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Synthesis and diastereoselective catalytic hydrogenation of optically active cyclobutyl α,β-dehydro-α-dipeptides

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Abstract—Optically active cyclobutyl (*Z*)-α,β-dehydro-α-dipeptides have been efficiently synthesized through the coupling of conveniently protected glycine or (*S*)-phenylalanine residues with a (*Z*)-dehydro-α-amino acid derivative prepared, in turn, from (−)-verbenone as a chiral precursor. The alternative use of (*R*,*R*)- and (*S*,*S*)-Et–duphos–Rh as the hydrogenation catalyst led to the stereoselective production of both diastereomeric saturated dipeptides in each case. Thus, the chirality of the catalyst employed has been shown to be the factor governing the configuration of the newly created stereogenic centre. Regarding structural features, both NMR and CD data establish a marked conformational bias for both the unsaturated and the saturated peptides synthesized herein.

1. Introduction

Small peptides containing α,β-unsaturated amino acid residues (dehydropeptides, DHPs) have been found in the structure of natural products with a variety of biological activities.[1–4] Chemical and structural studies of unsaturated peptides show interesting features for these compounds that could be responsible for the biological properties of some of them.[5] For instance, the binding abilities of peptide ligands towards metal ions is highly increased when a DHAA residue is incorporated into a di-, tri- or tetrapeptide.[6] Otherwise, the ability of α,β-DHAAs as β-turn inducers has been shown by conformational analysis of model peptides.[7] The (*Z*/*E*) stereochemistry of the double bond is a crucial factor for these structures and for the activity.[3]

The oxidative conversion of peptides into dehydro analogues has been proposed to account for the occurrence of α,β-DHAA residues in microbial peptides. In fact, a common biosynthetic pathway has been suggested for the formation of dehydro- and D-amino acids. They would not be directly incorporated into the peptides but derived from the L-isomer, after incorporation into a biosynthetic intermediate, through a dehydrogenation–hydrogenation sequence.[3b] In the laboratory, the most used methods for the synthesis of α,β-DHPs involve condensation[8] or β-elimination reactions.[9] In the latter cases, the introduction of suitable leaving groups and the availability of appropriate reagents is required.

DHAAs and DHPs, in turn, are valuable synthetic precursors for peptide surrogates constituted by unnatural amino acid residues. For instance, they can be reduced to afford saturated derivatives. The hydrogenation of α,β-DHPs both in homogeneous and heterogeneous phases has been described to proceed with variable stereoselectivity.[10] The asymmetric catalytic hydrogenation of achiral substrates has been accomplished by using chiral rhodium complexes.[11] Nevertheless, examples on the hydrogenation of chiral DHPs with chiral catalysts are very scarce in the literature.[12]

Among the peptides containing carbocyclic structural units, some natural or designed cyclobutyl peptides are relevant because of their interesting properties. For instance, the antibiotic X-1092 is a dipeptide produced by the microorganism *Streptomyces species X-1092*.[13] Moreover, the incorporation of the cyclobutane ring in conformationally constrained peptidomimetics has resulted in the production of biologically active deriva-

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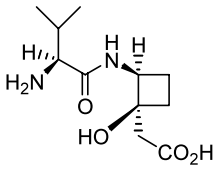
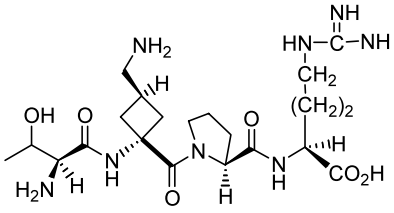


X-1092



Thr-[MOm^2]-Pro-Arg

tives such as the tuftsin analogue Thr-[MOm^2]-Pro-Arg, which exhibited high resistance to enzymatic hydrolysis as compared to tuftsin.[14]

With all these features in mind, we have accomplished the stereoselective synthesis of optically active cyclobutyl DHPs by direct coupling of glycine and (*S*)-phenylalanine, respectively, and a (*Z*)-dehydroamino acid obtained from (−)-verbenone as a chiral precursor (Scheme 1). Their hydrogenation using different chiral catalysts such as rhodium complexes with (*R*,*R*)-Me–duphos, and (*R*,*R*)- and (*S*,*S*)-Et-duphos has been investigated. For every case, we have considered the influence of the chirality in both the substrate and the catalyst on the configuration of the newly created stereogenic centre. The resultant cyclobutyl saturated dipeptides possess three or four asymmetric carbons with unambiguously determined absolute configuration.

