1018, 957, 860, 732, 605.

3.4. General procedure for reduction of oxime

Hydrated PtO_2 (12.5 mol %) was added to a solution of oxime **14** or **15** (usually 3 g, 1 equiv) in glacial acetic acid (usually 3 mL) and the mixture was stirred under H_2 (2 bar) at room temperature for six days. The solid was filtered off and powdered Zn (8 equiv) added to the solution, and concentrated under vacuum to 2 mL. The mixture was stirred at room temperature for 12 h then the solid was filtered off and washed with acetic acid. After concentration under reduced pressure, water was added and the aqueous solution was made basic with KOH pellets. The organic product was extracted with ethyl acetate and the organic extracts were dried (Na_2SO_4). The solvent was evaporated under vacuum affording pure amine.

3.4.1. Methyl 3α,7α-diacetyloxy-12α-amino-5β-cholan-24-oate 16. Yield 1 g, 81%. Mp 57–58 °C. $[\alpha]_D^{22} = +35.0$ (*c* 1.00, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$, δ): 0.70 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.95 (d, 3H, 21-CH_3), 0.90–2.40 (m, 28H, steroidal CH and CH_2), 2.00 (s, 3H, 3-CH_3CO), 2.04 (s, 3H, 7-CH_3CO), 3.16 (br t, 1H, 12-CH), 3.64 (s, 3H, CH_3OCO), 4.56 (m, 1H, 3-CH), 4.86 (br d, 1H, 7-CH). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.75, 17.39, 21.63, 21.84, 22.75, 23.35, 26.96, 27.72, 28.21, 28.87, 31.09, 31.28, 31.50, 34.59, 34.78, 34.95, 35.34, 38.62, 41.20, 41.98, 46.46, 47.96, 51.67, 53.97, 71.14, 74.28, 170.63 (acetate C=O), 170.78 (acetate C=O), 174.70 (24C=O). IR (KBr, cm^{-1}): 2949, 2868, 1734 (br C=O), 1710 (C=O), 1683, 1653, 1542, 1507, 1472, 1457, 1436, 1376, 1366, 1250, 1235, 1169, 1063, 1023, 928.

3.4.2. Methyl 3α,12α-diacetyloxy-7α-amino-5β-cholan-24-oate 17. Yield 1.60 g, 55%. Mp 58–60 °C. $[\alpha]_D^{22} = +68.0$ (*c* 1.00, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$, δ): 0.72 (s, 3H, CH_3), 0.79 (d, 3H, 21-CH_3), 0.89 (s, 3H, CH_3), 0.90–2.40 (m, 28H, steroidal CH and CH_2), 2.00 (s, 3H, 3-CH_3CO), 2.08 (s, 3H, 12-CH_3CO), 3.12 (br s, 1H, $CHNH_2$), 3.64 (s, 3H, CH_3OCO), 4.54 (m, 1H, 3-CH), 5.07 (br s, 1H, 12-CH). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.35, 17.72, 21.34, 21.54, 22.08, 22.69, 23.03, 25.33, 26.61, 27.39, 27.53, 30.95, 31.18, 31.43, 34.61, 34.82, 34.88, 37.21, 40.56, 42.82, 45.48, 47.54, 48.83, 51.73, 73.84, 74.99, 170.83 (acetate C=O), 170.92 (acetate C=O), 174.70 (24C=O), 177.02. IR (KBr, cm^{-1}): 2948, 2870, 1734 (br C=O), 1654, 1559, 1475, 1447, 1374, 1251, 1026, 717, 611, 583.

3.5. General procedure for prolinamide synthesis

To a solution of *N*-Boc protected proline (1.1 equiv) in anhydrous CH_2Cl_2, *N*-methylmorpholine (1.22 equiv) was added and the mixture cooled to −20 °C, after which isobutylchloroformate (1.1 equiv) was added. The reaction temperature was maintained at −20 °C for 5 min, then a CH_2Cl_2 solution of amine (1 equiv) was added dropwise over 15 min at 0 °C. The reaction mixture was stirred for 26 h. Finally, the reaction mixture was treated with HCl aq, $NaHCO_3$ aq, NaCl aq then dried over anhydrous Na_2SO_4. The organic phases were concentrated in vacuo and the residue purified by column chromatography to give the pure product.

3.5.1. Methyl 3α-acetyloxy-12α-*N*-(L-Boc-prolinoyl)amino-5β-cholan-24-oate 18a. Purified by chromatography (SiO_2, CH_2Cl_2/acetone 92:8). Yield 350 mg, 50%. Mp 68–70 °C. $[\alpha]_D^{22} = +31.0$ (*c* 1.00, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$, δ): 0.78 (s, 3H, CH_3), 0.86 (d, 3H, 21-CH_3), 0.90 (s, 3H, CH_3), 1.00–2.05 (m, 28H, steroidal CH and CH_2, and 3′ and 4′-CH_2 of Boc-Pro), 1.51 (s, 9H, Boc-3CH_3), 1.99 (s, 3H, CH_3CO), 3.47 (m, 2H, 5′-CH_2 of Boc-Pro), 3.64 (s, 3H, CH_3OCO), 4.16 (br s, 1H, 2'-CH of Boc-Pro), 4.34 (br d, 1H, 12-CH), 4.69 (m, 1H, 3-CH), 7.41 (br s, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.88, 17.32, 21.58, 23.37, 23.92, 24.67, 25.92, 26.30, 26.59, 26.88, 27.57, 28.76 (3CH_3Boc), 30.97, 31.32, 32.44, 34.24, 34.93, 35.14, 36.02, 41.85, 44.75, 47.33, 48.49, 50.94, 51.63, 52.87, 60.52, 74.24, 77.44, 80.63, 156.45 (C=O Boc), 170.63 (acetate C=O), 171.00 (amide C=O), 174.92 (24C=O). IR (KBr, cm^{-1}): 2959, 2858, 1767 (C=O), 1745 (C=O), 1734 (C=O), 1699 (C=O), 1678, 1649, 1633, 1554, 1537, 1520, 1509, 1453, 1245, 1161, 1020.

3.5.2. Methyl 3α-acetyloxy-12α-*N*-(D-Boc-prolinoyl)amino-5β-cholan-24-oate 18b. Purified by chromatography (SiO_2, CH_2Cl_2/acetone 90:10). Yield 266 mg, 49%. Mp 61–62 °C. $[\alpha]_D^{22} = +176.0$ (*c* 1.00, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$, δ): 0.77 (s, 3H, CH_3), 0.79 (d, 3H, 21-CH_3), 0.90 (s, 3H, CH_3), 0.95–2.60 (m, 28H, steroidal CH and CH_2, and 3′ and 4′-CH_2 of Boc-Pro), 1.52 (s, 9H, Boc-3CH_3), 2.01 (s, 3H, CH_3CO), 3.34 (m, 2H, 5′-CH_2 of Boc-Pro), 3.65 (s, 3H, CH_3OCO), 4.20 (br d, 1H, 2′-CH of Boc-Pro), 4.37 (br d, 1H, 12-CH), 4.67 (m, 1H, 3-CH), 7.57 (br s, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.77, 17.46, 21.75, 23.57, 23.96, 24.89, 26.22, 26.65, 26.92, 27.24, 27.73, 28.73 (3CH_3 Boc), 31.21, 31.31, 32.49, 34.55, 35.07, 36.25, 42.20, 44.86, 47.51, 48.80, 50.60, 51.72, 52.23, 60.59, 74.77, 74.24, 77.44, 80.65, 170.86 (acetate C=O), 171.05 (amide C=O), 174.82 (24C=O) 181.09 (C=O Boc). IR (KBr, cm^{-1}): 2950, 2862, 1738 (br C=O), 1694 (C=O), 1679, 1541, 1526, 1457, 1438, 1398, 1364, 1241, 1167, 1118, 1088, 1024, 980, 886.

3.5.3. Methyl 12α-hydroxy-3α-*N*-(L-Boc-prolinoyl)amino-5β-cholan-24-oate 19a. Purified by chromatography (SiO_2, CH_2Cl_2/acetone 87:13). Yield 180 mg, 20%. Mp 63–65 °C. $[\alpha]_D^{22} = +65.0$ (*c* 1.00, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$, δ): 0.69 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 0.98 (d, 3H, 21-CH_3), 1.00–2.00 (m, 28H, steroidal CH and CH_2, and 3′ and 4′-CH_2 of Boc-Pro), 1.51 (s, 9H, Boc-3CH_3), 1.99 (s, 3H, CH_3CO), 2.20 (m, 2H, 5′-CH_2 of Boc-Pro), 3.67 (s, 3H, CH_3OCO), 3.76 (m, 1H, 3-CH), 4.00 (br d, 1H, 12-CH), 4.18 (br s, 1H, 2′-CH of Boc-Pro). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.90, 17.60, 23.50, 23.80, 26.30, 27.10, 27.60, 28.00, 28.60, 28.90, (3CH_3Boc), 31.10, 31.30, 33.90, 34.30, 35.30, 36.00, 36.20, 42.60, 46.70, 47.50, 48.60, 49.40, 51.70, 73.40, 80.60,

128.70 (C=O Boc), 174.80 (2C methylester C=O, amide C=O). IR (KBr, cm^{-1}): 2956, 2862, 1774 (br C=O), 1730, 1656, 1570, 1566, 1510, 1456, 1450, 1420, 1270, 1190, 1170, 1087, 1069, 1038, 890.