2. Results and discussion

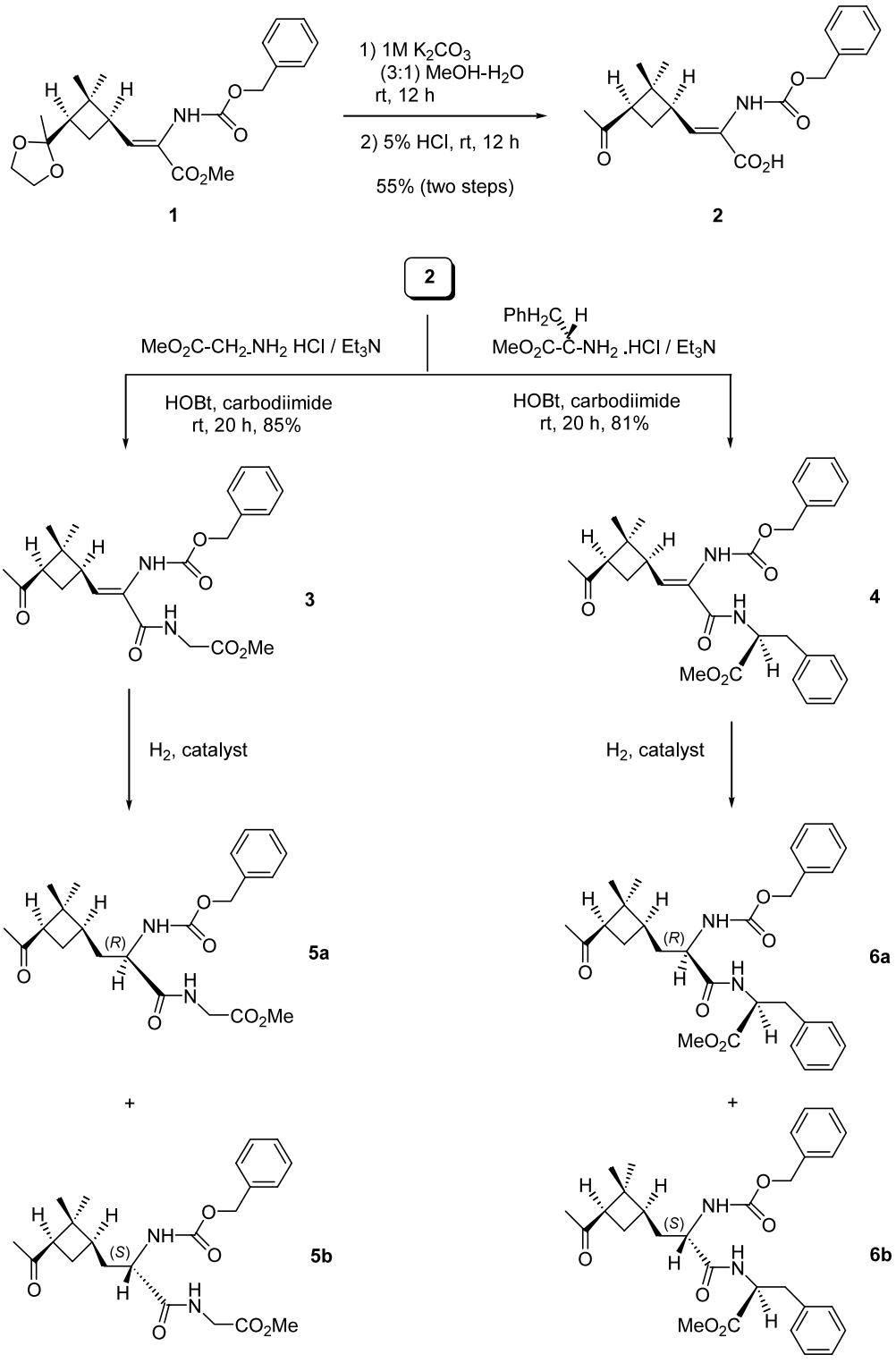
As previously reported, conveniently protected dehydro-amino acid **1** was synthesized by Wadsworth–Emmons condensation of a phosphonate, affording the amino acid function, and an aldehyde prepared from (−)-verbenone as the chiral precursor bearing the cyclobutane moiety. The (*Z*)-configuration of the double bond was established by NOE experiments.[15] Actually, mild saponification of the methyl ester was carried out using 1 M K_2CO_3 in (3:1) MeOH–H_2O at room temperature for 12 h. Subsequent treatment with 5% HCl gave the keto acid **2** (Scheme 1) without epimerization as verified by ^{1}H and ^{13}C NMR. Acid **2** was used for coupling with the other amino acids. Thus, reaction of **2** with glycine methyl ester, in the presence of 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide as dehydrating agent and hydroxybenzotriazole (HOBt) as a catalyst, at room temperature for 20 h afforded dipeptide **3** in 85% yield. Similarly, condensation of acid **2** with (*S*)-phenylalanine methyl ester gave dipeptide **4** in 81% yield. Both compounds were identified and fully characterized by their spectral data and physical constants.

The choice of the hydrogenation catalyst was envisioned on the basis of our preliminary results on the reduction of cyclobutyl dehydroamino acids derived from (−)-α-pinene and (−)-verbenone. We reported in an early communication that good diastereoselectivity was obtained when a (*S*,*S*)-Et-duphos-Rh was used. Moreover, hydrogenations promoted by such a catalyst were chemoselective, being compatible with the Cbz–amine protection. In addition, these reactions were completed much faster than the hydrogenations catalyzed by chiraphos–Rh (cf. 1 and 6 days, respectively) thus avoiding eventual epimerization of the stereogenic centers.[16] For this reason, we carried out the hydrogenation of dipeptides **3** and **4** in the presence of (*R*,*R*)- and (*S*,*S*)-Et-duphos-Rh in order to establish whether the configuration of the new stereogenic centre was induced by the chirality of the substrate and/or the catalyst. The use of (*R*,*R*)-Me–duphos–Rh was also investigated since this catalyst has been described to induce better stereoselectivity in the hydrogenation of sterically congested substrates than rhodium complexes based on phospholanes bearing bulkier alkyl groups.[12c,17]

Table 1 shows the results obtained. Both in the hydrogenation of **3** and **4**, (*R*)-diastereoisomers were produced as the major isomers when (*R*,*R*)-Et-duphos-Rh was employed as the catalyst (entries 1 and 4). A similar result was obtained when using (*R*,*R*)-Me–duphos–Rh for the reduction of **3** (entry 2). Alternatively, the (*S*)-diastereoisomers predominated in the reactions catalyzed by (*S*,*S*)-Et-duphos-Rh (entries 3 and 5). All these results show clearly that the chirality of the catalyst is the factor governing the diastereoselection.

Furthermore, the diastereomeric excesses (*de*) were higher for **6a**/**b** than for **5a**/**b**. This result suggests that the additional stereogenic centre in **4**, coming from (*S*)-phenylalanine, can cooperate in a double asymmetric induction. This hypothesis is also sustained by the fact that hydrogenation of **4** catalyzed by (*S*,*S*)-Et-duphos-Rh is slower and affords lower yield and lower *de* than the hydrogenation of the same substrate catalyzed by the enantiomeric rhodium complex (compare entries 4 and 5 in Table 1). Thus, substrate **4** and the (*R*,*R*)-catalyst are the matched pair while mismatching would occur with the (*S*,*S*)-catalyst.