3.5.4. Methyl 12α-hydroxy-3α-*N*-(D-Boc-prolinoyl)amino-5β-cholan-24-oate 19b. Purified by chromatography (SiO_2, CH_2Cl_2/acetone 87:13). Yield 420 mg, 42%. Mp 69–71 °C. $[\alpha]_D^{22} = +102.0$ (*c* 1.00, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$, δ): 0.67 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.96 (d, 3H, 21-CH_3), 1.00–2.00 (m, 28H, steroidal CH and CH_2, and 3′ and 4′-CH_2 of Boc-Pro), 1.52 (s, 9H, Boc-3CH_3), 2.01 (s, 3H, CH_3CO), 2.20 (m, 2H, 5′-CH_2 of Boc-Pro), 3.65 (s, 3H, CH_3OCO), 3.75 (m, 1H, 3-CH), 3.97 (br d, 1H, 12-CH), 4.18 (br d, 1H, 2′-CH of Boc-Pro). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.90, 17.60, 23.50, 23.80, 26.30, 27.20, 27.60, 28.10, 28.60, 28.90 (3CH_3Boc), 31.10, 31.30, 34.00, 34.30, 35.30, 35.90, 36.20, 42.60, 46.80, 47.50, 48.60, 49.30, 51.70, 61.30, 73.40, 80.60, 171.70 (Boc C=O), 174.80 (methylester C=O, amide C=O). IR (KBr, cm^{-1}): 2950, 2870, 1753 (br C=O), 1718, 1682, 1655, 1556, 1598, 1507, 1470, 1437, 1371, 1277, 1190, 1169, 1092, 1050, 1025, 898.

3.5.5. Methyl 3α,7α-diacetyloxy-12α-*N*-(D-Boc-prolinoyl)-amino-5β-cholan-24-oate 20a. Purified by flash chromatography (SiO_2, CH_2Cl_2/acetone 95:5). Yield 236 mg, 33%. Mp 63–64 °C $[\alpha]_D^{22} = +113.0$ (*c* 1.00, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$, δ): 0.78 (s, 3H, CH_3), 0.79 (d, 3H, 21-CH_3), 0.95 (s, 3H, CH_3), 0.95–2.60 (m, 28H, steroidal CH and CH_2, and 3′ and 4′-CH_2 of Boc-Pro), 1.52 (s, 9H, Boc-3CH_3), 2.06 (s, 3H, 3-CH_3CO), 2.19 (s, 3H, 7-CH_3CO), 3.36 (m, 2H, 5′-CH_2 of Boc-Pro), 3.68 (s, 3H, CH_3OCO), 4.28 (br d, 1H, 2′-CH of Boc-Pro), 4.47 (br d, 1H, 12-CH), 4.57 (m, 1H, 3-CH), 4.94 (br d, 1H, 7-CH) 8.04 (br s, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.33, 17.23, 21.56, 21.62, 22.88, 23.24, 24.86, 26.71, 27.10, 27.42, 28.47 (3CH_3 Boc), 28.85, 30.77, 30.91, 31.51, 34.49, 34.75, 35.01, 38.32, 41.13, 43.78, 44.65, 47.42, 48.25, 51.57, 51.67, 59.83, 70.75, 74.32, 80.59, 156.38 (C=O Boc), 170.53 (acetate C=O), 170.63 (acetate C=O), 170.78 (amide C=O), 174.57 (24C=O). IR (KBr, cm^{-1}): 2961, 1738, 1703, 1698, 1682, 1537, 1457, 1400, 1365, 1260, 1167, 1118, 1024, 935, 802.

3.5.6. Methyl 3α,12α-diacetyloxy-7α-(L-Boc-prolinoyl)-amino-5β-cholan-24-oate 21a. Purified by flash chromatography (SiO_2, CH_2Cl_2/acetone 90:10). Yield 260 mg, 50%. Mp 160–161 °C. $[\alpha]_D^{22} = +23.0$ (*c* 1.00, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$, δ): 0.72 (s, 3H, CH_3), 0.79 (d, 3H, 21-CH_3), 0.93 (s, 3H, CH_3), 0.90-2.40 (m, 28H, steroidal CH and CH_2 and 3′ and 4′-CH_2 of Boc-Pro), 1.49 (s, 9H, Boc-3CH_3), 2.02 (s, 3H, 3-CH_3CO), 2.15 (s, 3H, 12-CH_3CO), 3.39 (br s, 1H, 7-CH), 3.64 (s, 3H, CH_3OCO), 4.27 (br s, 1H, 2′-CH of Boc-Pro), 4.52 (m, 1H, 3-CH), 5.11 (br s, 1H, 12-CH) 6.93 (br s, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.51, 17.77, 21.62, 21.70, 23.05, 25.76, 27.29, 27.41, 28.73 (3CH_3Boc), 29.18, 29.49, 31.00, 31.39, 34.85, 34.91, 35.01, 36.79, 41.49, 44.17, 45.30, 47.51, 47.93, 51.73, 74.22, 75.51, 170.51 (acetate C=O), 170.80 (acetate C=O), 171.05 (amide C=O), 174.67 (24C=O), 181.09 (C=O Boc). IR (KBr, cm^{-1}): 2948, 2353, 1733 (br C=O), 1677, 1654, 1560, 1520, 1509, 1452, 1408, 1357, 1250, 1167, 1121, 1021, 802, 611.

3.5.7. Methyl 3α,12α-diacetyloxy-7α-(D-Boc-prolinoyl)-amino-5β-cholan-24-oate 21b. Purified by flash chromatography (SiO_2, CH_2Cl_2/acetone 90:10). Yield 170 mg, 32%. Mp 67–68 °C. $[\alpha]_D^{22} = +103.0$ (*c* 1.00, CH_2Cl_2). ^1H NMR (200 MHz, $CDCl_3$, δ): 0.76 (s, 3H, CH_3), 0.81 (d, 3H, 21-CH_3), 0.96 (s, 3H, CH_3), 0.90–2.40 (m, 28H, steroidal CH and CH_2 and 3′ and 4′-CH_2 of Boc-Pro), 1.54 (s, 9H, Boc-3CH_3), 2.03 (s, 3H, 3-CH_3CO), 2.18 (s, 3H, 12-CH_3CO), 3.32 (br s, 1H, 7-CH), 3.67 (s, 3H, CH_3OCO), 3.94 (br s, 1H, 2'-CH of Boc-Pro), 4.36 (br s, 1H, 12-CH) 4.54 (m, 1H, 3-CH), 6.93 ppm (br s, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.39, 17.61, 21.58, 22.74, 22.99, 25.79, 26.98, 27.24, 28.58, 29.30, 30.81, 31.02, 34.63, 34.79, 36.63, 41.35, 43.90, 45.32, 46.60, 47.35, 47.61, 51.61, 74.20, 75.40, 80.42, 170.37, 170.70, 171.18, 174.60, 198.11 ppm. IR (KBr, cm^{-1}): 2960, 2871, 1738 (br C=O), 1703 (C=O), 1679, 1654, 1526, 1506, 1457, 1437, 1398, 1368, 1245, 1162, 1118, 1083, 1022, 960, 887.

3.6. General procedure for deacetylation

The Boc protected derivative of the bis-acetylated cholic acid (1 equiv) was treated with MeONa solution 10% in MeOH (2.2 equiv) for 6 h at rt. The reaction was quenched in HCl aq, extracted with CH_2Cl_2 and dried over Na_2SO_4. The organic phases were concentrated in vacuo and the crude product was purified by column chromatography.

3.6.1. Methyl 3α,7α-dihydroxy-12α-*N*-(D-Boc-prolinoyl)-amino-5β-cholan-24-oate 22a. Purified by chromatography (SiO_2, CH_2Cl_2/acetone 1:1). Yield 210 mg, 46%. Mp 97–98 °C. $[\alpha]_D^{22} = +97.4$ (*c* 1.00, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$, δ): 0.77 (s, 3H, CH_3), 0.83 (d, 3H, 21-CH_3), 0.84 (s, 3H, CH_3), 0.90–2.40 (m, 28H, steroidal CH and CH_2, and 3′ and 4′-CH_2 of Boc-Pro), 1.45 (s, 9H, Boc-3CH_3), 3.37 (m, 2H, 5′-CH_2 of Pro), 3.44 (m, 1H, 3-CH) 3.63 (s, 3H, CH_3OCO), 3.83 (br s, 1H, 12-CH), 4.27 (br d, 1H, 7-CH), 7.10 (br d, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.69, 17.58, 19.53, 22.66, 23.43, 24.72, 26.07, 27.68, 27.87, 28.76, 30.55, 31.09, 31.15, 34.67, 35.03, 35.56, 39.66, 39.87, 41.58, 44.52, 44.64, 47.76, 48.73, 51.67, 52.19, 61.14, 68.20, 71.93, 80.97, 171.03 (Boc C=O), 174.74 (24C=O). IR (KBr, cm^{-1}): 2954, 2870, 1772 (br C=O), 1762, 1650, 1569, 1457, 1366, 1258, 1166, 1118, 1078, 1020, 982.