The (*R*)-configuration of **5a** was unambiguously assigned by X-ray structural analysis of a single crystal obtained from the mixture **5a**/**b** in ethanol.[18] Suitable crystals could not be obtained for **6a** and **6b**. Nevertheless, NMR studies allowed us to assign the configuration as well as the preferred conformation for these compounds. Thus, complete ^{1}H and ^{13}C resonance assignments were performed using gradient-based 2D techniques. NOESY experiments support the stereochemistry depicted in Figure 1 for these molecules showing (*R*)-configuration for the new stereogenic cen-



Scheme 1.

tre in **6a** and (*S*)-configuration in **6b**. It is noteworthy that **6a** contains an amino acid residue with (*S*)-configuration, proceeding from (*S*)-phenylglycine (L-amino acid), and the other one with (*R*)-configuration (D-amino acid), as the result of the catalyst induced diastereoselection.

The ^{1}H NMR spectra show very different chemical shifts for the two N*H* protons in each stereoisomer thus suggesting a marked conformational bias ($\Delta\delta$(Hb–Ha) = 1.21 ppm for **6a** and 0.98 ppm for **6b**). The NOESY data are in good agreement with the Chem3D optimized structures for these compounds (Fig. 1) in which the disposition of the two chains containing the benzyl carbamate and the second amino acid residue, respectively, are favored by the formation of intramolecular hydrogen bonds.

Table 1. Hydrogenation[a] of dehydropeptides **3** and **4** with catalysts [(COD)Rh(*R*,*R*)-Et-duphos)]OTf (*R*,*R*)-Et-**7**, [(COD)Rh(*S*,*S*)-Et-duphos)]OTf (*S*,*S*)-Et-**7**, and [(COD)Rh(*R*,*R*)-Me-duphos)]OTf (*R*,*R*)-Me-**8**, and diastereomeric excess of products[b]

Entry	Substrate	Catalyst	Product[c]	Configuration[d]	% *de*[e]	% Yield[f]
1	**3**	(*R*,*R*)-Et-**7**	**5a**	*R*	82	96
2	**3**	(*R*,*R*)-Me-**8**	**5a**	*R*	82	96
3	**3**	(*S*,*S*)-Et-**7**	**5b**	*S*	78	90
4	**4**	(*R*,*R*)-Et-**7**	**6a**	*R*	>99	88
5	**4**	(*S*,*S*)-Et-**7**	**6b**	*S*	90	82

[a] As MeOH solutions under 4 atm pressure for 1 day in entries 1–4 but 2 days in entry 5.
[b] Determined by ^{1}H NMR.
[c] Major diastereomer.
[d] Refers to the new stereogenic center in the major diastereomer.
[e] At 100% conversion.
[f] Total yield of the purified mixture **a**/**b**.