3.6.2. Methyl 3α,12α-dihydroxy-7α-*N*-(L-Boc-prolinoyl)-amino-5β-cholan-24-oate 23a. Yield 180 mg, 93%. Mp 104–108 °C. $[\alpha]_D^{22} = -3.6$ (*c* 1.00, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$, δ): 0.66 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.96 (d, 3H, 21-CH_3), 0.90–2.40 (m, 28H, steroidal CH and CH_2, and 3′ and 4′-CH_2 of Boc-Pro), 1.46 (s, 9H, Boc-3CH_3), 3.37 (m, 2H, 5′-CH_2 of Pro), 3.49 (m, 1H, 3-CH) 3.65 (s, 3H, CH_3OCO), 3.92 (br s, 1H, 12-CH), 3.92 (br s, 1H, 2′-CH of Pro), 4.18 (br d, 1H, 7-CH), 7.18 (br d, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.55, 17.27, 22.51, 23.21, 27.42, 27.68, 28.17, 28.55, 30.01, 30.86, 31.28, 32.30, 34.76, 35.40, 36.80, 39.86, 41.64, 42.24, 46.08, 46.65, 46.99, 47.17, 51.48, 59.99,

71.76, 73.06, 80.77, 171.17 (Boc C=O), 174.73 (24C=O). IR (KBr, cm^{-1}): 2963, 2880, 1780 (br C=O), 1702, 1684, 1539, 1401, 1370, 1260, 1162, 1118, 1087, 1048, 920.

3.7. General procedure for Boc-deprotection

A solution of amide in CH_2Cl_2 (5 mL) was treated with a large excess of TFA (2 mL) and stirred at rt for 15 h. The resulting mixture was washed with $NaHCO_3$5% and extracted with CH_2Cl_2 (3×30 mL). The organic phase was then washed with brine (3×50 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum affording chemical pure product.

3.7.1. Methyl 3α-acetyloxy-12α-*N*-(L-prolinoyl)amino-5β-cholan-24-oate 2a. Yield 250 mg, 94%. Mp 55–57 °C. $[\alpha]_D^{22} = +73.0$ (c 1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.79 (s, 3H, CH_3), 0.82 (d, 3H, 21-CH_3), 0.89 (s, 3H, CH_3), 0.90–2.40 (m, 28H, steroidal CH and CH_2, and 3′ and 4′-CH_2 of Pro), 1.98 (s, 3H, CH_3CO), 3.05 (m, 2H, 5′-CH_2 of Pro), 3.64 (s, 3H, CH_3OCO), 3.82 (br m, 1H, 2′-CH of Pro), 4.16 (br d, 1H, 12-CH), 4.68 (m, 1H, 3-CH), 8.18 (br d, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.85, 17.52, 21.58, 23.31, 23.99, 26.32, 26.42, 26.60, 26.92, 27.67, 31.10, 31.26, 32.35, 34.27, 34.93, 35.11, 36.05, 41.86, 44.82, 47.60, 48.97, 51.07, 51.69, 51.83, 60.85, 74.23, 170.66 (acetate C=O), 173.09 (amide C=O), 174.84 (24C=O). IR (KBr, cm^{-1}): 2940, 2867, 1733 (br C=O), 1655, 1517, 1450, 1383, 1361, 1244, 1194, 1166, 1100, 1022, 978, 805.

3.7.2. Methyl 3α-acetyloxy-12α-*N*-(D-prolinoyl)amino-5β-cholan-24-oate 2b. Yield 174 mg, quantitative. Mp 75-77 °C. $[\alpha]_D^{22} = +171.0$ (c 1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.79 (s, 3H, CH_3), 0.81 (d, 3H, 21-CH_3), 0.90 (s, 3H, CH_3), 0.90–2.40 (m, 28H, steroidal CH and CH_2, and 3′ and 4′-CH_2 of Pro), 2.02 (s, 3H, CH_3CO), 3.09 (m, 2H, 5′-CH_2 of Pro), 3.65 (s, 3H, CH_3OCO), 3.95 (br s, 1H, 2′-CH of Pro), 4.17 (br d, 1H, 12-CH), 4.67 (m, 1H, 3-CH), 8.14 (br d, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.87, 17.44, 21.74, 23.53, 23.99, 26.17, 26.30, 26.74, 26.91, 27.06, 27.77, 31.08, 31.16, 32.51, 34.34, 34.91, 35.12, 36.17, 42.04, 44.77, 47.68, 48.72, 50.98, 51.80, 52.11, 60.86, 74.52, 170.78 (acetate C=O), 172.80 (amide C=O), 174.98 (24C=O). IR (KBr, cm^{-1}): 2941, 2872, 1737 (br C=O), 1664, 1649, 1556, 1511, 1447, 1383, 1359, 1256, 1228, 1167, 1093, 1025, 975, 758.

3.7.3. Methyl 12α-acetyloxy-3α-*N*-(L-prolinoyl)amino-5β-cholan-24-oate 3a. Yield 150 mg, quant. Mp 68–72 °C. $[\alpha]_D^{22} = +36.0$ (c 1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0,67 (s, 3H, CH_3), 0,91 (s, 3H, CH_3), 0,96 (d, 3H, 21-CH_3), 1.00–2.00 (m, 28H, steroidal CH and CH_2, and 3′ and 4′-CH_2 of Pro), 1.98 (s, 3H, CH_3CO), 2.90 (m, 2H, 5′-CH_2 of Pro), 3.66 (s, 3H, CH_3OCO), 3.75 (m, 1H, 3-CH), 3.98 (br d, 1H, 12-CH). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.90, 17.60, 23.50, 23.80, 26.30, 27.20, 27.60, 28.00, 29.00, 30.80, 31.00, 33.50, 34.00, 34.30 35.30, 36.00, 36.20, 42.60, 46.70, 47.40, 47.60, 48.60, 48.90, 51.60 (OCH_3), 60.70 (C3), 73.40 (C12), 173.70 (amide C=O), 174.90 (24C=O). IR (KBr, cm^{-1}): 2939, 2858, 1734 (br C=O), 1713, 1678, 1648, 1633, 1628, 1537, 1522, 1507, 1456, 1431, 1376, 1260, 1189, 1169, 1099, 1069, 1038, 802.

3.7.4. Methyl 12α-hydroxy-3α-*N*-(D-prolinoyl)amino-5β-cholan- 24-oate 3b. Yield 360 mg, quantitative. Mp 80–85 °C. $[\alpha]_D^{22} = +54.0$ (c 1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.64 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.94 (d, 3H, 21-CH_3), 1.00–2.00 (m, 28H, steroidal CH and CH_2, and 3′ and 4′-CH_2 of Pro), 2.02 (s, 3H, CH_3CO), 2.95 (m, 2H, 5′-CH_2 of Pro), 3.67 (s, 3H, CH_3O-CO), 3.73 (m, 1H, 3-CH), 3.96 (br d, 1H, 12-CH). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.90, 17.50, 23.50, 23.80, 26.20, 27.20, 27.60, 28.00, 29.00, 30.80, 31.00, 33.50, 34.00, 34.20, 35.20, 35.90, 36.10, 42.60, 46.70, 47.40, 48.70, 48.90, 51.60 (OCH_3), 60.70 (C3), 73.40 (C12), 173.60 (amide C=O), 174.80 (24C=O). IR (KBr, cm^{-1}): 2939, 2858, 1734 (br C=O), 1718, 1678, 1648, 1638, 1632, 1542, 1522, 1507, 1457, 1437, 1371, 1260, 1189, 1169, 1099, 1069, 1038, 802.

3.7.5. Methyl 3α,7α-diacetyloxy-12α-(D-prolinoyl)amino-5β-cholan-24-oate 4a. Yield 30 mg, quantitative. Mp 70–71 °C. $[\alpha]_D^{22} = +69.5$ (c 1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.77 (s, 3H, CH_3), 0.82 (d, 3H, 21-CH_3), 0.90 (s, 3H, CH_3), 0.90–2.40 (m, 28H, steroidal CH and CH_2, and 3′ and 4′-CH_2 of Pro), 2.01 (s, 3H, 3-CH_3CO), 2.05 (s, 3H, 7-CH_3CO), 2.99 (m, 2H, 5′-CH_2 of Pro), 3.62 (s, 3H, CH_3OCO), 3.78 (dd, 1H, 2′-CH of Pro), 4.16 (br d, 1H, 12-CH), 4.52 (m, 1H, 3-CH), 4.88 (d, 1H, 7-CH), 8.19 (br d, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.44, 17.45, 21.65, 22.85, 23.36, 26.37, 26.63, 27.08, 27.53, 28.86, 30.76, 31.06, 31.38, 34.57, 34.77, 35.02, 35.26, 38.17, 41.04, 44.33, 44.66, 47.71, 48.71, 51.06, 51.64, 60.98, 70.95, 74.30, 170.22 (acetate C=O), 170.61 (acetate C=O), 173.79 (amide C=O), 174.64 (24C=O). IR (KBr, cm^{-1}): 2954, 2875, 1740 (br C=O), 1662, 1652, 1516, 1438, 1365, 1249, 1172, 1099, 1066, 1024, 968, 892.