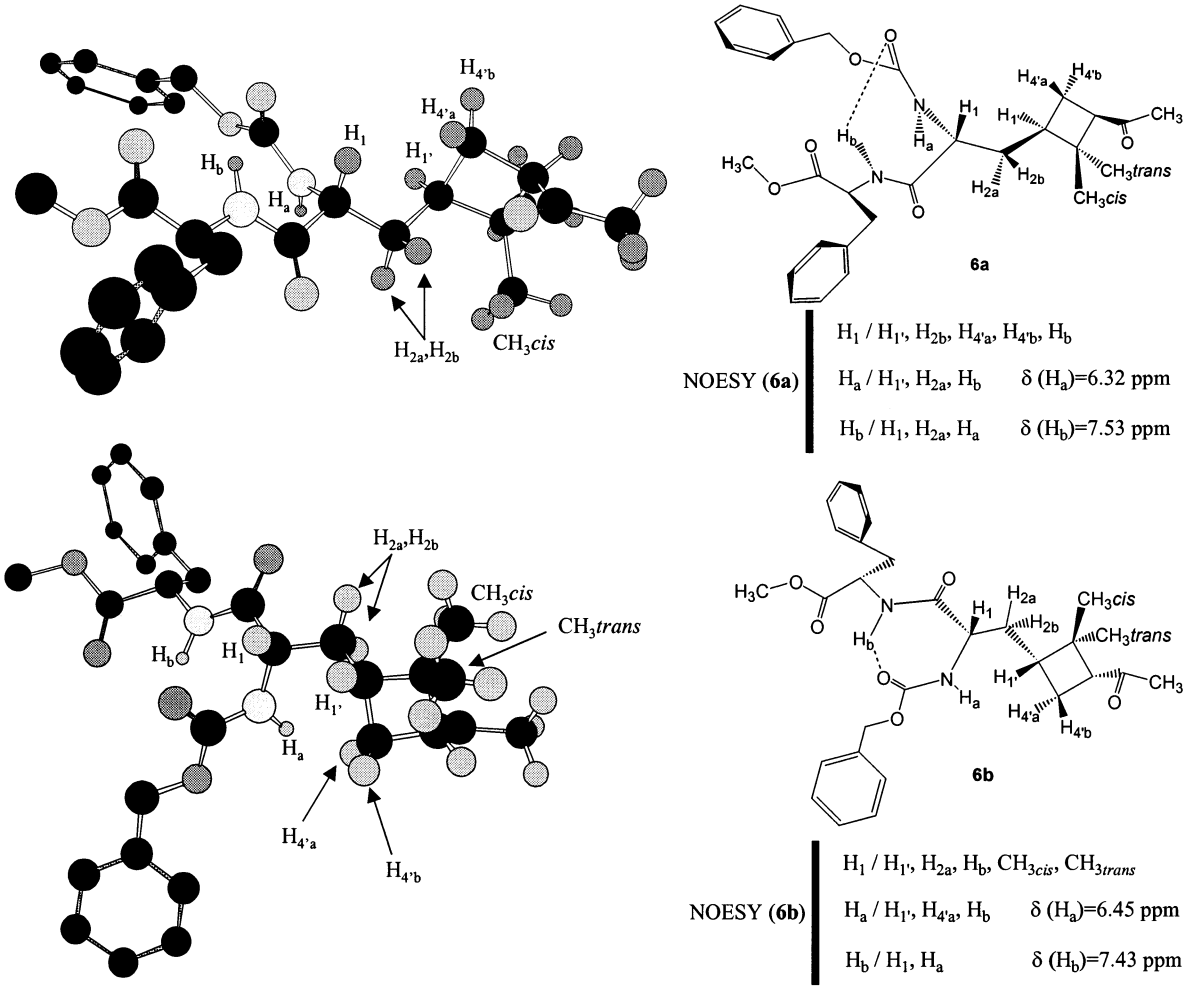


Figure 1. Chem3D optimized structures (left) and preferred conformations from NMR data (right) for peptides **6a** and **6b**.

The CD spectra between 340 and 220 nm for unsaturated DHPs **3** and **4**, and saturated **5b** and **6a**, in methanol, are shown in Figure 2. Two bands of similar shape are observed for **3** and **4**. Both compounds show a broad negative band centered at 290 nm, which could be related to a negative Cotton effect, and a positive band between 250 and 210 nm, being lower intensity for **3**. The CD spectrum of **6a** shows a broad negative band between 320 and 250 nm and a positive one with a maximum at 220 nm. Peptide **5b** shows two broad negative bands of different intensities between 320 and 250 and between 250 and 210 nm, respectively. The shapes of these CD spectra follow the same trend that those of some small peptides, which are reported to be β-turn inducers.[5a,7] Therefore, all these features, jointly with the NMR data, account for a remarkable conformational preference of these molecules.

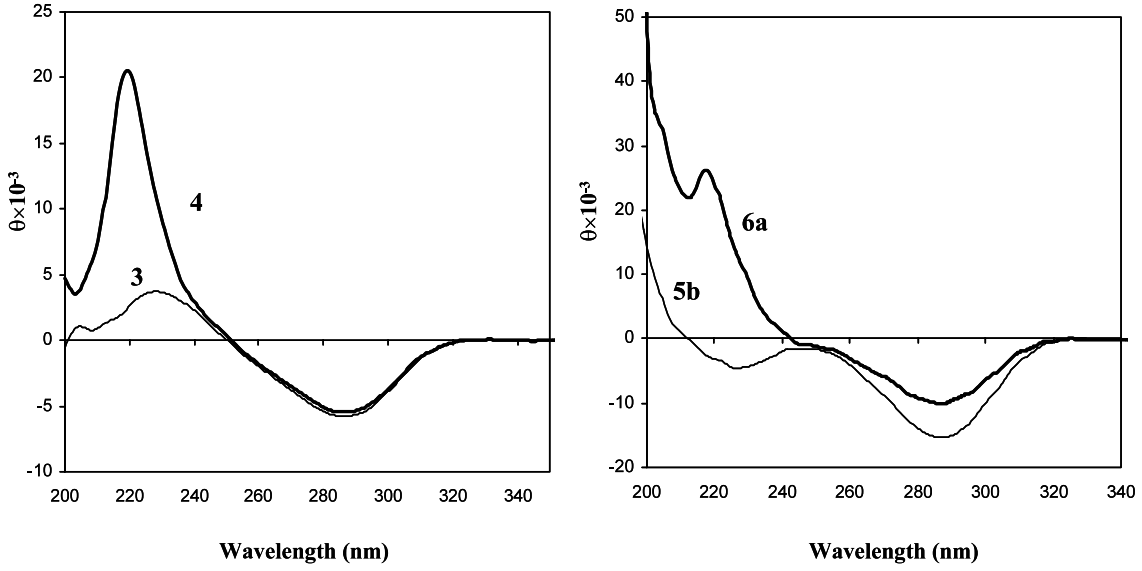


Figure 2. CD spectra (methanol) of DHPs **3** and **4** (left), and saturated peptides **5b** and **6a** (right).

3. Conclusions

The easy and efficient synthesis of cyclobutyl α,β-DHPs and their diastereoselective hydrogenation using duphos–Rh complexes has been achieved. We have shown that the configuration of the resultant stereogenic centre can be predicted by considering the chirality of the catalyst employed. A conformational bias can be assumed for the synthesized peptides on the basis of their NMR and CD spectroscopic data.

4. Experimental

4.1. General

Commercial (–)-verbenone (90% e.e.) was used as synthetic precursor without further purification. Compound **1** was synthesized as described in the literature.[15] Flash column chromatography was carried out on Baker-silica gel (400 mesh). Melting points were determined on a hot stage and are uncorrected. CD data were acquired on a spectropolarimeter equipped with a diode-array detector and measurements were made using methanol solutions contained in 1.0 nm pathlength cells. The CD spectra are the averages of four scans acquired over a 1 h-period with the base-lines subtracted. Standard ^{1}H and ^{13}C NMR spectra were recorded at 250 and 62.5 MHz, respectively, unless otherwise stated. Chemical shifts are given on the δ scale. Electron impact mass spectra were recorded at 70 eV.