3.7.6. Methyl 3α,7α-dihydroxy-12α-(D-prolinoyl)amino-5β-cholan-24-oate 4b. Yield 100 mg, quant. Mp 98–100 °C. $[\alpha]_D^{22} = +79.9$ (c 1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.81 (s, 3H, CH_3), 0.84 (d, 3H, 21-CH_3), 0.89 (s, 3H, CH_3), 0.90–2.40 (m, 28H, steroidal CH and CH_2, and 3′ and 4′-CH_2 of Pro), 2.98 (m, 2H, 5′-CH_2 of Pro), 3.45 (m, 1H, 3-CH) 3.66 (s, 3H, CH_3OCO), 3.78 (dd, 1H, 2′-CH of Pro), 3.87 (br s, 1H, 12-CH), 4.19 (br d, 1H, 7-CH), 8.17 (br d, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.68, 17.55, 22.98, 23.55, 26.56, 27.76, 28.04, 30.89, 31.26, 34.85, 35.24, 39.84, 41.64, 44.73, 47.71, 48.87, 51.69, 61.04, 68.22, 72.11, 173.91 (Boc C=O), 174.77 (24C=O). IR (KBr, cm^{-1}): 2957, 2925, 2869, 1739 (br C=O), 1648, 1523, 1448, 1382, 1261, 1198, 1170, 1078, 992, 801.

3.7.7. Methyl 3α,12α-diacetyloxy-7α-(L-prolinoyl)amino-5β-cholan-24-oate 5a. Yield 120 mg, 74%. Mp 78–79 °C. $[\alpha]_D^{22} = +84.0$ (c 1.00, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.73 (s, 3H, CH_3), 0.80 (d, 3H, 21-CH_3), 0.94 (s, 3H, CH_3), 0.90–2.40 (m, 28H, steroidal CH and CH_2 and 3′ and 4′-CH_2 of Pro), 2.03 (s, 3H, 3-CH_3CO), 2.13

(s, 3H, 12-CH_3CO), 3.08 (m, 2H, 5′-CH_2 of Pro) 3.60 (br s, 1H, 7-CH), 3.65 (s, 3H, CH_3OCO), 3.96 (br s, 1H, 2′-CH of Pro), 4.53 (m, 1H, 3-CH), 5.10 (br s, 1H, 12-CH) 7.95 (br s, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.48, 17.79, 21.62, 21.70, 23.00, 23.17, 25.70, 26.00, 26.08, 27.20, 27.35, 29.18, 31.05, 31.28, 32.03, 34.85, 34.88, 35.01, 35.74, 36.86, 41.46, 44.41, 45.17, 45.97, 47.51, 47.86, 51.74, 60.61, 74.28, 75.51, 76.47, 170.40 (acetate C=O), 170.50 (acetate C=O), 171.08 (amide C=O), 174.67 (24C=O). IR (KBr, cm^{-1}): 2925, 2861, 1739, 1734, 1730, 1718, 1700, 1696, 1684, 1675, 1669, 1663, 1653, 1647, 1636, 1559, 1534, 1517, 1499, 1457, 1437, 1395, 1375, 1026, 804.

3.7.8. Methyl 3α,12α-diacetyloxy-7α-(D-prolinoyl)amino-5β-cholan-24-oate 5b. Yield 121 mg, quantitative. Mp 80–82 °C. $[\alpha]_D^{22} = +131.0$ (c 1.00, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$, δ): 0.76 (s, 3H, CH_3), 0.83 (d, 3H, 21-CH_3), 0.96 (s, 3H, CH_3), 0.90–2.40 (m, 28H, steroidal CH and CH_2 and 3′ and 4′-CH_2 of Pro), 2.02 (s, 3H, 3-CH_3CO), 2.15 (s, 3H, 12-CH_3CO), 3.08 (m, 2H, 5′-CH_2 of Pro), 3.67 (s, 3H, CH_3OCO), 3.95 (br s, 1H, 7-CH) 3.96 (br s, 1H, 2′-CH of -Pro), 4.57 (m, 1H, 3-CH), 5.13 (br s, 1H, 12-CH) 8.12 (br s, NH amide) ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.34, 17.65, 21.44, 22.81, 25.52, 25.95, 26.94, 27.25, 29.01, 30.89, 31.07, 31.92, 34.65, 34.79, 35.42, 36.74, 41.26, 44.20, 45.08, 45.92, 47.24, 47.62, 51.62, 60.49, 74.19, 75.35, 170.43 (acetate C=O), 170.48, 188.63 (amide C=O), 174.62 (24C=O). IR (KBr, cm^{-1}): 2964, 2923, 2856, 1743, 1722, 1650, 1637, 1623, 1565, 1544, 1525, 1511, 1479, 1463, 1442, 1380, 1263, 1091, 883, 802.

3.7.9. Methyl 3α,12α-dihydroxy-7α-(D-prolinoyl)amino-5β-cholan-24-oate 5c. Yield 100 mg, quantitative. Mp 128–130 °C. $[\alpha]_D^{22} = +3.7$ (c 1.00, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$, δ): 0.67 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 0.96 (d, 3H, 21-CH_3), 0.90–2.40 (m, 28H, steroidal CH and CH_2 and 3′ and 4′-CH_2 of Pro), 3.28 (m, 2H, 5′-CH_2 of Pro), 3.64 (s, 3H, CH_3OCO), 3.92 (br s, 1H, 2′-CH of -Pro), 3.95 (br s, 1H, 7-CH) 4.89 (m, 1H, 3-CH), 5.45 (br d, 1H, 12-CH) 8.89 (br s, NH amide) ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.28, 17.40, 22.70, 23.56, 26.18, 27.91, 28.12, 28.23, 29.92, 31.02, 31.26, 31.44, 31.72, 32.56, 35.47, 35.96, 36.33, 37.11, 41.42, 42.25, 42.45, 46.89, 47.71, 48.06, 48.67, 51.68, 60.01, 72.54, 73.51, 166.90 (amide C=O), 174.81 (24C=O). IR (KBr, cm^{-1}): 2960, 2923, 2864, 1740, 1727, 1698, 1661, 1631, 1562, 1556, 1497, 1463, 1454, 1381, 1260, 1196, 1161, 1098, 1051, 1023, 804.

3.7.10. Methyl 3α-hydroxy-12α-*N*-(D-prolinoyl)amino-5β-cholan- 24-oate 2c. A solution of **2b** (174 mg, 0.32 mmol) in MeOH (8 mL) was treated with a large excess of concentrated HCl (0.2 mL) and stirred at rt for 24 h. The resulting mixture was washed with $NaHCO_3$5% and extracted with CH_2Cl_2 (3 × 30 mL). The organic phase was then washed with brine (3 × 50 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum to afford chemically pure product (yield 160 mg, quantitative). Mp 79–80 °C. $[\alpha]_D^{22} = +69.6$ (c 1.00, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$, δ): 0.81 (s, 3H, CH_3), 0.85 (d, 3H, 21-CH_3), 0.91 (s, 3H, CH_3), 0.90–2.40 (m, 28H, steroidal CH and CH_2, and 3′ and 4′-CH_2 of Pro), 3.04 (m, 2H, 5′-CH_2 of Pro), 3.62 (m, 1H, 3-CH), 3.67 (s, 3H, CH_3O-CO), 3.84 (br s, 1H, 2′-CH of Pro), 4.18 (br d, 1H, 12-CH), 8.21 (br d, NH amide). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 13.74, 17.39, 23.36, 23.88, 26.16, 26.31, 26.64, 27.08, 27.66, 29.78, 30.57, 30.77, 31.08, 31.17, 34.16, 34.93, 35.12, 36.07, 36.29, 42.07, 44.69, 47.58, 48.79, 50.97, 51.61, 51.76, 60.88, 71.73, 173.28 (24C=O), 174.78 (amide C=O). IR (KBr, cm^{-1}) 2923, 2862, 1741, 1654, 1647, 1560, 1522, 1449, 1583, 1310, 1261, 1167, 1097, 1034, 802.

3.8. General procedure for aldol reaction

The organocatalyst (10 mol %) was stirred in 0.5 mL of acetone for 1 h. *p*-Nitrobenzaldehyde (75.56 mg, 0.5 mmol) was added in 0.5 mL of acetone and the mixture was stirred at the desired temperature for 4 h. This solution was quenched in aqueous NH_4Cl (1 mL) and extracted with AcOEt (1 mL), then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified over a small column of silica gel (AcOEt/hexane 1:1) and after concentration, analyzed with HPLC (chiralcel AS, 1.0 mL/min flux, hexane/IPA 85:15) and ^1H NMR.

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