4.2. Hydrolysis of compound 1: synthesis of (1′*R*,3′*R*)-2-benzyloxycarbonylamino-3-(3′-acetyl-2′,2′-dimethylcyclobutyl)-(*Z*)-2-propenoic acid, 2

A mixture of ester **1** (187 mg, 0.4 mmol) and K_2CO_3 (308 mg, 2.2 mmol) in (3:1) MeOH–H_2O (2 mL) was stirred at rt for 12 h. Then, MeOH was removed under reduced pressure and the aqueous resultant solution was extracted with ether. Subsequently, 5% HCl was added to the aqueous phase to reach pH 2 and the resultant acid solution was stirred for 12 h. The solution was extracted with ether, the combined extracts were dried ($MgSO_4$) and solvent was removed to afford keto acid **2** (86 mg, 55% yield) as an oil that was characterized and used for coupling with other amino acids without further purification. $[\alpha]_D$ −20.9 (*c* 0.76, MeOH); IR (film): 3500–3000 (broad), 2958, 1704 cm^{-1}; ^{1}H NMR (acetone-d_6): 0.90 (s, 3H, *c*-2′-CH_3), 1.31 (s, 3H, *t*-2′-CH_3), 1.89–2.25 (complex absorption, 2H, $H_{4'a}$, $H_{4'b}$), 2.01 (s, 3H, COCH_3), 2.96–3.08 (complex absorption, 2H, $H_{1'}$, $H_{3'}$), 5.11 (s, 2H, PhCH_2), 6.59 (d, $J=8.8$ Hz, 1H, =C*H*), 7.26–7.41 (complex absorption, 5H, Ph), 7.54 (broad s, 1H, N*H*); ^{13}C NMR (acetone-d_6): 17.78 (*c*-2′-*C*H_3), 22.86 ($C_{4'}$), 29.23, 29.87 (*t*-2′-*C*H_3, and CO*C*H_3), 39.72 ($C_{1'}$), 45.06 ($C_{2'}$), 53.33 ($C_{3'}$), 66.15 (Ph*C*H_2), 127.53, 127.74, 127.77, 128.29 (6C, 5*C*$H_{aromatic}$, C_α), 137.41 (C_{ipso}), 137.55 (C_β), 154.52 (NH*C*O_2CH_2Ph), 164.97 (*C*O_2H), 205.91 (*C*OCH_3).

4.3. (1′*R*,3′*R*)-1-Benzyloxycarbonylamino-2-(3′-acetyl-2′,2′-dimethylcyclobutyl)-1-carboxylic acid *N*-methoxycarbonylmethyl-1-(*Z*)-enamide, 3

To a solution of acid **2** (85 mg, 0.2 mmol) in freshly distilled DMF (1.5 mL) dry TEA (34 μL, 0.2 mmol), methyl ester of glycine hydrochloride (46 mg, 0.4 mmol), HOBt (17 mg, 0.1 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (142 mg, 0.7 mmol) were subsequently added under a nitrogen atmosphere. The resultant mixture was light-protected and stirred at rt for 20 h. After this period of time, EtOAc (10 mL) was added and the solution was washed with saturated aqueous $NaHCO_3$ and dried over $MgSO_4$. The solvents were removed at reduced pressure and the residue was chromatographed on Baker-silica gel (1:1 CH_2Cl_2–EtOAc as eluent) to afford dipeptide **3** as an oil (87 mg, 85% yield). $[\alpha]_D$ −8.6 (*c*

0.58, MeOH); IR (film) 3306 (broad, NH), 2955, 1729, 1705, 1667 (C=O), 1635 cm^{-1}; ^1H NMR (acetone-d_6): 0.87 (s, 3H, *c*-2′-CH_3), 1.26 (s, 3H, *t*-2′-CH_3), 1.84–2.22 (complex absorption, 2H, $H_{4'a}$, $H_{4'b}$), 1.99 (s, 3H, COCH_3), 2.85–2.99 (complex absorption, 2H, $H_{1'}$, $H_{3'}$), 3.64 (s, 3H, CO_2CH_3), 3.98 (d, $J=5.8$ Hz, 2H, CH_2NH), 5.10 (s, 2H, PhCH_2O), 6.44 (d, $J=8.8$ Hz, 1H, =C*H*), 7.25–7.38 (complex absorption, 5H, Ph), 7.69 (broad s, 2H, 2N*H*); ^{13}C NMR (acetone-d_6): 18.56 (*c*-2′-*C*H_3), 23.68 ($C_{4'}$), 30.06, 30.65 (CO*C*H_3, *t*-2′-*C*H_3), 40.19 ($C_{1'}$), 41.75 (NH*C*H_2), 45.83 ($C_{2'}$), 52.05 ($C_{3'}$), 54.13 (*C*H_3CO_2), 67.07 (Ph*C*H_2O), 128.52, 128.61, 128.63 ($CH_{aromatic}$), 129.13 (C_α), 130.94 (C_β), 134.39 (C_{ipso}), 155.40 (PhCH$_2$O*C*ONH), 165.50 (-*C*ONHCH$_2$CO$_2$Me), 170.91 (*C*O_2Me), 206.87 (*C*OCH$_3$). HRMS: calcd for $C_{22}H_{28}N_2O_6$ (M): 416.4664; found: 416.1946. Calcd for $C_{21}H_{25}N_2O_5$ (M–CH_3O): 385.4326; found: 385.1785. Calcd for $C_{16}H_{18}N_2O_5$ (M–$C_6H_{10}O$): 318.1216; found: 318.1208.

4.4. (1′*S*,3′*R*)-1-Benzyloxycarbonylamino-2-(3′-acetyl-2′,2′-dimethylcyclobutyl)-1-carboxylic acid (1″*S*)-*N*-(1″-benzyl-1″-methoxycarbonyl)methyl-1-(*Z*)-enamide, 4

Compound **4** was prepared in 81% yield following the procedure described above for the synthesis of dipeptide **3**. Crystals, mp 131–135°C; $[\alpha]_D$ −30 (*c* 0.1, MeOH); IR (film): 3311 (broad, NH), 3954, 1739, 1700, 1667, 1633 cm^{-1}; ^1H NMR (acetone-d_6): 0.85 (s, 3H, *c*-2′-CH_3), 1.25 (s, 3H, *t*-2′-CH_3), 1.81–2.25 (complex absorption, 2H, $H_{4'a}$, $H_{4'b}$), 2.01 (s, 3H, COCH_3), 2.75–3.11 (complex absorption, 4H, $H_{1'}$, $H_{3'}$, PhCH_2), 3.63 (s, 3H, CO_2CH_3), 4.72 (m, 1H, C*H*CO$_2$Me), 5.09 (s, 2H, PhCH_2O), 6.34 (d, $J=8.9$ Hz, 1H, =C*H*), 7.15–7.40 (complex absorption, 11H, 2Ph, N*H*), 7.65 (broad s, 1H, N*H*); ^{13}C NMR (acetone-d_6): 18.31 (*c*-2′-*C*H_3), 23.41 ($C_{4'}$), 29.73, 30.36 (*t*-2′-*C*H_3, CO*C*H_3), 37.86 (*C*H_2Ph), 39.93 ($C_{1'}$), 45.11 ($C_{2'}$), 51.91 (CO_2CH_3), 53.86 ($C_{3'}$), 54.31 (C_2), 66.80 (Ph*C*H_2O), 127.18, 128.34, 128.84, 129.82 ($CH_{aromatic}$), 130.40 (C_5), 133.80 (C_6), 137.22 (C_{ipso}), 155.03 (NH*C*O_2CH$_2$Ph), 164.56 (*C*ONHCH), 172.16 (*C*O_2CH$_3$), 206.39 (*C*OCH$_3$). Anal. calcd for $C_{29}H_{34}N_2O_6$: C, 68.76; H, 6.77; N, 5.53. Found: C, 68.43; H, 6.68; N, 5.41.

4.5. General procedure for the catalytic hydrogenation of dehydropeptides 3 and 4

Substrates **3** and **4** were hydrogenated as the respective 0.1 M MeOH solutions under 2 atmospheres pressure in the presence of the catalyst (1:24 substrate/catalyst ratio) at rt for 1 day (2 days for the hydrogenation of **4** by using (*S*,*S*)-Et–duphos–Rh as a catalyst). Solvent was removed and the residue was chromatographed on Baker-silica gel (1:1 CH_2Cl_2–EtOAc as eluent).

4.6. (1*R*,1′*S*,3′*R*)- and (1*S*,1′*S*,3′*R*)-1-benzyloxycarbonylamino-2-(3′-acetyl-2′,2′-dimethylcyclobutyl)-1-carboxylic acid *N*-methoxycarbonylmethyl amide, 5a and 5b, respectively

Pure peptide **5a** was obtained by crystallization of the reaction mixture and was fully characterized. NMR spectroscopic data for oily **5b** were determined from enriched chromatographic fractions.

4.6.1. Compound 5a. Crystals, mp 75–79°C (from ethanol), $[\alpha]_D$ +32 (*c* 0.09, methanol); IR (film) 3321 (broad, NH), 2954, 1748, 1704, 1667 cm^{-1}; 500 MHz ^1H NMR (acetone-d_6): 0.81 (s, 3H, *t*-2′-CH_3), 1.24 (s, 3H, *c*-2′-CH_3), 1.40–2.22 (complex absorption, 5H, H_{2a}, H_{2b}, $H_{4'a}$, $H_{4'b}$, $H_{1'}$), 1.98 (s, 3H, COCH_3), 2.78–2.91 (m, 1H, $H_{3'}$), 3.66 (s, 3H, CO_2CH_3), 3.88–4.01 (complex absorption, 2H, NHCH_2), 4.14 (m, 1H, H_1), 5.02–5.12 (complex absorption, 2H, OCH_2), 6.46 (d, $J_{NH,H1}=7.7$ Hz, N*H*CO$_2$), 7.28–7.38 (complex absorption, 5H, Ph), 7.71 (broad s, 1H, N*H*); ^{13}C NMR (acetone-d_6): 17.57 (*c*-2′-*C*H_3), 23.72 ($C_{4'}$), 30.20, 30.36 (*t*-2′-*C*H_3, CO*C*H_3), 33.42 (C_2), 39.02 ($C_{1'}$), 41.45 (MeCO$_2$–*C*H$_2$–NH), 43.32 ($C_{2'}$), 52.09 ($C_{3'}$), 54.73 (CO_2CH_3), 54.99 (C_1), 66.77 (Ph*C*H_2O), 128.57, 128.59, 129.17 ($CH_{aromatic}$), 138.08 (C_{ipso}), 156.92 (NH*C*O_2CH$_2$Ph), 170.83 (*C*ONHCH$_2$), 173.20 (*C*O_2CH$_3$), 206.93 (*C*OCH$_3$); HRMS: calcd for $C_{22}H_{30}N_2O_6$ (M): 418.4822; found: 418.2112. Calcd for $C_{20}H_{27}N_2O_5$ (M–C_2H_3O): 375.4377; found: 375.1920.

4.6.2. Compound 5b. 500 MHz ^1H NMR (acetone-d_6): 0.84 (s, 3H, *t*-2′-CH_3), 1.26 (s, 3H, *c*-2′-CH_3), 1.40–2.22 (complex absorption, 5H, H_{2a}, H_{2b}, $H_{4'a}$, $H_{4'b}$, $H_{1'}$), 1.97 (s, 3H, COCH_3), 2.78–2.91 (m, 1H, $H_{3'}$), 3.66 (s, 3H, CO_2CH_3), 3.88–4.01 (complex absorption, 2H, NHCH_2), 4.10–4.16 (m, 1H, H_1), 5.02–5.12 (complex absorption, 2H, OCH_2), 6.53 (d, $J_{NH,H1}=8.2$ Hz, 1H, N*H*CO$_2$), 7.28–7.38 (complex absorption, 5H, Ph), 7.63 (broad s, 1H, N*H*); ^{13}C NMR(acetone-d_6): 17.24 (*c*-2′-*C*H_3), 24.26 ($C_{4'}$), 30.08, 30.38 (*t*-2′-*C*H_3, CO*C*H_3), 33.42 (C_2), 39.45 ($C_{1'}$), 41.45 (MeCO$_2$–*C*H$_2$–NH), 43.64 ($C_{2'}$), 52.09 ($C_{3'}$), 54.73 (CO_2CH_3), 55.04 (C_1), 66.77 (Ph*C*H_2O), 128.57, 128.59, 129.17 ($CH_{aromatic}$), 138.08 (C_{ipso}), 156.92 (NH*C*O_2CH$_2$Ph), 170.83 (*C*ONHCH$_2$), 173.20 (*C*O_2CH$_3$), 206.93 (*C*OCH$_3$).

4.7. (1*R*,1′*S*,3′*R*)- and (1*S*,1′*S*,3′*R*)-1-benzyloxycarbonylamino-2-(3′-acetyl-2′,2′-dimethylcyclobutyl)-1-carboxylic acid (1″*S*)-*N*-(1″-benzyl-1″-methoxycarbonyl)methyl amide, 6a and 6b, respectively

Pure compound **6a** was obtained by column chromatography of the reaction mixture and fully characterized. NMR spectroscopic data for **6b** were determined from enriched fractions.

4.7.1. Compound 6a. Oil, $[\alpha]_D$ −7.7 (*c* 1.2, MeOH); IR (film): 3307 (broad, NH), 2954, 1733, 1703, 1667 cm^{-1}; 500 MHz ^1H NMR (acetone-d_6): 0.77 (s, 3H, *t*-2′-CH_3), 1.21 (s, 3H, *c*-2′-CH_3), 1.41-2.10 (complex absorption, 5H, H_{2a}, H_{2b}, $H_{4'a}$, $H_{4'b}$, $H_{1'}$), 1.97 (s, 3H, COCH_3), 2.77 (m, 1H, $H_{3'}$), 2.96–3.16 (complex absorption, 2H, CH_2Ph), 3.66 (s, 3H, CO_2CH_3), 4.06 (m, 1H, H_1), 4.72 (m, 1H, $H_{1''}$), 5.03–5.11 (complex absorption, 2H, OCH_2Ph), 6.32 (d, $J_{NH,H1}=8.55$ Hz, 1H, N*H*CO$_2$), 7.11–7.41 (complex absorption, 10H, 2Ph), 7.53 (d, $J_{NH,H1''}=8.55$ Hz, 1H, CHCON*H*CH); ^{13}C NMR (acetone-d_6): 16.68 (*c*-2′-*C*H_3), 22.83 ($C_{4'}$), 29.65, 29.88 (*t*-2′-*C*H_3, CO*C*H_3), 33.39 (C_2), 37.29 (CH*C*H_2Ph), 38.59 ($C_{1'}$), 42.74 ($C_{2'}$), 51.41 (CO_2CH_3), 52.86 (C_1),

53.39 ($C_{3'}$), 54.27 ($C_{1''}$), 65.89 (Ph*C*H_2O), 126.60, 127.68, 128.26, 129.24 (10 *C*$H_{aromatic}$), 136.85, 137.26 (2 C_{ipso}), 155.97 (NH*C*$O_2$$CH_2$Ph, *C*ONHCH), 171.50 (*C*$O_2$$CH_3$), 206.08 (*C*O$CH_3$); HRMS: Calcd. for $C_{29}H_{36}N_2O_6$ (M): 508.2573; found: 508.2540. Calcd for $C_{27}H_{33}N_2O_5$ (M–C_2H_3O): 465.2389; found: 465.2385.

4.7.2. Compound 6b. 500 MHz ^{1}H NMR (acetone-d_6): 0.81 (s, 3H, *t*-2′-CH_3), 1.24 (s, 3H, *c*-2′-CH_3), 1.41–2.10 (complex absorption, 5H, H_{2a}, H_{2b}, $H_{4'a}$, $H_{4'b}$, $H_{1'}$), 1.97 (s, 3H, COCH_3), 2.77 (m, 1H, $H_{3'}$), 2.96–3.16 (complex absorption, 2H, CH_2Ph), 3.66 (s, 3H, CO_2CH_3), 4.06 (m, 1H, H_1), 4.72 (m, 1H, $H_{1''}$), 5.03–5.11 (complex absorption, 2H, OCH_2Ph), 6.45 (d, $J_{NH,H1'}$ = 7.85 Hz, 1H, N*H*CO_2), 7.11–7.41 (complex absorption, 10H, 2Ph), 7.43 (d, $J_{NH,H1''}$ = 7.90 Hz, 1H, CHCON*H*CH); ^{13}C NMR (acetone-d_6): 16.36 (*c*-2′-*C*H_3), 23.42 ($C_{4'}$), 29.65, 29.88 (*t*-2′-*C*H_3, CO*C*H_3), 32.39 (C_2), 37.29 (CH*C*H_2Ph), 38.09 ($C_{1'}$), 42.43 ($C_{2'}$), 51.41 (CO_2*C*H_3), 53.44 (C_1), 53.89 ($C_{3'}$), 54.53 ($C_{1''}$), 65.89 (Ph*C*H_2O), 126.60, 127.68, 128.26, 129.24 (10 *C*$H_{aromatic}$), 136.85, 137.26 (2 C_{ipso}), 155.97 (NH*C*$O_2$$CH_2$Ph, *C*ONHCH), 171.50 (*C*$O_2$$CH_3$), 206.08 (*C*O$CH_3$).

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References

1. (a) Givot, I. L.; Smith, T. A.; Abeles, R. H. *J. Biol. Chem.* **1969**, *244*, 6341; (b) Hanson, K. R.; Havier, E. A. *Arch. Biochem. Biophys.* **1970**, *141*, 1; (c) Wickner, R. B. *Ibid* **1969**, *244*, 6550.
2. Gupta, A.; Chauhan, V. S. *Biopolymers* **1990**, *30*, 395.
3. (a) Khoklov, A. S.; Lokshin, G. B. *Tetrahedron Lett.* **1963**, *4*, 1881; (b) Demain, A. L. In *Biosynthesis of Antibiotics*; Snell, J. F., Ed.; Academic Press: London and New York, 1966; p. 29; (c) Gross, E.; Morell, J. L. *J. Am. Chem. Soc.* **1967**, *89*, 2791; (d) Bycroft, B. W. *Nature (London)* **1969**, *224*, 595; (e) Gross, E.; Morell, J. L.; Craig, L. C. *Proc. Natl. Acad. Sci. USA* **1969**, *62*, 952; (f) Gross, E.; Kiltz, H. H. *Biochem. Biophys. Res. Commun.* **1973**, *50*, 559.
4. Wakamara, T.; Shimbo, K.; Sano, A.; Fukase, K.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 2044.
5. (a) Pieroni, O.; Montagnoli, G.; Fissi, A.; Merlino, S.; Ciardelli, F. *J. Am. Chem. Soc.* **1975**, *97*, 6820; (b) Ciajolo, M. R.; Tuzi, A.; Pratesi, C. R.; Fissi, A.; Pieroni, O. *Biopolymers* **1990**, *30*, 911.
6. Swiatek-Kozlowska, J.; Brasun, J.; Chruscinski, L.; Chruscinska, E.; Makowski, M.; Kozlowski, H. *New. J. Chem.* **2000**, *24*, 893.
7. Pietrzynski, G.; Rzeszotarska, B.; Ciszak, E.; Lisowski, M. *Polish J. Chem.* **1994**, *68*, 1015.
8. (a) Schmidt, U.; Lieberknecht, A.; Wild, J. *Synthesis* **1984**, 53; (b) Shin, C. N.; Takahashi, N.; Yonezawa, Y. *Chem. Phar. Bull.* **1990**, *38*, 2020.
9. (a) Andrusziewicz, R.; Czerwinski, A. *Synthesis* **1982**, 968; (b) Somekh, L.; Shanzer, A. *J. Org. Chem.* **1983**, *48*, 907; (c) Schmidt, U.; Lieberknecht, A.; Wild, J. *Synthesis* **1988**, 159; (d) Balsamini, C.; Duranti, E.; Mariani, L.; Salvatori, A.; Spadoni, G. *Synthesis* **1990**, 779; (e) Berti, F.; Eber, C.; Gardossi, L. *Tetrahedron Lett.* **1992**, *33*, 8145.
10. Lisichkina, I. N.; Ivanova, A. G.; Peregudov, A. S.; Belikov, V. M. *Russ. Chem. Bull., Int. Ed.* **2001**, *50*, 738.
11. (a) Meyer, D.; Poulin, J. C.; Kagan, H. B. *J. Org. Chem.* **1980**, *45*, 4680; (b) Yamagishi, T.; Yatagai, M.; Hatakeyama, H.; Hida, M. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 1897; (c) Ojima, I. *Pure Appl. Chem.* **1984**, *56*, 99.
12. See, for instance: (a) Takasaki, M.; Harada, K. *Chem. Lett.* **1984**, 1745; (b) Schmidt, U.; Lieberknecht, A.; Kazmaier, U.; Griesser, H.; Jung, G.; Metzger, J. *Synthesis* **1991**, 49; (c) Debenhamm, S. D.; Debenham, J. S.; Burk, M. J.; Toone, E. J. *J. Am. Chem. Soc.* **1997**, *119*, 9897.
13. (a) Adlington, R. M.; Baldwin, J. E.; Jones, R. H.; Murphy, J. A.; Parisi, M. F. *J. Chem. Soc., Chem. Commun.* **1983**, 1479; (b) Baldwin, K. A.; Adlington, R. M.; Parisi, M. F.; Ting, H.-H. *Tetrahedron* **1986**, *42*, 2575.
14. Gershonov, E.; Granoth, R.; Tzehoval, E.; Gaoni, Y.; Fridkin, M. *J. Med. Chem.* **1996**, *39*, 4833.
15. Moglioni, A. G.; García-Expósito, E.; Aguado, G. P.; Parella, T.; Branchadell, V.; Moltrasio, G. Y.; Ortuño, R. M. *J. Org. Chem.* **2000**, *65*, 3934.
16. Aguado, G. P.; Álvarez-Larena, A.; Illa, O.; Moglioni, A. G.; Ortuño, R. M. *Tetrahedron: Asymmetry* **2001**, *12*, 25.
17. (a) Albrecht, J.; Nagel, U. *Angew. Chem., Int. Ed. Engl.* **1996**, *24*, 407; (b) Burk, M. J.; Gross, M. F.; Harper, T. G. P.; Kalberg, C. S.; Lee, J. R.; Martínez, J. P. *Pure Appl. Chem.* **1996**, *68*, 37; (c) Burk, M. J.; Gross, M. E.; Martínez, J. P. *J. Am. Chem. Soc.* **1995**, *117*, 9375; (d) Burk, M. J.; Feaster, J. E.; Nugent, W. A.; Harlow, R. L. *J. Am. Chem. Soc.* **1993**, *115*, 10125; (e) Hoermer, R. S.; Askin, D.; Volante, R. P.; Reider, P. J. *Tetrahedron Lett.* **1998**, *39*, 3455.
18. Crystallographic details will be reported elsewhere.